ELSEVIER

Journal of Photochemistry and Photobiology A: Chemistry 135 (2000) 27–32

Journal of Photochemistry and Photobiology A:Chemistry

www.elsevier.nl/locate/jphotochem

Synthesis of 7-amino-4-trifluoromethyl-2-(1H)-quinolinone and its use as an antenna molecule for luminescent europium polyaminocarboxylates chelates

Jiyan Chen, Paul R. Selvin*

Loomis Laboratory of Physics, Physics Department and Center for Biophysics, 1110 W. Green Street, University of Illinois, Urbana, IL 61801, USA

Received 7 September 1999; received in revised form 7 March 2000; accepted 16 March 2000

Abstract

7-Amino-4-trifluoromethyl-2-(1H)-quinolinone (cs124-CF_3), has been synthesized and found to be an effective antenna molecule (or sensitizer) for europium, but not terbium, luminescence. Specifically, cs124-CF_3 has been covalently attached to the chelates diethylenetriaminepentaacetic acid (DTPA) and triethylenepentamine-hexanoic acid (TTHA). When bound to europium, excitation in the absorption band (<365 nm) of the cs124-CF_3 leads to characteristic europium luminescence: a sharply-spiked emission spectrum, and long-lived emission, including 1.19 ms excited-state lifetime for TTHA-CS(CF_3)-Eu^{3+} and 0.62 ms for DTPA-cs124-CF_3-Eu^{3+}. Emission from DTPA-cs124-CF_3-Eu^{3+} (TTHA-CS(CF_3)-Eu^{3+}) is about three times (1.7) brighter than that of the non-fluorinated parent compound, 7-amino-4-methyl-2-(1H)-quinolinone (cs124), which we have previously shown to be a valuable lanthanide sensitizer. The excitation of DTPA-cs124-CF_3-Eu^{3+} is red-shifted approximately 15 nm, enabling excitation at 355 and 365 nm, thereby matching two commonly available excitation sources. The chemical stability of DTPA-cs124-CF_3-Eu^{3+} is also excellent, in contrast to the similar compound, 7-amino-4-trifluoromethyl-2-coumarin. It is expected that cs124-CF_3 will be a valuable sensitizer for europium luminescence in time-resolved fluorescence assays, and will also aide in the design of future sensitizers. It may also be a useful fluorescent dye, independent of lanthanides. © 2000 Published by Elsevier Science S.A.

Keywords: Antenna molecule; Cs124-CF_3; Luminescent; Sensitizer; Lanthanide; Carbostyril; Time-resolved fluorescence

1. Introduction

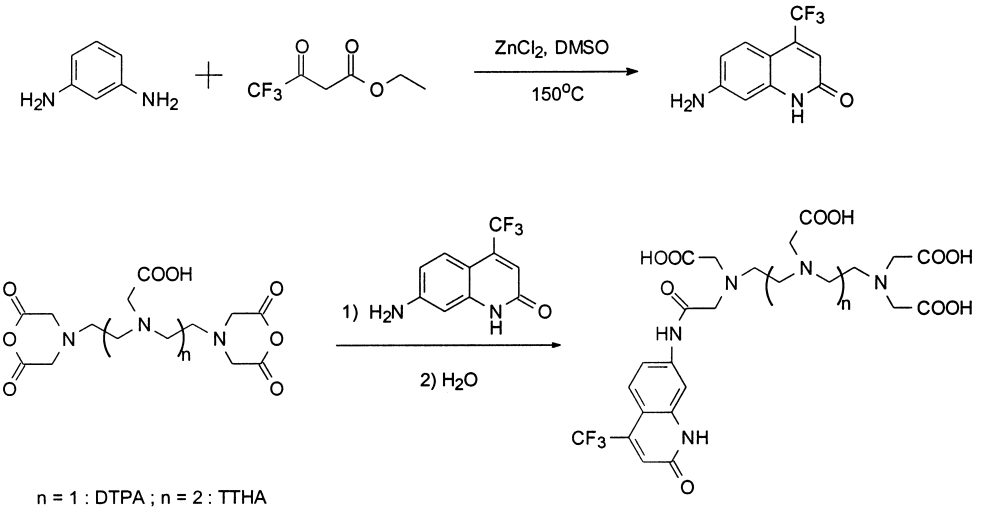
Luminescent lanthanide chelates display unusual spectroscopic characteristics, which include millisecond excited-state lifetime and sharply-spiked emission spectra. Through temporal and wavelength discrimination, picomolar or less concentrations can be measured, even in samples which contain significant short-lived autofluorescence (background fluorescence). Consequently, these probes are of considerable interest in clinical diagnostics, life-sciences, and drug-screening assays (reviewed in [1]).

The lanthanide ions have inherently extremely low absorption cross-sections ($<1\ M^{-1}\ cm^{-1}$) and hence generally require an organic sensitizer or antenna molecule to absorb the excitation light and then transfer the absorbed energy to a nearby lanthanide ion. To be a useful luminescent probe, the lanthanide must also be bound to a chelate that serves several roles, including: tightly binding the lanthanide; filling the lanthanide's coordination sphere to shield it from the quenching effects of water; providing a mechanism for covalent attachment of the sensitizer to keep the lanthanide and sensitizer in close proximity; providing a scaffold to create a reactive group for attachment of the luminescent complex to other macromolecules. The combination of chelate and sensitizer can increase lanthanide luminescent intensity greater than 10,000-fold.

Rational design of lanthanide sensitizers is difficult because the mechanism of energy transfer from sensitizer to lanthanide is complex, although energy transfer through the sensitizer's triplet state is often implicated [2–5]. Nevertheless, there are a number of different lanthanide antennas reported (see [1] for review), some of which are structurally distinct from the chelate, and others that are embedded in the chelate. To date all require UV-excitation. We have developed chelate–antenna complexes in which the chelate and sensitizer are readily synthesized separately and then coupled together, thereby allowing relatively independent optimization. The chelates are based on the widely used polyaminocarboxylate chelates such as diethylenetriaminepentaacetic acid (DTPA) and treiethylenetetraaminehexanoic acid (TTHA). We have shown

* Corresponding author. Tel.: +1-217-244-3371; fax: +1-217-244-7187.
E-mail address: selvin@uiuc.edu (P.R. Selvin)

1010-6030/00/$ – see front matter © 2000 Published by Elsevier Science S.A.
PII: S1010-6030(00)00280-X



Scheme 1. Syntheses of DTPA-cs124-CF_3 and TTHA-cs124-CF_3 as sensitizer-ligand complexes for Eu^{3+} sensitized luminescence.

7-amino-4-methyl-2-(1H)-quinolinone (trivial name carbostyril 124, or cs124), when attached to these chelates, is an efficient sensitizer for both terbium and europium luminescence [6], see Scheme 1. These and other lanthanide chelates–sensitizer complexes have been shown to be particularly excellent donors in resonance energy transfer measurements [7–14].

In this work, we set out to optimize the cs124. The goal was to produce brighter complexes with farther-red excitation. It has been recognized that for beta-diketone complexes of europium, introduction of a fluorine group dramatically increases brightness [15], and by polarizing the antenna molecule, such an electron-withdrawing group may lead to further-red excitation [16]. Here we report the syntheses and photophysical properties of 7-amino-4-trifluoromethyl-2-(1H)-quinolinone (cs124-CF_3) coupled to either DTPA or TTHA. This compound is two to four times brighter with europium (but not terbium) than cs124 and has 15 nm red-shifted excitation. These results suggest that the addition of further electron-withdrawing groups may lead to yet further improvements.

2. Experimental methods

2.1. Chemicals and materials

The following were purchased from Aldrich: diethylenetriaminepentaacetic acid dianhydride (caDPTA); triethylenetetraaminehexaacetic acid (TTHA); cs124; triethylamine (dried by activated molecular sieves before use); 1,3-phenylenediamine; ethyl 4,4,4-trifluoroacetoacetate; anhydrous pyridine; 1,3-dicyclohexylcarbodiimide (DCC); acetic anhydride; and C18-silica on glass TLC plates (150 um layer thick with fluorescent indicator). Glacial acetic acid, ammonium hydroxide, zinc chloride, and anhydrous ethyl ether were purchased from Fisher Scientific. Distilled and deionized water (18 MΩ cm^{-1}) was used throughout. All glassware was washed with a mixed acid solution and thoroughly rinsed with deionized, distilled water [17]. All plastic labware was purchased from Bio-Rad (metal-free). All chemicals were the purest grade available.

2.2. Purification

Reverse phase high-performance liquid chromatography was used to purify DTPA-cs124-CF_3 and TTHA-cs124-CF_3 at room temperature on a Waters Model 600 system with a Dynamax 60 Å C_{18} column (10 mm i.d. or 25 mm i.d. × 250 mm, Rainin, at 3 or 8 ml min^{-1}, respectively) using a linear gradient (solvent A: 0.1 M triethylammonium acetate, pH 6.5; solvent B: acetonitrile). Fractions were vacuum dried at room temperature and the powder stored at −80°C.

2.3. Spectroscopy

Time-resolved and gated luminescence measurements were made on a laboratory-built spectrometer described previously, employing a 5 ns excitation pulse at 337 nm followed by time-resolved detection of lanthanide emission [18,21]. Excited-state lifetimes can be measured, as well as time-delayed emission spectra. For emission spectra, luminescence was collected and integrated beginning 20 μs following the excitation pulse. UV–VIS absorption spectra were taken on Perkin-Elmer UV–VIS Lambda Bio 10 spectrometer. Corrected fluorescence emission spectra were taken on fluorimeter FluoroMAX-2 from ISA Instruments, Inc.

2.4. Synthesis

2.4.1. Triethylenetetraaminehexaacetic acid dianhydride (caTTHA).

The synthesis of caTTHA follows the procedure of Achour et al. with some modification [19]. TTHA (1.33 mg, 2.69 μmol) and DCC (1.11 mg, 2 eq) were sequentially added into 5 ml anhydrous pyridine. The reaction mixture was allowed to proceed at 40°C for 48 h with constant stirring. The reaction mixture was then filtered and washed with 30 ml of ethyl ether three times. The final light-brown solid product was further dried in a vacuum oven for overnight at 40°C. The caTTHA yield, ~60%, was estimated by the next step reaction, coupling with cs124.

2.4.2. 7-Amino-4-trifluoromethyl-2(1H)-quinolinone (cs124-CF_3) (Scheme 1)

To a 25 ml pear-shaped flask was added 1,3-phenylenediamine (1.0 g, 9.3 mmol), zinc chloride (1.4 g, 1.1 eq), 5 ml DMSO, and 1.4 ml ethyl 4,4,4-trifluoroacetoacetate (1 eq), sequentially. The reaction mixture was magnetically stirred and gradually heated to 140–150°C. The reaction was monitored on TLC with ethyl acetate as developing solvent, and the newly formed product appeared bright blue on TLC (R_f=0.8) when excited with λ=365 nm UV light. After 48 h, the final product was purified on silica gel with ethyl acetate as eluant to yield 0.94 g (45%). The ^{1}H NMR chemical shifts (*d*) of cs124-CF_3 in $CHCl_3$ are: 5.66 (2H, 7 amino), 6.48 (1H, 3H), 6.48 (1H, 8H), 6.68 (1H, 6H), 7.45 (1H, 5H), and 10.8 (broad, 1 amide). High resolution MS: 229.0588 (found), 229.0589 (MH^+, calc.), using the fast atom bombardment (FAB) as the ionization method.

2.4.3. DTPA-cs124-CF_3 and TTHA-cs124-CF_3 (Scheme 1)

The synthetic procedures of DTPA-cs124-CF_3 and TTHA-cs124-CF_3 are virtually the same as those of DTPA-cs124 and TTHA-cs124 [6], except extra caution was exercised in preventing long time light shining during the synthesis and subsequent manipulation. The desired products were purified on HPLC, and the final purified products are characterized by mass spectrometry. MS: DTPA-cs124-CF_3-H^+: 604.2 (calc.), 604.1 (found); TTHA-cs124-CF_3-H^+: 705.2 (calc.), 705.3 (found). These conjugated chelates were stored in at −80°C.

2.5. Addition of metal

$EuCl_3$ was added to the chelate in a 0.9:1 mole ratio at >0.1 mM concentration at pH 6–7, usually in 0.1 M TEAA pH 6.5 buffer. For the relative intensity measurements of DTPA-cs124-CF_3-Eu^{3+} versus DTPA-cs124-Eu^{3+} and TTHA-cs124-CF_3-Eu^{3+} versus TTHA-cs124-Eu^{3+}, the $EuCl_3$ was added as 0.25 eq to the corresponding chelates. Similar experiments using terbium instead of europium were performed but did not lead to terbium luminescence.

2.6. Titration of DTPA-cs124-CF_3 with $EuCl_3$

The extinction coefficient of the newly synthesized DTPA-cs124-CF_3 was measured by titration. A DTPA-cs124-CF_3 solution in 0.1 M TEAA pH 6.5 was gradually titrated with known concentration of $EuCl_3$, and the luminescent intensity was measured on a CCD. The DTPA-cs124-CF_3 concentration was calculated when the luminescent intensity increase reached its plateau. The 1:1 ratio of DTPA-cs124-CF_3 to Eu^{3+} was used for this complexation, which was confirmed by our earlier crystal structure study of DTPA-cs124-Eu^{3+} [20].

3. Results and discussion

3.1. Synthesis

The synthesis of cs124-CF_3 is analogous to that of cs124. The successful dehydration of TTHA to caTTHA is a key to attaching the chelate to cs124 and cs124-CF_3 with a specifically defined sensitizer position. Chemically the cs124-CF_3-DTPA and cs124-CF_3-TTHA derivatives are much more stable that those of similar 7-amino-4-trifluoromethylcoumarin derivatives, which we had previously synthesized but which were not chemically stable (unpublished results). This stability is presumably due to the less electrophilic nature of the endocyclic amide bond of cs124 than that of the ester bond in coumarin. The cs124-CF_3-DTPA and cs124-CF_3-TTHA did not show any noticeable degradation in pH 6.5 buffer for months. The identity and purity of cs124-CF_3 were confirmed by mass spectroscopy, NMR, and UV–VIS absorption. Its conjugate with DTPA and TTHA were confirmed by HPLC, mass spectroscopy UV–VIS absorption and when bound to europium, by lanthanide luminescence.

3.2. Absorption and emission spectra of cs124 versus cs124-CF_3

The absorption spectra of cs124-CF_3 bound to DTPA is shown in Fig. 1 and compared to cs124-DTPA. The absorption spectra are identical when bound to TTHA. The cs124-CF_3-chelate absorption maximum is 15 nm red-shifted compared to cs124-chelate and has a maximum extinction coefficient of 21,000 $M^{-1}\,cm^{-1}$ (at 341 nm), compared to a maximum of 12,000–14,000 $M^{-1}\,cm^{-1}$ (at 328 nm) for cs124. (Titrations with various lanthanide stocks to determine the absorption cross-section of cs124 give 12,000–14,000 $M^{-1}\,cm^{-1}$.) The extinction coefficient of cs124-CF_3 and of cs124 at 337 nm (nitrogen laser line), 351 nm (argon ion laser), 355 nm (Nd:YAG tripled), 365 nm (Hg line) are listed in Fig. 1. Note that cs124 has very little or no absorption at 351–365 nm, whereas the trifluoroderivative has strong absorption at

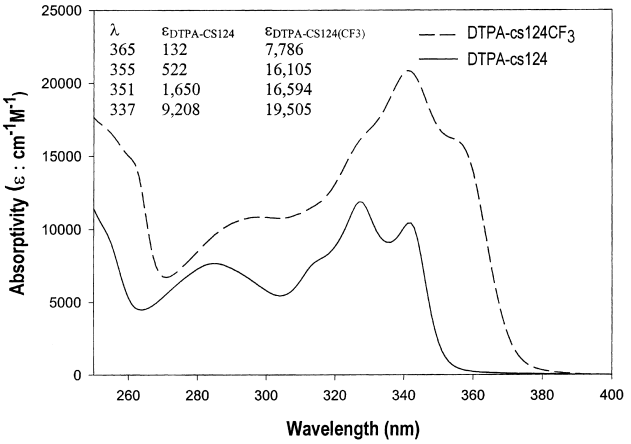


Fig. 1. Extinction coefficients of DTPA-cs124-CF_3 and DTPA-cs124. The DTPA-cs124-CF_3 and DTPA-cs124 samples were 10 μM, in 0.1 mM, pH 6.5 TEAA buffer, and the spectra were taken at room temperature.

these wavelengths. The emission spectra of free cs124 and free cs124-CF_3 (in methanol) are shown in Fig. 2. As expected, when the ring is polarized by introduction of a trifluoro group, the absorption and emission spectra are red-shifted [16].

3.3. Europium emission spectral shape

The relative intensities of the different europium emission lines are a strong function of chelate (compare DTPA versus TTHA (Fig. 3; see also [6]) but are identical when comparing the same chelates with cs124 or cs124-CF_3 (Fig. 3). This is to be expected since europium spectral shape is sensitive to the electric-field symmetry [6] surrounding the lanthanide, which is dominated by coordination from the chelate. The cs124 has been shown to coordinate to the europium only through the 7-amide bond in Eu-DTPA-cs124 [20], and in any case, cs124-CF_3 and cs124 are expected to have the

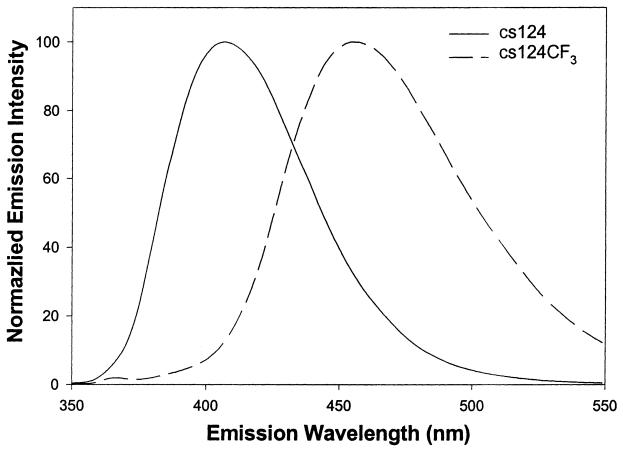


Fig. 2. Comparison of fluorescence emission of free cs124 and cs124-CF_3. The samples were 1 μM of cs124 and cs124-CF_3 in methanol. Corrected emission spectra were taken with λ_{ex}=330 nm, at room temperature.

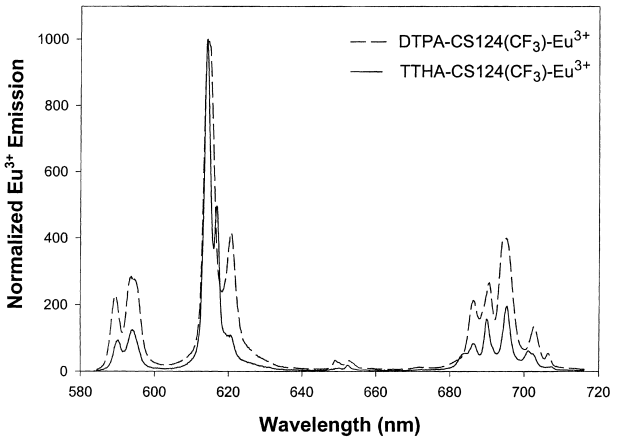


Fig. 3. The emission spectra of DTPA-cs124-CF_3-Eu^{3+} and TTHA-cs124-CF_3-Eu^{3+}. The samples were 1 μM of DTPA-cs124-CF_3-Eu^{3+} or TTHA-cs124-CF_3-Eu^{3+} in 0.1 mM, pH 6.5 triethylammoniumacetate buffer, and were excited by N_2 later at 337 nm with 40 Hz excitation frequency and ~5 μJ per pulse power. The luminescent emission was integrated for 1 s on a CCD detector. The dashed line is the emission of DTPA-cs124-CF_3-Eu^{3+}, and the solid line is the emission of TTHA-cs124-CF_3-Eu^{3+}. The spectra are identical to the corresponding DTPA-cs124-Eu^{3+} and cs124-TTHA compounds [6].

same ligation motif, resulting in identical europium emission spectral shapes.

3.4. Europium emission excited-state lifetime

The Eu^{3+} excited-state life-time is identical when comparing DTPA-cs124 to DTPA-cs124-CF_3 or when comparing TTHA-cs124 to TTHA-cs124-CF_3 (Fig. 4 and [6]). As previously shown, TTHA-based complexes have longer lifetimes, primarily because of the reduced numbers of

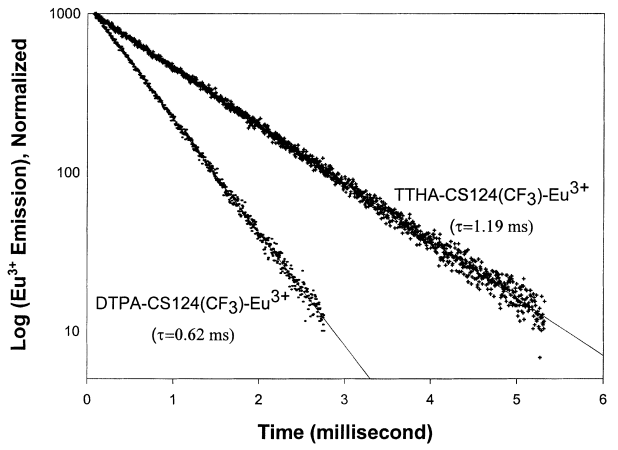


Fig. 4. Excited-state lifetimes of DTPA-cs124-CF_3-Eu^{3+} and TTHA-cs124-CF_3-Eu^{3+}. Data was acquired every 2 μs, digitally binned every 4 μs, and curve fit to a single exponential with TableCurve™. Curve-fits showed no residual structure and had r^2>0.9985. The excited-state lifetime of DTPA-cs124-CF_3-Eu^{3+} is 0.62 ms, and that of TTHA-cs124-CF_3-Eu^{3+} is 1.19 ms, identical to the lifetimes when using cs124 as sensitizer [6].

water ligated to europium [6]. We have previously shown that the excited-state lifetime of Eu-DTPA-cs124 is temperature independent and identical to Eu-DTPA (no sensitizer), proving that the cs124 sensitizer does not cause quenching of the europium. (A back-transfer of energy from the europium to the sensitizer would quench the Eu^{3+} and shorten its excited-state lifetime.) We can now conclude that cs124-CF_3 also does not cause quenching of europium excited state.

3.5. Relative intensity with respect to cs124 derivatives

Cs124CF_3-DTPA-Eu^{3+} and cs124-CF_3-TTHA-Eu^{3+} are 3 and 1.7 time brighter than their cs124 counterparts when excited at 337 nm. The europium brightness is the product of the absorption cross-section of the sensitizer times the efficiency of energy transfer from sensitizer to lanthanide times the lanthanide quantum yield. (The lanthanide quantum yield is the probability of europium emission given that the europium is excited.) From lifetime studies (see above) we can conclude that the Eu quantum yield is identical for europium complexes with cs124 or with cs124-CF_3. Cs124CF_3 has 1.8–2.1 times greater absorption at 337 nm than cs124. Consequently we can conclude that with DTPA, cs124-CF_3 is 3/(1.8–2.1)≈1.5 times more efficient at transferring energy to europium than is cs124. With TTHA, however, cs124-CF_3 is very similar or slightly less efficient (1.7/(1.8–2.1)≈0.8–1).

How can these results be understood? We can present a plausible, though not definitive explanation. We know that with DTPA, the crystal structure shows that the 7-amide group is ligated to the europium [20]. An electron withdrawing group such as trifluoromethyl in the aromatic ring (e.g. cs124-CF_3) may increase the negative charge on the carbonyl oxygen by shifting the equilibrium from NHC=O to NCO^-. This would increase the ligation strength and shorten the bond between the oxygen and the Eu^{3+}. (The CO_2 groups, in which the ligating oxygen is more negative than the equivalent NCO, have a shorter bond length than the NCO [20]). A stronger ligation would tend to increase energy transfer if the energy transfer process was through bond (such as electron transfer). A similar mechanism has been proposed to understand the increase in brightness of europium bound to β-diketones upon substitution of CF_3C=O for CH_3C=O [15].

The situation must be somewhat different for TTHA. We do not have a crystal structure of TTHA-cs124 although TTHA has nine coordinating groups in addition to the amide bond from cs124 or cs124-CF_3. The coordination number of Eu^{3+} is nine, and hence, it is completely filled (or nearly so see [6]) by the TTHA ligands. It is, therefore, possible that the 7-amide bond is not ligated to the europium and other energy-transfer mechanisms from sensitizer to europium are operable [4], or it is ligated some of the time but the modest increase in negative charge on the 7-amide oxygen does not significantly change the equilibrium between ligation of the amide oxygen and other TTHA ligating groups. (We favor the latter situation since this does not require postulating a different energy transfer mechanism between DTPA-sensitizer and TTHA-sensitizer.)

3.6. Photo-stability

Photophysically, cs124-CF_3-DTPA-Eu^{3+} is similar, though slightly less stable than cs124-DTPA-Eu^{3+}. After 10 min of continuos N_2 laser excitation (40 μJ per pulse, 40 Hz), cs124-CF_3-DTPA-Eu^{3+} (in 0.1 M aqueous triethylammonium acetate, pH 6.5, air-equilibrated) retains about 60% emission intensity, whereas cs124-DTPA-Eu^{3+} retains about 75%.

4. Conclusion

We have synthesized cs124-CF_3 and its DTPA and TTHA derivatives. These sensitizer-chelate compounds have been shown to have brighter Eu^{3+} emission and longer-wavelength excitation than their respective parent molecules, cs124-DTPA and TTHA derivatives. When Eu^{3+} (but not terbium) is chelated with these compounds, the complexes display europium characteristic sharply-spiked luminescent emission spectrum, and retain the long excited-state lifetime, including 1.19 ms excited-state lifetime for TTHA-cs124-CF_3-Eu^{3+} and 0.62 ms for DTPA-cs124-CF_3-Eu^{3+}. Because DTPA-cs124-CF_3-Eu^{3+} has significant absorption at 355 and 365 nm, whereas DTPA-cs124-Eu^{3+}/Tb^{3+} is non-absorbing at these wavelengths, selective excitation is possible for double-labeled samples. The chemical stability of DTPA-cs124-CF_3-Eu^{3+} is also excellent, in contrast to the same fluorination of 7-amino-4-methyl-2-coumarin. The further addition of electron-withdrawing groups may yield yet brighter complexes in the future that can be used in time-resolved fluorescence assays.

Acknowledgements

This work was supported by Amersham-Pharmacia under contract to P.R.S.

References

[1] P.G. Sammes, G. Yahioglu, Nat. Prod. Rep. 13 (1) (1996) 1–28.
[2] G.A. Crosby, R.E. Whan, R.M. Alire, J. Chem. Phys. 34 (1961) 743.
[3] A. Abusaleh, C. Meares, Photochem. Photobiol. 39 (1984) 763–769.
[4] W.R. Kirk, W.S. Wessels, F.G. Prendergast, J. Phys. Chem. 97 (1993) 10326–10340.
[5] B. Alpha, R. Ballardini, V. Balzani, J.-M. Lehn, S. Perathoner, N. Sabbatini, Photochem. Photobiol. 52 (1990) 299–306.
[6] M. Li, P.R. Selvin, J. Am. Chem. Soc. 117 (1995) 8132–8138.

[7] P.R. Selvin, T.M. Rana, J.E. Hearst, J. Am. Chem. Soc. 116 (1994) 6029–6030.
[8] P.R. Selvin, J.E. Hearst, in: Proc. Nat'l. Acad. Sci. Vol. 91, USA, 1994, pp. 10024–10028.
[9] M. Xiao, H. Li, G.E. Snyder, R. Cooke, R.G. Yount, P.R. Selvin, in: Proc. Nat'l. Acad. Sci. Vol. 95, USA, 1998, pp. 15309–15314.
[10] G. Mathis, Clin. Chem. 39 (1993) 1953–1959.
[11] G. Mathis, Clin. Chem. 41 (1995) 1391–1397.
[12] E. Heyduk, T. Heyduk, P. Claus, J.R. Wisniewski, J. Biol. Chem. 272 (1997) 19763–19770.
[13] E. Heyduk, T. Heyduk, Anal. Biochem. 248 (1997) 216–227.
[14] D.D. Root, in: Proc. Nat'l. Acad. Sci. USA, 1997, p. 94.
[15] N. Filipescu, W.F. Sager, F.A. Serafin, J. Phys. Chem. 68 (1964) 3324–3346.
[16] K. Nassau, The Physics and Chemistry of Color, Wiley, New York, 1983.
[17] R.C. Thiers, Methods Biochem. Anal. 5 (1957) 273–335.
[18] P.R. Selvin, IEEE J. Selected Top. Quantum Electron.: Lasers in Biol. 2 (1996) 1077–1087.
[19] B. Achour, J. Costa, R. Delgado, E. Garrigues, C.F.G.C. Geraldes, N. Korber, F. Nepveu, M.I. Prata, Inorg. Chem. 37 (1998) 2729–2740.
[20] P.R. Selvin, J. Jancarik, M. Li, L.W. Hung, Inorg. Chem. 35 (1996) 700–705.
[21] M. Xiao, P.R. Selvin, Rev. Sci. Inst. 70 (1999) 3877–3881.