Carbostyril Derivatives as Antenna Molecules for Luminescent Lanthanide Chelates

Pinghua Ge[†] and Paul R. Selvin*[,†,‡]

Department of Physics and Center for Biophysics and Computational Biology, University of Illinois, Urbana, Illinois 61801. Received April 6, 2004; Revised Manuscript Received June 18, 2004

Luminescent lanthanide complexes consisting of a lanthanide-binding chelate and organic-based antenna molecule have unusual emission properties, including millisecond excited state lifetimes and sharply spiked spectra, compared to standard organic fluorophores. We have previously used carbostyril (cs124, 7-amino-4-methyl-2(1*H*)-quinolinone) as an antenna molecule (Li and Selvin, *J. Am. Chem. Soc.*, 1995) attached to a polyaminocarboxylate chelate such as DTPA. Here, we report the chelate syntheses of DTPA conjugated with cs124 derivatives substituted on the 1-, 3-, 4-, 5-, 6-, and 8-position. Among them, the DTPA chelate of cs124-6-SO_3H has similar lifetime and brightness for both Tb^{3+} and Eu^{3+} compared to the corresponding DTPA−cs124 complexes, yet it is significantly more soluble in water. The Tb^{3+} complex of DTPA−cs124-8-CH_3 has significantly longer lifetime compared to DTPA−cs124 (1.74 vs 1.5 ms), indicating higher lanthanide quantum yield resulting from the elimination of back emission energy transfer from Tb^{3+} to the antenna molecule. Thiol-reactive forms of chelates were made for coupling to proteins. These lanthanide complexes are anticipated to be useful in a variety of fluorescence-based bioassays.

INTRODUCTION

Carbostyril (cs124, 7-amino-4-methyl-2(1*H*)-quinolinone) and its derivatives have been used as antenna molecules to absorb light and to transfer excitation energy to lanthanide ions, which significantly increases the fluorescence efficiency of lanthanides (*1*−*4*). To achieve this energy transfer, cs124 or its derivatives are attached to a chelate backbone (such as diethylenetriaminepentaacetic acid, DTPA), which is the site that ligates lanthanide ions. If placed in close range, energy transfer from cs124 (or derivatives) to lanthanide ions is achieved. The chelate backbone site can be further attached to either a thiol-reactive or amine-reactive group for attachment to biomolecules such as DNA and proteins. The advantages of such fluorescence complexes include high solubility in aqueous solution, long fluorescence lifetime (milliseconds), high quantum yields, large Stokes shifts, and sharply spiked emission spectra. These properties make lanthanide complexes attractive probes for fluorescence studies of biomolecules. Such complexes have been used in energy transfer experiments on the muscle protein myosin (*5*−*8*) and on ion channels (*9*).

Most of these lanthanide chelates have used carbostyril as the sensitizer (*10*−*14*). Rarely, a carbostyril derivative was used as a sensitizer (*15*). Because of complex photophysics, the mechanisms of energy transfer from sensitizer to lanthanides have not been fully understood (*16*−*19*). Therefore, it is difficult to rationally design the structures of carbostyril-based lanthanide sensitizers. Given the versatile synthetic possibilities of modifying the cs124 structure (Figure 1), it is interesting to explore how the structural modification can effect the chemical, physical, and photophysical properties of lanthanide chelates. We report the syntheses of new lanthanide chelates with carbostyril derivatives with substitutes on the 1-, 3-, 4-, 5-, 6-, and 8-position as sensitizer, their photophysical properties, and their reactivity to biomolecules.

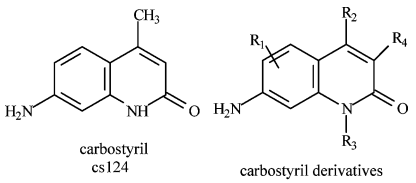

Figure 1. Structures of carbostyril and its derivatives.

EXPERIMENTAL METHODS

Chemicals and Materials. The following were purchased from Sigma-Aldrich: diethylenetriaminepentaacetic acid dianhydride (caDTPA), 3′-aminoacetanilide, 2,4-diaminobenzenesulfonic acid, 3,5-diaminobenzyl alcohol dihydrochloride, ethylenediamine (EDA), anhydrous dimethyl sulfoxide (DMSO, in sure seal bottle), and triethylamine (for reaction). DMSO and triethylamine were dried over activated molecular sieves before use. Glacial acetic acid, methanol, and triethylamine (for making triethylammonium acetate (TEAA) buffer) were purchased from Fisher Scientific. *β*-Maleimidopropionic acid hydrazide•TFA (EMPH•TFA) and *N*-hydroxysuccinimidyl bromoacetate were purchased from Molecular Biosciences (Boulder, CO). *N*-(*ϵ*-Maleimidocaproic acid) hydrazide (EMCH) was purchased from Pierce (Rockford, IL). Maltose binding protein (MBP) was a gift from Panvera Inc. (Madison, WI). Distilled and deionized (18 MΩ cm^{-1}) water was used throughout. All glassware was washed

* To whom correspondence should be addressed. Address: Department of Physics, 1110 W. Green Street, University of Illinois, Urbana, IL 61801. Phone: (217) 244-3371. Fax: (217) 244-7559. E-mail: selvin@uiuc.edu.

[†] Department of Physics.

[‡] Center for Biophysics and Computational Biology.

10.1021/bc049915j CCC: $27.50
Published on Web 08/20/2004

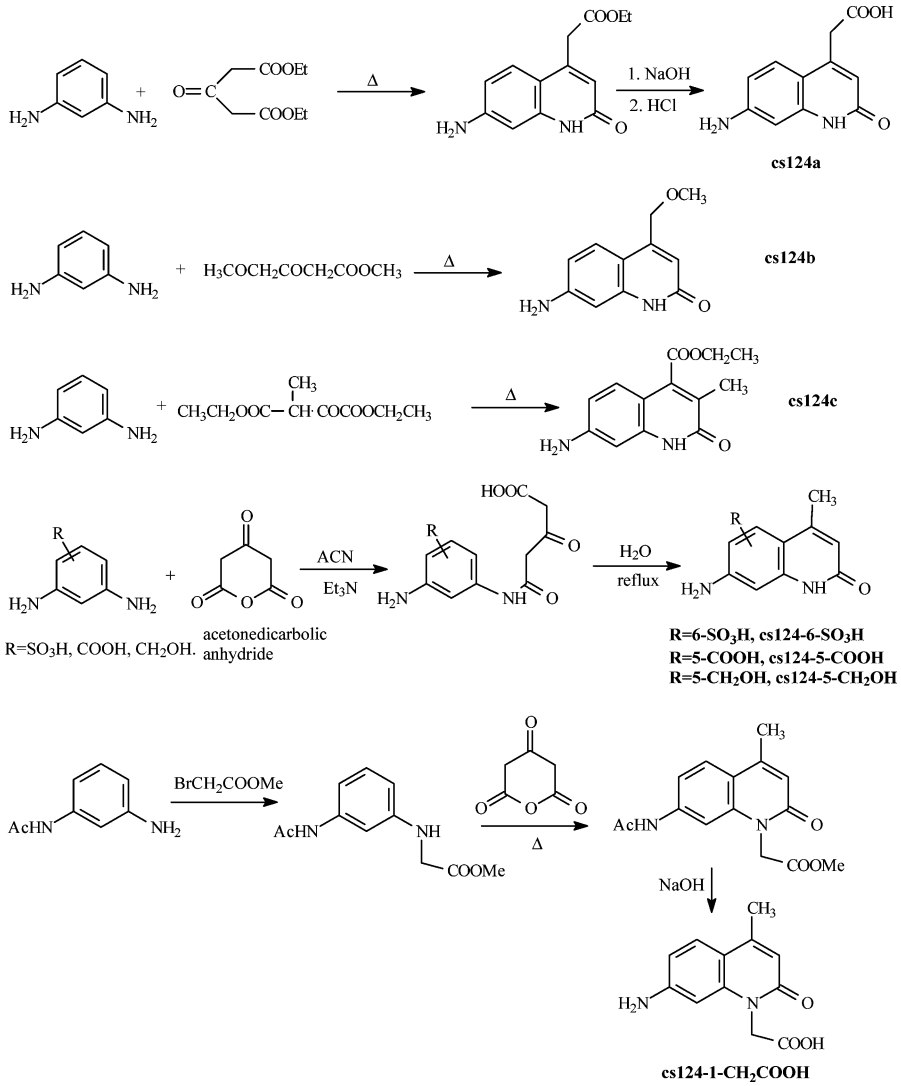

Figure 2. Syntheses and structures of 7-aminoquinolinone derivatives.

with a mixed acid solution and thoroughly rinsed with deionized, distilled water. All plastic labware was purchased from Bio-rad (metal-free). All chemicals were of the purest grade available.

Acetonedicarboxylic anhydride was synthesized according to published method (*20*). 7-Amino-4,8-dimethyl-2-(1*H*)-quinolinone (carbostyril 124-8-methyl, cs124-8-CH_3) was purchased from SPECS & BioSPECS (Rijswijk, The Netherlands). The syntheses of 7-amino-4,6-dimethyl-2-(1*H*)-quinolinone (carbostyril 124-6-methyl, cs124-6-CH_3) and 7-amino-4-methyl-6-sulfonic acid-2-(1*H*)-quinolinone (carbostyril 124-6-SO_3H, cs124-6-SO_3H) had been reported (*21*). In this report, a different synthetic method for cs124-6-SO_3H was used.

Purification. Reverse-phase high-performance liquid chromotography was performed at room temperature on a Waters model 600 system with a Dynamax 60 Å C_{18} column (10 or 25 mm i.d. × 250 mm, Rainin, at 8 mL/min, respectively) using a linear gradient (solvent A = 0.1 M triethylammonium acetate (TEAA), pH 6.5; solvent B = methanol or acetonitrile) over 40 min. The eluted fractions were monitored with a UV detector at a wavelength of 328 nm.

Spectroscopy. NMR spectra were recorded on a Varian Unity 400 spectrometer. 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 (*22*).

Syntheses of cs124 Derivatives (Figure 2). *Synthesis of 7-Amino-1,2-dihydro-2-oxo-4-quinoline Acetic Acid (cs124a).* 1,3-Diaminobenzene (200 mg, 1.85 mmol) was mixed with diethyl 1,3-acetonedicarboxylate (410 mg, 2.03 mmol). The mixture was heated at ∼160 °C for 24 h under N_2. A brown solid formed at the end. The solid was refluxed with acetone for 1 h. After the mixture was cooled to room temperature, the insoluble material was collected by filtration and washed with acetone. The collected solid was stirred with 5 mL of 10% NaOH for 5 h. Most of the solid dissolved. HCl and acetic acid were used to adjust the pH to ∼9. The solution was filtered through Celite. The filtrate was subjected to HPLC purification with a 20−40% linear gradient of MeOH/TEAA. The peak with the retention time of 12 min was collected. The solvent was removed to afford 7-amino-1,2-dihydro-2-oxo-4-quinoline acetic acid (trace amount, yield <1%). MS, *m/z*: 217 (M^- − 1, ESI). ^{1}H NMR (ppm, DMSO-d_6): 11.13 (s, 1H), 7.42 (d, 1H), 6.39 (d, 1H), 6.34 (s, 1H), 5.87 (s, 1H), 5.65 (s, br, 2H), 3.32 (s, 2H). ^{13}C NMR (ppm, DMSO-d_6): 171.72, 162.27, 151.06, 145.19, 141.00, 125.58, 116.11, 114.56, 110.51, 109.80, 96.74.

7-Amino-4-(methoxymethyl)-2(1H)-quinolinone (cs124b). 1,3-Diaminobenzene (200 mg, 1.85 mmol) was mixed with

methyl 4-methoxyacetoacetate (297 mg, 2.03 mmol). The mixture was heated at ~160 °C for 24 h under N_2, after which a brownish solid formed. The solid was washed with acetone and collected by filtration. The solid was further purified by recrystallization in EtOH to afford a pale-yellow solid, cs124b (232 mg, 69% yield). MS, *m/z*: 205 ($M^+ + 1$, ESI). Anal. Calcd for $C_{11}H_{12}N_2O_2$: C, 64.69; H, 5.92; N, 13.72. Found: C, 63.43; H, 5.93; N, 13.41. ^{1}H NMR (ppm, DMSO-d_6): 11.24 (s, 1H), 7.29 (d, 1H), 6.42 (d, 1H), 6.36 (s, 1H), 6.06 (s, 1H), 5.76 (s, 2H), 4.51 (s, 2H), 3.33 (s, 3H). ^{13}C NMR (ppm, DMSO-d_6): 162.35, 151.05, 147.25, 141.05, 124.96, 112.70, 110.46, 108.06, 96.78, 70.46, 57.99.

7-Amino-1,2-dihydro-2-oxo-3-methyl-4-quinoline Carboxylic Acid Ethyl Ester (cs124c). 1,3-Diaminobenzene (350 mg, 3.24 mmol) was mixed with diethyl oxalpropionate (719 mg, 3.56 mmol). The mixture was heated at ~160 °C for 24 h under N_2. A brownish solid formed and was dissolved in boiling ethyl acetate. The hot solution was cooled in an ice bath, causing yellow crystals to form. The solid was collected by filtration and further purified by passing through a silica gel column with ethyl acetate as the eluent to afford a yellow compound, cs124c (464 mg, 58% yield). MS, *m/z*: 247 ($M^+ + 1$, ESI). Anal. Calcd for $C_{13}H_{14}N_2O_3$: C, 63.40; H, 5.73; N, 11.38. Found: C, 63.15; H, 5.80; N, 11.07. ^{1}H NMR (ppm, acetone-d_6): 10.85 (s, br, 1H), 7.13 (d, 1H), 6.60 (s, 1H), 6.57 (d, 1H), 5.38 (s, br, 2H), 4.47 (q, 2H), 2.03 (s, 3H), 1.40 (t, 3H).

General Procedure for the Syntheses of cs124-6-SO_3H, cs124-5-COOH, and cs124-5-CH_2OH. To a suspension of substituted diaminobenzene dihydrochloride in anhydrous acetonitrile, 1 equiv of Et_3N was added at 0 °C under N_2. The mixture was stirred for 30 min. Acetonedicarboxylic anhydride, 1.2 equiv, was added to the above mixture. The reaction mixture was stirred for 8 h during which the temperature was warmed to room temperature. The organic solvent was removed under reduced pressure. The residue was dissolved in H_2O. $NaHCO_3$ was added until no CO_2 bubbles were released. The aqueous solution was refluxed under N_2 for 36 h. The solution was acidified with HCl and acetic acid to pH ~3−4. Precipitates formed during this process. The solution was allowed to stay overnight to complete the crystallization process. The solid was collected and further purified by recrystallization in ethanol.

cs124-6-SO_3H: yellow solid, 55% yield. MS, *m/z*: 253 ($M^- - 1$, ESI). ^{1}H NMR (ppm, DMSO-d_6): 11.16 (s, 1H), 7.73 (s, 1H), 6.38 (s, 1H), 5.95 (s, 1H), 2.27 (s, 3H).

cs124-5-COOH: white solid, 26% yield. MS, *m/z*: 219 ($M^+ + 1$, FAB). ^{1}H NMR (ppm, DMSO-d_6): 11.29 (s, 1H), 6.39 (s, 2H), 5.96 (s, 1H), 5.90 (s, 2H), 2.28 (s, 3H). ^{13}C NMR (ppm, DMSO-d_6): 172.08, 161.61, 149.79, 147.12, 141.68, 117.00, 109.82, 106.02, 97.56, 20.53.

cs124-5-CH_2OH: white solid, 18% yield. MS, *m/z*: 205 ($M^+ + 1$, FAB). ^{1}H NMR (ppm, DMSO-d_6): 11.15, (s, 1H), 6.58 (s, 1H), 6.30 (s, 1H), 5.88 (s, 1H), 5.73 (s, 2H), 3.34 (s, 2H), 2.54 (s, 3H). ^{13}C NMR (ppm, DMSO-d_6): 161.59, 149.88, 149.36, 142.65, 141.05, 116.60, 112.64, 108.76, 96.65, 63.28, 23.42.

Synthesis of cs124-1-CH_2COOH. 3′-Aminoacetanilide (500 mg, 3.33 mmol) was added to the aqueous solution containing 300 mg (3.57 mmol) of $NaHCO_3$. Methyl bromoacetate (560 mg, 3.66 mmol) was added to the mixture. The reaction mixture was refluxed for 4 h. The solution was cooled to room temperature and stirred overnight. The white solid of *N*-(3′-aminophenyl)glycine methyl ester was formed during the process. The solid was collected by filtration and dried. This solid was used for the next step without further purification.

The synthesized *N*-(3′-aminophenyl)glycine methyl ester (300 mg, 1.35 mmol) was mixed with acetonedicarboxylic anhydride (200 mg, 1.56 mmol) under N_2. The mixture was heated at ~160 °C for 24 h. The resulting deep-red solid was mixed with 3 mL of acetone. The mixture was refluxed for 30 min, then cooled to 0 °C. A precipitate formed during this process and was collected by filtration and washed with cold acetone to yield pale-reddish solid. The solid was further purified by recrystallization in EtOH to afford white 7-acetamide−cs124-1-CH_2COOCH_3 (152 mg, 39% yield). MS, *m/z*: 289 ($M^+ + 1$, FAB).

The obtained 7-acetamide−cs124-1-CH_2COOCH_3 was stirred in 10% NaOH for 8 h under N_2. A clear solution was formed. The solution was acidified with dilute HCl to pH ~3−4. A white precipitate formed during the process. The precipitate was collected and washed with H_2O. The solid was further purified by recrystallization in EtOH to afford 7-amino-*N*-(acetic acid)-2-quinolinone (47 mg, 37% yield). MS, *m/z*: 231 ($M^- - 1$, ESI). ^{1}H NMR (ppm, DMSO-d_6): 7.43 (d, 1H), 6.51 (d, 1H), 6.31 (s, 1H), 6.09 (s, 1H), 5.86 (s, br, 2H), 4.78 (s, 2H), 2.31 (s, 3H). ^{13}C NMR (ppm, DMSO-d_6): 169.85, 161.28, 151.66, 147.50, 141.11, 126.65, 113.51, 110.94, 109.90, 96.54, 43.07, 18.59.

Syntheses of DTPA-Based Lanthanide Chelates (Figure 3). *Gerneral Procedure for the Syntheses of Lanthanide Chelates from cs124 Derivatives.* An amount of 1 equiv of DTPA dianhydride was dissolved in a mixture of DMSO and 5 equiv of Et_3N under N_2. An amount of 0.7 equiv of cs124 derivative dissolved in DMSO was added slowly to the above solution with vigorous stirring. The reaction mixture was stirred at room temperature for 2 h. The solution was saved for the following steps.

If non-thiol-reactive chelates were to be synthesized, the above solution was quenched by addition of acetic acid and 0.1 M TEAA buffer (pH 6.5). The products were purified by reverse-phase HPLC with a linear gradient (typically 20−40% MeOH/TEAA, pH 6.5, over 40 min). The yield varies from ~20% to ~50%, as judged from the HPLC profiles (Table 1).

If the maleimide form of thiol-reactive chelates were to be synthesized, the above solution was transferred rapidly to a DMSO solution containing 1.3 equiv of EMPH·TFA (or EMCH) under N_2. The reaction mixture was stirred at room temperature for 4 h. Acetic acid and 0.1 M TEAA (pH 6.5) were added to quench the reaction. The product was purified by reverse-phase HPLC with a linear gradient (typically 20−40% MeOH/TEAA, pH 6.5, over 40 min). The yield varies from ~15% to 40%, as judged from the HPLC profiles (Table 2).

DTPA−cs124-8-CH_3−EDA-Br was prepared with a procedure similar to that used for DTPA−cs124−EDA-Br (*11*). DTPA dianhydride was reacted with cs124-8-CH_3 and EDA sequentially. The intermediate compound, DTPA−cs124-8-CH_3−EDA, was purified by HPLC. The pH of the concentrated aqueous solution of DTPA−cs124-8-CH_3−EDA was adjusted to ~8.5 by addition of Et_3N. *N*-Hydroxysuccinimidyl bromoacetate (3 equiv) was added to the solution. The reaction mixture was stirred at room temperature for 1 h. The product, DTPA−cs124-8-CH_3−EDA-Br, was purified by HPLC.

Addition of Metals. $TbCl_3$ or $EuCl_3$ was added to the chelate in a 1:2 molar ratio usually at >0.5 mM concentration at pH 6−7, usually in 0.1 M TEAA, pH 6.5 buffer, and allowed to stand for 30 min at room temperature before use.

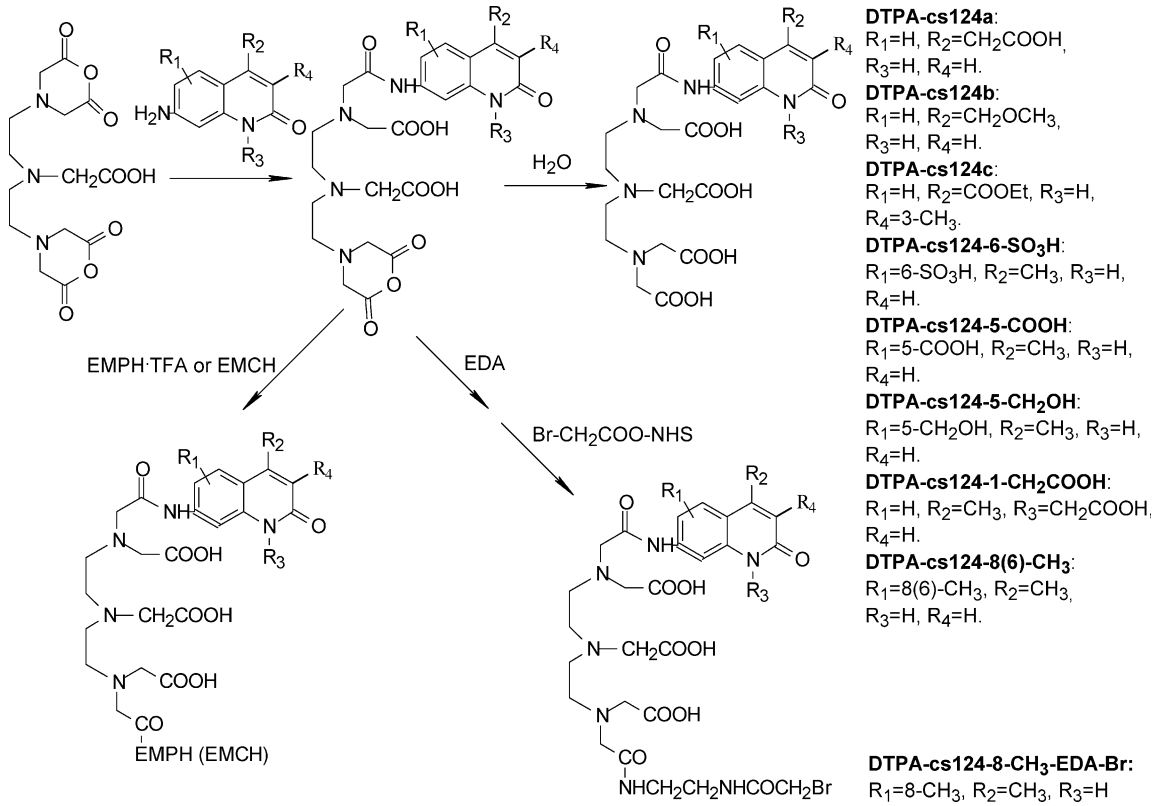

Figure 3. Syntheses of lanthanide chelates from cs124 derivatives.

Table 1. Non-Thiol Reactive Lanthanide Chelates

non-thiol-reactive DTPA chelates	linear gradients	retention time (min)	yield (%)	ESI-MS, $M^- - 1$ (m/z)
DTPA−cs124a	20−40% MeOH/TEAA	13.5	20	592
DTPA−cs124b	20−40% MeOH/TEAA	19	50	578
DTPA−cs124c	30−50% MeOH/TEAA	30.5	36	620
DTPA−cs124-6-SO_3H	30−50% MeOH/TEAA	13.5	20	628
DTPA−cs124-5-COOH	20−40% MeOH/TEAA	15.0	40	592
DTPA−cs124-5-CH_2OH	30−50% MeOH/TEAA	13.0	20	578
DTPA−cs124-1-CH_2COOH	30−50% MeOH/TEAA	13.5	40	606
DTPA−cs124-8-CH_3	30−50% MeOH/TEAA	13.4	30	562
DTPA−cs124-6-CH_3	20−40% MeOH/TEAA	17.2	40	562

Table 2. Thiol-Reactive Lanthanide Chelates

thiol-reactive chelates	linear gradients	retention time (min)	yield (%)	ESI-MS, $M^- - 1$ (m/z)
DTPA−cs124a−EMPH	20−40% MeOH/TEAA	20.6	25	757
DTPA−cs124-6-SO_3H−EMCH	30−50% MeOH/TEAA	19.5	40	835
DTPA−cs124-8-CH_3−EMCH	30−50% MeOH/TEAA	27.0	20	769
DTPA−cs124-8-CH_3−EDA-Br	30−50% MeOH/TEAA	21.5	95	725
DTPA−cs124-6-CH_3−EMCH	30−50% MeOH/TEAA	26.2	15	769
DTPA−cs124-5-CH_2OH−EMPH	30−50% MeOH/TEAA	18.3	15	743
DTPA−cs124-1-CH_2COOH−EMPH	30−50% MeOH/TEAA	16.5	30	771

Coupling to Biomolecules. The labeling conditions for conjugation to muscle protein (S1 fragment of smooth muscle myosin containing a single cysteine at position 208) have been described previously (*5*, *11*). The procedure for labeling Shaker K^+ ion channels in Xenopus oocytes has also been published (*9*). The maleimide chelates were coupled to muscle protein or to maltose binding protein (MBP, a 65 kDa protein containing a single cysteine) at a typical ratio of 10:1 with a protein concentration of ~15 μM. The coupling reaction was carried out on ice overnight in 20 mM 3-(*N*-morpholino)-propanesulfonic acid, pH 7.0, 5 mM $MgCl_2$. The excess lanthanide chelates were removed by passing the reaction mixture through a Sephadex G-50 size-exclusion column.

RESULTS AND DISCUSSION

Syntheses. The key step in synthesizing carbostyril and its derivatives is the formation of the 2-quinolinone ring. 1,3-Diaminobenzene and its derivatives reacted with β-diketone compounds to form intermediate β-diketone amido compounds, which further underwent ring closure to form the products. The compounds cs124a, cs124b, and cs124c were synthesized by reacting 1,3-diaminobenzene with the corresponding β-diketone esters at high temperature (usually >150 °C). A good yield was obtained for cs124b and cs124c, but a trace amount of cs124a was isolated after hydrolysis. The vast undesired byproducts were not identified.

Compounds cs124-6-SO_3H, cs124-5-COOH, cs124-5-CH_2OH, and cs124-1-CH_2COOH were synthesized with

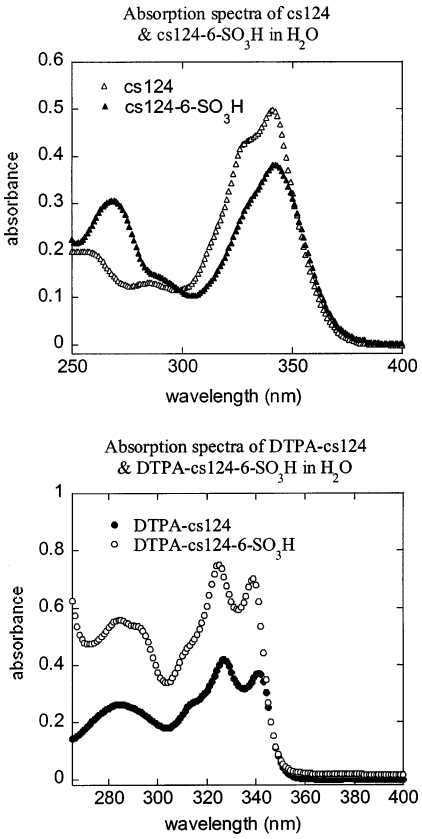

Figure 4. Comparison of the absorption spectra of cs124 and cs124-6-SO_3H and their lanthanide chelates.

a modified method. 1,3-Diaminobenzene derivatives were reacted with 1 equiv of acetonedicarboxylic anhydride at room temperature. The intermediates formed were then refluxed under milder conditions (100 °C) to undergo ring closure. The −COOH group in the intermediates from acetonedicarboxylic anhydride was easily lost to produce the 4-CH_3 group. The advantage of this method is that it does not require as high a temperature as the synthesis using β-diketone esters. This is especially important when the 1,3-diaminobenzene derivatives are not thermally stable. We note that acetonedicarboxylic anhydride had been used to synthesize quinolinone acetic acid compound (*20*). Our efforts in using this method to synthesize cs124a showed that mainly the −COOH depleted product, cs124, was formed.

The luminescent lanthanide chelates were synthesized in a way similar to that in previously reported work (*10*, *11*, *14*). DTPA dianhydride was reacted with cs124 derivatives to form monoanhydride intermediates (Figure 3). These intermediates can be either hydrolyzed or reacted with EMPH (or EMCH) to form thiol-reactive maleimide chelates. The chelates were purified by reverse-phase linear gradient HPLC. The syntheses are straightforward, and the yields are satisfactory.

Absorption Spectra. All of the synthesized cs124 derivatives and their DTPA lanthanide chelates exhibit similar or almost identical absorption spectra to those of cs124 and its DTPA lanthanide chelate. As an example, the UV−vis spectra of cs124-6-SO_3H and DTPA−cs124-6-SO_3H were compared to that of cs124 and DTPA−cs124 (Figure 4).

These absorption spectra were taken in aqueous media. Both of the absorption spectra of free cs124 and cs124-6-SO_3H exhibit the most intense absorption at 342 nm. A shoulder peak appears at ∼326 nm. After the coupling

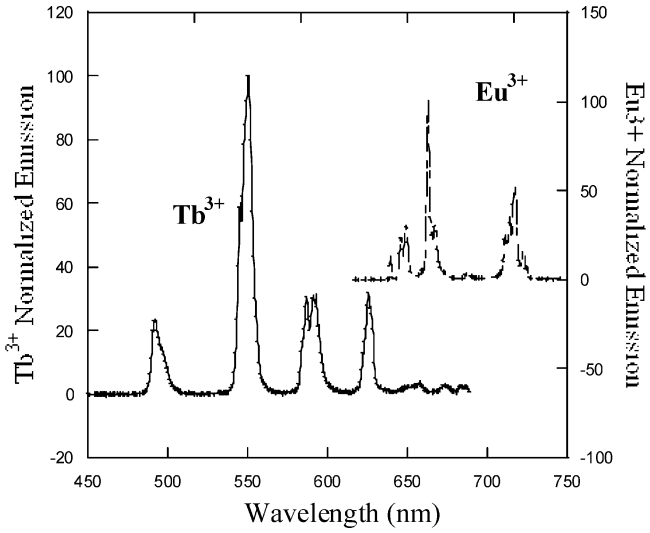

Figure 5. Normalized Tb^{3+} and Eu^{3+} emission spectra in DTPA−cs124-6-SO_3H in 0.1 M TEAA, pH 6.5, at a concentration of 1 μM. Tb^{3+} and Eu^{3+} display their characteristic sharply spiked spectra. Pulsed excitation was at 40 Hz, 3.5 μJ/pulse for Eu^{3+}, 3.0 μJ/pulse for Tb^{3+}. The integration time is 1 s for Tb^{3+} and 10 s for Eu^{3+}.

to DTPA, these two absorption peaks are well resolved, and the absorption at ∼326 nm becomes the most intense peak. These are the characteristic features of carbostyril and its DTPA lanthanide chelates. Such features were also observed for all cs124 derivatives and their lanthanide chelates.

Emission Spectroscopy of Lanthanide Complexes. All the Tb^{3+} and Eu^{3+} complexes exhibit their characteristic sharply spiked emission spectra as demonstrated by their DTPA−cs124-6-SO_3H complexes (Figure 5). Table 3 lists the excited-state lifetimes of Tb^{3+} and Eu^{3+} measured for their complexes with the chelates. Table 4 lists the excited state lifetimes of Tb^{3+} and Eu^{3+} measured for their complexes with the chelates labeled to proteins (smooth muscle myosin, maltose binding protein (MBP), and Xenopus oocytes). Generally, the Tb^{3+} and Eu^{3+} complexes of these chelates have emission brightness comparable to that of the benchmark chelate, DTPA−cs124. By introduction of hydrophilic groups to the sensitizers, the chelates derived from cs124a, cs124-6-SO_3H, cs124-5-COOH, cs124-5-CH_2OH, and cs124-1-CH_2COOH possess increased solubility in water, especially in the case of cs124-6-SO_3H. Although they show emission brightness comparable to that of the reference complex, DTPA−cs124, they often have shorter lifetimes. Contrary to this trend, Tb^{3+}−DTPA−cs124-6-SO_3H shows a slightly longer lifetime, and its brightness is about 62% that of Tb^{3+}−DTPA−cs124. Clearly, the introduction of 6-SO_3H on cs124 has no dramatic effect on the emission properties of its lanthanide complex.

The Tb^{3+} complexes with the chelates of 6- or 8-CH_3 cs124 derivatives, especially the 8-CH_3 derivative, have longer lifetimes than DTPA−cs124 (1.74 vs 1.53 ms). This suggests that a higher lanthanide quantum yield was achieved in these complexes. Increased lanthanide quantum yield could result from either a decrease in solvent quenching or a decrease in energy back transfer to the antenna. We measured the number of solvent molecules coordinated to the lanthanide ion in Tb^{3+}−DTPA−cs124-8-CH_3 using the method of Horrocks and Sudnick (*23*). Lifetimes of Tb^{3+}−DTPA−cs124 and Tb^{3+}−DTPA−cs124-8-CH_3 were measured in both H_2O and D_2O (Table 5). For both molecules, 1.1 H_2O molecules coordinated to

Table 3. Brightness and Lifetimes of Tb^{3+} and Eu^{3+} Complexes of DTPA−cs124 Derivative Chelates

chelate	relative brightness (%)	lifetimes (ms)
	Complexes with Tb^{3+}	
DTPA−cs124 (benchmark)	100	1.53
DTPA−cs124-6-SO_3H	62	1.58
DTPA−cs124-6-SO_3H−EMCH	72	1.86 76%; 0.44 24%
DTPA−cs124-8-CH_3	80	1.74
DTPA−cs124-8-CH_3−EMCH	58	2.16 82%; 0.92 18%
DTPA−cs124-8-CH_3−EDA-Br	64	1.68
DTPA−cs124-5-CH_2OH	79	0.93 45%; 0.65 55%
DTPA−cs124-5-CH_2OH−EMPH	37	0.82 85%; 0.37 15%
DTPA−cs124-5-COOH	36	0.82 24%; 0.50 76%
DTPA−cs124-6-CH_3	87	1.63
DTPA−cs124-6-CH_3−EMCH	4	1.85 59%; 0.25 41%
DTPA−cs124a	80	1.38
DTPA−cs124b	100	1.09
DTPA−cs124c	N/A	N/A
DTPA−cs124-1-CH_2COOH	77	1.29
DTPA−cs124-1-CH_2COOH−EMCH	20	1.43 74%; 0.41 26%
	Complexes with Eu^{3+}	
DTPA−cs124 ("benchmark")	100	0.61
DTPA−cs124-6-SO_3H	77	0.605
DTPA−cs124-6-SO_3H−EMCH	22	0.42 76%; 0.14 24%
DTPA−cs124-8-CH_3	100	0.603
DTPA−cs124-8-CH_3−EMCH	26	0.57 60%; 0.12 40%
DTPA−cs124-8-CH_3−EDA-Br	43	0.60
DTPA−cs124-5-CH_2OH	38	0.64 68%; 0.41 32%
DTPA−cs124-5-CH_2OH−EMPH	34	0.55 75%; 0.11 25%
DTPA−cs124-5-COOH	75	0.60
DTPA−cs124-6-CH_3	60	0.605
DTPA−cs124-6-CH_3−EMCH	N/A	N/A
DTPA−cs124a	52	0.60
DTPA−cs124b	173	0.60
DTPA−cs124c	152	0.53
DTPA−cs124-1-CH_2COOH	100	0.60
DTPA−cs124-1-CH_2COOH−EMCH	10	0.44 68%; 0.11 32%

Tb^{3+}, demonstrating that there is no difference in solvent quenching. Clearly, the higher quantum yield is achieved from the decrease of energy back transfer from excited Tb^{3+} to cs124-8-CH_3. Such lanthanide to ligands back energy transfer has been observed for other lanthanide complexes (*24*).

Interestingly, the thiol-reactive (maleimide form) chelates with 6- or 8-CH_3 cs124 derivatives exhibit significantly different emission properties. The Tb^{3+} complexes of DTPA−cs124-8-CH_3−EMCH is about 60% as bright as Tb^{3+}−DTPA−cs124 and is biexponential with an ∼80% long lifetime component (2.16 ms) and a ∼20% short lifetime component (0.92 ms). The Eu^{3+} complexes of DTPA−cs124-8-CH_3−EMCH is also biexponential with the brightness about 25% that of Eu^{3+}−DTPA−cs124. In contrast, the Tb^{3+} complex of DTPA−cs124-6-CH_3−EMCH is only 4% as bright as Tb^{3+}−DTPA−cs124 and is biexponential with a reduced population for the long lifetime component (1.85 59%, 0.25 41%). The Eu^{3+} complexes of DTPA−cs124-6-CH_3−EMCH is emission-silent.

The 3-CH_3 substituted chelate, DTPA−cs124c, is fluorescently effective only for Eu^{3+}. With Tb^{3+}, no emission was observed. This is consistent with our previous observation that with a substituent on the 3-position of cs124, its lanthanide chelate was fluorescent only for Eu^{3+} but not for Tb^{3+} (*25*).

In all cases, thiol-reactive chelates with the maleimide functional group are both biexponential and often dimmer compared to the benchmark chelate, DTPA−cs124. In contrast, DTPA−cs124-8-CH_3−EDA-Br is single exponential with both Tb^{3+} and Eu^{3+}. The intensity is about half of that of DTPA−cs124. Its Tb^{3+} complex has a longer lifetime than that of Tb^{3+}−DTPA−cs124 (1.68 vs 1.53 ms). After reaction to glutathione, the lifetimes remain single exponential and did not change.

The maleimide form of chelates have been successfully coupled to proteins and Xenopus oocytes cells. Both Tb^{3+} and Eu^{3+} complexes are biexponential. The Tb^{3+} complexes have a predominant long lifetime (>1 ms) component. Among them, the long lifetime components of Tb^{3+}−DTPA−cs124-8-CH_3−EMCH and Tb^{3+}−DTPA−cs124-6-SO_3H−EMCH have lifetimes significantly longer than the reference, Tb^{3+}−DTPA−cs124.

CONCLUSIONS

New light-harvesting quinolinone chromophors with the modification on the 1-, 3-, 4-, 5-, 6-, and 8-position of cs124 have been synthesized. Generally, their lanthanide chelates have similar brightness and lifetimes to the reference compound, DTPA−c124. The 6- or 8-methyl, especially 8-methyl, derived chelates have even longer lifetimes indicating higher lanthanide quantum yields. The 6-SO_3H derived chelate has similar luminescent properties to the reference compound while possessing dramatically increased water solubility. The thiol-reactive chelates made from these derivatives show promising physical and chemical properties.

ACKNOWLEDGMENT

This work is supported by NIH Grant AR44420, NSF Grant 9984841, and the U.S. Department of Energy, Division of Materials Sciences under Award No. DEFG02-91ER45439, through the Frederick Seitz Materials Re-

Table 4. Brightness and Lifetimes of Tb^{3+} and Eu^{3+} Complexes of Thiol-Reactive DTPA−cs124 Derivative Chelates Labeled to Proteins

chelate	protein	lifetimes (ms)
	Complexes with Tb^{3+}	
DTPA−cs124-8-CH_3−EMCH	smooth muscle myosin HC208[a]	2.19 77%; 1.21 23%
	Shaker K^+ channels in oocyte cells[b]	2.17 93%; 0.63 7%
DTPA−c124-6-SO_3H−EMCH	MBP[c]	1.86 78%; 0.72 22%
DTPA−c124-5-CH_2OH−EMPH	MBP[c]	0.97 79%; 0.36 21%
DTPA−c124-8-CH_3−EDA-Br	glutathione[d]	1.67
	Complexes with Eu^{3+}	
DTPA−c124-8-CH_3−EMCH	smooth muscle myosin HC208[a]	0.69 35%; 0.12 65%
DTPA−c124-6-SO_3H−EMCH	MBP[c]	0.42 45%; 0.14 55%
DTPA−c124-5-CH_2OH−EMPH	MBP[c]	0.63 40%; 0.19 60%
DTPA−c124-8-CH_3−EDA-Br	glutathione[d]	0.59

[a] Subfragment 1 (100 kDa) of smooth muscle myosin with a single cysteine at position 208 of the heavy chain. [b] Details of ion channel expression can be found in the following: Cha. (1999) *Nature 402*, 809−813. [c] Maltose binding protein. [d] Purified by HPLC after labeling.

Table 5. Lifetimes of Tb^{3+}−DTPA−c124 and Tb^{3+}−DTPA−c124-8-CH_3 Measured in H_2O and D_2O and the Number of H_2O Molecules Coordinated to Tb^{3+}

	lifetimes		
compd	H_2O (ms)	D_2O (ms)	no. of H_2O
Tb^{3+}−DTPA−c124	1.55	2.64	1.1
Tb^{3+}−DTPA−c124-8-CH_3	1.73	3.20	1.1

search Laboratory at the University of Illinois at Urbana−Champaign. We thank Baigen Mei, Marie Christenson, and Kurt Vogel from Panvera Corp. for the generous gift of MBP protein. The labeling experiments to oocytes expressing Shaker ion channels were done by David Posson. The mass spectrometry experiments were performed by the Mass Spectrometry Center, University of Illinois at Urbana−Champaign. The elemental analyses were performed by Microanalysis Laboratory, University of Illinois at Urbana−Champaign.

LITERATURE CITED

(1) Soini, E., and Lövgren, T. (1987) Time-resolved fluorescence of lanthanide probes and applications in biotechnology. *CRC Crit. Rev. Anal. Chem. 18*, 104−154.

(2) Sammes, P. G., and Yahioglu, G. (1996) Modern Bioassays using Metal Chelates as Luminescent Probes. *Nat. Prod. Rep. 13*, 1−28.

(3) Selvin, P. R. (2002). Principles and Biophysical Applications of Luminescent Lanthanide Probes. *Annu. Rev. Biophys. Biomol. Struct. 31*, 275−302.

(4) Selvin, P. R. (2003) Lanthanide-labeled DNA. *Topics in Fluorescence Spectroscopy, Vol 7*, (Lakowicz, J., Ed.) pp 177−212, Kluwer Academic/Plenum, New York.

(5) Burmeister-Getz, E., Cooke, R., and Selvin, P. R. (1998) Luminescence resonance energy transfer measurements in myosin. *Biohys. J. 75*, 2451−2458.

(6) Xiao, M., Li, H., Snyder, G. E., Cooke, R. G., Yount, R., and Selvin, P. R. (1998) Conformational changes between the active-site and regulatory light chain of myosin as determined by luminescence resonance energy transfer: The effect of nucleotides and actin. *Proc. Natl. Acad. Sci. U.S.A. 95*, 15309−15314.

(7) Chakrabarty, T., Xiao, M., Cooke, R., and Selvin, P. R. (2000) Structure and dynamics of the myosin dimer measured by fluorescence and luminescence resonance energy transfer. *Biohys. J. 78*, 233A.

(8) Xiao, M., Reifenberger, J. G., Wells, A. L., Baldacchino, C., Chen, L. Q., Ge, P., Sweeney, H. L., and Selvin, P. R. (2003) An actin-dependent conformational change in myosin. *Nat. Struct. Biol. 10*, 402−408.

(9) Cha, A., Snyder, G. E., Selvin, P. R., and Bezanilla, F. (1999) Atomic scale movement of the voltage sensing region in a potassium channel measured via spectroscopy. *Nature 402*, 809−813.

(10) Li, M., and Selvin, P. R. (1997). Amine-reactive forms of a luminescent DTPA chelate of terbium and europium: Attachment to DNA and energy transfer measurements. *Bioconjugate Chem. 8*, 127−132.

(11) Chen, J., and Selvin, P. R. (1999) Thiol-reactive luminescent lanthanide chelates. *Bioconjugate Chem. 10*, 311−315.

(12) Kapanidis, A. N., Ebright, Y. W., Ludescher, R. D., Chan, S., and Ebright, R. H. (2001) Mean DNA bend angle and distribution of DNA bend angles in the CAP-DNA complex in solution. *J. Mol. Biol. 312*, 453−68.

(13) Takalo, H., Mukkala, V.-M., Mikola, H., Liitti, P., and Hemmila, I. (1994) Synthesis of Europium(III) Chelates Suitable for Labeling of Bioactive Molecules. *Bioconjugate Chem. 5*, 278−282.

(14) Ge, P., and Selvin, P. (2003). Thio-reactive lanthanide chelates, II. *Bioconjugate Chem. 14*, 870−876.

(15) Chen, J., and Selvin, P. R. (2000) Synthesis of 7-amino-4-trifluoromethyl-2-(1*H*)-quinolinone and its use as an antenna molecule for luminescent europium polyaminocarboxyllates chelates. *J. Photochem. Photobiol., A 135*, 27−32.

(16) Crosby, G. A., Whan, R. E., and Alire, R. M. (1961) *J. Chem. Phys. 34* 743.

(17) Abusalch, A., and Meares, C. (1984). *Photochem. Photobiol. 39*, 763−769.

(18) Kirk, W. R., Wessels, W. S., and Prendergast, F. G. (1993) *J. Phys. Chem. 97*, 10326−10340.

(19) Alpha, B., Ballardini, R., Lehn, J.-M., Perathoner, S., and Sabbatini, N. (1990). *Photochem. Photobiol. 52*, 299−306.

(20) Kiang, A. K., Tan, S. F., Wong, W. S. (1971). Reactions of acetonedicarboxylic anhydride (3-oxoglutaric anhydride) and its mono- and diacetyl derivatives with amines. *J. Chem. Soc. C* (15), 2721−2726.

(21) Spathe, H. (1957) 7-Amino-2-hydroxy-4-methylquinolines. DE 958647 19570221.

(22) Xiao, M., and Selvin, P. R. (1999) An Improved instrument for measuring time-resolved lanthanide emission and resonance energy transfer. *Rev. Sci. Instrum. 70*, 3877−3881.

(23) Harrocks, W. D., Jr., and Sudnick, D. R. (1979) Lanthanide ion probes of structure in biology. Laser-induced luminescence decay constants provide a direct measure of the number of metal-coordinated water molecules. *J. Am. Chem. Soc. 123*, 7067−7073.

(24) Lis, S., Elbanowski, M., Makowska, B., Hnatejko, Z. (2002). Energy transfer in solution of lanthanide complexes. *J. Photochem. Photobiol. 150*, 223−247.

(25) Chen, J., and Selvin, P. R. Unpublished data.

BC049915J