Synthesis of Novel Near-Infrared Cyanine Dyes for Metal Ion Determination

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Four heptamethine cyanine dyes **10**, **19-21** containing an *ortho*-hydroxy-carboxy functionality for metal ion complexation and absorbing at λ max (methanol) 761 ±1 nm have been synthesized.

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A great deal of work has been devoted to the development of reagents for metal ion determination which is based on absorption or fluorescence derivatization upon complexation. Many studies have been reported on complexons and their metal complexes that absorb and fluoresce in the ultraviolet and visible regions. Such reagents have found limited applications in direct analysis of biological and environmental media where background interference is a major problem. For ultra trace determination of metal ions these techniques do not offer significant advantages over competing methods which utilize flame ionization and X-ray fluorescence [1-3].

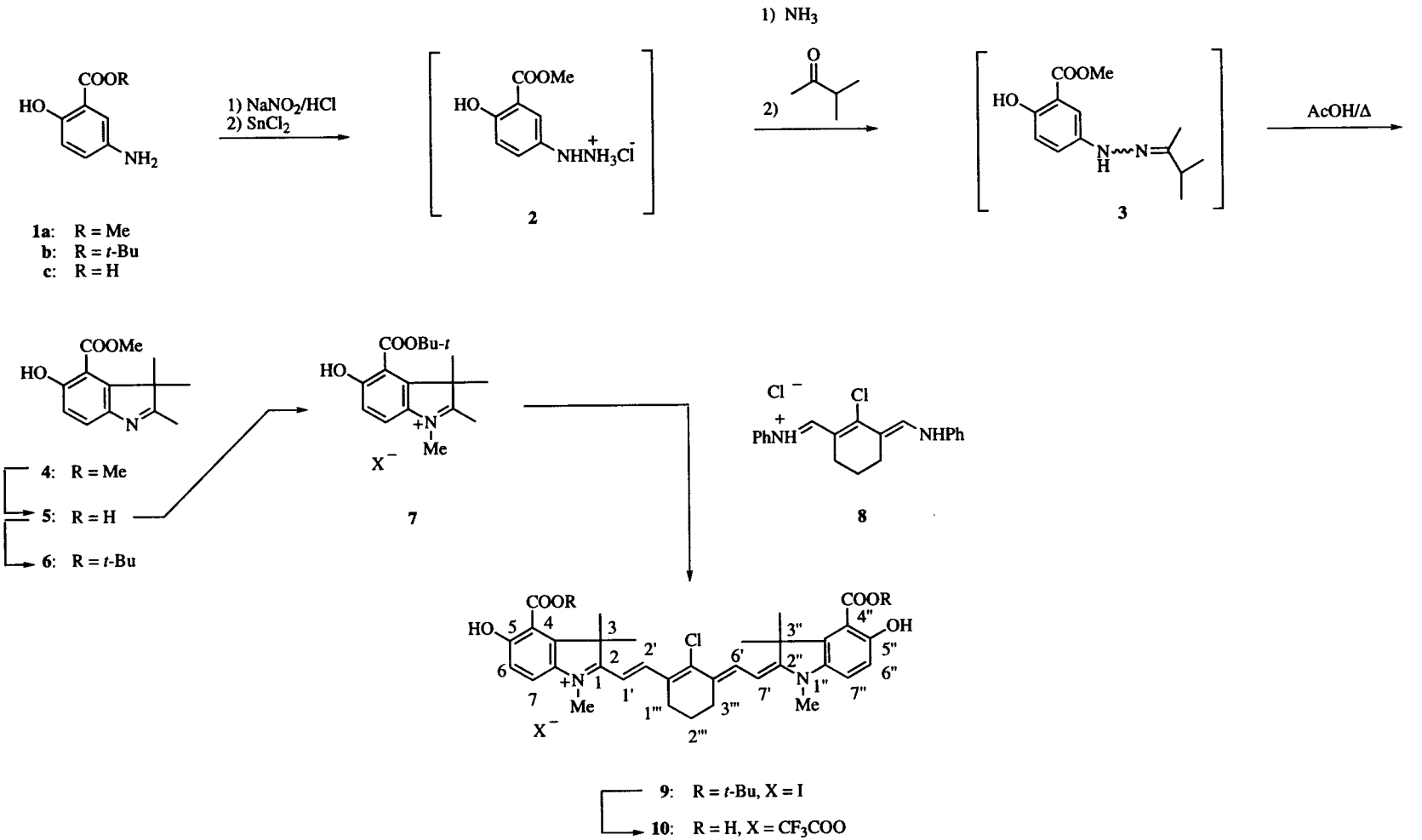
The background interference can be greatly reduced if not eliminated by using metal chelators which show electronic absorption and fluorescence in the near-infrared region above 700 nm. Previously we have demonstrated that a similar approach works well for bioanalytical techniques including ultra trace determination of proteins [4-6]. The high sensitivity has been achieved by using a near-infrared chromophore in conjunction with a commercially available and inexpensive GaAlAs diode laser emitting at 785 nm [7].

In this paper we report for the first time the design and synthesis of four metal ion complexing reagents (**10** in Scheme 1 and **19-21** in Scheme 2) which absorb strongly in the region of 720-800 nm with the maximum centered at 761 ±1 nm and show negligible absorption in the visible region. As such, these near-infrared dyes can be used in conjunction with the GaAlAs diode laser. Our preliminary studies have already indicated substantial changes in the absorption and fluorescence spectra of these dyes in the presence of a trace amount of aluminum ion [8]. Detailed analytical investigations with this and other ions will be reported in due course.

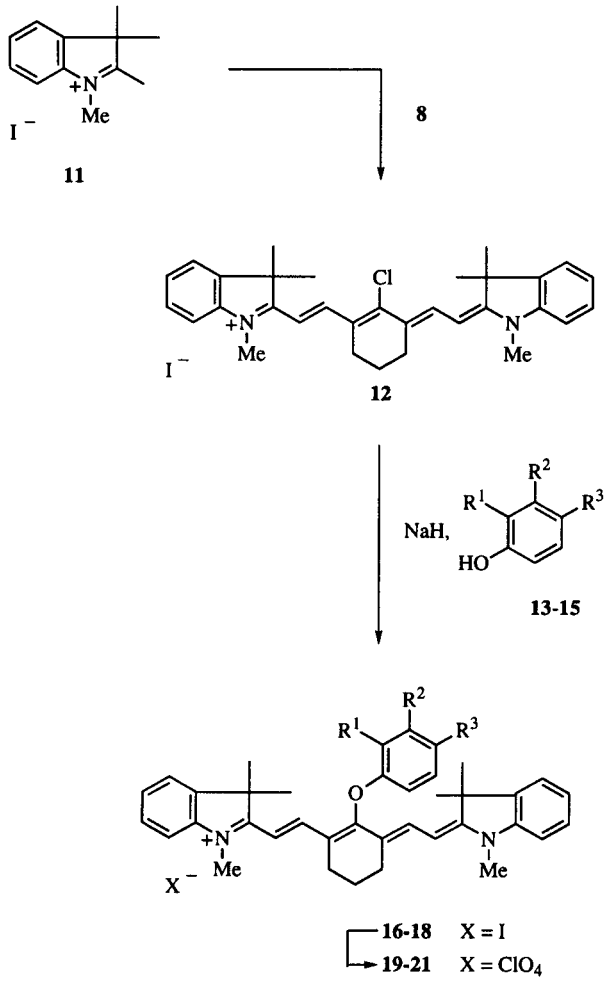
The reagents **10** and **19-21** contain a heptamethine cyanine chromophore. The dyes were designed with terminal indole derivatives and a central ring structure in the molecules to provide an increased photo and chemical stability in comparison to other cyanine dyes [7]. All compounds **10**, **19-21** contain an *ortho* carboxy-hydroxy functionality which is known to chelate metal ions with some degree of selectivity [1]. In **10** the metal chelating moiety is part of the heterocyclic unit of the molecule with the hydroxy group conjugated with the ring nitrogen atom. Apparently, this conjugation is decreased upon tying up nonbonding electrons on the oxygen atom upon metal ion complexation causing changes in spectral properties of the complex in comparison to those of a free dye. In **19-21** the metal chelating moiety is separated from the chromophore by a phenoxy bridge at the central position of the molecules. The oxygen atom of the phenoxy group is also strongly conjugated with the chromophore [4,5], and this conjugation is sensitive to conformational effects around the oxygen atom. Accordingly, the observed spectral changes of **19-21** in the presence of aluminum ion are apparently due to a different conformation of the complex in comparison to that of a free dye [8].

Synthesis of dye **10** is given in Scheme 1. Diazotization of the amino group of methyl 5-amino-2-hydroxybenzoate (**1a**) followed by reduction gave a hydrazine derivative **2** which, without purification, was allowed to react with 3-methyl-2-butanone to give a hydrazone **3**. Crude compound **3** was cyclized under acidic conditions to an indole ester **4**. Hydrolysis of **4** to a carboxylic acid **5** was followed by synthesis of a *tert*-butyl ester **6**. Quaternization of the ring nitrogen atom in **6** by the reaction with iodomethane furnished a key compound **7** which was allowed to react with a dialdehyde equivalent **8** to give dye **9**. Hydrolysis of the *tert*-butyl ester function in **9** under mild conditions completed this short and efficient synthesis of **10** containing the desired carboxy-hydroxy functionality. The intermediate products **2** and **3** were not purified because the chemistry involved, including a related cyclization [9,10], is well understood. On the other hand,

Scheme 1


1) NH3
1) NaNO2/HCl
2) SnCl2
2)
AcOH/Δ
1a: R = Me
b: R = t-Bu
c: R = H
2
3
4: R = Me
5: R = H
6: R = t-Bu
7
8
9: R = t-Bu, X = I
10: R = H, X = CF3COO

Scheme 2



Compound	R^1	R^2	R^3
13, 16	OH	CO_2Bu-*t*	H
14, 17	H	OH	CO_2Bu-*t*
15, 18	H	CO_2Bu-*t*	OH
19	OH	CO_2H	H
20	H	OH	CO_2H
21	H	CO_2H	OH

a further attempted simplification of this synthetic route to **10** by using a *tert*-butyl ester **1b** or an acid **1c** as a starting material was not successful. The ester **1b** was not stable under the strongly acidic conditions of the first step, and the resultant acid **1c** caused major solubility problems.

All new compounds **4-7**, **9**, and **10** were characterized by spectral methods and elemental analysis. In particular, the AB system in the ^1H nmr spectrum of **4** for two adjacent aromatic protons is fully consistent with the given structure. This coupling pattern is retained for **7**, **9**, and **10**. Also, the coupling constant of 14 Hz between H1'(H7') and H2'(H6') for the heptamethine moiety of **9** (resonances in deuteriochloroform at δ 6.10 and 8.26, respectively) is similar to that of other heptamethine cyanine dyes [4,5]. The ^1H nmr spectrum of **10** was taken in trifluoroacetic acid-*d* because this dye is not soluble in common organic solvents. We have shown recently that protons at positions 1',7' of heptamethine cyanine dyes rapidly undergo exchange with deuterium of the solvent under these conditions and, as a result, a singlet for H2'(H6') is normally observed [11]. Surprisingly, two one-proton singlets at δ 6.48 and 8.78 were observed in the ^1H nmr spectrum of **10** taken in trifluoroacetic acid-*d*. Other proton signals indicated an unsymmetrical structure of **10** in this solvent. This result can be explained in terms of a tight ion pair of a cationic chromophore and trifluoroacetate anion in **10**. Due to chemical shift values for **10**, which are typical for cyanine dyes, the formation of a covalent adduct of the cation with the trifluoroacetate anion is less likely. The near-infrared spectra of **9** and **10** taken in methanol were virtually identical.

The three-step synthesis of **19-21** involved condensation of an indolium iodide **11** with the reagent **8** to give a chloro-substituted dye **12** followed by replacement of the chlorine in **12** by the reaction of phenoxide ion derived from **13-15** and then hydrolysis of the ester function in the resulting dye **16-18**. It was reasoned that a dihydroxy derivative **13-15** would react at the less sterically hindered hydroxy group with **12**. This was confirmed by analysis of ^1H nmr spectra of **13-15** and products **16-18**. Thus, all compounds **13-18** showed a singlet at δ 11.1 ±0.5 attributed to the hydroxy group *ortho* to the *tert*-butoxycarbonyl function, but only substrates **13-15** gave a singlet at δ 5.2 ±0.5 for the second hydroxy group.

EXPERIMENTAL

All reagents **1**, **8**, **11** and dihydroxybenzoic acids, the precursors to **13-15**, were obtained from Aldrich. The ^1H nmr and ^{13}C nmr spectra were recorded at 30° at 300 MHz and 75 MHz, respectively, with tetramethylsilane as an internal reference. Proton-proton coupling constants smaller than 2 Hz are not reported. Fast-atom-bombardment mass spectra (fab-ms) were obtained in the presence of thioglycerol.

Methyl 5-Hydroxy-2,3,3-trimethyl-3*H*-indole-4-carboxylate (**4**).

A solution of methyl 5-amino-2-hydroxybenzoate (**1a**, 1.67 g, 10 mmoles) in hydrochloric acid (3*M*, 20 ml) was cooled to 0° and treated with a solution of sodium nitrite (0.76 g, 11 mmoles) in water (5 ml). The mixture was stirred at 0° for 10 minutes, filtered, and a cold filtrate was added dropwise with stirring to a solution of stannous chloride dihydrate (6.77 g, 30 mmoles) in concentrated hydrochloric acid (10*M*, 20 ml) at 0° to give 1.38 g (63%) of a precipitate of hydrazinium chloride **2** [^1H nmr (dimethyl sulfoxide-d_6): δ 3.90 (s, 3H), 6.97 (d, J = 9 Hz, 1H),

7.28 (dd, J = 9 Hz, J = 3 Hz, 1H), 7.48 (d, J = 3 Hz, 1H), 8.05 (br s, exchangeable, 1H), 10.17 (br s, exchangeable, 3H)]. A solution of the crude salt **2** (0.44 g, 2 mmoles) in aqueous ethanol (50%, 8 ml) was treated with an aqueous solution of ammonia (30%, 1 ml) and then 3-methyl-2-butanone (0.53 ml, 5 mmoles). The mixture was stirred at 23° for 1 hour and then extracted with dichloromethane. The extract was washed with water, dried over magnesium sulfate, and concentrated on a rotary evaporator. The residue of the crude hydrazone **3** was dissolved in glacial acetic acid (2 ml) and the solution was heated under reflux under a nitrogen atmosphere for 3 hours. Concentration of the mixture on a rotary evaporator was followed by a standard workup and then chromatography on a silica gel with hexanes/chloroform as an eluent to give 0.19 g (41%) of **4**. A sample of **4** crystallized from hexanes/dichloromethane had mp 110-111°; ^{1}H nmr (deuteriochloroform): δ 1.41 (s, 6H), 2.24 (s, 3H), 4.04 (s, 3H), 6.99 (d, J = 8.5 Hz, 1H), 7.65 (d, J = 8.5 Hz, 1H), 11.12 (s, exchangeable, 1H).

Anal. Calcd. for $C_{13}H_{15}NO_3$: C, 66.93; H, 6.48; N, 6.00. Found: C, 66.65, H, 6.43; N, 5.90.

5-Hydroxy-2,3,3-trimethyl-3*H*-indole-4-carboxylic Acid (**5**).

A solution of ester **4** (0.12 g, 0.52 mmole) and potassium hydroxide (0.2 g, 3.6 mmoles) in aqueous methanol (60%, 3 ml) was heated under reflux for 3 hours. After cooling the mixture was treated with diluted hydrochloric acid (5%) to *p*H 7, and the resultant precipitate of **5** was filtered and crystallized from aqueous methanol, yield 0.09 g (80%), mp 250-251° dec; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 1.33 (s, 6H), 2.15 (s, 3H), 6.84 (d, J = 8.5 Hz, 1H), 7.40 (d, J = 8.5 Hz, J = 3 Hz, 1H).

Anal. Calcd. for $C_{12}H_{13}NO_3$: C, 65.74; H, 5.98; N, 6.39. Found: C, 65.50; H, 5.92; N, 6.25.

tert-Butyl 5-Hydroxy-2,3,3-trimethyl-3*H*-indole-4-carboxylate (**6**).

A solution of acid **5** (0.44 g, 2 mmoles) and 1,1'-carbonyldiimidazole (0.32 g, 2 mmoles) in *N,N*-dimethylformamide (5 ml) was heated to 40° under a nitrogen atmosphere for 1 hour followed by treatment with *tert*-butyl alcohol (0.38 ml, 4 mmoles) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 0.3 ml, 2 mmoles) and stirring of the mixture at 40° for an additional 24 hours. After treatment with ether (50 ml) the mixture was washed with an aqueous solution of sodium hydrogen carbonate, dried over magnesium sulfate, and concentrated on a rotary evaporator. Chromatography on silica gel with hexanes/chloroform as an eluent was followed by crystallization of the resultant product **6** from aqueous methanol: yield 0.34 g (62%), mp 104-105°; ^{1}H nmr (deuteriochloroform): δ 1.47 (s, 6H), 1.72 (s, 9H), 2.23 (s, 3H), 6.96 (d, J = 8.5 Hz, 1H), 7.60 (d, J = 8.5 Hz, 1H), 11.35 (s, exchangeable, 1H).

Anal. Calcd. for $C_{16}H_{21}NO_3$: C, 69.79; H, 7.69; N, 5.09. Found: C, 69.50; H, 7.58; N, 5.05.

4-*tert*-Butoxycarbonyl-5-hydroxy-1,2,3,3-tetramethyl-3*H*-indolium Iodide (**7**).

A solution of **6** (0.28 g, 1 mmole) and methyl iodide (0.5 ml, 8 mmoles) in acetonitrile (5 ml) was allowed to stand at 23° for 20 hours. Concentration on a rotary evaporator was followed by crystallization of the residue from methanol/ether to give 0.38 g (92%) of indolium iodide **7**, mp 230-231°; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 1.52 (s, 6H), 1.57 (s, 9H), 2.68 (s, 3H), 3.89 (s, 3H), 7.07 (d, J = 9 Hz, 1H), 7.77 (d, J = 9 Hz, 1H), 10.70 (s, exchangeable, 1H).

Anal. Calcd. for $C_{17}H_{24}INO_3$: C, 48.93; H, 5.80; N, 3.36. Found: C, 48.72; H, 5.76; N, 3.27.

4-*tert*-Butoxycarbonyl-2-[4'-chloro-7'-(4"-*tert*-butoxycarbonyl-5"-hydroxy-1",3",3"-trimethylindolin-2"-ylidene)-3',5'-(propane-1"',3"'-diyl)-1',3',5'-heptatrien-1'-yl]-5-hydroxy-1,3,3-trimethyl-3*H*-indolium Iodide (**9**).

A reaction of indolium iodide **7** with *N*-[5-anilino-3-chloro-2,4-(propane-1',3'-diyl)-2,4-pentadien-1-ylidene]anilinium chloride (**8**) was conducted by using a general procedure [4,5]. The dye **9** was purified by chromatography on silica gel with chloroform/methanol (4:1) as an eluent and then crystallization from methanol/ether, yield 76%, mp >200° dec; ^{1}H nmr (deuteriochloroform): δ 1.75 (s, 18H), 1.90 (s, 12H), 1.97 (m, 2H), 2.74 (t, J = 6 Hz, 4H), 3.74 (s, 6H), 6.10 (d, J = 14 Hz, 2H), 7.10 (d, J = 8 Hz, 2H), 7.30 (d, J = 8 Hz, 2H), 8.26 (d, J = 14 Hz, 2H), 10.80 (s, exchangeable, 2H).

Anal. Calcd. for $C_{42}H_{52}IN_2O_6$: C, 62.45; H, 6.49; N, 3.47. Found: C, 62.32; H, 6.45; N, 3.42.

4-Carboxy-2-[4'-chloro-7'-(4"-carboxy-5"-hydroxy-1",3",3"-trimethylindolin-2"-ylidene)-3',5'-(propane-1"',3"'-diyl)-1',3',5'-heptatrien-1'-yl]-5-hydroxy-1,3,3-trimethyl-3*H*-indolium Trifluoroacetate (**10**).

A solution of dye **9** (0.10 g, 0.12 mmole) in trifluoroacetic acid (5 ml) was allowed to stand at 23° for 1 hour. Concentration on a rotary evaporator was followed by crystallization of the residue from methanol/ether to give 85 mg (94%) of an analytically pure dye **10**, mp >200° dec; ^{1}H nmr (trifluoroacetic acid-*d*): δ 1.95 (s, 6H), 2.08 (s, 6H), 2.11 (m, 2H), 2.81 (m, 4H), 4.16 (s, 6H), 6.48 (s, 1H), 7.47 (d, J = 7 Hz, 2H), 7.93 (d, J = 7 Hz, 1H), 7.96 (d, J = 7 Hz, 1H), 8.78 (s, 1H); nir: λ max methanol, 761 nm.

Anal. Calcd. for $C_{34}H_{36}ClN_2O_6^+ \cdot CF_3COO^-$: C, 60.29; H, 5.06; N, 3.91. Found: C, 59.98; H, 5.35; N, 4.08.

2-[4'-Chloro-7'-(1",3",3"-trimethylindolin-2"-ylidene)-3',5'-(propane-1"',3"'-diyl)-1',3',5'-heptatrien-1'-yl]-5-hydroxy-1,3,3-trimethyl-3*H*-indolium Iodide (**12**).

Condensation of an indolium iodide **11** with the reagent **8** was conducted by using a general procedure [4,5]. Crude dye **12** was crystallized from methanol/ether; mp >200° dec; ^{1}H nmr (deuteriochloroform): δ 1.73 (s, 12H), 1.95 (quintet, J = 6 Hz, 2H), 2.77 (t, J = 6 Hz, 4H), 3.77 (s, 6H), 6.26 (d, J = 14 Hz, 2H), 7.17-7.27 (m, 4H), 7.36-7.40 (m, 4H), 8.34 (d, J = 14 Hz, 2H); ^{13}C nmr (deuteriochloroform): δ 20.6, 26.7, 28.0, 32.6, 49.2, 101.6, 110.8, 122.1, 125.3, 127.7, 128.7, 140,8, 142.7, 144.3, 150.5, 172.8; nir: λ max methanol, 775 nm; hrms: (FAB) Calcd. for $C_{32}H_{36}ClN_2$: (M^+) m/z 483.2567: Found: m/z 483.2541.

Anal. Calcd. for $C_{32}H_{36}ClN_2^+ \cdot I^-$: C, 62.90; H, 5.94; N, 4.59. Found: C, 62.80; H, 5.98; N, 4.57.

tert-Butyl Dihydroxybenzoates **13-15**.

Esterification of a dihydroxybenzoic acid with *tert*-butyl alcohol was conducted and the ester was purified by using a general procedure described for **6** above.

tert-Butyl 2,3-Dihydroxybenzoate (**13**).

This compound was additionally purified by sublimation and

had mp 70-71°, yield 58%; ^{1}H nmr (deuteriochloroform): δ 1.61 (s, 9H), 5.64 (s, exchangeable, 1H), 6.76 (t, J = 8 Hz, 1H), 7.07 (d, J = 8 Hz, 1H), 7.31 (t, J = 8 Hz, 1H), 11.20 (s, exchangeable, 1H).

Anal. Calcd. for $C_{11}H_{14}O_4$: C, 62.84; H, 6.71. Found: C, 62.97; H, 6.77.

tert-Butyl 2,4-Dihydroxybenzoate (**14**).

This compound had mp 71-82°, yield 41%; ^{1}H nmr (deuteriochloroform): δ 1.59 (s, 9H), 5.64 (s, exchangeable, 1H), 6.32-6.38 (m, 2H), 7.67 (d, J = 8 Hz, 1H), 11.25 (s, exchangeable, 1H).

Anal. Calcd. for $C_{11}H_{14}O_4$: C, 62.84; H, 6.71. Found: C, 62.58; H, 6.73.

tert-Butyl 2,5-Dihydroxybenzoate (**15**).

This compound was additionally purified by sublimation and had mp 79-80°, yield 55%; ^{1}H nmr (deuteriochloroform): δ 1.60 (s, 9H), 4.74 (s, exchangeable, 1H): 6.85 (d, J = 9 Hz, 1H), 6.97 (dd, J = 9 Hz, J = 3 Hz, 1H), 7.23 (d, J = 3 Hz, 1H), 10.60 (s, exchangeable, 1H).

Anal. Calcd. for $C_{11}H_{14}O_4$: C, 62.84; H, 6.71. Found: C, 62.92; H, 6.75.

Dyes **16-18**.

A mixture of sodium hydride (60% in mineral oil, 40 mg, 1 mmole) and *tert*-butyl dihydroxybenzoate **13-15** (210 mg, 1 mmole) in anhydrous chloroform (30 ml) was stirred under a nitrogen atmosphere at 23° until evolution of hydrogen ceased (10 minutes) and then treated with a solution of dye **12** (244 mg, 0.4 mmole) in anhydrous *N,N*-dimethylformamide (10 ml). After stirring for an additional 7 hours at 23° the mixture was poured onto dry ice (5 g) and then concentrated on a rotary evaporator. Chromatography on silica gel with chloroform/methanol as an eluent (gradient, up to 20% of methanol) was followed by crystallization from methanol/ether.

2-[4'-(3""-*tert*-Butoxycarbonyl-2""-hydroxyphenoxy)-7'-(1",3",3"-trimethylindolin-2"-ylidene)-3',5'-(propane-1"',3"'-diyl)-1',3',5'-heptatrien-1'-yl]-1,3,3-trimethyl-3*H*-indolium Iodide (**16**).

This dye was obtained in a 76% yield, mp >200° dec; ^{1}H nmr (deuteriochloroform): δ 1.42 (s, 12H), 1.68 (s, 9H), 2.04 (quintet, J = 6 Hz, 2H), 2.78 (t, J = 6 Hz, 4H), 3.69 (s, 6H), 6.12 (d, J = 14 Hz, 2H), 6.78 (t, J = 8 Hz, 1H), 7.03 (d, J = 8 Hz, 1H), 7.11-7.25 (m, 6H), 7.36 (t, J = 7.5 Hz, 2H), 7.48 (d, J = 8 Hz, 1H), 7.93 (d, J = 14 Hz, 2H), 11.60 (s, exchangeable, 1H); ^{13}C nmr (deuteriochloroform): δ 21.1, 24.7, 27.7, 28.1, 32.3, 48.8, 83.8, 100.6, 110.5, 115.1, 119.0, 119.5, 121.8, 123.0, 123.7, 125.0, 128.6, 140.9, 141.6, 142.7, 148.0, 150.7, 164.9, 169.9, 172.3; ms: (FAB) m/z 657.4 (100%, $C_{43}H_{49}N_2O_4^+$); nir: λ max methanol, 763 nm.

Anal. Calcd. for $2C_{43}H_{49}N_2O_4^+ \cdot 2I^- \cdot 3H_2O$: C, 63.62; H, 6.46; N, 3.45. Found: C, 63.40; H, 6.25; N. 3.29.

2-[4'-(4""-*tert*-Butoxycarbonyl-3""-hydroxyphenoxy)-7'-(1",3",-3"-trimethylindolin-2"-ylidene)-3',5'-(propane-1"',3"'-diyl)-1',-3',5'-heptatrien-1'-yl]-1,3,3-trimethyl-3*H*-indolium Iodide (**17**).

This dye was obtained in an 84% yield, mp >200° dec; ^{1}H nmr (deuteriochloroform): δ 1.40 (s, 12H), 1.58 (s, 9H), 2.05 (quintet, J = 6 Hz, 2H), 2.77 (t, J = 6 Hz, 4H), 3.70 (s, 6H), 6.13 (d, J = 14 Hz, 2H), 6.57 (dd, J = 9 Hz, J = 3 Hz, 1H), 6.64 (d, J = 3 Hz, 1H), 7.12-7.25 (m, 6H), 7.36 (t, J = 8 Hz, 2H), 7.78 (d, J = 9 Hz, 1H), 7.81 (d, J = 14 Hz, 2H), 11.31 (s, exchangeable, 1H); ^{13}C nmr (deuteriochloroform): δ 21.0, 24.7, 27.9, 28.3, 32.5, 48.9, 83.1, 100.9, 103.1, 106.3, 108.7, 110.6, 122.0, 122.7, 125.1, 128.7, 132.7, 140.9, 141.4, 142.8, 162.8, 164.0, 164.5, 169.3, 172.5; ms: (FAB) m/z 657.4 (100%, $C_{43}H_{49}N_2O_4^+$); nir: λ max methanol, 765 nm.

Anal. Calcd. for $C_{43}H_{49}N_2O_4^+ \cdot I^- \cdot H_2O$: C, 64.33; H, 6.40; N, 3.49. Found: C, 64.52; H, 6.27; N, 3.51.

2-[4'-(3""-*tert*-Butoxycarbonyl-4""-hydroxyphenoxy)-7'-(1",3",3"-trimethylindolin-2"-ylidene)-3',5'-(propane-1"',3"'-diyl)-1',3',5'-heptatrien-1'-yl]-1,3,3-trimethyl-3*H*-indolium Iodide (**18**).

This dye was obtained in a 58% yield, mp >200° dec; ^{1}H nmr (deuteriochloroform): δ 1.41 (s, 12H), 1.65 (s, 9H), 2.04 (quintet, J = 6 Hz, 2H), 2.78 (t, J = 6 Hz, 4H), 3.70 (s, 6H), 6.15 (d, J = 14 Hz, 2H), 6.95 (t, J = 9 Hz, 1H), 7.11-7.25 (m, 7H), 7.36 (t, J = 8 Hz, 2H), 7.49 (d, J = 3 Hz, lH), 7.89 (d, J = 14 Hz, 2H), 10.61 (s, exchangeable, 1H); ^{13}C nmr (deuteriochloroform): δ 21.1, 24.7, 27.8, 28.2, 32.4, 48.8, 83.5, 100.6, 110.6, 114.1, 115.1, 119.2, 121.8, 122.0, 123.1, 125.0, 128.7, 140.7, 141.7, 142.7, 151.9, 156.9, 164.1, 168.7, 172.2; ms: (FAB) m/z 657.4 (100%, $C_{43}H_{49}N_2O_4^+$); nir: λ max methanol, 764 nm.

Anal. Calcd. for $2C_{43}H_{49}N_2O_4^+ \cdot 2I^- \cdot H_2O$: C, 65.06; H, 6.35; N, 3.53. Found: C, 64.83; H, 6.33; N, 3.49.

Dyes **19-21**.

A solution of a *tert*-butoxycarbonyl derivative **16-18** (0.16 g, 0.2 mmole) in trifluoroacetic acid (10 ml) was heated under reflux under a nitrogen atmosphere for 30 minutes. Concentration on a rotary evaporator was followed by chromatography of the residue on silica gel with chloroform/methanol (4:1) as an eluent. The resultant trifluoroacetate of a dye was dissolved in methanol (5 ml) and the solution was treated with a solution of perchloric acid in methanol (0.1 *M*, 5 ml) followed by the addition of ether (10 ml). The resultant precipitate of perchlorate **19-21** was crystallized from methanol/ether.

2-[4'-(3""-Carboxy-2""-hydroxyphenoxy)-7'-(1",3",3"-trimethylindolin-2"-ylidene)-3',5'-(propane-1"',3"'-diyl)-1',3',5'-heptatrien-1'-yl]-1,3,3-trimethyl-3*H*-indolium Perchlorate (**19**).

This dye was obtained in an 85% yield, mp >200° dec; ^{1}H nmr (deuteriochloroform): δ 1.41 (s, 12H), 2.02 (quintet, J = 6 Hz, 2H), 2.68 (t, J = 6 Hz, 4H), 3.54 (s, 6H), 5.00 (br, exchangeable, 2H), 5.94 (d, J = 14 Hz, 2H), 6.61 (t, J = 8 Hz, 1H), 8.83 (d, J = 8 Hz, 1H), 7.05 (d, J = 8 Hz, 2H), 7.19 (t, J = 8 Hz, 2H), 7.30-7.36 (m, 4H), 7.68 (d, J = 8 Hz, 1H), 8.06 (d, J = 14 Hz, 2H); nir: λ max methanol 760 nm; hrms: (FAB), Calcd. for $C_{39}H_{41}N_2O_4$ (M^+): m/z 601.3066. Found: m/z 601.3064.

Anal. Calcd. for $2C_{39}H_{41}N_2O_4^+ \cdot 2ClO_4^- \cdot H_2O$: C, 65.95; H, 5.96; N, 3.94. Found: C, 65.92; H, 6.02; N, 3.89.

2-[4'-(4""-Carboxy-3""-hydroxyphenoxy)-7'-(1",3",3"-trimethylindolin-2"-ylidene)-3',5'-(propane-1"',3"'-diyl)-1',3',5'-heptatrien-1'-yl]-1,3,3-trimethyl-3*H*-indolium Perchlorate (**20**).

This dye was obtained in an 89% yield, mp >200° dec; ^{1}H nmr (deuteriochloroform): δ 1.34 (s, 12H), 2.01 (quintet, J = 6 Hz, 2H), 2.66 (t, J = 6 Hz, 4H), 3.55 (s, 6H), 4.2 (br, exchangeable, 2H), 5.95 (d, J = 14 Hz, 2H), 6.54 (d, J = 8 Hz, 1H), 6.57 (s, 1H), 7.06 (d, J = 8 Hz, 2H), 7.16-7.24 (m, 4H), 7.33 (t, J = 8

Hz, 2H), 7.87 (d, J = 14 Hz, 2H), 8.02 (d, J = 8 Hz, 1H); nir: λ max methanol, 762 nm; hrms: (FAB), Calcd. for $C_{39}H_{41}N_2O_4$: (M^+) m/z 601.3066. Found: m/z 601.3113.

Anal. Calcd. for $2C_{39}H_{41}N_2O_4^+ \cdot 2ClO_4^- \cdot H_2O$: C, 65.95; H, 5.96; N, 3.94. Found: C, 66.13; H, 6.09; N, 3.83.

2-[4'-(3""-Carboxy-4""-hydroxyphenoxy)-7'-(1",3",3"-trimethyl-indolin-2"-ylidene)-3',5'-(propane-1"',3"'-diyl)- 1',3',5'-heptatrien-1'-yl]-1,3,3-trimethyl-3*H*-indolium Perchlorate (**21**).

This dye was obtained in a 75% yield, mp >200° dec; ^1H nmr (methanol-d_4): δ 1.38 (s, 12H), 2.03 (m, 2H), 2.74 (m, 4H), 3.57 (s, 6H), 6.10 (d, J = 14 Hz, 2H), 6.92 (d, J = 9 Hz, 1H), 7.17-7.26 (m, 5H), 7.36 (m, 4H), 7.54 (d, J = 3 Hz, 1H),8.02 (d, J = 14 Hz, 2H); nir: λ max methanol, 762 nm; hrms: (FAB), Calcd. for $C_{39}H_{41}N_2O_4$: (M^+) m/z 601.3066. Found: m/z 601.3058.

Anal. Calcd. for $2C_{39}H_{41}N_2O_4^+ \cdot 2ClO_4^- \cdot H_2O$: C, 65.95; H, 5.96; N, 3.94. Found: C, 65.57; H, 6.01; N, 3.85.

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[8] The analytical utility of **10** is clear if one looks at the absorption spectrum change in the presence of Al^{3+}: A = 0.26 (15 μ*M* dye, no Al^{3+}), A = 0.17 (15 μ*M* dye, 5 μ*M* Al^{3+}). Significant changes were observed regarding fluorescence intensities and fluorescence lifetime for all dyes **10**, **19-21**. In particular, the fluorescence lifetimes of **19** and **20** decrease in the presence of aluminum ion. The lifetime of **21** increases with complexation of aluminum ion, which may provide a better discriminator from other quenching effects or interferences. A complete discussion of analytical results will be reported elsewhere.

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