Water-Soluble pH-Sensitive 2,6-Bis(substituted ethylidene)-cyclohexanone/Hydroxy Cyanine Dyes that Absorb in the Visible/Near-Infrared Regions

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Three methods were used to synthesize a series of the title compounds. The ketones absorb in the visible region, and upon protonation (pH<6) they are converted to hydroxy-substituted heptamethine cyanines that show an intense absorption in the near-infrared region (>700 nm). The conversion is reversible and depends solely on pH conditions.

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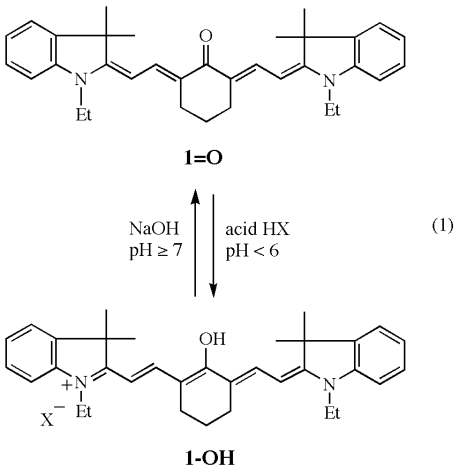
A decade ago we reported for the first time a novel pH-sensitive dye system **1=O/1-OH** shown in equation 1 [1]. Ketone **1=O** exhibits absorption in the visible region (λ_{max} = 535 nm), and upon protonation it is transformed into hydroxy cyanine **1-OH** with an intense absorption in the near-infrared region (λ_{max} = 709 nm). This change is reversible and depends solely on pH conditions. A related



dye (not shown) has been described by us more recently [2]. The search for new pH-sensitive dyes that absorb in the near-infrared region of the electromagnetic spectrum (>650 nm) has been stimulated by potential application of such indicators in bioanalytical chemistry. When complexed or covalently attached to a protein, the pH-sensitive label can act as a pH-dependent reporter molecule in a complex biological material by using non-invasive spectroscopic detection. The direct spectral measurements are possible because few natural products show absorption above 650 nm and, as a result, any biological medium is extensively penetrated by near-infrared radiation [3]. Recently, covalent and non-covalent labeling of biological macromolecules with a near-infrared chromophore has become a firmly established trend in bioanalytical research [3-6].

Due to their limited solubility, the two pH-sensitive dye systems mentioned above have found little application. In continuation of our research we now report for the first time the synthesis of a series of ketone/cyanine compounds that are water soluble. The previous chemistry [1,2] is evaluated, and an improved synthetic methodology is presented.

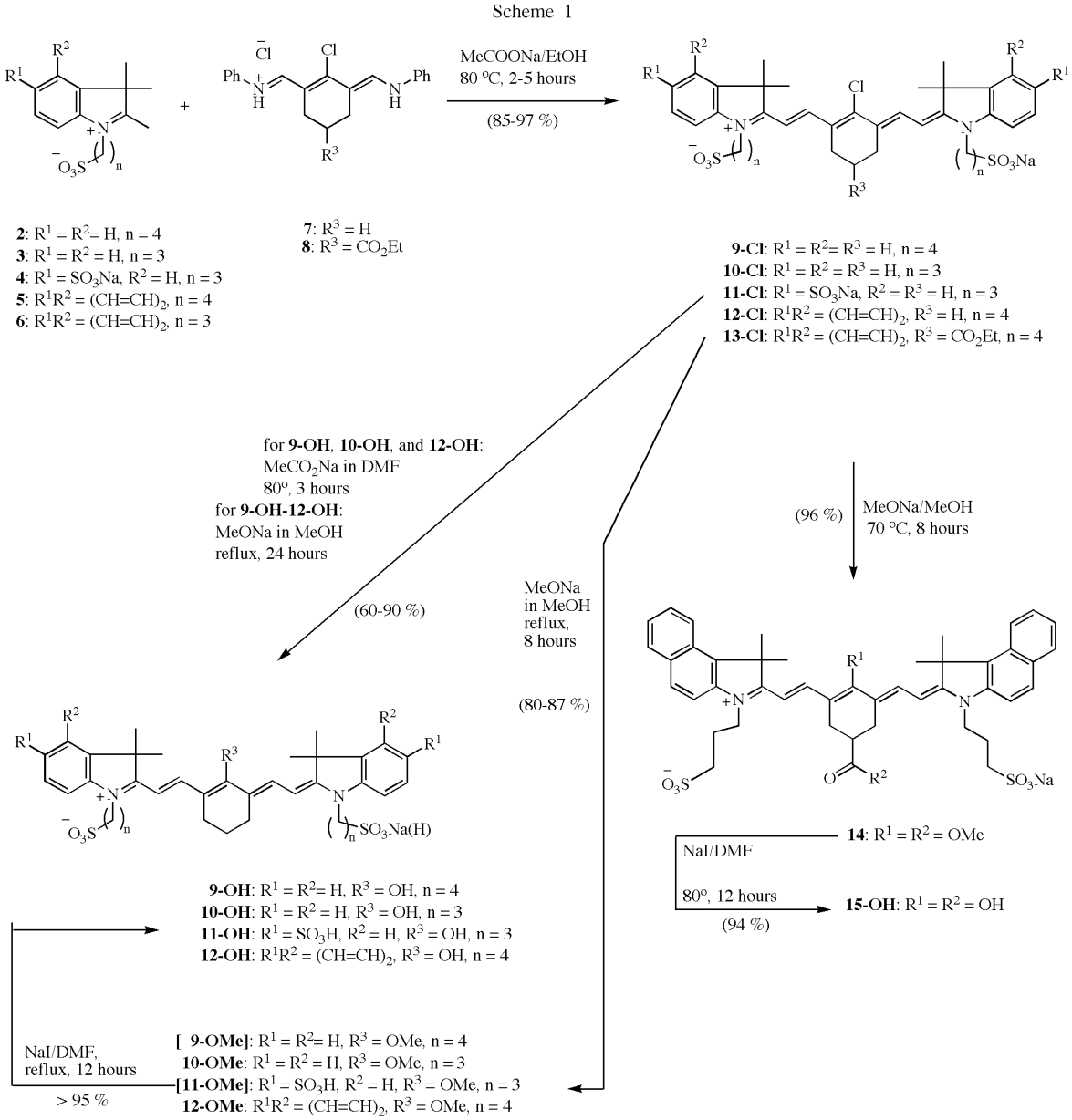
The structures of new pH-sensitive compounds are given in Scheme 1. For the sake of clarity of presentation, only the cyanine forms (**9-OH** - **12-OH, 15-OH**) are shown. As can be seen, all these dyes are N-substituted with hydrophilic sulfoalkyl groups, additional two sulfo groups are attached to the aromatic subunits of **10-OH**, and an additional carboxylic acid function is present in **15-OH**. Chloro cyanines **9-Cl** - **13-Cl** served as precursors to the final products. The cyanines **9-Cl** - **13-Cl** were efficiently synthesized by condensation of indolium derivatives **2-6** with Vilsmeier-Haack reagents **7** or **8**. This is a classical preparation that is conveniently conducted in ethanol in the presence of sodium acetate as a base [7]. The conversion of substrates **9-Cl** - **13-Cl** to the corresponding pH-sensitive dyes is summarized in Table 1 and discussed as follows.

The Sodium-Acetate Method.

It is important to note again that condensation of **2-6** with **7**, **8** in the presence of sodium acetate to give **8-Cl** - **13-Cl** was conducted in ethanol. On the other hand, a subsequent treatment of **9-Cl**, **10-Cl**, **12-Cl** with sodium acetate in *N,N*-dimethylformamide instead of ethanol gave the respective pH-sensitive dyes **9-OH**, **10-OH**, **12-OH**.

The yields ranged from 60% to 76% but the chloro polyanionic cyanine **11-Cl** could not be converted to the expected product **11-OH** under similar conditions. It can be suggested that the successful displacement reactions of chlorine involve an $S_{RN}1$ pathway to generate an acetoxy-substituted cyanine that is hydrolyzed to a ketone/hydroxy cyanine upon aqueous workup [2]. The $S_{RN}1$ reactions of chloro-substituted indolium heptamethine cyanines are quite common [8]. Consistent with this suggestion is a

Scheme 1



known ability of *N,N*-dimethylformamide, in contrast to ethanol, to promote a single-electron-transfer process (SET) of the $S_{RN}1$ pathway [9-11]. The acetate ion mediated reactions of the chloro cyanines were also inhibited in a mixed solvent of *N,N*-dimethylformamide and nitrobenzene (4:1). Nitrobenzene is a single-electron scavenger, and it interferes with the initial SET process of the $S_{RN}1$ pathway [9-11].

The Sodium Methoxide/Sodium Iodide Method.

The hydrophobic dye **1-OH** (eq. 1) has been obtained by us previously by treatment of the corresponding chloro cyanine with sodium methoxide followed by isolation and demethylation of the resultant methoxy-substituted cyanine by the reaction with iodide ion [1]. As part of this work, sulfo-substituted cyanines **9-OH** - **12-OH** and **15-OH** were obtained efficiently in a similar way. Importantly, compound **11-Cl** that is stable in the presence of sodium acetate (see above) was converted to **11-OH** by using this two-step methodology. In a simplified procedure two intermediate methoxy cyanines, **9-OMe** and **11-OMe**, were not purified and were subjected to the subsequent reaction with sodium iodide in a crude form. In the preparation of carboxy-substituted compound **15-OH**, the treatment of **13-Cl** with sodium methoxide resulted not only in displacement of the chlorine but also in transesterification of the ethoxycarbonyl function. The resultant dimethoxy derivative **14** was then allowed to react with sodium iodide to give the desired hydroxy acid **15-OH**.

Table 1

Electronic spectra [a] and yields of the cyclohexanone/cyanine coumpounds. [b]

Ketone	λ_{max} (ε) nm ($M^{-1}cm^{-1}$)	Cyanine	λ_{max} (ε) nm ($M^{-1}cm^{-1}$)	Yield (%) for the indicated synthetic method [c] MeCOONa	MeONa/NaI	MeONa
9=O	528 (39000)	**9-OH**	711 (170000)	76	80	82
10=O	528 (39000)	**10-OH**	711 (170000)	75	80	83
11=O	545 (38000)	**11-OH**	748 (190000)	0 [d]	65	90
12=O	550 (40000)	**12-OH**	750 (180000)	60	84	90
15=O	555 (47000)	**15-OH**	746 (120000)	_	94	_

[a] The spectra were taken in methanol. [b] The ketones and the corresponding hydroxy cyanines are denoted by the respective symbols =O and –OH. [c] The yields for a ketone and the corresponding cyanine were within 2% regardless of the isolation method. All yields are for the conversions starting with the chloro cyanines **9-Cl** – **13-Cl**. [d] Cyanine **10-Cl** is stable in the presence of sodium acetate in *N,N*-dimethylformamide.

The Sodium Methoxide Method.

It was observed that treatment of chloro-substituted cyanines with sodium methoxide, in addition to the major methoxy-substituted product, always produced a small amount of the corresponding pH-sensitive dye directly in the reaction medium. Obviously, the methoxy derivatives undergo demethylation by an S_N2 process with the methoxide ion acting as nucleophile. This observation led to simplification of the two-step synthesis discussed above. Specifically, by prolonging the reaction time of a chloro cyanine with sodium methoxide, the treatment of the intermediate methoxy cyanine with sodium iodide can be eliminated and the pH-sensitive dye is obtained directly in a one-pot procedure. As can be seen from Table 1, this simplified procedure provides pH-sensitive products in better yields than the two methods discussed above.

Isolation of pH-Sensitive Dyes.

In order to simplify presentation of this work only the hydroxy forms of the dyes are given in Scheme 1 and discussed in the text. However, either hydroxy cyanines or their respective ketones can be isolated depending on workup. Thus, acidification of the reaction mixtures causes precipitation of hydroxy cyanines, and dilution with ether of the mixtures causes precipitation of the corresponding ketones. The ketones are also converted to cyanines by crystallization from 2% perchloric acid in ethanol. Depending on the structure, the sulfo-substituted hydroxy cyanines are isolated either as sulfonic acid or sodium sulfonate derivatives. Examples are provided in the experimental section.

Structure Determination of pH-Sensitive Dyes.

Depending on the isolation method, the dyes were obtained in the hydroxy cyanine or aminodienone form. All isolated cyanines and ketones gave accurate combustion analysis results or hrms data that are fully consistent with the given molecular compositions. As already mentioned, the cyanine/ketone conversion in solution is pH dependent and fully reversible. Since both compounds **9-OH** and **9=O** were isolated in analytically pure forms, it was of interest to compare their spectral features in more detail. The ^1H nmr spectrum of **9-OH** is typical for heptamethine cyanines [7,8] in respect to chemical shifts and coupling patterns. The latter include a coupling constant for 14 Hz for two adjacent protons of the vinylene moieties of **9-OH**. As expected, deprotonation of cationic cyanine **9-OH** gives rise to upfield shifts of all corresponding proton signals in the resultant neutral ketone **9=O**.

In the ir spectrum of **9-OH** taken in a pellet of potassium bromide the absorption of the hydroxy group is obscured by that of water present in the crystalline sample. Nevertheless, the observation of an intense band around 1100 cm^{-1} for the carbon-oxygen stretching vibration, which is the strongest absorption in the spectrum, is indicative of the presence of a hydroxy group in the molecule of **9-OH**. By contrast, this absorption band is absent from the ir spectrum of the ketone **9=O**, and a new intense band is observed around 1590 cm^{-1} for the stretching vibration of the carbonyl group. This intense band clearly stands out from the aromatic and vinyl absorptions of medium intensities. In a similar way, the aromatic and vinyl absorptions of **9-OH** are also relatively weak. The rather low wavenumber for the vibration of a carbonyl group can be understood in terms of a highly conjugated structure of the aminodienone **9=O**. More specifically, the expected low vibrational frequency for the dienone system of **9=O** is additionally decreased by its conjugation with an amino moiety. Since aminodienone **9=O** and analogs reported from this laboratory are the first examples of a new class of compounds, no literature data is available for comparison.

Final Remarks.

Of the several approaches to conversion of readily available heptamethine chloro cyanines to pH-sensitive ketone/cyanine compounds, the one-pot reaction mediated by methoxide ion is the most efficient. In addition to near-

infrared applications, all cyanine compounds **9-OH** - **12-OH** and **15-OH** can also be used as pH indicators in the visible region. Thus, their cyanine form (pH 4) has an unpleasant gray-green color, due to a partial far-end visible absorption, and a beautiful pink color is observed for the ketone form (pH 7). Dramatic changes in color are observed with pH changes from 7 to 4, and the difference is clearly discernible at pH 6 by using the naked eye. Spectrophotometric titration of **15-OH** gave pKa = 4.5 ± 0.2 for the cyanine-ketone transition.

EXPERIMENTAL

Compounds **2**, **3**, **5-8**, **9-Cl**, and **10-Cl** were obtained by using the published procedures [4,12-15]. All new compounds had no defined mp decomposing above 200 °C. Unless indicated otherwise, ^{1}H nmr spectra were recorded at 400 MHz in dimethyl sulfoxide-d_6 with tetramethylsilane as an internal standard.

Sodium [1-(3-Sulfonatopropyl)-2,3,3-trimethyl-3*H*-indolium-5-sulfonate (**4**).

Alkylation of 2,3,3-trimethyl-3*H*-indole-5-sulfonic acid with 1,3-propane sultone was conducted by using a general procedure (toluene, reflux for 20 hours) [4,15]. After crystallization from methanol/ether the yield was 91%; ^{1}H nmr: δ 1.52 (s, 6H), 2.14 (quint, J = 7 Hz, 2 H), 2.63 (t, J = 7 Hz, 2H), 2.81 (s, 3H), 4.62 (t, J = 7 Hz, 2H), 7.80 (d, J = 8 Hz, 1 H), 7.96 (d, J = 8 Hz, 1H), 7.99 (s, 1H); hrms: (FAB, negative *m*-nitrobenzyl alcohol matrix), Calcd. for $C_{14}H_{18}NNaO_6S_2$, m/z 360.0576; observed m/z 360.0563.

Cyanine Dyes **11-Cl** - **13-Cl**.

Condensation of inner salt **4-6** with Vilsmeier-Haack reagent **7,8** was conducted in ethanol in the presence of sodium acetate by using a general procedure [1,4,14]. Products were crystallized from methanol/ether.

Sodium 3-[2-[4-Chloro-7-[3,3-dimethyl-5-(sodium sulfonato)-1-(3-sulfonatopropyl)-1*H*-indol-1-ium-2-yl]-3,5-(propane-1,3-diyl)-2,4,6-heptatrien-1-ylidene]-3,3-dimethyl-5-(sodium sulfonato)-1,2-dihydro-3*H*-indol-1-yl]propanesulfonate (**11-Cl**).

This compound was obtained in 85% yield; ^{1}H nmr: δ 1.68 (s, 12H), 1.84 (m, 2H), 2.04 (m, 4H), 2.60 (t, J = 6 Hz, 4 H), 2.75 (m, 4H), 4.38 (m, 4H), 6.54 (d, J = 14 Hz, 2H), 7.46 (d, J = 9 Hz, 2H), 7.68 (d, J = 9 Hz, 1H), 7.80 (s, 2H), 8.26 (d, J = 14 Hz, 2H); nir λ_{max} = 793 nm (ε = 2.6 x 10^5 $cm^{-1}M^{-1}$).

Anal. Calcd. for $C_{36}H_{40}ClN_2Na_3O_{12}S_4$•NaCl•6$H_2O$: C, 39.61; H, 4.80; N, 2.57; Cl, 6.50. Found: C, 39.29; H, 4.58; N, 2.65; Cl, 6.79.

Co-crystallization of **11-Cl** with an equimolar amount of sodium chloride from a homogenous ether solution can be interpreted in terms as a dye structure in which all four anionic sulfonate groups are neutralized by four sodium counter cations, and the cationic cyanine chromophore interacts with external chloride anion. This phenomenon is quite common [16].

Sodium 4-[2-[4- Chloro-7-[1,1-dimethyl-3-(4-sulfonatobutyl)-1*H*-benz[*e*]indol-3-ium-2-yl]-3,5-(propane-1,3-diyl)-2,4,6-heptatrien-1-ylidene]-1,1-dimethyl-1,2-dihydro-3*H*-benz[*e*]indol-3-yl]butanesulfonate (**12-Cl**).

This compound was obtained in 90% yield; ^{1}H nmr: δ 1.81 (m, 4H), 1.91 (m, 6H), 1.96 (s, 12H), 2.52 (t, J = 6 Hz, 4H), 2.78 (m, 4H), 4.36 (m, 4H), 6.41 (d, J = 14 Hz, 2H), 7.52 (t, J = 8 Hz, 2H), 7.65 (t, J = 8 Hz, 2H), 7.81 (d, J = 8 Hz, 2H), 8.07 (d, J = 8 Hz, 2H), 8.10 (d, J = 8 Hz, 2H), 8.29 (d, J = 8 Hz, 2H), 8.38 (d, J = 14 Hz, 2H).

Anal. Calcd. for $C_{46}H_{50}ClN_2NaO_6S_2$•2$H_2O$: C, 63.98; H, 6.42; N, 3.24. Found: C, 64.24; H, 6.69; N, 3.16.

Sodium 3-[2-[4-Chloro-7-[1,1-dimethyl-3-(3-sulfonatopropyl)-1*H*-benz[*e*]indol-3-ium-2-yl]-3,5-(2-(ethoxycarbonyl)propane-1,3-diyl)-2,4,6-heptatrien-1-ylidene]-1,1-dimethyl-1,2-dihydro-3*H*-benz[*e*]indol-3-yl]propanesulfonate (**13-Cl**).

This compound was obtained in 97% yield; ^{1}H nmr: δ 1.22 (t, J = 7 Hz, 3H), 1.97 (s, 12H), 2.12 (t, J = 7 Hz, 4H), 2.64 (t, J = 7 Hz, 4H), 2.80-3.30 (m, 5H), 4.15 (q, J = 7 Hz, 2H), 4.53 (t, J = 7 Hz, 4H), 6.54 (br d, J = 14 Hz, 2H), 7.36 (d, J = 8 Hz, 2H), 7.53 (t, J = 8 Hz, 2H), 7.66 (t, J = 8 Hz, 2H), 7.88 (d, J = 8 Hz, 2H), 8.07 (d, J = 8 Hz, 2H), 8.09 (d, J = 8 Hz, 2H), 8.29 (d, J = 8 Hz, 2H), 8.38 (br d, J = 14 Hz, 2H); nir: λ_{max} = 818 nm (ε = 3.2 x 10^5 $cm^{-1}M^{-1}$).

Anal. Calcd. for $C_{47}H_{50}ClN_2NaO_8S_2$•2$H_2O$: C, 60.73; H, 5.86; N, 3.01. Found: C, 60.70; H, 6.12; N, 3.19.

Cyanine Dyes **10-OMe**, **12-OMe** and **14**.

A solution of **10-Cl, 12-Cl** or **13-Cl** (2 mmoles) and sodium methoxide in methanol (0.5 *M*, 50 ml) was heated under reflux for 8 hours, and then the mixture was cooled and treated with solid carbon dioxide or hydrochloric acid (6 *M*, 5 ml). The solution was filtered and concentrated, and the residue of the resultant product, **10-OMe, 12-OMe** or **14**, was purified by silica gel chromatography eluting with methanol/chloroform (1:5). Then the dyes were crystallized from methanol/ether.

3-[2-[7-[3,3-3-Dimethyl-1-(3-sulfonatopropyl)-1*H*-indol-1-ium-2-yl]-4-methoxy-3,5-(propane-1,3-diyl)-2,4,6-heptatrien-1-ylidene]-3,3-dimethyl-1,2-dihydro-3*H*-indol-1-yl]propanesulfonic Acid (**10-OMe**).

Following workup with hydrochloric acid this propanesulfonic acid was obtained in 80% yield; ^{1}H nmr: δ 1.66 (s, 12H), 1.80 (m, 10H), 2.61(m, 4H), 3.95 (s, 3H), 4.16 (m, 4H), 6.21 (d, J = 14 Hz, 2H), 7.23 (m, 2H), 7.41 (m, 4H), 7.59 (d, J = 7 Hz, 2H), 8.01 (d, J = 14 Hz, 2H); nir λ_{max} = 760 nm (ε = 1.9 x 10^5 $cm^{-1}M^{-1}$).

Anal. Calcd. for $C_{37}H_{46}N_2O_7S_2$•4H_2O: C, 57.94; H, 7.10; N, 3.65. Found: C, 57.93; H, 7.08; N, 3.45.

Sodium 4-[2-[7-[1,1-Dimethyl-3-(4-sulfonatobutyl)-1*H*-benz[*e*]indol-3-ium-2-yl]-4-methoxy-3,5-(propane-1,3-diyl)-2,4,6-heptatrien-1-ylidene]-1,1-dimethyl-1,2-dihydro-3*H*-benz[*e*]indol-3-yl]butanesulfonate (**12-OMe**).

Following workup with carbon dioxide this sodium butanesulfonate was obtained in 87% yield; ^{1}H nmr: δ 1.80 (m, 10H), 2.53 (m, 4H), 2.64 (m, 4H), 4.00 (s, 3H), 4.28 (m, 4H), 6.24 (d, J = 14 Hz, 2H), 7.48 (t, J = 8 Hz, 2H), 7.63 (t, J = 8 Hz, 2H), 7.75 (d, J = 9 Hz, 2H), 8.04 (d, J = 9 Hz, 2H), 8.06 (d, J = 8 Hz, 2H), 8.10 (d, J = 14 Hz, 2H), 8.26 (d, J = 8 Hz, 2H); nir λ_{max} = 794 nm (ε = 2.0 x 10^5 $cm^{-1}M^{-1}$); hrms: (FAB, negative m-nitrobenzyl alcohol matrix), Calcd. for $C_{47}H_{53}N_2O_7S_2$ m/z 821.3294, observed m/z 821.3262.

Anal. Calcd. for $C_{47}H_{53}N_2NaO_7S_2$•5H_2O: C, 60.37; H, 6.79; N, 3.00. Found: C, 60.31; H, 6.65; N, 2.93.

3-[2-[7-[1,1-Dimethyl-3-(3-sulfonatopropyl)-1*H*benz[*e*]indol-3-ium-2-yl]-4-methoxy-3,5-(2-(methoxycarbonyl)propane-1,3-diyl)-2,4,6-heptatrien-1-ylidene]-1,1-dimethyl-1,2-dihydro-3*H*-benz[*e*]indol-3-yl]propanesulfonic Acid (**14**).

Following workup with hydrochloric acid this propanesulfonic acid was obtained in 96% yield; ^{1}H nmr: δ 1.96 (s, 12H), 2.09 (m, 4H), 2.63 (t, J = 7 Hz, 4H), 2.70 (m, 4H), 3.00 (m, 1H), 3.70 (s, 3H), 4.00 (s, 3H), 4.41 (t, J = 7 Hz, 4H), 6.36 (d, J = 14 Hz, 2H), 7.50 (t, J = 8 Hz, 2H), 7.65 (t, J = 8 Hz, 2H), 7.85 (d, J = 9 Hz, 2H), 8.05 (d, J = 9 Hz, 2H), 8.07 (d, J = 8 Hz, 2H), 8.15 (d, J = 14 Hz, 2H), 8.27 (d, J = 8 Hz, 2H); nir λ_{max} = 794 nm (ε = 2.2 x 10^5 $cm^{-1}M^{-1}$).

Anal. Calcd. for $C_{45}H_{53}N_2O_9S_2$•$5H_2O$: C, 58.74; H, 6.90; N, 3.04. Found: C, 58.40; H, 6.53; N, 2.80.

pH-Sensitive Dyes.

General.

All reactions described below were conducted under an atmosphere of dry nitrogen. The reaction progress was monitored by visible/near-infrared spectroscopy for aliquots diluted with methanol until absorption of the starting chloro cyanine disappeared. The procedures given below provide a ketone dye. The ketones were converted to the corresponding, analytically pure cyanines by crystallization from ethanolic solution of 2% perchloric acid. Alternatively, the hydroxy cyanine was precipitated directly from a crude mixture by stirring and dropwise acidification with 6 *M* aqueous hydrochloric acid. For sulfoalkyl-substituted hydroxy cyanines, depending on structure, either a sulfonic acid or sodium sulfonate derivative was precipitated upon acidification. The resultant precipitate was washed with ether. The yields for ketones and the corresponding cyanines were within 2% (Table 1). All products thus obtained were additionally crystallized by a dropwise dilution of a solution in methanol with *tert*-butyl methyl ether. In all cases the crystallization yields were greater than 96%. All products were dried at 50°C/0.5 mmHg.

The Sodium Acetate Method.

The previously published procedure [1,8] was modified as follows. A solution of a chloro cyanine (0.5 mmole) and sodium acetate (85 mg, 1 mmole) in anhydrous *N,N*-dimethylformamide (8 ml) was heated to 80° for 3 hours. After quenching with solid carbon dioxide, a crude mixture was filtered and the solution was concentrated by rotary evaporation. A ketone dye was isolated by silica gel flash chromatography eluting with methanol/dichloromethane, up to 20% of methanol.

The Sodium Methoxide/Sodium Iodide Method.

A solution of a chloro cyanine (2 mmoles) and sodium methoxide in methanol (0.5 *M*, 50 ml) was heated under reflux for 8 hours and then cooled and quenched with solid carbon dioxide. The solution was filtered and concentrated, and the crude methoxy-substituted cyanine thus obtained was then heated under reflux in *N,N*-dimethylformamide (25 ml) in the presence of sodium iodide (2 g, 13 mmoles) for 12 hours. After concentration, the residue was subjected to silica gel flash chromatography as described above to give a ketone. Methoxy-substituted intermediate cyanines **10-OMe**, **12-OMe**, and **14** were isolated by silica gel flash chromatography (see above) before the treatment with sodium iodide.

The Sodium Methoxide Method.

A solution of a chloro cyanine and sodium methoxide in methanol, as described above, was heated under reflux for 24 hours, and then cooled and quencheed with solid carbon dioxide, and filtered. Dropwise dilution of the methanolic solution with ether gave a precipitate of a ketone.

2,6-Bis[[1-(sodium 4-sulfonatobutyl)-3,3-dimethylindolin-2-ylidene]ethylidene]cyclohexanone (**9=O**).

This compound was also characterized as a cyanine derivative (see below); ^{1}H nmr: δ 1.55 (s, 12H), 1.57-1.70 (m, 10H), 2.40 (m, 8H), 3.72 (m, 4H), 5.46 (d, J = 14 Hz, 2H), 6.87 (t, J = 8 Hz, 2H), 6.90 (d, J = 8 Hz, 2H), 7.16 (d, J = 8 Hz, 2H), 7.29 (d, J = 8 Hz, 2H), 7.91 (d, J = 14 Hz, 2H); hrms: (FAB, negative m-nitrobenzyl alcohol matrix), Calcd. for $C_{38}H_{46}N_2O_7S_2Na$ (M^+-Na) m/z 729.2644, observed m/z 729.2646.

Sodium 4-[2-[7-[3,3-Dimethyl-1-(4-sulfonatobutyl)-3*H*-indol-1-ium-2-yl]-4-hydroxy-3,5-(propane-1,3-diyl)-2,4,6-heptatrien-1-ylidene]-3,3-dimethyl-1,2-dihydro-3*H*-indol-1-yl]butanesulfonate (**9-OH**).

This compound was also characterized in the keto form (see above); ^{1}H nmr: δ 1.61 (s, 12H), 1.64-1.72 (m, 10H), 2.50 (m, obscured by dimethyl sulfoxide signal), 4.01 (t, J = 6 Hz, 4H), 5.93 (d, J = 14 Hz, 2H), 7.10 (t, J = 8 Hz, 4H), 7.24 (d, J = 8 Hz, 2H), 7.31 (t, J = 8 Hz, 2H), 7.47 (d, J = 8 Hz, 2H), 8.19 (d, J = 14 Hz, 2H). hrms: (FAB, negative m-nitrobenzyl alcohol matrix), Calcd. for $C_{38}H_{46}N_2O_7S_2Na$ (M^+-H) m/z 729.2644, observed m/z 729.2660.

3-[2-[7-[3,3-Dimethyl-1-(3-sulfonatopropyl)-3*H*-indol-1-ium-2-yl]-4-hydroxy-3,5-(propane-1,3-diyl)-2,4,6-heptatrien-1-ylidene]-3,3-dimethyl-1,2-dihydro-3*H*-indol-1-yl]propanesulfonic Acid (**10-OH**).

The acidic workup gave an analytically pure compound; ^{1}H nmr: δ 1.66 (s, 12H), 1.70 (m, 2H), 2.02 (m, 4H), 2.58-2.63 (m, 8H), 4.31 (m, 4H), 6.35 (d, J = 14 Hz, 2H), 7.23 (t, J = 8 Hz, 2H), 7.40-7.47 (m, 4H), 7.57 (d, J = 8 Hz, 2H), 8.02 (d, J = 14 Hz, 2H).

Anal. Calcd. for $C_{36}H_{44}N_2O_7S_2$•$3H_2O$: C, 58.83; H, 6.86; N, 3.81. Found: C, 58.71; H, 6.83; N, 3.88.

3-[2-[7-[3,3-Dimethyl-5-sulfo-1-(3-sulfonatopropyl)-3*H*-indol-1-ium-2-yl]-4-hydroxy-3,5-(propane-1,3-diyl)-2,4,6-heptatrien-1-ylidene]-3,3-dimethyl-5-sulfo-1,2-dihydro-3*H*-indol-1-yl]-propanesulfonic Acid (**11-OH**).

This compound was obtained was obtained as a tetrasulfonic acid; ^{1}H nmr: δ 1.64 (s, 12H), 1.75 (m, 2H), 2.00 (m, 4H), 2.60 (m, 8H), 4.28 (m, 4H), 6.33 (d, J = 14 Hz, 2H), 7.37 (d, J = 8 Hz, 2H), 7.63 (d, J = 8 Hz, 2H), 7.73 (s, 2H), 7.99 (d, J = 14 Hz, 2H).

Anal. Calcd. for $2C_{36}H_{44}N_2O_{13}S_4$•$3H_2O$: C, 49.81; H, 5.46; N, 3.23. Found: C, 49.69; H, 5.85; N, 3.52.

2,6-Bis[[1-(sodium 4-sulfonatobutyl)-1,1-dimethyl-1,2-dihydro-3*H*-benz[*e*]indol-2-ylidene]ethylidene]cyclohexanone (**12=O**).

This compound was characterized as follows; ^{1}H nmr (methanol-d_4): δ 1.98 (m, 10H), 2.02 (s, 12H), 2.69 (m, 4H), 2.93 (m, 4H), 3.98 (m, 4H), 5.68 (d, J = 14 Hz, 2H), 7.31 (t, J = 8 Hz, 2H), 7.36 (d, J = 8 Hz, 2H), 7.51 (t, J = 8 Hz, 2H), 7.86 (m, 4H), 8.14 (d, J = 8 Hz, 2H), 8.36 (d, J = 14 Hz, 2H); hrms: (FAB, negative m-nitrobenzyl alcohol matrix), Calcd. for $C_{46}H_{50}N_2O_7S_2Na$ (M^+-Na) m/z 829.2957, observed m/z 829.2954.

Sodium 3-[2-[7-[1,1-Dimethyl-3-(3-sulfonatopropyl)-1*H*-benz[*e*]indol-3-ium-2-yl]-4-hydroxy-3,5-(2-carboxypropane-1,3-diyl)-2,4,6-heptatrien-1-ylidene]-1,1-dimethyl-1,2-dihydro-3*H*-benz[e]indol-3-yl]propanesulfonate (**15-OH**).

This compound was obtained by demethylation of 14; ^{1}H nmr: δ 1.95 (s, 12H), 2.11 (m, 4H), 2.70-3.10 (m, 9H), 4.41 (m, 4H), 6.18 (d, J = 13 Hz, 2H), 7.47 (t, J = 8 Hz, 2H), 7.63 (t, J = 8 Hz, 2H), 7.78 (d, J = 8 Hz, 2H), 8.02 (d, J = 8 Hz, 2H), 8.04 (d, J = 8 Hz, 2H), 8.25 (d, J = 8 Hz, 2H), 8.40 (d, J = 13 Hz, 2H).

Anal. Calcd. for $C_{43}H_{47}N_2NaO_9S_2 \bullet 6H_2O$: C, 55.47; H, 6.39; N, 3.01. Found: C, 55.18; H, 6.05, N. 2.73.

REFERENCES AND NOTES

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