NOVEL SYNTHETIC ROUTE TO pH-SENSITIVE 2,6-BIS(SUBSTITUTED ETHYLIDENE)CYCLOHEXANONE/HYDROXYCYANINE DYES THAT ABSORB IN THE VISIBLE/NEAR-INFRARED REGIONS

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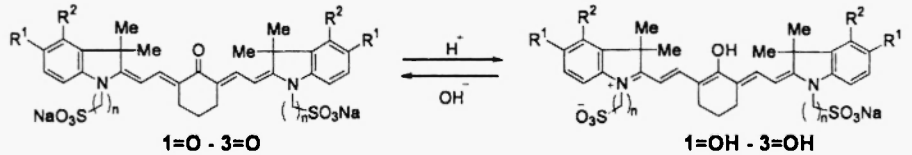
Abstract: Succinimide *N*-oxide anion-mediated reaction of heptamethine cyanines that are chloro substituted at the central position of the heptamethine moiety furnishes the title dyes in high yield (80-96%). The ketones absorb in the visible region, and upon protonation (pH<6) they are transformed into hydroxycyanines that show an intense absorption in the near-infrared region (>700 nm).

Introduction

Recently, non-covalent labeling of biological macromolecules with a near-infrared chromophore (λ_{max} >700 nm) has become a firmly established trend in bioanalytical research.[1] With the use of near-infrared pH-sensitive reporter molecules another dimension is added to bioanalytical research because the spectral properties of the labels change with hydrogen ion concentration. Examples of pH-sensitive dyes[2,3] (Scheme 1) are ketones[4] **1=O - 3=O** (λ_{max} = 528-550 nm), the protonation of which results in the formation of near-infrared cyanines[4] **1-OH - 3-OH** (λ_{max} = 711-750 nm). These dyes were obtained in modest yields by ipso substitution of the corresponding chlorocyanines in the sodium acetate or sodium methoxide mediated reactions. The treatment with MeCOONa in DMF at an elevated temperature produces an acetoxycyanine that undergoes hydrolysis to a ketone during workup. In the second method, an intermediate methoxycyanine undergoes demethylation in a nucleophilic substitution reaction with methoxide ion in methanol. A superior pH-sensitive dye **8=O** (structure in Scheme 2) was synthesized in a similar way,[5] albeit in the low yield of 26% (**8=O**, λ_{max} = 648 nm; **8-OH**, λ_{max} = 932 nm). The attempted conversions of **9-Cl**, **10-Cl**, **12-Cl**, and **13-Cl** (structures in Schemes 2-4) were all unsuccessful.[6]

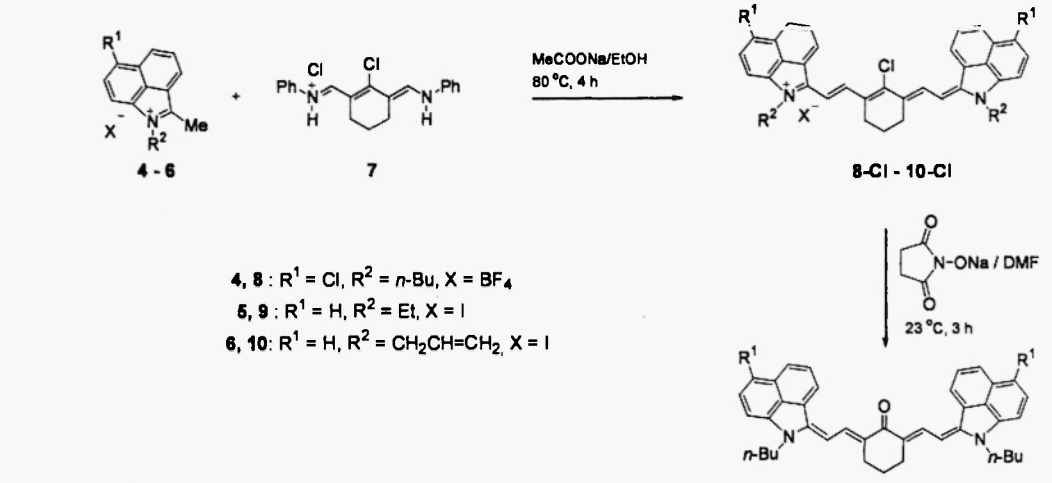


1=O - 3=O **1=OH - 3=OH**

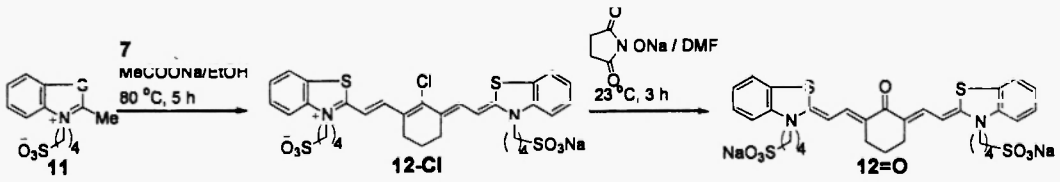
Scheme 1 **1**: $R^1 = R^2$ = H, n = 3; **2**: $R^1 = R^2$ = H, n = 4; **3**: R^1R^2 = $(CH=CH)_2$, n = 4

Results and Discussion

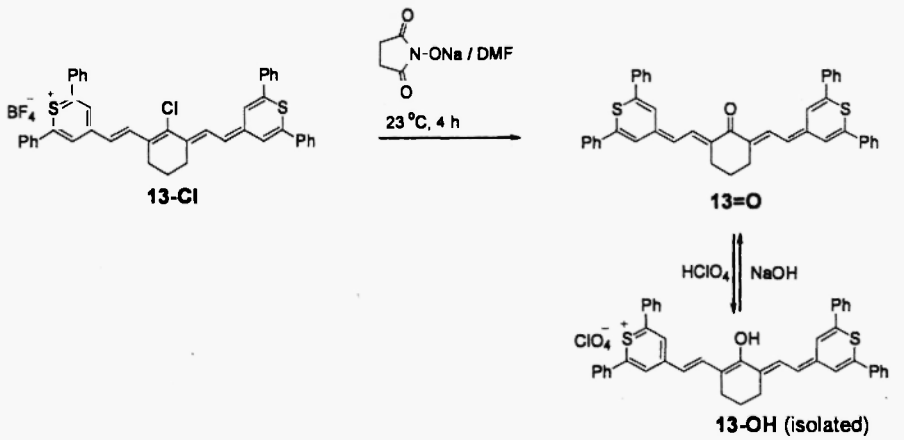
In this work, ketones of Scheme 1 and ketones **8=O - 10=O**, **12=O**, and **13=O** (Schemes 2-4) were obtained with high efficiency (yields **80-96%**) by treatment of the corresponding chlorocyanines with sodium succinimide *N*-oxide in DMF under mild conditions. Due to the high yields, chromatography was not required, and the ketones were obtained in an analytically pure form by precipitation from a crude mixture followed by single crystallization. Alternatively, a corresponding hydroxycyanine was precipitated from a crude mixture by stirring and dropwise acidification with perchloric acid as exemplified in Scheme 4. The isolated ketones could also be converted to the corresponding cyanines by crystallization from ethanolic solution of $HClO_4$. Conversely, basification of a



Scheme 2



Scheme 3



Scheme 4

Table 1. Spectral properties of new 2,6-disubstituted cyclohexanone/cyanine dyes[a]

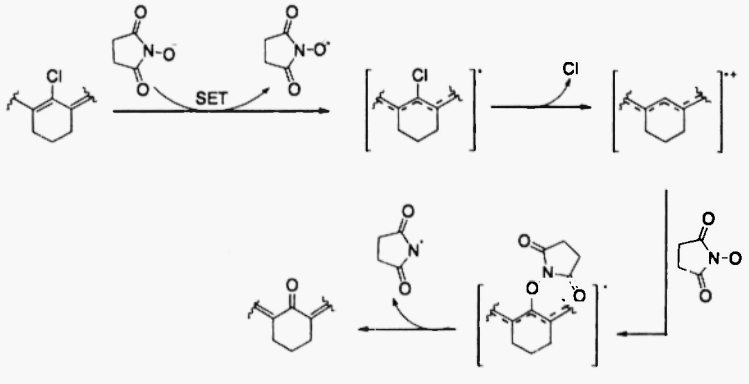
Ketone	λ_{max}, nm (ε), $M^{-1}cm^{-1}$	Cyanine	λ_{max}, nm (ε), $M^{-1}cm^{-1}$
9=O	644 (50800)	**9-OH**	917 (142000)
10=O	634 (51100)	**10-OH**	914 (142000)
12=O	656 (35000)	**12-OH**	739 (150000)
13=O	582 (54000)	**13-OH**	932 (200000)

[a]The electronic spectra were taken in MeOH (**9, 10**) and in EtOH (**12, 13**).

hydroxycyanine solution in DMF followed by treatment with ether caused precipitation of a ketone. The visible/near-infrared spectra of the new ketone/cyanine systems are given in Table 1.

Sodium succinimide *N*-oxide was generated by the reaction of *N*-hydroxysuccinimide with sodium hydride in anhydrous DMF. This step can be avoided by the use of *N*-hydroxysuccinimide in the presence of triethylamine. The penalty is an increased reaction time, up to three-fold for the conversion of chlorocyanines to ketone/cyanine dyes. Nevertheless, the two methodologies (NaH and Et_3N) give the final dyes with virtually identical efficiency.

The suggested mechanism for the succinimide *N*-oxide anion mediated transformation of chlorocyanines to ketones (Scheme 5) is based on the following observations. First, the ketones are formed directly in the anhydrous reaction medium, that is, their formation does not involve hydrolysis of an intermediate product during workup. This conclusion was derived from the observation of the characteristic color of the ketones, which became more intense as the reactions progressed, and confirmed by analysis of visible absorption spectra of the crude mixtures. Second, the reactions are strongly inhibited in the presence of nitrobenzene which is a single-electron scavenger.[7] For example, the yield of **12=O** was 96% in the absence of nitrobenzene, 40% for a 9:1 mixture of DMF/$PhNO_2$, and the



Scheme 5

synthesis of **12=O** was completely inhibited in a 1:1 mixture of DMF/$PhNO_2$ under otherwise identical conditions. The starting cyanine **12-Cl** constituted the remaining material balance. The mechanism of Scheme 5 is also consistent with

analysis of GC-MS spectra of crude mixtures, which showed the presence of succinimide, apparently drived from succinimidyl radical. Related free-radical reactions have previously been postulated.[8,9] Detailed mechanistic studies are in progress.

In summary, a novel, highly efficient conversion of readily available heptamethine chlorocyanines to pH-sensitive ketone/cyanine systems is described. The one-pot reaction is mediated by succinimide *N*-oxide anion. This reaction provides an easy access to pH-sensitive benz[*cd*]indolium (**8-10**) and thiopyrylium (**13**) derivatives with outstanding pH-dependent spectral properties. The most dramatic changes in absorption are observed for **13** ($\Delta\lambda_{max}$ = 350 nm) as the visible absorption of ketone under neutral or basic pH conditions is bathochromically shifted beyond 900 nm for the cyanine chromophore that is generated upon protonation of the ketone. In all cases the spectral changes are reversible and depend on pH conditions.

Experimental

General. Chlorocyanines **8-Cl** and **13-Cl** are commercial products. The preparations of **1-Cl - 3-Cl**,[3] **5**, **6**[10] and **7**[11] have been described previously. All new compounds had no defined mp decomposing above 200 °C. Unless indicated otherwise, ^{1}H NMR spectra were recorded at 400 MHz in DMSO-d_6 with TMS as an internal standard.

4-(2'-Methyl-3'-benzothiazolio)butanesulfonate (11). Alkylation of 2-methylbenzothiazole with 1,4-butane sultone was conducted by using a general procedure (toluene, reflux for 20h).[12] Compound **11** was crystallized from MeOH/ether. Yield 84%; ^{1}H NMR δ 1.76 (quint, J = 7 Hz, 2 H), 1.97 (quint, J = 7 Hz, 2 H), 2.53 (t, J = 7 Hz, 2 H, 3.20 (s, 3 H), 4.72 (t, J = 7 Hz, 2 H), 7.78 (t, J = 8 Hz, 1H), 7.87(t, J = 8 Hz, 1H), 8.38 (d, J = 8 Hz, 1 H), 8.39 (d, J = 8 Hz, 1H). Anal. Calcd for $C_{12}H_{15}NO_3S_2 \cdot 1/2H_2O$: C, 48.96; H, 5.48; N, 4.76. Found: C, 49.32; H, 5.33; N, 4.71.

Chlorocyanines 9-Cl, 10-Cl, 12-Cl. Condensation of salts **5**, **6**, **11** with **7** was conducted in the presence of MeCOONa by using a general procedure (EtOH, reflux for 5h).[3] The products were crystallized from MeOH/ether.

2-]4'-Chloro-7'-(1''-ethyl-1'',2''-dihydrobenz[*cd*]indol-2''-ylidene)-3',5'-(propane-1''',3'''-diyl)-1',3',5',-heptatrien-1'-yl]-1-ethylbenz[*cd*]indolium iodide (9-Cl): Yield 85 %; ^{1}H NMR δ 1.33 (m, 6 H), 2.33 (m, 2 H), 2.80 (m, 4 H), 4.40 (m, 4 H), 7.21 (d, J = 13 Hz, 2 H), 7.62 (m, 4 H), 7.84(m, 6 H), 8.31 (m, 2 H), 8.62 (d, J = 13 Hz, 2 H); NIR λ_{max} = 1025 nm (ε = 1.9 x 10^5 $cm^{-1}M^{-1}$). Anal. Calcd for $C_{36}H_{32}ClIN_2 \cdot 1/2H_2O$: C, 65.10; H, 4.97; N, 4.22. Found: C, 65.00; H, 4.90; N, 4.25.

1-Allyl-2-[7'-(1''-allyl-1'',2''-dihydrobenz[*cd*]indol-2''-ylidene)-4'-chloro-3',5'-(propane-1''',3'''-diyl)-1',3',5',-heptatrien-1'-yl]benz[*cd*]indolium iodide (10-Cl): Yield 80 %; ^{1}H NMR δ 1.95 (m, 2 H), 2.54 (m, 4 H), 5.10 (m, 4 H), 5.21 (m, 4 H), 6.05 (m, 2 H), 7.14 (d, J = 13 Hz, 2 H), 7.65 (m, 6 H), 7.82(m, 2 H), 8.08 (m, 2 H), 8.41 (m, 2 H), 8.62 (d, J = 13 Hz, 2 H); NIR λ_{max} = 1026 nm (ε = 1.2 x 10^5 $cm^{-1}M^{-1}$). Anal. Calcd for $C_{38}H_{32}ClIN_2 \cdot H_2O$: C, 65.41; H, 4.66; N, 4.02. Found: C, 65.47; H, 4.88; N, 4.02.

Sodium 4-]2'-[4''-chloro-7''-[1'''-(4''''-sulfonatobutyl)benzothiazol-3'''-ium-2'''-yl]-3'',5''-(propane-1''''',3'''''-diyl)-2'',4'',6''-heptatrien-1''-ylidene]-1',2'-dihydro-3'*H*-benzothiazol-1'-yl]butanesulfonate (12-Cl): Yield 84 %; ^{1}H NMR δ 1.79 (m, 10 H), 2.53 (m), 2.66 (m, 4 H), 4.39 (m, 4 H), 6.49 (d, J = 14 Hz, 2 H), 7.30 (t, J = 8 Hz, 2 H), 7.48 (t, J = 8 Hz, 2 H), 7.72 (m, 4 H), 7.91 (d, J = 8 Hz, 2 H); NIR λ_{max} = 804 nm (ε = 2.6 x 10^5 $cm^{-1}M^{-1}$). Anal. Calcd for $C_{32}H_{34}ClN_2NaO_7S_4 \cdot H_2O$: C, 51.43; H, 4.86; N, 3.75. Found: C, 51.68; H, 4.87; N, 4.10.

Dyes 1=O - 3=O, 8=O - 10=O, 12=O, 13=O, 13-OH. A solution of sodium succinimide *N*-oxide in anhydrous DMF (10 mL), obtained from *N*-hydroxysuccinimide (334 mg, 2.9 mmol) and sodium hydride (70 mg, 2.9 mmol), was treated with a solution of chlorocyanine (1 mmol) in anhydrous DMF (6 mL), and the mixture was stirred at 23 °C under a nitrogen

atmosphere for 3 h (dyes **1-Cl - 3-Cl, 8-Cl - 10-Cl**), 2 days (dye **12-Cl**) or 4 h (dye **13-Cl**). Alternatively, similar mixtures prepared from N-hydroxysuccinimide, chlorocyanines, and triethylamine (0.5 mL) were stirred at 23 °C under a nitrogen atmosphere for periods of time that were up to 3-fold longer. The reaction progress was monitored by visible/near-infrared spectroscopy. Dilution of the mixture with ether (30 mL) caused precipitation of ketone **1=O - 3=O, 8=O - 10=O, 12=O** or **13=O** with yields virtually identical for either procedure (NaH or Et_3N). All ketones were crystallized by a dropwise dilution of a solution in MeOH with *t*-BuOMe. In the preparation of hydroxycyanine **13-OH** the reaction mixture was stirred and slowly acidified by dropwise addition of aqueous 6 M $HClO_4$, which caused crystallization of the product in an analytically pure form. Crystallization of **13=O** from 1% ethanolic solution of $HClO_4$ also gave **13-OH**. The crystallization yield was virtually quantitative.

Compound, yield: **1=O**, 85%; **2=O**, 88%; **3=O**, 86%; **8=O**, 96%. The analytical data has been published.[3,5]

2,6-Bis[(1'-ethyl-1',2'-dihydrobenz[*cd*]indol-2'-ylidene)ethylidene]cyclohexanone (9=O): Yield 85%; ^{1}H NMR δ 1.36 (t, J = 7 Hz, 6H), 2.0 (m, 2H), 2.81 (m, 4H), 3.94 (q, J = 7 Hz, 4H), 6.03 (d, J = 13 Hz, 2H), 6.60 (d, J = 7 Hz, 2H), 7.25 (d, J = 8 Hz, 2H), 7.36 (t, J = 8 Hz, 2H), 7.62 (t, J = 8 Hz, 2H), 7.73 (d, J = 8 Hz, 2H), 8.28 (d, J = 8 Hz, 1H), 8.67 (d, J = 13 Hz, 2H). Anal. Calcd for $C_{36}H_{32}N_2O$•1/2 H_2O: C, 83.53; H, 6.37; N, 5.41. Found: C, 83.85; H, 6.36; N, 5.52.

2,6-Bis[(1'-allyl-1',2'-dihydrobenz[*cd*]indol-2'-ylidene)ethylidene]cyclohexanone (10=O): Yield 80%; ^{1}H NMR δ 1.96 (m, 2H), 2.76 (m, 4H), 4.50 (m, 4H), 5.24 (m, 4H), 5.96 (m, 2H), 6.00 (d, J = 13 Hz, 2H), 6.60 (d, J = 7 Hz, 2H), 7.25 (d, J = 8 Hz, 2H), 7.34 (t, J = 8 Hz, 2H), 7.62 (t, J = 8 Hz, 2H), 7.73 (d, J = 8 Hz, 2H), 8.26 (d, J = 8 Hz, 2H), 8.61 (d, J = 13 Hz, 2H). Anal. Calcd for $C_{38}H_{32}N_2O$•3/4 H_2O: C, 83.56; H, 6.13; N, 5.12. Found: C, 83.52; H, 6.04; N, 5.15.

2,6-Bis[3"-(sodium 4"-sulfonatobutyl)-2",3"-dihydrobenzothiazol-2"-ylidene]ethylidene]cyclohexanone (12=O): Yield 96%; ^{1}H NMR δ 1.72 (m, 12H), 2.56 (m, 2H), 3.93 (m, 4H), 5.57 (d, J = 13 Hz, 2H), 7.00 (t, J = 7 Hz, 2H), 7.17 (d, J = 7 Hz, 2H), 7.24 (t, J = 7 Hz, 2H), 7.34 (d, J = 13 Hz, 2H), 7.53 (d, J = 7 Hz, 2H). Anal. Calcd for $C_{32}H_{34}N_2Na_2O_7S_4$•$H_2O$: C, 51.18; H, 4.83; N, 3.73. Found: C, 51.39; H, 4.75; N, 3.68.

2,6-Bis[[(2,6-diphenyl-4*H*-thiopyran-4-ylidene)ethylidene]cyclohexanone (13=O): Yield 95%; ^{1}H NMR ($CDCl_3$) δ 1.85 (m, 2H), 2.68 (m, 4H), 6.09 (d, J = 14 Hz, 2H), 6.87 (s, 4H), 7.43 (m, 12H), 7.61 (m, 8H), 7.99 (t, J = 14 Hz, 2H). HR-FAB-MS (thioglycerol): Calcd for $C_{44}H_{35}OS_2$ (M^++1) *m/z* 643.2129, obsd *m/z* 643.2138.

4-[4'-Hydroxy-7'-(2",6"-diphenyl-4"*H*-thiopyran-4"-ylidene)-3',5'-(propane-1"',3"'-diyl)-1',3',5'-heptatrien-1'-yl]-2,6-diphenylthiopyrylium perchlorate (13-OH): Yield 95%; ^{1}H NMR δ 1.80 (m, 2H), 2.67 (m, 4H), 6.42 (br, 2H), 7.35-7.80 (m, 24H), 7.89 (br, 2H), broadening is due to strong aggregation. Anal. Calcd for $C_{44}H_{35}ClO_5S_2$: C, 71.10; H, 4.75. Found: C, 71.07; H, 4.85.

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References and Notes

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4. Note: the series of dyes with the same molecular framework and differing only in a substituent at the central position of the chromophore are denoted by the chemical symbol of the substituent. For example, compound **1-Cl** is a chloro-substituted cyanine, **1=O** is a ketone, and **1-OH** is a hydroxycyanine derived from this ketone by protonation

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