A NEW pH-SENSITIVE NEAR-INFRARED CHROMOPHORE

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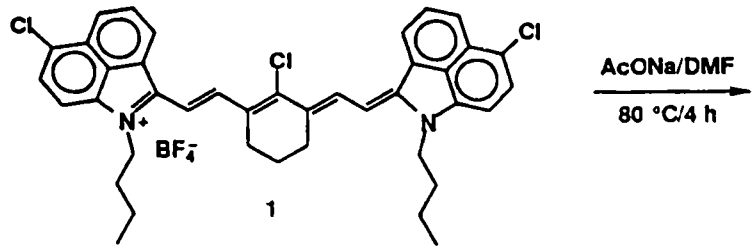
Abstract: The stable bis(aminodien)one system **2** (λ_{max} = 648 nm in MeOH) undergoes protonation at the oxygen atom to give a cyanine chromophore **2'** (λ_{max} = 932 nm). The transition is fully reversible and depends solely on pH conditions.

The red glow produced by a flashlight inside the mouth demonstrates that red light is penetrating in tissue. At near-infrared (NIR) wavelengths (700-1300 nm), multi-centimeter penetration of biological tissues is common. This unusual feature is a cornerstone of current, aggressive worldwide efforts to develop NIR imaging technology for *in vivo* applications (1-3). Central to such technology are NIR molecular probes that are chemically responsive to their tissue environment. For example, pH-sensitive NIR probes that change their spectral properties in a highly acidic environment of solid tumors are essential for the development of a novel imaging technology for detection of solid tumors. The NIR approach offers a potentially unparalleled sensitivity in comparison to other approaches because of the negligible background absorbance and fluorescence of biological tissues in the NIR region. The major barrier to the development of such NIR methodology has been the general lack of availability of suitable NIR compounds.

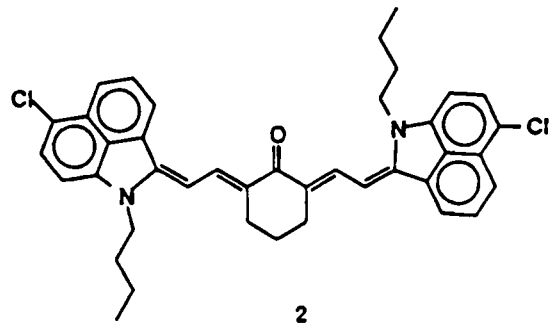
In the quest for pH-sensitive probes we have focused on bis(aminodien)ones, the visible absorption of which under neutral or basic pH conditions undergoes a large batochromic shift to the NIR region under low pH conditions (4,5). This large spectral shift is due to formation of a cyanine chromophore upon protonation. Unfortunately, all dyes of this class synthesized previously by us and others are highly unstable in solution (4-6).

In this paper we report for the first time a stable bis(aminodien)one system **2**. Compound **2** is easily prepared in one step from a commercially available cyanine dye **1** (7). Briefly, a mixture of **1** (200 mg, 0.27 mmol) and anhydrous AcONa (440 mg, 5.4 mmol) in anhydrous DMF (10 mL) was stirred under a nitrogen atmosphere at 80 °C for 4 h (4). After cooling, the mixture was treated with benzene/dichloromethane (1:1, 10 mL) and filtered, and the solution was then concentrated on a rotary evaporator. Flash chromatography on silica gel (2 g) eluting with dichloromethane and followed by crystallization from dichloromethane/methanol (1:1) gave **2** (45 mg, 26%) as a single diastereomer (8). Compound **2** gave good microanalysis and HR-FAB-MS results, and the suggested structure (Scheme) is fully consistent with ^{1}H NMR and ^{13}C NMR (9). Due to the *anti* stereochemistry of the two terminal heteroaromatic subunits in the planar conjugated molecule the pairs of methine protons C1'-H and C2'-H are slightly nonequivalent [δ 6.11 (broad d, J = 13 Hz,

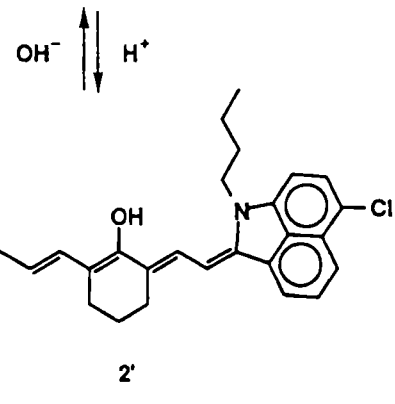


1

(stereochemistry unknown)



2



2'

2 H), 8.51 (broad d, J = 13 Hz, 2 H)] and the two methylene groups N-CH_2 exhibit distinctly different chemi shifts [δ 3.78 (t, J = 7.5 Hz, 2 H), 3.92 (t, J = 7.5 Hz, 2 H)]. In a similar way, the ^{13}C NMR spectrum of **2** show 40 well resolved signals for each carbon atom in the molecule. The central carbon of the carbonyl group resonates at δ 186.7. The electronic spectrum of **2** taken in methanol shows λ_{max} at 648 nm. Upon acidification of the methanol solution with diluted hydrochloric acid to about pH 3 this absorption maximum disappears and a new, much stronger absorption centered at 932 nm, apparently for a cyanine chromoph **2'**, is observed. This transition is fully reversible and the electronic spectrum depends solely on pH conditions. We noticed only slight changes in the electronic spectrum for a solution of **2** exposed to light and air for two days. In a similar way the ^1H NMR spectra of **2** taken immediately after dissolution in CD_2Cl_2 and after exposure to light and air for two days were virtually identical. This is an astonishing result becaus rapid photooxidation of this class of molecules is a quite general phenomenon rather than an exception (4

In summary we have found the first stable bis(aminodien)one/cyanine dye system. Quantitative absorption and fluorescent spectra of the **2/2'** system in various solvents and under various pH conditions will be published in due course. Water soluble analogs of **2** are strong candidates for use as pH-sensitive dyes in NIR imaging technology. Accordingly, we are synthesizing sulfonato-substituted derivatives of **2** to increase the solubility. Our previous research with cyanine dyes has shown that this modification has little effect on the absorption and fluorescence properties of the NIR chromophore (7,10,11).

References and Notes

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(6) For example, the bis(amionodien)one reported by us previously (refs. 4, 5) has been one of the more stable pH-sensitive dyes. When exposed to light and air it decomposed with a half-life of several hours.

(7) Dye **1** (commercial designation **IR-1048**) is available from Aldrich. It is highly rewarding to see in the recent Aldrich catalog the cyanine dyes that are synthesized by using chemistry developed in our laboratories: ref. 4 and L. Strekowski, M. Lipowska and G. Patonay, J. Org. Chem. *57*, 4578 (1992).

(8) Compound **2** is apparently formed by hydrolysis of an intermediate acetoxy derivative during workup. See the last reference in footnote (7) for the likely $S_{RN}1$ mechanism of the substitution reaction.

(9) 2,6-Bis[2'-[1"-butyl-6"-chlorobenz[*cd*]indol-2"(1"*H*)ylidene]ethylidene]cyclohexanone (**2**): mp > 116 °C (dec.); ^{1}H NMR (400 MHz, CD_2Cl_2): δ 0.97 (t, J = 7.5 Hz, 6 H), 1.44 (m, 4 H), 1.79 (quint, J = 7.5 Hz, 4 H), 1.95 (quint, J = 5.5 Hz, 2 H), 2.79 (t, J = 5.5 Hz, 4 H), 3.78 (t, J = 7.5 Hz, 2 H), 3.92 (t, J = 7.5 Hz, 2 H), 6.11 (br d, J = 13 Hz, 2 H), 6.55 (d, J = 8 Hz, 2 H), 7.39 (d, J = 8 Hz, 2 H), 7.75 (t, J = 8 Hz, 2 H), 7.93 (d, J = 8 Hz, 2 H), 8.22 (d, J = 8 Hz, 2 H), 8.51 (br d, J = 13 Hz, 2 H); ^{13}C NMR (75 MHz, CD_2Cl_2): δ 14.1, 14.2, 20.8, 20.9, 23.0, 26.8, 30.3, 30.5, 31.3, 40.6, 43.2, 101.6, 101.9, 102.2, 105.9, 120.7, 123.6, 123.8, 124.5, 125.0, 125.2, 126.7, 127.7, 128.1, 128.41, 128.44, 128.5, 128.8, 129.3, 129.7, 130.0, 130.2, 131.8, 132.5, 133.1, 139.5, 143.4, 146.9, 167.9, 186.7; HR-FAB-MS: Calcd for $C_{40}H_{39}(^{35}Cl)_2N_2O$ m/z 633.2439, obsd m/z 633.2456 (2.6 ppm error). Analysis for a sample dried at 25 °C/0.1 mmHg for 3 days. Calcd for $C_{40}H_{39}Cl_2N_2O$•1/2 H_2O: C, 74.76; H, 6.12,; N, 4.36. Found: C, 75.17; H, 6.30; N, 4.53 (the presence of water was also evident from the IR spectrum: ν 3450 cm^{-1}).

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