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The addition reaction of hydroxide or ethoxide ion with benzindolium heptamethine cyanine dyes

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Abstract

This paper pertains to a nucleophilic addition reaction at the C2 atom of a benz[c]indolium or 3,3-dimethyl-1H-benz[e]indolium subunit of the corresponding near-infrared heptamethine cyanine that contains a chlorine atom at the central meso position of the chromophore. An important finding is that the efficient $S_{RN}1$ replacement of the chloro substituent in such dyes is completely suppressed in the reactions (i) of hydroxide and ethoxide ions, both of which are poor single electron donors and (ii) conducted in aqueous alcohol, a medium that does not promote single electron transfer. The adducts produced were isolated and characterized by elemental analysis, ^{1}H NMR, and ^{13}C NMR. The NIR-absorbing parent dye is regenerated quantitatively upon treatment of the corresponding adduct with a weak acid, including silica gel.

Keywords: Cyanine; Heptamethine; IR-1048; Hydroxide; Ethoxide; Addition reaction; NMR

1. Introduction

Synthetic modification of indolium heptamethine cyanine dyes that contain a chloro substituent at the central position of the chromophore has recently become a cornerstone in the preparation of various functionalized derivatives for bioanalytical studies including near-infrared (NIR) labeling of biomolecules [1–4]. The NIR absorption of these chromophores can be fine-tuned to the outputs of the commercially available diode lasers by replacing the chloro group in a reaction with an appropriate nucleophile [1–3,5,6]. Depending on the electronic nature of the introduced substituent, a bathochromatic or hypsochromic shift in the absorption is obtained. An additional change in the absorption wavelength is observed with benzo-fused analogues such as **1** and **4** (Fig. 1). In contrast to the vast majority of other NIR heptamethine cyanines that undergo rapid photodegradation in the presence of molecular oxygen, compounds **1** and **4** are exceptionally stable towards photo-oxidation [3,6]. These dyes can also be stored in a neutral solution for an extended period of time without appreciable decomposition. On the other hand, little is known about their chemical properties under extreme pH conditions. This is an important issue because coupling of appropriately

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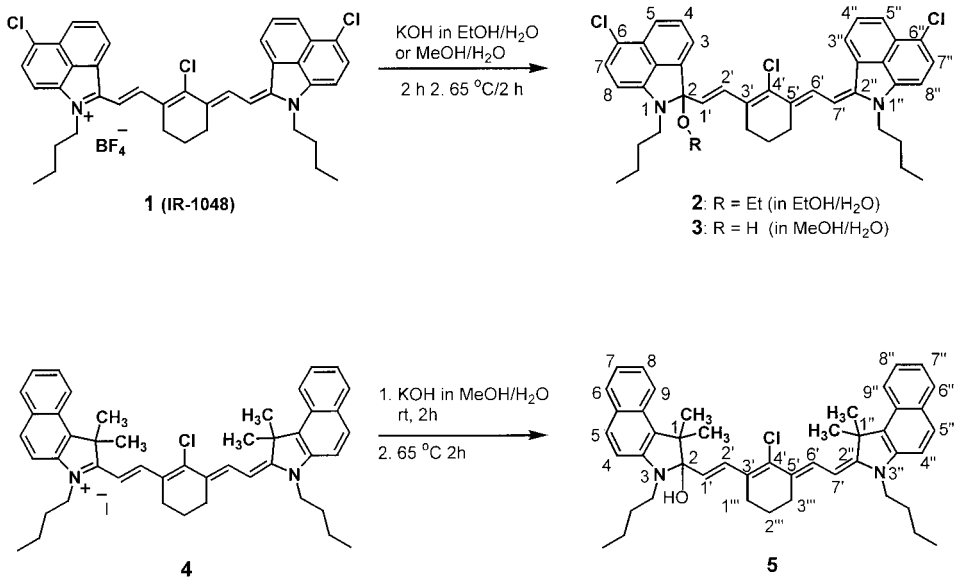

Fig. 1. Dyes **1** and **4** and their adducts **2**, **3**, **5** with ethoxide and hydroxcide ions. Note that the numbering scheme for the benzo-fused indole in **4**/**5** differs from that of the parent indole system as required by the IUPAC nomenclature [17].

functionalized dyes to biomolecules is conducted in a basic medium [4,7]. Accordingly, in this paper we have examined the reactivity of **1** and **4** in the presence of KOH in aqueous alcohol.

2. Results and discussion

The electronic spectra of **1** and **4** in MeOH show absorption maxima at 1048 and 820 nm, respectively, with a shoulder extending to the visible region. The absorption spectra are similar, in the first approximation, in an aqueous MeOH or aqueous EtOH solution. Heating a solution of **1** in aqueous EtOH in the presence of KOH resulted in a gradual disappearance of color, indicating that a chemical modification of the chromophore was taking place. Following a simple workup the ethoxy adduct **2** (Fig. 1) was isolated in high yield. Suprisingly, a similar reaction of **1** in aqueous MeOH produced the corresponding hydroxy derivative **3** as the sole product. An analogous reaction was observed for **4** in aqueous MeOH in the presence of KOH, which gave the hydroxy adduct **5**.

The solid samples of **2**, **3** and **5** are indefinitely stable when stored refrigerated in a dry container. They are also stable in an aprotic solution, such as in DMSO, but undergo a rapid decomposition to regenerate the NIR-absorbing parent dye quantitatively upon contact with dilute acid. Even silica gel is acidic enough to cause decomposition of these adducts to **1** and **4**.

The excellent microanalysis data for **2**, **3** and **5** are consistent with a purity level of at least 96%. This conclusion is consistent with the ^{1}H and ^{13}C NMR spectra, which showed the presence of the single adduct in each case. It should be noted that the presence of an additional component at the level of less than 4% is, normally, not detected by these spectral methods.

The presence of the ethoxy or hydroxy group in the adducts at the 2-position of the benzindole subunit was established by NMR, and the spectral analysis of **2** is discussed in detail for illustration. In this regard, the ^{1}H and ^{13}C NMR spectra of **2** showed unambiguously that the molecule lacks symmetry, thereby ruling out the presence of the ethoxy group at the central 4′-position. The lack of symmetry would be consistent with the location

of the ethoxy group at a dihydronaphthalene moiety of the heterocyclic subunit, at C2 of the heterocyclic subunit, or at C2′ of the polymethine chain of the molecule. However, ^{1}H and ^{13}C NMR spectra indicate the presence of two aromatic naphthalene moieties and four olefinic protons. This suggests that structure **2** is the only possibility. Consistent with this structural assignment are the relatively broad signals for O–CH_2 of the ethoxy group and 1′-H of the heptamethine chain in the ^{1}H NMR spectrum of 2. First, the protons of OCH_2 are expected to be anisochronous due to their proximity to the asymmetric C2 atom. For molecules with the methylene protons located β to the asymmetric atom, anisochrony is often small and results in broadening of the signals [8] rather than in clear resolution, as observed for **2**. Second, an additional broad resonance for 1′-H of **2** is attributed to weak coupling ($J \leqslant 0.5$ Hz) between O–CH_2 and 1′-H. Such long-range coupling depends on the relative stereochemistry of the protons and is frequently observed in ^{1}H NMR [9]. Indeed, the interaction suggested above was clearly demonstrated by using correlation spectroscopy (COSY). Using COSY, it was also possible to unambiguously assign most of the chemical shifts to the protons of **2**. The structural assignments for **3** and **5** were achieved in a similar fashion.

An interesting feature of the addition reaction is the formation of an ethoxy or hydroxy adduct in aqueous EtOH or MeOH, respectively. The formation of a hydroxy adduct with a benzothiazolium heptamethine cyanine has also been suggested previously for the reaction conducted in reagent-grade (non-anhydrous) MeOH [10]. This and our results can be explained in terms of reversibility of the addition reactions with ethoxide/hydroxide and methoxide/hydroxide ions. Such reversible additions at the C2 atom of the indolium heptamethine cyanines in a methanol-d_4 solution in the presence of a base have been postulated by us previously to account for the regioselective hydrogen–deuterium exchange at the positions 1′ and 7′ of the heptamethine chain in related indolium cyanines [11].

Thus, in order to understand the different outcomes of the two reactions not only the addition of alkoxide and hydroxide ions but also the dissociation of the adducts must be considered. The latter process must involve solvation of the leaving group before its dissociation from the adduct. Due to the steric congestion around the C2 atom and the relatively high hydrophobicity of the ethoxy substituent in **2**, the ethoxy group is solvated inefficiently. As a result, under the equilibrium conditions for the addition and dissociation of the ethoxide and hydroxide ions, the ethoxy adduct is present at high concentration. On the other hand, a much less hydrophobic methoxy group is solvated to a greater extent than the ethoxy substituent and, as such, is also a better leaving group. The high concentration of hydroxide ion in the mixture may also play a role in the formation of the hydroxy adduct.

Finally, we wish to address a dual reactivity of indolium heptamethine dyes that contain a nucleofugal group at the central position, such as the chlorine atom in **1** and **4**. We have shown previously that the nucleofugal group is easily and efficiently displaced by a number of nucleophiles that are good electron donors [1,2]. This reaction involves an $S_{RN}1$ pathway and is efficient in solvents, such as DMF, that promote the single electron transfer (SET) process [12]. It is important to note that substitution of the chlorine atom in **1** and **4** was not observed under the conditions of the current work. First, alcohols are poor solvents for SET reactions and water is even worse and, secondly, alkoxide and hydroxide ions are relatively poor single electron donors [12]. As a result, the anionic addition reaction of these nucleophiles with a dye chromophore is observed as the only process in the aqueous alcohol medium. This analysis of the reactivity of dyes **1** and **4** has important ramifications for future design, synthesis, and application of functionalized indolium heptamethine cyanines. Dyes **1**, **4**, and similar chloro-substituted chromophores are readily available, and the chlorine atom can be displaced by the reaction with nucleophiles under $S_{RN}1$ conditions. The derivatized dyes are stable at neutral pH in aqueous solvents and undergo a nucleophile addition reaction with the chromophore under basic aqueous conditions. However, the resultant adducts are quantitatively decomposed to the starting NIR dyes by weak acid.

Several adducts of nucleophiles with dyes containing a short polymethine moiety have been suggested or isolated previously [13,14]. Related reactions involved reduction of trimethine cyanines at the central position by treatment with Na BH_4 (a hydride ion transfer reagent) and the borohydride-mediated reduction of the iminium function in pentamethine and heptamethine cyanines [15]. However, this reduction is irreversible. The addition reaction of hydroxide ion with a heterocyclic portion of dyes followed by a ring opening reaction of the heterocyclic subunit have also been suggested [10, 16]. In this work we have shown for the first time that the nucleophile addition reaction with indolium heptamethine cyanines occurs at the C2 atom of the indolium moiety.

3. Experimental

Dye IR-1048 (**1**) was purchased from Aldrich and found to be pure by ^{1}H NMR. The ^{1}H NMR, including COSY, and ^{13}C NMR data were obtained on Varian instruments in DMSO-d_6 solutions with TMS as an internal standard at 30°C. VIS-NIR spectra were recorded on a Perkin Elmer LAMBDA 20 spectrophotometer in MeOH.

*3.1. 2-[7′-(3″-Butyl)-1,1-dimethyl-2″,3″-dihydro-1″*H*-benz[*e*]indol-2″-ylidene)-4′-chloro-3′,5′-(propane-1‴,3‴-diyl)-1′,3′,5′-heptatrien-1′-yl]-3-butyl-1,1-dimethyl-1*H*-benz[*e*]indolium iodide* (**4**)

This compound was prepared using a general procedure [1] and crystallized by slow addition of water to the solution in EtOH: yield 93%; ^{1}H NMR (300 MHz) δ0.96 (t, J=7 Hz, 6H, $CH_2CH_2CH_2\mathbf{CH_3}$), 1.46 (m, 4H, $CH_2CH_2\mathbf{CH_2}CH_3$), 1.79 (m, 4H, $CH_2\mathbf{CH_2}CH_2CH_3$), 1.91 (m, 2H, 2‴-$CH_2$), 1.96 (s, 12H, $C(CH_3)_2$), 2.76 (m, 4H, 1‴-CH_2 and 3‴-CH_2), 4.36 (t, J=7 Hz, 4H, $\mathbf{CH_2}CH_2CH_2CH_3$), 6.38 (d, J=14 Hz, 2H, 1′-H and 7′-H), 7.54 (t, J=8 Hz, 2H, Ar-H), 7.67 (t, J=8 Hz, 2H, Ar-H), 7.79 (d, J=8 Hz, 2H, Ar-H), 8.08 (d, J=8 Hz, 2 H, Ar-H), 8.11 (d, J=8 Hz, 2H, Ar-H), 8.31 (d, J=8 Hz, 2H, Ar-H), 8.38 (d, J=14 Hz, 2H, 2′-H and 6′-H); ^{13}C NMR (100 MHz) δ13.7, 19.5, 25.9, 27.0, 29.4, 43.9, 50.7, 101.2, 111.7, 122.2, 125.0, 126.2, 127.4, 127.8, 129.9, 130.4, 131.5, 133.6, 139.7, 140.6, 142.0, 173.4; VIS-NIR λ_{max}=820 nm.

Analysis. Calculated for $C_{46}H_{52}ClIN_2$: C, 69.48; H, 6.54; N, 3.52. Found: C, 69.59; H, 6.88; N, 3.60.

*3.2. A general procedure for the preparation of adducts **2**, **3** and **5***

A mixture prepared from a dye **1** or **4** (150 mg), EtOH or MeOH (4 ml), and a 10% solution of KOH in water (1 ml), was stirred and heated to 65°C for 2 h under a nitrogen atmosphere. The slurry was then cooled and poured onto ice (50 g). The resultant precipitate was filtered, washed with water and dried at 0.5 mmHg/40°C.

*3.3. 2-[7′-(1″-Butyl-6′-chloro-1″,2″-dihydrobenz[*cd*]indol-2″-ylidene)-4′-chloro-3′,5′-(propane-1‴,3‴-diyl)-1′,3′,5′-heptatrien-1′-yl]-1-butyl-6-chloro-2-ethoxy-1,2-dihydrobenz[*cd*]indole*

(**2**, from **1** in the presence of EtOH): yield 79% ; ^{1}H NMR (300 MHz) δ 0.90 (t, J=7 Hz, 3H, N–$CH_2CH_2CH_2\mathbf{CH_3}$), 0.93 (t, J=7 Hz, 3H, N–$CH_2CH_2CH_2\mathbf{CH_3}$), 1.07 (t, J=7 Hz, 3H, O–$CH_2\mathbf{CH_3}$), 1.36 (m, 4H, N–$CH_2CH_2\mathbf{CH_2}CH_3$), 1.67 (m, 4H, N–$CH_2\mathbf{CH_2}CH_2CH_3$), 1.74 (t, J=6 Hz, 2H, 2‴-CH_2), 2.45 (t, J=6 Hz, 2H, 3‴-CH_2), 2.68 (t, J=6 Hz, 2H, 1‴-CH_2), 2.75 (m, 2H, O–$\mathbf{CH_2}CH_3$), 3.37 (t, J=7 Hz, 2H, N–$\mathbf{CH_2}CH_2CH_2CH_3$), 3.90 (t, J=7 Hz, 2H, N–$\mathbf{CH_2}CH_2CH_2CH_3$), 6.08 (d, J=16 Hz, 1H, 7′-H), 6.24 (d, J=12 Hz, 1H, 2′-H), 6.34 (d, J=8 Hz, 1H, Ar-H), 6.66 (d, J=8 Hz, 1H, Ar-H), 7.14 (d, J=16 Hz, 1H, 6′-H), 7.39 (t, J=4 Hz, 2H, Ar-H), 7.42 (d, J=8 Hz, 1H, Ar-H), 7.55 (br d, J=12 Hz, 1H, 1′-H), 7.71 (d, J=8 Hz, 1H, Ar-H), 7.73 (d, J=8 Hz, 2H, Ar-H), 7.81 (d, J=8 Hz, 2H, Ar-H); ^{13}C NMR (100 MHz) δ 13.7, 13.8, 14.9, 19.5, 19.93, 20.9, 26.1, 26.9, 29.4, 30.5, 57.9, 62.4, 96.8, 99.9, 100.6, 100.9, 102.9, 114.1, 117.2, 119.8, 121.2, 121.4, 121.7, 123.3, 127.4, 127.4, 127.9, 128.2, 128.7, 129.3, 129.4, 129.9, 130.2, 131.2, 131.4, 132.3, 137.3, 139.1, 141.5, 142.6, 147.4; VIS-NIR λ_{max}=526 nm.

Analysis. Calculated for $C_{42}H_{43}Cl_3N_2O{\cdot}2H_2O$: C, 68.71; H, 6.45; N, 3.82. Found: C, 68.86; H, 6.06; N, 3.79.

3.4. 2-[7′-(1″-Butyl-6″-chloro-1″,2″-dihydrobenz[cd]indol-2″-ylidene)-4′-chloro-3′,5′-(propane-1‴,3‴-diyl)-1′,3′,5′-heptatrien-1′-yl]-1-butyl-6-chloro-2-hydroxy-1,2-dihydrobenz[cd]indole

(**3**, from **1** in the presence of MeOH): yield 90%; ^{1}H NMR (300 MHz) δ 0.90 (t, J=7 Hz, 3H, N-$CH_2CH_2CH_2\mathbf{CH_3}$), 0.93 (t, J=7 Hz, 3H, N–$CH_2CH_2CH_2\mathbf{CH_3}$), 1.37 (m, 4H, N–$CH_2CH_2\mathbf{CH_2}CH_3$), 1.63 (m, 4H, N–$CH_2\mathbf{CH_2}CH_2CH_3$), 1.73 (t, J=6 Hz, 2H, 2‴-CH_2), 2.45 (t, J=6 Hz, 2H, 3‴-CH_2), 2.69 (t, J=6 Hz, 2H, 1‴-CH_2), 3.39 (t, J=7 Hz, 2H, N–$\mathbf{CH_2}CH_2CH_2CH_3$), 3.92 (t, J=7 Hz, 2H, N–$\mathbf{CH_2}CH_2CH_2CH_3$), 6.05 (br d, J=16 Hz, 1H, 7′-H), 6.26 (d, J=13 Hz, 1H, 2′-H), 6.37 (d, J=8 Hz, 1H, Ar-H), 6.68 (d, J=8 Hz, 1H, Ar-H), 7.21 (d, J=16 Hz, 1H, 6′-H), 7.39 (m, 3H, Ar-H), 7.57 (br d, J=13 Hz, 1H, 1′-H), 7.72 (m, 3H, Ar-H), 7.82 (d, J=8 Hz, 2H, Ar-H); ^{13}C NMR (100 MHz) δ 14.1, 14.2, 19.9, 20.4, 26.5, 27.4, 29.9, 30.8, 41.7, 42.0, 50.4, 95.4, 97.4, 100.6, 101.0, 103.3, 114.7, 117.6, 120.3, 121.6, 121.8, 122.2, 123.7, 127.7, 128.0, 128.4, 128.6, 128.7, 129.0, 129.7, 129.8, 130.4, 130.6, 131.7, 132.7, 138.9, 141.9, 143.1, 147.9; VIS-NIR λ_{max}=512 nm.

Analysis. Calculated for $C_{40}H_{39}Cl_3N_2O{\cdot}1.5H_2O$: C, 68.91; H, 6.07; N, 4.02. Found: C, 68.54; H, 5.81; N, 3.95.

3.5. 2-[7′-(3″-Butyl-1, 1-dimethyl-2″,3″-dihydro-1″H-benz[e]indol-2″-ylidene)-4′-chloro-3′,5′-(propane-1‴,3‴-diyl)-1′,3′,5′-heptatrien-1′-yl]-3-butyl-2-hydroxy-1,1-dimethyl-2,3-dihydro-1H-benz[e]indole

(**5**, from **4** in the presence of MeOH): yield 93%; ^{1}H NMR (300 MHz) δ 0.96 (t, J=7 Hz, 3H, N–$CH_2CH_2CH_2\mathbf{CH_3}$), 1.44 (m, 4H, N–$CH_2CH_2\mathbf{CH_2}CH_3$), 1.63 (m, 4H, N–$CH_2\mathbf{CH_2}CH_2CH_3$), 1.79 (m, 2H, 2‴-$CH_2$), 1.88 (s, 3H, $C(CH_3)_2$), 1.96 (s, 9H, $C(CH_3)_2$), 2.64 (m, 2H, 3‴-CH_2), 2.76 (m, 2H, 1‴-CH_2), 3.85 (m, 2H, N–$\mathbf{CH_2}CH_2CH_2CH_3$), 4.30 (m, 2H, N–$\mathbf{CH_2}CH_2CH_2CH_3$), 6.10 (br d, J=14 Hz, 1H, 7′-H), 6.38 (d, J=14 Hz, 1H, 2′-H), 7.54 (d, J=14 Hz, 1H, 6′-H), 7.67 (m, 2H, Ar-H), 7.78 (m, 2H, Ar-H), 7.87 (m, 2H, Ar-H), 8.09 (t, J=8 Hz, 4H, Ar-H), 8.29 (m, 2H, Ar-H), 8.38 (br d, J=14 Hz, 1H, 1′-H); ^{13}C NMR (100 MHz) δ 13.6, 13.7, 19.5, 19.6, 27.3, 28.2, 29.2, 41.4, 43.4, 47.7, 50.3, 91.8, 99.1, 109.9, 111.5, 112.3, 120.7, 120.8, 121.4, 121.7, 122.1, 122.3, 123.7, 123.8, 124.6, 125.7, 126.8, 127.3, 127.5, 127.6, 128.3, 128.5, 129.1, 129.5, 129.6, 129.8, 129.9, 130.2, 11.2, 131.5, 132.9, 133.9, 139.8, 141.5, 163.3, 166.9; VIS-NIR λ_{max}=552 nm.

Analysis. Calculated for $C_{46}H_{53}ClN_2O{\cdot}3H_2O$: C, 74.75; H, 7.18; N, 3.79. Found: C, 74.88; H, 7.43; N, 3.84.

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