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Diphenylpolyene-cholesterol conjugates as fluorescent probes for microheterogeneous media

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ABSTRACT

Extrinsically conjugated fluorescent diphenylpolyene cholesterol derivatives are synthesized and spectroscopic investigations in homogeneous and aqueous micellar solutions are described. The emission of these cholesterol conjugates reveals characteristic intra-molecular charge transfer (ICT) behaviour in homogeneous solvents with a mono-exponential decay. Spectroscopic evidence in micellar aqueous solutions reveals a bi-exponential decay. This indicates the presence of two preferred locations of the cholesterol conjugated diphenylpolyenes sites of lower polarity and interfacial sites. The sensitivity of these fluorophores was utilized to determine the critical micelle concentrations.

1. Introduction

π-Conjugated materials based on dyes that fluoresce at various wavelengths and exhibit charge transfer are of great interest towards various optoelectronic [1–4] as well as diagnostic/analytical applications [5–9]. The unique modulation of the ground and excited state dynamics of diphenylpolyenes using variously substituted donor and acceptor groups have rendered them with potential applications in organic optoelectronic devices [3,10] and as photoresponsive materials [10,11]. Molecules having donor and acceptor groups on the aromatic ring can lead to strong intramolecular charge transfer (ICT) character responsible for environment sensitive emission behaviour [12]. For such systems, fluorescence quantum yield decreases with increase in solvent polarity accompanied by large bathochromic emission spectral shifts [13]. This unique behaviour can be utilized for fluorometric characterization of biological membranes [14]. Diphenylpolyenes substituted with suitable donor or acceptor groups have been examined as membrane probes in homogeneous and microheterogeneous environments [7,15,16]. A well-known example of an extensively studied non-covalent probe was diphenylhexatriene (DPH) which preferentially resides in the nonpolar regions of the cell membrane [17].

Biological membranes are composed of complex assemblies of lipids and proteins that allow many important cellular functions. Amphipathic cholesterol is an essential component of these biological membranes and has been used as a building block for creating photo responsive materials [18–20] and in bionanotechnology [21]. The rigid planar subunit of the rings and a flexible iso-octyl side chain 'tail' allows cholesterol to modulate various functions in the biological membrane organization. Many fluorescent cholesterol conjugates [22–27] containing fluorophores such as NBD [28,29], BODIPY [30,31], fluorescein [23] have been used to examine various functionalities of cholesterol in membranes. Functions such as cholesterol organization, trafficking, lipid interactions and modulation of activity of membrane proteins can be monitored by utilizing changes in fluorescence response with respect to their polar or non-polar environments.

In this paper, we report the synthesis and photophysical properties of two novel fluorescent diphenylpolyene-cholesterol analogues linked at the 3β-OH position. Due to the lack of 3-OH group these cholesterol probes lose the amphipathic property [14,32], but may show preference for partitioning into ordered micro domains and therefore can be a valuable tool for exploration of such dynamics. This structural design yields a fluorophore that can be monitored using non-invasive fluorescence methods. Presence of cholesterol also enables the fluorophores to be embedded in the biological hydrophobic environment rendering biosensing applications [33,34]. The fluorophores (**7**, **8**) and their cholesterol derivatives (**10**, **11**) (Scheme 1) that we utilized exhibit strong solvent dependent emission characteristics attributed to ICT behaviour [12]. We intended to tap into their emission properties and understand the feasibility of using these covalently linked cholesterol fluorophores for probing microenvironments.

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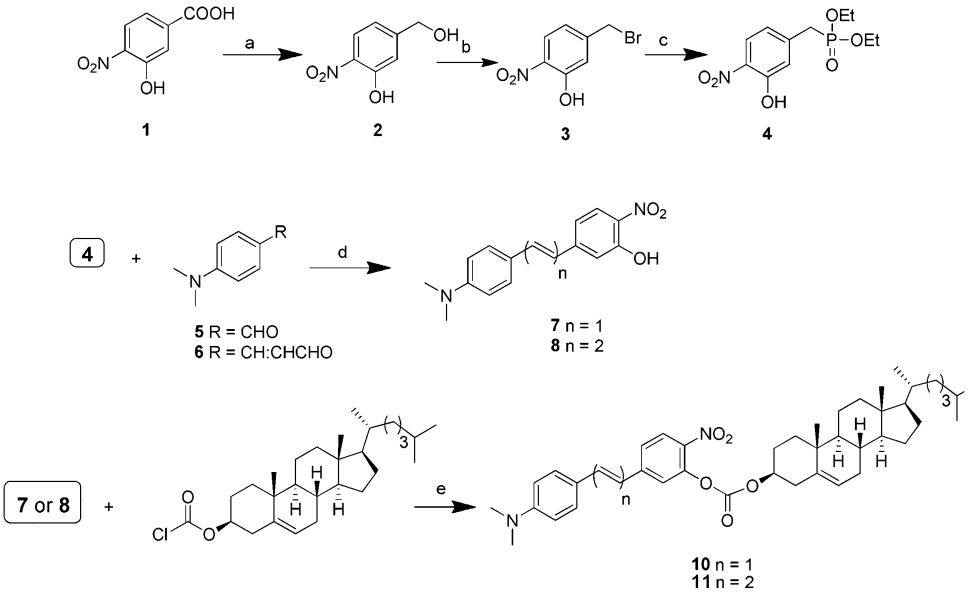

Scheme 1. Synthesis of cholesterol conjugated stilbene (**10**) and diphenylbutadiene (**11**). Reagents & conditions used: (a) $B(OMe)_3$, $BF_3{\cdot}Et_2O$, BH(Py), EDC, RT to 0 °C, 3 h; (b) CBr_4, DCM, PPh_3, 0 °C to RT, 3 h; (c) $P(OEt)_3$, DMF, 140 °C, 2 h; (d) NaH, THF, 0 °C to RT, 12 h and (e) pyridine, benzene, 80 °C, 24 h.

2. Experimental

2.1. Materials and methods

The chemicals, surfactants (CTAB, Triton X-100 and SDS) and other reagents used for this study were obtained from Aldrich, Alfa Aesar, Acros or S.D. fine chemicals Ltd. Solvents were dried using reported procedures before their use in synthesis and optical spectroscopic studies. Double distilled Millipore water was used to prepare solutions of the desired concentration. A 20 μL tetrahydrofuran (THF) solution of the fluorophore (1×10^{-4} M) was added to the surfactant solution maintaining a uniform dye-concentration. ^1H and ^{13}C NMR characterization was done using Bruker Avance 500 (500 MHz) spectrometer and accurate mass analysis was performed using Waters-synapt G2S (ESI-QToF) mass spectrometer.

UV–vis absorption spectra were recorded using Analytik Jena Specord 210 plus and steady state fluorescence emission studies were performed using Horiba Jobin Yvon fluolorog-3 spectrofluorimeter using a slit-width of 1 nm. The fluorescence quantum yields were determined using a reference solution with a known quantum yield of fluorescence [35] with similar optical density. Picosecond pulsed diode laser-based time-correlated single photon counting (TCSPC) instrument from Horiba Jobin Yvon IBH (UK) set at a magic angle at 54.7° was used to determine the fluorescence lifetimes. The excitation sources used were 406 nm and 440 nm with the corresponding *fwhm* of 249, 248 ps having resolutions of 7 and 14 ps/channel, respectively. 406 nm excitation was used for molecule (**10**) and 440 nm excitation was used for molecules (**7**), (**8**) and (**11**). The excitation wavelength corresponds to their observed absorption maxima. The number of channels per decay was 5000 for both resolutions. The decays were fitted by using IBH DAS v6.2 software in mono and biexponential models by deconvolution by iterative reconvolution. The error associated with the determination of lifetime studies is 0.1–1.5%.

2.2. Synthesis of fluorescent cholesterol conjugates

The synthesis of cholesterol analogues of diphenylpolyenes (**10**) and (**11**) was achieved in five steps (Scheme 1). Carboxylic group of 3-hydroxy, 4-nitrobenzoic acid (**1**) was reduced to the corresponding alcohol using trimethylborate and borane-pyridine complex in quantitative yields [36]. The hydroxyl group was replaced with bromide using tetrabromomethane (CBr_4) yielding a yellow crystalline solid (**3**) with 83% yield [37]. Phosphonate (**4**) was obtained upon treatment of (**3**) with triethylphosphite in dimethylformamide (DMF). The phosphonate ester (**4**) was then subjected to a reaction with suitable aldehyde, 4-dimethylaminobenzaldehyde yielding (**7**) and 4-dimethylaminocinnamaldehyde yielding (**8**) with sodium hydride (NaH) as a base and THF as a solvent [37]. The final step of conjugation with cholesterol was done utilizing cholesteryl chloroformate to yield the carbonate linked fluorescent sterol derivatives (**10**) and (**11**).

2.3. General procedure for the synthesis of diphenylpolyene derivatives (7) and (8)

To a solution of phosphonate (**4**) (1 mmol) in dry THF (5 mL) at 0 °C, NaH (2.5 mmol) was added under N_2 atmosphere. After stirring for five minutes, aldehyde (1 mmol) in dry THF (5 mL) was added drop wise. Stirring was continued for further 30 min while maintaining the temperature at 0 °C. The reaction was later allowed to stir at room temperature for 12 h and the mixture was poured into ice-cold water. Extraction with dichloromethane (DCM) and concentrating under reduced pressure yields a dark red residue, which on elution by column chromatography (silica gel, 15% ethyl acetate/petroleum ether) afforded the desired product as dark red solid.

2.3.1. (E)-5-(4-(dimethylamino)Styryl)-2-nitrophenol (7)

Yield: 30% (85 mg); dark red solid; ^1H NMR (500 MHz, $CDCl_3$) 3.03 (s, 6H), 6.71 (d, J = 8.5 Hz, 2H), 6.83 (d, J = 16.5 Hz, 1H), 7.09 (d, J = 9.0 Hz, 1H), 7.13 (s, 1H), 7.19 (d, J = 16.5 Hz, 1H), 7.44 (d, J = 8.5 Hz, 2H), 8.04 (d, J = 8.5 Hz, 1H), 10.79 (s, 1H). ^{13}C 125 MHz, $CDCl_3$) 40.3, 111.9, 115.9, 127.2, 128.6, 131.1, 141.1. HRMS [ESI] $[M-1]^-$ 283.1449.

2.3.2. 5-((1E,3E)-4-(4-(dimethylamino)phenyl)buta-1, 3-dienyl)-2-nitrophenol (8)

Yield: 27% (83 mg); dark red solid; ^1H NMR (500 MHz, $CDCl_3$) 3.03 (s, 6H), 6.51 (d, J = 15 Hz, 1H), 6.71 (d, J = 8.5 Hz, 2H), 6.83 (d, J = 16.5 Hz, 1H), 7.09 (d, J = 9.0 Hz, 1H), 7.13 (s, 1H), 7.19 (d, J = 16.5 Hz, 2H), 7.44 (d, J = 8.5 Hz, 2H), 8.04 (d, J = 8.5 Hz, 1H), 10.79 (s, 1H).