Dyes and Pigments 112 (2015) 290–297

Contents lists available at ScienceDirect

Dyes and Pigments

journal homepage: www.elsevier.com/locate/dyepig

New brightly coloured, water soluble, core-substituted naphthalene diimides for biophysical applications

Heather F. Higginbotham, Subashani Maniam, Steven J. Langford, Toby D.M. Bell*

School of Chemistry, Monash University, Wellington Road, Clayton, Victoria 3800, Australia

ARTICLE INFO

Article history:
Received 22 April 2014
Received in revised form
26 June 2014
Accepted 16 July 2014
Available online 30 July 2014

Keywords:
Naphthalene diimide
Red emission
Water soluble
Time-resolved fluorescence
Reverse micelle
Fluorescence correlation spectroscopy

ABSTRACT

Symmetrically and dissymmetrically core-substituted amino naphthalene diimides (cNDIs) with water-soluble substituents have been synthesised. The compounds display characteristic steady-state optical properties absorbing and emitting light in the visible part of the electromagnetic spectrum. Time-resolved fluorescence studies reveal long emission lifetimes of ~10 ns in chloroform, ~8 ns in methanol and ~4 ns in water. Suitability for use in biophysical applications is demonstrated by encapsulation into reverse micelles and liposomes. Fluorescence lifetime is shown to indicate cavity size in the reverse micelles and fluorescence correlation spectroscopy measurements of cNDI loaded liposomes successfully determined diffusion coefficients and hydrodynamic radii of the liposomes.

1. Introduction

Since the development of the first synthetic dyes in the mid-19th century, coloured molecules have found applications ranging from clothing to cellular imaging. The search for new dye compounds with improved and unusual properties is on-going and new applications for existing dyes are continually being found. Naphthalene diimides (NDIs) are a well-known class of functional molecules which typically absorb light in the UV region of the electromagnetic spectrum and are thus uncoloured. NDIs have, however, attracted the attention of researchers for many years who have sought to take advantage of the many useful properties of NDIs such as their electron deficient nature, rigid structure, air stability and solubility in a range of organic solvents. Significant recent interest in NDIs has been in the drive to create new and interesting molecules [1]. These aspects have led to NDIs being used in a variety of applications including in molecular pulleys and levers in rotaxanes and pseudo-rotaxanes [2], as molecular recognition indicators through pi–anion interactions [3], and in porous metal organic frameworks [4]. The high electron affinity of NDIs has also been taken advantage of in the design of donor–acceptor complexes [5,6] and organic semiconductors [7].

Substitution of NDIs on the core has seen the recent development of a whole new sub-class of NDIs, the core-substituted naphthalene diimides (cNDIs). Many of these molecules are intensely coloured with dramatically altered optical properties with respect to the parent NDI and are being pursued as novel and versatile fluorescent chromophores. One highly favourable aspect of cNDIs is that optical properties such as absorption and emission wavelengths can often be 'tuned' through the visible and near infrared regions of the electromagnetic spectrum by the nature and particularly, the number, of electron donating groups on the core [8–10]. One of the most common type of cNDIs, are the substituted amino naphthalene diimides (SANDIs), which have been investigated for use in fluorescence applications such as cationic and anionic sensors [11–15], as indicators for G-quaduplex in DNA [16–18], and in single molecule studies [19]. SANDIs have been identified as ideal small molecules for such applications due to their facile functionalisation, creating intramolecular electron poor and rich domains required for rapid regulation of fluorescence output. SANDIs are also bright, displaying high emission quantum yields and good photostability, even at the level of single molecules.

The small size of SANDIs makes them ideal candidates for water solubilisation creating further potential for use as biologically relevant materials. Emission in the red region of the electromagnetic spectrum [9] provides a further advantage by contrasting auto-fluorescence and diminishing light reabsorption from other biological materials. Additionally, SANDIs have already been shown

* Corresponding author. Tel.: +61 3 9905 4566; fax: +61 3 9905 4597.
E-mail address: toby.bell@monash.edu (T.D.M. Bell).

http://dx.doi.org/10.1016/j.dyepig.2014.07.018
0143-7208/

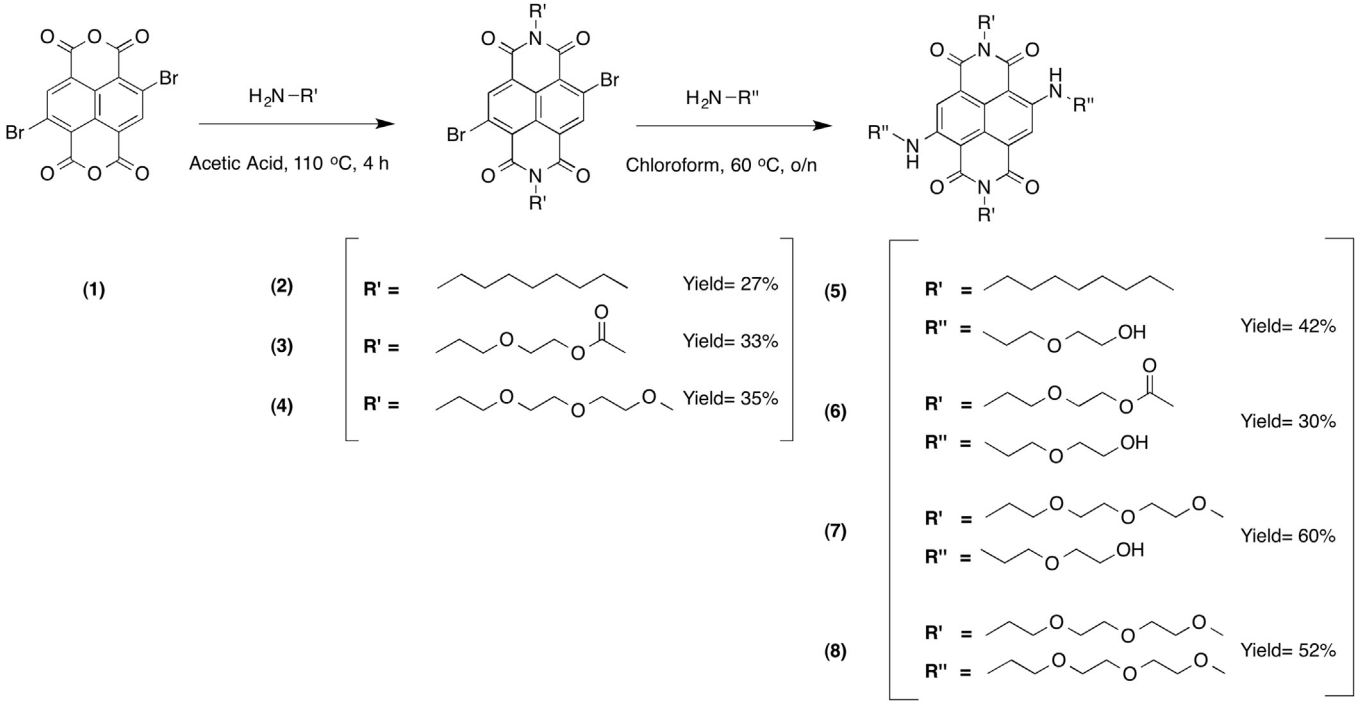
[10] to have synthetic flexibility in both the core and the imide positions making the synthesis of a diverse range of hydrophilic structures possible. Non-core substituted naphthalene diimides have already been shown to be water soluble through the substitution of hydrophilic groups at the imide positions [20–22]. These have included the use of imide substituted amino acids as solubilising units for self assembly [23], and in combinatorial libraries [5]. SANDIs have also been rendered water soluble through quaternary ammonium salts and used for the detection of singlet oxygen [24], while very recently, a series of phenolate functionalised, conjugated cNDIs has been described and characterised [25].

In pursuit of the goal of water soluble, red emitting dyes, a series of SANDI molecules has been synthesised (Scheme 1) and characterised. The SANDIs were synthesised from the common di-bromo starting material **(1)**, with imidation at the anhydride positions occurring *via* a substitution reaction under acidic conditions to allow for selective substitution in the imides without further substitution on the core [9]. This imidation reaction was followed by nucleophilic aromatic substitution to synthesise compounds **5**–**7**. A one-pot method was utilised to synthesise **8** with the choice of groups being made on the grounds that they were structurally straight forward and readily available. The photophysical properties, including fluorescence lifetimes, of these compounds were then determined in a range of solvents including water for compounds **6**–**8**. SANDIs **7** and **8** were then further examined in AOT reverse micelles and **7** in liposomes to investigate their potential and suitability for use in biophysical applications.

2. Experimental

2.1. General

1,4,5,8-naphthalene tetracarboxylic dianhydride was purchased from Tokyo Chemical Industries and used without further purification. 2-(2-aminoethoxy)ethanol (98%), tri(ethylene glycol) monomethyl ether, 1-octylamine (99%), 4-methylbenzene sulfonyl chloride, triphenylphosphine and sodium bis(2-ethylhexyl) sulfosuccinate (AOT) were purchased from Aldrich and used without further purification. Sodium azide was purchased from Ajax chemicals. Chloroform ($CHCl_3$, Merck spectrophotometric grade), methanol (MeOH, Merck GR grade) and water (Millipore) were used as received. ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded using a Bruker DRX 400 MHz Spectrometer (^1H taken at 400 MHz and ^{13}C taken at 100 MHz) or Bruker DRX 600 MHz Spectrometer (^{13}C taken at 125 MHz), using deuterated chloroform ($CDCl_3$) unless otherwise stated. The chemical shifts (δ) were calibrated with reference to the solvent peak in the spectrum. For ^1H NMR spectra, each resonance was assigned according to the following convention: chemical shift (δ), measured in parts per million (ppm), multiplicity, coupling constant (J), measured in Hz, number of protons and assignment. Multiplicities are given as (s) singlet, (d) doublet, (t) triplet, (q) quartet, (p) pentet or (m) multiplet. The ^{13}C NMR spectra were assigned a chemical shift (δ) measured in parts per million. High Resolution-Electrospray Ionisation Mass Spectrometry (HR-ESI) was completed on an Agilent Technologies 6220 Accurate- Mass TOF-time of flight instrument. Absorbance spectra were run on a Cary 100 Bio UV–visible spectrophotometer (Agilent technologies) and steady state fluorescence measurements were recorded on a Varian model Cary Eclipse fluorescence spectrophotometer (Agilent technologies) and corrected for detector efficiency. Fluorescence quantum yields were determined using Rhodamine 101 ($\Phi_f = 1.0$ in ethanol with 0.01% HCl) [26] as the standard. Solutions were prepared in thoroughly cleaned 1.0 cm quartz cuvettes. For quantum yield measurements absorbance maxima were less than 0.10 in order to reduce inner filter effects and samples were deoxygenated by bubbling with N_2 for 20 min immediately prior to measurement. Emission spectra were taken under identical conditions and the areas under the curve integrated in order to determine fluorescence quantum yields. Differences in the fraction of light absorbed and refractive index were accounted for using appropriate corrections.



Scheme 1. Two step, sequential imidation and core substitution reaction scheme for the synthesis of SANDIs **5**–**8**.