

**The preparation of BODIPY and porphyrin dyes and
their cyclodextrin inclusion complexes and Pluronic[®]
F-127 encapsulation micelles for use in PDT and
PACT**

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“If I have seen further it is by standing on the shoulders of Giants.”

– *Isaac Newton (1642–1727)*

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“Ke tseba ho kokobetsoa, ke tseba ho ateloa ke lintho; kahohle le nthong tsohle, ke ithutile ho khora le ho lapa, ho ba naleng le ho ba bofumeng. Nka etsa tsohle ka Krete ea mphang matla.”

–Philippians 4:12-13

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Abstract

Several novel BODIPY dyes ((4,4'-difluoro-1,7-tetramethyl-3,5-(3-dithiophene)-2,6-diiodo-8-(4-dimethylamino)-4-bora-3a,4a-diaza-s-indacene (**1c**), 4,4'-difluoro-1,7-tetramethyl-3,5-(3-dithiophene)-2,6-diiodo-8-(4-methylthio)-4-bora-3a,4a-diaza-s-indacene (**3c**) and 4,4'-difluoro-1,7-tetramethyl-3,5-(4-dibenzyloxybenzene)-2,6-diiodo-8-(4-methylbenzoate)-4-bora-3a,4a-diaza-s-indacene (**4c**)) and porphyrins (tetraacenaphthylporphyrin (**7a**) and Sn(IV) tetraacenaphthylporphyrin (**7b**)) were synthesized and characterized. Previously reported BODIPY dyes (4,4'-difluoro-1,7-tetramethyl-3,5-(2-dihydroxy)-2,6-diiodo-8-(4-bromo)-4-bora-3a,4a-diaza-s-indacene (**5**) and 4,4'-difluoro-1,7-tetramethyl-3,5-(2-dithiophene)-2,6-diiodo-8-(phenyl)-4-bora-3a,4a-diaza-s-indacene (**6**)) were also used. Pluronic® F-127 and cyclodextrins were used as solubilizing drug delivery agents for the synthesized BODIPY dyes. The encapsulation of BODIPY dyes with Pluronic® F-127 micelles improved the water solubility of the BODIPY **5**. Further modification of Pluronic® F-127 by coating with folate-functionalized chitosan for targeted delivery of BODIPY **1c** and **6** was explored. The BODIPY dyes and their encapsulation complexes exhibited significant inhibition of human MCF-7 breast cancer cell growth. When cyclodextrins were used as nanocarriers, the inclusion complexes of BODIPY **4c** with m β CD were found to enhance the water-solubility of the dye. Greater photoinactivation of *Staphylococcus aureus* was observed for the inclusion complexes when compared to the effect of solutions of non-complexed BODIPY **4c**. The cyclodextrin inclusion complexes of porphyrin **7b** with m β CD were also found to enhance the water-solubility of **7b**. When the photodynamic effect was evaluated, solutions of the porphyrin alone and their inclusion complexes were found to have significant photodynamic effects against human MCF-7 breast cancer cells.

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List of abbreviations

ADMA	Anthracene-9,10- <i>bis</i> -methylmalonate
BODIPY	Boron dipyrromethene
CD	Cyclodextrin
α CD	α -cyclodextrin
β CD	β -cyclodextrin
m β CD	Methyl β -cyclodextrin
γ CD	γ -cyclodextrin
CFU	Colony forming units
DCM	Dichloromethane
DDQ	2,3-dichloro-5,6-dicyano- <i>p</i> -benzoquinone
DLS	Dynamic light scattering
DMEM	Dulbecco's modified Eagle's medium
DMF	Dimethylformide
DMSO	Dimethylsofoxide
DPBF	1,3-diphenylisobenzofuran
DPBS	Dulbecco's phosphate-buffered saline

EDC	<i>N</i> -ethyl- <i>N'</i> -(3-dimethylaminopropyl)carbodiimide
EDG	Electron donating group
EtOH	Ethanol
EWG	Electron withdrawing group
FCS	Fetal calf serum
FDA	Food and drug administration
FT-IR	Fourier transform-Infrared
HOMO	Highest occupied molecular orbital
IC	Internal conversion
ICT	Intramolecular charge transfer
ISC	Intersystem crossing
LUMO	Lowest occupied molecular orbital
MALDI-TOF-MS	Matrix-assisted laser desorption/ionization time of flight mass spectroscopy
MOs	Molecular orbitals
NBS	<i>N</i> -bromosuccinimide
NIR	Near-infrared region

NIS	<i>N</i> -iodosuccinimide
NMR	Nuclear magnetic resonance
NPs	Nanoparticles
PACT	Photodynamic antimicrobial therapy
Pcs	Phthalocyanines
PDT	Photodynamic therapy
PS	Photosensitizer
ROS	Reactive oxygen species
TCSPC	Time-correlated single photon counting
TD-DFT	Time-dependent Density functional theory
TEA	Triethylamine
TEM	Transmission electron microscope
TFA	Trifluoroacetic acid
UV-vis	Ultraviolet-visible
XRD	X-ray diffraction
ZnPc	Zinc phthalocyanine

List of symbols

A	Absorbance
ϵ	Molar extinction coefficient
F	Fluorescence
λ	Wavelength
Φ_F	Fluorescence quantum yield
Φ_Δ	Singlet oxygen quantum yield
$^1\text{O}_2$	Singlet oxygen
$^3\text{O}_2$	Triplet oxygen
P	Phosphorescence
S_0	Ground state
S_1	Singlet excited state
T_1	First triplet state
τ_F	Fluorescence lifetime

Chapter 1: Introduction

1.1.Nanotechnology in medicine

Nanotechnology aims at engineering materials at a nanometer size range for various applications [1-3]. Nanomaterials can be atomic, molecular and macromolecular. Nanomedicine describes the use of nanomaterials in biological fields [3]. The properties of nanosized materials often make them superior for use in applications than their microscale counterparts [2, 4]. The interaction of nanomaterials with biological molecules at the nanoscale has opened the way to a wide range of applications [5-7]. In nanomedicine, the interaction of biomolecules and synthetic molecules is conducted in solvents/extracellular medium and *in vitro* in human cell lines with the aim of inferring the interactions that occur in physiological environments [3, 8, 9]. The nanomaterials are investigated for their diagnostic and therapeutic properties prior to *in vitro* or *in vivo* applications. The synthesized nanomaterials are designed to be recognized, captured and to accumulate in the target cells *in vitro*. The targeting of nanomaterials is crucial in drug delivery to enhance their biocompatibility and improve their accumulation in the target cells, thus increasing their efficacy [10-12]. Since nanomaterials are usually toxic at certain nanosize ranges, it is essential to explore their cytotoxicity before attempting to use them in biomedical applications [10-12].

Advances in modern medicine have led to the development of innovative technologies in treating various diseases such as cancer and bacterial infections [13-16]. The modalities used previously for treating bacterial diseases have been rendered ineffective in some cases, and as such, the development of alternative treatments has been the focus of a considerable amount of research [13-16]. Conventional cancer treatments such as chemotherapy and radiotherapy lack selectivity, efficacy and are toxic. Thus, photodynamic therapy (PDT) has emerged as an alternative treatment, which is clinically accepted for treatment of malignant cells [17-20]. A photosensitizer

(PS) dye preferentially accumulates in abnormal tissues which have large vasculature compared to normal tissues, and light of a specific wavelength in the visible region is focused to confine the damage to the targeted region [21-23]. When compared to other cancer treatment modalities, PDT is found to have high efficacy, low dark cytotoxicity and to be relatively non-invasive [21-23]. Some of the photosensitizers used in theranostics, an approach which combines nanoscience to achieve diagnostic and therapeutic treatment using agents such fluorescence-based bioimaging; rhodamines, porphyrins, and fluorescein are shown in **Figure 1** [24, 25].

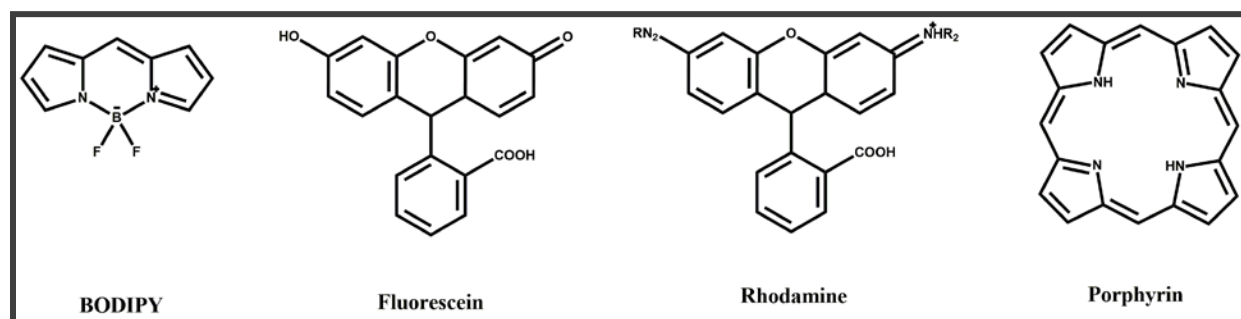


Figure 1. Structures of the BODIPY core, and fluorescein, Rhodamine and porphyrin dyes.

Photodynamic antimicrobial therapy (PACT) predates PDT in its application and has been accepted as an alternative treatment for bacterial diseases [21-23]. In a similar manner to PDT, a PS dye and visible region or ultraviolet light are used to kill bacteria through oxidative damage of the bacterial cell wall. Currently, PACT is used mainly in the treatment of oral infections and the disinfection of blood products, and has been found to be effective in destroying bacteria that have developed resistance to almost all antibiotics. Examples of these bacteria are *Neisseria gonorrhea* and *Staphylococcus aureus*. The main advantage of PACT as a potential treatment is that the photoinactivation of the bacteria can be carried out repeatedly without the bacteria developing resistance [24, 26].

The PS dyes of interest in this work are boron dipyrromethene (BODIPY) and tetraarylporphyrin dyes. BODIPY dyes have gained popularity due to their tunable properties, notably singlet oxygen quantum yields and are hence considered as being suitable for use in a new generation of photosensitizers for PDT and PACT [14, 27, 28]. The halogenation of the BODIPY dyes increases the production of $^1\text{O}_2$. The focus of the work will be to shift the absorption maxima of BODIPY dyes into the near-infrared (NIR) region to afford deeper penetration into the tumor cells, thus enhancing the efficacy of the PS drug molecules [25, 27, 29].

It has been reported that using Pluronic[®] polymer micelles and cyclodextrins as nanocarriers in drug delivery can increase the efficacy of drugs by decreasing their hydrophobicity, therefore making them biocompatible and stable in the physiological environment [16, 30-32]. The main goal of this study is, therefore, to devise novel strategies for using water-insoluble ultraviolet-visible or near infrared absorbing 2,6-dihalogenatedBODIPYs, 2,6-dihalogenated-3,5-divinyleneBODIPY dyes and a novel Sn(IV) tetraacenaphthylporphyrin in PDT studies by using Pluronic[®] F-127, β -cyclodextrin and/or methyl β -cyclodextrin as nanocarriers. This study will investigate whether these delivery systems can make the highly photostable BODIPY dyes suitable for use in PACT and/or PDT.

1.2. Structure and properties of BODIPY dyes

Boron dipyrromethenes (4,4'-difluoro-4-bora-**3a**,4a-diaza-s-indacenes), usually referred to colloquially as BODIPY dyes, are a class of fluorescent dyes which have gained popularity due to their versatile structure [33, 34]. The versatility allows the modification of the core structure for a wide range of applications including include fluorescent labeling [35], fluorescent switches [36], solar cells [37] and optical limiting [31].

The structure of BODIPY dyes is formed through the complexation of a dipyrromethene ligand with a disubstituted boron moiety, usually a BF_2 moiety [5, 7, 14, 33]. This is achieved using boron trifluoride diethyl etherate [34]. The dipyrromethene ligand is initially formed by linking two pyrrole molecules at the α -positions via a methine bridge. The complexation with BF_2 results in a constrained π -electron tricyclic system. This prevents isomerization of the dipyrromethene ligand [33-37]. The conjugation of the π -electrons on the rigid carbon-nitrogen backbone results in the exceptionally high fluorescence quantum yields [5, 7, 14, 33]. The BODIPY fluorophore has a planar core structure, and the resultant zwitterionic species possess an overall neutral charge. Different numbering systems have been used for the BODIPY core. The first systematic naming is derived from the structure of *s*-indacene where the carbons are numbered from 1 to 8, **Figure 2**. The most widely used atom naming system (**Figure 2**) refers to the C-8 atom as the *meso*-position, while the C-3 and C-5 atoms are referred to as the α -carbons and the C-1, C-2, C-6 and C-7 atoms are referred to as the β -carbons [38, 39].

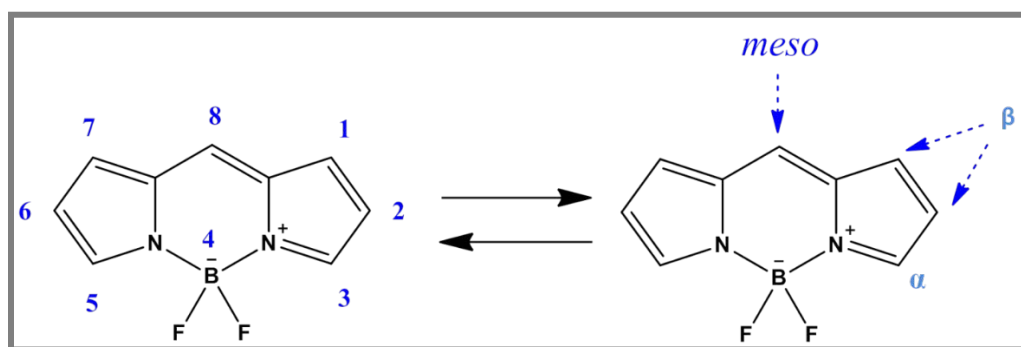


Figure 2. Naming and numbering conventions for 4,4'-difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY).

BODIPY dyes have been referred to as “porphyrin’s little sister” because the BODIPY structure resembles half the porphyrin molecular structure [38, 39]. However, BODIPYs do not follow the

Hückel's $4n+2$ aromaticity rule. The parent indacene structure of the BODIPY follows Hückel's rule, but the introduction of BF_2 disrupts the aromaticity [21, 40]. Further disruption is caused by the pyrrole nitrogens and the cross-linking. This results in a complete lifting of the degeneracies of the π -MOs, since the structure is characterized by its C_{2v} symmetry [21, 40]. The highest occupied molecular orbital (HOMO) and lowest occupied molecular orbital (LUMO) of BODIPY dyes are well separated from other π -MOs in energy terms. This separation results in the main spectral band of the dyes being dominated by the HOMO $\rightarrow$ LUMO transition [21, 40].

The most notable properties of BODIPY dyes and their derivatives are their rigid core structure, small Stokes shifts (ca. 10 nm), intense absorption in the visible region, narrow emission and excitation peaks which are responsible for the brightness of the dyes, high molar extinction coefficients (ranging from 40,000 to 80,000 $\text{M}^{-1}\cdot\text{cm}^{-1}$), high stability in multiple organic solvents, and high environment-independent fluorescence quantum yields [32, 33, 41]. Additionally, BODIPY dyes are resistant to self-aggregation and photobleaching. They also have fluorescence lifetimes within the nanosecond range that are insensitive to solvent polarity and pH [33, 41].

1.3. Synthetic background and methodologies

1.3.1. Background

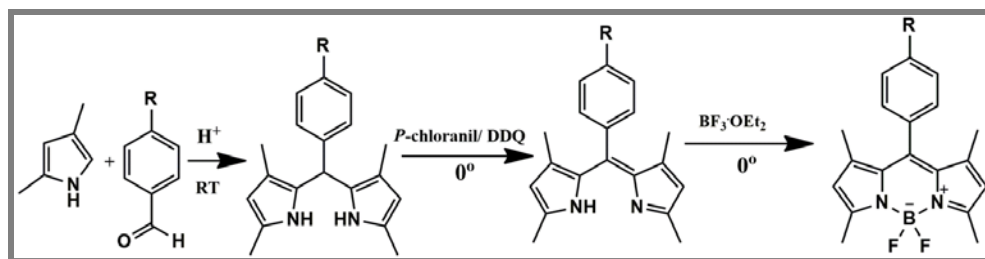
Treibs and Kreuzer were the first to synthesize BODIPY dyes serendipitously in 1968 through the reaction of 2,4-dimethylpyrrole and acetic anhydride in the presence of $\text{BF}_3\cdot\text{OEt}_2$ [21, 41]. However, minimal work was carried out on BODIPYs until the 1980s when their potential utility was recognized in cellular imaging due to their highly fluorescent nature [41]. BODIPY dyes have replaced fluoresceins as laser dyes [42], due to their higher photo-stability. In the mid-

1990s, the amount of research on BODIPYs increased significantly and subsequently became very extensive [21, 41, 43].

1.3.2. Synthesis of BODIPY core dyes

Different synthetic routes for the formation of dipyrromethenes have been derived from the chemistry of dipyrromethanes through condensation of two pyrroles with highly electrophilic carbonyl groups [44, 45]. The resultant dipyrromethenes are complexed with BF_2 in the presence of a non-nucleophilic base resulting in the preparation of BODIPY dyes in moderate and reproducible yields [44, 45]. The synthesis of the parent unsubstituted BODIPY core, has proven to be difficult compared to the synthesis of highly substituted BODIPY cores, especially at the *meso*-position [14, 43, 46].

The most commonly used procedure for the synthesis of BODIPY core dyes is an acid-catalyzed condensation reaction between an aldehyde and a highly electrophilic carbonyl compound, an acyl chloride or an acid anhydride. The acid-catalyzed condensation approach had previously been extensively explored in the context of porphyrin chemistry [41, 47]. The condensation of aldehydes and 2,4-dimethylpyrrole was used in this study. The synthetic procedure of the BODIPY is shown in **Scheme 1**. In the first step, the aldehyde and 2,4-dimethylpyrrole are reacted in the presence of trifluoroacetic acid (acidic media) to yield the dipyrromethane moiety. The use of the substituted 2,4-dimethylpyrrole results in high selectivity and the formation of BODIPYs in high yields [41, 47]. This is because the methyl substituents on the pyrrole prevent the polymerization of the pyrrole units into tripyrromethanes or porphyrins.



Scheme 1. The acid-catalyzed condensation reaction synthesis for BODIPY core (R = aldehyde substituent group).

The second step is the oxidation of the dipyrromethane using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) or *p*-chloranil to yield a relatively unstable dipyrromethene. To avoid the decomposition of this intermediate, boron trifluoride diethyl etherate ($BF_3 \cdot OEt_2$) is promptly added to complex the dipyrromethene with BF_2 in the presence of a tertiary amine. Triethylamine (TEA) is usually used, and this final step yields the BODIPY core dye [35, 48, 49]. The preferred organic solvent is dichloromethane (DCM), but the use of other organic solvents has been reported [21, 41]. A green chemistry synthetic procedure involving precipitation of the aryldipyrromethane from aqueous solution in relatively high yields has also been used [41, 50, 51].

1.3.3. Modifications of BODIPY core

The BODIPY chromophore can be modified through the addition of peripheral substituents. This alters the photochemical and photophysical properties of the dye depending on the nature of the substituents that are added. The modifications can be carried out through the addition of functional groups at the α , β , and *meso*-positions and by substitution of the two fluorine atoms [21]. The incorporation of suitable substituents can result in significant bathochromic shifts of the main spectral band [14, 24, 52]. As observed in **Figure 3**, symmetrical di-substituted

BODIPY dyes yield more red-shifted absorption and emission maxima than asymmetric mono-substituted dyes with similar substituents [14, 43, 53]. Substitution of the 1,3,5,7-tetramethylBODIPY core can result in a red-shift of the main absorption band. A significant bathochromic shift is typically observed upon addition of substituents at the 2,6-positions of the core when compared to changes in the substitution on aryl rings at the *meso*-position, since the *meso*-aryl ring lie orthogonal to the BODIPY core for steric reasons. It is noteworthy that the absorption maxima of highly substituted BODIPYs have larger bathochromic shifts than less substituted counterparts as shown in **Figure 3**. The greatest bathochromic shifts (although not shown in **Figure 3**) is observed when there is an extension of the π -system through modifications of methyl groups at the 1,3,5,7-positions [14, 43, 53].

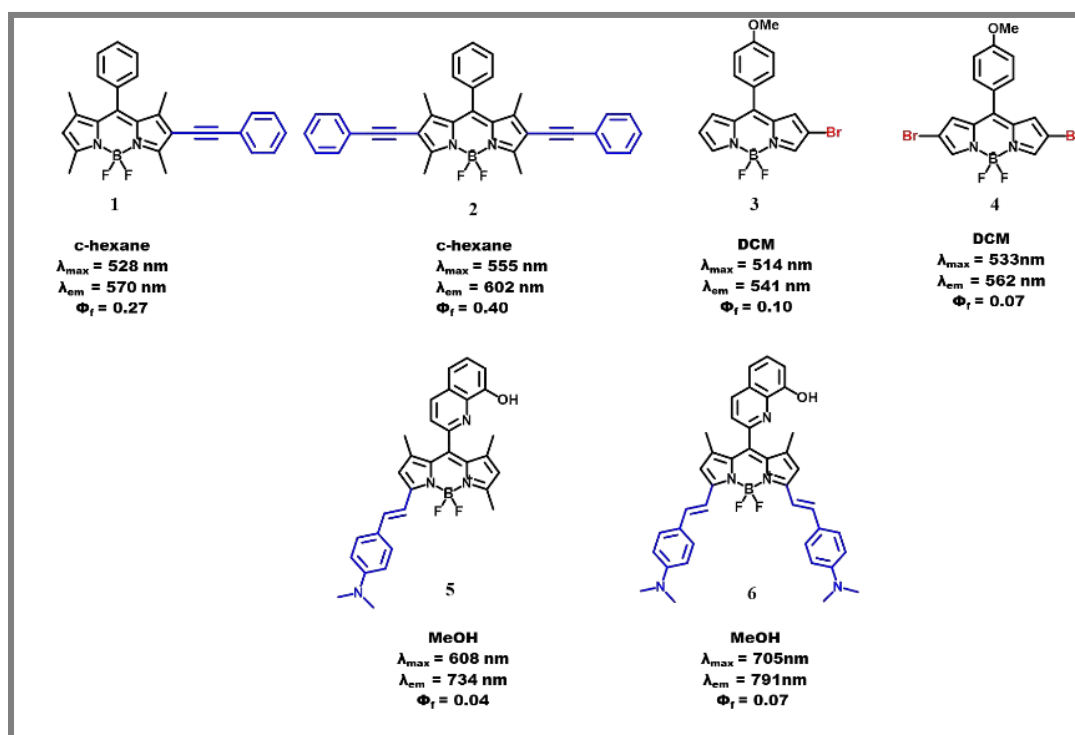


Figure 3. The effects of symmetry and substituents on the absorbance maxima (λ_{abs}), emission maxima (λ_{em}), and fluorescence quantum yield (Φ_f) values of a series of BODIPY dyes [14, 43, 53].

The extension of the BODIPY π -conjugation system results in a red-shift of the main absorption spectra into the NIR. The electronic and steric interactions of the substituents also greatly influence the emissive behavior of the dyes [23, 41, 43]. The brightness and absorptive properties of the BODIPY are influenced by the rigidity of the substituents, and the presence of electron-donating groups (EDG) and electron-withdrawing groups (EWG) on the core structure [14, 16, 54, 55].

The BODIPY chromophore can be modified to suit the desired application. The introduction of halogens to the BODIPY core enhances the production of ROS through enhanced intersystem crossing (ISC). A red shift of the main absorption band of the BODIPY into the near-infrared (NIR) region is necessary for PDT [41], since this region coincides with the optimal therapeutic window between 600–850 nm where there is minimal interference from biological chromophores such as blood, hemoglobin and water, making the dyes suitable for application in physiological environments [52, 56]. The versatility of the BODIPY chromophore has led to intensive research towards developing diverse BODIPY dyes for a variety of applications [41]. The modification of the BODIPY structure to enable their use in PDT and PACT will form the main focus of this dissertation.

1.3.4. Substitution at the meso-position

The functionalization of 1,3,5,7-tetramethylBODIPY dyes at the *meso*-position usually has a trivial effect on the spectroscopic properties of the dyes. Substituents at this position are introduced via the use of an aldehyde with the desired functional group in the first step of the BODIPY core synthesis [21]. The free rotation of the phenyl rings at the *meso*-position is prevented by the methyl substituents on the pyrrole, resulting in the phenyl groups being oriented

orthogonal to the BODIPY core (**Scheme 1**), which minimizes the electronic coupling of the core and the *meso*-substituents. The substitution at the *meso*-position is often vital for post-modification purposes [43, 52]. For example, halogen atoms can later be readily substituted with water solubilizing groups or other desired functionalities. In addition, aldehydes with functional groups such as carboxylic acids and amines can be used for conjugation with nanomaterials [41].

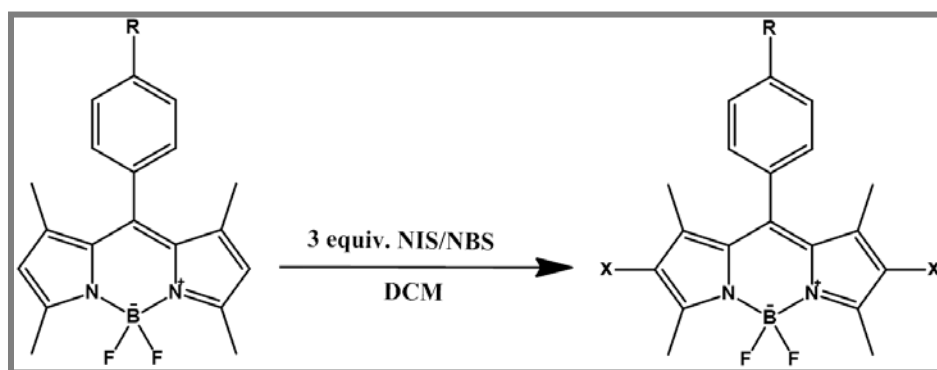
1.3.5. Functionalization at the 2,6-positions

1,3,5,7-TetramethylBODIPY dyes can be readily modified at the 2,6-positions. The addition of halogens at these positions enhances the suitability of dyes for use as PDT and PACT PS dyes. The 2,6-positions are predicted in theoretical calculations to have lower positive charges making these positions susceptible to electrophilic substitution or attack [14, 34]. The halogenation of the 2,6-positions is achieved through the substitution of the 2,6-protons with either iodine or bromine atoms [22, 24]. The planarity of the core structure is not disrupted upon bromination or iodination [22]. The introduction of either halogen atom causes a relatively small (< 50 nm) bathochromic shift in the absorption and emission maxima of the BODIPYs [14, 43].

Non-halogenated BODIPYs are highly fluorescent. Upon excitation of the PS dyes, the electrons are excited into the higher singlet states whereupon relaxation from the S_1 to the S_0 ; fluorescence is observed [14]. At this point, ISC to the triplet state is usually minimal. The introduction of halogens favours the spin-forbidden transition, which enhances ISC while the fluorescence is quenched since the T_1 state becomes more populated than the S_1 state [14, 21, 33]. This results from spin-coupling to heavy atoms and the phenomenon is referred to as the “heavy atom effect” [14, 21, 33].

Various halogenation routes have been utilized in BODIPY syntheses. These include palladium-catalyzed cross-coupling and S_NAr for 3,5-chlorinated BODIPYs. These synthetic routes were first developed by Boens et al. [43]. The most efficient halogenation procedure is that of using substituted pyrroles prior to halogenation [53]. The methyl groups of 1,3,5,7-tetramethylBODIPY core dyes direct the halogens to the desired positions upon halogenation. This direct regioselective halogenation of BODIPYs was recently achieved by Churchill and coworkers [43], while 2,6-dibromoBODIPYs were reported by Shinikubo and co-workers [22, 55].

In this work, the regioselective halogenation of the BODIPY core at 2,6-positions was achieved as shown in **Scheme 2** [25, 54]. For bromination, *N*-bromosuccinimide (NBS) was used while *N*-iodosuccinimide (NIS) was used for iodination to achieve dihalogenated BODIPY core [25, 54].



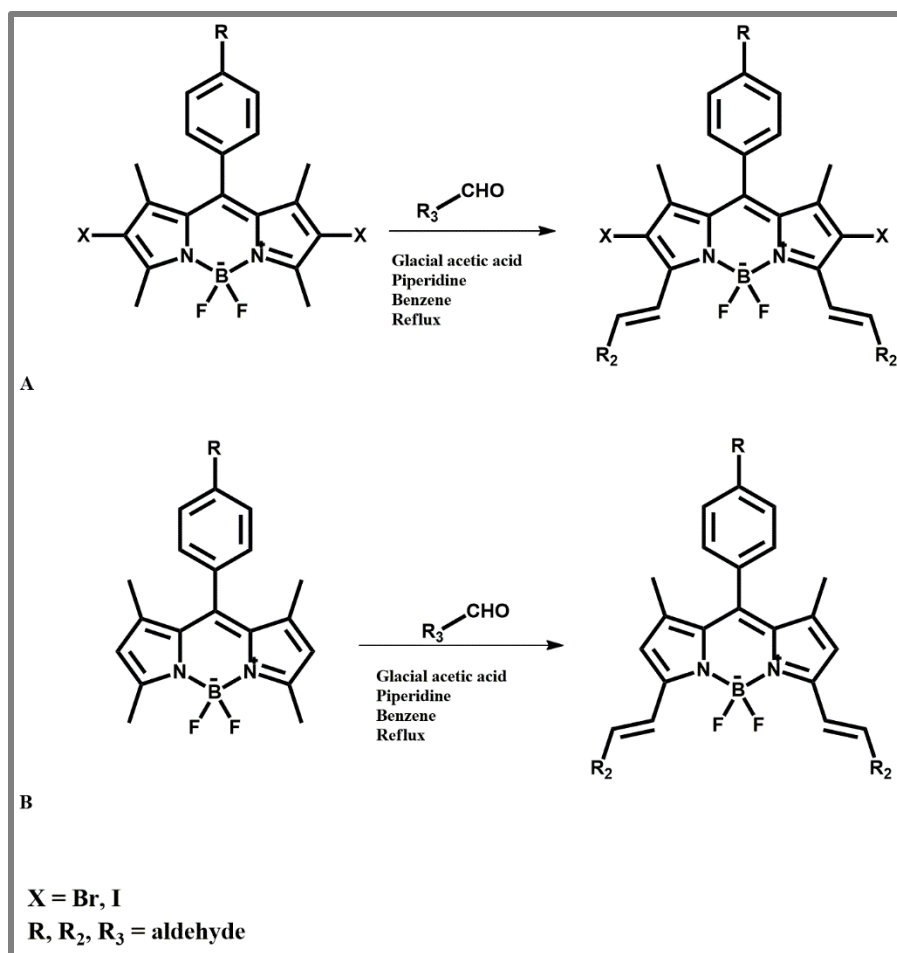
Scheme 2. The regioselective halogenation of the BODIPY core; X = Br or I.

1.3.6. Functionalization at the 1,3,5,7-positions

The fine-tuning of the properties of 1,3,5,7-tetramethylBODIPY core dyes has been achieved by functionalization at both the 2,6 or 1,3,5,7-positions [43], so that the absorption and emission maxima can shift from the UV-visible to the NIR region [14, 41, 43]. The functionalization of

the 1,3,5,7-positions can be readily achieved, since the methyl groups, which are typically incorporated at these positions due to the use of 2,4-dimethylpyrrole as a precursor, are highly nucleophilic [14, 41]. The high nucleophilicity makes the methyl groups prone to deprotonation under mild basic conditions. The methyl groups at 3,5-positions are more reactive than the 1,7-methyl groups in this regard [14, 41]. The π -conjugation system of the BODIPY core can be readily extended at the 3,5-positions through a Knoevenagel condensation to form vinylene groups at these positions [23, 25, 33]. Mono-, di-, tri- and tetravinylene-BODIPYs can be prepared depending on the desired application. The largest π -conjugation extension and bathochromic shifts are observed when tetravinylene-BODIPY dyes are synthesized, while the smallest are observed for monovinylene-BODIPYs [16, 49, 57].

3,5-DivinyleneBODIPY dyes typically absorb beyond 600 nm in the therapeutic window [52, 56]. These dyes can exhibit bathochromic shifts of over 100 nm of the main BODIPY core spectral and retain a highly rigid planar structure. The fluorescence quantum yields tend to decrease due to the rotational relaxation introduced by the aromatic substituents which result in non-radiative decay. Halogenation of 3,5-divinyleneBODIPY dyes at the 2,6-positions results in lower fluorescence quantum yields (Φ_F), and higher $^1\text{O}_2$ quantum yields (Φ_Δ) [16, 49, 57] for reasons explained in the previous section. **Scheme 3** shows the synthesis of 3,5-divinyleneBODIPY dyes via the Knoevenagel condensation of the appropriate aldehyde and either the halogenated (**A**) or non-halogenated (**B**) in dry benzene.



Scheme 3. The Knoevenagel condensation reaction for halogenated (A) and non-halogenated BODIPYs (B).

1.4. The absorption and emission spectra of BODIPY dyes

Meso-aryl-tetramethylBODIPY core dyes usually absorb at ca. 500 nm and emit at ca. 510 nm (**Figure 4**) [14]. The morphology of the BODIPY absorption spectra is independent of the concentration of the dye (up to 2×10^{-3} M). This shows that there are minimal intermolecular dye-dye interactions that could rise due to aggregation at high concentrations of the dye [36]. The narrow and intense band at ca. 500 nm can be attributed almost exclusively to the excitation of an electron from the HOMO to the LUMO as demonstrated by theoretical calculations [58,

59]. The lowest absorption band is attributed to the S_0 - S_1 (π - π^*) electron transition. A minor band at ca. 480 nm is a high energy shoulder of intensity that is assigned to a 0-1 vibrational translation [53, 54]. The broader and weaker band at ca. 350 nm has been assigned to the S_0 - S_2 (π - π^*) transition [34, 54, 59, 60]. Upon excitation of the PS dye to the S_1 or S_2 states, a narrow emission band is observed, which is an approximate mirror image of the main absorption band.

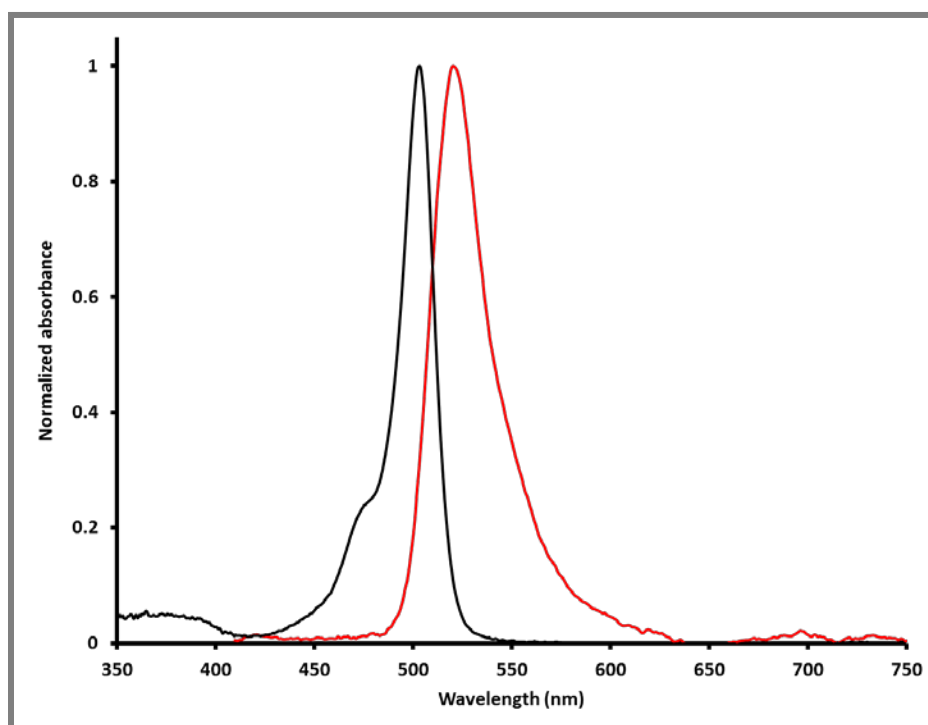


Figure 4. Typical normalized UV-visible absorption (black) and emission (red) spectra of a BODIPY core dye in DCM.

1.5. Photophysical and photochemical properties of BODIPY dyes

A Jablonski diagram (**Figure 5**) can be used to describe the physicochemical properties of porphyrin and BODIPY dyes and the mode of action of PDT and PACT. The PS dye is illuminated at a specific wavelength. The excited PS dye transitions from the ground state (S_0) to the lowest vibrational level of the first singlet excited state (S_1) via internal conversion where it

can undergo fluorescence and ISC [61, 62]. The PS dye requires the introduction of heavy atoms in its structure for enhanced rates of ISC into the first triplet excited state (T_1) which is a crucial process in PDT and PACT [63, 64]. In the T_1 state, the PS dye can interact with molecular dioxygen to produce reactive oxygen species (ROS) via type II (singlet oxygen; $^1O_2$) and type I (hydroxyl radical ($\bullet OH$), hydrogen peroxide (H_2O_2)) triplet state oxygen ($^3O_2$)) electron transfer reactions [14, 48, 65]. The ROS eradicate tumour cells through apoptosis and/or necrosis and disrupt bacterial cell walls through oxidative processes [13, 16, 61].

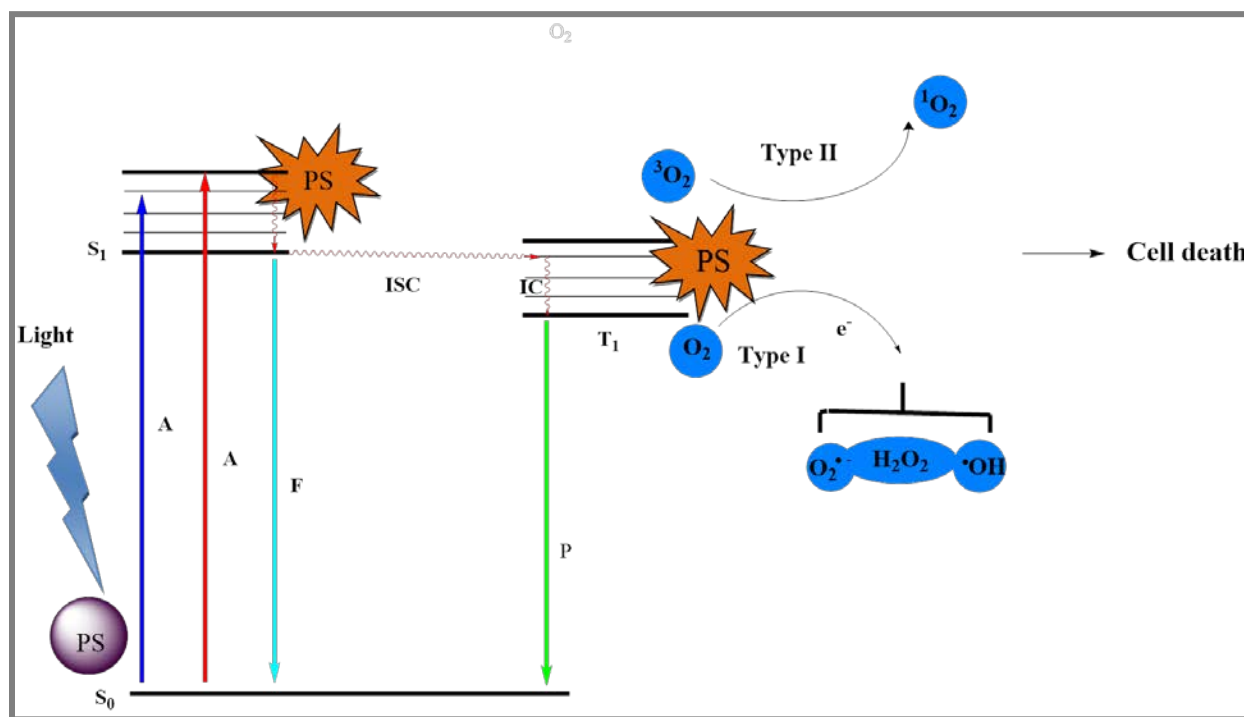


Figure 5. A modified Jablonski: A = absorbance, F = fluorescence, P = phosphorescence, IC = internal conversion, ISC = intersystem crossing, PS = photosensitizer, S_0 = ground state, S_1 = singlet excited state, T_1 = first triplet state.

1.5.1. The fluorescence quantum yields and lifetimes

Fluorescence is important in PDT and PACT as imaging techniques can be used for tumourous or bacterial cells. Fluorescence lifetime measurements can be used to quantify how long the PSs take before relaxation to the S_0 . Fluorescence is defined as the emission of light from excited atoms and molecules due to an electron transition from the lowest singlet excited state to the ground state ($S_1 \rightarrow S_0$) [36, 66]. This process usually follows Kasha's rule, which states that the emission normally occurs only from the lowest vibrational level of the S_1 [67]. The morphology of the emission band is independent of the excitation wavelength in this context. The emission spectrum is normally a mirror image of the absorption spectrum (**Figure 4**) due to the electronic states of dyes with rigid structures having similar potential energy surfaces [21, 22, 39].

A fluorescence quantum yield (Φ_F) value quantifies the efficiency of the fluorescence process, which can be very high for BODIPY dye with values of up to 1.0 [47, 55, 66]. The Φ_F value is determined by a comparative method where the integrated area under the emission spectrum of the dye is compared to that of a molecular dye with a known Φ_F value that acts as a standard. The Φ_F value of the sample is calculated using equation (1):

$$\Phi_F = \Phi_{F(\text{std})} \frac{F \cdot A_{\text{std}} \cdot \eta^2}{F_{\text{std}} \cdot A \cdot \eta_{\text{std}}^2} \quad (1)$$

where F and F_{std} are the integrated areas under the emission curves for the sample and standard; A and A_{std} are the absorbances of the sample and standard at the excitation wavelength; η and η_{std} are the refractive indices of the solvents used for the sample and standard, respectively. $\Phi_{F(\text{std})}$ is the known Φ_F value of the standard used. Examples of standards are Rhodamine 6G (Φ_F

= 0.95 in ethanol) [68-70], zinc phthalocyanine (ZnPc) (Φ_F = 0.20 in DMSO, 0.28 in ethanol) [43, 71].

The fluorescence lifetime (τ_F) is defined as the time it takes for the population of the S_1 to decline by $1/e$ due to the fluorescence process. The τ_F values are independent of the emission and excitation wavelengths used if the emission is from a locally excited state. The τ_F values of BODIPYs are typically not long-lived, but their absorbance and emission are known to be efficient [55, 72, 73]. The lifetime values are obtained from a decay curve from the time-correlated single photon counting (TCSPC) instrument [74]. A typical decay curve with residuals is shown in **Figure 6**.

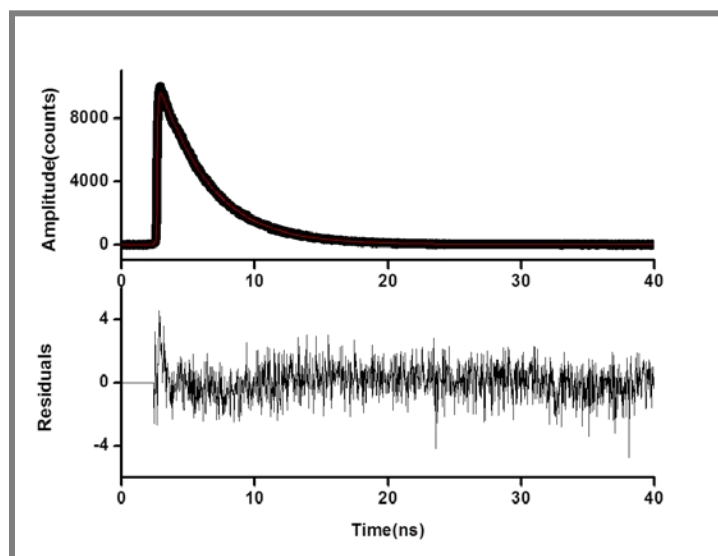


Figure 6. A typical fluorescence lifetime decay curve.

1.5.2. The singlet oxygen quantum yields

BODIPY dyes are highly fluorescent dyes with higher molar extinction coefficients than porphyrins [75, 76]. The BF_2 moiety of BODIPY dyes makes the core structure highly rigid [77].

This disrupts the delocalized π -system when the structure is viewed as that of an *s*-indacene so that there is no macrocyclic ring system comparable to that of porphyrins and the main spectral band is dominated by the HOMO $\rightarrow$ LUMO transition [21]. As such, BODIPY dyes do not have aromatic π -systems in contrast with porphyrins, which are heteroaromatic [78, 79]. Although the low rate of ISC has limited the use of BODIPY dyes in applications that require $^1\text{O}_2$ [14, 80], the structures of molecular dyes can be modified by enhancing the rate of ISC through the addition of heavy atoms to populate the T_1 state and thus enhance the generation of $^1\text{O}_2$ [81-83]. The focus of this study in this regard is the introduction of iodine atoms at the 2,6-positions to maximize the singlet oxygen quantum yield (Φ_Δ) values of a series of BODIPY dyes. This is achieved through the introduction of a central Sn(IV) ion in the context of the porphyrins studied.

Singlet oxygen quantum yields provide a quantitative measurement that is used to evaluate the efficiency of the PS to utilize light energy to convert molecular dioxygen in the ground state to $^1\text{O}_2$, since this cytotoxic species has a vital role in PDT and PACT [13, 42]. The Φ_Δ value can be quantified using two methods, the comparative chemical method and through the use of a germanium detector to study the phosphorescence of $^1\text{O}_2$ [84, 85]. The comparative chemical method was used in this study. Singlet oxygen scavengers such as 1,3-diphenylisobenzofuran (DPBF) and anthracene-9,10-*bis*-methylmalonate (ADMA) were used in this context [86, 87]. The generation of $^1\text{O}_2$ is quantified relative to the known Φ_Δ value of a standard such as zinc phthalocyanine (ZnPc). The mixed solution of the dye/standard and scavenger are irradiated at fixed time intervals. During the irradiation, the $^1\text{O}_2$ produced by the dye reacts with the scavenger and gradually degrades it. The effect of this process on the ground state absorption spectrum of the scavenger is used to monitor the production of $^1\text{O}_2$ from the change in the

absorption maxima intensity of the $^1\text{O}_2$ scavenger in solution. Equation (2) is used for the calculation of the Φ_{Δ} value [81, 88].

$$\Phi_{\Delta} = \Phi_{\Delta(\text{std})} \frac{\alpha_{\text{std}}}{\alpha_{\text{PS}}} \quad (2)$$

where $\Phi_{\Delta(\text{std})}$ is the singlet oxygen quantum yield value of the standard (Rose Bengal or ZnPc), and α_{PS} and α_{std} are the ΔA slopes of the photosensitizer and standard, respectively. This equation is only valid when the solvents and optical densities used are the same for both the sample and standard solutions.

The Φ_{Δ} values of PS dyes are solvent dependent. The Φ_{Δ} values of most PS dyes have been reported in organic solvents. In this work, the quantification of the Φ_{Δ} values in both organic solvents and water was attempted [88-90]. The accurate determination of the Φ_{Δ} value of PS dyes in aqueous media using ADMA is significantly more challenging to achieve because the dyes tend to have shorter triplet state lifetimes compared to the lifetimes in organic solvents [88-90].

1.6. Overview of porphyrins

Porphyrins are naturally occurring macrocyclic compounds which have a vital role in the metabolism of living organisms. The porphyrin ligand is a very stable heteroaromatic system that follows Huckel's rule for aromaticity. Synthetic porphyrins have been used in various applications [60-62]. As mentioned above, research on porphyrin analogues such as BODIPY dyes was inspired by porphyrin chemistry, and these analogues have properties that often closely parallel those of porphyrins [60-62]. The porphyrin structure comprises four pyrrole rings linked via methene bridges with an inner cavity size up to 3.7 Å [91], **Figure 7**. This makes porphyrins

excellent ligands for use in coordination chemistry. During coordination, the two protons on the pyrrole nitrogens are removed and replaced with negative charges [92]. A variety of metals with different coordination numbers can be chelated by porphyrins and the complexes formed are very stable.

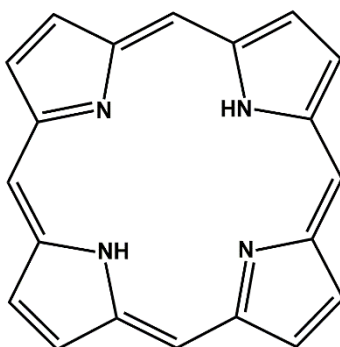


Figure 7. The macrocyclic structure of the porphyrin ligand.

The properties that make porphyrins attractive for use in applications are the highly symmetrical planar D_{4h} structure of metalloporphyrins, their photostability, versatility and unique electronic and optical properties [92, 93]. Since tissue penetration is affected by the wavelength of light used to excite the PS, the porphyrin absorption bands need to be shifted by modifying the structure at the *meso*- and/or β - positions. The insertion of a heavy central metal ion increases the rate of ISC. Porphyrin spectra are characterized by an intense absorption band at approximately 400 nm called the B (or Soret) band, and less intense bands at the red end of the visible region (550–750 nm) called the Q bands [62, 94]. The Q bands are useful for PDT, and a red-shift of their band maxima into the therapeutic window (600–850 nm) is the key goal explored in this study. However, it is essential that PSs absorb below ca. 800 nm in the therapeutic window due to the limited ability of the PS to transfer energy to $^3\text{O}_2$.

The functionalization of the porphyrins prepared in this work at the *meso*-positions with 5-acenaphthenecarboxaldehyde was inspired by pyrene-based cyclodextrin inclusion complexes, which have been studied previously [95, 96]. The motivation for adding the bulky *meso*-acenaphthyl groups was to expand the π -conjugation system with the hope of red shifting the Q-bands. Sn(IV) was inserted as a central heavy metal ion that is routinely used in porphyrin-based PSs with anti-tumor properties [97, 98]. A free-base tetraacenaphthylporphyrin (**7a**) and a Sn(IV) tetraacenaphthylporphyrin (**7b**) were synthesized for so that their PDT activity can be compared to a similar metalloporphyrin (Sn(IV) tetrathienylporphyrin) which was recently reported by Mack and coworkers [55], **Figure 8**. A Sn(IV) tetraphenylporphyrin control compound is referred to throughout as compound **8**. The effect of the *meso*-acenaphthyl groups on the optical properties and photodynamic activity was investigated in depth.

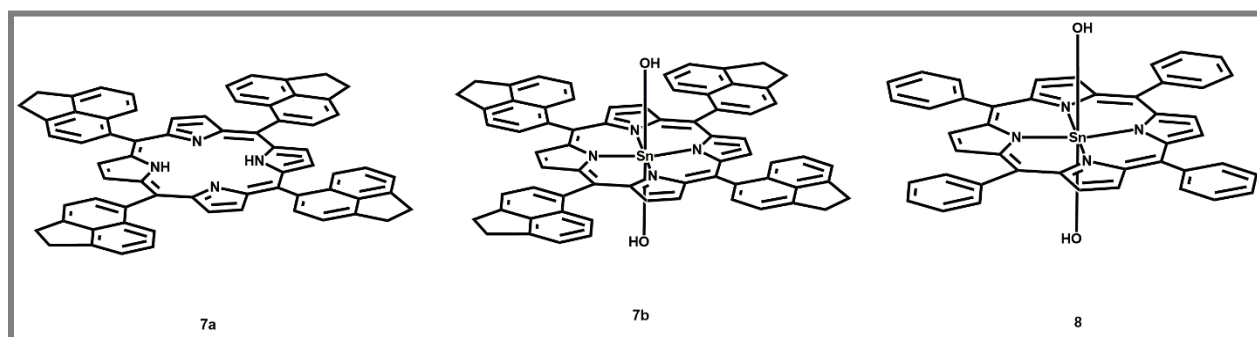


Figure 8. Molecular structures of tetraacenaphthylporphyrins **7a** and **7b** and the Sn(IV) tetraphenylporphyrin control complex **8**.

1.7. Drug delivery agents

1.7.1. Pluronic[®] micelles

The use of nanocarriers is crucial in drug delivery to enhance the efficacy and biocompatibility of drug molecules in physiological environments. Nanoparticles (NPs) have been successfully

conjugated to PS dyes such as phthalocyanines (Pcs) and porphyrins. These NPs include carbon nanotubes, and silicon, gold and silver NPs [26, 35]. Polymeric nanocarriers such as Pluronics, cyclodextrins, and liposomes can be used as transport agents for PS dyes. Since hydrophobicity limits the biocompatibility and efficacy of BODIPYs in physiological environments, solubilization through the use of Pluronic[®] F-127 micelles will be explored. The use of solubilizing agents such as PF-127 as a drug delivery agent is approved by the Food and Drug Administration (FDA) due to its aqueous solubility and biocompatibility in biological environments [67, 81, 87]. Pluronic[®] is a triblock copolymer with a hydrophobic polypropylene oxide (PPO) center unit and hydrophilic polyethylene oxide (PEO) end units (**Figure 9**) [67, 99]. Micelles are temperature dependent and form more readily as temperature increases due to the increase in solubility of the PPO unit of the copolymer. In the presence of a hydrophobic drug molecule in solution, PF-127 encapsulates the drug molecules as illustrated in **Figure 9**, making them water-soluble [99-101]. In this study, this approach will be used to form encapsulation complexes with hydrophobic BODIPY dyes.

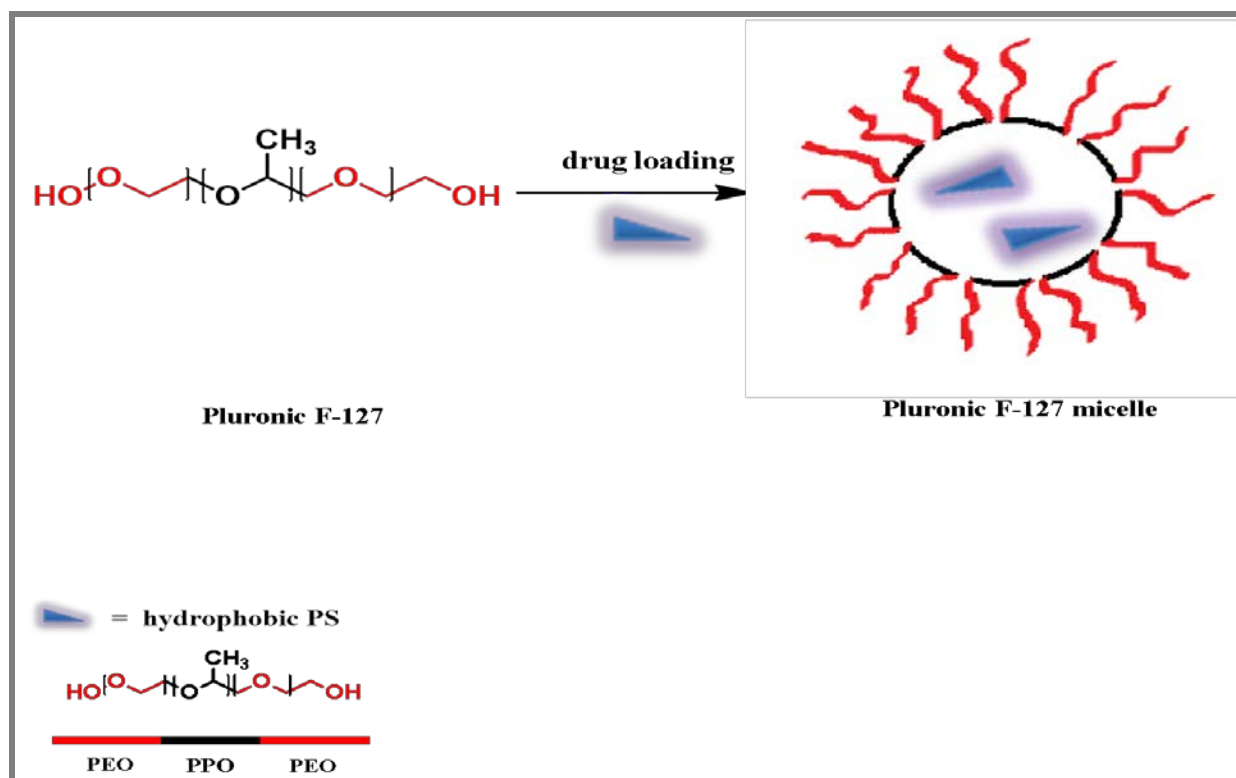


Figure 9. Schematic diagram of the micellation of Pluronic[®] F-127 in the presence of a hydrophobic molecule.

1.72. Folic acid functionalized chitosan

PF-127 micelles aggregate and are unstable when exposed to varying concentrations and temperatures. To address this limitation, the micelles can be coated with other polymers such as chitosan to stabilize the micelles and enable prolonged drug delivery [81]. Chitosan is a 2-amino-2-deoxy- β -D-glucan polymer with unique properties that make it suitable for pharmaceutical applications [37, 81]. The chitosan structure has free amino groups which encourage cross-linkages. Its muco-adhesive properties enhance the retention of the encapsulated drugs in their target substrates. Additionally, chitosan is more cationic than other polymers thus it is can easily target malignant cell membranes and form ionic linkages to anions [37, 81, 102]. The ease of

cross-linking allows chitosan to be functionalized with various molecules including targeting ligands such as folic acid [74]. Folic acid is important in this regard because most cancerous cells possess overexpressed folate receptors [103]. As such, folic acid can be used as targeting ligand to enhance the uptake of the drug molecules by tumor cells.

1.7.3. Cyclodextrins

Cyclodextrins (CDs) are FDA approved and have been used in the pharmaceutical industry to encapsulate vitamins, hormones and cancer drugs such as doxorubicin [104-106]. It has been reported that using β -cyclodextrins (β -CDs) as nanocarriers in drug delivery can increase the efficacy of drugs by decreasing their hydrophobicity, thus affording biocompatible and stable drugs in physiological environments [104-106]. CDs are cyclic oligosaccharides formed from the degradation of starch by *bacillus macaren*. The β -CD structure comprises seven glucopyranose units compared to six and eight units in α -CD and γ -CD, respectively. The glucopyranose units are linked by α -(1–4) bonds forming a truncated conical structure with a hydrophobic cavity and hydrophilic exterior with primary and secondary hydroxyl groups. **Figure 10** shows the β -CD chemical structure and a schematic diagram of the truncated β -CD cone [107].

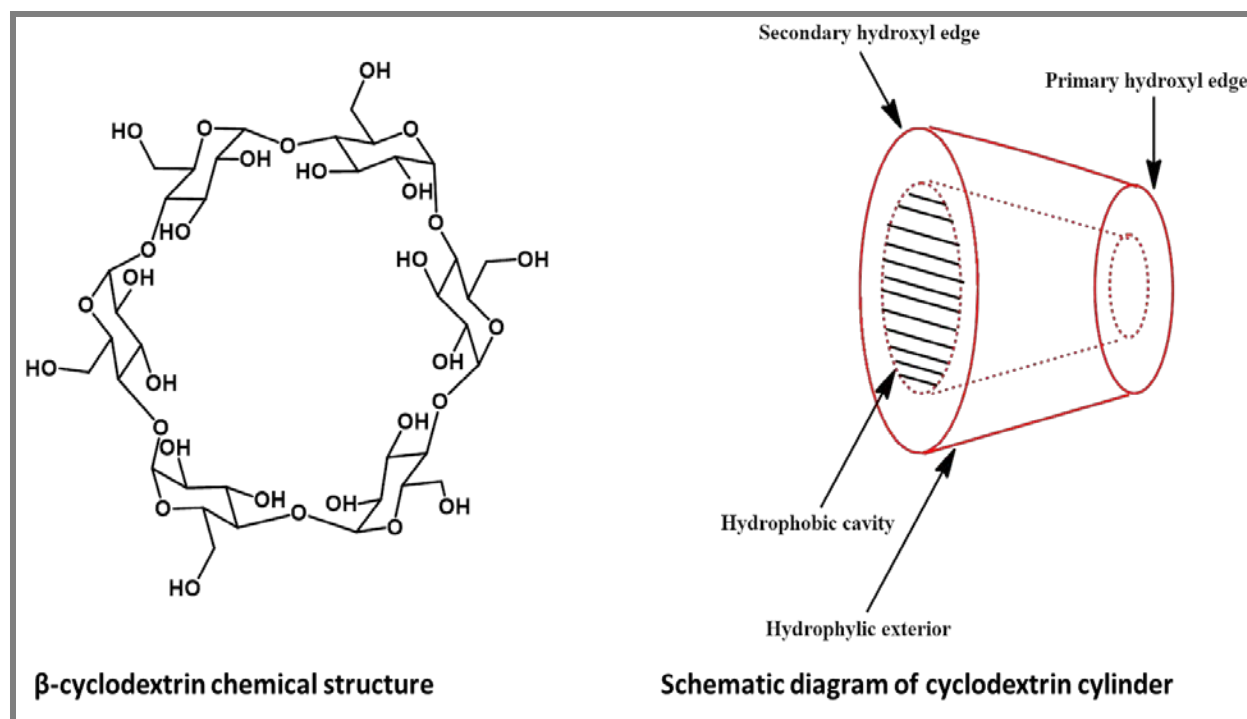


Figure 10. The chemical structure and molecular shape of β -cyclodextrin.

β -CDs are of particular interest due to their appropriate cavity size which enables them to encapsulate a variety of small organic guest molecules or functional groups to form inclusion complexes [107]. Derivatives of β -CD are more water soluble than the parent β -CD, and thus tend to enhance the water solubility of hydrophobic drugs and molecules [103, 108]. In the formation of inclusion complexes, the guest molecules replace the water molecules in the β -CD cavity and bind to the CDs non-covalently through hydrogen bonding, Van der Waals forces, hydrophobic interactions and other intermolecular forces. The non-covalent interactions maintain the properties of the complexed molecules and allow easy release of the molecules at the target site [102, 108, 109]. The use of β -CD and methyl- β -CD to form inclusion complexes with BODIPY and porphyrin dyes will be explored in this study.

1.8. Applications

1.8.1. Photodynamic therapy

Photodynamic therapy (PDT) is an FDA approved treatment for numerous types of cancer including lung, oral, esophageal and breast cancers [110, 111]. It is clinically proven to be effective and is relatively non-invasive. Light of a specific wavelength is used to excite a photoactive chemical, usually a PS dye, which then reacts with molecular oxygen to produce cytotoxic ROS which eradicate tumor cells [112, 113]. This mechanism is illustrated in **Figure 11**. When the same approach is applied to the treatment of bacteria, the therapy is referred to as photodynamic antimicrobial chemotherapy (PACT).

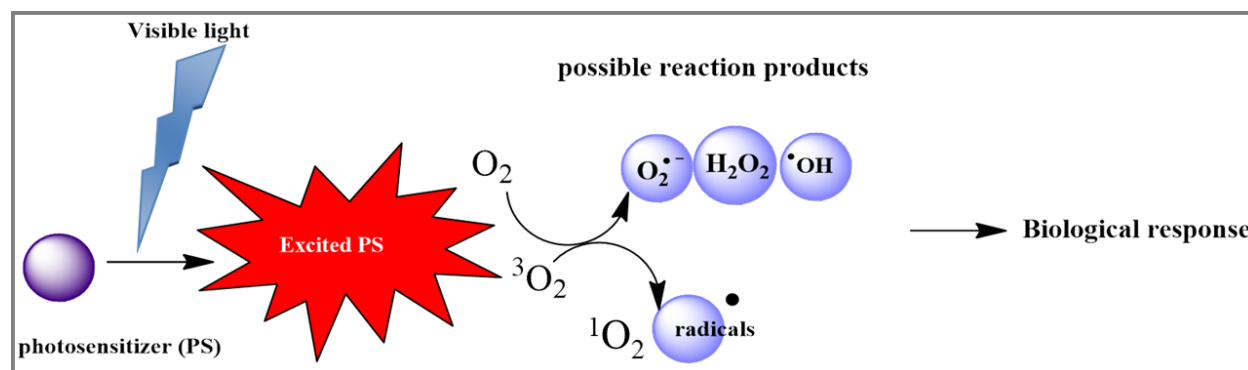


Figure 11. The mechanism of PDT and PACT.

When PDT is used to treat cancer patients, a maximum of four days is required, and the patients are usually outpatients [114, 115]. In addition, PDT is minimally invasive on healthy cells, due to the use of targeted laser light and PS dyes that selectively accumulate in the tumor. Conversely, chemotherapy is completed over several months, and is non-selective and toxic to normal cells [116, 117]. Radiotherapy is also time-extensive; completed over a period of seven weeks and also typically damages normal cells around tumorous cells [118]. Surgery is the

quickest treatment but the recovery time after the removal of the tumor is usually long [119, 120]. PDT is therefore rendered cost-effective and efficient compared to the other possible treatment methods.

The PDT treatment varies depending on the location of the infection. For dermal treatments, topical application of the PS is used; otherwise, the PS is administered intravenously into the patient. The PS dye can selectively accumulate in the target tumor cells over a period of 24–96 hours after which light of a specific wavelength is used to irradiate the PS dye [121-123]. The dyes used for PDT should be non-toxic in the absence of light, lipophilic and absorb at the red end of the visible region between 620–700 nm, or in some cases even further to the red in the NIR region [14, 124].

1.8.2. BODIPY and porphyrin dyes synthesized for photodynamic therapy

Four 3,5-divinyleneBODIPY dyes with halogens at the 2,6-positions (**1c**, **3c**, **4c** and **5**) were synthesized for evaluation as PDT agents, and BODIPY **6** was provided by Prof. Zhen Shen of Nanjing University, **Figure 12** [22]. Two novel porphyrin derivatives, **7a** and **7b**, were synthesized for evaluation as PDT agents. Porphyrin **8** [55] was used for PDT as a control to evaluate the effect of the acenaphthyl substituents of **7a** and **7b**.

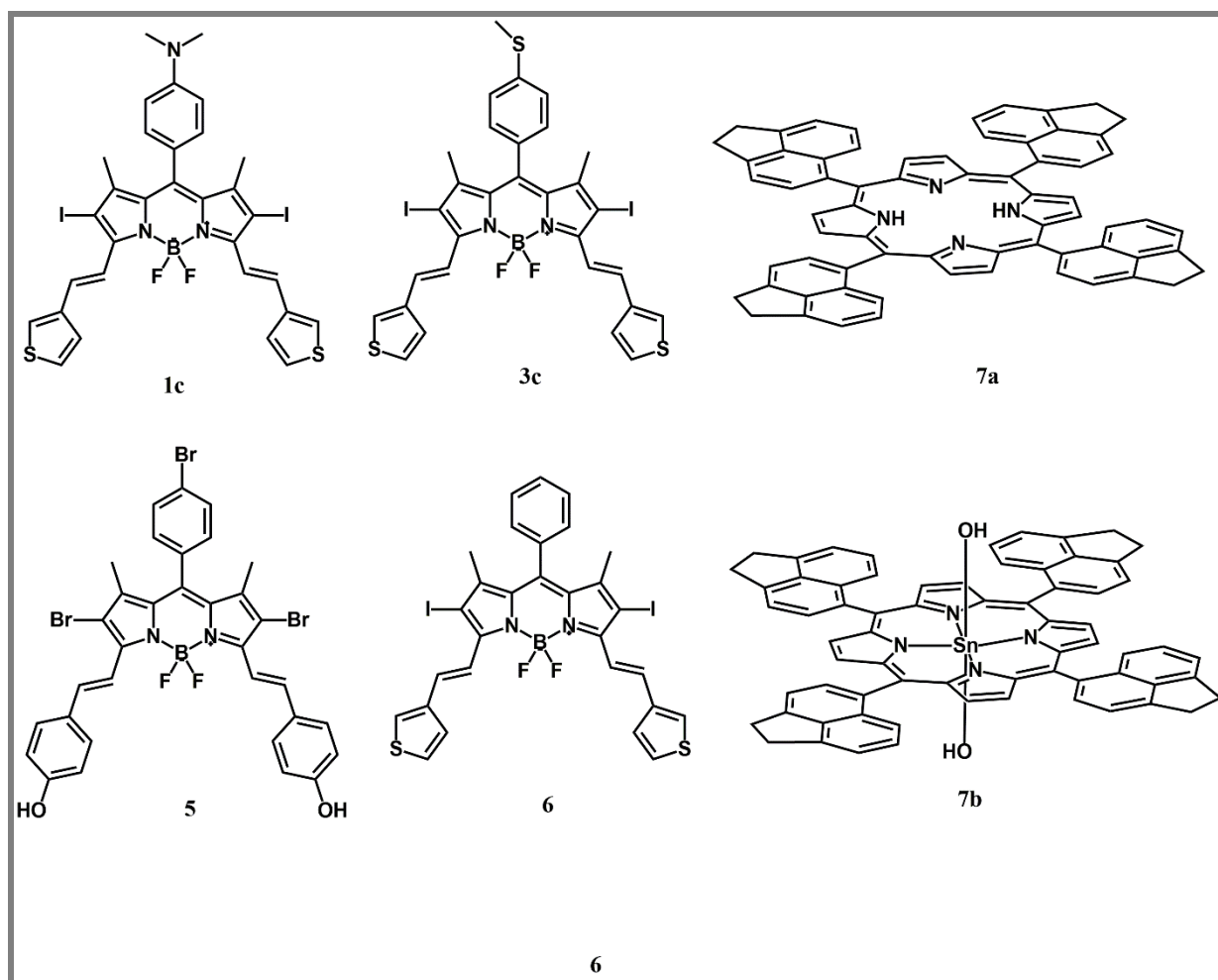


Figure 12. Photosensitizers used for PDT; BODIPY dyes **1c**, **3c**, **5** and **6**; porphyrins **7a** and **7b**.

1.8.3. Antimicrobial photoinactivation

PACT has been accepted as an alternative antimicrobial treatment since microbes are unable to develop resistance to it. This treatment is now often preferred over antibiotics due to the multidrug resistance of several gram-positive and gram-negative bacteria to almost all antibiotics [25, 31]. Examples of these pathogenic bacteria are *Enterococcus faecium*, *Neisseria gonorrhea*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* [25, 125]. *In vitro* studies have demonstrated the effectiveness of photoinactivation of various bacterial strains [21-23]. Due to

the nature of pathogenic microbes, the ideal PS dye is required to have low phototoxicity in the dark. Cationic charges tend to be necessary to enable the photoinactivation of gram-negative bacteria, while neutral PS dyes have been effective in eradicating gram-positive microbes [16]. PS dyes used for PACT can absorb in either the UV-visible or NIR regions [16, 90, 125]. The efficiency of the photoinactivation is primarily dependent on the Φ_{Δ} value of the PS dye. The mechanism of PACT in terms of the photophysical processes involved is essentially identical to that of PDT (**Figures 5 and 11**).

1.8.4. BODIPY dye synthesized for PACT

A 2,6-diiodinated-3,5-divinyleneBODIPY (**4c**) was synthesized for evaluation as PACT agent, **Figure 13**.

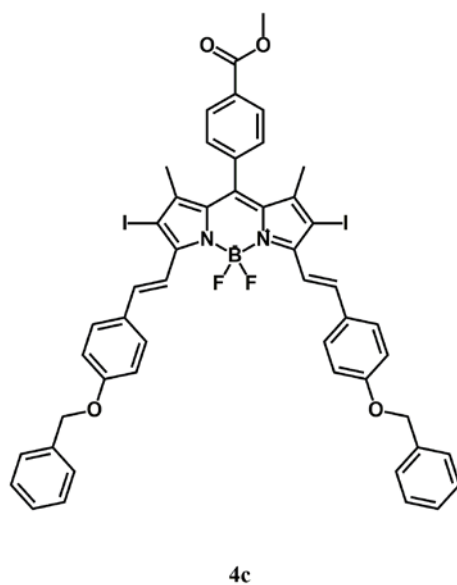


Figure 13. BODIPY dye **4c** synthesized for PACT studies.

1.9. Photosensitizers in PDT

Photofrin[®] was the first photosensitizer to be approved by the FDA in the 1990s [110]. It is a first-generation PS along with hematoporphyrin derivative (HpD). This drug formulation is comprised of mixtures of monomeric, dimeric and oligomeric porphyrin derivatives. Photofrin[®] was clinically approved for treatment of lung cancer and esophageal cancers amongst others. It has relatively weak absorption at 630 nm in the biological window but a very high Φ_{Δ} value of 0.89 which is desirable for PDT agents [110]. Unfortunately, Photofrin[®] leaves the skin photosensitized for prolonged periods and patients are required to avoid sunlight for up to ten weeks. This limitation motivated scientists to develop new PS dyes and derivatives of Photofrin[®] such as Photogem[®] [124]. Some of the clinically approved photosensitizers are listed in **Table 1**.

Table 1. Spectroscopic and physicochemical properties of photosensitizers in clinical trials and clinically approved PDT photosensitizers.

Photosensitizer	λ_{abs} (nm)	ϵ ($\text{M}^{-1} \text{cm}^{-1}$)	Φ_{Δ}	Ref.
Photophrin [®] (Porfimer sodium)	630	30,000	0.25 (PB + 1% TX100; 630 nm; oxygen depletion with FFA	[129]
Photofrin [®] (Hematoporphyrin)	630	1,170	0.28 (methylene blue, lysozyme inactivation)	[130]
Levulan [®] (Protoporphyrin IX)	635	5,000	0.54	

Laserphyrin® (Talaporfrin)	654 (PBS)	4,000 (PBS)	0.77 (D ₂ O, oxygen depletion with FFA)	[123]
Visudyne® (Verteporfrin)	668 (PBS + 2% TX100)	31,200 (PBS + 2% TX100)	0.82 (PB + 1% TX100; 692 nm; lysozyme inactivation; MB at 0.52)	[123]
Tookad® (Palladium bacteriopheophorbide (WST11))	763	88,000	0.50 (clinical trial for prostate cancer)	[131]
Photosens® (Aluminium phthalocyanine tetrasulfonated (AlPcS4))	676	200,000	0.38	[132]
Silicon phthalocyanine (Pc4)	675	200,000	Clinical trial	[132]

Second generation photosensitizers were motivated by the limitations of the first generation photosensitizers [49, 57]. The second generation photosensitizers include porphyrin analogue PS dyes such as phthalocyanines, chlorins and non-porphyrin photosensitizers like phenothiazines and curcuminoids [49, 57]. Some of these PS dyes were also found to have prolonged phototoxicity with porphyrin analogue dyes being generally better suited for use in PDT than

non-porphyrin based ones [49, 57]. Some of these have been clinically approved while others are currently in clinical trials [49, 57, 126] (**Table 1**). The use of porphyrins as PDT agents has continued to be studied, and various drug delivery strategies have been devised to limit the side-effects of these PS dyes [87, 127]. Following the recent work carried out by Mack and coworkers [55] on axially-ligated Sn(IV) tetrathienylporphyrin complexes, in which a marked red-shift of the porphyrin Q-bands into the therapeutic window and a significant PDT effect *in vitro* was observed [55], a novel Sn(IV) tetraacenaphthylporphyrin dye was synthesized in this study with the aim of shifting the Q-bands further to the red to improve the PDT activity.

The prolonged cytotoxicity of porphyrin-based PS dyes has led to researchers focusing on non-porphyrin-based PS dyes which are easier to synthesize and modify and have less significant side-effects [123, 128]. In comparison with porphyrins, only a minimal level of research has been carried out on the suitability of BODIPY dyes as a new class of PDT agents. Several review papers have covered various functionalities and derivatives of PDT-based BODIPY dyes with moderate to high Φ_{Δ} values [27, 123]. **Figure 14** shows some of the BODIPY dyes that have been studied as PDT agents and their spectroscopic properties. The potential utility of hydrophobic BODIPY dyes as PDT agents prompted the research in this study on the use of Pluronic[®] F-127 encapsulation micelles and cyclodextrin inclusion complexes as delivery agents, which will be the main focus of this thesis.

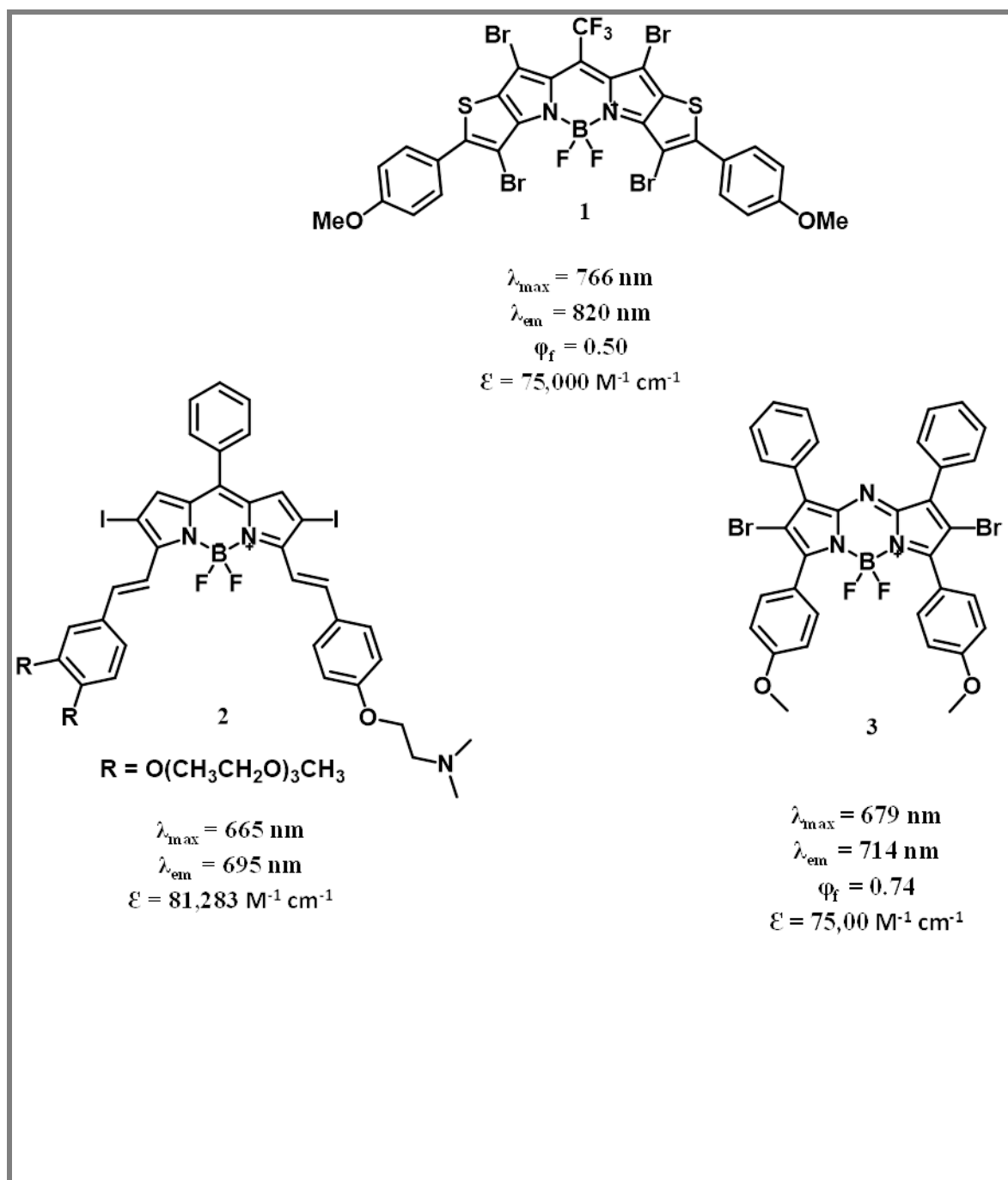


Figure 14. BODIPY (1 and 2) and aza-BODIPY (3) dyes that have previously been reported as PDT agents [26, 121].

1.10. Aims

The aims of this study can be summarized as follows:

1. Synthesis, characterization and photophysical studies of 2,6-diiodinated-3,5-divinyl-BODIPYs for photodynamic therapy.
2. Preparation of 2,6-diiodinated-3,5-divinyleneBODIPYs encapsulated in Pluronic[®] F-127 micelles to afford water-soluble dyes for photodynamic therapy.
3. Preparation of 2,6-diiodinated-3,5-divinyleneBODIPY/ β -CDs and γ -m β -CDs inclusion complexes to afford water-soluble dyes for photodynamic therapy studies.
4. Synthesis of novel tetraacenaphthylporphyrin derivatives and their encapsulation with β -CDs and m β -CDs.
5. *In vitro* photodynamic therapy studies on MCF-7 breast cancer cell with BODIPY and porphyrin dyes along with their cyclodextrin inclusion complexes or PF-127 micelles encapsulation complexes.
6. *In vitro* photodynamic antimicrobial therapy studies with a 2,6-iodinated BODIPY dye and its cyclodextrin inclusion complexes using *Staphylococcus aureus* as the bacterial strain of choice.
7. Molecular modeling studies to identify trends in the electronic structures and optical properties of the BODIPY and porphyrin dyes.

1.11. Publications:

Some of the results discussed in this thesis have already been published in peer-reviewed journals.

1. **N. Molupe**, B. Babu, D.O. Oluwole, E. Prinsloo, J.Mack, T. Nyokong. The investigation of *in vitro* dark cytotoxicity and photodynamic therapy effect of 2,6-dibromo-3,5-distyryl BODIPY dye encapsulated in Pluronic[®] F-127 micelles, *Journal of Coordination Chemistry*, (2018) 1-14.
2. B.P. Ngoy, **N. Molupe**, J. Harris, G. Fomo, J. Mack, T. Nyokong, Photophysical studies of 2,6-dibrominated BODIPY dyes substituted with 4-Benzylloxystyryl substituents, *Journal of Porphyrins and Phthalocyanines*, 21 (2017) 431-438.

Chapter 2: Experimental

2.1 Materials

2,4-Dimethylpyrrole, triethylamine (TEA), boron trifluoride diethyl etherate ($\text{BF}_3 \cdot \text{Et}_2\text{O}$), trifluoroacetic acid (TFA), 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ), 2,3,5,6-tetrachloro-*p*-benzoquinone (*p*-choranil), *N*-iodosuccinimide (NIS), *N*-bromosuccinimide (NBS), 4-(dimethylamino)benzaldehyde, 4-(pyridine)benzaldehyde, magnesium sulfate anhydride, 5-acenaphthenecarboxaldehyde, tin(II)chloride dehydrate, *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide (EDC), hydrochloride, folic acid, chitosan, 1,3-diphenylisobenzofuran (DPBF), anthracene-9,10-*bis*-methylmalonate (ADMA), 3-thiophenecarboxyaldehyde, 4-methylthiobenzaldehyde, methyl 4-formyl benzoate, sodium thiosulfate and ZnPc were obtained from Sigma-Aldrich. Pluronic[®] F-127, β -CD and methyl β -CD were purchased from Sigma-Aldrich and used without purification. All other reagents obtained from commercial suppliers were used without further purification unless otherwise stated.

4-Benzyloxybenzaldehyde was donated by Dr Bokolombe Ngoy, and its synthesis has been reported previously [47]. Hydrochloric acid, pyrrole, propionic acid, methanol, ammonia solution and the silica gel used for chromatography were purchased from Merck. Dimethylsulfoxide (DMSO), petroleum ether, ethyl acetate, glacial acetic acid and piperidine purchased from Sigma-Aldrich. Dichloromethane (DCM) was obtained from Saarchem. Ultrapure water was obtained from a Milli-Q Water System (Millipore Corp, Bedford, MA, USA).

All atmospheric air and moisture sensitive reactions were carried out under argon gas, and dry solvents were used. Cultures of MCF-7 cells were obtained from Cellonex[®]. Heat-inactivated

fetal calf serum (FCS) and 100 unit/mL penicillin-100 µg/mL streptomycin-amphotericin B were obtained from Biowest[®]. Dulbecco's phosphate-buffered saline (DPBS) and Dulbecco's modified Eagle's medium (DMEM) were obtained from Lonza[®]. Cell proliferation neutral red reagent (WST-1 assay) and trypan blue were obtained from Sigma-Aldrich. *Staphylococcus aureus* (ATCC 25923) was obtained from Davies Diagnostic. PBS solution (pH 7.4) was prepared using appropriate amounts of Na₂HPO₄ and NaOH in ultrapure water. Nutrient agar and agar bacteriological BBL Muller Hinton broth were purchased from Merck.

2.2. Equipment

- Ground state electronic absorption spectra were measured on a Shimadzu UV-2550 spectrophotometer.
- Fluorescence emission spectra were recorded on a Varian Eclipse spectrofluorimeter.
- Fluorescence lifetimes were measured using a time-correlated single photon counting (TCSPC) spectrometer (FluoTime 300, Picoquant GmbH).
- ¹H nuclear magnetic resonance (NMR) measurements were recorded on a Bruker AMX 600 MHz.
- Mass spectral data were acquired on a Bruker Auto-FLEX III Smartbeam MALDI-TOF mass spectrometer operated in positive ion mode using α-cyano-4-hydroxycinnamic acid as a matrix.
- Fourier transform-infrared (FT-IR) spectra were obtained from a Bruker Alpha FT-IR spectrometer with a universal attenuated total reflectance (ATR) sampling accessory.

- Monochromatic laser light between 400–700 nm from an Ekspla NT 342B-20-AW pulsed laser (2.0 mJ/5 ns, 20 Hz) was used for quantifying the singlet oxygen quantum yields of compounds with DPBF or ADMA as a $^1\text{O}_2$ quencher.
- Powder X-ray diffraction (XRD) data were obtained from a Bruker D8 Diffractometer fitted with a Lynx Eye detector under Cu K α ($\lambda=1.5405 \text{ \AA}$) with 192 s per step scanning rate. Zero background silicon wafer slide were used as sample holders.
- A Zeiss Libra model 120 transmission electron microscope operated at 90 kV was used to assess the sizes and morphologies of the micelles and the encapsulation complexes.
- Dynamic light scattering (DLS) and zeta-potential (ζ -potential) analyses were performed on a Malvern Zetasizer Nano series, Nano-ZS90.
- The Centre for High Performance Computing in Cape Town was used to carry out the theoretical calculations. All calculations were carried out using Gaussian 09 software package [133] running on an Intel/Linux. Beck, three-parameter, Lee-Yang-Parr (B3LYP) level of theory and SDD basis sets were used for the geometry optimization. The time-dependent DFT (TD-DFT) method was used to calculate the electronic absorption properties with the Coulomb attenuated B3LYP (CAM-B3LYP) functional and SDD basis sets. The molecular orbitals were visualized on Avogadro [134].
- The MCF-7 cells were cultured in 25 cm² vented flasks (Corning) then subcultured in 75 cm² vented flasks (Corning) in a humidified atmosphere incubator with 5% CO₂ at a physiological temperature of 37°C (Heal Force). A Zeiss-AxioVert. A Fluorescence LED (FL-LED) inverted microscope was used to view the cells under phase contrast. WST-1 cell proliferation neutral red reagent (Roche) was used to measure the cell viability through a Synergy 2 multi-mode microplate reader (BioTek®). PDT studies were

conducted using a Modulight[®] Medical Laser system (ML) 7710-680 fitted with a Thorlab M660L4 LED. A Fluorescence LED (FL-LED) inverted microscope was used to view the cells under phase contrast (Zeiss-AxioVert).

- The optical density of bacteria culture was determined using the Ledetect 96 microplate reader from Labxim Products.
- A China Medical Device RAU-530D autoclave was used to sterilize and autoclave nutrient broth and agar, PBS buffer and the apparatus used for bacterial and cancer studies.
- A Vortex mixer from Lasiec was used to mix the bacteria suspension
- A Hermle Z233M-2 centrifuge from Lasiec was used to harvest bacteria from the suspension.
- A Scan[®] 500 automatic colour colony counter was used for determination of colony forming units (CFU)/ml of bacteria.

2.3. Syntheses of BODIPY dyes

2.3.1. 4,4'-Difluoro-1,3,5,7-tetramethyl-8-(4-dimethylamino)-4-bora-3a,4a-diaza-s-indacene (**1a**)

4-(Dimethylamino)benzaldehyde (1.052 g, 7.053 mmol) and 2,4-dimethylpyrrole (1.342 g, 14.11 mmol) were dissolved in dry DCM (30 ml) under argon flow [38, 47]. TFA (0.4 ml) was added to the reaction mixture, and the reaction vessel was left to stir at room temperature. The reaction progress was monitored by TLC. When the aldehyde was consumed, the temperature was lowered to 0°C and *p*-chloranil (1.734 g, 7.053 mmol). This was added followed by stirring for 20 min at room temperature, TEA (4.282 g, 42.32 mmol) was added, and the mixture was left to stir for a further 10 min. At 0°C, BF₃·OEt₂ (10.17 g, 70.53 mmol) was added dropwise over 30 min. The mixture was stirred for 16 h. The solution was washed with deionized water, extracted with DCM then dried over anhydrous sodium thiosulphate. The solvent was removed *in vacuo*. The residue was purified by silica column chromatography by eluting with petroleum ether/ ethyl acetate (9:1).

1a: obtained as an orange crystalline solid in 73% yield: ¹H NMR (600 MHz, DMSO-*d*₆) δ 7.11 (d, *J* = 6.3 Hz, 2H), 6.85 (d, *J* = 7.12 Hz, 2H), 6.16 (s, 2H), 2.98 (s, 6H), 6.17 (s, 6H), 1.45 (s, 6H) ppm.

2.3.2. 4,4'-Difluoro-1,3,5,7-tetramethyl-8-(4-pyridine)-4-bora-3a,4a-diaza-s-indacene (**2a**)

The procedure outlined for **1a** was used with the following reagents [38, 47]: 4-(Pyridine)benzaldehyde (0.8795 g, 9.336 mmol) and 2,4-dimethylpyrrole (1.776 g, 18.67 g,

mmol), dry DCM (30 ml), TFA (0.4 ml), *p*-chloranil (2.296 g, 9.336 mmol), TEA (5.668 g, 56.02 mmol), $\text{BF}_3 \cdot \text{OEt}_2$ (13.24 g, 93.36 mmol).

2a: obtained as an orange crystalline solid in 76% yield. ^1H NMR (600 MHz, CDCl_3) δ 7.35 (s, 2H), 7.28 (s, 2H), 6.03 (s, 2H), 2.58 (s, 6H), 1.42 (s, 6H) ppm

2.3.3. 4,4'-Difluoro-1,3,5,7-tetramethyl-8-(4-methylthio)-4-bora-3a,4a-diaza-s-indacene (**3a**)

The procedure outlined for **1a** was used with the following reagents: 4-(Methylthio)benzaldehyde (0.8795 g, 9.336 mmol), 2,4-dimethylpyrrole (1.776 g, 18.67 g, mmol), TFA (0.4 ml), *p*-chloranil (2.296 g, 9.336 mmol), TEA (5.668 g, 56.02 mmol), $\text{BF}_3 \cdot \text{OEt}_2$ (13.24 g, 93.36 mmol).

3a: ^1H NMR (600 MHz, CDCl_3) δ 7.36 (d, J = 6.0 Hz, 2H), 7.22 (d, J = 6.0 Hz, 2H), 6.00 (s, 1H), 2.07 (s, 3H), 1.61 (s, 6H), 0.90 (s, 6H) ppm.

2.3.4. 4,4'-Difluoro-1,3,5,7-tetramethyl-8-(4-methylbenzoate)-4-bora-3a,4a-diaza-s-indacene (**4a**)

The procedure outlined in for **1a** was used with the following reagents: 4-Methylbenzoate (1.127 g, 6.325 mmol), 2,4-dimethylpyrrole (1.204 g, 12.65 mmol), TFA (0.4 ml), *p*-chloranil (1.555 g, 6.325 mmol), TEA (3.840 g, 37.95 mmol), $\text{BF}_3 \cdot \text{OEt}_2$ (8.976 g, 63.25 mmol).

4a: obtained as an orange crystalline solid in 81% yield. ^1H NMR (600 MHz, CDCl_3) δ 8.20 (d, J = 6.0 Hz, 2H), 7.43 (d, J = 6.0 Hz, 2H), 6.01 (s, 2H), 3.98 (s, 3H), 2.58 (s, 6H), 1.38 (s, 6H) ppm.

2.3.5. 4,4'-Difluoro-1,3,5,7-tetramethyl-2,6-diiodo-8-(4-dimethylamino)-4-bora-3a,4a-diaza-s-indacene (**1b**)

1a was iodinated following literature procedures [43, 47]. **1a** (0.5289 g, 1.227 mmol) was dissolved in 30 ml of dry DCM. The solution was degassed and 2 equiv. NIS (0.8281 g, 3.680 mmol) in DCM (5 ml) was added dropwise over 30 min with stirring. The reactions then proceeded for 8 h (monitored by TLC). The solution was washed with a 1 M sodium thiosulfate solution and the product was extracted with DCM. The extracted product solution was filtered through anhydrous magnesium sulfate then dried *in vacuo*. The products were isolated by silica column chromatography with ethyl acetate/ hexane (v/v; 1:4) used as the eluent.

1b: obtained as a dark pink crystalline product in 42% yield: ^1H NMR (600 MHz, CDCl_3) δ 7.03 (d, 6.21 Hz, 2H), 6.77 (d, 7.23 Hz, 2H), 3.04 (s, 6H), 2.63 (s, 6H), 1.50 (s, 6H) ppm. MALDI-TOF-MS: Calculated: 619.04 amu; Found: 618.34 m/z [M^+].

2.3.6. 4,4'-Difluoro-1,3,5,7-tetramethyl-2,6-diiodo-8-(4-pyridine)-4-bora-3a,4a-diaza-s-indacene (**2b**)

The iodination procedure outlined for **1b** was used. The following reagents were used: **2b** (0.4024 g, 1.096 mmol), NIS (0.7396 g, 3.287 mmol).

2b: obtained as a dark pink crystalline product in 73% yield. ^1H NMR (600 MHz, CDCl_3) δ 7.43 (s, 2H), 7.41 (s, 2H), 2.76(s, 6H), 1.53 (s, 6H) ppm. MALDI-TOF MS: Calculated: 576.95 amu; Found: 576.40 m/z [M^+].

2.3.7. 4,4'-Difluoro-1,3,5,7-tetramethyl-2,6-diiodo-8-(4-methylthio)-4-bora-3a,4a-diaza-s-indacene (**3b**)

The iodination procedure outlined for **1b** was used. The following reagents were used: **3a** (0.3011 g, 0.8135 mmol), NIS (0.5490 g, 2.440 mmol).

3b: obtained as a dark pink crystalline product in 65% yield. ^1H NMR (600 MHz, CDCl_3) δ 7.38 (d, $J = 7.2$ Hz, 2H), 7.18 (d, $J = 10.8$ Hz 2H), 2.58 (s, 3H), 1.47 (s, 6H), 1.27 (s, 6H) ppm. MALDI-TOF-MS: Calculated: 621.94 amu; Found: 623.04 m/z $[\text{M}+\text{H}]^+$.

2.3.8. 4,4'-Difluoro-1,3,5,7-tetramethyl-2,6-diiodo-8-(4-methylbenzoate)-4-bora-3a,4a-diaza-s-indacene (**4b**)

The iodination procedure outlined for **1b** was used. The following reagents were used: **4a** (0.5293 g, 1.385 mmol), NIS (0.9347 g, 4.155 mmol).

4b: obtained as a dark pink crystalline product in 78% yield. ^1H NMR (600 MHz, CDCl_3) δ 8.23 (d, $J = 6.0$ Hz, 2H), 7.41 (d, $J = 6.0$ Hz, 2H), 4.01 (s, 3H), 2.67 (s, 6H), 1.39 (s, 6H) ppm. MALDI-TOF MS: Calculated: 633.96 amu; Found: 635.98 m/z $[\text{M}+2\text{H}]^+$.

2.3.9. 4,4'-Difluoro-1,7-tetramethyl-3,5-(3-dithiophene)-2,6-diiodo-8-(4-dimethylamino)-4-bora-3a,4a-diaza-s-indacene (**1c**)

3,5-distyryl BODIPY was synthesized using a modified Knoevenagel condensation method [47, 71]. **1b** (0.1194 g, 0.1905 mmol) and 3 equiv. of 3-thiophenecarboxaldehyde (0.06408 g, 0.5715 mmol) were mixed and dissolved in dry benzene (20 ml). Glacial acetic acid (0.5 ml) and piperidine (0.5 ml) were added under reflux, and a Dean-Stark trap was used. The reaction was monitored by absorption spectroscopy until BODIPY **1b** was fully consumed. The mixture was

washed with 1 M hydrochloric acid and dried *in vacuo*. The residue was purified by silica chromatography using petroleum ether/ethyl acetate (4:1).

1c: obtained as a blue product in 40% yield. ^1H NMR (600 MHz, CDCl_3) δ 8.17 (d, $J = 16.8$ Hz, 2H), 7.54 (s, 8H), 7.41 (s, 4H), 3.18 (s, 6H), 1.51 (s, 6H) ppm. MALDI-TOF MS: Calculated: 807.30 amu; Found: 807.33 m/z [M^+].

2.3.10. 4,4'-Difluoro-1,7-tetramethyl-3,5-(3-dithiophene)-2,6-diiodo-8-(4-methylthio)-4-bora-3a,4a-diaza-s-indacene (**3c**)

3c was obtained following the procedure outlined in section 2.3.9. The reagents used were as follows: **3b** (0.1420 g, 0.2287 mmol), 3-thiophenecarboxaldehyde (0.0768 g, 0.6848 mmol), acetic acid (0.75 ml) and piperidine (0.75 ml).

3c: obtained as a green product in 39% yield. ^1H NMR (600 MHz, CDCl_3) δ 8.17 (d, $J = 16.4$ Hz, 2H), 7.53 (s, 6H), 7.40 (s, 4H), 7.21 (d, $J = 7.4$ Hz, 2H), 2.59 (s, 3H), 1.53 (s, 6H). MALDI-TOF MS: Calculated: 809.92 amu; Found: 810.25 m/z [M^+].

2.3.11. 4,4'-Difluoro-1,7-tetramethyl-3,5-(4-dibenzyloxybenzene)-2,6-diiodo-8-(4-methylbenzoate)-4-bora-3a,4a-diaza-s-indacene (**4c**)

4c was obtained following the procedure in section 2.3.9. The reagents used were as follows: **4b** (0.157 g, 0.2469 mmol), 4-benzyloxy benzaldehyde (0.1779 g, 0.7406 mmol), acetic acid (0.85 ml) and piperidine (0.85 ml).

4c: obtained as a green product in 91% yield. ^1H NMR (600 MHz, CDCl_3) δ 8.24 (d, $J = 7.0$, 2H), 8.16 (d, $J = 16.6$, 2H), 7.63 (t, $J = 15.1$ Hz, 6H), 7.48 (d, $J = 6.5$, 4H), 7.44 (d, $J = 7.3$, 6H), 7.37 (d, $J = 6.4$, 2H), 7.06 (d, $J = 7.5$, 4H), 5.16 (s, 4H), 4.02 (s, 3H), 1.59 (s, 6H). MALDI-TOF MS: Calculated: 1022.11 amu; Found: 1022.64 m/z [M^+].

2.3.12. 4,4'-Difluoro-1,7-tetramethyl-3,5-(4-hydroxyphenyl)-2,6-dibromo-8-(4-bromophenyl)-4-bora-3a,4a-diaza-s-indacene (**5**) and 4,4'-difluoro-1,7-tetramethyl-3,5-(2-thiophene)-2,6-dibromo-8-(phenyl)-4-bora-3a,4a-diaza-s-indacene (**6**).

BODIPY **5** was previously reported by Ngoy et al. [47] and BODIPY **6** was provided by Prof. Zhen Shen of Nanjing University [22].

5: obtained as a green product in 74%: ^1H NMR (600 MHz, THF- d_8) δ 8.31 (d, J = 16.6 Hz, 2H), 7.96 (d, J = 8.2 Hz, 2H), 7.79 (d, J = 16.6 Hz, 2H), 7.68 (d, J = 8.5 Hz, 5H), 7.56 (d, J = 8.2 Hz, 2H), 7.00 (d, J = 8.5 Hz, 5H), 1.67 (s, 6H) ppm. MALDI-TOF MS: Calculated: 770.9 amu; found: 771.1 m/z [M^+].

6: obtained as a green product in 78% yield. ^1H NMR (600 MHz, THF- d_8) δ 8.44 (d, J = 16.4 Hz, 2H), 7.62 (dd, J = 8.2, 6.7 Hz, 4H), 7.59–7.55 (m, 3H), 7.47–7.44 (m, 2H), 7.37 (d, J = 3.5 Hz, 2H), 7.13 (dd, J = 5.0, 3.7 Hz, 2H), 1.51 (s, 6H). MALDI-TOF MS: Calculated: 764.24 amu; Found: 764.45 m/z [M^+].

2.4. Synthesis of free-base and Sn(IV) tetraacenaphthylporphyrin

2.4.1. Free base tetraacenaphthylporphyrin (**7a**)

7a was synthesized following a modified literature procedure [73, 135]. 5-acenaphthenecarboxaldehyde (1.024 g, 5.620 mmol) and distilled pyrrole (0.390 ml, 5.620 mmol) in 200 mL of propionic acid were heated to reflux for 4 h. The reaction mixture was cooled and filtered then washed with cold methanol. The solid obtained was purified by silica column chromatography using chloroform as the eluent to give a solid crystalline product.

7a: obtained as a black solid in 80% yield. ^1H NMR (600 MHz, CDCl_3) δ 8.61–8.50 (m, 4H), 8.19 (dt, $J = 14.6, 7.7$ Hz, 4H), 7.63 (d, $J = 6.6$ Hz, 4H), 7.17–7.14 (m, 4H), 7.01–6.87 (m, 4H), 3.72–3.64 (m, 16H), –2.30 (s, 2H). MALDI-TOF MS: Calculated: 918.37 amu; Found: 919.79 $[\text{M}+\text{H}^+]$.

2.4.2. *Sn(IV) tetraacenaphthylporphyrin (7b)*

The metallation of porphyrin with tin was carried out by following a previously reported procedure [73]. **7a** (0.7192, 0.782 mmol) and $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ (0.1764 g, 1.564 mmol) were dissolved in 50 ml pyridine and refluxed for 12 h. The reaction mixture was cooled and ammonia solution (20 ml) carefully added then refluxed for an additional 1 h. The reaction mixture was cooled and 100 ml of water was added. The green precipitate formed was filtered and washed with water. The filter cake was dissolved in chloroform, filtered and dried to give **7b** as a green solid.

7b: obtained as a black solid in 83% yield. ^1H NMR (600 MHz, CDCl_3) δ 8.89–8.85 (m, 8H), 8.45–8.26 (m, 4H), 7.71–7.68 (m, 4H), 7.21–7.14 (m, 12H), 3.70 (dd, $J = 33.8, 4.6$ Hz, 16H). MALDI-TOF MS: Calculated: 1070.25 amu; Found: 1072.49 $[\text{M}+2\text{H}]^+$.

2.5. Functionalization of chitosan with folic acid

The functionalization procedure was adapted from Yang et al. [74]. Folic acid (1.3942 g, 3.159 mmol) and EDC (0.6055 g, 3.159 mmol) were dissolved in anhydrous DMSO (20 ml) and stirred at room temperature for 24 h to activate the carboxylic acid moiety of the folic acid. The activated folic acid was gradually added to 0.5% (w/v) chitosan in an acetic acid solution (0.1 M, pH 4.7) with constant stirring for 16 h in the dark to allow for amide bond formation between the folic acid and chitosan. The solution was adjusted to pH 9.0 (using 1.0 M aq. NaOH) then

centrifuged at 2500 rpm to precipitate out the folate-functionalized chitosan. The precipitate was dialyzed in phosphate buffered saline (PBS, pH 7.4) for 3 days then deionized water for 4 days. The folate-functionalized chitosan solid was obtained through freeze-drying. Yield: 76%. IR cm^{-1} : 1510 (amide I), 1602 (amide II).

2.5.1. Preparation of the Pluronic[®] F127 Micelles encapsulated BODIPY and BODIPY encapsulated in Folate-Functionalized Chitosan-Coated Pluronic[®] F127 Micelles

A previously reported thin-film hydration method was used with some modifications for the incorporation of dyes into Pluronic[®] F-127 micelles [81, 87]. Each dye was co-solubilized with Pluronic[®] F-127 (1/40: w/w) in DCM (5 ml), and the solution was sonicated for 15 min. Then, the solution was dried *in vacuo* for 1 h at 50 °C and dried for 12 h in a desiccator. The solid obtained was hydrated with Millipore water (5 ml) and sonicated for 15 min then further stirred for 2 h. The solution was filtered through a 0.2 μm filter to remove unloaded dye molecules. The solution was then freeze-dried for 2 days to obtain solid inclusions. Micelles with no encapsulated BODIPY dyes were prepared with a similar protocol.

For further modification with folate-functionalized chitosan, the dye-Pluronic[®] F-127 Micelles solution was not freeze dried. Instead, 0.04% (w/v) solution of sodium dodecyl sulfate (SDS) was added to the dye-micelle solution and left for 1 h to form closely packed micelles [81]. The solution was filtered through a 0.2 μm filter to remove unloaded dye molecules. Micelles with no encapsulated BODIPY dyes were prepared with a similar protocol [81].

The dye-micelle solution (core) was mixed slowly with 0.005% (w/v) solution of folate-functionalized chitosan in 0.4 M acetate buffer with pH 4.7 (dye-micelle solution: folate-functionalized chitosan solution = 1:1) for 20 min. The resultant solution was dialyzed for 3 days

against deionized water to remove excess SDS and BODIPY dye and the acetate buffer. The solution was then freeze-dried for 2 days to obtain solid inclusion complexes.

2.6. Preparation of solid inclusion complexes

2.6.1. Complexation methods used for BODIPY **4c** and Sn(IV) porphyrin **7b**/βCD inclusion complexes

Thermogravimetric analysis was first used to determine the water content of BODIPYs **4c**, **7b**, βCD and mβCD. Both dyes had 3% while βCD and mβCD had 13% and 0% water respectively, **Figure 15**. The method followed for the complexation of βCD and BODIPY dyes has been described by Miclea et al. [136] and Hedges [88], and was followed with some modifications. The amount of water in each sample was accounted for in calculating the molar ratios of the dye and cyclodextrin. Since CDs are spectroscopically transparent, UV-visible absorption spectroscopy can also be readily used in this context. Molar ratios of 1:3 for **4c** to either βCD or mβCD and 1:4 for **7b** to βCD or mβCD were used during the initial complexation.

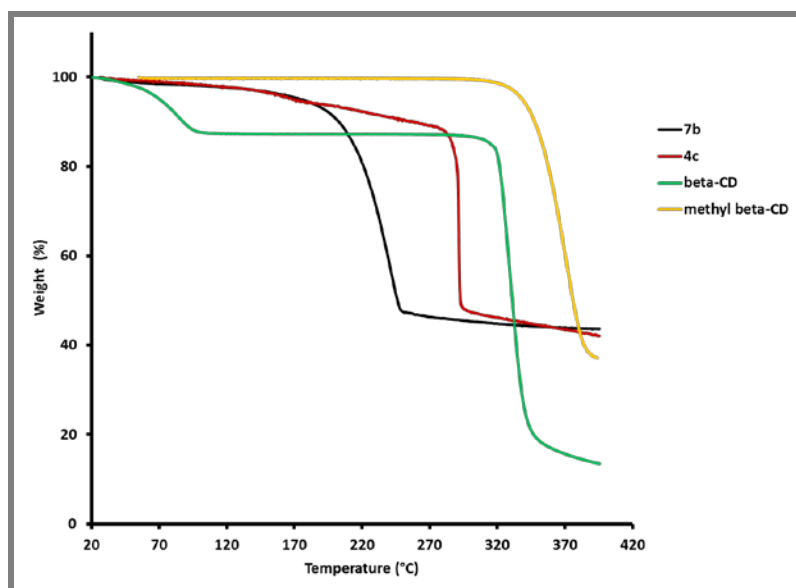


Figure 15. Thermogravimetric graphs showing the water content of BODIPYs **4c** and **7b**, β CD and m β CD.

Kneading method

β CD or m β CD was added to a ceramic mortar and wetted with a minimum amount of Millipore water and kneaded. The dye was added in small amounts. The slurry was kneaded for 30 min then recrystallized in water. The water was evaporated slowly and the crystals stored at room temperature for further use [136].

Ethanol-water system method

The modified complexation method was adapted from Miclea et al. [136]. β CD or m β -CD was dissolved in a minimum amount of water and heated up to 100°C. When the CDs were completely dissolved, BODIPY or porphyrin was dissolved in DMSO instead of ethanol and added gradually. The solutions were left to stir for 24–72 h then cooled to ambient temperature. The solutions were filtered and the product recrystallized in water. The solvents were allowed to

evaporate slowly, and the products were stored at room temperature for further use. Pictures of the complexation methods used are shown in **Figure 16**.

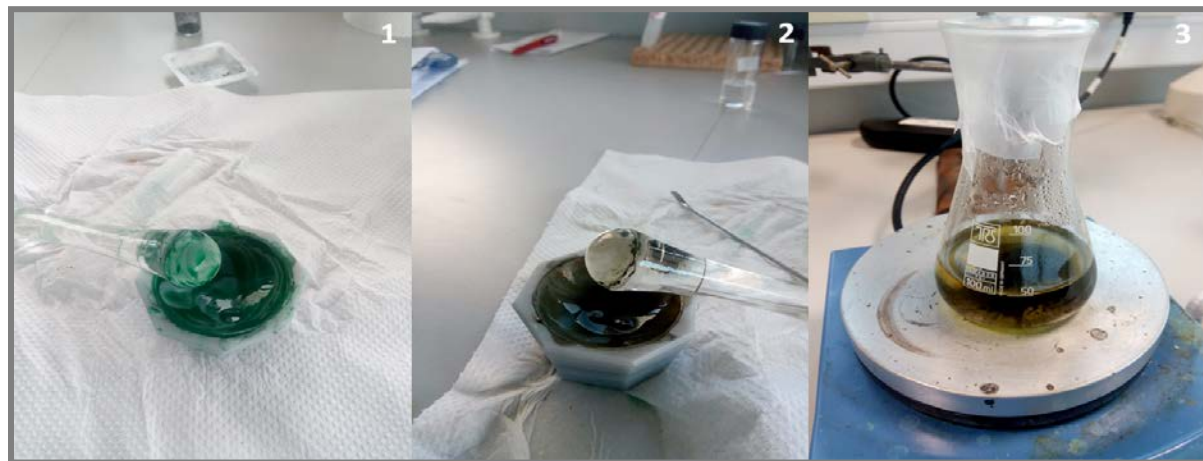


Figure 16. Pictures of BODIPY **4c** (1) and **7b** (2) with β CD (1 and 2 = Kneaded samples) and **7b** with β CD (3 = ethanol-water sample).

2.7. Phase solubility studies

The solubility studies were studied following a procedure reported by Hadžiabdić et al. [137]. The solutions with BODIPY dyes were prepared by mixing 3 mg of dye with Pluronic[®] F-127 at concentrations of 0, 5, 10, 15, 20, 25 and 30%. The samples were stirred at ambient conditions for 24 h for equilibrium to be reached. To confirm this, the samples were then stirred for a further 24 h, and the solubility of the dyes did not change. The equilibrated samples were filtered using a 0.2 μ m pore size membrane filter, and the concentration was determined with a Shimadzu UV-1601 spectrophotometer. For each experiment, three replicates were made. The solubility changes of the BODIPY dyes with the addition of various Pluronic[®] F-127 concentrations were plotted to obtain phase solubility diagrams and evaluate the stoichiometry, apparent stability constants ($K_{1:1}$) and complexation efficiencies (CE) of the resultant complexes.

The apparent stability constants were estimated from the linear line of the phase solubility curve using the following equation of Higuchi and Connors [138]:

$$K_{1:1} = \frac{\text{Slope}}{S_o(1-\text{Slope})} \quad (3)$$

Where $K_{1:1}$ is the apparent stability constant (L/mol), S_o is the saturation concentration of the drug molecule (BODIPY) in pure water and slope is the slope of the straight line from the linear equation. From the 1:1 BODIPY/Pluronic® F-127 encapsulation complexes, the complexation efficiency (CE) can be calculated from the slope of the phase-solubility diagram:

$$CE = \frac{\text{Slope}}{1-\text{Slope}} = S_o \cdot K_{1:1} \quad (4)$$

Where CE is the complexation efficiency and S_o is the saturation concentration of the drug molecule (BODIPY) in pure water.

2.8. Cell culture and in vitro studies on MCF-7 breast cancer cells

2.8.1. In vitro dark cytotoxicity

The cell culturing and *in vitro* studies were carried out as reported in the literature [55, 89]. *In vitro* cytotoxicity studies were performed in the dark by using human breast adenocarcinoma cell (MCF-7 cell) prior to *in vitro* photodynamic therapy studies. The MCF-7 cells were cultured using DMEM containing 4.5 g/L glucose with L-glutamine and Phenol red, supplemented with 10% (v/v) heat-inactivated FCS and 100 unit/mL penicillin-100 µg/mL streptomycin-amphotericin B. The cells were grown in 75 cm² vented flasks (Corning) and incubated at 37°C and 5% CO₂ with a humidified atmosphere. Routine subculturing using standard trypsinization was followed. Viable trypsinized cells were counted by following the trypan blue dye exclusion

assay (0.4%) with a hemocytometer. 10,000 cells/well were seeded in supplemented DMEM containing Phenol red in 96-well tissue culture plates (Porvair®). The cells were incubated at 37°C and 5% CO₂ for 24 h to facilitate cell attachment to the wells. The cells adhered cells were washed with 100 µl DPBS twice, followed by administration of 100 µl supplemented DMEM containing BODIPY 5 and BODIPY 5 encapsulated in Pluronic® F-127 micelles (5@PF127) at a range of different concentrations (5, 10, 20, 40 and 80 µg/mL). The encapsulation complexes of BODIPYs **3c** and **6** in folate-functionalized chitosan-coated Pluronic® F-127 micelles (**3c**@PF127@FC and **6**@PF127@FC) were prepared at concentrations of 3, 5, 10, 20 and 40 µg/mL. The stock concentrations of BODIPY dyes alone were prepared in 5% DMSO, followed by making the volume up with supplemented DMEM. The stock concentrations of BODIPY dyes in Pluronic® F127 micelles or functionalized chitosan-coated Pluronic® F127 micelles were prepared in Millipore water followed by making the volume up with supplemented DMEM. Placebo cells were incubated with supplemented DMEM only. After 24 h treatment, the wells were rinsed with 100 µL DPBS twice, supplemented DMEM with Phenol red was added and the plates re-incubated for 24 h. The WST-1 assay was used to quantify the viable cells in the monolayer of the cells treated with the drugs and the placebo cells, respectively. The quantification was carried out in accordance with the manufacturer's instructions using a Synergy 2 multi-mode microplate reader (BioTek®) at 450 nm. The percentage cell viability was determined using Eq. (5):

$$\% \text{cell viability} = \frac{\text{Absorbance sample at 450 nm}}{\text{Absorbance of control at 450 nm}} \times 100 \quad (5)$$

where the Absorbance sample at 450 nm is the absorbance value for the cells containing drugs, while Absorbance of control at 450 nm is the value for the placebo cells containing only supplemented DMEM with Phenol red.

A similar protocol was used for cells treated with porphyrin compounds **7a**, **7b** and **7b**/m β CD. A Sn tetraphenylporphyrin ((OH)₂SnTPP) control compound (**8**) was used to assess the effect of the acenaphthyl substituents of porphyrin **7b**. The MTT assay was used to assess the cytotoxicity of the porphyrin compounds on MCF-7 cancer cells. The details of the MTT assay protocol are outlined in 2.8.2.

2.8.2. *In vitro photodynamic therapy*

The MCF-7 cells treated for PDT were cultured and seeded into 96-well plates as described in the previous section. The stock concentrations of BODIPY dyes **3c**, **5**, and **6** were prepared in 5% DMSO, and the volume was made up with supplemented DMEM. The stock concentrations of **5**@PF127, **3c**@PF127@FC and **6**@PF127@FC were prepared in Millipore water, and the volume was made up with supplemented DMEM. Placebo cells were incubated with supplemented DMEM only. After 24 h treatment, the wells were rinsed with 100 μ L DPBS twice, supplemented DMEM with Phenol red was added, and the plates were re-incubated for 24 h. The plates were rinsed with 100 μ L PBS and Phenol red-free supplemented DMEM was added instead of supplemented DMEM with Phenol red. A fixed light dosimetry of 336 J/cm² was achieved by illuminating a set of plates for 20 min with a Thorlabs 660 nm LED. After illumination, the cells were rinsed with PBS then Phenol red-free supplemented DMEM was replaced with supplemented DMEM with Phenol red. Surviving cells were quantified after re-incubation with supplemented DMEM with Phenol red with the use of WST-1 assay after 24 h. Cell survival was expressed as a percentage of the values derived for the placebo cells following Eq. 5 above.

The MTT assay was used to assess the cytotoxicity of the porphyrin compounds on MCF-7 cancer cells [55]. Approximately 1×10^4 cells were seeded in 96-well plates in 100 μL media per well. Cells were grown for 24 h in a CO_2 incubator kept at 37°C . Concentrations (50, 25, 12.5, 6.25, 3.13, 0 $\mu\text{g/ml}$) of the compounds were dissolved in 1% DMSO then added to the cells. Incubation was continued for a further 24 h at 37°C in the CO_2 incubator. The medium was then replaced with 50 mM phosphate buffer (pH 7.4) containing 150 mM NaCl (PBS). The MCF-7 cancer cells plated in 96 wells and treated with the compounds were irradiated for 30 min using the illumination kit of a Modulight® 7710-680 Medical Laser fitted with a Thorlab M625L3 light emitting diode (LED) with an irradiance of 240 mW/cm^2 (measured with a Coherent FieldmaxII TOP energy/power meter fitted with a Coherent Powermax PM10 sensor). After irradiation, PBS was replaced with 10% DMEM and cells were left in the dark for 24 h followed by the addition of 25 μL of 4 mg mL^{-1} of MTT to each well then incubation for 3 h. The culture medium was discarded, and 200 μL of DMSO was added to dissolve the purple formazan crystals. A Synergy 2 multi-mode microplate reader (BioTek®) at an excitation wavelength of 540 nm was used to determine the number of viable cells after treatment. The cytotoxicity of the compounds was measured as the percentage ratio of the absorbance of the treated cells to the untreated controls. Nonlinear regression analysis (GraphPad Prism 5) was used to determine the IC_{50} values of the compounds.

2.9. Antimicrobial studies

Staphylococcus aureus was used for the PACT studies. *Staphylococcus aureus* was grown on agar plates following the manufacturer's specifications to obtain a colony of bacteria [90]. This colony was inoculated into nutrient broth then placed on a rotary shaker at 200 rpm in an incubator at 37°C overnight to enable bacteria growth. Aliquots of the bacteria culture were

transferred into freshly prepared broth (4 ml) and further incubated at 37°C to achieve a mid-logarithmic phase ($OD_{620\text{ nm}} \approx 0.6$). The broth culture was removed through centrifugation for 15 min at 3000 rpm to obtain the bacteria in its mid-logarithmic phase. The bacteria extract was washed three times with PBS to remove the nutrient broth then diluted to 1/1000 (v/v) of *Staphylococcus aureus*/PBS to obtain the working stock solution of *Staphylococcus aureus*.

PACT studies were carried out following a previously reported procedure [90]. For all experiments, *Staphylococcus aureus* suspensions were incubated for 30 min in an oven with a shaker at 37°C. 3 ml of the incubated *Staphylococcus aureus* suspension was irradiated at the absorption maxima of the main spectral band of the BODIPY in a 24 well plate. The other 3 ml of *Staphylococcus aureus* suspension was plated in a 24 well plate and kept in the dark. The treatment parameters used were as follows; spot diameter (1.5 cm), irradiance (1000 mW/cm^2), dose (2700 J/cm^2), power (mW) and treatment time (2700 s). After irradiation for 3 h at 45 min interval, 100 μL samples were inoculated onto agar plates and incubated at 37°C for 24 hours. A similar protocol was followed for the 24 well plates kept in the dark. The colony forming units (CFU)/ml of bacteria were counted using a Scan[®] 500 automatic colour colony counter.

Chapter 3: Characterization of BODIPY and porphyrin dyes

3.1. Syntheses and characterization of BODIPY dyes

This chapter describes the synthesis and characterization of a series of novel BODIPY dyes. These dyes were characterized by ^1H NMR, UV-visible absorption spectroscopy, mass spectrometry and other techniques where appropriate. The photophysical and photochemical properties were also analyzed.

3.1.1. Synthesis of BODIPY dyes

Four series of BODIPY dyes synthesized in this work are labeled as **1a-1c** (Figure 17), **2a-2b** (Figure 18), **3a-3c** (Figure 19) and **4a-4c** (Figure 20), respectively.

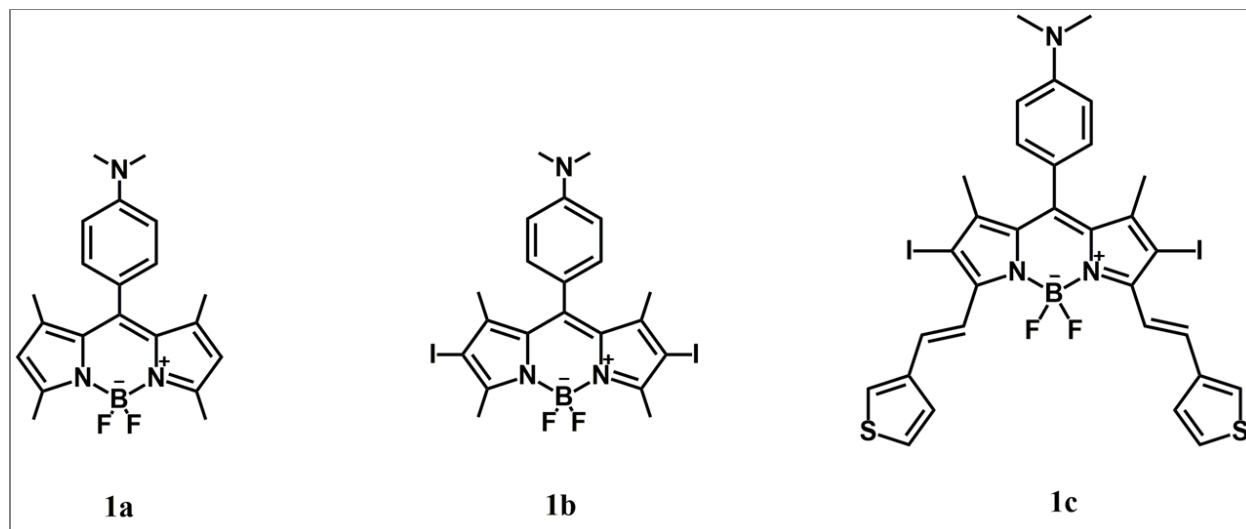


Figure 17. The structures of BODIPYs **1a-c**.

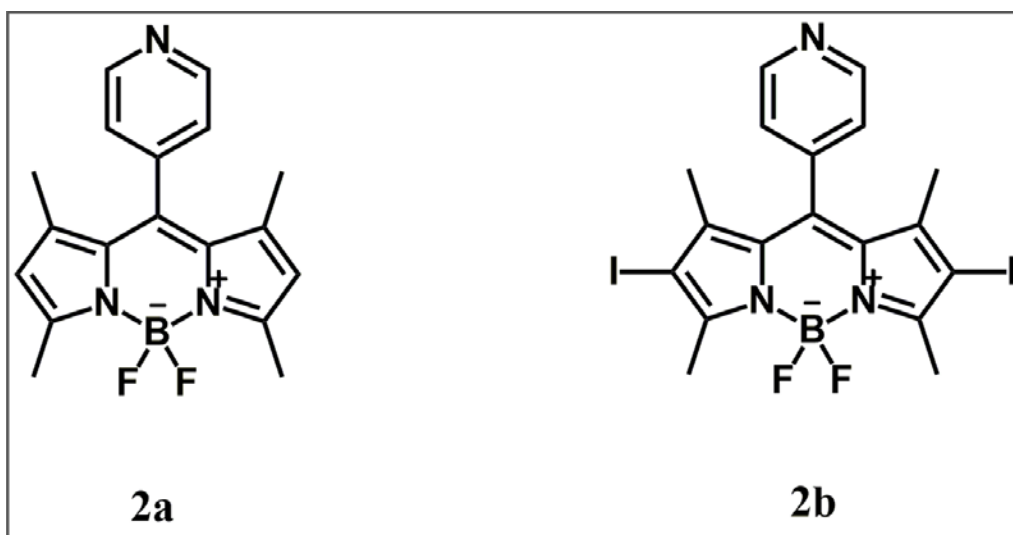


Figure 18. The structures of BODIPYs 2a-2b.

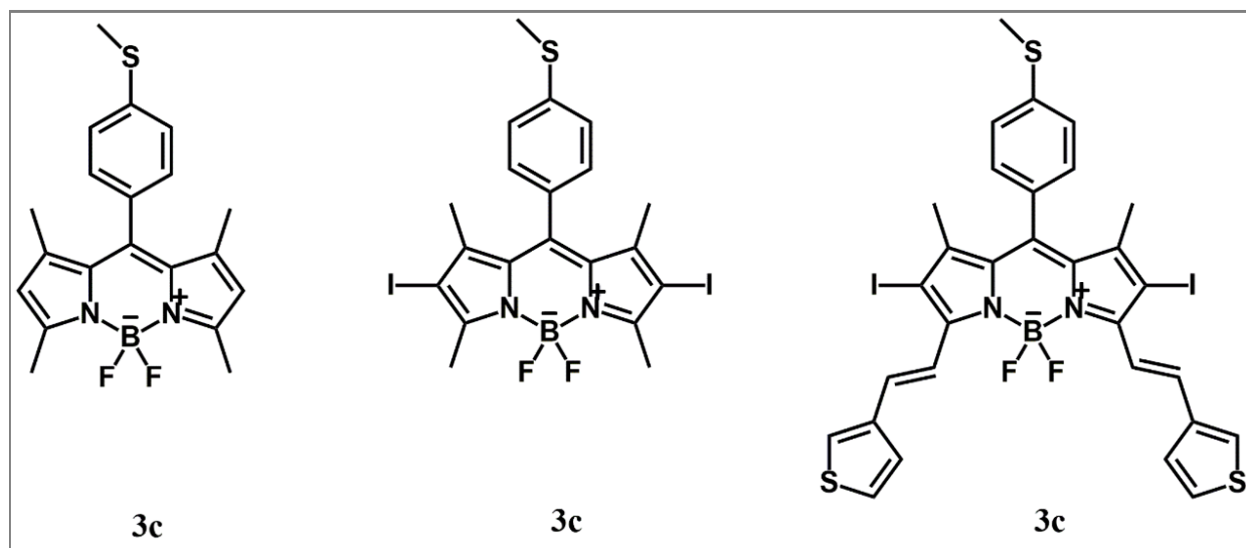


Figure 19. The structures of BODIPYs 3a-c.

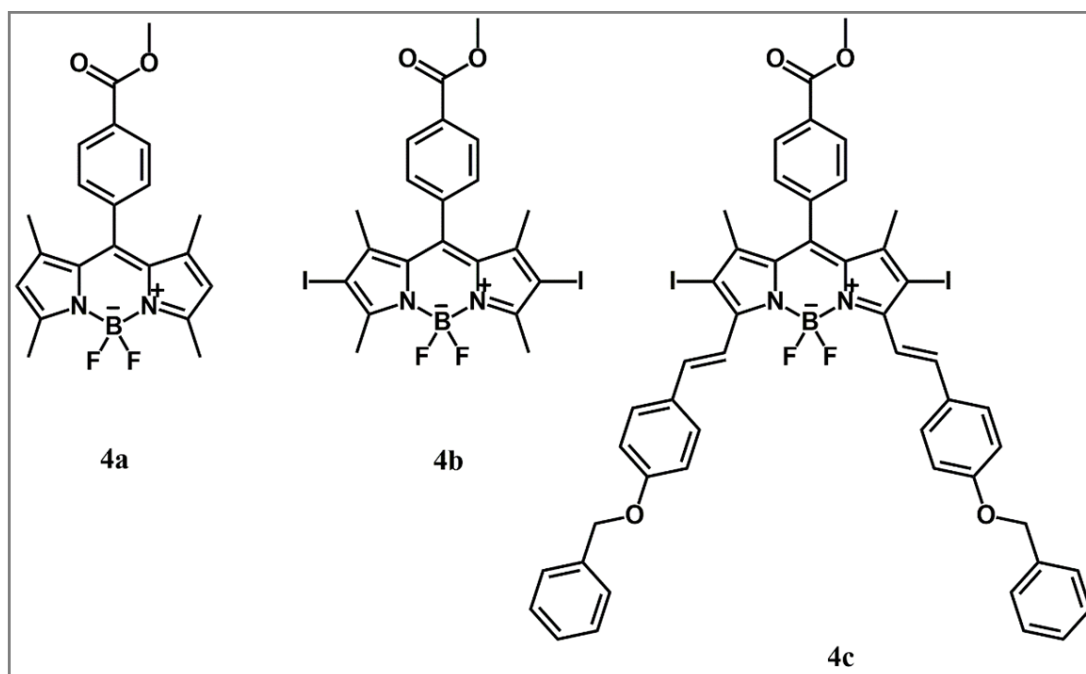


Figure 20. The structures of BODIPYs **4a-c**.

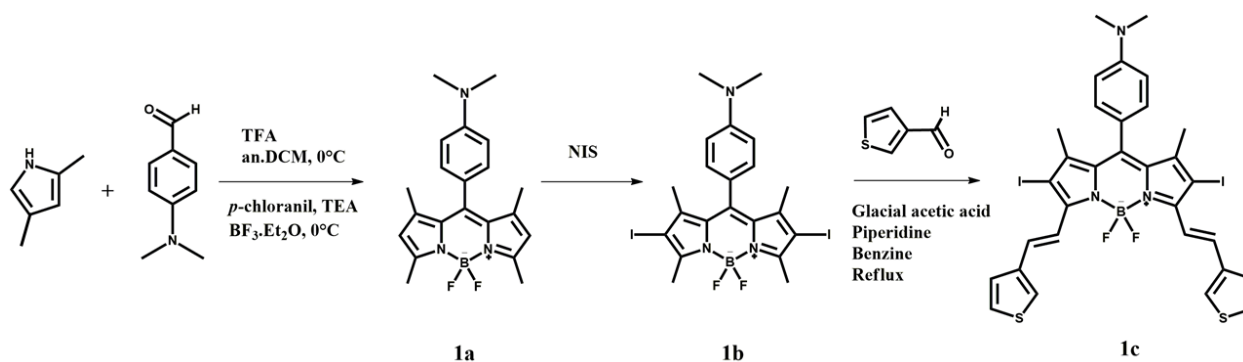
The synthetic route for **1a-c** is shown in **Scheme 4**. The BODIPY core dyes were synthesized using the commonly used three-step “one-pot” acid-catalyzed condensation reaction of the appropriate aldehyde and 2,4-dimethylpyrrole [39, 139]. TFA was used as a homogenous catalyst to speed up the condensation of the two pyrrole rings with the aldehyde through the protonation of the carbonyl oxygen [43, 140]. The resulting partial positive charge on the carbonyl carbon makes it prone to nucleophilic attack by the 2,6-dimethylpyrrole, and this results in the formation of dipyrromethane intermediates, which were immediately oxidized with *p*-chloranil to form more stable dipyrromethenes. TEA was used to neutralize or deprotonate the dipyrromethenes and complexation by BF₂ was subsequently achieved through the addition of BF₃·OEt₂ [140]. The target BODIPY dye (orange fraction) was isolated through column chromatography using ethyl acetate and hexane (1:9; v/v respectively) as the eluent. These

highly fluorescent dyes can be readily identified by thin layer chromatography under UV light

Figure 21. A.

The BODIPY dyes were further modified to enhance the rate of ISC through the electrophilic substitution of the protons at the 2,6-positions with iodines through the use of *N*-iodosuccinamide (NIS) under ambient conditions [43, 140]. The desired products were isolated and dark pink products collected (**Figure 21 B**).

Since application in PDT was envisaged, BODIPYs **1c**, **3c** and **4c** were selected as target dyes since the absorption bands maxima are shifted into the therapeutic window through the extension of the π -conjugation system at the 3,5-positions. This was achieved through the Knoevenagel condensation reactions of the corresponding 2,6-diiodoBODIPY dyes and the relevant aldehydes under reflux with the reagents shown in **Scheme 4**. The blue or green target dyes were isolated using ethyl acetate: hexane (1:4; v/v) as the eluent. **1a** exhibits a reddish fluorescent colour under UV light (**Figure 21 C**).



Scheme 4. The synthetic route for **1a**, **1b** and **1c**.

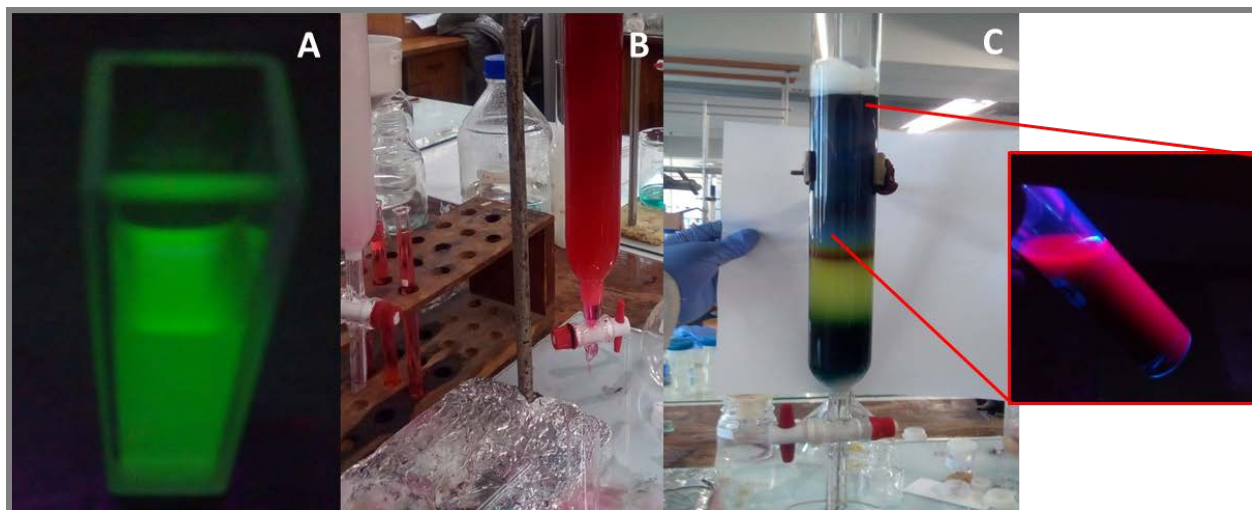


Figure 21. Pictures showing BODIPY **1a** under UV light (A), the isolation of **1b** by column chromatography (B), and the isolation of **1c** by column chromatography and the product under UV light (C).

3.1.2. Characterization

^1H NMR spectroscopy and MALDI-TOF MS were used to confirm the structures of the synthesized BODIPY dyes.

BODIPY 1a: the structure of **1a** was confirmed by ^1H NMR spectroscopy. All of the anticipated protons can be readily assigned. The 7.10–7.11 and 6.84–6.85 ppm doublets integrate to a total of four protons and are assigned to the *meso*-phenyl ring. A two proton singlet at 6.16 ppm corresponds to the two hydrogens at 2,6-positions of the BODIPY core structure. The six dimethylamino protons appear as a singlet at 2.98 ppm. The singlet at 2.44 ppm integrates to six methyl protons and is assigned to the 3,5-position methyls, while the six proton singlet at 1.45 ppm corresponds to the 1,7-position methyls.

BODIPY **2a**: in the ^1H NMR spectrum of **2a**, the *meso*-phenyl aromatic protons are observed as doublets at 7.34–7.35 and 7.27–7.28 ppm, and a two proton singlet is observed at 6.03 ppm due to the 2,6-position protons. The six proton singlets at 2.58 and 1.42 ppm can be assigned to the 3,5- and 1,7-position protons, respectively.

BODIPY **3a**: in the ^1H NMR spectrum of **3a**, the *meso*-phenyl aromatic protons are observed as doublets at 7.36–7.37 and 7.21–7.22 ppm, and a two proton singlet is observed at 6.00 ppm due to the 2,6-position protons. The six proton singlets at 1.61 and 0.90 ppm can be assigned to the 3,5- and 1,7-position protons, respectively, while the three proton singlet at 2.07 ppm corresponds to the methyl protons attached to the sulfur atom.

BODIPY **4a**: in the ^1H NMR spectrum of **3a**, the *meso*-phenyl aromatic protons are observed as doublets at 8.20–8.21 and 7.43–7.44 ppm, and a two proton singlet is observed at 6.01 ppm due to the 2,6-position protons. The three proton singlet at 3.98 ppm can be assigned to the methyl protons of the ester functional group. The six proton singlets at 2.58 and 1.38 ppm can be assigned to the 3,5- and 1,7-position protons, respectively.

Figure 22 shows the ^1H NMR spectrum of **4a** as an example of a typical BODIPY core spectrum. The color coding on the BODIPY core structure corresponds to those used for chemical shifts in the ^1H NMR spectrum.

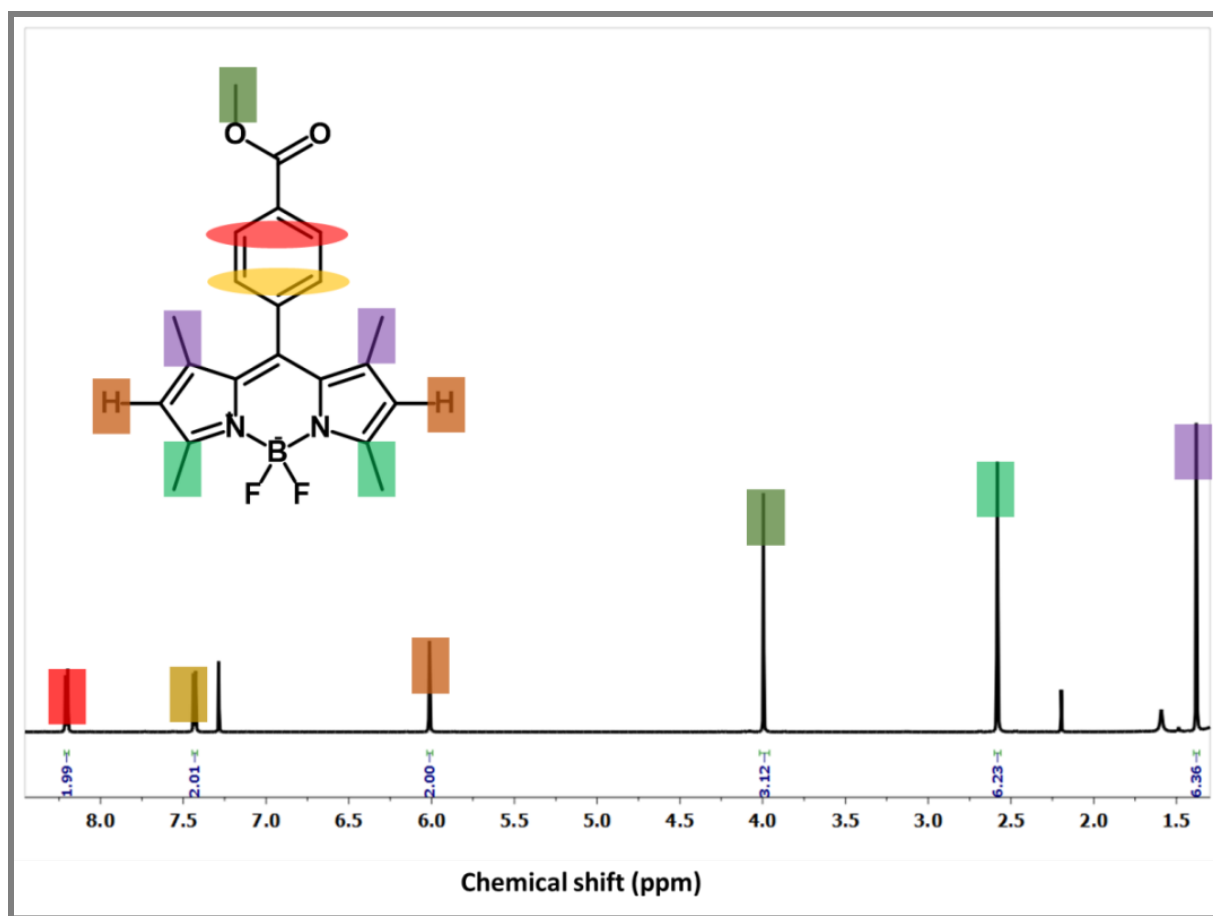


Figure 22. The ^1H NMR spectrum of BODIPY **4a** in CDCl_3 .

The structures of iodinated dyes **1b**, **2b**, **3b** and **4b** were confirmed through ^1H NMR spectroscopy and MALDI-TOF-MS. The only significant difference observed from the spectra of **1a**, **2a**, **3a** and **4a** described above is the complete disappearance of the two proton singlet at ca. 6.00 ppm due to iodination at the 2,6-positions. A typical example is provided by the ^1H NMR spectrum of **4b** (**Figure 23**). MALDI-TOF spectra further confirmed these structures. The following masses were obtained, **1b**; calculated: 619.04 amu; found: 618.34 m/z [M^+], **2b**; calculated: 576.95 amu; Found: 576.40 m/z [M^+], **3b**; calculated: 621.94 amu; found: 623.04 m/z [$\text{M}+\text{H}^+$] and **4b**; calculated: 633.96 amu; found: 635.98 m/z [$\text{M}+2\text{H}^+$]. **Figure 23** provides the MALDI-TOF mass spectrum of BODIPY **4b** as a typical example.

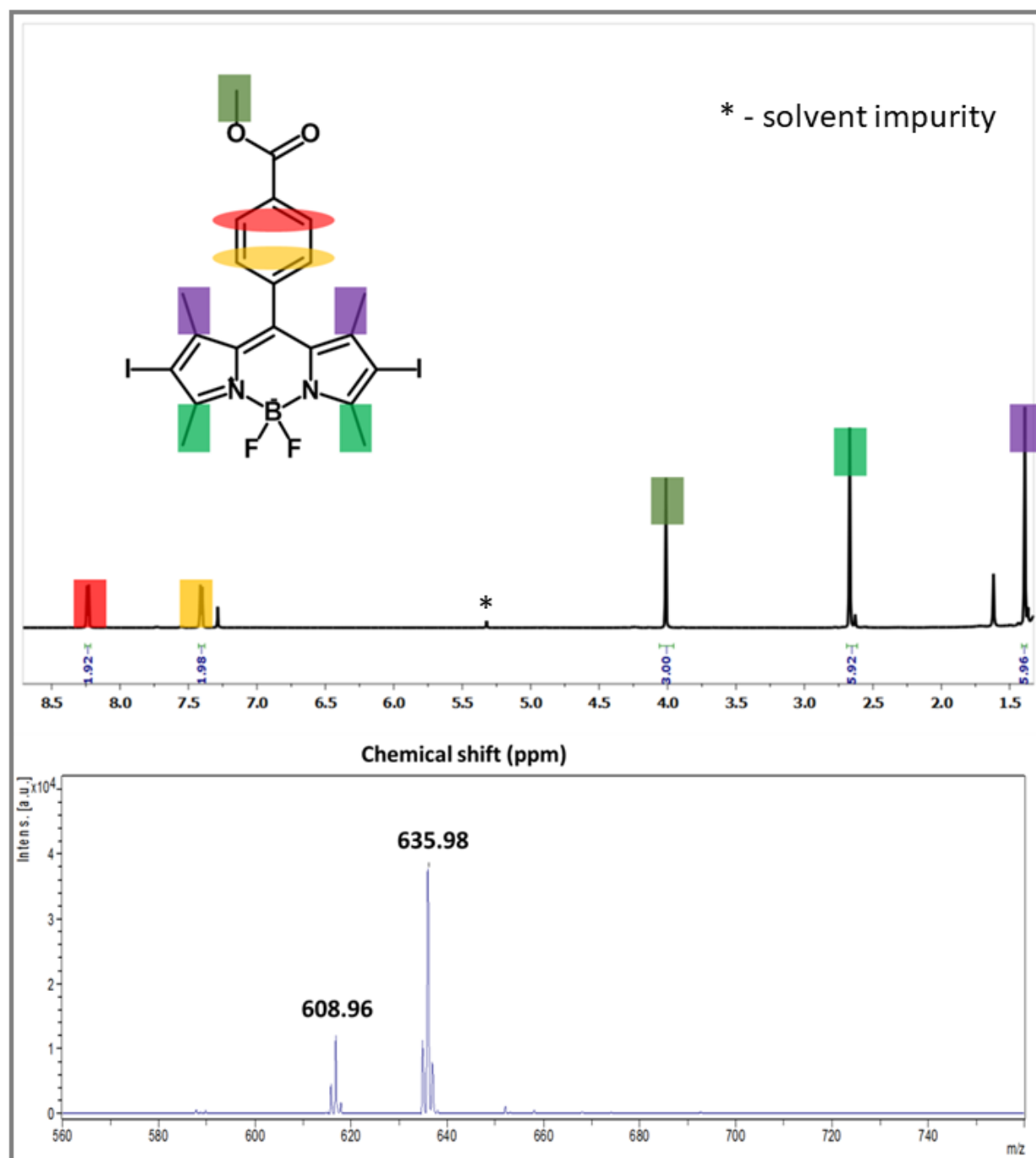


Figure 23. The ¹H NMR spectrum and MALDI-TOF spectrum of BODIPY **4b** in CDCl₃.

The structures of the synthesized 2,6-diiodo-3,5-distyrylBODIPY dyes (**1c**, **3c** and **4c**) were confirmed by ¹H NMR spectroscopy and MALDI-TOF MS. The anticipated number of protons

was observed in all of the spectra and the observed masses on the MALDI-TOF mass matched the calculated masses of the dyes.

BODIPY 1c: the additional protons observed in the ^1H NMR spectrum of **1c** were consistent with the intended vinylation of BODIPY **1b**. **Figure 24** provides a colour-coded assignment of the chemical shifts and the corresponding protons in the BODIPY structure. The two protons at 8.17 ppm correspond to the protons shaded grey on the terminal thiophenes. The eight proton signal at 7.54 ppm can be assigned to the four aromatic protons of the *meso*-phenyl ring and four protons on the thiophene rings (red shading). MALDI-TOF MS provided further confirmation of the structure of **1c**. The calculated mass of 807.30 amu is in close agreement with the parent peak at 807.33 m/z. The other major peak at 788.32 m/z corresponds to the loss of a fluorine atom.

BODIPYs 3c and 4c: similar trends were also observed for **3c** and **4c**. **Figures 25 and 26** provide the colour-coded chemical shift assignments in a similar manner to those of **1c** in **Figure 24**. The structures were also confirmed by MALDI-TOF MS. The calculated masses of 809.92 and 1022.11 amu are in close agreement with the observed parent peaks at 810.25 and 1022.64 m/z, respectively.

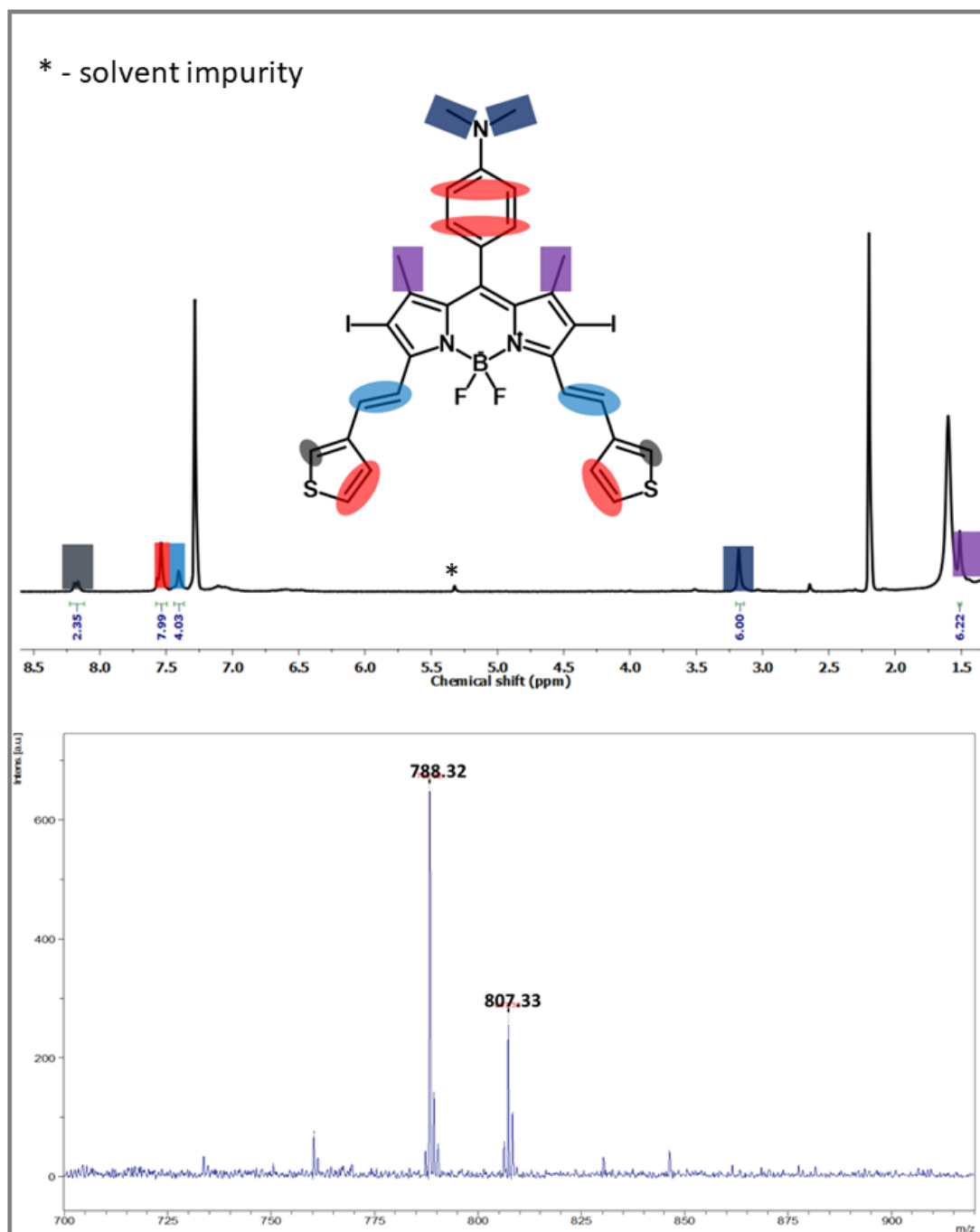


Figure 24. The ¹H NMR spectrum and MALDI-TOF mass spectrum of BODIPY 1c in CDCl₃.

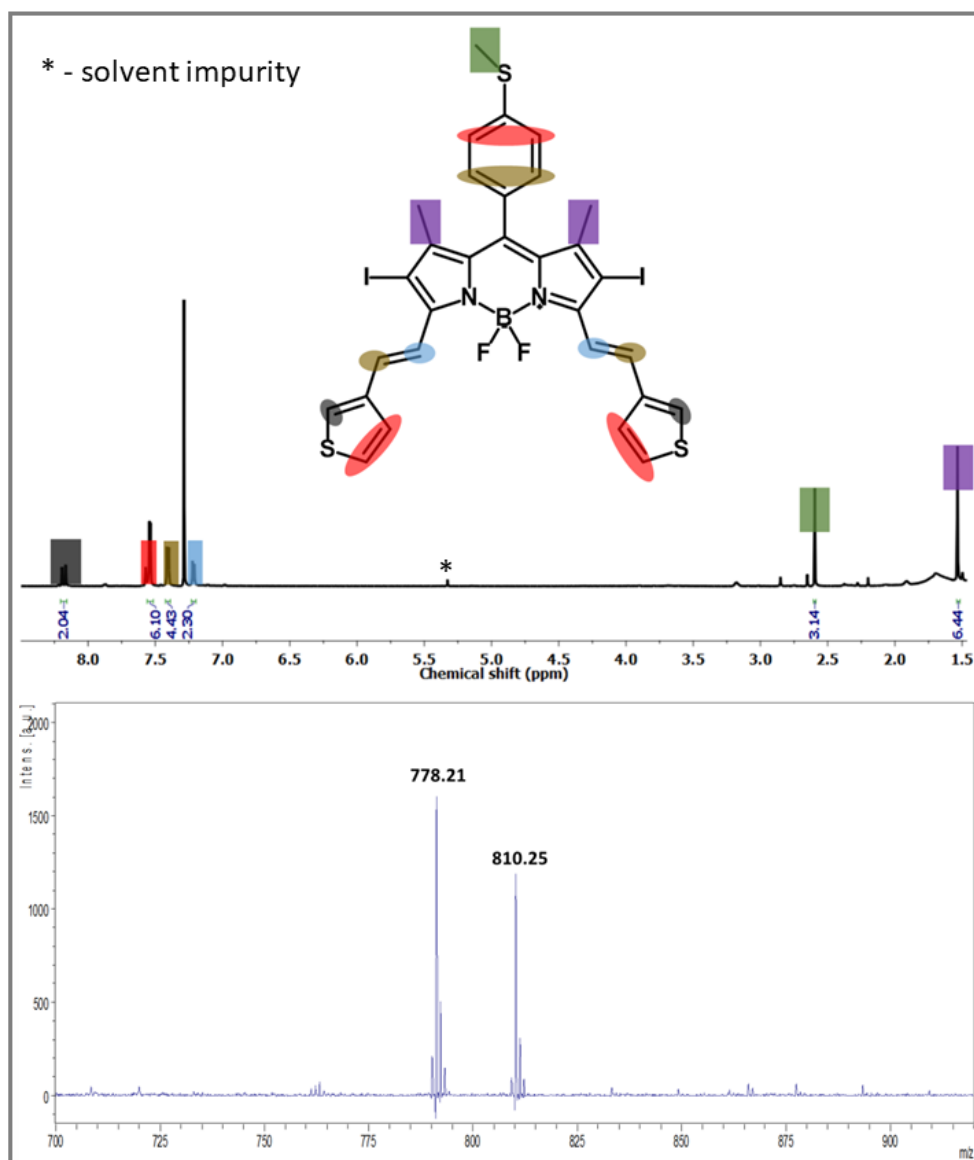


Figure 25. The ^1H NMR spectrum and MALDI-TOF mass spectrum of BODIPY 3c in CDCl_3 .

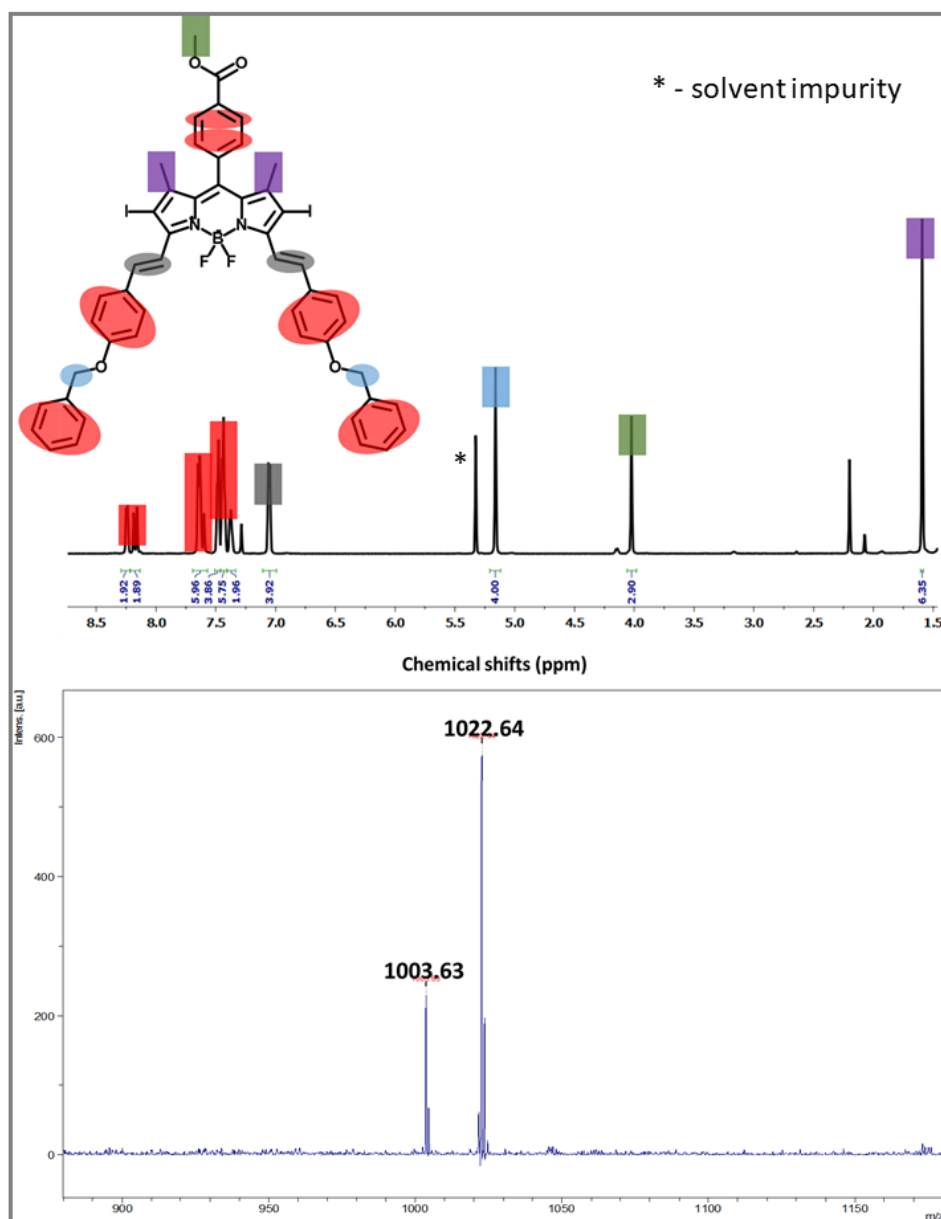


Figure 26. The ¹H NMR spectrum and MALDI-TOF mass spectrum of BODIPY **4c** in CDCl₃.

3.1.3. Optical spectroscopy

The ground state absorption spectra of **1a-c** and **2a,b** are shown in **Figure 27**. The UV-visible absorption spectra of **1a** and **2a** exhibit the characteristic bands of monomeric BODIPY dyes [141] and the main absorption bands, which arise from the $S_0 \rightarrow S_1$ transition, lie at 502 and 505

nm, respectively. A lower intensity should of intensity at ca. 420 nm is attributed to a weaker vibrational band associated with the $S_0 \rightarrow S_1$ transition [21, 142]. The different aryl-groups at the *meso*-position have no significant effect on the characteristic band of the BODIPYs as can be observed in the similarity of the two spectra of **1a** and **1b** since these rings lie orthogonal to the BODIPY core in the context of 1,3,5,7-tetramethyl dyes.

Upon iodination, the absorption maximum red shifts to 533 nm for **1b** and to 541 nm for **2b** with respect to the spectra of the parent dyes, proving that halogenation at the 2,6-positions was successful [143]. The red shift is induced by the introduction of heavy iodine atoms, which causes a relative destabilization of the highest occupied molecular orbital (HOMO) with respect to the lowest occupied molecular orbital (LUMO) that results in a narrowing of the HOMO–LUMO gap [27, 144]. A further red shift of the absorption maximum to ca. 641 nm is observed in the spectrum of **1c**. The reasons for these spectral changes will be described in greater depth in Chapter 8.

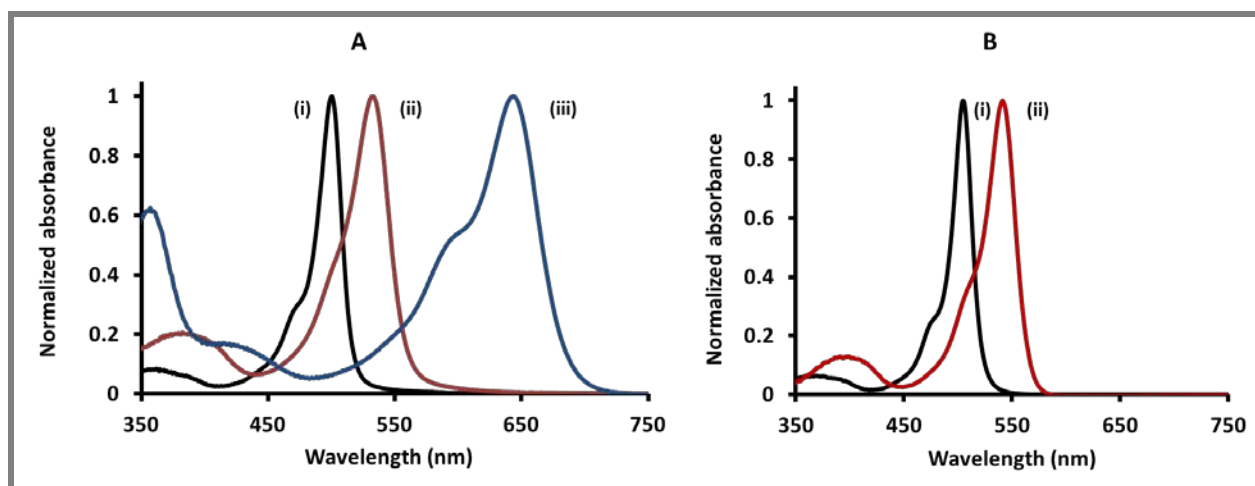


Figure 27. Ground state absorption spectra of (A); **1a** (i), **1b** (ii) and **1c** (iii) and (B); **2a** (i) and **2b** (ii). Solvent = DMSO.

The UV-visible absorption spectra of **3a-c** and **4a-c** are shown in **Figure 28 A** and **B**, respectively. The characteristic main absorption bands of the BODIPY core dyes are observed at 502 and 503 nm for **3a** and **4a**, respectively. Upon iodination at the 2,6-positions, the absorption maxima shift to 536 (**3b**) and 537 nm (**4b**), and a further red-shift towards the NIR region was observed upon vinylation due to the extension of the π -conjugation system [145]. The absorption maxima were observed to lie at 648 and 672 nm for **3c** and **4c**, respectively. The main spectral band of **4c** was found to be more red-shifted, due to the extension with 4-benzyloxystyryls rather than thienylvinylene groups at the 3,5-positions.

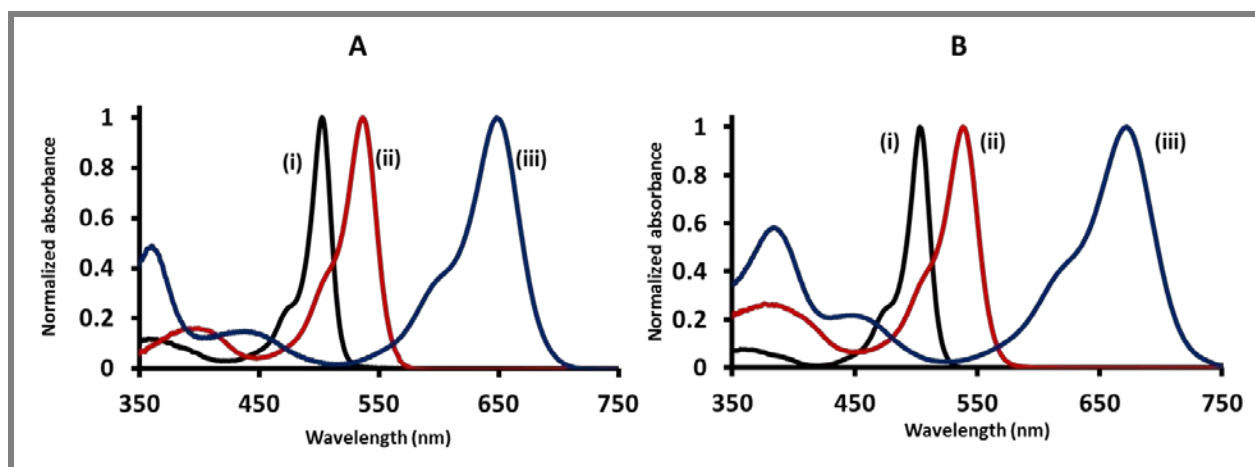


Figure 28. Ground state absorption spectra of (A); **3a** (i), **3b** (ii) and **3c** (iii) and (B); **4a** (i), **4b** (ii) and **4c** (iii). Solvent = DMSO.

The spectroscopic data for BODIPY dyes in water (polarity index = 10.2), DMSO (polarity index = 7.2) and ethanol (polarity index = 5.2) are summarized where appropriate in **Table 2** [101]. The study focused on polar solvents since these are used in the context of the envisaged applications, so the spectroscopic, photophysical and photochemical properties in non-polar solvents were not studied in depth. In general, there was no aggregation observed for the studies in the latter two solvents. However, there were minor bathochromic shifts of the absorption maxima with increasing solvent polarity. The molar extinction coefficients ($\log \epsilon$) values were obtained from plots of the absorption maxima versus concentration as shown in **Figure 29**. At concentrations less than $3.8 \times 10^{-6} \text{ mol.L}^{-1}$, the selected dyes exhibited no aggregation effects, since there was linear behavior according to the Beer-Lambert's law. The compounds have high $\log \epsilon$ values ranging from 4.11–5.70 in DMSO. These values were consistent with those reported previously for similar dyes [14].

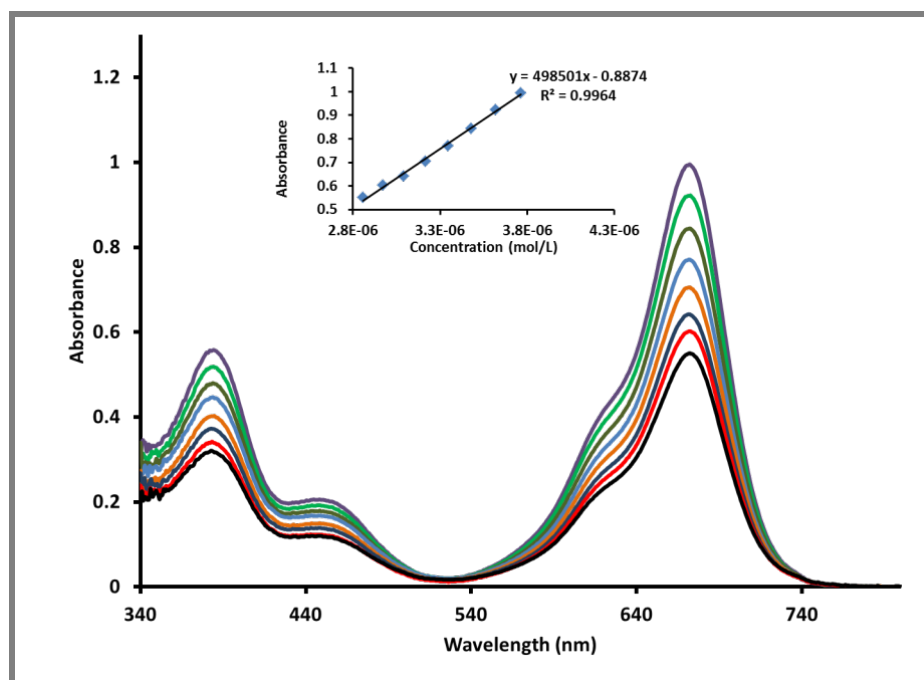


Figure 29. Ground state absorption spectra and absorption maxima vs concentration plot of **1c** for $\log \epsilon$ determination. Solvent = DMSO.

Table 2. The main absorption wavelengths of BODIPY series **1-4** and their molar extinction coefficients (log ϵ) values.

Dye	Solvent	λ_{abs} (nm)	Log ϵ
1a	DMSO	502	4.84
	ethanol	499	-
1b	DMSO	533	4.64
	ethanol	530	-
1c	DMSO	641	4.69
	ethanol	638	-
2a	DMSO	505	4.57
2b	DMSO	541	4.33
3a	DMSO	502	-
3b	DMSO	536	-
3c	DMSO	648	4.11
4a	DMSO	503	-
4b	DMSO	537	-
4c	DMSO	672	5.70

3.2. Photophysicochemical Studies of BODIPY dyes

3.2.1..Fluorescence studies

Dilute solutions of the dyes and standards (0.05–0.10 absorbance) were used to measure the photophysical properties in selected polar solvents. The BODIPY cores were excited at 480 nm, while the 3,5-divinyleneBODIPYs were excited at 510 nm. The absorbance, emission and excitation spectra of BODIPY dyes **1a**, **2a**, **3a** and **4a** are shown in **Figure 30**. For all of the compounds, the emission spectra are mirror images of the excitation spectra, and the excitation

spectra are almost identical to the absorbance spectra, further indicating that there is no significant aggregation of the dyes in solution. All these spectra contain narrow spectral bands, and there are small Stokes shift values as expected for BODIPY dyes [34, 146], due to their structural rigidity. Upon halogenation, low fluorescence quantum yields were derived for all of the 2,6-diodoBODIPY dyes (**1b**, **2b**, **3b** and **4b**), due to the anticipated heavy atom effect. The Φ_F values of the dyes are summarized in **Table 3**, along with the absorbance (λ_{abs}), excitation (λ_{exc}) and emission band maxima (λ_{em}), and Stokes shift values.

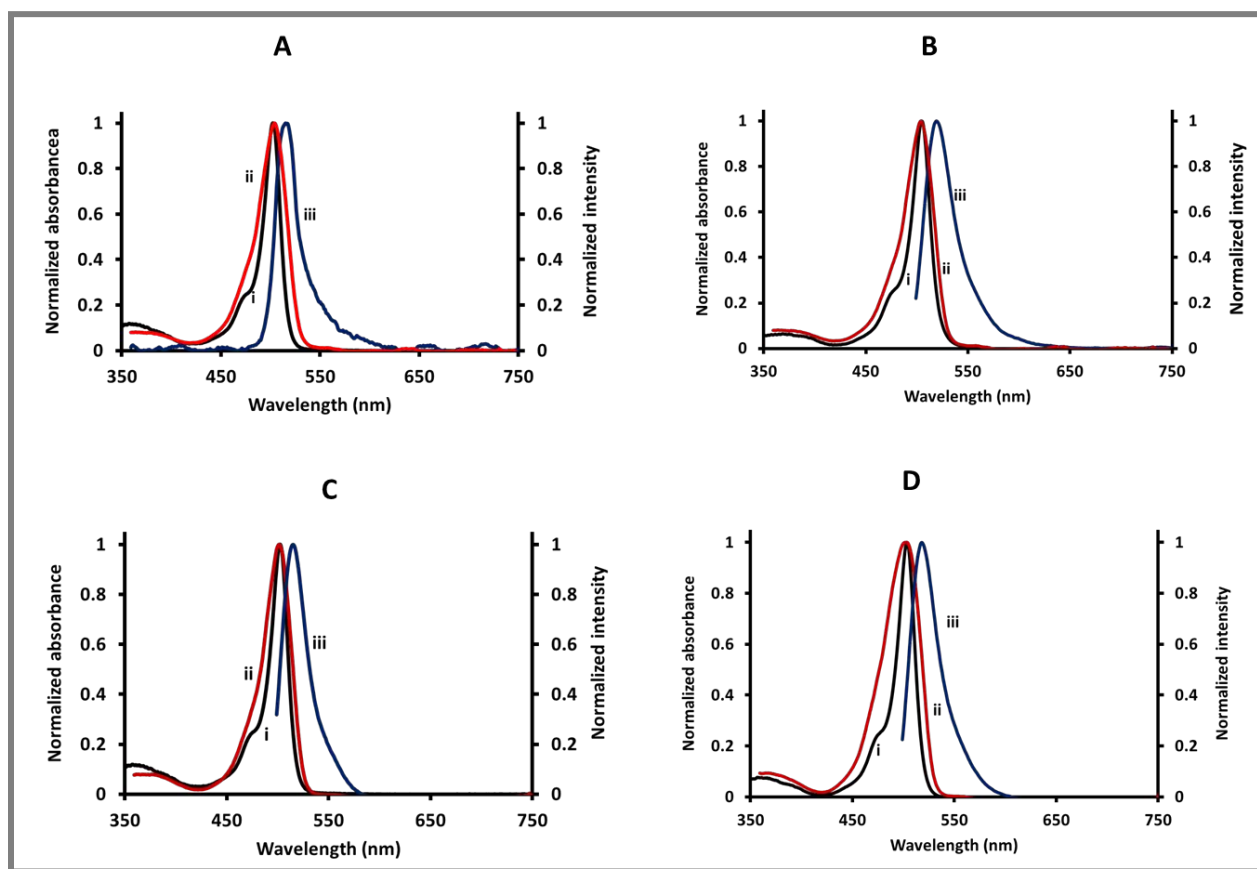


Figure 30. Ground state absorption (i), excitation (ii) and emission (iii) spectra of BODIPYs **1a** (A), **2a** (B), **3a** (C) and **4a** (D). Solvent = DMSO.

The fluorescence quantum yield (Φ_F) values of the dyes were obtained by using the comparative method. Rhodamine-6G and ZnPc were used as standards for the BODIPY core dyes and the 3,5-divinyleneBODIPYs, respectively. The calculated Φ_F values for these dyes are reported in **Table 3**.

Unmodified BODIPY dyes usually have high fluorescence quantum yields due to the rigid core structure and minimal ISC which results in very low triplet state quantum yields [36]. The Φ_F values of **1a** and **2a** (0.03) are smaller than those of **3a** and **4a** (0.11 and 0.09, respectively), probably due to intramolecular charge transfer (ICT) that occurs when the electron is promoted from the HOMO to the LUMO upon excitation of these BODIPYs, since the lone pairs on the nitrogen atoms of the dimethylamino group or pyridine ring can act as a donor of electronic charge while the BODIPY core acts as an acceptor [147, 148]. The introduction of iodine atoms at the 2,6-positions results in a decrease in the Φ_F value, due to the enhanced ISC from the S_1 to T_1 state. Upon vinylation of the 2,6-diiodoBODIPYs, the Φ_F values decreased to <0.01 (**Table 3**). The emission maxima were red-shifted upon iodination, and a still greater bathochromic shift was observed upon addition of the vinylene groups at the 3,5-positions in a similar manner to what is observed for the absorption bands.

The fluorescence lifetime (τ_F) values were determined using a TCSPC instrument. The τ_F value is independent of the excitation and emission wavelengths as emission occur from the lowest excited state following Kasha's rule. **Figures 31-33** show the fluorescence decay curves of BODIPYs **1c**, **3c** and **4c**, respectively. The τ_F values obtained for the synthesized dyes used in either PACT or PDT are summarized in **Table 3**. BODIPY dyes **1c**, **3c** and **4c** have one lifetime each, and the values are similar to those reported previously for similar BODIPY dyes [149, 150]. When the Φ_F values are very low, some of the τ_F values cannot be determined (**Table 3**).

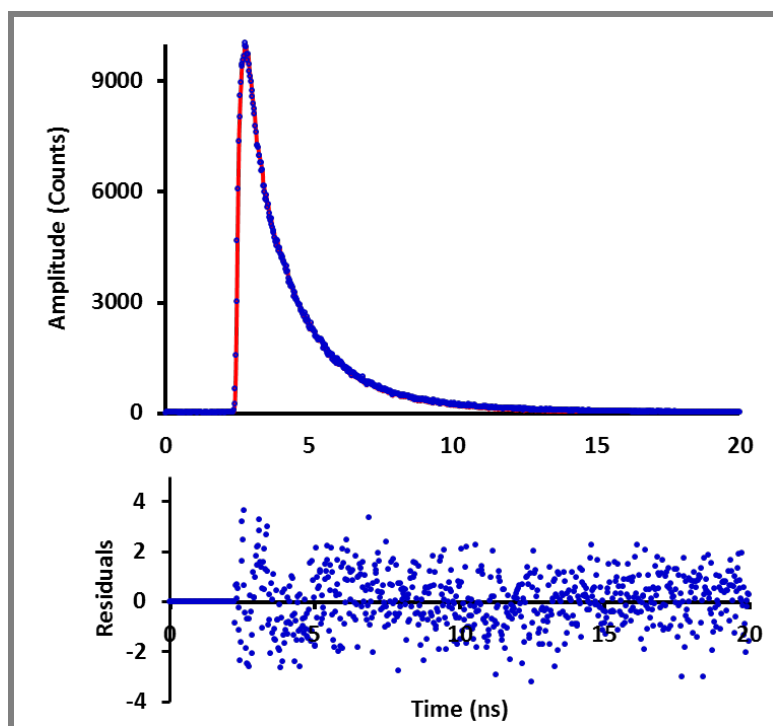


Figure 31. Fluorescence decay curve and residuals for BODIPY 1c. Solvent = DMSO.

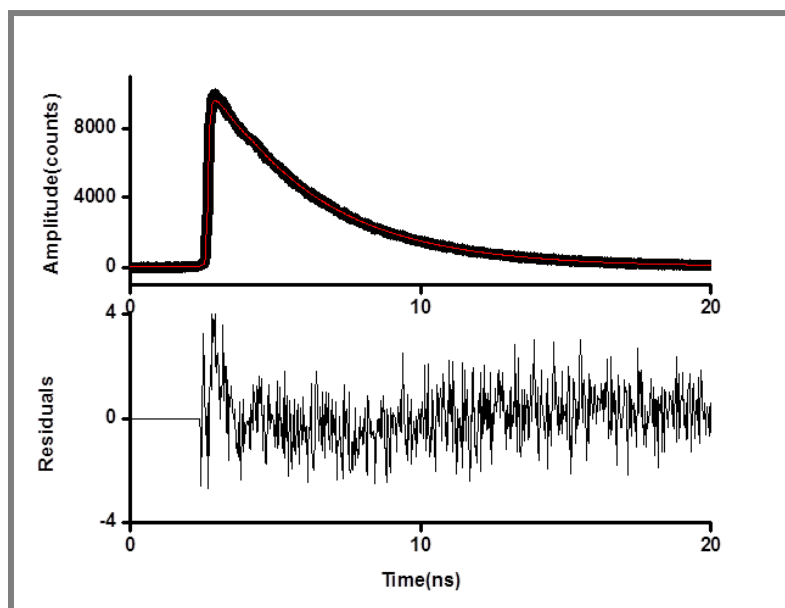


Figure 32. Fluorescence decay curve and residuals of BODIPY 3c. Solvent = DMSO.

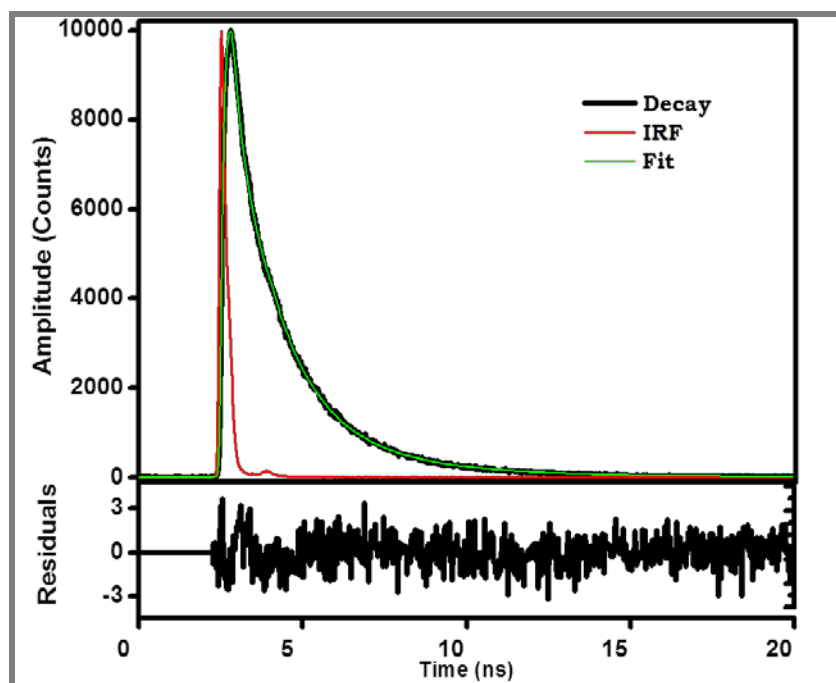


Figure 33. Fluorescence decay curve and residuals for BODIPY **4c**. Solvent = DMSO.

Table 3. Summary of the photophysical properties of BODIPYs **1a-4c**.

	Solvent	λ_{abs} (nm)	λ_{ex} (nm)	λ_{em} (nm)	Δ Stokes/ cm^{-1}	Φ_{F}	τ_{F} /ns
1a	DMSO	502	502	512	389	0.03	^a n.d.
1b	DMSO	533	-	-	-	^a n.d.	^a n.d.
1c	DMSO	641	642	665	515	<0.01	3.02
2a	DMSO	505	505	517	460	0.03	^a n.d.
2b	DMSO	541	-	-	-	^a n.d.	^a n.d.
3a	DMSO	502	502	512	389	0.11	-
3b	DMSO	536	536	558	736	0.01	-
3c	DMSO	648	648	669	484	<0.01	2.95
4a	DMSO	503	503	517	538	0.09	-
4b	DMSO	537	537	560	440	0.02	-
4c	DMSO	672	674	707	693	<0.01	2.83

^an.d. implies that the value is too low to be detectable.

3.3. Photochemical studies of BODIPY dyes

3.3.1. Singlet oxygen quantum yields

The singlet oxygen generation studies were carried out by using the comparative method through the use of 1,3-diphenylisobenzofuran (DPBF) as a singlet oxygen scavenger in the presence of a BODIPY, a porphyrin or a standard. The decrease in the DPBF absorbance band at 410 nm was recorded after appropriate irradiation intervals, and the Φ_{Δ} value of the photosensitizer was compared to that of the standard. The Φ_{Δ} value of a PS dye can be used to assess its potential for use in PDT or PACT. The Φ_{Δ} values of **1b** and **2b** were found to be relatively high ($\Phi_{\Delta} = 0.82$ and 0.79 in DMSO, respectively), while BODIPYs **1c**, **3c** and **4c** have lower Φ_{Δ} values of 0.40,

0.66 and 0.61, respectively, in ethanol. The decrease in the Φ_{Δ} values upon vinylation (**1c** and **3c**) and styrylation (**4c**) is probably due to enhanced non-radiative decay that occurs upon the extension of the π -conjugation system [48, 151, 152], since the dye structure becomes less rigid.

The Φ_{Δ} values of the BODIPY dyes were consistently found to be higher in ethanol than in DMSO. High Φ_{Δ} values were obtained due to efficient energy transfer between the T_1 state of the BODIPYs and ground state molecular dioxygen. **Table 4** shows a summary of the values for the dyes in DMSO and/or ethanol. **Figures 34-36** show the rate of DPBF degradation, which is proportional to the amount of $^1\text{O}_2$ produced by BODIPYs **1c**, **3c** and **4c** upon irradiation in DMSO. The Φ_{Δ} values of **5** were previously reported to be 0.10 and 0.16 in DMSO and ethanol, respectively [47]. BODIPY **6** was found to have a Φ_{Δ} value of 0.40 in DMSO. All of the dyes were highly photostable in this context, since no significant degradation was observed in the intensity of the main BODIPY spectral band during the irradiations.

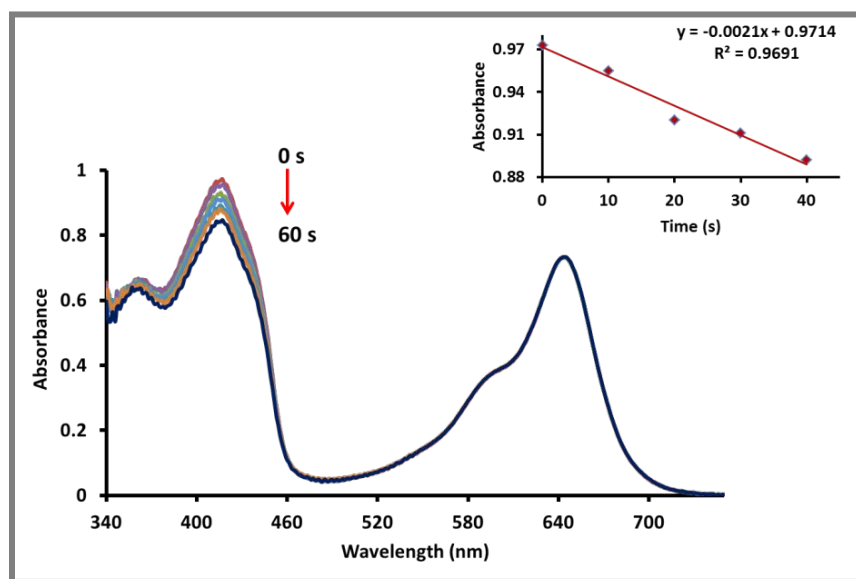


Figure 34. Ground state absorption spectra showing the photodegradation of DPBF in the presence of BODIPY **1c** at 10 s intervals. Solvent = DMSO.

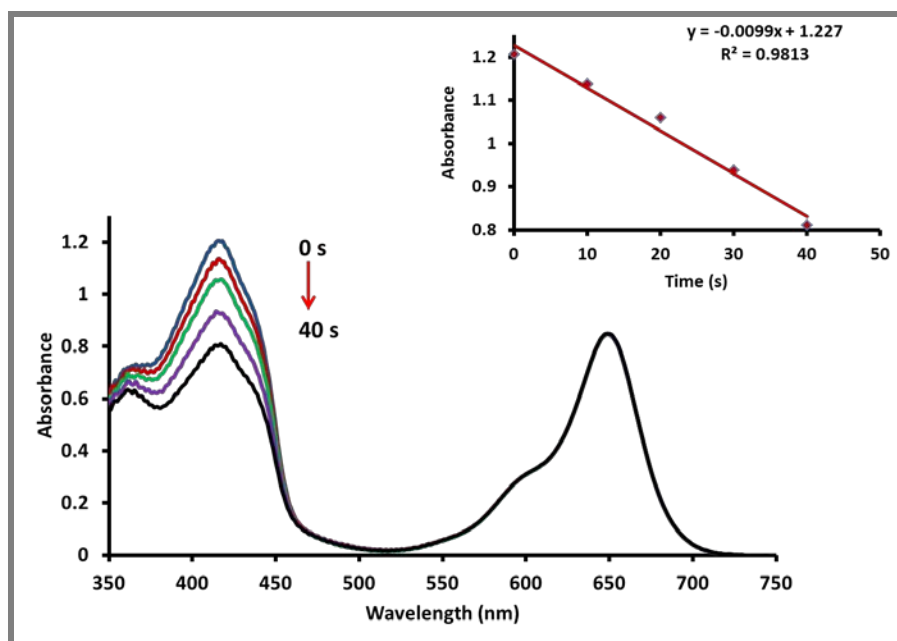


Figure 35. Ground state absorption spectra showing the photodegradation of DPBF in the presence of BODIPY 3c at 10 s intervals. Solvent = DMSO.

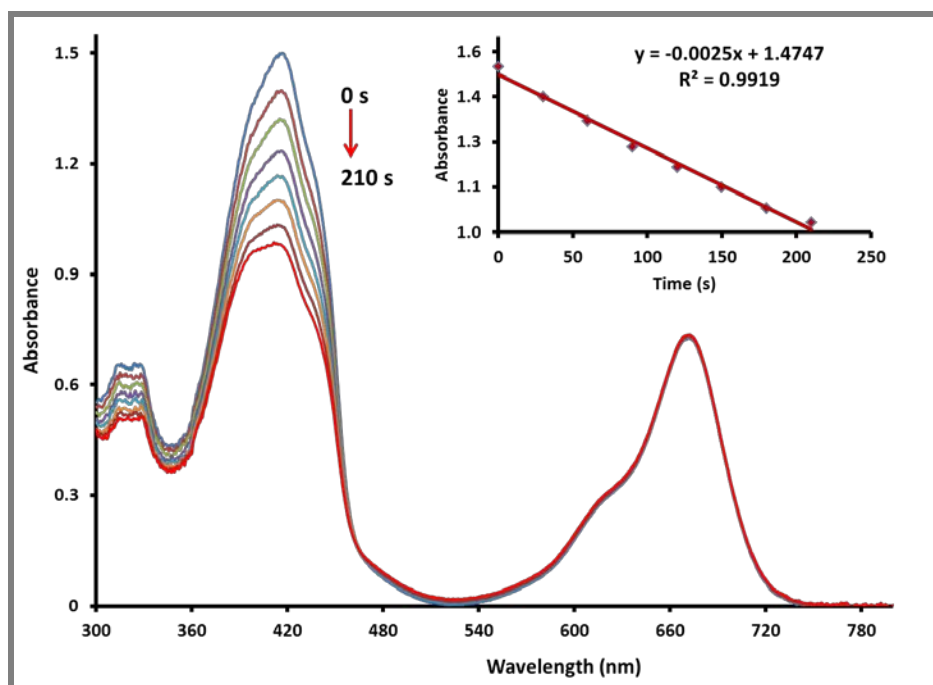


Figure 36. Ground state absorption spectra showing the photodegradation of DPBF in the presence of BODIPY **4c** at 30 s intervals. Solvent = DMSO.

Table 4. A summary of the Φ_{Δ} values for the iodinated BODIPY dyes.

Dye	Solvent	$\Phi_{\Delta} \pm 0.01$
1b	DMSO	0.82
2b	DMSO	0.79
3b	DMSO	0.76
4b	DMSO	0.63
1c	DMSO	0.13
	Ethanol	0.40
3c	DMSO	0.59
	Ethanol	0.66
4c	DMSO	0.52
	Ethanol	0.61
5	DMSO	0.10
	Ethanol	0.16
6	DMSO	0.40

3.4. Characterization of porphyrin dyes

This section describes the characterization of tetraacenaphthylporphyrins **7a** and **7b** (**Figure 37**). The dyes were characterized by ^1H NMR and UV-visible absorption spectroscopy and mass spectrometry. The photophysical and photochemical properties were also analyzed. The Sn(IV) tetraphenylporphyrin (**8**) (**Figure 37**) was previously synthesized and characterized by Mack and coworkers [55] and was used in this work as a control compound. This work sought to evaluate the potential of the Sn(IV) tetraacenaphthylporphyrin (**7b**) as a PDT agent following the work carried out by Mack and coworkers [55].

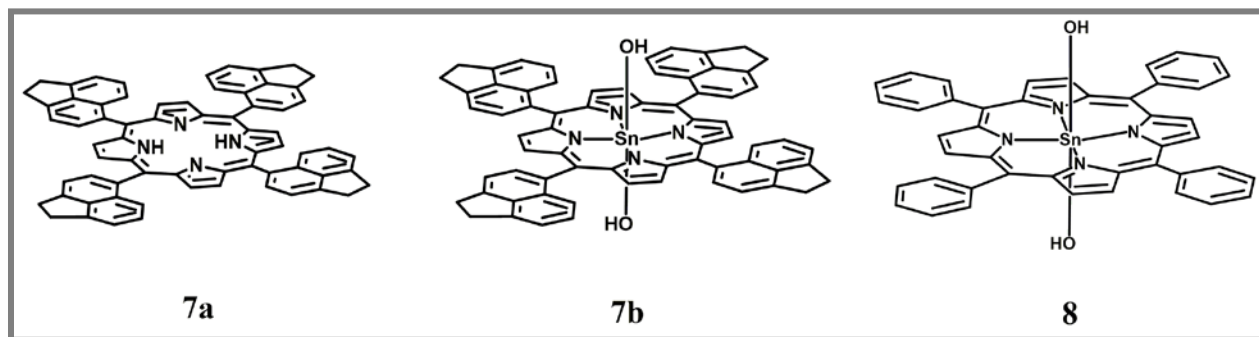


Figure 37. The structures of porphyrins **7a**, **7b** and **8**.

3.4.1. Characterization

The structure of free base porphyrin **7a** was confirmed by MALDI-TOF mass spectroscopy and ^1H NMR spectroscopy. The calculated mass of 918.37 amu matches the observed parent peak at 919.79 m/z, **Figure 38**. The protons obtained from ^1H NMR spectrum contained all 46 anticipated protons. The eight β -proton peaks lie between 8.61–8.50 ppm. The four proton signals between 8.19–8.22 ppm can be assigned to the aromatic part of the *meso*-acenaphthyl rings, along with those at 7.62–7.64 and 7.17–7.14 ppm. The sixteen proton signals resonating

between 3.72–3.64 ppm can be assigned to the $-\text{CH}_2$ protons of the *meso*-acenaphthyl rings [153]. A two proton peak resonating upfield at -2.30 ppm can be assigned to the two pyrrole hydrogens in the porphyrin cavity.

After the insertion of the Sn(IV) ion, further characterization was carried out. MALDI-TOF MS confirmed the structure of **7b**. The calculated mass of 1070.25 amu is in close agreement with the observed parent peak at 1072.49 m/z, **Figure 39**. The anticipated protons of the porphyrin ligand can again be readily identified in the ^1H NMR spectrum. The insertion of the Sn(IV) ion was confirmed by the disappearance of the signal for the two inner N-H protons at -2.30 ppm.

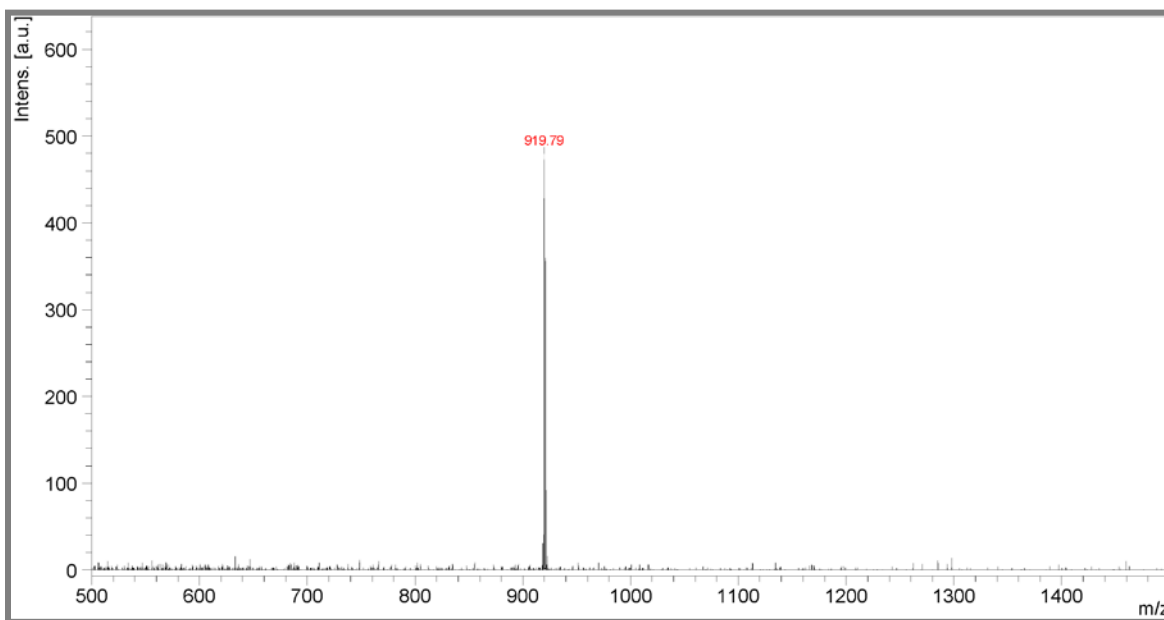


Figure 38. MALDI-TOF mass spectrum of **7a**.

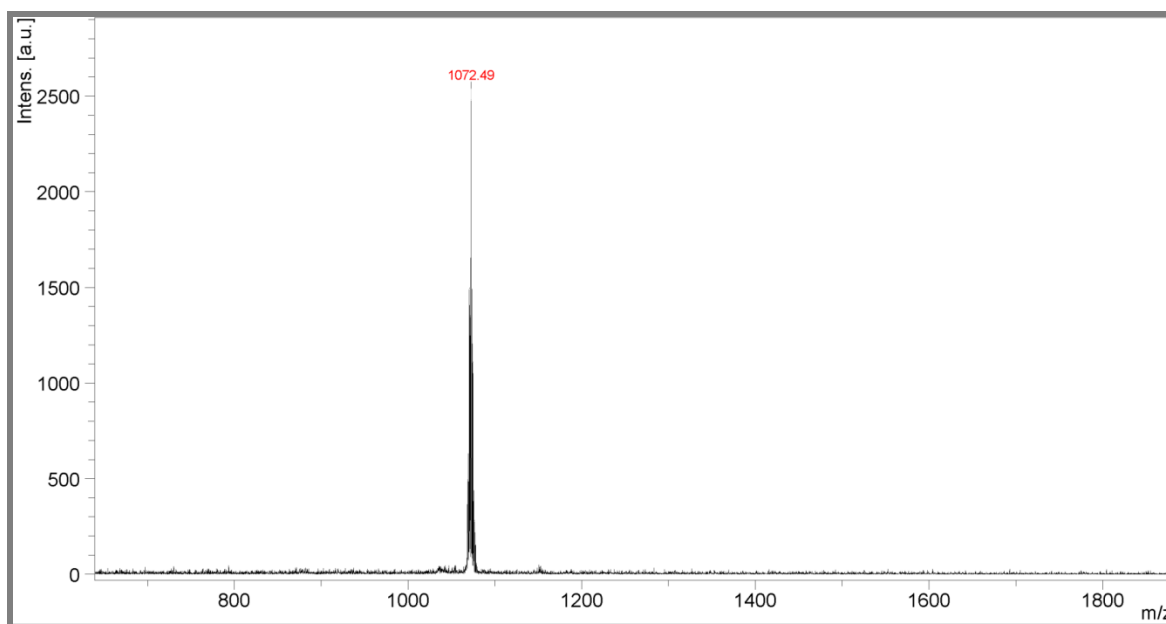


Figure 39. MALDI-TOF mass spectrum of **7b**.

3.5. Optical spectroscopy of porphyrin dyes

The characteristic bands of porphyrins were observed in the UV-visible absorption spectra for **7a** and **7b** (**Figure 40**). The B band of **7a** was observed at 427 nm, and there was a spectral shift to 432 nm upon metalation with Sn(IV). The Q-bands lie at 515, 547, 595 and 655 nm in the spectrum of **7a**. Upon metallation, two Q-bands were observed at 558 and 610 nm. The disappearance of two Q-bands confirms that the metallation of the free base porphyrin was successful. The log ϵ value for the B band of **7b** was calculated to be 4.64 in DMF. **Table 4** summarizes the optical properties of **7a** and **7b**.

The absorption spectra of **7a**, **7b** and **8** are shown in **Figure 41**. Porphyrin **7b** has the most red-shifted spectral band, and the lowest energy Q-band, was observed at 610 nm, and is hence red-shifted compared to that of **8** at 600 nm. This is important in the context of PDT studies reported

in Chapter 7. The reason for the observed red-shift of the **7b** spectral bands will be explored in greater depth through molecular modeling in Chapter 8.

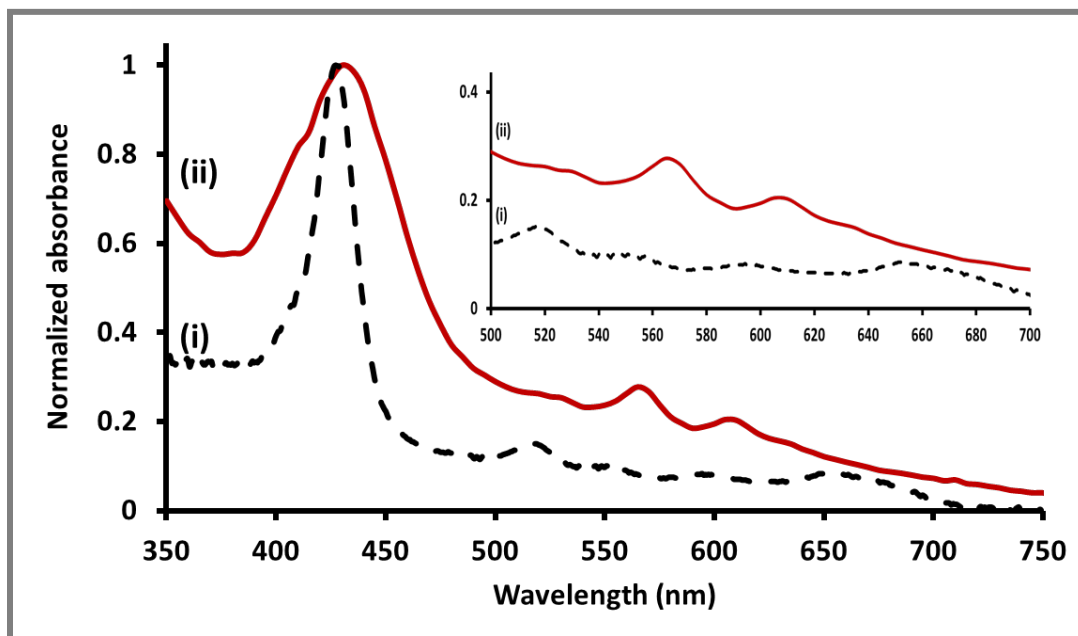


Figure 40. Ground state absorption spectra of **7a** and **7b**. Solvent = DMF.

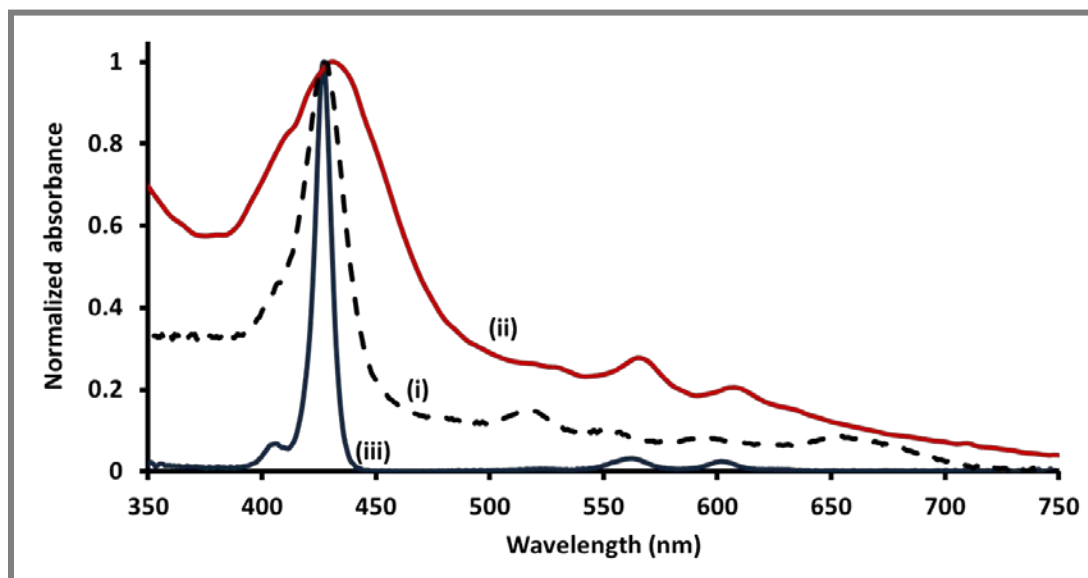


Figure 41. Ground state absorption spectra of **7a** (i), **7b** (ii) and **8** (iii). Solvent = DMF.

3.6. Photophysical properties of porphyrins

3.6.1. Fluorescence studies

The Φ_F value of **7a** was found to be 0.10 in DMF. The metallated porphyrin **7b** has a Φ_F value of less than 0.01 due to the enhanced rate of ISC, **Table 5**.

3.6.2. Singlet oxygen quantum yields

The Φ_Δ value of **7a** was low ($\Phi_\Delta = 0.15$) as would be expected for a free base porphyrin. Upon metallation, there was an increase in the Φ_Δ value to 0.36 in DMF, **Table 5**. The control compound **8** has a Φ_Δ value of 0.16.

Table 5. Summary of the spectroscopic and physicochemical properties of **7a**, **7b** and **8** in DMF.

	Q-bands/ nm	B-band/ nm	Φ_F	Φ_Δ
7a	515, 549, 595, 655	427	0.10	0.15
7b	570, 610	432	< 0.01	0.36
8	558, 600	427	---	0.16

3.7. Closing remarks

Novel BODIPY dyes that absorb within the range 600–700 nm were synthesized and characterized. The introduction of iodine atoms onto the BODIPY structures as heavy atoms enhanced the production of singlet oxygen. Through the addition of styryl and vinylene groups at the 3,5-positions, the main absorption bands of the BODIPY dyes were shifted towards the NIR region. The addition of styryls (BODIPY **4c**) and vinylene groups (BODIPY **1c** and **3c**) resulted in decreased singlet oxygen quantum yields relative to the iodinated BODIPY core dyes **1b**, **3b**,

and **4b**. This can be attributed to greater conformational flexibility and non-radiative decay after the extension of the π -conjugation system. When Knoevenegal condensation reactions with BODIPY **2b** and 3-thiophenecarboxyaldehyde were attempted to form **2c** in a similar manner to **1c** and **3c**, a red-shift of the absorption maximum of the crude product to ca. 640 nm was observed by UV-visible absorption spectroscopy, but further purification proved difficult.

Novel tetraacenaphthylporphyrins **7a** and **7b** were also synthesized and characterized. The metallated porphyrin analogue, **7b**, has a red-shifted lowest energy Q band relative to the analogous tetraphenylporphyrin complex, **8**, and a moderately high Φ_{Δ} value. The ability of these BODIPY and porphyrin dyes to produce singlet oxygen and their photostability makes the dyes potentially suitable for use as PS dyes in PACT and PDT.

Chapter 4: Pluronic® F-127 micelles encapsulation complexes

4.1. Pluronic® F-127 micelles encapsulation complexes

Three BODIPY dyes **1c**, **5** and **6** (**Figure 42**) were encapsulated in Pluronic® F127 micelles to enhance their solubility in water for applications in physiological environments, specifically PDT. **5** was encapsulated in Pluronic® F-127 micelles, while **1c** and **6** were encapsulated with folate-functionalized chitosan-coated Pluronic® F-127. The formation of BODIPY-Pluronic® micelles was confirmed by UV-visible spectroscopy since Pluronic® polymers are spectroscopically transparent. The micelles were then characterized by a series of different techniques.

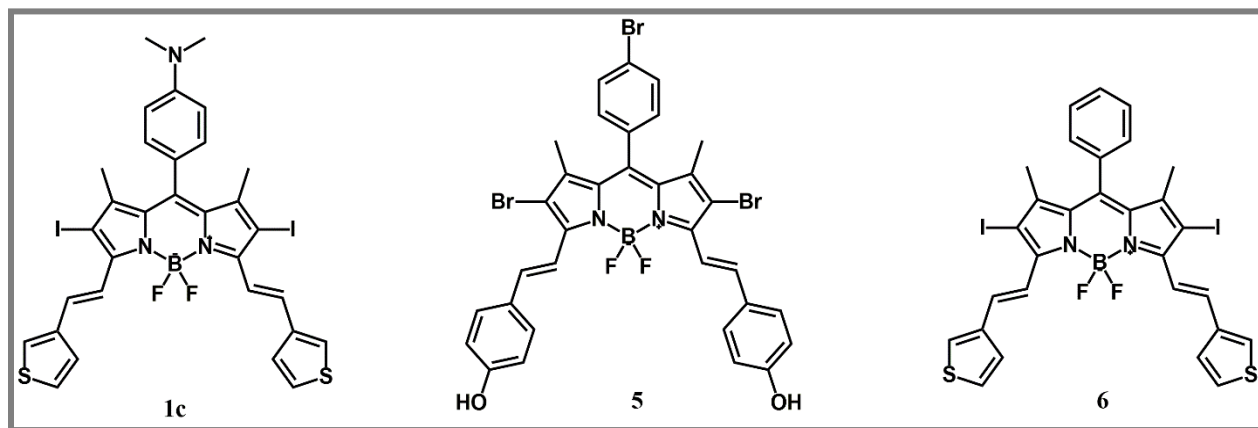


Figure 42. BODIPYs used for the preparation of encapsulation complexes with Pluronic® F-127 (**5**) and folate-functionalized chitosan-coated Pluronic® F-127 micelles (**1c** and **6**).

4.2. Characterization of BODIPY 5/ Pluronic® F-127 encapsulation complexes

4.2.1. Transmission electron microscope (TEM) images

Figure 43 shows the TEM micrographs of Pluronic® F-127 micelles before (A) and after (B) BODIPY **5** was encapsulated in the micelles (**5@PF127**). The plain Pluronic® F-127 micelles

were near spherical with a size range of 17.7–28.3 nm, and the encapsulation complexes had a similar morphology with an increased size range of 28.5–32.6 nm.

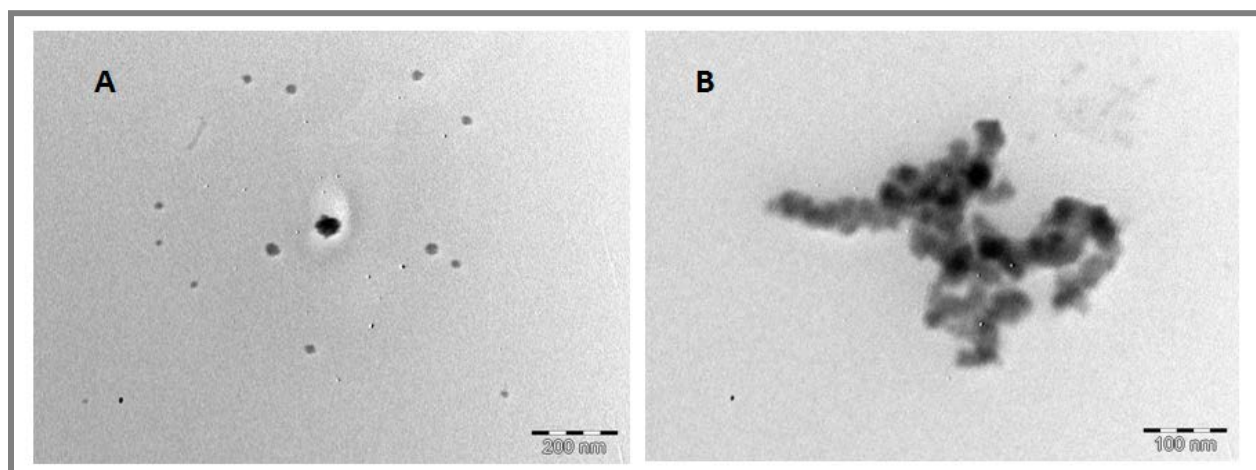


Figure 43. TEM micrographs for Pluronic® F-127 (A) and 5@PF127 (B) micelles. The Pluronic® F-127 micelles are relatively spherical with a size range of 17.7–28.3 nm, while the encapsulation complexes afforded similar morphology with a size range of 28.5–32.6 nm.

4.2.2. Dynamic light scattering (DLS) and Zeta (ζ) potential

DLS was used to determine the diameters of the micelles **Figure 44**, which is crucial in drug delivery since the micelles must be small enough to bypass the reticular endothelial system [87]. Their biocompatibility increases the efficacy of the photosensitizer drugs. The values obtained were 23.1 and 29.0 nm for Pluronic® F-127 (A) and 5@PF127 micelles (B), respectively, as shown in **Figure 44**.

ζ -potential analyses were conducted on the micelles, and phase solubility studies were carried out for the micellar solutions, **Table 6**. The ζ -potential is an indicator of stability. The values obtained for all samples are consistent with moderate stability, so the complexes can be expected to resist aggregation in water [103].

Table 6. Zeta potentials of complexes prepared for phase solubility studies and 5@PF127 in water.

BODIPY 5 (mg)	Pluronic® F-127 (%)	Mean ζ -potential (mV)
3	0	-8.08
3	5	-10.4
3	10	-16.0
3	15	-13.8
3	20	-12.5
3	25	-10.7
5@PF127	---	-24.2

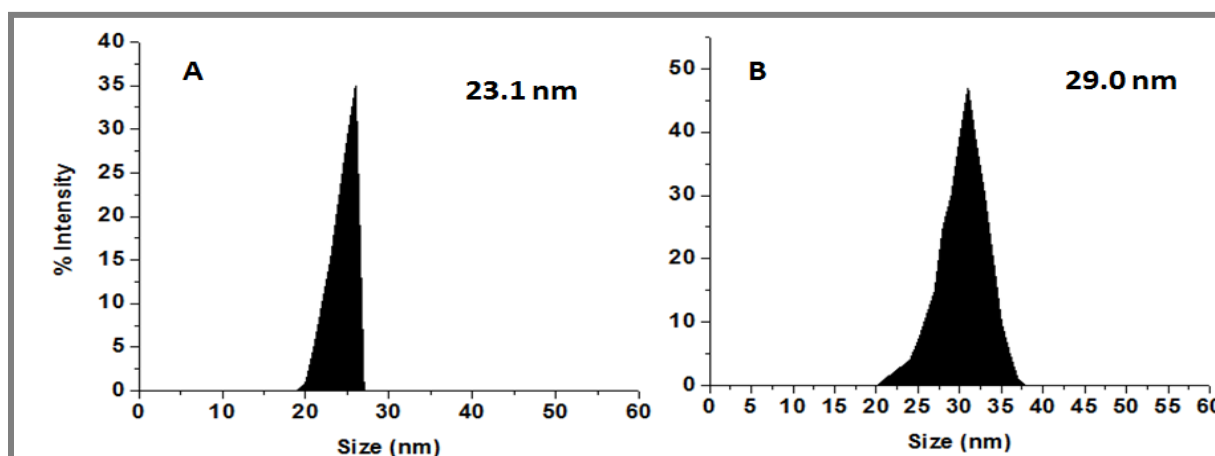


Figure 44. Dynamic light scattering of Pluronic® F-127 (A) and 5@PF127 (B) micelles. Solvent = water.

4.2.3. Determination of the location of BODIPY 5 in the Pluronic® F-127 micelles using UV-visible absorption spectroscopy

Pluronic® F-127 is spectroscopically transparent in the UV-visible absorption region in a similar manner to CDs, so the spectroscopic properties of the dyes are retained and the ratio of dye to PF-127 in the encapsulation complex samples can be calculated by UV-visible spectroscopy. Tehrani-Bagha and Holmberg highlighted that the location of the dye in micelles could be determined indirectly through UV-visible absorption spectroscopy [112]. This is done by comparing the absorption spectra of the dye in micellar solutions of solvents with variable solubilities. The results obtained for 5@PF127 are shown in **Figure 45**. The spectra in 50% (i) ethanol and 100% ethanol (ii) are very similar, while the main band in water (iii) is somewhat broadened and red-shifted due to aggregation, but the three spectra are broadly similar. When 5@PF127 was dissolved in petroleum ether, as a typical non-polar solvent, no absorbance intensity was observed due to a lack of solubility, and thus this spectrum is not shown in **Figure 45**. On the basis of these observations, it can be concluded that the dye is most likely located in the moderately polar palisade region of the micelles [112].

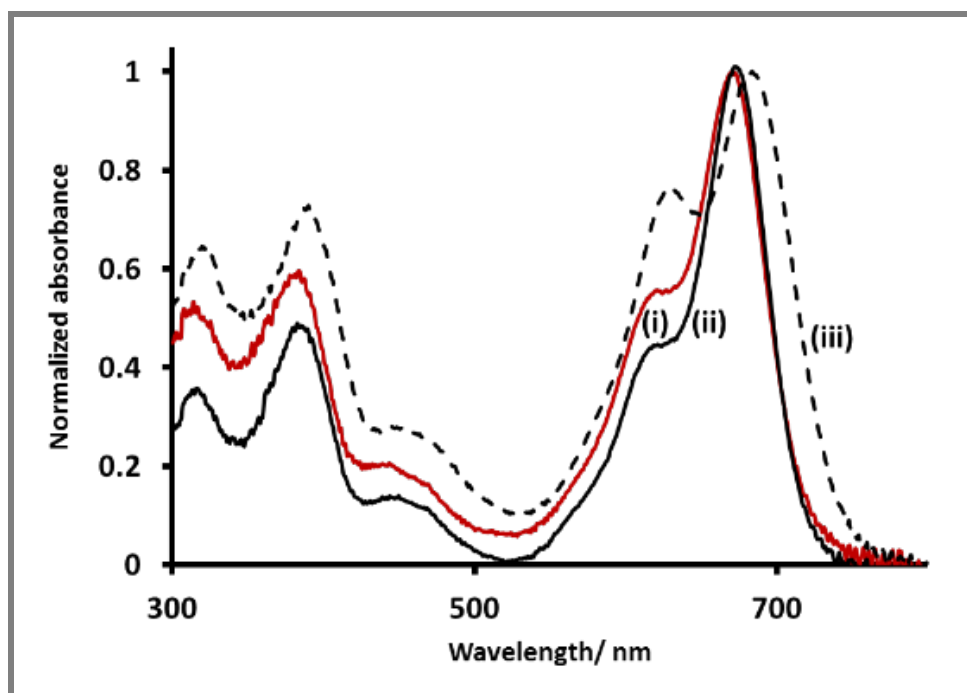


Figure 45. UV-visible absorption spectra of 5@PF127 in micellar solutions of different solvents; a water:ethanol mixture (1:1 v/v) (i), ethanol (ii) and water (iii).

4.2.4. Phase solubility studies

The phase-solubility studies were successfully conducted for BODIPY 5 in water over a range of Pluronic® F-127 concentrations. The results are shown in **Figure 46** and the summary listed in **Table 7**. The solubility of BODIPY 5 increased with increasing concentration of Pluronic® F-127 in water until the CMC (1.06×10^{-4} mol/L at 25 and 37 °C) was reached (**Figure 46**). The CMC values obtained are similar to values reported for Pluronic® F-127 in the literature [112, 154]. The linear increase in solubility of BODIPY 5 as a function of Pluronic® F-127 is characteristic of an A_N -type system which suggests the formation of water-soluble complexes. The slope of the positive linear line was less than one, indicating the formation of a complex at a 1:1 molar ratio of the BODIPY 5 guest molecule and the Pluronic® F-127 host in water.

The assumption of 1:1 complex formation enabled the calculation of the apparent stability constant of the binary complexes using the linear regression method with Equation 4 in chapter 1. High stability constants were obtained for the BODIPY 5 encapsulation complexes of Pluronic® F-127 (5.47 and 3.19 L/mol at 25 and 37 °C respectively), and this represents a possible limitation in drug release from the complex. Optimal stability constant values have been reported to lie in the 1–3 range [108]. At 25 °C, a CE value of 0.08 was calculated using Equation 5, showing that approximately 8 out of every 100 micelle molecules form complexes with BODIPY 5, while at 37 °C a CE value of 0.64 was derived, which suggests that 64 out of 100 micelles molecules form complexes with BODIPY 5.

Table 7. The summary of the phase solubility studies for BODIPY 5 with Pluronic® F-127 in water.

	Temp. (°C)	slope ^a	R ²	K _{1:1} (M ⁻¹) ^b	Log(K _{1:1})	CE ^b
BODIPY 5 and	25	0.0863	0.922	293600	5.47	0.079
Pluronic® F-127	37	0.0408	0.9997	1537	3.19	0.635

^aValues obtained directly from the phase solubility diagram. ^bValues calculated from phase solubility diagram data using Equations 4 and 5 in Chapter 1.

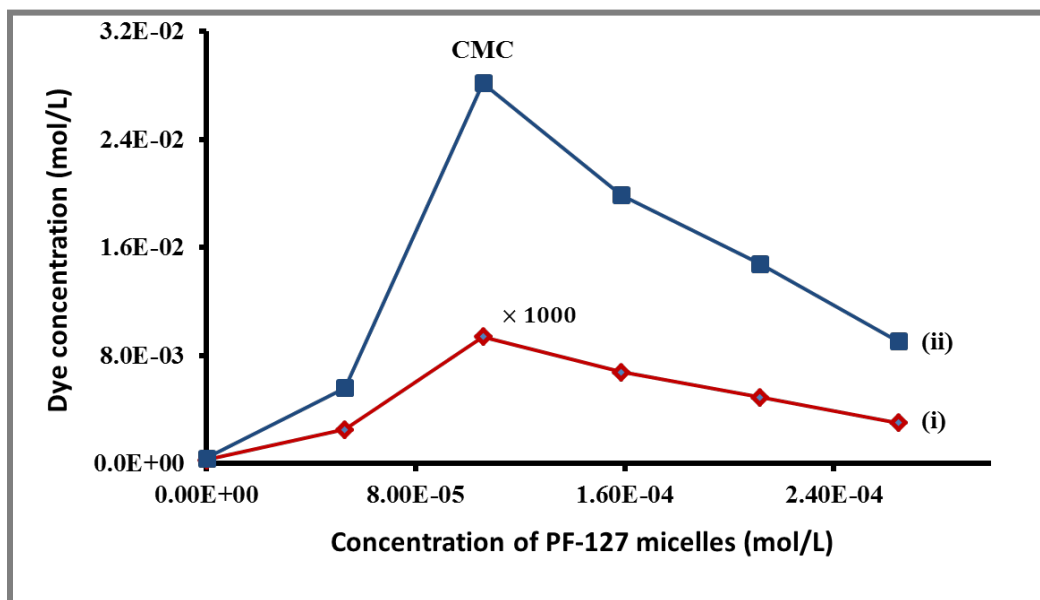


Figure 46. Phase-solubility diagram of BODIPY 5 with Pluronic® F-127 at different temperatures. Solvent = water.

4.2.5. Singlet oxygen quantum yields

The Φ_{Δ} value for 5@PF127 in water was estimated to be 0.30 by using ADMA as a singlet oxygen scavenger, **Figure 47**. The Φ_{Δ} quantum yield for 5 in ethanol was 0.16 [95]. The Φ_{Δ} value is hence higher in micellar-water solution. This is consistent with the literature, as it has been reported that upon encapsulation the polymer effectively supports the dye in its structure, and the Φ_{Δ} value can increase as a result [87].

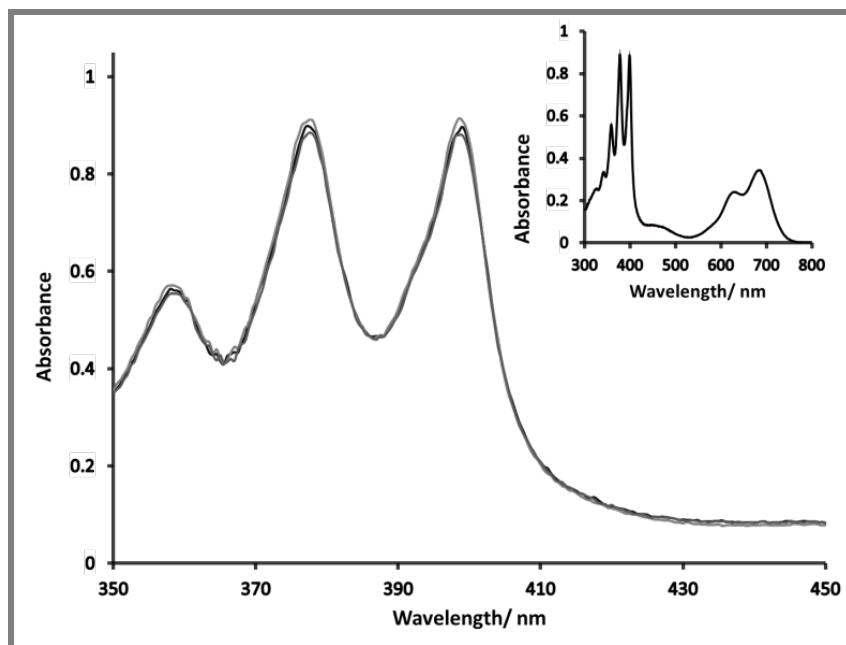


Figure 47. Ground state absorption spectra recorded for the determination of the Φ_{Δ} value of 5@PF127 in the presence of ADMA. Additional data sets were omitted for clarity. Solvent = water.

4.3. Characterization of BODIPY 1c and 6 encapsulation complexes of folate-functionalized chitosan-coated Pluronic® F-127 Micelles

The Φ_{Δ} value of BODIPY 1c was found to be 0.13 and 0.40 in DMSO and ethanol (**Table 4**), respectively, and that of BODIPY 6 was determined to be 0.40 in DMSO. The two dyes were chosen for inclusion in folate-functionalized chitosan-coated Pluronic® F-127 micelles due to their moderately high Φ_{Δ} values. The goal of this study was to explore whether micelles of this type can be successfully used in PDT activity studies with MCF-7 human breast cancer cell line and hence merit further in-depth study.

4.3.1. Fourier Transform-Infrared of folate-functionalized chitosan

FT-IR spectroscopy is a qualitative technique that can be used to determine the presence of functional groups and identify fingerprint signals for different compounds. In **Figure 48**, the chitosan FT-IR spectrum had a broad stretch between 3000 and 3500 cm^{-1} due to the overlap of -NH and -OH functional groups overlap due to hydrogen bonds. The C-H stretch was observed at 2873 cm^{-1} . The amide I (-N-H) peaks lie at 1640–1588 cm^{-1} , while the amide II and III stretches are observed at 1419 and 1375 cm^{-1} , respectively. The band at 1061 cm^{-1} arises from the vibration of C-O-C in the chitosan structure. Folic acid has a complex structure consisting of glutamic acid, *p*-aminobenzoic acid and heterocyclic pteridine. The broad peak between 3400–3600 cm^{-1} was attributed to the -OH and -NH groups of the glutamic and pterinic parts of folic acid, respectively. Intense peaks at 1689, 1602 and 1481 cm^{-1} arise from -C=O stretch, -NH bends and vibrations of the C-C bonds in the pterinic rings, respectively [109].

Figure 48 B shows the superimposed spectra of chitosan (black), folic acid (blue) and folate-functionalized chitosan (red). Upon functionalization, there was an apparent increase in the intensity of the broad band at 3000–3500 cm^{-1} due to the overlap of -OH and -NH vibrations. The bands associated with the carbonyl functional group of the folic acid are reduced in intensity upon functionalization. New peaks formed at 1510 and 1602 cm^{-1} associated with amide I and II bands, respectively, confirm the formation of the folate-functionalized chitosan.

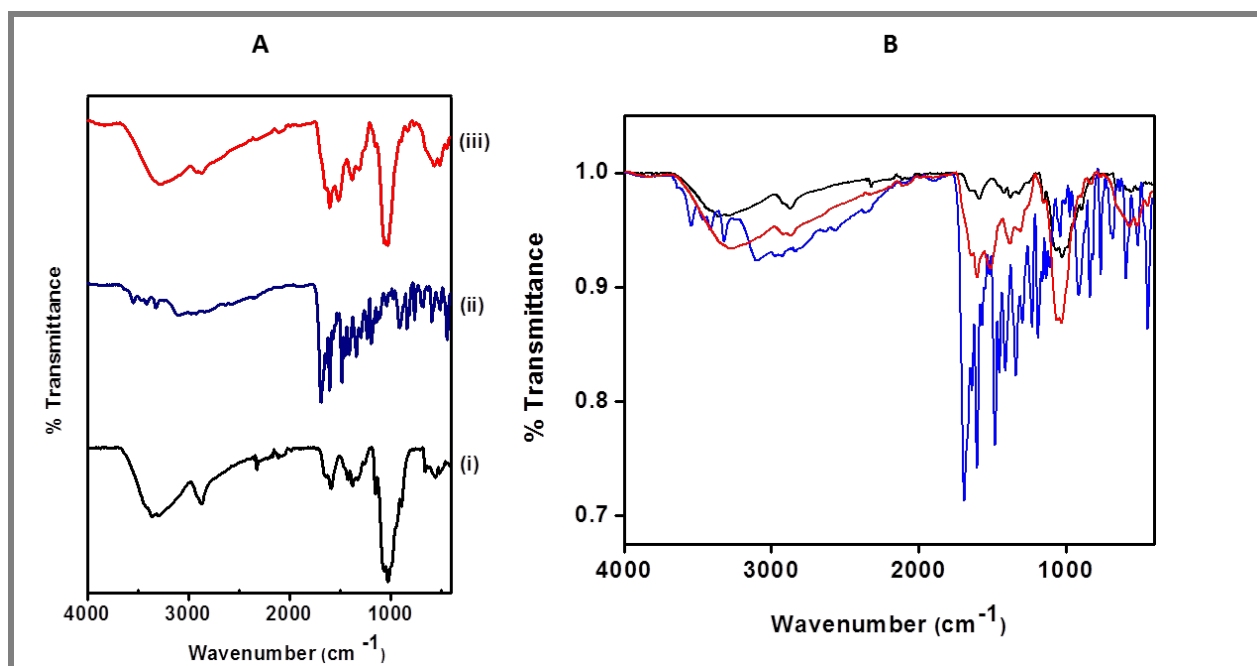


Figure 48. Infrared spectra of chitosan (i), folic acid (ii) and folate-functionalized chitosan (iii) (A), and for clarity the superimposed spectra of chitosan (black), folic acid (blue) and folate-functionalized chitosan (red) (B).

4.3.2. X-Ray Diffraction

The XRD technique was used to determine the crystalline behavior of the samples. The diffractograms are shown in **Figure 49**. The diffraction pattern of chitosan has an intense peak at $2\theta = 19.95^\circ$, and a broad peak was observed for folic acid at $2\theta = 10$ and 30° . The folate-functionalized chitosan showed a highly amorphous structure and hence a lack of crystallinity characterized by a peak at $2\theta^\circ$. The diffractogram of BODIPY **1c** and its encapsulation complex with Pluronic® F-127 micelles alone (**1c**@PF127) showed highly crystalline patterns. Upon coating of the **1c**@PF127 micelles with folate-functionalized chitosan to give **1c**@PF127@FC, there was a change from a crystalline form to an amorphous structure as observed in **Figure 49**. This indicates that there was a successful modification of the micelles in this regard. The

diffractograms for BODIPY **6**, **6**@PF127 and **6**@PF127@FC, followed the same trends as the ones shown for BODIPY **1c** in **Figure 49**.

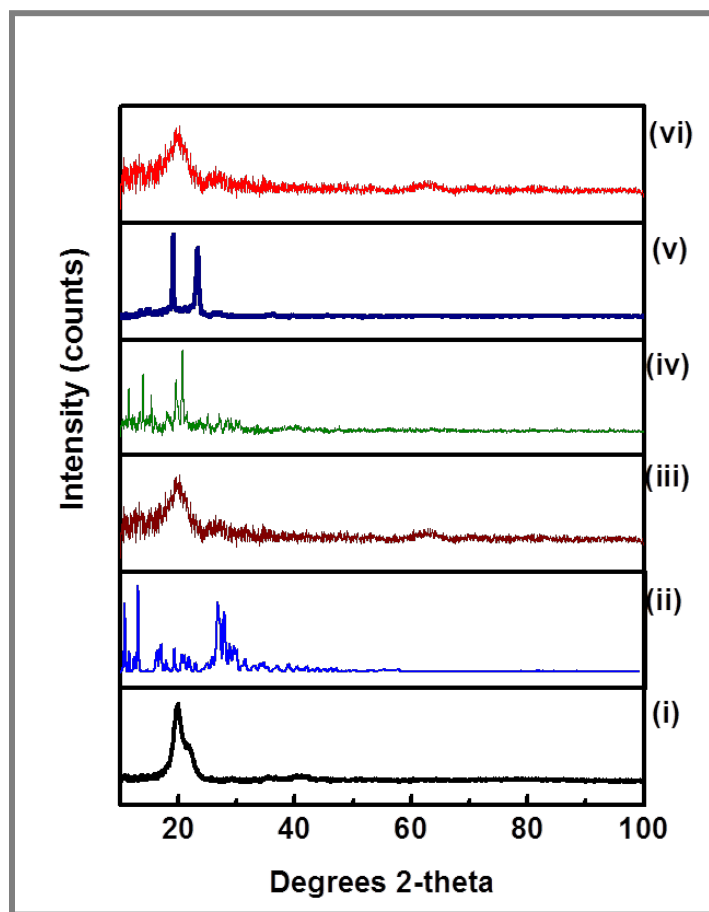


Figure 49. X-ray diffractograms of chitosan (i), folic acid (ii), folate-functionalized chitosan (iii), BODIPY **1c** (iv), **1c**@PF127 (v) and **1c**@PF127@FC (vi).

4.3.3. Transmission electron microscope

TEM was used to determine the morphology and sizes of the micelles prepared for this work to determine whether they are small enough to bypass the reticular endothelial system and are hence biocompatible. **Figure 50** shows the TEM micrographs of plain Pluronic® F-127 micelles (A), **1c**@PF127 (B) and **1c**@PF127@FC (C) micelles. The micelles appear to be reasonably

spherical in shape. Upon encapsulation of the dye with Pluronic® F-127 micelles, there was an increase in size from 6–12 (plain Pluronic® F-127 micelles) to 28–35 nm (**1c**@PF127). After coating with folate-functionalized chitosan, there was a further apparent increase to 52–60 nm (**1c**@PF127@FC). Similar trends were observed for **6**@PF127 (D) and **6**@PF127@FC (E) in **Figure 50**, where the micelles increased in size from 13–22 to 28–41 nm, respectively.

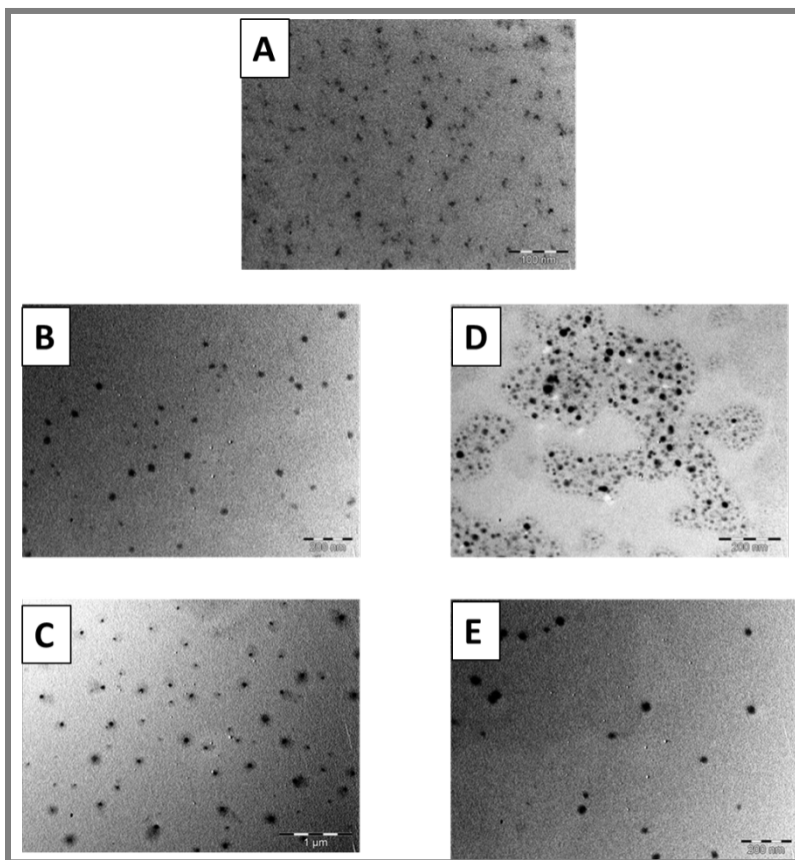


Figure 50. TEM micrographs of Pluronic® F-127 micelles (A), **1c**@PF127 (B), **1c**@PF127@FC (C), **6**@PF127 (D) and **6**@PF127@FC (E) micelles. The Pluronic® F-127 micelles are relatively spherical with a size range of 6–12 nm. Upon encapsulation of the dye there was an increase to 28–35 nm (**1c**@PF127), and a further apparent increase to 52–60 nm (**1c**@PF127@FC) after coating with folate-functionalized chitosan. Similar trends were observed for **6**@PF127 (D) and

6@PF127@PF (E), where the micelles increased from 13–22 to 28–41 nm, respectively. Solvent = water.

4.3.4. Dynamic light scattering

Dynamic light scattering (DLS) was used to determine the diameters of the micelles, **Figure 51**. The results obtained from DLS complement those derived from TEM. The values obtained were 11.7 nm for plain Pluronic® F-127 (A), 37.8 nm for **1c**@PF127 (B), 50.7 nm for **1c**@PF127@FC (C), 24.4 nm for **6**@PF127 (D) and 32.7 nm for **6**@PF127@FC (E) micelles (**Figure 51**). The overall trend observed was consistent with an increase in the size of micelles upon encapsulation of the hydrophobic PS dyes [87].

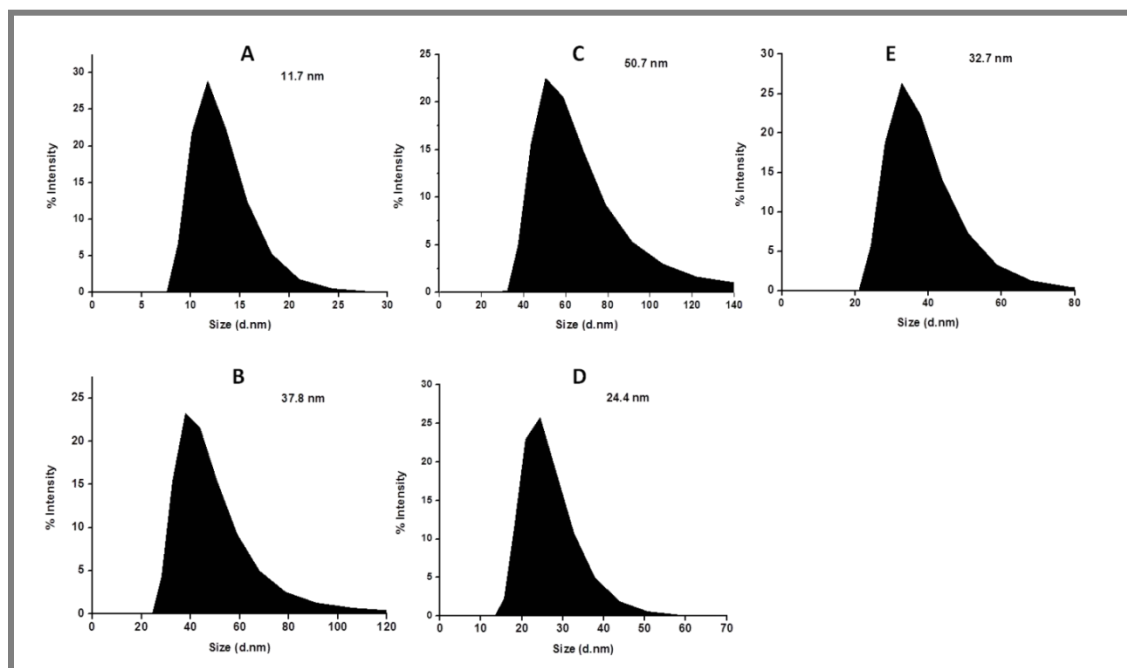


Figure 51. Dynamic light scattering of plain Pluronic® F-127 micelles (A), **1c**@PF127 (B), **1c**@PF127@FC (C), **6**@PF127(D) and **6**@PF127@FC. Solvent = water.

4.3.5. Determination of the location of dyes in Pluronic® F-127 micelles

The location of the dyes in the micelles was determined indirectly through UV-visible absorption spectroscopy through a comparison of the absorption spectra of the dye measured in micellar solutions in solvents of different polarities. The UV-visible absorption spectra of BODIPY **1c** in water and petroleum ether were found to be very similar, **Figure 52 A**. The main absorption bands lies at 636 nm in both. According to Tehrani-Bagha and Holmberg, the similarity in spectra measured in polar and non-polar solvents suggests that BODIPY **1c** is most likely located in the core region of the micelles [112]. The UV-visible absorption spectra of micellar solutions of BODIPY **6** in water, petroleum ether and ethanol have somewhat similar band morphologies. The main absorption bands were observed at 665 nm, with a slight red-shift when in water, **Figure 52 B**. There are, however, marked differences observed between 601–610 nm. This suggests that BODIPY **6** could be distributed between the core and palisade regions of the micelles.

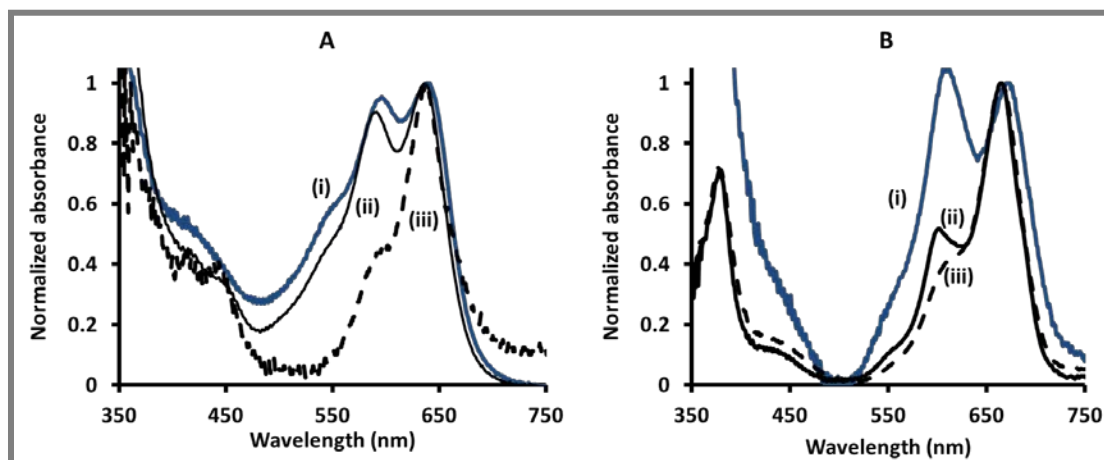


Figure 52. Ground state absorption spectra of BODIPY **1c** in Pluronic® F-127 micellar solutions in water (i), petroleum ether (ii) and ethanol (iii) (LEFT, A), and of BODIPY **6** in Pluronic® F-127 micellar solution of water (i), petroleum ether (ii) and ethanol (iii) (RIGHT, B).

4.3.6. Phase solubility studies

The phase-solubility studies for BODIPYs **1c** and **6** could not be successfully conducted due in part to limitations imposed by the low yield of **1c** and solvent solubility issues with **6**. Since the studies conducted for BODIPY **5** were successful and UV-visible absorption spectra have been recorded for various micellar solutions of **1c** and **6**, it can still be inferred that encapsulation by Pluronic® F-127 solubilizes BODIPY dyes at ambient temperatures and that their solubility is likely to be further enhanced at physiological temperatures.

4.3.7. Singlet oxygen quantum yields

Upon encapsulation with folate-functionalized chitosan coated Pluronic® F-127 micelles the Φ_{Δ} value that was calculated for **1c**@PF127@FC in water was estimated to be $\Phi_{\Delta} = 0.59$ when ADMA was used as a singlet oxygen scavenger. This is higher than the value of 0.40 that was obtained in ethanol (**Table 4**). It has been reported that the encapsulation of dyes with Pluronic® F-127 can increase the Φ_{Δ} values due to support of the dye by the polymer [155]. There may also be scope for changes in the photophysical and photochemical properties of the dye due to protonation of the dimethylamino moieties [156, 157]. In contrast, the Φ_{Δ} value for **6**@PF127@FC was estimated to decrease to 0.29 relative to the value of 0.40 that was obtained in DMSO (**Table 4**).

4.4. Closing remarks

The modification of the Pluronic® F-127 micelles with folate-functionalized chitosan was successful. The BODIPY dyes **1c** and **6** were successfully encapsulated with folate-functionalized chitosan chitosan-coated Pluronic® F-127 micelles and **5** with Pluronic® F-127 micelles. Stable spherical micelles were formed, with appropriate sizes (< 100 nm) for efficient

drug delivery. The singlet oxygen quantum yield values in water that were derived with ADMA as a scavenger demonstrated that $^1\text{O}_2$ can be generated in aqueous solution by the encapsulation micelles. The next step of the study was to examine their photodynamic therapy activity. This aspect of the study will be described in Chapter 7.

Chapter 5: Cyclodextrin inclusion complexes

5.1. Cyclodextrins inclusion complexes

The ease of formation of the cyclodextrin inclusion complexes of molecular dyes depends on the size of the hydrophobic dye molecule and the cavity size of CDs [24, 26]. Complexation does not occur if a molecule is too large to fit into the CD cavity, while weak interactions occur when the molecule is too small to fit in the CD cavity [35]. Relatively large molecules, such as porphyrins and styrylated BODIPY dyes, can still form complexes if parts of the molecule fit into the CD cavity. **Figure 53** shows the dyes used for preparation on inclusion complexes in this study. Inclusion complexes were prepared using BODIPY **4c** and porphyrin **7b** to evaluate the possibility of achieving enhanced water-solubility for these hydrophobic dyes in applications such as PDT and/or PACT. The complexes were prepared using different complexation methods; co-precipitation, kneading and the ethanol-water system [88, 136, 158]. The solid complexes were characterized by using UV-visible absorption, FT-IR and XRD spectroscopy.

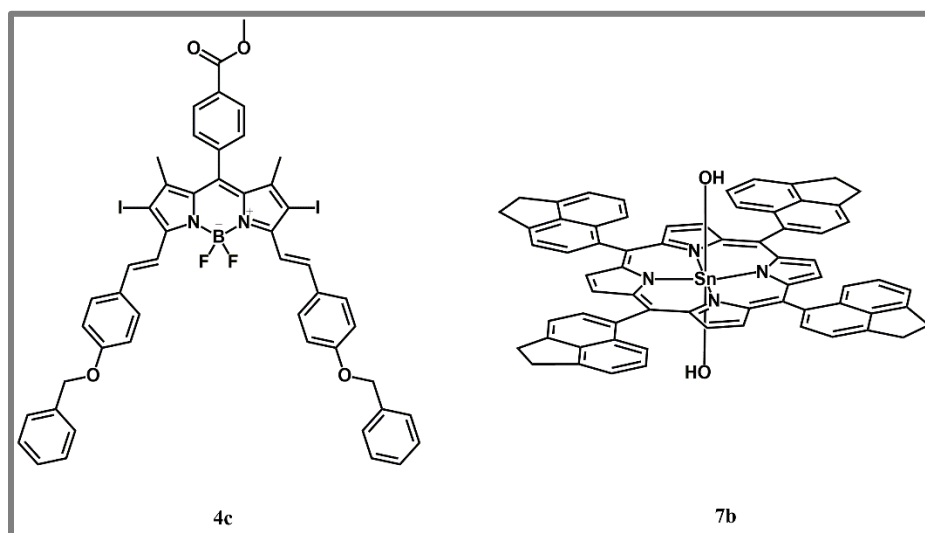


Figure 53. BODIPY **4c** and porphyrin **7b** used for the preparation of CDs inclusion complexes.

5.2. Characterization of **4c** and **7b** inclusion complexes with β CD and m β CD

The inclusion complexes of the BODIPY and porphyrin compounds were prepared using a procedure derived from the ethanol-water method described in Chapter 3, where DMSO was used to dissolve the dyes instead of ethanol [88, 136].

5.2.1. X-ray Powder Diffraction

The XRD diffractograms of **7b**, β CD, a physical mixture of **7b** and β CD and the **7b**/ β CD inclusion complex are shown in **Figure 54**. The X-ray diffractogram of β CD and porphyrin **7b** exhibited crystalline behavior characterized by sharp peaks at multiple diffraction angles. **7b** and β CD physical mixtures are consistent with superimposed diffractograms, while the inclusion complex **7b**/ β CD showed a completely different crystalline behavior from the parent dye and β CD. The new crystalline material had relatively intense peaks concentrated below 30° with some peak broadening. In a similar manner, the inclusion complex of BODIPY **4c** with β CD, **4c**/ β CD, was successfully formed and the crystalline structure observed by XRD was also found to be different from the parent materials in a similar manner.

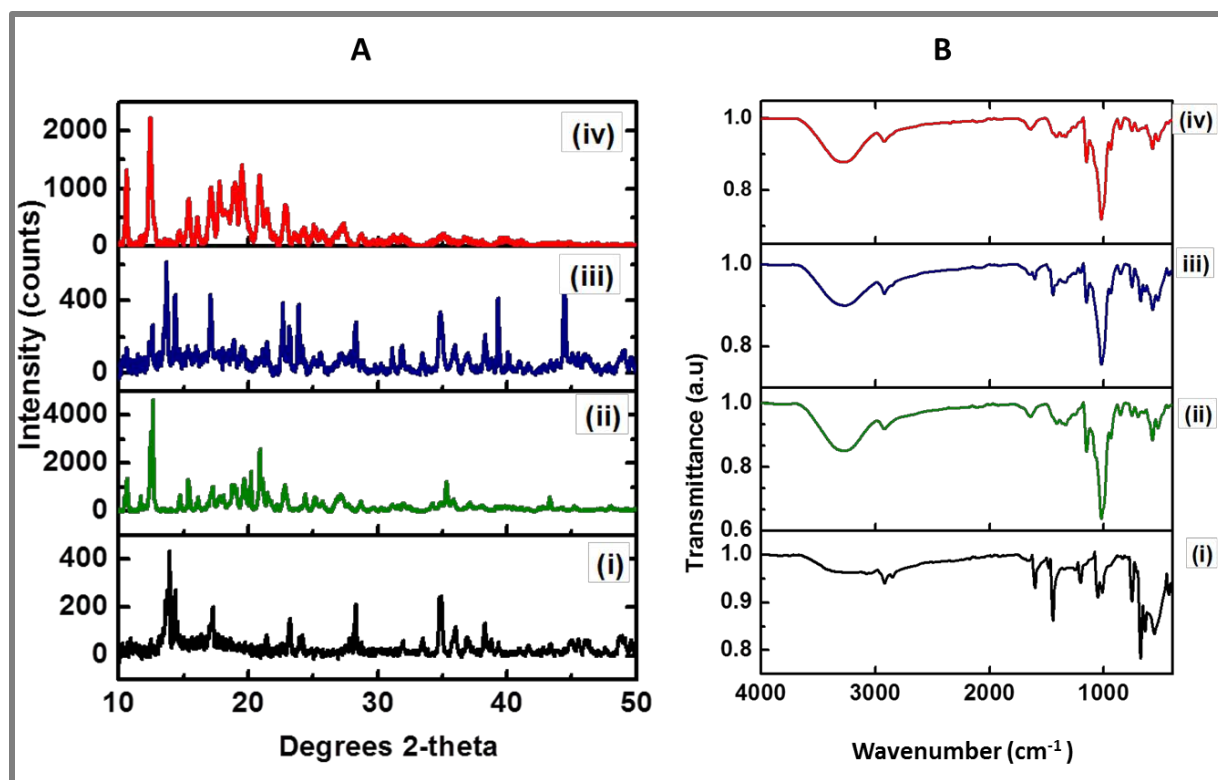


Figure 54. X-ray diffractograms of **7b** (i), β CD (ii), a physical mixture of **7b** and β CD (iii), and the **7b**/ β CD inclusion complex (iv) (LEFT, A), and the IR spectra of **7b** (i), β CD (ii), a physical mixture of **7b** and β CD (iii), and the **7b**/ β CD inclusion complex (iv) (RIGHT, B).

In a similar manner, the XRD diffractograms of **7b** (i), m β CD (ii), a physical mixture of **7b** and m β CD (iii) and **7b**/m β CD inclusion complex (iv) are shown in **Figure 54 A**. The diffractogram of m β CD has an amorphous peak at ca. 15°. **7b** and m β CD physical mixture diffractogram was again found to be a superimposition. The diffractogram of **7b**/m β CD showed a completely different crystalline behavior from the parent dye and no amorphicity from m β CD confirming the formation of an inclusion complex. The new crystalline material had relatively intense peaks below 30°. Similar trends for changes in crystallinity were observed by XRD. The inclusion complexes of BODIPY **4c** with m β CD (**4c**/m β CD).

The IR spectra of **7b** (i), m β CD (ii), a physical mixture of **7b** and m β CD (iii), and **7b**/m β CD inclusion complex (iv) are shown in **Figure 55 B**. The m β CD stretch at 3392 cm⁻¹ (OH) shifts to 3285 cm⁻¹ upon complexation with **7b**. There is also an increase in the intensity of this band. The C-H stretches of m β CD and **7b** in the 2930–2847 cm⁻¹ range are replaced with one stretch at 2925 cm⁻¹ upon formation of the **7b**/m β CD inclusion complex. Below 2000 cm⁻¹, the fingerprint region of the inclusion complex although unique, was still somewhat similar to the m β CD fingerprint region. Spectra for the physical mixtures of the dyes and m β CD were different from those of the inclusion complexes. Similar trends were also observed in **Figure 55 B**. This confirms the inclusion of **7b** into the cyclodextrins cavities. The formation of **4c**/m β CD inclusion complexes was confirmed in a similar manner by changes in the IR stretches and bends shifts.

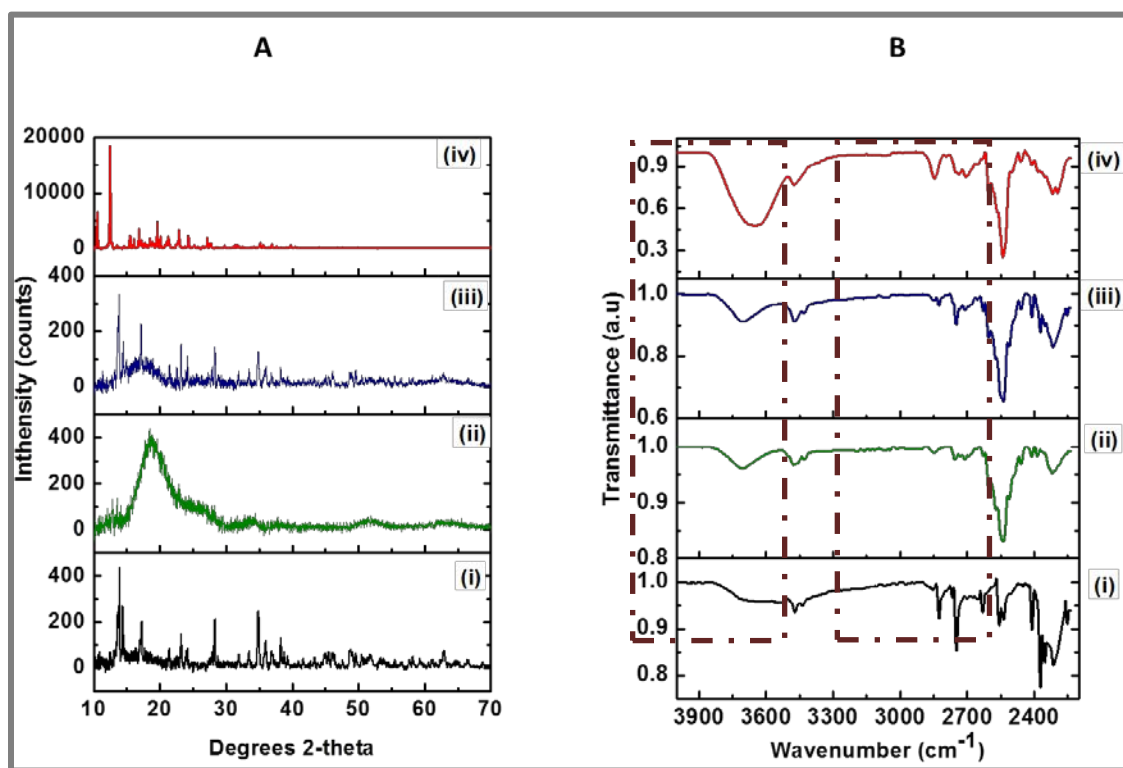


Figure 55. XRD diffractograms of **7b** (i), m β CD (ii), a physical mixture of **7b** and m β CD (iii), and the **7b**/m β CD inclusion complex (iv) (LEFT, A), and the overlaid IR spectra (RIGHT, B).

5.2.2. UV-visible absorption spectroscopy

The absorption spectra of the dyes before and after encapsulation with cyclodextrins are shown in **Figure 56**. There was a shift in the main absorption band of **4c** (**Figure 56 A**) from 672 nm (DMSO) to 705 nm when encapsulated with β CD, and to 710 nm, when encapsulated with $m\beta$ CD. For **7b**, the Soret band shifted from 430 nm (DMF) to 465 nm upon encapsulation with both β CD and $m\beta$ CD. The **7b**/ $m\beta$ CD inclusion complex had an enhanced Q-band at 570 nm. The solubility of the hydrophobic dyes was enhanced in water after the formation of inclusion complexes with cyclodextrins proving the successful formation of the inclusion complexes.

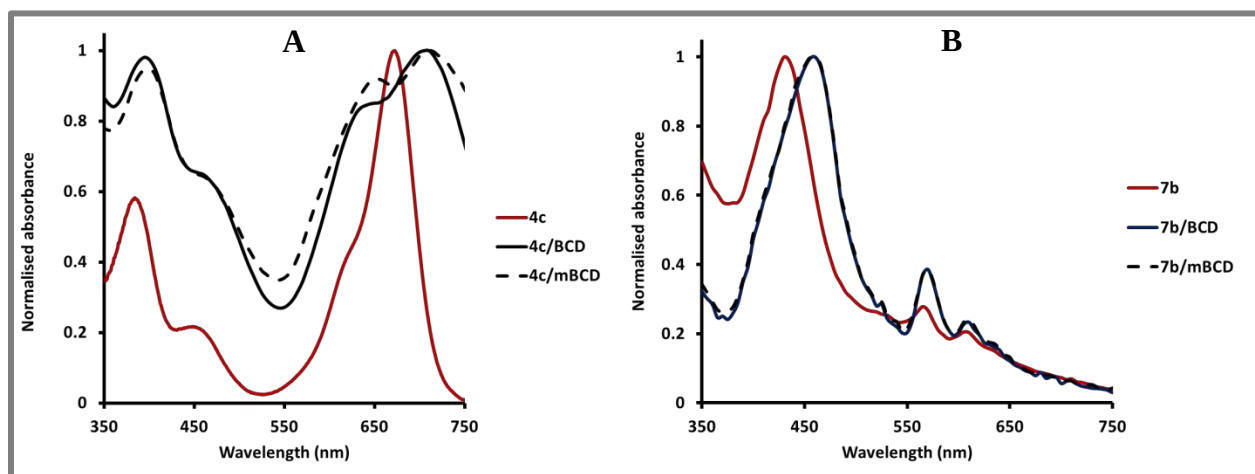


Figure 56. Ground state absorption spectra of **4c**, **4c**/ β CD and **4c**/ $m\beta$ CD (LEFT, A), and **7b**, **7b**/ β CD and **7b**/ $m\beta$ CD (RIGHT, B). Solvent = water for the complex, DMSO for **4c** and DMF for **7b**.

5.2.3. Singlet oxygen quantum yields

The Φ_{Δ} values for **4c**/ $m\beta$ CD and **7b**/ $m\beta$ CD in water were estimated to be 0.51 and 0.09, respectively, when ADMA was used as the singlet oxygen scavenger. The Φ_{Δ} value for **4c** in

DMF ($\Phi_{\Delta} = 0.52$) was the same as the value obtained for the **4c**/m β CD inclusion complex in water. In contrast, **7b**/m β CD had a lower value of $\Phi_{\Delta} = 0.09$, which is a marked decrease from the value of 0.36 calculated for **7b** in DMF. Lower and higher Φ_{Δ} values have been reported for similar dyes in water solubilizing agents have been reported previously in the literature [86, 87], since the photophysical of the dyes can be either enhanced or quenched upon inclusion.

5.3. Closing remarks

The porphyrin and BODIPY cyclodextrin inclusion complexes were successfully prepared and characterized. The β CD inclusion complexes were partially water-soluble, while the solubility of both dyes was greatly enhanced upon complexation with m β CD. Singlet oxygen measurements with ADMA demonstrated that $^1\text{O}_2$ can be generated by the inclusion complexes in aqueous solution. The complexation of the porphyrin and BODIPY dyes with cyclodextrin enhanced the water solubility photosensitizer dyes, so the next step was to determine their photodynamic therapy activity. This aspect of the study will be described in Chapter 7.

Chapter 6: Photodynamic antimicrobial therapy

6.1. Photodynamic Antimicrobial Chemotherapy (PACT)

This chapter describes the results of PACT studies for treatments of *Staphylococcus aureus* with BODIPY **4c** and **4c**/m β CD.

6.2. PACT studies on *Staphylococcus aureus*

PACT studies were carried out in aqueous solution with 0.2% DMF for both **4c** and **4c**/m β CD. **4c** is not soluble in water, while, in contrast, the **4c**/m β CD inclusion complex is soluble in aqueous solution. To enable direct comparison, both **4c** and **4c**/m β CD were initially dissolved in 0.2% DMF water solution then PBS was used to prepare stock solutions. 5 μ g/ml of each dye solution was used for the studies following optimization. The effect of 0.2% DMF in PBS on *Staphylococcus aureus* has been shown to be negligible [90]. PBS solution alone was used as a control and had no effect on the bacteria [67, 90]. Zero irradiation time was used to define 100% bacteria colony survival in **Figures 57** and **58**. Dark cytotoxicity studies demonstrated that treatment with the BODIPY alone had some a dark toxicity effect with a log reduction (LR) value of 0.24 after 180 min, **Figure 57**. In contrast, when **4c** is encapsulated by m β CD, this dark toxicity decreased markedly, and an LR value of 0.049 was obtained, **Figure 57**.

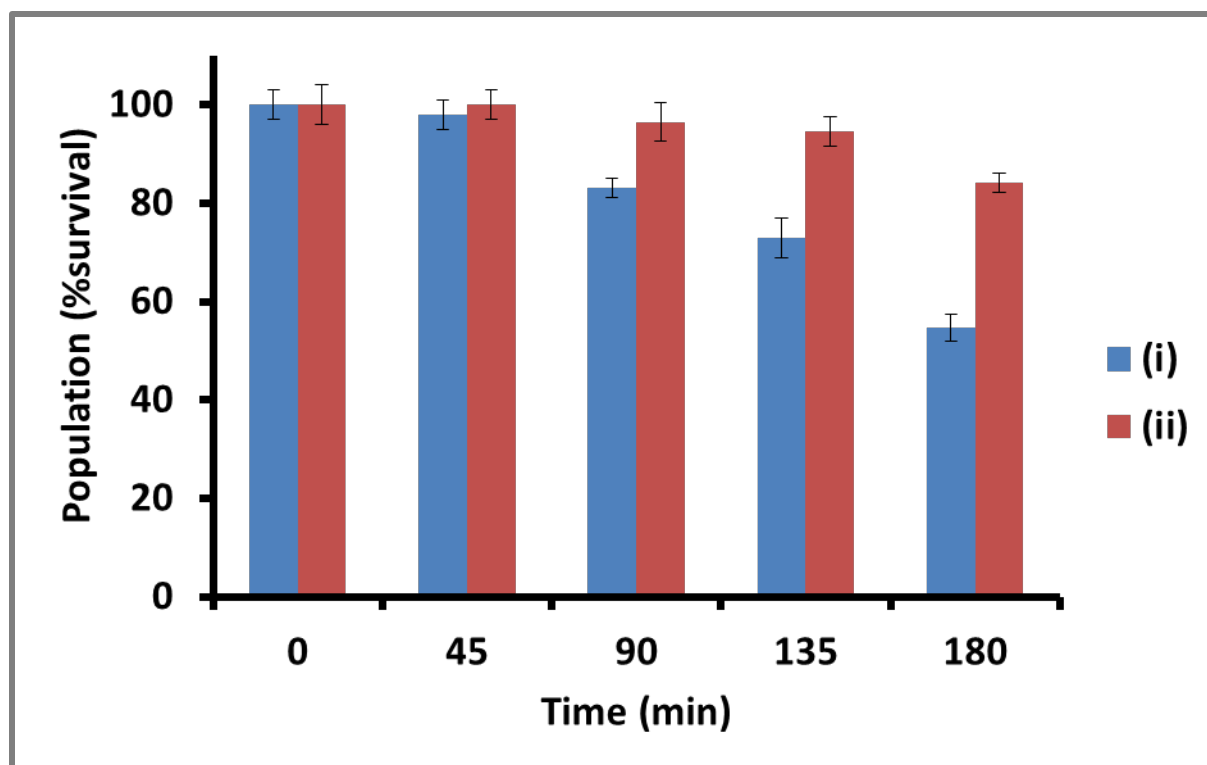


Figure 57. Dark cytotoxicity studies for *Staphylococcus aureus* during treatment with **4c** (i) and **4c/mβCD** (ii) in 0.2% DMF in PBS.

The PACT activities of BODIPY **4c** and **4c/mβCD** are shown in **Figure 58**, while **Figure 59** shows the remaining colonies of *Staphylococcus aureus* at zero control and after 180 min irradiation with **4c** and **4c/mβCD**. The survival of the bacteria colonies decreased with increasing irradiation time. 50% (LR = 0.21) of the bacteria colonies survived treatment with **4c** after 180 min. In contrast, **4c/mβCD** photoinactivation of *Staphylococcus aureus* resulted in 5% survival (LR = 1.28). The use of mβCD enhanced the water-solubility of **4c**, and this increased the photoinactivation of *Staphylococcus aureus*. A summary of the results is shown in **Table 8**.

Staphylococcus aureus is a gram-positive bacteria strain which can be readily photoinactivated by positively charged, negatively charged or neutral photosensitizers [113]. Neutral

photosensitizers such as BODIPY **4c** can easily penetrate the *Staphylococcus aureus* cell wall, which is comprised of a capsule and a thick peptidoglycan layer. Other gram-positive bacteria such as *Candida albicans* have thicker cell walls made up of polysaccharides and proteins which usually require treatment with positively charged photosensitizers for effective photoinactivation to occur [159]. The minimal dark toxicity of the inclusion complex confirmed that the observed inactivation was almost entirely due to the irradiation of the BODIPY dye at the absorption maxima and the subsequent production of $^1\text{O}_2$ *in vitro*.

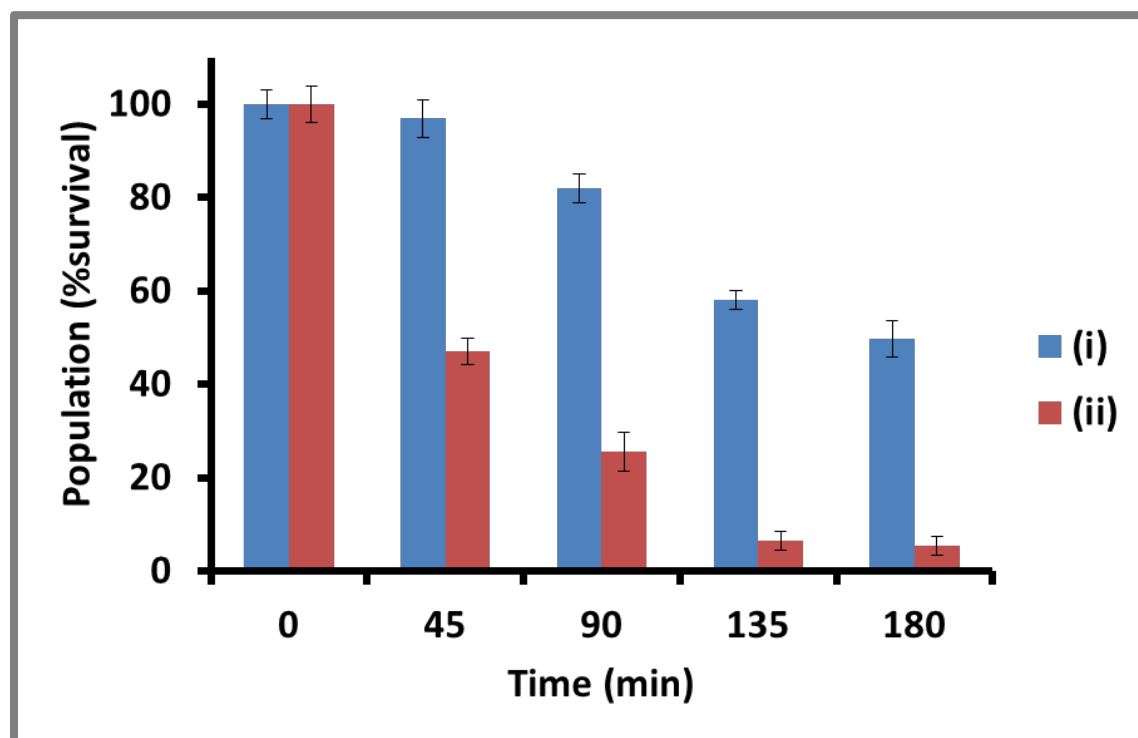


Figure 58. Survival bar graph for *Staphylococcus aureus* using **4c** (i) and **4c/mβCD** (ii) in 0.2% DMF in PBS.

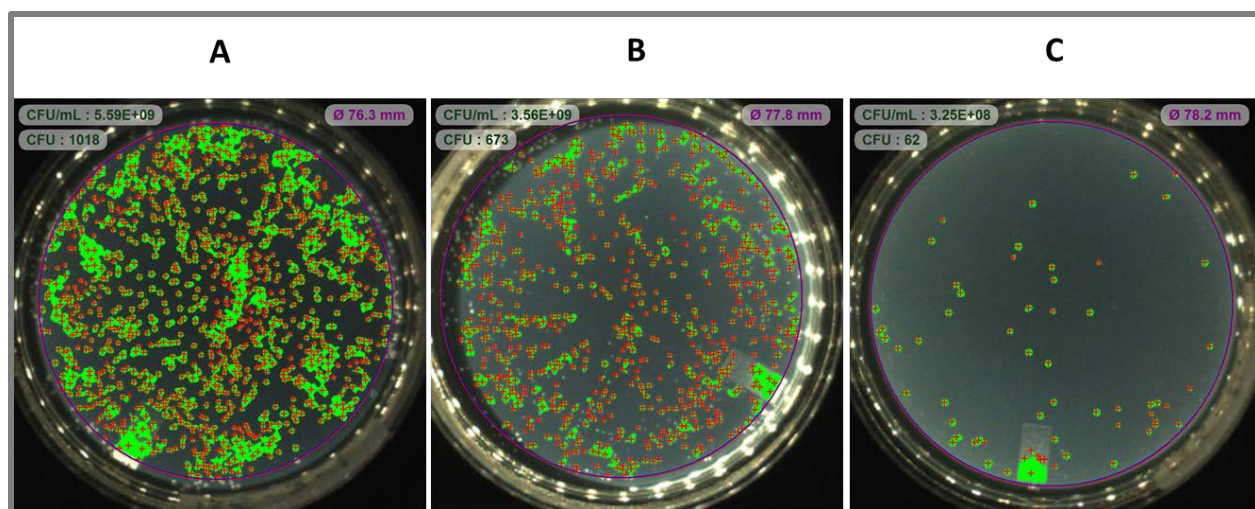


Figure 59. Images of *Staphylococcus aureus* at the zero control (A), *Staphylococcus aureus* with **4c** after 180 min irradiation (B) and *Staphylococcus aureus* with **4c**/m β CD at 180 min irradiation (C) showing the bacteria colonies.

Table 8. Photodynamic antimicrobial chemotherapy results.

	Population inactivation in solution (%)
4c	50.3
4c /m β CD	95.0

6.3. Closing remarks

The PACT activity of the BODIPY alone and in m β CD was evaluated by using *Staphylococcus aureus*, a gram-positive bacteria strain which can be photoinactivated by various charged or neutral photosensitizer dyes [37, 99]. The dye alone was insoluble in water and had low PACT activity. Upon encapsulation with m β CD, the solubility of the dye was enhanced, and the PACT activity increased from 50.3% photoinactivation to 95% photoinactivation. The dye alone

exhibited some dark toxicity, which was reduced markedly upon encapsulation with m β CD. These results demonstrate that further in-depth study of the use of the CD inclusion complexes of halogenated BODIPYs in PACT is merited.

Chapter 7: Photodynamic therapy

7.1. *In vitro* photodynamic therapy studies

This chapter describes the evaluation of the effectiveness of the dye molecules and their water-soluble encapsulation or inclusion complexes in the context of photodynamic therapy. *In vitro* studies were conducted using human breast adenocarcinoma MCF-7 cells. The WST-1 assay was used for the determination of percentage cell viability of the cells treated with BODIPY dyes and their encapsulation micelles, while the MTT assay was used for the porphyrins and their m β CD inclusion complex. The dark cytotoxicity of the compounds was also evaluated. The evaluation of the PDT activity was conducted through the calculation of the half maximal inhibitory concentration (IC₅₀) value, which is a measure of the effectiveness of a compound in inhibiting a particular biological behavior. The IC₅₀ values were used to quantify the concentration of the dyes or their complexes that is needed to inhibit the MCF-7 cell growth by 50% [160, 161]. A summary of the IC₅₀ values for all the compounds studied is provided in **Table 1**.

7.2. The investigation of *in vitro* dark cytotoxicity and photodynamic therapy effect of BODIPY 5 encapsulated in Pluronic® F-127 micelles

7.2.1. Photodynamic effect

The *in vitro* photodynamic activity of BODIPY 5 and 5@PF127 was determined using the WST-1 assay on human breast adenocarcinoma (MCF-7) cells following a protocol reported previously in the literature [87, 89]. The studies were carried out over a concentration range of 5–80 μ g/mL. BODIPY 5 was solubilized in water solution with 5.0 % DMSO, while 5@PF127 was dissolved in water. *In vitro* PDT studies were conducted using the illumination kit of a Modulight® Medical 7710-680 laser system fitted with a Thorlabs M660L4 660 nm light emitting diode (LED) that was found to provide an irradiance of 280 mW/cm² (Coherent FieldmaxII TOP energy/power meter

fitted with a Coherent Powermax PM10 sensor) in this context, to illuminate a 127.76×85.48 mm 96 well tissue culture plate for 20 min. No cytotoxicity was observed for DMSO and appropriate controls. **5** exhibited minimal dark toxicity at higher concentrations, while **5@PF127** showed no dark toxicity across the studied concentration range.

The photodynamic activity of BODIPY **5** (**Figure 60 A**) was found to be similar to that of **5@PF127** (**Figure 60B**), with IC_{50} values of 3.8 ± 1.3 and 3.2 ± 1.3 $\mu\text{g/ml}$, respectively. This demonstrated that the PDT activity of BODIPY **5** was retained upon encapsulation in the Pluronic[®] F-127 micelles. The statistically insignificant PDT activity (P^* value > 0.05) across the concentrations demonstrated that relatively low concentrations of **5@PF127** can be used for the eradication of MCF-7 cancer cells at an irradiation time of 20 min. Despite the insignificant difference in the PDT activity of **5** and **5@PF127**, this still demonstrates that encapsulation complexes of this type merit further in-depth investigation. The enhanced absorbance of light in the therapeutic window provided by BODIPY **5** could lead to increased light penetration into the tumor cells and thus higher efficacy of the drug molecule.

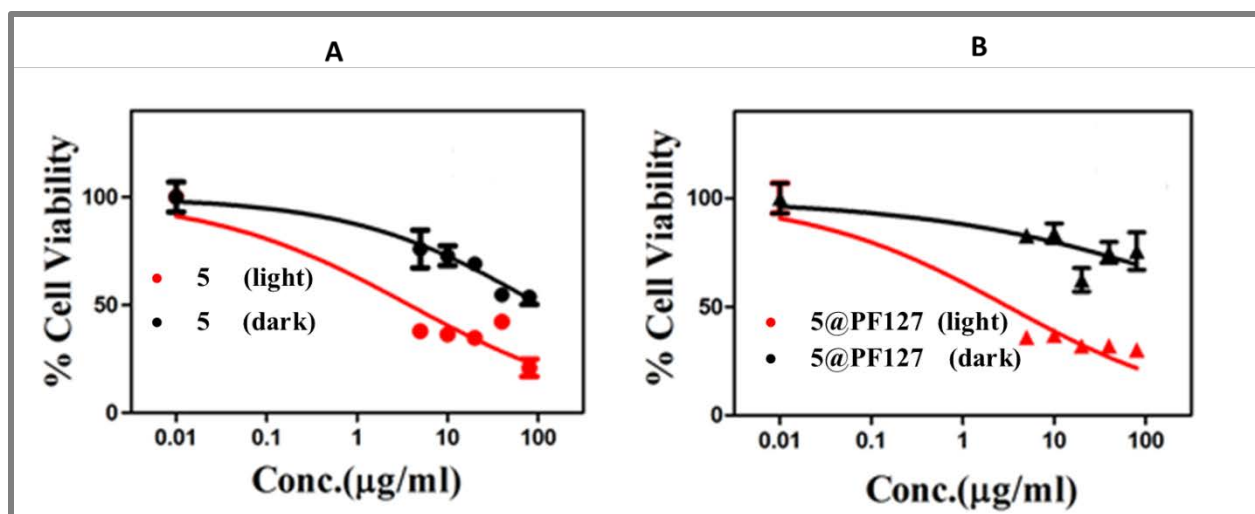


Figure 60. Cell viability plots showing the cytotoxic effect of BODIPY 5 and 5@PF127 on MCF-7 cancer cells in the dark (black symbols) and after irradiation (red symbols).

7.2.2. Cell imaging

The cell imaging provided in **Figure 61** shows the effect of BODIPY 5 and 5@PF127 on the MCF-7 cells before and after 20 min irradiation. The images for the control solutions (**Figures 61A** and **D**) show a high confluence of cells as anticipated based on the high percentage cell viabilities in **Figures 59**. After treatment with 80 $\mu\text{g/ml}$ of BODIPY 5 (**Figures 61B** and **E**) and 5@PF127 (**Figures 61C** and **F**), and subsequent irradiation for 20 min, a decrease in the MCF-7 cells confluence was observed as anticipated given the decrease in percentage cell viability in **Figures 59**.

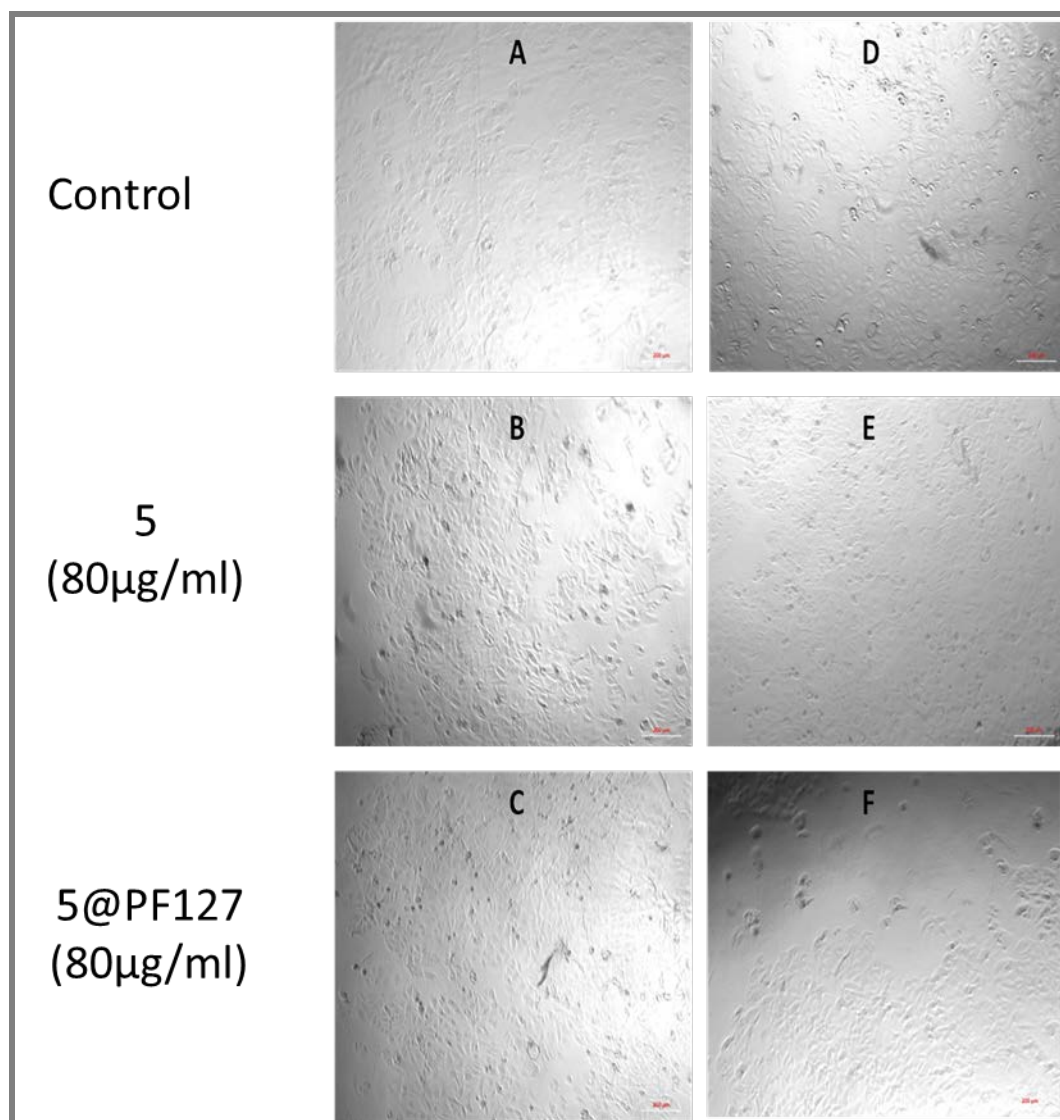


Figure 61. Microscopy images of the MCF-7 cells for the control (A), cells treated with BODIPY 5 at 80 µg/mL (B) and 5@PF127 at 80 µg/mL (C), and images after 20 min irradiation for the control (D), BODIPY 5 at 80 µg/mL (E) and 5@PF127 at 80 µg/mL (F). Scale = 200 µm.

7.3. The investigation of *in vitro* dark cytotoxicity and photodynamic therapy effect of BODIPYs **1c** and **6** encapsulated in folate-functionalized chitosan-coated Pluronic® F-127 micelles

7.3.1. Photodynamic effect

The *in vitro* studies mentioned in section 6.1.1. were followed for BODIPYs **1c** and **6** and their encapsulation complexes **1c**@PF127@FC and **6**@PF127@FC over a concentration range from 3–40 µg/ml. A Modulight® 7710-680 Medical Laser fitted with a Thorlab M660L4 light emitting diode (LED) with an irradiance of 280 mW/cm² was used, and a set of plates was irradiated for 20 min. Minimal dark cytotoxicity was observed for all of the compounds across the studied concentrations **Figures 62A-D**.

BODIPYs **1c** and **6** and their encapsulation complexes **1c**@PF127@FC and **6**@PF127@FC (**Figure 62**) exhibited no dark cytotoxicity. **1c**@PF127@FC was determined to have a higher Φ_{Δ} value than **1c** and was found to be more photoactive, with $IC_{50} = 20 \pm 1.3$ µg/mL. The higher photo-activity can be attributed to greater cellular uptake due to the folic acid targeting functionality. BODIPY **6** ($IC_{50} = 20 \pm 1.2$ µg/mL) was found to be more photoactive than its encapsulation complex **6**@PF127@FC ($IC_{50} > 50$ µg/mL). This activity is consistent with the lower Φ_{Δ} value that was derived for the micelles of BODIPY **6**. Another possible reason could be lower cellular uptake of the dye [162].

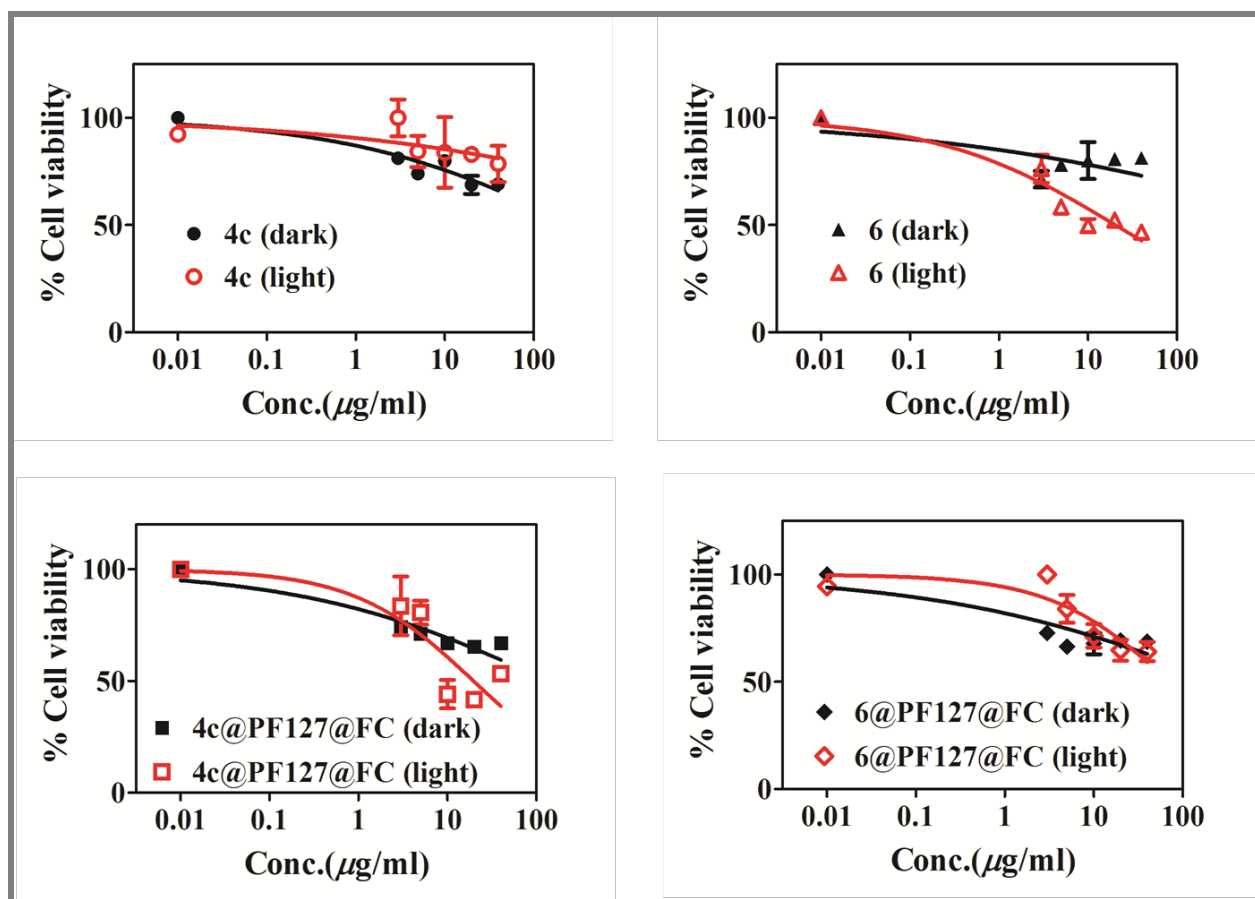


Figure 62. Cell viability plots showing the cytotoxic effect of BODIPYs **1c** and **6**, and the **1c@PF127@FC** and **6@PF127@FC** micelles on MCF-7 cancer cells in the dark (black symbols) and after irradiation (red symbols).

7.3.2. Cell imaging

The cell imaging provided in **Figure 63** shows the effect of BODIPY **1c** (B), **1c@PF127@FC** (C), **6** (C) and **6@PF127@FC** (D) on the MCF-7 cells in the dark. The image for the control solution (A) showed a high confluence of cells as anticipated, suggesting that in the absence of visible region light (500–700 nm), the BODIPY dyes and their encapsulation complexes exhibit minimal dark cytotoxicity. These observations are consistent with the high percentage cell viabilities that are shown in **Figure 62**.

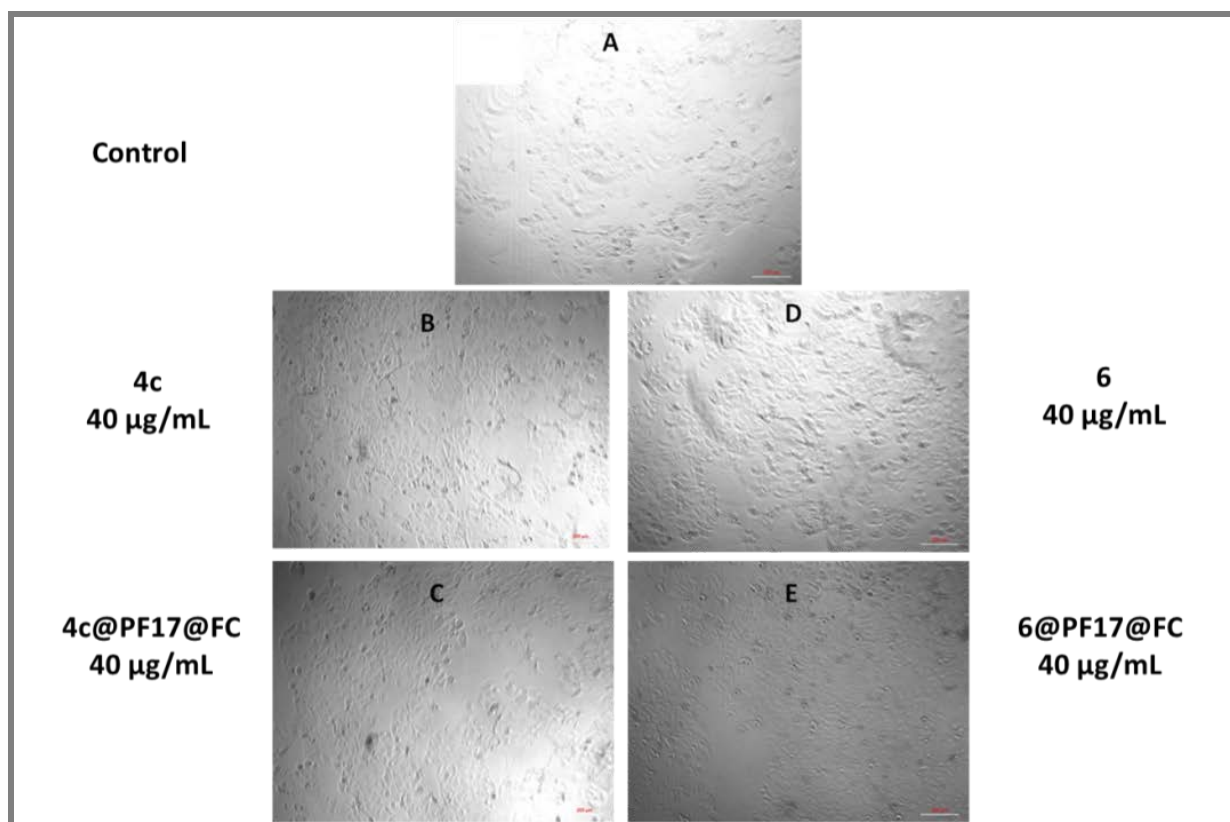


Figure 63. Microscopy images of the MCF-7 cells for the control (A), cells treated with BODIPY **1c** at 40 µg/mL (B), **1c@PF127@FC** at 80 µg/mL (C), BODIPY **6** at 80 µg/mL (D) and **6@PF127@FC** at 80 µg/mL (E). Scale = 200 µm.

The cell imaging in **Figure 64A-E** shows the effect of BODIPY **1c** (B), **1c@PF127@FC** (C), **6** (C) and **6@PF127@FC** (D) on the MCF-7 cells after 20 min irradiation. The image for the control solution (A) showed a high confluence of cells as anticipated based on the high percentage cell viabilities shown in **Figures 62A** and **B**. After treatment with the BODIPY dyes and their encapsulation complexes followed by irradiation for 20 min, a lower cell confluence was observed as would be anticipated based on the decrease in cell viability shown in **Figures 62**.

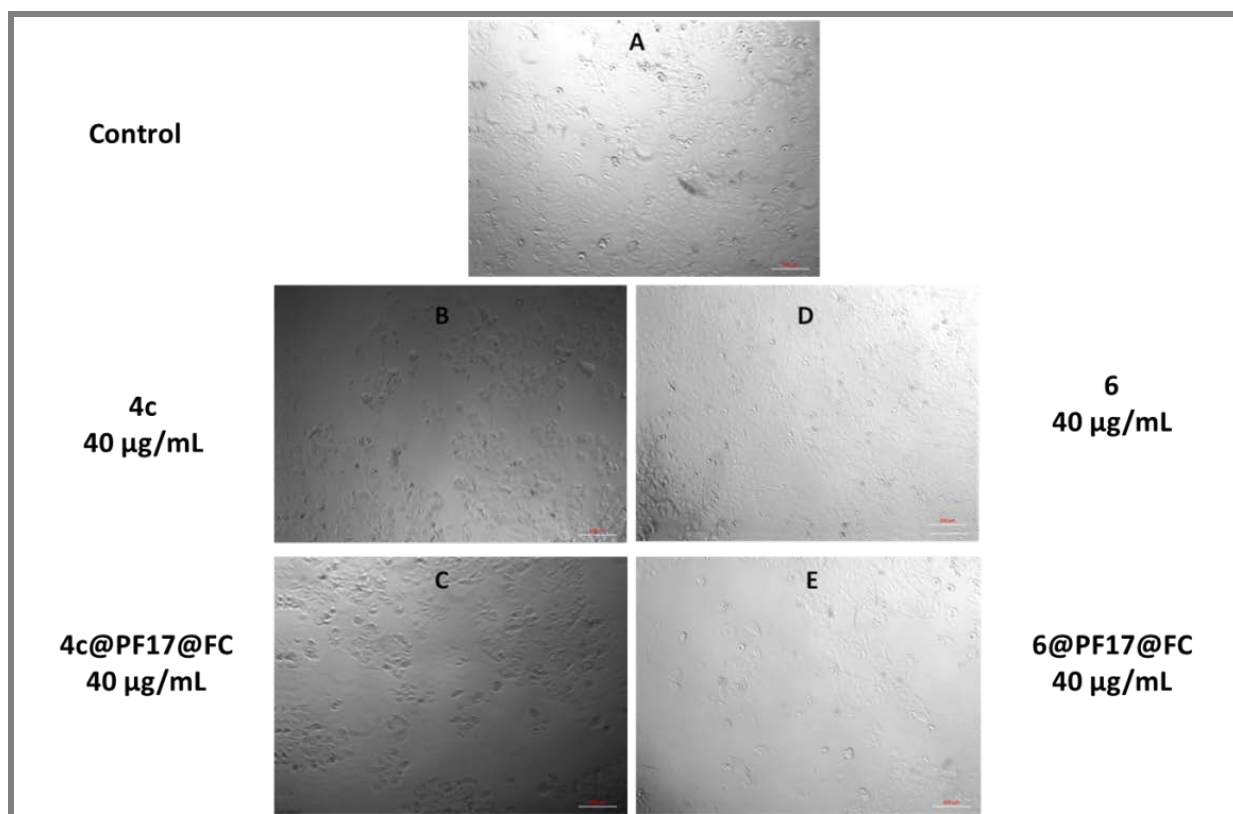


Figure 64. Microscopy images of the MCF-7 cells for the control (A), cells treated with BODIPY **1c** at 40 µg/mL (B) and **1c**@PF127@FC at 40 µg/mL (C), BODIPY **6** at 40 µg/mL (D) and **6**@PF127@FC at 40 µg/mL (E) after 20 min irradiation. Scale = 200 µm.

7.4. The investigation of *in vitro* dark cytotoxicity and photodynamic therapy effect of porphyrin **7a** and **7b** and **7c**/mβCD inclusion complex

The *in vitro* photodynamic activity of porphyrins **7a**, **7b** and **8** and the **7b**/mβCD inclusion complex was determined by using the MTT assay on human breast adenocarcinoma (MCF-7) cells following a protocol reported in the literature [163, 164]. The studies were carried out over a concentration range of 3.13–50 µg/mL. All of the compounds were solubilized in aqueous solution with 5.0% DMSO. A Modulight® 7710-680 Medical Laser fitted with a Thorlab M625L3 light emitting diode (LED) with an irradiance of 240 mW/cm² was used, and a set of plates were

illuminated for 30 min. 5.0% DMSO solution and appropriate controls were found to exhibit no cytotoxicity on the MCF-7 cells. No significant dark toxicity was observed across the studied concentration range, **Figure 65A-D**. Porphyrin **8** was studied to evaluate the effect of incorporating the *meso*-acenaphthyl substituents in the structures of **7a** and **7b**.

Porphyrin **7a** was found to have an $IC_{50} = 19 \pm 1.1 \mu\text{g/mL}$. The metallated derivative, **7b** had an $IC_{50} = 11 \pm 1.1 \mu\text{g/mL}$. This demonstrated that **7b** had a higher PDT activity which can be attributed to its higher Φ_{Δ} value compared to **7a**, which would be expected to make it more effective in eradicating cancer cells [55]. The encapsulated analogue, **7b**/m β CD exhibited decreased PDT activity ($IC_{50} = 21 \pm 1.1 \mu\text{g/mL}$). This can be attributed to lower cellular uptake of the hydrophobic dye in this context, thus resulting in a decrease in the photo-activity of the dye *in vitro* [32, 162]. It is noteworthy that porphyrin **8** had a lower Φ_{Δ} value of 0.16 (**Table 5**) but was found to have a better PDT activity ($IC_{50} = 7.2 \pm 1.1 \mu\text{g/mL}$) than **7b** ($\Phi_{\Delta} = 0.36$). Further studies on these porphyrins are merited, with the incorporation of bulky axial ligands to limit aggregation effects in a similar manner to what was achieved in the recent study by Mack and coworkers [55] on Sn(IV) tetrathienylporphyrins being an obvious next step.

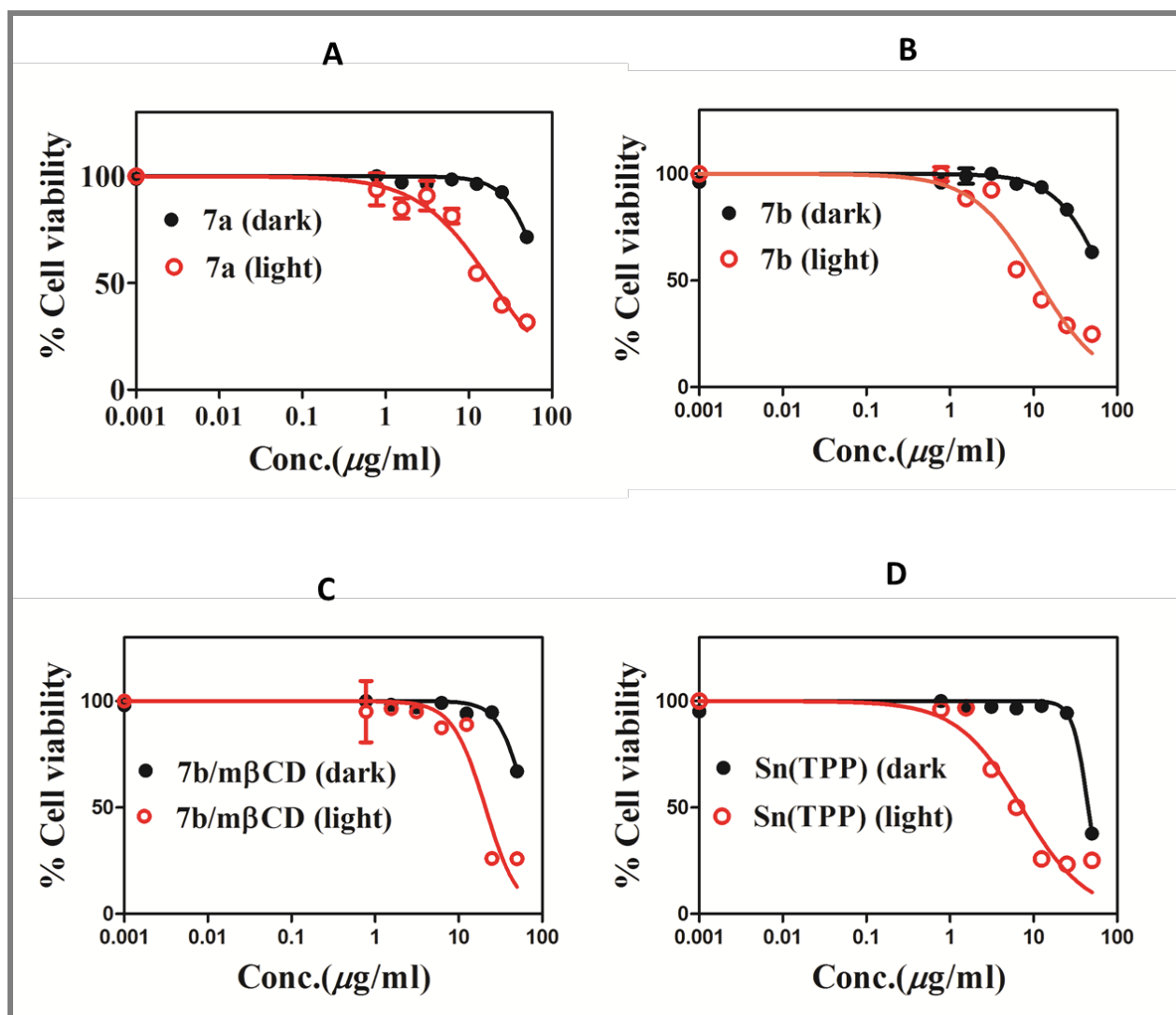


Figure 65. Cell viability plots showing the cytotoxic effect of porphyrin **7a** (A), **7b** (B), **7b/m β CD** (C) and porphyrin control complex **8** (D) on MCF-7 cancer cells in the dark (black symbols) and after irradiation (red symbols).

Table 8. IC₅₀ values of BODIPY dyes and porphyrins along with their encapsulation complexes and inclusion complexes.

Compound	IC ₅₀ (μg/ml) dark ^a	IC ₅₀ (μg/ml) light ^b
1c	> 50	> 50
1c@PF127@FC	> 50	20 ± 1.3
5	> 50	> 50
5@PF127	3.8 ± 1.3	3.2 ± 1.3
6	> 50	20 ± 1.2
6@PF127@FC	> 50	> 50
7a	79 ± 1.1	19 ± 1.1
7b	73 ± 1.1	11 ± 1.1
7b/mβCD	65 ± 1.1	21 ± 1.1
8	45 ± 1.0	7.2 ± 1.1

7.5. Closing remarks

2,6-Dihalogenated-3,5-vinyleneBODIPY dyes with moderately high singlet oxygen quantum yields were encapsulated in Pluronic[®] F-127 micelles (**5**) and folate-functionalized chitosan-coated Pluronic[®] F-127 micelles (**1c** and **6**). Novel tetraacenaphthylporphyrin porphyrins (**7a** and **7b**) and porphyrin **8** were studied along with **7b**/mβCD inclusion complexes in a similar manner. The dark cytotoxicity and photodynamic activity of the water-soluble complexes and their

respective dyes were investigated on the human breast adenocarcinoma MCF-7 cell line. All the dyes exhibited minimal dark toxicity and a significant level of PDT activity when applied alone after solubilizing with DMSO, and in water after encapsulation with Pluronic[®] F-127 or inclusion with m β CD. Sn(IV) tetraphenylporphyrin **8** was found to have a lower IC₅₀ value than tetraacenaphthylporphyrin complex **7b** despite having a lower Φ_{Δ} value and less red-shifted Q bands. Further structural modification of **7b** to include bulky axial ligands with suitable targeting groups may be required to limit aggregation effects and enhance cellular uptake.

The photo-activity of BODIPY **5** was retained upon encapsulation with the Pluronic[®] F-127 encapsulation micelles. There was an enhancement in the PDT activity of BODIPY **1c** upon encapsulation with folate-functionalized chitosan Pluronic[®] F-127 core micelles. These Pluronic[®] F-127 core-shell micelles are promising drug delivery agents for the treatment of cancer cells, for which folate receptors are excessive. The PDT activity of BODIPY **6** was higher than that of its encapsulation complexes, but the micelles still exhibited photo-cytotoxicity against MCF-7 cancer cells. Generally, the dyes and their complexes can be said to be promising PDT agents given the success of eradicating MCF-7 cancer cells at the studied concentrations. The use of micelle encapsulation complexes and cyclodextrin inclusion complexes merits further in-depth investigation.

Chapter 8: Molecular modeling

8.1. Molecular modeling

Molecular modeling has been used to investigate the structure-property relationships of molecules in biology, chemistry, material science and solid-state physics [165]. Time-dependent density functional theory (TD-DFT) calculations were used in this study to investigate the electronic structures and spectroscopic properties of porphyrins and BODIPY dyes. The electronic structures of porphyrins can be understood in terms of Gouterman's 4-orbital model [166, 167]. The main absorption bands of porphyrins emerge from the transitions between the four MOs derived from the doubly degenerate HOMOs and LUMOs of a 16 atom 18 π -electron system associated with the inner ligand perimeter. These transitions are affected by the structural modifications that are required to form a porphyrin ring from a $C_{16}H_{16}^{2-}$ parent hydrocarbon perimeter and by the nature of the metal center and substituents on the porphyrin ring. The transitions between the four frontier π -MOs result in the allowed B (or Soret) bands and the forbidden Q bands. In contrast, the main absorption band of BODIPY dyes usually arises almost exclusively from the HOMO $\rightarrow$ LUMO transition [165, 168], since there is no heteroaromatic macrocycle involved.

8.2. Structural geometric optimization and time-dependent density functional theory calculations

The Gaussian 09 software package [31] was used to optimize the geometries of a series of BODIPY dyes and porphyrin analogues by using the Beck, three-parameter, Lee-Yang-Parr (B3LYP) exchange-correlation functional with SDD basis sets. The electronic absorption properties were calculated by TD-DFT by using the Coulomb-Attenuated B3LYP (CAM-B3LYP) functional with SDD basis sets. The SDD basis sets provide appropriate approximations

for all atoms in a manner that is inclusive of the heavy atoms (bromine, iodine and tin) [165, 168]. The CAM-B3LYP functional was used in estimating the electronic absorption properties since it includes a long-range correction of the exchange potential. This correction integrates an increasing fraction of Hartree-Fock (HF) exchange with an increase in interelectronic separation [165, 168] and provides more accurate predictions of trends for transitions with significant charge transfer character.

8.2.1. BODIPY dyes used for PDT and PACT

BODIPY dyes are not aromatic when Hückel's $4N+2$ rule for aromaticity is applied, since there is no macrocycle involved. The BODIPY core structure has C_{2v} symmetry. Complexation by the BF_2 moiety in the indacene plane gives rise to the non-degeneracy of the π -MOs [152, 169]. The *meso*-substituents have little to no effect on the HOMO energies since the HOMO has nodal planes at this position [36, 152, 170]. This accounts for the similar calculated absorption maxima of BODIPY cores **1a**, **3a** and **4a**, **Table 9**. The *meso*-aryl rings of the BODIPYs are oriented perpendicular to the BODIPY indacene plane due to the methyl groups at 1,7-positions which cause steric hindrance. Due to this orientation, the delocalization of electrons in the π -system is disrupted [47, 168]. The trends observed in this regard are consistent with experimental data reported previously in the literature [47, 168].

In the context of Michl's perimeter model, the indacene plane of the BODIPY core structure can be compared to those of an aromatic $C_{12}H_{12}^{2-}$ parent hydrocarbon perimeter [152]. BODIPYs have properties similar to aromatic systems due to the rigid planar structure introduced by the BF_2 bridge that disrupts the aromatic π -system [14, 39]. In TD-DFT calculations, the $S_0 \rightarrow S_1$ transition is predicted to be mostly associated with the HOMO $\rightarrow$ LUMO one-electron transition.

Substituents introduced to the BODIPY core at various positions affect the energies of the HOMO and LUMO and thus result in changes in the HOMO–LUMO gap that cause significant shifts in the wavelength of the main spectral band [168].

The BODIPY dyes were modified through the introduction of iodine atoms at 2,6-position to enhance their suitability for PDT or PACT. This modification increased the rate of ISC to the triplet manifold and thus enhanced the production of singlet oxygen [168]. A second modification was required to shift the main spectral bands of the dyes to longer wavelengths into the heart of the biological window. This was achieved by extending the π -conjugation system through the addition of vinylene groups (**1c** and **3c**) and styryl groups (**4c**) at the 3,5-positions. The sequential modifications of the BODIPY chromophore were studied through molecular modeling of BODIPY dye series **1**, **3** and **4**. The MO energy changes arising from these modifications are shown in **Figure 68**.

Modifications at the 2,6-positions

The calculated wavelengths and oscillator strengths of the main spectral bands of BODIPYs **1a-4c** are tabulated in **Table 9**. **Figures 68-70** show the changes in the HOMO and LUMO energies upon structural modifications at the *meso*-position, the 2,6-positions and at the 3,5-positions. A general trend observed is that upon iodination of the BODIPY cores at the 2,6-positions, the entire stack of MOs is stabilized due to halogens having electron withdrawing inductive properties, **Figure 71**. The HOMOs undergo a relative destabilization, since they have larger MO coefficients at the 2,6-positions than the LUMOs and hence there is a larger mesomeric interaction with the lone pairs on the iodine atoms. The net destabilization of the HOMOs results

in a red-shift of the main spectral bands of **1b**, **2b**, **3b** and **4b** (**Figure 71**), since there is a narrowing of the HOMO–LUMO gap.

Modifications at the 3,5-positions

The iodinated BODIPY analogues have unevenly distributed MO coefficients at the 3,5-positions in the HOMO and LUMO as can be observed in **Figure 66-68** for **1b**, **3b** and **4b**, respectively. Upon addition of styryls, a greater destabilization of the HOMO is predicted due to the mesomeric interaction with the styryl π -system since it has larger MO coefficients at these positions. Hence there is a narrowing of the HOMO–LUMO gap and a red-shift of the main spectral band depending on the nature of the groups added, **Figure 69**. BODIPYs **1c** and **3c** have 3-thienylvinylene groups at the 3,5-positions, so the red shifts of the main spectral bands are almost the same. BODIPY **4c** has 4-benzyloxystyryls, which provide greater scope for extended π -conjugation and this results in a larger red-shift. The calculated spectral shift trends were found to be consistent with the experimental shifts (**Table 9**) [152]. The intense bands in the spectra of **1c**, **3c** and **4c** near the blue end of the absorption spectra are associated with transitions out of MOs localized on the styryl substituents as a result of a HOMO–2→LUMO one-electron transition [152]. Similar spectral trends are observed in the experimental spectra in the 350–450 nm region [22, 152].

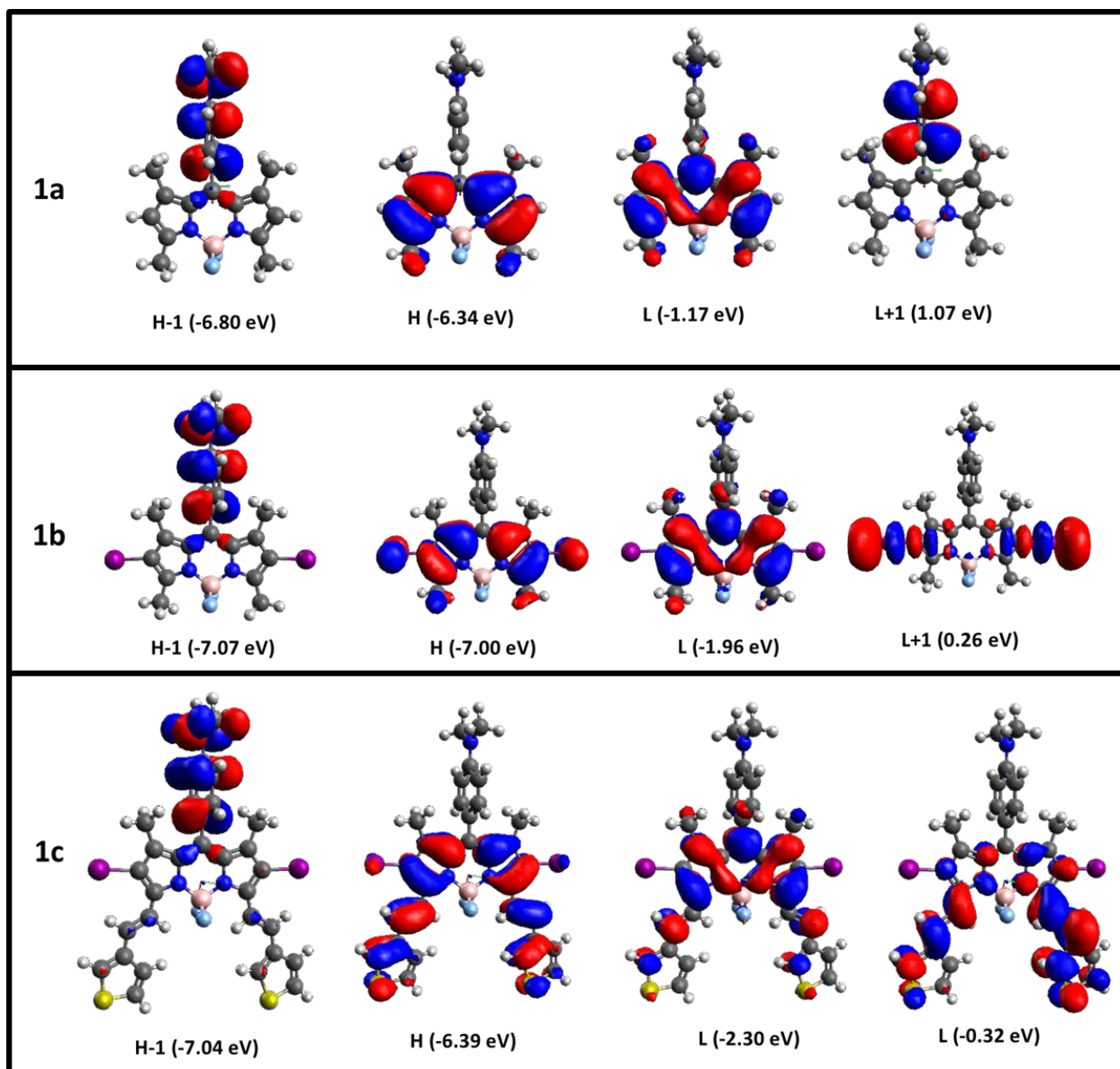


Figure 66. Nodal patterns (at an isosurface value of 0.02 a.u.) and energies of the frontier MOs of BODIPYs **1a**, **1b** and **1c** from TD-DFT calculations obtained by using the CAM-B3LYP functionals with SDD basis sets. HOMO and LUMO are denoted by H and L, respectively.

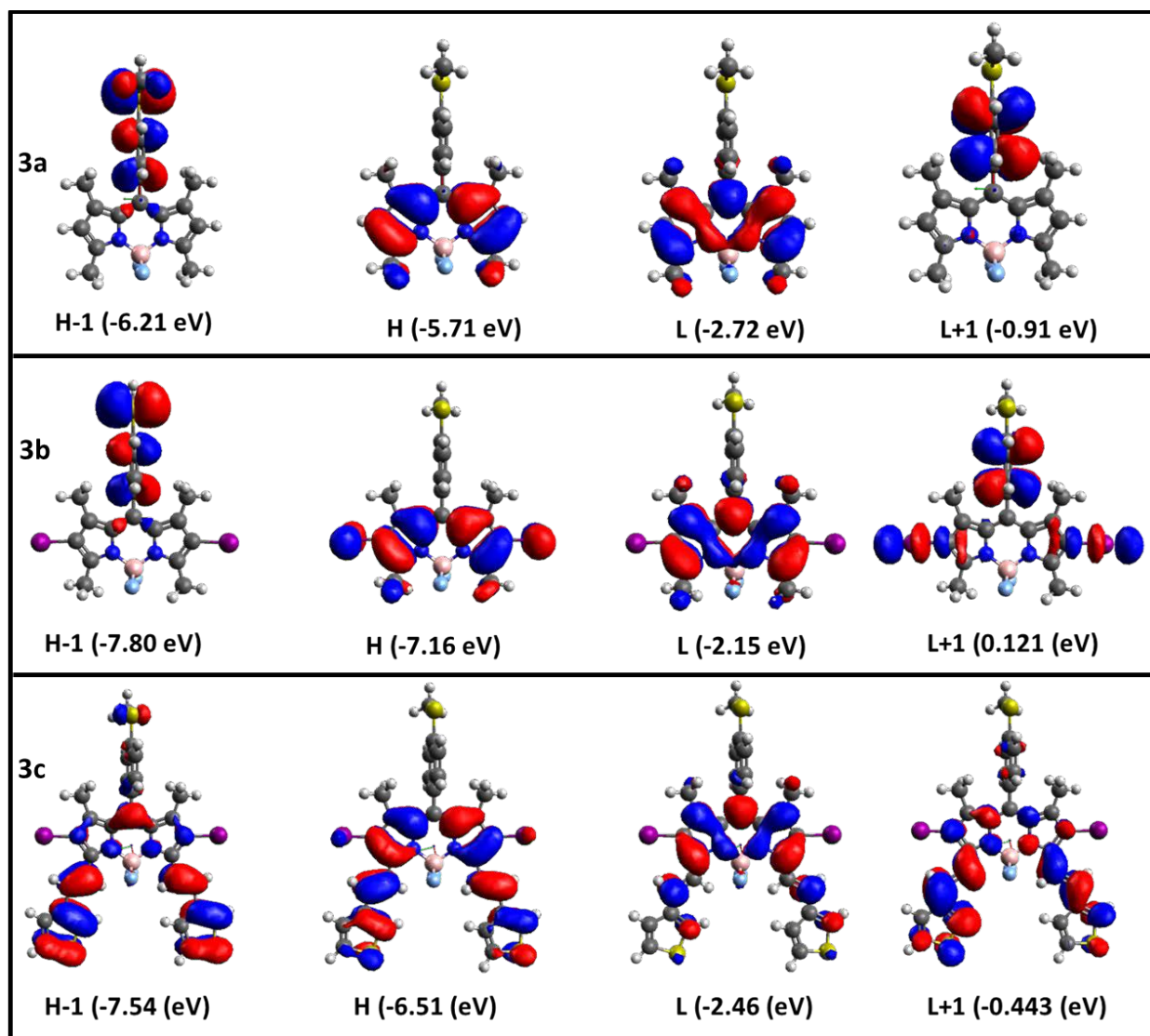


Figure 67. Nodal patterns (at an isosurface value of 0.02 a.u.) and energies of the frontier MOs of BODIPYs **3a**, **3b** and **3c** from TD-DFT calculations obtained by using the CAM-B3LYP functionals with SDD basis sets. HOMO and LUMO are denoted by H and L, respectively.

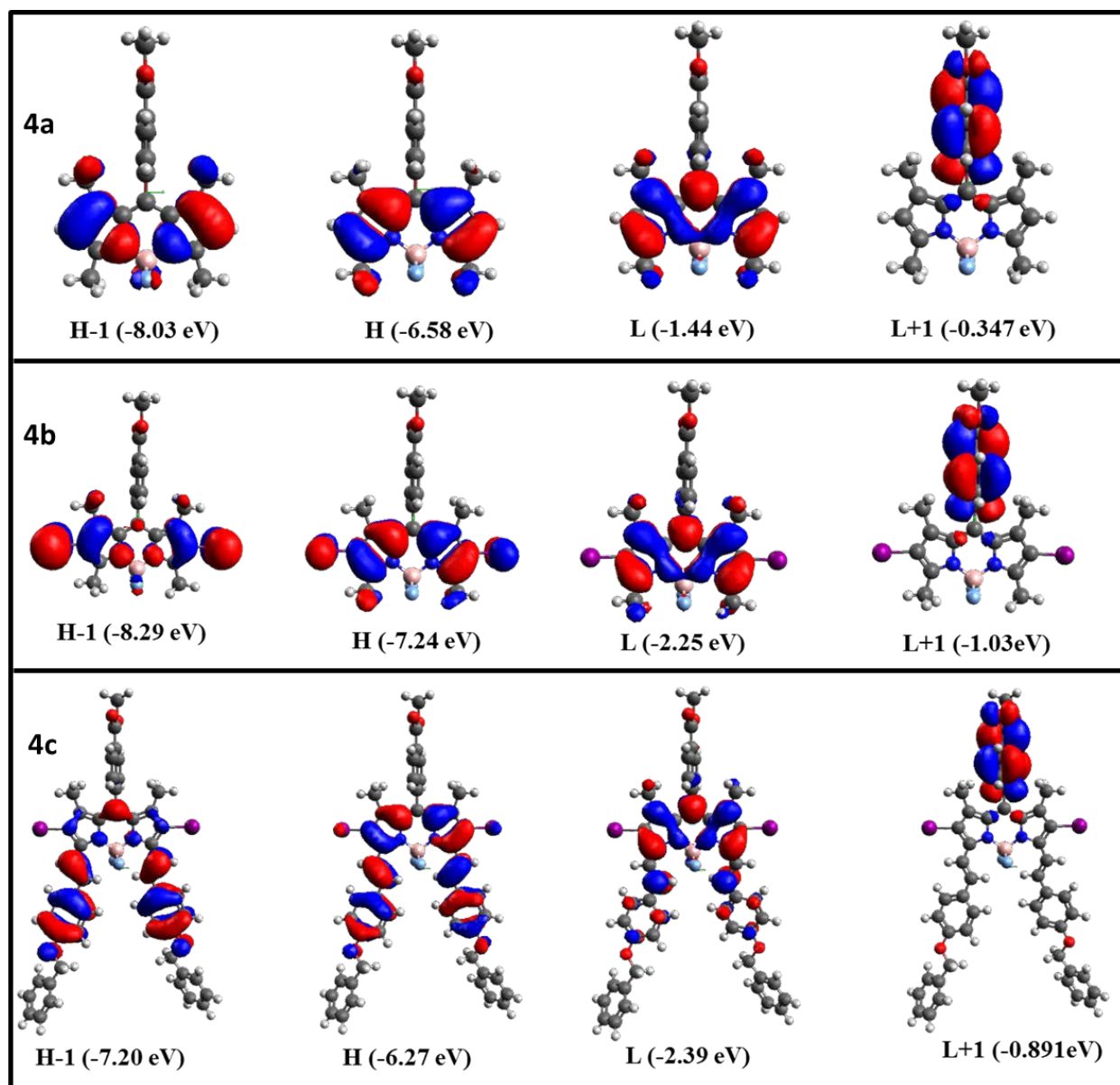


Figure 68. Nodal patterns (at an isosurface value of 0.02 a.u.) and energies of the frontier MOs of BODIPYs **4a**, **4b** and **4c** from TD-DFT calculations obtained by using the CAM-B3LYP functionals with SDD basis sets. HOMO and LUMO are denoted by H and L, respectively.

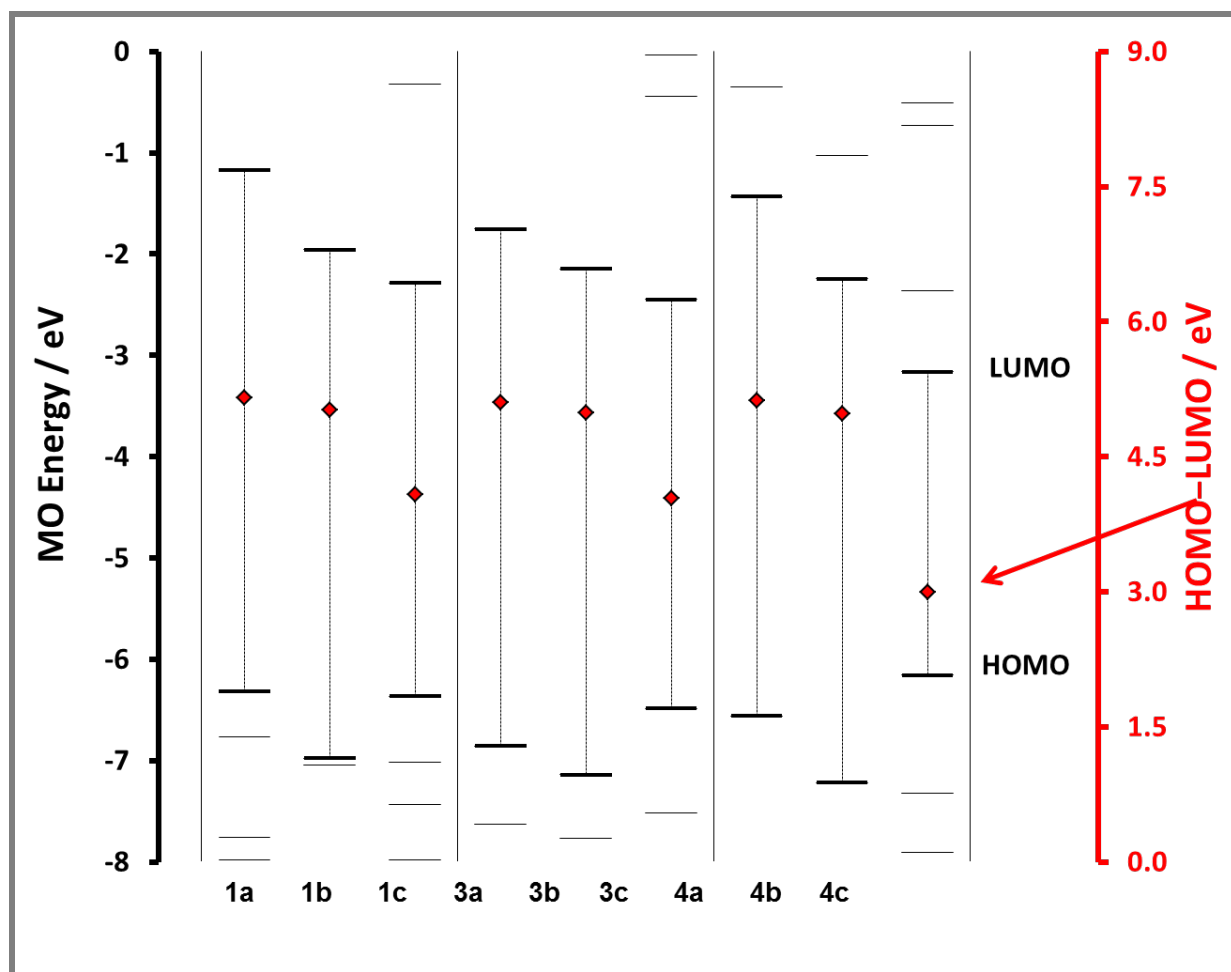


Figure 69. MO energies for **1a**, **1b**, **1c**, **3a**, **3b**, **3c**, **4a**, **4b** and **4c** obtained from TD-DFT calculations at the CAM-B3LYP/SDD level of theory. The calculated HOMO–LUMO gaps (red diamonds) are plotted against a secondary axis.

Simulated TD-DFT spectra

The simulated spectra of BODIPYs **1a**, **1b**, **1c**, **3a**, **3b**, **3c**, **4a**, **4b** and **4c** are shown in **Figure 70**. As explained in sections 7.1.2.1 and 7.1.2.2, the shift towards longer wavelengths rendered the synthesized dyes suitable for use in PDT since the main absorption bands lie within the therapeutic

window when a consistent over-estimation of the energy of the main spectral band relative to the experimental data is taken into account (Table 9).

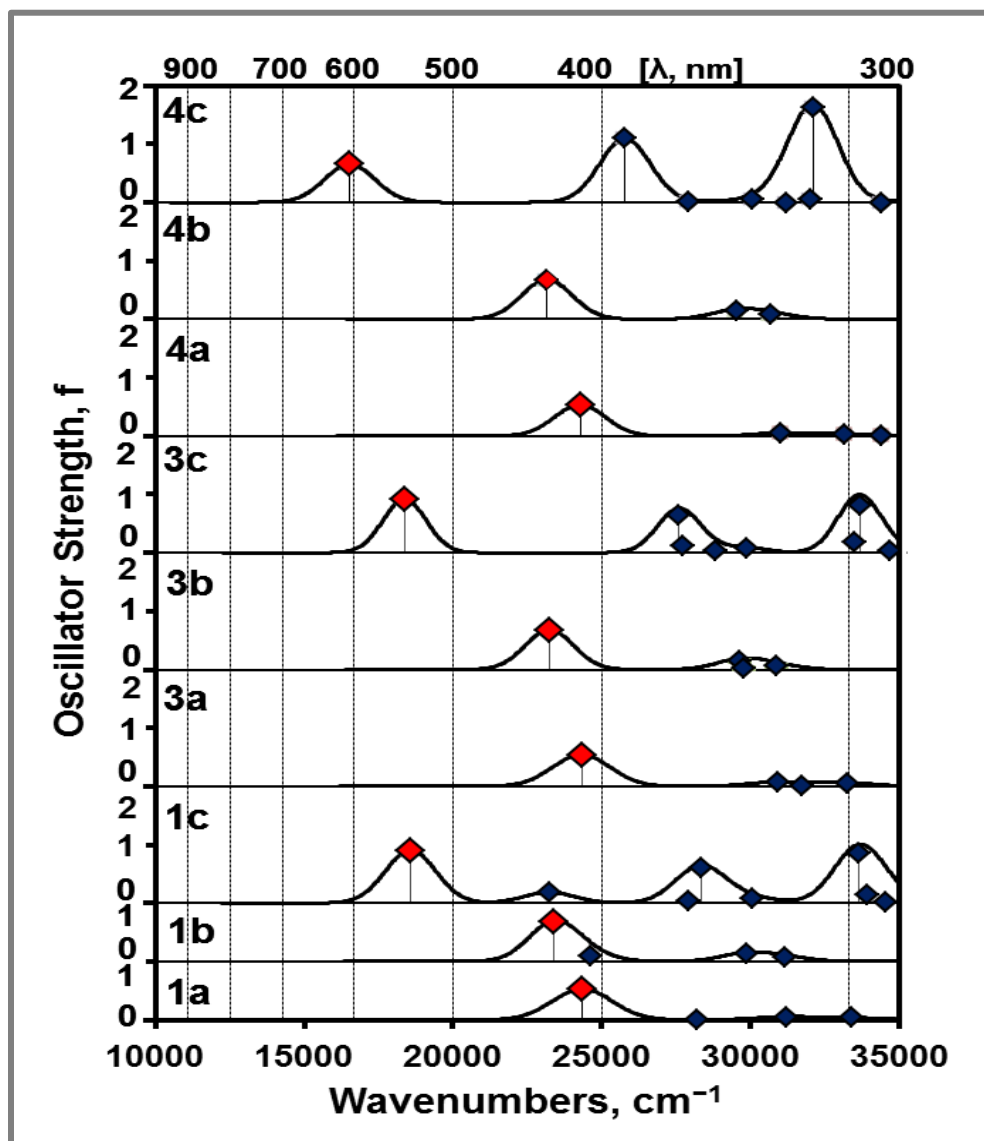


Figure 70. Calculated TD-DFT spectra of BODIPYs **1a-4c** at the CAM-B3LYP/SDD level theory. The red diamonds highlight the main spectral bands. The simulated spectra were generated from Chemcraft program with a fixed bandwidth of 2000 cm^{-1} .

Table 9. Experimental (λ_{exp}) and excitation wavelengths (λ_{cal}), calculated band energies (eV), calculated oscillator strengths (f) and wavefunctions (v_{calc}) of BODIPYs **1a-c**, **3a-c** and **4a-c**.

	# ^a	E (eV) ^b	λ_{exp} ^c	λ_{calc} ^d	v_{calc} ^e	f ^f	Wavefunction ^g
1a	1	3.03	502	411	24.4	0.53	H→L (97%);...
1b	1	2.90	533	427	23.4	0.67	H→L (97%);...
1c	1	2.30	641	538	18.6	0.90	H→L (97%);...
3a	1	3.02	502	411	24.3	0.54	H→L (96%);...
3b	1	2.88	536	430	23.2	0.68	H→L (97%);...
3c	1	2.27	648	544	18.4	0.92	H→L (97%);...
4a	1	3.01	503	412	24.3	0.54	H→L (97%);...
4b	1	2.87	537	432	23.2	0.68	H→L (97%);...
4c	1	2.05	672	605	16.5	0.65	H→L (96%);...

^a-Excited state numbers in increasing energy from the TD-DFT calculations. ^b – Calculated band energies (eV). ^c – Experimental absorption maxima wavelengths in nm (solvent = DMSO). ^d – Calculated wavelengths in nm. ^e – Calculated band energy (10^3 cm^{-1}). ^f – Calculated oscillator strengths. ^g – Wavefunctions of MOs involved in the transitions and their respective contributions on the basis of eigenvectors predicted by TD-DFT calculations. HOMO and LUMO are denoted by H and L respectively.

8.2.2. Porphyrins used for PDT

In the context of Michl's perimeter model, the four frontier π -MOs of the porphyrin molecule derived from the doubly degenerate HOMO and LUMO of the parent $\text{C}_{16}\text{H}_{16}^{2-}$ hydrocarbon perimeter are labeled as **a**, **s**, **-a** and **-s**, **Figure 71** [40]. This assignment depends on the presence of a nodal plane (**a** or **-a**) or large MO coefficients (**s** or **-s**) along the symmetry-defined y-axis. Using this assignment scheme, the electronic structures of porphyrins with different substituents and symmetries can be readily compared [40, 171]. When the predicted

MO energies of **7a** and **7b** are compared (**Figure 71**), there is a narrowing of the average HOMO–LUMO gap of **7b** relative to that of **7a** (**Figure 72**) when all four MOs derived from the HOMO and LUMO of the $C_{16}H_{16}^{2-}$ parent hydrocarbon perimeter are taken into account. These trends are also observed in the simulated spectra in **Figure 73** in terms of the red shift of the B band, and are consistent with the experimental spectral shifts as listed in **Table 10** and reported previously in the literature [40, 55, 171].

Porphyrins **7b** and **8** are both metallated with Sn(IV) and have hydroxyl axial ligands. The difference is in the substituents at the *meso*-positions, where **7b** and **8b** have acenaphthyl and phenyl groups, respectively. According to the TD-DFT calculations, the Q bands of **8** were expected to be more red-shifted than those of **7b** (**Figure 73**) and a slightly smaller average HOMO–LUMO gap is predicted for **7b** (**Figure 72**) when all four MOs derived from the HOMO and LUMO of the $C_{16}H_{16}^{2-}$ parent hydrocarbon perimeter are taken into account. However, the predicted trends were not observed experimentally (**Table 10**), since the Q bands of **7b** were more red-shifted (570 and 610 nm) than those of **8** (558 and 600 nm). This may be related to issues with the B3LYP optimized geometries, since they may not accurately predict the dihedral angles between the *meso*-aryl rings and the main porphyrin π -system, since this affects the relative energies of the **a**, **s**, **-a** and **-s** MOs and hence the magnitude of the HOMO–LUMO gap. A summary of the results is provided in **Table 10**.

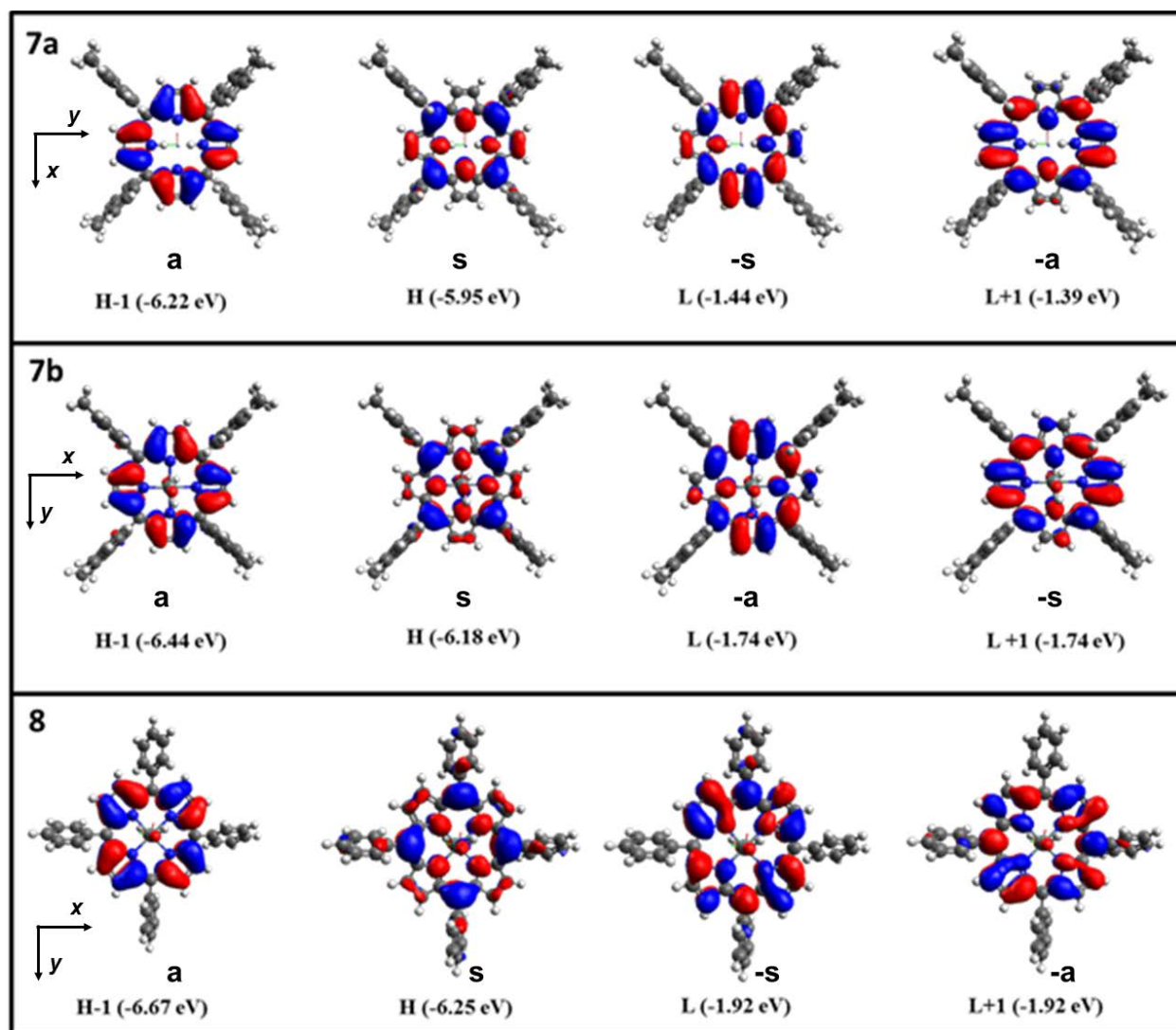


Figure 71. Nodal patterns (at an isosurface value of 0.02 a.u.) and energies of the frontier MOs of porphyrins **7a**, **7b** and **8** from TD-DFT calculations obtained by using the CAM-B3LYP functional with SDD basis sets.

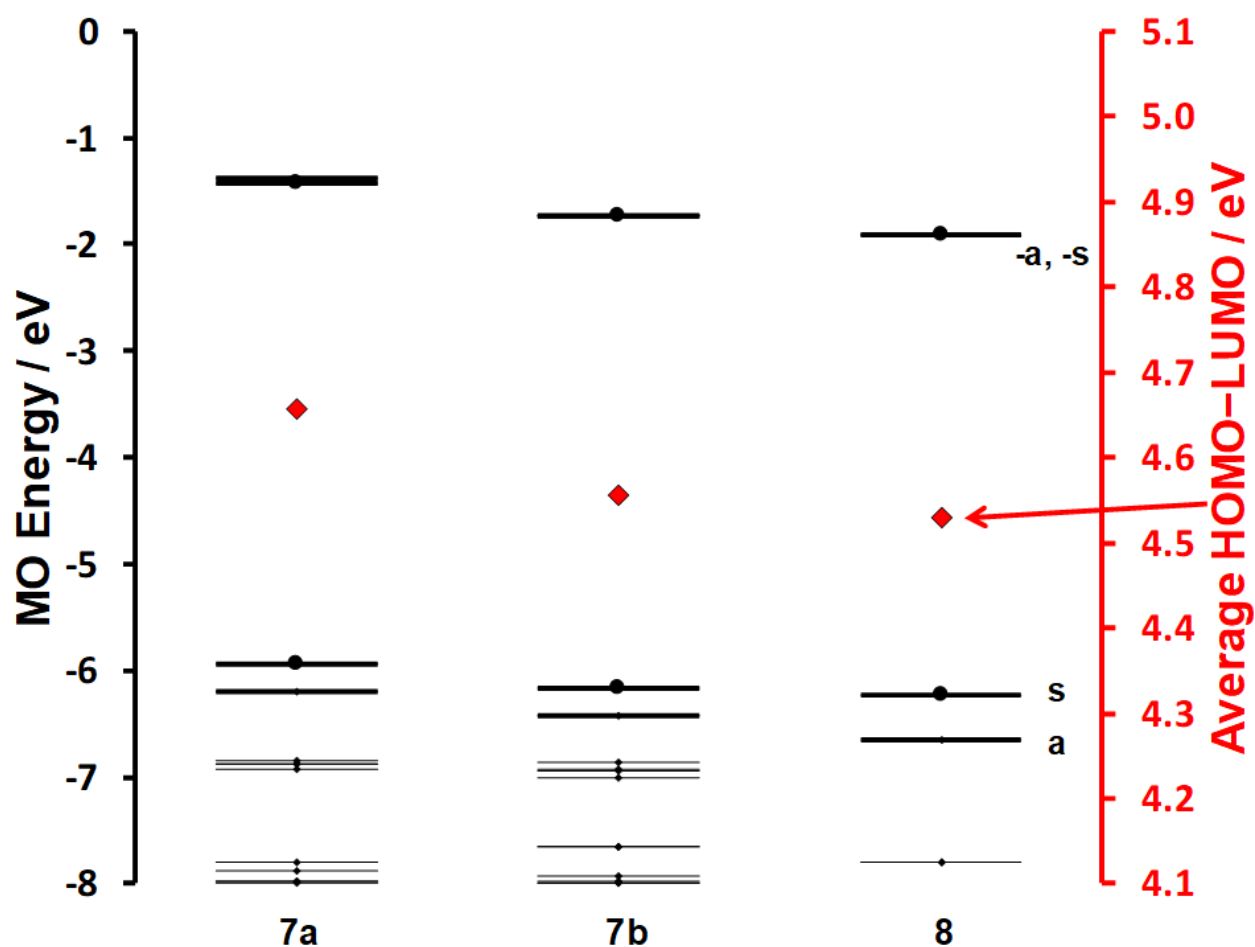


Figure 72. MO energies for the B3LYP optimized geometries of **7a**, **7b** and **8** obtained from TD-DFT calculations at the CAM-B3LYP/SDD level of theory. The **a**, **s**, **-a** and **-s** MOs are highlighted with thicker black lines. The **s** and **-s** MOs are denoted with black circles. The calculated average HOMO-LUMO gaps taking into account all four MOs derived from the HOMO and LUMO of the $C_{16}H_{16}^{2-}$ parent perimeter (red diamonds) are plotted against a secondary axis. Occupied MOs are highlighted with small black diamonds.

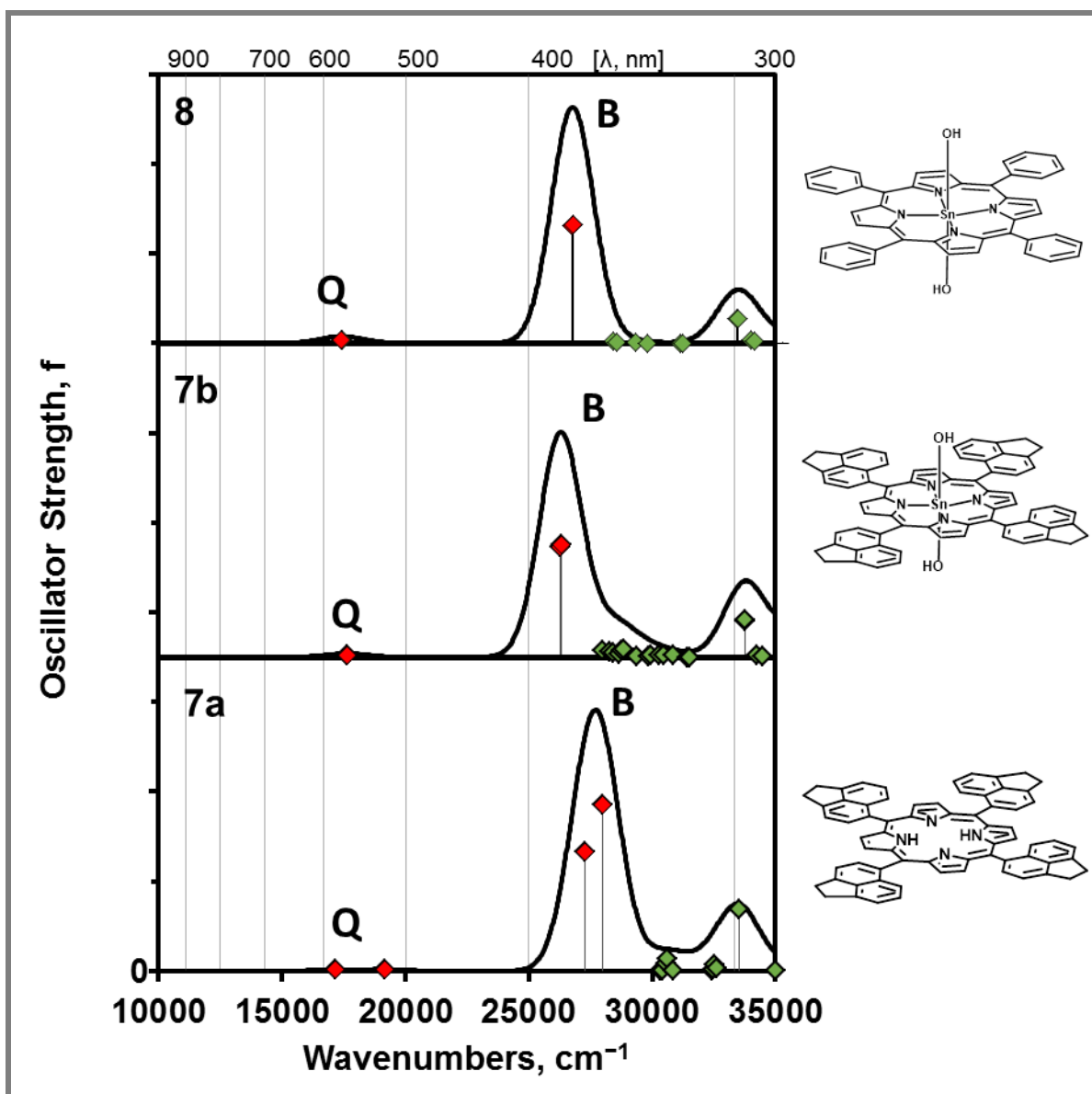


Figure 73. Calculated TD-DFT spectra of porphyrins **7a**, **7b** and **8** at the CAM-B3LYP/SDD level of theory. The red diamonds highlight the Q and B bands. The simulated spectra were generated from the Chemcraft program with a fixed bandwidth of 2000 cm^{-1} .

Table 10. Experimental (λ_{exp}) and electronic excitation wavelengths (λ_{cal}), calculated band energies (eV), calculated oscillator strengths (f) and wavefunctions (v_{calc}) of porphyrins **7a**, **7b** and **8**.

	Band	# ^a	E (eV) ^b	λ_{exp} ^c	λ_{cal} ^d	v_{calc} ^e	f ^f	Wavefunction= ^g
7a	Q	1	2.13	515, 547	582	17.2	0.02	62% s→-s; 37% a→-a; ...
	Q	2	2.37	595, 655	522	19.2	0.02	57% s→-a; 42% a→-s; ...
	B	3	3.38	427	367	27.3	1.33	58% a→-a; 31% s→-s; ...
	B	4	3.47		357	28.0	1.85	57% a→-s; 42% s→-a; ...
7b	Q	1	2.18	570	568	17.6	0.02	38% s→-a; 24% a→-s; 21% s→-s; 13% a→-a; ...
	Q	2	2.19	610	567	17.6	0.02	38% s→-s; 24% a→-a; 22% s→-a; 13% a→-s; ...
	B	3	3.26	432	381	26.3	1.23	40% a→-a; 25% s→-s; 15% a→-s; ...
	B	4	3.26		380	26.3	1.26	41% a→-s; 26% s→-a; 16% a→-a; ...
8	Q	1	2.16	558	574	17.4	0.04	63% s→-s; 35% a→-a; ...
	Q	2	2.16	600	574	17.4	0.04	63% s→-a; 35% a→-a; ...
	B	3	3.32	427	374	26.8	1.31	62% a→-a; 34% s→-s; ...
	B	4	3.32		373	26.9	1.32	62% a→-s; 34% s→-a; ...

^a-Excited state numbers in increasing energy from the TD-DFT calculations. ^b – Calculated band energies (eV). ^c – Experimental absorption maxima wavelengths in nm (solvent = DMF). ^d – Calculated wavelengths in nm. ^e – Calculated band energy (10^3 cm^{-1}). ^f – Calculated oscillator strengths. ^g – Wavefunctions of MOs involved in the transitions and their respective contributions on the basis of eigenvectors predicted by TD-DFT calculations.

8.3. Closing remarks

TD-DFT calculations were carried out to study the structure-property relationships and spectroscopic properties of the series of porphyrin and BODIPY dyes that were prepared in this study. The iodination of the BODIPY dyes and subsequent addition of vinylene groups (**1c** and **3c**) resulted in a pronounced shift of the absorption maxima towards the NIR region. The addition of the styryl groups (**4c**) resulted in a larger red shift due to the larger π -conjugation systems of the benzyloxy substituents that were used. The predicted properties of the BODIPY dyes were compared to those observed experimentally, and a better understanding of their

behavior was established. Molecular modeling aided in visualizing the structures and also in understanding the effects of different functional groups on the spectral trends. With the use of molecular modeling, the rational design of novel BODIPY dyes for PDT and PACT can be achieved.

The calculations for the porphyrin complexes were more problematic, however. In comparison to the Sn(IV) tetraphenylporphyrin **8**, the Q bands of **7b** were more red-shifted, but this trend was not predicted in the TD-DFT calculations. This suggests that TD-DFT calculations with the Gaussian 09 software package do not always accurately predict trends in the Q band region of the spectra of tetraarylporphyrins, since small changes in the relative energies of the **a**, **s**, **-a** and **-s** MOs can have a significant effect. The B3LYP optimized geometries may not accurately predict the dihedral angles formed between the *meso*-aryl rings and the main porphyrin π -system.

Chapter 9: Conclusion and future prospects

9.1. General conclusions

BODIPY dyes have been used in a variety of applications due to their ease of synthesis and versatility. Novel BODIPY dyes **1c**, **3c** and **4c**, were prepared as photosensitizer dyes for PDT studies using three sequential reaction mechanisms; namely acid catalyzed condensation, electrophilic substitution and Knoevenagel condensation reactions. Previously reported BODIPY dyes **5** and **6** were also studied in this context. The preparation of red shifted and singlet oxygen generating dyes for photodynamic therapy was successfully achieved. The dyes were also found to be photostable when exposed to UV-visible region light. The encapsulation of the hydrophobic BODIPY dyes **1c**, **5** and **6** with Pluronic[®] F-127 and **4c** with cyclodextrins, significantly improved their water solubility. In aqueous medium, the dyes retained their ability to generate singlet oxygen, although there was a decrease in singlet oxygen quantum yields for some of the photosensitizer dyes. When the PDT activity of the BODIPY dyes and their Pluronic[®] F-127 micelles encapsulation complexes was studied, they all exhibited significant inhibition of MCF-7 breast cancer cell growth. Further research into BODIPY dyes and their Pluronic[®] F-127 micelles encapsulation complexes in PDT is merited. BODIPY **4c** and its m β CD inclusion complexes were also found to eradicate *Staphylococcus aureus* colonies during PACT studies, so the further investigation of using cyclodextrins as drug delivery agents for BODIPY dyes should be pursued.

In the context of the porphyrins that were studied, the incorporation of *meso*-acenaphthyl rings (porphyrin **7b**) resulted in pronounced red shifts of the Q and B bands compared to the *meso*-thienyl rings (porphyrin **8**). However, **7b** showed some aggregation compared to **8** due to the bulky acenaphthyl rings which tend to π - π stack. All the porphyrins generated singlet oxygen, making them suitable for photodynamic therapy. The cyclodextrin inclusion complexes formed

with porphyrin **7b** enhanced the solubility of the dye. When the photodynamic activity of the porphyrins was evaluated on MCF-7 breast cancer cells, there was evidence of photocytotoxicity. Further research on the use of Sn(IV) tetraacenaphthoporphyrins and their cyclodextrin inclusion complexes for both PDT and PACT is clearly merited.

9.2. Future prospects

In the case of PDT for cancer, the utility of aza-BODIPY dyes, which are more red shifted derivatives of BODIPY dyes that often absorb beyond 700 nm deep within the therapeutic window, is worth exploring in this context. Since the use of Pluronic® F-127 and the modification of the polymer with folate-functionalized chitosan as solubilizing nanocarrier has been proven to work, research into aza-BODIPY dyes encapsulation complexes should also now be explored. The results of the preliminary PACT study suggest that the photoinactivation of both gram-negative bacterial strains such as *Escherichia coli* and gram-positive strains like *Staphylococcus aureus* should now be explored in depth. The efficiency of cationic, anionic and neutral BODIPY and aza-BODIPY dyes should also be studied in this context. BODIPYs **1b**, **2b**, **3b** and **4b** were found to have exceedingly high singlet oxygen quantum yields due to the presence of iodines, so these dyes are likely to be suitable for application in PACT. **1b** and **2b** can be readily studied as cationic photosensitizer dyes through quaternization with 1,3-propanesultone, while **3b** and **4b** would be studied as neutral photosensitizer dyes.

The porphyrins used in this work have shown their potential for use as PDT agents. Further synthesis of Sn(IV) tetraacenaphthenylporphyrins with bulky axial ligands to enhance the absorption and photophysicochemical properties could lead to better PDT efficiency. Suitable

targeting ligands could also be attached to the axial ligands to achieve more selective drug delivery.

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