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THESIS

INDOLENINE AND BENZOTHIAZOLE CYANINE DYES WITH AN INDOLE NUCLEUS AS FLUORESCENT DYES FOR DNA DETECTION: MICROWAVE-ASSISTED SOLVENT-FREE SYNTHESIS, SPECTRAL PROPERTIES AND THEORETICAL STUDIES

WILAWAN AMATAPITAKSAKUL

A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
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Wilawan Amatapitaksakul 2012: Indolenine and Benzothiazole Cyanine Dyes with an Indole Nucleus as Fluorescent Dyes for DNA Detection: Microwave-assisted Solvent-free Synthesis, Spectral Properties and Theoretical Studies. Master of Science (Chemistry), Major Field: Chemistry, Department of Chemistry. Thesis Advisor: Mr. Surachai Thachepan, Ph.D. 65 pages.

Indolenine and benzothiazole cyanine dyes were synthesized by condensation of 2,3,3-trimethylindolenine or 2-methyl benzothiazole with carboxaldehyde in the presence of triethylamine under solvent-free microwave irradiation. Characterization by FTIR, UV-Vis and ^{1}H-NMR spectroscopy confirmed that the synthesized products were indolenine and benzothiazole cyanine dyes. The products were obtained with high yield, suggesting that solvent-free synthesis of indolenine and benzothiazole cyanine dyes was a fast and efficient route. The absorption and fluorescence properties of the dyes were investigated both experimentally and theoretically. The calculations performed using a combination of time-dependent density functional theory (TD-DFT) and polarizable continuum model (PCM) helped us to interpret the behavior of electronic transition of indolenine and benzothiazole cyanine dyes and the calculated results were in good agreement with experimental values.

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Student's signature Thesis Advisor's signature

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I am specially appreciated my parents for continuously boosting my morale, encouraging me to overcome the obstacles and giving me financial assistance. Honestly speaking, I especially thank my colleagues and my friends for endorsing me and devoting their valuable time to helping me during my graduate study.

Wilawan Amatapitaksakul

May, 2012

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LIST OF ABBREVIATIONS

B3LYP	=	Becke-Lee, Yang and Parr correlation functional
DFT	=	Density Functional Theory
FTIR	=	Fourier Transform Infrared Spectroscopy
NMR	=	Nuclear Magnetic Resonance Spectroscopy
PESs	=	Potential Energy Surfaces
TD-DFT	=	Time-Dependent Density Functional Theory
UV-Vis	=	Ultraviolet–visible Spectrophotometry

INDOLENINE AND BENZOTHIAZOLE CYANINE DYES WITH AN INDOLE NUCLEUS AS FLUORESCENT DYES FOR DNA DETECTION: MICROWAVE-ASSISTED SOLVENT-FREE SYNTHESIS, SPECTRAL PROPERTIES AND THEORETICAL STUDIES

INTRODUCTION

Cyanine dyes

Cyanine dyes, first synthesized in 1856, came to the limelight for application in the field of photography and had now received wide attention due to their applications in photodynamic therapy. Cyanine dye is a class of polymethine dyes, which consisted planar, conjugated and open-chain (sometimes ring) systems of sp^2-hybridized carbon atoms with an odd number of methine groups and an even number of π electrons according to the general formula: $X\text{-}(CR)_n\text{-}X'$ with n=1, 3, 5, ... ; R-H or substituents X and X'= terminal chain atoms, e.g., N, O, P, S or atom groups, e.g., NR_2, CH=O (Panigrahi *et al.,* 2012).

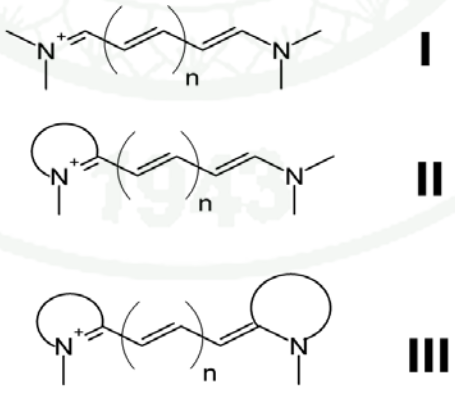


Figure 1 Types of cyanines (I = Open chain cyanines, II = Hemicyanines and III = Closed chain cyanines)

Source: Aktuell bei (2007)

Cyanine dyes present many properties such as large molar extinction coefficient, large tunable range of maximum absorption wavelength, high fluorescence efficiency, ease of synthesis, relatively high stability and act as the most important organic functional dyes in many processes of technological interest such as sensitizers in photography, sensitizers in solar cell, optical recording materials in laser disks, biomedical applications, photodynamic therapy, etc. In addition, a very attractive feature of cyanine dyes is its affinity for biological structures, especially DNA (Wang *et al.,* 2004), (Fu *et al.,* 2009).

These dyes are suggested to be used as fluorescent probes for DNA as they exhibit a dramatic enhancement in fluorescence intensity upon binding to DNA. Many researchers' efforts have been devoted to develop the syntheses and applications of cyanine dyes. Synthetic processes have been proposed extensively for monomethines, dimethines, trimethines, tetramethines, pentamethines, heptamethines, squarylium cyanines and other cyanine dyes.

Fluorescence

Fluorescence was observed in 1565 in the infusion of a certain type of wood. The chemical compound responsible for fluorescence is *matlanine*, the oxidation product of the flavonoids found in the wood. Fluorescence is the emission of light by a substance that has absorbed light or other electromagnetic radiation. The fluorescence occurs when an electron of a molecule, atom or nanostructure relaxes to ground state by emitting a photon of light after being excited to a higher quantum state. The absorbed radiation is in the ultraviolet region of the spectrum and the emitted light is in the visible region.

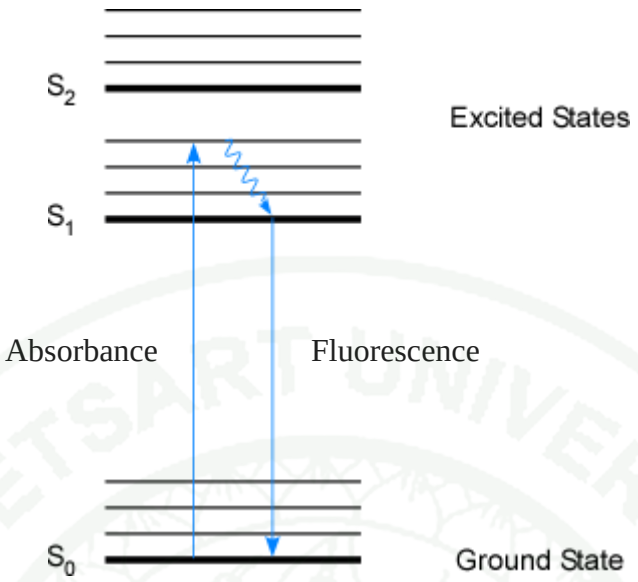


Figure 2 Jablonski diagram of fluorescence

Source: Dunnivant and Ginsbach (2008)

Fluorescence has many applications such as chemical sensors (fluorescence spectroscopy), fluorescent labelling, dyes, biological detectors and fluorescent lamps. The example of fluorescence application is a detection of nucleic acids in molecular biology. Le Pecq and Paoletti proposed the first fluorescence label for nucleic acids-ethidium bromide (Timtcheva *et al.,* 2000). Fluorescence probes are powerful tools for investigating molecular behavior in living tissues and cells. The detection of biological molecules in response to environmental change depend on fluorescence methods (Feng *et al.,* 2010), (Zheng *et al.,* 2011).

Microwave irradiation technique

Microwave technology has been used in organic chemistry since the mid-1980s and in the mid-1990s, the number of publications has increased significantly as the technique was the solvent-free technique, improved the safety aspects and shortened reaction times.

During microwave dielectric heating, microwave energy is introduced into the chemical reactor remotely and direct access by the energy source to the reaction vessel is obtained. The microwave radiation passes through the walls of the vessel and heats only the reactants and solvent, not the reaction vessel itself (Lidstrom *et al.,* 2001). Microwave irradiation presents a powerful tool toward organic reactions. Solvent-free microwave irradiation is well known as environmentally benign method, which offers several advantages including shorter reaction time, cleaner reaction profiles and simple experimental/product isolation procedures (Wang *et al.,* 2003). The advantages of this technique have been exploited in the context of multistep total synthesis and medicinal chemistry or drug discovery.

In this work, a microwave-assisted preparation of indolenine and benzothiazole cyanine dyes via condensation of 2,3,3-trimethylindolenine or 2-methylbenzothiazole with carboxaldehyde derivatives in the presence of triethylamine was investigated. This approach provided an attractive and environmentally friendly pathway to several useful synthetic indolenine and benzothaiazole cyanine dyes as it involved solvent-free synthesis. Furthermore, an investigation of spectral properties of the prepared indolenine and benzothiazole cyanine dyes was also carried out. UV-Vis absorption behaviour of indolenine and benzothiazole cyanine dyes was studied experimentally using UV-Vis spectrophotometer and theoretically using TD-DFT approach. The goal of the research presented herein is to investigate the synthesis, structural and spectral properties of indolenine and benzothiazole cyanine dyes, which can be potential candidates for future application in a number of fields.

OBJECTIVES

1. To synthesize and characterize indolenine and benzothiazole cyanine dyes using solvent-free microwave irradiation technique.

2. To investigate the structural and electronic properties of indolenine and benzothiazole cyanine dyes using quantum chemical calculations.

LITERATURE REVIEW

There are several methods for the synthesis of cyanine dyes each of which has different advantages and disadvantages. Classical synthesis of hemicyanine dyes with benzimidazole nucleus or with 4-linked pyridine nucleus is often carried out by refluxing a mixture of an aromatic aldehyde, a quaternary salt of benzimidazole or pyridine having reactive methyl group and a catalyst in organic medium (in 1949, 1964 and 1976).

In order to enhance the fluorescence probing of nucleic acids, some novel asymmetric analogues of thiazole orange with amide substituents in the benzothiazole moiety were synthesized. For the preparation of some neutral monomethine cyanine dyes, Deligeorgiev and coworker (2000) synthesized homodimeric asymmetric monomethine cyanine dyes based on the thiazole orange via an improved synthetic procedure. Qunolinium iodide and N,N,N',N'-tetramethyl-1,3-propanediamine, N,N,N',N'-tetra-methyl-1,6-hexanediamine, 1,4-diazabicyclo-[2,2,2]-octane or 1,40-bipyridine were refluxed in 2-methoxyethanol. After that the precipitated dye was filtered, dried and recrystallized from methanol. The products were characterized by ^{1}H-NMR, UV-Vis spectroscopy and photofluorescence spectroscopy.

Wang and coworker (2003) developed synthetic strategy, based on modified Vilsmeier reaction. They used the condensation reaction of indoline derivatives with substitutional organic pyridinium iodide or 4-diphenylamino-benzaldehyde, to generate methane cyanine dyes with asymmetrical structures. The products were characterized by ^{1}H-NMR, mass spectroscopy, UV/visible spectroscopy and an elementar vario EL III (made in Germany) analyzer.

In 2006, Romieu and coworker reported the synthesis of a new fluorescent pentamethine cyanine dye with an amino acid derivative. Other derivative with a trisulfonated linker led to a novel watersoluble near-infrared (NIR) dye suitable for labeling of biomolecules. Synthesis of the cyanine-based amino acid was

accomplished in five steps, starting from 2,2,3-trimethyl-1H-benz[e]indole as a common precursor for the two moieties.

Jiang and coworker (2007) synthesized unsymmetrical water-soluble cyanine dyes using poly(ethylene glycol) (PEG) as a soluble support. Loading and activation of sulfoindolenium to poly(ethylene glycol) were achieved via a simple strategy. Cyanine dyes were released by the attack of heterocyclic carbon nucleophile and the cleavage of PEG-bound hemicyanine. It was found the reaction in ethanol with reflux gave by far the best result. The products confirmed by ^{1}H-NMR spectroscopy and analyzed by HPLC and TLC.

In 2009, Reis and coworker synthesised several squarylium cyanine dyes from benzothiazole, benzoselenazole and quinoline. Synthesis of squarylium cyanine dyes was carried out by condensation of squaric acid and N-hexylbenzoazolium iodide in a refluxing mixture of n-BuOH/pyridine. The products were characterized by ^{1}H- and ^{13}C-NMR, IR and mass spectroscopy.

El-Shishtawy and coworker (2010) prepared novel monomethine cyanine dye from β-naphthothiazole and benzothiazole. The product foemed by the condensation of two sulfate heterocyclic quaternary salts was recrystallized from DMF. The dyes were characterized by ^{1}H- and ^{13}C-NMR, FTIR, ESIMS, elemental analysis, absorption and fluorescence spectroscopy. The cyanine dye was used a probe for the detection of ct-DNA in aqueous solution.

Kaloyanova and coworker (2011) synthesized sixteen new asymmetric monomethine cyanine dyes by the reaction of two heterocyclic quaternary salts, an alkylthio and a methyl group. The reaction was stirred at room temperature for 1–5 h. and the products were washed with diethyl ether, air-dried and finally recrystallized from methanol. The products were characterized by ^{1}H-NMR, UV-Vis spectroscopy and fluorescence spectroscopy. The spectral characteristics and interaction with double stranded DNA was also investigated.

The previously described preparation methods had substantial drawbacks, such as relatively strenuous reaction conditions, i.e., is refluxing reactants for several hours in organic solvents, a large amount organic solvents used, and the complexity of isolation of the products. Alternative to classical organic synthesis, microwave irradiation technique has been proposed for rapid synthesis of a variety of compounds. In recent years, microwave-accelerated solvent-free organic synthesis had provided faster reactions and high product yield could be obtained. Moreover, this technique was friendly to environment since no solvent was required.

Zhang and coworker (2008) demonstrated a fast and efficient reaction with high yield by the condensation of quaternary salts with 1H-indole-3-carbaldehyde in the presence of piperidine under solvent-free microwave irradiation. The products were identified by ^{1}H-NMR, IR, UV-Vis spectroscopy and elemental analysis. The absorption and fluorescence properties of the dyes in both free state and binded state with DNA or BSA were investigated. Significant enhancement of fluorescent quantum yield was observed for all dyes in the presence of DNA, with one compound demonstrating excellent sensitivity as a fluorescent probe.

In 2004 and 2009, Wang and coworker used solvent-free microwave radiation technique to synthesize several useful cyanine dyes. The absorption property of the prepared dyes was investigated both experimentally and theoretically. Calculations performed using a combination of time-dependent density functional theory (TD-DFT) and polarizable continuum model (PCM) gave π/π^* type absorption bands of the dyes. Multiple linear regression, applied to the theoretical absorption maxima in different solvents, fitted well with experimental data. Resonance frequency calculations were undertaken to simulate the IR spectra of the dyes. The calculated results were in good accordance with experimental values.

Yi-Le Fu and coworker (2009) synthesized four asymmetric monomethine indocyanine dyes by the condensation of indole quaternary salts with 2-methylthio quinoline quaternary salt in the presence of triethylamine under solvent-free, microwave irradiation. The effects of microwave power and irradiation time on yield

were examined. The products were identified by ^{1}H- and ^{13}C-NMR, IR, MS, UV-Vis spectroscopy and elemental analysis. The UV-Vis absorption of the dyes was investigated both experimentally and theoretically.

In 2010, Winstead and coworker synthesized heptamethine cyanine dyes using microwave assisted organic synthesis. The reported yields of cyanine dyes were in a range of 64-83% with a significant reduction in solvent used. The dyes were identified by ^{1}H- and ^{13}C-NMR, UV-Vis spectroscopy and fluorescence spectroscopy.

Liu and coworker (2011) synthesized merocyanine dyes via both conventional solvent method and a microwave-irradiation method using rhodanine, 2-methylthio quaternary salts and 1H-indole-3-carbaldehyde as starting materials. The structures of two dyes were characterized and analyzed by X-ray diffraction.

MATERIALS AND METHODS

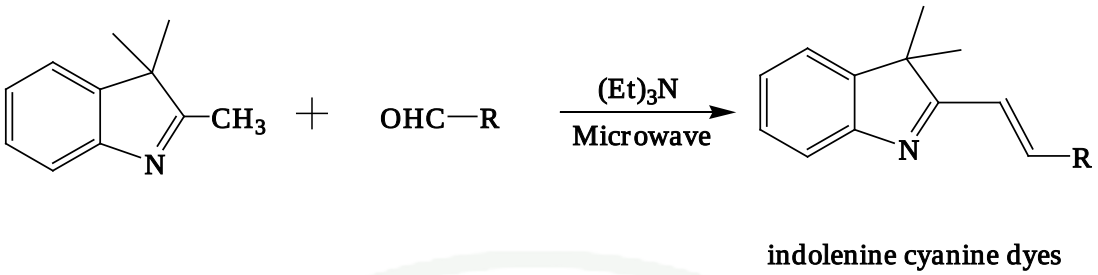
1. Synthesis and Characterization

1.1 Materials

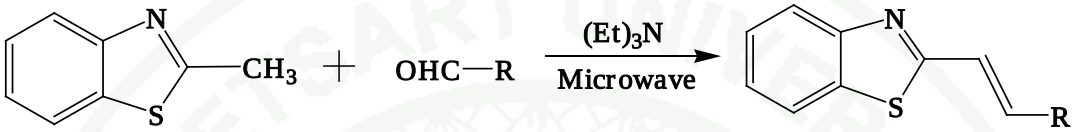
- 2,3,3-trimethylindolenine ($C_{11}H_{13}N$)
- 2-methylbenzothiazole (C_8H_7NS)
- Indole-3-carboxaldehyde (C_9H_7NO)
- 1-methylindole-3-carboxaldehyde ($C_{10}H_9NO$)
- N-Ethyl-3-carbazolecarboxaldehyde ($C_{15}H_{13}NO$)
- 2-Fluorenecarboxaldehyde ($C_{14}H_{10}O$)
- 2-thiophenecarboxaldehyde (C_5H_4OS)
- Triethylamine (Et_3N)
- Ethanol (C_2H_5OH)

1.2 Synthesis

Diagrams showing synthesis of indolenine cyanine dyes and benzothiazole cyanine dyes are presented in Figure 3.



(a)



(b)

Figure 3 diagrams showing synthesis of (a) indolenine and (b) benzothiazole cyanine dyes

A series of indolenine and benzothiazole cyanine dyes were synthesized using equal moles of 2,3,3-trimethylindolenine (or 2-methylbenzothaizole for benzothaiazole cyanine dyes) and carboxaldehyde derivatives (OHC-R, R= C_8H_6N, C_9H_8N, $C_{14}H_{12}N$, $C_{13}H_9$, C_4H_3S) with a few drops of triethylamine.

1.2.1 indolenine cyanine dyes (cpd I1, cpd I2 and cpd I3)

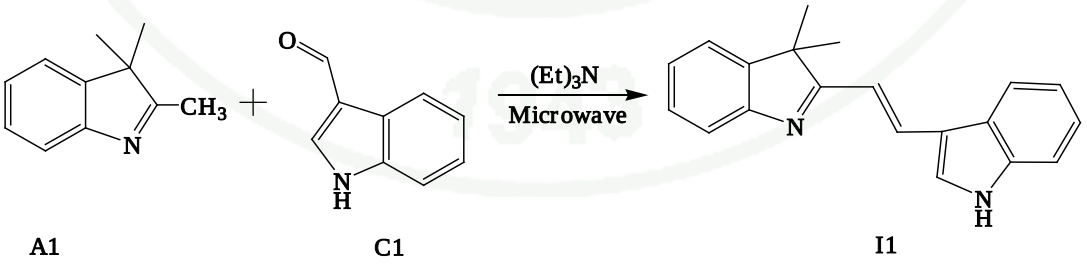


Figure 4 diagrams showing synthesis of indolenine cyanine dyes (cpd I1)

First, 2,3,3-Trimethylindolenine (A1) (0.1605 ml, 1 mmol), Indole-3-carboxaldehyde (C1) (0.1452 g, 1 mmol) and a few drops of triethylamine were mixed in a glass vial. After that, the reaction mixture was subjected to microwave irradiation. The yellow product was purified by recrystallization in ethanol.

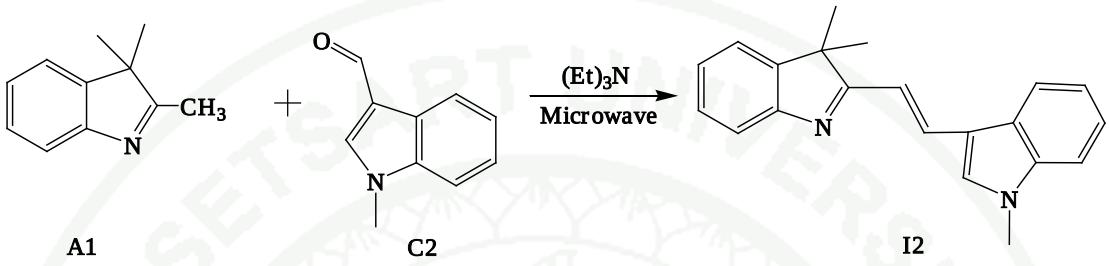


Figure 5 diagrams showing synthesis of indolenine cyanine dyes (cpd I2)

First, 2,3,3-trimethylindolenine (A1) (0.1605 ml, 1 mmol), 1-methylindole-3-carboxaldehyde (C2) (0.1592 g, 1 mmol) and a few drops of triethylamine were mixed in a glass vial. After that, the reaction mixture was subjected to microwave irradiation. The yellow product was purified by recrystallization in ethanol.

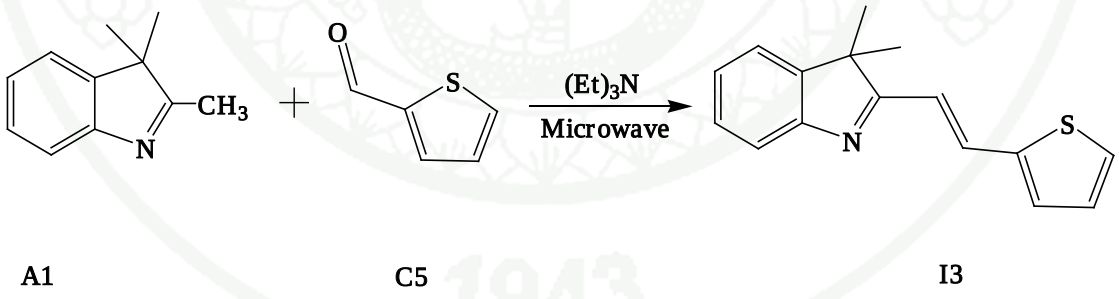


Figure 6 diagrams showing synthesis of indolenine cyanine dyes (cpd I3)

First, 2,3,3-Trimethylindolenine (A1) (0.1605 ml, 1 mmol), 2-thiophene-carboxaldehyde (C5) (0.0934 ml, 1 mmol) and a few drops of triethylamine were mixed in a glass vial. After that, the reaction mixture was subjected to microwave irradiation. The yellow product was purified by recrystallization in ethanol.

1.2.2 Synthesis of Benzothiazole Cyanine dyes (cpd B1, cpd B2 and cpd B3)

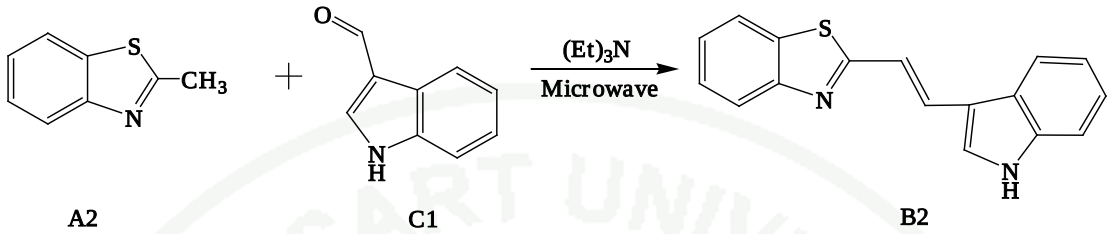


Figure 7 diagrams showing synthesis of benzothiazole cyanine dyes (cpd B1)

First, 2-Methylbenzothiazole (A2) (0.1272 ml, 1 mmol), Indole-3-carboxaldehyde (C1) (0.1452 g, 1 mmol) and a few drops of triethylamine were mixed in a glass vial. After that, the reaction mixture was subjected to microwave irradiation. The yellow product was purified by recrystallization in ethanol.

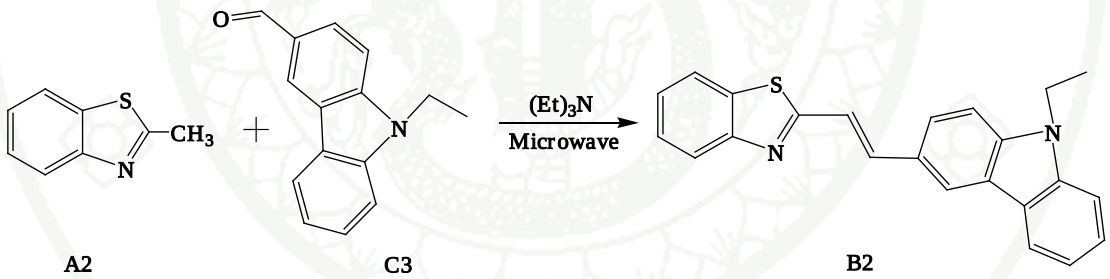
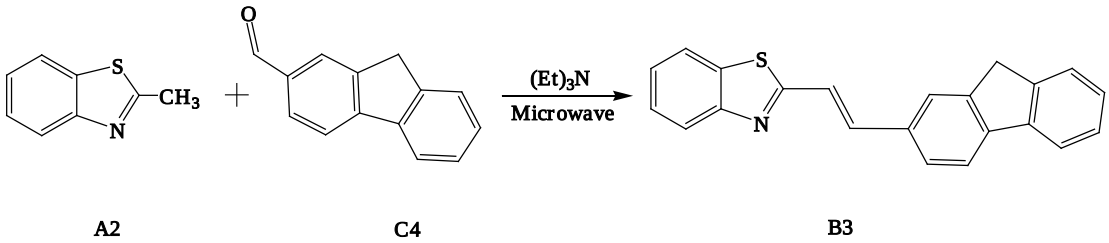


Figure 8 diagrams showing synthesis of benzothiazole cyanine dyes (cpd B2)

First, 2-Methylbenzothiazole (A2) (0.1272 ml, 1 mmol), N-Ethyl-3-carbazolecarboxaldehyde (C3) (0.2233 g, 1 mmol) and a few drops of triethylamine were mixed in a glass vial. After that, the reaction mixture was subjected to microwave irradiation. The yellow product was purified by recrystallization in ethanol.



A2 C4 B3

Figure 9 diagrams showing synthesis of benzothiazole cyanine dyes (cpd B3)

First, 2-Methylbenzothiazole (A2) (0.1272 ml, 1 mmol), 2-Fluorene-carboxaldehyde (C4) (0.1942 g, 1 mmol) and a few drops of triethylamine were mixed in a glass vial. After that, the reaction mixture was subjected to microwave irradiation. The yellow product was purified by recrystallization in ethanol.

1.3 Instruments

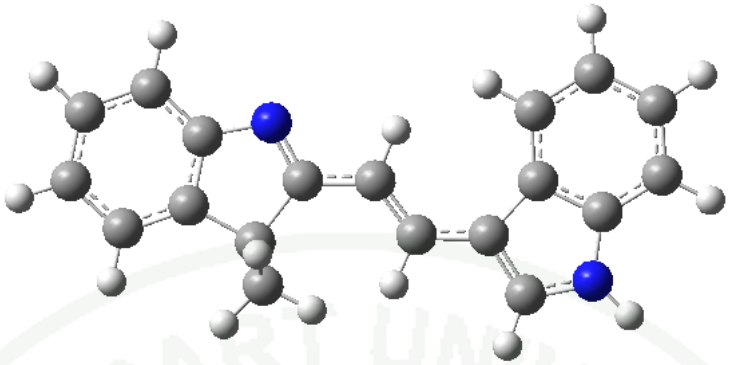
^{1}H-NMR analysis was carried out using a VARIAN ^{UNITY} INOVA spectrometer, operating at 400.00 MHz for ^{1}H. Samples were dissolved in deuterated chloroform ($CDCl_3$) with addition of tetramethylsilane (TMS) as an internal reference. FTIR analyses were recorded using a Perkin Elmer spectrometer model system-2000. Samples were measured using KBr disk method. UV-Vis measurements were performed using a Perkin Elmer Lambda 35 UV-Vis spectrophotometer. Samples were dissolved in ethanol and a quartz cell with 1 cm path length was used. Fluorescence measurements were performed using a Perkin Elmer Instruments LS55 fluorescence spectrophotometer. Samples were dissolved in ethanol and a quartz cell with 1 cm path length was used.

2. Quantum Chemical Calculation

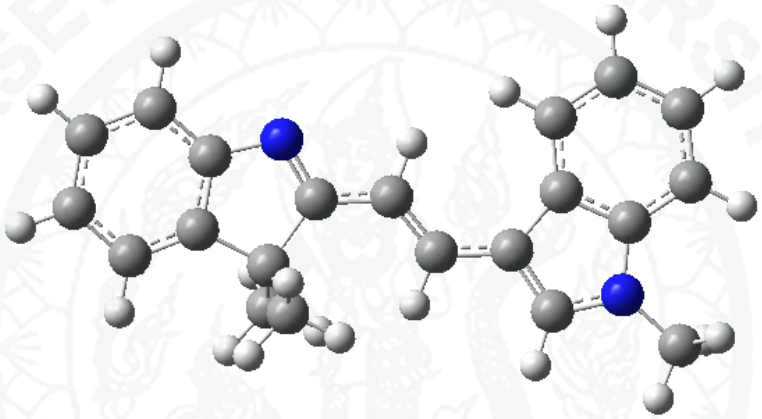
Quantum chemical calculation is the application of chemical, mathematical and computational skills to the approximations of chemistry problems. It uses computers to generate information such as properties of structure and dynamic molecular system. It also helps predict before running the actual experiments so that they can be better prepared for making observations. Theoretical studies are an important to approximate the electronic structure determinations, geometry optimizations, frequency calculations, transition structures, protein calculations, electron and charge distributions, potential energy surfaces (PES), rate constants for chemical reactions (kinetics), thermodynamic calculations heat of reactions and energy of activation of molecular system with common computer software. In addition, the experimental cannot be explained with the electron term. Computational quantum chemistry is one of the challenging tasks in calculating the electronic structure and predicting properties of variety of molecules. This approach can be provided molecular models and guide the design of novel molecules.

2.1 Method of Calculations

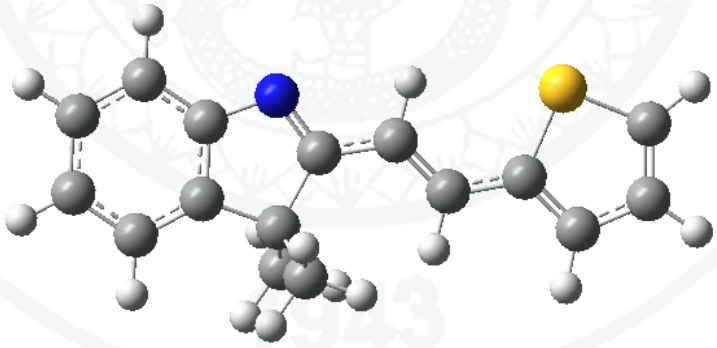
Starting geometries of indolenine and benzothiazole cyanine dyes were constructed by molecular modeling and their ground state geometry was determined by a full optimization using the density functional theory (DFT) level. The ground state geometries of indolenine and benzothiazole cyanine dyes were fully optimized using B3LYP/6-31G(d) calculations. The molecular structure of indolenine and benzothiazole cyanine dyes composed of -CH= group linking two the heterocyclic rings. All molecules are shown in Figure 10 and 11.



cpd I1

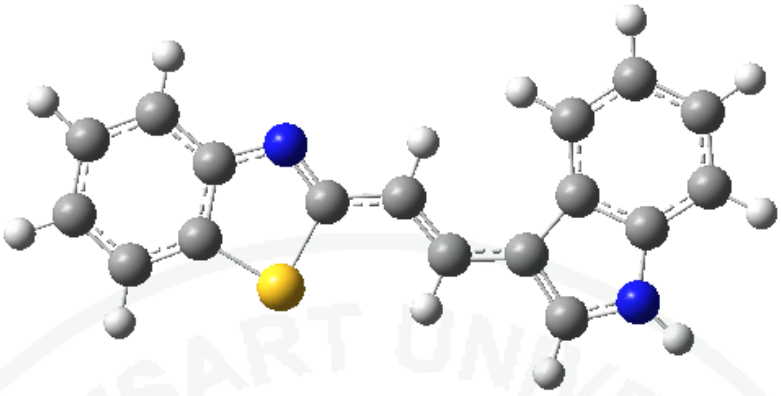


cpd I2

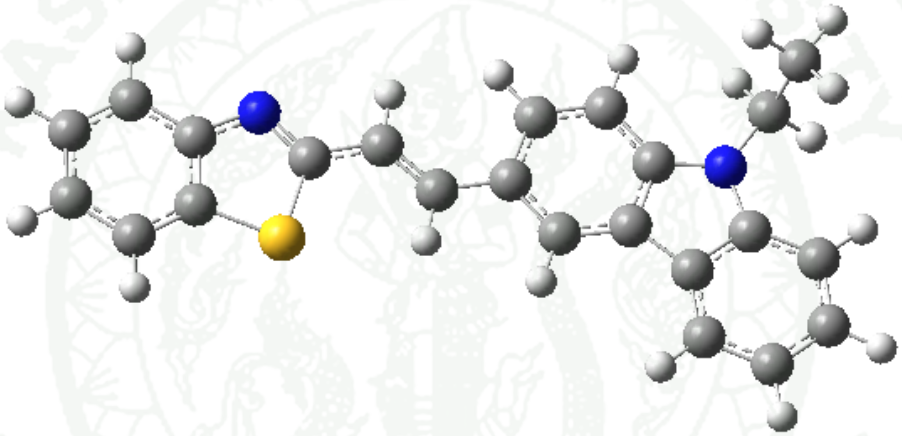


cpd I3

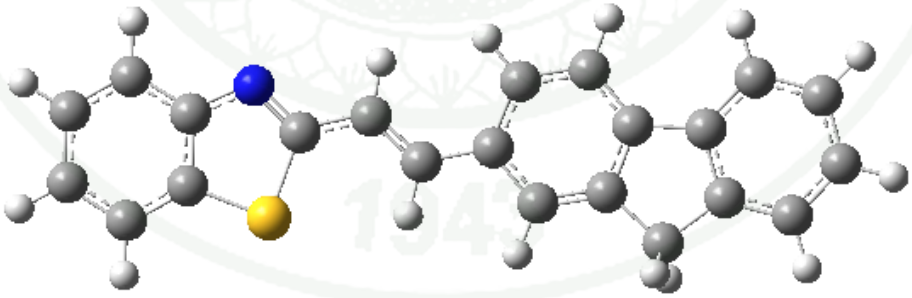
Figure 10 The structure of indolenine cyanine dyes from calculations.



cpd B1



cpd B2



cpd B1

Figure 11 The structure of benzothiazole cyanine dyes from calculations.

2.2 Conformation Analysis

In this study, the indolenine and benzothiazole cyanine dyes conformations were investigated. Bond lengths, bond angles and torsion angles were calculated using B3LYP method with 6-31G(d) basis set. The basis set 6-31G(d) has been used for geometries optimization. The torsion angles units (∠C1-C2-C3-C4) of indolenine and benzothiazole cyanine dyes were studies between two isomers.

.

2.3 Electronic Properties

2.3.1 Absorption properties

The electronic transitions (vertical excitation energies) were calculated by time-dependent density function theory (TDDFT) level with B3LYP method and the basis set 6-311G(d,p), which performed by single point calculations at the optimized geometries of the ground state. All calculations were performed using the Gaussian 09 program package of programs, revision B.01.

RESULTS AND DISCUSSION

1. Synthesis and Charaterization

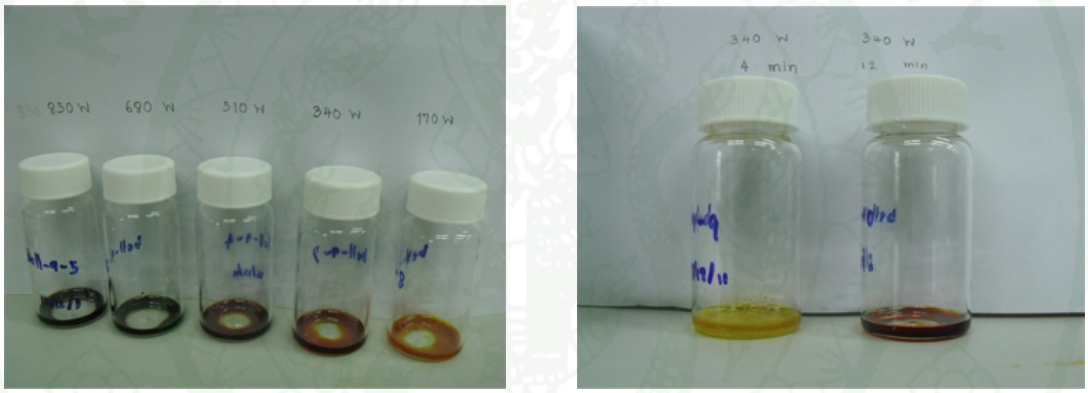
In general, the dimethine cyanine dye formation reaction proceeded efficiently and high to excellent yields of product could be obtained within short reaction time. The cyanine dye products were successfully synthesized in solid-state reaction as all reactants were in solid form and the reaction temperature was maintained below their melting points using microwave-assisted strategy.

1.1 Indolenine Cyanine dyes (cpd I1)

Indolenine cyanine dyes (cpd I1) was prepared by mixing equal moles of 2,3,3-trimethylindolenine (A1) and indole-3-carboxaldehyde (C1) with a few drops of triethylamine followed by microwave irradiation. The synthesized dye was purified by recrystallization in ethanol. ^{1}H-NMR and FTIR analyses of the obtained dyes confirmed the identity of indolenine cyanine dyes (cpd I1). The percentage yield of the dyes obtained from various reaction conditions were tabulated in Table 1. The utilized microwave power and irradiation time were optimized in order to maximize the percentage yield of the products as shown in Figure 12. Typically, the syntheses gave the product yield comparable to those obtained by heat reaction (El-Shishtawy *et al.*, 2010). It was found that the reaction yields increased with increasing irradiation time, but too long irradiation time produced large amount of heat, which induced thermal degradation of the products. The optimized microwave power and irradiation time for the synthesis were 510 Watt and 8 minutes respectively, which produced 92.26% yield of the dyes.

Table 1 The percentage yield of indolenine cyanine dyes (cpd I1) obtained from reaction using various microwave powers and irradiation times.

	Power (W)	Time (min)	Yield (%)
Solvent-free Microwave irradiation	170	8	58.49
	340	4	66.55
	340	8	74.58
	340	12	54.82
	510	8	92.26
	680	8	78.85
	850	8	64.18
Heat reaction (El-Shishtawy *et al.*, 2010)	-	540	34



(a) (b)

Figure 12 The various reaction conditions for synthesis (a) microwave powers and (b) irradiation times

The ^{1}H-NMR spectrum was shown in Appendix A. The ^{1}H-NMR spectrum of indolenine cyanine dyes (cpd I1) was easily obtained because it could be dissolved in *d*-chloroform ($CDCl_3$) at room temperature. The ^{1}H-NMR spectrum exhibited the chemical shift of methyl side chain in the region of 1.0-1.8 *ppm*. The double bond-*H* showed chemical shift in the region of 5.0-6.0 *ppm* and 9.3 *ppm*. The aromatic group (indole ring) signal appeared at 7.0-8.0 *ppm*. The nitrogen-*H* signal appeared at 10.0 *ppm*. The elemental composition of indolenine cyanine dyes (cpd I1) showed good agreement with those calculated from the expected empirical formula.

The FTIR spectrum was shown in Appendix B. The FTIR spectrum showed absorption bands assigned to C=C stretching at 1640 cm^{-1}, aromatic ring at 1450-1600 cm^{-1}, =C-H at 3100 cm^{-1}, C-H stretching at 2931 cm^{-1}, C-H bending at 759 cm^{-1}, C-N at1230 $cm^{-1}$and N-H at 3300 cm^{-1}.

1.2 Indolenine Cyanine dyes (cpd I2 and cpd I3)

Indolenine cyanine dyes (cpd I2 and cpd I3) was prepared by mixing equal moles of 2,3,3-trimethylindolenine (A1) and 1-methylindole-3-carboxaldehyde (C2)/ 2-thiophenecarboxaldehyde (C5) with a few drops of triethylamine followed by microwave irradiation. The synthesized dye was purified by recrystallization in ethanol. ^{1}H-NMR and FTIR analyses of the obtained dyes confirmed the identity of indolenine cyanine dyes (cpd I2 and cpd I3). The optimized microwave power and optimized irradiation time for the synthesis were 510 Watt and 8 minutes respectively. The percentage yield of the indolenine cyanine dyes (cpd I2 and cpd I3) obtained 90.07% yield and 89.76% yield, respectively.

The ^{1}H-NMR spectrum of indolenine cyanine dyes (cpd I2) was shown in Appendix A. The ^{1}H-NMR spectrum was easily obtained because it could be dissolved in *d*-chloroform ($CDCl_3$) at room temperature. The ^{1}H-NMR spectrum exhibited the chemical shift of alkyl side chain in the region of 1.2-1.6 *ppm* and 3.6-4.0 *ppm*. The double bond-*H* showed chemical shift in the region of 5.0-6.0 *ppm* and 8.2-8.6 *ppm*. The aromatic group (indole ring) signal appeared at 7.0-8.0 *ppm*. The elemental composition of indolenine cyanine dyes (cpd I2) showed good agreement with those calculated from the expected empirical formula.

The FTIR spectrum of indolenine cyanine dyes (cpd I2) was shown in Appendix B. The FTIR spectrum showed C=C stretching at 1641 cm^{-1}, aromatic ring at 1450-1600 cm^{-1}, =C-H at 3100 cm^{-1}, C-H stretching at 2931 cm^{-1}, C-H bending at 759 cm^{-1} and C-N at 1190 cm^{-1}.

The ^{1}H-NMR spectrum of indolenine cyanine dye (cpd I3) was shown in Appendix A. The ^{1}H-NMR spectrum was easily obtained because it could be dissolved in *d*-chloroform ($CDCl_3$) at room temperature. The ^{1}H-NMR spectrum exhibited the chemical shift of alkyl side chain in the region of 1.2-1.8 *ppm*. The double bond-*H* showed chemical shift in the region of 5.0-6.0 *ppm*. The aromatic group (indole ring and thiophene ring) signal appeared at 7.0-8.0 *ppm*. The elemental composition of indolenine cyanine dyes (cpd I3) showed good agreement with those calculated from the expected empirical formula.

The FTIR spectrum of indolenine cyanine dyes (cpd I3) was shown in Appendix B. The FTIR spectrum showed C=C stretching at 1654 cm^{-1}, aromatic ring at 1450-1600 cm^{-1}, =C-H at 3067 cm^{-1}, C-H stretching at 2931 cm^{-1}, C-H bending at 759 cm^{-1} and C-N at1197 cm^{-1}.

1.3 Benzothaizole Cyanine dyes (cpd B1, cpd B2 and cpd B3)

Benzothiazole cyanine dyes (cpd B1, cpd B2 and cpd B3) was prepared by microwave irradiation of a mixture of equal moles of 2-methylbenzothiazole (A2), Indole-3-carboxaldehyde (C1)/ carbazolecarboxaldehyde (C3)/ 2-Fluorene-carboxaldehyde (C4) and a few drops of triethylamine under solvent-free condition. The condition for the synthesis was microwave power at 510 Watt and irradiation time at 8 minutes. The product was purified by recrystallization in ethanol. The percentage yield of the benzothiazole cyanine dye (cpd B1, cpd B2 and cpd B3) obtained 93.54% yield, 87.57% yield and 90.34% yield, respectively. The benzothiazole cyanine dyes has yellow crystals. ^{1}H-NMR and FTIR analyses of the obtained dyes confirmed the identity of benzothiazole cyanine dyes (cpd B1, cpd B2 and cpd B3).

The ^{1}H-NMR spectrum of benzothiazole cyanine dye (cpd B1) was shown in Appendix A. The ^{1}H-NMR spectrum was easily obtained because it could be dissolved in acetone-d_6 at room temperature. The ^{1}H-NMR spectrum exhibited the chemical shift of double bond-*H* showed chemical shift in the region of 7.0-7.4 *ppm*.

The aromatic group (indole ring) signal appeared at 7.4-8.4 *ppm*. The nitrogen-*H* signal appeared at 10.0 *ppm*.

The FTIR spectrum of benzothiazole cyanine dye (cpd B1) was shown in Appendix B. The FTIR spectrum showed C=C stretching at 1637 cm^{-1}, aromatic ring at 1450-1600 cm^{-1}, =C-H at 3100 cm^{-1}, C-H stretching at 2892 cm^{-1}, C-H bending at 742 cm^{-1} and C-N at1150 cm^{-1}.

The ^{1}H-NMR spectrum of benzothiazole cyanine dyes (cpd B2) was shown in Appendix A. The ^{1}H-NMR spectrum was easily obtained because it could be dissolved in acetone-d_6 at room temperature. The ^{1}H-NMR spectrum exhibited the chemical shift of alkyl side chain in the region of 1.3-1.6 *ppm* and 4.0-4.8 *ppm*. The aromatic group (indole ring and carbazole ring) signal appeared at 7.0-8.4 *ppm*.

The FTIR spectrum of benzothiazole cyanine dyes (cpd B2) was shown in Appendix B. The FTIR spectrum showed C=C stretching at 1680 cm^{-1}, aromatic ring at 1450-1600 cm^{-1}, =C-H at 3053 cm^{-1}, C-H stretching at 2895 cm^{-1}, C-H bending at 750 cm^{-1} and C-N at 1149 cm^{-1}.

The ^{1}H-NMR spectrum of benzothiazole cyanine dyes (cpd B3) was shown in Appendix A. The ^{1}H-NMR spectrum was easily obtained because it could be dissolved in acetone-d_6 at room temperature. The ^{1}H-NMR spectrum exhibited the chemical shift of double bond-*H* in the region of 3.8-4.4 *ppm*. The aromatic group (indole ring and fluorene ring) signal appeared at 7.0-8.4 *ppm*.

The FTIR spectrum of benzothiazole cyanine dyes (cpd B3) was shown in Appendix B. The FTIR spectrum showed C=C stretching at 1680 cm^{-1}, aromatic ring at 1450-1600 cm^{-1}, =C-H at 3049 cm^{-1} and C-H bending at 730 cm^{-1}.

2. Absorption Properties

The absorption spectra of indolenine and benzothiazole cyanine dyes in ethanolic solution were depicted in Figure 13 and Figure 14, respectively. The spectra showed similar charecteristic pattern for cpd I1, cpd I2 and cpd I3 and for cpd B1, cpd B2 and cpd B3. Maximum absorption of the indolenine cyanine dyes appeared at 319 and 371 nm for cpd I1, 317 and 398 nm for cpd I2 and 283 and 359 nm for cpd I3 as shown in Figure 13. The important absorptions were 371 nm for cpd I1, 398 nm for cpd I2 and 359 nm for cpd I3 because absorption range about 400 nm was conjugate (C=C) bands. Maximum absorption of the benzothiazole cyanine dyes appeared at 295 nm for cpd B1, 331 nm for cpd B2 and 330 nm for cpd B3 as shown in Figure 14.

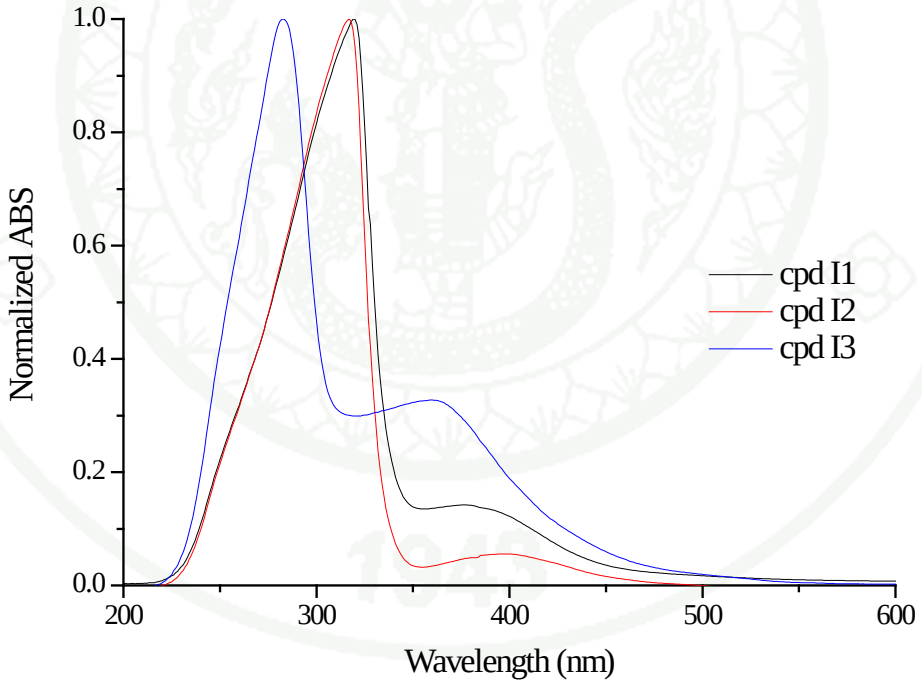


Figure 13 Absorption spectra of indolenine cyanine dyes in ethanolic solution.

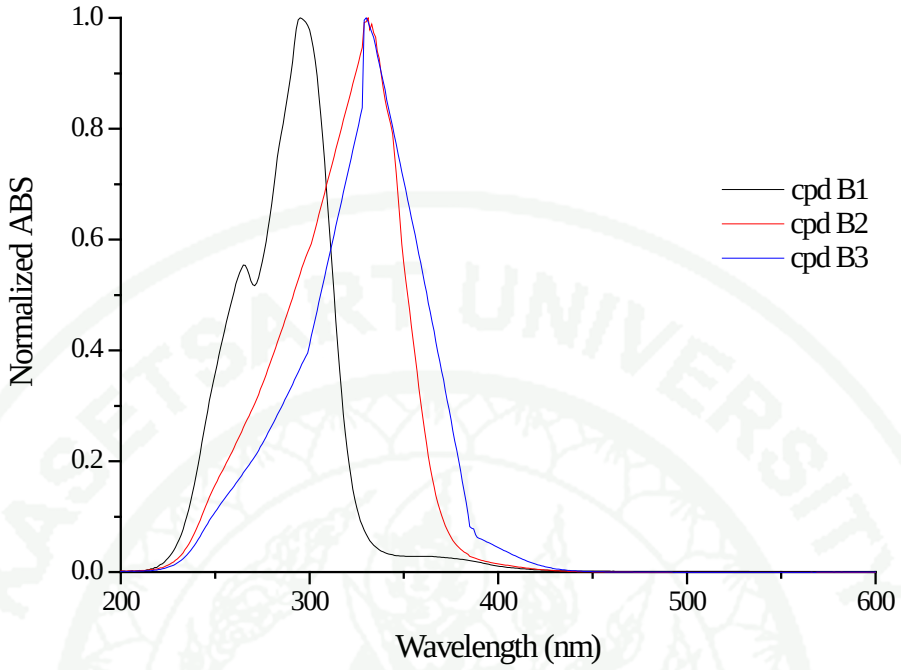


Figure 14 Absorption spectra of benzothiazole cyanine dyes in ethanolic solution.

Absorption spectra of indolenine cyanine dyes cpd I3 showed blue shift when compared to those of cpd I1 and cpd I2. The reason of this blue shift was structure of cpd I3 has π-π* conjugate less than cpd I1 and cpd I2 because cpd I3 has 1 hetrocyclic ring but cpd I1 and cpd I2 has 2 hetrocyclic ring. Similarly, absorption spectra of benzothiazole cyanine dyes represented blue shift of cpd B1 due to cpd B1 structure has 2 hetrocyclic ring but cpd B2 and cpd B3 has 3 hetrocyclic ring therefore π-π* conjugate of cpd B1 has less than cpd B2 and cpd B3.

3. Emission Properties

The emission spectra of indolenine and benzothiazole cyanine dyes in ethanolic solution at room temperature were shown in Figure 15 and 16, respectively. The spectra showed same charecteristic pattern for cpd I1, cpd I2 and cpd I3 and similary, same charecteristic pattern for cpd B1, cpd B2 and cpd B3. Maximum emission of the indolenine cyanine dyes appeared at 467 nm for cpd I1, 454 nm for cpd I2 and 424 nm for cpd I3 as shown in Figure 15. Maximum emission of benzothiazole cyanine dyes appeared at 450 nm for cpd B1, 460 nm for cpd B2 and 473 nm for cpd B3 as shown in Figure 16.

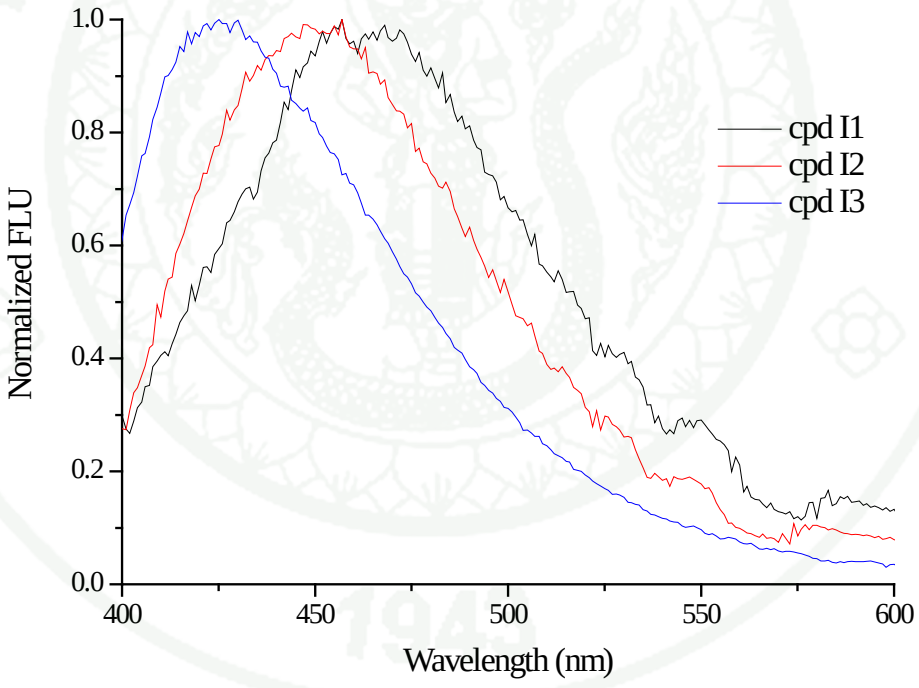


Figure 15 Emission spectra of indolenine cyanine dyes in ethanol.

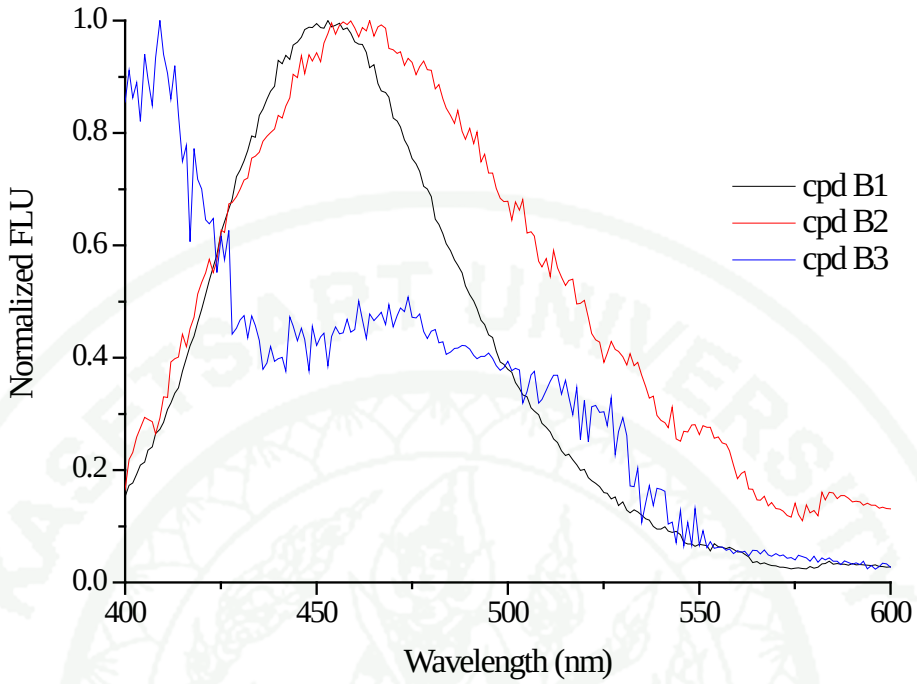


Figure 16 Emission spectra of benzothiazole cyanine dyes in ethanol.

The emission spectra of indolenine and benzothiazole cyanine dyes indicated yellow fluorescence because these dyes showed maximum emission in range 420-480 nm. Stokes shift is the difference between of absorption wavelength and emission wavelength (Stokes, 1958). Stokes shift of the synthesized cyanine dyes were shown in table 2. The stoke shift of benzothiazole cyanine dyes (about ≈129-155) are higher the stoke shift of indolenine cyanine dyes about ≈60 nm. The results indicated that the benzothiazole cyanine dyes structures can be rotated in the excited state more than indolenine cyanine dyes structures.

Table 2 Stokes shift of the synthesized indolenine and benzothiazole cyanine dyes.

	cpd I1	cpd I2	cpd I3	cpd B1	cpd B2	cpd B3
Stokes shift (nm)	96	56	65	155	129	143

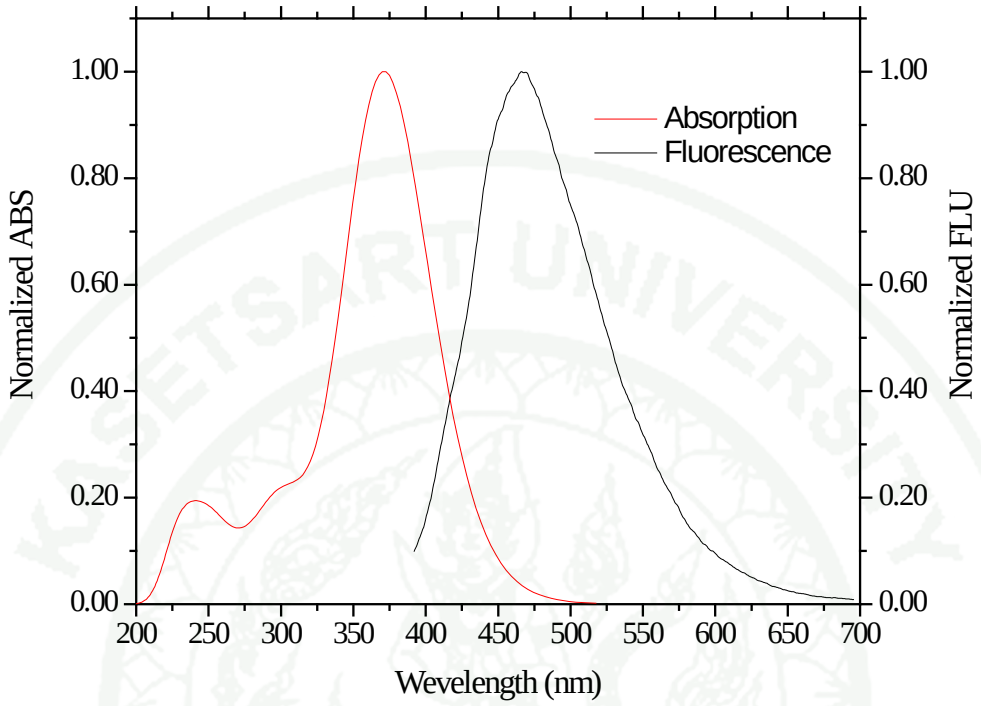


Figure 17 Absorption and emission spectra of indolenine cyanine dyes (cpd I1) in ethanolic solution.

Although the mechanism of the relaxation of indolenine and benzothiazole cyanine dyes in the excited state had not been fully understood, it was postulated that the isomerisation process was accompanied by fast consecutive reactions. The absorption spectra of indolenine cyanine dyes (cpd I1) in ethanolic solution for photoisomerization were depicted in Figure 18. In this study, quantum chemical calculations were used to examine photoisomerization in order to provide detailed insight into the process.

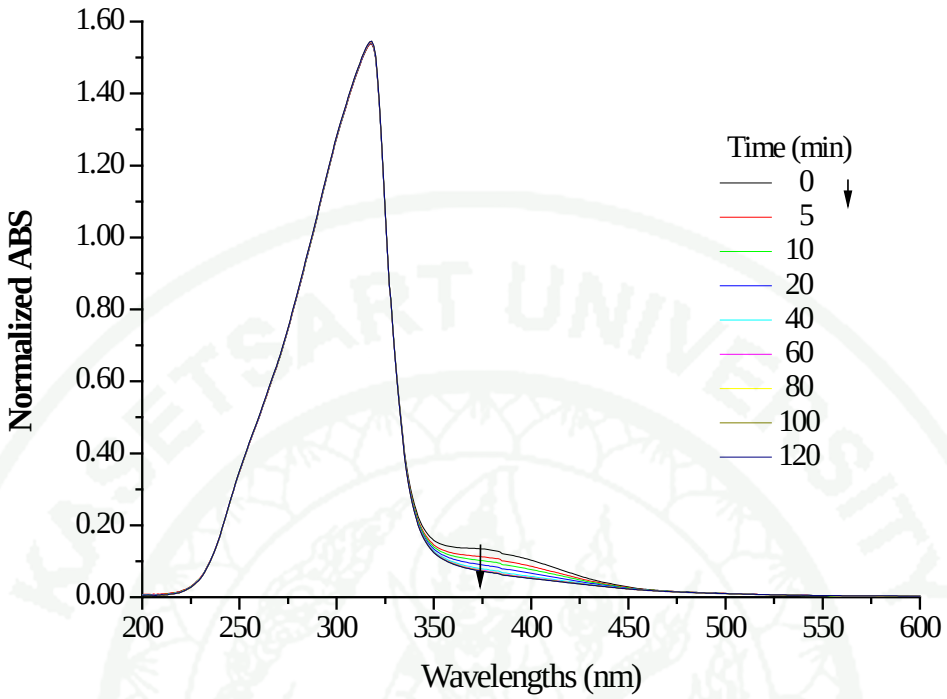


Figure 18 Example of photoisomerization of indolenine cyanine dyes (cpd I1) in ethanolic solution.

The photoisomerization process of indolenine cyanine dyes (cpd I1) can be observed when the dyes in ethanolic solution exposed to UV irradiation and the absortion spectra were recorded with different UV irradiation times. According to the result photoisomerization of indolenine cyanine dyes (cpd I1) was found that the absorption intenity decreased when increasing UV irradiation times. These results indicated that indolenine cyanine dyes (cpd I1) could change from *tran*-isomer to *cis*-isomer as *tran*-isomer was more stable than *cis*-isomer.

4. Quantum Chemical Calculations

4.1 Structural properties

The density function theory (DFT) method was used for the purpose of conformation analysis. It would be useful to examine structural parameters of indolenine and benzothiazole cyanine dyes using B3LYP method. Preliminary calculations of the dyes structures were performed by using DFT/TDDFT(B3LYP)/6-31G(d) method. Two isomers, *cis*- and *trans*-, were examined for the ground state optimized geometries (S_0) and level of the excited state (S_n) optimized geometries. In order to understand structural properties of the molecule, geometries of *cis*- and *trans*-isomers, bond lengths, bond angles and torsion angles, of indolenine and benzothiazole cyanine dyes were investigated.

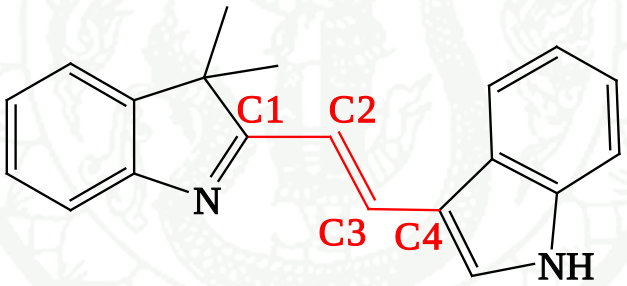


Figure 19 Structure of indolenine cyanine dyes (cpd I1) molecule, showing bond-lebelling of the backbone.

The C–C, C=C bond lengths and the torsion angles units of the optimized structures for the indolenine and benzothiazole cyanine dyes are listed in Table 3 and Table 4, respectively. The bond length values were considered as the distance between the carbon atoms on the main chain structure. In general, the calculated results showed that the C–C single bond lengths was shorter than the C=C double bond. The bond length and the torsion angles units was compared between indolenine and benzothiazole cyanine dyes structure based on optimized geometries of *cis*- and

trans- isomers, which bond length of both isomer was similar and the torsion angles units of *cis*- isomers was defined as 0^o while *trans*- isomers was defined as 180^o.

Table 3 Optimized bond lengths (Å) of indolenine cyanine dyes obtained from DFT/TDDFT(B3LYP)/6-31G(d) methods

Bond length (Å)	cpd I1		cpd I2		cpd I3	
	cis	trans	cis	trans	cis	trans
C1-C2	1.45	1.45	1.44	1.45	1.44	1.45
C2-C3	1.36	1.36	1.37	1.36	1.36	1.36
C3-C4	1.45	1.45	1.44	1.44	1.44	1.44
∠ C1-C2-C3-C4	0.00	180.00	0.00	180.00	0.00	180.00

Table 4 Optimized bond lengths (Å) of benzothiazole cyanine dyes obtained from DFT/TDDFT(B3LYP)/6-31G(d) methods

Bond length (Å)	cpd B1		cpd B2		cpd B3	
	cis	trans	cis	trans	cis	trans
C1-C2	1.46	1.44	1.46	1.45	1.46	1.45
C2-C3	1.35	1.35	1.35	1.35	1.35	1.35
C3-C4	1.47	1.44	1.47	1.46	1.47	1.46
∠ C1-C2-C3-C4	0.00	180.00	0.00	180.00	0.00	180.00

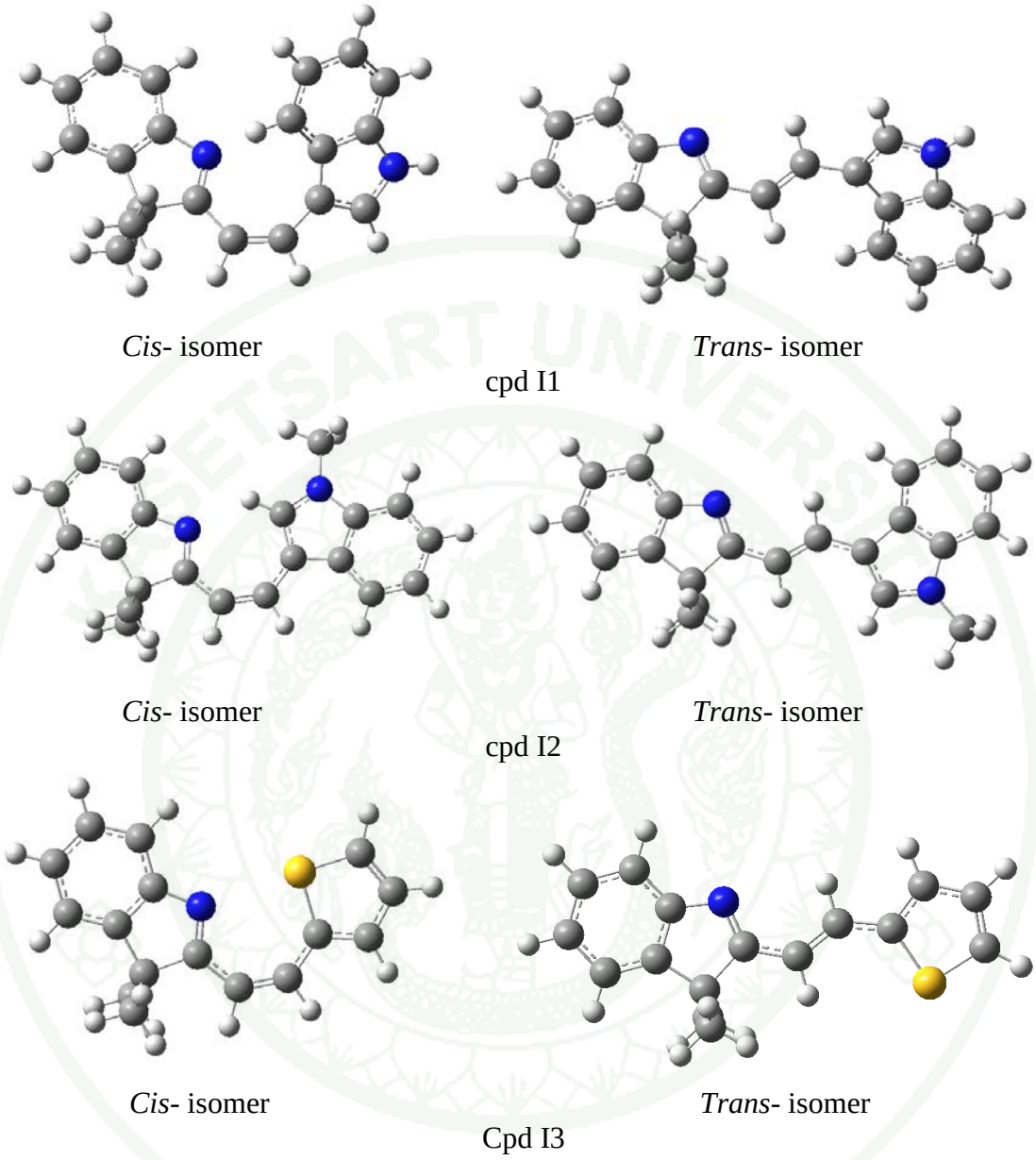


Figure 20 Structure of *cis*- and *trans*- isomers of indolenine cyanine dyes

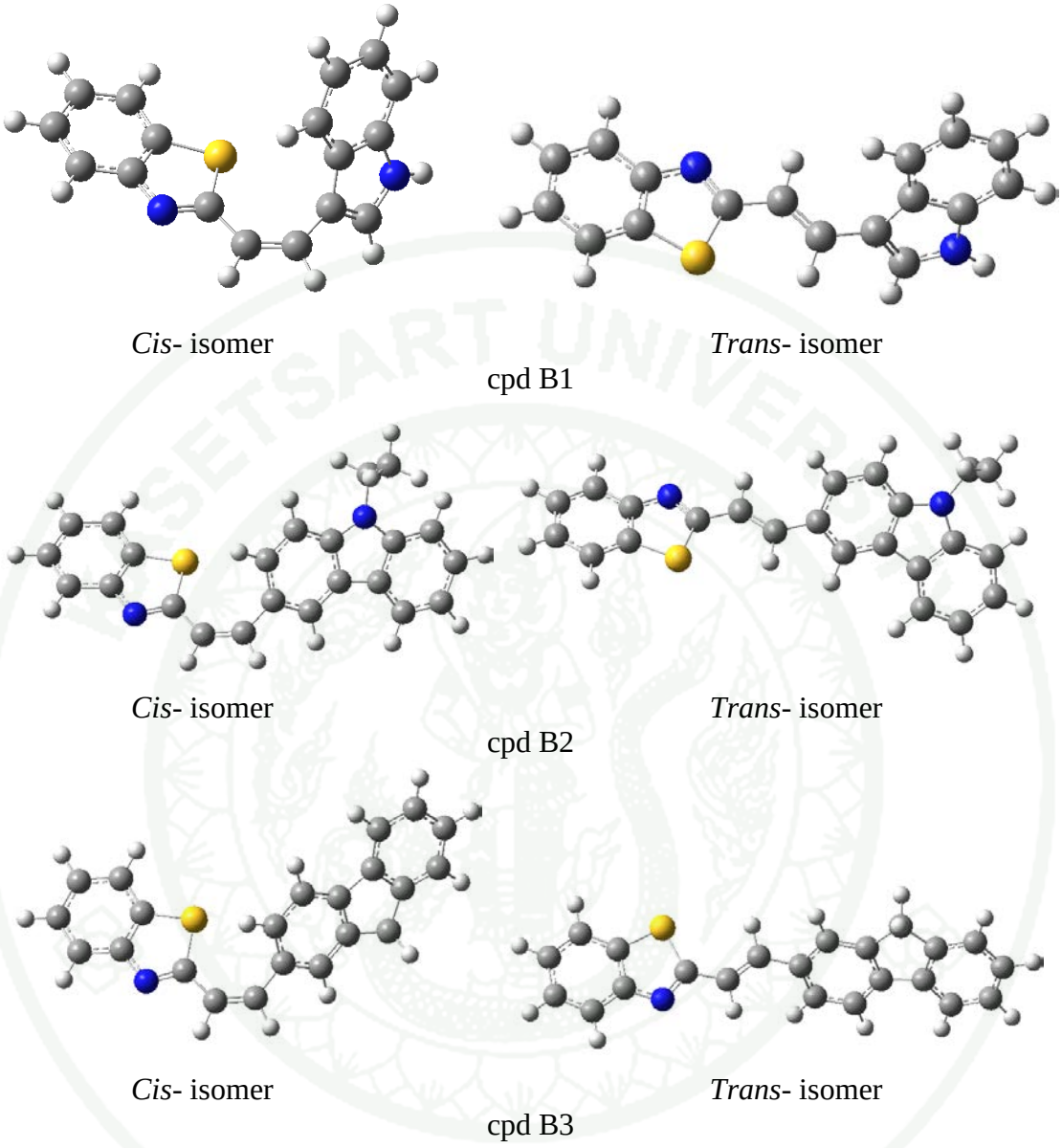


Figure 21 Structure of *cis*- and *trans*-isomers of benzothaizole cyanine dyes.

The potential energy surfaces (PESs) for photoisomerization process between *cis*- and *trans*-isomers were examined for both ground (S_0) and excited states (S_n) using the DFT/TDDFT-(B3LYP) method. The photoisomerization process was of particular interest because the transient absorption/fluorescence spectra apparently indicated that the absorption and emission processes involved photoisomerization. The PESs were examined by considering the isomerization occurring at vinylene double bonds, therefore the PESs were calculated by partial optimization with torsion

angle being fixed and all other degrees of freedom being relaxed. The torsion angle (θ) was defined as an angle between planes formed by the vinylene linkage units. The torsion angles (θ = ∠C1-C2-C3-C4 as shown in Table 3 and 4) for *cis*- and *trans*-isomers of indolenine and benzothiazole cyanine dyes were defined as 0^o and 180^o, respectively. All structures possessing various sizes of torsional angles from 0^o to 180^o with an increasing step of 30^o were partially optimized using DFT/TDDFT(B3LYP)/6-31G(d) for the ground (S_0) and excited (S_n) states.

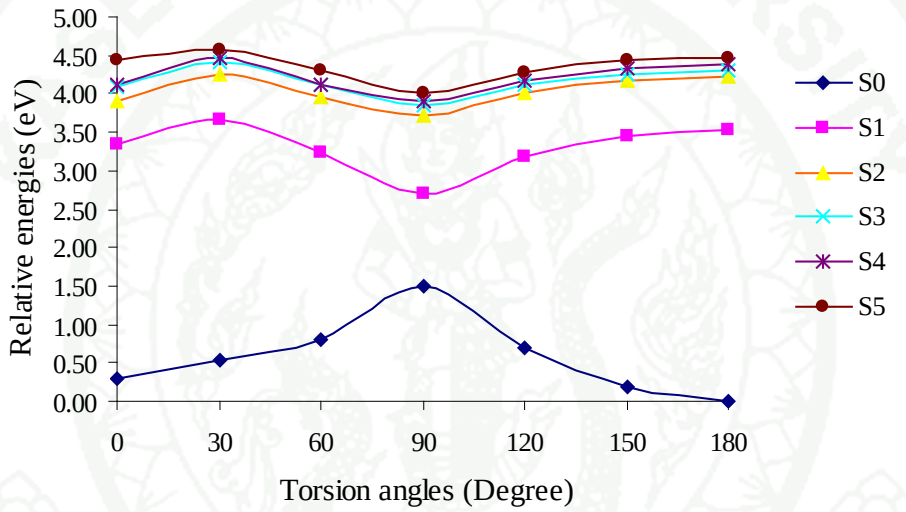


Figure 22 Potential energy surfaces for the ground (S_0) and excited(S_n) states of the indolenine cyanine dye (cpd I1) for photoisomerization process calculated by DFT/TDDFT(B3LYP).

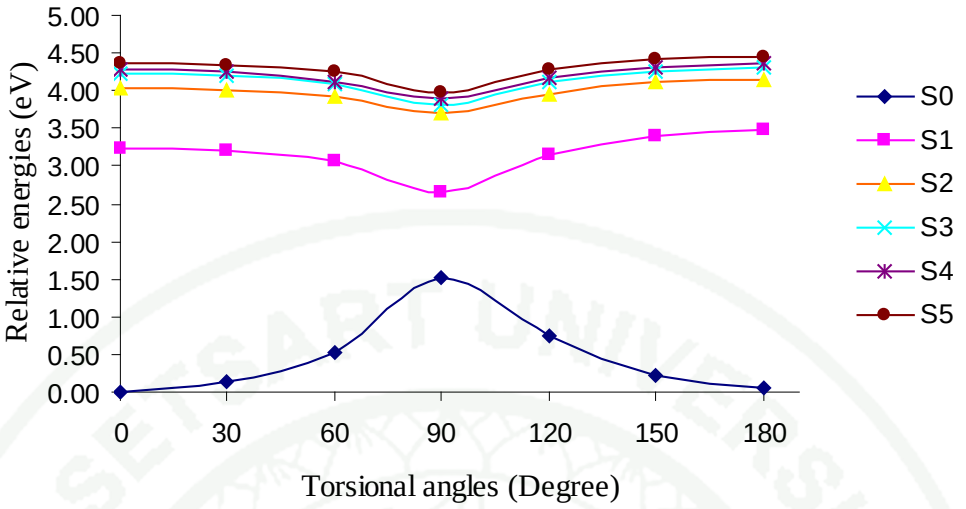


Figure 23 Potential energy surfaces for the ground (S_0) and excited (S_n) states of the indolenine cyanine dye (cpd I2) for photoisomerization process calculated by DFT/TDDFT(B3LYP).

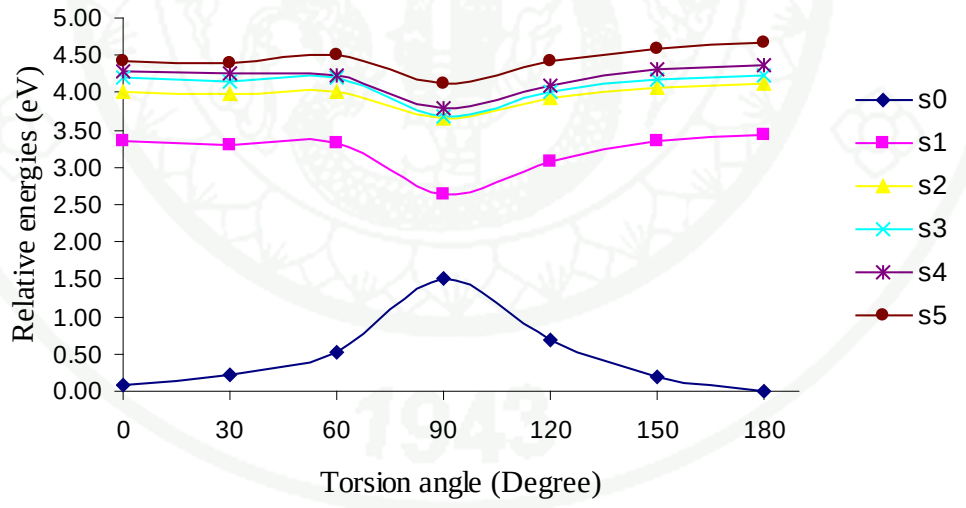


Figure 24 Potential energy surfaces for the ground (S_0) and excited (S_n) states of the indolenine cyanine dye (cpd I3) for photoisomerization process calculated by DFT/TDDFT(B3LYP).

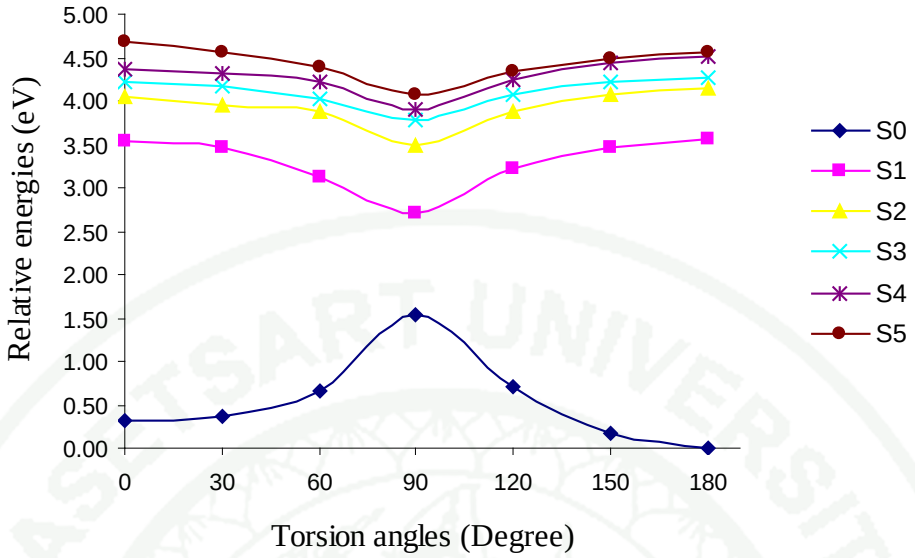


Figure 25 Potential energy surfaces for the ground (S_0) and excited (S_n) states of the benzothiazole cyanine dye (cpd B1) for photoisomerization process calculated by DFT/TDDFT(B3LYP).

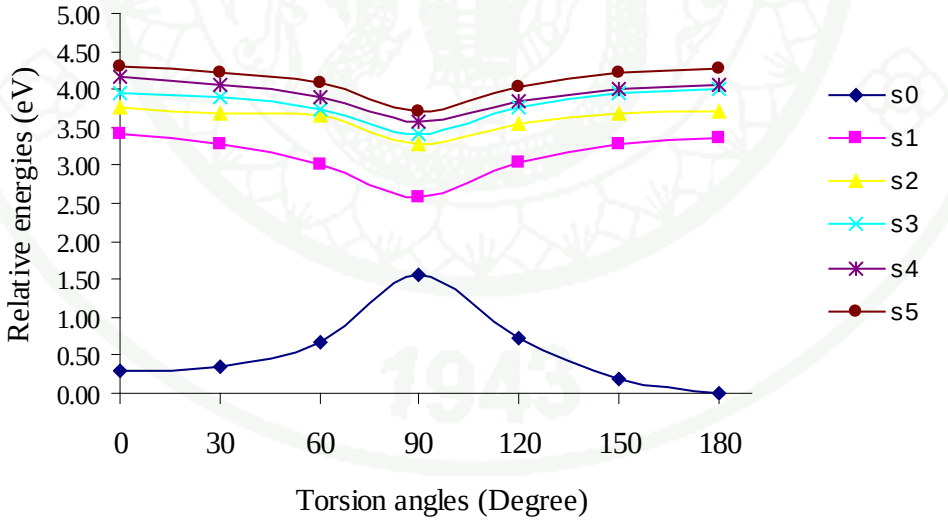


Figure 26 Potential energy surfaces for the ground (S_0) and excited (S_n) states of the benzothiazole cyanine dye (cpd B2) for photoisomerization process calculated by DFT/TDDFT(B3LYP).

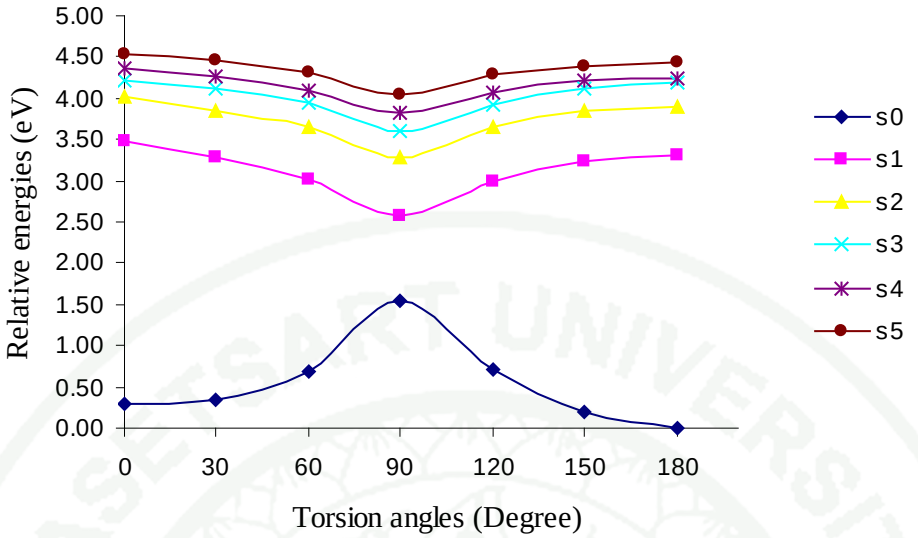


Figure 27 Potential energy surfaces for the ground (S_0) and excited (S_n) states of the benzothiazole cyanine dye (cpd B3) for photoisomerization process calculated by DFT/TDDFT(B3LYP).

In general, the PESs showed that two local minima existed, i.e. torsion angle of 0^o of the *cis*-isomer and 180^o of the *trans*-isomer of the indolenine and benzothiazole cyanine dyes. The PESs also indicated that the *trans*- isomer was more stable in a planar configuration by ~0.3 eV than the *cis*- isomer. This could be presumably geometry stabilization due to strong π-conjugation and low steric hindrance. The energy barrier for the *cis*- and *trans*- isomers to change to perpendicular conformation, i.e., at the torsion angle of 90^o was ~1.2 eV. The above results indicated that all isomers were interchangeable and the photoisomerization between two isomers was possible.

4.2 Electronic Properties

4.2.1 Absorption Properties

The electronic transitions were calculated within the framework of time-dependent density function theory (TDDFT) method employing the 6-311G(d,p) basis set. Vertical excitation energies were then performed by single point calculations at the optimized geometries of the ground state. The absorption wavelengths, oscillator strengths and excitation character of indolenine (cpd I1, cpd I2, cpd I3) and benzothiazole cyanine dyes (cpd B1, cpd B2, cpd B3) were shown in Table 5 and 6, respectively. It was found that the calculated excitation energies corresponding to absorption were underestimated when compared to the experimental results.

The calculated $S_0 \rightarrow S_1$ absorption energies, transition states (H denoted HOMO and L is LUMO) and oscillator strengths (*f*) of the cyanine dyes and the corresponding experimental values were presented in Table 5 and 6. All $S_0 \rightarrow S_1$ transition involved one-electron transition mainly from HOMO to LUMO (H→L), which distinguished the highest oscillator strength (π–π^* transition) of all states calculated. These calculated results showed that the maximum absorption of indolenine cyanine dyes at 368 nm for cpd I1, 374 nm for cpd I2 and 374 nm for cpd I3 while the maximum absorption from experimental observed at 371 nm for cpd I1, 398 nm for cpd I2 and 359 nm for cpd I3. The absorptions of dyes cpd I1 agreed well with the experimental spectra but those cpd I2 and cpd I3 slightly differed from the experimental data. The calculated maxima absorption of benzothiazole cyanine dyes displayed at 367 nm for cpd B1, 391 nm for cpd B2, and 389 nm for cpd B3 while the experimental absorption maxima were observed at 295 nm for cpd B1, 331 nm for cpd B2, and 330 nm for cpd B3. The dyes cpd B1, cpd B2 and cpd B3 gave slightly different absorption maxima from the experimental data.

Table 5 The lowest excitation energies in eV and oscillator strength (*f*) in solvent phase (ethanol) using DFT/TDDFT(B3LYP)/6-311G(d,p) method and experimental absorption wavelength in C_2H_5OH solution for the indolenine cyanine dye. HOMO and LUMO are denoted as H and L, respectively.

Electronic transitions	E_g (eV)	E_{ex} (nm)	*f*	Transition	Experimental λ_{abs} (nm)
cpd I1 trans-isomer					
$S_0 \rightarrow S_1$	3.36	368	1.2272	H→L	371
$S_0 \rightarrow S_2$	4.22	293	0.0007	H-1→L	-
$S_0 \rightarrow S_3$	4.35	284	0.0201	H-2→L	-
$S_0 \rightarrow S_4$	4.46	277	0.0000	H-4→L	-
$S_0 \rightarrow S_5$	4.47	277	0.0568	H→L+1	-
cpd I2 trans-isomer					
$S_0 \rightarrow S_1$	3.30	374	1.2895	H→L	398
$S_0 \rightarrow S_2$	4.16	297	0.0045	H→L+1	-
$S_0 \rightarrow S_3$	4.33	285	0.0286	H-1→L	-
$S_0 \rightarrow S_4$	4.41	280	0.0496	H-2→L	-
$S_0 \rightarrow S_5$	4.47	277	0.0004	H-4→L	-
cpd I3 trans-isomer					
S0→S1	3.30	374	1.1807	H→L	359
S0→S2	4.25	291	0.0213	H-1→L	-
S0→S3	4.26	290	0.0001	H-4→L	-
S0→S4	4.35	284	0.013	H-2→L	-
S0→S5	4.57	271	0.0649	H-3→L	-

Table 6 The lowest excitation energies in eV and oscillator strength (*f*) in solvent phase (ethanol) using DFT/TDDFT(B3LYP)/6-311G(d,p) method and experimental absorption wavelength in C_2H_5OH solution for the benzothiazole cyanine dye. HOMO and LUMO are denoted as H and L, respectively.

Electronic transitions	E_g (eV)	E_{ex} (nm)	*f*	Transition	Experimental λ_{abs} (nm)
cpd B1 trans-isomer					
$S_0 \rightarrow S_1$	3.37	367	1.2271	H→L	295
$S_0 \rightarrow S_2$	4.19	295	0.0434	H-2→L	-
$S_0 \rightarrow S_3$	4.20	294	0.0029	H-1→L	-
$S_0 \rightarrow S_4$	4.47	277	0.0832	H-3→L	-
$S_0 \rightarrow S_5$	4.52	274	0.064	H→L+1	-
cpd B2 trans-isomer					
$S_0 \rightarrow S_1$	3.16	391	1.4736	H→L	331
$S_0 \rightarrow S_2$	3.66	338	0.0242	H-1→L	-
$S_0 \rightarrow S_3$	3.92	316	0.12	H→L+1	-
$S_0 \rightarrow S_4$	4.07	304	0.0199	H-3→L	-
$S_0 \rightarrow S_5$	4.21	294	0.3172	H-2→L	-
cpd B3 trans-isomer					
S0→S1	3.18	389	1.7601	H→L	330
S0→S2	3.93	315	0.0663	H-1→L	-
S0→S3	4.17	297	0.0371	H-2→L	-
S0→S4	4.20	294	0.0142	H-3→L	-
S0→S5	4.38	282	0.0168	H-3→L	-

In order to understand the physical origin of optical transitions for the selected excitation energies, examination of the relevant (highest) occupied and the lowest unoccupied molecular orbitals would play a dominant role. The TDDFT (B3LYP)/6-311G(d,p) method was used to compute vertical electronic excitation

energies, the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO), absorption wavelength and oscillator strength (*f*) of all stereoisomers. The calculated energies and absorption wavelengths were compared with the experimental data in ethanol solution. The electronic transitions were described below in term of the contribution to the excited state to which the excitation took place.

The lowest excitation energy involved electronic excitation from the highest occupied molecular orbitals (HOMO) to the lowest unoccupied molecular orbital (LUMO). This transition possessed $\pi \rightarrow \pi^*$ character arising exclusively from $S_0 \rightarrow S_1$ electronic transition mainly composed of HOMO→LUMO transition.

The patterns of molecular orbitals involved the highest occupied molecular orbitals (HOMO) and the lowest occupied molecular orbitals (LUMO) for the indolenine and benzothiazole cyanine dyes were presented in Figure 28 and 29, respectively. In the HOMO of indolenine and benzothiazole cyanine dyes molecules, the electrons were localized on the backbone structure (C2–C3). The LUMO contained localization on the backbone structure (C1–C2, C3–C4). These results indicated that structure could rotate and the interchangeable structures of these compounds involved photoisomerization.

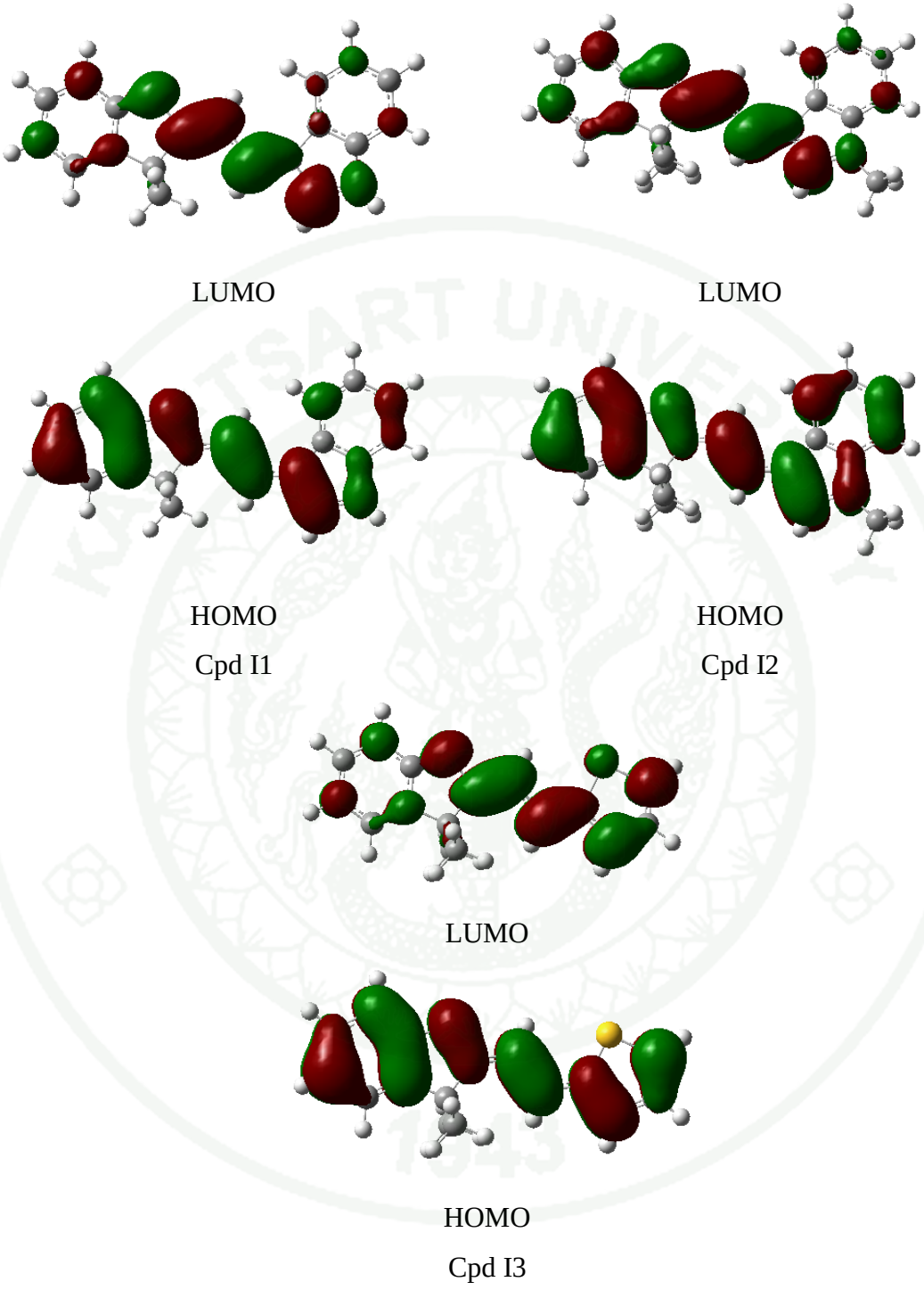


Figure 28 Pictures showing molecular orbitals of the indolenine cyanine dyes

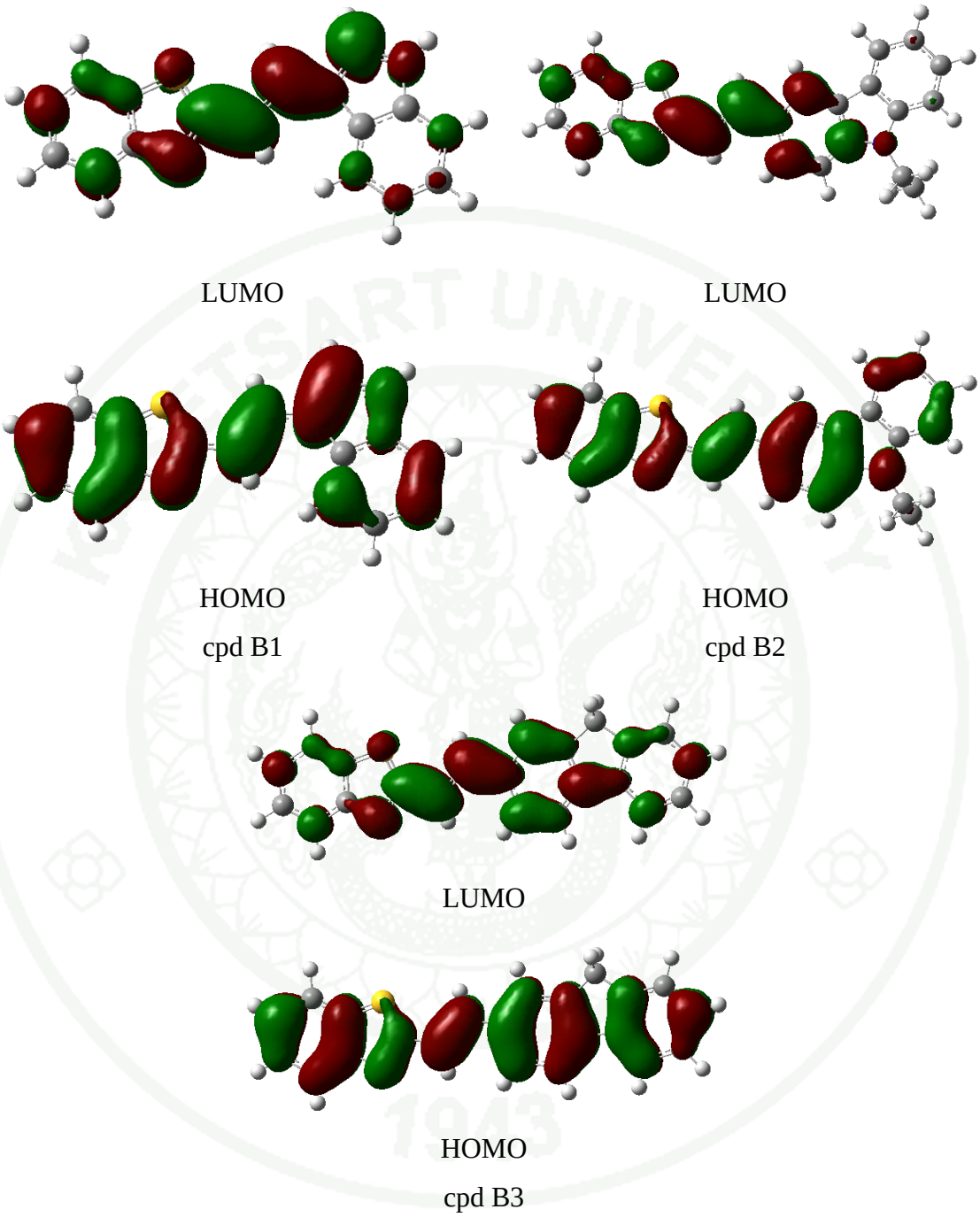


Figure 29 Pictures showing molecular orbitals of the benzothiazole cyanine dyes.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

The syntheses of indolenine and benzothiazole cyanine dyes were carried out. All compounds were synthesized via solvent-free microwave irradiation technique. The results indicated that the cyanine dye formation reactions were complete within short reaction time and with high to excellent yields.These cyanine dyes were characterized by ^{1}H-NMR, FTIR, UV-Vis and fluorescence measurements. Absorption and fluorescence spectra of the cyanine dye were measured.

The structural conformations and electronic properties of indolenine and benzothiazole cyanine dyes were investigated. The DFT/TDDFT(B3LYP)/6-311G(d,p) method was used for the systematic theoretical investigation of the conformation analysis. The absorption and fluorescence properties were investigated using TDDFT(B3LYP)/6-311G(d,p) method. The results indicated that calculated absorption spectra related well with the experimental data.

Recommendations

1. For experimental section, indolenine and benzothiazole cyanine dyes could be further purified by column chromatography.

2. Thermal analysis is recommended for measurement of thermal properties of cyanine dyes.

3. The application of cyanine dyes for DNA detection.

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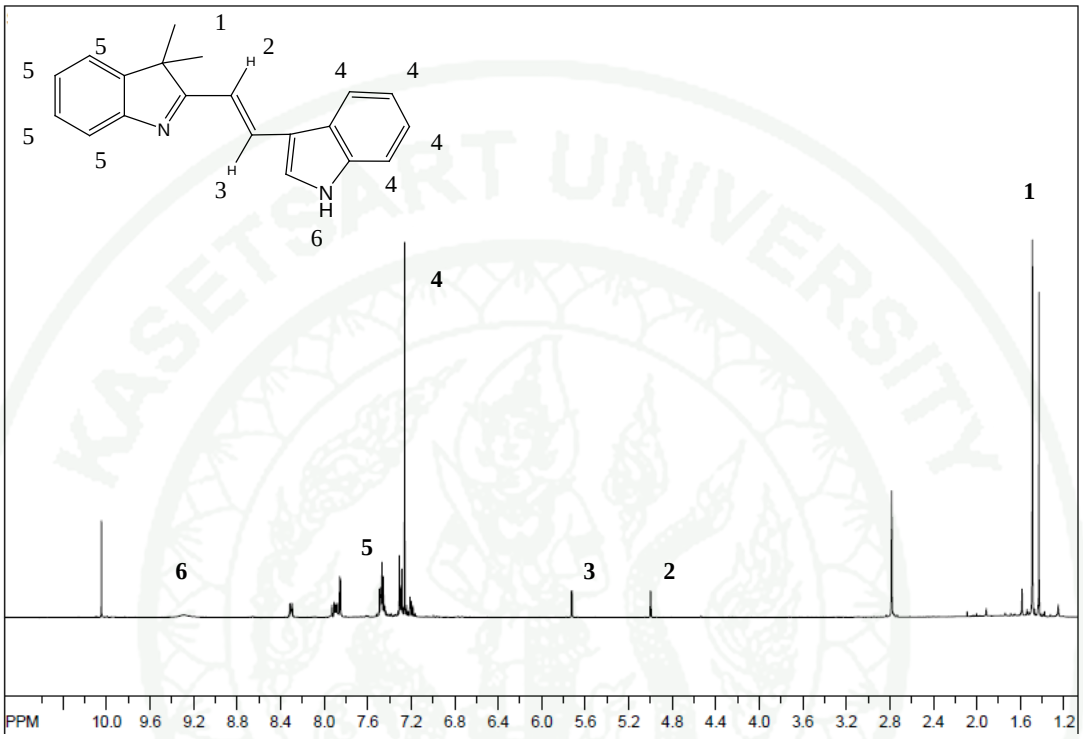
APPENDICES

Appendix A

Supporting information

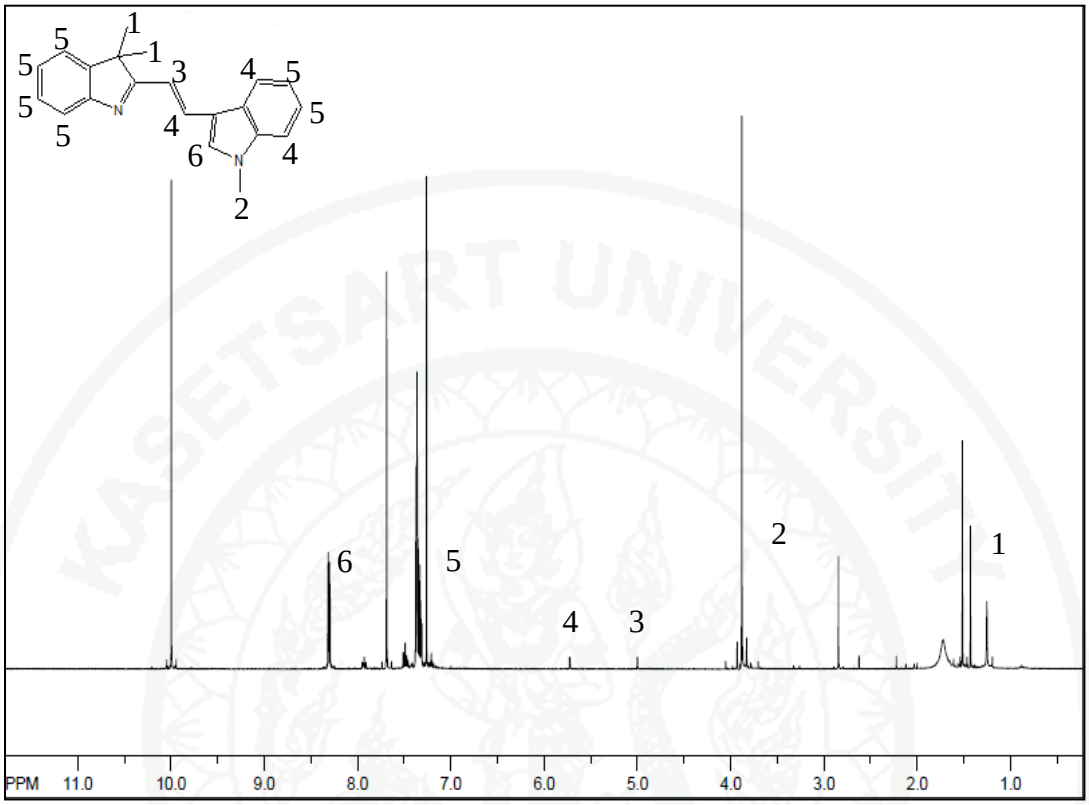
^{1}H-NMR spectrum

1. Indolenine cyanine dyes (cpd I1)



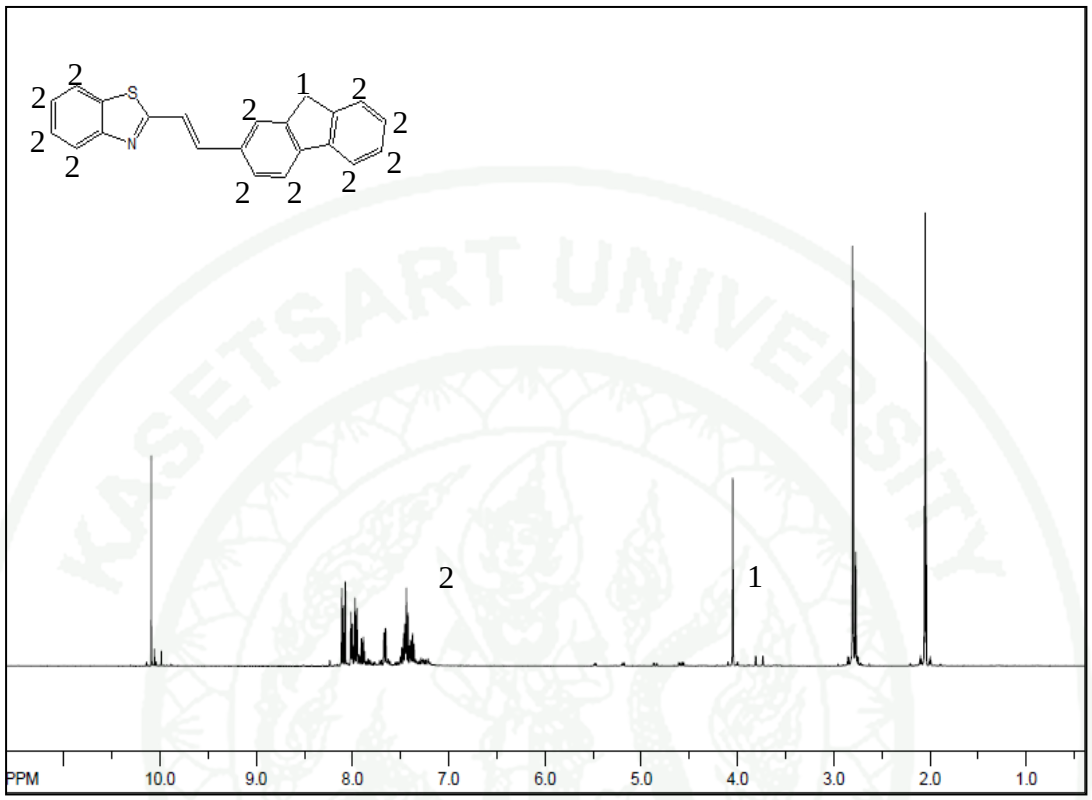
Appendix Figure A1 ^{1}H-NMR spectrum of indolenine cyanine dyes (cpd I1)

2. Indolenine cyanine dyes (cpd I2)



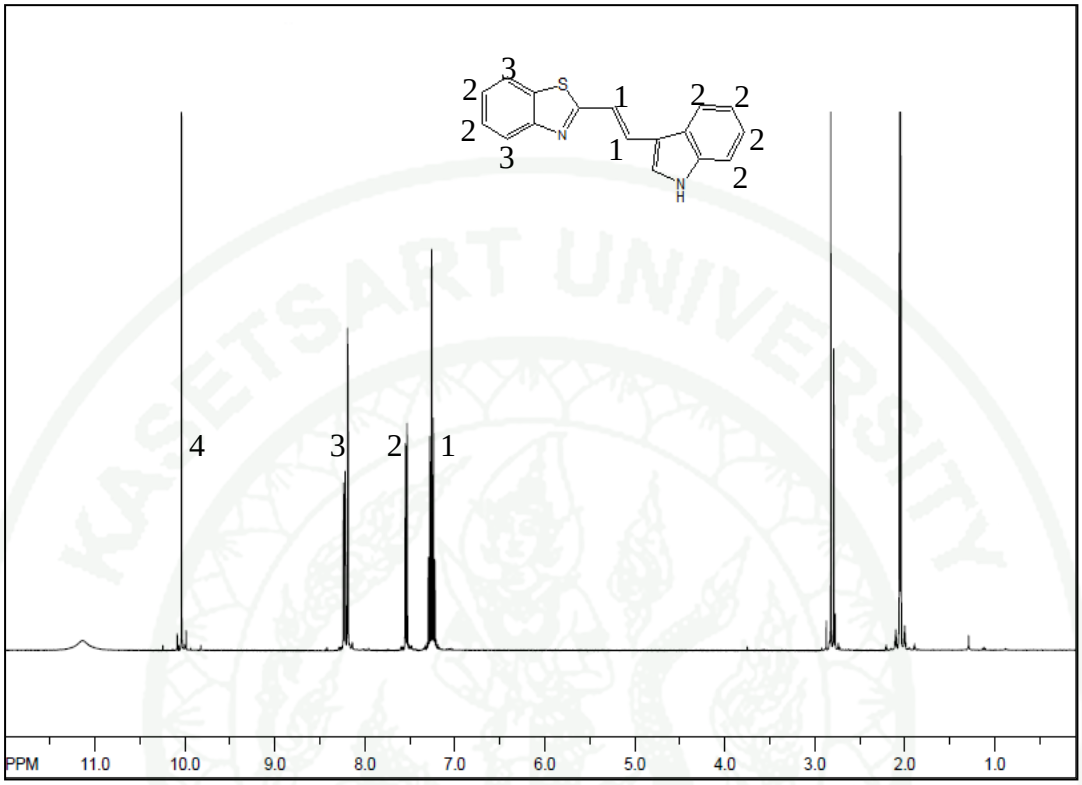
Appendix Figure A2 ^{1}H-NMR spectrum of indolenine cyanine dyes (cpd I1)

3. Indolenine cyanine dyes (cpd I3)



Appendix Figure A3 ^{1}H-NMR spectrum of indolenine cyanine dyes (cpd I3)

4. Benzothaiazole cyanine dyes (cpd B1)



Appendix Figure A4 ^{1}H-NMR spectrum of benzothaiazole cyanine dyes (cpd B1).

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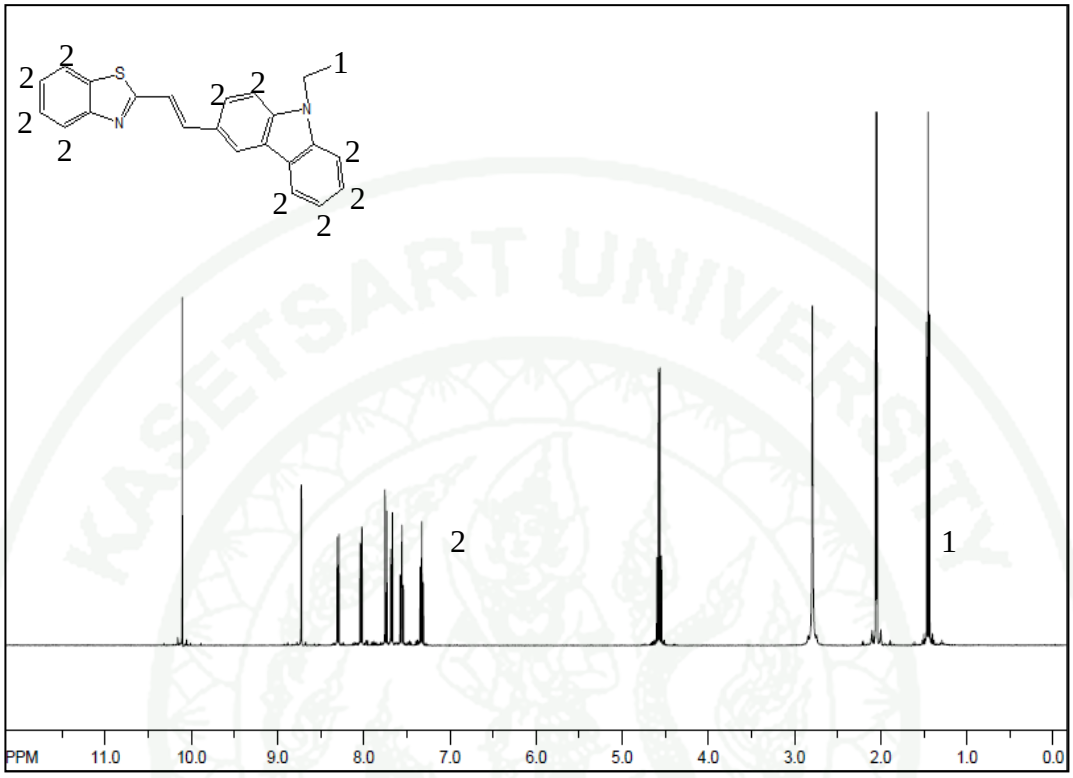
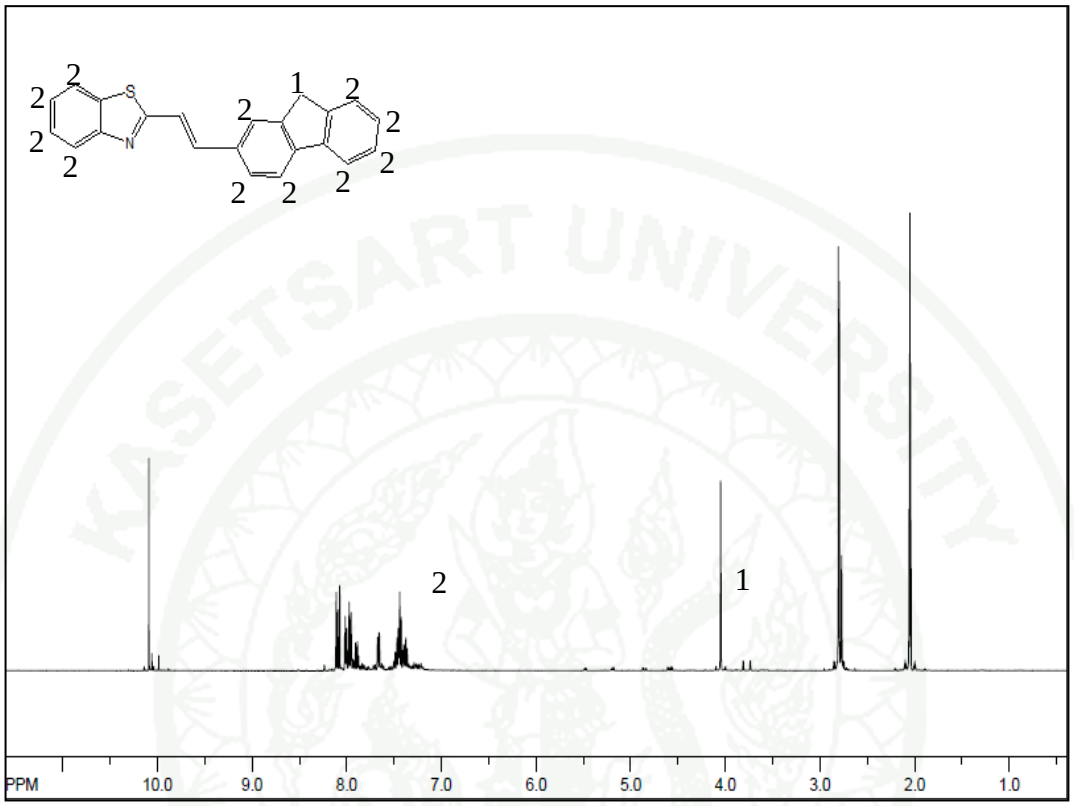
5. Benzothaiazole cyanine dyes (cpd B2)



Appendix Figure A5 ^{1}H-NMR spectrum of benzothaiazole cyanine dyes (cpd B2).

6. Benzothaiazole cyanine dyes (cpd B3)



Appendix Figure A6 ^{1}H-NMR spectrum of benzothaiazole cyanine dyes (cpd B3).

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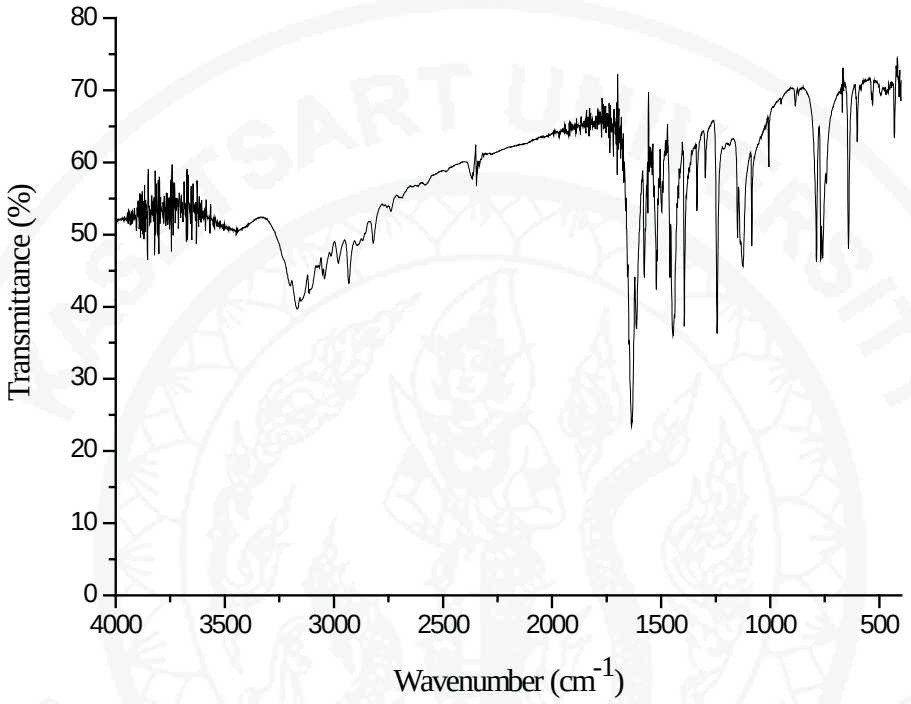
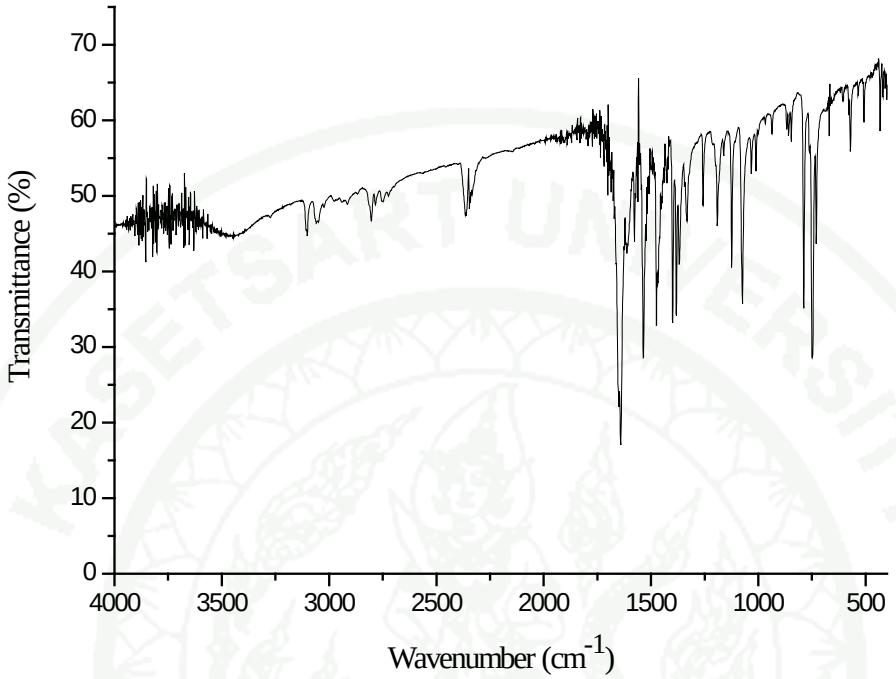
FTIR spectrum

1. Indolenine cyanine dyes (cpdI1)



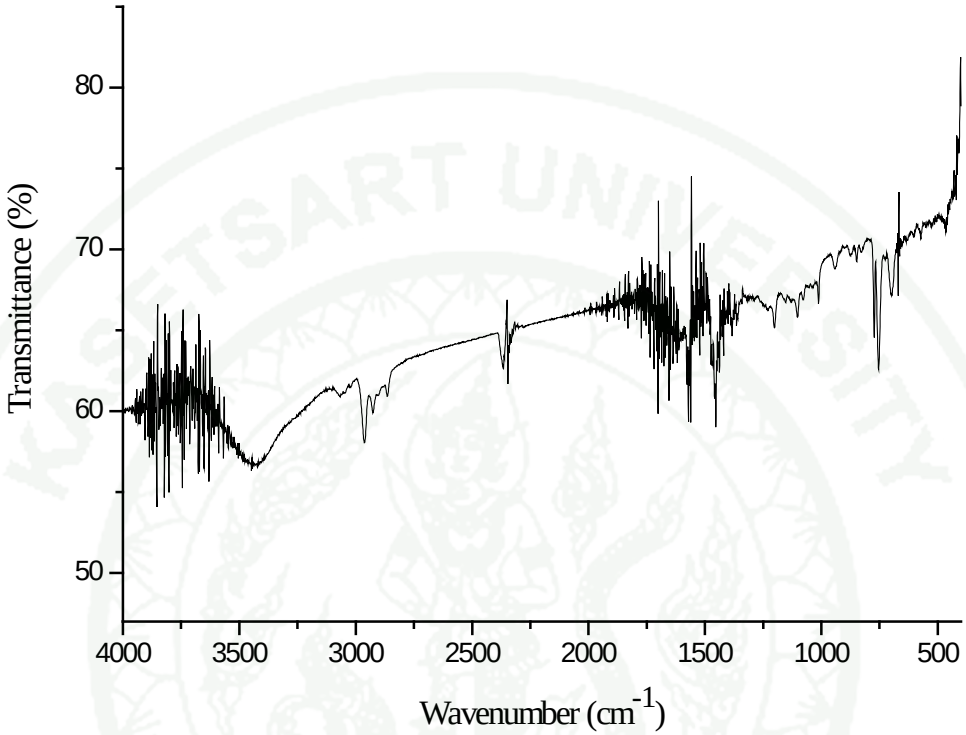
Appendix Figure A7 FTIR spectrum of indolenine cyanine dyes (cpd I1)

2. Indolenine cyanine dyes (cpd I2)



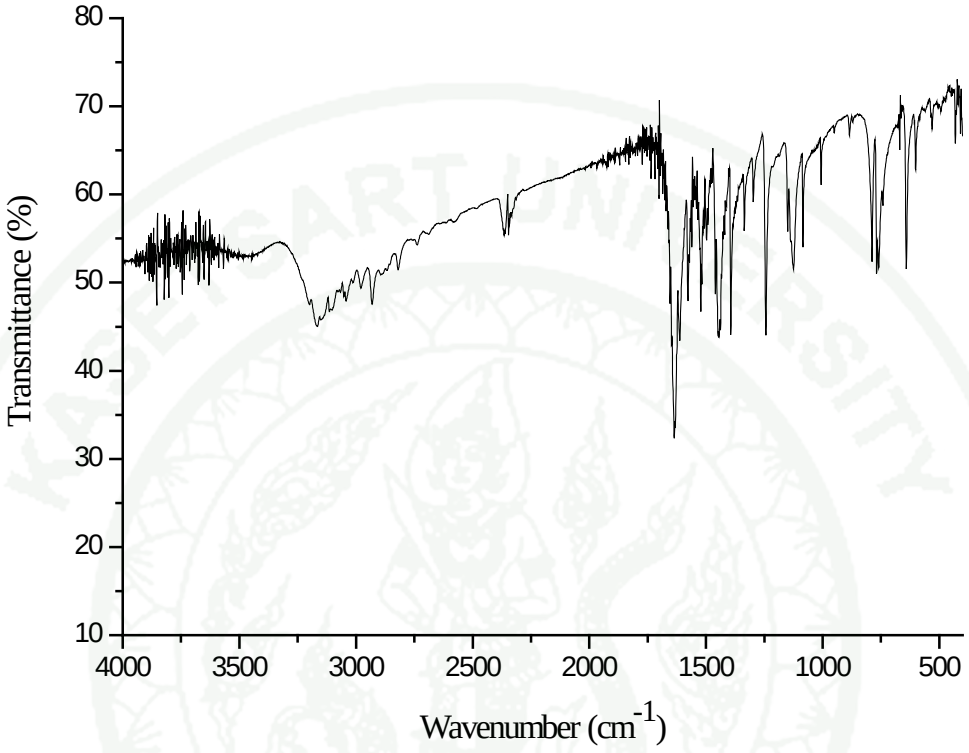
Appendix Figure A8 FTIR spectrum of indolenine cyanine dyes (cpd I2)

3. Indolenine cyanine dyes (cpd I3)



Appendix Figure A9 FTIR spectrum of indolenine cyanine dyes (cpd I3)

4. Benzothaiazole cyanine dyes (cpd B1)



Appendix Figure A10 FTIR spectrum of benzothaiazole cyanine dyes (cpd B1).

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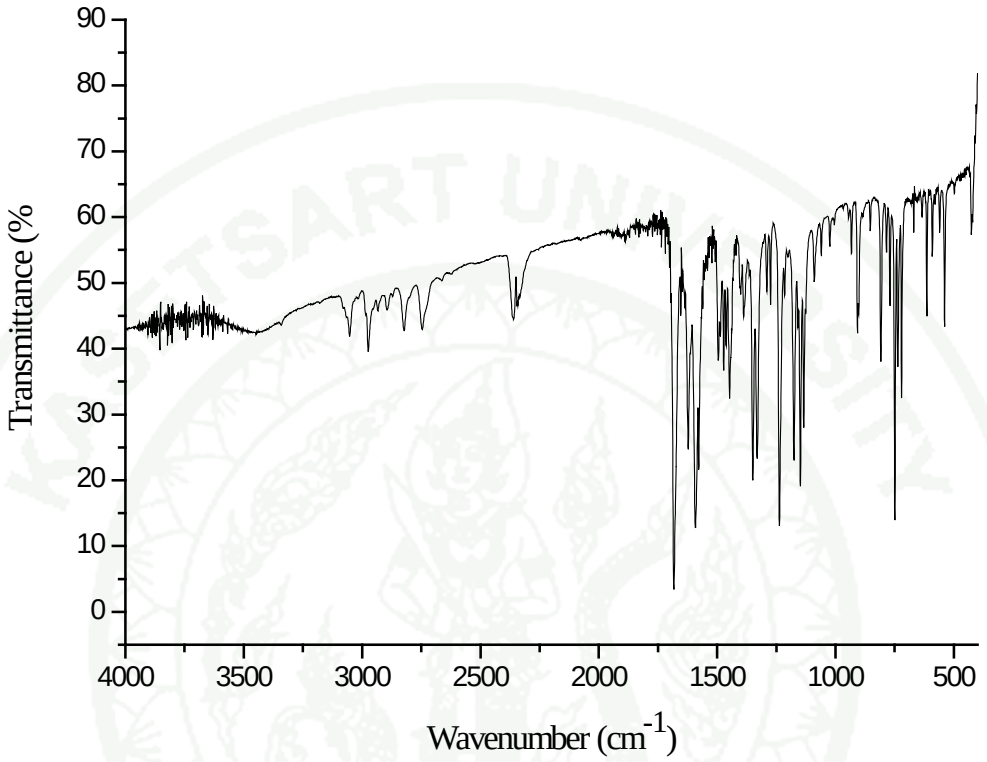
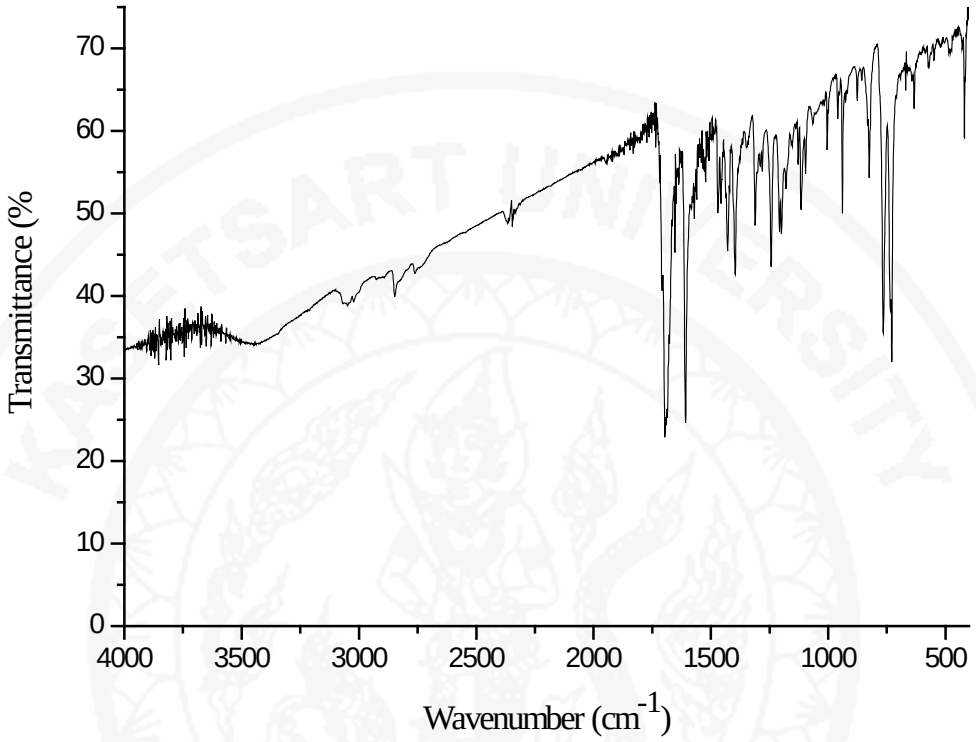
5. Benzothaiazole cyanine dyes (cpd B2)



Appendix Figure A11 FTIR spectrum of benzothaiazole cyanine dyes (cpd B2).

6. Benzothaiazole cyanine dyes (cpd B3)



Appendix Figure A12 FTIR spectrum of benzothaiazole cyanine dyes (cpd B3).

Appendix B

Presentation and proceeding

Proceedings

Wilawan Amatapitaksakul, Supa Hannongbua, Songwut Suramitr and Surachai Thachepan. **Microwave-assisted solvent-free synthesis of Indolenine cyanine dyes:Spectral properties and PCM-TD-DFT.** The 3rd Research Symposium on Petrochemical and Materials Technology and The 18th PPC Symposium on Petroleum, Petrochemicals, and Polymers ,Queen Sirikit National Convention Center, Bangkok, Thailand, 24 April 2012.

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