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(54) Title: [5]HELICENE DERIVATIVES AS MOLECULAR REPORTERS FOR DIAGNOSTIC APPLICATIONS AND METHODS OF SYNTHESIS THEREFOR

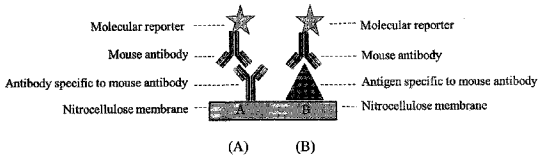


Figure 1

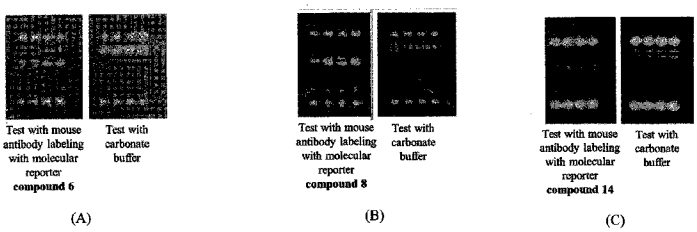
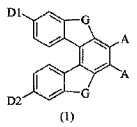


Figure 3



(57) Abstract: The present invention provides the methods of synthesis of [5]helicene compounds and the use of the said compounds conjugating with biomolecules to work as molecular reporter for diagnostic. The compounds in the present invention have the chemical structure illustrated in the formula (1): wherein G is a connecting group composes of 2 carbon atoms selected from the group consisting of ethane and ethylene; A is a separated or connected group selected from the group consisting of cyano and imide; Dl is selected from the group consisting of oxyalkanoic acid, oxyalkanal and oxyalkanesulfonate; and D2 has structure selected from the group consisting of hydroxyl, oxyalkanoic acid, oxyalkanal, alkyl oxyalkanoate, oxyalkanol and oxyalkanesulfonate. The compounds in the present invention compose of aromatic [5]helicene core comprising long π-conjugating system. The said compounds contain functional groups which able to link with biomolecules and they are soluble in water or other solvents that used in binding process with biomolecules.

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Moreover, owing to having proper chemical structure, the compounds in the present invention exhibit good fluorescent emission in wavelength of 425-675 um. When the said compounds connected with biomolecules, the biomolecules give good fluorescence and can be detected under ultraviolet radiation.

[5]HELICENE DERIVATIVES AS MOLECULAR REPORTERS FOR DIAGNOSTIC APPLICATIONS AND METHODS OF SYNTHESIS THEREFOR

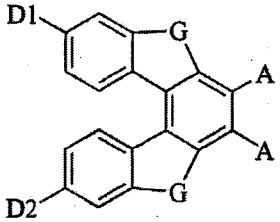
FIELD OF THE INVENTION

The present invention relates to chemistry and more particularly to organic dyes based on derivatives of [5]helicene, the use as molecular reporters for diagnostic applications and methods of synthesis therefor.

SUMMARY OF THE INVENTION

The present invention provides organic dyes based on [5]helicene derivative compounds for conjugating with biomolecules and the use thereof as reporter molecules in diagnostic for microbials, toxins and toxicants in samples from agricultural industry, food and environment.

The structure of [5]helicene derivative compounds in the present invention are represented by the following chemical formula (1):

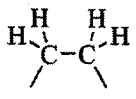


(1)

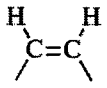
wherein

G is a connecting group composes of 2 carbon atoms selected from the group consisting of

- Ethane


; and

- Ethylene

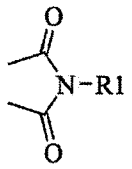


A is a separated or connected group selected from the group consisting of

- Cyano

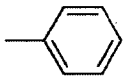
—CN ; and

- Imide

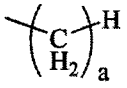


wherein R1 is selected from the group consisting of

- Phenyl

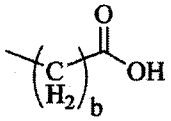
 ;

- Alkyl

 ;

when a is a number of carbon atoms in aliphatic hydrocarbon and a equals to 1 to 7; and

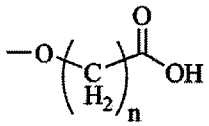
- Alkanoic acid



when b is a number of carbon atoms in aliphatic hydrocarbonand b equals to 1 to 7.

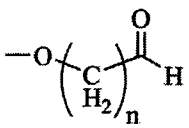
D1 is selected from the group consisting of

- Oxyalkanoic acid



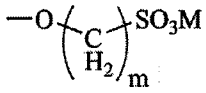
when n is a number of carbon atoms in aliphatic hydrocarbon and n equals to 1 to 7;

- Oxyalkanal



when n is a number of carbon atoms in aliphatic hydrocarbon and n equals to 1 to 7; and

- Oxyalkanesulfonate



when M is a metal atom selected from the group consisting of sodium and potassium,

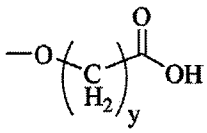
m is a number of carbon atoms in aliphatic hydrocarbon and m equals to 3 or 4.

D2 is selected from the group consisting of

- Hydroxy

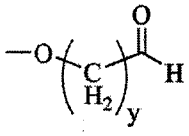
—OH ;

- Oxyalkanoic acid



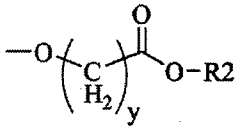
when y is a number of carbon atoms in aliphatic hydrocarbon and y equals to 1 to 7;

- Oxyalkanal



when y is a number of carbon atoms in aliphatic hydrocarbon and y equals to 1 to 7;

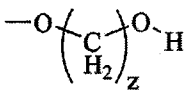
- Alkyl oxyalkanoate



when y is a number of carbon atoms in aliphatic hydrocarbonand y equals to 1 to 7,

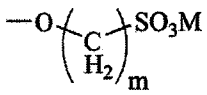
R2 is selected from the group consisting of methyl and ethyl group;

- Oxyalkanol



when z is a number of carbon atoms in aliphatic hydrocarbonand z equals to 2 to 8; and

- Oxyalkanesulfonate



when M is a metal atom selected from the group consisting of sodium and potassium,

m is a number of carbon atoms in aliphatic hydrocarbonand m equals to 3 or 4.

The derivative of [5]helicene compounds in the present invention compose of aromatic [5]helicene core comprising long π-conjugating system. The said compounds contain functional groups which able to link with biomolecules and they are soluble in water or other solvents that used in binding process with biomolecules. Moreover, owing to having proper chemical structure, the compounds in the present invention exhibit good fluorescent emission in wavelength of 425-675 nm. When the said compounds connected with biomolecules, the biomolecules give good fluorescence and can be detected under ultraviolet radiation.

The other embodiment in this invention is the synthetic method of [5]helicene compounds comprising: step a) An O-alkylation reaction of the [5]helicene compound in formula (**4**), selected from the [5]helicene compound in formula (**4**) wherein A1 is imide or the [5]helicene compound in formula (**4**) wherein A1 is cyano, with haloalkanoic acid alkyl ester (**I**) in the present of base 1 in organic solvent 1 to give [5]helicene compound (5) and/or compound (6) as intermediate; step b) A hydrolysis reaction of [5]helicene compound (5) or compound (6) using base 2 in organic solvent 2 at temperature in the range of 25-150 °C for 1-24 hours, follow by acidify with acid 1 to gain pH 0 to obtain [5]helicene compound (7) or compound (8) as intermediate; step c) An O-alkylation reaction of the intermediate compound containing OH group, selected from of [5]helicene compound (4), compound (5) or compound (7), with alkane sultone (**II**) at the present of base 3 in an organic solvent 3 to obtain [5]helicene compound (9), compound (10) or compound (11) as final product of intermediate; and step d) A reduction reaction of intermediate containing ester group, selected from [5]helicene compound (5), compound (6) or compound

(10), using diisobutylaluminum hydride, (DIBAL-H) in an organic solvent 4 to obtain [5]helicene compound (12), compound (13) and/or compound (14) and/or compound (15) or compound (16) as final product.

BACKGROUND OF THE INVENTION

Luminescent organic compounds are used as reporter molecules to give optical signal in biotechnology. The organic compounds are excited by a light source with a proper wavelength and give fluorescent light. The molecules can be attached to biomolecules, such as protein, antibody peptide or DNA, by covalent or non-covalent bonding specifically. When a luminescent organic compound is connected to a biomolecule, it can be commonly called a fluorophore. Mostly, reporter molecules comprise aromatic structure which make the molecules exhibit fluorescent emission.

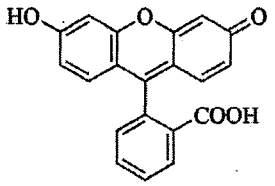
There are many uses of luminescent materials for diagnostic in biotechnology especially for mycotoxin analysis. Mycotoxins are organic compounds produced by fungi and toxic to human and animal. The contamination of mycotoxin can be found in all food production chain in agriculture process, including prehavesting, posthavesting food processing, storage and logistic. Since many toxins are chemical resistance and highly thermal stability, they can be accumulated in human bodies and having an affect on health in short and long term, although fungi are killed,

Due to high toxicity of some mycotoxins, many food administators set the standard for the allowed amont of toxins in foods. For consumers safety, diagnostic for mycotoxins are very important for not only consumers but also food producers.

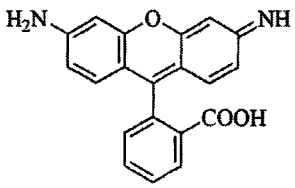
Mycotoxin diagnostic can be performed by immunochemical technique which composes of two main parts. An element that specifically binds with a target molecule is a selective recognition element. In case of the target molecule is mycotoxin, the recognition element can be antibody. The second element is reporter molecule which gives signal of the analysis. The example of commonly used reporter molecules include enzymes, radioactive compounds, nanoparticles, and fluorescent dyes or fluorophores. Due to different advantages of reporter molecules, objectives of analysis, methods of diagnostic and other considerations are needed to be taken into account for selecting a proper reporter molecule. For example, the fluorescent dyes or fluorophores give strong optical signal. Therefore, the analysis of a target molecule can be performed at very low concentration with high sensitivity. Nonetheless, the fluorescent compound can be prepared in large quantity with consistent properties in batch to batch synthesis.

One of the component in the development of diagnostic in biotechnology is reporter molecule. One of the key components of a diagnostic development in biotechnology is an effective reporter molecule. A fluorophore becomes a powerful reporter molecule for detection of multiple targets in many techniques such as in a multiplex real-time polymerase chain reaction and microarray technique. However, the usage of fluorophores is limited in the research only because an expensive detector is required to measure the fluorescent signal. Currently, fluorescent organic compounds are widely used as luminescent materials in many areas. Nevertheless, this type of fluorescent compounds was not suitable for linking to biorecognition element (e.g. antibody, peptide, and DNA) for diagnostic applications owing to their physiochemical properties: solubility and specific functional group for conjugation with the biorecognition element.

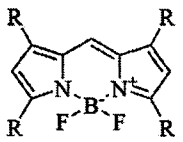
There are many fluorescent organic compounds or fluorophores widely used in biotechnology including fluorescein, rhodamine, BODIPY, squaraine and cyanine (Goncalves, Chem Rev 109(2009) 190–212; Kobayashi et al, Chem Rev 110(2010) 2620–2640; Gust, et al, Molecules 19(2014) 15824-15865) and their chemical structures are shown below.



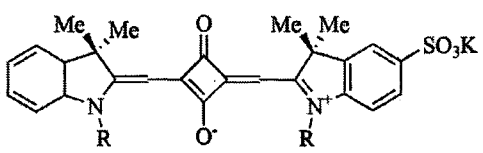
Fluorescein



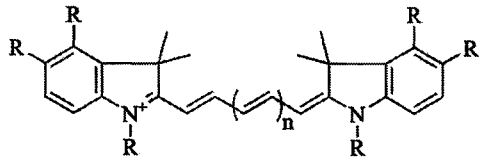
Rhodamine



BODIPY



Squaraine

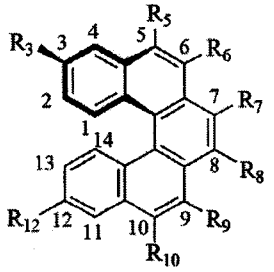


Cyanine

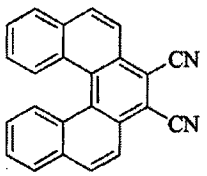
Fluorescent materials can be categorized in many ways such as emission wavelength or main chemical structure. Fluorescent compounds for diagnostic in biotechnology have been continuously developed to improve the desired properties for the application. The said characteristics include high molar extinction coefficient or molar absorptivity (ε), high fluorescence quantum efficiency (Φ_F), high chemical stability, thermal stability and photo stability or optical stability. The compounds also should be soluble in solvents especially in water which used for conjugating the compounds with biomolecules. Importantly, after binding fluorescent compounds with biomolecules, the activity of the biomolecules should not decrease significantly.

The important development of luminescent organic compound for biotechnology is the synthesis of new organic compound. Some new compounds give better certain properties while decrease other performances. For instance, organic dye with long wavelength emission is generaly a large molecule which less soluble than a small molecular dye. Moreover, when a large molecule conjugated with a biomolecule, it can possibly decreases the activity of the biomolecule. The optical properties such as absorption and emission are crucially taken into account for the development of luminescent organic dye. Many organic dyes, with small Stokes shift, absorb light in the visible region and emit light in the nearby excitation wavelength. However, an organic dye with a large Stokes shift is desired because the light from the diagnostic light source will not interfere the resulting emitted light from the reporter organic molecule. As a result, an organic dye with a large Stokes shift will provide a benefit to the design for a diagnostic test kit.

[5]Helicenes or pentahelicenes are hydrocarbon compounds compose of ortho-fused five aromatic rings resulting in helical out-of-plane structures. The unsubstituted derivatives give very low fluorescence quantum yields. The structure of [5]helicene is depicted below.



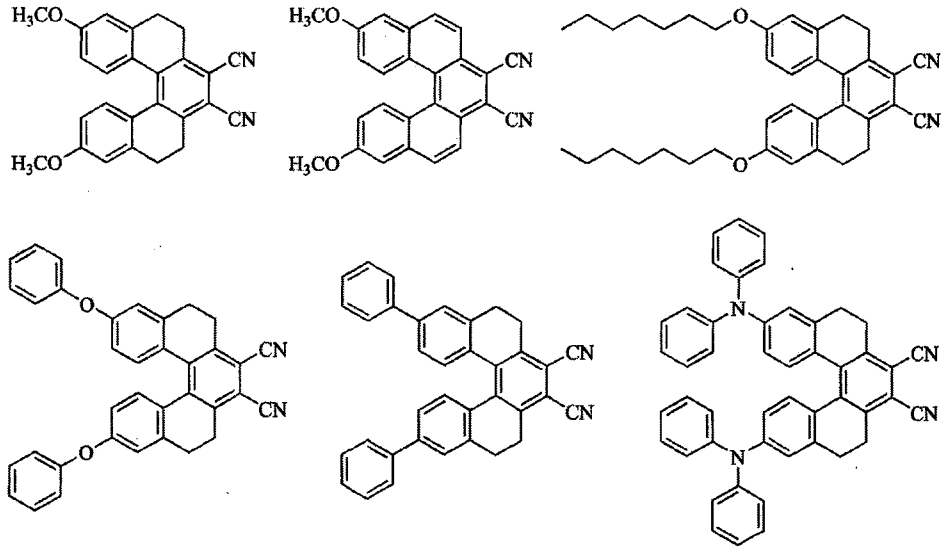
Depend on the substitutions, Rs, there are many synthetic pathways for preparation of [5]helicene compounds. 7,8-Dicyano[5]helicene compounds were prepared as precursors for phthalocyanine synthesis. [Sooksimuang et al, Porphyrins and Phthalocyanines 6(2002) 544-547 and Mandal et al, Porphyrins and Phthalocyanines 10(2006) 140-146] The studies showed the resulting phthalocyanines were soluble because of the out-of-plane structure of helicene. The studies, however, did not explain the optical properties of dicyano[5]helicene compounds. The representative of the said compound is demonstrated below.



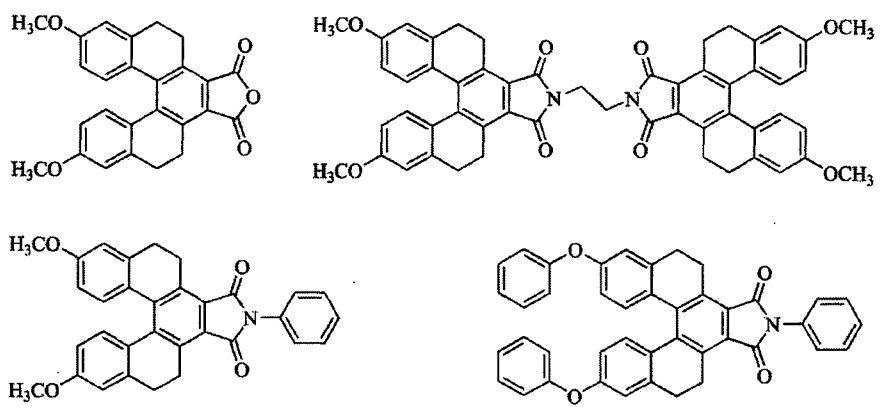
Because cyano is a good electron withdrawing group, the direction of electron delocalization within 7,8-dicyano[5]helicene molecule points toward cyano group. Thus,

addition of electron donating groups at proper positions provided many novel organic compounds that emit light at various wavelengths with high efficiency. Many compounds were prepared and utilized as emitting layer for organic light-emitting diode as described in the following inventions.

Thai patent applications number 0601006279, 1001001071, 1001001072, 1001001426 and 1101002049 showed the addition of various functional groups to derivatives of 5,6,9,10-tetrahydro-7,8-dicyano[5]helicene compound to gain compounds having different optical and thermal properties. The compounds in these applications have optical and optoelectronic charateristics suitable for emitting materials in organic light-emitting diode. The said compounds were utilized for blue, green and yellow diodes. The chemical structures of the compounds are demonstrated below.

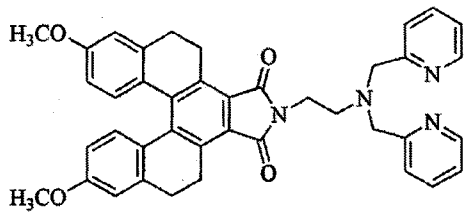


Moreover, [5]helicene derivative compounds were further developed to gain broader garmut emission by changing the electron withdrawing group from cyano to anhydride and imide as appeared in Thai patent applications number 0901003446, 1201005097, 1201005098 and 1501006011. The structures of the compounds are showed as folloing.



The compounds in above applications have good optical, optoelectronic and thermal charateristics suitable for emitting materials in organic light-emitting diode. Therefore, the said compounds were utilized for green diodes with good efficiencies.

2-(2-(bis(pyridin-2-yl-methyl)amino)ethyl)-7,12-dimethoxy-4,5,14,15-tetrahydro-1H-dinaphtho[2,1-e:1',2'-g]isoindole-1,3(2H)-dione, a [5]helicene derivative, was prepared and used as a chemical sensor. The molecule composes of an ionophore which able to analyze either copper or zinc ion specifically by applying different diagnostic conditions. [5]helicene fragment works as a fluorophore which makes the molecule emits fluorescent signal. The said compound was described in the Thai patent application number 1501003213 and the structure is depicted below.



Due to the [5] helicene derivative compounds described above have many good charateristics, i.e., various visible emission wavelengths with high efficiencies, high thermal stability and good chemical resistance, the said compounds meet criteria for emissive layer in organic light-emitting diode.

Though, the strucutures of the said [5]helicene compounds are further modified to present different structures and properties to meet requirements for reporter molecule in biotechnology.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Schematic of testing of the molecular reporter molecule conjugated to biomolecules such as antibody or protein.

Figure 2. Schematic of antibody spots on membrane (A), and the results interpretation (B).

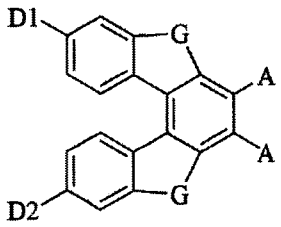
Figure 3. Testing of the molecular reporters in the present invention conjugated mouse antibody on membrane.

Figure 4. Schematic of testing of binding reactivity between molecular reporter the present invention conjugated antibody and antigen.

Figure 5. Binding reactivity of antibody conjugated with and without molecular reporter in the present invention.

DETAIL DESCRIPTION OF THE INVENTION

The present invention provides [5]helicene derivative compounds represented by the following chemical formula (**1**):

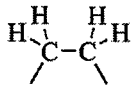


(1)

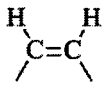
wherein

G is a connecting group composes of 2 carbon atoms selected from the group consisting of

- Ethane


; and

- Ethylene

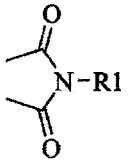


A is a separated or connected group selected from the group consisting of

- Cyano

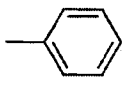
—CN ; and

- Imide

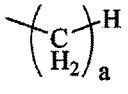


wherein R1 is selected from the group consisting of

- Phenyl

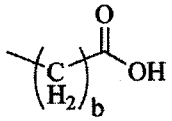

;

- Alkyl



when a is a number of carbon atoms in aliphatic hydrocarbon selected from the group consisting of straight chain and branch chain, and a equals to 1 to 7; and

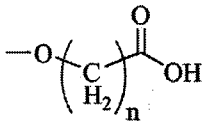
- Alkanoic acid



when b is a number of carbon atoms in aliphatic hydrocarbon selected from the group consisting of straight chain and branch chain, and b equals to 1 to 7.

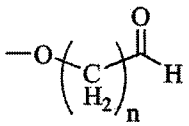
D1 is selected from the group consisting of

- Oxyalkanoic acid



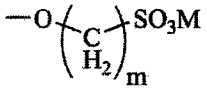
when n is a number of carbon atoms in aliphatic hydrocarbon selected from the group consisting of straight chain and branch chain, and n equals to 1 to 7;

- Oxyalkanal



when n is a number of carbon atoms in aliphatic hydrocarbon selected from the group consisting of straight chain and branch chain and n equals to 1 to 7; and

- Oxyalkanesulfonate



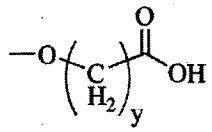
when M is a metal atom selected from the group consisting of sodium and potassium,

m is a number of carbon atoms in aliphatic hydrocarbon, selected from the group consisting of straight chain and branch chain, with a sulfonate end-group and m equals to 3 or 4.

D2 is selected from the group consisting of

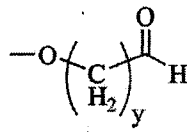
- Hydroxy

- Oxyalkanoic acid



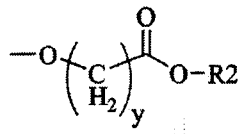
when y is a number of carbon atoms in aliphatic hydrocarbon selected from the group consisting of straight chain and branch chain, and y equals to 1 to 7;

- Oxyalkanal



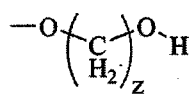
when y is a number of carbon atoms in aliphatic hydrocarbon selected from the group consisting of straight chain and branch chain, and y equals to 1 to 7;

- Alkyl oxyalkanoate



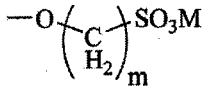
when y is a number of carbon atoms in aliphatic hydrocarbon selected from the group consisting of straight chain and branch chain, and y equals to 1 to 7,
R2 is selected from the group consisting of methyl and ethyl group;

- Oxyalkanol



when z is a number of carbon atoms in aliphatic hydrocarbon selected from the group consisting of straight chain and branch chain, and z equals to 2 to 8; and

- Oxyalkanesulfonate



when M is a metal atom selected from the group consisting of sodium or potassium,

m is a number of carbon atoms in aliphatic hydrocarbon, selected from the group consisting of straight chain and branch chain, with a sulfonate end-group and m equals to 3 or 4.

The other embodiment in this invention relates to [5]helicene derivative compounds represented in chemical formula (**1**) wherein

a) A is cyano; G is ethane; D1 is hydroxy; and D2 is selected from the group consisting of oxyalkanoic acid and oxyalkanal.

b) A is cyano; G is ethane; D1 is oxyalkanoic acid; and D2 is selected from the group consisting of hydroxy, oxyalkanoic acid and oxyalkanesulfonate.

c) A is cyano; G is ethane; D1 is oxyalkanal; and D2 is selected from the group consisting of hydroxy, alkyl oxyalkanoate, oxyalkanal and oxyalkanol.

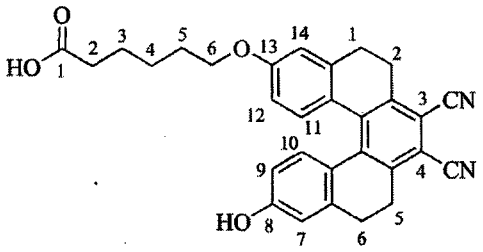
d) A is cyano; G is ethylene; D1 is oxyalkanoic acid; and D2 is selected from the group consisting of hydroxy, oxyalkanoic acid and oxyalkanesulfonate.

e) A is cyano; G is ethylene; D1 is oxyalkanal; and D2 is selected from the group consisting of hydroxy and oxyalkanol.

f) A is imide wherein R1 is selected from the group consisting of phenyl and alkyl; G is selected from the group consisting of ethane and ethylene; D1 is oxyalkanal; and D2 is selected from the group consisting of hydroxy, oxyalkanal, alkyl oxyalkanoate, oxyalkanol and oxyalkanesulfonate.

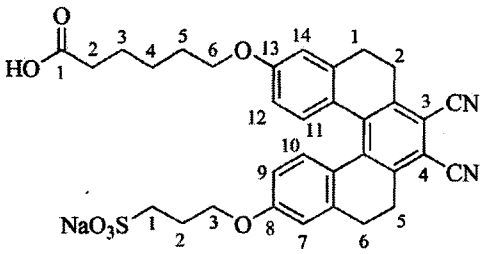
g) A is imide wherein R1 is alkanoic acid; G is ethane; D1 and D2 are oxyalkanesulfonate.

An example of [5]helicene derivative compounds, wherein G is ethane, A is cyano, D1 is 6 -oxyhexanoic acid and D2 is hydroxyl, is 6-((3,4-dicyano-8-hydroxy-1,2,5,6-tetrahydro dibenzo[c,g]phenanthren-13-yl)oxy)hexanoic acid namely compound **1** represented by the following structure:



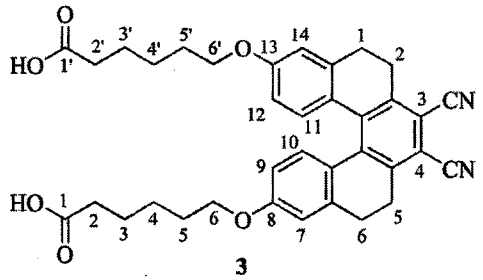
1

An example of [5]helicene derivative compounds, wherein G is ethane, A is cyano, D1 is 6 -oxyhexanoic acid and D2 is sodium 3-oxypropane-1-sulfonate, is sodium 3-((13-((5-carboxypentyl)oxy)-3,4-dicyano-1,2,5,6-tetrahydrodibenzo[c,g]phenanthren-8-yl)oxy) propane-1-sulfonate namely compound **2** represented by the following structure:



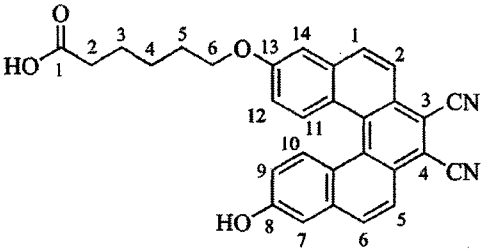
2

An example of [5]helicene derivative compounds, wherein G is ethane, A is cyano, D1 and D2 are 6-oxyhexanoic acid, is 6,6'-((3,4-dicyano-1,2,5,6-tetrahydrodibenzo[c,g]phenanthrene-8,13-diyl)bis(oxy))di hexanoic acid namely compound **3** represented by the following structure:



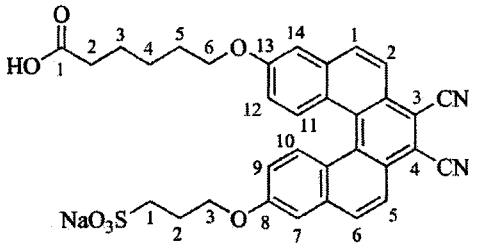
3

An example of [5]helicene derivative compounds, wherein G is ethylene, A is cyano, D1 is 6-oxyhexanoic acid and D2 is hydroxy, is 6-((3,4-dicyano-8-hydroxydibenzo[c,g]phenanthren-13-yl)oxy)hexanoic acid namely compound **4** represented by the following structure:



4

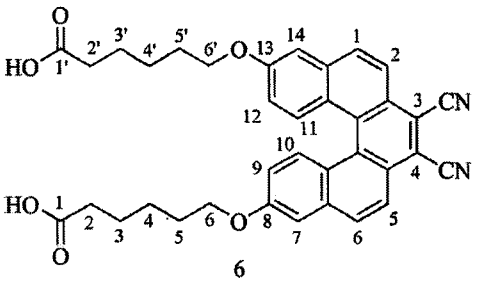
An example of [5]helicene derivative compounds, wherein G is ethylene, A is cyano, D1 is 6 -oxyhexanoic acid and D2 is sodium 3-oxypropane-1-sulfonate, is sodium 3-((8-((5-carboxypentyl)oxy)-3,4-dicyanodibenzo[c,g]phenanthren-13-yl)oxy)propane-1-sulfonate namely compound **5** represented by the following structure:



5

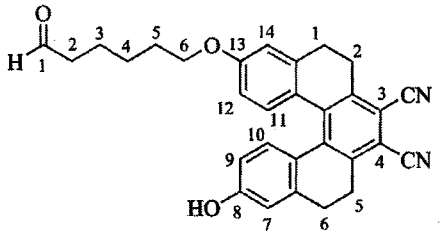
5

An example of [5]helicene derivative compounds, wherein G is ethylene, A is cyano, D1 and D2 are 6 -oxyhexanoic acid, is 6,6'-((3,4-dicyanodibenzo[c,g]phenanthrene-8,13-diyl)bis(oxy))dihexanoic acid namely compound **6** represented by the following structure:



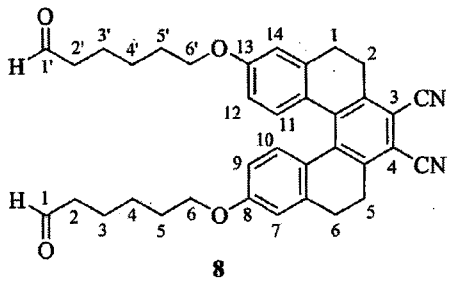
6

10 An example of [5]helicene derivative compounds, wherein G is ethane, A is cyano, D1 is 6 -oxyhexanal and D2 is hydroxy, is 8-hydroxy-13-((6-oxohexyl)oxy)-1,2,5,6-tetrahydro dibenzo[c,g]phenanthrene-3,4-dicarbonitrile namely compound **7** represented by the following structure:



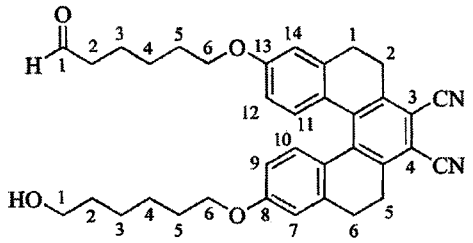
7

15 An example of [5]helicene derivative compounds, wherein G is ethane, A is cyano, D1 and D2 are 6-oxyhexanal, is 8,13-bis((6-oxohexyl)oxy)-1,2,5,6-tetrahydrodibenzo[c,g]phenanthrene-3,4-dicarbonitrile namely compound **8** represented by the following structure:



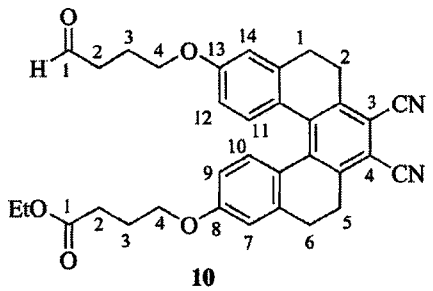
8

An example of [5]helicene derivative compounds, wherein G is ethane, A is cyano, D1 is 6-oxyhexanal and D2 is 6-oxyhexan-1-ol, is 8-((6-hydroxyhexyl)oxy)-13-((6-oxohexyl)oxy)-1,2,5,6-tetrahydrodibenzo[c,g]phenanthrene-3,4-dicarbonitrile namely compound **9** represented by the following structure:



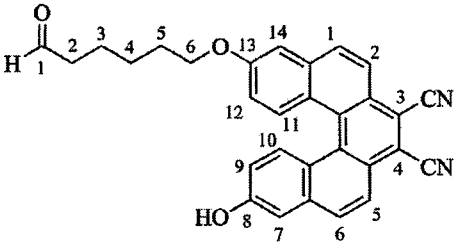
9

An example of [5]helicene derivative compounds, wherein G is ethane, A is cyano, D1 is 4-oxybutanal and D2 is ethyl 4-oxybutanoate, is ethyl 4-((3,4-dicyano-13-(4-oxobutoxy)-1,2,5,6-tetrahydrodibenzo[c,g]phenanthren-8-yl)oxy)butanoate namely compound **10** represented by the following structure:



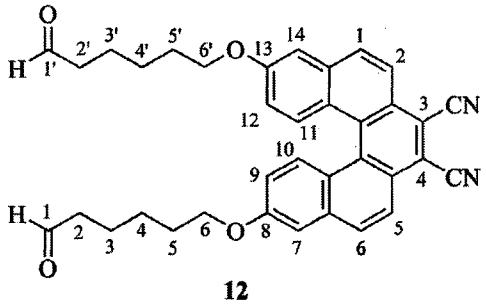
10

An example of [5]helicene derivative compounds, wherein G is ethylene, A is cyano, D1 is 6-oxyhexanal and D2 is hydroxy, is 8-hydroxy-13-((6-oxohexyl)oxy)dibenzo[c,g]phenanthrene-3,4-dicarbonitrile namely compound **11** represented by the following structure:



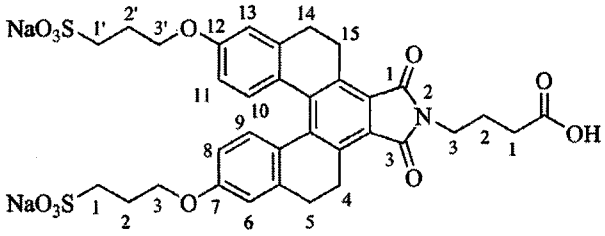
11

An example of [5]helicene derivative compounds, wherein G is ethylene, A is cyano, D1 and D2 are 6 -oxyhexanal, is 8,13-bis((6-oxohexyl)oxy)dibenzo[c,g]phenanthrene-3,4-dicarbonitrile namely compound **12** represented by the following structure:



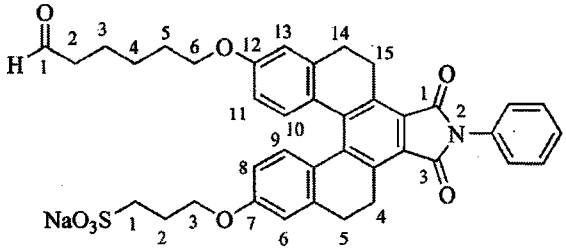
12

An example of [5]helicene derivative compounds, wherein G is ethane, A is imide wherein R1 is 4-butanoic acid, D1 and D2 are sodium 3-oxypropane-1-sulfonate, is sodium 3,3'-((2-(3-carboxypropyl)-1,3-dioxo-4,5,14,15-tetrahydro-1H-dinaphtho[2,1-e:1',2'-g]isoindole-7,12-diyl) bis(oxy))bis(propane-1-sulfonate) namely compound **13** represented by the following structure:



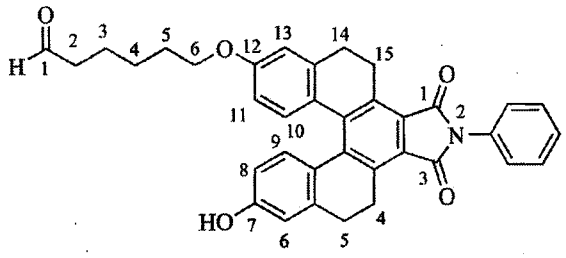
13

An example of [5]helicene derivative compounds, wherein G is ethane, A is imide wherein R1 is phenyl, D1 is 6-oxyhexanal and D2 is sodium 3-oxypropane-1-sulfonate, is sodium 3-((1,3-dioxo-1 2 - ((6 - oxohexyl)oxy)-2 - phenyl-4 ,5 ,1 4 ,1 5 - tetrahydro-1 H-dinaphtho[2 ,1 - e:1 ',2 '-g]isoindol-7 - yl)oxy)propane-1 - sulfonate namely compound **14** represented by the following structure:



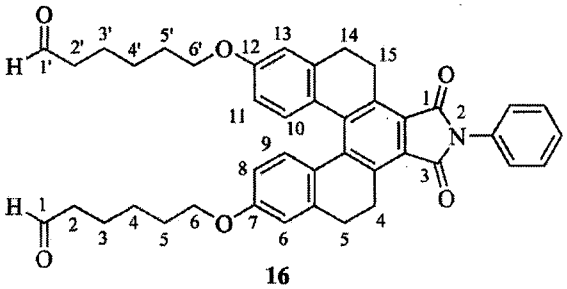
14

An example of [5]helicene derivative compounds, wherein G is ethane, A is imide wherein R1 is phenyl, D1 is 6-oxyhexanal and D2 is hydroxy, is 6-((7-hydroxy-1,3-dioxo-2-phenyl-4,5,14,15-hexahydro-1H-dinaphtho[2,1-e:1',2'-g]isoindol-12-yl)oxy)hexanal namely compound **15** represented by the following structure:



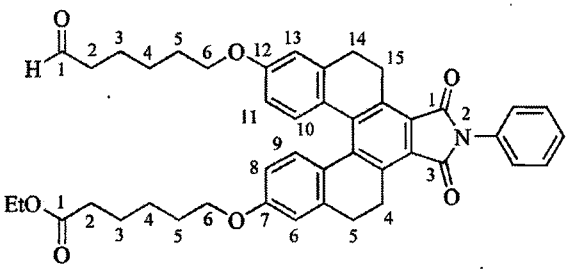
15

An example of [5]helicene derivative compounds, wherein G is ethane, A is imide wherein R1 is phenyl, D1 and D2 are 6 -oxyhexanal, is 6,6'-((1,3-dioxo-2-phenyl-2,3,4,5,14,15-hexahydro-1H-dinaphtho[2,1-e:1',2'-g]isoindole-7,12-diyl)bis(oxy))dihexanal namely compound **16** represented by the following structure:



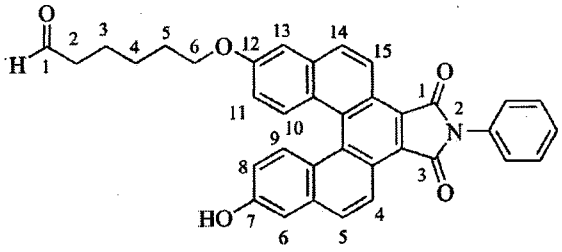
16

An example of [5]helicene derivative compounds, wherein G is ethane, A is imide wherein R1 is phenyl, D1 is 6-oxyhexanal and D2 is ethyl 6-oxyhexanoate, is ethyl 6-((1,3-dioxo-12-((6-oxohexyl)oxy)-2-phenyl-4,5,14,15-tetraahydro-1H-dinaphtho[2,1-e:1',2'-g] isoindol-7-yl)oxy) hexanoate namely compound **17** represented by the following structure:



17

An example of [5]helicene derivative compounds, wherein G is ethylene, A is imide wherein R1 is phenyl, D1 is 6 -oxyhexanal and D2 is hydroxy, is 6-((7-hydroxy-1,3-dioxo-2-phenyl-1H-dinaphtho[2,1-e:1',2'-g]isoindol-12-yl)oxy)hexanal namely compound **18** represented by the following structure:

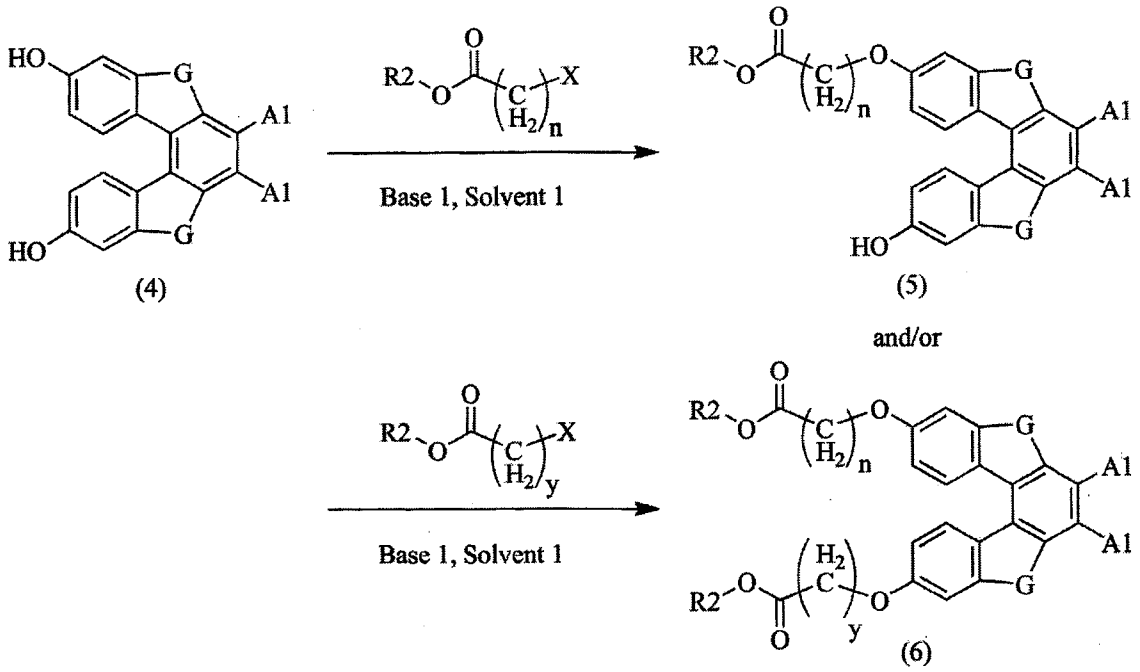


18

The derivative of [5]helicene compounds in the present invention compose of aromatic [5]helicene core comprising long π-conjugating system. Owing to having proper chemical structure, the compounds in the present invention exhibit good fluorescence. Moreover, the said compounds contain functional groups which able to link with biomolecule and they are soluble in water or other solvents that used in binding process with biomolecules. The said compounds give fluorescent emission in the range of 425-675 nm. When the said compounds connected with biomolecules such as protein, antibody and peptide, the biomolecules give good fluorescent emission which can be detected under ultraviolet radiation. Because of their good chemical and optical properties, the luminescent organic compounds in the present invention are suitable for utilizing as reporter molecules in diagnostic for microbials, toxins and toxicants in samples from agricultural industries, foods and environments

The methods of synthesis for [5]helicene derivative compounds in the present invention comprise steps of:

a.) An O-alkylation reaction (to incorporate an alkylester) of the [5]helicene compound in formula (**4**), selected from the [5]helicene compound in formula (**4**) wherein A1 is imide or the [5]helicene compound in formula (**4**) wherein A1 is cyano, with haloalkanoic acid alkyl ester (**I**) which is a primary alkyl halide containing a desired ester end group. The said reaction is performed in the present of base 1, as a catalyst, selected from the group consisting of sodium bicarbonate ($NaHCO_3$), potassium bicarbonate ($KHCO_3$), sodium carbonate (Na_2CO_3) and potassium carbonate (K_2CO_3) and the most effective is potassium carbonate (K_2CO_3). Also, the said alkylation is done in organic solvent 1 selected from the group consisting of dimethyl formamide, acetone, acetonitrile and mixture thereof wherein the most effective solvent is dimethyl formamide. The reaction is carried on at the temperature in the range of 60-160 °C for the period of 2-12 hours to give [5]helicene compound (**5**) and/or compound (**6**) as intermediate molecule containing at least one alkylester group. The reaction is depicted below.



wherein n and y are indepent at which

n is a number of carbon atoms of aliphatic hydrocarbon in haloalkanoic acid alkyl ester (**I**) reacting in the first alkylation reaction where n equals to 1 to 7 and the said hydrocarbon is selected from the group consisting of straight chain and branch chain.

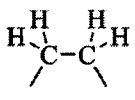
y is a number of carbon atoms of aliphatic hydrocarbon in haloalkanoic acid alkyl ester (**I**) reacting in the second alkylation reaction where y equals to 1 to 7 and the said hydrocarbon is selected from the group consisting of straight chain and branch chain.

X is halogen atom selected from the group consisting of chlorine(Cl), bromine(Br) and iodine(I).

R2 is alkyl group selected from the group consisting of methyl and ethyl.

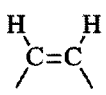
G is a connecting group composes of 2 carbon atoms selected from the group consisting of

- Ethane



; and

- Ethylene

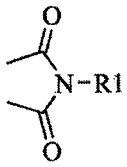


A1 is a separated or connected group selected from the group consisting of

- Cyano

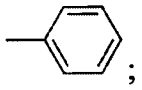
—CN ; and

- Imide

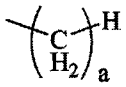


wherein R1 is selected from the group consisting of

- Phenyl

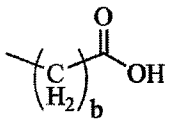


- Alkyl



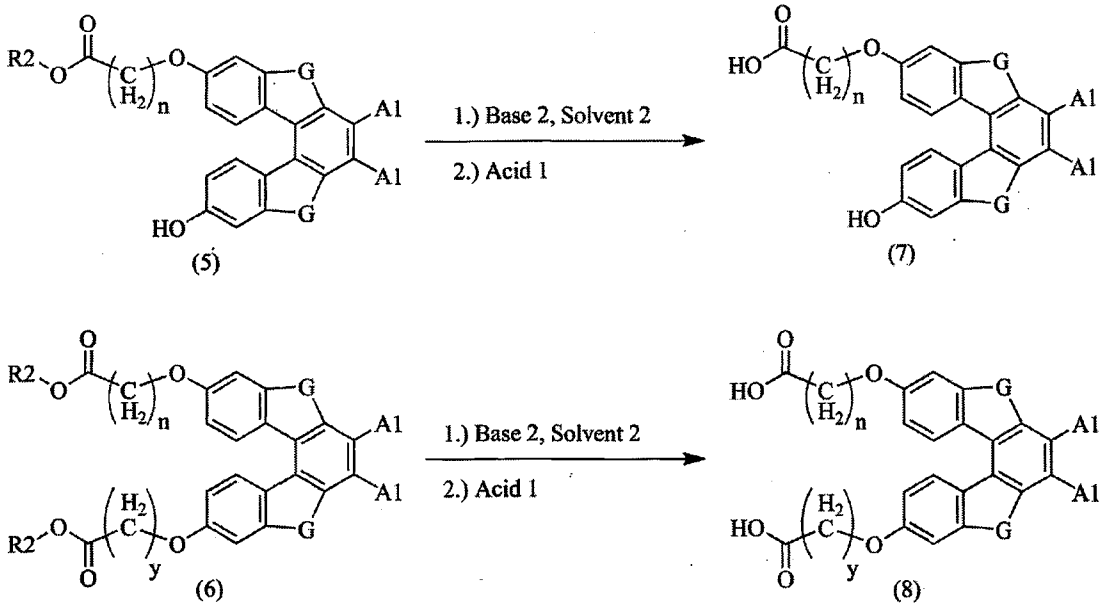
when a is a number of carbon atoms in aliphatic hydrocarbon selected from the group consisting of straight chain and branch chain, and a equals to 1 to 7; and

- Alkanoic acid

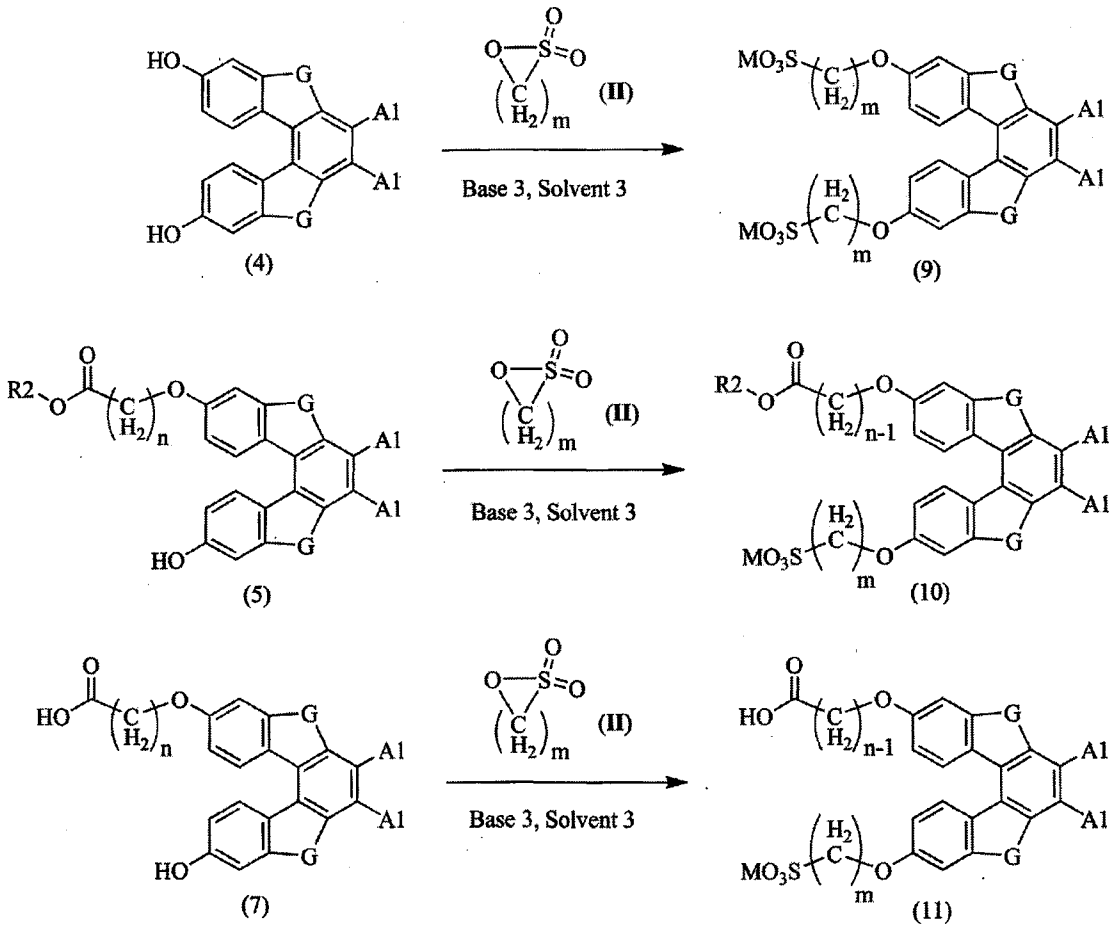


when b is a number of carbon atoms in aliphatic hydrocarbon selected from the group consisting of straight chain and branch chain, and b equals to 1 to 7.

b.) A hydrolysis reaction of [5]helicene compound (**5**) or compound (**6**) using base 2 selected from the group consisting of sodium hydroxide (NaOH), potassium hydroxide (KOH) and lithium hydroxide (LiOH). The reaction is performed in an organic solvent 2 selected from the group consisting of ethanol, methanol, tetrahydrofuran, dioxane, dichloromethane and a mixture thereof wherein the most effective solvent is ethanol. The said reaction is carried out at temperature in the range of 25-150 °C for 1-24 hours, follow by acidify with acid 1, selected from the group consisting hydrochloric acid and sulfuric acid, to gain pH 0 to obtain [5]helicene compound (**7**) or compound (**8**) which contains at least one carboxylic group as a final product or an intermediate. The reaction is depicted below.



c.) An O-alkylation reaction for addition of sulfonate group to the molecule and make the molecule soluble in aqueos media. The intermediate compound containing OH group, selected from of [5]helicene compound (**4**), compound (**5**) or compound (**7**) reacts with alkane sultone) (**II**) at the present of base 3 as a catalyst, selected from the group consisting of sodium hydroxide (NaOH), potassium hydroxide (KOH), sodium methoxide (NaOMe), potassium methoxide (KOMe), sodium ethoxide (NaOEt) and potassium ethoxide (KOEt) wherein the most effective is sodium ethoxide. An organic solvent 3 selected from the group consisting of methanol, ethanol, acetone and acetonitrile is used as solvent in this reaction and the most effective is ethanol. The reaction is carried out at temperature in the range of 25-80 °C for 6 to 120 hours to obtain [5]helicene compound (**9**), compound (**10**) or compound (**11**) as final product of intermediate. The reaction is presented as following;

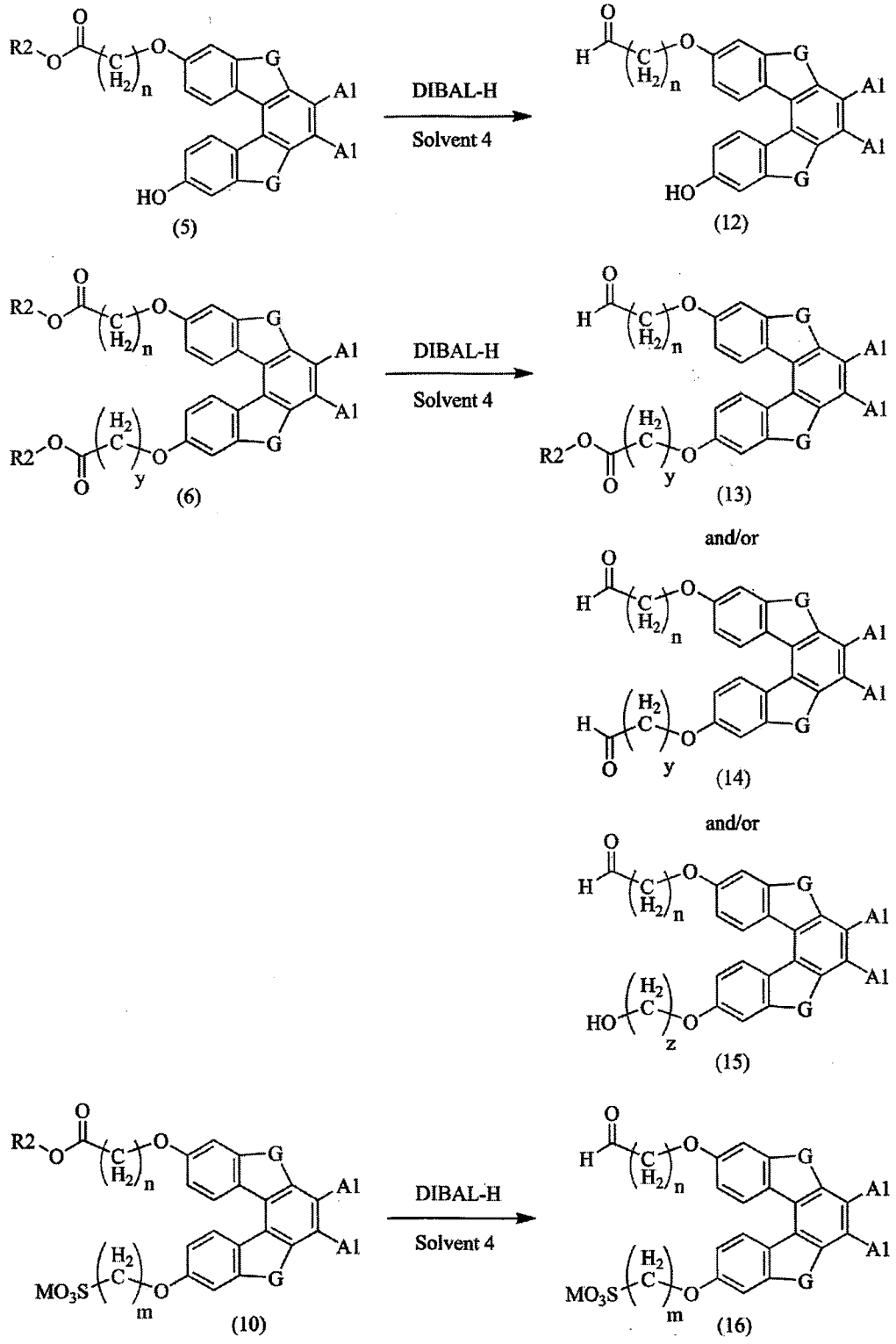


wherein

M is a metal atom selected from the group consisting of sodium and potassium,

m is a number of carbon atoms in aliphatic hydrocarbon, selected from the group consisting of straight chain and branch chain and m equals to 3 or 4.

d.) A reduction reaction of intermediate containing ester group, selected from [5]helicene compound (**5**), compound (**6**) or compound (**10**), using diisobutylaluminum hydride, (DIBAL-H). The reaction is performed in an organic solvent 4 selected from the group consisting of dichloromethane, tetrahydrofuran, toluene and a mixture thereof. The reaction is carried out at temperature in the range of -90 °C to room temperature for 1-4 hours to gain [5]helicene compound (**12**), compound (**13**) and/or compound (**14**) and/or compound (**15**) or compound (**16**) as final product.



wherein m, n, y and z are independent when

n is a number of carbon atoms in aliphatic hydrocarbon selected from the group consisting of straight chain and branch chain and n equals to 1 to 7,

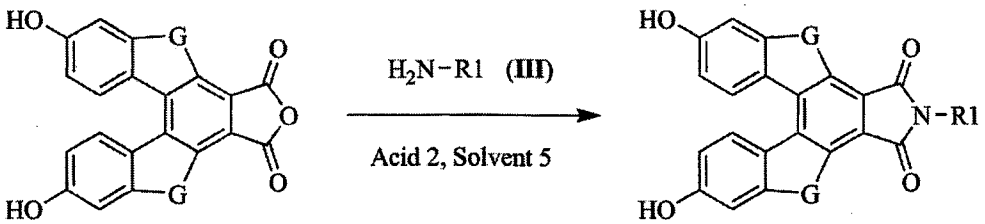
y number of carbon atoms in aliphatic hydrocarbon selected from the group consisting of straight chain and branch chain and y equals to 1 to 7,

z is a number of carbon atoms in aliphatic hydrocarbon selected from the group consisting of straight chain and branch chain and z equals to 1 to 7,

M is a metal atom selected from the group consisting of sodium or potassium,

m is a number of carbon atoms in aliphatic hydrocarbon, selected from the group consisting of straight chain and branch chain, with a sulfonate end-group and m equals to 3 or 4.

Furthermore, the synthesis of organic dyes based on [5]helicene derivative compounds in this invention comprises imidation reaction to make imide compound. The said reaction is done by reacting [5]helicene compound in formula (**2**) with a primary amine (**III**) in the present of acid 2 as a catalyst selected from the group consisting of acetic acid, hydrochloric acid and sulfuric acid and the most effective is acetic acid. The reaction is performed in an organic solvent 5 selected from the group consisting of dimethyl formamide, dimethyl sulfoxide, acetonitrile, toluene, benzene and a mixture thereof and the most effective solvent is dimethyl formamide. The reaction is carried out at temperature in the range of 80 to 160 °C for 2 to 12 hours to gain [5]helicene isoindole dione compound represented by the chemical formula (**3**) and the reaction is depicted as following.

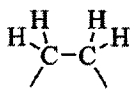


(2) (3)

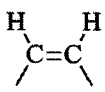
wherein

G is a connecting group composes of 2 carbon atoms selected from the group consisting of

- Ethane

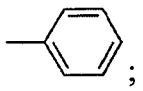

; and

- Ethylene

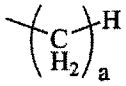


wherein R1 is selected from the group consisting of

- Phenyl

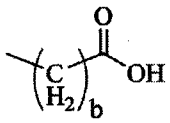


- Alkyl



when a is a number of carbon atoms in aliphatic hydrocarbon selected from the group consisting of straight chain and branch chain and a equals to 1 to 7; and

- Alkanoic acid



when b is a number of carbon atoms in aliphatic hydrocarbon selected from the group consisting of straight chain and branch chain and b equals to 1 to 7.

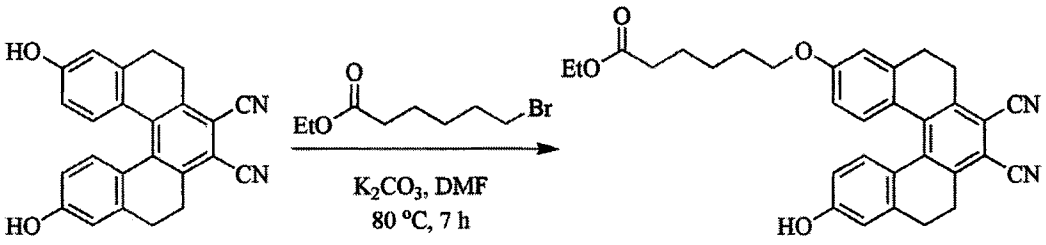
Nonetheless, the sequences of reaction steps for preparation method of [5]helicene derivative compounds are alterable to obtain desired products.

The synthesis method of [5]helicene derivative compounds in the present invention are demonstrated in the following examples:

Example 1

The synthesis of 6-((3,4-dicyano-13-hydroxy-1,2,5,6-tetrahydrodibenzo[c,g]phenanthren-8-yl)oxy)hexanoic acid) or compound **1**

Step a)

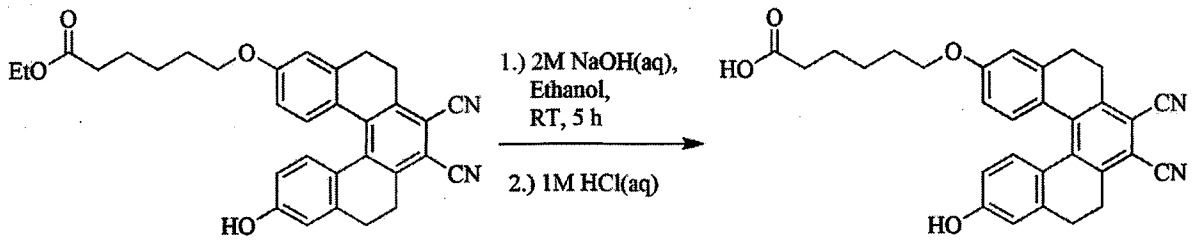


21 **23**

A mixture of 8,13-dihydroxy-1,2,5,6-tetrahydrodibenzo[c,g]phenanthrene-3,4-dicarbo nitrile, compound **21**, (1.00 g, 2.75 mmol), ethyl-6-bromohexanolate (0.61 g, 2.75 mmol), potassium carbonate (K_2CO_3) (0.46 g, 3.30 mmol) and 50 mL of DMF in a 100 mL round-bottom flask were stirred and heated at 80 °C under argon atmosphere for 7 h. After cooling to room

temperature, the reaction mixture was dumped into water (600 mL) with vigorous stirring for 1 h. The aqueous layer was extracted with ethyl acetate (200 mLx2). The organic layer was dried with Na_2SO_4 and removed to yield a crude product. The crude product was purified by normal phase column chromatography (silica gel, 20% to 50% EtOAc-Hexane) to give pure ethyl 6-((3,4-dicyano-13-hydroxy-1,2,5,6-tetrahydrodibenzo[c,g]phenanthren-8-yl)oxy)hexanoate, compound **23**, as yellow viscous liquid (0.8 g, 57% yield). This compound was used for the next step.

Step b)



23 **1**

6-((3,4-Dicyano-13-hydroxy-1,2,5,6-tetrahydrodibenzo[c,g]phenanthren-8-yl)oxy) hexa noate, compound **23**, (0.20 g, 0.39 mmol) was dissolved in 4.0 mL of ethanol and 1.5 mL of 2M NaOH aqueous solution was added. The solution was stirred at room temperature for 5 h. 1M HCl Aqueous solution was added to the reaction solution until pH equals to 0, resulting in a yellow-orange precipitation. The precipitated solid was washed with water and dried to gain pure product, 6 - ((3 ,4- dicyano-1 3 - hydroxy-1 ,2 ,5 ,6 - tetrahydrodibenzo[c,g]phenanthren-8 - yl)oxy)hexanoic acid) or compound **1**, as pale yellow solid (0.18 g, 95% yield).

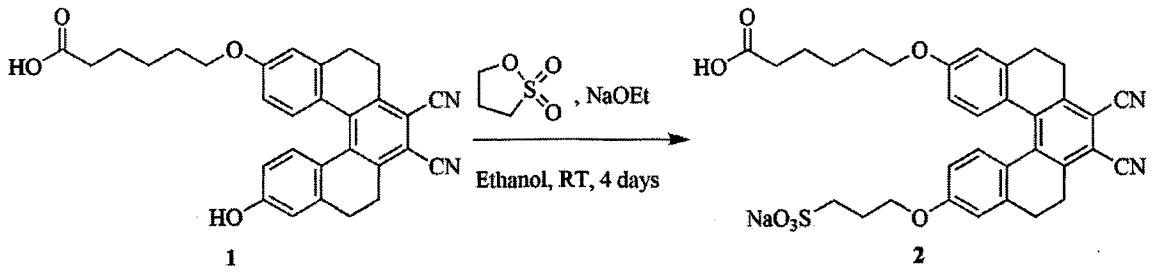
^{1}H NMR (500 MHz, MeOD-d_4): δ 7.10 (d, *J*=8.5 Hz, 1H), 7.01 (d, *J*=8.5 Hz, 1H), 6.85 (s, 1H), 6.72 (s, 1H), 6.51 (d, *J*=7.0 Hz, 1H), 6.38 (d, *J*=7.0 Hz, 1H), 3.96 (s, 2H), 3.23 (s, 2H), 2.87-2.80 (m, 4H), 2.58 (s, 2H), 2.18 (t, *J*=7.5 Hz, 2H), 1.77-1.75 (m, 2H), 1.65-1.62 (m, 2H), 1.50-1.48 (m, 2H) ppm.

^{13}C NMR (125 MHz, MeOD-d_4): δ 183.00, 161.82, 160.32, 145.44, 145.34, 142.83, 142.68, 139.35, 138.90, 132.83, 132.60, 126.99, 125.90, 117.10, 115.71, 114.97, 114.68, 114.17, 112.58, 112.37, 69.51(2×CH_2), 39.41(3×CH_2), 30.67, 30.10, 27.86, 27.66 ppm.

FT-IR (KBr): ν_{max} 3363, 3212, 2943, 2224, 1705, 1606, 1411, 1274, 1242, 863, 821 cm^{-1}.

Example 2

The synthesis of sodium 3-((13-((5-carboxypentyl)oxy)-3,4-dicyano-1,2,5,6-tetrahydrodibenzo[c,g]phenanthren-8-yl)oxy) propane-1-sulfonate or compound **2**



A mixture of 6-((3,4-dicyano-13-hydroxy-1,2,5,6-tetrahydrodibenzo[c,g]phenanthren-8-yl)oxy)hexanoic acid) or compound **1** (0.13 g, 0.27 mmol) in dry ethanol (5 mL) was stirred until all solids disappeared under argon atmosphere. A mixture of sodium ethoxide (0.040 g, 0.59 mmol) in 3 mL of dry ethanol was added dropwise and stirred for 1 h, causing the yellow solution to turn orange-brown. Then, 1,3 propanesultone (0.05 g, 0.44 mmol) in 2 mL of dry ethanol was added to the mixture. The reaction mixture was stirred at room temperature for 4 days. The reaction was followed by using TLC and precipitation of orange solid. Removing of ethanol by distillation under reduced pressure gave the crude product. The crude product was purified by column chromatography using reversed phased silica gel (50% to 100% MeOH:H_2O) to give pure product, sodium 3-((13-((5-carboxypentyl)oxy)-3,4-dicyano-1,2,5,6-tetrahydrodibenzo[c,g] phenanthren-8-yl)oxy) propane-1-sulfonate or compound **2**, as yellow-green solid (0.09 g, 44% yield).

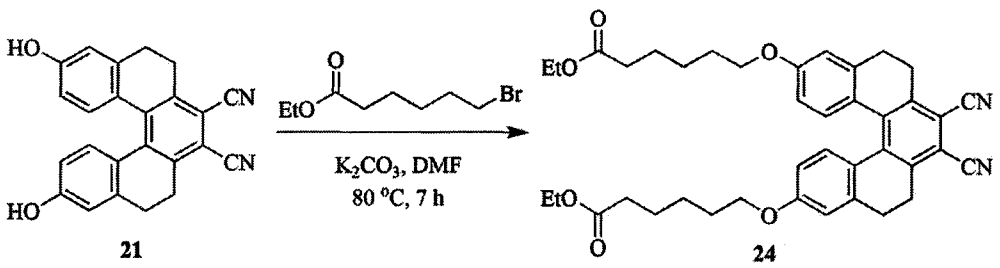
^{1}H NMR(500 MHz, MeOD-d_4): δ 7.10 (tt, *J*=7.8, 1.5 Hz, 2H), 6.90 (s, 1H), 6.87 (s, 1H), 6.54 (tt, *J*=10.3, 2.0 Hz, 2H), 4.11 (s, 2H), 3.97 (s, 2H), 3.30-3.25 (broad, 1H), 2.96 (t, *J*=7.5 Hz, 2H), 2.90 (s, 4H), 2.62 (s, 2H), 2.25-2.18 (m, 2H), 2.16 (t, *J*=7.5 Hz, 2H), 1.78-1.75 (m, 2H), 1.66-1.63 (m, 2H), 1.50-1.48 (m, 2H) ppm.

FT-IR(KBr): ν_{max} 3445, 2943, 2857, 2221, 1718, 1607, 1275, 1244, 1209, 1038, 853, 597 cm^{-1}.

Example 3

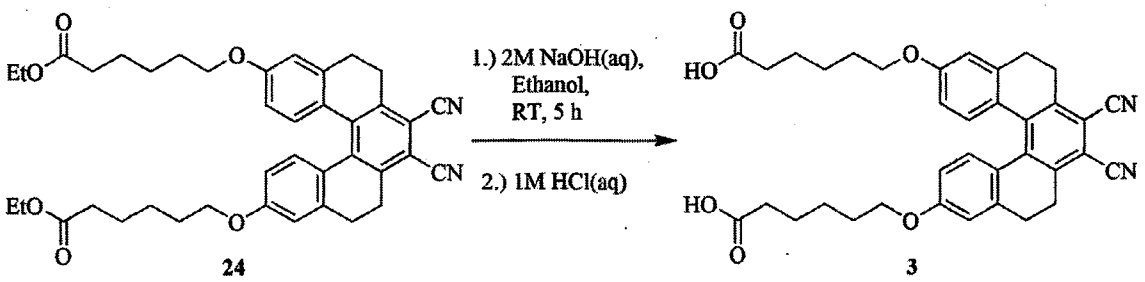
The synthesis of 6,6'-((3,4-dicyano-1,2,5,6-tetrahydrodibenzo[c,g] phenanthrene-8,13-diyl)bis(oxy))dihexanoic acid or compound **3**

Step a)



A mixture of 8,13-dihydroxy-1,2,5,6-tetrahydrodibenzo[c,g] phenanthrene-3,4-dicarbonitrile, compound **21**, (0.50 g, 1.37 mmol), ethyl-4-bromobutyrate (0.67 g, 3.00 mmol), potassium carbonate (K_2CO_3) (0.57 g, 4.13 mmol) and 15 mL of DMF were stirred and heated at 80°C under argon atmosphere for 7 h. After cooling to room temperature, the reaction mixture was dumped into water (600 mL) with vigorous stirring for 1 h. The aqueous layer was extracted with ethyl acetate (200 mLx2). The organic layer was dried with Na_2SO_4 and removed to yield a crude product. The crude product was purified by normal phase column chromatography (silica gel, 50% EtOAc-Hexane) to give pure diethyl 6,6'-((3,4-dicyano-1,2,5,6-tetrahydro dibenzo[c,g]phenanthrene-8,13-diyl)bis(oxy))dihexanoate, compound **24** as yellow solid (0.82 g, 92% yield).

Step b)



Diethyl 6,6'-((3,4-dicyano-1,2,5,6-tetrahydrodibenzo[c,g] phenanthrene-8,13-diyl) bis (oxy))dihexanoate, compound **24**, (0.29 g, 0.44 mmol) was dissolved in 5.0 mL of ethanol and 3 mL of 2M NaOH aqueous solution was added to the reaction mixture. The solution was stirred at room temperature for 5 h, after which ethanol was removed under reduced pressure. 1M HCl (aq) was added to the solution until pH equals to 0, resulting in an orange precipitate. The precipitate was washed with water (15 mL) and dried (0.24 g, 92% yield, mp. 172-173°C).

^{1}H NMR (500 MHz, MeOD-d_4): δ 7.08 (d, *J*=8.5 Hz, 2H), 6.86 (s, 2H), 6.51 (d, *J*=7.0, 2H), 4.12 (b s, 4H), 3.30-3.20 (b, 2H), 2.95-2.85 (b, 4H), 2.70-2.52 (b, 2H), 2.30 (t, *J*=7.0 Hz, 4H), 1.90-1.75 (m, 4H), 1.75-1.60 (m, 4H), 1.55-1.45 (m, 4H) ppm.

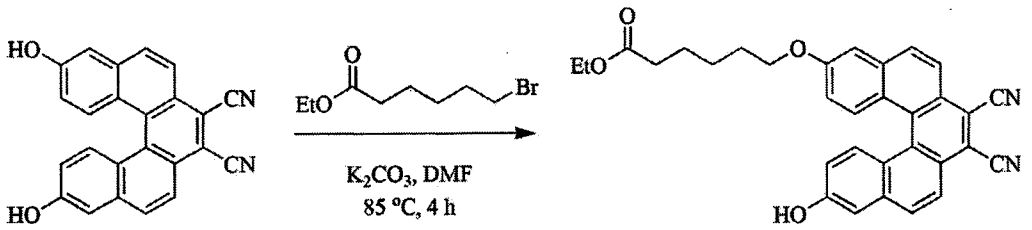
^{13}C NMR (125 MHz, MeOD-d_4): δ 177.55, 161.30, 144.96, 142.28, 138.54, 132.19, 126.48, 116.54, 114.24, 113.68, 112.21, 68.88, 34.87, 30.02, 29.64, 29.58, 26.75, 25.83 ppm.

FT-IR(KBr): ν_{max} 3462, 2943, 2910, 2221, 1707, 1607, 1275, 1244, 1108, 1095, 855, 809 cm^{-1}.

Example 4

The synthesis of 6-((3,4-dicyano-8-hydroxydibenzo[c,g]phenanthren-13-yl)oxy)hexanoic acid or compound **4**

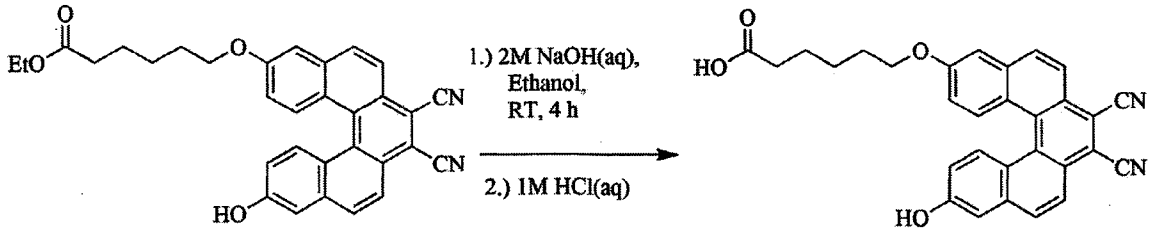
Step a)



22 25

A mixture of 8,13-dihydroxy-dibenzo[c,g]phenanthrene-3,4-dicarbonitrile, compound **22**, (1.16 g, 3.23 mmol), ethyl-6-bromohexanolate (0.73 g, 3.29 mmol), potassium carbonate (K_2CO_3) (0.68 g, 4.94 mmol) and 90 mL of DMF was stirred and heated at 85°C under argon atmosphere for 4 h. After cooling to room temperature, the reaction mixture was poured into 800 ml of water with vigorous stirring for 1 h. The aqueous layer was extracted with ethyl acetate. The organic layer was dried with anhydrous Na_2SO_4 and the solvent was removed to yield the crude product. The crude was purified by column chromatography (SiO_2, 25% to 50% EtOAc-Hexane) to give ethyl 6-((3,4-dicyano-13-hydroxydibenzo[c,g]phenanthren-8-yl)oxy)hexanoate or compound **25**, (0.64 g, 40% yield) as a brown solid.

Step b)



25 4

Ethyl 6-((3,4-dicyano-13-hydroxydibenzo[c,g] phenanthren-8-yl)oxy) hexanoate, compound **25**, (0.64 g, 1.32 mmol) in 10 ml of ethanol and 4 ml of 2M NaOH (aq) were mixed and stirred at room temperature under argon atmosphere for 4 h. After ethanol was removed under reduced pressure, the reaction mixture was acidified by adding 1M HCl dropwise until pH equals to 0 and orange solid was precipitated out from the solution. The solid was filtered, washed with water and dried. The corresponding product 6-((3,4-dicyano-13-hydroxydibenzo[c,g] phenanthren-8-yl)oxy)hexanoic acid or compound **4** was obtained as an orange solid (0.54 g, 87% yield).

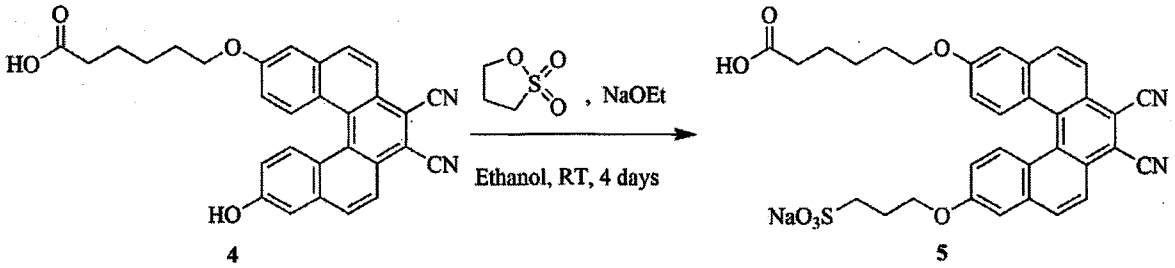
^{1}H NMR (500 MHz, MeOD-d_4): δ 8.11-8.06 (m, 5H), 8.02 (d, J=9.0 Hz, 1H), 7.43 (s, 1H), 7.30 (s, 1H), 6.90 (d, J=8.0 Hz, 1H), 6.84 (d, J=9.0 Hz, 1H), 4.19 (t, J= 6.5 Hz, 2H), 2.33 (t, J=7.0 Hz, 2H), 1.90 (quin, J=7.0 Hz, 2H), 1.73 (quin, J=7.0 Hz, 2H), 1.60 (quin, J=7.0 Hz, 2H) ppm.

^{13}C NMR (125 MHz, MeOD-d_4): δ 160.85, 159.59, 137.36, 137.14, 131.14, 131.75, 131.48, 131.36, 131.23, 131.01, 129.39, 129.16, 125.16, 124.86, 123.36, 123.22, 118.51, 118.25, 117.60, 117.36, 114.12, 113.78, 111.34, 109.06, 69.28, 35.84, 30.04, 26.91, 26.23 ppm.

FT-IR (KBr): ν_{max} 3222, 2922, 2223, 1708, 1614, 1565, 1490, 1354, 1236, 1186, 858, 529 cm^{-1}.

Example 5

The synthesis of sodium 3-((8-((5-carboxypentyl)oxy)-3,4-dicyanodibenzo [c,g]phenan thren-13-yl)oxy)propane-1-sulfonate or compound **5**



4 5

A mixture of 6-((3,4-dicyano-13-hydroxydibenzo[c,g]phenanthren-8-yl)oxy)hexanoic acid or compound **4** (0.70 g, 1.48 mmol) in dry ethanol (45 mL) was stirred until all solids disappeared under argon atmosphere. A mixture of sodium ethoxide (0.15 g, 2.22 mmol) in 10 mL of dry ethanol was added dropwise and stirred for 1 h, causing the yellow solution to turn orange-brown. Then, 1,3 propanesultone (0.19 g, 1.62 mmol) in 5 mL of dry ethanol was added to the mixture. The reaction mixture was stirred at room temperature for 4 days. The reaction was followed by using TLC and precipitation of orange solid. Removing of ethanol by distillation under reduced pressure gave the crude product. The crude product was purified by column chromatography using reversed phased silica gel (50% MeOH:H_2O) to give pure product, sodium 3-((8-((5-carboxypentyl)oxy)-3,4-dicyanodibenzo[c,g] phenanthren-13-yl)oxy) propane-1-sulfonate or compound **5**, as yellow-green solid (0.12 g, 27% yield).

^{1}H NMR (500 MHz, MeOD-d_4): δ 8.15-8.19 (m, 5H), 7.49 (d, J=2.3 Hz, 2H), 6.95-6.70 (m, 3H), 4.33 (t, J=6.5 Hz, 2H), 4.18 (t, J=6.5 Hz, 2H), 3.04 (t, J=7.5 Hz, 2H), 2.34 (quin, J=7.0 Hz, 2H), 2.20 (t, J=7.5 Hz, 2H), 1.89 (quin, J=8 Hz, 2H), 1.70 (quin, J=7.5 Hz, 2H), 1.57 (quin, J=8 Hz, 2H) ppm.

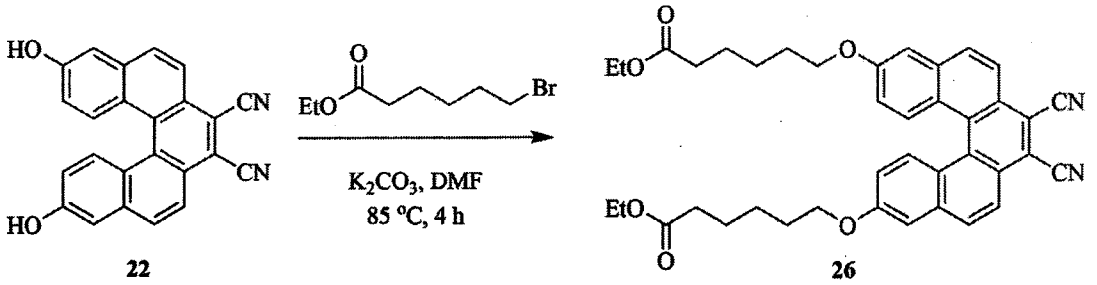
^{13}C NMR (125 MHz, MeOD-d_4): δ 161.09, 160.79, 137.28, 131.61, 130.92, 129.48, 128.63, 125.85, 125.70, 123.52, 123.40, 123.36, 118.79, 116.56, 109.30, 109.13, 69.46, 68.16, 39.14, 30.75, 30.18, 27.49, 27.23, 26.27 ppm

FT-IR (KBr): ν_{max} 3435, 2928, 2224, 1614, 1565, 1450, 1408, 1358, 1192, 1051, 861, 796, 671, 537, 459 cm^{-1}.

Example 6

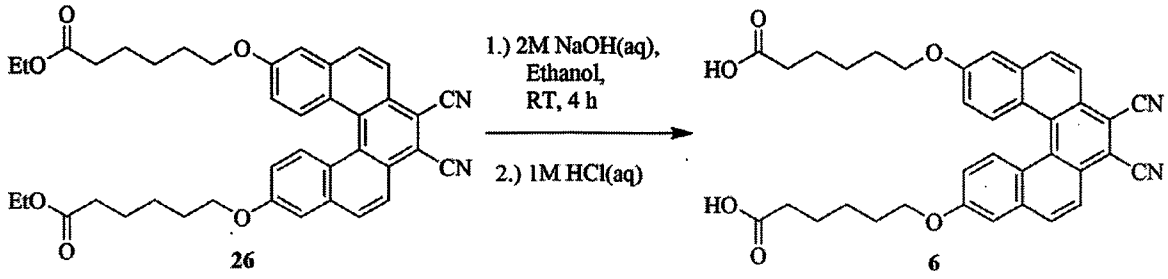
The synthesis of 6,6'-((3,4-dicyanodibenzo[c,g]phenanthrene-8,13-diyl)bis(oxy)) dihexanoic acid or compound **6**

Step a)



A mixture of 8,13-dihydroxy-dibenzo[c,g]phenanthrene-3,4-dicarbonitrile, compound **22**, (1.16 g, 3.23 mmol), ethyl-6-bromohexanolate (0.73 g, 3.29 mmol), potassium carbonate (K_2CO_3) (0.68 g, 4.94 mmol) and 90 mL of DMF was stirred and heated at 85°C under argon atmosphere for 4 h. After cooling to room temperature, the reaction mixture was poured into 800 ml of water with vigorous stirring for 1 h. The aqueous layer was extracted with ethyl acetate. The organic layer was dried with anhydrous Na_2SO_4 and the solvent was removed to yield the crude product. The crude was purified by column chromatography (SiO_2, 50% EtOAc-Hexane) to give diethyl 6,6'-((3,4-dicyano-dibenzo[c,g]phenanthrene-8,13-diyl)bis(oxy))dihexanoate, compound **26**, (0.59 g, 28% yield) as a yellow solid.

Step b)



Diethyl 6,6'-((3,4-dicyano-dibenzo[c,g] phenanthrene-8,13-diyl)bis(oxy)) dihexanoate, compound **26**, (0.20 g, 3.30 mmol) in 30 ml of ethanol and 10 ml of 2M NaOH (aq) were mixed and stirred at room temperature under argon for 4 h. After ethanol was removed under reduced pressure, 1M HCl was added dropwise into reaction until pH equals to 0 and orange solid was precipitated out from the solution. The solid was filtered, washed with water and dried.

The corresponding product, 6,6'-((3,4-dicyanodibenzo[c,g]phenanthrene-8,13-diyl)bis(oxy)) dihexanoic acid or compound **6** was obtained as orange solid (0.162 g, 83%yield).

^{1}H NMR (500 MHz, MeOD-d_4): δ 8.18 (d, J=7.5 Hz, 1H), 8.12 (d, J=2.0 Hz, 1H), 8.07 (d, J=4.0 Hz, 1H), 7.99 (d, J=9.0 Hz, 1H), 7.95 (s, 1H), 7.88 (d, J=8.5 Hz, 1H), 7.33-7.41 (m, 2H), 6.84-6.91 (m, 2H), 4.16 (q, J=6.3 Hz, 4H), 2.33 (q, J=7.0 Hz, 4H), 1.86 (quin, J=6.0 Hz, 4H), 1.70 (quin, J=7.0 Hz, 4H), 1.46 (quin, J=7.0 Hz, 4H) ppm.

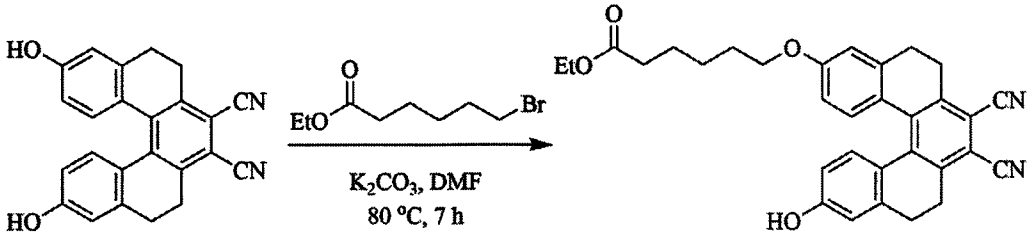
^{13}C NMR (125 MHz, DMSO-d_6): δ 174.63, 170.51, 158.76, 157.29, 135.28, 134.54, 130.44, 129.85, 129.71, 129.42, 128.60, 127.44, 125.62, 124.61, 124.36, 122.47, 122.03, 121.16, 117.34, 116.59, 115.56, 112.41, 108.08, 107.98, 67.52, 33.84, 28.45, 25.24, 24.39 ppm.

FT-IR (KBr): ν_{max} 3065, 2936, 2223, 1704, 1615, 1447, 1354, 1280, 1235, 1183, 849, 667, 539 cm^{-1}.

Example 7

The synthesis of 8-hydroxy-13-((6-oxohexyl)oxy)-1,2,5,6-tetrahydrodibenzo[c,g] phenan threne-3,4-dicarbonitrile or compound **7**

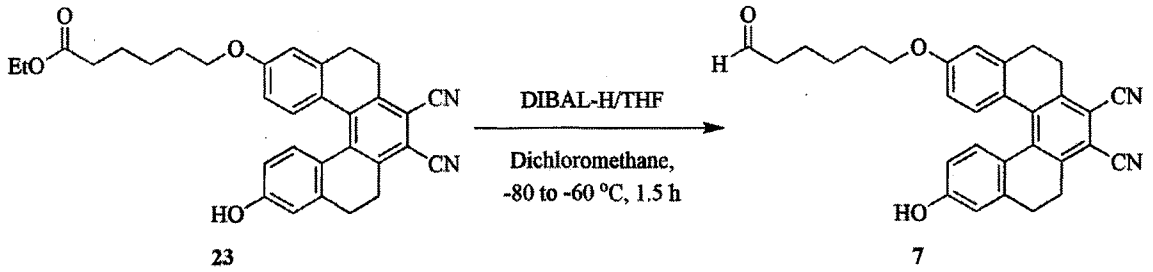
Step a)



21 **23**

A mixture of 8,13-dihydroxy-1,2,5,6-tetrahydrodibenzo[c,g] phenanthrene-3,4-dicarbo nitrile, compound **21**, (2.00 g, 5.50 mmol), ethyl 6-bromohexanoate (1.64 g, 7.35 mmol), potassium carbonate (K_2CO_3) (1.51 g, 10.95 mmol) and 180 mL of DMF in a 250 mL round-bottom flask were stirred and heated at 80 °C under argon atmosphere for 7 h. After cooling to room temperature, the reaction mixture was dumped into water (1000 mL) with vigorous stirring for 1 h. The aqueous layer was extracted with ethyl acetate (300 mLx2). The organic layer was dried with Na_2SO_4 and removed to yield a crude product. The crude product was purified by normal phase column chromatography (silica gel, 20% to 50% EtOAc-Hexane) to give pure ethyl 6-((3,4-dicyano-13-hydroxy-1,2,5,6-tetrahydrodibenzo[c,g]phenanthren-8-yl)oxy)hexanoate, compound **23**, as yellow solid (1.68 g, 57% yield). This compound was used for the next step.

Step b)



23 7

Ethyl 6-((3,4-dicyano-13-hydroxy-1,2,5,6-tetrahydrodibenzo[c,g] phenanthren-8-yl)oxy) hexanoate, compound **23**, (0.25 g, 0.49 mmole) was dissolved in CH_2Cl_2 (7 mL) and stirred at -80 to -60°C under argon for 15 min. Then a solution of 1M DIBAL-H in THF (4 mL) was added. The reaction was controlled temperature at -80 to -60°C under argon for 1.5 hours. The reaction was added dropwise methanol (10 mL) and water (15 mL) and continued to stir under argon for 30 min. The aqueous layer was extracted with dichloromethane (100 mLx2). The organic layer was dried with anhydrous Na_2SO_4 and removed under reduced pressure to yield crude product. The crude was purified by column chromatography (30% EtOAc-Hexane to 100% EtOAc) to give 8-hydroxy-13-((6-oxohexyl)oxy)-1,2,5,6-tetrahydrodibenzo[c,g]phenanthrene-3,4-dicarbo nitrile or compound **7** as yellow solid (0.057 g, 25% yield).

^{1}H NMR (500 MHz, $CDCl_3$): δ 9.77 (s, 1H), 7.10 (dd, J = 13.0, 8.5 Hz, 2H), 6.76 (dd, J = 13.0, 2.0 Hz, 2H), 6.45 (ddd, J = 13.0, 8.5, 2.0 Hz, 2H), 5.14 (s, 1H), 4.10-3.90 (m, 2H), 3.32 (d, J = 14.7 Hz, 2H), 2.95-2.80 (br s, 4H), 2.70-2.55 (m, 2H), 2.48 (t, J = 7.5 Hz, 2H), 1.85-1.75 (m, 2H), 1.75-1.62 (m, 2H), 1.55-1.45 (m, 2H) ppm.

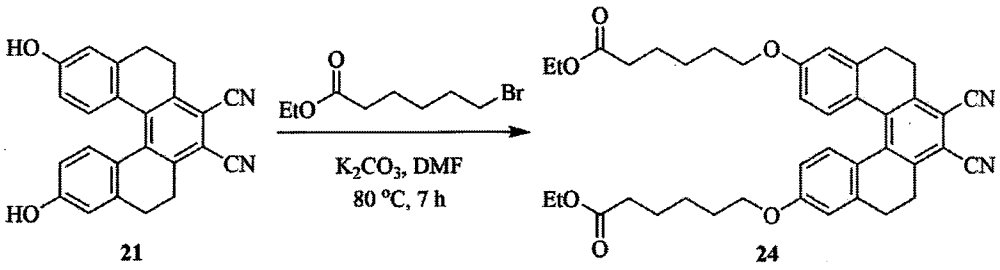
^{13}C NMR (125 MHz, $CDCl_3$): δ 202.52, 159.56, 156.23, 143.57, 143.53, 140.97, 140.65, 137.19, 137.02, 131.38, 131.15, 125.52, 125.19, 14.39, 113.51, 113.24, 112.56, 67.59, 43.77, 28.96, 28.81, 28.60, 28.47, 28.42, 25.67, 21.74 ppm.

FT-IR (KBr): ν_{max} 3368, 2943, 2861, 2723, 2223, 1713, 1605, 1577, 1545, 1498, 1273, 1242, 1091, 1019, 819, 609 cm^{-1}

Example 8

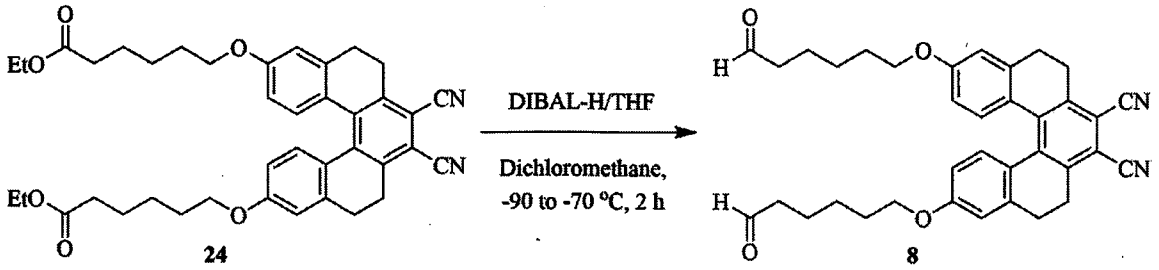
The synthesis of 8,13-bis((6-oxohexyl)oxy)-1,2,5,6-tetrahydrodibenzo[c,g]phenanthrene-3,4-dicarbonitrile or compound **8**

Step a)



A mixture of 8,13-dihydroxy-1,2,5,6-tetrahydrodibenzo[c,g]phenanthrene-3,4-dicarbo nitrile, compound **21**, (0.50 g, 1.37 mmol), ethyl-4-bromobutyrate (0.67 g, 3.00 mmol), potassium carbonate (K_2CO_3) (0.57 g, 4.13 mmol) and 15 mL of DMF were stirred and heated at 80°C under argon atmosphere for 7 h. After cooling to room temperature, the reaction mixture was dumped into water (600 mL) with vigorous stirring for 1 h. The aqueous layer was extracted with ethyl acetate (200 mLx2). The organic layer was dried with Na_2SO_4 and removed to yield a crude product. The crude product was purified by normal phase column chromatography (silica gel, 50% EtOAc-Hexane) to give pure diethyl 6,6'-((3,4-dicyano-1,2,5,6-tetrahydrodibenzo [c,g]phenanthrene-8,13-diyl)bis(oxy))dihexanoate, compound **24** as yellow solid (0.82 g, 92% yield).

Step b)



Diethyl 6,6'-((3,4-dicyano-1,2,5,6-tetrahydrodibenzo[c,g] phenanthrene-8,13-diyl)bis (oxy))dihexanoate or compound **24** (0.50 g, 0.77 mmole) was dissolved in dichloromethane, (CH_2Cl_2) (10 mL) and stirred at -90 to -70°C under argon atmosphere for 15 min. Then 1M of diisobutylaluminum hydride, DIBAL-H, solution in THF (8 mL) was added dropwise into the reaction mixture. The reaction was stirred at -90 to -70°C under argon atmosphere for 2 hours. The reaction was quenched by adding dropwise methanol (10 mL) and water (20 mL) at -90 to -70°C and continued stirring for 30 min. The aqueous layer was extracted with CH_2Cl_2 (50 mLx2). The organic layer was dried with anhydrous Na_2SO_4. Then the solvent was removed to yield the crude product. The crude was purified by column chromatography (SiO_2, 30% EtOAc-Hexane) to give 8,13-bis((6-oxohexyl)oxy)-1,2,5,6-tetrahydrodibenzo[c,g] phenanthrene-3,4-dicarbo nitrile or compound **8** as yellow solid (0.33 g, 76% yield, mp. 135-139 °C).

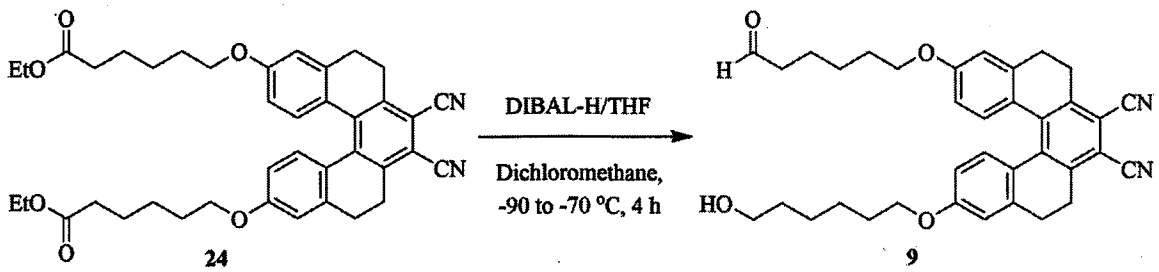
^{1}H NMR (500 MHz $CDCl_3$): δ 9.77 (s, 2H), 7.11 (d, J=9.0 Hz, 2H), 6.77 (d, J=1.5 Hz, 2H), 6.47 (dd, J=2.5, 9.0 Hz, 2H), 3.94 (s, 4H), 3.32 (d, J=15.0 Hz, 2H), 2.64 (s, 4H), 2.47 (t, d=7.5 Hz, 2H), 1.90-1.75 (m, 4H), 1.75-1.60 (ın, 4H), 1.55-1.45 (m, 4H)ppm.

^{13}C NMR (125 MHz, $CDCl_3$): δ 159.52, 143.52, 140.59, 137.10, 131.10, 125.21, 115.57, 113.11, 112.57, 111.40, 67.56, 43.76, 28.97, 28.81, 28.46, 25.66, 21.73 ppm.

FT-IR (KBr): ν_{max} 2942, 2908, 2856, 2722, 2221, 1722, 1606, 1541, 1502, 1274, 1244, 1109, 854, 820, 808 cm^{-1}.

Example 9

The synthesis of 8-((6-hydroxyhexyl)oxy)-13-((6-oxohexyl)oxy)-1,2,5,6-tetrahydro dibenzo[c,g]phenanthrene-3,4-dicarbonitrile or compound **9**



Diethyl 6,6'-((3,4-dicyano-1,2,5,6-tetrahydrodibenzo[c,g] phenanthrene-8,13-diyl)bis (oxy))dihexanoate or compound **24** (1.00 g, 1.54 mmole) was dissolved in dichloromethane, (CH_2Cl_2) (20 mL) and stirred at -90 to -70°C under argon atmosphere for 15 min. Then 1M of diisobutylaluminum hydride, DIBAL-H, solution in THF (15 mL) was added dropwise into the reaction mixture. The reaction was stirred at -90 to -70°C under argon atmosphere for 4 hours. The reaction was quenched by adding dropwise methanol (30 mL) and water (30 mL) at -90 to -70°C and continued stirring for 30 min. The aqueous layer was extracted with CH_2Cl_2 (200 mLx2). The organic layer was dried with anhydrous Na_2SO_4. Then the solvent was removed to yield the crude product. The crude was purified by column chromatography (SiO_2, 30% EtOAc-Hexane to 100% EtOAc) to give 8-((6-hydroxyhexyl)oxy)-13-((6-oxohexyl)oxy)-1,2,5,6-tetrahydrodibenzo[c,g]phenanthrene-3,4-dicarbonitrile or compound **9** as yellow solid (0.45 g, 47% yield).

^{1}H NMR (500 MHz, $CDCl_3$): δ 9.80 (s, 1H), 7.12 (dd, J=2.0, 8.5 Hz, 2H), 6.90 (s, 2H), 6.50 (dd, J=3.5, 8.5 Hz, 2H), 3.97 (s, 2H), 3.67 (t, J=6.5 Hz, 2H), 3.33 (d, J=14.0 Hz, 2H), 3.00-2.78 (m, 4H), 2.72-2.53 (m, 2H), 2.50 (t, J=6.5 Hz, 2H), 1.95-1.78 (m, 4H), 1.71 (quin, J=7.5 Hz, 2H), 1.62 (quin, J=7.0 Hz, 2H), 1.60-1.38 (m, 6H) ppm.

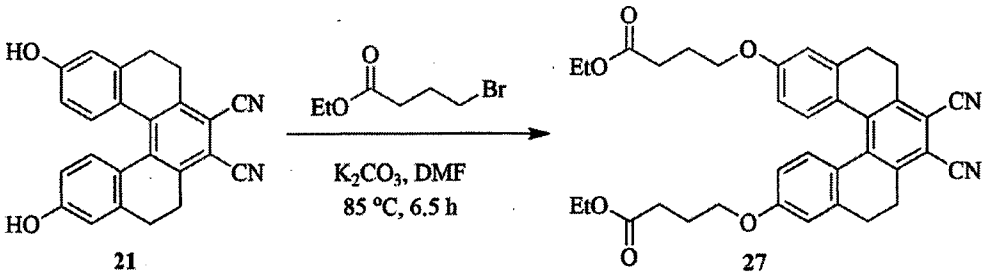
^{13}C NMR (125 MHz, $CDCl_3$): δ. 202.48, 159.61, 159.50, 143.51(2), 140.56(2), 137.13, 137.08, 131.08(2), 125.21, 125.11, 115.56(2), 113.11(2), 112.56, 111.32(2), 67.84, 67.55, 62.79, 43.73, 32.56, 29.13, 28.94, 28.79(2C), 28.45(2), 25.83(2), 25.49, 21.71 ppm.

FT-IR (KBr): ν_{max} 3390, 2937, 2858, 2222, 1717, 1606, 1542, 1500, 1387, 1272, 1243, 1011, 853, 611 cm^{-1}

Example 10

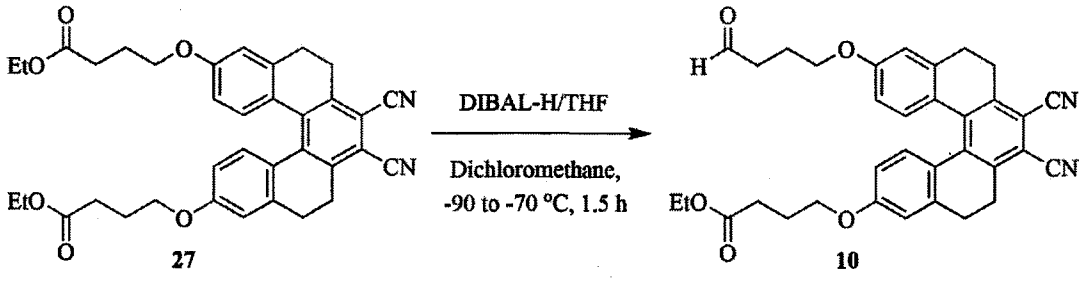
The synthesis of ethyl 4-((3,4-dicyano-13-(4-oxobutoxy)-1,2,5,6-tetrahydrodibenzo [c,g]phenanthren-8-yl)oxy)butanoate or compound **10**

Step a)



A mixture of 8,13-dihydroxy-1,2,5,6-tetrahydrodibenzo[c,g] phenanthrene-3,4-dicarbo nitrile or compound **21** (1.00 g, 2.75 mmol), ethyl-4-bromobutanoate (2.14 g, 10.99 mmol), potassium carbonate (K_2CO_3) (1.14 g, 8.25 mmol) and 35 mL of DMF were stirred and heated at 85°C under argon atmosphere for 6.5 hours. The reaction was cooled to room temperature and dumped into 800 mL of water. The aqueous layer was extracted with EtOAc (250 mLx2). The organic layer was dried with anhydrous Na_2SO_4 and the solvent was removed to yield the crude product. The crude was purified by column chromatography (SiO_2, 40% EtOAc-Hexane) to give diethyl 4,4'-((3,4-dicyano-1,2,5,6-tetrahydrodibenzo[c,g] phenanthrene-8,13-diyl)bis(oxy)) dibutyrate or compound **27** as yellow solid (1.13, 65% yield).

Step b)



diethyl 4,4'-((3,4-dicyano-1,2,5,6-tetrahydrodibenzo[c,g]phenanthrene-8,13-diyl)bis(oxy)) dibutyrate or compound **27** (0.60 g, 1.04 mmol) was dissolved in CH_2Cl_2 (25 mL) and stirred at -90 to -70°C under argon atmosphere for 15 min. 1M DIBAL-H in THF (9 mL) was added to the

reaction solution. The reaction was stirred at -90 to -70 °C under argon atmosphere for 1.5 hours. The reaction was quenched by adding dropwise of MeOH (10 mL) and water (15 mL) at -90 to -70 °C under argon for 30 min. The aqueous layer was extracted with CH_2Cl_2 (100 mLx2). The organic layer was dried with anhydrous Na_2SO_4 and the solvent was removed to yield the crude product. The crude was purified by column chromatography (SiO_2, 30% to 50% EtOAc-Hexane) to give ethyl 4-((3,4-dicyano-13-(4-oxobutoxy)-1,2,5,6-tetrahydrodibenzo[c,g] phenanthren-8-yl)oxy)butanoate or compound **10** as yellow solid (0.16 g, 29% yield).

^{1}H NMR (500 MHz, $CDCl_3$): δ 9.83 (s, 1H), 7.10 (dd, J=3.5, 8.5 Hz, 2H), 6.78 (s, 2H), 6.47 (t, J=3.5 Hz, 2H), 4.13 (q, J=7.0 Hz, 2H), 4.00 (s, 4H), 3.32 (d, J=14.5 Hz, 2H), 2.92-2.78 (m, 4H), 2.66 (t, J=7.0 Hz, 2H), 2.64-2.52 (br s, 2H), 2.50 (t, J=7.0 Hz, 2H), 2.20-2.05 (m, 4H), 1.25 (t, J=7.0 Hz, 3H) ppm.

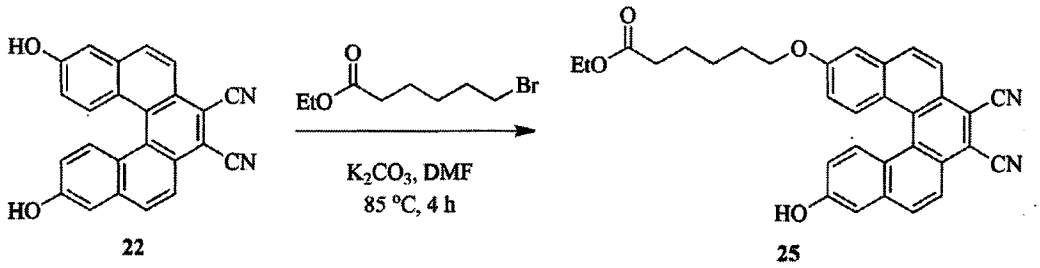
^{13}C NMR (125 MHz, $CDCl_3$): δ 173.13, 159.37, 159.19, 143.56(2), 140.66(2), 137.13, 137.05, 131.14(2), 125.49, 125.32, 115.55(2), 113.22, 113.17, 112.65, 112.53, 111.52, 111.49, 66.76, 66.68, 60.51, 40.52, 30.72, 28.82(2), 28.47(2), 24.56, 21.93, 14.23 ppm.

FT-IR (KBr): ν_{max} 3456, 2943, 2909, 2222, 1729, 1606, 1541, 1502, 1272, 1245, 1177, 1032, 856, 611 cm^{-1}.

Example 11

The synthesis of 8-hydroxy-13-((6-oxohexyl)oxy) dibenzo[c,g]phenanthrene-3,4-dicarbonitrile or compound **11**

Step a.)

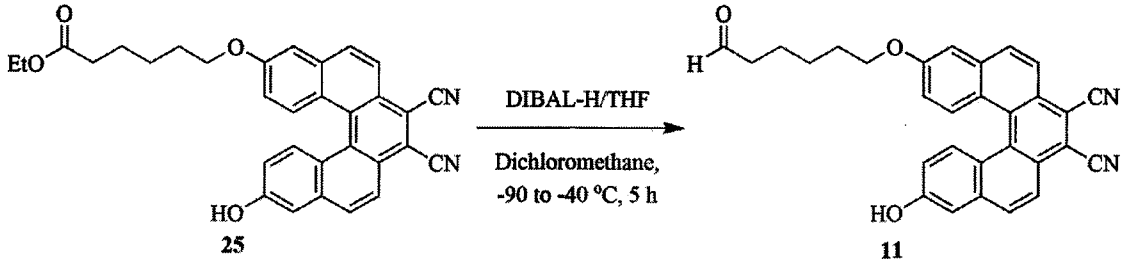


22 25

A mixture of 8,13-dihydroxy-dibenzo[c,g]phenanthrene-3,4-dicarbonitrile, compound **22**, (1.16 g, 3.23 mmol), ethyl-6-bromohexanolate (0.73 g, 3.29 mmol), potassium carbonate (K_2CO_3) (0.68 g, 4.94 mmol) and 90 mL of DMF was stirred and heated at 85°C under argon atmosphere for 4 h. After cooling to room temperature, the reaction mixture was poured into 800 ml of water with vigorous stirring for 1 h. The aqueous layer was extracted with ethyl acetate. The organic layer was dried with anhydrous Na_2SO_4 and the solvent was removed to yield the crude product. The crude was purified by column chromatography (SiO_2, 25% to 50% EtOAc-

Hexane) to give ethyl 6-((3,4-dicyano-13-hydroxydibenzo[c,g]phenanthren-8-yl)oxy)hexanoate or compound **25**, (0.64 g, 40% yield) as a brown solid.

Step b)



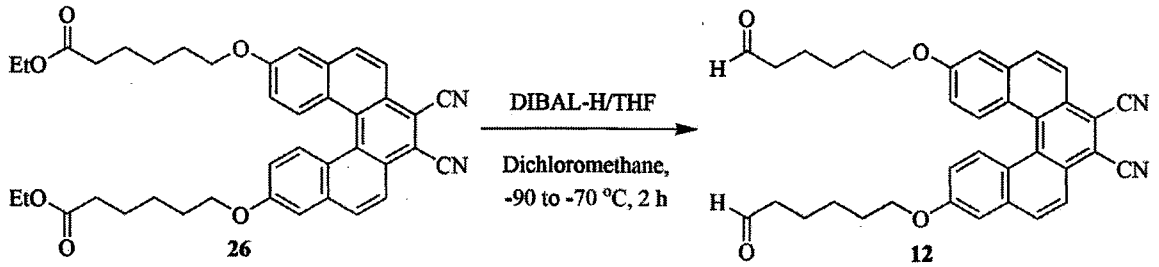
Ethyl 6-((3,4-dicyano-13-hydroxydibenzo[c,g] phenanthren-8-yl)oxy) hexanoate, compound **25**, (0.30 g, 0.60 mmole) was dissolved in CH_2Cl_2 (5 mL) and stirred at -90 to -40 °C under argon for 15 min. Then a solution of 1M DIBAL-H in THF (5 mL) was added dropwise. The reaction was controlled temperature at -90 to -40°C under argon for 5 hours. The reaction was added dropwise methanol (10 mL) and water (15 mL) and continued to stir under argon for 30 min. The aqueous layer was extracted with dichloromethane (50 mLx2). The organic layer was dried with anhydrous Na_2SO_4 and removed under reduced pressure to yield crude product. The crude was purified by column chromatography (30% EtOAc-Hexane to 100% EtOAc) to give 8-hydroxy-13-((6-oxohexyl)oxy) dibenzo[c,g]phenanthrene-3,4-dicarbonitrile or compound **11** as yellow solid (0.09 g, 36% yield).

^{1}H NMR (500 MHz, $CDCl_3$): δ 9.80 (s, 1H), 8.32-8.17 (m, 3H), 7.98 (dd, J=19.5, 9.0 Hz, 2H), 7.40-7.21 (m, 2H), 7.19-7.11 (m, 1H), 6.91 (t, J=11.0 Hz, 2H), 4.12 (dt, J=9.0, 6.5 Hz, 2H), 2.51 (t, J=7.5 Hz, 2H), 1.90 (quin, J=7.5 Hz, 2H), 1.76 (quin, J=7.0 Hz, 2H), 1.56 (quin, J=7.0 Hz, 2H) ppm.

FT-IR (KBr): ν_{max} 3366, 2927, 2860, 2741, 2223, 1713, 1612, 1565, 1491, 1446, 1354, 1277, 1235, 1179, 1013, 850, 828, 669, 529 cm^{-1}.

Example 12

The synthesis of 8,13-bis((6-oxohexyl)oxy)dibenzo[c,g]phenanthrene-3,4-dicarbonitrile or compound **12**



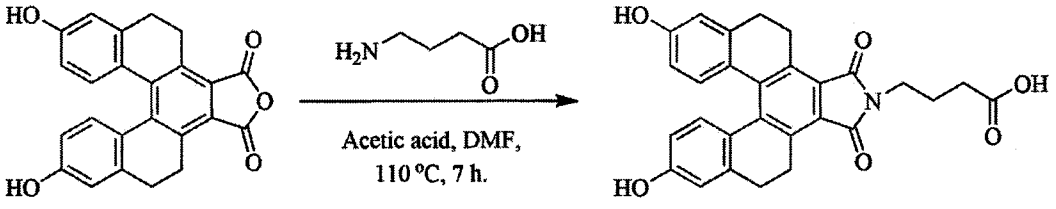
Diethyl 6,6'-((3,4-dicyano-dibenzo[c,g] phenanthrene-8,13-diyl)bis(oxy)) dihexanoate, compound **26**, (0.25 g, 0.39 mmole) was dissolved in CH_2Cl_2 (7 mL) and stirred at -90 to -70 °C under argon for 10 min. Then a solution of 1M DIBAL-H in THF (5 mL) was added dropwise. The reaction was controlled temperature at -90 to -70°C under argon for 2 hours. The reaction was added dropwise methanol (10 mL) and water (10 mL) and continued to stir under argon for 30 min. The aqueous layer was extracted with dichloromethane (50 mLx2). The organic layer was dried with anhydrous Na_2SO_4 and removed under reduced pressure to yield crude product. The crude was purified by column chromatography (30% EtOAc-Hexane) to give 8,13-bis((6-oxohexyl)oxy)dibenzo[c,g]phenanthrene-3,4-dicarbonitrile or compound **12** as yellow solid (0.06 g, 26% yield).

^{1}H NMR (500 MHz, $CDCl_3$): δ 9.70 (s, 2H), 7.25-7.10 (m, 3H), 6.75 (d, J=2.0 Hz, 2H), 6.65-6.50 (m, 1H), 6.52 (dd, J= 9.0, 2.0 Hz, 2H), 6.44 (t, J=10.5 Hz, 2H), 3.96 (s, 4H), 2.49 (t, d=7.5 Hz, 4H), 1.90-1.70 (m, 4H), 1.75-1.65 (m, 4H), 1.55-1.40 (m, 4H) ppm.

Example 13

The synthesis of sodium 3,3'-((2-(3-carboxypropyl)-1,3-dioxo-4,5,14,15-tetrahydro-1H-dinaphtho[2,1-e:1',2'-g]isoindole-7,12-diyl)bis(oxy))bis(propane-1-sulfonate) or compound **13**

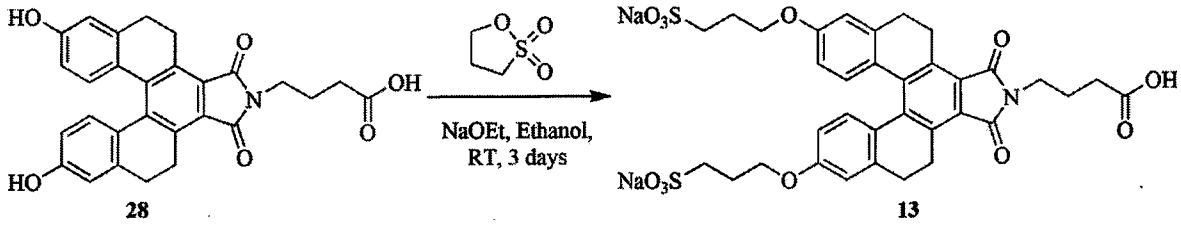
Step a)



19 **28**

A mixture of 7,12-dihydroxy-4,5,14,15-tetrahydronaphtho[2',1':3,4] phenanthro[1,2-c] furan-1,3-dione, compound **19**, (1.00 g, 2.6 mmol), γ-aminobutyric acid (0.04 g, 3.9 mmol), 4 mL of acetic acid and 30 mL of DMF was stirred under argon atmosphere and heated at 110 °C for 7 h. After cooling to room temperature, the reaction mixture was dumped into water (500 mL) with vigorous stirring for 1 h. The yellow solid was collected by vacuum filtration, washed with 100 mL of water, and 50 mL of CH_2Cl_2-Hexane (1:1) mixture to give pure product of 4-(7,12-dihydroxy-1,3-dioxo-1,3,4,5,14,15-hexahydro-2H-dinaphtho[2,1-e:1',2'-g]isoindol-2-yl) butanoic acid or compound **28** as a yellow solid (1.17 g, 96% yield).

Step b)



28 13

A mixture of 4-(7,12-dihydroxy-1,3-dioxo-1,3,4,5,14,15-hexahydro-2H-dinaphtho[2,1-e:1',2'-g]isoindol-2-yl)butanoic acid or compound **28** (0.20 g, 0.43 mmol) in dry ethanol (10 mL) was stirred until all solids disappeared under argon atmosphere. A mixture of sodium ethoxide (0.09 g, 1.28 mmol) in 5 mL of dry ethanol was added dropwise and stirred for 1 h, causing the yellow solution turn to orange-brown. Then, 1,3 propanesultone (0.11 g, 0.94 mmol) in 5 mL of dry ethanol was added to the mixture. The reaction mixture was stirred for 3 days. The reaction was followed by using TLC and precipitation of orange solid. The crude solid was collected by vacuum filtration and washed with dichloromethane. The crude product was dried in vacuum. The crude product was purified by column chromatography using reversed phased silica gel (1:1, MeOH:H_2O) to give pure product of 3,3'-((2-(3-carboxypropyl)-1,3-dioxo-4,5,14,15-tetrahydro-1H-dinaphtho[2,1-e:1',2'-g]isoindole-7,12-diyl)bis(oxy))bis(propane-1-sulfonate) or compound **13** as yellow-orange solid (0.07 g, 21%yield).

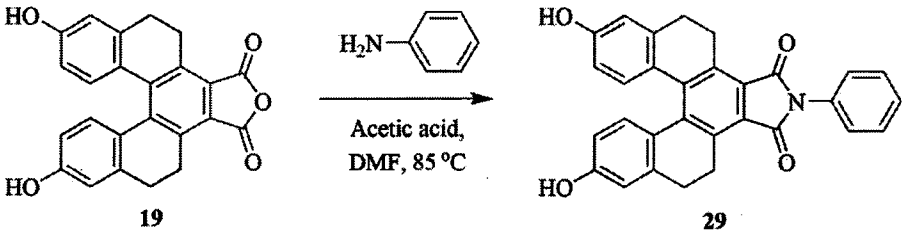
^{1}H NMR (500 MHz, DMSO-d_6): δ 7.01 (d, 2H), 6.89 (s, 2H), 6.54 (d, 2H), 4.02 (s, 4H), 3.90 (d, 2H), 2.82 (d, 4H), 2.53 (s, 6H), 2.33 (d, 2H), 1.96 (s, 4H), 1.86 (s, 2H), 1.69 (d, 2H), 1.19 (s, 1H) ppm.

FT-IR (KBr): 3448, 1754, 1695, 1597, 1573, 1392, 1181, 1112, 1046, 795, 615, 530 cm^{-1}.

Example 14

The synthesis of sodium 3-((1,3-dioxo-12-((6-oxohexyl)oxy)-2-phenyl-4,5,14,15-tetrahydro-1H-dinaphtho[2,1-e:1',2'-g]isoindol-7-yl)oxy)propane-1-sulfonate or compound **14**

Step a.)

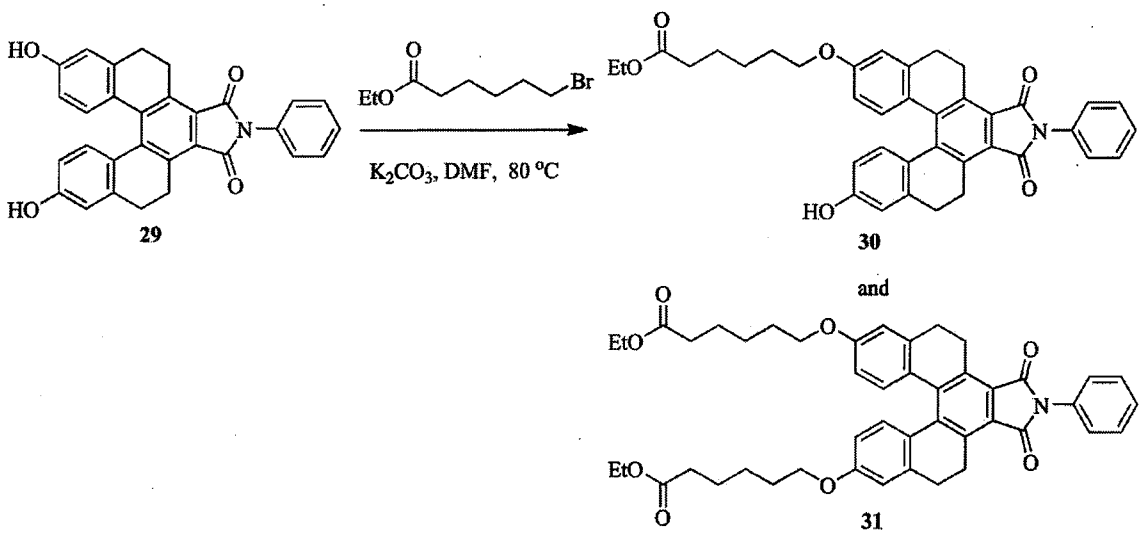


19 29

A mixture of 7,12-dihydroxy-4,5,14,15-tetrahydronaphtho[2',1':3,4] phenanthro[1,2-c] furan-1,3-dione, compound **19**, (3.00 g, 7.81 mmol), aniline (1.09 g, 11.71 mmol), 10 mL of acetic acid and 60 mL of DMF were stirred and heated at 85°C under argon atmosphere for 5 hours. After cooling to room temperature, the reaction mixture was dumped into water (800 mL)

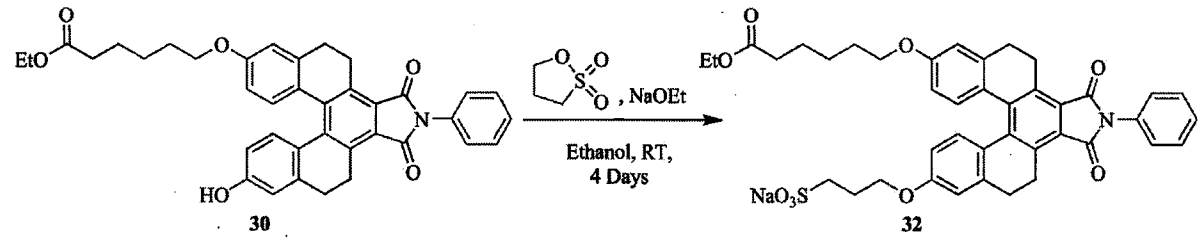
with vigorous stirring for 1 hour. The yellow solids were collected by vacuum filtration, washed with 500 mL of water, and 100 mL of mixed solvent CH_2Cl_2-Hexane (1:1) to give pure compound **29** as a yellow solid (3.19 g, 89% yield).

Step b)



A mixture of compound **29** (3.12 g, 6.80 mmol), ethyl-6-bromohexanoate (1.01 g., 4.53 mmol), potassium carbonate (K_2CO_3) (0.94 g, 6.80 mmol) and 140 mL of DMF were stirred and heated at 80 °C under argon atmosphere for 5 hours. The reaction was cooled to room temperature and dumbed into 800 mL of water. The aqueous layer was extracted with EtOAc (200 mLx2). The organic layer was dried with anhydrous Na_2SO_4 and removed to yield crude product. The crude was purified by column chromatography (SiO_2, 25% to 80% EtOAc-Hexane) to give compound **30** (1.49 g, 55% yield) and compound **31** (0.40 g, 24% yield) as yellow-orange solid.

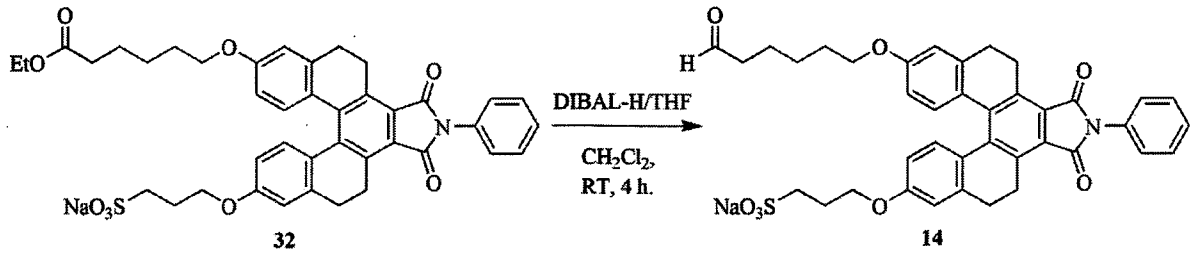
Step c.)



A mixture of compound **30** (1.11 g, 0.27 mmol) in dry ethanol (100 mL) was stirred until all solids disappeared under argon. A mixture of sodium ethoxide, NaOEt, (0.14 g, 2.03 mmol) in 5 mL of dry ethanol was added dropwise and stirred for 1 h, causing the orange solution to turn orange-brown. Then, 1,3 propanesultone (0.14 g, 2.03 mmol) in 5 mL of dry ethanol was added to the mixture. The reaction was stirred for 4 days (followed by TLC) until an orange product was precipitated out from the solution. Ethanol was removed under reduced pressure.

The crude product was purified by chromatography (revered-phase SiO_2, 25% to 50% EtOH:H_2O) to give compound **32** (1.03 g, 75% yield) as yellow solid.

Step d)



The compound **32** (0.15 g, 0.20 mmol) was dissolved in CH_2Cl_2 (6 mL) and stirred at room temperature under argon atmosphere for 15 minutes. Then 1M DIBAL in THF (0.5 mL) was added dropwise. The reaction was stirred at room temperature under argon atmosphere for 4 hours. The reaction was added methanol (6 mL) and water (10 mL) and stirred under argon atmosphere for 30 minutes. The solvent, then, was removed under reduced pressure. The crude product was purified by Revered-phase column chromatography (SiO_2, EtOH:H_2O, 1:1) to give sodium 3-((1,3-dioxo-12-((6-oxohexyl)oxy)-2-phenyl-4,5,14,15-tetrahydro-1H-dinaphtho[2,1-e:1',2'-g]isoindol-7-yl)oxy)propane-1-sulfonate or compound **14** as a yellow solid (71.00 mg, 50% yield).

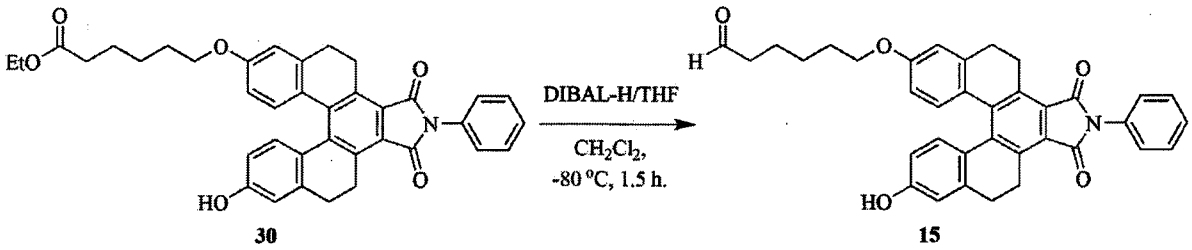
^{1}H NMR (500 MHz, DMSO-d_6): δ 7.49 (t, J=7.5, 2H), 7.39 (d, J=10.5, 3H), 7.05 (d, J=6.5, 2H), 6.91 (s, 2H), 6.55 (t, J=10.5, 2H), 4.12-4.00 (m, 2H), 4.00-3.88 (m, 4H), 2.98-2.70 (m, 4H), 2.52 (t, J=7.5, 2H), 2.45-2.30 (m, 2H), 1.96 (quin, J=7.0, 2H), 1.67 (quin, J=7.0, 2H), 1.48-1.28 (m, 6H) ppm.

^{13}C NMR (125 MHz, MeOD-d_4): δ 167.29, 158.59, 140.82, 137.59, 137.45, 132.12, 130.74, 130.68, 128.77, 127.93, 127.67, 125.69, 124.75, 113.02, 112.95, 112.49, 112.38, 67.37, 66.63, 60.62, 47.81, 32.46, 28.76, 28.12, 25.42, 25.32, 25.26, 23.80 ppm.

FT-IR (KBr): ν_{max} 3433, 2933, 2855, 1703, 1602, 1501, 1373, 1266, 1207, 1177, 1106, 1043, 761, 624 cm^{-1}.

Example 15

The synthesis of 6-((12-hydroxy-1,3-dioxo-2-phenyl-4,5,14,15-hexahydro-1H-dinaphtho [2,1-e:1',2'-g]isoindol-7-yl)oxy)hexanal or compound **15**



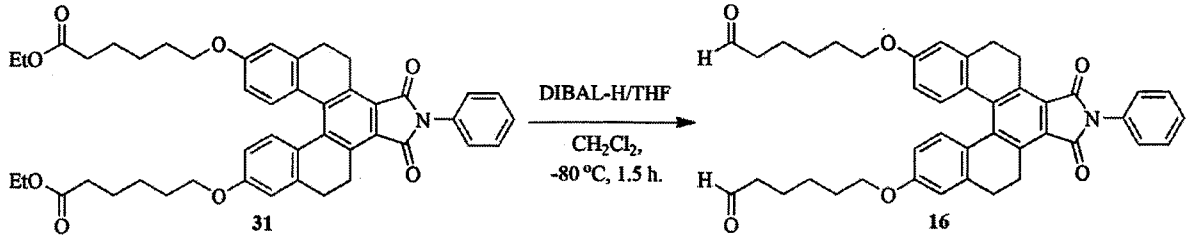
30 15

The compound **30** (0.07 g, 0.16 mmol) was dissolved in CH_2Cl_2 (6 mL) and stirred at -80 °C under argon atmosphere for 10 minutes. Then 1M DIBAL in THF (1.0 mL) was added dropwise. The reaction was stirred at -80 °C under argon atmosphere for 1.5 hours. The reaction was added methanol (20 mL) and water (10 mL) and stirred under argon atmosphere for 30 minutes. The solvent, then, was removed under reduced pressure. The crude product was purified by column chromatography (SiO_2, 20% to 50% EtOAc:Hexane) to give 6-((12-hydroxy-1,3-dioxo-2-phenyl-4,5,14,15-hexahydro-1H-dinaphtho[2,1-e:1',2'-g]isoindol-7-yl)oxy)hexanal or compound **15** as a yellowish brown solid (27 mg, 43% yield).

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.56-7.45 (m, 2H), 7.43-7.31 (m, 3H), 7.13 (dd, J=18.0, 7.5 Hz, 2H), 6.78 (d, J=18.0 Hz, 2H), 6.46 (dd, J=18.0, 7.5 Hz, 2H), 5.18 (s, 1H), 4.12-4.00 (m, 2H), 3.96 (s, 2H), 2.98-2.70 (br s, 4H), 2.60-2.40 (m, 2H), 1.71 (quin, J=7.0 Hz, 2H), 1.50 (quin, J=7.0 Hz, 2H), 1.23(quin, J=7.0 Hz, 2H) ppm.

Example 16

The synthesis of 6,6'-((1,3-dioxo-2-phenyl-2,3,4,5,14,15-hexahydro-1H-dinaphtho[2,1-e:1',2'-g]isoindole-7,12-diyl)bis(oxy))dihexanal or compound **16**



31 16

The compound **31** (0.23 g, 0.31 mmol) was dissolved in CH_2Cl_2 (10 mL) and stirred at -80 °C under argon atmosphere for 10 minutes. Then 1M DIBAL in THF (6.0 mL) was added dropwise. The reaction was stirred at -80 °C under argon atmosphere for 1.5 hours. The reaction was added methanol (20 mL) and water (10 mL) and stirred under argon atmosphere for 30 minutes. The solvent, then, was removed under reduced pressure. The crude product was purified by column chromatography (SiO_2, 20% to 50% EtOAc:Hexane) to give 6,6'-((1,3-dioxo-2-phenyl-2,3,4,5,14,15-hexahydro-1H-dinaphtho[2,1-e:1',2'-g] isoindole-7,12-diyl)bis(oxy))dihexanal or compound **16** as a yellowish brown solid (0.15g, 72% yield).

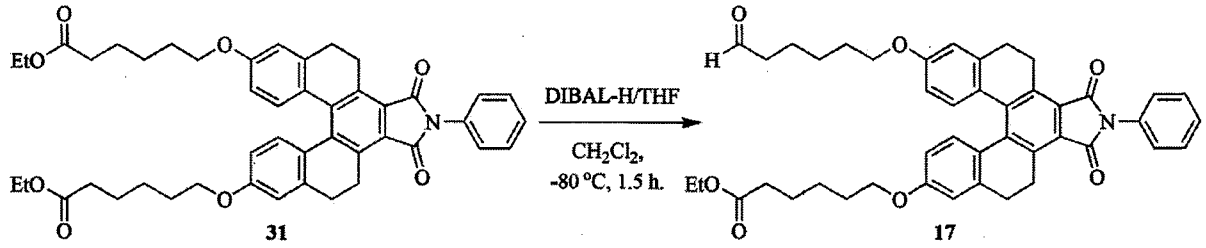
^{1}H NMR (500 MHz, $CDCl_3$): δ 9.78 (s, 2H), 7.50 (t, J=7.5 Hz, 2H), 7.45-7.32 (m, 3H), 7.15 (d, J=8.5 Hz, 2H), 6.80 (s, 2H), 6.48 (dd, J=8.5, 2.0 Hz, 2H), 4.20-4.05 (br s, 2H), 3.96 (s, 4H), 2.85 (s, 4H), 2.60-2.50 (br s, 2H), 2.47 (t, J=6.0 Hz, 4H), 1.80 (t, J=1.5 Hz, 4H), 1.71 (quin, J=7.5 Hz, 2H), 1.51 (quin, J=7.5 Hz, 2H), 1.24 (quin, J=7.5 Hz, 2H) ppm.

^{13}C NMR (125 MHz, $CDCl_3$): δ 202.45, 167.99, 158.91, 140.99, 138.41, 132.02, 131.31, 128.96, 127.83, 126.88, 126.44, 124.89, 112.99, 112.28, 67.48, 43.79, 29.04, 25.71, 24.25, 21.78 ppm.

FT-IR (KBr): ν_{max} 2942, 2842, 2867, 2717, 1761, 1704, 1603, 1501, 1466, 1378, 1264, 1261, 1177, 1103, 1027, 832, 696, 625 cm^{-1}.

Example 17

The synthesis of ethyl 6-((1,3-dioxo-12-((6-oxohexyl)oxy)-2-phenyl-4,5,14,15-tetrahydro-1H-dinaphtho[2,1-e:1',2'-g]isoindol-7-yl)oxy)hexanoate or compound **17**



31 17

The compound **31** (0.17 g, 0.23 mmol) was dissolved in CH_2Cl_2 (10 mL) and stirred at -80 °C under argon atmosphere for 10 minutes. Then 1M DIBAL in THF (3.0 mL) was added dropwise. The reaction was stirred at -80 °C under argon atmosphere for 1.5 hours. The reaction was added methanol (20 mL) and water (10 mL) and stirred under argon atmosphere for 30 minutes. The solvent, then, was removed under reduced pressure. The crude product was purified by column chromatography (SiO_2, 20% to 50% EtOAc:Hexane) to give ethyl 6-((1,3-dioxo-12-((6-oxohexyl)oxy)-2-phenyl-4,5,14,15-tetraahydro-1H-dinaphtho[2,1-e:1',2'-g]isoindol-7-yl) oxy)hexanoate or compound **17** as a yellowish brown solid (0.06g, 35% yield).

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.53-7.44 (m, 3H), 7.42-7.35 (m, 2H), 7.15 (d, J=8.5 Hz, 2H), 6.80 (s, 2H), 6.48 (d, J=8.5 Hz, 2H), 4.11 (q, J=7.0 Hz, 2H), 3.95 (s, 4H), 2.84 (s, 4H), 2.60-2.48 (br s, 2H), 2.32 (t, J=7.5 Hz, 4H), 1.79 (quin, J=7.5 Hz, 4H), 1.70 (quin, J=7.5 Hz, 4H), 1.61 (s, 2H), 1.51 (quin, J=7.5 Hz, 4H), 1.24 (quin, J=7.0 Hz, 2H) ppm.

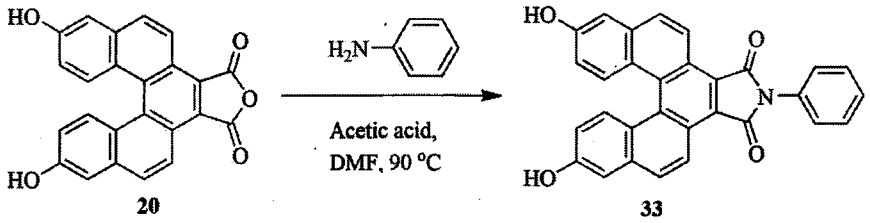
^{13}C NMR (125 MHz, $CDCl_3$): δ 202.47, 168.01, 158.96, 158.91, 140.98, 138.42, 132.03, 130.07, 126.45, 126.39, 124.87, 122.21, 113.00, 112.31, 67.57, 67.49, 60.28, 43.80, 34.24, 29.05, 28.95, 25.72, 25.66, 24.47, 24.26, 21.79, 14.25 ppm.

FT-IR (KBr): ν_{max} 2940, 2866, 1763, 1733, 1704, 1603, 1591, 1500, 1466, 1382, 1276, 1264, 1240, 1178, 1103, 1029, 845, 826, 625 cm^{-1}.

Example 18

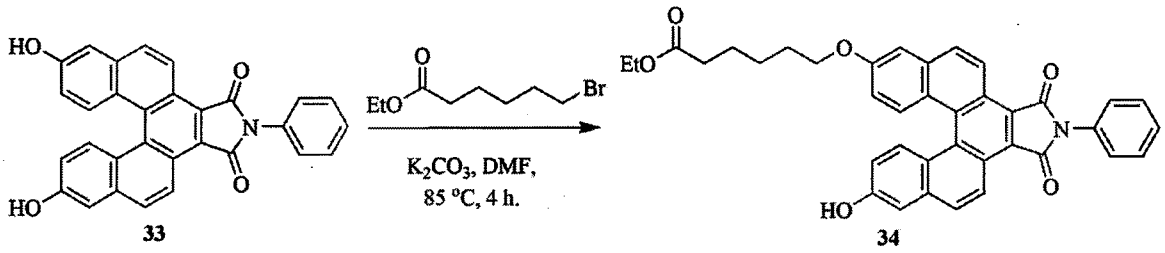
The synthesis of 6-((12-hydroxy-1,3-dioxo-2-phenyl-1H-dinaphtho[2,1-e:1',2'-g]isoindol-7-yl)oxy)hexanal or compound **18**

Step a)



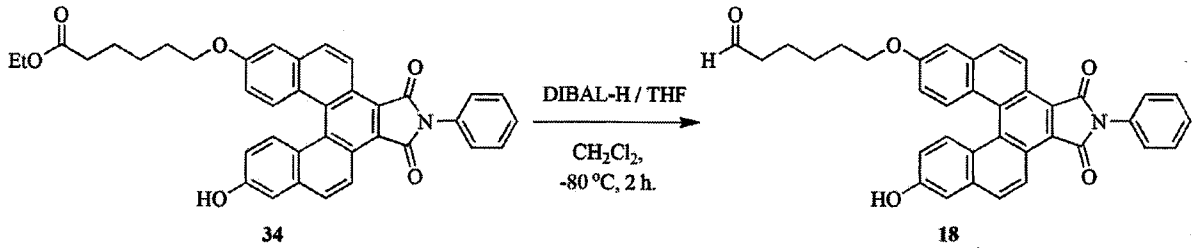
A mixture of 7,12-dihydroxytetrahydronaphtho[2',1':3,4] phenanthro[1,2-c]furan-1,3-dione, compound **20**, (1.00 g, 2.66 mmol), aniline (0.50 g, 5.32 mmol), 3 mL of acetic acid and 30 mL of DMF was stirred and heated at 90 °C under argon atmosphere for 8 hours 8. After cooling to room temperature, the reaction mixture was dumped into water (500 mL) with vigorous stirring for 1 hour. The yellow solids were collected by vacuum filtration, washed with 250 mL of water, and 100 mL of mixed solvent CH_2Cl_2-Hexane (1:1) to give pure compound **33** as a yellow solid (1.15 g, 96% yield) which will be used for the next step.

Step b)



A mixture of compound **33** (0.42 g, 0.92 mmol), ethyl-6-bromohexanoate (0.23 g., 1.05 mmol), potassium carbonate (K_2CO_3) (0.17 g, 1.26 mmol) and 40 mL of DMF was stirred and heated at 85 °C under argon atmosphere for 4 hours. The reaction was cooled to room temperature and dumbed into 500 mL of water. The aqueous layer was extracted with EtOAc (200 mLx2). The organic layer was dried with anhydrous Na_2SO_4 and removed to yield crude product. The crude was purified by column chromatography (SiO_2, 25% to 80% EtOAc-Hexane) to give compound **34** (0.16 g, 29% yield) as yellow-orange solid which will be used for the next step.

Step c)



34 18

The compound **34** (0.12 g, 0.20 mmol) was dissolved in CH_2Cl_2 (45 mL) and stirred at -80 °C under argon atmosphere for 15 minutes. Then 1M DIBAL in THF (2.5 mL) was added dropwise. The reaction was stirred at -80 °C under argon atmosphere for 2 hours. The reaction was added methanol (15 mL) and water (15 mL) and stirred under argon atmosphere for 30 minutes. The solvent, then, was removed under reduced pressure. The crude product was purified by column chromatography (SiO_2, 15% to 50% EtOAc:Hexane) to give 6-((12-hydroxy-1,3-dioxo-2-phenyl-1H-dinaphtho[2,1-e:1',2'-g]isoindol-7-yl)oxy)hexanal or compound **18** as an orange solid (25 mg, 23% yield).

^{1}H NMR (500 MHz, DMSO-d_6): δ 9.80 (s, 1H), 9.07 (s, 1H), 8.31 (d, J=8.0 Hz, 2H), 8.08 (s, 2H), 7.95-7.97 (m, 2H), 7.52 (s, 4H), 7.41 (s, 1H), 6.87 (d, J=8.0 Hz, 2H), 4.25 (t, J=5.5 Hz, 2H), 2.51 (t, J=7.0 Hz, 2H), 1.59 (quin, J=7.5, 2H), 1.25 (quin, J=7.5 Hz, 2H), 0.89 (quin, J=7.5 Hz, 2H) ppm.

^{13}C NMR (125 MHz, DMSO-d_6): δ 173.40, 172.75, 167.80, 166.00, 140.15, 135.19, 134.23, 131.34, 130.88, 129.48, 129.09, 128.80, 127.87, 126.89, 122.33, 116.64, 115.93, 110.57, 107.79, 67.77, 77.21, 38.90, 37.39, 31.92, 30.56, 29.71, 23.98, 22.69 ppm.

FT-IR (KBr): ν_{max} 3212, 2922, 2851, 1758, 1695, 1617, 1501, 1392, 1359, 1268, 1236, 1150, 1115, 858, 827, 742 cm^{-1}.

The use of the organic compounds in the present invention as molecular reporter by comjugating with biomolecule

After obtaining organic compounds as molecular reporters, the ability to conjugate the said compounds with biomolecules such as antibody or protein was investigated. The conjugation process depends on the binding groups on the said organic compounds. The molecular reporters in the present invention compose of two types of binding groups, i.e., carboxyl and aldehyde. The conjugated biomolecules were tested under ultraviolet irradiation. The conjugating ability testing are showed in the following examples.

Example 19

The testing of ability to conjugate a molecular reporter containing carboxyl group to biomolecule, in this case an antibody is used as a biomolecule representative.

The conjugation between molecular reporter and antibody, in order to obtain antibody containing molecular reporter could be tested by immobilization of antibody or protein that could bind to antibody containing molecular reporter on a nitrocellulose membrane. The antibody containing molecular reporter could be captured by the immobilized antibody or protein on the membrane. The detection system is illustrated in **Figure 1**.

The method of conjugation of synthesized molecular reporter containing carboxyl group which is used as a crosslinking group in the present invention consists of the following steps.

a) Prepare 10 mg/mL of molecular reporter (compound **6**) in dimethyl sulfoxide (DMSO).

b) Prepare 10 mg/mL of 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) in buffer solution containing 25 mM of 2-(N-morpholino)ethanesulfonic acid (MES buffer) pH 5.0.

c) Prepare 10 mg/mL of sulfo-N-hydroxysulfosuccinimide (sulfo-NHS) solution in buffer solution containing 25 mM of 2-(N-morpholino)ethanesulfonic acid (MES buffer), pH 5.0.

d) Prepare 1 mg/mL of antibody in phosphate-buffered saline (PBS) solution containing 1 mM potassium dihydrogen phosphate (KH_2PO_4), 154 mM sodium chloride, and 3 mM disodium hydrogen phosphate (Na_2HPO_4), pH 7.2.

e) Combine the solution with 10 µL each of step 1, 2 and 3, and incubate at room temperature for 15 minutes.

f) Combine the solution in step 5 with 50 µL of step 4, and incubate at room temperature for 2 hours.

g) Prepare protein solution to prevent non-specific binding by preparation 1% (w/v) of bovine serum albumin (BSA) in PBS solution.

h) Combine the solution in step 6 with 10 µL of step 7, and incubate at room temperature for 1 hour.

i) Keep a mixture solution at 4°C until use.

After conjugation of molecular reporter and mouse antibody, the signal of molecular reporter and binding reactivity of antibody are tested. In order to apply this molecular reporter conjugated antibody in diagnostic, it may be tested by using membrane and 96-well plates.

Example 20

The testing of ability to conjugate molecular reporter containing aldehyde group to biomolecules, in this case an antibody is used as an example biomolecule

The conjugation between molecular reporter and antibody, in order to obtain antibody containing molecular reporter could be tested by immobilization of antibody or protein that could bind to antibody containing molecular reporter on a nitrocellulose membrane. The antibody containing molecular reporter could be captured by the immobilized antibody or protein on the membrane. The detection system is illustrated in **Figure 1**.

The method of conjugation of the synthesized molecular reporter containing aldehyde group which was used as a crosslinking group in the present invention consists of the following steps.

a) Prepare 5 mg/mL of molecular reporter (compound **8** and **14**) in dimethyl sulfoxide (DMSO).

b) Prepare 1 mg/mL of biomolecule in phosphate-buffered saline (PBS) solution containing 1 mM potassium dihydrogen phosphate (KH_2PO_4), 154 mM sodium chloride, and 3 mM disodium hydrogen phosphate (Na_2HPO_4), pH 7.2, in this case, the mouse antibody is used as an example.

c) Prepare 5M of sodium cyanoborohydride in 1M of sodium hydroxide (NaOH) solution.

d) Prepare blocking solution by preparation of 3M ethanolamine in phosphate-buffered saline, pH 6.6.

e) Combine the solution with 10 µL of step 1, 100 µL of step 2, and 1.1 µL of step 3, and incubate at room temperature for 2 hours.

f) Combine the solution in step 5 with 2.4 µL of step 4, and incubate at room temperature for 15 minutes.

g) Centrifuge solution from step 6 by centrifuge machine at 10,000 round per minute for 5 minutes.

h) Supernatant was ollected and kept at 4 °C until use.

After conjugation of molecular reporter and mouse antibody, the signal of molecular reporter and binding reactivity of antibody are tested. In order to apply this molecular reporter conjugated antibody in diagnostic, it may be tested by using membrane and 96-well plate.

Example 21

The testing of conjugation of molecular reporter in this invention to antibody on membrane

Method of testing of conjugation molecular reporter in this invention to antibody on membrane consists of the following steps

a) Prepare spotting of 1 mg/mL antibody that could capture the molecular reporter conjugated antibody (**Figure 1A**), and 0.90 and 0.45 mg/mL of antigen that molecular reporter conjugated antibody could bind (**Figure 1B**) in carbonate buffer solution containing 50 mM carbonate and 50 % v/v glycerol, pH 9.6. Both of antibody and antigen are spotted on nitrocellulose membrane using an automatic microarrayer machine.

b) Keep the membranes from step 1 at 4°C for 12-16 hours.

c) Block non-specific binding with 2% (w/v) skimmed milk in PBS containing 0.05% Tween (PBST), and incubate at room temperature for 1 hour.

d) Wash the membranes from step 3 with 400 µL of PBST per membrane for three times

e) Add 18 µg/mL of antibody conjugating with molecular reporter (compound **6**, **8**, and **14**) for each membrane, and incubate at room temperature for 1 hour

f) Wash the membranes from step 5 with 400 µL/membrane of PBST for three times

Each spot on nitrocellulose membranes is illustrated in **Figure 2A**, and the interpreting results are shown in **Figure 2B**.

The membranes from step 6 were observed under ultraviolet light 250-450 nm. The results gave high signal when observed at 312 nm for compound **6**, and 8, whereas compound **14** showed high signals at 365 nm.

The results of antibody conjugating molecular reporter by membrane method were shown in **Figure 3**, where A) was compound **6**, B) was compound **8**, and C) was compound **14**. The spots of antibody capturing mouse antibody and antigen showed signals (Left), whereas the membranes adding buffer without molecular conjugated antibody showed negative results (Right)

Example 22

The testing of binding reactivity of antibody after conjugated with molecular reporter on 96-well plates

The binding reactivity of antibody conjugating molecular reporter (compound **6**, compound **8**, and compound **14**) to antigen could be tested with an immobilization of antigen on wells of

96-well plates. The molecular reporter conjugated antibody was added into the wells. The antibody would bind to the antigen coating wells. Horseradish peroxidase labeled antibody specific to mouse antibody was used as a reporter molecule for reporting binding reactivity. The results of molecular reporter conjugated antibody were compared with results of antibody without molecular reporter conjugation.

The method for testing binding reactivity of molecular reporter conjugated antibody and antigen in 96-well plate consists of the following steps.

a) Prepare 2 µg/mL of antigen in carbonate buffer containing 50 mM carbonate, pH 9.6, and immobilize antigen (100 µL/well) in 96-well plate.

b) Incubate 96-well plate in step 1 at 4°C for 12-16 hours.

c) Block non-specific binding with 300 µL/well of 2% bovine serum albumin in phosphate-buffered saline containing 0.05% Tween (PBST), and incubate at room temperature for 1 hour.

d) Wash 96-well plates in step 3 with 300 µL/well of PBST for 3 times.

e) Add 100 µL/ well of 2 µg/mL of antibody conjugating molecular reporter (compound **6**, compound **8**, and compound **14**), and incubate at room temperature for 1 hour.

f) Wash 96-well plates in step 5 with 300 µL/well of PBST for 3 times.

g) Add 100 µL/well of horseradish peroxidase labeled antibody specific to mouse antibody (dilution at 1:10,000), and incubate at room temperature for 1 hour.

h) Wash 96-well plates in step 7 with 300 µL/well of PBST for 3 times.

i) Add 100 µL/well of substrate solution for horseradish peroxidase which is 3,3',5,5'-Tetramethylbenzidine, and incubate at room temperature for 30 minutes.

j) Add 50 µL/well of sulfuric acid (H_2SO_4) for stop reaction.

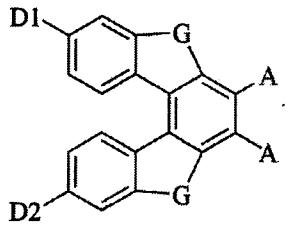
k) Measure signals at 450 nm.

The detection system of binding reactivity between antibody conjugating molecular reporter (compound **6**, compound **8**, and compound **14**) and antigen is illustrated in **Figure 4**. The results of antibody conjugating molecular reporter (compound **6**, compound **8**, and compound **14**) showed that antibody could bind to antigen. The molecular reporters did not affect the binding reactivity of antibody when were compared with antibody without any conjugation. The results showed in **Figure 5**, where (A) was compound **6**, (B) was compound **8**, and (C) was compound **14**.

CLAIMS

What is claimed is:

1. The [5]helicene derivative compounds represented by the following chemical formula (1)

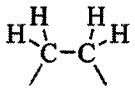


(1)

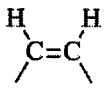
wherein

G is a connecting group composes of 2 carbon atoms selected from the group consisting of

- Ethane


; and

- Ethylene

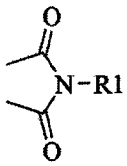


A is a separated or connected group selected from the group consisting of

- Cyano

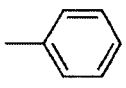
—CN ; and

- Imide

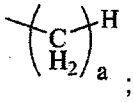


wherein R1 is selected from the group consisting of

- Phenyl

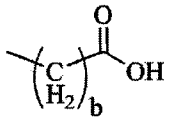

;

- Alkyl



when a is a number of carbon atoms in aliphatic hydrocarbon and a equals to 1 to 7; and

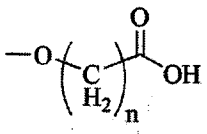
- Alkanoic acid



when b is a number of carbon atoms in aliphatic hydrocarbonand b equals to 1 to 7.

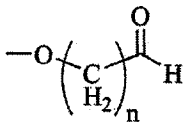
D1 is selected from the group consisting of

- Oxyalkanoic acid



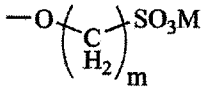
when n is a number of carbon atoms in aliphatic hydrocarbon and n equals to 1 to 7;

- Oxyalkanal



when n is a number of carbon atoms in aliphatic hydrocarbon and n equals to 1 to 7; and

- Oxyalkanesulfonate



when M is a metal atom selected from the group consisting of sodium and potassium,

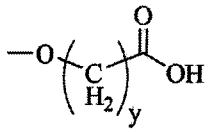
m is a number of carbon atoms in aliphatic hydrocarbon and m equals to 3 or 4.

D2 is selected from the group consisting of

- Hydroxy

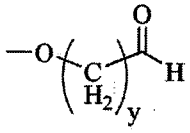
  —OH ;

- Oxyalkanoic acid



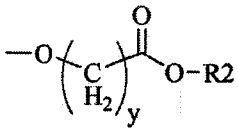
  when y is a number of carbon atoms in aliphatic hydrocarbon and y equals to 1 to 7;

- Oxyalkanal



  when y is a number of carbon atoms in aliphatic hydrocarbon and y equals to 1 to 7;

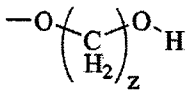
- Alkyl oxyalkanoate



  when y is a number of carbon atoms in aliphatic hydrocarbonand y equals to 1 to 7,

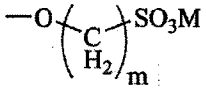
  R2 is selected from the group consisting of methyl and ethyl group;

- Oxyalkanol



  when z is a number of carbon atoms in aliphatic hydrocarbonand z equals to 2 to 8; and

- Oxyalkanesulfonate



when M is a metal atom selected from the group consisting of sodium and potassium,

m is a number of carbon atoms in aliphatic hydrocarbon and m equals to 3 or 4.

2. The [5]helicene derivative compound according to claim 1 wherein

a) A is cyano; G is ethane; D1 is hydroxy; and D2 is selected from the group consisting of oxyalkanoic acid and oxyalkanal.

b) A is cyano; G is ethane; D1 is oxyalkanoic acid; and D2 is selected from the group consisting of hydroxy, oxyalkanoic acid and oxyalkanesulfonate.

c) A is cyano; G is ethane; D1 is oxyalkanal; and D2 is selected from the group consisting of hydroxy, alkyl oxyalkanoate, oxyalkanal and oxyalkanol.

d) A is cyano; G is ethylene; D1 is oxyalkanoic acid; and D2 is selected from the group consisting of hydroxy, oxyalkanoic acid and oxyalkanesulfonate.

e) A is cyano; G is ethylene; D1 is oxyalkanal; and D2 is selected from the group consisting of hydroxy and oxyalkanol.

f) A is imide wherein R1 is selected from the group consisting of phenyl and alkyl; G is selected from the group consisting of ethane and ethylene; D1 is oxyalkanal; and D2 is selected from the group consisting of hydroxy, oxyalkanal, alkyl oxyalkanoate, oxyalkanol and oxyalkanesulfonate.

g) A is imide wherein R1 is alkanoic acid; G is ethane; D1 and D2 are oxyalkanesulfonate.

3. The [5]helicene derivative compound according to claim 1 or 2, wherein said alkyl chain of imide group is straight chain.

4. The [5]helicene derivative compound according to claim 1 or 2, wherein said alkyl chain of imide group is branched chain.

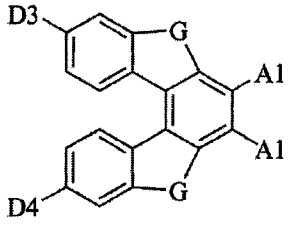
5. The [5]helicene derivative compound according to claim 1 or 2, wherein said alkanoic acid of imide group is straight chain.

6. The [5]helicene derivative compound according to claim 1 or 2, wherein said alkanoic acid of imide group is branched chain.

7. The [5]helicene derivative compound according to any one of claims 1 to 6, wherein said oxyalkanoic acid at the said D1 position is straight chain.

8. The [5]helicene derivative compound according to any one of claims 1 to 6, wherein said oxyalkanoic acid at the said D1 position is branched chain.

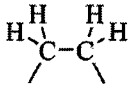
9. The [5]helicene derivative compound according to any one of claims 1 to 6, wherein said oxyalkanal at the said D1 position is straight chain.
10. The [5]helicene derivative compound according to any one of claims 1 to 6, wherein said oxyalkanal at the said D1 position is branched chain.
11. The [5]helicene derivative compound according to any one of claims 1 to 6, wherein said oxyalkanesulfonate at the said D1 position is straight chain.
12. The [5]helicene derivative compound according to any one of claims 1 to 6, wherein said oxyalkanesulfonate at the said D1 position is branched chain.
13. The [5]helicene derivative compound according to any one of claims 1 to 12, wherein said oxyalkanoic acid at the said D2 position is straight chain.
14. The [5]helicene derivative compound according to any one of claims 1 to 12, wherein said oxyalkanoic acid at the said D2 position is branched chain.
15. The [5]helicene derivative compound according to any one of claims 1 to 12, wherein said oxyalkanal at the said D2 position is straight chain.
16. The [5]helicene derivative compound according to any one of claims 1 to 12, wherein said oxyalkanal at the said D2 position is branched chain.
17. The [5]helicene derivative compound according to any one of claims 1 to 12, wherein said alkyl oxyalkanoate at the said D2 position is straight chain.
18. The [5]helicene derivative compound according to any one of claims 1 to 12, wherein said alkyl oxyalkanoate at the said D2 position is branched chain.
19. The [5]helicene derivative compound according to any one of claims 1 to 12, wherein said oxyalkanol at the said D2 position is straight chain.
20. The [5]helicene derivative compound according to any one of claims 1 to 12, wherein said oxyalkanol at the said D2 position is branched chain.
21. The [5]helicene derivative compound according to any one of claims 1 to 12, wherein said oxyalkanesulfonate at the said D2 position is straight chain.
22. The [5]helicene derivative compound according to any one of claims 1 to 12, wherein said oxyalkanesulfonate at the said D2 position is branched chain.
23. The intermediate compound for preparation of the compound according to any one of claims 1 to 22, having the chemical formula:



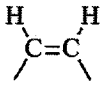
wherein

G is a connecting group composes of 2 carbon atoms selected from the group consisting of

- Ethane

 ; and

- Ethylene

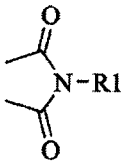


A is a separated or connected group selected from the group consisting of

- Cyano

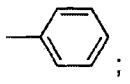
 ; and

- Imide

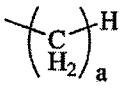


wherein R1 is selected from the group consisting of

- Phenyl

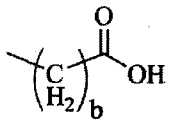
 ;

- Alkyl



when a is an aliphatic hydrocarbon containing 1 to 7 carbon atoms; and

- Alkanoic acid



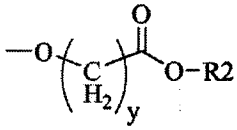
when b is an aliphatic hydrocarbon containing 1 to 7 carbon atoms.

D3 is selected from the group consisting of

- Hydroxy

—OH ; and

- Alkyl oxyalkanoate



when y is an aliphatic hydrocarbon containing 1 to 7 carbon atoms,

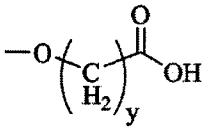
R2 is selected from the group consisting of methyl and ethyl group.

D4 is selected from the group consisting of

- Hydroxy

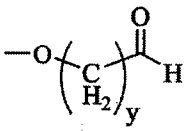
—OH ;

- Oxyalkanoic acid



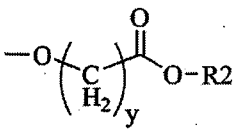
when y is an aliphatic hydrocarbon containing 1 to 7 carbon atoms;

- Oxyalkanal



when y is an aliphatic hydrocarbon containing 1 to 7 carbon atoms;

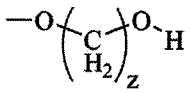
- Alkyl oxyalkanoate



when y is an aliphatic hydrocarbon containing 1 to 7 carbon atoms,

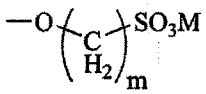
R2 is selected from the group consisting of methyl and ethyl group;

- Oxyalkanol



when z is an aliphatic hydrocarbon containing 2 to 8 carbon atoms; and

- Oxyalkanesulfonate



when M is a metal atom selected from the group consisting of sodium and potassium,

m is an aliphatic hydrocarbon containing to 3 or 4 carbon atoms.

24. The intermediate compound according to claim 23, wherein said alkanoic acid at A position of imide group is straight chain.
25. The intermediate compound according to claim 23, wherein said alkanoic acid at A position of imide group is branched chain.
26. The intermediate compound according to any one of claims 23 to 25, wherein said alkyl oxyalkanoate at D3 position of imide group is straight chain.
27. The intermediate compound according to any one of claims 23 to 25, wherein said alkyl oxyalkanoate at D3 position of imide group is branched chain.
28. The intermediate compound according to any one of claims 23 to 27, wherein said oxyalkanoic acid at D4 position of imide group is straight chain.
29. The intermediate compound according to any one of claims 23 to 27, wherein said oxyalkanoic acid at D4 position of imide group is branched chain.
30. The intermediate compound according to any one of claims 23 to 27, wherein said oxyalkanal at D4 position of imide group is straight chain.
31. The intermediate compound according to any one of claims 23 to 27, wherein said oxyalkanal at D4 position of imide group is branched chain.
32. The intermediate compound according to any one of claims 23 to 27, wherein said alkyl oxyalkanoate at D4 position of imide group is straight chain.
33. The intermediate compound according to any one of claims 23 to 27, wherein said alkyl oxyalkanoate at D4 position of imide group is branched chain.

34. The intermediate compound according to any one of claims 23 to 27, wherein said oxyalkanol at D4 position of imide group is straight chain.

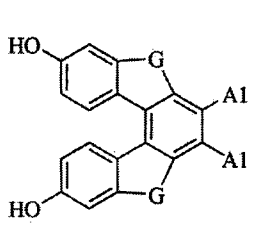
35. The intermediate compound according to any one of claims 23 to 27, wherein said oxyalkanol at D4 position of imide group is branched chain.

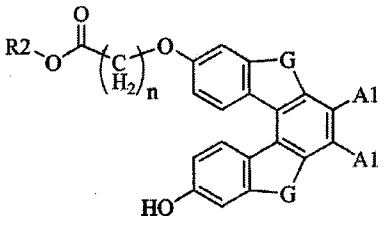
36. The intermediate compound according to any one of claims 23 to 27, wherein said oxyalkanesulfonate at D4 position of imide group is straight chain.

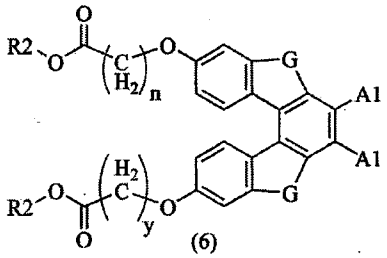
37. The intermediate compound according to any one of claims 23 to 27, wherein said oxyalkanesulfonate at D4 position of imide group is branched chain.

38. A method of preparation process of the [5]helicene derivative compound, comprising the steps of:

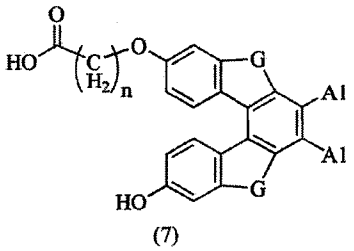
a) An O-alkylation reaction of the [5]helicene compound in formula (**4**), selected from the [5]helicene compound in formula (**4**) wherein A1 is imide or the [5]helicene compound in formula (**4**) wherein A1 is cyano, with haloalkanoic acid alkyl ester (**I**) in the present of base 1 in organic solvent 1 to give [5]helicene compound (**5**) and/or compound (**6**) as intermediate molecule.

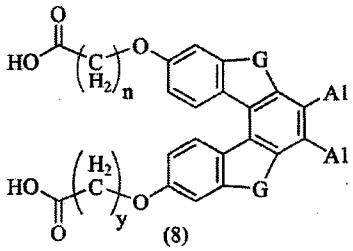

(4)


(5)

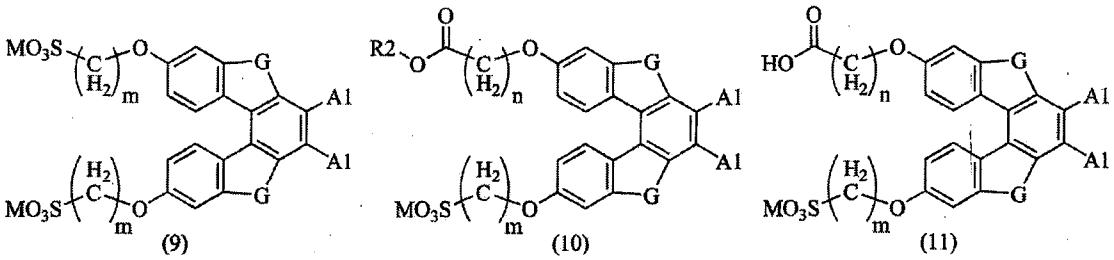

(6)

b) A hydrolysis reaction of [5]helicene compound (**5**) or compound (**6**) using base 2 in organic solvent 2 at temperature in the range of 25-150 °C for 1-24 hours, follow by acidify with acid 1 to gain pH 0 to obtain [5]helicene compound (**7**) or compound (**8**) as a final product or an intermediate.


(7)

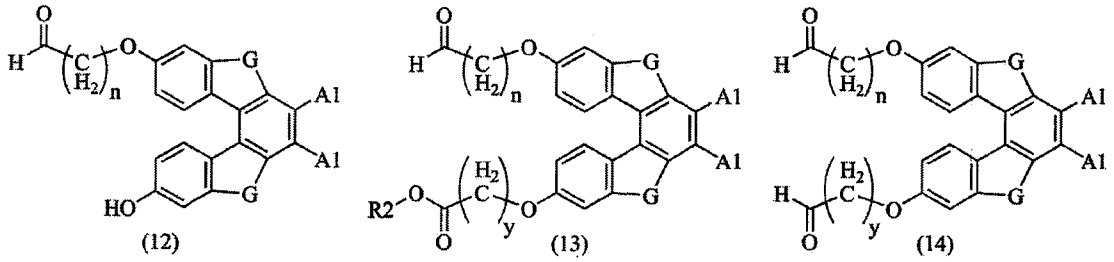

(8)

c) An O-alkylation reaction of the intermediate compound containing OH group, selected from of [5]helicene compound (**4**), compound (**5**) or compound (**7**), with alkane sultone (**II**) at the present of base 3 in an organic solvent 3 to obtain [5]helicene compound (**9**), compound (**10**) or compound (**11**) as final product of intermediate.

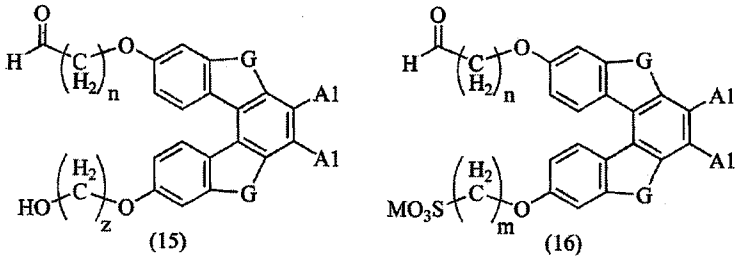


(9) (10) (11)

d) A reduction reaction of the intermediate containing ester group, selected from [5]helicene compound (**5**), compound (**6**) or compound (**10**), using diisobutylaluminum hydride, (DIBAL-H) in an organic solvent 4 to obtain [5]helicene compound (**12**), compound (**13**) and/or compound (**14**) and/or compound (**15**) or compound (**16**) as final product.



(12) (13) (14)



(15) (16)

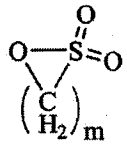
39. The method of preparation process of the [5]helicene derivative compound according to claim 38, wherein said base 1 is selected from the group consisting of sodium bicarbonate ($NaHCO_3$), potassium bicarbonate ($KHCO_3$), sodium carbonate (Na_2CO_3) and potassium carbonate (K_2CO_3)

40. The method of preparation process of the [5]helicene derivative compound according to claim 38 or 39, wherein the preferably base 1 is potassium carbonate (K_2CO_3).

41. The method of preparation process of the [5]helicene derivative compound according to any one of claims 38 to 40, wherein said organic solvent 1 is selected from the group consisting of dimethyl formamide, acetone, acetonitrile and a mixture thereof.

42. The method of preparation process of the [5]helicene derivative compound according to any one of claims 38 to 41, wherein the preferably organic solvent 1 is dimethyl formamide.

43. The method of preparation process of the [5]helicene derivative compound according to any one of claims 38 to 42, wherein said O-alkylation in step a) is carried out at temperature in the range of 60-160 °C for 2-12 hours.
44. The method of preparation process of the [5]helicene derivative compound according to any one of claims 38 to 43, wherein said base 2 is selected from the group consisting of sodium hydroxide (NaOH), potassium hydroxide (KOH) and lithium hydroxide (LiOH).
45. The method of preparation process of the [5]helicene derivative compound according to any one of claims 38 to 44, wherein the preferably base 2 is sodium hydroxide (NaOH).
46. The method of preparation process of the [5]helicene derivative compound according to any one of claims 38 to 45, wherein said organic solvent 2 is selected from the group consisting of ethanol, methanol, tetrahydrofuran, dioxane, dichloromethane and a mixture thereof.
47. The method of preparation process of the [5]helicene derivative compound according to any one of claims 38 to 46, wherein the preferably organic solvent 2 is ethanol.
48. The method of preparation process of the [5]helicene derivative compound according to any one of claims 38 to 47, wherein said acid 1 is selected from the group consisting of hydrochloric acid and sulfuric acid.
49. The method of preparation process of the [5]helicene derivative compound according to any one of claims 38 to 48, wherein the preferably acid 1 is hydrochloric acid.
50. The method of preparation process of the [5]helicene derivative compound according to any one of claims 38 to 49, wherein said alkane sultone (**II**) has the chemical formula:

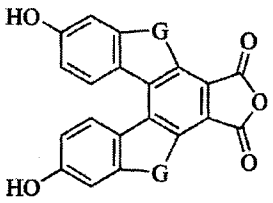


(**II**)

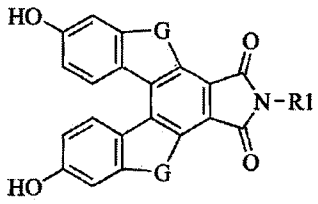
wherein m is a number of carbon atoms in aliphatic hydrocarbon, and m equals 3 or 4.

51. The method according to any one of claims 38 to 50, wherein said alkane sultone (**II**) is straight chain.
52. The method according to any one of claims 38 to 51, wherein said alkane sultone (**II**) is branched chain.
53. The method according to any one of claims 38 to 52, wherein said base 3 is selected from the group consisting of sodium hydroxide (NaOH), potassium hydroxide (KOH), sodium methoxide (NaOMe), potassium methoxide (KOMe), sodium ethoxide (NaOEt) and potassium ethoxide (KOEt).

54. The method according to any one of claims 38 to 53, wherein the preferably base 3 is sodium ethoxide (NaOEt).
55. The method according to any one of claims 38 to 54, wherein said organic solvent 3 is selected from the group consisting of methanol, ethanol, acetone and acetonitrile.
56. The method according to any one of claims 38 to 55, wherein the preferably organic solvent 3 is ethanol.
57. The method according to any one of claims 38 to 56, wherein said O-alkylation in step c) is carried out at temperature in the range of 25-80 °C for 6-120 hours.
58. The method according to any one of claims 38 to 57, wherein said organic solvent 4 selected from the group consisting of dichloromethane, tetrahydrofuran, toluene and a mixture thereof.
59. The method according to any one of claims 38 to 58, wherein said reduction in step d) is carried out at temperature in the range of -90 °C to room temperature for 1-4 hours.
60. The method according to any one of claims 38 to 59, wherein said O-alkylation reaction in step a) further comprises the imidation reaction between a [5]helicene compound in the formula (**2**) and a primary amine (**III**) at the present of acid 2 in an organic solvent 5 to obtain a [5]helicene isoindole dione compound in the formula (**3**)



(2)



(3)

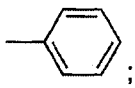
61. The method according to claim 60, wherein said primary amine has the formula (**III**)

$$H_2N\text{-}R1 \quad \textbf{(III)}$$

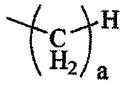
wherein

R1 is selected from the group consisting of

- Phenyl

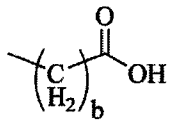

;

- Alkyl



when a is a number of carbon atoms in aliphatic hydrocarbon selected from the group consisting of straight chain and branch chain, and b equals to 1 to 7; and

- Alkanoic acid



when b is a number of carbon atoms in aliphatic hydrocarbon selected from the group consisting of straight chain and branch chain, and b equals to 1 to 7.

62. The method according to claim 60 or 61, wherein said acid 2 is selected from the group consisting of acetic acid, hydrochloric acid and sulfuric acid.
63. The method according to any one of claims 60 to 62, wherein the preferably acid 2 is acetic acid.
64. The method according to any one of claims 60 to 63, wherein said organic solvent 5 is selected from the group consisting of dimethyl formamide, dimethyl sulfoxide, acetonitrile, toluene, benzene and a mixture thereof.
65. The method according to any one of claims 60 to 64, wherein the preferably organic solvent 5 is dimethyl formamide.
66. The method according to any one of claims 60 to 65, wherein said imidation reaction is carried out at temperature in the range of 80-160 °C for 2-12 hours.
67. The use of the [5]helicene derivative compound according to any one of claims 1 to 37 as a molecular reporter for sensing microbial, toxin and toxicant in a sample.
68. The use of the [5]helicene derivative compound according to any one of claims 1 to 37 to crosslink with biomolecule as a molecular reporter for diagnosing microbial, toxin and toxicant in a sample.
69. The use of the [5]helicene derivative compound according to claim 68, wherein the biomolecule is selected from the group consisting of protein, antibody and peptide.

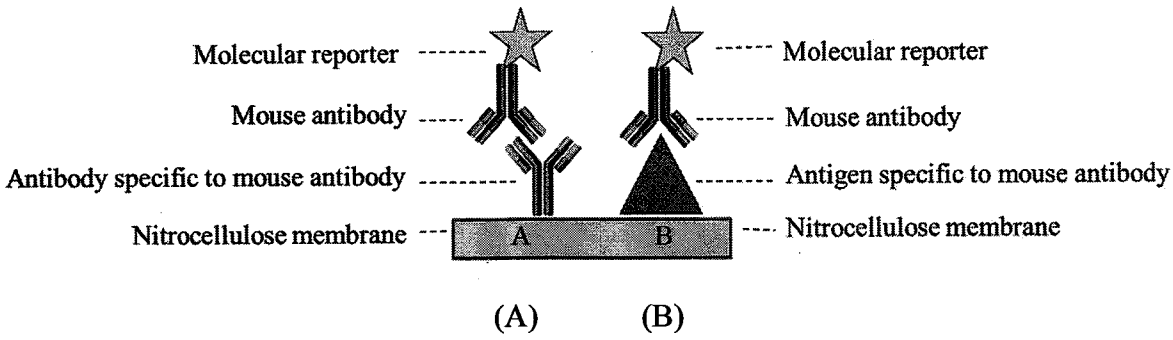

Molecular reporter
Mouse antibody
Antibody specific to mouse antibody
Nitrocellulose membrane
A
B
Molecular reporter
Mouse antibody
Antigen specific to mouse antibody
Nitrocellulose membrane
(A)
(B)

Figure 1

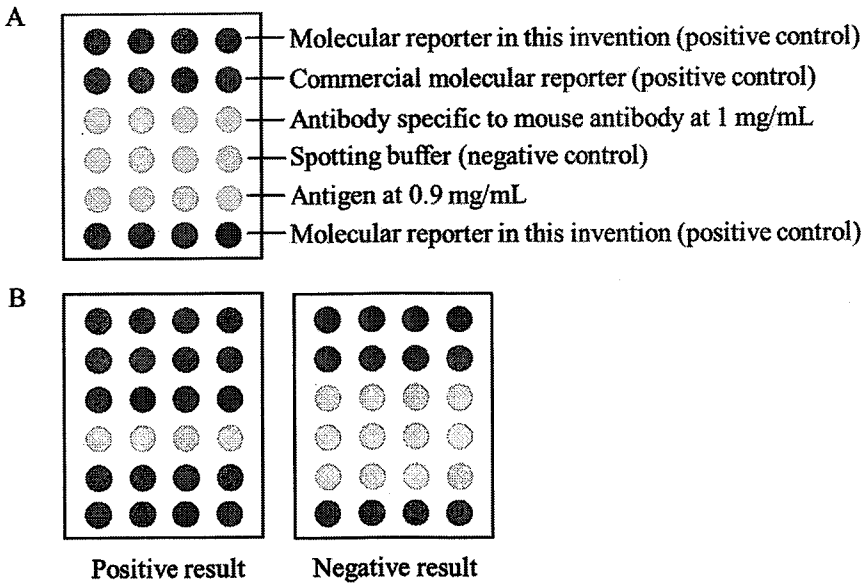
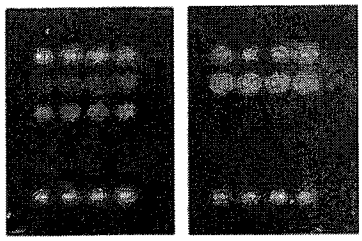

A
Molecular reporter in this invention (positive control)
Commercial molecular reporter (positive control)
Antibody specific to mouse antibody at 1 mg/mL
Spotting buffer (negative control)
Antigen at 0.9 mg/mL
Molecular reporter in this invention (positive control)
B
Positive result
Negative result

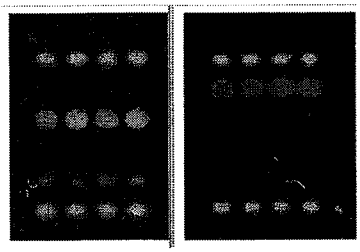
Figure 2



Test with mouse antibody labeling with molecular reporter **compound 6**

Test with carbonate buffer

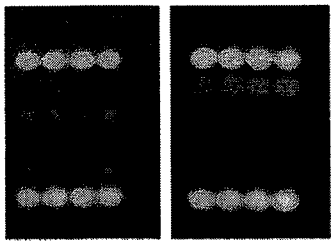
(A)



Test with mouse antibody labeling with molecular reporter **compound 8**

Test with carbonate buffer

(B)



Test with mouse antibody labeling with molecular reporter **compound 14**

Test with carbonate buffer

(C)

Figure 3

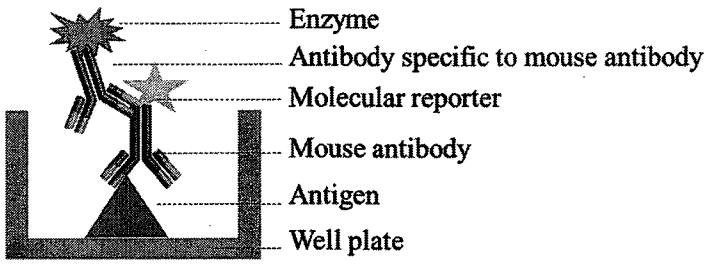
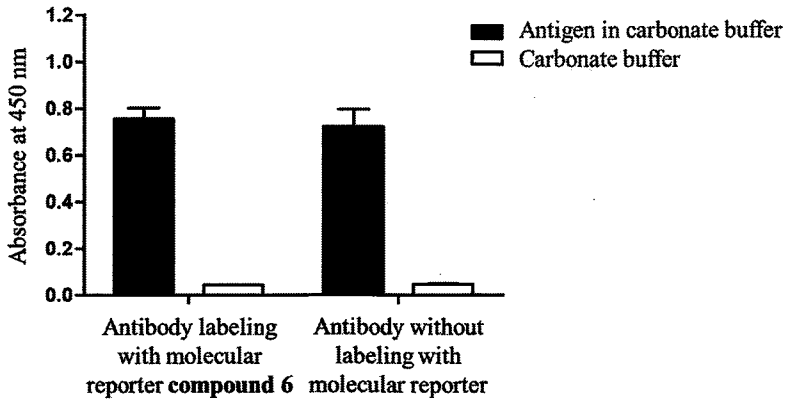
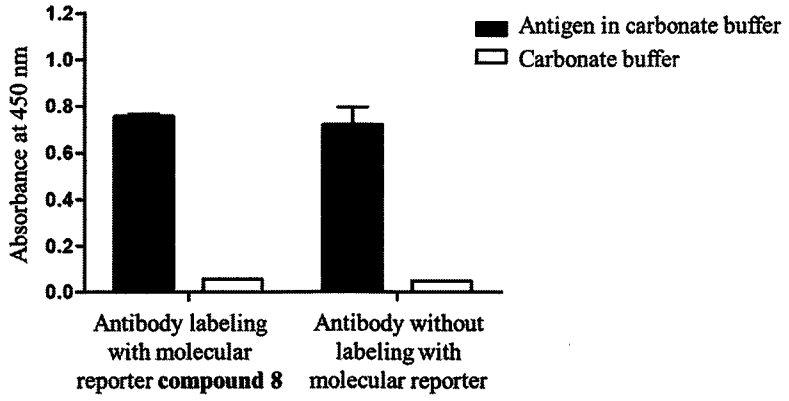

Enzyme
Antibody specific to mouse antibody
Molecular reporter
Mouse antibody
Antigen
Well plate

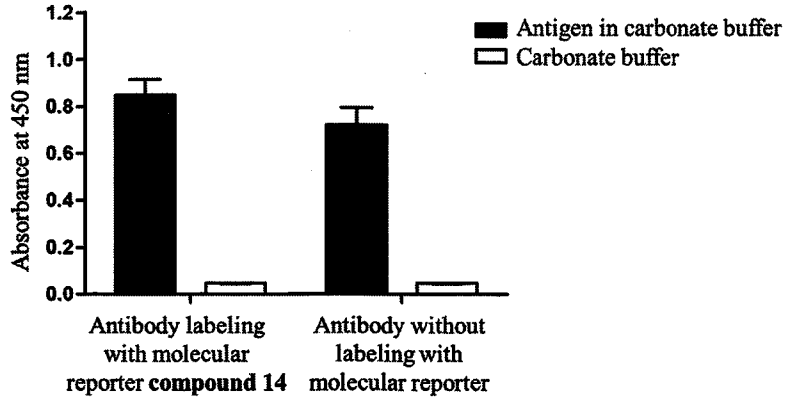
Figure 4


Absorbance at 450 nm
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Antigen in carbonate buffer
Carbonate buffer
Antibody labeling with molecular reporter compound 6
Antibody without labeling with molecular reporter

(A)


Absorbance at 450 nm
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Antigen in carbonate buffer
Carbonate buffer
Antibody labeling with molecular reporter compound 8
Antibody without labeling with molecular reporter

(B)


Absorbance at 450 nm
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Antigen in carbonate buffer
Carbonate buffer
Antibody labeling with molecular reporter compound 14
Antibody without labeling with molecular reporter

(C)

Figure 5

INTERNATIONAL SEARCH REPORT

International application No
PCT/TH2017/000072

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61B5/00 C07C255/54 C07D209/48 G01N33/58
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61B C07C C07D G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SAHASITHIWAT S ET AL: "3,12-Dimethoxy-7,8-dicyano-[5]helicene as a novel emissive material for organic light-emitting diode", SYNTHETIC METALS, ELSEVIER SEQUOIA, LAUSANNE, CH, vol. 160, no. 11-12, 1 June 2010 (2010-06-01), pages 1148-1152, XP027067776, ISSN: 0379-6779, DOI: 10.1016/J.SYNTHMET.2010.02.039 [retrieved on 2010-06-01] figure 1 introduction ----- -/--	1-69

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 December 2017	04/01/2018

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Delanghe, Patrick

1

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/TH2017/000072

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HISATAKA KOBAYASHI ET AL: "New Strategies for Fluorescent Probe Design in Medical Diagnostic Imaging", CHEMICAL REVIEWS, vol. 110, no. 5, 12 May 2010 (2010-05-12), pages 2620-2640, XP55429802, US ISSN: 0009-2665, DOI: 10.1021/cr900263j cited in the application figure 15 introduction -----	1-69

1

专利名称(译)	[5]螺旋衍生物作为诊断用途的分子报道分子及其合成方法		
公开(公告)号	EP3518746A1	公开(公告)日	2019-08-07
申请号	EP2017805303	申请日	2017-09-29
申请(专利权)人(译)	国家科学技术发展局		
当前申请(专利权)人(译)	国家科学技术发展局		
[标]发明人	SOOKSIMUANG THANASAT KAROONUTHAISIRI NITSARA CHARLERMROJ RATTHAPHOL SAHASITHIWAT SOMBOON PANCHAN WARAPORN MAKORNWATTANA MANLIKA PHUENGWAS SUDTIDA KANGKAEW LAONGDAO		
发明人	SOOKSIMUANG, THANASAT KAROONUTHAISIRI, NITSARA CHARLERMROJ, RATTHAPHOL SAHASITHIWAT, SOMBOON PANCHAN, WARAPORN MAKORNWATTANA, MANLIKA PHUENGWAS, SUDTIDA KANGKAEW, LAONGDAO		
IPC分类号	A61B5/00 C07C255/54 C07D209/48 G01N33/58		
CPC分类号	A61B5/0071 C07C255/54 C07C309/11 C07C2603/52 C07D209/58 G01N33/582		
优先权	1601005887 2016-09-30 TH 1601005888 2016-09-30 TH 1601005889 2016-09-30 TH 1601005890 2016-09-30 TH 1701005538 2017-09-22 TH 1701005608 2017-09-25 TH 1701005612 2017-09-25 TH 1701005613 2017-09-25 TH		
外部链接	Espacenet		

摘要(译)

本发明提供[5]螺环烯化合物的合成方法以及与生物分子共轭的所述化合物作为分子报道分子用于诊断的用途。本发明的化合物具有式（1）所示的化学结构：其中G是连接基团，其由选自乙烷和乙烯的2个碳原子组成;A是选自氰基和酰亚胺的分离或连接的基团;DI选自氧化烷酸，氧化烷醛和氧化烷烃磺酸盐;D2具有选自羟基，氧基链烷酸，氧化链烷酸，氧基链烷酸烷基酯，氧基链烷醇和氧基链烷磺酸盐的结构。本发明中的化合物由包含长π共轭体系的芳香族[5]螺环核组成。所述化合物含有能够与生物分子连接的官能团，并且它们可溶于水或用于与生物分子结合的其他溶剂中。而且，由于具有适当的化学结构，本发明的化合物在425-675nm的波长下表现出良好的荧光发射。当所述化合物与生物分子连接时，生物分子具有良好的荧光并且可以在紫外线照射下检测。