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Synthesis of PEG derivatives bearing aminophenyl and their application for liquid-phase synthesis of water-soluble unsymmetrical cyanine dyes

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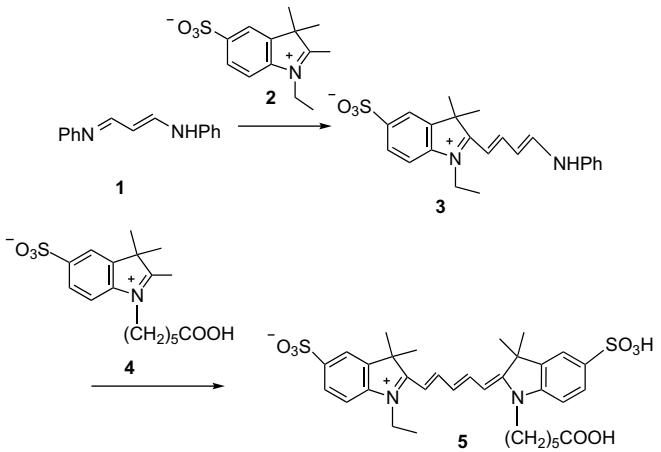
ABSTRACT

The synthesis and NMR characterization of soluble PEG-supported polymers were described, and their subsequent application for liquid-phase synthesis of water-soluble cyanine dyes was also studied. Nucleophilic substitution of tosylation of PEG **16** with 1,3-bis(4-nitrophenoxy)-2-propanol **15** under basic conditions, followed by nitro group reduction, gave PEG-bound aminophenyl **18**. Another PEG-bound aminophenyl **28** was prepared by condensation reaction of PEG-bound pentaerythritol **25** and 4-aminobenzoic acid followed by the cleavage of BOC group. Subsequent loading and activation of sulfoindoleninium to PEG derivatives **18** or **28** were achieved via simple strategies. Cyanine dyes were released by the attack of heterocyclic carbon nucleophile and the cleavage of PEG-bound hemicyanine without the chromatographic separation. The efficient, facile, and practical approaches appear to be robust and versatile strategies to deliver not only indocyanine dyes but also benzoindocyanine dyes.

1. Introduction

Cyanine dyes[1] have been found numerous applications such as photographic sensitizers,[2] nonlinear optical materials,[3] and more recently fluorescent probes for biomolecular labeling.[4–8] Among cyanine dyes, water-soluble sulfoindocyanine dye developed by Waggoner's[9] group is one of the most important type of dyes. The sulfoindocyanines have two or more SO_3^- groups to increase water solubility, which is crucial for the fluorophore to avoid dye aggregation and nonspecific binding to irrelevant components when it is applied to biological analysis as a probe in aqueous environment.[10] However, synthesis of this type of unsymmetrical water-soluble cyanine dyes is very difficult.

The preparation of unsymmetrical water-soluble dyes, such as pentamethine cyanine dye (Cy5) **5**, is traditionally accomplished by a stepwise condensation reaction of two nucleophilic heterocycles with a polyene-chain precursor (Scheme 1). In this process, contaminating symmetrical dye is also formed because hemicyanine intermediate **3** is liable to react with a second molecule of heterocycle **2**. Nontrivial chromatographic separation of unsymmetrical dye is required. Therefore, purification of the unsymmetrical dye is a particular problem in traditional solution synthesis.[11–13] Balasubramanian's group[14,15] achieved an improvement in solid-phase synthesis of cyanine dyes employing polystyrene as loading materials. They had developed two approaches to the solid-phase synthesis of trimethine and pentamethine cyanine dyes, which afforded products in high purity without the need for column chromatography. Although there was a big progress, the preparation of water-soluble cyanine dye with two or more SO_3^- groups was not successful. Therefore, water solubility of cyanine dyes synthesized by solid-phase synthesis was poor for biomolecular labeling.[16–18]



Scheme 1. Traditional synthesis of a water-soluble cyanine dye.

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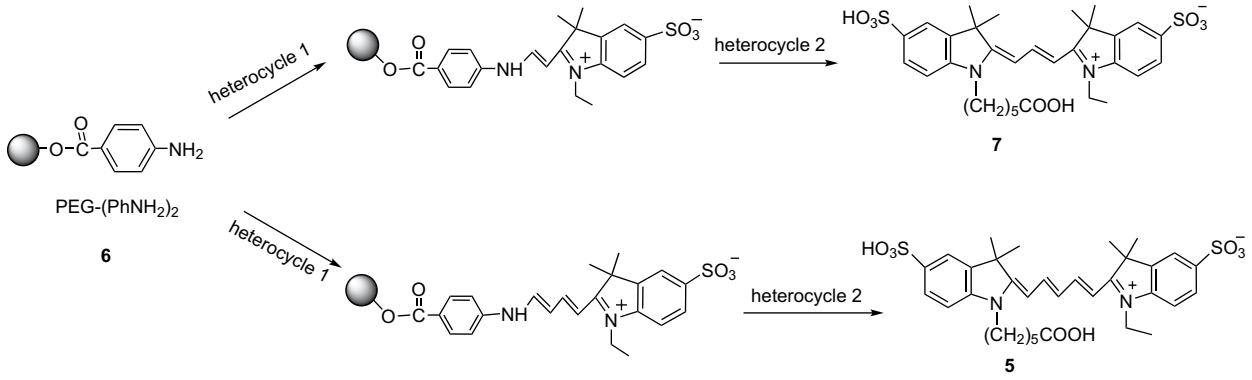


Scheme 2. Synthesis of cyanine dyes by using PEG–$(PhNH_2)_2$ support.

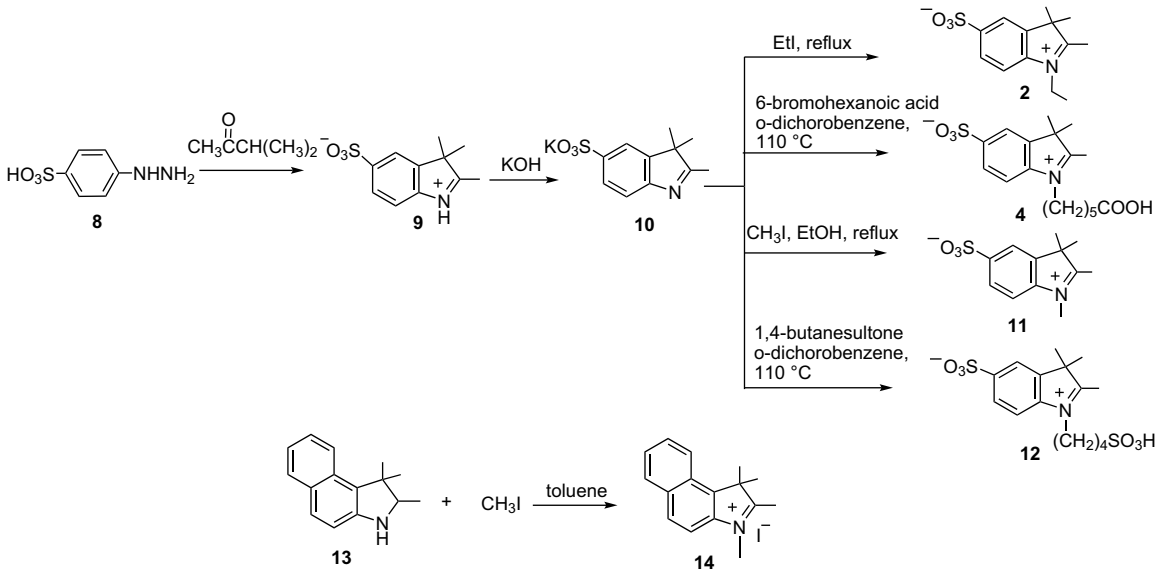
In recent years, the soluble polymers' supported liquid-phase organic synthesis (LPOS) has emerged as an alternative and powerful technique for the preparation of small heterocyclic libraries.[19–24] LPOS offers several unique advantages, for example, convenient products' purification can be achieved by simple filtration and washing. Reactions can be carried out in homogeneous solution. Meanwhile, the soluble polymer-bound species allow the use of routine analytical methods (NMR, TLC, or IR) to monitor the reaction process and to determine the structures of products attached to the polymer supports. Poly(ethylene glycol) (PEG) is one type of soluble polymer that exhibits solubility in a wide range of solvents including dichloromethane (CH_2Cl_2), DMF, toluene, THF, and CH_3OH at room temperature and can be precipitated from a solution by addition of diethyl ether, hexane, or isopropyl alcohol. Therefore, PEG and its derivatives are considered as ideal supports for LPOS.

In our previous published strategy,[25] we have reported the successful synthesis of PEG–$(PhNH_2)_2$ polymer (**6**) and its use in the synthesis of water-soluble unsymmetrical cyanine dyes **5** and **7** (Scheme 2). There are only two aminophenyl groups per PEG–$(PhNH_2)_2$ molecule. It suffers from a drawback, which is its low loading capacity of functional group. In the work presented here, we show some modified PEG derivatives that exhibit higher loading capacities compared to PEG–$(PhNH_2)_2$. A small array of water-soluble trimethine and pentamethine indocyanine dyes are also synthesized by these soluble polymer supports. Furthermore, the synthesis of other type of cyanine dye such as benzoindocyanine dye is also realized by LPOS.

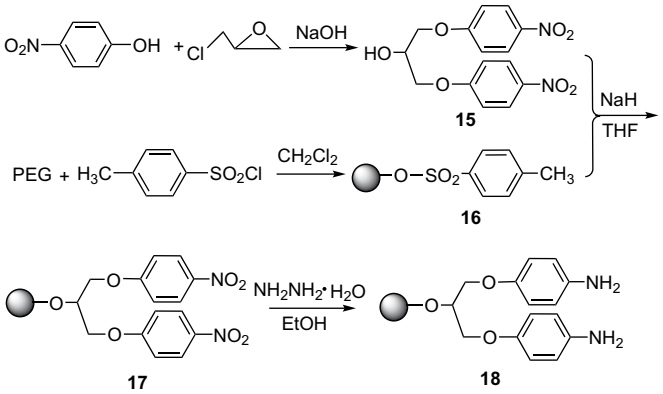
2. Results and discussion

Water solubility required for dyes can be achieved by appending a charged solubilizing functional group such as $-SO_3H$ to heterocycles of a dye (Scheme 3). Quarternization of sulfoindole nucleophile[26] with ethyl iodide or 6-bromohexanoic acid was investigated in our previous work.[25] Heterocycles **11** and **12** were synthesized by modification of literature procedure.[9] In order to investigate whether the polymer-supported methodology is suitable for the synthesis of other types of cyanine dyes, 1,2,3,3-tetramethyl-1*H*-benzo[*e*]indolinium iodide (**14**) was synthesized according to the literature procedure.[27]

Because each molecule of PEG–$(PhNH_2)_2$ possess only two attachment sites, represented by the terminal aminophenyl groups, two molecules of water-soluble cyanine dye could be obtained from a PEG–$(PhNH_2)_2$ molecule. A number of attempts to increase attachment sites of PEG through chemical modification have been realized.[28–30] In order to increase the efficient use of PEG derivative in LPOS, PEG–$(PhNH_2)_4$ **18** bearing four terminal aminophenyl groups was synthesized according to Scheme 4. Synthesis of PEG–$(PhNH_2)_4$ (**18**) was accomplished in four steps starting



Scheme 3. Synthesis of heterocycle compounds.



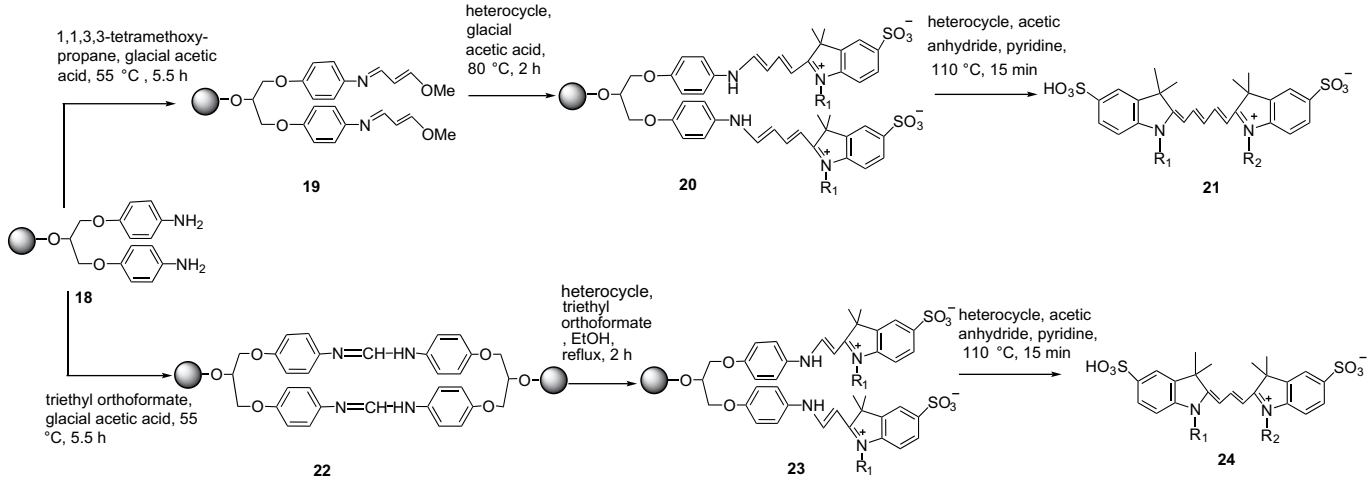
Scheme 4. Synthesis of PEG–$(PhNH_2)_4$.

from 4-nitrophenol and epichlorohydrin as common precursors. Firstly, 4-nitrophenol reacted with epichlorohydrin to afford 1,3-bis(4-nitrophenoxy)-2-propanol (**15**) in 38% yield. Tosylation of PEG was usually performed in pyridine under reflux.[31] However, a more efficient and simple tosylation of PEG using a water/NaOH/CH_2Cl_2 system was reported by Lee-Ruff group.[32] Etherification between **15** and **16** was performed in the presence of NaH in dry THF. Then, PEG derivative **17** was isolated by addition of the reaction mixture into diethyl ether, followed by filtration and washing of polymeric precipitate with diethyl ether to give reagent **17** in 90.6% yield, based on the weight of polymer isolate. The derivation of PEG could be confirmed by the change in chemical shifts of α-methylene protons of PEG. The chemical shift of the α-protons of PEG was δ 3.64 ppm in $CDCl_3$. For the tosylated PEG (**16**), this changed to δ 4.15 ppm and this changed to δ 4.24 ppm for the nitro-terminated polymer **17**. The nitro group was reduced with hydrazine hydrate to give the new polymer support **18**. The formation of arylamino was also confirmed by the ^{1}H NMR. After the nitro group was reduced for 24 h, the signals at δ 7.00 and 8.20 ppm of phenyl group in polymer **17** disappeared. The resonance signals at δ 6.57 and 6.70 ppm of phenyl group in polymer **18** indicated a complete transformation from arylnitro to arylamino. The modified PEG derivative **18** had four functional groups in one molecule, which should be twice as much as the functional groups of PEG–$(PhNH_2)_2$. The loading, determined by ^{1}H NMR method,[25] was 0.3 mmol/g, which is higher than that of PEG–$(PhNH_2)_2$.

To demonstrate the usefulness of the new soluble polymer support in the synthesis of water-soluble unsymmetrical cyanine dyes, we next investigated the synthesis of a small array of water-soluble trimethine and pentamethine indocyanine dyes using this polymer support (Scheme 5). Balasubramanian's group had described that immobilized aniline was converted to the immobilized imidate by reaction with triethyl orthoformate in the presence of $BF_3 \cdot OEt_2$.[15] However, products **19** and **22** were successfully prepared in glacial acetic acid in our system. The formation of conjugated C=N bond in their molecules was confirmed by sharp peaks at 1649 cm^{-1} or 1641 cm^{-1} in FTIR spectra. It was anticipated that their structures were similar to that of PEG–$(PhNH_2)_2$-bound derivatives described in our previous work.[25] Subsequent reaction to form PEG–$(PhNH_2)_4$-bound tetramethine hemicyanine (**20**) and PEG–$(PhNH_2)_4$-bound dimethine heminecyanine dye (**23**) was investigated by the reaction of **19** or **22** with various nucleophilic heterocycle compounds at 80 °C in HAc or ethanol. The peaks near 1183 cm^{-1} of sulfo group in the FTIR spectra of **20** and **23** indicated that the sulfonated heterocycles reacted with PEG derivative **19** or **22** in the loading reaction. Indeed, symmetrical dyes could be also formed in this step. However, it could be removed by using the appropriate conditions.[25] The unsymmetrical water-soluble dye formation steps namely cleavage of hemicyanines from support were investigated by employing substoichiometric quantity of another heterocycle. When the reaction was finished, the reaction mixture was cooled and the blue or red gummy product was precipitated with ethyl acetate. After the gummy product was purified by washing with CH_2Cl_2, blue powder **5** or red powder **7** could be obtained. Obviously, the advantages of both homogeneous reaction and easy isolation and purification of products coexisted in this protocol.

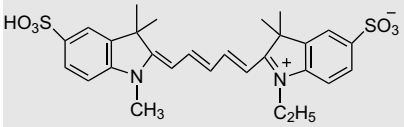
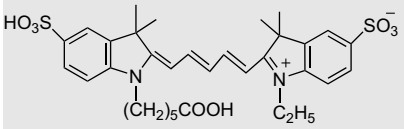
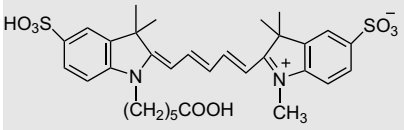
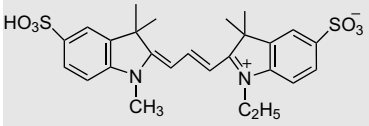
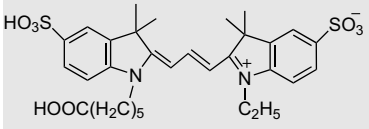
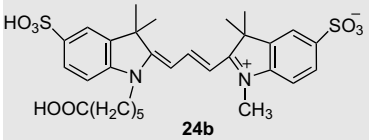
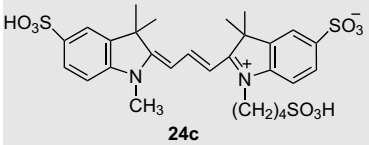
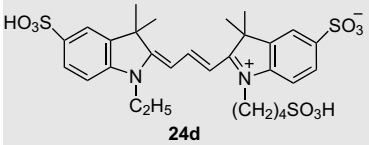
In order to test whether this protocol is suitable for the synthesis of other cyanine dyes, we synthesized eight water-soluble unsymmetrical dyes (Table 1). Products were characterized by ^{1}H NMR, ^{13}C NMR, and mass spectrometry.

Although the loading capacity of PEG–$(PhNH_2)_4$ is higher than that of PEG–$(PhNH_2)_2$, we tried to increase the loading capacity of functional groups for the synthesis of cyanine dyes. The attachment of dendrimeric units with alcohol functionalities has been reported and used in various medical and engineering applications and in combinatorial chemistry.[32–34] To increase the loading capacity of support, a favorable way is to increase the number of anchoring functional groups. A dendrimeric PEG derivative PEG–$(PhNH_2)_6$ (**28**) was developed base on pentaerythritol in Scheme 6. The branched polymer **25** was synthesized using pentaerythritol and tosylation of PEG, which was reported by Lee-Ruff group.[32] Then, 4-[(*tert*-butoxycarbonyl)amino]benzoic acid[35] was reacted with **25** to



Scheme 5. PEG–$(PhNH_2)_4$ supported synthesis of unsymmetrical water-soluble cyanine dyes.

Table 1
Synthesis of trimethine and pentamethine water-soluble dyes by using PEG–$(PhNH_2)_4$ as a support

Compound	Monomer 1 Heterocyclic 1	Monomer 2 Heterocyclic 2	Yield[a] (%)
 **21a**	**2**	**11**	26.0
 **5**	**2**	**4**	25.9
 **21b**	**11**	**4**	21.3
 **24a**	**2**	**11**	22.3
 **7**	**2**	**4**	19.0
 **24b**	**11**	**4**	20.8
 **24c**	**11**	**12**	19.2
 **24d**	**2**	**12**	20.5

[a] Yields are based on weight of heterocycle in cleavage step.

provide the corresponding product **27**. It showed strong peaks at δ 7.96, 7.45, and 1.53 ppm in its ^{1}H NMR spectrum, which we attributed to the presence of the phenyl and methyl groups. In addition, an IR spectroscopic peak at 1715 cm^{-1}, indicative of the C=O bond, was observed. Subsequent cleavage of BOC group under classical condition[36] (TFA/CH_2Cl_2) afforded a new scaffold of aniline **28**. Conventional ^{1}H NMR was also used as an essential tool for direct reaction monitoring and optimization. The loading capacities of PEGs were determined by ^{1}H NMR method and the following equation: functional group (mol) = $W_{is}A_an_{is}/W_aA_{is}n_aM_{is}$.[25] According to the W_a, W_{is}, A_a, and A_{is} in the experiments, the loading level was increased from 0.21 mmol/g for PEG–$(NH_2)_2$ to 0.44 mmol/g for PEG–$(NH_2)_6$. It was anticipated that **28** would be more efficient for the preparation of unsymmetrical dyes.

The reaction of **28** with triethyl orthoformate or 1,1,3,3-tetramethoxypropane in glacial acetic acid resulted in compound **32** or **29**, respectively. Subsequent treatment with 2-methyl substituted heterocyclic molecule led to formation of the desired PEG–$(PhNH_2)_6$-bound hemicyanine dyes **33** and **30**. The structures of **29**, **30**, **32**, and **33** were also similar to that of PEG–$(NH_2)_2$-bound derivatives (Scheme 7).

Unsymmetrical water-soluble dyes were also synthesized using the PEG–$(PhNH_2)_6$. Cyanine dyes (**5** and **7**) were firstly synthesized as described in PEG–$(PhNH_2)_4$ strategy, allowing a direct comparison of two synthetic routes. When we used the same quantity of PEG–$(PhNH_2)_4$ and PEG–$(PhNH_2)_6$, more amount of **5** and **7** was obtained using PEG-$(PhNH_2)_6$ strategy. It was obvious because there was more functional group existing in polymer support **28**. Therefore, the new approach enables more efficient preparation of trimethine cyanine dye (Cy3) and pentamethine cyanine dye (Cy5). Furthermore, another type of cyanine dye such as trimethine benzoindocyanine dye was also synthesized utilizing this polymer support (Table 2).

In summary, the simple and low cost preparation of modified PEG derivatives **18** and **28** was developed. Compared with previous PEG–$(PhNH_2)_2$, the loading level of these new polymer supports was improved with dendritic end groups. Aminophenyl groups of these polymers also permit them as soluble polymer supports in the synthesis of water-soluble cyanine dyes, which afford products without the need for nontrivial column chromatography separation. The versatility of both routes has been demonstrated by the synthesis of cyanine dyes including sulfonated indocyanine and benzoindocyanine dyes, which are of great importance in biological research field. Because the new PEG-supported polymers have more functional groups, the new approaches are preferred methods for the synthesis of water-soluble unsymmetrical cyanine dyes.

3. Experimental

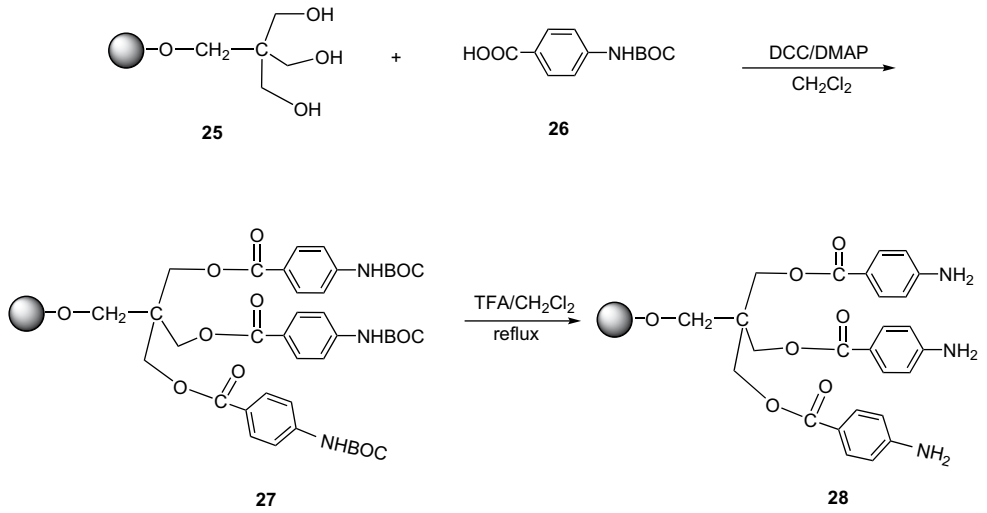
3.1. General methods

All chemicals used were of analytical grade. The melting points were determined using a WRS-113 digital melting point instrument (the thermometer was not corrected). The FTIR spectra were recorded on a Nicolet 170SX FT-IR spectrometer using KBr pellets. Analytical thin layer chromatography (TLC) was performed on silica gel plates, which was developed with a mixture of *n*-butanol/acetic acid/water (4/1/2). The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE300 spectrometer, chemical shifts were referenced internally to tetramethylsilane (TMS, δ=0.0) in parts per million. MS data were obtained on a Bruker Esquire 3000plus mass instrument and a Kratos PC Axima CFRplus MALDI-TOF Mass instrument.

3.2. Synthesis of PEG derivatives 17–23

*3.2.1. PEG-bound 1,3-bis(4-nitrophenoxy)-2-propether (**17**)*

1,3-Bis(4-nitrophenoxy)-2-propanol (0.651 g, 1.9 mmol) and NaH (0.062 g, 2.6 mmol) were dissolved in 30 mL of dry THF. The mixture was stirred at room temperature for 30 min. PEG tosylate (**16**) (1.39 g, 0.3 mmol) was then added to the solution and refluxed for 24 h. THF was then evaporated under reduced pressure. The brown residue was re-dissolved in water (5 mL) and extracted with CH_2Cl_2 (3×50 mL). The volume of CH_2Cl_2 was reduced by 95% under reduced pressure. The remaining CH_2Cl_2 solution was cooled to



Scheme 6. Synthetic procedure of PEG–$(PhNH_2)_6$.

0 °C, and 50 mL of diethyl ether were added. After 30 min, 1.45 g (90.6% yield) of yellow precipitate was collected by filtration. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.22 (d, 4H, J=8.8 Hz), 7.01 (d, 4H, J=8.8 Hz), 4.2–3.4 (m, PEG), 3.89 (m, 4H). IR (KBr): 3452, 2884, 1607, 1513, 1467, 1358, 1094 cm^{-1}.

*3.2.2. PEG-bound 1,3-bis(4-aminophenoxy)-2-propether (**18**)*

PEG-bound 1,3-bis(4-nitrophenoxy)-2-propether **17** (3.15 g, 0.68 mmol), activated carbon (0.122 g), $FeCl_3 \cdot 6H_2O$ (0.143 g), and ethanol (30 mL) were added in a three-necked flask. Hydrazine hydrate (80%) (10 mL) was then added to the mixture within 2 h. Afterward the reaction mixture was refluxed for 24 h, and ethanol was evaporated under reduced pressure. The remaining ethanol solution was cooled to 0 °C, and 100 mL of diethyl ether were added. The brown-black precipitate was collected by filtration. This precipitate was re-dissolved in CH_2Cl_2 and precipitated by addition of diethyl ether at 0 °C. Yield 2.08 g (96.5%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.78 (d, 4H, J=8.1 Hz), 6.58 (d, 4H, J=8.2 Hz), 4.2–3.4 (m, PEG), 3.89 (m, 4H). IR (KBr): 3430, 3386, 3318, 1511, 1463, 1237, 1032 cm^{-1}.

*3.2.3. PEG–$(PhNH_2)_4$-bound formamidine (**22**)*

To product **18** (1.05 g, 0.23 mmol) was added a solution of triethyl orthoformate (3.25 mL, 20 mmol) in glacial acetic acid (9 mL). The reaction mixture was heated at 55 °C for 5.5 h and then 30 mL of cold diethyl ether were added to the solution with vigorous stirring to produce a yellow precipitate, which was dissolved in CH_2Cl_2 and precipitated with diethyl ether for further purification. Yield 0.951 g (95.4%). IR (KBr): 3342, 2906, 1678, 1649, 1513, 1457, 1397, 1111 cm^{-1}.

*3.2.4. PEG–$(PhNH_2)_4$-bound 4-(3-methoxyallylideneamino)benzoic acid ester (**19**)*

To product **18** (1.02 g, 0.22 mmol) was added a solution of 1,1,3,3-tetramethoxypropane (3.7 mL, 22.2 mmol) in glacial acetic acid (9 mL). The reaction mixture was heated at 55 °C for 5.5 h and then 30 mL of cold diethyl ether were added to the solution with vigorous stirring to produce a dark yellow precipitate, which was dissolved in CH_2Cl_2 and precipitated with diethyl ether for further purification. Yield 0.996 g (91.7%). IR (KBr): 3435, 2884, 1704, 1641, 1512, 1463, 1350, 1111 cm^{-1}.

*3.2.5. PEG–$(PhNH_2)_4$-bound dimethine hemicyanine (**23**)*

A mixture of **22** (1.10 g, 0.23 mmol), 1,2,3,3-tetramethylindolenium-5-sulfonate (0.132 g, 0.52 mmol), triethyl orthoformate (0.50 mL, 3.05 mmol), and ethanol (5 mL) was stirred and heated to reflux under nitrogen atmosphere for 2.5 h and then 50 mL of cold diethyl ether were added to the mixture with vigorous stirring to produce an orange precipitate, in which a little of symmetrical dye was existed. Just as we described in previous work,[25] the symmetrical dye could be removed. Yield 1.21 g (93.1%). IR (KBr): 3433, 2884, 1671, 1639, 1516, 1463, 1183, 1110 cm^{-1}.

*3.2.6. PEG–$(PhNH_2)_4$-bound tetramethine hemicyanine (**20**)*

A mixture of **19** (1.01 g, 0.21 mmol), 1,2,3,3-tetramethylindolenium-5-sulfonate (0.111 g, 0.42 mmol), and glacial acetic acid (9 mL) was stirred and heated at 80 °C under nitrogen atmosphere for 2 h and then cold diethyl ether (30 mL) was added to the solution with vigorous stirring to produce a brown precipitate, in which a little of symmetrical dye existed. After the byproduct was removed by appropriate method,[25] product **20** was obtained. Yield 1.15 g (95.8%). IR (KBr): 3429, 2917, 1704, 1679, 1645, 1600, 1446, 1180, 1108 cm^{-1}.

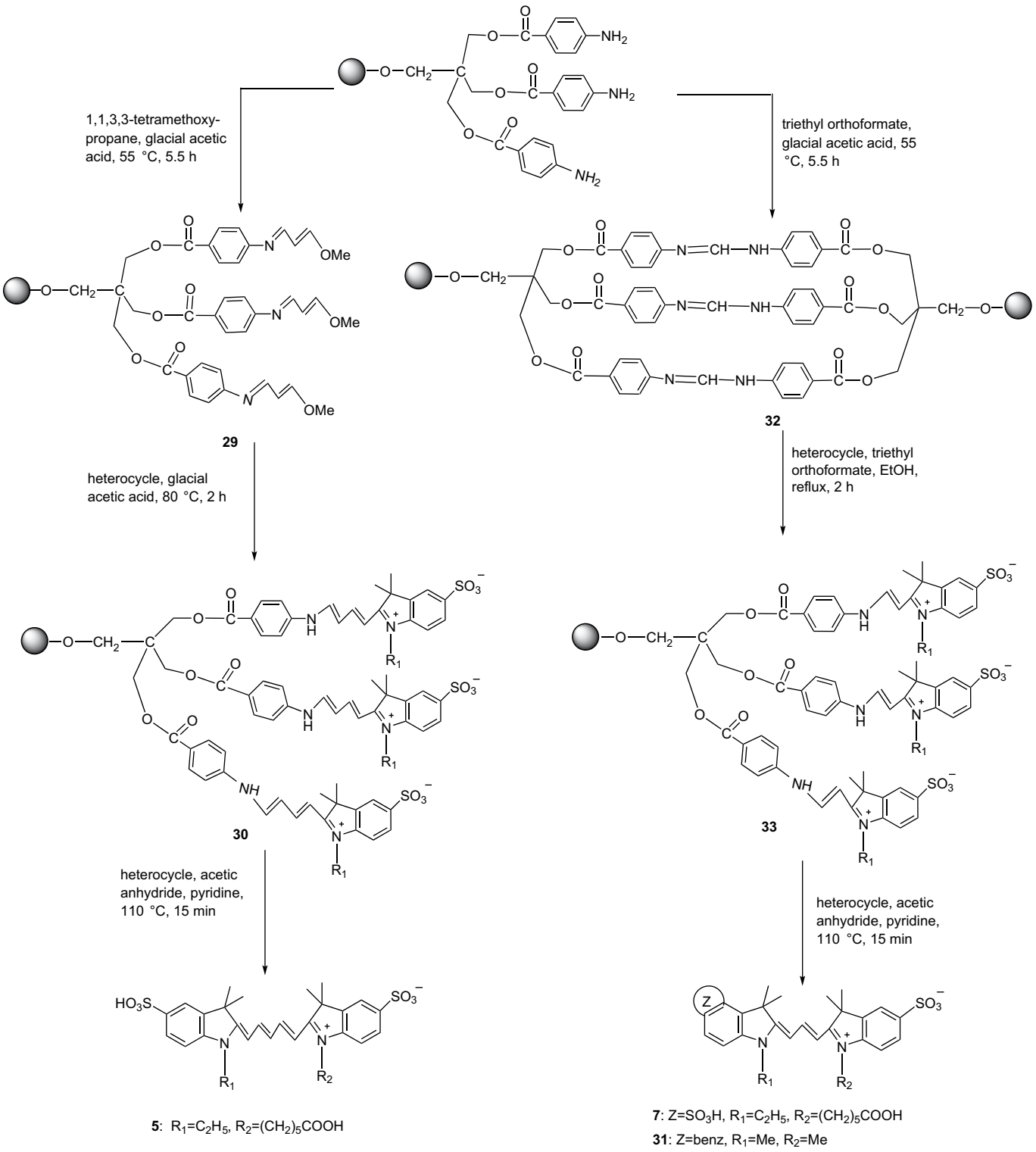
3.3. Synthesis of trimethine and pentamethine cyanine dyes using PEG–$(PhNH_2)_4$

*3.3.1. General procedure for the formation of trimethine cyanine dyes (**24**)*

To PEG–$(PhNH_2)_4$-bound dimethine hemicyanine **23** (0.833 g, 0.16 mmol) and 1-(ε-carboxypentynyl)-2,3,3-trimethylindoleninium-5-sulfonate **4** (0.058 g, 0.16 mmol) was added a solution of acetic anhydride (Ac_2O) (2 mL) and pyridine (1 mL). The mixture was stirred and heated at 110 °C under nitrogen atmosphere for 15 min. After the mixture was cooled, the red dye was precipitated with ethyl acetate. The gummy product **24b** was washed with CH_2Cl_2 until the red free powder was obtained. The red product was collected in 20.8% yield.

*Product **7***. Yield 19.0%. ^{1}H NMR (300 MHz, D_2O): δ 8.31 (t, 1H, β proton of the bridge, J=13.9 Hz), 7.73–7.64 (m, 4H, 4-H, 4′-H, 6-H, 6′-H), 7.18–7.15 (m, 2H, 7-H, 7′-H), 6.22–6.17 (m, 2H, α, α′ proton of the bridge), 3.94–3.89 (m, 4H, α-, α′-CH_2), 2.03 (t, 2H, –CH_2COOH, J=7.1 Hz), 1.64–1.15 (m, 21H, 3CH_2 groups, 1CH_3 and 2$(CH_3)_2$ groups). ^{13}C NMR (300 MHz, D_2O): δ 183.5, 175.8, 175.5, 151.9, 144.1, 143.7, 141.7, 141.5, 139.7, 139.60, 126.8, 119.8, 111.5, 111.3, 103.4, 49.3, 44.3, 39.6, 37.3, 27.1, 27.0, 26.6, 26.1, 25.6, 11.9. MS: m/z for $C_{31}H_{38}S_2O_8N_2$ $[M+Na]^+$ calcd 653.20, found 653.09.

*Product **24a***. Yield 22.3%. ^{1}H NMR (300 MHz, D_2O): δ 8.27 (t, 1H, β proton of the bridge, J=13.2 Hz), 7.71–7.63 (m, 4H, 4-H, 4′-H, 6-H, 6′-H), 7.14–7.06 (m, 2H, 7-H, 7′-H), 6.20–6.10 (m, 2H, α, α′ proton of



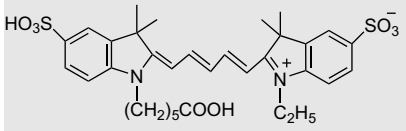
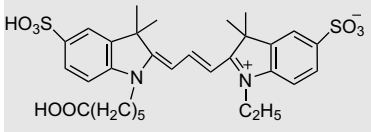
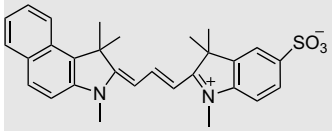
Scheme 7. Synthesis of water-soluble cyanine dyes by using PEG–$(PhNH_2)_6$ as a support.

the bridge), 3.90 (t, 2H, α-CH_2), 3.37 (s, 3H, CH_3), 1.78–1.15 (m, 15H, 1CH_3 groups and 2$(CH_3)_2$ groups). ^{13}C NMR (300 MHz, D_2O): δ 176.1, 175.3, 151.8, 144.5, 143.4, 141.5, 141.2, 139.7, 139.6, 126.7, 119.8, 119.6, 111.2, 103.4, 103.3, 49.2, 39.5, 31.3, 26.9, 11.5. MS: *m*/*z* for $C_{26}H_{30}S_2O_6N_2$ $[M-1]^-$ calcd 529.2, found 529.1.

Product ***24b***. Yield 20.8%. ^{1}H NMR (300 MHz, D_2O): δ 8.38 (t, 1H, β proton of the bridge, *J*=11.5 Hz), 7.78–7.70 (m, 4H, 4-H, 4′-H, 6-H, 6′-H), 7.23–7.71 (m, 2H, 7-H, 7′-H), 6.27–6.19 (m, 2H, α, α′ proton of the bridge), 3.95 (t, 2H, α-CH_2), 3.46 (s, 3H, CH_3), 2.12 (t, 2H, –CH_2COOH, *J*=6.9 Hz), 1.97–1.18 (m, 18H, 3CH_2 groups and 2$(CH_3)_2$ groups). ^{13}C NMR (300 MHz, D_2O): δ 182.2, 176.4, 176.3, 151.9, 144.7, 144.6, 144.0, 141.5, 141.3, 139.7, 126.8, 119.8, 111.5, 111.3, 103.6, 49.3, 44.2, 36.5, 31.3, 27.0, 26.6, 25.9, 25.2. MS: *m*/*z* for $C_{30}H_{36}S_2O_8N_2$ $[M-1]^-$ calcd 615.2, found 615.1.

Product ***24c***. Yield 19.2%. ^{1}H NMR (300 MHz, D_2O): δ 8.46 (t, 1H, β proton of the bridge, *J*=13.7 Hz), 7.79–7.73 (m, 4H, 4-H, 4′-H, 6-H,

Table 2
Synthesis of trimethine and pentamethine dyes by using PEG–$(PhNH_2)_6$ as a support

Compound	Monomer **1**	Monomer **2**	Yield[a] (%)
 **5**	**2**	**4**	26.1
 **7**	**2**	**4**	23.2
 **31**	**11**	**14**	18.5

[a] Yields are based on weight of heterocycle in cleavage step.

6′-H), 7.30–7.25 (m, 2H, 7-H, 7′-H), 6.33–6.26 (m, 2H, α, α′ proton of the bridge), 4.05 (t, 2H, α-CH_2), 3.53 (s, 3H, 1CH_3 groups), 2.87 (t, 2H, J=6.9 Hz, δ-CH_2), 1.86–1.65 (m, 16H, β-CH_2, γ-CH_2, and 2$(CH_3)_2$ groups). ^{13}C NMR (300 MHz, D_2O): δ 176.6, 175.6, 152.0, 144.7, 143.9, 141.4, 139.6, 139.5, 126.7, 119.8, 111.9, 103.8, 50.5, 49.2, 46.8, 31.2, 26.9, 25.6, 21.7. MS: m/z for $C_{28}H_{34}S_3O_9N_2$ $[M+1]^+$ calcd 639.1, found 639.1.

Product ***24d***. Yield 20.5%. ^{1}H NMR (300 MHz, D_2O): δ 8.27 (t, 1H, β proton of the bridge, J=13.2 Hz), 7.63–7.55 (m, 4H, 4-H, 4′-H, 6-H, 6′-H), 7.12–7.09 (m, 2H, 7-H, 7′-H), 6.16–6.09 (m, 2H, α, α′ proton of the bridge), 3.87 (m, 4H, α-, α′-CH_2), 2.70 (t, 2H, J=6.9 Hz, δ-CH_2), 1.68–1.08 (m, 19H, β-CH_2, γ-CH_2, 1CH_3 groups and 2$(CH_3)_2$ groups). MS: m/z for $C_{29}H_{36}S_3O_9N_2$ $[M-1]^-$ calcd 651.1, found 651.0.

3.3.2. *General procedure for the formation of pentamethine cyanine dyes (**21b**)*

To PEG–$(PhNH_2)_4$-bound tetramethine hemicyanine **20** (0.601 g, 0.10 mmol) and 1-(ε-carboxypentynyl)-2,3,3-trimethylindolenium-5-sulfonate **4** (0.038 g, 0.11 mmol) was added a solution of Ac_2O (2 mL) and pyridine (1 mL). The mixture was stirred and heated at 110 °C under nitrogen atmosphere for 15 min. After the mixture was cooled, the blue dye was precipitated with ethyl acetate. The gummy product **21b** was washed with CH_2Cl_2 until the blue free powder was obtained. The blue product was collected in 21.3% yield.

Product ***5***. Yield 25.9%. ^{1}H NMR (300 MHz, D_2O): δ 7.86–7.70 (m, 6H, 4-H, 4′-H, 6-H, 6′-H, β, β′ protons of the bridge), 7.27–7.25 (m, 2H, 7-H, 7′-H), 6.38 (dd, 1H, γ proton of the bridge, J=12.6, 12.5 Hz), 6.11–6.00 (m, 2H, α, α′ proton of the bridge), 4.00–3.95 (m, 4H, α-, α′-CH_2), 2.18 (t, 2H, –CH_2COOH, J=7.0 Hz), 1.72–1.27 (m, 21H, 3CH_2 groups, 1CH_3 and 2$(CH_3)_2$ groups). ^{13}C NMR (300 MHz, D_2O): δ 183.1, 173.9, 173.7, 154.3, 144.2, 143.7, 141.9, 141.8, 139.4, 139.3, 126.6, 119.8, 111.1, 110.9, 104.0, 103.8, 49.1, 49.0, 44.1, 39.4, 37.1, 26.8, 26.7, 26.1, 25.6, 11.7. MS: m/z for $C_{33}H_{40}S_2O_8N_2$ $[M]^+$ calcd 656.81, found 656.86; $[M+Na]^+$ calcd 679.80, found 679.90.

Product ***21a***. Yield 26.0%. ^{1}H NMR (300 MHz, DMSO): δ 8.36 (t, 2H, J=13.1 Hz, β, β′ protons of the bridge), 7.81 (s, 2H, 4-H, 4′-H), 7.64 (d, 2H, J=8.2 Hz, 6-H, 6′-H), 7.32 (m, 2H, 7-H, 7′-H), 6.56 (dd, 1H, γ proton of the bridge), 6.34–6.24 (m, 2H, α, α′ proton of the bridge), 4.13 (t, 2H, J=6.7 Hz, α-CH_2), 3.59 (s, 3H, CH_3 group), 1.69–1.24 (m, 15H, CH_3 group and 2$(CH_3)_2$ groups). ^{13}C NMR (300 MHz, DMSO): δ 174.1, 173.1, 154.8, 145.7, 145.6, 143.2, 142.0, 141.1, 140.8, 126.6, 120.4, 110.5, 110.3, 104.0, 103.5, 49.3, 39.1, 31.7, 27.5, 12.5. MS: m/z for $C_{28}H_{32}S_2O_6N_2$ $[M-1]^-$ calcd 555.2, found 555.1.

Product ***21b***. Yield 21.3%. ^{1}H NMR (300 MHz, D_2O): δ 7.88–7.66 (m, 6H, 4-H, 4′-H, 6-H, 6′-H, β, β′ protons of the bridge), 7.21–7.18 (m, 2H, 7-H, 7′-H), 6.38 (dd, 1H, γ proton of the bridge, J=12.2, 12.5 Hz), 6.11–5.98 (m, 2H, α, α′ proton of the bridge), 3.93 (t, 2H, α-CH_2), 3.49 (s, 3H, CH_3 group), 2.07 (t, 2H, –CH_2COOH), 1.79–1.31 (m, 18H, 3CH_2 groups and 2$(CH_3)_2$ groups). ^{13}C NMR (300 MHz, D_2O): δ 180.1, 173.8, 173.7, 154.2, 144.1, 143.7, 141.8, 141.7, 139.3, 139.2, 126.6, 119.8, 111.1, 111.0, 104.1, 103.9, 49.3, 49.0, 44.1, 37.0, 26.3, 25.7, 25.2, 22.9, 18.9. MS: m/z for $C_{32}H_{38}S_2O_8N_2$ $[M-1]^-$ calcd 641.2, found 641.1.

3.4. Synthesis of PEG derivatives 27–33

3.4.1. *PEG–$(PhNHBOC)_6$ (**27**)*

PEG-bound pentaerythritol (**25**) (1.53 g, 0.68 mmol) and 4-[(*tert*-butoxycarbonyl)amino]benzoic acid (1.95 g, 8.2 mmol) were dissolved in 45 mL of anhydrous CH_2Cl_2. *N*,*N*-Dimethyl aminopyridine (DMAP) (0.114 g, 1.2 mmol) was added to the solution as a catalyst and dicyclohexyl carbodiimide (DCC) (2.49 g, 11.7 mmol) was added as a coupling agent. The mixture was kept in a dry nitrogen environment at room temperature for 12 h. After the dicyclohexylurea (DCU) was filtered off, the filtrate was concentrated under reduced pressure, and then 50 mL of cold diethyl ether were added to the solution with vigorous stirring. Then the white precipitate was filtered and collected. The purified product **27** was dried in vacuum oven for 24 h. Yield 2.0 g (83.3%). IR (KBr): 3426, 2913, 1725, 1602, 1538, 1461 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.97 (d, 2H, J=8.4 Hz), 7.46 (d, 2H, J=8.3 Hz), 4.45–3.34 (m, PEG), 1.53 (s, 9H).

3.4.2. *PEG–$(PhNH_2)_6$ (**28**)*

Compound **27** (1.65 g, 0.46 mmol), trifluoroacetic acid (TFA) (4.5 mL), and CH_2Cl_2 (7 mL) were introduced in a flask. The mixture was refluxed for 10 h under magnetic stirring. After the TFA was neutralized by triethylamine, 50 mL of cold diethyl ether were added to the solution with vigorous stirring to produce a yellow precipitate. Yield 1.15 g (80.3%). IR (KBr): 3428, 2911, 1742, 1605, 1460 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.83 (d, 2H, J=7.3 Hz), 6.64 (d, 2H, J=7.1 Hz), 4.40–3.40 (m, PEG).

3.4.3. *PEG–$(PhNH_2)_6$-bound formamidine (**32**)*

A mixture of **28** (0.418 g, 0.14 mmol), triethyl orthoformate (1.5 mL, 9 mmol), and glacial acetic acid (5 mL) was stirred and heated at 55 °C for 5.5 h. Then 30 mL of cold diethyl ether were added to the solution with vigorous stirring to produce a yellow precipitate, which was dissolved in CH_2Cl_2 and precipitated with diethyl ether for further purification. Yield 0.302 g (85.7%). IR (KBr): 3430, 2885, 1698, 1635, 1603, 1461, 1111 cm^{-1}.

3.4.4. *PEG–$(PhNH_2)_6$-bound 4-(3-methoxyallylideneamino)benzoic acid ester (**29**)*

A mixture of **28** (0.522 g, 0.17 mmol), 1,1,3,3-tetramethoxypropane (2.1 mL, 12.6 mmol), and glacial acetic acid (5 mL) was stirred and heated at 55 °C for 5.5 h. Then 30 mL of cold diethyl ether were added to the solution with vigorous stirring to produce a dark yellow precipitate, which was dissolved in CH_2Cl_2 and precipitated with diethyl ether for further purification. Yield 0.493 g (82.1%). IR (KBr): 34 442, 2885, 1701, 1638, 1601, 1463, 1111 cm^{-1}.

3.4.5. *PEG–$(PhNH_2)_6$-bound dimethine hemicyanine (**33**)*

A mixture of **32** (0.320 g, 0.097 mmol), 1-ethyl-2,3,3-trimethylindolenium-5-sulfonate (0.096 g, 0.361 mmol), triethyl orthoformate (0.30 mL), and ethanol (5 mL) was stirred and heated to reflux under nitrogen atmosphere for 2 h. After the reaction was

finished, product **33** was obtained as the collecting of PEG–$(PhNH_2)_2$-bound dimethine hemicyanine described in Ref. 25. Yield 0.28 g (65.1%) IR (KBr): 3431, 2879, 1698, 1602, 1458, 1186, 1112 cm^{-1}.

*3.4.6. PEG–$(PhNH_2)_6$-bound tetramethine hemicyanine (**30**)*

A mixture of **29** (0.465 g, 0.14 mmol), 1-ethyl-2,3,3-trimethylindolenium-5-sulfonate (0.112 g, 0.422 mmol), and glacial acetic acid (5 mL) was stirred and heated at 80 °C under nitrogen atmosphere for 2 h. After the reaction was finished, product **30** was obtained as the collecting of PEG–$(PhNH_2)_2$-bound tetramethine hemicyanine described in Ref. 25. Yield 0.452 g (69.2%) IR (KBr): 3430, 2874, 1709, 1635, 1458, 1189, 1109 cm^{-1}.

3.5. Synthesis of cyanine dyes and benzoindocyanine dye using PEG–$(PhNH_2)_6$

*3.5.1. General procedure for the formation of trimethine dyes **7** and **31***

To PEG–$(PhNH_2)_6$-bound dimethine hemicyanine **33** (0.301 g, 0.065 mmol) and 1-(ε-carboxypentynyl)-2,3,3-trimethylindolenium-5-sulfonate **4** (0.046 g, 0.13 mmol) were added Ac_2O (1.5 mL) and pyridine (1 mL). The mixture was stirred and heated at 110 °C under nitrogen atmosphere for 15 min. After the mixture was cooled, the red free powder **7** was collected as we described above. The red product was collected in 23.2% yield.

*Product **31***. Yield 18.5%. ^1H NMR (300 MHz, DMSO-d_6): δ 8.46 (t, 1H, β proton of the bridge, J=12.7 Hz), 8.34–7.63 (m, 9H, Ar H), 6.54–6.15 (m, 2H, α, α′ proton of the bridge), 3.79 (s, 3H, CH_3 group), 3.64 (s, 3H, CH_3 group), 1.97 (s, 6H, $(CH_3)_2$ groups), 1.73 (s, 6H, $(CH_3)_2$ groups). MS: m/z for $C_{29}H_{30}S_2O_3N_2$ $[M-1]^-$ calcd 485.2, found 485.1.

*3.5.2. General procedure for the formation of pentamethine cyanine dyes **5***

To PEG–$(PhNH_2)_6$-bound tetramethine hemicyanine **30** (0.361 g, 0.075 mmol) and 1-(ε-carboxypentynyl)-2,3,3-trimethylindoleninium-5-sulfonate **4** (0.042 g, 0.12 mmol) was added a mixture of Ac_2O (1.5 mL) and pyridine (1 mL). The mixture was stirred and heated at 110 °C under nitrogen atmosphere for 15 min. After the mixture was cooled, the blue free powder **5** was collected as we described above. The blue product was collected in 26.1% yield.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2009.04.086.

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