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Synthesis and post-synthetic derivatization of a cyanine-based amino acid. Application to the preparation of a novel water-soluble NIR dye

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Abstract—An efficient synthesis of a new fluorescent amino acid derived from a pentamethylene cyanine dye is described. Selective derivatization of its amino group with an original trisulfonated linker has led to a novel water-soluble NIR dye suitable for covalent labeling of biomolecules.

1. Introduction

Cyanine dyes[1] are fluorescent derivatives, which have found numerous applications such as photographic sensitizers,[2] nonlinear optical materials,[3] and more recently fluorescent probes for biomolecular labeling.[4–6] In particular, their use in genetic analysis, DNA sequencing,[7,8] in vivo imaging[9] and proteomics[10] is growing. The appeal to this class of fluorophores derives from their straightforward syntheses,[11,12] a broad wavelength tunability, and particularly their high near-infrared (NIR) absorption and emission wavelengths, large molar extinction coefficients, and moderate fluorescence quantum yields. Therefore, many different polymethine cyanine derivatives have been synthesized during the last decade. Their preparation is traditionally accomplished by a stepwise condensation reaction of two nucleophilic aza-heterocycles (i.e., heterocyclic bases containing an activated methyl group) with a polyene-chain precursor (i.e., an unsaturated bisaldehyde or its equivalent, usually derivatized as Schiff base, in the presence of a catalyst). Structural diversity is reached through varying the polyene chain, the nitrogen substituents, or the heterocycles themselves. However, this general synthetic scheme is not compatible with a wide range of reactive groups, required on aza-heterocycles for a fine tuning of the solubility, reactivity, and spectroscopic properties of the corresponding cyanine dyes. Indeed, only functional groups such as carboxylic and sulfonic acids are completely inert toward the reagents and reaction conditions used for achieving the condensation reaction.[†] This major drawback can be bypassed by an alternative synthetic approach based on the preparation of a precursor of the target functionalized cyanine dye, a so-called 'convertible cyanine dye' and its subsequent post-synthetic chemical transformation to give the fluorophore bearing the desired reactive groups. This synthetic methodology was applied to the chemical derivatization of heptamethine cyanine dyes (Fig. 1a). The use of precursors bearing a chlorine atom at the central meso position,[13,14] which can be easily replaced by various nucleophiles (metal alcoholates,[15] amines,[16,17] and thiols[18,19]) through an $S_{RN}1$ mechanism, has enabled the synthesis of some NIR labels whose reactivity and optical properties are suitable for in vivo imaging[20]

Keywords: Cyanine dye; Fluorescent amino acid; Sulfonation.
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[†] N-Acetylation of the intermediate hemicyanine and high temperatures are often required to get the target cyanine dye in good yield. Thus, to avoid the formation of numerous byproducts, the presence of free amino or hydroxyl groups onto aza-heterocycles is not recommended.

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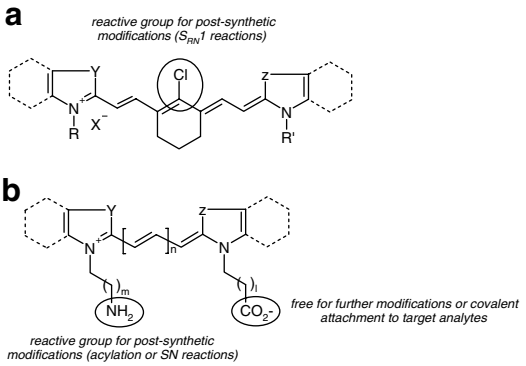


Figure 1. General structure of 'convertible cyanine dyes' already reported in the literature and studied in this letter. Y, Z = CR_2, NH, O, S, $(CH_2)_2$; R, R′ = alkyl; $n = 0–3$; $m = 3–5$; $l = 0–4$.

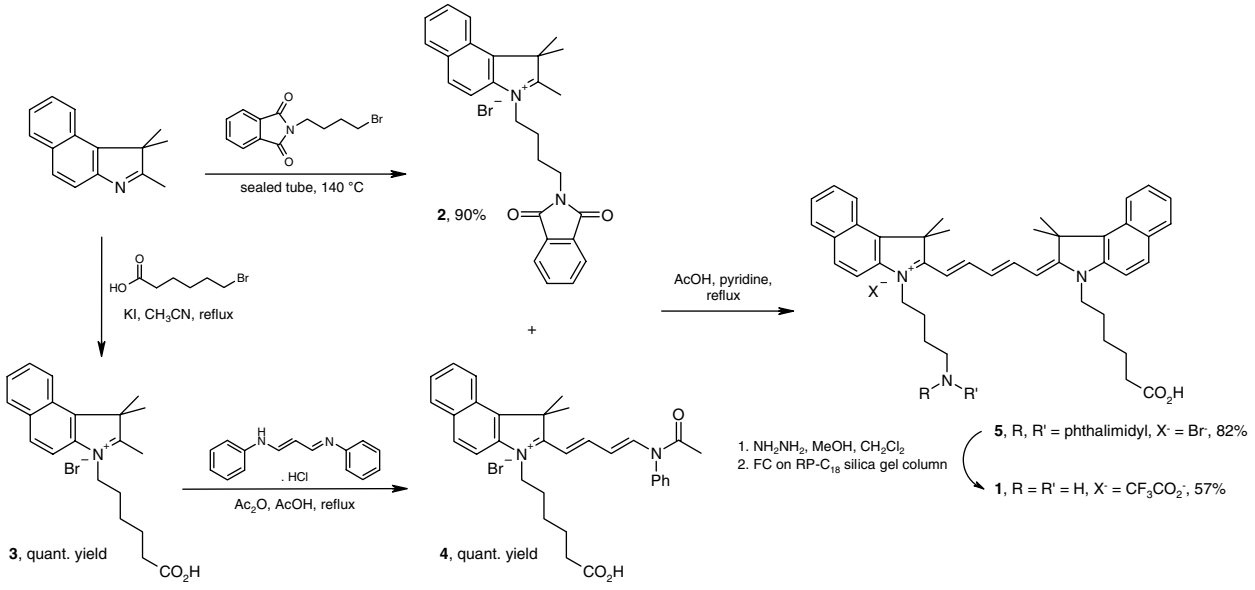
and DNA sequencing applications.[15] This efficient synthetic approach is currently used for the preparation of monocarboxylate functionalized NIR fluorophores suitable for bioconjugation to target analytes or biopolymers but restricted to heptamethine cyanine dyes containing a chloro-cyclohexenyl (or cyclopentenyl) moiety within their polyene chain. Therefore, it is interesting to explore an alternative post-synthetic approach applicable to a wider range of cyanine dyes. Furthermore, some applications of cyanine dyes such as DNA sequencing by means of energy transfer (ET) terminators[7] or fluorescent labeling of biopolymers through solid-phase synthesis techniques,[6] are possible only with heterobifunctional derivatives of these fluorophores.

To our knowledge, cyanine dyes bearing two orthogonal reactive groups have not yet been reported in the literature. Therefore, we thought of design the original cyanine-based amino acids whose amino group could be used either to link a second fluorescent unit to get efficient ET cassettes[7,21–23] or to introduce a further functional group to modulate the hydrophobic/hydrophilic character or the spectral properties of the cyanine core. The carboxylic acid group would be left free for the covalent attachment to biomolecules or related biomacromolecules.

In this letter, we present an original synthetic route to functionalized cyanine dyes based on the use of such a non natural amino acid as 'convertible cyanine dye' (Fig. 1b). The structure of this precursor is designed to get a scaffold bearing the two orthogonal reactive amino and carboxylic acid groups, which can be modified readily and selectively. As preliminary results, the first total synthesis of such cyanine-based amino acid is described. The utility and the reactivity of its aminoalkyl arm are demonstrated by the preparation of a novel water soluble analogue of pentamethine cyanine dye Cy 5.5, through an effective acylation reaction with an original trisulfonated linker.

2. Results and discussion

Synthesis of the cyanine-based amino acid **1** was accomplished in a highly efficient and convergent five step scheme, starting from 1,1,2-trimethyl-1*H*-benz[*e*]-indole[24,25] as a common precursor for the two moieties (Scheme 1). Firstly, this indole undergoes quaternization with (4-bromobutyl)phthalimide to afford the iminium quaternary salt **2** in 90% yield. The phthalimidobutyl group was selected as a precursor for the reactive amino linker side chain since it is completely compatible with the experimental conditions currently used for the synthesis of cyanine dyes, and its removal is achieved under neutral conditions (i.e., hydrazine monohydrate) having no deleterious effects toward the fragile and highly functionalized target fluorophore. Furthermore, the length of this linker arm is suitable to prevent an hypothetical Mannich type cyclization side reaction, which we already observed with the aminoethyl and aminopropyl derivatives at the monomeric indole level.[26] Such a cyclization, which would lead to the detrimental decomposition of the polyene chain, is prevented since the

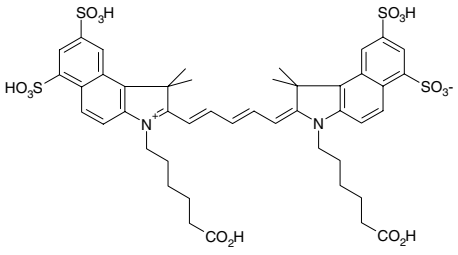


Scheme 1. Synthesis of the cyanine-based amino acid.

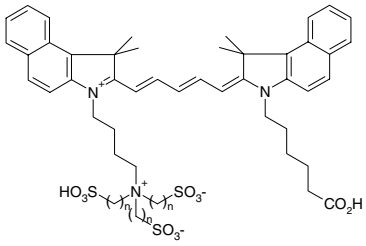
formation of a seven-membered ring is not favored under these conditions. The readily formed and already described second indole unit bearing a carboxylate group **3**[27] was treated with malonaldehyde dianilido hydrochloride in a (1/1) mixture of acetic acid and acetic anhydride under reflux to give **4** in quantitative yield.[‡] Reaction of **2** with **4** in a (1/1) mixture of acetic acid and pyridine under reflux furnished the pentamethine cyanine derivative **5**, which was isolated in good yield (82%) by silica gel chromatography. Finally, the removal of the phthalimide protecting group was achieved by treatment with a 10-fold excess of hydrazine monohydrate in a mixture of dichloromethane and methanol. The targeted cyanine-based amino acid **1** was purified by flash chromatography on an RP–C_{18} silica gel column (yield: 57%) and its structure was confirmed by detailed spectroscopic measurements, including MALDI-TOF mass spectrometry and NMR analyses.

To demonstrate the usefulness of the additive amino group introduced onto the cyanine scaffold and validate our post-synthetic derivatization approach, we next investigated the synthesis of an analogue of cyanine dye Cy 5.5 **6**.[§] Indeed, due to its spectroscopic properties, relative stability, and easy bioconjugation, this pentamethine cyanine dye, commercially available from GE Healthcare (formerly, Amersham-Biosciences), is one of the most popular fluorophores used as a marker in life science applications. This sulfobenzoindocyanine is synthesized through a low-yield (15% as described) synthetic route, which involves 6 steps from 6-amino-1,3-naphthalenedisulfonic acid.[28] Major synthetic difficulties are related to the poor solubility of this latter aminonaphthalene derivative, even in polar protic solvents. To circumvent this issue, it seemed interesting to introduce the sulfonate groups after completion of the cyanine synthesis. Thus, the cyanine-based amino acid **1** could be a suitable precursor and its free amino group a preferred site for the incorporation of some hydrophilic moieties. Furthermore, its structural features (two naphthalene rings and pentamethine chain) have been chosen to get the same spectral properties than cyanine dye Cy 5.5. Firstly, we tried to directly convert **1** into the sulfonated derivative **7** by successive alkylations of the amino group with 1,3-propanesulfone or sodium 2-bromoethanesulfonate.[29] However, all our attempts to get **7** failed.[¶] Amino acid **1** is either completely recovered or converted into decomposition products, which were not characterized.

Since the sulfonation of aromatic amines is well described in the literature,[30,31] we have explored the synthesis of an original polysulfonated linker derived from 3,5-diaminobenzoic acid and its subsequent covalent attachment to the aminoalkyl arm of **1** (Scheme 2). Reaction of 3,5-diaminobenzoic acid with a large excess of sodium 2-bromoethanesulfonate and KI in aq NaOH under reflux for 4 days gave trisulfonated derivative **8** as the major compound. This highly polar linker was purified by semi-preparative RP-HPLC and its structure was confirmed by detailed measurements, including ESI mass spectrometry and NMR analyses. Thereafter, benzoic acid **8** was quantitatively converted into the corresponding *N*-hydroxysuccinimidyl ester **9** by treatment with DCC and *N*-hydroxysuccinimide (NHS) in dry DMF. Finally, acylation of the amino group of **1** was achieved by treatment with a slight excess of **9** (1.2 equiv) in dry NMP in the presence of DIEA. Purification by semi-preparative RP-HPLC provided the Cy 5.5 analogue **10** in good yield (70%). The introduction of the trisulfonated linker onto the aminobutyl arm of cyanine **1** was confirmed by ^{1}H NMR and MALDI-TOF mass analyses. Furthermore, **10** was found to be perfectly soluble in water in the concentration range (1 μM–5 mM) suitable for biomolecular labeling applications.



6, cyanine dye Cy 5.5



7, n = 2-4

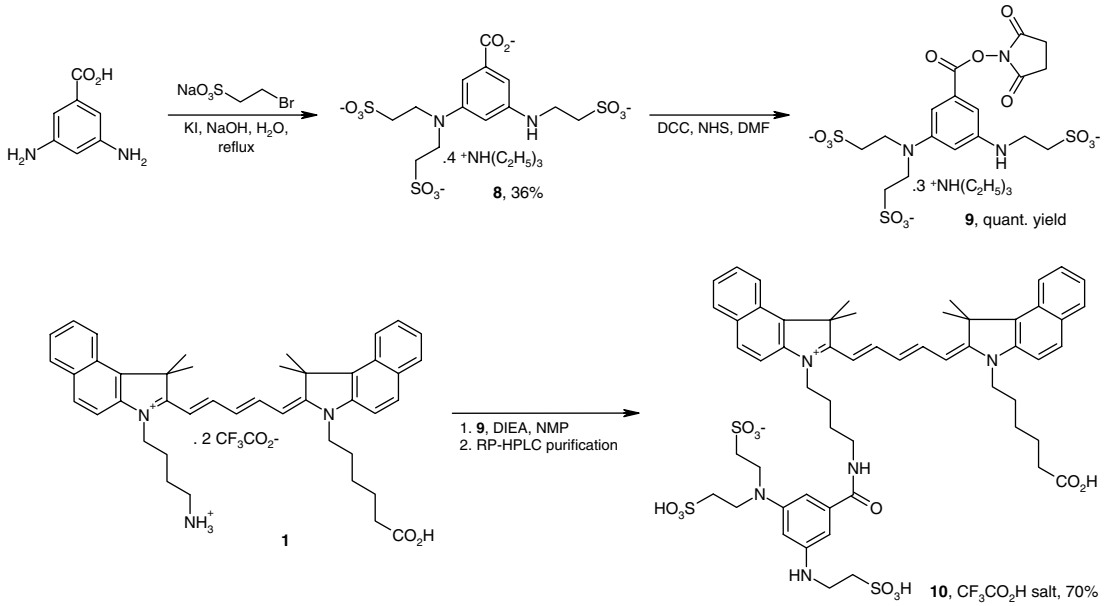
Spectral properties of cyanine dye Cy 5.5 and its analogue **10** in phosphate-buffered saline (PBS) are summarized in Table 1. As expected, **10** displays absorption and emission bands that closely match those of Cy 5.5. The lower quantum yield may be explained by the presence of the aryl group **8**. Indeed, aromatic amines are known to quench the excited state of some fluorophores by an electron transfer mechanism.[32,33] However, the fluorescence efficiency of **10** is compatible with its use as a donor in NIR molecular probes, which relies on the fluorescence resonance energy transfer (FRET) process.

In addition to these characterizations, we have checked that the reactivity of the free carboxylic acid function

[‡] No formation of symmetrical dicarboxylic acid cyanine dye was observed when the reaction mixture was heated under reflux for 1 h. The completion of this reaction was carefully monitored by UV–visible spectrometry: hemicyanine intermediate **4** and symmetrical dicarboxylic acid cyanine dye have an absorption maximum around 455 and 680 nm, respectively.

[§] An unsymmetrical derivative bearing the ethyl and carboxypentyl substituents is also commercially available but its synthesis is not described in the literature.

[¶] Reactions with 1,3-propanesultone were performed with the following bases: NaH, aq NaOH, K_2CO_3, DIEA and TEA in various solvents (i.e., CH_3CN, DMF, MeOH, NMP and THF) at room temperature or under reflux. Attempts of sulfonation with sodium 2-bromoethane sulfonate were achieved under the following conditions: KI, DIEA, DMF, 80 °C or reflux and aq NaOH, reflux.



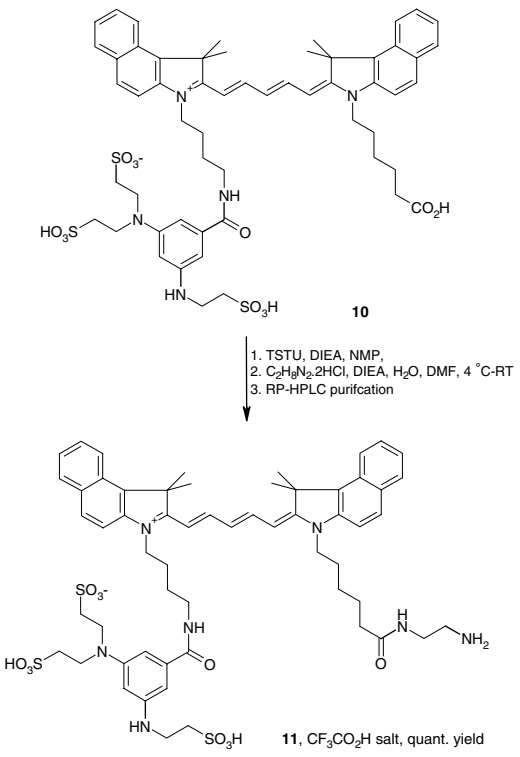
Scheme 2. Synthesis of the novel water-soluble NIR dye.

Table 1. Spectral properties of cyanine dye Cy 5.5 and its analogue **10** in PBS buffer

Cyanine dye	$\lambda_{max,abs}$ (nm)	$\lambda_{max,em}$ (nm)	Stokes shift (nm)	ε (dm^3 mol^{-1} cm^{-1})	Relative QY[b]
Cy 5.5[a]	674	694	20	195 000	0.23
10	680	710	30	197 000	0.05

[a] Values reported by Mujumdar et al.[28]

[b] Determined at 25 °C by using cyanine dye Cy 5.0 in PBS buffer (QY = 0.20, according to Ref. 37) as standard.



Scheme 3.

of **10** remains unchanged compared to monofunctional cyanine dyes such as Cy 3.0, Cy 5.0, or Cy 5.5. Thus, **10** was quantitatively converted into the amino derivative **11** by using the two-step procedure depicted in Scheme 3, usually applied with the traditional cyanine dyes.[34]

In conclusion, we have presented here the first synthesis of a fluorescent cyanine-based amino acid. This convertible fluorophore was employed to design a novel high yield synthetic route for the preparation of an original analogue of cyanine dye Cy 5.5. Furthermore, preliminary experiments have clearly shown that it is possible to convert this amino acid into the corresponding *N*-Fmoc building block suitable for both solid-phase and solution peptide synthesis. Indeed, there is a growing need to develop such fluorescent amino acid monomers that could be incorporated at selected position during the peptide assembly, especially for the synthesis of fluorescent peptide ligands and fluorogenic protease substrates.[‖]

3. Spectroscopic characterisations of 1, 8 and 10

3.1. Cyanine-based amino acid (1)

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04–7.27 (m, 14H), 6.68 (t, $J = 12.5$ Hz, 1H), 6.29 (d, $J = 13.5$ Hz, 1H), 6.16 (d, $J = 13.5$ Hz, 1H), 4.10 (bm, 2H), 4.00 (bm, 2H), 3.11 (bm, 2H), 2.32 (t, $J = 6.9$ Hz, 2H), 1.94 (s, 12H), 1.94–1.25 (m, 10H); ^{13}C NMR (75.5 MHz, $CDCl_3 + CD_3OD$) δ 180.2, 178.3 (2C), 156.4 (2C), 143.5 (2C), 138.2, 137.9,

[‖] To our knowledge, only two papers, which describe the synthesis of fluorescein-based amino acids, have addressed this issue.[35,36]

136.4 (2C), 135.1 (3C), 134.4 (3C), 132.4 (2C), 132.2, 129.8, 129.6, 129.5, 126.2 (2C), 114.7, 107.2 (2C), 55.5, 55.4, 48.4, 47.8, 43.3, 37.9, 31.7 (4C), 30.5, 28.9 (2C), 28.7 (2C); HPLC (*System A*): $t_R = 19.0$ min. UV/vis (recorded during the HPLC analysis): $\lambda_{max} = 674$ nm; MS (MALDI-TOF, positive mode): *m/z*: calcd for $C_{43}H_{49}N_3O_2$ 639.89, found: 640.45 $[M+H]^+$; elemental analysis calcd. (%) for $C_{43}H_{49}N_3O_2{\cdot}2C_2HF_3O_2$ (TFA)$\cdot 5H_2O$: C, 58.93; H, 6.42; N, 4.39; found: C, 59.08; H, 6.48; N, 4.49.

3.2. Trisulfonated linker (8)

^{1}H NMR (300 MHz, D_2O) δ 6.78 (s, 1H), 6.71 (s, 1H), 6.32 (s, 1H), 3.77 (m, 6H, N–CH_2–CH_2–SO_3^-), 3.11 (q, $J = 14.7$ Hz, $J = 7.1$ Hz, 30H, TEA and N–CH_2–CH_2–SO_3^-), 1.21 (t, $J = 7.2$ Hz, 36H, TEA); ^{13}C NMR (75.5 MHz, D_2O) δ 175.1, 148.8, 147.7, 138.7, 104.9, 104.2, 100.8, 49.8 (2C), 48.3 (4C), 48.1 (4C), 46.9 (12C), 39.9 (2C), 8.5 (12C); HPLC (*System B*): $t_R = 7.0$ min. UV/vis (recorded during the HPLC analysis): $\lambda_{max} = 229$, 334 nm; MS (ESI, negative mode): *m/z*: calcd for $C_{13}H_{20}N_2O_{11}S_3$ (acid form) 476.50, found: 157.93 $[M-3H]^{3-}$, 237.33 $[M-2H]^{2-}$, 475.20 $[M-H]^-$.

3.3. Water-soluble NIR dye (10)

^{1}H NMR (300 MHz, DMSO-d_6 + TFA-d_1) δ 8.56 (m, 2H), 8.47 (t, $J = 12.8$ Hz, 1H), 8.27 (m, 2H), 8.11 (m, 4H), 7.84–7.68 (m, 4H), 7.53 (m, 2H), 7.28 (br s, N*H*), 7.07 (br s, 1H, N*H*), 7.01 (br s, 1H, N*H*), 6.89 (br s, 1H, N*H*), 6.72 (t, $J = 12.5$ Hz, 1H), 6.44 (dd, 2H), 4.28 (m, 4H), 3.75 (m, 6H, N–CH_2–CH_2–SO_3^-), 2.83 (m, 2H), 2.72 (m, 6H, N–CH_2–CH_2–SO_3^-), 2.24 (t, $J = 7.2$ Hz, 2H), 1.99 (s, 12H), 1.83–1.01 (m, 10H). HPLC (*System A*): $t_R = 18.4$ min; MS (MALDI-TOF, positive mode): *m/z*: calcd for $C_{56}H_{66}N_5O_{12}S_3$ (acid form) 1097.37, found: 1098.89 $[M+H]^+$; MS (ESI, negative mode): *m/z*: 400.93 $[M-3H+TEA]^{3-}$, 601.87 $[M-2H+TEA]^{2-}$.

The chromatographic systems used for the analytical experiments are the following: *System A*: RP-HPLC (Thermo Hypersil GOLD C_{18} column, 5 μm, 4.6 × 150 mm) with CH_3CN and 0.1% aqueous trifluoroacetic acid (aq TFA, 0.1%, v/v, pH 2.0) as the eluents [80% aq TFA (5 min), linear gradient from 20% to 40% of CH_3CN (5 min) and 40% to 100% (50 min)] at a flow rate of 1.0 mL/min. Dual UV/visible detection was achieved at 260 and 650 nm. *System B*: RP-HPLC (Thermo Hypersil GOLD C_{18} column, 5 μm, 4.6 × 150 mm) with CH_3CN and triethylammonium acetate buffer (TEAA, 0.1 M, pH 7.0) as the eluents [100% TEAA (10 min), linear gradient from 0% to 60% (30 min) of CH_3CN] at a flow rate of 1.0 mL/min. Dual UV detection was achieved at 260 and 330 nm.

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References and notes

1. Mishra, A.; Behera, R. K.; Behera, P. K.; Mishra, B. K.; Behera, G. B. *Chem. Rev.* **2000**, *100*, 1973–2011.
2. Almeida, P. *Quimica* **1999**, *73*, 9–12.
3. Williams, D. J. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 690–703.
4. Gómez-Hens, A.; Aguilar-Caballos, M. P. *Trends Anal. Chem.* **2004**, *23*, 127–136.
5. Patonay, G.; Salon, J.; Sowell, J.; Strekowski, L. *Molecules* **2004**, *9*, 40–49.
6. Tung, C.-H. *Biopolymers* **2004**, *76*, 391–403.
7. Kumar, S. Fluorescent dye nucleotide conjugates for DNA sequencing. In *Modified Nucleosides: Synthesis and Applications*; Loakes, D., Ed.; Research Signpost: Trivandrum (India), 2002; pp 87–110.
8. Mitra, R. D.; Shendure, J.; Olejnik, J.; Krzymanska-Olejnik, E.; Church, G. M. *Anal. Biochem.* **2003**, *320*, 55–65.
9. Weissleder, R.; Ntziachristos, V. *Nat. Med.* **2003**, *9*, 123–128.
10. Tonge, R.; Shaw, J.; Middleton, B.; Rowlinson, R.; Rayner, S.; Young, J.; Pognan, F.; Hawkins, E.; Currie, I.; Davison, M. *Proteomics* **2001**, *1*, 377–396.
11. Mason, S. J.; Balasubramanian, S. *Org. Lett.* **2002**, *4*, 4261–4264.
12. Mason, S. J.; Hake, J. L.; Nairne, J.; Cummins, W. J.; Balasubramanian, S. *J. Org. Chem.* **2005**, *70*, 2939–2949.
13. Narayanan, N.; Patonay, G. *J. Org. Chem.* **1995**, *60*, 2391–2395.
14. Narayanan, N.; Strekowski, L.; Lipowska, M.; Patonay, G. *J. Org. Chem.* **1997**, *62*, 9387.
15. Flanagan, J. H., Jr.; Khan, S. H.; Menchen, S.; Soper, S. A.; Hammer, R. P. *Bioconjugate Chem.* **1997**, *8*, 751–756.
16. Song, F.; Peng, X.; Lu, E.; Zhang, R.; Chen, X.; Song, B. *J. Photochem. Photobiol. A: Chem.* **2004**, *168*, 53–57.
17. Peng, X.; Song, F.; Lu, E.; Wang, Y.; Zhou, W.; Fan, J.; Gao, Y. *J. Am. Chem. Soc.* **2005**, *127*, 4170–4171.
18. Strekowski, L.; Mason, C. J.; Lee, H.; Gupta, R.; Sowell, J.; Patonay, G. *J. Heterocycl. Chem.* **2003**, *40*, 913–916.
19. Song, F.; Peng, X.; Lu, E.; Wang, Y.; Zhou, W.; Fan, J. *Tetrahedron Lett.* **2005**, *46*, 4817–4820.
20. Hilderbrand, S. A.; Kelly, K. A.; Weissleder, R.; Tung, C.-H. *Bioconjugate Chem.* **2005**, *16*, 1275–1281.
21. Kumar, S.; Nampalli, S.; Finn, P. J.; Sudhakar Rao, T.; Chen, C.-Y.; Flick, P. K.; Fuller, C. W. *Nucleosides Nucleotides Nucleic Acids* **2003**, *22*, 1595–1598.
22. Sudhakar Rao, T.; Zhang, W.; Xiao, H.; Flick, P. K.; Kumar, S.; Nampalli, S. *Nucleosides Nucleotides Nucleic Acids* **2003**, *22*, 1443–1445.
23. Jiao, G.-S.; Thoresen, L. H.; Burgess, K. *J. Am. Chem. Soc.* **2003**, *125*, 14668–14669.
24. Portoghese, P. S.; Sultana, M.; Takemori, A. E. *J. Med. Chem.* **1990**, *33*, 1714–1720.
25. Kappe, T.; Fruhwirth, F.; Roschger, P.; Jocham, B.; Kremsner, J.; Stadlbauer, W. *J. Heterocycl. Chem.* **2002**, *39*, 391–397.
26. Clavé, G.; Bernardin, A.; Massonneau, M.; Renard, P.-Y.; Romieu, A. *Tetrahedron Lett.* **2006**, *47*, 6229–6233.

27. Hirata, T.; Kogiso, H.; Morimoto, K.; Miyamoto, S.; Taue, H.; Sano, S.; Muguruma, N.; Ito, S.; Nagao, Y. *Bioorg. Med. Chem.* **1998**, *6*, 2179–2184.
28. Mujumdar, S. R.; Mujumdar, R. B.; Grant, C. M.; Waggoner, A. S. *Bioconjugate Chem.* **1996**, *7*, 356–362.
29. Marvel, C. S.; Sparberg, M. S. *Org. Synth. Coll.* **1930**, *10*, 96–97.
30. Nunes de Jesús, M.; Hioka, N.; Ferreira da Costa, W.; Maionchi, F. *J. Phys. Org. Chem.* **2002**, *15*, 617–622, and references cited therein.
31. Ho, N.-H.; Weissleder, R.; Tung, C.-H. *Tetrahedron* **2006**, *62*, 578–585.
32. Kumbhakar, M.; Nath, S.; Mukherjee, T.; Pal, H. *J. Chem. Phys.* **2004**, *120*, 2824–2834.
33. Kumbhakar, M.; Nath, S.; Rath, M. C.; Mukherjee, T.; Pal, H. *Photochem. Photobiol.* **2004**, *79*, 1–10.
34. Gruber, H. J.; Kada, G.; Pragl, B.; Riener, C.; Hahn, C. D.; Harms, G. S.; Ahrer, W.; Dax, T. G.; Hohenthanner, K.; Knaus, H. G. *Bioconjugate Chem.* **2000**, *11*, 161–166.
35. Lohse, J.; Nielsen, P. E.; Harrit, N.; Dahl, O. *Bioconjugate Chem.* **1997**, *8*, 503–509.
36. Burchak, O. N.; Mugherli, L.; Chatelain, F.; Balakirev, M. Y. *Bioorg. Med. Chem.* **2006**, *14*, 2559–2568.
37. Mujumdar, R. B.; Ernst, L. A.; Mujumdar, S. R.; Lewis, C. J.; Waggoner, A. S. *Bioconjugate Chem.* **1993**, *4*, 105–111.