Available online at www.sciencedirect.com

ScienceDirect

Mendeleev Commun., 2021, **31**, 70–72

Mendeleev Communications

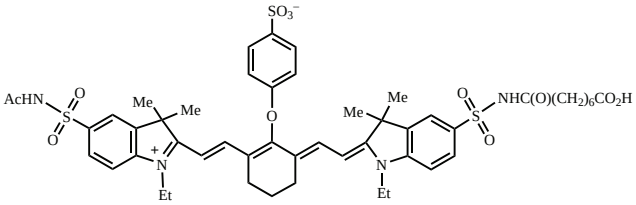
Derivatization of a rigid *meso*-substituted heptamethine cyanine dye

Valeriy E. Shershov, Viktoriya E. Kuznetsova, Rinat A. Miftakhov, Sergey A. Lapa, Andrey A. Stomahin, Edward N. Timofeev, Irina V. Grechishnikova, Alexander S. Zasedatelev and Alexander V. Chudinov*

V. A. Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, 119991 Moscow, Russian Federation. Fax: +7 499 135 1405; e-mail: chud@eimb.ru

DOI: 10.1016/j.mencom.2021.01.021

A novel electroneutral rigid *meso*-sulfophenyloxy substituted heptamethine dye was synthesized in six steps. Selective derivatization of one sulfonamide group with octanedioic acid introduced the carboxy end group attached through the hexamethylene linker, which provided the dye solubility in water. Absorbance of the dye in the near infrared region makes it promising for covalent labeling of biomolecules.



Keywords: electroneutral dyes, cyanine dyes, heptamethine dyes, *meso*-sulfophenyloxy dyes, sulfonamides, acetylation, *N*-acylsulfonamides, fluorescently labeled nucleotides.

Optical imaging techniques occupy a very important place in medical diagnostics.[1] One of the most significant improvements is to develop new fluorophores with improved chemical structure, photophysical properties and solubility characteristics.[2] Heptamethine cyanine dyes represent a wide class of near infrared (NIR) fluorescent charged chromophores absorbing in the region of low autofluorescence of biological samples.[3] Various structural modifications have been attempted to improve their overall photophysical properties.[4] These modifications include the attainment of symmetry in the heptamethine dye structure and rigidization of the polymethine chain in order to inhibit subsequent isomerization and increase stability.[5] Also, six-membered ring with *meso*-chlorine atom containing cyanine fluorophores has an improved performance in derivatization reactions.[6]

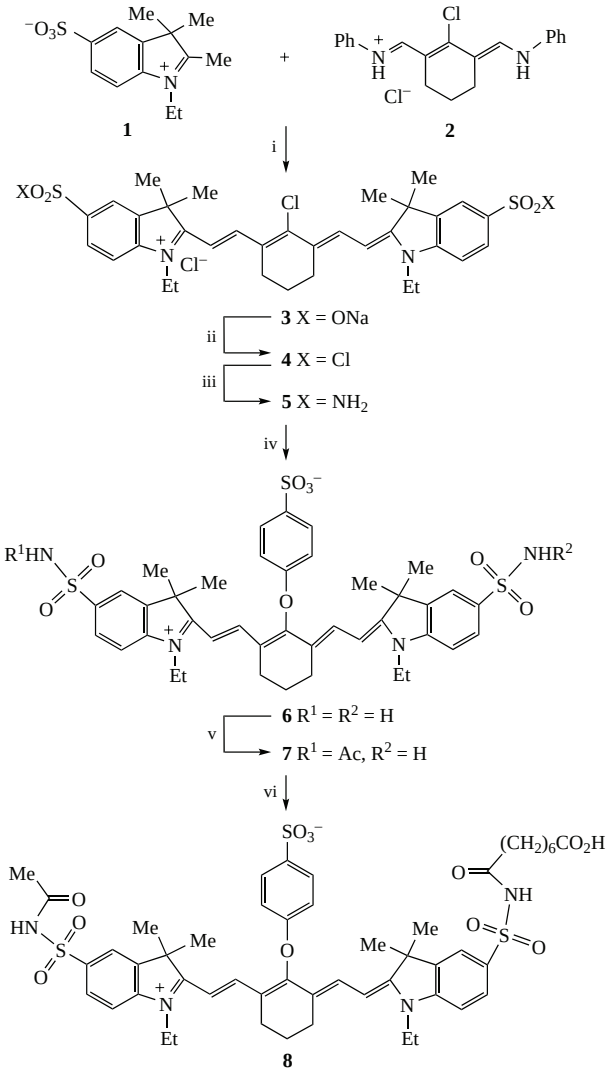
In continuation of our research on cyanine dye chemistry,[7] we focuse much attention on the synthesis of electroneutral rigid heptamethine dye with enhanced water solubility and stability. In this study, the synthetic route described in Scheme 1 provided a direct conversion of sulfo groups in cyanine dyes to the corresponding N-acetylated sulfonamides leading to a brighter, photo- and chemo stable fluorophore with a decreased tendency to aggregation. The sulfonamide group was introduced after the symmetrical disulfo dye was synthesized. This allowed us to accomplish an efficient synthetic protocol which required no complex purification methods for unstable hydrophobic substituted indolenine precursors. Symmetry factor simplifies the synthetic method by reducing by-product formation and also allows simple purification to be used for the water-soluble dyes.

In general, synthesis of the modified cyanine dye **8** was accomplished in six chemical steps starting from 1-ethyl-2,3,3-trimethylindolenin-1-ium-5-sulfonate **1** as a common building block for the two moieties and *meso*-chlorine substituted six-membered compound **2** as a part of the heptamethine linker (see Scheme 1). The symmetrical chlorine-cyanine dye **3** with a rigid polymethine chain was obtained from these reactants by heating them (60 °C) in ethanol–acetic anhydride mixture in the presence of potassium acetate as the catalyst[8] in a yield of ~43%.

Since the amidation reaction of arylsulfonic derivatives is well studied,[9] we tried a procedure where bis-sulfonamide **5** could be prepared by reaction of ammonium hydroxide with the corresponding sulfonyl chloride **4** as a reactive intermediate.[10] Bis-sulfonyl chloride **4** is best prepared by the reaction of disodium disulfonate **3** with phosphorus oxychloride as both chlorinating agent and solvent. Due to thermal instability of compound **4**, the reaction temperature must not be raised above 90 °C while the desired sulfonyl chloride **4** is formed at 80 °C within 2 h, the fraction of monochloride being luckily low. Excess $POCl_3$ can be readily distilled off, and the crude material was pure enough for the use at the next step. Tetraethylammonium analogue of salt **3** may be also utilized without significant decrease in yield or reaction efficiency. Addition of pyridine or triethylamine to scavenge the generated hydrochloric acid showed no improvement of conversion. The use of trimethyl phosphate as the solvent and phosphorus pentachloride gave lower yield of bis-sulfonyl chloride **4**, the destruction of the cyanine chromophore having occurred. Bis-sulfonamide **5** was prepared as the major compound by reaction of bis-sulfonyl chloride **4** with an excess of an ammonium hydroxide. Due to its solubility and convenience, acetonitrile has been chosen as the solvent at this step. The optimal reaction temperature and reaction time provided minimal formation of by-products.

The central chlorine atom in cyanine dye structure can be efficiently replaced by nucleophiles.[11] In our case, chloro dye **5** was reacted with disodium derivative of 4-hydroxybenzenesulfonic acid in DMSO at 70 °C to afford *meso*-sulfophenyloxy dye **6**. Interestingly, the nature of counter-anion in chloro dye **5** has a significant influence on the yield of the desired product **6**. To analyze the effects of anion selection on chloro cyanine dye **5**, six anions such as acetate, chloride, bromide, perchlorate, hydrocarbonate and tosylate with varying size and charge distributions were tested.[12] It was found that chloro dye **5** with non-covalently bound tosylate and chloride anion are good substrates for this reaction.

Bis-sulfonamide **6** was subjected to mono-acetylation with acetic anhydride at 50 °C in DMF in the presence of *N*,*N*-diiso-



Scheme 1 *Reagents and conditions*: i, EtOH, Ac_2O, AcOK, 60 °C, 7 h, then ion exchange (43%); ii, $POCl_3$, 80 °C, 2 h; iii, NH_3 aq., MeCN, –18 °C; iv, 4-$NaOC_6H_6SO_3Na$, DMSO, 70 °C, 9 h (14%); v, Ac_2O, DMF, 50 °C, 30 min (40%); vi, $HOOC(CH_2)_6COOH$, EDC, DMAP, DMF, DIPEA, N_2, 2 h (42%).

propylethylamine for 30 min (optimized conditions[13]), and product **7** was isolated in ~40% yield after a C18-RP column chromatography, while N,N′-diacetylated product was formed in trace amount. Major synthetic difficulties at this step are related to the poor solubility of compound **6** even in polar protic solvents. According to the literature,[14] sodium hydride was applied to make the sulfonamidic nitrogen atom more nucleophilic, however in the case of compound **6**, *N*,*N*′-diacetyl derivative was immediately (3 min) formed at room temperature.

The subsequent direct coupling of mono-acetylsulfonamide **7** with octane-1,8-dioic acid was carried out according to our previously published approach.[7(a)] The sulfonamide moiety undergoes acylation when significant excesses of carboxylic acid, EDC, DMAP and DIPEA were used.[15] In our hands, the desired product **8** was obtained in a yield of only about 42%. It should be noted that the usage of a large excess of DMAP results in the degradation of the chromophore dye system and decrease in the yield of product **8**. Also, the reaction must be carried out at room temperature because heating leads to decomposition of the chromophore and a sharp decrease in the yield of the desired product **8**.[†]

Table 1 Spectroscopic characteristics of heptamethine dyes.[a]

Dye	Solvent	λ_{abs}/nm	λ_{em}/nm	$\varepsilon/10^{-5}$ dm^3 mol^{-1} cm^{-1}	Φ_F (%)
IRDye 800	PBS	771	786	2.4 ± 0.03	14
	MeOH	773	789	3.0 ± 0.03	26
3	PBS	779	793	1.6 ± 0.02[b]	8
	MeOH	785	802	–	12
8	PBS	774	794	2.8 ± 0.02	14
	MeOH	776	795	3.1 ± 0.02	23

[a] ε – molar extinction coefficient; Φ_F – relative fluorescence quantum yield; PBS – 10 mM potassium phosphate buffer solution, 0.9% NaCl, pH 7.4. Reference standard IRDye® (Li-Cor), Φ_F 0.14 in PBS at 25 °C. [b] Molar extinction coefficient was published by Song *et al*.[16]

The results of spectroscopic studies of the synthesized dyes are given in Table 1. Among the parent cyanine dyes investigated, the commercially available cyanine dye IRDye 800 is one of the most popular fluorophores used as a marker in life science applications. The structural features of modified dye **8** are designed based on those of the standard IRDye 800, hence, their spectral properties are expected to be close.

In addition, other important requirements such as photo- and thermal stability were determined using our previously published procedure.[8] The photo-fading behavior of commercial IRDye 800 and modified dye **8** upon irradiation of their solutions with a 60-W light bulb are equal. After 6 h of irradiation, both dyes showed ~50% photo-fading. The thermal decomposition of IRDye 800 and dye **8** upon heating their 10–6 M solutions in Milli-Q water at 95 °C for 6 h showed about 50% chromophore destruction as well as photostability.

To demonstrate the usefulness of the additive N-acetylated sulfonamide linker with carboxyl end group into rigid *meso*-sulfophenyloxy substituted heptamethine dye structure, we investigated the enzymatic incorporation of fluorescently labeled nucleotide into DNA using microarray.[7] The synthesized dye–nucleotide conjugate is incorporated into DNA by Taq, Vent (exo-) and deep Vent (exo-) DNA-polymerases. The dye **8** was used to control thermal cleavage of oligonucleotide primers from agarose on a chip assay.

In conclusion, the post-synthetic derivatization of a rigid *meso*-substituted heptamethine cyanine dye by conversion of its sulfo group into an N-acylated sulfonamide linker with carboxyl end group has been accomplished. The spectral characteristics, thermal- and photo stabilities of the modified dye with geometrically balanced structure and neutral charge are equal to those of the commercial IRDye®800 with negative charge.

This study was supported by grant no. 17-04-02062 from the Russian Foundation for Basic Research and by the program ‘The Scholarship of the President of the Russian Federation for 2018–2020 to young scientists and post-graduate students conducting promising scientific research on modernization of the Russian economy’.

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2021.01.021.

References

1 (*a*) C. Shi, J. B. Wu and D. Pan, *J. Biomed. Opt.*, 2016, **21**, 50901; (*b*) H. Hyun, E. A. Owens, H. Wada, A. Levitz, G. Park, M. H. Park, J. V. Frangioni, M. Henary and H. S. Choi, *Angew. Chem., Int. Ed.*, 2015,

[†] For the syntheses and characteristics of compounds **1**–**8**, see Online Supplementary Materials.

54, 8648; (*c*) E. A. Owens, M. Henary, G. Fakhri and H. S. Choi, *Acc. Chem. Res*., 2016, **49**, 1731; (*d*) J. Kasprzak, D. Samsel, A. Borkowska and M. Kecik, *Acta Phys. Pol., A*, 2011, **120**, 678; (*e*) K. S. Levchenko, K. A. Chudov, E. V. Zinoviev, K. A. Lyssenko, A. N. Fakhrutdinov, D. U. Demin, N. O. Poroshin, A. A. Zhukova, P. S. Shmelin and E. P. Grebennikov, *Mendeleev Commun*., 2019, **29**, 301.

2 (*a*) H. Lee, J. C. Mason and S. Achilefu, *J. Org. Chem*., 2006, **71**, 7862; (*b*) T. Liu, S. Luo, Y. Wang, X. Tan, Q. Qi and C. Shi, *Drug Des., Dev. Ther.*, 2014, **8**, 1287; (*c*) S. M. Usama and K. Burgess, *Molecules*, 2018, **23**, 2900; (*d*) B. Chipon, G. Clave, C. Bouteiller, M. Massonneau, P.-Y. Renarda and A. Romieu, *Tetrahedron Lett.*, 2006, **47**, 8279; (*e*) S. Thavornpradit, S. M. Usama, G. K. Park, J. P. Shrestha, S. Nomura, Y. Baek, H. S. Choi and K. Burgess, *Theranostics*, 2019, **9**, 2856; (*f*) A. F. Mironov, K. A. Zhdanova and N. A. Bragina, *Russ. Chem. Rev*., 2018, **87**, 859; (*g*) K. A. Zhdanova, A. V. Ezhov, N. A. Bragina and A. F. Mironov, *Mendeleev Commun*., 2018, **28**, 626.

3 (*a*) C. L. Amiot, S. Xu, S. Liang, L. Pan and J. X. Zhao, *Sensors*, 2008, **8**, 3082; (*b*) G. Deng, S. Li, Z. Sun, W. Li, L. Zhou, J. Zhang, P. Gong and L. Cai, *Theranostics*, 2018, **8**, 4116; (*c*) K. Vus, U. Tarabara, A. Kurutos, O. Ryzhova, G. Gorbenko, V. Trusova, N. Gadjev and T. Deligeorgiev, *Mol. BioSyst*., 2017, **13**, 970.

4 (*a*) O. A. Okoh, M. E. Critchley, R. H. Bisby, C. L. Lawrence, M. Wainwright and R. B. Smith, *Color. Technol*., 2019, **135**, 305; (*b*) M. Henary, S. Paranjpe and E. A. Owens, *Heterocycl. Commun*., 2013, **19**, doi.org/10.1515/hc-2013-0026; (*c*) T. A. Podrugina, A. S. Pavlova, I. A. Doroshenko, V. A. Kuz'min, A. A. Kostyukov and A. A. Shtil', *Russ. Chem. Bull., Int. Ed*., 2018, **67**, 806 (*Izv. Akad. Nauk, Ser. Khim*., 2018, 806); (*d*) V. V. Prokhorov, S. I. Pozin, O. M. Perelygina and E. I. Mal'tsev, *Mendeleev Commun.*, 2019, **29**, 450.

5 N. S. James, Y. Chen, P. Joshi, T. Y. Ohulchanskyy, M. Ethirajan, M. Henary, L. Strekowski and R. K. Pandey, *Theranostics*, 2013, **3**, 692.

6 A. B. Braun, I. Wehl, D. K. Kölmel, U. Schepers and S. Bräse, *Chem. – Eur. J*., 2019, **25**, 7998.

7 (*a*) V. E. Shershov, V. E. Kuznetsova, S. A. Lapa, M. A. Spitsyn, T. O. Guseinov, Ya. V. Tkachev, A. S. Zasedatelev and A. V. Chudinov, *Mendeleev Commun*., 2017, **27**, 360; (*b*) M. A. Spitsyn, V. E. Kuznetsova, V. E. Shershov, M. A. Emelyanova, T. O. Guseinov, S. A. Lapa, T. V. Nasedkina, A. S. Zasedatelev and A. V. Chudinov, *Dyes Pigm.*, 2017, **147**, 199.

8 V. E. Shershov, M. A. Spitsyn, V. E. Kuznetsova, E. N. Timofeev, O. A. Ivashkina, I. S. Abramov, T. V. Nasedkina, A. S. Zasedatelev and A. V. Chudinov, *Dyes Pigm*., 2013, **97**, 353.

9 (*a*) G. Caputo and C. L. Della, *Patent EP 1491591 A1*, 2004; (*b*) L. Malet-Sanz, J. Madrzak, S. V. Leya and I. R. Baxendale, *Org. Biomol. Chem*., 2010, **8**, 5324.

10 A. Tačić, V. Nikolić, L. Nikolić and I. Savić, *Adv. Technol*., 2017, **6**, 58.

11 (*a*) J. H. Flanagan, S. H. Khan, S. Menchen, S. A. Soper and R. P. Hammer, *Bioconjugate Chem*., 1997, **8**, 751; (*b*) M. Lipowska, G. Patonay and L. Strekowski, *Synth. Commun*., 1993, **23**, 3087.

12 J. N. Gayton, S. Autry, R. C. Fortenberry, N. I. Hammer and J. H. Delcamp, *Molecules*, 2018, **23**, 3051.

13 (*a*) M. Berredjem, F. Bouchareb, S. A. Kaki, M. Dekhil and N.-E. Aouf, *Arabian J. Chem.*, 2017, **10**, S1095; (*b*) J. F. Riordan and B. L. Vallee, *Methods Enzymol*., 1967, **11**, 565.

14 M. Trávnícek and M. Potáček, *Molecules*, 1999, **4**, 238.

15 A. R. Massah, M. Dabagh, M. Afshar, A. R. Momeni, H. Aliyan and H. J. Naghash, *Turk. J. Chem*., 2007, **31**, 611.

16 F. Song, X. Peng, E. Lu, R. Zhang, X. Chen and B. Song, *J. Photochem. Photobiol., A*, 2004, **168**, 53.

17 A. Levitz, F. Marmarchi and M. Henary, *Molecules*, 2018, **23**, 226.

Received: 30th October 2020; Com. 20/6351