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Organic solvent-free synthesis of Cy39 iodide

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ABSTRACT

The iodide salt of the pyrylium dye Cy39 (Cy39I, **1**) has been suggested as an alternative of its perchlorate salt, which is widely used for biolabeling applications. A new efficient organic solvent-free procedure for synthesis of **1**, allowing for a greener approach to the cyanine dyes, is suggested. The unique combination of solubility and thermostability of both the starting materials and the target Cy39 salt made the performance of the synthesis in an aqueous solution as well as reusing of unreacted starting materials possible.

The pyrylium dye Cy39 perchlorate (Cy39ClO$_4$, **2**) is widely used for labeling amino-terminated molecules and surfaces such as aminoacids, proteins, DNA, and polymer particles with formation of cyanine dyes.[1–3] In 1962, Kelemen and Wizinger first reported the synthesis of the perchlorate and tetrafluoroborate salts of Cy39.[1b] However, the perchlorate salt **2** has never been properly described in the literature. This compound had previously been synthesized by condensation of 2,3-dimethylbenzo-1,3-thiazolium methosulfonate **3** with 2,6-dimethyl-4-pyrone **4** in acetic anhydride (Scheme 1), followed by sodium perchlorate precipitation.[1a] Unfortunately, the previous patent provides neither the yield nor characterization of the final product **3**.[1a] Examination of the literature also yields very little in terms of a complete procedure, including a publication of the tetraflouroborate salt of 2,3-dimethylbenzo-1,3-thiazolium.[1b] A tosylate salt of Cy39 has been studied as a DNA intercalating agent, but neither the procedure for synthesis nor characterization of the dye was reported.[4]

Herein, we suggest a simple and fast procedure[5] for the synthesis of the iodide salt of Cy39 **1** in an organic solvent-free aqueous solution, starting from easily available 2,3-dimethylbenzo-1,3-thiazolium iodide **6**[6] (Scheme 2).

The structure of **1** is consistent with its ^{1}H, ^{13}C NMR, and IR-spectra.[7] Additionally, **1** was converted to the cyanine dye D7, which was identical to the known compound (^{1}H NMR in DMSO-d_6).[3] We found that the known synthesis of the perchlorate **3**[1a] is reproducible, but the reaction product was significantly (ca. 30 mol %) contaminated by 2,3-dimethyl-1,3-thiazolium. We treated the crude **3** with hot water, filtered out, and dried on air to afford a pure sample with ^{1}H NMR that is identical to the spectrum of the iodide **1**. The yield of pure **3** (23%) was lower than the yield of pure iodide **1** according to our procedure (35%). Evaporation of combined organic filtrates yields a mixture of pure starting materials **5** and **6** that can be used again to produce additional amounts of **1**. A simplified solvent-free version of our procedure[8] has been introduced to the undergraduate laboratory chemistry curriculum, and allows instantaneous preparation of the salt **1** with an average yield of 20%.

The modified (the step of precipitation by NaClO$_4$ is omitted; the reaction product was treated with hot water, cooled, filtered, and washed with water to produce a pure sample) known procedure[1] for the synthesis of perchlorate **3** is also applicable for the iodide **1** (yield 45%); however, it takes 11 h and does not allow for the unreacted starting materials to be reused.

Success of the reaction is determined by solubility and thermostability of both the starting materials and the target Cy39 salt. The unique combination of these parameters takes place for the suggested synthesis of Cy39I in the presence of HClO$_4$. We found that variations in the structure of starting materials, catalysts, and reaction conditions detrimentally affected the yield of the product. For instance, attempts to apply these reaction conditions[5] to the methosulfonate salt **4**, followed by NaClO$_4$ precipitation, yielded **3** only at low yields, not exceeding 5%. Low solubility of 2,3-dimethyl-1,3-thiazolium perchlorate in water makes its separation from the reaction product difficult, and the low thermostability of 2,3-dimethyl-1,3-thiazolium methosulfonate **3** does not allow to bring the reaction to a practical extent of conversion. Replacement of HClO$_4$ with H$_2$SO$_4$ decreased the yield of Cy39I **1** to 15%, and the rest of the reaction mixture was unusable due to the presence of side products in significant amounts. Utilization of polyphosphoric acid (47 mg per 0.25 mmol of **4**) instead of HClO$_4$ was able to produce Cy39I **1** only at 25% if the 14-fold excess of 2,3-dimethylbenzo-1,3-thiazolium iodide **6** was taken. Lowering the reaction temperature from 190 °C to 150 °C led to the decrease in yield from 35% to 5%. Replacement of 2,3-dimethylbenzo-1,3-thiazolium iodide **6** with 1,2,3,3-tetramethyl-3a,7a-dihydro-3*H*-indolium iodide **7** produced only a dark mixture of unidentified compounds. Variation of the pyrone derivative was equally inefficient. Thus, no reaction had taken place between 2,3-dimethylbenzo-1,3-thiazolium iodide **6** and 4-oxo-4*H*-pyran-2,6-

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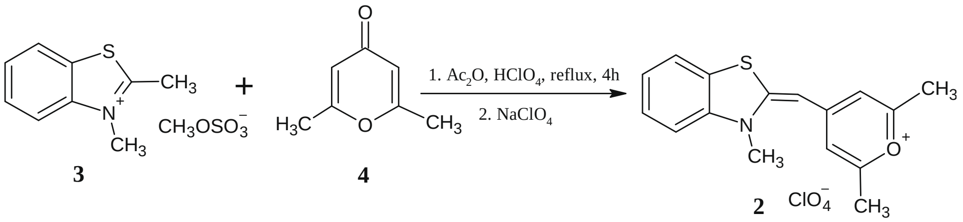
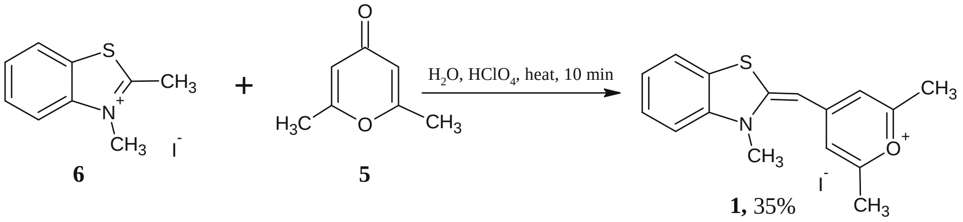


Scheme 1. Synthesis of Cy39 perchlorate **3**.



Scheme 2. Synthesis of Cy39 iodide **1**.

dicarboxylic acid **8** under the original conditions.[5] Finally, presence of the methyl group at the quaternary nitrogen in the benzothiazole derivative **3** proved to be essential for the successful condensation. Although the butyl analogue of Cy39I was detected by ^{1}H NMR in the reaction mixture of 3-butyl-2-methyl-1,3-benzothiazol-3-ium iodide **9** and 2,6-dimethyl-4-pyrone **4**, isolation of the pyrylium dye could not be easily performed due to the presence of significant amounts of side products. Therefore, the iodide **1** possesses a unique set of properties (solubility, stability, and reactivity) that makes the suggested aqueous process successful, practical, and waste-free.

Acknowledgments

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References and notes

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5. 2,3-Dimethylbenzo-1,3-thiazolium iodide (**6**, 1.04 g, 3.5 mmol), 2,6-dimethyl-4-pyrone (**5**, 0.31 g, 2.5 mmol), and 1 drop of 70% aqueous perchloric acid were heated and stirred with 1.0 ml of water in an open flask until water is evaporated (10 min). The temperature rose to 190 °C over 10 min. The mixture was cooled to room temperature, treated with 3 ml of ethanol, and filtered. The precipitate was thoroughly washed with water and dried to give 0.35 g (35%) of Cy39I (**1**) (decomposes at 280 °C). The filtrate was evaporated in vacuum to give a reusable mixture of starting compounds.
6. Buffa, R.; Zahradnik, P.; Foltinova, P. *Heterocycl. Commun.* **2001**, *7*, 331–336.
7. Compound **1**: ^{1}H NMR (200 MHz, methanol-d_4) δ 2.40 (s, 3H), 2.52 (s, 3H), 4.05 (s, 3H), 6.40 (s, 1H), 6.8 (s, 1H), 7.10 (s, 1H), 7.60 (dd, 1H), 7.75 (dd, 1H), 7.90 (d, 1H), 8.05 (d, 1H). ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.35 (s, 3H), 3.95 (s, 3H), 6.45 (s, 1H), 6.8 (s, 1H), 7.0 (s, 1H), 7.55 (dd, 1H), 7.70 (dd, 1H), 7.95 (d, 1H), 8.15 (d, 1H). ^{13}C NMR (DMSO-d_6) δ 20.06, 20.87, 35.21, 95.27, 106.59, 113.04, 115.44, 123.90, 126.29, 126.88, 129.44, 140.91, 150.03, 163.24, 165.18, 166.88. IR (Nujol) ν, cm^{-1} 1662, 1585, 1540 (conjugated double bond stretches).
8. 2,3-Dimethylbenzo-1,3-thiazolium iodide (**6**, 0.47 g), 2,6-dimethyl-4-pyrone (**5**, 0.20 g), and 1 drop of 70% aqueous perchloric acid were ground with a mortar and pestle, transferred to a test tube, and heated on a burner until the mixture turned into a deep-red liquid (1–2 min). The reaction mixture was cooled to ca. 50 °C, stirred with 10 mL of hot water, and filtered. The precipitate was washed with hot water (3 × 10 mL) and dried in air to produce the Cy39 iodide **1**. The typical yields in a teaching laboratory setting were about 20–30%.