

A Rapid and Eco-friendly Synthesis of 5(6)-Carboxy-Rhodamine 110 Isomers

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ABSTRACT: 5(6)-Carboxy-rhodamine 110 isomers were rapidly synthesized by condensation of 3-aminophenol and trimellitic anhydride using microwave irradiation as heating method. After esterification of the two isomers, separation and hydrolyzation provided target products, 5-carboxy-rhodamine 110 and 6-carboxy-rhodamine 110, in high yields. The results shows microwave irradiation can effectively reduce the reaction time and the demanding of condensed H_2SO_4 .

KEY WORDS: 5(6)-Carboxy-rhodamine 110, Eco-friendly, Microwave irradiation.

INTRODUCTION

Rhodamine, such as rhodamine B and rhodamine 6G, is a family of related fluorone dyes compounds. They are used as a dye or a dye laser gain medium in analysis chemistry, environmental chemistry, biological chemistry [1-8], and especially in the field of non-radioactive detection of biological analysis of modern molecular biology. Furthermore, rhodamine derivatives have been employed for the measurements of mitochondrial membrane potential [1,2], and also as enzymes fluorogenic substrates [3,4]. Now many new rhodamine derivatives, such as amino and/or acid rhodamine derivatives, rhodamine methyl ester and etc, have been designed and synthesized for special use [5-8].

The synthesized 5(6)-Carboxy-rhodamine 110 and its derivatives can readily react with a primary or secondary amine [9, 10]. In this time-consuming process, large quantities of condensed H_2SO_4 is employed as solvent, and inert gas protection is needed. Besides, the separation of the two isomers of 5(6)-carboxy-rhodamine 110 can not be avoided in many cases [11], but it is very difficult to separate them directly for their similar and high polarity [12,13]. Recently, esterification of 5(6)-arboxy-rhodamine 110 was proved to be an effective method for their separation by several authors [14], and further hydrolysis provided target products with relative high yield. But it was also a time-consuming process.

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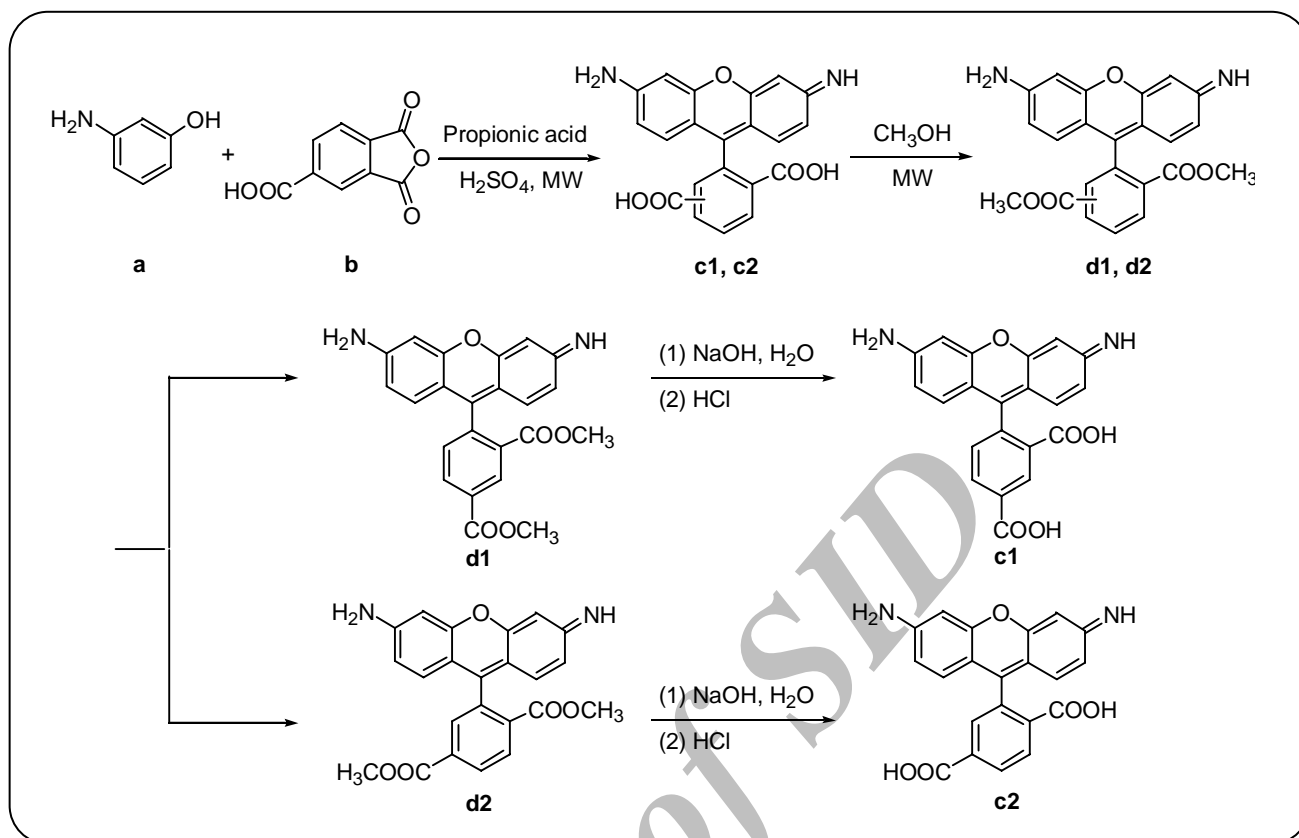


Fig. 1: Microwave-assisted synthesis of 5(6)-carboxy-rhodamine 110 isomers.

To develop an eco-friendly method which permits the synthesis of 5(6)-arboxy-rhodamine 110 rapidly with high purity is one of the challenges for organic chemists. Microwave irradiation accelerates the chemical reactions, and high yields are achieved within a short time. In this work, we introduce a microwave-assisted method to synthesis 5(6)-arboxy-rhodamine 110 in simple approach; the strategy has been shown in Fig. 1.

EXPERIMENTAL SECTION

All the reagents were A.R. grade, and were used without further purification. The reactions were performed on a rebuilt WP-750B Galanz microwave oven [15]. The system was equipped with magnetic stirrer bar which allows continuous stirring. The Anal and NMR were operated on Carlo Erba 1106 element analysis instrument and Varian INOVA-400 MHz nuclear magnetic resonance appearance. Mass spectrums were recorded on a Micromass Platform spectrometer using the direct-inlet system operating in the Electron Impact (EI) mode at 75 eV.

Synthesis of 5(6)-carboxy-rhodamine 110 methyl ester

2.40 g (22 mmol) 3-aminophenol (**a**) and 1.92 g (10 mmol) trimellitic anhydride (**b**) were dissolved in 15 mL propionic acid in a flask, and then 0.5 mL H_2SO_4 was added dropwise. The flask was placed into the microwave oven, and the oven was started under the pre-setted "power" and "time". After the reaction finished, cooled to room temperature and the solvent was removed by vacuum distillation, 30 mL methanol was added to the residue, and the flask was put into the microwave oven for irradiation under the pre-setted "power" and "time". Then the following separation was carried out by the reported method [14], providing 1.62 g 5-carboxy-rhodamine 110 methyl ester (**d1**) and 1.83 g 6-carboxy-rhodamine 110 methyl ester (**d2**) with total yield 85.8%.

Synthesis of 5(6)-carboxy-rhodamine 110

The method reported in [14] was used for the hydrolysis of 5(6)-carboxy-rhodamine 110 methyl ester. 5-carboxy-rhodamine 110 (**c1**) and 6-carboxy-rhodamine 110 (**c2**) were obtained with the yield of 95.8% and 94.3% respectively.

Spectral and analytical data of 5(6)-Carboxy-rhodamine 110 methyl ester and 5(6)-Carboxy-rhodamine

5-Carboxy-rhodamine methyl ester (d1)

¹H-NMR (400 MHz, CD₃OD) δ: 8.85 (s, 1H), 8.41 (d, J = 6.8 Hz, 1H), 7.53 (d, J = 6.8 Hz, 1H), 6.92 (d, J = 8.8 Hz, 1H), 6.82 (d, J = 8.8 Hz, 1H), 6.74 (d, J = 8.8 Hz, 1H), 6.66 (d, J = 8.8 Hz, 1H), 6.55~6.51 (m, 2H), 4.02 (s, 3H), 3.64 (s, 3H). Anal. Calcd. for C₂₃H₁₈N₂O₅: C 68.65, H 4.51, N 6.96; found C 68.60, H 4.47, N 6.90. MS (EI) m/z: 402 (M⁺)

6-Carboxy-rhodamine methyl ester (d2)

¹H-NMR (400 MHz, CD₃OD) δ: 8.43 (d, J = 7.2 Hz, 1H), 8.34 (d, J = 7.2 Hz, 1H), 7.98 (s, 1H), 7.01 (d, J = 9.6 Hz, 1H), 6.97 (d, J = 9.6 Hz, 1H), 6.83~6.80 (m, 4H), 3.98 (s, 3H), 3.62 (s, 3H). Anal. Calcd. for C₂₃H₁₈N₂O₅: C 68.65, H 4.51, N 6.96; found C 68.61, H 4.52, N 6.91. MS (EI) m/z: 402 (M⁺)

5-Carboxy-rhodamine 110 (c1)

¹H-NMR (400 MHz, CD₃OD) δ: 8.93 (s, 1H), 8.44 (d, J = 7.2 Hz, 1H), 7.59 (d, J = 7.2 Hz, 1H), 7.22 (d, J = 9.4 Hz, 2H), 7.01 (d, J = 9.4 Hz, 2H), 6.96~6.92 (m, 2H). Anal. Calcd. for C₂₁H₁₄N₂O₅: C 67.38, H 3.77, N 7.48; found C 67.45, H 3.77, N 7.44. MS (EI) m/z: 374 (M⁺)

6-Carboxy-rhodamine 110 (c2)

¹H-NMR (400 MHz, CD₃OD) δ: 8.56 (d, J = 7.6 Hz, 1H), 8.24 (d, J = 7.6 Hz, 1H), 7.86 (s, 1H), 7.19 (d, J = 9.6 Hz, 2H), 6.99~6.94 (m, 4H). Anal. Calcd. for C₂₁H₁₄N₂O₅: C 67.38, H 3.77, N 7.48; found C 67.35, H 3.78, N 7.42. MS (EI) m/z: 374 (M⁺)

RESULTS AND DISCUSSION

Firstly, the reaction was carried out at different power levels (power 1 to 5) without inert gas protection in order to select the appropriate power level. The results showed that the propionic acid solution could keep boiling at power 5, so the reaction was carried out at this power level. The reaction was monitored by TLC every 5 minutes. 20 minutes later, trimellitic anhydride could not be detected, and there was not any other point of byproducts on TLC. The results suggested that the removal of the inert gas protection in this experiment was reasonable due to the short reaction time.

The following esterification should employ Lewis acid as catalyst, so the residual of vacuum distillation, 5(6)-carboxy-rhodamine 110 and H₂SO₄, could be used in the next step directly without further separation. The power level of the esterification reaction was selected as power 3, and the reaction time also determined by TLC detection as 30 minutes.

Initially, the hydrolysis of 5(6)-carboxy-rhodamine 110 methyl esters was tested in the presence of NaOH in methanol. But it was found that the results were complex. So the hydrolysis was carried out using the traditional method [14].

CONCLUSIONS

In conclusion, a convenient and rapid microwave-assisted synthesis of 5(6)-carboxy-rhodamine 110 in high yields was established. Experimental results indicated that microwave heating has effectively reduced the total reaction time of the first two steps from about twenty hours to less than one hour, and, eco-friendly, the demanding for condensed H₂SO₄ was greatly reduced, which brings about considerable time saving as well as cost. This method can be applied into the synthesis of more rhodamine derivatives with rapid, economic and environmentally friendly characters. We also are confident that this method will find its use in preparative organic chemistry.

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REFERENCES

- [1] Leytus S.P., Patterson W.L., Mangel W.F., New class of Sensitive and Selective Fluorogenic Substrates for Serine Proteinases. Amino Acid and Dipeptide Derivatives of Rhodamine, *Biochem. J.*, **215**, p. 253 (1983).
- [2] Bernal S.D., Lampidis T.J., McIsaac R.M., Chen L.B., Anti-Carcinoma Activity in Vivo of Rhodamine-123, A Mitochondrialspecific Dye, *Science*, **222**, 4620, 169 (1983).
- [3] Leytus S.P., Melhado L.L., Mangel W.F., Rhodaminebased Compounds as Fluorogenic Substrates for Serine Proteases, *Biochem. J.*, **209**, p. 299 (1983).

- [4] Morliere P., Mangel W.F., Santus R., Huppe G., Reyftmann J.P., Kohen E., Interaction of Tetrapyrrolic Rings with Rhodamine 110 and 123 and with Rhodamine 110 Derivatives Bearing a Peptidic Side Chain, *Biochem. Biophys. Res. Commun.*, **146**, p. 107 (1987).
- [5] Nedelčev T., Račko D., Krupa I., Preparation and Characterization of a New Derivative of Rhodamine B with an Alkoxysilane Moiety, *Dyes Pigments*, **76**, p. 550 (2008).
- [6] Gee K.R., Weinberg E.S., Kozlowski D.J., Caged Q-Rhodamine Dextran: A New Photoactivated Fluorescent Tracer, *Bioorg. Med. Chem. Lett.*, **11**, p. 2181 (2001).
- [7] Yan F.Y., Chen L.G., Yan X.L., Ge F.Y., Duan E.H., Synthesis and Applications of Rhodamine Fluorescent Dyes, *Prog. Chem.*, **18**, p. 252 (2006).
- [8] Cai S.X., Zhang H.Z., Guastella J., Drewe J., Yang W., Weber E., Design and Synthesis of Rhodamine 110 Derivative and Caspase-3 Substrate for Enzyme and Cell-Based Fluorescent Assay, *Bioorg. Med. Chem. Lett.*, **11**, p. 39 (2001).
- [9] Hung S.C., Mathies R.A., Glazer A.N., Optimization of Spectroscopic and Electrophoretic Properties of Energy Transfer Primers, *Anal. Biochem.*, **252**, p. 78 (1997).
- [10] Edmundson A.B., Ely K.R., Herron J.N., A Search for Site-Filling Ligands in the Mcg Bence-Jones Dimer: Crystal Binding Studies of Fluorescent Compounds, *Mol. Immunol.*, **21**(7), p. 561 (1984).
- [11] Lee L.G., Spurgeon S.L., Heiner C.R., Benson S.C., New Energy Transfer Dyes for DNA Sequencing, *Nucl. Acids Res.*, **25**, p. 2816 (1997).
- [12] Menchen S.M., Fung S., 5- and 6-Succinimidyl-Carboxylate Isomers of Rhodamine Dyes, EP 0272007 (1988).
- [13] Corrie J.E., Craik J.S., Synthesis and Characterization of Pure Isomers of Iodoacetamidotetramethylrhodamine, *J. Chem. Soc. Perkin Trans.*, **1**, p. 2967 (1994).
- [14] Gao Y., Tian M., Zhang B., Zhang M., Li J.L., Shi Z., Synthesis, Separation and Characterization of 5(6)-Carboxy-Rhodamine 110 Isomers, *Chin. J. Org. Chem.*, **27**(2), p. 269 (2007).
- [15] Wei J.F., Shi X.Y., He D.P., Zhang Y., A Rapid Detosylation of N,N',N'', N'''-Tetra(p-Tosyl)-1,4,7,10-Tetraaza-Cyclododecane under Microwave Irradiation and Normal Pressure, *Chin. J. Org. Chem.*, **23**, 1142 (2003).