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Facile Synthesis of Rhodamine Esters using Acetyl Chloride in Alcohol Solution

Justin A. Ross [a] , Benjamin P. Ross [b] , Halina Rubinsztein-Dunlop [a] & Ross P. McGeary [b c]

[a] Centre for Biophotonics and Laser Science, School of Physical Sciences, University of Queensland, Brisbane, Queensland, Australia

[b] School of Molecular and Microbial Sciences, University of Queensland, Brisbane, Queensland, Australia

[c] School of Molecular and Microbial Sciences, and School of Pharmacy, University of Queensland, Brisbane, Queensland, Australia

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Facile Synthesis of Rhodamine Esters using Acetyl Chloride in Alcohol Solution

Justin A. Ross
Centre for Biophotonics and Laser Science, School of Physical Sciences, University of Queensland, Brisbane, Queensland, Australia

Benjamin P. Ross
School of Molecular and Microbial Sciences, University of Queensland, Brisbane, Queensland, Australia

Halina Rubinsztein-Dunlop
Centre for Biophotonics and Laser Science, School of Physical Sciences, University of Queensland, Brisbane, Queensland, Australia

Ross P. McGeary
School of Molecular and Microbial Sciences, and School of Pharmacy, University of Queensland, Brisbane, Queensland, Australia

Abstract: The rhodamines are a highly fluorescent class of compound used in many different fields of research, from the lasing medium in dye lasers to biological stains and markers for cellular drug resistance. In this study, esters (2–7) of rhodamine 110 (1) were conveniently prepared via the addition of acetyl chloride to a solution of the free acid (1) in the appropriate alcohol. This method conferred several advantages over previous preparations, namely that for low boiling alcohols, simple evaporation of the solution afforded the ester in quantitative yield with no need for purification. For higher boiling point alcohols, a method has been developed which allows the separation of longer chain esters from the alcohol solvent.

Keywords: Rhodamine, esterification, acetyl chloride, lipophilicity

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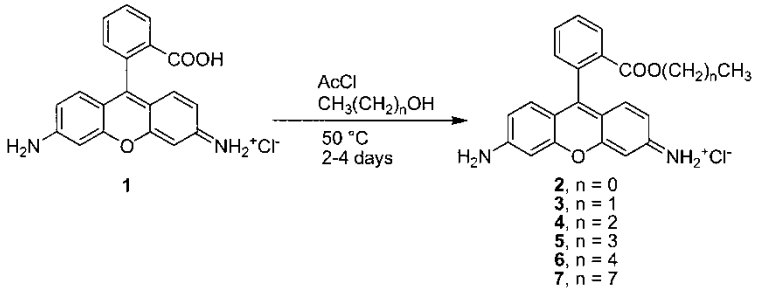
Address correspondence to Ross P. McGeary, School of Molecular and Microbial Sciences, University of Queensland, Brisbane, Queensland 4072, Australia. Tel: +61-7-3365-3955; Fax: +61-7-3346-3249;. E-mail: r.mcgeary@uq.edu.au

The rhodamines are a highly fluorescent class of compound used in many different fields of research, from the lasing medium in dye lasers to biological stains and markers for cellular drug resistance.[1–3] Rhodamine 123 **(2)**, the methyl ester of rhodamine 110 **(1)**, is a fluorescent lipophilic compound[4] with a positive charge delocalized across the xanthene rings. It is commonly used as a biological marker for mitochondria [5] and as a substrate for multidrug resistance transporter (MDT) assays.[6–9] The use of fluorescent substrates as MDT markers forms the basis of a convenient method of tracking the transport of substrates. We have synthesized a series of esters (**2**–**7**) of rhodamine 110 with increasing lipophilicity. Thus a rhodamine ester with similar lipophilic properties to a drug of interest could be chosen to mimic the drug in MDT assays. In this article we report the facile method by which esters **2**–**7** were prepared. The method conferred several advantages over previous preparations, namely that for low boiling alcohols, simple evaporation of the solution afforded the ester in quantitative yield with no need for purification.

Pal and coworkers synthesized the methyl **(2)** and *n*-butyl **(5)** esters of rhodamine 110 **(1)** by bubbling hydrogen chloride through a solution of rhodamine 110 **(1)** in methanol or *n*-butanol respectively.[10] The reaction mixtures were refluxed, and then the solvent was evaporated in vacuo to afford the crude ester, which was purified by column chromatography using silica gel. The disadvantages of this method are the requirement for gaseous hydrogen chloride and the need for two silica flash columns to purify the product.

Ramos and coworkers prepared a variety of rhodamine esters, including the methyl **(2)** and ethyl **(3)** esters of rhodamine 110 **(1)**, under Fischer conditions.[11] The rhodamine was dissolved in the appropriate alcohol solution containing 3% sulfuric acid, and this solution was heated with stirring at 50°C for 1–6 days. The solution was concentrated, and the ester was precipitated as a bromide, iodide, or perchlorate salt by treatment with a 14% aqueous solution of potassium iodide, potassium bromide, or sodium perchlorate respectively. In our hands, the synthesis of rhodamine esters using this method proved troublesome. Precipitation of the esters was not suitable for longer-chain alcohols because the aqueous and alcoholic phases were immiscible. For shorter-chain alcohols the aqueous and alcoholic phases were miscible; however, the addition of an aqueous phase to the reaction in the presence of strong acid resulted in some hydrolysis of the ester to the free carboxylic acid, which therefore contaminated the product.

We have developed an improved method for the preparation of rhodamine esters that utilizes anhydrous hydrogen chloride generated in situ by the addition of acetyl chloride (AcCl) to a mixture of the rhodamine free acid **(1)** in the appropriate alcohol (Scheme 1). After heating the stirred solution at 50°C for 2–4 days, evaporation of the solution in vacuo afforded the rhodamine esters (**2**–**6**) in quantitative yield without the need for purification. For the synthesis of rhodamine 110 octyl ester **(7)**, the solvent (*n*-octanol) was insufficiently volatile (bp 196°C) for complete removal in vacuo, and therefore



Scheme 1.

7 was isolated by flash column chromatography using neutral alumina. The use of silica for flash chromatography caused streaking of the product and gave a poor recovery of the ester.

In summary, we have developed a facile method for the preparation of rhodamine esters that utilizes anhydrous hydrogen chloride generated in situ by the addition of AcCl to a mixture of the rhodamine free acid in the appropriate alcohol. The method confers several advantages over previous preparations, namely that for low boiling alcohols, simple evaporation of the solvent affords the ester in quantitative yield with no need for purification. The synthesis of compound **7** illustrates a convenient method that can be used to isolate rhodamine esters from alcohols with high boiling points.

EXPERIMENTAL

Commercial reagents were used without further purification. Rhodamine 110 (**1**) was purchased from Fluka (Buchs, Switzerland) and acetyl chloride was purchased from Aldrich Chemical Company (Milwaukee, WI). Thin-layer chromatography (TLC) was performed on silica-gel 60 F_{254} aluminum sheets (Merck, Darmstadt, Germany). The solvent for TLC was *n*-butanol–water–ethanol, 9:2:1. Aluminum oxide 90 active neutral, 70–230 mesh (Merck, Darmstadt, Germany) was used for flash column chromatography. Reverse-phase High Performance Liquid Chromatography (HPLC) purification and analysis was carried out with a Shimadzu HPLC system (SCL-10A VP controller, LC-10AT VP pump, DGU-14A degasser, FCV-10AL VP solvent mixer and a SPD-10A VP UV detector) using an analytical (LiChrospher® 100, RP-18e, 250 × 4 mm, 5 μm) or semipreparative column (LiChrospher® 100, RP-18e, 250 × 10 mm, 10 μm). The mobile phase was solvent A: 0.025 M ammonium acetate in water; solvent B: 10% solvent A and 90% acetonitrile. For analytical HPLC, a linear gradient of 30–100% B over 70 min was employed at a flow rate of 1 mL/min. For semipreparative HPLC, a linear gradient of 20–100% B over 80 min was used with a flow

rate of 6 mL/min. The eluent was monitored at 510 nm [for detection of the product **(7)** and rhodamine-based impurities] and at 254 nm (for detection of nonrhodamine impurities).[10] Mass spectra (ES-MS) were recorded on a Perkin-Elmer Sciex API 3000 mass spectrometer operating in positive-ion electrospray mode using 0.1% formic acid in 90% acetonitrile/10% water. High-resolution mass spectra were run on a Finnigan MAT 900 XL-Trap instrument with a Finnigan API III electrospray source using methanol as solvent and polypropylene glycol as reference for accurate mass data acquired by electric sector scan (HRMS). ^{1}H NMR spectra were recorded on a Bruker AV-500 instrument (500 MHz), and ^{13}C NMR spectra were recorded on a Bruker AV-300 instrument (75 MHz). The following abbreviations were used to indicate the peak multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad.

General Procedure for the Preparation of Rhodamine Esters

The appropriate alcohol (7.5 mL) was added to rhodamine 110 (**1**, 30 mg, 0.082 mmol), and the mixture was stirred under an atmosphere of argon. Acetyl chloride (375 μL, 5.27 mmol) was added dropwise, and the solution was heated at 50°C for 2–4 days. When the reaction was 70–90% complete [analysis by TLC (*n*-butanol–water–ethanol 9:2:1)], additional acetyl chloride (75 μL, 1.05 mmol) was added to drive the reaction to completion. When TLC indicated no remaining starting material, the solution was evaporated in vacuo (water bath temperature <50°C to avoid degradation of the product) to afford the rhodamine ester **(2–6)**, a hygroscopic amorphous solid, in quantitative yield as determined by mass.

Data

Rhodamine 110 methyl ester (rhodamine 123) (2): Analytical HPLC t_R = 17.6 min, purity 96% (510 nm), 95% (254 nm). TLC R_f 0.49; ES-MS, m/z: 345 $[M+H]^+$; ^{1}H NMR (500 MHz, d_6-DMSO) δ 8.24 (1H, dd, J = 8.1 and 1.4 Hz), 8.10 (4H, br s), 7.90 (1H, dt, J = 7.6 and 1.4 Hz), 7.82 (1H, dt, J = 7.7 and 1.2 Hz), 7.49 (1H, dd, J = 7.7 and 1.4 Hz), 6.96 (2H, d, J = 9.4 Hz), 6.84–6.82 (4H, m), 3.57 (3H, s); ^{13}C NMR (75 MHz, d_6-DMSO) δ 165.0, 159.4, 158.7, 157.3, 133.5, 133.1, 131.5, 130.7, 130.4, 130.3, 129.3, 116.9, 112.8, 97.0, 52.3; UV-visible (H_2O): λ_{max} 500 nm; HRMS calcd. for $[M+H]^+$ 345.1239; found 345.1254.

Rhodamine 110 ethyl ester (3): Analytical HPLC t_R = 21.2 min, purity 97% (510 nm), 96% (254 nm). TLC R_f 0.51; ES-MS, m/z: 359 $[M+H]^+$; ^{1}H NMR (500 MHz, d_6-DMSO) δ 8.22 (1H, dd, J = 7.9 and 1.1 Hz), 8.14 (4H, br s), 7.89 (1H, dt, J = 7.5 and 1.4 Hz), 7.82 (1H, dt, J = 7.7 and 1.4 Hz), 7.49

(1 H, dd, $J = 7.6$ and 1.2 Hz), 6.97 (2 H, d, $J = 9.5$ Hz), 6.86–6.84 (4 H, m), 3.94 (2 H, q, $J = 7.1$ Hz), 0.84 (3 H, t, $J = 7.1$ Hz); ^{13}C NMR (75 MHz, d_6-DMSO) δ 164.7, 159.4, 158.5, 157.3, 133.1, 132.9, 131.5, 130.7, 130.3, 129.7, 116.9, 112.9, 97.0, 60.9, 13.3; UV-visible (H_2O): λ_{max} 501 nm; HRMS calcd. for $[M + H]^+$ 359.1381; found 359.1396.

Rhodamine 110 propyl ester (4): Analytical HPLC $t_R = 25.6$ min, purity 99% (510 nm), 99% (254 nm). TLC R_f 0.53; ES-MS, m/z: 373 $[M + H]^+$; ^{1}H NMR (500 MHz, d_6-DMSO) δ 8.23 (1 H, dd, $J = 7.9$ and 1.1 Hz), 8.11 (4 H, br s), 7.89 (1 H, dt, $J = 7.5$ and 1.3 Hz), 7.82 (1 H, dt, $J = 7.7$ and 1.3 Hz), 7.49 (1 H, dd, $J = 7.5$ and 1.1 Hz), 6.98 (2 H, d, $J = 9.0$ Hz), 6.86–6.82 (4 H, m), 3.86 (2 H, t, $J = 6.4$ Hz), 1.23 (2 H, sextet, $J = 6.9$ Hz), 0.59 (3 H, t, $J = 7.4$ Hz); ^{13}C NMR (75 MHz, d_6-DMSO) δ 165.0, 159.5, 158.6, 157.4, 133.1, 131.6, 130.8, 130.4, 129.8, 117.0, 112.9, 97.0, 66.7, 21.1, 10.0; UV-visible (H_2O): λ_{max} 501 nm; HRMS calcd. for $[M + H]^+$ 373.1552; found 373.1550.

Rhodamine 110 butyl ester (5): Analytical HPLC $t_R = 29.7$ min, purity 99% (510 nm), 99% (254 nm). TLC R_f 0.56; ES-MS, m/z: 387 $[M + H]^+$; ^{1}H NMR (500 MHz, d_6-DMSO) δ 8.22 (1 H, dd, $J = 7.8$ and 1.1 Hz), 8.15 (4 H, br s), 7.88 (1 H, dt, $J = 7.5$ and 1.3 Hz), 7.82 (1H, dt, $J = 7.7$ and 1.3 Hz), 7.49 (1H, dd, $J = 7.5$ and 0.98 Hz), 6.97 (2 H, d, $J = 9.2$ Hz), 6.86–6.84 (4 H, m), 3.89 (2 H, t, $J = 6.2$ Hz), 1.15 (2 H, m), 0.91 (2 H, m), 0.69 (3 H, t, $J = 7.3$ Hz); ^{13}C NMR (75 MHz, d_6-DMSO) δ 165.1, 159.5, 158.4, 157.3, 133.0, 132.9, 131.6, 130.9, 130.4, 129.8, 117.0, 112.9, 97.0, 65.0, 29.8, 18.5, 13.4; UV-visible (H_2O): λ_{max} 502 nm; HRMS calcd. for $[M + H]^+$ 387.1709; found 387.1715.

Rhodamine 110 pentyl ester (6): Analytical HPLC $t_R = 34.6$ min, purity 96% (510 nm), 95% (254 nm). TLC R_f 0.59; ES-MS, m/z: 401 $[M + H]^+$; ^{1}H NMR (500 MHz, d_6-DMSO) δ 8.22 (1 H, dd, $J = 7.8$ and 1.1 Hz), 8.16 (4 H, br s), 7.88 (1 H, dt, $J = 7.5$ and 1.3 Hz), 7.82 (1H, dt, $J = 7.7$ and 1.2 Hz), 7.48′ (1 H, dd, $J = 7.5$ and 0.98 Hz), 6.98 (2 H, d, $J = 9.0$ Hz), 6.87–6.84 (4 H, m), 3.87 (2 H, t, $J = 6.2$ Hz), 1.14 (2 H, m), 1.07 (2 H, m), 0.84 (2 H, m), 0.74 (3 H, t, $J = 7.4$ Hz); ^{13}C NMR (75 MHz, d_6-DMSO) δ 165.0, 159.5, 158.3, 157.3, 132.9, 132.8, 131.5, 130.8, 130.3, 130.3, 129.8, 116.9, 112.9, 97.0, 65.2, 30.6, 27.4, 21.7, 13.6; UV-visible (H_2O): λ_{max} 502 nm; HRMS calcd. for $[M + H]^+$ 401.1865; found 401.1871.

Rhodamine 110 octyl ester (7). Compound **7** was prepared by following the general procedure except the solvent (*n*-octanol) was not sufficiently volatile (bp 196°C) for evaporation in vacuo. Instead, compound **7** was isolated by flash column chromatography using neutral alumina. The reaction mixture was loaded onto the column, and the *n*-octanol was eluted with ethyl acetate and then 5% methanol in ethyl acetate. Further elution with methanol afforded the product (**7**), which was contaminated with a small amount of *n*-octanol. The molar ratio of rhodamine 110 octyl ester (**7**): *n*-octanol was ~4:1

by ^{1}H NMR, and the yield was 82% (corrected for *n*-octanol). A sample of this material was further purified by semipreparative reverse-phase HPLC to afford the product (**7**), an amorphous solid: analytical HPLC $t_R = 49.1$ min, purity 98% (510 nm), 96% (254 nm). TLC R_f 0.62; ES-MS, m/z: 443 $[M + H]^+$; ^{1}H NMR (500 MHz, d_6-DMSO) δ 8.21 (1H, dd, $J = 7.9$ and 1.2 Hz) overlapping with 8:21 (4 H, br s), 7.87 (1H, dt, $J = 7.5$ and 1.4 Hz), 7.81 (1 H, dt, $J = 7.7$ and 1.3 Hz), 7.47 (1 H, dd, $J = 7.6$ and 1.2 Hz), 6.94 (2 H, d, $J = 9.1$ Hz), 6.84–6.80 (4 H, m), 3.88 (2 H, t, $J = 6.1$ Hz), 1.28–1.01 (12 H, m), 0.86 (3 H, t, $J = 7.3$ Hz); UV-visible (H_2O): λ_{max} 503 nm; HRMS calcd. for $[M + H]^+$ 443.2335; found 443.2329.

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