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Scalable Regioselective Synthesis of Rhodamine Dyes

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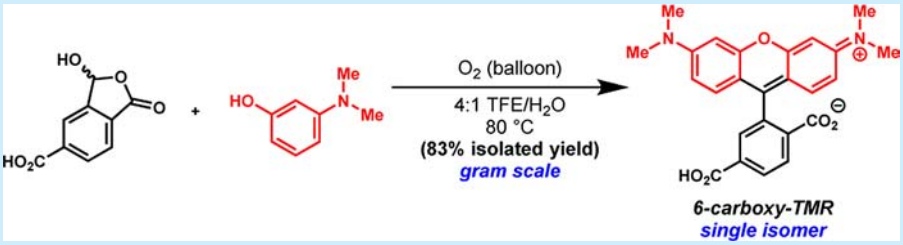
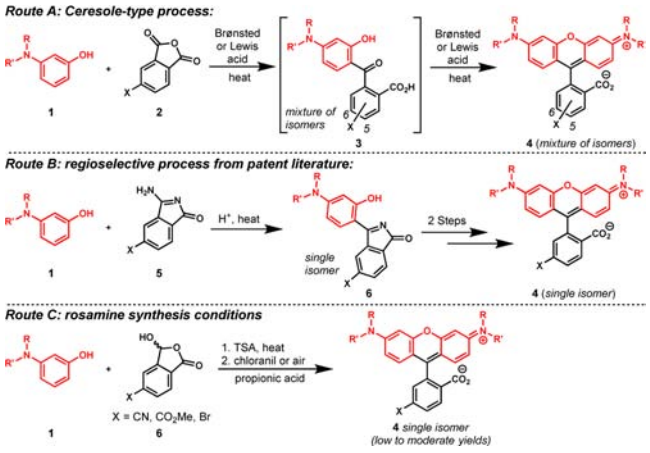
Supporting Information



ABSTRACT: A one-step, operationally simple protocol for the synthesis of isomerically pure rhodamine dyes from phthalaldehydic acids is reported. Using a mixture of 2,2,2-trifluoroethanol and water as reaction media allows for clean and efficient formation of various rhodamines as a single isomer. This method was successfully applied to the synthesis of several isomerically pure rhodamines, including 6-carboxytetramethylrhodamine and 6-carboxy-X-rhodamine (6-CXR) on gram scale. A simple, one-step, Pd-catalyzed hydroxycarbonylation approach to phthalaldehydic acids from appropriately substituted dihalobenzadehydes is also described.

Since the discovery of the first rhodamines[1] by Swiss chemist Maurice Ceresole[2] in 1887, this dye family has achieved prominence in many applications, in part due to its photochemical properties.[3] These dyes are widely used as tracer agents, laser dyes, labeling agents for biologics, etc. Remarkably, even a century after their discovery, rhodamines are still largely produced by variations of the original process reported by Ceresole. However, the relatively simple Ceresole process (Scheme 1 Route A) has significant drawbacks, e.g. harsh reaction conditions, the generation of difficult to separate isomers, and often low yields.[4] These constraints significantly limit the availability of isomerically pure dyes and pose a challenge for the efficient synthesis of novel rhodamines. In this letter we report an operationally simple protocol for synthesis of pure rhodamine dyes on a gram scale.

Scheme 1. Historical Examples of Synthetic Approaches to Rhodamines



In many applications, rhodamine dyes are chemically attached to another molecule or biomolecule via a carboxylate handle at the 5- or 6-position of the rhodamine core. Mixtures of isomers not only complicate synthetic and purification operations but also can give confounding results in some cases due to differences in physicochemical properties and bioactivity between two dye isomers.[4,5] Thus, widespread adoption of the classic synthesis combined with the broad use of rhodamines in biological research sparked numerous efforts to develop efficient separation and purification strategies for the isolation of isomerically pure rhodamines. Although purification approaches have achieved significant advances, they lack generality, which significantly increases cost.[6] Despite a dire need for a generally robust and efficient regioselective synthesis of this class of dyes, only recently have researchers started to address this problem.[4,7]

To our knowledge, the earliest examples to produce synthetically useful quantities of isomerically pure rhodamines were reported in the patent literature. The patent sources indicate that when 3-amino-1*H*-isoindol-1-one (**5**) is reacted with 3-aminophenol (**1**), a single isomer of **6** is produced,[8] which could be further elaborated to a single isomer of a rhodamine dye (Scheme 1 Route B).[9] The more recent report by Lavis and co-workers achieves synthesis of isomerically pure rhodamines from fluorescein ditriflate via Buchwald–Hartwig coupling.[10] Although this approach allows for efficient access to

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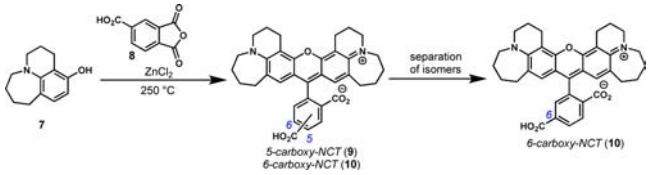
many rhodamines,[10,11] it is not applicable to rhodamines with cyclic backbones (X-rhodamine for example).

Another approach utilizes phthalaldehydic acids (2-carboxybenzaldehydes) under rosamine synthesis conditions[12] to access the rhodamine core regioselectively (Scheme 1 Route C). Considering that both benzaldehydes and 2-sulfobenzaldehydes are successfully used to synthesize single isomers of rosamines[12] and sulforadamines[13] respectively, it would seem surprising that phthalaldehydic acids have not been applied to the synthesis of rhodamines until recently.[7,14] Under rosamine synthesis conditions, phthalaldehydic acids delivered isomerically pure rhodamines in low to moderate yields. Furthermore, carboxyrhodamines such as 6-carboxytetramethylrhodamine (**17**) required a final deprotection step.[7]

We encountered the aforementioned shortcomings when attempting to scale up 6-carboxy-NCT (**10**).[15] 6-Carboxy-NCT is a carbon-homologated analogue of 6-carboxy-X-rhodamine (**19**). Due to its photophysical and biological properties, **10** is an acceptor dye of choice for intracellular BRET applications.[16] Isomerically pure 6-carboxy-NCT (**10**) was originally prepared by a variation of a classic process of condensing terephthalic anhydride (**8**) with tricyclic aminophenol **7**, followed by HPLC separation of the resulting isomers (Scheme 2).[15] Considering

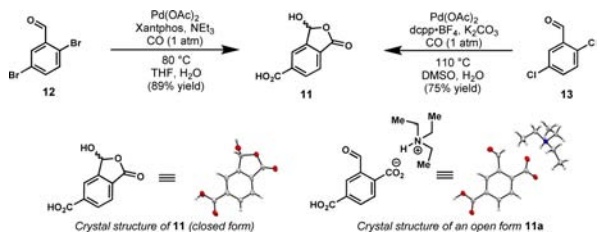
Scheme 2. Classic Approach to 6-Carboxy-NCT via Condensation of Terephthalic Anhydride with 7



that the commercially unavailable phenol **7** requires a multistep synthesis,[15] using a nonregioselective route to access a 6-carboxy-NCT (**10**) results in considerable loss of valuable material through formation of the undesired 5-carboxy-NCT (**9**).

To address this challenge, we investigated the rosamine synthesis conditions[7,12,14a] with phthalaldehydic acids (Scheme 1 Route C). Although many methods exist for synthesis of phthalaldehydic acids,[14b,17] we opted for Pd-catalyzed hydroxycarbonylation[18,19] of properly substituted, commercially available dihalobenzaldehydes (Scheme 3). Briefly, exposure of 2,5-dibromobenzaldehyde (**12**) to carbon monoxide (balloon) in wet THF in the presence of a base and catalytic amounts of $Pd(OAc)_2$/Xantphos yielded **11** in 89% yield. Similarly, exposure of 2,5-dichlorobenzaldehyde (**13**) to carbon monoxide (balloon) in wet DMSO in the presence of a base and catalytic amounts of $Pd(OAc)_2$/dcpp provided 4-carboxyphthalaldehydic acid (**11**) in 75% yield.[20] Interestingly, we noticed that, depending on conditions, **11** crystallized in both closed and open forms. Lactone **11** crystallized out of a heptane−EtOAc mixture, and carboxylate salt **11a** formed X-ray quality crystals from a water−acetonitrile mixture in the presence of triethylamine (Scheme 3). This result is consistent with prior evidence that a basic environment favors the ring-opened form of phthalaldehydic acid.[21]

Scheme 3. Pd-Catalyzed Hydroxycarbonylation of Dihalobenzaldehydes



Attempted condensation of tricyclic phenol **7** with 4-carboxyphthaladehydic acid (**11**) under rosamine synthesis conditions (Scheme 1 Route C) produced only small quantities of dye **10**. The reaction produced mostly lactone **14**, the product of the initial Friedel − Crafts condensation (Scheme 4

Scheme 4. Synthesis of 6-Carboxy-NCT from 4-Carboxyphthalaldehydic Acid

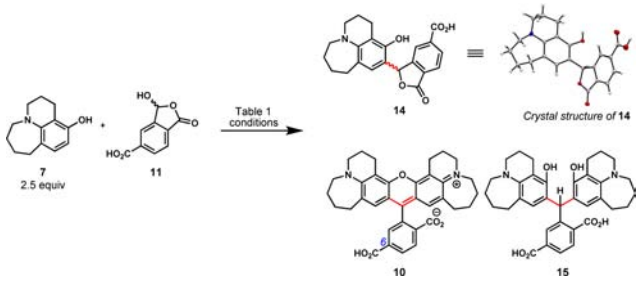


Table 1. Solvent Screen for Rhodamine Synthesis[a]

			yield, %[b]		
entry	solvent	additive	**14**	**10**	**15**
1	$EtCO_2H$	TSA	57(54)[c]	8	–
2	$EtCO_2H$	–	48(52)[c]	3	–
3	EtCN	TSA	55	trace[d]	–
4	EtCN	–	58	trace[d]	–
5	DMF	–	13	–	–
6	*i*PrOH	–	73	2	–
7	HFIP[e]	–	22	trace[d]	76(73)[c]
8	TFE	–	47	trace[d]	52
9	HFIP[f,e]	–	30	66	–
10	TFE[f]	–	8	88(85)[c]	–

[a]Reactions were run open to air, [**11**] = 0.03 M at 80 °C for 20 h. [b]Determined by ^{1}H NMR analysis of crude reaction mixture, using an internal standard. [c]Isolated yield. [d]Observed in HPLC analysis of crude reaction mixture only. [e]Reaction at 60 °C. [f]Reaction under O_2 balloon.

and Table 1 entry 1). As we investigated the reaction conditions, we found that, unlike rosamine synthesis with benzaldehydes, no Brønsted acid was required to complete the Friedel−Crafts reaction. When we attempted the reaction in neat propionic acid without any catalytic *p*-toluenesulfonic acid (TSA), the result was essentially the same (Table 1 entry 2). Furthermore, propionic acid was not required, as lactone **14** also formed in propionitrile in 58% yield with no added acid catalyst (Table 1 entry 4). We attribute these results to intramolecular activation of the aldehyde group of the phthalaldehydic open form (Scheme 5).[22]

To push the reaction beyond lactone **14**, we turned to the 1965 report by Reese and Sabet investigating the reactivity of phthalaldehydic acid toward indoles.[22] They reported that a

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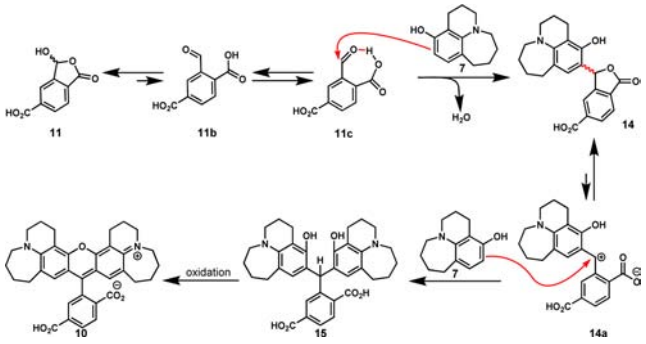
Scheme 5. Mechanistic Hypothesis for Rhodamine Formation from Phthalaldehydic Acid



base was required to achieve an addition of a second equivalent of indole to the intermediate lactone. In our case, using a base was not an option, as lactone **14** was unstable in the presence of a base. On the other hand, a more recent report mentioned formation of a small amount of bis-indolyl side-product when phthalaldehydic acid was allowed to react with indole in neutral H_2O.[23] Mindful of these reports, we hypothesized that solvent may have an effect on this process by possibly stabilizing the open form of the lactone via some form of benzhydrylium ion pair **14a** (Scheme 5).

The solvent screen (Table 1) revealed that fluorinated alcohols, 2,2,2-trifluoroethanol (TFE) and hexafluoro-2-propanol (HFIP), are excellent solvents for 6-carboxy-NCT synthesis from phthalaldehydic acid (Scheme 4 and Table 1). Both HFIP and TFE promoted two consecutive Friedel–Crafts condensations of **11** with 2 equiv of **7**, providing primarily triarylmethane **15** along with a trace amount of dye **10** when exposed to air (Table 1 entries 7 and 8). Due to the inefficient and irreproducible air oxidation of **15**, an oxygen atmosphere was used, which cleanly provided **10** in either solvent (Table 1 entries 9 and 10). We hypothesized that the unique physicochemical properties of the fluorinated alcohols,[24] such as high ionizing power,[25] low nucleophilicity,[26] and hydrogen-bond donating ability,[27] may promote the reactivity of the benzhydrylium ion **14a** (Scheme 5). For cost reasons (TFE is much cheaper than HFIP)[28] and its higher boiling point, we chose TFE over HFIP for our further studies.

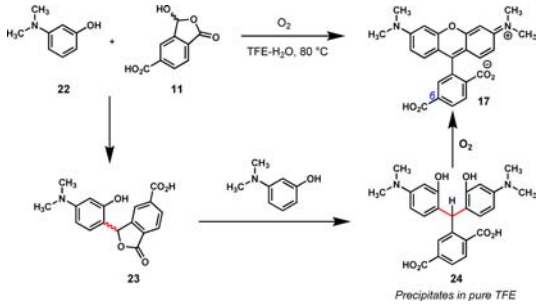
The optimized synthetic processes scaled up smoothly, providing 6-carboxy-NCT (**10**) in 85% isolated yield on gram scale (Table 2, entry 2). The gram-scale synthesis of commonly used dye 6-carboxytetramethylrhodamine (**17**) required additional optimization. The impurity profile for the reaction of **11** with commercial 3-dimethylaminophenol (**22**) varied greatly depending on the impurity profile of commercial **22**. When additionally purified **22**[29] was employed, intermediate triarylmethane **24** precipitated out of TFE, preventing efficient formation of dye **17** (Scheme 6). Through a cosolvent screen

Table 2. Synthesis of Selected Rhodamine Dyes[a]

entry	dye	solvent	yield, %[b]
1	5-carboxy-NCT (**9**)	4:1 TFE–H_2O	71
2	6-carboxy-NCT (**10**)	TFE	85[c]
3	5-carboxy-TMR (**16**)	TFE	78
4	6-carboxy-TMR (**17**)	4:1 TFE–H_2O	83[c]
5	5-CXR (**18**)	TFE	87
6	6-CXR (**19**)	4:1 TFE–H_2O	82[c]
7	6-Cl-NCT (**20**)	TFE	78
8	6-Br-NCT (**21**)	4:1 TFE–H_2O	75

[a]Dye structures are provided in Supporting Information. [b]Isolated yield after silica column purification. 18–48 h at 75–80 °C under oxygen balloon. [c]Gram scale.

Scheme 6. Synthesis of 6-Carboxy-TMR



we discovered that addition of 10% to 20% water by volume kept the reaction mixture homogeneous, providing **17** in 83% yield on a gram scale. The intermediate triarylmethanes of dyes **9**, **19**, and **21** (Table 2) also showed poor solubility in TFE, and addition of water was necessary to ensure efficient oxidation by molecular oxygen and minimize the amount of TFE needed in large-scale reactions. In general, reaction of various 3-aminophenols with 4- or 5-substituted phthalaldehydic acids in TFE or TFE–water under an oxygen atmosphere (balloon) cleanly provides isomerically pure rhodamines in high yields after simple silica column purification (Table 2).

Dyes (**10**, **17**, and **19**) crystallized from a DCM–MeOH solvent mixture yielded deep colored, X-ray quality crystals in the open zwitterionic form (Figure 1). The zwitterionic

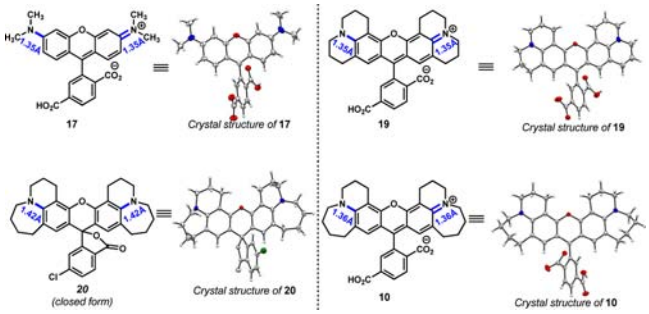


Figure 1. Crystal structures of some rhodamines.

structure allows for charge delocalization over the rhodamine core, resulting in partial double bond character, thus shortening the C–N bonds. Lengths of the aryl carbon–nitrogen bonds in crystal structures of **10**, **17**, and **19** were measured as 1.35–1.36 Å, consistent with reported carbon–nitrogen partial double bond length.[30] On the other hand, 6-Cl-NCT (**20**) formed colorless crystals from DMSO-d_6. X-ray analysis revealed the closed nonzwitterionic structure of **20** (Figure 1). The closed structure prevents charge delocalization, resulting in a typical aryl carbon–nitrogen single bond length of 1.42 Å.[30]

In summary, we developed a simple and efficient method for synthesis of rhodamine dyes from phthalaldehydic acids. Using TFE–water as a reaction medium and molecular oxygen as an oxidant allowed for one-step, operationally simple, and efficient access to a variety of rhodamines. We demonstrated the efficiency of this approach through a gram scale synthesis of

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several common rhodamines, including 6-carboxytetramethylrhodamine in 83% yield as a single isomer.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b02635.

Experimental procedures, characterization data, ^{1}H and ^{13}C NMR spectra data of new and previously reported compounds (PDF)
X-ray crystal structure data for **10** (CIF)
X-ray crystal structure data for **11** (CIF)
X-ray crystal structure data for **11a** (CIF)
X-ray crystal structure data for **19** (CIF)
X-ray crystal structure data for **20** (CIF)
X-ray crystal structure data for **14** (CIF)
X-ray crystal structure data for **17** (CIF)
X-ray crystal structure data for **S05** (CIF)
X-ray crystal structure data for **S09** (CIF)

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Notes

The authors declare no competing financial interest.

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