DOI: 10.1002/ange.201205369

Fluorescent Dyes

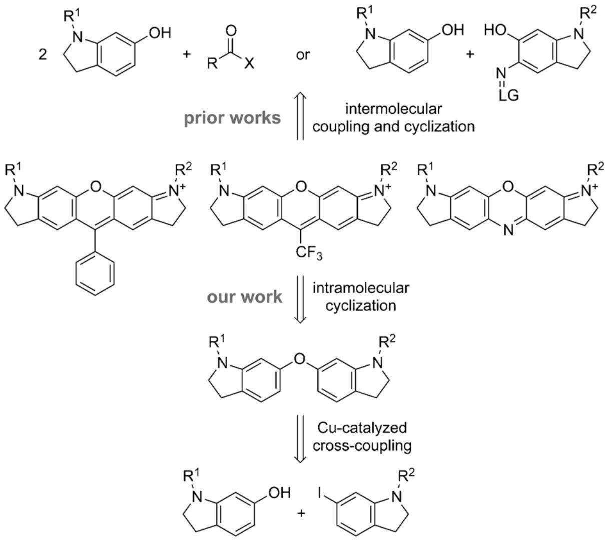
A Common Diaryl Ether Intermediate for the Gram-Scale Synthesis of Oxazine and Xanthene Fluorophores**

*Andrew V. Anzalone, Tracy Y. Wang, Zhixing Chen, and Virginia W. Cornish**

Recent advances in fluorescence spectroscopy have driven the demand for dyes with improved photophysical and fluorescence properties.[1] In addition to their development as cellular and single-molecule imaging tools, engineered fluorescent dyes have also been developed as environmental sensors that can provide information on local viscosity, pH value, solute concentration, and electrical potential.[2] Current and future developments in this field, especially those relevant to single-molecule and cellular imaging, depend on the synthesis of customized fluorescent dyes that emit in the red region of the visible spectrum, have high extinction coefficients and quantum yields, and display high photostability.[3]

Dyes from the oxazine and xanthene classes are among the best for these purposes, exemplified by commercial compounds ATTO-655 and Alexa Fluor-594, respectively.[3a,4] In the synthesis of derivatized or customized fluorophores, modification of commercially available dyes is often limited, either because of cumbersome functionalization of the parent compounds, or because of prohibitive costs to obtain sufficient quantities of dye for carrying out the necessary synthetic steps. Thus, the de novo synthesis of fluorescent dyes from basic organic building blocks is an essential aspect of technology development. Despite the obvious importance of these molecules and the evident need for improved synthetic methodologies, oxazines and xanthenes are still largely synthesized by using methods reported decades or more ago that do not take advantage of the efficiencies of modern chemical transformations.[5]

Here, we report a novel and scalable synthetic approach to widely used oxazine and xanthene fluorophores through a common diaryl ether intermediate (Scheme 1). Taking advantage of recent developments in transition-metal catalysis, we prepared electronically activated diaryl ethers to serve as tethered nucleophiles, reacting with a range of substrates to undergo cyclization to cationic fluorescent compounds (Scheme 1, our work). Final products were provided in good overall yields, were amenable to purification using standard silica-based normal-phase flash chromatography, and, significantly, could be prepared on gram scale.



Scheme 1. Retrosynthetic analysis for oxazine and xanthene fluorophores, highlighting the differences in approach between prior works and our work. LG = leaving group, X = H or RCO_2^-.

As part of our ongoing research program to develop improved technologies for in vivo super-resolution imaging, we sought to prepare derivatives of the commonly utilized oxazine ATTO-655.[4b] Because of the expense associated with obtaining large quantities of this dye, and the lack of commercial availability of other desirable analogues, we set out to synthesize the oxazines following previously described methods.[5b,c] These prior works rely on coupling a pair of aminophenol derivatives, one of which is substituted with an electrophilic nitroso or diazo functionality, by heating the two components in acidic medium (Scheme 1, prior works). Though the aminophenol intermediates are readily prepared, the final coupling and cyclization reactions frequently result in low yields (≈15%), and necessitate the use of preparative-scale reverse-phase high-performance liquid chromatography (prep-HPLC) in order to obtain material of satisfactory purity. In our hands, the quantities obtained by this method proved insufficient to reasonably carry out the remaining steps in the synthesis of our final targets, and we concluded that scaling up the process to obtain the desired quantities was impractical. As a result, we concluded that the existing approach was not a viable synthetic route and chose to pursue alternative synthetic strategies.

Within the oxazine core structure, we identified a previously unexplored diaryl ether disconnection, which is

---

[*] A. V. Anzalone, T. Y. Wang, Z. Chen, Prof. V. W. Cornish
Department of Chemistry, Columbia University
550 West 120th Street, MC 4854, New York, NY 10027 (USA)
E-mail: vc114@columbia.edu

[**] Financial support was provided by the NIH (GM090126, GM087519, GM091804). A.V.A. is supported by Columbia University College of Physicians and Surgeons Medical Scientist Training Program (NIH T32 GM007367). We are grateful to Dr. D. Romanini for editing the manuscript, Prof. S. A. Snyder and R. L. Gonzalez for helpful discussions, and Prof. W. Min for graciously offering the use of his spectroscopy equipment.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201205369.

© 2013 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

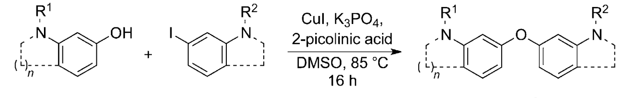
exploited in the retrosynthetic analysis shown in Scheme 1. We envisioned that a step-wise coupling and cyclization process would proceed in higher overall yield compared to the classical coupling strategy and, more importantly, would lead to fewer by-products in the final step. Thus, isolation and purification could be carried out using standard flash-chromatography techniques amenable to gram-scale operations. In pursuit of this synthetic strategy, it was necessary to examine methods for synthesizing the diaryl ether in a versatile and robust fashion.

In addition to the more traditional dihydroquinoline and tetrahydroquinoline derivatives, we also chose to explore an indoline-based scaffold to study this synthetic sequence. The precursor indoles provide a large number of building blocks from which to start, and we were also interested in the photophysical properties of the resulting oxazine products, which are not well described for the indoline class. Preparation of the indoline coupling partners proved to be straightforward by Gribble reduction and alkylation of the commercially available indole starting materials (see the Supporting Information, Scheme S1).[6]

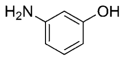
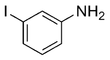
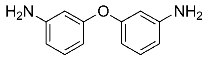
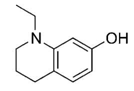
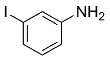
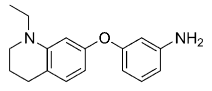
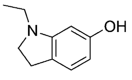
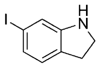
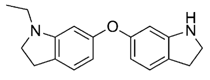
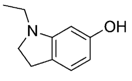
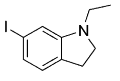
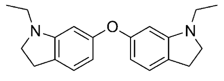
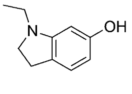
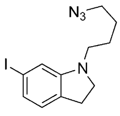
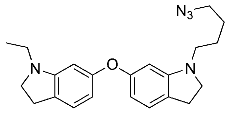
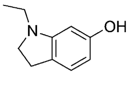
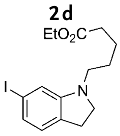
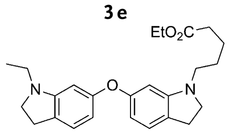
The Ullmann ether synthesis poses a potential route to the diaryl ether structural motif by copper(I)-promoted reaction between phenols and aryl halides.[7] Although attractive in theory, the classical reaction conditions typically employ strong base, stoichiometric quantities of copper, and heating at temperatures higher than 200 °C. Additionally, the reaction is known to be highly substrate dependent and, even when successful, commonly results in only modest yields. To overcome these limitations and eliminate the harsh reaction conditions, we chose to explore a more contemporary method that utilizes palladium catalysis.[8] Unexpectedly, coupling between phenol **1c** and its corresponding aryl-triflate resulted predominantly in reaction at C5 of the indoline in a Heck-type manner, producing the biaryl phenol derivative as the major product (Scheme S2). Thus, the palladium-catalyzed coupling reaction does not appear to be a useful method for synthesizing diaryl ethers when electron-rich carbon nucleophiles, such as those that exist in our system, are present.

We next turned to recent developments in ligand-assisted copper-catalyzed coupling reactions.[9] Based on work reported by Buchwald and co-workers, the coupling between phenol **1b** and 3-iodoaniline (**2a**) provided the diaryl ether **3b** in 82 % yield when carried out in the presence of catalytic copper iodide (10 mol %) and 2-picolinic acid (20 mol %) at 85 °C in DMSO (Table 1, entry 2). This catalytic system offers the advantage of orthogonality with the aniline functional group. As opposed to aryl iodides, aryl bromides were found to be very poor substrates in our system, with nearly no product formation, even at elevated temperatures (180 °C) and increased catalyst loadings (50 mol %). Nonetheless, aryl iodides were readily prepared from the corresponding commercially available aryl bromides by employing a copper-promoted halogen exchange reaction.[10] Couplings carried out under the aforementioned conditions provided a range of substituted diaryl ethers in good yields (Table 1). Following these coupling reactions, anilines **3a** and **3b** were transformed to their dihydroquinoline derivatives through a modified Skraup reaction, and subsequently alkylated and reduced where appropriate (see the Supporting Information).

Table 1: Copper(I)-catalyzed couplings between phenols and aryl iodides to furnish diaryl ethers.[a]



1a–c 2a–e 3a–f

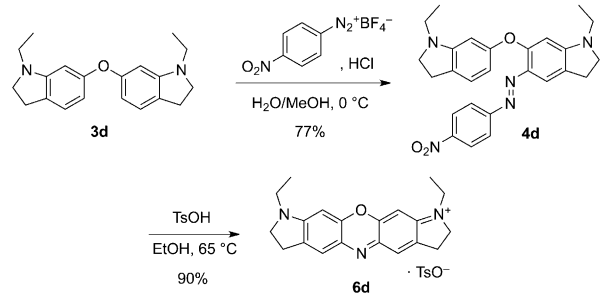
Entry	Phenol	Aryl iodide	Diaryl ether	Yield [%]
1	 **1a**	 **2a**	 **3a**	90
2	 **1b**	 **2a**	 **3b**	82
3	 **1c**	 **2b**	 **3c**	88
4	 **1c**	 **2c**	 **3d**	80
5	 **1c**	 **2d**	 **3e**	77
6	 **1c**	 **2e**	 **3f**	80

[a] Conditions: phenol (1.2 equiv), copper(I) iodide (10 mol %), 2-picolinic acid (20 mol %), K_3PO_4 (2.0 equiv), DMSO, 85 °C, 24 h. DMSO = dimethyl sulfoxide.

Having established a reliable means of synthesizing the critical diaryl ether intermediates, we next explored conditions to convert these compounds to their corresponding oxazine dyes. This transformation was readily accomplished by reaction of diaryl ether **3d** with one equivalent of 4-nitrobenzene diazonium tetrafluoroborate, followed by heating the corresponding diazene **4d** with *p*-toluenesulfonic acid (TsOH) to 65 °C in ethanol (Scheme 2). The latter process proceeded with near-quantitative conversion and, following simple silica-based flash chromatography to remove the liberated *p*-nitroaniline, we obtained the oxazine tosylate salt in high purity and 90 % yield. Implementing this strategy, several substituted oxazine dyes with various spectroscopic properties were synthesized in good to excellent yields from the corresponding diazene compounds (Table 2). Of note, over one gram of oxazine **6f** was prepared by this method in 94 % yield, thus demonstrating the scalability of our approach (Table 2, entry 6). Additionally, **6g**, which is the sulfonated analogue of **6c** and resembles commercially available ATTO-655, was synthesized in good yield by this method.

To further expand the scope of this methodology, we examined other popular fluorescent dyes for similar diaryl

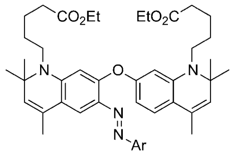
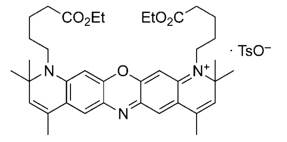
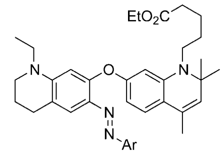
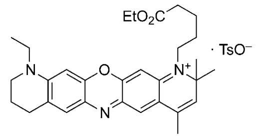
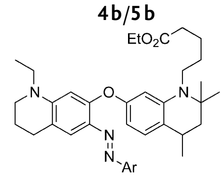
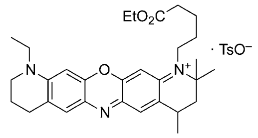
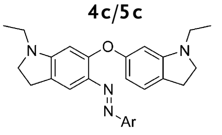
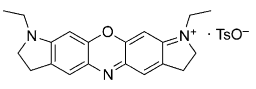
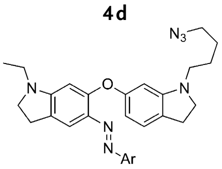
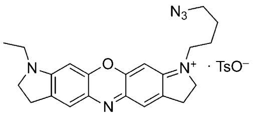
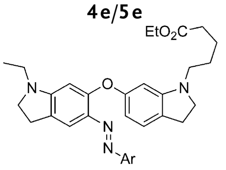
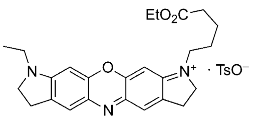
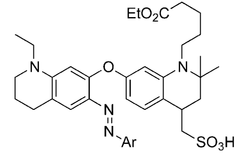
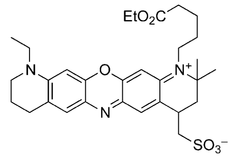
© 2013 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim



Scheme 2. Reaction sequence for conversion of diaryl ethers to oxazine dyes.

Table 2: Synthesis of substituted oxazine dyes.[a]

Diazene $\xrightarrow[\text{EtOH, 65 °C}]{\text{TsOH}}$ Oxazine tosylate salt
4a–f 6a–f

Entry	Diazene[b,c]	Oxazine	Yield [%]
1	 **4a**	 **6a**	74
2	 **4b/5b**	 **6b**	85
3	 **4c/5c**	 **6c**	87
4	 **4d**	 **6d**	90
5	 **4e/5e**	 **6e**	78
6[d]	 **4f/5f**	 **6f**	94
7	 **4g/5g**	 **6g**	83

[a] Conditions: TsOH (3.0 equiv), EtOH, 65 °C, 4–8 h. [b] Ar = *p*-NO_2Ph. [c] Diazenes were obtained and used as regioisomeric mixtures to cyclize to a single oxazine product. [d] Reaction conducted on a gram-scale.

ether disconnections, and identified xanthene dyes, exemplified by rhodamine and rosamine, as potential targets (Scheme 1). The classical method to synthesize these aminoxanthenes requires heating two equivalents of an aminophenol with one equivalent of a carboxylic acid anhydride or aldehyde under acidic conditions ($ZnCl_2$ or H_2SO_4), often at high temperatures (Scheme 1, prior works).[5a] When this strategy is employed with derivatized analogues, the reactions typically result in modest to poor yields, and isolation of the products is difficult by conventional purification methods.[11] We sought to prepare these fluorescent dyes from our diaryl ethers in an analogous manner to the synthesis of the oxazine dyes. After screening several Lewis acid catalysts and reaction conditions, we found that $Ga(OTf)_3$ catalyzed the tandem-Friedel–Crafts acylation–cyclization reaction between the aforementioned diaryl ethers and aromatic acid chlorides to provide the corresponding xanthene dyes in modest to good yields with substantial amounts of recoverable starting materials (Table 3).[12]

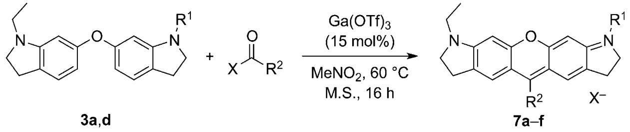
Supplementing the reaction with additional $Ga(OTf)_3$ did not lead to further product formation, suggesting that a byproduct formed in the course of the reaction inhibited the progression of the reaction. Possibly, this was due to substrate inactivation by HCl, which is generated by the Friedel–Crafts acylation and from hydrolysis of the acid chloride (water is generated in the cyclization step). Interestingly, in the case of the *p*-Me_2N-substituted derivative (Table 3, entry 3), nearly full consumption of starting material was observed, suggesting that the *p*-Me_2N group may be capable of buffering the reaction to allow increased conversion. Higher conversions of starting material could be obtained by resubjecting the crude product mixture (following workup) to the initial reaction conditions.

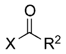
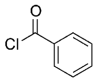
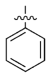
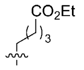
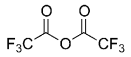
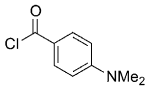
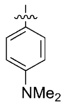
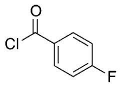
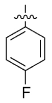
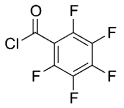
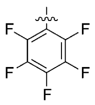
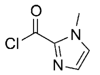
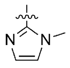
Of note, this synthetic strategy allows the synthesis of asymmetrically functionalized xanthene dyes, which are not accessible by the classical coupling strategy. Additionally, several dyes were prepared in significantly higher yields compared to traditional syntheses. For example, compound **7b**, an asymmetric xanthene, was synthesized in 83% yield simply by reaction of the diaryl ether with 2.2 equivalents of trifluoroacetic anhydride at room temperature without a Lewis acid catalyst. By comparison, rhodamine 700, a similar dye that bears the trifluoromethyl substituent at the *meso* carbon atom, is reported to be prepared in only 5% yield from aminophenol derivatives.[13] Although this current work is limited to the synthesis of rosamines with fully substituted anilines, it may be possible to synthesize mono- or nonalkylated analogues by minor modification of this synthetic approach.

Figure 1 shows the absorption and fluorescence spectra for several of the oxazine and xanthene dyes synthesized. Notably, the increased conjugation of oxazines **6a** and **6b** results in significant red-shifted absorption and fluorescence. Similarly, xanthenes **7b**, **7e**, and **7f**, which possess electron-

© 2013 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

Table 3: Tandem catalytic Friedel–Crafts acylation/cylization reaction for the synthesis of xanthene fluorophores.[a]



Entry	R1		R2	Prod.	Yield [%][b]
1	Et			**7a**	56 (92)
2[c,e]				**7b**	83
3[d]	Et			**7c**	77 (82)
4	Et			**7d**	48 (89)
5	Et			**7e**	53 (93)
6[d]	Et			**7f**	36 (81)

[a] Conditions: acid chloride (8 equiv), Ga(OTf)$_3$ (15 mol%), MeNO$_2$, 60 °C, 4 Å molecular sieves (M.S.), 16 h. [b] Yields in brackets based on recovered starting material. [c] No Lewis acid catalyst required, TFAA (2.2 equiv) in CH$_2$Cl$_2$ at RT for 12 h. [d] Acid chloride (5 equiv) was used. [e] Reaction conducted on a gram-scale.

withdrawing side chains, also display red-shifted spectra. The p-Me$_2$N-substituted rosamine **7c** displays pH-dependent fluorescence, with a near 30-fold increase in fluorescence intensity under acidic conditions (data not shown). Spectral properties of the dyes in aqueous solution are provided in Table 4.

In summary, we have established a high-yielding, scalable synthetic route for the preparation of the widely used oxazine- and xanthene-based fluorescent dyes from a common diaryl ether intermediate. Compared to previously existing synthetic methodologies, our work provides a versatile means for preparing these fluorescent dyes and eliminates the need for tedious and expensive purifications. Following this synthetic approach, a number of oxazine and xanthene fluorophores were synthesized and characterized. With proper synthetic planning, we believe this to be a general and widely applicable approach to the synthesis of derivatized oxazine and xanthene dyes, thus possibly facilitating the development of novel fluorophores and probes with unique properties.

Table 4: Spectral properties of fluorescent dyes in H$_2$O.[a]

Dye	$\lambda_{\max abs}$ [nm]	$\varepsilon_{\max}$ [M^{-1} cm^{-1}]	λ_{fluor} [nm]	Fwhm[b] [nm]	Φ_f
6a	703	50000	717	42	0.08
6b	682	69000	696	44	0.09
6c	664	67000	681	45	0.24
6d	648	66000	661	43	0.11
6g	663	97000	677	43	0.20
7a	553	58000	576	49	0.19
7b	616	61000	643	90	0.08
7c	559	79000	585	53	0.14
7d	555	60000	575	47	0.21
7e	579	68000	602	44	0.27
7f	581	57000	600	42	0.24

[a] Measurements were taken in H$_2$O, with the exception of **7c**, which was measured in aqueous HCl (50 mM). [b] Full-width at half-maximum height.

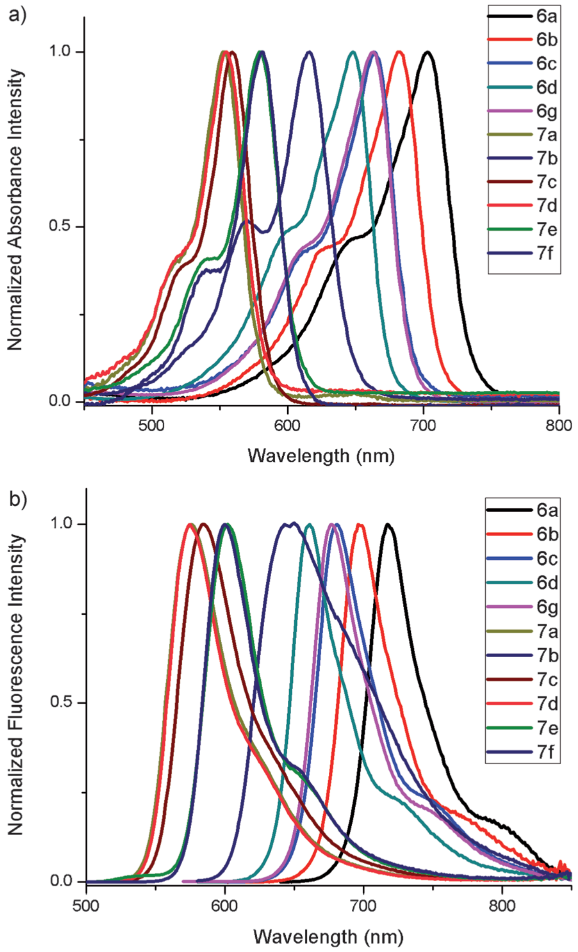


Figure 1. a) Absorbance and b) fluorescence spectra of various oxazine and xanthene derivatives. Spectra were obtained in H$_2$O, with the exception of **7c**, which was obtained in aqueous HCl (50 mM).

Received: July 7, 2012
Revised: September 25, 2012
Published online: November 21, 2012

Keywords: copper · cyclization · domino reaction · dyes/pigments · fluorescence

---

[1] a) P. Tinnefeld, M. Sauer, *Angew. Chem.* **2005**, *117*, 2698–2728; *Angew. Chem. Int. Ed.* **2005**, *44*, 2642–2671; b) B. Huang, M.

Bates, X. Zhuang, *Annu. Rev. Biochem.* **2009**, *78*, 993 – 1016; c) M. Fernández-Suárez, A. Y. Ting, *Nat. Rev. Mol. Cell Biol.* **2008**, *9*, 929 – 943.

[2] a) M. A. Haidekker, T. P. Brady, D. Lichlyter, E. A. Theodorakis, *J. Am. Chem. Soc.* **2006**, *128*, 398 – 399; b) S. Charier, O. Ruel, J.-B. Baudin, D. Alcor, J.-F. Allemand, A. Meglio, L. Jullien, *Angew. Chem.* **2004**, *116*, 4889 – 4892; *Angew. Chem. Int. Ed.* **2004**, *43*, 4785 – 4788; c) G. Grynkiewicz, M. Poenie, R. Y. Tsien, *J. Biol. Chem.* **1985**, *260*, 3440 – 3450; d) F. Yu, P. Li, G. Li, G. Zhao, T. Chu, K. Han, *J. Am. Chem. Soc.* **2011**, *133*, 11030 – 11033; e) E. Fluhler, V. G. Burnham, L. M. Loew, *Biochemistry* **1985**, *24*, 5749 – 5755.

[3] a) G. T. Dempsey, J. C. Vaughan, K. H. Chen, M. Bates, X. Zhuang, *Nat. Methods* **2011**, *8*, 1027 – 1036; b) J. Fölling, V. Belov, R. Kunetsky, R. Medda, A. Schönle, A. Egner, C. Eggeling, M. Bossi, S. W. Hell, *Angew. Chem.* **2007**, *119*, 6382 – 6386; *Angew. Chem. Int. Ed.* **2007**, *46*, 6266 – 6270.

[4] a) M. Heilemann, S. van de Linde, A. Mukherjee, M. Sauer, *Angew. Chem.* **2009**, *121*, 7036 – 7041; *Angew. Chem. Int. Ed.* **2009**, *48*, 6903 – 6908; b) R. Wombacher, M. Heidbreder, S. van de Linde, M. P. Sheetz, M. Heilemann, V. W. Cornish, M. Sauer, *Nat. Methods* **2010**, *7*, 717 – 719.

[5] a) M. Beija, C. A. M. Afonso, J. M. G. Martinho, *Chem. Soc. Rev.* **2009**, *38*, 2410 – 2433; b) A. Kanitz, H. Hartmann, *Eur. J. Org. Chem.* **1999**, 923 – 930; c) S. M. Pauff, S. C. Miller, *Org. Lett.* **2011**, *13*, 6196 – 6199.

[6] G. W. Gribble, P. D. Lord, J. Skotnicki, S. E. Dietz, J. T. Eaton, J. L. Johnson, *J. Am. Chem. Soc.* **1974**, *96*, 7812 – 7814.

[7] a) F. Ullmann, P. Sponagel, *Ber. Dtsch. Chem. Ges.* **1905**, *38*, 2211 – 2212; b) J. S. Sawyer, *Tetrahedron* **2000**, *56*, 5045 – 5065.

[8] C. H. Burgos, T. E. Barder, X. Huang, S. L. Buchwald, *Angew. Chem.* **2006**, *118*, 4427 – 4432; *Angew. Chem. Int. Ed.* **2006**, *45*, 4321 – 4326.

[9] D. Maiti, S. L. Buchwald, *J. Am. Chem. Soc.* **2009**, *131*, 17423 – 17429.

[10] A. Klapars, S. L. Buchwald, *J. Am. Chem. Soc.* **2002**, *124*, 14844 – 14845.

[11] G. Y. Mitronova, V. N. Belov, M. L. Bossi, C. A. Wurm, L. Meyer, R. Medda, G. Moneron, S. Bretschneider, C. Eggeling, S. Jakobs, S. W. Hell, *Chem. Eur. J.* **2010**, *16*, 4477 – 4488.

[12] a) G. A. Olah, O. Farooq, S. M. F. Farnia, J. A. Olah, *J. Am. Chem. Soc.* **1988**, *110*, 2560 – 2565; b) S. Kobayashi, I. Komoto, J.-i. Matsuo, *Adv. Synth. Catal.* **2001**, *343*, 71 – 74; c) G. K. S. Prakash, T. Mathew, G. A. Olah, *Acc. Chem. Res.* **2012**, *45*, 565 – 577.

[13] S. Clunas, J. M. D. Strory, J. E. Rickard, D. Horsley, C. R. Harrington, C. M. Wischik, WO2010/067078, **2010**.

© 2013 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim