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Modular synthesis of 4-aminocarbonyl substituted 1,8-naphthalimides and application in single molecule fluorescence detection

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Robust methodology to install amide, carbamate, urea and sulfonamide functionality to the 1,8-naphthalimide scaffold has been developed and exemplified. New benzamidonaphthalimide 6, synthesised using this approach, was found to be sensitive to base whereupon fluorescence emission strongly increases (>10-fold) and red-shifts (>4000 cm^{-1}). The optical properties of deprotonated 6 allow for single molecule fluorescence detection, the first example of such behaviour from this class of fluorophore.

In light of their photophysical properties, including high fluorescence quantum yields and inherent photostability, amine substituted 1,8-naphthalimides are extensively used as colourimetric and fluorescent probes.[1] While less common, amide and carbamate substituted 1,8-naphthalimides have also been employed for sensing and as intracellular probes.[2] Hydrolysis of amide or carbamate containing 1,8-naphthalimides to the corresponding amine elicits a bathochromic shift of emission maxima (from λ_{em} ~470 to ~550 nm), a property exploited in the design of ratiometric probes. In addition, Tian et al. showed that a 4-benzamide substituted 1,8-naphthalimide can be deprotonated to effect red-shifted emission (λ_{em} = 583 nm, MeCN) that enabled the detection of fluoride.[3] This sensing mechanism also functions in carbamates and has been applied to the detection of carbon dioxide.[4] Importantly, the 1,8-naphthalimide is also a common scaffold in medicinal chemistry with scriptaid, amonafide, and their derivatives possessing potent anti-cancer activity.[5]

Despite the demonstrated utility of 1,8-naphthalimide-based fluorescent probes, the synthesis of functionalised 1,8-naphthalimides remains remarkably underdeveloped with a dependence on highly functional group specific, linear, approaches. For example, the typical synthesis of an amide begins with 4-nitro- or 4-azido-1,8-naphthalic anhydride (Figure 1), the latter requiring an S_NAr step from 4-bromo-1,8-naphthalic anhydride. In each case formation of the imide and a reduction step (usually involving stoichiometric $SnCl_2$, PPh_3 or NaSH) are required to give the 4-amino analogue. This amine is a poor nucleophile and can only be modified to accommodate one extra substituent, a fact highlighted by a lack of lactam derivatives in the literature. In addition, converting the 4-amino moiety to a carbamate relies on the use of toxic triphosgene to generate the intermediate carbamoyl chloride.[2]

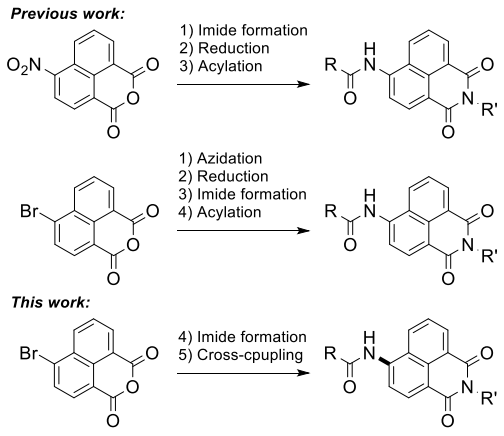

Figure 1. Summary of traditional approaches and current work.

While amine substituted 1,8-naphthalimide derivatives can be directly accessed using copper and palladium-mediated cross-coupling reactions,[6] for carboxamide substituents, examples are limited to the introduction of phthalimide using stoichiometric copper(I)[7] and the single example of palladium-catalysed bisarylation of urea reported by Fabrizzi *et al*.[8] With the diverse utility of 1,8-naphthalimides in medicinal chemistry and fluorescence spectroscopy there remains a need for methodology that can efficiently attach functional groups to the 1,8-naphthalimide core.

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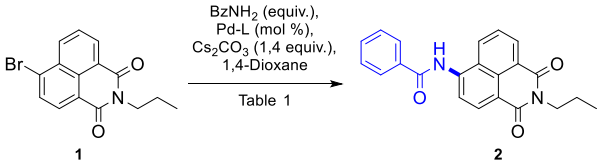
This communication details a high-yielding, modular method that directly attaches a variety of functionalities to the 1,8-naphthalimide scaffold. This method allows ready access to underrepresented derivatives including benzamides, lactams and sulphonamides, as well as commonly encountered analyte responsive triggers and cellular localisation moieties. Of considerable interest the new benzamide analogues have been shown to be readily detectable at the level of single molecule (SM). This opens the way for 1,8-naphthalimides to be used in applications such as SM-FRET and SM-sensing.

Xantphos is compatible with 1,8-naphthalimide amination[6a] and fluorophore synthesis,[9] and as such we sought inspiration from the seminal work by Yin and Buchwald.[10] In preliminary experiments, the treatment of 4-bromo-*N*-propyl-1,8-naphthalimide (**1**) (Entry 1, Table 1) with benzamide in the presence of $Pd(OAc)_2$ (1.0 mol%) and Xantphos (1.5 mol%) led to the consumption of starting material after 20 h at 100 °C. Complete reaction occurred despite conducting the reactions 10-fold more dilute than previously reported,[10] a requirement for the poorly soluble starting material. After completion of the reaction, dilution using H_2O led to precipitation and easy isolation of the secondary benzamide **2** in good yield (71%).

Table 1. Conditions for amidation.

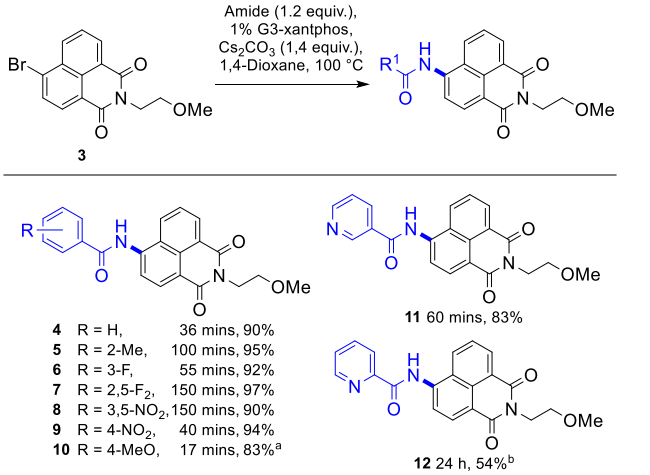


Entry	$BzNH_2$ (equiv.)	Pd-L	Pd (mol%)	Temp (°C)	Time (h)	Yield (%)
1	1.2	$Pd(OAc)_2$/ Xantphos	1.0[a]	100	20	71
2	1.2	G3-Xantphos	1.0	100	2.5	93
3	2.0	G3-Xantphos	1.0	100	0.25	90
4	1.2	G3-Xantphos	0.5	100	6.5	76
5	1.0	G3-Xantphos	1.0	100	7	82
6	1.2	G3-Xantphos	1.0	80	23	82
7	1.5	G3-Xantphos	1.0	80	22	78

a) $Pd(OAc)_2$ (1.0 mol%), Xantphos (1.5 mol%).

In an effort to enhance the reaction rate the palladacycle precatalyst G3-Xantphos was investigated. The G3-precatalyst offers a number of advantages including ease of preparation (and also commercial availability) and insensitivity to both air and moisture.[11] The Pd to Xantphos ratio is controlled and H_2O is not required for reduction of Pd(II), which can negate the potential for competitive hydroxylation.[12] Repeating the trial reaction using 1 mol% G3-Xantphos led to the consumption of starting material in 2.5 h (Entry 2) providing **2** in an isolated yield of 93% after precipitation. When two equivalents of benzamide were employed (Entry 3) a yield of 90% was obtained after 0.25 h. Lower G3-Xantphos loading (0.5 mol%, Entry 4) or use of equimolar amounts of benzamide (Entry 5) also gave secondary amides in very good yields (76 and 82%, respectively) but with longer reaction times of (>6.5 h). Conditions involving an equimolar ratio of reagents are particularly advantageous if two synthetically valuable substrates are to be coupled. When performing the reaction at 80 °C (Entry 6) longer reaction times were required (23 h) however a high yield (82%) was again obtained. Increasing the amount of coupling partner (1.5 equiv. of benzamide, Entry 7) failed to improve the reaction rate (22 h).

With methodology in hand, substrate scope could be investigated. A small series of aryl carboxamides were synthesised from 4-bromo imide **3** (Scheme 1). In general, yields were greater than 90% when 1.2 equivalents of neutral (**4**–**5**) and electron deficient benzamides (**6**–**9**) were used as coupling partners. The 4-methoxybenzamide was incorporated in 83% but two equivalents were required to achieve full conversion within a suitable timeframe.



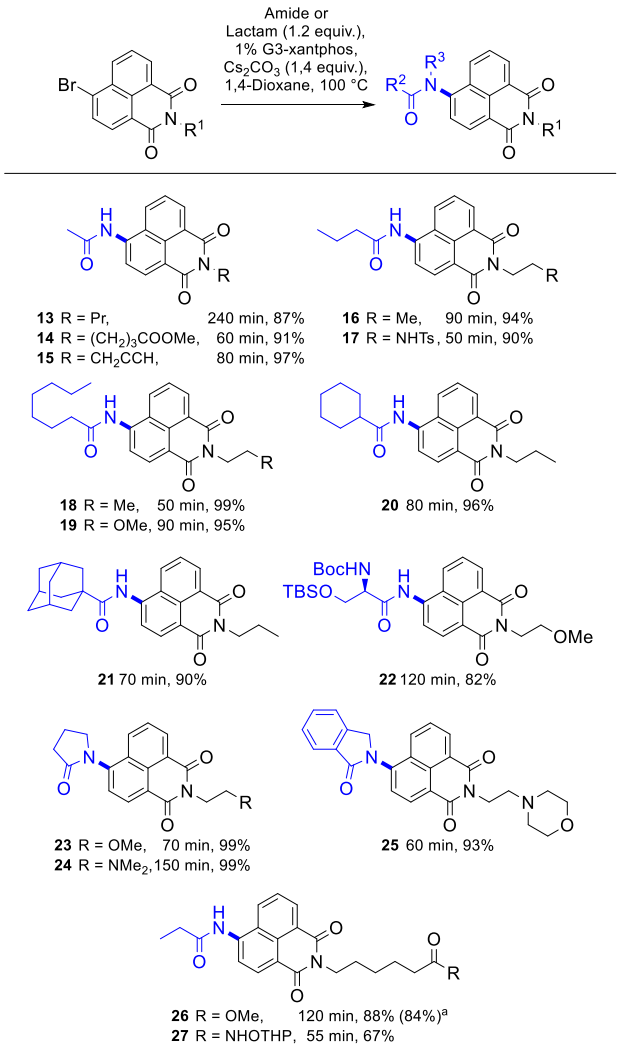
Scheme 1. Conditions: Bromide (0.25 or 0.5 mmol, 0.1 M) Amide (1.2 equiv)., G3-Xantphos (1 mol%), Cs_2CO_3 (1.4 equiv.), 100 °C; a) Amide (2.0 equiv.); b) G3-Xantphos (2 mol%).

Nicotinamide was also coupled to the 1,8-naphthalimide scaffold to give **11** in very good yield (83%). In contrast, the best yield achieved using picolinamide was 54%, a reaction that required 2 mol% of G3-Xantphos. Increasing the equivalents of amide was detrimental in this instance. The long reaction time (24 h) also suggested that the reaction may be hindered by potential chelation of the substrate to palladium.

Simple aliphatic primary amides were coupled smoothly to a number of 4-bromo-1,8-naphthalimides (Scheme 2). Linear aliphatic amides (**13**–**19**) were installed in yields greater than 87%. Cyclohexanecarboxamide (**20**) and 1-adamantanecarboxamide (**21**) were also incorporated in excellent yields (96 and 90%, respectively). Lactams, 1-pyrrolidinone and isoindolin-1-one were coupled to give tertiary amides (**23**–**24**), again, in near quantitative yields. To date there are only two reported structures of 1,8-naphthalimides bearing a lactam at the 4-position; neither of which contain any preparative information.[13] Secondary amides (e.g. *N*-methyl benzamide) failed to give any product.

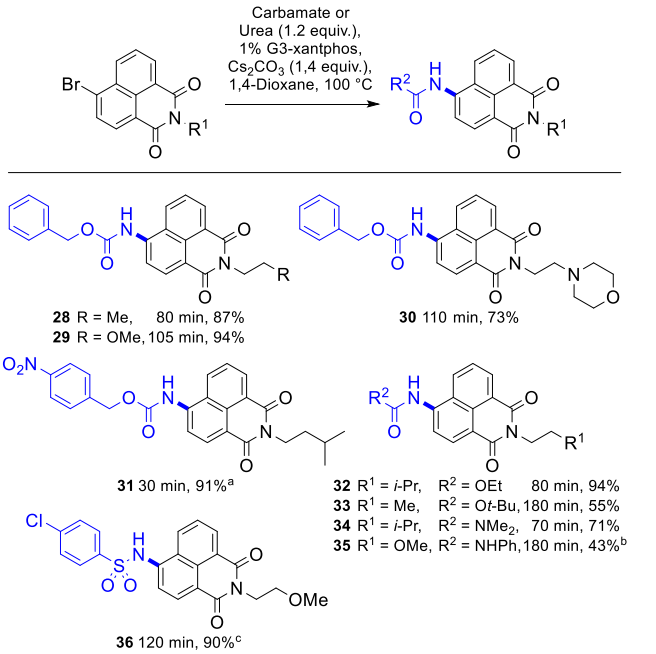
This methodology was compatible with a number of functional groups important in both medicinal chemistry and fluorescence sensing and labelling applications. Methyl ester

(**14**) and terminal alkyne (**15**) were well tolerated, as were tertiary amines, which facilitates access to amonafide derivatives (such as **24**) and lysosome targeting groups (for example **25**). Similarly, the secondary sulphonamide recently reported by Tang[14] as an endoplasmic reticulum targeting group was compatible with these conditions giving **17** in 90% yield. dos Santos' fluoride trigger[15] could also be incorporated using these reaction conditions highlighting that acid sensitive protecting groups are also well tolerated. Here the D-serine amide bearing *tert*-butyldimethylsilyl ether and secondary *tert*-butoxycarbonyl groups was incorporated to give **22** in 82% yield. A THP-protected hydroxamic acid was also accommodated by these conditions giving rise to a protected scriptaid analogue[5b] **27** in moderate yield (67%).



Scheme 2. Conditions: Bromide (0.25 or 0.5 mmol, 0.1 M) Amide (1.2 equiv.), G3-Xantphos (1 mol%), Cs_2CO_3 (1.4 equiv.), 100 °C; a) Bromide (1.5 mmol).

To circumvent the use of triphosgene in the synthesis of carbamate derivatives, benzyl carbamate was directly attached to the 1,8-naphthalimide scaffold (**28**–**30**, Scheme 3) in very good (73% for **30**) to excellent yields (94% for **28**). The 4-nitrobenzyl carbamate group (synthesised using CDI, see ESI), was also coupled to give **31** in 91% yield. This approach gave access to probes that contain an established trigger for the cellular monitoring of nitroreductase[5b] and hydrogen sulfide.[16] Ethyl and *tert*-butyl carbamates also underwent smooth coupling giving **32** and **33** in 94 and 55% yield, respectively.



Scheme 3. Conditions: Bromide (0.25 or 0.5 mmol, 0.1 M) R^2-NH_2 (1.2 equiv)., G3-Xantphos (1 mol%), Cs_2CO_3 (1.4 equiv.), 100 °C; a) G3-Xantphos (2 mol%); b) *N*-Phenylurea (2.0 equiv.); c) Sulfonamide (2.0 equiv.).

The coupling of 1,1-dimethylurea afforded **34** in reasonable yield (71%) after purification by column chromatography. *N*-Phenylurea could also be incorporated to give the 1,8-naphthalimide **35**, albeit in moderate yield (43%). We also trialled phenylthiourea as a coupling partner to access analogues described by Gale *et al*. as transmembrane chloride transporters,[17] however no appreciable conversion was detected. Incorporation of 4-chlorobenzenesulfonamide was successful with **36** isolated in excellent yield (90%). Examples of sulfonamides appended to 1,8-naphthalimide scaffold are rare[18] presumably owing to the difficulty of the transformation.

Optical characterisation of a number of the compounds reported herein was undertaken with photophysical data compiled (see ESI, Table S1). Of particular interest is benzamide **6** which showed a dramatic >10-fold increase in fluorescence emission on addition of NaOH to a solution in DMSO (Figure 2a). Neutral **6** shows an absorption maximum at 357 nm and weak emission at 461 nm (Φ_F = 0.05). On addition of NaOH (~5–20 equivalents), a new absorption grows at 486 nm with emission shifting to 575 nm, a bathochromic shift of >4000 cm^{-1}, and the quantum yield increases to 0.69 upon full deprotonation with excess NaOH (>100 equivalents). Time-resolved fluorescence measurements show that emission from benzamide **6** in DMSO (monitored at 470 nm) is short-lived and dominated by a decay component with a lifetime of 0.56 ns (Figure 2b). Addition of excess NaOH leads to emission (monitored at 570 nm) with a

significantly increased lifetime of 6.91 ns, a value similar to other reported 1,8-naphthalimides.[19]

Based on the favourable optical properties of the deprotonated form of **6** which gives a molecular brightness ($\varepsilon_M \times \Phi_F$) of 16,300 M^{-1} cm^{-1}, additional experiments were undertaken to see if single molecules could be detected by fluorescence. Compound **6** was embedded in a film of PVA spincoated from a highly dilute (~nM) aqueous solution of **6** containing 1% PVA and excess NaOH to ensure deprotonation. Single molecules were readily visualised using widefield microscopy (λ_{ex} = 532 nm, Figure 2c) and while not exceptionally bright (signal to noise ~2–3) they were comparable to core substituted naphthalene diimides imaged under similar conditions.[20] Promisingly, SMs of **6** often stayed visible for over a minute and showed some long lived reversible fluorescent intermittency (or "blinks") under ambient conditions (see ESI for video), suggesting favourable photostability and that further investigation into this as yet unexplored aspect of 1,8-naphthalimide photophysics is warranted.

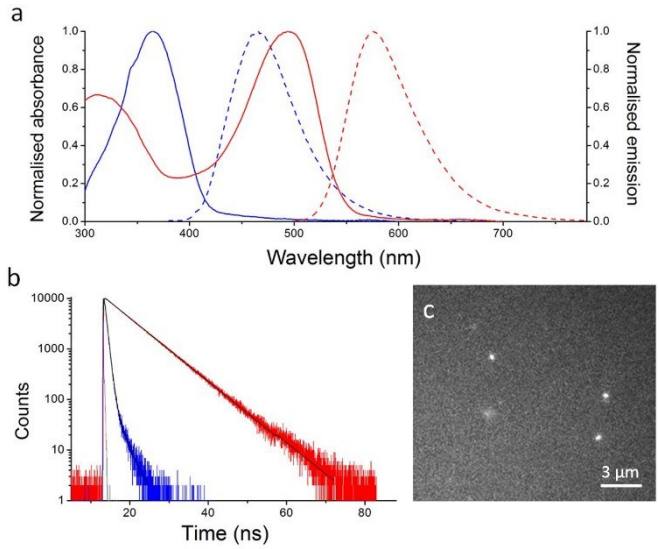


Figure 2. a) Normalised steady state absorption spectra (solid lines) and fluorescence emission spectra (dashed lines) of **6** in DMSO (red) and in DMSO with excess NaOH (blue). b) Time-resolved fluorescence decay profiles of **6** in DMSO (red) and DMSO with excess NaOH (blue) with fitted functions (black lines) and instrument response function (grey). c) Widefield fluorescence image (λ_{ex} = 532 nm) of single molecules of **6** embedded in a basified poly(vinyl alcohol) film (scale bar 3 µm).

In summary a robust general method for the functionalisation of 1,8-naphthalimides has been developed. Using this method the installation of amides, lactams, carbamates, ureas and sulphonamides has been accomplished in high yield. Furthermore, using one of the newly constructed fluorophores, the first instance of single molecule fluorescence from a 1,8-naphthalimide has been observed. It is envisaged that this work will be of widespread use to those active in the field of 1,8-naphthalimide chemistry.

Conflicts of interest

The Authors declare no conflict of interest.

Notes and references

1. (a) T. D. Ashton, K. A. Jolliffe and F. M. Pfeffer, *Chem. Soc. Rev.*, 2015, **44**, 4547. (b) R. M. Duke, E. B. Veale, F. M. Pfeffer, P. E. Kruger and T. Gunnlaugsson, *Chem. Soc. Rev.*, 2010, **39**, 3936. (c) J. Pancholi, D. J. Hodson, K. Jobe, G. A. Rutter, S. M. Goldup and M. Watkinson, *Chem. Sci.*, 2014, **5**, 3528. (d) X. Sun, Q. Xu, G. Kim, S. E. Flower, J. P. Lowe, J. Yoon, J. S. Fossey, X. Qian, S. D. Bull and T. D. James, *Chem. Sci.*, 2014, **5**, 3368.
2. (a) X. Ao, S. A. Bright, N. C. Taylor and R. B. P. Elmes, *Org. Biomol. Chem.*, 2017, **15**, 6104. (b) L. Cui, Y. Zhong, W. Zhu, Y. Xu, Q. Du, X. Wang, X. Qian and Y. Xiao, *Org. Lett.*, 2011, **13**, 928.
3. B. Liu and H. Tian, *J. Mater. Chem.*, 2005, **15**, 2681.
4. X. Zhang, S. Lee, Y. Liu, M. Lee, J. Yin, J. L. Sessler and J. Yoon, *Sci. Rep.*, 2014, **4**, 4593/1.
5. (a) S. Banerjee, E. B. Veale, C. M. Phelan, S. A. Murphy, G. M. Tocci, L. J. Gillespie, D. O. Frimannsson, J. M. Kelly and T. Gunnlaugsson, *Chem. Soc. Rev.*, 2013, **42**, 1601. (b) C. L. Fleming, T. D. Ashton, C. Nowell, M. Devlin, A. Natoli, J. Schreuders and F. M. Pfeffer, *Chem. Commun.*, 2015, **51**, 7827. (c) V. Gaur, T. Connor, A. Sanigorski, S. D. Martin, C. R. Bruce, D. C. Henstridge, S. T. Bond, K. A. McEwen, L. Kerr-Bayles, T. D. Ashton, C. Fleming, M. Wu, L. S. Pike Winer, D. Chen, G. M. Hudson, J. W. R. Schwabe, K. Baar, M. A. Febbraio, P. Gregorevic, F. M. Pfeffer, K. R. Walder, M. Hargreaves and S. L. McGee, *Cell Reports*, 2016, **16**, 2802.
6. (a) C. L. Fleming, T. D. Ashton and F. M. Pfeffer, *Dyes Pigments*, 2014, **109**, 135. (b) R. Umeda, H. Nishida, M. Otono and Y. Nishiyama, *Tetrahedron Lett.*, 2011, **52**, 5494.
7. M. V. Ramakrishnam Raju, E. Chandra Prakash, H.-C. Chang and H.-C. Lin, *Dyes Pigments*, 2014, **103**, 9.
8. (a) D. Esteban-Gomez, L. Fabbrizzi and M. Licchelli, *J. Org. Chem.*, 2005, **70**, 5717. (b) D. Esteban-Gomez, L. Fabbrizzi, M. Licchelli and D. Sacchi, *J. Mater. Chem.*, 2005, **15**, 2670.
9. J. B. Grimm and L. D. Lavis, *Org. Lett.*, 2011, **13**, 6354.
10. J. Yin and S. L. Buchwald, *Org. Lett.*, 2000, **2**, 1101.
11. N. C. Bruno, M. T. Tudge and S. L. Buchwald, *Chem. Sci.*, 2013, **4**, 916.
12. C. L. Fleming, T. D. Nalder, E. H. Doeven, C. J. Barrow, F. M. Pfeffer and T. D. Ashton, *Dyes Pigments*, 2016, **126**, 118.
13. WO2006027628A2, 2006.
14. H. Xiao, P. Li, X. Hu, X. Shi, W. Zhang and B. Tang, *Chem. Sci.*, 2016, **7**, 6153.
15. A. C. Goncalves, N. C. Sato, H. M. Santos, J. L. Capelo, C. Lodeiro and A. A. dos Santos, *Dyes Pigments*, 2016, **135**, 187.
16. L. Zhang, W.-q. Meng, L. Lu, Y.-S. Xue, C. Li, F. Zou, Y. Liu and J. Zhao, *Sci. Rep.*, 2014, **4**, 5870.
17. S. N. Berry, V. Soto-Cerrato, E. N. W. Howe, H. J. Clarke, I. Mistry, A. Tavassoli, Y.-T. Chang, R. Perez-Tomas and P. A. Gale, *Chem. Sci.*, 2016, **7**, 5069.
18. (a) S. Inada, F. Hojo and M. Okazaki, *J. Syn. Org. Chem. Jpn*, 1973, **31**, 648. (b) C. Dong, C.-Q. Zhou, J.-W. Yang, T.-C. Liao, J.-X. Chang, C.-X. Yin and W.-H. Chen, *RSC Adv.*, 2015, **5**, 32990. (c) Y. Singh, A. Misra and K. Misra, *Ind. J. Chem.*, 2002, **41B**, 1238.
19. (a) L. Bekere, D. Gachet, V. Lokshin, W. Marine and V. Khodorkovsky, *Beilstein J. Org. Chem.*, 2013, **9**, 1311. (b) J. Ren, X.-L. Zhao, Q.-C. Wang, C.-F. Ku, D.-H. Qu, C.-P. Chang and H. Tian, *Dyes Pigments*, 2005, **64**, 179.
20. (a) T. D. M. Bell, S. Yap, C. H. Jani, S. V. Bhosale, J. Hofkens, F. C. De Schryver, S. J. Langford and K. P. Ghiggino, *Chem. Asian J.*, 2009, **4**, 1542. (b) S. Maniam, R. P. Cox, S. J. Langford and T. D. M. Bell, *Chem. Eur. J.*, 2015, **21**, 4133.