ChemComm

COMMUNICATION

View Article Online
View Journal

Published on 23 October 2014. Downloaded by University of Illinois at Chicago on 31/10/2014 08:49:22.

Cite this: DOI: 10.1039/c4cc07807a

Received 3rd October 2014,
Accepted 23rd October 2014

DOI: 10.1039/c4cc07807a

www.rsc.org/chemcomm

One-pot synthesis of dibenzo[*b*,*h*][1,6]naphthyridines from 2-acetylaminobenzaldehyde: application to a fluorescent DNA-binding compound†

Kentaro Okuma,* Tomohiro Koga, Saori Ozaki, Yutaro Suzuki, Kenta Horigami, Noriyoshi Nagahora, Kosei Shioji, Masatora Fukuda and Masanobu Deshimaru

Dibenzo[*b*,*h*][1,6]naphthyridines were synthesized in one pot by reacting 2-acetylaminobenzaldehyde with methyl ketones under basic conditions *via* four sequential condensation reactions. This method was also applied to the synthesis of 1,2-dihydroquinolines. 6-Methyl-1,6-dibenzonaphthyridinium triflates showed strong fluorescence, and the fluorescence intensities were changed upon intercalation into double-stranded DNA.

Cyclic polyaza compounds, such as quinolines, dihydroquinolines, naphthyridines, benzonaphthyridines, and phenanthridines, are important structural components of naturally occurring alkaloids and synthetic analogues possessing interesting biological activities.[1,2] Ethidium bromide (EtBr) and the analogues of phenanthridine and benzonaphthyridine are used for the visualization of nucleic acids in agarose gels. Those dyes produce red-orange fluorescence under ultraviolet light and show enhanced fluorescence when bound to double-stranded DNA. Because of their fluorescence properties, those dyes are used as DNA intercalating agents.[3] In this regard, the development of efficient routes for the preparation of phenanthridines and benzonaphthyridines is of great importance in synthetic organic, pharmaceutical, and material chemistry.[4] It is well known that benzaldehyde reacts with acetophenone under basic conditions to afford chalcone.[5] We have reported a one-pot synthesis of quinolines and 2-arylquinoline *N*-oxides that involved reacting 2-nitrochalcones with Sn/HCl or Zn/HCl, the intermediates of which were 2-aminochalcones, or the Friedländer reaction.[6] Methods for the synthesis of dibenzo[*b*,*h*][1,6]naphthyridines (**1**) include the reaction of quinolines with 2-aminobenzoic acid or 2-aminoacetophenone[7] and the reaction of quinolinones with 2-aminoacetophenones,[8] all of which are based on the Friedländer reaction that proceeds at a high reaction temperature (160–180 °C). Recently, three-component reactions that yielded naphthyridine derivatives were reported.[9] Although 2,2,4-trimethyl-[1,2]dihydroquinoline was obtained by reacting aniline with acetone and iodine,[10] there is no report on the direct synthesis of dibenzo[*b*,*h*][1,6]naphthyridines **1** from easily available starting materials, such as 2-aminobenzaldehydes. Those results prompted us to look into ways for the synthesis of dibenzo[*b*,*h*][1,6]naphthyridines **1** and their alkylation. Our synthetic plan consists of four sequential condensation reactions (aldol, imination, aza-Morita–Baylis–Hillman, and intramolecular imination) of 2-acetylaminobenzaldehyde (**2**) with methyl ketones (**3**), as shown in Fig. 1. In this communication, we report the synthesis of **1**, their fluorescence properties, and their DNA intercalation properties.

2-Acetylaminobenzaldehyde **2** was synthesized by reducing 2-nitrobenzaldehyde with Sn/HCl or Fe/HCl and subsequently adding acetic anhydride.[11] Treatment of **2** with 2 eq. of 4-methylacetophenone **3a** in the presence of aq NaOH (5 M solution, 2 eq.) in refluxing EtOH gave 2-(2-acetylaminophenyl)-3-(4-methylbenzoyl)-1,2-dihydroquinoline (**4a**) in 80% yield.[12] The structure of **4a** was confirmed by ^{1}H NMR, ^{13}C NMR, and MS analyses. The ^{1}H NMR spectrum of **4a** showed a singlet at 6.06 ppm, which unambiguously indicated the existence of a methine proton, along with peaks of 13 aromatic protons. Other reactions were carried out in a similar manner (55–99% yields, Scheme 1, Table S1, ESI†).

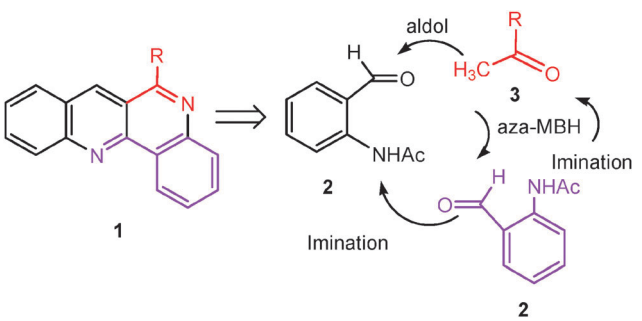
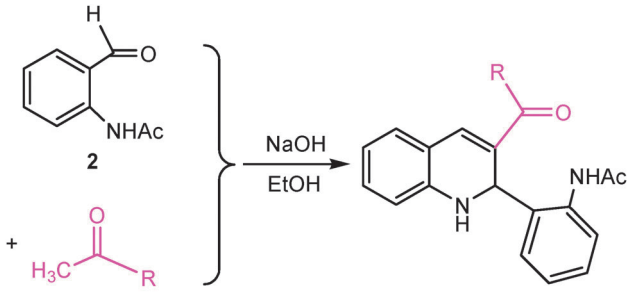


Fig. 1 Retrosynthetic analysis of dibenzonaphthyridine **1**.

Department of Chemistry, Faculty of Science, Fukuoka University, Jonan-ku, Fukuoka 814-0180, Japan. E-mail: kokuma@fukuoka-u.ac.jp

† Electronic supplementary information (ESI) available. CCDC 1003324 and 1003843. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c4cc07807a

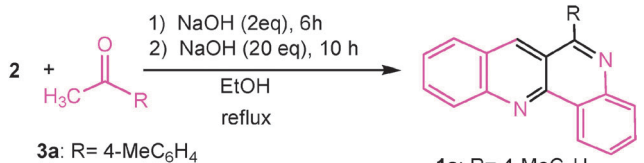
Published on 23 October 2014. Downloaded by University of Illinois at Chicago on 31/10/2014 08:49:22.



3a: R= 4-MeC_6H_4
3b: R= Ph
3c: R= 4-$MeOC_6H_4$
3d: R= 4-ClC_6H_4
3e: R= 4-BrC_6H_4
3f: R= 4-$NO_2C_6H_4$
3g: R= 1-naphthyl
3h: R= CH_3CH_2
3i: R= 2-pyridyl
3j: R= 2-thienyl

4a: R= 4-MeC_6H_4
4b: R= Ph
4c: R= 4-$MeOC_6H_4$
4d: R= 4-ClC_6H_4
4e: R= 4-BrC_6H_4
4f: R= 4-$NO_2C_6H_4$
4g: R= 1-naphthyl
4h: R= CH_3CH_2
4i: R= 2-pyridyl
4j: R= 2-thienyl

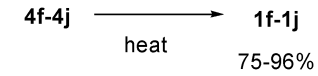
Scheme 1 Reaction of 2-acetylaminobenzaldehyde **2** with **3**.



3a: R= 4-MeC_6H_4
3b: R= Ph
3c: R= 4-$MeOC_6H_4$
3d: R= 4-ClC_6H_4
3e: R= 4-BrC_6H_4
3f: R= 4-$NO_2C_6H_4$
3g: R= 1-naphthyl
3h: R= CH_3CH_2
3i: R= 2-pyridyl
3j: R= 2-thienyl

1a: R= 4-MeC_6H_4
1b: R= Ph
1c: R= 4-$MeOC_6H_4$
1d: R= 4-ClC_6H_4
1e: R= 4-BrC_6H_4
1f: R= 4-$NO_2C_6H_4$
1g: R= 1-naphthyl
1h: R= CH_3CH_2
1i: R= 2-pyridyl
1j: R= 2-thienyl

Scheme 2 One-pot synthesis of dibenzo[*b*,*h*][1,6]naphthyridines **1**.



Scheme 3 Thermal transformation of **4f–4j**.

Compound **4e** was finally determined by X-ray crystallographic analysis (Fig. S5, ESI†). Compound **4c** was gradually oxidized in a CH_2Cl_2 solution to give the corresponding quinoline **4c**′ after 24 h at rt.

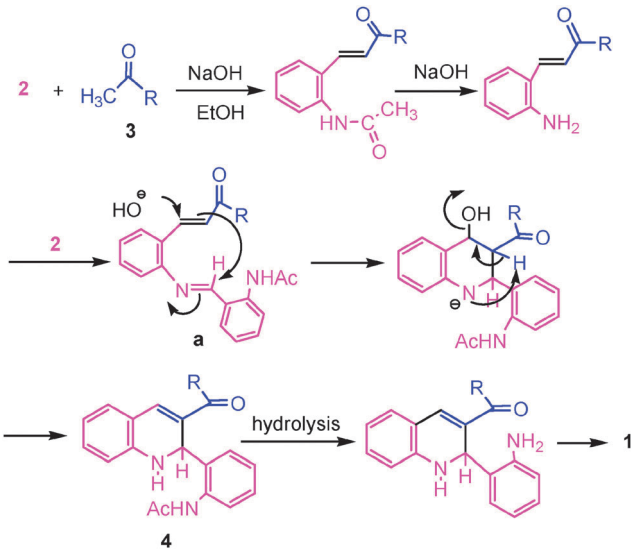
It is well known that the reaction of 2-aminobenzaldehyde with acetophenones gives 2-arylquinolines *via* the Friedländer reaction.[13] However, there is no report on the synthesis of 1,2-dihydroquinolines by reacting 2-aminobenzaldehyde derivatives with acetophenones. There are several methods for the synthesis of dihydroquinolines.[1,9] Recent examples include the $AuCl_3/AgSbF_6$-catalyzed intramolecular allylic amination of 2-tosylaminophenylprop-1-en-3-ols,[14] the electrocyclization of *N*-methyl-2-hydroxyalkylanilines,[15] the intramolecular cyclization of *o*-(1-hydroxy-2-alkenyl)phenyl isocyanides in the presence of $BF_3{\cdot}Et_2O$ as a catalyst,[16] and the tandem Michael-aldol reaction of *N*-tosyl-2-aminobenzaldehyde with an α,β-unsaturated carbonyl compound.[17] A synthesis conducted under mild basic conditions is expected to offer more advantages than the above-mentioned methods. In the method developed in this study, readily available **2** and **3** were used as substrates.

If the *N*-acetylamino group of **4** could be hydrolyzed and condensed with a carbonyl group, it would be possible to synthesize dibenzo[*b*,*h*][1,6]naphthyridines **1**. Because only aq NaOH was used as the base for the synthesis of dihydroquinolines **4**, we reacted **2** with **3** in the presence of NaOH hoping that **1** would be obtained in a one-pot operation (Scheme 2). Treatment of **2a** with **3a** in the presence of 20 M NaOH (1 eq.) in refluxing ethanol for 6 h furnished polymeric products. When 2 eq. of 5 M NaOH was added to refluxing ethanol followed by 20 eq. of 20 M NaOH, and then reflux was conducted for 10 h, naphthyridine **1a** was formed in 70% yield. Under similar conditions, naphthyridines **1b–j** were synthesized as well (Scheme 2, Table S2, ESI†). However, as the yields of **1f–1j** were low (21–23%), aerobic oxidation, deacetylation, and imination of **4** at an elevated temperature (~230 °C) were performed, and the yields of **1f–1j** were improved to 75–96% (Scheme 3, Table S3, ESI†).

The structure of **1a** was confirmed by ^1H NMR, ^{13}C NMR, MS, and elemental analyses. As single crystals of **1a** were obtained by reaction with 4-methylacetophenone, X-ray crystallographic analysis was performed (Fig. S6, ESI†).[18]

The reaction is speculated to proceed as follows: the aldol reaction of benzaldehyde **2** with acetophenone **3** furnished chalcone, which underwent hydrolysis and imination with **2** to give imine **a**. Intramolecular aza-Morita–Baylis–Hillman cyclization of imine **a** produced dihydroquinoline derivative **4**. Under specific conditions (20 eq. NaOH in refluxing ethanol for 10 h), dihydroquinoline **4** was hydrolyzed, oxidized, and intramolecularly condensed to give naphthyridine **1** (Scheme 4).

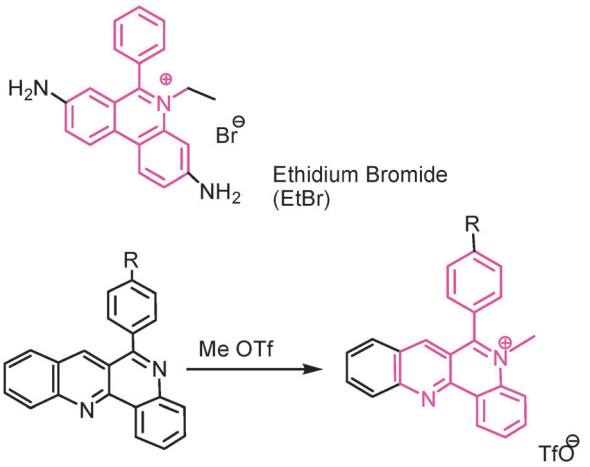
Previously, dibenzo[*b*,*h*][1,6]naphthyridines **1** were synthesized in two steps: the reaction of formylquinolin-2-one with aniline and the reaction of this imine with polyphosphoric acid.[19] 1,6-Dibenzonaphthyridin-6-ones were synthesized by



Scheme 4 Plausible reaction mechanism.

This journal is © The Royal Society of Chemistry 2014

Published on 23 October 2014. Downloaded by University of Illinois at Chicago on 31/10/2014 08:49:22.



Scheme 5 Synthesis of dibenzonaphthyridinium triflates **5a–c**.

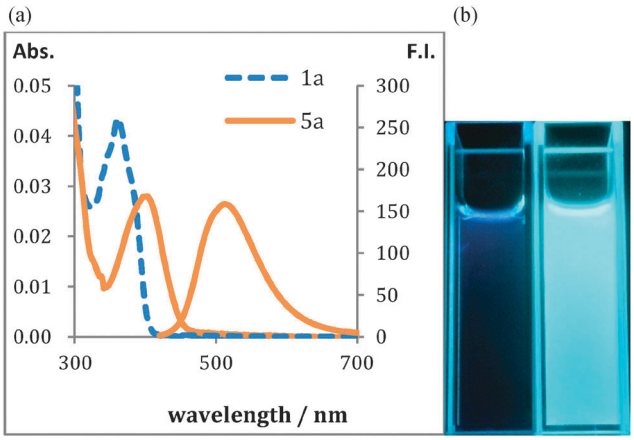


Fig. 2 (a) UV/vis and fluorescence spectra of **1a** (5 μM, EtOH, excitation at 360 nm) and **5a** (5 μM, EtOH, excitation at 400 nm). (b) Photograph of EtOH solution of **1a** (left, 0.1 mM) and **5a** (right, 0.1 mM) under 365 nm light irradiation.

reaction with diethyl 2-(3-methylbut-2-enyl)malonate with anilines in refluxing diphenyl ether, but the yields were very low at 2–4%.[20] The present reaction requires only one step using commercially available substituted methyl ketones **3** and *N*-acetyl *o*-aminobenzaldehyde **2**, which was synthesized from *o*-nitrobenzaldehyde. Thus, this method provides a versatile alternative for the synthesis of substituted naphthyridines in a one-pot operation.

If dibenzonaphthyridines **1** were selectively methylated at 6-position, their structures would be very similar to that of EtBr, a well-known DNA intercalating agent. Then, the alkylation of those compounds with methyl triflate was carried out (Scheme 5). As expected, the methylation proceeded regioselectively at 6-position to afford *N*-methylation products (**5a–c**) almost quantitatively.

EtBr is characterized by its intrinsic fluorescence. In contrast, the fluorescence properties of methylated naphthyridines **5a–c** remain unexplored. Therefore, we examined the differences in spectroscopic properties between **1a** and **5a**. Although dibenzonaphthyridine **1a** did not show significant fluorescence, methylated dibenzonaphthyridine **5a** exhibited strong fluorescence (Fig. 2a and b). The fluorescence quantum yield of **5a** is 0.15 (0.17 for **5b** and 0.18 for **5c**; see Fig. S1, ESI†).

We then examined the intercalation property of **5b** with the expectation that it would behave as an EtBr-like DNA staining reagent. As shown in Fig. 3a, a titration experiment revealed a gradual decrease in the fluorescence intensity of **5b** in response to the increasing concentration of plasmid DNA. This result can be explained by considering the ability of the nitrogen-containing planar ring to coordinate to DNA, because the structure of the ring system is very similar to that of EtBr. Interestingly, such a negative effect of the intercalation on the fluorescence is in contrast to the characteristics of EtBr, whose fluorescence is strongly enhanced by the intercalation into DNA. The difference in fluorescence properties between **5b** and EtBr was visually confirmed when DNA fragments electrophoresed on acrylamide gels were stained with those two reagents. When **5b**-stained gel was observed under UV irradiation, DNA

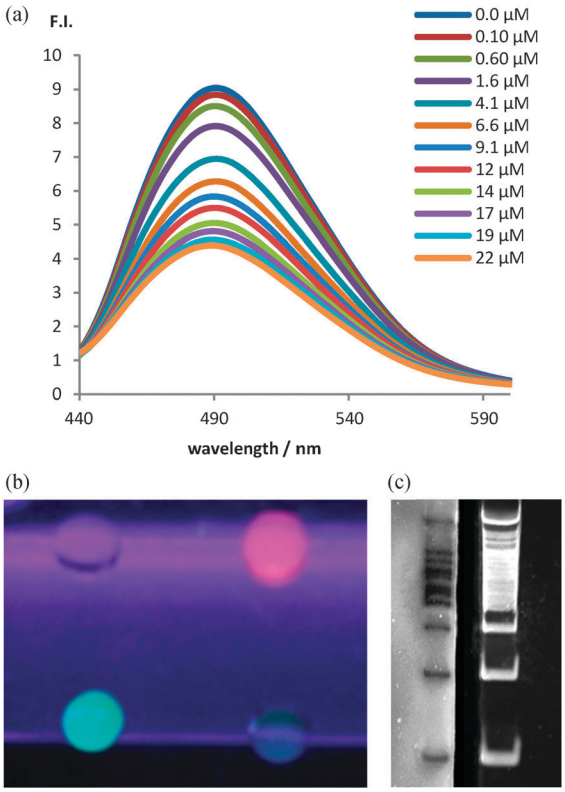


Fig. 3 Change in fluorescence of compound **5b** upon intercalation into DNA. (a) Fluorescence spectra of 0.10 μM serial solutions of **5b** containing the indicated concentrations of 31mer oligonucleotide, spanning from 440 nm to 600 nm (excitation at 370 nm). (b) Difference in DNA-intercalation-induced fluorescence between **5b** and EtBr. Upper, EtBr (0.5 μM in water); lower, compound **5b** (0.5 μM in 0.1% DMSO in water); left, without DNA; right, containing 7.5 mg mL^{-1} pUC-18 plasmid DNA. Fluorescence was observed by excitation with 254 nm UV light. (c) DNA staining of **5b**. 100 bp DNA ladder fragments electrophoresed on 8% acrylamide gels were stained with **5b** (left) and EtBr (right). The excitation conditions are the same as those for (b).

fragments were visible as dark bands against the bright background of the gel (Fig. 3b and c). As typical DNA intercalators have

Published on 23 October 2014. Downloaded by University of Illinois at Chicago on 31/10/2014 08:49:22.

planar aromatic rings, such as EtBr and acridinium derivatives,[21,22] the interaction mode of **5a** is predicted as an intercalation. The fluorescence of **5a** was quenched with increasing DNA concentration, as observed in several intercalators. Therefore, one of the possible mechanisms for this quenching is the photo-induced electron-transfer reaction between the excited compound and the nucleic bases.[22,23]

We have synthesized dibenzo[*b*,*h*][1,6]naphthyridines in one pot by reacting 2-acetylaminobenzaldehyde with acetophenone under basic conditions. This method was also applied to the synthesis of 1,2-dihydroquinolines. 6-Methyl-1,6-dibenzonaphthyridinium triflates showed significant fluorescence and intercalated into double-stranded DNA. Further studies on the novel features of those reagents are in progress.

Notes and references

1 For reviews, see: M. Balasubramanian and J. G. Keay, Pyridines and their benzo derivatives: application, in *Comprehensive Heterocyclic Chemistry II*, ed. A. P. Katrizky, V. W. Rees and E. F. Scriven, Pergamon, Oxford, 1996, vol. 5, pp. 245–300; *Name Reactions in Heterocyclic Chemistry*, ed. J. J. Li, Wiley, Hoboken, 2005, ch. 9; J. P. Michael, *Nat. Prod. Rep.*, 2003, **20**, 476–493; A. R. Katritzky, S. Rachwal and B. Rachwal, *Tetrahedron*, 1996, **52**, 1503.

2 For recent examples, see: K. Makino, O. Hara, Y. Takiguchi, T. Katano, Y. Asakawa, K. Hatano and Y. Hamada, *Tetrahedron Lett.*, 2003, **44**, 8925; P. D. Pohlhaus, R. K. Bowman and J. S. Johnson, *J. Am. Chem. Soc.*, 2004, **126**, 2294; C. S. Yi and S. Y. Yun, *J. Am. Chem. Soc.*, 2005, **127**, 17000; X.-Y. Liu, P. Ding, J.-S. Huang and C.-M. Che, *Org. Lett.*, 2007, **9**, 2645; S.-L. Cui, J. Wang and Y.-G. Wang, *Tetrahedron*, 2008, **64**, 487.

3 L. Moran, M.-E. Mirault, A. Tissières, J. Lis, P. Schedl, S. Artavanis-Tsakonas and W. Gehring, *Cell*, 1979, **17**, 1; D. Reha, M. Kabelác, F. Ryjácek, J. Sponer, J. E. Sponer, M. Elstner, S. Suhai and P. Hobza, *J. Am. Chem. Soc.*, 2003, **124**, 3366.

4 H. Kimura, K. Torikai and I. Ueda, *Chem. Pharm. Bull.*, 2009, **57**, 393; R. B. Woodward and E. C. Kornfeld, *J. Am. Chem. Soc.*, 1948, **70**, 2508; M. Kratzel and A. Weigl, *Monatsh. Chem.*, 1998, **129**, 967.

5 For a recent review, see: T. T. Dao, H. J. M. Linthorst and R. Verpoorte, *Phytochem. Rev.*, 2011, **10**, 397. For an example, see: F. Toda, K. Tada and K. Hamai, *J. Chem. Soc., Perkin Trans. 1*, 1990, 3207.

6 K. Okuma, J. Seto, N. Nagahora and K. Shioji, *J. Heterocycl. Chem.*, 2010, **47**, 1372; K. Okuma, S. Ozaki, J. Seto, N. Nagahora and K. Shioji, *Heterocycles*, 2010, **81**, 935; K. Okuma, K. Hirano, C. Shioga, N. Nagahora and K. Shioji, *Bull. Chem. Soc. Jpn.*, 2013, **86**, 615.

7 A. A. Avestisyan, I. L. Aleksanyan and L. P. Ambartsumyan, *Russ. J. Org. Chem.*, 2007, **43**, 1052; M. Manoj and K. J. Rajendra Prasad, *Synth. Commun.*, 2012, **42**, 434.

8 J. T. Braunholtz and F. G. Mann, *J. Chem. Soc.*, 1958, 3368.

9 M. Sellstedt and F. Almqvist, *Org. Lett.*, 2011, **13**, 5278; H. Wu, W. Lin, Y. Wan, H. Q. Xin, D. Q. Shi, Y. H. Shi, R. Yuan, R. C. Bo and W. Yin, *J. Comb. Chem.*, 2010, **12**, 31.

10 W. R. Vaughan, *Org. Synth., Coll.*, 1955, **3**, 329.

11 P. Cohn and L. Springer, *Monatsh. Chem.*, 1903, **24**, 87; C. L. Diedrich, D. Haase, W. Saak and J. Christoffers, *Eur. J. Org. Chem.*, 2008, 1811.

12 A typical reaction is as follows. To a solution of **2** (2.2 mmol) and **3a** (0.50 mmol) in EtOH was added 5 M aq. NaOH (0.24 mL, 1.2 mmol). After refluxing for 8 h, the reaction mixture was concentrated and left to stand for 2 h to yield orange crystals. Recrystallization from EtOH gave orange crystals of **4a** (0.41 mmol). Mp 234–236 °C.

13 For a review, see: C.-C. Cheng and S.-J. Yan, The Friedländer synthesis of quinolines, *Organic Reactions*, Wiley, Hoboken, 1982, vol. 28. For an example, see: Q. Zhao, C.-Y. Jiang, M. Shi, F.-Y. Li, T. Yi, Y. Cao and C.-H. Huang, *Organometallics*, 2006, **25**, 3631.

14 P. Kothandaraman, S. J. Foo and P. W. H. Chan, *J. Org. Chem.*, 2009, **74**, 5947.

15 D. Migneault, M. A. Bernstein and C. K. Lau, *Can. J. Chem.*, 1995, **73**, 1506.

16 K. Kobayashi, S. Nagato, M. Kawakita, O. Morikawa and H. Konishi, *Chem. Lett.*, 1995, **24**, 575.

17 K. Makino, O. Harada, Y. Takiguchi, T. Katano, Y. Asakawa, K. Hatano and Y. Hamada, *Tetrahedron Lett.*, 2003, **44**, 8925.

18 The structures of **4a** and **1a** were confirmed by spectroscopic and X-ray crystallographic analyses. Crystallographic data for **4a**: CCDC 1003324: monoclinic space group $P2_1/n$, a = 13.424(16) Å, b = 9.834(11) Å, c = 16.368(19) Å, α = 90°, β = 113.546(7), γ = 93.981(4), Z = 4, R_1 = 0.0902, wR_2 = 0.2539. Compound **1a**: CCDC 1003843: monoclinic space group $C2/c$, a = 36.980(18) Å, b = 5.904(3) Å, c = 15.103(7) Å, α = 90°, β = 90.257(9)°, γ = 90°. V = 3297(3) Å^3, Z = 8, R_1 = 0.0707, wR_2 = 0.1356. CCDC contains the supplementary crystallographic data for compound **1a**.

19 T. Suresh, T. Dhanabal, R. N. Kumar and P. S. Mohan, *Indian J. Chem.*, 2005, **44B**, 2375.

20 R. Oels, R. Storer and D. S. Young, *J. Chem. Soc., Perkin Trans. 1*, 1977, 2546.

21 B. A. D. Neto and A. A. M. Lapis, *Molecules*, 2009, **14**, 1725.

22 C. Bohne, K. Faulhabe, B. Giese, A. Häfner, A. Hofmann, H. Ihmels, A. K. Köhler, S. Perä, F. Schneider and M. A. L. Sheepwash, *J. Am. Chem. Soc.*, 2005, **127**, 76.

23 M. Sirajuddin, S. Ali and A. Badshah, *J. Photochem. Photobiol., B*, 2013, **124**, 1.

 This journal is © The Royal Society of Chemistry 2014