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Synthesis of mono-(*p*-dimethylamino)styryl-containing BOPHY dye for a turn-on pH sensor†

Xin-Dong Jiang[a],*, Yajun Su[a], Shuai Yue[a], Chen Li[a], Haifeng Yu[a], Han Zhang[b], Chang-Liang Sun[c] and Lin-Jiu Xiao[a]

Mono-substitutional bis(difluoroboron)1,2-bis((1H-pyrrol-2-yl)methylene)hydrazine (BOPHY) **3a** with a (*p*-dimethylamino)styryl group in α-position was confirmed to be synthesized by the Knoevenagel-type condensation. Dimethylamino-containing BOPHY dye **3a** is almost non-fluorescent by the ICT effect. Upon the protonation of the tertiary
10 amine function of **3a**, the strong fluorescence ($\Phi_f = 0.98$) was released and the fluorescence intensity was dramatically increased by one thousand folds. BOPHY **3a** as a pH probe can be used.

1. Introduction

In recent decades, 4,40-difluoro-4-bora-3a,4a-diaza-sindacene
15 (abbreviated as BODIPY) dyes have received increased attention because of their high fluorescence quantum yields, high absorption coefficients, good solubility in organic solvents, and excellent thermal and photochemical stabilities.[1] Since the discovery of the excellent photochemical properties of BODIPY,
20 various modifications on the BODIPY dye **1** (Fig. 1a) including the modification on the core or aza-BODIPY[2], have been widely explored for sensors, laser dyes, light harvesters, organic light emitting diodes, and sensitizers for solar cells and so forth.[3] Therefore, the BODIPY dyes have been attracting increasing
25 interests in the design and synthesis of these novel fluorescent dyes to date.

Ziegler et. al recently presented another type of unique pyrrole-BF$_2$-based fluorophore bis(difluoroboron)1,2-bis((1H-pyrrol-2-yl)methylene)hydrazine (BOPHY) **2** (Fig. 1a).[4] The new fluorescent BOPHY dye **2** can be successfully obtained by the
35 reaction of pyrrole-2-carboxaldehyde with hydrazine, and followed by complexation with Et$_3$N-BF$_3$·Et$_2$O, based on the traditional classical method (Fig. 1b).[4] The aesthetic symmetric structure is composed of four rings at the same plane, including two BF$_2$ units in six-membered chelate rings in the center and
40 two pyrrole units on the periphery (Fig. 1).[4] The fluorescence quantum yield for the unmodified BOPHY **2** is so high to near to be 100%.[4] Since the new BOPHY dye **2** has a grid structure, excellent optical properties and is similar to BODIPY **1**, these urge us to investigate the BOPHY structural motif for a
45 functionalization. Our recent research interest lies in the novel BODIPY/aza-BODIPY family of fluorescent dyes and their application.[5] During preparation of this manuscript, modifications on BOPHY dyes, including BOPHYs **3a** and **3b**, were reported by Jiao and Hao group (Fig. 2).[6] However, the synthesis of **3b**
50 was not shown, and no study of BOPHY as a pH fluorescent probe was explored either. Therefore, we herein communicate our studies on a (*p*-dimethylamino)-styryl-containing BOPHY **3a** as a turn-on fluorescent probe for pH (Fig. 2).

Fig. 1 The core structure of BODIPY and BOPHY.

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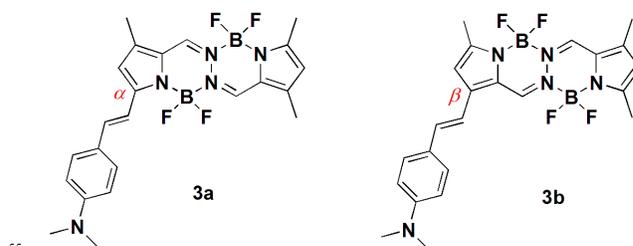
Fig. 2 Structure of BOPHY **3a** and **3b**.

2. Experimental section

2.1. General

^{1}H NMR spectra were recorded on a Bruker AVANCE III 500 MHz spectrometer. ^{1}H NMR chemical shifts (δ) are given in ppm
5 downfield from Me$_4$Si, determined by chloroform (δ = 7.26 ppm). ^{13}C NMR spectra were recorded on a Bruker AVANCE III 125 MHz spectrometer. ^{13}C NMR chemical shifts (δ) are reported in ppm with the internal CDCl$_3$ at δ 77.0 ppm as standard. Toluene solvents were distilled over CaH$_2$. Merck silica gel 60
10 was used for the column chromatography. All pH measurements were performed with a PHS-3E pH meter.

Fluorescence spectra were recorded on FluoroSENS spectrophotometer. UV/Vis spectra were recorded on UV-2550 spectrophotometer at room temperature. The refractive index of
15 the medium was measured by 2 W Abbe's refractometer at 20 °C. The fluorescence quantum yields (Φ_f) of the BOPHY system was calculated using the following relationship (equation 1):

$$\Phi_f = \Phi_{ref} F_{sampl} A_{ref} n^2_{sampl} / F_{ref} A_{sampl} n^2_{ref} \qquad (1)$$

Here, F denotes the integral of the corrected fluorescence
20 spectrum, A is the absorbance at the excitation wavelength, ref and sampl denote parameters from the reference and unknown experimental samples, respectively. The reference systems used was rhodamine 6G in methanol as standard (Φ_f = 0.78, in air equilibrated water and deaerated solutions) for **3a-H$^+$**.
25 The MO calculations were performed at the DFT level, and the frontier molecular orbitals of BOPHY **3a** and **3a-H$^+$** at the MP2/6-31G* level with Gaussian 03.

2.2. Synthesis of BOPHY 3a

4-N,N-dimethylbenzaldehyde (33 mg, 0.22 mmol), compound
30 **4** (40 mg, 0.12 mmol) (see following Scheme 1), AcOH (0.5 mL), and piperidine (0.5 mL) were stirred for 24 h at 95 °C in dry toluene (6 mL) in the presence of a small amount of activated 4 Å molecular sieves. The mixture was cooled to room temperature, quenched with water, extracted with CH$_2$Cl$_2$, washed with brine,
35 dried over Na$_2$SO$_4$, evaporated and purified by TLC to afford BODIHY **3a** (15 mg, 28%) as red solids. ^{1}H NMR (500 MHz, CDCl$_3$): δ (ppm) 7.96 (s, 1H), 7.85 (s, 1H), 7.46 (d, J = 8.5 Hz, 2H), 7.22 (d, J = 12.5 Hz, 2H), 6.71 (d, J = 6.0 Hz, 2H), 6.68 (s, 1H), 6.17 (s, 1H), 3.03 (s, 6H), *2.49 (s, 3H), 2.35 (s, 3H), 2.33 (s,*
40 *3H)*. ^{13}C NMR (125 MHz, CDCl$_3$): δ (ppm) 151.6, 151.0, 150.2, 140.7, 140.1, 137.7, 133.7, 132.4, 128.9, 124.4, 124.0, 123.3, 118.2, 114.5, 112.8, 111.9, 110.9, 40.2, 14.1, 11.2, 11.1. HRMS-MODAI (ESI) Calcd. for C$_{23}$H$_{25}$N$_5$B$_2$F$_4$ [M + H]$^+$: 470.2305, found 470.2305.

3. Results and discussion

3.1 synthsis and Characterization of BOPHY 3a

BOPHY dyes **3a** and **3b** with a (*p*-dimethylamino)styryl group in α/β-position of the pyrrole (Fig. 2), were found to occur in the text and the supporting information in Jiao and Hao's paper,
50 respectively.[6] It is illusive to which one was synthesized in the Knoevenagel-type condensation. Therefore, we curiously repeated this reaction from BOPHY **4**[4,6] via the Knoevenagel-type condensation (Scheme 1).[7] We successfully synthesized the mono-dimethylaminostyryl BOPHY **3a** (Scheme 1). Though the
55 use of 10 eq. 4-dimethylaminobenzaldehyde, no bis-

dimethylamino styryl BOPHY was observed.

The structure of dye **3a** was confirmed by ^{1}H NMR spectrum. Due to the shield from the full effect of the applied field by their surrounding electrions,[8] the chemical shift of H$_\alpha$ is well-known to
60 be lower than that of H$_\beta$ in ^{1}H NMR spectrum in dye **3a** and **4** (Scheme 1). By the measure of the ^{1}H NMR spectrum of **3a** using a Bruker AVANCE III 500 MHz spectrometer, three sets of distinct hydrogen signals of the methyl group (δ = 2.49 (s, 3H$_c$), *2.35 (s, 3H$_a$), 2.33 (s, 3H$_b$)* ppm in CDCl$_3$) were showed (Fig. 3
65 and Scheme 1). Therefore, the logical structure was thought to be the dye **3a** not **3b** which should have two sets of hydrogen signals in the *low* field and a set in the *high* field (Scheme 1 and Fig. 2).
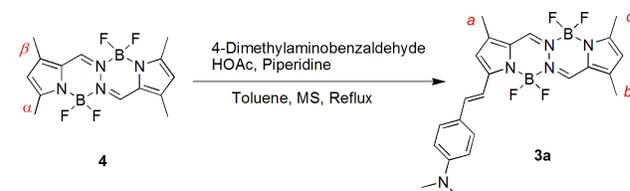
70 **Scheme 1** Synthesis of the BOPHY dye **3a**.
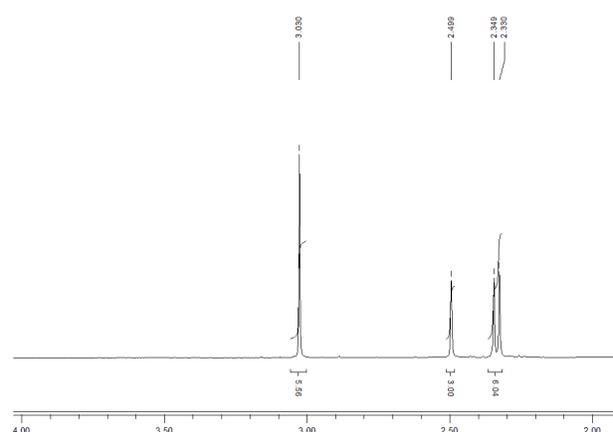
Fig. 3 Partial [1]NMR spectra of the BOPHY dye **3a**.

3.2 A turn-on fluorescent probe for pH

The most popular strategy for pH-responsive fluorescent sensors takes advantage of intramolecular charge transfer (ICT) or the photoinduced electron transfer (PET).[9] Dimethylamino group is one of the fragments frequently used for the purpose of
80 ICT. The BODIPY is a family of very widely used fluorescent dye for potential applications and often designed for pH probes, wherein the excited state of the fluorophore can be quenched by the electron transfer from electron donating amine to the fluorophore.[10] Upon recognition of a proton, the electron transfer
85 is "switched off" and in turn the emission of fluorescence is "switched on". To the best of our knowledge, a functionalization of BOPHY dye for a pH probes has not been documented. Therefore, we continue to explore the response of the dimethylamino-containing BOPHY dye **3a** to pH value.
90 Photoimage of **3a** were taken under normal room illumination and UV light, and notable changes of relatively vivid bright colors of **3a** with the pH can be easily observed with naked eye (Fig. 4). The mechanism of BOPHY **3a** to pH value is same to that of the above described BODIPY (Fig. 5). Upon addition of
95 hydrochloric acid to BOPHY **3a** with a -NMe$_2$ group as a pH-

sensitive functionality, **3a** was protonated at relatively low pH value.[11] A stepwise decrease of the absorption intensity was observed in the 543 nm band of **3a**, and this peak disappeared completely at 1 M (Fig. 6). The formation of a new band at 504 nm was first observed in pH 3, and another new peak subsequently arose at 481 nm (Fig. 6). The absorption intensity of **3a–H**[+] reached the maximum when 4 M HCl was used, and the absorption intensity was subsequently decreased at 6 and 8 M HCl. The absorption band of **3a–H**[+] is blue-shifted by about 50 nm compared to that of **3a**. The fluorescence quantum yield of **3a** in CH_3CN/H_2O (1:1, v/v) is very low (Φ_f = 0.01 at pH 7) due to the ICT effect; however, with decreasing pH the twin emission maxima were shifted to 532, 573 nm (Fig. 7a). A dramatic increase in fluorescence intensity at 532 nm by 1200 folds (Φ_f = 0.98 when treated with HCl to 4 M) (Fig. 7b).

pH   **0**   **1**   **1.5**   **2**   **2.5**   **3**   **3.5**   **4**   **7**
Fig. 4 Photograph of solutions of 5 μM BOPHY dye **3a** at pH 0, 1, 1.5, 2, 2.5, 3, 3.5, 4 and 7 in CH_3CN/H_2O (1:1, v/v) under normal room illumination (upper row) and under UV irradiation (bottom row), respectively.
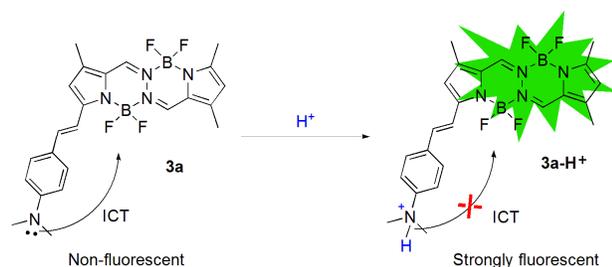
Fig. 5 Protonation of BOPHY **3a** to genarate **3a-H**[+] and release the strong fluorescence by switching off the ICT effect.

Moreover, the molecular geometries of BOPHY **3a** and **3a-H**[+] were optimized using density functional theory (DFT) at the MP2/6-31G* level.[12] The calculated HOMO and LUMO orbital energy levels were summarized in Fig 8. Upon excitation of the BOPHY fluorophore, an electron of the highest occupied molecular orbital (HOMO) is promoted to the lowest unoccupied molecular orbital (LUMO), which enables ICT from the donor (amino nitrogen atom) to that of the BOPHY fluorophore, causing fluorescence quenching of the latter. Upon the protonation of **3a**, ICT is no longer possible, and the fluorescence intensity of the probes was enhanced. The protonation of BOPHY **3a** (**3a-H**[+]: λ_{abs} = 481, 504 nm) resulted in a remarkable hypsochromic shift compared to that (λ_{abs} = 540 nm) of the BOPHY **3a**. It is due to the increase in the HOMO–LUMO band

gap (2.65 ev) for the lowest energy absorption bands observed for **3a-H**[+] relative to that (2.53 ev) of **3a** by MO calculations (Fig. 8). However, the energy level of frontier orbitals in **3a-H**[+] was lower than that of **3a** (Fig. 8).
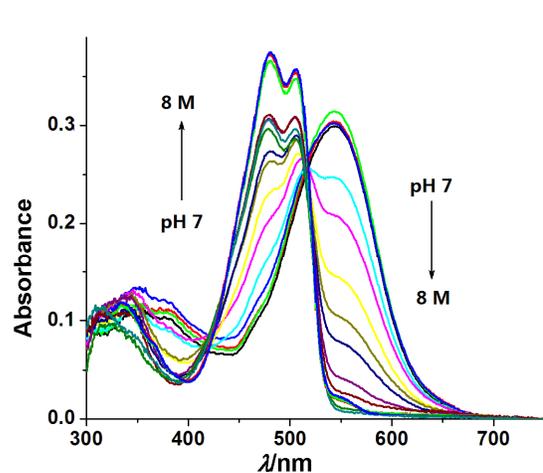
Fig. 6 Absorption spectra (pH 7, 5, 4, 3.5, 3. 2.8, 2.5, 2.2, 2.0, 1.5, 1 and 1 M, 2 M, 4 M, 6 M, 8 M of HCl) of 5 μM dye **3a** in CH_3CN/H_2O (1:1, v/v) as a function of pH.
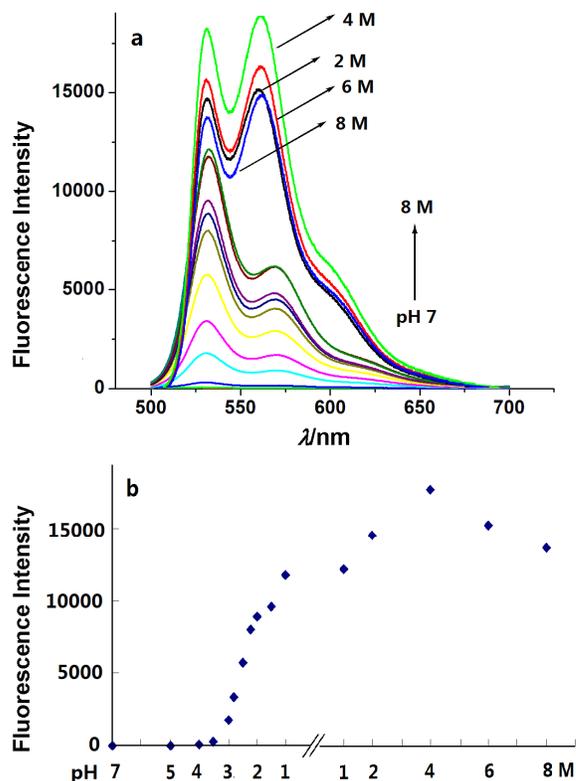
Fig. 7 (a) Corresponding fluorescence spectra (pH 7, 5, 4, 3.5, 3. 2.8, 2.5, 2.2, 2.0, 1.5, 1 and 1 M, 2 M, 4 M, 6 M, 8 M of HCl, λ_{ex} = 490 nm) of 5 μM dye **3a** in CH_3CN/H_2O (1:1, v/v) as a function of pH. (b) Fluorescence titration responses of **3a** towards HCl at 532 nm.
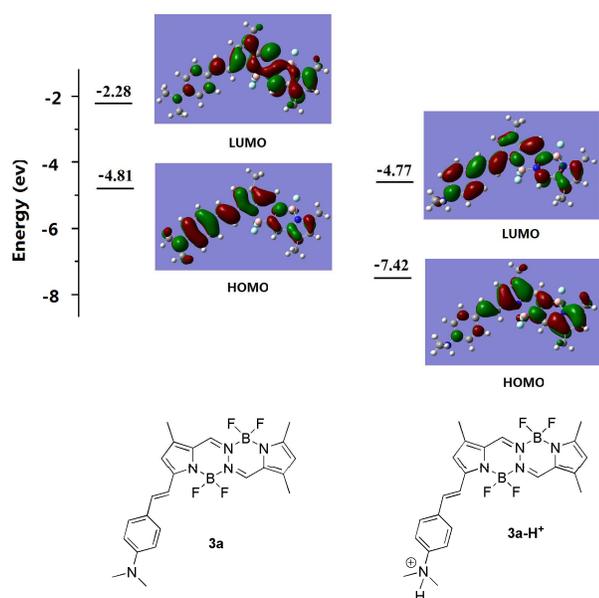
Fig. 8 The frontier molecular orbitals (MOs) of BOPHY **3a** and **3a-H$^+$**. The energy levels of the MOs are shown (ev). Calculations are based on ground state geometry by DFT at the MP2/6-31G* level with Gaussian 03. HOMO/LUMO (ev) = −4.81/−2.28 for **3a**; HOMO/LUMO (ev) = −7.42/−4.77 for **3a-H$^+$**.

4. Conclusions

Mono-substitutional BOPHY **3a** with a (*p*-dimethylamino)styryl group in α-position was confirmed to be synthesized by a Knoevenagel-type condensation, and no dis substitutional BOPHY with a (*p*-dimethylamino)styryl group was observed. BOPHY **3a** is almost non-fluorescent by ICT effect. Upon the protonation of the tertiary amine function of **3a**, the strong fluorescence ($\Phi_f = 0.98$) was released and the florescence intensity was dramatically increased by 1200 folds. Based on MO calculations the increase in the HOMO–LUMO band gap (2.65 ev) for the lowest energy absorption bands observed for **3a-H$^+$** relative to that (2.53 ev) of **3a**, and the energy level of frontier orbitals in **3a-H$^+$** was lower than that of **3a**. BOPHY **3a** as a pH probe can be used. Further efforts for development of probes for thiol[5b,g] based on BOPHY dyes in biotechnology are ongoing in our lab.

Acknowledgements

Notes and references

aCollege of Applied Chemistry, Shenyang University of Chemical Technology, Shenyang, 110142, China. E-mail: xdjiang@syuct.edu.cn; Tel: +86 024 89387219.

bSchool of Materials Science and Chemical Engineering, Tianjin University of Science & Technology, Tianjin, 300457, China

cCenter of Physical and Chemistry Test, Shenyang University of Chemical Technology, Shenyang 110142, China.

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1 (*a*) A. Loudet and K. Burgess, *Chem. Rev.*, 2007, **107**, 4891; (*b*) L. Yuan, W. Lin, K. Zheng, L. He and W. Huang, *Chem. Soc. Rev.*, 2013, **42**, 622; (*c*) R. Weissleder and M. J. Pittet, *Nature*, 2008, **452**, 580; (*d*) L. Yuan, W. Lin, S. Zhao, W. Gao, B. Chen, L. He and S. Zhu, *J. Am. Chem. Soc.*, 2012, **134**, 13510; (*e*) Z. Zhang, B. Xu, J. Su, L. Shen, Y. Xie and H. Tian, *Angew. Chem., Int. Ed.*, 2011, **50**, 11654; (*f*) X. Chen, Y. Zhou, X. Peng and J. Yoon, *Chem. Soc. Rev.*, 2010, **39**, 2120; (*g*) Y. Yang, Q. Zhao, W. Feng and F. Li, *Chem. Rev.*, 2013, **113**, 192; (*h*) N. Boens, V. Leen and W. Dehaen, *Chem. Soc. Rev.* 2012, **41**, 1130; (*i*) H. Peng, W. Chen, Y. Cheng, L. Hakuna, R. M. Strongin and B. Wang, *Sensors*, 2012, **12**, 15907; (*j*) L. Yuan, W. Lin, K. Zheng, L. He and W. Huang, *Chem. Soc. Rev.,* 2013, **42**, 622; (*k*) H. Jung, X. Chen, J. Kim and J. Yoon, *Chem. Soc. Rev.*, 2013, **42**, 6019; (*l*) G. Ulrich, A. Harriman and R. Ziessel, *Angew. Chem., Int. Ed.*, 2008, **47**, 1184; (*m*) A. Bessette and G. S. Hanan, *Chem. Soc. Rev.*, 2014, **43**, 3342; (*n*) H. Lu, J. Mack, Y. Yang and Z. Shen, *Chem. Soc. Rev.*, 2014, **43**, 4778.

2 (*a*) M. A. T. Rogers, *J. Chem. Soc.*, 1943, 596; (*b*) E. B. Knott, *J. Chem. Soc.*, 1947, 1196.

3 (*a*) M. A. T. Rogers, *Nature*, 1943, **151**, 504; (*b*) M. A. T. Rogers, *J. Chem. Soc.*, 1943, 590; (*c*) J. Killoran, L. Allen, J. Gallagher, W. Gallagher and D. F. O'Shea, *Chem. Commun.*, 2002, 1862; (*d*) A. Gorman, J. Killoran, C. O'Shea, T. Kenna, W. M. Gallagher and D. F. O'Shea, *J. Am. Chem. Soc.*, 2004, **126**, 10619; (*e*) L. Jiao, Y. Wu, Y. Ding, S. Wang, P. Zhang, C. Yu, Y. Wei, X. Mu and E. Hao, *Chem. Asian J.*, 2014, **9**, 805; (*f*) S. O. McDonnell, M. J. Hall, L. T. Allen, A. Byrne, W. M. Gallagher and D. F. O'Shea, *J. Am. Chem. Soc.*, 2005, **127**, 16360; (*g*) M. J. Hall, L. T. Allen and D. F. O'Shea, *Org. Biomol. Chem.*, 2006, **4**, 776; (*h*) J. Killoran and D. F. O'Shea, *Chem. Commun.*, 2006, 1503; (*i*) H. Lu, S. Shimizu, J. Mack, Z. Shen and N. Kobayashi, *Chem.-Asian J.*, 2011, **6**, 1026; (*k*) V. F. Donyagina, S. Shimizu, N. Kobayashi and E. A. Lukyanets, *Tetrahedron Lett.*, 2008, **49**, 6152; (*l*) Y. Wu, C. Cheng, L. Jiao, C. Yu, S. Wang, Y. Wei, X. Mu and E. Hao, *Org. Lett.*, 2014, **16**, 748.

4 I.-S. Tamgho, A. Hasheminasab, J. T. Engle, V. N. Nemykin, and C. J. Ziegler, *J. Am. Chem. Soc.*, 2014, **136**, 5623.

5 (*a*) X.-D. Jiang, J. Zhang, T. Furuyama and W. Zhao, *Org. Lett.*, 2012, **14**, 248; (*b*) X.-D. Jiang, J. Zhang, X. Shao and W. Zhao, *Org. Biomol. Chem.*, 2012, **10**, 1966; (*c*) X.-D. Jiang, H. Zhang, Y. Zhang and W. Zhao, *Tetrahedron*, 2012, **68**, 9795; (*d*) X.-D. Jiang, Y. Fu, T. Zhang and W. Zhao, *Tetrahedron Lett.*, 2012, **53**, 5703; (*e*) X.-D. Jiang, R. Gao, Y. Yue, G.-T. Sun and W. Zhao, *Org. Biomol. Chem.*, 2012, **10**, 6861; (*f*) R. Kang, X. Shao, F. Peng, Y. Zhang, G.-T. Sun, W. Zhao and X.-D. Jiang, *RSC Adv.*, 2013, **3**, 21033; (*g*) J. Zhang, X.-D. Jiang, X. Shao, J. Zhao, Y. Su, D. Xi, H. Yu, S. Yue, L.-J. Xiao and W. Zhao, *RSC. Adv.*, 2014, **4**, 54080; (*h*) X.-D. Jiang, D. Xi, J. Zhao, H. Yu, G.-T. Sun and L.-J. Xiao, *RSC. Adv.*, 2014, **4**, 60970; (*i*) P. Shi, X.-D. Jiang, R. Gao, Y. Dou and W. Zhao, *Chin. Chem. Lett.*, 2014, DOI: 10.1016/j.cclet.2014.05.035.

6 C. Yu, L. Jiao, P. Zhang, Z. Feng, C. Cheng, Y. Wei, X. Mu and E. Hao, *Org. Lett.*, 2014, **16**, 1952.

7 (*a*) Q. Zheng, G. Xu and P. N. Prasad, *Chem. Eur. J.*, 2008, **14**, 5812; (*b*) D. P. Wang, Y. Shiraishi and T. Hirai, *Chem. Commun.*, 2011, **47**, 2673; (*c*) T. Bura, R. Pascal, G. Ulrich and R. Ziessel, *J. Org. Chem.*, 2011, **76**, 1109; (*d*) S. Zhu, J. Zhang, G. Vegesna, F. Luo, S. A. Green and H. Liu, *Org. Lett.*, 2011, **13**, 438.

8 (a) H. R. Westover, *J. Chem. Educ.*, 1967, **44**, 486; (b) D. Parker, *Chem. Rev.*, 1991, **91**, 1441; (c) K. C. Wong, *J. Chem. Educ.*, 2014, **91**, 1103.

9 (*a*) Y. Chen, D. Qi, L. Zhao, W. Cao, C. Huang and J. Jiang, *Chem. Eur. J.*, 2013, **23**, 7342; (*b*) C. Thivierge, J. Han, R. M. Jenkins and K. Burgess, *J. Org. Chem.*, 2011, **76**, 5219; (*c*) A. W. Czarnik, *Acc. Chem. Res.*, 1994, **27**, 302; (*d*) W. Rettig, *Angew. Chem. Int. Ed.*, 1986, **25**, 971; (*e*) B. Tang, X. Liu, K. Xu, H. Huang, G. Yang and L.

An, *Chem. Commun.*, 2007, 3726; (*f*) W. F. Jager, T. S. Hammink, O. van den Berg and F. C. Grozema, *J. Org. Chem.*, 2010, **75**, 2169☐

10 (*a*) S. Hoogendoorn, K. L. Habets, S. Passemard, J. Kuiper, G. A. van der Marel, B. I. Florea and H. S. Overkleeft, *Chem. Commun.*, 2011,

5   **47**, 9363; (*b*) P. Song, X. Chen, Y. Xiang, L. Huang, Z. Zhou, R. Wei and A. Tong, *J. Mater Chem.*, 2011, **21**, 13470; (*c*) X. Gao, Y. Zhang and B. Wang, *Org. Lett.*, 2003, **5**, 4615; (*d*) B. Tang, F. Yu, P. Li, L. Tong, X. Duan, T. Xie and X. Wang, *J. Am. Chem. Soc.*, 2009, **131**, 3016; (*e*) X. Ma, Y. Wang, T. Zhao, Y. Li, L.-C. Su, Z. Wang, G.

10 Huang, B. D. Sumer and J. Gao, *J. Am. Chem. Soc.*, 2014, **136**, 11085; (*f*) S. Grunder, P. L. McGrier, A. C. Whalley, M. M. Boyle, C. Stern and J. F. Stoddart, *J. Am. Chem. Soc.*, 2013, **135**, 17691; (*g*) A. Thottiparambil, P. R. Anil Kumar and L. Chakkumkumarath, *RSC Adv.*, 2014,**4**, 56063.

15 11 (a) S. O. McDonnell and D. F. O'Shea, *Org. Lett.*, 2006, **8**, 3493; (b) K. Rurack, M. Kollmannsberger and J. Daub, *Angew. Chem. Int. Ed.*, 2001, **40**, 385.

12 M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A., Jr. Montgomery, R.

20 E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui, K. Morokuma, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J.

25 Cioslowski, J. V. Ortiz, A. D. Baboul, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, C. Gonzalez, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chem, M. W. Wong, J. L. Andres, C. Gonzalez, M. Head-Gordon, E. S.

30 Replogle, J. A. Pople, *Gaussian 03*, Gaussian Inc.: Pittsburgh, PA, 2003.