

crystalline state. The six-membered C1–C6 ring adopts a quinoidal structure with marked bond alternation, while the other six-membered C8–C13 ring adopts a benzene-like structure. The unsymmetrical structures of **BF2** and **BF3** are probably due to crystal packing effects, *i.e.*, the formation of intermolecular hydrogen bonds between the hydroxy group in the phenol moiety of one molecule and the carbonyl group of the *p*-quinone methide moiety of an adjacent molecule (Fig. S1 and S3, ESI†).

Under basic conditions, bora-fluoresceins showed characteristic absorption and fluorescence in the NIR region (Fig. 3 and Table 1). **BF2** exhibited a broad absorption band with the maximum wavelength (λ_{abs}) of 538 nm ($\epsilon = 0.84 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$) in acetonitrile. Upon addition of 1,8-diazabicyclo [5.4.0]undec-7-ene (DBU) as the Brønsted base to the acetonitrile solution of **BF2**, a significantly red-shifted absorption band emerged at $\lambda_{\text{abs}} = 851 \text{ nm}$ ($\epsilon = 1.90 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$). This absorption band in the NIR region was attributed to the deprotonated form of **BF2**, which stems from the equilibrium at the phenolic hydroxy group. This notion is consistent with the behavior of other heteroatom-substituted fluorescein analogues, for which similar spectral changes have been observed (Fig. S7, ESI†). Under basic conditions, deprotonated **BF2** exhibited NIR fluorescence with the maximum at $\lambda_{\text{em}} = 907 \text{ nm}$, while the fluorescence quantum yield is low ($\Phi_{\text{F}} = 0.003$).

A comparison of **BF2** with other heteroatom-substituted analogues clearly demonstrated the impact of the tricoordinate boron atom on the electronic structure. The λ_{abs} of **BF2** (851 nm in acetonitrile) under basic conditions is significantly red-shifted relative to those of the corresponding oxygen (**OF**: 524 nm; $\Delta\tilde{\nu} = 7330 \text{ cm}^{-1}$), dimethylsilyl (**SiF**: 612 nm; $\Delta\tilde{\nu} = 4560 \text{ cm}^{-1}$), and phosphine oxide (**POF**: 624 nm; $\Delta\tilde{\nu} = 3730 \text{ cm}^{-1}$) analogues. Meanwhile, the molar absorption coefficient of **BF2** ($\epsilon = 1.90 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$) is about one-fifth that of **OF**, while their Stokes shifts are comparable. In the fluorescence spectra, deprotonated **BF2** exhibits the most red-shifted fluorescence in the NIR region, whereas **OF**, **SiF**, and **POF** only exhibit fluorescence in the visible-to-red region. It is noteworthy that the NIR absorption and fluorescence of **BF2** are even further red-shifted relative to those of the naphthofluorescein derivative **NAF D** (Fig. 1a), where the fluorescein skeleton is extended with two naphthalenes.²⁰ These comparisons clearly

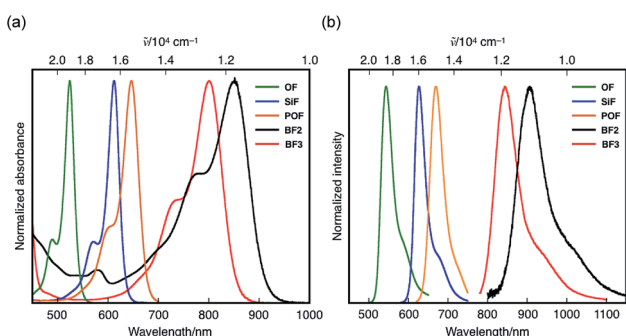


Fig. 3 (a) Absorption and (b) fluorescence spectra of heteroatom-substituted fluorescein dyes in the presence of DBU in acetonitrile.

Table 1 Photophysical properties of bora-fluoresceins and relevant analogues under basic conditions^a

Dye	λ_{abs} [nm]	ϵ [$10^4 \text{ M}^{-1} \text{ cm}^{-1}$]	λ_{em} [nm]	Stokes shift [cm^{-1}]
BF2	851	1.90	907	730
BF3	801	3.40	843	620
OF	524	9.95	544	700
SiF	612	12.3	627	390
POF	646	8.43	669	530
NAF D ^{b,c}	802	9.60	839	550

^a Measured after addition of an excess amount of DBU in acetonitrile with a sample concentration of 1.2 to $3.2 \times 10^{-5} \text{ M}^{-1}$. ^b Ref. 20. ^c Reported as a Cs salt in acetonitrile.

show that the tricoordinate boron atom significantly perturbs the electronic structure of the fluorescein skeleton.

To elucidate the significant influence of the boron atom on the photophysical properties of the fluorescein dyes, the electronic structure of the deprotonated **BF2** was theoretically compared to those of **OF**, **SiF**, and **POF** by DFT calculations at the B3LYP/6-31+G(d) level of theory (Fig. 4). A remarkable difference was observed for their LUMO levels, where the specific orbital interaction between the fluorescein π -skeleton and the substituent at the 10-position plays a crucial role. **POF** has a low-lying LUMO due to the $\sigma^*-\pi^*$ interaction between the P–Ph bond and the fluorescein skeleton, in addition to the inductive effect of the P=O moiety. Compared to the LUMO in **POF** that of **BF2** lies even lower by 0.23 eV. The low-lying LUMO level of **BF2** was attributed to the effective orbital interaction between the vacant p orbital on the boron atom and the π^* orbital of the fluorescein skeleton. In contrast to the significant

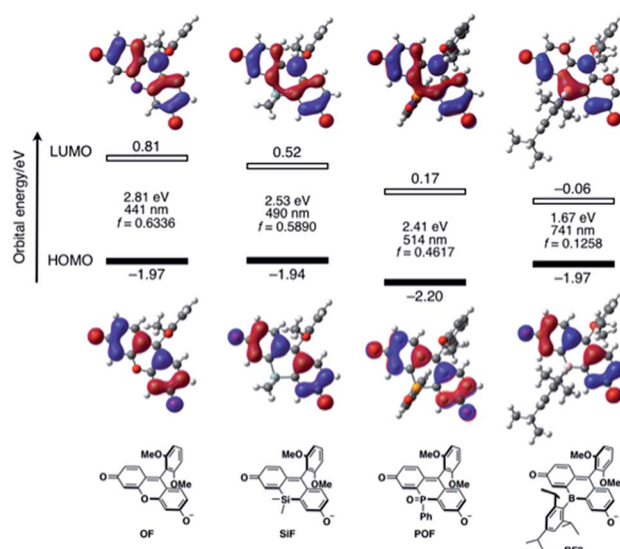


Fig. 4 Energy diagrams and Kohn–Sham plots of the HOMOs and LUMOs for the deprotonated forms of the fluorescein dyes **OF**, **SiF**, **POF**, and **BF2** calculated at the B3LYP/6-31+G(d) level of theory. The results of TD-DFT calculations at the same level of theory are also shown.



dependence of the LUMOs on the substituent at the 10-position of the fluorescein skeleton, the HOMOs are delocalized over the fluorescein skeleton with a node at the 10-position, regardless of the substituent. Consequently, **BF2** has a comparable HOMO level with those of the other analogues, except for **POF**, which has a slightly lower-lying HOMO due to the inductive effect of the P=O moiety. Overall, **BF2** exhibits the narrowest HOMO–LUMO gap in this series of fluorescein analogues.

TD-DFT calculations at the same level of theory suggested that deprotonated **BF2** exhibits a much red-shifted absorption maximum in the NIR region than the other fluorescein analogues (Fig. 4). The calculated oscillator strength for **BF2** is also significantly reduced compared to those of the other derivatives, which is consistent with the experimentally observed smallest molar absorption coefficient of **BF2**. This difference in the oscillator strength should be related to the contribution of the substituent at the 10-position to the LUMO, *i.e.*, the overlap between the HOMO and LUMO should decrease with increasing contribution of the substituent at the 10-position. Consequently, **BF2** has the smallest oscillator strength. Importantly, the narrow HOMO–LUMO gap observed in **BF2** is no longer retained once the vacant p orbital on the boron atom is occupied by a Lewis base externally added to form a tetra-coordinate species (*vide infra*) (Fig. S14, ESI†). Therefore, only tricoordinate boron species that ensure the effective orbital interaction through the vacant p orbital on the boron atom can attain NIR-absorption and fluorescence.

The response to Lewis bases, such as fluoride ions, is a characteristic feature of boron-containing π -conjugated compounds, and should endow the bora-fluoresceins with unusual properties. Indeed, **BF2** exhibits drastic multi-stage color changes from purple to yellow and blue upon addition of tetra-*n*-butylammonium fluoride (TBAF). These color changes were monitored by UV-vis absorption spectroscopy. Upon addition of TBAF to an acetonitrile solution of **BF2** (2.8×10^{-5} M), the broad absorption band at $\lambda_{\text{abs}} = 538$ nm diminished with the

concomitant emergence of a new absorption band at $\lambda_{\text{abs}} = 436$ nm (Fig. 5a). Successive addition of excess TBAF resulted in the appearance of an intense and sharp absorption band at $\lambda_{\text{abs}} = 585$ nm, which is reminiscent of the deprotonation of fluorescein dyes (Fig. 5b). Ultimately, the thus obtained mixture shows intense red fluorescence with the fluorescence maximum at $\lambda_{\text{em}} = 595$ nm with a high quantum yield of $\Phi_{\text{F}} = 0.89$.

These changes can be interpreted in terms of a complexation of a fluoride ion at the boron center, followed by deprotonation of the phenolic hydroxy group in the fluorescein skeleton (Fig. 5c). Specifically, **BF2** initially incorporates a fluoride ion to form tetra-coordinate borate $[\text{BF2} \cdot \text{F}]^-$. With increasing TBAF concentration, subsequent deprotonation by the basic fluoride ion from $[\text{BF2} \cdot \text{F}]^-$ generates borate fluorescein anion $[\text{BF2} \cdot \text{F}]^{2-}$.^{56–58} For a detailed study on the electronic structure of $[\text{BF2} \cdot \text{F}]^-$ and $[\text{BF2} \cdot \text{F}]^{2-}$, TD-DFT calculations were performed at the B3LYP/6-31+G(d) level of theory (Fig. S14, ESI†). The absorption bands observed at 436 nm for $[\text{BF2} \cdot \text{F}]^-$ and 585 nm for $[\text{BF2} \cdot \text{F}]^{2-}$ should be ascribed to π – π^* transitions, which mainly consist of the HOMO–LUMO transitions in both complexes. It should be noted that the LUMOs of these complexes are delocalized over the fluorescein skeleton with a subtle contribution from the boron moiety, which stands in stark contrast to the orbital interaction observed in the LUMO of neutral **BF2** bearing a tricoordinate boron atom. Moreover, complexation with a fluoride ion switches the role of the boron moiety in the π -conjugated system from a π -electron-accepting unit to a strong σ -donating unit, giving rise to the red-shifted absorption of $[\text{BF2} \cdot \text{F}]^{2-}$ compared to that of deprotonated **OF**. In this sense, the boron moiety in the fluorescein skeleton enables multi-stage electronic structure changes accompanied by drastic color changes in the visible-to-NIR region.

BF2 underwent complexation not only with fluoride ions, but also with weak neutral Lewis bases such as pyridine. It should be noted that in this case Lewis acid–base complexation predominates over the deprotonation of the phenolic hydroxy group (Fig. S11, ESI†). However, the binding constant of **BF2** with pyridine in acetonitrile is low ($K = 31 \text{ M}^{-1}$) due to the steric congestion around the boron center induced by the bulky tri-*i*-propylphenyl group. In this context, we discovered that the Lewis acidity of the boron center was further enhanced by planarization of the *B*-phenyl group without the loss of chemical stability. Upon treatment of *B*-phenyl-planarized **BF3** with DBU in acetonitrile, deprotonated **BF3** showed red-shifted absorption at $\lambda_{\text{abs}} = 801$ nm, which is similar to the behavior of **BF2**. The hypsochromic shift of 730 cm^{-1} relative to the absorption of **BF2** (851 nm) is mainly due to the electron-donating effect of the methylene bridges in **BF3** (Fig. S13, ESI†). Deprotonated **BF3** exhibits NIR fluorescence ($\lambda_{\text{em}} = 843$ nm) with an improved fluorescence quantum yield ($\Phi_{\text{F}} = 0.03$). In contrast, upon treatment with pyridine, **BF3** showed a new hypsochromically shifted absorption band at 445 nm accompanied by a drastic color change from purple to yellow (Fig. S12, ESI†). On the basis of the UV-vis spectral changes, the binding constant of **BF3** toward pyridine was determined ($K = 2.1 \times 10^3 \text{ M}^{-1}$), which indicated that the complexation ability of **BF3** toward pyridine is higher than that of **BF2** by two orders of magnitude.

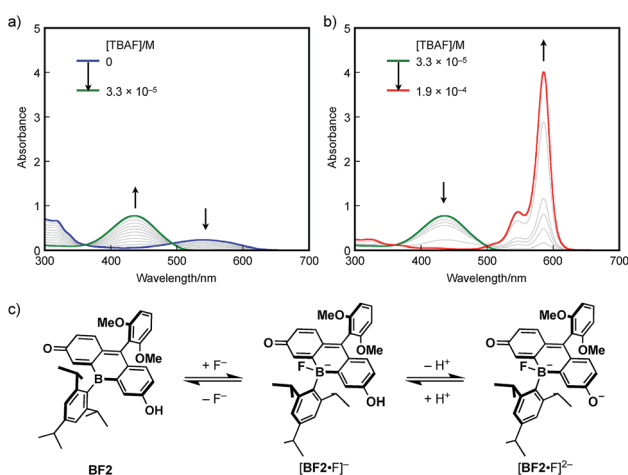


Fig. 5 UV-vis absorption spectral changes of **BF2** in acetonitrile (2.8×10^{-5} M) upon addition of TBAF: (a) $[\text{TBAF}] = 0$ to $3.3 \times 10^{-5} \text{ M}^{-1}$ and (b) 3.3×10^{-5} to $1.9 \times 10^{-4} \text{ M}^{-1}$. (c) Equilibrium between neutral **BF2**, borate $[\text{BF2} \cdot \text{F}]^-$, and borate fluorescein anion $[\text{BF2} \cdot \text{F}]^{2-}$.



Conclusions

In summary, we have successfully synthesized bora-fluoresceins **BF1–3**, which contain a tricoordinate boron atom at the 10-position in the fluorescein skeleton. The deprotonated forms of bora-fluoresceins exhibit absorption and fluorescence in the NIR region. In particular, deprotonated **BF2** shows absorption and emission maxima at 851 and 907 nm, respectively. These values are the most red-shifted of all hitherto reported heteroatom-substituted fluorescein dyes, demonstrating the significant impact of the boron atom on the electronic structure of fluorescein dye. The Lewis acidity of the boron atom offers a unique switching mechanism for the photophysical properties in addition to the Brønsted acid–base equilibrium on the fluorescein skeleton. Namely, the complexation of a Lewis base at the boron center of the bora-fluoresceins results in a significant hypsochromic shift of the absorption and emission. Consequently, bora-fluoresceins show multi-stage changes in their absorption and fluorescence properties, which is a noticeably different behavior from that of other heteroatom-substituted fluoresceins. The results of this study not only provide insight into the design principles of xanthene dyes, but also demonstrate the utility of tricoordinate boron atoms in the development of NIR dyes.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was supported by JSPS KAKENHI grants 18H03909 and 18H05261. N. A. thanks the JSPS for a Research Fellowship for Young Scientists. The authors also thank Prof. Dr T. Sasamori (Nagoya City Univ.) and Dr M. Hirai (Nagoya Univ.) for their help with the X-ray crystallographic analysis. The synchrotron X-ray crystallography experiment was performed at the BL40XU beamline of SPring-8 with the approval of the Japan Synchrotron Radiation Research Institute (JASRI; proposal 2017B1179).

Notes and references

- 1 T. Terai and T. Nagano, *Curr. Opin. Chem. Biol.*, 2008, **12**, 515–521.
- 2 L. D. Lavis and R. T. Raines, *ACS Chem. Biol.*, 2008, **3**, 142–155.
- 3 H. Kobayashi, M. Ogawa, R. Alford, P. L. Choyke and Y. Urano, *Chem. Rev.*, 2010, **110**, 2620–2640.
- 4 X. Chen, T. Pradhan, F. Wang, J. S. Kim and J. Yoon, *Chem. Rev.*, 2012, **112**, 1910–1956.
- 5 J. Chan, S. C. Dodani and C. J. Chang, *Nat. Chem.*, 2012, **4**, 973–984.
- 6 H. Zheng, X.-Q. Zhan, Q.-N. Bian and X.-J. Zhang, *Chem. Commun.*, 2013, **49**, 429–447.
- 7 L. D. Lavis and R. T. Raines, *ACS Chem. Biol.*, 2014, **9**, 855–866.
- 8 X. Li, X. Gao, W. Shi and H. Ma, *Chem. Rev.*, 2014, **114**, 590–659.
- 9 J. Yin, Y. Hu and J. Yoon, *Chem. Soc. Rev.*, 2015, **44**, 4619–4644.
- 10 R. Weissleder, *Nat. Biotechnol.*, 2001, **19**, 316–317.
- 11 R. Weissleder and V. Ntziachristos, *Nat. Med.*, 2003, **9**, 123–128.
- 12 J. V. Frangioni, *Curr. Opin. Chem. Biol.*, 2003, **7**, 626–634.
- 13 K. Kiyose, H. Kojima and T. Nagano, *Chem.–Asian J.*, 2008, **3**, 506–515.
- 14 L. Yuan, W. Lin, K. Zheng, L. He and W. Huang, *Chem. Soc. Rev.*, 2013, **42**, 622–661.
- 15 Z. Guo, S. Park, J. Yoon and I. Shin, *Chem. Soc. Rev.*, 2014, **43**, 16–29.
- 16 L. G. Lee, G. M. Berry and C.-H. Chen, *Cytometry*, 1898, **10**, 151–164.
- 17 K. Xu, B. Tang, H. Huang, G. Yang, Z. Chen, P. Li and L. An, *Chem. Commun.*, 2005, 5974–5976.
- 18 M. Sibrian-Vazquez, J. O. Escobedo, M. Lowry, F. R. Fronczek and R. M. Strongin, *J. Am. Chem. Soc.*, 2012, **134**, 10502–10508.
- 19 E. Azuma, N. Nakamura, K. Kuramochi, T. Sasamori, N. Tokitoh, I. Sagami and K. Tsubaki, *J. Org. Chem.*, 2012, **77**, 3492–3500.
- 20 K. Sezukuri, M. Suzuki, H. Hayashi, D. Kuzuhara, N. Aratani and H. Yamada, *Chem. Commun.*, 2016, **52**, 4872–4875.
- 21 J. Arden-Jacob, J. Frantzeskos, N. U. Kemnitzer, A. Zilles and K. H. Drexhage, *Spectrochim. Acta, Part A*, 2001, **57**, 2271–2283.
- 22 J. B. Grimm, A. J. Sung, W. R. Legant, P. Hulamm, S. M. Matlosz, E. Betzig and L. D. Lavis, *ACS Chem. Biol.*, 2013, **8**, 1303–1310.
- 23 K. Kolmakov, V. N. Belov, C. A. Wurm, B. Harke, M. Leutenegger, C. Eggeling and S. W. Hell, *Eur. J. Org. Chem.*, 2010, 3593–3610.
- 24 M. Fu, Y. Xiao, X. Qian, D. Zhao and Y. Xu, *Chem. Commun.*, 2008, 1780–1782.
- 25 T. Egawa, Y. Koide, K. Hanaoka, T. Komatsu, T. Terai and T. Nagano, *Chem. Commun.*, 2011, **47**, 4162–4164.
- 26 Y. Kushida, T. Nagano and K. Hanaoka, *Analyst*, 2015, **140**, 685–695.
- 27 T. Ikeno, T. Nagano and K. Hanaoka, *Chem.–Asian J.*, 2017, **12**, 1435–1446.
- 28 Y. Koide, Y. Urano, K. Hanaoka, T. Terai and T. Nagano, *ACS Chem. Biol.*, 2011, **6**, 600–608.
- 29 H. Nie, J. Jing, Y. Tian, W. Yang, R. Zhang and X. Zhang, *ACS Appl. Mater. Interfaces*, 2016, **8**, 8991–8997.
- 30 A. Fukazawa, S. Suda, M. Taki, E. Yamaguchi, M. Grzybowski, Y. Sato, T. Higashiyama and S. Yamaguchi, *Chem. Commun.*, 2016, **52**, 1120–1123.
- 31 X. Chai, X. Cui, B. Wang, F. Yang, Y. Cai, Q. Wu and T. Wang, *Chem.–Eur. J.*, 2015, **21**, 16754–16758.
- 32 X. Zhou, R. Lai, J. R. Beck, H. Li and C. I. Stains, *Chem. Commun.*, 2016, **52**, 12290–12293.
- 33 X. Chai, J. Xiao, M. Li, C. Wang, H. An, C. Li, Y. Li, D. Zhang, X. Cui and T. Wang, *Chem.–Eur. J.*, 2018, **24**, 14506–14512.



- 34 T. Hirayama, A. Mukaimine, K. Nishigaki, H. Tsuboi, S. Hirose, K. Okuda, M. Ebihara and H. Nagasawa, *Dalton Trans.*, 2017, **46**, 15991–15995.
- 35 M. R. Detty, P. N. Prasad, D. J. Donnelly, T. Ohulchanskyy, S. L. Gibson and R. Hilf, *Bioorg. Med. Chem.*, 2004, **12**, 2537–2544.
- 36 J. Liu, Y.-Q. Sun, H. Zhang, H. Shi, Y. Shi and W. Guo, *ACS Appl. Mater. Interfaces*, 2016, **8**, 22953–22962.
- 37 G. Dejouy, M. Laly, I. E. Valverde and A. Romieu, *Dyes Pigm.*, 2018, **159**, 262–274.
- 38 B. Calitree, D. J. Donnelly, J. J. Holt, M. K. Gannon, C. L. Nygren, D. K. Sukumaran, J. Autschbach and M. R. Detty, *Organometallics*, 2007, **26**, 6248–6257.
- 39 Y. Koide, M. Kawaguchi, Y. Urano, K. Hanaoka, T. Komatsu, M. Abo, T. Terai and T. Nagano, *Chem. Commun.*, 2012, **48**, 3091–3093.
- 40 M. W. Kryman, G. A. Schamerhorn, K. Yung, B. Sathyamoorthy, D. K. Sukumaran, T. Y. Ohulchanskyy, J. B. Benedict and M. R. Detty, *Organometallics*, 2013, **32**, 4321–4333.
- 41 M. W. Kryman, G. A. Schamerhorn, J. E. Hill, B. D. Calitree, K. S. Davies, M. K. Linder, T. Y. Ohulchanskyy and M. R. Detty, *Organometallics*, 2014, **33**, 2628–2640.
- 42 L. V. Lutkus, H. E. Irving, K. S. Davies, J. E. Hill, J. E. Lohman, M. W. Eskew, M. R. Detty and T. M. McCormick, *Organometallics*, 2017, **36**, 2588–2596.
- 43 N. Shimomura, Y. Egawa, R. Miki, T. Fujihara, Y. Ishimaru and T. Seki, *Org. Biomol. Chem.*, 2016, **14**, 10031–10036.
- 44 X. Zhou, L. Lesiak, R. Lai, J. R. Beck, J. Zhao, C. G. Elowsky, H. Li and C. I. Stains, *Angew. Chem., Int. Ed.*, 2017, **56**, 4197–4200.
- 45 C. D. Entwistle and T. B. Marder, *Angew. Chem., Int. Ed.*, 2002, **41**, 2927–2931.
- 46 C. D. Entwistle and T. B. Marder, *Chem. Mater.*, 2004, **16**, 4574–4585.
- 47 F. Jäkle, *Coord. Chem. Rev.*, 2006, **250**, 1107–1121.
- 48 S. Yamaguchi and A. Wakamiya, *Pure Appl. Chem.*, 2006, **78**, 1413–1424.
- 49 F. Jäkle, *Chem. Rev.*, 2010, **110**, 3985–4022.
- 50 A. Wakamiya and S. Yamaguchi, *Bull. Chem. Soc. Jpn.*, 2015, **88**, 1357–1377.
- 51 A. Escande and M. J. Ingleson, *Chem. Commun.*, 2015, **51**, 6257–6274.
- 52 Y. Ren and F. Jäkle, *Dalton Trans.*, 2016, **45**, 13996–14007.
- 53 L. Ji, S. Griesbeck and T. B. Marder, *Chem. Sci.*, 2017, **8**, 846–863.
- 54 E. von Grotthuss, A. John, T. Kaese and M. Wagner, *Asian J. Org. Chem.*, 2018, **7**, 37–53.
- 55 Z. Zhou, A. Wakamiya, T. Kushida and S. Yamaguchi, *J. Am. Chem. Soc.*, 2012, **134**, 4529–4532.
- 56 K. H. Lee, H.-Y. Lee, D. H. Lee and J.-I. Hong, *Tetrahedron Lett.*, 2001, **42**, 5447–5449.
- 57 H. Tong, G. Zhou, L. Wang, X. Jing, F. Wang and J. Zhang, *Tetrahedron Lett.*, 2003, **44**, 131–134.
- 58 D. A. Jose, P. Kar, D. Koley, B. Ganguly, W. Thiel, H. N. Ghosh and A. Das, *Inorg. Chem.*, 2007, **46**, 5576–5584.

