RSC Chemical Biology

ROYAL SOCIETY OF CHEMISTRY

COMMUNICATION

View Article Online
View Journal | View Issue

Open Access Article. Published on 05 May 2022. Downloaded on 4/20/2026 11:35:28 AM.
This article is licensed under a Creative Commons Attribution-NonCommercial 3.0 Unported Licence.

Check for updates

Cite this: *RSC Chem. Biol.*, 2022, 3, 698

Received 25th February 2022,
Accepted 27th April 2022

DOI: 10.1039/d2cb00062h

rsc.li/rsc-chembio

# [4 + 2] and [2 + 4] cycloaddition reactions on single- and double-stranded DNA: a dual-reactive nucleoside†

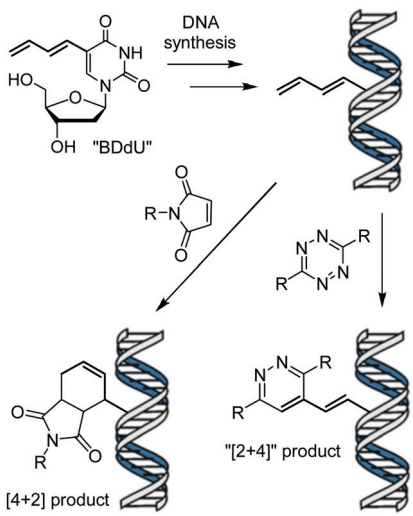
Anna Bujalska,[a] Kaleena Basran[b] and Nathan W. Luedtke *[ab]

**Here we report dual reactivity of diene-modified duplex DNA containing 5-(1,3-butadienyl)-2′-deoxyuridine "BDdU". Normal-electron demand [4 + 2] cycloaddition proceeded upon addition of a maleimide, whereas inverse-electron demand [2 + 4] cycloaddition occurred upon addition of a tetrazine to give a novel, photoswitchable product.**

Bioorthogonal "Click" reactions[1] are widely used for bioconjugation of modified nucleic acids in vitro[2] and *in vivo*.[3] Post-synthetic labeling of nucleic acids using nucleophilic addition,[4] normal-electron-demand Diels–Alder reactions (DA),[5] and strain-promoted inverse-electron-demand Diels–Alder reactions (invDA)[6] enable numerous bioanalytical, material and diagnostic techniques. The introduction of bulky modifications onto nucleosides and nucleotides is common practice for such labeling strategies, however, the resulting steric hindrance can interfere with duplex stability and/or nucleoside metabolism.[7] The development of new strategies utilizing smaller, non-interfering bioorthogonal functional groups therefore remains a high priority.

Terminal alkenes have recently emerged as minimalistic modifications that can serve as reactive groups in catalyst-free bioconjugation reactions *in vitro*,[8] on cell surfaces,[9] in whole cells[10] and animals.[11] In 2014, our group reported the metabolic incorporation of 5-vinyl-2′-deoxyuridine (VdU) into cellular DNA,[10b] and later synthetic oligonucleotides,[4g] as well as its modification by means of invDA reactions with tetrazines. Herein, we report the synthesis and reactivity of 5-(1,3-butadienyl)-2′-deoxyuridine (BDdU) bearing a 1,3-butadienyl moiety attached to C5 position of 2′-deoxyuridine. We postulated that the expansion of the conjugated π system should increase the HOMO energy level of the molecule, allowing for improved HOMO–LUMO overlap[5c] and increased tetrazine reactivity as compared to VdU. In addition, the inclusion of a butadienyl group was envisioned to allow dual modality of DNA modification in both inverse- and normal-electron-demand Diels–Alder cycloaddition reactions with electron-poor dienes or dienophiles, respectively (Scheme 1).

To synthesize 5-(1,3-butadienyl)-2′-deoxyuridine (BDdU, **2**), we utilized a Stille cross coupling reaction between bromovinyl deoxyuridine (BVDU, **1**) and tri-*n*-butylvinylstanne (Scheme 2), the resulting BDdU nucleoside was obtained in a 54% yield and fully characterized by $^{1}$H NMR, $^{13}$C NMR, and high resolution ESI MS (see ESI†). To prepare materials for DNA synthesis, BDdU (**2**) was treated with DMTrCl to give the corresponding DMTr-protected nucleoside (**3**) which was subsequently reacted with chlorophosphoramidite under basic conditions to give BDdU phosphoramidite (**4**) in a 28% overall isolated yield.




Scheme 1 DNA containing 5-(1,3-butadienyl)-2′-deoxyuridine (BDdU) can react as a diene or dienophile upon addition of a maleimide or tetrazine, respectively.

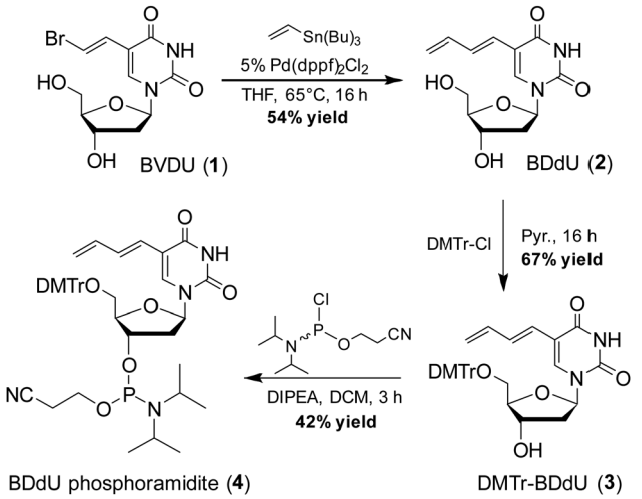
[a] *Department of Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland*
[b] *Department of Chemistry, McGill University, 801 Sherbrooke St. West, Montréal, Québec, H3A 0B8, Canada. E-mail: nathan.luedtke@mcgill.ca*

† Electronic supplementary information (ESI) available. See DOI: https://doi.org/10.1039/d2cb00062h



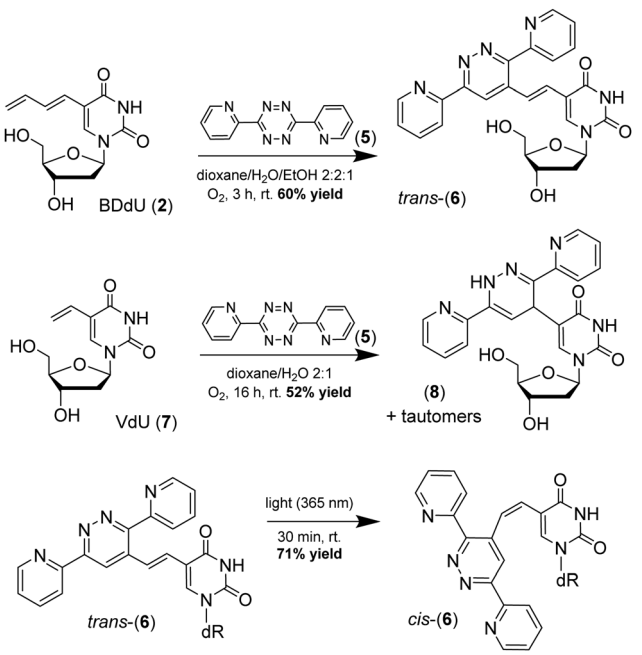
© 2022 The Author(s). Published by the Royal Society of Chemistry

Open Access Article. Published on 05 May 2022. Downloaded on 4/20/2026 11:35:28 AM.
This article is licensed under a Creative Commons Attribution-NonCommercial 3.0 Unported Licence.




Scheme 2 Synthesis of BDdU nucleoside and phosphoramidite where THF = tetrahydrofuran, Pyr. = pyridine, DMTrCl = 4,4′-dimethoxytriphenylmethyl chloride, and DIPEA = *N*,*N*-diisopropylethylamine. See the ESI† for the synthesis and characterization of these compounds.

To investigate the chemical reactivity of BDdU in an inverse-electron demand Diels–Alder reaction, BDdU (**2**) and 3,6-di-2-pyridyl-1,2,4,5-tetrazine were reacted in an aqueous solvent mixture, reaching completion after 3 hours with a 60% isolated yield of the oxidized product (**6**) containing a *trans* alkene (Scheme 3). In contrast, when the same reaction was conducted using VdU (**7**) under ambient conditions, the main product was the dihydrodiazine product (**8**).[10b] To evaluate their relative reaction rates, a series of BDdU-tetrazine reactions were conducted under pseudo-first-order conditions by monitoring the consumption of tetrazine (**5**) upon addition of a large excess of VdU or BDdU. The progress of each reaction was tracked by the disappearance of tetrazine's absorbance maximum at 530 nm (see Fig. S1, ESI†). The invDA cycloaddition reaction between BDdU (**2**) and tetrazine (**5**) exhibited an apparent, second-order reaction rate constant ($k$) = 7.4 × $10^{-2}$ $M^{-1}$ $s^{-1}$. This value was over 3-fold higher than that of VdU-tetrazine (**5**) with a rate constant ($k$) = 2.1 × $10^{-2}$ $M^{-1}$ $s^{-1}$ (Fig. S1, ESI†).




Scheme 3 The reaction between BDdU (**2**) and 3,6-di-2-pyridyl-1,2,4,5-tetrazine in the presence of ambient oxygen gives diazine (**6**), whereas VdU (**7**) gives mostly dihydrodiazine (**8**).[10b]

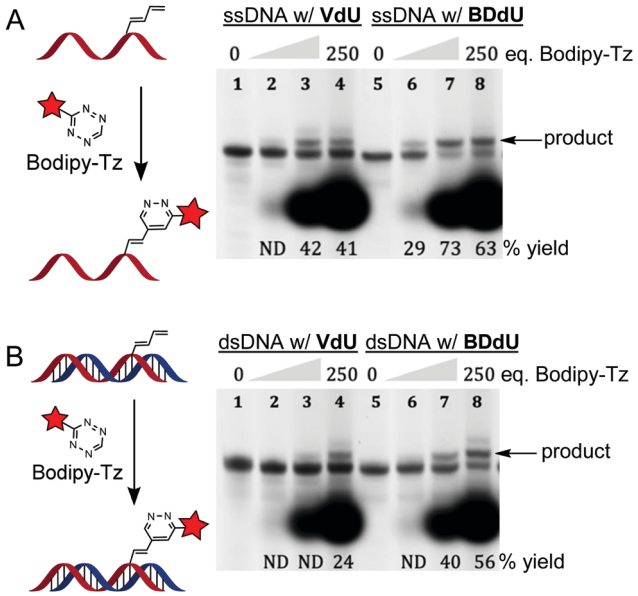
The framework of oxidized product **6** resembles photoswitchable *trans*-stilbenes which have been reported to be fluorescent molecular rotors[12] that can undergo UV-light mediated isomerization to give their corresponding *cis* isomers.[13] Indeed, irradiation of a solution of *trans*-**6** with 365 nm UV light at room temperature for 15 minutes resulted in the formation of two sets of peaks by $^1$H NMR that were assigned according to the coupling constants at 16 Hz for *trans* and 12 Hz for the *cis* isomer (see Fig. S4, ESI†). This *trans-cis* isomerization resulted in the steady-state formation of 71% of the *cis* isomer after 30 min of irradiation. *Trans-cis* photoswitching was accompanied by changes in the molecule's absorbance and fluorescence spectra (Fig. S2 and S3, ESI†).

To evaluate product formation in DNA, we synthesized oligonucleotides modified with BDdU (**2**) or VdU (**7**) using standard, solid-phase supported synthesis. The VdU phosphoramidite was synthesized according to published procedures.[4g,14] We introduced BDdU (**2**) or VdU (**7**) into oligonucleotides using standard phosphoramidite chemistry and purified the products using HPLC (Fig. S6, ESI†). The modified single-stranded (ss) or double stranded (ds) DNA was reacted with a fluorescent, BODIPY-tetrazine conjugate "Bodipy-Tz" (Jena Bioscience) and analyzed using denaturing gel electrophoresis. Both ss and ds DNA containing BDdU exhibited a higher reactivity towards Bodipy-Tz than VdU in both ssDNA and dsDNA (Scheme 4). The products for each reaction were confirmed using MALDI-TOF-MS (Table S1, ESI†).

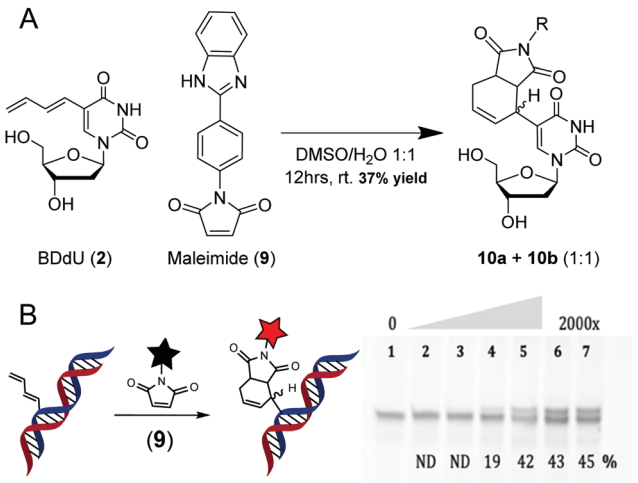
Oligonucleotides containing VdU exhibited moderate yields (41%) in single-stranded DNA (ssDNA) treated with a 250-fold excess of tetrazine for 2 hours at 37 °C. There was a drop to 24% conversion for the same reaction in double-stranded DNA (dsDNA) modified with VdU (**7**) after 2 hours of incubation with an excess of Bodipy-Tz. In contrast, the invDA reaction on oligonucleotides containing BDdU (**2**) was less impacted by being ssDNA or dsDNA. The conversion after 2 hours of incubation with 250-fold excess of tetrazine was 63% and 56% for ssDNA and dsDNA, respectively. These results suggest that BDdU (**2**) is more reactive towards tetrazines as compared to VdU (**7**) due to both electronic and steric effects in DNA.

To evaluate its reactivity towards maleimides, BDdU (**2**) and the fluorogenic maleimide (**9**) were incubated in a mixture of DMSO and water for 12 h at room temperature (Scheme 5). The reaction afforded a 1 : 1 diastereomeric mixture of products (**10a** + **10b**) with a 37% overall isolated yield. The progress of the reaction could be monitored according to fluorescence increases over time ($\lambda_{ex}$ = 310 nm, $\lambda_{em}$ = 366 nm, Fig. S5c, ESI†). Reactions conducted by adding **9** to BDdU-containing DNA gave a modest yield of approximately 45% for both the single-stranded and duplex DNA (Scheme 5 and Fig. S7, ESI†).

Open Access Article. Published on 05 May 2022. Downloaded on 4/20/2026 11:35:28 AM.
This article is licensed under a Creative Commons Attribution-NonCommercial 3.0 Unported Licence.




Scheme 4 Reactions of oligonucleotides modified with VdU (**7**) or BDdU (**2**) with Bodipy-Tz on single- (A) and double-stranded DNA (B). Oligonucleotides were incubated with a 10-, 100- or 250-fold excess of Bodipy-Tz for 2 h at 37 °C in 10 mM sodium cacodylate buffer (pH 7.4). The gels (22.5% PAGE) were stained with SYBR Gold in 1X TBE buffer and scanned using Typhoon FLA 9500. See ESI† for Bodipy-Tz structure.




Scheme 5 A. Reaction between BDdU nucleoside (**2**) and fluorogenic maleimide (**9**). B. Reactions between BDdU-containing duplex DNA with a 10-, 100-, 250-, 500-, 1000- or 2000-fold excess of **9** for 16 hours at 37 °C in 10 mM sodium cacodylate buffer (pH 7.4). The gels (22.5% denaturing PAGE) were stained with SYBR Gold solution in TBE buffer and scanned using Typhoon FLA 9500. Reaction yields are indicated at the bottom of the gel. The product identity for the DNA was confirmed using MALDI-TOF-MS (Table S1, ESI†).

In summary, here we report the synthesis of a new, base-conjugated butadiene (BDdU) phosphoramidite and its incorporation into DNA where it exhibits dual reactivity, undergoing normal-electron demand [4 + 2] cycloaddition upon addition of a maleimide, or inverse-electron demand [2 + 4] cycloaddition upon addition of a tetrazine. In both cases, the reaction can be fluorogenic, where a fluorescence-quenching maleimide[15] or a tetrazine is consumed in the reaction.[6j] Preliminary results suggest that BDdU will show good stability in the presence of cellular thiols (Fig. S8, ESI†), however, it may require installation of a membrane-permeable nucleotide triester "pro-phosphate" group to be metabolically incorporated into cellular DNA.[7e]

BDdU exhibited faster reaction rates with tetrazines as compared to VdU. These results demonstrate that a nucleobase-conjugated diene can exhibit a faster tetrazine reaction rate and give a product that is more prone to oxidation as compared to a simple terminal alkene. Following initial product formation and oxidation, *trans*-(**6**) undergoes a photoisomerization reaction upon irradiation with 365 nm light to give *cis*-(**6**). The future development of light-mediated control of nucleobase structure and function may enable new strategies for manipulating biological processes.[16]

## Conflicts of interest

There are no conflicts to declare.

## Acknowledgements

The authors would like to thank the University of Zurich, McGill University, the Natural Sciences and Engineering Research Council of Canada (grant #05048), and the Canada Foundation for Innovation/John R. Evans Leaders Fund (#39168/253033) for funding.

## Notes and references

1 (*a*) V. V. Rostovtsev, L. G. Green, V. V. Fokin and B. K. Sharpless, *Angew. Chem., Int. Ed.*, 2002, **41**(14), 2596–2599; (*b*) C. W. Tornøe, C. Christensen and M. Meldal, *J. Org. Chem.*, 2002, **67**(9), 3057–3064.

2 (*a*) J. Gierlich, G. A. Burley, P. M.-E. Gramlich, D. M. Hammond and T. Carell, *Org. Lett.*, 2006, **8**(17), 3639–3642; (*b*) F. Seela, V. R. Sirivolu and P. Chittepu, *Bioconjugate Chem.*, 2008, **19**(1), 211–224; (*c*) A. H. El-Sagheer and T. Brown, *Chem. Soc. Rev.*, 2010, **39**(4), 1388; (*d*) E. Paredes and S. R. Das, *ChemBioChem*, 2011, **12**(1), 125–131; (*e*) I. Ivancová, D.-L. Leone and M. Hocek, *Curr. Opin. Chem. Biol.*, 2019, **52**, 136–144.

3 (*a*) A. Salic and T. J. Mitchison, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**(7), 2415–2420; (*b*) A. B. Neef and N. W. Luedtke, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**(51), 20404–20409; (*c*) P. Shieh, V. T. Dien, B. J. Beahm, J. M. Castellano, T. Wyss-Coray and C. R. Bertozzi, *J. Am. Chem. Soc.*, 2015, **137**(22), 7145–7151.

4 (*a*) H. Eberhard, F. Diezmann and O. Seitz, *Angew. Chem., Int. Ed.*, 2011, **50**(18), 4146–4150; (*b*) J. Dadová, P. Orság, R. Pohl, M. Brázdová, M. Fojta and M. Hocek, *Angew. Chem., Int. Ed.*, 2013, **52**(40), 10515–10518; (*c*) A. Sánchez, E. Pedroso and A. Grandas, *Chem. Commun.*, 2013, **49**(3), 309–311; (*d*) L. L.-G. Carrette, E. Gyssels, N. De Laet and A. Madder, *Chem.*


© 2022 The Author(s). Published by the Royal Society of Chemistry

Open Access Article. Published on 05 May 2022. Downloaded on 4/20/2026 11:35:28 AM.
This article is licensed under a Creative Commons Attribution-NonCommercial 3.0 Unported Licence.

*Commun.*, 2016, **52**(8), 1539–1554; (*e*) J. Matyašovský, P. Perlíková, V. Malnuit, R. Pohl and M. Hocek, *Angew. Chem., Int. Ed.*, 2016, **55**(51), 15856–15859; (*f*) A. Olszewska, R. Pohl, M. Brázdová, M. Fojta and M. Hocek, *Bioconjugate Chem.*, 2016, **27**(9), 2089–2094; (*g*) A. Naik, J. Alzeer, T. Triemer, A. Bujalska and N. W. Luedtke, *Angew. Chem., Int. Ed.*, 2017, **56**(36), 10850–10853.

5 (*a*) V. Borsenberger and S. Howorka, *Nucleic Acids Res.*, 2009, **37**(5), 1477–1485; (*b*) C. Paris, O. Brun, E. Pedroso and A. Grandas, *Molecules*, 2015, **20**(4), 6389–6408; (*c*) B. Milián-Medina and J. Gierschner, *Wiley Interdiscip. Rev. Comput. Mol. Sci.*, 2012, **2**, 513–524.

6 (*a*) J. Schoch, M. Wiessler and A. Jäschke, *J. Am. Chem. Soc.*, 2010, **132**(26), 8846–8847; (*b*) J. Šečkutė, J. Yang and N. K. Devaraj, *Nucleic Acids Res.*, 2013, **41**(15), e148; (*c*) P. N. Asare-Okai, E. Agustin, D. Fabris and M. Royzen, *Chem. Commun.*, 2014, **50**(58), 7844–7847; (*d*) A. M. Pyka, C. Domnick, F. Braun and S. Kath-Schorr, *Bioconjugate Chem.*, 2014, **25**(8), 1438–1443; (*e*) H. Wu, B. T. Cisneros, C. M. Cole and N. K. Devaraj, *J. Am. Chem. Soc.*, 2014, **136**(52), 17942–17945; (*f*) C. Domnick, F. Eggert and S. Kath-Schorr, *Chem. Commun.*, 2015, **51**(39), 8253–8256; (*g*) X. Ren, A. H. El-Sagheer and T. Brown, *Analyst*, 2015, **140**(8), 2671–2678; (*h*) K. Wang, D. Wang, K. Ji, W. Chen, Y. Zheng, C. Dai and B. Wang, *Org. Biomol. Chem.*, 2015, **13**(3), 909–915; (*i*) M. Merkel, S. Arndt, D. Ploschik, G. B. Cserép, U. Wenge, P. Kele and H.-A. Wagenknecht, *J. Org. Chem.*, 2016, **81**(17), 7527–7538; (*j*) N. W. Luedtke and M. O. Loehr, *Angew. Chem., Int. Ed.*, 2022, e202112931.

7 (*a*) F. Seela, E. Feiling, J. Gross, F. Hillenkamp, N. Ramzaeva, H. Rosemeyer and M. Zulauf, *J. Biotechnol.*, 2001, **86**(3), 269–279; (*b*) A. B. Neef, L. Pernot, V. N. Schreier, L. Scapozza and N. W. Luedtke, *Angew. Chem., Int. Ed.*, 2015, **54**(27), 7911–7914; (*c*) A. Hottin and A. Marx, *Acc. Chem. Res.*, 2016, **49**(3), 418–427; (*d*) V. Raindlová, M. Janoušková, M. Slavíčková, P. Perlíková, S. Boháčová, N. Milisavljevič, H. Šanderová, M. Benda, I. Barvík, L. Krásný and M. Hocek, *Nucleic Acids Res.*, 2016, **44**(7), 3000–3012; (*e*) M. Tera, S. M.-K. Glasauer and N. W. Luedtke, *ChemBioChem*, 2018, **19**(18), 1939–1943.

8 (*a*) H. Bußkamp, E. Batroff, A. Niederwieser, O. S. Abdel-Rahman, R. F. Winter, V. Wittmann and A. Marx, *Chem. Commun.*, 2014, **50**(74), 10827–10829; (*b*) A. R. Kore, B. Yang and B. Srinivasan, *Tetrahedron Lett.*, 2015, **56**(6), 808–811.

9 (*a*) A. Niederwieser, A.-K. Späte, L. D. Nguyen, C. Jüngst, W. Reutter and V. Wittmann, *Angew. Chem., Int. Ed.*, 2013, **52**(15), 4265–4268; (*b*) Y.-J. Lee, Y. Kurra, Y. Yang, J. Torres-Kolbus, A. Deiters and W. R. Liu, *Chem. Commun.*, 2014, **50**(86), 13085–13088; (*c*) A.-K. Späte, V. F. Schart, S. Schöllkopf, A. Niederwieser and V. Wittmann, *Chem. – Eur. J.*, 2014, **20**(50), 16502–16508.

10 (*a*) Q. Li, T. Dong, X. Liu and X. Lei, *J. Am. Chem. Soc.*, 2013, **135**(13), 4996–4999; (*b*) U. Rieder and N. W. Luedtke, *Angew. Chem., Int. Ed.*, 2014, **53**(35), 9168–9172; (*c*) J. M. Holstein, D. Stummer and A. Rentmeister, *Chem. Sci.*, 2015, **6**(2), 1362–1369; (*d*) H. Wu, S. C. Alexander, S. Jin and N. K. Devaraj, *J. Am. Chem. Soc.*, 2016, **138**(36), 11429–11432.

11 Y. Wu, G. Guo, J. Zheng, D. Xing and T. Zhang, *ACS Sens.*, 2019, **4**(1), 44–51.

12 A. Karimi, R. Börner, G. Mata and N. W. Luedtke, *J. Am. Chem. Soc.*, 2020, **142**(34), 14422–14426.

13 D. Schulte-Frohlinde, H. Blume and H. Güsten, *J. Phys. Chem.*, 1962, **66**(12), 2486–2491.

14 K. Fujimoto, S. Matsuda, N. Takahashi and I. Saito, *J. Am. Chem. Soc.*, 2000, **122**(23), 5646–5647.

15 J. Guy, K. Caron, S. Dufresne, S. W. Michnick, W. G. Skene and J. W. Keillor, *J. Am. Chem. Soc.*, 2007, **129**(39), 11969–11977.

16 (*a*) S. Ogasawara, *ChemBioChem*, 2014, **15**, 2652–2655; (*b*) K. Hüll, J. Morstein and D. Trauner, *Chem. Rev.*, 2018, **118**(21), 10710–10747; (*c*) M. L. Hammill, G. Islam and J. P. Desaulniers, *ChemBioChem*, 2020, **21**(16), 2367–2372; (*d*) Y. Wu, Z. Yang and Y. Lu, *Curr. Opin. Chem. Biol.*, 2020, **57**, 95–104; (*e*) L. Yang, D. Trentini, H. Kim, J. Y. Sul, J. H. Eberwine and I. J. Dmochowski, *ChemPhotoChem*, 2021, **5**(10), 940–946.

© 2022 The Author(s). Published by the Royal Society of Chemistry