


 Cite this: *RSC Adv.*, 2024, 14, 31820

High-efficiency detection of primary amine-based chiral molecules by a facile aldimine condensation reaction†

 Yang Yu,^{‡*a} Aiyang Shi,^{‡b} Tongtong Wang,^{‡b} Tiefeng Wang^a and Fei Xu^a

Detection of chiral molecules in a high-efficiency way is very important to meet the demands for chiral analysis in drug testing, asymmetric synthesis, etc. Herein, we have developed a novel route to realize the rapid determination of concentration and configuration of primary amine-based chiral molecules. An aldehyde functionalized acid & base-sensitive fluorane dye (R–C) was used as the active agent to be reacted with the chiral molecules through an aldimine condensation reaction. After the mixing operation, concentration and configuration of the detected chiral molecule could be readily read from the UV-vis absorption spectra and CD spectra, respectively.

 Received 31st August 2024
 Accepted 4th October 2024

DOI: 10.1039/d4ra06291d

rsc.li/rsc-advances

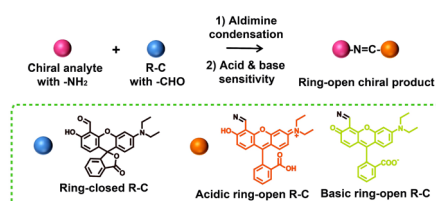
Detection of chiral molecules (concentration and configuration) is of great significance in asymmetric synthesis, pharmaceutical analysis, etc.^{1–7} With the constant progress of science and technology, numerous methods for chiral molecular detection have been developed, such as nuclear magnetic resonance (NMR),^{8,9} chiral chromatography^{10,11} and spectral sensing assays,^{12–16} etc. Among the current detection schemes, spectral analysis has been regarded as one of the most competitive qualitative/quantitative analysis techniques, due to its unique advantages of facile operation, intuitive observation and low solvent consumption.^{17–20}

Since most common chiral molecules (analytes) haven't the ability of absorption in ultraviolet (UV) and visible (vis) bands, traditional absorption spectral instruments cannot detect any identifiable signal.^{21,22} To solve this problem, the chiral analytes have been used to produce specific absorption characteristics by linking them with some suitable chromophores through covalent or non-covalent interactions.^{23,24} Based on this method, the absorption intensity detected by the UV-vis absorption spectrometer can be used to determine the concentration of chiral analytes.²⁵ And, the circular dichroism (CD) absorption signal measured by CD spectrometer can identify the configuration of chiral analytes directly.^{26–31}

So far, various functional materials have been developed to be combined with chiral analytes, so that to generate recognizable absorption signals.^{32,33} For example, detected chiral

signal could be generated after the interaction between the chiral analytes and some polymers³⁴ or supramolecular assemblies³⁵ (hydrogen bonds, electrostatic interaction, etc.). In this way, high-sensitivity detection can be realized, but the poor repeatability cannot be ignored and applicable range of detectable concentration is usually narrow. In another kind of material system, some organic molecules³⁶/oligomers³⁷ with active functional groups or some specific ionic compounds³⁸ can react with chiral analytes to form new products with the formation of apparent spectral absorption. With this method, the repeatability and applicability of the detection on chiral analytes could be ensured.³⁹ However, the detection sensitivity and the operability still need to be improved.^{40,41} According to the material design principle, exploring a functional molecule with sensitive reaction sites, easy synthesis processes and high molar absorption efficiencies are the key parameters to optimize the detection sensitivity and promote the application.

Therefore, in this work, we have designed a novel fluorane dye-based molecular sensor (R–C, 6'-(diethylamino)-3'-hydroxy-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthene]-4'-carbaldehyde) with the functional group of aldehyde to detect the primary amines-based chiral molecules (Scheme 1). Based on the aldimine condensation reaction,^{42–47} the chiral analytes could react



Scheme 1 Schematic diagram of the chiral detection mechanism between R–C and the chiral molecules with –NH₂.

^aCollege of Advanced Materials Engineering, Jiaxing Nanhu University, 314001, Jiaxing, P. R. China. E-mail: yuyang@jxnhu.edu.cn

^bSmart Materials for Architecture Research Lab, Innovation Center of Yangtze River Delta, Zhejiang University, Jiaxing, 314100, P. R. China

 † Electronic supplementary information (ESI) available: Experimental details and supplementary figure for devices. See DOI: <https://doi.org/10.1039/d4ra06291d>

‡ These authors contributed equally.



with the R-C to form new chiral products. And, due to the exhibited acidity or alkalinity of chiral analytes, the new product has a strong absorption in vis band induced by the ring-open pattern of pH-sensitive dye^{48–50} (R-C). According to this mechanism, the concentration of chiral analytes could be easily determined by the UV-vis absorption spectra, while the configuration could be easily read from the CD spectra.

Firstly, the R-C was prepared through a two-step organic synthesis process (Scheme S1 and Fig. S1†). And, its acid & base-responsive property was researched. As shown in Fig. 1a and b, the R-C was transparent without any absorption at vis band. When R-C was treated with acid/base (trifluoroacetic acid, TFA/tetrabutyl ammonium hydroxide, TBAOH), the characteristic absorption spectra appeared with the formation of various colors (orange in acidic state, deep yellow in alkaline state). This meant that the R-C has the potential to identify the chiral analytes which could exhibit acidity or alkalinity.

Apart from the absorption ability, the formation of new chiral product is another necessary condition for the chiral analytes, especially for the absolute configuration. Thus, the reaction feasibility between the R-C and the compounds with primary amines ($-NH_2$) has been verified (Fig. 1c). The appeared change of UV-vis absorption spectra in the mixture of R-C and the primary amines-based chiral molecule (taking the butylamine as the example) meant the formation of a new product, compared with the UV-vis absorption spectra of R-C and butylamine (BA), respectively. In addition, from the results of ¹H nuclear magnetic resonance (NMR) spectra (no. 1 and no. 2 hydrogen (H) had apparent shifts due to the presence of intramolecular hydrogen bond), it could be concluded that a “Schiff base” structure was generated by the condensation reaction between R-C and BA (Fig. 1d and S2†). Therefore, the R-C had a potential to be used as a sensitive sensor for the detection of primary amines-based chiral molecules which could exhibit the property of acid or base, according to the above experimental results.

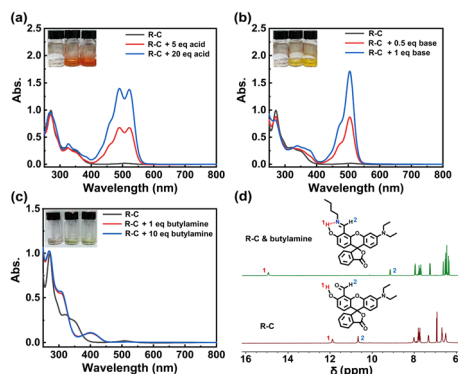


Fig. 1 UV-vis absorption spectra of 1.0×10^{-4} M R-C in CH_3CN and when it was mixed with various eq. (a) acid (TFA), (b) base (TBAOH), and (c) butylamine (BA), respectively. Inset pictures: corresponding photographs of these functional solutions. ($d = 1$ cm) (d) ¹H NMR spectra of R-C and the mixture of R-C with BA in deuterated dimethyl sulfoxide (DMSO).

After the feasibility verification, we then researched the detection effects of R-C for the primary amines-based chiral molecules. Since the most amino acids have $-NH_2$ and the ability of exhibiting acidity or alkalinity, they were suitable to be reacted with R-C as the detected chiral compounds. Taking the L-cysteine (L-Cys) as an example of chiral analyte, the detection of its concentration and configuration were validated. As shown in Fig. 2a, b and S3,† a linear response ($R^2 > 99.7\%$) in the absorption intensity change could be observed with the addition of L-Cys (1×10^{-3} M– 1×10^{-2} M) in the solution of 1×10^{-3} M R-C in acetonitrile (CH_3CN). This meant that the R-C could be used as a sensor to detect the L-Cys according to a specific linear relationship. In addition, by changing the concentration of R-C, L-Cys with other concentration range could be detected effectively (Fig. S4†).

Based on the experimental results, it could be concluded that the detection range of L-Cys could be from 4×10^{-4} M to 1×10^{-2} M. And, even the concentration of L-Cys was as low as 3×10^{-4} M, it could be detected by the R-C with an apparent absorption change in the visible band, indicating the high sensitivity of our prepared system (Fig. S5†). In addition, the limit of quantitation (LOQ) was calculated as 1.14×10^{-4} M, which further proved this viewpoint.

Apart from the concentration of the chiral analyte, the configuration is also very important to be determined. According to the aldimine condensation reaction, the L-Cys could be combined with R-C through a covalent bond ($-C=N-$), accompanied by the formation of the new chiral reaction product. Thus, we measured chiral absorption property of the mixture systems composed of R-C and L-Cys/D-Cys (Fig. 2c and d). Specific absorption of acidic ring-open R-C was observed, accompanied by the appearance of totally symmetrical CD signals, indicating that the new chiral products with specific CD absorption features were prepared effectively. Therefore, according to the above results, R-C could be applied as an effective qualitative and quantitative detection material for the typical primary amines-based chiral molecules with acidity or alkalinity.

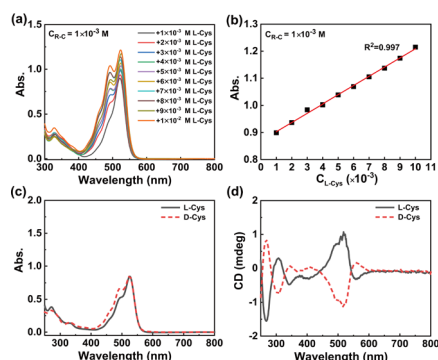


Fig. 2 (a) UV-vis absorption spectra and (b) corresponding absorption values at 522 nm of the mixture of 1.0×10^{-3} M R-C in CH_3CN and L-Cys with various concentrations in deionized water. (c) UV-vis absorption and (d) CD spectra of the mixture of 1.0×10^{-3} M R-C in CH_3CN and 1.0×10^{-3} M L-Cys or D-Cys in deionized water, respectively. ($d = 2$ mm).



Then, the reaction mechanism was researched in detail. The reaction product prepared using the combination of R-C and L-Cys was determined by the ^1H NMR spectra. As revealed in Fig. 3, with the addition of L-Cys, no. 2 H exhibited an apparent shift, which meant the formation of $-\text{N}=\text{C}$. It was similar with the reaction result between R-C and BA (Fig. 1d), indicating the successful occurrence of aldimine condensation reaction. In addition, compared with the ^1H NMR spectra of acidified and alkalinized R-C, the L-Cys showed similar results in the chemical shifts of H with acidified R-C (Fig. S6 †). Thus, the absorption change was due to the production of acidified ring-open R-C grafted with L-Cys. The similar spectral absorption changing results proved this viewpoint (Fig. 1a and 2a).

In addition, the apparent molecular ion peak of the mass spectral result of the mixture of R-C and L-Cys kept the similar with the molecular weight of their condensation product (Fig. S7 †). This result could further confirm that the chiral sensing mechanism in this system was based on the condensation reaction between R-C and L-Cys. According to these experimental results, the reaction process was depicted in Fig. S8 † . R-C with $-\text{CHO}$ was reacted by L-Cys with $-\text{NH}_2$ to produce a new chiral product composed of acidic ring-open R-C and L-Cys, accompanied with apparent absorption changes in UV-vis and CD spectra.

Based on the design concept and reaction mechanism, the primary amines-based chiral molecules exhibiting acidity or alkalinity could react with R-C to form a chiral product with strong absorption in the visible band. This meant that most amino acids could be detected in this way. Thus, two typical amino acids (L/D-glutamic acid, L/D-Glu; L/D-histidine, L/D-His) were tried here. As demonstrated in Fig. 4, apparent absorption changes and symmetric CD spectra could be induced by the addition of various chiral analytes with the opposite configurations. Considering the features of this system, other chiral molecules with both primary amines and acidity/alkalinity, such as aniline derivatives, saccharides, *etc.* could also be facily detected in this way. As shown in Fig. S9 † , a typical

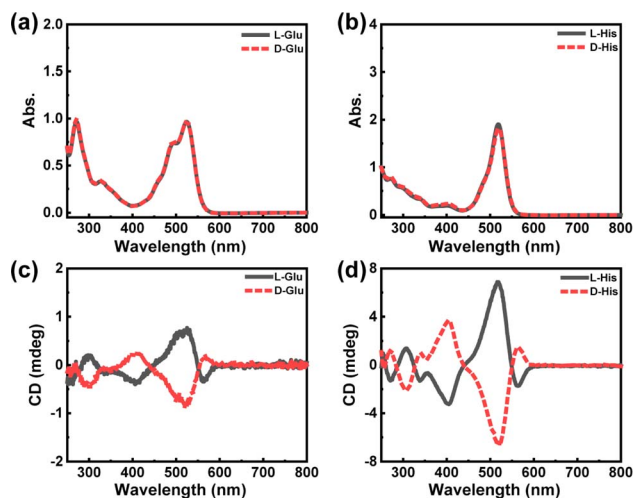


Fig. 4 (a) UV-vis absorption and (c) CD spectra of the mixture of 1.0×10^{-3} M R-C in CH_3CN and 1.0×10^{-3} M L-Glu or D-Glu in deionized water, respectively. (b) UV-vis absorption and (d) CD spectra of the mixture of 1.0×10^{-3} M R-C in CH_3CN and 1.0×10^{-3} M L-His or D-His in deionized water, respectively. ($d = 2$ mm).

compound with $-\text{NH}_2$ ((S)-(-)- α -methylbenzylamine, S(-)- α -MBA) was taken as the chiral analyt to be reacted with R-C. From the spectral measurement result, the concentration of S(-)- α -MBA could be determined by the absorption value effectively, which was similar with the detection results of L-Cys. This phenomenon indicated the great scalability of this detection system.

In conclusion, we have efficiently developed a facile method to achieve the rapid detection (concentration & configuration) of the primary amines-based chiral molecules exhibiting acidity or alkalinity. Through a facile aldimine condensation reaction, a pH-sensitive dye (R-C) could react with chiral analytes, which could exhibit the property of acid or base, so that to form a new chiral product with strong absorption effects in the visible band. The optical changes could be easily detected by the UV-vis absorption and CD spectra. It is worth mentioning that the R-C was only a typical representative molecule, which could be used as an effective chiral sensor. According to the features of various chiral analytes, different chiral molecular sensors could be designed and prepared according to the chiral sensing mechanism proposed in this work. In a word, this unique exploration opens a new route to achieve the high-efficiency chiral detection and undoubtedly will promote the development of the novel chiral analysis materials.

Experimental section

Experimental methods are available in the ESI †

Data availability

The authors confirm that the data supporting the findings of this study are available within the article [and/or its ESI materials]. †

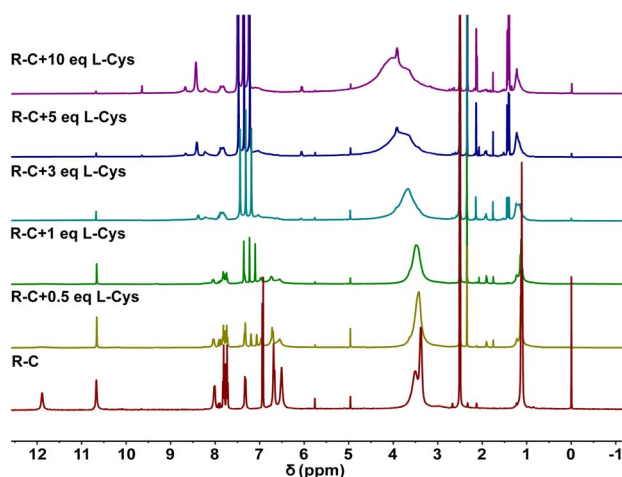


Fig. 3 ^1H NMR spectra of 1.2×10^{-2} M R-C and when it was mixed with various eq. L-Cys in deuterated dimethyl sulfoxide (DMSO).



Author contributions

Yang Yu: conceptualization, methodology, writing – original draft, resources, funding acquisition; Aiyan Shi: validation, software, data curation, investigation, writing – review & editing; Tongtong Wang: writing – review editing, funding acquisition; Tiefeng Wang: project administration, resources; Fei Xu: data curation.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

We thank the General Scientific Research Project of Department of Education of Zhejiang Province (Y202454574), Youth Science and Technology Talent Special Project of Jiaying (2024AY40017), the 2022 Ministry of Education “Chunhui Program” Co-Operative Scientific Research Project of Ministry of Education of China (No. HZKY20220195), the Public Welfare Research Plan of Jiaying (2023AY11016), and the Start-Up Funds of Jiaying Nanhu University (No. QD61220019 and No. QD61220017) for their financial supports.

Notes and references

- 1 Y. Liu, Z. Wu, D. W. Armstrong, H. Wolosker and Y. Zheng, *Nat. Rev. Chem.*, 2023, **7**, 355–373.
- 2 H.-L. Qian, S.-T. Xu and X.-P. Yan, *Anal. Chem.*, 2023, **95**, 304–318.
- 3 M. L. Solomon, A. A. E. Saleh, L. V. Poulidakos, J. M. Abendroth, L. F. Tadesse and J. A. Dionne, *Acc. Chem. Res.*, 2020, **53**, 588–598.
- 4 C. Hao, L. Xu, H. Kuang and C. Xu, *Adv. Mater.*, 2020, **32**, 1802075.
- 5 P. Anzenbacher Jr, S. M. George and A. R. Sartori, *Chem.*, 2024, **10**, 2345–2348.
- 6 E. G. Shcherbakova, T. D. James and P. Anzenbacher Jr, *Nat. Protoc.*, 2020, **15**, 2203–2229.
- 7 S. Sheykhi, L. Mosca, M. Pushina, K. Dey and P. Anzenbacher Jr, *Chem. Commun.*, 2020, **56**, 8964–8967.
- 8 L. T. Kuhn, K. Motiram-Corral, T. J. Athersuch, T. Parella and M. Pérez-Trujillo, *Angew. Chem., Int. Ed.*, 2020, **59**, 23615–23619.
- 9 G. Gu, Z. Xu, L. Wen, J. Liang, C. Wang, X. Wan and Y. Zhao, *JACS Au*, 2023, **3**, 1348–1357.
- 10 D. Wu, C. Ma, G.-C. Fan, F. Pan, Y. Tao and Y. Kong, *J. Sep. Sci.*, 2022, **45**, 325–337.
- 11 W. Wang, Y. Zhang, B. Tang, H. Hou, S. Tang and A. Luo, *J. Chromatogr. A*, 2022, **1675**, 463150.
- 12 M. Quan, X.-Y. Pang and W. Jiang, *Angew. Chem., Int. Ed.*, 2022, **61**, e202201258.
- 13 F. Fan, C. Zhong, Z. Zhang, S. Li and S. Chang, *Nanoscale Adv.*, 2021, **3**, 4790–4798.
- 14 L. Pu, *Chem. Commun.*, 2022, **58**, 8038–8048.
- 15 Y. Chen, F. Zhao, J. Tian, L. Jiang, K. Lu, Y. Jiang, H. Li, S. Yu, X. Yu and L. Pu, *J. Org. Chem.*, 2021, **86**, 9603–9609.
- 16 L. Pu, *Angew. Chem., Int. Ed.*, 2020, **59**, 21814–21828.
- 17 J. H. Han, Y.-C. Lim, R. M. Kim, J. Lv, N. H. Cho, H. Kim, S. D. Namgung, S. W. Im and K. T. Nam, *ACS Nano*, 2023, **17**, 2306–2317.
- 18 H. Zhu, Q. Li, Z. Gao, H. Wang, B. Shi, Y. Wu, L. Shangguan, X. Hong, F. Wang and F. Huang, *Angew. Chem., Int. Ed.*, 2020, **59**, 10868–10872.
- 19 R. M. Kim, J.-H. Huh, S. Yoo, T. G. Kim, C. Kim, H. Kim, J. H. Han, N. H. Cho, Y.-C. Lim, S. W. Im, E. Im, J. R. Jeong, M. H. Lee, T.-Y. Yoon, H.-Y. Lee, Q.-H. Park, S. Lee and K. T. Nam, *Nature*, 2022, **612**, 470–476.
- 20 S. A. Valenzuela, H. S. N. Crory, C.-Y. Yao, J. R. Howard, G. Saucedo, A. P. d. Silva and E. V. Anslyn, *Angew. Chem., Int. Ed.*, 2021, **60**, 13819–13823.
- 21 D. S. Hassan and C. Wolf, *Nat. Commun.*, 2021, **12**, 6451.
- 22 A. Prabodh, D. Bauer, S. Kubik, P. Rebmann, F. G. Klärner, T. Schrader, L. D. Bizzini, M. Mayor and F. Biedermann, *Chem. Commun.*, 2020, **56**, 4652–4655.
- 23 S. Huang, H. Yu and Q. Li, *Adv. Sci.*, 2021, **8**, 2002132.
- 24 G. A. Hembury, V. V. Borovkov and Y. Inoue, *Chem. Rev.*, 2008, **108**, 1–73.
- 25 C. Wolf and K. W. Bentley, *Chem. Soc. Rev.*, 2013, **42**, 5408–5424.
- 26 Y. Yu, G. Yang, S. Zhang, M. Liu, S. Xu, C. Wang, M. Li and S. X.-A. Zhang, *ACS Nano*, 2022, **16**, 148–159.
- 27 Y. Xiao, A. Shi, G. Yang, Y. Yu, Q. Nie, S. Qi, C. Xiang and T. Zhang, *Small*, 2024, 2404913.
- 28 J. R. Howard, J. R. Shuluk, A. Bhakare and E. V. Anslyn, *Chem*, 2024, **10**, 2074–2088.
- 29 A. Sripada, F. Y. Thanzeel and C. Wolf, *Chem*, 2022, **8**, 1734–1749.
- 30 J. R. Howard, A. Bhakare, Z. Akhtar, C. Wolf and E. V. Anslyn, *J. Am. Chem. Soc.*, 2022, **144**, 17269–17276.
- 31 J. Lim, M. Guo, S. Choi, S. J. Miller and E. V. Anslyn, *Chem. Sci.*, 2023, **14**, 5992–5999.
- 32 J. S. S. K. Formen, J. R. Howard, E. V. Anslyn and C. Wolf, *Angew. Chem., Int. Ed.*, 2024, **63**, e202400767.
- 33 J. Guo, X. Hou, J. Hu, Y. Geng, M. Li, H. Wang, I. Wang and Q. Luo, *Chem. Commun.*, 2023, **59**, 9157–9166.
- 34 S. Ma, B. Zhao and J. Deng, *ACS Cent. Sci.*, 2023, **9**, 1409–1418.
- 35 J.-Z. Liu, X.-Y. Chai, J. Huang, R. S. Li, C. M. Li, J. Ling, Q.-E. Cao and C. Z. Huang, *Anal. Chem.*, 2024, **96**, 4282–4289.
- 36 C. Wolf and K. W. Bentley, *Chem. Soc. Rev.*, 2013, **42**, 5408–5424.
- 37 Y. Wang, Y. Chen, H. Bian, Y. Sun, L. Zhu and D. Xia, *Sens. Actuators, B*, 2021, **341**, 130044.
- 38 D. Wu, Q. Yin, P. Cai, X. Zhao and Y. Pan, *Anal. Chim. Acta*, 2017, **962**, 97–103.
- 39 L. A. Warning, A. R. Miandashti, L. A. McCarthy, Q. Zhang, C. F. Landes and S. Link, *ACS Nano*, 2021, **15**, 15538–15566.
- 40 M. Hu, H.-T. Feng, Y.-X. Yuan, Y.-S. Zheng and B. Z. Tang, *Coord. Chem. Rev.*, 2020, **416**, 213329.
- 41 M. M. Moein, *Talanta*, 2021, **224**, 121794.



- 42 G. Yang, Y. Yu, B. Yang, T. Lu, Y. Cai, H. Yin, H. Zhang, N. Zhang, L. Li, Y.-M. Zhang and S. X.-A. Zhang, *Angew. Chem., Int. Ed.*, 2021, **60**, 2018–2023.
- 43 G. Yang, Z. Yao, X. Yang, Y. Xie, P. Duan, Y.-M. Zhang and S. X.-A. Zhang, *Adv. Sci.*, 2022, **9**, 2202636.
- 44 A. Bade, P. Yadav, L. Zhang, R. N. Bypaneni, M. Xu and T. E. Glass, *Angew. Chem., Int. Ed.*, 2024, e202406401.
- 45 M. R. Smith, L. Zhang, Y. Jin, M. Yang, A. Bade, K. D. Gillis, S. Jana, R. N. Bypaneni, T. E. Glass and H. Lin, *ACS Cent. Sci.*, 2023, **9**, 980–991.
- 46 L. Zhang, X. A. Liu, K. D. Gillis and T. E. Glass, *Angew. Chem., Int. Ed.*, 2019, **58**, 7611–7614.
- 47 C. Yin, F. Huo, N. P. Cooley, D. Spencer, K. Bartholomew, C. L. Barnes and T. E. Glass, *ACS Chem. Neurosci.*, 2017, **8**, 1159–1162.
- 48 J. Li, G. Yang, A. Shi, D. Han, M. Han, C. Xiang, T. Zhang and H. Wang, *Adv. Opt. Mater.*, 2024, **12**, 2400156.
- 49 A. Shi, H. Wang, G. Yang, C. Gu, C. Xiang, L. Qian, J. W. Y. Lam, T. Zhang and B. Z. Tang, *Angew. Chem., Int. Ed.*, 2024, **63**, e202409782.
- 50 J. Sun, M. Han, G. Yang, Y. Wang, W. Fang, A. Shi, C. Xiang, J. Wang, T. Zhang and H. Wang, *Adv. Opt. Mater.*, 2024, 2402080.

