

A Vibration-Induced Emission-Based Ratiometric Sensor for Detection of Anions in Aqueous Solution

Stephen M. Butler,^[a] Nikki A. Tzioumis,^[a] and Katrina A. Jolliffe*^[a]

Sensors that respond to anions via vibration-induced emission (VIE) have recently emerged as effective tools for discrimination between closely related carboxylate species in non-competitive, organic solvents. However, the utility of this sensing mechanism has not yet been demonstrated for anions in aqueous media. Here, we prepared two sensors, **monoZnDPA-DPAC** and **ZnDPA-**

DPAC, with either one or two anion-binding motifs. These systems are capable of binding to anions in water. Dual emission via VIE is maintained in solvent mixtures of up to 70% water. **ZnDPA-DPAC** provides a unique, ratiometric response to citrate and phosphate, which was used for the accurate quantification of aqueous solutions of these anions.

1. Introduction

The *N,N'*-diphenyl-dihydrodibenzo[*a,c*]phenazine (DPAC) motif is a highly versatile fluorophore that displays unique emission properties.^[1] Compounds based on this scaffold exhibit dual emission bands due to vibration-induced emission (VIE) (Figure 1).^[2] In the ground state, the DPAC unit sits in a bent conformation, due to steric hindrance between the phenyl “arms” and phenanthrene motif. However, in the excited state, rotation of the “arms” and subsequent planarization of the DPAC core typically occurs. This conformational change leads to a second, lower energy emission band. Consequently, the DPAC fluorophore is highly sensitive to temperature and freedom of movement, and DPAC-based sensors have found use in various applications, including as molecular thermometers,^[3] viscosity sensors,^[4] polymer and gelation sensors,^[5] sensors for cell membranes and cell wall properties,^[6] and as supramolecular sensors for various analytes.^[7]

Seminal work by Tian and co-workers demonstrated that through the use of a covalent tether to interrupt the excited-state planarization of functionalized DPAC derivatives, the emission maxima of these molecules can be precisely tuned across a range from 400 to 613 nm.^[8] The groups of Stang and Chou,^[9] and of Sessler and Tian,^[7c] expanded on this work by demonstrating that non-covalent interactions may be used to tune the emission of DPAC derivatives in dichloromethane and in

acetonitrile respectively. In both these cases, supramolecular assembly of macrocycles comprised of dicarboxylates with a DPAC-based recognition unit led to a broad range of emission wavelengths. These systems displayed impressive discrimination between highly similar dicarboxylate species based on their length: a challenging feat. However, sensing and identification of dicarboxylate species in aqueous media remains a challenge.^[10] The platinum(II) phosphine/carboxylate complexes employed by Stang and Chou are likely too unstable to operate in aqueous media, and Sessler and Tian’s calixarenes do not display sufficiently high binding affinities with carboxylates to be effective in this medium. A recently reported DPAC-based ratiometric sensor for sugars was shown to operate in mixtures of up to 20% water/methanol.^[7a] However, the sensitivity for sugar detection was low. Tian and co-workers have also reported the use of a DPAC-based ratiometric sensor for mercury ions that operates in 70% water mixtures.^[7b] In this case, the reported mechanism is due to the formation of nano-aggregates. As far as we are aware, there are no reported VIE-based sensors for anions that operate in aqueous media.

Zinc(II) complexes of dipicolylamine (DPA) ligands are highly stable in aqueous solution and have been widely applied as high-affinity recognition units for carboxylates and phosphates.^[11] ZnDPA groups are also highly amenable for use in fluorescent sensors, as the *d*¹⁰ metal center does not typically interfere with the excited state of fluorophores. In this work, we set out to incorporate ZnDPA complexes into the DPAC scaffold, to assess the suitability of VIE for dual-color sensing of anions in highly competitive aqueous media.


2. Results and Discussion

2.1. Sensor Design and Synthesis

We designed two ZnDPA-based DPAC sensors, incorporating either one or two ZnDPA units: **monoZnDPA-DPAC** and **ZnDPA-DPAC**. DFT modeling of **ZnDPA-DPAC** indicated that C4–C8 dicarboxylates can bridge between the two ZnDPA groups

[a] S. M. Butler, N. A. Tzioumis, K. A. Jolliffe
School of Chemistry and ARC Centre of Excellence for Innovations in Peptide
and Protein Science, University of Sydney, Sydney, Australia
E-mail: kate.jolliffe@sydney.edu.au

 Supporting information for this article is available on the WWW under
<https://doi.org/10.1002/chem.202500610>

 © 2025 The Author(s). Chemistry – A European Journal published by
Wiley-VCH GmbH. This is an open access article under the terms of the
[Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which
permits use and distribution in any medium, provided the original work is
properly cited, the use is non-commercial and no modifications or
adaptations are made.

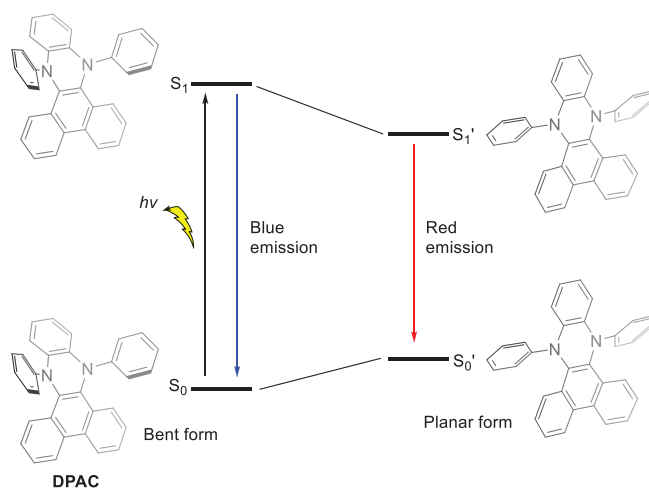


Figure 1. The DPAC motif undergoes vibration-induced emission (VIE). Excited state planarization leads to a lower energy excited state from which a second, longer wavelength emission can originate.

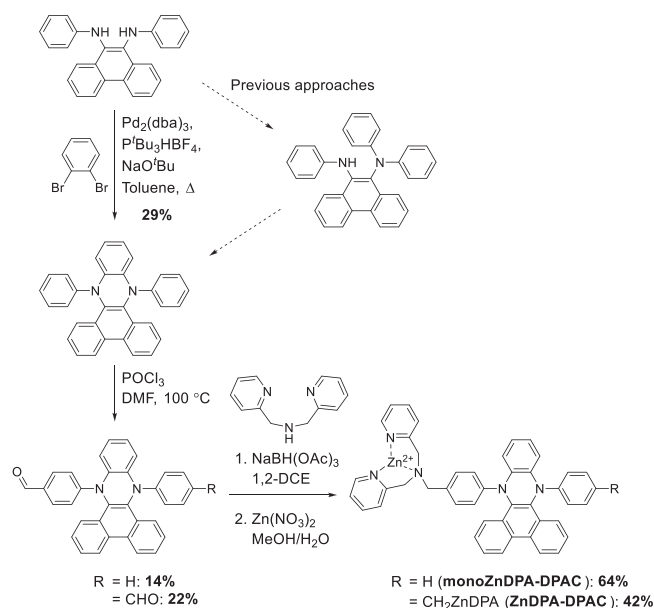
of **ZnDPA-DPAC**, forming a supramolecular macrocycle that restricts the movement of the DPAC “arms” (Figures S3–S6), and their ability to planarize in the excited state. **monoZnDPA-DPAC** was prepared as a control compound to distinguish anion recognition via restricted planarization from other possible fluorescence mechanisms.

In our hands, the previously published synthetic routes to access the DPAC core gave inconsistent results, and the separation of multiple, low-polarity products proved challenging. In these methods, the “top” aryl ring is introduced by a Ullmann^[12] or Buchwald–Hartwig^[13] cross-coupling, followed by C–H amination to form the sterically challenged final ring. The final C–H amination is performed under high temperatures and cyclization occurs at whichever of the *N*-aryl substituents is most activated. Consequently, we developed a double-Buchwald–Hartwig approach to install the final DPAC ring system in a single step (Scheme 1). Unlike the C–H amination approaches applied in previous syntheses, this approach also has the advantage of providing precise control over the site of cyclization.

Vilsmeier–Haack formylation of the DPAC core and subsequent reductive amination with dipicolylamine, followed by complexation with $\text{Zn}(\text{NO}_3)_2$ afforded the final sensors. Zinc complexation was confirmed by the emergence of diastereotopic character of the DPA methylene proton resonances.

2.2. Spectral Changes in Response to Solvent

Both **ZnDPA-DPAC** and **monoZnDPA-DPAC** displayed excitation and emission spectra in DMSO solution that are consistent with the VIE properties of previously reported DPAC sensors (Figure S7).^[14] The maximal excitation wavelength was 360 nm, and two emission peaks were observed at $\lambda_{\text{max}} = 440$ and 613 nm, respectively (Figure 2). Both compounds displayed multi-color solvatochromism, with emissive properties that are highly dependent on the solvent composition. Both emission bands of **monoZnDPA-DPAC** were quenched with increasing water



Scheme 1. Synthesis of **ZnDPA-DPAC** sensors. Formation of the DPAC core was achieved through a double-Buchwald–Hartwig coupling.

fraction, then an emission band at 505 nm appeared above 70% water. The intensity of this signal increased dramatically as the water fraction was further increased. For **ZnDPA-DPAC**, the longer wavelength emission was quenched as the water content of the solvent was increased, and beyond 70% water content only the blue emission band at 440 nm was observed. Slight enhancement of the long wavelength emission could be achieved by increasing the temperature (Figure S8). This observation is consistent with a VIE mechanism.

Time-course experiments also indicated that solvent composition had a significant impact on the photostability of both **ZnDPA-DPAC** and **mono-ZnDPA-DPAC**. Oxidative degradation of DPAC derivatives in aqueous solution has been previously reported and leveraged as a strategy for controlled drug release.^[14] We also observed slow aerobic oxidation of DPAC to form phenanthrenequinone when samples were stored under air. Solutions of both **ZnDPA-DPAC** and **mono-ZnDPA-DPAC** in DMSO maintained consistent fluorescence intensity following continuous irradiation with 360 nm light for 1000 s (Figures S9–S10). However, increasing the water content had a deleterious effect on the photostability of both compounds. In both 1:1 and 1:9 mixtures of DMSO:H₂O, **mono-ZnDPA-DPAC** was more photostable than **ZnDPA-DPAC**, suggesting that the ZnDPA group has an impact on sensor stability. Intermittent irradiation of the solutions of the sensors in 1:1 or 1:9 DMSO:H₂O, consistent with conditions used in the fluorescence experiments below, did not significantly impact the fluorescence intensity of the sensors (Figure S11).

2.3. Response of Sensors to Carboxylates

Initial screening with **ZnDPA-DPAC** was performed with 5 equivalents of carboxylate anions as their tetrabutylammonium (TBA)

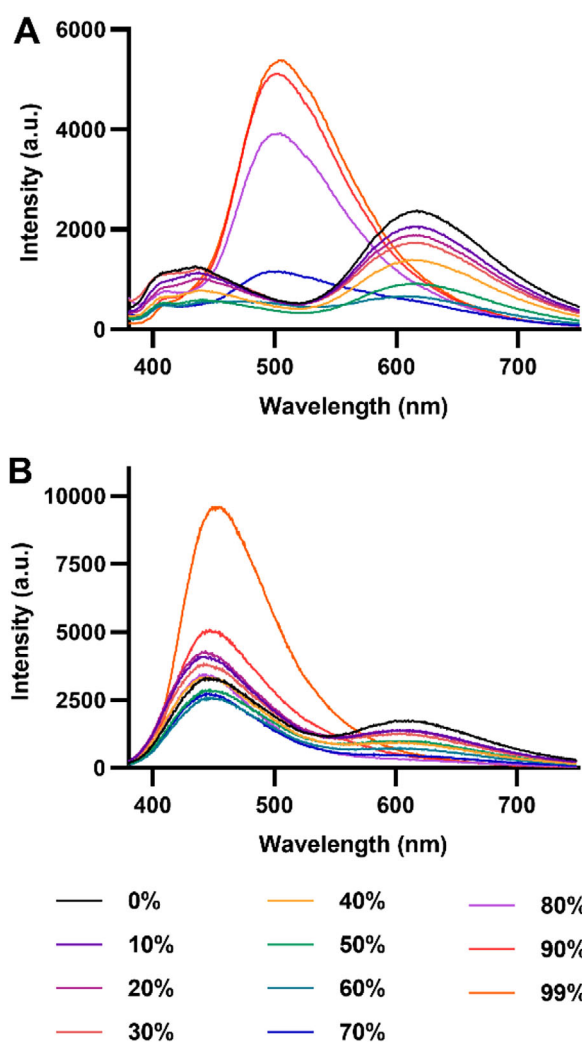


Figure 2. Spectra of: A. **monoZnDPA-DPAC** and; B. **ZnDPA-DPAC** probes (2 μ M) in response to changing solvent composition. The percentage of water in a DMSO/water solvent mixture is indicated in the legend. λ_{ex} = 360 nm.

salts. A solvent mixture of 1:1 DMSO:H₂O was used to ensure that both emission peaks were present, and the sensors were sufficiently photostable. The monocarboxylate anions acetate and benzoate had little effect on the intensity of either emission band (Figure 3). However, the addition of dicarboxylates, which were predicted to bridge between both ZnDPA units, led to quenching of the long wavelength emission band at 613 nm. The intensity of the short wavelength, 440 nm band was largely unaffected by the presence of carboxylate species, and there was no clear trend linking carboxylate length and emission wavelength or intensity. However, the tricarboxylate citrate elicited a unique response, with enhancement of emission at 440 nm and concomitant quenching of the band at 613 nm (Figure 3).

To confirm that the changes in emission observed are due to the engagement of each anion with both ZnDPA units, rather than by aggregation (c.f. Tian's mercury sensor),^[7b] screens were also performed with the one-armed sensor, **monoZnDPA-DPAC** (Figure S12). The addition of mono- and di-carboxylate anions had minimal effect on either emission peak at up to 1000

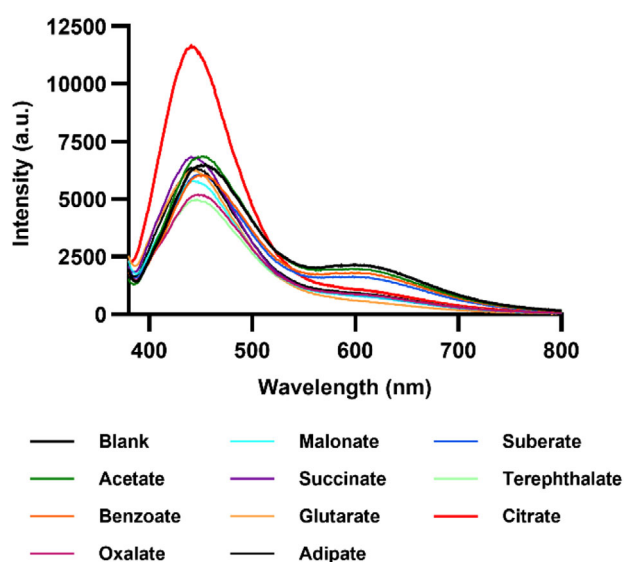


Figure 3. Response of **ZnDPA-DPAC** (20 μ M) in 1:1 DMSO:H₂O in the presence of 5 equivalents of carboxylate anions. All anions were added as their tetrabutylammonium salts. λ_{ex} = 360 nm.

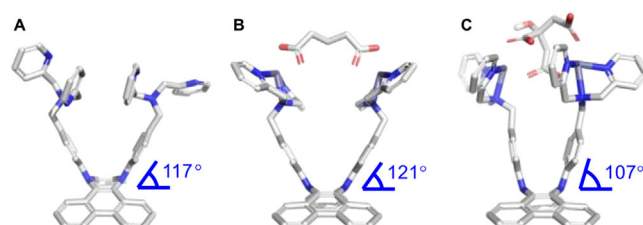


Figure 4. Minimized ground-state structures of A. **DPA-DPAC**; B. **ZnDPA-DPAC** with glutarate; C. **ZnDPA-DPAC** with citrate. Calculations in vacuum, with B3LYP-6-31G*. The average angle between the "arms" and DPAC core is shown.

equivalents. The addition of up to 200 equivalents of citrate also had no effect; however, at higher citrate concentrations, a slight fluorescence enhancement of both peaks was observed. This observation is consistent with the abstraction of the zinc (II) cation from the DPA ligands at high citrate concentrations. The addition of higher equivalents of mono- and di-carboxylate anions had little effect on the emission profile of **ZnDPA-DPAC** in both 1:1 and 1:9 DMSO:H₂O (Figures S13, S14).

The unique response of **ZnDPA-DPAC** to the presence of citrate may be rationalized by analysis of the modeled structures of the sensor with the carboxylates tested. The ground state sensor, in the absence of an anion, is predicted to sit in a conformation where each "arm" sits at an angle of 117° from the plane of the DPAC core (Figure 4A). These calculations are consistent with those previously obtained by Tian and co-workers (c.f. 118°).^[8] Monocarboxylates and short dicarboxylates (C2, C3) are predicted to interact with only one of the two ZnDPA units each, rather than bridging across both, and so do not lead to a large change in emission profile (Figure S2). Long dicarboxylates form a flexible bridge across both binding groups and hence restrict the planarization of the "arms" leading to suppression of the red emission (Figures 4B, S3–S5). In contrast, citrate is predicted to

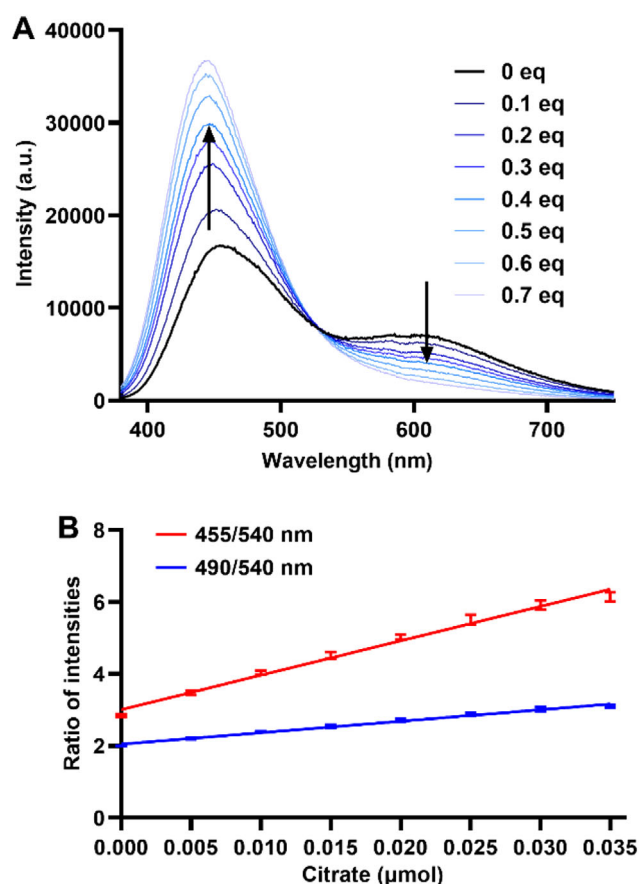


Figure 5. A. Emission spectra of ZnDPA-DPAC (20 μM) in 1:1 DMSO:H₂O on the addition of aliquots of sodium citrate ($\lambda_{\text{ex}} = 360 \text{ nm}$); B. Calibration curves generated from analysis of spectra at 455, 490, and 540 nm. Linear regression of the data was used to generate the solid lines. Error bars show the deviation across three replicates.

form a close interaction with both ZnDPA units, leading to a contraction of the angle of the “arms” to 107°, and enhancement of the blue emission (Figures 4C, 56).

2.4. Quantification of Anions

Given the unique response of ZnDPA-DPAC to the presence of citrate, we sought to further explore its potential as a ratiometric citrate sensor. Citrate is a vital intermediate in cellular metabolism,^[15] has been proposed as a biomarker for prostate cancer,^[16] and low citrate levels in urine are indicated in nephrolithiasis.^[17] Although a range of citrate sensors have been previously developed,^[18] single-component ratiometric sensors that allow quantification of citrate concentration are rare.^[18e,k,l]

Titration of ZnDPA-DPAC with citrate in 1:1 DMSO:H₂O confirmed that citrate elicits a robust ratiometric response, with changes in intensity to both the short and long wavelength emissions (Figure 5A). An inflection point in the emission was reached at ~0.7 equiv. of citrate, indicative of 1:1 sensor: citrate stoichiometry up until this point, followed by the formation of a 1:2 complex at higher equivalents of citrate. Plots of the ratio of ZnDPA-DPAC emissions at either 455/540 or 490/540 nm could

be fitted to a linear calibration curve in the range of 0–0.7 eq of citrate, with $R^2 > 0.98$ (Figure 5B). To assess the robustness of this response, we used ZnDPA-DPAC to quantify the amount of citrate in unknown samples. Following appropriate dilution to the linear range of the calibration, the concentration of aqueous solutions of sodium citrate could be accurately determined with high precision and accuracy (Table S1). The limit of detection (LoD) for a 20 μM solution of ZnDPA-DPAC was 1.9 nM (Table S2).

To demonstrate the versatility of ZnDPA-DPAC, we also applied this sensor to the quantification of aqueous samples of dihydrogen phosphate. In a similar manner to the response to citrate, ZnDPA-DPAC also provides a robust response to the presence of phosphate species in 1:1 DMSO:H₂O (Figures S15, S16). Upon addition of dihydrogen phosphate, a large enhancement of the emission at ~450 nm was observed, and a decrease in intensity of the longer wavelength emission. An isosbestic point was found at 540 nm, indicating 1:1 binding. Quantification of unknown samples of sodium dihydrogen phosphate could be performed with similar precision and accuracy to that obtained for citrate (LoD = 2.1 nM, Tables S1, S2). Hence, ZnDPA-DPAC may be applied for the detection and quantification of a range of anions in aqueous solution.

3. Conclusion

We constructed two water-soluble DPAC-based sensors with ZnDPA binding units: the first VIE-based anion sensors capable of operating in an aqueous solution. Although both sensors form high-affinity interactions with a range of anions, dual emission is observed in solvent mixtures up to ~70% water only, and photostability is impaired in high water content mixtures. A sensor with only a single ZnDPA unit, **monoZnDPA-DPAC**, produced only minor changes in fluorescence intensity in response to anions. In contrast, **ZnDPA-DPAC** bearing two ZnDPA units, provided a ratiometric response to di- and tri-carboxylate and phosphate anions in 1:1 DMSO:H₂O. Citrate displayed a unique response among the carboxylate anions tested. **ZnDPA-DPAC** was successfully used to quantify the concentration of aqueous solutions of citrate and dihydrogen phosphate, with minimal sample preparation. This work demonstrates that VIE-based anion sensors are not limited to use in non-competitive organic solvents and may be applied to highly sensitive and accurate quantification of anions in aqueous media.

Supporting Information

The authors have cited additional references within the Supporting Information.^[19]

Acknowledgments

This work was supported by the Australian Research Council (CE200100012) and facilitated by access to Sydney Analytical, a core research facility at the University of Sydney.

Open access publishing facilitated by The University of Sydney, as part of the Wiley - The University of Sydney agreement via the Council of Australian University Librarians.

Conflict of Interests

The authors declare no conflicts of interest.

Keywords: anion recognition · citrate · DPAC · fluorescent chemosensor · vibration induced emission

- [1] Z. Zhang, G. Sun, W. Chen, J. Su, H. Tian, *Chem. Sci.* **2020**, *11*, 7525.
- [2] W. Huang, L. Sun, Z. Zheng, J. Su, H. Tian, *Chem. Commun.* **2015**, *51*, 4462.
- [3] a) L. Shi, W. Song, C. Lian, W. Chen, J. Mei, J. Su, H. Liu, H. Tian, *Adv. Opt. Mater.* **2018**, *6*, 1800190; b) J. Chen, Y. Wu, X. Wang, Z. Yu, H. Tian, J. Yao, H. Fu, *Phys. Chem. Chem. Phys.* **2015**, *17*, 27658.
- [4] S. Wang, B. Zhou, N. Wang, C. Yu, N. Yang, J. Chen, Q. Wu, C. Zhang, L. Li, W. Huang, *Chin. Chem. Lett.* **2020**, *31*, 2897.
- [5] a) G. Sun, H. Zhou, Y. Liu, Y. Li, Z. Zhang, J. Mei, J. Su, *ACS Appl. Mater. Interfaces* **2018**, *10*, 20205; b) Y. Zhang, Y. Li, H. Wang, Z. Zhang, Y. Feng, Q. Tian, N. Li, J. Mei, J. Su, H. Tian, *ACS Appl. Mater. Interfaces* **2019**, *11*, 39351; c) F. Gu, T. Jiang, X. Ma, *ACS Appl. Mater. Interfaces* **2021**, *13*, 43473.
- [6] a) C. Liang, Q. Huang, H. Zheng, M. Duan, X. Cheng, J. Chen, Q. Li, Z. Zhang, C. Fan, H. Tian, J. Shen, *J. Am. Chem. Soc.* **2024**, *146*, 34341; b) X.-L. Hu, H.-Q. Gan, Q. Li, M. Li, D. Chen, J. L. Sessler, H. Tian, X.-P. He, *J. Am. Chem. Soc.* **2023**, *145*, 8917.
- [7] a) J. Ramos-Soriano, S. J. Benitez-Benitez, A. P. Davis, M. C. Galan, *Angew. Chem.* **2021**, *133*, 17017; b) H. Zhou, J. Mei, Y.-A. Chen, C.-L. Chen, W. Chen, Z. Zhang, J. Su, P.-T. Chou, H. Tian, *Small* **2016**, *12*, 6542; c) W. Chen, C. Guo, Q. He, X. Chi, V. M. Lynch, Z. Zhang, J. Su, H. Tian, J. L. Sessler, *J. Am. Chem. Soc.* **2019**, *141*, 14798; d) G. Sun, J. Pan, Y. Wu, Y. Liu, W. Chen, Z. Zhang, J. Su, *ACS Appl. Mater. Interfaces* **2020**, *12*, 10875; e) Z. Zong, Q. Zhang, D.-H. Qu, *CCS Chem.* **2024**, *6*, 774.
- [8] W. Chen, C.-L. Chen, Z. Zhang, Y.-A. Chen, W.-C. Chao, J. Su, H. Tian, P.-T. Chou, *J. Am. Chem. Soc.* **2017**, *139*, 1636.
- [9] Z. Zhou, D.-G. Chen, M. L. Saha, H. Wang, X. Li, P.-T. Chou, P. J. Stang, *J. Am. Chem. Soc.* **2019**, *141*, 5535.
- [10] S. M. Butler, K. A. Jolliffe, *Org. Biomol. Chem.* **2020**, *18*, 8236.
- [11] a) H. T. Ngo, X. Liu, K. A. Jolliffe, *Chem. Soc. Rev.* **2012**, *41*, 4928; b) C. Bravin, E. Badetti, F. A. Scaramuzzo, G. Licini, C. Zonta, *J. Am. Chem. Soc.* **2017**, *139*, 6456; c) C. Bravin, G. Mason, G. Licini, C. Zonta, *J. Am. Chem. Soc.* **2019**, *141*, 11963.
- [12] H. Wang, Z. Zhang, H. Zhou, T. Wang, J. Su, X. Tong, H. Tian, *Chem. Commun.* **2016**, *52*, 5459.
- [13] X. Li, C. Zhang, C. Wang, W. Ye, Q. Zhang, Z. Zhang, J. Su, Y. Chen, H. Tian, *Chem. Commun.* **2020**, *56*, 2260.
- [14] K. Lu, Y. Wang, H. Zhang, C. Tian, W. Wang, T. Yang, B. Qi, S. Wu, *J. Med. Chem.* **2022**, *65*, 9193.
- [15] P. Icard, L. Poulain, H. Lincet, *Biochim. Biophys. Acta, Rev. Cancer* **2012**, *1825*, 111.
- [16] L. Galey, A. Olanrewaju, H. Nabi, J.-S. Paquette, F. Pouliot, É. Audet-Walsh, *Nat. Rev. Urology* **2024**, *21*, 573.
- [17] J. M. Zuckerman, D. G. Assimos, *Rev. Urol.* **2009**, *11*, 134.
- [18] a) A. Akdeniz, M. G. Caglayan, P. Anzenbacher, Jr., *Chem. Commun.* **2016**, *52*, 1827; b) S. M. Butler, M. Hountondji, S. N. Berry, J. Tan, L. Macia, K. A. Jolliffe, *Org. Biomol. Chem.* **2023**, *21*, 8548; c) L. Fabbri, F. Foti, A. Taglietti, *Org. Lett.* **2005**, *7*, 2603; d) Y. Gao, G. Huang, Z. Ou, Z. Wang, B. Ju, Y. Li, X. Wang, S. Yin, *New J. Chem.* **2015**, *39*, 8948; e) C. G. Mdel, F. Oton, A. Espinosa, A. Tarraga, P. Molina, *Org. Biomol. Chem.* **2015**, *13*, 1429; f) T. Jiang, N. Lu, Y. Hang, J. Yang, J. Mei, J. Wang, J. Hua, H. Tian, *J. Mater. Chem. C* **2016**, *4*, 10040; g) C. Liu, Y. Hang, T. Jiang, J. Yang, X. Zhang, J. Hua, *Talanta* **2018**, *178*, 847; h) Z.-H. Liu, S. Devaraj, C.-R. Yang, Y.-P. Yen, *Sens. Actuators, B* **2012**, *174*, 555; i) A. Metzger, E. V. Anslyn, *Angew. Chem., Int. Ed.* **1998**, *37*, 649; j) A. Metzger, V. M. Lynch, E. V. Anslyn, *Angew. Chem., Int. Ed.* **1997**, *36*, 862; k) D. Parker, J. Yu, *Chem. Commun.* **2005**, 3141; l) K. Rajalakshmi, Y.-S. Nam, M. Selvaraj, Y. Lee, K.-B. Lee, *Sens. Actuators, B* **2018**, *259*, 90; m) M. M. Rhaman, M. H. Hasan, A. Alamgir, L. Xu, D. R. Powell, B. M. Wong, R. Tandon, M. A. Hossain, *Sci. Rep.* **2018**, *8*, 286; n) H. Tavallali, M. A. Karimi, O. Espergham, *Int. J. Environ. Anal. Chem.* **2019**, *99*, 776; o) S. Zhuo, J. Gong, P. Zhang, C. Zhu, *Talanta* **2015**, *141*, 21.
- [19] X. Zhu, H. Du, *Org. Lett.* **2015**, *17*, 3106.

Manuscript received: February 16, 2025

Revised manuscript received: April 3, 2025

Version of record online: April 22, 2025