

Institute of Organic Chemistry
Polish Academy of Sciences

DISSERTATION

in the form of a coherent thematic series of articles published in scientific journals

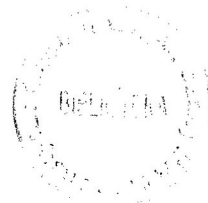
The synthesis and photophysical properties of novel indolizine-based fluorophores

Jaqueline Stella Araujo Badarò M.Sc.

Supervisor: prof. dr hab. Daniel T. Gryko

Warsaw 2025

A-21 A-21-6
K-c-121
K-c-125
K-c-124
K-c-130



B. Onp.
470/26

Biblioteka Instytutu Chemii Organicznej PAN

O-B.470/26



10000000118139

Doctoral thesis completed as part of the project:



UNIA EUROPEJSKA
EUROPEJSKI FUNDUSZ
ROZWOJU REGIONALNEGO



*“New generation of fluorescent probes for stimulated emission depletion
microscopy”*

Realizowanego w ramach programu TEAM

Fundacji na rzecz Nauki Polskiej

Numer grantu: POIR.04.04.00-00-3CF4/16-00-TEAM/2016-3/22



Acknowledgements

The four years I spent in Poland were not only an academic chapter of my life but also a complete experience of growth that allowed me to develop both as a chemist and as a person. For these years and this opportunity, I am immensely grateful to Prof. Daniel T. Gryko, who was for me not only a supervisor but also a mentor, and without whom the life I live today would be very different. He gave me a chance at a time when I had completely lost faith in the future. He always encouraged and supported me when I set ambitious goals, but he also showed understanding when I was too hard on myself and failed to meet my own expectations. I truly looked up to him as a supervisor because he always brought to the table not only his undoubtedly profound scientific knowledge but also his personality, irony, humanity, and compassion—qualities too often overlooked in academia. Working with him was, for me, a truly joyful and inspiring experience.

I am equally grateful to the pillars of Group X—Zenia, Mariusz, Olena, and Beata—for the time and patience they dedicated to teaching me everything I know today about lab experiments, purification and characterization methods, photophysical experiments and calculations, and much more. I also wish to acknowledge everyone else in the group who lent me a hand whenever I struggled with basic, or less basic, chemistry problems.

My heartfelt gratitude goes to Beata once again, as well as to Paula, Łukasz K., Kamil Sz., and Bartek, for sharing with me lab 37 (the best one, if I may say). We talked, laughed, shared; sometimes I even danced and sang, and in that cheerful and sometimes chaotic environment we performed excellent chemistry. This atmosphere led me to an *Angewandte* publication—the best way to close this chapter and proof that productivity has nothing to do with working in a rigid and overly serious environment.

I also wish to express my sincere appreciation to all the collaborators of my published papers: Dr. B. Koszarna and M. Perkowska for their help in the synthesis of the compounds; Dr. M. H. E. Bousquet, Prof. D. Jacquemin, and Dr. I. Deperasińska for the theoretical calculations; Dr. E. T. Ouellette for the single-crystal X-ray diffraction (SCXRD) measurements; Dr. O. Morawski for the fluorescence decay experiments; Dr. A. Wrzosek, Prof. A. Szewczyk, D. V. Kolygina, J. Park, K. Kandere-Grzybowska, and Prof. B. Grzybowski for confocal imaging of the fluorescent compounds in living cells; and finally, B. Godlewski for joining me in writing the review and, once again, Prof. D. T. Gryko for supervising all my work.

I gratefully acknowledge FNP-TEAM for funding my PhD, the MSCA for allowing me to spend some time in Malaysia, and NAWA for supporting me during a research visit in France. Most importantly, I also thank all IChO members—especially the administration and analysis teams—for their daily commitment and assistance, which allow research to run smoothly.

To all of you mentioned above: this thesis and my publications exist only because of your support.

I am deeply grateful also to the friends and colleagues who shared even a small part of this journey with me: Gana and Katia for being my PhD buddies; Ahmad and Yasemin for being among the first friends I made; Meme, Grace, and Franco for becoming good friends in such a short time; my friends

in Warsaw: Arturo, Vishali, Deepshikha, Shantanu, Souvik, and Mohad for the joyful moments together; Paola, Emiliano, Monica, and Debora for allowing me to always have a bit of Italy with me in Poland; and last, but most importantly, Kitty, for being the first person I met at IChO and, since day one, my best friend.

Finally, I am grateful to those who, even from afar, never felt distant: my friends in Italy—Miriam, Elisabetta, Ludovica, Silvia, Vanessa, Fabrizio and Gabriele; my family in the USA and Brazil, especially my parents, for always supporting me; and Federico, for being my person and feeling so close no matter how far.

Abstract in English

In the quest to develop small fluorescent molecules for bioimaging applications, this work focused on a deeper understanding of π -expanded indolizines; the improvement of existing pathways for the synthesis of known cores; the development of novel strategies for new ones; the rationalization of the relationship between substituents and photophysical properties; and, finally, the investigation of promising candidates for bioimaging applications.

Around this topic, three articles and a review were published, all of which are included in this dissertation.

The first project I worked on led to the publication "*The Kröhnke synthesis of benzo[a]indolizines revisited: towards small, red light emitters*", in which I described the improvement of the synthetic methodology and the broadening of the scope for obtaining **benzo[a]indolizines**, a class of π -expanded indolizines first described by Kröhnke in the 70s, and the rationalization of their photophysical properties in relation to the different substituents introduced.

Kröhnke described the synthesis of benzo[a]indolizines starting from pyridinium salts and 1-chloro-2,4,6-trinitrobenzene in two steps, using first an inorganic base, K_2CO_3 in $CHCl_3$, followed by an organic base, piperidine, in DMSO. This methodology led to a limited number of products in relatively low yield.

The first change I implemented over the proposed path was the substitution of the highly explosive 1-chloro-2,4,6-trinitrobenzene with the less hazardous 4-chloro-3,5-dinitrobenzene derivatives, yielding three products in 20–30% yield. The second was the identification of a more suitable combination of base and solvent to avoid the isolation step of the intermediate. After a series of attempts using different bases and solvents to enhance the yields, I was able to reduce the number of synthetic operations, increase the yields, and accelerate the reactivity of the pyridinium salt and the 4-chloro-3,5-dinitrobenzene derivatives by employing Cs_2CO_3 and sulfolane. This led to the isolation of eleven different products in yields ranging from 5 to 80%. Further functionalization afforded two additional derivatives.

Once a more efficient synthetic pathway was established, attention was turned to the photophysical investigation. The analysis of the photophysical properties based on the different substituents and their positions allowed for a rationalization of the structure-photophysics relationship of this class of fluorophores (fig 1).

In the second project I followed the same general approach and worked on optimizing the methodology for the synthesis of **2-oxo-2H-pyrano[2,3-b]indolizine-3-carboxylates**, first proposed by Kakehi in the 90s, and on investigating their photophysical properties. This work led to the following publication: "*Strongly fluorescent indolizine-based coumarin analogs*".

Starting from pyridinium salts and diethyl 2-(ethoxymethylene)malonate Kakehi obtained products in one-pot reaction after one week using K_2CO_3 as base in EtOH. I instead switched the base to Cs_2CO_3 and used a diverse array of pyridinium salts, preparing 8 products in 5-50% yield. Further functionalization was performed, and five additional structures were obtained.

With a plethora of 2-oxo-2H-pyrano[2,3-b]indolizine-3-carboxylates available, the photophysical investigation and rationalization of the fluorescent properties was carried out (representatives displayed in fig. 1). Among those, the styryl dye was selected for H9c2 cell line staining and demonstrated, after permeabilization with digitonin, selective localization in the nuclei.

The last project extended beyond the improvement and expansion of previous works and resulted in the discovery a completely new class of fluorophores which culminated with recently published article: *The Hybrid of Indolizine and Merocyanine – A New Class of Organelle-specific Dyes*.

In this work, starting from indolizin-ol intermediates and 2,3,5,6-tetrafluoro-4-hydroxybenzaldehyde in xylene, I obtained six hybrids polymethine dyes which I called **indolizine-merocyanines (IndMers)**, in a one-pot procedure with yields ranging from 26-90%. The developed methodology was also tested with different aldehydes of similar characteristics, generating two additional heretofore structures: a V-shaped xanthene dye and a cyanine type dye.

A comprehensive photophysical investigation was carried out on the obtained structures (representatives are shown in fig. 1) providing a broad understanding of their structure-photophysics relationship. In addition, for some selected structures, photostability and solubility in aqueous media were investigated.

Among those compounds one showed emission in the NIR region, solubility in aqueous media, high cell permeability in HT1080 fibrosarcoma and MRC5 normal human lung fibroblast cells, and context dependent accumulation in mitochondria and RNA rich nucleoli when compared to MitoTracker™ Green FM and SYTO RNASelect™ respectively suggesting our success in developing a new dye for bioimaging.

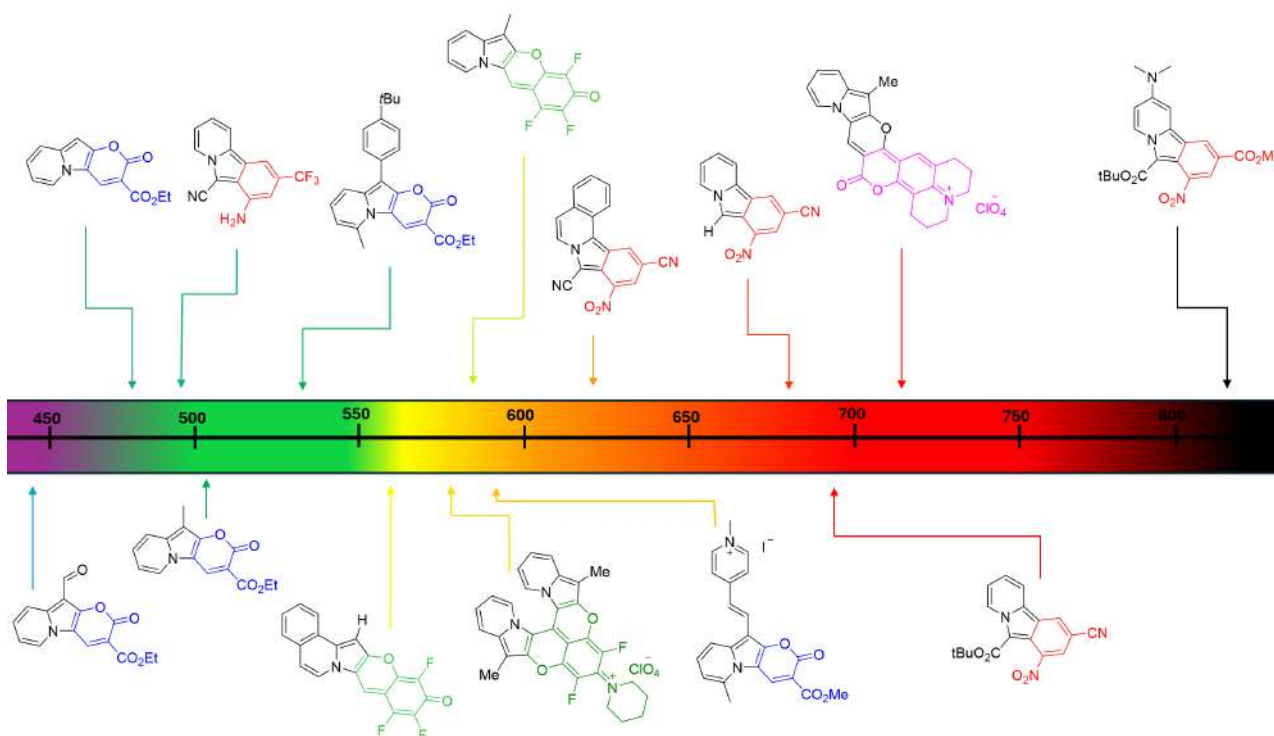


Figure 1: Structure and visual comparison of the fluorescence emission of some representatives of benzo[a]indolizines, pyrano[b]indolizines and IndMers in DCM.

Streszczenie w języku polskim

Celem niniejszej rozprawy doktorskiej jest pogłębienie wiedzy dotyczącej indolizyn o rozszerzonym układzie sprzężonym π , traktowanych jako potencjalni kandydaci do zastosowań w bioobrazowaniu. Zakres badań obejmował zarówno udoskonalanie istniejących ścieżek syntezy znanych rdzeni indolizynowych, jak i opracowanie nowych strategii syntetycznych prowadzących do wcześniej nieopisanych struktur. Szczególny nacisk położono na racjonalizację wpływu podstawników na właściwości fotofizyczne otrzymywanych związków, co umożliwiło identyfikację struktur o szczególnie obiecujących właściwościach do dalszych zastosowań biologicznych. Przedstawione wyniki stanowią istotny wkład w rozwój chemii indolizyn oraz w projektowanie nowych sond fluorescencyjnych dla celów bioobrazowania.

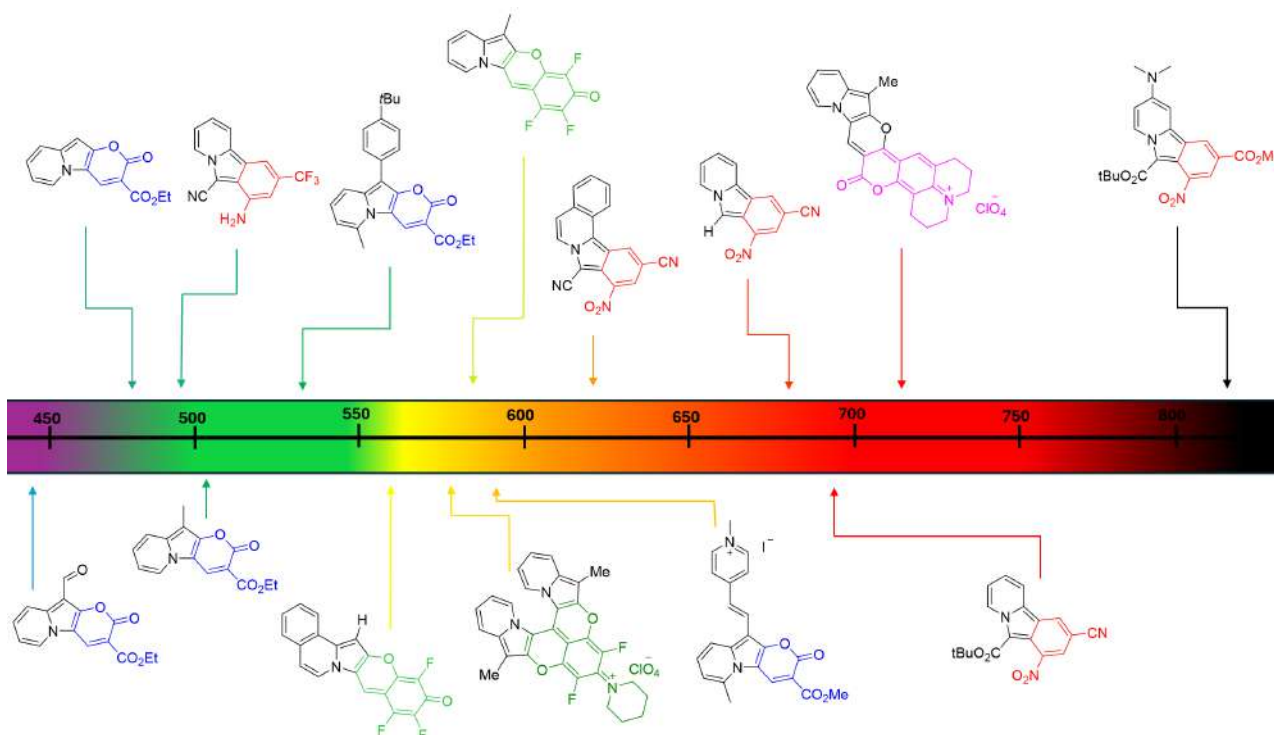
W ramach tego tematu opublikowano trzy artykuły oraz jedną pracę przeglądową, które zostały w całości włączone do niniejszej dysertacji.

Pierwszy projekt, którym się zajmowałam, zaowocował publikacją: „The Kröhnke synthesis of benzo[a]indolizines revisited: towards small, red light emitters”, w której opisałam zoptymalizowaną metodologię syntezy oraz rozszerzyłam bibliotekę **benzo[a]indolizyn**. Przedstawiłam również racjonalizację ich właściwości fotofizycznych w zależności od wprowadzonych podstawników. Synteza benzo[a]indolizyn – klasy indolizyn o rozszerzonym układzie sprzężonym π – została po raz pierwszy opisana przez Kröhnkego w latach 70. Opierała się ona na dwuetapowej reakcji soli pirydyniowych z 1-chloro-2,4,6-trinitrobenzenem, w której pierwszy etap prowadzono w CHCl_3 z użyciem nieorganicznej zasady K_2CO_3 , a drugi w DMSO z piperydyną pełniącą rolę zasady. Metodologia ta ograniczała się do otrzymania kilku produktów ze stosunkowo niskimi wydajnościami. Pierwszą modyfikacją, którą wprowadziłam w syntezie, było zastąpienie silnie wybuchowego 1-chloro-2,4,6-trinitrobenzenu mniej niebezpiecznymi pochodnymi 4-chloro-3,5-dinitrobenzenu, co pozwoliło mi uzyskać trzy produkty z wydajnościami na poziomie 20–30%. Drugą istotną zmianą było opracowanie bardziej odpowiedniego układu zasada–rozpuszczalnik, eliminującego konieczność izolowania związku pośredniego. Po przeprowadzeniu szeregu prób z różnymi zasadami i rozpuszczalnikami, dzięki zastosowaniu Cs_2CO_3 w sulfolanie, udało mi się zmniejszyć liczbę etapów syntetycznych, zwiększyć wydajności oraz skrócić czas reakcji soli pirydyniowej z pochodnymi 4-chloro-3,5-dinitrobenzenu. Pozwoliło mi to wyizolować jedenaście różnych produktów w wydajnościach od 5% do 80%, a dalsza funkcjonalizacja umożliwiła otrzymanie dwóch dodatkowych pochodnych. Po opracowaniu bardziej efektywnej ścieżki syntetycznej skupiłam się na badaniach właściwości fotofizycznych otrzymanych związków. Analiza wpływu różnych podstawników oraz ich położenia na właściwości fotofizyczne pozwoliła mi zracjonalizować zależności pomiędzy strukturą a fotofizyką tej klasy fluoroforów (rys. 1).

W drugim projekcie skupiłam się na optymalizacji metodologii syntezy estrów **2-okso-2H-pirano[2,3-b]indolizyno-3-karboksylowych**, zaproponowanej po raz pierwszy przez Kakehiego w latach 90., oraz na zbadaniu właściwości fotofizycznych otrzymanych pochodnych. Prace te doprowadziły do publikacji pt. „Strongly fluorescent indolizine-based coumarin analogs”. Kakehi przeprowadził reakcję wybranych soli pirydyniowych z dietylo-2-(etoksymetyleno)malonianem w EtOH, stosując K_2CO_3 jako zasadę. Zastępując węglan potasu Cs_2CO_3 , udało mi się skrócić czas reakcji, a stosując zróżnicowany zestaw soli pirydyniowych, uzyskałam osiem produktów o wydajnościach 5–50%. Następnie przeprowadziłam dalszą funkcjonalizację, otrzymując pięć dodatkowych struktur. Dysponując szeroką gamą estrów 2-okso-2H-pirano[2,3-b]indolizyno-3-karboksylowych, przeprowadziłam badania fotofizyczne oraz racjonalizację ich właściwości fluorescencyjnych (przykłady pokazano na rys. 1). Wśród

nich barwnik styrylowy został wybrany do barwienia linii komórkowej H9c2 i wykazał, po permeabilizacji digitoniną, selektywną lokalizację w jądrach komórkowych.

Ostatni projekt wykraczał poza udoskonalanie i rozwijanie wcześniejszych prac, prowadząc do odkrycia zupełnie nowej klasy fluoroforów. Wyniki tej pracy zostały opisane w publikacji pt. „The Hybrid of Indolizine and Merocyanine – A New Class of Organelle-specific Dyes”. W ramach tego projektu przeprowadziłam reakcję „one pot” indolizynoli, otrzymanych z soli pirydyniowych, z 2,3,5,6-tetrafluoro-4-hydroksybenzaldehydem w ksylenie, uzyskując sześć hybrydowych barwników polimetynowych (z wydajnościami 26–90%), które **nazwałam indolizyno-merocyjaninami (IndMers)**. Opracowaną metodologię rozszerzyłam również na reakcję z innymi aldehydami o podobnych właściwościach, uzyskując dwie nowe, dotychczas nieopisane struktury: barwnik ksantenowy w kształcie litery V oraz barwnik typu cyjanina. Dla otrzymanych struktur przeprowadziłam kompleksowe badania fotofizyczne (przykłady przedstawiono na rys. 1), które pozwoliły ustalić zależności między strukturą a właściwościami fotofizycznymi tych związków. Ponadto, dla wybranych struktur zbadalam fotostabilność i rozpuszczalność w środowisku wodnym. Co ważne jeden z otrzymanych związków charakteryzował się szczególnie korzystnymi właściwościami, w tym emisją w zakresie NIR, rozpuszczalnością w wodzie, wysoką przenikalnością do komórek HT1080 (fibrosarcoma) oraz MRC5 (normalne ludzkie fibroblasty płucne), a także zależną od kontekstu akumulacją w mitochondriach i jąderkach bogatych w RNA (w porównaniu odpowiednio z MitoTracker™ Green FM i SYTO RNaselect™). Świadczy to o tym, że udało mi się opracować nowy barwnik do bioobrazowania.



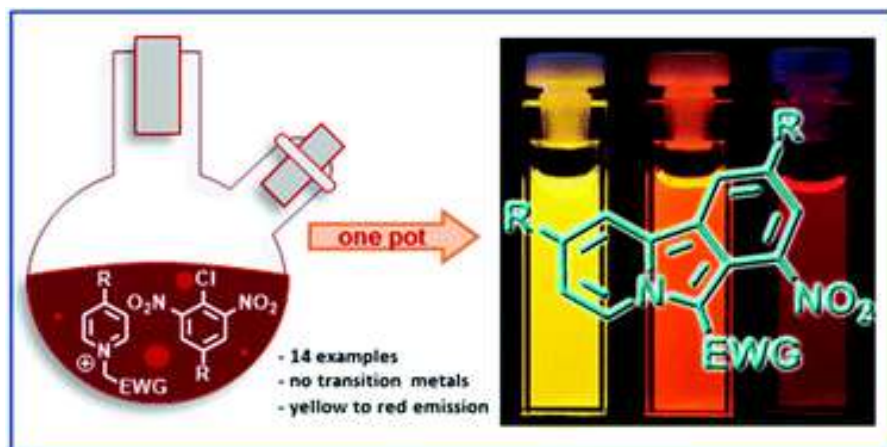
Rysunek 1. Struktura i wizualne porównanie emisji fluorescencyjnej wybranych przedstawicieli benzo[a]indolizyn, pirano[b]indolizyn oraz IndMerów w DCM.

Table of Contents:

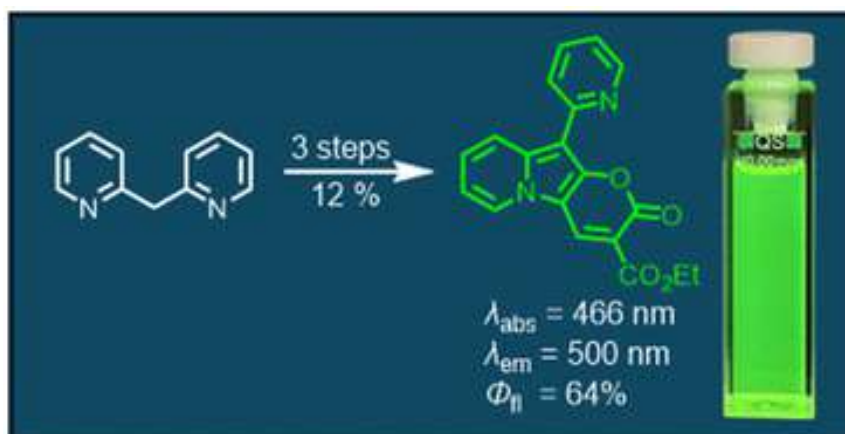
List of Publications for the Doctoral Dissertation	10
Participation in Conferences	11
Aims and Objectives	12
1. Introduction	14
1.1. Indolizines	14
1.2. Reactivity of indolizines	16
1.3. π -Expanded indolizines	17
1.4. π -Extended indolizines	20
2. Results and Discussion	22
2.1. Synthesis and Photophysical Properties of Substituted Benzo[a]indolizines	22
2.2. Synthesis and Photophysical Properties of Substituted Pyrano[2,3-b]indolizin-2-ones	28
2.3. Synthesis and Photophysical Properties of Substituted Indolizine-merocyanines (IndMer)	33
3. Summary and Conclusions	37
References	39
Original Publications and Authors' Contribution Declarations	44

List of Publications for the Doctoral Dissertation

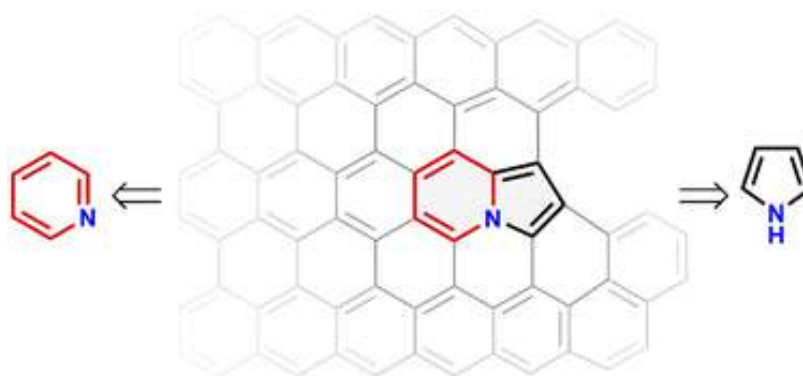
1. **Badaro, J. S. A.**; Koszarna, B.; Bousquet, M. H. E.; Ouellette, E. T.; Jacquemin, D.; Gryko, D. T. 'The Kröhnke synthesis of benzo[a]indolizines revisited: towards small, red light emitters', *Org. Chem. Front.* 2022, 9, 1861-1874. <https://doi.org/10.1039/D2QO00097K>



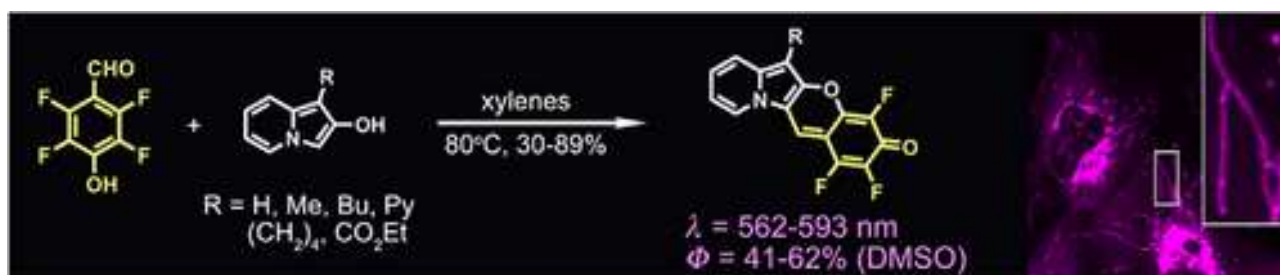
2. **Badaro, J. S. A.**; Wrzosek, A; Morawski, O.; Szewczyk, A; Deperasinska, I; Gryko, D. T. 'Strongly fluorescent indolizine-based coumarin analogs' *Org. Chem. Front.*, 2024, 11, 6627-6641. <https://doi.org/10.1039/D4QO01216J>



3. **Badaro, J. S. A.**; Godlewski, B.; Gryko, D. T., 'Advances in the synthesis of indolizines and their π -expanded analogues: update 2016-2024', *Org. Chem. Front.*, 2025, 12, 2860-2907. <https://doi.org/10.1039/D4QO02082K>



4. **Badaro, J. S. A.**; Koszarna, B.; Perkowska, M.; Kandere-Grzybowska, K.; Kolygina, D. V.; Morawski, O.; Park, J.; Grzybowski, B.; Deperasinska, I.; Gryko, D. T., 'The Hybrid of Indolizine and Merocyanine - A New Class of Organelle-specific Dyes', *Angew. Chem. Int. Ed.*, 2025, e202508044. <https://doi.org/10.1002/ange.202508044>



Participation in Conferences

The 19th international symposium on novel aromatic compounds (ISNA 2019)
 Warsaw, 3rd-8th July 2022.

'The Kröhnke synthesis of benzo[*a*]indolizines revisited: towards small, red light emitters'

Aims and Objectives

A cell is a key functional unit of life. All forms of life rely on cellular processes to maintain normal functions, and changes in cell function induced by metabolic disturbances, physicochemical damage or infection may cause disease. Along with advances in fluorescence microscopy and *in-vitro* cell culture, live-cell imaging has become an essential tool in modern biology for the study of molecular and cellular events by means of specifically targeted fluorescent labels and sensors. Fluorescence imaging has a wide range of benefits not offered by other imaging modalities, derived from its combination of high specificity, high sensitivity, and high spatio-temporal resolution. Fluorescent dyes are widely used as indispensable markers and sensors in biology-related optical microscopy. At the same time, current dyes do not perfectly fulfill many requirements for use in fluorescence microscopy. They lack one or more of the following properties: (1) photostability; (2) large Stokes shift; (3) large fluorescence quantum yield (particularly in the near-infrared [NIR] region) and (4) cell permeability.

As far as fluorescence microscopy is concerned, cell biologists are entirely focused on rhodamines, coumarins, fluoresceins, cyanines and BODIPYs mostly because of reasonable combination of favorable photophysical properties.^[1] My central hypothesis was that small electron-rich aromatic heterocycle, indolizine, can be a suitable platform to prepare alternative dyes with suitable combination of photophysical properties. In the quest for developing small fluorescent molecules for bioimaging applications, indolizines, despite being discovered more than 100 years ago,^[2] were set aside. According to PubMed, the peak in the number of publications on indolizines was reached in 2012 (around 1500 articles). In contrast, in the same year, publications on fluoresceins nearly doubled those on indolizines and has continued to grow to date.^[3] This discrepancy has created a vicious cycle: the limited development of indolizine scaffolds and the insufficient fine-tuning of their properties have restricted their adoption in bioimaging, which in turn has further discouraged efforts to optimize them.

Several factors contributed to this stagnation^[4]

1) Photophysical limitations

Many indolizines absorb and emit in the blue region of the UV spectra, which is not ideal for bioimaging due to strong background autofluorescence, limited tissue penetration and potential photodamage. Additionally, their quantum yield and photostability tend to be inferior compared to those of established fluorophores.

2) Chemical stability

The large electron density of indolizines makes them highly chemically reactive, allowing them to undergo multiple reactivity pathways as described in §1.2 and shown in Scheme 4. This reactivity undermines their stability in biological environments compared to more robust dye families.

3) Synthetic accessibility and tunability

Although indolizines have been known for more than a century, their synthetic chemistry remains underdeveloped. Routes to expanded indolizines are especially scarce, as they typically rely on pre-formed expanded pyridines or pyrroles, which restricts structural diversity and limits opportunities for systematic fine-tuning.

4) Limited commercial development and biological applications assessment

Because the field has long been dominated by BODIPYs, cyanines, rhodamines, and fluoresceins, indolizines and their expanded analogues received little attention. This lack of investment left key practical issues, such as aqueous solubility and stability under physiological conditions, largely unresolved, further discouraging their adoption in bioimaging.

The general aim of my PhD research was to address these limitations and expand the understanding of indolizine as fluorescent scaffold, with the ultimate goal of improving their 'photophysical performance' and bioimaging applicability. Along these lines my work focused on π -expanded indolizines which intrinsically exhibit bathochromically shifted absorption/emission and enhanced stability due to greater electron delocalization. At the same time they maintain the appealing features of the parent indolizine: small size, rigidity, electron density and the ability to form push-pull systems through fine-tuned substituents.

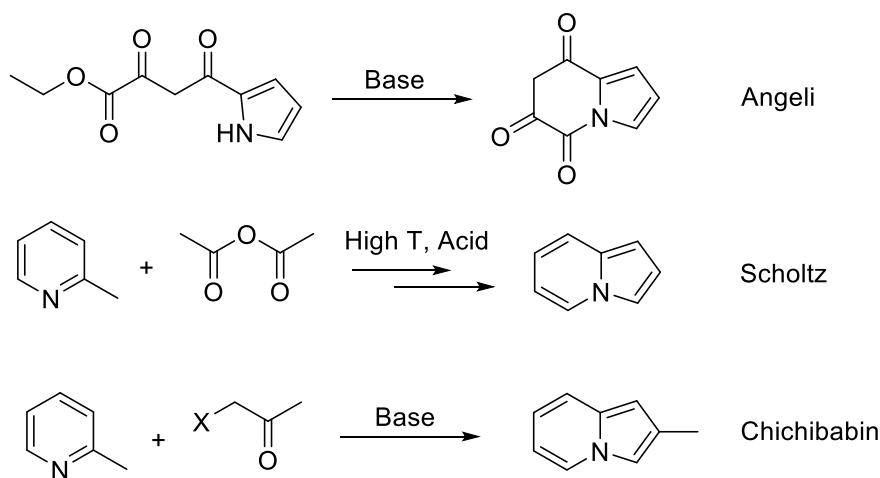
Specific objectives of my thesis were:

- 1) To expand the knowledge and understanding of π -expanded indolizines by improving existing synthetic methodologies and developing new synthetic paths for the synthesis of two classes: benzo[*a*]indolizines and pyrano[2,3-*b*]indolizines, both reported in the last century as promising fluorophores, but obtained in low yield and with limited scope and characterization.
- 2) To conduct a comprehensive study of the absorption, emission and stability profiles, therefore building a foundation for their rational design, guided structural modification and optimization through tailored substituents.
- 3) To develop a new class of polymethine dyes possessing indolizine as an electron donor i.e. replacing classical dialkylamino group in merocyanine type of structures with indolizine. Consequently, I aimed at entirely new type of π -expanded indolizine dyes, leveraging on the insights acquired during the realization of the first two objectives. Simultaneously my goal were functional dyes with properties compatible with fluorescence bioimaging requirements.

1. Introduction

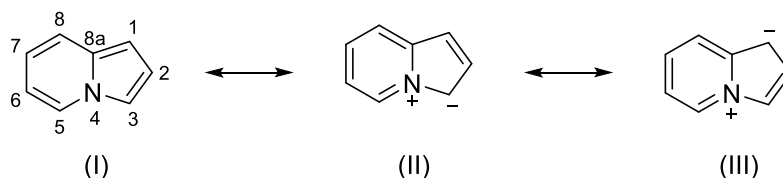
1.1. Indolizines

The first indolizine derivative was synthesized by A. Angeli in 1890, *via* the intramolecular cyclization of the ethyl pyrrolyl pyruvate under basic condition, and he suggested that the fully unsaturated system, later isolated by Scholtz^[5], should be named “pyrindole” as it is an isomer of the already known indole.^[2] However, it was only with the methodology proposed by Chichibabin in the early 20th century that indolizines, also known as “pyrrolo[1,2-*a*]pyridine”, started to become more popular.^[6] He developed a base-mediated, straightforward, versatile and efficient method starting from α -picoline and α -halogenated ketones that became the go-to strategy for indolizines synthesis for decades and is up to now the most known and used (Scheme 1).



Scheme 2: First synthesis of indolizines

Structurally, indolizines have a 10π delocalized electron system composed of an electron-rich pyrrole-type five-membered ring and an electron-deficient six-membered ring. Different naming and numbering systems were used since their discovery, but for this Thesis the name “indolizine” will be used and the numbering system adopted by the American Chemical Society will be the standard. Unsubstituted indolizine have a resonance energy of 0.29 kcal/mol and is best represented by a resonance hybrid of three structures (I), (II), and (III) (Scheme 2).^[7]



Scheme 3: Numbering and resonance structures of indolizine

Although not naturally occurring in their aromatic form, these compounds have been encountered in a few cases as π -expanded derivatives. More commonly, however, they are found as saturated alkaloids isolated from plants, animals, and microbes, where they exhibit potent biological activity (Figure 1).^[7–12]

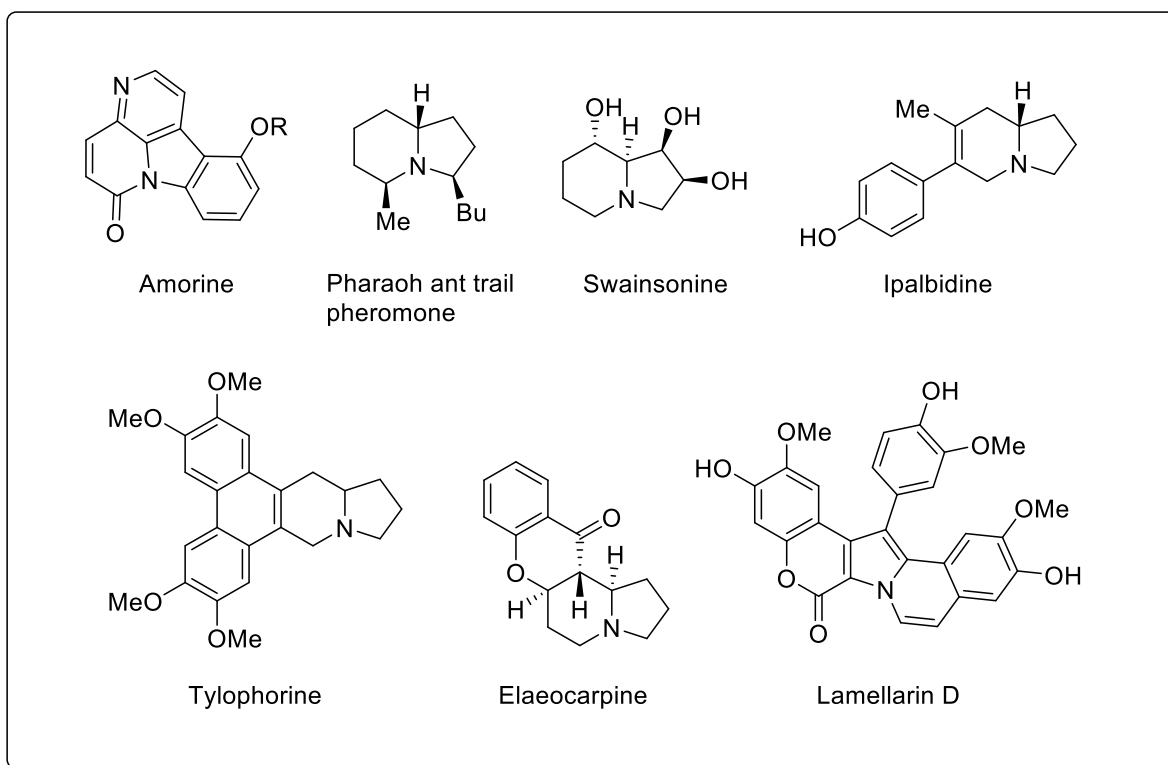
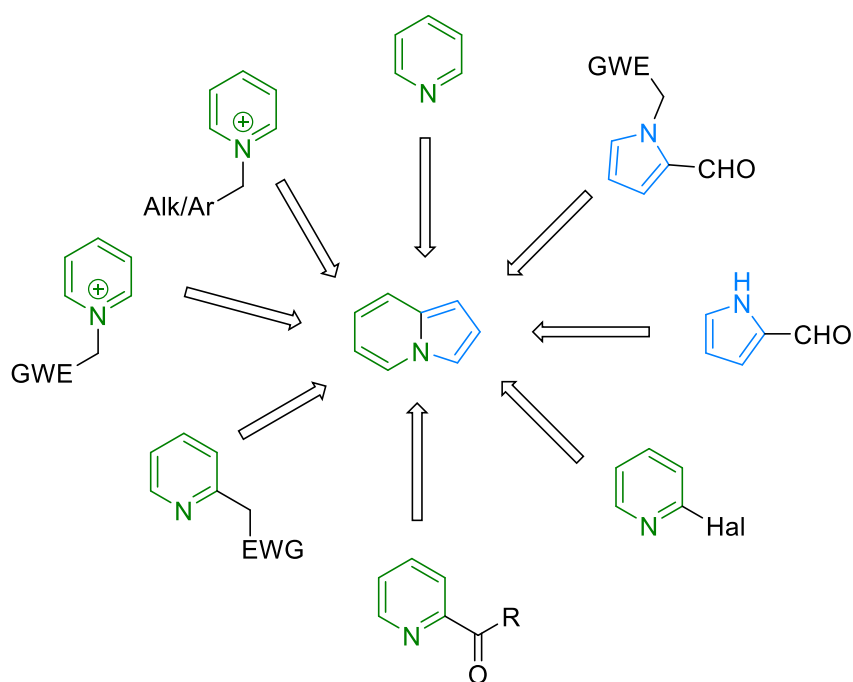


Figure 1: Naturally available partially or fully saturated indolizines

Typical methodologies for their synthesis can be classified into methods starting from Pyridine, and methods starting from pyrroles (Scheme 3), the latter being the least explored and only recently more popular. This is because pyrrole-based building blocks face significant challenges due to their higher cost, lower availability, and reduced stability. Despite these limitations, their unique reactivity and potential provide promising opportunities for indolizine synthesis in a fashion complementary to the classic pyridine-based methods.^[13–25]

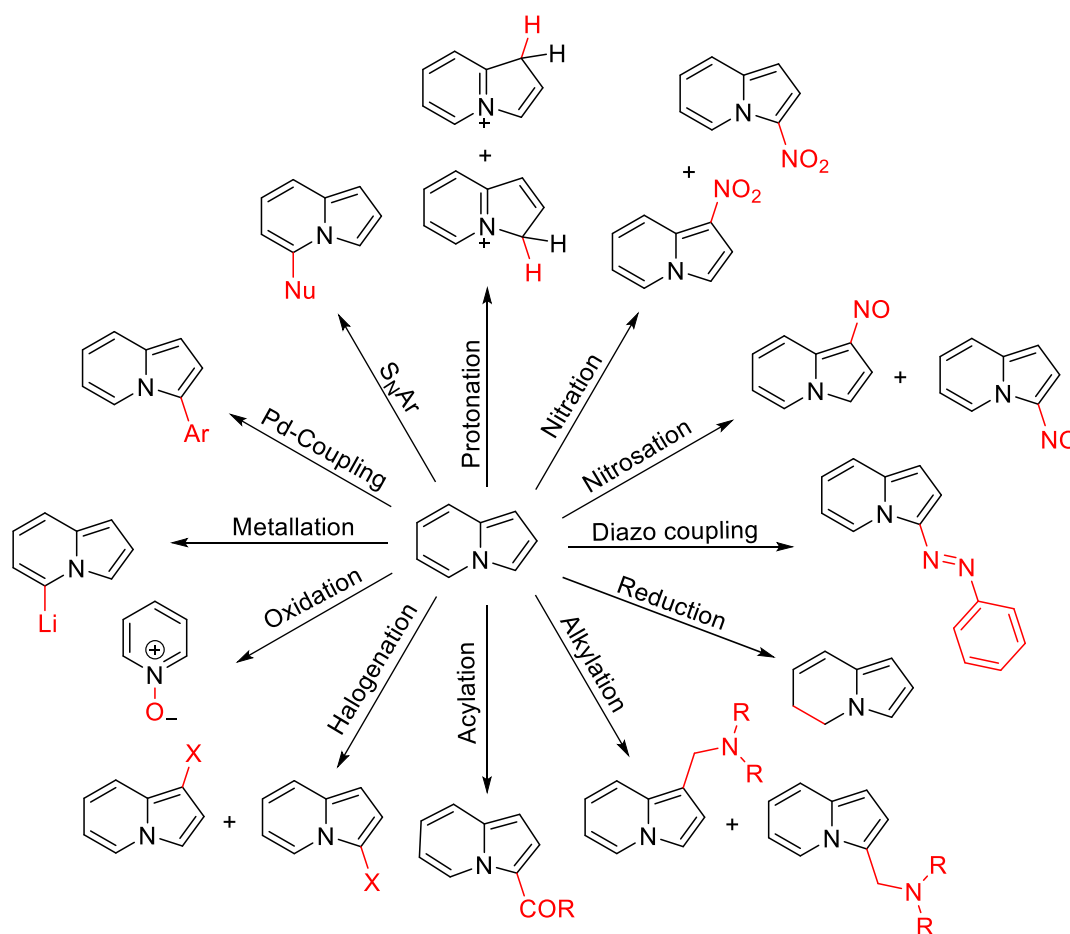


Scheme 3: Indolizine synthesis from different pyridines and pyrroles

1.2. Reactivity of indolizines

Indolizines exhibit sensitivity to air and light. They demonstrate high reactivity *via* electrophilic substitution and infrequently could also undergo nucleophilic attack. They produce a colorful reaction with Ehrlich's reagent and are highly fluorescent.^[26] Density Functional Theory (DFT) calculations indicate that the highest occupied molecular orbital (HOMO) is situated on the pyrrole-type ring, with the highest electron density located at C-3, making it the preferred site for electrophilic attack, followed by C-1.^[27] Resonance structures corroborate these findings, with structure II (Scheme 2) being the more stable configuration due to lower energy charge separation. Conversely, the lowest unoccupied molecular orbital (LUMO) is positioned on the pyridine-type ring.

Classical reactions occurring at C-1 and/or C-3 were well documented in the last century and have been further developed recently. These include protonation,^[28–30] nitration,^[31,32] nitrosation,^[32,33] and diazo coupling,^[32] as well as alkylation,^[34–36] acylation,^[37–39] and halogenation.^[40–42] Oxidation^[43,44] and reduction^[5,45,37,46] reactions are also feasible. However, oxidation typically results in ring opening and "decomposition," while reduction leads to a saturated system crucial for alkaloid synthesis. Coupling reactions at position C-3 are possible via usually palladium-catalyzed coupling.^[27] Metalation of indolizines^[47,48] serves as a viable functionalization approach, permitting the introduction of various substituents at C-5, including halogens and providing access to less common substituted indolizines.^[48] Additionally, reactivity at C-5 can involve a nucleophilic attack if an electron-withdrawing group is present and functions as a leaving group.^[49]



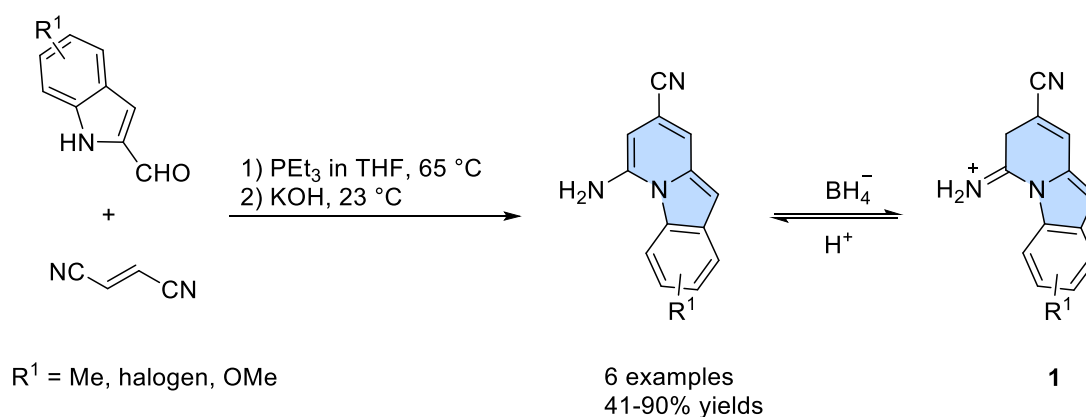
Scheme 4: Reactivity of indolizines

1.3. π -Expanded indolizines

As previously mentioned, expanded indolizines have been discovered in nature and have demonstrated promising antibiotic and cytotoxic activities, both in their natural environment and in medical applications.^[50] In addition to their biological relevance, these compounds exhibit intriguing photophysical properties, making them excellent candidates for development in photoluminescent materials^[51,52], solar cells^[53], and fluorescent dyes for bioimaging.^[54,55] From a synthetic point of view, in many cases the π -expansion is achieved simply by using quinolines^[56–58] or isoquinolines^[59–62] as starting materials. However, in the context of this introductory chapter, attention will be focused on more structurally complex and less conventional systems, those more closely related to the scaffolds studied in my own research. Particular emphasis will be placed on their photophysical behavior, which will later be critically compared with the properties of the indolizines developed in this work. The extensive overview of the recent developments in the synthesis of π -expanded indolizines can be found in the review of which I am co-author and which is part of this PhD-Thesis.

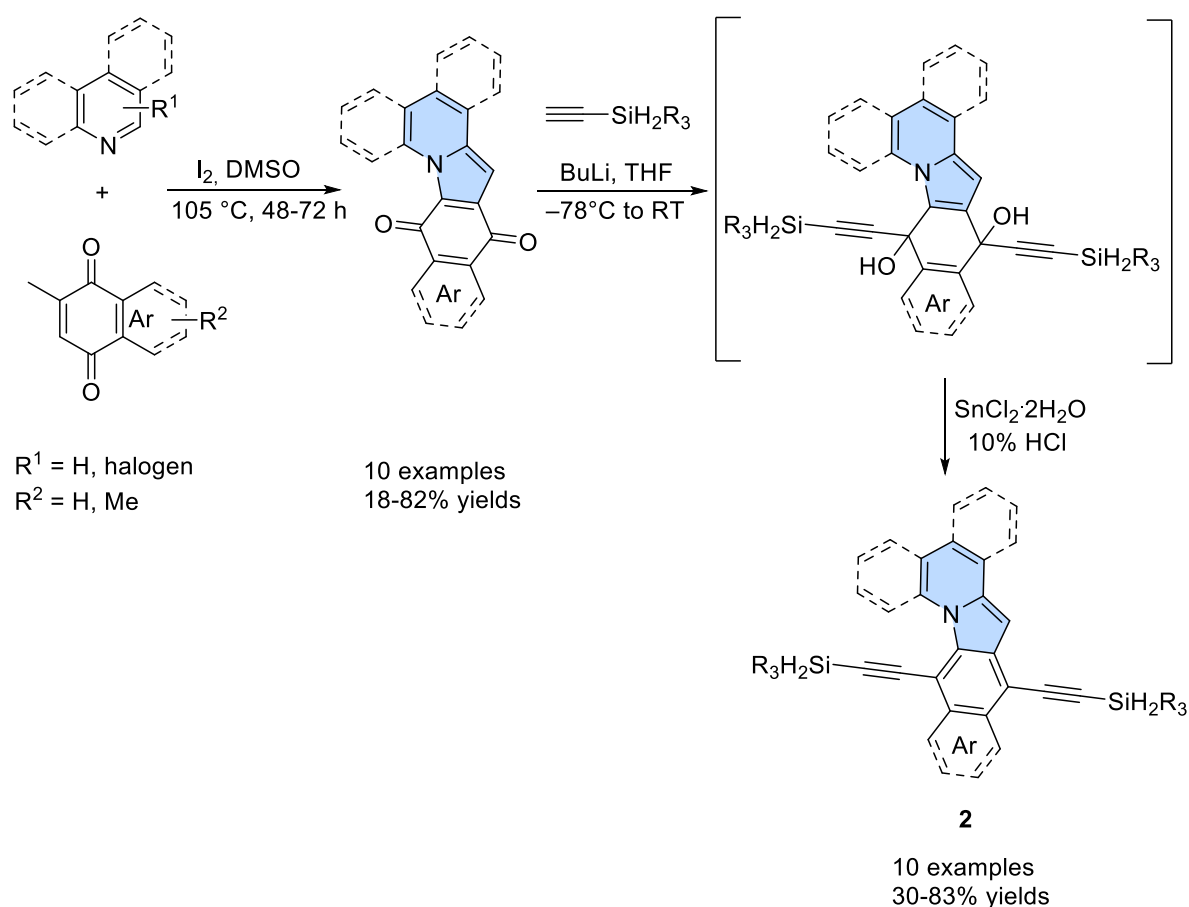
1.3.1. Benzo-indolizines

In 2016, Outlaw *et al.* developed a new method to synthesize 6-amino-8-cyanobenzo[1,2-*b*]indolizines from substituted indoles and fumaronitrile *via* a Wittig mechanism followed by deprotonation of the indole N-H (Scheme 5).^[63] This strategy promotes cyclization towards the formation of indolizines rather than carbazoles and works well with both electron-donating and electron-withdrawing groups at the 4-position of the indole. A comparison of the optical properties of these newly synthesized 6-amino-8-cyanobenzo[1,2-*b*]indolizines (**1**) with those of 6,9,10-trimethyl-pyrido[1,2-*a*]indole, previously synthesized by Saxton *et al.* in the 1950s,^[64] reveals a bathochromic shift in absorption. This red-shift is attributed to the presence of amino and cyano substituents in the benzoindolizine system. Compounds absorb light at 270, 340, and 440 nm, and emit fluorescence between 490 and 520 nm, with an average fluorescence quantum yield of 10%, while trimethyl-pyrido[1,2-*a*]indole displays peaks at 210 and 320 nm. Furthermore, the 6-amino-8-cyanobenzo indolizines demonstrate pH-sensitive fluorescence: protonation breaks aromaticity and causes a hypsochromic (blue) shift in emission. Upon the addition of trifluoroacetic acid (TFA), a new equilibrium leads to increased emission at 440 nm and a higher quantum yield of 20%, corresponding to a non-aromatic protonated form.



Scheme 5: Synthesis of pH-sensitive benzo[1,2-*b*]indolizines

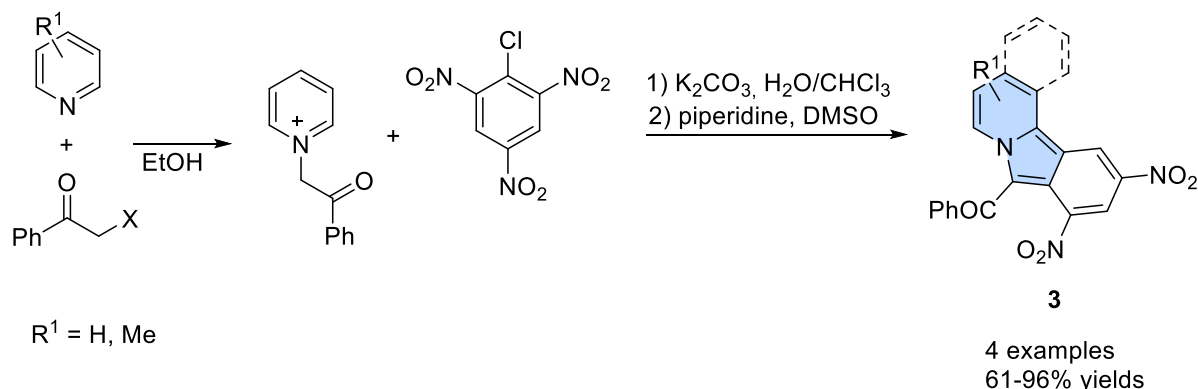
Even more expanded and red shifted benzo[1,2-*b*]indolizine derivatives (**2**) were recently reported by Gawel and co-workers, who proposed an improved method for synthesizing indolizinequinones^[51] from methylquinones, iodine, and pyridine derivatives in DMSO, building upon Delcanale's earlier protocol.^[65,66] The resulting indolizinequinones were further derivatized in a one-pot process to produce substituted π -expanded indolizines. The synthetic route involves the initial addition of acetylene derivatives to quinone *via* a *n*-butyllithium-mediated reaction, forming an intermediate that then undergoes reductive elimination using SnCl₂ in 10% aqueous HCl (Scheme 6). The final compounds display notable photophysical properties, with absorption and emission maxima in the visible region. The most pronounced bathochromic shift was observed for a π -extended system with six fused aromatic rings ($\lambda_{\text{abs}} = 594 \text{ nm}$, $\lambda_{\text{em}} = 621 \text{ nm}$, $\Phi_{\text{fl}} = 17\%$).



Scheme 6: Synthesis of π -expanded indolizines from quinones.

Long before the recent developments in benzo[1,2-*b*]indolizines, a different structural isomer, benzo[*a*]indolizine (**3**), had already been explored. In 1966, Kröhnke *et al.*, described the synthesis of these red fluorescent derivatives using a three-step methodology (Scheme 7).^[67] The sequence begins with the quaternization of pyridine using (Hal)CH₂(EWG) reagents, followed by a nucleophilic aromatic substitution (S_NAr) of the resulting pyridinium salts with 1-chloro-2,4,6-trinitrobenzene, and concludes with a 1,5-electrocyclization, which forms the five-membered central ring. The mechanism of the last key step relies on the formation of vinylpyridinium ylides which are prone toward 1,5-electrocyclization. Notably, despite the structural similarity to benzo[*b*]indolizines,

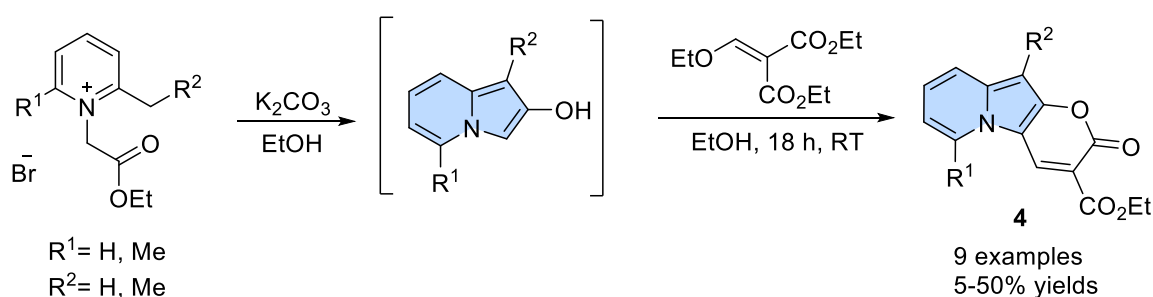
benzo[*a*]indolizines have remained relatively unexplored and were only recently brought back into focus.



Scheme 7: Synthesis of benzo[*a*]indolizines from picryl chloride

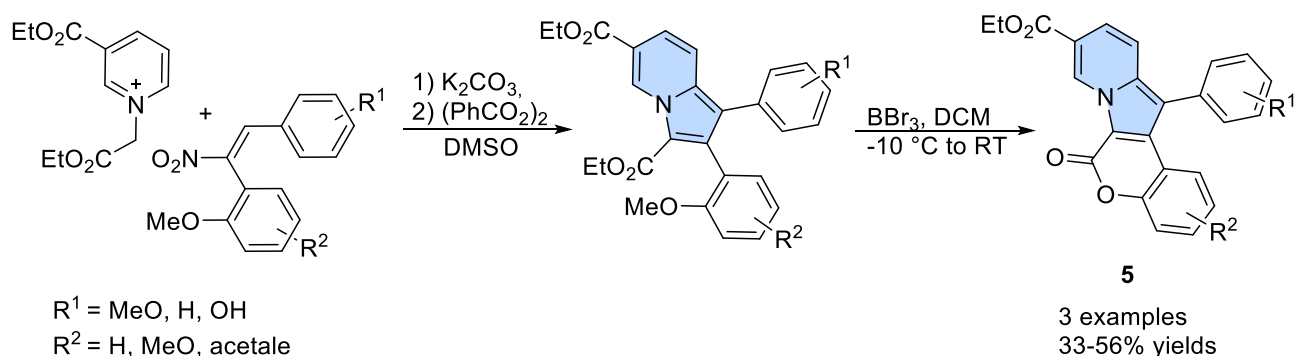
1.3.2. Pyrano-indolizinones and coumarin-indolizines

In the 80s, Kakehi *et al.* synthesized a new class of molecules, pyrano[2,3-*b*]indolizin-2-ones (**4**), which can be considered as analogues of coumarins and benzocoumarins (Scheme 8).^[68] Their synthetic route comprised four steps: (1) quaternization of pyridines with ethyl 2-bromoacetate; (2) Dieckmann condensation to form the indolizin-2-ol intermediate; (3) Friedel-Crafts reaction of the intermediate with diethyl 2-(ethoxymethylene)malonate; (4) final intramolecular cyclization *via* transesterification with the formation of the lactone ring. It should be noted that the last three steps occur in one pot. Interestingly, the authors reported strong fluorescence for these compounds, although this property was not investigated further.



Scheme 8: Synthesis of pyrano-indolizinones from pyridinium salts

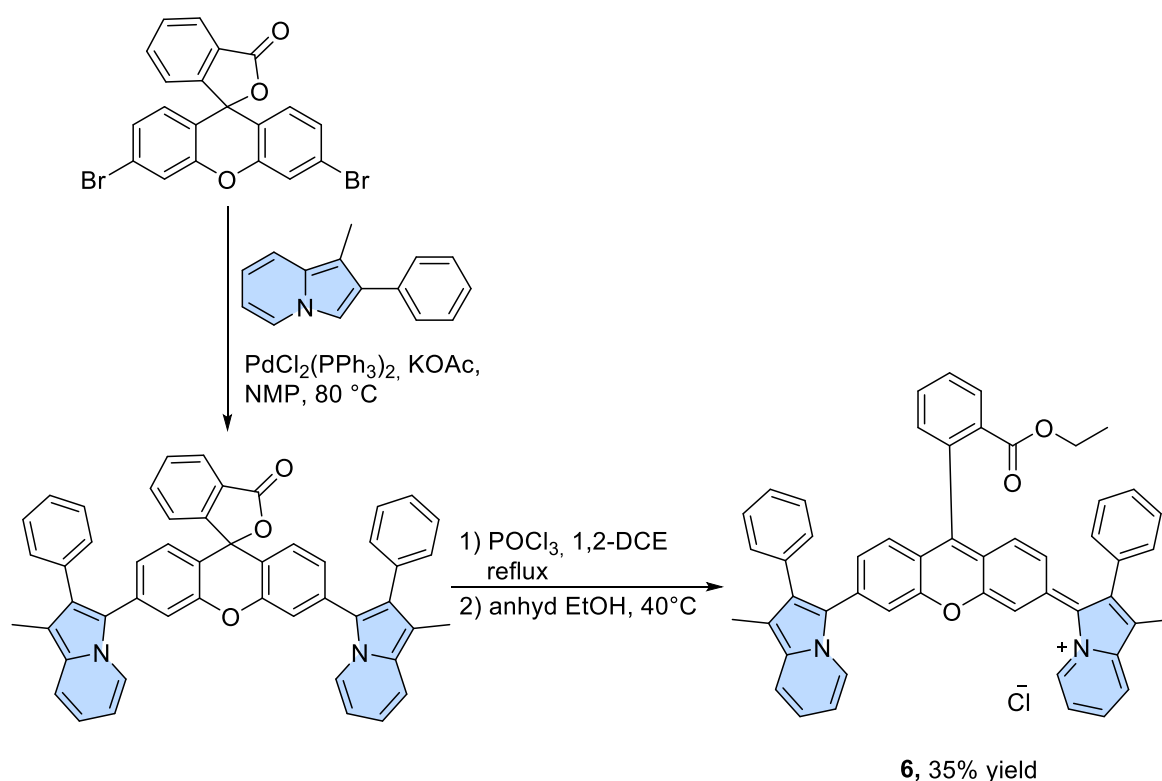
A coumarin-fused indolizine was recently reported by Rusanov *et al.*, who developed a novel synthetic methodology for the construction of pentacyclic lamellarin analogues (**5**) lacking the typical E-ring (Scheme 9).^[69] The key step involved generating an indolizine intermediate *via* a [3 + 2] cycloaddition reaction. This reaction used pyridinium ylides, α -nitrostilbenes, and benzoyl peroxide as an oxidant. Subsequent transformations included O-demethylation of the ortho-methoxy group and a final lactonization step, leading to the formation of a coumarin-fused indolizine product. The authors also reported a preliminary biological evaluation of these new lamellarin analogues; however, no data on their emissive properties were provided.



Scheme 9: Synthesis of coumarin-indolizines

1.4. π -Extended indolizines

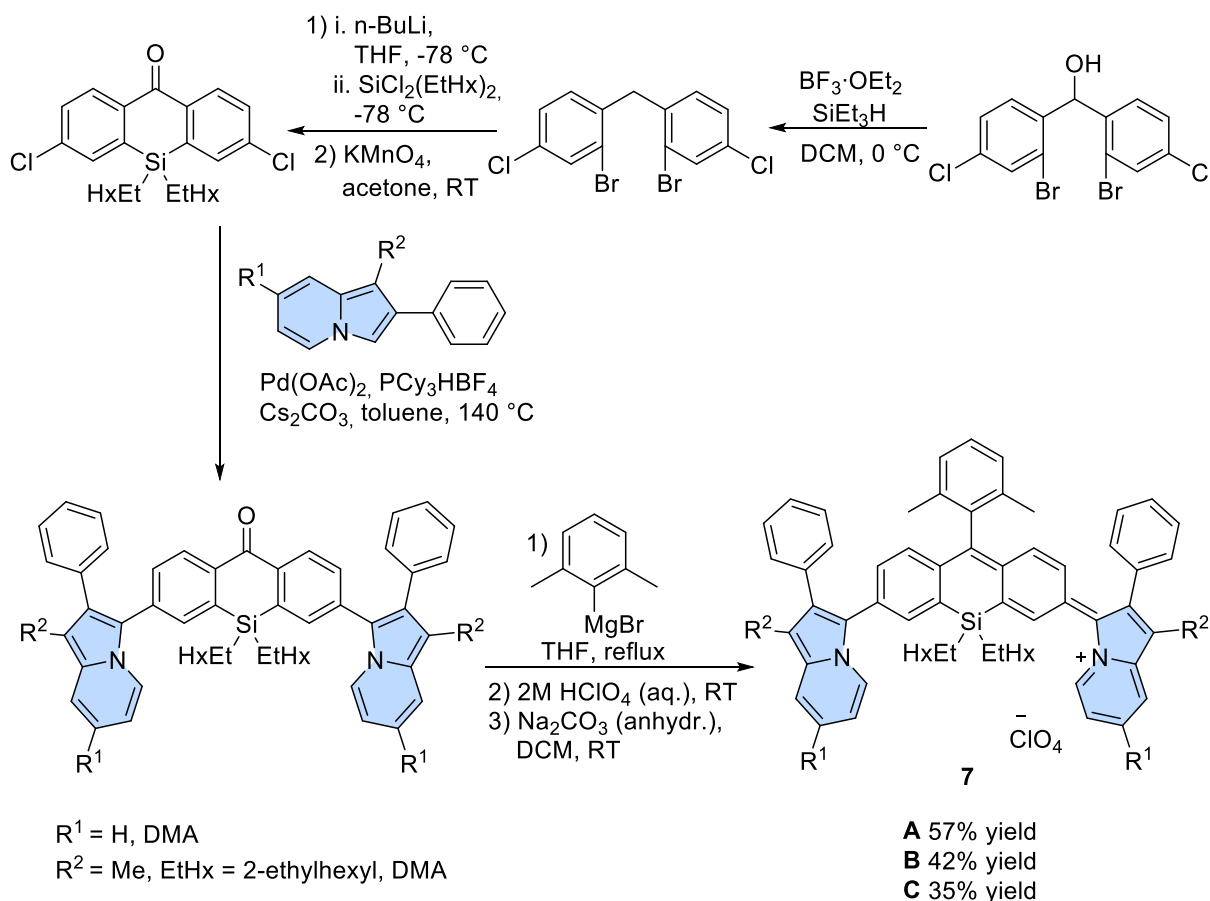
Another creative use of indolizines is their incorporation into extended π -systems through π -conjugation. Over the past decade, significant advancements were made by Delcamp's group in the development of extended-indolizine dyes capable of operating in the short-wave infrared (SWIR) region (Scheme 10).^[70] A key breakthrough was the development of xanthene-indolizine dyes with NIR-II (1000–1700 nm) emission that was achieved via a successful C-H bond functionalization strategy. This approach enabled the coupling of an electron-rich indolizine donor with an electron-deficient xanthene core, yielding the dye **RhIndz (6)**. The optimal conditions for this transformation involved $\text{PdCl}_2(\text{PPh}_3)_2$ as the catalyst, KOAc as the base, and NMP as the solvent at 110 °C. The fluorescent properties of RhIndz were recorded as follows: ($\lambda_{\text{abs}} = 920 \text{ nm}$, $\lambda_{\text{em}} = 1092 \text{ nm}$, $\Phi_{\text{fl}} = 0.03\%$).



Scheme 10: Synthesis of xanthene dyes possessing indolizines as donors (RhIndz)

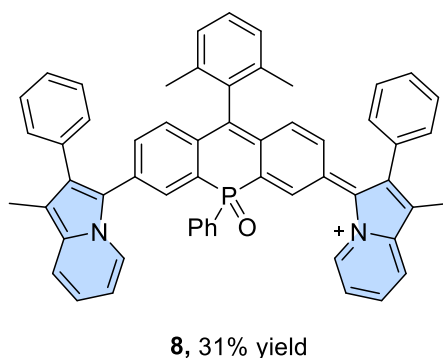
Building on this platform, in 2024 the same research group achieved a bathochromic shift of absorption and emission by substituting the xanthene oxygen atom with silicon and introducing a

dimethylaminoaryl substituent on the indolizine core (Scheme 11). This led to the synthesis of three fluorophores **SiRos** (**7**) through a sequence involving deoxygenation, silylation-cyclization, oxidation, and palladium-catalyzed C–H activation to install the indolizine donors.^[71] Final conversion to the desired perchlorate salts was completed via a Grignard reaction followed by acidic work-up. These dyes (**SiRos1300**, **SiRos1550** and **SiRos1700**) exhibit λ_{em} of 1,300 nm, 1,557 nm, and 1,700 nm in DCM, with quantum yields of 0.0056%, 0.0025%, and 0.0011%, respectively.



Scheme 11: Synthesis of **SiRos1300**, **SiRos1550** and **SiRos1700**

To further explore the effect of heteroatom substitution, Delcamp *et al.* later replaced the bridging oxygen with a phosphoryl group (P=O), yielding **PRos1450** (**8**), which emits at 1,450 nm red-shifted relative to **SiRos1300**.^[72] The synthetic route mirrored that used for the previous analogues, except that in this case, PhPCl₂ was used as the phosphorus source in the synthesis of the xanthene ring.

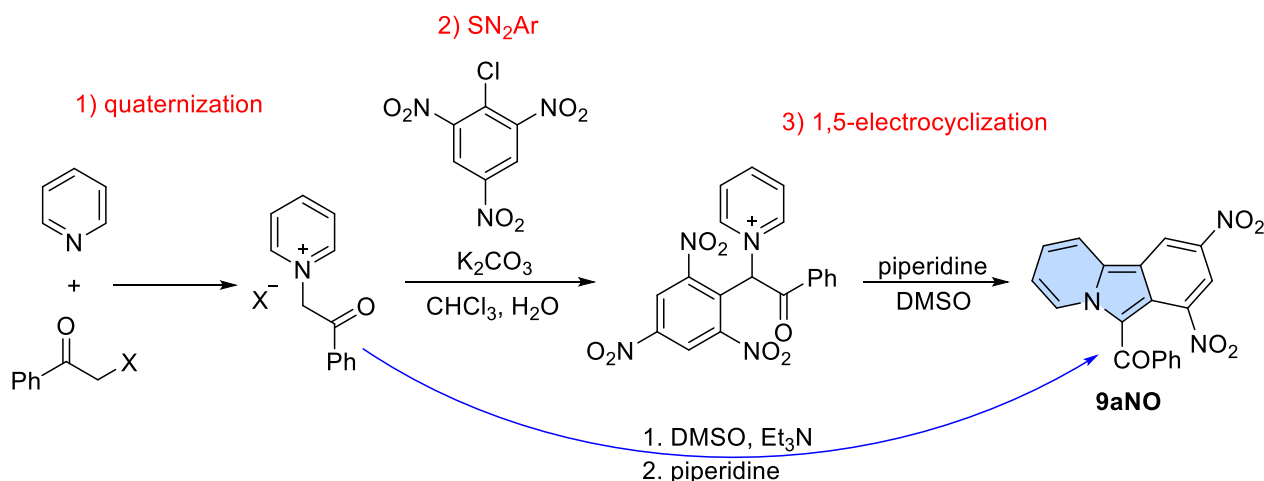


Scheme 12: Synthesis of **PRos1450**

2. Results and Discussion

2.1. Synthesis and Photophysical Properties of Substituted Benzo[a]indolizines

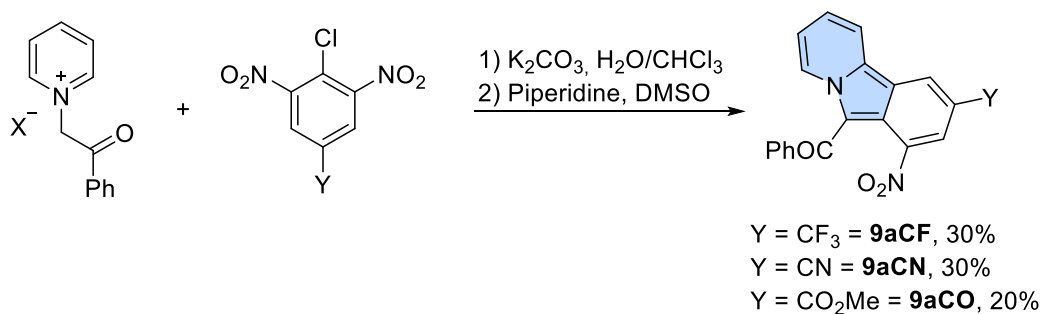
My first project focused on revisiting Kröhnke's synthesis of benzo[a]indolizines and investigating their photophysical properties.^[73] I chose to focus on this specific isomer of benzo-indolizines due to its promising fluorescent properties, briefly mentioned by the authors, and because it is significantly less explored compared to other isomers. In Kröhnke's work the synthesis (Scheme 13) involved the use of pyridinium salts and 1-chloro-2,4,6-trinitrobenzene, a compound known for its explosive nature in a two-step methodology. The first step was carried out with K_2CO_3 in $CHCl_3$, followed by a second step using piperidine in DMSO.^[67] The obtained structure was decorated with two nitro groups on the benzene ring and one ketone on the pyrrole moiety. From a photophysical perspective, such electron-withdrawing groups are generally responsible for hypsochromic shifts in both absorption and emission, as well as a quench in fluorescence emission due to enhanced intersystem crossing (ISC). Nevertheless, authors observed in structure **9aNO** a red fluorescence which was worth investigating further.



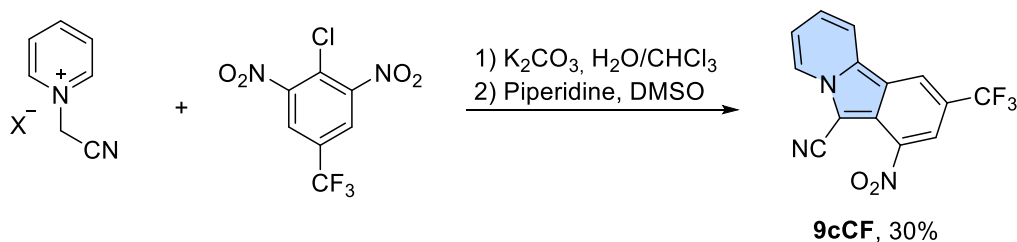
Scheme 13: Kröhnke's synthesis of Benzo[a]indolizines

Taking it into account, my whole research focus was devoted to obtaining benzo[a]indolizines bearing different substituents, using tailored building blocks to investigate how structural modifications influence their optical properties.

Initially, three derivatives (**9aCF**, **9aCN**, **9aCO**) incorporating CO_2Me , CN and CF_3 in position Y were obtained in 20-30% yield (Scheme 14). This was possible by following Kröhnke's first methodology and substituting the 1-chloro-2,4,6-trinitrobenzene with the less dangerous 4-chloro-3,5-dinitrobenzene derivatives. These structures, however, exhibited weak fluorescence indicating a limited ability to modulate the photophysical properties through variations at position Y alone and suggesting a significant influence of the substituent at position R^1 , which in all three cases was a carbonyl group (COPh). Thus, I decided to try modulating the properties by replacing the COPh group at position R^1 with a cyano group (CN) (Scheme 15). Indeed, this change resulted in a novel compound obtained in 30% yield (**9cCF**) and showing a visibly higher fluorescence emission, thereby confirming the critical role of the substituent at position R^1 in tuning the photophysical behavior of the benzo[a]indolizine scaffold.



Scheme 14: Kröhnke's method for the synthesis of benzo[a]indolizines



Scheme 15: Kröhnke's method for the synthesis of **9cCF**

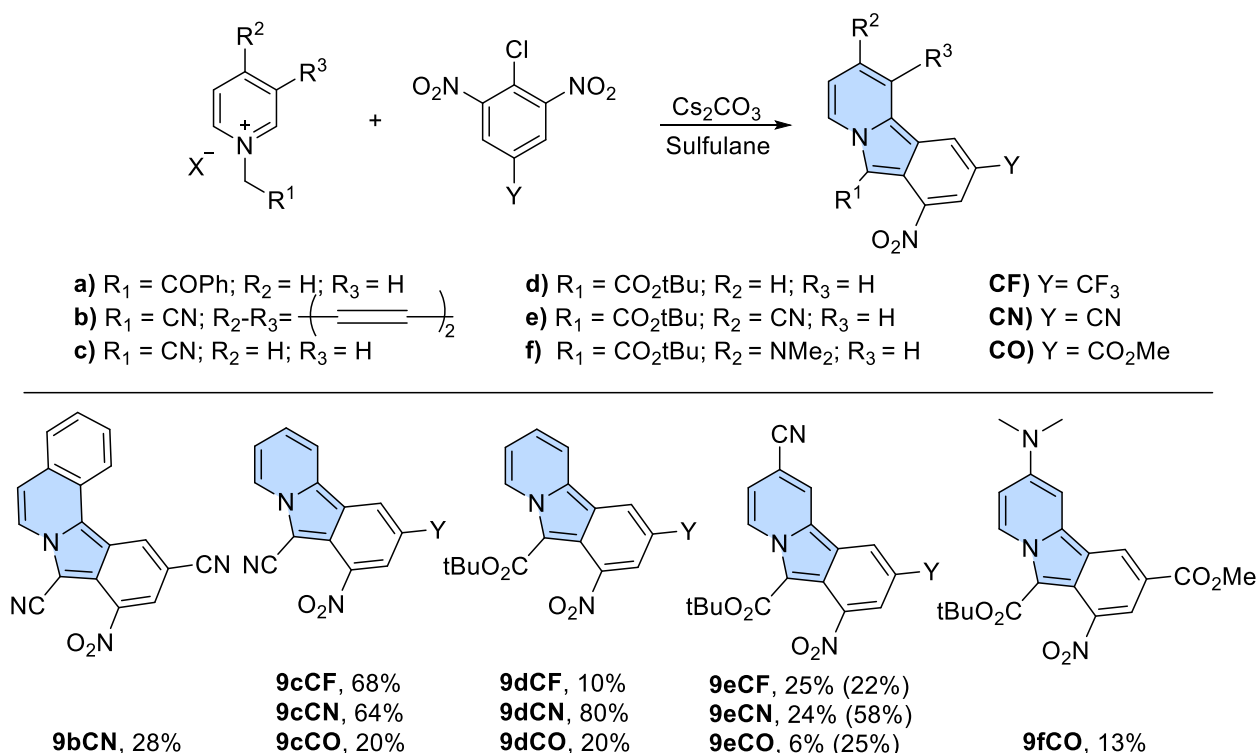
From this point onwards, I focused on improving the synthetic methodology with the aim of increasing products' yields and reducing the number of synthetic steps. Compound **9cCF** was selected as a model for optimization studies. A few years after the initial publication on this topic, an improved protocol was proposed, which employed a one-pot strategy using Et₃N and piperidine added sequentially in DMSO (Scheme 13, blue arrow).^[74] Building upon this approach, I tested various organic bases such as DBU and piperidine, as well as inorganic bases including K₂CO₃, KOtBu, CsOPiv, and Cs₂CO₃, the latter proving to be the most effective. In addition, several polar aprotic solvents were screened, including DMSO, DMPU, NMP, and sulfolane. Sulfolane was ultimately selected due to its superior performance under the chosen reaction conditions. Interestingly, increasing the reaction temperature and duration did not lead to further improvements in yield. Nevertheless, with the optimized conditions, I was able to improve the yield of **9cCF** from the previously reported 30% to 68%.

Compounds **9cCN** and **9cCO** were synthesized, followed by the derivatives **9dCF**, **9dCN**, and **9dCO** obtained by introducing a different electron-withdrawing group (CO₂tBu) at position R¹. Each substituent at position Y influenced the reaction outcome, highlighting not only their effect on the photophysical properties but also on the chemical reactivity (Scheme 16).

Next, an additional substituent was introduced into the system at position R², employing 4-substituted pyridines. Although this modification proved detrimental to reactivity, it still allowed the isolation of compounds **9eCF**, **9eCN**, **9eCO**, with yields around 20% and **9fCO** in 13% yield. Pyridine with EWG in position R² were less reactive, but what turned out to be even more interesting is that also pyridine with EDG showed the same behavior.

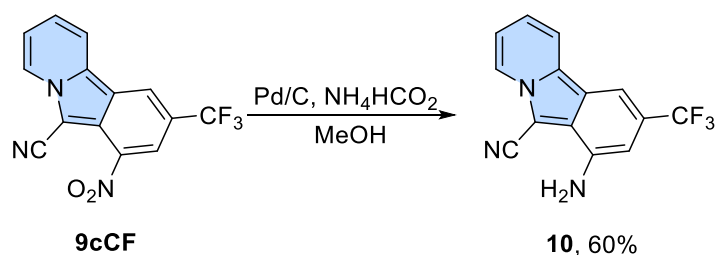
To improve the reactivity of the 4-substituted pyridines a new reaction condition was investigated using **9eCN** as the model. The use of *tert*-butylimino-tri(pyrrolidino)phosphorane (BTPP) DMSO proved to be optimal for this dye but not so efficient for **9eCF** and **9eCO** (Scheme 16). These results reaffirmed the criticality of the system to substituent effects in the *para* position of the pyridine ring.

Alternative approaches were explored to check the reactivity on the benzene ring. However, neither 1-chloro-2,4-dinitro-6-trifluoromethylbenzene nor 2-chloro-3,5-dinitropyridine led to the formation of the desired products. Similarly, less activated C-Hal bond like those in 2,4-dinitrofluorobenzene or 2,4-dichloro-1,5-dinitrobenzene, also proved ineffective, suggesting that the presence of two NO₂ in ortho position relative to Cl is crucial for reactivity, limiting the number of suitable starting materials.

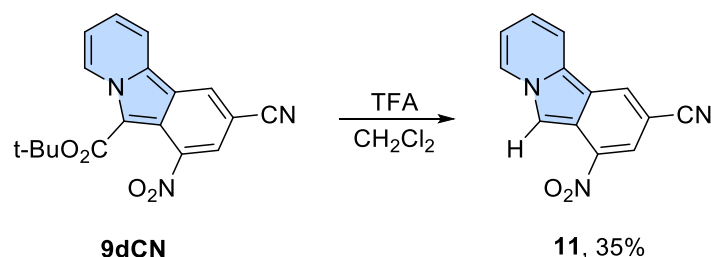


Scheme 16: Scope of benzo[a]indolizines

Additional modifications to the available products were possible due to the presence of reactive functional groups such as NO₂ and CO₂t-Bu. A classic reduction of the nitro group to amino group in **9cCF** led to structure **10**. While in a different transformation, a reverse Friedel-Crafts reaction TFA mediated was used to cut out the ester group in compound **9dCN**, affording structure **11** with a free R¹ position (Schemes 17 and 18)



Scheme 17: Post-functionalization of **9cCF**



Scheme 18: Post-functionalization of **9dCN**

To unravel the photophysical properties of the synthesized benzo[*a*]indolizine derivatives, I recorded absorption and emission spectra for all the obtained structures in DCM and toluene and compared them to the parent benzo[*a*]indolizines exhibiting fluorescence quantum yield of 14% in DCM with $\lambda_{\text{abs}} = 368 \text{ nm}$ and $\lambda_{\text{em}} = 498 \text{ nm}$.^[75] Most of the new compounds displayed λ_{abs} between 400–500 nm and emission localized in the orange-red region of the visible spectrum with Φ_{fl} ranging from negligible to 60%. These results demonstrate a significant bathochromic shift relative to the unsubstituted benzo[*a*]indolizine core and its isomer, benzo[1,2-*b*]indolizine (**2-3**), indicating a strong influence of both substituents and the position of the nitrogen atom within the core on their photophysical behavior. A marked solvatochromism was observed when moving from toluene to DCM consistent with an intramolecular charge transfer (ICT) process that was later confirmed by computational studies.

A comparative analysis of molar absorptivity (solid lines) and normalized emission spectra (dashed lines) for **9aCO**, **9cCO**, **9dCO**, **9eCO**, and **9fCO** (represented in pink, orange, green, blue, and black, respectively) in toluene revealed the impact of substituents on the pyridine and pyrrole rings, while maintaining fixed CO₂Me and NO₂ groups on the benzene moiety. Notably, the introduction of an electron-donating group (EDG) such as NMe₂ on the six-membered ring resulted in the largest Stokes shift and the lowest fluorescence quantum yield (<0.1%). Conversely, replacing the COPh group at position R¹ with a cyano group **9cCO** led to the smallest Stokes shift and the highest Φ_{fl} (0.58%) (Fig. 2a).

A similar comparison was conducted for compounds **9cCF**, **9cCN**, and **9cCO** (blue, red, and orange), all bearing a CO₂tBu group at position R¹ and varying substituents at position Y, in DCM. The results showed minimal variation in their photophysical properties, confirming that the substituent at position R¹ plays a critical role, while the effect of substituents at position Y is negligible (Fig. 2b). This was further supported by the comparison of **9dCN** (orange) and its deprotected analog **11** (purple), where a significant bathochromic shift in λ_{abs} was observed, whereas the emission wavelength remained largely unchanged (Fig. 2c). For both compounds, photostability measurements were performed in comparison to Rhodamine 6G. The results showed that both compounds are less stable than Rhodamine 6G but notably lower stability of **11** supported the already known behavior of indolizine-based dyes, confirming that position R¹ is a hotspot for electron density and highly reactive. (Fig 3)

Another particularly interesting observation emerged from this photophysical analysis: contrary to expectations, the NO₂ group, typically associated with fluorescence quenching, contributed to a bathochromic shift in both absorption and emission spectra, along with relatively large fluorescence quantum yields. When comparing compound **9cCF** with its reduced analog **10** (bearing an NH₂ group instead of NO₂), a drop in emission intensity (38% vs 26%) and a hypsochromic shift of both λ_{abs} (488 vs 377 nm) and λ_{em} (603 vs 495 nm) were observed.

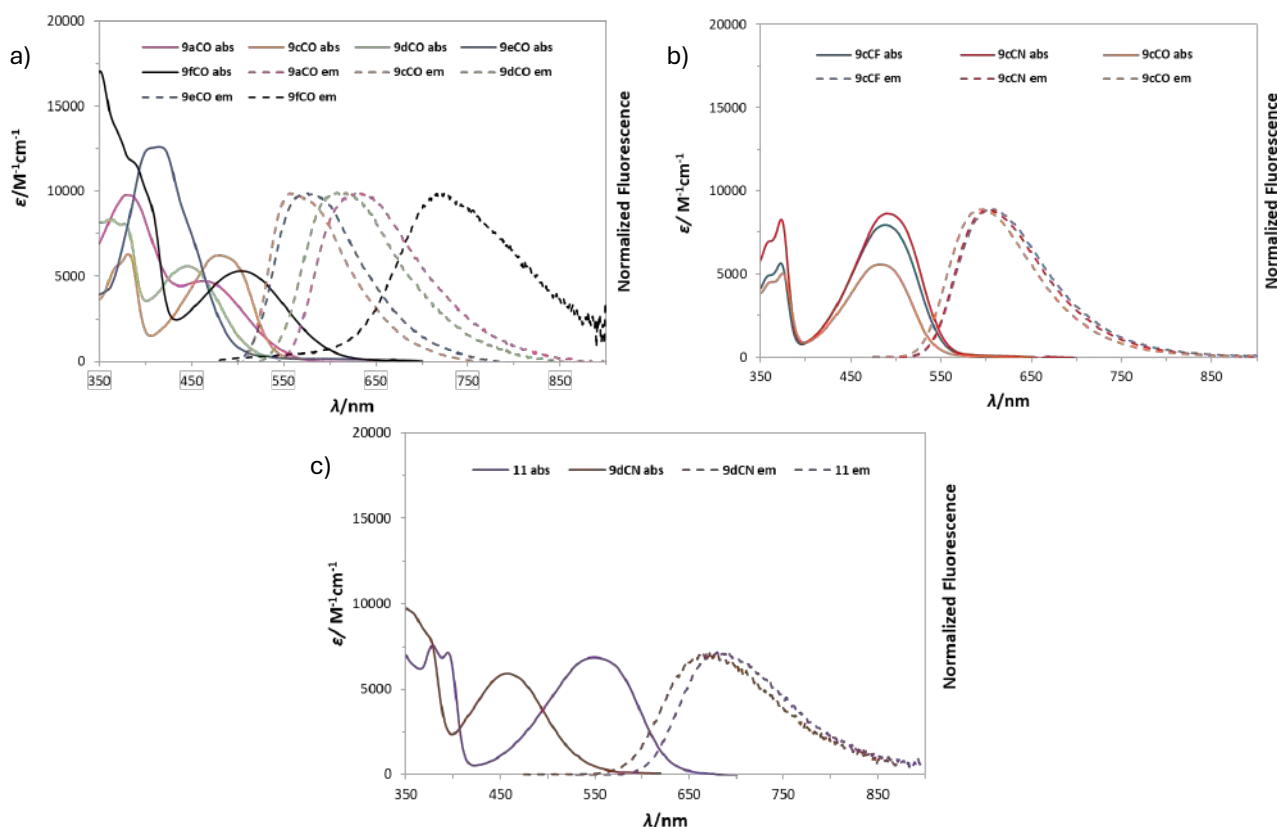


Figure 2: a) Absorption and emission spectra of dyes **9aCO**, **9cCO**, **9dCO**, **9eCO**, and **9fCO** (pink, orange, green, blue, and black) in toluene, b) Absorption and emission spectra of **9cCF**, **9cCN**, and **9cCO** in DCM (blue, red, and orange) c) Absorption and emission spectra of **11** and **9dCN** in DCM (purple and brown)

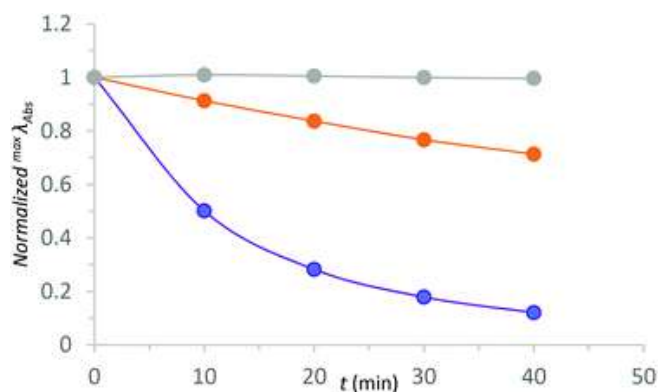


Figure 3: Photostability of compound **11** compared to **9dCN** and Rhodamine 6G

Theoretical calculations carried out by collaborators corroborated my experimental findings and confirmed the limited influence of substituents at position Y. Moreover, the calculations highlighted the key role of the NO_2 , which acts as a strong electron-withdrawing group (EWG) and facilitates intramolecular charge transfer (ICT) with the electron-rich pyrrole ring, functioning as an electron-donating group (EDG). As expected, the donor strength of the pyrrole was modulated by the substituent at position R^1 . Surprisingly, however, substituents typically classified as EWGs, such as CN, CPh, and CO_2tBu , exhibited donor-like behavior in this molecular framework, acting as secondary donors and participating in ICT.

In addition, simulated fluorescence quantum yields Φ_{fi} aligned well with the experimental data and provided further insight into structure-property relationships. The calculations explained the

dramatic drop in fluorescence efficiency observed for compounds bearing bulky groups at position R¹. These substituents induce significant distortion from planarity, which enhances intersystem crossing (ISC) due to a reduced singlet–triplet (S-T) energy gap and increased spin–orbit coupling (SOC), particularly evident in compound **9fCO**.

Conversely, derivatives from series **9b** and **9c**, bearing smaller EWGs, exhibited relatively large S-T gaps (> 0.3 eV) and negligible SOC, supporting their higher fluorescence efficiency. The remaining compounds displayed intermediate photophysical behavior, consistent with moderate structural distortion and partial SOC contribution.

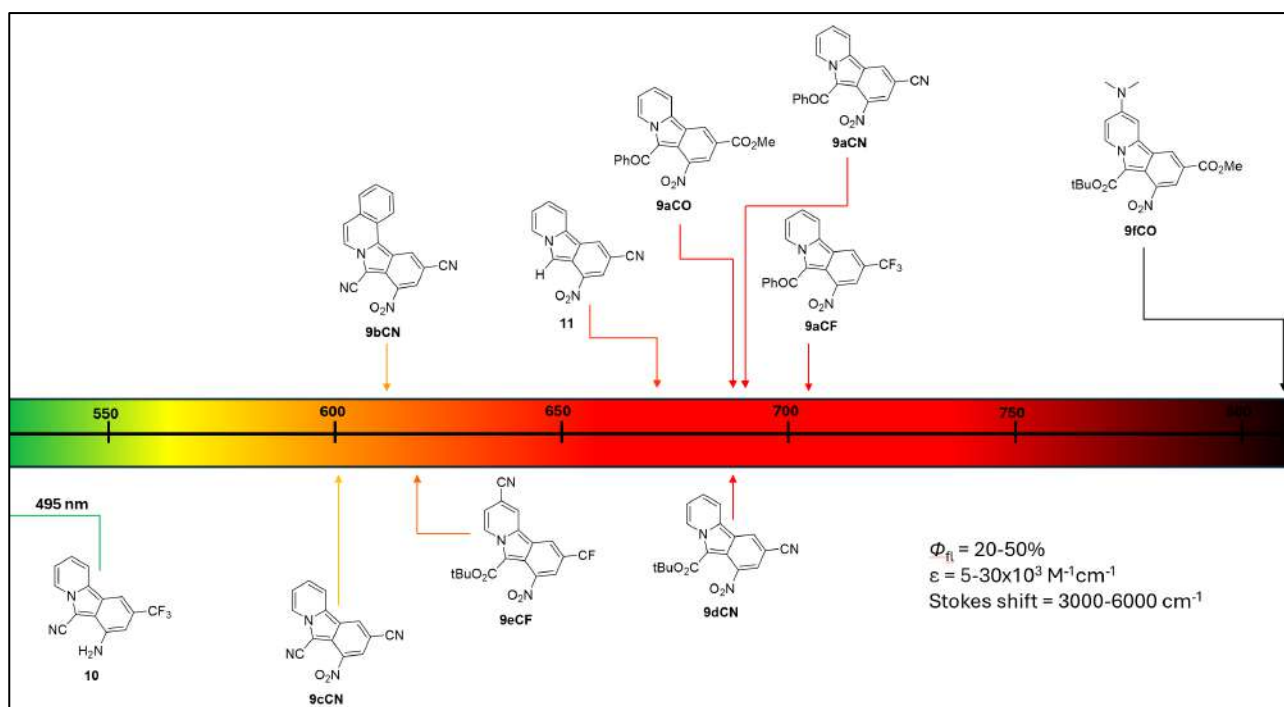
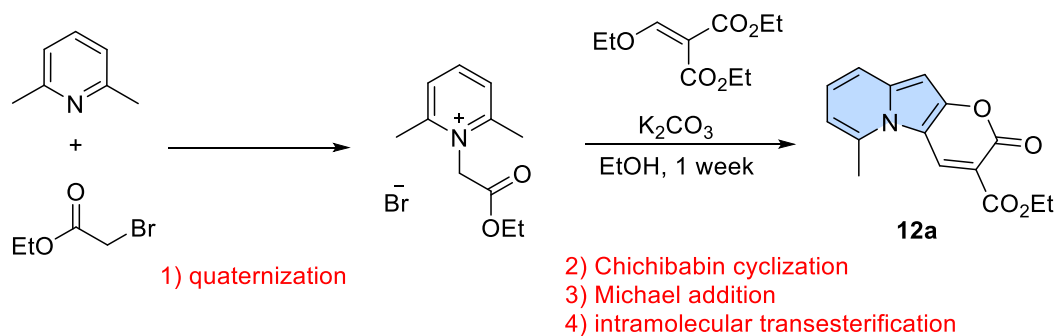


Figure 4: Visual comparison of benzo[a]indolizine derivatives' emission in DCM.

2.2. Synthesis and Photophysical Properties of Substituted Pyrano[2,3-*b*]indolizin-2-ones

The second project I worked on followed the same vision; I wanted to further investigate another type of nearly forgotten, but extremely promising class of π -expanded indolizines. Between the 80s and the 90s Kakehi and co-workers developed different methodologies to synthesize derivatives of 2-oxo-2H-pyrano[2,3-*b*]indolizine-3-carboxylates, structures that can be considered analogs of coumarins exhibiting interesting fluorescent properties and potential applicability in bioimaging. Although authors proposed different paths, all involved long time reaction (7 days) and afforded relatively low product yields (38%), thus opening the doors to methodology improvement and photophysical investigations.

The proposed reaction mechanism involved the synthesis of pyridinium salts, a Dieckman condensation to obtain the indolizin-ol intermediate followed by a Friedel-Crafts reaction, and a last step being an intramolecular transesterification to obtain the lactone ring. The last three steps occur in one pot (Scheme 19).

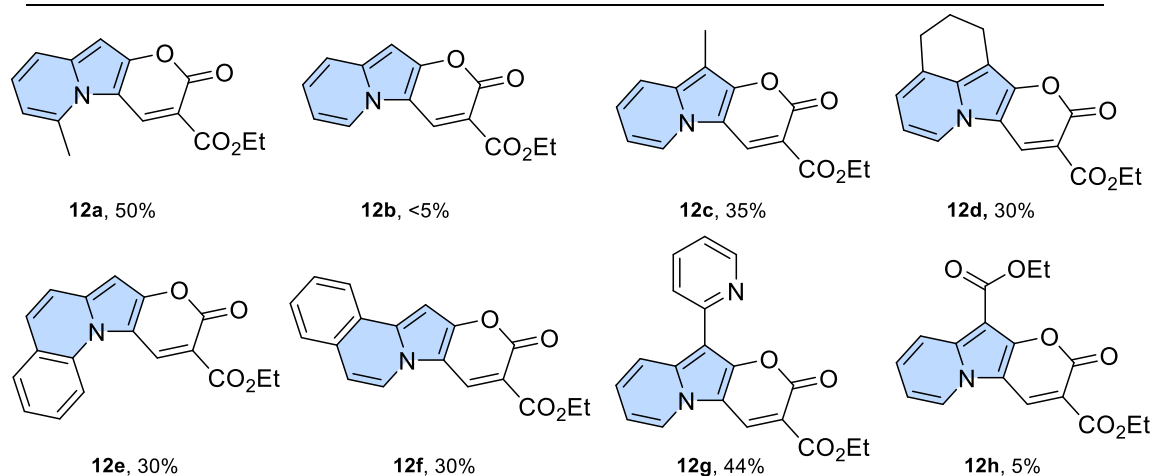
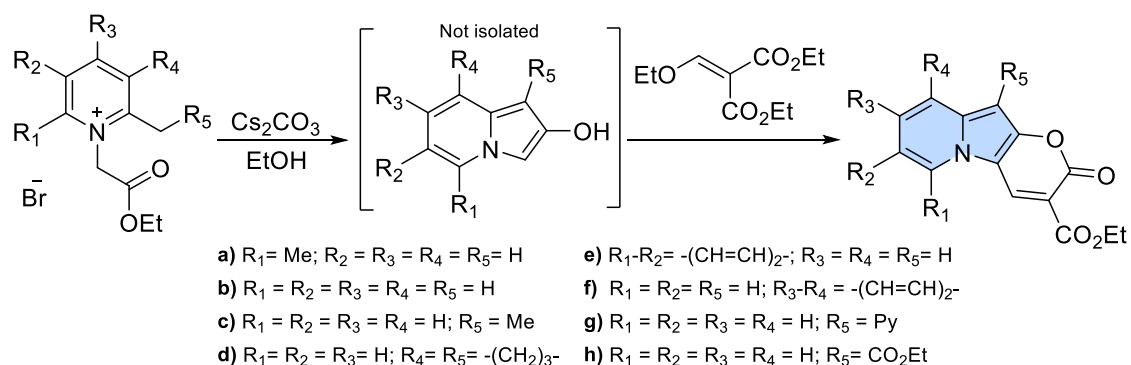


Scheme 19: Kakehi's synthesis of pyrano[2,3-*b*]indolizin-2-ones

For the development of an improved reaction condition compound **12a** was used as a model. Initial changes of the solvent, (DMF instead of EtOH) and on the base (NaOEt instead of K_2CO_3) did not lead to the expected outcome and caused me to rethink my plan. Considering that the mechanism involved the formation of a critical intermediate I thought that the best would be to allow sufficient time for the first reaction to happen before the addition of the malonate. Consequently, in this 2-steps approach different bases were studied in the classical solvent used (EtOH), and I found the best to be Cs_2CO_3 affording the product **12a** in 50% yield.

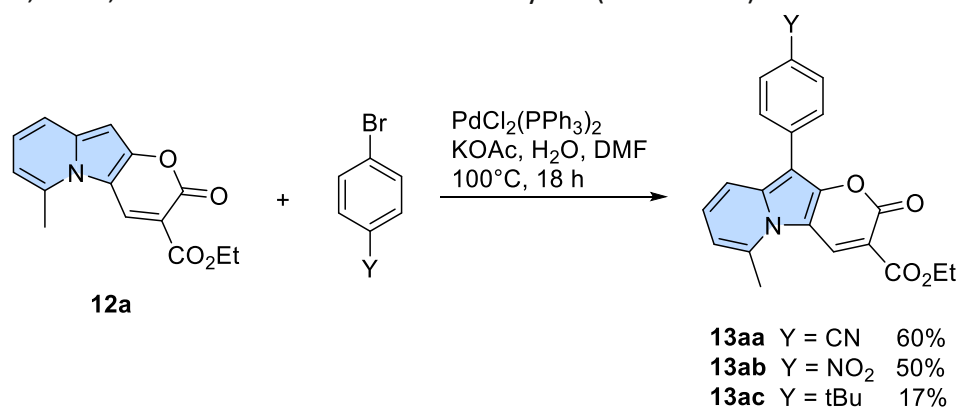
Using this optimal condition, I evaluated the substrate scope and a total of eight new structures were synthesized starting from different pyridinium salts. 'Formal' picolinium salts reacted as expected and yielded pyrano-indolizines (**12a**, **12b**, **12c**) possessing a hydrogen or an alkyl substituent in position R^5 (Scheme 20). Similar but cyclic pyridinium salts such as 2,3-cyclopentenopyridinium and 5,6,7,8-tetrahydroquinolinium were also tested, but giving two different outcomes. The first one failed to give the product while the latter generated the expected product **12d** in 30% yield. Reactivity was also good for expanded pyridinium salts like 2-methylquinolinium and 1-methylisoquinolinium and two expanded products with free R^5 position (**12e**, **12f**) were achieved in 30 and 28% yield. Lastly, with the aim of having EWG in position C-5 α -picolines with EWG (2-Py and CO_2Et) in the methylene group were used and products **12g** and **12h** were obtained in 44% and 5% yield respectively. Differently from the previous cases the first reaction step, the formation of the indolizin-ol happened, without the need of a base, right after

the quaternization. Interestingly, both EWG and EDG in para position to the pyridine prevented the synthesis of the final product.



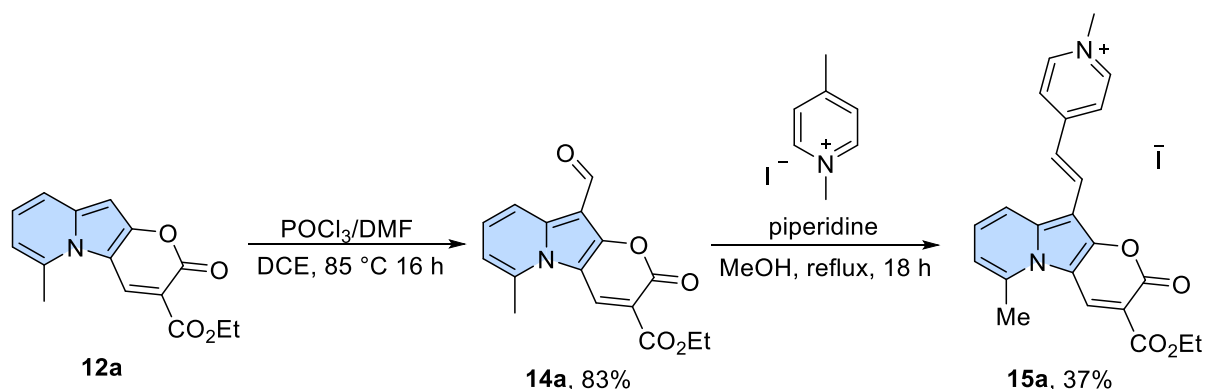
Scheme 20: Scope of pyrano[2,3-*b*]indolizin-2-ones

In order to achieve a broader variety of substituents in C-5, I used structure **12a** for further functionalization. The highly electron-rich carbon was a good candidate for direct arylation and products **13aa**, **13ab**, **13ac** were obtained in 17-60% yield (Scheme 21).



Scheme 21: Post-functionalization of dye **12a**

Then, I also envisioned in the Vilsmeier-Haack formylation the opportunity not only to get a different EWG, but mostly the chance to obtain a new styryl dye through a sequent Knoevenagel condensation (Scheme 22), with potential for cell staining. In these last steps compounds **14a** and **15a** were isolated in 83 and 37% yields respectively.



Scheme 22: Post-functionalization of dye **12a**

For this class of compounds Uv-Vis absorption and emission were measured in toluene, DCM and DMSO and compared with indolo-coumarins like 11-benzyl-7-hydroxyisochromeno[4,3-*b*]indol-5-one ($\Phi_{\text{fl}} = 42\%$ in DCM, with $\lambda_{\text{abs}} = 382$ and $\lambda_{\text{em}} = 496$) and methyl 2-oxo-4-(pyrrolidin-1-yl)-2,4a,5,9b-tetrahydropyrano[3,2-*b*]indole-3-carboxylate ($\lambda_{\text{abs}} = 335$ in EtOH). Results showed a significant bathochromic shift for absorption (420-470 nm) while emission remained in the same range (440-520 nm), and a solvatochromism emerged when moving from apolar to polar solvents.

The distinctly different fluorescence emissions of compounds **12a**, **12b**, and **12c**, clearly discernible to the naked eye and supported by the recorded data, prompted me to begin analyze the impact of substituent positions on this core structure, even for simple groups such as methyl. In fact, having Me in R^5 (**12c**) caused a bathochromic shift compared to **12b** while the same group in R^1 (**12a**) caused a hypsochromic shift of bands. These findings were later rationalized by my colleague as a consequence of the largest change in HOMO energy for **12c** with methyl in R^5 (Fig. 5a).

Interestingly, the expansion of the π -system which usually is responsible for shifts towards the red region, in these structures was dependent on the actual direction of the expansion. Dye **12f** has hypsochromically shifted emission compared to dye **12b**, and *vice versa* **12e** displayed a slight shift of the signals towards lower energies (Fig. 5b), computational calculations computed energies for these two systems as such: $E_{\text{LUMO}}(\mathbf{12e}) < E_{\text{LUMO}}(\mathbf{12f})$.

Further investigations into the effect of different substituents in R^5 on the photophysics clarified the role of EWG in **14a** (CHO $\lambda_{\text{abs}} = 426$ nm and $\lambda_{\text{em}} = 448$ nm), EDG in **12c** (Me $\lambda_{\text{abs}} = 468$ nm and $\lambda_{\text{em}} = 494$ nm), and the π -extension of **13aa** (4-CN $\lambda_{\text{abs}} = 453$ nm and $\lambda_{\text{em}} = 490$ nm), and **13ac** (4-*t*Bu $\lambda_{\text{abs}} = 457$ nm and $\lambda_{\text{em}} = 514$ nm). (Fig. 5c) The presence of the expanded system shifts the absorption and emission to the blue region and an even stronger effect on the same direction is given by the presence of the aldehyde. All the small changes on the spectra caused by the different substituents in R^5 were explained with computational investigation by a slight modification of the HOMO and LUMO of the substituted molecules compared to the parent dye **12b**.

When recording the data for **13ab** (4-NO₂ $\lambda_{\text{abs}} = 452$ nm and $\lambda_{\text{em}} = 491/642$ nm), I noticed an increase in the Φ_{fl} in apolar solvents (toluene) compared to the other structure, and the presence of a second peak in the emission in both DCM and DMSO, showing two emission bands overlapping, one at the 'expected' wavelength (491 nm) and another one around 642 nm. To rule out the possibility of the second signal being caused by impurities I recorded excitation spectra and confirmed that both peaks were coming from the investigated molecule. I then hypothesized that this structure, composed of a strong electron donor (2-oxo-pyrano[2,3-*b*]indolizine) and a strong electron acceptor (nitrophenyl), could undergo intramolecular charge transfer (ICT) in polar solvents. This process may

lead to the formation of two distinct excited states, depending on the molecular geometry, thereby enabling two possible emission pathways. This hypothesis was then confirmed by experiments on fluorescence decay (τ_f) and computational simulations which confirmed the presence of both a low emissive and a TICT emissive states.

Finally, I investigated the optical properties of the dye **15a** ($\lambda_{\text{abs}} = 509$ nm and $\lambda_{\text{em}} = 575$ nm), a potential cell probe, compared to its precursor **14a** (Fig. 5d). By adding a conjugated pyridinium system, a large red shift on the emission was observed which was later rationalized by my colleague as a consequence of a qualitative change of LUMO from localized on the main indolizine core to localized on the substituent, the same situation was envisioned for **13ab**.

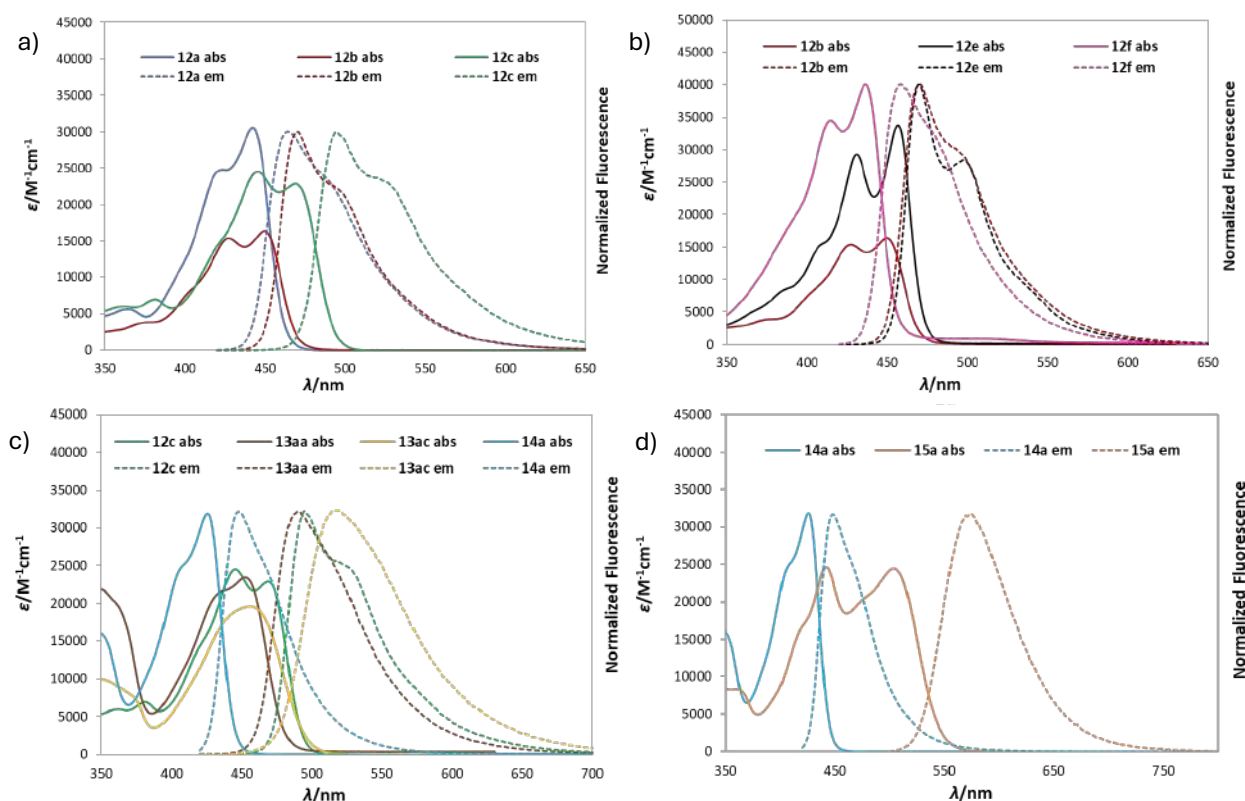


Figure 5: a) Absorption (solid line) and normalized emission (dashed line) of **12a**, **12b**, **12c** (blue, red, green) b) Absorption (solid line) and normalized emission (dashed line) of **12b**, **12e** and **12f** (red, black, pink) c) Absorption (solid line) and normalized emission (dashed line) of **12c**, **13aa**, **13ac** and **14a** (green, brown, yellow and light blue) d) Absorption (solid line) and normalized emission (dashed line) of **14a** and **15a** (light blue, orange).

Preliminary cell staining experiments were carried out by collaborators on rat embryonic cardiomyoblast-derived cells H9c2 for dye **15a**, but the styryl dye alone was unable to permeate the cell membrane. Therefore, digitonin was required to facilitate its entry. Once inside the cell, however, compound **15a** exhibited remarkable behavior: it accumulated in the nucleus and along the nuclear membrane, as confirmed by co-localization with the standard nuclear stain Hoechst 33342. Interestingly, unlike the standard dye, **15a** appeared to selectively stain specific subnuclear structures, suggesting potential for more refined nuclear imaging.

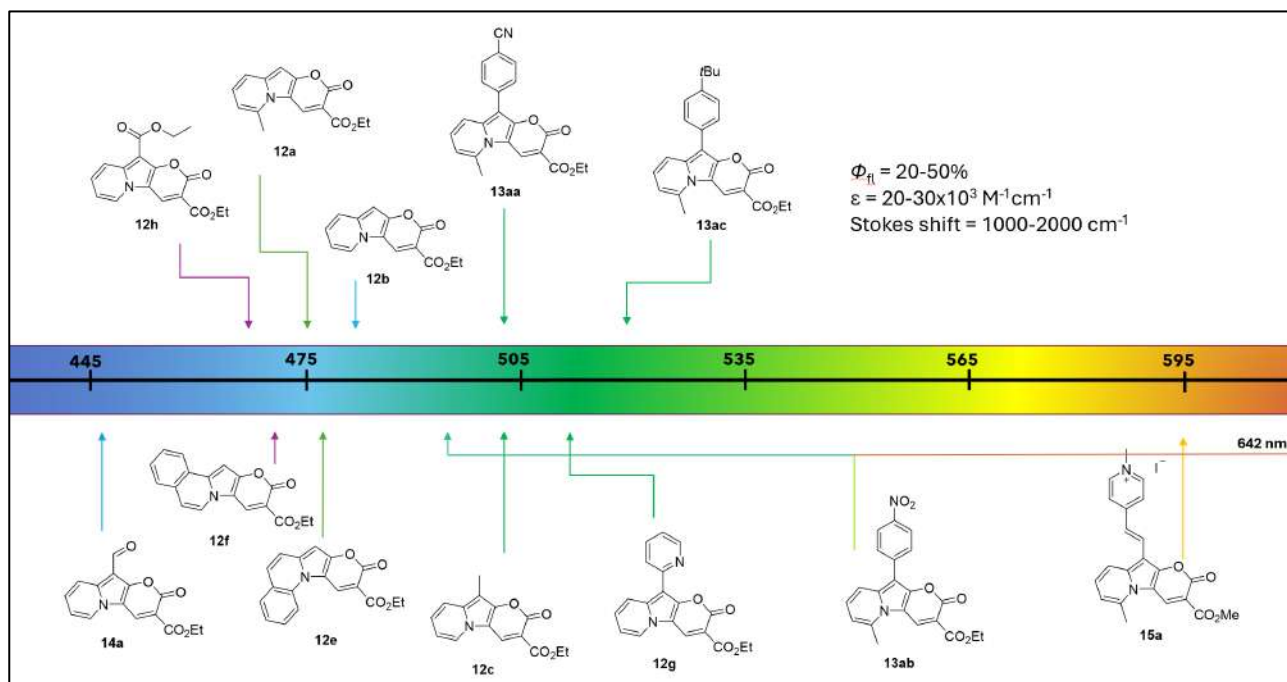
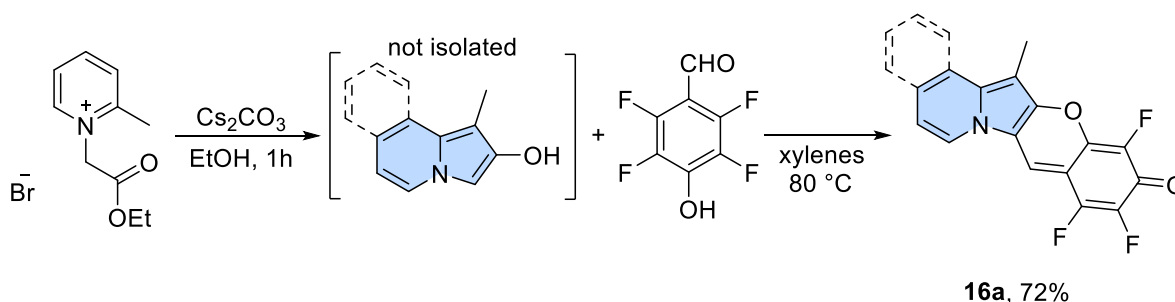


Figure 6: Visual comparison of pyrano[b]indolizines' emission in DCM

2.3. Synthesis and Photophysical Properties of Substituted Indolizine-merocyanines (IndMer)

While working on the second project, the idea that the indolizin-ol intermediate could serve as an electron-donating building block for the synthesis of xanthene-like systems emerged. Additionally, the recent work of Delcamp on π -extended rhodol-indolizines **8** emitting in the near-infrared (NIR) region further inspired the conceptual development of this project. In this context hydroxyindolizines should behave in a similar manner to the electron-rich phenols typically used for the synthesis of systems like rhodols but thanks to its stronger electron-donating character, push the optical properties of these new fused systems towards the red region of the UV-vis spectrum compared to classical rhodols.

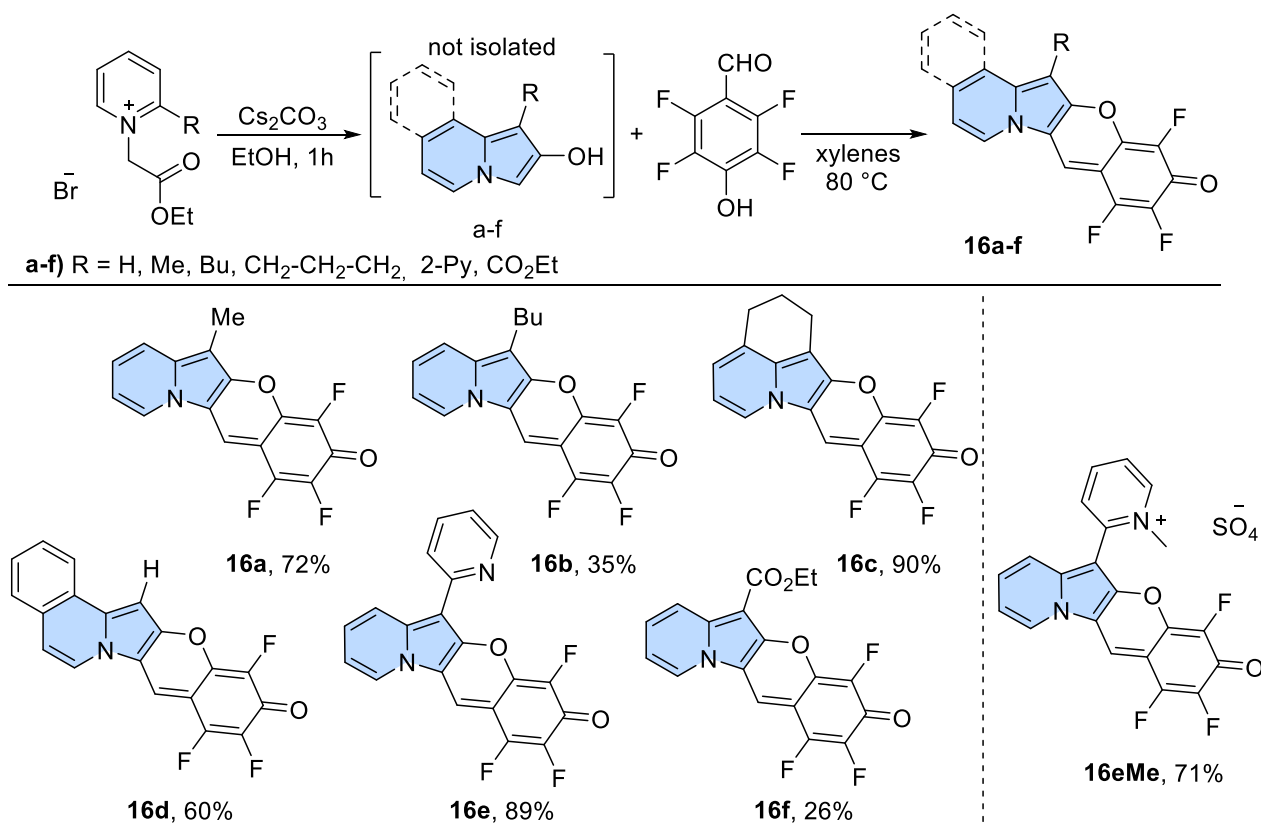
I employed a Friedel-Crafts alkylation/nucleophilic aromatic substitution, which was previously discovered in our group for the reaction of 2,3,5,6-tetrafluoro-4-hydroxybenzaldehyde with aminophenols.^[76] Instead, I used substituted indolizin-ols with the above mentioned aldehyde in xylenes at 80 °C and compound **16a** (IndMer) was obtained in 72% yield.



Scheme 23: Synthesis of IndMer **16a**

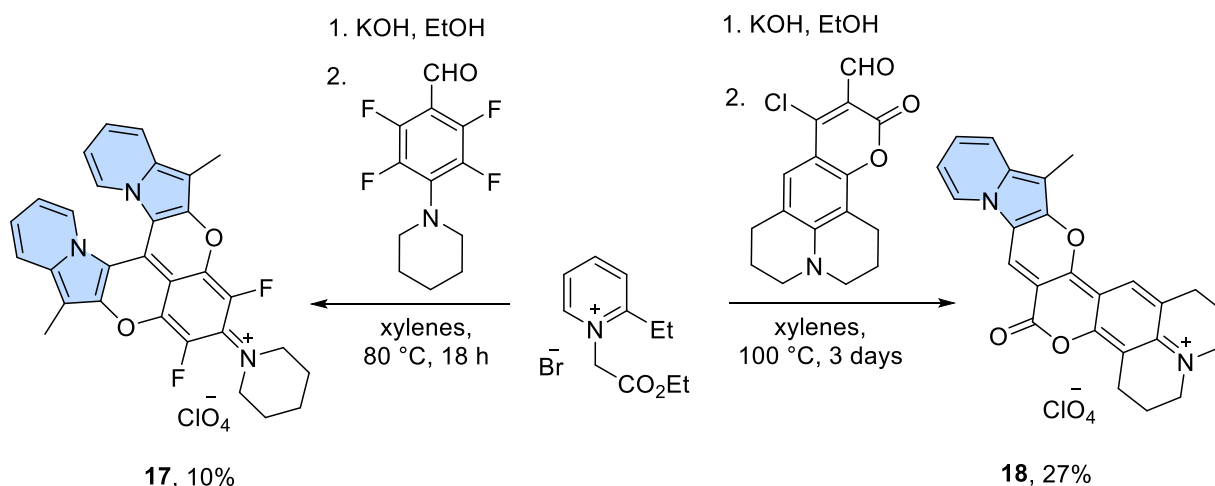
The scope of the reaction was assessed by changing the substituents on the indolizine core and 5 additional derivatives were obtained with both electron-withdrawing and slightly electron-donating group in R in yields from 26 to 90% and isolated *via* precipitation thanks to their poor solubility (Scheme 24). The only exception was compound **16d** which was designed to improve the solubility and was purified *via* chromatography. Additionally, a post functionalization on **16e** was performed, quaternization of the pyridyl group led to product **16eMe**.

Using the same methodology other products (ImpMer in 24-62 % yields) were achieved through the reaction of 2-hydroxyimidazo[1,2-*a*]pyridine and 2,3,5,6-tetrafluoro-4-hydroxybenzaldehyde in similar conditions (xylenes, 100-150 °C). However, since the focus of this thesis are indolizines, those structures will not be discussed further but were worth mentioning to validate the success and versatility of the presented methodology.



Scheme 24: Synthesis and scope of indolizine-merocyanines (*IndMer*)

In order to verify the compatibility of this methodology with different aldehydes having similar characteristics (a halogen atom in vicinal position to CHO, and an electron-deficient character of the aromatic ring) I used 2,3,5,6-tetrafluoro-4-(piperidin-1-yl)benzaldehyde and a coumarin-based aldehyde (Scheme 25). Both aldehydes reacted with 2-hydroxyindolizines affording however completely different products, the first one led to a V-shaped bis-xanthene dye **17** in 10% yield with the incorporation of two indolizines and one aldehyde, while the latter afforded a cyanine-type dye **18** in 27% yield.



Scheme 25: Synthesis of two alternative indolizine-cyanine hybrids **17** and **18** from aldehydes **N** and **C**.

I then performed photophysical characterization with UV-Vis absorption and emission spectra recorded in DCM, THF, DMSO, and for some selected representatives, in aqueous media

(H₂O+DMSO). A comparison was made with classical rhodols and merocyanine dyes and a slight bathochromic shift was observed, but no solvatochromism. This feature was later explained by collaborators who computed a large dipole moment in the ground S₀ state which changes little in the excited S₁. Consequently, no difference in the solute-solvent interaction energies was observed. Moreover, a comparison between the dyes showed no significant influence of the group in position R suggesting that the main properties come from the core structure (Fig. 7a). Stokes shifts are rather small, compatible with the rigidity of the structure and the negligible solvent influence. Surprisingly, for almost all the dyes Φ_{fl} was above 50% even in polar solvent such as DMSO. Structures **17** and **18** have a larger Stokes Shift 50-80 nm DCM, but a decrease in Φ_{fl} (32 and 2%) compared to the previous systems.

More interesting properties were encountered for dye **18** ($\lambda_{\text{abs}}^{\text{max}} = 624 \text{ nm}$ and $\lambda_{\text{em}}^{\text{max}} = 705 \text{ nm}$) (Fig. 7b) which could be compared to the rhodol-fused coumarin **19** ($\lambda_{\text{abs}}^{\text{max}} = 586 \text{ nm}$ $\lambda_{\text{em}}^{\text{max}} = 625 \text{ nm}$) obtained by Bardi *et al.*^[76], and showing intriguing possible applicability for cell imaging (Fig. 8).

This compound showed good solubility and photostability in aqueous media, comparable to Rhodamine 6G, which guided further experiments in cells to assess its potential for organelle visualization.

Confocal microscopy studies performed on compound **18**, by our collaborators in Korea, in HT1080 fibrosarcoma and MRC5 normal human lung fibroblast cells showed high cell permeability and context dependent accumulation in mitochondria and RNA-rich nucleoli when compared to MitoTracker™ Green FM and SYTO RNASelect™ respectively, in both MRC5 and HT1080 cells.

These findings align with the recent reports of single probes exhibiting dual localization, which allows for studying mitochondrial damage and monitoring mitochondrial state in live cells under both physiological and stress conditions.

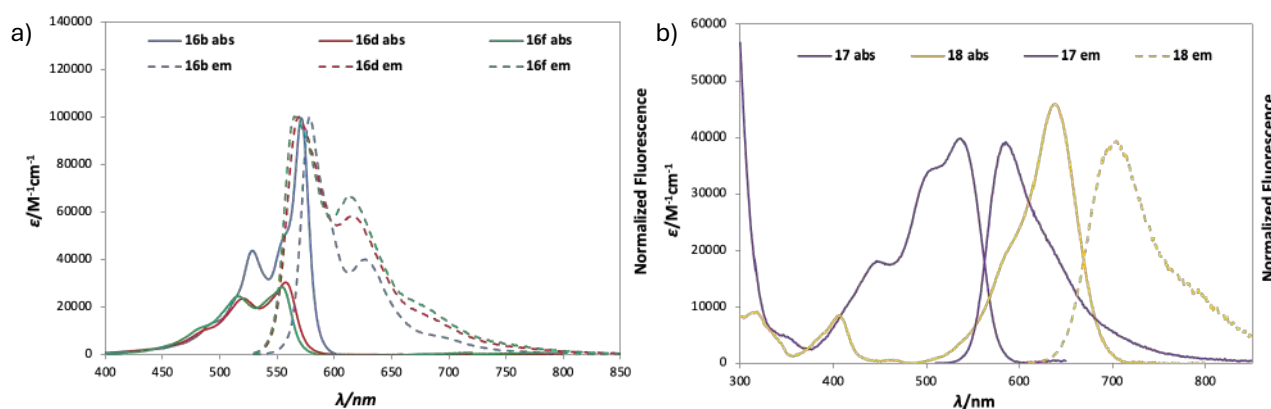


Figure 7: a) UV-vis absorption spectra (solid lines) and fluorescence spectra (dashed lines) of compounds **16b**, **16d**, and **16f** measured in DCM. b) UV-vis absorption spectra (solid lines) and fluorescence spectra (dashed lines) of compounds **17** and **18** measured in DCM.

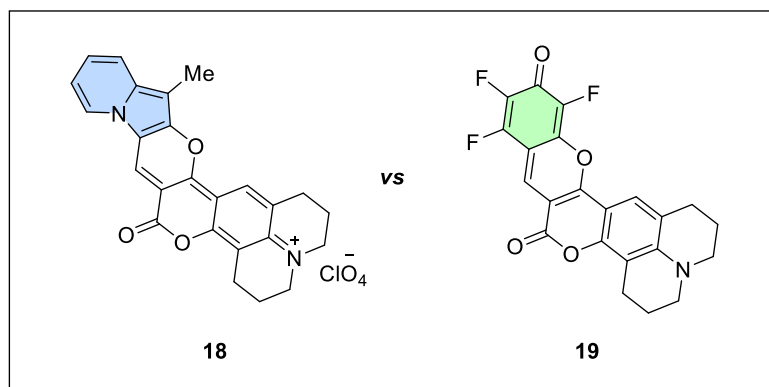


Figure 8: Structural comparison between an indolizine fused coumarin and a rhodol fused coumarin

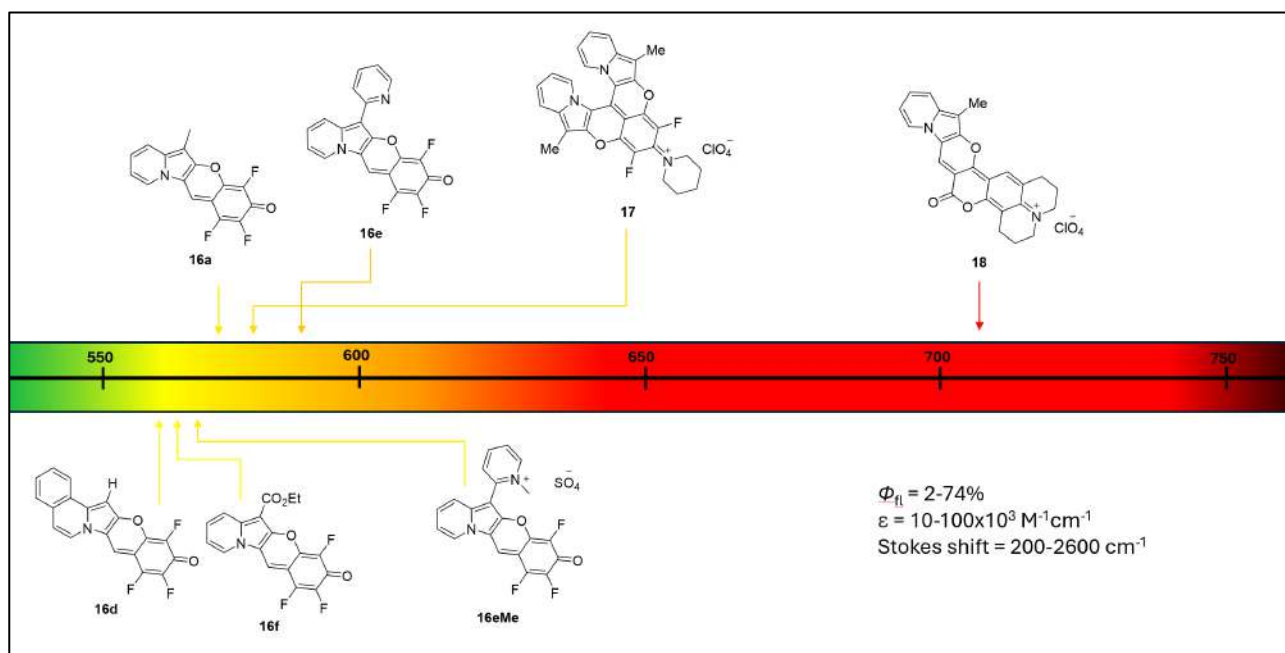


Figure 9: Visual comparison of emission of the *IndMer* derivatives in DCM.

3. Summary and Conclusions

In conclusion, over the past years, I have improved two existing methodologies for the synthesis of pyrano- and benzo-indolizines, discovered a novel approach for constructing an entirely new indolizine-fused xanthene system, and explored their photophysical properties.

In the first two cases, I succeeded in shortening the reaction time and eliminating a purification step by implementing a one-pot procedure, thereby saving both time and laboratory resources. These optimizations led to higher yields and allowed for significantly broader substrate (and product) scope. My equally important contribution to the field was the comprehensive photophysical characterization of the resulting structures, along with a rational analysis of how different substituents, positioned around the indolizine core, moderate these properties.

The final project, in contrast, involved not only a comprehensive photophysical investigations but also the discovery of a completely novel type of polymethine dyes which are at the same time π -expanded indolizines. This work was made possible by a deeper understanding of the reactivity and handling of highly reactive indolizin-ol intermediates gained during the second project. It also stemmed from the successful adaptation of a straightforward methodology, originally developed by our group in recent years for the synthesis of rhodols, by employing 2,3,5,6-tetrafluoro-4-hydroxybenzaldehyde as key component.

All the methodologies proposed in my projects for the synthesis of the indolizine core start from pyridines and rely on classical organic chemistry approaches, demonstrating their continued relevance even after decades from their development. The last two projects followed a Chichibabin-type mechanism, involving the formation of a pyridinium ylide that facilitates closure of the pyrrole ring, followed by π -system expansion. In contrast, the first project featured a final step of 1,5-electrocyclization, which was responsible for the formation of the indolizine core. This pericyclic reaction was enabled by prior extension of the conjugated system, made possible through a nucleophilic aromatic substitution ($\text{S}_{\text{N}}2\text{Ar}$) involving electron-deficient benzene derivatives and pyridinium salts via formation of a vinylpyridinium ylides. Yields were generally good across all reactions, averaging between 50% and 70%, with variations largely depending on the specific starting pyridine and its intrinsic reactivity.

Considering the photophysical properties of the synthesized products, their $\lambda_{\text{abs}}^{\text{max}}$ and $\lambda_{\text{em}}^{\text{max}}$ span the entire visible spectrum, from the blue to the red region. Pyrano-indolizines are the most blue-shifted, benzo-indolizines are the most red-shifted, and IndMer structures exhibit intermediate properties (Fig. 10). From a general point of view, any of the considered structural expansion led to a red shift in the photophysical properties and an increase in the fluorescence compared to the parent indolizine which has $\lambda_{\text{abs}} = 368 \text{ nm}$ and $\lambda_{\text{em}} = 498 \text{ nm}$ with Φ_{fl} of 14% in DCM.

When considering the impact of the substituents on the optical properties, substituents at position R^6 of the benzo-indolizines have a greater impact than those at position R^1 of the pyrano-indolizines and IndMer. In the latter cases, changes in the electronic nature of the substituent moderately influenced the photophysics. This observation is supported by computational studies, which showed that variations in the HOMO and LUMO energies of the pyrano-indolizine and IndMer derivatives are small, indicating limited electronic delocalization through those positions. While in the case of benzo-indolizines, the different size and character of substituents in R^6 change the geometry of the molecule thus changing their orbital energies.

This work also highlights the influence of isomerism, as expanded *[a]*indolizines having λ_{abs} around 500 nm and λ_{em} in the red region exhibit a greater red shift than the expanded *[b]*indolizine counterparts presented in the introduction, such as structure **1** ($\lambda_{\text{abs}} = 440$ nm, $\lambda_{\text{em}} = 490$ and 520 nm) and structure **2** ($\lambda_{\text{abs}} = 594$ nm, $\lambda_{\text{em}} = 621$ nm, $\Phi_{\text{fl}} = 17\%$).

Lastly, the solvatochromism observed for IndMer is less pronounced compared to the other discussed cores, due to the minimal increase in dipole moment upon excitation to the S_1 state relative to the S_0 state. As a result, the differential stabilization by solvents is limited, leading to a smaller solvent-dependent shift in the absorption and emission spectra.

To sum up, with my PhD research, I contributed to advancing the knowledge and understanding of the photophysical properties of two largely overlooked classes of compounds: **benzo[*a*]indolizines** and **pyrano[*b*]indolizines**. These scaffolds can be synthesized efficiently through short, one-pot procedures. In addition, I developed a straightforward methodology for the synthesis of a completely novel structure (**IndMer**) and provided a comprehensive analysis of its optical properties.

Out of the plethora of synthesized and characterized molecules few interesting structures stood out for their photophysical properties and ability to enable organelle visualization. Among them, compounds **15a** and **18** exhibited the most promising features.

Altogether, these findings highlight the significant potential of this class of fluorophores for bioimaging applications, as demonstrated by cell studies performed by our collaborators.

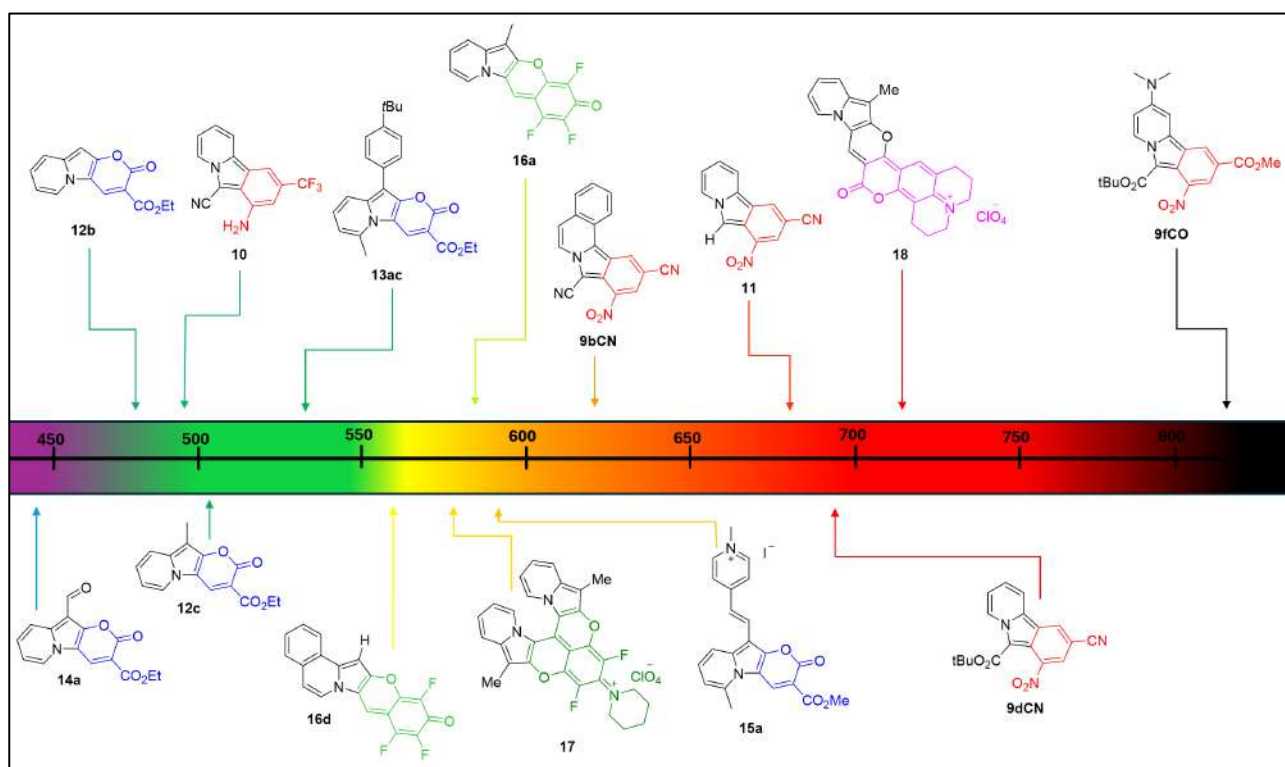


Figure 10: Visual comparison of the fluorescence emission of some representatives of **benzo[*a*]indolizines**, **pyrano[*b*]indolizines** and **IndMers** in DCM

References

- [1] E. Kim, Y. Lee, S. Lee, S. B. Park, "Discovery, Understanding, and Bioapplication of Organic Fluorophore: A Case Study with an Indolizine-Based Novel Fluorophore, Seoul-Fluor" *Acc. Chem. Res.* **2015**, *48*, 538–547.
- [2] A. Angeli, "Ueber die Einwirkung des Oxaldiäthylesters auf das Pyrrolmethylnketon" *Berichte Dtsch. Chem. Ges.* **1890**, *23*, 1793–1797.
- [3], "PubMed," can be found under <https://pubmed.ncbi.nlm.nih.gov/> (accessed 23 September 2025), **n.d.**
- [4] Y. Lee, *Systematic Exploration of Indolizine-Based Small Fluorescent Molecules: Synthesis, Analysis and Application*, Springer, Singapore, **2018**.
- [5] M. Scholtz, "Die Einwirkung von Essigsäureanhydrid auf α -Picolin" *Berichte Dtsch. Chem. Ges.* **1912**, *45*, 734–746.
- [6] A. E. Tschitschibabin, "Tautomerie in der Pyridin-Reihe" *Berichte Dtsch. Chem. Ges. B Ser.* **1927**, *60*, 1607–1617.
- [7] W. Flitsch in *Compr. Heterocycl. Chem.*, Elsevier, **1984**, pp. 443–495.
- [8] J. W. Daly, "Thirty Years of Discovering Arthropod Alkaloids in Amphibian Skin" *J. Nat. Prod.* **1998**, *61*, 162–172.
- [9] J. P. Michael, "Indolizidine and quinolizidine alkaloids" *Nat. Prod. Rep.* **2008**, *25*, 139–165.
- [10] G. S. Singh, "Recent progress in synthesis and bioactivity studies of indolizines" *Eur. J. Med. Chem.* **2011**.
- [11] C. Sandeep, K. N. Venugopala, M. A. Khedr, M. Attimarad, B. Padmashali, R. S. Kulkarni, R. Venugopala, B. Odhav, "Review on Chemistry of Natural and Synthetic Indolizines with their Chemical and Pharmacological Properties" *J. Basic Clin. Pharm.* **2017**, *8*.
- [12] C. Bailly, "Anticancer Properties of Lamellarins" *Mar. Drugs* **2015**, *13*, 1105–1123.
- [13] E. T. Borrows, D. O. Holland, "The chemistry of the pyrrocolines and the octahydropyrrocolines" *Chem. Rev.* **1948**, *42*, 611–643.
- [14] H. R. Ing, R. C. Elderfield (Ed.), *Heterocyclic Compounds*, WILEY, **1952**.
- [15] T. Uchida, K. Matsumoto, "Methods for the Construction of the Indolizine Nucleus*" *Synthesis* **1976**, *1976*, 209–236.
- [16] H. L. Blewitt, *Special Topics in Heterocyclic Chemistry*, Wiley, **n.d.**
- [17] F. J. Swinbourne, J. H. Hunt, G. Klinkert in *Adv. Heterocycl. Chem.*, Elsevier, **1979**, pp. 103–170.
- [18] B. Sadowski, J. Klajn, D. T. Gryko, "Recent advances in the synthesis of indolizines and their π -expanded analogues" *Org. Biomol. Chem.* **2016**, *14*, 7804–7828.
- [19] J. Hui, Y. Ma, J. Zhao, H. Cao, "Recent advances in the synthesis of indolizine and its derivatives by radical cyclization/cross-coupling" *Org. Biomol. Chem.* **2021**, *19*, 10245–10258.
- [20] S. Ghosh, K. Biswas, "Microwave-assisted synthesis of indolizine derivatives: Recent developments: A review (2003-present)" *Synth. Commun.* **2024**, *54*, 505–525.
- [21] A. A. Nevskaya, A. D. Zinoveva, E. V. Van der Eycken, L. G. Voskressensky, "Synthetic Strategies for the Construction of Indolizines and Indolizine-fused Compounds: Thienindolizines and Indolizinoindoles" *Asian J. Org. Chem.* **2023**, *12*, e202300359.
- [22] Priyanka, P. Rani, Kiran, J. Sindhu, "Indolizine: A Promising Framework for Developing a Diverse Array of C–H Functionalized Hybrids" *ChemistrySelect* **2023**, *8*, e202203531.
- [23] X. Liu, H. Fu, Q. Hu, H. Cao, "Recent Advances on the Construction of Functionalized Indolizine and Imidazo[1,2-a]pyridine Derivatives" *Chem. Rec.* **2024**, *24*, e202400135.

- [24] J. Huang, C. Li, X. Li, G. Li, Z. Yang, Y. Yu, Y. Xie, H. Huang, F. Yu, Z. He, "Recent Advances in the Development of Indolizine Scaffolds: Synthetic Methodology and Mechanistic Insights" *Chin. J. Chem.* **2025**, *43*, 315–348.
- [25] J. S. A. Badaro, B. Godlewski, D. T. Gryko, "Advances in the synthesis of indolizines and their π -expanded analogues: update 2016–2024" *Org. Chem. Front.* **2025**, *12*, 2860–2907.
- [26] C. R. de Souza, A. C. Gonçalves, M. F. Z. J. Amaral, "RECENT SYNTHETIC DEVELOPMENTS AND REACTIVITY OF AROMATIC INDOLIZINES" *Targets Heterocycl. Syst. Vol 20* **2016**, 365.
- [27] C.-H. Park, V. Ryabova, I. V. Seregin, A. W. Sromek, V. Gevorgyan, "Palladium-Catalyzed Arylation and Heteroarylation of Indolizines" *Org. Lett.* **2004**, *6*, 1159–1162.
- [28] M. Fraser, S. McKenzie, D. H. Reid, "Nuclear magnetic resonance. Part IV. The protonation of indolizines" *J. Chem. Soc. B Phys. Org.* **1966**, 44–48.
- [29] M. Fraser, A. Melera, B. B. Molloy, D. H. Reid, "651. Nuclear magnetic resonance. Part I. The structure of indolizinium salts" *J. Chem. Soc. Resumed* **1962**, 3288–3294.
- [30] W. L. F. Armarego, "C-1 and C-3 protonation of indolizines" *J. Chem. Soc. B Phys. Org.* **1966**, 191–194.
- [31] E. T. Borrows, D. O. Holland, J. Kenyon, "238. The chemistry of the pyrrocolines. Part III. Nitration" *J. Chem. Soc. Resumed* **1946**, 1077–1083.
- [32] J. A. Hickman, D. G. Wibberley, "Indolizines. Part V. The synthesis of 3-amino- and 3-acetamido-indolizines and their precursors, the 3-azo-, -nitroso-, -nitro-, and -acetyl-indolizines" *J. Chem. Soc. Perkin 1* **1972**, 2954–2958.
- [33] E. T. Borrows, D. O. Holland, J. Kenyon, "237. The chemistry of the pyrrocolines. Part II. Nitroso-derivatives of some substituted pyrrocolines" *J. Chem. Soc. Resumed* **1946**, 1075–1077.
- [34] W. B. Harrell, "Mannich bases from 1,2-diphenylindolizine: Ephedrine and methamphetamine as amine components" *J. Pharm. Sci.* **1970**, *59*, 275–275.
- [35] Y.-Z. Zhang, F.-T. Sheng, Z. Zhu, Z.-M. Li, S. Zhang, W. Tan, F. Shi, "Organocatalytic C3-functionalization of indolizines: synthesis of biologically important indolizine derivatives" *Org. Biomol. Chem.* **2020**, *18*, 5688–5696.
- [36] A. F. Kassir, N. Rad, P. Klochowicz, J. M. Granda, "Diversity-oriented chiral phosphoric acid catalyzed alkylation of indolizines with amins" *Org. Chem. Front.* **2024**, *11*, 7186–7198.
- [37] M. L. Bode, P. T. Kaye, R. George, "Indolizine studies. Part 3. Synthesis and dynamic NMR analysis of indolizine-2-carboxamides" *J. Chem. Soc. Perkin 1* **1994**, 3023–3027.
- [38] H. Liu, S. Ren, C. Ma, G. Shi, Y. Li, G. Duan, Y. Ge, "Copper-Promoted Direct Decarboxylative C3-Acylation of Electron-Rich Indolizines Using α -Keto Acids" *ChemistrySelect* **2022**, *7*, e202104426.
- [39] K. Watanabe, N. Terao, T. Niwa, T. Hosoya, "Direct 3-Acylation of Indolizines by Carboxylic Acids for the Practical Synthesis of Red Light-Releasable Caged Carboxylic Acids" *J. Org. Chem.* **2021**, *86*, 11822–11834.
- [40] E. T. Borrows, D. O. Holland, "127. The chemistry of the pyrrocolines. Part V. The action of Grignard reagents and of iodine on 3-acetyl-2-phenylpyrrocoline" *J. Chem. Soc. Resumed* **1947**, 670–672.
- [41] L. Sun, X. Zhang, Z. Li, J. Ma, Z. Zeng, H. Jiang, "A Versatile C–H Halogenation Strategy for Indole Derivatives under Electrochemical Catalyst- and Oxidant-Free Conditions" *Eur. J. Org. Chem.* **2018**, *2018*, 4949–4952.
- [42] J.-B. Xia, S.-L. You, "Synthesis of 3-Haloindolizines by Copper(II) Halide Mediated Direct Functionalization of Indolizines" *Org. Lett.* **2009**, *11*, 1187–1190.

- [43] O. Diels, K. Alder, "Synthesen in der hydroaromatischen Reihe. XVII. Mitteilung. („Dien-Synthesen" stickstoffhaltiger Heteroringe. 5. Dien-Synthesen des Pyridins, Chinolins, Chinaldins und Isochinolins.)" *Justus Liebigs Ann. Chem.* **1932**, 498, 16–49.
- [44] H. Zheng, H. Xiong, C. Su, H. Cao, H. Yao, X. Liu, "Photoinduced successive oxidative ring-opening and borylation of indolizines with NHC–boranes" *RSC Adv.* **2022**, 12, 470–474.
- [45] O. Diels, R. Meyer, "Synthesen in der hydroaromatischen Reihe. XXI. „Dien-Synthesen" stickstoffhaltiger Heteroringe. 8. Über den Verlauf der Dien-Synthese des Pyridins in methylalkoholischer Lösung" *Justus Liebigs Ann. Chem.* **1934**, 513, 129–145.
- [46] J. T. Kim, V. Gevorgyan, "Selective Partial Reduction of Various Heteroaromatic Compounds with Bridgehead Nitrogen via Birch Reduction Protocol" *J. Org. Chem.* **2005**, 70, 2054–2059.
- [47] M. Renard, J. Gubin, "Metallation of 2-phenylindolizine" *Tetrahedron Lett.* **1992**, 33, 4433–4434.
- [48] A. G. Kuznetsov, A. A. Bush, V. B. Rybakov, E. V. Babaev, "An Improved Synthesis of Some 5-Substituted Indolizines Using Regiospecific Lithiation" *Mol. J. Synth. Chem. Nat. Prod. Chem.* **2005**, 10, 1074–1083.
- [49] E. V. Babaev, N. I. Vasilevich, A. S. Ivushkina, "Efficient synthesis of 5-substituted 2-aryl-6-cyanoindolizines via nucleophilic substitution reactions" *Beilstein J. Org. Chem.* **2005**, 1, 9.
- [50] M. V. R. Reddy, M. R. Rao, D. Rhodes, M. S. T. Hansen, K. Rubins, F. D. Bushman, Y. Venkateswarlu, D. J. Faulkner, "Lamellarin α 20-Sulfate, an Inhibitor of HIV-1 Integrase Active against HIV-1 Virus in Cell Culture" *J. Med. Chem.* **1999**, 42, 1901–1907.
- [51] A. Pareek, M. Y. Mehboob, M. Cieplak, M. Majdecki, H. Szabat, K. Noworyta, P. Połczyński, M. Morawiak, P. S. Sharma, C. Foroutan-Nejad, P. Gawet, "Indoloindolizines: The Complete Story of a Polycyclic Aromatic Scaffold from Theoretical Design to Organic Field-Effect Transistor Applications" *J. Am. Chem. Soc.* **2025**, DOI 10.1021/jacs.4c16189.
- [52] D. B. Granger, Y. Mei, K. J. Thorley, S. R. Parkin, O. D. Jurchescu, J. E. Anthony, "Synthesis and Electrical Properties of Derivatives of 1,4-bis(trialkylsilyl)ethynylbenzo[2,3-b:5,6-b']diindolizines" *Org. Lett.* **2016**, 18, 6050–6053.
- [53] D. Miao, C. Aumaitre, J.-F. Morin, "Photochemical synthesis of π -extended ullazine derivatives as new electron donors for efficient conjugated D–A polymers" *J. Mater. Chem. C* **2019**, 7, 3015–3024.
- [54] S. Yi, D. Kim, W. Cho, J. H. Lee, J. H. Kwon, J. Kim, S. B. Park, "Rational Design of Pyrido[3,2-b]indolizine as a Tunable Fluorescent Scaffold for Fluorogenic Bioimaging" *JACS Au* **2024**, 4, 2896–2906.
- [55] B. Zhou, M. Guo, Q. Pan, M. Zhou, L. Xu, Y. Rao, K. Wang, B. Yin, J. Zhou, J. Song, "Rhodium-catalyzed annulation of pyrrole substituted BODIPYs with alkynes to access π -extended polycyclic heteroaromatic molecules and NIR absorption" *Org. Chem. Front.* **2021**, 8, 868–875.
- [56] M. He, N. Chen, J. Wang, S. Peng, "Rhodium-Catalyzed Regiodivergent [3 + 2] and [5 + 2] Cycloadditions of Quinolinium Ylides with Alkynes" *Org. Lett.* **2019**, 21, 5167–5171.
- [57] Y. Zhang, W. Wang, J. Sun, Y. Liu, "TEMPO-catalyzed decarboxylation reactions for the synthesis of 1,2-unsubstituted indolizines" *J. Heterocycl. Chem.* **2020**, 57, 210–217.
- [58] S. Tiwari, D. S. Rawat, "Regiodivergent Synthesis of Densely Functionalized Indolizines via (2 + 2 + 1) Cycloaddition" *J. Org. Chem.* **2023**, 88, 6805–6815.
- [59] B. Shen, B. Li, B. Wang, "Rh(III)-Catalyzed Oxidative Annulation Leading to Substituted Indolizines by Cleavage of C(sp²)–H/C(sp³)–H Bonds" *Org. Lett.* **2016**, 18, 2816–2819.

- [60] J.-Q. Zhang, D. Hu, J. Song, H. Ren, “[3 + 2]-Annulation of gem-Difluoroalkenes and Pyridinium Ylides: Access to Functionalized 2-Fluoroindolizines” *J. Org. Chem.* **2021**, DOI 10.1021/acs.joc.0c03041.
- [61] J. Zheng, X. He, H. Xu, H. Liu, W. Yang, “A formal [3 + 2] annulation reaction of propargyl sulfonium compounds and N-ylides: access to pyrrolo[2,1-a]quinolines, pyrrolo[2,1-a]phthalazines and indolizines” *Org. Biomol. Chem.* **2020**, *18*, 8867–8875.
- [62] S. Dong, J. Huang, H. Sha, L. Qiu, W. Hu, X. Xu, “Copper-catalyzed formal [1 + 2 + 2]-annulation of alkyne-tethered diazoacetates and pyridines: access to polycyclic indolizines” *Org. Biomol. Chem.* **2020**, *18*, 1926–1932.
- [63] V. K. Outlaw, J. Zhou, A. E. Bragg, C. A. Townsend, “Unusual blue-shifted acid-responsive photoluminescence behavior in 6-amino-8-cyanobenzo[1,2-b]indolizines” *RSC Adv.* **2016**, *6*, 61249–61253.
- [64] R. Robinson, J. E. Saxton, “611. A synthesis of substituted 2 : 3-benzpyrrocolines” *J. Chem. Soc. Resumed* **1950**, 3136–3140.
- [65] A. Citterio, M. Fochi, A. Marion, A. Mele, R. Sebastiano, M. Delcanale, “ChemInform Abstract: Synthesis of Naphtho[2,3-b]indolizine-6,11-dione Derivatives by Iodine Oxidation of 2-Alkyl-1,4-naphthoquinones in the Presence of Substituted Pyridines.” *ChemInform* **1999**, *30*, DOI 10.1002/chin.199907163.
- [66] E. F. Pratt, R. G. Rice, R. W. Luckenbaugh, “Reactions of Naphthoquinones with Malonic Ester and its Analogs. III. 1-Substituted Phthaloyl- and Phthaloylbenzopyrrocolines¹” *J. Am. Chem. Soc.* **1957**, *79*, 1212–1217.
- [67] W. Augstein, F. Kröhnke, “Synthesen des Benzo[a]- und des Naphtho[2.3-b]indolizin-Ringsystems” *Justus Liebigs Ann. Chem.* **1966**, 697, 158–170.
- [68] A. Kakehi, S. Ito, K. Nakanishi, K. Watanabe, M. Kitagawa, “Preparation of New Nitrogen-bridged Heterocycles. A Facile Synthetic Method of Pyrano[2,3-b]indolizinone Derivatives” *Bull Chem Soc Jpn* **1980**, *53*, 1115–1120.
- [69] D. A. Rusanov, S. Mutasim Alfadul, E. Yu. Portnyagina, E. A. Silyanova, N. A. Kuznetsov, K. E. Podpovetny, A. V. Samet, V. V. Semenov, M. V. Babak, “Toward ‘E-Ring-Free’ Lamellarin Analogues: Synthesis and Preliminary Biological Evaluation” *ChemBioChem* **2023**, *24*, e202300161.
- [70] C. S. L. Rathnamalala, J. N. Gayton, A. L. Dorris, S. A. Autry, W. Meador, N. I. Hammer, J. H. Delcamp, C. N. Scott, “Donor–Acceptor–Donor NIR II Emissive Rhodindolizine Dye Synthesized by C–H Bond Functionalization” *J. Org. Chem.* **2019**, *84*, 13186–13193.
- [71] W. E. Meador, E. Y. Lin, I. Lim, H. C. Friedman, D. Ndaleh, A. K. Shaik, N. I. Hammer, B. Yang, J. R. Caram, E. M. Sletten, J. H. Delcamp, “Silicon-RosIndolizine fluorophores with shortwave infrared absorption and emission profiles enable in vivo fluorescence imaging” *Nat. Chem.* **2024**, *16*, 970–978.
- [72] M. A. Saucier, N. A. Kruse, B. E. Seidel, N. I. Hammer, G. S. Tschumper, J. H. Delcamp, “Phospha-RosIndolizine Dye with Shortwave Infrared (SWIR) Absorption and Emission” *J. Org. Chem.* **2024**, *89*, 9092–9097.
- [73] J. S. A. Badaro, B. Koszarna, M. H. E. Bousquet, E. T. Ouellette, D. Jacquemin, D. T. Gryko, “The Kröhnke synthesis of benzo[a]indolizines revisited: towards small, red light emitters” *Org. Chem. Front.* **2022**, *9*, 1861–1874.
- [74] D. Reuschling, F. Kröhnke, “Ringschlüsse unter HNO₂-Abspaltung und C-C-Verknüpfung, II. Synthese neuer Ringsysteme” *Chem. Ber.* **1971**, *104*, 2103–2109.
- [75] S. M. McDonald, S. K. Mellerup, C. Barran, X. Wang, S. Wang, “Binding Modes and Reactivity of Pyrido[2,1- a]isoindole as a Neutral Carbon Donor with Main-Group and Transition-Metal Elements” *Organometallics* **2017**, *36*, 4054–4060.

- [76] B. Bardi, K. V. Vygranenko, B. Koszarna, O. Vakuliuk, Ł. Dobrzycki, D. T. Gryko, F. Terenziani, A. Painelli, "Novel Method for the Synthesis of Merocyanines: New Photophysical Possibilities for a Known Class of Fluorophores" *Chem Eur J* **2023**, 29, e202300979.

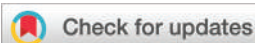


Original Publications and Authors' Contribution Declarations

RESEARCH ARTICLE

View Article Online

View Journal | View Issue

Cite this: *Org. Chem. Front.*, 2022, **9**, 1861The Kröhnke synthesis of benzo[*a*]indolizines revisited: towards small, red light emitters†Jaqueline S. A. Badaro,^a Beata Koszarna,^a Manon H. E. Bousquet,^b Erik T. Ouellette,^{c,d} Denis Jacquemin^b * and Daniel T. Gryko^a *

Benzo[*a*]indolizines with an ordered arrangement of various electron-withdrawing substituents (NO₂, CF₃, CN, CO₂R and COPh) were prepared directly from pyridinium salts and chloronitroarenes, allowing for refined control of the photophysical properties. Facile entry into almost unknown isoindolo[1,2-*a*]isoquinolines is disclosed to demonstrate the potential of this method. The rational manipulation of the substituents makes it possible to obtain electron-deficient dyes (HOMO $\approx$ -5.6 eV) with yellow, orange to red emission, large Stokes shifts (up to 9000 cm⁻¹) and fluorescence quantum yields reaching 0.58. Strong emission in spite of the presence of an NO₂ group has been rationalized by a large singlet-triplet energy gap combined with small spin-orbit couplings, hence small intersystem crossing. The ability to substitute various electron-withdrawing groups at multiple positions on this heterocyclic skeleton offers an unprecedented opportunity to study their effect on the fate of the molecules in the excited state.

Received 20th January 2022,

Accepted 9th February 2022

DOI: 10.1039/d2qo00097k

rsc.li/frontiers-organic

Introduction

Indolizines, although known since the 19th century,^{1–4} have always been overshadowed by regioisomeric indoles. Only during the last two decades was their chemistry rejuvenated, likely because of their potential as fluorophores,^{5–11} which is especially strong for π -expanded indolizines. In recent years, multiple new methods for indolizine synthesis have been revealed,^{12–19} nicely complementing older approaches.^{20–24}

There are a few basic classes of benzoindolizines: pyrrolo[1,2-*a*]quinolines,^{25–36} pyrrolo[2,1-*a*]isoquinoline,^{37–40} benzo[*b*]indolizine^{41–48} and benzo[*a*]indolizines (pyrido[2,1-*a*]isoindoles)^{49–61} (Fig. 1). The latter ones attracted our particular attention since they are considered as π -expanded isoindoles, which are rarely encountered. However, benzo[*a*]indolizines are reported to be very unstable under ambient conditions.⁴⁹ To overcome this instability issue, adding several electron-withdrawing groups (EWGs) could be an advantageous strat-

egy. At the same time, the presence of electron-withdrawing groups would polarize the π -system and induce bathochromic shifts in both the absorption and emission spectra.⁶² Taking this into account, we reasoned that a one-pot reaction with the potential to assemble a π -expanded indolizine core decorated with several EWGs would be an appreciated addition to the existing repertoire of benzo[*a*]indolizines synthetic methodologies. Inspired by a legoTM-type philosophy, our target strategy was to build a final scaffold from easily available building blocks.

In light of all these requirements, we identified the Kröhnke method for the synthesis of benzo[*a*]indolizines from pyridines, ω -bromoacetophenones, and picryl chloride (Scheme 1)^{52,63,64} as the most promising starting point. This methodology consists of three steps: (1) quaternization of pyridine with (Hal)CH₂(EWG) reagents; (2) *C*-arylation of the

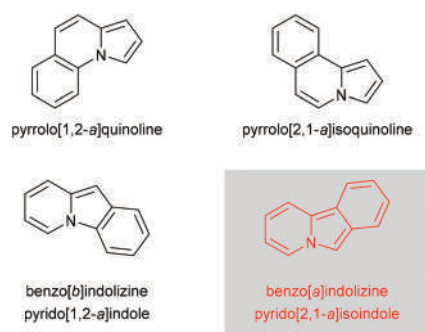


Fig. 1 Structures of various benzoindolizines.

^aInstitute of Organic Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw, Poland. E-mail: dtgryko@icho.edu.pl

^bCEISAM Lab—UMR 6230, CNRS, University of Nantes, Nantes, France.

E-mail: Denis.Jacquemin@univ-nantes.fr

^cDepartment of Chemistry, University of California, Berkeley, 420 Latimer Hall, Berkeley, CA, USA

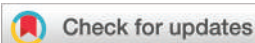
^dChemical Sciences Division, Lawrence Berkeley National Laboratory, 1 Cyclotron Road, Berkeley, CA, USA

† Electronic supplementary information (ESI) available: Synthetic optimization studies, crystallographic data, electrochemical and computational details, copies of NMR spectra. CCDC 2132628. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/d2qo00097k

RESEARCH ARTICLE

View Article Online

View Journal | View Issue

Cite this: *Org. Chem. Front.*, 2024, **11**, 6627

Strongly fluorescent indolizine-based coumarin analogs†

Jaqueline S. A. Badaro, ^a Antoni Wrzosek, ^b Olaf Morawski, ^c Adam Szewczyk, ^{*b} Irena Deperasińska ^{*c} and Daniel T. Gryko ^{*a}

It is now possible to prepare 2-oxo-2*H*-pyrano[2,3-*b*]indolizine-3-carboxylates with an ordered arrangement of various substituents directly from pyridinium salts and diethyl 2-(ethoxymethylene)malonate, allowing for refined control of their photophysical properties. Facile entry into some previously unknown derivatives is disclosed to demonstrate the potential of this method. The use of substituted picolinium salts, as well as further functionalization of the pyrrole ring, permitted easy introduction of new moieties upon the dye, which enabled fine-tuning of the photophysical properties. The obtained dyes possess absorption and emission spectrum in the blue–green region and fluorescence quantum yields reaching 92%. The parent 2-oxo-pyrano[2,3-*b*]indolizine-3-carboxylate turned out to be an electron-deficient system with a low-lying LUMO, an electronic transition energy of 2.7 eV and possessing a large oscillator strength. Almost complete overlap of the HOMO and LUMO in the 2-oxo-pyrano[2,3-*b*]indolizine core is responsible for the large fluorescence quantum yields for almost all prepared derivatives. The reason for maintaining the large emission intensity in polar solvents is that the increase in the dipole moment is accompanied with a significant change in its orientation in space. Fluorescence imaging studies have proven that 2-oxo-pyrano[2,3-*b*]indolizines penetrate the membrane of living cells. A positively charged analog was synthesized and used to stain intracellular organelles in the H9c2 cell line. This compound did not penetrate the cell membrane, however after permeabilization, it specifically stained the nucleus.

Received 3rd July 2024,
Accepted 10th September 2024

DOI: 10.1039/d4qo01216j

rsc.li/frontiers-organic

Introduction

Pyrano[2,3-*b*]indolizin-2-ones are a unique class of heterocycles that, contrary to their analogues pyrano[3,2-*b*]indol-2-ones^{1–12} and pyrano[2,3-*b*]indol-2-ones,^{13–22} have been greatly ignored after they were synthesized by Kakehi *et al.* in the 80s^{23–25} (together with their isomers pyrano[3,2-*a*]indolizin-2-ones)^{25,26} (Fig. 1). However, their distinct structure, composed of a fused tricyclic system, in which a pyran ring is fused to an indolizine moiety, offers a wide range of opportunities for the design and development of novel compounds, exhibiting immense potential for applications in various fields, ranging from medicinal chemistry to materials science. Structurally, pyrano[2,3-*b*]indolizin-2-ones can be considered analogues of coumarins^{27–34} and benzocoumarins.^{35–42}

The synthesis of pyrano[2,3-*b*]indolizines was investigated by Kakehi and co-workers mainly from 1980 to 1998, driven by the need to establish an efficient and reliable methodology. Numerous synthetic routes have been explored, involving the use of pyridinium salts as starting materials and the formation of various intermediates, *e.g.* 3-bis(methylthio)methylene-2,3-dihydroindolizin-2-ones,²³ 2,3-dihydroindolizin-2-ones,²⁴ 3-vinylindolizines²⁵ as well as the hydrolysis of pyrano[2,3-*b*]indolizine-2-imines.⁴³ These synthetic efforts have resulted in the successful preparation of a diverse array of pyrano[2,3-*b*]

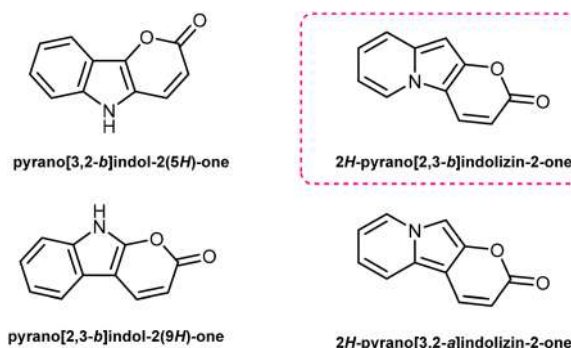


Fig. 1 Pyrano-indol-2-ones and pyrano-indolizin-2-ones.

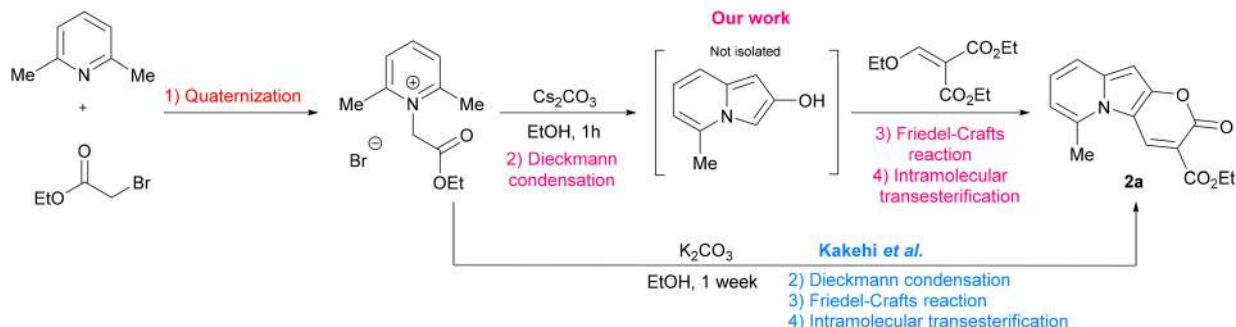
^aInstitute of Organic Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw, Poland. E-mail: dtgryko@icho.edu.pl

^bNencki Institute of Experimental Biology, Polish Academy of Sciences, Pasteur 3, 02-093 Warsaw, Poland. E-mail: a.szewczyk@nencki.gov.pl

^cInstitute of Physics, Polish Academy of Sciences, Al. Lotników 32/46, 02-668 Warsaw, Poland. E-mail: deper@ifpan.edu.pl

†Electronic supplementary information (ESI) available: NMR, UV/vis, fluorescence spectra and computational details. See DOI: <https://doi.org/10.1039/d4qo01216j>





Scheme 1 Synthesis of ethyl 6-methyl-2-oxo-pyrano[2,3-*b*]indolizine-3-carboxylate.

indolizine derivatives, expanding the chemical space available for further exploration and application.

In the context of search for novel fluorescent platforms we resolved to explore 2-oxo-pyrano[2,3-*b*]indolizine-3-carboxylates because of the easily available starting materials and the strong fluorescence emission claimed by the authors in their paper, but not sufficiently explored.²⁴ The Kakehi's synthesis (Scheme 1) consisted of four steps: (1) quaternization of 2,6-dimethylpyridine with ethyl 2-bromoacetate; (2) Dieckmann condensation to form the 5-methylindolizin-2-ol intermediate; (3) Friedel-Crafts reaction of the intermediate with diethyl 2-(ethoxymethylene)malonate; (4) final intramolecular cyclization *via* transesterification with the formation of the lactone ring and additional ethanol elimination. It is noteworthy that the last three steps occur in one pot. However, the drawback of this methodology is that a long reaction time (7 days) is required, and the yield is relatively poor (38%). By solving the synthetic drawbacks and shedding light on the properties of this class of compounds, we aimed to provide a comprehensive understanding of this intriguing heterocyclic system, thus paving the way for further research and development of novel compounds with enhanced properties and biological application.

Results and discussion

Synthesis

Optimization. Our investigation started with the synthesis of the dye **2a** (Scheme 2), and we used this reaction as a model for the optimization studies, Table 1. First, we attempted to decrease the reaction time by changing the solvent to DMF (entry 2), but the expected product was not detected; instead, **2i** was obtained. Then we tried NaOEt as base (entry 3), but the product was observed only in trace amounts. At this point, before proceeding to test alternative bases, we hypothesized that sequential addition of reagents, allowing first the formation of the 5-methylindolizin-2-ol intermediate (Scheme 2) and then the following steps, could be the key to increasing the yield. Different bases were evaluated (entries 4–6) and we eventually found that the use of Cs₂CO₃ along with sequential addition of the reagents turned out to be pivotal to obtain a yield of 50% (entry 6). Interestingly, Cs₂CO₃ turned out to be

the best base to promote the cyclization. This result can be partly attributed to the increased basicity strength *versus* that of K₂CO₃. And it is in line with some recently developed synthetic approaches.^{44,45–47}

Scope. Having established the optimal reaction conditions (Cs₂CO₃, EtOH, RT, 18 h), the substrate scope was evaluated. As shown in Scheme 2, different 'formal' picolinium salts, prepared according to literature procedure (**1a–f**)^{48–51} and used as such, reacted with Cs₂CO₃ to form the indolizin-2(3*H*)-one intermediate that, after addition of diethyl 2-(ethoxymethylene)malonate, successfully gave pyranoindolizines possessing a hydrogen or an alkyl substituent in position R⁵ (**2a**, **2b**, **2c**) in 5–50% yield.

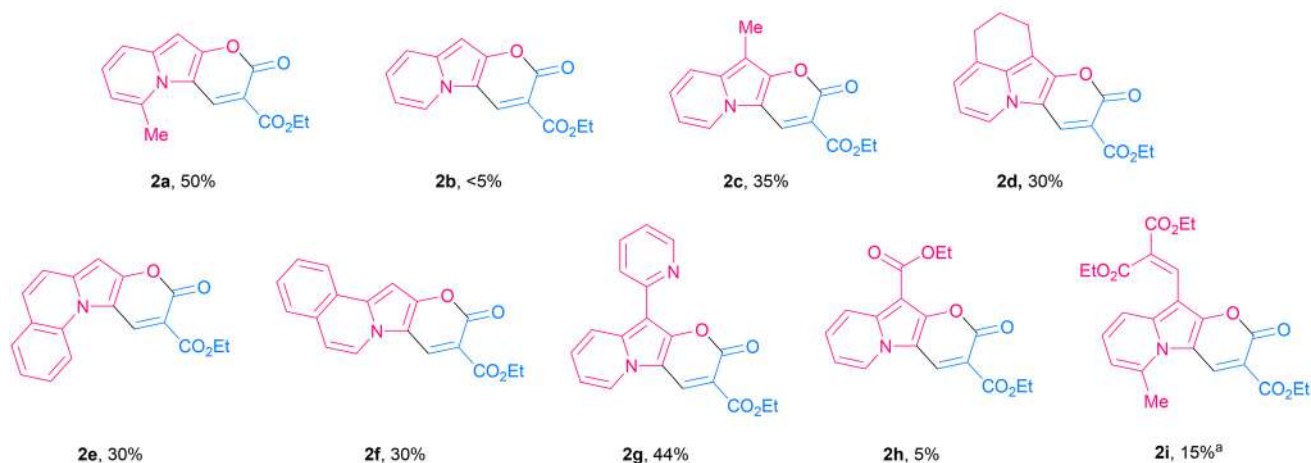
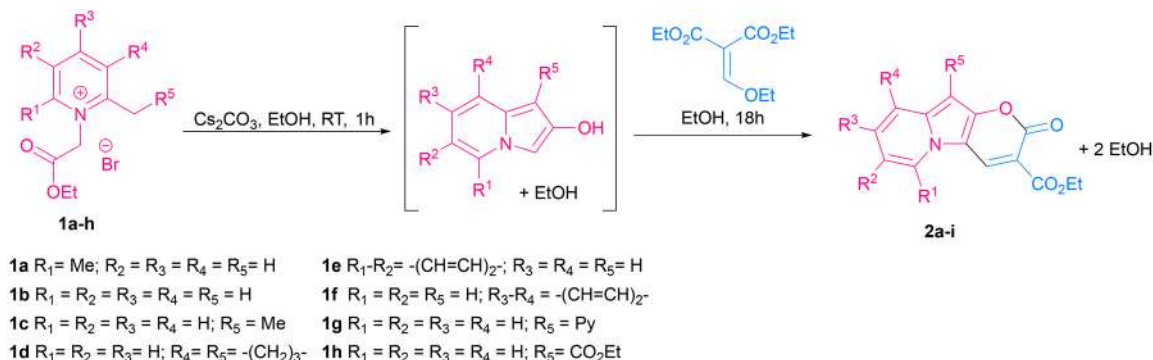
Consistent with what was observed by Kakehi and co-workers in 1986, the reaction with 2,3-cyclopentenopyridinium salts did not lead to the indolizine product,⁵² while the reaction with a 5,6,7,8-tetrahydroquinolinium salt led to the expected product **2d** in 30% yield. 2-Methylquinolinium and 1-methylisoquinolinium salts also reacted nicely and gave π -expanded indolizine products with a free R⁵ position (**2e**, **2f**) in 30 and 28% yield, respectively, while 3-methylisoquinolin-2-ium salt did not react as expected.

Additionally, α -picoline derivatives (**1g** and **1h**) bearing EWG groups (2-Py and CO₂Et) attached to the methylene group (R⁵) furnished the target product (**2g**, **2h**) in 44% and 5% yield respectively. It is noteworthy that in those cases, due to the highly acidic character of the proton at the α -position, the quaternization of the pyridine with ethyl 2-bromoacetate led to a 3-substituted indolizin-2-ol directly, even in the absence of base. In such cases the base was added later, only to promote the last two steps: nucleophilic substitution and transesterification.

Meanwhile, the reaction from a 4-bromopyridinium salt and diethyl 2-(ethoxymethylene)malonate did not produce the expected product, probably due to a less reactive indolizin-2-ol intermediate. Similarly, the reaction with a 4-dimethylaminopyridinium salt did not give the target product, in this case probably due to the difficulty in the formation of the indolizine intermediate caused by the effect of the electron-donating group, which turned the proton in the α -position less acidic, thus the reagent is less prone to cyclization.

Functionalization. The structure of indolizine **2a** brings about exciting possibilities to create π -expanded, polarized





Scheme 2 Scope of 2-oxo-pyrano[2,3-*b*]indolizine-3-carboxylate. ^a Obtained by simultaneous addition of malonate.

Table 1 Conditions for the optimization of 6-methyl-2-oxo-pyrano[2,3-*b*]indolizine-3-carboxylate

Entry	Base	Solvent	<i>T</i> /°C	Time/h	Yield %
1 ^a	K ₂ CO ₃	EtOH	RT	7 days	38
2 ^b	K ₂ CO ₃	DMF	RT	18 h	15% of 2i
3 ^b	NaOEt	EtOH	RT	18 h	Traces
4 ^c	KOH	EtOH	RT	18 h	30
5 ^c	LiOH	EtOH	RT	18 h	15
6 ^c	Cs ₂ CO ₃	EtOH	RT	18 h	50

^a Kakehi's methodology. ^b Simultaneous addition of malonate.

^c Postponed addition of malonate.

derivatives. Among a few possibilities, the presence of a highly electron-rich carbon β to the nitrogen was appealing to exploit (Scheme 3). To explore these possibilities, three additional derivatives (**3aa**, **3ab**, **3ac**) were obtained by direct arylation. As expected, bromoarenes with EWG in the *para* position, such as CN or NO₂, were more reactive in the direct arylation and the products were obtained in good yields (60% and 50%). In contrast, the bromoarene bearing an EDG, such as *t*Bu, gave the product **3ac** in poor yield (17%).

The Vilsmeier–Haack formylation also successfully occurred at the electron-rich position, resulting in the formation of **4a** in 83% yield.

To probe the potential of this class of dyes, Knoevenagel condensation was carried out between **4a** and 1,4-dimethylpyridin-1-ium iodide (**5**) (Scheme 3) forming a new styryl^{53–55} dye **6a**, which was obtained in 37% yield and later evaluated for cell staining (*vide infra*).

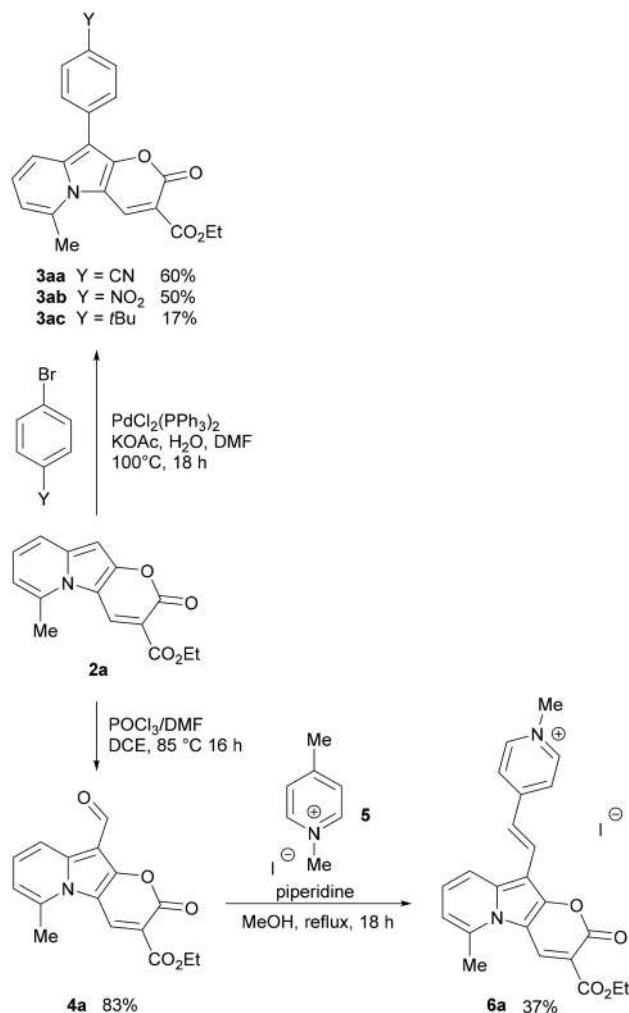
Considering the principles of green chemistry,⁵⁶ the reactions leading to compounds **2a–2i** and **4a** are considered to have moderate atom economy (AE) efficiency, in the range of 40–60%. Meanwhile, direct arylation products (**3aa–3ac**) and Knoevenagel condensation were obtained with AE efficiency above 80%, which is considered optimal according to the above principles. For all reactions, the reaction mass efficiency is very low, less than 10%, mainly due to the significant impact of the solvent in the calculation.

Photophysical properties

UV-Vis absorption and emission spectra were recorded in non-polar toluene (dielectric constant $\epsilon = 2.38$), moderately polar DCM ($\epsilon = 8.93$), and polar DMSO ($\epsilon = 46.45$) for all dyes, except **6a**, which was measured only in DCM and DMSO and will be discussed separately and correlated to its precursor **4a**.

A comparison between the photophysical parameters for 2-oxo-pyrano[2,3-*b*]indolizine-3-carboxylates and those available for similar structures (Fig. 2) such as 11-benzyl-7-hydroxyisochromeno[4,3-*b*]indol-5-one⁹ ($\Phi_{\text{fl}} = 42\%$ in DCM, with $\lambda_{\text{abs}} =$





Scheme 3 Synthesis of **3aa**, **3ab**, **3ac** and **4a** from **2a**, and synthesis of **6a** from **4a**.

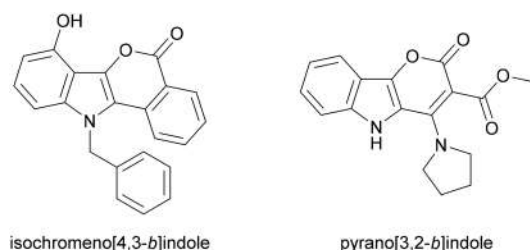


Fig. 2 Indoles structures used as reference to discuss photophysical properties of synthesized dyes.

382 and λ_{em} 496) or methyl 2-oxo-4-(pyrrolidin-1-yl)-2,5-dihydropyrano[3,2-*b*]indole-3-carboxylate⁵⁷ (λ_{abs} = 335 in EtOH) reveals a significant bathochromic shift for absorption, while emission and fluorescence quantum yield (Φ_{f}) remain in the same range.

The collected data of compounds in DCM are presented in Table 2 and the spectra of selected dyes are compared in

Table 2 Photophysical data of synthesized 2-oxo-pyrano[2,3-*b*]indolizine derivatives in DCM

Cmp	Solv	λ_{abs} [nm]	λ_{em} [nm]	$\Delta \nu$ [cm ⁻¹]	ϵ [M ⁻¹ cm ⁻¹]	Φ_{f}
2a	DCM	446	464	900	30 500	0.71
2b	DCM	449	470	1000	16 300	0.77
2c	DCM	468	494	1100	22 900	0.61
2d	DCM	467	499	1400	24 600	0.64
2e	DCM	456	470	700	39 700	0.73
2f	DCM	436	458	1100	39 700	0.33
2g	DCM	466	500	1500	17 600	0.92
2h	DCM	433	452	1000	29 200	0.81
2i	DCM	446	485	1800	21 000	0.25
3aa	DCM	453	490	1700	22 928	0.64
3ab	DCM	452	491/642	6500	27 700	0.03
3ac	DCM	457	514	2400	19 600	0.65
4a	DCM	426	448	1200	31 800	0.77
6a^a	DCM	509	575	2300	24 400	0.44

Fluorescence quantum yields measured with Coumarin 153 in EtOH as reference (Φ_{f} = 0.55). ^a Fluorescence quantum yield measured with Rhodamine 6G in EtOH as reference (Φ_{f} = 0.95).

Fig. 4, while the remaining data for the other solvents are available in Table S5 in ESI.† Absorption maxima (λ_{abs}) of the dyes obtained are located within 420–470 nm in all solvents, while the emission maxima (λ_{em}) falls between 440 and 520 nm for almost all of them and is more bathochromically shifted in polar solvents (solvatochromism). It is worth noting that compound **3ab** represents an exception to this trend in both DCM and DMSO, showing two emission bands overlapping, one at the ‘expected’ wavelength (491 nm) and another one around 642 nm. Excitation spectra were recorded to prove that both signals come from the same molecule and are not due to impurities in the sample. This property can be explained by hypothesizing that this structure, constituting of a strong donor, 2-oxo-pyrano[2,3-*b*]indolizine, and a strong acceptor, nitrophenyl, can undergo internal charge transfer (ICT) in polar solvents and generate two possible excited states according to the geometry and consequently allow for two possible emitting paths.

The fluorescence quantum yields (Φ_{f}) are in most cases on the same level regardless of the solvent and they vary among the studied 2-oxopyrano[3,2-*b*]indolizines from 0.92 for dye **2g** in DCM to being barely detectable for **3ab** in DCM and DMSO due to the preferred non-emissive decay (Fig. 3). Exceptions to this trend are **2c**, **2d**, **2g** that show lower fluorescence in toluene; and **3ab**, which shows a reverse trend by having the highest Φ_{f} in the less polar solvent. The comparison of the photophysical properties of **2a**, **2b** and **2c** (Fig. 4A), which vary their position of a methyl group (or is absent), reveals that this alkyl group at position R⁵ shifts both absorption and emission bathochromically (**2c** vs. **2b**) by about 20 nm, while the same group at position R¹ slightly shifts both signals hypsochromically (**2a** vs. **2b**).

Moreover, different electronic character of the substituent in R⁵ (Fig. 4B) influences both λ_{abs} and λ_{em} . If CHO is present (**4a**), λ_{abs} = 426 nm and λ_{em} = 448 nm, while if Me is present (**2c**), λ_{abs} = 468 nm and λ_{em} = 494. The presence of a



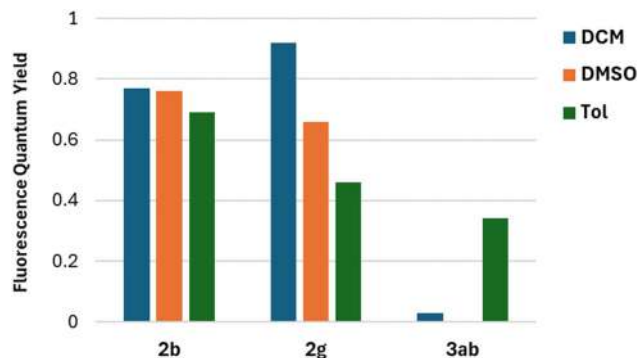


Fig. 3 Comparison of Fluorescence quantum yield in different solvents for representative compounds **2b**, **2g** and **3ab**.

π -extended system, as in **3aa** and **3ac**, slightly blue shifts the absorption. Additionally, having a *t*Bu group in the *para* position (**3ac**) red shifts the emission, consequently increasing the Stokes shift (2400 cm^{-1}). Interestingly, the effect of the π -expansion of the heterocyclic core strongly depends on the position of the fused benzene ring. In the case of dye **2f** a blue shift is observed accompanied with a decrease of the Φ_f (0.33 vs. 0.77) compared to **2b** (Fig. 4C).

The addition of a methylpyridinium conjugated through an alkene to the 2-oxo-pirano[2,3-*b*]indolizine system (**6a**) allowed for an increased dipole moment that translates to a bathochromic shift of 83 nm for λ_{abs} and almost 130 nm for λ_{em} when compared to **4a** (Fig. 4D). Even though a decrease in the fluorescence quantum yield (0.44 vs. 0.77) was observed comparing **6a** and **4a**, the emission is still high suggesting that, in comparison to **3ab**, the decay through a radiative channel is favoured.

Fluorescence kinetic studies were performed for selected compounds: **2a** representing “typical” 2-oxo-pyrano[2,3-*b*]indolizine, **3ab** exhibiting dual fluorescence and salt **6a**. In all solvents fluorescence of dyes **2a** and **6a** decays exponentially (Fig. S11†) with decay times, τ_F , about 3.5 and 2.0 ns respectively (Table S6†). For both compounds τ_F changes slightly with solvent's polarity (represented by dielectric constant, ϵ) and the change is parallel to that of Φ_f , – for **2a** both τ_F and Φ_f grow with increasing ϵ whereas for **6a** they decrease (Table S6†). Consequently, the radiative rate constant $k_r = \Phi_f / \tau_F$ of the $S_1 \rightarrow S_0$ transition remains constant and amounts to $1.3 \times 10^8\text{ s}^{-1}$ and $2.1 \times 10^8\text{ s}^{-1}$ respectively (Table S6†). Such high value is typical for the fully allowed $\pi\pi^*$ transitions. For dye **3ab** kinetic traces are complex. In non-polar toluene fluorescence decays exponentially (Fig. S12a†) with characteristic time 2.62 ns (Table S7†). Radiative rate is $8.8 \times 10^7\text{ s}^{-1}$, much less than values obtained for dyes **2a** and **6a**. For polar solvents where dual fluorescence from LE and CT states is observed emission decays are non-exponential and depend on wavelength of observation. For DCM the high energy band of the LE state with maximum at 487 nm two components are clearly visible in the decay trace (Fig. S12b†), and deconvolution yields 20 and 356 ps decay times. Intensity of emission origi-

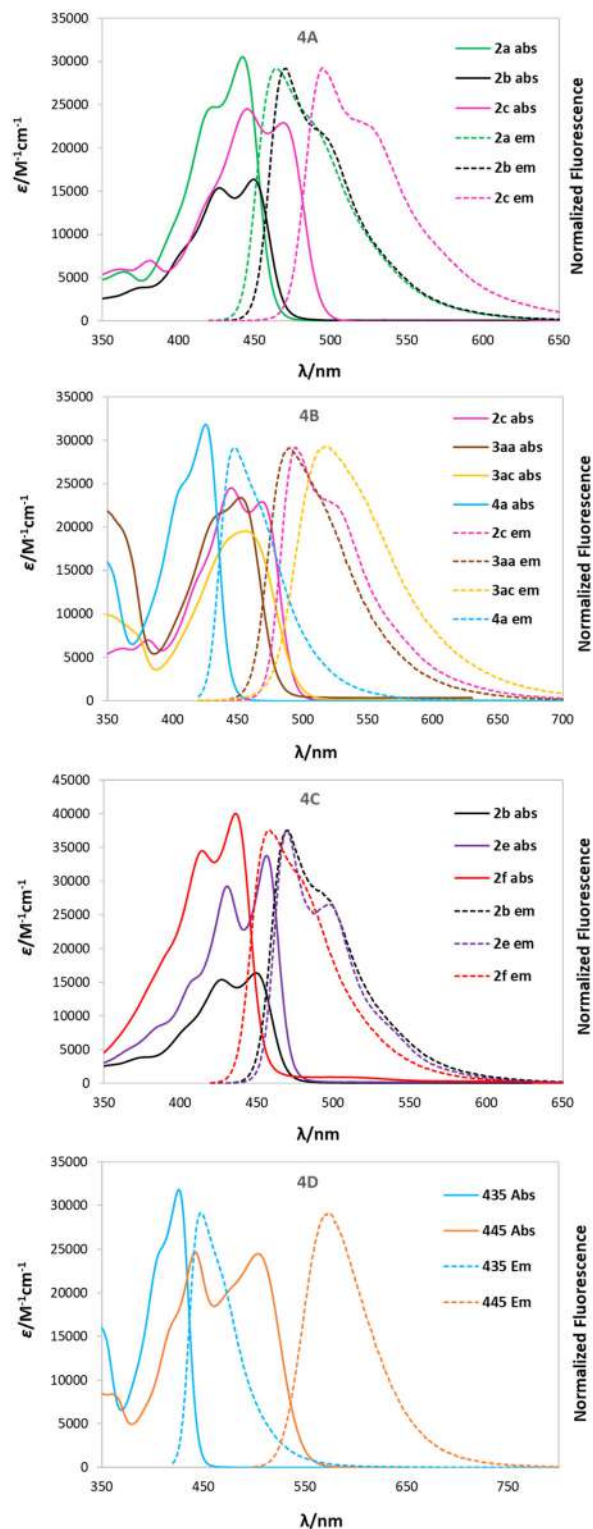


Fig. 4 Absorption and emission spectra of some representative 2-oxo-pyrano[2,3-*b*]indolizine-3-carboxylate derivatives in DCM. (A) Normalized absorption (solid line) and normalized emission (dashed line) of **2a**, **2b**, **2c** (green, black, pink) (B) normalized absorption (solid line) and normalized emission (dashed line) of **2c**, **3aa**, **3ac** and **4a** (pink, brown, yellow and light blue) (C) normalized absorption (solid line) and normalized emission (dashed line) of **2b**, **2e** and **2f** (black, purple, red) (D) normalized absorption (solid line) and normalized emission (dashed line) of **4a** and **6a** (light blue, orange).



inating from low energy CT band with maximum at 700 nm grows at early times and reaches maximum much later than the LE profile (Fig. S12b†). This points to precursor (LE) – successor (CT) scheme and suggests that that initial population of the CT state is null. Indeed, deconvolution reveals grow time of 55 ps and decay time of 356 ps (Table S7†). Negative amplitude of the grow time is comparable to the (positive) amplitude of the decay time what confirms that CT state is not directly excited and is populated from the LE state. This provides experimental proof for the TICT concept hypothesised below. Long component of the LE profile has same decay time as decay time of CT trace, pointing to thermalization of both states. However, grow time of CT is a bit longer than the short decay time of LE so one may expect presence of a transition state or a slow relaxation to emissive CT structure, so the simple two states model used commonly for TICT can be not adequate. In more polar DMSO kinetic profiles are faster (Fig. S12c†) and the LE decay time decreases down to 15 ps (Table S7†) pointing to the increase of the intramolecular CT rate constant. Formation of TICT enhances also non-radiative relaxation, hence the Φ_{fl} decreases by two orders of magnitude.

Theoretical analysis

The DFT and TDDFT O3LYP/6-31G(d,p) calculations were performed to corroborate the experimental data. The choice of the O3LYP functional allows for the best reconstruction of the spectral data in comparison to our attempts using other functionals (Table S1†). Calculations included the optimization of molecular structures in their ground (S_0) and lowest electronic excited (S_1) states along with the determination of the corresponding absorption and fluorescence energies. Corresponding data for the molecules in the three solvents, toluene, DCM and DMSO, are collected in Table S2.† The calculated dipole moments for the S_0 and S_1 states are summarized in Table S3.† The two highest occupied (HOMO's) and two lowest unoccupied molecular orbital (LUMO's) energy levels are shown in the diagram in Fig. S1,† while the values of HOMO and LUMO energies are given and compared in Table S4.† More detailed results of calculations are in the ESI.†

Dye **2b** lacking any substituents can be considered a model chromophore for this family of heterocycles (Scheme 2). This is a molecule with planar geometry in both S_0 , and S_1 , states. The electronic transition between them has a π - π^* character and essentially it is described by a single HOMO/LUMO electronic configuration. A diagram of the calculated electronic states of heterocycle **2b** with a short analysis is presented in ESI, Fig. S2.†

According to the computational results, the geometry of **2b** in the excited state undergoes relatively small changes. This result is consistent with the small Stokes shift observed experimentally. Small changes in geometry are manifested by small Franck–Condon factors (Fig. S3†) which well reproduce the experimental spectrum (Fig. 1). The small values of the calculated spin–orbit coupling elements (H_{so}) between the excited S_1 state and the energetically close triplet states (Fig. S2†) together with the small Franck–Condon factors are indicative

of relatively small rate constants of non-radiative processes,^{58,59} which in the experiment (Table 2) is visible as significant values of Φ_{fl} achieved for dye **2b**.

The calculation results show also, that **2b** is a molecule with a large dipole moment, increasing in the excited state (9.6 D for S_0 state and 12 D in S_1 – Table S3†). In this light, slight red-shifts of its electronic spectra (both observed, Table 2 and Table S5† and calculated, Table S2†) as the solvent polarity increases may seem surprisingly small. This phenomenon, has also been observed in the case of various other molecules,^{60,61} and can be explained with the use of classical theory of the solvent effect,^{60,61} and is related to the fact that dipole moments are vector quantities (and not scalars). When the increase in the dipole moment in the S_1 state (relative to the S_0 state) is accompanied by a change of orientation in space, the solvent effect is smaller than expected based on values alone (see Fig. S4†).

Fig. S2† shows the shapes of the HOMO and LUMO of molecule **2b**, providing high oscillator strength for the transition between the S_0 and S_1 states. They will constitute a reference orbital in the presentation of results for other 2-oxopyrano[3,2-*b*]indolizines.

The substitution of molecule **2b** causes changes in the energy of the molecular orbitals (Fig. S1†), including changes in the spacing between them (Table S4†). This is reflected by the shifts of the electronic spectra, since the electronic transition has an initial and final state, the magnitude of this shift depends on the energy changes in both states.

In Fig. 5, correlation between the absorption $S_0 \rightarrow S_1$ energy and the energy difference of the HOMO and LUMO for the studied 2-oxopyrano[2,3-*b*]indolizines is presented. This correlation demonstrates that transitions between the S_0 and S_1 states for all molecules, as in parent dye **2b**, are described by a single HOMO/LUMO electronic configuration. At the same

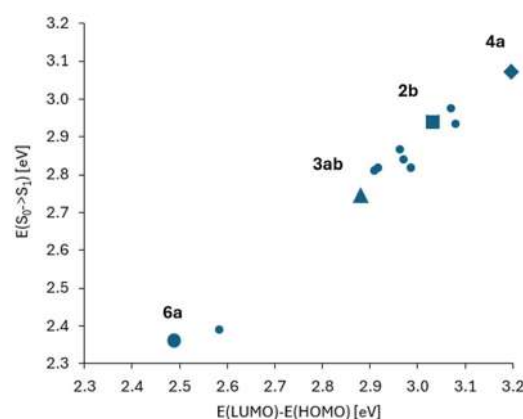


Fig. 5 Correlation between the absorption energy and the energy difference of HOMO and LUMO orbitals (numerical data in Tables S2 and S4†). The point corresponding to molecule **2b** is marked with a square. Two distant points (for lower energies, i.e. with red shift in comparison to **2b**) correspond to molecules **3ab** and **6a**. These two molecules also differ from the others in large values and changes of the dipole moment between S_0 and S_1 states (Table S3†).



time, two groups of molecules can be distinguished, the first with moderate changes in the HOMO/LUMO energy gaps and excitation energies (in relation to **2b**), and the other group of two molecules with large changes in these quantities.

Fig. 6 (part of the diagram in Fig. S1†) illustrates the variation of the $E(\text{HOMO})-E(\text{LUMO})$ gap and shows the shapes of these orbitals for four representatives of the studied family of 2-oxopyrano[2,3-*b*]indolizines: **2b** – as a model chromophore, **3ac** – a dye with observed red-shift in the absorption and fluorescence spectra (relative to the spectra of **2b**), **4a** – with a blue-shift of the spectra and dye **6a** – as a molecule with large red-shift of electronic spectra (see also Fig. 4).

Based on Fig. 6, it can be concluded that the effect of substitution of molecule **2b** produces two qualitatively different effects depending on the electron affinity of the substituent. In the case of compounds **3ac** and **4a**, this is a modification effect, *i.e.* their HOMO and LUMO are the modified (to a larger or smaller extent) HOMO and LUMO of molecule **2b**, but this is no longer the case of the LUMO of molecule **6a**. Although the HOMO of **6a** is still a modified HOMO of molecule **2b**, the LUMO is the orbital located on the substituent (while the orbital located on core **2b** lies above it, as shown in Fig. 6).

In addition to the diagram shown on Fig. 6, two other diagrams are shown in the ESI (Fig. S5 and S6†): HOMO/LUMO energy changes from methylation of the dye **2b** in different positions (**2a** vs. **2c**) and with π -extension (**2e** vs. **2f**) of **2b**. Methylation of **2b** at the R^5 position causes the largest change in HOMO energy leading to a red shift of the **2c** spectra.

In contrast to the substitution with a single bond, the π -expansion of **2b** becomes a problem not only at the level of

the HOMOs, but also at the level of the (so far untouched) LUMO of molecule **2b**.

Therefore, the reason for the large red shift of the **6a** (and **3ab**) spectra is a qualitative change of LUMO from localized on **2b** to localized on the substituent. The $S_0 \rightarrow S_1$ transition becomes, instead of the modified **2b** excitation, intramolecular charge-transfer between the core and the substituent.

Both orbitals are extended into the attached ring. The energy effect depends on the geometry in which the expansion is carried out ($E_{\text{LUMO}}(\mathbf{2e}) < E_{\text{LUMO}}(\mathbf{2f})$).

The calculation results for excited states indicate that most of the considered molecules retain features characteristic of parent dye **2b**. The fluorescence energies of the molecules optimized in the excited state are not very far from the absorption energies, and these transitions are described by high oscillator strengths (Table S1†). Although these results are in agreement with the experiments, (small Stokes shift, large emission efficiencies) these facts may seem surprising at first. This is because in these dyes there is a convenient relaxation path, which is a rotation around a single bond connecting the substituents with the heterocyclic core **2b**. We can expect two effects to play a role: a difference between the dihedral angles corresponding to the energy minimum in the ground and excited states, as well as the formation of rotational conformers. Differences between geometries optimized in the ground and excited states do indeed exist. For example, in systems containing a phenyl substituent at position R^5 , such as **3aa**, in the ground state, the aromatic ring makes an angle of 41.3° with the **2b** plane, while in the optimized excited state it makes an angle of 30.7° ; a similar rotation occurs in **3ac**, from 35.9° to 29.5° . However, they do not have a major impact on the spectroscopic properties because the LUMO is not extended to the substituent (Fig. 6). The exceptions are two molecules: **3ab** and **6a**, because their LUMOs are located on the substituent (Fig. 6, S7 and S8†). In both cases, the dark rotational conformers were optimised in the excited state (Table S1†). These conformers are characterised by zero oscillator strength for $S_1 \rightarrow S_0$ transition at the perpendicular arrangement of the planes of the heterocyclic core and the substituent ring. The difference between **6a** and **3ab** is that the dark form of **3ab** is the only form optimized in the excited state in polar DMSO. This result is consistent with experimental: while **6a** in this solvent emits with significant efficiency, the emission yield of **3ab** is extremely low. This means that the relaxation of **6a** to the dark state is slower than the decay *via* the radiative channel or inhibited by a barrier due to a more complex link between substituent and the heterocyclic core.

In turn, the bright form of **3ab** is experimentally observed and optimised in calculations for the S_1 state, in a non-polar medium only. Fig. S8† shows a diagram of electronic states of isolated molecule **3ab** with both optimised forms of the excited state. This is a diagram typical of TICT systems.⁶²

Overall, it is worth to acknowledge the consistency between experimental and theoretical results. The ratio of radiative rate constant of **2a** and **6a**, $1.3/2.1 = 0.62$ is very similar to the ratio

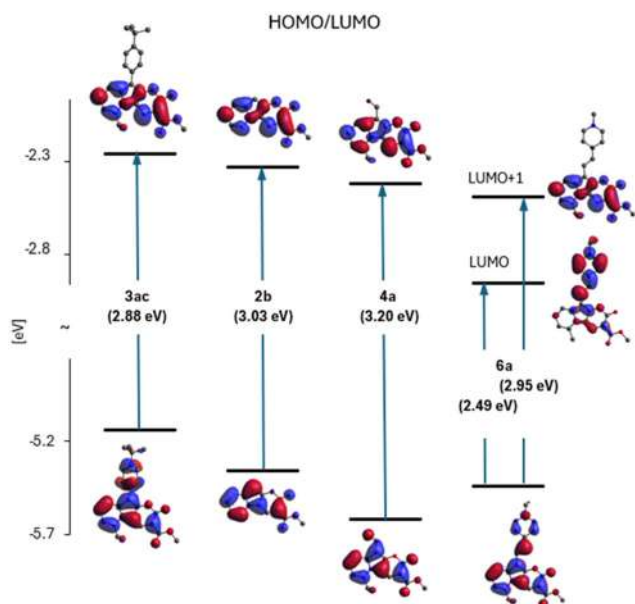


Fig. 6 Diagram of orbital energies: **2b** as the model heterocycle and its three derivatives, **3ac**, **4a** and **6a** in DMSO. The numbers in brackets indicate the size of the HOMO/LUMO energy gaps. In the case of **6a** two LUMOs are present in the considered energy region and in contrary to previous cases lowest of them is localised on substituent.



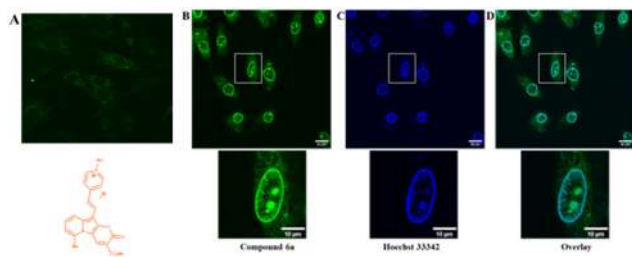


Fig. 7 Intracellular localization of compound **6a** in H9c2 cells detected using confocal fluorescence microscopy. (A) Image of the cells incubated with dye **6a** in a non-permeabilized cell membrane. Lower diagram structure of the dye. (B) Fluorescence of cells with digitonin ($100 \mu\text{g ml}^{-1}$) permeabilized membrane. (C) Fluorescence of permeabilized cell co-incubated with $0.5 \mu\text{M}$ Hoechst 33342 recorded with a 405 nm excitation wavelength and emission ranges from 420–520 nm. (D) Overlay of B and C. Lower pictures represents zoom upper square marked cells for B, C and D. The fluorescence of **6a** was recorded with a 473 nm excitation wavelength and emission ranges from 510–610 nm.

oscillator strength calculated for them, 0.64:1.06 (Tables S1 and S7†). Also, the radiative rate obtained experimentally for **3ab** is two/three times lower than for **2a/6a**, exactly like the oscillator strength calculated for **3ab** and **2a/6a** (Tables S1 and S7†). For **3ab** the theoretically predicted TICT mechanism is fully confirmed by fluorescence kinetic study. These as well as other correspondence between theory and experiment supports confidence for the HOMO and LUMO overlap statements.

Imaging studies

To prove the potential applicability of 2-oxo-pyrano[2,3-*b*]indolizines as a fluorescent probes, incubation of rat embryonic cardiomyoblast-derived cells H9c2 with selected dyes **2g**, **2c**, **4a** and **6a** was performed. The results showed that, neutral dyes from this family (*i.e.* **2g**, **2c** and **4a**) penetrate the living cell membrane and, as they are uncharged, accumulate nonspecifically in a wide range of cellular structures (Fig. S13†). In the case of dye **6a** which is a quaternary pyridinium salt, imaging experiments did not lead to accumulation of the dye intracellularly (Fig. 7A). To promote the access to the cytosol, H9c2 were permeabilized with digitonin ($100 \mu\text{g ml}^{-1}$). Addition of digitonin to the incubation medium resulted in permeabilization of the cell membrane and the successful penetration of the dye into the cytosol and cell organelles (Fig. 7B). It turned out that under these conditions, compound **6a**, as seen in the presented image (Fig. 7B), the dye was accumulated in the structure of the nucleus and the nuclear membrane. In order to compare the dye **6a** with the classical cell nuclear stain, co-staining with Hoechst 33342 was performed (Fig. 7C). It appears that the fluorescence of these two dyes overlaps except for some intranuclear structures, which are more strongly stained by pyranoindolizine **6a** (Fig. 7C and D). Additional intranuclear structures more strongly stained by the dye **6a**

may prove useful in distinguishing specific cell nuclear structures.

Conclusions

In conclusion, the synthesis of pyrano[2,3-*b*]indolizin-2-ones from picolinium salts and diethyl 2-(ethoxymethylene) malonate under basic conditions could be optimized to reach 50% yields for the one-pot procedure. The presence of an electron-rich pyrrolic position can be harnessed to open the avenue towards various novel derivatives. The presence of different substituents on the starting picoline as well as post-functionalization allowed for detailed studies on their impact on the photophysical properties in polar and non-polar solvents. It was found that the fluorescence intensity is large for all studied pyrano[2,3-*b*]indolizin-2-ones regardless of the substitution pattern which originates from the almost complete overlap of the HOMO and LUMO. Combined experimental and computational study enabled to conclude that pyrano[2,3-*b*]indolizin-2-one core can be considered as an electron-acceptor and in most of the derivatives examined in this work, the consequence of π -expansion was the extension of the HOMO and the modification of the electronic transition energy in the range of 0.2 eV. We discovered that a simultaneous increase in the dipole moment and change of its orientation in space is the reason for maintaining the large emission intensity as well as the small Stokes shift and solvatochromism, in polar solvents. Studies on the staining of the H9c2 cell line with exemplary 2-oxo-pyrano[2,3-*b*]indolizines has proven that uncharged dyes easily penetrate the living cells' membrane. On the other hand styryl derivative **6a** does not, it specifically stains structures associated with the cell nucleus of permeabilized cells. All reported findings may serve as the guide enabling modulation of this class of molecules towards the design of improved dyes for cell imaging applications.

Experimental section

General synthetic information

All reagents and solvents were purchased from commercial sources and used as received. Reactions with moisture and oxygen sensitive compounds were performed under an argon atmosphere. The progress of reactions was monitored by thin-layer chromatography (TLC), performed on aluminum foil plates, covered with silica gel 60 F₂₅₄ (Merck). Pure products were achieved by means of column chromatography with silica gel 60 (230–400 mesh). The identity and purity of final products were established by ^1H and ^{13}C NMR spectrometry as well as by MS-spectrometry (APCI-MS or ESI-MS). All reported ^1H NMR and ^{13}C NMR spectra were recorded on Varian AM 500 MHz, or Varian AM 600 MHz spectrometers. Chemical shifts (ppm) were determined with TMS as internal reference; *J* values are given in Hz. All melting points were measured with EZ-Melt apparatus.



Synthesis of precursors for pyrano[2,3-*b*]indolizin-2-ones

Pyridinium salts **1a**,⁴⁸ **1b**,⁴⁹ **1c**,⁵⁰ **1d**,⁴⁸ **1e**,⁵¹ **1f**,⁵⁰ and bis(2-pyridyl)methane **0g**⁶³ were synthesized according to previously published procedures, and spectroscopic data are consistent.

1-(2-Ethoxy-2-oxoethyl)-2,6-dimethylpyridin-1-ium bromide (1a). ¹H NMR (600 MHz, DMSO-*d*₆) δ 8.46 (t, *J* = 7.9 Hz, 1H), 7.99 (d, *J* = 7.9 Hz, 2H), 5.62 (s, 2H), 4.26 (q, *J* = 7.1 Hz, 2H), 2.78 (s, 6H), 1.25 (t, *J* = 7.1 Hz, 3H).

1-(2-Ethoxy-2-oxoethyl)-2-methylpyridin-1-ium bromide (1b). ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.01 (dd, *J* = 6.2, 1.3 Hz, 1H), 8.61 (td, *J* = 7.8, 1.5 Hz, 1H), 8.15 (d, *J* = 7.9 Hz, 1H), 8.08 (ddd, *J* = 7.7, 6.0, 1.5 Hz, 1H), 5.73 (s, 2H), 4.26 (q, *J* = 7.1 Hz, 2H), 2.77 (s, 3H), 1.26 (t, *J* = 7.1 Hz, 3H).

1-(2-Ethoxy-2-oxoethyl)-2-ethylpyridin-1-ium bromide (1c). ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.07 (d, *J* = 5.4 Hz, 1H), 8.65 (td, *J* = 7.9, 1.5 Hz, 1H), 8.15 (d, *J* = 7.9 Hz, 1H), 8.11–8.07 (m, 1H), 5.78 (s, 2H), 4.23 (q, *J* = 7.1 Hz, 2H), 3.05 (q, *J* = 7.4 Hz, 2H), 1.28 (t, *J* = 7.4 Hz, 3H), 1.24 (t, *J* = 7.1 Hz, 3H).

1-(2-Ethoxy-2-oxoethyl)-5,6,7,8-tetrahydroquinolin-1-ium bromide (1d). ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.90 (d, *J* = 6.1 Hz, 1H), 8.42 (d, *J* = 7.9 Hz, 1H), 7.97 (dd, *J* = 7.9, 6.2 Hz, 1H), 5.70 (s, 2H), 4.23 (q, *J* = 7.1 Hz, 2H), 3.00–2.97 (m, 4H), 1.90–1.84 (m, 2H), 1.76–1.70 (m, 2H), 1.23 (t, *J* = 7.1 Hz, 3H).

1-(2-Ethoxy-2-oxoethyl)-2-methylquinolin-1-ium bromide (1e). ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.31 (d, *J* = 8.5 Hz, 1H), 8.48–8.50 (m, 2H), 8.30–8.23 (m, 2H), 8.03 (t, *J* = 7.5 Hz, 1H), 6.12 (s, 2H), 4.28 (q, *J* = 7.1 Hz, 2H), 3.12 (s, 3H), 1.27 (t, *J* = 7.1 Hz, 3H).

2-(2-Ethoxy-2-oxoethyl)-1-methylisoquinolin-2-ium bromide (1f). ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.80 (d, *J* = 8.8 Hz, 1H), 8.70 (d, *J* = 6.9 Hz, 1H), 8.49 (d, *J* = 6.9 Hz, 1H), 8.33 (d, *J* = 8.1 Hz, 1H), 8.27 (dd, *J* = 7.5, 7.5 Hz, 1H), 8.08 (ddd, *J* = 8.4, 6.8, 1.3 Hz, 1H), 5.91 (s, 2H), 4.25 (q, *J* = 7.1 Hz, 2H), 3.21 (s, 3H), 1.25 (t, *J* = 7.1 Hz, 3H).

1-(Pyridin-2-yl)indolizin-2-ol (1g). In a round bottom flask, bis(2-pyridyl)methane (1.70 g, 10 mmol) was mixed with ethyl 2-bromoacetate (1.11 mL, 10 mmol) in ethanol and heated at 60 °C overnight. The pyridinium salt was not possible to isolate since the reaction spontaneously proceeded to the cyclization. After confirming the formation of the indolizine on TLC with Ehrlich's reagent, the solvent was evaporated, and the crude was used for the next reaction.

Ethyl 2-oxo-2,3-dihydroindolizine-1-carboxylate (1h). In a round bottom flask, ethyl 2-(pyridin-2-yl)acetate (1.61 mL, 10 mmol) was mixed with ethyl 2-bromoacetate (1.11 mL, 10 mmol) in ethanol and heated at 60 °C overnight. The pyridinium salt was not possible to isolate since the reaction spontaneously proceeded to the cyclization. After confirming the formation of the indolizine on TLC with Ehrlich's reagent, solvent was evaporated, and the crude was used for the next reaction.

Di(pyridin-2-yl)methane (0g). ¹H NMR (600 MHz, DMSO-*d*₆) δ 8.45 (ddd, *J* = 4.9, 1.9, 0.9 Hz, 2H), 7.64 (td, *J* = 7.6, 1.9 Hz, 2H), 7.26 (dt, *J* = 7.9, 1.2 Hz, 2H), 7.16 (ddd, *J* = 7.7, 4.9, 1.2 Hz, 2H), 4.23 (s, 2H).

General procedure for the synthesis of 2-oxo-2H-pyrano[2,3-*b*]indolizine-3-carboxylates (condition A)

In a 20 mL Schlenk flask, pyridinium salt (6 mmol, 1 equiv.) and Cs₂CO₃ (6 mmol, 1 equiv.) were added and dissolved in 5 mL of ethanol. After 1–2 hours a color change in the mixture was observed and fluorescence, typical for indoles, was visible when irradiated with UV lamp. Diethyl 2-(ethoxymethylene)malonate (6 mmol, 1 equiv.) was added, and the reaction mixture was stirred overnight at room temperature. After completion, the mixture was extracted with DCM, and the combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, AcOEt/hexane 1 : 1).

Ethyl 6-methyl-2-oxo-2H-pyrano[2,3-*b*]indolizine-3-carboxylate (2a). According to the general procedure (condition A), 1-(2-ethoxy-2-oxoethyl)-2,6-dimethylpyridin-1-ium bromide (500 mg, 1.82 mmol), Cs₂CO₃ (594 mg, 1.82 mmol) and diethyl 2-(ethoxymethylene)malonate (0.369 mL, 1.82 mmol) were reacted affording **2a** (250 mg, 50%) as yellow/orange crystals. *R*_f = 0.48 (SiO₂, AcOEt/hexane, 3 : 1); m.p. 189.0 °C. ¹H NMR (500 MHz, CDCl₃) δ 8.97 (s, 1H), 7.46 (d, *J* = 8.8 Hz, 1H), 7.25 (dd, *J* = 8.8, 7.0 Hz, 1H), 6.77 (d, *J* = 7.0 Hz, 1H), 6.43 (s, 1H), 4.41 (q, *J* = 7.1 Hz, 2H), 2.89 (s, 3H), 1.41 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 165.8, 158.3, 153.7, 142.1, 138.4, 138.1, 126.9, 117.0, 114.5, 112.7, 100.8, 89.0, 61.2, 22.0, 14.5 ppm. HRMS (ESI): *m/z* calcd for C₁₅H₁₃NO₄Na, 294.0742 [M + Na]⁺; found, 294.0741.

Ethyl 2-oxo-2H-pyrano[2,3-*b*]indolizine-3-carboxylate (2b). According to the general procedure (condition A), 1-(2-ethoxy-2-oxoethyl)-2-methylpyridin-1-ium bromide (0.500 mg, 2.77 mmol), Cs₂CO₃ (904 mg, 2.77 mmol) and diethyl 2-(ethoxymethylene)malonate (0.561 mL, 2.77 mmol) were reacted affording **2b** (20 mg, 5%) as yellow crystals. *R*_f = 0.32 (SiO₂, AcOEt/hexane, 3 : 1); m.p. 199.5 °C. ¹H NMR (500 MHz, CDCl₃) δ 8.81 (s, 1H), 8.27 (d, *J* = 6.9 Hz, 1H), 7.50 (d, *J* = 9.0 Hz, 1H), 7.26–7.20 (m, 1H), 6.90 (t, *J* = 6.9 Hz, 1H), 6.33 (s, 1H), 4.41 (q, *J* = 7.1 Hz, 2H), 1.41 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 165.1, 158.8, 152.9, 140.5, 133.7, 126.3, 124.6, 119.4, 113.0, 111.2, 101.9, 88.2, 61.3, 14.5. HRMS (ESI): *m/z* calcd for C₁₄H₁₁NO₄Na, 280.0586 [M + Na]⁺; found, 280.0583.

Ethyl 10-methyl-2-oxo-2H-pyrano[2,3-*b*]indolizine-3-carboxylate (2c). According to the general procedure (condition A), 1-(2-ethoxy-2-oxoethyl)-2-ethylpyridin-1-ium bromide (0.200 mg, 1.03 mmol), Cs₂CO₃ (335 mg, 1.03 mmol) and diethyl 2-(ethoxymethylene)malonate (0.208 mL, 1.03 mmol) were reacted affording **2c** (100 mg, 35%) as orange crystals. *R*_f = 0.54 (SiO₂, AcOEt/hexane, 3 : 1); m.p. 215.8 °C. ¹H NMR (500 MHz, CDCl₃) δ 8.76 (s, 1H), 8.23 (dt, *J* = 6.9, 1.1 Hz, 1H), 7.45 (dt, *J* = 9.0, 1.2 Hz, 1H), 7.21 (ddd, *J* = 9.0, 6.8, 1.1 Hz, 1H), 6.86 (td, *J* = 6.9, 1.2 Hz, 1H), 4.40 (q, *J* = 7.1 Hz, 2H), 2.30 (s, 3H), 1.41 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 165.3, 159.2, 150.7, 139.7, 133.1, 125.6, 124.5, 117.5, 112.6, 110.8, 100.8, 96.6, 61.1, 14.5, 6.2 ppm. HRMS (ESI): *m/z* calcd for C₁₅H₁₃NO₄Na, 294.0742 [M + Na]⁺; found, 294.0739.



Ethyl 8-oxo-5,6-dihydro-4*H*,8*H*-pyrano[2',3':4,5]pyrrolo[3,2,1-*ij*]quinoline-9-carboxylate (2d). According to the general procedure (**condition A**), 1-(2-ethoxy-2-oxoethyl)-5,6,7,8-tetrahydroquinolin-1-ium bromide (250 mg, 1.13 mmol), Cs₂CO₃ (370 mg, 1.13 mmol) and diethyl 2 (ethoxymethylene)malonate (0.229 mL, 1.13 mmol) were reacted affording **2d** (110 mg, 30%) as orange/red crystals. *R*_f = 0.48 (SiO₂, AcOEt/hexane, 3 : 1); m.p. 244.1 °C. ¹H NMR (600 MHz, CDCl₃) δ 8.76 (s, 1H), 8.05 (dd, *J* = 6.9, 0.8 Hz, 1H), 6.90 (dd, *J* = 6.9, 0.8 Hz, 1H), 6.79 (t, *J* = 6.8 Hz, 1H), 4.40 (q, *J* = 7.1 Hz, 2H), 2.93–2.86 (m, 4H), 2.03 (p, *J* = 6.1 Hz, 2H), 1.41 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (151 MHz, CDCl₃) δ 165.4, 159.3, 148.0, 139.5, 133.6, 130.8, 121.8, 121.5, 113.4, 111.5, 99.9, 98.3, 61.1, 27.4, 22.0, 19.8, 14.5 ppm. HRMS (ESI): *m/z* calcd for C₁₇H₁₅NO₄Na, 320.0899 [M + Na]⁺; found, 320.0902.

Ethyl 9-oxo-9*H*-pyrano[2',3':4,5]pyrrolo[1,2-*a*]quinoline-10-carboxylate (2e). According to the general procedure (**condition A**), 1-(2-ethoxy-2-oxoethyl)-2-methylquinolin-1-ium bromide (200 mg, 0.645 mmol), Cs₂CO₃ (210 mg, 0.645 mmol) and diethyl 2-(ethoxymethylene)malonate (0.130 mL, 0.645 mmol) were reacted affording **2e** (60 mg, 30%) as yellow crystals. *R*_f = 0.56 (SiO₂, AcOEt/hexane, 2 : 1); m.p. not measured, decomposed around 280 °C. ¹H NMR (500 MHz, CDCl₃) δ 9.36 (s, 1H), 8.32 (d, *J* = 8.5 Hz, 1H), 7.82 (dd, *J* = 7.9, 1.3 Hz, 1H), 7.77–7.72 (m, 1H), 7.54–7.48 (m, 2H), 7.41 (d, *J* = 9.2 Hz, 1H), 6.48 (s, 1H), 4.46 (q, *J* = 7.1 Hz, 2H), 1.45 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 165.5, 157.9, 154.3, 140.0, 138.0, 134.6, 130.6, 130.1, 127.8, 125.1, 124.3, 117.8, 115.6, 114.2, 103.5, 91.7, 61.6, 14.5 ppm. HRMS (APCI): *m/z* calcd for C₁₈H₁₃NO₄, 307.0845 [M⁺]; found, 307.0847.

Ethyl 10-oxo-10*H*-pyrano[2',3':4,5]pyrrolo[2,1-*a*]isoquinoline-9-carboxylate (2f). According to the general procedure (**condition A**), 2-(2-ethoxy-2-oxoethyl)-1-methylisoquinolin-2-ium bromide (250 mg, 0.806 mmol), Cs₂CO₃ (263 mg, 0.806 mmol) and diethyl 2-(ethoxymethylene)malonate (0.163 mL, 0.806 mmol) were reacted affording **2f** (70 mg, 28%) as yellow crystals. *R*_f = 0.64 (SiO₂, AcOEt/hexane, 3 : 1); m.p. 261.2 °C. ¹H NMR (600 MHz, CDCl₃) δ 8.79 (d, *J* = 0.8 Hz, 1H), 8.11–8.08 (m, 1H), 8.01 (d, *J* = 7.1 Hz, 1H), 7.74–7.69 (m, 1H), 7.65–7.58 (m, 2H), 7.11 (d, *J* = 7.2 Hz, 1H), 6.84 (s, 1H), 4.41 (q, *J* = 7.1 Hz, 2H), 1.41 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (151 MHz, CDCl₃) δ 165.0, 158.8, 152.9, 138.6, 134.0, 129.6, 129.2, 128.7, 127.6, 124.3, 121.0, 113.2, 112.5, 103.3, 88.8, 61.4, 14.4 ppm. HRMS (ESI): *m/z* calcd for C₁₈H₁₃NO₄Na, 330.0742 [M + Na]⁺; found, 330.0746.

Ethyl 2-oxo-10-(pyridin-2-yl)-2*H*-pyrano[2,3-*b*]indolizine-3-carboxylate (2g). According to the general procedure (**condition A**), 1-(pyridin-2-yl)indolizin-2(3*H*)-one (250 mg, 1.19 mmol), Cs₂CO₃ (387 mg, 1.19 mmol) and diethyl 2-(ethoxymethylene)malonate (0.240 mL, 1.19 mmol) were reacted affording **2g** (177 mg, 44%) as yellow/orange crystals. *R*_f = 0.37 (SiO₂, AcOEt/hexane, 2 : 1); m.p. 252.6 °C. ¹H NMR (500 MHz, CDCl₃) δ 9.02 (d, *J* = 9.1 Hz, 1H), 8.83 (s, 1H), 8.67–8.64 (m, 1H), 8.33 (d, *J* = 6.8 Hz, 1H), 8.23 (d, *J* = 8.1 Hz, 1H), 7.78 (td, *J* = 7.8, 1.9 Hz, 1H), 7.41 (ddd, *J* = 9.1, 6.9, 1.2 Hz, 1H), 7.16 (ddd, *J* = 7.5, 4.8, 1.1 Hz, 1H), 7.03 (td, *J* = 6.9, 1.3 Hz, 1H), 4.42 (q, *J* = 7.1 Hz,

2H), 1.43 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 164.8, 158.4, 151.4, 150.4, 148.9, 139.5, 136.7, 133.4, 127.9, 124.3, 122.6, 122.1, 120.9, 114.3, 111.1, 101.7, 101.2, 61.3, 14.5. HRMS (ESI): *m/z* calcd for C₁₉H₁₅N₂O₄, 335.1032 [M + H]⁺; found, 335.1031.

Diethyl 2-oxo-2*H*-pyrano[2,3-*b*]indolizine-3,10-dicarboxylate (2h). According to the general procedure (**condition A**), ethyl 2-oxo-2,3-dihydroindolizine-1-carboxylate generated *in situ* (404 mg, 2.00 mmol), Cs₂CO₃ (652 mg, 2.00 mmol) and diethyl 2 (ethoxymethylene)malonate (0.404 mL, 2.00 mmol) were reacted affording **2h** (35 mg, 5%) as yellow crystals. *R*_f = 0.43 (SiO₂, AcOEt/hexane, 3 : 1); m.p. 227.9 °C. ¹H NMR (500 MHz, CDCl₃) δ 8.82 (s, 1H), 8.43 (d, *J* = 8.8 Hz, 1H), 8.37 (d, *J* = 6.8 Hz, 1H), 7.50 (t, *J* = 8.8 Hz, 1H), 7.13 (t, *J* = 6.8 Hz, 1H), 4.42 (q, *J* = 24.5 Hz, 2H), 4.40 (q, *J* = 24.5 Hz, 2H), 1.43 (t, *J* = 21.3, Hz, 3H), 1.40 (t, *J* = 21.3, Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 164.5, 162.4, 157.6, 152.3, 140.8, 134.0, 129.1, 124.6, 120.4, 115.1, 110.8, 104.2, 93.8, 61.5, 60.5, 14.5, 14.4 ppm. HRMS (ESI): *m/z* calcd for C₁₇H₁₅NO₆Na, 352.0797 [M + Na]⁺; found, 352.0798.

General procedure for the synthesis of 2-oxo-2*H*-pyrano[2,3-*b*]indolizine-3-carboxylates (**condition B**)

In a 20 mL Schlenk flask, pyridinium salt (6 mmol, 1 equiv.), potassium carbonate (6 mmol, 1 equiv.) and diethyl 2-(ethoxymethylene)malonate (6 mmol, 1 equiv.) were added and dissolved in 10 mL of DMF. The reaction mixture changed color and was stirred for 24 hours at room temperature. After completion, the mixture was extracted with DCM, and the combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, AcOEt/hexane 1 : 1).

Diethyl 2-((3-(ethoxycarbonyl)-6-methyl-2-oxo-2*H*-pyrano[2,3-*b*]indolizin-10-yl)methylene)malonate (2i). According to the general procedure (**condition B**), 1-(2-ethoxy-2-oxoethyl)-2,6-dimethylpyridin-1-ium bromide (450 mg, 2.3 mmol), potassium carbonate (320 mg, 2.3 mmol) and diethyl 2-(ethoxymethylene)malonate (0.467 mL, 2.3 mmol) were reacted affording **2i** (100 mg, 15%) as yellow/orange crystals. *R*_f = 0.45 (SiO₂, AcOEt/hexane, 3 : 1); m.p. 210.5 °C. ¹H NMR (600 MHz, CDCl₃) δ 8.92 (s, 1H), 7.77 (s, 1H), 7.65 (d, *J* = 8.6 Hz, 1H), 7.36 (dd, *J* = 8.9, 7.1 Hz, 1H), 6.85 (d, *J* = 7.1 Hz, 1H), 4.53 (q, *J* = 7.1 Hz, 2H), 4.35 (q, *J* = 7.1 Hz, 2H), 4.25 (q, *J* = 7.2 Hz, 2H), 2.86 (s, 3H), 1.34 (t, *J* = 7.2 Hz, 3H), 1.32 (t, *J* = 7.2 Hz, 3H), 1.28 (t, *J* = 7.2 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 166.7, 165.1, 165.1, 156.5, 151.5, 141.6, 138.9, 138.1, 128.9, 128.7, 124.7, 116.4, 115.1, 113.3, 102.9, 96.9, 62.4, 61.6, 61.5, 22.1, 14.4, 14.2, 14.0 ppm. HRMS (APCI): *m/z* calcd for C₂₃H₂₂NO₈, 442.1345 [M – H][–]; found, 440.1350.

General procedure for direct arylation

A Schlenk flask flushed with argon, was charged with ethyl 6-methyl-2-oxo-2*H*-pyrano[2,3-*b*]indolizine-3-carboxylate (0.55 mmol, 1 equiv.), aryl bromide (0.66 mmol, 1.2 equiv.), potassium acetate (1.11 mmol, 2 equiv.), NMP (3 mL) and PdCl₂(PPh₃)₂ (0.027 mmol, 0.05 equiv.). The solution was



stirred at 100 °C for 10 min. Afterwards, water (1.11 mmol, 2 equiv.) was added, and the heating was continued at the same temperature overnight. Then, the mixture was cooled to room temperature and extracted with DCM. The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, AcOEt/hexane 1 : 1).

Ethyl 10-(4-cyanophenyl)-6-methyl-2-oxo-2H-pyrano[2,3-*b*]indolizine-3-carboxylate (3aa). According to the general procedure for direct arylation, ethyl 6-methyl-2-oxo-2H-pyrano[2,3-*b*]indolizine-3-carboxylate (0.55 mmol, 1 equiv.), 4-bromobenzonitrile (0.66 mmol, 1.2 equiv.), potassium acetate (1.11 mmol, 2 equiv.), NMP (3 mL), PdCl₂(PPh₃)₂ (0.027 mmol, 0.05 equiv.), and water (1.11 mmol, 2 equiv.) were made to react affording **3aa** (120 mg, 60%) as greenish yellow crystals. *R*_f = 0.45 (SiO₂, AcOEt/hexane, 3 : 1); m.p. 306.1 °C. ¹H NMR (600 MHz, CDCl₃) δ 9.06 (s, 1H), 7.84–7.81 (m, 2H), 7.80–7.77 (m, 3H), 7.38 (dd, *J* = 8.9, 7.0 Hz, 1H), 6.89 (d, *J* = 7.0 Hz, 1H), 4.42 (q, *J* = 7.1 Hz, 2H), 2.96 (s, 3H), 1.42 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (151 MHz, CDCl₃) δ 165.3, 157.4, 150.5, 139.4, 138.9, 138.3, 135.1, 132.7, 129.0, 128.0, 118.9, 115.6, 115.4, 112.5, 110.4, 102.3, 101.4, 61.5, 22.3, 14.4 ppm. HRMS (APCI): *m/z* calcd for C₂₂H₁₅N₂O₄, 371.1032 [M – H][–]; found, 371.1036.

Ethyl 6-methyl-10-(4-nitrophenyl)-2-oxo-2H-pyrano[2,3-*b*]indolizine-3-carboxylate (3ab). According to the general procedure for direct arylation, ethyl 6-methyl-2-oxo-2H-pyrano[2,3-*b*]indolizine-3-carboxylate (0.55 mmol, 1 equiv.), 1-bromo-4-nitrobenzene (0.66 mmol, 1.2 equiv.), potassium acetate (1.11 mmol, 2 equiv.), NMP (3 mL), PdCl₂(PPh₃)₂ (0.027 mmol, 0.05 equiv.), and water (1.11 mmol, 2 equiv.) were made to react affording **3ab** (25 mg, 50%) as orange crystals. *R*_f = 0.45 (SiO₂, AcOEt/hexane, 3 : 1); m.p. 284.3 °C. ¹H NMR (500 MHz, CDCl₃) δ 9.07 (s, 1H), 8.36 (d, *J* = 8.8 Hz, 2H), 7.89 (d, *J* = 8.8 Hz, 2H), 7.83 (d, *J* = 8.9 Hz, 1H), 7.40 (dd, *J* = 9.0, 7.0 Hz, 1H), 6.91 (d, *J* = 7.1 Hz, 1H), 4.43 (q, *J* = 7.1 Hz, 2H), 2.97 (s, 3H), 1.42 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 165.3, 157.3, 150.7, 146.3, 139.4, 139.0, 138.3, 137.2, 129.0, 128.2, 124.3, 115.8, 115.5, 112.5, 102.5, 101.1, 61.5, 22.3, 14.4 ppm. HRMS (APCI): *m/z* calcd for C₂₁H₁₅N₂O₆, 391.0930 [M – H][–]; found, 391.0932.

Ethyl 10-(4-(*tert*-butyl)phenyl)-6-methyl-2-oxo-2H-pyrano[2,3-*b*]indolizine-3-carboxylate (3ac). According to the general procedure for direct arylation, ethyl 6-methyl-2-oxo-2H-pyrano[2,3-*b*]indolizine-3-carboxylate (0.20 mmol, 1 equiv.), 1-bromo-4-(*tert*-butyl)benzene (0.26 mmol, 1.2 equiv.), potassium acetate (0.40 mmol, 2 equiv.), NMP (3 mL), PdCl₂(PPh₃)₂ (0.010 mmol, 0.05 equiv.), and water (0.40 mmol, 2 equiv.) were made to react affording **3ac** (14 mg, 17%) as orange/brown crystals. *R*_f = 0.56 (SiO₂, AcOEt/hexane, 2 : 1); m.p. 241.6 °C. ¹H NMR (600 MHz, CD₂Cl₂) δ 8.93 (s, 1H), 7.71 (d, *J* = 8.7 Hz, 1H), 7.57–7.51 (m, 2H), 7.51–7.46 (m, 2H), 7.24 (dd, *J* = 8.9, 6.9 Hz, 1H), 6.76 (d, *J* = 7.0 Hz, 1H), 4.27 (q, *J* = 7.1 Hz, 2H), 2.84 (s, 3H), 1.32–1.29 (m, 12H). ¹³C NMR (126 MHz, CDCl₃) δ 165.7, 158.1, 150.3, 140.1, 138.5, 137.9, 128.9, 128.5, 127.0, 126.8, 125.9, 116.1, 114.9, 112.5, 103.3, 101.1, 61.2, 34.7, 31.3, 22.2,

14.5 ppm. HRMS (APCI): *m/z* calcd for C₂₅H₂₆NO₄, 404.1862 [M + H]⁺; found, 404.1861.

Procedure for the synthesis of styryl dye

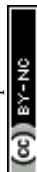
Ethyl 10-formyl-6-methyl-2-oxo-2H-pyrano[2,3-*b*]indolizine-3-carboxylate (4a). In a round bottom flask, POCl₃ (0.280 mL, 3 mmol) was added to dry DMF (0.310 mL, 4 mmol), at 0 °C. The ice bath was removed and the solution was stirred for 30 min. A solution of ethyl 6-methyl-2-oxo-2H-pyrano[2,3-*b*]indolizine-3-carboxylate (0.250 g, 0.92 mmol) in dry DCE (5 mL) was added and the mixture was stirred at 85 °C for 16 hours. After cooling to room temperature, an aqueous solution of saturated sodium acetate (3 mL) was added and heated again for 1 hour at 100 °C, then the reaction mixture was cooled to room temperature and the water-organic layer was extracted with DCM. The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. The crude product was purified by column chromatography (silica, AcOEt/hexane 1 : 1) affording **4a** (230 mg, 83%) as yellow crystals. *R*_f = 0.40 (SiO₂, AcOEt/hexane, 3 : 1); m.p. 241.4 °C. ¹H NMR (500 MHz, CDCl₃) δ 10.29 (s, 1H), 9.03 (s, 1H), 8.54 (d, *J* = 8.8 Hz, 1H), 7.60 (dd, *J* = 8.8, 7.2 Hz, 1H), 7.05 (d, *J* = 7.2 Hz, 1H), 4.43 (q, *J* = 7.1 Hz, 2H), 2.99 (s, 3H), 1.43 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 181.8, 164.8, 156.6, 155.8, 140.6, 139.1, 139.0, 131.5, 118.5, 118.0, 112.2, 103.7, 102.3, 61.7, 22.1, 14.4 ppm. HRMS (APCI): *m/z* calcd for C₁₆H₁₂NO₅, 298.0714 [M – H][–]; found, 298.0715.

4-Methylpyridinium iodide (5). Synthesis was conducted according to literature.⁶⁴ ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.81 (d, *J* = 6.4 Hz, 2H), 7.94 (d, *J* = 6.2 Hz, 2H), 4.26 (s, 3H), 2.58 (s, 3H).

(*E*)-4-(2-(3-(Methoxycarbonyl)-6-methyl-2-oxo-2H-pyrano[2,3-*b*]indolizin-10-yl)vinyl)-1-methylpyridin-1-ium iodide (6a). In a round bottom flask, 4-methylpyridinium iodide (18 mg, 0.17 mmol), ethyl 10-formyl-6-methyl-2-oxo-2H-pyrano[2,3-*b*]indolizine-3-carboxylate (51 mg, 0.17 mmol) and piperidine (0.017 mL) were dissolved in MeOH (4 mL) and refluxed overnight. After cooling down to room temperature, a precipitate is formed, filtered and washed with MeOH to afford ethyl 10-formyl-6-methyl-2-oxo-2H-pyrano[2,3-*b*]indolizine-3-carboxylate as red crystals **6a** (25 mg, 37%). M.p. 250.7 °C. ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.94 (s, 1H), 8.78 (d, *J* = 6.3 Hz, 2H), 8.34–8.19 (m, 4H), 7.68 (t, *J* = 7.9 Hz, 1H), 7.31 (d, *J* = 16.0 Hz, 1H), 7.20 (d, *J* = 7.1 Hz, 1H), 4.23 (s, 3H), 3.81 (s, 3H), 2.95 (s, 3H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 164.9, 156.7, 153.4, 151.7, 145.0, 141.4, 141.2, 139.5, 130.5, 129.0, 123.0, 121.2, 117.4, 115.6, 113.8, 101.1, 99.2, 52.5, 47.0, 21.5 ppm. HRMS (ESI): *m/z* calcd for C₂₂H₁₉N₂O₄, 375.1345.1501 [M⁺]; found, 375.1348.

Photophysical measurements

HPLC grade solvents were purchased from Sigma-Aldrich and used as obtained. For optical studies, solutions of molecules at low concentrations were used to avoid dimerization or reabsorption effects. All absorption and fluorescence spectra were taken at room temperature (21 °C). A Shimadzu UV/VIS-NIR



spectrophotometer, model UV-3600i Plus, was used for absorption spectra measurement. Fluorescence spectra were recorded with an FS5 spectrofluorometer from Edinburgh Instruments. Fluorescence quantum yields (Φ_f) of molecules in solvents at 21 °C were determined using Coumarin 153 in EtOH ($\Phi_f = 0.38$) and Rhodamine 6 g in EtOH ($\Phi_f = 0.95$) as references. Solutions of low absorbance ($A < 0.1$) were used to avoid reabsorption or concentration quenching. Refractive index correction for solvents have been performed in the calculations of quantum yields. Molar absorption coefficient, ϵ , was calculated from the absorbance, A , of a solution of the given molar concentration, c , in a cuvette of length, l , according to Lambert Beer formula:

$$A = c \epsilon l$$

Fluorescence kinetics studies were performed using the time correlated single photon counting technique. A mode-locked Coherent Mira-HP picosecond laser pumped by a Verdi 18 laser was used for excitation. The fundamental pulses of the Mira laser were up-converted to 387.5 nm. The temporal width of the excitation pulses was ~280 fs and the instrument response function (IRF) about 40 ps. Fluorescence was dispersed with a 0.25 m Jarrell-Ash monochromator and detected with a HMP-100-07 hybrid detector coupled to an SPC-150 PC module, (Becker&Hickl GmbH). Fluorescence decays were analyzed with deconvolution software using a nonlinear least squares procedure with the Marquardt algorithm. A standard χ^2 test and Durbin-Watson (DW) parameter along with residual and autocorrelation function plots were used to assess the quality of a fit. The estimated accuracy for the determination of decay time was below 10 ps.

Computational details

The calculations were performed using the Gaussian 16 program.⁶⁵ The solvent effect was introduced in PCM procedure. The SOC elements were also calculated using the Orca program.⁶⁶

Biological studies

The rat embryonic cardiomyoblast-derived H9c2 cells were loaded with the fluorophore at the final concentration of 500 nM in DMEM medium supplemented with 10% foetal bovine serum, 2 mM glutamine, 100 U ml⁻¹ penicillin, and 100 µg ml⁻¹ streptomycin, and incubated at 37 °C in a humidified atmosphere containing 5% CO₂ for 15 to 60 minutes. After that, the incubation medium was replaced with FluoroBrite™ DMEM. The measurements were performed with the use of an Olympus IX83 confocal microscope with the silicone objective 60× UPLSAPO 60XS. Registered data were transferred to the ImageJ and analysed for presentation.

Author contributions

Conceptualization: D. T. G. and J. S. A. B.; investigation: J. S. A. B., A. W., I. D.; supervision: D. T. G., A. S.; visualization:

J. S. A. B., A. W., I. D.; writing – original draft: J. S. A. B., A. W., I. D.; writing – review and editing: D. T. G., J. S. A. B.

All the authors discussed the results and commented on the manuscript.

Data availability

The data supporting this article have been included as part of the ESI.†

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

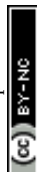
This work was supported by the Polish National Science Center, Poland (grants OPUS 2020/37/B/ST4/00017 and HARMONIA 2018/30/M/ST5/00460). This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement no 101007804. The work was financially supported by the Foundation for Polish Science (TEAM POIR.04.04.00-00-3CF4/16-00). Calculations were performed at the Interdisciplinary Center for Mathematical and Computational Modeling (ICM) University of Warsaw under computational allocation G95-1734. We thank Joseph Milton for amending the manuscript.

References

- 1 K. Sun, S. Jin, S. Fang, R. Ma, X. Zhang, M. Gao, W. Zhang, T. Lu and D. Du, N-Heterocyclic carbene-catalyzed formal [3 + 3] annulation of alkynoic acid esters with indolin-3-ones: access to functionalized pyrano[3,2-b]indol-2-ones, *Org. Chem. Front.*, 2019, **6**, 2291–2295.
- 2 X.-Y. Meng, M.-Y. Sun, F.-J. Zhao, Y.-J. Dang, B. Jiang and S.-J. Tu, Domino Reaction of 2,2-Dihydroxyindene-1,3-dione with Aromatic Amines: Efficient Synthesis of Isochromeno[4,3-b]indol-5(11H)-one Derivatives, *Synthesis*, 2014, 3207–3212.
- 3 N. S. Masterova, S. Yu. Ryabova, L. M. Alekseeva, M. I. Evstratova, S. S. Kiselev and V. G. Granik, Novel pyrano[3,2-b]indole derivatives: synthesis and some properties, *Russ. Chem. Bull.*, 2010, **59**, 637–641.
- 4 N. Monakhova, J. Korduláková, A. Vocat, A. Egorova, A. Lepioshkin, E. G. Salina, J. Nosek, E. Repková, J. Zemanová, H. Jurdáková, R. Górová, J. Roh, G. Degiacomi, J. C. Sammartino, M. R. Pasca, S. T. Cole, K. Mikušová and V. Makarov, Design and Synthesis of Pyrano[3,2-b]indolones Showing Antimycobacterial Activity, *ACS Infect. Dis.*, 2021, **7**, 88–100.



- 5 A. P. Gaywood and H. McNab, Methylene Meldrum's Acid Derivatives of Indoxyl and Their Cyclization Reactions under Flash Vacuum Pyrolysis Conditions, *Synthesis*, 2010, 1361–1364.
- 6 P. C. Unangst and R. E. Brown, Indole esters as heterocyclic synthons. II. Preparation of 1,3-oxazino[5,6-b]indoles and 3-substituted-pyrano[3,2-b]indoles, *J. Heterocycl. Chem.*, 1984, **21**, 283–288.
- 7 H. K. Kadam and S. G. Tilve, Unique synthesis of isochromenoindolone via reductive-oxidative cyclisation approach, *Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem.*, 2019, **58**, 104–108.
- 8 X. Zhang, W. Hou, D. Zhang-Negrerie, K. Zhao and Y. Du, Hypervalent Iodine-Mediated Intramolecular trans-Aminocarboxylation and Oxoaminocarboxylation of Alkynes: Divergent Cascade Annulations of Isocoumarins under Metal-Free Conditions, *Org. Lett.*, 2015, **17**, 5252–5255.
- 9 S. Pathak, D. Das, A. Kundu, S. Maity, N. Guchhait and A. Pramanik, Synthesis of 4-hydroxyindole fused isocoumarin derivatives and their fluorescence “Turn-off” sensing of Cu(II) and Fe(III) ions, *RSC Adv.*, 2015, **5**, 17308–17318.
- 10 J. L. Bullington and J. H. Dodd, Synthesis of tetrahydroindeno[1,2-b]indol-10-ones and their rearrangement to [2]benzopyrano[4,3-b]indol-5-ones, *J. Org. Chem.*, 1993, **58**, 4833–4836.
- 11 A. Sugimoto, K. Sakamoto, Y. Fujino, Y. Takashima and M. Ishikawa, Synthesis and Inhibitory Effect on Platelet Aggregation of 2-Phenyl-1 (2H)-phthalazinone Derivatives, *Chem. Pharm. Bull.*, 1985, **33**, 2809–2820.
- 12 A. J. Frew, G. R. Proctor and J. V. Silverton, Reactions of the sodium salts of some heterocyclic β -ketoesters with dimethyl acetylenedicarboxylate, *J. Chem. Soc., Perkin Trans. 1*, 1980, 1251–1256.
- 13 A. Arcadi, S. Cacchi, F. Marinelli and P. Pace, 5-Alkyl-5-[2-(o-iodophenylcarbamoyl)vinyl] Derivatives of Meldrum's Acid as Substrates for the Intramolecular Heck Reaction: Application to the Synthesis of Carbazoles, *Synlett*, 1993, 743–744.
- 14 N. L. Nam and I. I. Grandberg, Condensation of oxindole with acetoacetic ester and acetylacetone, *Chem. Heterocycl. Compd.*, 2006, **42**, 1010–1011.
- 15 W.-Z. Zhang, M.-W. Yang and X.-B. Lu, Carboxylative cyclization of substituted propenyl ketones using CO₂: transition-metal-free synthesis of α -pyrones, *Green Chem.*, 2016, **18**, 4181–4184.
- 16 G. H. Ko, C. Maeng, H. Jeong, S. H. Han, G. U. Han, K. Lee, H. C. Noh and P. H. Lee, Rhodium(III)-Catalyzed Sequential C–H Activation and Cyclization from N-Methoxyarylamides and 3-Diazooxindoles for the Synthesis of Isochromenoindolones, *Chem. – Asian J.*, 2021, **16**, 3179–3187.
- 17 S. Park, J. Lee, K. J. Shin, E. Oh and J. H. Seo, Consecutive One-Pot versus Domino Multicomponent Approaches to 3-(Diarylmethylene)oxindoles, *Molecules*, 2017, **22**, 503.
- 18 Q. Chen, Y. Teng and F. Xu, Lanthanide Silylamide-Catalyzed Synthesis of Pyrano[2,3-b]indol-2-ones, *Org. Lett.*, 2021, **23**, 4785–4790.
- 19 A. S. Kumar and R. Nagarajan, Cyclization Routes for the Synthesis of Functionalized Pyrano[2,3-b]indolones, Pyrazolo[3,4-b]indoles, and Furo[2,3-b]indoles, *Synthesis*, 2013, 1235–1246.
- 20 M. Abdel-Megid, A versatile molecule for the synthesis of some new polynuclear bioactive heterocyclic systems, *Int. J. Chem.*, 2006, **16**, 149–157.
- 21 R. Frutos-Pedreño and J.-A. García-López, 2-Arylacetamides as Versatile Precursors for 3-Aminoisocoumarin and Homophthalimide Derivatives: Palladium-Catalyzed Cascade Double Carbonylation Reactions, *Adv. Synth. Catal.*, 2016, **358**, 2692–2700.
- 22 X. Peng, Y. Huang, W. Wang, S. Li, G. Hao, S. Ren and Y. R. Chi, N-Heterocyclic Carbene-Catalyzed Remote Enantioselective C–C Bond Formation via 1,6-Addition with Formyl Enynes, *ACS Catal.*, 2024, **14**, 2127–2133.
- 23 A. Takehi, S. Ito, K. Nakanishi, K. Watanabe and M. Kitagawa, Preparation of New Nitrogen-bridged Heterocycles. A Facile Synthetic Method of Pyrano[2,3-b]indolizinone Derivatives, *Bull. Chem. Soc. Jpn.*, 1980, **53**, 1115–1120.
- 24 A. Takehi, S. Ito, K. Watanabe, M. Kitagawa, S. Takeuchi and T. Hashimoto, Preparation of new nitrogen-bridged heterocycles. Synthesis and some reactions of 2,3-dihydroindolizin-2-one derivatives, *J. Org. Chem.*, 1980, **45**, 5100–5104.
- 25 A. Takehi, S. Ito, S. Murakami and H. Sano, Preparation of New Nitrogen-bridged Heterocycles. 8. Syntheses of Some Fused Indolizine Derivatives via the Acid-catalyzed Cyclizations of Functionalized 1- and 3-Vinylindolizines, *Bull. Chem. Soc. Jpn.*, 1984, **57**, 548–552.
- 26 A. Takehi, S. Ito and K. Matsubara, Preparation of New Nitrogen-Bridged Heterocycles. 39. One-Pot Synthesis of 2H-Pyrano[3,2-a]indolizin-2-one Derivatives, *Bull. Chem. Soc. Jpn.*, 1995, **68**, 2409–2415.
- 27 G. Jones, W. R. Jackson and A. M. Halpern, Medium effects on fluorescence quantum yields and lifetimes for coumarin laser dyes, *Chem. Phys. Lett.*, 1980, **72**, 391–395.
- 28 J. A. Van Gompel and G. B. Schuster, Photophysical behavior of ester-substituted aminocoumarins: a new twist, *J. Phys. Chem.*, 1989, **93**, 1292–1295.
- 29 B. B. Raju and S. M. B. Costa, Photophysical properties of 7-diethylaminocoumarin dyes in dioxane–water mixtures: hydrogen bonding, dielectric enrichment and polarity effects, *Phys. Chem. Chem. Phys.*, 1999, **1**, 3539–3547.
- 30 M. Sulpizi, P. Carloni, J. Hutter and U. Rothlisberger, A hybrid TDDFT/MM investigation of the optical properties of aminocoumarins in water and acetonitrile solution, *Phys. Chem. Chem. Phys.*, 2003, **5**, 4798–4805.
- 31 K. Azuma, S. Suzuki, S. Uchiyama, T. Kajiro, T. Santa and K. Imai, A study of the relationship between the chemical structures and the fluorescence quantum yields of coumarins, quinoxalinones and benzoxazinones for the devel-



- opment of sensitive fluorescent derivatization reagents, *Photochem. Photobiol. Sci.*, 2003, **2**, 443–449.
- 32 M. El-Kemary and W. Rettig, Multiple emission in coumarins with heterocyclic substituents, *Phys. Chem. Chem. Phys.*, 2003, **5**, 5221–5228.
 - 33 X. Liu, Z. Xu and J. M. Cole, Molecular Design of UV-vis Absorption and Emission Properties in Organic Fluorophores: Toward Larger Bathochromic Shifts, Enhanced Molar Extinction Coefficients, and Greater Stokes Shifts, *J. Phys. Chem. C*, 2013, **117**, 16584–16595.
 - 34 M. Cavazzini, S. Quici, S. Orlandi, C. Sissa, F. Terenziani and A. Painelli, Intimately bound coumarin and bis(alkylaminostyryl)benzene fragments: synthesis and energy transfer, *Tetrahedron*, 2013, **69**, 2827–2833.
 - 35 D. Kim, S. Singha, T. Wang, E. Seo, J. H. Lee, S.-J. Lee, K. H. Kim and K. H. Ahn, In vivo two-photon fluorescent imaging of fluoride with a desilylation-based reactive probe, *Chem. Commun.*, 2012, **48**, 10243–10245.
 - 36 I. Kim, D. Kim, S. Sambasivan and K. H. Ahn, Synthesis of π -Extended Coumarins and Evaluation of Their Precursors as Reactive Fluorescent Probes for Mercury Ions, *Asian J. Org. Chem.*, 2012, **1**, 60–64.
 - 37 D. Kim, Q. P. Xuan, H. Moon, Y. W. Jun and K. H. Ahn, Synthesis of Benzocoumarins and Characterization of Their Photophysical Properties, *Asian J. Org. Chem.*, 2014, **3**, 1089–1096.
 - 38 Y. W. Jun, H. R. Kim, Y. J. Reo, M. Dai and K. H. Ahn, Addressing the autofluorescence issue in deep tissue imaging by two-photon microscopy: the significance of far-red emitting dyes, *Chem. Sci.*, 2017, **8**, 7696–7704.
 - 39 Y. J. Reo, Y. W. Jun, S. W. Cho, J. Jeon, H. Roh, S. Singha, M. Dai, S. Sarkar, H. R. Kim, S. Kim, Y. Jin, Y. L. Jung, Y. J. Yang, C. Ban, J. Joo and K. H. Ahn, A systematic study on the discrepancy of fluorescence properties between in solutions and in cells: super-bright, environment-insensitive benzocoumarin dyes, *Chem. Commun.*, 2020, **56**, 10556–10559.
 - 40 Y. Jung, N. K. Park, S. Kang, Y. Huh, J. Jung, J. K. Hur and D. Kim, Latent turn-on fluorescent probe for the detection of toxic malononitrile in water and its practical applications, *Anal. Chim. Acta*, 2020, **1095**, 154–161.
 - 41 A. Mukhopadhyay, T. Hossen, I. Ghosh, A. L. Koner, W. M. Nau, K. Sahu and J. N. Moorthy, Helicity-Dependent Regiodifferentiation in the Excited-State Quenching and Chiroptical Properties of Inward/Outward Helical Coumarins, *Chem. – Eur. J.*, 2017, **23**, 14797–14805.
 - 42 A. Mukhopadhyay, V. K. Maka and J. N. Moorthy, Remarkable influence of ‘phane effect’ on the excited-state properties of cofacially oriented coumarins, *Phys. Chem. Chem. Phys.*, 2017, **19**, 4758–4767.
 - 43 A. Kakehi, S. Ito, H. Suga, S. Takano and K. Hirata, Preparation of New Nitrogen-Bridged Heterocycles. 45. Smooth Hydrolysis of 6-Membered Heterocyclic 2-Imines with Partial Aromaticity, *Chem. Pharm. Bull.*, 1998, **46**, 1632–1634.
 - 44 C. Galli, “CESIUM ION EFFECT” AND MACROCYCLIZATION. A CRITICAL REVIEW, *Org. Prep. Proced. Int.*, 1992, **24**, 285–307.
 - 45 S. S. Bhalodiya, M. P. Parmar, D. B. Upadhyay, C. D. Patel, D. P. Vala, D. Rajani and H. M. Patel, Cs₂CO₃-promoted one-pot synthesis of novel tetrahydrobenzofuran-4(2H)-ones: *In vitro* antimicrobial, antimalarial activity and *in silico* docking study, *Results Chem.*, 2024, **7**, 101304.
 - 46 P. A. Khardina, E. M. Buev, V. S. Moshkin and V. Y. Sosnovskikh, Novel synthesis of 2,3-dihydrobenzofuran-3-amines from salicylic aldehydes: Trimethylsilyl as traceless activating group, *Tetrahedron Lett.*, 2024, **139**, 154992.
 - 47 P. Li, Y. Ji, W. Chen, X. Zhang and L. Wang, The facile synthesis of 2-bromoindoles via Cs₂CO₃-promoted intramolecular cyclization of 2-(gem-dibromovinyl)anilines under transition-metal-free conditions, *RSC Adv.*, 2012, **3**, 73–78.
 - 48 J. Ezquerra and J. Alvarez-Builla, 2-Methylpyridinium salts as 1,4-dinucleophiles. II. Westphal condensation with substituted pyridinium substrates, *J. Heterocycl. Chem.*, 1986, **23**, 1151–1157.
 - 49 Z. Chen, S. Zhang, X. Qi, S. Liu, Q. Zhang and Y. Deng, Fluorescent quinolininium ionic liquids (salts) with unexpectedly high quantum yields up to >99%, *J. Mater. Chem.*, 2011, **21**, 8979–8982.
 - 50 T. S. Chinta Rao, S. Saha, G. B. Raolji, B. Patro, P. Risbood, M. J. Difilippantonio, J. E. Tomaszewski and S. V. Malhotra, Microwave assisted Westphal condensation and its application to synthesis of sempervirine and related compounds, *Tetrahedron Lett.*, 2013, **54**, 487–490.
 - 51 J. V. Greenhill, H. Loghmani-Khouzani and D. J. Maitland, Tautomerism in ketomethylquinolines. Part 2. Further results on 2-ketomethylquinolines, *J. Chem. Soc., Perkin Trans. 1*, 1991, 2831–2840.
 - 52 A. Kakehi, S. Ito and T. Yotsuya, Preparation of New Nitrogen-Bridged Heterocycles. XIII.: Syntheses of Some Tricyclic and Tetracyclic Indolizine Derivatives with Antiallergic Activity, *Chem. Pharm. Bull.*, 1986, **34**, 2435–2442.
 - 53 S. Wu, Q. Cao, X. Wang, K. Cheng and Z. Cheng, Design, synthesis and biological evaluation of mitochondria targeting theranostic agents, *Chem. Commun.*, 2014, **50**, 8919–8922.
 - 54 A. S. Brown, L.-M. Bernal, T. L. Micotto, E. L. Smith and J. N. Wilson, Fluorescent neuroactive probes based on stilbazolium dyes, *Org. Biomol. Chem.*, 2011, **9**, 2142–2148.
 - 55 Y. Liu, B. Wang, J.-T. Hou, P. Xie, W. Li and S. Wang, Molecular engineering and bioimaging applications of C2-alkenyl indole dyes with tunable emission wavelengths covering visible to NIR light, *Bioorg. Chem.*, 2023, **141**, 106905.
 - 56 12 Principles of Green Chemistry, <https://www.acs.org/greenchemistry/principles/12-principles-of-green-chemistry.html>, (accessed 31 July 2024).
 - 57 G. Kobayashi, Y. Matsuda, R. Natsuki, Y. Tominaga, T. Okamura and A. Itamura, Studies on Indole Derivatives. XVII. The Synthesis of Indoxyl Derivatives. (3) The



- Reactions of 3-Hydroxy-2-(1-methylthioviny)-indole Derivatives with Amines, *Yakugaku Zasshi*, 1973, **93**, 964–970.
- 58 R. R. Valiev, V. N. Cherepanov, G. V. Baryshnikov and D. Sundholm, First-principles method for calculating the rate constants of internal-conversion and intersystem-crossing transitions, *Phys. Chem. Chem. Phys.*, 2018, **20**, 6121–6133.
- 59 A. Manian, R. A. Shaw, I. Lyskov, W. Wong and S. P. Russo, Modeling radiative and non-radiative pathways at both the Franck–Condon and Herzberg–Teller approximation level, *J. Chem. Phys.*, 2021, **155**, 054108.
- 60 A. Kowski, On the Estimation of Excited-State Dipole Moments from Solvatochromic Shifts of Absorption and Fluorescence Spectra, *Z. Naturforsch., A: Phys. Sci.*, 2002, **57**, 255–262.
- 61 Ł. Kielesiński, I. Deperasińska, O. Morawski, K. V. Vygranenko, E. T. Ouellette and D. T. Gryko, Polarized, V-Shaped, and Conjoined Biscoumarins: From Lack of Dipole Moment Alignment to High Brightness, *J. Org. Chem.*, 2022, **87**, 5961–5975.
- 62 Z. R. Grabowski, K. Rotkiewicz and W. Rettig, Structural Changes Accompanying Intramolecular Electron Transfer: Focus on Twisted Intramolecular Charge-Transfer States and Structures, *Chem. Rev.*, 2003, **103**, 3899–4032.
- 63 G. Dyker and O. Muth, Synthesis of Methylene- and Methine-Bridged Oligopyridines, *Eur. J. Org. Chem.*, 2004, 4319–4322.
- 64 K. Senthil, S. Kalainathan and A. R. Kumar, Effect of additives on the large-size growth of 4-N,N-dimethylamino-4-N-methyl stilbazolium naphthalene-2-sulfonate (DSNS) single crystal: an efficient stilbazolium derivative NLO crystal with potential terahertz wave properties, *CrystEngComm*, 2014, **16**, 9847–9856.
- 65 M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson, H. Nakatsuji, X. Li, M. Caricato, A. V. Marenich, J. Bloino, B. G. Janesko, R. Gomperts, B. Mennucci, H. P. Hratchian, J. V. Ortiz, A. F. Izmaylov, J. L. Sonnenberg, D. Williams-Young, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. A. Montgomery, J. E. Peralta Jr., F. Ogliaro, M. J. Bearpark, J. J. Heyd, E. N. Brothers, K. N. Kudin, V. N. Staroverov, T. A. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. P. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, J. M. Millam, M. Klene, C. Adamo, R. Cammi, J. W. Ochterski, R. L. Martin, K. Morokuma, O. Farkas, J. B. Foresman and D. J. Fox, *Gaussian 16 (Revision A.03)*, Gaussian Inc., Wallingford CT, 2016.
- 66 F. Neese, Software update: the ORCA program system, version 4.0, *Wiley Interdiscip. Rev.: Comput. Mol. Sci.*, 2018, **8**, e1327.



REVIEW

View Article Online

View Journal | View Issue



Cite this: *Org. Chem. Front.*, 2025, **12**, 2860

Advances in the synthesis of indolizines and their π -expanded analogues: update 2016–2024

Jaqueline S. A. Badaro, , Bartosz Godlewski and Daniel T. Gryko *

Indolizine (pyrrolo[1,2-*a*]pyridine) is an isomer of indole. The diversity of synthetic approaches leading to the indolizine skeleton is unrivalled, compared to many other heterocycles of this size. Increasing availability of densely functionalized pyrrole derivatives makes it possible to expand the scope of indolizine synthesis from these substrates. In this article, we describe progress in the development of new strategies that lead to indolizine and its π -expanded analogs during the period 2016–2024. Developments of the past decade combined the optimization of known strategies with the discovery of entirely new approaches. First, we discuss the synthetic pathways leading to the indolizine core from pyridine, followed by describing methods starting from pyrrole derivatives. Finally, we focus on π -expanded indolizines, also describing their optoelectronic properties. Although certain synthetic limitations exist, the newly developed methodologies provide impetus for numerous explorations that use indolizines. We anticipate that our review will help motivate further advances in indolizine synthesis.

Received 6th November 2024,

Accepted 31st January 2025

DOI: 10.1039/d4qo02082k

rsc.li/frontiers-organic

10th anniversary statement

In 2019, I published a key paper in *Organic Chemistry Frontiers* (*Org. Chem. Front.*, 2019, **6**, 2939–2948). It revealed new conditions for a multicomponent reaction leading to 1,4-dihydropyrrolo[3,2-*b*]pyrroles, which significantly expanded the substrate scope (both amines and aldehydes) and improved the yields of the heterocyclic products. Eventually, this paper opened new horizons for these exceptionally electron-rich functional dyes. I wish *Organic Chemistry Frontiers* a successful and prosperous second decade of existence!

1. Introduction

Indolizines entered the chemical world in 1890.¹ This isomer of indole has long been overshadowed by indole itself, which took prime position due to its overwhelming presence in the structures of both alkaloids and synthetic, biologically active compounds. Classical methods furnishing the indolizine skeleton, such as the Chichibabin reaction,² are well established in the literature. The simplicity of the indolizine core and the presence of a bridging nitrogen atom continued to attract attention from chemists, who developed a plethora of synthesis strategies, which started from either pyridine derivatives or substituted pyrroles. The subject was reviewed in the past^{3–8} and quite recently specialized review articles appeared as well,^{9–13} followed by major review.¹⁴ Originally interest in the indolizine scaffold was more related to its dehydrogenative derivatives, which are present in some alkaloids.¹⁵ Over time, the picture has changed and in recent decades interest shifted

towards optoelectronic-type applications.^{16,17} Indolizines' intrinsically strong emission of violet–blue light was intensively exploited by Park's group (Seoul-Fluor dyes);^{18,19} this sparked broader research interest that eventually provided impetus for further development. This shift of interest seamlessly translated into the emergence of π -expanded indolizines as a distinct research direction. It turned out that structural modifications enabled a bathochromic shift of the emission to the deep red region and beyond. Here, we present a comprehensive picture of progress in the synthesis of indolizines that has occurred from 2016 to now (Scheme 1).

2. Methods based on pyridine derivatives

The synthesis of indolizines from pyridine derivatives is the most widely recognized approach found in the literature. Pyridine derivatives are readily available, commercially accessible building blocks that are typically bench-stable, air-stable solids. These characteristics make them highly convenient substrates for the construction of indolizine frameworks.

Institute of Organic Chemistry of the Polish Academy of Sciences, 01-224 Warsaw, Poland. E-mail: dtgryko@icho.edu.pl

Polymethine Dyes

Hybrid of Indolizine and Merocyanine—A New Class of Organelle-Specific Dyes

Jaqueline S. A. Badaro, Beata Koszarna, Maja Perkowska, Kristiana Kandere-Grzybowska, Diana V. Kolygina, Olaf Morawski, Juhee Park, Bartosz Grzybowski,* Irena Deperasińska,* and Daniel T Gryko*

Dedicated to Prof. Jonathan Lindsey on his 70th birthday

Abstract: Unlocking the potential of electron-rich 2-hydroxyindolizines led to the discovery of a new class of dyes—indolizine-merocyanines (IndMer) and indolizine-cyanines. Tandem Friedel–Crafts alkylation followed by intramolecular nucleophilic aromatic substitution afforded structurally diverse dyes, as both the nucleophilic and electrophilic partners can be broadly modified. A convergent fragment coupling strategy allowed rapid access to these π -conjugated merocyanines in three steps from pyridines. Uniform distribution of the HOMO and LUMO combined with negligible change of dipole moment upon excitation is responsible for the intense orange or red emission of this new family of reasonably photostable dyes in a broad range of solvents. The new merocyanine dyes have the potential to target a variety of organelles—both uncharged and positively charged indolizine–merocyanines localize a subset of cellular lysosomes, positively charged indolizine–cyanine hybrid accumulates in mitochondria, while coumarin–merocyanine shows context-dependent localization to mitochondria and RNA-rich nucleoli of the living cells.

Introduction

Fluorescein, rhodamine, and rhodol were all discovered in the 19th century, and over the course of 140 years, extensive effort

has been devoted to advancing the photophysical properties of these ubiquitous dyes through structural modifications. In particular π -expansion^[1–7] and replacement of the xanthene oxygen atom bridge with silicon,^[8–11] phosphorus,^[12,13] sulfur,^[14] or carbon^[15–18] in scaffolds of rhodamine,^[19–22] fluorescein,^[23,24] and rhodol^[25] have proven popular and effective (Figure 1).

Other notable modifications encompass altering the size of the middle ring^[26,27] and replacing dialkylamino groups as a donor with indolizine,^[28,29] allowing further modulation of the photophysical properties.

The key application of xanthene dyes is fluorescence imaging, which has become a powerful tool for molecular biology due to its merits of real-time monitoring capability, non-invasive nature, and high spatiotemporal resolution.^[30,31] Compared to classical fluorescence microscopy, fluorescence imaging in the near-infrared window (NIR, 750–1700 nm) allows for both deeper tissue penetration and a higher signal-to-noise ratio for noninvasive in vivo imaging at subcentimeter tissue depths, due to decreased scattering.^[32–35] Advancements in this imaging technique have demanded more stringent and challenging properties to be included in dyes, which currently available dyes do not entirely fulfill. Indeed, NIR dyes lack a large fluorescence quantum yield (Φ_f) and photostability.^[36,37,38] Some of the modern, highly modified xanthene dyes possess emission in NIR often with reasonable quantum yield.^[39,40]

In the context of addressing the above challenges, an interesting question emerges about possible modifications of electron-donating moiety especially by an electron-rich aromatic heterocycle. The hypothesis underlying our current work is that by incorporating the entire indolizine^[41] moiety

[*] J. S. A. Badaro, Dr. B. Koszarna, M. Perkowska, Prof. B. Grzybowski, Prof. D. T. Gryko
Institute of Organic Chemistry of Polish Academy of Sciences, Kasprzaka 44/52, Warsaw 01-224, Poland
E-mail: grzybor72@unist.ac.kr
dtgryko@icho.edu.pl

Dr. K. Kandere-Grzybowska, Dr. D. V. Kolygina, J. Park, Prof. B. Grzybowski
Center for Algorithmic and Robotized Synthesis, Institute for Basic Science (IBS), Ulsan 44919, Republic of Korea

Dr. K. Kandere-Grzybowska
Department of Biomedical Engineering, Ulsan National Institute of Science and Technology (UNIST), 50 UNIST-gil, Ulsan 44919, Republic of Korea

Prof. B. Grzybowski
Department of Chemistry, Ulsan National Institute of Science and Technology (UNIST), 50 UNIST-gil, Ulsan 44919, Republic of Korea

Dr. O. Morawski, Dr. I. Deperasińska
Institute of Physics, Polish Academy of Sciences, Al. Lotników 32/46, Warsaw 02-668, Poland
E-mail: deper@ifpan.edu.pl

Additional supporting information can be found online in the Supporting Information section

© 2025 The Author(s). Angewandte Chemie International Edition published by Wiley-VCH GmbH. This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial License](#), which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.



Institute of Organic Chemistry
Polish Academy of Sciences

Jaqueline Stella Araujo Badaró

+48 22 343 20 37

jaqueline.badaró@icho.edu.pl

Warsaw 28th September 2025

I declare that my contribution to the following publication consisted of:

1. **Badaró, J. S. A.**; Koszarna, B.; Bousquet, M. H. E.; Ouellette, E. T.; Jacquemin, D.; Gryko, D. T. 'The Kröhnke synthesis of benzo[a]indolizines revisited: towards small, red light emitters', Org. Chem. Front. 2022, 9, 1861-1874. <https://doi.org/10.1039/D2QO00097K>
2. **Badaró, J. S. A.**; Wrzosek, A; Morawski, O.; Szewczyk, A; Deperasinska, I; Gryko, D. T. 'Strongly fluorescent indolizine-based coumarin analogs' Org. Chem. Front., 2024, 11, 6627-6641. <https://doi.org/10.1039/D4QO01216J>
3. **Badaró, J. S. A.**; Koszarna, B.; Perkowska, M.; Kandere-Grzybowska, K.; Kolygina, D. V.; Morawski, O.; Park, J.; Grzybowski, B.; Deperasinska, I.; Gryko, D. T., 'The Hybrid of Indolizine and Merocyanine - A New Class of Organelle-specific Dyes', Angew. Chem. Int. Ed. 2025, 64, doi.org/10.1002/anie.202508044.

co-development of research concepts, synthesis, characterization, absorption and fluorescence measurements, photostability measurements, interpretation of results and preparation of the manuscript.

4. **Badaró, J. S. A.**; Godlewski, B.; Gryko, D. T., 'Advances in the synthesis of indolizines and their π -expanded analogues: update 2016-2024', Org. Chem. Front., 2025, 12, 2860-2907. <https://doi.org/10.1039/D4QO02082K>

Writing and editing of the text of one of the chapters, drawing figures for one of the chapters and revising the final version of the manuscript.

Yours sincerely

Jaqueline Stella Araujo Badaró



Warsaw 25th September 2025

I declare that my contribution to the following publications consisted of:

- › Badaro, J. S. A.; Koszarna, B.; Bousquet, M. H. E.; Ouellette, E. T.; Jacquemin, D.; **Gryko, D. T.** 'The Kröhnke synthesis of benzo[a]indolizines revisited: towards small, red light emitters', *Org. Chem. Front.* **2022**, *9*, 1861-1874. <https://doi.org/10.1039/D2QO00097K>

Co-development of research concepts, interpretation of results and preparation of the final version of the manuscript.

- › Badaro, J. S. A.; Wrzosek, A.; Morawski, O.; Szewczyk, A.; Deperasińska, I.; **Gryko, D. T.** 'Strongly fluorescent indolizine-based coumarin analogs' *Org. Chem. Front.*, **2024**, *11*, 6627–6641."

Co-development of research concepts, interpretation of results and preparation of the final version of the manuscript.

- › Badaro, J. S. A.; Godlewski, B.; **Gryko, D. T.**, 'Advances in the synthesis of indolizines and their π -expanded analogues: update 2016–2024', *Org. Chem. Front.*, **2025**, *12*, 2860-2907. <https://doi.org/10.1039/D4QO02082K>".

Development of the review concept, writing the introduction and summary/outlook as well as editing of the final version of the manuscript.

- › Badaro, J. S. A.; Koszarna, B.; Perkowska, M.; Kandere-Grzybowska, K.; Kolygina, D. V.; Morawski, O.; Park, J.; Grzybowski, B.; Deperasińska, I.; **Gryko, D. T.**, 'The Hybrid of Indolizine and Merocyanine – A New Class of Organelle-specific Dyes', *Angew. Chem. Int. Ed.* **2025**, *64*, doi.org/10.1002/anie.202508044.

Co-development of research concepts, interpretation of results and preparation of the final version of the manuscript.

Yours sincerely



Warsaw 23rd September 2025

I declare that my contribution to the following publications consisted of:

1. J. S. A. Badaro, B. Koszarna, M. H. E. Bousquet, E. T. Ouellette, D. Jacquemin, D. T. Gryko; *"The Krohnke synthesis of benzo[a]indolizines revisited: towards small, red light emitters"*; [Org. Chem. Front.](#), **2022**, *9*, 1861-1874
2. J. S. A. Badaro, B. Koszarna, M. Perkowska, K. Kandere-Grzybowska, D. V. Kolygina, O. Morawski, J. Park, B. Grzybowski, I. Deperasińska, D. T. Gryko; *"The Hybrid of Indolizine and Merocyanine – A New Class of Organelle-specific Dyes"*; *Angewandte Chemie*, 2025, In Press

I participated in absorption and fluorescence measurements, supervised laboratory work, and contributed to manuscript drafting.

Yours sincerely



Institute of Organic Chemistry
Polish Academy of Sciences

Maja Perkowska

+48 737 389 032

maja.perkowska@icho.edu.pl

Instytut Chemii Organicznej PAN

Marcina Kasprzaka 44/52

01-224 Warsaw

Warsaw 25rd September 2025

Statement

I declare that my contribution to the following publication consisted of:

Jaqueline S. A. Badaro, Beata Koszarna, Maja Perkowska, Kristiana Kandere-Grzybowska, Diana V. Kolygina, Olaf Morawski, Juhee Park, Bartosz Grzybowski, Irena Deperasińska and Daniel T Gryko, *Angewandte Chemie*, 2025, *In Press*, "The Hybrid of Indolizine and Merocyanine – A New Class of Organelle-specific Dyes".

I participated in the synthesis, absorption and fluorescence measurements of some reported dyes. I was also involved in measuring the melting temperatures of the dyes and creating the graphical abstract.

Yours sincerely

Maja Perkowska



Instytut Chemii Organicznej
Polskiej Akademii Nauk

Bartosz Godlewski
bartosz.godlewski@icho.edu.pl
Institute of Organic Chemistry PAS
Kasprzaka 44/52, 01-224 Warsaw, Poland

Warsaw, 23.09.2025

STATEMENT

I hereby declare that my contribution to publication:

“*Advances in the Synthesis of Indolizines and Their π -Expanded Analogues: Update 2016–2024*”; J. S. A. Badaro, B. Godlewski and D. T. Gryko, *Org. Chem. Front.*, 2025, **12**, 2860–2907

is as follows:

writing and editing the text of one of the chapters, drawing figures for one of the chapters and revising the final version of the manuscript.

Bartosz Godlewski



INSTYTUT FIZYKI POLSKIEJ AKADEMII NAUK
INSTITUTE OF PHYSICS, POLISH ACADEMY OF SCIENCES

02-668 WARSZAWA, Al. LOTNIKÓW 32/46
fax: (48-22) 843-0926; <http://info.ifpan.edu.pl>

dr hab. Irena Deperasińska

e-mail: deper@ifpan.edu.pl

Warszawa, 23.09.2025

Declaration

I declare that my contribution to the following publications:

1. **Jaqueline S. A. Badaro** et al., The Hybrid of Indolizine and Merocyanine – A New Class of Organelle-specific Dyes, *accepted for publication in Angewandte Chemie International Edition*

2. **Jaqueline S. A. Badaro** et al., Strongly Fluorescent Indolizine-based Coumarin Analogs, *Org. Chem. Front.*, 2024, 11, 6627

consisted of performing DFT calculations of the electronic transition energies in compounds **3a**, **3d**, **3Ng**, **5** in the case of the first publication and **2b**, **2a**, **2c**, **2e**, **2f**, **4a**, **3aa**, **3ac**, **3ab** and **6a** in the case of the second publication.

J. Deperasińska



INSTYTUT FIZYKI POLSKIEJ AKADEMII NAUK
INSTITUTE OF PHYSICS, POLISH ACADEMY OF SCIENCES

02-668 WARSZAWA, Al. LOTNIKÓW 32/46
fax: (48-22) 843-0926; <http://info.ifpan.edu.pl>

dr hab. Olaf Morawski

e-mail: morawo@ifpan.edu.pl

Warszawa, 23.09.2025

Declaration

I declare that my contribution to the following publications:

1. **Jaqueline S. A. Badaro** et al., The Hybrid of Indolizine and Merocyanine – A New Class of Organelle-specific Dyes, *accepted for publication in Angewandte Chemie International Edition*
2. **Jaqueline S. A. Badaro** et al., Strongly Fluorescent Indolizine-based Coumarin Analogs, *Org. Chem. Front.*, 2024, 11, 6627

consisted of performing measurements of all fluorescence decay profiles for all compounds in both publications as well as performing for them deconvolution calculations to obtain decay times.

Dr. Antoni Wrzosek

A.Wrzosek@nencki.edu.pl

Ref. Contribution Letter

Warsaw, 23/09/2025

To whom it may concern

I hereby declare that my contribution to the publication:

Jaqueline S. A. Badaro, Antoni Wrzosek, Olaf Morawski, Adam Szewczyk, Irena Deperasińska and Daniel T. Gryko. *Org. Chem. Front.*, 2024,**11**, 6627-6641. **"Strongly Fluorescent Indolizine-based Coumarin Analogs"**

I performed the confocal microscopy measurements for this work. I participated in the proofreading of the section on confocal microscopy studies.

Sincerely,



Dr. Antoni Wrzosek

Prof. Adam Szewczyk

A.Szewczyk@nencki.edu.pl

Ref. Contribution Letter

Warsaw, 23/09/2025

To whom it may concern

I hereby declare that my contribution to the publication:

Jaqueline S. A. Badaro, Antoni Wrzosek, Olaf Morawski, Adam Szewczyk, Irena Deperasińska and Daniel T. Gryko. *Org. Chem. Front.*, 2024,**11**, 6627-6641. **"Strongly Fluorescent Indolizine-based Coumarin Analogs"**

I participated in the proofreading of the section on confocal microscopy studies.

Sincerely,



Prof. Adam Szewczyk

Manon Bousquet
mhe.bousquet@gmail.com

Ref. **Contribution Letter**

Nantes, 20/09/2025

To whom it may concern,

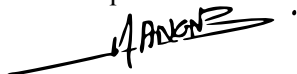
I hereby declare that my contribution to the publications below:

1. **Jaqueline S. A. Badaro**, Beata Koszarna, Manon H. E. Bousquet, Erik T. Ouellette, Denis Jacquemin and Daniel T. Gryko. *Org. Chem. Front.*, 2022,**9**, 1861-1874. “**The Kröhnke synthesis of benzo[a]indolizines revisited: towards small, red light emitters**”

I realized the theoretical part of this work and wrote the theoretical section of this manuscript.

With best regards.

Manon Bousquet



September 22nd, 2025

To whom it may concern,

I hereby declare that my contribution to the publications listed below is as follows:

1. Badaro, J. S. A.; Koszarna, B.; Bousquet, M. H. E.; **Ouellette, E. T.**; Jacquemin, D.; Gryko, D. T. "The Kröhnke synthesis of benzo[*a*]indolizines revisited: towards small, red light emitters." *Org. Chem. Front.* **2022**, 9, 1861-1874.

I collected single-crystal x-ray diffraction (SCXRD) measurements, analyzed data, and developed structural solutions of relevant compounds from the manuscript.

Sincerely,



Erik T. Ouellette

Prof. Denis Jacquemin
Denis.Jacquemin@univ-nantes.fr

Ref. **Contribution Letter**

Nantes, 19/09/2025

To whom it may concern,

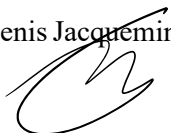
I hereby declare that my contribution to the publications below:

1. **Jaqueline S. A. Badaro**, Beata Koszarna, Manon H. E. Bousquet, Erik T. Ouellette, Denis Jacquemin and Daniel T. Gryko. *Org. Chem. Front.*, 2022, **9**, 1861-1874. “**The Kröhnke synthesis of benzo[a]indolizines revisited: towards small, red light emitters**”

I supervised the theoretical part of this work and contributed to write the theoretical section of this manuscript. I was involved in the proof checking of the full manuscript.

With best regards.

Prof. Denis Jacquemin





STATEMENT

September 22, 2025

I hereby declare that my contributions to the publication listed below is as follows:

Jaqueline S. A. Badaro, Beata Koszarna, Maja Perkowska, Kristiana Kandere-Grzybowska, Diana V. Kolygina, Olaf Morawski, Juhee Park, Bartosz Grzybowski, Irena Deperasińska, and Daniel T Gryko. The Hybrid of Indolizine and Merocyanine – A New Class of Organelle-specific Dyes. *Angew. Chem. Int. Ed.* In Press, 2025.

I designed and performed confocal imaging of the fluorescent compounds in living cells, specifically for Figure S10, Figure S11, Figure S12 ab, Figure S13, Figure S14, Fig S15 (with MRC5 cells), Figure S16 ab, Figure S17 (with MRC5 cells), Figure S18, Figure S19, and Figure S20. I analyzed the corresponding images and produced the figures related to confocal imaging. I contributed to the Materials and Methods part for ESI section 6 Imaging.

Sincerely,

Diana V. Kolygina
Senior Researcher

A handwritten signature in black ink, appearing to read 'DVK', is placed over the printed name and title of the researcher.

Center for Algorithmic and Robotized Synthesis, Institute for Basic Science (IBS)



STATEMENT

September 22, 2025

I hereby declare that my contribution to the publication listed below is as follows:

Jaqueline S. A. Badaro, Beata Koszarna, Maja Perkowska, Kristiana Kandere-Grzybowska, Diana V. Kolygina,¹ Olaf Morawski, Juhee Park, Bartosz Grzybowski, Irena Deperasińska and Daniel T Gryko, *Angewandte Chemie*, 2025, In Press, *The Hybrid of Indolizine and Merocyanine – A New Class of Organelle-specific Dyes*

For the S21 data, I performed cytotoxicity assays using multiple cell lines and various compounds, analyzed the IC50 values, and provided a detailed description of the experimental methods.

Sincerely,

Juhee Park

Senior Research Engineering Staff, IBS CARS



STATEMENT

September 22, 2025

I hereby declare that my contributions to the publication listed below is as follows:

Jaqueline S. A. Badaro, Beata Koszarna, Maja Perkowska, Kristiana Kandere-Grzybowska, Diana V. Kolygina, Olaf Morawski, Juhee Park, Bartosz Grzybowski, Irena Deperasińska, and Daniel T Gryko. The Hybrid of Indolizine and Merocyanine – A New Class of Organelle-specific Dyes. *Angew. Chem. Int. Ed.* In Press, 2025.

I designed experiments and supervised the work related to confocal imaging of the dyes in living cells. Specifically, I performed confocal imaging for Fig 6, Fig S12c,d, Fig S15, Fig S16c,d, Fig S17 (experiments with HT1080 cells), and analyzed the corresponding images. I co-wrote the Materials and Methods section for ESI Section 6 Imaging, and contributed text to the manuscript for section on fluorescent imaging.

Sincerely,

A handwritten signature in blue ink, appearing to read 'K. Kandere-Grzybowska', is written over a large, faint circular watermark of the IBS logo.

Kristiana Kandere-Grzybowska
Adjunct Professor, UNIST
Senior Research Fellow, IBS CARS

Ulsan National Institute of Science and Technology
IBS Center for Algorithmic and Robotized Synthesis
50, UNIST-gil, Ulsan 44919, Republic of Korea

STATEMENT

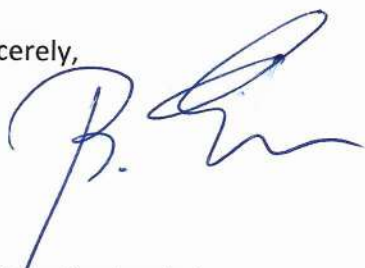
September 29, 2025

I hereby declare that my contribution to the publication listed below is as follows:

Jaqueline S. A. Badaro, Beata Koszarna, Maja Perkowska, Kristiana Kandere-Grzybowska, Diana V. Kolygina, Olaf Morawski, Juhee Park, Bartosz Grzybowski, Irena Deperasińska and Daniel T Gryko, *Angewandte Chemie*, 2025, In Press, *The Hybrid of Indolizine and Merocyanine – A New Class of Organelle-specific Dyes*

I supervised the work performed by the other coauthors from UNIST related to confocal imaging of the dyes and revised the manuscript.

Sincerely,



Bartosz Grzybowski

Distinguished Professor, Department of Chemistry, UNIST
Director, Center for Algorithmic and Robotized Synthesis (CARS),
Institute for Basic Science

O-B.470/26



10000000118139