THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

Electrophilic, Activation-Free Fluorogenic Reagent for Labeling Bioactive Amines

Citation for published version:

Sintes, M, De Moliner, F, Caballero-Lima, D, Denning, DW, Read, ND, Kielland, N, Vendrell, M & Lavilla, R 2016, 'Electrophilic, Activation-Free Fluorogenic Reagent for Labeling Bioactive Amines', *Bioconjugate chemistry*. <https://doi.org/10.1021/acs.bioconjchem.6b00245>

Digital Object Identifier (DOI):

[10.1021/acs.bioconjchem.6b00245](https://doi.org/10.1021/acs.bioconjchem.6b00245)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Peer reviewed version

Published In:

Bioconjugate chemistry

Publisher Rights Statement:

Author's peer-reviewed manuscript as accepted for publication

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

An Electrophilic, Activation-free Fluorogenic Reagent for Labeling Bioactive Amines

Miquel Sintes,[†] Fabio De Moliner,[‡] David Caballero-Lima,[¥] David W. Denning,[¥] Nick D. Read,[¥] Nicola Kielland,^{*,†} Marc Vendrell^{*,‡} and Rodolfo Lavilla^{*,†,§}

[†] Laboratory of Organic Chemistry, Faculty of Pharmacy, University of Barcelona, Barcelona Science Park, Baldiri Reixac 10-12, 08020, Spain.

[‡] MRC/UoE Centre for Inflammation Research, University of Edinburgh. 47 Little France Crescent, EH16 4TJ Edinburgh, United Kingdom.

[¥] Manchester Fungal Infection Group, Institute of Inflammation and Repair, University of Manchester. CTF Building, Grafton St., M13 9NT Manchester, United Kingdom.

[§] CIBER-BBN, Networking Centre on Bioengineering, Biomaterials and Nanomedicine, Barcelona Science Park, Baldiri Reixac 10-12, 08028 Barcelona, Spain.

ABSTRACT: Herein we report the preparation of BODIPY mesoionic acid fluorides through a short sequence involving an isocyanide multicomponent reaction as the key synthetic step. These novel BODIPY acid fluorides are water-stable electrophilic reagents that can be used for the fluorescent derivatization of amine-containing biomolecules using mild and activation-free reaction conditions. As a proof of principle, we have labelled the antifungal natamycin and generated a novel fluorogenic probe for imaging a variety of human and plant fungal pathogens, with excellent selectivity over bacterial cells.

INTRODUCTION

Electrophilic tags have enabled the functionalization of nucleophilic metabolites and biomolecules for *in vitro* profiling and/or imaging studies.^[1] Some of the first examples of electrophilic labels include dansyl chloride,^[2] Sanger^[3] and Edman reagents.^[4] Since then, the reactivity and application of different electrophilic groups for tagging purposes has been reported. For instance, the group of Cravatt pioneered the use of electrophiles in activity-based protein profiling.^[5,6] Likewise, the HaloTag technology has become a broad and powerful tool to fluorescently label proteins.^[7] In the context of small molecule labeling, most electrophilic tagging protocols rely on a variety of reactions, including conjugate additions and S_NAR , as well as the conventional formation of amides from carboxylic acids and amines with participation of a variety of coupling reagents.^[9-20] These conjugation reactions proceed with high yields but render side products (e.g. NHS in the case of succinidimyl esters) and, to some extent, are sensitive to small amounts of water. In order to overcome these limitations, we have developed a new reagent to fluorescently label amine-containing bioactive compounds using a simple, one-step transformation with an electrophilic BODIPY derivative.

Our groups have recently described the synthesis of dipolar acid fluorides through a multicomponent reaction (MCR)^[21-23] involving isoquinolines, isocyanides and trifluoroacetic acid anhydride (TFAA).^[24] This process provides an efficient path to structures otherwise inaccessible through classical synthesis. Interestingly, dipolar acid fluorides display remarkable resistance to water hydrolysis while maintaining high reactivity against amine nucleophiles. We have employed isoquinoline dipolar acid fluorides (**1**) for imaging intracellular histamine^[25]

as well as for tagging oligonucleotides (Scheme 1),^[26] but their short excitation and emission wavelength hamper their application for cell imaging studies. The derivatization of the BODIPY structure using MCR chemistry has proven an excellent strategy for the generation of unconventional architectures with unique spectral properties.^[27-28] We envisaged that the conjugation of dipolar acid fluorides to the BODIPY fluorescent scaffold, which has excellent photophysical and permeability properties,^[29-34] would render useful electrophilic BODIPY dyes for rapid and activation-free labeling of amine-containing bioactive compounds.

Scheme 1. Conjugation of blue-fluorescent acid fluorides **1** to different bioactive compounds.

RESULTS AND DISCUSSION

We designed the synthesis of BODIPY dipolar acid fluorides (**2**, Scheme 2) in two consecutive steps. First, the conjugation of 4-isoquinoline boronic acid to BODIPY aryl iodides via Suzuki coupling to render suitable fluorescent isoquinolines (**3**, Scheme 2). Secondly, BODIPY-isoquinoline conjugates were used in MCRs with TFAA and isocyanides to yield the corresponding BODIPY dipolar acid fluorides (Scheme 2).

Scheme 2. Retrosynthetic analysis for BODIPY dipolar acid fluorides (**2**).

Since *m*- and *p*-iodinated BODIPY dyes have been reported to perform differently in some conjugation reactions,^[35] we performed MCRs using the two regioisomers. We isolated both *p*- and *m*-BODIPY derivatives (**4a** and **4b**, respectively),^[35,36] and successfully coupled them to 4-isoquinoline boronic acid under conventional Suzuki cross-coupling conditions (Scheme 3).^[37] The adducts **3a** (*para*, 83%) and **3b** (*meta*, 90%) were obtained in excellent yields, and were subjected to MCR to obtain the corresponding dipolar acid fluorides (**2**). We employed two nucleophilic isocyanides (i.e. cyclohexyl and benzyl) to explore the effect of different groups in the final acid fluorides. Quinolines **3a** and **3b** reacted with the different isocyanides and TFAA in DCM, using relative stoichiometry 1:2:3 and yielding a series of BODIPY dipolar acid fluorides (**2a-d**, Scheme 3) in good yields (for synthetic details and characterization, see Electronic Supporting Information (ESI)). Notably, the formation of Arndtsen-dipoles (**5a** and **5b**, Scheme 3) was only observed when benzyl isocyanide was used.^[38]

Scheme 3. Synthesis of a collection of BODIPY dipolar acid fluorides (**2a-d**).

A major limitation of many fluorescent labeling reagents is their potential hydrolysis in aqueous media, which is particularly relevant when tagging bioactive molecules. Previous mesoionic acid fluorides showed excellent resistance to hydrolysis^[25]; hence we assessed the chemical integrity of the BODIPY dipolar acid fluorides in aqueous media. HPLC-MS analysis confirmed their high stability in aqueous environments, even under basic conditions (Figure S1 in ESI). This unique feature represents an advantage over other carbonyl-containing

labelling reagents (e.g. succinidimyl esters) for reduced fluorescence background and purification steps.

We then evaluated the fluorescence properties of the different BODIPY acid fluorides. All derivatives displayed typical excitation and emission wavelengths of the BODIPY structure (Table 1 and Figure S4 in ESI), with higher extinction coefficients observed for *m*- over *p*-derivatives. No significant differences in the wavelength maxima or the fluorescence quantum yields were detected between *p*- and *m*-derivatives (i.e., **2a** vs. **2b**, **2c** vs. **2d**), only with slightly higher quantum yields in cyclohexyl over benzyl derivatives (i.e., **2a** vs. **2c**, **2b** vs. **2d**). These results correlate with our analysis of the structural and energetic arrangements of the molecular orbitals for these compounds (Figure S2 in ESI). In these studies, we observed that both *p*- and *m*-cyclohexyl derivatives **2a** and **2b** showed a preferential parallel disposition of the BODIPY and isoquinoline cores linked through the perpendicular phenyl spacer, with energy diagrams of the frontier orbitals showing minimal differences between regioisomers (Figure S3 in ESI). All BODIPY dipolar fluorides exhibited high fluorescence quantum yields in the green region of the spectrum, making them useful molecules for the fluorescence labeling of amine-containing compounds.

Table 1. Spectral properties of BODIPY dipolar acid fluorides in EtOH.

Compound	$\lambda_{\text{abs.}}$ (nm)	$\lambda_{\text{em.}}$ (nm)	ϵ (M ⁻¹ cm ⁻¹)	QY ^a
2a	502	514	16,100	0.56
2b	502	514	85,500	0.59
2c	501	512	11,400	0.45
2d	502	514	66,100	0.50

^a Determined using fluorescein in basic EtOH as standard (QY: 0.92).^[17]

In view of the high extinction coefficient and quantum yield of the BODIPY dipolar acid fluoride **2b**, we decided to examine its application as a labeling reagent for natamycin, a naturally-occurring antifungal agent with high affinity and selectivity for fungal cells. Natamycin blocks fungal growth by binding specifically to ergosterol without permeabilizing the membrane where it inhibits vacuole fusion at the priming phase and interferes with membrane protein functions. As it has been reported for other antimicrobial drugs,^[39] we envisaged that fluorescent analogues of natamycin could be used as probes for selective imaging of fungal infection sites. The complex polyene macrolide structure of natamycin includes several functional groups (e.g. epoxides, alcohols, carboxylic acids, amines), which demands highly specific labeling reagents for its derivatization (Figure 1). Notably, the exclusive reactivity of the acid fluoride **2b** against amine groups enabled the preparation of the fluorescent analogue of natamycin **6** (Figure 1) by simply mixing the acid fluoride **2b** with natamycin in DMF at 40 °C, without any prior activation or additional reagent. The conjugation reaction was completed in several hours, and we isolated the conjugate **6** in high purity (>95%) and synthetic yield (80%). Furthermore, the conjugation reactions between natamycin and other BODIPY acid fluorides proceeded with similarly good results (for details and characterization data, see derivatives **7** and **8** in ESI), which validates our approach as a general methodology for labeling amine-containing biomolecules using activation-free reaction conditions. The efficient derivatization of natamycin with different acid fluorides opens the possibility of extending the

chemical diversity of this set of labelling reagents by using various isocyanides to tune their selectivity without compromising their performance. To the best of our knowledge, these are the first fluorescent derivatives of natamycin described to date.

Figure 1. Synthesis of a fluorescent analogue of natamycin under mild and activation-free reaction conditions. Inset) Pictograms of solutions of **6** (50 μ M) in 1,4-dioxane (left) and PBS (right) upon irradiation with a 365 nm UV-lamp.

The spectral characterization of compound **6** corroborated that the fluorescent conjugate retained the excitation and emission maxima wavelengths of the BODIPY acid fluoride **2b** (Figure S5 in the SI). Because electron-rich BODIPY derivatives have been reported as environmentally-sensitive dyes,^[35,40,41] we assessed the fluorescence emission of compound **6** in different environments. Natamycin strongly binds to ergosterol, which is a key component of the membranes of fungal cells, thus we determined the fluorescence quantum yields of compound **6** in mixtures of 1,4-dioxane and PBS (phosphate buffer saline) as an indication of its behaviour in lipid membranes and intracellular environments, respectively.^[42,43] Notably, compound **6** displayed around 70-fold higher fluorescence emission in 1,4-dioxane, which mimics the dielectric constant of hydrophobic environments found in lipid bilayers (Figure 1 and Figure S6 in ESI).^[44] This feature suggests that BODIPY acid fluorides might be used as reagents for the fluorogenic labeling of biomolecules that preferentially localize in hydrophobic environments, such as cellular membranes.

In order to examine the application of compound **6** as a fluorescent probe for imaging fungal cells, we incubated the natamycin analogue **6** with several filamentous human and plant fungal pathogens -namely *Fusarium solani*, *F. oxysporum* and *Aspergillus flavus*-, and acquired fluorescence images of the different species using live cell confocal microscopy. As shown in Figure 2, compound **6** brightly stained the three fungal species since ergosterol, the binding target of natamycin, is present in the plasma membranes of all fungal pathogens, except the highly co-evolved lung pathogen *Pneumocystis* spp.. Furthermore, we analysed the staining of compound **6** in the

different species over time (Figure 2d). In this kinetic study, we observed that the binding of compound **6** at fungal cells increased sharply in the first minutes and reached saturation after 20 minutes, which asserts the potential of compound **6** for the rapid identification of fungal cells using fluorescence readouts.

Figure 2. Fluorescence images of fungal cells after incubation with the natamycin analogue **6** (5 μ M) for 20 min. a) *F. solani*, b) *F. oxysporum*, c) *A. flavus*. Scale bar: 20 μ m. d) Time-course analysis of the fluorescence emission of compound **6** in the different strains (*F. oxysporum* (red), *A. flavus* (green), *F. solani* (blue)).

Figure 3. Compound **6** brightly stains fungal cells with high selectivity over bacterial cells. Brightfield and fluorescence images of *F. oxysporum* (a, b) and *Pseudomonas aeruginosa* (c, d) after incubation with compound **6** (5 μ M) for 20 min. Scale bar: 10 μ m.

Finally, we assessed the selectivity of compound **6** as an imaging probe for fungal cells over other microbes (e.g. bacteria) which do not contain ergosterol in their membrane structures. We incubated compound **6** in both fungi (*F. oxysporum*) and bacteria (*Pseudomonas aeruginosa*) under the same experimental conditions, and acquired fluorescence images by confocal microscopy. As shown in Figure 3, compound **6** brightly stained fungal cells (*F. oxysporum*) whereas no staining was observed in bacterial cells. These results confirm that the derivatization of natamycin with the BODIPY acid fluoride **2b** did not impair the binding of natamycin for ergosterol in fungal cells, and assert the potential of the BODIPY analogue **6** as a fluorescent agent for selective imaging of fungal infection sites.

CONCLUSIONS

In conclusion, we have developed a new class of mesoionic BODIPY acid fluorides as highly reactive fluorescent labeling reagents for amine-containing biomolecules. These new reagents display excellent fluorescence properties in the green region of the spectrum as well as high stability in aqueous media and basic conditions. As a proof-of-concept, we have modified the complex polyene structure of the antifungal drug natamycin to produce the first fluorescent analogues of natamycin described to date. Notably, the conjugation between the BODIPY acid fluorides and natamycin was performed in very mild conditions, without the need for protective groups or activation reagents, and without producing any side products. The resulting BODIPY-natamycin conjugates have been used for selective imaging of fungal cells using fluorescence microscopy, and showed broad applicability to a variety of fungal species and very high selectivity over bacterial cells. Altogether, these mesoionic BODIPY acid fluorides represent a new methodology for the non-invasive labeling of biomolecules and will open many opportunities in the development of optimal fluorescence imaging probes.

ASSOCIATED CONTENT

Supporting Information

Experimental procedures, spectral and characterization data (NMR, HRMS) and additional biological assays. The Supporting Information is available free of charge on the ACS Publications website.

AUTHOR INFORMATION

Corresponding Authors

* Nicola Kiehländ, nicola.kiehländ@gmail.com

* Marc Vendrell, marc.vendrell@ed.ac.uk

* Rodolfo Lavilla, rlavilla@pcb.ub.es

Notes

The authors declare no competing financial interest.

ACKNOWLEDGEMENTS

D. C.-L., D. W. D and N. D. R acknowledge the support of the Global Action Fund for Fungal Infection (104684). M.V. acknowledges the support of the Medical Research Council, the Marie Curie Integration Grant (333847), and the Biotechnology and Biological Sciences Research Council (BB/M025160/1). R.L. acknowledges the support of DGICYT-Spain (CTQ2015- 67870-P), Generalitat de Catalunya (2014SGR137).

REFERENCES

(1) For a recent article, see: Sunbul, M., Nacheva, L., and Jäschke, A. (2015) Proximity-Induced Covalent Labeling of Proteins with a

Reactive Fluorophore-Binding Peptide Tag. *Bioconjugate Chem.* 26, 1466-1469.

(2) Bartzatt, R. (2001) Dansylation of hydroxyl and carboxylic acid functional groups. *J. Biochem. Biophys. Methods*, 47, 189-195 and references therein.

(3) Sanger, F. (1945) The free amino groups of insulin. *Biochem. J.* 39, 507-515.

(4) Edman, P., Högfeldt, E., Sillén, L. G., and Kinell, P.-O. (1950) Method for determination of the amino acid sequence in peptides. *Acta Chem. Scand.* 4, 283-293.

(5) Weerapana, E., Simon, G. M., and Cravatt, B. F. (2008) Disparate proteome reactivity profiles of carbon electrophiles. *Nat. Chem. Biol.* 4, 405-407.

(6) Nomura, D. K., Dix, M. M., and Cravatt, B. F. (2010) Activity-based protein profiling for biochemical pathway discovery in cancer. *Nat. Rev. Cancer* 10, 630-638.

(7) England, C. G., Luo, H., and Cai, W. (2015) HaloTag technology: a versatile platform for biomedical applications *Bioconjugate Chem.* 26, 975-986.

(8) Shannon, D. A., Banerjee, R., Webster, E. R., Bak, D. W., Wang, C., and Weerapana, E. (2014) Investigating the proteome reactivity and selectivity of aryl halides. *J. Am. Chem. Soc.* 136, 3330-3333.

(9) Morales-Sanfrutos, J., Lopez-Jaramillo, F. J., Hernandez-Mateo, F., and Santoyo-Gonzalez, F. (2010) Vinyl sulfone bifunctional tag reagents for single-point modification of proteins *J. Org. Chem.* 75, 4039-4047.

(10) McDonald, R. I., Guiling, J. P., Mukherji, S., Curtis, E. A., Lee, W. I., and Liu, D. R. (2014) Electrophilic activity-based RNA probes reveal a self-alkylating RNA for RNA labeling. *Nat. Chem. Biol.* 10, 1049-1054.

(11) Xiao, J., Broz, P., Puri, A. W., Deu, E., Morell, M., Monack, D. M., and Bogoy, M. (2013) A coupled protein and probe engineering approach for selective inhibition and activity-based probe labeling of the caspases. *J. Am. Chem. Soc.* 135, 9130-9138.

(12) Chen, Z., Jing, C., Gallagher, S. S., Sheetz, M. P., and Cornish, V. W. (2012) Second-generation covalent TMP-tag for live cell imaging. *J. Am. Chem. Soc.* 134, 13692-13699.

(13) Galonic, D. P., Ide, N. D., van der Donk, W. A., and Gin, D. Y. (2005) Aziridine-2-carboxylic acid-containing peptides: application to solution- and solid-phase convergent site-selective peptide modification. *J. Am. Chem. Soc.* 127, 7359-7369.

(14) Holm, L., Moody, P., and Howarth, M. (2009) Electrophilic antibodies forming covalent bonds to protein targets. *J. Biol. Chem.* 284, 32906-32913.

(15) Banerjee, A., Panosian, T. D., Mukherjee, K., Ravindra, R., Gal, S., Sackett, D. L., and Bane, S. (2010) Site-specific orthogonal labeling of the carboxy terminus of alpha-tubulin. *ACS Chem. Biol.* 5, 777-785.

(16) Fauq, A. H., Kache, R., Khan, M. A., and Vega, I. E. (2006) Synthesis of acid-cleavable light isotope-coded affinity tags (ICAT-L) for potential use in proteomic expression profiling analysis. *Bioconjugate Chem.* 17, 248-254.

(17) Sinha, B., Cao, Z., Murray, T. F., and Aldrich, J. V. (2009) Discovery of dermorphin-based affinity labels with subnanomolar affinity for mu opioid receptors *J. Med. Chem.* 52, 7372-7375.

(18) Larda, S. T., Pichugin, D., and Prosser, R. S. (2015) Site-Specific Labeling of Protein Lysine Residues and N-Terminal Amino Groups with Indoles and Indole-Derivatives. *Bioconjugate Chem.* 26, 2376-2383.

(19) Vendrell, M., Samanta, A., Yun, S. W., and Chang, Y.-T. (2011) Synthesis and characterization of a cell-permeable near-infrared fluorescent deoxyglucose analogue for cancer cell imaging. *Org. Biomol. Chem.* 9, 4760-4762.

(20) Dong, J., Krasnova, L., Finn, M. G., and Sharpless, K. B. (2014) Sulfur(VI) fluoride exchange (SuFEx): another good reaction for click chemistry. *Angew. Chem. Int. Ed.* 53, 9430-9448.

(21) For an overview on MCRs see: Zhu, J., Wang, Q., and Wang, M.-X. Eds. (2015) *Multicomponent Reactions in Organic Synthesis* Wiley-VCH, Weinheim.

(22) Hulme, C., Ayaz, M., Martinez Ariza, G., Medda, F., and Shaw, A. (2015) Recent Advances in Multicomponent Reaction Chemistry:

Applications in Small Molecule Drug Discovery, *Small Molecule Medicinal Chemistry. Strategies and Technologies* (Czechitzky, W., and Hamley, P., Eds.) pp 145-187, Chapter 6, Wiley, Weinheim.

(23) Levi, L., and Müller, T. J. J. (2016) Multicomponent syntheses of functional chromophores. *Chem. Soc. Rev.* 45, 2825-2846.

(24) Arévalo, M. J., Kielland, N., Masdeu, C., Miguel, M., Isambert, N., and Lavilla, R. (2009) Multicomponent Access to Functionalized Mesoionic Structures Based on TFAA Activation of Isocyanides: Novel Domino Reactions. *Eur. J. Org. Chem.* 617-625.

(25) Kielland, N., Vendrell, M., Lavilla, R., and Chang, Y.-T. (2012) Imaging histamine in live basophils and macrophages with a fluorescent mesoionic acid fluoride. *Chem. Commun.* 48, 7401-7403.

(26) Pérez-Rentero, S., Kielland, N., Lavilla, R., and Eritja, R. (2010) Synthesis and Properties of Oligonucleotides Carrying Isoquinoline Imidazo[1,2-a]azine Fluorescent Units. *Bioconjugate Chem.* 21, 1622-1628.

(27) Vázquez-Romero, A., Kielland, N., Arévalo, M. J., Preciado, S., Mellanby, R., Feng, Y., Lavilla, R., and Vendrell, M. (2013) Multicomponent Reactions for de Novo Synthesis of BODIPY Probes: In vivo Imaging of Phagocytic Macrophages. *J. Am. Chem. Soc.* 135, 16018-16021.

(28) Ramírez-Ornelas, D. E., Alvarado-Martínez, E., Bañuelos, J., López Arbeloa, I., Arbeloa, T., Mora-Montes, H. M., Pérez-García, L. A., and Peña-Cabrera, E. (2016) FormylBODIPYs: Privileged Building Blocks for Multicomponent Reactions. The case of the Passerini Reaction. *J. Org. Chem.*, 81, 2888-2898.

(29) Myochin, T., Hanaoka, K., Komatsu, T., Terai, T., and Nagano, T. (2012) Design strategy for a near-infrared fluorescence probe for matrix metalloproteinase utilizing highly cell permeable boron dipyrromethene. *J. Am. Chem. Soc.* 134, 13730-13737.

(30) Michel, B. W., Lippert, A. R., and Chang, C. J. (2012) A reaction-based fluorescent probe for selective imaging of carbon monoxide in living cells using a palladium-mediated carbonylation. *J. Am. Chem. Soc.* 134, 15668-15671.

(31) Dodani, S. C., Leary, S. C., Cobine, P. A., Winge, D. R., and Chang, C. J. (2011) A targetable fluorescent sensor reveals that copper-deficient SCO1 and SCO2 patient cells prioritize mitochondrial copper homeostasis. *J. Am. Chem. Soc.* 133, 8606-8616.

(32) Isik, M., Ozdemir, T., Turan, I. S., Kolemen, S., and Akkaya, E. U. (2013) Chromogenic and fluorogenic sensing of biological thiols in aqueous solutions using BODIPY-based reagents. *Org. Lett.* 15, 216-219.

(33) Gavande, N., Kim, H. L., Doddareddy, M. R., Johnston, G. A. R., Chebib, M., and Hanrahan, J. R. (2013) Design, Synthesis, and Pharmacological Evaluation of Fluorescent and Biotinylated Antagonists of p1 GABAC Receptors. *ACS Med. Chem. Lett.* 4, 402-407.

(34) Komatsu, T., Urano, Y., Fujikawa, Y., Kobayashi, T., Kojima, H., Terai, T., Hanaoka, K., and Nagano, T. (2009) Development of 2,6-carboxy-substituted boron dipyrromethene (BODIPY) as a novel scaffold of ratiometric fluorescent probes for live cell imaging. *Chem. Commun.* 7015-7017.

(35) Mendive-Tapia, L., Zhao, C., Akram, A. R., Preciado, S., Albericio, F., Lee, M., Serrels, A., Kielland, N., Read, N. D., Lavilla, R., et. al. (2016) Spacer-free BODIPY fluorogens in antimicrobial peptides for direct imaging of fungal infection in human tissue. *Nat. Commun.* 7, 10940, doi: 10.1038/ncomms10940.

(36) Tahtaoui, C., Thomas, C., Rohmer, F., Klotz, P., Duportail, G., Mély, Y., Bonnet, D., and Hibert, M. (2007) Convenient method to access new 4,4-dialkoxy- and 4,4-diaryloxy-diaza-s-indacene dyes: Synthesis and spectroscopic evaluation. *J. Org. Chem.* 72, 269-272.

(37) Lakshmi, V., Ravikanth, M. (2014) Synthesis of Hexasubstituted Boron-Dipyrromethenes Having a Different Combination of Substituents. *Eur. J. Org. Chem.* 5757-5766.

(38) St. Cyr, D. J., Martin, N., and Arndtsen, B. A. (2007) Direct Synthesis of Pyrroles from Imines, Alkynes, and Acid Chlorides: An Isocyanide-Mediated Reaction. *Org. Lett.* 9, 449-452.

(39) van Oosten, M., Schafer, T., Gazendam, J.A.C., Ohlsen, K., Tsompanidou, E., de Goffau, M.C., Harmsen, H. J. M., Crane, L. M., Lim, E., Francis, K. P., et. al. (2013) Real-time in vivo imaging of invasive- and biomaterial-associated bacterial infections using fluorescently labelled vancomycin. *Nat. Commun.* 4, 2584, doi: 10.1038/ncomms3584.

(40) Er, J. C., Tang, M. K., Chia, C. G., Liew, H., Vendrell, M., and Chang, Y.-T. (2013) MegaStokes BODIPY-triazoles as environmentally sensitive turn-on fluorescent dyes. *Chem. Sci.* 4, 2168-2176.

(41) Zhang, L., Er, J. C., Jiang, H., Li, X., Luo, Z., Ramezani, T., Feng, Y., Tang, M. K., Chang, Y.-T., and Vendrell, M. (2016) A highly selective fluorogenic probe for the detection and in vivo imaging of Cu/Zn superoxide dismutase. *Chem. Commun.* DOI: 10.1039/C6CC00095A.

(42) Grimm, J. B., Sung, A. J., Legant, W. R., Hulamm, P., Matlosz, S. M., Betzig, E., and Lavis, L. D. (2013) Carbofluoresceins and Carborhodamines as Scaffolds for High-Contrast Fluorogenic Probes. *ACS Chem. Biol.* 8, 1303-1310.

(43) Butkevich, A. N., Mitronova, G. Y., Sidenstein, S. C., Klocke, J. L., Kamin, D., Meineke, D. N. K., D'Este, E., Kraemer, P. T., Danzl, J. G., Belov, V. N., et. al.. (2016) Fluorescent Rhodamines and Fluorogenic Carbopyronines for Super-Resolution STED Microscopy in Living Cells. *Angew. Chem. Int. Ed. Engl.* 55, 3290-3294.

(44) Huang, W., and Levitt, D.G. (1977) Theoretical calculation of the dielectric constant of a bilayer membrane. *Biophys. J.* 17, 111-128.