



## Catalyst-free thia-Diels-Alder click reaction for the labeling of peptides for imaging applications

Timothé Maujean, Patrice Marchand, Patrick Wagner, Stéphanie Riché, Frédéric Boisson, Nicolas Girard, Julie Karpenko, Dominique Bonnet, Mihaela Gulea

### ► To cite this version:

Timothé Maujean, Patrice Marchand, Patrick Wagner, Stéphanie Riché, Frédéric Boisson, et al.. Catalyst-free thia-Diels-Alder click reaction for the labeling of peptides for imaging applications. Phosphorus, Sulfur, and Silicon and the Related Elements, 2023, 198 (6), pp.532-535. 10.1080/10426507.2023.2165659 . hal-04195673

**HAL Id: hal-04195673**

**<https://hal.science/hal-04195673>**

Submitted on 16 Oct 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Catalyst-free thia-Diels-Alder click reaction for the labeling of peptides for imaging applications

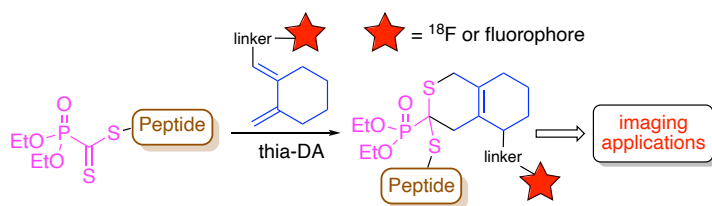
Timothé Maujean,<sup>1</sup> Patrice Marchand,<sup>2</sup> Patrick Wagner,<sup>1</sup> Stéphanie Riché,<sup>1</sup> Frédéric Boisson,<sup>2</sup> Nicolas Girard,<sup>1</sup> Julie Karpenko,<sup>1</sup> Dominique Bonnet,<sup>1\*</sup> and Mihaela Gulea<sup>1\*</sup>

<sup>1</sup>Université de Strasbourg, CNRS, LIT UMR 7200, 67000, Strasbourg, France

<sup>2</sup>Université de Strasbourg, CNRS, IPHC, UMR 7178, 67000, Strasbourg, France

dbonnet@unistra.fr, gulea@unistra.fr

## GRAPHICAL ABSTRACT



**Abstract.** A thia-Diels-Alder reaction between a phosphonodithioester and a highly reactive exocyclic diene for the chemoselective labeling of peptides under catalyst-free mild conditions is described. An efficient method to introduce the dithioester function into a model tripeptide was first developed and then applied to two bioactive peptides, PSMA and apelin-13. The tripeptide-dithioester was used to optimize the cycloaddition with dienes bearing fluorine-18 or a fluorophore. Then, the cycloaddition of the PSMA-dithioester with the <sup>18</sup>F-diene led to a new PSMA-radiotracer, which was used for in vivo Positron Emission Tomography (PET) imaging. In the same way, a fluorescent apelin-13 probe was obtained from the apelin-dithioester and a squaraine-linked diene and used for in cellulo optical imaging.

**Keywords:** Click chemistry, Peptides, Dithioesters, thia-Diels-Alder, Fluorine-18, Fluorophores

## INTRODUCTION

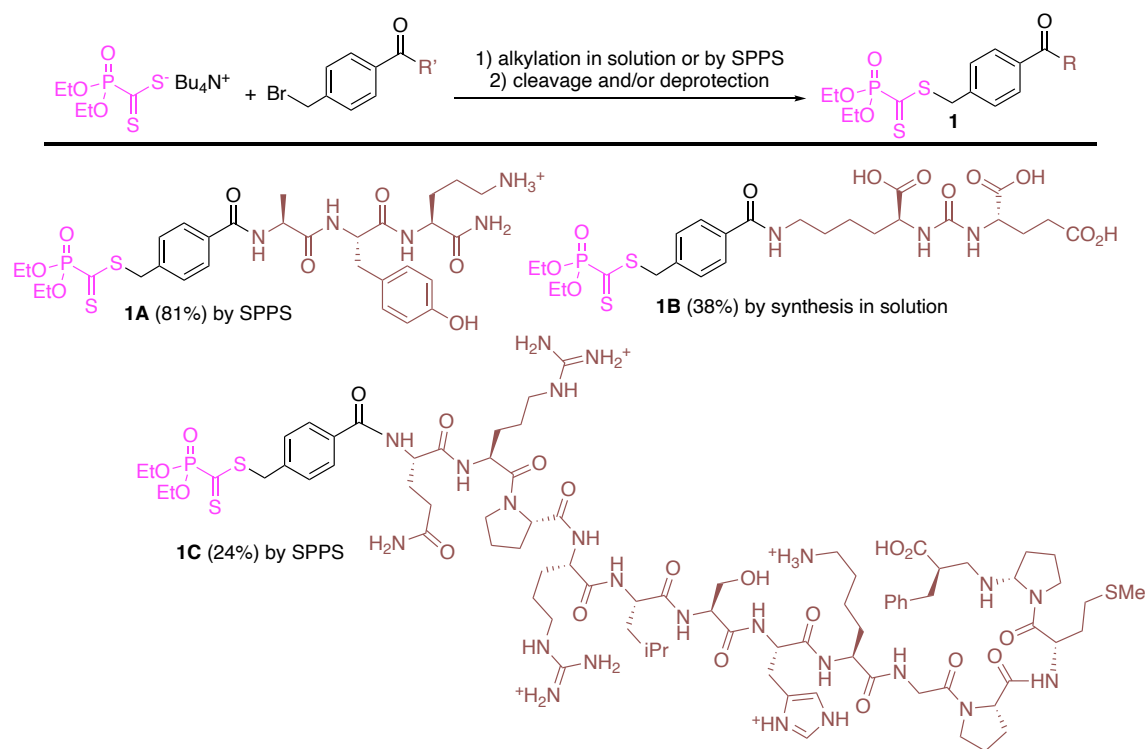
Peptides are biomolecules found in all living organisms and are widely used for medical applications as therapeutic or targeting agents, and also as diagnostic tools. This is why the interest of their labeling is growing, as testified by a large number of reviews in this topic.<sup>1</sup> Click reactions<sup>2</sup> represent an efficient method for the chemoselective labeling of peptides in mild conditions. Amongst these reactions, the Copper-Catalyzed Alkyne-Azide cycloaddition (CuAAC) remains the most used system despite the *in vivo* toxicity of the Cu(I) catalyst that may get trapped by the peptide at the end of the reaction. Alternatives such as the Strain-promoted Azide-Alkyne cycloaddition (SPAAC) or the Inverse Electron Demand Diels-Alder reaction (IEDDA) have been developed to overcome this issue, but still remain scarcely represented in the literature.

In this context, our recent work focused on the use of a thia-Diels-Alder (thia-DA) reaction between a phosphonodithioester and a diene for the chemoselective labeling of peptides, under catalyst-free mild conditions, for imaging applications. Herein are presented selected results of this work dealing with the development of the method and its application to the labeling of bioactive peptides with either fluorine-18 for *in vivo* Positron Emission Tomography (PET)<sup>3</sup> or fluorophores for *in cellulo* optical imaging.<sup>4</sup>

## RESULTS AND DISCUSSION

Based on our previous studies,<sup>5</sup> and on those of Barner-Kowollik and co-workers in the polymers field,<sup>6</sup> phosphonodithioformate was selected as the heterodienophile in the thia-DA reaction as it possesses good reactivity and stability, and an easy access. Moreover, the characterization of substrates and products by <sup>31</sup>P NMR spectroscopy is advantageous. Phosphonodithioester peptide derivatives **1A-C** were prepared by *S*-benzylation of the diethyl phosphonodithioformate tetrabutylammonium salt with the corresponding alkylating precursors (Scheme 1). The tripeptide-dithioester **1A** containing an AYK-NH<sub>2</sub> residue (AYK: alanine-tyrosine-lysine) was the model used to develop the solid-phase peptide synthesis (SPPS) procedure, which was also applied to the preparation of peptide-dithioester **1C**. The latter was based on the structure of apelin-13, a peptide containing thirteen amino acids and multiple nucleophilic functions (one amine, two guanidines, one imidazole, one alcohol) and which is an agonist of apelin G protein-coupled receptor (apelinR).<sup>7</sup> Starting from a Rink amide resin and using a Fmoc/*t*Bu strategy and after final cleavage and deprotection steps, it was possible to obtain **1A** and **1C** in respectively 81% and 24% overall

yield. This strategy prevented undesired thioacylation of the amino functions present on the peptide by its isolation as its trifluoroacetic acid ammonium salt. Finally, pseudopeptide-phosphonodithioester **1B** derived from the PSMA (prostate-specific membrane antigen) ligand, which contains a KuE-residue (KuE: lysine-urea-glutamate), was synthesized in solution with 38% overall yield (5 steps).



**Scheme 1.** Phosphonodithioesters **1A-C** prepared and used as heterodienophiles in this study.

Then, tripeptide-dithioester **1A** was used as a model to develop and optimize both the radiofluorination and the labeling with fluorophores, while dithioesters **1B** and **1C** derived from the bioactive peptides were labeled respectively with fluorine-18 (for *in vivo* PET imaging) and with a fluorophore (for live-cell optical imaging).

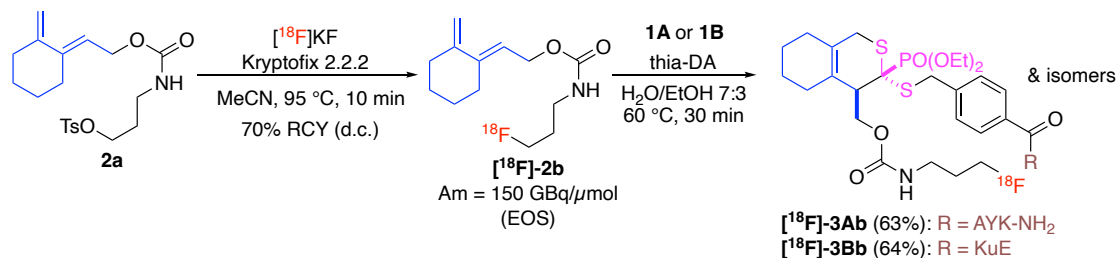
Due to the short-lived  $^{18}\text{F}$ -radioisotope, which is characterized by a half-life ( $t_{1/2}$ ) of 109.8 min, the choice of the structure of the dienic partner was crucial to reach high kinetics of the cycloaddition. Thus, we designed and synthesized a highly reactive exocyclic diene whose reaction with the phosphonodithioester has a second order rate constant of around  $4.3 \text{ M}^{-1}.\text{s}^{-1}$ , which is compatible with the  $^{18}\text{F}$ -labeling. A *para*-nitrophenyl carbonate exocyclic diene derivative was used as the

common precursor to access the suitable dienes partners **2** for labeling, via the formation of a carbamate link by reaction with the corresponding amines (Scheme 2).



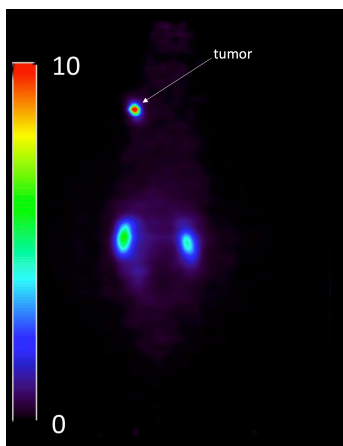
**Scheme 2.** Synthesis of the carbamate exocyclic dienes **2** used in the study

The radiolabeling reaction conditions were adapted on a fully automated apparatus to produce, starting from dithioester **1A** and diene [ $^{18}\text{F}$ ]-**2b** (obtained by radiofluorination from tosylate **2a**), the desired radiolabeled peptide [ $^{18}\text{F}$ ]-**3Ab** in  $54 \pm 6\%$  ( $n = 2$ ) radiochemical yield after purification (Scheme 3). The same conditions were used with PSMA-dithioester **1B** to obtain the purified radiolabeled PSMA ligand [ $^{18}\text{F}$ ]-**3Bb** in  $44 \pm 6\%$  radiochemical yield ( $n = 5$ ). Both automated syntheses were performed within 2 h (purification and formulation included) and the radiofluorinated peptides were obtained with high molar activities of  $80 \pm 20$  GBq/ $\mu\text{mol}$  ( $n = 3$ , EOS).



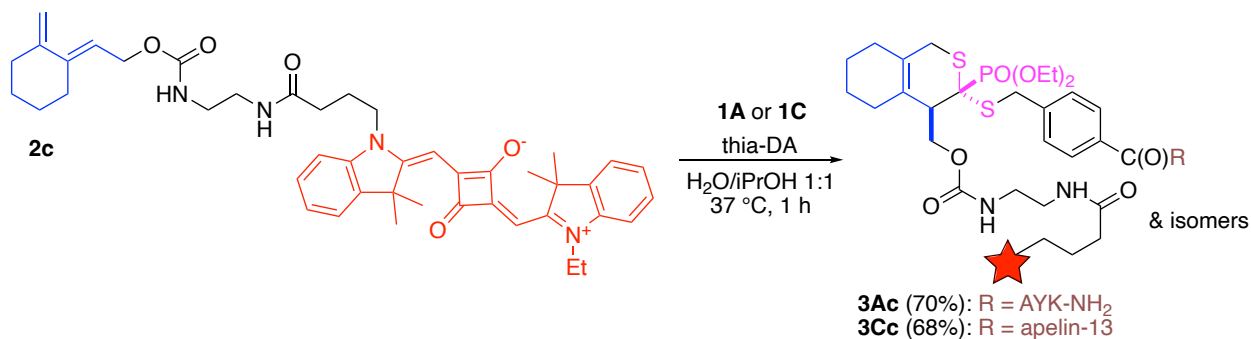
**Scheme 3.** Automated radiosynthesis of [ $^{18}\text{F}$ ]-**3Ab** and [ $^{18}\text{F}$ ]-**3Bb**.

The PSMA-ligand [ $^{18}\text{F}$ ]-**3Bb** was then used for *in vivo* experiments on mice bearing LNCap tumors. By imaging the mouse after injection of around 9 MBq of [ $^{18}\text{F}$ ]-**3Bb**, the vascularized tumor was efficiently detected (Figure 1). The specificity of [ $^{18}\text{F}$ ]-**6** was evaluated on LNCap cells in competition with 2-PMPA (a known inhibitor of PSMA receptors) and confirmed that the specificity of the ligand towards the PSMA receptor is preserved after the formation of the dihydrothiopyran cycloadduct.



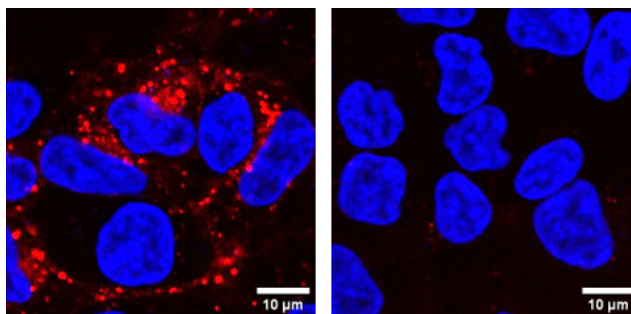
**Figure 1.** Anterior coronal view of mouse bearing LNCap tumor (placed back of the right shoulder) 60 min after i.v. injection; white arrow pointing to the tumor. Scale bar from 0 to 10% ID/g.

To synthesize the diene partner for the peptide-labeling by a fluorophore, we selected the squaraine dye, which displays emission wavelength in the far-red region ( $\lambda_{\text{abs}} \approx 635 \text{ nm}$ ,  $\lambda_{\text{em}} \approx 645 \text{ nm}$ ), and was described and used by us for GPCRs imaging in living cells.<sup>8</sup> It is characterized by a high photostability, a strong molar extinction coefficient ( $\approx 350\,000 \text{ M}^{-1}\text{cm}^{-1}$ ), and fluorogenic properties with a quantum yield 5 to 20 times lower in water than in 1,4-dioxane. Starting from the *para*-nitrophenyl carbonate exocyclic diene (illustrated in Scheme 2) and the suitable amine derivative, the desired diene-squaraine **2c** (Scheme 4) was obtained in 93% yield. Under optimized reaction conditions, the thia-Diels-Alder reaction between tripeptide-phosphonodithioester **1A** and diene **2c** led within one hour to the expected labeled peptide **3Ac** in 70% yield (Scheme 4). Applied to dithioester **1C** derived from apelin-13, the reaction with **2c** led to the desired peptide-probe **3Cc** in 68% yield. This result demonstrated again the efficiency and compatibility of our cycloaddition with the presence of the various functions of a complex peptide sequence.



**Scheme 4.** Synthesis of squaraine-labeled peptides **3Ac** and **3Cc**.

To demonstrate the applicability of the new probe **3Cc**, live-cell imaging of apelinR was performed by laser-scanning confocal microscopy on living HEK cells overexpressing apelinR (Figure 2). Due to its fluorogenic character, **3Cc** allowed visualization of apelinR under no-wash conditions with a high signal-to-noise ratio (Figure 2, left). Due to its agonist character **3Cc** fully internalized apelinR already after 15 min of incubation at 37 °C. On the other hand, no staining of apelinR was observed in the presence of a large excess of non-fluorescent apelin-13 (Figure 2, right), which confirmed the high specificity of **3Cc** for apelinR.



**Figure 2.** Cells incubated with **3Cc** (left) or its mixture with apelin-13 (right) for 15 min at 37 °C prior to the imaging. Fluorescence of squaraine is shown in red; nuclei stained with Hoechst 33342 are shown in blue.

## CONCLUSIONS

In conclusion, we developed a new fast click-reaction for peptides labeling. This consists of a thia-Diels-Alder reaction involving a phosphonodithioester-functionalized peptide as the heterodienophile and a highly reactive *s-cis* constrained exocyclic diene bearing fluorine-18 or the squaraine as a fluorophore. Using this cycloaddition, a model tripeptide containing reactive  $\text{NH}_2$  and OH functions and a PSMA ligand were efficiently radiolabeled in mild conditions within 2 hours (purification and formulation included) and the conjugates obtained in high molar activities and radiopurities. The PSMA-radiotracer [ $^{18}\text{F}$ ]-**3Bb** was used for the *in vivo* detection of small size tumors in xenografted mice. The thia-Diels-Alder click reaction was then successfully extended to the labeling by the squaraine fluorophore of the model tripeptide, as well as of apelin-13. The fluorescent apelin-13 probe **3Cc** was employed for the no-wash imaging of apelinR in living HEK cells overexpressing this receptor. This method represents a highly efficient and straightforward approach to the chemoselective labeling of bioactive peptides for further imaging applications.

## ACKNOWLEDGMENTS

This work is part of the Interdisciplinary Thematic Institutes IMS and InnoVec, ITI 2021-2028 program of the University of Strasbourg, CNRS, and Inserm, supported by IdEx Unistra (ANR-10-IDEX-0002) and SFRI-STRAT'US project (ANR-20-SFRI-0012). T.M. was supported by a fellowship from the ENS Paris-Saclay. We thank Dr. Nicolas Humbert (LBP, UMR 7021) for his expertise on the spectrophotometers and fluorometers, and the platforms PACSI (GDS 3670), PIQ-QuEst (Faculty of Pharmacy, Strasbourg), and CYRCé for technical support.

## REFERENCES

1. (a) Fani, M.; Maecke, H. R.; *Eur. J. Nucl. Med. Mol. Imaging* **2012**, 39, 11; (b) Krecisz, P.; Czarnecka, K.; Krolicki, L.; Mikiciuk-Olasik, E.; Szymanski, P. *Bioconjugate Chem.* **2021**, 32, 25. (c) Jacobson, O.; Kiesewetter, D. O.; Chen, X. *Bioconjugate Chem.* **2015**, 26, 1; (d) Olberg, D. E.; Hjelstuen, O. K. *Curr. Top. Med. Chem.* **2010**, 10, 1669; (e) Richter, S.; Wuest, F. *Molecules*, **2014**, 19, 20536; (f) Mendive-Tapia, L.; Wang, J.; Vendrell, M. J. *Pep. Sci.* **2020**, e24181; (g) Maity, D. *Beilstein J. Org. Chem.* **2020**, 16, 2971; (h) Cheng, Z.; Kuru, E.; Sachdeva, A.; Vendrell, M. *Nat. Rev. Chem.* **2020**, 4, 275.
2. Kolb, H. C.; Finn M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, 40, 2004.
3. Maujean, T.; Marchand, P.; Wagner, P.; Riché, S.; Boisson, F.; Girard, N.; Bonnet, D.; Gulea, M. *Chem. Commun.* **2022**, 58, 11151.
4. Maujean, T.; Wagner, P.; Valencia, C.; Riché, S.; Iturrioz, X.; Villa, P.; F.; Girard, N.; Karpenko, J.; Gulea, M. Bonnet, D. *Bioconjugate Chem.* **article ASAP**, DOI: 10.1021/acs.bioconjchem.2c00500.
5. (a) Heras, M.; Gulea, M.; Masson, S. *Chem. Commun.* **2001**, 611; (b) Heras, M.; Gulea, M.; Masson, S.; Philouze, C. *Eur. J. Org. Chem.* **2004**, 160; (c) Dentel, H.; Chataigner, I.; Lohier, J-F.; Gulea, M. *Tetrahedron* **2012**, 68, 2326.
6. (a) Sinnwell, S.; Inglis, A. J.; Davis, T. P.; Stenzel M. H.; Barner-Kowollik C. *Chem. Commun.* **2008**, 2052; (b) Beloqui, A.; Mane, S. R.; Langer, M.; Glassner, M.; Bauer, D. M.; Fruk, L.; Barner-Kowollik, C.; Delaittre, G. *Angew. Chem. Int. Ed.* **2020**, 59, 19951.
7. (a) O'Carroll, A. M.; Lolait, S. J.; Harris, L. E.; Pope, G. R. *J. Endocrinol.* **2013**, 219, 13; (b) Kleinz, M. J.; Davenport, A. P. *Pharmacol. Ther.* **2005**, 107, 198; (c) Yamaleyeva, L. M.; Shaltout, H.; Varagic, J. *Curr. Opin. Nephrol. Hypertens.* **2016**, 25, 396; (d) Wysocka, M. B.; Pietraszek-Gremplewicz, K.; Nowak, D. *Front Physiol.* **2018**, 9, 557.
8. Karpenko, J.; Klymchenko, A. S.; Gioria, S.; Kreder, R.; Shulov, I.; Villa, P.; Mély, Y.; Hibert, M.; Bonnet, D. *Chem. Commun.* **2015**, 51, 2960.