Chemoselective P-S coupling of aminophospholes to peptides under mild conditions

Miyanou Rosales-Hurtado,[1] Laure Liénart,[1] Sébastien Clément,[2] Jean-Alain Fehrentz,[1] Jean-Louis Banères,[1] Florine Cavelier,[1] Emmanuelle Rémond.[1]

[1]Pôle Chimie Balard, IBMM - UMR 5247, 1919, route de Mende, 34293 MONTPELLIER cedex 5 ; [2]Pôle Chimie Balard, ICGM - UMR 5253, 1919, route de Mende, 34293 MONTPELLIER cedex 5

✉ emmanuelle.remond@umontpellier.fr ; florine.cavelier@umontpellier.fr

https://doi.org/10.17952/37EPS.2024.P1027

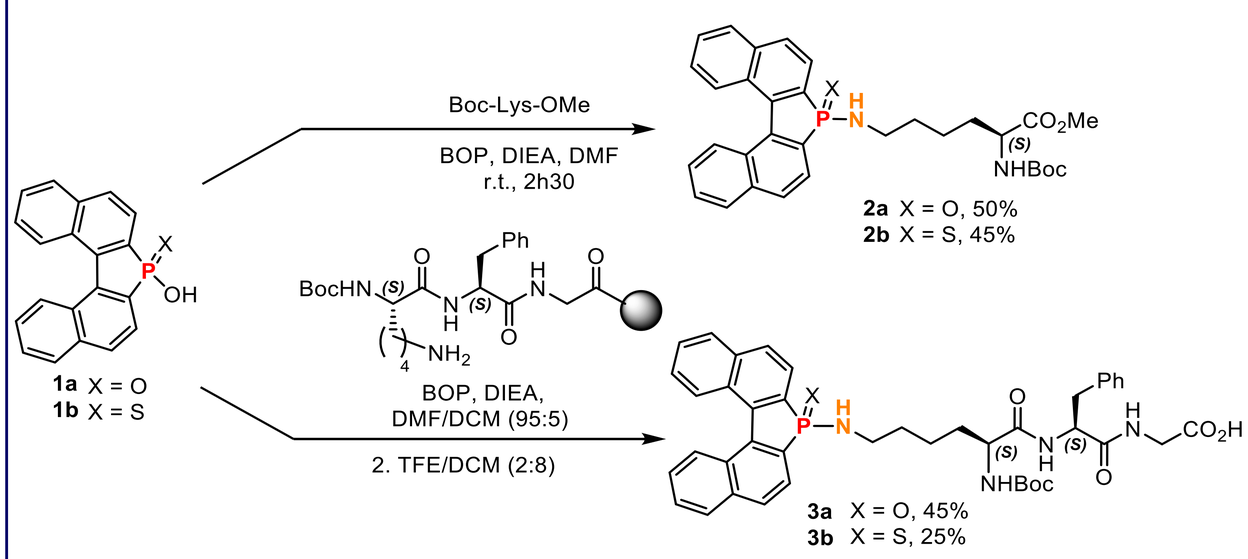
INTRODUCTION

Phospholes are dienes bridged with a tetrahedral phosphorus atom, which offer excellent resistance to photobleaching, and the phosphorus center can be easily transformed into derivatives with adjustable spectroscopic properties.[1] However, despite their low molecular weight, their absence of toxicity, and high photochemical stability compared to other fluorophores, the use of fluorescent phospholes in bioimaging is rare.[2] Strategies required for the chemistry and functionalization of phospholes are often associated with harsh conditions that cannot be used for biolabeling. To tackle this problem, we explored the attachment of phosphole-based fluorophores to peptides through the phosphorus atom under mild conditions, by P-heteroatom bond formation.[3]

COUPLING P-HYDROXYPHOSPHOLES OXIDE OR SULFIDE

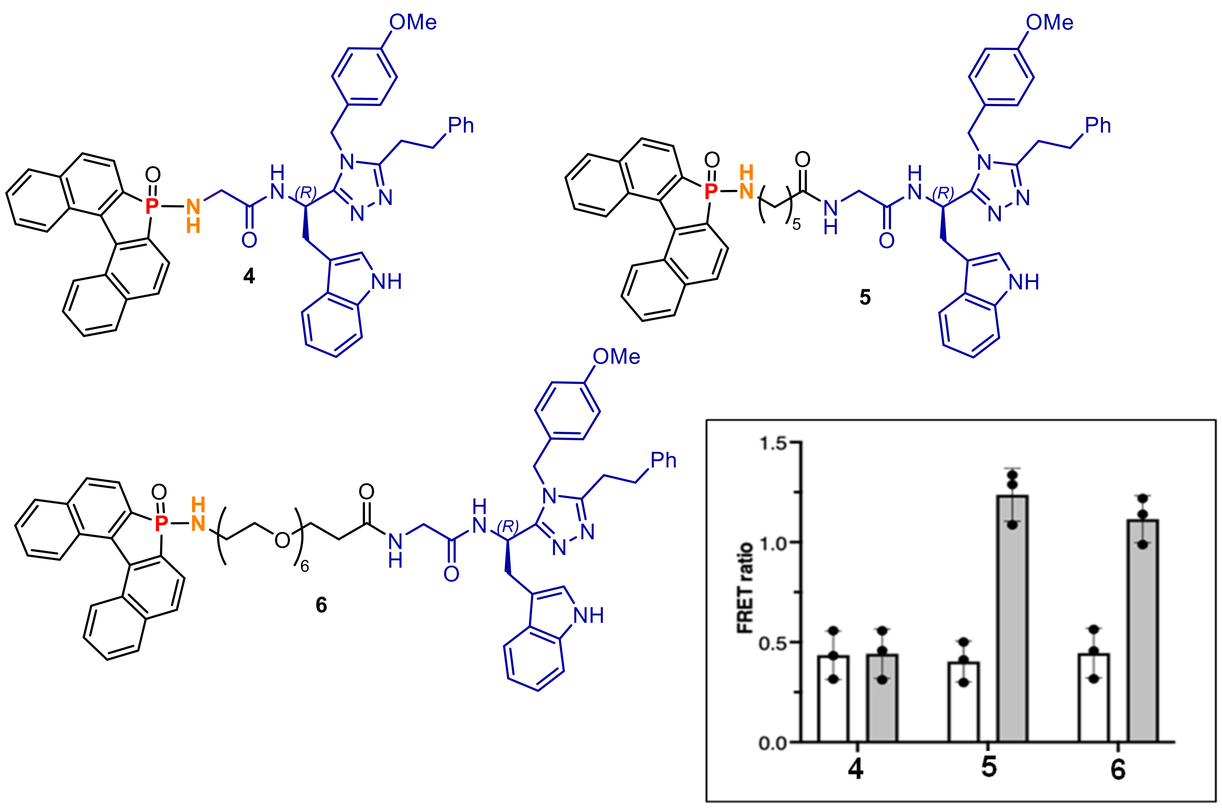
We have recently reported the synthesis of P-hydroxyphosphole-oxide or sulfide by trapping the dibromobinaphthyl dianion with a dichlorophosphine reagent (Cl_2POMe or Cl_2PNEt_2), followed by oxidation or sulfuration of the P-center. Finals compounds **1a,b** were obtained by saponification or acid hydrolysis.[4]

Labeling amino acids and peptides in solution or on solid support



Labeling JMV2959 and FRET assays

This strategy was used to label JMV2959,[5] an antagonist of the Growth Hormone Secretagogue Receptor type 1a (GHSR1a), to the glycyl group or through spacers (Figure 1).



- Binaphtylphosphole oxide: fluorophore donor (λ_{abs} 380 nm; λ_{em} 464 nm)
- Recombinant GHSR labeled with AF488 dye: fluorophore acceptor (λ_{em} 525 nm)

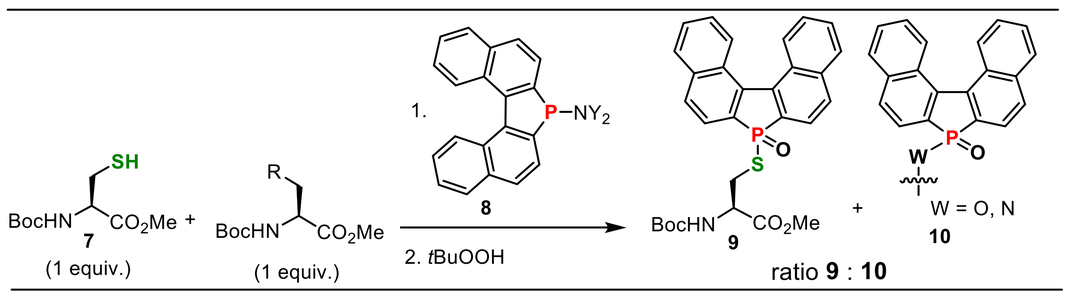
Figure 1. Structure of conjugates **4-6** and FRET ratio with the labeled receptor GHSR1a

➡ **FRET-based binding assays demonstrate that the presence of a spacer is required to maintain high binding affinity (Figure 1).**

CHEMOSELECTIVE COUPLING OF P-AMINOPHOSPHOLES

Alternatively, we have successfully developed a new method for coupling aminophospholes to biomolecules under neutral and reagent-free conditions.

Competitive experiments between amino acids



	ratio **9** : **10**
R = —OH, **Boc-Tyr-OMe**	>99 : -
R = —OH, **Boc-Ser-OMe**	98 : 2
R= —NH_2, **Boc-Lys-OMe**	91 : 9

➡ **High selectivity of coupling to cysteine P-S >> P-N > P-O**

Chemoselective labeling of cysteinylpeptides

Grafting aminophospholes onto cysteinyl pentapeptides which contain tyrosine, serine, or lysine residues, exhibit high chemoselectivity at the cysteine position. Only one product was observed in UPLC analysis.

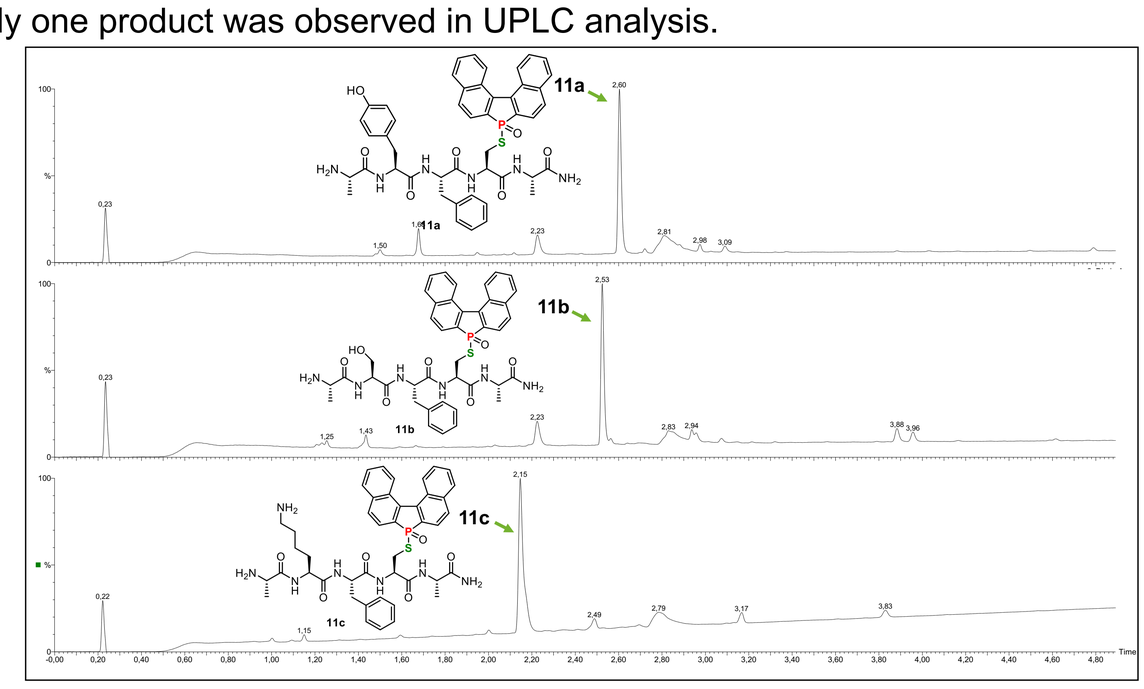


Figure 2: UPLC analysis of crude product **11a-c**

This innovative methodology has been applied for labeling peptide of interest such as Ghrelin [1-8]-Cys9, the natural ligand of GHSR1a.[6]

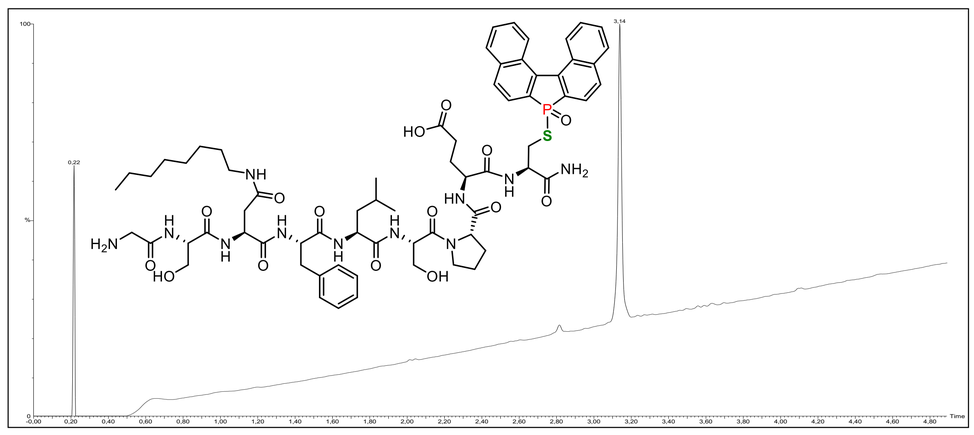


Figure 3: UPLC chromatogram of pure binaphtylphosphole Ghrelin[1-8]Cys9 conjugate

CONCLUSION

We have developed new classes of fluorophores derived of phospholes, the P-hydroxyphosphole-oxide or -sulfide and P-aminophospholes, for coupling to amino acids or peptides by formation of P-N or P-S bond, respectively, under mild conditions with high chemoselectivity. This latter strategy is currently explored for labeling proteins as well as other biomolecules.

References: (1) Y. Asok, *et al*. *Acc. Chem. Res.* **2023**, 56, 536−547; (2) M. Schenk, *et al*. *ChemBioChem* **2024**, e202300857; (3) J. Vantourout *et al. J.Am. Chem. Soc*. **2020**, 17236−17242; (4) E. Rémond, *et al*., *Chem. Eur. J*. **2022**, 28, e202201526; (5) A. Moulin, *et al*., *Amino Acids* **2013**, *44*, 301-314; (6) T.D. Müller, *et al*. *Mol. Metab*. **2015**, 4, 437–460.

Acknowledgments: We thank Dr. Ludovic MAILLARD for HPLC preparative apparatus and Pierre SANCHEZ for UPLC/MS analysis.