

## Introduction

In nature several compounds with heterocyclic groups, such as porphyrins, flavins, and pteridines, behave like sensitizers. In particular, pteridines are tetra-azo-naphthalenes that are substituted in position 6. This substituent conditions the photochemical and photophysical behavior of each compound. Also, they are water-soluble compounds with low affinity for lipid membranes, therefore we have recently developed new lipophilic pteridine derivatives with the aim of achieving a greater interaction with membranes and greater photoactivity on lipids.<sup>1,2</sup> Affinity for lipid membranes has been achieved by attaching aliphatic chains to photosensitizers by a nucleophilic substitution reaction ( $S_N2$ ) using as reagents 1-iododecane and the sensitizer. In particular, pterin (Ptr), lumazine (Lum) and 6-carboxypterin (Cap) in *N,N*-dimethylformamide or *N,N*-dimethylacetamide as a solvent, obtaining a set of alkylated compounds. In the case of Ptr and Lum, alkylation occurs in the pyrimidine ring, while in Cap the reaction occurs in the carboxylic group. On the other hand, pteric acid (Pte), like folic acid, undergoes photooxidation giving as a product 6-formylpterin (Fop), which photochemically oxidizes to Cap (Fig.1).<sup>3</sup> Taking this into account, in this work we chose Pte as reagent to alkylate, which has a carboxylic group in its substituent. The hypothesis is to obtain alkylated derivatives of Pte that may bind to lipid membranes and, under irradiation, may release Fop to the medium, which is an efficient water-soluble photosensitizer.

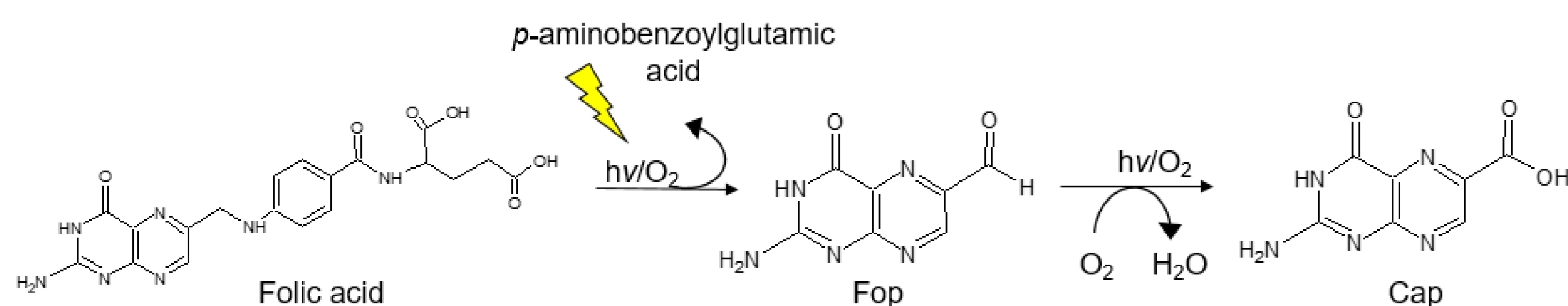


Fig 1: Photooxidation of Folic acid

## Synthesis

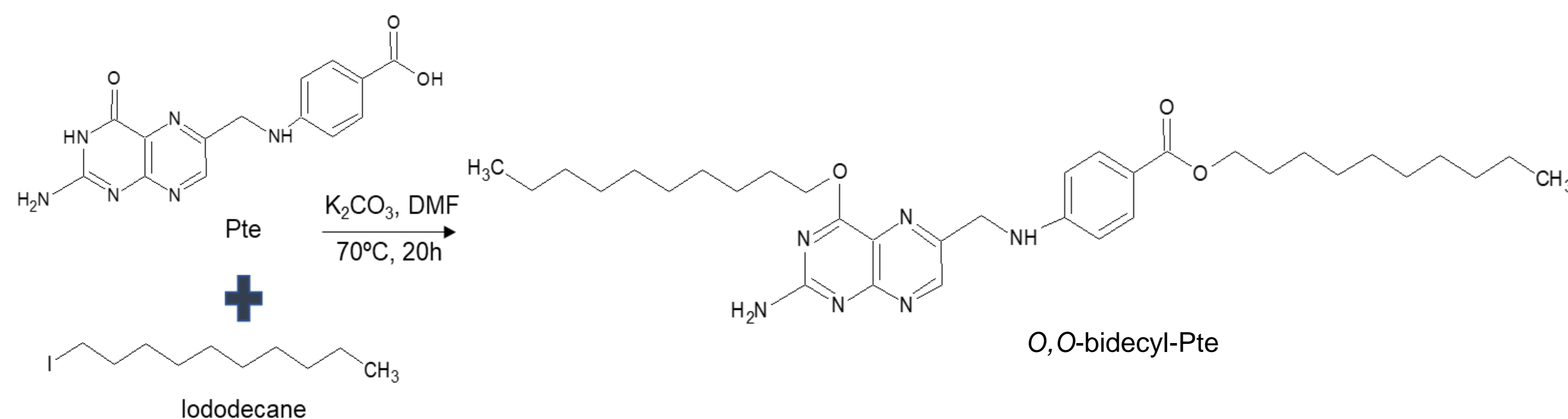
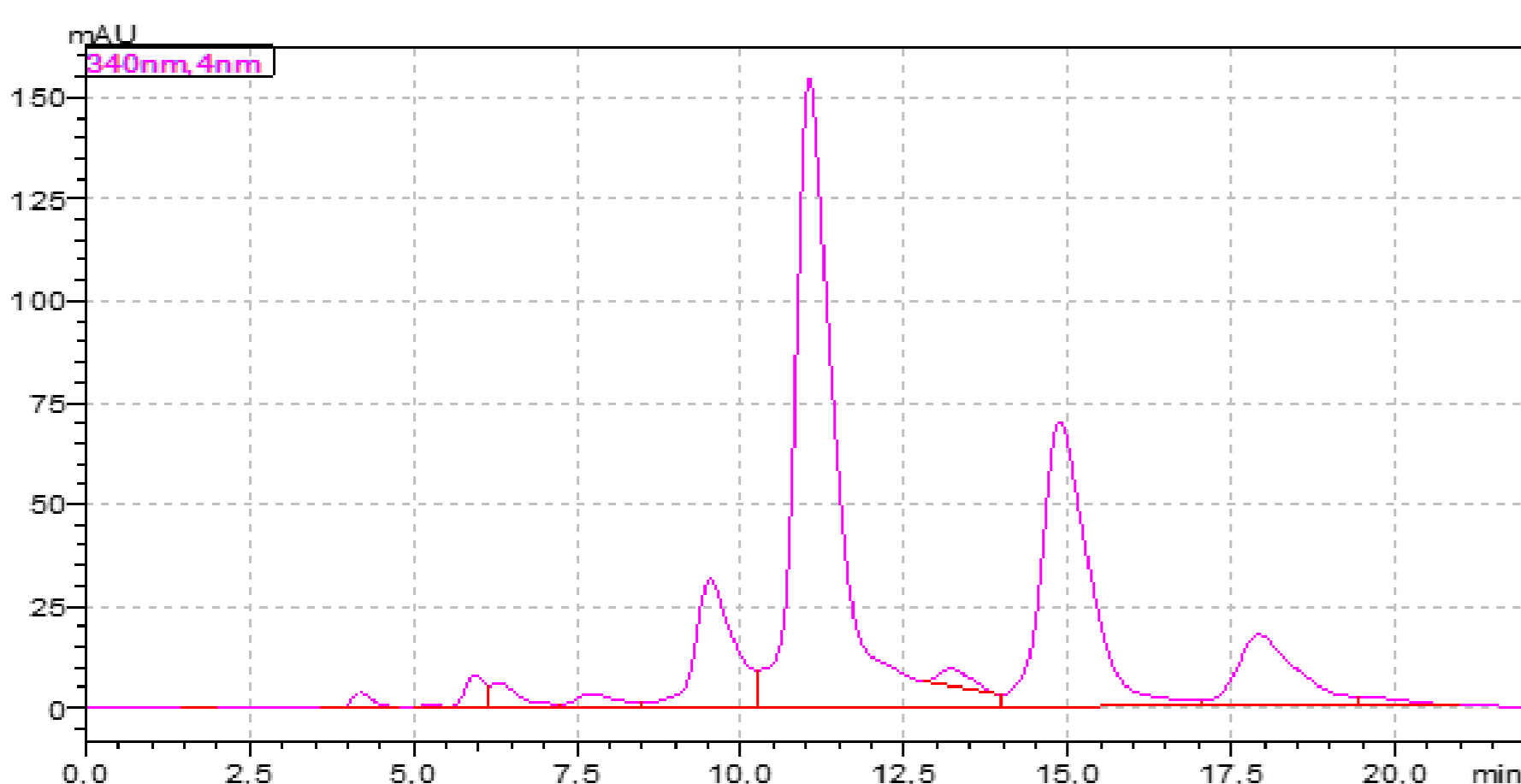


Fig 2: Reaction scheme between Pte:  $H_2CO_3$ :  $IC_{10}$  molar ratio 1: 1: 2

The addition of alkyl chains (C10) to Pte was achieved by nucleophilic substitution ( $S_N2$ ) reaction with Pte and Iododecane. Several products were formed during the reaction, the major product was selected and purified by liquid-liquid extraction, then flash chromatography (200-400 mesh silica gel) followed by HPLC purification.

## O,O-bidecyl-Pte purification

A) Reaction Mix



B) Purified O,O-bidecyl-Pte

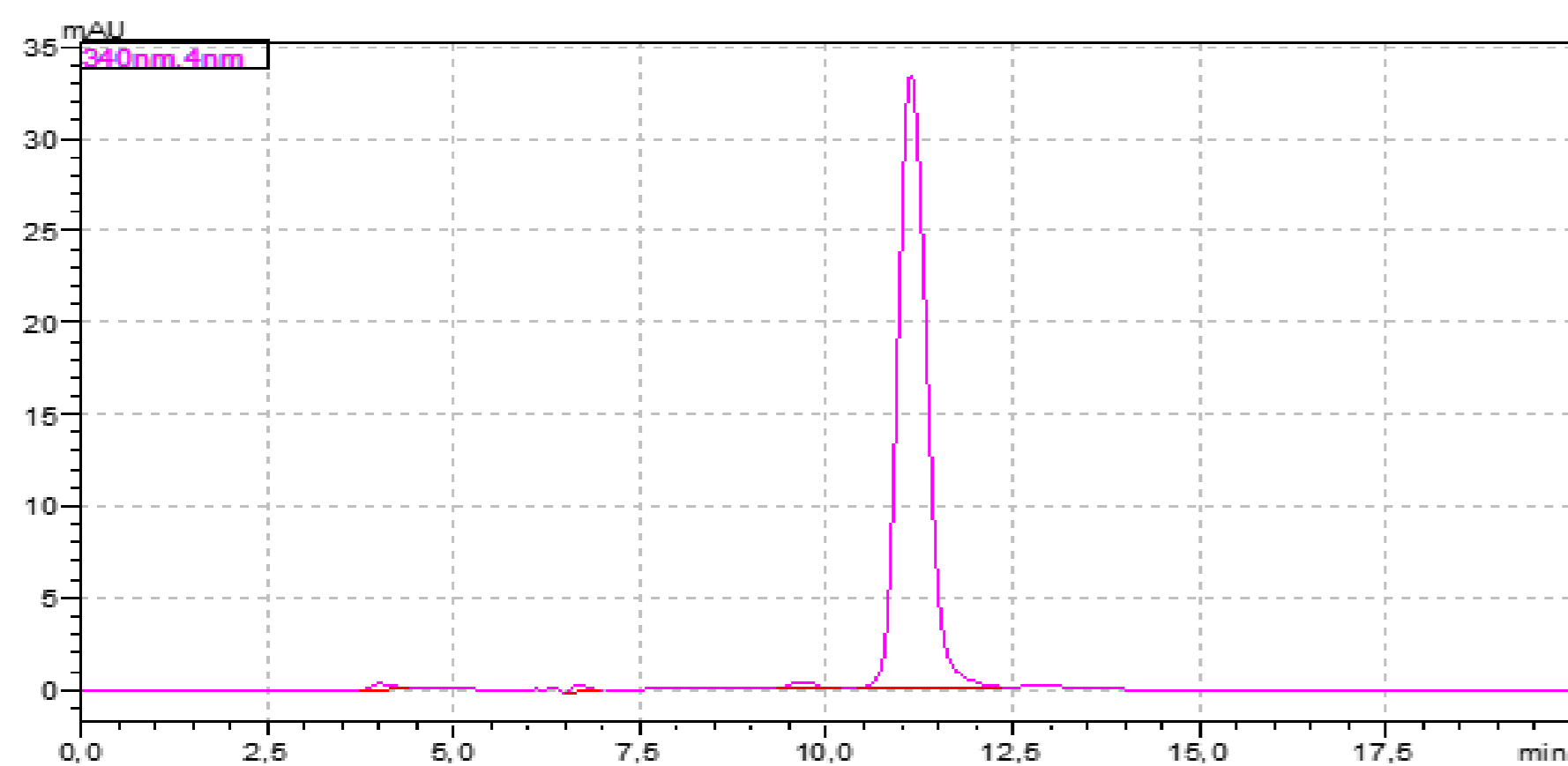


Fig 3: Chromatograms show A) the reaction mixture and B) O,O-bidecyl-Pte after purification. Experimental conditions are specified in the methodology section

## O,O-bidecyl-Pte photolysis

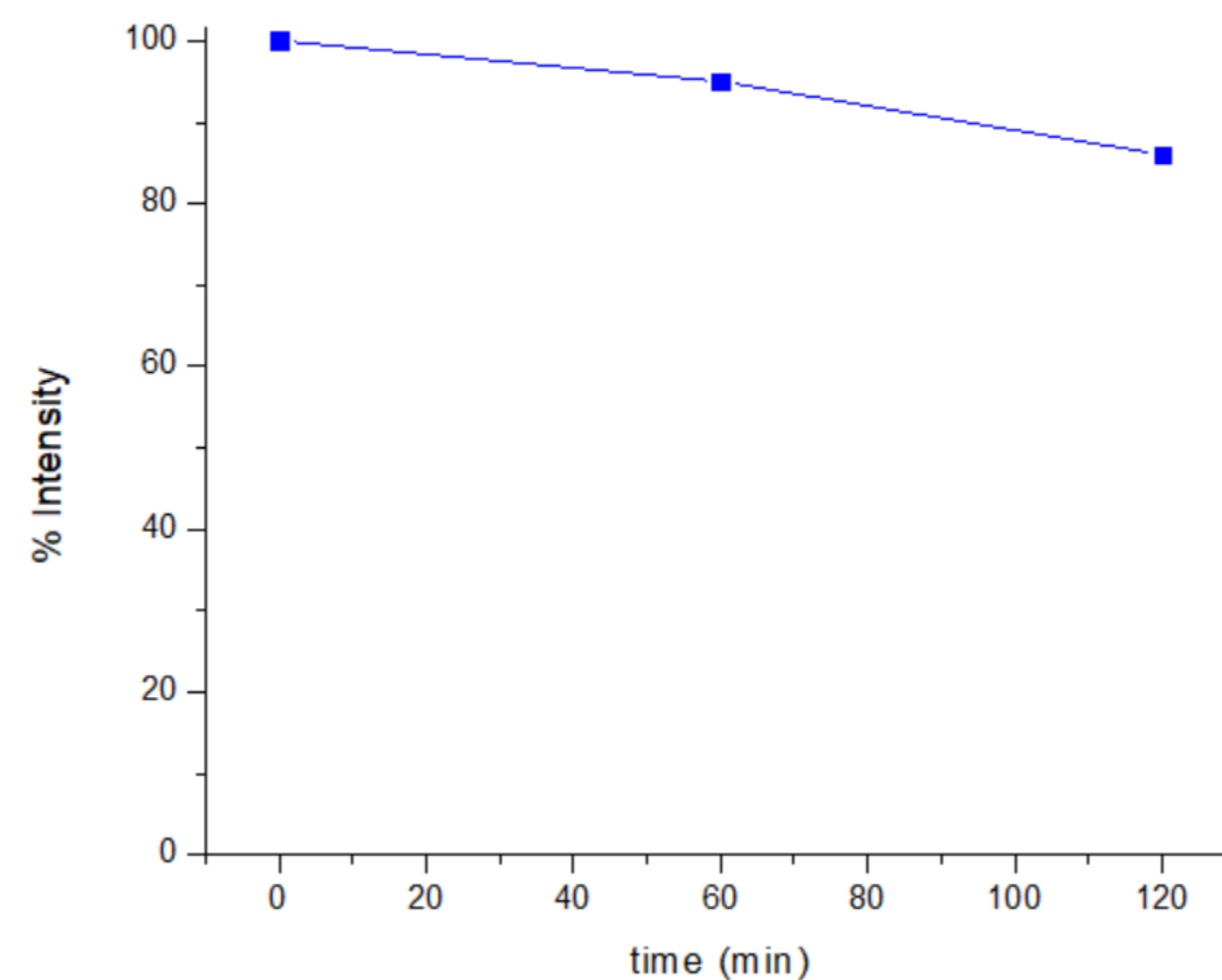


Fig 4: O,O-bidecyl-Pte % intensity versus irradiation time in min. O,O-bidecyl-Pte is 14% consumed after 2 continuous hours of irradiation. The solutions were irradiated and analyzed by HPLC under the conditions indicated in the methodology section.

## Methodology

### HPLC

A Shimadzu HPLC apparatus with PDA and fluorescence detector was employed with a Synergi Polar-RP column (ether-linked phenyl phase with polar end-capping,  $150 \times 4.6$  mm,  $4 \mu m$ , Phenomenex) for product separation. The mobile phase was methanol, and the runs were carried out with a flow of  $0.3 \text{ mL min}^{-1}$

### Mass Spectrometry

Agilent Technologies 6220 Accurate-Mass TOF LC/MS

### Proton NMR:

400 MHz by Bruker Nuclear Magnetic Resonance

### Spectrophotometry UV-Vis

Shimadzu UV-1800, Optical path 1 cm

### Fluorescence

Single-Photon-Counting equipment FL3 TCSPC-SP (Horiba Jobin Yvon)

### Fluorescence Quantum Yield ( $\Phi_F$ )

Reference: pterin  $\Phi_F$ : 0.27

$\lambda_{exc}$ : 365 nm

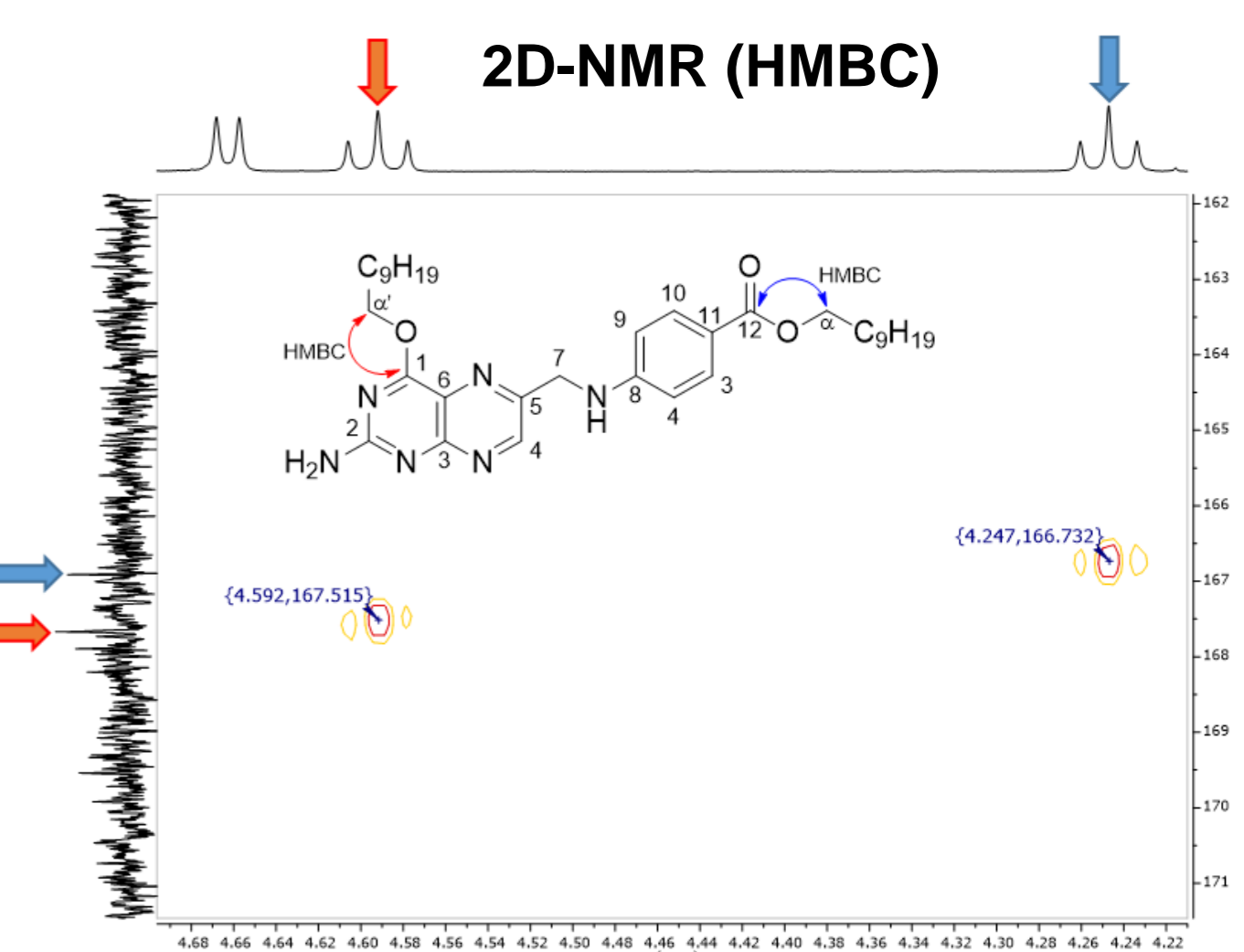
### Photolysis

Rayonet RPR lamp. Maximum emission 365nm

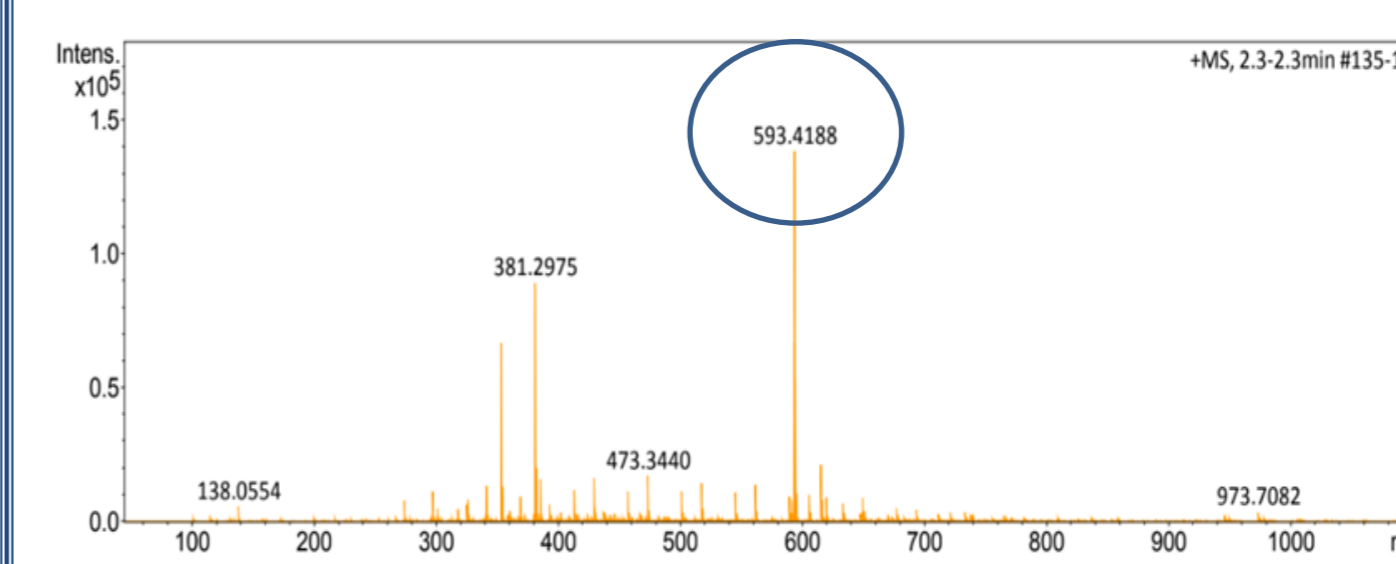
## Structural Characterization

$^1H$  and  $^{13}C$  NMR data of O,O-bidecyl-Pte

Spectra	O,O-bidecyl-pte
$^1H$ NMR	(500 MHz, $CDCl_3$ ) $\delta$ 8.89; 7.93 (d,2H); 5.32 (s,H);4.69 (d,2H); 4.62 (t,2H); 4.27 (t,2H); 1.59 (s,H); 0.90 (t,3H).
$^{13}C$ NMR	(126 MHz, $CDCl_3$ ) $\delta$ 150.10, 148.41, 131.59, 112.02, 68.61, 64.54, 46.71, 31.90, 29.56, 29.33, 28.85, 28.47, 26.09, 25.88, 22.69, 14.12.



### Mass spectrometry

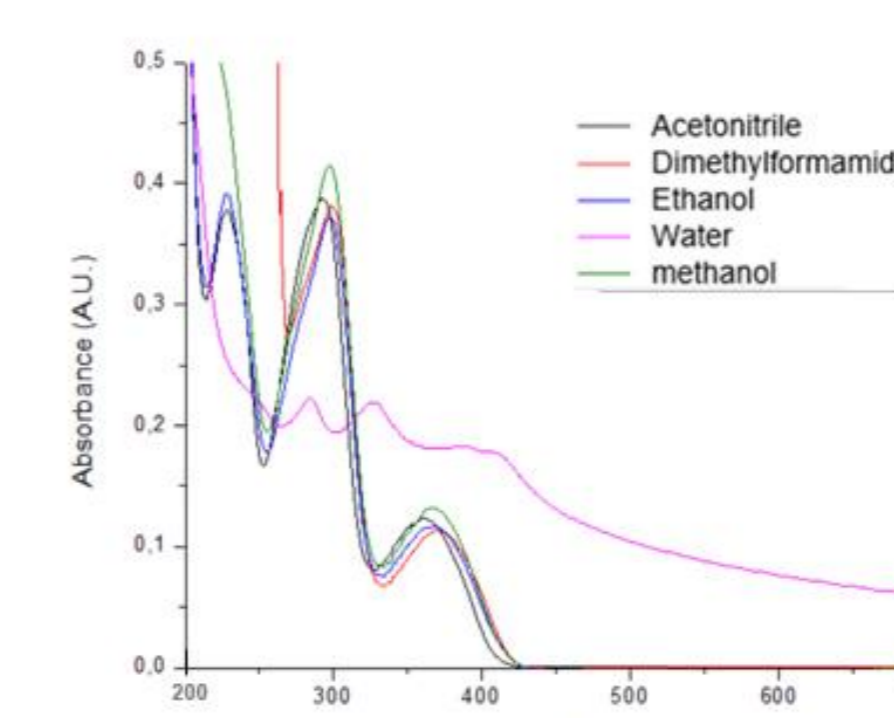


HRMS data of O,O-bidecyl-Pte. Molecular formula, observed and calculated mass and mass error

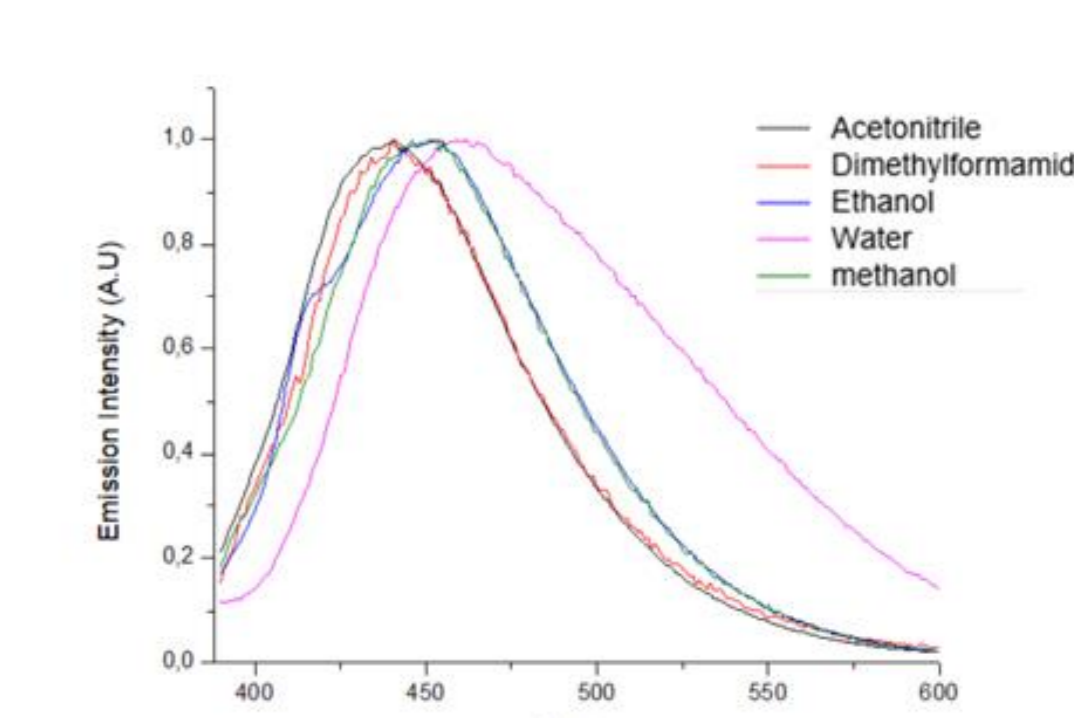
Molecular formula [M+H] <sup>+</sup>	$C_{24}H_{35}N_6O_3$
Calculated mass/Da	593.4173
Observed mass/Da	593.4188
$\Delta/mDa$	1.5

## Spectroscopic properties

A) Absorption spectra



B) Emission spectra



O,O-bidecyl-Pte	$\lambda_{max}$ absorption/nm	$\lambda_{max}$ emission/nm
Acetonitrile	229/300/360	435
Dimethylformamide	229/301/360	435
Ethanol	229/301/360	460
Water	295/326/400	470
Methanol	229/298/360	460

C) Fluorescence quantum yield  $\Phi_F \pm SD$

0.0024  $\pm$  0.002

A) Absorbance spectra in different solvents for O,O-bidecyl-Pte.  
B) Normalized Emission spectra in different solvents for O,O-bidecyl-Pte.  
C) O,O-bidecyl-Pte Fluorescence quantum yield in Methanol using Pterin as reference.

## Conclusions

- O,O-bidecyl-Pte was successfully synthesized and characterized by bidimensional NMR and mass spectroscopy.
- The absorbance and emission spectra were measured in different solvents for O,O-bidecyl-Pte.
- The O,O-bidecyl-Pte is photochemically stable after 2hr irradiation.
- The O,O-bidecyl-Pte presented a low fluorescence quantum yield.

## References

- 1) Vignoni, M., et al., Mol. Pharm., 15, 798 (2018).
- 2) Walalawela, N., et al., Photochem. Photobiol., 95(5), 1160 (2019).
- 3) Dántola, M. L., Photochem. Photobiol. Sci., 9, 1604 (2010)

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