

CERTIFICATE OF ATTENDANCE

We hereby confirm that **Anna Fülöp** has participated in our conference ChemCYS 2018 (Chemistry Conference for Young Scientists), organised in Blankenberge, Belgium, on February 21-23, 2018, by Jong-KVCV, the youth division of the Royal Flemish Chemical Society.

She contributed to our conference with following presentation:

New type of red fluorescent calcium sensor for two-photon imaging - Poster presentation in the session of Organic and Organometallic Chemistry

For Jong-KVCV,

Karel Haesevoets,
President of ChemCYS 2018



New type of red fluorescent calcium sensor for two-photon imaging

A. Fülöp^{1,2}, Z. Mucsi², M. Bojtár¹, E. Kovács³

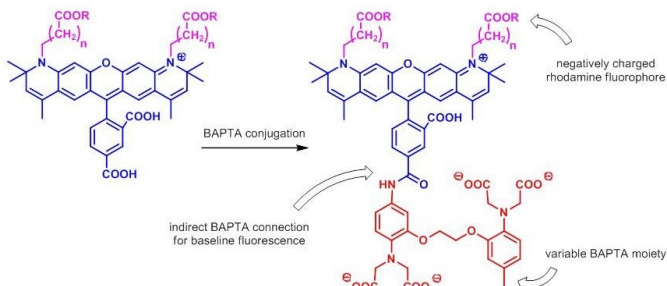
1. Department of Organic Chemistry and Technology, Budapest University of Technology and Economics, Budafoki út 8., 1111 Budapest, Hungary

2. Femtonics Kft., Tűzoltó u. 59., 1094 Budapest, Hungary

3. Research Centre for Natural Sciences, Hungarian Academy of Sciences, Magyar tudósok körútja 2., 1117 Budapest, Hungary

The calcium ion has an essential role in numerous processes in the living organism. The well known fluorescent calcium sensors are able to sense the free calcium ion in the intracellular space, following-up the time and space dependent changing of cellular Ca^{2+} ion concentration. The optical observation in a neuron shows direct information about the neuronal activities. The two-photon microscope technology is suitable for imaging of three dimensional tissue so these experiments have also become applicable *in vivo*.

Many fluorescent calcium indicator is known, but there is not a good sensor with right properties for two-photon *in vivo* imaging. The most frequently used ion chelating molecule is the 1,2-bis(*o*-aminophenoxy) ethane-*N,N,N',N'*-tetraacetic acid (BAPTA). Combining this receptor with a fluorescent dye results a calcium sensor. Our goal is to find a new fluorophore with right fluorescent properties: intensive red emission, good two-photon absorption, and good water solubility. In the literature the ATTO 590 fluorophore has one of the best two-photon properties, so we aimed to repair it's other properties. Because of the positive charge of this dye, it can accumulate in the mitochondria. We introduced carboxyl groups into the molecule to remove its positive charge and avoid accumulation. With this fluorophore we can get a new, red calcium sensor for two photon imaging.



NEW TYPE OF RED FLUORESCENT CALCIUM SENSOR FOR TWO-PHOTON IMAGING

Anna Fülöp^{1,2}, Zoltán Mucsi², Márton Bojtár¹, Ervin Kovács³

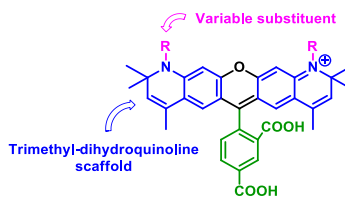
¹Department of Organic Chemistry and Technology, Budapest University of Technology and Economics, Budafoki út 8., 1111 Budapest, Hungary ; ²Femtonics Kft., Tűzoltó u. 59., 1094 Budapest, Hungary; ³Research Centre for Natural Sciences, Hungarian Academy of Sciences, Magyar tudósok körútja 2., 1117 Budapest, Hungary

INTRODUCTION

Calcium ion has an essential role in numerous processes in living organisms. In particular, intracellular Ca^{2+} concentration in neurons provides direct information about their activity. Two-photon microscope technology combined with fluorescent calcium sensors is an efficient method to monitor neurological processes for *in vivo* applications.

Numerous fluorescent calcium indicators are known in the literature, however, a sensor with the right properties for two-photon *in vivo* imaging is lacking. The most frequently used ion chelating molecule is 1,2-bis-(*o*-aminophenoxy) ethane-*N,N,N',N'*-tetraacetic acid (BAPTA). Combining this receptor with a fluorescent dye results in a calcium sensor.

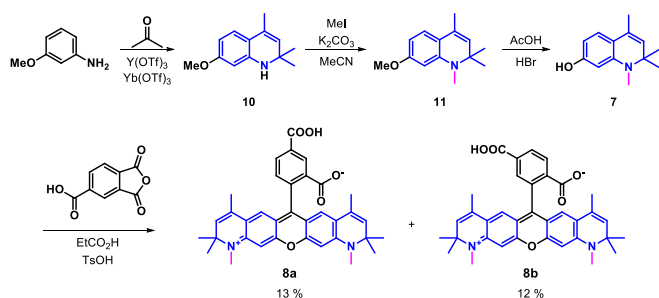
Our goal was to find a new fluorophore with optimal spectroscopic properties (intensive red emission, large two-photon absorption) and enhanced water solubility.



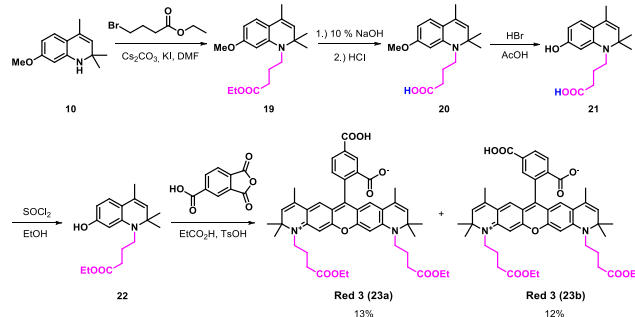
substituent:
 R = Me name: ATTO 590 methyl-derivative
 Et ATTO 590
 $(\text{CH}_2)_n\text{-COO}^-$ Red 1 - 3

The ATTO 590 dye has one of the largest two-photon absorption amongst rhodamine dyes, so we aimed to improve this fluorophore. To avoid accumulation and enhance water solubility, carboxyl groups were introduced onto the main trimethyl-dihydroquinoline scaffold.

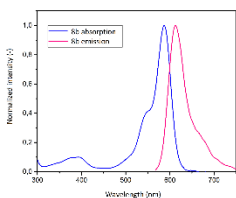
SYNTHESIS OF ATTO590 METHYL DERIVATIVE



SYNTHESIS OF ATTO590 CARBOXYL DERIVATIVE

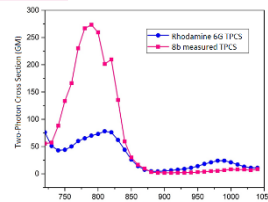
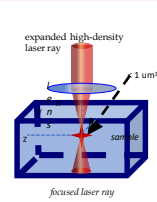
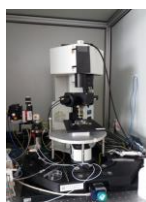


SPECTROSCOPIC PROPERTIES

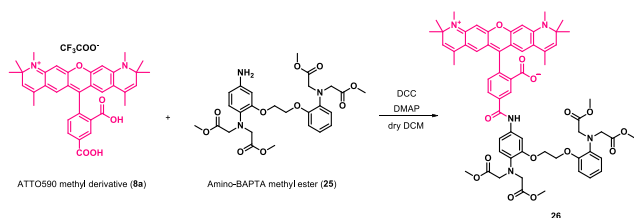


Name	Absorption maximum (λ_{ab})	Emission maximum (λ_{em})	Stokes shift ($\Delta\lambda = \lambda_{em} - \lambda_{ab}$)	Quantum yield (QY)
8a	587	615	28	0,69
8b	587	611	24	0,76
24a	592	621	29	0,55
24b	592	618	26	0,77
ATTO 590	593	622	29	0,80
Cresyl	603	621	17	0,54

TWO PHOTON MEASUREMENTS



SYNTHESIS OF THE FIRST CALCIUM SENSOR



SUMMARY

An efficient synthetic route was developed for rhodamine dyes with dihydroquinoline structure. Carboxyl groups were successfully conjugated onto the basic scaffold obtaining a fluorophore with ideal spectral properties. According to these results, this fluorophore is suitable to combine with the BAPTA receptor. Based on these findings, we have synthesized the first fluorescent Ca^{2+} sensor constructed from this fluorophore.



REFERENCES

- [1] Göppert-Mayer, M., *Ann. Phys.* **1931**, 401, 273–294
- [2] Oheima, M.; Hoff, M.; Feltz, A.; Zamaleeva, A.; Malle, J., *Biochimica et Biophysica Acta*, **2014**, 1482, 2284-2306
- [3] Tsien, R. Y., *Calcium as a Cellular Regulator*, Oxford University Press, New York, **1999**, 28–54.
- [4] Belov, V. N.; Bossi, M. L.; Fölling, J.; Boyarskiy, V. P.; Hell, S. W., *Chem. - Eur. J.*, **2009**, 15 (41), 10762–10776
- [5] de Reguardati, S.; Pahapill, J.; Mikhailov, A.; Stepanenko, A., *Opt. Express*, **2016**, 24 (8), 9053-9066

This project was supported by
 NTP-SZKOLL-17-0017
 KFI_16-1-2016-0177
 NVKP_16-1-2016-0043
 Hungarian tenders.