Technology Offer

PONy Dyes – Fluorescent Dyes with Phosphorus Substituents

Ref.-No.: 0707-5297-BC

Fluorescent dyes are widely used as indispensable markers in biology, optical microscopy, and analytical chemistry. In particular, the sensitive and stable imaging of cellular components depends on the favourable combination of chemical, biological and physical factors. The availability and proper choice of fluorescent dyes is a key factor to success in the entire labelling and imaging procedure. Due to their superior brightness and photostability, synthetic dyes represent an attractive alternative to fluorescent proteins.
Dyes with compact structures and a zero net charge (neutral or zwitterionic molecules with a short charge separation distance) are known to penetrate the outer plasma membrane of living cells and may be used as fluorescent labels in biology, optical microscopy and material science. Fluorescent dyes with increased Stokes shifts offer an advantage of using more flexible imaging schemes. In this case two fluorescent labels with small and large Stokes shifts can be combined in one experiment and imaged separately. Therefore, the discovery of new labels is a vital task in modern biology related natural science.

Technology

While searching for new fluorophores suitable for nanoscale imaging of intracellular targets, various organic dyes with electrophilic conjugated systems were found to react with nucleophilic phosphorus(III) reagents (see figure 1) to form phosphonylated leuco bases. Upon oxidation, these intermediates provide new fluorophores (PONy dyes) with red-shifted absorption and emission maxima and increased Stokes shifts as compared to the precursor dyes. The versatility of phosphorus addition at the sp^2-carbon, combined with the wide availability of functionally substituted P(III) reagents, offers an extended array of conceivable PONy dyes with broadly varying properties (for examples, see table 1).

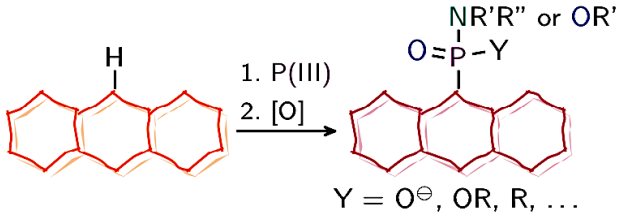

Figure 1: Addition of neutral or anionic P(III) nucleophiles to cationic or highly polarized fluorophores results in "PONy" dyes with "grown-up" Stokes shift and red-shifted absorption and emission bands.

Table 1: Comparison of photophysical properties the example PONy dyes (compare figure 2) and their precursors; λ_{abs}^{max}: wavelength of maximum absorption, ε: absorption coefficient at λ_{abs}^{max}, λ_{em}^{max}: wavelength of maximum emission, Φ_{fl}: fluorescence quantum yield, $\Delta\tilde{\nu}$: Stokes shift, τ: fluorescence lifetime.

dye	M [g/mol]	λ_{abs}^{max} [nm]	ε [mol/g cm]	λ_{em}^{max} [nm]	Φ_{fl}	$\Delta\tilde{\nu}$ [cm^{-1}]	τ [ns]	solvent
methylacridine orange	280	495	71000	524	0.30	1118	1.7	MeOH
A1	416	535	38000	595	0.10	1885	1.4	PBS
coumarin 6	350	457	51000	501	0.63	1922	3.2	MeCN
C7	430	419	22000	613	0.04	7554	1.2, 0.3[a]	PBS
pyronin Y	267	546	77000	569	0.36	740	1.8	PBS
P1	330	590	59000	627	0.33	1000	1.8	PBS

[a] Biexponential fluorescence decay.

Max-Planck-Innovation GmbH
Amalienstr. 33
80799 München
www.max-planck-innovation.de

Comparison of the UV-vis spectra indicates that PONy dyes absorb and emit at longer wavelengths and possess larger Stokes shifts than the parent dyes. Additionally, PONy dyes are particularly attractive due to their low molecular masses (typically $M < 500$ Da) and orange to near-infrared emission. These dyes can be prepared in cationic or zwitterionic forms, making them promising candidates for the development of cell-permeant fluorescent markers for living cells. The fluorescence lifetimes of PONy dyes vary and generally do not exceed 3 ns. Their sensitivity to the nature of the solvent may allow using the PONy-derived probes in fluorescence lifetime imaging or as polarity sensors. Example structures for different PONy dye classes are given in figure 2.

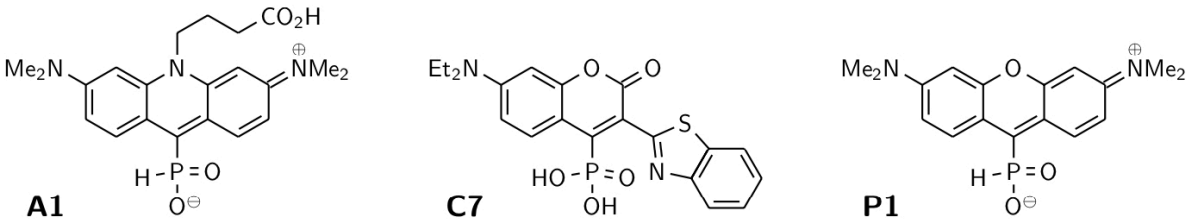

Figure 2: Examples illustrating the structural diversity of stable PONy dyes synthesised according to the reaction scheme in figure 1; **A1**: substituted acridinium salt, **C7**: substituted coumarin, **P1**: substituted

The versatility of the proposed transformation is demonstrated by the facile functionalizations of the commercial fluorophores Atto 495 and Pyronin Y. Using the phosphinylation/oxidation chemistry, Atto 495 was converted into an orange emitting dye **A1** applicable in immunolabelling (figure 2). Pyronin Y gave dye **P1**. Additionally, the red-emitting coumarin dye **C7** with huge Stokes shift was prepared. The transformations required only few synthetic steps and provided functional derivatives of the PONy dyes (e. g., **A1** and **P1**) in zwitterionic form with a very short charge separation distance – which is known to favour intact membrane permeability in living cells.

Summary

- neutral or zwitterionic dyes with a very short charge separation
- low molecular mass and compact structures
- bathochromic and bathofluoric shifts of absorption and emission bands
- possibility to introduce additional functional groups
- increased Stokes shifts with sufficient emission efficiency

Patent Information

EP patent application filed in February 2017.

US patent application filed in February 2018.

Contact

Dr Bernd Ctortecka

Senior Patent- & License Manager
Physicist

Phone: +49 (0)89 / 29 09 19 - 20
eMail: ctortecka@max-planck-innovation.de

Max-Planck-Innovation GmbH
Amalienstr. 33
80799 München
www.max-planck-innovation.de