Diaminoterephthalates – Fluorescent Dyes for Life Sciences

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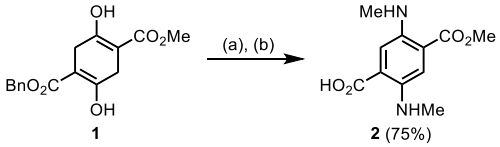
Diaminoterephthalates can be synthesized from succinylsuccinates *via* bis-enamines by oxidation with air. Different functional groups, so called effector groups, can then be attached to the scaffold by amide coupling reactions, resulting in a toolkit consisting of many different derivatives. For example, it was possible to attach cyclooctyne and maleimide residues to the dye, acting as reactive moieties for ligation with azides or thiols. Therefore, this bifunctionalized dye can act as a cross-linker between different proteins.

Preparation of Diaminoterephthalates

Fluorescent dyes are important tools for many applications in biology. They can, for example, act as chemosensors by reacting selectively to certain ions in the cell or indicate conformational changes or the position of a functional group inside of a protein. As an example of a fluorophore, diaminoterephthalate **2** could be synthesized from succinylsuccinate **1** in good yields (Scheme 1). After the cleavage of the benzyl protecting group, different effector groups could be attached to the dye, resulting in a versatile toolkit of many different derivatives. By cleavage of the methyl protecting group and subsequent amide coupling reactions, bifunctionalized compounds could be synthesized, acting as cross-linkers between different proteins.[1]

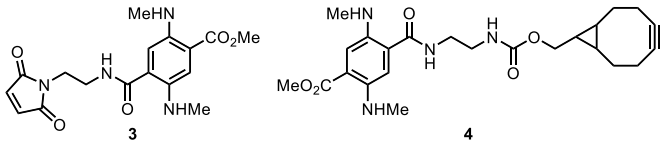


1 2 (75%)

Scheme 1. Synthesis of diaminoterephthalate **2**. (a) $MeNH_2$, AcOH, air, toluene, reflux, 3 d; (b) Pd/C, 1 atm H_2, THF, rt, 2 h.

Monofunctionalized Dyes

Two diaminoterephthalic acid derivatives **3** and **4** (Figure 1) were synthesized as reactive probes for mapping Ca^{2+}-sensitive regions in the neuronal Ca^{2+} sensor GCAP2 by site-specific fluorescence labeling. The maleimide functionalized probe **3** was used for specific binding to a single Cys residue of the protein. The congener **4** equipped with a cyclooctyne residue was used for ligating the protein on its 12-azidododecanoic acid residue (which is a substitute for a native myristoyl residue) near the *N*-terminus of GCAP2.[2]

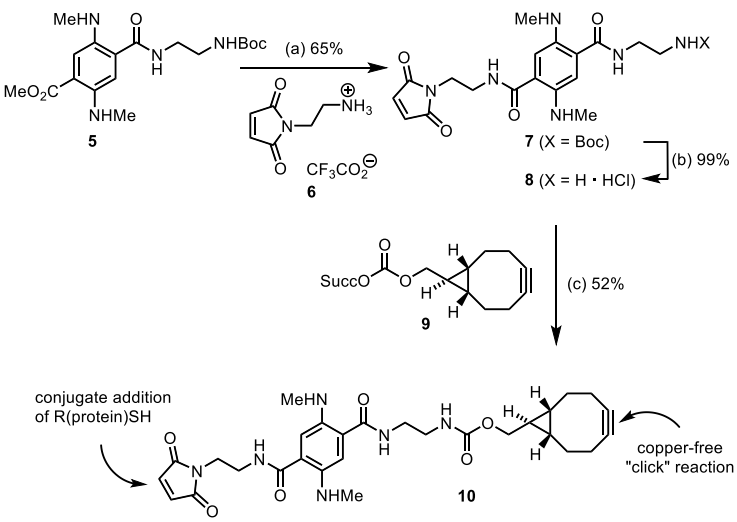


3 4

Figure 1. Two diaminoterephthalate dyes with reactive maleimide **3** and cyclooctyne **4** residues.

Bifunctionalized Dye

Furthermore, a bifunctional dye **10** with both, maleimide and cyclooctyne residues was developed as probe suited to cross-link two proteins. This fluorescent dye could be a versatile new tool for studying protein-protein interactions. The synthesis started from compound **5**, which was prepared from acid **2** by an amide coupling reaction with HATU and DIPEA in 81% yield. The methyl ester was saponified with KOH and the maleimide group **6** was installed using the same amide coupling reaction with HATU and DIPEA in one step. Compound **7** could be obtained in a yield of 65%. The Boc protecting group was cleaved with AcCl in MeOH, giving the amine **8** in 99% yield. The cyclooctyne residue could then be attached to the dye by a S_Nt reaction, using the commercially available derivative **9** and DIPEA as a base. The target compound **10** could be obtained in a yield of 52% (Scheme 2).[3]



5 6 7 (X = Boc) 8 (X = H · HCl) 9 10

Scheme 2. Preparation of maleimide-cyclooctyne-functionalized dye **5**. (a) i) KOH, THF/H_2O, rt, 21 h; ii) compound **6**, HATU, DIPEA, CH_2Cl_2, rt, 18 h; (b) AcCl, MeOH, 0°C→rt, 18 h; (c) compound **9**, DIPEA, acetone, 50°C, 18 h.

This bifunctionalized dye was now used for cross-linking of for example two different proteins. The maleimide function reacts selectively with thiols of for example a cysteine residue. The cyclooctyne moiety can react with azides in a so called copper-free “click” reaction. Thus, proteins containing a myristoyl residue can be labeled with a fluorescent marker without using any copper catalyst.[3]

[1] R. Pflantz, J. Christoffers, *Chem. Eur. J.* **2009**, *15*, 2200–2209.
[2] S. Sulmann, M. Wallisch, A. Scholten, J. Christoffers, K.-W. Koch, *Biochemistry* **2016**, *55*, 2567–2577.
[3] M. Wallisch, S. Sulmann, K.-W. Koch, J. Christoffers, *Chem. Eur. J.* **2017**, in press (doi: 10.1002/chem.201700774).