Bifunctional Diaminoterephthalic Acid Derivatives as New Tools for Life Sciences

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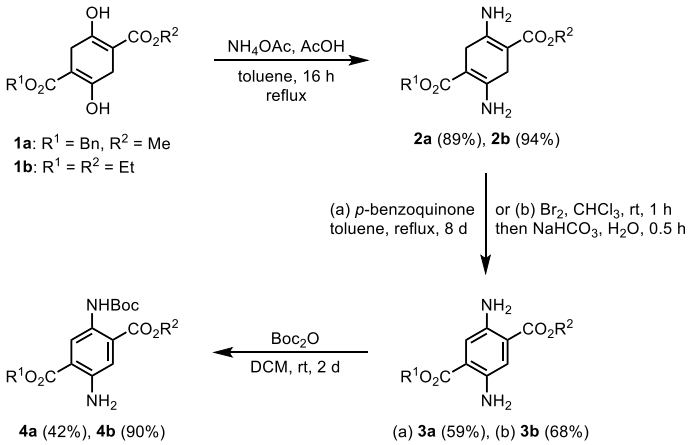
As a first step to bifunctional fluorescence dyes, three diaminoterephthalate precursors were synthesized which represent the different combinations of orthogonally protected amine or acid linkers attached to the scaffold. Starting from these precursors it is possible to access a variety of bifunctional fluorescence dyes with different “effector groups”.

Preparation of Diaminoterephthalate Scaffolds

Diaminoterephthalates are intensively colored and highly fluorescent materials. Due to their special constitution including two amino and acid moieties, they can be used as scaffolds for the attachment of different “effector groups”.

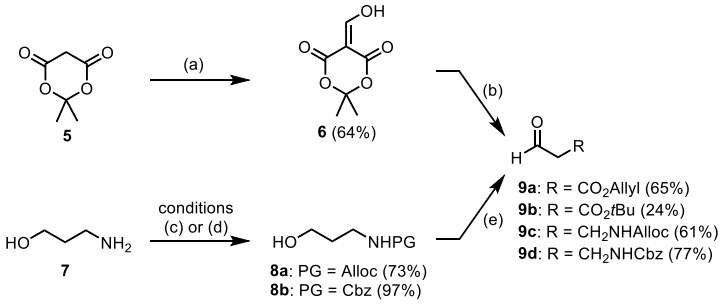
The target compounds can be synthesized starting from succinylsuccinates **1a**–**b** by aminolysis to the corresponding enamines **2a**–**b** followed by oxidation. The resulting diaminoterephthalates **3a**–**b** are then selectively protected at one of the two amino groups furnishing orthogonally protected scaffolds **4a**–**b**.[1]



Scheme 1. Synthesis of orthogonal protected diaminoterephthalate scaffolds **4a** and **4b**.

Linker Synthesis

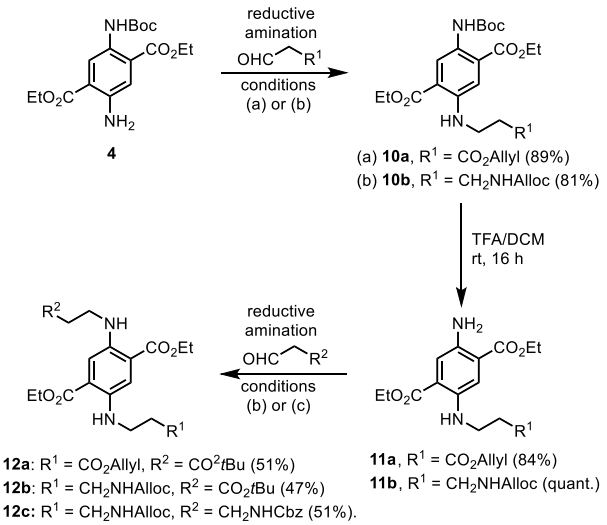
Linkers **9a**–**d** were synthesized either by treating formyl Meldrum’s acid **6** with the corresponding alcohol or by Swern oxidation of protected aminopropanols **8a** and **8b**.



Scheme 2. Synthesis of linkers **9a**–**d**. (a) $HC(OMe)_3$, 85°C, 3 h then HCl-H_2O, rt, 30 min; (b) *t*BuOH or AllylOH, toluene, N_2, 80°C, 90 min; (c) AllocCl, Na_2CO_3, MeCN, H_2O, 0°C to rt, 18 h; (d) CbzCl, NaOH-H_2O, DCM, rt, 4 h; (e) $COCl_2$, DMSO, NEt_3, -78°C, 4 h.

Synthesis of Precursors for Bifunctional Dyes

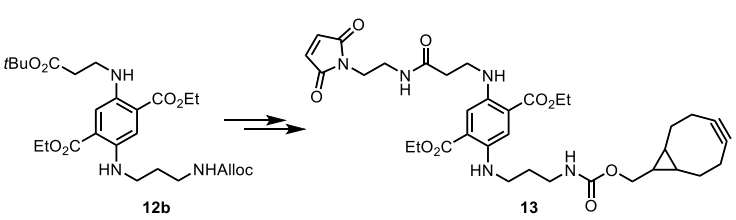
The attachment of the first protected aldehyde linker was accomplished by reductive amination. After deprotection of the Boc-group the second linker was attached, delivering three different precursors **12a**–**c** for the synthesis of bifunctionalized fluorescent dyes.[2] These precursors possess the three different combinations of orthogonally protected acid and amine groups.



Scheme 3. Synthesis of precursors for bifunctional dyes **12a**-**c**. (a) $NaBH(OAc)_3$, AcOH, N_2, DCM, 1 h; (b) $NaBH_3CN$, $ZnCl_2$, DCM, 0°C to rt, 16 h; (c) $NaBH(OAc)_3$, N_2, 0°C to rt.

Synthesis of a Bifunctional Dye

Starting from precursor **12b** we are able to synthesize the bifunctional dye **13** by subsequent deprotection and amide coupling first with maleimide and then with a cyclooctyne building block. The maleimide moiety is used for the attachment to thiol groups in proteins, the cyclooctyne undergoes click reaction with azide group.[3]



Scheme 4. An example for a bifunctional dye **13** with maleimide and cyclooctyne “effector groups” starting from precursor **12b**.

[1] R. Pflantz, J. Christoffers, *Chem. Eur. J.* **2009**, *15*, 2200–2209.
[2] L. Freimuth, J. Christoffers, *Chem. Eur. J.* **2015**, *21*, 8214–8221.
[3] S. Sulmann, M. Wallisch, A. Scholten, J. Christoffers, K.-W. Koch, *Biochemistry* **2016**, *55*, 2567-2577.