7-Nitroindoline-Based Photocleavable Crosslinkers

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Statement of purpose: Photodegradable hydrogels have gained interest as scaffolds for photolithography, and as tunable cell culture platforms[1-3]. Most strategies involve the crosslinking of hydrogel-forming polymers and modified PEGs with *o*-nitrobenzyl-based crosslinkers capable of undergoing photolysis by user-programmed multiphoton excitation[4-6]. Recent studies have shown that the length of the crosslinker is an important variable to control the mechanical and viscoelastic properties of the biomaterial, which can ultimately affect the growth and proliferation of cells on its surface[7-8]. *o*-Nitrobenzyl moieties produce aldehyde or ketone byproducts upon photolysis, which can react with matrix or cellular amines resulting in undesired crosslinking/modification by Schiff base formation. Here we describe the synthesis of new photocleavable crosslinkers suitable for crosslinking amine-containing polymers, which do not produce aldehyde or ketone photolysis products. We utilized photoreactive *N*-acyl-7-nitroindoline (NA7N) moieties [9,11] and N-thiocarbamoyl-7-nitroindoline (NT7N) moieties (unpublished data), which undergo photolysis with near UV light (λ = 350 nm), or with femtosecond laser light (λ = 710 nm). The two-photon absorption capability of compounds with NA7N moieties has been described [12-14]. Our crosslinkers, which differ in length and in solubility, can potentially produce hydrogels that meet certain mechanical and structural demands, and that can be photochemically modified. Such materials could be useful as new bioinks and matrices for cell growth.

Methods, materials, and analytical procedures used: We have synthesized six nitroindoline-based crosslinkers of different lengths with photolabile NA7N or NT7N moieties (**Figure 1**, compounds **A-F**). The common synthetic precursor for all of them is nitroindoline **1**, produced by the Suzuki cross-coupling of 5-bromo-7-nitroindoline and a boronic ester (unpublished data). To demonstrate the crosslinking capability and photolytic property of one crosslinker, *N*-hydroxysuccinimde ester **B** was used to crosslink a test amine followed by photolysis using UV light (**Figure 2**). In addition, the photolysis of model NT7N **J** was also studied (**Figure 3**).

Results: Crosslinker **B** reacts with two equivalents of benzylamine to produce diamide **G** (**Figure 2**). Upon illumination with UV light at 350 nm in 1% water in acetonitrile, photolytic cleavage produces nitroindoline **H** and carboxylic acid **I**. Photolysis was also observed for a model NT7N **J**, in which nitrosoindoline (**K**), a thiol, and CO_2 were obtained upon illumination with light (**Figure 3**).

Conclusions: Six novel photocleavable 7-nitroindoline-based crosslinkers with different photoreactive moieties and linker lengths were synthesized. The crosslinking capability of NA7N-containing crosslinker **B**, and the photoreactivity of the resulting crosslinked compound **G** were demonstrated. To prove the photocleavability of NT7Ns, model compound **J** was also successfully photolyzed. Our data suggest that the crosslinkers **A-F** may be suitable for crosslinking amine-containing polymers (*e.g.* gelatin or chitin), and that the crosslinked materials may undergo photolysis in a similar manner as the NA7N and NT7N model compounds studied here. This work has implications for new functional biomaterials for biophotolithography and tissue engineering applications.

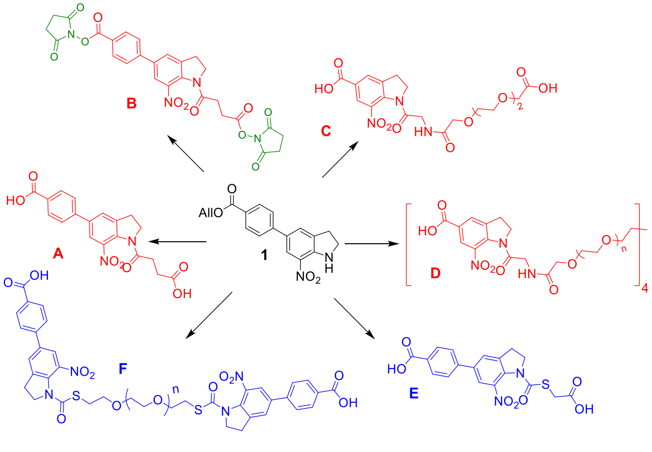

Figure 1: Synthesis of 7-nitroindoline-based crosslinkers.

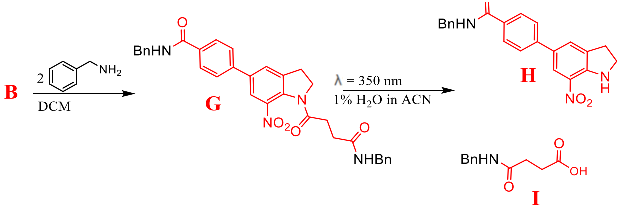

Figure 2: Model crosslinking reaction using crosslinker B, and subsequent photolysis to produce a 7-nitroindoline and a carboxylic acid.

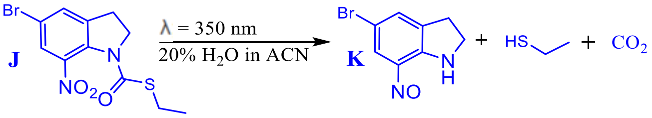

Figure 3: Photolysis of model thiocarbamate **J**, resulting in the formation of nitrosoindoline **K**, a thiol and CO_2 as photolysis products.

References:

1. Kloxin, A. M. et al. Nat Protoc. 2010; 5: 1867.
2. Tibbitt, M. W. et al. Soft Matter. 2010, 6, 5100.
3. Tamura, M. et al. PLoS One. 2017; 12: e179372.
4. Yanagawa, F. et al. Adv Healthcare Mater. 2015; 4: 246.
5. Tamura, M. et al. Sci. Rep. 2015, 5, 15060.
6. Zhao, H. et al. Macromolecules. 2012; 45: 1723.
7. Zaragoza, J. et al. J. Phys. 2017; 790: 1-6
8. Shengchang, T. et al. Prog. Mater. Sci. 2020, doi: 10.1016/j.pmatsci.2020.100738
9. Patchornik, A. et al. J Am Chem Soc. 1970; 92: 6333.
10. Amit, B. et al. J Am Chem Soc. 1976; 98: 843
11. Del Castillo, P. et al. (Unpublished data)
12. Matsuzaki, M. et al. Nature Neurosci. 2001; 4: 1086.
13. Ornelas, A. et al. Org. Biomol. Chem. 2018; 16: 1000.
14. Hatch, K. A. et al. Biomed Opt Express. 2016; 7: 4654.