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Synthesis of a new hydrophilic *o*-nitrobenzyl photocleavable linker suitable for use in chemical proteomics

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Abstract—Linkers currently used in solid phase synthesis are generally short and hydrophobic, limiting their usefulness in biological systems. Herein, we describe a facile synthesis of a long, hydrophilic, *o*-nitrobenzyl photocleavable linker, suitable for constructing affinity supports for use in chemical proteomics. The rates of photolysis of the linker on exposure to UV light emitting diodes are reported.

In chemical proteomics[1] and reverse chemical proteomics,[2] a target molecule is linked to some form of tag (such as biotin,[3] a fluorophore[4,5] or a solid support[6]), which can be used to isolate a single protein or a family of proteins from an entire proteome. Since Merrifield's pioneering work on solid phase organic synthesis in 1963,[7] there has been an exponential increase in the range of cleavable linkers that are available to immobilise small molecules onto solid supports.[8,9] The incorporation of new photolabile,[10] traceless[11] and safety catch[12] linkers allows the orthogonal cleavage of different reagents from the same solid phase, greatly increasing the range of reactions possible. However, as these linkers were designed for use in organic solvents, they are generally hydrophobic, and are often quite short, thereby limiting their usefulness in biological systems.

This letter describes the synthesis and characterisation of a long, hydrophilic, photocleavable linker **7**, suitable for generating affinity supports for use in chemical proteomics studies. Poly(ethylene glycol) (PEG) is an ideal backbone for constructing linkers for use in aqueous media due to its hydrophilicity, chemical stability and biocompatibility.[13] Monodisperse PEGs, such as tetra(ethylene glycol) (TEG), allow discrete, fixed-length linkers to be synthesised and characterised easily, while polydisperse PEGs can be used to generate linkers with a statistical distribution of lengths, thereby increasing chemical diversity. The *o*-nitrobenzyl group has been incorporated in a wide variety of photolabile linkers[14–16] and protecting groups[17,18] due to its chemical stability and rapid cleavage by exposure to UV light around 365 nm.

The rate limiting step in the photolysis of the *o*-nitrobenzyl group is the abstraction of a benzylic proton by the photoactivated nitro group, so the presence of a methyl group on the benzylic carbon increases the acidity of this proton and has been shown to increase the rate of photolysis considerably.[19] Therefore, to set up a secondary *o*-nitrobenzylic centre, 2-nitroterephthalic acid was reduced to the diol **2** with BH_3·THF then oxidised to the dialdehyde **3** with PCC (Scheme 1). Attempts to methylate **3** using MeMgBr were unsuccessful, yielding a mixture of products including methylation of the aromatic ring and nitro group, as reported previously in the literature.[20] $MeTiCl_3$,[21,22] which is more selective than the standard Grignard reagents, gave the desired secondary diol **4** exclusively. Activation of **4** with *N*,*N*′-disuccinimidyl carbonate to form the dicarbonate **5**, followed by coupling with mono-Boc protected TEG diamine (Boc–NH–TEG–NH_2) **1**, gave the Boc-protected secondary *o*-nitrobenzyl linker **6**, which is suitable for long-term storage. When required, **6** was deprotected with TFA, yielding the free diamine **7**.

A dilute solution of **6** in chloroform-*d* was exposed to UV light ($\lambda_{max} = 365$ nm) from a pyrex-filtered 16 W mercury vapour fluorescent lamp and the photolysis

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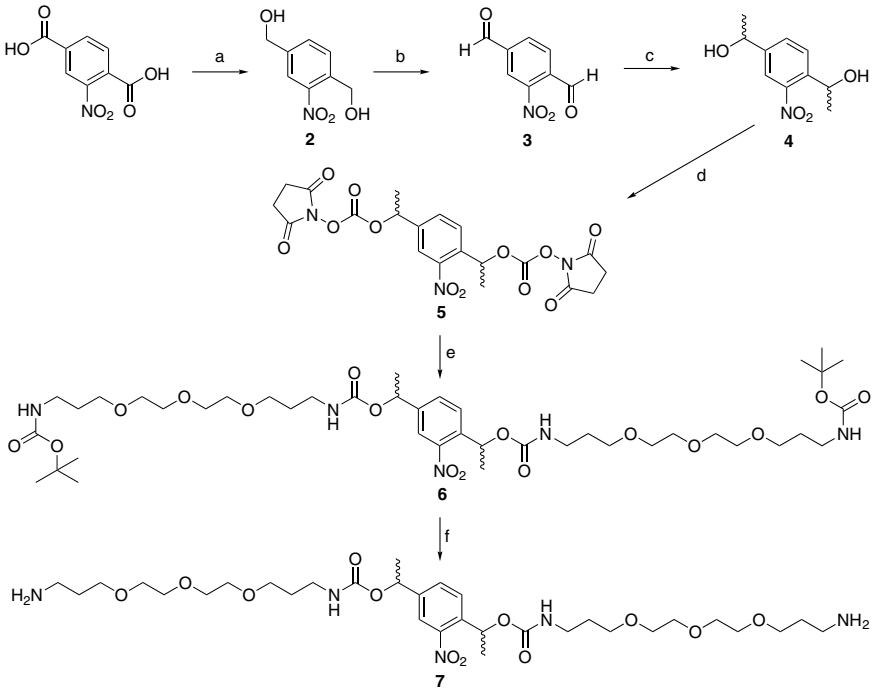


Scheme 1. Reagents and conditions: (a) BH_3·THF/THF, 0 °C, 1 h then 40 °C, 18 h, 92%, (b) PCC/DCM, 25 °C, 4 h, 93%; (c) $MeTiCl_3/Et_2O$, −30 °C, 3 h, 65%; (d) DSC, Et_3N, DMAP (cat.)/MeCN, 25 °C, 18 h, 65%; (e) Boc–NH–TEG–NH_2 **1**, TEA/MeCN, 25 °C, 18 h, 82%; (f) 20% TFA/$CHCl_3$, 25 °C, 30 min, quant.

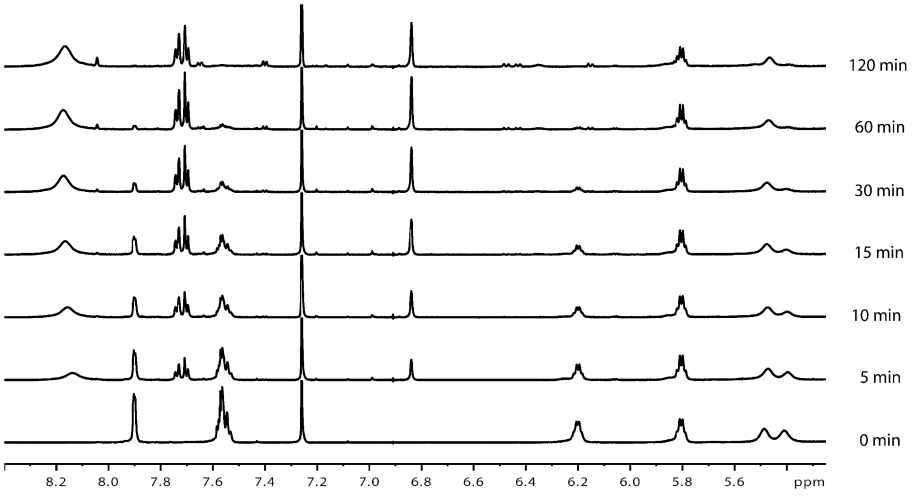
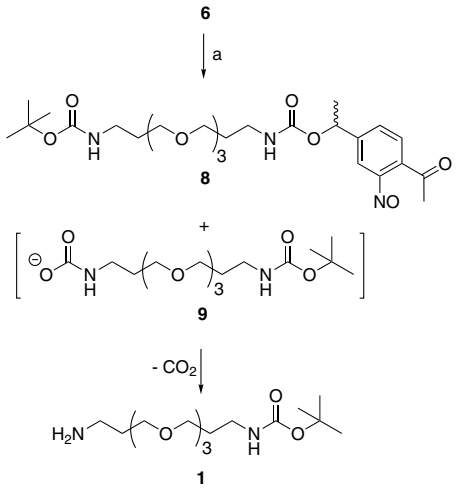


Figure 1. Photolysis of **6** by exposure to UV light ($\lambda_{max} = 365$ nm) from a pyrex-filtered 16 W mercury vapour fluorescent lamp, as followed by ^{1}H NMR.

was followed by NMR spectroscopy (Fig. 1). The reaction proceeded rapidly, with the majority of the linker cleaved after 60 min of exposure. Analysis of the photolysis products by NMR and MS revealed that the reaction yields the expected nitrosoketone **8**, as well as the starting material **1**, following spontaneous decarboxylation of the corresponding carbamate **9** (Scheme 2). The data obtained were fitted to a first order exponential equation (Fig. 2) using least squares regression and the pseudo first order rate constant for the photolysis reaction was found to be 1.0×10^{-3} s^{-1}, corresponding to a half-life of 12 min.

Mercury vapour lamps emit high intensity radiation over a wide range of wavelengths, including a considerable amount of heat and hence may cause undesirable photooxidation and/or thermal damage to biological systems. Conversely, UV light emitting diodes (LEDs), which have recently become commercially available, emit UV light in a focused beam over a narrow (~30 nm) bandwidth, with virtually no dissipation of heat. In addition, each LED is small enough to be positioned directly over one well of a 96-well microtitre plate, allowing the UV light to be pointed directly at the surface of the well or its contents. UV LEDs are



Scheme 2. Reagents and conditions: (a) hν (365 nm)/$CDCl_3$, 25 °C, 2 h, quant.

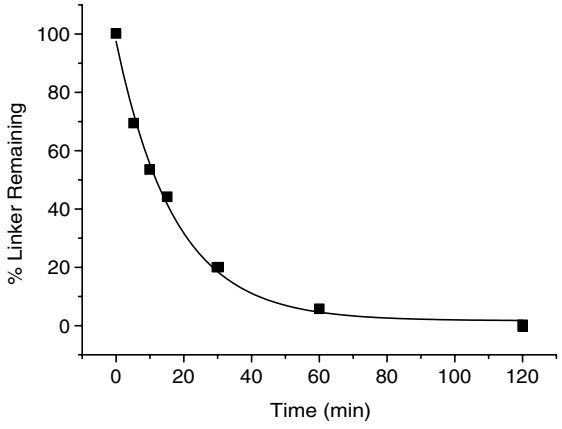


Figure 2. Rate of photolysis of **6** by exposure to UV light ($\lambda_{max} = 365$ nm) from a pyrex-filtered 16 W mercury vapour fluorescent lamp was determined by integrating the NMR signal corresponding to H3 (δ 7.90).

now available in a range of different wavelengths from 350 to 400 nm, although power output is still quite low at the shorter wavelengths. Therefore, two different UV LEDs, with emission maxima of 370 nm (1 mW) and 395 nm (6 mW), were investigated for their ability to photolyse the *o*-nitrobenzyl group.

Dilute solutions of **6** in acetonitrile were exposed to UV light from either a 370 or a 395 nm UV LED and the optical density of each solution at 240 nm was measured and plotted as a function of time (Fig. 3). The pseudo first order rate constants for the reaction were found to be $1.9 \times 10^{-4}\ s^{-1}$ ($t_{1/2} = 61$ min) using the 370 nm LED and $2.5 \times 10^{-4}\ s^{-1}$ ($t_{1/2} = 46$ min) using the 395 nm LED. Clearly, these rates of photolysis are considerably slower than were obtained using the mercury vapour lamp, although given that the radiant flux from the UV LEDs is several orders of magnitude less than from the lamp, the rates of photolysis obtained were impressive.

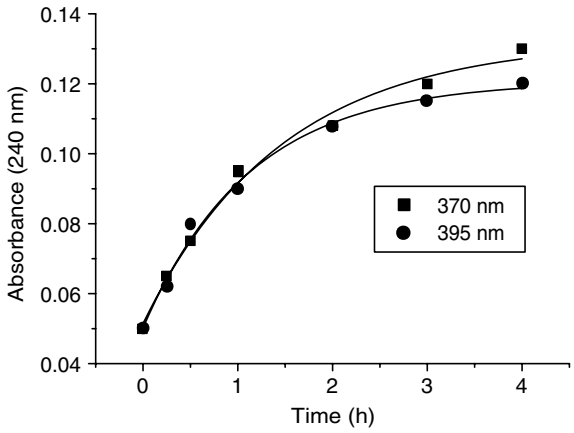


Figure 3. Optical density of a solution of **6** at 240 nm after exposure to UV light from either a 370 nm (1 mW) or a 395 nm (6 mW) UV LED.

In conclusion, we have described a facile synthesis of a new hydrophilic *o*-nitrobenzyl photocleavable linker with utility in many areas of chemical biology for the reversible linking of macromolecules, combinatorial libraries and natural products to solid phases, fluorophores or other tags. We have also presented a novel cleavage strategy using recently available UV diodes that are small, give off no heat and have narrow emission profiles. These diodes are suitable for high throughput devices due to their small size and wide range of spectral characteristics.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2005.09.077.

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