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An Acid- and Base-Stable *o*-Nitrobenzyl Photolabile Linker for Solid Phase Organic Synthesis

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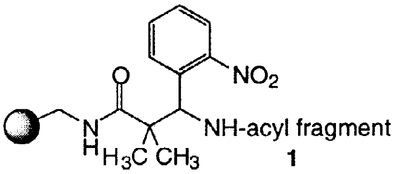
Abstract: The synthesis of an *o*-nitrobenzyl photolabile linker (**1**) from *o*-nitrobenzaldehyde is described, and the efficiency of its light-mediated (365 nm) cleavage is found to be comparable to related, previously developed systems. In contrast, **1** is shown to be stable to acid, base, and Lewis acid/amine combinations while the previously developed linker **2** is shown to degrade under the latter two conditions. © 1998 Elsevier Science Ltd. All rights reserved.

Chemical genetics[1] provides a framework to explore the cellular and physiological functions of proteins using low molecular weight organic compounds ("small molecules"). Its development will rely upon the ability of synthetic chemists to synthesize small molecules both reminiscent of natural products and compatible with miniaturized cell-based assays.



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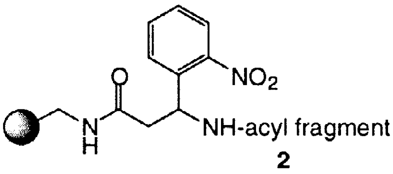

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These assays will often require light-sensitive linkers in order to release a resin-bound small molecule into a solution.[1,2] Many commonly used photolinkers are based on the *o*-nitrobenzyl functionality, which is readily incorporated and undergoes cleavage following ultraviolet (UV) irradiation.[3,4,5] Photolinkers containing this functionality, however, are not compatible with many useful synthetic reactions.[4] For example, in the course of a solid phase synthesis that used the 3-amino-3-(2'-nitrophenyl)propionic acid linker (**2**) developed by Brown *et al.*,[3] low recovery of product was observed following sequential reactions using Lewis acid/amine combinations [e.g., $Yb(OTf)_3$, Me_2NH] and UV light. From the solution phase of these reactions, prior to UV irradiation, we isolated and characterized (TLC, FAB-MS, ^1H NMR) the carboxamide corresponding to the acyl fragment, implicating β-elimination as the cause of low recovery upon photocleavage. To overcome some of these limitations, we have developed a simple synthesis of a 3-amino-3-(2'-nitrophenyl)-2,2-dimethylpropionic acid linker (**1**) that does not undergo acid or base-catalysed β-elimination. The cleavage efficiency of **1** is comparable to its parent **2** and it has increased stability to acid, base, and Lewis acid/amine conditions. The synthesis of **1** is described in Scheme 1. Imine **3** was synthesized in two steps (87% yield) from commercially available 2-nitrobenzaldehyde by a modification of a reported procedure.[6] Amino ester **4** was formed by addition of a pre-cooled solution (-78 °C) of **3** to the lithum enolate of methyl isobutyrate.

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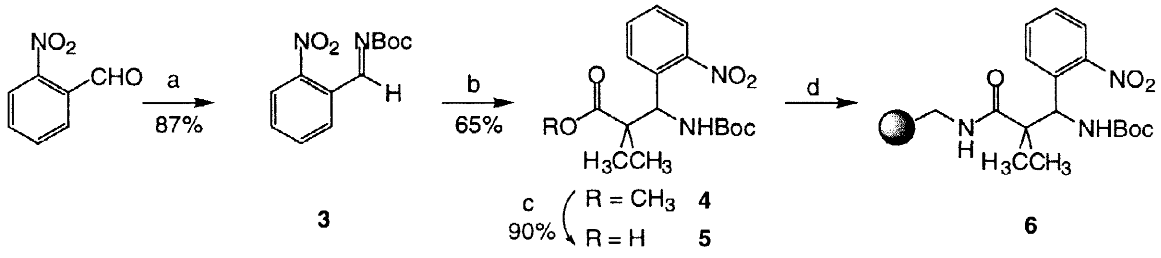
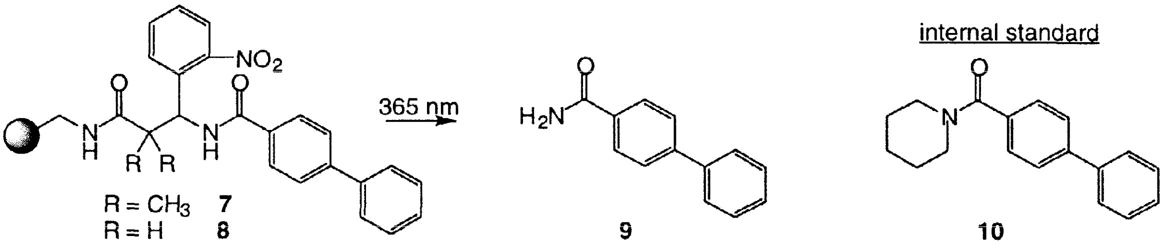
Recrystallization from 1:1 EtOAc/hexanes provided **5** in 90% yield.[7] Acid **5** was coupled to Tentagel S NH_2 (0.29 meq/g) with HATU.[8]



Scheme 1: (a) i. *t*-$BuOCONH_2$ (1.5 equiv), $NaSO_2Ph$ (2.5 equiv), HCOOH (2 equiv), 2:1 H_2O/MeOH, 3 crops over 60 h; ii. K_2CO_3, THF, reflux 12 h; (b) i. *i*-Pr_2NH, BuLi, THF, -78 °C, then methyl isobutyrate, 30 min; ii. **3**, -78 °C, 2 min, then AcOH/THF; (c) LiOH (10 equiv), MeOH/H_2O, 60 °C; (d) Tentagel S NH_2, **5** (1.6 equiv), HATU (1.5 equiv), *i*-Pr_2NEt (4 equiv), 3:1 DMF/CH_2Cl_2, 12 h.

To demonstrate the chemical stability of **1**, we developed a system by which cleavage of a chemically inert chromophore from **1** could be monitored quantitatively. 4-Phenylbenzoic acid was coupled to linkers **1** and **2** on Tentagel S NH_2 resin to give **7** and **8** (0.26 meq/g), respectively. Photocleavage[9] in acetonitrile (for solubility) at 365 nm released the chromophore, 4-phenylbenzamide (**9**), which was quantitated spectrophotometrically during HPLC[10] by using an internal standard, 1-(4-phenylbenzoyl)-piperdine (**10**).



Following UV irradiation, the rate of release of carboxamide **9** from test resins **7** and **8** was determined to be slightly greater with linker **1** (resin **7**) relative to linker **2** (resin **8**). It was of concern that the yields of **9** after 8 h photolysis in acetonitrile are 35% and 31% of loading capacity for **7** and **8**, respectively. The low efficiency observed here is similar to that reported by Brown, *et al.* for the cleavage of Fmoc-Arg(Tos)-NH_2 from **2** in methanol (~40% yield after 7 h).[3] The yield is reported to increase to ~70% after 7 h photolysis in 4:1 H_2O/MeOH.[3] In order to determine the cleavage efficiency of **1** in this water-methanol solvent system, **1** was derivatized with the water soluble isonicotinamide chromophore. Photolysis in 4:1 H_2O/MeOH and quantitation of the released isonicotinamide by spectrophotometric comparison with an internal standard during HPLC showed 86% cleavage from **1** (acyl= isonicotinoyl) after 4 h photolysis. It appears that an aqueous solvent mixture significantly improves the yield of the photocleavage, consistent with Brown, *et al.*[3]

In order to evaluate whether **1** has the expected increased chemical stability relative to **2** under conditions that promote β-elimination, resins **7** and **8** were subjected to Lewis acid/amine, basic, and acidic reaction conditions.[11] Photolysis of resin that had been subjected to these reagents provided clear demonstration of the relative stabilities of these linkers. Figure 2 shows HPLC traces resulting from these studies. In each case, the internal standard (**10**) reflects a concentration of 5 mM and corresponds to the rightmost peak. The stability of each linker was quantitated as percent recovery of the chromophore (**9**) relative to an unreacted control (data shown beneath the trace).

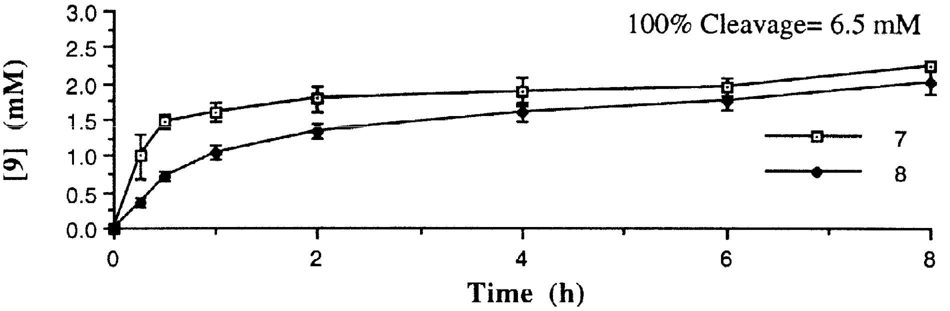


Figure 1. Comparative photocleavage time course of **7** and **8** in CH_3CN. 3-6 experiments are averaged for each time point; error bars corresponding to one standard deviation are provided.

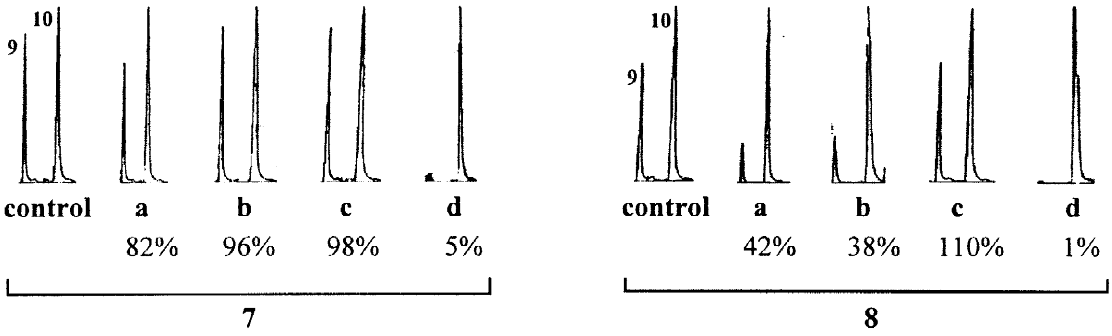


Figure 2. HPLC analysis of photochemically released compound **9** after treatment of **7** and **8** with the following reagents: (**a**) 50 mM $Yb(OTf)_3$, 200 mM $(CH_3)_2NH$, THF; (**b**) 200 mM NaOMe, MeOH; (**c**) 200 mM TsOH, THF; (**d**) 100 mM SmI_2, 10% MeOH-THF. The percent recovery of **9** relative to the corresponding control (labeled **9** in the control traces) is given beneath the trace.

The Lewis acid/amine combination that led to β-elimination in the solid phase synthesis with linker **2** (see above) is shown to be compatible with **1**; no β-elimination product was observed in the solution phase of the reaction by TLC or FAB-MS. As illustrated in Figure 2a, exposure to 50 mM $Yb(OTf)_3$ and 200 mM Me_2NH in THF for 24 h gave 82% recovery of **9** from resin **7** while low yields were observed with **8**.[12] However, the 82% yield resulting from photolysis of **7** after the reaction indicates an additional mechanism other than β-elimination leading to less than quantitative recovery upon photocleavage.

Linker **1** is also compatible with strong base while **2** is degraded by strongly basic conditions. Ninety-six percent recovery from **7** and 38% recovery from **8** were observed following treatment with 200 mM NaOMe in methanol at 50 °C for 24 h (Figure 2b). Treatment of **7** and **8** with 200 mM *p*-toluenesulfonic acid (TsOH) acid in THF at 50 °C for 24 h (Figure 2c) illustrates that both linkers are stable to acidic conditions.

Reducing agents, however, reveal a limitation in both **7** and **8** due to the reactivity of the nitrophenyl functionality. This is demonstrated by treatment of **7** and **8** with 100 mM SmI_2 in 10% MeOH-THF for 5 h. Figure 2d shows virtually no photocleavage from either linker after the reaction. The IR spectrum of **7** is consistent with reduction of the aryl nitro functionality to the corresponding aryl amine as revealed by loss of the diagnostic aromatic nitro N-O stretching peak at 1531 cm^{-1}.

We have demonstrated the synthesis of a new photolabile linker (**1**) for use in solid phase synthesis that exhibits high cleavage efficiency as well as stability to acid, base and Lewis acid/base combinations. Development of photolabile linkers that are stable to multiple synthetic procedures, including methodology relying on strong reduction conditions, is currently under investigation.

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7. For **5:** mp 166-167 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) (major rotamer) δ 7.87 (d, J = 8.0 Hz, 1H); 7.73 (d, J = 7.8 Hz, 1H); 7.60 (t, J = 7.5 Hz, 1H); 7.43 (t, J = 7.5 Hz, 1H); 7.28 (br s, 1H); 5.81 (d, J = 7.2 Hz, 1H); 1.40 (s, 3H); 1.31 (s, 9H); 1.12 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 179.4, 157.4, 149.9, 134.7, 132.7, 129.9, 128.3, 124.4, 82.1, 55.5, 46.7, 28.0, 24.9, 20.1; IR (KBr) 3306, 3246, 3098, 2978, 1700, 1651, 1529 cm^{-1}; HRMS calcd for $C_{16}H_{22}N_2O_6$ $(M+Na)^+$ 361.1376, found 361.1362.
8. Procedure for **6**: In an oven dried vial under N_2, **5** (157 mg, 0.464 mmol) and HATU (165 mg, 0.435 mmol) were dissolved in dry DMF (3.75 mL) followed by addition of *i*-Pr_2NEt (151 µL, 0.867 mmol). After 5 min, the solution was added to Tentagel S NH_2 resin (Rapp Polymere) (1.0 g, 0.29 mmol) in an oven dried vial under N_2, pre-swelled with dry CH_2Cl_2 (1.25 mL), and the vial was agitated for 12 h. IR (KBr) 1655 (br), 1531 cm^{-1}. HATU= O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
9. Photolysis was performed on 3 mg resin in 120 µL degassed solvent containing 0.6 µmol of internal standard in 500 µL polypropylene Eppendorf tubes under a UVP High Intensity Longwave UV Lamp at a measured intensity of 30 mW/cm^2.
10. HPLC was performed on a Nest Group (Southborough, MA) Hypersil C18 100 Å 3 µ 4.6 mm x 6 cm column. A flow rate of 3 mL/min and a 3 min gradient of 0-50% CH_3CN in water/0.1% TFA, constant 0.1% MeOH followed by a 4 min gradient of 50-70% CH_3CN in water/0.1% TFA, constant 0.1% MeOH was used. Absorbance was measured at 254 nm.
11. The isonicotinamide chromophore was not used in these studies due to the reactivity of the isonicotinic ring under the test conditions.
12. All solid phase reactions were performed in oven dried 2 mL vials on 10 mg of resin under N_2.