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Two Useful Photolabile Surfaces for Solid-Phase Synthesis

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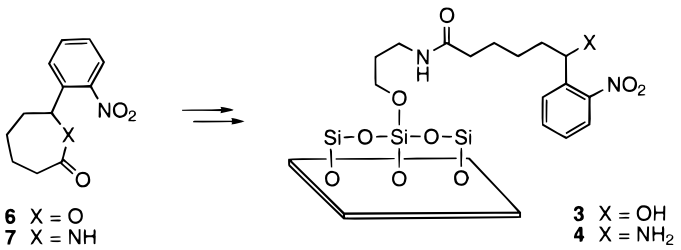
ABSTRACT



***o*-Nitrobenzyl-based photolabile surfaces 3 and 4 have been synthesized from 2-phenylcyclohexanone and aminopropylsiloxane-grafted controlled pore glass. The procedures are simple and inexpensive and generate materials whose minimally functionalized reactive chromophore should tolerate a range of subsequent chemistries. Time- and solvent-dependent analysis of photodegradation demonstrates a performance comparable to related photolabile systems.**

We are attempting to develop an artificial analogy to small molecule, processive biosynthesis: one that operates through synthetic restructuring of functionalized polymers. In these experiments, surface-bound oligopeptides are arrayed as starting materials for synthesis rather than being individual end points. Therefore, a format which enables efficient assembly of immobilized, unprotected peptides and also provides for versatility in their manipulation is vital. Because radicals, carbenoids, and active oxidants will play a role in this chemistry, commonly used organic polymer matrices are not attractive solid supports. Moreover, the system is designed to interface with whole-cell and receptor-based activity screens. Synthetic linkers degradable with acid and/or base can complicate this objective which, in other contexts, has been accomplished using photolabile inserts and product release using light.[1] The same tactic seemed appropriate for the current application, and we first employed a material formed from aminopropylsiloxane-grafted controlled pore glass (**1**, amino-CPG) and *o*-nitrobenzylamine **2** (Figure 1) for solid-phase peptide synthesis. This combination performs well; however, our synthesis of **2**[2] is time-consuming and inefficient. Herein, we describe syntheses and preliminary performance of related photolabile reagents **3** and **4**: materials which retain a reactive chromophore of relatively high

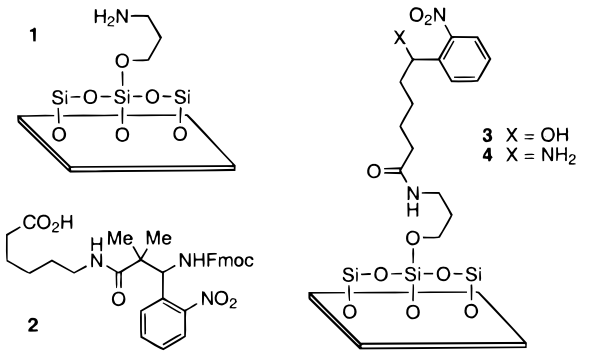


Figure 1.

(1) The preparation and utility of photolabile linkers has been reviewed: (a) Gordon, K.; Balasubramanian, S. *J. Chem. Technol. Biotechnol.* **1999**, *74*, 835−851. (b) Backes, B. J.; Ellman, J. A. *Curr. Opin. Chem. Biol.* **1997**, *1*, 86−93. (c) Lloyd-Williams, P.; Albericio, F.; Giralt, E. *Tetrahedron* **1993**, *49*, 11065−11133. (d) Pallai, V. N. R. *Synthesis* **1980**, 1−26.

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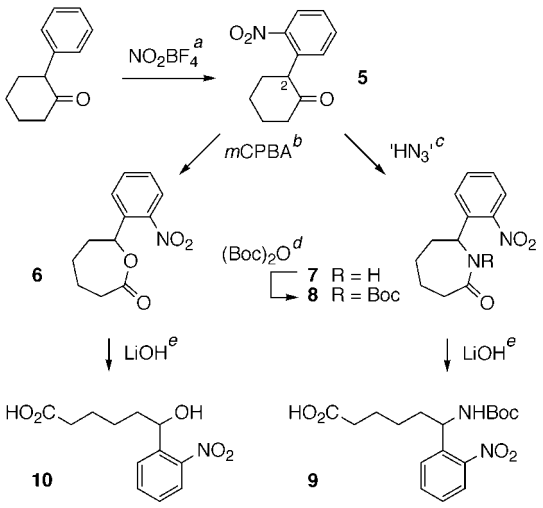
oxidation potential (in comparison to commercial veratryl-based systems) and are produced concisely from a common precursor.

Commercial 2-phenylcyclohexanone is nitrated with 85% nitronium tetrafluoroborate to afford an excess of 2′ isomer **5** (Scheme 1).[3] Purified **5** is then either converted to

Scheme 1[a]



[a] (a) NO_2BF_4, CH_3NO_2, 10 °C, 15 min (46% + 12% 4′ isomer); (b) *m*-CPBA, Na_2HPO_4, CH_2Cl_2, rt, 3 h (99%); (c) H_2SO_4, NaN_3, PhH, rt, 12 h (66%); (d) $(Boc)_2O$, DMAP, THF, rt (91%); (e) LiOH, THF/H_2O (82% for **9**, 93% for **10**).

caprolactone **6** via Baeyer−Villiger oxidation (*m*-CPBA) or to caprolactam **7** via Schmidt rearrangement (H_2SO_4, NaN_3). In both instances, ring expansion proceeds regioselectively with migration of the more substituted cyclohexanone α-carbon (C_2).[4] Lactone **6** is saponified and the resultant hydroxy acid (**10**) transferred onto the surface of amino-CPG (Biosearch Technologies) through amidation (TBTU,[5] DIPEA, HOBt). The composite formed (**3**) is functionally identical to a known polystyrene-based resin (six steps from *o*-nitrobenzaldehyde)[6] although the current preparation is advantageous in that sensitive organometallic reagents and protecting group manipulations are not required. To complete the amine congener of **3**, lactam **7** is N-acylated with di-*tert*-butyl dicarbonate and the formed imide (**8**) treated with LiOH to generate ω-carbamoyl carboxylic acid **9**. Amidation of **9** with amino-CPG (TBTU, DIPEA, HOBt) followed by exposing the resin to anhydrous CF_3CO_2H gives target material **4**. On a larger (10−15 g) scale, the conversion of **5** to **9** is carried out without intermediate purifications.

The density of reactive sites presented by batches of **3** (50−60 μmol/g)[7] and **4** (120−125 μmol/g) is determined by acylation with *N*-Fmoc-Gly-OH and quantifying the dibenzofulvene released upon DBU treatment (2% volume in DMF).[8] Photolyses are performed as stirred resin suspensions (borosilicate vials) in degassed solvent (vide infra) using a 450-W medium-pressure mercury vapor lamp positioned 5−8 cm from the reaction vessel. No attempt is made to attenuate power levels[9] although a long-pass filter (cut-on 348 nm, Oriel Corp.) is routinely utilized.

A cited advantage of veratryl-based photolabile inserts is an increased photocleavage rate, due to higher molar absorptivity, at wavelengths (>350 nm) desirable for combinatorial chemistry applications.[10] Interestingly, photophysical measurements in solution indicate that this increase in absorptivity can, in certain instances, be offset by a marked decrease in quantum yield.[11] Immobilization introduces additional variables. For example, Holmes has demonstrated that the photodecomposition of **11** (Hg(Xe) arc) occurs with $t_{1/2}$ = 0.66 min in pH 7.4 phosphate-buffered saline (Figure 2).[10b] In contrast, a solid-phase analogy of the process (i.e., photoinduced release of **13** from **12**) requires 3 h to reach 95% conversion. For comparison, resin **4** loaded with *N*-Fmoc-Gly-OH evolves *N*-Fmoc-Gly-NH_2 on photolysis (*p*-dioxane, Hg vapor lamp, ≥348 nm) wherein yield peaks at 75% after roughly 90 min (Figure 3).[12] In *p*-dioxane, the

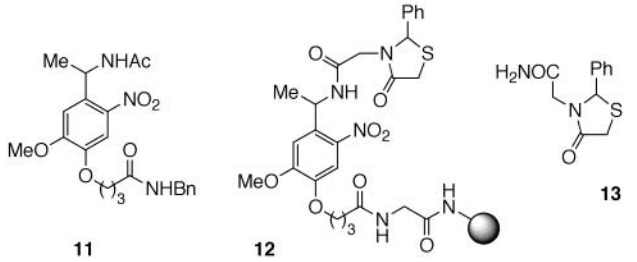


Figure 2.

(2) Compound **2** is prepared from known 2,2-dimethyl-3-(*tert*-butoxycarbonyl)amino-3-(2′-nitrophenyl)propionic acid. Sternson, S. M.; Schreiber, S. L. *Tetrahedron Lett.* **1998**, *39*, 7451−7454.

(3) The use of 95% NO_2BF_4 (Aldrich) results in lower regioselectivity (ortho:para = 1.4:1). The 85% reagent (Aldrich) is contaminated primarily with $NOBF_4$. Reaction mixtures using 95% NO_2BF_4 can be doped with 10 mol % of $NOBF_4$ to reconstitute, in part, a more selective nitration mixture. Nonselective nitration of 2-phenylcyclohexanone has been described: Prager, R. H.; Tippett, J. M.; Ward, A. D. *Aust. J. Chem.* **1978**, *31*, 1989−2001.

(4) The conversion of **5** to **7** generates trace byproducts, none of which account for more than 5% of unrecovered mass. It is possible that one of these materials is a regioisomeric lactam.

(5) TBTU = *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate, DIPEA = *N*,*N*-diisopropylethylamine, HOBt = *N*-hydroxybenzotriazole.

(6) Rodebaugh, R.; Fraser-Reid, B.; Geysen, H. M. *Tetrahedron Lett.* **1997**, *38*, 7653−7656.

(7) The discrepancy in available sites for CPG derivatized with **9** and **10** is unexplained. In solution, the condensation of **10** with *n*-$BuNH_2$ (TBTU, DIPEA, HOBt, DMF) affords the corresponding hydroxy amide in 82% isolated yield. Self-condensation and/or re-lactonized products are not detected. Moreover, there is no significant difference in immobilized yields using **9** or **10** on organic polymer supports (Tentagel, Argopore).

(8) Newcomb, W. S.; Deegan, T. L.; Miller, W.; Porco, J. A., Jr. *Technical Bulletin 012*; Argonaut Technologies Inc.: San Carlos, CA. This information can be downloaded from www.argotech.com.

(9) 983 mW/cm^2 at the 366 nm line (50 cm distance from the lamp).

(10) (a) Holmes, C. P.; Jones, D. G. *J. Org. Chem.* **1995**, *60*, 2318−2319. (b) Holmes, C. P. *J. Org. Chem.* **1997**, *62*, 2370−2380.

(11) Krafft, G. A.; Randall Sutton, W.; Cummings, R. T. *J. Am. Chem. Soc.* **1988**, *110*, 301−303.

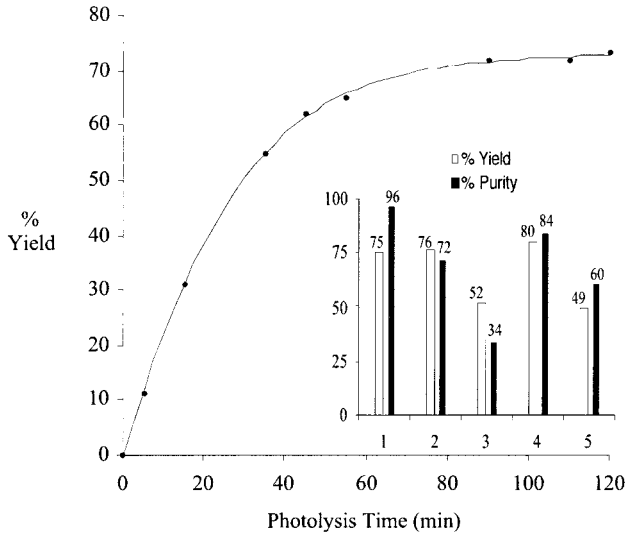


Figure 3. Time and solvent-dependent photorelease of Fmoc-Gly-NH_2 from **4** acylated with Fmoc-Gly-OH (450W Hg vapor lamp, rt, ≥348 nm). Aliquots (5 μL) of reaction solution (10 mg of resin suspended in 0.5 mL of degassed *p*-dioxane) removed by syringe and analyzed by HPLC (4.6 × 250 mm Vydac C_{18}, linear gradiant 40 → 60% 1:1 *i*-PrOH/CH_3CN in H_2O at 0.5 mL min^{-1}) using Fmoc-Gly-OH (Bachem) as external standard. Inset: The same photochemical experiment with varying solvents and product analysis after 2 h of irradiation: lane 1, *p*-dioxane; lane 2, 4:1 H_2O/MeOH; lane 3, CH_3CN; lane 4, 1:1 THF/H_2O; lane 5, THF.

purity of isolated product is >95% (^{1}H NMR and HPLC), although both yield and purity do vary with reaction medium. Of the solvents examined, *p*-dioxane has proven most generally effective (Figure 3). Hydroxyl-presenting particles **3** loaded with *N*-Fmoc-Gly-OH release unchanged acid in 77% yield (85% purity) after 2.0 h of photolysis in *p*-dioxane. This contrasts with the 12−24 h required to similarly photodegrade acylated **10** immobilized on grafted polystyrene (THF/H_2O).[6]

Solid-phase peptide synthesis on resin **4** is facile and efficient. Using Fmoc-protected monomers and TBTU-mediated couplings, semiautomated syntheses of four- and five-residue oligomers accommodate the incorporation of all proteinogenic and designed residues thus far examined. The glass matrix tolerates a corrosive reagent mixture (95% CF_3CO_2H, 5% thioanisole, 5% 1:1 $HS(CH_2)_2SH/H_2O$) used for side-chain deprotections. Protected Leu-enkephalin-amide (YGGFL-NH_2) is synthesized on **4** and, following exposure to the above mixture (3h, rt), photoreleased in 40% yield (93% per step average) and 88% purity (HPLC, Vydac C_{18}).

The combination of an inorganic matrix and a minimally functionalized linking group has advantages in our experiments that should also be useful in other applications. The resins load at low density, there are no issues of solvent-dependent swelling, nor is there a need to protect **3**/**4** from normal working light. The extent of their chemical and mechanical stability remains to be determined although strong aqueous base (NaOH, KOH) should be avoided. Finally, the use of **9** and **10** is not limited to immobilization on glass. The simplicity and low cost of synthesizing each from 2-phenylcyclohexanone will hopefully facilitate their use with other solid supports in the range of solid-phase chemistry now being explored by the community at large.

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Supporting Information Available: Experimental procedures, characterization data, and ^{1}H NMR spectra for **5**−**10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) It is common to detect residual dibenzofulvene, accounting for ~15% of the original loading capacity, when photolyzed glass particles (dark red after 2 h of irradiation) are re-subjected to DBU treatment.