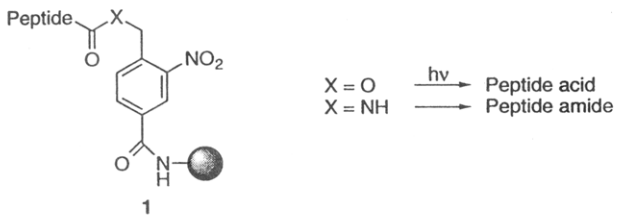
# Reagents for Combinatorial Organic Synthesis: Development of a New *o*-Nitrobenzyl Photolabile Linker for Solid Phase Synthesis

Christopher P. Holmes* and David G. Jones

*Affymax Research Institute, 4001 Miranda Avenue, Palo Alto, California 94304*

*Received February 9, 1995*

The use of a photolabile molecule as a linker for the cleavage of peptides from solid supports has received considerable attention during the last two decades. It is widely recognized that photolysis offers a mild method of cleavage which complements traditional acidic or basic cleavage techniques in routine use today.[1] *o*-Nitrobenzyl support **1** derived from 4-(bromomethyl)-3-nitrobenzoic




**1**

acid has been the most widely employed photolabile support for the generation of both peptide acids and amides.[2] Photolabile amide protecting groups for C-termini of peptides which rely on the same basic *o*-nitrobenzyl chemistry have also been reported.[3] The rapidly growing field of combinatorial organic synthesis[4] involving libraries of peptides and small molecules has markedly renewed interest in the use of photolabile linkers for the release of both ligands and tagging molecules.[5]

The original photolabile support **1**, though useful, does suffer from several limitations. For example, workers have been unable to obtain high yields of methionine-containing peptides from support **1** without substantial contamination with methionine sulfoxide.[2b,6] One solution has been to employ methionine sulfoxide throughout the peptide assembly and to subsequently reduce back to methionine to avoid any ambiguities associated with partial oxidation,[6] but this clearly detracts from the usefulness of the technique. Support **1** also suffers from unduly slow cleavage kinetics, with typical photolysis times in organic solvents ranging from 12 to 24 h.[1,2,6] Moreover, photolysis of **1** generates a reactive and chromogenic nitroso aldehyde on the support which can trap liberated compounds and may act as an internal light filter to slow the rate of cleavage.[7] Pillai and co-workers have described an α-methyl-*o*-nitrobenzyl support designed to eliminate formation of the nitroso-aldehyde, but they observed inefficient release of peptides longer than five residues due to poor swelling of the resin.[8] In the course of optimizing the photolithographic synthesis of both peptides[9] and oligonucleotides[10] we had occasion to explore the use of a variety of *o*-nitrobenzyl compounds as photolabile protecting groups.[11,12] The most useful among these were the 6-nitroveratryl derived protecting groups, which incorporate two additional alkoxy groups onto the benzene ring. Introduction of an α-methyl onto the benzylic carbon serves to greatly facilitate the photolytic cleavage with >350 nm UV light and results in the formation of a nitroso ketone. We anticipated that incorporation of these salient features into linker **1** would lead to a support with improved properties and now wish to report the synthesis and application of a new photolinker based on α-methyl-6-nitroveratrylamine designed for the release of peptide- and small molecule-amides from a solid support in aqueous environments.

One of the most successful methods of preparing photolabile amide-generating supports is to couple the linker as a suitably protected amino acid, thereby affording maximum control over the chemistry and level of substitution of the support.[1] We thus designed and prepared amino acid **7** with the 9-fluorenylmethoxycarbonyl (Fmoc) protecting group through a seven-step sequence starting with acetovanillone (Scheme 1). Of particular note is that the procedure does not require any chromatography. All intermediates in Scheme 1 are crystalline and it can be carried out in preparative scale to afford ample quantities of **7** in roughly 55% overall yield. Alternative protecting group strategies in addition to Fmoc are also accessible by derivatizing intermediate **6** with other common amine protecting groups.

With linker **7** in hand we set out to explore its use as an amide-generating linker for both peptides and small molecules. Coupling of **7** to commercially available amino supports proceeded with high conversion as judged by conventional Kaiser test to afford the corresponding photolabile supports. Despite the support's sensitivity toward photolytic cleavage, it can be handled without any special precautions other than avoiding direct exposure to sunlight or UV light. We routinely use subdued laboratory lights and store the supports and linker **7** in foil-wrapped vials.

The first use of the linker involved synthesis and cleavage of a support-bound 4-thiazolidinone.[13] The stability of both linker **7** and 4-thiazolidinone **9** toward typical TFA deprotection conditions was examined by

(1) An excellent review on solid phase peptide synthesis which also provides a comprehensive account of the use of photolabile supports has recently appeared: Lloyd-Williams, P.; Albericio, F.; Giralt, E. *Tetrahedron* **1993**, *49*, 11065−11133.

(2) (a) Rich, D. H.; Gurwara, S. K. *J. Am. Chem. Soc.* **1975**, *97*, 1575−1579. (b) Hammer, R. P.; Albericio, F.; Gera, L.; Barany, G. *Int. J. Peptide Protein Res.* **1990**, *36*, 31−45.

(3) (a) Henriksen, D. B.; Breddam, K.; Buchardt, O. *Int. J. Peptide Protein Res.* **1993**, *41*, 169−180. (b) Ramesh, D.; Wieboldt, R.; Billington, A. P.; Carpenter, B. K.; Hess, G. P. *J. Org. Chem.* **1993**, *58*, 4599−5605. (c) Pillai, V. N. R.; Mutter, M.; Bayer, E. *Tetrahedron Lett.* **1979**, 3409−3412.

(4) (a) Gallop, M. A.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gordon, E. M. *J. Med. Chem.* **1994**, *37*, 1233−1251. (b) Gordon, E, M.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gallop, M. A. *J. Med. Chem.* **1994**, *37*, 1385−1401.

(5) (a) Ohlmeyer, M. H. J.; Swanson, R. N.; Dillard, L. W.; Reader, J. C.; Asouline, G.; Kobayashi, R.; Wigler, M.; Still, C. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 10922−10926. (b) Lebl, M.; Pátek, M.; Krchnák, V.; Hruby, V. J.; Salmon, S. S.; Lam, K. S. Int. *J. Peptide Protein Res.* **1993**, *41*, 210−203.

(6) (a) LLoyd-Williams, P.; Albericio, F.; Giralt, E. Int. *J. Peptide Protein Res.* **1991**, *37*, 58−60. (b) Lloyd-Williams, P.; Gairí, M; Albericio, F.; Giralt, E. *Tetrahedron* **1993**, *49*, 10069−10078.

(7) Patchnornik, A.; Amit, B.; Woodward, R. B. *J. Am. Chem. Soc.* **1970**, *92*, 6333−6335.

(8) Pillai generated an α-methyl *o*-nitrobenzyl linker directly on polystyrene via a four-step procedure of Friedel−Crafts acylation with acetyl chloride, reduction with $NaBH_4$, conversion of the secondary alcohol to the corresponding bromide, and final nitration with nitric acid. He postulates that overnitration of the support led to its poor swelling properties: Ajayaghosh, A; Pillai, R. *Tetrahedron* **1988**, *44*, 6661−6666.

(9) Fodor, S. P. A.; Read, J. L.; Pirrung, M. C.; Stryer, L.; Lu, A. T.; Solas, D. *Science* **1991**, *251*, 767−773.

(10) Pease, A. C.; Solas, D.; Sullivan, E. J.; Cronan, M. T.; Holmes, C. P.; Fodor, S. P. A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 5022−5026.

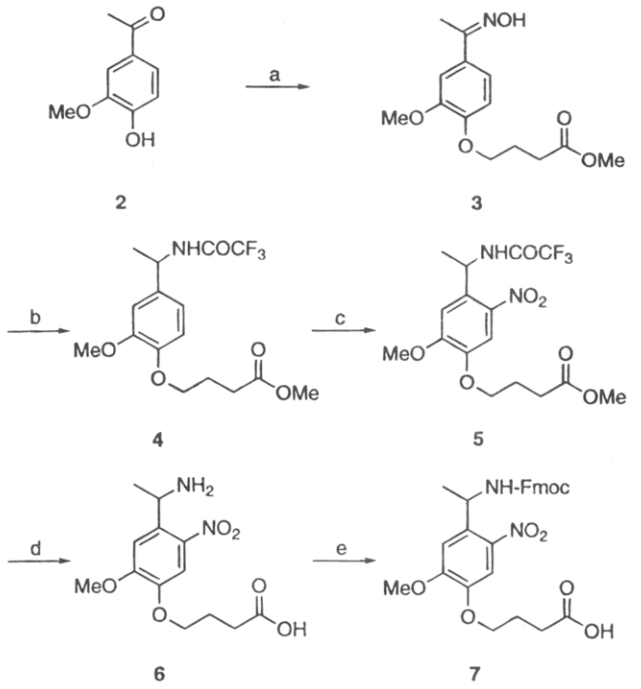
(11) Holmes, C. P.; Kiangsoontra, B. In *Peptides: Chemistry, Structure and Biology (Proceedings of the 13th American Peptide Symposium)*; Hodges, R. S., Smith, J. A., Eds.; ESCOM: Leiden, 1994; pp 110−112.

(12) For a review on the use of photolabile protecting groups see: Pillai, V. N. R. *Synthesis* **1980**, 1−26.

(13) Holmes, C. P.; Chinn, J. P.; Look, G. C.; Gallop, M. A.; Gordon, E. M. *J. Am. Chem. Soc.*, submitted.

0022-3263/95/1960-2318$09.00/0 

**Scheme 1**




[a] Reagents: (a) (i) $Br(CH_2)_3CO_2Me$, $K_2CO_3$, DMF, (ii) $H_2NOH \cdot HCl$, pyridine/$H_2O$; (b) (i) $H_2$, Pd/C, HOAc, (ii) TFAA, pyridine (80% for four steps); (c) $HNO_3$ (86%); (d) NaOH, MeOH; (e) Fmoc-Cl, $H_2O$/*p*-dioxane (81% for 2 steps).

incubating support **8** with a standard TFA-scavenger cocktail containing phenol, thioanisole, water, ethanedithiol, and TFA for 2 h at room temperature. Analysis of the support by fast $^{13}C$ NMR[14] indicated that both components were stable to these conditions. Photolytic cleavage in pH 7.4 phosphate-buffered saline (PBS) containing 5% DMSO (simulating a cleavage cocktail appropriate for transfer to a biological assay) was performed by irradiating for 3 h with 365 nm UV light. The liberated 4-thiazolidinone was obtained in 95% purity and >90% yield (Figure 1). The use of less support also shortens the time required for complete cleavage due to minimization of light-scattering and shadowing effects; we have been able to achieve complete cleavage with 1−2 h of irradiation *from single beads of resin*. The use of linker **7** to assemble and cleave peptides was additionally examined. Thus, a cholecystokinin peptide (H-Met-Gly-Trp-Met-Asp-Phe-$NH_2$), chosen as model peptide because it contains two methionines and a tryptophan residue, was prepared in a stepwise fashion and was subjected to photolytic cleavage conditions. The support-bound peptide was found to cleave with 1 h of irradiation in the presence of hydrazine as scavenger[15] to afford the desired CCK peptide in 87% purity (75% yield) (Figure 2). Comparison of the released product with the three possible methionine sulfoxide products independently prepared indicated that less than 4% of oxidized product was present. The principal HPLC peak at 18.8 min comigrated with authentic CCK peptide, and its identity was confirmed by mass spectroscopy.

In conclusion, we have described a new amide-generating *o*-nitrobenzyl cleavable linker based on α-methyl-6-nitroveratrylamine which offers significant advantages over the previously reported photolabile linkers. The rapid cleavage kinetics permits the direct release of compounds into aqueous environments and should serve to enhance high throughput screening techniques which rely on the release of molecules directly into the assay medium. Linker **7** should also find broad application in the field of solid phase organic synthesis where mild orthogonal methods of release are required.[16]

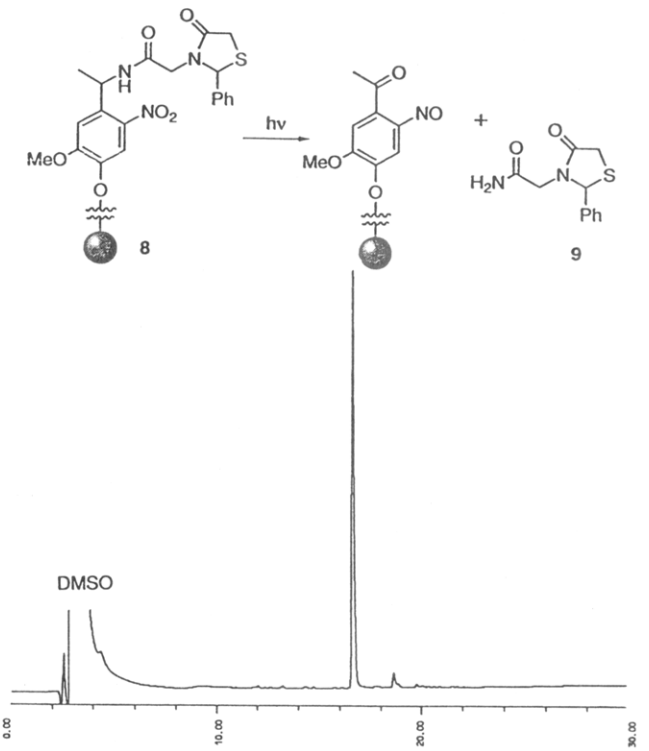



**Figure 1.** HPLC trace (220 nm) of 4-thiazolidinone **9** released upon photolysis in pH 7.4 PBS buffer containing 5% of DMSO.

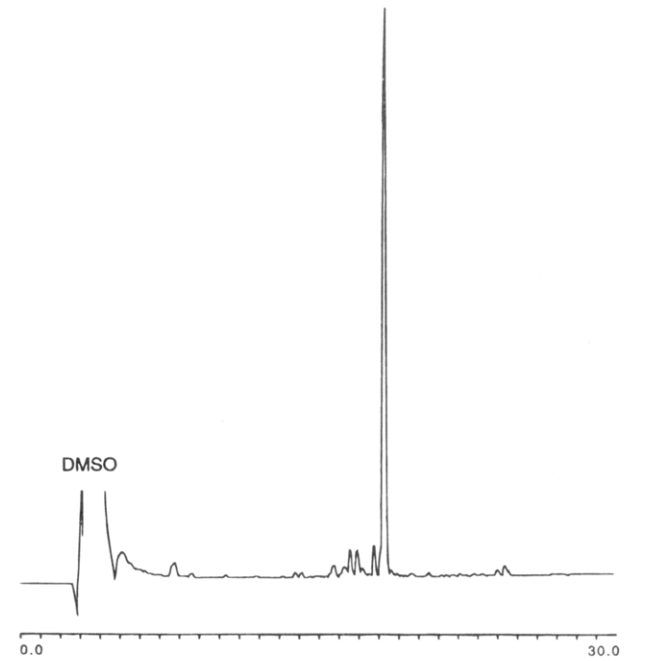



**Figure 2.** HPLC trace (220 nm) of H-Met-Gly-Trp-Met-Asp-Phe-$NH_2$ released upon photolysis in pH 7.4 PBS buffer containing 50% of DMSO.

**Supplementary Material Available:** Experimental procedures for the preparation of compounds **3**−**7** and general photolysis conditions (7 pages).

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(14) Look, G. C.; Holmes, C. P.; Chinn, J. P.; Gallop, M. A. *J. Org. Chem.* **1994**, *59*, 7588−7590.

(15) The use of scavengers during photolysis of *o*-nitrobenzyl groups is well documented; see refs 3 and 7.

(16) The authors wish to thank Mark Gallop and Eric Gordon for helpful discussions and Lun-Cong Dong for the use of hydrazine as scavenger.