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Sequentially Photocleavable Protecting Groups in Solid-Phase Synthesis

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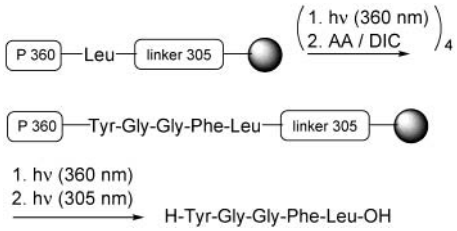
ABSTRACT



A sequential solid-phase peptide synthesis was developed using both photolabile linker and protecting groups. The chromatic sequential lability between a *tert*-butyl ketone-derived linker (sensitive to irradiation at 305 nm) and a nitroveratryloxycarbonyl (NVOC) group (sensitive at 360 nm) was exploited to prepare Leu-Enkephalin in a 55% overall yield. This new strategy allows the preparation of peptides in essentially neutral medium, by avoiding the use of common deprotection reagents such as trifluoroacetic acid or piperidine.

Solid-phase organic synthesis (SPOS) has dramatically increased in importance in the last four decades.[1] It is now the main strategy in most of the automated synthesis schemes, for both parallel and combinatorial approaches.[2] The relevant substrate is attached to the solid support via a linker throughout the synthetic process; essentially, this link should be robust and withstand a large spectrum of reagents. At the end of the sequence, usually quite harsh conditions, basic or acidic, trigger the release of the modified compound. Photorelease is an attractive alternative to aggressive reagents.[3] Such linkers are usually derived from known photolabile groups (e.g., **1** or **2**).[4,5] We recently described **3**, which contains a robust linker with very efficient photocleavage.[6] However, the development of a linker cleavable under very mild conditions is only of interest if all the other transformations in the sequence are equally mild. For example, in peptide synthesis, the deprotection of the N-terminus of the growing chain requires basic (Fmoc removal) or acidic (Boc removal) conditions. We recently described a new protecting strategy based on the group differentiation by light of specific wavelengths (*chromatic orthogonality*).[7,8] We show here how such a strategy could be used in peptide synthesis, with both linker *and* temporal protecting groups removed photochemically at different wavelengths. All these transformations can, in principle, be carried out at neutral pH.

Preliminary Photolysis Experiments. The negligible absorbance of the photosensitive *tert*-butyl ketone **2** above 330 nm prompted us to check whether it could be possible to remove the more sensitive nitroveratrole protecting group

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(1) (a) Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149−2154. (b) Merrifield, R. B. *Science* **1965**, *165*, 178−185.
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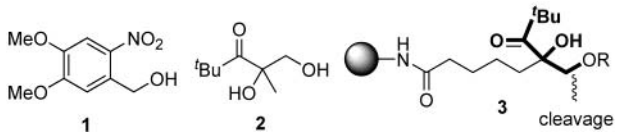
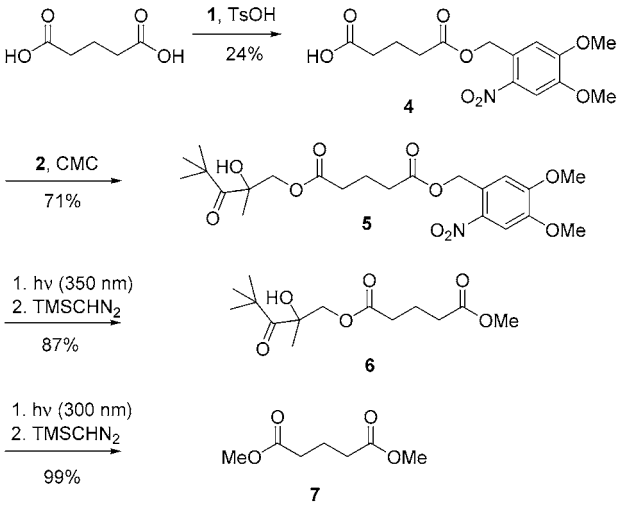
Scheme 1. Photolabile Linkers



1 photolytically at a longer wavelength. Hence, we prepared the diester **5**, by first esterifying glutaric acid with alcohol **1** under acidic catalysis (24%)[9] and then coupling **4** with **2** using *N*-cyclohexyl-*N*′-(2-morpholinoethyl)carbodiimide methyl-*p*-toluenesulfonate (CMC) (Scheme 2). The photolysis

Scheme 2. Sequential Photolysis of Diesters



of the diester **5** at 360 nm smoothly gave the monomethylester **6** (87%) after treatment with diazomethane (together with 2% totally photolyzed **7**). A second photolysis at 300 nm gave the dimethyl glutarate **7** in quantitative yield after methyl ester formation.

With this encouraging modulated lability,[10] we confirmed that the same strategy could also be applied to the solid phase, by using the glutamic acid derivative **8**, attached to the TentaGel-bound photosensitive linker **3**. Hence, *N*,*N*′-diisopropylcarbodiimide-mediated (DIC) coupling of **8** to the resin was followed by the cleavage of the Fmoc group with piperidine (in DMF, 25 °C, 30 min); titration of the resulting dibenzofulvene by UV spectroscopy indicated a resin loading of 0.3 mmol/g. As expected, the photolysis at 360 nm of **9** followed by esterification with a solution of trimethylsilyl diazomethane in hexane smoothly led to methylester **10**. Final release from the resin by irradiation at 335 nm gave **11** in a 45% overall yield (from initial compound **8**) (Scheme 3). The identity was determined by HPLC and electrospray mass spectrometry. Clearly, the presence of the resin does not have a detrimental effect on the photolytic processes.

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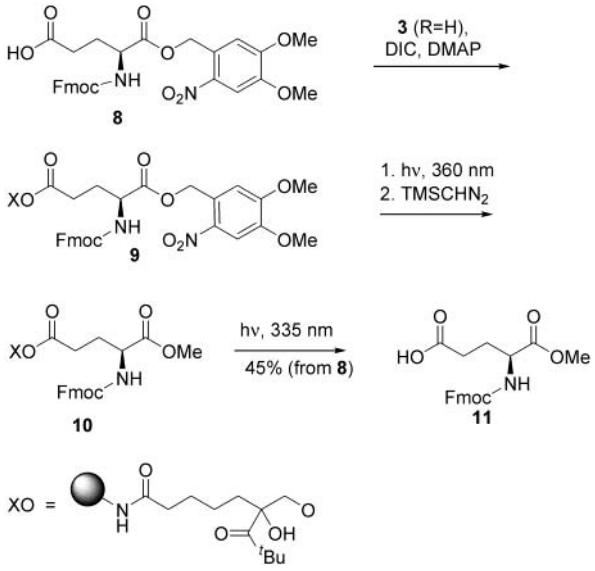
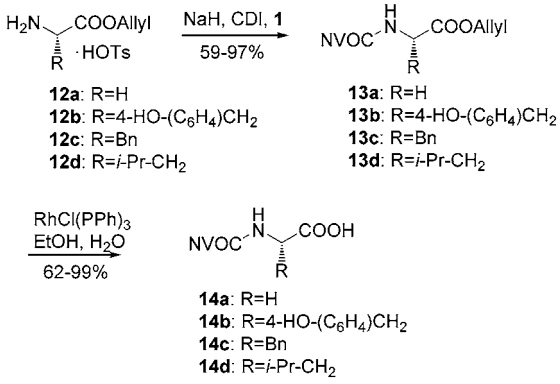
Scheme 3. Photolysis of Resin-Bound Amino Acids



Application to the Sequential Peptide Synthesis. All the requirements for sequential solid-phase peptide synthesis were now fulfilled. We chose Leu-Enkephalin as a test substrate for its well-known characterization data and our prior experience.[5] In addition, the presence of UV-absorbing amino acids (Tyr) would be a test for the scope of our strategy (an authentic sample of Leu-Enkephalin showed less than 1% degradation after irradiation at 305 nm for 60 min, as determined by HPLC). Leu-Enkephalin (H-Tyr-Gly-Gly-Phe-Leu-OH) is an endorphin pentapeptide isolated from brain, with morphin-like activity.[11]

Preparation of N-Protected Amino Acids. The commercially available amino acid hydrotosylates **12a**−**d** were protected with the nitroveratryloxycarbonyl group (NVOC) according to our previously published procedure (Scheme 1).[12] Thus, neutralization of the salts with sodium hydride in dichloromethane (25 °C, 30 min) was followed by acylation with a mixture of 2-nitroveratrole **1** and *N*,*N*′-carbonyldiimidazole (CDI) to give carbamates **13a**−**d**. The

Scheme 4. Preparation of N-Protected Amino Acids

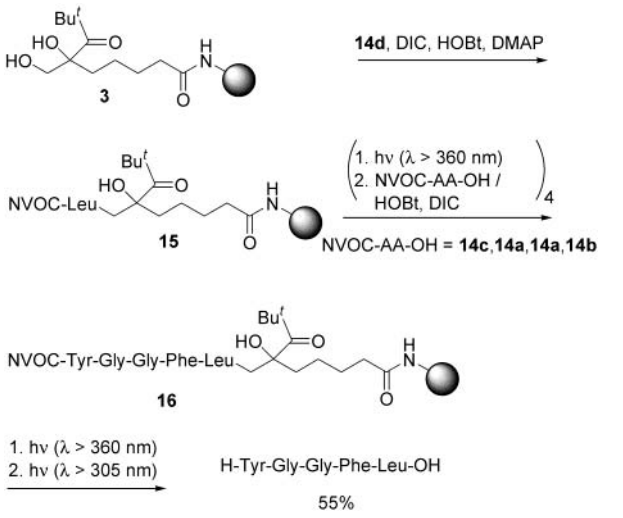


12a: R=H
12b: R=4-HO-(C_6H_4)CH_2
12c: R=Bn
12d: R=*i*-Pr-CH_2

13a: R=H
13b: R=4-HO-(C_6H_4)CH_2
13c: R=Bn
13d: R=*i*-Pr-CH_2

14a: R=H
14b: R=4-HO-(C_6H_4)CH_2
14c: R=Bn
14d: R=*i*-Pr-CH_2

Scheme 5. Preparation of Leu-Enkephalin



free acids were obtained by the Rh-catalyzed hydrolysis of the allyl esters (EtOH−H_2O).[13,14]

Sequential Synthesis of Leu-Enkephalin. The TentaGel-bound linker **3** was coupled with NVOC-Leu-OH **14d** (DIC, HOBt, DMAP cat., rt, 20 h). Exposure to 360 nm light (Osram 500 W high-pressure mercury lamp with a 360 nm cutoff filter) for 3−4 h led to a significant darkening of the solution and mediocre deprotection. Such poor results have already been observed and were attributed to the subsequent reaction of the released amine and the aldehyde photoproduct of NVOC.[4a] To circumvent this side-reaction, we trapped in situ the aldehyde as soon as it was produced, by using solvents containing 0.5% semicarbazide hydrochloride for all our photolyses at 360 nm. After 20 min of irradiation, the solvent was filtered and analyzed for the presence of side products. The process was repeated until completion of the deprotection. The same sequence was repeated four times using NVOC-amino acids **14c**, **14a**, **14a**, and **14b**.[15] The concentration of free Leu-Enkephalin in the last photodeprotection sample was below the detection limit of the electrospray mass spectrometer. After each coupling step, complete acylation was checked by picric acid monitoring.[16] Final cleavage photolysis was performed by irradiation at higher energy (Osram 500W high-pressure mercury lamp, 305 nm cut off filter) in a 4:1 THF−water mixture (16 °C, 30 min). Leu-Enkephalin was released in a yield of 55% and in a purity of 92%. Shorter peptides were formed in quantities of less than 1%. This absence of deletion in the sequence is very important, since it proves the near quantitative yield for each individual step.

In summary, we were able to synthesize a pentapeptide in an iterative scheme, without the need for chemical deprotection steps in both the substrate elaboration and final release phases. This allowed the handling of sensitive side chains, since the whole protocol operates under essentially neutral conditions.

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Supporting Information Available: Typical experimental procedures and characterization data for selected compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) A similar route was also used, with the ^{t}butyl ester hydrochlorides as starting materials, replacing the final saponification by acidic hydrolysis (CF_3COOH). Overall yields were comparable.

(15) Experimental details on the synthesis on a solid support and photorelease of Leu-Enkephalin are available as Supporting Information.

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