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Photo-Triggered Selective C-Terminal N-Methylamidative Cleavage of Polyethyleneglycol-Bound Peptides[1]

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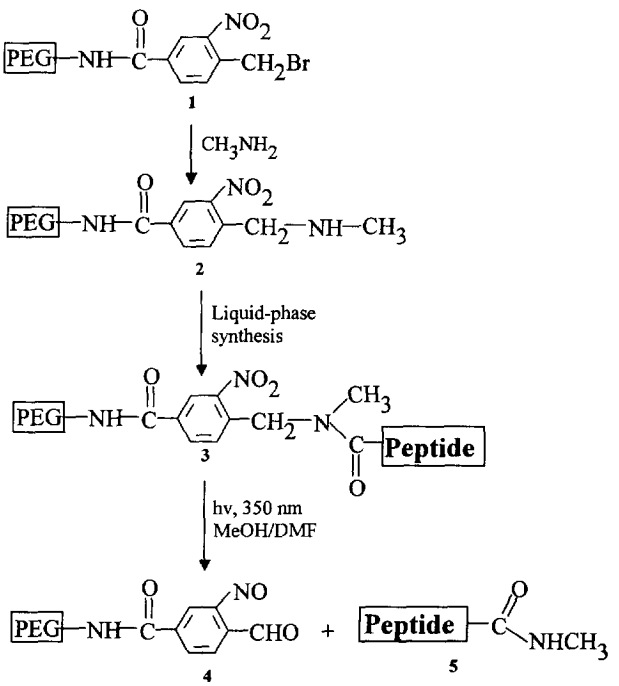
Abstract : *A novel liquid-phase method has been designed for the photolytic release of the attached peptides, directly as their C-terminal N-methylamides, under mild conditions in excellent yields and purity. The present approach provides fully side-chain protected peptide N-methylamides, useful for segment condensation and facilitate photo-controlled release of biologically active peptides for therapeutic applications.*

C-Terminal modification of peptides without affecting the side-chain protecting groups and chiral centres has been a great challenge to peptide chemists. In this context, synthesis of peptide N-alkylamides have attracted much attention due to their enhanced biological activity compared to the naturally occuring analogues.[2,3] C-Terminal modified peptides are also used for structure-activity relationship studies and conformational studies.[4,5] However, there are not many reports pertaining to the efficient synthesis of peptide N-alkylamides under mild conditions.[6] The classical solid-phase approach towards the synthesis of peptide N-alkylamides suffers from several limitations.[7-9] For example, with the existing methods, it is difficult to synthesize fully side-chain protected peptide N-alkylamides which are useful for the synthesis of complex peptides by segment condensation method. On the other hand, due to the enhanced biological properties and therapeutic value of peptide N-alkylamides, a suitable method for their controlled release for *invivo* application would be of great interest. With these goals, we now report here a conceptually novel and mild strategy for the synthesis of peptide N-alkylamides.

Photolytic cleavage has been widely accepted as a mild method for the removal of polymer-bound peptides in solid- and liquid-phase peptide synthesis.[10] *o*-Nitrobenzylic photo-rearrangement is the most exploited reaction for this purpose.[11,12] Taking advantage of *o*-nitrobenzylic photochemistry, we have earlier reported procedures for the solid-phase synthesis of C-terminal peptide amides.[13,14] However, the poor swelling of the polystyrene-based peptide resin in alcoholic solvents under heterolytic reaction condition has decreased the efficiency of the photolytic cleavage. Therefore, we have designed a viable liquid-phase method for the synthesis of peptide N-methylamides on a biocompatable PEG support which may be suitable

for controlled release applications. For this purpose, 3-nitro-4-bromomethylbenzamido PEG (**1**) was prepared from amino PEG (Mn, 6000) according to a reported procedure.[15,16] PEG **1** was subsequently reacted with anhydrous methylamine to get the required 3-nitro-4-methylaminomethylbenzamido PEG (**2**) in almost quantitative conversion. Elemental analysis gave 0.25 mmol/g of amino group with no detectable amount of bromine. In this way, the aminomethyl group which has a dual role as amino acid anchor group as well as a later reagent for the N-methyl amidative peptide cleavage, could be incorporated to the PEG support prior to the peptide synthesis.



Scheme 1

The synthetic utility of the new PEG support is illustrated with the synthesis of some representative peptide N-methylamides, adopting the standard liquid-phase procedures (Scheme 1). Our initial attempt in this direction was to synthesize a simple tripeptide N-methylamide, Boc-Leu-Ala-Val-$NHCH_3$, containing a hindered amino acid valine as the C-terminal residue. The peptides were assembled following the standard Fmoc-amino acid coupling procedures. Each coupling step was monitored for completion by a semiquantitative ninhydrin test and the amino acid loading was determined spectrophotometrically from the amount of the liberated Fmoc group. In all peptide synthesis, the end coupling was performed using either Boc- or Z-amino acid instead of Fmoc-amino acid, inorder to prevent light absorption by Fmoc group under

photolytic conditions of the peptide removal. The N-methylamidative peptide cleavage was accomplished by irradiating (350 nm) a solution of the PEG-peptide in methanol containing 5 % DMF. In this way, the tripeptide N-methylamide, Boc-Leu-Ala-Val-$NHCH_3$ was obtained in 87 % yield over 90 % purity. The crude peptide was further purified by semipreparative Hplc on Vydac C-18 column using gradient elution with aqueous methanol. The purified peptide amide was characterized by spectral, elemental and amino acid analyses.[17]

After successful synthesis of the tripeptide amide, our next goal was to synthesize a peptide N-methylamide with a benzyl ester side-chain protecting group which are difficult to synthesize by conventional cleavage using methyl amine due to the simultaneous amidation of the side-chain functional group. For this purpose, a tetrapeptide was assembled on the PEG **2**. With the help of our photo-triggered indirect amidative cleavage, we could selectively amidate the C-terminal residue, leading to the tetrapeptide N-methylamide, Boc-Ala-Asp(OBzl)-Ala-Val-$NHCH_3$ in 86 % photocleavage yield. ^1H NMR analysis of the tetrapeptide amide clearly indicated that the side-chain benzyl ester protection remained intact. Elemental and amino acid analyses were in agreement with the peptide sequence.[18] Finally, inorder to check the viability of our strategy in designing complex peptide N-methylamides with several side-chain protecting groups, we carried out the liquid-phase assembling of a nonapeptide N-methylamide, Z-Cys(Bzl)-Tyr(Bzl)-Phe-Glu-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-$NHCH_3$. On photolytic removal, the nonapeptide N-methylamide was obtained in 80 % yield.[19]

In conclusion, the above described strategy offers a simple and convenient method for the selective C-terminal N-methylamidative removal of PEG-bound peptides, making use of the well-known *o*-nitrobenzylic photo-rearrangement. In addition to this, the described method may find wide use in photo(optical fibre)-triggered controlled release of biologically active synthetic peptide N-alkylamides, directly from a biocompatable PEG support, for *invivo* therapeutic applications.

Acknowledgements

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17. Boc-Leu-Ala-Val-$NHCH_3$; mp. 188-190 °C; IR (KBr): 1650 (amide), 1710 cm^{-1} (urethane): ^1H NMR (270 MHz, $CDCl_3$): δ 1.45 (s, 9 H, Boc), 0.95 (t, 6 H, $C_\delta H$, Leu), 1.37-1.42 (m, $C_\gamma H$ & $C_\beta H$, Leu), 1.25 (d, 3 H, $C_\beta H$, Ala), 2.2 (b, $C_\beta H$, Val), 2.8 (d, 3 H, CH_3), 4.13 (b, 1 H, $C_\alpha H$, Ala), 4.3 (t, 1 H, CαH, Val), 4.4 (q, 1 H, CαH, Leu), 5.06 (d, 1 H, NH, Ala), 6.68 (b, 1 H, NH), 6.9 (d, 1 H, NH, Leu), 7.0 (d, 1 H, NH, Val); Anal. Calcd. for $C_{20}H_{38}N_4O_5$: C, 57.95; H, 9.17; N, 13.52. Found: C, 57.50; H, 9.14; N, 13.97. Amino acid analysis: Leu, 1.09; Ala, 1.05; Val, 1.0.
18. Boc-Ala-Asp(OBzl)-Leu-Ala-Val-$NHCH_3$; mp. 178-180 °C; Anal. Calcd.for $C_{34}H_{54}N_6O_9$: C, 59.13; H, 7.82; N, 12.17. Found: C, 59.0; H, 7.79; N, 12.32. Amino acid analysis: Ala, 2.03; Asp, 0.98; Leu, 1.0; Val, 0.97.
19. Satisfactory spectral, elemental and amino acid analyses could be obtained for the nonapeptide N-methylamide.

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