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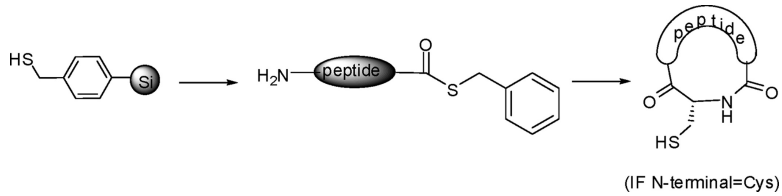

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Reports

High Throughput Synthesis of Peptide α-Thioesters Through the Use of "Volatilizable" Support

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Native chemical ligation has facilitated the total synthesis of small- and medium-sized proteins and cyclic peptides and has thus opened the world of proteins to the tools of organic chemistry.[1] Native chemical ligation relies on the combination of two steps. In the first step, a C-terminal peptide α-thioester is reacted with an N-terminal cysteine peptide. The second step involves a rapid intramolecular S- to N-shift forming an amide bond at the ligation site. Recently, the scope of ligation methods has expanded from N-terminal cysteine to noncysteine residues by employing auxiliary thiols, thus increasing the applicability of the approach.[2] C-terminal peptide α-thioesters are key intermediates in all of these methodologies. This need for C-terminal peptide α-thioesters has stimulated the development in our laboratory of simple and efficient synthetic methods for this class of peptides.

Currently there are several reported methods for the solid-phase synthesis of peptide α-thioesters. Previous studies report strategies for the synthesis of peptide α-thioesters using both Boc and Fmoc cheminstry. For Boc solid-phase chemisty *p*-methylbenzhydrylamine (MBHA) resin must first be converted to a mercaptan linker resin by coupling 3-mercaptopropionic acid to MBHA prior to the peptide synthesis.[3] For Fmoc chemistry, peptide α-thioesters are commonly accessed by utilizing a "safety-catch" sulfonamide resin.[4] In this method the sulfonamide must be activated by alkylation before the fully protected peptide is cleaved from the resin with an excess of a thiol nucleophile. The fully protected peptide thioester is then deprotected with concentrated trifluoroacetic acid in solution. Other approaches such as the backbone amide linker (BAL) strategy rely on anchoring the first amino acid ester on an aldehyde-functionalized resin by reductive amination.[5] After elongating the peptide sequence at the N-terminal, the C-terminal ester-protected group must be removed to release the carboxyl group and then coupled with the amino acid thioester, prior to cleavage from the solid support. Though there are different synthetic methods developed for different types of resin solid supports, the methods available thus far for the solid-phase synthesis of peptide α-thioesters are neither as simple nor

Scheme 1. Synthesis of Mercaptomethylphenyl-Functionalized Silica Gel

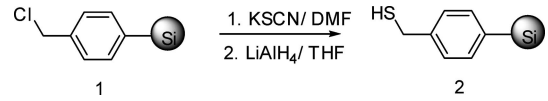

Table 1. Peptide α-Thioester Synthesized on Volatilizable Mercaptomethylphenyl-Functionalized Silica Support

entry	sequence	purity (%)[a]	yield (%)[b]
5a	H-Phe-Ala-SBzl	90	100
5b	H-Met(O)-Leu-SBzl	93	88
5c	H-Gly-Phe-Leu-SBzl	93	82
5d	H-Phe-Leu-Ala-SBzl	85	91
5e	H-Phe-Leu-Leu-SBzl	83	96
5f	H-Ala-Phe-Leu-Leu-SBzl	84	95
5g	H-Ala-Phe-Leu-Ala-SBzl	90	90
5h	H-Gly-Asn-Leu-Phe-Leu-SBzl	85	92
5i	H-Val-Val-Leu-Asn-Leu-Asn-Phe-SBzl	80	82
5j	H-Ala-Pro-Ile-Ala-Phe-Phe-Gly-SBzl	81	83
6a	H-Ala-Lys-Phe-Ala-SBzl	91	87
6b	H-Thr-Gly-Val-Phe-Ala-SBzl	80	86
6c	H-Ala-Arg-Gly-Leu-Phe-SBzl	78	97
6d	H-Ala-Trp-Phe-Ala-SBzl	70	92
6e	H-Gly-Asp-Tyr-Gly-Gly-Phe-Ser-Leu-Tyr-Lys-Ala-SBzl	65	60
8a	H-Cys-Gly-Gly-Phe-Leu-SBzl	84	90
8b	H-Cys-Tyr-Gly-Gly-Phe-Leu-SBzl	80	85

[a] Purity (in percent) is determined by the peak area of HPLC at 214 nm. [b] Yields (in percent) are based on the weight of crude product and are relative to the substitution of the resin.

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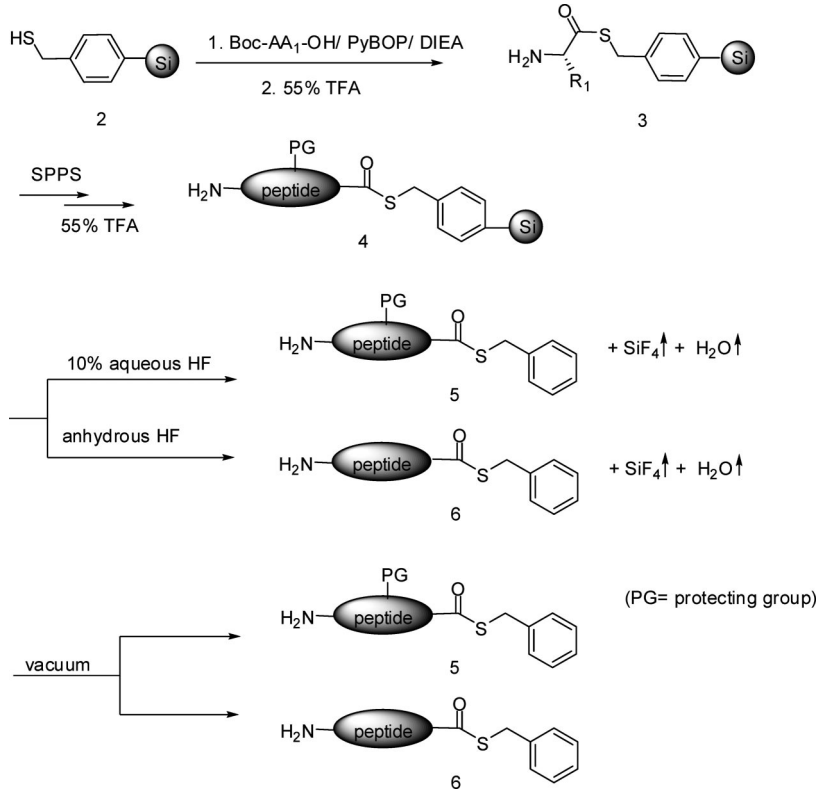
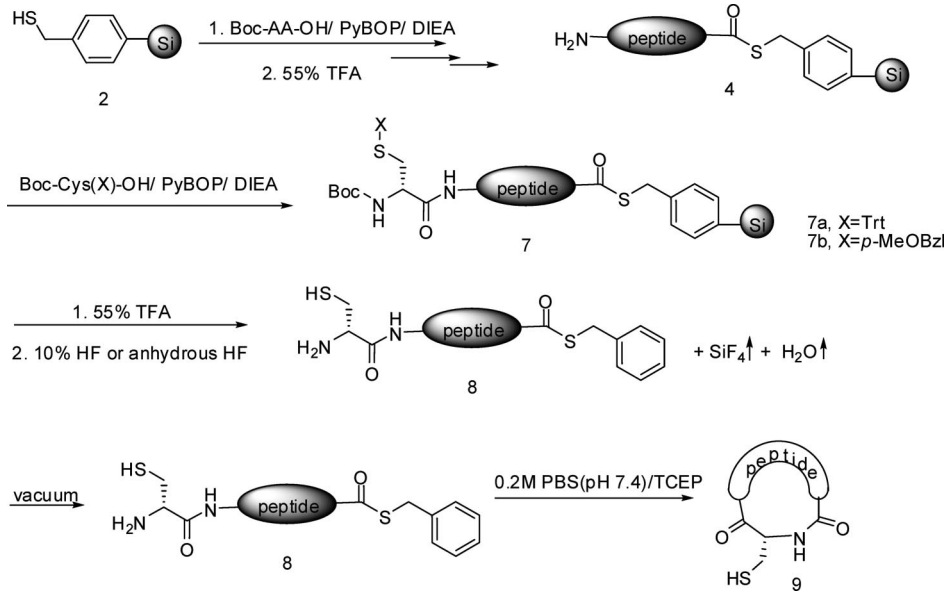
Scheme 2. Synthesis of Benzyl Peptide α-Thioester on "Volitilizable" Mercaptomethylphenyl-Functionalized Silica Support



Scheme 3. Synthesis of Cyclic Peptide on Volitilizable Mercaptomethylphenyl-Functionalized Silica Support



efficient as the current techniques for the synthesis of peptide acids and peptide amides. Furthermore, there exists no specific resin for the solid-phase synthesis of peptide α-thioesters. Therefore the development of an improved methodology and/or unique resin for the synthesis of peptide α-thioesters would be of clear value.

Here, we report a simple and efficient synthetic approach for the production of peptide α-thioesters on a novel mercaptomethylphenyl-functionalized silica gel. The synthetic approach eliminates the need to convert commercially available resin prior to synthesis. Additionally, the new approach eliminates the postcleavage workups such as exhaustive extraction, filtration and purification needed with previously reported methods. The desired peptide α-thioesters were obtained with high yields and purities.

The mercaptomethylphenyl-functionalized silica gel was synthesized by the on-resin substitution and reduction of chloromethylphenyl-functionalized silica gel[6] as shown in Scheme 1. In order to eliminate the truncation products produced by failed coupling reactions, the loading of the chloromethylphenyl group is optimized by performing a surface dilution with a phenyl group. This was accomplished by treating the silica gel with a mixture of *p*-chloromethylphenyltrimethoxysilane and phenyltrimethoxysilane at re-

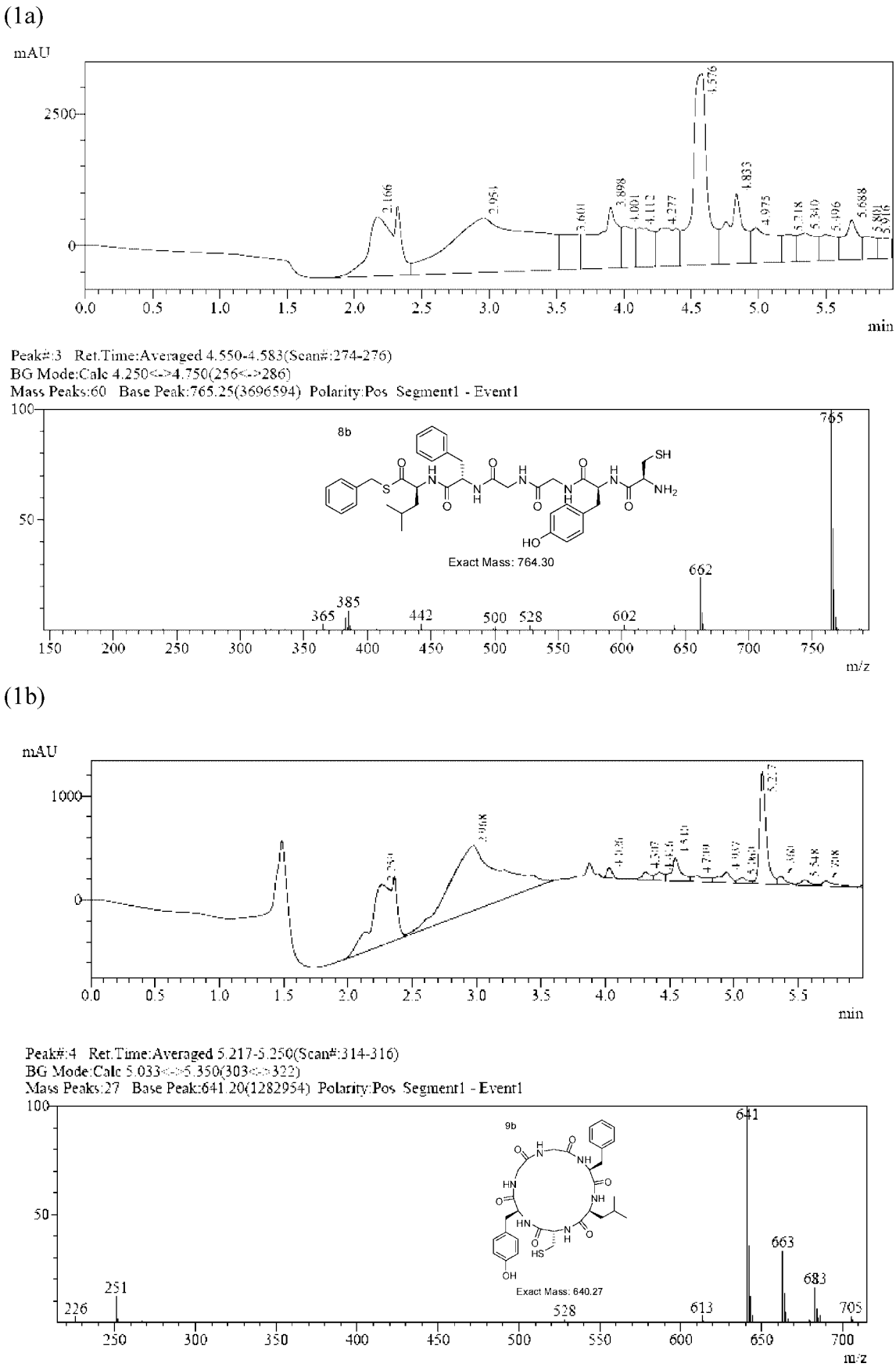


Figure 1. (a) LC-MS of the crude peptide thioester H-Cys-Tyr-Gly-Gly-Phe-Leu-SBzl (8b). (b) LC-MS of the native ligation product (9b).

flux in toluene to form the desired chloromethylphenyl-functionalized silica gel 1. The phenyl-functional group on the silica gel is unreactive to the typical peptide synthesis approaches used and is decomposed through Si−O−Si and

Si-phenyl bond cleavage into the volatile constituents (SiF_4, water and benzene) under the influence of aqueous or anhydrous hydrogen fluoride (Scheme 2). It was determined that the optimal molar ratio of *p*-chloromethylphenytrimethoxysilane to phenyltrimethoxysilane was 1:1. The *p*-chloromethylphenyl-functionalized silica gel was then treated with potassium thiocyanate in DMF at 65 °C for 24 h. After washing with DMF and DCM, the functionalized silica gel was reduced with lithium aluminum hydride in THF at room temperature for 24 h to yield the desired mercaptomethylphenyl-functionalized silica gel 2.

Parallel solid-phase synthesis of peptide thioesters were carried out using the "teabag" approach.[7] Boc amino acids (4 equiv) preactivated with PyBOP (4 equiv) and DIEA (6 equiv) in DMF for 5 min were added to the mercaptomethylphenyl-functionalized silica gel 2 and shaken at room temperature overnight. After washing with DMF, the Boc group was removed with 55% TFA in DCM to yield the resin bound peptide α-thioester 3 as shown in Scheme 2. Stepwise peptide synthesis was carried out using a standard PyBOP/ DIEA coupling protocol. After peptide chain elongation, the resin bound peptide 4 was treated with 10% aqueous hydrofluoric acid for 1 h at room temperature. Following solvent removal by lyophilization (HF and tetrafluorosilane are readily trapped and/or decomposed by in-line traps containing solid CaO), the desired side chain protected peptide α-thioesters 5 were obtained as the sole products in the reaction vessel in excellent yields and purities (Table 1, 5a−j). In a separate experiment, support-bound 4 was treated with anhydrous HF for 2 h at 0 °C. Following evaporation of the anhydrous HF with a gaseous nitrogen stream, the unprotected peptide α-thioester 6 was obtained in excellent yield and purity following lyophilization (Table 1, 6a−e).

Special consideration was given to the synthesis of peptide α-thioesters containing tryptophan. A commonly used Boc-protected tryptophan for peptide synthesis is Boc-Trp(For)-OH, which utilizes a formyl (For) group for the protection of the indole functionality. However, the formyl group is stable to acid cleavage and typically needs to be removed prior to peptide cleavage using 10% piperidine. Unfortunately treatment with piperidine has the undesired effect of decomposing the thioester by aminolysis. Therefore Boc-Trp(Mts)-OH is employed in the synthesis of peptide α-thioesters containing tryptophan. The mesitylene-2-sulfonyl (Mts) group is removed during the anhydrous HF cleavage and the unprotected peptide α-thioesters synthesized utilizing Boc-Trp(Mts)-OH provides good result(6d).

The N-terminal cysteine peptide was also synthesized on the mercaptomethylphenyl-functionalized silica gel. The synthesized N-terminal cysteine peptide α-thioesters were utilized for the formation of cyclic peptides through native ligation as shown in Scheme 3. Boc-Cys(Trt)-OH was coupled to the N-terminal prior to cleavage of the peptide with 10% hydrofluoric acid. The Trt- and Boc- groups were removed simultaneously with a mixture of 5% TIS and 55% TFA in DCM. Cleavage with anhydrous HF gave the fully unprotected N-terminal cysteine peptide α-thioester. After removal of the solvents by lyophilization, the linear peptide α-thioesters having an N-terminal cysteine were obtained with high yields and purities (Table 1, 8a−b; Figure 1a). Intramolecular native chemical ligation/cyclization was generated with the crude peptides in a 0.2 M phosphate buffer (pH 7.4) containing 3 equiv of tris(carboxyethyl)phosphine (TCEP) overnight[8] (Figure 1b).

In summary, we present here a novel approach for the synthesis of peptide α-thioester on functionalized mercaptomethylphenyl silica gel. The synthetic process is straightforward and efficient. After the final cleavage, which decomposes and volatilizes the solid support, only the desired protected or unprotected peptide α-thioester remained in the vessel. This approach is promising for the automated synthesis of peptide α-thioesters and cyclic peptides.

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Supporting Information Available. Experimental methods; LC-MS of the products; NMR data of the representative products. This material is available free of charge via the Internet at http://pubs.acs.org.

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