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Solid-Phase Staudinger Ligation from a Novel Core-Shell-Type Resin: A Tool for Facile Condensation of Small Peptide Fragments

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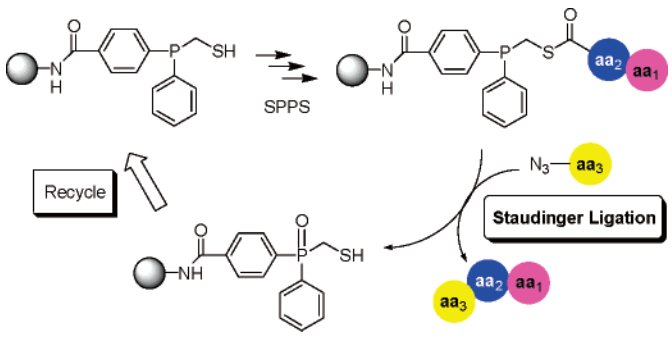
ABSTRACT



Solid-phase Staudinger ligation of small peptides was performed on a novel core-shell-type resin. Solid-phase Staudinger ligation was mediated by synthetic solid-supported phosphinothiol, which was readily prepared by a straightforward synthetic route. This protocol afforded final peptide products in excellent yields and purities and thus could provide the opportunity to facilitate a simple manipulation for condensation of peptide fragments. In particular, the resulting resin could be recycled in a successful manner.

The chemical synthesis approach to protein engineering has attracted a great deal of attention because of its generality and flexibility in the incorporation of noncoded moieties.[1] For the total chemical synthesis of proteins, the ligation of synthetic peptides is an essential step, and the most common route of accomplishing this is native chemical ligation, which is widely utilized for the preparation of peptides containing over 50 residues.[2] In a typical native chemical ligation, the thiolate of an *N*-terminal cysteine residue of one peptide attacks the *C*-terminal thioester of the other peptide, ultimately leading to the synthesis of the final peptide through S → N acyl migration (Figure 1a). However, a fatal drawback of this method is the requirement that there be a cysteine residue at the ligation junction. To overcome this limitation, more general ligation strategies have been proposed,[3] and in this respect, the protocol based on the Staudinger reaction

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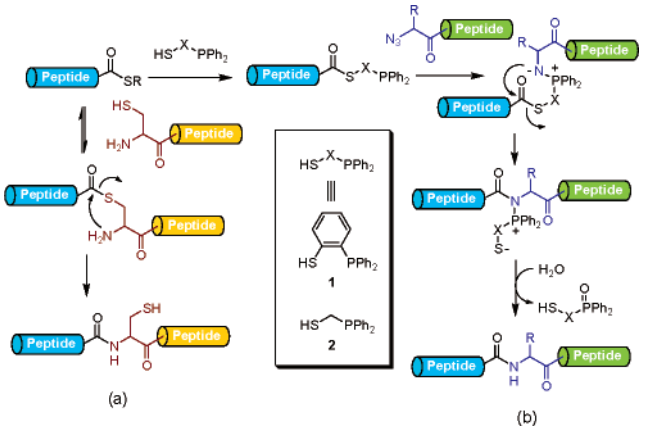

Figure 1. Schematic rationale of peptide fragment condensation via (a) native chemical ligation and (b) Staudinger ligation.

has particularly interesting features for the formation of peptide bonds.[4] The Staudinger ligation does not rely on the presence of a cysteine or other specific residue at the *N*-terminus of the peptide fragment. In addition, this method is traceless, in the sense that no residual groups from the phosphinothiol involved as a mediator remain in the peptide product. This reaction probably proceeds via the intramolecular rearrangement of an iminophosphorane intermediate to give an amidophosphonium salt, which is in turn hydrolyzed to yield an amide and *o*-(diphenylphosphinyl)benzenethiol (Figure 1b). There are two types of phosphinothiol which can be used to mediate Staudinger ligation (**1** and **2** in Figure 1). Since the isolated yields of the Staudinger ligations using **1** are too low in some cases, **2** was developed more recently.[4c,5] Although **2** has shown an appropriate level of performance in various ligation reactions, all of the synthetic steps for **2** are complicated and the overall yields are unsatisfactory. Furthermore, additional purification steps are required in order to isolate both the thioester precursor prior to ligation and the final peptide obtained after ligation.

These factors prompted us to exploit a readily removable and reusable solid-supported phosphinothiol for Staudinger ligation. Also, given that, in this case, Staudinger ligation would be performed in the aqueous phase, water-compatible TentaGel resin and CLEAR resin were chosen as candidate supports. Following the coupling of 4-bromophenylacetic acid onto the amino-functionalized resin, an excess of magnesium was added to the reaction mixture to form resin-bound phenylmagnesium bromide, which could act as a Grignard reagent. However, the resins failed to endure the harsh reaction conditions. In particular, during the insertion of magnesium on CLEAR resin, unknown products were released, which were probably the decomposition products of the resin backbone.

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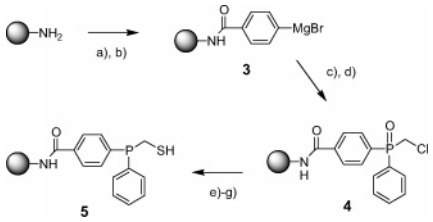
Recently, we developed a highly cross-linked core−shell type (HiCore) resin from aminomethyl polystyrene (AM PS) resin.[6] Its highly cross-linked rigid core and poly(ethylene glycol) (PEG) shell provide it with chemical and physical stability and facilitate the easy access of large sized molecules to the functional groups of the resin, which are located on the surface of the resin beads (Figure 2b).

Figure 2. Cross-sectional images of the FITC-coupled resins viewed through a confocal microscope: (a) TentaGel resin (0.30 mmol NH_2/g); (b) HiCore resin (0.32 mmol NH_2/g).

As anticipated, when the HiCore resin coupled with 4-bromophenylacetic acid was treated with magnesium, no severe damage was observed on the resin beads. Subsequently, the reaction between **3** and chloromethylphosphonic dichloride followed by another solid-phase Grignard reaction with phenylmagnesium bromide were carried out to afford resin-bound phosphinoxide **4**.[7] After displacement with thioacetic acid in the presence of TEA, the resulting phosphinoxide was reduced by using trichlorosilane, and, finally the remaining acetyl group was removed by hydrazinolysis to furnish the target solid-supported phosphinothiol **5** (Scheme 1).

Scheme 1. Preparation of Solid-Supported Phosphinothiol **5**[a]



[a] Key: (a) 4-bromophenylacetic acid, BOP, DIPEA, NMP; (b) Mg, THF, reflux; (c) chloromethylphosphonic dichloride, THF, reflux; (d) PhMgBr, THF, reflux; (e) thioacetic acid, TEA, reflux; (f) $SiHCl_3$, THF, reflux; (g) $NH_2NH_2 \cdot H_2O$, DMF, 60 °C.

However, the final loading level (0.063 mmol/g) of the resin-bound phosphinothiol was substantially decreased, as compared to the initial loading level (0.31 mmol/g) of the amino group. This led us to search for a method of minimizing the matrix effect of the polymer backbone in

(6) Kim, H.; Cho, J. K.; Chung, W. J.; Lee, Y. S. *Org. Lett.* **2004**, *6*, 3273.

(7) To prevent undesired cross-linking, excess of chloromethylphosphonic dichloride (5 equiv) was used.

the magnesium insertion step (Scheme 1b)), which occurs between the two solid phases, i.e., magnesium and the resin. Therefore, spacers with four different lengths were introduced onto the HiCore resin by using ϵ-aminocaproic acid (E) and/or β-alanine (B), and the supported phosphinothiols were prepared on each spacer-attached resin. We found that the longest BEBE spacer exhibited the best result (the stepwise coupling yield for BEBE spacer was 92%, overall yield 65%; see Table 1).[8]

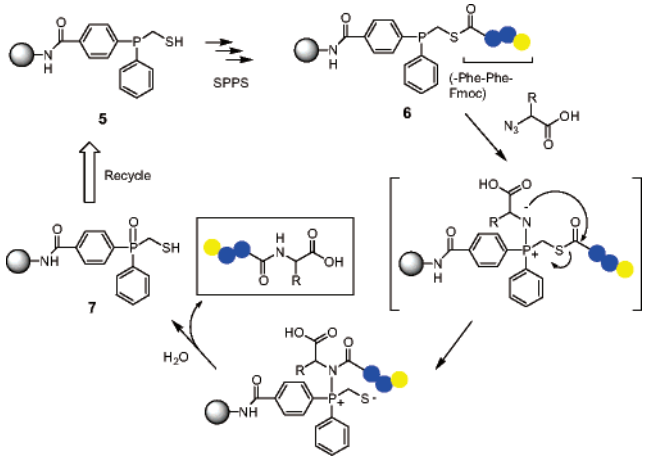
Table 1. Spacer Effect on Final Loading Level of Thiol Groups

spacer	no spacer	E	BE	EBE	BEBE
mmol of SH/g[a]	0.063	0.091	0.12	0.14	0.17

[a] The loading level of the thiol groups on resin was determined by trityl titration and confirmed by the Fmoc photometric test after the coupling of Fmoc amino acid.

As the other component for the Staudinger ligation, azido acids were synthesized using the diazo transfer reaction with triflyl azide.[9] Triflyl azide was prepared by the biphasic reaction of sodium azide and triflyl anhydride and then combined with 15 different amino acids, viz. Gly, Ala, Leu, Ile, Val, Phe, Asn, Gln, Asp(^{t}Bu), Ser(^{t}Bu), Thr(^{t}Bu), Met, Tyr(^{t}Bu), Lys(^{t}Boc), and His, respectively.[10] Solid-phase Staudinger ligation was performed with **5**. Initially, the dipeptide (Fmoc-Phe-Phe-OH) was assembled by solid-phase peptide synthesis on **5** (Fmoc-strategy). Then, solid-phase Staudinger ligations were performed between **5** and the synthetic azido acids (Scheme 2). To minimize the contamination of the released peptides, <1 equiv of azido acid was added to **6**, and the reaction mixture was stirred until azido acid was no longer observed on TLC.

Scheme 2. Strategy for Solid-Phase Staudinger Ligation Using Solid-Supported Phosphinothiol and Recycling of the Resin



All of the reactions were completed within 24−40 h in THF/H_2O (3:1) at rt, and the final tripeptides were obtained in quantitative yields. Regardless of the properties of the azido acids, the purities of the ligation products were excellent, thus dispensing for the need for any chromatography step (Figure 3a). Another noticeable advantage of the solid-phase Staudinger ligation is the reuse of the resin-bound phosphinothiol. The resulting resin-bound phosphine oxide **7** can be readily recovered from the ligation product by simple filtration and reduced with an excess of trichlorosilane. The loading levels of the thiol group of the regenerated resin **5** were equal to the original loading level. Resin **5** was recycled five times and showed consistent performance in terms of the ligation yield and the purity of the product (Figure 3b).

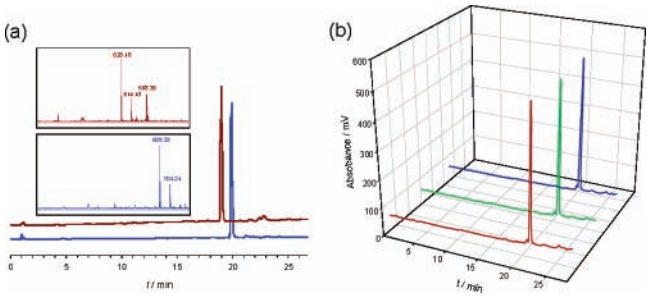


Figure 3. (a) HPLC traces and MALDI-TOF MS of representative crude products obtained by solid-phase Staudinger ligation: Fmoc-Phe-Phe-Ala-OH (red, *m/z* 628.48 $[M + Na]^+$, 644.43 $[M + K]^+$, 668.39 $[M - H + Na + K]^+$); Fmoc-Phe-Phe-Met-OH (blue, *m/z* 688.33 $[M + Na]^+$, 704.24 $[M + K]^+$); (b) HPLC profiles of the crude product (Fmoc-Phe-Phe-Ile-OH) obtained using regenerated resin-bound phosphinothiol: first reuse (red), second reuse (green), fifth reuse (blue).

In conclusion, we successfully performed the solid-phase Staudinger ligation on HiCore resin, which was recycled five times without any change in performance. Due to its physical and chemical stability, the backbone structure of the HiCore resin remained unchanged under harsh reaction conditions. Moreover, the surface localized functionalities allowed for the Staudinger ligation to be easily accomplished in the solid phase.

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Supporting Information Available: Experimental procedures for the preparation of triflyl azide, azido acids, solid supported phophinothiol, solid-phase Staudinger ligation of small peptides and their analytical data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) The rather low overall yield (65%) might be due to the proton exchange between the resin-bound Grignard reagent and the relatively acidic protons in the amide bonds of the spacer, which caused the phenylmagnesium bromide to decompose to phenyl group. However, this side reaction did not influence the subsequent reaction steps.

(9) For detailed experimental conditions for preparation of 15 azido acids and their characterization data, see the Supporting Information.

(10) Fmoc-Phe-Phe-OH was selected considering the sensitive detection on TLC and HPLC.