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Apply peptide C-terminal semicarbazides to peptide segment coupling using transfer active ester condensation technology

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Abstract—Peptide C-terminal semicarbazides are used as the starting materials in transfer active ester condensation technology to prepare HOCt active esters intermediates, which react with other peptide segments or reagents to afford long chain peptides, branch peptides and peptide C-terminal derivatives. The semicarbazido derivatives afford reliable results and avoid side reactions efficiently.

Transfer active ester condensation (TAEC) technology has been applied to the syntheses of long chain peptides, branch peptides as well as peptide C-terminal derivatives by using peptide hydrazide (peptide-$NHNH_2$) as the starting material. Besides maintaining the advantages of the traditional azide method (AM) with respect to the use of minimal side chain protection in convergent peptide synthesis, TAEC method has the advantages of high coupling yield, fast reaction rate and mild reaction condition.[1–3] By transfer of the hydrazide group (–$NHNH_2$) to the 1-hydroxy-7-azabenzotriazole (HOAt) or ethyl 1-hydroxy-1*H*-1,2,3-triazole-4-carboxylate (HOCt) active ester via an azide intermediate, TAEC method can circumvent the problems faced in AM, such as low reaction temperature, limitation on solvent choice, low yield and strong acid condition.[4] TAEC method is superior to the AM when applied to convergent synthesis of proteins.

When peptide-$NHNH_2$ was used as the starting material in TAEC, the produced active ester might react with the remaining –$NHNH_2$ to produce the unexpected side products in some instances. The main reason was that –$NHNH_2$ could be a nucleophile to attack the produced active ester. To avoid this side reaction, peptide C-terminal semicarbazide (peptide-$NHNHCONH_2$) was used as the starting materials in TAEC technology since the semicarbazido (–$NHNHCONH_2$) at the C-terminal of the peptide was much less nucleophilic.

The peptide-$NHNHCONH_2$ was synthesized with Fmoc solid phase peptide synthesis (SPPS) strategy (Scheme 1). Fmoc-OSu (1) was with semicarbazide hydrochloride to provide Fmoc-semicarbazide reagent 2 (Fmoc-$NHNHCONH_2$; MS: 298.12 $[M+1]^+$, MW: 297.31) under base condition. Intermediate 2 was treated with tricyclic linker-resin (3) under the condition of benzene/TsOH to prepare the Fmoc-NHNHCONH-tricyclic linker-NH-resin (4), which was used for the synthesis of the expected peptide C-terminal semicarbazide derivatives (7) by Fmoc SPPS method. The X-group in synthesized product (7) could be Fmoc-, Ac- or other protecting groups depending on the choice of the related amino acid derivatives at the last coupling step. Boc-group could be used as X since it would be stable when the synthetic peptide was cleaved from the tricyclic linker-resin under mild acid condition (5% TFA/DCM).[5] The results of the synthetic X-peptide-$NHNHCONH_2$ derivatives were summarized in Table 1.

Scheme 2 shows the TAEC process by using X-peptide-$NHNHCONH_2$ as the starting material. Compound 7 was converted to the HOCt active ester intermediate under HOCt/DMF/*t*-BuONO condition at room temperature, and then reacted with another reagent or peptide segment in DMF/DIEA to afford the target products (8a, 8b and 8c, see Table 2). As the key reagent in TAEC, HOCt served as an acid to facilitate the

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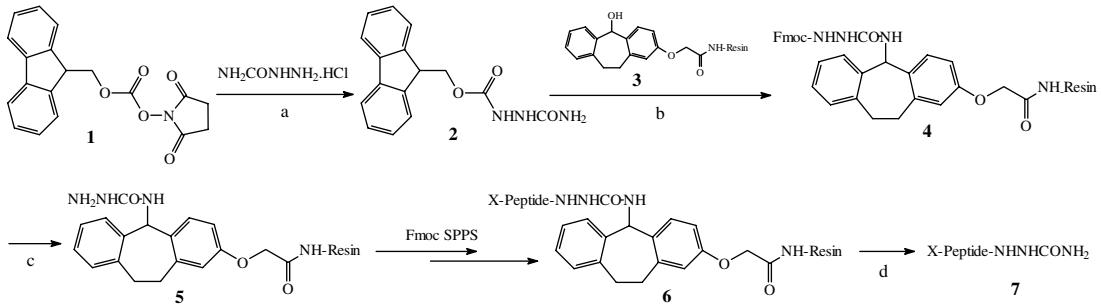
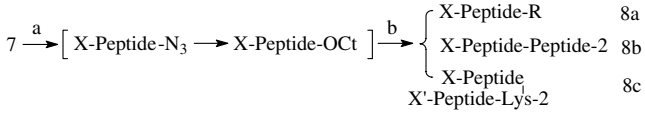


Scheme 1. Synthesis o f peptide C-terminal semicarbazide derivatives. Reagents: (a) DMF/DIEA; (b) Benzene/TsOH; (c) Piperidine/DMF; (d) TFA/DCM; (X could be Fmoc-, Ac- or other protecting group in 7).

Table 1. Synthetic peptide C-terminal semicarbazide derivatives in Scheme 1

Product	Sequence of the X-Peptide-$NHNHCONH_2$	HPLC (min)	Purity[a] (%)	MS $[M+1]^{+}$[b]	Calculated
7-1	Fmoc-Arg-Glu-Ile-$NHNHCONH_2$	19.2	95	696.3	696.35
7-2	Fmoc-Arg-Glu-Ile-Gly-Glu-Pro-Thr-Pro-Val-$NHNHCONH_2$	19.8	88	1276.6	1276.63
7-3	Fmoc-Leu-His-Leu-Val-Leu-Arg-Leu-Arg-Gly-Gly-$NHNHCONH_2$	23.2	92	1412.8	1412.83
7-4	Ac-Arg-Glu-Ile-Gly-Glu-Pro-Thr-Pro-$NHNHCONH_2$	15.8	86	997.5	997.53
7-5	Fmoc-Ala-Ala-Leu-Glu-Ser-Leu-Ile-Asn-Val-Ser-Gly-$NHNHCONH_2$	23.6	90	1352.4	1352.48
7-6	Fmoc-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-$NHNHCONH_2$	23.8	90	1236.6	1236.59

[a] HPLC analysis purity.
[b] FAB-MS was used to measure the MS of the products.



Scheme 2. TAEC process. Reagents and condition: a. HOCt/DMF/*t*-Bu-ONO/RT; b. DMF/DIEA/Y (8a. Y = Bzl-SH, MeO(Me)NH; 8b. Y = Peptide-2; 8c. Y = Peptide-Lys-2).

transformation of $-NHNHCONH_2$ to the azide group ($-N_3$), then replaced the $-N_3$ group to give the HOCt active ester intermediate (X-peptide-OCt), which was an efficient intermediate in the peptide segment coupling, and reliable for chiral product synthesis.[6,7] Low reaction temperature and strong acid such as hydrochloric acid used in the typical AM were not used in TAEC process, which provided a reliable and convenient way for peptide segment coupling under mild conditions. The side chain functional groups including hydroxyl (–OH), carboxyl (–COOH), imidazole (=NH) and guanidino [$-NHC(NH_2)=NH$] in both the peptide active ester and the added peptide segment (peptide-2) did not demand protection since the produced X-peptide-OCt could not react with these groups in TAEC process. But the amino ($-NH_2$) and thiol (–SH) groups should be protected in TAEC process.

When excess MeO(Me)NH or Bzl-SH were added into the reaction system, peptide C-terminal *N*-methyl-*N*-methoxy amides (8a-1 and 8a-2 in Table 2) and peptide thioester (8a-3) were produced in 82% (8a-1), 78% (8a-2) and 85% (8a-3) yields, respectively, in HPLC analysis. Peptide thioester was an important intermediate used in chemical ligation proteins synthesis;[8–14] peptide

Table 2. Segment coupling product using TAEC method

Product	Sequence of the produced peptide derivatives	HPLC (min)	Yield[a] (%)	MS $[M+1]^{+}$[b]	Calculated
8a-1	Fmoc-Arg-Glu-**Ile-N**$(CH_3)OCH_3$	21.6	82	682.4	682.36
8a-2	Fmoc-Arg-Glu-Ile-Gly-Glu-Pro-Thr-Pro-**Val-N**$(CH_3)OCH_3$	22.2	78	1262.6	1262.64
8a-3	Fmoc-Arg-Glu-Ile-Gly-Glu-Pro-Thr-Pro-**Val-S**$CH_2C_6H_6$	25.9	85	1325.6	1325.64
8b-1	Fmoc-Arg-Glu-Ile-Gly-Glu-Pro-Thr-Pro-**Val-Gly**-Glu-Ala-Pro-Asn-Ala-Leu-Leu-OH	21.4	68	1986.0	1986.01
8b-2	Fmoc-Glu-Ser-Thr-Leu-His-Leu-Val-Leu-Arg-**Leu-Val**-Gly-Glu-Ala-Pro-Asn-Ala-Leu-Leu-OH	20.3	65	2268.2	2268.25
8c-1	Ac-Arg-Glu-Ile-Gly-Glu-Pro-Thr-Pro Fmoc-Ala-Gly-Lys-Gln-Leu-Glu-Asp-Gly-OH	19.2	70	1961.9	1961.93
8c-2	Fmoc-Leu-His-Leu-Val-Leu-Arg-Leu-Arg-Gly-Gly Fmoc-Ala-Gly-Lys-Gln-Leu-Glu-Asp-Gly-OH	25.2	75	2377.3	2377.25

[a] Coupling product yield in HPLC analysis.
[b] FAB-MS was used to measure the MS of the products.

C-terminal *N*-methyl-*N*-methoxy amide was a valuable precursor for further synthesis of corresponding peptide aldehyde.[15,16] If other reagents such as R-OH, R-NH_2 and R_1R_2NH were used to react with X-peptide-OCt, peptide esters and peptide amides could be obtained.[3]

The X-peptide-OCt could couple with other peptide fragment (peptide-2) to produce long chain peptide derivatives. A peptide with a free $-NH_2$ at N-terminal could produce the linear product (8b-1 and 8b-2 in Table 2), in which X-peptide-OCt reacted with the free $-NH_2$ at the N-terminal. If the peptide segment contained only a free ε-NH_2 on the side chain of Lys, branch peptide (8c-1 and 8c-2) could be synthesized since the reaction occurred on the ε-NH_2 only.

Compared to peptide-$NHNH_2$, peptide-$NHNHCONH_2$ could also be used as the starting material in peptide segment coupling and special peptide C-terminal derivative preparation, while peptide-$NHNHCONH_2$ could avoid the side reactions that happened on $-NHNH_2$ group since the $-NHNHCONH_2$ group was much less nucleophilic. So peptide-$NHNHCONH_2$ was a type of valuable material for protein synthesis in TAEC technology.

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