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Transfer allyl esters to thioesters in solid phase condition: synthesis of peptide thioesters by Fmoc chemistry

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Abstract—A method to transfer allyl esters to thioesters under a solid phase condition has been developed to synthesize peptide thioesters. A Fmoc chemistry has been applied to synthesize the peptide allyl esters, which are selectively transferred to the expected peptide thioesters under solid phase synthesis conditions successfully.

Since developed by Kent and co-workers in 1994,[1] native chemical ligation method has been used to synthesize various polypeptides and proteins such as interleukin-8, OMTKY3, microbial ribonuclease barnase, active human type II secretory phospholipase A_2, lymphotactin and other peptides.[1–9] This method was also applied for the synthesis of polymer-modified erythropoiesis protein (SEP), a product with significant biological activities.[10] In all of these achievements, peptide thioesters were used as the key intermediates, which always played the important role in the native chemical ligation technology.

Preparation of peptide thioesters in solid phase peptide synthesis (SPPS) is restricted to *tert*-butoxycarbonyl (Boc) associated methods largely because peptide thioesters can be aminolysed by piperidine, a base, that is commonly used to remove 9-fluorenylmethoxycabonyl (Fmoc) group in Fmoc-associated SPPS methods.[8] To take the advantage of Fmoc SPPS such as mild de-protection and cleavage conditions, which are suitable for the preparation of acidic sensitive peptides including glycopeptides, sulfated and phosphorylated peptides,[11] it is always important and valuable to explore new methods to synthesize peptide thioesters using Fmoc SPPS.

Some useful methods have been developed to make peptide thioesters using Fmoc chemistry. For example, by using 3-carboxypropanesulfonamide as a safety-catch linker, peptide thioethers were prepared in the presence of Fmoc group.[12,13] Special cleavage reagents such as $AlMe_2Cl$/EtSH, $AlMe_3$/EtSH were available for the peptide thioesters synthesis in Fmoc chemistry.[14,15] Other methods including transfer active ester condensation (TAEC),[16] masked trithioortho esters,[17] backbone amide linker,[18] base sensitive Fmoc (2-F) groups replacing Fmoc group[19] and DBU/HOBt deblocking agent were also used for peptide thioester synthesis in Fmoc methodology.[20] All of these approaches showed their advantages and features, but also had their limitations such as not suitable for scale up synthesis, need of special reagents, resins or linkers.

In this letter, we wish to report a method to transfer allyl ester to thioester (TAETE) under a solid phase synthesis condition. Three trifunctional Fmoc amino acids with their –COOH group at the C-terminal protected by allyl (–All) group, for example, Fmoc–Lys–OAll, Fmoc–Glu–OAll and Fmoc–Asp–OAll (Fmoc–AA–OAll) were used as the key starting materials; the side chain functional groups in these Fmoc–AA–OAll were reacted with different resins and located as the C-terminal amino acids in the produced peptide thioesters that were synthesized by normal Fmoc method.

The details of TAETE were shown in Scheme 1. The side chain group (–NH_2 or –COOH) of the Fmoc–AA–OAll was linked to a resin, which could be 2-chlorotrityl chloride-Resin, Wang-Resin or *p*-NO_2–C_6H_4–OCOO–Wang-Resin. The produced Fmoc–AA(Y–Resin)–OAll was used for the peptide synthesis under normal Fmoc

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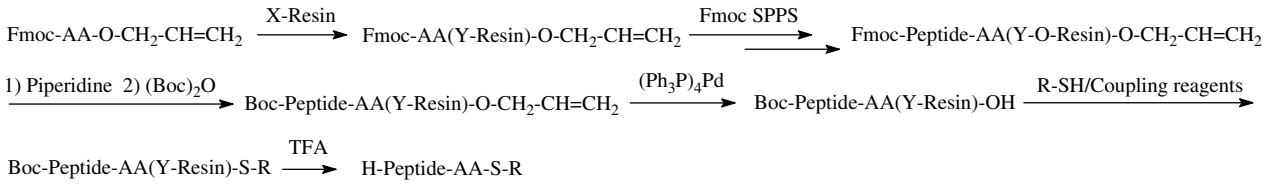

Scheme 1. The general route of TAETE. AA = Lys; Asp; Glu. X–Resin = 2-Chlorotrityl chloride-Resin; Wang-Resin; *p*-NO_2–C_6H_4–OCOO–Wang-Resin; Y–Resin = –CH_2COO–Wang-Resin; –CH_2CH_2COO–Wang-Resin; –$CH_2CH_2CH_2CH_2$NHCOO–Wang-Resin; –$CH_2CH_2CH_2CH_2$NH-2-Chlorotrityl-Resin. R = –$CH_2CH_2CO_2$Et; –$CH_2CH_2CO_2$Me; –$CHMe_2$.

Table 1. Products synthesized with Fmoc–Lys(2-chlorotrityl resin)–OAll

Product	Structure	HPLC (min)	MS (MW)
1	Fmoc–Ala-Phe-Ile-Lys–$OCH_2CH{=}CH_2$	18.50	741.0 (739.81)
2	Fmoc–Ala-Phe-Ile-Lys–OH	16.74	700.0 (699.83)
3	Fmoc–Ala-Phe-Ile-Lys–S–$(CH_2)_2COOCH_3$	18.62	803.0 (802.00)

SPPS conditions. –Y was the group that was produced by the side chain group in Fmoc–AA–OAll contacting with the linker on the resin. After the synthesis was completed and the N-terminal was protected with Boc group, the –All group at the peptide C-terminal was removed selectively with tetrakis (triphenylphosphine)-palladium(0) [$(Ph_3P)_4Pd$] to produce free –COOH group, which was transferred to thioester group by coupling with thio reagents under solid phase condition. The expected peptide thioesters could be obtained when the products were cleaved from the resin by TFA.

Fmoc–Lys–OAll was reacted with 2-chlorotrityl chloride resin in DMF/DIEA to produce Fmoc–Lys(2-chlorotrtyl resin)–OAll, which was used for the model peptide thioester synthesis under Fmoc solid phase conditions successfully. The expected product and intermediates were obtained and identified with HPLC and MS (Table 1). The racemization of the C-terminal amino acid in the peptide thioester was minimal and only 2.8% of D–H–Lys–OH was found in the final product. The yield of peptide thioester (product **3** in Table 1) was 92% since the transformations of the allyl ester to the thioester were carried out under SPPS conditions.

Different conditions were investigated to remove the –All group selectively and the results are compared. Even though $(Ph_3P)_4Pd/PhSiH_3$ condition has been used to remove –All group widely,[21] some impurities were generated in the model studies. $(Ph_3P)_4Pd/NHMe_2$. BH_3 are powerful reagents for the –All removal,[22] but the expect product could not be obtained in the model experiment. Under the condition of $(Ph_3P)_4Pd$/AcOH/*N*-methyl morpholine/DCM, the –All group could be removed selectively, and the yield of the expected product was almost quantitative.

Different thio reagents were used to react with Fmoc–Lys(2-chlorotrityl resin)–OAll under various coupling conditions, then the expected products were cleaved from the resin with TFA (Table 2). Even though the thioesters could be obtained under different conditions, HS–$(CH_2)_2CO_2$Et/PyBOP/DIEA/1 h was the best among others because HS–$CHMe_2$ required long reaction time, DIC did not afford a high yield, PyAOP produced Fmoc–Lys–pyrrolidine amide instead of the thioester except when 2,4,6-collidine was used to replace DIEA, and the yield was low in synthesis of large peptide thioester when 2,4,6-collidine was used.

Fmoc–Lys–OAll could also react with *p*-nitrophenyl carbonate-Wang-Resin in DMF/DIEA to produce Fmoc–Lys(CO–O–Wang-Resin)–OAll, which was available for peptide thioesters especially long sequence syntheses in Fmoc chemistry. The results of some products and their intermediates synthesized with this resin were summarized in Table 3, in which the yields of the peptide thioesters (product **6** and **9**) were 84% and 89%, respectively.

Both Fmoc–Glu–OAll and Fmoc–Asp–OAll could link with Wang resin under the conditions of methyllimidazole (MeIm)/1-(mesitylene-2-sulfonyl)-3-nitro-1*H*-1,2,4-

Table 2. Yields of Fmoc–Lys–S–R at different coupling conditions[a]

–R	–Ra[b]	–Rb[b]	–Ra[c]	–Rb[c]	–Rb	–Ra[c]	–Ra[c]
Coupling condition (h)	DIC/ DCM (1)	DIC/ DCM (1)	PyAOP/ B_2/DCM (1)	PyBOP/B_2/ DCM (1)	PyBOP/B_1/ DCM (1)	PyAOP/ B_2/DCM (3)	PyAOP/ B_2/CuCl[d] (2)
Yield (%)	46	31	48	88	96	52	44

[a] Products cleaved from Resin: Fmoc–Lys–S–$(CH_2)_2CO_2$Et (HPLC: 16.3 min MS: 485.0, MW: 484.61); Fmoc–Lys–S–$CHMe_2$ (HPLC: 16.7 min, MS: 427.0, MW: 426.57).

[b] The main impurity at these conditions is Fmoc–Lys–OH (HPLC: 12.6, MS: 369.0, MW: 368.4).

[c] The main impurity at these conditions is Fmoc–Lys–pyrrolidine amide (HPLC: 13.7 min, MS: 422.0, MW: 421.53).

[d] DMF–DCM (1:1) are the solvents; –Ra = –$CHMe_2$; –Rb = –$(CH_2)_2CO_2$Et; B_1 = DIEA; B_2 = 2,4,6-collidine.

Table 3. Products synthesized with Fmoc–Lys(CO–O–Wang-Resin)–OAll

Product	Peptide	HPLC (min)	MS (MW)
4	H–Ser-Pro-Tyr-Ser-Ser-Asp-Thr-Thr-Pro-Cys(Bu^t)^{10}-Cys(Bu^t)-Phe-Ala-Tyr-Ile-Ala-Arg-Pro-Leu-Pro^{20}-Arg-Ala-His-Ile-Lys-Glu-Tyr-Phe-Tyr-Thr^{30}-Ser-Gly-Lys–O–CH_2–CH=CH_2	14.6	3923 (3921.09)
5	H–Ser-Pro-Tyr-Ser-Ser-Asp-Thr-Thr-Pro-Cys(Bu^t)^{10}-Cys(Bu^t)-Phe-Ala-Tyr-Ile-Ala-Arg-Pro-Leu-Pro^{20}-Arg-Ala-His-Ile-Lys-Glu-Tyr-Phe-Tyr-Thr^{30}-Ser-Gly-Lys–OH	14.3	3882 (3881.03)
6	H–Ser-Pro-Tyr-Ser-Ser-Asp-Thr-Thr-Pro-Cys(Bu^t)^{10}-Cys(Bu^t)-Phe-Ala-Tyr-Ile-Ala-Arg-Pro-Leu-Pro^{20}-Arg-Ala-His-Ile-Lys-Glu-Tyr-Phe-Tyr-Thr^{30}-Ser-Gly-Lys–S–$C_2H_4CO_2Et$	14.8	3999 (3997.21)
7	H–Cys(Acm)-Pro-Leu-Gln-Leu-His-Val-Asp-Lys-Ala^{10}-Val-Ser-Gly-Leu-Arg-Ser-Leu-Thr-Thr-Leu^{20}-Leu-Arg-Ala-Leu-Gly-Ala-Gln-Lys–O–CH_2–CH=CH_2	15.5	3100 (3098.79)
8	H–Cys(Acm)-Pro-Leu-Gln-Leu-His-Val-Asp-Lys-Ala^{10}-Val-Ser-Gly-Leu-Arg-Ser-Leu-Thr-Thr-Leu^{20}-Leu-Arg-Ala-Leu-Gly-Ala-Gln-Lys–OH	14.83	3060 (3058.73)
9	H–Cys(Acm)-Pro-Leu-Gln-Leu-His-Val-Asp-Lys-Ala^{10}-Val-Ser-Gly-Leu-Arg-Ser-Leu-Thr-Thr-Leu^{20}-Leu-Arg-Ala-Leu-Gly-Ala-Gln-Lys–S–CH_2CH_2COOEt	16.08	3177 (3174.93)

triazole (MSNT)/DCM to produce Fmoc–Glu(O–Wang-Resin)–OAll and Fmoc–Asp(O–Wang-Resin)–OAll. These two resins were also suitable for peptide thioesters synthesis under Fmoc conditions. The yields of the peptide thioesters in Table 4 (product **12** and **15**) were 92% and 86%, respectively.

When Fmoc–Glu–OAll and Fmoc–Asp–OAll linked to Rink linker-Resin by using HBTU/DIEA/DMF as the coupling reagents, the produced resins, Fmoc–Glu-(Rink-linker-Resin)–OAll and Fmoc–Glu(Rink-linker-Resin)–OAll, both were available for the syntheses of peptide-Gln-thioester and peptide-Asn-thioester because amide group could be produced when the peptide was cleaved from the Rink linker-Resin.

In conclusion, by using Fmoc–Lys–OAll, Fmoc–Glu–OAll and Fmoc–Asp–OAll as the key starting materials, TAETE method has been established to synthesis peptide thioesters under Fmoc SPPS conditions. This method is available for the synthesis of peptide thioester with long amino acid sequence and takes the advantages of Fmoc SPPS. Further work on using this technology to synthesis proteins, branch and cyclic peptides will be reported in due course.

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Table 4. Products synthesized with Fmoc–Glu(Wang-Resin)–OAll and Fmoc–Asp(Wang resin)–OAll

Product	Peptide	HPLC (min)	MS (MW)
10	H–Cys(Acm)-Ile-Thr-Ala-Asp–O–CH_2–CH=CH_2	9.65	633.2 (632.77)
11	H–Cys(Acm)-Ile-Thr-Ala-Asp–OH	6.98	593.0 (591.68)
12	H–Cys(Acm)-Ile-Thr-Ala-Asp–S–CH_2–CH_2COOEt	11.22	709.0 (707.88)
13	H–Ala-Pro-Pro-Arg-Leu-Leu-Ile-Cys(Acm)-Asp-Ser^{10}-Arg-Val-Leu-Glu-Arg-Tyr-Leu-Leu-Glu-Ala^{20}-Lys-Glu-Ala-Glu-Lys-Ile-Thr-Thr-Gly-Cys(Acm)^{30}-Ala-Glu–O–CH_2–CH=CH_2	14.48	3658.5 (3658.3)
14	H–Ala-Pro-Pro-Arg-Leu-Leu-Ile-Cys(Acm)-Asp-Ser^{10}-Arg-Val-Leu-Glu-Arg-Tyr-Leu-Leu-Glu-Ala^{20}-Lys-Glu-Ala-Glu-Lys-Ile-Thr-Thr-Gly-Cys(Acm)^{30}-Ala-Glu–OH	12.93	3618.0 (3618.2)
15	H–Ala-Pro-Pro-Arg-Leu-Leu-Ile-Cys(Acm)-Asp-Ser^{10}-Arg-Val-Leu-Glu-Arg-Tyr-Leu-Leu-Glu-Ala^{20}-Lys-Glu-Ala-Glu-Lys-Ile-Thr-Thr-Gly-Cys(Acm)^{30}-Ala-Glu–S–$C_2H_4CO_2Et$	14.79	3734.0 (3734.4)

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