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Enabling N-to-C Ser/Thr Ligation for Convergent Protein Synthesis via Combining Chemical Ligation Approaches

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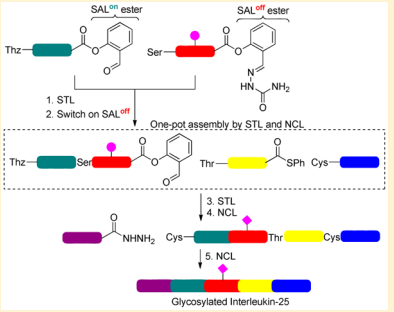
Supporting Information

ABSTRACT: In this article, Ser/Thr ligation$^{on/off}$ has been realized to enable N-to-C successive peptide ligations using a salicylaldehyde semicarbazone (SAL^{off}) group by in situ activation with pyruvic acid of the peptide SAL^{off} ester into the peptide salicylaldehyde (SAL^{on}) ester. In addition, a peptide with a C-terminal thioester and N-terminal Ser or Thr as the middle peptide segment can undergo one-pot Ser/Thr ligation and native chemical ligation in the N-to-C direction. The utility of this combined ligation strategy in the N-to-C direction has been showcased through the convergent assembly of a human cytokine protein sequence, GlcNAcylated interleukin-25.



INTRODUCTION

Effective methods and strategies in chemical ligation development have enabled many proteins to be synthetically accessible.[1,2] Peptide ligation usually relies on a chemoselective reaction to join two side-chain unprotected peptide segments.[1] Because of the poor solubility or aggregation problem of the peptide segments, one often needs to try to disconnect the target peptide at different sites, and the synthesis requires multiple ligations.[3] Generally, two strategies have been adopted for successive ligations: C-to-N direction ligation[4] and N-to-C direction ligation,[5] which are complementary and have been used in native chemical ligation (NCL) for chemical synthesis of complex proteins. C-to-N successive ligations utilize a temporary protecting group at the N-terminus. Thiazolidine (Thz) was first introduced to NCL effectively,[2b,4d,l] followed by other developments.[3,4] To realize N-to-C successive ligations is more challenging, as intramolecular cyclization tends to be faster than the intermolecular ligation.[5c,f,g] To this end, Kent's kinetic NCL,[5c] Liu's hydrazide-based NCL,[5o,v−x,z,ab] Melnyk's SEA-based NCL,[5l,u] and Bode's KAHA ligation[4n,o,5ag] have been developed.

Ser/Thr ligation (STL) was introduced into protein chemical synthesis as an alternative ligation strategy (Scheme 1a).[6] STL involves merger of a side-chain unprotected peptide segment containing a C-terminal salicylaldehyde (SAL) ester and another peptide segment with an N-terminal Ser or Thr residue. The chemoselective reaction between the peptide SAL ester and 1,2-hydroxylamine group of Ser or Thr leads to the formation of an *N,O*-benzylidene acetal linked intermediate, which undergoes acidolysis to afford a natural peptidic Xaa-Ser/Thr linkage. This method does not involve the use of unnatural amino acids, and it is simple to operate. Ser/Thr ligation provides a complementary method for protein chemical synthesis and semisynthesis.[6b−g] However, the successive assembly of peptide fragments in the N-to-C direction in STL has not been realized to date. In this report, we introduce the STL$^{on/off}$ concept to enable N-to-C multiple ligations. In addition, we demonstrate that this novel N-to-C STL can be combined with NCL for the assembly of three peptide segments in a one-pot fashion for convergent protein synthesis.

RESULTS AND DISCUSSION

Synthetic Design. To realize N-to-C Ser/Thr ligation, the C-terminal SAL ester of the peptide fragment B has to be temporarily masked by a functional moiety to become a peptide SAL^{off} ester (Scheme 1b). Peptide SAL^{off} ester must ideally be inert during the first ligation to avoid oligomerization or cyclization of fragment B. Activation, ideally simple, of the peptide SAL^{off} ester into the corresponding peptide SAL^{on} ester subsequently allows the second ligation with the third segment, Ser/Thr-Peptide fragment C-*CO*-R^2 (Scheme 1b, STL 2). We conjectured that a functional moiety capable of temporarily masking the aldehyde group in the C-terminal SAL^{on} ester of the peptide fragment B during the ligation and readily being removed after the ligation to regenerate SAL^{on} ester would be the key to implement this STL$^{on/off}$ concept. The central challenge is to find the temporary aldehyde-masking group to enable reversible activation and deactivation of the peptide SAL ester between SAL^{on} and SAL^{off}. However, to identify such an orthogonal masking group is not trivial. The functional moiety has to fulfill the following criteria: (1) the group needs to be stable under the TFA conditions employed during global deprotection to generate side-chain unprotected peptides; (2) it has to be stable under the STL conditions and resistant to side

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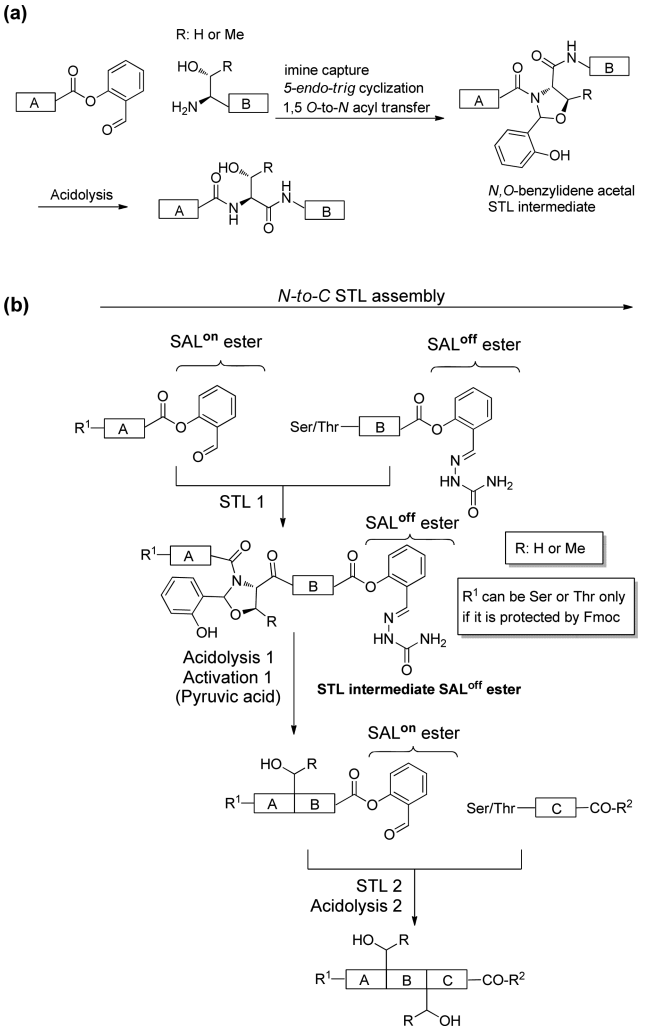
Scheme 1. (a) Principle of Ser/Thr Ligation. (b) Synthetic Logic in Developing N-to-C Ser/Thr Ligation[a]

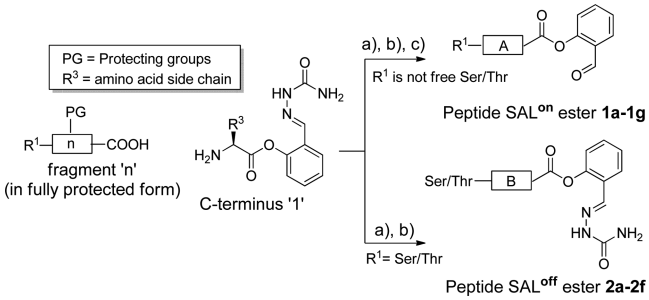


[a]The first step is a Ser/Thr ligation between peptide SAL^{on} ester fragment A and peptide fragment B, during which the semicarbazone (SAL^{off}) acts as a masked aldehyde group. Activation of SAL^{off} into SAL^{on} by in situ addition of pyruvic acid during acidolysis allows tandem ligation to be performed with the Ser/Thr fragment C, where R^2 can be either SAL^{off}, OH, or SR^3.

reactions, such as hydrolysis and cyclization; (3) the activation should be conducted under nonbasic, nonoxidative, and nonreductive conditions to regenerate the aldehyde group easily and mildly. From our screening, we eventually identified that a semicarbazide could be employed to mask the aldehyde group of the peptide SAL ester, the resultant peptide SAL semicarbazone ester is stable under TFA conditions and inert under the STL condition to act as SAL^{off} ester, and more importantly, the aldehyde group of this SAL^{off} ester can be restored to become an SAL^{on} ester under acidic conditions in the presence of pyruvic acid (Scheme 1b).[7]

Peptide SAL^{on} and SAL^{off} Ester Synthesis by 'n+1' Strategy. To devise a practical synthesis of both side-chain-unprotected peptide SAL^{on} and peptide SAL^{off} esters for N-to-C STL, here we introduced the operationally simple 'n+1' strategy under the Sakakibara conditions,[2f,8] which have been used extensively in generating peptide thioesters. Under these conditions, the epimerization at the C-terminal amino acids during the coupling was not observed.[9] The optimized process is as follows: first, the peptide fragment n in its protected form was assembled through Fmoc-SPPS using 2-chlorotrityl chloride resin and cleaved under the mild condition of CH_2Cl_2/AcOH/trifluoroethanol (TFE) in the side-chain protected form. The C-terminus '1' was obtained through coupling between a Boc amino acid (*BocHN*-Xaa-*COOH*) and salicylaldehyde semicarbazone followed by HCl-mediated Boc removal during which the semicarbazone moiety was intact. The 'n+1' reaction was carried out between the 'n' and '1' using EDC and HOOBt as coupling reagents in $CHCl_3$/TFE.[2f,8] Global deprotection by TFA gave the side-chain unprotected peptide SAL^{off} esters in 25−88% yield after RP-HPLC purification, while with the addition of pyruvic acid to the global deprotection condition, side-chain unprotected peptide SAL^{on} esters were obtained. Using this method, a series of model peptide SAL^{off} and SAL^{on} esters were successfully obtained (Table 1). We reasoned the success of the

Table 1. Peptide SAL^{on} Esters 1a−g and SAL^{off} Esters 2a−f Synthesis Using 'n+1' Strategy and Yields[a]



entry	fragment n	C-ter 1	no. of aa	product	yield (%)
1	*BocHN*-VIGGVGN-*COOH*	A	8	**1a**	88
2	*BocHN*-VIGGVGN-*COOH*	Y	8	**1b**	69
3	*BocHN*-VIGGVGN-*COOH*	V	8	**1c**	70
4	*BocHN*-VIGGVGN-*COOH*	S	8	**1d**	83
5	*FmocHN*-SEHDKTA-*COOH*	Y	8	**1e**	39
6	*BocHN*-ZIGGVGN-*COOH*	Y	8	**1f**	25
7	*FmocHN*-SARKLLQDI-*COOH*	M	10	**1g**	31
8	*BocHN*-TLHAPTD-*COOH*	Y	8	**2a**	61
9	*BocHN*-TLHAPTD-*COOH*	S	8	**2b**	72
10	*BocHN*-TLHAPTD-*COOH*	A	8	**2c**	66
11	*BocHN*-TLHAPTD-*COOH*	V	8	**2d**	69
12	*BocHN*-TNSYRKVLGQ-*COOH*	L	11	**2e**	49
13	*BocHN*-SARKLLQDI-*COOH*	M	10	**2f**	47

[a]Conditions: (a) n+1 strategy: fragment n (1.0 equiv), C-terminus 1 (3.0 equiv), EDC (3.0 equiv), HOOBt (3.0 equiv), $CHCl_3$/TFE, 15 mM, rt, 3 h; (b) 95% TFA/5% H_2O, rt, 1−2 h; (c) regeneration of the aldehyde: 100.0 equiv of pyruvic acid, rt, 3 h. Z = Thz.

regeneration of aldehyde was due to the formation of the more stable pyruvic acid semicarbazone under the acidic condition, which drove the hydrolysis equilibrium of SAL^{off} semicarbazone to the SAL^{on} side.[7d] The epimerization issue at the C-terminal amino acids during the 'n+1' coupling was studied by comparing the coupling reaction product H_2N-VIGGVGNA-*CO*-SAL^{off} ester and H_2N-VIGGVG*n*A-*CO*-SAL^{off} ester obtained, respectively, from epimeric H_2N-VIGGVGN-*COOH* and H_2N-VIGGVG*n*-*COOH* (*n* is Asn in the D-configuration) as the 'n' fragments. No epimerization at the C-

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terminal Asn site during the 'n+1' coupling was observed (Figure 1).

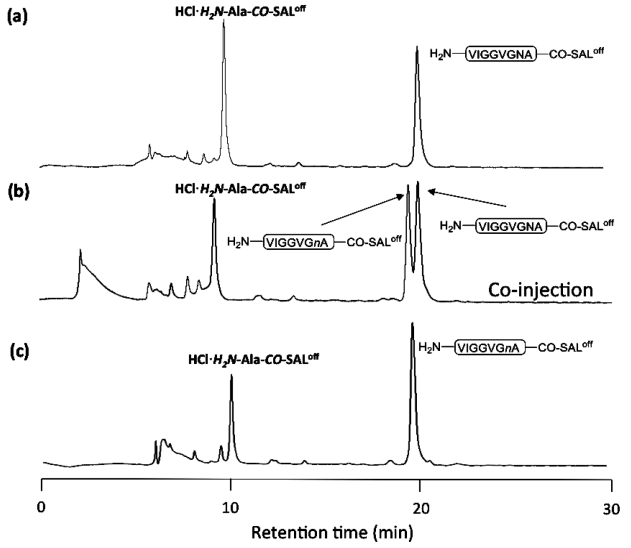


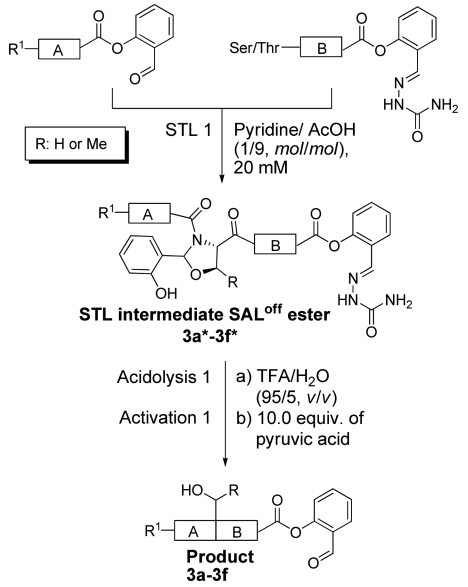
Figure 1. Study of epimerization issue in the 'n+1' process. (a, c) HPLC trace of the crude peptide SAL^{off} esters, H_2N-VIGGVGNA-*CO*-SAL^{off} and H_2N-VIGGVG*n*A-*CO*-SAL^{off}, prepared from two epimeric peptides H_2N-VIGGVGN-*COOH* and H_2N-VIGGVG*n*-*COOH*, respectively. (b) Co-injection of the two SAL^{off} esters. *n* is Asn in the D-configuration.

N-to-C STL Study. With the successful methodology in hand, we were now poised to investigate the extension of the peptide SAL^{off} ester on the application of N-to-C STL (Table 2). To this end, we studied the ligation using random-sequence model peptide fragment A-SAL^{on} esters **1a−f** and peptide fragment B-SAL^{off} esters **2a−d** (Scheme 1b, STL 1). Ligation between peptide fragment A-SAL^{on} esters and peptide fragment B-SAL^{off} esters in pyridine/acetic acid buffer was left to proceed for 2−9 h to give peptide fragment A+B STL intermediate SAL^{off} esters **3a*−f***. LCMS results suggested that the ligation proceeded smoothly without any side reactions, such as cyclization or polymerization. To perform tandem ligations, the ligation intermediates were directly subjected to one-pot acidolysis to generate natural peptide linkage and switch on the SAL^{off} esters to give SAL^{on} esters **3a−f** by addition of pyruvic acid.

Next, we continued to investigate the N-to-C convergent ligation involving more than three segments (Scheme 1b, STL 2). Peptide fragment C-SAL^{off} ester **2c** was subjected to ligation with peptide SAL^{on} ester **3a** (Figure 2). A clean formation of the target ligation intermediate was observed within 4 h. Then the intermediate was subjected to acidolysis, and the SAL^{off} ester was switched on to yield SAL^{on} ester **4a**. Peptide SAL^{on} ester **4a** could undergo convergent STL with a Ser/Thr peptide generated by traditional C-to-N STL or this N-to-C STL. In this example, peptide SAL^{on} ester **3e** was ligated with peptide **5**, which upon Fmoc removal afforded peptide **4b**. With peptides **4a** and **4b** in hand, the convergent STL was examined and the ligation between **4a** and **4b** proceeded cleanly under STL condition to generate the product peptide **6** after acidolysis and RP-HPLC purification.

One-Pot STL and NCL. Having demonstrated the success in the convergent STL enabled by the N-to-C STL, we

Table 2. STL between Peptide Fragment A-SAL^{on} Esters 1a−f and Peptide Fragment B-SAL^{off} Esters 2a−d in the N-to-C Direction To Generate Peptide Fragment A+B-SAL^{on} Esters 3a−f and Yields



entry	peptide fragment A	peptide fragment B	product	sequence of product	yield (%)
1	**1a**	**2a**	**3a**	VIGGVGNATLHAPTDY	39
2	**1b**	**2b**	**3b**	VIGGVGNYTLHAPTDS	30
3	**1c**	**2c**	**3c**	VIGGVGNVTLHAPTDA	34
4	**1d**	**2d**	**3d**	VIGGVGNSTLHAPTDV	42
5	**1e**	**2b**	**3e**	*FmocHN*-SEHDKTAYTLHAPTDS	62
6	**1f**	**2b**	**3f**	ZIGGVGNYTLHAPTDS	47

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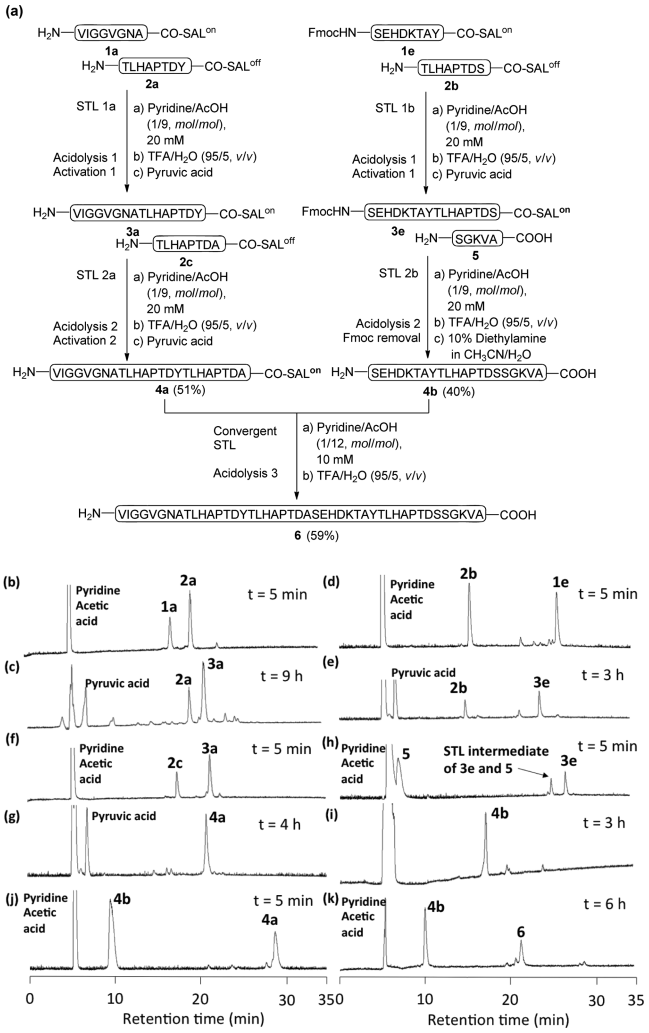

Figure 2. Convergent STL between model peptide SAL^{on} esters and Ser peptides. (a) Production of both model peptide SAL^{on} ester and Ser peptide by N-to-C STL strategy and the convergent STL. STL 1a: **1a** (1.0 equiv) and **2a** (1.5 equiv). STL 2a: **3a** (1.0 equiv) and **2c** (1.0 equiv). STL 1b: **1e** (1.0 equiv) and **2b** (1.5 equiv). STL 2b: **3e** (1.0 equiv) and **5** (2.0 equiv). Convergent STL: **4a** (1.0 equiv) and **4b** (1.5 equiv). (b) HPLC trace for the STL 1a between **1a** and **2a** at t = 5 min. (c) HPLC trace for the STL 1a between **1a** and **2a** at t = 9 h. (d) HPLC trace for the STL 1b between **1e** and **2b** at t = 5 min. (e) HPLC trace for the STL 1b between **1e** and **2b** at t = 3 h. (f) HPLC trace for the STL 2a between **3a** and **2c** at t = 5 min (g) HPLC trace for the STL 2a between **3a** and **2c** at t = 4 h. (h) HPLC trace for the STL 2b between **3e** and **5** at t = 5 min. (i) HPLC trace for the STL 2b between **3e** and **5** at t = 3 h (Fmoc removed product **4b**). (j) HPLC trace for the convergent STL between **4a** and **4b** at t = 5 min. (k) HPLC trace for the convergent STL between **4a** and **4b** at t = 6 h.

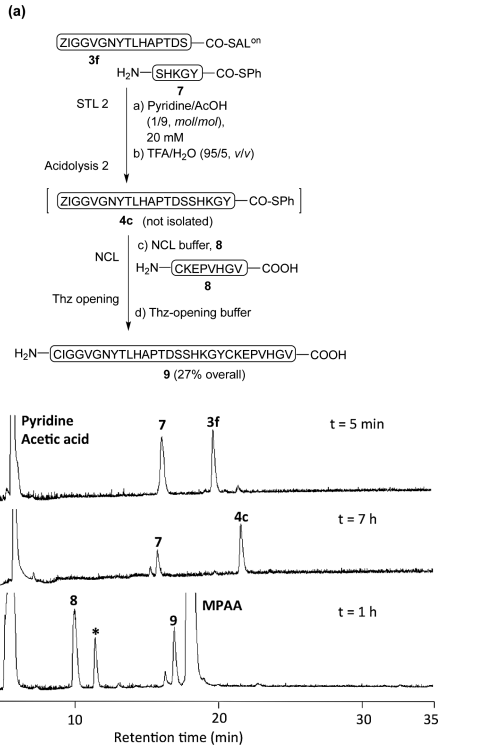

Figure 3. (a) One-pot three-segment STL and NCL. (b) HPLC trace for the STL 2 between **3f** and **7** (t = 5 min). (c) HPLC trace for the STL 2 between **3f** and **7** (t = 7 h). (d) HPLC trace for the NCL between **4c** and **8** (t = 1 h) followed by Thz opening. *: product between excess **7** and **8**. NCL buffer: guanidine·HCl (6 M), Na_2HPO_4 (0.2 M), tris(2-carboxyethyl)phosphine hydrochloride (TCEP·HCl) (0.02 M), 4-mercaptophenylacetic acid (MPAA) (0.2 M), pH 6.8−7.2. Thz-opening buffer: $MeONH_2$·HCl (0.3 M), TCEP·HCl (0.5 M), pH 4.0−4.5.

expanded our interest in one-pot ligation with STL and NCL. We hypothesized that the STL inert peptide SAL^{off} ester could be replaced by a thioester and remained stable during STL (Scheme 1b, STL 2, R^2 = SPh) due to the acidic conditions in STL with pyridine acetic acid buffer (pH 3.8−6.2).[10] To ascertain whether the thioester is compatible and does not pose any side effect to STL, a model Ser peptide thioester **7** was allowed to ligate in pyridine acetic acid buffer with the N-terminal Thz peptide SAL^{on} ester **3f** (Figure 3a). Notably, LCMS showed that the ligation proceeded cleanly and generated the corresponding peptide thioester **4c** in 7 h after acidolysis. To further probe whether this method could be applied to generate an N-terminal Cys peptide following NCL in one-pot manner, the peptide thioester **4c** was not isolated. Instead, an N-terminal Cys peptide **8** in phosphate buffer was added to the crude peptide thioester. LCMS analysis showed that NCL between **4c** and **8** underwent smoothly and completed within 1 h to give the desired product (Figure 3b−d). In situ conversion of Thz to Cys by treatment with methoxyamine hydrochloride yielded the N-terminal Cys peptide **9**. We have demonstrated that the development of the N-to-C STL strategy has opened the door to perform one-pot, three-segment STL and NCL. These strategies enable the two ligation methods to be performed in the N-to-C direction owing to the compatibility of the peptide thioester with STL conditions and the ability to activate the SAL^{off} group in situ with pyruvic acid after the first ligation.

Synthetic Application to Interleukin-25. With these successes in hand, we continued to explore the application of STL$^{on/off}$ in the convergent protein chemical synthesis. We chose cytokine human interleukin (IL)-25 (also known as IL-17E) to demonstrate the ability of the use of both Ser/Thr- and Cys-mediated chemical ligations to streamline the synthesis of an *N*-linked glycoprotein. This synthetic study could also serve as a proof of concept for the feasibility of the successive N-to-C ligation with combined STL and NCL approaches on protein level. IL-25 is a distinct member of the IL-17 cytokine family which induces IL-4, IL-5, and IL-13 expression and promotes pathogenic T helper 2 cell responses in various organs.[11] A

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study has shown that IL-25 is able to cause apoptosis of IL-25R, expressing breast cancer cells without toxicity to nonmalignant cells.[12] This discovery renders IL-25 a promising target for the development of new antitumor drugs for breast cancer. The ability to gain access to fully synthetic IL-25 with defined glycan structures would allow a better understanding on the binding of this glycoprotein to its receptor. Processed human IL-25 contains 145 amino acids with 10 cysteines, four of which form two disulfide bonds and an *N*-linked glycan at Asn104 (Figure 4).[13]

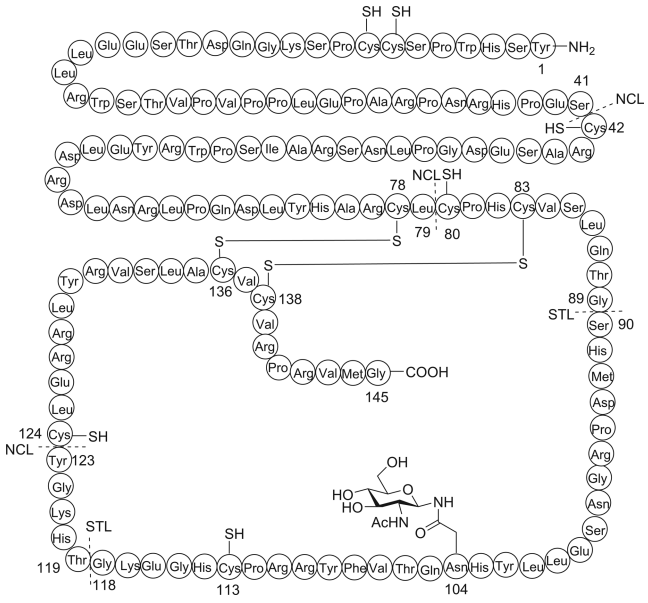

Figure 4. Structure of interleukin (IL)-25. The dash line shows the disconnection site for ligations.

We performed its synthesis by using convergent strategy between the left-hand fragment IL-25 (1−79) and the right-hand fragment IL-25 (80−145), which were based on the consideration of manageable sequence length. IL-25 (1−79) could be readily obtained through NCL between IL-25 (1−41) and IL-25 (42−79), while the synthetic strategy for the glycosylated IL-25 (80−145) required careful design. Of the six Cys residues on the IL-25 (80−145), two of them are the first and fourth amino acid at the N-terminal (Cys80 and Cys83), while the remaining four (i.e., Cys113, Cys124, Cys136, and Cys138) are located at the C-terminal region, which are potential sites for performing NCL. However, Cys113 and Cys138 are next to Val and Pro, respectively, which are sterically hindered amino acid residues leading to poor ligation efficiency.[1f,k] Thus, of the four potential sites for NCL, only Cys124 and Cys136 are considerable in terms of manageable peptide length and ligation efficiency. In addition, there is a paucity of Cys or Ala (for the NCL-desulfurization approach) residue for disconnection in the vicinity of the glycosylation site, Asn104.

First, we attempted the synthesis of IL-25 (80−145) through NCL between IL-25 (80−123) and IL-25 (124−145). However, the 44-amino acid long IL-25 (80−123) glycopeptide fragment was extremely difficult to be prepared under the standard Fmoc-SPPS conditions, leading to a messy mixture of unidentified truncated products. Thus, NCL alone is not sufficient for the synthesis of IL-25 protein.

Our second attempt was to employ C-to-N STL at Gly118-Thr119 and Gly89-Ser90 to shorten the glycopeptide fragment in an attempt to overcome the problematic SPPS we encountered. However, the synthesis of IL-25 (80−145) was still unsuccessful due to the extremely low solubility of the C-terminal region, IL-25 (119−145). The low solubility of IL-25(119−145) made the purification very difficult in less than 0.5% yield.

In the end, we envisioned that the success of this synthesis requires the C-terminal region of IL-25 (119−145) to be further fragmentized to increase its solubility and ligation efficiency. Additional disconnection site Tyr123-Cys124 was made on the basis of the preliminary result in the success of the synthesis of IL-25 (124−145). We conjectured that ligations might be performed in the N-to-C direction to circumvent the occurrence of the low solubility sequence IL-25 (119−145).

With the modified strategy, the synthesis of IL-25 (80−145) began with the syntheses of the requisite fragments via Fmoc-SPPS strategy, including N-terminal Thz peptide SAL^{on} ester IL-25 (80−89) (**12**), N-terminal Ser glycopeptide SAL^{off} ester IL-25 (90−118) (**13**), N-terminal Thr peptide thioester IL-25 (119−123) (**15**),[2f,8] and Cys peptide IL-25 (124−145) (**16**) (see Figure 5a and the SI). Peptide **12** (1.5 equiv) was first ligated with glycopeptide SAL^{off} ester **13** (1.0 equiv) in pyridine acetic acid buffer (5 mM, 1/6, mol/mol) for 6 h. Following the completion of the ligation as monitored by LCMS, the STL intermediate SAL^{off} ester was treated with TFA and pyruvic acid to generate the native amide bond to afford the peptide SAL^{on} ester IL-25 (80−118) (**14**) in 40% yield after RP-HPLC purification (Figure 5b). Compound **14** then underwent another STL with peptide thioester **15** (1.5 equiv) under STL conditions (5 mM, pyridine/acetic acid, 1/6, mol/mol). The ligation finished in 4 h, and the peptide thioester **17** was successfully generated after acidolysis (Figure 5c). Without any purification steps, TFA was blown off under a stream of condensed air followed by the addition of **16** (3.0 equiv) in phosphate buffer to the crude reaction mixture containing **17**. Careful LCMS analysis suggested that the NCL between **16** and **17** was completed within 2 h to afford the desired ligated product. No side reaction was observed except for the formation of ligated product of **15** and **16** due to the excess amount of **15** present in the reaction mixture. Finally, Thz group was removed using methoxyamine in one pot to generate IL-25 (80−145) **18** in 20% yield after RP-HPLC purification. Subsequently, **18** was treated with 10% aqueous hydrazine in the presence of mercaptoethanol and dithiothreitol leading to the complete removal of the acetate groups on GlcNAc to give **19** in 51% yield after purification. With this N-to-C ligation strategy, we successfully synthesized the right-hand fragment **19** and overcame the problem of the low solubility sequence at the C-terminus.

The synthesis of fragment **20** adopted the peptide hydrazide method.[5w] Peptide hydrazides **10** and **11** were generated by Fmoc-SPPS on hydrazine 2-chlorotrityl chloride resin. **10** in phosphate buffer was first converted to a thioester by in situ $NaNO_2$ activation and thiolysis, and then reacted with **11** to afford **20** at pH 7.0 within 6 h (Figure 5d). After purification, peptide hydrazide **20** and the glycopeptide **19** were subjected to convergent NCL. LCMS analysis suggested a full-length glycosylated IL-25 (**21**) was generated in 16 h (Figure 5e). The linear **21** bearing *N*-linked GlcNAc was purified by RP-HPLC to give homogeneous material confirmed by LCMS and SDS-PAGE (Figure 5f,g). This accomplishment marked the first

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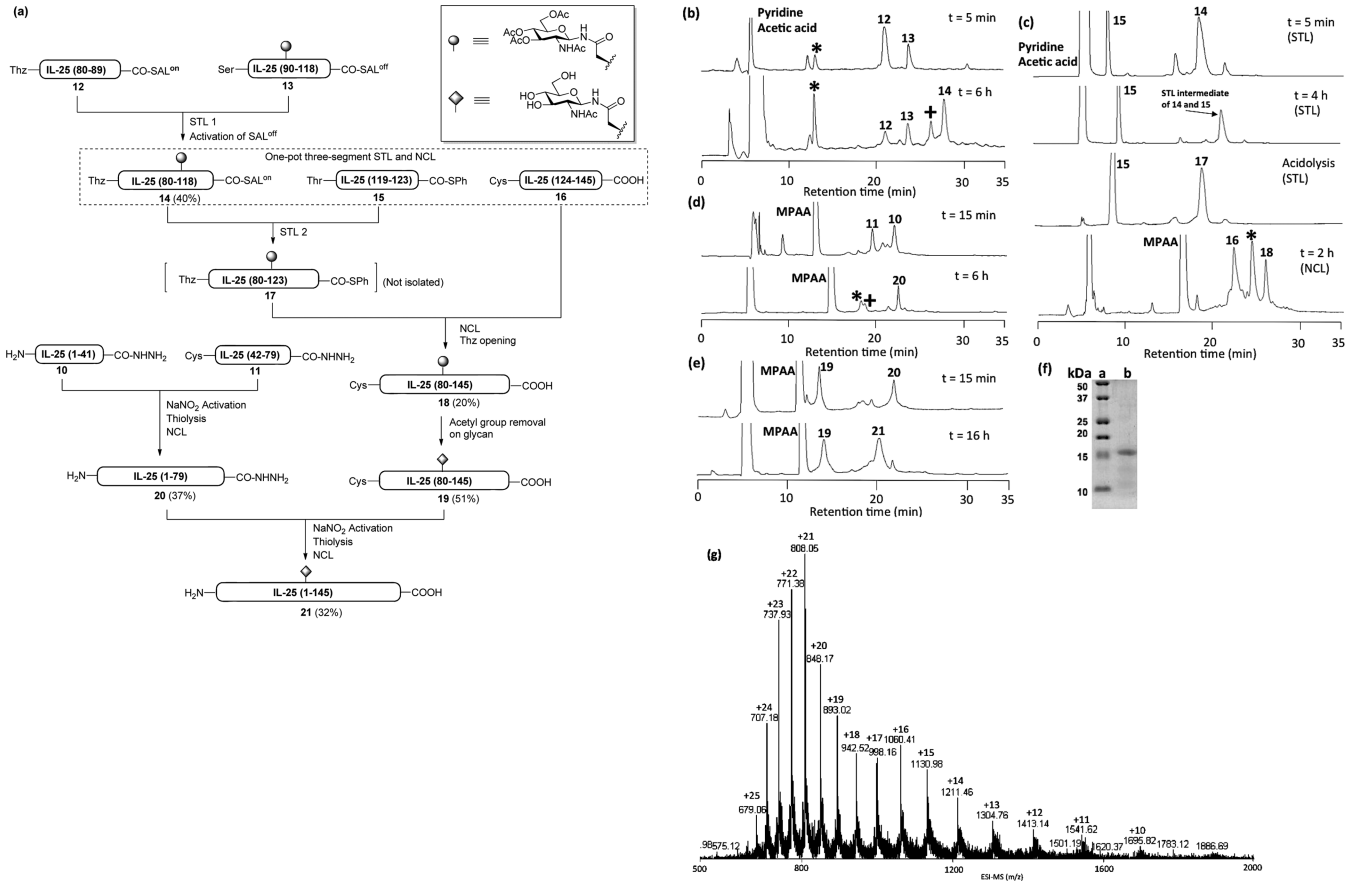

Figure 5. Convergent synthesis of the glycosylated IL-25 using N-to-C Ser/Thr ligation and one-pot three-segment combined ligations method. (a) Synthetic route. (b) HPLC trace for the STL 1 between **12** and **13** (t = 5 min and 6 h). *: hydrolysis of **12**. +: Oxidation of Met92 of **14.** (c) One-pot, three-segment ligations between **14** and **15** (STL, t = 5 min and 4 h) and **16** (NCL, t = 2 h). *: product between excess **15** and **16**. (d) HPLC trace for the NCL between **10** and **11** (t = 15 min and 6 h). **10**: in the form of MPAA ester. *: unreacted **10** with all S^{t}Bu removed from Cys. +: hydrolysis of **10** with all S^{t}Bu removed from Cys. (e) HPLC trace for the NCL between **19** and **20** (t = 15 min and 16 h). **19**: in the form of MPAA ester. (f) SDS-PAGE analysis. Lane a: marker. Lane b: the synthetic IL-25 **21**. (g) ESI-MS spectrum of **21**.

synthesis of fully deprotected IL-25 with a GlcNAc installed at Asn104 for further installation of complex glycan by enzymatic approach. Although the current work has only involved GlcNAc as a representative *N*-linked model glycan, this demonstration of feasibility through the modular glycoform assembly presented herein opens the door to the application of this strategy to the synthesis of a library of homogeneous glycosylated IL-25 via chemical combined ligation strategy. The obtained full-length processed IL-25 (**21**) was subjected to several oxidative folding buffer to obtain the three-dimensional structure involving the formation of two disulfide bonds Cys78-Cys136 and Cys83-Cys138 out of the 10 Cys residues. Unfortunately, under all of the folding conditions we attempted,[16] no desired folded IL-25 was observed, likely due to the cysteine disulfide mis-formation. The failed folding of the synthetic IL-25 suggests a future synthetic route requiring orthogonal cysteine protection.

CONCLUSION

In summary, new methods that enable N-to-C successive peptides ligations have been developed that include N-to-C multiple STL ligations via modulating STL$^{on/off}$ and one-pot STL−NCL tandem ligation. A key feature of the new STL$^{on/off}$ strategy is to make use of an STL−inert SAL semicarbazone ester which could be removed to generate the aldehyde bearing SAL ester after STL. In addition, a new and operationally simple method to prepare the peptide salicylaldehyde ester has been developed. These strategies have been utilized to accomplish the synthesis of a full-length glycosylated interleukin-25. We believe the compatibility of STL and NCL will provide a versatile approach for the future convergent synthesis of proteins of many kinds.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.6b04238.

Experimental procedures; spectral and other characterization data (PDF)

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Notes

The authors declare no competing financial interest.

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