

Cysteine-Free Peptide and Glycopeptide Ligation by Direct Aminolysis**

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Native chemical ligation (NCL) is an extremely useful technique for the synthesis of peptide and protein targets.^[1,2] The method, which relies on the chemoselective condensation of a C-terminal peptide thioester with a peptide containing an N-terminal cysteine residue to afford a native peptide bond, has been implemented successfully in the total synthesis of hundreds of proteins to date.^[3] Furthermore, NCL has also been applied effectively to the synthesis of glycoproteins.^[4] Although NCL has proved to be an extremely powerful technique, certain limitations still exist. The most obvious is the requirement for a cysteine residue at the ligation junction. Alanine^[5] and phenylalanine^[6] disconnections were realized recently by the use of desulfurization techniques and can therefore be added to the NCL repertoire.^[7] The combined abundance of cysteine, alanine, and phenylalanine in protein sequences is still relatively low, and as such, there is a high probability that a particular target protein does not contain one of these amino acids at a synthetically viable position. An alternative strategy has been the development of cysteine-free ligation techniques, whereby a thiol-containing auxiliary is incorporated at the N terminus.^[8,9] These methods have proven useful for the synthesis of glycopeptides;^[10,11] however, the use of such auxiliaries appears to be limited to ligation sites with amino acid side chains of low steric bulk.

Recently, we reported an alternative peptide-ligation strategy for the synthesis of glycopeptides: sugar-assisted ligation (SAL). This method utilizes a glycopeptide in which the carbohydrate is derivatized with a mercaptoacetate auxiliary.^[12,13] While investigating the mechanism of SAL, we discovered that ligation reactions could proceed in an intermolecular fashion between an N-terminal amine of a glycopeptide and a C-terminal peptide thioester in the absence of the thiol auxiliary, albeit at a lower rate and with significant quantities of hydrolyzed thioester.^[14] This observation suggests that the previously reported ligation reactions

may proceed by a direct aminolysis pathway in conjunction with the intramolecular cyclization mechanism proposed previously.

The direct coupling of a C-activated peptide with a peptide containing a free amine was reported by Kemp and co-workers in the 1970s.^[15,16] In these studies, activated C-terminal peptide esters (for example, *N*-ethylsalicylamide and *p*-nitrophenyl esters) were used to couple peptides efficiently in the absence of an N-terminal cysteine residue, a thiol auxiliary, or exogenous activating reagents. Reactions were conducted in dimethyl sulfoxide or *N,N*-dimethylformamide (DMF) and gave ligation products in high yields. The limited solubility and potential racemization of peptides under these conditions have prevented the exploitation of this method. Clearly, a method that could overcome these drawbacks would prove extremely useful for the synthesis of peptides and glycopeptides that can not be otherwise synthesized by NCL or SAL owing to the lack of a suitable ligation junction.

In 1981, Blake reported a strategy based on silver-ion mediation to facilitate peptide ligations via peptide thioacids.^[17] The procedure was modified by Aimoto and co-workers, who used a thioester as the acyl donor.^[18,19] The latter method exploits the reactivity of a peptide thioester, which, in the presence of silver(I) and a suitable activating agent, such as 1-hydroxy-1*H*-benzotriazole (HOBt) or 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (HOObt), reacts with the N-terminal amine of a peptide to afford the desired ligation product.^[19] Recently, Danishefsky and co-workers reported an extension of this method, whereby the thioester was masked as a protected *o*-thiol-containing phenolic ester.^[20] Unfortunately, the reaction conditions caused epimerization of the C-terminal thioester residue, and therefore only non-epimerizable amino acids could be incorporated at this position (glycine or proline). Additionally, in contrast to NCL, the method lacked chemoselectivity in the presence of cysteine and lysine residues, which required protection with acetamidomethyl (Acm) and 1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl (ivDde) groups, respectively. We were therefore interested in pursuing a general cysteine- and auxiliary-free ligation strategy for the construction of peptides and glycopeptides without the use of activating agents. The strategy should be applicable to a range of peptide thioesters without causing epimerization of the C-terminal residue. We believed that this goal was possible through the use of suitable buffer conditions to facilitate the direct aminolysis of a peptide and a C-terminal peptide thioester (Scheme 1).

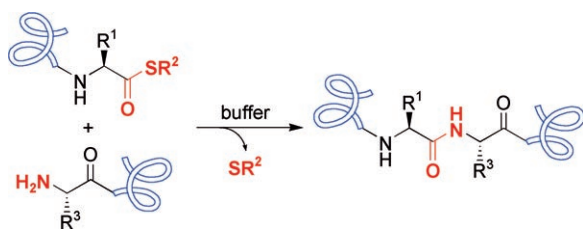
In initial studies, we investigated the direct coupling of a model peptide thioester **1** containing a C-terminal glycine

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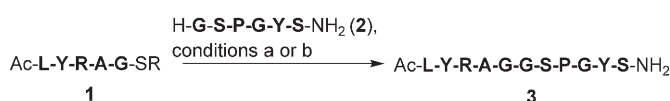
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Supporting information for this article is available on the WWW under <http://www.angewandte.org> or from the author.



Scheme 1. Proposed cysteine-free ligation reaction (R^1 and R^3 are amino acid side chain functionalities).

residue with a peptide **2** bearing an N-terminal glycine residue by using the standard NCL buffer (conditions a, Scheme 2).^[2,3] Under these conditions, we observed the formation of the desired product **3**; however, the yield was unacceptably low (40%) as a result of competing thioester hydrolysis in the aqueous media.



Scheme 2. Ligation of **1** and **2** under different conditions: a) **2** (14 mM), **1** (9 mM), Gn·HCl (6 M), potassium phosphate (0.1 M), pH 8.5, PhSH (2% v/v), 37°C, 48 h (40%); b) **2** (14 mM), **1** (9 mM), 4:1 v/v NMP/buffer: Gn·HCl (6 M), HEPES (1 M), pH 8.5, PhSH (2% v/v), 37°C, final pH value: 7.5, 48 h (89%). R = (CH₂)₂CONH₂; Gn·HCl = guanidine hydrochloride, HEPES = 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid, NMP = N-methylpyrrolidinone.

To suppress hydrolysis and increase the ligation yield, we investigated a range of different solvent conditions. A general strategy to prevent hydrolysis is the addition of cosolvents. We examined various cosolvents in combination with a number of suitable buffers. As the reaction relies on direct attack at the thioester by a deprotonated amine, the buffer should operate at pH 7.0–8.5. Buffers that contain nucleophilic amines (e.g. tris(hydroxymethyl)aminomethane (Tris)) could not be used owing to competing reactivity (see the Supporting Information). Commonly used buffers that fulfill these criteria include potassium phosphate, HEPES, and imidazole, each of which was mixed with guanidine hydrochloride (Gn·HCl) as the denaturant. N-Methylpyrrolidinone (NMP) was chosen as a suitable cosolvent. The buffers were incubated in the presence of Ac-LYR- AA^1 -S(CH₂)₂CONH₂, which contains a C-terminal alanine residue, so that any hydrolysis and epimerization instigated by the buffer could be assessed (see the Supporting Information). The use of potassium phosphate, HEPES, and imidazole in combination with 6 M Gn·HCl in the absence of an organic cosolvent caused 80–100% hydrolysis of the thioester in 48 h; thus, these conditions were not suitable for the cysteine-free ligation reaction (see Figure S1 in the Supporting Information). A 4:1 v/v mixture of NMP and HEPES or imidazole buffer drastically suppressed hydrolysis of the thioester (<15% after 48 h) with no detectable epimerization of the C-terminal alanine residue (see Figure S1 in the Supporting Informa-

tion). We selected the following ligation buffer: 4:1 v/v NMP: 6 M Gn·HCl, 1 M HEPES, pH 8.5, as it displayed the most suitable buffering range for the direct aminolysis reaction and had been used successfully to suppress the hydrolysis of peptide thioesters in previous ligation studies.^[14] Under these conditions, the ligation reaction between the peptide thioester **1** and peptide **2** reached completion within 48 h with minimal thioester hydrolysis, and the desired product **3** was isolated in 89% yield (Scheme 2 and Table 1, entry 1).

Table 1: Scope of the cysteine-free aminolysis reaction (R = (CH₂)₂CONH₂).

H-AA ² -S-P-G-Y-S-NH ₂ 4:1 v/v NMP: buffer ^[a] , 2% vol. PhSH, 37 °C, final pH = 7.3–7.6 Ac-L-Y-R-A-AA ¹ -SR → Ac-L-Y-R-A-AA ¹ -AA ² -X-P-G-Y-S-NH ₂				
Entry	Peptide thioester AA ¹	Peptide AA ²	(Glycosyl) amino acid X	Yield [%] ^[b]
1 ^[c]	Gly	Gly	Ser	89
2 ^[c]	Ala	Gly	Ser	quant.
3 ^[c]	His	Gly	Ser	quant.
4 ^[d]	Gly	His	Ser	57
5 ^[d]	Ala	His	Ser	40
6 ^[d]	His	His	Ser	41
7 ^[d]	Gly	Ala	Ser	66
8 ^[d]	Ala	Ala	Ser	54
9 ^[d]	His	Ala	Ser	37
10 ^[c]	Gly	Asp	Ser	quant.
11 ^[c]	His	Asp	Ser	89
12 ^[c]	Gly	Glu	Ser	quant.
13 ^[c]	Ala	Glu	Ser	quant.
14 ^[c]	His	Glu	Ser	90
15 ^[d]	Gly	Tyr	Ser	39
16 ^[d]	Ala	Tyr	Ser	34
17 ^[d]	His	Tyr	Ser	12
18 ^[c]	Gly	Gly	Ser(β-GlcNAc)	98
19 ^[c]	Gly	Gly	Thr(α-GalNAc)	65

[a] Buffer: Gn·HCl (6 M), HEPES (1 M), pH 8.5. [b] Yield of the isolated product. [c] Reaction time: 48 h. [d] Reaction time: 96 h.

To study the efficiency of this cysteine-free ligation reaction in the presence of amino acids other than glycine, peptides with a range of N-terminal residues (Gly, His, Ala, Asp, Glu, and Tyr) and peptide thioesters with a range of C-terminal residues (Gly, Ala, and His) were synthesized by solid-phase peptide synthesis (SPPS; see the Supporting Information). These peptides were ligated in the mixed-solvent system (Table 1). The results show a general trend, whereby the N-terminal amino acid of the peptide appears to be more influential than the substitution of the C-terminal thioester component on the yield of the aminolysis reaction. When glycine was the N-terminal amino acid (Table 1, entries 1–3), the ligation reactions were very efficient and ligation products were obtained in quantitative yield for peptide thioesters containing a C-terminal alanine or histidine residue. The reaction yields were also excellent (89–100%) for the ligation of peptides with N-terminal aspartic acid and

glutamic acid residues (Table 1, entries 10–14). The incorporation of an N-terminal alanine residue resulted in diminished ligation yields (Table 1, entries 7–9). Peptides containing sterically demanding N-terminal residues were still able to undergo direct aminolysis reactions in satisfactory yields. Specifically, a peptide with an N-terminal histidine residue reacted with a range of thioesters to give the desired products in 40–57% yield (Table 1, entries 4–6), whereas lower yields were observed when a peptide with an N-terminal tyrosine residue was used (entries 15–17). All reactions were carried out at a final pH value of 7.3–7.6 (as a result of the acidity of the peptides, which were prepared as trifluoroacetate salts) and appeared to be free of epimerization. The ligation products were obtained with intact stereochemical integrity, as deduced from the presence of a single peak in their HPLC trace under analytical conditions suitable for the resolution of epimerized peptides.

Having demonstrated the efficiency of the direct aminolysis reaction for the synthesis of peptides, we next investigated the ligation of unprotected glycopeptides. Ligation reactions between thioester **1** and glycopeptides containing Ser- β -GlcNAc and Thr- α -GalNAc gave the desired ligation products in good yields (Table 1, entries 18 and 19). Thus, the direct aminolysis reactions also appear to be efficient when glycans are present on the peptide backbone.

The presence of internal cysteine residues is known to be problematic in peptide ligation reactions as a result of the reversible formation of unproductive thioesters and thiolactones and consequent lowering of the reaction rate.^[21] The current strategy employed to overcome this issue relies on the protection of internal cysteine residues with an Acm group.^[5,20–22] We were interested in assessing the efficiency of our ligation reaction in the presence of unprotected internal cysteine residues to streamline synthetic efforts and prevent additional protection and deprotection steps in the synthetic sequence. To this end, the six hexameric peptides **4–9** were synthesized by SPPS (see the Supporting Information). These constructs contain cysteine residues at all possible positions along the backbone. Peptide **4** has an N-terminal cysteine residue, which was expected to undergo an NCL reaction with a peptide thioester, whereas peptide **9** contains a cysteine residue at the C terminus (Table 2).

Peptides **4–9** were treated with the peptide thioester **1**, which contains a C-terminal glycine residue, in the mixed-

solvent system. With the exception of peptide **7**, the ligation reactions proceeded in good yields (69–93%) which were comparable with the yield observed in the NCL reaction of **4** (94%; Table 2, entry 1). We performed kinetic studies to gain insight into the relative rates of these ligation reactions in the presence and absence of internal cysteine residues (see Figure S2 in the Supporting Information). These studies showed that although NCL is faster than direct aminolysis, reactions still proceeded at synthetically useful rates. Furthermore, these rates suggest that ligation reactions proposed previously to occur via large-ring (> 21-membered-ring) SAL transition states may actually proceed through direct intermolecular aminolysis.^[14]

Having established that the ligation tolerates the incorporation of unprotected cysteine residues, we synthesized a series of peptides based on **8** with N-terminal glycine, histidine, alanine, aspartic acid, and glutamic acid residues (see the Supporting Information). These peptides were ligated under the mixed-solvent conditions with peptide thioesters containing C-terminal glycine, alanine, and histidine residues (see Table S1 in the Supporting Information for ligation reactions and yields). These reactions followed similar general trends and afforded the products in comparable yields to those described in Table 1. Thus, the presence of unprotected cysteine residues does not have a marked effect on ligation efficiency.

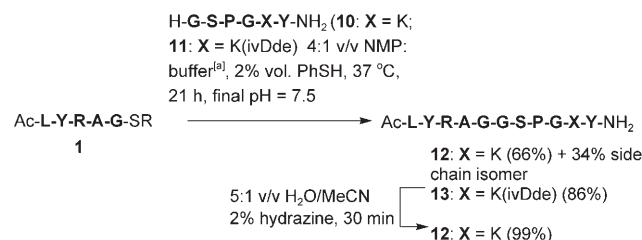
As a result of the lower reaction rate displayed by the cysteine-free ligation relative to that of NCL, we anticipated chemoselectivity issues in the presence of the free ϵ -amino side chain of lysine. To establish whether this concern was founded, we synthesized peptide **10** with an internal lysine residue (see the Supporting Information). The treatment of this peptide with peptide thioester **1**, which contains a C-terminal glycine residue, gave a 2:1 regioisomeric mixture of the desired ligation product **12** (66%) and the undesired product with an acylated lysine side chain (34%; Scheme 3). Attempts to bias the formation of the desired ligation product by modifying the pH value of the buffer and the concentration of the reaction mixture were unsuccessful. In a similar manner to that described by Danishefsky and co-workers,^[20] we used the ivDde group for orthogonal protection of the ϵ -amino functionality. The lysine(ivDde)-containing peptide **11** was ligated to the peptide thioester **1** in 86% yield, and the ivDde protecting group was subsequently removed in high yield by hydrazinolysis.

Owing to our interest in the synthesis of glycopeptides and glycoproteins, our vision for this method extended to the

Table 2: Ligation of peptides containing cysteine residues ($R = (CH_2)_2CONH_2$).^[a]

$Ac-L-Y-R-A-G-SR \xrightarrow{4-9} Ac-L-Y-R-A-G-peptide$ 1		
Entry	Peptide	Yield [%] ^[b]
1 ^[c]	H- C SPGYS-NH ₂ (4)	94
2 ^[c]	H- G PCGYS-NH ₂ (5)	69
3 ^[c]	H- G SCGYS-NH ₂ (6)	93
4 ^[d]	H- G SPCYS-NH ₂ (7)	44
5 ^[c]	H- G SPG C Y-NH ₂ (8)	84
6 ^[c]	H- G SPG C Y-NH ₂ (9)	87

[a] Reaction conditions: 4:1 v/v NMP/buffer: Gn-HCl (6 M), HEPES (1 M), pH 8.5, PhSH (2% v/v), 37°C, final pH value: 7.3–7.6. [b] Yield of the isolated product. [c] Reaction time: 48 h. [d] Reaction time: 96 h.

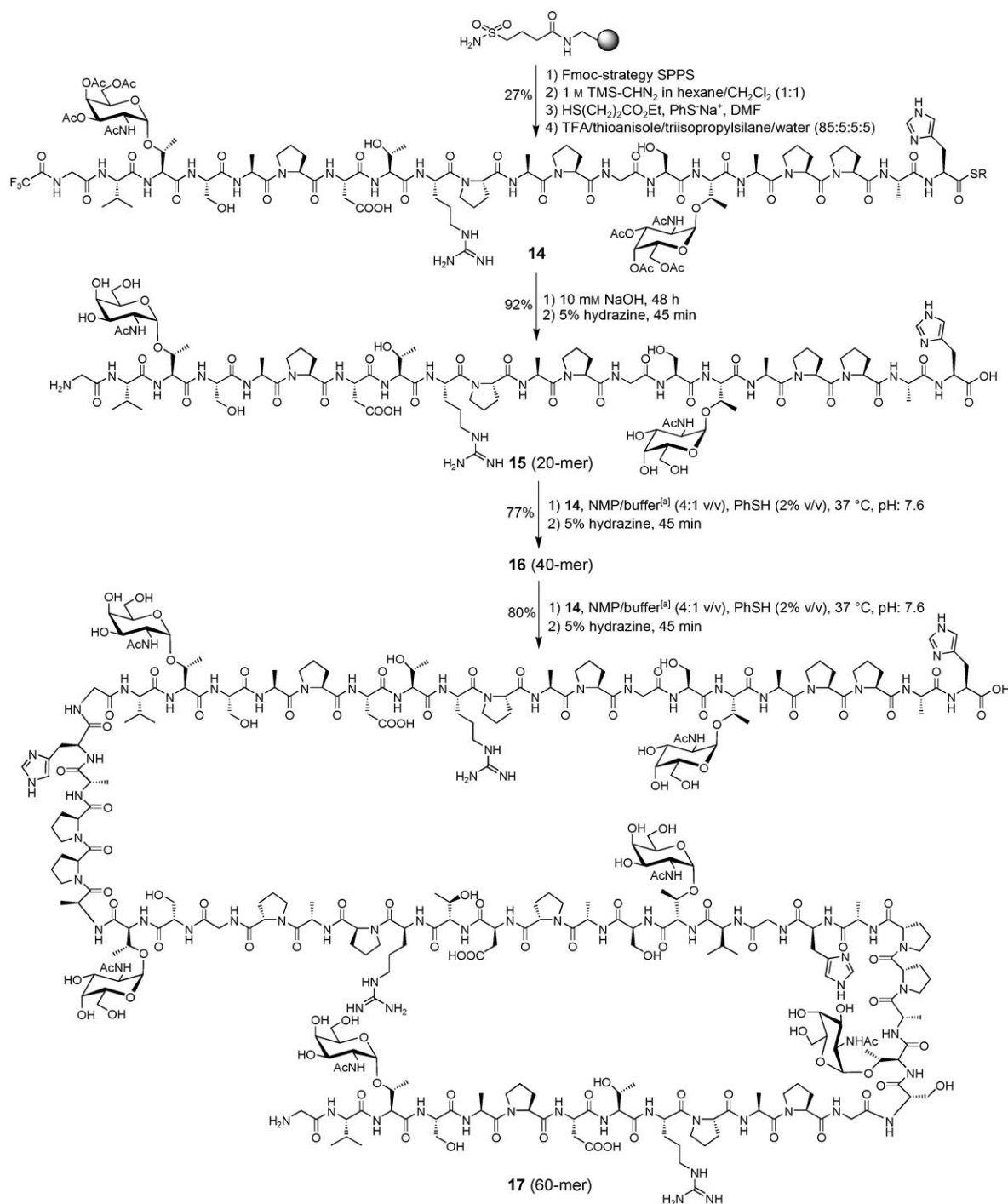


Scheme 3. Chemoselectivity of the cysteine-free aminolysis reaction in the presence of an internal lysine residue. [a] Buffer: Gn-HCl (6 M), HEPES (1 M), pH 8.5. $R = (CH_2)_2CONH_2$.

synthesis of a homogeneous glycopeptide of biological relevance. We chose to synthesize a native trimeric 60-mer section of the cancer-associated MUC1 tandem-repeat glycoprotein. MUC1 is a heavily *O*-glycosylated glycoprotein that is present at the interface between epithelial cells and their extracellular matrix.^[23] The extracellular domain consists of tandem repeating units comprising twenty amino acid residues. In cancer, MUC1 is overexpressed and aberrantly glycosylated with truncated glycans, resulting from down-regulation of glycosyltransferases. Therefore, specific incor-

poration of cancer-associated glycans into the MUC1 repeating unit is recognized as a promising target for production of immunostimulating antigens and the development of cancer vaccines.^[24,25]

As an initial demonstration, the T_N antigen (α -GalNAc) was attached to two threonine residues within the 20-mer repeat unit. The key glycopeptide thioester fragment **14** was synthesized on sulfamylbutyryl resin by using an activation and thiol-release strategy (see the Supporting Information).^[4] Trifluoroacetamide-protected glycine was coupled as the



Scheme 4. Synthesis of a 60-mer MUC1 repeat glycopeptide by application of the cysteine-free aminolysis. [a] Buffer: Gn-HCl (6 M), HEPES (1 M), pH 8.5. R = (CH₂)₂CO₂Et; Fmoc = 9-fluorenylmethoxycarbonyl, TFA = trifluoroacetic acid, TMS = trimethylsilyl.

N-terminal amino acid to facilitate sequential fragment condensations under the direct aminolysis conditions without generating cyclization and polymerization by-products. After complete assembly on the resin, activation of the sulfonamide linkage with trimethylsilyldiazomethane^[26] and release from the solid support upon treatment with ethyl-3-mercaptopropionate gave the desired 20-mer diglycopeptide thioester **14** in 27% yield (see the Supporting Information). The treatment of **14** with dilute aqueous NaOH for 48 h followed by brief hydrazinolysis gave the fully deprotected diglycopeptide **15** (92% yield), which was ligated with the protected glycopeptide thioester **14** under the conditions for direct aminolysis (Scheme 4). After 60 h, the reaction had reached completion (as determined by LC-MS), and the ligation mixture was treated with 5% hydrazine to deacetylate the glycans and remove the N-terminal trifluoroacetamide group. After purification by HPLC, the desired dimeric 4.6-kDa tetraglycopeptide of MUC1 was isolated in 77% yield. The MUC1 dimer **16** was submitted to a subsequent ligation with the 20-mer glycopeptide thioester **14** to afford, after hydrazinolysis and HPLC purification, the trimeric 6.9-kDa MUC1 repeat hexaglycopeptide **17** (1.2 mg) in 80% yield, as verified by analytical HPLC, LC-MS, and MALDI-TOF mass spectrometry. The efficiency with which these large glycopeptide fragments can be constructed by the direct aminolysis method demonstrates clearly its potential for the production of complex peptides and even proteins, with or without post-translational modifications.

In summary, we have described the discovery of an effective new method for the assembly of peptides and glycopeptides by direct aminolysis in the absence of an N-terminal auxiliary or cysteine residue. Ligation reactions are performed in a mixed-solvent system containing *N*-methylpyrrolidinone and guanidine hydrochloride/HEPES buffer. This medium allows reactions to proceed at reasonable rates with minimal hydrolysis of the thioester component. The ligation products were isolated in good yields, and the reactions proceeded without epimerization. The method proved to be general, as demonstrated by the use of a wide range of amino acid residues at the ligation junction. Furthermore, the method is chemoselective in the presence of all naturally occurring side chains, with the exception of lysine, which must be protected.

This method clearly shows potential for application in the synthesis of a host of native (glyco)peptide and (glyco)protein targets that can not be constructed by other currently available ligation techniques. Additionally, the buffer system may prove useful in preventing thioester hydrolysis in other ligation reactions (e.g. NCL). To demonstrate its value for the production of more-complex targets, the method was implemented successfully in the synthesis of a 6.9-kDa section of the cancer-associated MUC1 glycoprotein, which contains six glycosylation sites. Current efforts in our laboratory are focused on the use of the direct aminolysis reaction for the construction of more-complex MUC1 glycopeptides by incorporating other cancer-associated glycans, such as the Thomsen-Friedenreich antigen (T antigen), sialyl T, and GloboH. Furthermore, we are attempting to synthesize

larger oligomeric MUC1 constructs by extending the iterative ligation strategy reported here, with the intention of producing new immunogenic glycopeptides for the production of immunostimulating antigens. It is hoped that these constructs may ultimately aid in the development of cancer vaccines.

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- [1] T. Wieland, E. Bokelmann, L. Bauer, H. U. Lang, H. Lau, *Justus Liebigs Ann. Chem.* **1953**, 583, 129–149.
- [2] P. E. Dawson, T. W. Muir, I. Clarklewis, S. B. H. Kent, *Science* **1994**, 266, 776–779.
- [3] P. E. Dawson, S. B. H. Kent, *Annu. Rev. Biochem.* **2000**, 69, 923–960.
- [4] Y. Shin, K. A. Winans, B. J. Backes, S. B. H. Kent, J. A. Ellman, C. R. Bertozzi, *J. Am. Chem. Soc.* **1999**, 121, 11684–11689.
- [5] B. L. Pentelute, S. B. H. Kent, *Org. Lett.* **2007**, 9, 687–690.
- [6] D. Crich, A. Banerjee, *J. Am. Chem. Soc.* **2007**, 129, 10064–10065.
- [7] Other cysteine residues in the peptide or protein must be protected when these desulfurization methods are used.
- [8] J. Offer, C. N. C. Boddy, P. E. Dawson, *J. Am. Chem. Soc.* **2002**, 124, 4642–4646.
- [9] D. W. Low, M. G. Hill, M. R. Carrasco, S. B. H. Kent, P. Botti, *Proc. Natl. Acad. Sci. USA* **2001**, 98, 6554–6559.
- [10] D. Macmillan, D. W. Anderson, *Org. Lett.* **2004**, 6, 4659–4662.
- [11] B. Wu, J. H. Chen, J. D. Warren, G. Chen, Z. H. Hua, S. J. Danishefsky, *Angew. Chem.* **2006**, 118, 4222–4231; *Angew. Chem. Int. Ed.* **2006**, 45, 4116–4125.
- [12] A. Brik, Y. Y. Yang, S. Ficht, C. H. Wong, *J. Am. Chem. Soc.* **2006**, 128, 5626–5627.
- [13] A. Brik, S. Ficht, Y. Y. Yang, C. S. Bennett, C. H. Wong, *J. Am. Chem. Soc.* **2006**, 128, 15026–15033.
- [14] S. Ficht, R. J. Payne, A. Brik, C. H. Wong, *Angew. Chem.* **2007**, 119, 6079–6083; *Angew. Chem. Int. Ed.* **2007**, 46, 5975–5979.
- [15] D. S. Kemp, Z. W. Bernstein, G. N. McNeil, *J. Org. Chem.* **1974**, 39, 2831–2835.
- [16] D. S. Kemp, S. L. H. Choong, J. Pekaar, *J. Org. Chem.* **1974**, 39, 3841–3847.
- [17] J. Blake, *Int. J. Pept. Protein Res.* **1981**, 17, 273–274.
- [18] S. Aimoto, N. Mizoguchi, H. Hojo, S. Yoshimura, *Bull. Chem. Soc. Jpn.* **1989**, 62, 524–531.
- [19] S. Aimoto, *Biopolymers* **1999**, 51, 247–265.
- [20] G. Chen, Q. Wan, Z. Tan, C. Kan, Z. Hua, K. Ranganathan, S. J. Danishefsky, *Angew. Chem.* **2007**, 119, 7527–7531; *Angew. Chem. Int. Ed.* **2007**, 46, 7383–7387.
- [21] T. Durek, V. Y. Torbeev, S. B. H. Kent, *Proc. Natl. Acad. Sci. USA* **2007**, 104, 4846–4851.
- [22] Y. Y. Yang, S. Ficht, A. Brik, C. H. Wong, *J. Am. Chem. Soc.* **2007**, 129, 7690–7701.
- [23] S. Dziadek, H. Kunz, *Synlett* **2003**, 1623–1626.
- [24] M. A. Tarp, H. Clausen, *Biochim. Biophys. Acta* **2008**, 1780, 546–563.
- [25] S. Dziadek, D. Kowalczyk, H. Kunz, *Angew. Chem.* **2005**, 117, 7798–7803; *Angew. Chem. Int. Ed.* **2005**, 44, 7624–7630.
- [26] S. Mezzato, M. Schaffrath, C. Unverzagt, *Angew. Chem.* **2005**, 117, 1677–1681; *Angew. Chem. Int. Ed.* **2005**, 44, 1650–1654.