Solid-Phase Synthesis

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9-Fluorenylmethoxycarbonyl-Based Solid-Phase Synthesis of Peptide α-Thioesters

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peptide segment coupling · peptide thioester · peptides · solid-phase synthesis

Dedicated to Professor Horst Kunz on the occasion of his 70th birthday

Peptide thioesters play a key role in convergent protein synthesis strategies such as native chemical ligation, traceless Staudinger ligation, and Ag^{+}-mediated thioester ligation. The Boc-based solid-phase synthesis provides a very reliable access to peptide thioesters. However, the acid lability of many peptide modifications and the requirements of most parallel peptide synthesizers call for the milder Fmoc-based solid-phase synthesis. The Fmoc-based synthesis of peptide thioesters is more cumbersome and typically proceeds with lower yields than the synthesis of peptide acids and peptide amides. The success of native chemical ligation and related technologies has sparked intensive research effort devoted to the development of new methods. The recent progress in this rapidly expanding field is reviewed.

1. Introduction

The advent of powerful ligation reactions that allow the construction of functional proteins by assembly of peptide segments provided a major impetus to chemical protein sciences.[1,2] Among the existing methods that allow chemoselective peptide coupling, the native chemical ligation introduced by Kent and co-workers is particularly noteworthy.[3] In this powerful reaction, an unprotected C-terminal peptide thioester reacts with an N-terminal cysteine residue of a second unprotected peptide segment to yield a native amide bond in the formed product. However, peptide thioesters are also required for other segment coupling reactions such as the extended native chemical ligation,[2,4] the Ag^{+}-mediated thioester ligation,[5] and the traceless Staudinger ligation.[6] Moreover, the reactivity of the thioester moiety is used in protein modification and immobilization strategies in peptide- and protein-array techniques.[7]

Despite the versatility, the synthesis of peptide thioesters is often challenging and time consuming. Typically, peptide thioesters are successfully synthesized by using Boc/Bzl chemistry.[8] However, the repeated use of TFA means that peptide thioesters with acid-sensitive modifications such as O-glycosidic and/or phosphate residues are hardly accessible. Furthermore, the corrosive character of TFA complicates automated parallel synthesis in standard peptide synthesizers. Alternatively, the milder Fmoc/*t*Bu strategy may be used, but the sensitivity of the thioester moiety to piperidine, which is used for cleavage of the Fmoc group, seems to limit the Fmoc-based synthesis of peptide thioesters. Nevertheless, several ingenious developments made in the last few years have led to a variety of methods, which efficiently circumvent the problem of aminolysis.

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2. Fmoc-Based Solid-Phase Synthesis of Peptide α-Thioesters

2.1. Direct Synthesis of Peptide α-Thioesters using Non-Nucleophilic Reaction Mixtures for Fmoc Cleavage

The undesired cleavage of the peptide thioester can be reduced when the nucleophilic piperidine is replaced by less nucleophilic bases for the cleavage of the Fmoc group.[9] For example, a mixture of 25% 1-methylpyrrolidine, 2% hexamethyleneimine, and 2% HOBt in NMP/DMSO (1:1) was used in the synthesis of a 25mer peptide thioester, which was linked to the resin through a tertiary alkyl thiol. Primary thioester linkages are prone to cleavage during removal of the Fmoc group. Guo and co-workers showed that a 0.88:1 ratio of DBU/HOBt provided a useful rate of Fmoc cleavage, yet presented little harm to thioester linkages.[10] Nevertheless,

the aminolysis of the thioester linkage especially during the first two to three synthesis cycles remains problematic.[11]

2.2. Thioesterification of Fully Protected Peptide Acids

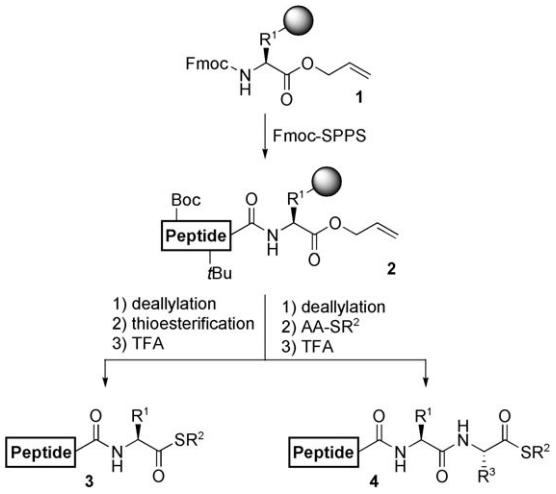
The aminolytic cleavage of the thioester linkage can be avoided by introducing the thioester moiety after the assembly of the peptide chain. A widely used approach relies on the direct conversion of fully protected peptide acids into the corresponding thioesters in solution. Generally, the peptide is synthesized on highly acid-sensitive resins such as chlorotrityl or HMPB resins.[12] After liberation of the fully protected peptide acid under mildly acidic conditions, thioesterification is usually performed by using thiols and activating agents. The method has been applied in the synthesis of functional protein domains, synthetic proteins, and cyclopeptides.[13] The drawbacks of this method are the limited solubility of the fully protected peptides and the frequently observed epimerization at the C terminus.[14] Recently, Flemer reported a synthesis protocol for direct thioesterification in situ, which may present a solution to the solubility problem.[15] A three-step sequence based on the reaction of fully protected peptide acids with tosylisocyanate, N-alkylation of the resulting *N*-tosyl peptide amides, and thiolysis has been reported.[16]

2.3. Conversion of Resin-Bound Peptide Allyl Esters into Peptide α-Thioesters

The on-resin conversion of peptide acids into thioesters avoids solubility problems. The peptide is linked to the resin through a side chain (see Section 2.3.1) or the backbone (see Section 2.3.2), while the C-terminal carboxylic acid is protected as an α-allyl ester. After assembly of the peptide, deallylation of the C terminus is followed by thioesterification on the solid support. This can be achieved directly by reaction with a thiol or indirectly by coupling with an amino acid thioester. However, direct thioesterification is often accompanied by a higher risk of epimerization at the C terminus. Detachment of the thioester and removal of the side-chain protecting groups is finally achieved by acidic treatment.

2.3.1. Side-Chain Anchored peptide α-Allyl Esters as Precursors for Peptide Thioesters

Nakahara et al. used a fluoride- and acid-sensitive silyl linker for the side-chain anchoring of glycosylated and nonglycosylated serine and threonine allyl esters.[17] After Fmoc-based solid-phase synthesis and deallylation, the side-chain-linked peptides were modified at the C terminus by on-resin fragment coupling or thioesterification (Scheme 1). The approach was expanded to the anchoring of lysine, glutamic



Scheme 1. On-resin thioesterification of side-chain-anchored peptide allyl esters. AA = amino acid.

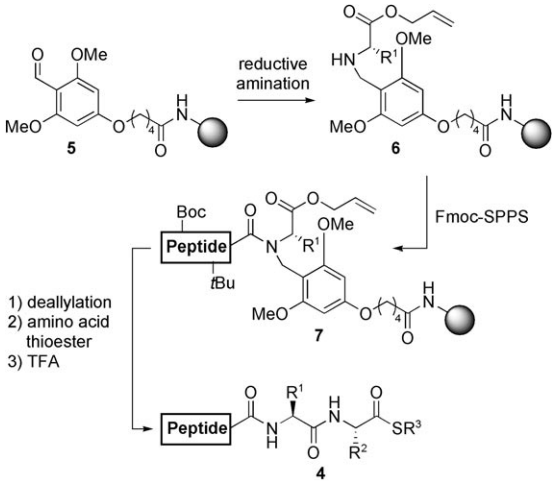
and aspartic acid, cysteine, and tyrosine to Wang or chlorotrityl resin.[18] Ficht et al. reported the linkage of aspartic and glutamic acid to Rink amide resin, thereby resulting in an asparagine or glutamine residue after acidolytic cleavage.[19] In a similar approach, the side chains of serine, threonine, tyrosine, and cysteine were anchored to (4-methoxyphenyl)-methylpolystyrene resin, which allowed the synthesis of a glycopeptide thioester fragment of erythropoietin (EPO; 1–28).[19] Side-chain anchoring was applied in the synthesis of cyclic peptides by on-resin native chemical ligation.[20]

Franziska Mende studied chemistry at the Humboldt University Berlin (diploma 2004), where she obtained her PhD in 2010, working under the supervision of Prof. Oliver Seitz. Her thesis was concerned with the development of an Fmoc-based peptide thioester synthesis with a self-purifying effect and its application in the parallel automated synthesis of peptide thioesters.

Oliver Seitz obtained his PhD from the University of Mainz in 1995. After postdoctoral research at the Scripps Research Institute in La Jolla with Prof. Chi-Huey Wong, he moved to the University of Karlsruhe. In 2000, he became group leader at the Max-Planck Institute of Molecular Physiology in Dortmund, and in 2003 he was appointed Full Professor at the Humboldt University Berlin. His research interests include peptide and nucleic acid chemistry, molecular diagnostics, DNA/RNA-directed chemistry, and the biomolecular control of protein–protein and protein–nucleic acid interactions.

2.3.2. Backbone-Anchored Peptide α-Allyl Esters as Precursors for Peptide Thioesters

The backbone amide linker (BAL) offers an alternative to side-chain anchoring. Originally, the BAL concept was designed for the solid-phase organic synthesis of small molecules.[21] Barany and co-workers first demonstrated the application of the BAL strategy in the Fmoc-based synthesis of peptide thioesters.[22] The α-amino group of the C-terminal amino acid is coupled to tris(alkoxy)benzaldehyde resin **5** by reductive amination (Scheme 2). Elongation of the peptide

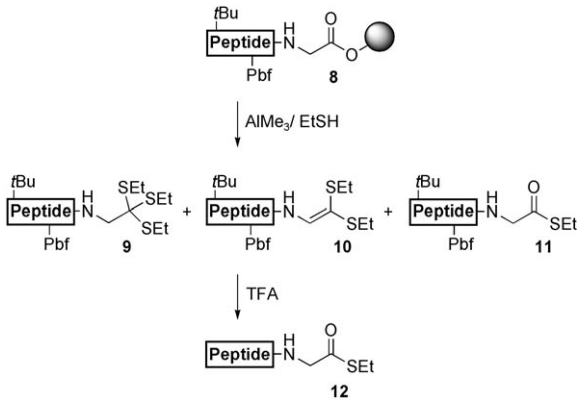


Scheme 2. Backbone amide linker strategy.

chain yields peptide **7**, which after deallylation is used for the coupling of an amino acid thioester. In contrast to the side-chain-anchoring strategy, the backbone amide linkage is independent of the peptide sequence. However, a significant limitation of this method is the low yield in the coupling of the second amino acid to the secondary amine of the linker. Moreover, formation of diketopiperazines during removal of the Fmoc group from the second amino acid was observed. Diketopiperazine formation is significantly reduced when the thioester functionality is masked as a trithioorthoester.[23]

2.4. Conversion of Resin-Bound Peptide Esters into Peptide α-Thioesters by Using Alkyl Aluminum Thiolates

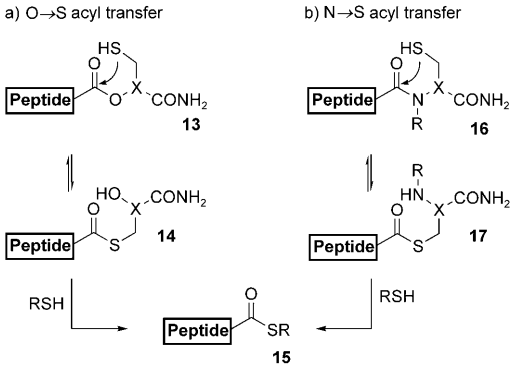
Hilvert and co-workers used alkyl aluminum thiolates to cleave resin-bound peptide esters. This reaction yields a mixture of fully protected peptide tri(thioorthoesters) **9**, ketene dithioacetals **10**, and peptide thioesters **11** (Scheme 3).[24] The product mix was completely converted into **12** by treatment with TFA. The method is restricted to C-terminal glycine because chiral amino acids would undergo epimerization under these conditions. Care has to be taken to avoid aspartimide formation and the conversion of protected aspartic and glutamic acid side chains into thioesters.



Scheme 3. Conversion of resin-bound peptide esters into peptide thioesters by treatment with alkyl aluminum thiolates.

2.5. Solid-Phase Synthesis of Peptide α-Thioesters by O→S and N→S Acyl Shift

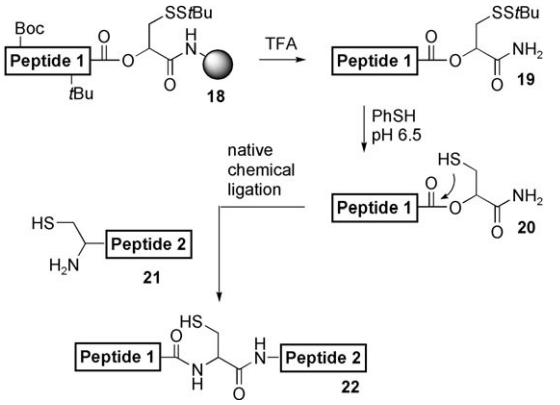
The last five years have witnessed an explosion in the number of methods which involve the Fmoc-based solid-phase synthesis of peptide thioesters by using intramolecular O→S or N→S acyl-transfer reactions. In this approach, peptide esters or peptide amides are connected to the solid support through a thiol-functionalized building block (Scheme 4). After cleavage from the resin, the thiol group serves as an internal nucleophile. The peptide thioesters **14** and **17** are formed in an equilibrium reaction, and these can be intercepted by thiol-exchange reactions to furnish peptide thioesters **15**.



Scheme 4. β-Mercapto-substituted a) peptide esters and b) N-alkylated peptide amides undergo partial O→S or N→S acyl transfer. X = organic structure.

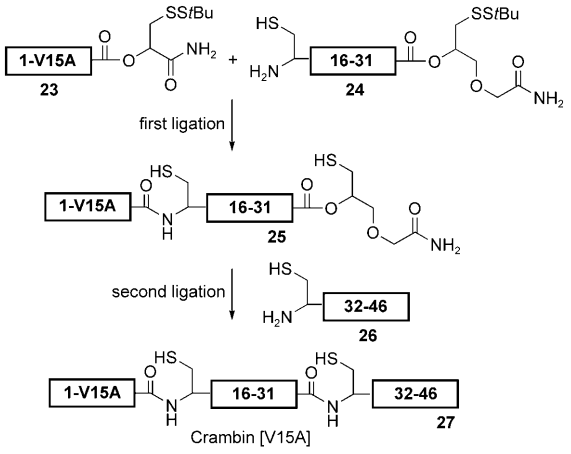
2.5.1. Methods Based on O→S Acyl Transfer

The peptide thioester synthesis based on O→S acyl transfer was introduced by Danishefsky and co-workers.[25] Fully protected peptide acids were converted in solution into disulfide-protected α-2-mercaptophenyl esters. The resulting peptide segments were directly used in native chemical ligation. Botti et al. were the first to report an anchor based on a peptide ester that served as a latent thioester in solid-

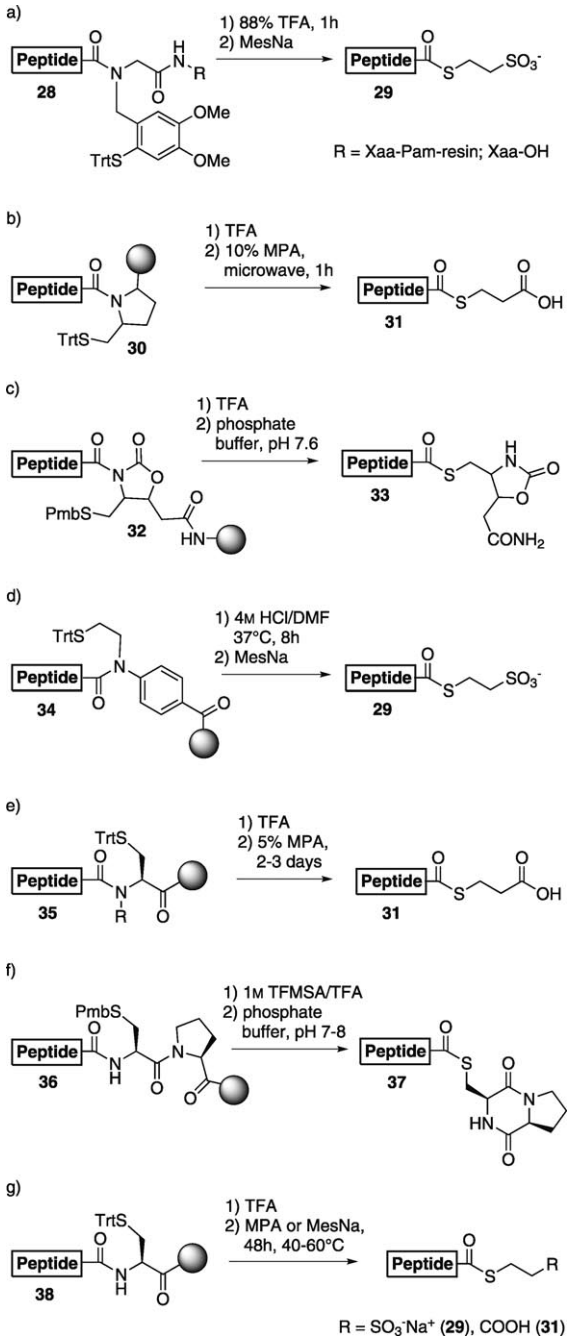


Scheme 5. The peptide-2-mercapto-carboxyamide ester **19** is directly used in native chemical ligation.

phase synthesis. They prepared peptide ester **18**, which bears a disulfide-protected thiol functionality in the β position. After acidolytic release from the resin, segment **19** was used in the ligation with cysteinyl peptide **21** (Scheme 5).[26] The reductive conditions of native chemical ligation led to cleavage of the disulfide, which set the stage for the O→S acyl transfer in **20**. Hydrolysis of the ester (10–20%) during ligation was a major problem. Nonetheless, this approach allowed the successful synthesis of cyclic peptide thioesters as well as phosphorylated and acetylated histone H2B.[27] Recently, Liu and co-workers tuned the reactivity of the O-alkyl ester units to allow a kinetically controlled sequential ligation of three peptide fragments **23**, **24**, and **26** (Scheme 6) for the synthesis of the 46mer polypeptide [V15A]crambin **27**.[28]



Scheme 6. Synthesis of [V15A]crambin by kinetically controlled ligation by using masked peptide thioesters with different reactivity.[28]



Scheme 7. Fmoc-based solid-phase synthesis of peptide thioesters based on N→S acyl transfer: a) Dmmb-mediated peptide thioester synthesis, b) β-mercapto-methylated proline linker, c) mercapto-methylated oxazolidine linker, d) *N*-mercaptoethylaniline linker, e) *N*-alkyl cysteine mediated peptide thioester synthesis, f) CPE-based peptide thioester synthesis, g) reverse native chemical ligation.

2.5.2. Methods Based on N→S Acyl Transfer

Vorherr et al. observed the N→S acyl shift as a side reaction when the Dmmb group was used as a ligation auxiliary at a N-terminal glycine residue.[29] Based on this finding, the Dmmb-mediated synthesis of peptide thioesters was developed (Scheme 7a).[30] After deprotection of the trityl-protected mercapto group in **28**, an acid-induced N→S acyl shift leads to partial formation of a peptide thioester, which is intercepted by an external thiol such as MesNa. This rearrangement can be performed in solution or on-resin. The acyl transfer was performed at 37 °C for 3 h to increase the reaction rate. The method was used in the synthesis of a 41mer segment of TRF2 (telomere repeat-binding factor 2) as well as phosphorylated and methylated peptide thioesters.[31]

Hojo, Nakahara, and co-workers investigated a mecaptomethylated proline derivative (Scheme 7b).[32] The acyl shift in **30** is induced upon treatment with 10–40% of aqueous MPA. Microwave irradiation is needed to achieve acceptable reaction rates. partial cleavage of an Asp-Ser bond was observed under these conditions. The α-mercaptomethylated *N*-peptidyloxazolidinones **32** provide significantly higher reactivity (Scheme 7c).[33] These compounds can be used directly in ligation reactions, but are also sensitive to piperidine, thus requiring the use of non-nucleophilic reagent mixtures for removal of the Fmoc group in SPPS. A major drawback of this method is the epimerization of the C-terminal amino acid during cleavage of the Fmoc group. The peptide anilide derivatives **34** are described as more robust but less-reactive linkages (Scheme 7d).[34] The N→S acyl transfer is achieved by treatment with 4 M HCl/DMF in the presence of 1% TCEP for 8 h at 37 °C. Finally, thiolysis with MesNa induces the release of thioester **29**.

Hojo reported the *N*-alkyl cysteine linker in **35** which provides peptide thioesters without C-terminal epimerization (Scheme 7e).[35] The N→S acyl migration and transthioesterification is triggered by acidic treatment with 5% mercaptopropionic acid for two to three days at room temperature. The approach was used in the synthesis of the 95mer chemokine CCL27 and the glycosylated murine pro-opiomelanocortin (1–74).[36] Very recently, Dawson, Brik, and co-workers succeeded in the total synthesis of an ubiquitin thioester by native chemical ligation.[37] During the synthesis, the C-terminal thioester moiety was masked as a protected *N*-methylcysteine amide and installed, in the last step, by treatment with MPA.

Kawakami and Aimoto reported that the cysteine-proline ester (CPE) in **36** serves as an auto-activating unit (Scheme 7f).[38] It was proposed that the cysteine amine liberated upon N→S acyl transfer is trapped in a subsequent irreversible reaction that leads to the formation of the diketopiperazine **37**. The reaction sequence proceeds at ambient temperature and about pH 7, and is complete after 24 h. The Cys-Pro unit has to be tethered to the solid support as a dipeptide to prevent diketopiperazine formation during the peptide assembly.

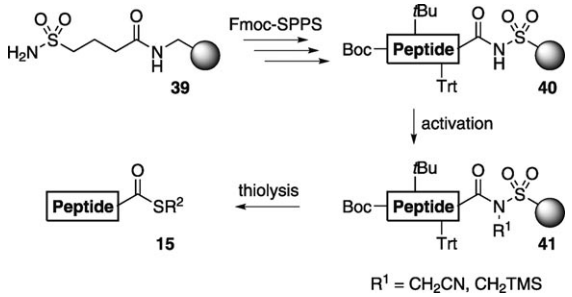
Macmillan and co-workers observed that peptides containing internal Gly-Cys, His-Cys, and Cys-Cys units decompose on exposure to MPA to yield the Gly, His, or Cys thioester fragments (Scheme 7g).[39] This reverse native chemical ligation was optimized and used in the synthesis of a 33 amino acid long neoglycopeptide thioester of EPO (1–33).[40] The optimized His-Cys motif was cleaved within 48 h at increased temperatures (60 °C). Sequence internal cysteine residues require protection during the acid-induced thioesterification.

2.6. The "Safety-Catch" Principle in the Solid-Phase Synthesis of Peptide α-Thioesters

The design of "safety-catch" linkers includes a chemical modification reaction, which increases the reactivity of the peptide acyl group. The desired peptide thioesters are obtained upon nucleophilic cleavage of the activated peptidyl linkage.

2.6.1. Solid-Phase Synthesis of Peptide α-Thioesters at the Sulfonamide Linker

A widely used method in the solid-phase synthesis of peptide thioesters involves Ellman's modification (**39**) of Kenner's sulfonamide linker (Scheme 8).[41] After elongation



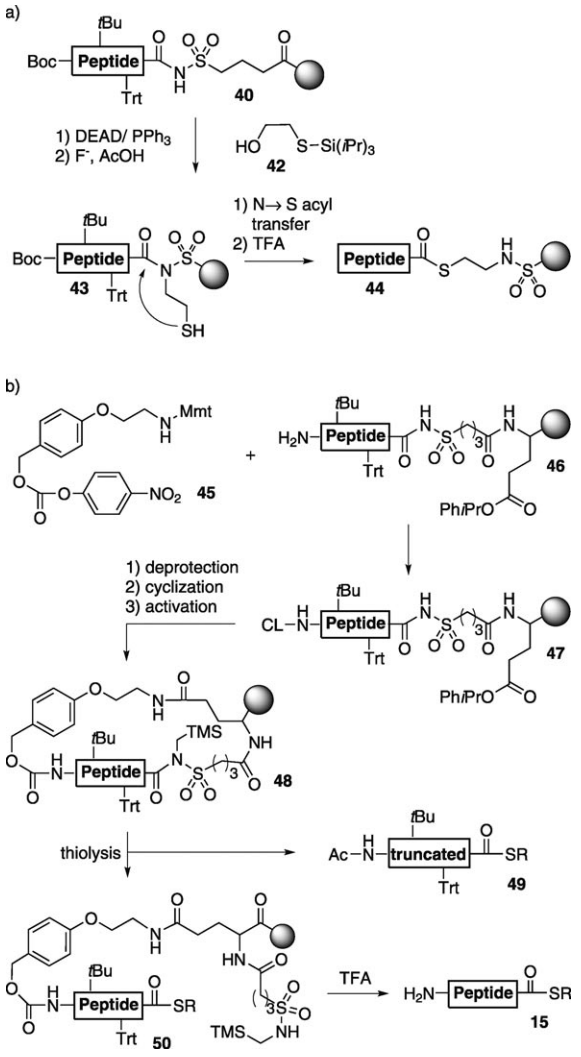
Scheme 8. Fmoc-based peptide thioester synthesis on the sulfonamide "safety-catch" resin.

of the peptide chain, the peptidyl–sulfonamide bond in **40** is alkylated in a reaction with iodoacetonitrile or trimethylsilyldiazomethane. The *N*-alkyl-*N*-acyl-sulfonamide **41** is vulnerable to nucleophilic attack. Thiolysis provides fully protected peptide thioesters, which are treated with TFA for global deprotection.[42]

Various peptide thioesters of up to 35 amino acids in length have been prepared by using this approach.[43,44] Shortcomings to be considered include the risk of epimerization during long-lasting loading reactions, side reactions during the activation process, and aggregation of the fully protected peptide thioesters.[45,46] The use of amino acid fluorides and the coupling of amino thioacids to resin-bound sulfonylazides present solutions to the epimeriaztion problem,[47] while side reactions during activation are minimized when trimethylsilyldiazomethane is used for the alkylation.[48] Unverzagt and co-workers observed the undesired acetylation of the acyl sulfonamide during the capping step.[44]

The two-step approach comprised of alkylation of the sulfonamide bond and subsequent nucleophilic cleavage offers opportunities for reaction design. For example, polymer-bound peptide thioesters were accessed in a reaction sequence involving a) a Mitsunobu reaction with *S*-silylated mercaptoethanol **42** for alkylation of the sulfonamide **41**, b) fluoride-mediated deprotection of the thiol group (→**43**), and c) subsequent intramolecular thiolysis (Scheme 9a).[48] Treatment of the side-chain-protected peptide thioester resin with TFA afforded unprotected peptide thioesters in 20–25% overall yield when the Rink resin was used. Omission of the Rink linker allowed the synthesis of resin-bound peptide thioester **44**, which was subsequently applied in a native chemical ligation on the solid support.

We recently reported the automated parallel synthesis of peptide thioesters with a self-purifying effect (Scheme 9b).[49] The peptide chain was assembled on a carboxy-functionalized

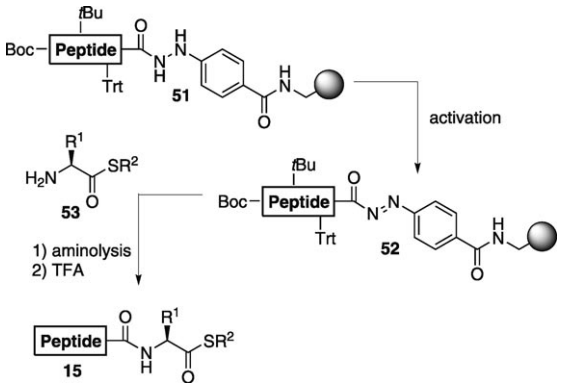


Scheme 9. a) Synthesis of resin-bound peptide thioesters by intramolecular N→S acyl transfer. b) Fmoc-based synthesis of peptide thioesters with a self-purification effect.

sulfonamide resin. N-terminal modification of the full-length peptide **46** with the cyclization linker **45** marks the resin-bound peptide for a backbone-to-side-chain cyclization. Acetylation after failed amino acid coupling reactions prevents the cyclization linker being introduced at truncated peptide sequences. Thus, only the full-length product is amenable to cyclization. Subsequent alkylation of the *N*-acyl sulfonamide furnished the activated peptide sulfonamide bond in **48**. The macrocycle is opened upon thiolysis. The formed peptide thioester **50** remains attached to the solid support, while the noncyclic, acetylated truncation products **49** are expelled into solution. The acid lability of the cyclization linker enables the liberation of the unprotected peptide thioester **15**, which occurs simultaneously with deprotection of the side chain in the presence of TFA. This approach provided crude product of high purity. The 20–40 amino acid peptide thioesters were applied in ligation reactions without further HPLC purification and used in the construction of an SH3-domain array.

2.6.2. Solid-Phase Synthesis of Peptide α-Thioesters at an Aryl Hydrazine Linker

The oxidation of acyl hydrazides to the more-reactive acyl diazenes is the basis for the Fmoc-based synthesis of peptide thioesters on the aryl hydrazine linker **51** (Scheme 10).[50] The peptidyl diazene **52** is formed upon mild oxidation with



Scheme 10. Fmoc-based synthesis of peptide thioesters on a hydrazine resin.

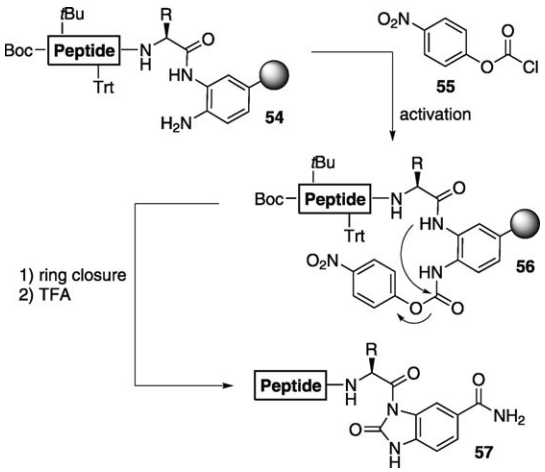
N-bromosuccinimide. Direct thiolysis of activated **52** is not feasible because mercaptans would induce reduction of the diazene moiety. Thus, aminolysis with amino acid thioesters **53** is used. The final TFA treatment is performed in solution. Several model peptides have been prepared in 70–90% yield, and a 23-residue peptide thioester fragment of the N-terminal SH3 domain of *c*-Crk adaptor protein was prepared. Cysteine and methionine can undergo side reactions in the oxidative step.

2.6.3. Peptide α-Aryl Benzimidazolones as Precursors for Peptide α-Thioesters

Blanco-Canosa and Dawson recently introduced a new safety-catch principle.[51] The assembly of the peptide chain is performed on a 3,4-diaminobenzoyl linker (Scheme 11). Acylation of the amino group in **54** by 4-nitrophenyl chloroformate (**55**) initiates a spontaneous ring closure in **56**. In the presence of mercaptans the resulting *N*-acylbenzimidazolone **57** is readily converted into a thioester and can, therefore, be applied directly in native chemical ligation. However, capping by acetylation should be avoided to prevent acylation of the unprotected aniline-amino group in **54**. The synthesis of a 29-residue peptide-benzimidazolone succeeded in 36% yield. The usefulness of the approach has been demonstrated in the total chemical synthesis of HIV-1 Tat protein,[52] the second type 1 repeat of thrombospondin-1 (TSR2),[53] and in the semisynthesis of the N-terminal domain of the anthrax lethal factor.[54]

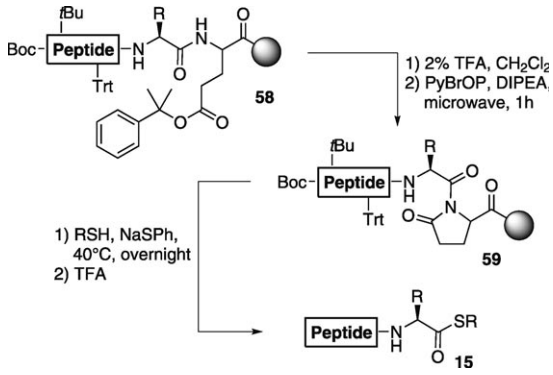
2.6.4. Backbone Pyroglutamylimides as Precursors for Peptide α-Thioesters

Conde-Frieboes, Hoeg-Jensen, and co-workers designed the peptide linker **58**, which is activated for nucleophilic



Scheme 11. Synthesis of peptide benzimidazolones as highly reactive precursors for peptide thioesters.

scission by formation of a pyroglutamylimide structure (Scheme 12).[55] The solid-phase assembly of the peptide is followed by selective acidolysis of the phenyl isopropyl ester



Scheme 12. Pyroglutamylimide formation activates the peptide backbone for thiolytic cleavage.

in **58**. Activation of the side-chain carboxy group with PyBrOP under basic conditions and microwave irradiation results in the formation of the pyroglutamylimide in **59**. This structure is susceptible to thiolysis. Increased temperatures and long reaction times are needed to obtain the peptide thioesters in 45–60% yields because of the relatively low reactivity of the pyroglutamylimide.

3. Conclusion

In this Minireview we have reviewed the recent state-of-the-art in the Fmoc-based solid-phase synthesis of peptide thioesters. Conceptually new developments involve latent thioesters, which draw upon peptide amide or peptide acid structures that undergo intramolecular O→S or N→S acyl transfer. The equilibrium of this rearrangement reaction typically lies on the side of the ester or amide structure. However, external mercaptans intercept the *S*-acyl products and shift the equilibrium in favor of the peptide thioester. Tuning the reactivity of the latent thioester allows kinetically controlled two- or three-step ligation reactions.

Among the many methods introduced, those that rely on side-chain anchoring, backbone amide, or safety-catch sulfonamide linkers have probably been used most frequently. The clever design of reaction sequences, in which safety-catch principles are combined with additional chemical reactions offers interesting opportunities. For example, synthesis of the peptide thioester at the novel 3,4-diaminobenzoyl "safety-catch" linker involves an activation step (acylation) and a subsequent O→N acyl transfer that yields highly reactive peptide benzimidazolones, which undergo rapid thioesterification. The use of the established sulfonamide linker in combination with on-resin macrocyclization and thiolytic ring opening enabled selective detachment of full-length peptide thioesters. This method with "self-purification" eliminates a major bottleneck, which is HPLC purification of the prepared peptide thioesters.

Reaction steps in solution present a major limitation in automated synthesis. Thus, in an ideal method the entire set of reactions would be performed on the solid support. Only a few methods fulfill this requirement. These include methods based on side-chain or backbone-amide linkages as well as on sulfonamide linkages that are either combined with intramolecular N→S acyl transfer or with a sequence of macrocyclization and ring-opening thiolysis reactions. The new diaminobenzoic acid linker also falls in this category, because the peptidyl benzimidazolone obtained after cleavage with TFA forms a peptide thioester under the conditions of native chemical ligation.

The progress that has been achieved in the Fmoc-based solid-phase synthesis of peptide thioesters is enormous. There are a variety of methods that provide reliable access to peptide thioesters that are up to 40 amino acids long. The improved methods can be applied in virtually any peptide synthesis laboratory. However, a literature survey reveals that the Fmoc-based synthesis of 60–80 residue peptide thioesters has not been reported. Despite the progress made, the current methods are still lacking behind the simpler methods used in the synthesis of peptide acids and peptide amides. Undoubtedly, further research effort will provide the necessary improvements, either by the development of new methods and/or by the design of better reaction sequences. This will not only facilitate the chemical synthesis of modified peptide thioesters but provide a major boost for the chemical synthesis of proteins and protein sciences in general.

4. Abbreviations

BAL	backbone amide linker
Boc	*tert*-butoxycarbonyl
Bzl	benzyl
CPE	cysteine-proline ester
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DEAD	diethylazodicarboxylate
DIPEA	*N*,*N*-diisopropylethylamine

DMF	dimethylformamide
Dmmb	4,5-dimethoxy-2-mercaptobenzyl
DMSO	dimethylsulfoxide
EPO	erythropoietin
Fmoc	9-fluorenylmethoxycarbonyl
HMPB	4-(4-hydroxymethyl-3-methoxyphenoxy)-butyric acid
HOBt	hydroxybenzotriazole
HPLC	high-performance liquid chromatography
MPA	mercaptopropionic acid
MesNa	sodium 2-sulfanylethanesulfonate
Mmt	monomethoxytrityl
NMP	*N*-methylpyrrolidone
Pbf	2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl
Pmb	*para*-methoxybenzyl
PyBrOP	bromo tris(pyrrolidino)phosphonium hexafluorophosphate
SPPS	solid-phase peptide synthesis
TCEP	tris(2-carboxyethyl)phosphine
TFA	trifluoroacetic acid
TMS	trimethylsilyl
Trt	trityl

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