Bioconjugate Chemistry

Review

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Phenylthiocarbamate or *N*-Carbothiophenyl Group Chemistry in Peptide Synthesis and Bioconjugation

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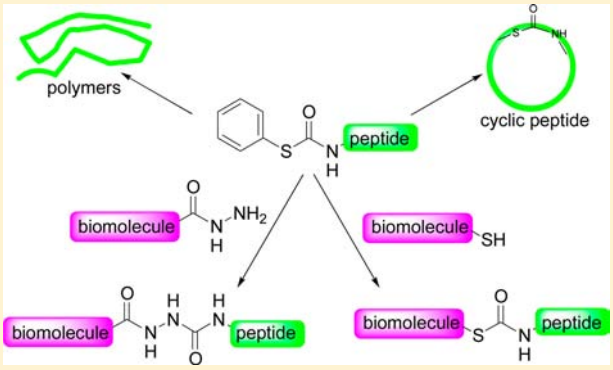
ABSTRACT: The design of novel chemoselective and site-specific ligation methods provides new tools for obtaining complex scaffolds, peptidomimetics, and peptide conjugates. The chemistry of the *N*-phenylthiocarbonyl group has led to several developments in peptide ligation chemistry and peptide bioconjugation during the last 10 years. The aim of this review is to provide an overview of this emerging field.



INTRODUCTION

The design of novel chemoselective and site-specific ligation methods provides new tools for obtaining complex scaffolds, peptidomimetics, and peptide conjugates. The entry of the *N*-phenylthiocarbonyl group (Figure 1) into the peptide field began in the late 1940s, but its potential as a thioester surrogate for the design of chemoselective ligation methods has been realized only recently. The aim of this short review is to highlight the main features of phenylthiocarbonyl peptide **1** chemistry and its potential for obtaining novel peptide scaffolds and peptide conjugates.

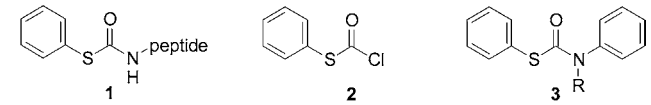


Figure 1. Structure of *N*-phenylthiocarbamate peptide **1**, phenylthiochloroformate **2**, and *S*-phenyl phenylcarbamothioate **3**.

Apart from the introduction, the review is composed of six main sections. The first section gives a historical overview of the field and presents some important chemical properties of the *N*-phenylthiocarbonyl group. The second section describes the synthesis of phenylthiocarbonyl peptides **1** using Fmoc solid-phase peptide synthesis (SPPS) and commercially available phenylthiochloroformate **2**. The third section presents the interest of the *N*-phenylthiocarbonyl group for the preparation of peptide polymers. The fourth section is focused on thiocarbamate chemoselective ligation, whereas the fifth section describes the potential of azaGly ligation for accessing azaGly peptides or conjugates. Lastly, the final section is devoted to the synthesis of cyclic peptide scaffolds.

HISTORY AND CHEMICAL PROPERTIES OF PHENYLTHIOCARBAMATES

Phenylthiochloroformate **2** was first described by Rivier in 1907.[1] Rivier also reported the reaction of phenylthiochloroformate **2** with aniline, which led to the isolation of *S*-phenyl phenylcarbamothioate product **3a**. In the same paper, the author noticed that *S*-phenyl phenylcarbamothioate **3a** decomposed into phenylisocyanate and thiophenol upon heating or at room temperature in aqueous alkali. In contrast, compound **3b** proved to be stable in aqueous alkali. Note that the hydrolysis of *S*-phenyl dialkylcarbamothioates has been used to obtain thiophenols.[2]

The entry of the phenylthiocarbonyl group into the peptide field began with the report of Ehrensvärd in 1947, who suggested that the phenylthiocarbonyl group might overcome some limitations of the benzyloxycarbonyl (Cbz) group when used as an amine protecting group during peptide synthesis.[3] In particular, the work of Rivier suggested that phenylthiocarbonyl derivatives of α-amino acids or peptides might be selectively deprotected in aqueous alkalis, whereas hydrogenolysis of the Cbz group was complicated by the simultaneous hydrogenation of some peptide functional groups.

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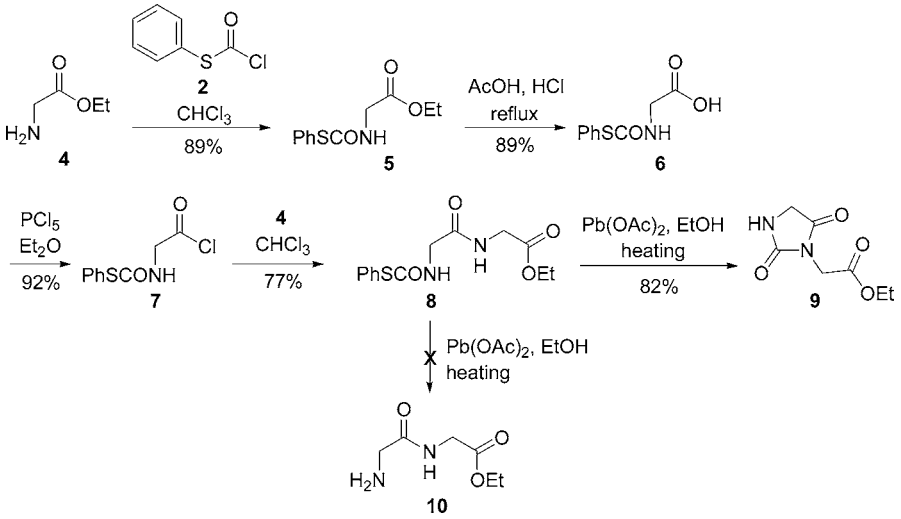
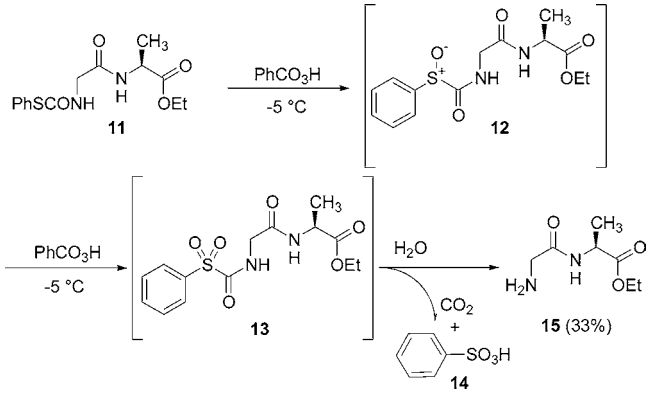
Scheme 1. Deprotection of *N*-Phenylthiocarbonyl Dipeptides in the Presence of Lead Diacetate Leads Mainly to Hydantoin Formation[6]



The phenylthiocarbonyl group could be introduced on the α-amino group of glycine ethyl ester **4** to produce derivative **5** (Scheme 1). The acid-catalyzed hydrolysis of the ester functionality successfully yielded *N*-phenylthiocarbonyl glycine **6**. Activation of the carboxylic acid function with phosphorus pentachloride yielded acid chloride **7**, which was reacted with glycine ethyl ester **4** to produce *N*-phenylthiocarbonyl diglycine ethyl ester **8**.[4,5] Ehrensvärd claimed that the deprotection of *N*-phenylthiocarbonyl amino acids or dipeptides could be performed by heating the compounds in 70% ethanol in the presence of lead diacetate. However, 5 years later, Lindenmann and co-workers established that treatment of *N*-phenylthiocarbonyl diglycine ethyl ester **8** with lead diacetate in 70% ethanol at 80−85 °C yielded essentially ethyl hydantoin-3-acetate **9** instead of expected dipeptide **10**.[6] Hydantoin formation was confirmed with other dipeptides. The authors concluded that the phenylthiocarbonyl protecting group had "little practical significance as a tool in peptide chemistry".

Following the work of Lindenmann and co-workers,[6] Kollonitsch and co-workers showed that the phenylthiocarbonyl group can be split off from various *N*-phenylthiocarbonyl dipeptides using perbenzoic acid at −5 °C (Scheme 2).[7] The reaction presumably proceeds through the formation of a thiocarbamate sulfoxide intermediate **12**,[8,9] which is further oxidized by the peracid into thiocarbamate sulfone **13**. The latter decomposes spontaneously in the presence of water, with production of carbon dioxide and benzenesulfonic acid **14**. This deprotection procedure has not been used very much, probably because of the poor compatibility of perbenzoic acid with many functional groups present in peptides. In support of the mechanism proposed in Scheme 2, the S oxidation of *S*-alkylthiocarbamates by peracids is known to yield thiocarbamate sulfone products similar to **13**.[9−11] This point will be discussed later in the section devoted to thiocarbamate ligation.

Scheme 2. Removal of *N*-Phenylthiocarbonyl Group by Treatment with Perbenzoic Acid[7]



The work of Lindenmann and co-workers shows that the removal of the *N*-phenylthiocarbonyl group is problematic when the intermediate isocyanate can react intramolecularly with a nucleophile in a 1,5 relationship relative to the carbonyl. This is typically the case in Scheme 1, where the activated carbonyl reacts with an amide nitrogen through a five-membered ring intermediate to yield a hydantoin product.[6] The same authors showed that treatment of *N*-phenylthiocarbonylglycine carbobenzyloxyhydrazide PhSCO-Gly-NHNHCOOBn with lead acetate yielded a 2-carbobenzyloxy-3,6-dioxohexahydro-1,2,4-triazine product, which was formed in this case through a six-membered ring intermediate.

Similarly, Degani and co-workers showed that *S*-phenylthiocarbonyl derivatives of cysteinyl peptides undergo a cyclization reaction by nucleophilic attack of the cysteine amide nitrogen on the *S*-phenylthiocarbonyl group (Scheme 3).[12] The resulting 2-keto-3-acylthiazolidine intermediate **17** is then cleaved by hydroxide ion to yield peptide acid **18** and 2-ketothiazolidine product **19**. Interestingly, the authors screened a series of carbonic acid derivatives as S-acylating agents, among which, phenylthiochloroformate **2** proved to be the most efficient for peptide cleavage. In essence, this cyclization reaction is homologous to the cleavage of cystine peptides by cyanide,[13] a process that involves the intermediary of an *S*-cyanocysteinyl residue, which yields a 2-imino-3-acylthiazolidine after cyclization.

The phenylthiocarbonyl group can be used advantageously as an amine protecting group when such an intramolecular cyclization process cannot occur. In this case, treatment of the *N*-phenylthiocarbonyl derivative with base yields the intermediate isocyanate, which can be trapped by an external nucleophile. For example, treatment of *N*-phenylthiocarbonyl-

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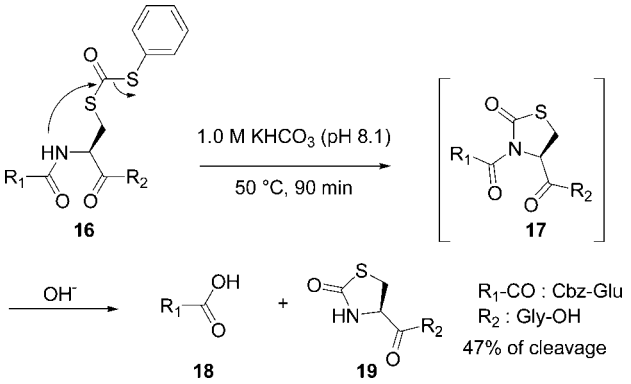
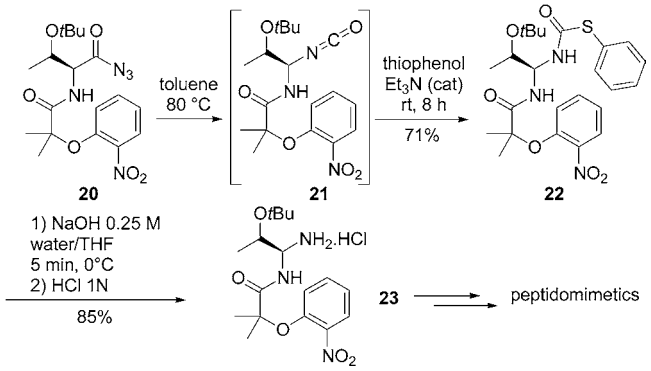
Scheme 3. Specific Cleavage of Peptides at Cysteinyl Residues[12]



protected nucleosides with sodium benzyl alkoxide yielded the corresponding *N*-Cbz-protected nucleosides.[14,15] In another application, exposure of *N*-phenylthiocarbonyl derivatives of *gem*-diaminoalkanes with aqueous base yielded the amine products in good yield (Scheme 4).[16] This deprotection

Scheme 4. Synthesis of the *gem*-Diaminoalkane 23 Derived from *O*-*tert*-Butyl Threonine and Protected by a 2-Methyl-2-(2′-nitrophenoxy)propionyl Group (MNP)[16,18]



procedure was exploited by Gazerro and co-workers for the synthesis of peptidomimetics starting from *N*-[2-methyl-2-(2′-nitrophenoxy)propionyl] α-amino acid azides such as **20**.[17,18]

To summarize, the instability of the *N*-phenylthiocarbonyl group in the presence of aqueous alkali, various primary or secondary amines such as piperidine,[19−22] or very popular alkylating reagents such as diazomethane[23,24] as well as the paucity of methods for removing it[7,25] have discouraged the routine use of this protecting group for the stepwise or block synthesis of native peptides in solution. Apart from the synthesis of peptide polymers, which is presented later, the main applications of the *N*-phenylthiocarbonyl group in peptide chemistry exploit its thioester surrogate properties for setting up chemoselective ligations, as will be discussed in the last sections of this review.

FMOC SOLID-PHASE SYNTHESIS OF PHENYLTHIOCARBONYL PEPTIDES

In early studies discussed in the preceding section, the phenylthiocarbonyl group was introduced onto α-amino acids[3,6,7,26] or peptides[12] using phenylthiochloroformate **2** and solution chemistry. Therefore, the functionality of the studied peptides was limited because of the need to work with protected peptides. Recent studies have shown that the phenylthiocarbonyl group can be easily introduced into peptides using phenylthiochloroformate **2** and Fmoc-SPPS,[27] provided that it is installed in the last stage of synthesis to avoid exposure of the phenylthiocarbamate group to the piperidine used for removing the Fmoc group.[19,20] The introduction of a phenylthiocarbonyl group on the N-terminus of peptides is described in Scheme 5. Triethylamine can be used as base when hydantoin formation is not favored. This is typically the case when the second amino acid residue, isoleucine in peptide **25**, is bulky. When hydantoin formation can be a potential issue, as in the case of lysine dendrimers **27** and **28**, triethylamine must be replaced by a weaker base such as *N*-methylmorpholine and the reaction time must be kept as short as possible.[27]

The phenylthiocarbonyl group can also be introduced exclusively on the side chain of a lysine residue, as shown in Scheme 6. For this, the side-chain amino group of the lysine residue must be orthogonally protected to enable the selective unmasking of the ε-amino group on the solid phase after the peptide elongation step. The example provided in Scheme 6 makes use of methyltrityl protection,[28] which can be selectively removed by washing with 1% TFA in dichloromethane.[29] Contrary to what has been observed during the synthesis of N-terminal phenylthiocarbonyl peptides, hydantoin formation cannot occur during the reaction of phenylthiochloroformate **2** with internal lysine residues, so isolation of peptides such as **30** shows no particular difficulties. The thiol group of the N-terminal cysteine residue is protected with a *tert*-butylsulfenyl group to avoid any intramolecular or intermolecular thiol−thiol ester exchange during workup, purification, or storage.

SYNTHESIS OF POLYMERS

The synthesis of polymers made of amino acids has various potential applications such as the preparation of biomimetic biomaterials derived from natural proteins.[30] One well-known method for producing peptide polymers is the polymerization of *N*-carboxy α-amino acid anhydrides, which was introduced by Leuchs at the beginning of the 20th century.[31] The tendency of *N*-carboxy α-amino acid anhydrides to oligomerize was noticed by Leuchs himself,[32] but the potential of *N*-carboxy α-amino acid anhydrides for giving access to large peptide polymers was established in 1947 with the work of Woodward and Schramm.[33] The field has been reviewed.[34]

Soon after the work of Woodward and Schramm, Noguchi introduced a novel method for producing peptide polymers based on the chemistry of the *N*-phenylthiocarbonyl group (Schemes 7 and 8).[26] The polymerization of *N*-carboxythiophenyl-derivatized amino acids or peptides occurs by melting (Scheme 7, method 1) or by heating in an organic solvent in the presence of pyridine (Scheme 8, method 2).

In the absence of solvent, *N*-carbothiophenyl amino acids start to decompose at 120−130 °C. Decomposition is a fast process at 150 °C. Under these conditions, the *N*-phenylthiocarbamate group yields isocyanate derivatives such as **33**, which react with the C-terminal carboxylic acid group of another peptide molecule to produce mixed anhydride intermediate **34** (Scheme 7, method 1).[36,37] The latter was believed to yield amide product **35** after elimination of carbon dioxide. This is indeed a mode of decomposition of mixed anhydrides of type **34**. However, it has been known since the work of Naegli and co-workers[38,39] that another mode of decomposition can occur, leading to the formation of symmetrical anhydride **36** and urea **37**, which, on heating to

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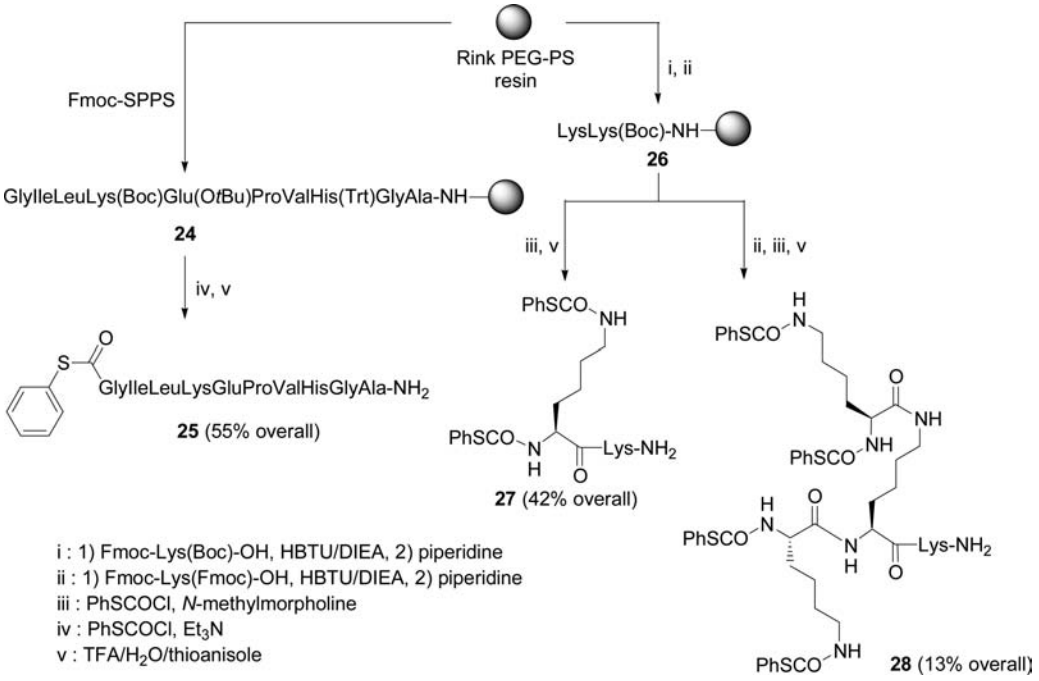
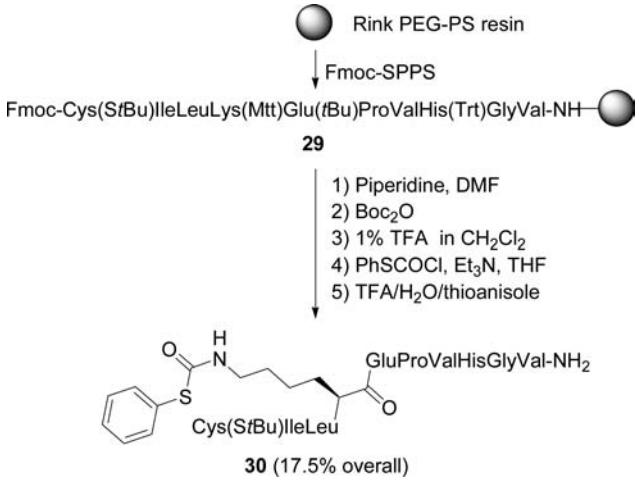
Scheme 5. Synthesis of Phenylthiocarbonyl Peptides Using Fmoc-SPPS[27]



Scheme 6. Synthesis of Peptides Featuring the Phenylthiocarbonyl Group on a Lysine Side Chain[27]



high temperatures (>135 °C), can react with each other, again yielding amide **35** with the loss of carbon dioxide.

Noguchi noticed that polymerization of *N*-carbothiophenyl α-amino acids at high temperatures (>130 °C) yielded polypeptides with a low degree of polymerization because of the occurrence of side reactions. For example, polymerization of *N*-carbothiophenyl-L-nitroarginine in dimethyl sulfoxide at 120 °C for 14 h resulted in a degree of polymerization of only 5−10.[40] Thus, an alternative procedure for inducing the polymerization of *N*-carbothiophenyl α-amino acids was developed (Scheme 8, method 2).[26,35] In method 2, the *N*-phenylthiocarbamate group of **32** reacts directly with the carboxylate function of **31** to again produce mixed anhydride intermediate **34**. The transient formation of an isocyanate intermediate of type **33** (Scheme 7) could be ruled out in this case because, in a control experiment, *N*-carbothiophenyl-D,L-alanine ethylester was found to be stable in ethanol at reflux.[35,41,42] Interestingly, method 2 enabled the synthesis of polypeptides with a high degree of polymerization by minimizing the occurrence of side reactions (135.000 g/mol for polyglycine[26] and 22.400 g/mol for poly-D,L-alanine[26]). The use of a mixture of *N*-carbothiophenyl α-amino acids yielded copolymers such as copoly-(glycine, D,L-alanine) with a degree of polymerization of 89 (11.500 g/mol).[26,43]

Interestingly, this method also enabled the polymerization of *N*-carbothiophenyl dipeptides (dioxane or benzene, pyridine, 80 °C), thereby giving better control of the copolymer structure and periodicity.[26,35,44,45] In particular, polymerization of *N*-carbothiophenyl-ε-aminocaproyl-D,L-alanine yielded poly-ε-aminocaproyl-D,L-alanine endowed with reversible heat coagulation properties.[45] The fate of the N-terminal phenylthiocarbonyl group was not discussed by Noguchi and coworkers. It presumably evolves into a hydantoin product[6] when the kinetics of the amide bond forming reaction are slowed because of the decrease of the reactant concentration and because of the decrease of the diffusion rate as a result of the viscosity of the polymer mixture.

SYNTHESIS OF BIOCONJUGATES USING THIOCARBAMATE CHEMOSELECTIVE LIGATION

Chemoselective ligation methods are important tools for obtaining various peptide conjugates or scaffolds. In particular, thiol-based ligation methods[46] that enable the formation of a thioether,[47−51] disulfide,[50,52] or thiazolidine[53−55] bond between unprotected peptides or biomolecules are very popular because of their efficiency, the ease of introducing a thiol group in peptides using cysteine residue, and, lastly, the possibility of combining some of these techniques with other chemoselective ligation methods.[56−61] Thiocarbamate ligation[27] belongs to the family of thiol-based chemoselective ligation methods and is the subject of this section. Interestingly, the thiocarbamate bond is considered to be a peptide bond bioisostere.[62,63] This, combined with the specific features of the S-CO-NH group chemistry or biochemistry, makes thiocarbamate ligation of

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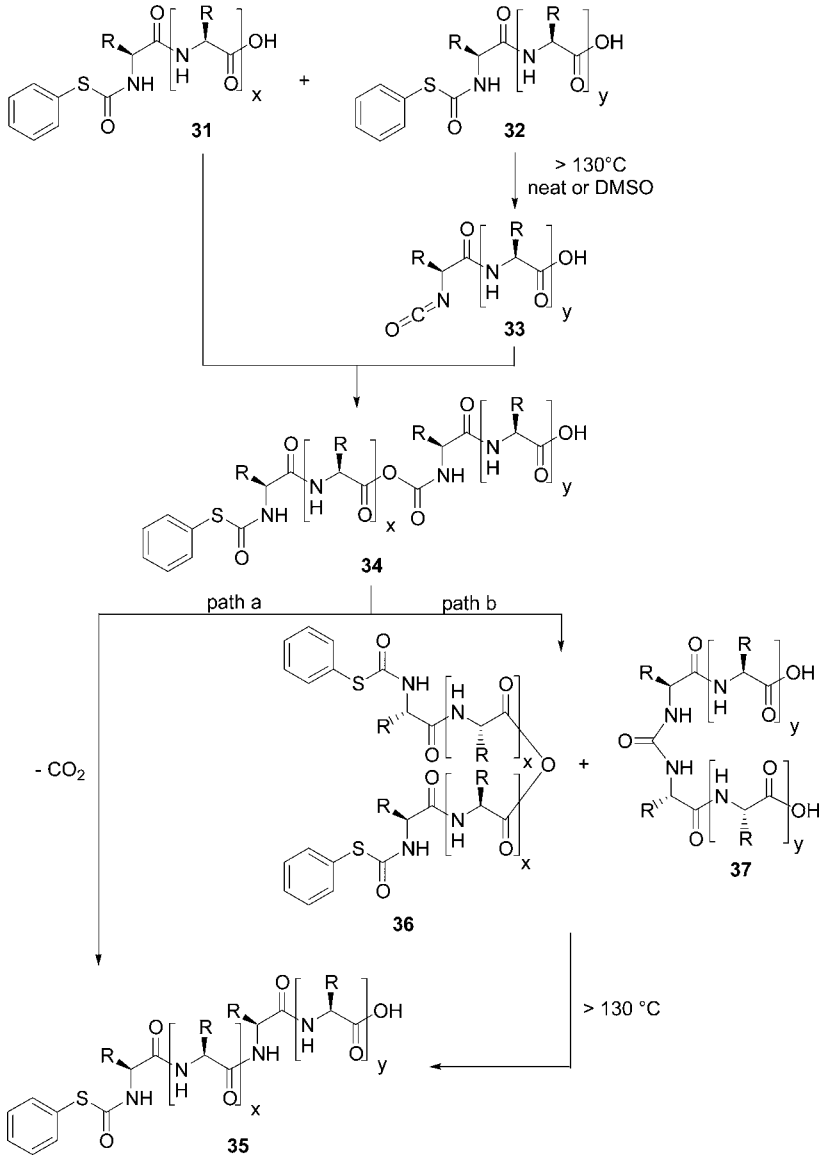
Scheme 7. Synthesis of Polymers Using *N*-Carbothiophenyl Derivatives of Amino Acids or Peptides, Method 1 (>130 °C)[35]



potential interest for building molecules and modulating their physicochemical or biological properties.

The development of thiocarbamate ligation was stimulated by pioneering studies on thioester ligation by Kent and co-workers (Scheme 9).[64,65] Thioester ligation is based on the reaction of peptide thiocarboxylate **38** with bromoacetyl peptide **39** to yield a thioester-linked peptide, **40**.

One significant limitation of the method is the sensitivity of the thioester bond toward hydrolysis at neutral pH or in the presence of nucleophiles. For example, Kent and co-workers noticed that a thioester bond of the type -$NHCH_2COSCH_2CO$- displayed a half-life of 2 h at pH 7.5.[64] However, the thioester analogue of HIV-1 protease reported in their seminal paper was stable in the pH range 4 to 6. Similar results were reported by Tam and co-workers using model thioester peptide Gly-SCH_2-CO-Ala-Lys-Ala.[46] Thus, thioester ligation is best carried out at mildly acidic pH (pH ∼ 4.3) to avoid the hydrolysis of the target product during the ligation reaction. At this pH, the thioacid is mainly in the thiocarboxylate form because of the low pK_a of thioacids and is a better nucleophile than the thiol group of cysteine so that the thiocarboxylate group can be selectively alkylated at pH 4 in the presence of cysteine residues.[66] In contrast, an alkylthiocarbamate bond is stable in a wide pH range (2−7.5). The ligation can be performed in pH 7.4 phosphate buffer or in an organic solvent such as *N*,*N*-dimethylformamide (DMF) in the presence of triethylamine, whereas HPLC monitoring and purification can be done as usual using acidic water−acetonitrile gradients.

Another significant limitation of thioester ligation is the limited access to the thioacid component because of the sensitivity of the thioacid functionality to various nucleophiles, oxidants, and electrophilic reagents. In particular, the Fmoc-SPPS of peptide thioacids is highly challenging, although significant advances have been realized during the past few years.[67,68] In contrast, *N*-phenylthiocarbonyl peptides are easily prepared using Fmoc-SPPS as discussed before.

The principle of the thiocarbamate ligation is presented in Scheme 10. The process is based on the reaction of a thiol such as a peptide featuring a cysteine residue with a phenylthiocarbamate component. Basically, it is a thiol−thioester exchange that proceeds efficiently in water at neutral pH. The

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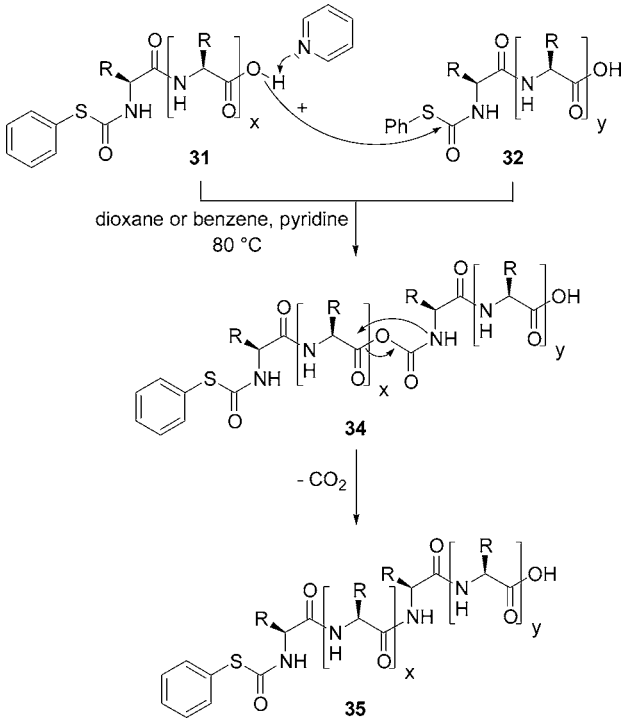
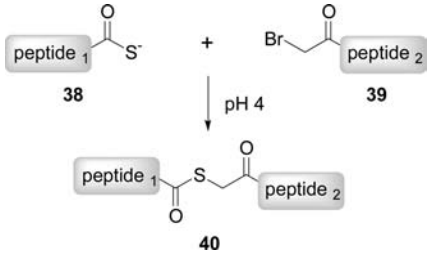
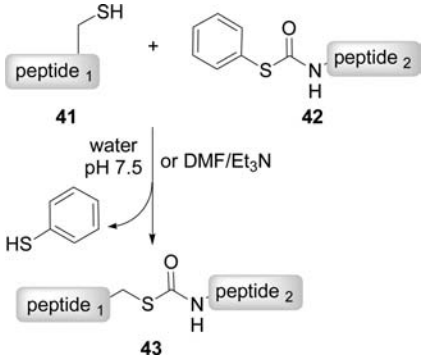
Scheme 8. Synthesis of Polymers Using *N*-Carbothiophenyl Derivatives of Amino Acids or Peptides, Method 2 (80 °C)[26,35]



Scheme 9. Thioester Ligation[64,65]



Scheme 10. Thiocarbamate Ligation[27]

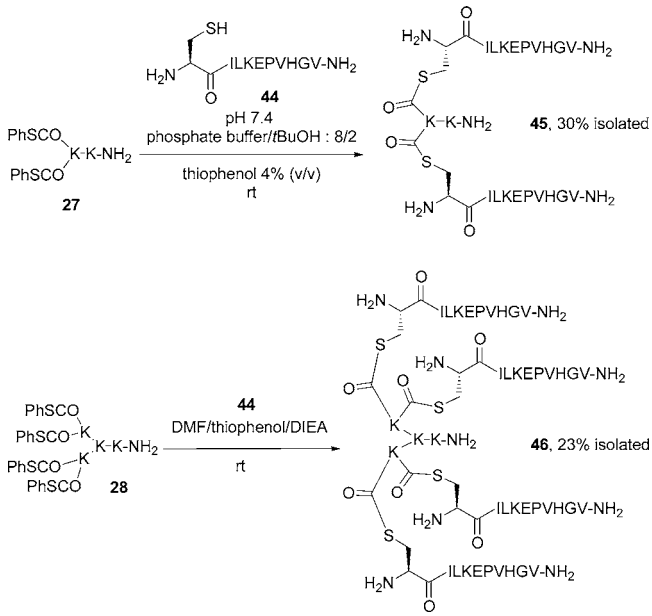


reaction has been performed in the presence of an excess of thiophenol to minimize the oxidation of the cysteine residue by molecular oxygen. However, recent studies have shown that this is not mandatory because of the high kinetic rates for the ligation in most cases.

N,N-Dialkyl-*S*-thiocarbamate compounds have been intensively developed as pesticides. Consequently, examination of this literature gives a lot of information on the stability of this bond toward various chemicals, in the environment, and in living organisms. *S*-Phenylthiocarbamates of type **42** are highly reactive toward alkylthiols. In contrast, *S*-ethyl dipropylcarbamothioate $(n\mathrm{Pr})_2\mathrm{NCOSEt}$ is resistant to hydrolysis and does not participate significantly in transthiocarbamoylation in the presence of thiophenol[69] or glutathione.[70] *N,N*-Diethyl-*S*-*p*-chlorobenzylthiocarbamate, also called thiobencarb (*p*-$\mathrm{ClPhCH_2SCONEt_2}$), is oxidized by aqueous chlorine into thiobencarb sulfoxide, *p*-$\mathrm{ClPhCH_2S(O)CONEt_2}$. Moreover, various *N,N*-dialkyl-*S*-thiocarbamates are oxidized by peracids into sulfoxide or sulfone thiocarbamate derivatives, as discussed earlier (Scheme 2). These derivatives are also the main metabolites formed in living organisms.[8−11,69−72] Unlike the starting *N,N*-dialkyl-*S*-thiocarbamates, the sulfoxide or sulfone derivatives formed by chemical or metabolic S-oxidation are potent carbamoylation agents that react efficiently with cysteine or glutathione. Thus, the formation of thiocarbamate-linked conjugates might find use in the design of prodrug systems.

The efficiency of thiocarbamate ligation is illustrated in Scheme 11 with the synthesis of model multiple antigenic

Scheme 11. Application of the Thiocarbamate Ligation to the Synthesis of Peptide Dendrimers[27]



peptides (MAPs[54,55,73]) built on a divalent or tetravalent lysine dendrimer core, the preparation of which was presented in the section devoted to the synthesis of *N*-phenylthiocarbonyl peptides using SPPS.[27] Divalent dendrimer **45** was assembled in phosphate buffer at neutral pH, whereas the synthesis of tetravalent dendrimer **46** required the use of DMF to solubilize the starting phenylthiocarbonyl lysinyl core **28**. Note that the thiocarbamate ligation product does not rearrange by *S,N*-acyl shift, as is observed for thioesters during the native chemical ligation (NCL) reaction,[74−76] even under forcing conditions. Thus, peptide dendrimer **46** features four amino groups in addition to those displayed by the peptide chains. This certainly improves the solubility of the ligation product in aqueous buffer.

Given the ease of introducing the *N*-phenylthiocarbonyl functionality on peptides and the thiol group on various biomolecules or modifiers, thiocarbamate ligation constitutes an

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interesting chemoselective reaction for obtaining well-defined conjugates.

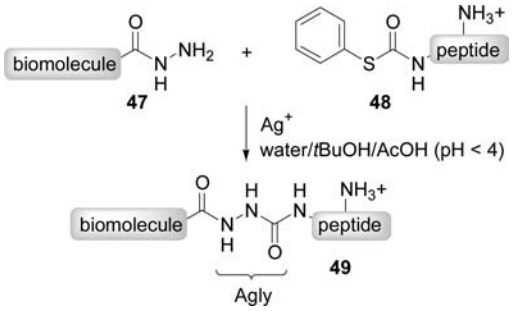
AzaGLY LIGATION: SYNTHESIS OF azaGLY PEPTIDES AND azaGLY-LINKED LIPOPEPTIDES

In an aza-amino acid, the α-carbon is replaced by a nitrogen atom.[77,78] Azapeptides are peptide analogues in which at least one α-amino acid residue is substituted by an aza-amino acid residue. Azapeptides exhibit propensity to adopt a β-turn geometry.[79−83] Aza-amino acid scan has been used to study the relationship between β-turn secondary structure and biological activity.[84,85] Moreover, azapeptides were shown to be more stable in biological medium compared to native peptides, and for this reason, they have been used to improve the stability and bioavailability of peptide drugs.[86]

Glycine is a frequent amino acid in peptides and proteins (7.4% in vertebrates) and is the most flexible among the coded amino acids. The replacement of Gly by azaGly (Agly) induces significant conformational constraints in the peptide chain, a property that has been used for improving the stability and/or potency of bioactive peptides.[87] Thus, methods enabling the synthesis of Agly peptides are of great interest because bioactive peptides very often feature one or several glycine residues. Moreover, the bioactivity and properties of bioconjugates are often dependent on the flexibility and/or stability of the linker regions.[88] Here again, incorporation of an Agly residue can be used to achieve the desired properties.

Agly can be incorporated into peptides using well-developed SPPS methods.[84,85,89−91] Alternatively, the preparation of large Agly peptides or conjugates is best carried out in solution using chemoselective ligation methods. Agly ligation between a hydrazide **47** and an N-terminal phenylthiocarbonyl peptide **48** in the presence of silver ions offers a potential solution to this problem (Scheme 12).[92]

Scheme 12. Agly Ligation[92]



AzaGly ligation is reminiscent of the so-called thioester method developed by Aimoto and co-workers for protein total synthesis.[93,94] This method relies on the activation of peptide thioesters by silver ions for the coupling of fully or minimally protected peptide segments. Besides silver ions, other metal ions such as Hg^{2+} or Tl^{3+} were shown to promote the activation and hydrolysis of phenylthiocarbamates in dilute aqueous acid solution.[25]

Two critical parameters for Agly chemoselective ligation are the use of a high proportion of *tert*-butanol as cosolvent (80%) and the control of the apparent pH, which must be around 4. The role of the cosolvent is to minimize hydrolysis of the *N*-phenylthiocarbonyl group by lowering the concentration of water in the reaction mixture. Another advantage of using a *tert*-butanol/water mixture for the reaction is the possibility to solubilize hydrophobic compounds such as lipids. The apparent pH must be kept around 4 to ensure a good *in situ* protection of ε-amino groups by protonation, which is essential for the chemoselectivity,[95,96] whereas the hydrazide component remains nucleophilic because of its low pK_a (3.24 for acetylhydrazine). The different examples presented in Scheme 13 illustrate the potential of the method for the synthesis of azaGly peptides or lipopeptides with an azaGly linker between the lipid moiety and the peptide. The mildness of the experimental conditions enabled the incorporation of unsaturated lipidic or cholesterol moieties.

SYNTHESIS OF CYCLIC PEPTIDE SCAFFOLDS

The importance of peptide cyclization for studying peptide conformation, for creating new structures, or for developing peptide therapeutics is well-established. Cyclization enables one to rigidify the structure and to improve the interaction with the molecular target, the biological activity,[97] or the stability.[98,99] The synthesis of cyclic peptides can be challenging, and various methods have been designed to affect peptide macrocyclization.[100] For a given peptide sequence, it is also of interest to vary the bond formed in the cyclization reaction because its chemical and conformational properties are expected to affect the properties of the target cyclic scaffold. One important method for producing cyclic peptides is the NCL reaction[74,101,102] between a β-amino thiol group such as a cysteinyl residue and a thioester group or thioester surrogate.[103,104]

The *N*-phenylthiocarbonyl group chemistry has been used for the synthesis of tail-to-side chain cyclic peptides using thiocarbamate ligation, as shown in Scheme 14.[27] For this, an internal lysine residue was modified by an *N*-phenylthiocarbonyl group while a cysteine residue was placed N-terminally. The *tert*-butylsulfenyl group protecting the N-terminal cysteine residue of peptide **30** was removed *in situ* during the cyclization process because of the mild reducing properties of thiophenol present in the reaction mixture. Corresponding cyclic scaffold **53** was isolated successfully after HPLC purification. As mentioned earlier, the thiocarbamate ligation product does not rearrange by *S*,*N*-acyl shift as observed for thioesters during the NCL reaction, even under forcing conditions. Consequently, this method easily enables one to vary the size of the cyclic part of the molecule and its position within the peptide by modifying the position of the lysine residue bearing the *N*-phenylthiocarbonyl group and of the cysteine residue. In contrast, cyclization using NCL has so far been restricted to the preparation of tail-to-head or side chain-to-head cyclized peptides by varying the position of the cysteine residue (N-terminal cysteine,[101] cysteine on a lysine side chain[105]), whereas the thioester functionality was on the C-terminus.

The *N*-phenylthiocarbonyl group also enabled the synthesis of tail-to-head cyclic peptides such as peptide **55** using a carbamate bond forming process catalyzed by a nonribosomal peptide cyclase (Scheme 15).[106] Cyclase-catalyzed hydrolysis of the *N*-phenylthiocarbonyl group of peptide **54** was mentioned as a significant side reaction (cyclization/hydrolysis ratio of 0.78), which could be minimized by using *o*-methyl (cyclization/hydrolysis ratio of 0.73) or, even better, *p*-methoxyphenylthiocarbonyl derivatives (cyclization/hydrolysis ratio of 0.64). In the control experiment in the absence of cyclase, peptide **54** evolved into a different cyclic peptide by reaction of the *N*-phenylthiocarbonyl group with diaminopropionic acid residue at position three to give a urea bond. In this

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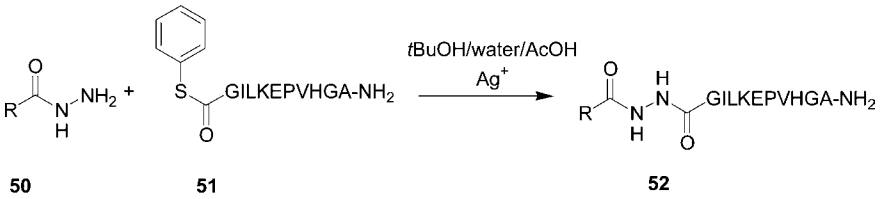
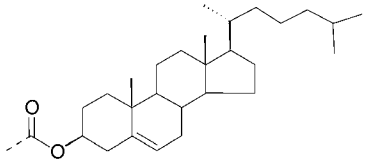
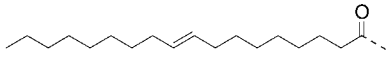
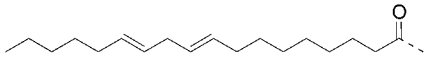
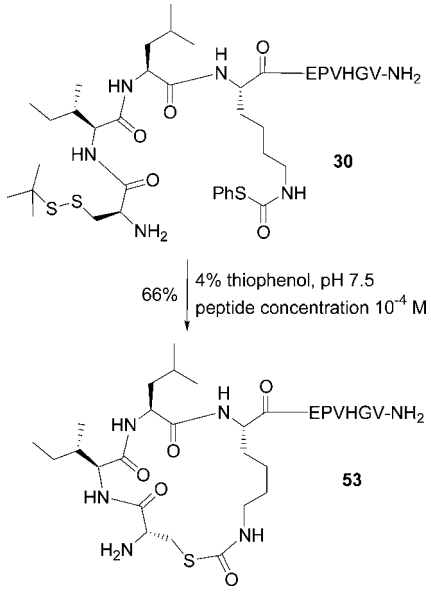
Scheme 13. Synthesis of azaGly Peptides or azaGly-Linked Conjugates[92]



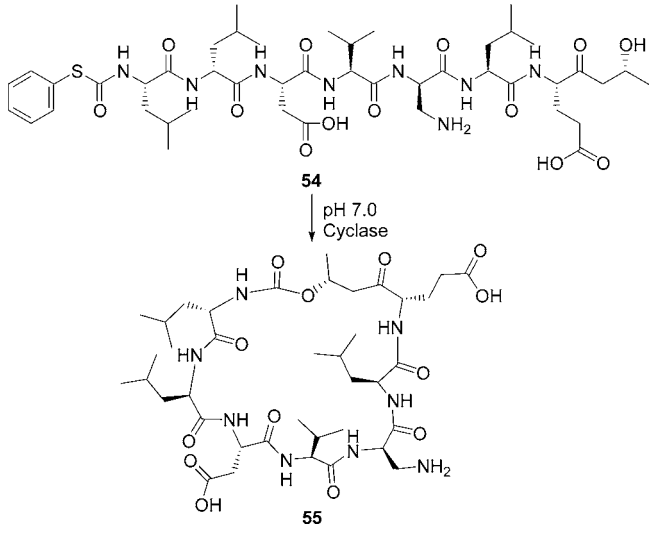
50 51 52

R-CO	Yield for ligation product **52** (%)
	39
	36
	33
	43
Ac-FSLKEPAVHQG---	48
Ac-FSLKEPAVHQA---	41

Scheme 14. Synthesis of a Cyclic Peptide Using Thiocarbamate Ligation[27]



Scheme 15. Nonribosomal Peptide Cyclase Catalyzed the Formation of a Cyclic Peptide with Formation of a Carbamate Bond[106]



case, hydrolysis of the *N*-phenylthiocarbonyl group was not significant. This spontaneous intramolecular urea bond forming reaction has not been studied so far and might represent an interesting entry to novel cyclic peptide scaffolds.

■ CONCLUSIONS

The phenylthiocarbonyl group can be easily incorporated into peptides on α- or ε-amino groups using commercially available phenylthiochloroformate and standard Fmoc-SPPS protocols. *N*-Phenylthiocarbonyl peptides can be purified using standard HPLC procedures and are stable upon storage. Nevertheless, the *N*-phenylthiocarbonyl group is an active thiol ester that can participate in a variety of useful chemical transformations, enabling the synthesis of dendrimers, cyclic peptides, or peptide conjugates. Recent synthetic applications have exploited its thioester surrogate properties. In particular, the *N*-phenylthiocarbonyl group participates efficiently in a thiol−thioester exchange reaction with alkylthiols to yield *S*-alkylthiocarbamate-linked scaffolds. Alternately, it can react chemoselectively

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with hydrazide nucleophiles upon activation with silver ions to form an azaGly bond. Another interesting application examined the activation of the *N*-phenylthiocarbonyl group by non-ribosomal peptide cyclases to produce peptides cyclized through the formation of a carbamate bond. We believe that the ease of synthesis of *N*-phenylthiocarbonyl peptides will certainly stimulate novel synthetic developments and biological applications.

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Notes

The authors declare no competing financial interest.

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