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Synthesis of Peptide Thioacids at Neutral pH Using Bis(2-sulfanylethyl)amido Peptide Precursors

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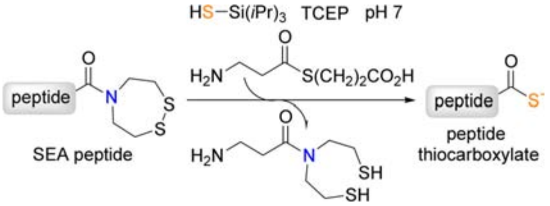
ABSTRACT



Reaction of bis(2-sulfanylethyl)amido (SEA) peptides with triisopropylsilylthiol in water at neutral pH yields peptide thiocarboxylates. An alkylthioester derived from β-alanine was used to trap the released bis(2-sulfanylethyl)amine and displace the equilibrium toward the peptide thiocarboxylate.

C-terminal peptide thioacids are used in a variety of useful chemoselective reactions and therefore constitute an important class of peptide derivatives.[1] For example, selective activation of C-terminal peptide thioacids by silver ion,[2a] Sanger and Mukaiyama reagents,[2b] arylsulfonamides,[2c] bromoacetic acid,[2d] or oxidants[2e] enables the block synthesis of peptides. The nucleophilic properties of the thioacid group toward bromoalkyl,[3a] disulfide,[3b] isocyanate or isothiocyanate,[3c] aziridine,[3d] isonitrile,[3e] or azide functionalities[4] were also used for designing various useful native or peptidomimetic amide bond-forming reactions.

The development of synthetic methods giving access to C-terminal peptide thioacids is thus an important goal. It is also challenging because of the sensitivity of thioacids to nucleophiles, oxidants, and various electrophilic reagents. Peptide thioacids can be produced by the Boc[5a,b] or Fmoc[5c] solid-phase peptide synthesis (SPPS) methods. The thioester linkage linking the peptide to the solid support was cleaved with strong acids[5] or by hydrothiolysis[5c,d] in aqueous base to release the peptide thioacid in solution. An alternative is to use soluble peptide thioester precursors to generate the peptide thioacid by hydrothiolysis[6] or by base-induced elimination from *S*-(9-fluorenylmethyl)[7] or

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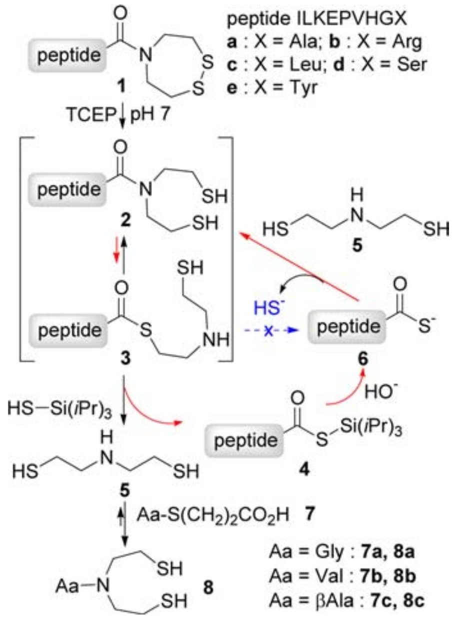
10.1021/ol402601j

S-(2-cyanoethyl)[8] thiocarboxylates. The need for a milder access to peptide thioacids led us to develop a method allowing the synthesis of these useful peptide derivatives in water at neutral pH.

The principle of the method is described in Scheme 1 and exploits the masked thioester properties of bis(2-sulfanylethyl)amido (SEA) peptides **1**,[9] which can be produced by Fmoc SPPS. Reduction of SEA peptide **1** with tris(2-carboxyethyl)phosphine (TCEP) at neutral pH generates dithiol peptide **2**, which equilibrates in situ with the transient thioester peptide **3**. At the beginning of this work, we expected that hydrothiolysis of peptide **3** with NaSH could yield directly target thiocarboxylate **6**. However, reaction of model peptide **1a** with an excess of NaSH in pH 7 sodium phosphate buffer in the presence of TCEP and 4-mercaptophenylacetic acid[10] (MPAA) to catalyze the exchange reaction failed to give any peptide thiocarboxylate **6a**. We next examined if triisopropylsilylthiol could serve as a hydrogen sulfide anion surrogate. Indeed, we reasoned that the thiol−thioester exchange reaction between peptide **3** and triisopropylsilylthiol could yield peptide thiocarboxylate **6** after in situ hydrolysis of intermediate triisopropylsilyl thioester **4**. Gratifyingly, reaction of peptide **1a** with triisopropylsilylthiol, TCEP, and MPAA at pH 7 yielded thiocarboxylate **6a**, albeit with an HPLC yield of only ~30−35% after 20 h (Figure 1a).[11] Note that the conversion and rate of thiocarboxylate formation was significantly lower in the absence of MPAA.[12]

LC−MS analysis of the reaction mixture in the presence of MPAA showed essentially two peaks corresponding to unreacted dithiol peptide **2a** and thiocarboxylate **6a**. Unexpectedly, longer reaction times resulted in the reverse process, that is an increase of peptide **2a** proportion in the mixture to the detriment of thiocarboxylate **6a** (Figure 1a). This phenomenon can be understood by considering (i) the capacity of thiocarboxylate **6** to react efficiently with amino thiol **5** to give peptide **2** plus hydrogen sulfide ion,[13] (ii) the fact that the latter does not react with peptide **2** to give thiocarboxylate **6** as discussed before, and (iii) the concentration of triisopropylsilylthiol is limited. Under these conditions, the cyclic process highlighted in red in Scheme 1 is fueled with triisopropylsilylthiol and produces hydrogen sulfide ion, which presumably escapes from the reaction mixture as hydrogen sulfide (pK_a 7.05). It runs until the concentration of triisopropylsilylthiol is not high enough to sustain the production of thiocarboxylate **6**. Interestingly, feeding the reaction again with triisopropylsilylthiol after 49 h allowed us to maintain the proportion of peptide thiocarboxylate **6a** in the mixture, in accordance with the proposed mechanism (Figure 1a). We noticed also that prolonged reaction times resulted in the appearance of an organic phase, which by sequestering triisopropylsilylthiol might amplify the decrease of triisopropylsilylthiol concentration in the aqueous phase and thus the reversal of the reaction. The use of guanidinium hydrochloride as an additive (3.7 M) in addition to *t*-BuOH solved this problem and retarded the reversal of peptide thiocarboxylate formation (Figure 1a).

Scheme 1. Conversion of SEA Peptides into Peptide Thioacids by Reaction with Triisopropylsilyl Thiol



With this knowledge, we reasoned that capturing amino thiol **5** would favor the formation of target thiocarboxylate **6**. We used for this the capacity of alkylthioesters **7** to react with *β*-amino thiol **5** to give amides **8** by analogy with the native chemical ligation (NCL) of peptide alkylthioesters with Cys peptides.[14] Interestingly, the use of 2.3 equiv of Gly-S$(CH_2)_2CO_2H$ **7a**[15] resulted in a significant increase in the yield of thiocarboxylate **6a** (Figure 1b).[16] LC−MS

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[11] Triisopropylsilyl thioester **4a** was not detected by LC−MS in the reaction mixture. The presence of thiocarboxylate **6a** was demonstrated by alkylation experiments with iodoacetamide and LC−MS detection of the formed peptide thioester ILKEPVHGA-SCH_2CONH_2.

[12] In the absence of MPAA, we also observed the formation of side products arising from the desulfurization of dithiol peptide **2a**.

[13] Reaction of purified peptide **6a** (4.8 mM) with amino thiol **5** (4.8 mM) at pH 7 (30% *t*-BuOH) and 37 °C in the presence of TCEP (30 mM) and MPAA (60 mM) cleanly furnished peptide **2a** with a half-life of ~15 h (see Figure S29 in the Supporting Information). As discussed in ref 2e, the capacity of thioacids to acylate amines might proceed through the oxidation of the thioacid into an diacyl disulfide by traces of molecular oxygen.

[14] Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. *Science* **1994**, *266*, 776–779.

[15] Thioesters **7a**−**c** were prepared according to the method of Hojo et al. Hojo, H.; Aimoto, S. *Bull. Soc. Chem. Jpn* **1991**, *64*, 111–117.

[16] A similar kinetic profile was obtained by using 3.3 equiv of **7a**.

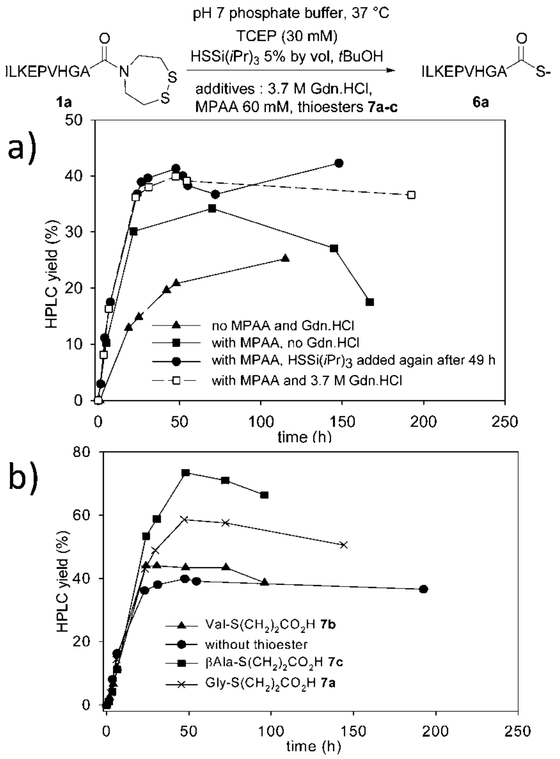

Figure 1. Time course of the conversion of SEA peptide **1a** into thiocarboxylate **6a** (detection at 215 nm).

analysis of the mixture showed also the formation of amide **8a** in accordance with the mechanism proposed in Scheme 1 (see the Supporting Information). In contrast, no significant improvement in the yield of thiocarboxylate **6a** was observed by using Val-S(CH$_2$)$_2$CO$_2$H **7b** as trapping agent. The fact that valine thioester **7b** is a poor trapping agent compared to glycine analogue **7a** is due presumably to the reduced accessibility of the valine carbonyl group. Given the reversibility of amide **8** formation (Scheme 1), we reasoned that the ideal thioester trapping agent **7** should react efficiently with amino thiol **5** such as Gly thioester **7a**, but the formed amide **8** should be a poor amino thiol **5** donor.

Kinetic studies with C-terminal SEA peptides established that ligation with Cys peptides proceeded faster for Gly as C-terminal residue than for other amino acid residues.[9g] Figure 2 shows the time course of the ligation of peptide ILKEPVHGG-SEA **9** with CILKEPVHGV-NH$_2$ **10** at pH 7 in the presence of TCEP and MPAA (200 mM each). In other words, SEA peptides featuring a C-terminal Gly residue are good amino thiol **5** donors. In contrast, the reactivity of peptide Ac-Asp(SEA)-ILKEPVHGA-NH$_2$ **11** featuring a SEA group on the side chain of an aspartate residue was significantly altered, maybe due to the lack of an amido group in α position relative to the SEA carbonyl group (Figure 2).[9d] We also noticed that the kinetic rate for the reaction of peptide ILKEPVHG-βAla-SEA **12** with Cys peptide **10** was very similar to those obtained for Asp(SEA) peptide **11**. These results show that the kinetic profile for peptides **11** and **12** is dictated by the 3-aminopropionamide structure of Asp(SEA) or βAla-SEA moieties rather than by their position within the peptide structure. The important consequence of these observations is that βAla-SEA amide **8c** is probably a poor amino thiol **5** donor compared to Gly-SEA amide **8a**.

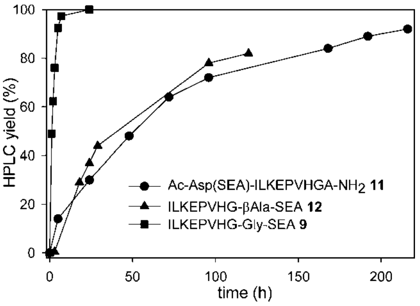

Figure 2. Time course of the ligation of peptides **9**, **11**, or **12** with CILKEPVHGV-NH$_2$ at pH 7 and 37 °C (TCEP 200 mM, MPAA 200 mM).

We thus evaluated the relevance of βAla-S(CH$_2$)$_2$CO$_2$H **7c** as trapping agent. Gratifyingly, the use of 2.3 equiv of **7c** led to a net increase in the yield of thiocarboxylate **6a** compared to Gly analogue **7a**. The best yield was obtained after 48 h of reaction at 37 °C (Figure 1b).

These experimental conditions proved to be efficient for different C-terminal amino acids such as Ala, Arg, Leu, Ser, and Tyr as shown in Table 1. In each case, LC–MS analysis of the reaction mixtures showed the formation of amide **8c** in parallel to those of thiocarboxylates **6a**–**e** (Figure 3). Using these conditions, thiocarboxylate **6a** was isolated in 45% yield (52% corrected, entry 1, Table 1). Note that the purification of thiocarboxylates **6a**–**e** was carried out by HPLC using triethylamine–acetate pH 6.5 buffer, which to the contrary of 0.1% TFA water/acetonitrile eluent preserves the peptide thiocarboxylates from partial degradation during the lyophilization step.[17] Figure 3 shows the LC–MS analysis of the crude thiocarboxylate **6e** as a typical example.

Table 1. Synthesis of Thiocarboxylates **6a**–**e**[a]

entry	thiocarboxylate	X	isolated yield (%)
1	**6a**	Ala	45 (52% corrected)
2	**6b**	Arg	44
3	**6c**	Leu	27
4	**6d**	Ser	32
5	**6e**	Tyr	47

[a] Peptide **1** (4.1 mM), thioester **7c** (9.4 mM), TCEP (27 mM), and MPAA (56 mM) in 0.1 M pH 7.1 sodium phosphate buffer, 30% *t*BuOH, 3.7 M Gdn.HCl, 37 °C under nitrogen atmosphere.

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(17) Contrary to thiocarboxylates, thioacids hydrolyze upon storage; see ref 3d and: Mitin, Yu, V.; Zapevalova, N. P. *Int. J. Pept. Protein Res.* **1990**, *35*, 352–356.

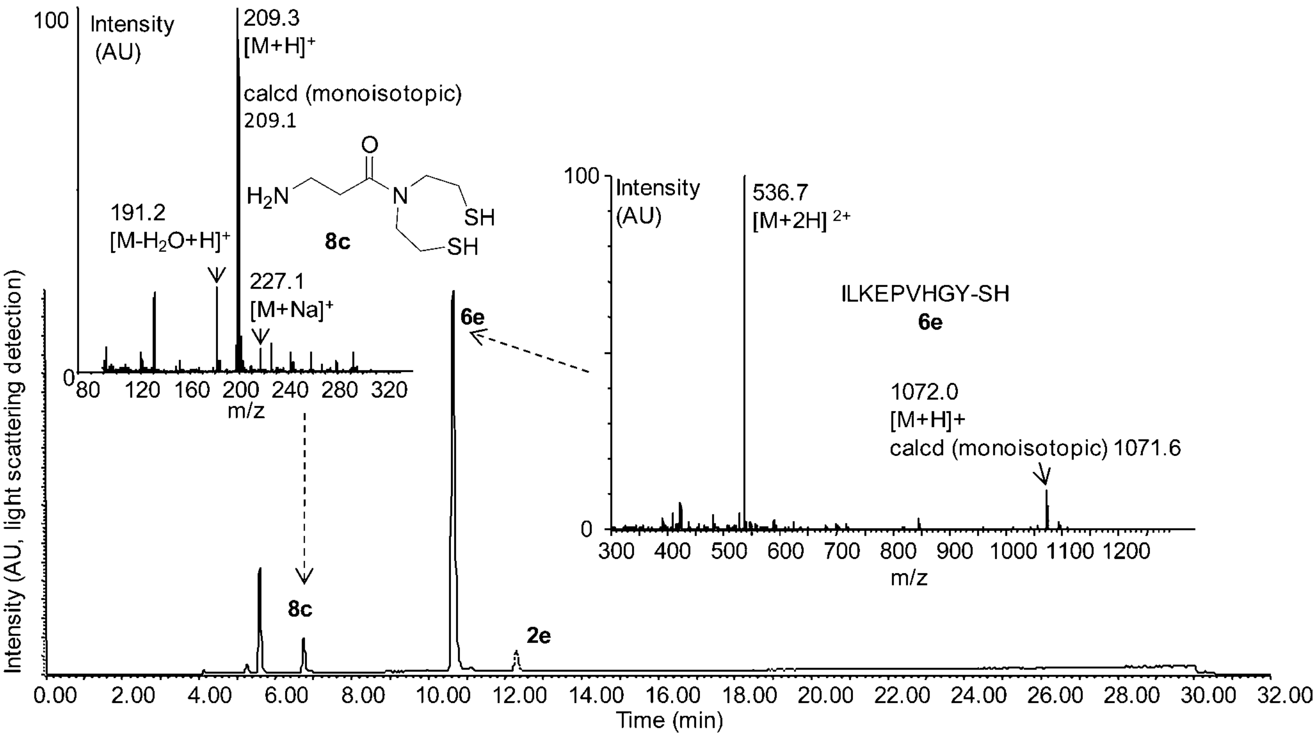
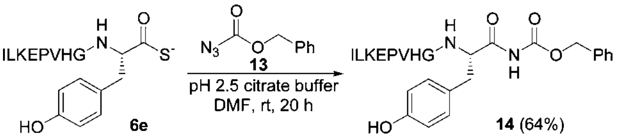


Figure 3. Synthesis of peptide ILKEPVHGY-SH **6e**. LC–MS analysis of the crude reaction mixture (after extraction of MPAA).

Scheme 2. Synthesis of Imide **14**



Finally, the functionality of thiocarboxylates **6** was verified by the chemoselective imide ligation of peptide **6e** with azidoformiate **13** at pH 2.5 to give imide **14** (64% isolated, Scheme 2).[4f,g] Moreover, chiral GC–MS analysis of the imide ligation product **14** after acid hydrolysis indicated a D-Tyr content of only 0.28% and thus that racemization of C-terminal Tyr residue was negligible for both the exchange and imide ligation process.

In conclusion, C-terminal SEA peptides can be converted into the corresponding thiocarboxylates at pH 7 by reaction with triisopropylsilylthiol in the presence of an alkylthioester derivative of β-alanine. The reaction is very mild and racemization free and should facilitate the access to these highly valuable peptide derivatives.

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Supporting Information Available. Experimental procedures and characterization data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.