Note

Facile Preparation of Peptides with C-Terminal *N*-Alkylamide *via* Radical-Initiated Dethiocarboxylation

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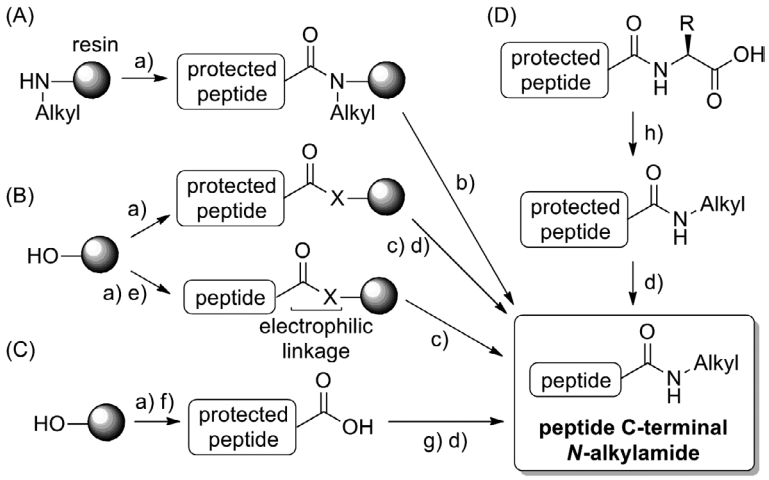
A new synthetic method has been developed to prepare peptides bearing a C-terminal *N*-alkylamide from peptide thioacids *via* a radical-initiated dethiocarboxylation process. This method enables the introduction of various alkyl groups to C-terminal amides simply by replacing the amino acid building block. Its application to the preparation of anti-cancer drug ABT-510 is also reported.

Key words C-terminal *N*-alkylamide; peptide thioacid; dethiocarboxylation

Peptides with C-terminal modifications have attracted much attention as pharmaceutical agents and biological tools. Peptides bearing a C-terminal *N*-alkylamide have been investigated extensively because of its pronounced effects on the biological activity and metabolic stability of the parent peptides,[1–5] and they are usually prepared from commercially available amino acid building blocks using an on- or an off-resin protocol. For the on-resin protocol, the peptides are typically constructed on an *N*-alkylamino resin. This protocol therefore requires preparing an alkylamino resin, followed by the coupling of the C-terminal amino acid on the less reactive secondary amine[6] (Chart 1A). To avoid the coupling of the first amino acid on the secondary amine, an alternative method employing on-resin aminolysis of an electrophilic peptide-resin linkage was reported[7,8] (Chart 1B). Compared with the on-resin protocol, the off-resin protocol is much more straightforward and involves the condensation of a C-terminal carboxylic acid of a peptide with an amine (Chart 1C). However, this protocol requires protecting almost all of the reactive functional groups present on the substrates, except for the C-terminal carboxylic acid, followed by the deprotection of those groups after the condensation. Yoshimi and colleagues[9] and several others[10–12] recently reported a new off-resin protocol for converting side-chain-protected peptides to the corresponding peptide alkylamides *via* a photo-induced radical decarboxylation reaction (Chart 1D). However, these methods require the protection of the non-C-terminal side-chains to avoid the occurrence of decarboxylation and photo-induced side reactions. Therefore, we speculated that the C-terminal selective decarboxylation of peptides in the presence of other carboxylic acids without photo-irradiation would provide practical method for preparing peptide alkylamides, and avoid laborious deprotection after the decarboxylation.

We previously reported a synthetic method for preparing peptide thioacids using *N*-sulfanylethylanilide (SEAlide)[13–19] (Chart 2). SEAlide peptide **1**, which was constructed using Fmoc solid-phase peptide synthesis (SPPS), was successfully converted to peptide thioacid **2** *via* a phosphate-mediated *N–S* acyl transfer reaction, followed by a hydrothiolysis step. During that study, we unexpectedly found that peptide thioacid **2** could be converted to the C-terminal *N*-alkylamidated peptide **3** under radical desulfurization conditions, which have



a) Solid-phase peptide synthesis (SPPS); b) Global deprotection (deprotection and cleavage from the resin); c) Aminolysis; d) Deprotection; e) Deprotection on the resin; f) Cleavage from the resin; g) Condensation with an alkyl amine; h) Photo-induced radical decarboxylation.

Chart 1. Preparation of Peptides Bearing a C-Terminal *N*-Alkylamide

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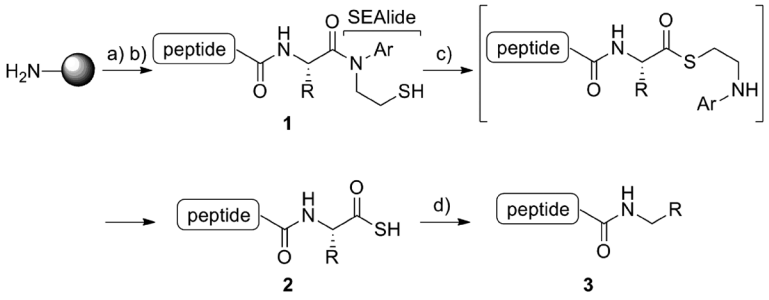
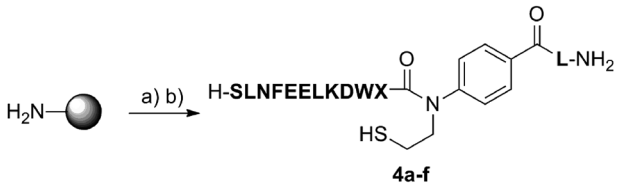


a) Fmoc SPPS; b) Global deprotection; c) *N*–*S* acyl transfer followed by hydrothiolysis in the presence of phosphate (one pot); d) Radical-initiated dethiocarboxylation.

Chart 2. Preparation of Peptides Bearing a C-Terminal *N*-Alkylamide Using *N*-Sulfanylethylanilide (SEAlide)



	X
a	Gly
b	Ala
c	Ser
d	Lys
e	Arg
f	Tyr

Reagents and conditions: a) Fmoc SPPS; b) Trifluoroacetic acid (TFA)–*m*-cresol–thioanisole–1,2-ethanedithiol (EDT)–H_2O (80 : 5 : 5 : 2.5 : 7.5 (v/v)); c) 120 mM $(NH_4)_2S$–100 mM tris(2-carboxyethyl)phosphine hydrochloride (TCEP·HCl)–250 mM Na ascorbate–1 M Na phosphate buffer (pH 7).

Chart 3. Preparation of Peptide Thioacids *via* Corresponding SEAlide Peptides

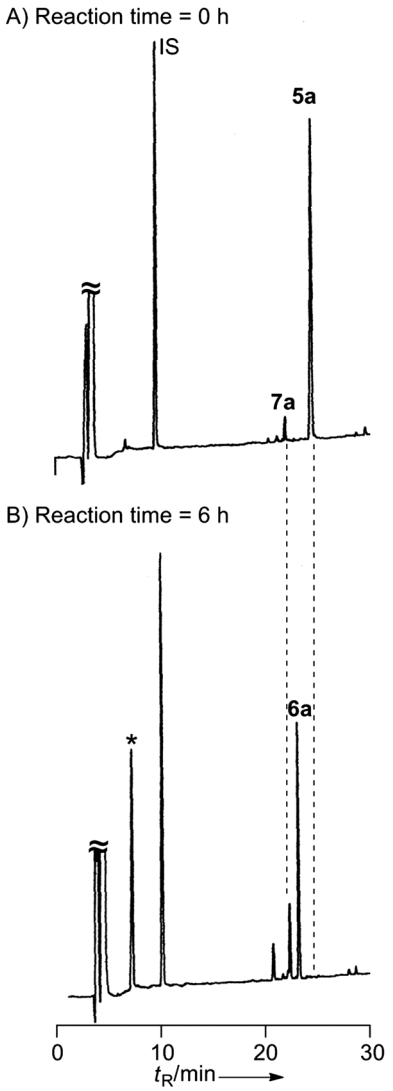


Fig. 1. HPLC Monitoring of the Dethiocarboxylation of Thioacid **5a** (Table 1, Entry 1)

Internal standard (IS)=benzamide. The asterisk indicates the presence of a non-peptidic compound, which was derived from the buffer.

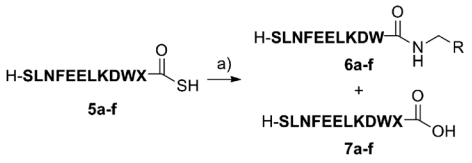
been widely used for conversion of cysteine to alanine.[20] Notably, the reaction did not require protecting the amino acid side chains, except for the cysteine residue, which should be protected to avoid conversion to alanine. The radical dethiocarboxylation step could therefore be used to prepare non-protected peptides without a cysteine. With this in mind, we investigated this method as an efficient strategy for forming peptide alkylamides *via* the radical-initiated dethiocarboxylation of suitable peptide thioacids.

Peptide thioacids **5a**–**f** bearing various C-terminal amino acids were initially prepared from the corresponding SEAlide peptides **4a**–**f** *via* Fmoc SPPS followed by hydrothiolysis, as shown in Chart 3.[19] We synthesized derivatives of N-terminal human RFamide-related peptide-1 (hRFRP-1) (1–11)[21] consisting of non-protected residues, some of which are not compatible with previous methods.[9–12]

The radical dethiocarboxylation of peptide thioacid **5a** was initially investigated under the reaction conditions typically used for the desulfurization of a cysteine residue. The progress of this reaction was monitored by HPLC (Fig. 1), and the results are summarized in Table 1. The results revealed that the peptide thioacid **5a** containing unprotected Asp, Glu, Lys and Trp residues was successfully converted to the corresponding amide **6a** within 6 h in high yield (Table 1, entry 1). Notably, the hydrolyzed carboxylic acid **7a** was characterized as the major byproduct of this reaction.

The dethiocarboxylation protocol was subsequently applied to peptides **5b**–**f** bearing a wide range of typical functional groups on the side chain of their C-terminal amino thioacids (Table 1, entries 2–6). Acidic/nucleophilic C-terminal residues (*e.g.*, Asp, Glu/Asn, Gln) were not examined in the current study because of issues associated with preparing the corresponding peptide thioacids using SEAlide.[19] In the case of the Ala-containing thioacid **5b**, the corresponding ethylamide **6b**

Table 1. Preparation of Peptides Bearing a C-Terminal *N*-Alkylamide from the Corresponding Peptide Thioacids



	X	R
a	Gly	H
b	Ala	Me
c	Ser	CH_2OH
d	Lys	$(CH_2)_4NH_2$
e	Arg	$(CH_2)_3NHC(=NH)NH_2$
f	Tyr	4-hydroxybenzyl

Entry	Peptide	Reaction time (h)	Ratio of **6** (%)[b]	Ratio of **7** (%)[c]
1	**5a**	6	74	26
2	**5b**	6	94	6
3	**5c**	4	100	0
4[d]	**5d**	4	100	0
5	**5e**	2	92	8
6	**5f**	6	92	8

Reagents and conditions: *a*) 0.9 mM **5**, 5 M guanidine·HCl, 0.1 M Na phosphate, 9 mM glutathione, 34 mM TCEP·HCl, 21 mM VA-044, 37°C, pH 7. The ratios of **6** and **7** were determined by HPLC separation (detection at 220 nm) and subjected to the following calculation: (integ.=peak area). *b*) Ratio of **6**=integ. **6**/(integ. **5**+integ. **6**+integ. **7**)×100; *c*) Ratio of byproduct **7**=(integ. **7**)/(integ. **5**+integ. **6**+integ. **7**)×100; *d*) A small peak its retention time was identical to that of **5d** was observed by HPLC. However, MS analysis suggested that this peak was not **5d** but some other peptide derivative (LRMS: Calcd for $[\mathbf{5d}+2H]^{2+}$, 712.9; Obs. 640.3^{2+}; Relative peak area after 4 h of the reaction: **6d**–the peptide derivative=94 : 6).

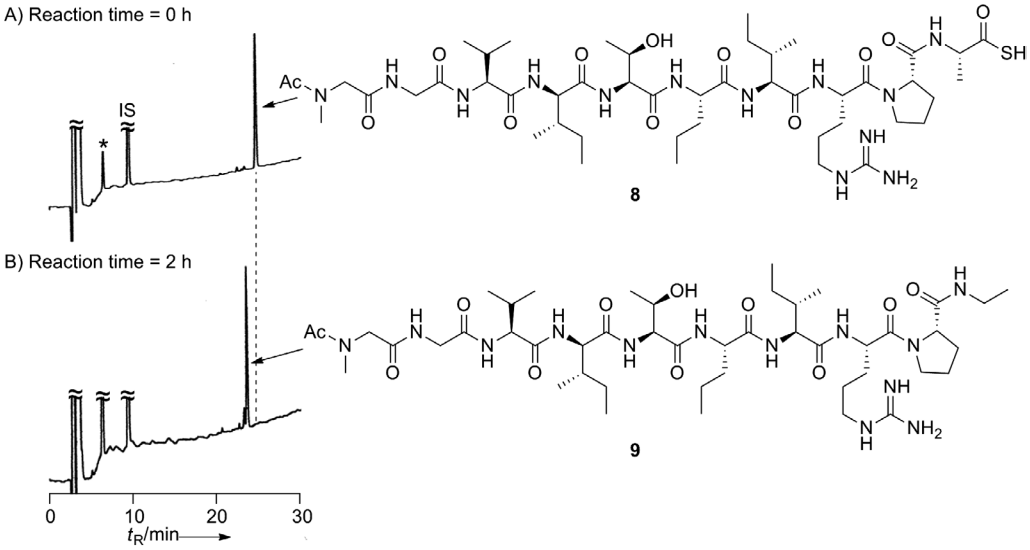


Fig. 2. Preparation of ABT-510 (**9**) Using Our Radical-Induced Dethiocarboxylation Process

Internal standard (IS)=benzamide. The asterisk indicates the presence of a non-peptidic compound, which was derived from the buffer.

was obtained in high yield. However, the hydrolysis reaction of **5b** occurred at a slower rate than that of **5a**, presumably because of steric hindrance from the methyl group. Higher yields were also observed for thioacids **5c**–**f** bearing Ser, Lys, Arg and Tyr residues at their C-terminal, respectively, compared with the yield of **5a** (Table 1, entries 3–6). These results therefore demonstrate that our method is reliable for preparing the C-terminally alkylated peptides.

Finally, to test the utility of our newly developed dethiocarboxylation reaction, we investigated its application to synthesizing anticancer agent ABT-510 bearing a C-terminal ethylamide[22] (Fig. 2). The precursor thioacid **8**, which was prepared by the hydrothiolysis of the corresponding SEAlide peptide **S1** (Fig. S1 in the Supplementary Materials section),[19] was successfully converted to ABT-510 **9** by the dethiocarboxylation in a high isolated yield of 47%. This result therefore demonstrates that our method is practical for preparing C-terminally alkylated peptides.

In conclusion, we have developed a new method to prepare peptide bearing a C-terminal *N*-alkylamide from the corresponding peptide thioacids *via* a dethiocarboxylation reaction. This method not only allows for the introduction of various alkyl groups to the C-terminal amide of peptides by replacing the amino acid building block, but also sheds light on the utility of thioacids. We believe that this dethiocarboxylation process represents facile and reliable reaction to prepare peptides bearing a C-terminal *N*-alkylamide.

Experimental

General Methods For analytical HPLC separations, a Cosmosil $5C_{18}$-AR-II analytical column (Nacalai Tesque, 4.6×250 mm, flow rate 1.0 mL/min) was employed, and eluting products were detected by UV at 220 nm. Mobile phases consisting of 0.1% (v/v) TFA in H_2O (solvent A) and 0.1% TFA (v/v) in acetonitrile (solvent B) were used for the elution of the HPLC system with a linear gradient of solvent A in solvent B over 30 min. Characterization data for the peptides are shown in the Supplementary Materials section.

Preparing C-Terminal Peptide Thioacids 5 and 8 Peptide thioacids **5** and **8** were prepared from the corresponding SEAlide derivatives according to a previously reported method.[19)]

Preparing Peptides with C-Terminal *N*-Alkylamide 6 and 9 To peptide thioacid **5** or **8** (0.2 μmol) was added a 6 M solution of guanidine hydrochloride–0.1 M Na phosphate buffer (200 μL, pH 7) containing 10 mM of glutathione and 40 mM of TCEP·HCl. The resulting mixture was treated sequentially with an aqueous solution of VA-044 (200 mM, 25.2 μL) and a 1% aqueous benzamide solution (10 μL), and the resulting reaction mixture was incubated at 37°C. Two microliter aliquots of the reaction mixture were periodically withdrawn and analyzed using analytical HPLC to monitor the progress of the reaction.

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Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

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