Dynamic Article Links 🕟

# Organic & Biomolecular Chemistry

Cite this: Org. Biomol. Chem., 2011, 9, 4653

# *N*-Methyl-phenacyloxycarbamidomethyl (Pocam) group: a novel thiol protecting group for solid-phase peptide synthesis and peptide condensation reactions<sup>†</sup>

Hidekazu Katayama,\* Yoshiaki Nakahara and Hironobu Hojo\*

*Received 17th February 2011, Accepted 4th April 2011* DOI: 10.1039/c1ob05253e

In the so-called thioester method for the condensation of peptide segments, protecting groups for amino and thiol groups are required for chemoselective ligation. In this study, we developed a novel thiol protecting group, *N*-methyl-phenacyloxycarbamidomethyl (Pocam). We used it for protection of cysteine side chains, and synthesized Pocam-containing peptides and peptide thioesters. These were condensed by the thioester method. After the condensation reaction, Pocam groups were cleaved by Zn/AcOH treatment. At the same time, the azido group, which was used for the protection of lysine side chains, was also converted to an amino group, demonstrating that this protecting group strategy simplified the deprotecting reaction after the peptide condensation reaction to only one step.

# Introduction

The solid-phase peptide synthesis (SPPS) method is generally limited to a length of approximately less than 50 residues long. To synthesize long peptide chains, methods for the condensation of peptide segments, such as native chemical ligation,<sup>1</sup> traceless Staudinger ligation<sup>2</sup> and thioester method,<sup>3</sup> have been developed. Although a cysteine residue is usually required at the ligation point in the native chemical ligation method, there is no limitation at the ligation point in the thioester method. In return, protecting groups for amino and thiol groups are required for the chemoselective condensation. The tert-butoxycarbonyl (Boc) group was traditionally used for the amino protecting group.<sup>3</sup> Recently, we demonstrated that the azido group acted as an effective amino protecting group for SPPS and the peptide condensation reaction by the thioster method.<sup>4</sup> In our previous study, we achieved the total chemical synthesis of a large glycoprotein using this azidobased strategy.<sup>5</sup> Until now, the acetamidomethyl (Acm) group was generally used for the protection of a thiol group in peptide condensation reactions. This protecting group is stable under acidic and basic conditions used for 9-fluorenylmethoxycarbonyl (Fmoc)-SPPS method, and specifically removed by silver ion treatment under weakly basic conditions.6

After the peptide condensation reaction by the thioester method, the protecting groups on the amino and thiol groups must be removed. Until now, two deprotection steps were needed; for example, Zn/AcOH and silver ion treatments for azido and Acm groups, respectively. Since the conditions of the deprotecting reactions, such as solvent and pH, were quite different from each other, purification step(s) would have been necessary between the deprotection steps, and the yield of the final product reduced. If there is a thiol protecting group which is cleaved simultaneously with amino protecting group, deprotection steps would be simplified to only one step and the yield might be higher.

In our azido-based strategy, an azido group was converted to an amino group by reduction using Zn/AcOH.<sup>4,5</sup> Since this reaction can reduce disulfide bonds, disulfide-type protecting groups such as *tert*-butylthio can be used for such purpose. Several preparation methods for peptide thioester compatible with Fmoc-SPPS have been developed.<sup>7,8</sup> In these methods however, an excess amount of thiol compound is usually used, and disulfide bond cannot remain intact under such conditions. Therefore, a novel thiol protecting group that is cleaved by Zn/AcOH but stable under reducing conditions by thiol compounds is required. In this study, we tried to develop a novel thiol protecting group with such characteristics.

# **Results and discussion**

# Design of the thiol protecting group

We considered that the protected aminomethyl group might act as a thiol protecting group similarly to the Acm group. The removal of the amino protecting group generates a thiohemiaminal structure, and the aminomethyl part is spontaneously cleaved due to its lability. Since we used an azido group as an amino protecting group for Lys side chain, it was likely that the azido group was also valid for the protection of aminomethyl portion. However, the azidomethyl group on sulfur atom is labile even under trifluoroacetic acid (TFA) acidic conditions (data not

Department of Applied Biochemistry, Faculty of Engineering, Tokai University, Hiratsuka, Hiratsuka, Kanagawa 259-1292, Japan. E-mail: katay@ tokai-u.jp, hojo@keyaki.cc.u-tokai.ac.jp; Fax: +81 463 50 2075; Tel: +81 463 50 2075

<sup>&</sup>lt;sup>†</sup> Electronic supplementary information (ESI) available: RP-HPLC chromatograms and <sup>1</sup>H-NMR spectra of compounds **1**, **5** and **7**, and Supplementary figures. See DOI: 10.1039/c1ob05253e

shown). Therefore, it is difficult to use it as a thiol protecting group, and another amino protecting group that could be removed by Zn reduction was desired.

It is widely known that the phenacyl group is an efficient protecting group for carboxylic acid and can be removed by the reducing reaction with Zn powder under acidic conditions.<sup>9</sup> It was likely that the phenacyloxycarbonyl (Poc) group might be a good candidate for our purpose. To confirm that Poc group acts efficiently as an amino protecting group, we synthesized Fmoc-Lys(Poc)-OH and checked its characteristics.

#### Stability of the phenacyloxycarbonyl group on a Lys side chain

The synthetic route to Fmoc-Lys(Poc)-OH (1) is shown in Scheme 1. 2-Hydroxyacetophenone (2) was treated with disuccinimidyl carbonate in DMF for 2 h, and then Fmoc-Lys-OH was added to this solution, giving the desired product 1 in 30% yield.



Scheme 1 Synthesis of Fmoc-Lys(Poc)-OH.

This lysine derivative 1 was introduced to a model peptide, Ser-Phe-Lys-Tyr-Glu, and the stability of the Poc group was tested. Starting from Fmoc-Glu(OBu')-Wang resin, the peptide chain was elongated by the N,N'-dicyclohexylcarbodiimide (DCC)-1hydroxybenzotriazole (HOBt) method. After the peptide chain assembly, the peptide was cleaved off from the resin by a TFA cocktail treatment. On the reversed-phase (RP)-HPLC chromatogram, two peaks were observed: one (peptide 3) is minor, and the other (peptide 4) is major with a longer retention time than 3 (See supplementary Figure<sup>†</sup>). MALDI-TOF mass analysis revealed that peptide 3 showed a protonated molecular ion peak at m/z835.7, which coincided well with the calculated value of the desired Poc-containing peptide. On the other hand, peptide 4 showed a protonated ion peak at m/z 817.6, which was smaller than that of 3 by 18 Da. In the <sup>1</sup>H-NMR spectra of 3, a singlet peak at a chemical shift of 5.33 ppm was observed, which arose from the methylene group in the phenacyl moiety. On the other hand, this signal disappeared in the spectrum of 4. These results suggest that the cyclized product was generated under the acidic conditions by the proposed mechanism shown in Scheme 2. It has been demonstrated that a similar reaction was observed in phenacyloxycarbamide by heating without solvent.<sup>10</sup> Although various TFA cocktails were tested, this undesirable side reaction could not be suppressed, suggesting that the Poc group is not suitable for protecting the amino group of primary amines.

On the other hand, if the reaction mechanism shown in Scheme 2 is correct, the Poc group should be still valid for protecting secondary amines, because the nitrogen atom at the intermediate lacks nucleophilicity and the reaction could not proceed. Therefore, it was likely that *N*-methyl-phenacyloxycarbamidomethyl group might be a good candidate for the thiol protecting group.



Scheme 2 Proposed mechanism of cyclization at the Poc group.

#### Synthesis of $N^{\alpha}$ -Fmoc-*S*-(*N*-methylphenacyloxycarbamidomethyl)cysteine

 $N^{\alpha}$ -Fmoc-S-(N-methyl-phenacyloxycarbamidomethyl)cysteine [Fmoc-Cys(Pocam)-OH, 5] was synthesized by the procedure shown in Scheme 3. The carbonyl group of 2 was protected by ethylene glycol to form ethylene acetal 6 in 55% yield. Compound 6 was treated with disuccinimidyl carbonate followed by methylamine, to give compound 7 in 68% yield. The amido nitrogen of 7 was then hydroxymethylated by HCHO treatment generating hydroxymethylated compound 8, and then cysteine was reacted in neat TFA. At this stage, the ethylene acetal structure was partly decomposed to a carbonyl group, and a mixture of compound 9 and 10 was obtained. Since these could not be separated by silica gel chromatography, we used them as a mixture to the next reaction. The Fmoc group was introduced to the amino group by Fmoc-OSu treatment, and the product was purified by silica gel chromatography. The acetal structure was almost completely lost, and only Fmoc-Cys(Pocam)-OH 5 was obtained. The yield was 72% over the three steps.



Scheme 3 Synthesis of Fmoc-Cys(Pocam)-OH.

#### Preparation of Pocam-containing peptide

Using this Cys derivative **5**, we tried to synthesize growth-blocking peptide (GBP) by the thioester method. GBP was originally isolated from the larval hemolymph of the host armyworm, *Pseudaletia separata*, whose development is halted in the last larval instar stage from parasitization by the parasitoid wasp, *Cotesia hariyai*.<sup>11</sup> To firstly check the characteristics of the Pocam group, we introduced **5** into the peptide segment GBP(11–25) (**11**), by ordinary Fmoc-SPPS. Starting from Fmoc-Gln(Trt)-CLEAR Acid resin, the peptide chain was elongated manually by DCC–HOBt strategy. At Lys<sup>20</sup> and Cys<sup>19</sup> sites, Fmoc-Lys(N<sub>3</sub>)-OH and Fmoc-Cys(Pocam)-OH were used as building blocks, and the protected peptide resin was successfully obtained. When

crude peptide was cleaved off from the resin by Reagent K<sup>12</sup> treatment at room temperature for 2 h, the Pocam group on the Cys residue was partly removed (*ca.* 70%, based on the peak area on the RP-HPLC chromatogram). To suppress the removal of the Pocam group, various conditions were tested. When the TFA cocktail was used at 4 °C for 4 h, the Pocam group almost remained and the other protecting groups, such as Bu<sup>4</sup>, Trt, 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pbf) and 2,4-dimethoxybenzyl (Dmb) groups, were clearly removed by the TFA treatment (See Supplementary Figure†). The desired peptide **11** was obtained in 8.9% yield.

To develop the deprotecting conditions for the Pocam group, peptide **11** was treated with various conditions (Fig. 1). As expected, the Pocam group was removed by Zn powder treatment in aqueous AcOH solution, and the azido group of Lys side chain was also converted to amino group at the same time, giving a completely deprotected peptide **12**. Interestingly, the Pocam group was easily removed by TFA treatment at 50 °C for 1 h without decomposition of the azido group, generating partly deprotected



**Fig. 1** (A) RP-HPLC elution profiles of GBP(11–25). (a) Purified peptide **11**. Observed: m/z 2093.6; calcd: 2093.9 for (M+H)<sup>+</sup>. (b) After Zn powder treatment in 50% aqueous AcOH for 1 h. Observed: m/z 1862.6; calcd: 1862.9 for (M+H)<sup>+</sup>. (c) After TFA cocktail (TFA/H<sub>2</sub>O/TIS, 93/5/2) treatment at 50 °C for 1 h. Observed: m/z 1888.4; calcd: 1888.8 for (M+H)<sup>+</sup>. (d) After reduction by TCEP in 50 mM phosphate buffer (pH 7.0) for 20 h. Observed: m/z 2067.4; calcd: 2067.9 for (M+H)<sup>+</sup>. Column: Mightysil RP-18 GP (4.6 × 150 mm), eluent: 0.1% TFA in aqueous acetonitrile at a flow rate of 1 ml/min. (B) Structure of peptides **11**, **12**, **13** and **14**.

azido-peptide **13**. In addition, Tris(2-carboxyethyl)phosphine (TCEP) treatment at a neutral pH reduced only the azido group without decomposition of the Pocam group, giving peptide **14**. These results suggest that this protecting group might be useful for site-specific modification of proteins synthesized by the thioester method.

#### Synthesis of growth-blocking peptide

GBP was synthesized by the method shown in Scheme 4. The N-terminal segment of GBP, GBP(1-10) thioester, was synthesized by Fmoc-SPPS and N-alkylcysteine (NAC)-assisted thioesterification reaction.8 Fmoc-(Et)Cys(Trt)-OH was introduced to H-Arg(Pbf)-Arg(Pbf)-NH-resin by the DCC-HOBt method. After Fmoc removal by piperidine treatment, Fmoc-Gly-OH was condensed using O-(7-azabenzotriazol-1-yl)-1,1,3,3tetramethyluronium hexafluorophosphate (HATU) as a condensation reagent, and then the peptide chain was elongated manually by the DCC-HOBt method. After the peptide chain assembly, the crude peptide was cleaved from the resin by the TFA cocktail treatment at 4 °C for 4 h, and dissolved in 50% aqueous acetonitrile solution containing 5% AcOH. 4-Mercaptophenylacetic acid (MPAA) was added to the solution at a concentration of 5%. The thioesterification reaction was almost complete within 20 h, giving the desired peptide thioester 15 in 2.0% yield (Scheme 4).



Scheme 4 Synthetic route to peptide 15.

The peptide segments, **11** and **15**, were condensed by the Ag<sup>+</sup>free thioester method (Scheme 5).<sup>13</sup> The segments were mixed and dissolved in dimethyl sulfoxide (DMSO) containing 2% 3hydroxy-3,4-dihydro-4-oxo-1,2,3-benzotriazine (HOObt) and 2% N,N-diisopropylethylamine (DIEA), and the reaction mixture was held at room temperature. The N-terminal segment **15** was exhausted within 4 h, and an additional peak corresponding to the desired product **16** appeared in RP-HPLC chromatogram (Fig. 2). After the condensation reaction, Fmoc group at the N-terminus was removed by adding piperidine to give peptide **17**. During condensation and Fmoc-removal steps, no decomposition of the



Scheme 5 Synthetic route to GBP. (a) 2% HOObt/2% DIEA/DMSO, rt, 4 h. (b) 5% piperidine, rt, 30 min. (c) Zn powder in 50% aqueous AcOH solution, rt, 1 h. (d) 10% DMSO/50 mM phosphate buffer (pH 7.0), rt, 20 h.



Fig. 2 RP-HPLC elution profiles of peptide condensation and deprotection. (a) Coupling reaction mixture of 11 and 15 (0 h). (b) Four hours after the coupling reaction. (c) Reaction mixture after piperidine treatment. (d) Reaction mixture after Zn/AcOH treatment. Column: Mightysil RP-18 GP ( $4.6 \times 150$  mm), eluent: 0.1% TFA in aqueous acetonitrile at a flow rate of 1 ml/min. Asterisks indicate non-peptidic components.

Pocam groups was observed. The Pocam groups were subsequently cleaved by Zn powder treatment in an aqueous AcOH solution. At the same time, the azido group at the Lys side chain was converted into an amino group without significant side reaction, and the linear GBP 18 was successfully obtained in 31% yield. Finally, the disulfide bond was formed by oxidation in a phosphate buffer containing 10% DMSO, giving the desired product 19 in 52% yield (Fig. 3).

### Synthesis of a-conotoxin SI

To demonstrate the usefulness of Pocam group for the selective disulfide formation, we synthesized  $\alpha$ -conotoxin SI as a model



**Fig. 3** RP-HPLC elution profiles of oxidation reaction of GBP. (a) 0 h. (b) 20 h. Column: Mightysil RP-18 GP ( $4.6 \times 150$  mm), eluent: 0.1% TFA in aqueous acetonitrile at a flow rate of 1 ml/min.



Scheme 6 Synthetic route for  $\alpha$ -conotoxin SI. (a) 2% HOObt/2% DIEA/DMSO, rt, 4 h. (b) 5% piperidine, rt, 30 min. (c) Zn powder in 50% aqueous AcOH solution, rt, 1 h. (d) 10% DMSO/50 mM phosphate buffer (pH 7.0), rt, 20 h. (e) I<sub>2</sub> in CH<sub>3</sub>OH/H<sub>2</sub>O containing HCl, rt, 15 min.

(Scheme 6).  $\alpha$ -Conotoxin SI is a small peptide neurotoxin isolated from the venom of fish-hunting cone snail, *Conus striatus*, and consists of 13 amino acid residues with four Cys forming two disulfide bonds.<sup>14</sup> This sequence was separated into two segments, 1–8 and 9–13, and these segments were prepared by ordinary Fmoc-SPPS.

Starting from Fmoc-Rink Amide MBHA resin, the peptide chain was elongated manually by the DCC–HOBt method, giving the desired protected peptide resin corresponding to the 9–13 sequence. At Cys<sup>13</sup>, Fmoc-Cys(Pocam)-OH was used as a building block. After the TFA cocktail treatment at 4 °C for 4 h, the crude peptide was separated by RP-HPLC, giving the C-terminal segment **20** in 11% yield.

The N-terminal segment **21** was also synthesized by Fmoc-SPPS and NAC-assisted thioesterification reaction. As in the synthesis of peptide **15**, two Arg and NAC residues were sequentially introduced to Rink Amide MBHA resin, and then Fmoc-Gly-OH was condensed using HATU as a coupling reagent. The (a)

(b)

c)

(d)

0

Relative absorbance at 220 nm

peptide chain was elongated by the DCC–HOBt method. Fmoc-Cys(Acm)-OH was used at Cys<sup>2</sup> and Cys<sup>7</sup>, and Fmoc-Cys(Pocam)-OH was introduced at Cys<sup>3</sup>. After cleaving the crude peptide from the resin by the TFA cocktail treatment at 4 °C for 4 h, the thioesterification reaction was carried out in 50% aqueous acetonitrile solution containing 5% AcOH and 5% MPAA at room temperature for 20 h, giving the desired peptide thioester **21** in 8.0% yield.

The peptide segments, **20** and **21**, were mixed and dissolved in DMSO containing 2% HOObt and 2% DIEA (Scheme 6). The N-terminal peptide thioester **21** was almost exhausted within 4 h, and an additional peak corresponding to the desired product **22** appeared in the RP-HPLC chromatogram (Fig. 4). After the condensation reaction, the Fmoc group at the N-terminus was removed by adding piperidine to give peptide **23**. The Pocam and azido groups were subsequently cleaved by Zn powder treatment in an aqueous AcOH solution, and the linear  $\alpha$ -conotoxin SI **24** 

20

20

20

24

10

23

21

22



Time (min)

20

was successfully obtained in 66% yield. During condensation and deprotection reactions, no significant side reaction was observed, and the product was generated in good yield. The disulfide bond between  $Cys^3$ – $Cys^{13}$  was formed by oxidation in a phosphate buffer containing 10% DMSO, giving desired product **25** in 64% yield (Fig. 5). Finally, the second disulfide bond between  $Cys^2$ – $Cys^7$  was formed by iodine oxidation. Peptide **25** was dissolved in water, and was added dropwise to I<sub>2</sub> solution in methanol containing HCl. After 15 min, the reaction was quenched with ascorbic acid, and the product was purified by RP-HPLC, to give the final product **26** in 88% yield.



Fig. 5 RP-HPLC elution profiles of disulfide formation reactions of  $\alpha$ -conotoxin SI. (a) Before disulfide formation. (b) After DMSO oxidation for 20 h. (c) After iodine oxidation. Column: Mightysil RP-18 GP (4.6 × 150 mm), eluent: 0.1% TFA in aqueous acetonitrile at a flow rate of 1 ml/min.

# Conclusions

Acetonitrile (%)

10

30

We developed a novel thiol protecting group, Pocam. We chemically synthesized Fmoc-Cys(Pocam)-OH and introduced it into peptides and peptide thioesters, indicating that the Pocam group was compatible with Fmoc-SPPS and NAC-assisted thioesterification reactions. It was useful for peptide condensation by the thioester method, and also for selective disulfide formation. The Pocam group was cleaved simultaneously with azido group by reduction using Zn/AcOH, demonstrating that the deprotection reaction after peptide condensation by the thioester method, which conventionally took two or more steps, was simplified to only one step.

# Experimental

# General

Fmoc-(Et)Cys(Trt)-OH<sup>8,15</sup> and Fmoc-Lys(N<sub>3</sub>)-OH<sup>4</sup> were prepared by previously described methods. MALDI-TOF mass spectra were recorded using a Voyager-DE PRO spectrometer (Applied Biosystems, CA). Amino acid composition was determined using a LaChrom amino acid analyzer (Hitachi, Tokyo, Japan) after hydrolysis with a 6 M HCl solution at 150 °C for 2 h in a vacuumsealed tube.

### Fmoc-Lys(Poc)-OH 1

2-Hydroxyacetophenone (140 mg, 1.0 mmol), disuccinimidyl carbonate (260 mg, 1.0 mmol), 4-dimethylaminopyridine (12 mg, 0.10 mmol) and N,N-diisopropylethylamine (DIEA, 170 µl, 1 mmol) were dissolved in N,N-dimethylformamide (DMF, 4 ml), and stirred at room temperature for 1 h. To this solution, Fmoc-Lys-OH hydrochloride (370 mg, 1.0 mmol) was added and the solution was stirred overnight. The reaction mixture was diluted with EtOAc, washed with 1 M HCl and brine, and dried over Na<sub>2</sub>SO<sub>4</sub>. After filtration and concentration in vacuo, the residue was chromatographed on silica gel with toluene/EtOAc/AcOH (50/50/1) to give Fmoc-Lys(Poc)-OH 1 (160 mg, 30%, colorless solid). R<sub>f</sub> 0.15 (Toluene/EtOAc/AcOH, 50/50/1). Near UV in CH<sub>3</sub>OH:  $\lambda_{max} = 251$  nm ( $\varepsilon$  11 900), 264 nm ( $\varepsilon$  11 800). <sup>1</sup>H-NMR  $(CDCl_3): \delta 7.87 - 7.13 \text{ (m, 13H, Ar)}, 5.91 \text{ (d, 1H, } J = 8.0 \text{ Hz}, \text{N}\alpha H),$ 5.48 (t, 1H, J = 5.6, N $\varepsilon$ H), 5.25 [s, 2H, C(=O)CH<sub>2</sub>], 4.38–4.10 (m, 4H,  $C\alpha H$ , >CH–CH<sub>2</sub>–O-), 3.20 (m, 2H,  $C\epsilon H$ ), 1.87 (m, 1H, С*βH*), 1.75 (m, 1H, C*βH*), 1.54–1.44 (m, 4H, C*γH*, C*δH*). <sup>13</sup>С-NMR (CDCl<sub>3</sub>): δ 193.9 (COOH), 175.8 (Ph–CO), 156.4 and 156.1  $[NH-C(O)-O], 67.0 (>C-CH_2-O), 66.3 (CO-CH_2-O), 53.6 (C\alpha),$ 47.0 (>C-CH<sub>2</sub>-O), 40.4 ( $C\varepsilon$ ), 31.4 ( $C\beta$ ), 29.0 ( $C\delta$ ), 22.0 ( $C\gamma$ ). MALDI-TOF mass, found: m/z 552.9, calcd: 553.2 for (M+Na)<sup>+</sup>.

#### Synthesis of the model peptide containing Poc group

Fmoc-Glu(OBu')-Wang resin (0.55 mmol/g, 36 mg, 20 µmol) was swelled in N-methylpyrrolidone (NMP) for 30 min, and was treated with 20% piperidine/NMP for 5 and 15 min. After washing with NMP, Fmoc-Tyr(Bu')-OBt, which was prepared by mixing Fmoc-Tyr(Bu')-OH (0.1 mmol), 1 M DCC/NMP (100 µl) and 1 M HOBt/NMP (100 µl) at room temperature for 30 min, was added and the reaction mixture was vortexed at 50 °C for 1 h. The resin was washed with NMP and 50% dichloromethane (DCM)/CH<sub>3</sub>OH, treated with 10% Ac<sub>2</sub>O/5% DIEA/NMP for 5 min, and washed with NMP. Fmoc-Lys(Poc)-OH, Fmoc-Phe-OH and Fmoc-Ser(Bu')-OH were sequentially introduced to the resin by the same manner as for Tyr residue, and H-Ser(Bu')-Phe-Lys(Poc)-Tyr(Bu')-Glu(OBu')-OCH<sub>2</sub>-resin (49 mg) was obtained. The resin was treated with 95% aqueous TFA solution (1 ml) at room temperature for 2 h. TFA was removed under nitrogen stream and the peptide was precipitated with diethyl ether. After washing twice with ether, the precipitant was dried under vacuum. The crude peptide was separated by RP-HPLC on a Mightysil RP-18 GP column with a linear gradient of acetonitrile containing 0.1% TFA.

#### N-Methyl-O-phenacylcarbamate ethylene acetal 7

2-Hydroxyacetophenone (540 mg, 4.0 mmol) was mixed with ethylene glycol (2.0 ml) in dichloromethane (2 ml) and 4-N HCl/1,4dioxane (1 ml). The solution was stirred at room temperature for 2 d. The solvent was removed under reduced pressure, and the residue was chromatographed on silica gel with toluene/EtOAc (3/1) to give compound 6 (400 mg, 55%). Then, 6 (290 mg, 1.6 mmol) was mixed with disuccinimidyl carbonate (410 mg, 1.6 mmol), 4-dimethylaminopyridine (20 mg, 0.16 mmol) and DIEA (280 µl, 1 mmol) in DMSO (3 ml), and the solution was stirred at room temperature for 1 h. To this solution, methylamine hydrochloride (110 mg, 1.6 mmol) and DIEA (280 µl, 1.6 mmol) was added, and the mixture was stirred for 1 h. The solution was diluted with EtOAc, washed with 1 M HCl and brine, and dried over Na<sub>2</sub>SO<sub>4</sub>. After filtration and concentration in vacuo, the residue was chromatographed on silica gel with toluene/EtOAc (3/1) to give compound 7 (260 mg, 68%, colorless amorphous).  $R_{\rm f}$ 0.23 (Toluene/EtOAc, 3/1). Near UV in CH<sub>3</sub>OH:  $\lambda_{max} = 260$  nm ( $\varepsilon$ 1,600). <sup>1</sup>H-NMR (CDCl<sub>3</sub>): δ 7.52–7.28 (m, 5H, Ar), 4.96 (brs, 1H, NH), 4.32 [s, 2H, C(=O)CH<sub>2</sub>], 4.07 (m, 2H, -O-CH<sub>2</sub>-CH<sub>2</sub>-O-), 3.86 (m, 2H,  $-O-CH_2-CH_2-O-$ ), 2.74 (d, 3H, J = 5.2 Hz,  $CH_3$ ). <sup>13</sup>C-NMR (CDCl<sub>3</sub>): δ156.6 (*C*=O), 139.3, 128.5, 128.1, 126.0 and 126.0 [Ph-C(O)<sub>2</sub>, Ar], 66.8 (>C-CH<sub>2</sub>-O), 65.2 (O-CH<sub>2</sub>-CH<sub>2</sub>-O), 27.5 (N-CH<sub>3</sub>).

#### Fmoc-Cys(Pocam)-OH 5

Compound 7 (260 mg, 1.1 mmol) was dissolved in dimethoxyethane (1.5 ml), and 36% aqueous formaldehyde solution (0.50 ml) and Na<sub>2</sub>CO<sub>3</sub> (120 mg1.1 mmol) were added. After stirring at room temperature for 2 h, the mixture was diluted with EtOAc, washed with H<sub>2</sub>O and brine, and dried over Na<sub>2</sub>SO<sub>4</sub>. After filtration and concentration, hydroxymethylated product 8 was used in the next reaction without further purification. Compound 8 was mixed with cysteine hydrochloride (350 mg, 2.0 mmol) and dissolved in TFA (2 ml). The reaction mixture was stirred at room temperature for 30 min. After TFA was removed under reduced pressure, the residue was chromatographed on silica gel with CHCl<sub>3</sub>/CH<sub>3</sub>OH/AcOH (90/10/1). During the reaction and purification steps, ethylene acetal portion was decomposed in part, and the cysteine derivatives 9 and 10 were obtained as a mixture. Since these could not be separated on the silica gel chromatography, we used them in the next reaction as a mixture. The yield of the mixture was 370 mg. To the mixture of 9 and 10, Fmoc-OSu (370 mg, 1.1 mmol) was added in DMF (5 ml) containing DIEA (170  $\mu$ l, 1.0 mmol), and the solution was stirred at room temperature for 2 h. The solution was then diluted with EtOAc, washed with 1 M aqueous HCl and brine, and dried over Na<sub>2</sub>SO<sub>4</sub>. After filtration and concentration in vacuo, the residue was chromatographed on silica gel with toluene/EtOAc/AcOH (50/50/1). During the reaction and purification steps, the ethylene acetal portion was almost completely decomposed, and only Fmoc-Cys(Pocam)-OH 5 was obtained (440 mg, 73% in 3 steps) as colorless solid. Rf 0.18 (Toluene/EtOAc/AcOH, 50/50/1). Near UV in CH<sub>3</sub>OH:  $\lambda_{max} = 261$  nm ( $\varepsilon$  6,200), 277 nm ( $\varepsilon$  5,400) <sup>1</sup>H-NMR was measured as a mixture of conformers in DMSO-d<sub>6</sub>:  $\delta$ 7.93–7.29 (m, 13H, Ar), 5.43 [s, 1.2H, C(=O)CH<sub>2</sub>], 5.38 [s, 0.8H, C(=O)CH2], 4.71-4.45 (m, 2H, N-CH2-S), 4.29-4.19 (m, 4H,

Cα*H*, >C*H*–C*H*<sub>2</sub>–O-), 3.35 (brs, 3H, C*H*<sub>3</sub>), 3.12–2.83 (m, 2H, Cβ*H*). <sup>13</sup>C-MNR (DMSO-d<sub>6</sub>): δ 193.6 and 193.3 (COOH), 172.3 (Ph–C=O), 67.5 (CO–CH<sub>2</sub>–O), 65.9 (>CH–CH<sub>2</sub>–O), 54.0 (Cα), 51.3 and 50.3 (N–CH<sub>2</sub>–S), 46.7 (>CH–CH<sub>2</sub>–O), 33.1 and 32.8(N–CH<sub>3</sub>), 31.8 and 31.5 (Cβ). MALDI-TOF mass, found: *m*/*z* 571.3, calcd: 571.2 for (M+Na)<sup>+</sup>

#### Fmoc-[Cys(Pocam)<sup>7</sup>]-GBP(1-10)-SC<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>COOH 15

Fmoc-Rink Amide MBHA resin (0.34 mmol/g, 150 mg, 50 µmol) was swelled in NMP for 30 min, and was treated with 20% piperidine/NMP for 5 and 15 min. After washing with NMP, Fmoc-Arg(Pbf)-OBt, which was prepared by mixing Fmoc-Arg(Pbf)-OH (0.2 mmol), 1 M DCC/NMP (200 µl) and 1 M HOBt/NMP (200 µl) at room temperature for 30 min, was added and the reaction mixture was vortexed at 50 °C for 1 h. The resin was washed with NMP and 50% dichloromethane (DCM)/CH<sub>3</sub>OH, treated with 10% Ac<sub>2</sub>O/5% DIEA/NMP for 5 min, and washed with NMP. Another Arg residue was introduced to the resin by the same manner, and the resin was washed with NMP. After Fmoc group was removed by 20% piperidine/NMP treatment at room temperature for 5 and 15 min, the resin was washed with NMP. Fmoc-(Et)Cys(Trt)-OBt, which was prepared by mixing Fmoc-(Et)Cys(Trt)-OH (0.1 mmol), 1 M DCC/NMP (150 µl) and 1 M HOBt/NMP (150 µl) at room temperature for 30 min, was added to the resin, and the mixture was vortexed at 50 °C for 1 h. The resin was washed with NMP and 50% DCM/CH<sub>3</sub>OH, treated with 10% Ac<sub>2</sub>O/5% DIEA/NMP for 5 min, and washed with NMP. After Fmoc group was removed by 20% piperidine/NMP treatment at room temperature for 5 and 15 min, the resin was washed with NMP. Fmoc-Gly-OH (0.5 mmol) and HATU (0.5 mmol) was dissolved in NMP (1 ml) containing DIEA (170 µl, 1 mmol), and the mixture was added to the resin. The reaction mixture was vortexted at 50 °C for 1 h. After washing with NMP, the peptide chain was elongated by ordinary Fmoc-based SPPS. The amino acids (0.2 mmol each) were activated by mixing with 1 M DCC/NMP (0.2 ml) and 1 M HOBt/NMP (0.2 ml) at room temperature for 30 min, and the coupling reaction was carried out at 50 °C for 1 h. After elongation, Fmoc-Glu(OBu')-Asn(Trt)-Phe-Ser(Bu')-Gly-Gly-Cys(Pocam)-Val-Ala-Gly-(Et)Cys(Trt)-Arg(Pbf)-Arg(Pbf)-NHresin (280 mg) was obtained. A part of the resin (20 mg) was treated with a TFA cocktail (TFA/thioanisole/H2O/phenol/ triisopropylsilane, 82.5/5/5/2.5, 500 µl) at 4 °C for 4 h. TFA was removed under nitrogen stream and the peptide was precipitated with diethyl ether. After washing twice with ether, the precipitant was dried under vacuum. The crude peptide was dissolved in 50% CH<sub>3</sub>CN/5% AcOH/H<sub>2</sub>O (1 ml), and MPAA (50 mg) was added to the solution. After the overnight reaction at room temperature, the crude peptide was separated by RP-HPLC on a Mightysil RP-18 GP column with a linear gradient of acetonitrile containing 0.1% TFA, to give peptide thioester 15 (74 nmol, 2.0% yield). MALDI-TOF mass, found: *m/z* 1839.5, calcd: 1839.5 for (M+Na)<sup>+</sup>. Amino acid analysis: Asp<sub>0.92</sub>Ser<sub>0.86</sub>Glu<sub>1.08</sub>Gly<sub>3</sub>Ala<sub>1.00</sub>Val<sub>0.92</sub>Phe<sub>0.91</sub>.

# [Cys(Pocam)<sup>19</sup>, Lys(N<sub>3</sub>)<sup>20</sup>]-GBP(11-25) 11

Fmoc-Gln(Trt)-CLEAR Acid resin (0.41 mmol/g, 120 mg, 50  $\mu$ mol) was swelled in NMP for 30 min, and treated with 20 %

piperidine/NMP for 5 and 15 min. After washing with NMP, the peptide chain was elongated by Fmoc-SPPS. The amino acids (0.20 mmol each) were activated by mixing with 1 M DCC/NMP (0.2 ml) and 1 M HOBt/NMP (0.2 ml) at room temperature for 30 min, and the coupling reaction was carried out at 50 °C for 1 h, except that Fmoc-Asp(OBu<sup>t</sup>)-(Dmb)Gly-OH dipeptide unit (52 mg, 0.1 mmol) was used as a building block at Asp<sup>16</sup>-Gly<sup>17</sup> site. After elongation, H-Tyr(Bu<sup>*t*</sup>)-Met-Arg(Pbf)-Thr(Bu<sup>*t*</sup>)-Pro-Asp(OBu<sup>t</sup>)-(Dmb)Gly-Arg(Pbf)-Cys(Pocam)-Lys(N<sub>3</sub>)-Pro-Thr(Bu')-Phe-Tyr(Bu')-Gln(Trt)-OCH2-resin (260 mg) was obtained. A part of the resin (20 mg) was treated with the TFA cocktail (500 µl) at 4 °C for 4 h. TFA was removed under nitrogen stream and the peptide was precipitated with diethyl ether. After washing twice with ether, the precipitant was dried under vacuum. The crude peptide was separated by RP-HPLC on a Mightysil RP-18 GP column with a linear gradient of acetonitrile containing 0.1% TFA, to give peptide 11 (340 nmol, 8.9% yield). MALDI-TOF mass, found: m/z 2094.2, calcd: 2093.9 for (M+H)<sup>+</sup>. Amino acid analysis: Asp<sub>0.98</sub>Thr<sub>1.94</sub>Glu<sub>1.08</sub>Pro<sub>1.84</sub>Gly<sub>1</sub>Met<sub>0.82</sub>Tyr<sub>1.96</sub>Phe<sub>1.00</sub>Lys<sub>0.50</sub>Arg<sub>2.02</sub>.

# Linear GBP 18

Peptides 15 (74 nmol) and 11 (100 nmol) were mixed and dissolved in DMSO (40 µl) containing 2% HOObt/2% DIEA, and incubated at room temperature for 4 h. Piperidine (3 µl) was then added to this solution and the reaction mixture was kept at room temperature for 30 min. The crude peptide was precipitated by addition of 20 times volume of diethyl ether, washed twice with ether and dried under vacuum. The precipitant was dissolved in 50% aqueous AcOH (500 µl), and an excess amount of Zn powder was added to the solution. The mixture was vortexed at room temperature for 1 h. After filtration to remove Zn powder, the desired product 18 was purified by RP-HPLC using a Mightysil RP-18 GP column with a linear gradient of acetonitrile containing 0.1% TFA. The isolated yield of 18 was 31% (23 nmol). MALDI-TOF mass, found: m/z 2783.8, calcd: 2784.2 for (M+H)<sup>+</sup>. Amino acid analysis: Asp<sub>2,13</sub>Thr<sub>2,00</sub>Ser<sub>1,01</sub>Glu<sub>2,16</sub>Pro<sub>2,00</sub>Gly<sub>4</sub>Ala<sub>1,08</sub>Val<sub>1,02</sub>Met<sub>0,97</sub>Tyr<sub>2,47</sub>-Phe<sub>2.10</sub>Lys<sub>1.04</sub>Arg<sub>2.13</sub>.

# **GBP 19**

Peptide 18 (22 nmol) was dissolved in 400  $\mu$ l of 10% DMSO/50 mM phosphate buffer (pH 7.0), and the solution was kept at room temperature for 24 h. The crude peptide was separated by RP-HPLC using a Mightysil RP-18 GP column with a linear gradient of acetonitrile containing 0.1% TFA to give peptide 19 (11 nmol, 52%). MALDI-TOF mass, found: m/z 2781.9, calcd: 2782.2 for (M+H)<sup>+</sup>. Amino acid analysis: Asp<sub>1.91</sub>Thr<sub>1.95</sub>Ser<sub>1.01</sub>Glu<sub>2.10</sub>Pro<sub>1.93</sub>Gly<sub>4</sub>Ala<sub>0.99</sub>Val<sub>0.95</sub>Met<sub>0.73</sub>Tyr<sub>2.33</sub>-Phe<sub>2.16</sub>Lys<sub>1.08</sub>Arg<sub>2.06</sub>.

# Fmoc-[Cys(Acm),<sup>2,7</sup> Cys(Pocam)<sup>3</sup>]-α-conotoxin SI(1–8)-SC<sub>6</sub>-H<sub>4</sub>CH<sub>2</sub>COOH 21

Fmoc-Rink Amide MBHA resin (0.34 mmol/g, 150 mg, 50 µmol) was swelled in NMP for 30 min, and was treated with 20% piperidine/NMP for 5 and 15 min. After washing with NMP, Fmoc-Arg(Pbf)-OBt, which was prepared by mixing Fmoc-Arg(Pbf)-OH (0.2 mmol), 1 M DCC/NMP (200 µl) and 1 M

HOBt/NMP (200 µl) at room temperature for 30 min, was added and the reaction mixture was vortexed at 50 °C for 1 h. The resin was washed with NMP and 50% dichloromethane (DCM)/CH<sub>3</sub>OH, treated with 10% Ac<sub>2</sub>O/5% DIEA/NMP for 5 min, and washed with NMP. Another Arg residue was introduced to the resin by the same manner, and the resin was washed with NMP. After Fmoc group was removed by 20% piperidine/NMP treatment at room temperature for 5 and 15 min, the resin was washed with NMP. Fmoc-(Et)Cys(Trt)-OBt, which was prepared by mixing Fmoc-(Et)Cys(Trt)-OH (0.1 mmol), 1 M DCC/NMP (150 µl) and 1 M HOBt/NMP (150 µl) at room temperature for 30 min, was added to the resin, and the mixture was vortexed at 50 °C for 1 h. The resin was washed with NMP and 50% DCM/CH<sub>3</sub>OH, treated with 10% Ac<sub>2</sub>O/5% DIEA/NMP for 5 min, and washed with NMP. After Fmoc group was removed by 20% piperidine/NMP treatment at room temperature for 5 and 15 min, the resin was washed with NMP. Fmoc-Gly-OH (0.5 mmol) and HATU (0.5 mmol) was dissolved in NMP (1 ml) containing DIEA (170 µl, 1 mmol), and the mixture was added to the resin. The reaction mixture was vortexted at 50 °C for 1 h. After washing with NMP, the peptide chain was elongated by the ordinary Fmoc-based SPPS. The amino acids (0.2 mmol each) were activated by mixing with 1 M DCC/NMP (0.2 ml) and 1 M HOBt/NMP (0.2 ml) at room temperature for 30 min, and the coupling reaction was carried out at 50 °C for 1 h. After elongation, Fmoc-Ile-Cys(Acm)-Cys(Pocam)-Asn(Trt)-Pro-Ala-Cys(Acm)-Gly-(Et)Cys(Trt)-Arg(Pbf)-Arg(Pbf)-NH-resin (270 mg) was obtained. A part of the resin (20 mg) was treated with the TFA cocktail (500 µl) at 4 °C for 4 h. TFA was removed under nitrogen stream and the peptide was precipitated with diethyl ether. After washing twice with ether, the precipitant was dried under vacuum. The crude peptide was dissolved in 50% CH<sub>3</sub>CN/5% AcOH/H<sub>2</sub>O (1 ml), and MPAA (50 mg) was added to the solution. After the overnight reaction at room temperature, the crude peptide was separated by RP-HPLC on a Mightysil RP-18 GP column with a linear gradient of acetonitrile containing 0.1% TFA, to give peptide thioester 21 (300 nmol, 8.0% yield). MALDI-TOF mass, found: m/z 1521.7, calcd: 1521.5 for (M+Na)<sup>+</sup>. Amino acid analysis: Asp<sub>0.94</sub>Pro<sub>0.95</sub>Gly<sub>1</sub>Ala<sub>1.00</sub>Ile<sub>0.50</sub>.

#### [Cys(Pocam),<sup>13</sup> Lys(N<sub>3</sub>)<sup>10</sup>]-α-conotoxin SI(9–13) 20

Fmoc-Rink Amide MBHA resin (0.34 mmol/g, 88 mg, 30 µmol) was swelled in NMP for 30 min, and treated with 20 % piperidine/NMP for 5 and 15 min. After washing NMP, the peptide chain was elongated by the Fmoc-SPPS. The amino acids (0.12 mmol each) were activated by mixing with 1 M DCC/NMP (0.15 ml) and 1 M HOBt/NMP (0.15 ml) at room temperature for 30 min, and the coupling reaction was carried out at 50 °C for 1 h. After elongation, Boc-Pro-Lys(N<sub>3</sub>)-Tyr(Bu')-Ser(Bu<sup>1</sup>)-Cys(Pocam)-NH-resin (120 mg) was obtained. A part of the resin (20 mg) was treated with the TFA cocktail (500 µl) at 4 °C for 4 h. TFA was removed under nitrogen stream and the peptide was precipitated with diethyl ether. After washing twice with ether, the precipitant was dried under vacuum. The crude peptide was separated by RP-HPLC on a Mightysil RP-18 GP column with a linear gradient of acetonitrile containing 0.1%TFA, to give peptide 20 (580 nmol, 11% yield). MALDI-TOF

mass, found: m/z 827.4, calcd: 827.3 for  $(M+H)^+$ . Amino acid analysis: Ser<sub>0.67</sub>Pro<sub>1.06</sub>Tyr<sub>1</sub>Lys<sub>0.57</sub>.

# [Cys(SH),<sup>3,13</sup> Cys(Acm)<sup>2,7</sup>]-α-conotoxin SI 24

Peptides **21** (280 nmol) and **20** (400 nmol) were mixed and dissolved in DMSO (150  $\mu$ l) containing 2% HOObt/2% DIEA, and incubated at room temperature for 4 h. Piperidine (8  $\mu$ l) was then added to this solution and the reaction mixture was kept at room temperature for 30 min. The crude peptide was precipitated by addition of 20 times volume of diethyl ether, washed twice with ether and dried under vacuum. The precipitant was dissolved in 50% aqueous AcOH (1.0 ml), and an excess amount of Zn powder was added to the solution. The mixture was vortexed at room temperature for 1 h. After filtration to remove Zn powder, the desired product **24** was purified by RP-HPLC using a Mightysil RP-18 GP column with a linear gradient of acetonitrile containing 0.1% TFA. The isolated yield of **24** was 66% (190 nmol). MALDI-TOF mass, found: m/z 1499.8, calcd: 1499.6 for (M+H)<sup>+</sup>. Amino acid analysis: Asp<sub>0.92</sub>Ser<sub>0.59</sub>Pro<sub>1.95</sub>Gly<sub>1</sub>Ala<sub>0.97</sub>Ile<sub>0.58</sub>Tyr<sub>1.04</sub>Lys<sub>0.88</sub>.

#### [Cys(Acm)<sup>2,7</sup>]-α-conotoxin SI 25

Peptide **24** (190 nmol) was dissolved in 1.0 ml of 10% DMSO/ 50 mM phosphate buffer (pH 7.0), and the solution was kept at room temperature for 24 h. The crude peptide was separated by RP-HPLC using a Mightysil RP-18 GP column with a linear gradient of acetonitrile containing 0.1% TFA to give peptide **25** (120 nmol, 64%). MALDI-TOF mass, found: m/z 1497.7, calcd: 1497.6 for (M+H)<sup>+</sup>. Amino acid analysis: Asp<sub>0.87</sub>Ser<sub>1.24</sub>Pro<sub>2.00</sub>Gly<sub>1</sub>Ala<sub>0.94</sub>Ile<sub>0.94</sub>Tyr<sub>0.57</sub>Lys<sub>1.10</sub>.

#### a-Conotoxin SI 26

Peptide **25** (120 nmol) was dissolved in H<sub>2</sub>O (1.0 ml) and added dropwise to CH<sub>3</sub>OH (5.0 ml) containing 20 mM I<sub>2</sub>/CH<sub>3</sub>OH (200 µl) and 6 M HCl (67 µl) within 5 min with mixing. After the resultant solution was stirred for another 15 min, the reaction was terminated by adding aqueous ascorbic acid solution. The product was purified by RP-HPLC using a Mightysil RP-18 GP column with a linear gradient of acetonitrile containing 0.1% TFA to give peptide **26** (100 nmol, 88%). MALDI-TOF mass, found: m/z 1353.3, calcd: 1353.5 for (M+H)<sup>+</sup>. Amino acid analysis: Asp<sub>0.84</sub>Ser<sub>0.81</sub>Pro<sub>2.07</sub>Gly<sub>1</sub>Ala<sub>0.95</sub>Ile<sub>0.76</sub>Tyr<sub>1.10</sub>Lys<sub>0.99</sub>.

# Acknowledgements

We thank Mr. Paul Singleton for the critical reading of this manuscript. This work was supported in part by Grant-in-Aid for Scientific Research from the Ministry of Education, Sports, Science and Technology of Japan (Nos. 22880033 and 00209214).

#### Notes and references

- 1 P. E. Dawson, T. W. Muir, I. Clark-Lewis and S. B. H. Kent, *Science*, 1994, **266**, 776–779.
- 2 B. L. Nilsson, L. L. Kiessling and R. T. Raines, *Org. Lett.*, 2000, 2, 1939–1941; B. L. Nilsson, L. L. Kiessling and R. T. Raines, *Org. Lett.*, 2001, 3, 9–12.
- 3 H. Hojo and S. Aimoto, *Bull. Chem. Soc. Jpn.*, 1991, **64**, 111–117; S. Aimoto, *Biopolymers*, 1999, **51**, 247–265.

- 4 H. Katayama, H. Hojo, T. Ohira and Y. Nakahara, *Tetrahedron Lett.*, 2008, **49**, 5492–5494.
- 5 H. Katayama, H. Hojo, I. Shimizu, Y. Nakahara and Y. Nakahara, Org. Biomol. Chem., 2010, 8, 1966–1972.
- 6 D. F. Veber, J. Milkowski, S. Varga, R. Denkewalter and R. Hirschmann, J. Am. Chem. Soc., 1972, 94, 5456–5461; M. Yoshida, K. Akaji, T. Tatsumi, S. Iinuma, Y. Fujiwara, T. Kimura and Y. Kiso, Chem. Pharm. Bull., 1990, 38, 273–275.
- 7 S. Futaki, K. Sogawa, J. Maruyama, T. Asahara, M. Niwa and H. Hojo, Tetrahedron Lett., 1997, 38, 6237-6240; X. Li, T. Kawakami and S. Aimoto, Tetrahedron Lett., 1998, 39, 8669-8672; Y. Shin, K. A. Winans, B. J. Backes, S. B. H. Kent, J. A. Ellman and C. R. Bertozzi, J. Am. Chem. Soc., 1999, 121, 11684-11689; J. Alsina, T. S. Yokumu, F. Albericio and G. Barany, J. Org. Chem., 1999, 64, 8761-8769; R. Ingenito, E. Bianchi, D. Fattori and A. Pessi, J. Am. Chem. Soc., 1999, 121, 11369-11374; A. B. Clippingdale, C. J. Barrow and J. D. Wade, J. Pept. Sci., 2000, 6, 225-234; D. Swinnen and D. Hilvert, Org. Lett., 2000, 2, 2439-2442; A. R. Mezo, R. P. Cheng and B. Imperiali, J. Am. Chem. Soc., 2001, 123, 3885-3891; R. R. Flavell, M. Huse, M. Goger, M. Trester-Zedlitz, J. Kuriyan and T. W. Muir, Org. Lett., 2002, 4, 165-168; J. Brask, F. Albericio and K. J. Jensen, Org. Lett., 2003, 5, 2951-2953; J. A. Camarero, B. J. Hackel, J. J. de Yoreo and A. R. Mitchell, J. Org. Chem., 2004, 69, 4145-4151; J. D. Warren, J. S. Miller, S. J. Keding and S. J. Danishefsky, J. Am. Chem. Soc., 2004, 126, 6576-6578; P. Botti, M. Villain, S. Manganiello and H. Gaertner, Org. Lett., 2004, 6, 4861-4864; T. Kawakami, M. Sumida, K. Nakamura, T. Vorherr and S. Aimoto, Tetrahedron Lett., 2005, 46, 8805-8807; N. Oliver, J.-B. Behr, O. El-Mahdi, A. Blanpain and O. Melnyk, Org. Lett., 2005, 7, 2647-2650; Y. Ohta, S. Itoh, A. Shigenaga, S. Shintaku, N. Fujii and A. Otaka, Org. Lett., 2006, 8, 467-470; F. Nagaike, Y. Onuma, C. Kanazawa, H. Hojo,
- A. Ueki, Y. Nakahara and Y. Nakahara, Org. Lett., 2006, 8, 4465–4468;
  T. J. Hogenauer, Q. Wang, A. K. Sanki, A. J. Gammon, C. H. L. Chu, C. M. Kaneshiro, Y. Kajihara and K. Michael, Org. Biomol. Chem., 2007, 5, 759–762;
  T. Kawakami and S. Aimoto, Chem. Lett., 2007, 36, 76–77;
  J. B. Blanco-Canosa and P. E. Dawson, Angew. Chem., Int. Ed., 2008, 47, 6857–6861;
  J. Kang, J. P. Richardson and D. Macmillan, Chem. Commun., 2009, 407–409;
  S. Tsuda, A. Shigenaga, K. Bando and A. Otaka, Org. Lett., 2009, 11, 823–826;
  A. P. Tofteng, K. K. Sorensen, K. W. Conde-Frieboes, T. Hoeg-Jensen and K. J. Jensen, Angew. Chem., Int. Ed., 2009, 48, 7411–7414;
  J. Kang, N. L. Reynolds, C. Tyrrell, J. R. Dorin and D. Macmillan, Org. Biomol. Chem., 2009, 7, 4918–4923.
- 8 H. Hojo, Y. Onuma, Y. Akimoto, Y. Nakahara and Y. Nakahara, *Tetrahedron Lett.*, 2007, 48, 25–28; H. Hojo, Y. Onuma, Y. Akimoto, Y. Nakahara and Y. Nakahara, *Tetrahedron Lett.*, 2007, 48, 1299.
- 9 D. Hagiwara, M. Neya and M. Hashimoto, *Tetrahedron Lett.*, 1990, 31, 6539–6542.
- 10 R. Melnicky, L. Kvapil, P. Slézar, M. Grepl, J. Hlavác, A. Lycka and P. Hradil, J. Heterocycl. Chem., 2008, 45, 1437–1443.
- 11 Y. Hayakawa, J. Biol. Chem., 1990, 265, 10813–10816; Y. Hayakawa, J. Biol. Chem., 1991, 266, 7982–7984; Y. Hayakawa and Y. Yasuhara, Insect Biochem. Mol. Biol., 1993, 23, 225–231.
- 12 D. S. King, C. G. Fields and G. B. Fields, Int. J. Pept. Protein Res., 1990, 36, 255–266.
- 13 H. Hojo, Y. Murasawa, H. Katayama, T. Ohira, Y. Nakahara and Y. Nakahara, Org. Biomol. Chem., 2008, 6, 1808–1813.
- 14 G. C. Zafaralla, C. Ramilo, W. R. Gray, R. Karlstrom, B. M. Olivera and L. J. Cruz, *Biochemistry*, 1988, 27, 7102–7105.
- 15 Y. Nakahara, I. Matsuo, Y. Ito, R. Ubagai, H. Hojo and Y. Nakahara, *Tetrahedron Lett.*, 2010, 51, 407–410.