Tetrahedron
journal homepage: www.elsevier.com

Synthesis of lactam-bridged cyclic peptides using sequential olefin metathesis and diimide reduction reactions

Keisuke Aihara, Tsubasa Inokuma, Chiaki Komiya, Akira Shigenaga and Akira Otaka*

Institute of Health Bioscience and Graduate School of Pharmaceutical Sciences, Tokushima University, Tokushima 770-8505, Japan

ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

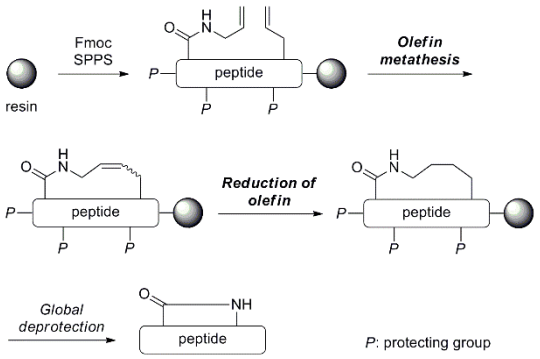
Keywords:
Lactam-bridged peptide
Cyclic peptide
Ring-closing metathesis
Fmoc solid-phase peptide synthesis
Diimide reduction

ABSTRACT

A new approach has been developed for the synthesis of lactam-bridged cyclic peptides. Following the introduction of *N*-allyl glutamine and α-allylglycine into the peptide backbone, the side chains of these residues were subjected to a cyclization reaction by ring-closing metathesis (RCM). Reduction of the resulting peptide bearing olefin moiety was achieved using diimide, which was generated *in situ* from *o*-nitrobenzenesulfonyl hydrazine and piperidine, gave the corresponding saturated cyclic peptides.

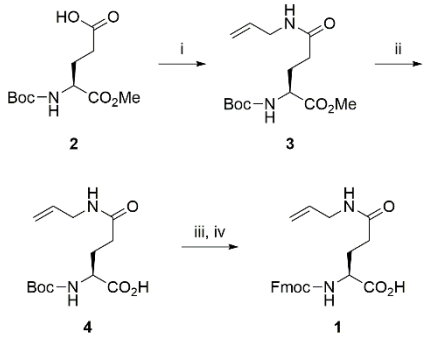
1. Introduction

Protein-protein interactions (PPIs) play important roles in numerous biological signaling networks, including cell cycle, proliferation and metabolism activities, and the effective regulation of these interactions could allow us to develop a deeper understanding of their role in biological phenomena and discover novel molecular targets for therapeutic systems.[1] PPIs can be controlled using a variety of different ligands, which can interact with the surfaces involved in the formation of the PPIs and prevent the proteins from coming together. Among the ligands used in this context, peptides possessing an analogous amino acid sequence to that of the site of PPI are appropriate candidates.[2] Peptide ligands of this type can be readily prepared by solid-phase peptide synthesis (SPPS) and purified by standard reverse phase HPLC. Furthermore, chemical modifications such as the attachment of a fluorophore[3] or post-translational modifications that effectively mimic the actions of biological systems[4] can be installed at the intended site. Compared with small molecule ligands, peptide ligands have a much wider molecular surface, and are therefore better suited to modulating PPIs. However, the stability profiles of peptide ligands are generally lower than those of small molecule ligands.[5] Furthermore, the three-dimensional structures of peptide fragments do not always reflect the structures of the protein surfaces involved in PPIs because of the flexibility of short peptides. One potential solution to these problems would involve the use of a cyclized peptide, which would allow for the fixation of the active conformer. Furthermore, it was envisaged that the use of unnatural cyclized bonding would prevent these compounds from being degraded by endogenous enzymes. Over the years, a wide variety of methodologies have been reported for the preparing cyclic peptides using side-to-side chain bridging techniques, as evidenced by reports pertaining to disulfide formation,[6] lactamization,[7] all-hydrocarbon linkage (stapled peptide).[8] Among the route already reported for the construction of cyclic peptides, we decided to focus on lactam-bridged cyclic peptides. It was envisaged that lactam-bridged peptides would exhibit much longer periods of activity than the corresponding disulfide peptides because the chemical stability of a lactam bond is generally superior to that of a disulfide.



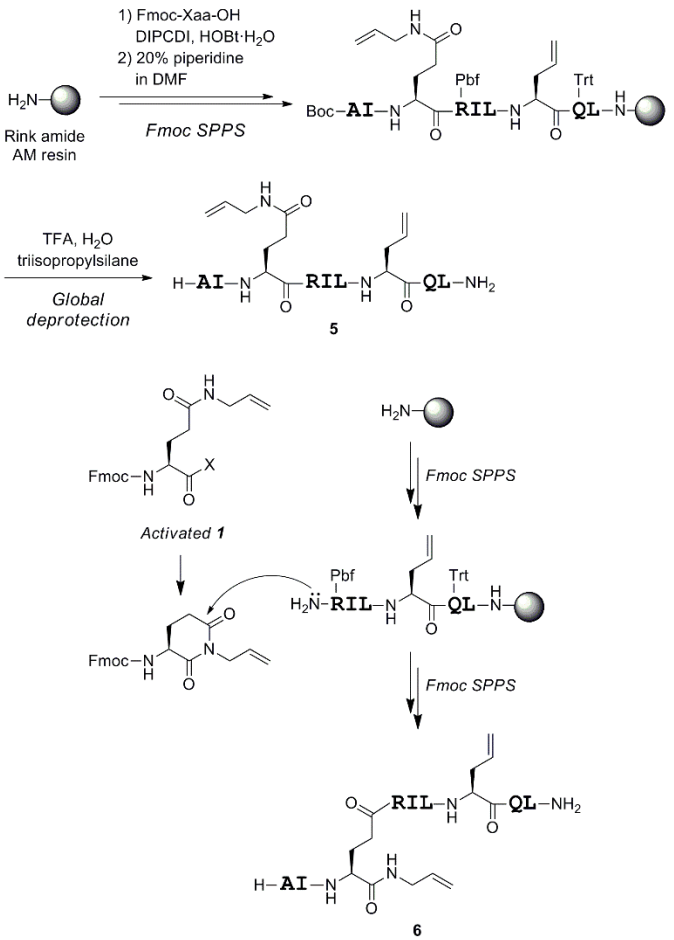
Scheme 1. Synthetic route to the lactam-bridged cyclic peptide.

* Corresponding author. Tel.: +81-88-633-9534; fax: +81-88-633-9505; e-mail: aotaka@tokushima-u.ac.jp



Scheme 2. Reagents and conditions: (i) 3-amino-1-propene, EDC·HCl, DMAP, CH_2Cl_2, 80%; (ii) LiOH·H_2O, THF, MeOH, H_2O, 85%; (iii) TFA, H_2O; (iv) Fmoc-OSu, Na_2CO_3, THF, H_2O, 61% (two steps).

It was also envisioned that the amide linkage of the lactam moiety would be more hydrophilic in nature than the corresponding the stapled peptide or disulfide linkage, and that this would result in an increase in the aqueous solubility of the resulting peptide ligands, which would offer significant benefits in terms of the biological activity of these compounds in a living system. It has recently been reported that lactam-bridged GLP-1 analogues exhibit strong receptor binding properties and



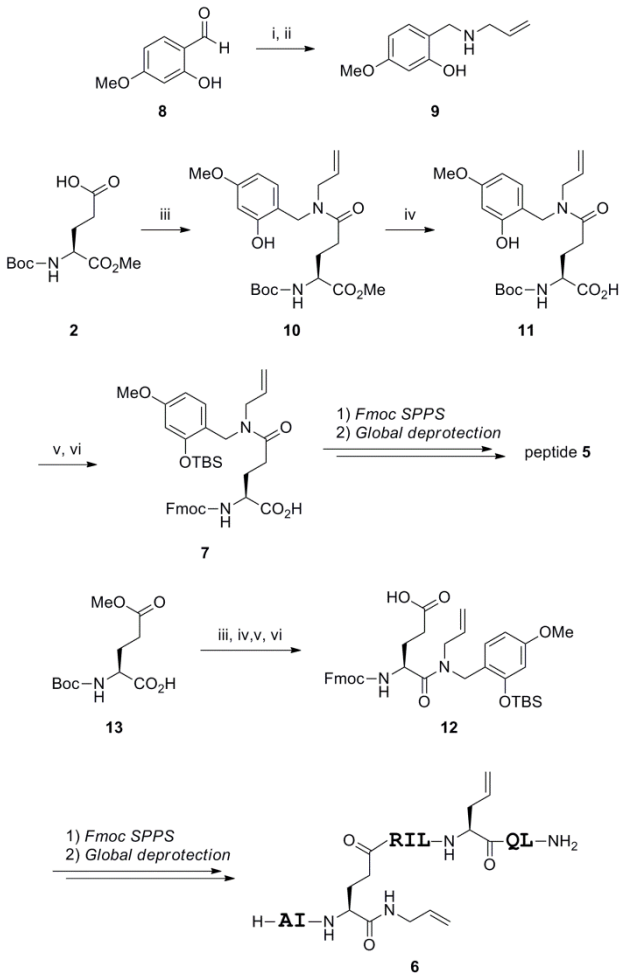
Scheme 3. Outline of the Fmoc SPPS used for the preparation of **5** and proposed mechanism of the generation of the byproduct.

remarkable stability towards enzymatic degradation.[9] Although the macrolactamization of the amino group of a lysine residue with the carboxyl group of a glutamic acid or aspartic acid residue has been used as a straightforward approach for preparing the cyclized lactam-bridged peptide, the selective on-resin removal of the side chain protecting groups of the lysine and glutamic acid residues required by the macrolactamization, followed by an amide bond forming reaction, has been widely used.[10] On the other hand, in the case of stapled peptides alternative to the lactam bridged peptides, the hydrocarbon bridge can be formed by Ru-mediated ring-closing metathesis (RCM) between two olefin moieties.[11] It is noteworthy that RCM represents a highly chemoselective C–C bond forming reaction that has no effect on any of the other sites of peptide except for the olefin moieties. The incorporation of staples to form a cyclized peptide therefore only requires the inclusion of olefin-bearing building blocks at the appropriate sites of the peptide chain. With this in mind, it was envisaged that the RCM reaction of a peptide bearing *N*-allyl glutamine and α-allylglycine residues would provide an efficient and protection-free method for the synthesis of cyclized lactam peptides (Scheme 1). It was also envisaged that the reduction of the olefin moiety resulting from the RCM reaction would provide access to another cyclized peptide with a different conformation to that of the RCM product, and therefore increase the repertoire of this important classes of molecules.

2. Results and discussion

2.1. Synthesis of the *N*-allyl glutamine derivative and its subsequent incorporation into a peptide

N-Allyl glutamine derivative **1** was prepared from Boc-Glu-OMe **2**[12] (Scheme 2). The *N*-allyl amide **3** was initially synthesized by the condensation of **2** with 3-amino-1-propene.



Scheme 4. Reagents and conditions: Synthesis of **7**: (i) 3-amino-1-propene, AcOH, $MgSO_4$, CH_2Cl_2; (ii) $NaBH_4$, MeOH, CH_2Cl_2, 92% (two steps); (iii) **9**, DCC, CH_2Cl_2, 90%; (iv) LiOH·H_2O, THF, MeOH, H_2O, 94%; (v) TBSOTf,

2,6-lutidine; (vi) Fmoc-OSu, Na_2CO_3, THF, H_2O, 88% (two steps). Synthesis of **12**: (iii) 76%; (iv) 88%; (v), (vi) 62% (two steps).

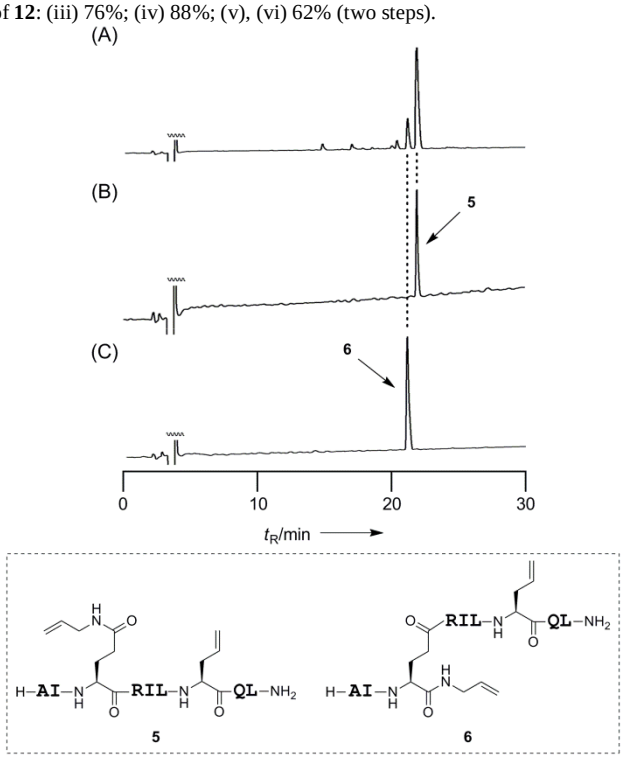
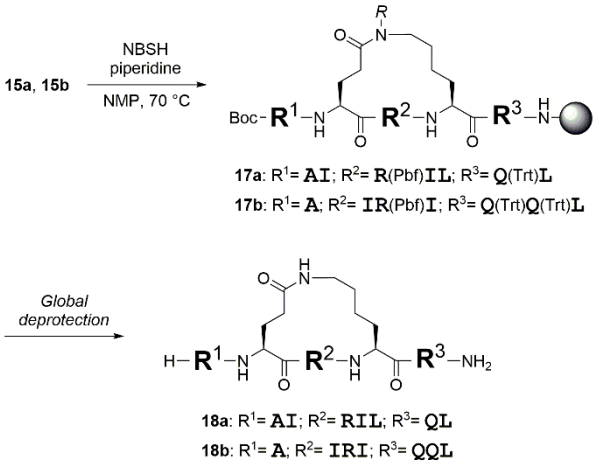


Fig. 1. HPLC profiles of crude peptides prepared by using **1** (A), **7** (B), and **12** (C) after global deprotection. HPLC conditions: Cosmosil $5C_{18}$-AR-II column, linear gradient of 0.1% (v/v) TFA/MeCN in 0.1% (v/v) TFA aq, 15–40% over 30 min.

Hydrolysis of the methyl ester in **3** gave the corresponding carboxylic acid **4**, followed by the deprotection of Boc group and subsequent protection of the resulting amine with Fmoc group to afford the *N*-allyl glutamine derivative **1**. The *N*-allyl glutamine derivative containing peptide **5** was then synthesized using Fmoc-SPPS on Rink amide AM resin. The coupling of the Fmoc amino acids (Fmoc-Xaa-OH) (5.0 eq.) was performed using diisopropylcarbodiimide (DIPCDI) (5.0 eq.) and 1-hydroxybenzotriazole monohydrate (HOBt·H_2O) (5.0 eq.) in DMF, and the Fmoc groups were removed with 20% piperidine in DMF (Scheme 3). However, the global deprotection of the completed resin with the concomitant release of the peptide gave two peptide products (**5** and **6**), which both had the same mass value of the desired product **5** (Fig. 1(A)). Although it was not possible to correctly identify which of these two peptides was the desired material at this stage, the formation of two different products was attributed to the γ-to-α-carbonyl transfer of the allyl amine unit during the acylation of **1** (Scheme 3).

2.2. Synthesis of *N*-allyl-*N*-Hmb glutamine derivatives and incorporation into peptide

It was expected that the installation of a suitable protecting group on the nitrogen atom of the side chain of the amide moiety of the glutamine derivatives would prevent the production of the undesired byproduct **6**. Sheppard et al.[13] reported the use of a 2-hydroxy-4-methoxybenzyl (Hmb) group as a protecting group for minimizing the formation of aspartimide during the incorporation of asparagine residues. Inspired by this report, we synthesized the Fmoc-protected-*N*-allyl-*N*-Hmb glutamine derivative **7** to suppress the formation of the glutarimide that we believed to be responsible for the generation of the undesired side product (Scheme 4). The reductive amination of 2-hydroxy-4-methoxy benzaldehyde **8** with 3-amino-1-propene gave Hmb amine derivative **9**, which was coupled with Boc-Glu-OMe **2** to afford **10**. The subsequent hydrolysis of the methyl ester of **10** gave the corresponding carboxylic acid **11** in excellent yield.



Scheme 5. Reduction of resin-supported peptides using NBSH.

The replacement of the Boc group with an Fmoc group, followed by the TBS protection of the phenol moiety of the Hmb group gave **7**. The olefin-bearing Fmoc amino acid **7** could also be applied to standard Fmoc SPPS to give a single product **5**, which was identical to the major product formed from the Fmoc-SPPS using **1** (Fig. 1(B)).The glutamine derivative **12**, which has the allyl and Hmb groups on the carboxylic acid group of its main chain, was synthesized from Boc-L-glutamic acid γ-methyl ester **13** and incorporated by Fmoc SPPS. This strategy resulted in a single product, which was identical to the byproduct **6** shown in Scheme 3 (Fig. 1(C)). Based on this result, it was concluded that the side reaction occurred as a consequence of an intramolecular imidation during the coupling reaction.

2.3. RCM and diimide reduction of peptides

The RCM reaction was initially attempted using the resin-bound fully-protected peptide **14a**, which is an intermediate in the formation of **5** (Table 1). When the reaction was conducted in the presence of 20 mol% of Grubbs 2nd generation catalyst in 1,2-dichloroethane at 70 °C for 60 min, followed by the global deprotection and cleavage of the peptide from resin, the desired RCM product **15a** was isolated in a low yield of only 24% (Table 1, entry 1). Pleasingly, the yield of the reaction increased significantly to 97% when *o*-dichlorobenzene was used as the solvent at a higher temperature (Table 1, entry 2). The use of the Hoveyda-Grubbs 2nd generation catalyst under the same conditions gave a slightly lower yield of the desired product (Table 1, entry 3). When compound **14b** bearing a different amino acid sequence to that of **14a** was used as the substrate, and reacted with 20 mol% of Grubbs 2nd generation catalyst in 1,2-dichloroethane at 70 °C for 60 min, the corresponding RCM product was formed in low yield (Table 1, entry 4). Even at an elevated temperature of 120 °C in the presence of the same catalyst, the cyclized peptide was obtained in only moderate yield (Table 1, entry 5). Contrary to expectation, the Hoveyda-Grubbs 2nd generation catalyst, which afforded moderate conversion in the reaction of **14a**, resulted in a much higher yield of **15b** (Table 1, entry 6). With the cyclized resin-bound peptides **15a** and **15b** in hand, we proceeded to investigate the reduction of the olefin moiety. Several previous studies from the literature have indicated that the hydrogenation of an olefin moiety on the solid-phase over a heterogeneous catalyst could be difficult to achieve.

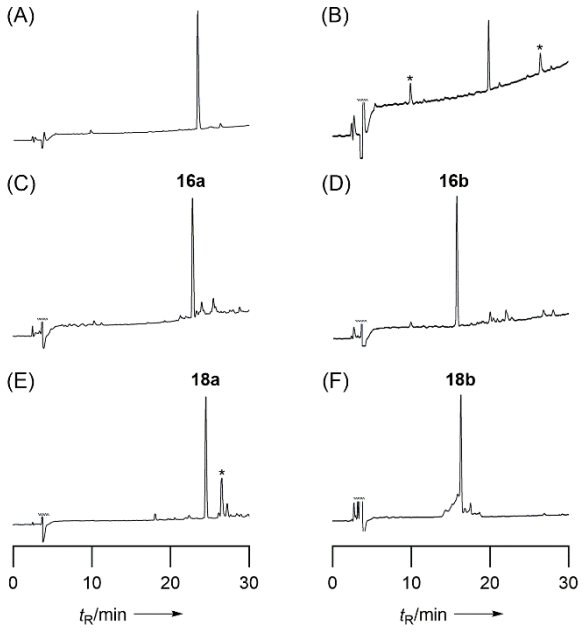


Fig. 2. HPLC monitoring of the olefin metathesis reaction, followed by the reduction of the resulting olefin; (A) and (B) linear peptides cleaved from **14a** and **14b**, respectively; (C) and (D) lactam bridged peptides cleaved from **15a** (Table 1, entry 2) and **15b** (Table 1, entry 6), respectively, following the olefin metathesis; (E) and (F) lactam bridged peptides cleaved from **17a** and **17b**, respectively, following the reduction of the olefin; HPLC conditions: Cosmosil $5C_{18}$-AR-II column, linear gradient of 0.1% (v/v) TFA/MeCN in 0.1% (v/v) TFA aq, 5–45% over 30 min. *non-peptidic compound.

With this in mind, we employed a homogenous reduction methodology involving a diimide species, which could be prepared *in situ* from a suitable sulfonohydrazine and Brønsted base.[14] The reaction of the resin-bound cyclic peptide **15a** with *o*-nitrobenzenesulfonyl hydrazine (NBSH) and piperidine at 70 °C gave **17a** (Scheme 5). The progress of the reaction was monitored by HPLC analysis by collecting an aliquot of the reaction mixture after the global deprotection and cleavage of the peptide from the resin (Fig. 2). Given that the reduction did not proceed to completion within 1 h, the same reaction was performed a second time after washing the resin with NMP. The reduction reaction had to be repeated several times in this way to reach completion. HPLC purification of the crude peptide gave the pure peptide **18a** in 20% isolated yield based on the starting resin. Peptide **16b** containing a different amino acid sequence was also converted to the corresponding saturated cyclized peptide **18b** in 15% isolated yield under the same conditions. These yields were similar to those obtained yields, which were in cases using allyl ester and alloc group as side chain protecting groups.

2.4. Synthesis of indomethacin conjugated lactam-bridged peptide

Next, we examined the possibility of the combination of our strategy and the conventional methods using allyl group. Following abovementioned Fmoc SPPS protocol, the resin-bound fully-protected peptide **19** was prepared. Then, alloc group was deprotected and indomethacin was coupled. The obtained product **20** was subjected to the RCM reaction, followed by NBSH reduction on resin. (Scheme 6) These reactions proceeded smoothly and afford the desired peptide **21** in 9% isolated yield based on starting resin (Fig. 3). This result shows our approach is compatible with conventional method and provides new orthogonal synthetic strategy toward the site selective modification of lactam-bridged peptides.

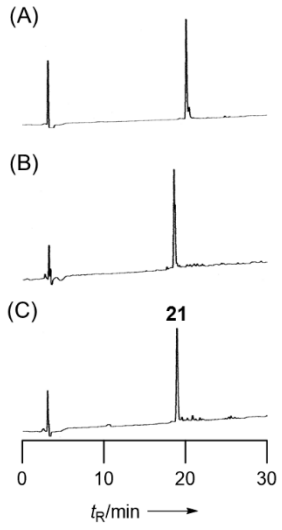


Fig. 3. HPLC monitoring of the olefin metathesis reaction, followed by the reduction of the resulting olefin; (A) Peptides cleaved from **19**; (B) Peptides cleaved from **20** after the olefin metathesis; (C) Peptides cleaved after the reduction of the olefin; HPLC conditions: Cosmosil $5C_{18}$-AR-II column, linear gradient of 0.1% (v/v) TFA/MeCN in 0.1% (v/v) TFA aq, 10–50% over 30 min.

3. Conclusion

In conclusion, we have developed a new synthetic method for the formation of lactam-bridged peptides via the cyclization of lysine and glutamic acid residues. This new method involves a RCM reaction between two olefinic amino acid residues, followed by the reduction of the resulting double bond with diimide. Notably, this method avoided the need for the selective cleavage of protecting groups on the lysine and glutamic acid residues. This methodology therefore represents a new option for the synthesis of various lactam-bridged cyclic peptides. To further highlight the overall utility of this newly developed methodology, we are currently investigating the synthesis of multi-bridged peptides and evaluating their biological properties. This work will be reported in due cause.

4. Experimental section

4.1. General methods

For column chromatography, Silica Gel 60 N (KANTO CHEMICAL CO., INC., spherical, neutral, particle size 63–210 μm) was employed. Mass spectra were recorded on a Waters MICROMASS® LCT PREMIERTM (ESI-TOF). IR spectra were measured on a JASCO FT–IR 6200 spectrometer. NMR spectra were measured using a Bruker AV400N or a JEOL GSX300 spectrometer. Chemical shifts were calibrated to the solvent signal. For HPLC separation, a Cosmosil $5C_{18}$-AR-II analytical column (Nacalai Tesque, 4.6 × 250 mm, flow rate 1 mL/min) or a Cosmosil $5C_{18}$-AR-II semi-preparative column (Nacalai Tesque, 10 × 250 mm, flow rate 3.0 mL/min) was employed and eluting products were detected by UV at 220 nm. A solvent system consisting of 0.1% (v/v) TFA aqueous solution (solvent A) and 0.1% (v/v) TFA in MeCN (solvent B) was used for HPLC elution. Optical rotation was determined on JASCO P2200 polarimeter.

4.2. Synthesis of glutamine derivatives

*4.2.1. 4-Allylcarbamoyl-2-tert-butoxycarbonylamino-butyric acid methyl ester (**3**).*

To a solution of carboxylic acid **2** (318 mg, 1.22 mmol) in CH_2Cl_2 (10.0 mL) were added 3-amino-1-propene (98.0 µL, 2.43 mmol), DMAP (14.9 mg, 0.122 mmol) and EDC·HCl (280 mg, 2.43 mmol) at 0 ºC. After being stirred for 3 h at room temperature, the reaction mixture was concentrated. The residue was extracted with EtOAc, and the extract was washed with 5% (w/v) $KHSO_4$ aq. and brine. The obtained organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (hexanes/EtOAc = 1:1 (v/v)) to yield amide **3** (293 mg, 0.977 mmol, 80%) as a white powder: $[\alpha]^{18}_D$ – 15.8 (*c* 1.55, MeOH); IR ($CHCl_3$): ν_{max}, cm^{-1}: 922, 992, 1451, 1530, 1659, 1712, 1742, 2979, 3082, 3309.; ^1H NMR ($CDCl_3$, 400 MHz) δ = 1.42 (9H, s), 1.91–2.00 (1H, m), 2.11–2.26 (1H, m), 2.24–2.35 (2H, m), 3.72 (3H, s), 3.86 (2H, dddd *J* = 5.7, 5.7, 1.5 and 1.5 Hz), 4.27 (1H, m), 5.11 (1H, ddt, *J* = 10.3, 1.5 and 1.5 Hz), 5.18 (1H, ddt, *J* = 17.1, 1.5 and 1.5 Hz), 5.28–5.42 (1H, br d, *J* = 6.5 Hz), 5.82 (1H, ddt, *J* = 16.9, 10.5 and 5.7 Hz), 6.17 (1H, br s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 28.4, 28.9, 32.6, 42.1, 52.5, 53.2, 80.2, 116.4, 134.2, 155.9, 171.9, 172.9; HRMS (ESI–TOF) *m/z* calcd for $C_{14}H_{24}KN_2O_5$ ($[M + K]^+$): 339.1322, found: 339.1319.

*4.2.2. 4-Allylcarbamoyl-2-tert-butoxycarbonylamino-butyric acid (**4**).*

Ester **3** (200 mg, 0.666 mmol) was dissolved in THF (2.00 mL), MeOH (1.00 mL) and H_2O (1.00 mL). To the solution was added $LiOH·H_2O$ (55.0 mg, 1.33 mmol) at 0 ºC. After 2 h stirring at room temperature, the solution was acidified with 1 M HCl aq. The reaction mixture was extracted with EtOAc and the extract was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography (hexanes/EtOAc = 1:2 (v/v)) to yield carboxylic acid **4** (162 mg, 0.566 mmol, 85%) as a white powder: $[\alpha]^{19}_D$ – 3.86 (*c* 2.21, MeOH); IR ($CHCl_3$): ν_{max}, cm^{-1}: 1452, 1535, 1698, 2980, 3089, 3330.; ^1H NMR ($CDCl_3$, 400 MHz) δ = 1.43 (9H, s), 1.93–2.10 (1H, m), 2.12–2.24 (1H, m), 2.28–2.54 (2H, m), 3.87 (2H, dd, *J* = 5.7 and 5.0 Hz), 4.15–4.35 (1H, m), 5.13 (1H, d, *J* = 10.4 Hz), 5.19 (1H, d, *J* = 16.9 Hz), 5.62 (1H, br d, *J* = 6.8 Hz), 5.81 (1H, ddt, *J* = 16.9, 10.4 and 5.7 Hz), 6.73 (1H, br s), 8.78 (1H, br s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 28.4, 29.2, 32.6, 42.6, 53.1, 80.5, 116.8, 133.7, 156.2, 173.6, 174.2; HRMS (ESI-TOF) *m/z* calcd for $C_{13}H_{22}KN_2O_5$ ($[M + K]^+$): 325.1166, found: 325.1151.

*4.2.3. 4-Allylcarbamoyl-2-(9H-fluoren-9-ylmethoxycarbonylamino)-butyric acid (**1**).*

To Boc derivative **4** (45.0 mg, 0.157 mmol) were added TFA (2.00 mL) and H_2O (0.200 mL) at 0 ºC. The reaction mixture was stirred at room temperature for 2 h and concentrated to remove TFA. Resulting concentrate was diluted with THF (5.00 mL) and 10% (w/v) Na_2CO_3 aq. (5.00 mL). After addition of Fmoc-OSu (53.0 mg, 0.157 mmol), the solution was stirred at room temperature for 12 h and then concentrated. The concentrate was extracted with EtOAc, and the organic phase was washed with 1 M HCl aq. and brine, dried over $MgSO_4$, filtered and concentrated. The obtained residue was purified by column chromatography (hexanes/EtOAc = 1/1 (v/v)) to yield Fmoc derivative **1** (39.0 mg, 95.5 µmol, 61%) as a white powder: $[\alpha]^{19}_D$ –7.82 (*c* 0.515, MeOH); IR (KBr): ν_{max}, cm^{-1}: 936, 991, 1447, 1542, 1650, 1692, 2943, 3296.; ^1H NMR (DMSO-d_6, 400 MHz) δ = 1.69–1.85 (1H, m), 1.58–1.98 (1H, m), 2.20–2.35 (2H, m), 3.64–3.80 (2H, br m), 4.00 (1H, dt, *J* = 5.9 and 8.4 Hz), 4.17–4.37 (3H, m), 5.04 (1H, ddt, *J* = 10.3, 1.7 and 1.6 Hz), 5.12 (1H, ddt, *J* = 17.3, 1.7 and 1.7 Hz), 5.78 (1H, ddt, *J* = 17.3, 10.3 and 5.1 Hz), 7.33 (2H, t, *J* = 7.4 Hz), 7.42 (2H, t, *J* = 7.4 Hz), 7.52 (1H, d, *J* = 5.9 Hz), 7.73 (2H, d, *J* = 7.4 Hz), 7.89 (2H, d, *J* = 7.4 Hz), 8.04 (1H, t, *J* = 5.7 Hz); ^{13}C NMR (DMSO-d_6, 75 MHz) δ = 27.3, 30.3, 40.8, 46.7, 54.1, 65.7, 115.0, 120.1, 125.3, 127.1, 127.6, 135.2, 140.7, 143.8, 143.9, 156.0, 171.2, 173.8; HRMS (ESI-TOF) *m/z* calcd for $C_{23}H_{24}KN_2O_5$ ($[M + K]^+$): 447.1322, found: 447.1335.

*4.2.4. 2-Allylaminomethyl-5-methoxy-phenol (**9**).*

To a solution of 2-hydroxy-4-methoxybenzaldehyde **8** (2.00 g, 13.1 mmol) in CH_2Cl_2 (40.0 mL) were added $MgSO_4$ (7.91 g, 65.7 mmol), allylamine (1.97 mL, 26.3 mmol) and AcOH (0.400 mL), and the mixture was stirred at room temperature for 20 min. It was then filtered to remove $MgSO_4$ and concentrated *in vacuo*. The obtained residue was diluted with MeOH (40.0 mL). To the solution was added $NaBH_4$ (991 mg, 26.2 mmol) at 0 ºC and the resulting mixture was stirred at room temperature. After 1 h, the reaction was quenched with saturated $NaHCO_3$ aq. The reaction mixture was concentrated and then diluted with EtOAc. The obtained organic phase was washed with saturated $NaHCO_3$ aq. and brine, dried over $MgSO_4$, filtered and concentrated. The obtained crude material of **9** (2.33 g, 12.1 mmol, 92%) as pale yellow oil was used for a next reaction without further purification: IR ($CHCl_3$): ν_{max}, cm^{-1}: 1385, 1445, 1592, 1620, 2836, 2935.; ^1H NMR ($CDCl_3$, 400 MHz) δ = 3.30 (2H, ddd, *J* = 6.1, 1.5 and 1.5 Hz), 3.74 (3H, s), 3.93 (2H, s), 5.19 (1H, ddt, *J* = 10.3, 1.5 and 1.3 Hz), 5.22 (1H, ddt, *J* = 17.1, 1.5, 1.3 Hz), 5.90 (1H, ddt, *J* = 17.1, 10.3 and 6.1 Hz), 6.33 (1H, dd, *J* = 8.3 and 2.5 Hz), 6.45 (1H, d, *J* = 2.5 Hz), 6.57 (2H, br s), 6.88 (1H, d, *J* = 8.3 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz, 50 ºC) δ = 50.8, 51.4, 55.3, 102.1, 105.0, 114.8, 117.3, 129.0, 135.1, 159.4, 160.5; HRMS (ESI-TOF) *m/z* calcd for $C_{11}H_{15}KNO_2$ ($[M + K]^+$): 232.0740, found: 232.0747.

*4.2.5. 4-[Allyl-(2-hydroxy-4-methoxy-benzyl)-carbamoyl]-2-tert-butoxycarbonylamino-butyric acid methyl ester (**10**).*

To a solution of carboxylic acid **2** (1.10 g, 4.21 mmol) and **9** (885 mg, 4.58 mmol) in CH_2Cl_2 (10.0 mL) was added a solution of DCC (869 mg, 4.21 mmol) in CH_2Cl_2 (10.0 mL) at 0 ºC. The reaction mixture was stirred at room temperature. After 2 h, precipitated *N*,*N'*-dicyclohexylurea was filtered off and the filtrate was concentrated. The residue was purified by column chromatography (hexanes/EtOAc = 1/1 (v/v)) to yield amide **10** (1.65 g, 3.78 mmol, 90%) as colorless oil: $[\alpha]^{19}_D$ –10.1 (*c* 1.02, MeOH); IR ($CHCl_3$): ν_{max}, cm^{-1}: 1365, 1441, 1507, 1618, 1712, 1743, 2977, 3342, 3728.; ^1H NMR ($CDCl_3$, 400 MHz) δ = 1.38 (9H, s), 1.89–2.02 (1H, m), 2.11–2.23 (1H, m), 2.35 (1H, ddd, *J* = 16.3, 7.9 and 6.5 Hz), 2.42 (1H, dt, *J* = 17.3 and 6.5 Hz), 3.68 (3H, s), 3.71 (3H, s), 3.87 (2H, d, *J* = 5.2 Hz), 4.19–4.33 (1H, m), 4.28 (1H, d *J* = 14.8 Hz), 4.35 (1H, d, *J* = 14.8 Hz), 5.17 (1H, d, *J* = 17.3 Hz), 5.25 (1H, d, *J* = 10.4 Hz), 5.27–5.36 (1H, br m), 5.74 (1H, ddt, *J* = 17.3, 10.4 and 5.2 Hz), 6.31 (1H, dd, *J* = 8.25 and 2.6 Hz), 6.43 (1H, d, *J* = 2.6 Hz), 6.91 (1H, d, *J* = 8.25); ^{13}C NMR ($CDCl_3$, 75 MHz, 50 ºC) δ = 27.8, 28.3, 29.0, 46.4, 49.8, 52.2, 53.4, 55.2, 80.0, 102.8, 105.7, 114.7, 117.7, 131.8, 132.0, 155.5, 157.6, 161.6, 172.6, 174.4; HRMS (ESI-TOF) *m/z* calcd for $C_{22}H_{32}N_2NaO_7$ ($[M + Na]^+$): 459.2107, found: 459.2086.

*4.2.6. 4-[Allyl-(2-hydroxy-4-methoxy-benzyl)-carbamoyl]-2-tert-butoxycarbonylamino-butyric acid (**11**).*

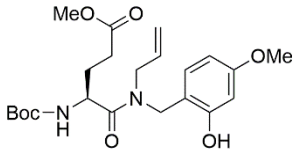
To a solution of **10** (900 mg, 2.06 mmol) in THF (6.40 mL) and MeOH (2.40 mL) was added a solution of $LiOH·H_2O$ (173 mg, 4.12 mmol) in H_2O (1.60 mL) at 0 ºC. The reaction mixture was

stirred at room temperature for 1 h and quenched with 5% (w/v) $KHSO_4$ aq. The solution was extracted with EtOAc, and the organic phase was washed with 5% (w/v) $KHSO_4$ aq. followed by brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography ($CHCl_3$/MeOH = 100/1 (v/v)) to yield **11** (821 mg, 1.94 mmol, 94%) as a white powder: $[\alpha]^{19}_D$ –2.44 (*c* 1.02, MeOH); IR ($CHCl_3$): ν_{max}, cm^{-1}: 929, 963, 1610, 1713, 2877, 2980, 3013, 3325.; ^1H NMR ($CDCl_3$, 400 MHz) δ = 1.40 (9H, s), 1.94–2.11 (1H, m), 2.14–2.31 (1H, m), 2.42 (1H, dt, *J* = 16.2 and 6.9 Hz), 2.47–2.60 (1H, m), 3.73 (3H, s), 3.90 (2H, br d, *J* = 4.5 Hz), 4.15–4.29 (1H, m), 4.32 (1H, d, *J* = 15.1 Hz), 4.38 (1H, d, *J* = 15.1 Hz), 5.19 (1H, d, *J* = 17.2 Hz), 5.27 (1H, d, *J* = 10.4 Hz), 5.48 (1H, br d, *J* = 7.1 Hz), 5.75 (1H, ddt, *J* = 17.2, 10.4 and 4.5 Hz), 6.34 (1H, dd, *J* = 8.4 and 2.5 Hz), 6.46 (1H, d, *J* = 2.5 Hz), 6.93 (1H , d, *J* = 8.4 Hz), 9.17–9.42 (1H, br s); ^{13}C NMR ($CDCl_3$, 75 MHz, 50 ºC) δ = 27.8, 28.3, 29.2, 46.2, 49.8, 53.0, 55.3, 80.3, 102.6, 105.8, 114.4, 118.0, 131.5, 132.1, 155.9, 157.3, 161.5, 174.9, 175.2; HRMS (ESI-TOF) *m/z* calcd for $C_{21}H_{30}KN_2O_7$ ($[M + K]^+$): 461.1690, found: 461.1697.

*4.2.7. 4-{Allyl-[2-(tert-butyl-dimethyl-silanyloxy)-4-methoxy-benzyl]-carbamoyl}-2-(9H-fluoren-9-ylmethoxycarbonylamino)-butyric acid (**7**).*

To a solution of Boc derivative **11** (2.25 g, 5.09 mmol) in CH_2Cl_2 (50.0 mL) were added TBSOTf (4.67 mL, 20.3 mmol) and 2,6-lutidine (3.56 mL, 30.5 mmol) at 0 ºC. The reaction mixture was stirred at 0 ºC for 1 h and at room temperature for additional 2 h. The solution was concentrated *in vacuo* and then diluted with 10% (w/v) Na_2CO_3 aq. (10.0 mL). The solution was neutralized with 2 M NaOH aq. (5.00 mL) at 0 ºC, and THF (10.0 mL) and Fmoc-OSu (1.89 g, 5.60 mmol) were added to the solution. After 14 h stirring at room temperature, the mixture was acidified with 1 M HCl aq. and extracted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$, filtrated and concentrated. The residue was purified by column chromatography ($CHCl_3$/MeOH = 200/1 – 20/1 (v/v)) to yield **7** (2.96 g, 4.49 mmol, 88%) as a white powder: $[\alpha]^{19}_D$ 17.3 (*c* 1.05, $CHCl_3$); IR ($CHCl_3$): ν_{max}, cm^{-1}: 978, 1446, 1505, 1610, 1721, 2953.; ^1H NMR ($CDCl_3$, 300 MHz, mixture of rotamers, 50 ºC) δ = 0.25 (3.6H, s), 0.26 (2.4H, s), 1.00 (5.4H, s), 1.00 (3.6H, s), 1.90–2.11 (1H, m), 2.17–2.38 (1H, m), 2.50–2.72 (1H, m), 2.87–3.11 (1H, m), 3.73 (1.2H, s), 3.75 (1.8H, s), 3.82–3.97 (2H, br m), 4.10–4.50 (5H, m), 4.56–4.72 (1H, dd) 5.07–5.24 (2H, m), 5.63–5.85 (1H, m), 6.12 (1H, br), 6.39 (1H, dd, *J* = 7.5 and 2.4 Hz), 6.50 (1H, dd, *J* = 8.3 and 2.4 Hz), 6.89 (0.4H, d, *J* = 7.5 Hz), 7.11 (0.6H, d, *J* = 8.3 Hz), 7.30 (2H, t, *J* = 7.3 Hz), 7.39 (2H, t, *J* = 7.3 Hz), 7.53–7.54 (2H, m), 7.76 (2H, d, *J* = 7.4 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz, mixture of rotamers) δ = –4.0, –4.0, –3.6, 25.7, 25.9, 27.7, 27.9, 28.4, 29.3, 46.3, 47.1, 49.8, 53.1, 53.6, 55.3, 67.3, 102.6, 105.8, 114.3, 114.4, 118.0, 120.0, 125.2, 125.3, 127.2, 127.8, 131.4, 131.5, 132.1, 141.3, 143.7, 143.9, 156.0, 156.5, 157.4, 161.6, 174.9, 175.2; HRMS (ESI-TOF) *m/z* calcd for $C_{37}H_{46}N_2NaO_7Si$ ($[M + Na]^+$): 681.2972, found: 681.3302.

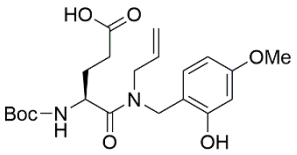
*4.2.8. 4-[Allyl-(2-hydroxy-4-methoxy-benzyl)-carbamoyl]-4-tert-butoxycarbonylamino-butyric acid methyl ester (**19**).*



19

Amide **19** was prepared from carboxylic acid **13** in a manner similar to that described for **10**. Colorless oil (496 mg, 1.14 mmol, 76%): $[\alpha]^{18}_D$ –21.8 (*c* 1.09, $CHCl_3$); IR ($CHCl_3$): ν_{max}, cm^{-1}: 961, 1367, 1442, 1507, 1619, 1710, 1736, 2838, 2977.; ^1H NMR ($CDCl_3$, 400 MHz, mixture of rotamers) δ = 1.40 (9H, s), 1.66–1.84 (1H, m), 1.88–2.04 (1H, m), 2.29–2.51 (2H, m), 3.65 (3H, s), 3.75 (3H, s), 4.01–4.21 (2H, m), 4.33 (1H, d, *J* = 14.8 Hz), 4.41 (2H, d, *J* = 14.8 Hz), 4.62 (1H, br t, *J* = 8.8 Hz), 5.25–5.39 (3H, m), 5.86 (1H, ddt, *J* = 16.9, 10.6 and 5.2 Hz), 6.33–6.41 (1H, m), 6.47 (1H, d, *J* = 2.6 Hz), 6.96 (1H, d, *J* = 8.4 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz, mixture of rotamers) δ = 28.4, 28.8, 29.5, 46.2, 49.7, 50.0, 51.7, 55.4, 55.4, 80.2, 102.2, 102.9, 105.7, 106.0, 114.2, 115.0, 118.7, 119.7, 131.0, 132.1, 132.3, 133.3, 157.5, 157.6, 160.8, 161.8, 173.3, 174.5; HRMS (ESI-TOF) *m/z* calcd for $C_{22}H_{32}N_2NaO_7$ ($[M + Na]^+$): 459.2107, found: 459.2108.

*4.2.9. 4-[Allyl-(2-hydroxy-4-methoxy-benzyl)-carbamoyl]-4-tert-butoxycarbonylamino-butyric acid (**20**).*



20

Carboxylic acid **20** was prepared from amide **19** in a manner similar to that described for **11**. A white powder (66.9 mg, 0.158 mmol, 88%): $[\alpha]^{18}_D$ –40.3 (*c* 0.388, MeOH); IR ($CHCl_3$): ν_{max}, cm^{-1}: 1366, 1441, 1508, 1618, 1708, 2926, 2976, 3505, 3658.; ^1H NMR (DMSO-d_6, 400 MHz, mixture of rotamers) δ = 1.28, (1.6H, br s) 1.36 (2.7H, s), 1.37 (4,7H, s), 1.60–1.73 (1H, m), 1.74–1.89 (1H, m), 2.21 (1H, t, *J* = 6.9 Hz), 2.26 (1H, t, *J* = 6.9 Hz), 3.67 (3H, s), 3.71–4,66 (5H, m), 4.97–5.13 (0.7H, m), 4.13–5.29 (1.3H, m), 5.52–5.74 (0.3H, m), 5.76–5.93 (0.7H, m), 6.27–6.45 (2H, m), 6.88–7.09 (2H, m), 9.60 (1H, br s); ^{13}C NMR ($CDCl_3$, 75 MHz, mixture of rotamers) δ = 26.8, 27.1, 27.9, 28.2, 29.8, 29.9, 43.0, 44.2, 46.5, 48.9, 49.6, 54.9, 78.2, 101.2, 104.3, 104.5, 115.3, 115.6, 116.0, 117.1, 122.4, 129.4, 129.7, 133.2, 133.7, 155.4, 155.5, 156.3, 159.0, 159.6, 171.6, 172.7, 174.0; HRMS (ESI-TOF) *m/z* calcd for $C_{21}H_{30}N_2NaO_7$ ($[M + Na]^+$): 445.1951, found: 445.1963.

*4.2.10. 4-{Allyl-[2-(tert-butyl-dimethyl-silanyloxy)-4-methoxy-benzyl]-carbamoyl}-4-(9H-fluoren-9-ylmethoxycarbonylamino)-butyric acid (**12**).*

Fmoc derivative **12** was prepared from Boc derivative **20** in a manner similar to that described for **7**. A white powder (62.2 mg, 94.4 μmol, 62%): $[\alpha]^{19}_D$ –21.9 (*c* 1.00, $CHCl_3$); IR ($CHCl_3$): ν_{max}, cm^{-1}: 936, 978, 1505, 1583, 1612, 1712, 2858, 2931, 3015, 3286.; ^1H NMR (DMSO-d_6, 300 MHz, 100 ºC) δ = 0.24 (6H, s), 0.98 (9H, s), 1.74–2.02 (2H, m), 2.30 (2H, t, *J* = 2.9 Hz), 3.70 (3H, s), 3.84–4.03 (2H, br m), 4.17–4.25 (1H, br m), 4.25–4.36 (2H, br m), 4.39–4.51 (2H, br m), 4.51–4.60 (1H, br m), 5.07 (1H, s), 5.11 (1H, s), 5.75 (1H, br m), 6.36 (1H, d, *J* = 2.4 Hz), 6.48 (1H, dd, *J* = 2.4 and 8.4 Hz), 6.99 (1H, br d, *J* = 8.4 Hz), 7.06–7.23 (1H, br m), 7.31 (2H, td, *J* = 7.3 and 2.2 Hz) 7.41 (2H, t, *J* = 7.3 Hz), 7.68 (2H, dd, *J* = 7.5 and 2.2 Hz), 7.86 (2H, d, *J* = 7.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz, 50 ºC, mixture of rotamers) δ = –3.9, –3.8, 18.6, 26.1, 28.7, 29.0, 29.7, 29.9, 43.3, 45.3, 47.6, 48.4, 49.3, 50.7, 50.8, 55.5, 67.4, 105.8, 106.0, 106.6, 106.9, 117.5, 117.9, 118.7, 119.7, 120.2, 125.4, 127.3, 127.9, 130.0, 132.7, 133.0, 141.6, 144.1, 144.2, 154.6, 154.9, 156.3, 156.7, 160.1, 160.4, 172.2, 172.7, 176.8, 176.8; HRMS (ESI-TOF) *m/z*

calcd for $C_{37}H_{46}N_2NaO_7Si$ ([M + Na]$^+$): 681.2972, found: 681.3004.

4.3. General procedures for peptide synthesis

4.3.1. Solid-phase peptide synthesis

Peptides were synthesized on Rink Amide AM resin (0.62 mmol amine/g) using standard Fmoc SPPS.
Coupling condition: Fmoc-Xaa-OH (5.0 eq.), DIC (5.0 eq.), and HOBt·H_2O (5.0 eq.) in DMF, 1.5 h, at room temperature.
TFA cleavage: TFA/TIPS/H_2O (95:2.5:2.5 (v/v), 50 μL/1 mg resin), 1.5 h, at room temperature.

4.3.2. Olefin metathesis

Completed dried resin (20.0 mg, 12.4 μmol) was swollen in degassed solvent (1,2-dichloroethane or *o*-dichlorobenzene (620 μL)) for 10 min. A solution of Grubbs catalyst (Grubbs 2nd generation catalyst (2.11 mg, 2.48 μmol) or Hoveyda 2nd generation catalyst (1.55 mg, 2.48 μmol) in solvent (1,2-dichloroethane or *o*-dichlorobenzene (620 μL)) was added to the resin. The reaction was monitored by HPLC after TFA cleavage. The reaction progress was quantified based on peak areas (= A) of HPLC as follow.

Conversion (%) = ($A_{\text{cyclized product}}$) / [($A_{\text{cyclized product}}$ + $A_{\text{substrate peptide}}$)] × 100

4.3.3. Reduction of resin-supported peptide using NBSH

Hydrogenation of a resin-supported peptide was carried out according to a literature[15]. A solution of 0.7 M NBSH and 1.4 M piperidine in NMP (200 μL) was added to dried resin (20.0 mg, 12.4 μmol) and the reaction mixture was heated at 70 ºC. The reaction was monitored by HPLC after TFA cleavage. When the reduction had been still incomplete, resin was washed with NMP and a fresh reagent was added for further reaction. This procedure was repeated four times.

4.4. Preparation of model peptides 5 and 13

Peptides were synthesized according to the section *"Solid-phase peptide synthesis"*

*Fmoc SPPS using **1***
Peptide **6** (minor peak): retention time = 20.9 min (Analytical HPLC conditions: linear gradient of solvent B in solvent A, 15 to 40% over 30 min); LRMS (ESI-TOF) *m/z* calcd for $C_{51}H_{92}N_{15}O_{11}$ ([M + H]$^+$): 1090.7, found: 1090.3.
Peptide **5** (major peak): retention time = 21.5 min (Analytical HPLC conditions: linear gradient of solvent B in solvent A, 15 to 40% over 30 min); LRMS (ESI-TOF) *m/z* calcd for $C_{51}H_{92}N_{15}O_{11}$ ([M + H]$^+$): 1090.7, found: 1090.4.
*Fmoc SPPS using **7***
Peptide **5**: a white lyophilized powder (4.4 mg, 3.31 μmol, 33%); retention time = 21.9 min (Analytical HPLC conditions: linear gradient of solvent B in solvent A, 15 to 40% over 30 min); retention time = 29.3 min (Semi-preparative HPLC conditions: A linear gradient of solvent B in solvent A, 15% to 40% over 30 min); LRMS (ESI-TOF) *m/z* calcd for $C_{51}H_{92}N_{15}O_{11}$ ([M + 2H]$^{2+}$): 545.9, found 545.9.
*Fmoc SPPS using **12***
Peptide **6**: a white lyophilized powder (3.2 mg, 2.44 μmol, 24%); retention time = 21.0 min (Analytical HPLC conditions: linear gradient of solvent B in solvent A, 15 to 40% over 30 min); retention time = 25.5 min (Semi-preparative HPLC conditions: A linear gradient of solvent B in solvent A, 15% to 40% over 30 min); LRMS (ESI-TOF) *m/z* calcd for $C_{51}H_{92}N_{15}O_{11}$ ([M + 2H]$^{2+}$): 545.9, found 545.8.

4.5. Preparation of resin-supported peptide 15a and 15b

Olefin metathesis was performed according to the section "*Olefin metathesis*".
16a: a white lyophilized powder (3.4 mg, 2.63 μmol, 26%); retention time = 23.2 min (Analytical HPLC conditions: A linear gradient of solvent B in solvent A, 15% to 40% over 30 min); retention time = 25.8 min (Semi-preparative HPLC conditions: A linear gradient of solvent B in solvent A, 18% to 38% over 30 min);
LRMS (ESI-TOF) *m/z* calcd for $C_{49}H_{88}N_{15}O_{11}$ ([M + H]$^+$): 1062.7, found 1062.4.
16b: a white lyophilized powder (2.9 mg, 2.22 μmol, 22%); retention time = 15.8 min (Analytical HPLC conditions: A linear gradient of solvent B in solvent A, 15% to 40% over 30 min); retention time = 21.2 min (Semi-preparative HPLC conditions: A linear gradient of solvent B in solvent A, 10% to 35% over 30 min); LRMS (ESI-TOF) *m/z* calcd for $C_{48}H_{85}N_{16}O_{12}$ ([M + H]$^+$): 1077.7, found 1077.4.

4.6. Preparation of peptide 18a and 18b

Hydrogenation and cleavage from the resin were carried out according to the section "*Reduction of resin-supported peptide using NBSH*"
18a: a white lyophilized powder (3.2 mg, 2.5 μmol, 20%); retention time = 24.4 min (Analytical HPLC conditions: A linear gradient of solvent B in solvent A, 15% to 40% over 30 min); retention time = 24.1 min (Semi-preparative HPLC conditions: A linear gradient of solvent B in solvent A, 10% to 30% over 30 min); LRMS (ESI-TOF) *m/z* calcd for $C_{49}H_{91}N_{15}O_{11}$ ([M + 2H]$^{2+}$): 532.9, found: 532.9.
18b: a white lyophilized powder (2.4 mg, 1.8 μmol, 15%); retention time = 16.3 min (Analytical HPLC conditions: A linear gradient of solvent B in solvent A, 15% to 40% over 30 min); retention time = 28.9 min (Semi-preparative HPLC conditions: A linear gradient of solvent B in solvent A, 15% to 40% over 30 min); LRMS (ESI-TOF) *m/z* calcd for $C_{48}H_{88}N_{16}O_{12}$ ([M + 2H]$^{2+}$): 540.7, found: 540.4.

4.7. Preparation of Peptide 21

The resin-bound fully-protected peptide **19** was prepared according to the section *"Solid-phase peptide synthesis"*. The obtained **19** (10.0 μmol) was added to a solution of $Pd(PPh_3)_4$ (34.7 mg, 30.0 μmol), *N*-methylmorpholine (11.0 μL, 0.10 mmol), AcOH (22.0 μL, 0.385 μmol) in $CHCl_3$ (407 μL). After 6 h shaking at room temperature, the resin was washed with CH_2Cl_2, 20% (v/v) piperidine/DMF and DMF respectively. Indomethacin was coupled with generated amine using the general coupling condition of Fmoc amino acids mentioned above. The obtained resin-bound fully-protected peptide **20** was subjected to olefin metathesis, followed by NBSH reduction according to the section *"Olefin metathesis"* and *"Reduction of resin-supported peptide using NBSH"*. The completed resin was globally deprotected and peptide **21** was obtained after HPLC purification.
Peptide **21**: an off-white lyophilized powder (1.7 mg, 0.885 μmol, 9%); retention time = 19.7 min (Analytical HPLC conditions: linear gradient of solvent B in solvent A, 20 to 60% over 30 min); retention time = 26.7 min (Semi-preparative HPLC conditions: A linear gradient of solvent B in solvent A, 25% to 45% over 30 min); LRMS (ESI-TOF) *m/z* calcd for $C_{79}H_{125}ClN_{20}O_{19}$ ([M + 2H]$^{2+}$): 846.5, found 846.2.

Acknowledgments

This research was supported in part by a Grant-in-Aid for Scientific Research (KAKENHI). K.A. is grateful to the Japanese Society for the Promotion of Science (JSPS) for a research fellowship. α-Allylglycine was provided as a gift from Nagase & Co., Ltd.

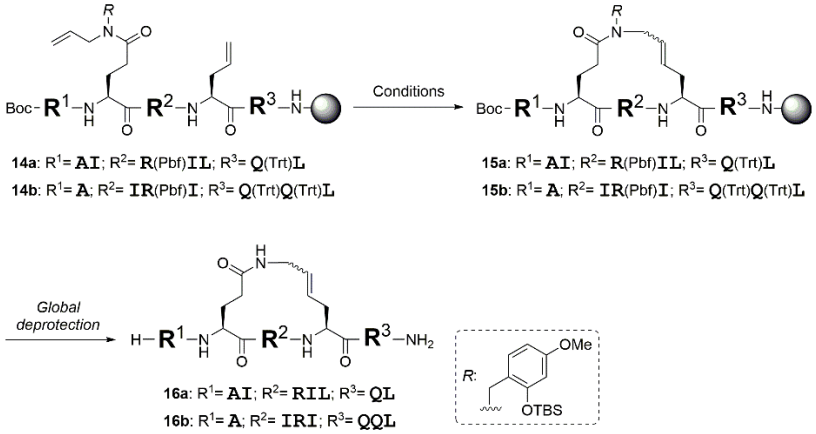
References and notes

1. Ruffner, H.; Bauer, A.; Bouwmeester, T. *Drug Discov. Today* **2007**, *12*, 709–716; Wells, J. A.; McClendon, C. L. *Nature* **2007**, *450*, 1001–1009.
2. Hruby, V. J. *Nat. Rev. Drug Discovery* **2002**, *1*, 847–858; Benyamini, H.; Friedler, A. *Future Med. Chem.* **2010**, *2*, 989–1003.
3. Sainlos, M.; Imperiali, B. *Nat. Protoc.* **2007**, *2*, 3210–3218; Luković, E.; González-Vera, J. a.; Imperiali, B. *J. Am. Chem. Soc.* **2008**, *130*, 12821–12827; Loving, G.; Imperiali, B. *J. Am. Chem. Soc.* **2008**, *130*, 13630–13638.
4. McGinty, R. K.; Kim, J.; Chatterjee, C.; Roeder, R. G.; Muir, T. W. *Nature* **2008**, *453*, 812–816; Dancy, B. C. R.; Ming, S. a.; Papazyan, R.; Jelinek, C. a.; Majumdar, A.; Sun, Y.; Dancy, B. M.; Drury, W. J.; Cotter, R. J.; Taverna, S. D.; Cole, P. a. *J. Am. Chem. Soc.* **2012**, *134*, 5138–5148; Bertran-vicente, J.; Serwa, R. A.; Schu, M.; Schmieder, P.; Krause, E.; Hackenberger, C. P. R. **2014**, *136*, 13622 −13628.
5. Torchilin, V. P.; Lukyanov, A. N. *Drug Discov. Today* **2003**, *8*, 259–266; Powell, M. F.; Stewart, T.; Otvos Jr, L.; Urge, L.; Gaeta, F. C. A; Sette, A.; Arrhenius, T.; Thomson, D.; Soda, K.; Colon, S. M. *Pharm. Res.* **1993**, *10*, 1268–1273.
6. Jackson, D. Y.; King, D. S.; Chmielewski, J.; Singh, S.; Schultz, P. G. *J. Am. Chem. Soc.* **1991**, *113*, 9391–9392.
7. Houston, M. E.; Patricia Campbell, a.; Lix, B.; Kay, C. M.; Sykes, B. D.; Hodges, R. S. *Biochemistry* **1996**, *35*, 10041–10050.
8. Walensky, L. D.; Kung, A. L.; Escher, I.; Malia, T. J.; Barbuto, S.; Wright, R. D.; Wagner, G.; Verdine, G. L.; Korsmeyer, S. J. *Science* **2004**, *305*, 1466–1470; Walensky, L. D.; Bird, G. H. *J. Med. Chem.* **2014**, *57*, 6275–6288.
9. Miranda, L. P.; Winters, K. A.; Gegg, C. V.; Patel, A.; Aral, J.; Long, J.; Zhang, J.; Diamond, S.; Guido, M.; Stanislaus, S.; Ma, M.; Li, H.; Rose, M. J.; Poppe, L.; Véniant, M. M. *J. Med. Chem.* **2008**, *51*, 2758–2765; Murage, E. N.; Schroeder, J. C.; Beinborn, M.; Ahn, J. M. *Bioorganic Med. Chem.* **2008**, *16*, 10106–10112; Day, J. W.; Ottaway, N.; Patterson, J. T.; Gelfanov, V.; Smiley, D.; Gidda, J.; Findeisen, H.; Bruemmer, D.; Drucker, D. J.; Chaudhary, N.; Holland, J.; Hembree, J.; Abplanalp, W.; Grant, E.; Ruehl, J.; Wilson, H.; Kirchner, H.; Lockie, S. H.; Hofmann, S.; Woods, S. C.; Nogueiras, R.; Pfluger, P. T.; Perez-Tilve, D.; DiMarchi, R.; Tschöp, M. H. *Nat. Chem. Biol.* **2009**, *5*, 749–757; Murage, E. N.; Gao, G.; Bisello, A.; Ahn, J. M. *J. Med. Chem.* **2010**, *53*, 6412–6420.
10. Taylor, J. W. *Biopolymer* **2002**, *66*, 49–75; Cantel, S.; Isaad, A. L. C.; Scrima, M.; Levy, J. J.; DiMarchi, R. D.; Rovero, P.; Halperin, J. A.; D'Ursi, A. M.; Papini, A. M.; Chorev, M. *J. Org. Chem.* **2008**, *73*, 5663–5674; Oliveira, V. X.; Fázio, M. A.; Silva, A. F.; Campana, P. T.; Pesquero, J. B.; Santos, E. L.; Costa-Neto, C. M.; Miranda, A. *Regul. Pept.* **2011**, *172*, 1–7; Frankiewicz, L.; Betti, C.; Guillemyn, K.; Tourwé, D.; Jacquot, Y.; Ballet, S. *J. Pept. Sci.* **2013**, *19*, 423–432.
11. Kim, Y.-W.; Grossmann, T. N.; Verdine, G. L. *Nat. Protoc.* **2011**, *6*, 761–771.
12. Cluzeau, J.; Oishi, S.; Ohno, H.; Wang, Z.; Evans, B.; Peiper, S. C.; Fujii, N. *Org. Biomol. Chem.* **2007**, *5*, 1915–1923.
13. Quibell, M.; Owen, D.; Packman, L. C.; Johnson, T. *J. Chem. Soc., Chem. Commun.* **1994**, 2343–2344; Packman, L. C. *Tetrahedron Lett.* **1995**, *36*, 7523–7526; Offer, J.; Quibell, M.; Johnson, T. *J. Chem. Soc., Perkin Trans. 1* **1996**, 175–182; Nicolás, E.; Pujades, M.; Bacardit, J.; Giralt, E.; Albericio, F. *Tetrahedron Lett.* **1997**, *38*, 2317–2320; Wahlström, K.; Planstedt, O.; Undén, A. *Tetrahedron Lett.* **2008**, *49*, 3921–3924.
14. Myers, A. G.; Zheng, B.; Movassaghi, M. *J. Org. Chem.* **1997**, *62*, 7507; Buszek, K. R.; Brown, N. *J. Org. Chem.* **2007**, *72*, 3125–3128; García-Aranda, M. I.; González-Muñiz, R.; Teresa García-López, M.; De Jesús Pérez Vega, M. *Eur. J. Org. Chem.* **2009**, 24, 4149–4157; García-Aranda, M. I.; Marrero, P.; Gautier, B.; Martín-Martínez, M.; Inguimbert, N.; Vidal, M.; García-López, M. T.; Jiménez, M. A.; González-Muñiz, R.; Pérez de Vega, M. J. *Bioorg. Med. Chem.* **2011**, *19*, 1978–1986; Schafmeister, C. E.; Po, J.; Verdine, G. L.; *J. Am. Chem. Soc.* **2000**, *122*, 5891–5892.

Supplementary Material

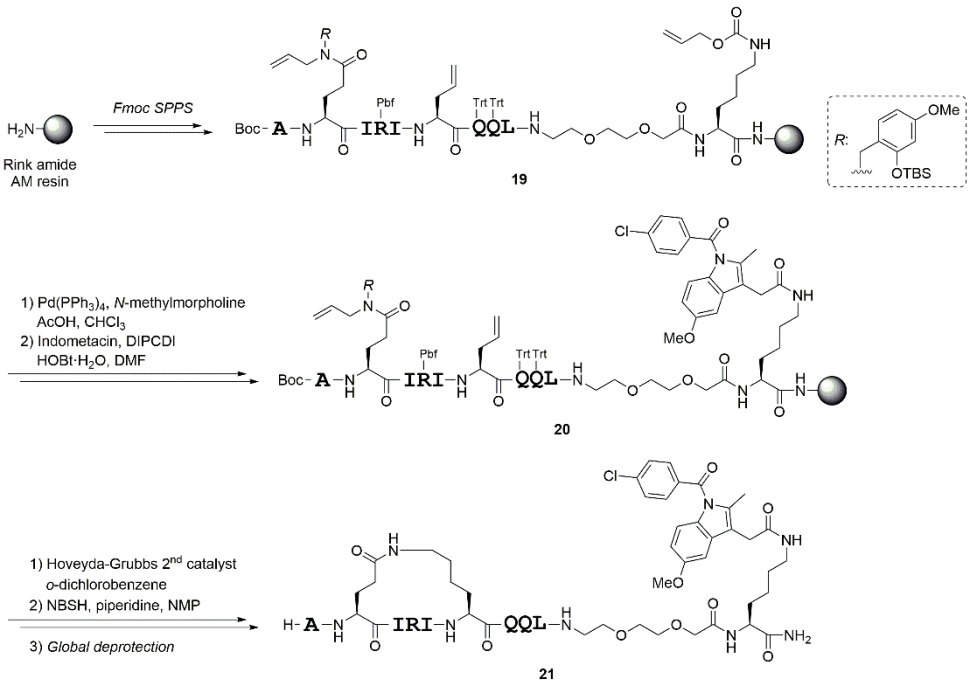
Supplementary data related to this article can be found at http: ####

Table 1. Reaction conditions for the RCM reaction.

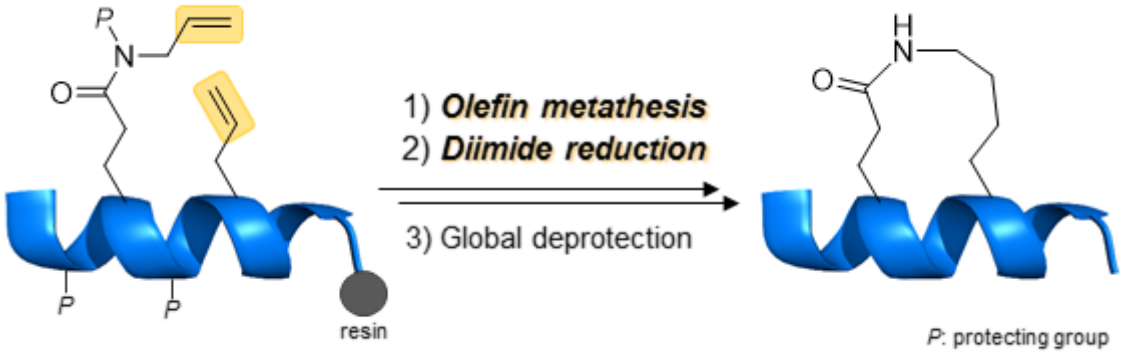


Entry	Peptide	Catalyst[a]	Solvent	Temperature (ºC)	Time (min)	Conversion[b] (%)
1	**14a**	Grubbs 2nd	1,2-dichloroethane	70	60	24
2	**14a**	Grubbs 2nd	*o*-dichlorobenzene	120	10	97
3	**14a**	Hoveyda-Grubbs 2nd	*o*-dichlorobenzene	120	10	70
4	**14b**	Grubbs 2nd	1,2-dichloroethane	70	60	9
5	**14b**	Grubbs 2nd	*o*-dichlorobenzene	120	10	45
6	**14b**	Hoveyda-Grubbs 2nd	*o*-dichlorobenzene	120	10	92

[a] 20 mol% of Grubbs catalyst was used.; [b] Conversion was determined by HPLC separation and peak areas (=A) of cyclized peptide **16a**, **16b** ($=A_{\text{cyclized product}}$) detected at 220 nm as a fraction of the sum of the unreacted peptide ($A_{\text{cyclized product}} + A_{\text{substrate peptide}}$).



Scheme 6. Synthesis of indomethacin-conjugated lactam-bridged peptide.



Graphical Abstract