Organic & Biomolecular Chemistry

PAPER

View Article Online
View Journal

Cite this: DOI: 10.1039/c6ob02391f

A rapid and clean synthetic approach to cyclic peptides *via* micro-flow peptide chain elongation and photochemical cyclization: synthesis of a cyclic RGD peptide†

Yuto Mifune, Hiroyuki Nakamura and Shinichiro Fuse*

Received 3rd November 2016,
Accepted 9th November 2016

DOI: 10.1039/c6ob02391f

www.rsc.org/obc

A cyclic RGD peptide was efficiently synthesized based on micro-flow, triphosgene-mediated peptide chain elongation and micro-flow photochemical macrolactamization. Our approach enabled a rapid (amidation for peptide chain elongation <5 s, macrolactamization <5 min) and clean (only one column chromatographic separation) synthesis of a cyclic peptide.

Introduction

Cyclic peptides are important as drugs and tools in chemical biology,[1,2] and their decreased level of conformational freedom enhances their metabolic stability and binding affinity compared with linear peptides. In general, cyclic peptides **4** are chemically synthesized *via* a two-stage approach: peptide chain elongation and cyclization (Fig. 1). The linear peptide **2** is prepared from the protected C-terminal amino acid **1** *via* repetitive amide bond formation using an *N*-protected amino acid and deprotection. After the deprotection at the C-terminal carboxyl group of the prepared linear peptide **2**, macrolactamization of **3** affords the desired cyclic peptide **4**. This conventional approach has several drawbacks. (1) The remaining/generated compounds in the deprotection step (**2** to **3**) often cannot be removed from the cyclization precursor **3**. Free amino and carboxyl groups make precursor **3** poorly soluble against a variety of solvents, and this complicates the removal of the impurities. These can inhibit the subsequent key step of macrolactamization.[3] (2) Macrolactamization (**3** to **4**) requires an excess amount of coupling reagents to accelerate the activation of carboxylic acids because high dilution conditions are usually employed in order to avoid the undesired intermolecular reactions. A great deal of waste originates from excess amounts of coupling reagents, which complicates the purification of the cyclization product **4**.[4] (3) Coupling reagents, such as carbodiimides, uronium salts or phosphonium salts,[5] are usually used for amide bond formations (**1** to **2** and **3** to **4**). The use of these low-atom-economy reagents generate much waste, and tedious purification steps to remove them are usually required after each amide bond formation.[6] Therefore, the development of a rapid, clean and practical synthetic approach for cyclic peptides remains highly important.

In terms of the key macrolactamization,[4,7] a native chemical ligation (NCL) and its modified approach have been developed into a clean and mild cyclization method.[8–13] This approach requires neither the addition of coupling reagents nor an isolation of the poorly soluble precursor **3**. However, the precursors must have either a cysteine[8,9] or a serine/threonine[10] residue at their N-terminus. Several cyclization-cleavage approaches have been reported using oxime-, thioester-, sulfonamide-, hydrazine- or catechol-linkers in solid-phase peptide synthesis.[14] These approaches do not require the isolation of **3**. However, the oxime- and thioester-linkers are applicable only to Boc chemistry due to their high reactivity against nucleophiles. The use of sulfonamide-, hydrazine- and catechol-linkers requires an excess amount of activating reagents and an extended reaction time (≥1 day) for cyclization, and these linkers also have a limited scope of amino acids and protecting groups.

Nicolaou and coworkers reported 5-acyl-7-nitroindoline as a photoactivatable linker, and they demonstrated an intramolecular cyclization-cleavage approach for the synthesis of 7-membered heterocycles.[15,16] This approach is attractive because the cyclization precursor does not contain the free carboxyl group, which simplifies the removal of impurities from the cyclization precursor. Moreover, the addition of an excess amount of activating reagent is unnecessary for photochemical macrolactamization. However, this approach usually requires an extended reaction time (6–12 h), and it has yet to be used for the macrolactamization of peptides.

Laboratory for Chemistry and Life Science, Institute of Innovative Research, Tokyo Institute of Technology, 4259 Nagatsuta-cho, Midori-ku, Yokohama 226-8503, Japan. E-mail: sfuse@res.titech.ac.jp

†Electronic supplementary information (ESI) available: A micro-flow reactor setup and NMR spectra of products. See DOI: 10.1039/c6ob02391f

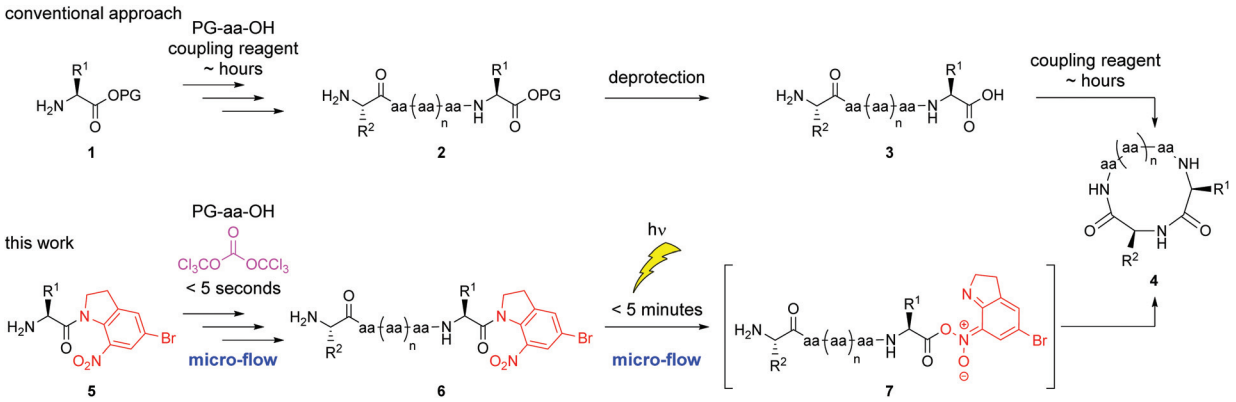
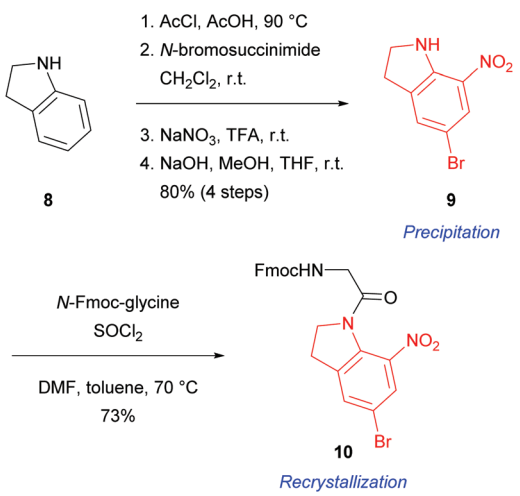


Fig. 1 Conventional synthetic approach and our approach for a cyclic peptide. PG = protecting group; aa = amino acid.

We demonstrated an efficient micro-flow photochemical synthesis of vitamin D and its analogues, along with α-aryl esters.[17] The narrow reaction space in a micro-flow reactor enhanced the penetration efficiency of light and reduced the reaction time.[18] We envisaged that the use of micro-flow technology would enable rapid photochemical cyclization (Fig. 1). In addition, we planned to use our originally developed triphosgene-mediated micro-flow amide bond formation for peptide chain elongation.[19] Herein, we report a rapid and clean synthesis of a cyclic RGD peptide, which is a highly potent and selective antagonist for the $\alpha_v\beta_3$ integrin receptor.[20]

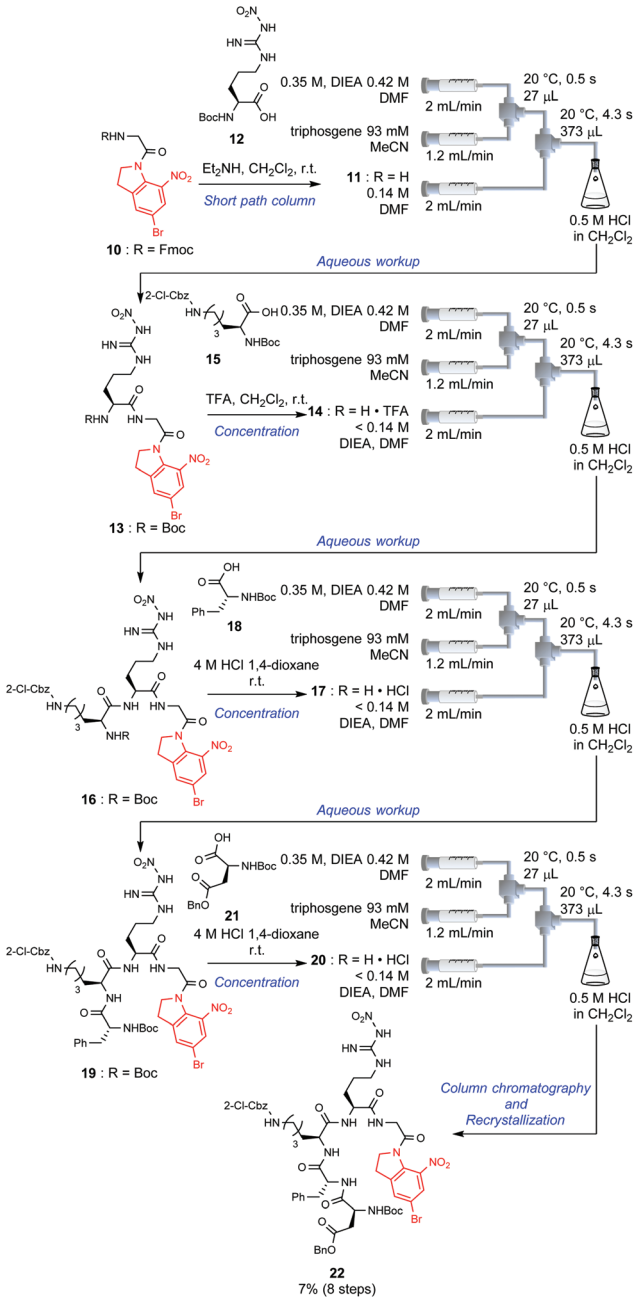
Results and discussion

We selected readily available 5-bromo-7-nitroindoline (Bni)[16a,b,f,21] as a photoactivatable group. Bni-protected Fmoc-glycine **10**, a precursor of peptide chain elongation, was efficiently prepared from indoline (**8**) as shown in Scheme 1. The sequence of acetylation/regioselective bromination/nitrization/removal of the acetyl group and the following simple precipitation afforded the pure Bni **9** in 4 steps with 80% yield. An acylation with *N*-Fmoc-glycine and thionyl chloride, followed by recrystallization, provided Bni-protected *N*-Fmoc-glycine **10** in 73% yield.



Scheme 1 Synthesis of Bni-protected *N*-Fmoc-glycine **10**.

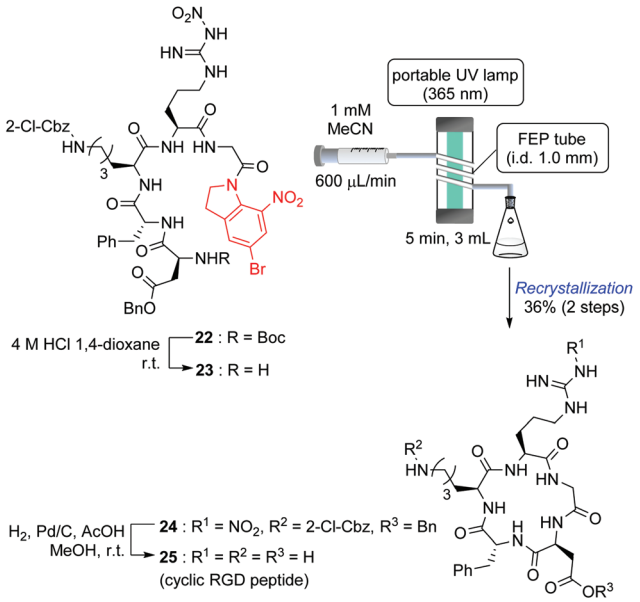
Peptide chain elongation was performed using our developed micro-flow amide bond formation[19] and batch deprotection (Scheme 2). The Fmoc group of the glycine derivative **10** was removed under basic conditions. After removing the non-polar compounds that originated from the Fmoc group *via* short-path chromatography, micro-flow amide bond formation with N_α-Boc-N_ω-nitro-L-arginine (**12**) was carried out. We connected two T-shaped micro-mixers with Teflon® tubing. A solution of carboxylic acid **12** and *N*,*N*-diisopropylethylamine (DIEA) in DMF was introduced into the first micro-mixer with a syringe pump. A solution of triphosgene in MeCN was also introduced into the first micro-mixer with a syringe pump. After the generation of symmetric anhydride *in situ* (<0.5 s), a solution of amine **11** was introduced into the second micro-mixer with a syringe pump to accomplish amidation (<4.3 s). The reaction temperature was maintained at 20 °C by immersing the micro-mixers into a water bath. The reaction was quenched by pouring the mixture into a 0.5 M solution of HCl in CH_2Cl_2. The HCl salt of DIEA and unreacted carboxylic acid **12** was separated by simple aqueous workup to afford a fairly pure protected dipeptide **13**. The removal of the Boc group of the protected dipeptide **13** was performed under acidic conditions. In the subsequent micro-flow amidation with N_α-Boc-N_ε-2-chlorocarbobenzoxy (2-Cl-Cbz)-L-lysine (**15**), the TFA salt of amine **14** was basified with DIEA before introduction into the micro-mixer. After aqueous workup, the crude protected tripeptide **16** was treated with 4 M HCl in 1,4-dioxane to provide the HCl salt of amine **17**. Next, micro-flow amidation with *N*-Boc-D-phenylalanine (**18**) was performed under the same conditions as described above. The final coupling of tetrapeptide **20** with *N*-Boc-L-aspartic acid 4-benzyl ester (**21**) afforded a crude mixture of the protected pentapeptide **22**. After silica-gel column chromatography and recrystallization, the desired pure pentapeptide **22**, which did not contain



Scheme 2 Peptide chain elongation based on micro-flow amide bond formation and batch deprotection.

epimers, was obtained in 8 steps with 7% yield from protected glycine **10**. Although the observed yield was moderate, this result was obtained in only one trial with no optimization of the reaction conditions. In addition, it should be noted that the desired pentapeptide **22** was obtained using only one-column chromatographic purification.

Next, we performed the intramolecular cyclization of a Bni-protected linear peptide by irradiation using a micro-flow reactor (Scheme 3). A fluorinated ethylene propylene copolymer (FEP) tube (inner diameter: 1.0 mm) was tightly wrapped



Scheme 3 Intramolecular cyclization of Bni-protected pentapeptide and global deprotection.

around a 9 W portable UV lamp (wavelength: 365 nm) and connected with PEEK tubing.[17c,d] After removal of the Boc group of the protected pentapeptide **22**, a syringe pump was used to introduce a 1 mM solution of amine **23** in MeCN into the FEP tube, which was then irradiated for 5 min. The desired protected cyclic pentapeptide **24** was readily obtained after recrystallization in 36% yield (2 steps). The subsequent hydrogenation of the protected pentapeptide **24** afforded a cyclic RGD peptide (**25**) in a quantitative yield. The observed spectra of the cyclic RGD peptide (**25**) were in good agreement with the previously reported data.[20c,h]

Conclusions

In summary, we demonstrated a rapid and clean synthesis of a cyclic RGD peptide. Our originally developed triphosgene-mediated micro-flow amide bond formation enabled a rapid (amidation <5 s) and clean (only one column chromatographic separation) synthesis of the linear protected pentapeptide **22**. A rapid intramolecular cyclization (<5 min) was achieved by irradiation of the Bni-protected pentapeptide **23** in a micro-flow reactor. Our developed process will allow the rapid and practical preparation of a variety of biologically active cyclic peptides.

Experimental

General techniques

NMR spectra were recorded on Bruker Biospin AVANCE II 400 (400 MHz for ^{1}H, 100 MHz for ^{13}C)and Bruker Biospin AVANCE III HD 500 (500 MHz for ^{1}H, 125 MHz for ^{13}C) instruments in

the indicated solvent. Chemical shifts are reported in units of parts per million (ppm) relative to the signal (0.00 ppm) for internal tetramethylsilane for solutions in $CDCl_3$ (7.26 ppm for ^1H, 77.0 ppm for ^{13}C) or DMSO (2.50 ppm for ^1H, 39.5 ppm for ^{13}C) or D_2O (4.79 ppm for ^1H, 21.0 ppm for ^{13}C using acetic acid as an internal standard). Multiplicities are reported by using the following abbreviations: s; singlet, d; doublet, t; triplet, q; quartet, m; multiplet, br; broad, *J*; coupling constants in Hertz (Hz). IR spectra were recorded on a JASCO FT/IR-4100. Only the strongest and/or structurally important peaks are reported as the IR data given in cm^{-1}. Optical rotations were measured with a Rudolph Research Analytical AUTOPOL® IV. HRMS (ESI-TOF) were recorded on a Bruker micrOTOF II.

All reactions were monitored by thin-layer chromatography carried out on 0.25 mm E. Merck silica gel plates (60F-254) with UV light, and visualized by 10% ethanolic phosphomolybdic acid or 0.5% ninhydrin *n*-butanol solution. Flash column chromatography was performed on silica gel PSQ 60B purchased from Fuji Silysia Chemical Ltd. DIEA was distilled from ninhydrin and KOH.

Synthesis of 5-bromo-7-nitroindoline (Bni) (9)

To a solution of indoline (**8**) (5.00 mL, 44.5 mmol) in acetic acid (66.8 mL), acetyl chloride (18.7 mL) was added at room temperature. After being stirred at 90 °C for 1.5 h, the reaction mixture was cooled and concentrated *in vacuo*. The residue was used for the next reaction without further purification.

To a solution of crude *N*-acetyl indoline (3.22 g, 20.0 mmol, 1.00 equiv.) in CH_2Cl_2 (40.0 mL), *N*-bromosuccinimide (3.92 g, 22.0 mmol, 1.10 equiv.) was added at room temperature. After being stirred at the same temperature for 30 min, the reaction mixture was diluted with CH_2Cl_2, washed with water, saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was used for the next reaction without further purification.

To a solution of crude aryl bromide (4.64 g, 19.3 mmol, 1.00 equiv.) in TFA (39.0 mL), sodium nitrate (1.80 g, 21.2 mmol, 1.10 equiv.) was added at 0 °C. After being stirred at room temperature for 30 min, the reaction mixture was poured into ice cold water. The precipitate was filtered, washed with water, dried under vacuum, and used for the next reaction without further purification.

To a solution of crude amide (5.50 g, 19.3 mmol, 1.00 equiv.) in MeOH (19.0 mL) and THF (97.0 mL), sodium hydroxide (1.30 g, 32.5 mmol, 1.68 equiv.) was added at 0 °C. After being stirred at room temperature for 30 min, one third of THF was removed *in vacuo*. The resultant mixture was poured into ice cold water. The precipitate was filtered, washed with water, and dried under vacuum to give 5-bromo-7-nitroindoline (**9**) (3.89 g, 16.0 mmol, 4 steps 80%) as an orange solid.

^1H NMR (400 MHz, $CDCl_3$): δ 7.89 (s, 1H), 7.24 (s, 1 H), 6.79 (brs, 1H), 3.90 (t, J = 8.4 Hz, 2H), 3.18 (t, J = 8.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 148.0, 136.0, 132.5, 128.9, 124.2, 107.0, 46.9, 27.9; IR (KBr): 3369, 2352, 1621, 1581, 1507, 1482, 1392, 1317, 1284, 1230, 1157, 965, 861, 763 cm^{-1}.

Synthesis of Bni-protected *N*-Fmoc-glycine 10

To a solution of *N*-Fmoc-glycine (5.23 g, 17.6 mmol, 1.10 equiv.) in toluene (80.0 mL), thionyl chloride (2.30 mL, 32.0 mmol, 2.00 equiv.) and two drops of DMF were added dropwise at room temperature. After being stirred at 70 °C for 1.5 h, 5-bromo-7-nitroindoline (**9**) (3.89 g, 16.0 mmol, 1.00 equiv.) was added at room temperature. After being stirred at 70 °C for 17 h, the reaction mixture was cooled and concentrated *in vacuo*. The residue was recrystallized from EtOAc/hexane to give Bni-protected *N*-Fmoc-glycine **10** (6.09 g, 11.7 mmol, 73%) as a light yellow solid.

^1H NMR (400 MHz, $CDCl_3$): δ 7.70 (d, J = 8.0 Hz, 2H), 7.68 (s, 1H), 7.55 (d, J = 7.2 Hz, 2H), 7.43 (s, 1H), 7.32 (t, J = 7.4 Hz, 2H), 7.25 (t, J = 7.6 Hz, 2H), 5.96 (br, 1H), 4.31 (d, J = 6.8 Hz, 2H), 4.17–4.09 (m, 5H), 3.10 (t, J = 8.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.1, 156.3, 143.6, 141.0, 140.5, 138.4, 133.1, 131.6, 127.6, 127.0, 125.1, 125.0, 119.8, 116.7, 67.1, 48.6, 46.9, 44.0, 28.7; IR (KBr): 3422, 3328, 3068, 3016, 2948, 2894, 2360, 1716, 1685, 1539, 1459, 1218, 742 cm^{-1}; mp 120–122 °C; HRMS (ESI-TOF): calcd for $[C_{25}H_{20}BrN_3O_5 + Na]^+$ 544.0479, found 544.0487.

Removal of the Fmoc group

To a solution of Bni-protected *N*-Fmoc-glycine **10** (731 mg, 1.40 mmol) in CH_2Cl_2 (2.8 mL), diethylamine (2.8 mL) was added at room temperature. After being stirred at the same temperature for 1 h, the reaction mixture was concentrated *in vacuo*. The residue was purified by short path column chromatography on silica gel (10% MeOH in CH_2Cl_2) and used for the next reaction without further purification.

General procedure for removal of the Boc group

To a crude peptide, TFA/CH_2Cl_2 (1/3, 6.0 mL) or 4 M HCl 1,4-dioxane (2.0–2.5 mL) was added at room temperature. After being stirred at the same temperature for 40–60 min, the reaction mixture was concentrated *in vacuo*. The residue was used for the next reaction without further purification.

General procedure for micro-flow amide bond formation

A solution of carboxylic acid (0.35 M) and DIEA (0.42 M) in DMF (flow rate: 2.0 mL min^{-1}) and a solution of triphosgene (0.093 M) in MeCN (flow rate: 1.2 mL min^{-1}) were introduced into a T-shape mixer 1 (inner diameter: 0.25 mm) at 20 °C with the syringe pumps. The resulting mixture was passed through a reaction tube 1 (inner diameter: 0.8 mm, length: 54 mm, volume: 27 μL, reaction time: 0.5 s) at the same temperature. Then, the resulting mixture and a solution of amine (<0.14 M) and DIEA that was added to adjust the pH to 8–9 in DMF (flow rate: 2.0 mL min^{-1}) were introduced to a T-shape mixer 2 (inner diameter: 0.25 mm) at 20 °C. The resulting mixture was passed through a reaction tube 2 (inner diameter: 0.8 mm, length: 742 mm, volume: 373 μL, reaction time: 4.3 s) at the same temperature. The resulting mixture was poured into a 0.5 M HCl and CH_2Cl_2 suspension at room temperature. The aqueous layer was extracted twice with CH_2Cl_2. The com-

bined organic layer was washed with 1 M HCl, twice with saturated aqueous $NaHCO_3$ and with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was used for the next reaction without further purification.

Pentapeptide 22

Purification conditions: column chromatography on silica gel (5% MeOH in CH_2Cl_2), then recrystallization from EtOH/CH_2Cl_2/Et_2O/hexane, 120 mg, 0.0960 mmol, 8 steps 7% from Bni-protected *N*-Fmoc-glycine **10**

Yellow solid; ^{1}H NMR (500 MHz, $CDCl_3/CD_3OD$ = 1/1): δ 8.01 (brd, J = 5.0 Hz, 1H), 7.93 (brd, J = 5.0 Hz, 1H), 7.85 (br, 2H), 7.74 (s, 1H), 7.61 (s, 1H), 7.42–7.17 (m, 14H), 6.65 (brd, J = 7.0 Hz, 1H), 6.55 (br, 1H), 5.17 (s, 2H), 5.10–5.05 (m, 2H), 4.55–4.46 (m, 2H), 4.41 (br, 1H), 4.34–4.08 (m, 5H), 3.30–3.16 (m, 4H), 3.14–3.04 (m, 3H), 2.99 (dd, J = 8.0, 12.5 Hz, 1H), 2.82 (dd, J = 6.5, 17.5 Hz, 1H), 2.77 (dd, J = 6.5, 17.5 Hz, 1H), 1.99–1.90 (m, 1H), 1.83–1.72 (m, 2H), 1.71–1.52 (m, 3H), 1.48–1.35 (m, 11H), 1.18–1.08 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3/CD_3OD$ = 1/1): δ 172.5, 172.2, 172.1, 170.6, 167.1, 158.8, 156.6, 155.7, 155.7, 140.2, 138.8, 135.7, 134.9, 133.8, 132.8, 132.4, 131.4, 128.7, 128.6, 128.5, 128.5, 128.0, 127.8, 127.6, 127.4, 126.4, 126.3, 124.5, 116.2, 79.7, 66.1, 63.0, 54.9, 53.6, 52.3, 50.2, 48.6, 41.8, 39.9, 39.8, 36.5, 35.5, 30.0, 28.4, 28.2, 27.8, 27.3, 24.0, 22.2; IR (KBr): 3286, 3087, 2932, 2362, 2343, 1699, 1636, 1538, 1459, 1257, 1165 cm^{-1}; $[\alpha]_D^{28}$ = +6.73 (c 1.12, $CHCl_3$); mp 154–157 °C; HRMS (ESI-TOF): calcd for $[C_{55}H_{66}BrClN_{12}O_{15} + Na]^+$ 1271.3535, found 1271.3548.

Intramolecular cyclization

To pentapeptide **22** (30.3 mg, 0.0242 mmol), 4 M HCl 1,4-dioxane (1.5 mL) was added at room temperature. After being stirred at the same temperature for 40 min, the reaction mixture was concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2, and the organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was used for the next reaction without further purification.

A solution of crude amine **23** in MeCN (24.0 mL) was introduced into the tube reactor (FEP tube, inner diameter: 1.0 mm, length: 3821 mm, volume: 3.00 mL) at room temperature with a syringe pump (flow rate: 600 μL min^{-1}) and irradiated with a 9 W UV lamp (wavelength: 365 nm) for 5 min. The resultant mixture was collected over 40 min and concentrated *in vacuo*. The residue was recrystallized from MeOH to give the cyclic pentapeptide **24** (7.8 mg, 0.00860 mmol, 2 steps 36%) as a colorless solid.

^{1}H NMR (500 MHz, DMSO): δ 8.54 (brs, 1 H), 8.39 (br, 1H), 8.12 (d, J = 8.0 Hz, 1H), 8.08 (d, J = 7.0 Hz, 1H), 8.02 (d, J = 7.0 Hz, 1H), 7.54 (d, J = 7.5 Hz, 1H), 7.50–7.46 (m, 2H), 7.43–7.27 (m, 8H), 7.24 (t, J = 7.0 Hz, 2H), 7.17 (d, J = 7.0 Hz, 1H), 7.13 (d, J = 7.0 Hz, 2H), 5.09 (s, 2H), 5.06 (s, 2H), 4.70 (ddd, J = 8.0, 8.0, 7.0 Hz, 1H), 4.45 (ddd, J = 7.0, 7.0, 7.0 Hz, 1H), 4.17 (ddd, J = 7.0, 7.0, 7.5 Hz, 1H), 4.03 (dd, J = 7.5, 14.5 Hz, 1H), 3.94–3.87 (m, 1H), 3.23 (dd, J = 3.5, 14.5 Hz, 1H), 3.14 (br, 2H), 2.97–2.75 (m, 5H), 2.54 (dd, J = 5.5, 16.0 Hz, 1H), 1.76–1.65 (m, 1H), 1.59–1.20 (m, 7H), 1.08–0.92 (m, 2H); ^{13}C NMR (125 MHz, DMSO): δ 172.0, 171.2, 170.5, 169.9, 169.7, 169.6, 159.2, 155.8, 137.2, 136.1, 134.6, 132.3, 129.8, 129.7, 129.3, 129.1, 128.4, 128.1, 128.0, 127.8, 127.3, 126.3, 65.6, 62.6, 54.6, 54.4, 51.9, 48.8, 43.3, 40.2, 40.2, 37.4, 35.2, 30.9, 28.7, 28.7, 24.8, 22.7; IR (KBr): 3284, 3071, 2937, 2364, 2337, 1643, 1542, 1261 cm^{-1}; $[\alpha]_D^{28}$ = −38.1 (c 0.156, $CHCl_3/CH_3OH$ = 1/1); mp 206–209 °C; HRMS (ESI-TOF): calcd for $[C_{42}H_{51}ClN_{10}O_{11} + Na]^+$ 929.3320, found 929.3318.

Synthesis of a cyclic RGD peptide (25)

To a solution of the cyclic pentapeptide **24** (3.0 mg, 0.0033 mmol, 1.0 equiv.) in MeOH (0.4 mL) and acetic acid (0.4 mL), 10% Pd/C (8.8 mg, 0.0083 mmol, 2.5 equiv.) was added at room temperature under argon. After being stirred at the same temperature for 18 h under H_2, the reaction mixture was filtered through a pad of Celite and concentrated *in vacuo* to give a cyclic RGD peptide (**25**) (4.2 mg, quant.) as a colorless solid.

^{1}H NMR (500 MHz, D_2O): δ 7.46–7.22 (m, 5H), 4.71 (dd, J = 7.0, 7.0 Hz, 1H), 4.57 (dd, J = 5.0, 10.0 Hz, 1H), 4.40 (dd, J = 7.0, 7.0 Hz, 1H), 4.20 (d, J = 15.0 Hz, 1H), 3.88 (br, 1H), 3.48 (d, J = 14.5 Hz, 1H), 3.19 (br, 2H), 3.12 (dd, J = 5.5, 13.0 Hz, 1H), 2.95 (dd, J = 12.0, 12.0 Hz, 1H), 2.87 (t, J = 8.0 Hz, 2H), 2.70 (dd, J = 7.0, 15.5 Hz, 1H), 2.56 (dd, J = 5.5, 14.5 Hz, 1H), 1.92–1.82 (m, 1H), 1.74–1.60 (m, 2H), 1.58–1.39 (m, 5H), 0.96–0.78 (m, 2H); ^{13}C NMR (125 MHz, D_2O): δ 178.3, 173.2, 172.5, 171.5, 171.5, 170.3, 155.7, 135.0, 128.3, 127.9, 126.2, 54.6, 53.9, 51.4, 49.7, 42.8, 39.5, 38.1, 36.6, 35.7, 28.7, 26.5, 25.0, 23.4, 21.2; HRMS (ESI-TOF): calcd for $[C_{27}H_{41}N_9O_7 + H]^+$ 604.3202, found 604.3202.

Acknowledgements

This work was partially supported by a Grant-in-Aid for JSPS Research Fellow, a Grant-in-Aid for Young Scientists (B), Scientific Research on Innovative Areas 2707 Middle molecular strategy form MEXT (no. 15H05849), and a Naito Foundation Natural Science Scholarship.

Notes and references

1 (*a*) V. J. Hruby, *Nat. Rev. Drug Discovery*, 2002, **1**, 847; (*b*) Y. Hamada and T. Shioiri, *Chem. Rev.*, 2005, **105**, 4441; (*c*) M. Katsara, T. Tselios, S. Deraos, G. Deraos, M.-T. Matsoukas, E. Lazoura, J. Matsoukas and V. Apostolopoulos, *Curr. Med. Chem.*, 2006, **13**, 2221; (*d*) D. J. Craik, D. P. Fairlie, S. Liras and D. Price, *Chem. Biol. Drug Des.*, 2013, **81**, 136; (*e*) P. Thapa, M. J. Espiritu, C. Cabalteja and J.-P. Bingham, *Int. J. Pept. Res. Ther.*, 2014, **20**, 545; (*f*) A. Tapeinou, M.-T. Matsoukas, C. Simal and T. Tselios, *Biopolymers*, 2015, **104**, 453.

2 (*a*) E. M. Driggers, S. P. Hale, J. Lee and N. K. Terrett, *Nat. Rev. Drug Discovery*, 2008, **7**, 608; (*b*) E. Marsault and

M. L. Peterson, *J. Med. Chem.*, 2011, **54**, 1961; (*c*) J. Mallinson and I. Collins, *Future Med. Chem.*, 2012, **4**, 1409.

3 Simultaneous removal of alloc and allyl protecting groups from the cyclization precursor using Pd (0) and 1,3-dimethylbarbituric acid (DMBA) was performed, however, DMBA could not be removed by column chromatographic separations. The remaining DMBA inhibited macrolactamization. See: S. Fuse, H. Koinuma, A. Kimbara, M. Izumikawa, Y. Mifune, H. He, K. Shin-ya, T. Takahashi and T. Doi, *J. Am. Chem. Soc.*, 2014, **136**, 12011.

4 S. Jiang, Z. Li, K. Ding and P. P. Roller, *Curr. Org. Chem.*, 2008, **12**, 1502.

5 (*a*) S.-Y. Han and Y.-A. Kim, *Tetrahedron*, 2004, **60**, 2447; (*b*) C. A. G. N. Montalbetti and V. Falque, *Tetrahedron*, 2005, **61**, 10827; (*c*) E. Valeur and M. Bradley, *Chem. Soc. Rev.*, 2009, **38**, 606; (*d*) A. El-Faham and F. Albericio, *Chem. Rev.*, 2011, **111**, 6557.

6 (*a*) D. J. C. Constable, P. J. Dunn, J. D. Hayler, G. R. Humphrey, J. L. Leazer, Jr., R. J. Linderman, K. Lorenz, J. Manley, B. A. Pearlman, A. Wells, A. Zaks and T. Y. Zhang, *Green Chem.*, 2007, **9**, 411; (*b*) V. R. Pattabiraman and J. W. Bode, *Nature*, 2011, **480**, 471.

7 J. S. Davies, *J. Pept. Sci.*, 2003, **9**, 471.

8 (*a*) L. Zhang and J. P. Tam, *J. Am. Chem. Soc.*, 1997, **119**, 2363; (*b*) J. A. Camarero and T. W. Muir, *Chem. Commun.*, 1997, 1369.

9 After NCL-based macrolactamization, the cysteine residue was converted to an alanine residue *via* desulfurization. See: L. Z. Yan and P. E. Dawson, *J. Am. Chem. Soc.*, 2001, **123**, 526.

10 For serine and threonine ligation, see: C. T. T. Wong, H. Y. Lam, T. Song, G. Chen and X. Li, *Angew. Chem., Int. Ed.*, 2013, **52**, 10212.

11 The use of acyl transfer auxiliary in NCL-based macrolactamization enabled the cyclization of peptides containing various amino acids on their N-terminus, however premodification of peptides is required. See: J. Offer, C. N. C. Boddy and P. E. Dawson, *J. Am. Chem. Soc.*, 2002, **124**, 4642.

12 NCL-based macrolactamization of peptides containing selenocysteine at their N-terminus was reported. Selenocysteine could be converted into cysteine or alanine. See: R. Quaderer and D. Hilvert, *Chem. Commun.*, 2002, 2620.

13 Decarboxylative macrolactamization between α-ketoacids and hydroxylamines without external reagents was reported. However, premodification of amino acids is necessary. See: (*a*) T. Fukuzumi, L. Ju and J. W. Bode, *Org. Biomol. Chem.*, 2012, **10**, 5837; (*b*) F. Rohrbacher, G. Deniau, A. Luther and J. W. Bode, *Chem. Sci.*, 2015, **6**, 4889.

14 (*a*) G. Ösapay, A. Profit and J. W. Taylor, *Tetrahedron Lett.*, 1990, **31**, 6121; (*b*) M. Xu, N. Nishino, H. Mihara, T. Fujimoto and N. Izumiya, *Chem. Lett.*, 1992, 191; (*c*) L. S. Richter, J. Y. K. Tom and J. P. Burnier, *Tetrahedron Lett.*, 1994, **35**, 5547; (*d*) L. Yang and G. Morriello, *Tetrahedron Lett.*, 1999, **40**, 8197; (*e*) C. Rosenbaum and H. Waldmann, *Tetrahedron Lett.*, 2001, **42**, 5677; (*f*) G. T. Bourne, S. W. Golding, R. P. McGeary, W. D. F. Meutermans, A. Jones, G. R. Marshall, P. F. Alewood and M. L. Smythe, *J. Org. Chem.*, 2001, **66**, 7706.

15 K. C. Nicolaou, B. S. Safina and N. Winssinger, *Synlett*, 2001, 900.

16 The 7-nitroindoline derivatives were used as the photoactivatable carboxyl protecting groups in peptide synthesis. See: (*a*) Sh. Pass, B. Amit and A. Patchornik, *J. Am. Chem. Soc.*, 1981, **103**, 7674; (*b*) C. M. Kaneshiro and K. Michael, *Angew. Chem., Int. Ed.*, 2006, **45**, 1077; (*c*) J.-L. Débieux and C. G. Bochet, *J. Phys. Org. Chem.*, 2010, **23**, 272; (*d*) J.-L. Débieux and C. G. Bochet, *Chem. Sci.*, 2012, **3**, 405; (*e*) C. G. Bochet, *Chimia*, 2013, **67**, 896; (*f*) B. A. F. Le Bailly, L. Byrne and J. Clayden, *Angew. Chem., Int. Ed.*, 2016, **55**, 2132.

17 (*a*) S. Fuse, N. Tanabe, M. Yoshida, H. Yoshida, T. Doi and T. Takahashi, *Chem. Commun.*, 2010, **46**, 8722; (*b*) S. Fuse, Y. Mifune, N. Tanabe and T. Takahashi, *Org. Biomol. Chem.*, 2012, **10**, 5205; (*c*) Y. Mifune, S. Fuse and H. Tanaka, *J. Flow Chem.*, 2014, **4**, 173; (*d*) S. Fuse, Y. Otake, Y. Mifune and H. Tanaka, *Aust. J. Chem.*, 2015, **68**, 1657.

18 For recent reviews on micro-flow photochemical synthesis, see: (*a*) M. Oelgemöller and O. Shvydkiv, *Molecules*, 2011, **16**, 7522; (*b*) J. P. Knowles, L. D. Elliott and K. I. Booker-Milburn, *Beilstein J. Org. Chem.*, 2012, **8**, 2025; (*c*) Y. Su, N. J. W. Straathof, V. Hessel and T. Noël, *Chem. – Eur. J.*, 2014, **20**, 10562.

19 (*a*) S. Fuse, N. Tanabe and T. Takahashi, *Chem. Commun.*, 2011, **47**, 12661; (*b*) S. Fuse, Y. Mifune and T. Takahashi, *Angew. Chem., Int. Ed.*, 2014, **53**, 851; (*c*) S. Fuse, Y. Mifune, N. Tanabe and T. Takahashi, *Synlett*, 2014, 2087.

20 (*a*) M. Aumailley, M. Gurrath, G. Müller, J. Calvete, R. Timpl and H. Kessler, *FEBS Lett.*, 1991, **291**, 50; (*b*) M. Gurrath, G. Müller, H. Kessler, M. Aumailley and R. Timpl, *Eur. J. Biochem.*, 1992, **210**, 911; (*c*) R. Haubner, R. Gratias, B. Diefenbach, S. L. Goodman, A. Jonczyk and H. Kessler, *J. Am. Chem. Soc.*, 1996, **118**, 7461; (*d*) R. Haubner, D. Finsinger and H. Kessler, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 1374; (*e*) X. Dai, Z. Su and J. O. Liu, *Tetrahedron Lett.*, 2000, **41**, 6295; (*f*) M. Schottelius, B. Laufer, H. Kessler and H.-J. Wester, *Acc. Chem. Res.*, 2009, **42**, 969; (*g*) S. Liu, *Bioconjugate Chem.*, 2009, **20**, 2199; (*h*) J. Paleček, G. Dräger and A. Kirschning, *Synthesis*, 2011, 653; (*i*) C. Mas-Moruno, R. Fraioli, F. Rechenmacher, S. Neubauer, T. G. Kapp and H. Kessler, *Angew. Chem., Int. Ed.*, 2016, **55**, 7048.

21 Y. Hiroshi, O. Takashi, O. Tatsuo, Y. Kentaro, S. Naoko, N. Takeshi, O. Yoichi, K. Nozomu and K. Kyosuke, *US Pat*, US5721246(A), 1998.

