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Total Synthesis of Malformin C, an Inhibitor of Bleomycin-Induced G2 Arrest

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Abstract Total synthesis of a fungal cyclic peptide, malformin C, recently rediscovered as a G2 checkpoint inhibitor was completed. Our synthesis involved a convergent approach with respect to a linear pentapeptide, cyclization, and oxidative disulfide formation.

Keywords total synthesis, malformin C, G2 checkpoint inhibitor, anti-cancer reagent, cyclic peptide, disulfide

Introduction

Spontaneous and chemical damage to DNA induces signal transduction pathways called checkpoints, which delay cell cycle progression and repair of DNA [1]. DNA damage in normal human cells can be repaired in both the G1 and G2 phases. In contrast, most cancer cells can restore DNA damage only in the G2 phase due to mutations in genes for the G1 checkpoint. Therefore, an inhibitor of the G2 checkpoint in cancer cells is expected to be a selective potentiator of DNA-damaging agents and thus useful for the treatment of cancer.

Recently, malformins A1 and C (Fig. 1), isolated from the culture broth of *Aspergillus niger* FKI-2342, were found to abrogate the bleomycin-induced G2 arrest in Jurkat cells with IC_{50} values of 480 nM and 0.9 nM, respectively [2]. Malformin A1 is a bicyclic pentapeptide containing one L-isoleucine, one D-leucine, one L-valine, and two D-cysteines, while malformin C has L-leucine instead of L-isoleucine. Interestingly, this slight structural difference of the side-chains has an influence on the inhibitory activity. Consequently, malformin C is a promising candidate as a potentiator of anti-cancer agents. Although malformins are also known to show a variety of activities such as inducing root curvatures and malformations in plants, antibacterial activity and enhanced fibrinolytic activity [3~6], the interesting bioactivity of malformin C, as well as its bicyclic structure with a disulfide-bond bridge, prompted us to study the synthesis of this class of cyclic peptides. Herein, we describe a synthesis of malformin C.

Results and Discussion

We planned to undertake the synthesis of malformin C *via* a convergent approach, involving preparation and coupling of tripeptide (**1**) and dipeptide (**2**) to allow creation of linear

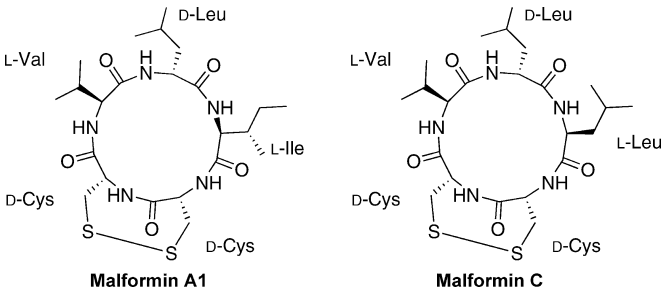

Fig. 1 Structures of malformins A1 and C.

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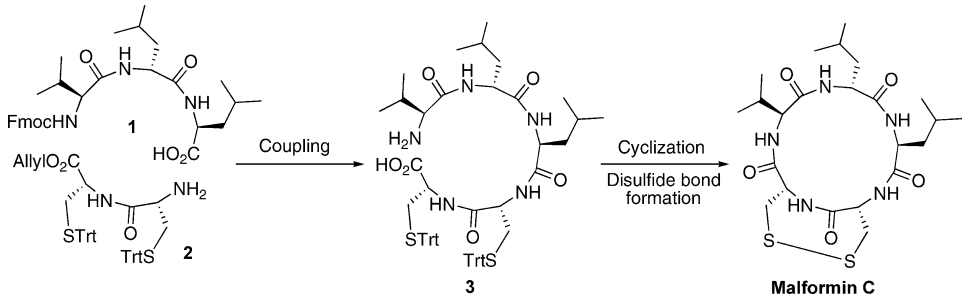
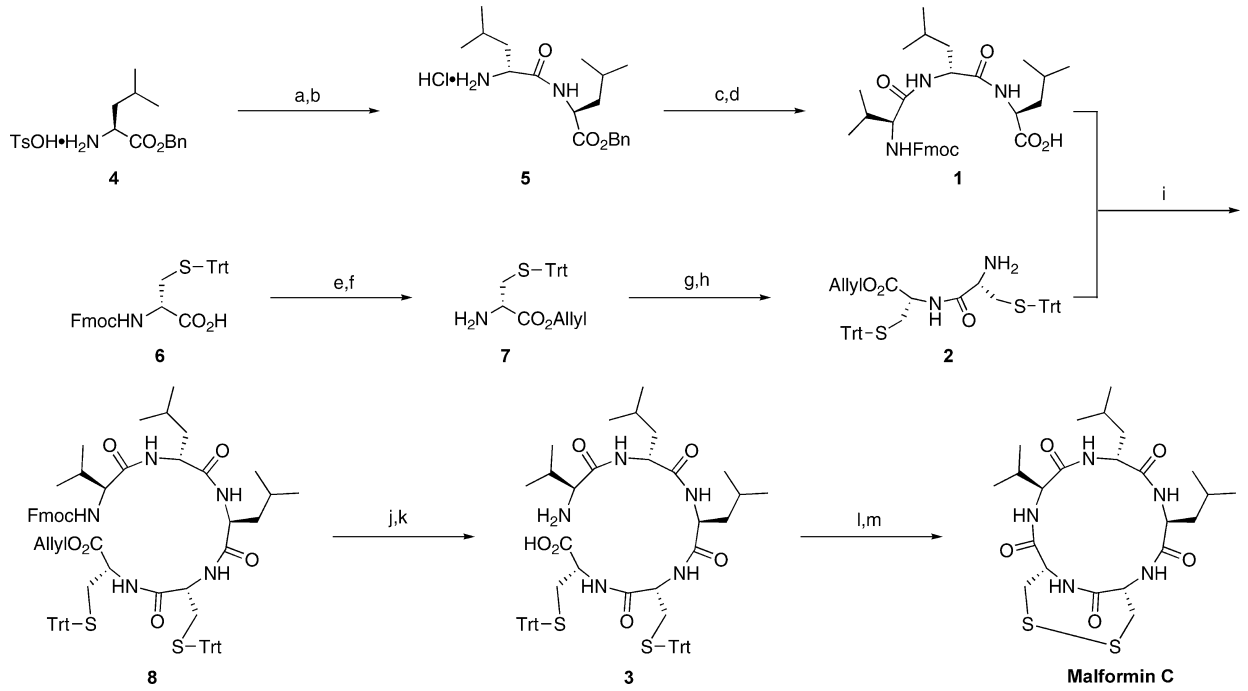
pentapeptide (**3**), which should undergo cyclization and oxidative disulfide formation (Scheme 1). The less hindered amide bond between D-cysteine and L-valine was chosen for cyclization because the cyclization position between D-leucine and L-isoleucine has already been investigated in the stepwise synthesis of malformin A1 [7].

Total synthesis of malformin C is shown in Scheme 2. Coupling of L-leucine benzyl ester (**4**) and Boc-D-leucine, followed by removal of the Boc group with 4.0 N HCl/dioxane provided dipeptide (**5**), which was subjected to condensation of Fmoc-L-valine. Hydrogenolysis of the benzyl group proceeded in a catalytic amount of $Pd(OH)_2$ at 40°C to give tripeptide **1**. Heating was necessary to dissolve the Fmoc protected tripeptide in ethyl acetate. D-*S*-Tritylcystein allyl ester (**7**) was prepared from commercially available Fmoc-D-*S*-tritylcystein (**6**) by allylation of the carboxylic acid, followed by deprotection of the Fmoc group. After coupling of **6** and **7**, dipeptide **2** was obtained by deprotection of the Fmoc group with piperidine. Coupling of **1** and **2** using HBTU/HOBt



Scheme 1 Plan for synthesis of malformin C



Scheme 2 Synthesis of malformin C

a) Boc-D-Leu-OH, EDCI, HOBt, DIPEA, CH_2Cl_2, rt, 98%; b) 4.0 N HCl/dioxane, 0°C; c) Fmoc-L-Val-OH, EDCI, HOBt, DIPEA, CH_2Cl_2/DMF (4/1), rt; d) H_2, $Pd(OH)_2$, EtOAc, 40°C, 86% in 3 steps; e) Cs_2CO_3, AllylBr, DMF, rt; f) piperidine, CH_2Cl_2, 0°C, 93% in 2 steps; g) Fmoc-D-Cys(Trt)-OH **6**, EDCI, HOBt, DIPEA, CH_2Cl_2, rt; h) piperidine, CH_2Cl_2, 0°C, 72% in 2 steps; i) HBTU, HOBt, NMM, CH_2Cl_2/DMF (4/1), rt, 93%; j) piperidine, CH_2Cl_2, 0°C; k) 1.0 N NaOH, THF, rt, 81% in 2 steps; l) HATU, HOAt, NMM, CH_2Cl_2 (1.0 mM), 0°C, 69%; m) I_2, DMF, rt, 85%. DIPEA=diisopropylethylamine, EDCI=1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, HATU=*O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, HBTU=*O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, HOAt=1-hydroxy-7-azabenzotriazole, HOBt=1-hydroxybenzotriazole, NMM=*N*-methylmorphorine.

provided linear peptide (**8**) in 93% yield. Cyclization precursor **3** was obtained by removal of the Fmoc group, followed by hydrolysis of the Allyl group with aq NaOH. In the deprotection of the Allyl group, palladium-catalyzed reaction with dimedone resulted in poor yield. With linear peptide **3** in hand, we examined the effect of conditions on cyclization. After several attempts, combination of HATU and HOAt under high dilution conditions (1.0 mM) at 0°C afforded the cyclic peptide in 69% yield. Other coupling reagents such as HBTU/HOBt and PyBop reduced the yields (44 and 34%). Finally, oxidative disulfide formation with iodine in DMF smoothly proceeded, resulting in malformin C in 85% yield after silica gel chromatography. Its yield was reduced to 26% when 10% MeOH/CH_2Cl_2 was used as a solvent system. Spectra data of the synthetic malformin C were found to be identical to those of the natural product.

In conclusion, we have demonstrated a synthesis of malformin C, a fungal cyclic peptide, in 9 steps and 30% overall yield. The results could be exploited to generate malformin derivatives for elucidation of the structure-activity relationships. Further studies on *in vivo* activities and solid phase synthesis of malformin derivatives are in progress.

Experimental

Reagents were purchased at highest commercial quality and used without further purification, unless otherwise specified. Reactions were monitored by TLC using Merck $F60_{254}$ silica gel plates. Spots were visualized with UV light (254 nm) and stained with phosphomolybdic acid and ninhydrin. Silica gel chromatography was performed on a Merck Kieselgel 60 (Art. 1.09385).

FT-IR spectra were recorded in KBr pellets on a Horiba FT-210 spectrometer. Mass spectra were recorded on a JEOL JMS-700V Mass Spectrometer. ^{1}H-NMR spectra were recorded on a JEOL JNM-EX270 spectrometer in $CDCl_3$ or pyridine-d_5. ^{1}H-NMR spectral data are reported as follows: chemical shifts relative to $CHCl_3$ (7.26 ppm) or pyridine (8.71 ppm), integration, multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br=broad) and coupling constant. Optical rotation was obtained with a JASCO DIP-370 polarimeter. ^{13}C-NMR spectral data are reported in ppm relative to $CHCl_3$ (77.0 ppm) or pyridine (135.5 ppm). Melting points were measured with a Yanaco micromelting point apparatus.

Benzyl (*N*-*tert*-Butoxycarbonyl-D-leucinyl)-L-leucinate
To a solution of **4** (3.27 g, 9.63 mmol) in CH_2Cl_2 (80 ml) were added *N*-Boc-D-Leu (2.0 g, 8.02 mmol), DIPEA (3.07 ml, 17.6 mol), HOBt (2.60 g, 19.3 mmol) and EDCl (1.85 g, 9.63 mmol) at 0°C, and the mixture was stirred at room temperature for 2 hours. The reaction was quenched with H_2O, and the resulting mixture was extracted with $CHCl_3$. The organic extracts were washed with saturated aq NH_4Cl, saturated aq $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc=5/1 to 4/1) to give the dipeptide (3.40 g, 98%) as a white solid. mp 91°C; $[\alpha]_D^{22}$ +22.4 (*c* 1.0, $CHCl_3$); IR (KBr) cm^{-1}; 3332, 2958, 1725, 1685, 1652, 1542, 1523, 1168, 752, 701; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 7.32～7.19 (5H, m), 6.63 (1H, s), 5.07 (2H, dd, *J*=8.2, 10.7 Hz), 4.85 (1H, s), 4.57 (1H, dt, *J*=4.8, 8.4 Hz), 4.08～4.00 (1H, m), 1.60～1.38 (6H, m), 1.36 (9H, s), 0.84～0.82 (12H, m); ^{13}C-NMR (67.2 MHz, $CDCl_3$) δ 172.5, 172.4, 155.5, 135.2, 128.2, 128.0, 127.8, 79.4, 66.5, 52.8, 50.5, 40.9, 40.7, 28.0, 24.5, 22.6, 21.7, 21.4; MS (FAB) *m*/*z* 435.2859 $[M+H]^+$ (calcd for $C_{24}H_{39}O_5N_2$: 435.2859 [M+H]).

Benzyl D-Leucinyl-L-leucinate Hydrochloride (5)
To a solution of dipeptide (3.40 g, 7.82 mmol) was added 4.0 N HCl/dioxane (39 ml) at 0°C, and the mixture was stirred at same temperature for 2 hours. The reaction mixture was concentrated to give **5** as a white solid. The title compound was used for subsequent reaction without further purification.

Benzyl *N*-9-Fluorenylmethyloxycarbonyl-L-valinyl-D-leucinyl-L-leucinate
To a solution of **5** (7.82 mmol) in CH_2Cl_2/DMF (4/1, 78 ml) were added *N*-Fmoc-L-Val (3.19 g, 9.39 mmol), DIPEA (8.18 ml, 46.9 mmol), HOBt (2.11 g, 10.6 mmol) and EDCl (3.0 g, 10.6 mmol) at 0°C, and the mixture was stirred at room temperature for 4 hours. The reaction was quenched with H_2O, and the resulting mixture was extracted with $CHCl_3$. The organic extracts were washed with saturated aq NH_4Cl, saturated aq $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was recrystallized from $CHCl_3$ and hexane to give the tripeptide (5.30 g, quant.) as a white solid. mp 145～150°C; $[\alpha]_D^{23}$ +16.2 (*c* 1.0, $CHCl_3$); IR (KBr) cm^{-1}; 3299, 2956, 1720, 1691, 1643, 1535, 1247, 1029, 732; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 7.75 (2H, d, *J*=7.6 Hz), 7.57 (2H, d, *J*=7.3 Hz), 7.42～7.30 (9H, m), 6.80 (1H, d, *J*=6,9 Hz), 6.39 (1H, d, *J*=7.9 Hz), 5.37 (1H, d, *J*=7.9 Hz), 5.08 (2H, dd, *J*=10.0, 14.5 Hz), 4.62～4.42 (3H, m), 4.31 (1H, t, *J*=8.6 Hz), 4.20 (1H, t, *J*=6.8 Hz), 3.87 (1H, t, *J*=7.1 Hz), 2.15 (1H, m), 1.75～1.47 (6H, m), 0.93 (6H, d, *J*=6.3 Hz), 0.91 (6H, d,

J=5.8 Hz), 0.84 (6H, d, J=5.3 Hz); ^{13}C-NMR (67.5 MHz, $CDCl_3$) δ 172.6, 171.8, 169.2, 156.6, 143.8, 141.2, 135.4, 128.5, 128.3, 127.7, 127.1, 125.0, 120.0, 67.0, 53.1, 51.5, 50.9, 47.1, 42.0, 40.5, 30.7, 24.8, 24.2, 23.1, 22.7, 21.8, 21.3, 19.2, 18.0; MS (FAB) m/z 656.3711 $[M+H]^+$ (calcd for $C_{39}H_{50}O_6N_3$: 656.3700 [M+H]).

_N_-9-Fluorenylmethyloxycarbonyl-L-valinyl-D-leucinyl-L-leucine (1)

$Pd(OH)_2$ (20% Pd on carbon) (21.3 mg, 30.4 μmol) was activated in EtOAc (3.0 ml) under H_2 atmosphere at room temperature for 30 minutes. The tripeptide (99.7 mg, 152 μmol) was added to the mixture. After stirring at 40°C for 6 hours, the reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated. The residue was purified by flash column chromatography on silica gel ($CHCl_3$ to $CHCl_3$/MeOH=10/1) to give **1** (74.2 mg, 86%) as a light yellow solid. mp 105°C; $[\alpha]_D^{22}$ +6.8 (c 1.0, $CHCl_3$); IR (KBr) cm^{-1}; 3288, 2958, 1712, 1668, 1650, 1573, 1515, 1230, 759, 740; ^{1}H-NMR (270 MHz, Pyridine-d_5) δ 9.65 (1H, d, J=8.3 Hz), 9.00 (1H, d, J=7.3 Hz), 8.65 (1H, d, J=8.9 Hz), 7.71 (2H, d, J=7.6 Hz), 7.65 (2H, d, J=7.6 Hz), 7.40～7.16 (4H, m), 5.24～5.12 (2H, m), 4.72 (1H, dd, J=6.9, 10.2 Hz), 4.46 (2H, dd, J=7.3, 15.8 Hz), 4.30 (1H, t, J=6.7 Hz), 2.49～2.36 (1H, m), 2.12～1.83 (6H, m), 1.16 (3H, d, J=6.9 Hz), 1.11 (3H, d, J=6.6 Hz), 0.90～0.84 (12H, m); ^{13}C-NMR (67.2 MHz, Pyridine-d_5) δ 173.3, 173.1, 158.1, 144.7, 141.9, 128.3, 127.7, 126.0, 125.9, 120.7, 120.6, 80.0, 67.2, 62.8, 52.6, 48.0, 41.9, 41.5, 31.0, 25.5, 25.4, 23.7, 23.4, 22.4, 21.5, 20.1, 19.6; MS (FAB) m/z 588.3059 $[M+Na]^+$ (calcd for $C_{32}H_{43}O_6N_3Na$: 588.3050 [M+Na]).

Allyl _N_-9-Fluorenylmethyloxycarbonyl-D-_S_-tritylcysteinate

To a solution of **6** (586 mg, 1.00 mmol) in H_2O/MeOH (1/1, 10 ml) was added Cs_2CO_3 (196 mg, 0.6 mmol) at 0°C. After stirring at room temperature for 1 hour, the reaction mixture was concentrated. The residue was dissolved in DMF (2.0 ml), and then allyl bromide (173 μl, 2.0 mmol) was added at 0°C. The mixture was stirred at room temperature for 9.5 hours. The reaction mixture was diluted with EtOAc and washed with H_2O. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc=5/1 to 3/1) to give the allyl ester (624 mg, quant.) as a white solid. mp 55°C; $[\alpha]_D^{26}$ −14.3 (c 1.0, $CHCl_3$); IR (KBr) cm^{-1}; 3415, 3060, 1727, 1509, 1446, 1334, 1186, 1051, 742, 701; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 7.77 (2H, d, J=7.3 Hz), 7.61 (2H, d, J=7.3 Hz), 7.42～7.18 (19H, m), 5.88 (1H, m), 5.34～5.23 (2H, m), 4.62 (2H, d, J=5.3 Hz), 4.43～4.35 (3H, m), 4.23 (1H, t, J=7.1 Hz), 2.68 (1H, dd, J=6.3, 12.5 Hz), 2.65 (1H, dd, J=4.6, 12.5 Hz); ^{13}C-NMR (67.2 MHz, $CDCl_3$) δ 170.1, 155.5, 144.2, 144.1, 143.7, 143.6, 141.1, 131.3, 129.4, 127.9, 127.6, 127.0, 126.8, 125.0, 119.8, 118.5, 66.9, 66.0, 60.2, 52.9, 46.9, 33.9; MS (FAB) m/z 648.2196 $[M+Na]^+$ (calcd for $C_{40}H_{35}O_4NSNa$: 648.2185 [M+Na]).

Allyl D-_S_-Tritylcysteinate (7)

A mixture of the allyl ester (297 mg, 475 μmol) in piperidine/CH_2Cl_2 (1/4, 4.8 ml) was stirred at 0°C for 3 hours. The reaction was quenched with H_2O, and the resulting mixture was extracted with $CHCl_3$. The organic extracts were washed with saturated aq NH_4Cl and brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc=10/1 to 1/1) to give **7** (178 mg, 93%) as a yellow oil; $[\alpha]_D^{21}$ −44.7 (c 1.0, $CHCl_3$); IR (KBr) cm^{-1}; 3058, 1735, 1488, 1444, 1174, 742, 700; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 7.45～7.18 (15H, m), 5.93～5.78 (1H, m), 5.27 (1H, dd, J=1.3, 13.7 Hz), 5.22 (1H, dd, J=1.3, 6.3 Hz), 4.56 (2H, d, J=5.6 Hz), 3.24 (1H, dd, J=5.0, 7.6 Hz), 2.62 (1H, dd, J=5.0, 12.6 Hz), 2.54 (1H, dd, J=7.6, 12.6 Hz); ^{13}C-NMR (67.2 MHz, $CDCl_3$) δ 173.2, 144.4, 131.6, 129.4, 127.8, 126.6, 118.3, 66.7, 65.5, 53.7, 36.7; MS (FAB) m/z 426.1500 $[M+Na]^+$ (calcd for $C_{25}H_{25}O_2NSNa$: 426.1504 [M+Na]).

Allyl _N_-9-Fluorenylmethyloxycarbonyl-D-_S_-tritylcysteinyl-D-_S_-tritylcysteinate

To a solution of **7** (38.7 mg, 95.9 μmol) in CH_2Cl_2 (0.96 ml) were added _N_-Fmoc-D-(_S_-Trt)-Cys (67.4 mg, 115 μmol), DIPEA (100 ml, 575 μmol), HOBt (25.9 mg, 192 μmol) and EDCl (36.8 mg, 192 μmol) at 0°C, and the mixture was stirred at room temperature for 4 hours. The reaction was quenched with H_2O, and the resulting mixture was extracted with $CHCl_3$. The organic extracts were washed with saturated aq NH_4Cl, saturated aq $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc=10/1) to give the dipeptide (82.9 mg, 89%) as a white solid. mp 74°C; $[\alpha]_D^{23}$ −6.6 (c 1.0, $CHCl_3$); IR (KBr) cm^{-1}; 3390, 3054, 1737, 1681, 1488, 1444, 742, 700; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 7.77 (2H, t, J=6.6 Hz), 7.55 (2H, s), 7.44～7.13 (34H, m), 6.33 (1H, d, J=7.6 Hz), 5.90～5.73 (1H, m), 5.27 (1H, dd, J=1.3, 13.7 Hz), 5.18 (1H, dd, J=1.3, 6.3 Hz), 4.98 (1H, d, J=6.3 Hz), 4.54 (1H, d, J=5.0 Hz), 4.47～4.29 (3H, m), 4.18 (1H, t, J=7.1 Hz), 3.76 (1H, dd, J=5.8, 7.3 Hz), 2.65 (2H, dd, J=6.0, 8.1 Hz), 2.58 (2H, t, J=5.0 Hz); ^{13}C-NMR (67.2 MHz, $CDCl_3$) δ 169.7, 169.3, 155.7, 144.3, 144.1, 143.8, 143.6, 141.2,

131.2, 129.5, 129.3, 128.0, 127.9, 127.7, 127.0, 126.8, 125.0, 119.9, 118.8, 67.3, 66.7, 66.2, 53.8, 51.3, 47.0, 33.5; MS (FAB) *m/z* 971.3588 $[M+H]^+$ (calcd for $C_{62}H_{55}O_5N_2S_2$: 971.3552 [M+H]).

Allyl D-*S*-Tritylcysteinyl-D-*S*-tritylcysteinate (2)

A mixture of the dipeptide (67.5 mg, 69.5 μmol) in piperidine/CH_2Cl_2 (1/4, 0.70 ml) was stirred at 0°C for 1.5 hours. The reaction was quenched with H_2O, and the resulting mixture was extracted with $CHCl_3$. The organic extracts were washed with saturated aq NH_4Cl and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc=5/1 to 1/1) to give **2** (42.1 mg, 81%) as a white solid. mp 60~63°C; $[\alpha]_D^{21}$ −20.6 (*c* 1.0, $CHCl_3$); IR (KBr) cm^{-1}; 3374, 3014, 1743, 1673, 1492, 1432, 1182, 742, 692, 669; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 7.54~7.16 (30H, m), 5.89~5.75 (1H, m), 5.28~5.15 (2H, m), 4.55 (2H, d, *J*=5.6 Hz), 4.43 (1H, dt, *J*=5.5, 8.0 Hz), 2.95 (1H, dd, *J*=4.0, 8.9 Hz), 2.75 (1H, dd, *J*=4.0, 13.2 Hz), 2.62~2.53 (3H, m); ^{13}C-NMR (67.2 MHz, $CDCl_3$) δ 172.5, 169.6, 144.3, 144.0, 131.2, 129.3, 129.2, 127.7, 126.5, 118.3, 66.7, 66.3, 65.8, 53.6, 50.7, 36.9, 33.6; MS (FAB) *m/z* 749.2853 $[M+H]^+$ (calcd for $C_{47}H_{45}O_3N_2S_2$: 749.2872 [M+H]).

Allyl *N*-9-Fluorenylmethyloxycarbonyl-L-valinyl-D-leucinyl-L-leucinyl-D-*S*-tritylcysteinyl-D-*S*-tritylcysteinate (8)

To a solution of **1** (31.8 mg, 562 μmol) and **2** (35.1 mg, 469 μmol) in CH_2Cl_2/DMF (4/1, 0.47 ml) were added NMM (13.6 μl, 122 μmol), HOBt (9.3 mg, 609 μmol) and HBTU (23.1 mg, 609 μmol) at 0°C, and the mixture was stirred at room temperature for 2.5 hours. The reaction was quenched with H_2O, and the resulting mixture was extracted with $CHCl_3$. The organic extracts were washed with saturated aq NH_4Cl, saturated aq $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc=20/1 to 1/1) to give **8** (56.5 mg, 93%) as a white solid. mp 142°C; $[\alpha]_D^{22}$ +2.2 (*c* 1.0, $CHCl_3$); IR (KBr) cm^{-1}; 3392, 3060, 2923, 1735, 1631, 1527, 1500, 1448, 1218, 761, 742, 700, 669, 617; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 7.76 (2H, d, *J*=7.3 Hz), 7.58 (2H, d, *J*=7.3 Hz), 7.43~7.19 (34H, m), 6.96~6.90 (2H, m), 6.67 (1H, d, *J*=8.3 Hz), 5.82~5.66 (2H, m), 5.20~5.13 (2H, m), 4.50~4.14 (9H, m), 3.91 (1H, t, *J*=7.1 Hz), 2.82 (1H, dd, *J*=8.1, 12.8 Hz), 2.72 (1H, dd, *J*=7.9, 12.8 Hz), 2.55 (2H, dt, *J*=4.6, 12.0 Hz), 2.22~2.07 (1H, m), 1.65~1.42 (6H, m), 0.97~0.77 (18H, m); ^{13}C-NMR (67.2 MHz, $CDCl_3$) δ 172.5, 172.1, 171.8, 170.3, 169.5, 156.5, 144.5, 144.2, 144.1, 143.7, 143.6, 141.1, 131.1, 129.5, 129.4, 129.3, 128.4, 127.9, 127.8, 127.5, 127.0, 126.7, 125.0, 119.8, 118.3, 67.0, 66.9, 66.8, 65.9, 52.6, 52.1, 51.7, 51.2, 46.9, 39.5, 39.3, 38.6, 33.5, 33.3, 30.3, 24.6, 24.5, 22.8, 22.7, 22.0, 21.7, 19.2, 18.1; MS (FAB) *m/z* 1296.5887 $[M+H]^+$ (calcd for $C_{79}H_{86}O_8N_5S_2$: 1296.5918 [M+H]).

Allyl L-Valinyl-D-leucinyl-L-leucinyl-D-*S*-tritylcysteinyl-D-*S*-tritylcysteinate

A mixture of **8** (52.4 mg, 404 μmol) in piperidine/CH_2Cl_2 (1/4, 0.80 ml) was stirred at 0°C for 1 hour. The reaction was quenched with H_2O, and the resulting mixture was extracted with $CHCl_3$. The organic extracts were washed with saturated aq NH_4Cl and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel ($CHCl_3$ to $CHCl_3$/MeOH=50/1) to give the amine (38.7 mg, 89%) as a white solid. mp 193~195°C; $[\alpha]_D^{26}$ −7.6 (*c* 1.0, $CHCl_3$); IR (KBr) cm^{-1}; 3262, 3054, 2956, 1747, 1633, 1538, 1442, 1170, 742, 692; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 7.62 (1H, d, *J*=4.2 Hz), 7.42~7.18 (30H, m), 6.99~6.90 (3H, m), 5.85~5.71 (1H, m), 5.24~5.12 (2H, m), 4.51~4.23 (6H, m), 4.04 (1H, d, *J*=5.0 Hz), 3.05 (1H, s), 2.84 (1H, dd, *J*=8.7, 12.5 Hz), 2.67 (1H, dd, *J*=6.9, 12.5 Hz), 2.56 (1H, dd, *J*=4.6, 13.4 Hz), 2.48 (1H, dd, *J*=4.6, 12.9 Hz), 1.75~1.44 (7H, m), 0.92~0.81 (18H, m); ^{13}C-NMR (67.2 MHz, $CDCl_3$) δ 175.9, 172.6, 171.6, 170.5, 169.9, 144.4, 144.2, 131.2, 129.5, 129.4, 127.9, 126.7, 118.6, 67.0, 66.7, 66.0, 60.0, 53.0, 51.8, 51.7, 51.2, 39.6, 39.4, 33.7, 33.1, 30.9, 24.7, 24.6, 23.0, 22.7, 22.0, 21.7, 19.6, 16.2; MS (FAB) *m/z* 1074.5270 $[M+H]^+$ (calcd for $C_{64}H_{76}O_6N_5S_2$: 1074.5237 [M+H]).

L-Valinyl-D-leucinyl-L-leucinyl-D-*S*-tritylcysteinyl-D-*S*-tritylcysteine (3)

To a solution of the amine (38.7 mg, 360 μmol) in THF/H_2O (4/1, 0.72 ml) was added NaOH (2.16 mg, 540 μmol) at 0°C, and the mixture was stirred at 0°C for 1 hour. The reaction temperature was warmed to room temperature. After stirring at the same temperature for 5 hours, the reaction was quenched with 0.1 N HCl (540 μl), and the resulting mixture was extracted with $CHCl_3$. The organic extracts were concentrated, and the residue was purified by flash column chromatography on silica gel ($CHCl_3$ to $CHCl_3$/MeOH=10/1) to give **3** (34.0 mg, 91%) as a white solid. mp 158~164°C; $[\alpha]_D^{26}$ +3.7 (*c* 1.0, $CHCl_3$); IR (KBr) cm^{-1}; 3297, 3058, 1958, 1731, 1660, 1531, 1492, 1444, 1241, 742, 700; ^{1}H-NMR (270 MHz, Pyridine-d_5) δ 10.5 (1H, d, *J*=8.3 Hz), 9.77 (1H, d, *J*=8.0 Hz), 9.17 (1H, d, *J*=7.6 Hz), 8.57 (1H, d, *J*=7.5 Hz), 7.67~7.17 (30H, m), 5.27~4.91 (3H, m), 4.82 (1H, dd,

J=6.9, 13.5 Hz), 4.25 (1H, dd, J=6.3, 11.6 Hz), 3.40 (1H, dd, J=3.8, 12.0 Hz), 3.27～2.94 (3H, m), 2.51～2.42 (1H, m), 2.14～2.01 (2H, m), 1.96～1.79 (4H, m), 1.21～0.86 (18H, m); ^{13}C-NMR (67.2 MHz, Pyridine-d_5) δ 175.0, 173.8, 173.4, 169.9, 169.7, 145.8, 145.7, 145.6, 145.3, 143.7, 130.2, 130.1, 130.0, 129.9, 128.8, 128.6, 128.4, 128.3, 128.2, 128.0, 127.1, 127.0, 126.8, 123.9, 123.5, 123.1, 66.9, 66.4, 64.2, 59.6, 54.4, 54.1, 52.1, 42.3, 40.7, 31.0, 25.8, 25.2, 23.1, 23.0, 22.9, 22.5, 19.0, 18.9; MS (FAB) m/z 1034.4921 $[M+H]^+$ (calcd for $C_{61}H_{72}O_6N_5S_2$: 1034.4924 [M+H]).

***Cyclo*(-L-valinyl-D-leucinyl-L-leucinyl-D-*S*-tritylcysteinyl-D-*S*-tritylcysteinyl-)**

To a solution of **3** (100.0 mg, 96.7 μmol) in CH_2Cl_2 (97 ml) were added NMM (64.8 μl, 580 μmol), HOAt (39.5 mg, 290 μmol) and HATU (110.3 mg, 290 μmol), and then the mixture was stirred at 0°C for 7 hours. The resulting mixture was concentrated, and the residue was purified by flash column chromatography on silica gel ($CHCl_3$ to $CHCl_3$/MeOH=200/1) to give the cyclicpentapeptide (67.6 mg, 69%) as a white solid. mp >300°C; $[\alpha]_D^{21}$ −10.1 (c 2.0, $CHCl_3$/MeOH=10/1); IR (KBr) cm^{-1}; 3268, 3054, 2956, 1639, 1538, 744, 698; ^{1}H-NMR (270 MHz, Pyridine-d_5) δ 10.1 (1H, d, J=8.6 Hz), 10.0 (1H, d, J=6.3 Hz), 9.89 (1H, d, J=8.6 Hz), 8.70 (1H, d, J=7.2 Hz), 8.05 (1H, d, J=9.6 Hz), 7.68～7.51 (12H, m), 7.29～7.16 (18H, m), 5.01 (1H, dd, J=8.7, 15.7 Hz), 4.89 (1H, dt, J=4.2, 9.4 Hz), 4.79～4.69 (2H, m), 4.63 (1H, t, J=9.6 Hz), 3.84 (1H, dd, J=9.1, 11.8 Hz), 2.97 (1H, dd, J=4.6, 12.2 Hz), 2.73～2.61 (2H, m), 2.39 (1H, dq, J=6.9, 12.7 Hz), 2.03～1.91 (2H, m), 1.83～1.66 (4H, m), 1.03 (3H, d, J=6.6 Hz), 1.00 (3H, d, J=6.6 Hz), 0.93 (3H, d, J=6.3 Hz), 0.84 (3H, d, J=6.6 Hz), 0.80 (3H, d, J=6.3 Hz), 0.76 (3H, d, J=6.3 Hz); ^{13}C-NMR (67.2 MHz, Pyridine-d_5) δ 175.1, 173.3, 172.4, 170.7, 169.9, 145.5, 145.1, 130.0, 129.7, 129.0, 128.3, 128.2, 127.0, 126.8, 67.0, 66.6, 59.7, 54.0, 53.8, 53.3, 51.7, 41.1, 39.7, 34.1, 33.2, 27.6, 25.0, 24.9, 22.8, 22.7, 22.2, 21.6, 19.9, 18.9; MS (FAB) m/z 1038.4683 $[M+Na]^+$ (calcd for $C_{61}H_{69}O_5N_5S_2Na$: 1038.4638 [M+Na]).

Malformin C

A solution of I_2 (45.5 mg, 179 μmol) in DMF (3.0 ml) was added to a solution of the cyclic pentapeptide (60.8 mg, 59.8 μmol) in DMF (3.0 ml) at room temperature. After stirring at room temperature for 30 minutes, 1.0% aq $Na_2S_2O_3$ was added to the reaction mixture which was then extracted with EtOAc. The organic extracts were washed with H_2O, dried over Na_2SO_4, filtered, and concentrated. The residue was treated with TFA/CH_2Cl_2 (1/1, 6.0 ml) and triisopropylsilane (49.0 μl, 239 μmol), the resulting mixture was stirred at room temperature for 1 hour. After concentration, the residue was purified by flash column chromatography on silica gel ($CHCl_3$ to $CHCl_3$/MeOH=30/1) to give malformin C (27.0 mg, 85%) as a white solid. mp >300°C; $[\alpha]_D^{22}$ −24.8 (c 1.0, $CHCl_3$/MeOH=10/1); IR (KBr) cm^{-1}; 3432, 2962, 1648, 1536, 1201; ^{1}H-NMR (270 MHz, 1 drop d-TFA in $CDCl_3$) δ 5.10 (1H, dd, J=5.3, 9.9 Hz), 4.49 (1H, t, J=3.0 Hz), 4.41 (1H, dt, J=8.4, 6.3 Hz), 4.11～4.05 (2H, m), 3.84 (1H, dd, J=3.0, 15.5 Hz), 3.19～3.31 (3H, m), 2.03 (1H, dt, J=6.6, 11.2 Hz), 1.74～1.49 (6H, m), 1.00～0.89 (18H, m); ^{13}C-NMR (67.2 MHz, 1 drop d-TFA in $CDCl_3$) δ 177.1, 176.5, 173.2, 171.3, 61.1, 54.6, 53.8, 53.4, 53.2, 46.8, 45.7, 38.7, 38.6, 27.5, 25.0, 24.7, 22.1, 22.0, 21.5, 21.2, 18.8, 18.1; MS (FAB) m/z 530.2468 $[M+H]^+$ (calcd for $C_{23}H_{40}O_5N_5S_2$: 530.2471 [M+H]).

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