The Journal of Antibiotics (2009) 62, 681–686

www.nature.com/ja

ORIGINAL ARTICLE

Solid-phase synthesis and biological activity of malformin C and its derivatives

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We accomplished the solid-phase total synthesis of malformin C, which is adaptable for the easy preparation of various derivatives. A solid-phase total synthesis of malformin C was achieved by on-resin macrolactamization and disulfide bond formation, with concurrent cleavage from the resin. Antimalarial and antitrypanosomal activities were examined, which helped elucidate partial structure–activity relationships. Results indicate that the disulfide bond is essential and branched amino acids are also crucial components if the compound is to exhibit potent antimalarial and antitrypanosomal properties.
The Journal of Antibiotics (2009) **62,** 681–686; doi:10.1038/ja.2009.100; published online 30 October 2009

Keywords: antimalarial activity; antitrypanosomal activity; cyclic pentapeptide; disulfide bond; malformins; solid-phase synthesis

INTRODUCTION

Malformins, produced from *Aspergillus niger*, are a group of cyclic pentapeptides with a disulfide bond formed from two cysteine thiols. The compounds express various biological activities, such as inducing root curvatures and malformations in plants,[1–4] antibacterial activity against Gram-positive and Gram-negative bacteria,[5–7] enhanced fibrinolytic activity[8] and inhibitory activity of the G_2 checkpoint.[9] Owing to their unique structures and diverse biological profiles, several processes for the synthesis of malformins have been examined.[10–13] More recently, our research group has found that malformin C[14,15] exhibits bioactivity against malarial parasites and trypanosomes, with IC_{50} values of 70 and 1.6 ng ml^{-1}, respectively.

Malaria, caused by *Plasmodium* species, occurs in more than 90 countries worldwide, and it was estimated that there were over 247 million clinical cases of malaria and 881 000 malaria-caused deaths in 2006.[16] Many antimalarial agents have been developed, but widespread drug resistance has rendered many of them ineffective, with the current exception of artemisinin and its derivatives. More recently, resistance to members of the antimalarial artemisinin class of drugs is being reported from Southeast Asia, especially from the Cambodia region, which is causing significant alarm and concern for the future of this class of drugs (http://sciencenow.sciencemag.org/cgi/content/full/2009/729/3). Therefore, development of new, safe and potent antimalarial drugs, with new modes of action and structural features, is urgently required.

Human African trypanosomiasis (HAT), also known as sleeping sickness, is recognized as one of Africa's most neglected diseases and is a significant cause of mortality and morbidity in sub-Saharan Africa. The World Health Organization (WHO) estimated that, in 2000, approximately 300 000 Africans were affected by the disease, a figure much larger than the 27 000 cases diagnosed and treated that year. Owing to increased surveillance activities in the past 7 years, recent estimates indicate that there are now approximately 50 000–70 000 cases of HAT annually. In 2007, the number of new cases reported had actually decreased to 10 769 (http://www.who.int/trypanosomiasis_african/disease/en/index.html). Currently, only four drugs, including pentamidine, are registered for the treatment of HAT. All four are unsatisfactory, as they cannot be administered orally and are hampered by severe toxicity and increasing resistance of the parasites.

Consequently, there is a pressing need for new antitrypanosomal drugs that have new structures and mechanisms of action and are both safe and effective. The global need for such antimalarial and antitrypanosomal drugs led us to develop a new methodology for the preparation of malformin analogs to elucidate structure–activity relationships. We had already completed a total synthesis of malformin C in liquid-phase sequences,[13] but sought to discover a solid-phase synthesis that would provide the advantages of speeding up reactions, allow the use of a large amount of reagent and allow all remaining reagents and side products to be removed easily by washing with solvent, as well as create the possibility of rapidly synthesizing analogs with different amino acids. In this study, we report the total synthesis of malformin C using a solid-phase route, easily adaptable for analog preparation and for evaluation of the antimalarial and antitrypanosomal activities of malformin C and various analogs.

RESULT AND DISCUSSION

Our retrosynthetic analysis of malformin C is shown in Scheme 1. We focused on the development of a solid-phase strategy that would

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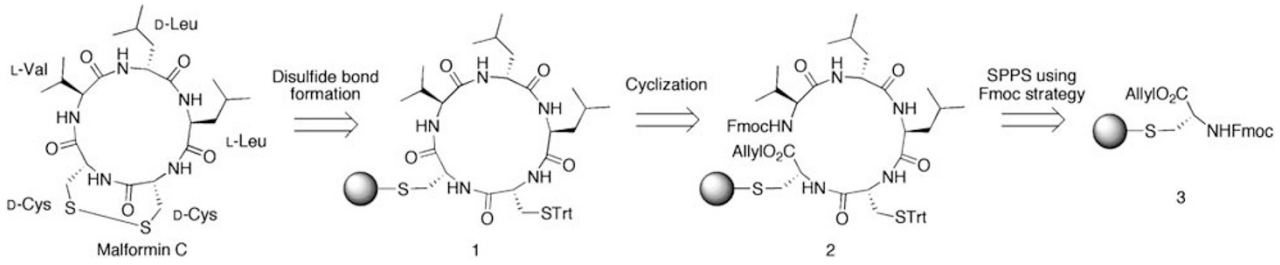
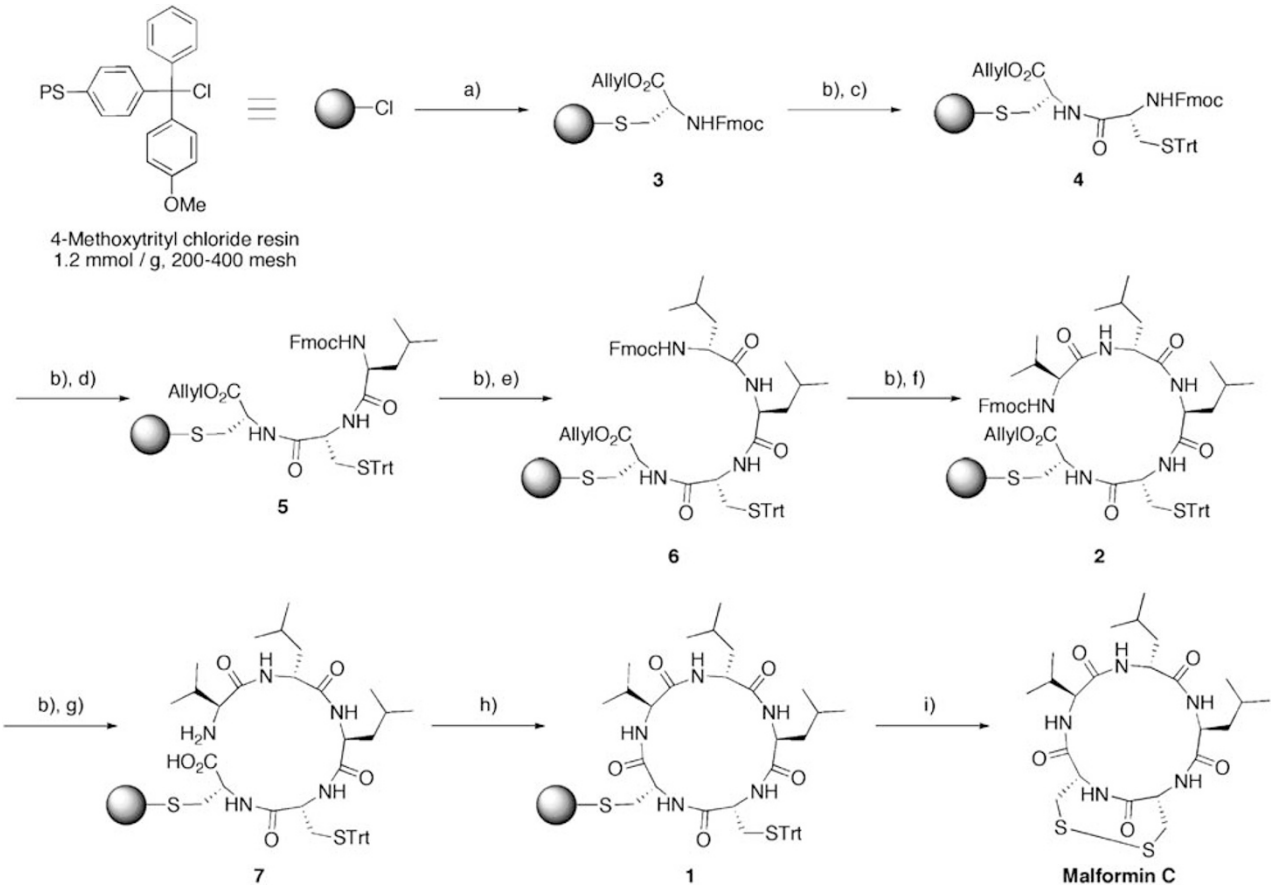
Received 13 July 2009; revised 23 September 2009; accepted 28 September 2009; published online 30 October 2009



Scheme 1 Retrosynthetic analysis of malformin C with resin.



Scheme 2 Solid-phase synthesis of malformin C; (**a**) Fmoc-D-Cys-OAllyl, *i*-Pr_2NEt, CH_2Cl_2; (**b**) 20% piperidine/DMF; (**c**) Fmoc-D-Cys(*S*-Trt)-OH, PyBOP, *i*-Pr_2NEt, DMF; (**d**) Fmoc-L-Leu-OH, PyBOP, *i*-Pr_2NEt, DMF; (**e**) Fmoc-D-Leu-OH, PyBOP, *i*-Pr_2NEt, DMF; (**f**) Fmoc-L-Val-OH, PyBOP, *i*-Pr_2NEt, DMF; (**g**) $Pd(PPh_3)_4$, 5,5-dimethyl-1,3-hexanedione, THF; (**h**) HBTU, HOBt, *i*-Pr_2NEt, 20% DMF/CH_2Cl_2; (**i**) I_2, DMF, 15% overall yield of malformin C from **3**.

enable the preparation of various malformin analogs. Malformin C could be synthesized through oxidative disulfide formation of the cyclic peptide (**1**) in which one of two Cys residues is protected with a trityl (Trt) group and the other is bound on a Trt linker. Compound **1** could be prepared by selective deprotection and subsequent on-resin cyclization of the pentapeptide (**2**). We chose the allyl ester for C-terminal protection of the peptide chain because it can be tolerant under 9-fluorenylmethyloxycarbonyl (Fmoc) and *tert*-butoxycarbonyl deprotection conditions and removed by Pd-catalyzed chemistry without interference with *S*-Trt functionalities. The amide linkage between D-cysteine and L-valine residues was chosen for on-resin macrolactamization. The peptide chain could be elongated by the Fmoc solid-phase peptide synthesis method.

We used 4-methoxytrityl chloride linker, which is useful for immobilization of thiol groups.[17,18] As shown in Scheme 2, Fmoc-D-Cys-OAllyl was loaded through the thiol group onto the 4-methoxytrityl chloride resin to yield the cysteine derivative (**3**). The loading yield was estimated to be quantitative by acid cleavage from the resin with 50% trifluoroacetic acid (TFA)/CH_2Cl_2. The four amino acid derivatives, Fmoc-D-Cys(*S*-Trt)-OH, Fmoc-L-Leu-OH, Fmoc-D-Leu-OH and Fmoc-L-Val-OH, were sequentially introduced, using 20% piperidine/*N*,*N*′-dimethylformamide (DMF) for deprotection of the Fmoc group and benzotriazole-l-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP) as a coupling reagent, to yield **2**. After deprotection of the Fmoc group and Allyl group of **2**, cyclization of the linear peptide (**7**) was performed, with

O-benzotriazole-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluroniumhexafluorophosphate (HBTU) and 1-hydroxybenzotriazole (HOBt) as coupling reagents, to yield **1**. Other coupling reagents such as diphenylphosphoryl azide and *N*,*N*′-diisopropylcarbodiimide did not result in the cyclic compound. Finally, oxidative disulfide formation with iodine in DMF,[19] and then release from the resin by treatment with 50% TFA/CH_2Cl_2 and triisopropylsilane,[20] resulted in malformin C in 15% overall yield for 13 steps after silica gel chromatography. The melting point, $[\alpha]_D$, IR, ^1H and ^{13}C NMR and high-resolution mass spectra were found to be identical to those of the natural product. It is assumed that the relatively low yield is because of the poor solubility of malformin C in organic solvents. Although **1** was obtained in almost quantitative yield, the crude yield of malformin C was moderate. Thus, in the final step, oxidative conditions might cause undesired intermolecular interactions with the solid support, resulting in a relatively low yield. Oxidation by the liquid phase of the dithiol compound obtained from resin treatment with 50% TFA/CH_2Cl_2 and triisopropylsilane was not productive; thus, malformin C was synthesized using the above-mentioned method in spite of the low yield.

To demonstrate the flexibility of this methodology, malformins A1,[21–23] we also synthesized A2[24] and B2[25–27] (in 6, 13 and 7% yield, respectively). Spectroscopic data of synthetic malformins were identical to references. Synthesis of unnatural malformin (**8**), which introduced less hindered L-Ala instead of the L-Leu of malformin C, was performed to examine the effect of the L-Leu residue on antimalarial and antitrypanosomal activities. Because the bioactivity of malformins A1 and C is significantly different, because of the impact of the L-Leu residue on the inhibitory activity on G_2 checkpoint,[9] sulfide (**9**) and thiol (**10**) were synthesized to investigate the importance of the disulfide bond in antiparasitic activity (Table 1). The synthetic compounds were purified by silica gel column chromatography, characterized by HPLC and ESI-MS, and subjected to assay for antimalarial and antitrypanosomal activities.

Assay results are summarized in Table 1. Artemether,[28] an effective antimalarial reagent, showed activity with an IC_{50} value of 2.3 ng ml^{-1}, and pentamidine,[29] an effective antitrypanosomal reagent, showed activity with an IC_{50} value of 1.58 ng ml^{-1}. Malformin C has antitrypanosomal activity with an IC_{50} value of 1.6 ng ml^{-1} equal to that of pentamidine, and antimalarial activity with an IC_{50} value of 70 ng ml^{-1}, a little lower than that of artemether. However, **9** and **10** exhibited reduced activity against both parasites. Thus, the disulfide bond of malformin C seems to be necessary for antiparasitic activity. To compare antitrypanosomal and antimalarial activities, we calculated the selectivity index (SI: antitrypanosomal activity (IC_{50} for the GUTat 3.1 strain)/antimalarial activity (IC_{50} for the K1 strain)) (Table 1). What is interesting is that the SIs of malformin A1, A2 and **8** were reversed with malformin C. Therefore, both activities of malformins A1, A2 and **8**, incorporating amino acid residues instead of the L-Leu of malformin C, were different by substituent, suggesting that L-Leu residues were important for the activity. A comparison of **8** with others indicates that a degree of the bulk at the position of L-Leu of malformin C is necessary for antimalarial activity, whereas malformin B2 showed the strongest activity against *Plasmodium falciparum*. These correlations suggest that the replacement of the D-Leu moiety of malformin C to a smaller amino acid residue may express better potency for antimalarial activity. Considering the bioactivity results of malformin C and B2, it would seem that L-Leu is necessary for antitrypanosomal activity.

In conclusion, we demonstrated a solid-phase synthesis of malformin C and used this to produce a variety of derivative compounds. As the result of the synthesis of these compounds, we obtained substantial information with respect to the structure–activity relationships of antimalarial and antitrypanosomal activities. We also discovered that malformin B2 exhibits more potent antimalarial activity than does malformin C.

EXPERIMENTAL SECTION

General

Reagents of the highest commercial quality were purchased and were used without further purification, unless otherwise specified. Reactions were monitored by TLC using Merck $F60_{254}$ silica gel plates (Merck, Tokyo, Japan). Spots were visualized with UV light (254 nm) and stained with phosphomolybdic acid. 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 (Horiba, Kyoto, Japan). Mass spectra were recorded on a JEOL JMS-700V (Jeol, Tokyo, Japan) or JMS-T100LP Mass Spectrometer (Jeol). ^1H NMR spectra were recorded at 270, 300 or 400 MHz and ^{13}C NMR spectra were recorded at 67.5, 75 or 100 MHz on JEOL JNM-EX270 (270 MHz), MERCURY-300 (300 MHz), Varian XL-400 (400 MHz) or Varian UNITY-400 (400 MHz) spectrometers (Varian, Tokyo, Japan) in $CDCl_3$ or DMSO-d_6. ^1H NMR spectral data are reported as follows: chemical shifts relative to $CHCl_3$ (7.26 p.p.m.) or dimethyl sulfoxide (DMSO) (2.49 p.p.m.), 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 (Jasco, Tokyo, Japan). ^{13}C NMR spectral data are reported in p.p.m. relative to $CHCl_3$ (77.0 p.p.m.) or DMSO (39.7 p.p.m.). Melting points were measured with a Yanaco micro melting point apparatus (Yanaco, Kyoto, Japan). HPLC analysis was conducted on a Hitachi ELITE LaChrom (Hitachi, Tokyo, Japan) (Column; Senshu Pak PEGASIL ODS 4.6ϕ×250 mm with a flow rate of 1.0 ml min^{-1}. Mobile phase was 0.05% TFA in 45% MeCN/H_2O).

Allyl *N*-9-fluorenylmethyloxycarbonyl-D-cysteinate

To a solution of Fmoc-D-Cys(*S*-Trt)-OAllyl (5.35 g, 8.56 mmol) in CH_2Cl_2 (85.6 ml) was added triethylsilane (4.1 ml, 25.7 mmol) and dropwise TFA (1.98 ml, 25.7 mmol) at room temperature (RT) under N_2 atmosphere. After stirring at RT for 2 h, the reaction mixture was concentrated. The residue was purified by flash chromatography on silica gel (hexane/EtOAc=10/1 to hexane/EtOAc=5/1) to yield the thiol (3.01 g, 92%) as a white solid. The spectra data corresponded to the known substance.[30]

Allyl *N*-9-fluorenylmethyloxycarbonyl-D-*S*-4-methoxytrityl resin cysteinate (3)

4-Methoxytrityl chloride resin (30.0 mg, 1.2 mmol g^{-1}) was swollen with CH_2Cl_2 for 30 min at RT. Subsequently, Fmoc-D-Cys-OAllyl (27.6 mg, 72 µmol) was dissolved in CH_2Cl_2 (0.72 ml), and the resin and *N*,*N*-diisopropylethylamine (DIPEA) (37.6 µl, 216 µmol) were added. The mixture was then agitated for 3 h at RT. MeOH was added to cap any unreacted site, and the reaction mixture was agitated for an additional 10 min. The resin was then washed with DMF (3×2 min), CH_2Cl_2/MeOH=10/1 (2 min) and CH_2Cl_2 (3×2 min).

General procedure for deprotection of the Fmoc group

The resin was swollen with DMF (1.5 ml) for 30 min, filtered and treated with 20% piperidine in DMF (1.5 ml) for 1 h at RT. The mixture was filtered and washed with DMF (3×2 min), CH_2Cl_2/MeOH=10/1 (2 min) and CH_2Cl_2 (3×2 min).

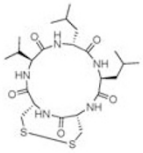
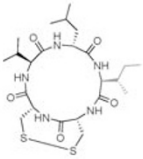
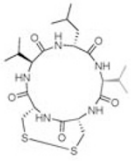
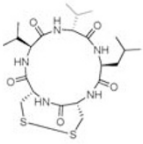
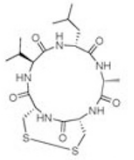
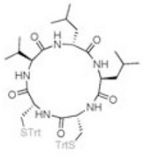
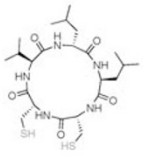
General procedure for elongation of the peptide chain

The resin was swollen with DMF for 30 min at RT. Fmoc-amino acid (2.0 equivalents) was then dissolved in DMF (0.72 ml); thereafter, PyBOP (3.0 equivalents, 56.2 mg, 108 µmol), DIPEA (6.0 equivalents, 37.6 µl, 216 µmol) and the resin were added. The mixture was subsequently agitated for 3 h at RT. The resin was filtered and washed with DMF (3×2 min), CH_2Cl_2/MeOH=10/1 (2 min) and CH_2Cl_2 (3×2 min).

General procedure for deprotection of the Allyl group

The resin was swollen with tetrahydrofuran (THF) (1.5 ml) for 1 h at RT under N_2 atmosphere, and excess THF was drained using a syringe. A solution of

Table 1 Structure of synthetic compounds, result of assay and synthetic yields

Compounds	IC_{50} (μg/ml)		Selectivity Index (SI) *2 / *1	Synthetic yields
	Anti-malarial activity*1	Anti-trypanosomal activity*2		
Malformin C 	0.07	0.0016	0.023	15%
Malformin A1 	0.056	0.19	3.4	6%
Malformin A2 	0.095	0.56	5.9	13%
Malformin B2 	0.019	0.0052	0.27	7%
Unnatural malformin (**8**) 	0.24	0.70	2.9	3%
(**9**) 	>12.5	1.52	<0.12	35%*3
(**10**) 	3.8	0.17	0.045	66%
Existing medication				
Artemether	0.0023	—	—	—
Chloroquine	0.184	—	—	—
Pentamidine	—	0.00158	—	—

*1 *Plasmodium falciparum* K1, *2 *Trypanosoma brucei brucei* GUTat 3.1
*3 prepared by liquid-phase synthesis[13]

$Pd(PPh_3)_4$ (62.4 mg, 54 μmol) and 5,5-dimethyl-1,3-hexanedione (50.5 mg, 360 μmol) in THF (1.5 ml) was added to the resin, and the mixture was agitated for 1 h at RT under N_2 atmosphere. The resin was filtered and washed with THF (4×2 min), DMF (4×2 min), CH_2Cl_2 (4×2 min), 0.02 M sodium *N*,*N*-diethyldithio carbamate trihydrate in DMF (3×15 min), DMF (5×2 min) and CH_2Cl_2 (3×2 min).

General procedure for cyclization of linear peptide

The resin was swollen with DMF/CH_2Cl_2=1/4 for 30 min at RT. Next, HBTU (3.0 equivalents, 41 mg, 108 μmol) was dissolved in DMF/CH_2Cl_2=1/4 (0.72 ml), after which HOBt (3.0 equivalents, 16.5 mg, 108 μmol), DIPEA (6.0 equivalents, 37.6 μl, 216 μmol) and the resin were added. The mixture was then agitated for 3 h at RT, after which it was filtered and washed with DMF (3×2 min), CH_2Cl_2/MeOH=10/1 (2 min) and CH_2Cl_2 (3×2 min).

General procedure for synthesis of malformins

The resin was swollen with DMF for 30 min at RT under N_2 atmosphere. A solution of I_2 (5.0 equivalents, 45.7 mg, 180 μmol) and triisopropylsilane (4.0 equivalents, 29.5 μl, 144 μmol) in DMF (1.5 ml) was added to the resin, and the mixture was agitated for 1 h at RT under N_2 atmosphere. The resin was filtered and cleaved from solid phase by treatment with 50% TFA/CH_2Cl_2 for 1 h. The product was filtered, washed with CH_2Cl_2 and the combined filtrates were concentrated. The residue was purified by flash column chromatography on silica gel ($CHCl_3$ to $CHCl_3$/MeOH=30/1) to yield malformins as white solids (2.9 mg, 15% of malformin C, 1.2 mg, 6% of malformin A1, 2.3 mg, 13% of malformin A2, 1.5 mg, 7% of malformin B2, 0.6 mg, 3% of **9**).

Malformin C. The ^{1}H-NMR data and HPLC analysis of solid-phase synthetic malformin C corresponded to the previously synthetic one.

Malformin A1. m.p.>300 °C; $[\alpha]_D^{29}$−42.2 (c 0.10, 2-methoxyethanol); IR (NaCl) cm^{-1}: 3266, 2958, 1633, 1535; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.84 (1H, d, *J*=4.2 Hz), 8.59 (1H, d, *J*=6.9 Hz), 7.95 (1H, d, *J*=8.9 Hz), 7.39 (1H, d, *J*=9.4 Hz), 7.10 (1H, d, *J*=10.8 Hz), 4.70 (1H, dt, *J*=4.4, 11.0 Hz), 4.46 (1H, dt, *J*=6.1, 9.3 Hz), 3.95 (1H, dd, *J*=3.2, 7.1 Hz), 3.92 (1H, dd, *J*=9.0, 10.3 Hz), 3.86 (1H, dd, *J*=6.8, 10.5 Hz), 3.50 (1H, dd, *J*=3.7, 14.6 Hz), 3.11–3.24 (3H, m), 2.03 (1H, dq, *J*=6.7, 13.3 Hz), 1.65–1.72 (1H, m), 1.35–1.59 (4H, m), 1.09–1.18 (1H, m), 0.88 (3H, d, *J*=6.6 Hz), 0.85 (3H, d, *J*=6.6 Hz), 0.80–0.82 (9H, m), 0.77 (3H, d, *J*=6.9 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.9, 173.0, 172.7, 170.5, 169.7, 58.8, 58.0, 52.9, 52.3, 50.3, 46.2, 45.2, 33.9, 30.4, 26.8, 24.7, 24.4, 22.7, 21.7, 19.7, 18.7, 14.9, 9.9; MS (ESI+) *m/z* 530.2450 $[M+H]^+$ (530.2470 calcd for $C_{23}H_{40}O_5N_5S_2$ [M+H]).

Natural malformin A1[23,25]. $[\alpha]_D^{29}$ −39.0 (2-methoxyethanol); ^{1}H NMR (DMSO-d_6) δ 8.87 (1H, d, *J*=4.03 Hz), 8.61 (1H, d, *J*=6.60 Hz), 7.96 (1H, d, *J*=8.79 Hz), 7.41 (1H, d, *J*=9.16 Hz), 7.13 (1H, d, *J*=10.99 Hz), 4.74 (1H, dt, *J*=4.40, 10.99 Hz), 4.49 (1H, dt, *J*=6.23, 9.16 Hz), 4.01 (1H, dt, *J*=3.67, 6.60 Hz), 3.95 (1H, d, *J*=9.16 Hz), 3.90 (1H, dd, *J*=6.60, 10.26 Hz), 3.53 (1H, dd, *J*=3.30, 14.65 Hz), 3.28 (2H, m), 3.17 (1H, dd, *J*=3.3, 14.29 Hz), 2.06 (1H, m), 1.72 (1H, m), 1.58 (1H, m), 1.54 (1H, m), 1.40 (2H, m), 1.17 (1H, m), 0.79–0.92 (18H, m); ^{13}C NMR (DMSO-d_6) δ 173.7, 172.7, 172.4, 170.4, 169.6, 58.6, 57.8, 52.8, 52.3, 50.3, 46.1, 45.0, 40.6, 34.0, 26.8, 24.6, 24.3, 22.5, 21.6, 19.4, 18.4, 14.7, 9.8; HRMS *m/z* 529.2411 $[M^+]$ (529.2392 calcd for $C_{23}H_{39}O_5N_5S_2$ [M]).

Malformin A2. m.p.>300 °C; $[\alpha]_D^{28}$ −29.6 (c 0.10, 2-methoxyethanol); IR (NaCl) cm^{-1}: 3286, 2962, 1660, 1536, 1174; ^{1}H NMR (400 MHz, 1 drop *d*-TFA in $CDCl_3$) δ 7.98 (1H, d, *J*=5.9 Hz), 7.15–7.30 (3H, m), 7.03 (1H, d, *J*=8.3 Hz), 5.00 (1H, dt, *J*=3.6, 11.3 Hz), 4.42 (1H, m), 4.02 (1H, dd, *J*=7.5, 10.7 Hz), 3.86 (1H, dd, *J*=3.0, 15.3 Hz), 3.72 (1H, dt, *J*=3.3, 6.7 Hz), 3.36 (1H, dd, *J*=11.6, 15.6 Hz), 3.15–3.25 (2H, m), 2.09 (1H, dq, *J*=6.6, 13.1 Hz), 1.99 (1H, dq, *J*=6.7, 13.4 Hz), 1.51–1.65 (3H, m), 1.05 (3H, d, *J*=6.6 Hz), 0.99 (3H, d, *J*=6.1 Hz), 0.91–0.96 (12H, m), ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.82 (1H, d, *J*=3.9 Hz), 8.54 (1H, d, *J*=6.8 Hz), 7.88 (1H, d, *J*=8.5 Hz), 7.41 (1H, d, *J*=9.2 Hz), 7.13 (1H, d, *J*=9.3 Hz), 4.70 (1H, dt, *J*=4.5, 10.9 Hz), 4.45 (1H, dt, *J*=6.3, 9.0 Hz), 3.97 (1H, dd, *J*=3.1, 6.9 Hz), 3.91 (1H, dt, *J*=6.0, 10.1 Hz), 3.77 (1H, dd, *J*=6.8, 9.9 Hz), 3.50 (1H, dd, *J*=3.3, 15.1 Hz), 3.11–3.24 (3H, m), 2.02 (1H, dq, *J*=6.9, 13.8 Hz), 1.83 (1H, dq, *J*=6.7, 13.3 Hz), 1.55 (1H, dt, *J*=7.1, 13.6 Hz), 1.37 (2H, m), 0.89 (3H, d, *J*=6.3 Hz), 0.88 (3H, d, *J*=6.2 Hz), 0.85 (3H, d, *J*=6.7 Hz), 0.80–0.82 (9H, m); ^{13}C NMR (100 MHz, 1 drop *d*-TFA in $CDCl_3$) δ 175.7, 175.6, 174.2, 172.4, 171.1, 62.1, 60.5, 54.6, 52.8, 52.6, 47.1, 45.8, 29.7, 29.0, 27.2, 24.9, 22.1, 21.7, 19.2, 19.1, 19.0, 18.3; ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.4, 173.9, 173.1, 171.1, 170.2, 60.5, 59.3, 53.3, 52.8, 50.8, 46.6, 45.6, 29.4, 29.0, 27.4, 24.9, 23.1, 22.2, 20.1, 19.9, 19.4, 19.1; MS (FAB) *m/z* 516.2316 $[M+H]^+$ (516.2314 calcd for $C_{22}H_{38}O_5N_5S_2$ [M+H]).

Natural malformin A2[23]. ^{1}H NMR (DMSO-d_6) δ 8.75 (1H, d, *J*=4.28 Hz), 8.46 (1H, d, *J*=6.71 Hz), 7.73 (1H, d, *J*=8.54 Hz), 7.43 (1H, d, *J*=9.15 Hz), 7.15 (1H, d, *J*=10.99 Hz), 4.73 (1H, dt, *J*=4.89, 10.99 Hz), 4.47 (1H, dt, *J*=6.72, 9.15 Hz), 3.99 (1H, dt, *J*=3.66, 7.32 Hz), 3.94 (1H, dd, *J*=9.15, 9.77 Hz), 3.82 (1H, dd, *J*=7.32, 9.77 Hz), 3.52 (1H,m), 3.25 (2H, m), 3.20 (1H, m), 2.04 (1H, m), 1.86 (1H, m), 1.60 (1H, m), 1.41 (2H, m), 0.92 (3H, d, *J*=4.88 Hz), 0.90 (3H, d, *J*=6.71 Hz), 0.89 (3H, d, *J*=6.11 Hz), 0.85 (3H, d, *J*=6.72 Hz), 0.84 (3H, d, *J*=6.71 Hz); HRMS *m/z* 515.2227 $[M^+]$ (515.2235 calcd for $C_{22}H_{37}O_5N_5S_2$ [M]).

Malformin B2. m.p.>300 °C; $[\alpha]_D^{29}$ −47.7 (c 0.050, 2-methoxyethanol); IR (NaCl) cm^{-1}: 3266, 2925, 1671, 1546; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.92 (1H, d, *J*=4.3 Hz), 8.54 (1H, d, *J*=6.5 Hz), 8.27 (1H, d, *J*=9.1 Hz), 7.18 (1H, d, *J*=9.6 Hz), 6.94 (1H, d, *J*=11.2 Hz), 4.66 (1H, dt, *J*=3.8, 11.1 Hz), 4.18 (1H, dt, *J*=6.1, 9.4 Hz), 4.11 (1H, dd, *J*=7.5, 9.2 Hz), 3.89–3.94 (2H, m), 3.43–3.45 (1H, m), 3.23–3.26 (1H, m), 3.05–3.12 (2H, m), 1.94–2.04 (1H, m), 1.66–1.75 (1H, m), 1.44–1.58 (3H, m), 0.84 (3H, d, *J*=6.3 Hz), 0.76–0.80 (15H, m); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.8, 173.1, 172.0, 170.7, 169.7, 58.6, 57.3, 52.8, 52.4, 51.6, 46.0, 45.2, 30.3, 29.0, 26.3, 24.0, 22.9, 21.5, 19.8, 19.0, 18.7, 18.5; MS (ESI+) *m/z* 516.2326 $[M+H]^+$ (516.2314 calcd for $C_{22}H_{38}O_5N_5S_2$ [M+H]).

Natural malformin B2[25,27]. $[\alpha]_D^{25}$−49.5 (*c* 0.73, 2-methoxyethanol); ^{1}H NMR (DMSO-d_6) δ 9.01 (1H, d, *J*=4.15 Hz), 8.61 (1H, d, *J*=6.35 Hz), 8.34 (1H, d, *J*=8.54 Hz), 7.26 (1H, d, *J*=9.52 Hz), 7.00 (1H, d, *J*=10.98 Hz), 4.73 (1H, dt, *J*=3.66, 10.99 Hz), 4.23 (1H, dt, *J*=6.1, 9.28 Hz), 4.17 (1H, dd, *J*=8.06, 8.79 Hz), 3.98 (1H, m), 3.97 (1H, dd, *J*=8.54, 10.5 Hz), 3.53 (1H, m), 3.30 (1H, dd, *J*=11.48, 14.89 Hz), 3.16 (1H, m), 3.13 (1H, m), 2.05 (1H, m), 1.77 (1H, m), 1.58 (1H, m), 1.54 (1H, m), 1.25 (1H, m), 0.80–0.95 (18H, m); ^{13}C NMR (DMSO-d_6) δ 173.8, 173.2, 172.0, 170.8, 169.7, 58.7, 57.3, 52.8, 52.4, 51.6, 46.0, 45.2, 38.7, 30.3, 26.3, 24.0, 23.0, 21.5, 19.8, 19.0, 18.8, 18.5; HRMS *m/z* 515.2231 $[M^+]$ (515.2235 calcd for $C_{22}H_{37}O_5N_5S_2$ [M]).

Cyclo(-D-cysteinyl-D-cysteinyl-L-valinyl-D-leucinyl-L-alanyl-), cyclic (1→2)-disulfide (8). m.p.>300 °C; $[\alpha]_D^{27}$−28.8 (c 0.10, 2-methoxyethanol); IR (NaCl) cm^{-1}: 3286, 2958, 1652, 1538; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.75 (1H, d, *J*=2.9 Hz), 8.55 (1H, d, *J*=5.9 Hz), 7.69 (1H, d, *J*=8.5 Hz), 7.47 (1H, d, *J*=9.0 Hz), 7.28 (1H, d, *J*=10.9 Hz), 4.68 (1H, dt, *J*=3.0, 7.7 Hz), 4.39 (1H, dt, *J*=4.8, 9.7 Hz), 4.14–4.21 (1H, m), 3.94 (1H, dd, *J*=3.3, 6.8 Hz), 3.91 (1H, dd, *J*=2.4, 10.2 Hz), 3.49 (1H, dd, *J*=3.3, 15.0 Hz), 3.19 (2H, d, *J*=8.4 Hz), 3.14 (1H, dt, *J*=3.0, 15.2 Hz), 1.99 (1H, dq, *J*=5.8, 12.9 Hz), 1.63–1.56 (1H, m), 1.39–1.46 (2H, m), 1.14 (3H, d, *J*=7.1 Hz), 0.87 (3H, d, *J*=6.6 Hz), 0.84 (3H, d, *J*=6.6 Hz), 0.81–0.82 (6H, m); ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.0, 173.4, 172.8, 171.0, 169.8, 58.9, 52.9, 52.5, 50.3, 48.9, 46.2, 45.3, 29.0, 27.3, 24.4, 23.0, 21.3, 19.6, 18.7, 16.2; MS (ESI+) *m/z* 510.1814 $[M+Na]^+$ (510.1820 calcd for $C_{20}H_{33}O_5N_5S_2Na$ [M+Na]).

Cyclo(-D-cysteinyl-D-cysteinyl-L-valinyl-D-leucinyl-L-leucinyl-) (10). The resin was swollen with CH_2Cl_2 for 30 min at RT. A solution of 50% TFA/CH_2Cl_2 (1.5 ml) and triisopropylsilane (4.0 equivalents, 29.5 μl, 144 μmol) was added to the resin and the mixture was agitated for 1 h at RT. The product was filtered and washed with CH_2Cl_2, and the filtrate was combined and concentrated. The residue was purified by flash column chromatography on silica gel ($CHCl_3$ to $CHCl_3$/MeOH=10/1) to yield dithiol compound **10** as a white solid (13.6 mg, 66%); m.p.>300 °C; $[\alpha]_D^{23}$ −5.0 (c 0.05, 2-methoxyethanol); IR (KBr) cm^{-1}: 3280, 2962, 1637, 1546; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.66 (1H, d, *J*=8.6 Hz), 8.62 (1H, d, *J*=8.1 Hz), 8.54 (1H, d, *J*=6.6 Hz), 7.56 (1H, d, *J*=6.5 Hz), 7.48 (1H, d, *J*=9.2 Hz), 4.40 (1H, dt, *J*=5.0 Hz), 4.10–4.21 (3H, m), 3.94 (1H, t, *J*=9.7 Hz), 2.79–2.88 (1H, m), 2.59–2.73 (3H, m), 2.44 (1H, t, *J*=8.7 Hz), 2.11 (1H, t, *J*=8.6 Hz), 1.92–2.01 (1H, m), 1.37–1.59 (6H, m), 0.89 (3H, d, *J*=6.7 Hz), 0.87 (3H, d, *J*=6.9 Hz), 0.79–0.85 (12H, m); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 173.1, 172.3, 171.0, 170.1, 168.7, 58.8, 56.5, 56.3, 52.3, 50.4, 40.7, 39.1, 26.9, 25.9, 24.5, 24.2, 23.0, 22.5, 22.4, 21.5, 19.5, 18.8; MS (ESI+) *m/z* 554.2442 $[M+Na]^+$ (554.2447 calcd for $C_{23}H_{41}O_5N_5S_2Na$ [M+Na]).

ACKNOWLEDGEMENTS

This work was supported in part by funds from the Drugs for Neglected Diseases initiative (DND*i*); a grant for the All Kitasato Project Study (AKPS); and by the 21st Century COE Program, Ministry of Education, Culture, Sports, Science and Technology. We also thank Ms A Nakagawa, Dr K Nagai and Ms N Sato (School of Pharmaceutical Sciences, Kitasato University) for the various instrumental analyses.

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