Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 2315–2318

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Antimicrobial Activity of Novel Globomycin Analogues

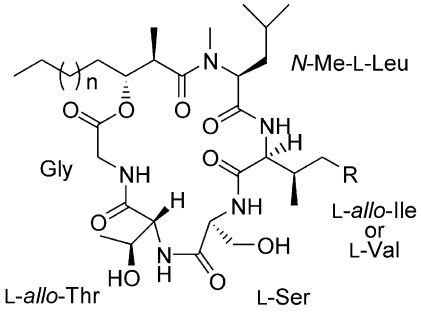
Toshihiro Kiho,[a] Mizuka Nakayama,[a] Kayo Yasuda,[b] Shunichi Miyakoshi,[b] Masatoshi Inukai[b] and Hiroshi Kogen[a,*]

[a]*Exploratory Chemistry Research Laboratories, Sankyo Co., Ltd., Tokyo 140–8710, Japan*
[b]*Lead Discovery Research Laboratories, Sankyo Co., Ltd., Tokyo 140–8710, Japan*

Received 11 March 2003; revised 22 April 2003; accepted 22 April 2003

Abstract—Globomycin, a signal peptidase II inhibitor, and its derivatives show potent antibacterial activity against Gram-negative bacteria. The synthesis and antimicrobial activity of novel globomycin analogues are reported. One of the analogues showed a more potent activity against Gram-negative bacteria than globomycin and also exhibited antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA).

Globomycin (**1a**)[1] and its congeners, SF-1902 A_2–A_5,[2] isolated by different researchers as antibiotics against only Gram-negative bacteria are 19-membered cyclic depsipeptides.[1,2] The major component, **1a**, has only been proven to be a specific inhibitor of signal peptidase II, a prolipoprotein-processing enzyme,[3] that processes the acylated precursor form of lipoprotein into apolipoprotein and a signal peptide in *Escherichia coli*.[4] Inhibition of signal peptidase II leads to the accumulation of the lipoprotein precursor in the cytoplasmic membrane and consequently to the death of the cell.[5] Signal peptidase II represents an attractive target because the mechanism is different from currently available drugs. Previously, we reported the absolute structure of **1a** obtained by X-ray analysis and the first asymmetric total synthesis of **1a** and SF-1902A_5 (**1b**).[1d,e] Now, we wish to report the structure–activity relationships (SARs) of synthetic new globomycin analogues that have potent inhibitory activity against Gram-negative bacteria. Structurally, these congeners were constructed from four natural amino acids, one *N*-methyl amino acid and a β-hydroxy-α-methyl carboxylic acid. Naturally occurring globomycin congeners are as shown in Figure 1. The minor congeners, SF-1902 A_3 and A_{4b}, have an L-Val in place of L-*allo*-Ile, and the other congeners, SF-1902 A_2, A_{4a} and A_{4b}, have a shorter or longer alkyl side chain in the fatty acid unit than that of **1a**. It was reported that the antibacterial activity is quite sensitive to the length of the alkyl side chain, either in a fatty acid or in an amino acid.[2] The congeners which have a longer side chain, **1b**, SF-1902 A_{4a} and A_{4b}, are more potent than **1a** (MIC: **1a**, 6.25 μg/mL; **1b**, 1.56 μg/mL against *E. coli* NIHJ JC-2). However, SF-1902



	R	n
Globomycin (**1a**)	CH_3	3
SF-1902 A_5 (**1b**)	CH_3	5
SF-1902 A_{4a}	CH_3	4
SF-1902 A_2	CH_3	1
SF-1902 A_3	H	3
SF-1902 A_{4b}	H	5

Figure 1. Structure of naturally occurring globomycin (**1a**) and its congeners.

*Corresponding author. Tel.: +81-3-3492-3131; fax: +81-3-5436-8570; e-mail: hkogen@shina.sankyo.co.jp

0960-894X/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00432-3

A_2 with the shortest side chain showed the weakest activity.[2b]

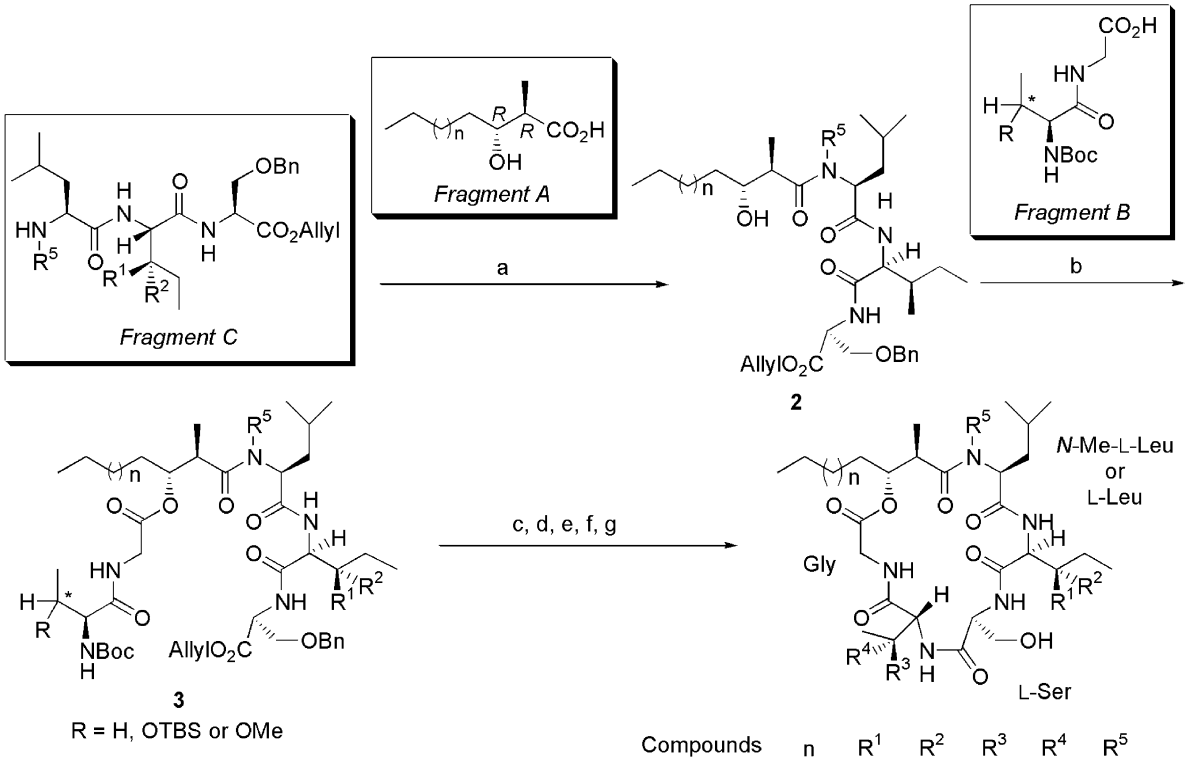
Furthermore, the congeners containing an L-Val, SF-1902 A_3 and A_{4b}, are less active compared with **1a** and **1b**, respectively. In this paper, we focused on the length of the alkyl side chain, the stereochemistry of *allo*-type amino acids, the hydroxyl group in L-*allo*-Thr and the *N*-methyl group.

Synthesis

The novel synthetic analogues (**1c–1i**) were prepared by the convergent macrolactamization method using three fragments[6] (*Fragment A*, *B* and *C*) as shown in Scheme 1. *Fragment A* was prepared by an *anti*-selective boron aldol reaction followed by hydrolysis.[7] *Fragments B* and *C* were synthesized from commercially available protected amino acids with PyBOP.[8] *Fragment C* was condensed with *Fragment A* mediated by DEPC[8] to give the acylated tripeptide **2**. The esterification of **2** was performed with DIPC[8] and *Fragment B* under Keck's condition[11] to afford a fully protected *seco*-acid **3**. Sequential deprotections of TBS, allyl and Boc group in **3** provided macrocyclization precursors. The macrolactamization was performed by HATU[8] or TBTU[8] to give *O*-Bn derivatives under highly diluted conditions. Finally, the removal of the benzyl group by hydrogenolysis yielded novel globomycin analogues (**1c–1i**). *N*-Demethyl derivative, **1i**, only exists as a single isomer in CD_3OD, which is different from other analogues.[1d,e,13]

Antibacterial Activity

Antibacterial activities of the synthetic globomycin analogues (**1a–1i**) against Gram-negative bacteria are summarized in Table 1. As a result, **1c**[14] possessing the longest alkyl side chain shows the most potent activity among the analogues (MIC: **1a**, 12.5 μg/mL; **1b**, 3.13 μg/mL; **1c**, 1.56 μg/mL against *E. coli* SANK 70569). The length of the alkyl side chain greatly affects the



[a]Reagents and conditions:
(a) DEPC, Et_3N, THF, 0°C to rt
(b) DIPC, CSA, DMAP, CH_2Cl_2, 0°C to rt
(c) TBAF, AcOH
(d) cat $Pd(PPh_3)_4$, morpholine
(e) TFA, CH_2Cl_2
(f) HATU or TBTU, *i*-Pr_2NEt
(g) H_2, $Pd(OH)_2$

Compounds		n	R^1	R^2	R^3	R^4	R^5
globomycin	**1a**	3	Me	H	OH	H	Me
SF-1902 A_5	**1b**	5	Me	H	OH	H	Me
	1c	7	Me	H	OH	H	Me
	1d	3	H	Me	OH	H	Me
	1e	3	Me	H	H	OH	Me
	1f	3	H	Me	H	OH	Me
	1g*	3	Me	H	H	H	Me
	1h*	3	Me	H	OMe	H	Me
	1i	3	Me	H	OH	H	H

* Condition c was not used.

Scheme 1.[a] Synthesis of globomycin (**1a**) and its analogues.

Table 1. Antibacterial spectrum of globomycin (**1a**), SF-1902 A_5 (**1b**) and the synthetic analogues (**1c**–**1i**)

Organisms	MIC (μg/mL)								
	1a	**1b**	**1c**	**1d**	**1e**	**1f**	**1g**	**1h**	**1i**
Escherichia coli SANK 70569 (NIHJ JC-2)	12.5	3.13	1.56	12.5	>100	>100	25	25	>100
Escherichia coli SANK 72290	12.5	3.13	1.56	12.5	>100	>100	25	25	>100
Salmonella enteritidis SANK 72390	25	6.25	3.13	50	>100	>100	100	50	>100
Klebsiella pneumoniae SANK 72490	25	3.13	1.56	25	>100	>100	50	50	>100
Enterobacter cloacae 846	6.25	1.56	1.56	6.25	100	>100	12.5	12.5	100
Enterobacter cloacae SANK 72690	50	12.5	3.13	50	>100	>100	>100	100	>100
Serratia marcescens SANK 72790	100	12.5	3.13	>100	>100	>100	>100	>100	>100
Proteus vulgaris SANK 72890	>100	>100	>100	>100	>100	>100	>100	>100	>100
Morganella morganii SANK 72990	>100	>100	>100	>100	>100	>100	>100	>100	>100
Pseudomonas aeruginosa SANK 73090	>100	>100	>100	>100	>100	>100	>100	>100	>100
Pseudomonas aeruginosa SANK 73190	>100	>100	>100	>100	>100	>100	>100	>100	>100
Pseudomonas aeruginosa SANK 3719	>100	>100	>100	>100	>100	>100	>100	>100	>100

antibacterial activity. Four-carbon increase in the fatty acid side chain enhanced the activity by 4- to 8-fold compared with **1a**. Therefore, it may be possible to produce a more potent inhibitor.

With regard to stereoisomers, the activity of **1d** diminished and the activity of **1e** and **1f** were completely lost. In particular, the stereochemistry of the hydroxyl group in L-Thr is quite important for the activity. Compound **1e** was inactive although the deoxy derivative **1g** and methyl ether derivative **1h** retained their activity. Therefore, the hydroxyl group in L-*allo*-Thr is not essential for the activity.[15] Finally, *N*-demethyl derivative **1i** also lost its activity.

Surprisingly, **1c** showed moderate activity against all Gram-positive bacteria tested such as *Staphylococcus aureus* (MRSA) (MIC = 12.5 μg/mL) even though **1a** and **1b** were almost inactive as shown in Table 2. This is the first example that the antibacterial spectrum of globomycin analogues was expanded to also include Gram-positive bacteria. These results suggest that lipoproteins are essential for not only Gram-negative bacteria but also Gram-positive bacteria and signal peptidase II inhibitors would probably be effective against most bacteria. Finding such an inhibitor would lead to development of a new class of antibiotics. Finally, the antifungal activity of these analogues was tested. However, no activity was observed against *Candida albicans*, *Candida glabrata* and *Aspergillus clavatus*.

In summary, we disclosed the SAR of synthetic new globomycin analogues and succeeded in producing a promising antibiotic, which shows activity against not only Gram-negative bacteria but also Gram-positive bacteria. Now, further investigations on SARs are currently underway.

Table 2. Antibacterial spectrum of globomycin (**1a**–**1c**) against Gram-positive bacteria

Organisms	MIC (μg/mL)		
	1a	**1b**	**1c**
Staphylococcus aureus SANK 70668	>100	50	6.25
Staphylococcus aureus SANK 71790	>100	50	6.25
Staphylococcus aureus SANK 71890[a]	>100	50	12.5
Enterococcus faecalis SANK 71990	>100	100	12.5

[a]MRSA.

Measurement of Antibacterial Activity

Bacteria were inoculated on Nutrient Agar (Eiken Chemical Co., Ltd.) and the MIC was determined by the agar dilution method.[16]

References and Notes

1. (a) Taxonomy of producing organism and fermentation: Inukai, M.; Enokita, R.; Torikata, A.; Nakahara, M.; Iwado, S.; Arai, M. *J. Antibiot*. **1978**, *31*, 410. (b) Isolation and physico-chemical and biological characterization: Inukai, M.; Nakajima, M.; Osawa, M.; Haneishi, T.; Arai, M. *J. Antibiot*. **1978**, *31*, 421. (c) Structural determination of **1a**: Nakajima, M.; Inukai, M.; Haneishi, T.; Terahara, A.; Arai, M. *J. Antibiot*. **1978**, *31*, 426. (d) Kogen, H.; Kiho, T.; Nakayama, M.; Furukawa, Y.; Kinoshita, T.; Inukai, M. *J. Am. Chem. Soc*. **2000**, *122*, 10214. (e) Kiho, T.; Nakayama, M.; Kogen, H. *Tetrahedron* **2003**, *59*, 1685.
2. (a) Omoto, S.; Suzuki, H.; Inouye, S. *J. Antibiot*. **1979**, *32*, 83. (b) Omoto, S.; Ogino, H.; Inouye, S. *J. Antibiot*. **1981**, *34*, 1416.
3. Pugsley, A. P. *Microbiol. Rev*. **1993**, *57*, 50.
4. (a) Inukai, M.; Takeuchi, M.; Shimizu, K.; Arai, M. *J. Antibiot*. **1978**, *31*, 1203. (b) Hussain, M.; Ichihara, S.; Mizushima, S. *J. Biol. Chem*. **1980**, *255*, 3707. (c) Dev, I. K.; Harvey, R. J.; Ray, P. H. *J. Biol. Chem*. **1985**, *260*, 5891. (d) Ichihara, S.; Hussain, M.; Mizushima, S. *J. Biol. Chem*. **1981**, *256*, 3125. (e) Witke, C.; Götz, F. *FEMS Microl. Lett*. **1995**, *126*, 233. (f) Braun, B.; Wu, H. C. In *Bacterial Cell Wall*; Ghuysen, J. M., Hakenbeck, R., Eds.; Elsevier: Amsterdam, 1994; Chapter 14.
5. Cavard, D. *Arch. Microbiol*. **1998**, *171*, 50.
6. Synthesis of three fragments, *Fragment A*, *B* and *C*, were performed by the method described in refs 1d and 1e.
7. Abiko, A.; Liu, J.-F.; Masamune, S. *J. Am. Chem. Soc*. **1997**, *119*, 2586.
8. PyBOP:[9] (benzotriazolyloxy) tris(pyrrolydino)phosphonium hexafluorophosphate, DEPC:[10] diethylcyanophosphate, DIPC; diisopropylcarbodiimide, HATU:[12] *O*-(7-azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, TBTU:[12] 2-(1*H*-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate.

9. Coste, J.; Le-Nguyen, D.; Castro, B. *Tetrahedron Lett*. **1990**, *31*, 205.
10. Shioiri, T.; Yokoyama, Y.; Kasai, Y.; Yamada, S. *Tetrahedron* **1976**, *32*, 2211.
11. Boden, E. P.; Keck, G. E. *J. Org. Chem*. **1985**, *50*, 2394.
12. (a) Carpino, L. A.; El-Faham, A. *J. Org. Chem*. **1994**, *59*, 695. (b) Carpino, L. A. *J. Am. Chem. Soc*. **1993**, *115*, 4397. (c) Carpino, L. A.; El-Faham, A. *J. Org. Chem*. **1995**, *60*, 3561. (d) Humphrey, J. M.; Chamberlin, A. *R. Chem. Rev*. **1997**, *97*, 2243.
13. ^{1}H NMR suggested that other analogues (**1a**–**1h**) exist as a mixture of rotational isomers in solution.
14. The spectrum data for compound **1c**. The minor conformer is marked with an asterisk. ^{1}H NMR [500 MHz, $CDCl_3$, 16 mM, two rotamers (major/minor = 5.3/1)] δ ppm: 0.85–0.97 (m, 15H), 1.10 (d, 15/6H, J = 6.8 Hz), 1.15* (d, 3/6H, J = 7.1 Hz), 1.13–1.42 (m, 21H), 1.48–1.57 (m, 1H), 1.62–1.71 (m, 3H), 1.76–1.88 (br, 2H), 2.02–2.10* (m, 1/6H), 2.10–2.16 (m, 1H), 2.18–2.23 (m, 5/6H), 2.78* (s, 3/6H), 3.11–3.18 (m, 1H), 3.21 (s, 15/6H), 3.63 (br s, 5/6H), 3.75 (dd, 5/6H, J = 4.1, 17.2 Hz), 3.85* (dd, 1/6H, J = 4.0, 18.0 Hz), 3.94 (s, 10/6H), 3.98* (s, 2/6H), 4.01–4.17 (m, 2H), 4.20–4.40 (m, 2H), 4.53 (dd, 1H, J = 4.3, 7.4 Hz), 4.77* (dd, 1/6H, J = 4.5, 9.4 Hz), 4.90* (d, 1/6H, J = 9.9 Hz), 5.06–5.10 (m, 5/6H), 6.92* (d, 1/6H, J = 9.1 Hz), 7.11 (d, 5/6H, J = 7.4 Hz), 7.37* (br s, 1/6H), 7.41* (d, 1/6H, J = 8.1 Hz), 7.53* (br t, 1/6H, J = 3.1 Hz), 7.62 (d, 5/6H, J = 4.4 Hz), 7.68 (br m, 10/6H); ^{13}C NMR (125 MHz, $CDCl_3$, 33 mM, both rotamers) δ ppm: 11.6, 12.3*, 14.1, 14.6, 14.8*, 15.0, 18.9, 19.1*, 21.9, 22.66, 22.72*, 23.0*, 24.3, 24.8*, 25.2, 25.9*, 26.9*, 27.1, 29.3, 29.42, 29.49, 29.56, 29.59, 29.7*, 31.3, 31.9, 36.6, 37.3*, 38.1, 38.4*, 39.2*, 40.1, 40.5, 41.1, 56.2*, 56.6, 57.7, 57.8*, 59.1, 59.2*, 60.7*, 61.5, 66.9, 67.2*, 68.0, 76.4, 77.2, 77.9*, 168.8, 170.3, 170.7, 170.9*, 171.0*, 173.3, 173.4*, 174.6*, 174.7, 177.0; IR (KBr) cm^{-1}: 3326, 2959, 2927, 2856, 1758, 1656, 1545, 1466, 1377, 1196; HRMS m/z $(M+H)^+$ calcd 712.4861, found 712.4849. Anal. calcd for $C_{36}H_{65}N_5O_9{\cdot}H_2O$: C, 59.24; H, 9.25; N, 9.59. Found: C, 58.97; H, 8.93; N, 9.48; $[\alpha]_D^{26} = +19.0$ (c 0.50, CH_3OH).
15. Diacetylation of **1a** in L-Ser and L-*allo*-Thr residue diminished the activity.[2b] *O*-Bn or *O*-Me globomycin derivatives in L-Ser residue were inactive against *E. coli* SANK 70569 tested only (MIC > 50 μg/mL).
16. National Committee for Clinical Laboratory Standards. Standard Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. Approved Standard (M7-A5); NCCLS: Villanova, 2000; p 7.