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Synthesis and Anti-*Helicobacter pylori* Activity of Pyloricidin Derivatives

II. The Combination of Amino Acid Residues in the Dipeptidic Moiety and its Effect on the Anti-*Helicobacter pylori* Activity

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The novel natural antibiotics pyloricidin A, B and C, consisting of a common (2*S*,3*R*,4*R*,5*S*)-5-amino-2,3,4,6-tetrahydroxyhexanoyl-β-D-phenylalanine moiety and a terminal peptidic moiety (pyloricidin A: L-valine-L-valine-L-leucine; pyloricidin B: L-valine-L-leucine; pyloricidin C: L-leucine), exhibit potent and highly selective anti-*Helicobacter pylori* activity. In order to develop more potent compounds and to investigate structure activity relationships for the peptidic moiety with regard to the combination of amino acids, a series of derivatives with various dipeptidic moieties were prepared and evaluated for their anti-*H. pylori* activity. The combination of the two amino acids in the moiety was found to have a significant effect on the activity; the compound with Nva–Abu showed excellent anti-*H. pylori* activity with an MIC value of 0.013 μg/ml against *H. pylori* TN2. In addition, this compound was found to show 60% clearance of *H. pylori* from infected Mongolian gerbils upon repetitive oral administration (10 mg/kg, b. i. d. for 7 days).

Helicobacter pylori, a Gram-negative bacterium, plays a crucial role in the pathogenesis of gastric and duodenal ulcers.[1~7] Therefore, eradication of this organism is of great importance in treating peptic ulcers. In our program to develop therapeutically useful anti-*H. pylori* agents, promising lead compounds, pyloricidin A, B and C, were discovered in Takeda's pharmaceutical discovery center.[8,9]

In order to investigate structure activity relationships, initially, we carried out chemical modification of the common (2*S*,3*R*,4*R*,5*S*)-5-amino-2,3,4,6-tetrahydroxyhexanoyl-β-D-phenylalanine moiety and revealed that this structural unit is the minimum component required to express anti-*H. pylori* activity.[10] Next, we focused on the terminal peptidic moiety. A series of pyloricidin B and pyloricidin C derivatives, in which the terminal L-valine and L-leucine were substituted with various amino acids, were prepared and evaluated for their anti-*H. pylori* activity. As a result, it was clear that the L-valine and L-leucine residues were replaceable with other α-L-amino acids and the activity was largely associated with the amino acids introduced; the allylglycine ((allyl)Gly) derivative **1A** exhibited the most potent activity, which was 60-fold

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greater than that of the lead compound pyloricidin C.[11] Based on these findings, we speculated that the fixed L-leucine residue in the pyloricidin B derivatives could be substituted with other amino acids. Furthermore, considering that the anti-*H. pylori* activity of pyloricidin B derivatives is generally superior to that of pyloricidin C, we anticipated that more potent derivatives might be prepared by elongation of the peptidic moiety of **1A** with an appropriate amino acid. Therefore, we prepared a series of derivatives with X–(allyl)Gly (X=norvaline (Nva), Ile, Met, (allyl)Gly, Asn and *S*-methylcysteine (Cys(Me)) as the terminal dipeptidic moiety. In addition, to elucidate how the combination of amino acids in the peptidic moiety affects the anti-*H. pylori* activity, a series of derivatives, possessing the dipeptides Nva–Y (Y=Ala, (*S*)-2-aminobutyric acid (Abu), Nva, Gln and Met), were also prepared. In this paper, we report the synthesis and anti-*H. pylori* activity of these derivatives as well as the *in vivo* clearance of *H. pylori* by the repetitive oral administration of the selected compound **2aC** to Mongolian gerbils infected with *H. pylori* TN2GF4.

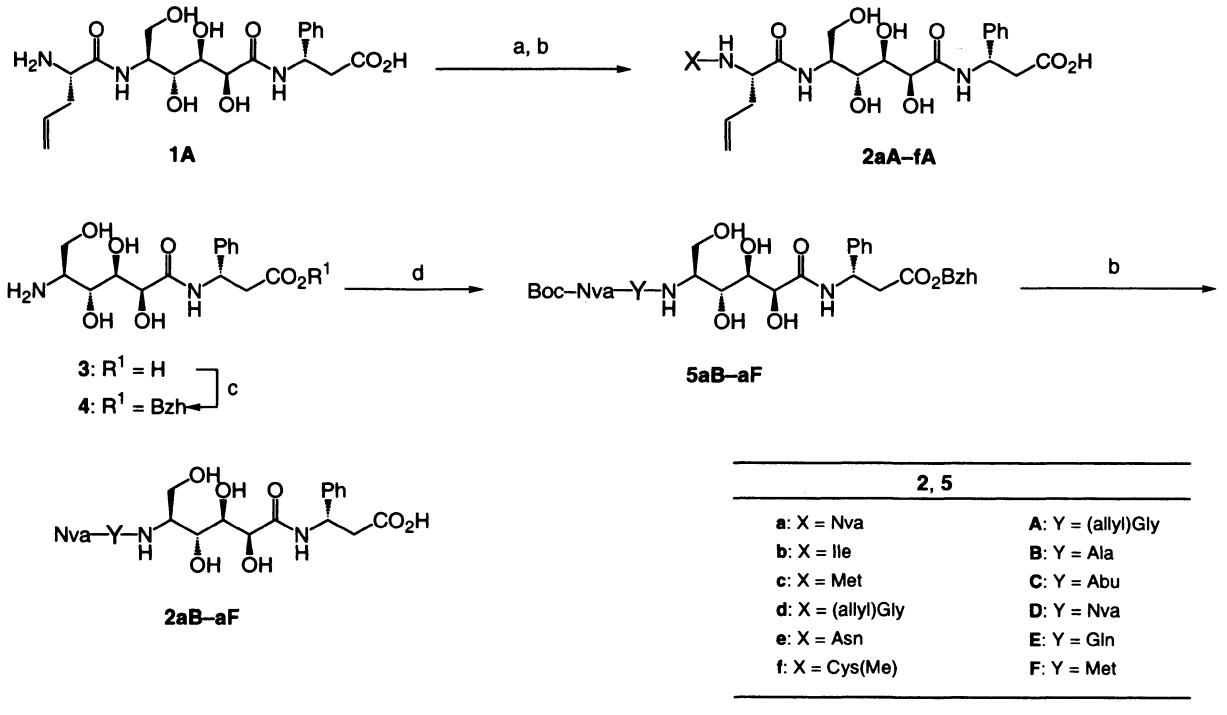
Chemistry

The derivatives **2aA~fA** and **2aB~aF**, bearing X–(allyl)Gly (X=Nva, Ile, Met, (allyl)Gly, Asn and Cys(Me)) and Nva–Y (Y=Ala, Abu, Nva, Gln and Met), were prepared as shown in Scheme 1. The derivatives **2aA~fA** were obtained by the condensation of the *N*-hyroxysuccinimide esters (Boc–X–OSu) derived from the Boc protected amino acids (Boc–X) with the (allyl)Gly derivative **1A**,[11] followed by removal of the Boc protecting groups. The derivatives **2aB~aF** were prepared as follows. The Boc protected dipeptides (Boc–Nva–Y) were coupled with the benzhydryl ester **4** using water soluble carbodiimide hydrochloride (WSC·HCl) and 1-hydroxybenzotriazole (HOBt) to give **5aB~aF**, which were treated with 4N HCl/EtOAc to afford the desired compounds **2aB~aF**.

Results and Discussion

Table 1 shows the anti-*H. pylori* activity (minimum inhibitory concentrations (MICs)) of the derivatives

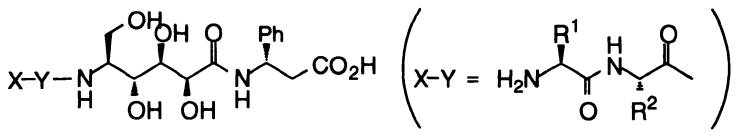
Scheme 1[a].



[a] Reagents and conditions : (a) Boc-X-OSu (X = Nva, Ile, Met, (allyl)Gly, Asn and Cys(Me)), Et_3N/DMF; (b) TFA or 4N HCl/EtOAc; (c) (i)1N HCl/MeOH, (ii) Ph_2CN_2/MeOH; (d) Boc-Nva-Y (Y = Ala, Abu, Nva, Gln and Met), HOBt, WSC•HCl, iPr_2EtN/DMF.

prepared in this work along with pyloricidin B, pyloricidin C and their derivatives (**1a~f**, **1A~F**) as reference compounds. The anti-*H. pylori* activity was evaluated against four strains of *H. pylori* (NCTC11637, CPY433, TN2 and TN58). The TN2GF4 strain which derived from the TN2 strain was used for *in vivo* clearance experiments, therefore, we focused our studies on the anti-*H. pylori* activity against TN2. In our previous work,[11] six pyloricidin B derivatives **1a~f**, bearing Nva, Ile, Met, (allyl)Gly, Asn and Cys(Me), showed efficient anti-*H. pylori* activity (MIC: 0.05~0.2 μg/ml against TN2). Therefore, we adopted their terminal amino acids to construct the dipeptidic moiety of the new series of derivatives **2aA~fA**, anticipating improved activity over that of **1A**. However, contrary to our expectation, none of these derivatives displayed more potent activity than **1A** and only derivatives **2aA** and **2dA** maintained equal activity to that of **1A**. In order to more clearly elucidate the effect of the combination of amino acids on the activity, a series of derivatives with Nva–Y (Y=Ala, Abu, Nva, Gln and Met) were prepared. In this series of derivatives, the terminal Nva was fixed, because the Nva derivatives **1a** and **2aA** had shown strong activity. On the other hand, the amino acids Y were selected, taking into account the efficient activity of the pyloricidin C derivatives **1B~F**. Interestingly, the activity varied depending on the dipeptidic moiety, and the derivative **2aC**, bearing Nva–Abu, showed the most potent activity with an MIC value of 0.013 μg/ml against TN2. The potency of **2aC** against TN2 was 30-fold greater than that of **2aB**, containing Nva–Ala. It should be noted that subtle structural differences made to the α-side chain of Y resulted in large differences in activity. These

Table 1. Anti-*H. pylori* activity of pyloricidin derivatives against four strains of *H. pylori*.



Compound	X (R^1)	Y (R^2)	MIC (μg/ml)[a]			
			NCTC11637	CPY433	TN2	TN58
Pyloricidin B	Val (isopropyl)	Leu	0.05	0.39	0.2	0.1
Pyloricidin C	–	Leu	0.39	3.13	0.78	1.56
1a	Nva (Pr)	Leu	0.025	0.1	0.05	0.025
1b	Ile (*sec*-Bu)	Leu	0.05	0.2	0.1	0.1
1c	Met (CH_2CH_2SMe)	Leu	0.1	0.2	0.1	0.1
1d	(allyl)Gly (allyl)	Leu	0.05	0.2	0.1	0.05
1e	Asn (CH_2CONH_2)	Leu	0.1	0.39	0.2	0.1
1f	Cys(Me) (CH_2SMe)	Leu	0.05	0.2	0.1	0.1
1A	–	(allyl)Gly (allyl)	<0.006	0.1	0.025	0.025
1B	–	Ala (Me)	0.2	1.56	0.78	0.39
1C	–	Abu (Et)	0.025	0.39	0.39	0.025
1D	–	Nva (Pr)	0.05	0.39	0.39	0.78
1E	–	Gln ($CH_2CH_2CONH_2$)	0.05	1.56	0.39	0.1
1F	–	Met (CH_2CH_2SMe)	0.78	0.78	0.39	0.1
2aA	Nva	(allyl)Gly	0.013	0.2	0.05	0.05
2bA	Ile	(allyl)Gly	0.05	0.39	0.2	0.1
2cA	Met	(allyl)Gly	0.05	0.39	0.1	0.1
2dA	(allyl)Gly	(allyl)Gly	0.013	0.05	0.025	0.025
2eA	Asn	(allyl)Gly	0.1	0.2	0.1	0.05
2fA	Cys(Me)	(allyl)Gly	0.05	0.39	0.2	0.1
2aB	Nva	Ala	0.1	0.78	0.39	0.2
2aC	Nva	Abu	<0.006	0.05	0.013	0.025
2aD	Nva	Nva	0.025	0.2	0.1	0.1
2aE	Nva	Gln	0.05	0.2	0.1	0.1
2aF	Nva	Met	0.05	0.39	0.2	0.1

[a] Minimum inhibitory concentrations (MICs) were determined by the agar dilution method in brucella agar with a bacterial suspension of about 10^6 cfu/ml.

Table 2. Anti-*H. pylori* activity of **2aC**, AMPC, CAM and MNZ against 54 strains of *H. pylori*.

	MIC (μg/ml)[a]				
	Range	MIC_{50}[b]	MIC_{90}[c]	TN352	TN309
2aC	0.013 – 0.1	0.025	0.05	0.1	0.025
AMPC	<0.006 – 0.39	0.025	0.2	0.1	0.013
CAM	0.013 – 100	0.05	50	100	0.1
MNZ	0.78 – 100	3.13	6.25	3.13	100

[a] Minimum inhibitory concentrations (MICs) were determined by the agar dilution method in brucella agar with a bacterial suspension of about 10^6 cfu/ml, [b] MIC required to inhibit 50% of strains, [c] MIC required to inhibit 90% of strains.

observations allowed us to speculate that each amino acid in the peptidic moiety affects the conformation of the other, which might reflect the potency of the anti-*H. pylori* activity.

So far, the best results for eradication of *H. pylori* have been achieved by combination therapy, involving concomitant administration of a proton pump inhibitor and antimicrobial agents (clarithromycin (CAM) and metronidazole (MNZ), or CAM and amoxicillin (AMPC)). However, recently, GOTOH *et al.* reported that of 63 clinical isolates of *H. pylori*, 9.5% and 7.9% of the strains displayed drug resistance to CAM and MNZ, respectively.[12] VAN ZWET *et al.* also reported the emergence of an AMPC resistant strain.[13] In addition, *H. pylori* strains show a high degree of diversity at the genetic level,[14] which might cause a difference in the susceptibility of strains to antimicrobial agents. Therefore, any anti-*H. pylori* agent with therapeutic usefulness should have potent activity against various strains, including the drug resistant strains. Accordingly, we evaluated the anti-*H. pylori* activity of **2aC** against 54 clinical isolates including the CAM resistant strain (TN352) and the MNZ resistant strain (TN309) and compared its potency with those of AMPC, CAM and MNZ. The range of MICs and the concentrations of agents required to inhibit 50% and 90% of strains are shown in Table 2. Compound **2aC** was found to show potent anti-*H. pylori* activity against TN352 and TN309 with MIC values of 0.1 μg/ml and 0.025 μg/ml, respectively. Furthermore, compound **2aC** displayed excellent activity against all strains, within a narrow range of MICs (0.013~0.1 μg/ml). From these results, it is clear that compound **2aC** is effective against various strains including the strains acquiring resistance to CAM or MNZ.

Next, we assessed the antibacterial selectivity of **2aC**. Table 3 shows antibacterial activity of **2aC** against various microorganisms. Compound **2aC** did not show any inhibitory activity up to 100 μg/ml, which suggests that the antibacterial activity of **2aC** is highly specific for *H. pylori*. Considering that pyloricidin A, B and C also showed highly specific anti-*H. pylori* activity, it is speculated that the antibacterial selectivity observed in pyloricidin antibiotics is attributed to the common (2*S*,3*R*,4*R*,5*S*)-5-amino-2,3,4,6-tetrahydroxyhexanoyl-β-D-phenylalanine part. CAM, MNZ and AMPC have broad antibacterial spectra and therefore affect gastrointestinal microflora, which would cause gastrointestinal side effects.[15] As the antibacterial spectra of **2aC** is restricted to *H. pylori*, its oral use is expected to have less side effects.

On the basis of the anti-*H. pylori* activity, we selected **2aC** as a test compound for the *in vivo* clearance of *H. pylori*. MATSUMOTO *et al.* reported that Mongolian gerbils developed gastritis with severe inflammation from 4 weeks after *H. pylori* infection,[16] which is a common feature of *H. pylori*-associated gastritis in humans. Therefore, we carried out *in vivo* clearance experiments using Mongolian gerbils infected with *H. pylori* TN2GF4 for 4 weeks. Table 4 shows the clearance rate and the bacterial recovery after repetitive oral administration of **2aC** (10 mg/kg, twice a day for 7 days). Complete clearance of *H. pylori* was achieved in 3 of the 5 animals (clearance rate: 60%) and a significant decrease of bacterial recovery was observed in the remaining animals. From these results, it was confirmed that compound **2aC** could effectively eradicate *H. pylori* from infected Mongolian gerbils by oral administration. These findings together with the antibacterial profiles described above suggest that compound **2aC** is a promising agent for the treatment of *H. pylori* infection.

Table 3. Antibacterial activity of **2aC** against various microorganisms.

Organism	Strain	MIC (μg/ml) **2aC**
Staphylococcus aureus	FDA 209P	>100
Staphylococcus epidermidis	IFO 3762	>100
Streptococcus pneumoniae	Type I	>100
Enterococcus faecalis	IFO 12580	>100
Escherichia coli	NIHJ JC-2	>100
Enterobacter cloacae	CS4495	>100
Proteus vulgaris	IFO 3988	>100
Citrobacter freundii	IFO 12681	>100
Serratia marcescens	IFO 16484	>100
Klebsiella pneumoniae	IFO 3321	>100
Morganella morganii	IFO 3168	>100
Pseudomonas aeruginosa	IFO 3445	>100

[a] Minimum inhibitory concentrations (MICs) were determined by the agar dilution method using Mueller–Hinton agar supplemented with 5% horse serum.

Table 4. Effect of repetitive oral administration of **2aC** against gastric infection caused by *H. pylori* TN2GF4 in Mongolian gerbils.

Compound	Dose (mg / kg)[a]	Clearance rate (number of gerbils cleared infection / total number)	Bacterial recovery (log cfu / gastric wall)[b]
Vehicle control[c]	0	0 / 5 (0)	6.15 ± 0.15
2aC	10	3 / 5 (60)	3.31 ± 1.23*

[a] b. i. d. for 7 days starting 4 weeks after the bacterial challenge.

[b] Bacterial counts less than $10^{1.48}$ cfu were regarded as $10^{1.48}$ to calculate the mean. Values are means ± standard error.

[c] The control was a 0.5% methylcellulose solution.

*: $p<0.05$ vs vehicle control by Dunnett's test.

Conclusion

We prepared a series of pyloricidin derivatives containing various dipeptidic moieties and evaluated them for their anti-*H. pylori* activity. The combination of the two amino acids was found to have a significant effect on the activity; each amino acid in the moiety affects the conformation of the other, which reflects the potency of the activity. In this study, we discovered a promising compound **2aC** bearing Nva–Abu as the dipeptidic moiety. Compound **2aC** showed excellent anti-*H. pylori* activity with an MIC value of 0.013 μg/ml against *H. pylori* TN2, and it was effective against CAM or MNZ resistant strains. Furthermore, compound **2aC** was found to show 60% clearance of *H. pylori* from infected Mongolian gerbils upon its repetitive

oral administration (10 mg/kg, b. i. d. for 7 days).

Experimental

Melting points were determined using a Yanagimoto melting point apparatus and are uncorrected. IR spectra were measured with a JASCO IR-810 or SHIMADZU FTIR-8200 spectrometer. ^{1}H-NMR spectra were recorded on a Varian Gemini-200 spectrometer with tetramethylsilane or 2,2-dimethyl-2-silapentane-5-sulfonate sodium salt (DSS) as an internal standard. The optical rotations were recorded with a JASCO DIP-181 or DIP-370 digital polarimeter. Chromatographic separations were carried out on Silica gel 60 (0.040~0.063 or 0.063~0.200 mm, E. Merck) or high porous polymer (MCI gel, Mitsubishi kasei) using the indicated eluents.

N-[(2*S*,3*R*,4*R*,5*S*)-5-(L-Norvalyl-L-allylglycyl)amino-2,3,4,6-tetrahydroxyhexanoyl]-*β*-D-phenylalanine (**2aA**)

To a solution of *N*-*tert*-butoxycarbonyl-L-norvaline (54 mg, 0.25 mmol) in CH_3CN (1 ml) were added *N*-hydroxysuccinimide (29 mg, 0.25 mmol) and *N*,*N*′-dicyclohexylcarbodiimide (52 mg, 0.25 mmol) at 0°C. After being stirred at room temperature for 3 hours, the reaction mixture was filtered and the filtrate was concentrated under reduced pressure. To a solution of the residue in DMF (8 ml) were added Et_3N (0.035 ml) and compound **1A** (110 mg, 0.25 mmol). The reaction mixture was stirred at room temperature for 20 hours and then concentrated. The residue was suspended in 4 N HCl/EtOAc (10 ml) at room temperature for 1 hour. After removal of solvent, the residue was chromatographed on MCI gel (HP-20SS) with H_2O and then 20% CH_3CN in H_2O as eluent and recrystallized from MeOH-Et_2O to afford **2aA** (56 mg, 42%) as a colorless solid.

Compounds **2bA**~**fA** were prepared in a similar manner to that employed for the preparation of **2aA**, using Boc-Ile (**2bA**), Boc–Met (**2cA**), Boc–(allyl)Gly (**2dA**), Boc–Asn (**2eA**) and Boc–Cys(Me) (**2fA**), respectively. Their yields and physicochemical data are listed in Table 5.

N-[(2*S*,3*R*,4*R*,5*S*)-5-(L-Norvalyl-L-alanyl)amino-2,3,4,6-tetrahydroxyhexanoyl]-*β*-D-phenylalanine (**2aB**)

A solution of **5aB** (160 mg, 0.205 mmol) in 4 N HCl/EtOAc (5 ml) was stirred at room temperature for 1 hour. The resulting precipitates were collected by filtration and chromatographed on MCI gel (CHP-20P) with H_2O and then 10% CH_3CN in H_2O as eluent and recrystallized from MeOH-EtOAc to afford **2aB** (73 mg, 65%) as a colorless solid.

A series of compounds **2aC**~**aF** were prepared in a similar manner to that employed for the preparation of **2aB** and their yields and physicochemical data are listed in Table 5.

N-[(2*S*,3*R*,4*R*,5*S*)-5-Amino-2,3,4,6-tetrahydroxyhexanoyl]-*β*-D-phenylalanine Diphenylmethyl Ester Hydrochloride (**4**)

N-[(2*S*,3*R*,4*R*,5*S*)-5-Amino-2,3,4,6-tetrahydroxyhexanoyl]-*β*-D-phenylalanine **3**[8] (3.40 g, 10.0 mmol) was dissolved in 1 N HCl (11 ml)-MeOH (10 ml). This solution was evaporated to dryness and the residue was dissolved in MeOH (100 ml). Diphenyldiazomethane (3.88 g, 20 mmol) was added to the solution and the mixture was stirred at room temperature for 1.5 hours, and then concentrated. The residue was washed with Et_2O to give **4** (5.28 g, 97%) as a colorless solid: mp 143~145°C; IR (KBr) 3266, 1721, 1667, 1528 cm^{-1}; ^{1}H-NMR (CD_3OD, TMS) δ 3.03 (1H, dd, J=6.0, 15.8 Hz), 3.15 (1H, dd, J=6.0, 15.8 Hz), 3.55~3.60 (1H, m), 3.70~3.95 (4H, m), 4.25~4.30 (1H, m), 5.45 (1H, t, J=6.0 Hz), 6.73 (1H, s), 7.10~7.35 (15H, m); $[\alpha]_D^{20}$ −84.8° (*c* 0.10, MeOH); *Anal* Calcd for $C_{28}H_{32}N_2O_7 \cdot 1.0HCl \cdot 0.5H_2O$: C 60.70, H 6.19, N 5.06. Found: C 60.97, H 5.94, N 5.06.

N-[(2*S*,3*R*,4*R*,5*S*)-5-[(*N*-*tert*-Butoxycarbonyl-L-norvalyl)-L-alanyl]amino-2,3,4,6-tetrahydroxyhexanoyl]-*β*-D-phenylalanine Diphenylmethyl Ester (**5aB**)

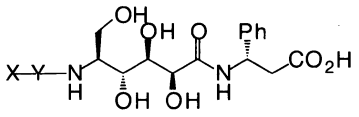
To a solution of *N*-(*N*-*tert*-butoxycarbonyl-L-norvalyl)-L-alanine (145 mg, 0.504 mmol), **4** (250 mg, 0.459 mmol) in DMF (3 ml) were added 1-ethyl-3-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (116 mg, 0.605 mmol), HOBt (82 mg, 0.605 mmol) and ethyldiisopropylamine (0.088 ml, 0.50 mmol) at 0°C. After being stirred at this temperature for 1 hour, the reaction mixture was warmed to room temperature and stirred for 18 hours. After removal of solvent under reduced pressure, the residue was extracted with EtOAc-H_2O. The organic layer was washed with 5% aqueous citric acid, saturated aqueous $NaHCO_3$ and brine, and then dried over Na_2SO_4 and concentrated. The residue was recrystallized from EtOAc to give **5aB** (281 mg, 79%) as a colorless solid.

Compounds **5aC**~**aF** were prepared by a similar procedure to that used for the preparation of **5aB**. Their yields and physicochemical data are listed in Table 6.

Determination of Minimum Inhibitory Concentrations (MICs)

The MICs were determined by an agar dilution method. Bacterial suspensions of approximately 10^6 cfu/ml were

Table 5. Physicochemical data of pyloricidin derivatives **2aA**~**aF**.

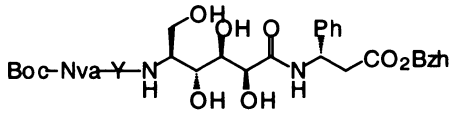


compd X Y	yield (%) mp(°C) solv.[a]	Formula	Analysis (%) Calcd (Found) C	H	N	^{1}H-NMR δ	IR (KBr) cm^{-1}	$[\alpha]_D$ (°C) c (solv)[e]
2aA	42	$C_{25}H_{38}N_4O_9$	53.95	7.24	10.07	0.86 (3H, t, J = 7.0Hz), 1.20–1.80 (4H, m), 2.20–2.90 (4H,m), 3.30–5.90	3300	-57.5°
Nva	142–143	•1.0H_2O	(53.68	7.38	10.25)	(12H, m),7.10–7.45 (5H, m),7.54 (1H, d, J =7.8Hz), 8.28 (1H, d, J=12.8Hz)[b]	1649	{23}
(allyl)Gly	M-EE						1530	0.10 (M)
2bA	43	$C_{26}H_{40}N_4O_9$	53.87	7.48	9.67	0.75–0.95 (6H, m),0.95–1.80 (3H, m),2.10–2.90 (4H, m),3.10–4.50 (8H, m),	3350	-55.6°
Ile	133–135	•1.5H_2O	(54.04	7.33	9.45)	4.95–5.90 (4H, m), 7.15–7.20 (5H, m)[b]	1660	{23}
(allyl)Gly	M-EE						1640	0.10 (M)
							1535	
2cA	47	$C_{25}H_{38}N_4O_9S$	51.01	6.85	9.52	2.10 (3H, s), 2.00–2.30 (2H, m), 2.40–2.80 (6H, m), 3.60–4.00 (4H, m),	3350	-56.2°
Met	127–128	•1.0H_2O	(50.83	6.78	9.20)	4.10–4.50 (4H, m),5.10–5.30 (2H, m),5.33 (1H, t, J=5.2Hz), 5.70–6.00 (1H,	1650	{23}
(allyl)Gly	M-EE					m), 7.20–7.50 (5H, m)[c]	1530	0.10 (M)
2dA	39	$C_{25}H_{36}N_4O_9$	54.14	6.91	10.10	2.40–2.74 (6H, m),3.50–3.75 (3H, m),3.80–3.90 (1H, m),3.98–4.10 (1H, m),	3300	-96.1°
(allyl)Gly	181–182	•1.0H_2O	(54.13	6.96	10.12)	4.14–4.24 (1H, m),4.26–4.32 (1H, m),4.34–4.48 (1H, m),5.05–5.30 (5H, m),	1645	{20}
(allyl)Gly	M-EE					5.60–5.80 (2H, m), 7.20–7.50 (5H, m)[d]	1522	0.10 (W)
2eA	50	$C_{24}H_{35}N_5O_{10}$	46.16	5.51	10.35	2.46–2.54 (2H, m), 2.79–3.02 (4H, m), 3.54–3.72 (3H, m), 3.84 (1H, d, J=	3300	-62.8°
Asn	132–134	•1.0TFA	(46.18	5.46	10.41)	10.0Hz), 4.17–4.44 (4H, m), 5.10–5.29 (3H, m), 5.70–5.73 (1H, m), 7.25–	1674	{25}
(allyl)Gly	M-EE	•0.5H_2O				7.35 (5H, m)[d]	1539	0.13 (W)
2fA	39	$C_{24}H_{36}N_4O_9S$	47.91	6.87	9.31	2.45–2.60 (2H,m),2.65–2.80 (5H, m),3.20–3.40 (2H, m),3.55–3.70 (3H, m),	3300	-65.3°
Cys(Me)	131–132	•2.5H_2O	(48.23	6.86	9.21)	3.75–3.90 (1H, m),4.15–4.26 (1H,m),4.28–4.32 (1H, m),4.35–4.50 (2H, m),	1651	{20}
(allyl)Gly	M-EE					5.10–5.25 (3H, m), 5.60–5.80 (1H, m), 7.20–7.45 (5H, m)[d]	1539	0.11 (W)
2aB	65	$C_{23}H_{36}N_4O_9$	47.96	7.00	9.73	0.85 (3H, t, J = 7.8Hz), 1.20–1.50 (2H, m), 1.40 (3H, d, J = 7.0Hz), 1.70–	3166	-53.2°
Nva	162–164	•1.0HCl	(48.26	6.73	9.53)	1.90 (2H, m), 2.91 (2H, d, J =6.6Hz), 3.60–4.50 (8H, m),5.20–5.35 (1H, m),	1661	{20}
Ala	M-EA	•1.5H_2O				7.20–7.45 (5H, m)[d]	1532	0.10 (W)
2aC	90	$C_{24}H_{38}N_4O_9$	52.93	7.40	10.29	0.80–1.00(6H, m), 1.20–1.45 (2H, m), 1.55–1.90 (4H, m), 2.68 (2H, d, J =	3300	-84.0 °
Nva	147–149	•1.0H_2O	(52.93	7.28	10.03)	7.0Hz), 3.52–3.74 (3H, m),3.81–4.00 (2H, m), 4.16–4.33 (3H, m),5.14 (1H,	1651	{20}
Abu	M-EA					t, J = 7.0Hz), 7.20–7.40 (5H, m)[d]	1531	0.10 (W)
2aD	93	$C_{25}H_{40}N_4O_9$	53.75	7.58	10.03	0.70–1.00 (6H, m), 1.20–1.40 (4H, m), 1.60–1.90 (4H, m), 2.92 (2H, d, J=	3300	-44.8°
Nva	159–160	•1.0H_2O	(54.03	7.65	9.82)	7.0Hz), 3.50–4.00 (8H, m), 5.20–5.35 (1H, m), 7.20–7.50 (5H, m)[d]	1659	{20}
Nva	M-EA						1534	0.10 (W)
2aE	92	$C_{25}H_{39}N_5O_{10}$	51.89	6.97	12.10	0.85 (3H, t, J=7.4Hz), 1.20–1.45 (2H, m), 1.70–2.10 (4H, m),2.20–2.40 (2H,	3281	-46.7°
Nva	150–151	•0.5H_2O	(51.83	7.04	11.83)	m), 2.92 (2H, d, J =7.0Hz), 3.50–3.75 (3H, m),3.80–3.90 (1H, m),3.90–4.05	1661	{20}
Gln	M-EA					(1H, m), 4.10–4.40 (3H, m), 5.20–5.35 (1H, m), 7.20–7.40 (5H, m)[d]	1534	0.10 (W)
2aF	92	$C_{25}H_{40}N_4O_9S$	50.83	7.17	9.49	0.86 (3H, t, J =7.5Hz), 1.20–1.50 (2H, m), 1.65–1.90 (2H, m), 1.95–2.30 (2H,	3300	-38.7°
Nva	143–144	•1.0H_2O	(50.90	7.04	9.29)	m), 2.04 (3H, s), 2.40–2.70 (2H, m), 2.93 (2H, d, J =6.6Hz), 3.50–4.35 (8H,	1659	{20}
Met	M-EA					m), 5.20–5.40 (1H, m), 7.20–7.50 (5H, m)[d]	1534	0.10 (W)

a) recrystallization solvent: M = methanol, EE = ethyl ether, EA = ethyl acetate, *b)* in DMSO-d_6, *c)* in CD_3OD, *d)* in D_2O, *e)* $[\alpha]_D$ values were measured in MeOH (M) or H_2O (W).

applied to the brucella agar plates supplemented with 7% horse blood containing twofold serial dilutions of test compounds using a multiinoculator delivering 5 μl samples. The plates were incubated at 37°C in a microaerobic atmosphere containing 5% O_2, 10% CO_2 and 85% N_2. MICs were defined as the lowest concentrations of the compounds preventing visible bacterial growth after four days of incubation.

Table 6. Physicochemical data of pyloricidin derivatives **5aB~aF**.



compd	Y yield (%) mp(°C)[a]	Formula	Analysis (%) Calcd (Found) C	H	N	^{1}H-NMR δ (CD_3OD)	IR (KBr) cm^{-1}	$[\alpha]_D^{b)}$
5aB	Ala 79 174–175	$C_{41}H_{54}N_4O_{11}$ •0.5H_2O	62.50 (62.49)	7.04 (7.00)	7.11 (7.24)	0.92 (3H, t, *J* = 7.4Hz), 1.38 (3H, d, *J* = 7.4Hz), 1.43 (9H, s), 1.50–1.80 (4H, m), 2.95–3.20 (2H, m), 3.60–3.75 (3H, m), 3.85–4.05 (2H, m), 4.10–4.25 (1H, m), 4.30–4.40 (2H, m), 5.44 (1H, t, *J* = 6.6Hz), 6.73 (1H, s), 7.10–7.40 (15H, m)	3339 1723 1663 1518	-45.4°
5aC	Abu 85 160–162	$C_{42}H_{56}N_4O_{11}$ •0.5H_2O	62.91 (63.04)	7.16 (7.03)	6.99 (7.13)	0.80–0.96 (6H, m), 1.20–1.30 (2H, m), 1.43 (9H, s), 1.58–1.88 (4H, m), 3.05 (1H, dd, *J* = 7.0, 15.6Hz), 3.10 (1H, dd, *J* = 7.0, 15.6Hz), 3.63–3.71 (3H, m), 3.88–4.04 (2H, m), 4.16–4.31 (3H, m), 5.43 (1H, t, *J* = 7.0Hz), 6.73 (1H, s), 7.15–7.30 (15H, m)	3300 1720 1650 1520	-46.9°
5aD	Nva 79 135–136	$C_{43}H_{58}N_4O_{11}$ •1.0H_2O	62.60 (62.84)	7.33 (6.98)	6.79 (6.55)	0.80–1.00 (6H, m), 1.40–1.90 (8H, m), 1.42 (9H, s), 2.95–3.20 (2H, m), 3.60–3.75 (3H, m), 3.85–4.05 (2H, m), 4.10–4.25 (1H, m), 4.30–4.40 (2H, m), 5.44 (1H, t, *J* = 6.0Hz), 6.73 (1H, s), 7.05–7.40 (15H, m)	3316 1723 1663 1518	-42.4°
5aE	Gln 77 111–112	$C_{43}H_{57}N_5O_{12}$ •0.5H_2O	61.12 (60.88)	6.92 (6.94)	8.29 (8.31)	0.92 (3H, t, *J* = 7.2Hz), 1.30–1.80 (4H, m), 1.43 (9H, s), 1.90–2.20 (2H, m), 2.25–2.45 (2H, m), 2.95–3.20 (2H, m), 3.60–3.75 (3H, m), 3.80–4.05 (2H, m), 4.15–4.25 (1H, m), 4.30–4.45 (2H, m), 5.44 (1H, t, *J* = 6.6Hz), 6.73 (1H, s), 7.05–7.40 (15H, m)	3287 1719 1647 1530	-43.0°
5aF	Met 67 123–124	$C_{43}H_{58}N_4O_{11}S$ •1.0H_2O	60.26 (60.39)	7.06 (6.82)	6.54 (6.41)	0.92 (3H, t, *J* = 7.4Hz), 1.40–1.80 (2H, m), 1.43 (9H, s), 1.85–2.30 (4H, m), 2.08 (3H, s), 2.40–2.70 (2H, m), 3.00–3.20 (2H, m), 3.60–3.75 (3H, m), 3.85–4.05 (2H, m), 4.15–4.25 (1H, m), 4.28–4.34 (1H, m), 4.40–4.55 (1H, m), 5.44 (1H, m), 6.73 (1H, s), 7.05–7.40 (15H, m)	3301 1721 1655 1512	-39.9°

a) recrystallization solvent is EtOAc, *b)* $[\alpha]_D$ values were measured in MeOH (*c* = 0.1) at 20°C.

In Vivo Clearance of *H. pylori* TN2GF4

Five-week-old MON/Jms/Gbs Slc Mongolian gerbils (SLC Japan Inc., Shizuoka, Japan) were inoculated intragastrically with $10^{7.61}$ cfu of *H. pylori* TN2GF4. Four weeks after infection, compound **2aC** was administered orally at a dose of 10 mg/kg twice a day for 7 days in the form of a solution in 0.5% aqueous methylcellulose. One day after administration of the final dose, the animals were killed and the stomachs were removed. Each stomach was homogenized with Brucella broth and serial dilutions were plated on modified Skirrow's medium. The plates were incubated for 4 days at 37°C under microaerobic conditions in GasPak jars. The viable cell counts for each gastric wall were calculated by counting the number of colonies on the agar plates.

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