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

I. Structure-activity Relationships on the Terminal Peptidic Moiety

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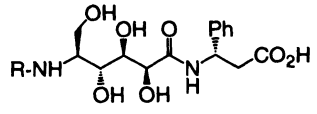
The novel natural antibiotics pyloricidin A, B and C possess potent and highly selective antibacterial activity against *Helicobacter pylori*. In order to investigate the structure activity relationships for the terminal peptidic moiety, a series of pyloricidin B and pyloricidin C derivatives, bearing various amino acids in the moiety, were prepared and evaluated for their anti-*H. pylori* activity. The derivatives bearing α-D-, β- and γ-amino acids or peptidemimetics showed drastically decreased activity. On the other hand, the derivatives with α-L-amino acids were found to maintain the activity. Among the derivatives prepared in this work, the allylglycine derivative **2s** showed the most potent anti-*H. pylori* activity, with an MIC value of less than 0.006 μg/ml against *H. pylori* NCTC11637, which is 60-fold greater than the activity of the lead compound pyloricidin C.

Helicobacter pylori, a Gram-negative bacterium inhabiting the mucus layer of human gastric epithelium, is regarded as a major cause of gastric and duodenal ulcers[1,2]. Therefore, eradication of this organism is effective to cure peptic ulcers. Although combination therapy with proton pump inhibitors and antibacterial agents is prevalent for the purpose of eradicating *H. pylori*[3~5], single agent therapy is preferable because of better patient compliance and fewer side effects[6,7]. It is therefore important to develop effective and safe anti-*H. pylori* agents. In the course of the search for anti-*H. pylori* agents, novel antibiotics, pyloricidin A, B and C, were isolated from the fermentation broth of *Bacillus* sp. HC-70[8]. Pyloricidin A, B and C possess unique structures 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) as shown in Fig. 1. In addition, pyloricidin A, B and C showed potent and highly selective

Fig. 1.



Pyloricidin A: R = L-Val-L-Val-L-Leu
Pyloricidin B: R = L-Val-L-Leu
Pyloricidin C: R = L-Leu

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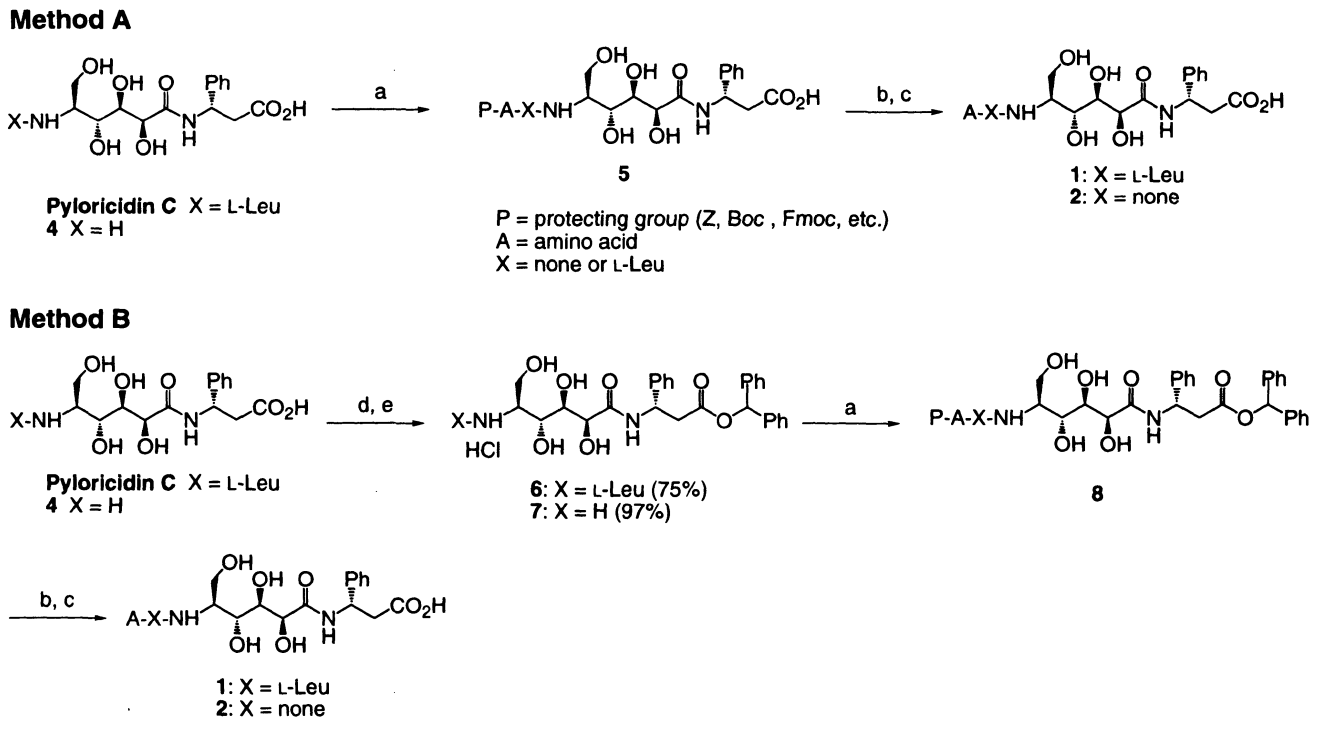
Scheme 1.[a]



[a] *Reagents and conditions* : (a) P-A-OSu or P-A-ONB, Et_3N, DMF; (b) deprotection (P = Boc, *tert*-Bu, 4N HCl/EtOAc or TFA; P = carbobenzoxy (Z), benzyl, H_2, 10% Pd/C; P = 9-fluorenylmethoxycarbonyl (Fmoc), piperidine); (c) purification by MCI gel column chromatography and recrystallization; (d) 1N HCl (1.1 equiv); (e) diphenyldiazomethane, MeOH.

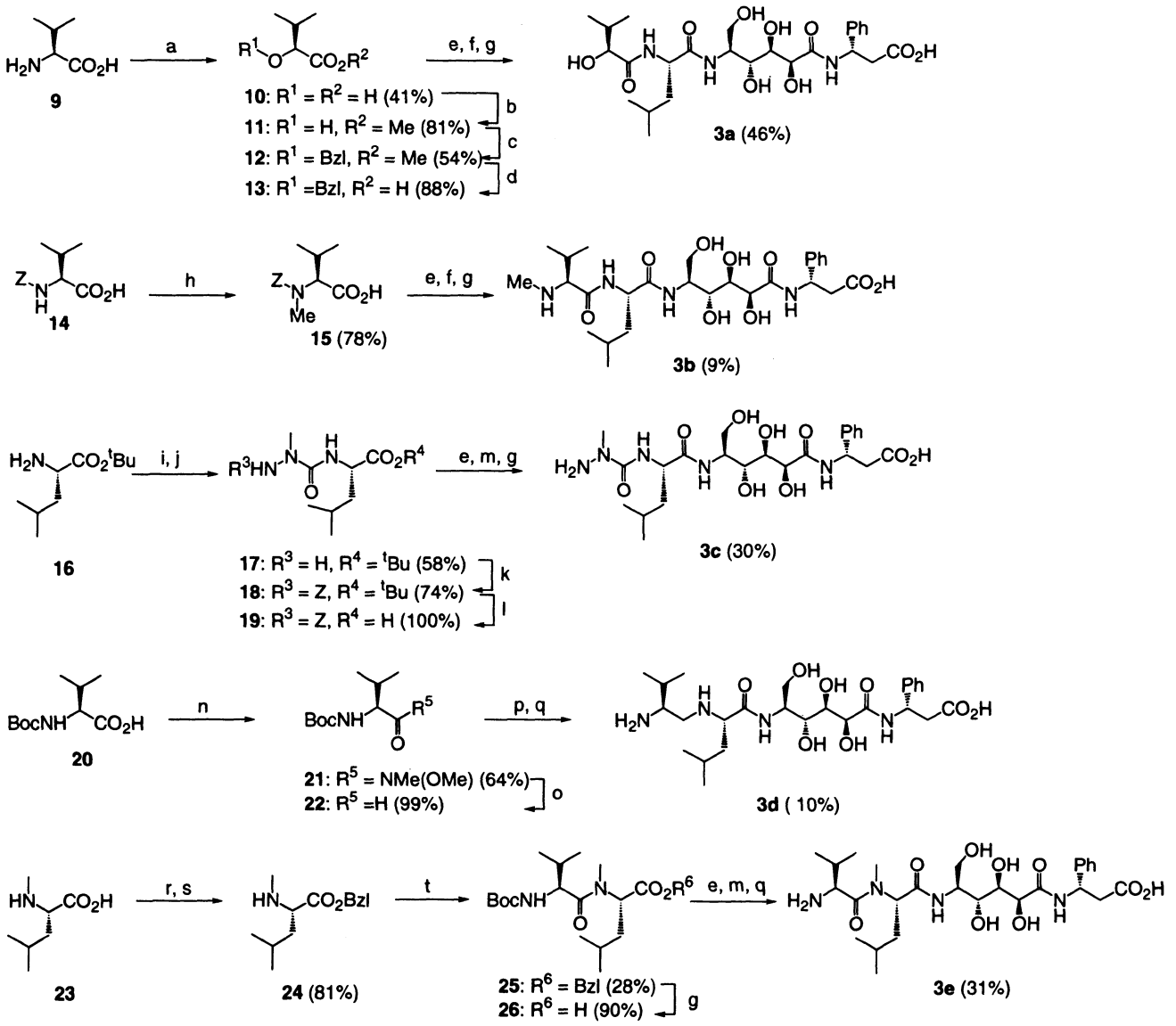
anti-*H. pylori* activity[9]. Therefore, we selected them as lead compounds for a therapeutically useful anti-*H. pylori* agent.

We have already investigated the structure activity relationships for the (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 of the pyloricidin antibiotics required to express anti-*H. pylori* activity[10]. Based on this finding, we speculated that the remaining peptidic moiety controls the potency of the activity and more potent derivatives might be obtained through its modification. In order to investigate the detailed structure activity relationships for the 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. In this paper, we describe the synthesis and structure activity relationships of pyloricidin derivatives modified at the terminal peptidic moiety.

Chemistry

The pyloricidin B derivatives **1** and the pyloricidin C derivatives **2**, bearing a variety of amino acids, were prepared by two general methods, as shown in Scheme 1. One method used direct condensation of the *N*-hydroxysuccinimide (HOSu) or *N*-hydroxy-5-norbornene-2,3-dicarboximide (HONB)[11] esters derived from protected amino acids (P-As) with pyloricidin C or (2*S*,3*R*,4*R*,5*S*)-5-amino-2,3,4,6-tetrahydroxyhexanoyl-β-D-phenylalanine **4**[8] (Method A). The other method featured the condensation of P-As with the benzhydryl esters **6** or **7** derived from pyloricidin C or **4** (Method B). Although both condensation reactions were carried out without protection of the hydroxyl groups, the formation of the *O*-acylated by-products was not observed. Deprotection of **5** or **8** and subsequent purification by column chromatography on MCI gel with acetonitrile-water as the eluent was followed by recrystallization to give the desired compound **1** or **2**.

Scheme 2.[a]



[a] *Reagents and conditions* : (a) $NaNO_2$, 2N H_2SO_4, H_2O; (b) concd H_2SO_4, MeOH, reflux; (c) benzyl 2,2,2-trichloroacetimidate, trifluoromethanesulfonic acid; (d) 1N NaOH, MeOH; (e) HOSu, DCC; (f) **Pyloricidin C**, Et_3N; (g) H_2, 10% Pd/C, MeOH; (h) MeI, NaH, THF; (i) chloro 4-nitrophenylformate, 4-(dimethylamino)pyridine (DMAP), CH_2Cl_2; (j) methylhydrazine; (k) carbobenzoxy chloride (Z-Cl), DMAP, CH_2Cl_2; (l) TFA; (m) **4**, Et_3N, DMF; (n) MeNH(OMe)•HCl, benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate, Et_3N, MeCN; (o) $LiAlH_4$, diethyl ether; (p) **6**, $NaBH_3CN$, MeOH; (q) 4N HCl/EtOAc; (r) benzyl alcohol, *p*-toluenesulfonic acid, toluene, reflux; (s) 1N NaOH; (t) Boc-L-Val, diethyl cyanophosphonate, Et_3N, DMF.

A series of derivatives (**3a**~**e**) bearing peptide mimetics were prepared as shown in Scheme 2. The (*S*)-hydroxy isovaleric acid derivative **3a** was prepared by the condensation of pyloricidin C with (*S*)-benzyloxy isovaleric acid **13**, which was derived from L-valine according to the method of Li *et al.*[12], and followed by deprotection of the benzyl group. *N*-Methyl-L-valine derivative **15** was coupled with pyloricidin C, followed by removal of the carbobenzoxy (Z) group to give **3b**. The azadipeptide[13] **18** was prepared by the successive treatment of the L-leucine

tert-butyl ester **16** with chloro 4-nitrophenylformate and methylhydrazine, followed by protection of the amino group. Removal of the *tert*-butyl group of **18** with TFA gave **19**, which was condensed with **4** and deprotected to give the azapeptide derivative **3c**. Reductive amination of the aldehyde **22**[14] derived from Boc-L-valine **20** with **6** and subsequent removal of the Boc and benzhydryl protecting groups gave the pseudo peptide derivative **3d**. The benzyl ester **24** of *N*-methyl-L-leucine was condensed with Boc-L-valine to give **25**, which was converted to **26** by catalytic hydrogenation. The carboxylic acid **26** was condensed with **4**, followed by removal of the Boc group to give the *N*-methyl-L-leucine derivative **3e**.

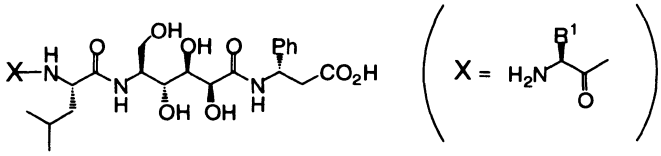
Results and Discussion

In the previous evaluation of the anti-*H. pylori* activity of natural pyloricidins, pyloricidin B was found to have strong activity[9]. Therefore, we initially focused on pyloricidin B and examined structure activity relationships for its terminal peptidic moiety. Table 1 shows the minimum inhibitory concentrations (MICs) of the pyloricidin B derivatives, bearing various amino acids or peptidemimetics in the terminal position, against four strains of *H. pylori* (NCTC11637, CPY433, TN2 and TN58). As reference, the activity of pyloricidin B is also listed. Introduction of D-amino acids (D-Ala and D-Val, **1a** and **1b**), β-alanine (**1c**) and γ-aminobutyric acid (**1d**) resulted in remarkable decrease of the activity. On the other hand, replacement of the terminal L-valine with other natural α-L-amino acids (**1e**~**q**) was found to maintain the activity. Among the derivatives tested, the alanine derivative **1f**, leucine derivative **1g**, isoleucine derivative **1h**, methionine derivative **1k**, asparagine derivative **1m** and glutamine derivative **1n** showed comparable activity against the four strains to that of pyloricidin B. Whereas, the derivatives with basic or acidic amino acids (Orn (**1o**), Lys (**1p**) and Glu (**1q**)) showed weak activity compared with pyloricidin B. In addition, the glycine derivative **1e**, phenylalanine derivative **1i**, proline derivative **1j** and serine derivative **1l** also showed decreased activity. These findings suggested that the activity was closely associated with the terminal amino acids and the structure of α-side chains (R^1s) significantly affected the potency of the activity; alkyl groups or alkylamido groups seemed to promote anti-*H. pylori* activity. For optimization of the length of R^1, a series of derivatives **1r**~**t**, bearing unnatural amino acids with linear alkyl side chains ranging from C_2 to C_4, were prepared. It was clear that the activity of these derivatives was variable depending on the length of R^1 and the norvaline derivative (**1s**, R^1=propyl) displayed more than 2-fold greater activity than that of pyloricidin B. Based on this finding, we synthesized the derivatives, bearing linear side chains of 2~3 carbon atoms containing sulfur, oxygen, fluoro and cyano, and compared their activity with **1s**. Changing of propyl group into allyl (**1u**), $-CH_2SMe$ (**1v**), or $-CH_2OMe$ (**1w**) groups resulted in slightly decreased activity. However, the derivatives containing an electron withdrawing group, such as fluoro (**1x**) or cyano (**1y**), in the side chains showed significantly less potent activity than that of **1s**.

Subsequently, we examined the anti-*H. pylori* activity of a series of derivatives containing peptide mimetic moieties in the terminal position. Modification of L-valine by conversion into (*S*)-hydroxy isovaleric acid **3a** or by methylation (**3b**), resulted in a decrease of activity, which suggested that presence of the terminal NH_2 group is important for the activity. Azapeptides are peptidemimetics derived by replacement of an α-CH group in a peptide chain with a nitrogen atom. Compound **3c**, which is an azapeptide analogue of the alanine derivative **1f**, did not show anti-*H. pylori* activity. Furthermore, modification at the peptide linkage by removal of the carbonyl oxygen (**3d**), or methylation on amide nitrogen (**3e**) also lowered the activity, which might be attributed to the conformational changes resulting from modification on the dipeptide framework. From these results, it is assumed that the structure of the terminal peptidic moiety, including the terminal NH_2 group, α-CH group and amido linkage, must be important to express anti-*H. pylori* activity.

Finally, we examined the anti-*H. pylori* activity of the pyloricidin C derivatives in which the terminal L-leucine was replaced with various amino acids (Table 2). Because pyloricidin C possesses only L-leucine in the terminal position, pyloricidin C was regarded as a suitable compound for studying the relationship between the terminal amino acids and the anti-*H. pylori* activity. Similar to the pyloricidin B derivatives, introduction of D-leucine (**2a**) or γ-aminobutyric acid (**2b**) resulted in decrease of activity. Of the derivatives possessing natural amino acids (**2c**~**k**), the valine derivative **2d**, isoleucine derivative **2e**, proline derivative **2g**, ornithine derivative **2j** and glutamic acid derivative **2k** showed weak activity, which suggests that the presence of bulky or polar groups in the α-side chain R^2 is unfavorable for the activity. A series of derivatives **2l**~**s**, with linear side chains, showed a similar trend in their activity to that of pyloricidin B derivatives; the side chains of 2~3 carbon atoms were favorable and the introduction of a fluoro atom reduced activity. Surprisingly,

Table 1. Antibacterial activity of pyloricidin B derivatives against 4 strains of *H. pylori*.

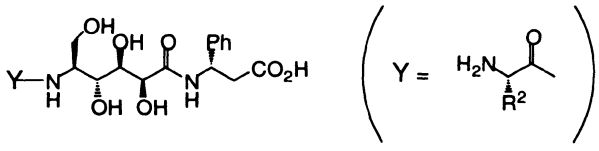


Compound	X(R^1)	MIC (μg / ml)[a]			
		NCTC11637	CPY433	TN2	TN58
Pyloricidin B	Val	0.05	0.39	0.2	0.1
1a	D-Ala	3.13	6.25	>6.25	6.25
1b	D-Val	50	>6.25	>6.25	>6.25
1c	β-Ala	25	>6.25	>6.25	>6.25
1d	GABA[b]	6.25	>6.25	>6.25	6.25
1e	Gly	0.39	0.78	3.13	0.78
1f	Ala	0.1	0.1	0.2	0.1
1g	Leu	0.1	0.39	0.2	0.1
1h	Ile	0.05	0.2	0.1	0.1
1i	Phe	0.2	0.78	1.56	0.39
1j	Pro	0.1	0.78	0.78	0.78
1k	Met	0.1	0.2	0.1	0.1
1l	Ser	0.2	0.78	0.39	0.39
1m	Asn	0.1	0.39	0.2	0.1
1n	Gln	0.1	0.39	0.39	0.2
1o	Orn	0.39	0.2	1.56	0.2
1p	Lys	0.2	0.78	0.78	0.39
1q	Glu	0.2	1.56	0.78	0.78
1r	Abu[c] (Et)	0.05	0.2	0.2	0.1
1s	Nva[d] (Pr)	0.025	0.1	0.05	0.025
1t	Nle[e] (*n*-Bu)	0.2	0.78	0.39	0.2
1u	($CH_2CH{=}CH_2$)	0.05	0.2	0.1	0.05
1v	(CH_2SMe)	0.05	0.2	0.1	0.1
1w	(CH_2OMe)	0.05	0.2	0.1	0.05
1x	($CH_2CH_2CF_3$)	0.39	1.56	1.56	0.39
1y	(CH_2CN)	0.39	1.56	0.78	0.39
3a	*f*	3.13	>6.25	3.13	3.13
3b	*f*	1.56	6.25	>6.25	3.13
3c	*f*	>6.25	>6.25	>6.25	>6.25
3d	*f*	>6.25	>6.25	>6.25	6.25
3e	*f*	6.25	>6.25	>6.25	6.25

[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] γ-aminobutyryl, [c] (2*S*)-2-aminobutyryl, [d] norvalyl [(2*S*)-2-aminopentanoyl], [e] norleucyl [(2*S*)-2-aminohexanoyl], [f] See Scheme 2.

the allylglycine derivative **2s** exhibited an excellent anti-*H. pylori* activity with an MIC value of less than 0.006 μg/ml against *H. pylori* NCTC11637, which was 60-fold greater than that of pyloricidin C. These results prompted us to prepare a series of derivatives **2t**~**w** with analogous α-side chains to the allyl group. A shift of the double bond (**2t**) reduced activity, and the introduction of a methyl group (**2u**) resulted in about 20-fold decreased activity against NCTC11637 compared to **2s**. Replacement of the double bond with a triple bond (**2v**) had little effect on the activity,

Table 2. Antibacterial activity of pyloricidin C derivatives against 4 strains of *H. pylori*.



Compound	Y (R^2)	MIC (μg / ml)[a]			
		NCTC11637	CPY433	TN2	TN58
Pyloricidin C	Leu	0.39	3.13	0.78	1.56
2a	D-Leu	>6.25	>6.25	>6.25	6.25
2b	GABA[b]	>6.25	>6.25	>6.25	6.25
2c	Ala	0.2	1.56	0.78	0.39
2d	Val	0.78	6.25	3.13	1.56
2e	Ile	6.25	>6.25	>6.25	3.13
2f	Phe	0.39	3.13	0.78	0.78
2g	Pro	>6.25	>6.25	>6.25	3.13
2h	Met	0.78	0.78	0.39	0.1
2i	Gln	0.05	1.56	0.39	0.1
2j	Orn	6.25	>6.25	>6.25	3.13
2k	Glu	6.25	>6.25	6.25	1.56
2l	Abu[c] (Et)	0.025	0.39	0.39	0.025
2m	Nva[d] (Pr)	0.05	0.39	0.39	0.78
2n	Nle[e] (*n*-Bu)	0.2	1.56	0.78	0.2
2o	($CH_2CH_2CH_2F$)	0.78	>6.25	3.13	1.56
2p	($CH_2CH_2CF_3$)	0.78	1.56	0.78	0.2
2q	(CH_2OCH_3)	0.1	0.39	0.78	0.05
2r	(CH_2SCH_3)	0.013	0.78	0.39	0.025
2s	($CH_2CH{=}CH_2$)	<0.006	0.1	0.025	0.025
2t	($CH_2{=}CHCH_3$) *trans*	0.025	0.78	0.2	0.05
2u	($CH_2C(Me){=}CH_2$)	0.1	3.13	0.78	0.2
2v	($CH_2C{\equiv}CH$)	0.013	0.1	0.1	0.025
2w	($CH_2C{\equiv}N$)	3.13	3.13	3.13	0.39

[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] γ-aminobutyryl, [c] (2*S*)-2-aminobutyryl, [d] norvalyl [(2*S*)-2-aminopentanoyl], [e] norleucyl [(2*S*)-2-aminohexanoyl].

but conversion of the triple bond into a nitrile group (**2w**) drastically decreased activity. From these observations, it was concluded that the activity is closely associated with the steric bulkiness and electronic nature of the α-side chain R^2.

Conclusion

In order to investigate the structure activity relationships for the terminal peptidic moiety, a series of pyloricidin B and 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 we speculated, the activity was closely associated with the terminal peptidic moiety. The derivatives bearing α-D-, β- and γ-amino acids or peptide mimetics, showed drastically decreased activity, while derivatives with α-L-amino acids in place of L-valine or L-leucine were found to maintain the activity. Among them, the allylglycine derivative **2s** showed the most potent anti-*H. pylori* activity, with an MIC value of less than 0.006 μg/ml against *H. pylori* NCTC11637.

Table 3. Physicochemical data of pyloricidin derivatives **1a**~**y**.

Compd	amino acid method yield(%)	mp(°C) solv.[β]	Formula	Analysis (%) Calcd (Found) C	H	N	^{1}H-NMR δ	IR (KBr) cm^{-1}	$[\alpha]_D$ (°C) c (solv)
1a	Boc-D-Ala A 66	205 M-EA	$C_{24}H_{38}N_4O_9$ •1.0HCl •0.2H_2O	50.87 (50.84	7.01 7.06	9.89 9.64)	0.92–1.01 (6H, m), 1.51 (3H, d, J=7.0Hz), 1.58–1.73 (3H, m), 2.66–2.71 (2H, m), 3.60–3.74 (2H, m), 3.91–4.02 (2H, m), 4.09–4.36 (4H, m), 5.33 (1H, m), 7.25–7.40 (5H, m)[g]	3216 1726 1655	-82.0° {20} 1.0 (H_2O)
1b	Z-D-Val B 44	208–209 M-EE	$C_{26}H_{42}N_4O_9$ •1.0H_2O	54.53 (54.19	7.74 7.51	9.78 9.92)	0.93 (3H, d, J = 6.0Hz), 1.00 (3H, d, J = 6.0Hz), 1.06 (3H, d, J = 7.0Hz), 1.11 (3H, d, J = 7.0Hz), 1.60–1.80 (3H, m), 2.00–2.30 (1H, m), 2.59 (1H, dd, J = 9.8, 13.6Hz), 2.71 (1H, dd, J = 4.8, 13.6Hz), 3.55–3.90 (4H, m), 4.00–4.30 (4H, m), 5.35 (1H, dd, J = 4.8, 9.8Hz), 7.15–7.45 (5H, m)[g]	3390 1642 1550	-140.1° {25} 0.10 (MeOH)
1c	Z-β-Ala A 72	220–221 M-W	$C_{24}H_{38}N_4O_9$ •2.5H_2O	50.43 (50.52	7.58 7.42	9.80 9.82)	0.80–1.00 (6H, m), 1.54–1.74 (3H, m), 2.64–2.74 (4H, m), 3.21–3.25 (2H, m), 3.71–3.80 (2H, m), 3.83–3.93 (2H, m), 4.15 (1H, m), 4.24–4.31 (2H, m), 5.36 (1H, t, J = 6.6Hz), 7.21–7.40 (5H, m)[g]	3245 1651 1537	-84.7° {20} 0.2 (H_2O)
1d	Z-GABA[a] A 67	158–160 M-EA	$C_{25}H_{40}N_4O_9$ •2.5H_2O	51.27 (50.92	7.74 7.73	9.57 9.84)	0.92 (3H, d, J=5.8Hz), 0.96 (3H, d, J=6.0Hz), 1.61–1.80 (3H, m), 1.91–2.01 (2H, m), 2.32 (1H, m), 2.46 (1H, q, J=5.8Hz), 2.65 (2H, d, J=7.0Hz), 2.92–3.04 (2H, m), 3.66–3.78 (3H, m), 3.89 (1H, d, J=9.6Hz), 4.15 (1H, t, J= 6.2Hz), 4.29 (2H, m), 5.32 (1H, t, J = 7.0Hz), 7.20–7.40 (5H, m)[g]	3264 1651 1534	-73.0° {20} 0.15 (H_2O)
1e	Z-Gly B 62	165–166 M-EE	$C_{23}H_{36}N_4O_9$ •1.5H_2O	51.20 (51.27	7.29 7.30	10.38 10.21)	0.94 (3H, d, J=6.2Hz), 0.99 (3H, d, J=6.2Hz), 1.10–1.85 (3H, m), 2.66 (2H, d, J = 7.6Hz), 3.50–4.40 (9H, m), 5.33 (1H, t, J = 7.6Hz), 7.10–7.45 (5H, m)[g]	3350 1645 1559	-98.3° {24} 0.10 (MeOH)
1f	Z-Ala A 62	160–162 M-EA	$C_{24}H_{38}N_4O_9$ •1.5H_2O	52.07 (51.87	7.46 7.72	10.12 10.22)	0.93–1.00 (6H, m), 1.55 (3H, d, J = 7.4Hz), 1.60–1.80 (3H, m), 2.68 (2H, d, J = 6.6Hz), 3.65–3.72 (3H, m), 3.86–3.93 (2H, m), 4.15–4.36 (3H, m), 5.31 (1H, t, J= 6.6Hz), 7.22–7.41 (5H, m)[g]	3237 1651 1532	-75.2° {20} 1.0 (H_2O)
1g	Z-Leu A 37	156–157 M-W	$C_{27}H_{44}N_4O_9$ •1.5H_2O	54.44 (54.48	7.95 8.05	9.41 9.21)	0.80–1.00 (12H, m), 1.00–1.90 (6H, m), 2.60–2.80 (2H, m), 3.20–5.30 (9H, m), 7.10–7.60 (5H, m)[g]	3376 1688 1636 1520	-54.7° {24} 0.10 (DMSO)
1h	Z-Ile A 37	152–153 M-W	$C_{27}H_{44}N_4O_9$ •1.8H_2O	53.95 (54.02	7.98 8.18	9.32 9.22)	0.70–1.80 (18H, m), 2.60–2.90 (2H, m), 3.00–5.30 (9H, m), 7.10–7.50 (5H, m)[g]	3378 1686 1630 1518	-89.1° {24} 0.10 (H_2O)
1i	Z-Phe A 74	135–138 M-EA	$C_{30}H_{42}N_4O_9$ •2.0H_2O	56.41 (56.42	7.26 7.15	8.77 8.83)	0.94 (3H, d, J=6.4Hz), 0.97 (3H, d, J=7.8Hz), 1.60–1.80 (3H, m), 2.72 (2H, d, J=6.6Hz), 3.05 (1H, dd, J=8.0, 14.4Hz), 3.30 (1H, dd, J = 4.2, 14.4Hz), 3.66–3.78 (3H, m), 3.91 (1H, m), 4.02 (1H, m), 4.22 (1H, t, J =6.6Hz), 4.30–4.36 (2H, m), 5.33 (1H, t, J = 6.6Hz), 7.20–7.39 (10H, m)[g]	3214 1649 1526	-46.9° {20} 1.0 (H_2O)
1j	Z-Pro B 22	171–172 M-W	$C_{26}H_{40}N_4O_9$ •1.0H_2O	54.72 (54.69	7.42 7.57	9.82 9.63)	0.80–1.00 (6H, m), 1.40–2.20 (7H, m), 2.60–5.30 (13H, m), 7.15–7.45 (5H, m), 7.59 (1H, d, J=8.8Hz), 8.15–8.30 (2H, m)[h]	3340 1638 1541	-122.6° {25} 0.10 (H_2O)
1k	Boc-Met A 34	190–191 M-EA	$C_{26}H_{42}N_4O_9S$ •3.0H_2O	48.74 (48.61	7.55 7.40	8.74 8.82)	0.84–0.91 (6H, m), 1.45–1.99 (5H, m), 2.03 (3H, s), 2.47–2.56 (2H, m), 2.69–2.75 (2H, m), 3.40–4.00 (6H, m), 4.13 (1H, s), 4.37 (1H, m), 5.21 (1H, m), 7.21–7.38 (5H, m), 7.49 (1H, d, J = 8.8Hz), 8.18 (1H, m)[h]	3362 1684 1624 1570	-47.8° {23} 0.10 (DMSO)
1l	Z-Ser(Bzyl) A 17	159–160 M-EE	$C_{24}H_{38}N_4O_{10}$ •2.0H_2O	49.82 (49.73	7.32 7.36	9.68 9.71)	0.94 (3H, d, J=5.8Hz), 0.98 (3H, d, J=5.8Hz), 1.60–1.85 (3H, m), 2.67 (2H, d, J = 6.6Hz), 3.50–4.50 (10H, m), 5.33 (1H, t, J = 6.6Hz), 7.15–7.50 (5H, m)[g]	3300 1645 1550	-86.3° {23} 0.10 (MeOH)
1m	Z-Asn A 46	150–155 M-EA	$C_{25}H_{39}N_5O_{10}$ •1.0H_2O	51.10 (51.28	7.03 7.19	11.92 11.95)	0.92–0.99 (6H, m), 1.60–1.80 (3H, m), 2.69 (2H, d, J=7.0Hz), 2.80 (1H, dd, J= 7.4, 16.8Hz), 2.95 (1H, dd, J =5.2, 16.8Hz), 3.67–3.75 (3H, m), 3.88 (1H, m), 4.07–4.22 (2H, m), 4.30–4.40 (2H, m), 5.33 (1H, t, J = 7.0Hz), 7.20–7.41 (5H, m)[g]	3300 1661 1539	-71.2° {20} 0.10 (H_2O)

Table 3. Continued

Compd	amino acid method yield(%)	mp(°C) solv.[f]	Formula	Analysis (%) Calcd (Found) C	H	N	^{1}H-NMR δ	IR (KBr) cm^{-1}	$[\alpha]_D$ {°C} c (solv)
1n	Z-Gln A 43	125–128 M-EA	$C_{26}H_{41}N_5O_{10}$ •1.0H_2O	51.90 (51.65	7.20 7.13	11.64 11.34)	0.93–1.02 (6H, m), 1.60–1.80 (3H, m), 2.10–2.20 (2H, m), 2.48–2.60 (2H, m), 2.91–2.98 (2H, m), 3.68–4.55 (8H, m), 5.42 (1H, m), 7.29–7.37 (5H, m)[g]	3200 1732 1667 1532	-75.3° {22} 0.10 (H_2O)
1o	Z-Orn(Z) A 60	157–158 M-EA	$C_{26}H_{43}N_5O_9$ •1.0H_2O	53.14 (53.34	7.72 7.75	11.92 11.81)	0.92–0.98 (6H, m), 1.63–1.72 (7H, m), 2.65 (2H, d, J=6.6Hz), 2.90–2.92 (2H, m), 3.44 (1H, m), 3.65–3.75 (3H, m), 3.88 (1H, dd, J=1.8, 9.8Hz), 4.19 (1H, dt, J=1.8, 6.2Hz), 4.31 (1H, d, J=1.2Hz), 4.45 (1H, t, J=7.4Hz), 5.32 (1H, t, J=6.6Hz), 7.20–7.41 (5H, m)[g]	3300 1649 1532	-80.8° {20} 0.10 (H_2O)
1p	Z-Lys(Boc) A 70	133–134 M-EA	$C_{27}H_{45}N_5O_9$ •2.0HCl •3.5H_2O	45.06 (45.04	7.56 7.50	9.73 9.60)	0.97 (3H, d, J=6.0Hz), 1.00 (3H, d, J=6.0Hz), 1.40–2.00 (10H, m), 2.74 (1H, m), 2.93–2.99 (2H, m), 3.61–3.73 (3H, m), 3.84–4.00 (2H, m), 4.22–4.46 (3H, m), 5.37 (1H, m), 7.23–7.41 (5H, m)[g]	3300 1678 1532	-48.5° {20} 1.0 (MeOH)
1q	Z-Glu(^{t}Bu) A 61	140–143 M-EA	$C_{26}H_{40}N_4O_{11}$ •2.0H_2O	50.32 (50.09	7.15 6.89	9.03 9.19)	0.70 (3H, d, J=6.2Hz), 0.74 (3H, d, J=8.0Hz), 1.38–1.60 (3H, m), 1.70–2.05 (2H, m), 2.21–2.35 (2H, m), 2.40–2.64 (2H, m), 3.44–3.47 (3H, m), 3.63–3.68 (2H, m), 3.97 (1H, t, J=7.6Hz), 4.09–4.15 (2H, m), 5.12 (1H, m), 6.98–7.17 (5H, m)[g]	3241 1659 1535	-46.9° {20} 1.0 (MeOH)
1r	Boc-Abu[b] A 57	151 M-EA	$C_{25}H_{40}N_4O_9$ •1.0HCl •0.5EtOAc	52.21 (52.13	7.30 7.08	9.02 9.32)	0.94–1.09 (9H, m), 1.62–1.72 (3H, m), 1.84–1.98 (2H, m), 2.69 (2H, d, J=6.4Hz), 3.68–3.83 (3H, m), 4.19–4.43 (5H, m), 5.32 (1H, t, J=6.4Hz), 7.22–7.43 (5H, m)[g]	3252 1657 1530	-80.8° {20} 0.1 (H_2O)
1s	Z-Nva[c] A 37	152 M	$C_{26}H_{42}N_4O_9$ •3.0H_2O	51.30 (51.23	7.95 7.98	9.20 9.06)	0.93–1.02 (9H, m), 1.44–1.52 (2H, m), 1.62–1.74 (3H, m), 1.81–1.90 (2H, m), 2.69 (2H, d, J=6.2Hz), 3.68–3.83 (3H, m), 4.18–4.47 (5H, m), 5.32 (1H, t, J=6.2Hz), 7.19–7.43 (5H, m)[g]	3248 1657 1532	-64.5° {20} 0.1 (H_2O)
1t	Boc-Nle[d] A 74	145–146 M-EA	$C_{27}H_{43}N_4O_9$ •1.0HCl •1.5H_2O	51.30 (51.52	7.65 7.41	8.86 8.83)	0.91–1.00 (9H, m), 1.37–1.50 (4H, m), 1.63–1.77 (3H, m), 1.87–1.94 (2H, m), 2.82–2.86 (2H, m), 3.64–3.72 (2H, m), 3.85–3.95 (2H, m), 4.09–4.23 (2H, m), 4.30–4.41 (2H, m), 5.37 (1H, t, J=6.6Hz), 7.23–7.42 (5H, m)[g]	3258 1657 1530	-64.8° {20} 0.1 (H_2O)
1u	Boc-L-allylglycine A 21	148–149 M-EA	$C_{26}H_{40}N_4O_9$ •2.5H_2O	52.25 (52.01	7.59 7.51	9.37 9.40)	0.84–0.90 (6H, m), 1.43–1.64 (3H, m), 2.12–2.48 (2H, m), 2.69–2.75 (2H, m), 3.40–4.09 (6H, m), 4.13 (1H, s), 4.38 (1H, m), 5.00–5.28 (3H, m), 5.76 (1H, m), 7.21–7.40 (5H, m), 7.49 (1H, d, J=8.6Hz), 8.13 (1H, d, J=7.6Hz), 8.28 (1H, d, J=10.8Hz)[h]	3368 1686 1628	-50.2° {20} 0.10 (H_2O)
1v	Boc-Cys(Me) A 34	140–141 W	$C_{25}H_{40}N_4O_9S$ •2.5H_2O	48.61 (48.56	7.34 7.01	9.07 8.81)	0.84–0.91 (6H, m), 1.46–1.73 (3H, m), 2.05 (3H, s), 2.55–2.88 (4H, m), 3.40–4.09 (6H, m), 4.13 (1H, s), 4.38 (1H, m), 5.22 (1H, m), 7.21–7.38 (5H, m), 7.50 (1H, d, J=8.4Hz), 8.14–8.25 (2H, m)[h]	3376 1688 1630	-68.4° {23} 0.10 (DMSO)
1w	Z-Ser(Me) A 41	141–142 M-EA	$C_{25}H_{40}N_4O_{10}$ •1.5H_2O	51.45 (51.60	7.43 7.22	9.60 9.80)	0.85 (3H, d, J=6.0Hz), 0.88 (3H, d, J=6.0Hz), 1.40–1.70 (3H, m), 2.70–2.80 (2H, m), 3.25 (3H, s), 3.10–5.30 (10H, m), 7.10–7.60 (5H, m), 8.00–8.40 (2H, m)[h]	3374 1686 1644 1530	-93.9° {23} 0.10 (DMSO)
1x	**27**[e] A 20	126–127 M-EA	$C_{26}H_{39}N_4O_9F_3$ •1.5H_2O •0.5EtOAc	49.48 (49.61	6.82 6.83	8.24 8.37)	0.75–0.81 (6H, m), 1.42–1.60 (3H, m), 1.98–2.22 (4H, m), 2.59 (2H, d, J=6.6Hz), 3.43–3.59 (3H, m), 3.73–3.95 (2H, m), 4.08–4.36 (3H, m), 5.06 (1H, t, J=6.6Hz), 7.15–7.35 (5H, m)[i]	3289 1657 1531	-63.7° {20} 0.50 (H_2O)
1y	Boc-Ala(CN) A 16	127–130 M-EA	$C_{25}H_{37}N_5O_9$ •1.0H_2O	52.72 (52.69	6.90 6.82	12.30 12.25)	0.93–0.99 (6H, m), 1.61–1.82 (3H, m), 2.74–2.95 (4H, m), 3.62–3.92 (5H, m), 4.17–4.46 (3H, m), 5.37 (1H, t, J=6.6Hz), 7.26–7.38 (5H, m)[g]	3287 1653 1530	-70.8° {20} 0.10 (H_2O)

a) GABA = γ-aminobutyric acid, *b)* Abu = (2*S*)-2aminobutyric acid, *c)* Nva = L-norvaline, *d)* Nle = L-norleucine, *e)* structure of protected amino acids **27** is (2*S*)-2-(benzyloxycarbonylamino)-5,5,5-trifluoropentanoic acid, *f)* recrystallization solvent: M = methanol, EE = ethyl ether, EA = ethyl acetate, W = water, *g)* in CD_3OD, *h)* in DMSO-d_6, *i)* in D_2O.

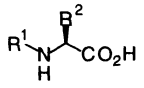
Table 4. Physicochemical data of pyloricidin derivatives **2a**~**w**.

Compd	amino acid method yield(%)	mp(°C) solv.[b]	Formula	Analysis (%) Calcd (Found) C	H	N	^{1}H-NMR δ	IR (KBr) cm^{-1}	$[\alpha]_D$ {°C} c (solv)
2a	Z-D-Leu A 8	149–150 M-EE	$C_{21}H_{33}N_3O_8$ •2.8H_2O	49.85 (49.88	7.69 7.55	8.31 8.13)	0.90–1.10 (6H, m), 1.60–1.85 (3H, m), 2.65 (2H, d, J=5.8Hz), 3.60–3.95 (5H, m), 4.30–4.40 (2H, m), 5.32 (1H, t, J=5.8Hz), 7.10–7.50 (5H, m)[g]	3350 1645 1560	-81.7° {25} 0.10 (MeOH)
2b	Z-GABA[a] A 34	147–150 M-EA	$C_{19}H_{29}N_3O_8$ •1.5H_2O	50.21 (50.55	7.10 6.67	9.25 9.42)	1.74–1.90 (2H, m), 2.27–2.35 (2H, m), 2.58 (2H, d, J=6.9Hz), 2.80–2.91 (2H, m), 3.46–3.76 (4H, m), 4.11–4.22 (2H, m), 5.06 (1H, t, J = 6.9Hz), 7.22–7.26 (5H, m)[i]	3300 1645 1539	-80.3° {20} 0.11 (H_2O)
2c	Z-Ala A 64	168–169 M-EE	$C_{18}H_{27}N_3O_8$ •1.0H_2O •0.5Et_2O	51.27 (51.64	7.31 7.24	8.97 9.36)	1.20 (3H, d, J=7.0Hz), 2.60–2.80 (2H, m), 3.30–4.20 (7H, m), 5.10–5.30 (1H, m), 7.70–7.90 (5H, m), 8.30–8.50 (1H, m)[h]	3300 1647 1535	-87.7° {25} 0.10 (DMSO)
2d	Z-Val B 36	147–155 M-EA	$C_{20}H_{31}N_3O_8$ •1.0H_2O	52.28 (52.18	7.24 7.36	9.14 9.13)	0.85–1.10 (6H, m), 2.10 (1H, m), 2.65 (2H, d, J = 6.9Hz), 3.54–3.81 (5H, m), 4.19–4.26 (2H, m), 5.07 (1H, t, J=6.9Hz), 7.24 (5H, br s)[i]	3258 1667 1537	-56.7° {20} 0.10 (H_2O)
2e	Z-Ile A 11	146–148 M-EE	$C_{21}H_{33}N_3O_8$ •0.5H_2O	54.30 (54.22	7.38 7.42	9.05 8.98)	0.70–0.95 (6H, m), 1.20–1.90 (3H, m), 2.50–3.00 (2H, m), 3.10–4.20 (7H, m), 5.10–5.30 (1H, m), 7.10–7.40 (5H, m), 7.90 (1H, d, J = 8.0Hz), 8.30 (1H, d, J = 8.0Hz)[h]	3300 1659 1537	-108.1° {21} 0.10 (H_2O)
2f	Z-Phe A 51	183–187 M-EA	$C_{24}H_{31}N_3O_8$ •0.5H_2O	57.82 (57.55	6.47 6.47	8.43 8.15)	2.59 (2H, d, J = 6.4Hz), 2.94 (1H, dd, J = 9.0, 14.0Hz), 3.18 (1H, dd, J= 5.4, 14.0Hz), 3.44–3.76 (4H, m), 4.09–4.21 (3H, m), 5.08 (1H, t, J = 6.4Hz), 7.17–7.25 (10H, m)[i]	3248 1653 1530	-60.2° {20} 0.10 (H_2O)
2g	Z-Pro A 40	136–139 M-EA	$C_{20}H_{29}N_3O_8$ •1.2H_2O	52.10 (52.29	6.86 6.70	9.11 8.91)	1.85–1.98 (3H, m), 2.34 (1H, m), 2.58 (2H, d, J = 7.0Hz), 3.16–3.32 (2H, m), 3.48–3.77 (4H, m), 4.18–4.30 (3H, m), 5.06 (1H, J = 7.0Hz), 7.21–7.26 (5H, m)[i]	3300 1651 1539	-101.5° {20} 0.10 (H_2O)
2h	Boc-Met A 62	146–148 M-EE	$C_{20}H_{31}N_3O_8S$ •0.5H_2O	49.78 (49.84	6.68 6.97	8.71 8.52)	2.12 (3H, s), 2.20 (2H, m), 2.62 (2H, t, J = 7.3Hz), 2.74 (2H, d, J = 6.9Hz), 3.60–3.94 (4H, m), 4.16 (1H, t, J = 6.7Hz), 4.33–4.40 (2H, m), 5.20 (1H, t, J=6.9Hz), 7.30–7.48 (5H, m)[i]	3266 1655 1530	-65.9° {23} 0.12 (H_2O)
2i	Boc-Gln B 49	151 M-EA	$C_{20}H_{30}N_4O_9$ •1.0H_2O	49.18 (49.37	6.60 6.41	11.47 11.31)	2.08–2.21 (2H, m), 2.37–2.44 (2H, m), 2.97 (2H, d, J=6.6Hz), 3.67–3.90 (4H, m), 4.00–4.10 (1H, m), 4.30–4.37 (2H, m), 5.31 (1H, t, J = 6.6Hz), 7.33–7.39 (5H, m)[i]	3300 1663 1534	-75.7° {20} 0.10 (H_2O)
2j	Z-Orn(Boc) B 47	143–145 M-A	$C_{20}H_{32}N_4O_8$ •2.0HCl •1.5H_2O	43.17 (43.33	6.70 6.55	10.07 10.15)	1.50–2.30 (4H, m), 2.76–6.00 (12H, m), 7.10–7.50 (5H, m), 8.10–8.50 (2H, m)[h]	3300 1655 1528	-46.9° {21} 0.10 (MeOH)
2k	Z-Glu(^{t}Bu) B 48	116–117 EA	$C_{20}H_{29}N_3O_{10}$ •1.0HCl •3.0H_2O •0.5EtOAc	43.60 (43.55	6.65 6.27	6.93 6.97)	1.80–2.30 (4H, m), 2.70–3.00 (2H, m), 3.50–6.00 (8H, m), 7.10–7.50 (5H, m)[h]	3400 1721 1655	-39.0° {20} 0.10 (MeOH)
2l	Boc-Abu[b] B 86	168 EA	$C_{19}H_{29}N_3O_8$ •1.0HCl •1.0H_2O	47.35 (47.54	6.69 6.56	8.72 8.37)	1.05 (3H, t, J = 7.6Hz), 1.85–1.96 (2H, m), 2.74 (2H, d, J = 6.4Hz), 3.68–3.91 (5H, m), 4.30–4.33 (2H, m), 5.32 (1H, t, J= 6.4Hz), 7.24–7.42 (5H, m)[g]	3300 1669 1539	-71.9° {20} 0.10 (H_2O)
2m	Z-Nva[c] B 60	146–147 M-EA	$C_{20}H_{31}N_3O_8$ •1.0H_2O	52.28 (51.98	7.24 7.38	9.14 9.22)	0.79 (3H, t, J = 6.8Hz), 1.23 (2H, m), 1.73 (2H, m), 2.61 (2H, d, J=7.0Hz), 3.53–3.92 (5H, m), 4.17–4.24 (2H, m), 5.06 (1H, t, J = 7.0Hz), 7.17–7.23 (5H, m)[i]	3280 1684 1514	-64.7° {20} 0.10 (H_2O)
2n	Boc-Nle[d] B 35	133–134 M-EE	$C_{21}H_{33}N_3O_8$ •1.0HCl	51.27 (51.28	6.97 6.83	8.54 8.41)	0.80–1.00 (3H, m), 1.20–1.45 (4H, m), 1.50–1.80 (2H, m), 2.60–3.00 (2H, m), 3.20–5.40 (8H, m), 7.20–7.50 (5H, m), 7.90–8.30 (2H, m)[h]	3300 1655 1528	-69.7° {21} 0.10 (MeOH)
2o	**28**[e] A 25	144–146 M-EA	$C_{20}H_{30}N_3O_8F$ •0.5H_2O	51.28 (51.61	6.67 6.51	8.97 9.10)	1.80–2.15 (3H, m), 2.30–2.55 (1H, m), 2.69 (2H, d, J=6.6Hz), 3.33–3.42 (2H, m), 3.60–3.87 (4H, m), 4.28–4.40 (3H, m), 5.17 (1H, t, J = 6.6Hz), 7.32–7.34 (5H, m)[i]	3285 1651 1537	-108.0° {20} 0.10 (H_2O)
2p	**27**[e] A 42	132–134 M-EA	$C_{20}H_{28}N_3O_8F_3$ •1.0H_2O	46.78 (46.50	5.89 5.80	8.18 8.21)	1.95–2.30 (4H, m), 2.58 (2H, d, J=6.8Hz), 3.49–3.78 (4H, m), 3.98 (1H, m), 4.18–4.25 (2H, m), 5.06 (1H, t, J = 6.8Hz), 7.21–7.24 (5H, m)[i]	3283 1655 1535	-66.1° {20} 0.10 (H_2O)
2q	Z-Ser(Me) B 39	140–142 M-EE	$C_{19}H_{29}N_3O_9$ •0.2H_2O	51.05 (50.99	6.63 6.75	9.40 9.23)	2.60–2.90 (2H, m), 3.42 (3H, s), 3.50–4.00 (7H, m), 4.30–4.45 (2H, m), 5.30–5.45 (1H, m), 7.20–7.50 (5H, m)[g]	3300 1655 1527	-80.4° {21} 0.10 (MeOH)

Table 4. Continued

Compd	amino acid method yield(%)	mp(°C) solv.[f]	Formula	Analysis (%) Calcd (Found) C	H	N	^{1}H-NMR δ	IR (KBr) cm^{-1}	$[\alpha]_D$ (°C) c (solv)
2r	Boc-Cys(Me) A 46	138–141 M	$C_{19}H_{29}N_3O_8S$ •0.5H_2O	48.71 (48.68)	6.45 6.64	8.97 8.79)	2.05 (3H, s), 2.56–2.86 (4H, m), 3.40–3.80 (5H, m), 4.05 (1H, m), 4.13 (1H, s), 5.22 (1H, m), 7.19–7.37 (5H, m), 7.84 (1H, d, J = 8.8Hz), 8.24 (1H, d, J = 8.8Hz)[h]	3268 1661 1532	-64.4° {23} 0.40 (MeOH)
2s	Boc-L-allylglycine A 56	135–136 EA-EE	$C_{20}H_{29}N_3O_8$ •1.0HCl •1.0H_2O	48.63 (48.55	6.53 6.57	8.51 8.42)	2.40–2.80 (4H, m), 3.65–3.80 (3H, m), 3.85–4.00 (2H, m), 4.20–4.40 (2H, m), 5.20–5.40 (3H, m), 5.70–5.95 (1H, m), 7.20–7.50 (5H, m)[g]	3330 1676 1541	-75.6° {23} 0.10 (MeOH)
2t	**29**[e] B 50	120 M-EA	$C_{20}H_{29}N_3O_8$ •1.0HCl •1.0H_2O	48.63 (48.93	6.53 6.79	8.51 8.56)	1.75–1.78 (3H, m), 2.98 (2H, d, J=6.6Hz), 3.64–3.79 (2H, m), 3.88–4.04 (2H, m), 4.30–4.38 (2H, m), 4.55–4.58 (1H, m), 5.33 (1H, t, J=6.6Hz), 5.58–5.62 (1H, m), 6.15–6.18 (1H, m), 7.30–7.50 (5H, m)[g]	3300 1653 1532	-65.0° {20} 0.10 (H_2O)
2u	**30**[e] B 58	137–138 M-EE	$C_{21}H_{31}N_3O_8$ •1.0H_2O	53.49 (53.66	7.05 7.13	8.91 8.84)	1.70 (3H, s), 2.04 (1H, dd, J = 9.8, 14.0Hz), 2.43 (1H, dd, J= 4.0, 14.0Hz), 2.60–2.90 (2H, m), 3.00–5.40 (10H, m), 7.10–7.20 (5H, m)[h]	3350 1660 1550	-74.4° {23} 0.10 (MeOH)
2v	Fmoc-L-propargylglycine B 4	135–136 M-C	$C_{20}H_{27}N_3O_8$		*j)*		1.30–1.80 (2H, m), 2.65–2.90 (2H, m), 3.00–5.40 (8H, m), 7.15–7.40 (5H, m), 7.75 (1H, d, J = 8.8Hz), 8.18 (1H, d, J = 8.8Hz)[h]	3300 1650 1528	-63.2° {23} 0.10 (MeOH)
2w	Boc-Ala(CN) A 12	129–131 M-EA	$C_{19}H_{26}N_4O_8$ •1.5H_2O	49.03 (49.28	6.28 6.04	12.04 12.19)	2.60 (2H, d, J=6.4Hz), 3.05 (2H, d, J=6.0Hz), 3.48–3.79 (4H, m), 4.13–4.26 (3H, m), 5.18 (1H, t, J= 6.4Hz), 7.15–7.35 (5H, m)[g]	3275 1651 1539	-86.6° {22} 0.12 (H_2O)

a) GABA = γ-aminobutyric acid, *b)* Abu = (2*S*)-2aminobutyric acid, *c)* Nva = L-norvaline, *d)* Nle = L-norleucine, *e)* structure of protected amino acids **27**~**30** are indicated below, *f)* recrystallization solvent: M = methanol, EE = ethyl ether, EA = ethyl acetate, C = chloroform, A = acetonitrile, *g)* in CD_3OD, *h)* in DMSO-d_6, *i)* in D_2O, *j)* HR-MS (FAB) *m/z* 438.1862 (calcd for $C_{20}H_{28}N_3O_8$ (M+H)$^+$: 438.1877).



27[15]: R^1 = Z, R^2 = $CH_2CH_2CF_3$
28[15]: R^1 = Z, R^2 = $CH_2CH_2CH_2F$
29[16]: R^1 = Boc, R^2 = $CH{=}CHCH_3$
30: R^1 = Fmoc, R^2 = $CH_2C(CH_3){=}CH_2$

From these result, we concluded that the terminal peptidic moiety of pyloricidins must play a critical role in controlling the potency of the anti-*H. pylori* activity.

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 MCI gel (CHP-20P, HP-20 ss, Mitsubishi kasei) using the indicated eluents.

General Synthetic Procedure of Pyloricidin B and Pyloricidin C Derivatives

The pyloricidin B and pyloricidin C derivatives (**1** and **2**) were prepared by the following general methods (Method A and Method B). The *N*-protected amino acids used for the preparation of **1** and **2**, except for **27**~**29** (Table 4), were commercially available. Compounds **27**~**29** were prepared according to the reported methods[15,16].

Method A: A mixture of *N*-protected amino acid (1.0 mmol), HOSu (115 mg, 1.0 mmol) or HONB (215 mg, 1.2 mmol) and DCC (206 mg, 1.0 mmol) in CH_3CN (10 ml) was stirred at 0°C for 30 minutes, then at room temperature for 3 hours before being filtered. The filtrate was added to a stirred solution of pyloricidin C (456 mg, 1.0 mmol) or **4** (342 mg, 1.0 mmol) and Et_3N (0.139 ml, 1.0 mmol) in DMF (50 ml) at 0°C. After being stirred at room temperature for 2~72 hours, the reaction mixture was acidified with 1 N HCl and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4, filtered and then concentrated. Finally, the protective groups were removed by the conventional methods. When the Boc or *tert*-butyl protective group was used, the residue was treated with 4 N HCl/EtOAc or TFA at room temperature for 2 hours. When the benzyloxycarbonyl or benzyl protective group was used, the residue was hydrogenated over 10% palladium on charcoal at room temperature for a few hours. When 9-fluorenylmethoxycarbonyl (Fmoc) protective group was used, the residue was treated with 20% piperidine/DMF (v/v) for 10~30 minutes. The crude product was purified by column chromatography on MCI gel (CHP-20P, HP-20SS) with H_2O and then 5~40% CH_3CN in H_2O as eluent and recrystallized to afford the desired compounds.

Method B: A mixture of *N*-protected amino acid (1.0 mmol), HOSu (115 mg, 1.0 mmol) or HONB (215 mg, 1.2 mmol) and DCC (206 mg, 1.0 mmol) in CH_3CN (10 ml) was stirred at 0°C, then at room temperature for 3 hours before being filtered. The filtrate was added to a stirred solution of **6** (657 mg, 1.0 mmol) or **7** (545 mg, 1.0 mmol) and Et_3N (0.139 ml, 1.0 mmol) in DMF (50 ml) at 0°C. After being stirred at room temperature for 2~72 hours, the reaction mixture was concentrated. The residue was diluted with EtOAc and water. The organic layer was separated and then washed with 5% aqueous citric acid, saturated aqueous $NaHCO_3$ and brine before being dried over Na_2SO_4, filtered and concentrated. The residue was then subjected to deprotection and purification in a similar manner to that described for method A, to afford the desired compounds. The physicochemical data of **1a**~**y** and **2a**~**w** are listed in Table 3 and Table 4.

N-[(2*S*,3*R*,4*R*,5*S*)-5-[*N*-[(2*S*)-2-Hydroxy-3-methylbutyryl]-L-leucyl]amino-2,3,4,6-tetrahydroxyhexanoyl]-*β*-D-phenylalanine (**3a**) and *N*-[(2*S*,3*R*,4*R*,5*S*)-5-[*N*-(*N*-Methyl-L-valyl)-L-leucyl]amino-2,3,4,6-tetrahydroxyhexanoyl]-*β*-D-phenylalanine (**3b**)

These compound were prepared from pyloricidin C and (2*S*)-2-benzyloxy-3-methylbutyric acid **13** or *N*-benzyloxycatbonyl-*N*-methyl-L-valine **15** according to Method A. Their yields and physicochemical data are as follows. **3a**: 46%, colorless solid; mp 159~160°C (MeOH - Et_2O); IR (KBr) 3350, 1649, 1530 cm^{-1}; ^{1}H-NMR (CD_3OD, TMS) δ 0.80~1.10 (12H, m), 1.50~2.20 (4H, m), 2.77 (2H, d, J=7.4 Hz), 3.50~4.60 (8H, m), 5.39 (1H, t, J=7.4 Hz), 7.10~7.50 (5H, m); $[\alpha]_D^{23}$ −95.3 (*c* 0.10, MeOH); *Anal* Calcd for $C_{26}H_{41}N_3O_{10}\cdot 1.6H_2O$: C 53.43, H 7.62, N 7.19. Found: C 53.29, H 7.33, N 7.28; **3b**: 9%, colorless solid; mp 195~197°C (MeOH - Et_2O); IR (KBr) 3339, 1660, 1550 cm^{-1}; ^{1}H-NMR (CD_3OD, TMS) δ 0.90~1.10 (12H, m), 1.55~1.80 (3H, m), 2.00~2.30 (1H, m), 2.52 (3H, s), 2.73 (2H, d, J=6.6Hz), 3.40~3.50 (1H, m), 3.60~4.00 (3H, m), 4.20~4.60 (3H, m), 5.32 (1H, t, J=6.6Hz), 7.20~7.50 (5H, m); $[\alpha]_D^{24}$ −67.4 (*c* 0.10, DMSO); *Anal* Calcd for $C_{27}H_{44}N_4O_9\cdot 0.5H_2O$: C 56.14, H 7.85, N 9.70. Found: C 55.84, H 7.73, N 9.51.

N-[(2*S*,3*R*,4*R*,5*S*)-5-[*N*-[(1-Methylhydrazino)carbonyl]-L-leucyl]amino-2,3,4,6-tetrahydroxyhexanoyl]-*β*-D-phenylalanine (**3c**)

To a stirred solution of **19** (420 mg, 0.89 mmol) in CH_3CN (20 ml) were added HOSu (113 mg, 0.98 mmol) and DCC (183 mg, 0.89 mmol) at 0°C. After being stirred at room temperature for 2 hours, the reaction mixture was filtered. The filtrate was added to a solution of **4** (609 mg, 1.78 mmol) and Et_3N (0.25 ml, 1.79 mmol) in DMF (30 ml) and the mixture was stirred at room temperature for 18 hours. After removal of solvent under reduced pressure, the residue was acidified with 0.1 N HCl (50 ml) and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4 and concentrated. A mixture of the residue and 10% palladium on charcoal (150 mg) and MeOH (30 ml) was vigorously stirred at room temperature under an atmosphere of hydrogen. After filtration of the reaction mixture, the filtrate was concentrated and the residue was chromatographed on MCI gel (CHP-20P, H_2O then CH_3CN - H_2O, 1 : 10) and recrystallized from MeOH - EtOAc to give **3c** (48 mg, 30%) as a colorless solid: mp 115~120°C; IR (KBr) 3322, 1698, 1651, 1528 cm^{-1}; ^{1}H-NMR (D_2O, DSS) δ 0.93~0.99 (6H, m), 1.57~1.76 (3H, m), 2.83 (1H, dd, J=6.8, 15.8 Hz), 2.94 (1H, dd, J=6.8, 15.8 Hz), 3.10 (3H, s), 3.67~3.74 (3H, m), 3.90~3.93 (1H, m), 4.16~4.28 (2H, m), 4.30~4.32 (1H, m), 5.39 (1H, t, J=6.8 Hz), 7.24~7.41 (5H, m); $[\alpha]_D^{20}$ −64.2 (*c* 0.10, H_2O); *Anal* Calcd for $C_{23}H_{37}N_5O_9\cdot 0.5H_2O$: C 51.48, H 7.14, N 13.05. Found: C 51.70, H 7.04, N 12.70.

N-[(2*S*,3*R*,4*R*,5*S*)-5-[*N*-[(2*S*)-2-Amino-3-methylbutyl]-L-leucyl]amino-2,3,4,6-tetrahydroxyhexanoyl]-β-D-phenylalanine (**3d**)

To a mixture of **22** (100 mg, 0.50 mmol) and **6** (278 mg, 0.45 mmol) in MeOH (10 ml) was added sodium cyanoborohydride (63 mg, 1.0 mmol) at 0°C. The mixture was stirred at 0°C for 2 hours and then warmed to room temperature and stirred for 18 hours. After removal of solvent under reduced pressure, the residue was chromatographed on silica gel (EtOAc - MeOH, 20 : 1) and the obtained material was dissolved in 4 N HCl/EtOAc (10 ml). After being stirred at room temperature for 1 hour, the mixture was concentrated. The residue was chromatographed on MCI gel (HP-20SS, H_2O then CH_3CN - H_2O, 1 : 5) and recrystallized from MeOH - Et_2O to give **3d** (25 mg, 10%) as a colorless solid: mp 156~157°C; IR (KBr) 3400, 1626, 1518 cm^{-1}; ^{1}H-NMR (DMSO-d_6+TFA, TMS) δ 0.80~1.20 (12H, m), 1.40~1.80 (3H, m), 1.85~2.10 (1H, m), 2.70~2.90 (2H, m), 3.00~3.10 (2H, m), 3.20~3.60 (4H, m), 3.70~3.80 (1H, m), 3.90~4.10 (1H, m), 4.15~4.35 (2H, m), 5.15~5.35 (1H, m), 7.20~7.40 (5H, m); $[\alpha]_D^{24}$ −87.5 (*c* 0.10, 1 N HCl); *Anal* Calcd for $C_{26}H_{44}N_4O_8 \cdot 1.5H_2O$: C 55.01, H 8.35, N 9.87. Found: C 54.81, H 8.32, N 9.73.

N-[(2*S*,3*R*,4*R*,5*S*)-5-[*N*-Methyl-*N*-(L-valyl)-L-leucyl]-amino-2,3,4,6-tetrahydroxyhexanoyl]-β-D-phenylalanine (**3e**)

To a solution of **26** (240 mg, 0.70 mmol) and HOSu (88 mg, 0.76 mmol) in CH_3CN (20 ml) was added DCC (151 mg, 0.73 mmol) and the mixture was stirred at room temperature for 3 hours and then filtered. The filtrate was added to a solution of **4** (239 mg, 0.70 mmol) and Et_3N (0.097 ml, 0.70 mmol) in DMF (70 ml). After being stirred at room temperature for 16 hours, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in 4 N HCl/EtOAc (20 ml) and the solution was stirred at room temperature for 1 hour. After removal of solvent under reduced pressure, the residue was chromatographed on MCI gel (HP-20SS, H_2O then CH_3CN - H_2O, 1 : 5) and recrystallized from MeOH - Et_2O to give **3e** (123 mg, 31%) as a colorless solid: mp 142~143°C; IR (KBr) 3330, 1653, 1530 cm^{-1}; ^{1}H-NMR (DMSO-d_6, TMS) δ 0.70~1.00 (12H, m), 1.00~2.20 (4H, m), 2.50~2.80 (2H, m), 2.86 (3/2H, s), 2.89 (3/2H, s), 3.00~5.30 (9H, m), 7.10~7.40 (5H, m); $[\alpha]_D^{23}$ −119.4 (*c* 0.10, MeOH); *Anal* Calcd for $C_{27}H_{44}N_4O_9 \cdot 0.5H_2O$: C 56.14, H 7.85, N 9.70. Found: C 56.30, H 7.95, N 9.66.

N-[(2*S*,3*R*,4*R*,5*S*)-5-(L-Leucyl)amino-2,3,4,6-tetrahydroxyhexanoyl]-β-D-phenylalanine Benzhydryl Ester Hydrochloride (**6**)

To a solution of pyloricidin C (2.00 g, 4.4 mmol) in H_2O (50 ml) was added 1 N HCl (4.83 ml) and the mixture was concentrated. The residue was recrystallized from MeOH - Et_2O to give a colorless solid. To a solution of this solid in MeOH (40 ml) was added a solution of diphenyldiazomethane (1.45 g, 7.4 mmol) in MeOH (20 ml). After being stirred at room temperature for 4 hours, acetic acid (0.1 ml) was added to the reaction mixture. After removal of solvent, the residue was crystallized from hexane - Et_2O to give **6** (2.05 g, 75%) as a colorless solid: mp 175~176°C; IR (KBr) 3353, 1734, 1680, 1663, 1541 cm^{-1}; ^{1}H-NMR (CD_3OD, TMS) δ 1.00 (3H, *J*=5.6 Hz), 1.02 (3H, d, *J*=5.6 Hz), 1.60~1.80 (3H, m), 3.04 (1H, dd, *J*=7.6, 16.0 Hz), 3.15 (1H, dd, *J*=5.4, 16.0 Hz), 3.55~4.40 (7H, m), 5.43 (1H, dd, *J*=5.4, 7.6 Hz), 6.73 (1H, s), 7.10~7.40 (15H, m); $[\alpha]_D^{24}$ −47.7 (*c* 0.10, MeOH); *Anal* Calcd for $C_{34}H_{43}N_3O_8 \cdot 1.0HCl \cdot 1.0H_2O$: C 60.39, H 6.86, N 6.21. Found: C 60.60, H 6.74, N 6.17.

N-[(2*S*,3*R*,4*R*,5*S*)-5-Amino-2,3,4,6-tetrahydroxyhexanoyl]-β-D-phenylalanine Benzhydryl Ester Hydrochloride (**7**)

Compound **7** was prepared from **4** by a similar procedure to that used for the preparation of **6** as a colorless solid in 97% yield: 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.

(2*S*)-2-Hydroxy-3-methylbutyric Acid (**10**)

To a stirred solution of L-valine (16.0 g, 137 mmol) in H_2O (100 ml) were added dropwise 2 N H_2SO_4 (75 ml) and 2 N $NaNO_2$ (75 ml) while keeping temperature at 0°C. The mixture was stirred at 0°C for 3 hours, then warmed to room temperature and stirred for 24 hours. The reaction mixture was extracted with EtOAc and the extract was washed with brine, dried over Na_2SO_4 and concentrated. The residue was crystallized with hexane to give **10** (6.65 g, 41%) as a colorless solid: ^{1}H-NMR (CD_3OD, TMS) δ 0.93 (3H, d, *J*=6.8 Hz), 1.08 (3H, d, *J*=6.8 Hz), 2.05~2.30 (1H, m), 4.16 (1H, d, *J*=3.6 Hz).

Methyl (2*S*)-2-Hydroxy-3-methylbutyrate (**11**)

To a stirred solution of **10** (5.00 g, 42 mmol) in MeOH (50 ml) was added concentrated H_2SO_4 (1 ml) and the mixture was refluxed for 4 hours. After being cooled to room temperature, the mixture was diluted with H_2O and extracted with EtOAc. The extract was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated to give **11** (4.55 g, 81%) as a colorless syrup: ^{1}H-NMR (CD_3OD, TMS) δ 0.87 (3H, d, J=7.0 Hz), 1.02 (3H, d, J=7.0 Hz), 2.00~2.20 (1H, m), 2.68 (1H, d, J=6.2 Hz), 3.80 (3H, s), 4.04 (1H, dd, J=3.4, 6.2 Hz).

Methyl (2*S*)-2-Benzyloxy-3-methylbutyrate (**12**)

To a stirred solution of **11** (800 mg, 6 mmol) in cyclohexane (6 ml) - CH_2Cl_2 (3 ml) were added benzyl 2,2,2-trichloroacetimidate (1.13 ml, 6.0 mmol) and trifluoromethanesulfonic acid (0.08 ml, 0.9 mmol) and the mixture was stirred at room temperature for 20 minutes. After filtration of the reaction mixture, the filtrate was washed successively with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated. The residue was chromatographed on silica gel (hexane - EtOAc, 19 : 1) to give **12** (732 mg, 54%) as a colorless syrup: ^{1}H-NMR (CD_3OD, TMS) δ 0.95 (3H, d, J=6.6 Hz), 0.98 (3H, d, J=6.6 Hz), 1.90~2.20 (1H, m), 3.71 (1H, d, J=5.8 Hz), 3.75 (3H, s), 4.38 (1H, d, J=11.8 Hz), 4.71 (1H, d, J=11.8 Hz), 7.20~7.40 (5H, m).

(2*S*)-2-Benzyloxy-3-methylbutyric Acid (**13**)

To a solution of **12** (333 mg, 1.50 mmol) in MeOH (6 ml) was added 1 N NaOH (3 ml) and the mixture was stirred at room temperature for 18 hours. The reaction mixture was acidified with 1 N HCl (6 ml) and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4 and concentrated to give **13** (274 mg, 88%) as a colorless syrup: ^{1}H-NMR (CD_3OD, TMS) δ 1.01 (3H, d, J=6.6 Hz), 1.04 (3H, d, J=6.6 Hz), 2.00~2.30 (1H, m), 3.80 (1H, d, J=5.2 Hz), 4.49 (1H, d, J=11.4 Hz), 4.73 (1H, d, J=11.4 Hz), 7.20~7.50 (5H, m).

N-Benzyloxycarbonyl-*N*-methyl-L-valine (**15**)

To a stirred solution of *N*-benzyloxycarbonyl-L-valine (2.51 g, 10 mmol) and iodomethane (0.69 ml, 11 mmol) in THF (30 ml) was added NaH (60% oil suspension, 1.20 g, 30 mmol) at 0°C and the mixture was stirred at room temperature for 24 hours and then concentrated. The residue was diluted with Et_2O - water. The aqueous layer was acidified with 1 N HCl and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4 and concentrated. The residue was crystallized with Et_2O - hexane to give **15** (2.08 g, 78%) as a colorless amorphous solid: ^{1}H-NMR ($CDCl_3$, TMS) δ 0.80~1.20 (6H, m), 2.10~2.40 (1H, m), 2.95 (3H, s), 4.34 (1H, d, J=10.6 Hz), 5.18 (2H, s), 7.20~7.50 (5H, m).

N-[(1-Methylhydrazino)carbonyl]-L-leucine *tert*-Butyl Ester (**17**)

To a stirred mixture of chloro 4-nitrophenylformate (450 mg, 2.23 mmol) and 4-(dimethylamino)pyridine (817 mg, 6.69 mmol) in CH_2Cl_2 (20 ml) was added L-leucine *tert*-butyl ester (500 mg, 2.23 mmol) and the mixture was stirred at room temperature for 1 hour. Methylhydrazine (0.14 ml, 2.6 mmol) was added to the reaction mixture and stirred at room temperature for 3 hours. After removal of solvent under reduced pressure, the residue was chromatographed on silica gel (EtOAc) to give **17** (336 mg, 58%) as a pale yellow syrup: IR (KBr) 1732, 1661, 1561 cm^{-1}; ^{1}H-NMR ($CDCl_3$, TMS) δ 0.95 (6H, d, J=6.2 Hz), 1.46 (9H, s), 1.51~1.78 (3H, m), 3.15 (3H, s), 3.64 (2H, br s), 4.33~4.35 (1H, m), 6.70 (1H, d, J=9.2 Hz).

N-[(2-Benzyloxycarbonyl-1-methylhydrazino)carbonyl]-L-leucine *tert*-Butyl Ester (**18**)

To a solution of **17** (320 mg, 1.23 mmol) in CH_2Cl_2 (20 ml) were added 4-(dimethylamino)pyridine (540 mg, 4.42 mmol) and carbobenzoxy chloride (0.54 ml, 3.9 mmol) and the mixture was stirred at room temperature for 3 hours. After removal of solvent, the residue was acidified with 0.1 N HCl (50 ml) and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4 and concentrated. The residue was chromatographed on silica gel (hexane - EtOAc, 3 : 1) to give **18** (482 mg, 74%) as a pale yellow syrup: IR (KBr) 1682, 1533 cm^{-1}; ^{1}H-NMR ($CDCl_3$, TMS) δ 0.86 (6H, d, J=6.2 Hz), 1.42 (9H, s), 1.40~1.60 (3H, m), 3.10 (3H, s), 4.35~4.37 (1H, m), 5.17 (2H, s), 7.35~7.50 (5H, m).

N-[(2-Benzyloxycarbonyl-1-methylhydrazino)carbonyl]-L-leucine (**19**)

A solution of **18** (470 mg, 0.89 mmol) in TFA (5.0 ml) was stirred at room temperature for 1 hour and then concentrated under reduced pressure to give **19** (420 mg, 100%) as a pale yellow amorphous solid: IR (KBr) 1780, 1732, 1539 cm^{-1}; ^{1}H-NMR ($CDCl_3$, TMS) δ 0.85~0.91 (6H, m), 1.48~1.61 (3H, m), 3.11 (3H, s), 4.42~4.45 (1H, m), 5.18 (2H, s), 5.42~5.45 (1H, m), 7.17~7.41 (5H, m).

tert-Butyl (1*S*)-1-[[Methoxy(methyl)amino]carbonyl]-2-methylpropylcarbamate (**21**)

To a stirred solution of *N*-*tert*-butoxycarbonyl-L-valine

(4.34 g, 20.0 mmol) and *N,O*-dimethylhydroxylamine hydrochloride (1.95 g, 20.0 mmol) in CH_3CN (100 ml) were added benzotriazol-1-yloxytris(dimethylamino)-phosphonium hexafluorophosphate (8.84 g, 20.0 mmol) and Et_3N (5.6 ml, 40.0 mmol). After being stirred at room temperature for 1 hour, the reaction mixture was acidfied with 10% aqueous citric acid and extracted with EtOAc. The extract was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated. The residue was chromatographed on silica gel (hexane - EtOAc, 2 : 1) to give **21** (3.34 g, 64%) as a colorless syrup: ^{1}H-NMR ($CDCl_3$, TMS) δ 0.91 (3H, d, J=6.8 Hz), 0.96 (3H, d, J=6.8 Hz), 1.44 (9H, s), 1.80~2.15 (1H, m), 3.22 (3H, s), 3.78 (3H, s), 4.50~4.70 (1H, m), 5.05~5.25 (1H, m).

tert-Butyl (1*S*)-1-Formyl-2-methylpropylcarbamate (**22**)

To a stirred solution of **21** (520 mg, 2.0 mmol) in Et_2O (20 ml) was added lithium aluminum hydride (87 mg, 2.5 mmol) at 0°C and the mixture was stirred for 30 minutes. To the reaction mixture was added 5% aqueous potassium hydrogensulfate and extracted with EtOAc. The extract was washed with 1 N HCl, saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated to give **22** (400 mg, 99%) as a colorless syrup: ^{1}H-NMR ($CDCl_3$, TMS) δ 0.94 (3H, d, J=7.0 Hz), 1.03 (3H, d, J=7.0 Hz), 1.45 (9H, s), 2.15~2.45 (1H, m), 4.20~4.35 (1H, m), 5.00~5.20 (1H, m), 9.65 (1H, s).

N-Methyl-L-leucine Benzyl Ester (**24**)

A mixture of *N*-methyl-L-leucine (1.03 g, 7.10 mmol), benzyl alcohol (7.0 ml, 68 mmol) and *p*-toluenesulfonic acid monohydrate (1.62 g, 8.50 mmol) in toluene (42 ml) was refluxed for 3 hours. After removal of solvent under reduced pressure, the residue was diluted with water and Et_2O. The aqueous layer was made basic with 1 N NaOH and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated to give **24** (1.36 g, 81%) as a colorless syrup: IR (neat) 3390, 1730, 1450, 1240, 1165 cm^{-1}; ^{1}H-NMR ($CDCl_3$, TMS) δ 0.89 (3H, d, J=6.0 Hz), 0.92 (3H, d, J=6.0 Hz), 1.40~1.80 (3H, m), 2.35 (3H, s), 3.24 (1H, t, J=7.2 Hz), 5.18 (2H, s), 7.20~7.50 (5H, m).

N-(*N*-*tert*-Butoxycarbonyl-L-valyl)-*N*-methyl-L-leucine Benzyl Ester (**25**)

To a solution of *N*-*tert*-butoxycarbonyl-L-valine (650 mg, 3.0 mmol) and **24** (705 mg, 3.0 mmol) in DMF (10 ml) were added diethyl cyanophosphonate (612 mg, 3.75 mmol) and Et_3N (1.04 ml, 7.50 mmol) and the mixture was stirred at room temperature for 45 hours. The reaction mixture was diluted with water and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was chromatographed on silica gel (hexane - EtOAc, 6 : 1) and recrystallized from hexane - EtOAc to give **25** (362 mg, 28%) as a colorless solid: IR (KBr) 3279, 1736, 1696, 1636 cm^{-1}; ^{1}H-NMR ($CDCl_3$, TMS) δ 0.70~1.00 (12H, m), 1.20~2.00 (13H, m), 2.96 (3/2H, s), 2.99 (3/2H, s), 4.30~5.50 (4H, m), 7.20~7.50 (5H, m); $[\alpha]_D^{23}$ −67.8 (*c* 0.10, MeOH); *Anal* Calcd for $C_{24}H_{38}N_2O_5$: C 66.33, H 8.81, N 6.45. Found: C 66.25, H 8.85, N 6.52.

N-(*N*-*tert*-Butoxycarbonyl-L-valyl)-*N*-methyl-L-leucine (**26**)

A mixture of **25** (350 mg, 0.81 mmol) and 10% palladium on charcoal (50 mg) and MeOH (10 ml) was stirred vigorously under an atmosphere of hydrogen at room temperature for 18 hours. After filtration of the reaction mixture, the filtrate was concentrated to give **26** (249 mg, 90%) as a colorless solid: mp 56~58°C; IR (KBr) 3328, 1719, 1645, 1611 cm^{-1}; ^{1}H-NMR ($CDCl_3$, TMS) δ 0.80~1.05 (12H, m), 1.43 (9H, s), 1.20~1.60 (1H, m), 1.70~2.10 (3H, m), 3.05 (3H, s), 4.30~4.60 (2H, m), 5.10~5.40 (1H, m); $[\alpha]_D^{23}$ −64.8 (*c* 0.10, MeOH); *Anal* Calcd for $C_{17}H_{32}N_2O_5$: C 59.28, H 9.36, N 8.13. Found: C 58.98, H 9.07, N 8.05.

Determination of Minimum Inhibitory Concentrations (MICs)

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

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