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Total Synthesis of Novel Antibiotics Pyloricidin A, B and C and Their Application in the Study of Pyloricidin Derivatives

ATSUSHI HASUOKA[a,*], YUJI NISHIKIMI[a], YUTAKA NAKAYAMA[a], KEIJI KAMIYAMA[a], MASAFUMI NAKAO[b], KEN-ICHIRO MIYAGAWA[c], OSAMU NISHIMURA[d] and MASAHIKO FUJINO[e]

[a] Medicinal Chemistry Research Laboratories I, Pharmaceutical Research Division, Takeda Chemical Industries, Ltd., 2-17-85, Juso-Honmachi, Yodogawa-ku, Osaka 532-8686, Japan
[b] Vaccine Group, Marketing Division, Takeda Chemical Industries, Ltd., 4-1-1, Doshomachi, Chuo-ku, Osaka 540-8645, Japan
[c] Pharmaceutical Discovery Center, Pharmaceutical Research Division, Takeda Chemical Industries, Ltd., 2-17-85, Juso-Honmachi, Yodogawa-ku, Osaka 532-8686, Japan
[d] Pharmaceutical Research Division, Takeda Chemical Industries, Ltd., Wadai 10, Tsukuba, Ibaraki 300-4293, Japan
[e] Takeda Chemical Industries, Ltd., 4-1-1, Doshomachi, Chuo-ku, Osaka 540-8645, Japan

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The novel natural antibiotics pyloricidin A, B and C, which possess potent and highly selective anti-*Helicobacter pylori* activity, were synthesized from D-galactosamine as a chiral template for the common (2*S*,3*R*,4*R*,5*S*)-5-amino-2,3,4,6-tetrahydroxyhexanoic acid moiety. The synthetic strategy, using 2-amino-2-deoxyuronic acid derivatives as key intermediates, was also useful to prepare a series of derivatives modified at the β-D-phenylalanine and with altered stereochemistry on the 5-amino-2,3,4,6-tetrahydroxyhexanoic acid moiety. From the drastic decrease of their anti-*H. pylori* activity, it was clear that the β-D-phenylalanine part and the stereochemistry of the 5-amino-2,3,4,6-tetrahydroxyhexanoic acid moiety were significant for the activity.

Helicobacter pylori is a Gram-negative bacterium that was isolated from the mucus layer of human gastric epithelium in 1983.[1] Since its discovery, continuous research has been made to investigate the relationships between infection with this organism and peptic ulcers. As a result, it has been widely accepted that *H. pylori* infection is a major cause of gastric and duodenal ulcers and eradication of this organism results in a drastic decrease in the recurrence rate in peptic ulcer patients.[2,3] Recent research has revealed that *H. pylori* infection is also associated with gastric cancer.[4~6] Therefore, eradication of *H. pylori* has become an important topic in the field of gastroenterology. In 1994, the National Institute of Health consensus conference concluded that all ulcer patients with *H. pylori* infection should be treated with eradication therapy.[7]

H. pylori has susceptibility to a variety of antimicrobial agents,[8~10] however, successful eradication could not be achieved by single administration of these agents.[11] Accordingly, dual therapies or triple therapies, concomitant administration of antimicrobial agents and a proton pump inhibitor, are prevalent for the purpose of eradicating *H. pylori*, and eradication rates of over 80% have been reported.[11~13] However, single agent therapy is preferable for patient compliance and fewer side effects.

In our program to develop an efficient anti-*H. pylori* agent, the novel antibiotics pyloricidin A, B and C were discovered from the fermentation broth of *Bacillus* sp. HC-70 in Takeda's pharmaceutical discovery center. They possess unique structures consisting of a novel (2*S*,3*R*,4*R*,5*S*)-5-amino-2,3,4,6-tetrahydroxyhexanoic acid and several amino acids (L-valine, L-leucine and β-D-

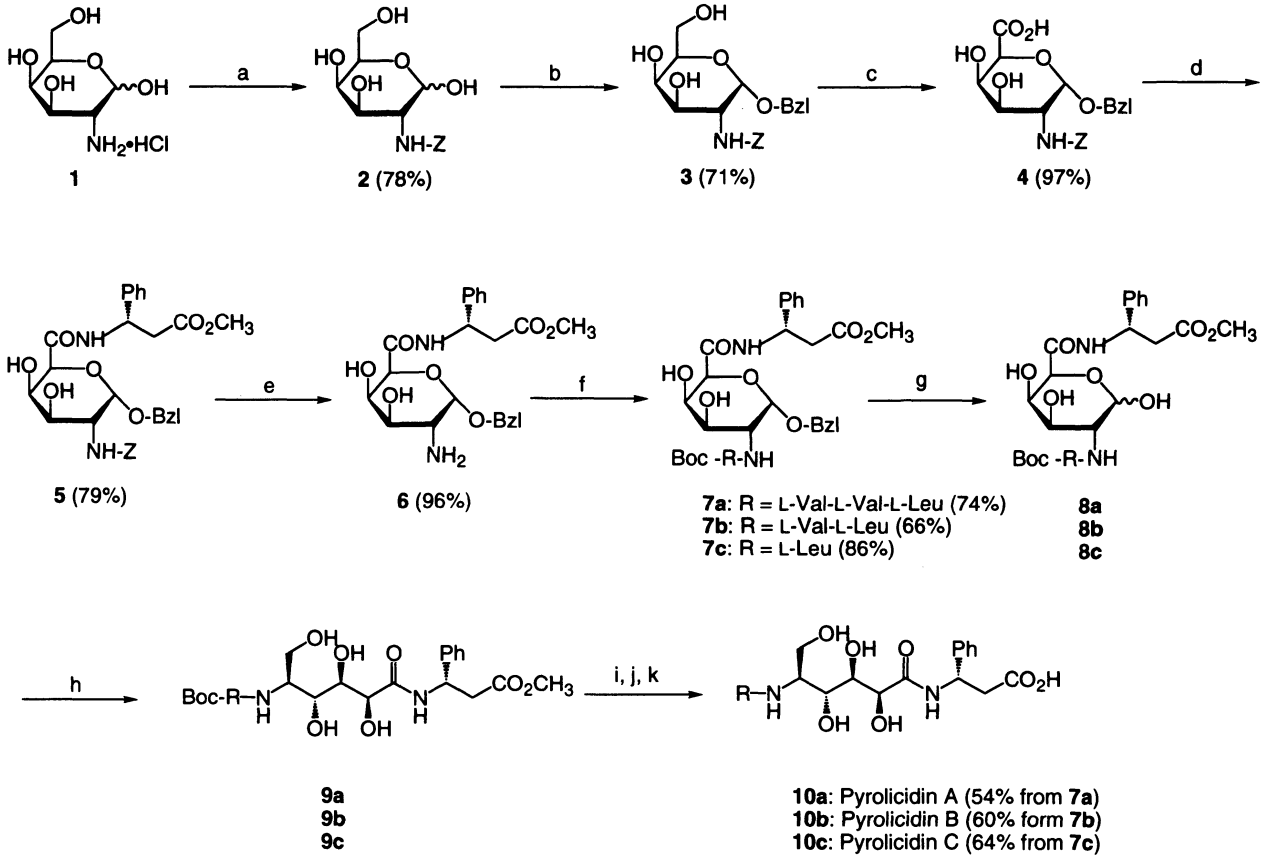
* Corresponding author: Hasuoka_Atsushi@takeda.co.jp

phenylalanine).[14] In addition, they were found to have potent and highly selective anti-*H. pylori* activity.[15] From these findings, they were expected to eradicate *H. pylori* without disturbing the gastrointestinal microflora. Their unique structures and antibacterial profiles made them important lead compounds for therapeutically useful anti-*H. pylori* agents. In order to investigate structure activity relationships on the common (2*S*,3*R*,4*R*,5*S*)-5-amino-2,3,4,6-tetrahydroxyhexanoyl-β-D-phenylalanine moiety, it was required to develop a convenient synthesis of pyloricidin A, B and C which would be applicable for the preparation of modified derivatives. In this paper, we wish to describe the total synthesis of pyloricidin A, B and C, as well as their application in the study of pyloricidin derivatives.

Chemistry

The synthesis of pyloricidin A, B and C is shown in Scheme 1. We selected D-galactosamine as a chiral template for the (2*S*,3*R*,4*R*,5*S*)-5-amino-2,3,4,6-tetrahydroxyhexanoyl moiety, because it had all the correct stereocenters required to construct the moiety. Benzyl 2-carbobenzyloxyamino-2-deoxy-α-D-galactopyranosiduronic acid **4**, prepared from D-galactosamine **1** in three steps by the method of HEYNS *et al.*,[16] was coupled with β-D-phenylalanine methyl ester[17] using 1,3-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt)[18] to give **5**. Hydrogenolysis of **5** over 10% palladium on charcoal resulted in selective removal of the carbobenzoxy (Z) group. Condensation of **6** with Boc-L-Val-L-Val-L-Leu, Boc-L-Val-L-Leu and Boc-L-Leu by the DCC-HOBt method gave **7a**, **7b** and **7c**, respectively. Removal of the anomeric

Scheme 1.[a]



[a] Reagents and conditions : (a) carbobenzoxy chloride (Z-Cl), $NaHCO_3$ in H_2O; (b) AcCl (0.4 equiv), benzyl alcohol; (c) O_2, Pt black, $NaHCO_3$ in H_2O; (d) β-D-phenylalanine methyl ester, 1-hydroxybenzotriazole (HOBt), 1,3-dicyclohexylcarbodiimide (DCC) in DMF; (e) H_2, 10% Pd-C (f) Boc-R (R = L-Val-L-Val-L-Leu, L-Val-L-Leu or L-Leu), HOBt, DCC in DMF; (g) H_2, 10% Pd-C in MeOH-H_2O (pH 2.0); (h) $NaBH_4$ in MeOH; (i) TFA; (j) (i)1N NaOH; (ii) 1N HCl; (k) purification by CHP-20P column chromatography.

benzyl groups of **7a~c** was effected by hydrogenolysis in an acidic media (pH 2.0) to give **8a~c**, which were subsequently treated with sodium borohydride to afford the protected pyloricidins **9a~c**. Finally, removal of Boc protecting group with TFA and saponification followed by purification by high porous polymer (MCI gel CHP-20P) column chromatography and recrystallization afforded **10a~c**.

Utilizing the synthetic procedure described above, pyloricidin C derivatives, in which the β-D-phenylalanine was replaced with glycine (**13a**), β-alanine (**13b**), DL-phenylglycine (**13c**), D-phenylalanine (**13d**), (*S*)-3-amino-3-(4-methylphenyl)propionic acid (**13e**, β-D-Phe(4-Me)-OH)[19] or . β-L-phenylalanine (**13f**), were successfully prepared as shown in Scheme 2.

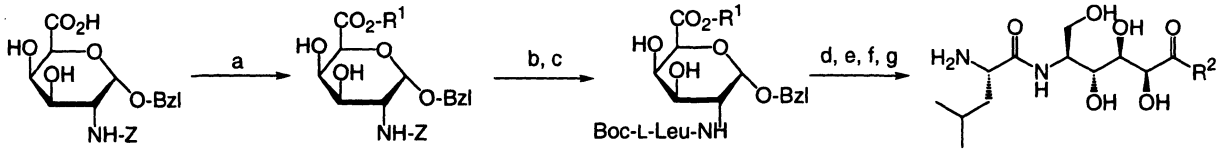
The epimer **21** of pyloricidin C, with an (*S*)-configuration at the C-3 position on the 5-amino-2,3,4,6-tetrahydroxyhexanoyl moiety, was also prepared as shown in Scheme 3. D-Glucosamine **14** was transformed into the mixture of α- and β-2-amino-2-deoxyglucuronic acids **17**, which was coupled with β-D-phenylalanine *tert*-butyl ester. The products were purified by silica gel column chromatography to afford the α-anomer **18α** and the β-anomer **18β** in 40% and 47% yields, respectively, and **18α** was converted to **21** by a similar procedure to that employed for the preparation of **10c**.

Results and Discussion

The physicochemical data of **10a~c** are listed in Table 1. The $[\alpha]_D$ values of chemically synthesized pyloricidin A, B and C (**10a~c**) were −87.7°, −68.9° and −68.3°, respectively, which were in agreement with those of the natural pyloricidin A, B and C (pyloricidin A, $[\alpha]_D^{24}$ −89° (*c* 0.53, 0.1 N HCl); pyloricidin B, $[\alpha]_D^{24}$ −69° (*c* 0.50, 0.1 N HCl); pyloricidin C, $[\alpha]_D^{24}$ −67° (*c* 0.55, 0.1 N HCl)).[14] ^{1}H-NMR spectra and the HPLC retention times of **10a~c** were in complete accord with those of natural pyloricidin A, B and C. Previously, the absolute configuration of the 5-amino-2,3,4,6-tetrahydroxyhexanoic acid moiety was determined by X-ray diffraction and Mosher's method[20] applied to the derivatives with that moiety (Fig. 1).[14] The agreement of physicochemical properties of **10a~c** with those of natural pyloricidin A, B and C confirmed their chemical structures, including the absolute configuration of the 5-amino-2,3,4,6-tetrahydroxyhexanoic acid moiety.

In order to investigate 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, the pyloricidin C derivatives, **13a~f** and **21**, were synthesized and evaluated for their anti-*H. pylori* activity. For the preparation of the glycine derivative **13a**, at first we examined the procedure involving alkaline hydrolysis of pyloricidin C and condensation of the resulting carboxylic acid **23** with Gly-OMe (Scheme 4). However, this procedure was unsuccessful, because the yield of **22** was low (*ca.* 40%) and the γ-lactone derivative

Scheme 2.[a]



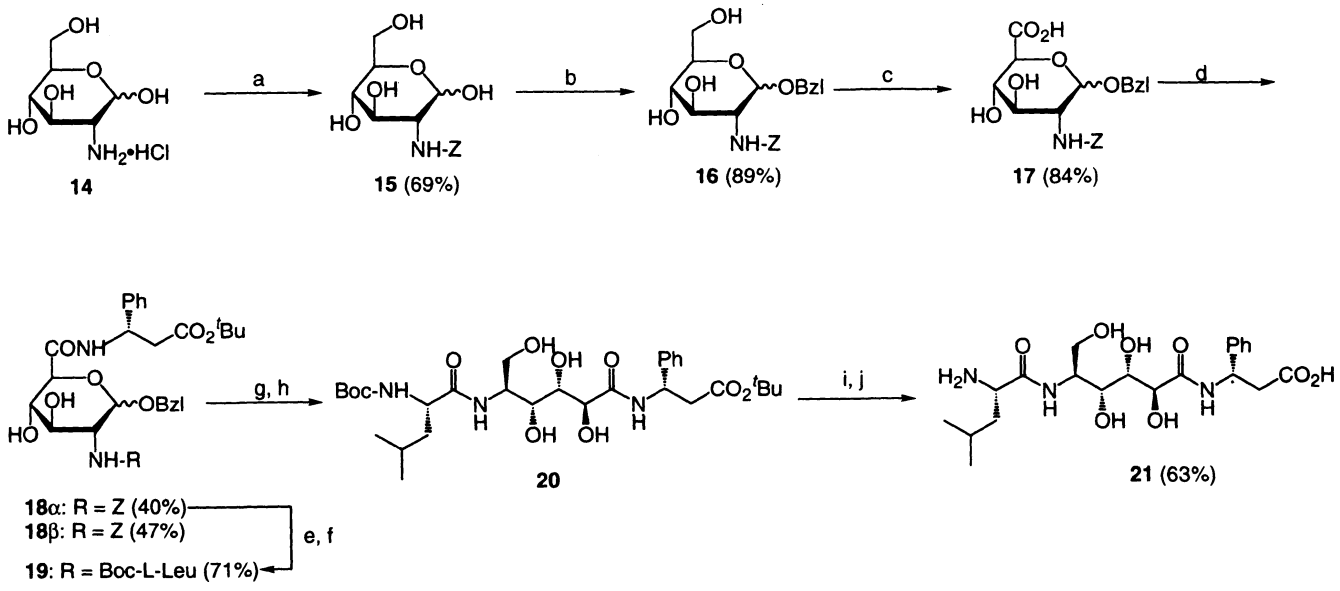
11a: R^1 = Gly-OEt (55%)
11b: R^1 = β-Ala-OMe (53%)
11c: R^1 = DL-Phg-OMe (67%)
11d: R^1 = D-Phe-OMe (68%)
11e: R^1 = β-D-Phe(4-Me)-OEt (64%)
11f: R^1 = β-L-Phe-O^{t}Bu (59%)

12a (66%)
12b (76%)
12c (68%)
12d (80%)
12e (72%)
12f (83%)

13a: R^2 = Gly-OH (81%)
13b: R^2 = β-Ala-OH (72%)
13c: R^2 = DL-Phg-OH (73%)
13d: R^2 = D-Phe-OH (80%)
13e: R^2 = β-D-Phe(4-Me)-OH (75%)
13f: R^2 = β-L-Phe-OH (82%)

[a] Reagents and conditions : (a) Gly-OEt, β-Ala-OMe, DL-Phg-OMe, D-Phe-OMe, β-D-Phe(4-Me)-OEt, β-L-Phe-O^{t}Bu, HOBt, DCC in DMF; (b) H_2, 10% Pd-C in MeOH (c) Boc-L-Leu, HOBt, DCC in DMF; (d) H_2, 10% Pd-C in MeOH-H_2O (pH 2.0~3.0); (e) $NaBH_4$ in MeOH; (f) in the case of **12a~e**: (i) TFA, (ii) 1N NaOH, (iii) 1N HCl; in the case of **12f**: TFA; (g) purification by CHP-20P column chromatography.

Scheme 3.[a]



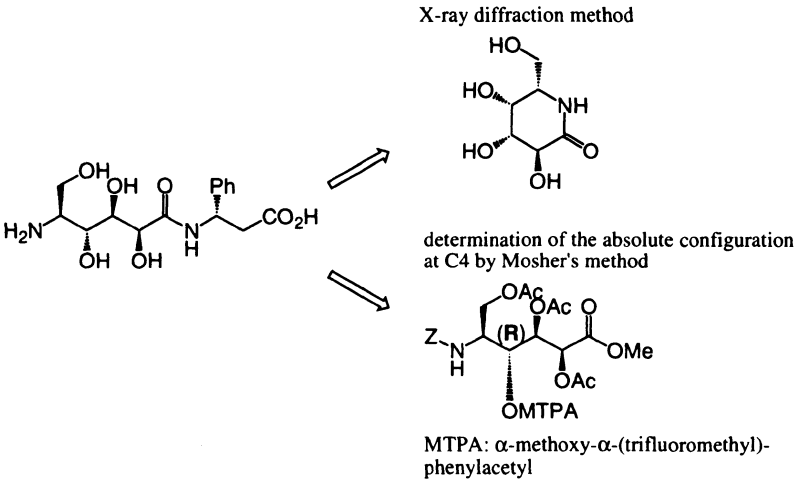
[a] Reagents and conditions : (a) Z-Cl, $NaHCO_3$ in H_2O; (b) AcCl (0.3 equiv), benzyl alcohol; (c) O_2, Pt black, $NaHCO_3$ in H_2O; (d) β-D-Phe-O^{t}Bu, HOBt, DCC in DMF; (e) H_2, 10% Pd-C in MeOH; (f) Boc-L-Leu, HOBt, DCC in DMF; (g) H_2, 10% Pd-C in MeOH-H_2O (pH 3.0); (h) $NaBH_4$ in MeOH; (i) TFA; (j) purification by CHP-20P column chromatography.

Table 1. Physicochemical data of **10a~c**.

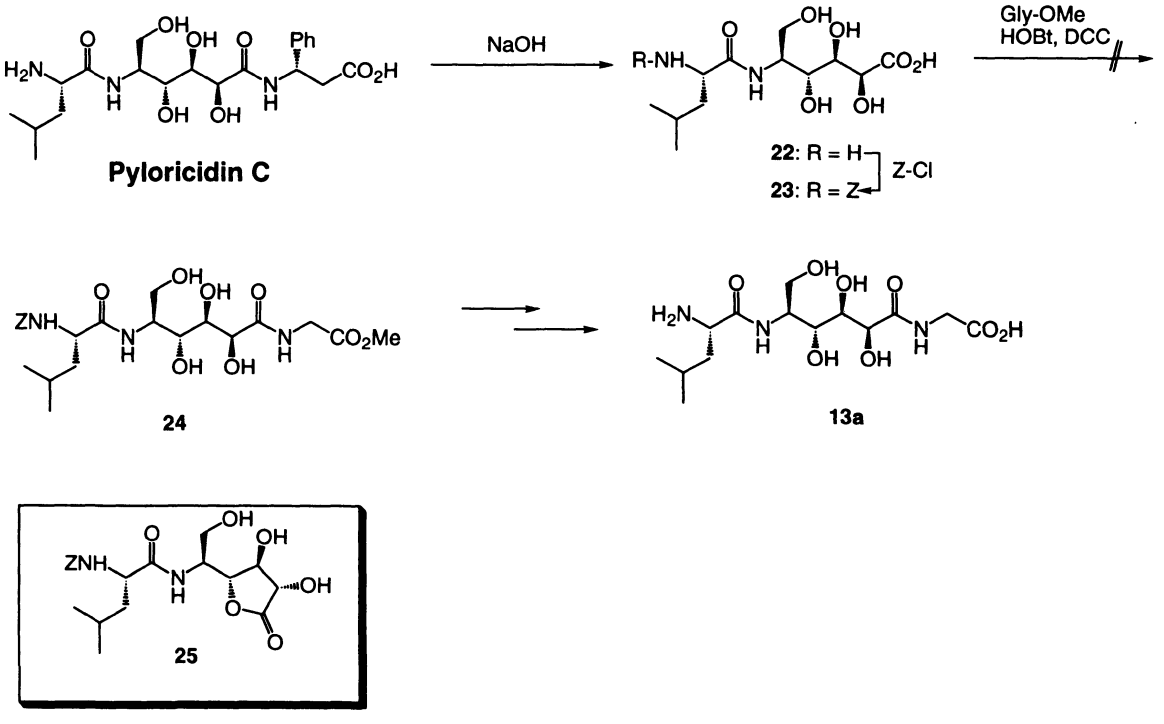
Compd.	mp(°C)[a]	Formula	Analysis (%) Calcd (Found) C	H	N	^{1}H-NMR (D_2O) δ	IR (KBr) cm^{-1}	$[\alpha]_D$ {°C} c (solv)	HPLC retention time[b] (min)
10a	200-201	$C_{31}H_{51}N_5O_{10}$ •2.0H_2O	53.98 (54.22)	8.04 (7.99)	10.15 (10.20)	0.80–1.05 (18H, m), 1.50–1.75 (3H, m), 1.90–2.08 (1H, m), 2.10–2.25 (1H, m), 2.98 (2H, d, J = 7.0Hz), 3.55–3.74 (3H, m), 3.80–3.95 (2H, m), 4.10–4.27 (2H, m), 4.35–4.50 (2H, m), 5.32 (1H, t, J = 7.0Hz), 7.30–7.50 (5H, m)	3303 2959 1632 1539	-87.7° {25} 0.11 (0.1N HCl)	9.5[c]
10b	186-187	$C_{26}H_{42}N_4O_9$ •2.5H_2O	52.07 (51.96)	7.90 (8.13)	9.34 (9.07)	0.85–1.10 (12H, m), 1.50–1.70 (3H, m), 2.10–2.30 (1H, m), 3.00 (2H, d, J = 7.4Hz), 3.60–4.00 (5H, m), 4.15–4.32 (1H, m), 4.35–4.42 (1H, m), 4.42–4.55 (1H, m), 5.34 (1H, t, J = 7.4Hz), 7.30–7.50 (5H, m)	3372 2965 1626 1520	-68.9° {27} 0.11 (0.1N HCl)	13.3
10c	210-211	$C_{21}H_{33}N_3O_8$ •0.5H_2O	54.30 (54.27)	7.38 (7.31)	9.05 (9.06)	0.85–1.05 (6H, m), 1.50–1.85 (3H, m), 2.70 (2H, d, J = 7.0 Hz), 3.60–3.80 (3H, m), 3.81–3.86 (1H, m), 3.90–4.10 (1H, m), 4.25–4.40 (2H, m), 5.16 (1H, t, J = 7.0 Hz), 7.20–7.45 (5H, m)	3372 2969 1664 1543	-68.3° {27} 0.10 (0.1N HCl)	10.5

[a] recrystallized from EtOH–H_2O, [b] HPLC analysis of **10a~c** was carried out on Inertsil ODS-3® 4.6 x 250 mm column (GL Sciences Inc.) under following conditions. mobile phase, acetonitrile-20mM phosphate buffer (10 : 90); temperature, room temperature (*ca.* 25°C); flow rate, 1.0ml/ml; UV detection at 214nm, [c] mobile phase, acetonitrile-20mM phosphate buffer (25 : 75).

Fig. 1.



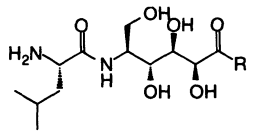
Scheme 4.



25, instead of the desired compound **24**, was found to be the major product of the condensation reaction. Therefore we prepared **13a**~**f** by the synthetic procedure described above for pyloricidin C (**10c**). In addition, this synthetic procedure was applicable to the synthesis of the epimer **21** of pyloricidin C, using D-glucosamine as the starting material. On the basis of these results, we concluded that the synthetic strategy *via* the 2-amino-2-deoxyuronic acid derivative **4** (or **17**) was convenient and effective not only for the preparation of pyloricidin A, B and C but also for pyloricidin derivatives.

The anti-*H. pylori* activity of **13a**~**f** and **21** against four

Table 2. Antibacterial activity of **10c** and pyloricidin C derivatives (**13a~f, 21**) against four strains of *H. pylori*.



Compound	R	MIC (μg / ml)[a]			
		NCTC11637	CPY433	TN2	TN58
10c	β-D-Phe-OH	0.2	3.13	0.78	1.56
13a	Gly-OH	>6.25	>6.25	>6.25	>6.25
13b	β-Ala-OH	>6.25	>6.25	>6.25	>6.25
13c	DL-Phg-OH	>6.25	>6.25	>6.25	>6.25
13d	D-Phe-OH	1.56	6.25	6.25	1.56
13e	β-D-Phe(4-Me)-OH	6.25	>6.25	>6.25	>6.25
13f	β-L-Phe-OH	12.5	>6.25	>6.25	>6.25
21	*b*	12.5	>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] see Scheme 3.

clinical isolates (NCTC11637, CPY433, TN2 and TN58) is shown in Table 2. As a reference, the anti-*H. pylori* activity of synthetic pyloricidin C (**10c**) is also listed. Compound **10c** displayed potent anti-*H. pylori* activity in the range of 0.2~3.13 μg/ml. On the other hand, the replacement of the β-D-phenylalanine with other amino acids (**13a**, **b**, **c** and **d**) resulted in marked decrease of the activity. In addition, introduction of a methyl group into the benzene ring (**13e**) or inversion of the chiral center (**13f**) also lowered the activity. Furthermore, the epimer **21** showed weak activity. These results indicated that the β-D-phenylalanine part was essential and the stereochemistry of the 5-amino-2,3,4,6-tetrahydroxyhexanoic acid moiety was also important for anti-*H. pylori* activity. Considering the fact that (2*S*,3*R*,4*R*,5*S*)-5-amino-2,3,4,6-tetrahydroxyhexanoyl-β-D-phenylalanine (pyloricidin D), which was isolated from the fermentation broth of *Bacillus* sp. HC-72, maintained the anti-*H. pylori* activity (1.0 μg/ml against NCTC11637),[15] this structural unit can be regarded as the minimum component of the pyloricidin antibiotics required to express anti-*H. pylori* activity.

Conclusion

We achieved the total synthesis of the novel antibiotics pyloricidin A, B and C from D-galactosamine as a chiral template for the (2*S*,3*R*,4*R*,5*S*)-5-amino-2,3,4,6-tetrahydroxyhexanoic acid moiety. The synthetic strategy employed in this work was also useful for the preparation of pyloricidin derivatives. The anti-*H. pylori* activity of the pyloricidin derivatives (**13a~f** and **21**) revealed that the β-D-phenylalanine part and the stereochemistry on the 5-amino-2,3,4,6-tetrahydroxyhexanoic acid moiety were significant for 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. FAB-mass spectra were measured with a JEOL JMS-AX505W mass spectrometer. 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 CHP-20P, Mitsubishi kasei corporation) using the indicated eluents.

2-Carbobenzyloxyamino-2-deoxy-D-galactopyranose (**2**)

To a solution of D-galactosamine hydrochloride **1** (10.0 g, 46.4 mmol) in H_2O (300 ml) were added $NaHCO_3$ (11.7 g, 139 mmol) and carbobenzoxy chloride (8.7 g, 51.0 mmol). After being stirred at room temperature for 18 hours, the reaction mixture was extracted with THF - EtOAc (2 : 1, 300 ml×3). The organic layer was washed with brine, dried over Na_2SO_4, filtered and then concentrated under reduced pressure. The residue was recrystallized from EtOH to give **2** (11.3 g, 78%) as a colorless solid: mp 173~175°C; IR (KBr) 3308, 1684, 1549 cm^{-1}; ^{1}H-NMR (CD_3OD, TMS) δ 3.70~4.10 (6H, m), 5.09 (2H, s), 5.15 (1H, d, J=3.4 Hz), 7.20~7.45 (5H, m); *Anal* Calcd for $C_{14}H_{19}NO_7$: C 53.67, H 6.11, N 4.47. Found: C 53.49, H 6.04, N 4.47.

Benzyl 2-Carbobenzyloxyamino-2-deoxy-α-D-galactopyranoside (**3**)

A mixture of **2** (6.0 g, 19.2 mmol) and acetyl chloride (0.5 ml, 7.0 mmol) in benzyl alcohol (50 ml) was stirred at 80°C for 1.5 hours. After being cooled to room temperature, Et_3N (1.1 ml, 7.7 mmol) and hexane (1000 ml) were added to the reaction mixture and the resulting precipitates were collected. The crude product was recrystallized from EtOH to give **3** (5.47 g, 71%) as a colorless solid: mp 197~198°C; IR (KBr) 3333, 1682, 1547 cm^{-1}; ^{1}H-NMR (CD_3OD, TMS) δ 3.65~4.10 (6H, m), 4.50 (1H, d, J=12.2 Hz), 4.75 (1H, d, J=12.2 Hz), 4.91 (1H, d, J=3.8 Hz), 5.07 (2H, s), 7.20~7.45 (10H, m).

Benzyl 2-Carbobenzyloxyamino-2-deoxy-α-D-galactopyranosiduronic Acid (**4**)

A suspension of **3** (1.00 g, 2.47 mmol), $NaHCO_3$ (208 mg, 2.47 mmol) and platinum black (1.0 g) in H_2O (140 ml) was vigorously stirred and heated at 80°C under a continuous O_2 stream for 5 hours. After being cooled to room temperature, the mixture was filtered and the filtrate was concentrated to *ca.* 20 ml. The resulting aqueous solution was acidified with 1 N HCl and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4, filtered and concentrated. The residue was recrystallized from EtOH to give **4** (1.00 g, 97%) as a colorless solid: mp 223~224°C; IR (KBr) 3322, 1715, 1674, 1541 cm^{-1}; ^{1}H-NMR (CD_3OD, TMS) δ 3.84 (1H, dd, J=3.4, 11.0 Hz), 3.94~4.10 (1H, m), 4.20~4.26 (1H, m), 4.38~4.44 (1H, m), 4.55 (1H, d, J=12.0 Hz), 4.70 (1H, d, J=12.0 Hz), 5.01 (1H, d, J=3.4 Hz), 5.06 (2H, s), 6.80 (1H, d, J=9.0 Hz), 7.15~7.45 (10H, m); *Anal* Calcd for $C_{21}H_{23}NO_8$: C 60.43, H 5.55, N 3.36. Found: C 60.19, H 5.40, N 3.28.

N-(Benzyl 2-Carbobenzyloxyamino-2-deoxy-α-D-galactopyranosiduronyl)-β-D-phenylalanine Methyl Ester (**5**)

To a stirred mixture of **4** (1.00 g, 2.40 mmol), β-D-phenylalanine methyl ester (550 mg, 3.07 mmol) and HOBt (415 mg, 3.07 mmol) in DMF (15 ml) was added DCC (633 mg, 3.07 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 and then concentrated under reduced pressure. The residue was extracted with EtOAc and H_2O. The organic layer was washed with 5% aqueous citric acid, saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated. The residue was recrystallized from EtOAc to give **5** (1.10 g, 79%) as a colorless solid: mp 169~170°C; IR (KBr) 3318, 1696, 1651, 1539 cm^{-1}; ^{1}H-NMR (CD_3OD, TMS) δ 2.90 (1H, dd, J=6.7, 15.0 Hz), 3.03 (1H, J=6.7, 15.0 Hz), 3.61 (3H, s), 3.70~3.85 (1H, m), 4.00~4.24 (3H, m), 4.55 (1H, d, J=12.0 Hz), 4.65 (1H, J=12.0 Hz), 5.00~5.10 (3H, m), 5.43 (1H, t, J=6.7 Hz), 7.10~7.50 (15H, m); High-resolution FAB-MS *m/z* 579.2331 (calcd for $C_{31}H_{35}N_2O_9$ $(M+H)^+$: 579.2343); $[\alpha]_D^{23}$ +56.6 (*c* 0.17, MeOH); *Anal* Calcd for $C_{31}H_{34}N_2O_9 \cdot 0.5H_2O$: C 63.36, H 6.00, N 4.77. Found: C 63.40, H 5.88, N 4.70.

N-(Benzyl 2-Amino-2-deoxy-α-D-galactopyranosiduronyl)-β-D-phenylalanine Methyl Ester (**6**)

A suspension of **5** (500 mg, 0.89 mmol) and 10% palladium on charcoal (150 mg) in MeOH (40 ml) was vigorously stirred under an atmosphere of hydrogen at room temperature for 1 hour. The mixture was filtered and the filtrate was concentrated under reduced pressure to dryness to give **6** (366 mg, 96%) as a colorless amorphous solid: IR (KBr) 3300, 1742, 1655, 1545 cm^{-1}; ^{1}H-NMR (CD_3OD, TMS) δ 2.80~3.10 (3H, m), 3.55~3.70 (4H, m), 4.10~4.20 (2H, m), 4.60 (1H, d, J=12.0 Hz), 4.65 (1H, d, J=12.0 Hz), 5.11 (1H, d, J=3.8 Hz), 5.42 (1H, t, J=6.4 Hz), 7.15~7.50 (10H, m); FAB-MS *m/z* 445.1969 (calcd for $C_{23}H_{29}N_2O_7$ $(M+H)^+$: 445.1975); $[\alpha]_D^{22}$ +8.17 (*c* 0.17, MeOH).

N-[Benzyl 2-[(*N*-*tert*-Butoxycarbony-L-valyl-L-valyl-L-leucyl)amino]-2-deoxy-α-D-galactopyranosiduronyl]-β-D-phenylalanine Methyl Ester (**7a**)

To a stirred mixture of **6** (183 mg, 0.412 mmol), Boc-L-Val-L-Val-L-Leu (177 mg, 0.412 mmol) and HOBt (61 mg, 0.45 mmol) in DMF (5 ml) was added DCC (93 mg, 0.45 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 and then concentrated under reduced pressure. The residue was extracted with EtOAc and brine. The organic layer was washed with 5% aqueous citric acid, saturated aqueous $NaHCO_3$ and brine and then dried over Na_2SO_4, filtered and concentrated. The residue was chromatographed on silica gel with EtOAc as an eluent and recrystallized from EtOAc to give **7a** (260 mg, 74%) as a colorless solid: mp 224~226°C; IR (KBr) 3320, 2930, 1636, 1520 cm^{-1}; ^{1}H-NMR (CD_3OD, TMS) δ 0.85~1.00 (18H, m), 1.05~2.00 (5H, m), 1.45 (9H, s), 2.90 (1H, dd, J=7.2, 15.8 Hz), 3.02 (1H, dd, J=7.2, 15.8 Hz), 3.40~3.55 (1H, m), 3.61 (3H, s), 3.80~3.96 (2H, m), 4.10~4.20 (2H, m), 4.24~4.34 (1H, m), 4.42~4.54 (1H, m), 4.58 (2H, s), 5.09 (1H, d, J=3.6 Hz), 5.43 (1H, t, J=7.2 Hz), 7.15~7.45 (10H, m); $[\alpha]_D^{24}$ −9.3 (*c* 0.10, MeOH); *Anal* Calcd for $C_{44}H_{65}N_5O_{12}\cdot 1.2H_2O$: C 60.22, H 7.74, N 7.98. Found: C 60.30, H 7.73, N 7.96.

Compound **7b** and **7c** were synthesized by the similar procedure employed for the synthesis of **7a** using Boc-L-Val-L-Leu and Boc-L-Leu. **7b**: colorless solid (66%); mp 180~183°C (EtOAc); IR (KBr) 3316, 2959, 1694, 1645, 1532 cm^{-1}; ^{1}H-NMR (CD_3OD, TMS) δ 0.90~1.05 (12H, m), 1.10~1.90 (4H, m), 1.45 (9H, s), 2.90 (1H, dd, J=6.8, 15.4 Hz), 3.02 (1H, dd, J=6.8, 15.4 Hz), 3.61 (3H, s), 3.80~3.92 (2H, m), 4.10~4.20 (2H, m), 4.22~4.36 (1H, m), 4.44~4.56 (1H, m), 4.59 (2H, s), 5.10 (1H, d, J=3.6 Hz), 5.42 (1H, t, J=6.8 Hz), 7.15~7.40 (10H, m); $[\alpha]_D^{24}$ +11.6 (*c* 0.10, MeOH); *Anal* Calcd for $C_{39}H_{56}N_4O_{11}\cdot 0.5H_2O$: C 61.16, H 7.50, N 7.32. Found: C 61.11, H 7.42, N 7.40. **7c**: colorless solid (86%); mp 162~163°C (EtOAc); IR (KBr) 3300, 1742, 1651, 1539 cm^{-1}; ^{1}H-NMR (CD_3OD, TMS) δ 0.85~1.00 (6H, m), 1.45 (9H, s), 1.20~1.80 (3H, m), 2.90 (1H, dd, J=7.0, 16.0 Hz), 3.25 (1H, dd, J=7.0, 16.0 Hz), 3.61 (3H, s), 3.80~3.92 (1H, m), 4.05~4.35 (4H, m), 4.55~4.70 (2H, m), 5.13 (1H, d, J=3.6 Hz), 5.43 (1H, t, J=7.0 Hz), 7.15~7.50 (10H, m); $[\alpha]_D^{24}$ +20.0 (*c* 0.17, $CHCl_3$); *Anal* Calcd for $C_{34}H_{47}N_3O_{10}$: C 62.08, H 7.20, N 6.39. Found: C 61.83, H 7.23, N 6.62.

N-[(2*S*,3*R*,4*R*,5*S*)-2,3,4,6-Tetrahydroxy-5-(L-valyl-L-valyl-L-leucyl)aminohexanoyl]-β-D-phenylalanine (**10a**)

A solution of **7a** (150 mg, 0.175 mmol) in MeOH (10 ml)-H_2O (5 ml) was adjusted to pH 2.0 with 1 N HCl and then 10% palladium on charcoal (150 mg) was added to the solution. The mixture was vigorously stirred under an atmosphere of hydrogen at room temperature for 18 hours and filtered. The filtrate was neutralized with 1 N NaOH and evaporated to dryness under reduced pressure. The residue was dissolved in MeOH (15 ml) and sodium borohydride (13 mg, 0.35 mmol) was added to the solution. After being stirred at room temperature for 30 minutes, the mixture was evaporated under reduced pressure. The residue was dissolved in TFA (5 ml) and the solution was stirred at room temperature for 30 minutes and then evaporated. The residue was dissolved in H_2O (3 ml)-1 N NaOH (0.5 ml) and stirred at room temperature for 1 hour and then neutralized with 1 N HCl. After removal of solvent under reduced pressure, the residue was purified by CHP-20P (20 ml) column chromatography with H_2O (150 ml), CH_3CN-H_2O (1 : 10) as an eluent and recrystallized from EtOH-H_2O to give **10a** (65 mg, 54%) as a colorless solid. Compound **10b** and **10c** were obtained by the similar procedure employed for the preparation of **10a** in 60 and 64% yields. Their physicochemical data are listed in Table 1.

N-(Benzyl 2-Carbobenzyloxyamino-2-deoxy-α-D-galactopyranosiduronyl)glycine Ethyl Ester (**11a**)

To a stirred mixture of **4** (150 mg, 0.36 mmol), glycine ethyl ester hydrochloride (65 mg, 0.47 mmol), HOBt (64 mg, 0.47 mmol) and ethyldiisopropylamine (61 mg, 0.47 mmol) and DMF (5 ml) was added DCC (97 mg, 0.47 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 and then concentrated under reduced pressure. The residue was extracted with EtOAc and brine. The organic layer was washed with 5% aqueous citric acid, saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and then concentrated. The residue was chromatographed on silica gel with EtOAc as an eluent and recrystallized from isopropyl ether to give **11a** (100 mg, 55%) as a colorless solid. Compound **11b~f** were obtained by the similar procedure employed for the preparation of **11a** using β-Ala-OMe, DL-Phg-OMe, D-Phe-OMe, β-D-Phe(4-Me)-OEt and β-L-Phe-O^{t}Bu (purchased from OXFORD ASYMMETRY). β-D-Phe(4-Me)-OEt was prepared according to the reported method.[19] The physicochemical data are as follows. ^{1}H-NMR (CD_3OD, TMS) δ 1.23 (3H, t, J=7.2 Hz), 2.36 (3H, s), 2.97 (1H, dd, J=6.8, 16.8 Hz), 3.10 (1H, dd, J=6.8, 16.8 Hz), 4.14 (2H, q, J=7.2 Hz), 4.67 (1H, t, J=6.8 Hz), 7.25~7.37 (4H, m); $[\alpha]_D^{22}$ +7.07 (*c* 0.14, MeOH); EI-MS *m/z* 207.1258 (calcd

Table 3. Physicochemical data of **11**, **12** and **13**.

Compd	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)[d]
11a	55 174–175 PE	$C_{25}H_{30}N_2O_9$	59.75 (59.74	6.02 6.21	5.57 5.47)	1.29 (3H, t, J = 7.0Hz), 3.80–4.40 (8H, m), 4.57 (1H, d, J = 12.0Hz), 4.73 (1H, d, J = 12.0Hz), 4.90 (1H, d, J = 3.4Hz), 5.08 (2H, s), 7.20–7.40 (10H, m)[b]	3326 1736 1676 1543	+48.7° {26} 0.10 (M)
11b	53 163–164 PE	$C_{25}H_{30}N_2O_9$ •0.25H_2O	59.22 (59.13	6.06 6.37	5.53 5.84)	2.58 (2H, t, J = 6.2Hz), 3.51 (2H, t, J = 6.2Hz), 3.60–3.70 (1H, m), 3.69 (3H, s), 3.75–3.88 (1H, m), 3.95–4.08 (1H, m), 4.15–4.24 (1H, m), 4.55 (1H, d, J = 12.4Hz), 4.65 (1H, d, J = 12.4Hz), 5.00–5.10 (3H, m), 7.20–7.45 (10H, m)[b]	3326 1732 1694 1539	+70.4° {26} 0.10 (M)
11c	67 100–105 PE	$C_{30}H_{32}N_2O_9$	63.82 (63.61	5.71 5.69	4.96 4.87)	3.73 (1/2 x 3H, s), 3.76 (1/2 x 3H, s), 3.82 (1H, dd, J = 2.8, 11.0Hz), 4.00–4.30 (3H, m), 4.55–4.75 (2H, m), 5.00–5.15 (3H, m), 5.52 (1/2 x 1H, s), 5.54 (1/2 x 1H, s), 7.15–7.50 (15H, m)[b]	3300 1732 1682 1520	+68.8° {26} 0.10 (M)
11d	68 155–157 EA-H	$C_{31}H_{34}N_2O_9$	64.35 (64.39	5.92 5.95	4.84 4.95)	3.07 (1H, dd, J = 7.8, 13.0Hz), 3.25 (1H, dd, J = 6.0, 13.0Hz), 3.60–3.90 (1H, m), 3.74 (3H, s), 3.95–4.10 (1H, m), 4.15–4.20 (2H, m), 4.50 (1H, d, J = 12.0Hz), 4.63 (1H, J = 12.0Hz), 4.70–4.90 (1H, m), 5.06 (2H, s), 5.10 (1H, d, J = 3.6Hz), 7.10–7.40 (15H, m)[b]	3324 1736 1694 1537	+89.6° {23} 0.10 (M)
11e	64 150–151 PE	$C_{33}H_{38}N_2O_9$ •0.25H_2O	64.85 (64.85	6.35 6.42	4.58 4.55)	1.16 (3H, t, J= 7.4Hz), 2.33 (3H, s), 2.90 (1H, dd, J = 6.6, 15.8Hz), 2.97 (1H, dd, J = 6.6, 15.8Hz), 3.82 (1H, dd, J = 3.4, 11.0Hz), 3.95–4.24 (5H, m), 4.54 (1H, d, J= 12.2Hz), 4.64 (1H, d, J = 12.2Hz), 5.06 (1H, d, J = 3.4Hz), 5.07 (2H, s), 5.37 (1H, t, J = 6.6Hz), 7.10–7.40 (14H, m)[b]	3320 1726 1696 1534	+59.1° {26} 0.10 (M)
11f	59 92–93 EA-EE	$C_{34}H_{40}N_2O_9$	65.79 (65.76	6.50 6.84	4.51 4.75)	1.37 (9H, s), 2.84 (2H, d, J= 7.2Hz), 3.85 (1H, dd, J= 2.8, 11.0Hz), 4.00–4.32 (3H, m), 4.56 (1H, d, J = 12.4Hz), 4.72 (1H, d, J = 12.4Hz), 4.90 (1H, d, J = 2.8Hz), 5.08 (2H, s), 5.41 (1H, t, J = 7.2Hz), 7.20–7.50 (15H, m)[b]	3333 1724 1670 1530	+83.2° {23} 0.10 (M)
12a	66 196–199 PE	$C_{28}H_{43}N_3O_{10}$	57.82 (57.64	7.45 7.41	7.22 7.60)	0.85–1.00 (6H, m), 1.29 (3H, t, J=7.2Hz), 1.45 (9H, s), 1.46–1.80 (3H, m), 3.88 (1H, dd, J = 3.8, 11.6Hz), 4.10–4.35 (8H, m), 4.56 (1H, d, J = 11.8Hz), 4.72 (1H, d, J= 11.8Hz), 5.12 (1H, d, J = 3.8Hz), 7.20–7.45 (5H, m)[b]	3326 1743 1688 1651 1532	+45.1° {26} 0.10 (M)
12b	76 193–195 PE	$C_{28}H_{43}N_3O_{10}$ •1.25H_2O	55.66 (55.75	7.59 7.68	6.95 6.99)	0.85–1.00 (6H, m), 1.45 (9H, s), 1.50–1.80 (3H, m), 2.59 (2H, t, J = 7.0Hz), 3.51 (2H, t, J = 7.0Hz), 3.69 (3H, s), 3.85 (1H, dd, J = 3.2, 11.4Hz), 4.05–4.30 (4H, m), 4.55 (1H, d, J= 12.0Hz), 4.65 (1H, d, J= 12.0Hz), 5.09 (1H, d, J= 3.2Hz), 7.20–7.45 (5H, m)[b]	3326 1732 1651 1532	+52.6° {26} 0.10 (M)
12c	68 113–115 PE	$C_{33}H_{45}N_3O_{10}$ •0.5H_2O	60.72 (60.87	7.10 7.02	6.44 6.51)	0.85–1.05 (6H, m), 1.45 (9H, s), 1.40–1.80 (3H, m), 3.73 (1/2 x 3H, s), 3.76 (1/2 x 3H, s), 3.86 (1H, dd, J = 3.4, 11.0Hz), 4.05–4.35 (4H, m), 4.55–4.80 (2H, m), 5.12–5.20 (1H, m), 5.53 (1/2 x 1H, s), 5.54 (1/2 x 1H, s), 7.20–7.50 (10H, m)[b]	3303 1744 1668 1520	+41.8° {26} 0.10 (M)
12d	80 178–179 PE	$C_{34}H_{47}N_3O_{10}$ •0.5H_2O	61.25 (61.04	7.26 7.36	6.30 6.28)	0.85–1.00 (6H, m), 1.20–1.80 (3H, m), 1.44 (9H, s), 3.08 (1H, dd, J = 7.4, 13.2Hz), 3.25 (1H, dd, J=5.4, 13.2Hz), 3.75 (3H, s), 3.85 (1H, dd, J=3.4, 11.8Hz), 4.05–4.30 (5H, m), 4.45 (1H, d, J = 11.4Hz), 4.60 (1H, d, J= 11.4Hz), 5.05 (1H, d, J =3.4Hz), 7.15–7.40 (10H, m)[b]	3330 1742 1686 1657 1530	+34.4° {23} 0.10 (M)
12e	72 155–156 PE	$C_{36}H_{51}N_3O_{10}$ •0.5H_2O	62.23 (62.49	7.54 7.52	6.05 6.03)	0.85–1.00 (6H, m), 1.16 (3H, t, J=7.0Hz), 1.45 (9H, s), 1.40–1.80 (3H, m), 2.33 (3H, s), 2.88 (1H, dd, J= 6.8, 15.4Hz), 2.98 (1H, dd, J = 6.8, 15.4Hz), 3.85 (1H, dd, J= 3.2, 11.0Hz), 4.00–4.35 (6H, m), 4.55 (1H, d, J = 12.0Hz), 4.65 (1H, d, J = 12.0Hz), 5.12 (1H, d, J = 3.2Hz), 5.38 (1H, t, J = 6.8Hz), 7.10–7.40 (9H, m)[b]	3324 1703 1661 1520	+35.7° {26} 0.10 (M)
12f	83 100–105 PE	$C_{37}H_{53}N_3O_{10}$	63.50 (63.21	7.63 7.89	6.00 6.67)	0.85–1.00 (6H, m), 1.20–1.80 (3H, m), 1.37 (9H, s), 1.45 (9H, s), 2.84 (2H, d, J = 7.0Hz), 3.88 (1H, dd, J= 3.0, 11.0Hz), 4.05–4.35 (4H, m), 4.55 (1H, d, J = 11.6Hz), 4.70 (1H, d, J = 11.6Hz), 5.15 (1H, d, J =3.0Hz), 5.41 (1H, t, J= 7.0Hz), 7.20–7.50 (10H, m)[b]	3293 1725 1668 1523	+54.4° {23} 0.10 (M)
13a	81 157 E	$C_{14}H_{27}N_3O_8$	46.02 (45.90	7.45 7.41	11.50 11.30)	0.85–1.00 (6H, m), 1.50–1.80 (3H, m), 3.60–4.00 (7H, m), 4.25–4.40 (2H, m)[c]	3400 1661 1539	-36.5° {25} 0.10 (W)
13b	72 150 E	$C_{15}H_{29}N_3O_8$	47.48 (47.45	7.70 7.68	11.08 10.98)	0.85–1.00 (6H, m), 1.45–1.80 (3H, m), 2.35 (2H, t, J = 6.6Hz), 3.30–3.50 (2H, m), 3.55–4.00 (5H, m), 4.20–4.35 (2H, m)[c]	3400 1651 1539	-26.0° {25} 0.10 (W)
13c	73 150–151 E-PE	$C_{20}H_{31}N_3O_8$ •HCl •0.25H_2O	49.79 (50.02	6.79 6.88	8.71 8.41)	0.80–1.00 (6H, m), 1.40–1.80 (3H, m), 3.55–4.00 (5H, m), 4.20–4.40 (2H, m), 5.13–5.16 (1H, m), 7.20–7.50 (5H, m)[c]	3350 1655 1526	-57.9° {23} 0.10 (W)

Table 3. (Continued).

Compd	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)[d]
13d	80 215–220 M-EA	$C_{21}H_{33}N_3O_8$ •1.5H_2O	52.27 (52.15	7.52 7.28	8.71 8.52)	0.90–0.98 (6H, m), 1.58–1.72 (3H, m), 2.95–3.00 (1H, m), 3.15–3.20 (1H, m), 3.61–3.88 (4H, m), 4.00–4.05 (1H, m), 4.24–4.33 (2H, m), 4.45–4.48 (1H, m), 7.21–7.34 (5H, m)[c]	3245 1663 1535	-45.6° {23} 0.10 (W)
13e	75 214–215 M-EA	$C_{22}H_{35}N_3O_8$ •1.0H_2O	54.20 (54.16	7.65 7.60	8.62 8.63)	0.98–1.02 (6H, m), 1.60–1.85 (3H, m), 2.27 (3H, s), 2.67 (2H, d, J = 6.4Hz), 3.65–3.74 (3H, m), 3.85–3.90 (2H, m), 4.24–4.31 (2H, m), 5.27 (1H, t, J =6.4Hz), 7.09 (2H, d, J = 8.0Hz), 7.26 (2H, d, J = 8.0Hz)[b]	3308 1664 1537	-62.2° {20} 0.07 (W)
13f	82 148–152 M-EA	$C_{21}H_{33}N_3O_8$ •1.25H_2O	52.76 (52.71	7.49 7.21	8.79 8.62)	0.86–0.93 (6H, m), 1.60–1.85 (3H, m), 2.76 (2H, d, J= 5.9Hz), 3.59–4.07 (5H, m), 4.30–4.35 (1H, m), 4.40–4.43 (1H, m), 5.23 (1H, t, J = 5.9Hz), 7.38–7.40 (5H, m)[c]	3300 1651 1555	+27.2° {20} 0.10 (W)

a) recrystallization solvent: E = ethanol, M = methanol, H = hexane, EA = ethyl acetate, EE = ethyl ether, PE = isopropyl ether, *b*) in CD_3OD, *c*) in D_2O, *d*) $[\alpha]_D$ values were measured in MeOH (M) or H_2O (W).

for $C_{12}H_{17}NO_2$ $(M)^+$: 207.1259). The physicochemical data of **11a**~**f** are listed in Table 3.

N-[Benzyl 2-[(*N*-*tert*-Butoxycarbony-L-leucyl)amino]-2-deoxy-α-D-galactopyranosiduronyl]glycine Ethyl Ester (**12a**)

A suspension of **11a** (170 mg, 0.34 mmol) and 10% palladium on charcoal (80 mg) in MeOH (10 ml) was vigorously stirred under an atmosphere of hydrogen at room temperature for 1 hour. The mixture was filtered and the filtrate was concentrated under reduced pressure and the residue was dissolved in DMF (5 ml). To the solution were added Boc-L-Leu (84 mg, 0.34 mmol), HOBt (50 mg, 0.37 mmol) and DCC (76 mg, 0.37 mmol). After being stirred at room temperature for 2 hours, the reaction mixture was concentrated under reduced pressure. The residue was extracted with EtOAc and H_2O. The organic layer was washed with 5% aqueous citric acid, saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and then concentrated. The residue was chromatographed on silica gel with EtOAc as an eluent and recrystallized from isopropyl ether to give **12a** (130 mg, 66%) as a colorless solid. Compound **12b**~**f** were obtained by the similar procedure employed for the preparation of **12a**. Their physicochemical data are listed in Table 3.

N-[(2*S*,3*R*,4*R*,5*S*)-5-L-Leucylamino-2,3,4,6-tetrahydroxyhexanoyl]glycine (**13a**)

A solution of **12a** (60 mg, 0.10 mmol) in MeOH (5 ml)-H_2O (1 ml) was adjusted to pH 2.0 with 1 N HCl and then 10% palladium on charcoal (50 mg) was added to the solution. The mixture was vigorously stirred under an atmosphere of hydrogen at room temperature for 5 hours and filtered. The filtrate was neutralized with 1 N NaOH and evaporated to dryness under reduced pressure. The residue was dissolved in MeOH (5 ml) and sodium borohydride (10 mg, 0.26 mmol) was added to the solution. After being stirred at room temperature for 30 minutes, the mixture was evaporated under reduced pressure. The residue was dissolved in TFA (5 ml) and the solution was stirred at room temperature for 30 minutes and then evaporated. The residue was dissolved in H_2O (1 ml)-1 N NaOH (0.5 ml) and stirred at room temperature for 1 hour and then neutralized with 1 N HCl. After removal of solvent under reduced pressure, the residue was purified by CHP-20P (20 ml) column chromatography with H_2O (150 ml) as an eluent and recrystallized from EtOH to give **13a** (30 mg, 81%) as a colorless solid. Compound **13b**~**f** were obtained by the similar procedure employed for the preparation of **13a**. Their physicochemical data are listed in Table 3.

2-Carbobenzyloxyamino-2-deoxy-D-glucopyranose (**15**)

To a solution of D-glucosamine hydrochloride **14** (7.0 g, 33 mmol) in H_2O (200 ml) were added $NaHCO_3$ (8.20 g, 97.5 mmol) and carbobenzoxy chloride (5.54 g, 32.5 mmol). After being stirred at room temperature for 2 hours, the reaction mixture was extracted with THF-EtOAc (2 : 1, 300 ml×2). The extract was washed with brine, dried over Na_2SO_4, filtered and then concentrated under reduced pressure. The residue was recrystallized from isopropyl

ether to give **15** (7.0 g, 69%) as a colorless solid: mp 215~217°C; IR (KBr) 3354, 1684, 1549 cm^{-1}; ^{1}H-NMR (CD_3OD, TMS) δ 3.35~3.90 (6H, m), 5.05~5.20 (3H, m), 7.20~7.50 (5H, m); $[\alpha]_D^{23}$ +65.1 (*c* 0.10, MeOH); *Anal* Calcd for $C_{14}H_{19}NO_7$: C 53.67, H 6.11, N 4.47. Found: C 53.68, H 5.94, N 4.47.

Benzyl 2-Carbobenzyloxyamino-2-deoxy-D-glucopyranoside (**16**)

A mixture of **15** (2.50 g, 8.00 mmol) and acetyl chloride (0.17 ml, 2.4 mmol) in benzyl alcohol (20 ml) was stirred at 80°C for 1 hour. After being cooled to room temperature, Et_3N (0.56 ml, 4.0 mmol), hexane (100 ml) and Et_2O (100 ml) were added to the reaction mixture and the resulting precipitates were collected. The crude product was recrystallized from EtOH to give **16** (2.85 g, 89%) as a colorless solid: mp 164~165°C; IR (KBr) 3310, 1694, 1539 cm^{-1}; ^{1}H-NMR (CD_3OD, TMS) δ 3.35~4.00 (6H, m), 4.40~5.20 (5H, m), 7.20~7.50 (10H, m); $[\alpha]_D^{23}$ +48.3 (*c* 0.10, MeOH); *Anal* Calcd for $C_{21}H_{25}NO_7 \cdot 0.5H_2O$: C 61.16, H 6.35, N 3.40. Found: C 60.85, H 6.16, N, 3.56.

Benzyl 2-Carbobenzyloxyamino-2-deoxy-D-glucopyranosiduronic Acid (**17**)

A suspension of **16** (1.00 g, 2.50 mmol), $NaHCO_3$ (312 mg, 3.72 mmol) and platinum black (1.0 g) in H_2O (150 ml) was vigorously stirred and heated at 60°C under a continuous O_2 stream for 5 hours. After being cooled to room temperature, the mixture was filtered and the filtrate was concentrated to *ca.* 20 ml. The resulting aqueous solution was acidified with 1 N HCl and extracted with EtOAc - THF (1 : 1). The extract was washed with brine and dried over Na_2SO_4, filtered and concentrated. The residue was crystallized with isopropyl ether to give **17** (870 mg, 84%) as a pale yellow amorphous solid: IR (KBr) 3300, 1701, 1545 cm^{-1}; ^{1}H-NMR (CD_3OD, TMS) δ 3.40~3.80 (4H, m), 4.40~5.20 (5H, m), 7.20~7.40 (10H, m); $[\alpha]_D^{23}$ +32.2 (*c* 0.10, MeOH); *Anal* Calcd for $C_{21}H_{23}NO_8 \cdot 0.5H_2O$: C 59.15, H 5.67, N 3.28. Found: C 59.30, H 5.47, N, 3.29.

N-(Benzyl 2-Carbobenzyloxyamino-2-deoxy-α-D-glucopyranosiduronyl)-β-D-phenylalanine *tert*-Butyl Ester (**18**α)

To a stirred mixture of **17** (500 mg, 1.20 mmol), β-D-phenylalanine *tert*-butyl ester (329 mg, 1.56 mmol) and HOBt (210 mg, 1.56 mmol) in DMF (10 ml) was added DCC (321 mg, 1.56 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 and then concentrated. The residue was extracted with EtOAc and brine. The organic layer was washed with 5% aqueous citric acid, saturated aqueous $NaHCO_3$ and brine and then dried over Na_2SO_4, filtered and concentrated. The residue was chromatographed on silica gel with EtOAc - hexane (2 : 1) as an eluent and recrystallized from EtOAc to give **18**α (300 mg, 40%) and **18**β (350 mg, 47%) as a colorless solid. **18**α: mp 104~107°C; IR (KBr) 3325, 1716, 1661, 1520 cm^{-1}; ^{1}H-NMR (CD_3OD, TMS) δ 1.38 (9H, s), 2.81 (2H, d, J=6.8 Hz), 3.40~3.75 (3H, m), 4.02 (1H, d, J=10.0 Hz), 4.54 (1H, d, J=12.2 Hz), 4.72 (1H, d, J=12.2 Hz), 5.00 (1H, d, J=2.0 Hz), 5.06 (2H, s), 5.40 (1H, t, J=6.8 Hz), 7.20~7.40 (15H, m); $[\alpha]_D^{23}$ +66.3 (*c* 0.10, MeOH); *Anal* Calcd for $C_{34}H_{40}N_2O_9 \cdot 0.25H_2O$: C 65.32, H 6.53, N 4.48. Found: C 65.04, H 6.76, N 4.50. **18**β: mp 167~169°C; IR (KBr) 3314, 1716, 1661, 1537 cm^{-1}; ^{1}H-NMR (DMSO-d_6, TMS) δ 1.33 (9H, s), 2.72 (2H, d, J=8.2 Hz), 3.20~3.70 (4H, m), 4.40 (1H, d, J=5.0 Hz), 4.51 (1H, d, J=12.4 Hz), 4.78 (1H, d, J=12.4 Hz), 4.98 (1H, d, J=12.4 Hz), 5.07 (1H, d, J=12.4 Hz), 5.20~5.40 (1H, m), 7.20~7.50 (15H, m); $[\alpha]_D^{23}$ −32.7 (*c* 0.10, MeOH); *Anal* Calcd for $C_{34}H_{40}N_2O_9$: C 65.79, H 6.50, N 4.51. Found: C 65.69, H 6.41, N, 4.49.

N-[Benzyl 2-[(*tert*-Butoxycarbony-L-leucyl)amino]-2-deoxy-α-D-glucopyranosiduronyl]-β-D-phenylalanine *tert*-Butyl Ester (**19**)

A suspension of **18**α (150 mg, 0.24 mmol) and 10% palladium on charcoal (50 mg) in MeOH (20 ml) was vigorously stirred under an atmosphere of hydrogen at room temperature for 2 hours. The mixture was filtered and the filtrate was concentrated under reduced pressure and the residue was dissolved in DMF (5 ml). To the solution were added Boc-L-Leu (78 mg, 0.31 mmol), HOBt (49 mg, 0.36 mmol) and DCC (59 mg, 0.29 mmol) and the mixture was stirred at room temperature for 18 hours and concentrated. The residue was extracted with EtOAc and H_2O. The organic layer was washed with 5% aqueous citric acid, saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and then concentrated. The residue was chromatographed on silica gel with EtOAc - hexane (2 : 1) as an eluent and crystallized with isopropyl ether to give **19** (120 mg, 71%) as a colorless amorphous solid: IR (KBr) 3331, 2934, 1721, 1688, 1647, 1531 cm^{-1}; ^{1}H-NMR (CD_3OD, TMS) δ 0.85~1.00 (6H, m), 1.20~1.80 (3H, m), 1.38 (9H, s), 1.44 (9H, s), 2.82 (2H, d, J=7.0 Hz), 3.50~4.20 (5H, m), 4.55 (1H, d, J=12.0 Hz), 4.70 (1H, d, J=12.0 Hz), 5.05 (1H, d, J=3.2 Hz), 5.40 (1H, t, J=7.0 Hz), 7.20~7.45 (10H, m); $[\alpha]_D^{24}$ +12.6 (*c* 0.10, MeOH).

N-[(2*S*,3*S*,4*R*,5*S*)-5-L-Leucylamino-2,3,4,6-tetrahydroxyhexanoyl]-β-D-phenylalanine (**21**)

A solution of **19** (110 mg, 0.157 mmol) in MeOH (10 ml)-H_2O (1 ml) was adjusted to pH 3.0 with 1 N HCl and then 10% palladium on charcoal (60 mg) was added to the solution. The mixture was vigorously stirred under an atmosphere of hydrogen at room temperature for 18 hours and filtered. The filtrate was neutralized with 1 N NaOH and evaporated to dryness under reduced pressure. The residue was dissolved in MeOH (5 ml) and sodium borohydride (10 mg, 0.26 mmol) was added to the solution. After being stirred at room temperature for 30 minutes, the mixture was evaporated under reduced pressure. The residue was dissolved in TFA (5 ml) and the solution was stirred at room temperature for 1 hour and then evaporated. The residue was dissolved in H_2O (0.5 ml) and neutralized with 1 N NaOH and then 1 N HCl (0.5 ml) was added to the solution. After removal of solvent under reduced pressure, the residue was purified by CHP-20P (20 ml) column chromatography with H_2O (150 ml), CH_3CN-H_2O (1 : 10) as an eluent and recrystallized from MeOH-isopropyl ether to give **21** (46 mg, 63%) as a colorless solid: mp 149~151°C; IR (KBr) 3300, 1659, 1541 cm^{-1}; ^{1}H-NMR (D_2O, DSS) δ 0.90~1.05 (6H, m), 1.60~1.85 (3H, m), 2.72 (2H, d, J=7.2 Hz), 3.56~3.80 (3H, m), 3.88~4.06 (2H, m), 4.05~4.22 (2H, m), 5.14 (1H, t, J=7.2 Hz), 7.20~7.40 (5H, m). $[\alpha]_D^{25}$ −34.0 (*c* 0.10, H_2O); *Anal* Calcd for $C_{21}H_{33}N_3O_8 \cdot 0.5MeOH \cdot 0.5H_2O$: C 53.74, H 7.55, N 8.74. Found: C 53.78, H 7.44, N 8.56.

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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