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Synthesis of An Advanced Precursor to the Amino Epimer of (+)-Pyloricidins

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Summary. A consice synthesis of an advanced intermediate for the amino epimer of (+)-pyloricidins (the antipode of the natural ones) starting from *D*-galactose is presented.

Keywords. Amino acids; Azides; Carbohydrates; Drugs; Acylation.

Introduction

Pyloridin A, B, C, and D (**1a**–**1d**, isolated from *Bacillus* sp. HC-70 and HC-72) were reported [1] for the first time in 2001 by *Takeda* scientists. These compounds show significant activity against *Helicobacter pylori* [2], a *Gram*-negative bacterium that is a major cause for human gastric and duodental ulcers [3] and probably [4] also a promoting factor for gastric cancer.

Immediately after the report on the isolation and structure determination, the *Takeda* chemists disclosed their total syntheses [5] of pyloricidins using *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. They have also conducted investigations [6] on the structure-activity relationship, but mainly by altering the peptide chain at the amino group. In medicinal chemistry it is usually of interest to know what consequences in terms of activity might follow from a change of the configurations of a chiral drug. Herein we wish to report on the synthesis of an advanced intermediate, which on a few further manipulations would provide amino epimers of (+)-pyloricidins (the antipodes of the natural ones).

Results and Discussions

Our synthesis made full use of the stereogenic centers in the inexpensive chiral pool compound *D*-galactose. As shown in Scheme 1, *D*-galactose was first converted to its allyl acetal [7] by stirring at 60°C in allyl alcohol containing acetyl

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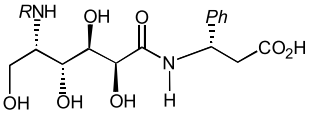
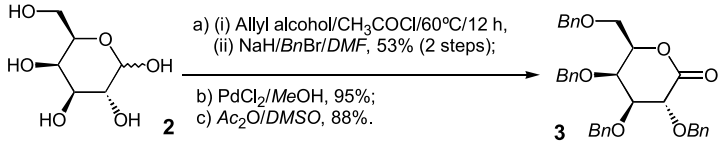
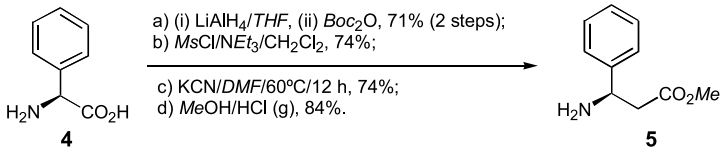


Pyloricidin A (**1a**): *R* = *L-Val-L-Val-L-Leu-*
Pyloricidin B (**1b**): *R* = *L-Val-L-Ile-L-Leu-*
Pyloricidin C (**1c**): *R* = *L-Leu-*
Pyloricidin D (**1d**): *R* = H

Fig. 1. Structures of pyloricidins



Scheme 1



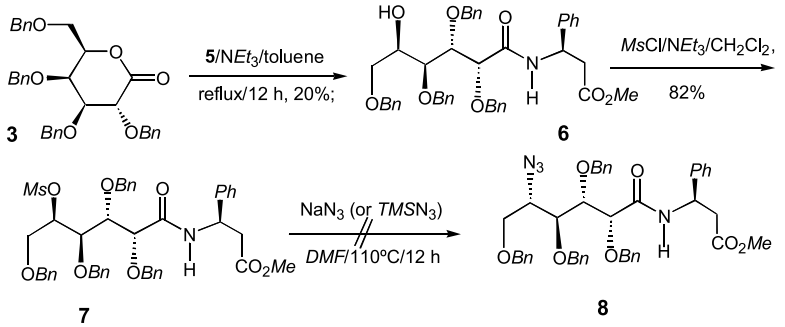
Scheme 2

chloride. The crude tetraol thus obtained was then converted [8] to the tetrabenzyl ether with NaH/*Bn*Br in 53% overall yield. The allyl protecting group was readily removed in *Me*OH in the presence of $PdCl_2$ following the work of *Liaigre* and coworkers [9]. An oxidation with acetyl anhydride in *DMSO* as previously reported by *Overkleeft et al.* [10] afforded (*2R,3S,4S,5R*)-3,4,5-tris(benzyloxy)-6-(benzyloxymethyl)tetrahydropyran-2-one (**3**).

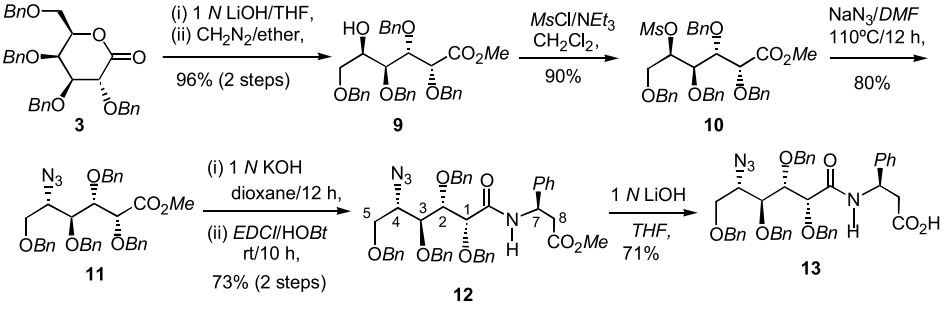
The β-amino acid was prepared via the route in Scheme 2. (*R*)-Phenylglycine (**4**) was reduced [11] to the corresponding aminol with $LiAlH_4$ and the amino group was protected [12] with Boc_2O (71.3% over two steps). The additional carbon was introduced by converting [13] the alcohol into the mesylate and treatment [14] with KCN. Methanolysis in HCl gas saturated *Me*OH with concurrent removal of the *Boc* protecting group gave methyl (*R*)-3-amino-3-phenylpropionate (**5**) [15] in 84% yield.

Lactone **3** was treated (Scheme 3) with **5** in refluxing toluene in the presence of a base such as pyridine, *DMAP*, or N*Et*$_3$, which led to methyl (*2R,3S,4S,5R*)-3-phenyl-3-(2,3,4,6-tetrakis(benzyloxy)-5-hydroxy-hexanoylamino)propionate (**6**) but only in rather low (20–30%) yields. Further transformation into the corresponding mesylate **7** proceeded smoothly. However, all efforts to introduce the azido functionality failed. Direct substitution with either NaN_3 or *TMS*N_3 resulted in a complex mixture, while an attempt to prepare the triflate (instead of the mesylate) of **6** led to formation of a lactam. Due to these difficulties we decided to adopt a longer route.

Thus, as shown in Scheme 4, the lactone **3** was converted to methyl (*2R,3S,4S,5R*)-(+)-2,3,4,6-tetrakis(benzyloxy)-5-hydroxyhexanoate (**9**) *via* base-



Scheme 3



Scheme 4

catalyzed hydrolysis followed by treatment with diazomethane. The hydroxyl-group in **9** was then mesylated with *Ms*Cl in the presence of N*Et*$_3$ in 90% yield. Interestingly, with a methoxy group instead of an amino acid at the carboxylic carbonyl carbon, the previously difficult reaction with NaN_3 (*ca.* 3 equiv.) became rather facile. After 12 h at 110°C in *DMF*, the desired methyl (*2R,3S,4S,5S*)-3-(5-azido-2,3,4,6-tetrakis(benzyloxy)-hexanoate (**11**) was obtained in 80% yield.

The methyl ester **11** was then readily hydrolyzed with 1 *N* KOH in 1,4-dioxane. The resulting acid was activated with *EDCI* [16] and further treated with **5** in the presence of *Bt*OH [16] to give methyl (*2R,3S,4S,5S*)-3-(5-azido-2,3,4,6-tetrakis (benzyloxy)hexanoylamino)-3-phenylpropionate (**12**), which is expected to yield the amino epimer of the antipodes of natural pyloricidins on reduction of the azido group, attaching a proper peptide chain, and removal of the benzyl protecting group. To ensure feasibility of cleavage of the terminal methyl ester in the presence of amido functionality, hydrolysis of **12** was also briefly examined. Indeed, on treament with 1 *N* LiOH in *THF* the methyl ester could be readily hydrolyzed into the free carboxylic acid **13** in 70% yield after neutralization.

Experimental

The ^{1}H NMR spectra were taken on either a Varian Mercury 300 (300 MHz) or a Bruker Avance 300 (300 MHz). The FT-IR spectra were measured on a Nicolet Avatar 360. EI-MS were recorded on an HP 5989A. ESI-MS spectra were taken on a PE Mariner API-TOF or an Agilent Technologies LC/MSD SL instrument. ESI-HRMS data were measured on a Bruker APEXIII 7.0 Tesla FT-MS spectrometer.

Optical rotations were measured on a Perkin Elmer Polarimeter 341. Elemental analyses were done using an Elementar Vario EL III autoanalyzer. Results of elemental analysis were found to agree favourably with the calculated values.

Methyl (2R,3S,4S,5R)-(+)-2,3,4,6-Tetrakis(benzyloxy)-5-hydroxy-hexanoate (**9**, $C_{35}H_{38}O_7$)

Aqueous LiOH (1 *N*, 2.4 cm^3) was added to a solution of 620 mg of **3** (1.16 mmol) in 5 cm^3 of *THF* stirred at 0°C and the stirring was continued at the same temperature until TLC showed disappearance of **3**. The reaction mixture was acidified with 1 *N* HCl to $pH = 2$–3, extracted with 3×30 cm^3 of CH_2Cl_2, washed with brine, and dried over anhydrous Na_2SO_4. After removal of the drying agent and the solvent, the residue was dissolved in diethyl ether and treated with ethereal CH_2N_2 at ambient temperature. When TLC showed complete reaction, the reaction mixture was evaporated to dryness on a rotary evaportaor and the residue was chromatographed on silica gel (*EtOAc*:*n*-hexane = 1:4) to give 460 mg of **9** as a colorless oil (71% yield), along with 200 mg of recovered **8** (0.31 mmol).

Data for **9**: $[\alpha]_D^{20} = +14.1°$ cm^2 g^{-1} ($c = 0.70$, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.40$–7.15 (m, 20H), 4.87 (d, $J = 11.4$ Hz, 1H, part of AB system 1), 4.69 (d, $J = 11.0$ Hz, 1H, part of AB system 1), 4.60 (d, $J = 11.9$ Hz, 1H, part of AB system 2), 4.52 (d, $J = 11.9$ Hz, 1H, part of AB system 2), 4.44 (d, $J = 11.8$ Hz, 1H), 4.42–4.29 (several sharp lines for 2 benzylic CH_2, 4H), 4.20 (dd, $J = 3.1$, 8.1 Hz, 1H), 4.11 (br q, $J = 7.0$ Hz, 1H), 3.84 (dd, $J = 1.3$, 8.1 Hz, 1H), 3.62 (s, 3H), 3.54 (dd, $J = 6.5$, 9.4 Hz, 1H), 3.84 (dd, $J = 6.2$, 9.7 Hz, 1H), 2.53 (d, $J = 7.8$ Hz, OH) ppm; FT-IR (film): $\bar{\nu} = 3494$ (br), 1754, 1101, 738 cm^{-1}; EI-MS: m/z (%) = 91 (100), 181 (13); ESI-MS: 571.3 ($[M + H]^+$).

Methyl (2R,3S,4S,5S)-(+)-5-azido-2,3,4,6-tetrakis(benzyloxy)hexanoate (**11**, $C_{35}H_{37}N_3O_6$)

*Ms*Cl (0.16 cm^3, 13.46 mmol) was added to a solution of 980 mg of **9** (1.72 mmol) in 15 cm^3 of dry CH_2Cl_2 containing 0.4 cm^3 of NEt_3 and stirred at 0°C. The stirring was continued at the same temperature until TLC showed disappearance of **9**. The reaction mixture was diluted with *EtOAc*, washed with brine, and dried over anhydrous Na_2SO_4. After removal of the drying agent and the solvent the residue was chromatographed on silica gel (*EtOAc*:*n*-hexane = 1:3) to afford 1.190 g of **10** as a colorless oil (90% yield): ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.40$–7.20 (m, 20 H), 5.14 (dt, $J = 6.3$, 4.2 Hz, 1H), 4.83 (d, $J = 10.9$ Hz, 1H), 4.73 (d, $J = 10.3$ Hz, 1H, part of AB system), 4.58 (d, $J = 10.6$ Hz, 1H, part of AB system), 4.54–4.30 (many sharp lines for 3 benzylic CH_2, 6H), 4.17 (dd, $J = 7.1$, 3.3 Hz, 1H), 3.99 (dd, $J = 7.3$, 3.8 Hz, 1H), 3.75 (dd, $J = 10.7$, 6.7 Hz, 1H), 3.69 (s, 3H), 3.65 (dd, $J = 10.6$, 4.0 Hz, 1H) ppm.

The above mesylate (390 mg, 0.60 mmol) was dissolved in 8 cm^3 of *DMF*. NaN_3 (140 mg, 2.15 mmol) was added. The mixture was heated to 110°C with stirring over night before cooling to ambient temperature, it was diluted with water, and extracted with 3×60 cm^3 of *EtOAc*. The combined *EtOAc* phases were washed with brine and dried over anhydrous Na_2SO_4. After removal of the drying agent and the solvent, the residue was chromatographed on silica gel (1:4 *EtOAc*:*n*-hexane = 1:4) to afford 283 mg of **11** as a colorless oil (80% yield): $[\alpha]_D^{20} = +8.0°$ cm^2 g^{-1} ($c = 1.30$, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.40$–7.15 (m, 20H), 4.80 (d, $J = 11.7$ Hz, 1H), 4.61 4.19 (many sharp lines for the 4 benzylic CH_2, 8H), 4.11 (m, 1H), 4.07 (dd, $J = 7.9$, 3.1 Hz, 1H), 3.80 (dd, $J = 2.9$, 7.9 Hz, 1H), 3.66 (s, 3H), 3.66–3.60 (m, 2H) ppm; FT-IR (film): $\bar{\nu} = 2101$, 1751, 1455, 1101, 698 cm^{-1}; EI-MS: m/z (%) = 91 (100), 181 (14); ESI-MS: 618.2 ($[M + Na]^+$).

Methyl (3R)-(+)-3-((2R,3S,4S,5S)-5-azido-2,3,4,6-tetrakis(benzyloxy)hexanoylamino)-3-phenylpropionate (**12**, $C_{44}H_{46}N_4O_7$)

Aqueous KOH (1 *N*, 0.43 cm^3) was added to a solution of 253 mg of **11** (0.43 mmol) in 5 cm^3 of 1,4-dioxane. After stirring for 1.5 h, another portion of KOH (1 *N*, 0.43 cm^3) was introduced. The mixture was then stirred at ambient temperature over night before it was diluted with 20 cm^3 of H_2O, acidified

with 1 *N* HCl to *pH* = 2–3, and extracted with 3 × 30 cm^3 of *EtOAc*. The combined *EtOAc* phases were washed with brine and dried over anhydrous Na_2SO_4. After removal of the drying agent and the solvent, the residue was dissolved in 5 cm^3 of dry CH_2Cl_2. (*R*)-*β*-Amino-phenylacetic acid (100 mg, 0.35 mmol) was added with cooling (ice-H_2O), followed by 75 mg of HO*Bt* (0.55 mmol). Two minutes later, 110 mg of *EDCI* (0.57 mmol) were introduced. The bath temperature was allowed to rise naturally to ambient temperature. About 10 h later, the reaction mixture was diluted with *EtOAc*, washed with aq. NH_4Cl and brine, and dried over anhydrous Na_2SO_4. After removal of the drying agent and the solvent, the residue was chromatographed on silica gel (*EtOAc*:*n*-hexane = 1:4) to give 190 mg of **12** as a colorless oil (73% yield from **11**): ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.73$ (d, $J = 8.5$ Hz, NH), 7.40–7.15 (m, 25H, aromat), 5.32 (br q, $J = 6.7$ Hz, H-7), 4.75 (d, $J = 11.5$ Hz, H-1), 4.60 (d, $J = 11.8$ Hz, 1H, part of AB system), 4.57 (d, $J = 11.5$ Hz, 1H, part of AB system), 4.50–4.20 (several sharp lines for the benzylic CH_2, 5H), 4.33 (dd, $J = 11.4$, 4.2 Hz, H-2), 4.16–4.08 (m, H-4 and 1H from the benzylic CH_2), 3.86 (dd, $J = 8.3$, 2.5 Hz, H-3), 3.64 (dd, $J = 10.2$, 4.0 Hz, H-5), 3.58 (br d, $J = 10.9$ Hz, H-5), 3.48 (s, OCH_3), 2.73 (dd, $J = 15.9$, 5.3 Hz, H-8), 2.44 (dd, $J = 15.8$, 6.8 Hz, H-8) ppm (an arbitary numbering system as shown in Scheme 4 was adopted for easier assignment, which was assisted by a COSY spectrum); FT-IR (film): $\bar{\nu} = 3405$ (br), 2100, 1738, 1676, 1092, 737 cm^{-1}. EI-MS: m/z (%) = 91 (100), 121 (10); ESI-MS: 743.3 ($[M + H]^+$); ESI-HRMS: calcd. for $C_{44}H_{47}O_7N_4$ ($[M+H]^+$) 743.3433; found 743.3439; $[\alpha]_D^{20} = +5.6^\circ$ cm^2 g^{-1} ($c = 1.39$, $CHCl_3$).

(3R)-(+)-3-((2R,3S,4S,5S)-5-azido-2,3,4,6-tetrakis(benzyloxy)hexanoylamino)-3-phenylpropionic acid (**13**, $C_{43}H_{44}N_4O_7$)

Aqueous LiOH (1 *N*, 0.24 cm^3) was added to a solution of 183 mg of **12** (0.25 mmol) in 2 cm^3 of *THF*. The mixture was stirred at ambient temperature until TLC showed disappearance of **12**. The reaction mixture was diluted with 20 cm^3 of H_2O, acidified with 1 *N* HCl to *pH* = 2–3, and extracted with 3 × 40 cm^3 of *EtOAc*. The combined *EtOAc* phases were washed with brine and dried over anhydrous Na_2SO_4. After removal of the drying agent and the solvent the residue was chromatographed on silica gel (CH_2Cl_2/*Me*OH = 20:1) to afford 165 mg of **13** as a colorless oil (71% yield): ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.62$ (d, $J = 8.3$ Hz, 1H), 7.40–7.15 (m, 25H), 5.30 (m, 1H), 4.75 (d, $J = 10.2$ Hz, 1H), 4.57–4.22 (several sharp lines for benzylic CH_2, 7H), 4.34 (dd, $J = 4.3$, 10 Hz, 1H), 4.10 (dd, $J = 1.8$, 8.3 Hz, 1H), 4.12–4.09 (several lines for benzylic CH_2, 1H), 3.85 (dd, $J = 2.0$, 8.3 Hz, 1H), 3.63 (dd, $J = 10.7$, 4.3 Hz, 1H), 3.56 (br d, $J = 10.2$ Hz, 1H), 2.73 (dd, $J = 16.2$, 5.4 Hz, 1H), 2.43 (dd, $J = 7.2$, 16.4 Hz, 1H) ppm; FT-IR (film): $\bar{\nu} = 2930$ (br), 2100, 1720, 1671, 1011, 737 cm^{-1}; ESI-MS: 729.3 ($[M + H]^+$); ESI-HRMS: calcd. for $C_{43}H_{44}O_7N_4Na$ ($[M + Na]^+$) 751.3102; found 751.3114; $[\alpha]_D^{20} = +6.0^\circ$ cm^2 g^{-1} ($c = 2.1$, $CHCl_3$).

Acknowledgments

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