Synthesis of (3*S*, 4*S*)- and (3*S*, 4*R*)-4-Amino-3-hydroxy-6-methylheptanoic Acid, and Their *N*-(Acetyl-L-valyl-L-valyl) Derivatives

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Natural (−)(3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid (**10a**) present in pepstatins, specific inhibitors of acid proteases, and its (+)(3*S*,4*R*) diastereomer (**10b**) were synthesized starting from 3-deoxy-1,2-*O*-isopropylidene-α-D-*erythro*-pentodialdo-1,4-furanose (**1**) through highly stereoselective and stereospecific routes. The partial peptide of pepstatin Ac, *N*-(acetyl-L-valyl-L-valyl)-(3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid (**15a**) and its (3*S*,4*R*) diastereomer (**15b**) were synthesized *via* azide-coupling of the *t*-butyl esters of **10a** and **10b** with acetyl-L-valyl-L-valine azide, respectively. Compound **15a** inhibited proteolysis by pepsin, however, the diastereomer **15b** showed no inhibition against pepsin.

A new amino acid, (−)4-amino-3-hydroxy-6-methylheptanoic acid[1b](abbreviated (−)AHMHA) which has been isolated from the hydrolyzates of all kinds of pepstatins,[1a–1e] specific inhibitors of acid proteases, was first synthesized together with its (−)diastereomer from L-leucine by Morishima *et al.*[2] Recently, we reported in a communication[3] that the natural (−)AHMHA was identical with (3*S*,4*S*)AHMHA (**10a**), through stereospecific syntheses of all four diastereomers of AHMHA from the appropriate deoxy-sugar derivatives. The synthetically determined absolute configuration 3(*S*), 4(*S*) of natural (−)AHMHA was consistent with the result of the X-ray diffraction study on the natural amino acid which was independently performed by Nakamura *et al.*[4]

The recent studies on pepstatin by Umezawa *et al.*[5,6] have revealed the interesting relationships between the structures of pepstatins and some partial peptides containing natural (−)AHMHA and their inhibition activities against acid proteases. In connection with these studies, it was considered to be important to evaluate the stereochemical effect concerning to the 4(*S*) configuration of natural AHMHA, which may play its role in the enzyme-inhibition activity in the peptide chains of pepstatins or their partial peptides. This paper presents the details of the previously reported synthesis[3] of natural (−)(3*S*,4*S*)AHMHA(**10a**) and its diastereomer (+)(3*S*, 4*R*)AHMHA(**10b**), and the preparation of *N*-(acetyl-L-valyl-L-valyl)-(3*S*,4*S*)AHMHA(**15a**) which is a partial peptide of pepstatin Ac,[1c,1e]) and its diastereomer, *N*-(acetyl-L-valyl-L-valyl)-(3*S*,4*R*)AHMHA (**15b**).

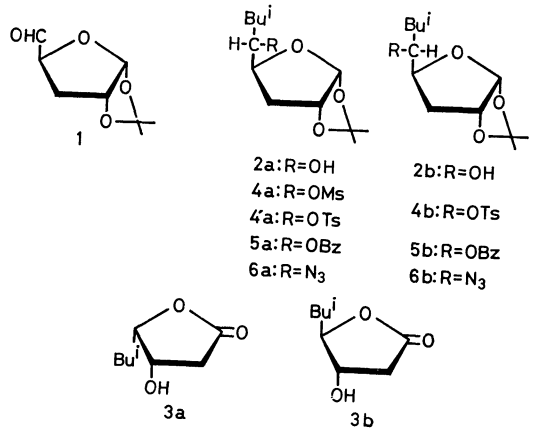

Chart 1.

For the purpose of the stereospecific synthesis of the natural (3*S*,4*S*)AHMHA(**10a**) and its diastereomer (3*S*,4*R*)AHMHA (**10b**), two kinds of diastereomeric deoxy-sugar derivatives, *i.e.*, 3-deoxy-5-*C*-isobutyl-1,2-*O*-isopropylidene-β-L-*lyxo*-pentofuranose(**2a**) and 3-deoxy-5-*C*-isobutyl-1,2-*O*-isopropylidene-α-D-*ribo*-pentofuranose(**2b**) were selected as the starting materials.

The Grignard reaction of 3-deoxy-1,2-*O*-isopropylidene-α-D-*erythro*-pentodialdo-1,4-furanose[7] (**1**) with isobutylmagnesium bromide in ether yielded a crude crystalline addition product (91% yield), which was recrystallized from petroleum ether to afford the pure isomer **2a** in a 67% yield. The 5(*S*) configuration of **2a** was confirmed by the fact that hydrolysis of **2a** followed by oxidation with periodate-hypoiodite[8] gave (3*S*,4*S*)-3,4-dihydroxy-6-methylheptanoic acid-1,4-lactone(**3a**), the 4(*S*) configuration of which was determined by application of Hudson's lactone rule[9] on the rotational change ($\Delta[\alpha]_D^{MeOH}$ (Lactone−potassium salt)=−36 °) of **3a** measured by Witkop's method.[10]

In the following fashion it was ascertained that the Grignard reaction product contained the diastereomer (**2b**) of **2a** as a minor product and that in this case the Grignad reaction was highly stereoselective (about 91%). The minor product **2b** which was concentrated in the mother liquor of the recrystallization of the Grignard reaction product could be isolated in a form of its 5-*O*-benzoyl derivative(**5b**) which was easily separable by silica gel chromatography from the isomeric 5-benzoate(**5a**).

The compound **2a** was converted with methylsulfonyl chloride in pyridine to 5-mesylate (**4a**), which was treated with sodium benzoate in dimethylformamide (DMF) at 140 to 150 °C to afford **5b** in a 52% yield. De-*O*-benzoylation of **5b** with sodium methoxide in methanol gave **2b** in an 81% yield. The 5(*R*) configuration of **2b** was confirmed by the same procedure as that in the case of **2a**; hydrolysis of **2b** followed by oxidation gave (3*S*,4*R*)-3,4-dihydroxy-6-methylheptanoic acid-1,4-lactone(**3b**) which showed a rotational change $\Delta[\alpha]_D^{MeOH}$ +24 °. Compounds **2a** and **2b** could not be separated by tlc in any of the solvent systems examined. The PMR spectra of **2a** and **2b** were apparently distinguishable in the region at δ 3.63 to 4.40, which contains the signals attributed to C–4 and C–5 protons.

Treatment of 5-tosylate (**4b**) prepared from **2b** with sodium azide in DMF at 90 to 97 °C gave 5-azido-3,5-dideoxy-5-*C*-isobutyl-1,2-*O*-isopropylidene-β-L-*lyxo*-pentofuranose (**6a**) as a sole product in an 85% yield. The configuration of C–5 in **6a** was assumed to be "S" because a facile S_N2 displacement of the 5-tosyloxy group by azide ion would proceed under the similar condition to that for the S_N2 reaction of the 5-mesyloxy group in **4a** by benzoate ion. Based on the same principle, the isomeric 5-tosylate **4'a** was treated with sodium azide in DMF at 87 to 95 °C to afford the epimeric (5*R*)-azido compound (**6b**) in an 81% yield.

Hydrolysis of **6a** with 50% aqueous acetic acid, followed by oxidation with sodium periodate gave (3*S*,4*S*)-4-azido-3-formyloxy-6-methylheptanal (**7a**) as a practically pure syrup in a 96% yield. In the same manner the compound **6b** afforded the isomeric aldehyde (**7b**) in a 98% yield.

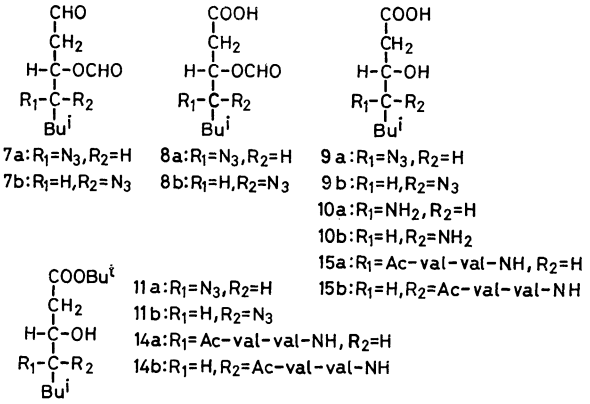

Chart 2.

Oxidations of **7a** and **7b** with a solution of chromium trioxide in acetic acid and pyridine[11] at room temperature afforded (3*S*,4*S*)-4-azido-3-formyloxy-6-methylheptanoic acid (**8a**) and its isomer **8b** as colorless syrup in 76% and 63% yields, respectively. De-*O*-formylations of **8a** and **8b** with dilute hydrogen chloride in aqueous dioxane at room temperature gave the corresponding azido hydroxy acids, **9a**(syrup) and **9b**(crystal) in 72% and 58% overall yields from **7a** and **7b**, respectively. The diastereomeric **9a** and **9b** were stable and their structures were confirmed by elemental analyses, IR, and PMR spectroscopy. By the use of the chromium trioxide solution, instead of the hypoiodite reagent which was previously used[3] the yields of the synthetic steps, **7a**→**9a** and **7b**→**9b** were remarkably improved (about twice).

Hydrogenolysis of **9a** on palladium black in methanol gave (−)(3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid (**10a**) in a 93% yield. The synthetic amino acid **10a** was proved to be identical with natural (−)AHMHA by mixture mp, optical rotation, PMR, IR, and high performance liquid chromatography. Hydrogenolysis of **9b** gave the diastereomeric (+)(3*S*,4*R*)AHMHA (**10b**, 81% yield), whose high performance liquid chromatogram showed a single peak having a longer elution time than that of **10a**.

The modified azide coupling according to Rudinger[12] between the free amino acid **10a** and *N*-acetyl-L-valyl-L-valine azide yielded only a small amount of condensation product, *N*-(acetyl-L-valyl-L-valyl)-(3*S*,4*S*)AHMHA (**15a**), probably because of poor solubility of **10a** in DMF. From this result it was decided to use *t*-butyl ester of **10a** for the azide coupling reaction.

t-Butylations of **8a** and **8b** with isobutene and a catalytic amount of sulfuric acid in dichloromethane, followed by de-*O*-formylations with sodium hydroxide in aqueous dioxane afforded the isomeric azidohydroxy acid *t*-butyl esters, **11a** and **11b** in 57% and 58% yields, respectively. Hydrogenolysis of **11a** on palladium black gave *t*-butyl ester of **10a** in quantitative yield, which was immediately coupled with the acid azide prepared from *N*-acetyl-L-valyl-L-valine hydrazide (**13**) by the Rudinger modification to afford *N*-(acetyl-L-valyl-L-valyl)-(3*S*,4*S*)AHMHA *t*-butyl ester (**14a**) in a 74% yield. Treatment of **14a** with trifluoroacetic acid yielded **15a** in a 94% yield. In the same method, the *t*-butyl ester **10b** prepared from **11b** was coupled with the acid azide. Resulting peptide ester (**14b**, 75%) was de-*t*-butylated to give the isomeric peptide (**15b**, 93%).

The synthetic partial peptide **15a**, which contains natural AHMHA, inhibited proteolysis of pepsin at the concentration of 6 μg/ml, in contrast, the diastereomeric peptide **15b** showed no inhibition against pepsin even the high concentration of 250 μg/ml. From this results it is noteworthy that the enzyme-inhibitory activities of pepstatins and their partial peptides may be reflected by the (*S*) configuration of the 4-amino group of AHMHA present in their peptide chains.

Experimental

Melting points were determined on a Mitamura Riken micro hot-stage and uncorrected. IR spectra were taken on a Hitachi IPI–2 spectrometer. PMR spectra were recorded on a Varian A–60D and HA–100D spectrometers using TMS as internal and external standards. Specific rotations were determined with a Zeiss Photoelectric Precision Polarimeter. TLC was performed on WAKOGEL B–5 and silica gel column chromatography on WAKOGEL C-200 which was activated at 110 °C for 1 hr. The following solvent systems were used: A_1 [benzene-ethyl acetate (12 : 1)], A_2[*ibid.* (50 : 1)], A_3[*ibid.* (3 : 1)], A_4[*ibid.* (1 : 1)], B_1[hexane-ethyl acetate (6 : 1)], B_2[*ibid.* (20 : 1)], B_3[*ibid.* (30 : 1)], B_4[*ibid.* (10 : 1)], C[hexane-acetone (6 : 1)], D_1 [hexane-butanone-acetic acid (10 : 1 : 0.17)], D_2[*ibid.* (20 : 2 : 1)], E_1[Chloroform-methanol (30 : 1)] E_2[*ibid.* (20 : 1)], F[chloroform-methanol-acetic acid (80 : 10 : 1)] and G [butanol-ethanol-chloroform-17% aqueous ammonia (7 : 3 : 3 : 3)]. High performance liquid chromatography (HPLC) was carried out on a Waters Associates Liquid Chromatograph ALC 201(RI–Detector) employing an Aminex A–7 (Bio–Rad) column (2.2×600 mm) with 0.1 M pyridine-acetate buffer (pH 5.00) in a flow rate of 0.1 ml/min at 95 °C. In general, all concentrations were carried out under reduced pressure below 40 °C

*1) 3-Deoxy-5-*C*-isobutyl-1,2-*O*-isopropylidene-β-*L*-lyxo-pentofuranose (**2a**).* A solution of 3-deoxy-1,2-*O*-isopropylidene-α-D-*erythro*-pentodialdo-1,4-furanose[7] (**1**) (5.28 g, 31 mmol) in dry ether (240 ml) was added dropwise with stirring over a period of 10 min to an ether solution of iso-

butylmagnesium bromide prepared from magnesium turnings (6.01 g, 247 mmol) and isobutyl bromide (26.6 ml, 247 mmol) in dry ether (100 ml). The mixture which was stirred for 40 min at room temperature, was decomposed with cold water (200 ml), and neutralized with ice-cooled 10% sulfuric acid. The aqueous layer was extracted with ether (200 ml × 4). The combined ether layers were washed with saturated aqueous NaCl, dried (Na_2SO_4), and evaporated to afford a crystalline solid (6.40 g, 91%). Recrystallization from petroleum ether gave pure material (**2a**): yield 4.70 g (67%), mp 62.5—63.0 °C, $[\alpha]_D^{19}$ −33° (*c* 1.86, chloroform), δ ($CDCl_3$), 0.93 and 0.95 (each d, $(CH_3)_2CH$, J=6.4 Hz], 1.33 and 1.52 [each s, $(CH_3)C$], 3.63 (dq, H-5, $J_{5,6}$=9.0 Hz, $J_{5,6}$=4.0 Hz), 4.20 (quintet, H-4, $J_{4,5}$=5.2 Hz, $J_{3,4}$=10.2 Hz, $J_{3',4}$=5.2 Hz), 4.82 (dt, H-2, $J_{2,3}$=3.9 Hz, $J_{2,3'}$=~0.5 Hz), and 5.90 (d, H-1, $J_{1,2}$=3.8 Hz).

Found: C, 62.73; H, 9.45%. Calcd for $C_{12}H_{22}O_4$: C, 62.58; H, 9.63%.

*2) 5-O-Benzoyl-3-deoxy-5-C-isobutyl-1,2-O-isopropylidene-β-L-lyxo-pentofuranose (**5a**).* Benzoyl chloride (0.11 ml, 0.94 mmol) was added to a solution of **2a** (108 mg, 0.47 mmol) in pyridine (1 ml), and the mixture was kept at room temperature overnight. It was then diluted with ice-cooled water (6 ml) and extracted with chloroform (3 ml × 4). The chloroform extracts were washed successively with saturated aqueous $KHSO_4$ (10 ml), saturated aqueous $NaHCO_3$ (5 ml), and saturated aqueous NaCl (10 ml), dried and evaporated. The syrupy residue (154 mg) immediately crystallized: recrystallization from ether afforded the pure benzoate **5a** (127 mg, 81%); mp 108.5—109 °C, $[\alpha]_D^{13}$ −43° (*c* 2.28, chloroform).

Found: C, 68.56; H, 7.94%. Calcd for $C_{19}H_{26}O_5$: C, 68.24; H, 7.84%.

*3) 3-Deoxy-5-C-isobutyl-1,2-O-isopropylidene-5-O-methanesulfornyl-β-L-lyxo-pentofuranose (**4a**).* To a solution of **2a** (2.64 g, 11.6 mmol) in dry pyridine (26.4 ml) was added methyl-sulfonyl chloride (1.8 ml, 23.2 mmol) and the mixture was set aside overnight at room temperature. Workup in the same manner as described in Exp. 2 afforded a practically pure sample of 5-mesylate **4a** (3.66 g, 100%) as a pale yellow syrup. Silica gel column chromatography of the sample (85 mg) with the solvent A_1 gave an analytical sample of **4a** (72 mg, 85%) as a colorless syrup: $[\alpha]_D^{13}$ −49° (*c* 2.7, chloroform).

Found: C, 50.30; H, 7.70; S, 10.56%. Calcd for $C_{13}H_{24}O_6S$: C, 50.63; H, 7.84; S, 10.40%.

*4) 3-Deoxy-5-C-isobutyl-1,2-O-isopropylidene-5-O-p-toluenesulfonyl-β-L-lyxo-pentofuranose (**4'a**).* Tosylation of **2a** was performed in a usual manner to afford **4'a** as a colorless syrup in a 80% yield: $[\alpha]_D^{13}$ −32° (*c* 2.1 chloroform).

Found: C, 59.51; H, 7.33; S, 8.17%. Calcd for $C_{19}H_{28}O_6S$: C, 59.35; H, 7.34; S; 8.34%.

*5) 5-O-Benzoyl-3-deoxy-5-C-isobutyl-1,2-O-isopropylidene-α-D-ribo-pentofuranose (**5b**).* A solution of **4a** (764 mg, 2.48 mmol) in DMF (55 ml) containing sodium benzoate (1.07 g, 7.43 mmol) was heated at 140—150 °C for 2 hr; tlc (solvent B_1) then revealed the absence of **4a**. The precipitare deposited was filtered off and washed with ethyl acetate. The filtrate and washings were diluted with cold water (100 ml) and was extracted with ethyl acetate (50 ml × 3). The combined extracts were washed with saturated aqueous NaCl (30 ml × 2), dried and evaporated. The syrupy residue (808 mg) was purified through a silica gel column (120 g, 3 × 40 cm) with the solvent B_2 to afford a pure sample of **5b** (405 mg, 52%): $[\alpha]_D^{21}$ +13° (*c* 1.31, chloroform).

Found: C, 67.99; H, 7.83%. Calcd for $C_{19}H_{26}O_5$: C, 68.24; H, 7.84%.

*6) 3-Deoxy-5-C-isobutyl-1,2-O-isopropylidene-α-D-ribo-pentofuranose (**2b**).* To a solution of **5b** (900 mg, 2.7 mmol) in dry methanol (4.5 ml) was added 1.32 M methanolic sodium methoxide (4.07 ml, 5.4 mmol) and the mixture was kept for 5 hr at room temperature. An excess of sodium methoxide was neutralized with 20% acetic acid; the solution was evaporated and the residue was extracted with chloroform (20 ml × 3). The extracts were washed with saturated aqueous NaCl (20 ml × 2), dried and evaporated to give a crystalline mass (620 mg) wihch was recrystallized from petroleum ether: yield of pure **2b** 500 mg (81%), mp 56—57 °C, $[\alpha]_D^{15}$ −4° (*c* 1.38, chloroform), δ ($CDCl_3$), 0.92 and 0.95 [each d, $(CH_3)_2CH$, J=6.3 Hz], 1.33 and 1.52 [each s, $(CH_3)_2C$], 3.8—4.4 (m, H-4 and H-5), 4.80 (dq, H-2, $J_{2,3}$=6.0 Hz, $J_{2,3'}$=0.7 Hz) and 5.89 (d, H-1, $J_{1,2}$=3.8 Hz).

Found: C, 62.27; H, 9.45%. Calcd for $C_{12}H_{22}O_4$: C, 62.58; H, 9.63%.

*7) 3-Deoxy-5-C-isobutyl-1,2-O-isopropylidene-5-O-p-toluenesulfonyl-α-D-ribo-pentofuranose (**4b**).* The tosylation of **2b** was performed in a usual manner to afford **4b** as a colorless syrup: $[\alpha]_D^{15}$ −1° (*c* 1.0, chloroform).

Found: C, 59.47; H, 7.28; S, 8.49%. Calcd for $C_{19}H_{28}O_6S$: C, 59.35; H, 7.34; S, 8.34%.

*8) (3S,4S)-3,4-Dihydroxy-6-methylheptanoic Acid-1,4-lactone (**3a**).* Treatment of **2a** (202 mg) with 20% acetic acid at 120 °C for 20 min gave a syrup of 3-deoxy-5-C-isobutyl-L-lyxo-pentose (165 mg). The free sugar was dissolved in acetone (3 ml) and to the solution was added a solution of sodium metaperiodate (645 mg) in water (6.3 ml). After standing at room temperature for 20 min, the precipitate was filtered off and acetone was removed from the filtrate by evaporation. The aqueous layer was extracted with chloroform and the extract was dried and evaporated to afford a syrup (165 mg), which was immediately subjected to the next oxidation reaction. To a solution of the product (163 mg) in dioxane (4 ml) and water (1.6 ml) was added a solution of K_2CO_3 (855 mg) and $KHCO_3$ (618 mg) in water (6 ml) and then a solution of iodine (730 mg) and KI (912 mg) in water (0.8 ml), and the mixture was stirred for 1 hr. The excess of iodine was reduced with solid sodium thiosulfate and then the solution was neutralized with 10% sulfuric acid, extracted with ether (10 ml × 3), and the extracts were washed with saturated aqueous NaCl, dried and evaporated. The residual syrup (81 mg) was purified by silica gel chromatography with the solvent C to afford a pure sample of **3a** a colorless liquid: yield 31 mg (23%). Optical rotation in methanol; $[\alpha]_D$ −81°, $[\alpha]_{436}$ −167°, and $[\alpha]_{365}$ −266° (*c* 1.12, 21 °C). Optical rotation in methanol-0.1 M aqueous KOH (1 : 1 vol); $[\alpha]_D$ −45°, $[\alpha]_{436}$ −89°, and $[\alpha]_{365}$ −130° (*c* 0.56, 21 °C). IR: $\nu_{max}^{CCl_4}$ 3480 (OH) and 1775 cm^{-1} (γ-lactone).

Found: C, 60.71; H, 8.79%. Calcd for $C_8H_{14}O_3$: C, 60.74; H, 8.92%.

*9) (3S,4R)-3,4-Dihydroxy-6-methylheptanoic Acid-1,4-lactone (**3b**).* The title compound **3b** was obtained from **2b** (176 mg) in the same procedure as described in Exp. 8; colorless liquid, yield 49 mg (42%). Optical rotation in methanol; $[\alpha]_D$ +41°, $[\alpha]_{436}$ +76°, and $[\alpha]_{365}$ +105° (*c* 1.73, 18 °C). Optical rotation in methanol-0.1 M aqueous KOH (1 : 1 vol); $[\alpha]_D$ +17°, $[\alpha]_{436}$ +34°, and $[\alpha]_{365}$ +57° (*c* 0.87, 18 °C). IR; $\nu_{max}^{CCl_4}$ 3480 (OH) and 1775 cm^{-1} (γ-lactone).

Found: C, 60.57; H, 8.73%. Calcd for $C_8H_{14}O_3$: C, 60.74; H, 8.92%.

10) Isolation of the Minor Product **2b** *produced in the Grignard Reaction of* **1** *with Isobutylmagnesium Bromide.* A crude product (898 mg) of the Grignard reaction, which was obtained in the same manner as described in Exp. 1, was chromatographed through a silica gel column (100 g, 2.8×38 cm) with the solvent A_3. The effluent containing the reaction product was collected in the fore-run, the main fraction, and the after-run, which gave crystal I (pure **2a**, 36 mg), crystal II (**2a** contaminated with a small amount of **2b**, 660 mg), and crystal III (a 4 : 3 mixture of **2a** and **2b**, 18 mg), respectively. The ratio of **2a** to **2b** in the mixture was estimated by PMR analysis. The crystals II and III were combined and recrystallized from petroleum ether to afford pure **2a** (485 mg, 72% yield). The mother liquor was evaporated and the residual solid (143 mg, *ca.* 4 : 3 mixture of **2a** and **2b**) was benzoylated with benzoyl chloride (0.15 ml) in pyridine (1.41 ml). The product was chromatographed on a silica gel column (30 g, 2.2×20 cm) with the solvent B_2 to give **5b** (76 mg, syrup) as the faster-moving component, a mixture of **5a**+**5b** (21 mg, syrup), and **5a** (95 mg, mp 107—108.5 °C) as the slower-moving component. From these results, the content of **2b** in the original Grignard reaction product may be estimated as about 9%.

*11) 5-Azido-3,5-dideoxy-5-*C*-isobutyl-1,2-*O*-isopropylidene-β-*L*-lyxo-pentofuranose (**6a**).* A solution of **4b** (1.17 g, 3.05 mmol) in dry DMF (26 ml) was heated at 90—97 °C with sodium azide (646 mg, 9.93 mmol) for 2.5 hr, and then poured into cold water (130 ml). The mixture saturated with NaCl was extracted with chloroform (50 ml×3). The extracts were washed with saturated aqueous NaCl (80 ml), dried and evaporated. The residual syrup (742 mg) was purified through a silica gel column (90 mg, 2.8×38 cm) with the solvent A_2 to afford **6a** as a colorless syrup. Yield 657 mg (85%); $[\alpha]_D^{18}$ −61° (*c* 0.35, chloroform); $\nu_{max}^{CCl_4}$ 2140 cm^{-1} (N_3); δ ($CDCl_3$), 0.94 and 0.96 [each d, $(CH_3)_2CH$ J=6.0 Hz), 1.33 and 1.51 [each s, $(CH_3)_2C$], 3.08—3.40 (m, H-5), 4.28 (quintet, H-4, $J_{4,5}$=5.0 Hz, $J_{3,4}$=5.0 Hz, $J_{3',4}$=10.0 Hz), 4.78 (t, H-2, $J_{2,3}$=5.0 Hz, $J_{2,3'}$=1.0 Hz) and 5.89 (d, H-1, $J_{1,2}$=3.8 Hz).

Found: C, 56.70; H, 8.29; N, 16.71%. Calcd for $C_{12}H_{21}O_3N_3$: C, 56.45; H, 8.29; N, 16.46%.

*12) 5-Azido-3,5-dideoxy-5-*C*-isobutyl-1,2-O-isopropylidene-α-*D*-ribo-pentofuranose (**6b**).* The tosylate **4'a** (2.17 g, 5.66 mmol) was treated with sodium azide (1.13 g, 17.4 mmol) in dry DMF (40 ml) at 87—95 °C for 1.7 hr. The S_N2 reaction product (1.63 g) was isolated by the procedure of Exp. 11, and purified by chromatography on silica gel (90 g) with the solvent B_3 to afford **6b** as a colorless syrup. Yield 1.17 g (81%); $[\alpha]_D^{20}$ −31° (*c* 1.88, chloroform); $\nu_{max}^{CCl_4}$ 2140 cm^{-1} (N_3); δ ($CDCl_3$), 0.97 [d, $(CH_3)_2CH$, J=6.0 Hz], 1.35 and 1.52 [each s, $(CH_3)_2C$], 3.60—3.95 (m, H-5), 4.30 (dq, H-4), 4.81 (dq, H-2, $J_{2,3}$=5.5 Hz, $J_{2,3'}$=1.0 Hz), and 5.89 (d, H-1, $J_{1,2}$=3.7 Hz).

Found: C, 56.70; H, 8.26; N, 16.48%. Calcd for $C_{12}H_{21}O_3N_3$: C, 56.45; H, 8.29; N, 16.46%.

*13) (3S,4S)-4-Azido-3-formyloxy-6-methylheptanal (**7a**).* Treatment of **6a** (589 mg) in 50% aqueous acetic acid (10 ml) at 115—125 °C for 20 min, followed by concentration afforded a pale red syrup (497 mg), which was dissolved in acetone (5.6 ml) and treated with a solution of sodium metaperiodate (1.48 g) in water (12.5 ml). After standing at room temperature for 15 min, the reaction mixture was concentrated to an aqueous residue, which was extracted with chloroform (10 ml×3). The extracts were washed with saturated aqueous NaCl, dried and evaporated to give **7a** as a pale yellow syrup; yield 470 mg (96%). This sample appeared as a practically pure material by tlc (solvent A_4) and PMR: $\nu_{max}^{CCl_4}$ 2100 (N_3) and 1730 cm^{-1} (CHO and OCHO); δ ($CDCl_3$), 8.28 (s, OCHO) and 10.07 (dd, CH_2CHO).

*14) (3S,4R)-4-Azido-3-formyloxy-6-methylheptanal (**7b**).* By the procedure described in Exp. 13, **7b** was obtained from **6b** in a 98% yield: $\nu_{max}^{CCl_4}$ 2100 (N_3) and 1728 cm^{-1} (CHO and OCHO); δ ($CDCl_3$), 8.25 (s, OCHO) and 10.05 (dd, CH_2CHO).

*15) (3S,4S)-4-Azido-3-hydroxy-6-methylheptanoic Acid (**9a**).* A solution of **7a** (470 mg) in a 28.7 ml of chromium trioxide solution[11] (CrO_3 1.1 g in acetic acid 3.3 ml and pyridine 1.1 ml) was allowed to stand at room temperature for 3 hr. The reaction mixture diluted with cold water (160 ml) was extracted with ether (30 ml×4) and the extracts were washed with saturated aqueous NaCl (20 ml×2). The dried ether solution was evaporated to afford a pale green syrup (526 mg). The syrup was chromatographed on a silica gel column (63 g, 2.3×36 cm) with the solvent D_1 to give (3*S*,4*S*)-4-azido-3-formyloxy-6-methylheptanoic acid **8a** (382 mg, 76%) as a colorless syrup; δ ($CDCl_3$), 8.21 (s, OCHO) and 9.60 (COOH). The syrup (382 mg) was dissolved in a mixture of conc. HCl (3.3 ml) and 50% (V/V) aqueous dioxane (56 ml) and the solution was kept at room temperature for 2 hr. The reaction mixture was concentrated and extracted with ether (10 ml×3). The extracts were washed with saturated aqueous NaCl (10 ml), dried and evaporated to afford **9a** (318 mg, 72% based on **7a**). A sample (27 mg) of this product was purified by silica gel chromatography with the solvent D_2 to give the pure sample (21 mg) of **9a** as a syrup: $[\alpha]_D^{21}$ −19° (*c* 0.57, chloroform); $\nu_{max}^{CCl_4}$ 3500 (OH), 2800—2500 (COOH), 2120 (N_3), and 1720 cm^{-1} (COOH); δ ($CDCl_3$), 0.97 and 0.98 (each d, $(CH_3)_2CH$, J=6.2 Hz), 1.13—2.0 (m, H-5, H-5', H-6, and OH), 2.57 (dd, H-2, $J_{2,3}$=4.7 Hz), 2.77 (dd, H-2', $J_{2',3}$=7.9 Hz, $J_{2,2'}$=16.7 Hz), 3.29 (dq, H-4, $J_{4,5}$=8.7 Hz, $J_{4,5'}$=5.0 Hz), 4.08 (dq, H-3, $J_{3,4}$=3.5 Hz), and 6.3 (broad, COOH).

Found: C, 47.85; H, 7.47; N, 21.22%. Calcd for $C_8H_{15}O_3N_3$: C, 47.75; H, 7.51; N, 20.88%.

*16) (3S,4R)-4-Azido-3-hydroxy-6-methylheptanoic Acid (**9b**).* By the procedure described in Exp. 15, **7b** (96 mg) was treated with the chromium trioxide solution to afford (3*S*,4*R*)-4-azido-3-formyloxy-6-methylheptanoic acid (**8b**) (65.2 mg, 63%) as a colorless syrup. A sample (31 mg) of **8b** was then de-*O*-formylated with hydrogen chloride in aqueous dioxane. The product (25.5 mg, 92%, mp 50—55 °C) was purified by silica gel chromatography (solvent D_2) and thereafter was recrystallized from petroleum ether to give a pure sample (16 mg) of **9b**: mp 60.5—61 °C; $[\alpha]_D^{15}$ +1° (*c* 2.82, chloroform); ν_{max}^{KBr} 3380 (OH), 2800—2500 (COOH), 2130 (N_3), and 1725 cm^{-1} (COOH); δ ($CDCl_3$), 0.96 and 0.98 (each d, $(CH_3)_2CH$, J=6.2 Hz), 1.25—1.50 (m, H-5 and H-5'), 1.55—2.0 (m, H-6 and OH), 2.63 (d, H-2 and H-2', $J_{2,3}$=$J_{2',3}$=6.0 Hz), 3.54 (quintet, H-4, $J_{4,5}$=4.5 Hz, $J_{4,5'}$=9.1 Hz), 4.07 (dt, H-3, $J_{3,4}$=4.5 Hz), and 6.5 (broad, COOH).

Found: C, 47.52; H, 7.37; N, 21.17%. Calcd for $C_8H_{15}O_3N_3$: C, 47.75; H, 7.51; N, 20.88%.

*17) (−)(3S,4S)AHMHA (**10a**).* A solution of **9a** (110 mg) in methanol (10 ml) and water (3 ml) was vigorously stirred for 30 min in a hydrogen atmosphere with palladium black. The crystalline product was washed with dry ether and dry ethanol to afford **10a** (97 mg, 93%) whose HPLC showed a single peak having the same elution time (137 min) as that of natural (−)AHMHA. An analytical sample was obtained by recrystallization from water-ethanol: mp 201—203 °C (decomp.) (lit,[1b] mp 199—

201 °C (decomp.)]; $[\alpha]_D$ −20 °, $[\alpha]_{546}$ −28 °, $[\alpha]_{436}$ −52 °, $[\alpha]_{405}$ −62 °, $[\alpha]_{365}$ −75 ° (*c* 0.64, water, 16 °C) [lit.[1b] $[\alpha]_D^{20}$ −18.9 ° (*c* 0.424, water), lit,[2] $[\alpha]_{541}$ −20 °, $[\alpha]_{436}$ −34 °, $[\alpha]_{405}$ −40 °, $[\alpha]_{365}$ −49 ° (*c* 1, water, 21 °C)]. The IR (KBr) and PMR (D_2O) spectra were identical with those of authentic sample* of natural (−)AHMHA.

Found: C, 54.79; H, 9.78; N, 7.93%. Calcd for $C_8H_{17}O_3N$: C, 54.83; H, 9.78; N, 7.99%.

*18) (+) (3S,4R)AHMHA (**10b**).* A solution of **9b** (58 mg) in methanol (6 ml) and water (1.2 ml) was hydrogenolyzed on palladium black. The crystalline product was washed with dry ether and dry ethanol to give **10b** (41 mg, 81%) whose HPLC showed a single peak which has a longer retension time (140 min) than that of **10a**. Recrystallization from water-ethanol afforded an analytical sample: colorless prisms, mp 202.5—203.5 °C (decomp.); $[\alpha]_D$ +20°, $[\alpha]_{546}$ +23°, $[\alpha]_{436}$ +46°, $[\alpha]_{405}$ +53 °, $[\alpha]_{365}$ +68° (*c* 0.66, water, 16°C).

Found: C, 54.58; H, 9.54; N, 7.76%. Calcd for $C_8H_{17}O_3N$: C, 54.83; H, 9.78; N, 7.99%.

*19) t-Butyl (3S,4S)-4-Azido-3-hydroxy-6-methylheptanoate (**11a**).* A sample (392 mg) of 4-azido-3-formyloxy-6-methylheptanoic acid (**8a**) (Exp. 15) was dissolved in dichloromethane (3.3 ml) and cooled to −40 °C. To the solution was added isobutene (2.2 ml) and conc. H_2SO_4 (0.033 ml), and the mixture was allowed to warm to room temperature. After standing for 3 hr, the reaction mixture was neutralized with triethylamine and evaporated. The residue was dissolved in ethyl acetate (15 ml) and washed with saturated aqueous NaCl, dried and evaporated to give a syrup (438 mg). To a solution of the syrup (398 mg) in dioxane (7 ml) was added 0.2 M NaOH (7 ml), and the mixture was stirred for 30 min at room temperature, evaporated, the residue was extracted with ethyl acetate (6 ml × 4). The ethyl acetate layers were washed with saturated aqueous NaCl, dried and evaporated. The oily product (358 mg) was chromatographed on silica gel (40 g) with the solvent B_4 to afford a pure sample of **11a**: colorless oil, yield 225 mg (57% based on **7a**); $[\alpha]_D^{15}$ −25° (*c* 2.62, chloroform).

Found: C, 56.09; H, 9.00; N, 16.24%. Calcd for $C_{12}H_{23}O_3N_3$: C, 56.01; H, 9.01; N, 16.33%.

*20) t-Butyl (3S,4R)-4-Azido-3-hydroxy-6-methylheptanoate (**11b**).* By the same procedure as in the preparation of **11a**, a sample (421 mg) of **8b** (Exp. 16) was *t*-butylated and the crude *t*-butyl ester (372 mg) was de-*O*-formylated. The oily product (429 mg) was purified by silica gel column (50 g) with the solvent B_4 to afford a pure sample of **11b**; yield 274 mg (58%), $[\alpha]_D^{26}$ +7° (*c* 1.72, chloroform).

Found: C, 56.19; H, 8.83; N, 16.58%. Calcd for $C_{12}H_{23}O_3N_3$: C, 56.01; H, 9.01; N, 16.33%.

*21) N-Acetyl-L-valyl-L-valine Methyl Ester (**12**).* *N*-Benzyloxycarbonyl-L-valyl-L-valine methyl ester[13] (2.3 g, 6.32 mmol) was hydrogenolyzed on palladium black in methanol (20 ml). The reduction product was acetylated in methanol (20 ml) with triethylamine (0.88 ml, 6.32 mmol) and acetic anhydride (1.18 ml, 12.64 mmol) in a usual manner to afford a crystalline solid (1.71 g). It was recrystallized from ethyl acetate; yield 1.47 g (86%), mp 147—150 °C. This sample was again recrystallized from ethyl acetate to give a pure sample of **12**, mp 155—157 °C, $[\alpha]_D^{32}$ −65° (*c* 1.07, methanol).

Found: C, 57.32; H, 8.90; N, 10 28%. Calcd for $C_{13}H_{24}O_4N_2$: C, 57,33; H, 8.88; N, 10.29%.

*22) N-Acetyl-L-valyl-L-valine Hydrazide (**13**).* A mixture of **12** (1.3 g), methanol (12 ml), and 80% hydrazine hydrate (5.9 ml) was kept overnight at room temperature. The crystalline hydrazide separated was collected by filtration and washed with methanol, chloroform, and water, successively, and dried; yield 1.14 g (88%). Analytical sample was obtained by recrystallization from DMF-water; mp 280 °C (decomp.), $[\alpha]_D^{31}$ −45° (*c* 0.5, dimethylsulfoxide).

Found: C, 53.09; H, 8.70; N, 20.71%. Calcd for $C_{12}H_{24}O_3N_4$: C, 52.92; H, 8.88; N, 20.57%.

*23) N-(Acetyl-L-valyl-L-valyl)-(3S,4S)AHMHA t-Butyl Ester (**14a**).* Isoamyl nitrite (0.056 ml, 0.015 mmol) and 3.93 M HCl in tetrahydrofuran (0.31 ml, 1.21 mmol) was added to a solution of **13** (106 mg, 0.388 mmol) in DMF (0.5 ml) cooled to −20 °C and the mixture was stirred at −20 °C for 30 min to make a clear solution. To this solution containing acetyl-L-valyl-L-valine azide was added triethylamine (0.223 ml, 1.6 mmol) and a solution of (3*S*,4*S*) AHMHA *t*-butyl ester, which was prepared from **11a** (99.6 mg, 0.388 mmol) by catalytic hydrogenolysis on palladium black, in DMF (0.6 ml). After stirring at −20 °C for 4 hr and standing overnight in a refregerator, the solvents were removed. The residue was triturated with water to give a crystalline product which was collected by filtration and washed with water; yield 183 mg (74%). Silica gel chromatography (solvent E_1) of the product (183 mg) gave a practically pure sample of **14a** (98 mg). Recrystallization from methanol-water afforded an analytical sample; mp 219—220 °C, $[\alpha]_D^{16}$ −85° (*c* 0.55, methanol).

Found: C, 60.92; H, 9.43; N, 9.04%. Calcd for $C_{24}H_{45}O_6N_3$: C, 61.12; H, 9.62; N, 8.91%.

*24) N-(Acetyl-L-valyl-L-valyl)-(3S,4R)AHMHA t-Butyl Ester (**14b**).* The *t*-butyl ester of **10b** obtained by hydrogenolysis of **11b** (103 mg) was coupled with acetyl-L-valyl-L-valine azide prepared from **13** (109 mg) by the procedure described in Exp. 23 to afford the product **14b** (141 mg, 75%) which appeared as a homogeneous material on tlc (solvent E_2). A sample (30 mg) of this product was purified by silica gel chromatography (solvent E_2) and by recrystallization from ethanol to give a pure sample (16 mg); mp 215 °C ~ (sublimated), $[\alpha]_D^{17}$ −43° (*c* 0.5, methanol).

Found: C, 61.25; H, 9.49; N, 8.90%. Calcd for $C_{24}H_{45}O_6N_3$: C, 61.12; H, 9.62; N, 8.91%.

*25) N-(Acetyl-L-valyl-L-valyl)-(3S,4S)AHMHA (**15a**).* A solution of **14a** (63 mg) in trifluoroacetic acid (1.5 ml) was allowed to stand for 25 min at room temperature and then evaporated to dryness. The syrupy residue was treated with ether to afford **15a** as a crystalline solid (52 mg, 94%). Silica gel chromatography (solvent F) of the product, followed by recrystallization from methanol-water gave an analytical sample; mp 226—227 °C, $[\alpha]_D^{13}$ −100° (*c* 0.68, methanol).

Found:. C, 57.68; H, 8.97; N, 10.28%. Calcd for $C_{20}H_{37}O_6N_3$: C, 57.81; H, 8.98; N, 10.11%.

*26) N-(Acetyl-L-valyl-L-valyl)-(3S,4R)AHMHA (**15b**).* Treatment of **14b** (144 mg) with trifluoroacetic acid (1.2 ml) for 20 min at room temperature afforded **15b** as a crystalline solid (118 mg, 93%) which appeared to be homogeneous on tlc (solvent F and G). Recrystallization from methanol-water gave a pure sample; mp 215—217 °C, $[\alpha]_D^{15}$ −40° (*c* 0.21, methanol).

Found: C, 57.57; H, 8.77; N, 10.09%. Calcd for $C_{20}H_{37}O_6N_3$: C, 57.81; H, 8.98; N, 10.11%.

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