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SYNTHESIS OF THE RACEMIC AND OPTICALLY ACTIVE FORMS OF GIZZEROSINE, THE INDUCER OF GIZZARD EROSION IN CHICKS

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Abstract—The racemate and both the (*R*)- and (*S*)-forms of gizzerosine [2-amino-9-(4-imidazolyl)-7-azanonanoic acid] were synthesised, and the (*S*)-isomer was identified as the toxic substance in fish meal causing severe gizzard erosion (black vomit) in chicks.

A serious disease named "black vomit" is a big problem in poultry production all over the world.[1] The disease is accompanied by gizzard erosion or ulceration, and known to be caused by brown fish meal in the diet.[1] Okazaki *et al.*[2] recently isolated from 10 kg of heated mackerel meal 2 mg of a compound which caused severe gizzard erosion in chicks within a week when fed to them at the level of 2.2 ppm in the diet (or *ca* 50 μg/day). This toxic compound was named gizzerosine.[2] Extensive spectral studies by Okazaki *et al.* on the isolated material was concluded by the proposal of its structure as 2-amino-9-(4-imidazolyl)-7-azanonanoic acid **1a** with an unknown absolute configuration at C-2.[2] Shortly afterwards, this proposal was confirmed by an unambiguous synthesis of (±)-**1a** by us.[3] Herein we describe in detail the synthesis of (±)-**1a**, (*R*)-**1a** and (*S*)-**1a**, which enabled us to test the toxicities of these materials in chicks.

Our synthesis of (±)-gizzerosine **1a** started from 4-methyl-3-pentenyl bromide **2**, which was readily obtainable from 2-cyclopropyl-2-propanol according to Julia *et al.*[4] Treatment of **2** with NaCN in DMSO gave a nitrile **3**, whose alkaline hydrolysis furnished **4**. Reduction of **4** with LAH yielded an alcohol **5a**. The corresponding tosylate **5b** was treated with NaI in acetone to give an iodide **6** in 57% overall yield from the starting cyclopropane derivative. Alkylation of diethyl acetaminomalonate with **6** in the presence of NaOEt in EtOH afforded **7** in 86% yield. This was oxidised with MCPBA to give an oily epoxide **8**. Treatment of **8** with HIO_4–$2H_2O$ in ether–THF for a short period gave a crude aldehyde **9** in quantitative yield from **7**. Ozonolysis (O_3 followed by treatment with Me_2S) of **7** also furnished **9**, although in a less pure state. The aldehyde **9** was rather unstable presumably due to its ease of cyclisation to give by-products.

Reductive amination of **9** with histamine dihydrochloride and $NaBH_3CN$ in MeOH[5] gave a diester **10** as a crude oil in 87% yield. This was hydrolysed with HCl aq to give (±)-**1a**–3HCl as a crude gum in 82% yield. This gum was purified to give pure (±)-**1a** by the procedure used for the isolation of **1a** from fish meal.[2] The synthetic (±)-**1a** was identical with the isolated authentic gizzerosine on the basis of chromatographic and spectral comparisons including TLC (cellulose powder), ^{1}H-NMR, ^{13}C-NMR and EI-MS (as the corresponding Me ester diacetamide). (±)-Gizzerosine dihydrochloride (±)-**1a**–2HCl was found to be

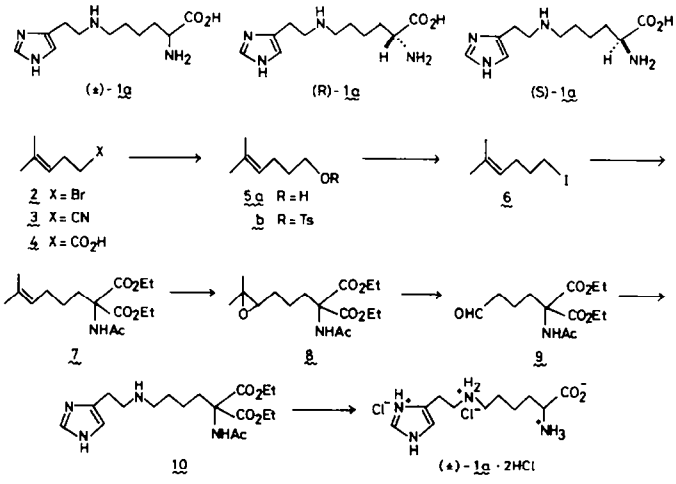

Fig. 1. Synthesis of (±)-gizzerosine.

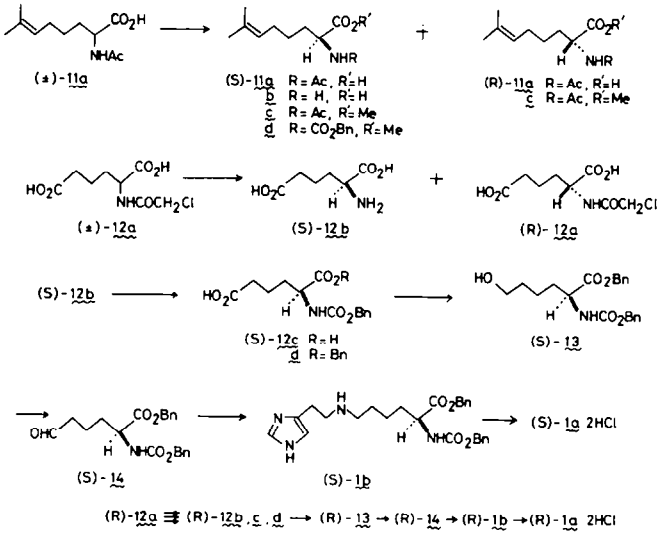

Fig. 2. Synthesis of the enantiomers of gizzerosine.

crystalline, m.p. 253–256° (dec), and stable at room temp. This was therefore used for the bioassay. The addition of 6.25 ppm of (±)-**1a**–2HCl in experimental diets for chicks caused severe erosion of their gizzards. The activity, however, was about one third as strong as the authentic **1a** (not **1a**–2HCl) suggesting that only one enantiomer of **1a** is bioactive. To clarify this, both the enantiomers of **1a** were synthesised as described below.

We first attempted to employ the enantiomers of **11a** as the starting material. Alkaline hydrolysis of the ester groups of **7** was followed by acidification and decarboxylation to give (±)-**11a**. Treatment of (±)-**11a** with *Aspergillus* amino acylase[6] smoothly effected the enantioselective hydrolysis of (*S*)-**11a** to (*S*)-**11b**, leaving (*R*)-**11a** intact. Acetylation of (*S*)-**11b** with Ac_2O and NaOH aq[7] gave (*S*)-**11a**. Both (*R*)- and (*S*)-**11a** were converted to the corresponding Me esters, (*R*)- and (*S*)-**11c**, by treatment with CH_2N_2. Another derivative (*S*)-**11d** was also prepared from (*S*)-**11b** in the conventional manner. Conversion of (*R*)- and (*S*)-**11c** as well as (*S*)-**11d** to the aldehyde similar to **14** was attempted by successive treatments of them with MCPBA and HIO_4–$2H_2O$. No useful result, however, was obtained due to the instability of the aldehydes. Their cyclisation to piperidine-type compounds seemed to have taken place.

At this stage we became aware of a timely publication of Tice and Ganem[8] on the synthesis of (*S*)-**14**. So as to prepare both the enantiomers of **14**, enzymatic resolution of (±)-**12a** was carried out as described by Greenstein *et al.*[9] except that we used *Aspergillus* amino acylase[6] instead of acylase I as originally used by the American workers.[9] We were thus able to prepare sufficient quantities of (*S*)-**12b** and (*R*)-**12a** by the enzymatic reaction, and (*R*)-**12a** was converted to (*R*)-**12b** in the usual manner.[9]

(*S*)-2-Aminoadipic acid **12b** was then benzyloxycarbonylated to give (*S*)-**12c**.[10] This was converted to the known half benzyl ester (*S*)-**12d** by the method of Baldwin *et al.*[11] As reported by Tice and Ganem, (*S*)-**12d** was reduced with BH_3–THF to (*S*)-**13**.[8] The Swern oxidation of (*S*)-**13** with $(COCl)_2$–DMSO yielded (*S*)-**14**,[8] which was immediately employed for the next stage. Reductive amination of (*S*)-**14** with histamine dihydrochloride was effected with $NaBH_3CN$ and molecular sieves 3Å in MeOH to give (*S*)-**1b** in 69% yield from (*S*)-**13**. Hydrogenation of (*S*)-**1b** over Pd–C was followed by acidification of the product with dil HCl to pH 5. The resulting (*S*)-gizzerosine dihydrochloride was recrystallised from aq MeOH to give pure (*S*)-**1a**–2HCl, m.p. 251–252° (dec), $[\alpha]_D^{22}$ + 14.3° (3 N HCl), in 33% yield from (*S*)-**1b**. Similarly (*R*)-**12b** yielded (*R*)-**1a**–2HCl, m.p. 252–254° (dec), $[\alpha]_D^{22}$ − 13.0° (3 N HCl). The TLC (Merck silanised SiO_2), IR (KBr disc), ^{1}H-NMR and ^{13}C-NMR data of (*R*)- and (*S*)-**1a**–2HCl were indistinguishable with those of (±)-**1a**–2HCl. The only discernible difference between the racemate and the optically active forms was a small variation in the intensities of their IR absorption bands at 1115 and 1135 cm^{-1} when measured as KBr discs. The bioassay of both the enantiomers of **1a**–2HCl was carried out by Dr M. Sugahara and Mr T. Masumura of C. Itoh Feed Mills Co., Ltd. The addition of 3 or 6 ppm of (*S*)-**1a**–2HCl in experimental diets for chicks caused severe gizzard erosion, while the same amount of (*R*)-**1a**–2HCl did not cause any damage in chicks. This clearly indicated that the absolute configuration of the isolated gizzerosine is *S*.

In summary the racemic and optically active forms of 2-amino-9-(4-imidazolyl)-7-azanonanoic acid (gizzerosine) **1a** were synthesised and (*S*)-**1a** was shown to be the compound which causes gizzard erosion in chicks. The biological aspects of this work will be published separately in due course.

EXPERIMENTAL

All b.ps and m.ps were uncorrected. IR spectra were measured on a Jasco A-102 spectrometer. ^{1}H-NMR spectra were recorded at 60 MHz with TMS as an internal standard on a Hitachi R-24A spectrometer unless otherwise stated. ^{13}C-NMR spectra were recorded at 25 MHz on a Jeol FX-100

spectrometer. Optical rotations were measured on a Jasco DIP-140 polarimeter.

5-Methyl-4-hexenenitrile **3**

To a stirred suspension of NaCN (20 g) in DMSO (200 ml) was added **2** (50 g) at room temp. Shortly afterwards, an exothermic reaction took place and the mixture became homogeneous. Gradually the ppt of NaBr separated from the soln. The mixture was left to stand overnight at room temp, diluted with ice–water and extracted with n-pentane. The pentane soln was washed with water and brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was distilled to give 29.5 g (88.3%) of **3**, b.p. 71°/16 mm, n_D^{22} 1.4365; ν_{max} (film) 2240 (m), 1670 (w), 835 (m), 815 (m) cm^{-1}; δ (CCl_4) 1.66 (3H, s), 1.75 (3H, s), 2.20–2.50 (4H), 5.15 (1H). (Calc for $C_7H_{11}N$: C, 77.01; H, 10.16; N, 12.83. Found: C, 76.70; H, 10.13; N, 12.66%.)

5-Methyl-4-hexenoic acid **4**

To a stirred soln of KOH (50 g) in ethylene glycol (180 ml) and H_2O (20 ml) was added **3** (29.3 g), and the mixture was stirred and heated under reflux for 7.5 hr. After cooling, the mixture was diluted with ice–water, acidified with conc HCl (*ca* 100 ml) and extracted with ether. The ether soln was washed with water and brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was distilled *in vacuo* to give 32.5 g (94.5%) of **4**, b.p. 118–120°/25 mm, n_D^{21} 1.4434; ν_{max} (film) ~3600–~2400 (m), 1710 (s), 935 (m) cm^{-1}; δ (CCl_4) 1.60 (3H, s), 1.66 (3H, s), 2.15–2.50 (4H), 5.08 (1H), 12.02 (1H, s). (Calc for $C_7H_{12}O_2$: C, 65.70; H, 9.54. Found: C, 65.59; H, 9.44%.)

5-Methyl-4-hexen-1-ol **5a**

A soln of **4** (32.5 g) in dry ether (100 ml) was added dropwise to a stirred and ice-cooled suspension of LAH (9.5 g) in dry ether (700 ml) at 5–10°. The stirring was continued for 6 hr at room temp. Excess LAH was destroyed by the gradual addition of H_2O to the ice-cooled and stirred mixture. It was then poured into ice and dil HCl. The ether layer was separated and the aq layer was extracted with ether. The combined ether soln was washed with water, $NaHCO_3$ aq and brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was distilled to give 25.6 g (88.6%) of **5a**, b.p. 78–80°/20 mm, n_D^{20} 1.4456; ν_{max} (film) 3350 (s), 1670 (w), 1060 (s) cm^{-1}; δ (CCl_4) 1.30–1.50 (2H, m), 1.60 (3H, s), 1.68 (3H, s), 1.70–2.30 (2H, m), 3.52 (2H, t, J = 6 Hz), 3.62 (1H, s), 5.12 (1H, t, J = 7 Hz). (Calc for $C_7H_{14}O$: C, 73.63; H, 12.36. Found: C, 73.51; H, 12.36%.)

5-Methyl-4-hexenyl tosylate **5b**

p-TsCl (56 g) was added to a stirred and ice-cooled soln of **5a** (25.6 g) in dry C_5H_5N (150 ml). The mixture was left to stand overnight in a refrigerator. It was then poured into ice–water and extracted with ether. The ether soln was washed with dil HCl, water, $NaHCO_3$ aq and brine, dried ($MgSO_4$) and concentrated *in vacuo* to give 64 g (quantitative) of **5b**, ν_{max} (film) 1360 (s), 1190 (s), 1175 (s), 960 (m), 925 (m), 810 (m), 660 (m) cm^{-1}. This was employed in the next step without further purification.

5-Methyl-4-hexenyl iodide **6**

To a stirred soln of NaI (50 g) in acetone (250 ml) was added **5b** (64 g) in acetone (50 ml). Immediately after the addition, an exothermic reaction took place and NaOTs began to precipitate. The mixture was left to stand overnight at 30–40°. After cooling, it was poured into water and extracted with n-pentane. The pentane soln was washed with water and brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was distilled to give 38.7 g (77% from **5a**) of **6**, b.p. 85–86°/21 mm, n_D^{21} 1.5097; ν_{max} (film) 1670 (w), 1200 (s), 830 (m) cm^{-1}; δ (CCl_4) 1.62 (3H, s), 1.68 (3H, s), 1.70–2.30 (4H, m), 3.12 (2H, t, J = 7 Hz), 5.04 (1H, t, J = 6 Hz). (Calc for $C_7H_{13}I$: C, 37.52; H, 5.85. Found: C, 38.03; H, 6.00%.)

Ethyl 2-acetamino-2-ethoxycarbonyl-7-methyl-6-octenoate **7**

Diethyl acetaminomalonate (35 g) was added to a stirred soln of NaOEt (from 3.7 g of Na) in abs EtOH (110 ml). To this was added **6** (38.5 g) with stirring at room temp. The mixture was stirred and heated under reflux for 6.5 hr. EtOH was removed *in vacuo*. The residue was diluted with ice–water and extracted with ether. The ether soln was washed with water and brine, dried ($MgSO_4$) and concentrated *in vacuo* to give 50 g of an oil. This was triturated with pet ether to give 43 g (86%) of **7** as crystals. An analytical sample was recrystallised from EtOAc–pet ether to give needles, m.p. 43.0–43.5°; ν_{max} (Nujol) 3300 (m), 1760 (m), 1745 (s), 1740 (s), 1645 (s), 1515 (m), 1260 (m), 1205 (m), 1195 (m) cm^{-1}; δ ($CDCl_3$) 1.26 (6H, t, J = 7 Hz), 1.58 (3H, s), 1.68 (3H, s), 2.05 (3H, s), 4.28 (4H, q, J = 7 Hz), 5.10 (1H, t, J = 6 Hz), 6.86 (1H, br s). (Calc for $C_{16}H_{27}O_5N$: C, 61.32; H, 8.68; N, 4.47. Found: C, 61.25; H, 8.51; N, 4.46%.)

Ethyl 2-acetamino-6,7-epoxy-2-ethoxycarbonyl-7-methyloctanoate **8**

MCPBA (2.06 g; 85% purity) was added portionwise to a stirred and ice-cooled soln of **7** (3.13 g) in CH_2Cl_2 (50 ml) at 0–5°. The mixture was stirred for 3 hr at 0–5°. It was then washed with Na_2CO_3 aq, dried ($MgSO_4$) and concentrated *in vacuo* to give 3.3 g (quantitative of **8**; ν_{max} (film) ~3400 (m), 1745 (s), 1680 (s), 1270 (s), 1210 (s), 1195 (s) cm^{-1}; δ ($CDCl_3$) 1.20 (6H, s), 1.30 (6H, t, J = 6 Hz), 2.05 (3H, s), 4.28 (4H, q, J = 6 Hz), 6.90 (1H, br); MS: *m/z* 329 (M^+, $C_{16}H_{27}O_6N$ = 329).

Ethyl 2-acetamino-2-ethoxycarbonyl-6-oxohexanoate **9**

(*a*) *From* **8**. A soln of **8** (3.3 g) in ether (100 ml) was added to a soln of HIO_4–$2H_2O$ (2.5 g) in THF (20 ml) with stirring and ice-cooling at 0–5°. The mixture was stirred for 10 min at 0–5° and the supernatant organic soln was separated by decantation. The solid precipitates of HIO_3 were washed thoroughly with ether. The combined THF–ether soln was washed with $NaHCO_3$ aq and brine, dried ($MgSO_4$) and concentrated *in vacuo* to give 2.9 g (quantitative) of **9**; ν_{max} (film) 3400 (m), 2730 (w), 1740 (s), 1670 (s), 1195 (s) cm^{-1}; δ ($CDCl_3$) 1.23 (6H, t, J = 6 Hz), 2.04 (3H, s), 4.24 (4H, q, J = 6 Hz), 6.85 (1H, br), 9.80 (1H, s). This compound was so unstable that it had to be used immediately in the next step.

(*b*) *From* **7**. O_3 was bubbled into a stirred and cooled soln of **7** (3.13 g) in MeOH (60 ml) in the presence of solid $NaHCO_3$ (10 g) for 1.5 hr at −78°. After bubbling N_2 into the mixture to remove excess O_3, Me_2S (1.47 ml or 1.24 g) was added to the stirred mixture at −78°. The temp was gradually raised over 2.5 hr to room temp. The mixture was then concentrated *in vacuo*. The residue was diluted with water and extracted with ether. The ether soln was washed with water and brine, dried ($MgSO_4$) and concentrated *in vacuo* to give 1.0 g (34.5%) of crude **9**. This was immediately employed for the reductive amination to give **10**.

Ethyl 2-acetamino-2-ethoxycarbonyl-9-(4-imidazolyl)-7-azanonanoate **10**

To a stirred soln of **9** (1.0 g, 3.5 mmol) and histamine–2HCl (1.8 g, 10 mmol) in MeOH (25 ml) was added $NaBH_3CN$ (200 mg, 3.5 mmol), and the mixture was stirred overnight at room temp. It was then acidified with conc HCl to pH < 2 and concentrated *in vacuo*. The residue was dissolved in water (10 ml) and extracted with ether (15 ml) to remove neutral impurities. The aq layer was made alkaline (pH > 10) with K_2CO_3 aq, saturated with NaCl and extracted several times with $CHCl_3$. The $CHCl_3$ soln was dried ($MgSO_4$) and concentrated *in vacuo* to give 1.3 g (98%) of oily crude **10**; ν_{max} (film) 3400 (m), 3150 (br m), 2450 (m), 1740 (s), 1660 (s), 1500 (m), 1460 (m), 1440 (m), 1365 (m), 1290 (m), 1260 (m), 1205 (s), 1095 (m), 1015 (m) cm^{-1}. This was employed in the next step without further purification.

(±)-2-Amino-9-(4-imidazolyl)-7-azanonanoic acid dihydrochloride [(±)-gizzerosine dihydrochloride] (±)-**1a**–2HCl

The crude **10** (1.0 g) was dissolved in HCl aq (conc HCl–H_2O, 2:1, 18 ml) and the soln was stirred and heated under reflux for 7 hr. It was then concentrated *in vacuo* to give 637 mg (59%) of crude (±)-**1a**–3HCl. This was chromatographed over Amberlite IR CG-50 (N-ethylmorpholine form, 10 cm × 2.4

cm). After washing the column with 2 M N-ethylmorpholine (250 ml), (±)-**1a** was eluted with 3 M NH_3 aq (250 ml) to give *ca* 300 mg of (±)-**1a**. This was further purified by preparative TLC over acid-washed cellulose powder (development with i-PrOH–28% NH_3 aq–H_2O, 16:3.5:4). When detected with diazotised sulfanilic acid in 10% Na_2CO_3, (±)-**1a** showed orange–red colour at $R_f \sim 0.60$. The spot was eluted with i-PrOH–28% NH_3 aq–H_2O (16:1:4) to give 150 mg of pure (±)-**1a**. The synthetic (±)-**1a** was identical with the isolated gizzerosine on the basis of chromatographic and spectral comparisons: TLC (cellulose powder: i-PrOH–28% NH_3 aq–H_2O, 16:3.5:4): R_f 0.58; ^{1}H-NMR (400 MHz, D_2O) δ 1.47 (2H, m), 1.76 (2H, dt, $J_1 = 16$, $J_2 = 8$ Hz), 1.90 (2H, dt, $J_1 = 10$, $J_2 = 7$ Hz), 3.12 (2H, t, J = 8 Hz), 3.16 (2H, t, J = 8 Hz), 3.38 (2H, t, J = 8 Hz), 3.75 (1H, t, J = 7 Hz), 7.35 (1H, s), 8.55 (1H, s); ^{13}C-NMR (100 MHz, D_2O) δ 22.4, 23.7, 25.9, 30.7, 47.4, 48.0, 55.5, 117.2, 132.9, 136.7, 175.3; EI-MS (as Me ester diacetamide) *m/z* 338.19496 (M^+, calc for $C_{16}H_{26}O_4N_4$: 338.19540). The free amino acid (±)-**1a** was dissolved in water and acidified with HCl aq to pH 5.2. The soln was concentrated *in vacuo*. The residue was recrystallised from MeOH–H_2O (5:1) to give (±)-**1a**–2HCl as fine prisms, m.p. 253–256° (dec); ν_{max} (Nujol) 3130 (s), 2780 (s), 2450 (s), 1640 (s), 1605 (s), 1525 (s), 1495 (s), 1355 (m), 1335 (m), 1235 (m), 1095 (m), 1055 (m), 960 (m), 835 (m), 795 (m), 720 (m) cm^{-1}; ^{1}H-NMR (100 MHz, D_2O, DOH = δ 4.8) δ 1.30–2.10 (6H, m), 3.00–3.50 (6H, m), 3.75 (1H, t, J = 6 Hz), 7.39 (1H, s), 8.75 (1H, s); ^{13}C-NMR (25 MHz, D_2O, dioxane = δ 67.4 as an external standard) δ 21.9, 22.3, 25.8, 30.6, 46.4, 48.1, 55.2, 117.7, 129.0, 134.7, 175.2. (Calc for $C_{11}H_{22}O_2N_4Cl_2$: C, 42.18; H, 7.08; N, 17.88. Found: C, 42.13; H, 7.00; N, 17.68%.)

(±)-2-*Acetamino-7-methyl-6-octenoic acid* (±)-**11a**

To a soln of KOH (8.0 g, 140 mmol) in THF (50 ml) and water (50 ml) was added **7** (18.8 g, 60 mmol). The mixture was stirred overnight at room temp. The mixture first deposited crystals and then became homogeneous. After stirring and heating under reflux for 30 min, the mixture was concentrated *in vacuo* to remove THF. AcOH (30 ml) was added to the residue to acidify it. The mixture was stirred and heated under reflux for 30 min and concentrated *in vacuo*. The residue was diluted with water and extracted with EtOAc. The EtOAc soln was washed with brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was dissolved in a small amount of ether and left to stand in a refrigerator to give 10.1 g (78.9%) of (±)-**11a**. An analytical sample was recrystallised from acetone–pet ether to give prisms, m.p. 88–89°; ν_{max} (Nujol) 3345 (s), 1705 (s), 1620 (s), 1555 (s), 1265 (m), 1245 (m), 1215 (m) cm^{-1}; δ ($CDCl_3$) 1.60 (3H, s), 1.68 (3H, s), 2.08 (3H, s), ~4.60 (1H, m), 5.20 (1H, t, J = 7 Hz), 6.08 (1H, d, J = 7 Hz), 11.30 (1H, s). (Calc for $C_{11}H_{19}O_3N$: C, 61.94; H, 8.98; N, 6.57. Found: C, 62.10; H, 8.90; N, 6.23%.)

Enzymatic resolution of (±)-**11a**. *Aspergillus* amino acylase (Tokyo Kasei Co., 2.0 g) was added to a soln of (±)-**11a** (9.15 g) in water (600 ml) whose pH had been adjusted to pH 7.1 by the addition of 3 N NaOH. After the addition of a trace amount of $CoCl_2$ (7×10^{-4} M; 20 ml), the soln was left to stand at 37° for 36 hr. After 24 hr, 1.0 g of the acylase was added. At the end of the incubation period, the separated (*S*)-**11b** was collected on a filter. The filtrate was concentrated *in vacuo* to a vol of 50 ml. The ppt (*S*)-**11b** was collected. The combined (*S*)-**11b** (3.2 g, 87.7%) was recrystallised from water (500 ml) to give pure (*S*)-**11b** as needles, m.p. 250–255° (dec), $[\alpha]_D^{20}$ +30.7° (*c* = 0.567, N HCl); ν_{max} (Nujol) 1580 (s), 1515 (s), 1405 (m), 840 (w) cm^{-1}. (Calc for $C_9H_{17}O_2N$: C, 63.13; H, 10.00; N, 8.18. Found: C, 63.09; H, 10.10; N, 8.22%.) The filtrates after removing (*S*)-**11b** were combined and concentrated *in vacuo* to a vol of 50 ml, acidified with HCl aq and extracted with EtOAc. The EtOAc soln was washed with brine, dried ($MgSO_4$) and concentrated *in vacuo* to give 4.0 g (87.0%) of (*R*)-**11a**. This was recrystallised from EtOAc–pet ether to give prisms, m.p. 95–96°, $[\alpha]_D^{20}$ −9.07° (*c* = 1.036, 99% EtOH); ν_{max} (Nujol) 3340 (s), 1705 (s), 1625 (s), 1555 (s), 1245 (m) cm^{-1}. (Calc for $C_{11}H_{19}O_3N$: C, 61.94; H, 8.98; N, 6.57. Found: C, 62.19; H, 9.02; N, 6.65%.) The ^{1}H-NMR spectrum of (*R*)-**11a** was identical with that of (±)-**11a**.

(S)-2-*Acetamino-7-methyl-6-octenoic acid* (*S*)-**11a**

A soln of NaOH (3.0 g) in water (35 ml) was prepared. A portion (7 ml) of this soln was added to a stirred and ice-cooled suspension of (*S*)-**11b** (3.5 g) in water (100 ml) to dissolve **11b**. To the stirred and ice–salt-cooled soln of (*S*)-**11b** were added simultaneously and portionwise Ac_2O (6.5 g) and the rest of the NaOH aq over 10 min at −10 to −5°. The soln was further stirred for 30 min at room temp, acidified with HCl aq and extracted with EtOAc. The EtOAc soln was washed with brine, dried ($MgSO_4$) and concentrated *in vacuo* to give 3.9 g (89.9%) of (*S*)-**11a**. Recrystallisation of this from EtOAc–ether–pet ether yielded prisms, m.p. 95–96°, $[\alpha]_D^{22}$ +9.60° (*c* = 0.823, 99% EtOH). (Calc for $C_{11}H_{19}O_3N$: C, 61.94; H, 8.98; N, 6.57. Found: C, 62.24; H, 9.14; N, 6.64%.) The IR and ^{1}H-NMR spectra of (*S*)-**11a** were identical with those of (*R*)-**11a**. The optical purities of (*R*)- and (*S*)-**11a** were found to be ~100% by measuring the ^{1}H-NMR spectra of the corresponding Me esters, (*R*)- and (*S*)-**11c**, in the presence of a chiral shift reagent Eu(hfc)$_3$ in $CDCl_3$. The 3H singlets due to $C\underline{H}_3CONH$— and —$CO_2C\underline{H}_3$ protons of (±)-**11c** split into pairs of singlets in the presence of Eu(hfc)$_3$, while the signals remained as singlets in the case of (*R*)- and (*S*)-**11c** even in the presence of Eu(hfc)$_3$ (= *tris*[3-(heptafluoropropylhydroxymethylene)-*d*-camphorato]europium).

Enzymatic resolution of (±)-2-*chloroacetaminohexanedioic acid* (±)-**12a**

According to Greenstein *et al.*,[9] (±)-**12a** (159.8 g) was enantioselectively hydrolysed with amino acylase. Instead of acylase I powder,[9] *Aspergillus* amino acylase (Tokyo Kasei Co., 6.5 g) was used. $CoCl_2$ (10 mg) was also added to the mixture. (*S*)-2-Aminohexanedioic acid (*S*)-**12b** (52.0 g, 96%) was obtained, m.p. 189–190°, $[\alpha]_D^{20}$ +23.1° (*c* = 1.08, 5 N HCl) [lit.[9] $[\alpha]_D^{25}$ +25.0° (*c* = 2, 5 N HCl)]; ν_{max} (KBr) 1670 (s), 1580 (s), 1500 (s) cm^{-1}. Intact (*R*)-**12a** (59.1 g, 74% after recrystallisation from EtOAc) was also obtained, m.p. 105–106°, $[\alpha]_D^{21}$ −9.2° (*c* = 0.58, acetone); ν_{max} (KBr) 3270 (s), 1715 (s), 1640 (s), 1260 (s) cm^{-1}. (Calc for $C_6H_{12}O_5NCl$: C, 40.43; H, 5.09; N, 5.81. Found: C, 40.37; H, 5.10; N, 5.81%.)

(R)-2-*Aminohexanedioic acid* (*R*)-**12b**

Hydrolysis of (*R*)-**12a** (58 g) with HCl aq in the conventional manner gave 37.7 g (96%) of (*R*)-**12b**, m.p. 187–188°, $[\alpha]_D^{21.5}$ −21.5° (*c* = 0.48, 5 N HCl) [lit.[9] $[\alpha]_D^{25}$ −25.0° (*c* = 2, 5 N HCl)]. Its IR spectrum was identical with that of (*S*)-**12b**.

2-*Benzyloxycarbonylaminohexanedioic acid* **12c**

(*a*) (S)-*Isomer*. According to the procedure of Claesen *et al.*[10] (*S*)-**12b** (53.3 g) gave 84.8 g (92%) of (*S*)-**12c**, m.p. 131–132° (lit.[10] m.p. 136–136.5°), $[\alpha]_D^{21}$ +14.8° (*c* = 1.90, EtOH–2 N NaOH, 9:1) [lit.[10] $[\alpha]_D$ +17° (*c* = 2, EtOH–2 N NaOH, 9:1)]; $[\alpha]_D^{22.5}$ −4.7° (*c* = 1.04, acetone); ν_{max} (KBr) 3340 (s), 1680 (s), 1530 (s), 1280 (s) cm^{-1}.

(*b*) (R)-*Isomer*. In the same manner, (*R*)-**12b** (31.2 g) gave 47 g (87%) of (*R*)-**12c**, m.p. 129.5–130.5° (lit.[10] m.p. 136–136.5°), $[\alpha]_D^{21.5}$ −13.0° (*c* = 2.01, EtOH–2 N NaOH, 9:1) [lit.[10] $[\alpha]_D$ −17° (*c* = 2, EtOH–2 N NaOH, 9:1)]; $[\alpha]_D^{22.5}$ +4.8° (*c* = 1.04, acetone). Its IR spectrum was identical with that of (*S*)-**12c**.

2-*Benzyloxycarbonylaminohexanedioic acid* 1-*benzyl ester* **12d**

(*a*) (S)-*Isomer*. According to the procedure of Baldwin *et al.*[11] (*S*)-**12c** (11.1 g) gave 6.6 g (45%) of (*S*)-**12d**, m.p. 89.5–90° (lit.[11] m.p. 90–92°), $[\alpha]_D^{22}$ −13.0° (*c* = 1.94, acetone) [lit.[11] $[\alpha]_D^{25}$ −13.3° (*c* = 2, acetone)]; ν_{max} (KBr) 1745 (s), 1715 (s), 1360 (s), 1180 (s) cm^{-1}; ^{13}C-NMR (25 MHz, $CDCl_3$) δ 20.4, 31.7, 33.2, 53.7, 67.3, 128.0, 128.2, 128.3, 128.5, 128.6, 135.2, 136.1, 156.1, 172.1, 178.3. ^{1}H-NMR data of (*S*)-**12d** coincided with those reported by Baldwin *et al.*[11]

(*b*) (R)-*Isomer*. In the same manner, (*R*)-**12c** (15 g) gave 9.4 g (47%) of (*R*)-**12d**, m.p. 89–89.5° (lit.[11] m.p. 92–94°), $[\alpha]_D^{22}$ +14.3° (*c* = 2.00, acetone) [lit.[11] $[\alpha]_D^{25}$ +13.5° (*c* = 2,

acetone)]. Its IR, ^{1}H- and ^{13}C-NMR spectra were identical with those of (*S*)-**12d**.

Benzyl 6-hydroxy-2-benzyloxycarbonylaminohexanoate **13**

(*a*) (S)-*Isomer*. According to Tice and Ganem,[8] (*S*)-**12d** (5.5 g) gave 4.4 g (83%) of (*S*)-**13**, n_D^{23} 1.5339, $[\alpha]_D^{23} - 3.9°$ (c = 1.06, $CHCl_3$); ν_{max} (film) 3360 (s), 1720 (s) cm^{-1}. Its ^{1}H-NMR spectrum was in agreement with the published data.[8]

(*b*) (R)-*Isomer*. In the same manner, (*R*)-**12d** (5.5 g) gave 4.7 g (89%) of (*R*)-**13**, n_D^{22} 1.5328, $[\alpha]_D^{22} + 4.6°$ (c = 1.03, $CHCl_3$); CI-MS (isobutane): m/z 372 ($M^+ + 1$). The IR and ^{1}H-NMR spectra of (*R*)-**13** was identical with those of (*S*)-**13**.

Benzyl 6-oxo-2-benzyloxycarbonylaminohexanoate **14**

According to the procedure of Tice and Ganem,[8] both the enantiomers of **13** (4 g) were oxidised to give both the enantiomers of **14** as crude gums. TLC (SiO_2, EtOAc–n-hexane, 1:1): R_f 0.4. These were immediately used in the next step.

Benzyl 2 - benzyloxycarbonylamino - 9 - (4 - imidazolyl) - 7 - azanonanoate **1b**

(*a*) (S)-*Isomer*. Crude (*S*)-**14** (obtained from 4 g of (*S*)-**13**) was dissolved in dry MeOH (60 ml). To this were added histamine 2HCl (7.72 g), $NaBH_3CN$ (800 mg) and molecular sieves 3Å (1 g). The mixture was stirred overnight at room temp and filtered. The filtrate was concentrated *in vacuo* and the residue was diluted with water (300 ml). The mixture was adjusted to pH 2 with HCl aq and washed with $CHCl_3$–MeOH (3:1, 100 ml × 3). The aq layer was adjusted to pH 8 by the addition of solid K_2CO_3 and extracted with $CHCl_3$ (100 ml × 4). The extract was washed with brine, dried ($MgSO_4$) and concentrated *in vacuo* to give 3.6 g [69% from (*S*)-**13**] of (*S*)-**1b**, ν_{max} (film) 3320 (m), 1720 (br s), 1260 (s), 1215 (s), 750 (s) cm^{-1}; TLC (Merck silanised SiO_2, Art 5747; MeOH–28% NH_3 aq–H_2O, 4:1:1; detected with diazotised sulfanilic acid reagent) R_f 0.4. This was employed in the next step without further purification.

(*b*) (R)-*Isomer*. In the same manner, starting from 4 g of (*R*)-**13**, 3.7 g (71% from (*R*)-**13**) of (*R*)-**1b** was obtained. Its TLC behaviour and IR spectrum were identical with those of (*S*)-**1b**.

Gizzerosine dihydrochloride **1a**–2HCl

(*a*) (S)-*Isomer*. 10% Pd–C (1 g) was added to a soln of (*S*)-**1b** (3.6 g) in EtOH–THF–H_2O (3:1:1, 200 ml). The mixture was shaken under H_2 for 1 hr. Then 10% Pd–C (1 g) and conc HCl (0.5 ml) were added to the mixture and the hydrogenation was continued. After 1 hr, 10% Pd–C (1 g) and conc HCl (0.5 ml) were again added and the hydrogenation was continued for another 1 hr. The catalyst was filtered off and the filtrate was concentrated *in vacuo*. The residue was diluted with water and the soln was adjusted to pH 5 by the addition of 3 N HCl. A small amount of Norit was added to the mixture and filtered off. The filtrate was concentrated *in vacuo*. MeOH was added to the residual syrup and the resulting white solid was collected on a filter. This was recrystallised from aq MeOH to give 0.9 g (33%) of (*S*)-**1a**–2HCl, m.p. 251–252° (dec), $[\alpha]_D^{22} + 10.3°$ (c = 1.28, H_2O); $[\alpha]_D^{22} + 14.3°$ (c = 0.95, 3 N HCl); ν_{max} (KBr) 3140 (s), 2970 (s), 2810 (s), 2460 (m), 2200–1800 (w, br), 1640 (s), 1605 (s), 1525 (s), 1465 (s), 1400 (s), 1355 (m), 1335 (m), 1235 (w), 1185 (w), 1130 (w), 1115 (w), 1095 (w), 1075 (w), 1020 (w), 960 (m), 905 (w), 870 (w), 840 (m), 795 (m), 755 (w), 720 (m), 660 (w) cm^{-1}; TLC (under the same conditions as described for **1b**) R_f 0.90. (Calc for $C_{11}H_{22}O_2N_4Cl_2$: C, 42.18; H, 7.08; N, 17.88. Found: C, 42.20; H, 6.94; N, 17.72%.) The ^{1}H- and ^{13}C-NMR spectra of (*S*)-**1a**–2HCl were identical with those of (±)-**1a**–2HCl.

(*b*) (R)-*Isomer*. In the same manner as described above, (*R*)-**1b** (3.7 g) gave 1.25 g (44.6%) of (*R*)-**1a**–2HCl, m.p. 252–254° (dec), $[\alpha]_D^{22} - 9.1°$ (c = 1.26, H_2O); $[\alpha]_D^{22} - 13.0°$ (c = 1.03, 3 N HCl). Its chromatographic (TLC) and spectral (IR, ^{1}H-NMR and ^{13}C-NMR) properties were identical with those of (*S*)-**1a**–2HCl. (Calc for $C_{11}H_{22}O_2N_4Cl_2$: C, 42.18; H, 7.08; N, 17.88. Found: C, 42.32; H, 6.96; N, 17.72%.)

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