Synthesis of D-Alloisoleucine from L-Isoleucine and from (*S*)-2-Methylbutan-1-ol. Synthesis of Isostatine

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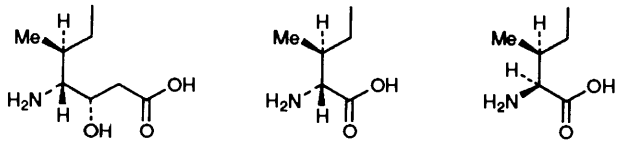
Three different synthetic routes to the uncommon α-amino acid D-alloisoleucine were studied. The first was based on the stereospecific inversion of configuration of the C-2 stereogenic carbon of L-isoleucine. The second involved acetylation of L-isoleucine with epimerisation at the C-2 carbon, giving a mixture of L-isoleucine and D-alloisoleucine, which was resolved enzymically with hog kidney acylase. In a new approach, an epimeric mixture of L-isoleucine and D-alloisoleucine was synthesized from (*S*)-2-methylbutan-1-ol and was again resolved enzymically. The D-alloisoleucine produced in these syntheses was subsequently transformed into the γ-amino acid isostatine.

The β-hydroxy-γ-amino acid isostatine [(3*S*,4*R*,5*S*)-4-amino-3-hydroxy-5-methylheptanoic acid **1**] is a constituent of the powerful antineoplastic agent Didemnin B.[1] It is related to the β-hydroxy-γ-amino acid statine, found in the proteinase inhibitor pepstatin,[2] and also to dolaisoleuine, a constituent of another potent antineoplastic substance, dolastatin 10.[3] Of the reported syntheses of isostatine,[4–7] most use the commercially available but expensive† amino acid D-alloisoleucine[8] [D-alle, (2*R*,3*S*)-isoleucine **2**] as starting material. Schmidt[7] has described a synthesis of isostatine from L-isoleucine [L-Ile, (2*S*,3*S*)-isoleucine **3**], which is, however, rather lengthy and, since the first step proceeds in a yield of only 55%, expensive in terms of the L-Ile required.

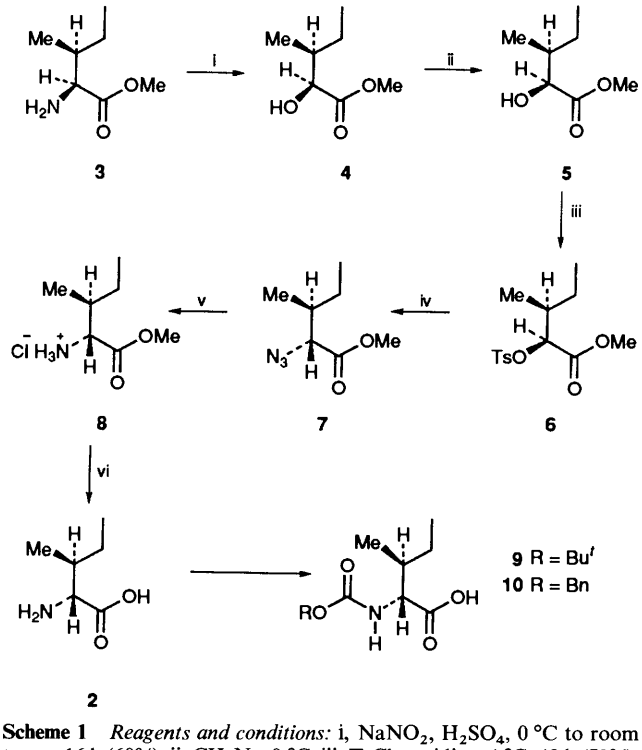


1 **2** **3**

D-alle itself is found in certain peptide antibiotics[9] and is also of interest for the synthesis of peptide mimetics. Since there is increasing interest in the potential therapeutic use of peptides and peptide mimetics in general, and of the Didemnins in particular,[10] economical methods for the synthesis of D-alle are desirable. Synthesis of D-alle from L-Ile may be brought about either by stereospecific inversion[7] of the stereogenic carbon at C-2 or by epimerisation at this same carbon atom followed by resolution of the epimeric mixture.[11,12] A novel synthesis of D-alle from the much cheaper (*S*)-2-methylbutan-1-ol may be carried out by using a method recently described by Corey,[13] allowing an alternative, non-amino acid, starting material to be used. Three syntheses of D-alle and its conversion into isostatine are described herein.

Results and Discussion

Diazotisation and hydrolysis of the amino group of L-Ile **3** proceeded with retention of configuration,[14,15] and provided the basis for a stereospecific synthesis of D-alle **2** (Scheme 1). These reactions afforded hydroxy acid **4** in 59% yield. Esterification of acid **4** with diazomethane gave hydroxy ester **5**, which on treatment with toluene-*p*-sulfonyl chloride in pyridine yielded tosyl ester **6**. Nucleophilic substitution with azide ion gave azide **7**, which was reduced to the amine hydrochloride **8** by catalytic hydrogenation. Saponification then furnished D-alle **2** (>99% enantiomeric and diastereoisomeric purity) as evidenced by amino acid analysis and by GLC analysis of the N-trifluoroacetylated isobutyl ester derivative using a chiral column.‡[16]



Scheme 1 *Reagents and conditions:* i, $NaNO_2$, H_2SO_4, 0 °C to room temp., 16 h (60%); ii, CH_2N_2, 0 °C; iii, TsCl, pyridine, 4 °C, 48 h (72%); iv, NaN_3, DMF, 50 °C, 30 h; v, H_2/Pd, 1 atm, room temp., 18 h (80%); vi, 1 mol dm^{-3} NaOH, water, room temp., 6 h (95%)

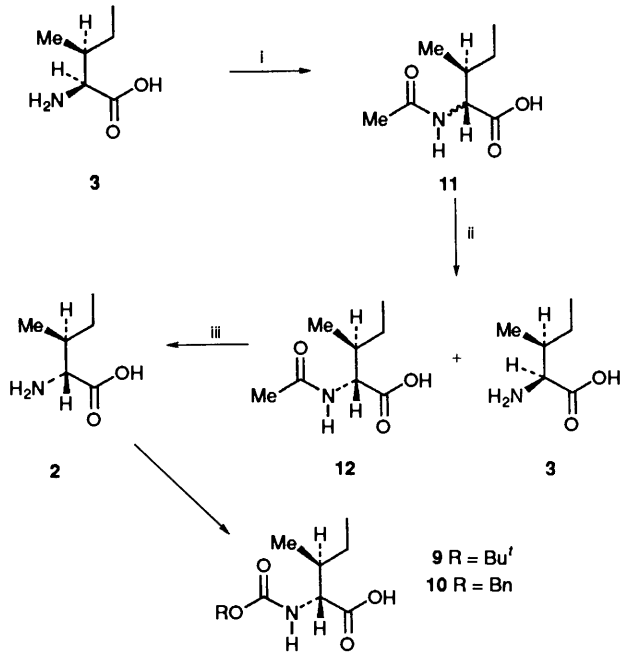
Although such a route leads to optically pure D-alle, it requires 6 steps from L-Ile and is only moderately efficient in terms of the conversion of L-Ile into D-alle.

† The price of D-alle is between 60 and 90 times that of L-Ile.

‡ Chirasil-Val column, 110 °C isothermal run. Under these conditions the D-allo diastereoisomer derivative is cleanly separated from the other three.

More economical are syntheses which allow all the L-Ile to be converted into its D-allo epimer. The selective enzymic hydrolysis of enantiomeric N-acetyl amino acids by acylases[17] provides the basis for a synthesis of D-aIle from L-Ile which should, in principle, allow all L-Ile to be converted into D-aIle by recycling. Acetylation of commercial L-Ile **3** with acetic anhydride and sodium hydroxide at 140 °C for 30 h yielded, after work-up, a 46:54 mixture **11** of acetyl-L-Ile and acetyl-D-aIle as judged by amino acid analysis of a hydrolysate of the mixture.

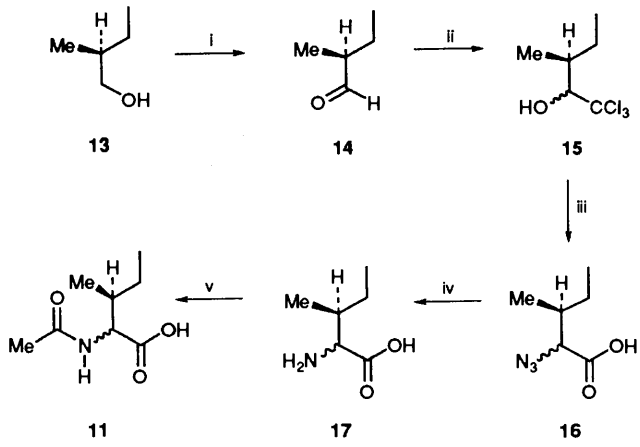
Epimerisation of the amino acid presumably proceeds either *via* the azlactone or by abstraction of a proton. The yield for this reaction was only 60%, probably due to some hydrolysis of the N-acetylated derivative under the reaction conditions. Non-acetylated amino acid can, however, be recovered on work-up and recycled. The N-acetyl epimers were then treated with hog kidney acylase[18] at pH 8 and 38 °C for 4 days, leading to selective hydrolysis of acetyl-L-Ile to L-Ile, leaving acetyl-D-aIle unchanged. Acetyl-D-aIle **12** (>90% of theoretical) was then isolated by simple extraction with ethyl acetate, L-Ile **3** remaining in the aqueous phase (Scheme 2).



Scheme 2 *Reagents and conditions:* i, Ac_2O, NaOH, 130 °C, 30 h (60%); ii, hog kidney acylase, pH 8, 38 °C, 4 days (90%); iii, 4 mol dm^{-3} HCl, reflux, 4 h (98%)

The progress of this enzymic hydrolysis can be monitored by standard, reversed-phase HPLC (C_{18} column; 15% acetonitrile in water; isocratic run; detection at 220 nm) and amino acid analysis provides a simple, highly sensitive method for judging the diastereoisomeric purity of the amino acids in the organic and aqueous phases. Hydrolysis of acetyl-D-aIle **12** to D-aIle **2** was brought about, without detectable epimerisation, by treatment with 4 mol dm^{-3} hydrochloric acid at reflux, for 4 h. D-aIle of greater than 99% enantiomeric and diastereoisomeric purity was obtained, as judged by amino acid analysis and gas chromatography using a chiral column, of the N-trifluoroacetylated isobutyl ester.*[16] The advantage of this approach is that the L-Ile recovered from the aqueous phase can be recycled, making it possible, by repeated epimerisation and resolution of the N-acetyl derivatives, for all L-Ile to be converted into D-aIle. The procedure is also amenable to scale-up to the multigram level.

* See footnote ‡ on previous page.



Scheme 3 *Reagents and conditions:* i, PCC/Al_2O_3, 2 h; ii, $CHCl_3$–KOH–MeOH–DMF, −10 to 5 °C, 2 h (76%); iii, NaOH, NaN_3, 24 h (45%); iv, H_2/Pd–C, 1 atm, room temp., 18 h (90%); v, Ac_2O, NaOH, 0 °C, 90 min (96%)

A novel synthesis of the mixture of acetyl-L-Ile and acetyl-D-aIle epimers, compounds **11**, was carried out by using a variation of the recently described reaction sequence[13] outlined in Scheme 3.

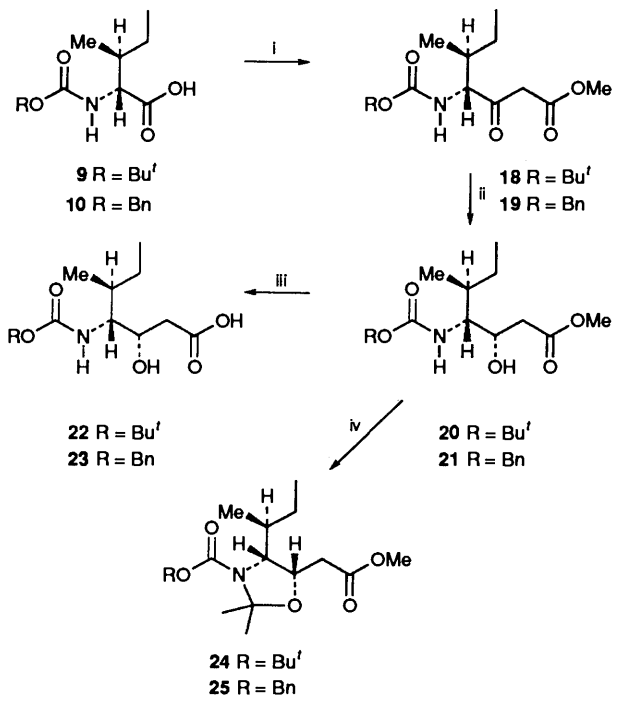
Commercial (*S*)-2-methylbutan-1-ol **13** was oxidised with pyridinium chlorochromate (PCC) adsorbed on alumina[19] and, without isolation of the aldehyde **14**, the solution was treated with a mixture of chloroform and sodium hydroxide in methanol–dimethylformamide (DMF) (2:7). Work-up afforded the trichloro alcohol **15**. Treatment of this with sodium hydroxide and sodium azide in 1,2-dimethoxyethane (DME)–water (4:1) gave an epimeric mixture of azides **16**. Catalytic reduction afforded a 64:36 mixture **17** of L-Ile and D-aIle, as judged by amino acid analysis of the hydrolysate. Acetylation then furnished the mixture of epimers **11**, which were resolved as before by use of hog kidney acylase. Since in this case the L-Ile produced can also be recycled, eventually allowing all amino acid material to be converted into the desired D-allo epimer, this method provides a synthesis of D-aIle from inexpensive starting materials.

The D-aIle **2** produced in these syntheses was protected at the amino function with the *tert*-butoxycarbonyl (Boc) group[20] to give Boc-D-aIle **9** or with the benzyloxycarbonyl (Z) group[21] to give Z-D-aIle **10**, respectively (Scheme 2).

Construction of the isostatine skeleton was accomplished by activation of the carboxylic acid of Boc-D-aIle **9** with carbonyldiimidazole (CDI) followed by reaction with the lithium enolate of methyl acetate,[5] to give keto ester **18**. Reduction of the ketone with potassium boranuide (borohydride) in methanol proceeded stereospecifically in accord with precedent,[5] and gave alcohol **20** (Scheme 4).

The stereochemistry of the reduction product was confirmed by treatment of a solution of compound **20** in DMF with 2-methoxypropene in the presence of toluene-*p*-sulfonic acid (PTSA), which gave the hemiaminal **24** which exhibited a value of 5.5 Hz for the coupling constant between the hydrogens at C-3 and C-4, indicating a *cis*-relationship. Saponification of the methyl ester **20** then gave *N*-Boc-isostatine **22**, whose spectroscopic and optical rotation data were identical with those reported.[5,7] An analogous sequence of reactions was carried out starting from Z-D-aIle **10**, which led to the formation of the hitherto unreported *N*-Z-isostatine **23**. The stereochemistry of the reduction of ketone **19** to alcohol **21** was confirmed by formation of the hemiaminal **25**.

The most efficient of the syntheses of D-aIle reported here is that based on acetylation with epimerisation of L-Ile, followed



Scheme 4 *Reagents and conditions:* i, CDI, AcOMe, LDA, −78 °C (57%); ii, KBH_4, MeOH, 10 min (83%); iii, 1 mol dm^{-3} NaOH, 1,4-dioxane (95%); iv, CH_2=C(OMe), PTSA

by enzymic resolution of the epimers. The synthesis of D-allo Ile in this way allows this amino acid to be produced economically and provides a short and efficient (seven steps overall from L-Ile) synthesis of isostatine, which compares favourably with those previously reported.

Experimental

All organic solutions were dried over sodium sulfate. Chemical shifts are quoted in δ-values downfield from tetramethylsilane. *J*-Values are given in Hz. All m.p.s were measured on a Kofler block apparatus and are uncorrected. Optical rotation data {$[\alpha]_D$-values given in units of 10^{-1} deg cm^2 g^{-1}} were measured with a Perkin-Elmer 241 MC polarimeter and amino acid analyses were carried out on a Beckmann 6300 system. GLC was carried out on a Hewlett-Packard 5890 apparatus using a Chirasil-Val column, and HPLC was performed using a Shimadzu apparatus and a Nucleosil C_{18} column (25 × 0.4 cm).

(2S,3S)-2-*Hydroxy*-3-*methylpentanoic Acid* **4**.—Sulfuric acid (50 cm^3 of a 4 mol dm^{-3} aq. solution, 0.20 mmol) was added dropwise to a stirred suspension of L-Ile **3** (10.1 g, 76.7 mmol) in water (100 cm^3) until all the solid had dissolved. The solution was cooled to 0 °C and aq. sodium nitrite (150 cm^3 of a 2 mol dm^{-3} solution, 0.30 mmol) and the remainder of the H_2SO_4 solution were added simultaneously over a period of 60 min. The resulting solution was kept at 0 °C for 2 h, brought to room temperature, and left for 16 h. Extraction with ethyl acetate (4 × 50 cm^3), followed by drying and removal of solvent, gave an off-white solid. Crystallisation from diethyl ether–hexane gave the hydroxy acid **4** as needles (6.00 g, 59%), m.p. 55.5–57 °C (lit.,[22] 47–49 °C); $[\alpha]_D$ +2.55 (*c* 2, water) (lit.,[23] +3.9); ν_{max}(KBr disc)/cm^{-1} 3600–3300, 2965, 2940, 2890, 1730, 1245 and 1135; δ_H(200 MHz; D_2O) 0.83 (3 H, t, *J* 7.3), 0.91 (3 H, d, *J* 7.0), 1.05–1.25 (1 H, m), 1.25–1.45 (1 H, m), 1.70–1.90 (1 H, m) and 4.11 (1 H, d, *J* 4.3); δ_C(50 MHz; $CDCl_3$) 14.77 (CH_3), 18.39 (CH_3), 26.72 (CH_2), 41.94 (CH), 77.70 (CH) and 182.75 (C); *m/z* (CI, NH_3) 167 [(M + N_2H_7)$^+$, 93%] and 150 [(M + NH_4)$^+$, 100] (Found: C, 54.2; H, 9.4. Calc. for $C_6H_{12}O_3$: C, 54.5; H, 9.2%).

Methyl (2S,3S)-2-*Hydroxy*-3-*methylpentanoate* **5**.—A solution of diazomethane (approx. 0.48 mol dm^{-3} solution in diethyl ether) was added slowly to a vigorously stirred solution of hydroxy acid **4** (8.41 g, 63.6 mmol) in diethyl ether (100 cm^3) until the solution remained pale yellow in colour. Removal of solvent gave the crude hydroxy ester as an oil (8.95 g, 95%), which was used without further treatment. It had ν_{max}(film)/cm^{-1} 3600–3200, 2950, 2920, 2870, 1740, 1260 and 1160; δ_H(200 MHz, $CDCl_3$) 0.89 (3 H, t, *J* 7.3), 0.97 (3 H, d, *J* 6.9), 1.13–1.50 (2 H, m), 1.70–1.90 (1 H, m), 3.09 (1 H, br s, removable with D_2O), 3.78 (3 H, s) and 4.09 (1 H, d, *J* 4); δ_C(75 MHz; $CDCl_3$) 11.71 (CH_3), 15.38 (CH_3), 23.71 (CH_2), 39.09 (CH), 52.31 (CH_3), 74.76 (CH) and 175.43 (C); *m/z* (CI, NH_3) 181 [(M + N_2H_7)$^+$, 20%] and 164 [(M + NH_4)$^+$, 100]; *m/z* (EI) 129 [(M − OH)$^+$, 34%], 101 [(M − OH − CO)$^+$, 100] and 87 [(M − CO_2Me)$^+$, 76].

Methyl (2S,3S)-3-*Methyl*-2-(p-*tolyl-sulfonyloxy*)*pentanoate* **6**.—Hydroxy ester **5** (2.10 g, 14.4 mmol) was dissolved in pyridine (7 cm^3) at 0 °C and toluene-*p*-sulfonyl chloride (3.91 g, 20.5 mmol) was added in batches during 15 min. The mixture was kept at 4 °C for 48 h. Dichloromethane (15 cm^3) was added and this solution was washed with dil. (10% v/v) hydrochloric acid (4 × 10 cm^3). The aqueous extracts were washed with dichloromethane (4 × 15 cm^3) and the organic phases were combined. Washing successively with saturated aq. sodium hydrogen carbonate and brine, followed by drying and removal of solvent gave the tosyl ester **6** as a yellow oil (2.96 g, 72%); $[\alpha]_D$ −29.3 (*c* 1, $CHCl_3$); ν_{max}(film)/cm^{-1} 2950, 2920, 2860, 1755, 1365, 1185, 1170 and 1090; δ(200 MHz; $CDCl_3$) 0.83 (3 H, t, *J* 7.4), 0.87 (3 H, d, *J* 7.0), 1.06–1.31 (1 H, m), 1.35–1.56 (1 H, m), 1.84–2.04 (1 H, m), 2.45 (3 H, s), 3.62 (3 H, s), 4.67 (1 H, d, *J* 5.5), 7.35 (2 H, d, *J* 8.7) and 7.80 (2 H, d, *J* 8.4); δ_C(75 MHz; $CDCl_3$) 11.00 (CH_3), 14.71 (CH_3), 21.67 (CH_3), 24.12 (CH_2), 37.40 (CH), 52.21 (CH_3), 81.53 (CH), 128.11 (CH), 129.71 (CH), 133.19 (C), 145.03 (C) and 168.86 (C); *m/z* (CI) 335 [(M + N_2H_7)$^+$, 9%] and 318 [(M + NH_4)$^+$, 100]; *m/z* (EI) 155 [($C_7H_7O_2S$)$^+$, 84%] and 91 [(C_7H_7)$^+$, 100].

D-*Alloisoleucine Hydrochloride Methyl Ester* **8**.—Sodium azide (0.79 g, 12.2 mmol) was added to a stirred solution of tosyl ester **6** (2.22 g, 7.38 mmol) in DMF (7 cm^3) and the resulting mixture was kept at 50 °C for 30 h. After the mixture had been partitioned between ethyl acetate and water (1:1; 20 cm^3) the aqueous phase was extracted with ethyl acetate (4 × 5 cm^3). The combined organic phases were dried and removal of solvent gave a DMF solution of azide **7** (2.53 g); ν_{max}(film)/cm^{-1} 2950, 2920, 2860, 2100, 1740, 1260 and 1200; δ_H(200 MHz; $CDCl_3$) 0.93 (3 H, d, *J* 6.25), 0.94, (3 H, t, *J* 6.25), 1.19–1.59 (2 H, m), 1.86–2.87 (1 H, m), 3.81 (3 H, s) and 3.90 (1 H, d, *J* 5.0), plus peaks corresponding to DMF: 2.88 (3 H, s), 2.96 (3 H, s) and 8.01 (1 H, s); δ_C(75 MHz; $CDCl_3$) 11.45 (CH_3), 14.75 (CH_3), 26.40 (CH_2), 37.45 (CH), 52.41 (CH_3), 66.39 (CH) and 170.85 (C), plus peaks corresponding to DMF: 31.81 (CH_3), 36.88 (CH_3) and 162.95 (CH); *m/z* (EI) 129 [(M − N_3)$^+$, 1%], 97 [(M − HCO_2Me − N)$^+$, 8] and 83 [(M − HCO_2Me − N_2)$^{\cdot+}$, 100].

This solution (0.68 g, 3.95 mmol) in a mixture of 10 mol dm^{-3} hydrochloric acid–ethanol (1:7.5; 8.5 cm^3) was hydrogenated in the presence of 10% Pd–C (90 mg) for 18 h. The suspension was filtered through Celite, which was washed well with ethanol. Solvent was removed to give a solid (0.57 g, 79%), m.p. 117–119 °C (lit.,[7] 123 °C); $[\alpha]_D$ −21.7 (*c* 0.75, water) (lit.,[7] −22.5); ν_{max}(KBr disc)/cm^{-1} 3140–2760, 1735, 1500, 1440, 1225 and

1115; δ_H(200 MHz; D_2O) 0.77 (3 H, t, *J* 7.4), 0.80 (3 H, d, *J* 7.0), 1.02–1.44 (2 H, m), 1.87–2.07 (1 H, m), 3.68 (3 H, s) and 3.97 (1 H, d, *J* 3.9); δ_C(75 MHz; D_2O) 13.81 (CH_3), 16.67 (CH_3), 27.89 (CH_2), 38.62 (CH), 56.58 (CH_3), 60.16 (CH) and 173.67 (C).

D-*Alloisoleucine* **2**.—Aq. sodium hydroxide (3.5 cm^3 of a 1.2 mol dm^{-3} solution, 4.13 mmol) was added to a mixture of methyl ester **8** (0.24 g, 1.33 mmol) in water (10 cm^3) and the reaction mixture was maintained at room temperature for 6 h. Aq. 2 mol dm^{-3} hydrochloric acid was added (to pH 2, measured using indicator paper). Solvent was removed to give a yellow solid (0.41 g). It had δ_H(300 MHz; D_2O) 0.78 (3 H, t, *J* 7.2), 0.79 (3 H, d, *J* 6.9), 1.07–1.37 (2 H, m), 1.86–2.00 (1 H, m) and 3.77 (1 H, d, *J* 5.4); δ_C(75 MHz; D_2O) 10.90 (CH_3), 13.38 (CH_3), 25.13 (CH_2), 35.40 (CH), 57.33 (CH) and 172.60 (C). Amino acid analysis showed a single peak at 28.24 min, corresponding to D-alloisoleucine.

N-*Acetyl*-L- *and* -D-*allo-isoleucine* **11**.—L-Ile **3** (10.10 g, 76.9 mmol) was dissolved in aq. sodium hydroxide (460 cm^3 of a 1 mol dm^{-3} solution, 460 mmol). Acetic anhydride (212 cm^3, 2.28 mol) was added, and the mixture was refluxed for 30 h. After cooling of the mixture and acidification to pH 1 with conc. hydrochloric acid, extraction with ethyl acetate (4 × 250 cm^3) followed by washing of the combined organic phases with brine (4 × 350 cm^3), drying and removal of solvent gave a solid, which was crystallised from water (8.00 g, 60%). Amino acid analysis indicated a 46:54% mixture **11** of acetyl-L-Ile/acetyl-D-aIle; δ_H(200 MHz; D_2O) 0.97–1.07 (2 × 6 H, m), 1.20–1.70 (2 × 2 H, m), 1.86–2.19 (2 × 1 H, m), 2.09 (3 H, s, acetyl-L-Ile), 2.10 (3 H, s, acetyl-D-aIle), 4.46 (1 H, d, *J* 5.6), 4.62 (1 H, d, *J* 4.6) and 5.40 (2 × 1 H, br s); *m/z* (CI) 208 [$(M + N_2H_7)^+$, 5%], 191 [$(M + NH_4)^+$, 52)], 174 [$(M + H)^+$, 2)] and 161 (100).

Acetyl-D-*alloisoleucine* **12** *from Mixture* **11**.—The mixture **11** (0.95 g, 5.48 mmol) was dissolved in water (60 cm^3) and the solution was adjusted to pH 8 with 1 mol dm^{-3} aq. lithium hydroxide. Hog kidney acylase I (20 mg, 82.8 U of acylase $mmol^{-1}$ acetyl-Ile) was added and the solution was stirred at 38 °C under nitrogen for 96 h. Conc. hydrochloric acid was added dropwise (to pH 4), activated charcoal (250 mg) was added, and the mixture was heated at 80 °C for 5 min. The suspension was filtered through Celite and washed successively with water and methanol. Acidification of the filtrate with conc. hydrochloric acid to pH 1 and extraction with ethyl acetate (4 × 25 cm^3) followed by drying and removal of solvent gave acetyl-D-alloisoleucine as a solid **12** (0.45 g, 90%), whose spectroscopic data were identical with those of an authentic sample.

Acetyl-D-*alloisoleucine* **12** *from* D-*aIle* **2**.—D-aIle (100 mg, 0.76 mmol) was dissolved in aq. sodium hydroxide (0.76 cm^3 of a 1 mol dm^{-3} solution, 0.76 mmol) at 0 °C and more aq. sodium hydroxide (0.15 cm^3 of a 1 mol dm^{-3} solution, 0.15 mmol) and acetic anhydride (15 mm^3, 0.16 mmol) were added. The addition of sodium hydroxide (15 mm^3, 0.16 mmol) and acetic anhydride (15 mm^3, 0.16 mmol) was repeated five times at intervals of 10 min and the alkalinity of the solution was maintained, if necessary, by adjustment with aq. sodium hydroxide. After 90 min, the pH of the solution was adjusted to 2 with conc. hydrochloric acid, which precipitated the title product as a solid, which was crystallised from water (95 mg, 72%); m.p. 158–161 °C (lit.,[24] 156 °C); $[\alpha]_D$ −20.5 (*c* 2, EtOH) (lit.,[21] −21.5); ν_{max}(KBr disc)/cm^{-1} 3330, 2955, 2920, 2870, 1700, 1615, 1555, 1265 and 1145; δ_H(200 MHz; CD_3OD) 0.92 (3 H, d, *J* 5.9), 0.93 (3 H, t, *J* 5.9), 1.10–1.54 (2 H, m), 1.87–2.10 (1 H, m), 2.00 (3 H, s), 4.52 (1 H, d, *J* 4.7), 4.92 (1 H, br s) and 5.48 (1 H, d, *J* 0.9); *m/z* (CI) 208 [$(M + N_2H_7)^+$, 5%], 191 [$(M + NH_4)^+$, 100] and 174 [$(M + H)^+$, 18].

D-*Alloisoleucine Hydrochloride*.—Acetyl-D-alloisoleucine **12** (0.25 g, 1.45 mmol) was refluxed for 2 h with hydrochloric acid (3 cm^3 of a 2 mol dm^{-3} solution, 6 mmol). Solvent was removed to give a solid (0.24 g, 98%); δ_H(200 MHz; D_2O) 0.92 (3 H, t, *J* 7.2), 0.94 (3 H, d, *J* 7.3), 1.17–1.54 (2 H, m), 1.97–2.20 (1 H, m) and 3.94 (1 H, d, *J* 5). Amino acid analysis showed a single peak at 28.24 min, corresponding to D-alloisoleucine.

L-/D-*Alloisoleucine* **17**.—(*S*)-2-Methylbutan-1-ol **13** (0.50 g, 5.71 mmol) was dissolved in chloroform (30 cm^3), PCC adsorbed on alumina[19] (17.4 g, 14.2 mmol) was added and the mixture stirred at room temperature for 2 h under nitrogen. The mixture was filtered and the resulting black solid was washed with chloroform (5 cm^3). This solution of (*S*)-2-methylbutanal **14**[25] was immediately introduced into a flask under nitrogen. The solution had δ_H(200 MHz; $CDCl_3$) 0.92 (3 H, t, *J* 7.5), 1.06 (3 H, d, *J* 7.0), 1.29–1.54 (1 H, m), 1.61–1.84 (1 H, m), 2.14–2.36 (1 H, m) and 9.60 (1 H, d, *J* 1.9); δ_C(50 MHz; $CDCl_3$) 11.35 (CH_3), 12.87 (CH_3), 23.53 (CH_2), 47.77 (CH) and 205.44 (CH); *m/z* (EI) 86 ($M^{\bullet+}$, 4%), 85 [$(M - H)^+$, 24], 71 [$(M - Me)^+$, 31] and 57 [$(M - CHO)^+$, 100].

DMF (7 cm^3) was added and the solution was cooled to −10 °C and a solution of potassium hydroxide (0.25 g, 4.53 mmol) in methanol (2.2 cm^3) was added over a period of 90 min. This mixture was stirred at between −10 and +5 °C for 2 h. Hydrochloric acid (4 cm^3 of a 2 mol dm^{-3} solution, 8 mmol) was added dropwise and the solution was partitioned between chloroform (10 cm^3) and brine (20 cm^3). The aqueous phase was extracted with chloroform (4 × 10 cm^3) and the combined organic phases were washed successively with brine, 5% aq. sodium hydrogen carbonate and brine, and was dried. Solvent was removed and gave the trichloro product **15** as a brown oil (0.89 g, 76%); δ_H(200 MHz; $CDCl_3$) 0.95 (3 H, t, *J* 7.4, diastereoisomer 1), 0.97 (3 H, t, *J* 7.4, diastereoisomer 2), 1.09 (3 H, d, *J* 6.8, diastereoisomer 1), 1.15 (3 H, d, *J* 7.0, diastereoisomer 2), 1.30–1.66 (2 × 2 H, m), 1.81–1.99 (1 H, m, diastereoisomer 1), 2.03–2.30 (1 H, m, diastereoisomer 2), 3.96 (1 H, d, *J* 5, diastereoisomer 1) and 4.05 (1 H, br s, diastereoisomer 2); δ_C(50 MHz; $CDCl_3$) 11.92 (CH_3, diastereoisomer 1), 12.11 (CH_3, diastereoisomer 2), 18.47 (CH_3, diastereoisomer 1), 19.25 (CH_3, diastereoisomer 2), 23.76 (CH_2, diastereoisomer 1), 29.94 (CH_2, diastereoisomer 2), 36.63 (CH, diastereoisomer 1), 37.58 (CH, diastereoisomer 2), 84.78 (CH, diastereoisomer 1), 86.66 (CH, diastereoisomer 2), 104.90 (CH, diastereoisomer 1) and 105.15 (CH, diastereoisomer 2); *m/z* (EI) 117 [$(M - Cl_2OH)^+$, 12%], 111 (100), 87 [$(M - CCl_3)^+$, 75], 86 [$(M - CHCl_3)^{\bullet+}$, 89] and 82 [$(M - Cl_3 - OH)^{\bullet+}$, 15].

A solution of sodium hydroxide (170 mg, 4.30 mmol) and sodium azide (130 mg, 2.06 mmol) in water (8 cm^3) was added to a solution of trichloro compound **15** (200 mg, 0.99 mmol) in 1,2-dimethoxyethane (2 cm^3), at 0 °C. The mixture was allowed to come to room temperature and was stirred for 24 h. The solution was washed with diethyl ether (4 × 3 cm^3) and the organic phase was extracted with 5% aq. sodium hydroxide (4 × 4 cm^3). The combined aqueous phases were acidified at 0 °C with 10% hydrochloric acid (to pH 1) and were extracted with ethyl acetate (4 × 7 cm^3). Drying, followed by removal of solvent gave (2*R*/*S*,3*S*)-2-azido-3-methylpentanoic acid **16** as a yellow oil (70 mg, 45%); δ_H(200 MHz; $CDCl_3$) 0.91–1.09 (2 × 6 H, m), 1.25–1.46 (2 H, m, diastereoisomer 1), 1.46–1.68 (2 H, m, diastereoisomer 2), 1.93–2.15 (2 × 1 H, m), 3.83 (1 H, d, *J* 5.9, diastereoisomer 1), 4.00 (1 H, d, *J* 4.6, diastereoisomer 2) and 7.40–8.00 (2 × 1 H, br s); δ_C(50 MHz; $CDCl_3$) 11.85 (CH_3, diastereoisomer 1), 12.11 (CH_3, diastereoisomer 2), 15.23 (CH_3, diastereoisomer 1), 16.54 (CH_3, diastereoisomer 2), 25.54

(CH_2, diastereoisomer 1), 27.08 (CH_2, diastereoisomer 2), 37.82 (CH, diastereoisomer 1), 38.10 (CH, diastereoisomer 2), 66.71 (CH, diastereoisomer 1), 67.73 (CH, diastereoisomer 2), 176.75 (C, diastereoisomer 1) and 177.37 (C, diastereoisomer 2); *m/z* (EI) 115 [$(M - N_3)^+$, 3%], 97 [$(M - HCO_2H - N)^+$, 17] and 83 [$(M - HCO_2H - N_2)^{\bullet+}$, 100]; *m/z* (CI) 234 (100%), 192 [$(M + N_2H_7)^+$, 13], 175 [$(M + NH_4)^+$, 14] and 158 [$(M + H)^+$, 14].

A solution of azide **16** (190 mg, 1.18 mmol) in ethyl acetate–methanol (1:1; 6 cm^3) was hydrogenated in the presence of 10% Pd–C (40 mg) for 18 h. The solvent was removed and hot water (80 °C; 6 cm^3) was added and the suspension was filtered through Celite, which was washed well with hot water. Solvent was removed to give compound **17** as a solid (140 mg, 90%). Amino acid analysis indicated a 64:36 mixture of L-Ile/D-aIle. It had δ_H(300 MHz; D_2O) 0.68 (3 H, t, *J* 6.3, L-Ile), 0.75 (3 H, d, *J* 7.2, L-Ile), 0.75 (3 H, t, *J* 7.0, D-aIle), 0.82 (3 H, d, *J* 7.2, D-aIle), 0.95–1.17 (2 H, m, L-Ile), 1.17–1.35 (2 H, m, D-aIle), 1.44–1.64 (1 H, m, L-Ile), 1.73–1.93 (1 H, m, D-aIle), 3.48 (1 H, d, *J* 3.5, L-Ile) and 3.56 (1 H, d, *J* 3.5, D-aIle); *m/z* (EI) 131 ($M^{\bullet+}$, 1%), 114 [$(M - NH_3)^{\bullet+}$, 22], 85 [$(M - HCO_2H)^{\bullet+}$, 76] and 83 [$(M - HCO_2H - H_2)^{\bullet+}$, 100].

Acetyl-L-/-D-*alloisoleucine* **11**.—The mixture **17** (93 mg, 0.71 mmol) was dissolved in aq. sodium hydroxide (1.42 cm^3 of a 1 mol dm^{-3} solution, 1.42 mmol) at 0 °C. Aq. sodium hydroxide (0.14 cm^3 of a 1 mol dm^{-3} solution, 0.14 mmol) followed by acetic anhydride (24 mm^3, 0.25 mmol) was added. Addition of sodium hydroxide (0.14 cm^3, 0.14 mmol) and acetic anhydride (24 mm^3, 0.25 mmol) was repeated four times at intervals of 10 min and the alkalinity of the solution was maintained, if necessary, by adjustment with aq. sodium hydroxide. After 90 min, the solution was adjusted to pH 2 with conc. hydrochloric acid and was extracted with ethyl acetate (4 × 10 cm^3). The combined extracts were dried and the solvent was removed to give title mixture as a pale yellow oil (48 mg); δ_H(200 MHz; CD_3OD) 0.95–1.10 (2 × 6 H, m), 1.23–1.72 (2 × 2 H, m), 1.80–2.15 (2 × 1 H, m), 2.09 (3 H, s, *N*-Ac-L-Ile), 2.10 (3 H, s, *N*-Ac-D-aIle), 4.45 (1 H, d, *J* 5.3, *N*-Ac-L-Ile), 4.61 (1 H, d, *J* 4.3, *N*-Ac-D-aIle) and 5.01 (2 × 1 H, br s).

N-(tert-*Butoxycarbonyl*)-D-*alloisoleucine* **9**.—Di-*tert*-butyl carbonate (0.50 g, 2.29 mmol) was added to a solution of compound **8** (0.20 g, 1.52 mmol) in 1,4-dioxane (3 cm^3), water (1.5 cm^3), and aq. sodium hydroxide (1.5 cm^3 of a 1 mol dm^{-3} solution, 1.5 mmol) at 0 °C. The mixture was allowed to reach room temperature and was stirred for 2 h before being cooled to 0 °C and 5% aq. potassium hydrogen sulfate was added (to pH 4). The aqueous phase was extracted with ethyl acetate (4 × 3 cm^3) and the combined organic phases were washed with brine. Drying followed by removal of solvent gave *compound* **9** as a solid (90 mg), m.p. 66–68 °C; $[\alpha]_D$ −15.6 (*c* 1.11, $CHCl_3$); δ_H(200 MHz; $CDCl_3$) 0.80–1.04 (6 H, m), 1.19–1.31 (2 H, m), 1.45 (9 H, s), 1.73–2.03 (1 H, m), 4.33 (1 H, m), 4.95 (1 H, d, *J* 8.7) and 9.0–9.2 (1 H, br s); ν_{max}(film)/cm^{-1} 3500, 3000, 1720, 1710, 1650, 1520 and 1500; *m/z* (CI) 249 [$(M + NH_4)^+$, 100%] and 232 [$(M + 1)^+$, 22] (Found: C, 57.6; H, 9.1; N, 6.0. $C_{11}H_{21}NO_4$ requires C, 57.1; H, 9.1; N, 6.0%).

N-(*Benzyloxycarbonyl*)-D-*alloisoleucine* **10**.—D-aIle (2.50 g, 19.0 mmol) and *N*-benzyloxycarbonyltrinorborn-5-ene-2,3-dicarboximide (6.58 g, 20.9 mmol) were dissolved in (1:1) tetrahydrofuran(THF)–water (200 cm^3) and the solution was cooled to 0 °C. Triethylamine (6 cm^3, 41.00 mmol) was added dropwise during 30 min and the mixture was allowed to come to room temperature and was stirred for 24 h. The amount of solvent was reduced by one-half by rotatory evaporation and then the remainder was extracted with diethyl ether (3 × 30 cm^3). The combined ethereal extracts were washed with saturated aq. sodium hydrogencarbonate and the combined aqueous phases were adjusted to pH 3–4 by addition of 2 mol dm^{-3} potassium hydrogen sulfate. Extraction with diethyl ether (3 × 60 cm^3) followed by washing of the combined organic phases with brine, drying, and removal of solvent gave the crude *product* **10** as an oil (5 g, 99%), which was used without further treatment. It had $[\alpha]_D$ −8.8 (*c* 1.6, $CHCl_3$); ν_{max}(film)/cm^{-1} 3500–3000, 3000–2800, 1720, 1520, 1450, 1420, 1350, 1300 and 1100; δ_H(200 MHz; CD_3OD) 0.97–1.03 (6 H, m), 1.20–1.60 (2 H, m), 2.00–2.20 (1 H, m), 4.40 (1 H, br s), 5.19 (2 H, s), 7.22 (1 H, br s) and 7.43–7.44 (5 H, m); *m/z* (CI, NH_3) 283 [$(M + NH_4)^+$, 100%] (Found: C, 63.5; H, 7.1; N, 5.2. $C_{14}H_{18}NO_4$ requires C, 63.6; H, 6.8; N, 5.3%).

Methyl (4R,5S)-4-(tert-*Butoxycarbonylamino*)-5-*methyl*-3-*oxoheptanoate* **18**.—*N*-(*tert*-Butoxycarbonyl)-D-aIle (1.90 g, 8.23 mmol) as a solution in dry THF (10 cm^3), was added to a stirred suspension of CDI (4.00 g, 24.7 mmol) in THF (6 cm^3) at 0 °C, under argon. The mixture was allowed to come to room temp. over a period of 5 h. The resulting solution was cooled to 0 °C and a solution of the lithium enolate of methyl acetate, prepared by adding methyl acetate (2.30 cm^3, 28.84 mmol) to a solution of lithium diisopropylamide (LDA) [from butyllithium (18.0 cm^3 of a 1.6 mol dm^{-3} solution in hexanes, 28.84 mmol) and diisopropylamine (4.47 cm^3, 31.7 mmol) in THF (5 cm^3), at −78 °C under argon], was added *via* cannula. The reaction mixture was allowed to come to 0 °C over a period of 120 min and was quenched with saturated aq. ammonium chloride and extracted with dichloromethane. The extracts were washed successively with 5% aq. hydrochloric acid, 5% aq. sodium hydrogen carbonate, and brine. Drying followed by removal of solvent gave an oil (2.04 g). Chromatography [silica gel; ethyl acetate–hexanes (1:4)] yielded the *product* **18** as a solid (1.36 g, 57%), m.p. 41–43 °C; $[\alpha]_D$ −32 (*c* 1.01, $CHCl_3$); ν_{max}-($CHCl_3$)/cm^{-1} 3480–3200, 3000–2840, 1750, 1720 and 1700; δ_H(200 MHz; $CDCl_3$) 0.78 (3 H, d, *J* 6.8), 0.97 (3 H, t, *J* 8), 1.38–1.50 (2 H, m), 1.45 (9 H, s), 1.90–2.04 (1 H, m), 3.55 (2 H, s), 3.74 (3 H, s), 4.42–4.51 (1 H, m) and 5.00 (1 H, d, *J* 9); δ_C(50 MHz; $CDCl_3$) 11.85 (CH_3), 13.87 (CH_3), 26.78 (CH_2), 28.27 (CH_3), 35.97 (CH), 46.59 (CH_2), 52.44 (CH_3), 62.60 (CH), 80.02 (C), 157.50 (C), 167.50 (C) and 202.35 (C); *m/z* 288 [$(M + H)^+$, 24%] and 305 [$(M + NH_4)^+$, 100] (Found: C, 58.5; H, 9.3; N, 4.8. $C_{14}H_{25}NO_5$ requires C, 58.5; H, 8.8; N, 4.9%).

Methyl (4R,5S)-4-(*Benzyloxycarbonylamino*)-5-*methyl*-3-*oxoheptanoate* **19**.—*N*-Benzyloxycarbonyl-D-aIle **10** (0.91 g, 3.43 mmol) as a solution in dry THF (15 cm^3), was added to a suspension of CDI (1.10 g, 6.85 mmol) in dry THF (10 cm^3) at 0 °C under argon. The mixture was allowed to reach room temperature and after 4 h the resulting solution was cooled to 0 °C and a solution of the lithium enolate of methyl acetate, prepared by adding methyl acetate (0.95 cm^3, 12 mmol) to a solution of LDA [from butyllithium (7.49 cm^3 of a 1.6 mol dm^{-3} solution in hexanes, 12.0 mmol) and diisopropylamine (1.86 cm^3, 13.00 mmol) in dry THF (10 cm^3), at −78 °C under argon] was added *via* cannula. The reaction mixture was allowed to come to 0 °C over a period of 2 h and was quenched with saturated aq. ammonium chloride and extracted with dichloromethane. The extracts were washed successively with 5% aq. hydrochloric acid, 5% aq. sodium hydrogen carbonate, and brine. Drying followed by removal of solvent gave an oil, which was purified by flash chromatography [silica; ethyl acetate–hexanes (1:4)] yielded the *product* **19** as a clear oil (0.41 g, 37%); $[\alpha]_D$ −38.2 (*c* 1.17, $CHCl_3$); ν_{max}(film)/cm^{-1} 3500–3100, 3050–2800, 1750, 1730, 1720, 1520, 1450, 1390, 1240 and 1150; δ_H 0.77 (3 H, d, *J* 6.8), 0.90–1.05 (3 H, m), 1.30–1.55 (2 H, m), 1.90–2.60 (1 H, m), 3.55 (2 H, s), 3.73 (3 H, s), 4.55–4.65 (1 H, m),

5.10 (2 H, s), 5.40 (1 H, d, *J* 9) and 7.34–7.35 (5 H, m); *m/z* (CI, NH_3) 339 [(M + NH_4)$^+$, 100%] (Found: C, 63.4; H, 7.3; N, 4.4. $C_{17}H_{23}NO_5$ requires C, 63.5; H, 7.1; N, 4.4%).

(3S,4R,5S)-N-(tert-*Butoxycarbonyl*)*isostatine Methyl Ester* **20**.—Keto ester **18** (1.34 g, 4.66 mmol) was dissolved in methanol (30 cm^3) at 0 °C. Potassium borohydride (1.32 g, 24.4 mmol) was added to the stirred solution and after 15 min the reaction was quenched with 5% aq. hydrochloric acid and extracted with diethyl ether. The extract was washed successively with 5% aq. hydrochloric acid, 5% aq. sodium hydrogen carbonate, and brine. Drying followed by removal of solvent and chromatography of the resulting oil [silica gel; ethyl acetate–hexanes (1:4)] gave the *product* **20** as an oil (1.12 g, 83%); $[\alpha]_D$ −1.5 (*c* 1.17, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3600–3100, 3000–2870, 1730, 1720, 1700, 1680 and 1530; δ_H(200 MHz; $CDCl_3$) 0.85–1.00 (6 H, m), 1.10–1.50 (2 H, m), 1.44 (9 H, s), 1.85–2.00 (1 H, m), 2.40–2.70 (2 H, m), 3.15–3.25 (1 H, d), 3.55–3.72 (1 H, m), 3.72 (3 H, s), 3.81–4.00 (1 H, m) and 4.41 (1 H, d, *J* 9); δ_C(50 MHz; $CDCl_3$) 12.08 (CH_3), 13.46 (CH_3), 27.44 (CH_2), 28.70 (CH_3), 34.29 (CH), 39.33 (CH_2), 52.14 (CH_3), 57.33 (CH), 69.40 (CH), 79.73 (C), 156.63 (C) and 174.06 (C); *m/z* 290 [(M + H)$^+$, 100%] and 307 [(M + NH_4)$^+$, 28] (Found: C, 58.4; H, 9.5; N, 4.8. $C_{14}H_{27}NO_5$ requires C, 58.1; H, 9.3; N, 4.8%).

(3S,4R,5S)-N-(*Benzyloxycarbonyl*)*isostatine Methyl Ester* **21**.—Keto ester **19** (0.78 g, 2.46 mmol) was dissolved in methanol (40 cm^3) at 0 °C. Potassium borohydride (0.26 g, 5.92 mmol) was added to the stirred mixture and after 15 min the reaction was quenched with 5% aq. hydrochloric acid and extracted with diethyl ether. The extract was washed successively with 5% aq. hydrochloric acid, 5% aq. sodium hydrogen carbonate, and brine. Drying followed by removal of solvent and chromatography of the resulting oil [silica gel; ethyl acetate–hexanes (1:4)] gave the *product* **21** as a solid (0.77 g, 96%), m.p. 80–82 °C; $[\alpha]_D$ −4.64 (*c* 1.58, $CHCl_3$); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3600–3200, 3100–2880, 1750–1700 and 1550–1500; δ_H(200 MHz; $CDCl_3$) 0.81–0.98 (6 H, m), 1.10–1.45 (2 H, m), 1.90–2.05 (1 H, m), 2.50–2.65 (2 H, m), 3.65–3.80 (1 H, m), 3.70 (3 H, s), 3.85–4.00 (1 H, m), 4.65 (1 H, d, *J* 9.7), 5.10 (2 H, s) and 7.36 (5 H, br s); δ_C(50 MHz; $CDCl_3$) 12.20 (CH_3), 13.70 (CH_3), 27.58 (CH_2), 34.36 (CH_2), 38.96 (CH), 52.37 (CH_3), 57.72 (CH), 67.46 (CH), 69.41 (CH_2), 128.57 (CH), 128.70 (CH), 129.05 (CH), 137.50 (C), 157.22 (C) and 174.19 (C); *m/z* 324 [(M + H)$^+$, 37%] and 341 [(M + NH_4)$^+$, 100] (Found: C, 63.0; H, 8.3; N, 4.3. $C_{17}H_{25}NO_5$ requires C, 63.1; H, 7.8; N, 4.3%).

(3S,4R,5S)-N-(tert-*Butoxycarbonyl*)*isostatine* **22**.—Ester **20** (0.83 g, 2.87 mmol) was dissolved in 1,4-dioxane (5 cm^3), aq. sodium hydroxide (4 cm^3 of a 1 mol dm^{-3} solution, 4 mmol) was added, and the mixture was stirred for 4 h at room temperature. Hydrochloric acid (1 mol dm^{-3}) was added (to pH 6) and the dioxane was removed by rotatory evaporation. The pH was adjusted to 3 by addition of 1 mol dm^{-3} hydrochloric acid and the solution was extracted with ethyl acetate (4 × 5 cm^3). Drying and removal of solvent gave an oily semi-solid (0.72 g, 91%), $[\alpha]_D$ −5.2 (*c* 2.6) [lit.,[7] −8.7 (*c* 2.4, $CHCl_3$)]; ν_{max}(film)/cm^{-1} 3600–3200, 1700, 1525, 1460, 1430–1360, 1250 and 1175; δ_H(200 MHz; $CDCl_3$) 0.85 (3 H, d, *J* 6.6), 0.90 (3 H, t, *J* 7.12), 1.21–1.40 (2 H, m), 1.44 (9 H, s), 1.80–2.00 (1 H, m), 2.41–2.75 (2 H, m), 3.52–3.71 (1 H, m), 3.85–4.01 (1 H, m) and 4.57 (1 H, d, *J* 10.2); δ_H(50 MHz; $CDCl_3$) 12.17 (CH_3), 13.49 (CH_3), 27.39 (CH_2), 28.80 (CH_3), 34.45 (CH), 39.26 (CH_2), 57.86 (CH), 69.25 (CH), 81.17 (C), 157.78 (C) and 177.30 (C); *m/z* 293 [(M + NH_4)$^+$, 100%] and 276 [(M + H)$^+$, 32] (Found: C, 55.7; H, 9.4; N, 4.8. Calc. for $C_{13}H_{25}NO_5$: C, 56.7; H, 9.2; N, 5.1%).

(3S,4R,5S)-N-(*Benzyloxycarbonyl*)*isostatine* **23**.—Ester **21** (18 mg, 0.05 mmol) was dissolved in (1:1:1) THF–water–MeOH (2 cm^3) at 0 °C, lithium hydroxide (3.5 mg, 0.08 mmol) was added, and the mixture was stirred for 4 h at 0 °C. The volume was reduced by one-half by rotatory evaporation and the pH was adjusted to 3 by addition of 1 mol dm^{-3} hydrochloric acid. Extraction with ethyl acetate (4 × 5 cm^3) followed by washing with brine (4 × 5 cm^3), drying and removal of solvent gave an oil (16 mg, 98%), $[\alpha]_D$ −2.54 (*c* 1.15, $CHCl_3$); ν_{max}(film)/cm^{-1} 3500–3200, 1720, 1700, 1550, 1460, 1450 and 1250; δ_H(200 MHz; $CDCl_3$) 0.84 (6 H, m), 1.10–1.40 (2 H, m), 1.80–2.00 (1 H, m), 2.40–2.70 (2 H, m), 3.65–3.80 (1 H, m), 3.90–4.00 (1 H, m), 4.80 (1 H, d, *J* 10), 5.09 (2 H, m) and 7.35–7.40 (5 H, m); δ_C(50 MHz; $CDCl_3$) 11.31 (CH), 14.78 (CH), 26.97 (CH), 33.89 (CH), 34.66 (CH), 39.15 (CH), 76.17 (CH), 77.19 (CH), 126.99 (CH), 127.67 (CH), 128.56 (C), 159.67 (C) and 173.14 (C); *m/z* (FAB) 310, [(M + 1)$^+$, 55%] and 260 (100).

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