Synthesis of derivatives of (2*S*,4*S*)-4-hydroxy-2,5-dimethyl-3-oxohexanoic acid, a constituent of the didemnins

Isabel González, Gemma Jou, Josep Maria Caba, Fernando Albericio, Paul Lloyd-Williams* and Ernest Giralt*

Department of Organic Chemistry, University of Barcelona, E-08028 Barcelona, Spain

The syntheses of the two protected derivatives 7 and 16 of (2*S*,4*S*)-4-hydroxy-2,5-dimethyl-3-oxohexanoic acid, a constituent of the didemnin family of antineoplastic macrocyclic depsipeptides are described. The preparation of 7 was carried out by modification of a previously reported synthetic route whereas the use of derivative 16 represents a novel approach to the management of this sub-unit. Removal of the carboxy protecting groups from 7 or 16, followed by amide bond formation with derivatives of (*S*)-leucine, and oxidation in the cases of compounds deriving from 16, generates the diastereoisomeric intermediates 11, 22 and 23. In each of these either of the protecting groups can be removed in the presence of the other, allowing them to be elaborated further at either terminus. Previous work indicates that diastereoisomeric mixtures of such intermediates can, in principle, be used to obtain optically pure didemnins.

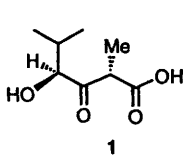
Introduction

(2*S*,4*S*)-4-Hydroxy-2,5-dimethyl-3-oxohexanoic acid **1** is one of the sub-units of the didemnins, macrocyclic depsipeptides isolated from a marine tunicate by Rinehart,[1] which are currently undergoing clinical evaluation as potential antitumour agents.[2,3] The simplest member of the series, didemnin A, has the structure **2** and others differ only in the type of side-chain appended to the (R)-N(Me)-Leu residue. The synthesis of all members of this interesting family of compounds requires that routes to suitably protected derivatives of **1** be devised and a number of approaches have been described.[4–8] Here we report on our own studies on the manipulation of **1** for the preparation of intermediates in the synthesis of the didemnins.

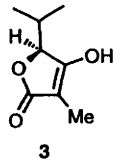
Results and discussion

(2*S*,4*S*)-4-Hydroxy-2,5-dimethyl-3-oxohexanoic acid **1** does not exist as such since spontaneous intramolecular cyclization gives the tetronic acid **3**, isolated in the preliminary structure elucidation studies on the didemnins.[9] Such cyclization must be borne in mind when designing protected derivatives of **1** since, unless the carboxylic acid is protected with a sufficiently poor leaving group, attempts to remove the protecting group of the alcohol will lead directly to the formation of **3**. Furthermore, the stereogenic carbon atom at C-2 of protected **1** is easily epimerized so that the most practical approach is to work with a mixture of diastereoisomers at this position. Previous work has demonstrated that optically pure didemnins can be obtained from such diastereoisomeric mixtures.[8]

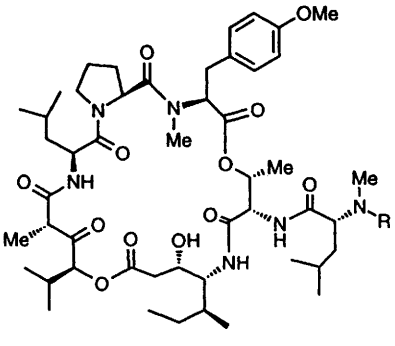
One of the more attractive ways of managing **1** is to use the known derivative **7**, which can be prepared from (*S*)-valine. The key steps in the reported[8] synthesis are the diethylphosphonocyanide-mediated condensation of **5** with Meldrum's acid, followed by transesterification with benzyl alcohol to give **6**. However, since the first of these transformations could not be made to proceed satisfactorily, an alternative preparation of **6** from **5** was developed, as shown in Scheme 1. Treatment of the amino acid with aqueous nitrous acid led to diazotization and hydrolysis with retention of configuration,[10–12] in accord with precedent,[13] affording hydroxy acid **4**. Reaction of this with an excess of *tert*-butyldimethylsilyl chloride followed by hydrolysis gave **5** in 66% yield from (*S*)-valine.



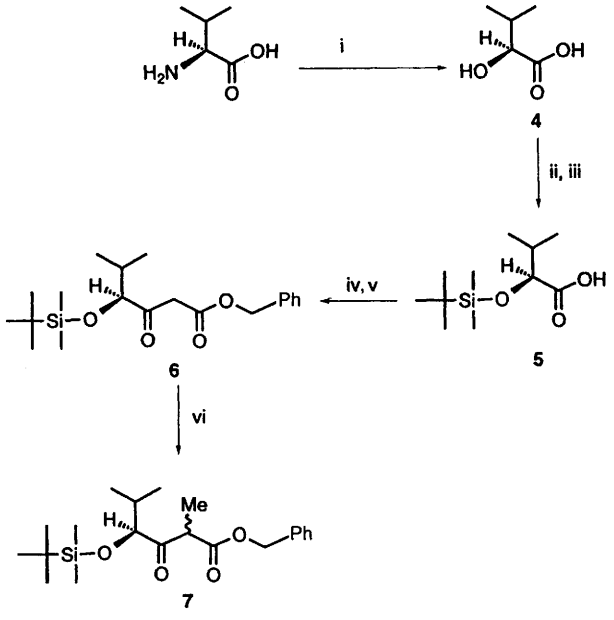
1



3



2

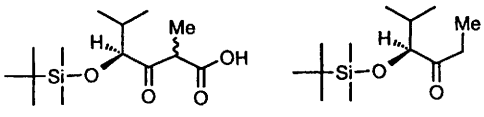


Scheme 1 *Reagents and conditions:* i, $NaNO_2$, H_2SO_4, 50%; ii, *tert*-butyldimethylsilyl chloride, imidazole, dimethylformamide; iii, K_2CO_3, MeOH, 66%; iv, carbonyldiimidazole; v, lithium diisopropylamide, benzyl acetate, 53%; vi, lithium diisopropylamide, methyl iodide, 68%

Attempted condensation of **5** and Meldrum's acid, using either diethylphosphonocyanide or carbonyldiimidazole to activate the carboxylic acid, led only to very low and poorly reproducible yields of the desired product. Condensation of **5**, activated with carbonyldiimidazole, with the lithium enolate of methyl propionate was also unsatisfactory, again reflecting what is presumably the steric hindrance and electronic deactivation operating at the carbonyl group of this ester. Much better results were obtained using the lithium enolate of benzyl acetate which was condensed successfully with **5** to give **6**, albeit only in moderate (53%, after chromatography), but reproducible, yield that was not improved by an increase in the excess of reagent or temperature nor by longer reaction times. Methylation of **6** to furnish both diastereoisomers of **7** required careful control of the reaction conditions, since a delicate balance between mono-, di- and non-methylated products was established. Best results were obtained on treatment of **6** with 1 equiv. of *lithium diisopropylamide* in the presence of a 13-fold excess of methyl iodide at 0 °C.

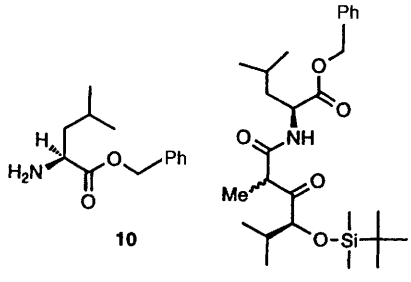
Attempts to remove the *tert*-butyldimethylsilyl group from **7**, using tetrabutylammonium fluoride or dilute aqueous hydrofluoric acid, in order to liberate the alcohol for esterification with suitably protected isostatine derivatives,[14] led to the formation of **3** quantitatively. In general, esterification at the hydroxy group of esters of **1** can only be accomplished by their prior conversion into compounds having a poor leaving group at the carboxy terminus and one alternative, directly applicable in the synthesis of the didemnins, is amide bond formation using suitably protected derivatives of (*S*)-leucine.

Hydrogenolysis of solutions of **7** in the presence of 10% Pd–C led to clean removal of the benzyl group but, since the β-keto acid **8** is decarboxylated easily, attempts at its isolation by filtering off the catalyst and solvent removal gave only the ketone **9**.



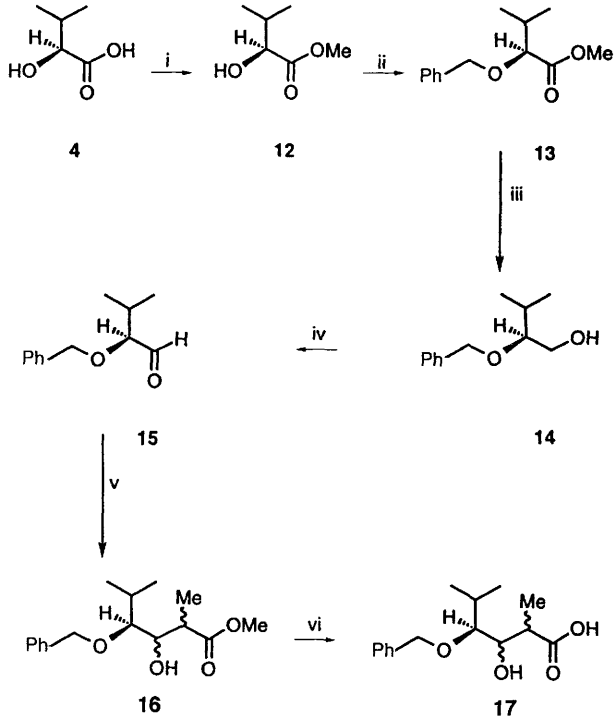
8 **9**

This facile decarboxylation complicates synthetic operations involving this intermediate but clean amide bond formation at the *C*-terminus could be brought about by hydrogenolysis of **7** in tetrahydrofuran followed by addition of the previously filtered solution of **8** to a solution of *N*-[(1*H*-benzotriazol-1-yl)-(dimethylamino)methylene]-*N*-methylmethanaminium tetrafluoroborate (TBTU) in tetrahydrofuran, prior to addition of (*S*)-leucine derivatives such as **10**. This method gave reproducible yields in excess of 60% after chromatography, for the formation of **11**, in which the hydroxy and carboxy functional groups are protected in an orthogonal[15] fashion so that, in each case, either one can be removed in the presence of the other, allowing elaboration at either terminus.



10 **11**

The rapid decarboxylation of compounds such as **8** may be avoided by working with derivatives of **1** in which the ketone is reduced. After coupling leucine derivatives to the carboxylic acid terminus, the ground oxidation level can then be restored by transformation of the alcohol into the ketone. This represents a new approach to the incorporation of **1** into synthetic intermediates and is outlined in Scheme 2.



4 **12** **13** **15** **14** **16** **17**

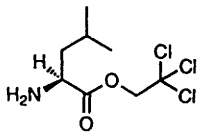
Scheme 2 *Reagents and conditions:* i, MeOH, H_2SO_4, 47%; ii, benzyl 2,2,2-trichloroacetimidate, trifluoromethanesulfonic acid, 60%; iii, $LiAlH_4$, Et_2O, 85%; iv, $(COCl)_2$, dimethyl sulfoxide, Et_3N, 90%; v, methyl propionate, lithium diisopropylamide, hexamethylphosphoric triamide, 66%; vi, LiOH, tetrahydrofuran–H_2O–MeOH (1:1:1), 91%

The (*S*)-valine-derived[13] hydroxy acid **4** was converted into its methyl ester **12**, the hydroxy group of which was protected as a benzyl ether **13**, by treatment with benzyl 2,2,2-trichloroacetimidate in 60% yield after chromatography. Reduction with lithium aluminium hydride, followed by Swern oxidation[16] of **14**, gave the aldehyde[17] **15**. Aldol condensation of **15** with the lithium enolate of methyl propionate required strict control of the reaction conditions but alcohol **16** was obtained in acceptable yield (66%, after chromatography) on addition of 1.5 equiv. of the enolate to **15** in the presence of hexamethylphosphorotriamide at −78 °C, the temperature being allowed to rise to −50 °C before quenching. The alcohol **16**, obtained as a mixture of 4 diastereoisomers (approximate proportions, 50:20:15:15), may also be considered to be a derivative of **1** and can be used to prepare synthetic intermediates similar to those derived from **7**.

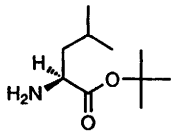
Saponification gave the hydroxy acid **17** which, unlike the β-keto acid **8**, is not readily decarboxylated: it is a stable white solid that can be manipulated without special precautions. Condensation of **17** with (*S*)-leucine derivatives **18** or **19**, again using TBTU, furnished **20** and **21** in excellent yield. Oxidation of the alcohols to the corresponding ketones with pyridinium chlorochromate adsorbed on alumina,[18] then gave the intermediates **22** and **23**, each as a mixture of two diastereoisomers which, in common with **11**, can be deprotected at either terminus for further synthetic elaboration.

Conclusions

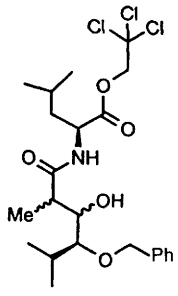
Both **7** and **16** are useful protected derivatives of **1**. Although each can be prepared from (*S*)-valine, **7** requires fewer steps than **16** but the advantage of the latter resides in the ease with which the hydroxy acid **17** can be manipulated in comparison



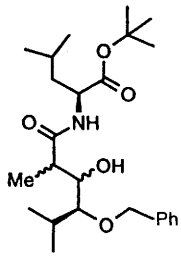
18



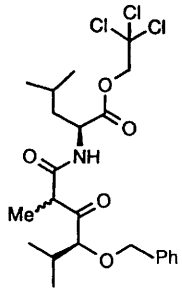
19



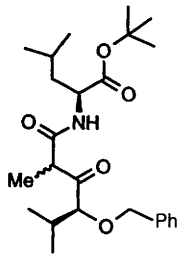
20



21



22



23

with the β-keto acid **8**. Although both these derivatives of **1** give rise to diastereoisomeric synthetic intermediates this causes no problems in practice and precedent indicates that optically pure didemnins can be prepared using such diastereoisomers. The application of **7** and **16** in the total synthesis of the didemnins is currently underway in our laboratory.

Experimental

All organic solutions were dried over sodium sulfate. Chemical shifts are quoted in δ values downfield from tetramethylsilane and J values are given in Hz. All melting points are uncorrected. Optical rotation data were measured with a Perkin-Elmer 241 MC polarimeter; values are recorded in units of 10^{-1} deg cm^2 g^{-1}. HPLC was performed using a Shimadzu apparatus and a Nucleosil C18 column (25 × 0.4 cm, 10 μm, 120 Å).

(2*S*)-2-Hydroxy-3-methylbutanoic acid 4

(*S*)-Valine (200 g, 1.71 mol) was suspended in water (500 cm^3) at 0 °C and aq. sulfuric acid (4 mol dm^{-3}; 470 cm^3, 3.64 mol) was added dropwise until complete dissolution. The remainder of the sulfuric acid together with aqueous sodium nitrite (4 mol dm^{-3}; 470 cm^3, 1.82 mol) were then added simultaneously over 120 min. The mixture was allowed to attain room temperature after which it was stirred for 24 h and then extracted with ethyl acetate (6 × 150 cm^3). The combined extracts were washed with saturated brine (3 × 100 cm^3), dried, filtered and evaporated to give a white solid which upon recrystallization from diethyl ether–hexanes, gave the product **4** as prisms (90.8 g, 45%), mp 68–70 °C (lit.,[13] 65–66 °C); $[\alpha]_D$ +15.4 (*c* 2.1, $CHCl_3$) [lit.,[13] +19.0 (*c* 1, $CHCl_3$)]; ν_{max}(film)/cm^{-1} 3428, 2973, 2400, 1719, 1470, 1393, 1376, 1298, 1260, 1215, 1131, 1107, 1055 and 1030; δ_H(200 MHz, $CDCl_3$) 0.92 (3 H, d, *J* 7), 1.05 (3 H, d, *J* 6.95), 2.05–2.20 (1 H, m), 4.15 (1 H, d, *J* 3.6) and 7.30 (1 H, br s); δ_C(50 MHz, $CDCl_3$) 16.34, 19.26, 32.47, 75.31 and 179.83; *m*/*z* 237 [(2M + H)$^+$, 100%] and 119 [(M + H)$^+$, 94%] (Found: C, 50.7; H, 8.4. Calc. for $C_5H_{10}O_3$: C, 50.83; H, 8.53).

(2*S*)-2-*tert*-Butyl(dimethyl)silyloxy-3-methylbutanoic acid 5

The hydroxy acid **4** (2.58 g, 21.8 mmol), *tert*-butyl(dimethyl)-silyl chloride (8.15 g, 52.5 mmol) and imidazole (7.12 g, 104 mmol) were dissolved in dry dimethylformamide (10 cm^3) under nitrogen and the mixture was stirred at room temperature for 22 h. It was then diluted with ethyl acetate (500 cm^3) and washed with saturated aqueous citric acid (3 × 100 cm^3), saturated aqueous sodium hydrogen carbonate (3 × 100 cm^3) and saturated brine (3 × 100 cm^3) and dried. Solvent removal gave an oil (5.26 g) whose spectroscopic data corresponded with those of *tert*-butyldimethylsilyl (2*S*)-2-*tert*-butyl(dimethyl)-silyloxy-3-methylbutanoate; $[\alpha]_D$ −33.9 (*c* 1.2, CH_2Cl_2); ν_{max}(film)/cm^{-1} 2960, 2933, 2861, 1740, 1717, 1472, 1364, 1254, 1190, 1148 and 1070; δ_H(200 MHz, $CDCl_3$) 0.01 (3 H, s), 0.03 (3 H, s), 0.25 (3 H, s), 0.26 (3 H, s), 0.89 (9 H, s), 0.92 (9 H, s), 0.82–0.94 (6 H, m), 1.93–2.10 (1 H, m) and 3.89 (1 H, d, *J* 5.1); δ_C(50 MHz, $CDCl_3$) −4.85, 16.80, 17.57, 18.28, 19.17, 25.52, 25.77, 32.73, 77.49 and 173.72; *m*/*z* 347 [(M + H)$^+$, 100%] and 364 [(M + NH_4)$^+$, 13%]. Potassium carbonate (7.5 g, 54.3 mmol) in water (65 cm^3) was added to a solution of this ester in methanol (200 cm^3) at 0 °C and the mixture stirred for 135 min. The solution was adjusted to pH 4 (indicator paper) by addition of 10% aqueous citric acid after which it was extracted with ethyl acetate (3 × 200 cm^3). The combined extracts were dried and evaporated to give an oil that was purified by chromatography (silica gel, ethyl acetate–hexanes, gradient elution) to afford the acid **5** as a colourless oil (3.36 g, 66% from **4**); $[\alpha]_D$ −16.5 (*c* 0.98, CH_2Cl_2); ν_{max}(film)/cm^{-1} 3450–3000, 2961, 2932, 2861, 1725, 1472, 1252, 1188, 1151 and 1072; δ_H(200 MHz, $CDCl_3$) 0.10 (3 H, s), 0.12 (3 H, s), 0.91–1.00 (15 H, m), 2.01–2.08 (1 H, m) and 4.09 (1 H, d, *J* 3.7); δ_C(50 MHz, $CDCl_3$) −4.66, 17.14, 18.81, 19.26, 26.16, 33.31, 77.14 and 177.20; *m*/*z* 250 [(M + NH_4)$^+$, 100%] and 233 [(M + H)$^+$, 21%] [Found: (M − Bu^t)$^+$, 175.0794. Calc. for $C_{11}H_{24}O_3Si$: (M − Bu^t)$^+$, 175.0791].

Benzyl (4*S*)-4-*tert*-butyl(dimethyl)silyloxy-5-methyl-3-oxohexanoate 6

A solution of carbonyldiimidazole (1.08 g, 6.6 mmol) in dry tetrahydrofuran (9 cm^3) was added to a solution of the acid **5** (771 mg, 3.3 mmol) dissolved in dry tetrahydrofuran (1 cm^3) at 0 °C under nitrogen. The mixture was stirred for 2.5 h during which time it was allowed to come to room temperature. After cooling to −78 °C, the mixture was treated with a solution of the lithium enolate of benzyl acetate [formed by adding butyllithium (1.6 mol dm^{-3} solution in hexanes; 7.25 cm^3, 11.6 mmol) to a solution of diisopropylamine (1.97 cm^3, 13.9 mmol) in dry tetrahydrofuran (1.5 cm^3) at −78 °C, stirring of the mixture for 15 min, and then treatment with benzyl acetate (1.69 cm^3, 11.6 mmol), added dropwise over 1 h], was added and the mixture was allowed to come to room temperature over 3 h. After re-cooling to 0 °C, the mixture was treated with saturated aqueous ammonium chloride (40 cm^3) to quench the reaction and extracted with dichloromethane (3 × 50 cm^3). The combined extracts were washed with 5% aqueous hydrochloric acid (2 × 50 cm^3), 5% aqueous sodium hydrogen carbonate (2 × 50 cm^3) and saturated brine (2 × 50 cm^3), dried and evaporated to give an oil that was purified by chromatography (silica gel, ethyl acetate–hexanes, 1:9). This gave the ester **6** as a colourless oil (639 mg, 53%); $[\alpha]_D$ −22.3 (*c* 0.50 MeOH), [lit.,[8] −19.8 (*c* 0.53 MeOH)]; ν_{max}(film)/cm^{-1} 2960, 2860, 1752, 1654, 1472, 1256, 1216 and 1081; δ_H(200 MHz, $CDCl_3$) 0.01 (3 H, s), 0.04 (3 H, s), 0.91 (9 H, s), 0.80–0.97 (6 H, m), 1.83–1.99 (1 H, m), 3.61 (2 H, s), 3.78 (1 H, d, *J* 5), 5.16 (2 H, s) and 7.34 (5 H, br s); δ_C(50 MHz, $CDCl_3$) −4.99, −4.83, 17.54, 18.10, 18.54, 25.78, 32.75, 45.00, 67.00,

83.36, 128.14, 128.34, 128.56, 128.61, 134.97 and 166.97; *m/z* 382 [$(M + NH_4)^+$, 100%] and 365 [$(M + H)^+$, 20%] [Found: $(M - Bu^t)^+$, 307.1376. Calc. for $C_{20}H_{32}O_4Si$: $(M - Bu^t)^+$, 307.1366].

Benzyl (2*RS*,4*S*)-4-*tert*-butyl(dimethyl)silyloxy-2,5-dimethyl-3-oxohexanoate 7

The ester **6** (170 mg, 0.5 mmol) in dry tetrahydrofuran (1 cm^3) was added to a solution of lithium diisopropylamide, prepared by adding butyllithium (1.6 mol dm^{-3} solution in hexanes; 313 mm^3, 0.5 mmol) dropwise to diisopropylamine (70 mm^3, 0.5 mmol) in dry tetrahydrofuran (1 cm^3) under nitrogen at 0 °C. The mixture was stirred for 5 min after which it was treated with methyl iodide (0.41 cm^3, 6.5 mmol) and stirred at 0 °C for 1 h; it was then allowed to come to room temperature when it was stirred for a further 20 h. The mixture was then cooled to 0 °C, treated with saturated aqueous ammonium chloride (3 cm^3), to quench the reaction and extracted with ethyl acetate–toluene (1:1, 2 × 10 cm^3). The combined organic phases were washed with water (3 × 5 cm^3) and saturated brine (3 × 5 cm^3), dried, filtered and evaporated to give an oil. This was purified by chromatography (silica gel, ethyl acetate–hexanes, 0.4:9.6) to give the two diastereoisomers of the ester **7** (ratio *ca.* 1:1) as a colourless oil (120 mg, 68%); ν_{max}(film)/cm^{-1} 3090–3000, 2959–2859, 1750, 1725, 1499, 1456, 1389, 1319, 1254, 1190, 1072 and 1007; δ_H(200 MHz, $CDCl_3$) 0.01 (3 H, s), 0.04 (3 H, s), 0.89 (9 H, s), 0.79–0.99 (6 H, m), 1.32 (3 H, d, *J* 7.4), 1.90–2.12 (1 H, m), 3.90–4.07 (2 H, m), 5.11 (2 H, s), 7.32 (5 H, br s); δ_C(50 MHz, $CDCl_3$) −5.20, −4.94, −4.83, −4.74, 13.67, 13.75, 17.08, 17.22, 18.13, 18.86, 18.97, 25.72, 31.49, 32.36, 47.14, 47.39, 66.85, 67.05, 82.59, 83.19, 128.21, 128.39, 128.46, 128.52, 135.27, 135.47, 170.10, 170.30, 206.85 and 208.37; *m/z* 379 [$(M + H)^+$, 100%] [Found: $(M - Bu^t)^+$, 321.1525. Calc. for $C_{21}H_{34}O_4Si$: $(M - Bu^t)^+$, 321.1522].

(4*S*)-4-Isopropyl-2-methyltetronic acid 3

Tetrabutylammonium tetrafluoroborate (420 mg, 1.32 mmol) was added to a solution of the ester **7** (250 mg, 6.58 × 10^{-4} mol) in dry tetrahydrofuran (4 cm^3) and the mixture stirred for 45 min at room temperature. It was then diluted with water (15 cm^3) and extracted with dichloromethane (3 × 10 cm^3). The combined extracts were washed with saturated brine (1 × 5 cm^3), dried, filtered and evaporated to give an oil. This was purified by chromatography (silica gel, ethyl acetate–hexanes, 3:7) to afford the tetronic acid **3** as a colourless oil (50 mg, 47%); $[\alpha]_D$ −56.2 (*c* 0.53, CH_2Cl_2) [lit.,[4] −61.6 (*c* 1.10)]; ν_{max}(film)/cm^{-1} 3000–2860, 1730, 1700–1590, 1410, 1380, 1360, 1300, 1230, 1100 and 1010; δ_H(200 MHz, $CDCl_3$) 0.82 (3 H, d, *J* 6.8), 1.12 (3 H, d, *J* 7), 1.75 (3 H, d, *J* 1.06), 2.17–2.34 (1 H, m) and 4.66–4.68 (1 H, m); *m/z* 174 [$(M + NH_4)^+$, 100%].

(2*RS*,4*S*)-4-*tert*-Butyl(dimethyl)silyloxy-2,5-dimethyl-3-oxohexanoyl leucine benzyl ester 11

The ester **7** (85 mg, 0.225 mmol) in dry tetrahydrofuran (0.6 cm^3) was added to a suspension of 10% palladium-on-charcoal (10 mg) in dry tetrahydrofuran (0.5 cm^3) and the mixture hydrogenolysed for 90 min at room temperature and atmospheric pressure. The reaction mixture was cooled to −10°C (ice–salt bath) and filtered rapidly through Celite, washing with dry tetrahydrofuran (2 cm^3), onto a solution of *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethanaminium tetrafluoroborate (TBTU) (92 mg, 1.08 mmol) and 1-hydroxybenzotriazole (32 mg, 1.02 mmol) in dry tetrahydrofuran at −10 °C (2 cm^3). Diisopropylethylamine (38 mm^3, 0.225 mmol) was added to the mixture followed, after 2 min, by (*S*)-leucine benzyl ester (75 mg, 0.337 mmol) and diisopropylethylamine (38 mm^3, 0.225 mmol). The reaction mixture was allowed to come to 0 °C over 90 min and then to room temperature over a further 90 min; it was then stirred for 19 h and finally evaporated. The residue was dissolved in ethyl acetate (20 cm^3) and the solution washed with 10% aqueous potassium hydrogen sulfate (2 × 5 cm^3), 10% aqueous sodium hydrogen carbonate (2 × 5 cm^3) and saturated brine (2 × 5 cm^3) and then dried, filtered and evaporated. This gave an oil which was purified by chromatography (silica gel, ethyl acetate–hexanes, 1:9) to afford the two diastereoisomers of product **11** (ratio *ca.* 1:1) as a white solid (69 mg, 63%), mp 102–104 °C; ν_{max}(film)/cm^{-1} 3480–3220, 3080–3020, 2980–2840, 1770–1700, 1770–1640, 1350, 1460, 1390–1330, 1255, 1190, 1150, 1070, 1025 and 1005; δ_H(200 MHz, $CDCl_3$) 0.00–0.04 (6 H, m), 0.85–0.93 (21 H, m), 1.36 (3 H, s), 1.39 (3 H, s), 1.50–1.79 (3 H, m), 1.83–2.11 (1 H, m), 3.8–4.08 (2 H, m), 4.50–4.68 (1 H, m), 5.18–5.90 (2 H, br s), 6.78 (1 H, d, *J* 6.8), 6.82 (1 H, d, *J* 6.8) and 7.34 (5 H, br s); δ_C(50 MHz, $CDCl_3$) −5.33, −4.91, 16.69, 17.07, 18.00, 18.53, 18.62, 18.93, 21.33, 21.04, 24.05, 25.47, 31.01, 31.47, 40.53, 40.88, 48.10, 50.51, 66.59, 83.93, 83.17, 127.71, 128.04, 135.17, 169.07, 171.95 and 209.52; *m/z* 509 [$(M + NH_4)^+$, 25%] and 492 [$(M + H)^+$, 100%] (Found: C, 65.9; H, 9.4; N, 2.8. $C_{27}H_{45}NO_5Si$ requires C, 65.94; H, 9.22; N, 2.85%); reversed-phase HPLC R_t 19.7 and 21.7 min, linear gradient from 10% B to 100% B over 20 min, followed by elution at 100% B for 10 min, where A is water–0.045% trifluoroacetic acid and B is acetonitrile–0.036% trifluoroacetic acid, at a flow rate of 1 cm^3 min^{-1}.

Methyl (2*S*)-2-hydroxy-3-methylbutanoate 12

(2*S*)-2-Hydroxy-3-methylbutanoic acid **4** (10 g, 84.70 mmol) was dissolved in methanol (100 cm^3) containing concentrated sulfuric acid (2 cm^3) and was refluxed for 13 h and then allowed to cool. Solvent was then removed and the resulting oil was dissolved in diethyl ether (450 cm^3) and the solution washed with saturated aqueous sodium hydrogen carbonate (2 × 100 cm^3), water (3 × 100 cm^3) and saturated brine (2 × 100 cm^3), dried and evaporated. This gave an oil that was purified by chromatography (silica gel, hexanes–ethyl acetate, gradient elution) to give the hydroxy ester **12** as a colourless oil (5.25 g, 47%); $[\alpha]_D$ +23.6 (*c* 1.2, $CHCl_3$) [lit.,[17] +18.4 (*c* 1.2, $CHCl_3$)]; ν_{max}(film)/cm^{-1} 3533, 2971, 2881, 1746, 1650, 1468, 1439, 1372, 1300, 1212, 1138 and 1028; δ_H(200 MHz, $CDCl_3$) 0.87 (3 H, d, *J* 6.8), 1.02 (3 H, d, *J* 7), 1.95–2.15 (1 H, m), 2.86 (1 H, d, *J* 6.2), 3.81 (3 H, s) and 4.05 (1 H, dd, *J* 3.6, *J* 6.2); δ_C(50 MHz, $CDCl_3$) 16.45, 19.07, 32.51, 52.59, 75.50 and 175.69; *m/z* 150 [$(M + NH_4)^+$, 100%] [Found: $(M)^{\bullet+}$, 132.0781. Calc. for $C_6H_{12}O_3$: $(M)^{\bullet+}$, 132.0785].

Methyl (2*S*)-2-benzyloxy-3-methylbutanoate 13

Benzyl 2,2,2-trichloroacetimidate (10.45 cm^3, 56.24 mmol) and trifluoromethanesulfonic acid (0.75 cm^3, 8.48 mmol) were added to a solution of hydroxy ester **12** (7.40 g, 56 mmol) in a mixture of hexanes–dichloromethane (2:1; 100 cm^3) at 0 °C. The reaction mixture was allowed to reach room temperature and after 4 h was cooled to 0 °C when benzyl 2,2,2-trichloroacetimidate (2.00 cm^3, 10.70 mmol) and trifluoromethanesulfonic acid (0.15 cm^3) were added. The reaction mixture was again allowed to attain room temperature and after 18 h was filtered. The filtrate was diluted with dichloromethane (200 cm^3), washed with saturated aqueous sodium hydrogen carbonate (2 × 25 cm^3) and saturated brine (2 × 25 cm^3), dried, filtered and evaporated to give an oil. This was purified by chromatography (silica gel, ethyl acetate–hexanes, 1:49), to afford the ester **13** as a colourless oil (7.46 g, 60%); $[\alpha]_D$ +67.6 (*c* 2.82, CH_2Cl_2) [lit.,[17] +53.9 (*c* 2.68, CH_2Cl_2); ν_{max}(film)/cm^{-1} 2966, 2877, 1750, 1497, 1455, 1436, 1269, 1202, 1144, 1075 and 1019; δ_H(200 MHz, $CDCl_3$) 0.95 (3 H, d, *J* 6.6), 0.97 (3 H, d, *J* 7), 2.00–2.20 (1 H, d, *J* 6.6), 3.75 (3 H, s), 4.37 (1 H, d, *J* 10), 4.70 (1 H, d, *J* 10) and 7.34–7.36 (5 H, m); δ_C(50 MHz, $CDCl_3$) 17.81, 18.78, 31.66, 51.59, 72.53, 83.36, 127.76, 127.96, 128.22 and 128.33; *m/z* 445 [$(2M + H)^+$, 100%] and 223 [$(M + H)^+$, 14%] [Found: $(M)^{\bullet+}$, 222.1247. Calc. for $C_{13}H_{18}O_3$: $(M)^{\bullet+}$, 222.1256].

(2*S*)-2-Benzyloxy-3-methylbutan-1-ol 14

The ester **13** (980 mg, 4.39 mmol) in anhydrous diethyl ether (5 cm^3) was added dropwise to a suspension of lithium aluminium hydride (555 mg, 14.50 *mmol*) in anhydrous diethyl ether (2 cm^3) under nitrogen at 0 °C and the mixture was stirred for 60 min. It was then treated with 5% aqueous hydrochloric acid at 0 °C until the pH was *ca.* 6 (indicator paper). The aqueous phase was then separated and extracted with diethyl ether (3 × 50 cm^3) and the combined organic phase and extracts were then washed with 5% aqueous sodium hydrogen carbonate (3 × 50 cm^3). In turn, combined aqueous phases were extracted with diethyl ether (3 × 50 cm^3) and the combined extracts washed with saturated aqueous brine (3 × 20 cm^3), dried, filtered and evaporated. This gave an oil which was purified by chromatography (silica gel, ethyl acetate–hexanes, 1:4) to afford the alcohol **14** as a colourless oil (720 mg, 85%); $[\alpha]_D$ +9.7 (*c* 2.35, $CHCl_3$) [lit.,[17] +10.8 (*c* 2.30, $CHCl_3$)]; ν_{max}(film)/cm^{-1} 3422, 2962, 2932, 2875, 1559, 1497, 1455, 1387, 1071 and 1028; δ_H(200 MHz, $CDCl_3$) 0.93 (3 H, d, *J* 6.9), 0.99 (3 H, d, *J* 6.8), 1.86–2.06 (3 H, m), 3.26 (1 H, td, J_1 6, J_2 3.5), 3.55–3.78 (2 H, m), 4.55 (1 H, d, *J* 10), 4.65 (1 H, d, *J* 10) and 7.34–7.37 (5 H, m); δ_C(50 MHz, $CDCl_3$) 18.18, 18.78, 29.08, 61.76, 72.46, 85.03, 127.78 and 128.49; *m/z* 212 [$(M + NH_4)^+$, 100%] [Found: $(M)^{\bullet+}$, 194.1309. Calc. for $C_{12}H_{18}O_2$: $(M)^{\bullet+}$, 194.1307].

(2*S*)-2-Benzyloxy-3-methylbutanal 15

Dimethyl sulfoxide (0.96 cm^3, 13.47 mmol) in dichloromethane (2 cm^3) was added to freshly distilled oxalyl chloride (0.56 cm^3, 6.47 mmol) in dichloromethane (2 cm^3) at −60 °C and the resulting solution stirred for 12 min. The alcohol **14** (1.05 g, 5.39 mmol) in dichloromethane (4 cm^3) was then added to the mixture after which it was stirred for 15 min before diisopropylethylamine (5.49 cm^3, 32.34 mmol) was also added. After the mixture had been allowed to attain room temperature, it was diluted with dichloromethane (100 cm^3), washed with saturated brine, (2 × 20 cm^3), dried, filtered and evaporated. This gave the crude aldehyde **15** as a yellow oil (940 mg, 90%), which was used without further treatment; $[\alpha]_D$ −81.1 (*c* 2.49, CH_2Cl_2); ν_{max}(film)/cm^{-1} 3066, 3033, 2965, 2876, 1732, 1497, 1454, 1369, 1261, 1207, 1072 and 1028; δ_H(200 MHz, $CDCl_3$) 1.01 (3 H, d, *J* 6.8), 0.99 (3 H, d, *J* 6.9), 2.00–2.20 (1 H, m), 3.48 (1 H, dd, J_1 5.7, J_2 2.7), 4.49 (1 H, d, *J* 11.8), 4.69 (1 H, d, *J* 11.8), 7.36 (5 H, m) and 9.66 (1 H, d, *J* 2.7); δ_C(50 MHz, $CDCl_3$) 17.59, 18.41, 29.95, 72.81, 88.07, 127.90, 127.95, 128.43 and 204.49; *m/z* 210 [$(M + NH_4)^+$, 100%] [Found: $(M)^{\bullet+}$, 192.1149. Calc. for $C_{12}H_{16}O_2$: $(M)^{\bullet+}$, 192.1150].

Methyl (2*RS*,3*RS*,4*S*)-4-benzyloxy-3-hydroxy-2,5-dimethylhexanoate 16

Butyllithium (1.6 mol dm^{-3} solution in hexanes; 2.35 cm^3, 3.76 mmol) was added to a solution of diisopropylamine (0.58 cm^3, 4.11 mmol) in dry tetrahydrofuran (0.5 cm^3), under nitrogen at −78 °C and the mixture stirred for 30 min. Methyl propionate (0.36 cm^3, 3.76 mmol) was then added to the mixture over 20 min and followed after continued stirring for a further 35 min, first by the aldehyde **15** (503 mg, 2.62 mmol) in dry tetrahydrofuran (2.5 cm^3), added over 10 min and then hexamethylphosphorotriamide (0.8 cm^3, 4.57 mmol). This mixture was stirred for 2 h at −78 °C followed by a further 2 h 30 min at −50 °C. The reaction was quenched by the addition of saturated aqueous ammonium chloride (20 cm^3) to the mixture which was then extracted with dichloromethane (4 × 25 cm^3). The combined extracts were washed with saturated brine (2 × 10 cm^3), dried, filtered and evaporated to give the crude product as a yellow oil. This was purified by chromatography (silica gel, ethyl acetate–hexanes, 18:82) to give the four diastereoisomers of the alcohol **16** (ratio *ca.* 50:20:15:15) as a colourless oil (0.49 g, 66%); ν_{max}(film)/cm^{-1} 3600–3200, 2957, 2876, 1734, 1456, 1497, 1364, 1200, 1173, 1068; δ_H(200 MHz, $CDCl_3$) 0.98–1.04 (6 H, m), 1.14 (3 H, d, *J* 7.1), 1.22 (3 H, d, *J* 7.1), 1.26 (3 H, d, *J* 7.0), 1.32 (3 H, d, *J* 7.3), 1.6 (1 H, br s), 1.90–2.12 (1 H, m), 2.00–2.22 (1 H, m), 2.55–2.70 (1 H, m), 2.60–2.70 (1 H, m), 2.76–2.90 (1 H, qd, J_1 7.26, J_2 2.6), 2.78–2.92 (1 H, qd, J_1 7.09, J_2 2.9), 2.6 (1 H, br s), 3.09–3.22 (1 H, dd, J_1 5.92, J_2 2.56), 3.16–3.21 (1 H, dd, J_1 6.54, J_2 1.84), 3.24–3.27 (1 H, dd, J_1 5.30, J_2 4.3), 3.24–3.30 (1 H, dd, J_1 7.8, J_2 3.18), 3.53 (3 H, s), 3.64 (3 H, s), 3.65 (3 H, s), 3.66 (3 H, s), 3.60–3.90 (1 H, m), 4.50–4.70 (2 H, m) and 7.25–7.35 (5 H, m); δ_C(75 MHz, $CDCl_3$) 10.9, 14.21, 15.69, 16.72, 17.89, 18.31, 18.68, 18.76, 18.92, 19.76, 20.21, 29.32, 29.79, 29.91, 39.03, 40.64, 44.06, 51.53, 51.59, 51.71, 71.30, 72.24, 73.28, 73.59, 74.22, 74.26, 74.82, 75.01, 76.58, 77.01, 77.43, 82.87, 83.63, 83.98, 86.87, 127.50, 127.53, 127.55, 127.67, 127.81, 128.24, 128.33, 138.00 and 138.51; *m/z* 289 [$(M + NH_4)^+$, 100%] [Found: $(M)^{\bullet+}$, 280.1668. $C_{16}H_{24}O_4$ requires $(M)^{\bullet+}$, 280.1675]; reversed-phase HPLC R_t 8.85, 9.3, 9.9 min, linear gradient from 50% B to 70% B over 20 min, where A is water–0.045% trifluoroacetic acid and B is acetonitrile–0.036% trifluoroacetic acid, at a flow rate of 1 cm^3 min^{-1}.

(2*RS*,3*RS*,4*S*)-4-Benzyloxy-3-hydroxy-2,5-dimethylhexanoic acid 17

Lithium hydroxide (0.95 g, 22 mmol) was added to a solution of the hydroxy ester **16** (1.26 g, 4.51 mmol) in tetrahydrofuran–water–methanol (1:1:1; 18 cm^3) at 0 °C and the mixture was stirred for 8 h at 0 °C. The volume of solvent was reduced to one-third by rotatory evaporation and water (30 cm^3) was added as replacement. The resulting solution was washed with ethyl acetate (2 × 10 cm^3) and the combined organic phases were washed with saturated aqueous sodium hydrogen carbonate (2 × 10 cm^3). The combined aqueous phases were brought to pH 2–3 (indicator paper) by addition of 1 mol dm^{-3} aqueous potassium hydrogen sulfate and extracted with ethyl acetate (3 × 20 cm^3). The combined extracts were washed with saturated brine (1 × 10 cm^3), dried, filtered and evaporated to give the four diastereoisomers of the hydroxy acid **17** (ratio 50:20:15:15) as a white semi-solid (1.09 g, 91%); ν_{max}(film)/cm^{-1} 3855, 3367, 2965, 2940, 2860, 1699, 1559, 1497, 1455, 1283, 1263, 1211, 1088 and 1067; δ_H(300 MHz, $CDCl_3$) 0.99–1.08 (6 H, m), 1.16 (3 H, d, *J* 6.9), 1.23 (3 H, d, *J* 6.8), 1.28 (3 H, d, *J* 7.2), 1.34 (3 H, d, *J* 7.2), 2.00–2.20 (1 H, m), 2.60–2.70 (1 H, m), 2.80–2.90 (1 H, m), 3.20–3.35 (1 H, m), 3.68–3.78 (1 H, m), 3.85–3.90 (1 H, m), 4.06–4.17 (1 H, m), 4.53–4.75 (2 H, m) and 7.31–7.35 (5 H, m); δ_C(75 MHz, $CDCl_3$) 10.57, 12.86, 15.34, 16.85, 17.46, 18.08, 19.03, 19.90, 20.12, 29.37, 29.70, 30.14, 39.67, 40.07, 43.71, 71.18, 71.95, 74.29, 74.45, 74.55, 74.59, 83.82, 84.22, 86.09, 127.56, 127.62, 127.74, 127.81, 128.29, 128.36, 128.42, 138.00, 138.40, 180.37, 181.53 and 181.87; *m/z* 284 [$(M + NH_4)^+$, 100%] [Found: $(M)^{\bullet+}$, 266.1521. $C_{15}H_{22}O_4$ requires $(M)^{\bullet+}$, 266.1518]; reversed-phase HPLC R_t 11.8 min, linear gradient from 20% B to 100% B over 20 min, where A is water–0.045% trifluoroacetic acid and B is acetonitrile–0.036% trifluoroacetic acid, at a flow rate of 1 cm^3 min^{-1}.

***N*-*tert*-Butoxycarbonylleucine 2,2,2-trichloroethyl ester**

4-Dimethylaminopyridine (0.73 g, 6.00 mmol), 2,2,2-trichloroethanol (1.39 cm^3, 14.40 mmol) and a solution of dicyclohexylcarbodiimide (2.97 g, 14.40 mmol) in dichloromethane (12 cm^3) were added to a solution of *N*-*tert*-butoxycarbonylleucine (3.00 g, 12.00 mmol) in dichloromethane (30 cm^3) at 0 °C and the mixture stirred for 18 h at room temperature under nitrogen. The mixture was then filtered, and evaporated and the resulting solid dissolved in ethyl acetate (100 cm^3). This solution was washed with 10% aqueous citric acid (2 × 20 cm^3), saturated aqueous sodium hydrogen carbonate (2 × 20 cm^3), and saturated brine (2 × 20 cm^3), dried, filtered and evaporated to give an oil. This was purified by chromatography (silica, ethyl acetate–hexanes, 35:65) to afford the *product* as a

white solid (4.05 g, 93%); mp 53–54 °C; $[\alpha]_D$ −38.4 (*c* 0.93, MeOH); ν_{max}(film)/cm^{-1} 3500–3250, 2980, 2920, 1770, 1720, 1500, 1470, 1450, 1440, 1390, 1370, 1270, 1250, 1150, 1050 and 1030; δ_H(300 MHz, $CDCl_3$) 0.95 (6 H, d, *J* 6), 1.43 (9 H, s), 1.50–1.83 (3 H, m), 4.38–4.47 (1 H, m), 4.63 (1 H, d, *J* 11.7) and 4.89 (1 H, d, *J* 11.7); δ_C(50 MHz, $CDCl_3$) 22.26, 23.29, 25.29, 28.79, 41.86, 52.59, 74.74, 81, 155 and 173; *m*/*z* 381.20 [(M′ + NH_4)$^+$, 34%], 379.3 [(M + NH_4)$^+$, 36%] and 149 (100%) (Found: C, 43.1; H, 6.1; N, 3.9. $C_{13}H_{22}Cl_3NO_4$ requires C, 43.05; H, 6.11; N, 3.86%).

(2*RS*,3*RS*,4*S*)-*N*-(4-Benzyloxy-3-hydroxy-2,5-dimethylhexanoyl)leucine trichloroethyl ester 20

A 40% solution of trifluoroacetic acid in dichloromethane (20 cm^3) was added to a solution of *N*-*tert*-butoxycarbonylleucine 2,2,2-trichloroethyl ester (2.86 g, 7.90 mmol) in dichloromethane (20 cm^3) at room temperature and the mixture stored for 20 min before evaporation. Addition of diethyl ether (5 cm^3) to the residue followed by evaporation was thrice repeated to give a white solid. This was dissolved in acetonitrile (5 cm^3) and the solution added to a stirred solution of the hydroxy acid **17** (1.06 g, 4.00 mmol) in acetonitrile (10 cm^3) at 0 °C. *N*-[(1*H*-Benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethanaminium tetrafluoroborate (TBTU) (1.52 g, 4.00 mmol), 1-hydroxybenzotriazole (0.61 g, 4.00 mmol) and then, dropwise, diisopropylethylamine (2.02 cm^3, 11.9 mmol) were added to the mixture which was then stirred at 0 °C under nitrogen for 2 h before evaporation. The resulting off-white solid was dissolved in ethyl acetate (70 cm^3) and the solution washed with 5% aqueous potassium hydrogen sulfate (3 × 10 cm^3), 5% aqueous sodium hydrogen carbonate (3 × 10 cm^3) and saturated brine (3 × 10 cm^3), dried, filtered and evaporated to afford an oil. This was purified by chromatography (silica gel, ethyl acetate–hexanes, 1:4) to afford the four diastereoisomers of product **19** (ratio *ca.* 45:25:20:10) as a colourless semi-solid (1.84 g, 90%); ν_{max}(film)/cm^{-1} 3280, 2959, 2917, 2883, 2851, 1765, 1648, 1645, 1541, 1497, 1456, 1273, 1180 and 1146; δ_H(300 MHz, $CDCl_3$) 0.91–1.09 (9 H, m), 1.22 (3 H, d, *J* 6.9), 1.23 (3 H, d, *J* 6.9), 1.25 (3 H, d, *J* 6.9), 1.33 (3 H, d, *J* 7.2), 1.50–1.90 (3 H, m), 1.94–2.20 (1 H, m), 2.40–2.49 (1 H, m), 2.60–2.69 (1 H, m), 2.61–2.68 (1 H, qd, J_1 7, J_2 2.4), 2.68–2.78 (1 H, qd, J_1 8.3, J_2 3), 2.81 (1 H, m, *J* 6.5), 2.89 (1 H, d, *J* 5.7), 3.25–3.33 (1 H, m), 3.27–3.31 (1 H, m), 3.28–3.32 (1 H, dd, J_1 8.1, J_2 2.7), 3.44–3.48 (1 H, m), 3.49 (1 H, d, *J* 2.7), 3.55–3.65 (1 H, m), 3.62–3.70 (1 H, m), 3.75–3.83 (1 H, m), 3.89 (1 H, d, *J* 9), 3.93–3.96 (1 H, m), 4.51–4.59 (5 H, m), 5.99 (1 H, d, *J* 8.1), 6.20 (1 H, d, *J* 8), 6.76 (1 H, d, *J* 8) and 7.34–7.38 (5 H, m); δ_C(75 MHz, $CDCl_3$) 11.02, 13.20, 15.75, 16.32, 16.52, 16.97, 17.00, 17.80, 19.01, 19.88, 19.99, 20.28, 21.55, 21.69, 22.69, 22.77, 24.87, 29.22, 29.50, 29.79, 30.33, 40.21, 40.53, 40.81, 40.92, 40.99, 43.81, 44.90, 50.32, 50.44, 50.49, 71.28, 71.80, 73.51, 73.80, 73.95, 74.11, 74.22, 74.33, 74.43, 74.66, 74.78, 83.36, 84.29, 86.37, 127.18, 127.43, 127.57, 127.70, 127.82, 128.35, 128.43, 128.51, 138.46, 139.16, 171.32, 174.84, 175.00, 176.78 and 177.34; *m*/*z* (FAB) 514.2 [(M″ + H)$^+$, 34%], 512.2 [(M′ + H)$^+$, 95%] and 510.3 [(M + H)$^+$, 100%] (Found: C, 54.1; H, 6.8; N, 2.9. $C_{23}H_{34}Cl_3NO_5$ requires C, 54.07; H, 6.71; N, 2.74%); reversed-phase HPLC R_t 18.38 min, linear gradient from 20% B to 100% B over 20 min followed by elution with 100% B for 10 min, where A is water–0.045% trifluoroacetic acid and B is acetonitrile–0.036% trifluoroacetic acid, at a flow rate of 1 cm^3 min^{-1}.

(2*RS*,3*RS*,4*S*)-*N*-(4-Benzyloxy-3-hydroxy-2,5-dimethylhexanoyl)leucine *tert*-butyl ester 21

Leucine *tert*-butyl ester hydrochloride (173 mg, 0.8 mmol) in dry acetonitrile (1 cm^3) was added to the hydroxy acid **17** (103 mg, 0.4 mmol) in dry acetonitrile (0.5 cm^3) at 0 °C, and *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethanaminium tetrafluoroborate (TBTU) (147 mg, 0.4 mmol), 1-hydroxybenzotriazole (59 mg, 0.4 mmol) and diisopropylethylamine (0.20 cm^3, 1.1 mmol) were added to the resulting solution. The mixture was stirred at 0 °C for 3 h, after which it was evaporated and the residue dissolved in ethyl acetate (3 cm^3). The solution was washed with 5% aqueous potassium hydrogen sulfate (2 × 3 cm^3), 5% aqueous sodium hydrogen carbonate (2 × 3 cm^3) and saturated brine (2 × 3 cm^3), dried, filtered and evaporated to give an oil. This was purified by chromatography (silica gel, ethyl acetate–hexanes, 1:4) to afford the four diastereoisomers of the alcohol **21** (ratio *ca.*, 65:10:10:5) as a white solid (128 mg, 76%), mp 70–72 °C; ν_{max}(film)/cm^{-1} 3600–3300, 2960, 1636, 1366 and 1123; δ_H(200 MHz, $CDCl_3$) 0.87–1.03 (12 H, m), 1.18 (3 H, d, *J* 7.2), 1.20 (3 H, d, *J* 7.2), 1.21 (3 H, d, *J* 7.0), 1.30 (3 H, d, *J* 7.0), 1.42 (9 H, s), 1.43 (9 H, s), 1.43 (9 H, s), 1.44 (9 H, s), 1.22–1.60 (3 H, m), 1.86–2.02 (1 H, m), 1.88–2.19 (1 H, m), 2.05–2.22 (1 H, m,), 2.33–2.46 (1 H, m), 2.36 (1 H, qd, J_1 = J_2 7.0), 2.38–2.52 (1 H, br s), 2.59 (1 H, qd, J_1 7.2, J_2 2.2), 2.66 (1 H, qd, J_1 7.0, J_2 2.6), 2.86–3.08 (1 H, br s), 3.24 (1 H, dd, J_1 7.8, J_2 3.2), 3.26 (1 H, dd, J_1 5.2, J_2 3.4), 3.27 (1 H, dd, J_1 8.2, J_2 2.6), 3.37 (1 H, dd, J_1 = J_2 5.4), 3.55 (1 H, m), 3.66 (1 H, dd, J_1 = J_2 5.4), 3.79 (1 H, dd, J_1 6.2, J_2 3.4), 3.91 (1 H, dd, J_1 8.2, J_2 2.2), 4.38 (1 H, td, J_1 8.0, J_2 5.4), 4.44 (1 H, td, J_1 8.0, J_2 5.6), 4.38–4.76 (3 H, m), 4.60–4.77 (2 H, d, *J* 11.4), 4.62 (2 H, d, *J* 11), 6.00 (1 H, d, *J* 8.4), 6.09 (1 H, d, *J* 8.0), 6.64 (1 H, d, *J* 8.0), 6.64 (1 H, d, *J* 8.4) and 7.26–7.38 (5 H, m); δ_C(50 MHz, $CDCl_3$) 10.92, 13.58, 15.62, 16.37, 16.48, 16.95, 17.11, 17.80, 19.17, 19.82, 20.00, 20.33, 21.93, 21.95, 21.99, 22.03, 22.68, 22.70, 22.75, 24.88, 24.92, 27.92, 29.23, 29.56, 29.74, 30.16, 40.20, 40.81, 41.28, 41.59, 41.66, 43.86, 45.06, 50.98, 51.01, 51.05, 71.34, 72.03, 73.72, 74.14, 74.22, 74.36, 74.58, 74.89, 81.60, 81.89, 81.91, 82.00, 83.13, 83.90, 84.22, 86.32, 127.37, 127.41, 127.57, 127.64, 127.70, 127.81, 128.30, 128.41, 138.47, 138.60, 139.03, 172.08, 172.17, 174.65, 175.00, 176.56 and 177.08; *m*/*z* 436 [(M + H)$^+$, 100%] (Found: C, 68.8; H, 9.5; N, 3.4. $C_{25}H_{41}NO_5$ requires C, 68.93; H, 9.49; N, 3.22%).

(2*RS*,4*S*)-*N*-(4-Benzyloxy-2,5-dimethyl-3-oxohexanoyl)leucine trichloroethyl ester 22

Pyridinium chlorochromate adsorbed on alumina[18] (1.04 g, 8.44 × 10^{-4} mol) was added to a solution of the alcohol **20** (220 mg, 4.22 × 10^{-4} mol) in dichloromethane (6 cm^3) and the mixture stirred vigorously in a covered flask for 36 h. Filtration followed by evaporation gave a yellow oil which was purified by chromatography (silica gel, ethyl acetate–hexanes, 1:4) to afford the two diastereoisomers of product **22** (ratio *ca.* 1:1) as a semi-solid (156 mg, 72%); ν_{max}(film)/cm^{-1} 2961, 2938, 2875, 1763, 1725, 1653, 1522, 1492, 1400, 1369, 1273, 1236 and 1146; δ_H(200 MHz, $CDCl_3$) 0.87–0.99 (12 H, m), 1.37 (3 H, d, *J* 7.12), 1.39 (3 H, d, *J* 7.18), 1.50–1.70 (3 H, m), 2.00–2.20 (1 H, m), 3.68 (1 H, d, *J* 4.4), 3.73 (1 H, d, *J* 3.9), 3.87–3.98 (1 H, two q, *J* 7.18), 4.38–4.92 (5 H, m), 6.71 (1 H, d, *J* 7.26), 6.75 (1 H, d, *J* 8.5) and 7.33–7.35 (5 H, m); δ_C(75 MHz, $CDCl_3$) 16.08, 16.33, 17.61, 17.78, 18.94, 18.99, 21.51, 21.67, 22.71, 22.76, 24.86, 24.92, 30.46, 40.60, 40.79, 41.04, 49.01, 49.38, 50.72, 50.77, 50.88, 72.89, 73.54, 74.24, 74.27, 74.35, 74.39, 89.29, 90.06, 127.76, 127.95, 128.05, 128.48, 128.52, 138.00, 168.96, 169.03, 170.89, 170.99, 210.47 and 210.98; *m*/*z* (FAB) 512.2 [(M + H)$^+$, 50%], 510.2 [(M′ + H)$^+$, 100%] and 508.2 [(M″ + H)$^+$, 85%] (Found: C, 54.0; H, 6.4; N, 2.6. $C_{23}H_{32}Cl_3NO_5$ requires C, 54.29; H, 6.34; N, 2.75%); reversed-phase HPLC R_t 13.77 and 14.45 min, linear gradient from 50% B to 100% B over 20 min, where A is water–0.045% trifluoroacetic acid and B is acetonitrile–0.036% trifluoroacetic acid, at a flow rate of 1 cm^3 min^{-1}.

(2*RS*,4*S*)-*N*-(4-Benzyloxy-2,5-dimethyl-3-oxohexanoyl)leucine *tert*-butyl ester 23

Pyridinium chlorochromate adsorbed on alumina[18] (350 mg, 0.3 mmol) was added to a solution of the alcohol **21** (39 mg, 0.09

mmol) in dichloromethane (1.6 cm^3) and the mixture was stirred vigorously for 24 h in a covered flask. Filtration followed by evaporation gave a yellow oil that was purified by chromatography (silica gel, ethyl acetate–hexanes, 1:5) to afford the two diastereoisomers of the product **22** (ratio *ca.* 1:1) as a semi-solid (23 mg, 59%); ν_{max}(film)/cm^{-1} 3000–2875, 1722, 1675, 1510, 1440, 1370, 1225, 1215, 1145 and 1060; δ_H(200 MHz, $CDCl_3$) 0.82–0.92 (12 H, m), 1.32, (3 H, d, $J_1 = J_2$ 7) 1.34 (3 H, d, $J_1 = J_2$ 7), 1.40 (9 H, s), 1.41 (9 H, s), 1.30–1.60 (3 H, m), 2.00–2.14 (1 H, m), 3.66 (1 H, dd, J_1 4.8, J_2 4.2), 3.69 (1 H, dd, J_1 4.8, J_2 4.2), 3.83 (1 H, 2q, $J_1 = J_2$ 7), 3.87 (1 H, 2q, $J_1 = J_2$ 7), 4.30–4.68 (3 H, m), 6.55 (1 H, 2d, J_1 8, J_2 6), 6.62 (1 H, 2d, J_1 8, J_2 6) and 7.26–7.37 (5 H, m); δ_C(50 MHz, $CDCl_3$) 15.67, 16.13, 17.53, 17.64, 18.99, 21.75, 21.93, 22.74, 24.83, 24.86, 27.87, 27.90, 30.31, 30.36, 41.18, 41.53, 49.16, 49.67, 51.34, 51.40, 72.85, 73.11, 81.71, 89.17, 89.68, 127.84, 128.35, 137.38, 168.53, 168.59, 171.50, 171.61, 210.06 and 210.95; *m/z* 451 [$(M + NH_4)^+$, 45%] and 434 [$(M + H)^+$, 100%] (Found: C, 69.5; H, 9.2; N, 3.2. $C_{25}H_{39}NO_5$ requires C, 69.25; H, 9.07; N, 3.23%).

Acknowledgements

The financial support for this work was provided by PharmaMar SA, Madrid, CICYT grant no. PB92-0864 and the Generalitat de Catalunya (Centre de Referència en Biotecnologia).

References

1 K. L. Rinehart, J. B. Gloer, R. G. Hughes, H. E. Renis, J. P. McGovren, E. B. Swynenberg, D. A. Stringfellow, S. L. Kuentzel and L. H. Li, *Science*, 1981, **212**, 933.
2 J. A. Stewart, J. B. Low, J. D. Roberts and A. Blow, *Cancer*, 1991, **68**, 2550.
3 J. H. Malfetano, J. A. Blessing and A. J. Jacobs, *Am. J. Clin. Oncol. (CCT)*, 1993, **16**, 47.
4 W. R. Ewing, K. L. Bhat and M. M. Joullié, *Tetrahedron*, 1986, **42**, 5863.
5 K. L. Rinehart, V. Kishore, S. Nagarajan, R. J. Lake, J. B. Gloer, F. A. Bozich, K.-M. Li, R. E. Maleczka, W. L. Todsen, M. H. G. Munro, D. W. Sullins and R. Sakai, *J. Am. Chem. Soc.*, 1987, **109**, 6846.
6 P. Jouin, J. Poncet, M.-N. Dufour, I. Maugras, A. Pantaloni and B. Castro, *Tetrahedron Lett.*, 1988, **29**, 2661.
7 U. Schmidt, M. Kroner and H. Griesser, *Tetrahedron Lett.*, 1988, **29**, 3057.
8 Y. Hamada, Y. Kondo, M. Shibata and T. Shioiri, *J. Am. Chem. Soc.*, 1989, **111**, 669.
9 K. L. Rinehart, J. B. Gloer, J. C. Cook, S. A. Mizsak and T. A. Scahill, *J. Am. Chem. Soc.*, 1981, **103**, 1857.
10 P. Brewster, F. Hiron, E. D. Hughes, C. K. Ingold and P. A. D. S. Rao, *Nature (London)*, 1950, **166**, 179.
11 K. Koga, C. C. Wu and S.-I. Yamada, *Tetrahedron Lett.*, 1971, 2287.
12 H. G. Lerchen and H. Kunz, *Tetrahedron Lett.*, 1985, **26**, 5257.
13 G. Losse and G. Bachmann, *Chem. Ber.*, 1964, **97**, 2671.
14 P. Lloyd-Williams, P. Monerris, I. Gonzalez, G. Jou and E. Giralt, *J. Chem. Soc., Perkin Trans. 1*, 1994, 1969.
15 G. Barany and R. B. Merrifield, *J. Am. Chem. Soc.*, 1977, **99**, 7363.
16 A. J. Mancuso, S.-L. Huang and D. Swern, *J. Org. Chem.*, 1978, **43**, 2480.
17 W.-R. Li, W. R. Ewing, B. D. Harris and M. M. Joullié, *J. Am. Chem. Soc.*, 1990, **112**, 7659.
18 Y.-S. Cheng, W.-L. Liu and S.-H. Chen, *Synthesis*, 1980, 223.

Paper 5/07523H
Received 17th *November* 1995
Accepted 1st *February* 1996