Pergamon

Tetrahedron, Vol. 52, No. 4, pp. 1361-1378, 1996

0040-4020/96 $15.00 + 0.00

0040-4020(95)00963-9

Synthesis of Novel Potassium Selective Valinomycins

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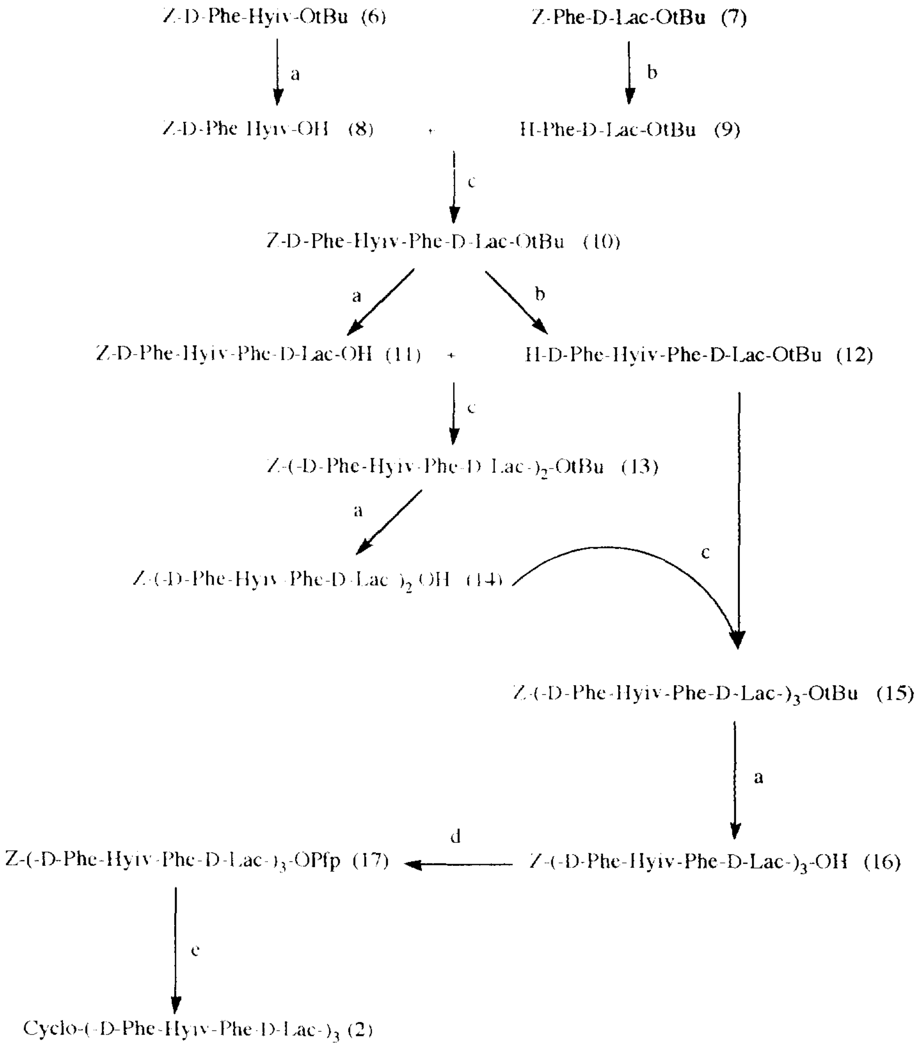
Abstract: The synthesis is described of three aryl substituted valinomycins, in which the aryl groups are placed around the poles of the cyclodepsipeptide. In one synthesis a valinomycin is prepared carrying a pendant hydroxyphenyl residue. In this synthesis the use of the acetate functionality to protect the phenolic site in tyrosine is developed. The spectroscopic and electrochemical evidence indicating the potential importance of such modified valinomycins in the design of improved ion selective electrodes is discussed.

Valinomycin (1), cyclo-(-L-Val-D-Hyiv-D-Val-L-Lac-)$_3$, an ionophore antibiotic, first isolated[1] in 1955, has attracted considerable attention because it displays the best potassium to sodium selectivity[2] of all known potassium complexones. This favorable selectivity offers important advantages in the use of valinomycin in the analysis of potassium ion concentrations in biofluids. Such analyses[3] are required rapidly in intensive care- and dialysis- units, and in the course of surgical operations such as open-heart surgery. The incorporation of valinomycin into an inert poly(vinylchloride) matrix has permitted[4] the development of a potassium ion selective electrode. Similarly ion selective field effect transistors have been developed[5] based on valinomycin PVC membranes. The possibility of the use of ion selective optodes based on valinomycin[6] has been investigated. In all these potential applications a severe limitation[7] is the leaching of valinomycin away from the membrane. This leaching in the case of ion selective electrodes provokes an undesired drift in measured potentials. This leaching would be prevented if the valinomycin were covalently bound to the membrane. The problem has been recognised[8] for sometime, but has recently been addressed in two different ways. In the development of ion selective CHEMFETS potassium selective hemispherands carrying remote functionality have been attached covalently to membranes. This study[9] was based on the recognition that valinomycin has limited possibilities for attachment to a membrane. However the resulting device is limited by the lower potassium selectivity of the hemispherands. In a patent[10] there is a report of the use of modified valinomycins carrying side-chain functionality. By incorporation of a lysine residue attachment of a valinomycin to a membrane is possible. Since early Russian synthetic studies[11], which produced a range of modified valinomycins, there has been little investigation of the possible synthesis of sidechain modified valinomycins. Their successful synthesis could lead to marked improvement in the clinical analysis of potassium ion by electrochemical methods. The great potential in the alternative of analysis of potassium ion by using optodes[6] adds to the interest in the synthesis of modified valinomycins. The sidechains might carry chromophores sensitive to the presence of potassium in the central cavity. Hence there is a need to study efficient routes to modified valinomycins. In an earlier communication[12] we have reported the use of the pentafluorophenyl ester method of coupling to permit the key cyclisation affording the 36-membered ring of modified valinomycins, and we describe in the preceeding paper[13] the background leading to this synthetic strategy. In this paper we describe the application of this methodology to the synthesis of a series of aryl substituted valinomycins, the successful synthesis of a modified valinomycin carrying a remote phenolic functionality, and the electrochemical evidence that the sidechain modified valinomycins retain an exceptional selectivity for potassium ion.

Valinomycin (1), cyclo-(-L-Val-D-Hyiv-D-Val-L-Lac-)$_3$, adopts the shape of a hollow cylinder inside which cations can fit. The side chain residues of the individual amino- and hydroxy-acids are situated either at the entrances to the cavity or around the equatorial belt. With a desire to synthesise modified valinomycins carrying a sensing group, appropriate to either electrical or optical sensing, or functionalised to permit covalent binding to electrode materials, there are two sites for these modifying groups. They may be placed close to the apertures, or around the belt, away from the cavity entrances. Introduction of the aryl groups close to the entrances requires their incorporation into amino-acid residues, and conversely they are placed around the belt by incorporation into hydroxy-acid residues.A key question in the design of modified valinomycins is the possibility that situation of the modifying groups close to the apertures may modify cation binding characteristics relative to situating the groups around the equatorial belt. The synthetic chemistry described in this paper leads to a series of substituted valinomycins with the aryl residues, for example as in (2), situated close to the cavity entrances. In the following paper the synthesis is described of the second series where the aryl residues, as in (3), are located around the belt. Another target (4) is a valinomycin carrying a tyrosine residue, which can be used as a site of possible attachment to electrodes or solid supports. The synthesis of the tyrosine (4) and the acetyl protected tyrosine (5) are also reported in this paper. Phenylalanine or tyrosine were chosen for the situation of aryl groups at the entrances and α-hydroxyhydrocinnamic acid was incorporated to place phenyl groups around the belt. The targets (cyclo-(-D-Phe-L-Hyiv-L-Phe-D-Lac-)$_3$ (2) and cyclo-(-D-Ala-L-Hyhc-L-Val-D-Hyhc-)$_3$ (3) are related to valinomycin (1), by in the first case, the replacement of valine residues by phenylalanine residues and, in the second case, by replacement of hydroxyisovaleric and lactic acid residues by α-hydroxyhydrocinnamic acid residues, and replacement of a valine residue by alanine. The latter replacement was made to lessen possible

Scheme 1



Reagents: a) TFA, CH_2Cl_2, r.t.; b) H_2, Pd/C (10%), MeOH, r.t.; c) DCC, DMAP, NMM, CH_2Cl_2, 0°C; b) PfpOH, DCC, CH_2Cl_2, 0°C; e) H_2, Pd/C (5%), DMAP, Dioxan, Ethanol, 90°C.

adverse steric interactions and it has been established earlier[2] that such a substitution facilitates cation binding.

The synthetic strategy required the elaboration of didepsipeptide fragments to afford linear dodecadepsipeptides, which were then cyclised through the appropriate pentafluorophenyl esters. The two syntheses incorporating phenylalanine and tyrosine are shown in Schemes 1 and 2 respectively. The

Scheme 2

Z-Tyr (Ac)-OH (**18**) + H-D-Lac-OtBu

$\downarrow$ a

Z-Tyr (Ac)-D-Lac-OtBu (19)

$\searrow$ b

Z-D-Phe-Hyiv-OH (8) + H-Tyr (Ac)-D-Lac-OtBu (20)

$\downarrow$ a

Z-D-Phe-Hyiv-Tyr (Ac)-D-Lac-OtBu (21)

$\searrow$ b

Z-(-D-Phe-Hyiv-Phe-D-Lac-$)_2$-OH (14) + H-D-Phe-Hyiv-Tyr (Ac)-D-Lac-OtBu (22)

$\downarrow$ a

Z-(-D-Phe-Hyiv-Phe-D-Lac-$)_2$-D-Phe-Hyiv-Tyr (Ac)-D-Lac-OtBu (23)

$\downarrow$ c

Z-(-D-Phe-Hyiv-Phe-D-Lac-$)_2$-D-Phe-Hyiv-Tyr-D-Lac-OtBu (24)

$\downarrow$ d

Z-(-D-Phe-Hyiv-Phe-D-Lac-$)_2$-D-Phe-Hyiv-Tyr-D-Lac-OH (25)

$\downarrow$ e

Z-(-D-Phe-Hyiv-Phe-D-Lac-$)_2$-D-Phe-Hyiv-Tyr-D-Lac-OPfp (26)

$\downarrow$ f

Cyclo-(-D-Phe-Hyiv-Phe-D-Lac-$)_2$-D-Phe-Hyiv-Tyr-D-Lac-) (4) $\xrightarrow{g}$ 4 (Ac) (5)

Reagents: a) DCC, DMAP, CH_2Cl_2, 0°C; b) H_2, Pd /C (10%), EtOAc, 0°C; c) Imidazole, MeCN, H_2O, 0°C; d) TFA, CH_2Cl_2, r.t.; e) PfpOH, DCC, DMAP, CH_2Cl_2, 0°C; f) H_2, Pd /C (5%), DMAP, dioxan, EtOH, 90°C; g) Ac_2O, DMAP, EtOAc, r.t.

construction of the linear fragments was achieved via protection of carboxylate and amino groups by t-butyl ester and benzyloxycarbonyl groups respectively. Fragments were coupled either using dicyclohexylcarbodiimide or via pentafluorophenyl esters. Thus in Scheme 1 from the dipeptide fragments (6) and (7) via intermediates (8 - 16) the dodecadepsipeptide (17) was readily obtained. In Scheme 2 O-acetyl-N-benzyloxycarbonyl L tyrosine (18) was coupled with D-lactic acid t-butyl ester, and the dipeptide (19), via intermediates (20 - 25), was extended to afford the dodecapeptide (26). A number of points concerning the build up of the linear dodecapeptides deserve mention. The introduction of a tyrosine residue (Scheme 2) necessitated a strategy for the protection of the phenolic hydroxyl group. Previous synthetic studies[14] have been based on silyl, methyl and benzyl ethers and benzyloxycarbonyl esters. Many of these protecting groups are incompatible with our use of benzyloxycarbonyl protection at nitrogen and protection of the carboxyl function as a t-butyl ester. As the removal of a protecting group from the phenolic site demanded very mild conditions to avoid ester hydrolysis at other sites, we were interested in the development of the simple acetate group as a potential method of protection of the phenolic site. It has previously been established[15] that there is a substantial rate difference in the imidazole promoted hydrolysis of phenyl acetate relative to ethyl acetate. The potential of the acetate protection was checked by study of the hydrolysis of ester (18). The acetate group could be readily removed without cleavage of the t-butyl or peptide esters. Following this and other successful model studies, the value of the acetate protecting group was demonstrated by the ability to carry forward the dipeptide intermediate (19) having protection of the phenolic site as an acetyl group, via the dipeptide (20), and the tetrapeptide intermediates (21) and (22) to give the dodecapeptide (23). The acetate protecting group was successfully selectively removed from the peptide (23) with imidazole in aqueous acetonitrile to give the phenol (24). The free phenolic functionality did not interfere in the subsequent conversion of the linear dodecapeptide via intermediate (25) to the cyclisation precursor (26).

The efficient method of cyclisation of pentafluorophenyl esters was applied to the linear precursors (17), and (26). By continuous injection over 24 hours the 36-membered ring was readily constructed to afford the target cyclododecapeptide (2) in 84% yield. Injection over 6 hours gave the target (2) in 48% yield. The phenolic target (4) was obtained in a satisfactory 58% yield by injection over 10 hours. In order to study the effect of the phenolic group on the behaviour of the valinomycins the ester (5) was prepared from the phenol (4) by conventional acetylation.

The syntheses described in this and the following paper provide the first access to aryl substituted valinomycins. It is particularly important that both targets (2) and (3) have been prepared, as this has enabled us to study the electrochemical properties of both series, and in part, by comparison with valinomycin (1), to conclude that the positioning of the phenyl residues about either the equator or the poles of valinomycin (1) does not greatly affect metal ion complexation. Similarly the synthesis of the phenol (4) and the acetate (5) has enabled us to study both the properties of remotely functionalised valinomycins and their attachment directly to electrodes. Here it may be noted that the ability of the cyclic peptides (2-5) to complex with metal ions is scarcely diminished relative to valinomycin (1). The stability constants of the cation complexes of the peptides (2-5) are very similar to those of valinomycin both in their order and approximate magnitude. The ease of binding metal ions for (2-5) increases in the series $Rb^+ = K^+ > Cs^+ > Na^+ > Li^+$. The stability constant K_{ML} of (4) and (5) with potassium ion is about a factor of 10 lower than with valinomycin (1). Neither the position of the aryl residues nor the presence of a phenolic site greatly interfere with metal ion complexation. Clearly these results facilitate the design of new efficient potassium ion sensors.

Experimental

General experimental details are described elsewhere[13].

Benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleric acid t-butyl ester (6).

Benzyloxycarbonyl D-phenylalanine (4.50g, 15.0mmol), L-α-hydroxyisovaleric acid t-butyl ester (2.40g, 13.8mmol) and dimethylaminopyridine (0.19g, 1.6mmol) were dissolved in dichloromethane (90ml) and cooled to -40°C. A solution of dicyclohexylcarbodiimide (3.10g, 15.0mmol) in dichloromethane (10ml) was added, and the resulting mixture was stirred for 20h. The precipitate of urea was filtered off, solvent was removed under reduced pressure and the residue was purified by chromatography on silica gel (light petroleum ether : diethyl ether, 3-1) to yield benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleric acid t-butyl ester (6) (5.27g, 84%) as an oil. $[\alpha]_D$ -24 (c 1.60 chloroform). IR ($CHCl_3$) (cm^{-1}) 3450 w (N-H stretch) 1745, 1740 & 1735 s (C=O stretch) 1515 s (N-H bend). δH (270MHz, solvent $CDCl_3$, standard Me_4Si) 0.93 (3H, d, J 7Hz, CH_3 Hyiv) 0.94 (3H, d, J 7Hz, CH_3 Hyiv) 1.44 (9H, s, $(CH_3)_3$ t-butyl) 2.18 (1H,m, CH i-propyl) 3.09 (1H, ABX, J 6 & 14Hz, CH_2 Phe) 3.22 (1H, ABX, J 6.5 & 14Hz, CH_2 Phe) 4.67 (1H, d, 4.5Hz, O-CH) 4.76 (1H, m, N-CH) 5.08 (2H, s, O-CH_2) 5.24 (1H, d, J 8Hz, NH) 7.1-7.35 (10H, c, C_6H_5). δC (68MHz, solvent $CDCl_3$, standard Me_4Si) 17.24 - 18.85 (CH_3 Hyiv) 28.05 (CH_3 t-butyl) 30.10 (CH i-propyl) 38.26 (CH_2 Phe) 54.98 (N-CH) 66.99 (O-CH_2) 78.24 (O-CH) 82.33 (C t-butyl) 127.17 - 128.18 - 128.24 - 128.60 - 128.70 - 129.40 (CH phenyl) 135.85 - 136.37 (C phenyl) 155.63 (C=O carbamate) 168.13 - 171.34 (C=O).

Benzyloxycarbonyl L-phenylalanyl D-lactic acid t-butyl ester (7).

To a solution of benzyloxycarbonyl L-phenylalanine (6.30g, 21.0mmol), D-lactic acid t-butyl ester (2.56g, 17.5mmol) and dimethylaminopyridine (0.3g, 2.5mmol) in dichloromethane (50ml) at -40°C, was added dicyclohexylcarbodiimide (4.34g, 21.0mmol) in dichloromethane (5ml). The mixture was stirred for 14h, and the precipitate of urea was filtered off. The solvent was removed and the residue was purified by chromatography on silica gel (light petroleum ether : diethyl ether, 3-1) to yield as a solid benzyloxycarbonyl L-phenylalanyl D-lactic acid t-butyl ester (7) (7.39g, 99%). mp 60-61°C (hexane/diethylether). $[\alpha]_D$ +32 (c 1.70 chloroform). IR ($CHCl_3$) (cm^{-1}) 3450 m (N-H stretch) 1740 & 1730 s (C=O stretch) 1510 s (N-H bend). δH (270MHz, solvent $CDCl_3$, standard Me_4Si) 1.40 (3H, d, J 7Hz, CH_3 Lac) 1.44 (9H, s, $(CH_3)_3$ t-butyl) 3.11 (1H, ABX, J 7 & 14Hz, CH_2 Phe) 3.16 (1H, ABX, J 7 & 14Hz, CH_2 Phe) 4.73 (1H, m, N-CH) 4.91 (1H, q, 7Hz, O-CH) 5.08 (2H, s, O-CH_2) 5.35 (1H, d, J 8Hz, NH) 7.1-7.35 (10H, c, C_6H_5). δC (68MHz, solvent $CDCl_3$, standard Me_4Si) 16.79 (CH_3 Lac) 27.89 (CH_3 t-butyl) 38.17 (CH_2 Phe) 54.85 (N-CH) 67.87 (O-CH_2) 70.01 (O-CH) 82.28 (C t-butyl) 127.08 - 128.05 - 128.13 - 128.51 - 128.55 - 129.36 (CH phenyl) 135.84 136.36 (C phenyl) 155.54 (C=O carbamate) 169.21 - 170.88 (C=O). $C_{24}H_{29}NO_6$ requires C 67.4; H 6.8; N 3.3%, Found C 67.5; H 6.8; N 3.2%.

Benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleric acid (8).

A solution of benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleric acid t-butyl ester (6) (1.29g, 2.75mmol) in dichloromethane (10ml) was treated with trifluoroacetic acid (10ml) for 5h at room temperature. The solvent was removed to afford benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleric acid (8) (1.13g, 100%) as an oil. $[\alpha]_D$ -14 (c 1.30 chloroform). IR ($CHCl_3$) (cm^{-1}) 3450 m (N-H stretch) 3000 br (O-H stretch) 1735 s (C=O stretch) 1510 s (N-H bend). δH (270MHz, solvent $CDCl_3$, standard Me_4Si) 0.95 (6H,d, J 7Hz, CH_3 Hyiv) 2.25 (1H, m, CH i-propyl) 3.09 (1H, ABX, J 6 & 14Hz, CH_2 Phe) 3.19 (1H, ABX, J 7 & 14Hz, CH_2 Phe) 4.75 (1H, dd, J 6 & 7Hz, N-CH) 4.88 (1H, d, J 4Hz, O-CH) 5.05 & 5.08 (2H, m, O-CH_2) 5.32 (1H, d, J 8Hz, NH) 7.1-7.3 (10H, c, C_6H_5). δC (68MHz, solvent $CDCl_3$, standard Me_4Si) 17.07 - 18.84 (CH_3 Hyiv) 30.05 (CH i-propyl) 37.99 (CH_2 Phe) 55.02 (N-CH) 67.42 (O-CH_2) 77.45 (O-CH) 127.34 - 128.25 - 128.39 - 128.64 - 128.81 - 129.34 (CH phenyl) 135.51 - 135.97 (C phenyl) 156.11 (C=O carbamate) 171.52 - 173.81 (C=O).

L-Phenylalanyl D-lactic acid t-butyl ester (9).

A mixture of benzyloxycarbonyl L-phenylalanyl D-lactic acid t-butyl ester (7) (1.02g, 2.4mmol) and palladium on charcoal (10%) (300mg) in ethyl acetate (50ml) was hydrogenated for 5h. The catalyst was filtered off and the solvent was removed to yield L-phenylalanyl D-lactic acid t-butyl ester (9) (0.69g, 99%) as an oil. δH (270MHz, solvent $CDCl_3$, standard Me_4Si) 1.41 (3H, d, J 7Hz, CH_3 Lac) 1.46 (9H, s, $(CH_3)_3$ t.butyl) 1.73 (2H, br, NH_2) 2.90 (1H, ABX, J 5.5 & 13.5Hz, CH_2 Phe) 3.09 (1H, ABX, J 7.5 & 13.5Hz, CH_2 Phe) 3.80 (1H, dd, J 5.5 & 7.5Hz, N-CH) 4.93 (1H, q, J 7Hz, O-CH) 7.2-7.3 (5H, c, C_6H_5).δC (68MHz, solvent $CDCl_3$, standard Me_4Si) 16.81 (CH_3 Lac) 27.91 (CH_3 t-butyl) 40.67 (CH_2 Phe) 55.64 (N-CH) 69.29 (O-CH) 82.06 (C t-butyl) 126.75 - 128.49 - 129.29 (CH phenyl) 137.21 (C phenyl) 169.64 - 174.40 (C=O).

Benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactic acid t-butyl ester (10).

To a solution of benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleric acid (8) (5.23g, 13.1mmol), L-phenylalanyl D-lactic acid t-butyl ester (9) (2.56g, 8.7mmol) and dimethylaminopyridine (0.16mg, 1.3mmol) in dichloromethane (25ml) was added dicyclohexylcarbodiimide (2.70g, 13.1mmol) in dichloromethane (5ml) at -40°C. The mixture was stirred for 3.5 days, the dicyclohexylurea was filtered off, the solvent removed and the residue was chromatographed on silica gel (light petroleum ether : diethyl ether, 1-1) to give benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactic acid t-butyl ester (10) (5.05g, 86%) as an oil. $[\alpha]_D$ -2 (c 3.24 chloroform). IR ($CHCl_3$) (cm^{-1}) 3450 & 3380 m (N-H stretch) 2990 & 2975 (C-H stretch) 1745 & 1685 s (C=O stretch) 1510 m (N-H bend). δH (270MHz, solvent $CDCl_3$, standard Me_4Si) 0.53 (3H, d, J 7.5Hz, CH_3 Hyiv) 0.55 (3H, d, J 7.5Hz, CH_3 Hyiv) 1.40 (3H, d, J 7Hz, CH_3 Lac) 1.42 (9H, s, $(CH_3)_3$ t-butyl) 2.09 (1H, m, CH i-propyl) 3.0-3.25 (4H, c, CH_2 Phe) 4.54 (1H, q, J 7Hz, O-CH Lac) 4.8-4.95 (3H, c, N-CH & O-CH Hyiv) 5.04 & 5.17 (2H, AB, J 12Hz, O-CH_2) 5.38 (1H, d, J 6.5Hz, NH carbamate) 7.10 (1H, d, J 8.5Hz, NH amide) 7.15-7.35 (15H, c, C_6H_5). δC (68MHz, solvent $CDCl_3$, standard Me_4Si) 16.13 - 16.79 - 18.46 (CH_3 Hyiv & Lac) 27.97 (CH_3 t-butyl) 30.07 (CH i.propyl) 37.21 - 37.54 (CH_2 Phe) 53.52 - 55.90 (N-CH) 67.53 (O-CH_2) 69.98 (O-CH Lac) 78.89 (O-CH Hyiv) 82.20 (C t-butyl) 126.94 - 127.42 - 128.29 - 128.37 - 128.57 - 128.62 - 128.90 - 129.26 - 129.34 (CH phenyl) 135.42 - 136.04 - 136.64 (C phenyl) 156.28 (C=O carbamate) 169.07 - 169.54 - 170.67 - 171.37 (C=O).

Benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactic acid (11).

A solution of benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactic acid t-butyl ester (10) (0.31g, 0.46mmol) in dichloromethane (10ml) and trifluoroacetic acid (10ml) was kept at room temperature for 6h. The solvent was removed under reduced pressure to yield benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactic acid (11) (0.28g, 99%) as an oil. $[\alpha]_D$ -19 (c 2.80 chloroform) IR ($CHCl_3$) (cm^{-1}) 3450 & 3350 w (N-H stretch) 1740 & 1690 s (C=O stretch) 1515 s (N-H bend). δH (270MHz, solvent $CDCl_3$, standard Me_4Si) 0.43 (3H, d, J 6.5Hz, CH_3 Hyiv) 0.45 (3H, d, J 6.5Hz, CH_3 Hyiv) 1.45 (3H, d, J 7Hz, CH_3 Lac) 2.06 (1H, m, CH i-propyl) 3.0-3.3 (4H, c, CH_2 Phe) 4.50 (1H, q, J 6.5Hz, O-CH Lac) 4.85-5.2 (5H, c, N-CH, O-CH Hyiv & O-CH_2) 5.61 (1H, d, J 6.5Hz, NH carbamate) 7.1-7.4 (15H, c, C_6H_5) 7.63 (1H, d, J 8Hz NH amide). δC (68MHz, solvent $CDCl_3$, standard Me_4Si) 15.74 - 16.56 - 18.28 (CH_3 Hyiv & Lac) 30.02 (CH i-propyl) 36.50 - 37.45 (CH_2 Phe) 53.87 - 56.14 (N-CH) 67.83 (O-CH_2) 69.40 (O-CH Lac) 78.65 (O-CH Hyiv) 127.17 - 127.62 - 128.19 - 128.54 - 128.71 - 128.74 - 128.88 - 128.03 - 129.21 (CH phenyl) 134.97 - 135.66 136.15 (C phenyl) 156.90 (C=O carbamate) 170.39 - 171.48 - 171.93 - 174.43 (C=O).

D-Phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactic acid t-butyl ester (12).

A solution of benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactic acid t-butyl ester (10) (4.50g, 6.67mmol), palladium on charcoal (10%) (1.5g) and acetic acid (0.80g, 13.34mmol) in ethyl acetate (40ml) was hydrogenated for 9h. Filtration and evaporation of solvent under reduced pressure yielded D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactic acid t-butyl ester (12) (3.58g, 99%) as an oil. $[\alpha_D]$ -18 (c 2.7, $CHCl_3$) δH (270MHz, solvent $CDCl_3$, standard Me_4Si) 0.75 (3H, d, J 7Hz, CH_3 Hyiv) 0.81 (3H, d, J 7Hz, CH_3 Hyiv) 1.42 (3H, d, J 7Hz, CH_3 Lac) 1.45 (9H, s, $(CH_3)_3$ t-butyl) 2.14 (1H, m, CH i-propyl) 2.38 (2H, br, NH_2) 2.85-3.2 (4H, c, CH_2 Phe) 3.77 (1H, m, N-CH) 4.9-5.0 (3H, c, N-CH, O-CH Hyiv & O-CH Lac) 7.00 (1H, d, J 8Hz, NH amide) 7.2-7.3 (10H, c, C_6H_5). δC (68MHz, solvent $CDCl_3$, standard Me_4Si) 16.92 - 16.98 - 18.68 (CH_3 Hyiv & Lac) 27.99 (CH_3 t-butyl) 30.33 (CH i-propyl) 37.68 - 40.51 (CH_2 Phe) 52.69 - 55.81 (N-CH) 70.15 (O-CH Lac) 78.65 (O-CH Hyiv) 82.52 (C t-butyl) 127.03 - 127.13 - 128.61 - 128.75 - 129.37 - 129.49 (CH phenyl) 136.14 - 137.11 (C phenyl) 169.11 - 169.57 - 170.85 - 173.65 (C=O)

Benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactic acid t-butyl ester (13).

Benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactic acid (11) (3.74g, 6.04mmol) and D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactic acid t-butyl ester (12) (3.38g, 6.20mmol) and dimethylaminopyridine (80mg, 0.6mmol) dissolved in dichloromethane (40ml), and the solution cooled to -5°C. Dicyclohexylcarbodiimide (1.32g, 6.04mmol) was added and the mixture was stirred for 3 days. The urea precipitate was filtered off and the solvent removed. The residue was purified by chromatography on silica gel (light petroleum ether-diethyl ether, 1-1) to give benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovalyl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactic acid t-butyl ester (13) (3.80g, 55%) as an oil. $[\alpha]_D$ -13 (c 0.66 chloroform). IR ($CHCl_3$) (cm^{-1}) 3450 & 3350 m (N-H stretch) 2990 & 2970 m (C-H stretch) 1750 & 1670 s (C=O) 1530 (N-H bend) δH (270MHz, solvent $CDCl_3$, standard Me_4Si) 0.41 (3H, d, J 7Hz, CH_3 Hyiv) 0.44 (3H, d, J 7Hz, CH_3 Hyiv) 0.47 (3H, d, J 7Hz, CH_3 Hyiv) 0.59 (3H, d, J 7Hz, CH_3 Hyiv) 1.19 (3H, d, J 7Hz, CH_3 Lac)

1.37 (3H, d, J 7Hz, CH_3 Lac) 1.42 (9H, s, $(CH_3)_3$ t-butyl) 2.08 (2H, m, CH i-propyl) 3.0-3.25 (8H, m, CH_2 Phe) 4.4-4.55 (3H, c, N-CH) 4.75-4.9 (4H, c, N-CH, O-CH Hyiv & O-CH Lac) 4.94 & 5.12 (2H, AB, J 12Hz, O-CH_2) 5.24 (1H, q, J 7Hz, O-CH Lac) 5.87 (1H, d, J 5.5Hz, NH carbamate) 7.15-7.35 (25H, c, C_6H_5) 7.71 (1H, d, J 8.5Hz, NH amide) 7.74 (1H, d, J 8.5Hz, NH amide) 7.80 (1H, d, J 6Hz, NH amide). δC (68MHz, solvent $CDCl_3$, standard Me_4Si) 15.87 - 16.59 - 16.76 - 17.05 - 18.27 - 18.32 (CH3 Hyiv & Lac) 27.87 (CH3 t-butyl) 29.81 - 29.87 (CH i-propyl) 36.16 - 36.60 - 36.89 - 37.11 (CH_2 Phe) 53.77 - 55.11 - 55.41 - 56.27 (N-CH) 67.46 (O-CH_2) 69.84 70.43 (O-CH Lac) 78.43 - 78.62 (O-CH Hyiv) 81.84 (C t-butyl) 126.59 - 127.00 - 127.42 - 128.02 - 128.11 - 128.32 - 128.58 - 128.62 - 128.87 - 129.17 - 129.23 (CH phenyl) 135.15 - 135.82 - 136.24 - 136.40 - 137.13 (C phenyl) 156.74 (C=O carbamate) 169.56 - 169.63 - 169.98 - 170.38 - 170.74 - 170.81 - 171.36 - 171.96 (C=O).

Benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactic acid (14).

A solution of benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactic acid t-butyl ester (13) (220mg, 0.19mmol) in dichloromethane (5ml) and trifluoroacetic acid (5ml) was kept at room temperature for 7.5h. Removal of solvent afforded benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L–α-hydroxyisovaleryl L-phenylalanyl D-lactic acid (14) (200mg, 97%) as an oil. $[\alpha]_D$ -32 (c 1.76 chloroform). IR ($CHCl_3$) (cm^{-1}) 3350 m (N-H stretch) 2950 w (C-H stretch) 1760, 1720 & 1670 s (C=O) 1540 & 1530 s (N-H bend). δH (270MHz, solvent $CDCl_3$, standard Me_4Si) 0.38 (3H, d, J 6.5Hz, CH_3 Hyiv) 0.43 (3H, d, J 6.5Hz, CH_3 Hyiv) 0.49 (3H, d, J 6.5Hz, CH_3 Hyiv) 0.56 (3H, d, J 6.5Hz, CH_3 Hyiv) 1.25 (3H, d, J 7Hz, CH_3 Lac) 1.41 (3H, d, J 7Hz, CH_3 Lac) 2.04 (2H, c, CH i-propyl) 3.0-3.2 (8H, c, CH_2 Phe) 4.45 (1H, m, N-CH) 4.55 (2H, c, N-CH) 4.76 (1H, m, N-CH) 4.9-5.2 (6H, c, O-CH_2, O-CH Hyiv & O-CH Lac) 5.90 (1H, d, J 6Hz, NH carbamate) 7.2-7.35 (25H, c, C_6H_5) 7.65 (1H, d, J 6.5Hz, NH amide) 7.73 (2H, d, J 6Hz, NH amide). δC (68MHz, solvent $CDCl_3$, standard Me_4Si) 15.74 - 16.09 - 16.78 - 17.34 - 18.30 - 18.38 (CH_3 Hyiv & Lac) 29.77 - 29.85 (CH i-propyl) 35.94 - 36.39 - 36.81 - 37.17 (CH_2 Phe) 53.55 - 54.63 - 55.37 - 56.33 (N-CH) 67.65 (O-CH_2) 69.22 - 71.03 (O-CH Lac) 78.56 - 79.04 (O-CH Hyiv) 126.74 - 127.13 - 127.24 - 127.50 - 128.02 - 128.19 - 128.44 - 128.48 - 128.86 - 128.71 - 128.80 - 128.86 - 129.23 - 129.29 - 129.34 (CH phenyl) 135.15 135.87 - 135.92 - 136.24 - 137.06 (C phenyl) 156.97 (C=O carbamate) 169.99 - 170.02 - 170.41 - 170.80 - 170.91 - 171.82 - 172.35 - 172.90 (C=O).

Benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L–α hydroxyisovaleryl L-phenylalanyl D-lactic acid t-butyl ester (15).

Dicyclohexylcarbodiimide (91mg, 0.44mmol) was added to a solution of benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactic acid (14) (361mg, 0.33mmol), and D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactic acid t-butyl ester (12) (238mg, 0.44mmol) and dimethylaminopyridine (8mg, 67μmol) in dichloromethane (10ml) at 0°C. The mixture was stirred for 11h, the urea precipitate was filtered off and the solvent removed under reduced pressure. The residue was purified by chromatography on silica gel (light petroleum : ether, 1::2) to give the title compound (15) (254mg, 53%) as an oil. $[\alpha]_D$ -18 (c 2.4 chloroform). IR ($CHCl_3$) (cm^{-1}) 1540 s (N-H bend) 1670 s (C=O stretch) 1720 m (C=O stretch) 1755 s (C=O stretch) 3340 m (N-H stretch) 3440 w (N-H stretch). δH (270MHz, solvent $CDCl_3$, standard Me_4Si) 0.39 (3H, d, J 7Hz, Me Hyiv) 0.45-0.5 (9H, c, Me Hyiv) 0.55 (3H, d, J 7Hz, Me Hyiv) 0.64 (3H, d, J 7Hz, Me Hyiv) 1.07 (3H, d, J 7Hz, Me Lac) 1.16 (3H, d, J 7Hz, Me Lac) 1.38 (3H, d, J 7Hz, Me Lac) 1.43 (9H, s, Me t-butyl) 2.0-2.1 (3H, c, CH i-propyl) 3.0-3.3 (12H, c, CH_2 Phe) 4.23 (1H, m, N-CH) 4.3-4.55 (4H, c, N-CH) 4.74 (1H, m, N-CH) 4.8-5.25 (8H, c, O-CH Hyiv, O-CH Lac & O-CH_2) 5.84 (1H, d, J 7Hz, NH carbamate) 7.05-7.35 (35H, c, C_6H_5) 7.65-7.75 (2H, c, NH amide) 7.86 (1H, d, J 5.5Hz, NH amide) 7.98 (1H, d, J 5.5Hz, NH amide) 8.10 (1H, d, J 5.5Hz, NH amide) δC (68MHz, solvent $CDCl_3$, standard Me_4Si) 15.94 - 16.00 - 16.66 - 16.89 - 17.12 - 18.35 - 18.42 (Me Hyiv & Lac) 27.94 (Me t-butyl) 29.79 - 29.88 - 30.00 (CH i-propyl) 35.96 - 36.27 - 36.65 - 36.71 - 36.95 - 37.09 (CH_2 Phe) 53.83 - 55.05 - 55.35 - 55.54 - 55.91 - 56.39 (N-CH) 67.58 (O-CH_2) 69.89 - 70.37 - 70.53 (O-CH Lac) 78.07 - 78.52 - 78.66 (O-CH Hyiv) 81.97 (C t-butyl) 126.59 - 126.91 - 126.97 - 127.08 - 127.16 - 127.48 - 128.16 - 128.34 - 128.45 - 128.57 - 128.61 - 128.67 - 128.74 - 128.94 - 129.21 - 129.33 (CH phenyl) 135.18 - 135.81 - 136.13 - 136.36 - 136.54 - 136.76 - 137.31(C phenyl) 156.82 (C=O carbamate) 169.62 - 169.73 - 169.80 - 169.96 - 170.48 - 170.74 - 170.83 - 170.90 - 171.52 - 171.75 - 172.09 (C=O).

Benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D phenylalanyl L–α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactic acid (16).

A solution of benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactic acid t-butyl ester (15) (210mg, 0.13mmol) in dichloromethane (5ml) and trifluoroacetic acid (5ml) was kept at room temperature for 7h. Evaporation of solvent under reduced pressure gave the title compound (16) (203mg, 100%) as an oil. $[\alpha]_D$ -26 (c 4.3 chloroform). IR ($CHCl_3$) (cm^{-1}) 1540 s (N-H bend) 1670 s (C=O stretch) 1720 m (C=O stretch) 1755 s (C=O stretch) 3340 m (N-H stretch) 3440 w (N-H stretch). δH (270MHz, solvent $CDCl_3$, standard Me_4Si) 0.35 (3H, d, J 7Hz, Me Hyiv) 0.4-0.6 (15H, c, Me Hyiv) 1.21 (3H, d, J 7Hz, Me Lac) 1.25 (3H, d, J 7Hz, Me Lac) 1.42 (3H, d, J 7Hz, Me Lac) 1.95-2.15 (3H, c, CH i-propyl) 3.0-3.3 (12H, c, CH_2 Phe) 4.4-5.25 (14H, c, N-CH, O-CH Hyiv, O-CH Lac & O-CH_2) 5.99 (1H, d, J 5.5Hz, NH carbamate) 7.1-7.4 (35H, c, C_6H_5) 7.81 (1H, d, J 6Hz, NH amide) 7.85-7.9 (2H, c, NH amide) 7.96 (1H, d, J 6Hz, NH amide) 8.04 (1H, d, J 5.5Hz, NH amide). δC (68MHz, solvent $CDCl_3$, standard Me_4Si) 15.64 - 15.99 - 16.06 - 16.71 - 17.09 - 17.15 - 18.32 (Me Hyiv & Me Lac) 29.78 - 29.98 - 30.02 (CH i-propyl) 35.94 -35.99 - 36.26 - 36.55 - 36.83 - 37.09 (CH_2 Phe) 54.06 - 55.11- 55.44 - 55.49 - 56.26 (N-CH) 67.66 (O-CH_2) 69.38 - 70.76 - 70.89 (O-CH Lac) 78.43 (O-CH Hyiv) 126.72 - 127.03 - 127.18 - 127.23 - 127.42 - 128.28 - 128.39 - 128.48 - 128.61 - 128.64 - 128.70 128.74 - 128.80 - 128.90 - 129.24 - 129.27 - 129.30 (CH phenyl) 135.32 - 135.85 - 135.95 - 136.14 - 136.60 - 137.15 (C phenyl) 157.00 (C=O carbamate) 169.85 - 170.49 - 170.84 - 170.90 - 171.03 171.21 - 171.27 - 171.63 - 171.85 - 171.99 - 172.19 (C=O).

Benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α hydroxyisovaleryl L-phenylalanyl D-lactic acid pentafluorophenyl ester (17).

A solution of benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactic acid (16) (220mg, 0.14mmol), pentafluorophenol (263mg, 1.43mmol) and dimethylaminopyridine (17mg, 0.14mmol) in dichloromethane (5ml) was treated with dicyclohexylcarbodiimide (32mg, 0.15mmol) at 0°C. The mixture was stirred for a further 17h. The solvent was removed and the residue was purified by chromatography on silica gel (light petroleum : ether, 1 : 2) to yield the title compound (17) (160mg, 65%) as an oil. $[\alpha]_D$ -21 (c 1.8 chloroform). IR ($CHCl_3$) (cm^{-1}) 1540 s (N-H bend) 1670 s (C=O stretch) 1720 m (C=O stretch) 1755 s (C=O stretch) 1800 w (C=O stretch) 3340 m (N-H stretch) 3440 w (N-H stretch). δH (270MHz, solvent $CDCl_3$, standard Me_4Si) 0.37 (3H, d, J 7Hz, Me Hyiv) 0.4-0.5 (9H, c, Me Hyiv) 0.57 (3H, d, J 7Hz, Me Hyiv) 0.65 (3H, d, J 7Hz, Me Hyiv) 1.07 (3H, d, J 7Hz, Me Lac) 1.16 (3H, d, J 7Hz, Me Lac) 1.62 (3H, d, J 7Hz, Me Lac) 1.95-2.1 (3H, c, CH i-propyl) 3.0-3.3 (12H, c, CH_2 Phe) 4.21 (1H, m, N-CH) 4.35-4.5 (4H, c, N-CH) 4.74 (1H, m, N-CH) 4.83 (1H, d, J 3.5Hz, O-CH Hyiv) 4.91 (1H, d, J 3.5Hz, O-CH Hyiv) 4.95-5.2 (5H, c, O-CH Hyiv, O-CH Lac & O-CH_2) 5.26 (1H, q, J 7Hz, O-CH Lac) 5.67 (1H, d, J 6Hz, NH carbamate) 7.1-7.4 (35H, c, C_6H_5) 7.71 (1H, d, J 6Hz, NH amide) 7.85-7.95 (2H, c, NH amide) 8.05 (1H, d, J 5Hz, NH amide) 8.15 (1H, d, J 6.5Hz, NH amide). δC (68MHz, solvent $CDCl_3$, standard Me_4Si) 15.90 - 16.03 - 16.55 - 16.88 - 17.08 - 18.35 - 18.38 - 18.41 (Me Hyiv & Me Lac) 29.78 - 29.88 - 30.04 (CH i-propyl) 36.04 - 36.29 - 36.60 - 36.68 - 36.72 - 37.21 (CH_2 Phe) 54.03 - 55.05 - 55.54 - 55.83 - 55.98 - 56.37 (N-CH) 67.68 (O-CH_2) 68.77 - 70.27 - 70.51 (O-CH Lac) 78.10 - 78.63 (O-CH Hyiv) 126.74 - 127.03 - 127.14 - 127.21 - 127.56 - 128.16 - 128.45 - 128.48 - 128.60 - 128.70 - 128.80 - 129.01 - 129.24 - 129.33 (CH phenyl) 135.15 - 135.82 - 136.17 - 136.33 - 136.41 - 136.74 - 137.15 (C phenyl) 156.83 (C=O carbamate) 166.88 - 169.88 - 170.06 - 170.18 - 170.58 - 170.85 - 170.93 - 171.00 - 171.78 - 171.80 - 172.09 (C=O).

Cyclo D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl (2).

A solution of benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-actyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactic acid pentafluorophenyl ester (17) (91mg, 53μmol) in 1,4-dioxan (20ml) was injected continuously over a period of 24h into a well stirred mixture of dimethylaminopyridine (6.5mg, 53μmol), palladium on charcoal (5%) (1.0g), absolute ethanol (5ml) and 1,4-dioxan (250ml) at 90°C, whilst passing hydrogen through the solution. The mixture was filtered, the solvent was removed and the residue chromatographed on silica gel (light petroleum : ether, 3 : 2) to give the title compound (2) (62mg, 84%) as an oil. $[\alpha]_D$ -79 (c 2.4 chloroform). δH (270MHz, solvent $CDCl_3$, standard Me_4Si) 0.37 (9H, d, J 7Hz, Me Hyiv) 0.47 (9H, d, J 7Hz, Me Hyiv) 1.38 (9H, d, J 7Hz, Me Lac) 1.97 (3H, c, CH i-propyl) 3.1-3.3 (12H, c, CH_2) 4.53 (3H, m, N-CH) 4.69 (1H, m, N-CH) 4.73 (3H, d, J 3.5Hz, O-CH Hyiv) 5.24 (3H, q, J 7Hz, O-CH Lac) 7.15-7.3 (30H, c, C_6H_5) 7.98 (3H, d, J 7Hz, NH) 8.00 (3H, d, J 9.5Hz, NH). δC (68MHz, solvent $CDCl_3$, standard Me_4Si) 16.25 - 16.94 - 18.26 (Me Hyiv & Me Lac) 30.05 (CH i-propyl) 35.84 36.35 (CH_2 Phe) 53.91 - 55.72 (N-CH) 70.83 (O-CH Lac) 79.25 (O-CH Hyiv) 126.91 - 127.21 - 128.61 - 128.85 - 129.30 - 129.39 (CH phenyl) 136.05 - 136.70 (C phenyl) 170.08 - 170.70 - 171.79 - 172.19 (C=O). Found M^+, 1398 FAB.$C_{78}H_{90}N_6O_{18}$ requires M^+, 1398.

O-Acetyl-N-benzyloxycarbonyl L-tyrosine (18).

A solution of N-benzyloxycarbonyl L-tyrosine (1.0g, 3.2mmol), acetic anhydride (0.42g, 4.1mmol) and dimethylaminopyridine (0.50g, 4.1mmol) in ethyl acetate was stirred at room temperature for 5h then washed with dilute hydrochloric acid (2x100ml), brine (2x100ml), dried (anhydrous Na_2SO_4) and the solvent removed to yield O-acetyl-N-benzyloxycarbonyl L-tyrosine (18) (1.19g, 100%). $[\alpha]_D$ +46 (c 1.73 chloroform) IR

($CHCl_3$) (cm^{-1}) 3450 w (N-H stretch) 1760 & 1735 s (C=O) 1510 m (N-H bend).δH (270MHz, solvent $CDCl_3$, standard Me_4Si) 2.26 (3H, s, $COCH_3$) 3.05 (1H, ABX, J 5 & 14Hz, CH_2 Tyr) 3.18 (1H, ABX, J 6 & 14Hz, CH_2 Tyr) 4.66 (1H, m, N-CH) 5.08 (2H, s, O-CH_2) 5.40 (1H, d, J 8Hz, NH) 6.97 (2H, d, J 8Hz, C_6H_4O) 7.13 (2H, d, J 8Hz, C_6H_4O) 7.31 (5H, c, C_6H_5). δC (68MHz, solvent $CDCl_3$, standard Me_4Si) 21.17 (CH_3 $COCH_3$) 37.09 (CH_2 Tyr) 54.63 (N-CH) 67.27 (O-CH_2) 121.76 - 130.47 (CH Tyr) 128.21 - 128.31 - 128.60 (CH phenyl) 133.37 - 149.83 (C Tyr) 136.13 (C phenyl) 156.04 (C=O carbamate) 169.77(C=O COCH3) 175.72 (C=O).$C_{19}H_{19}NO_6$ requires M^+ 357, CI (ammonia) (M & $NH_4)^+$ 375; 100%.

O-Acetyl-N-benzyloxycarbonyl L-tyrosyl D-lactic acid t-butyl ester (19).

A solution of O-acetyl-N-benzyloxycarbonyl L-tyrosine (18) (1.99g, 5.6mmol), D-lactic acid t-butyl ester (1.00g, 6.8mmol) and dimethylaminopyridine (0.08g, 0.6mmol) in dichloromethane (30ml) was cooled to -40°C and dicyclohexylcarbodiimide (1.15g, 5.6mmol) in dichloromethane (3ml) was added. The mixture was stirred for 18h, and the precipitate of urea was filtered off. The solvent was removed and the residue was purified by chromatography on silica gel (light petroleum ether : diethyl ether, 2-1) to yield O-acetyl-N-benzyloxycarbonyl L-tyrosyl D-lactic acid t-butyl ester (19) (2.01g, 74%) as an oil. IR ($CHCl_3$) (cm^{-1}) 3450 w (N-H stretch) 3000 w (C-H stretch) 1740 s (C=O) 1510 m (N-H bend). δH (270MHz, solvent $CDCl_3$, standard Me_4Si) 1.40 (3H, d, J 7Hz, CH_3 Lac) 1.44 (9H, s, $(CH_3)_3$ t-butyl) 2.27 (3H, s, $COCH_3$) 3.0-3.1 (2H, m, CH_2 Tyr) 4.71 (1H, m, N-CH) 4.91 (1H, q, J 7Hz, O-CH) 5.08 (2H, m, O-CH_2) 5.34 (1H, d, J 8Hz, NH) 6.99 (2H, d, J 8.5Hz, C_6H_4O) 7.14 (2H, d, J 8.5Hz, C_6H_4O) 7.33 (5H, c, C_6H_5). δC (68MHz, solvent $CDCl_3$, standard Me_4Si) 16.82 (CH_3 Lac) 21.18 (CH_3 $COCH_3$) 27.94 (CH_3 t-butyl) 37.61 (CH_2 Tyr) 54.83 (N-CH) 66.99 (O-CH_2) 70.11 (O-CH) 82.38 (C t-butyl) 121.67 -130.40 (CH Tyr) 128.15 - 128.21 - 128.55 (CH phenyl) 133.48 - 149.80 (C Tyr) 136.38 (C phenyl) 155.56 (C=O carbamate) 169.20 - 169.44 - 170.80 (C=O). $C_{26}H_{31}NO_8$ requires M^+ 485 CI+ (ammonia) (M & NH4$)^+$ 503; 100%

O-Acetyl L-tyrosyl D-lactic acid t-butyl ester (20).

A mixture of palladium on charcoal (10%) (0.5g) and O-acetyl-N-benzyloxycarbonyl L-tyrosyl lactic acid t-butyl ester (19) (1.70g, 3.5mmol) in ethyl acetate (15ml) was hydrogenated for 4.5h. The catalyst was filtered off and the solvent was removed to afford as an oil O-acetyl L-tyrosyl D-lactic acid t-butyl ester (20) (1.22g, 99%). δH (270MHz, solvent $CDCl_3$, standard Me_4Si) 1.41 (3H, d, J 7Hz, CH_3 Lac) 1.46 (9H, s, $(CH_3)_3$ t.butyl) 2.29 (3H, s, $COCH_3$) 2.57 (2H, br, NH_2) 2.94 (1H, ABX, J 6 & 13.5Hz, CH_2 Tyr) 3.11 (1H, ABX, J 7.5 & 13.5Hz, CH_2 Tyr) 3.81 (1H, dd, J 6 & 7.5Hz, N-CH) 4.93 (1H, q, J 7Hz, O-CH) 7.02 (2H, d, J 8.5Hz, C_6H_4O) 7.24 (2H, d, J 8.5Hz C_6H_4O). δC (68MHz, solvent $CDCl_3$, standard Me_4Si) 16.89 (CH_3 Lac) 21.21 (CH_3 $COCH_3$) 28.01 (CH_3 t-butyl) 39.92 (CH_2 Tyr) 55.57 (N-CH) 69.56 (O-CH) 82.33 (C t-butyl) 121.73 - 130.45 (CH Tyr) 134.73 - 149.63 (C Tyr) 169.64 - 169.72 173.89 (C=O).

Benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleryl-O-Acetyl L-tyrosyl D-lactic acid t-butyl ester (21).

Dicyclohexylcarbodiimide (1.08g, 5.2mmol) in dichloromethane (5ml) was added to a solution of benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleric acid (8) (2.08g, 5.2mmol), O-acetyl L-tyrosyl lactic acid t-butyl ester (20) (1.31g, 3.7mmol) and dimethylaminopyridine (0.06g, 0.5mmol) in dichloromethane (20ml) at -40°C. The mixture was stirred for 17h, the dicyclohexylurea was filtered off, the solvent removed and the residue was chromatographed on silica gel (light petroleum ether-diethyl ether, 1-2) to give benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleryl O-acetyl L-tyrosyl lactic acid t-butyl ester (21)

(2.08g, 76%) as an oil. $[\alpha]_D$ -4 (c 7.60 chloroform). IR (CHCl$_3$) (cm^{-1}) 3450 & 3340 w (N-H stretch) 2990 & 2975 w (C-H stretch) 1745, 1725 & 1690 s (C=O stretch) 1510 s (N-H bend). δH (270MHz, solvent CDCl$_3$, standard Me$_4$Si) 0.49 (3H, d, J 6.5Hz, CH$_3$ Hyiv) 0.51 (3H, d, J 6.5Hz, CH$_3$ Hyiv) 1.39 (3H, d, J 7Hz, CH$_3$ Lac) 1.41 (9H, s, (CH$_3$)$_3$ t-butyl) 2.08 (1H, m, CH i-propyl) 2.22 (3H, s, COCH$_3$) 3.0-3.25 (4H, c, CH$_2$ Phe & Tyr) 4.49 (1H, m, N-CH Phe) 4.75-5.0 (3H, c, N-CH Tyr, O-CH Hyiv & O-CH Lac) 5.03 & 5.18 (2H, AB, J 12Hz, O-CH$_2$) 5.76 (1H, m, NH carbamate) 6.96 (2H, d, J 8.5Hz, C$_6$H$_4$O) 7.16 (1H, d, J 6.5Hz, NH amide) 7.18 (2H, d, J 8.5Hz, C$_6$H$_4$O) 7.2-7.4 (10H, c, C$_6$H$_5$). δC (68MHz, solvent CDCl$_3$, standard Me$_4$Si) 15.87 - 16.58 - 18.25 (CH$_3$ Hyiv & Lac) 21.00 (CH$_3$ COCH$_3$) 27.79 (CH$_3$ t-butyl) 29.84 (CH i-propyl) 36.20 - 37.19 (CH$_2$ Phe & Tyr) 53.47 - 55.98 (N-CH) 67.30 (O-CH$_2$) 69.86 (O-CH Lac) 78.57 (O-CH Hyiv) 82.03 (C t-butyl) 121.48 - 130.18 (CH Tyr) 128.05 - 128.16 - 128.39 - 128.45 - 128.68 - 129.10 (CH phenyl) 134.21 - 149.49 (C Tyr) 135.35 - 135.95 (C phenyl) 156.37 (C=O carbamate) 169.31 - 169.42 - 170.38 171.40 (C=O). C$_{40}$H$_{48}$N$_2$O$_{11}$ requires M$^+$ 732, FAB (M & H)$^+$ 733; 3%.

D-Phenylalanyl L-α-hydroxyisovaleryl O-Acetyl L-tyrosyl D-lactic acid t-butyl ester (22).

To a solution of benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleryl O-acetyl-L-tyrosyl D-lactic acid t-butyl ester (21) (210mg, 0.29mmol) in ethyl acetate (20ml) was added palladium on charcoal (10%) (100mg) and acetic acid (0.04g, 0.66mmol). The mixture was hydrogenated for 13h. Filtration and evaporation of solvent under reduced pressure yielded D-phenylalanyl L-α-hydroxyisovaleryl O-acetyl L-tyrosyl D-lactic acid t-butyl ester (22) (160mg, 93%) as an oil. Traces of D-phenylalanyl L-α-hydroxyisovaleryl L-tyrosyl D-lactic acid t-butyl ester in which the acetyl protecting group was no longer in place were also observed. δH (270MHz, solvent CDCl$_3$, standard Me$_4$Si) 0.80 (3H, d, J 7Hz, CH$_3$ Hyiv) 0.82 (3H, d, J 7Hz, CH$_3$ Hyiv) 1.42 (3H, d, J 7Hz, CH$_3$ Lac) 1.46 (9H, s, (CH$_3$)$_3$ t-butyl) 2.14 (1H, m, CH i-propyl) 2.25 (3H, s, COCH$_3$) 2.85-3.2 (4H, c, CH$_2$ Phe & Tyr) 3.32 (2H, br, NH$_2$) 3.74 (1H, m, N-CH) 4.9-5.0 (3H, c, N-CH, O-CH Hyiv & O-CH Lac) 6.72 (2H, d, J 8Hz, C$_6$H$_4$O) 6.99 (2H, d, J 8Hz, C$_6$H$_4$O) 7.05 (1H, d, J 8Hz, NH amide) 7.15-7.3 (5H, c, C$_6$H$_5$). δC (68MHz, solvent CDCl$_3$, standard Me$_4$Si) 16.79 - 16.85 - 18.51 (CH$_3$ Hyiv & Lac) 21.01 (CH$_3$ COCH$_3$) 27.81 (CH$_3$ t-butyl) 30.15 (CH i-propyl) 36.69 - 40.35 (CH$_2$ Phe & Tyr) 52.47 - 55.58 (N-CH) 69.98 (O-CH Lac) 78.33 (O-CH Hyiv) 82.49 (C t-butyl) 121.55 - 130.35 (CH Tyr) 126.84 - 128.58 - 129.19 (CH phenyl) 133.59 - 149.62 (C Tyr) 136.82 (C phenyl) 169.16 - 169.33 - 169.56 - 170.48 - 173.79 (C=O).

Benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L α-hydroxyisovaleryl O-acetyl-L-tyrosyl D-lactic acid t-butyl ester (23).

Dicyclohexylcarbodiimide (140mg, 0.68mmol) was added to a solution of benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactic acid (14) (710mg, 0.65mmol), and D-phenylalanyl L-α-hydroxyisovaleryl O-acetyl-L-tyrosyl D-lactic acid t-butyl ester (22) (300mg, 0.50mmol) and dimethylaminopyridine (80mg, 0.65mmol) in dichloromethane (10ml) at -40°C. The mixture was stirred for 3 days, the urea precipitate was filtered off and the solvent removed under reduced pressure. The residue was purified by chromatography on silica gel (light petroleum ether- diethyl ether, 1-2) to give benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl O-acetyl-L-tyrosyl D-lactic acid t-butyl ester (23) (650mg, 78%) as an oil. $[\alpha]_D$ -12 (c 1.79 chloroform). IR (CHCl$_3$) (cm^{-1}) 3540 & 3340 m (N-H stretch) 2990 & 2975 w (C-H stretch) 1760

& 1670 s (C=O) 1550 & 1525 m (N-H bend). δH (270MHz, solvent $CDCl_3$, standard Me_4Si) 0.40 (3H, d, J 7Hz, CH_3 Hyiv) 0.45-0.5 (9H, c, CH_3 Hyiv) 0.55 (6H, c, CH_3 Hyiv) 1.09 (3H, d, J 7Hz, CH_3 Lac) 1.18 (3H, d, J 7Hz, CH_3 Lac) 1.39 (3H, d, J 7Hz, CH_3 Lac) 1.43 (9H, s, $(CH_3)_3$ t-butyl) 2.05 (3H, c, CH i-propyl) 2.23 (3H, s, $COCH_3$) 3.0-3.4 (12H, c, CH_2 Phe & Tyr) 4.3-5.2 (14H, c, N-CH, O-CH Hyiv, O-CH Lac & O-CH_2) 5.94 (1H, d, J 7Hz, NH carbamate) 6.72 (2H, d, J 8Hz, C_6H_4O) 6.98 (2H, d, J 8Hz, C_6H_4O) 7.0-7.4 (30H, c, C_6H_5) 7.73 (2H, c, NH amide) 7.82 (1H, d, J 6Hz, NH amide) 7.88 (1H, d, J 6Hz, NH amide) 8.03 (1H, d, J 6Hz, NH amide). δC (68MHz, solvent $CDCl_3$, standard Me_4Si) 15.93 - 16.02 - 16.62 - 16.75 - 16.82 - 16.89 - 17.07 18.30 - 18.35 (CH_3 Hyiv & Lac) 21.13 (CH_3 $COCH_3$) 27.89 (CH3 t-butyl) 29.71 - 29.85 - 29.98 (CH i-propyl) 35.97 - 36.07 - 36.29 - 36.62 - 36.71 - 37.09 (CH_2 Phe & Tyr) 53.72 - 55.02 - 55.32 - 55.50 - 55.83 - 56.33 (N-CH) 67.52 (O-CH_2) 69.92 - 70.34 - 70.50 (O-CH Lac) 78.07 - 78.52 78.66 (O-CH Hyiv) 82.18 (C t-butyl) 121.40 - 130.27 (CH Tyr) 126.97 - 127.06 - 127.13 - 127.44 - 128.09 - 128.18 - 128.39 128.42 - 128.55 - 128.62 - 128.81 - 128.90 - 129.19 (CH phenyl) 134.87 - 149.40 (C Tyr) 135.19 - 135.82 - 136.10 - 136.30 - 136.42 - 136.70 (C phenyl) 156.82 (C=O carbamate) 169.52 - 169.57 169.65 - 169.74 - 169.83 - 169.93 - 170.52 - 170.74 - 170.87- 171.10 - 171.73 - 171.89 (C=O).

Benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-tyrosyl D-lactic acid t-butyl ester (24).

Benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl O-acetyl-L-tyrosyl D-lactic acid t-butyl ester (23) (100mg, 60μmol) and imidazole (0.5g, 7.34mmol) were dissolved in a solution of acetonitrile (4ml) and water (1ml) and stirred for 21h at room temperature. Distilled water (15ml) was added and the solution extracted with diethyl ether (3x20ml). The organic layer was then washed with dilute hydrochloric acid (2x10ml) and brine (10ml), and the solvent removed under reduced pressure. The residue was purified by chromatography on silica gel (light petroleum ether-diethyl ether, 1-2) to yield benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-tyrosyl D-lactic acid t-butyl ester (24) (68mg, 70%). $[\alpha]_D$ -11 (c 1.92 chloroform). IR ($CHCl_3$) (cm^{-1}) 3340 m (N-H stretch) 2990 & 2975 w (C-H stretch) 1760 & 1670 s (C=O) 1550 & 1525 m (N-H bend).δH (270MHz, solvent $CDCl_3$, standard Me_4Si) 0.45-0.50 (9H, c, CH_3 Hyiv) 0.55 (6H, d, J 7Hz, CH_3 Hyiv) 0.60 (3H, d, J 7Hz, CH_3 Hyiv) 1.08 (3H, d, J 7Hz, CH_3 Lac) 1.18 (3H, d, J 7Hz, CH_3 Lac) 1.40 (3H, d, J 7Hz, CH_3 Lac) 1.43 (9H, s, $(CH_3)_3$ t-butyl) 2.06 (3H, c, CH i-propyl) 3.0-3.2 (12H, c, CH_2 Phe & Tyr) 4.3-4.8 (6H, c, N-CH) 4.85-5.2 (8H, c, O-CH Hyiv, O-CH Lac & O-CH_2) 5.89 (1H, d, NH carbamate) 6.70 (2H, d, J 8Hz, C_6H_4O) 7.04 (2H, d, J 8Hz, C_6H_4O) 7.15-7.35 (30H, c, C_6H_5) 7.62 (1H, d, J 7.5Hz NH amide) 7.79 (1H, d, J 5.5Hz NH amide) 7.86 (1H, d, J 5.5Hz NH amide) 7.82 (1H, d, J 5Hz NH amide) 8.09 (1H, d, J 5.5Hz NH amide). δC (68MHz, solvent $CDCl_3$, standard Me_4Si) 15.94 - 16.29 - 16.75 - 16.92 - 17.07 - 18.32 - 18.39 (CH_3 Hyiv & Lac) 27.91 (CH_3 t-butyl) 29.72 - 29.82 - 29.97 (CH i-propyl) 36.00 - 36.09 - 36.30 - 36.63 - 36.85 - 37.11 (CH_2 Phe & Tyr) 53.98 - 54.92 - 55.06 - 55.39 - 55.46 - 56.34 (N-CH) 67.56 (O-CH_2) 69.95 - 70.60 (O-CH Lac) 78.27 - 78.60 - 78.71 (O-CH Hyiv) 82.09 (C t-butyl) 115.44 - 130.31 (CH Tyr) 126.97 - 127.08 - 127.17 - 127.46 - 128.12 - 128.42 - 128.60 - 128.64 - 128.74 - 128.91 - 129.20 - 129.29 (CH phenyl) 127.99 - 155.42 (C Tyr) 135.18 - 135.81 - 136.03 - 136.27 - 136.37 - 136.63 (C phenyl) 156.84 (C=O carbamate) 169.70 - 169.73 - 169.95 - 170.02 - 170.64 - 170.77 - 170.80 - 170.87 - 171.33 - 171.81 - 172.18 (C=O). $C_{90}H_{106}N_6O_{22}$ requires M^+ 1623. FAB $(M \& H)^+$ 1624; 1%

Benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-tyrosyl D-lactic acid (25).

A solution of benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α–hydroxyisovalyl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-tyrosyl D-lactic acid t-butyl ester (24) (143mg, 86μmol) in dichloromethane (3ml) and trifluoroacetic acid (3ml) was kept at room temperature for 7h. Evaporation of solvent under reduced pressure gave benzyloxycarbonyl D-phenylalanyl L–α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-tyrosyl D-lactic acid (25) (134mg, 100%) as an oil. IR ($CHCl_3$) (cm^{-1}) 3350 m (N-H stretch) 2990 & 2975 w (C-H stretch) 1760 & 1670 s (C=O) 1550 & 1525 m (N-H bend). δH (270MHz, solvent $CDCl_3$, standard Me_4Si) 0.40-0.60 (18H, c, CH_3 Hyiv) 1.15 (3H, d, J 7Hz, CH_3 Lac) 1.18 (3H, d, J 7Hz, CH_3 Lac) 1.43 (3H, d, J 7Hz, CH_3 Lac) 2.06 (3H, c, CH i-propyl) 3.0-3.25 (12H, c, CH_2 Phe & Tyr) 4.4-4.8 (6H, c, N-CH) 4.85-5.2 (8H, c, O-CH Hyiv, O-CH Lac & O-CH_2) 5.87 (1H, d, NH carbamate) 6.71 (2H, d, J 8Hz, C_6H_4O) 7.02 (2H, d, J 8Hz, C_6H_4O) 7.2-7.5 (30H, c, C_6H_5) 7.66 (1H, d, J 7Hz NH amide) 7.74 (1H, d, J 7Hz NH amide) 7.77 (1H, d, J 7Hz NH amide) 7.79 (1H, d, J 6.5Hz NH amide) 8.00 (1H, d, J 7Hz NH amide). δC (68MHz, solvent $CDCl_3$, standard Me_4Si) 15.97 - 16.07 - 16.16 - 16.78 - 16.96 - 17.11 18.38 (CH_3 Hyiv & Lac) 29.82 - 29.90 - 30.04 (CH i-propyl) 36.00 - 36.09 - 36.35 - 36.62 - 36.91 - 37.22 (CH_2 Phe & Tyr) 53.84 - 54.43 - 55.06 - 55.34 - 55.50 - 56.34 (N-CH) 67.71 (O-CH_2) 69.58 - 70.93 - 71.13 (O-CH Lac) 78.59 - 78.69 - 79.24 (O-CH Hyiv) 115.59 - 130.54 (CH Tyr) 127.13 - 127.21 - 127.29 - 127.36 - 127.52 - 128.25 - 128.58 - 128.70 - 128.74- 128.83 - 128.88 - 128.98 - 129.03 - 129.30 (CH phenyl) 127.46 - 155.19 (C Tyr) 135.43 - 135.85 - 135.91 - 136.18 - 136.49 136.99 (C phenyl) 157.04 (C=O carbamate) 170.31 - 170.39 - 170.47 170.81 - 170.96 - 171.09 - 171.13 - 171.27 - 171.92 - 172.05 172.15 - 172.21 (C=O). $C_{86}H_{98}N_6O_{22}$ requires M^+ 1567, FAB $(M \& H)^+$ 1568; 1% $(M \& Na)^+$ 1590; 2%

Benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-tyrosyl D-lactic acid pentafluorophenyl ester (26).

A solution of benzyloxycarbonyl D-phenylalanyl L–α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-tyrosyl D-lactic acid (25) (473mg, 302μmol), pentafluorophenol (555mg, 3.0mmol) and dimethylaminopyridine (37mg, 302μmol) in dichloromethane (5ml) was treated with dicyclohexylcarbodiimide (93mg, 453μmol) at 0°C. The mixture was stirred for 18.5h. The solvent was removed and the residue was purified by chromatography on silica gel (light petroleum ether-diethyl ether, 1-1) to give benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-tyrosyl D-lactic acid pentafluorophenyl ester (26) (230mg, 44%) as an oil. $[\alpha]_D$ -20 (c 0.4 chloroform). IR ($CHCl_3$) (cm^{-1}) 3430 & 3330 m (N-H stretch) 1760 & 1670 s (C=O) 1525 m (N-H bend) δH (270MHz, solvent $CDCl_3$, standard Me_4Si) 0.40-0.50 (9H, c, CH_3 Hyiv) 0.56 (6H, d, J 7Hz, CH_3 Hyiv) 0.61 (3H, d, J 7Hz, CH_3 Hyiv) 1.06 (3H, d, J 7Hz, CH_3 Lac) 1.18 (3H, d, J 7Hz, CH_3 Lac) 1.65 (3H, d, J 7Hz, CH_3 Lac) 2.06 (3H, c, CH i-propyl) 3.0-3.25 (12H, c, CH2 Phe & Tyr) 4.29 (1H, m, N-CH) 4.4-4.5 (4H, c, N-CH) 4.55 (1H, m, N-CH) 4.85 (1H, d, J 3.5Hz, O-CH Hyiv) 4.91 (1H, d, J 3.5Hz, O-CH Hyiv) 4.95 & 5.03 (2H, AB, J 12Hz, O-CH_2) 5.05-5.2 (3H, c, O-CH Hyiv & O-CH Lac) 5.28 (1H, q, J 7Hz, O-CH Lac) 5.69 (1H, d, J 5.5Hz, NH carbamate) 6.71 (2H, d, J 8Hz, C_6H_4O) 7.04 (2H, d, J 8Hz, C_6H_4O) 7.15-7.4 (30H, c, C_6H_5) 7.70 (1H, d, J 6.5Hz NH amide) 7.74 (1H, d, J 6Hz NH amide) 7.87 (1H, d, J 5.5Hz NH amide) 7.92 (1H, d, J 5.5Hz NH amide) 8.11 (1H, d, J 5Hz NH amide). δC (68MHz, solvent $CDCl_3$, standard Me_4Si) 15.97 - 16.12 - 16.65 - 16.86 - 17.05 - 18.34 (CH_3 Hyiv & Lac) 29.78 - 29.87 - 29.97 (CH i-propyl) 35.69 - 36.01 - 36.24 - 36.62 - 36.75 - 37.15 (CH_2 Phe & Tyr) 54.29 - 55.03 - 55.48 - 55.60 - 55.70 - 56.37 (N-CH) 67.62 (O-CH2) 68.73 - 70.43 - 70.51 (O-CH Lac) 78.28 - 78.63 (O-CH Hyiv) 115.45 - 130.34 (CH Tyr) 127.01 - 127.13 - 127.23 - 127.51 - 128.12 - 128.44 - 128.58 - 128.67 - 128.80 - 128.90 - 128.97 - 129.09 - 129.23 - 129.30 (CH phenyl) 155.48 (C Tyr) 135.18 - 135.85 - 136.07 - 136.30 - 136.66 (C phenyl) 156.86 (C=O carbamate) 166.87 - 169.75 - 169.89 - 170.06 - 170.14 - 170.85 - 170.97 - 171.50 - 171.89 - 172.17 (C=O). $C_{92}H_{97}N_6O_{22}F_5$ requires M^+ 1733, FAB $(M \& H)^+$ 1734; 1% $(M \& Na)^+$ 1756; 1%

Cyclo D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-tyrosyl D-lactyl (4).

A solution of benzyloxycarbonyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-tyrosyl D-lactic acid pentafluorophenyl ester (26) (80mg,·43μmol), in 1,4-dioxan (25ml) was injected continuously over a period of 10h into a well stirred mixture of dimethylaminopyridine (6mg,·49μmol), palladium on charcoal (5%)·(1.0g), absolute ethanol (3ml) and 1,4-dioxane (100ml) at 90°C, whilst passing hydrogen through the solution. The reaction mixture was then stirred overnight under nitrogen, filtered, the solvent removed and the residue chromatographed on silica gel (light petroleum ether·: diethyl ether, 1-1). Cyclo [D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-tyrosyl D-lactyl] (4) (39mg, ·58%) was isolated as an oil. $[\alpha]_D$ -67° (c 0.6 chloroform). IR ($CHCl_3$) (cm^{-1}) 3600 w (free O-H stretch) 3420 & 3325 m (N-H stretch) 1740 & 1655 s (C=O) 1535 & 1515 s (N-H bend). δH (270MHz, solvent $CDCl_3$, standard Me_4Si) 0.37 (9H, c, CH_3 Hyiv) 0.47 (9H, c, CH_3 Hyiv) 1.37 (9H, d, J 7Hz, CH_3 Lac) 1.95 (3H, c, CH i-propyl) 3.0-3.3 (12H, c, CH_2 Phe & Tyr) 4.53 (3H, c, N-CH) 4.67 (3H, c, N-CH) 4.73 (3H, d, J 3.5Hz, O-CH Hyiv) 5.22 (3H, q, J 7Hz, O-CH Lac) 6.74 (2H, d, J 8Hz, C_6H_4O) 7.12 (2H, d, J 8Hz, C_6H_4O) 7.2-7.4 (25H, c, C_6H_5) 7.95 (3H, d, J 7.5Hz NH amide) 7.97 (3H, d, J 9.5Hz NH amide). δC (68MHz, solvent $CDCl_3$, standard Me_4Si) 16.26 - 16.98 - 18.26 (CH_3 Hyiv & Lac) 30.06 (CH i-propyl) 35.84 - 36.40 (CH_2 Phe & Tyr) 53.97 - 55.68 (N-CH) 70.87 (O-CH Lac) 79.27 (O-CH Hyiv) 115.49 - 130.52 (CH Tyr) 126.92 - 127.23 - 128.62 - 128.85 - 129.29 - 129.37 (CH phenyl) 127.10 - 155.13 (C Tyr) 136.00 - 136.65 (C phenyl) 170.08 - 170.78 - 171.85 - 172.15 (C=O). $C_{78}H_{90}N_6O_{19}$ requires M^+ 1414, FAB $(M \& H)^+$ 1415; 20%, $(M \& H_2O)^+$ 1432; 20%. $(M \& Na)^+$ 1437; 15%.

Cyclo D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl O-acetyl L-tyrosyl D-lactyl (5).

A solution of cyclo·[D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-tyrosyl D-lactyl] (4) (25mg,·18μmol), acetic anhydride (2mg, 21μmol) and dimethylaminopyridine (3mg,·21μmol) in ethyl acetate (5ml) was stirred at room temperature for 2h then washed with dilute hydrochloric acid (2x2ml), brine (2x3ml), dried (benzene). The solvent removed and the residue chromatographed on silica gel (light petroleum ether-diethyl ether,1-1) to yield cyclo-[D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl L-phenylalanyl D-lactyl D-phenylalanyl L-α-hydroxyisovaleryl O-acetyl L-tyrosyl D-lactyl] (5) (12mg, 59%). δH (270MHz, solvent $CDCl_3$, standard Me_4Si) 0.37 (9H, d, J 7Hz, CH_3 Hyiv) 0.48 (9H, d, J 7Hz, CH_3 Hyiv) 1.40 (9H, d, J 7Hz, CH_3 Lac) 1.97 (3H, c, CH i-propyl) 2.27 (3H, s, $COCH_3$) 3.1-3.3 (12H, c, CH_2 Phe & Tyr) 4.54 (3H, c, N-CH) 4.68 (3H, c, N-CH) 4.73 (3H, d, J 3.5Hz, O-CH Hyiv) 5.22 (3H, c, O-CH Lac) 7.00 (2H, d, J 8Hz, C_6H_4O) 7.2-7.4 (27H, c, C_6H_4O & C_6H_5) 7.98 (3H, d, J 8Hz NH amide) 8.02 (3H, d, J 9Hz NH amide). δC (68MHz, solvent $CDCl_3$, standard Me_4Si) 16.27 - 16.92 - 16.95 - 16.98 - 18.26 (CH_3 Hyiv & Lac) 21.27 (CH_3 $COCH_3$) 30.07 (CH i-propyl) 35.86 - 36.35 (CH_2 Phe & Tyr) 53.95 - 55.71 (N-CH) 70.82 - 70.99 (O-CH Lac) 79.31 (O-CH Hyiv) 121.76 - 130.41 (CH Tyr) 126.94 - 127.24 - 128.64 - 128.87 - 129.30 - 129.39 (CH phenyl) 134.33 - 149.69 (C Tyr) 136.03 - 136.66 (C phenyl) 169.60 (C=O acetoxy) 170.09 - 170.75 - 170.80 - 171.80 - 171.82 - 171.86 - 172.18 - 172.24 - 172.27 C=O). $C_{78}H_{90}N_6O_{19}$ requires M^+ 1457, FAB $(M \& H)^+$ 1458; 2% $(M \& H_2O)^+$ 1475; $(M \& Na)^+$ 1480; 2%

Acknowledgements

We thank Mrs. J. Street and Dr. G. J. Langley for recording nmr spectra and for collecting mass spectra respectively.

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(*Received in UK* 24 *August* 1995; *revised* 1 *November* 1995; *accepted* 2 *November* 1995)