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Applications of Pentafluorophenylester Coupling in the Synthesis of Cyclodepsipeptides Related to Valinomycins

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Abstract: A number of strategies of synthesis of cyclic depsipeptides are examined including cyclisation protocols based on pentafluorophenyl esters. The alternatives of amine protection as benzyloxycarbonyl or t-butoxycarbonyl derivatives are examined and optimum results are obtained by release of the amino functionality by in situ hydrogenolysis. The value of these protocols in relation to the ring size of the target peptide is studied.

The synthesis of cyclodepsipeptides provides routes to valinomycins, which are used in potassium ion selective electrodes[1], and to analogues of diverse biologically active peptides[2]. Such syntheses require the linear assembly of a depsipeptide via formation of ester and amide bonds. The subsequent cyclisation to afford the macrocycle is typically the most difficult synthetic step. As recently stated[3] 'Cyclisation is generally the limiting step in the synthesis of cyclic peptides.' If the linear assembly is via solid phase synthesis cyclisations have then been performed both by on resin cyclisations[4] and after resin cleavage[5].In the synthesis of valinomycins a number of different cyclisation strategies have been used with limited success. Although cyclodepsipeptides have been synthesized in solution via cyclisations[6] to make an ester bond, there has been a preference to form an amide bond in the cyclisation step. By choosing a mode of cyclisation with relatively minor steric hindrance around the centres of cyclisation, for example formation of the L-Lac-L-Val bond, the acid chloride methodology[7] permitted a 51% yield to be obtained. Related cyclisations[8] at more sterically demanding centres, for example the D-Hyiv-D-Val bond, proceeded in lower yields (about 20%). The phosphite mixed anhydride methodology gave valinomycin in 56% yield but was markedly less effective in sterically unfavourable cases. The adverse steric factors can arise either from steric hindrance about the reacting centres, or from a combination of units of unfavourable configuration in the linear depsipeptide. The alternation of centres of L and D configuration in valinomycin represents a particularly favourable situation for cyclisation. The length of the depsipeptide chain also affects the cyclisation. Smaller chains[9] are more difficult to cyclise; polymerisation can be competitive. There is therefore a need to develop better methods of cyclisation, which might be used, both in the construction of valinomycins, where the factors of chain length and relative configuration combine to offer the prospect of high yielding routes to macrocycles, and also in the construction of other cyclodepsipeptides presenting greater synthetic challenges. In this paper the application of the activated ester method of coupling, using pentafluorophenyl esters, is described. The method is tested with linear depsipeptides having highly

unfavourable length and sequence of absolute configuration. Two strategies using either protection of amines as benzyloxycarbonyl or t-butoxycarbonyl derivatives have been examined. The former is shown to be very satisfactory, and in the following papers[10] has been used in the synthesis of valinomycin analogues, thus permitting cyclisations in unprecedented high yield (up to 84%).

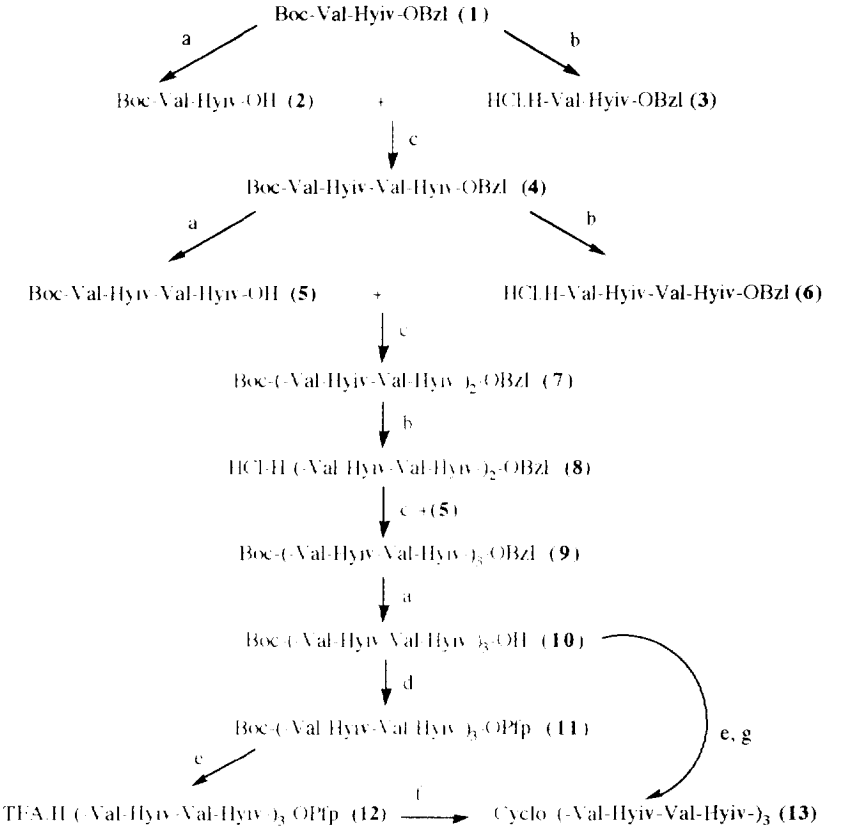
In the synthesis of cyclic peptides a number of different esters have been studied to permit carboxyl group activation in the formation of amide bonds. For example long reaction times are needed with nitrophenyl esters[11] and yields can be low. The group of Schmidt[12] has recently achieved considerable success in the use of pentafluorophenyl esters in the synthesis of cyclic peptides. Polymerisation has been minimized by release of the amino functionality by *in situ* hydrogenolysis. The appropriate protection to be used in such a hydrogenolysis is with the benzyloxycarbonyl group. Hence we have examined the role of this method of protection in the cyclisation of linear depsipeptides activated as the pentafluorophenyl esters. A series of linear depsipeptides, having terminal benzyloxycarbonyl protection have been studied. In order to assess the value of this procedure of *in situ* deprotection, we have studied a second mode of cyclisation. We have prepared via protection with the t-butoxycarbonyl group a related Boc-protected linear depsipeptide pentafluoroester, thus permitting a cyclisation to be studied in which the amine is first released by selective hydrolysis and then later cyclised. The advantage of the *in situ* procedure in the synthesis of complex depsipeptides is clearly demonstrated, and this strategy is further developed in the following paper in the synthesis of novel substituted valinomycins.

Three series of linear depsipeptides having benzyloxycarbonyl or t-butoxycarbonyl protection were chosen for study. A number of factors influenced the choice as substrates of the three series of peptides. The proposed strategies dictated the choice of the pentafluorophenyl ester as an activating group, and an N-terminal benzyloxycarbonyl or t-butoxycarbonyl group protection. The choice of the esters was made to permit the effect of the chain length of the peptide to be studied. Amino-acids and hydroxy-acids of the same configuration were chosen as this represents the more challenging problem. Cyclisations of peptide fragments with alternating configuration are known to be more easy. Acids having an L configuration were more readily available. By choosing valine and hydroxyisovaleric acid as the fragments particularly adverse steric interactions were expected, thus presenting a major test of the methodology.

The synthesis of the linear peptides was achieved without difficulty by solution coupling, as shown in Schemes 1 and 2. In Scheme 1 the series involving t-butoxycarbonyl protection was advanced from the known didepsipeptide fragment (1) obtained from L-valine and L-α-hydroxyisovaleric acid t-butyl ester[13]. Elaboration of this fragment (1) using t-butoxycarbonyl protection at nitrogen through the intermediates (2-9) afforded the linear dodecadepsipeptide (10) ready for possible conversion through intermediates (11) and (12) to the target cyclododecadepsipeptide (13). The alternative strategy for synthesis of the depsipeptide (13) is shown in Scheme 2, where elaboration of the didepsipeptide fragment (14) is based on benzyloxycarbonyl protection at nitrogen.

The investigation of the influence of chain length on the efficiency of cyclisation led to further cyclisations using benzyloxycarbonyl protection. These are also shown in Scheme 2. Thus elaboration through intermediates (15-23) afforded the linear pentafluorophenol esters (24-26), which were used in order to study the ease of cyclisation to give the tetradepsipeptide (27), the octadepsipeptide (28) and the hexadecadepsipeptide (29), in addition to the study of formation of the dodecadepsipeptide (13). In both series the elaboration of the didepsipeptide fragments proceeded without difficulty. It may be noted that to obtain the highest yields in steps involving benzyloxycarbonyl deprotection via hydrogenolysis required the direct bubbling of hydrogen into the reaction mixtures. The importance of this measure increased for peptides of higher molecular weight. In the coupling it was also observed that yields decreased slightly in the formation of the peptides of higher molecular

Scheme 1



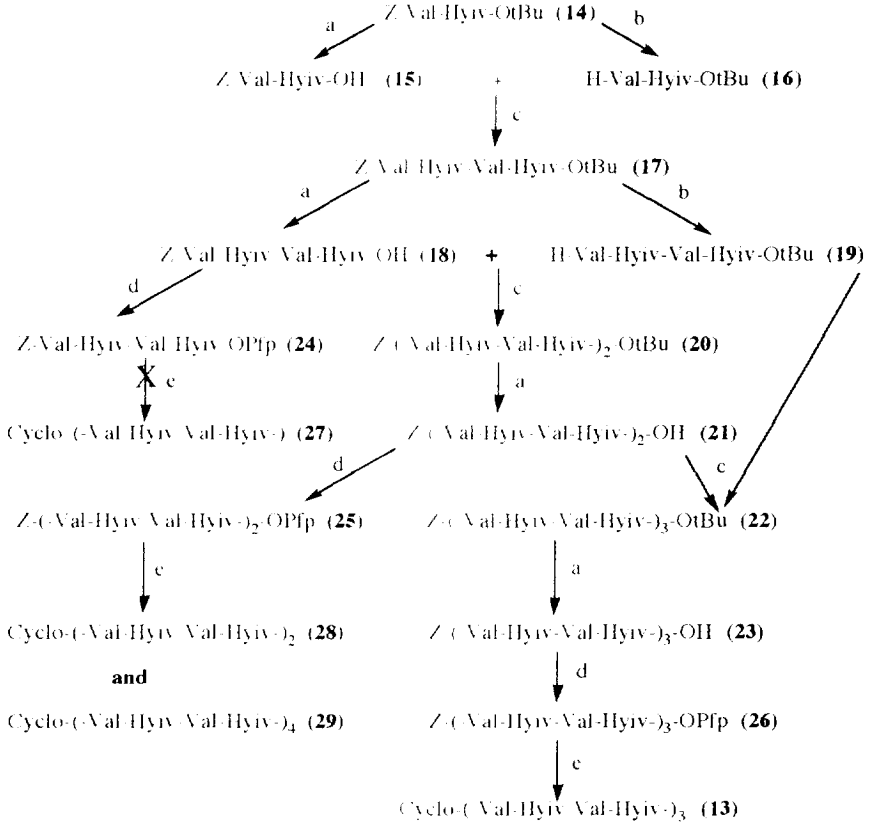
Reagents: a) H_2, Pd-C (10%), MeOH, r.t.; b) HCl, EtOAc, 0°C; c) DCC, DMAP, NMM, CH_2Cl_2, 0°C; d) PfPOH, DCC, DMAP, CH_2Cl_2, 0°C; e) TFA, CH_2Cl_2, r.t.; f) DMAP, dioxan, EtOH, 90°C; g) DPPA, NEt_3, DMF, -25°C

weight, and it was found that the presence of acid, as in the first series, improved the coupling efficiency. Related observations[14] have been made previously.

The first series of attempted cyclisations were made with the Boc-protected linear dodecadepsipeptide (10) using a variety of traditional methods. Hydrolysis of the Boc-protected peptide (10) and attempted cyclisation by reaction with thionyl chloride, a route via the acid chloride, known[7,15] to be effective for synthesis of valinomycins having a more favourable sequence of configurations, failed. Polymer formation was suspected. Similarly hydrolysis of peptide (10) and attempted DCC coupling failed. Application of the mixed anydride methodology using diphenylphosphoryl azide, as described in the experimental section, gave the desired cyclopeptide (13) in 3% yield. In comparison the activated ester route of cyclisation was explored via the pentafluorophenyl ester (11), and the product of selective hydrolysis, the aminoester (12). The cyclopeptide (13) could be prepared by this pentafluorophenyl ester coupling. However the yields were poor. Using very pure

dioxane, high dilution and long injection times (36 hours) a 14% yield was obtained. Therefore the use of pentafluorophenyl esters in the synthesis of cyclododecadepsipeptides of challenging configuration is an improvement on earlier methods, but in combination with Boc-protection, is an inefficient procedure.

Scheme 2



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; d) PfpOH, DCC, DMAP, CH_2Cl_2, 0°C, e) H_2, Pd/C DMAP, dioxan / ethanol, 90°C

The alternative of benzyloxycarbonyl protection permits the synthesis of these cyclodepsipeptides in much improved yield. By injection of the appropriate linear depsipeptide into a dioxane solution containing dimethylaminopyridine, into which hydrogen is bubbled, cyclodepsipeptides are obtained. In contrast to the 14% yield obtained from the aminoester (12), the cyclodepsipeptide (13) was isolated in 70% yield. Therefore this result shows that the methodology of Schmidt[12] is applicable to the synthesis of cyclodepsipeptides of favorable ring size, but adverse configuration, even where the coupling is likely to be hindered by adverse steric interactions. We have examined the effectiveness of the methodology in cases of both unfavourable ring size and adverse configuration. In the case of the linear tetradepsipeptide ester (24) these constraints are so severe that no cyclic product was obtained. In the case of the octadepsipeptide fragment (25) cyclisation afforded two

depsipeptides. The cyclohexadecadepsipeptide (29) was observed under all conditions to be the major product and the cyclooctadecadepsipeptide (28) was the minor product. In an injection of the ester (25) over 24 hours the peptides (28) and (29) were obtained in 4 and 26% yields respectively.

Our results establish that the technique of in situ deprotection of benzyloxycarbonyl derivatives to afford cyclodepsipeptides efficiently generates dodecadepsipeptides even in cases chosen to present unusually adverse factors. However under comparable conditions cyclotetradepsipeptides cannot be prepared. The relative inefficiency of prior deprotection of Boc-protected linear fragments has determined our approach to the synthesis of a series of novel valinomycins reported in the following paper. In view of our interest in the sensing applications of the peptides reported in this paper, the structural and electrochemical properties of the cyclic peptides and their metal ion complexes are discussed elsewhere.

Experimental

The solvents used were dried and purified using the procedures described by Perrin. Light petroleum ether refers to petroleum ether (bp. 40-60°C). Products were purified using flash column chromatography. Analytical thin layer chromatography was carried out on 0.25mm precoated silica gel plates (ALUGRAM SIL G/UV254). Melting points were determined with an Electrothermal melting point apparatus and are uncorrected. Optical rotations were measured at 25°C on an Optical Activity AA 100 polarimeter using a 5cm path length cell. Infrared spectra were recorded on a Perkin-Elmer 298 spectrometer in sodium chloride cells (thickness 0.1mm) in chloroform solutions. The relative degree of absorption is indicated (s:strong, m:medium, w:weak, br:broad). ^{1}H Nuclear magnetic resonance spectra were recorded at 270MHz on a Jeol JNM-GX270 spectrometer. The NMR spectra are reported as position (δH), relative integration, multiplicity (s:singlet, d:doublet, t:triplet, q:quartet, sept:septet, m:multiplet, c:complex, br:broad, AB:AB system, ABX:ABX system), coupling constant (J, Hz), and assignment of the protons. ^{13}C Nuclear magnetic resonance spectra were recorded at 68MHz on a Jeol JNM-GX270 and are reported as position (δC), level of protonation and assignment of the carbon signal. Mass spectra were recorded on a VG Analytical 70-250-SE mass spectrometer. The fast atom bombardment (FAB) spectra were recorded using metanitrobenzyl alcohol (MNBA) as a matrix. The chemical ionisation (CI^+) spectra were recorded using ammonia as the ionisation gas. The electron ionisation (EI+) spectra were recorded with an ionisation potential of 70eV. Elemental analyses were carried out at University College, London.

t-Butoxycarbonyl L-valyl L-α-hydroxyisovaleric acid benzyl ester (1)

To a cold solution of t-butoxycarbonyl L-valine (10.50g, 48.4mmol), L-α-hydroxyisovaleric acid benzyl ester (9.50g, 45.7mmol) and dimethylaminopyridine (1.05g, 8.5mmol) in dichloromethane (50ml), was added dicyclohexylcarbodiimide (9.97g, 48.4mmol). The reaction mixture was stirred for 4h at room temperature, the urea precipitate was filtered off and solvent was evaporated in vacuo. The residue was chromatographed on silica gel (light petroleum : ether, 3:2) to give the title compound (1) (16.00g, 86%) as an oil. $[\alpha]_D$ -24 (c 1.0 chloroform). IR ($CHCl_3$) (cm^{-1}) 1520 s (N-H bend) 1735 s (C=O stretch) 3450 m (N-H stretch). δH (360MHz, solvent $CDCl_3$, standard Me_4Si) 0.90 (3H, d, J 7Hz, Me i-propyl) 0.94 (3H, d, J 7Hz, Me i-propyl) 0.99 (6H, d, J 7Hz, Me i-propyl) 1.44 (9H, s, t-butyl) 2.2-2.35 (2H, c, CH i-propyl) 4.34 (1H, dd, J 4 & 9Hz, N-CH) 4.91 (1H, d, J 4.5Hz, O-CH) 5.08 (1H, d, J 9Hz, NH) 5.15 & 5.17 (2H, AB, J 12.5Hz, O-CH_2) 7.32 (5H, s, C_6H_5). δC (90MHz, solvent $CDCl_3$, standard Me_4Si) 17.16 - 17.27 - 18.7 - 18.94 (Me i-propyl) 28.36 (Me t-butyl) 30.22 - 31.26 (CH i-propyl) 58.32 (N-CH) 66.91 (O-CH_2) 77.37 (O-CH) 79.91 (C t-butyl) 128.45 - 128.58 (CH phenyl) 135.39 (C phenyl) 155.68 (C=O carbamate) 169.09 - 172.16 (C=O).

t-Butoxycarbonyl L-valyl L-α-hydroxyisovaleric acid (2).

t-Butoxycarbonyl L-valyl L-α-hydroxyisovaleric acid benzyl ester (1) (4.96g, 12.18mmol) in methanol (40ml) was hydrogenated in the presence of palladium on charcoal (10%) (0.57g) for 20h, the reaction mixture was filtered and solvent was removed. The residue was dissolved in ether, and the ethereal solution was washed several times with dilute sodium carbonate. The aqueous extract was acidified with hydrochloric acid and was extracted with chloroform. The chloroform phase was dried (Na_2SO_4) and solvent was removed under reduced pressure to yield the title compound (2) (3.49g, 90%) as an oil. m.p. 115-117°C (ether/hexane) $[\alpha]_D$ -12 (c 2.4 chloroform). IR ($CHCl_3$) (cm^{-1}) 1500 s (N-H bend) 1725 s (C=O stretch) 1745 s (C=O stretch) 3000 br (O-H stretch) 3450 m (N-H stretch). δH (360MHz, solvent $CDCl_3$, standard Me_4Si) 0.9-1.05 (12H, c, Me i-propyl) 1.43 (9H, s, t-butyl) 2.2-2.35 (2H, c, CH i-propyl) 4.32 (1H, dd, J 4 & 9Hz N-CH) 4.90 (1H, d, J 3.5Hz, O-CH) 5.14 (1H, d, J 9Hz, NH). δC (90MHz, solvent $CDCl_3$, standard Me_4Si) 17.04 - 17.22 - 18.75 - 18.92 (Me i-propyl) 28.28 (Me t-butyl) 30.04 - 31.12 (CH i-propyl) 58.40 (N-CH) 77.00 (O-CH) 80.07 (C t-butyl) 172.09 - 173.43 (C=O). Found C, 56.8; H, 8.6; N, 4.2. $C_{15}H_{27}NO_6$ requires C, 56.8; H, 8.5; N,4.4%

L-Valyl L-α-hydroxyisovaleric acid benzyl ester hydrochloride (3).

Hydrogen chloride gas was passed through a solution of t-butoxycarbonyl L-valyl L-α-hydroxyisovaleric acid benzyl ester (1) (2.90g, 7.12mmol) in ethyl acetate (30ml) for 1h. Evaporation of solvent under reduced pressure gave the title compound (3) (2.30g, 94%) as a white solid. m.p. 115-117°C (ethyl acetate / hexane) $[\alpha]_D$ -24 (c 1.0 chloroform). IR ($CHCl_3$) (cm^{-1}) 1515 m (N-H bend) 1605 w (N-H bend) 1740 s (C=O stretch) 3000 br (N-H stretch). δH (360MHz, solvent $CDCl_3$, standard Me_4Si 0.93) (3H, d, J 7Hz, Me i-propyl) 0.98 (3H, d, J 7Hz, Me i-propyl) 1.15 (3H, d, J 7Hz, Me i-propyl) 1.16 (3H, d, J 7Hz, Me i-propyl) 2.25 (1H, m, CH i-propyl) 2.51 (1H, m, CH i-propyl) 4.11 (1H, d, J 4Hz, N-CH) 5.00 (1H, d, J 3.5Hz, O-CH) 5.12 & 5.16 (2H, AB, J 12Hz, O-CH_2) 7.25 (5H, s, C_6H_5). δC (90MHz, solvent ($CDCl_3$, standard Me_4Si) 17.20 - 17.78 - 18.32 - 18.61 (Me i-propyl) 30.05 - 30.23 (CH i-propyl) 58.36 (N-CH) 67.13 (O-CH_2) 78.35 (O-CH) 128.51 - 128.53 - 128.61 (CH phenyl) 135.18 (C phenyl) 168.56 - 168.71 (C=O). Found C, 59.5; H, 7.65; N, 3.95. $C_{17}H_{26}NO_4Cl$ requires C, 59.4; H, 7.55; N, 4.05 %

t-Butoxycarbonyl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid benzyl ester (4).

Dicyclohexylcarbodiimide (1.40g, 6.80mmol) was added to a solution of t-butoxycarbonyl L-valyl L-α-hydroxyisovaleric acid (2) (1.71g, 5.41mmol), L-valyl L-α-hydroxyisovaleric acid benzyl ester hydrochloride (3) (2.26g, 6.60mmol), dimethylaminopyridine (210mg, 1.72mmol) and N-methylmorpholine (0.73ml, 6.60mmol) in dichloromethane (30ml) at 0°C. The reaction was stirred for a further 9h at room temperature, the precipitate was filtered off and solvent was removed under reduced pressure. The residue was purified by chromatography on silica gel (light petroleum : ethyl acetate, 2:3) to yield the title compound (4) (3.32g, 100%) as a white solid. m.p. 76-78°C (hexane) $[\alpha]_D$ -29 (c 2.4 chloroform). IR ($CHCl_3$) (cm^{-1}) 1520 s (N-H bend) 1690 s (C=O stretch) 1745 s (C=O stretch) 3340 w (C=O stretch) 3450 m (N-H stretch). δH (360MHz, solvent $CDCl_3$, standard Me_4Si) 0.9-1.1 (24H, c, Me i-propyl) 1.47 (9H, s, t-butyl) 2.2-2.4 (4H, c, CH i-propyl) 4.31 (1H, dd, J 4 & 8Hz, N-CH) 4.66 (1H, dd, J 4.5 & 9Hz, N-CH) 4.92 (1H, d, J 4.5Hz, O-CH) 5.02 (1H, d, J 8.5Hz, NH carbamate) 5.06 (1H, d, J 4Hz, O-CH) 5.15 & 5.18 (2H, AB, J 12.5Hz, O-CH_2) 6.78 (1H, d, J 8.5Hz, NH amide) 7.34 (5H, s, C_6H_5). δC (90MHz, solvent $CDCl_3$, standard Me_4Si)

17.14 - 17.23 - 17.43 - 17.64 - 18.66 - 18.72 - 18.88 - 19.28 (Me i-propyl) 28.39 (Me t-butyl) 30.25 - 30.71 - 30.85 - 31.20 (CH i-propyl) 56.79 - 58.89 (N-CH) 66.95 (O-CH_2) 77.50 - 79.06 (O-CH) 80.00 (C t-butyl) 128.44 - 128.47 - 128.60 (CH phenyl) 135.50 (C phenyl) 155.80 (C=O carbamate) 168.90 - 169.01 - 171.23 171.45 (C=O). Found C, 63.1; H, 8.3; N, 4.5. $C_{32}H_{50}N_2O_9$ requires C, 63.35; H, 8.25; N, 4.6 %

t-Butoxycarbonyl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid (5).

A solution of t-butoxycarbonyl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid benzyl ester (4) (1.95g, 3.20mmol) in methanol (20ml) was hydrogenated over palladium on charcoal (10%) (130mg) at atmospheric pressure for 48h, the catalyst was filtered off and solvent was removed under reduced pressure. The residue was taken up in dilute sodium carbonate and washed with ether. The aqueous phase was acidified with citric acid and extracted with chloroform. The chloroform phase was washed with brine, dried (Na_2SO_4) and solvent was removed to yield the title compound (5) (1.36g, 82%) as an oil. IR ($CHCl_3$) (cm^{-1}) 1520 s (N-H bend) 1690 s (C=O stretch) 1730 s (C=O stretch) 1750 s (C=O stretch) 3000 br (O-H stretch) 3340 w (C=O stretch) 3450 m (N-H stretch). δH (360MHz, solvent $CDCl_3$, standard Me_4Si) 0.9-1.1 (24H, c, Me i-propyl) 1.45 (9H, s, t-butyl) 2.25-2.4 (4H, c, CH i-propyl) 4.31 (1H, dd, J 4 & 8Hz, N-CH) 4.65 (1H, dd, J 4.5 & 8.5Hz, N-CH) 4.92 (1H, d, J 4Hz, O-CH) 5.05-5.1 (2H, m, O-CH & NH carbamate) 6.89 (1H, d, J 8.5Hz, NH amide). δC (90MHz, solvent $CDCl_3$, standard Me_4Si) 17.10 - 17.24 - 17.41 - 17.69 - 18.72 - 18.77 - 18.86 - 19.27 (Me i-propyl) 28.38 (Me t-butyl) 30.12 - 30.72 - 30.81 - 31.20 (CH i-propyl) 57.06 - 58.89 (N-CH) 77.24 - 79.09 (O-CH) 80.30 (C t-butyl) 156.24 (C=O carbamate) 169.40 - 171.12 - 171.49 - 172.68 (C=O).

L-Valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid benzyl ester hydrochloride (6).

A solution of t-butoxycarbonyl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid benzyl ester (4) (2.66g, 4.39mmol) in ethyl acetate (40ml) was treated with hydrogen choride gas for 3h at 0°C. Evaporation of solvent under reduced pressure afforded the title compound (6) (2.30g, 97%) as an oil. $[\alpha]_D$ - 35 (c 0.5 chloroform). IR ($CHCl_3$) (cm^{-1}) 1520 s (N-H bend) 1690 s (C=O stretch) 1745 s (C=O stretch) 3340 w (C=O stretch) 3450 m (N-H stretch). δH (360MHz, solvent $CDCl_3$, standard Me_4Si) 0.9-1.15 (18H, c, Me i-propyl) 1.16 (3H, d, J 7Hz, Me i-propyl) 1.18 (3H, d, J 7Hz, Me i-propyl) 2.2-2.35 (3H, c, CH i-propyl) 2.52 (1H, m, CH i-propyl) 4.05 (1H, br, N-CH) 4.62 (1H, dd, J 5 & 8.5Hz, N-CH) 4.90 (1H, d, J 4Hz, O-CH) 4.99 (1H, d, J 5.5Hz, O-CH) 5.15 & 5.18 (2H, AB, J 12Hz, O-CH_2) 6.86 (1H, d, J 8.5Hz, NH) 7.3-7.4 (5H, c, C_6H_5). δC (90MHz, solvent $CDCl_3$, standard Me_4Si) 17.18 - 17.96 - 18.24 - 18.29 - 18.57 - 18.64 - 18.94 (Me i-propyl) 30.16 - 30.29 - 30.74 - 31.10 (CH i-propyl) 57.16 - 58.67 (N-CH) 67.04 (O-CH_2) 77.63 - 80.84 (O-CH) 128.48 - 128.53 - 128.64 (CH phenyl) 135.39 (C phenyl) 168.21 - 168.37 - 169.02 - 171.51 (C=O).

t-Butoxycarbonyl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid benzyl ester (7).

To a solution of t-butoxycarbonyl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid (5) (2.49g, 4.80mmol), L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid benzyl ester hydrochloride (6) (2.72g, 5.00mmol), dimethylaminopyridine (410mg, 3.36mmol) and N-methylmorpholine (0.55ml, 6.04mmol) in dichloromethane (50ml) at 0°C was added dicyclohexylcarbodiimide (1.17g, 5.68mmol). The reaction mixture was stirred at room temperature for 15h, the precipitate of urea was filtered off

and solvent was removed under reduced pressure. The residue was purified by chromatography on silica gel (light petroleum : ethyl acetate, 2:3) to yield the title compound (7) (4.10g, 85%) as a white solid. m.p. 132-134°C (ether) $[\alpha]_D$ -56 (c 1.0 chloroform). IR ($CHCl_3$) (cm^{-1}) 1520 s (N-H bend) 1690 s (C=O stretch) 1745 s (C=O stretch) 3340 m (C=O stretch) 3450 w (N-H stretch). δH (360MHz, solvent $CDCl_3$, standard Me_4Si) 0.95-1.1 (48H, c, Me i-propyl) 1.49 (9H, s, t-butyl) 2.2-2.45 (8H, c, CH i-propyl) 4.21 (1H, dd, J 4 & 7.5Hz, N-CH) 4.45 (1H, dd, J 5 & 5.5Hz, N-CH) 4.53 (1H, dd, J 6 & 7Hz, N-CH) 4.62 (1H, dd, J 5 & 8.5Hz, N-CH) 4.93 (1H, d, J 4Hz, O-CH) 5.02 (1H, d, J 8.5Hz, NH carbamate) 5.05-5.1 (3H, c, O-CH) 5.15 & 5.18 (2H, AB, J 12Hz, O-CH_2) 6.91 (1H, d, J 8.5Hz, NH amide) 7.05 (1H, d, J 7.5Hz, NH amide) 7.13 (1H, d, J 7Hz, NH amide) 7.35 (5H, s, C_6H_5). δC (90MHz, solvent $CDCl_3$, standard Me_4Si) 17.11 - 17.17 - 17.21 - 17.61 - 17.86 - 18.02 - 18.11 - 18.67 18.71 - 18.81 - 18.87 - 18.99 - 19.23 - 19.28 - 19.32 (Me i-propyl) 28.44 (Me t-butyl) 30.31 - 30.37 - 30.43 - 30.60 - 30.68 - 30.77 - 31.00 (CH i-propyl) 57.01 - 57.84 - 58.14 - 59.29 (N-CH) 66.92 (O-CH_2) 77.48 - 79.10 - 79.14 - 79.26 (O-CH) 80.30 (C t-butyl) 128.45 - 128.60 (CH phenyl) 135.53 (C phenyl) 156.20 (C=O carbamate) 169.03 - 169.12 - 169.61 - 169.98 - 170.13 - 170.42 - 171.13 - 171.22 (C=O). Found C, 61.95; H, 8.45; N, 5.5. $C_{52}H_{84}N_4O_{15}$ requires C, 62.15; H, 8.4; N, 5.5 %

L-Valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid benzyl ester hydrochloride (8).

A stream of hydrogen chloride gas was passed through a solution of t-butoxycarbonyl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid benzyl ester (7) (2.00g, 1.99mmol) in ethyl acetate (30ml) at 0°C for 3h. Solvent was removed under reduced pressure to give the title product (8) (1.71g, 91%) as an oil. $[\alpha]_D$ -43 (c 2.0 chloroform). IR ($CHCl_3$) (cm^{-1}) 1520 s (N-H bend) 1685 s (C=O stretch) 1750 s (C=O stretch) 3440 w (N-H stretch). δH (360MHz, solvent $CDCl_3$, standard Me_4Si) 0.95-1.1 (42H, c, Me i-propyl) 1.15-1.25 (6H, br, Me i-propyl) 2.2-2.4 (7H, c, CH i-propyl) 2.55 (1H, br, CH i-propyl) 4.05 (1H, br, N-CH) 4.55 (1H, br, N-CH) 4.59 (1H, dd, J 5 & 7Hz, N-CH) 4.65 (1H, dd, J 4.5 & 8.5Hz, N-CH) 4.92 (1H, d, J 4Hz, O-CH) 5.05-5.1 (3H, c, O-CH) 5.15 & 5.18 (2H, AB, J 12Hz, O-CH_2) 6.75 (1H, d, J 9Hz, NH amide) 6.85 (1H, br,NH amide) 7.25 (1H, br, NH amide) 7.38 (5H, s, C_6H_5). δC (90MHz, solvent $CDCl_3$, standard Me_4Si) 17.07 - 17.17 - 17.25 - 17.61 - 17.74 - 17.86 - 18.18 - 18.26 - 18.36 - 18.46 - 18.64 - 18.87 - 19.22 - 19.28 (Me i-propyl) 30.19 - 30.50 - 30.69 - 30.73 - 31.08 (CH i-propyl) 56.79 - 57.39 - 57.95 58.63 (N-CH) 66.96 (O-CH_2) 77.49 - 79.05 - 79.17 - 80.75 (O-CH) 128.43 - 128.48 - 128.59 (CH phenyl) 135.32 (C phenyl) 167.79 - 168.84 - 168.87 - 169.04 - 169.32 - 170.65 - 170.82 - 171.28 (C=O).

t-Butoxycarbonyl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid benzyl ester (9).

To a solution of t-butoxycarbonyl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid (5) (1.38mg, 2.67mmol), L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid benzyl ester hydrochloride (8) (2.26g, 2.50mmol), dimethylaminopyridine (110mg, 0.90mmol) and N-methylmorpholine (0.25ml, 2.05mmol) in dichloromethane (40ml) at 0°C was added dicyclohexylcarbodiimide (0.70g, 3.40mmol). The reaction mixture was stirred at room temperature for 16h, the precipitate of urea was filtered off and solvent was removed under reduced pressure. The residue was chromatographed on silica gel (light petroleum : ethyl acetate, 2:3) to afford the title

compound (9) (2.51g, 71%) as a white solid, m.p. 146-148°C (ethanol/water) $[\alpha]_D$ -63 (c 1.2 chloroform). IR ($CHCl_3$) (cm^{-1}) 1515 s (N-H bend) 1670 s (C=O stretch) 1745 s (C=O stretch) 3320 m (C=O stretch) 3450 w (N-H stretch). δH (360MHz, solvent $CDCl_3$, standard Me_4Si) 0.9-1.15 (72H, c, Me i-propyl) 1.49 (9H, s, t-butyl) 2.2-2.45 (12H, c, CH i-propyl) 4.16 (1H, dd, J 4 & 7Hz, N-CH) 4.25-4.4 (3H, c, N-CH) 4.48 (1H, dd, J 6 & 7Hz, N-CH) 4.59 (1H, dd, J 5.5 & 8.5Hz, N-CH) 4.94 (1H, d, J 4Hz, O-CH) 5.0-5.1 (6H, c, O-CH & NH carbamate) 5.15 & 5.17 (2H, AB, J 12Hz, O-CH_2) 7.0-7.05 (2H, br, NH amide) 7.2-7.45 (8H, c, NH amide & C_6H_5). δC (90MHz, solvent $CDCl_3$, standard Me_4Si) 16.94 - 17.08 - 17.17 - 17.63 - 17.99 - 18.24 - 18.39 - 18.43 18.66 - 18.76 - 18.89 - 18.98 - 19.01 - 19.10 - 19.19 - 19.24 - 19.26 - 19.30 (Me i-propyl) 28.42 (Me t-butyl) 29.68 - 29.81- 29.84 - 29.99 - 30.06 - 30.27 - 30.44 - 30.59 - 30.69 - 30.81 (CH i-propyl) 57.07 - 58.20 - 58.78 - 58.94 - 59.05 - 59.45 (N-CH) 66.86 (O-CH_2) 77.33 - 78.95 - 79.01 - 79.07 - 79.26 (O-CH) 80.70 (C t-butyl) 128.40 - 128.45 - 128.58 (CH phenyl) 135.50 (C phenyl) 156.50 (C=O carbamate) 169.24 - 169.35 - 169.81 - 170.01 - 170.07 - 170.16 - 170.25 - 170.66 - 170.74 - 170.97 - 171.17 (C=O). Found C, 61.35; H, 8.55; N, 6.05. $C_{72}H_{118}N_6O_{21}$ requires C, 61.65; H, 8.4; N, 6.0 %

t-Butoxycarbonyl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid (10).

A mixture of t-butoxycarbonyl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid benzyl ester (9) (514mg, 0.37mmol) in methanol (15ml) was hydrogenated over palladium on charcoal (5%) (170mg) at atmospheric pressure for 48h. The catalyst was filtered off, solvent was removed under reduced pressure and the residue was purified by chromatography on silica gel (light petroleum : ethyl acetate, 3:2) to afford the title compound (10) (437mg, 90%) as an oil. $[\alpha]_D$ -53 (c 0.9 chloroform). IR ($CHCl_3$) (cm^{-1}) 1515 s (N-H bend) 1670 s (C=O stretch) 1745 s (C=O stretch) 3310 m (C=O stretch) 3440 w (N-H stretch). δH (360MHz, solvent $CDCl_3$, standard Me_4Si) 0.9-1.1 (72H, c, Me i-propyl) 1.48 (9H, s, t-butyl) 2.2-2.4 (12H, c, CH i-propyl) 4.17 (1H, dd, J 4.5 & 7Hz, N-CH) 4.25-4.4 (3H, c, N-CH) 4.44 (1H, dd, J 6 & 7Hz, N-CH) 4.51 (1H, dd, J 6.5 & 6.5Hz, N-CH) 4.9-5.1 (7H, c, O-CH & NH carbamate) 6.85-7.35 (5H, br, NH amide). δC (90MHz, solvent $CDCl_3$, standard Me_4Si) 16.82 - 16.90 - 17.04 - 17.42 - 17.98 - 18.12 - 18.23 - 18.52 - 18.58 - 18.65 - 18.83 - 18.91 - 19.00 - 19.07 (Me i-propyl) 28.23 (Me t-butyl) 29.53 - 29.70 - 29.74 - 29.85 - 29.92 - 30.06 - 30.30 30.43 (CH i-propyl) 57.71 - 58.83 - 58.68 - 58.85 - 59.27 (N-CH) 78.94 - 78.98 - 79.13 (O-CH) 80.50 (C t-butyl) 156.40 (C=O carbamate) 169.68 - 169.77 - 169.87 - 169.93 - 169.98 - 170.04 - 170.46 - 170.57 - 170.81 (C=O).

t-Butoxycarbonyl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid pentafluorophenyl ester (11).

Dicyclohexylcarbodiimide (90mg, 0.44mmol) was added to a solution of t-butoxycarbonyl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid (10) (367mg, 0.29mmol), pentafluorophenol (77mg, 0.42mmol) and dimethylaminopyridine (20mg, 0.17mmol) in dichloromethane (10ml) at 0°C. The reaction mixture was stirred at room temperature for 24h, the precipitate was filtered off and solvent was removed under reduced pressure. The residue was purified by chromatography on silica gel (light petroleum : ethyl acetate, 3:2) to give the title compound (11) (276mg, 64%) as an oil. IR ($CHCl_3$) (cm^{-1}) 1525 s (N-H

bend) 1670 s (C=O stretch) 1750 s (C=O stretch) 1800 w (C=O stretch) 3320 m (C=O stretch) 3460 w (N-H stretch). δH (360MHz, solvent $CDCl_3$, standard Me_4Si) 0.9-1.15 (72H, c, Me i-propyl) 1.49 (9H, s, t-butyl) 2.25-2.5 (12H, c, CH i-propyl) 4.16 (1H, dd, J 4 & 6.5Hz, N-CH) 4.25-4.35 (3H, c, N-CH) 4.43 (1H, dd, J 6 & 6.5Hz, N-CH) 4.61 (1H, dd, J 6 & 8.5Hz, N-CH) 4.96 (1H, d, J 6.5Hz, NH carbamate) 5.02 (1H, d, J 4Hz, O-CH) 5.04 (1H, d, J 3.5Hz, O-CH) 5.06 (1H, d, J 4Hz, O-CH) 5.09 (1H, d, J 4Hz, O-CH) 5.11 (1H, d, J 4Hz, O-CH) 5.21 (1H, d, J 4.5Hz, O-CH) 7.19 (1H, d, J 8Hz, NH amide) 7.28 (1H, d, J 7Hz, NH amide) 7.3-7.5 (3H, br, NH amide). δC (90MHz, solvent $CDCl_3$, standard Me_4Si) 16.98 - 17.09 - 17.18 - 17.65 - 18.18 - 18.38 - 18.47 - 18.52 - 18.65 - 18.87 - 18.94 - 19.02 - 19.09 - 19.14 - 19.23 - 19.31 (Me i-propyl) 28.45 (Me t-butyl) 29.72 - 29.83 - 29.86 - 29.98 - 30.02 - 30.30 - 30.49 - 30.53 - 30.63 - 30.74 (CH i-propyl) 57.21 - 58.57 - 58.71 - 59.04 - 59.17 - 59.49 (N-CH) 76.71 - 78.97 - 79.08 - 79.13 - 79.31 (O-CH) 80.58 (C t-butyl) 156.50 (C=O carbamate) 165.84 - 169.62 - 169.81 - 170.01 - 170.12 - 170.18 - 170.46 - 170.84 - 170.97 - 171.05 (C=O).

L-Valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid pentafluorophenyl ester trifluoroacetate (12).

A solution of t-butoxycarbonyl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxy-isovalericacid pentafluorophenyl ester (11) (276mg, 0.19mmol) in dichloromethane (15ml) and trifluoroacetic acid (15ml) was kept at room temperature for 2h. The solvent was removed under reduced pressure to afford the title compound (12) (270mg, 97%) as an oil. IR ($CHCl_3$) (cm^{-1}) 1525 s (N-H bend) 1670 s (C=O stretch) 1750 s (C=O stretch) 1800 w (C=O stretch) 3320 m (C=O stretch) 3460 w (N-H stretch). δH (270MHz, solvent $CDCl_3$, standard Me_4Si) 0.9-1.1 (66H, c, Me i-propyl) 1.11 (3H, d, J 7Hz, Me i-propyl) 1.13 (3H, d, J 7Hz, Me i-propyl) 2.2-2.5 (12H, c, CH i-propyl) 3.98 (1H, br, N-CH) 4.3-4.5 (4H, c, N-CH) 4.63 (1H, dd, J 6 & 8Hz, N-CH) 4.95-5.1 (5H, c, O-CH) 5.17 (1H, d, J 4.5Hz, O-CH) 7.0-7.4 (5H, c, NH amide). δC (68MHz, solvent $CDCl_3$, standard Me_4Si) 16.98 - 17.04 - 17.09 - 17.58 - 17.66 - 17.96 - 18.04 - 18.13 - 18.23 - 18.33 - 18.36 - 18.49 - 18.71 - 18.79 - 18.87 - 19.04 - 19.18 - 19.27 (Me i-propyl) 30.02 - 30.14 - 30.37 - 30.44 - 30.61 30.77 (CH i-propyl) 57.12 - 58.37 - 58.42 - 58.62 - 58.72 - 58.75 (N-CH) 76.86 - 79.20 - 79.25 - 79.32 (O-CH) 165.86 - 169.80 - 170.23 - 170.39 - 170.45 - 170.54 - 170.67 - 171.27 (C=O).

Benzyloxycarbonyl L-valyl L-α-hydroxyisovaleric acid t-butyl ester (14)

To a solution of benzyloxycarbonyl L-valine (4.52g, 18.0mmol) and L-α-hydroxyisovaleric acid t-butyl ester (3.70g, 17.1mmol) in dichloromethane (50ml) at 0°C was added dimethylaminopyridine (0.50g, 4.0mmol) and dicyclohexylcarbodiimide (3.81g, 18.5mmol). The reaction mixture was stirred at room temperature for 24h and the urea precipitate was then filtered off. The solvent was removed and the residue was purified by chromatography on silica gel (light petroleum : ether, 3-1) to give the title compound (14) (6.90g, 99%) as an oil. $[\alpha]_D$ -21 (c 1.7 chloroform). IR ($CHCl_3$) (cm^{-1}) 1520 s (N-H bend) 1735 s (C=O stretch) 3450 m (N-H stretch). δH (360MHz, solvent $CDCl_3$, standard Me_4Si) 0.95-1.1 (12H, c, Me i-propyl) 1.45 (9H, s, Me t-butyl) 2.20 (1H, m, CH i-propyl) 2.30 (1H, m, CH i-propyl) 4.40 (1H, dd, J 4.5 & 9Hz, N-CH) 4.72 (1H, d, J 4.5Hz, O-CH) 5.10 (2H, s, O-CH2) 5.38 (1H, d, J 9Hz, NH) 7.3-7.35 (5H, c, C_6H_5). δC (90MHz, solvent $CDCl_3$, standard Me_4Si) 17.30 - 17.47 - 18.68 - 18.99 (Me i-propyl) 28.10 (Me t-butyl) 30.21 - 31.33 (CH i-propyl) 59.09 (N-CH) 67.05 ($O-CH_2$) 78.10 (O-CH) 82.07 (C t-butyl) 128.07 - 128.13 - 128.54 (CH phenyl) 136.61 (C phenyl) 156.28 (C=O carbamate) 168.17 - 171.70 (C=O).

Benzyloxycarbonyl L-valyl L-α-hydroxyisovaleric acid (15).

Benzyloxycarbonyl L-valyl L-α-hydroxyisovaleric acid t-butyl ester (14) (14.3g, 35.1mmol) was dissolved in dichloromethane (40ml). Trifluoroacetic acid (30ml) was added and the mixture was then left at room temperature for 10h. The solvent was removed and the residue was taken up in dilute sodium carbonate solution. The aqueous solution was washed with ether and acidified with dilute hydrochloric acid. Extraction with ether followed by drying (Na_2SO_4) and removal of solvent afforded the title compound (15) (12.3g, 100%) as an oil. $[\alpha]_D$ -9 (c 1.6 chloroform). IR ($CHCl_3$) (cm^{-1}) 1515 s (N-H bend) 1730 s (C=O stretch) 3000 br (N-H stretch) 3440 m (N-H stretch). δH (360MHz, solvent $CDCl_3$, standard Me_4Si) 0.9-1.1 (12H, c, Me i-propyl) 2.2-2.35 (2H, c, CH i-propyl) 4.42 (1H, dd, J 4 & 9Hz, N-CH) 4.91 (1H, d, J 4Hz, O-CH) 5.11 (2H, s, O-CH_2) 5.47 (1H, d, J 9Hz, NH) 7.3-7.4 (5H, c, C_6H_5). δC (90MHz, solvent $CDCl_3$, standard Me4Si) 17.06 - 17.24 - 18.74 - 18.92 (Me i-propyl) 30.06 - 31.22 (CH i-propyl) 58.95 (N-CH) 64.24 (O-CH_2) 74.11 (O-CH) 128.10 - 128.22 - 128.56 (CH phenyl) 136.30 (C phenyl) 156.57 (C=O carbamate) 171.92 - 173.62 (C=O).

L-Valyl L-α-hydroxyisovaleric acid t-butyl ester (16).

Benzyloxycarbonyl L-valyl L-α-hydroxyisovaleric acid t-butyl ester (14) (14.0g, 34.0mmol) was dissolved in absolute methanol (100ml) and palladium on charcoal (10%) (1.4g) was then added to the solution. The mixture was hydrogenated for 5h, the catalyst was filtered off and solvent was removed under reduced pressure to afford the title compound (16) (9.30g, 99%) as an oil. IR ($CHCl_3$) (cm^{-1}) 1515 m (N-H bend) 1605 w (N-H bend) 1740 s (C=O stretch) 3000 br (N-H stretch). δH (60MHz, solvent $CDCl_3$, standard Me_4Si) 0.9-1.1 (12H, c, Me i-propyl) 1.4 (9H, s, Me t-butyl) 1.9-2.45 (2H,c, CH i-propyl) 3.5 (1H, d, J 5Hz, N-CH) 4.75 (1H, d, J 5Hz, O-CH). δC (90MHz, solvent $CDCl_3$, standard Me_4Si) 17.15 - 17.32 - 18.52 - 18.75 (Me i-propyl) 27.89 (Me t-butyl) 30.01 - 31.44 (CH i-propyl) 59.38 (N-CH) 77.63 (O-CH) 81.70 (C t-butyl) 168.08 - 173.33 (C=O).

Benzyloxycarbonyl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid t-butyl ester (17).

Benzyloxycarbonyl L-valyl L-α-hydroxyisovaleric acid (15) (12.0g, 34.3mmol) and L-valyl L-α-hydroxyisovaleric acid t-butyl ester (16) (9.30g, 34.0mmol) were dissolved in dichloromethane (180ml). Dimethylaminopyridine (0.41g, 3.3mmol) and N-methylmorpholine (7.4ml, 67.7mmol) were added and the mixture cooled down to 0°C. A solution of dicyclohexylcarbodiimide (7.63g, 37.0mmol) in dichloromethane (40ml) was added and the reaction mixture was stirred for 12h at room temperature. The urea precipitate was filtered off and solvent was removed. The residue was dissolved in ether and washed successively with dilute hydrochloric acid, sodium carbonate and brine. The organic layer was dried (Na_2SO_4) and solvent was removed. The residue was purified by suction flash chromatography (light petroleum : ether, 24:1) to give the title compound (17) (16.7g, 81%) as an oil. $[\alpha]_D$ -25 (c 1.7 chloroform). IR ($CHCl_3$) (cm^{-1}) 1515 s (N-H bend) 1690 s (C=O stretch) 1745 s (C=O stretch) 3340 w (N-H stretch) 3450 m (N-H stretch). δH (360MHz, solvent $CDCl_3$, standard Me_4Si) 0.9-1.05 (24H, c, Me i-propyl) 1.45 (9H, s, Me t-butyl) 2.20 (1H, m, CH i-propyl) 2.25-2.35 (3H, c, CH i-propyl) 4.40 (1H, dd, J 4.5 & 8.5Hz, N-CH) 4.65 (1H, dd, J 4.5 & 9Hz, N-CH) 4.71 (1H, d, J 4.5Hz, O-CH) 5.06 (1H, d, J 4.5Hz, O-CH) 5.10 & 5.13 (2H, AB, J 12Hz, O-CH_2) 5.29 (1H, d, J 9Hz, NH carbamate) 6.67 (1H, d, J 7.5Hz, NH amide) 7.3-7.4 (5H, c, C6H5). δC (90MHz, solvent $CDCl_3$, standard Me_4Si) 17.26 - 17.30 - 17.41 - 17.76 - 18.68 - 18.73 - 19.02 - 19.28 (Me i-propyl)

28.13 (Me t-butyl) 30.24 - 30.78 - 30.90 - 31.30 (CH i-propyl) 56.87 - 59.45 (N-CH) 67.24 (O-CH2) 78.17 - 79.29 (O-CH) 82.08 (C t-butyl) 128.14 - 128.24 - 128.60 (CH phenyl) 136.46 (C phenyl) 156.51 (C=O carbamate) 168.17 - 168.71 - 171.11 171.24 (C=O).

Benzyloxycarbonyl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid (18).

A solution of benzyloxycarbonyl L-valyl L-α-hydroxyisovaleric L-valyl L-α-hydroxyisovaleric acid t-butyl ester (17) (8.53g, 12.9mmol) in dichloromethane (25ml) was treated with trifluoroacetic acid (20ml) at 0°C for 8h. The solvent was then removed and the residue was taken up in dilute sodium carbonate solution. The aqueous solution was washed with ether, acidified with dilute hydrochloric acid and extracted again with ether. The second ether phase was dried (Na_2SO_4) and removal of solvent afforded the title compound(18) (7.70g, 99%) as an oil. $[\alpha]_D$ -22 (c 1.1 chloroform) IR ($CHCl_3$) (cm^{-1}) 1520 s (N-H bend) 1690 s (C=O stretch) 1730 s (C=O stretch) 1750 s (C=O stretch) 3000 br (O-H stretch) 3340 w (N-H stretch) 3450 m (N-H stretch). δH (360MHz, solvent $CDCl_3$, standard Me_4Si) 0.9-1.05 (24H, c, Me i-propyl) 2.25-2.4 (4H, c, CH i-propyl) 4.39 (1H, dd, J 4.5 & 8.5Hz, N-CH) 4.66 (1H, dd, J 4.5 & 8.5Hz, N-CH) 4.92 (1H, d, J 4Hz, O-CH) 5.07 (1H, d, J 4.5Hz, O-CH) 5.10 & 5.13 (2H, AB system, J 12Hz, O-CH_2) 5.33 (1H, d, J 8.5Hz, NH carbamate) 6.92 (1H, d, J 8.5Hz, NH amide) 7.34 (5H, s, C_6H_5). δC (90MHz, solvent $CDCl_3$, standard Me_4Si) 17.01 - 17.07 - 17.28 - 17.68 - 18.87 - 18.97 - 19.35 (Me i-propyl) 30.08 - 30.59 - 30.63 - 31.16 (CH i-propyl) 57.04 - 59.33 (N-CH) 67.45 (O-CH_2) 77.15 - 79.05 (O-CH) 128.28 - 128.47 - 128.70 (CH phenyl) 135.98 (C phenyl) 156.92 (C=O carbamate) 169.59 - 171.10 - 171.21 - 173.42 (C=O).

L-Valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid t-butyl ester (19).

Benzyloxycarbonyl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid t-butyl ester (17) (8.10g, 13.4mmol) was dissolved in absolute methanol (60ml). Palladium on charcoal (10%) (800mg) was then added to the solution. The mixture was hydrogenated at atmospheric pressure for 5h. The reaction mixture was filtered and solvent was removed to afford the title compound (19) (6.19g, 98%) as an oil. $[\alpha]_D$ -47 (c 1.7 chloroform). IR ($CHCl_3$) (cm^{-1}) 1525 s (N-H bend) 1685 s (C=O stretch) 1750 s (C=O stretch) 3000 br (N-H stretch). δH (360MHz, solvent $CDCl_3$, standard Me_4Si) 0.95-1.1 (24H, c, Me i-propyl) 1.46 (9H, s, Me t-butyl) 2.1-2.4 (4H, c, CH i-propyl) 3.46 (1H, d, J 5.5Hz, N-CH) 4.66 (1H, dd, J 4 & 9Hz, N-CH) 4.70 (1H, d, J 4.5Hz, O-CH) 5.10 (1H, d, J 4.5Hz, O-CH) 6.82 (1H, d, J 9Hz, NH). δC (90MHz, solvent $CDCl_3$, standard Me_4Si) 17.29 - 17.32 - 17.45 - 17.65 - 18.69 - 18.81 - 19.03 - 19.39 (Me i-propyl) 28.10 (Me t-butyl) 30.20 - 30.70 - 31.35 - 31.95 (CH i-propyl) 56.72 - 60.61 (N-CH) 78.18 - 78.68 (O-CH) 82.11(C t-butyl) 168.19 - 169.12 - 171.41 - 173.64 (C=O).

Benzyloxycarbonyl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid t-butyl ester (20).

To a solution of benzyloxycarbonyl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid (18) (6.54g, 11.9mmol), L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid t-butyl ester (19) (5.10g, 10.8mmol), dimethylaminopyridine (131mg, 1.07mmol) and N-methylmorpholine (1.3ml, 11.9mmol) in dichloromethane (90ml) at 0°C was added dicyclohexylcarbodiimide (2.44g, 11.9mmol) in dichloromethane (20ml) and the reaction mixture was stirred for 16h at room temperature. The urea precipitate was filtered off and the solution was washed successively with dilute hydrochloric acid, sodium carbonate and brine. The organic phase was dried (Na_2SO_4) and the solvent was removed. The residue was purified by suction flash chromatography (light petroleum : ether, 24 : 1) to give as a solid the title compound (20) (7.49g, 69%). m.p.

115-117°C (ethanol/water) $[\alpha]_D$ -46 (c 1.0 chloroform). IR ($CHCl_3$) (cm^{-1}) 1520 s (N-H bend) 1690 s (C=O stretch) 1755 s (C=O stretch) 3340 m (N-H stretch) 3450 w (N-H stretch). δH (360MHz, solvent $CDCl_3$, standard Me_4Si) 0.9-1.1 (48H, c, Me i-propyl) 1.46 (9H, s, Me t-butyl) 2.21 (1H, m, CH i-propyl) 2.25-2.4 (7H, c, CH i-propyl) 4.31 (1H, dd, J 4.5 & 7.5Hz, N-CH) 4.48 (1H, dd, J 5 & 7Hz, N-CH) 4.57 (1H, dd, J 6.5 & 8Hz, N-CH) 4.62 (1H, dd, J 5 & 8.5Hz, N-CH) 4.71 (1H, d, J 4.5Hz, O-CH) 5.05-5.1 (3H, c, O-CH) 5.12 & 5.14 (2H, AB, J 12Hz, O-CH_2) 5.33 (1H, d, J 8Hz, NH carbamate) 6.82 (1H, d, J 8.5Hz, NH amide) 6.93 (1H, d, J 8Hz, NH amide) 6.99 (1H, d, J 7.5Hz, NH amide) 7.35 (5H, s, C_6H_5). δC (90MHz, solvent $CDCl_3$, standard Me_4Si) 17.01 - 17.12 - 17.20 - 17.30 - 17.52 - 17.88 - 17.96 - 18.66 - 18.71 - 18.76 - 18.82 - 19.07 - 19.26 - 19.31 (Me i-propyl) 28.10 (Me t-butyl) 30.21 - 30.43 - 30.54 - 30.63 - 30.70 - 30.77- 31.09 (CH i-propyl) 56.93 - 57.62 - 57.96 - 59.70 (N-CH) 67.36 (O-CH2) 78.08 - 79.14 - 79.18 - 79.22 (O-CH) 81.99 (C t-butyl) 128.10 - 128.38 - 128.65 (CH phenyl) 136.17 (C phenyl) 157.08 (C=O carbamate) 168.27 - 168.91 - 169.46 - 169.73 - 170.24 - 170.51 - 170.75 - 171.18 (C=O). Found C, 62.0; H, 8.35; N, 5.45. $C_{52}H_{84}N_4O_{15}$ requires C, 62.15; H, 8.4; N, 5.5 %

Benzyloxycarbonyl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid (21).

A solution of benzyloxycarbonyl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid t-butyl ester (20) (6.40g, 6.37mmol) in dichloromethane (30ml) and trifluoroacetic acid (25ml) was kept at room temperature for 3h. The solvent was removed and the residue was dissolved in ether. Addition of dilute sodium carbonate solution with stirring gave a precipitate which was filtered off and successively washed with ether and water. The residue was vigorously stirred with a mixture of dilute hydrochloric acid and chloroform until complete dissolution. The organic layer was dried (Na_2SO_4) and solvent was removed to give the title compound (21) (5.27g, 87%) as a solid. m.p. 115-117°C (ethanol/water) $[\alpha]_D$ -45 (c 1.1 chloroform). IR ($CHCl_3$) (cm^{-1}) 1520 s (N-H bend) 1690 s (C=O stretch) 1750 s (C=O stretch) 3340 m (N-H stretch) 3450 w (N-H stretch). δH (270MHz, solvent $CDCl_3$, standard Me_4Si) 0.9-1.1 (48H, c, Me i-propyl) 2.2-2.4 (8H, c, CH i-propyl) 4.28 (1H, dd, J 4 & 7.5Hz, N-CH) 4.43 (1H, dd, J 6 & 6.5Hz, N-CH) 4.50 (1H, dd, J 6.5 & 7.5Hz, N-CH) 4.60 (1H, dd, J 6.5 & 8.5Hz, N-CH) 4.93 (1H, d, J 4Hz, O-CH) 5.05-5.1 (3H, c, O-CH) 5.12 & 5.14 (2H, AB, J 12Hz, O-CH_2) 5.31 (1H, d, J 7.5Hz, NH carbamate) 7.03 (1H, d, J 8.5Hz, NH amide) 7.05-7.1 (2H, c, NH amide) 7.35 (5H, s, C_6H_5). δC (68MHz, solvent $CDCl_3$, standard Me_4Si) 16.74 - 16.96 - 17.00 - 17.06 - 17.43 - 17.89 - 18.05 - 18.7 18.82 - 18.97 - 19.18 - 19.22 - 19.30 (Me i-propyl) 30.12 - 30.20 30.27 - 30.42 - 30.55 - 30.62 - 30.86 (CH i-propyl) 57.34 - 58.08 58.34 - 59.66 (NCH) 67.46 (O-CH_2) 77.21 - 78.96 - 79.06 (O-CH) 128.11 - 128.49 - 128.68 (CH phenyl) 135.76 (C phenyl) 157.15(C=O carbamate) 169.82 - 170.02 - 170.24 - 170.34 - 170.40 - 170.65 170.87 - 172.12 (C=O). Found C, 60.4; H, 8.05; N, 5.75.$C_{8}H_{76}N_4O_{15}$ requires C, 60.75; H, 8.05; N, 5.9 %

Benzyloxycarbonyl L-valyl L-α-hydroxyisovaleryl L-valyl L-α–hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid t-butyl ester (22).

A solution of dicyclohexylcarbodiimide (0.44g, 2.12mmol) in dichloromethane (5ml) was added to a solution of benzyloxycarbonyl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid (21) (2.01g, 2.12mmol), L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid t-butyl ester (19) (1.00g, 2.12mmol), dimethylaminopyridine (26mg,

0.21mmol) and N-methylmorpholine (0.24ml, 2.18mmol) in dichloromethane (25ml) at 0°C and the reaction mixture was stirred for 16h at room temperature. The urea precipitate was filtered off and solvent was removed. The residue was purified by chromatography on silica gel (light petroleum : ether, 4:1) to afford the title compound (22) (1.94g, 65%) as a white solid. m.p. 137-139°C (ethanol/water) $[\alpha]_D$ -53 (c 1.4 chloroform). IR ($CHCl_3$) (cm^{-1}) 1515 s (N-H bend) 1680 s (C=O stretch) 1745 s (C=O stretch) 3320 m (N-H stretch) 3450 w (N-H stretch). δH (270MHz, solvent $CDCl_3$, standard Me_4Si) 0.9-1.1 (72H, c, Me i-propyl) 1.46 (9H, s, Me t-butyl) 2.15-2.45 (12H, c, CH i-propyl) 4.25 (1H, dd, J 4 d & 7Hz, N-CH) 4.3-4.45 (3H, c, N-CH) 4.50 (1H, dd, J 5 & 8Hz, N-CH) 4.59 (1H, dd, J 5 & 9Hz, N-CH) 4.72 (1H, d, J 4Hz, O-CH) 5.05-5.15 (7H, c, O-CH & O-CH_2) 5.26 (1H, d, J 7Hz, NH carbamate) 6.94 (1H, d, J 7Hz, NH amide) 7.1-7.25 (4H, c, NH amide) 7.36 (5H, s, C6H5). δC (68MHz, solvent $CDCl_3$, standard Me_4Si) 16.62 - 16.73 - 16.80 - 16.91 - 17.18 - 17.41 - 17.93 - 18.08 - 18.17 - 18.26 - 18.67 - 18.76 - 18.90 - 18.99 - 19.10 - 19.15 19.21 - 19.30 (Me i-propyl) 27.98 (Me t-butyl) 29.74 - 29.81 - 30.04 - 30.12 - 30.35 - 30.48 - 30.65 - 30.83 (CH i.propyl) 56.85 - 57.93 - 58.60 - 58.79 - 58.85 - 59.73 (N-CH) 67.46 (O-CH_2) 77.75 - 78.74 - 78.79 - 78.83 - 78.86 - 78.96 (O-CH) 81.87 - (C t.-butyl) 128.07 - 128.53 - 128.68 (CH phenyl) 136.67 (C phenyl) 157.23 (C=O carbamate) 168.41 - 169.23 - 169.77 - 169.93 - 170.04 - 170.11 - 170.26 - 170.45 - 170.58 - 170.69 - 171.08 (C=O). Found C, 61.55; H, 8.45; N, 6.05.$C_{72}H_{118}N_6O_{21}$ requires C, 61.6; H, 8.45; N, 6.0 %

Benzyloxycarbonyl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid (23).

A solution of benzyloxycarbonyl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid t-butyl ester (22) (1.94g, 1.38mmol) in dichloromethane (25ml) was treated with trifluoroacetic acid (10ml) for 4h at room temperature. The solvent was removed and the residue was taken up in dilute sodium carbonate. The sodium salt which came out of solution was washed successively with water and ether, and was then stirred with a mixture of chloroform and dilute hydrochloric acid until complete dissolution. The chloroform layer was dried (Na_2SO_4) and the solvent removed to yield as a solid the title compound (23) (1.53g, 82%). m.p. 139-141°C (ethanol/water) $[\alpha]_D$ -53 (c 1.6 chloroform). IR ($CHCl_3$) (cm^{-1}) 1515 s (N-H bend) 1680 s (C=O stretch) 1745 s (C=O stretch) 3320 m (N-H stretch) 3450 w (N-H stretch). δH (270MHz, solvent $CDCl_3$, standard Me_4Si) 0.85-1.1 (72H, c, Me i-propyl) 2.2-2.45 (12H, c, CH i-propyl) 4.2-4.55 (6H, c, N-CH) 4.97 (1H, d, J 4Hz, O-CH) 5.0-5.1 (5H, c, O-CH) 5.13 & 5.15 (2H, AB, J 12Hz, O-CH_2) 5.29 (1H, d, J 7Hz, NH carbamate) 7.15-7.3 (5H, c, NH amide) 7.36 (5H, s, C_6H_5). δC (68MHz, solvent $CDCl_3$, standard Me_4Si) 16.62 - 16.81 - 17.09 - 17.44 - 18.10 - 18.27 - 18.40 - 18.67 - 18.81 - 18.85 - 18.91 - 18.97 - 19.10 - 19.16 - 19.29 (Me i-propyl) 29.79 - 29.99 - 30.20 - 30.35 - 30.46 - 30.53 - 30.64 (CH i-propyl) 57.71 - 58.52 - 58.83 - 58.97 - 59.06 - 59.80 (N-CH) 67.48 (O-CH2) 77.37 - 78.89 - 79.03 (O-CH) 128.06 - 128.54 - 128.68 (CH phenyl) 135.66 (C phenyl) 157.31 (C=O carbamate) 169.79 - 169.95 - 170.02 - 170.48 - 170.56 - 170.76 - 170.85 - 170.92 - 171.33 (C=O) Found C, 60.3; H, 8.15; N, 6.05. $C_{68}H_{110}N_6O_{21}$ requires C, 60.6; H, 8.2; N, 6.25 %

Benzyloxycarbonyl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid pentafluorophenyl ester (24).

A solution of dicyclohexylcarbodiimide (0.73g, 3.53mmol) in dichloromethane (5ml) was added to a solution of benzyloxycarbonyl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid (18) (1.62g,

2.94mmol), pentafluorophenol (0.65g, 3.53mmol) and dimethylaminopyridine (43mg, 0.35mmol) in dichloromethane (20ml) at 0°C. The reaction mixture was stirred at room temperature for 12h, the precipitate of urea filtered off and the solvent was removed under reduced pressure. The residue was purified by chromatography on silica gel (light petroleum : ether, 3:2) to give the title compound (24) (1.31g, 62%) as a colourless oil. IR ($CHCl_3$) (cm^{-1}) 1520 s (N-H bend) 1690 s (C=O stretch) 1750 s (C=O stretch) 1800 m (C=O stretch) 3330 w (N-H stretch) 3450 m (N-H stretch). δH (360MHz, solvent $CDCl_3$, standard Me_4Si) 0.9-1.1 (18H, c, Me i-propyl) 1.13 (3H, d, J 7Hz, Me i-propyl) 1.14 (3H, d, J 7Hz, Me i-propyl) 2.25-2.4 (3H, c, CH i-propyl) 2.44 (1H, m, CH i-propyl) 4.40 (1H, dd, J 4.5 & 8.5Hz, N-CH) 4.71 (1H, dd, J 4.5 & 8.5Hz, N-CH) 5.10 (1H, d, J 5Hz, O-CH) 5.07 & 5.17 (2H, AB, J 12Hz, O-CH_2) 5.16 (1H, d, J 4Hz, O-CH) 5.33 (1H, d, J 8.5Hz, NH carbamate) 6.84 (1H, d, J 7.5Hz, NH amide) 7.3-7.4 (5H, c, C_6H_5). δC (90MHz, solvent $CDCl_3$, standard Me_4Si) 17.09 - 17.13 - 17.36 - 17.65 - 18.46 - 18.75 - 18.93 - 19.25 (Me i-propyl) 30.47 - 30.75 - 31.17 (CH i-propyl) 56.81 - 59.49 (N-CH) 67.29 (O-CH2) 76.92 - 79.15 (O-CH) 128.15 - 128.29 - 128.61 (CH phenyl) 136.33 (C phenyl) 156.51 (C=O carbamate) 165.65 169.06 - 171.05 - 171.24 (C=O). δF (339Hz, solvent $CDCl_3$, standard C_6D_6) -0.03 (2F, dd, J 18 & 24Hz, meta) 4.72 (1F, t, J 24Hz, para) 10.11 (2F,d, J 18Hz, ortho).

Benzyloxycarbonyl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid pentafluorophenyl ester (25).

A solution of dicyclohexylcarbodiimide (0.44g, 2.12mmol) in dichloromethane (5ml) was added to a solution of benzyloxycarbonyl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid (21) (2.00g, 2.12mmol), pentafluorophenol (0.47g, 2.55mmol) and dimethylaminopyridine (26mg, 0.21mmol) in dichloromethane (20ml) at 0°C. The reaction mixture was stirred at room temperature for 15h, the precipitate of urea was filtered off and solvent was removed under reduced pressure. The residue was purified on silica gel (light petroleum : ether, 1:1) to give the title compound (25) (1.64g, 70%) as a solid. m.p. 133-135°C (ethanol/water). $[\alpha]_D$ -48 (c 1.7 chloroform). IR ($CHCl_3$) (cm^{-1}) 1520 s (N-H bend) 1690 s (C=O stretch) 1750 s (C=O stretch) 1800 w (C=O stretch) 3340 m (N-H stretch) 3450 w (N-H stretch). δH (360MHz, solvent $CDCl_3$, standard Me_4Si) 0.9-1.1 (42H, c, Me i-propyl) 1.11 (3H, d, J 7Hz, Me i-propyl) 1.13 (3H, d, J 7Hz, Me i-propyl) 2.25-2.5 (8H, c, CH i-propyl) 4.29 (1H, dd, J 4 & 8Hz, N-CH) 4.41 (1H, dd, J 4 & 8Hz, N-CH) 4.49 (1H, dd, J 4 & 8Hz, N-CH) 4.65 (1H, dd, J 4 & 8Hz, N-CH) 5.05-5.15 (5H, c, O-CH & O-CH_2) 5.18 (1H, d, J 4Hz, O-CH) 5.29 (1H, d, J 8Hz, NH carbamate) 7.01 (1H, d, J 8.5Hz, NH amide) 7.02 (1H, d, J 7.5Hz, NH amide) 7.05 (1H, d, J 7Hz, NH amide) 7.3-7.4 (5H, c, C_6H_5). δC (90MHz, solvent $CDCl_3$, standard Me_4Si) 16.93 - 17.05 - 17.12 - 17.52 - 17.93 - 18.06 - 18.44 - 18.77 - 18.80 - 18.88 - 19.01 - 19.23 - 19.28 (Me i-propyl) 30.28 - 30.40 30.48 - 30.60 - 30.66 - 30.75 - 30.84 (CH i-propyl) 57.01 - 58.07 - 58.32 - 59.76 (N-CH) 67.42 (O-CH_2) 76.81 - 79.09 - 79.25 (O-CH) 128.10 - 128.44 - 128.66 (CH phenyl) 135.83 (C phenyl) 157.08 (C=O carbamate) 165.81 - 169.32 - 169.95 - 170.03 - 170.12 - 170.33 - 170.67 - 171.13 (C=O). δF (339Hz, solvent $CDCl_3$, standard C_6D_6) -0.21 (2F, dd, J 18 & 22Hz, meta) 4.41 (1F, t, J 22Hz, para) 10.11(2F,d, J 18Hz, ortho). Found C, 58.2; H, 6.75; N, 5.05. $C_{54}H_{75}N_4O_{15}F_5$ requires C, 58.15; H, 6.8; N, 5.0 %

Benzyloxycarbonyl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid pentafluorophenyl ester (26).

A solution of dicyclohexylcarbodiimide (0.44g, 2.12mmol) in dichloromethane (5ml) was added to a

solution of benzyloxycarbonyl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid (23) (1.49g, 1.10mmol), pentafluorophenol (0.26g, 1.44mmol) and dimethylaminopyridine (13mg, 0.11mmol) at 0°C and the reaction mixture stirred for 24h at room temperature. The precipitate of urea was filtered off and the solvent was removed. The residue was purified by chromatography on silica gel (light petroleum : ether, 1:1) to give as a solid the title compound (26) (1.20g, 71%). m.p. 146-148°C (ethanol/water) $[\alpha]_D$ -50 (c 1.9 chloroform). IR ($CHCl_3$) (cm^{-1}) 1520 s (N-H bend) 1680 s (C=O stretch) 1750 s (C=O stretch) 1800 w (C=O stretch) 3320 m (N-H stretch) 3450 w (N-H stretch). δH (270MHz, solvent $CDCl_3$, standard Me_4Si) 0.9-1.15 (72H, c, Me i-propyl) 2.2-2.45 (12H, c, CH i-propyl) 4.24 (1H, dd, J 4 & 7Hz, N-CH) 4.3-4.35 (3H, c, N-CH) 4.43 (1H, dd, J 6 & 7Hz, N-CH) 4.61 (1H, dd, J 6 & 8Hz, N-CH) 5.0-5.2 (7H, c, O-CH & $O-CH_2$) 5.19 (1H, d, J 4Hz, O-CH) 5.26 (1H, d, J 7Hz, NH carbamate) 7.1-7.3 (5H, c, NH amide) 7.36 (5H, s, C_6H_5). δC (68MHz, solvent $CDCl_3$, standard Me_4Si) 16.65 - 16.75 - 16.84 - 16.90 - 17.06 - 17.48 - 18.12 - 18.26 - 18.33 - 18.45 - 18.86 - 18.95 - 19.05 - 19.16 - 19.25 - 19.32 (Me i-propyl) 29.70 - 29.80 - 29.84 - 30.01 - 30.40 - 30.50 - 30.63 (CH i-propyl) 57.06 - 58.51 - 59.01 - 59.12 - 59.85 (N-CH) 67.48 ($O-CH_2$) 76.56 - 77.32 - 78.77 - 78.92 - 79.03 (O-CH) 128.06 - 128.53 - 128.69 (CH phenyl) 135.71 (C phenyl) 157.33 (C=O carbamate) 165.84 - 169.63 - 169.78 - 169.91 - 170.05 - 170.11 - 170.45 - 170.49 - 170.73 - 170.88 - 171.00 (C=O). δF (339Hz, solvent $CDCl_3$, standard C_6D_6) - 0.27 (2F, dd, J 17 & 22Hz, meta) 4.32 (1F, t, J 22Hz, para) 10.05 (2F,d, J 17Hz, ortho). Found C, 58.9; H, 7.3; N, 5.65. $C_{74}H_{110}N_6O_{21}F_5$ requires C, 58.7; H, 7.3; N, 5.55 %

Cyclo L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl (28) and Cyclo L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl (29).

Benzyloxycarbonyl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxy isovaleryl L-valyl L-α-hydroxyisovaleric acid pentafluorophenyl ester (25) (510mg, 0.46mmol) in 1,4-dioxane (10ml) was slowly added over a period of 24h to a well stirred mixture of dimethylaminopyridine (56mg, 0.46mmol) and palladium on charcoal (5%) (1.5g) in 1,4-dioxane (250ml) and absolute ethanol (5ml). During this period the temperature was kept at 90°C and hydrogen was bubbled through the reaction mixture. The catalyst was filtered off and the solvent removed. The residue was dissolved in ether and the precipitate which formed was filtered off. The ether solution was washed with dilute hydrochloric acid, dried ($MgSO_4$) and the solvent removed under reduced pressure. The residue was chromatographed on silica gel with a gradient of polarity (from light petroleum : ether, 1:1 to neat ether) to give the cyclic compounds (monomer (28) 20mg, 5%. dimer (29) 95mg, 26%). Monomer (28). m.p. 233-235°C (ethanol) $[\alpha]_D$ -120 (c 1.1 chloroform). IR ($CHCl_3$) (cm^{-1}) 1525 s (N-H bend) 1680 s (C=O stretch) 1745 s (C=O stretch) 3310 m (N-H stretch) 3440m (N-H stretch). δH (270MHz, solvent $CDCl_3$, standard Me_4Si) 0.97 (12H, d, J 7Hz, Me i-propyl) 0.97 (12H, d, J 7Hz, Me i-propyl) 0.98 (12H, d, J 7Hz, Me i-propyl) 1.04 (12H, d, J 7Hz, Me i-propyl) 2.25-2.4 (8H, c, CH i-propyl) 4.66 (4H, dd, J 6 & 8.5Hz, N-CH) 4.97 (4H, d, J 6Hz, O-CH) 6.68 (4H, d, J 8.5Hz, NH).δC (90MHz, solvent $CDCl_3$, standard Me_4Si) 17.70 - 18.13 - 18.90 - 19.57 (Me i-propyl) 29.74 - 30.42 (CH i-propyl) 57.95 (N-CH) 80.09 (O-CH) 169.05 - 170.71 (C=O). Found C, 60.15; H, 8.5; N, 6.8 %; M^+, 796 FAB.$C_{40}H_{68}N_4O_{12}$ requires C, 60.3; H, 8.6; N, 7.0 %; M, 796 Dimer.(29) m.p. 128-130°C (ether/hexane) $[\alpha]_D$ -46 (c 0.4 chloroform). IR ($CHCl_3$) (cm^{-1}) 1530 s (N-H bend) 1550 s (N-H bend) 1660 s

(C=O stretch) 1740 s (C=O stretch) 3320 m (N-H stretch) 3410 w (N-H stretch). δH (270MHz, solvent CDCl3, standard Me_4Si) 0.94 (24H, d, J 7Hz, Me i-propyl) 0.95 (24H, d, J 7Hz, Me i-propyl) 0.98 (24H, d, J 7Hz, Me i-propyl) 1.02 (24H, d, J 7Hz, Me i-propyl) 2.32 (16H, m, CH i-propyl) 4.58 (8H, dd, J 5 & 8Hz, N-CH) 5.06 (8H, d, J 5Hz, O-CH) 6.82 (8H, d, J 8Hz, NH). δC (90MHz, solvent $CDCl_3$, standard Me_4Si) 17.23 - 17.82 - 18.75 - 19.40 (Me i-propyl) 30.61 - 30.67 (CH i-propyl) 57.52 (N-CH) 79.16 (O-CH) 169.57 - 170.62 (C=O). Found C, 59.85; H, 8.5; N, 7.05 %; M^+, 1592 FAB. $C_{80}H_{136}N_8O_{24}$ requires C, 60.3; H, 8.6; N, 7.0 %; M^+, 1592

Cyclo L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl (13).

Method A. A solution of t-butoxycarbonyl L-valyl L-α-hydroxisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α hydroxyisovaleric acid (10) (340mg, 0.26mmol) in ethyl acetate (50ml) was saturated with hydrogen chloride gas. It was kept at room temperature for 2h, and then solvent was removed under reduced pressure. The residue was dissolved in N,N-dimethylformamide (52ml) and the pH adjusted to 9 by adding triethylamine. The solution was cooled down to -25°C and diphenylphosphoryl azide (57μl, 0.26mmol) was added with stirring. The mixture was kept at -25°C for 72h and then ethyl acetate (200ml) was added. The resulting organic phase was washed several times with water, dried ($MgSO_4$) and the solvent removed. The oily residue was purified by chromatography on silica gel with a gradient of polarity (from light petroleum : ether, 1:1 to neat ether). Qualitative analysis of each fraction was performed by NMR spectroscopy and all fractions that gave the correct NMR spectra were collected to yield the title compound (13) (9mg, 3%) as a solid.

Method B. A solution of L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid pentafluorophenyl ester trifluoroacetate (12) (355mg, 0.24mmol) in 1-4,dioxane (20ml) was added slowly over a period of 36h to a well stirred mixture of dimethylaminopyridine (116mg, 0.95mmol), absolute ethanol (6ml) and 1,4-dioxan (250ml) at 92°C. The solvent was removed under reduced pressure and the residue dissolved in ethyl acetate. The organic extract was washed successively with dilute hydrochloric acid and water. The organic solution was dried ($MgSO_4$), the solvent removed and the residue purified in the same way as above to yield the title compound (13)(41mg, 14%) as a solid.

Method C. A solution of benzyloxycarbonyl L-valyl L-α-hydroxyisovaleryl L-valyl L-α–hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleryl L-valyl L-α-hydroxyisovaleric acid pentafluorophenyl ester (26) (400mg, 0.26mmol) in 1-4,dioxane (20ml) was added slowly over a period of 6h to a well stirred mixture of dimethylaminopyridine (32mg, 0.26mmol) and palladium on charcoal (5%) (1.2g) in 1-4,dioxane (300ml) and absolute ethanol (6ml) at 90°C. Throughout this period a stream of hydrogen was passed through the reaction mixture. The catalyst was filtered off and the solvent removed. The residue was purified in the same way as above to yield the title compound (13) (222mg, 70%) as a white solid. m.p. 230-232°C (ethanol/water) $[\alpha]_D$ -19 (c 1.4 chloroform). IR ($CHCl_3$) (cm^{-1}) 1520 s (N-H bend) 1690 s (C=O stretch) 1745 s (C=O stretch) 3320 w (N-H stretch) 3450 m (N-H stretch). δH (270MHz, solvent $CDCl_3$, standard Me_4Si) 0.94 (18H, d, J 7Hz, Me Val) 0.96 (18H, d, J 7Hz, Me Hyiv) 0.99 (18H, d, J 7Hz, Me Hyiv) 1.02 (18H, d, J 7Hz, Me Val) 2.26 (6H, m, CH i-propyl Hyiv) 2.39 (6H, m, CH i-propyl Val) 4.81 (6H, dd, J 3.5 & 9.5Hz, N-CH) 4.87 (6H, d, J 5.5Hz, O-CH) 6.39 (6H, d, J 9.5Hz, NH). δC (90MHz, solvent $CDCl_3$, standard Me_4Si) 16.87 (Me Val) 17.48 - 18.59 (Me Hyiv) 19.41 (Me Val)

30.63 (CH i-propyl Hyiv) 31.68 (CH i-propyl Val) 55.99 (N-CH) 79.72 (O-CH) 168.71 (C=O amide) 171.17 (C=O ester). Found C, 59.95; H, 8.4; N, 6.85 %; M^+, 1196 FAB.$C_{60}H_{102}N_6O_{18}$ requires C, 60.3; H, 8.6; N, 7.0 %; M^+ 1195.

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