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Synthesis of Phenyl Substituted Valinomycins

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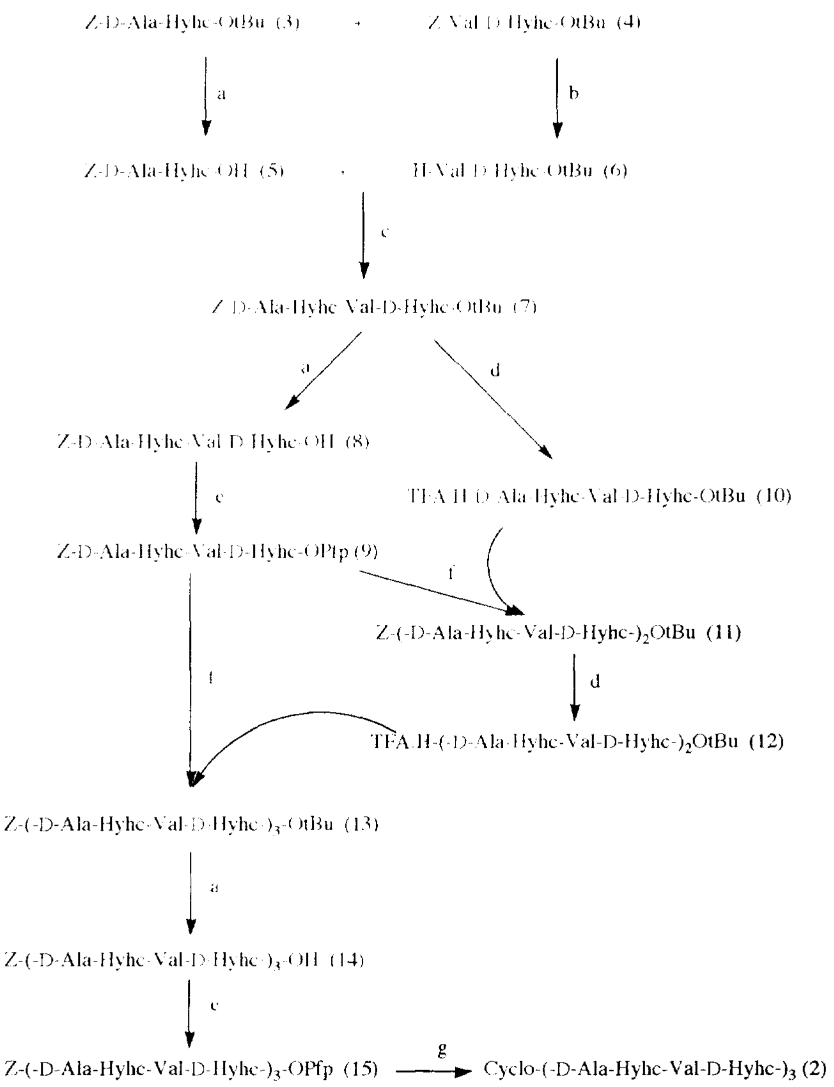
Abstract: *The synthesis is described of a modified valinomycin, which incorporates phenyl groups located around the exterior belt. The synthesis is accomplished by a build up of linear fragments using both dicyclohexylcarbodiimide- and pentafluoroester- methods of coupling. The final cyclisation is accomplished using the pentafluoroester protocol. The modified valinomycin is shown to be an effective ligand for complexation with potassium ion. Both nmr and electrochemical studies show that the modified valinomycin has similar properties to the parent valinomycin, and hence phenyl substitution around the periphery does not disrupt the network of hydrogen bonds which influence the conformation of valinomycins.*

In the two preceding papers[1,2] we have described the development of an efficient protocol for the key cyclisation step leading to cyclodepsipeptides and the use of this protocol in the synthesis of modified valinomycins. In this earlier paper by incorporation of aryl residues into the aminoacid building blocks, valinomycins have been synthesized carrying aryl residues close to the apertures of the valinomycin cavity. The alternative of incorporation of the aryl residues into the hydroxyacid moiety is likely to lead to modified valinomycins where the aryl residues are located away from the apertures. If the normal conformation[3] of the potassium complex of naturally occurring valinomycin (1) were to be adopted the aryl residues should be placed around the equator rather than the poles. As the conformation of natural valinomycin (1) in the form of the potassium complex is largely determined by the ligand metal ion interaction, and the network of hydrogen bonds[4] around the equator, i.e. the region where the aryl residues might be placed, it is important to establish whether such aryl substitution disrupts this network of hydrogen bonds, or alters the characteristics of metal ion binding. In the potassium ion complex of naturally occurring valinomycin the isopropyl residues of the valine residues screen the entrances of the cavity. Six amide hydrogen atoms and the six corresponding carbonyl oxygens form a belt of hydrogen bonds around the equator. The rigidity and stability of the potassium ion valinomycin complex derives in substantial part from this network of hydrogen bonds. In this paper the synthesis is described of a modified valinomycin (2) where the phenyl residues are incorporated in the hydroxyacid fragments. By nmr and electrochemical studies it is clearly established that such phenyl substitution does not interfere with the binding of metal ions or seriously modify the stability of the complexes. Hence in this and the preceding paper[2] the basis is laid for the synthesis of two series of modified valinomycins, which might act as potassium ion sensors by attachment to resins via a functionality located in the aryl residues.

The synthesis of the modified valinomycin (2) is shown in Scheme 1. The dipeptide fragments (3) and (4) were advanced through intermediates (5-14) in order to prepare the linear dodecapeptide (15). The coupling of the acid (5) with the amine (6) using dicyclohexylcarbodiimide proceeded in a satisfactory 92% yield. The later couplings to afford the dodecadepsipeptide were however not carried out using the diimide methodology. Instead the pentafluorophenyl ester (9) was coupled with the appropriate fragments (10) and (12). A difficulty was observed in the attempted dicyclohexylcarbodiimide coupling of the amine salt (10) with acids. The origin of the problem was traced to the instability of the free amine of the trifluoroacetate salt (10). Under conditions of attempted coupling of the free amine decomposition products were observed. Cleavage of the ester bond to afford D-alanine and a tridepsipeptide fragment easily occur. This process is clearly initiated by the terminal amino group and we attribute the unusual facility of this process to the relative lack of steric hindrance at this site. However the trifluoroacetate salt (10) is stable. The salts (10) and (12) were obtained by reductive cleavage of the benzyloxycarbonyl derivatives (7) and (11) in ethyl acetate containing 10% trifluoroacetic acid. These conditions avoided the destruction of the t-butyl ester group but permitted the isolation in near quantitative yield of the trifluoroacetate salts (10) and (12). These were then efficiently coupled with the pentafluoroester (9) under conditions which did not lead to cleavage to afford D-alanine. The coupled products, the octadepsipeptide (11) and the dodecadepsipeptide (13) were obtained in 64 and 59% yield respectively. The cyclisation of the linear dodecadepsipeptide was achieved via the pentafluorophenylester protocol. Thus the ester (14) was cyclized to afford the target cyclic dodecadepsipeptide (2) in 24% yield. This yield is substantially lower than the 84% yield[2] achieved in the preparation of the related modified valinomycin carrying the phenyl groups at the entrance to the aperture rather than round the equator. Two factors are likely to have lowered the yield of cyclisation. One is the possible cleavage of the terminal D-alanine residue as discussed above. However in view of the better yields (64 and 59%) obtained in coupling linear fragments under similar conditions, it is likely that a second factor is leading to reduced yields. The location of the aryl residues may profoundly affect the transition states for cyclisation and we suspect that adverse conformational factors contribute to the lowering of the observed yield.

The possibility that the phenyl residues, placed around the equator in the modified valinomycin (2) might modify the characteristics of ion binding was studied by nmr and by measurement of the stability constants associated with the binding of alkali metal ions. The ^1H and ^{13}C spectra of both the depsipeptide (2) and the potassium ion complex were simplified by an effective threefold symmetry. The observed HN-αCH vicinal coupling constants for D-Val (7.8Hz) and L-Val (6.4Hz) are similar to those observed for the D-Ala (6.5Hz) and L-Val (7.0Hz) residues in the modified valinomycin (2). Similar results were observed in the potassium ion complex of the modified valinomycin (2). Further spectroscopic evidence for the similar behaviour of the valinomycins (1) and (2) on complexation with potassium ion is seen from the ^{13}C nmr data. In both valinomycin (1) and the modified valinomycin (2) the resonances of the metal free ligand at 171-173ppm are displaced to 173-178ppm. Hence nmr evidence suggests that the conformation of the modified valinomycin (2) is not affected by phenyl substitution, and it may be assumed that phenyl substitution does not affect the network of hydrogen bonds which stabilize the conformations of valinomycins. These results are confirmed by electrochemical measurements across a membrane incorporating either valinomycin (1) or the modified valinomycin (2). The characteristics of metal ion transport shown by the two ligands is very similar. The selectivity order for both is $Rb^+>K^+>Cs^+>Na^+$ and both depsipeptides very efficiently bind potassium ions. Hence the position of the phenyl residues around the belt does not interfere with the process of metal ion complexation. A series of such aryl substituted valinomycins can be developed as sensors without concerns for the possible adverse influence of the aryl residues on either the conformation of the metal free ligands, or the process of metal ion complexation.

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;
d) H_2, Pd/C (10%), TFA, EtOAc, r.t.; e) PfPOH, DCC, CH_2Cl_2, 0°C; f) DMAP, dioxan, 90°C;
g) H_2, Pd/C (5%), DMAP, dioxan, EtOH, 90°C.

Experimental

General experimental details are described elsewhere[1].

Benzyloxycarbonyl D-alanyl L-α-hydroxyhydrocinnamic acid t-butyl ester (3)

To a solution of benzyloxycarbonyl D-alanine (5.32g, 23.9mmol), L-α-hydroxyhydrocinnamic acid t-butyl ester (4.82g, 22.7mmol) and dimethylaminopyridine (0.26g, 2.2mmol) in dichloromethane (80ml) at 0°C was added dicyclohexylcarbodiimide (4.92g, 23.9mmol). The mixture was kept at room temperature for 24h., the precipitate of dicyclohexylurea was filtered off, the solvent removed and the residue purified by suction chromatography (light petroleum : ether, 4-1) to give the title compound (3) (8.16g, 84%) as an oil, $[\alpha_D]$ -7 (c 1.2, chloroform) IR ($CHCl_3$) (cm^{-1}) 1520 s , 1735 s, 3450 m; δH (270MHz, solvent $CDCl_3$, standard Me_4Si) 1.29 (3H, d, J 7, Me), 1.40 (9H, s, Me, t-Bu), 3.09 and 3.15 (2H, ABX, J 4.5, 9, and 14.5, CH_2 Hyhc), 4.45 (1H, m, NCH), 5.09 (2H, s, OCH_2), 5.12 (1H, ABX, J 4.5 and 9, OCH), 5.30 (1H, d, J 7.5, NH) and 7.2-7.4 (10H, c, C_6H_5) δC (68MHz, solvent $CDCl_3$, standard Me_4Si) 18.60 (Me, Ala), 27.91 (Me, t-butyl), 37.27 (CH_2 Hyhc) 49.64 (N-CH), 66.90 (OCH_2), 74.00 (OCH), 82.68 (C t-butyl), 127.05 - 128.11 - 128.18 - 128.44 - 129.39 (CH phenyl) 135.86 - 136.30 (C phenyl) 155.43 (C=O carbamate) and 168.00 and 172.08 (C=O).

Benzyloxycarbonyl L-valyl D-α-hydroxyhydrocinnamic acid t-butyl ester (4).

To a solution of benzyloxycarbonyl L-valine (1.24g, 4.96mmol), D-α-hydroxyhydrocinnamic acid t-butyl ester (1.00g, 4.50mmol) and dimethylaminopyridine (55mg, 0.45mmol) in dichloromethane (30ml) at 0°C was added dicyclohexylcarbodiimide (1.02g, 4.96mmol). The mixture was stirred at room temperature for 24h, the precipitate of dicyclohexylurea was filtered off, the solvent removed and the residue purified by chromatography on silica gel (light petroleum : ether, 3-1) to give as a solid the title compound (4) (1.48g, 72%). m.p. 77-79°C (hexane). $[\alpha]_D$ +6 (c 1.2 chloroform). IR ($CHCl_3$) (cm^{-1}) 1510 s (N-H bend) 1735 s (C=O stretch) 3450 m (N-H stretch). δH (270Mhz, solvent $CDCl_3$, standard Me_4Si) 0.66 (3H, d, J 7Hz, Me Val) 0.87 (3H, d, J 7Hz, Me Val) 1.40 (9H, s, Me t-butyl) 2.06 (1H, m, CH i-propyl) 3.07 & 3.15 (2H, ABX, J 4.5, 9 & 14Hz, CH_2 Hyhc) 4.36 (1H, dd, J 4.5 & 9Hz, N-CH) 5.08 (2H, s, O-CH_2) 5.14 (1H, ABX, J 4.5 & 9Hz, O-CH) 5.23 (1H, d, J 9Hz, NH) 7.2-7.35 (10H, c, C_6H_5). δC (68Mhz, solvent $CDCl_3$, standard Me_4Si) 17.00 - 18.84 (Me Val) 27.81 (Me t-butyl) 31.14 (CH i-propyl) 37.23 (CH_2 Hyhc) 58.90 (N-CH) 66.92 (O-CH_2) 74.02 (O-CH) 82.50 (C t-butyl) 126.95 - 128.07 - 128.11 - 128.40 - 128.47 - 129.24 (CH phenyl) 135.86 - 136.28 (C phenyl) 156.03 (C=O carbamate) 168.00 - 171.15 (C=O) Found C, 68.8; H, 7.3; N, 3.1.$C_{26}H_{33}NO_6$ requires C, 68.55; H, 7.3; N, 3.05 %

Benzyloxycarbonyl D-alanyl L-α-hydroxyhydrocinnamic acid (5).

A solution of benzyloxycarbonyl D-alanyl L-α-hydroxyhydrocinnamic acid t-butyl ester (3) (9.54g, 22.34mmol) in dichloromethane (40ml) was treated with trifluoroacetic acid (40ml) for 6h. The solvent was removed and the residue dissolved in aqueous sodium carbonate solution. It was then washed with ether. The aqueous solution was acidified with dilute hydrochloric acid and extracted with ether. The latter ethereal extract was dried (Na_2SO_4) and the solvent removed under reduced pressure to yield the title compound (5) (7.91g, 95%) as an oil. $[\alpha]_D$ -6 (c 3.3 chloroform). IR ($CHCl_3$) (cm^{-1}) 1515 s (N-H bend) 1730 s (C=O stretch) 3000 br (O-H stretch) 3440 m (N-H stretch). δH (270Mhz, solvent $CDCl_3$, standard Me_4Si) 1.23 (3H, d, J

7.5Hz, Me) 3.13 & 3.24 (2H, ABX, J 3.5, 9 & 14Hz, CH_2 Hyhc) 4.43 (1H, m, N-CH) 5.06 & 5.08 (2H, AB, J 12.5Hz, OCH_2) 5.31 (1H, ABX, J 3.5 & 9Hz, O-CH) 5.38 (1H, d, J 7.5Hz, NH)7.15-7.35 (10H, c, C_6H_5).δC (68Mhz, solvent $CDCl_3$, standard Me_4Si) 18.25 (Me) 37.09 (CH_2 Hyhc) 49.59 (N-CH) 67.18 (O-CH_2) 73.24 (O-CH) 127.20 - 128.19 - 128.30 - 128.58 - 129.32 (CH phenyl) 135.59 - 136.07 (C phenyl) 155.79 (C=O carbamate) 172.22 - 173.12 (C=O).

L·Valyl D-α-hydroxyhydrocinnamic acid t-butyl ester (6).

To a solution of benzyloxycarbonyl L-valyl D-α-hydroxyhydrocinnamic acid t-butyl ester (4) (6.93g, 15.2mmol) in absolute ethanol (80ml) was added palladium on charcoal (10%) (700mg). The mixture was hydrogenated for 12h, then filtered and solvent was removed to afford the title compound (6) (4.89g, 100%) as an oil. $[\alpha]_D$ +6 (c 1.2 chloroform). IR ($CHCl_3$) (cm^{-1}) 1515 s (N-H bend) 1605 w (N-H bend) 1740 s (C=O stretch) 3410 w (N-H stretch) 3520 w (N-H stretch). δH (270Mhz, solvent $CDCl_3$, standard Me_4Si) 0.74 (3H, d, J 7Hz, Me Val) 0.92 (3H, d, J 7Hz, Me Val) 1.41 (9H, s, Me t-butyl) 2.05 (1H, m, CH i-propyl) 3.23 & 3.51 (2H, ABX, J 4.5, 9 & 14Hz, CH_2 Hyhc) 3.51 (1H, d, J 4.5Hz, N-CH) 5.16 (1H, ABX, J 4.5 & 9Hz, O-CH) 7.2-7.35 (10H, c, C_6H_5). δC (68Mhz, solvent $CDCl_3$, standard Me_4Si) 17.15 - 18.83 (Me Val) 27.86 (Me t-butyl) 30.85 (CH i-propyl) 37.30 (CH_2 Hyhc) 59.16 (N-CH) 74.08 (O-CH) 82.59 (C t-butyl) 126.98 - 128.43 - 129.32 (CH phenyl) 135.87 (C phenyl) 168.11 - 172.07 (C=O).

Benzyloxycarbonyl D-alanyl L-α-hydroxyhydrocinnamyl L-valyl D-α-hydroxy hydrocinnamic acid t-butyl ester (7).

A solution of dicyclohexylcarbodiimide (1.56g, 7.59mmol) in dichloromethane (5ml) at 0°C was added to a solution of benzyloxycarbonyl D-alanyl L-α-hydroxyhydrocinnamic acid (5) (2.81g, 7.59mmol), L-valyl D-α-hydroxyhydrocinnamic acid t-butyl ester (6) (2.03g, 6.32mmol), dimethylaminopyridine (77mg, 0.63mmol) and N-methylmorpholine (0.83ml, 7.59mmol) in dichloromethane (55ml) at 0°C. The mixture was stirred for 12h and the precipitate of urea was filtered off. The residue was purified by chromatography on silica gel (light petroleum : ether, 1-1) to afford the title compound (7) (3.95g, 92%) as a solid. m.p. 97-99°C (ether / hexane) $[\alpha]_D$ +10 (c 1.2 chloroform). IR ($CHCl_3$) (cm^{-1}) 1515 s (N-H bend) 1690 m (C=O stretch) 1745 s (C=O stretch) 3340 w (N-H stretch) 3450 m (N-H stretch). δH (270Mhz, solvent $CDCl_3$, standard Me_4Si) 0.58 (3H, d, J 7Hz, Me Val) 0.70 (3H, d, J 7Hz, Me Val) 1.22 (3H, d, J 7.5Hz, Me Ala) 1.37 (9H, s, Me t-butyl) 2.03 (1H, m, CH i-propyl) 3.0-3.3 (4H, c, CH_2 Hyhc) 4.34 (1H, m, N-CH Ala) 4.53 (1H, dd, J 5 & 9Hz, N-CH-Val) 5.01 & 5.06 (2H, AB, J 12.5Hz, O-CH_2) 5.11 (1H, ABX, J 5 & 9Hz, O-CH) 5.46 (1H, ABX, J 4 & 8Hz, O-CH) 5.49 (1H, d, J 7Hz, NH carbamate) 6.70 (1H, d, J 9Hz, NH amide) 7.1-7.4 (15H, c, C_6H_5). δC (68Mhz, solvent $CDCl_3$, standard Me_4Si) 17.27 - 17.46 - 18.70 (Me Ala & Val) 27.79 (Me t-butyl) 30.69 (CH i-propyl) 37.21 - 37.51 (CH_2 Hyhc) 46.73 (N-CH Ala) 56.90 (N-CH Val) 67.02 (O-CH_2) 74.11 - 74.74 (O-CH) 82.60 (C t-butyl) 126.96 - 128.08 - 128.16 - 128.27 - 128.39 - 128.47 - 129.27 - 129.65 (CH phenyl) 135.63 - 135.80 - 136.11 (C phenyl) 155.78 (C=O carbamate) 168.12 - 168.39 - 170.65 - 171.63 (C=O). Found C, 67.65; H, 6.8; N, 4.15.$C_{38}H_{46}N_2O_9$ requires C, 67.65; H, 6.85; N, 4.15 %

Benzyloxycarbonyl D-alanyl L-α-hydroxyhydrocinnamyl L-valyl D-α-hydroxy hydrocinnamic acid (8).

A solution of benzyloxycarbonyl D-alanyl L-α-hydroxyhydrocinnamyl L-valyl D-α-hydroxyhydrocinnamic acid t-butyl ester (7) (1.25g, 1.85mmol) in dichloromethane (10ml) and trifluoroacetic acid (10ml) was left at room temperature for 5h. Removal of solvent afforded the title product (8) (1.14g, 100%) as an oil. $[\alpha]_D$ -3 (c

1.5 chloroform). IR ($CHCl_3$) (cm^{-1}) 1515 s(N-H bend) 1690m(C=O stretch) 1740 s (C=O stretch) 3340 w (N-H stretch) 3450 m (N-H stretch).δH (270Mhz, solvent $CDCl_3$, standard Me_4Si) 0.59 (3H, d, J 7Hz, Me Val) 0.66 (3H, d, J 7Hz, Me Val) 1.19 (3H, d, J 7.5Hz, Me Ala) 2.00 (1H, m, CH i-propyl) 3.0-3.3 (4H, c, CH_2 Hyhc) 4.25 (1H, m, N-CH Ala) 4.42 (1H, dd, J 6 & 8Hz, N-CH Val) 4.99 & 5.05 (2H, AB, J 12Hz, O-CH_2) 5.29 (1H, ABX, J 3.5 & 9.5Hz, O-CH) 5.38 (1H, d, J 7Hz, NH carbamate) 5.45 (1H, ABX, J 4 & 8Hz, O-CH) 6.83 (1H, d, J 8Hz, NH amide) 7.1-7.4 (15H, c, C_6H_5). δC (68Mhz, solvent $CDCl_3$, standard Me_4Si) 17.43 - 17.57 - 18.75 (Me Ala & Val) 30.26 (CH i-propyl) 37.06 - 37.65 (CH_2 Hyhc) 49.81 (N-CH Ala) 57.50 (N-CH Val) 67.43 (O-CH_2) 73.69 - 74.82 (O-CH) 127.12 - 128.20 - 128.38 - 128.49 - 128.57 - 128.62 - 129.28 - 129.66 (CH phenyl) 135.53 - 135.84 - 135.89 (C phenyl) 156.25 (C=O carbamate) 169.39 - 170.90 - 171.72 - 171.80 (C=O). found $M^{+\cdot}$ 618 (FAB) $C_{34}H_{38}N_2O_9$ requires M^+, 618.

Benzyloxycarbonyl D-alanyl L-α-hydroxyhydrocinnamyl L-valyl D-α-hydroxy hydrocinnamic acid pentafluorophenyl ester (9).

A solution of dicyclohexylcarbodiimide (0.54g, 2.62mmol) in ethyl acetate (10ml) was added to a solution of pentafluorophenol (1.44g, 7.86mmol) in ethyl acetate (10ml) at 0°C. After reaction for 10min the mixture was added to a solution of benzyloxycarbonyl D-alanyl L-α-hydroxyhydrocinnamyl L-valyl D-α-hydroxy-hydrocinnamic acid (8) (1.62g, 2.62mmol) in ethyl acetate (20ml) at 0°C. The mixture was stirred for 4h, the precipitate was filtered off. The solvent removed and the residue chromatographed on silica gel (light petroleum : ether, 2-1) to give as an oil the title compound (9) (1.57g, 76%). $[\alpha]_D$ +9 (c 2.8 chloroform). IR ($CHCl_3$) (cm^{-1}) 1525 s (N-H bend) 1690 m (C=O stretch) 1720 s (C=O stretch) 1760 s (C=O stretch) 1800 m (C=O stretch) 3350 w (N-H stretch) 3450 m (N-H stretch). δH (270Mhz, solvent $CDCl_3$, standard Me_4Si) 0.65 (3H, d, J 7Hz, Me Val) 0.73 (3H, d, J 7Hz, Me Val) 1.21 (3H, d, J 7Hz, Me Ala) 2.05 (1H, m, CH i-propyl) 3.05-3.4 (4H, c, CH_2 Hyhc) 4.28 (1H, m, N-CH Ala) 4.51 (1H, dd, J 5.5 & 8.5Hz, N-CH Val) 4.97 & 5.05 (2H, AB, J 12Hz, O-CH_2) 5.24 (1H, d, J 6.5Hz, NH carbamate) 5.45-5.5 (2H, c, O-CH) 6.83 (1H, d, J 8.5Hz, NH amide) 7.15-7.35 (15H, c, C_6H_5) δC (68Mhz, solvent $CDCl_3$, standard Me_4Si) 17.35 - 17.67 - 18.78 (Me Ala & Val) 30.70 (CH i-propyl) 37.19 37.57 (CH_2 Hyhc) 49.98 (N-CH Ala) 57.34 (N-CH Val) 67.26 (O-CH_2) 73.15 - 74.70 (O-CH) 127.10 - 127.60 - 128.18 - 128.35 - 128.51- 128.61 - 129.83 - 129.36 - 129.69 (CH phenyl) 134.64 - 135.78 136.07 (C phenyl) 155.97 (C=O carbamate) 165.63 - 168.81 - 170.77 - 171.92 (C=O).

D-Alanyl L-α-hydroxyhydrocinnamyl L-valyl D-α-hydroxyhydrocinnamic acid t-butyl ester trifluoroacetate (10).

To a solution of benzyloxycarbonyl D-alanyl L-α-hydroxyhydrocinnamyl L-valyl D-α–hydroxyhydrocinnamic acid t-butyl ester (7) (820mg, 1.22mmol) in ethyl acetate (36ml) and trifluoroacetic acid (4ml) was added palladium on charcoal (10%) (250mg). The mixture was hydrogenated for 4h. The catalyst was filtered off and the solvent removed to yield the title compound (10) (795mg, 100%) as an oil. $[\alpha]_D$ -4 (c 2.3 chloroform). IR ($CHCl_3$) (cm^{-1}) 1530 m (N-H bend) 1680 m (C=O stretch) 1730 s (C=O stretch) 1745 s (C=O stretch) 1760 s (C=O stretch) 3000 br (N-H & O-H stretch). δH (270Mhz, solvent $CDCl_3$, standard Me_4Si) 0.60 (3H, d, J 6.5Hz, Me Val) 0.70 (3H, d, J 6.5Hz, Me Val) 1.33 (3H, d, J 7Hz, Me Ala) 1.39 (9H, s, Me t-butyl) 1.98 (1H, m, CH i-propyl) 2.95-3.25 (4H, c, CH_2 Hyhc) 4.15 (1H, br, N-CH Ala) 4.4 (1H, br, N-CH Val) 5.12 (1H, ABX, J4.5 & 9Hz, O-CH) 5.36 (1H, br, O-CH) 7.06 (1H, d, J8Hz, NH) 7.1-7.3 (15H, c, C_6H_5) δC (68Mhz, solvent $CDCl_3$, standard Me_4Si) 15.48 - 17.48 - 18.65 (Me Ala & Val) 27.82 (Me t-butyl) 30.50 (CH i-propyl) 37.28 - 37.60 (CH_2) 49.14 (N-CH Ala) 57.67 (N-CH Val) 74.36 - 76.04 (O-CH) 83.25 (C t-butyl)

127.17 - 127.33 - 128.58 128.65 - 129.29 - 129.50 (CH phenyl) 135.00 - 135.66 (C phenyl) 168.65 - 168.20 - 169.50 - 170.97 (C=O).

Benzyloxycarbonyl D-alanyl L-α-hydroxyhydrocinnamyl L-valyl D-α-hydroxy hydrocinnamyl D-alanyl L-α-hydroxyhydrocinnamyl L-valyl D-α-hydroxyhydrocinnamic acid t-butyl ester (11).

To a solution of benzyloxycarbonyl D-alanyl L-α-hydroxyhydrocinnamyl L-valyl D-α-hydroxyhydrocinnamic acid pentafluorophenyl ester (9) (198mg, 0.25mmol) and D-alanyl L-α-hydroxyhydrocinnamyl L-valyl D-α-hydroxyhydrocinnamic acid t-butyl ester trifluoroacetate (10) (194mg, 0.30mmol) in 1,4-dioxane (10ml) at 95°C was added dimethylaminopyridine (36mg, 0.30mmol). The mixture was stirred for 1h and filtered. The solvent was removed and the residue purified by chromatography on silica gel (light petroleum : ether, 1-2) to afford the title compound (11) (186mg, 64%) as an oil.$[\alpha]_D$ +4 (c 1.2 chloroform). IR ($CHCl_3$) (cm^{-1}) 1540 s (N-H bend) 1670 m (C=O stretch) 1715 s (C=O stretch) 1755 s (C=O stretch) 3340 m (N-H stretch) 3450 w (N-H stretch). δH (270Mhz, solvent $CDCl_3$, standard Me_4Si) 0.49 (3H, d, J 7Hz, Me Val) 0.66 (3H, d, J 7Hz, Me Val) 0.69 (3H, d, J 7Hz, Me Val) 0.73 (3H, d, J 7Hz, Me Val) 1.15 (3H, d, J 7Hz, Me Ala) 1.28 (3H, d, J 7Hz, Me Ala) 1.35 (9H, s, Me t-butyl) 1.92 (1H, m, CH i-propyl) 2.09 (1H, m, CH i-propyl) 2.9-3.35 (8H, c, CH_2 Hyhc) 3.90 (1H, dd, J 7 & 8Hz, N-CH Val) 4.14 (1H, m, N-CH Ala) 4.34 (1H, m, N-CH Ala) 4.48 (1H, dd, J 5.5 & 8.5Hz, N-CH Val) 4.94 & 5.01 (2H, AB, J 12.5Hz, O-CH_2) 5.08 (1H, ABX, J 5 & 8.5Hz, O-CH) 5.4-5.6 (4H, c, O-CH & NH carbamate) 7.1-7.3 (27H, c, C_6H_5 & NH amide) 7.62 (1H, d, J 6Hz, NH amide). δC (68Mhz, solvent $CDCl_3$, standard Me_4Si) 16.32 - 16.63 - 17.79 - 18.59 - 18.66 - 18.78 (Me Ala & Val) 27.82 (Me t-butyl) 29.05 - 30.63 (CH i-propyl) 37.25 - 37.35 - 37.53 (CH_2 Hyhc) 48.84 - 49.97 (N-CH Ala) 57.47 - 59.48 (N-CH Val) 67.24 (O-CH2) 74.14 - 74.30 - 74.44 - 74.63 (O-CH) 82.35 (C t-butyl) 126.79 - 126.87 - 127.00 - 128.11 - 128.26 - 128.34 - 128.37 - 128.41 - 128.52 - 129.40 - 129.50 - 129.69 - 129.71 (CH phenyl) 135.71 - 135.97 - 136.01 - 136.11 - 136.37 (C phenyl) 156.33 (C=O carbamate) 168.25 - 168.82 - 169.47 - 170.12 - 170.62 - 170.65 - 171.32 - 172.06 (C=O)

D-Alanyl L-α-hydroxyhydrocinnamyl L-valyl D-α-hydroxyhydrocinnamyl D-alanyl L-α-hydroxy hydrocinnamyl L-valyl D-α-hydroxyhydrocinnamic acid t-butyl ester trifluoroacetate (12).

A mixture of benzyloxycarbonyl D-alanyl L-α-hydroxyhydrocinnamyl L-valyl D-α-hydroxyhydrocinnamyl D-alanyl L–α-hydroxyhydrocinnamyl L-valyl D-α-hydroxyhydrocinnamic acid t-butyl ester (11) (186mg, 0.16mmol), ethyl acetate (9ml), trifluoroacetic acid (1ml) and palladium on charcoal (10%) (100mg) was hydrogenated for 5h. The catalyst was filtered off and the solvent removed to yield the title compound (12) (181mg, 99%) as an oil. $[\alpha]_D$ -1 (c 1.2 chloroform). IR ($CHCl_3$) (cm^{-1}) 1530 s (N-H bend) 1680 s (C=O stretch) 1715 m (C=O stretch) 1755 s (C=O stretch) 3000 br (N-H & O-H stretch) 3340 m (N-H stretch).δC (68Mhz, solvent $CDCl_3$, standard Me_4Si) 15.68 - 15.73 - 17.73 - 18.40 - 18.75 - 19.18 (Me Ala & Val) 27.81 (Me t-butyl) 29.75 - 30.21 (CH i-propyl) 37.29 - 37.55 - 37.73 (CH_2) 48.99 - 49.38 (N-CH Ala) 57.89 - 60.39 (N-CH Val) 74.34 - 75.05 - 75.39 - 75.97 (O-CH) 82.87 (C t-butyl) 127.04 - 127.30 - 128.48 - 128.55 - 129.29 - 129.36 - 129.42 - 129.62 - (CH phenyl) 135.00 - 135.02 - 135.87 - 136.10 (C phenyl) 168.45 - 169.93 - 170.29 - 170.35 - 170.94 - 170.47 - 172.86 (C=O)

Benzyloxycarbonyl D-alanyl L-α-hydroxyhydrocinnamyl L-valyl D-α-hydroxyhydro cinnamyl D-alanyl L-α-hydroxyhydrocinnamyl L-valyl D–α-hydroxyhydrocinnamyl D-alanyl L-α-hydroxyhydrocinnamyl L-valyl D–α–hydroxyhydrocinnamic acid t-butyl ester (13).

To a solution of benzyloxycarbonyl D-alanyl L-α-hydroxyhydrocinnamyl L-valyl D–α-hydroxyhydrocinnamic acid pentafluorophenyl ester (9) (139mg, 0.17mmol) and D-alanyl L-α-hydroxyhydrocinnamyl L-valyl D-α-hydroxyhydrocinnamyl D-alanyl L-α–hydroxyhydrocinnamyl L-valyl D-α-hydroxyhydrocinnamic acid t-butyl ester trifluoroacetate (12) (186mg, 0.17mmol) in 1,4-dioxane (10ml) was added dimethylaminopyridine (36mg, 0.30mmol) at 93°C. The mixture was stirred for 1h, the precipitate was filtered off and solvent removed. The residue was purified by chromatography on silica gel (light petroleum : ether, 1-2) to give the title compound (13) (140mg, 59%) as an oil. $[\alpha]_D$ +11 (c 2.8 chloroform). IR ($CHCl_3$) (cm^{-1}) 1540 s (N-H bend) 1670 m (C=O stretch) 1715 m (C=O stretch) 1755 s (C=O stretch) 3340 m (N-H stretch) 3450 w (N-H stretch) δH (270Mhz, solvent $CDCl_3$, standard Me_4Si) 0.45 (3H, d, J 6.5Hz, Me Val) 0.56 (3H, d, J 6.5Hz, Me Val) 0.66 (3H, d, J 6.5Hz, Me Val) 0.69 (3H, d, J 6.5Hz, Me Val) 0.7 0.75 (6H, c, Me Val) 1.15 (3H, d, J 7Hz, Me Ala) 1.29 (3H, d, J 7Hz, MeAla) 1.31 (3H, d, J 7Hz, Me Ala) 1.35 (9H, s, Me t-butyl) 1.8-2.15 (3H, c, CH i-propyl) 2.85-3.3 (12H, c, CH_2 Hyhc) 3.83 (1H, dd, J 7 & 8Hz, N-CH·Val) 3.93 (1H, dd, J 7 & 8Hz, N-CH·Val) 4.1-4.35 (3H, m, N-CH Ala) 4.46 (1H, dd, J 5.5 & 8.5Hz, N-CH·Val) 4.91 & 5.00 (2H, AB, J 12Hz, O-CH_2) 5.08 (1H, ABX, J 5 & 8.5Hz, O-CH) 5.3-5.6 (7H, c, O-CH & NH carbamate) 7.1-7.3 (37H, c, C_6H_5 & NH amide) 7.60 (1H, d, J 6Hz, NH amide) 7.81 (1H, d, J 6Hz, NH amide) 7.86 (1H, d, J 6Hz, NH amide). δC (68Mhz, solvent $CDCl_3$, standard Me_4Si) 15.83 - 16.43 - 16.52 - 17.93 - 18.53 - 18.66 - 18.79 - 18.87 - 18.92 (Me Ala & Val) 27.82 (Me t-butyl) 28.77 - 29.20 - 30.53 (CH i-propyl) 37.27 - 37.41 - 37.47 (CH_2 Hyhc) 49.06 - 49.16 50.01 (N-CH Ala) 57.60 - 59.56 - 59.73 (N-CH Val) 67.23 (O-CH_2) 74.11 - 74.33 - 74.49 - 74.56 (O-CH) 82.33 (C t-butyl) 126.74 - 126.78 - 126.85 - 126.90 - 126.93 - 127.04 - 128.06 - 128.31 - 128.35 - 128.38 - 128.42 - 128.45 - 128.52 - 129.30 - 129.42 - 129.47 - 129.59 - 129.75 (CH phenyl) 135.66 - 135.97 - 136.04 - 136.07 - 136.10 - 136.20 - 136.49 (C phenyl) 156.44 (C=O carbamate) 168.26 - 168.95 - 169.69 - 170.21 - 170.29 - 170.51 - 170.57 - 170.85 - 171.03 - 171.20 - 171.29 - 172.24 (C=O).

Benzyloxycarbonyl D-alanyl L-α-hydroxyhydrocinnamyl L-valyl D-α-hydroxy hydrocinnamyl D-alanyl L-α-hydroxyhydrocinnamyl L·valyl D–α-hydroxyhydrocinnamyl D-alanyl L-α-hydroxyhydrocinnamyl L-valyl D-α-hydroxyhydrocinnamic acid (14).

A solution of benzyloxycarbonyl D-alanyl L-α-hydroxyhydrocinnamyl L-valyl D-α-hydroxyhydrocinnamyl D-alanyl L-α-hydroxyhydrocinnamyl L-valyl D-α-hydroxyhydrocinnamyl D-alanyl L-α-hydroxyhydro cinnamyl L-valyl D–α-hydroxyhydrocinnamic acid t-butyl ester (13) (240mg, 0.14mmol) in dichloromethane (5ml) and trifluoroacetic acid (5ml) was kept at room temperature for 5h. Removal of solvent under reduced pressure afforded the title compound (14) (230mg, 99%) as an oil. $[\alpha]_D$ +7 (c 2.6 chloroform). IR ($CHCl_3$) (cm^{-1}) 1540 s (N-H bend) 1670 m (C=O stretch) 1715 m (C=O stretch) 1755 s (C=O stretch) 3340 m (N-H stretch) 3450 w (N-H stretch). δH (270Mhz, solvent $CDCl_3$, standard Me_4Si) 0.45 (3H, d, J 6.5Hz, Me Val) 0.55 (3H, d, J 6.5Hz, Me Val) 0.65-0.7 (9H, c, Me Val) 0.71 (3H, d, J 6.5Hz, Me Val) 1.16 (3H, d, J 7Hz, Me Ala) 1.25-1.3 (6H, c, Me Ala) 1.8-2.1 (3H, c, CH i-propyl) 2.85-3.3 (12H, c, CH_2 Hyhc) 3.8-4.45 (6H, c, N-CH) 4.91 & 5.01 (2H, AB, J 12Hz, O-CH_2) 5.25-5.5 (6H, c, O-CH) 5.80 (1H, d, J 7Hz, NH carbamate) 7.05-7.4 (37H, c, C_6H_5 & NH amide) 7.57 (1H, d, J 7.5Hz, NH amide) 7.83 (1H, d, J 6Hz, NH amide) 7.86 (1H, d, J 7Hz, NH amide) δC (68Mhz, solvent $CDCl_3$, standard Me_4Si) 15.90 - 16.27 - 16.63 - 17.72 - 18.53 - 18.74 - 18.82 (Me Ala & Val) 28.87 - 29.19 - 30.24 (CH i-propyl) 37.17 - 37.22 - 37.50 (CH_2 Hyhc)

48.84 - 49.08 -50.00 (N-CH Ala) 57.50 - 59.45 - 59.66 (N-CH Val) 67.23 (O-CH_2) 73.75 - 74.41 - 74.49 - 74.62 - 74.85 (O-CH) 126.97 - 128.05 - 128.47 - 129.33 - 129.40 - 129.43 - 129.50 - 129.60 (CH phenyl) 135.58 - 135.81 - 135.91 - 135.95 - 136.01 - 136.10 - 136.15 (C phenyl) 156.51 (C=O carbamate) 169.47 - 170.19 - 170.44 - 170.49 - 170.57 - 170.63 - 170.91 - 171.04 - 171.17 - 171.35 (C=O). found $M^{+\cdot}$ 1550 (FAB) $C_{86}H_{98}N_6O_{21}$ requires M^+, 1550.

Benzyloxycarbonyl D-alanyl L-α-hydroxyhydrocinnamyl L-valyl D-α-hydroxy hydrocinnamyl D-alanyl L-α-hydroxyhydrocinnamyl L-valyl D-α-hydroxyhydrocinnamyl D-alanyl L-α-hydroxyhydrocinnamyl L-valyl D-α-hydroxyhydrocinnamic acid penta-fluorophenyl ester (15).

A solution of pentafluorophenol (82mg, 0.45mmol) in ethyl acetate (1ml) was added to a solution of dicyclohexylcarbodiimide (31mg, 0.15mmol) in ethyl acetate (1ml). The solution was kept at 0°C for 10min and was then added to a solution of benzyloxycarbonyl D-alanyl L-α-hydroxyhydrocinnamyl L-valyl D-α-hydroxyhydrocinnamyl D-alanyl L-α-hydroxyhydrocinnamyl L-valyl D-α-hydroxyhydrocinnamyl D-alanyl L-α-hydroxyhydrocinnamyl L-valyl D–α-hydroxyhydrocinnamic acid (14) (230mg, 0.15mmol) in ethyl acetate (3ml) at 0°C. The mixture was stirred for 4h, solvent was removed and the residue was purified by chromatography on silica gel (light petroleum : ether, 1-2) to give the title compound (15) (114mg, 45%) as an oil. $[\alpha]_D$ +5 (c 1.9 chloroform). IR ($CHCl_3$) (cm^{-1}) 1540 s (N-H bend) 1670 s (C=O stretch) 1715 m (C=O stretch) 1755 s (C=O stretch) 1800 w (C=O stretch) 3340 m (N-H stretch) 3450 w (N-H stretch). δH (270Mhz, solvent $CDCl_3$, standard Me_4Si) 0.45 (3H, d, J 6.5Hz, Me Val) 0.53 (3H, d, J 6.5Hz, Me Val) 0.66 (3H, d, J 6.5Hz, Me Val) 0.73 (3H, d, J 6.5Hz, Me Val) 0.75-0.8 (6H, c, Me Val) 1.14 (3H, d, J 7Hz, Me Ala) 1.25-1.3 (6H, c, Me Ala) 1.9-2.1 (2H, c, CH i-propyl) 2.17 (1H, m, CH i-propyl) 2.85-3.35 (12H, c, CH_2 Hyhc) 3.8-3.9 (2H, c, N-CH) 4.05-4.25 (3H, c, N-CH) 4.42 (1H, dd, J 6.5 & 8Hz, N-CH Val) 4.91 & 5.01 (2H, AB, J 12Hz, O-CH_2) 5.3-5.6 (7H, c, O-CH & NH carbamate) 7.1-7.35 (36H, c, C_6H_5 & NH amide) 7.45 (1H, d, J 8Hz, NH amide) 7.61 (1H, d, J 6Hz, NH amide) 7.79 (1H, d, J 6Hz, NH amide) 7.95 (1H, d, J 5Hz, NH amide). δC (68Mhz, solvent $CDCl_3$, standard Me_4Si) 15.79 - 16.09 - 16.50 - 18.23 - 18.56 - 18.82 - 18.92 - 19.04 (Me Ala & Val) 28.77 - 29.16 - 30.30 (CH i-propyl) 35.15 - 37.24 - 37.28 - 37.48 (CH_2 Hyhc) 49.20 - 49.48 - 50.05 (N-CH Ala) 57.99 59.80 (N-CH Val) 67.30 (O-CH_2) 73.06 - 74.26 - 74.30 - 74.34 74.52 (O-CH) 126.79 - 126.82 - 126.94 - 127.10 - 127.40 - 128.11 - 128.31 - 128.38 - 128.42 - 128.49 - 128.55 - 128.70 - 129.33 - 129.39 - 129.49 - 129.59 - 129.67 - 129.75 (CH phenyl) 134.84 - 135.68 - 135.97 - 136.11 - 136.21 - 136.27 - 136.50 (C phenyl) 156.47 (C=O carbamate) 165.71 - 169.43 - 170.02 - 170.28 - 170.39- 170.58 - 170.62 - 170.84 - 171.04 - 171.47 - 172.34 (C=O).

Cyclo D-alanyl L-α-hydroxyhydrocinnamyl L-valyl D-α-hydroxyhydrocinnamyl D-alanyl L-α-hydroxyhydrocinnamyl L-valyl D–α-hydroxyhydrocinnamyl D-alanyl L-α-hydroxy hydrocinnamyl L-valyl D-α-hydroxyhydrocinnamyl (2).

A solution of benzyloxycarbonyl D-alanyl L-α-hydroxyhydrocinnamyl L-valyl -α-hydroxyhydrocinnamyl D-alanyl L-α-hydroxyhydrocinnamyl L-valyl D-α-hydroxyhydrocinnamyl D-alanyl L-α-hydroxy- hydrocinnamyl L-valyl D-α–hydroxyhydrocinnamic acid pentafluorophenyl ester (15) (114mg, 66μmol) in 1,4-dioxane (15ml) was injected over a period of 48h to a mixture of 1,4-dioxane (250ml), absolute ethanol (5ml), dimethylaminopyridine (8mg, 66μmol) and palladium on charcoal (5%) (1g) at 92°C. Throughout this period the mixture was hydrogenated. The catalyst was filtered off and the solvent removed. The residue was purified by chromatography on silica gel (light petroleum : ether, 1-1) to give as an oil the title compond (2) (22mg, 24%).

$[\alpha]_D$ -12 (c 1.9 chloroform). δH (270Mhz, solvent $CDCl_3$, standard Me_4Si) 0.49 (9H, d, J 6.5Hz, Me Val) 0.74 (9H, d, J 6.5Hz, Me Val) 1.28 (9H, d, J 7Hz, Me Ala) 1.98 (3H, m, CH i-propyl) 2.9-3.35 (12H, c, CH_2) 3.79 (3H, dd, J 6.5 & 10Hz, N-CH val) 4.32 (3H, m, N-CH Ala) 5.21 (3H,ABX, J 4.5 & 7Hz, O-CH) 5.30 (3H, ABX, J 3.5 & 8.5Hz,O-CH) 7.15-7.3 (30H, c, C_6H_5) 7.61 (3H, d,J 7Hz, NH) 7.74 (3H, d, J 6.5Hz, NH). δC (68Mhz, solvent $CDCl_3$, standard Me_4Si) 15.57 (Me Ala) 18.92 - 19.18 (Me Val) 28.44 (CH i-propyl) 37.04 - 37.44 (CH_2) 48.50 (N-CH Ala) 59.77 (N-CH Val) 74.95 - 75.06 (O-CH) 126.87 - 126.94 - 128.44 - 129.55 - 129.66 (CH phenyl) 136.10 136.50 (C phenyl) 170.38 - 170.77 - 170.80 - 171.21 (C=O) found $M^{+\cdot}$ 1398 (FAB) $C_{78}H_{90}N_6O_{18}$ requires M^+, 1398.

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