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SYNTHESIS OF TWO LINEAR OCTAPEPTIDE FRAGMENTS OF CYCLOSPORIN BY STEPWISE AND FRAGMENT CONDENSATION STRATEGIES

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Abstract: The Octapeptide Z-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t was prepared by stepwise elongation starting from H-(Me)Val-OBu^t. Diphenylphosphinic mixed anhydrides were preferentially used throughout as they gave high yields of optically homogeneous products in this extensively N-methylated peptide. The above peptide and the octapeptide Z-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-(Me)Thr(Bu^t)-Abu-Sar-OBu^t were also prepared by the fragment consensation approach employing a variety of coupling methods. Ultimately, it was clear that the stepwise assembly gave the highest yield, and most homogeneous product.

The cyclosporins are a group of potent immunosuppressive compounds which have been isolated from Tolypocladium inflatum Gams,[1] the general structure of the cyclosporins, based on X-ray crystallographic[2] and nmr data[3] being shown in Figure 1.

Cyclosporin A contains seven relatively hydrophobic, N-methylated amino acids, including N-methyl leucine and N-methyl valine. In addition, all cyclosporins contain the unusual "C_9" amino acid (4R)-4-[(E)-2- butenyl]-4,N-dimethyl-L-threonine) (MeBmt); this residue has the same optical configuration as threonine and has been synthesised in stereochemically pure form.[4-6] The majority of the other residues are invariant; however, a number of variations are known at position 2 and this gives rise to the range of known cyclosporins.[7]

As the immunosuppressive property of cyclosporin A is known[8,9] to be intimately connected with the nature of the residues at positions one and two, we chose to attempt modification of the cyclosporin structure at these positions. An additional impetus for modification at position-2 was the knowledge that the norvaline-2 analogue of cyclosporin is considerably less nephrotoxic than cyclosporin A itself, which has an aminobutyric acid residue at this position[10].

The total synthesis of cyclosporins has been reported by two groups,[9,11,12] and in both, the same general route using the fragment condensation approach with final cyclisation between the alanine residues has been used. These syntheses were facilitated using t-butyloxycarbonyl for amino group protection and a benzyl ester at the C-terminus. In a recent subsequent paper[13] the merits of benzyloxycarbonyl and 9-fluorenylmethoxycarbonyl have been contrasted with the use of t-butyloxycarbonyl for amino group protection. These groups in combination with the bis(-2- oxo-3-oxazolidinyl)phosphinic chloride[13,14] coupling reagent provided a means of

All amino acids are of the L-configuration unless otherwise specified, and nomenclature follows IUPAC-IUB Joint commission on biochemical nomenclature (JCBN) Nomenclature and symbolism for amino acids and peptides 1983.

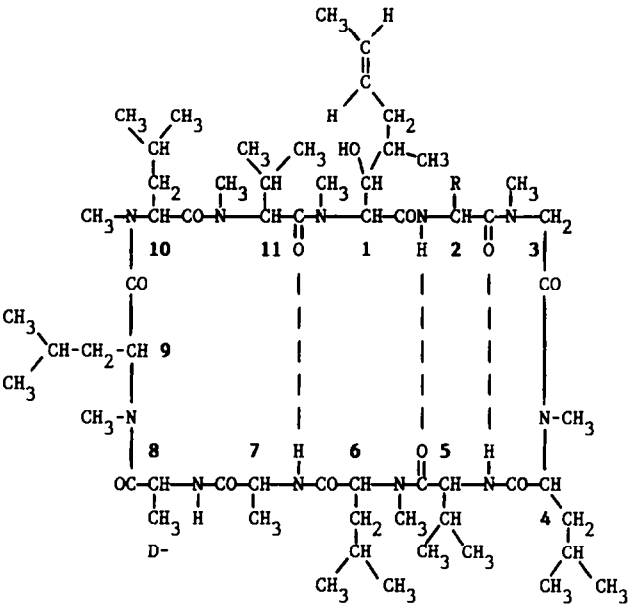


Figure 1 Structural formula of cyclosporin A (R = CH_2-CH_3).

assembling fragments of cyclosporins which was comparable to our own.[15]

In the current paper we describe the synthesis of the octapeptides (1) and (2) using both the fragment coupling and stepwise elongation approach.

Z-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t

(1)

Z-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-(Me)Thr(Bu^t)-Abu-Sar-OBu^t

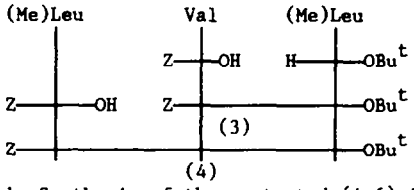
(2)

The synthesis of such peptides in optically pure form is not straightforward as the factors which influence racemisation are complex,[15-17] and, there is an increased tendency with N-methylated amino acids towards racemisation. Recently we have demonstrated[15] that the diphenylphosphinic (Dpp) mixed anhydrides are advantageous for the coupling of N-methyl amino acids when benzyloxycarbonyl is used for amino group protection. This protecting group was generally removed by catalytic hydrogenolysis using palladium on charcoal catalyst. In the initial work, the use of phenyl esters for carboxyl group protection was explored, but it was rapidly apparent that many side reactions occurred in the synthesis of N-methylated peptides using this protection, particularly the formation of diketopiperazine with the concomitant expulsion of phenoxide. In the final strategy the tert-butyl ester protecting group of Anderson[18] and Roeske[19] was used; also, tert-butyl ether protection was used for the side chain hydroxyl group of threonine and hydroxyproline derivatives, as it is widely believed that better yields may be obtained when side chain hydroxyl groups are blocked. The tert-butyl ether and ester groups remain unaffected during peptide bond formation and are easily removed by acidolysis at the appropriate point in the synthesis without the use of basic conditions which might give rise to racemisation.

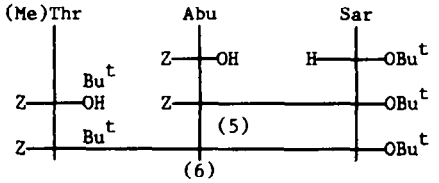
N-methyl leucine, N-methyl valine and N-methyl threonine which were required for this work were prepared by the N-methylation procedure reported by Benoiton[20,21]. The N-methylation of leucine and valine was straightforward; however, difficulties were encountered with the synthesis of N-methyl threonine. Mass spectrometry of the product of N-methylation of Z-Thr(OBu^t)-OH showed two intense peaks at m/z 324 and 310, corresponding to the methylated and

non-methylated threonine derivative respectively. The proton n.m.r. spectrum of the product showed a broad signal at δ 5.8 due to the NH resonance,this being in addition to the signal at δ 3.1 which was due to the N-methyl group protons. Thus only partial N-methylation had occured in line with previous observations by McDermott and Benoiton.[20] This difficulty may be attributed to steric hindrance of the side chain, as on attempted re-methylation of the partially methylated compound no change in the spectroscopic data was observed. Purified Z-(Me)Thr(Bu^t)-OH was finally obtained by separating the two compounds by silica gel chromatography.

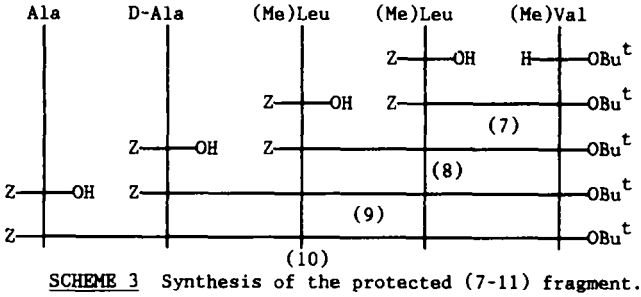
The octapeptides (1) and (2) were initially synthesised by the fragment condensation approach, octapeptide (1) being simultaneously synthesised using a stepwise procedure. The use of the fragment condensation approach was investigated, as it would reduce the length of time taken for the synthesis since the two subfragments could be prepared in parallel; also the fragment condensation approach had been used successfully by other workers.[9,11,12] The protected tripeptides (4) and (6) and the protected pentapeptide (10) were prepared by stepwise build-up according to the routes shown in Schemes 1 - 3. A variety of reaction conditions were studied,[15] and it was ultimately found that the diphenylphosphinic mixed anhydride procedure gave superior results in terms of both yield and optical homogeneity.



SCHEME 1 Synthesis of the protected (4-6) fragment.



SCHEME 2 Synthesis of the protected (1-3) fragment



SCHEME 3 Synthesis of the protected (7-11) fragment.

In order to carry out the fragment condensation between fragments (4) and (10) the tert- butyl ester protection was removed from the protected tripeptide (4) by treatment with 90% TFA over two hours, giving the peptide acid (11). The pentapeptide (10) was then hydrogenolysed over palladium charcoal giving the amino component (12) which was subsequently coupled to the peptide acid (11). Coupling of the fragments was investigated using the Dpp mixed anhydride method and the Castro reagent [(benzotriazol-1-yl)oxy]tris(dimethylamino)phosphonium hexafluorophosphate,[22] the results being summarised in Table 1.

The octapeptide (2) was also synthesised by a fragment condensation approach utilising the sub-fragments (10) and (6). The protected tripeptide (6) was hydrogenolysed to give the amino component (13) which was then condensed with the peptide acid (14) derived from the protected

pentapeptide (10) by treatment with 90% trifluoroacetic acid. In addition to the two coupling reagents mentioned above, di-isopropyl-carbodiimide in the presence of N-hydroxybenzotriazole was also evaluated, the results again being summarised in Table 1.

The Castro reagent (BOP) was investigated, as it was thought that this would provide a synthesis of optically pure peptide, as the rapid reaction is thought to lead to the formation of the hydroxybenzotriazolyl ester which may preclude racemisation. Alternative evidence,[17] however, suggests that considerable racemisation can occur when hydroxybenzotriazolyl active esters are formed by the conventional reaction between DCCI and hydroxybenzotriazole particularly when long reaction times are permitted.

The results shown in Table 1 indicate that in the preparation of octapeptides (1) and (2) the use of the BOP reagent in DMF led to a heterogeneous product as indicated by hplc and the observed optical rotations were lower than those obtained by other methods. Surprisingly, the diphenyl phosphinic mixed anhydride procedure employing activation at -20°C for one hour led to a single peak on hplc with a higher optical rotation than had been achieved by other methods. The octapeptide (2), which was obtained by the di-isopropyl-carbodiimide procedure, showed at least three poorly resolved peaks on hplc. These results are similar to earlier findings[23]

TABLE 1 Coupling methods used for the octapeptides (1) and (2).

Peptide	Coupling method[+]	Rection Time(h)	Yield %	$[\alpha]_D^{24}$ (c)	
(1)	BOP/DMF	24	77	-127 (1)	2 Peaks on hplc. unresolved by chromatography.
(1)	DppCl/THF[a]	42	27	-134 (1)	One peak on hplc.
(2)	BOP/DMF	24	77	-83	Heterogeneous on
(2)	BOP/DMF	48	43	(1)	hplc, tlc
(2)	BOP/DMF	64	52		Unresolved by
(2)	Diisopropyl-carbodiimide/-HOBt	120	10		chromatography.
(2)	DppCl/THF[a]	18	25	-98 (1)	Single peak on hplc.

[+] Activation temperature was 0°C except where indicated [a] -20°; a one hour activation period was used for all couplings.

which indicate that condensation of the peptide fragments containing leucine at the C-terminus gives rise to racemisation when DCCI/hydroxybenztriazole is used for activation.

Generally, low yields were encountered in the fragment coupling as indicated in Table 1, although somewhat higher yields were obtained using the BOP reagent. The various difficulties encountered using the fragment condensation strategy led us to consider a total stepwise

synthesis of the octapeptide (1).

The octapeptide (1) was prepared from the pentapeptide (10) shown in Scheme 3 by further sequential elongation with Z-(Me)Leu-OH, Z-Val-OH and Z-(Me)Leu-OH to give the hexa-, hepta- and octapeptides (15), (16) and (1) respectively. The diphenylphosphinic mixed anhydride procedure[15,24] was used for chain extension, and a 1.2-fold excess of the acylating component was employed in order to maximise the yield. The efficiency of these couplings is exemplified by the yields obtained, (96, 75 and 94% respectively) and by the fact that single peaks were observed on hplc of each peptide. No indication of the presence of diastereoisomers was evident from the hplc or ^{1}H nmr, although it is accepted that low levels of racemisation could conceivably pass undetected.

The high yields and overall level of purity suggest that diphenylphosphinyl chloride is particularly efficient as a coupling reagent when the point of coupling involves sterically hindered and/or N-methylated residues. In addition, as has been found previously,[15,24] that no "wrong-way" opening of the mixed anhydride was observed; thus this mixed anhydride procedure is particularly advantageous for the assembly of extensively N-methylated peptide such as cyclosporin A. The procedure thus permits stepwise assembly of large fragments of cyclosporin A and provides an alternative to the fragment condensation approach.

Acknowledgements

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EXPERIMENTAL

The general chromatographic and spectroscopic methods were those described previously,[15] except that:- hplc was carried out using a Spectra Physics SP8700 fitted with a 25 x 0.46 cm ODS Chromopak C18 column eluting with CH_3CN/H_2O gradients at a flow rate of 1.5 cm^3/min. Gel filtration was carried out using Sephadex LH20 or Sephadex G10 eluting with DMF and water respectively. Fractions were monitored by uv absorbance at 280 nm (Unicord III) or by optical rotation (Thorn-Bendix 143D polarimeter). In some cases column eluant was also monitored by hplc at 230 nm.

Amino acid derivatives.

Z-Ala-OH, Z-Val-OH, Z-Abu-OH, Z-Sar-OH, Z-Thr-OH and Z-Leu-OH were synthesised from the corresponding free amino acids using the methods of Bergmann[25] and Carter[26] the physical data was generally acceptably close to that found in the literature[27]; Z-Ala-OH, m.p., 85 - 86°C, $[\alpha]_D^{25}$ - 18.6° (c 1.0, CH_3OH); Z-D-Ala-OH, m.p., 86 - 87°C, $[\alpha]_D^{25}$ + 18.6° (c1, CH_3OH); Z-Val-OH, m.p., 60 - 61°C, $[\alpha]_D^{25}$ - 4.2°, (c 1.5, CH_3OH); Z-Abu-OH, m.p., 74 - 75°C; $[\alpha]_D^{22}$ - 17.8° (c 1.1, CH_3OH), Z-Sar-OH oil; Z-Thr-OH, m.p., 102 - 104°C; $[\alpha]_D^{25}$ - 3.0° (c 1.0, CH_3OH), and Z-Leu-OH, oil; $[\alpha]_D^{25}$ - 18.9°, (c 1.0, CH_3OH).

Z-(Me)Thr(Bu^t)-OH, Z-(Me)Leu-OH and Z-(Me)Val-OH were synthesised from the corresponding protected amino acids using the method of Cheung and Benoiton.[21] Z-(Me)Thr(Bu^t)-OH, oil, $[\alpha]_D^{25}$ + 9.8° (c 1.4, CH_3OH), $C_{17}H_{25}NO_5$ (C, H, N); Z-(Me)Leu-OH, m.p., 74 - 75°C $[\alpha]_D^{25}$ - 25.8°C, (c 1.0, C_2H_5OH), $C_{15}H_{21}NO_4$, (C, H, N); Z-(Me)Val-OH, m.p., 69 - 70°C, $[\alpha]_D^{25}$ - 86.4°, (c 5.5, C_2H_5OH), $C_{14}H_{19}NO_4$ (C, H, N).

Z-Sar-OBu^t, Z-Thr(Bu^t)-ONb, Z-(Me)Val-OBu^t and Z-(Me)Leu-OBu^t were prepared from the corresponding protected amino acids or N-methyl amino acids using the method of Anderson[18] and Roeske.[19] Z-Sar-OBu^t oil; Z-Thr(Bu^t)-ONB, m.p., 54 - 55°C, $[\alpha]_D^{20}$ - 7.3 (c 1.0, CH_3OH); Z-(Me)Val-OBu^t, m.p., 39°C, $[\alpha]_D^{25}$ - 67.2°, (c 1.0, $CHCl_3$), Z-(Me)Leu-OBu^t, m.p., 40°C, $[\alpha]_D^{24}$ - 32.8° (c 1.0, $CHCl_3$).

H-(Me)Leu-OBu^t and H-(Me)Val-OBu^t

Z-(Me)Leu-OBu^t and Z-(Me)Val-OBu^t were each dissolved in methanol and hydrogenolysed in the presence of 10% Pd/C catalyst for twenty-four hours. The catalyst was removed by filtration and the filtrate evaporated to give an oil which was subsequently purified by fractional distillation (0.5 mmHg).

H-(Me)Val-OBu^t, colourless oil, (89% yield), b.p., 44°C. Calculated for $C_{10}H_{21}NO_4$; C, 63.96; H, 11.30; N, 7.49. Found: C, 63.51; H, 11.10; N, 7.2%; δ_H (220 MHz, $CDCl_3$), 0.94 (6H, d, β-C$\underline{H}_3$), 1.47 (9H, s, OBu^t), 1.87 (1H, m, β-C$\underline{H}$), 2.39 (3H, s, N-C$\underline{H}_3$), 2.77 (1H, d, α-C-$\underline{H}$), and 7.44 (1H, br.d., N-$\underline{H}$), m/z 187 ($\underline{M}^+$, EI).

H-(Me)Leu-OBu^t Colourless oil, (71% yield), b.p., 48°C. Calculated for $C_{11}H_{23}NO_4$: C, 65.51; H, 11.52; N, 6.95. Found: C, 65.82; H, 11.35; N, 7.01%; δ_H (220 MHz, $CDCl_3$), 0.91 (6H, m, γ-C$\underline{H}_3$), 1.41 (2H, m, β-C$\underline{H}_2$-), 1.48 (9H, s, OBu^t), 1.73 (1H, m, γ-C$\underline{H}$), 2.37 (1H, t, α-C-$\underline{H}$), and 7.32 (1H, br.d., N$\underline{H}$), m/z 201 ($\underline{M}^+$, EI).

General procedures

General tert-butylation of C-terminal carboxyl group and hydroxy side chain of amino acids

The N-benzyloxycarbonyl-N-methyl amino acid (0.21 mol) was dissolved in dichloromethane (300 cm^3), in a pressure vessel containing concentrated H_2SO_4 (5 cm^3). The butylation vessel was cooled to -40 to -60°C, and condensed isobutylene gas (450 cm^3) was added with gentle stirring. The vessel was then sealed and the reaction mixture stirred at room temperature for five days. The vessel was then cooled to -10°C, the seal removed and excess isobutylene allowed to evaporate. The solution was allowed to reach 0°C and then brought to pH 9 with sodium carbonate (25g/150 cm^3 H_2O). The solvent was removed by evaporation and the oily residue was dissolved in ethyl acetate, washed successively with 1M $NaHCO_3$, and water then dried over anhydrous Na_2SO_4. Solvent evaporation gave the tert-butylated product which was generally purified by silica gel chromatography using one of a variety of solvent systems.

Coupling using the diphenylphosphinic mixed anhydride method

A solution of the N-protected amino acid (1.1 equiv.) and NMM (2 equiv.) in THF (2.4 mM/cm^3) was cooled to -20°C and DppCl (1.1 equiv.) added. The reaction mixture was stirred for ten to twenty minutes at -20°C and a pre-cooled solution of the amino component (1 equiv.) in THF (2.4 mM/cm^3) added. The suspension was stirred for one to two hours at -20°C and at ambient temperature for 24 - 48 hours. The solvent was evaporated and the residue taken up into ethyl acetate, washed successively with brine, 1M $KHSO_4$, 1M $NaHCO_3$ and water. The organic phase was dried over Na_2SO_4 and evaporated to give a residual oil or solid containing trace amounts of diphenylphosphinic acid. Products were generally purified by silica gel chromatography using a variety of solvent systems.

Coupling using the DICI/HOBt method

Diisopropylcarbodiimide (DICI) (1 equiv.) in DMF or THF (1 mM/2.5 cm^3) was added to a mixture of the N-protected amino acid or peptide (1 equiv.), the amino component (1 equiv.), HOBt (1 equiv.) and NMM (1 equiv.), in DMF or THF at 0°C. The mixture was stirred at 0°C for one hour and at room temperature for 12 - 20 hours. The precipitate was removed by filtration and the filtrate washed successively with 1M $KHSO_4$, $NaHCO_3$ and water, then dried over Na_2SO_4 and evaporated to give an oil or solid which was generally purified by silica gel chromatography using various solvent systems or by chromatography on Sephadex LH20 eluting with DMF.

Coupling using Castro reagent [Bt-OP(NMe_2)$_3^+$ PF_6^-]

Benzotriazol-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorphosphate (1.2 equiv.) was added to a solution of N-protected amino acid or peptide (1 equiv.), the amino component (1 equiv.), NMM (2 - 3 equiv.) in DMF or THF (1 mM/5 cm^3) at 0°C, and the reaction mixture stirred at room temperature for 24 - 72 hours. The residue, after solvent evaporation was purified by silica gel chromatography or on a Sephadex LH20 column eluting with DMF.

Scheme 1

Z-Val-(Me)Leu-OBu^t (3)

Z-L-Val-OH (36.84g, 0.14 mM) in THF (60 cm^3) was activated with DppCl (34.9g, 0.14 mM) in THF (60 cm^3) and NMM (18.7 cm^3, 0.14 mM) and coupled with H-(Me)Leu-OBu^t (24.3g, 0.12 mM) in THF (60 cm^3) according to the general procedure. The mixture was stirred at -20°C for one hour and room temperature for twenty-four hours. The solvent was evaporated and the residue worked up as described in the general method, final purification on silica gel eluting with EtOAc/DCM (1/2) gave the title compound (51.2g, 91%), m.p., 54 - 56°C, $[\alpha]_D^{23}$ - 67° (c 1.0, CH_3OH). Calculated for $C_{24}H_{38}N_2O_5$: C, 66.33; H, 8.81; N, 6.45. Found: C, 66.36; H, 8.98; N, 6.46%; δ_H (220 MHz, $CDCl_3$), 0.91 (6H, m, γ-CH_3, (Me)Leu, 1.02 (6H, m, β-CH_3, Val), 1.45 (9H, s, OBu^t), 1.51 - 1.70 (2H, m, β-CH_2, (Me)Leu), 1.91 - 2.10 (2H, m, β-CH, Val, γ-CH, (Me)Leu), 3.05 (3H, s, N-CH_3), 4.51 - 4.61 (1H, m, α-CH, Val), 5.08 (2H, s, PhCH_2), 5.21 - 5.30 (1H, m, α-CH(Me)Leu), 5.63 (1H, d, N-H Val) and 7.37 (5H, s, ArH), m/z 434 (M^+, DCI), R_t 12.2 min.

Z-(Me)Leu-Val-(Me)Leu-OBu^t (4)

a) Hydrogenolysis

Z-Val-(Me)Leu-OBu^t (3) (38g, 85 mM) in methanol (150 cm^3) was hydrogenolysed over 10% Pd/C (5g) at atmospheric pressure for twenty-four hours. The catalyst was removed by filtration and the solvent evaporated to give a white foam (22.5g, 93%), $[\alpha]_D^{22}$ - 25.3°C, (c 1.0, CH_3OH); δ_H (220 MHz, $CDCl_3$), 0.88 - 1.09 (12H, m, γ-CH_3 (Me)Leu, β-CH_3 Val), 1.45 (9H, s, OBu^t), 1.63 - 1.92 (3H, m, β-CH_2, γ-CH (Me)Leu), 2.47 (1H, m, β-CH Val), 2.97 (3H, s, N-CH_3), 3.80 (1H, m, α-C-H Val), 5.38 (1H, t, α-C-H (Me)Leu), and 7.29 (2H, br., NH_2), m/z 300 (M^+, DCI).

b) Coupling

Z-(Me)Leu-OH (23.3g, 84 mM) in THF (30 cm^3) was activated using DppCl (19.9 g, 84 mM) in THF (30 cm^3) and NMM (9.36 cm^3, 84 mM) and coupled to H-Val-(Me)Leu-OBu^t (21g, 70 mM) in THF (20 cm^3) according to the general coupling procedure, being stirred at -20°C for one hour and at room temperature for twenty-four hours. The residue, after solvent evaporation was worked-up as described in the general method and purified by silica gel chromatography eluting with CH_2Cl_2/EtOAc (3:1). Evaporation of the appropriate fractions gave (4) as a colourless oil, (33.2g, 85%), $[\alpha]_D^{23}$ - 93.4° (c 1.0, CH_3OH). Calculated for $C_{31}H_{51}N_3O_6$: C, 66.26; H, 9.15; N, 7.48. Found: C, 66.53; H, 9.25; N, 7.59%; δ_H (220 MHz, $CDCl_3$), 0.69 - 1.12 (18H, m, γ-CH_3-(Me)Leu, β-CH_3 Val), 1.33 (9H, s, OBu^t), 1.64 - 1.91 (6H, m, β-CH_2, γ-CH (Me)Leu), 2.05 (1H, m, β-C-H (Me)Val), 2.87 (3H, s, N-CH_3), 3.03 (3H, s, N-CH_3), 4.71 - 4.79 (1H, m, α-CH Val), 5.15 - 5.25 (4H, m, PhCH_2, α-CH (Me)Leu), 6.61 - 6.70 (1H, br.s., N-H Val), and 7.38 (5H, s, ArH), m/z 561 (M^+, DCI), R_t 13.0 min.

Scheme 2

Z-Abu-Sar-OBu^t (5)

Pivaloyl chloride (4.2 cm^3, 33.8 mM) was added dropwise to a solution of Z-Abu-OH (8.0g, 33.8 mM) in THF (85 cm^3) and NMM (3.7 cm^3, 33.8 mM) at -18°C, and a total of 20 minutes allowed

for the formation of the mixed anhydride. A pre-cooled solution of H-Sar-OBu^t (2.9g, 20 mM) in THF (85 cm^3) was then added, and the reaction mixture stirred at room temperature for twenty-two hours. The solvent was evaporated and the residue dissolved in ethyl acetate. The organic phase was washed successively with saturated $NaHCO_3$, 10% H_3PO_4, $NaHCO_3$ and brine. Solvent evaporation after drying (Na_2SO_4), gave a residue which was subjected to silica gel chromatography eluting with EtOAc/$CHCl_3$ (1:3). Evaporation of the appropriate fractions gave the title compound (5) as an oil, (4.3g, 59%); δ_H (220 MHz, $CDCl_3$), 1.0 (3H, t, CH_3 Abu), 1.5 (9H, 2s, OBu^t), 1.6 - 1.9 (2H, m, β-CH_2 Abu), 3.1 (3H, 2s, N-CH_3), 3.6 - 4.4 (2H, d x d, α-CH Sar), 4.6 - 4.8 (1H, m, α-CH Abu), 5.1 (2H, s, PhCH_2), 5.9 (1H, br.d., NH), and 7.3 (5H, s, ArH); m/z 365; R_t 10.6 min.

Z-(Me)Thr(Bu^t)-Abu-Sar-OBu^t (6)

Z-(Me)Thr(Bu^t)-OH (1.1g, 3.5 mM) in CH_2Cl_2 (10 cm^3) was activated using DppCl (0.2g, 3.5 mM) in CH_2Cl_2 (5 cm^3), and NMM (0.4 cm^3, 3.5 mM), the resulting mixed anhydride being coupled by the general procedure to H-Abu-Sar-OBu^t (0.7g, 3.5 mM) which had been obtained by hydrogenolysis of Z-Abu-Sar-OBu^t (5), the reaction mixture being stirred at -20°C for one hour and at room temperature for twenty-two hours. The product was worked-up as described in the general method, and the residue obtained was chromatographed on a silica gel column eluting with CH_2Cl_2/EtOAc (1:3). Evaporation of the appropriate fractions gave the title compound as a white gum (0.9g, 69%); $[\alpha]_D^{20}$ - 44.7° (c 1.2, CH_3OH). Calculated for $C_{28}H_{45}N_3O_7$: C, 62.80; H, 8.41; N, 7.85. Found : C, 62.56; H, 8.51; N, 7.81; δ_H (220 MHz, $CDCl_3$), 0.80 - 0.90 (3H, m, CH_3 Abu), 1.01 - 1.10 (3H, d, β-CH_3 (Me)Thr), 1.21 - 1.30 (9H, s, OBu^t (Me)Thr), 1.41 - 1.50 (9H, s, OBu^t Sar), 1.51 - 1.89 (2H, m, β-CH_2 Abu), 3.01 - 3.21 (6H, 2s, N-CH_3), 3.60 - 4.35 (2H, d x d, α-CH_2 Sar), 4.21 - 4.32 (1H, m, β-CH(Me)Thr), 4.71 - 4.82 (1H, m, α-CH (Me)Thr), 4.85 - 5.00 (1H, m, α-CH Abu), 5.20 (2H, s, PhCH_2), 7.40 (5H, s, ArH), and 7.51 1H, br.d., NH Abu); m/z 536 (M+1, FAB); R_t 12.0 min.

Scheme 3

Z-(Me)Leu-(Me)Val-OBu^t (7)

Z-(Me)Leu-OH (22.26g, 84 mM) in THF (25 cm^3) was activated with NMM (9.36 cm^3, 84 mM) and DppCl (19.92g, 84 mM) in THF (25 cm^3), and coupled with H-(Me)Val-OBu^t (13.09g, 70 mM) in THF (25 cm^3), according to the general procedure, being stirred at -20°C for one hour and at room temperature for twenty-four hours. Solvent evaporation gave a residue which was washed in the usual way, the resulting oil being purified on a silica gel column eluting with EtOAc/DCM (1:2). Evaporation of the appropriate fractions gave the title compound as a colourless oil (31.43g, 81%), $[\alpha]_D^{23}$ - 141.8° (c 1.0, CH_3OH), lit.,[12] $[\alpha]_D^{20}$ - 136.5°, (c 1.0, $CHCl_3$). Calculated for: $C_{26}H_{42}N_2O_5$: C, 66.90; H, 9.00; N, 9.25. Found: C, 66.60; H, 9.05; N, 9.08; δ_H (220 MHz, $CDCl_3$), 0.68 - 1.10 (12H, m, β-CH_3 (Me)Val, γ-CH_3 (Me)Leu), 1.42 (9H, s, OBu^t), 1.59 - 1.82 (3H, m, β-CH_2, γ-CH (Me)Leu), 2.20 (1H, m, β-C-H (Me)Val), 2.91 (3H, s, N-CH_3), 2.82 - 3.02 (3H, 2s, N-CH_3 Conf.), 4.29 - 4.80 (1H, d x d, α-CH (Me)Val), 5.02 (1H, m, α-CH (Me)Leu), 5.25 (2H, s, PhCH_2), and 7.61 (5H, s, ArH), m/z 448 (M^+, DCI), R_t 12.2 min.

Z-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (8)

a) Hydrogenolysis

Z-(Me)Leu-(Me)Val-OBu^t (7) (17.92g, 0.04 mM) in methanol (100 cm^3) was hydrogenolysed in the presence of 10% Pd/C catalyst (1g) at atmospheric pressure, for twenty-four hours. The catalyst was removed by filtration and the solvent evaporated to give the title compound as an oily foam (11.07g, 90%), $[\alpha]_D^{23}$ - 123.6° (c 1.0, CH_3OH), lit.,[12] $[\alpha]_D^{20}$ - 118° (c 1.0, $CHCl_3$); δ_H (220 MHz, $CDCl_3$), 0.84 - 1.10 (12H, m, β-CH_3 (Me)Val, γ-CH_3 (Me)Leu), 1.26 (3H, m, β-CH_2, γ-CH (Me)Leu), 1.42 (9H, s, OBu^t), 1.91 (1H, m, β-C-H (Me)Val), 2.29 (3H, s, N-CH_3), 2.60 (1H, br.s., NH), 3.02 (3H, s, N-CH_3), 3.47 - 3.58 (1H, d x d, α-C-H), and 3.80 - 4.9 (1H, d x d, α-C-H); m/z 314 (M^+, DCI).

b) Coupling

Z-(Me)Leu-OH (10.04g, 36 mM) in THF (10 cm^3) was activated with DppCl (8.52g, 36 mM) in THF (10 cm^3) and NMM (4.08 cm^3, 36 mM) and coupled with H-(Me)Leu-(Me)Val-OBu^t (9.42g, 30 mM) in THF (10 cm^3) by the general DppCl method. The reaction mixture was stirred at -20°C for two hours and at room temperature for twenty-four hours and worked-up as described in the general procedure. The residue obtained was purified by silica gel chromatography eluting with EtOAc/DCM (1:2). Solvent evaporation of the appropriate fractions gave the title compound as a sticky white foam (14.85g, 83%), $[\alpha]_D^{23}$ - 173.5° (c 1.0, CH_3OH), lit.,[12] $[\alpha]_D^{20}$ - 165.5° (c 1.0, $CHCl_3$). Calculated for: $C_{32}H_{53}N_3O_6$: C, 66.73; H, 9.28; N, 7.29. Found: C, 66.86; H, 9.41; N, 7.53%; δ_H (220 MHz, $CDCl_3$), 0.73 - 1.09 (18H, m, β-CH_3 (Me)Val, γ-CH_3 (Me)Leu), 1.48 (9H, s, OBu^t), 1.23 - 1.87 (6H, m, β-CH_2, γ-CH (Me)Leu), 2.18 (1H, m, β-CH (Me)Val), 2.82 - 2.85 (3H, s, N-CH_3 Conf.), 2.87 (8H, 2s, NCH_3), 4.15 - 4.76 (1H, d x d, α-CH (Me)Val), 5.03 (1H, t, α-CH (Me)Leu), 5.21 (2H, s, PhCH_2), 5.56 (1H, t, α-CH (Me)Leu), and 7.34 (5H, s, ArH); m/z 576 (M+1, FAB), R_t 13.6 min.

Z-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (9)

a) Hydrogenolysis

Z-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (8) (11.52g, 20 mM) in methanol (100 cm^3) was hydrogenolysed in the presence of 10% Pd/C catalyst for twenty-four hours. The solution, after removal of the catalyst by filtration, was evaporated to give the title compound as a sticky foam (7.84g, 89%); $[\alpha]_D^{23}$ (c 1.3, CH_3OH); δ_H (220 MHz, $CDCl_3$), 0.74 - 1.14 (18H, m, β-CH_3 (Me)Val, γ-CH_3 (Me)Leu), 1.45 (9H, s, OBu^t), 1.52 - 1.94 (6H, m, β-CH_2, γ-CH (Me)Leu), 2.19 (1H, m, β-CH (Me)Val), 2.48 (3H, s, N-CH_3), 3.10 (3H, s, N-CH_3), 3.12 (3H, s, N-CH_3), 3.88 (2H, m, α-CH), 4.79 (1H, d, α-CH), and 6.37 (1H, br.d., NH); m/z 442 (M+1, FAB).

b) Coupling

Z-D-Ala-OH (4.01g, 18 mM) in THF (6 cm^3) was activated by DppCl (4.25g, 18 mM) in THF (6 cm^3) and NMM (1.97 cm^3, 18 mM), and the resulting mixed anhydride coupled to H-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (6.61g, 15 mM) in THF (6 cm^3). After stirring at -20°C for two

hours and at ambient temperature for thirty hours the residue was worked-up as described in the general DppCl procedure and purified by silica gel chromatography eluting with EtOAc/DCM (1:2). Solvent evaporation of the appropriate fractions gave the title compound as a colourless oil, (6.8g, 80%); $[\alpha]_D^{24}$ - 137.7° (c 1.0, CH_3OH). Calculated for: $C_{35}H_{58}N_4O_7$. C, 64.90; H, 9.04; N, 8.66. Found: C, 65.00; H, 9.02; N, 8.67%; δ_H (220 MHz, $CDCl_3$), 0.73 - 1.11 (18H, m, β-CH_3 (Me)Val, γ-CH_3 (Me)Leu), 1.35 (3H, d, CH_3 D-Ala), 1.42 (9H, s, OBu^t), 1.48 - 1.82 (6H, m, β-CH_2, γ-CH (Me)Leu), 2.17 (1H, m, β-CH (Me)Val), 2.78 - 2.98 (6H, 4s, NCH_3 Conf.), 3.02 (3H, s, N-CH_3), 4.69 (1H, d x d, α-CH D-Ala), 4.74 (1H, m, α-CH (Me)Val), 5.11 (2H, s, $PhCH_2$-), 5.03 - 5.66 (2H, m, α-CH (Me)Leu), 5.72 (1H, d, NH D-Ala), and 7.39 (5H, s, ArH); m/z 646 (M^+, DCI), R_t 13.0 min.

Z-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (10)

a) Hydrogenolysis

Z-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (9) (6.42g, 10 mM) in methanol (50 cm^3) was hydrogenolysed in the presence of 10% Pd/C catalyst for twenty-four hours. The catalyst was removed by filtration and the filtrate evaporated to give the title compound as a sticky white foam, (4.76g, 93%); $[\alpha]_D^{25}$ - 126° (c 1.2, CH_3OH); δ_H (220 MHz, $CDCl_3$), 0.77 - 1.11 (18H, m, β-CH_3 (Me)Val, γ-CH_3 (Me)Leu), 1.16 - 1.27 (3H, d, CH_3 D-Ala), 1.15 (9H, s, OBu^t), 1.34 - 1.83 (6H, m, β-CH_2, γ-CH (Me)Leu), 2.07 (2H, br.s., NH_2), 2.18 (1H, m, β-CH (Me)Val), 2.87, 2.94, 3.01 (9H, series of singlets, N-CH_3), 3.85 - 3.99 (1H, q, α-CH D-Ala), 4.72 (1H, d, α-CH (Me)Val), and 4.55 (2H, 2t, α-CH (Me)Leu); m/z 512 (M+1, FAB).

b) Coupling

Z-Ala-OH (2.40g, 10.8 mM) in THF (4 cm^3) was activated using DppCl (2.55g, 10.8 mM) in THF (4 cm^3) and NMM (1.2 cm^3, 10.8 mM) and coupled to H-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (4.68g, 9 mM) in THF (4 cm^3) according to the standard procedure. The reaction mixture was stirred at -20°C for one hour and at ambient temperature for twenty-four hours. The residue obtained after work-up by the general method was purified by silica gel chromatography using CH_2Cl_2/EtOAc (2:1) as eluant. Evaporation of the appropriate fractions gave the title compound as a white solid (5.18g, 88%); m.p., 52 - 53°C; $[\alpha]_D^{25}$ - 168° (c 1.1, $CHCl_3$). Calculated for $C_{38}H_{63}N_5O_8$: C, 63.55; H, 8.86; N, 9.75. Found: C, 63.34; H, 8.96; N, 9.97%; δ_H (220 MHz, $CDCl_3$), 0.78 - 1.04 (18H, m, β-CH_3 (Me)Val, γ-CH_3 (Me)Leu), 1.31 (3H, d, CH_3 D-Ala), 1.39 (3H, d, CH_3 L-Ala), 1.46 (9H, s, OBu^t), 1.49 - 1.98 (6H, m, β-CH_2, γ-CH (Me)Leu), 2.19 (1H, m, β-CH (Me)Val), 2.79 - 3.03 (9H, series of singlets, N-CH_3), 4.31 (1H, m, α-CH L-Ala), 4.74 (1H, d x d, α-CH (Me)Val), 4.85 (1H, m, α-CH D-Ala), 5.07 (2H, s, $PhCH_2$), 5.49 - 5.56 (2H, m, α-CH (Me)Leu), 5.66 (1H, d, NH D-Ala), 7.07 (1H, d, NH L-Ala), and 7.30 (5H, s, ArH); m/z 717 (M+1, FAB), R_t 13.0 min.

Synthesis of Z-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (1) using the fragment condensation strategy

Z-(Me)Leu-Val-(Me)Leu-OH (11) (0.25g, 0.44 mM) in THF (6 cm^3) was activated using DppCl (0.15g, 0.60 mM) in THF (5 cm^3) and NMM (0.07 cm^3, 0.60 mM) and coupled to H-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (12) (0.27g, 0.45 mM) in THF (5 cm^3) according to the general DppCl coupling method. The reaction mixture was stirred at 0 to -5°C for one hour and at room temperature for forty-two hours. The residue, after solvent evaporation was applied to a Sephadex LH20 column eluting with DMF. Evaporation of the appropriate fractions gave the title compound as a white foam (0.13g, 27%); m.p., 65 - 67°C; $[\alpha]_D^{20}$ - 134°C (c 1.0, CH_3OH). Calculated for $C_{57}H_{98}N_8O_{11}$: C, 63.87; H, 9.22; N, 10.48. Found : C, 63.59; H, 9.29; N, 10.33; the ^1H (250 Mhz) spectrum was essentially the same as that obtained below from material produced by stepwise assembly; m/z 1073 (M+1, FAB); R_t 13.6 min.

Synthesis of the octapeptide Z-Ala-D-Ala-(Me)Leu-Val-(Me)Leu-(Me)Thr(Bu^t)-Abu-Sar-OBu^t (2) by the fragment condesation approach

Z-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OH (14) (0.4g, 0.6 mM) in THF (6 cm^3) was activated using DppCl (0.2g, 0.6 mM) in THF (3 cm^3) and NMM (0.1 cm^3, 0.9 mM) and coupled to H-(Me)Thr-(Bu^t)-Abu-Sar-OBu^t (13) (0.3g, 0.6 mM) in THF (6 cm^3) according to the general DppCl coupling procedure. The reaction mixture was stirred at -20°C for half an hour, -10°C for half an hour and at room temperature overnight. The residue, after solvent evaporation, was applied on a Sephadex LH20 column eluting with DMF. Evaporation of the appropriate fractions gave the title compound as a white foam (0.2g, 25%); m.p., 85 - 87°C; $[\alpha]_D^{20}$ - 98° (c 1.0, CH_3OH). Calculated for $C_{54}H_{91}N_8O_{12}.H_2O$: C, 61.05; H, 8.82; N, 10.55; Found : C, 61.38; H, 8.89; N, 10.46%; δ_H (250 MHz, $CDCl_3$), 0.74 - 1.04 (21H, m, β-CH_3 (Me)Val, γ-CH_3 (Me)Leu and Abu), 1.14 (9H, s, OBu^t (Me)Thr), 1.15 (3H, d, β-CH_3 (Me)Thr), 1.29 - 1.32 (6H, d x d, CH_3 of Ala, D-Ala), 1.44 (9H, s, OBu^t (Me)Val), 1.49 - 1.80 (6H, m, β-CH_2 of Abu, (Me)Leu), 1.82 - 2.00 (2H, m, γ-CH (Me)Leu), 2.35 - 2.42 (1H, m, β-CH (Me)Val), 2.88 - 3.14 (15H, series of singlets, N-CH_3), 3.19 (1H, d, α-CH Sar), 3.50 - 3.53 (1H, m, β-CH (Me)Thr), 4.23 - 4.40 (1H, m, α-CH Ala), 4.50 - 4.89 (4H, m, α-CH), 4.94 - 5.10 (1H, m, α-CH Abu), 5.12 (2H, s, Ph-CH_2), 5.15 - 5.42 (2H, m, α-CH (Me)Leu), 6.55 (1H, d, NH D-Ala), 6.91 (1H, d, NH L-Ala), 7.36 (5H, s, ArH), and 8.01 (1H, br.s., NH-Abu); m/z 1044 (M^+, CI), R_t 12.0 min.

Synthesis of octapeptide (1) by the stepwise procedure.

Z-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (15).

a) Hydrogenolysis

Z-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (6) (29.86g, 50 mM) was dissolved in methanol (200 cm^3) and hydrogenolysed in the presence of 10% Pd/C catalyst (2.7g), at atmospheric pressure for twenty-four hours. The catalyst was removed by filtration and the filtrate evaporated to give the title compound as a foam, (23.6g, 95%); m.p., 70 - 71°C; $[\alpha]_D^{24}$ - 195.4° (c 1.2, CH_3OH); δ_H (220 MHz, $CDCl_3$), 0.76 - 1.09 (18H, m, β-CH_3 (Me)Val, γ-CH_3 (Me)Leu), 1.22 (3H, d, CH_3-D-Ala), 1.36 (3H, d, CH_3 Ala), 1.45 - 1.48 (9H, 2s, OBu^t conf.), 1.54 - 2.09 (6H, m, β-CH_2, γ-CH (Me)Leu), 2.20 (1H, m, β-CH (Me)Val), 2.83 - 3.03 (9H, series of singlets, N-CH_3), 3.58 (1H,

br.s., NH D-Ala), 3.75 - 3.86 (1H, d x d, α-CH (Me)Val), 4.72 - 4.90 (2H, m, α-CH Ala, D-Ala), 5.56 (2H, 2t, α-CH (Me)Leu), and 7.33 - 7.89 (2H, br.m., NH_2); m/z 583 (M+1, FAB).

b) Coupling

Z-(Me)Leu-OH (16.65g, 59.7 mM) in THF (40 cm^3) was activated using DppCl (14.1g, 59.7 mM) in THF (40 cm^3) and NMM (5.46 cm^3, 59.7 mM) and coupled to H-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (29g, 49.7 mM) in THF (30 cm^3), according to the general procedure. The reaction mixture was stirred at -20°C for three hours and at ambient temperature for thirty hours. The reaction was worked-up as described for the general DppCl method, and the residue obtained was purified on a silica gel column eluting with CH_2Cl_2/EtOAc (2:1). Solvent evaporation of the appropriate fractions gave the title compound as a white solid (36.1g, 96%); m.p., 47 - 48°C; $[\alpha]_D^{25}$ - 173° (c 1.0, CH_3OH). Calculated for $C_{45}H_{76}N_6O_9$: C, 63.91; H, 9.10; N, 9.95; Found: C, 64.12; H, 9.32; N, 9.80%; δ_H (250 MHz, $CDCl_3$), 0.76 - 1.03 (24H, β-CH_3 (Me)Val, γ-CH_3 (Me)Leu), 1.25 - 1.34 (6H, d x d, CH_3 D-Ala, Ala), 1.44 (9H, s, OBu^t), 1.77 (3H, m, γ-CH (Me)Leu), 1.58 - 1.85 (6H, m, β-CH_2 (Me)Leu), 2.17 (1H, m, β-CH (Me)Val), 2.77 - 3.00 (9H, series of singlets, -NCH_3), 4.39 (1H, t, α-CH L-Ala), 4.71 (1H, d, α-CH (Me)Val), 4.63 - 4.87 (2H, m, α-CH D-Ala, (Me)Leu), 5.14 (2H, s, $PhCH_2$), 5.39 - 5.50 (2H, m, α-CH (Me)Leu), 6.57 (1H, br.d., NH D-Ala), 6.88 (1H, br.s., NH L-Ala), and 7.38 (5H, s, ArH); m/z 844 (M^+, DCI), R_t 13.0 min.

Z-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (16)

a) Hydrogenolysis

Z-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (15) 35.8g, 42.4 mM) in methanol (200 cm^3) was hydrogenolysed in the presence of 10% Pd/C catalyst at one atmosphere for thirty hours. The catalyst was removed by filtration and the filtrate evaporated to give the title compound as a white foam, (28.3g, 94%), m.p., 50 - 52°C; $[\alpha]_D^{25}$ - 155.4° (c 1.2, CH_3OH). Calculated for $C_{37}H_{70}N_6O_6$: C, 62.39; H, 9.93; N, 11.82. Found: C, 61.98; H, 9.79; N, 11.88%; δ_H (250 MHz, $CDCl_3$), 0.76 - 1.03 (24H, m, β-CH_3 (Me)Val, γ-CH_3 (Me)Leu), 1.24 - 1.36 (6H, d x d, CH_3 Ala, D-Ala), 1.45 (9H, s, OBu^t), 1.58 - 1.90 (6H, m, β-CH_2 (Me)Leu), 1.98 (3H, m, γ-CH (Me)Leu), 2.17 (1H, m, β-CH (Me)Val), 2.76 - 2.99 (12H, series of singlets, NCH_3), 4.03 (1H, br.d., -NH (Me)Leu), 4.44 - 5.50 (5H, m, α-CH), 6.26 (1H, br.d., NH Ala), 8.39 (1H, br.d., NH D-Ala); m/z 710 (M^+, DCI).

b) Coupling

Z-Val-OH (11.45g, 45.6 mM) in THF (30 cm^3) was activated using DppCl (10.79g, 45.6 mM) in THF (30 cm^3) and NMM (5.0 cm^3, 45.6 mM) and the resulting mixed anhydride coupled to H-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (27g, 38 mM) in THF (30 cm^3), as described for the general DppCl coupling procedure. The reaction mixture was stirred at -20°C for two hours and at room temperature for forty-five hours. The reaction was worked-up as described in the general procedure and the residue subjected to chromatography on silica gel eluting with CH_2Cl_2/EtOAc (2:1). Evaporation of the solvent gave the title compound as a white foam, (26.9g, 75%), m.p., 69.5°C; $[\alpha]_D^{25}$ - 143.1° (c 1.1 CH_3OH). Calculated for $C_{50}H_{85}N_7O_{10}$: C, 63.58; H, 9.08; N, 10.38. Found: C, 63.36; H, 9.05; N, 10.48%; δ_H (250 MHz, $CDCl_3$), 0.71 - 1.06 (30H, m, β-CH_3 Val, (Me)Val, γ-CH_3 (Me)Leu), 1.31 - 1.34 (6H, d x d, CH_3 Ala, D-Ala), 1.44 (9H, s, OBu^t), 1.47 - 1.60 (3H, m, γ-CH (Me)Leu), 1.58 - 1.84 (6H, m, β-CH_2 (Me)Leu, 1.99 (1H, m, β-CH Val), 2.14 (1H, m, β-CH (Me)Val), 2.76 - 3.01 (12H, series of singlets -NCH_3), 4.36 (1H, m, α-CH Ala), 4.46 (1H, m, α-CH Val), 4.68 (1H, d, α-CH (Me)Val), 4.61 - 4.84 (1H, m, α-CH D-Ala), 5.03 - 5.13 (4H, m, $PhCH_2$, α-CH (Me)Leu), 5.34 - 5.45 (2H, m, α-CH (Me)Leu), 6.58 (1H, br.d., NH D-Ala), 6.82 - 7.01 (2H, br.m., NH L-Ala, Val), and 7.23 (5H, s, ArH); m/z 943 (M^+, DCI); R_t 13.6 min.

Z-(Me)Leu-Val-(Me)Leu-L-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (1)

a) Hydrogenolysis

Z-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (16) (26g, 27.5 mM) in methanol (100 cm^3) was hydrogenolysed in the presence of 10% Pd/C catalyst for two days. Evaporation of the solvent, after removal of the catalyst, gave the title compound as a white foam, (20.96g, 94%); m.p., 71 - 72°C; $[\alpha]_D^{24}$ - 138.9° (c 1.2, CH_3OH). Calculated for $C_{42}H_{29}N_7O_8$: C, 62.25; H, 9.84; N, 12.10. Found : C, 62.20; H, 9.65; N, 12.23%; δ_H (250 MHz, $CDCl_3$), 0.71 - 1.06 (30H, m, γ-CH_3 (Me)Leu, β-CH_3 Val, (Me)Val), 1.23 - 1.33 (6H, 2d, CH_3-Ala, D-Ala), 1.44 (9H, s, OBu^t), 1.40 - 1.61 (3H, m, γ-CH (Me)Leu), 1.50 - 1.84 (6H, m, β-CH_2 (Me)Leu), 1.25 - 1.99 (2H, m, β-CH Val, (Me)Val), 2.11 (2H, br., NH_2), 2.78 - 3.01 (12H, series of singlets NCH_3), 4.38 (1H, m, α-CH Ala), 4.45 - 5.44 (6H, α-CH (Me)Leu, (Me)Val, Val, D-Ala), 6.68 (1H, br.d., NH D-Ala), and 6.95 (1H, br.d., NH L-Ala); m/z 809 (M^+, DCI).

b) Coupling

Z-(Me)Leu-OH (8.56g, 30.7 mM) in THF (10 cm^3) was activated using DppCl (7.26g, 30.7 mM) in THF (10 cm^3) and NMM (3.37 cm^3, 30.7 mM) and was coupled to H-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (20.7g, 25.6 mM) in THF (50 cm^3), according to the general coupling procedure. The reaction mixture was stirred at -20°C for two hours and at ambient temperature for forty-five hours. The solvent was evaporated and the reaction worked-up as described in the general procedure. The residue was subjected to chromatography on silica gel eluting with EtOAc/CH_2Cl_2 (1:1). Evaporation of the appropriate fractions gave the title compound as a white solid, (23g, 94%); m.p., 75°C; $[\alpha]_D^{24}$ - 133.1° (c 1.2, CH_3OH). Calculated for $C_{57}H_{98}N_8O_{11}$: C, 63.87; H, 9.22; N, 10.48; Found: C, 63.74; H, 9.23; N, 10.33%; δ_H (250 MHz, $CDCl_3$, 0.76 - 1.04 (36H, m, β-CH_3 Val, (Me)Val, γ-CH_3 (Me)Leu), 1.31 - 1.34 (6H, d x d, CH_3 Ala, D-Ala), 1.44 (9H, s, OBu^t), 1.47 - 1.60 (3H, m, γ-CH(Me)Leu), 1.61 - 1.70 (8H, m, β-CH_2 (Me)Leu), 1.98 (1H, m, β-CH Val), 2.15 (1H, m, β-CH (Me)Val), 2.79 - 3.06 (15H, series of singlets, NCH_3), 4.39 - 4.49 (1H, m, α-CH Ala), 4.69 - 4.95 (3H, m, α-CH D-Ala, Val, (Me)Val), 5.03 - 5.23 (4H, m, α-CH (Me)Leu, $PhCH_2$), 5.29 - 5.52 (2H, m, α-CH (Me)Leu), 6.50 (1H, br.d., NH D-Ala), 6.51 (1H, br.d., NH Val), 6.84 (1H, br.d., NH L-Ala), and 7.28 (5H, s, ArH); m/z 1072 (M^+, DCI), R_t 13.6 min.

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