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SYNTHESIS OF LINEAR UNDECAPEPTIDE PRECURSORS OF CYCLOSPORIN ANALOGUES

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Abstract: Eleven protected undecapeptide precursors of analogues of Cyclosporin have been prepared. The protected peptides were prepared by stepwise elongation of the Z-(4-11)-OBu^t using the diphenyl- phosphinic mixed anhydride method; yields in the region 60-90% of optically pure peptides were obtained. A number of fragment condensation approaches were investigated, however at best, using DppCl/NMM, only a 39% yield of chromatographically homogeneous material could be obtained.

The potent immunosuppressive cyclosporin[1-3] is at the centre of our current investigations. In the previous paper[4] application of the diphenyl phosphinic mixed anhydride procedure to the synthesis of extensively N-methylated fragments of cyclosporin A was discussed. Synthesis of the protected (4-11) octapeptide fragment was achieved by fragment condensation and by stepwise elongation. The latter method proved to be particularly successful using diphenyl phosphinic mixed anhydrides, and yields of 96, 75 and 94% were achieved for the addition of the last three residues in the octapeptide sequence.

As the method had previously proved to be successful in the synthesis of the octapeptide, it was felt that good yields would also be achieved for the addition of the last three residues to complete the linear undecapeptide sequence of a cyclosporin analogue.

As the synthesis of a small number of cyclosporins have been previously reported[5,6] using the fragment condensation approach, it was felt that continued stepwise extension would allow maximum variation in the incorporation of residues at positions-1 and -2 in the cyclic structure of cyclosporin. It is clear from the limited biological data which is available[6,7] that the nature of the residue at position 1 is particularly crucial and we, along with other workers[6], considered that the synthesis of the threonine-1 cyclosporin might reveal the extent to which the side chain of the C-9 amino acid was required for activity. It was also felt that useful information would be provided by the synthesis of analogues in which the second residue had been substituted by alternative hydrophobic residues, as the known norvaline-2 cyclosporin is noticably less nephrotoxic than cyclosporin A itself.[9] Limited molecular modelling studies prompted the preparation of the trans-4-hydroxyproline-1 analogue of cyclosporin, as the cyclic nature of the proline residue gave an additional degree of rigidity to the cyclic cyclosporin structure and the trans-4-hydroxyl group appeared to be placed such that it could participate in interactions similar to those which would be experienced by the hydroxyl group of the C-9 amino acid. Diaminobutyric acid was also incorporated at position one, as this would place an amino

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.

group close to the position normally occupied by the hydroxyl group in the C-9 amino acid. With these thoughts in mind a number of linear undecapeptide precursors of the final cyclic analogues of cyclosporin were prepared by the stepwise extension procedure.

The octapeptide (1) which had been previously prepared[4] by stepwise elongation using the diphenyl phosphinic mixed anhydride procedure was hydrogenolysed and the resulting material

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

(1)

coupled with Z-Sar-OH, again employing Dpp chloride for activation, using THF as the solvent and N-methyl morpholine as the base at -20°C; a bright yellow colouration was observed during the coupling. In contrast to some of the high yields that had been obtained using similar procedures, on this occasion only a 64% yield of chromatographically homogeneous material could be obtained. In this instance the bright yellow colouration which was produced in the reaction mixture was probably due to the formation of an oxazalonium salt which acted as the acylating species. The normal chemical and spectroscopic characterisations confirmed the nature of the nonapeptide (2).

The benzyloxycarbonyl nonapeptide-butyl ester (2) was then hydrogenolysed in the usual way, and individual couplings carried out with Z-Abu-OH, Z-Thr(Bu^t)-OH, Z-Nva-OH and Z-Nle-OH, in each case the diphenylphosphinic mixed anhydride procedure being used for activation. The resulting protected decapeptides were then obtained after work-up in the usual way and the yields were generally high, although in the case of the threonine analogue a rather lower yield was observed which was attributed to the steric hindrance encountered in the coupling of the protected threonine derivative. The decapeptides (3-6) showed a single peak on hplc at the retention times indicated in Table 1 and the optical rotations obtained were consistent for each compound in a number of preparations. The compounds were subsequently characterised by mass spectrometry, proton nmr and combustion analysis.

The decapeptides (3-6) were then extended to give the linear undecapeptide sequences of the compounds shown in Table 2. The (Me)Ser-1 analogues (7-10) were prepared by coupling with Z-(Me)Ser(Bu^t)-OH (11) employing the diphenylphosphinic mixed anhydride procedure for activation. The amino acid derivative (11) was successfully obtained in 96% yield by carrying out the N-methylation at 4°C for seventy hours without any β-elimination. In contrast, other workers[6,9,10] have experienced considerable difficulty with β-elimination and in general the use

TABLE 1 Synthesis of the fully protected decapeptides.

	YIELD %	M.P. °C	Hplc R_t (min)	$[\alpha]_D^{25}$ c=1,MeOH
Z-Abu-(3-11)-OBu^t (3)	90	83-84	13.2	-168.2
Z-Thr(Bu^t)-(3-11)-OBu^t (4)	48	85	14.1	-159.5
Z-Nva-(3-11)-OBu^t (5)	86	85-86	13.8	-164.0
Z-Nle-(3-11)-OBu^t (6)	77	83-84	11.8	-122.6

of low temperatures appears to reduce elimination appreciably. Also, in the synthesis of Boc-(Me)Ser(Bzl)-OH[6] the use of dimethoxyethane in place of THF gave much higher yields of the required derivative due to a decrease in the extent of β-elimination. Similarly in the present work the use of low temperatures (0-5°C), and use of a stoicheometric amount of sodium hydride limited the production of dehydroamino acid to less than 7%.

The diphenylphosphinic mixed anhydride method generally gave rise to good coupling yields as indicated in table 2 (compounds 7-10), there was some variation in yields but they were all considered to be satisfactory.

The protected threonine undecapeptides (12-15) were then prepared employing the same coupling procedure. In this case, the yields of chromatographically homogeneous products were

consistently over 64%.

Z-(Me)Thr(Bu^t)-OH was prepared by the procedure of Benoiton[9,10] and the derivative Fmoc-(Me)Thr(Bu^t)-OH (16), which was required for the preparation of peptide (15) was synthesised from the benzyloxycarbonyl derivative following hydrogenolysis. The Fmoc protecting group was satisfactorily introduced by reaction with Fmoc chloride over a period of forty minutes at pH 8.7. The derivative (16) was homogeneous by hplc, had a satisfactory CHN analysis and a fully interpretable proton nmr spectrum; the molecular ion was also evident by CI mass spectrometry. It was therefore concluded that there had been no tendency in this case to form Fmoc dipeptide derivatives[11] during preparation of the Fmoc amino acid derivative. The Fmoc protected peptide (15) was prepared as it was intended to prepare [(Me)Thr-1, Abu-2] cyclosporin by azide cyclisation using DPPA[12] to allow comparison with the cyclisation of the free peptide which would be generated from the similar derivative (14).

TABLE 2 Synthesis of protected undecapeptides.

	Yield%	M.p. °C	Hplc R_t (min)	$[\alpha]_D^{24}$ (c), MeOH
Z-(Me)Ser(Bu^t)-Nle-(3-11)-OBu^t (7)	75	72-73	15.8	-145.2 (1.5)
Z-(Me)Ser(Bu^t)-Nva-(3-11)-OBu^t (8)	73	70	15.2	-151.4 (1.2)
Z-(Me)Ser(Bu^t)-Thr(Bu^t)-(3-11)-OBu^t (9)	52	80	16.0	-136.4 (1)
Z-(Me)Ser-Abu-(3-11)-OBu^t (10)	41	80-81	13.5	-144 (1.3)
Z-(Me)Thr(Bu^t)-Nva-(3-11)-OBu^t (12)	65	70-71	14.8	-175.4 (1)
Z-(Me)Thr(Bu^t)-Nle-(3-11)-OBu^t (13)	64	-	15.7	-150.14 (1)
Z-(Me)Thr(Bu^t)-Abu-(3-11)-OBu^t (14)	86	92 - 93	12.2	-164.0 (1)
Fmoc-(Me)Thr(Bu^t)-Abu-(3-11)-OBu^t (15)	69	94-96	15.2	-121.8 (1.1)
Boc-Dab(Fmoc)-Abu-(3-11)-OBu^t (17)	81	109-110	11.7	-130.7 (0.8)
Z-Hyp(Bu^t)-Abu-(3-11)-OBu^t (19)	92	94-95	11.4	-128 (1)
Z-Hyp(Bu^t)-Nle-(3-11)-OBu^t (20)	68	95	11.8	-140.4 (1)

The protected [Dab-1, Abu-2] undecapeptide (17) was prepared by condensation of the product of hydrogenolysis of decapeptide (3) with Boc-Dab(Fmoc)-OH (18). Here again, the diphenylphosphinic mixed anhydride procedure was used and a highly satisfactory yield (81%) of the homogeneous protected undecapeptide (17) was obtained. The side chain amino protecting group was introduced by reaction of 9-fluorenyl-succinimidyl carbonate with the copper (II) complex of diaminobutyric acid. The resulting intermediate H-Dab(Fmoc)-OH was subsequently treated with di-tert-butyl-dicarbonate over twenty-four hours to give the Boc-Dab(Fmoc)-OH (18).

The two hydroxyproline peptides (19) and (20) were prepared using the diphenyl phosphinic mixed anhydride procedure for the coupling of Z-Hyp(Bu^t)-OH (21) with the suitably amino deprotected decapeptide. The hydroxyproline derivative (21) was prepared by reaction of isobutylene with Z-Hyp-ONb in the presence of sulphuric acid, followed by alkaline hydrolysis of the nitrobenzyl ester.

The two hydroxy proline undecapeptides (19) and (20) were obtained in high yield, the [Hyp-1, Abu-2] peptide (19) giving the highest yield observed in this series (92%); as has been found on all other occasions there was no evidence of racemisation as evidenced by hplc.

As part of the current studies the fragment condensation approach was also investigated for the preparation of undecapeptides; and in the pilot study synthesis of the peptide (22) was

examined by coupling between (Me)Leu-6 and alanine-7.[4] A number of coupling procedures were adopted including the use of diphenyl phosphinic mixed anhydrides as previously it had been shown that phosphinic mixed anhydrides are a potentially effective means of activation for fragment couplings.[13] Two other methods of fragment coupling were also evaluated as indicated in Table 3. From the Table it is clear that on no occasion was the fragment coupling approach completely satisfactory, as yields of no more than 39% were achievable, although the DppCl/NMM

TABLE 3 The fragment condensation synthesis of
Z-(Me)Leu-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-(Me)Thr(Bu^t)-Abu-Sar-OBu^t (22)

	Yield %	$[\alpha]_D^{25}$ (c), MeOH	
Castro reagent/NMM	32	-82 (1)	Heterogeneous on hplc/tlc
DppCl/NMM	39	-93 (1)	One peak on hplc
DppCl	13	-85 (1)	Heterogeneous on hplc/tlc
HONSu	30	-80 (1)	Heterogeneous on hplc/tlc

coupling did give a homogeneous product. These results, in conjunction with those found in the preparation of the octapeptides described in a previous paper[4], indicate that fragment condensation is not the most efficient route to the linear undecapeptide sequences, although this may be influenced by the nature of the C-terminal residue which is undergoing activation.

The fully protected undecapeptides shown in Table 2 were subsequently deprotected prior to cyclisation to form analogues of cyclosporin. The full details of these deprotections and cyclisations are presented in a subsequent paper.

The total synthesis of the eleven linear undecapeptides shown in Table 2 demonstrates conclusively that stepwise assembly of relatively large N-methylated peptides using the diphenylphosphinic mixed anhydride procedure is an efficient method of obtaining such compounds. Furthermore, the yields obtained make the method competitive with the fragment condensation approach, and the homogeneity of the products as indicated by hplc and corroborated by nmr suggest that minimal levels of racemisation are encountered.

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EXPERIMENTAL

Product purity was routinely checked by tlc and hplc, using the systems and detection methods outlined previously[4]. The general spectroscopic techniques and other generally applied experimental procedures have also been similarly detailed.

Amino acid derivatives.

Z-(Me)Ser(Bu^t)-OH (11)

Z-Ser(Bu^t)-OH (12g, 407 mM) and methyl iodide (20 cm^3, 320 mM) were dissolved in freshly distilled THF (120 cm^3) at 0°C, and sodium hydride dispersion (5.28g, 120 mM) was added cautiously with gentle stirring. The suspension, protected from the atmosphere by a drying tube was stirred at 4°C for seventy hours and the reaction worked-up in the usual way. The title compound was obtained as an oil (12.07g, 96%); $[\alpha]_D^{25}$ - 4.1° (c 1.2, CH_3OH); Calculated for $C_{16}H_{22}NO_5$: C, 62.14; H, 7.44; N, 4.53. Found: C, 61.82; H, 7.58; N, 4.19%; δ_H ($CDCl_3$, 220 MHz), 1.15 (9H, s, OBu^t), 3.01 (3H, s, N-CH_3), 3.71 - 3.90 (2H, m, β-CH_2), 4.65 - 4.85 (1H, m, α-CH), 5.13 (2H, s, CH_2-Ph), 7.34 (5H, s, ArH), and 9.25 (1H, br.s., COOH); m/z 309 (M^+, DCI).

Fmoc-(Me)Thr(Bu^t)-OH (16)

10% Na_2CO_3 (5 cm^3) was added to a solution of H-(Me)Thr(Bu^t)-OH (2g, 10.5 mM) in water, followed by dioxan (16 cm^3). The mixture was cooled to 0°C and Fmoc.Cl (2.7g, 10.5 mM) added over a period of forty minutes, while maintaining the pH at 8.7, by addition of 10% Na_2CO_3. The ice-bath was removed half an hour after the addition of Fmoc.Cl was completed, after which

time it was poured into water (500 cm^3). After washing with ether (x 3) the aqueous layer was cooled and acidified with 1M $KHSO_4$, extracted into EtOAc, washed with water and brine. Solvent evaporation gave a foam which was purified on a silica gel column eluting with EtOAc/CH_2Cl_2 1:8. Evaporation of the appropriate fractions afforded the title compound as a white foam (Z_g, 45%); m.p., 44 - 46°C; $[\alpha]_D^{20}$ + 12.8° (c 1, CH_3OH). Calculated for $C_{24}H_{29}NO_5$: C, 70.07; H, 7.10; N, 3.40. Found : C, 70.11; H, 7.22; N, 3.49%; δ_H (220 MHz, $CDCl_3$), 1.20 (9H, s, OBu^t), 1.05 (3H, d, β-CH_3), 3.05 (3H, d, N-CH_3), 4.10 - 4.20 (2H, m, α,β-CH), and 7.21 - 7.82 (8H, m, ArH); m/z 411 (M^+, CI), R_t 11.0 min (λ 278).

H-Dab(Fmoc)-OH

Cupric sulphate (1.4g, 5.5 mM) in (5.5 cm^3) water was added to a stirred solution of H-Dab-OH.2HCl (2g, 0.5 mM) and NaOH (0.8g, 0.02 mM) in water (44 cm^3), and the resulting mixture stirred for one hour. 9-Fluorenyl succinimide carbonate (3.2g) was then added dropwise with vigorous stirring and the resulting mixture stirred at room temperature for three days. The precipitate resulting was filtered, washed with water and dried. Excess ethylene diamine tetraacetic acid disodium salt was then added to one litre of boiling water, and the suspension kept at boiling point for ten minutes. The product was filtered, washed with water and dried in high vacuo over phosphorus pentoxide, (3.4g, 94%), m.p., 166°C.

Boc-Dab(Fmoc)-OH (18)

H-Dab-(Fmoc)-OH (3.4g, 0.01 mM) was added to a solution of Na_2CO_3 (0.7g) in water (5.1 cm^3) and dioxan/water (20 cm^3/9.9 cm^3) and stirred with cooling (ice-salt bath). Di-tert-butyl carbonate (2.4g, 11 mM) was then added and the mixture stirred at 0°C for one hour and room temperature for twenty-two hours. Dioxane was then evaporated and the residue basified to pH 10 with aqueous Na_2CO_3. After extraction into EtOAc, to remove excess di-tert-butyl carbonate, the aqueous layer was acidified with 1M $KHSO_4$. Extraction into EtOAc, washing the organic layer with water and brine, drying ($MgSO_4$) and solvent evaporation gave a white solid. This was recrystallised from Et_2O to give the title compound (2.2g, 50%); m.p., 79 - 80°C; $[\alpha]_D^{20}$ - 12.7°C (c 1, CH_3OH). Calculated for $C_{24}H_{28}N_2O_6$: C, 65.46; H, 6.36; N, 6.36. Found : C, 65.67; H, 6.38; N, 5.94%; δ_H (250 MHz, $CDCl_3$), 1.5 (10H, s, OBu^t and H-CH_2-), 1.9 - 2.2 (2H, m, β-CH_2), 3.2 - 3.7 (2H, m, γ-CH_2), 4.3 - 4.6 (3H, m, α-CH and CH_2 of Fmoc), 5.4 (1H, d, α-NH), 5.6 (1H, br.s., NH), and 7.3 - 7.8 (8H, m, ArH); m/z 341 (M+1, FAB), R_t 7.0 min.

Z-Hyp(Bu^t)-ONB

Isobutylene (660 cm^3) and concentrated H_2SO_4 (6.6 cm^3) were added to a solution of Z-Hyp(OH)-ONB (65g, 0.16 mol) in CH_2Cl_2 (660 cm^3). The butylation vessel was stoppered and the reaction mixture gently stirred for five days. The product was then washed with water and brine, after addition of 2M Na_2CO_3 (50 ml). Solvent evaporation gave a yellow oil, (30g, 41%); $[\alpha]_D^{20}$ - 18.3° (c, 1.2, CH_3OH); δ_H (250 MHz, $CDCl_3$), 1.16 (9H, s, Bu^t), 2.05 - 2.30 (2H, m, β-CH_2), 3.29 - 3.45 (1H, m, δ-CH), 3.73 - 3.82 (1H, m, δ-CH), 4.24 - 4.31 (1H, m, γ-CH), 4.45 - 4.59 (1H, m, α-CH), 5.12 (2H, s, CH_2), 5.29 2H, s, -CH_2-Ph-ONB), 7.35 (5H, s, ArH), 7.37 - 7.53 (2H, d x d, ArH), and 8.08 - 8.22 (2H, d x d, ArH); m/z 456 (M^+, CI), R_t 10.0 min.

Z-Hyp(Bu^t)-OH (21)

2M NaOH (778 cm^3) was added to a solution of Z-Hyp(Bu^t)-ONB (30g, 0.07M) in acetone (527 cm^3) and water (132 cm^3), and the mixture stirred at room temperature for two and a half hours. The solvent was removed in vacuo and the residue washed with ether. The aqueous layers were cooled and acidified with 1M $KHSO_4$, and extracted with EtOAc. The organic layer was then washed with water and brine and dried over Na_2SO_4. Solvent evaporation gave a solid, which was recrystallised from ether/petroleum-ether, to give the title compound (18g, 86%); m.p., 76 - 78°C; $[\alpha]_D^{20}$ - 29.1° (c 1, CH_3OH); calculated for $C_{17}H_{23}NO_5$: C, 63.55; H, 7.17; N, 4.36. Found: C, 63.56; H, 7.23; N, 4.19%; δ_H (250 MHz, $CDCl_3$), 1.17 (9H, s, OBu^t), 2.12 - 2.33 (2H, m, β-CH_2), 3.26 - 3.36 (1H, m, δ-CH), 3.67 - 3.80 (1H, m, δ-CH), 4.25 - 4.43 (1H, m, γ-CH), 4.45 - 4.52 (1H, m, α-CH), 5.12 (2H, s, Ph-CH_2-), 7.34 (5H, s, ArH), and 9.0 (1H, br.s., -COOH); m/z 321 (M^+, EI), R_t 8.0 min.

Protected nonapeptide

Z-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (2)

Z-Sar-OH (16.56g, 74.28 mM) in THF (24 cm^3) was activated using DppCl (17.55g, 74.28 mM) in THF (24 cm^3) and NMM (7.13 cm^3, 74.28 mM) at -20°C and coupled to H-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (57.66g, 61.89 mM) in THF (60 cm^3) as described for the general DppCl method. The reaction mixture was stirred at -15°C for two hours and at ambient temperature for forty-five hours, and was worked-up in the usual way. The residue was purified on a silica gel column eluting with EtOAc/CH_2Cl_2 (1:1) to give the title compound as a white solid, (45.36g, 64%); m.p., 64°C; $[\alpha]_D^{24}$ - 131.5°C (c 1.1, CH_3OH). Calculated for $C_{60}H_{103}N_9O_{12}$: C, 63.05; H, 9.09; N, 11.03. Found : C, 63.11; H, 9.14; N, 10.88%; δ_H (250 MHz, $CDCl_3$, 2 confs.), 0.76 - 1.06 (36H, m, β-CH_3, Val, (Me)Val, γ-CH_3 (Me)Leu), 1.27 - 1.36 (6H, d x d, CH_3 Ala,D-Ala), 1.44 (9H, s, OBu^t), 1.52 - 1.87 (12H, m, β-CH_2, -CH (Me)Leu), 2.04 - 2.17 (2H, m, β-CH, Val, (Me)Val), 2.80 - 3.07 (18H, series of s, N-CH_3), 4.01 - 4.11 (2H, d x d, CH_2, Sar), 4.36 (1H, α-CH, Ala), 4.65 - 4.72 (2H, m, α-CH, Val, (Me)Val), 4.68 - 5.07 (3H, m, α-CH, D-Ala, (Me)Leu), 5.12 (2H, s, Ph-CH_2-), 5.45 (2H, m, α-CH, (Me)Leu), 6.50 (1H, br.d., NH, D-Ala), 6.63 (1H, br.d., NH Val), 6.82 (1H, br.d., NH Ala), 7.31 (5H, s, ArH); m/z 1141 (M^+, DCI), R_t 12.0 min.

a) Hydrogenolysis

H-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t

Z-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (14.5,12.71mM) in methanol (150 cm^3) was hydrogenolysed at atmospheric pressure in the presence of 10% Pd/C catalyst for twenty-four hours. The catalyst was removed by filtration and the solvent evaporated to dryness. The residue was triturated with ether to afford the title compound as a white solid, (12.8g, 97%); m.p., 96 - 97°C; $[\alpha]_D^{23}$ - 154.2° (c 1, CH_3OH). Calculated for $C_{52}H_{97}N_9O_{10}$: C, 61.19; H, 9.70; N, 12.50. Found : C, 61.56; H, 9.73; N, 12.02%; δ_H (250 MHz, $CDCl_3$), 1.04 -

1.76 (36H, m, γ-CH_3, (Me)Leu; β-CH_3, Val, (Me)Val), 1.29 - 1.46 (6H, d x d, CH_3, Ala, D-Ala), 1.43 (9H, s, OBu^t), 1.52 - 1.86 (12H, m, β-CH_2, γ-CH, (Me)Leu), 2.15 - 2.45 (3H, m, β-CH, Val, (Me)Val, NH Sar), 2.74 - 3.09 (18H, series of s, N-CH_3), 4.32 - 4.37 (2H, d x d, CH_2 Sar), 4.34 (1H, m, α-CH, Ala), 4.51 - 5.52 (7H, m, α-CH), 6.59 (1H, br.d., NH, D-Ala), 6.72 (1H, br.d., NH Val), and 6.84 (1H, br.d., NH Ala); m/z 1007 (M^+, DCI).

Protected decaptides (Table 1).

Z-Abu-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (3)

Z-Abu-OH (4.4g, 18.8 mM) in THF (8 cm^3) was activated using DppCl (4.5g, 18.8 mM) in THF (5 cm^3) and NMM (2.1g, 18.8 mM) at -20°C and coupled to H-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (12.8g, 15.7 mM) according to the general DppCl procedure. The reaction mixture was stirred at -15°C for two hours and at ambient temperature for forty-five hours. The reaction was worked-up as described in the usual way and the residue purified on a silica gel column eluting with EtOAc/DCM (1:2). Evaporation of the appropriate fractions gave the title compound as a white foam (14.1g, 90%); m.p., 83 - 84°C; $[\alpha]_D^{24}$ - 168.2° (C 1.1, CH_3OH). Calculated for $C_{64}H_{110}N_{10}O_{13}$: C, 62.58; H, 9.02; N, 11.40. Found : C, 62.29; H, 9.09; N, 11.28%; δ_H (250 MHz, $CDCl_3$, 2 confs.), 0.75 - 1.06 (39H, m, β-CH_3 Val, (Me)Val, γ-CH_3 (Me)Leu, β-CH_3 Abu), 1.25 - 1.35 (6H, d x d CH_3 Ala, D-Ala), 1.48 (9H, s, OBu^t), 1.53 - 1.86 (12H, m, β-CH_2, γ-CH (Me)Leu), 1.91 (2H, m, β-CH_2 Abu), 2.02 - 2.15 (2H, m, β-CH Val, (Me)Val), 2.66 - 3.26 (18H, series of s, N-CH_3), 4.26 - 4.30 (2H, d x d, CH_2 Sar), 4.35 (1H, m, α-CH-Ala), 4.50 - 4.90 (3H, m, α-CH (Me)Val, D-Ala, Val), 4.94 - 5.07 (3H, m, α-CH Abu and (Me)Leu), 5.08 (2H, s, Ph-CH_2-), 5.46 (2H, m, α-CH (Me)Leu), 5.90 (1H, br.d., NH D-Ala), 6.55 (1H, br.d., NH Val), 6.71 (1H, br.d., NH Ala), and 7.33 (5H, s, ArH); R_t 13.2 min.; m/z 1225 (M^+, DCI).

Z-Thr(Bu^t)-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (4)

NMM (1.1 cm^3, 9.9 mM, 2.5 equiv.) was added to a stirred solution of Z-Thr(Bu^t)-OH (1.4g, 4.4 mM, 1 equiv.) in THF (10 cm^3) and the mixture stirred at -20°C. DppCl (1.4g, 6 mM, 1.5 equiv.) in THF (4 cm^3) was then added and the resulting mixture stirred at -20°C for twenty minutes.

A pre-cooled solution of H-Abu-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (4g, 4 mM, 1 equiv.) in THF (10 cm^3) was then added and the reaction mixture stirred at -20°C for one hour, 0 - 5°C for one hour and room temperature for four days. The product was worked-up as described for the general DppCl procedure, to yield an oil which was purified on a silica gel column eluting with EtOAc. Evaporation of the appropriate fractions gave the title compound as a white foam (2.5g, 48%); m.p., 85°C; $[\alpha]_D^{20}$ - 159.5° (c 1, CH_3OH). Calculated for $C_{68}H_{118}N_{10}O_{14}$: C, 62.87; H, 9.09; N, 10.79. Found : C, 62.48; H, 9.26; N, 10.71%; δ_H (250 Mhz, $CDCl_3$ 0.78 - 1.07 (36H, m, CH_3 of (Me)Val, (Me)Leu, Val), 1.13 (3H, d, β-CH_3 of Thr), 1.21 (9H, s, Thr, OBu^t), 1.28 - 1.38 (6H, m, CH_3 of Ala, D-Ala), 1.44 (9H, s, (Me)Val, OBu^t), 1.52 - 1.83 (10H, m, β-CH_2 of (Me)Leu and Abu), 1.85 - 2.32 (6H, m, β-CH of (Me)Val, Val and γ-CH of (Me)Leu), 2.80 - 3.09 (18H, series of s, N-CH_3), 3.35 (1H, d, α-CH, Sar), 3.45 - 3.50 (1H, m, β-CH of Thr), 4.41 - 4.52 (1H, m, α-CH, Ala), 4.70 - 4.92 (7H, m, α-CH), 5.20 (1H, d, α-CH, Thr), 5.14 (2H, s, Ph-CH_2), 5.31 - 5.56 (2H, m, α-CH), 6.21 (1H, d, NH Thr), 6.50 (1H, d, NH D-Ala), 6.92 - 7.02 (2H, m, NH Val, L-Ala), 7.35 (5H, s, ArH); m/z 1298 (M^+, CI), R_t 14.1 min.

Z-Nva-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (5)

Z-Nva-OH (1.79g, 7.14 mM) in THF (5 cm^3) was activated using DppCl (2.11g, 8.92 mM) in THF (5 cm^3) and NMM (1.30 cm^3, 8.92 mM) at -20°C and coupled to H-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (6.10g, 5.95 mM) as described in the general DppCl method. The reaction mixture was stirred at -20°C for two hours and at ambient temperature for thirty-six hours, and the residue worked-up in the usual way. Purification on a silica gel column eluting with EtOAc/DCM (2:1) gave the title compound as a white foam (6.4g, 86%); m.p., 85 - 86°C; $[\alpha]_D^{24}$ - 164°, (c 1, CH_3OH). Calculated for $C_{65}H_{112}N_{10}O_{13}$, C, 62.85; H, 9.10; N, 11.28. Found : C, 62.69; H, 9.21; N, 10.94%; δ_H (250 MHz, $CDCl_3$, 2 confs.), 0.77 - 1.03 (39H, m, β-CH_3 (Me)Val, Val, γ-CH_3(Me)Leu, CH_3 Nva), 1.29 - 1.31 (10H, d x d, CH_3 Ala, D-Ala, β, CH_2 Nva), 1.44 (9H, s, OBu^t), 1.51 - 1.81 (12H, m, β-CH_2, γ-CH of (Me)Leu), 2.05 - 2.24 (2H, m, β-CH Val, (Me)Val), 2.70 ; 3.28 (18H, series of s, N-CH_3), 4.28 - 4.35 (2H, d x d, CH_2 Sar), 4.35 (1H, m, α-CH Ala), 4.38 (1H, t, α-CH Nva), 4.66 - 5.06 (4H, m, α-CH), 5.10 (2H, s, $PhCH_2$), 5.46 - 5.52 (2H, m, α-CH (Me)Leu), 5.89 - 5.92 (1H, br.d., NH Nva), 6.58 (1H, br.d., NH D-Ala), 6.78 (1H, br.d., NH Val), 6.80 - 6.95 (1H, br.d., NH Ala), and 7.34 (5H, s, ArH), R_t 13.8 min.; m/z 1241 (M^+, DCI).

Z-Nle-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (6)

Z-Nle-OH (1.4g, 5.2 mM) in THF (5 cm^3) was activated using DppCl (1.5g, 6.2 mM) in THF (5 cm^3) and NMM (1.1g, 10.4 mM) at -20°C and coupled to H-Sar-(Me)Leu-(Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (4.8g, 4.7 mM) as described in the general DppCl coupling procedure. The reaction mixture was stirred at -20°C for two hours and at room temperature for forty- six hours. After work-up as described in the general DppCl procedure, the residue was purified on a silica gel column eluting with EtOAc/DCM (3:1), to give the title compound as a white solid, (4.6g, 77%); m.p., 83 - 84°C; $[\alpha]_D^{24}$ -122.6°, (c 1, CH_3OH). Calcualted for $C_{66}H_{114}N_{10}O_{13}$: C, 63.11; H, 9.15; N, 11.05. Found : C, 62.94; H, 9.16; N, 10.88%; δ_H (220 MHz, $CDCl_3$, 2 conf.), 0.78 - 1.03 (39H, m, β-CH_3 (Me)Val and Val, γ-CH_3 of (Me)Leu, CH_3-Nle), 1.23 - 1.31 (10H, m, CH_3 Ala, D-Ala, β,γ,δ-CH_2 Nle), 1.44 (9H, s, OBu^t), 1.53 - 1.80 (12H, m, β-CH_2, γ-CH of (Me)Leu), 2.04 - 2.15 (2H, m, β-CH Val, (Me)Val), 2.71 - 3.31 (18H, series of s, N-CH_3), 4.28 - 4.33 (2H, d x d, CH_2 Sar), 4.36 (1H, m, α-CH Ala), 4.39 (1H, t, α-CH Nle), 4.65 - 5.01 (5H, m, α-CH), 5.20 (2H, s, $PhCH_2$), 5.50 - 5.52 (2H, m, α-CH (Me)Leu), 5.92 (1H, br.d., NH Nle), 6.59 (1H, br.d., NH Ala), 6.78 (1H, br.d., NH Val), 6.81 - 6.95 (1H, br.d., NH D-Ala) and 7.34 (5H, s, ArH), R_t 11.8 min.; m/z 1225 (M^+, DCI).

Hydrogenolysis of decapeptides (3) - (6).

H-Abu-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t

Z-Abu-(3-11)-OBu^t (3) (2.45g, 2 mM) in CH_3OH (50 cm^3) was hydrogenolysed in the presence of 10% Pd/C catalyst for forty-eight hours. The catalyst was removed by filtration and the filtrate evaporated to give the title compound as a foam (2.12g, 97%); m.p., 92 - 94°C; $[\alpha]_D^{24}$ - 143° (c 1, CH_3OH); m/z 1092 (M+1, FAB).

H-Thr(Bu^t)-Sar-(Me)Leu-Val-(Me)Leu-L-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t

(10%) Pd/C catalyst (0.21g) was added to a stirred solution of Z-Thr-(Bu^t)-(3-11)-OBu^t (4) (2.1g, 1.6 mM) in MeOH (30 cm^3) and the mixture hydrogenolysed for forty-six hours. The catalyst was removed by filtration and the filtrate evaporated to dryness to give the title compound (1.7g, 88%); m.p., 88 - 90°C; $[\alpha]_D^{20}$ - 168°; (c 0.8, CH_3OH). Calculated for $C_{60}H_{112}N_{10}O_{12}.H_2O$: C, 60.90; H, 9.64; N, 11.84. Found : C, 61.16; H, 9.66; N, 11.63%; m/z 1166 (M+1, FAB).

H-Nva-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(MeOLeu-(Me)Val-OBu^t

Z-Nva-(3-11)-OBu^t (5) (4.96g, 4 mM) in MeOH (50 cm^3) was hydrogenolysed at atmospheric pressure in the presence of 10% Pd/C catalyst (0.2g). The catalyst was removed by filtration and the filtrate evaporated to give the title compound as a white foam (4.47g, 94%); $[\alpha]_D^{24}$ - 170°, (c 1, CH_3OH); δ_H (250 MHz, $CDCl_3$), 0.74 - 1.08 (39H, m, γ-CH_3 (Me)Leu, β-CH_3 Val, (Me)Val, CH_3 Nva), 1.23 - 1.31 (10H, m, CH_3 Ala, D-Ala, β-CH_2 Nva), 1.45 (9H, s, OBu^t), 1.53 - 1.85 (12H, m, β-CH_2, γ-CH (Me)Leu), 2.20 - 2.41 (2H, m, β-CH Val, (Me)Val), 2.79 - 3.20 (18H, series of s, NCH_3), 4.21 (2H, br.s., NH_2), 4.34 - 4.39 (2H, d x d, CH_2 Sar), 4.41 - 5.52 (9H, m, α-CH), 6.61 (1H, br.d., NH D-Ala), 6.74 (1H, br.d., NH Val), and 6.85 (1H, br.d., NH Ala); m/z 1107 (M+1, FAB).

H-Nle-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t

Z-Nle-(3-11)-OBu^t (6) (5.0g, 4 mM) in MeOH (50 cm^3) was hydrogenolysed in the presence of 10% Pd/C catalyst (0.2g) under atmospheric pressure for forty-eight hours. The catalyst was removed by filtration and the filtrate evaporated to give the title compound as a white solid, (4.9g, 95%); m.p., 84 - 86°C; $[\alpha]_D^{23}$ - 162.2°, (c 1, CH_3OH); δ_H (250 MHz, $CDCl_3$), 0.75 - 1.08 (39H, m, γ-CH_3 (Me)Leu, β-CH_3 Val, (Me)Val, CH_3 Nle), 1.24 - 1.31 (12H, m, CH_3 Ala, D-Ala, β, ,δ-CH_2 Nle), 1.45 - 1.48 (9H, s, OBu^t), 1.54 - 1.85 (12H, β-CH_2, γ-CH (Me)Leu), 2.21 - 2.42 (2H, m, β-CH Val, (Me)Val), 2.79 - 3.20 (18H, series of s, N-CH_3), 4.20 (2H, br.s., -NH_2), 4.34 - 4.39 (2H, d x d, α-CH Sar), 4.40 - 5.52 (9H, m, α-CH), 6.61 (1H, br.d., NH D-Ala), 6.75 (1H, br.d., NH Val), and 6.83 (1H, br.d., NH-Ala); m/z 1121 (M+1, FAB).

Protected undecapeptides (Table 2).

Z-(Me)Ser(Bu^t)-Nle-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (7)

To a solution of Z-(Me)Ser(Bu^t)-OH (11) (0.46g, 1.5 mM) in THF (2 cm^3) and NMM (0.35 cm^3, 1.3 mM) at -20°C was added a cooled solution of DppCl (0.37g, 1.5 mM) in THF (5 cm^3). The suspension was stirred at -20°C for ten minutes and then treated with a pre-cooled solution of H-Nle-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (1.44g, 1.3 mM). The reaction mixture was stirred for two hours at -20°C and thirty-six hours at room temperature and worked-up in the usual way to yield a crude solid. The residue was purified on a silica gel column eluting with EtOAc-DCM (3:1). Evaporation of the appropriate fractions afforded the title compound as a white solid (1.52g, 75%), m.p., 72 - 73°C; $[\alpha]_D^{24}$ - 145.2° (c 1.5, CH_3OH). Calculated for $C_{74}H_{129}N_{11}O_{15}$: C, 62.88; H, 9.23; N, 10.90. Found : C, 62.97; H, 9.33; N, 10.40%; δ_H (250 MHz, $CDCl_3$, 2 confs.), 0.73 - 1.03 (39H, β-CH_3 Val, (Me)Val, γ-CH_3 (Me)Leu, CH_3 Nle), 1.12 (9H, s, OBu^t (Me)Ser), 1.22 - 1.32 (12H, m, CH_3 Ala, D-Ala, β, δ ,-CH_2 Nle), 1.38 - 1.44 (9H, 2s, OBu^t, conf.), 1.54 - 1.62 (4H, m, γ-CH (Me)Leu), 1.63 - 1.81 (8H, m, β-CH_2- (Me)-Leu), 2.04 - 2.16 (2H, m, β-CH Val, (Me)Val), 2.70 - 3.30 (21H, series of s, NCH_3), 3.84 (2H, t, β-CH_2 (Me)Ser), 4.27 - 4.32 (2H, d x d, α-CH_2 Sar), 4.35 (1H, m, α-CH Ala), 4.40 (1H, t, α-CH Nle), 4.65 (2H, m, α-CH Val, (Me)Val), 4.69 - 4.86 (3H, m, α-CH D-Ala, (Me)Leu), 5.13 (2H, s, $PhCH_2$), 5.46 (2H, m, α-CH (Me)Leu), 5.51 (1H, t, α-CH (Me)Ser), 6.89 - 7.10 (1H, br.d., NH, D-Ala), 7.15 - 7.20 (1H, br.d., NH Val), 7.34 (5H, s, ArH), 7.62 - 7.78 (1H, br.d., NH-Ala), and 7.89 - 8.07 (1H, br.d., NH Nle); R_f 15.8 min.; m/z 1412 (M^+, DCI).

Z-(Me)Ser(Bu^t)-Nva-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (8).

DppCl (0.38g, 1.5 mM) in THF (5 cm^3) was added to a stirred solution of Z-(Me)Ser(Bu^t)-OH (11) (0.46g, 1.5 mM) in THF (2 cm^3) and NMM (0.36 cm^3, 1.3 mM) at -20°C, and the suspension stirred at this temperature for twenty minutes. A pre-cooled solution of H-Nva-Sar-(Me)Leu-Val-(Me)Leu-Ala-D- Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (1.43g, 1.3 mM) in THF (5 cm^3) was then added to the above mixture and the total mixture stirred at -20°C for two hours and at ambient temperature for thirty-five hours. The reaction was worked-up as described for the general DppCl procedure and the residue purified on a silica gel column eluting with EtOAc/DCM. Solvent evaporation of the appropriate fractions afforded the title compound as a white foam (1.41g, 73%); m.p., 70°C; $[\alpha]_D^{25}$ - 151.4° (c 1.4, CH_3OH). Calculated for $C_{73}H_{127}N_{11}O_{15}$: C, 62.66; H, 9.15; N, 11.01. Found : C, 62.31; H, 9.26; N, 10.64%; δ_H (250 MHz, $CDCl_3$, 2 confs.), 0.72 - 1.03 (39H, m, γ-CH_3 (Me)Leu, β-CH_3 Val and (Me)Val, CH_3 Nva), 1.13 (9H, s, OBu^t, (Me)Ser), 1.21 - 1.32 (10H, d x d, CH_3 Ala, D-Ala, β,γ,-CH_2 Nva), 1.44 (9H, 2s, OBu^t, conf.), 0.53 - 1.61 (4H, m, γ-CH (Me)Leu), 1.62 - 1.80 (8H, m, β-CH_2 (Me)Leu), 2.03 - 3.06 (1H, m, β-CH Val), 2.11 - 2.16 (1H, m, β-CH (Me)Val), 2.69 - 3.31 (21H, series of s, N-CH_3), 3.83 (2H, t, β-CH_2 (Me)Ser), 4.26 - 4.31 (2H, d x d, CH_2 Sar), 4.34 - 4.40 (1H, m, α-CH Ala), 4.40 (1H, t, α-CH Nva), 4.64 - 4.65 (2H, d x d, α-CH (Me)Val, α-CH Val), 4.69 - 5.08 (3H, m, α-CH (Me)Leu, α-CH D-Ala), 5.12 (2H, s, $PhCH_2$), 5.45 (2H, m, α-CH (Me)Leu), 5.52 (1H, t, α-CH- (Me)Ser), 6.88 - 7.09 (1H, br.d., NH D-Ala), 7.14 - 7.21 (1H, br.d., NH Val), 7.33 (5H, s, ArH), 7.61 - 7.77 (1H, br.d., NH Ala), and 7.90 - 8.08 (1H, br.d., NH Nva); R_f 15.2 min; m/z 1398 (M^+, DCI).

Z-(Me)Ser(Bu^t)-Thr(Bu^t)-Sar-(Me)Leu-(Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Val-OBu^t (9)

N.M.M. (0.4 cm^3, 3.6 mM, 2.5 equiv.) was added to a stirred solution of Z-(Me)Ser(Bu^t)-OH (11) (0.5g, 1.6 mM, 1.1 equiv.) in THF (5 cm^3) at -20°C. DppCl (0.5g, 2.2 mM, 1.5 equiv.) in THF (2 cm^3) was then added and the mixture stirred at -20°C for twenty minutes, after which time H-Thr(Bu^t)-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (1.7g, 1.4 mM, 1

equiv.) in THF (5 cm^3) was added. The resulting mixture was stirred at -20°C for one hour, 0 - 5°C for half an hour and room temperature for four days. The product was worked-up as described in the general DppCl procedure to give an oil, which was purified on a silica gel column eluting with EtOAc. Evaporation of the appropriate fractions afforded the title compound as a white foam (1.1g, 52%); m.p., 80°C; $[\alpha]_D^{20}$ - 136.4° (c 1.0, CH_3OH). Calculated for $C_{76}H_{133}N_{11}O_{16}$: C, 62.68; H, 9.14; N, 10.58. Found : C, 62.61; H, 9.31; N, 10.19%; δ_H (250 MHz, $CDCl_3$), 0.98 - 1.10 (36H, m, CH_3 of (Me)Val, (Me)Leu, Val), 1.18 (9H, s, Thr(Bu^t)), 1.21 - 1.25 (12H, m, (Me)Ser (Bu^t), and Thr-CH_3), 1.29 - 1.31 (6H, d x d, CH_3 of L-Ala, D-Ala), 1.54 - 1.96 (12H, m, β-CH_2 and γ-CH of (Me)Leu), 2.05 - 2.32 (2H, m, β-CH of (Me)Val, Val), 2.79 - 3.28 (21H, series of s, N-CH_3), 3.22 (1H, d, α-CH Sar), 3.65 - 3.75 (1H, m, β-CH of Thr), 4.05 - 4.10 (2H, m, β-CH_2 of (Me)Ser), 4.25 - 4.35 (1H, m, α-CH L-Ala), 4.53 - 4.99 (4H, m, α-CH of D-Ala, Val, (Me)Val, Sar), 5.02 - 5.20 (4H, m, α-CH of (Me)Ser, Thr, and Ph-CH_2-), 5.48 - 5.58 (4H, m, α-CH of (Me)Leu), 6.92 - 7.27 (3H, m, N-H), 7.28 (5H, s, Ar-H), and 7.31 (1H, d, NH L-Ala); m/z 1457 (M+1, FAB); R_t 16 min.

Z-(Me)Ser(Bu^t)-Abu-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (10)

N.M.M. (0.9 cm^3, 9.8 mM, 2.5 equiv.) was added to a solution of Z-(Me)Ser(Bu^t)-OH (1.21g, 3.9 mM, 1 equiv.) in THF (10 cm^3) and the mixture was stirred at -20°C. DppCl (1.38g, 5.9 mM, 1.5 equiv.) in THF (5 cm^3) was then added and the resulting mixture stirred at -20°C for twenty minutes. A pre-cooled solution of H-Abu-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (3.6g, 3.3 mM, 1 equiv.) in THF (5 cm^3) was added and the reaction mixture stirred at -20°C for one hour, 0 - 5°C for one hour and room temperature for four days. The product was worked-up as described for the general DppCl method, to yield an oil which was purified on a silica gel column eluting with EtOAc/CH_2Cl_2 (1:3); evaporation of the appropriate fractions gave the title compound as a white solid (1.9g, 41%); m.p., 80 - 81°C; $[\alpha]_D^{20}$ - 144° (c 1.3, CH_3OH). Calculated for $C_{72}H_{125}N_{11}O_{15}$: C, 62.47; H, 9.04; H, 11.14. Found : C, 62.34; H, 9.15; N, 10.94%; δ_H (250 MHz, $CDCl_3$), 0.77 - 1.02 (39H, m, CH_3 of Abu, Val, (Me)Leu, (Me)Val), 1.17 (9H, s, (Me)Ser, (Bu^t)), 1.24 - 1.30 (6H, d x d, CH_3 of L-Ala, D-Ala), 1.44 (9H, s, (Me)Val-(OBu^t)), 1.36 - 2.26 (14H, m, β-CH_2 of (Me)Leu and Abu, γ-CH of (Me)Leu), 2.18 - 2.22 (2H, m, β-CH of Val and (Me)Val), 2.81 - 3.49 (18H, series of s, N-CH_3), 3.12-3.80 (2H,dxd, α-CH Sar), 3.52 - 3.77 (2H, m, β-CH_2 of (Me)Ser), 3.80 (1H, d, α-CH Sar), 4.28 - 4.48 (1H, m, α-CH L-Ala), 4.67 - 4.72 (1H, d, α-CH Val), 4.72 - 4.76 (1H, d, α-CH (Me)Val), 4.79 - 4.96 (2H, m, α-CH, D-Ala and Abu), 5.05 - 5.15 (5H, m, Ph-CH_2- and α-CH of (Me)Leu, (Me)Ser), 5.48 - 5.52 (1H, m, α-CH (Me)Leu), 6.90 - 7.11 (2H, 2s, N-H), 7.27 (5H, s, ArH), 7.85 - 8.28 (2H, 2s, -NH); m/z 1384 (M+1, FAB); R_t 13.5 min.

Z-(Me)Thr(Bu^t)-Nva-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)-Val-OBu^t (12)

Z-(Me)Thr(Bu^t)-OH (0.39g, 1.20 mM) in THF (2 cm^3) was activated using DppCl (0.31g, 1.25 mM) in THF (2 cm^3) and NMM (0.3 cm^3, 2.5 mM) at -20°C and coupled to H-Nva-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (1.30g, 1.17 mM) in THF (5 cm^3) according to the general DppCl method. The reaction mixture was stirred at -20°C for two hours and at ambient temperature for forty-five hours, and then worked-up in the usual way. The residue was purified on a silica gel column eluting with EtOAc to give the title compound as a white solid (0.75g, 65%), m.p., 70 - 71°C; $[\alpha]_D^{25}$ - 175.4°, (c 1, CH_3OH). Calculated for $C_{74}H_{129}N_{11}O_{15}$: C, 62.88; H, 9.20; N, 10.90. Found : C, 63.02; H, 9.38; N, 10.70%; δ_H (250 MHz, $CDCl_3$, 2 confs.), 0.72 - 1.02 (39H, m, γ-CH_3 (Me)Leu, β-CH_3 Val, (Me)Val and CH_3 Nva), 1.09 - 1.25 (9H, s, OBu^t), 1.09 - 1.25 (3H, d, β-CH_3 (Me)Thr), 1.25 - 1.35 (12H, m, CH_3 Ala, D-Ala, β,γ,δ-CH_2 Nva), 1.46 - 1.48 (9H, 2s, OBu^t, Conf.,), 1.52 - 1.85 (12H, m, β-CH_2, γ-CH (Me)Leu), 2.03 - 2.16 (2H, β-CH-Val, (Me)Val), 2.70 - 3.14 (21H, series of s, N-CH_3), 4.19 (1H, m, β-CH- (Me)Thr), 4.28 - 4.36 (2H, d x d, CH_2 Sar), 4.39 (1H, m, α-CH Ala), 4.44 (1H, t, α-CH Nva), 4.63 - 4.70 (2H, m, α-CH Val, (Me)Val), 4.67 - 5.08 (3H, m, α-CH D-Ala, (Me)Leu), 5.12 (2H, s, $PhCH_2$), 5.45 (2H, m, α-CH (Me)Leu), 6.88 - 7.09 (1H, br.d., NH D-Ala), 7.13 - 7.18 (1H, br.d., NH Val), 7.33 (5H, s, ArH), 7.61 - 7.78 (1H, br.d., NH Ala), and 7.88 - 8.05 (1H, br.d., NH Nva); R_t 14.8 min.; m/z 1411 (M^+, DCI).

Z-(Me)Thr(Bu^t)-Nle-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (13)

Z-(Me)Thr(Bu^t)-OH (0.40g, 1.25 mM) in THF (2 cm^3) was activated using DppCl (0.31g, 1.24 mM) in THF (2 cm^3) and NMM (0.3 cm^3, 2.50 mM) at -20°C, and coupled to H-Nle-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (1.38g, 1.24 mM) in THF (2 cm^3), according to the general DppCl method. The reaction mixture was stirred at -20°C for two hours and ambient temperature for forty-five hours, and then worked-up as described for the general DppCl method. The residue was purified on a silica gel column eluting with EtOAc/DCM (1:1), to give the title compound as a white foam (0.71g, 64%); $[\alpha]_D^{24}$ - 150.4° (c 1.2, CH_3OH). Calculated for $C_{75}H_{131}N_{11}O_{15}$: C, 63.11; H, 9.26; N, 10.80. Found : C, 63.00; H, 9.52; N, 11.03%; δ_H (250 MHz, $CDCl_3$, 2 confs.), 0.73 - 1.02 (39H, m, γ-CH_3 (Me)Leu, β-CH_3 (Me)Val, Val and CH_3 Nle), 1.08 - 1.25 (12H, m, β-CH_3 (Me)Thr, OBu^t (Me)Thr), 1.25 - 1.35 (12H, m, CH_3 Ala, D-Ala, CH_2 Nle), 1.46 - 1.49 (9H, 2s, OBu^t, conf.), 1.52 - 1.85 (12H, m, β-CH_2 and γ-CH (Me)Leu), 2.04 - 2.16 (2H, m, β-CH Val, (Me)Val), 2.70 - 3.14 (21H, series of s, N-CH_3), 4.19 (1H, m, β-CH (Me)Thr), 4.29 - 4.37 (2H, d x d, CH_2 Sar), 4.39 (1H, m, α-CH Ala), 4.44 (1H, t, α-CH- Nle), 4.64 (3H, m, α-CH), 4.68 - 5.07 (3H, m, α-CH D-Ala, (Me)Leu), 5.12 (2H, s, $PhCH_2$), 5.46 (2H, m, α-CH (Me)Leu), 6.8 - 7.09 (1H, br.d., NH D-Ala), 7.14 - 7.19 (1H, br.d., NH Val), 7.33 (5H, s, ArH), 7.62 - 7.79 (1H, br.d., NH Ala), and 7.89 - 8.06 (1H, br.d., NH Nle); R_t 15.7 min; m/z 1425 (M^+, DCI).

Z-(Me)Thr(Bu^t)-Abu-Sar-(Me)Leu-Val-(Me)Leu-L-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (14)

N.M.M. (1.0 cm^3, 6.5 mM, 2.5 equiv.) was added to a solution of Z-(Me)Thr(Bu^t)-OH (1g, 3.1 mM, 1.2 equiv.) in THF (10 cm^3), and the mixture stirred at -20°C. DppCl (0.9g, 3.9 mmol, 1.5 equiv.) in THF (5 cm^3) was then added, and the resulting mixture stirred at -20°C for twenty minutes. A pre-cooled solution of H-Abu-Sar-(Me)Leu-Val-(Me)Leu-L-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (2.8g, 2.6 mM, 1 equiv.) in THF (10 cm^3) was added and the reaction mixture was stirred at -20°C for one hour, 0°C for one hour and room temperature for two days. The product

was worked up as described for the general DppCl procedure, to yield an oil which was purified on a silica gel column eluting with EtOAc. Evaporation of the appropriate fractions gave the title compound as a white foam, (3.1g, 86%); m.p., 92 - 93°C; $[\alpha]_D^{20}$ - 164 (c 1.0, MeOH). Calculated for $C_{73}H_{126}N_{11}O_{15}$: C, 62.66; H, 9.01; N, 11.02. Found : C, 62.60; H, 9.07; N, 11.03%; δ_H (250 MHz, $CDCl_3$), 0.77 - 1.12 (39H, CH_3 of Abu, Val, (Me)Leu, (Me)Val), 1.13 - 1.19 (3H, d, γ-CH_3 (Me)Thr), 1.20 (9H, s, (Me)Thr(Bu^t)), 1.29 - 1.32 (6H, d x d, CH_3 of L-Ala and D-Ala), 1.44 (9H, s, (Me)Val(OBu^t)), 1.52 - 1.89 (10H, m, β-CH_2 of Abu, (Me)Leu), 1.84 - 2.22 (6H, m, β-CH of Val and (Me)Val and γ-CH (Me)Leu), 2.72 - 3.34 (21H, series of s, N-CH_3), 3.21 (1H, d, α-CH Sar), 4.19 - 4.32 (1H, qt, β-CH (Me)Thr), 4.33 - 4.41 (1H, m, α-CH Ala), 4.50 - 4.90 (4H, m, α-CH of Sar, (Me)Val, D-Ala and Val), 4.94 - 5.09 (3H, m, α-CH Abu, α-CH (Me)Leu), 5.12 (2H, s, Ph-CH_2), 5.15 (1H, d, α-CH (Me)Thr), 5.49 - 5.69 (2H, m, α-CH (Me)Leu), 6.80 - 6.93 (2H, 2s, N-H), 7.35 (5H, s, ArH), 7.67 - 7.99 (2H, 2s, -NH); m/z 1398 (M^+, CI); R_t 12.2 min.

Fmoc-(Me)Thr(Bu^t)-Abu-Sar-(Me)Leu-Val-(Me)Leu-L-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (15)

Triethylamine (0.25 cm^3, 1.78 mM, 1.5 equiv.) was added to a stirred solution of Fmoc(Me)Thr(Bu^t)-OH (16) (0.73g, 1.78 mM, 1.5 equiv.) in THF (15 cm^3) at -20°C. DppCl (0.42g, 1.78 mM, 1.5 equiv.) in THF (2 cm^3) was then added and the mixture stirred at -20°C for twenty minutes, after which time H-Abu-Sar-(Me)Leu-Val-(Me)Leu-L-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (1.27g, 1.16 mM, 1 equiv.) in THF (5 cm^3) was added. The resulting mixture was stirred at -20°C for one hour, 0 - 5°C for half an hour and and room temperature for four days. The product was worked-up as described in the general DppCl procedure to give an oil which was purified on a silica gel column eluting with CH_2Cl_2/MeOH 12:1. Evaporation of the appropriate fractions gave the title compound as a white foam (1.11g, 69%); m.p., 94 - 96°C; $[\alpha]_D^{20}$ - 121° (c 1.0, CH_3OH); δ_H ($CDCl_3$, 220 MHz). Calculated for $C_{80}H_{131}N_{11}O_{15}$: C, 64.65; H, 8.82; N, 10.37. Found : C, 64.51; H, 8.93; N, 10.43%; δ_H ($CDCl_3$, 250 MHz), 0.77 - 1.10 (39H, m, CH_3 of Abu, Val, (Me)Val, (Me)Leu)), 1.11 - 1.28 (12H, m, CH_3(Me)Thr, (Me)Thr(Bu^t)), 1.29 - 1.30 (6H, d x d, CH_3 Ala, D-Ala), 1.44 (9H, s, (Me)Val-OBu^t), 1.53 - 1.72 (10H, m, β-CH_2 of (Me)Leu Abu), 1.73 - 2.20 (6H, m, β-CH, Val, (Me)Val), 2.79 - 3.21 (21H, series of s, N-CH_3), 3.22 (1H, d, α-CH Sar), 3.92 (1H, m, β-CH, (Me)Thr), 4.12 - 5.02 (9H, m, α-CH), 5.12 (2H, s, Fmoc-CH_2), 5.34 - 5.62 (2H, m, α-CH), 6.61 - 6.92 (2H, m, N-H), and 7.23 - 7.80 (9H, m, ArH, N-H); m/z 1486 (M+1, FAB); R_t 15.2 (λ 280 nm).

Boc-Dab(Fmoc)-Abu-Sar-(Me)Leu-Val-(Me)Leu-L-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (17)

N.M.M. (0.5 cm^3, 4.4 mM, 2.5 equiv.) was added to a solution of Boc-Dab(Fmoc)-OH (18) (0.8g, 1.9 mM, 1.1 equiv.) in THF (5 cm^3), and the mixture stirred at -20°C. DppCl (0.6g, 2.1 mM, 1.5 equiv.) in THF (3 cm^3) was then added and the resulting mixture stirred at -20°C for twenty minutes. A pre-cooled solution of H-Abu-Sar-(Me)Leu-Val-(Me)Leu-L-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (1.9g, 1.7 mM, 1 equiv.) in THF (10 cm^3) was added and the reaction mixture stirred at -20°C for one hour, 0°C for one hour and room temperature for three days. The solvent was evaporated and the residue worked-up as described in the usual way. Purification of the product on a silica gel column eluting with MeOH/EtOAc 1:5 afforded the title compound as a white foam (2.1g, 81%); m.p., 109 - 110°C; $[\alpha]_D^{20}$ - 130.7° (C 0.8, MeOH). Calculated for $C_{80}H_{130}N_{12}O_{16}/H_2O$: C, 62.66; H, 8.62; N, 10.97. Found : C, 62.90; H, 8.75; N, 10.92%; δ_H (250 MHz, $CDCl_3$), 0.79 - 1.05 (39H, m, CH_3 of Val, (Me)Val, (Me)Leu, Abu), 1.27 - 1.29 (6H, m, CH_3 of L-Ala, D-Ala), 1.46 (9H, s, (Me)Val(OBu^t)), 1.47 - 2.15 (16H, m, β-CH_2 of (Me)Leu, Abu, Dab, γ-CH of (Me)Leu), 2.17 - 2.23 (2H, m, β-CH of Val, (Me)Val), 2.77 - 3.28 (18H, series of s, N-CH_3), 4.19 - 4.48 (4H, m, γ-CH_2 of Dab, α-CH of Sar, α-CH of L-Ala), 4.71 - 4.90 (5H, m, α-CH of Sar, Val, (Me)Val, D-Ala, Abu), 5.01 (2H, s, Fmoc-CH_2-), 5.02 - 5.27 (5H, m, α-CH of Dab, (Me)Leu), 5.47 - 5.49 (1H, m, Dab-NH), 6.01 (1H, d, Dab-NH), 6.90 - 7.21 (2H, 2s, -NH), 7.25 - 7.28 (8H, m, ArH), and 7.31 - 8.10 (2H, 2s, -NH); m/z 1514 (M+1, FAB); R_t 11.7 min.

Z-Hyp(Bu^t)-Abu-Sar-(Me)Leu-Val-(Me)Leu-L-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (19)

N.M.M. (1.3 cm^3, 12.3 mM, 2.5 equiv.) was added to a solution of Z-Hyp(Bu^t)-OH (21) (1.7g, 5.3 mM, 1 equiv.) in THF (10 cm^3) and the mixture stirred at -20°C. DppCl (1.7g, 7.4 mM, 1.5 equiv.) in THF (5 cm^3) was then added and the resulting mixture stirred at -20°C for twenty minutes. A pre-cooled solution of H-Abu-Sar-(Me)Leu-Val-(Me)Leu-L-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (5.1g, 4.9 mM, 1 equiv.) in THF (5 cm^3) was added and the reaction mixture stirred at -20°C for one hour, 0 - 15°C for one hour and room temperature for two days. The product was worked-up as described for the general DppCl method to yield a yellow oil, which was purified on a silica gel column eluting with CH_2Cl_2/EtOAc (3:1). Evaporation of the appropriate fractions afforded the title compound as a white foam (6g, 92%); m.p., 94 - 95°C; $[\alpha]_D^{20}$ - 128° (c 1.0, CH_3OH). Calculated for $C_{73}H_{125}N_{11}O_{15}$: C, 62.80; H, 8.96; N, 11.04. Found : C, 62.41; H, 9.02; N, 10.69%; δ_H (250 MHz, $CDCl_3$), 0.74 - 1.03 (39H, m, CH_3 of Abu, Val, (Me)Leu, (Me)Val), 1.17 (9H, 2s, Hyp (Bu^t)), 1.27 - 1.34 (6H, d x d, CH_3 D-Ala, L-Ala), 1.45 (9H, s, (Me)Val-OBu^t), 1.46 - 2.01 (10H, m, β-CH_2 of Abu, (Me)Leu), 2.02 - 2.36 (8H, m, β-CH of Val, (Me)Val, Hyp and γ-CH of (Me)Leu), 2.93 - 3.30 (21H, series of s, N-CH_3), 3.11 - 3.12 (1H, dxd, α-CH Sar), 3.23 - 3.80 (2H, d x d, δ-CH_2 of Hyp), 3.99 - 4.25 (1H, m, γ-CH of Hyp), 4.32 - 4.43 (1H, m, α-CH of L-Ala), 4.49 - 4.94 (5H, m, α-CH of Val, (Me)Val, Sar, Abu and D-Ala), 5.13 (2H, s, Ph-CH_2), 5.14 - 5.31 (4H, m, α- CH (Me)Leu), 5.48 - 5.52 (1H, m, α-CH of Hyp), 7.14 - 7.30 (2H, 2s, NH of D-Ala,Val), 7.34 (1H, s, NH L-Ala), 7.35 (5H, s, ArH), and 8.10 (1H, s, NH Abu); m/z 1396 (M+1, FAB); R_t 11.4 min.

Z-Hyp(Bu^t)-Nle-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OBu^t (20)

Z-Hyp(Bu^t)-OH (21) (1.29g, 4 mM) in THF (5 cm^3), NMM (1.2 cm^3, 10.8 mM) and was activated with DppCl (1.73g, 7.3 mM) in THF (5 cm^3) at -20°C, and coupled to H-Nle-Sar-((Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val- OBu^t (4.1g, 3.7 mM) in THF (15 cm^3) according to the general DppCl procedure. The reaction mixture was stirred for two hours at -20°C and forty-five hours at room temperature. The reaction was worked-up in the usual way and the residue obtained was purified on a silica gel column eluting with EtOAc/DCM (2:1). Evaporation of the appropriate fractions gave the title compound as a white foam, (4.1g, 68%); m.p., 95°C; $[\alpha]_D^{24}$ - 140.4° (c 1.1, CH_3OH). Calculated for $C_{75}H_{130}N_{11}O_{15}$: C, 63.15; H, 9.19; N, 10.80.

Found : C, 63.02; H, 9.05; N, 11.09%; δ_H (250 MHz, $CDCl_3$, 2 conf.), 0.77 - 1.03 (39H, m, β-CH_3 Val, (Me)Val, CH_3 Nle, CH_3 (Me)Leu), 1.14 - 1.17 (9H, 2s, Hyp(Bu^t), conf.), 1.21 - 1.35 (12H, CH_2-Ala, D-Ala, β,γ and δ-CH_2 Nle), 1.44 - 1.46 (9H, 2s, OBu^t, conf.), 1.61 - 1.68 (8H, m, β-CH_2 (Me)Leu), 2.01 - 2.09 (1H, m, β-CH Val), 2.13 - 2.25 (2H, m, β-CH- (Me)Val, β-CH- Hyp), 2.93 - 3.30 (18H, series of s, N-CH_3), 3.23 - 3.80 (2H, d x d, δ-CH_2- Hyp), 3.99 - 4.25 (1H, m, γ-CH Hyp), 4.24 - 4.34 (2H, d x d, CH_2 Sar), 4.37 (1H, m, α-CH Ala), 4.44 (1H, t, α-CH Nle), 4.50 - 4.65 (3H, m, α-CH (Me)Val, Val, Hyp), 4.72 - 4.76 (2H, m, α-CH (Me)Leu), 5.12 - 5.15 (1H, t, α-CH-D- Ala), 5.13 (2H, s, $PhCH_2$), 5.43 - 5.52 (2H, m, α-CH (Me)Leu), 6.90 - 7.19 (2H, br.d., NH Val, NH D-Ala), 7.34 (5H, s, ArH), 7.64 (1H, br.d., NH Ala), and 7.88 - 8.07 (1H, br.d., NH Nle); R_t 11.8 min.; m/z 1424 (M^+, DCI).

Fragment condensation synthesis of (22)

(a) Diphenyl phosphinic mixed anhydride procedure

Z-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-(Me)Thr(Bu^t)-Abu-Sar-OBu^t (22)

N.M.M. (7 x 10^{-3} cm^3, 0.06 mM) was added to a solution of Z-(Me)Leu-Val-(Me)Leu-OH[4] (32 mg, 0.06 mM) in THF (2 cm^3) and the mixture stirred at -20°C. DppCl (15 mg, 0.06 mM) in THF (2 cm^3) was then added and the mixture stirred at -20°C for twenty minutes. A solution of H-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-(Me)Thr(Bu^t)-Abu-Sar-OBu^t[4] (58 mg, 0.06 mM) in THF (1 cm^3) was then added and the reaction mixture stirred at room temperature for twenty-two hours. The solvent was evaporated and the residue applied on a Sephadex LH20 column eluting with DMF. Evaporation of the appropriate fractions gave the title compound as a white foam (34 mg, 39%); m.p., 80 - 83°C; $[\alpha]_D^{20}$ - 93° (c 1.0, CH_3OH). Calculated for $C_{73}H_{127}N_{11}O_{15}\cdot H_2O$: C, 61.88; H, 9.18; N, 10.87. Found : C, 62.19; H, 9.12; N, 11.10%; δ_H (250 MHz, $CDCl_3$), 0.77 - 1.04 (39H, m, CH_3 of Abu, Val, (Me)Leu, (Me)Val), 1.15 (3H, s, CH_3 (Me)Thr), 1.16 (3H, d, -CH_3 D-Ala), 1.25 (9H, s, Bu^t), 1.34 (3H, d, CH_3 L-Ala), 1.45 (9H, s, OBu^t), 1.59 - 1.89 (10H, m, β-CH_2 of Abu, (Me)Leu), 1.90 - 2.50 (6H, m, β-CH of Val, (Me)Val, and γ-CH of (Me)Leu), 2.9 - 3.16 (21H, series of s, NCH_3), 3.24 (1H, d, α-CH Sar), 3.51 - 3.54 (1H, m, β-CH (Me)Thr), 4.33 - 5.01 (9H, m, α-CH), 5.12 (s, Ph-CH_2-), 5.20 - 5.52 (2H, m, α-CH), 7.00 - 7.30 (2H, 2s, -NH), 7.35 (5H, s, Ar-H), and 7.43 - 7.60 (2H, 2s, NH); m/z 1398 (M+1, FAB); R_t 14 min.

(b) Using Castro reagent

Z-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-(Me)Thr(Bu^t)-Abu-Sar-OBu^t (22)

N.M.M. (4.1 x 10^{-3} cm^3, 0.38 mM, 2.2 equiv.) and Castro reagent BOP (82 mg, 0.19 mM, 1.1 equiv.) were added to a stirred solution of Z-(Me)Leu-Val-(Me)Leu-OH[4] (94 mg, 0.19 mmol, 1.1 equiv.) and H-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-(Me)Thr(Bu^t)-Abu-Sar-OBu^t[4] (151 mg, 0.17 mM, 1.0 equiv.) at 0°C, in DMF (2 cm^3). The reaction mixture was allowed to warm to room temperature and stirred for forty-four hours. The total reaction mixture was applied on a Sephadex LH20 column eluting with DMF. Evaporation of the appropriate fractions gave the title compound as a white foam (75 mg, 32%); $[\alpha]_D^{20}$ - 82° (c 1.0 CH_3OH).

(c) Using DPPA

Z-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-(Me)Thr(Bu^t)-Abu-Sar-OBu^t (22)

A solution of DPPA (diphenyl phosphorazidate) (23.3 mg, 0.09 mmol, 1.1 equiv.) in THF (5 cm^3) was added dropwise to a solution of H-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-(Me)Thr(Bu^t)-Abu-Sar-OBu^t[4] (77 mg, 0.09 mM, 1.1 equiv.) and Z-(Me)Leu-Val-(Me)Leu-OH[4] in THF (5 cm^3) at -20°C. The reaction mixture was stirred at -20°C for one hour and then at 0°C for forty-two hours. The residue, after solvent evaporation, was purified on a Sephadex LH20 column eluting with DMF. Evaporation of the appropriate fractions gave the title compound as a white foam (15 mg, 13%); $[\alpha]_D^{20}$ - 85° (c 1.0 CH_3OH).

(d) Using DCCI/HONSu

Z-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-(Me)Thr(Bu^t)-Abu-Sar-OBu^t (22)

Z-(Me)Leu-Val-(Me)Leu-OH[4] (64.6 mg, 0.13 mM, 1 equiv.), HONSu (16.2 mg, 0.14 mM, 1.1 equiv.) and DCCI (31.7 mg, 0.15 mM, 1.2 equiv.) were added to a stirred solution of H-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-(Me)Thr(Bu^t)-Abu-Sar-OBu^t[4] (116 mg, 0.13 mM, 1 equiv.) in THF (10 cm^3) at -5°C. The reaction mixture was stirred at -5°C for half an hour and then at 0°C for four days. The solvent was evaporated and the residue purified on a Sephadex LH20 column eluting with DMF. Evaporation of the appropriate fractions gave the title compound as a white foam (54 mg, 30%); $[\alpha]_D^{20}$ - 80° (c 1.0, MeOH).

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