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SYNTHETIC STUDIES OF CYCLOSPORIN ANALOGUES

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Abstract: The syntheses of eleven analogues of Cyclosporin are described; the analogues which contain (Me)Thr, (Me)Ser, Hyp and Dab at position-1 and Abu, Nva, Nle and Thr at position-2 were prepared by stepwise assembly of the undecapeptide fragments followed by cyclisation with a variety of reagents. The highest yields were obtained using the Castro reagent,and in the best case, (Hyp^1,Abu^2) cyclosporin, a yield of 65% was obtained.

The cyclic undecapeptide cyclosporin A is a particularly potent immunosuppressive agent which has been isolated from the fungal species Tolypocladium Inflatum gams.[1] Since 1978[2] the predominant use of Cyclosporin has been for the prevention of rejection in transplantation surgery. Cyclosporin A (marketed under the trade name Sandimmune) is used clinically for a number of purposes to bring about the suppression of the immune system. although it may also be useful in the treatment of malaria,[3] diabetes[4] and AIDS.[5] The compound has been well characterised by chemical degradation and by X-ray crystallographic[6] and nmr studies.[7] The structure based on these studies is shown in Figure 1.

The cyclosporins contain seven N-methylated residues and the unique C-9 amino acid (4R)-4-[(E)-2-butenyl]-4-N-dimethyl-L-threonine (MeBmt). All the residues have the 2S configuration, except for the alanine residue at position 8 which has the 2R configuration, and achiral sarcosine at position 3. The unusual C-9 amino acid is thought to be central to the activity of the immunosuppressant[8] and has been synthesised in stereochemically pure form.[9-11]

Many of the residues are invariant and in the natural cyclosporins the only major variation is at position 2, which gives rise to a number of minor cyclosporins.[12,13] The norvaline-2 analogue of cyclosporin is of particular interest, as it is considerably less nephrotoxic than cyclosporin A,[14] and it is this nephrotoxicity associated with cyclosporin A which is one of the major drawbacks of current therapy using this drug.

Synthetic analogues of the immunosuppressive cyclosporin[15-17] can threfore provide otherwise unobtainable information regarding the features of cyclosporin which are required for its biological activity. For this reason the synthesis of a number of analogues of cyclosporin has been carried out by cyclisation of the deprotected undecapeptide sequences. In our work, which has previously been described in outline,[18] cyclisation between residues

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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.

one and eleven has been examined, thus allowing maximum modification of the residue at position one with the minimum of disturbance to the synthesis.

In contrast to the fragment condensation approach employed by other workers[16,17] a sequential assembly of the total linear undecapeptide sequence permitting variation at

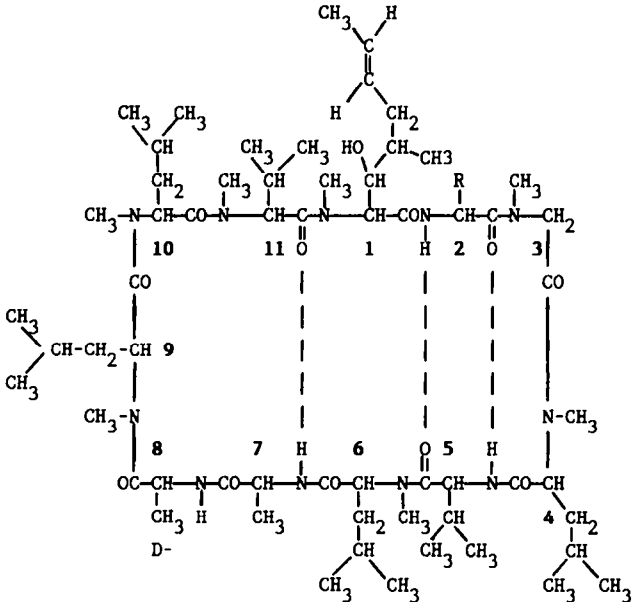

Figure 1 Structural formula of cyclosporin A (R = CH_2-CH_3).

positions one and two at a later stage in the synthesis has been adopted. Thus, a number of linear undecapeptide precursors were prepared[19] starting from a large stock of protected octapeptide.[20]

In addition to the cyclisation reactions which result in the formation of cyclic undecapeptides, the current paper also describes the deprotection, but not the cyclisation, of the protected nonapeptide and the natural Abu-2 decapeptide sequence.

Z-(3-11)-OBu^t (1) and Z-Abu-(3-11)-OBu^t (2) were both treated with 90% TFA to achieve removal of the t-butyl ester protecting group, this yielded the corresponding protected peptide acids (1a) and (2a). These acids were then hydrogenolysed in the presence of 10% palladium charcoal to give the corresponding free peptides (1b) and (2b) which were required for biological evaluation. High yields of the free peptide sequences were obtained and the products were characterised by the usual spectroscopic methods. The other decapeptide sequences: Z-Nva-(3-11)-OBu^t, Z-Nle-(3-11)-OBu^t and Z-Thr(Bu^t)-(3-11)-OBu^t, which had previously been prepared by the stepwise procedure[19] were not completely deprotected, as the free peptides were not required for biological studies.

The linear undecapeptides indicated in Table 1 were prepared by stepwise assembly using the diphenylphosphinic mixed anhydride procedure as indicated previously.[19,20] The compounds (3) - (12) were treated with 90% TFA to remove the butyl ester and/or ether protecting groups; the resulting peptide acids (3a) - (12a) were then hydrogenolysed in the presence of 10% palladium/charcoal to give the free undecapeptides (3b) - (12b) which were required for the preparation of the cyclosporin analogues. A detailed investigation of the cyclisation of the H-(Me)Thr-Abu-(3-11)-OH (3b) was carried out and the results are summarised in Table 2.

The synthesis of this analogue was also investigated using the azide method for cyclisation. The intermediates employed in the azide cyclisation are outlined in Table 3; here it can be seen that compound (3) may be treated with TFA to remove the t-butyl protection, subsequently esterified with diazomethane, hydrazinolysed with hydrazine

hydrate to give the corresponding hydrazide, then deprotected by hydrogenolysis and cyclised. A similar route using the corresponding Fmoc protected undecapeptide,[19] which ultimately gave the same free peptide hydrazide was also investigated. On both occasions a very low yield of cyclic material (ca.5%) was achieved.

TABLE 1 Undecapeptide precursors and cyclosporin analogues prepared.

PROTECTED UNDECAPEPTIDE	COMPOUND ( )*	CYCLOSPORIN ANALOGUE
Z-(Me)Thr(Bu^t)-Abu-(3-11)-OBu^t	(3)	(13)
Z-(Me)Ser(Bu^t)-Abu-(3-11)-OBu^t	(4)	(14)
Z-Hyp(Bu^t)-Abu-(3-11)-OBu^t	(5)	(15)
Boc-Dab(Fmoc)-Abu-(3-11)-OBu^t	(6)	(16)
Z-(Me)Thr(Bu^t)-Nva-(3-11)-OBu^t	(7)	(17)
Z-(Me)Ser(Bu^t)-Nva-(3-11)-OBu^t	(8)	(18)
Z-(Me)Thr(Bu^t)-Nle-(3-11)-OBu^t	(9)	(19)
Z-(Me)Ser(Bu^t)-Nle-(3-11)-OBu^t	(10)	(20)
Z-Hyp(Bu^t)-Nle-(3-11)-OBu^t	(11)	(21)
Z-(Me)Ser(Bu^t)-Thr(Bu^t)-(3-11)-OBu^t	(12)	(22)

In the text * a: indicates the benzyloxycarbonyl peptide acid, b:indicates totally deprotected peptide.

TABLE 2 Cyclisation of H-(Me)Thr-Abu-(3-11)-OH (3b).

REAGENT	SOLVENT	REACTION TIME	% YIELD	MELTING POINT (°C)	$[\alpha]_D^{20}$ (c) MeOH	Hplc/tlc
DCCI/HOBt/NMM	CH_2Cl_2	4 days	42	105-106	-110° (1)	Heterogeneous
DCCI/HOBt/NMM	CH_2Cl_2	6 days	32	103-105	-122° (1)	Broad peak on hplc. Homogeneous on tlc.
DCCI/HONSu/NMM	CH_2Cl_2	6 days	32	107-108	-96° (1)	Heterogeneous
DppCl/NMM	THF	5 days	6			Heterogeneous
BOP/DMAP	CH_2Cl_2	5 days	10	165-167	-121° (1)	Homogeneous
Dppa	DMF	4 days	7	164-176	-101° (1)	Heterogeneous

TABLE 3 Intermediates employed in the azide cyclisation preparation.

A-Abu-Sar-(4-11)-B			YIELD	M.P.	$[\alpha]_D$
A		B	%	°C	(c 1,MeOH)
Z(Me)Thr(Bu^t)	(3)	OBu^t	68	95	-170.4
Z-(Me)Thr		OH	94	134-136	-130
Z-(Me)Thr		OMe	91	Oil	-149.5
Z-(Me)Thr		$NHNH_2$	59	110-111	-123.5
H-(Me)Thr		$NHNH_2$	85		
[(Me)Thr^1]-Cyclosporin analogue (13)			ca.5%	163-166	-102
H-(Me)Thr		$NHNH_2$	87		
Fmoc-(Me)Thr		$NHNH_2$	61		-117.3
Fmoc-(Me)Thr		OMe	87	oil	-139.3
Fmoc-(Me)Thr		OH	89		
Fmoc-(Me)Thr(Bu^t)		OBu^t	69	94-96	-121.8

The best results, in terms of optical purity and yield were obtained by cyclising the fully deblocked linear undecapeptide (3b) using the BOP reagent of Castro;[21] these findings were similar to those of the Sandoz Group[16] who had investigated cyclisation between the D- and L-alanine residues. In the current work the difficult cyclisation between two N-methylated residues has been studied and it has been found that higher yields (> 60%) could only be obtained at very low concentrations of the linear undecapeptide. However, it had been accepted that this might be the case from the outset, since the current objective was to generate a large number of analogues by variation at the first and second position. Thus cyclisation at this point provided the most rapid route to these compounds in contrast to the fragment condensation approach with cyclisation between the alanine residues described by Wenger[16] and Rich.[17]

The interesting series of analogues containing hydroxyproline at position one gave considerably higher yields on cyclisation and therefore a detailed investigation of the cyclisation of the compound (5b) was carried out. The results of the study shown in Table 4, indicated that once again the Castro Reagent was superior, giving a 65% yield of

TABLE 4 Cyclisation of H-Hyp-Abu-(3-11)-OH (5b)

REAGENT	SOLVENT	REACTION TIME	% YIELD	MELTING POINT °C	$[\alpha]_D^{20}$ MeOH (c)	Hplc/tlc
$Pr(PO_2)_3$ /DMAP	CH_2Cl_2	4 days	17	130-135	-150 (1)	Heterogeneous
BOP/DMAP	CH_2Cl_2	3 days	65	166-168	-240 (1)}	Homogeneous
	CH_2Cl_2	3 days	20		}	
	CH_2Cl_2	4 days	9		}	
	CH_2Cl_2	2 days	30		}	

homogeneous material (hplc); it was again found that high dilution was necessary in order to reduce polymerisation. The results indicate that concentration rather than time was the important factor, as increasing the reaction time did not increase the yield.

The higher yield achieved for the cyclisation of the hydroxyproline analogue was most probably due to the conformational rigidity of the hydroxyproline skeleton, thus leading to greater nucleophilicity of the imino nitrogen. A summary of the cyclosporin analogues which have been prepared is presented in Table 5, and therefore only the more interesting features of the preparation of these analogues are discussed as many of the steps and reactions are repetitive.

Cyclisation of H-(Me)Ser-Abu-(3-11)-OH (4b) using Castro Reagent in dichloromethane, led to the isolation of both a major and minor cyclic product. The hplc indicated closely similar retention times and the R_f on tlc was also similar. The mass spectroscopic and nmr details for the compound were virtually identical; however, the melting point and optical rotation values were considerably different. Although at this stage there is no conclusive evidence, it is believed that these two compounds are diastereomers and they have been produced by racemisation of the C-terminal methyl valine during the cyclisation. Similar racemisation has been observed by other workers[16] and in that case the nature of the diastereomers was proven.

TABLE 5 Cyclosporin analogues.

A^1,B^2-CYCLOSPORIN		COMPOUND	YIELD %	MP. °C	HPLC (R_t) min.	$[\alpha]_D$ (c) MeOH, *$CHCl_3$
A	B					
(Me)Thr	Abu	(13)	10	165-167	9.4	-121 (1)
(Me)Ser	Abu	(14)	15	212-214	11.4	-144 (1)
Hyp	Abu	(15)	30	166-168	10.2	-240 (1)
Dab(Fmoc)	Abu	(16)	37	156-157	13.4	-190 (0.9)
Dab	Abu	(16a)	13	160-164	12.1	-181 (1.1)*
(Me)Thr	Nva	(17)	20	102-104	11.0	-147 (1)*
(Me)Ser	Nva	(18)	20	112-114	11.6	-136 (1)*
(Me)Thr	Nle	(19)	14	94-95	11.2	-168 (1)*
(Me)Ser	Nle	(20)	16	108-110	11.4	-141 (1)
Hyp	Nle	(21)	26	145	10.8	-152.4 (1)
(Me)Ser	Thr	(22)	5	144-146	11.7	-117 (1)

Cyclisation using the BOP reagent of Castro in the presence of DMAP was highly effective, giving [Hyp^1,Abu^2]-cyclosporin (15) and [Hyp^1,Nle^2]-cyclosporin (21) in reasonable yields; the high level of purity of these products following chromatography on silica gel eluting with EtOAc/MeOH is demonstrated by the hplc chromatograms which are shown in Figure 2.

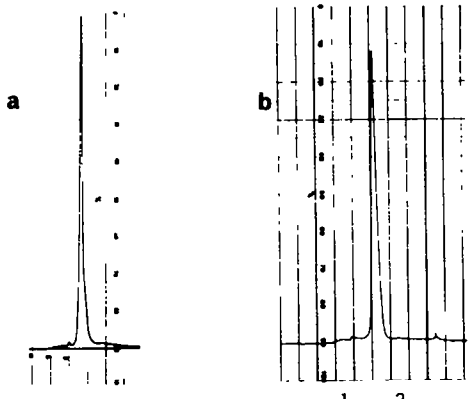

FIGURE 2 Hplc chromatograms of (a) [Hyp^1,Abu^2]-cyclosporin (15) and (b) [Hyp^1,Nle^2]-cyclosporin (21).

Fast atom bombardment mass spectrometry was found to be a particularly useful technique for the characterisation of the cyclic products and the spectra of the [$(Me)Ser^1$,Abu^2]-analogue (14) and the [$Dab(Fmoc)^1$,Abu^2]-analogue (16) are given in Figure 3 as examples. In both these spectra the molecular ion was clearly visible and little fragmentation was observed; very similar spectra were obtained with native cyclosporin A.

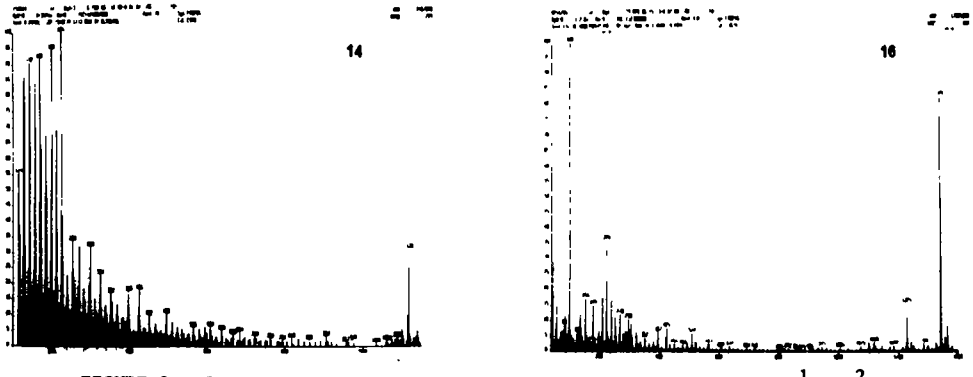

FIGURE 3 Fast atom bombardment mass spectra of [$(Me)Ser^1$,Abu^2]-cyclosporin (14) and [$Dab(Fmoc)^1$,Abu^2]-cyclosporin (16).

The (Me)Ser^1-Thr^2 analogue (22) was obtained in a rather low yield (5%) and on this occasion it is believed that the very poor coupling was due to increased steric hindrance brought about by the proximity of the serine and threonine residues. The FAB spectrum of the analogue confirms the molecular ion, but the nmr spectrum was difficult to assign completely. Further characterisation is currently being carried out on this compound and detailed analytical data will be presented in a subsequent paper.

Similarly, it was found that although the cyclisation of the free undecapeptide H-Dab(Fmoc)-Abu-(3-11)-OH (6b) proceeded satisfactorily to give the protected cyclosporin analogue (16) it was found to be difficult to remove the side chain Fmoc protection from this compound cleanly to give (16a); the hplc and FAB mass spectrum were satisfactory, however a detailed assignment of the nmr spectrum could not be made.

The nmr spectra of the two hydroxyproline analogues (15) and (21) are shown in Figure 4 in comparison with that of native cyclosporin A. The close similarity between the NH protons involved in hydrogen bonding which gives rise to the rigid β-sheet-like structure of cyclosporin is clearly evident in all three spectra, however a detailed study of the effect of introducing the relatively rigid cyclic hydroxyproline structure has not yet been made. From the similarity in the spectra it may be inferred, though not conclusively proven, that the introduction of the rather rigid hydroxyproline residue at position one does not dramatically alter the conformation of the molecule, although the downfield shift of the N-H proton of Abu may indicate a slight change in conformation.

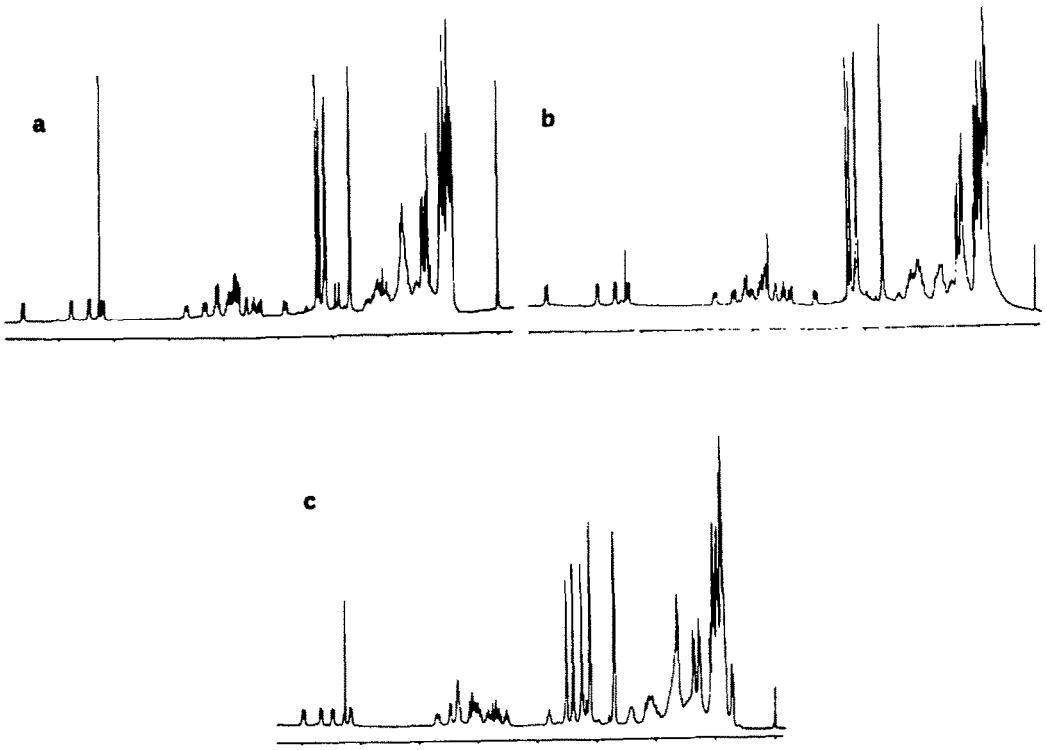

FIGURE 4 ^{1}H Nmr spectra (250 MHz) in $CDCl_3$ of (a) [Hyp^1,Abu^2]-cyclosporin (15) (b) [Hyp^1,Nle^2]-cyclosporin (21) and (c) natural cyclosporin A.

The results presented in this paper conclusively demonstrate that cyclosporin

analogues may be synthesised by stepwise assembly to the corresponding protected (1-11) undecapeptide followed by deprotection and cyclisation. It has been demonstrated that the Castro reagent (BOP) gives the most reliable yield of product when used at high dilution, and interestingly this finding is in common with that described by other workers[16,17] for cyclisation between the alanine-7 and the D-alanine-8 residues.

Here, and in the preceding papers,[18-20] it has been demonstrated that the successful synthesis of the linear sequence of this complex cyclic peptide was best accomplished by the use of the diphenylphosphinic mixed anhydride procedure for coupling the relatively hindered N-methylated amino acids.

The biological data, which relates to the compounds which have been prepared, is not presented in the current work but will subsequently be published elsewhere.

Acknowledgements

We acknowledge the generous financial support which has been provided by the British Technology Group (BTG) which has enabled us to carry out this interesting work.

EXPERIMENTAL

Product purity was routinely checked by tlc and hplc, using the systems and detection methods outlined previously.[22] The general spectroscopic techniques and other generally applied experimental procedures have also been similarly detailed.

Nonapeptide

H-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OH (1b)

10% Palladium/charcoal catalyst (0.2g) was added to a stirred solution of Z-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OH (1a) (1.8g, 1.7 mM) in CH_3OH (50 cm^3) and the mixture hydrogenolysed for eighteen hours. The catalyst was removed by filtration and the filtrate evaporated to dryness. The residue was applied to Sephadex G10, eluting with water; evaporation of the appropriate fractions giving the title compound as a white solid (1.4g, 91%); m.p., 103 - 105°C; $[\alpha]_D^{20}$ -111° (c 1.0, CH_3OH); m/z 951 (M^+, CI).

Decapeptide

Z-Abu-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OH (2a)

Z-Abu-(3-11)-OBu^t (2) (1.23g, 1 mM) was treated with 90% TFA (5 cm^3) for two hours at room temperature and overnight at 0°C. Excess TFA was removed *in vacuo* and the residue chromatographed on a silica gel column eluting with 95% EtOAc/5% MeOH. Evaporation of the appropriate fractions gave the title compound as a white solid (0.92g, 75%); $[\alpha]_D^{24}$ - 85.2° (c 1.1, CH_3CH_2OH); δ_H (250 MHz, $CDCl_3$), 0.78 - 1.19 (39H, m, β-CH_3 (Me)Val, Val, γ-CH_3 (Me)Leu, β-CH_3 Abu), 1.24 - 1.42 (6H, d x d, CH_3 Ala, D-Ala), 1.58 - 1.92 (8H, m, β-CH_2, γ-CH (Me)Leu), 2.05 - 2.25 (2H, m, β-CH (Me)Val, Val), 2.71 - 3.22 (18H, series of s, N-CH_3), 3.97 - 4.03 (2H, d x d, CH_2 Sar), 4.25 - 4.88 (7H, m, α-CH), 5.12 (2H, s, $PhCH_2$), 5.42 - 5.58 (2H, m, α-CH), 6.01 (1H, br.d., NH D-Ala), 6.19 (1H, br.d., NH Val), 7.10 (1H, br.d., NH Ala), 7.33 (5H, s, ArH), 7.47 (1H, br.d., NH Abu), and 9.44 (1H, br.s., -COOH); m/z 1172 (M^+, DCI).

H-Abu-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OH (2b)

Z-Abu-(3-11)-OH (2a) (0.85g, 0.72 mM) in CH_3OH (20 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 *in vacuo*. The residue obtained was chromatographed on a Sephadex G10 column eluting with water. Evaporation of the appropriate fractions gave the title compound as a white solid (0.63g, 84%); m.p., 131 - 132°C; $[\alpha]_D^{24}$ - 110°, (c 1.2, CH_3OH); m/z 1036 (M+1, FAB).

Linear undecapeptides

Z-(Me)Thr-Abu-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OH (3a)

(10 cm^3) TFA (in 90% acetic acid) was added to a cooled (0°C) solution of Z-(Me)Thr(Bu^t)-Abu-(3-11)-OBu^t (3) (2g, 1.4 mM) and stirred for four hours at room temperature. TFA was then evaporated and the residue purified on a silica gel column eluting with CH_2Cl_2/MeOH (15:1). Solvent evaporation of the appropriate fractions afforded the title compound as a white foam (1.7g, 94%); m.p., 134 - 136°; $[\alpha]_D^{20}$ - 130° (c 1.0, CH_3OH). Calculated for $C_{65}H_{111}N_{11}O_{15}\cdot 3H_2O$: C, 58.25; H, 8.74; N, 11.50. Found : C, 57.75; H, 8.30; N, 11.08%; δ_H (250 MHz, $CDCl_3$), 0.77 - 1.07 (39H, m, β-CH_3 Val, (Me)Val, γ-CH_2 Abu, (Me)Leu), 1.14 (3H, d, CH_3 (Me)Thr), 1.25 - 1.32 (6H, d, CH_3 Ala, D-Ala), 1.58 - 1.91 (12H, m, β-CH_2, γ-CH (Me)Leu), 2.29 (2H, m, β-CH_2 Abu), 2.32 - 2.47 (2H, β-CH (Me)Val, Val), 2.70 - 3.27 (21H, series of s, N-CH_3), 4.18 (1H, m, β-CH (Me)Thr), 4.30 - 4.39 (2H, d, CH_2 Sar), 4.51 - 5.02 (8H, m, α-CH), 5.17 (2H, s, $PhCH_2$), 5.48 - 5.67 (2H, m, α-CH), 6.93 (1H, br.d., NH, D-Ala), 7.41 (1H, br.s., NH Val), 7.30 (5H, s, ArH), 7.41 (1H, br.d., NH Ala), 7.52 (1H, br.d., NH Abu), and 8.67 (1H, br.s., -COOH); m/z 1287 (M+1, FAB); R_t 7.8 min.

H-(Me)Thr-Abu-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OH (3b)

10% Pd/C catalyst (0.1g) was added to a stirred solution of Z-(Me)Thr -Abu-(3-11)-OH (3a) (1.7g, 1.3 mM) in CH_3OH (20 cm^3) and hydrogenolysed for twenty-three hours. The catalyst was filtered and the filtrate evaporated to dryness. The residue was applied on a Sephadex G10 column eluting with water. Evaporation of the appropriate fractions gave the title compound as a white foam (1.4g, 93%); m.p., 134 - 136°C; $[\alpha]_D^{20}$ - 129° (c 1.0, CH_3OH). Calculated for $C_{57}H_{105}N_{11}O_{13}\cdot 5H_2O$; C, 55.12; H, 9.27; N, 12.41. Found: C, 54.84; H, 8.97; N, 12.09%; m/z 1151 ($\underline{M}^+$, DCI).

Z-(Me)Ser-Abu-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OH (4a)

90% TFA (5 cm^3) was added to Z-(Me)Ser(Bu^t)-Abu-(3-11)-Bu^t (4) (1.8g, 1.3 mM) and stirred at room temperature for four hours. The residue (after evaporating TFA under vacuo) was applied directly 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 solid (1.3g, 79%); m.p., 112 - 114°C; $[\alpha]_D^{20}$ - 127° (c 0.9, CH_3OH). Calculated for $C_{64}H_{109}N_{11}O_{15}\cdot H_2O$: C, 57.96; H, 8.68; N, 11.62. Found : C, 57.63; H, 8.31; N, 11.20%; m/z 1272 ($\underline{M}^+$, DCI); R_t 17.0 min.

H-(Me)Ser-Abu-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OH (4b)

10% Pd/C catalyst (0.12g) was added to a stirred solution of Z-(Me)Ser-Abu-(3-11)-OH (4a) (1.2g, 0.9 mM) in CH_3OH (30 cm^3) and the mixture hydrogenolysed for twenty-four hours. The catalyst was removed by filtration and the filtrate evaporated to give the title compound (1.03g, 96%); m.p., 135 - 137°C; $[\alpha]_D^{20}$ - 142° (c 1.1, CH_3OH). Calculated for $C_{56}H_{103}N_{11}O_{13}$; C, 59.10; H, 9.76; N, 13.54. Found : C, 59.25; H, 9.67; N, 13.40%; m/z 1138 ($\underline{M}^+$, CI).

Z-Hyp-Abu-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OH (5a)

90% TFA (10 cm^3) was added to Z-Hyp(Bu^t)-Abu-(3-11)-OBu^t (5) (4.3g, 3.1 mM) and the mixture stirred at room temperature for four hours. TFA was removed under vacuo and the residue purified on a silica gel column eluting with CH_2Cl_2:MeOH (15:1). Evaporation of the appropriate fractions gave the title compound as a white solid (2.6g, 65%); m.p., 124 - 125°C; $[\alpha]_D^{20}$ - 143.5° (c 0.6, CH_3OH). Calculated for $C_{65}H_{109}N_{11}O_{15}\cdot 2H_2O$: C, 59.14; H, 8.57; N, 11.68. Found : C, 59.18; H, 8.34; N, 11.16%; m/z 1283 ($\underline{M}^+$, CI); R_t 9.2 min.

H-Hyp-Abu-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OH (5b)

10% Pd/C catalyst was added to a stirred solution of Z-Hyp-Abu-(3-11)-OH (5a) (2.6g, 2 mM), and the mixture hydrogenolysed for twenty-three hours. The catalyst was removed by filtration and the filtrate evaporated to dryness to afford the title compound (2.2g, 96%); m.p., 140 - 141°C; $[\alpha]_D^{20}$ - 161.9° (c 1.0, CH_3OH); m/z 1149 ($\underline{M}^+$, EI).

H-Dab(Fmoc)-Abu-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OH.TFA (6b)

90% TFA (4 cm^3) was added to Boc-Dab(Fmoc)-Abu-(3-11)1-OBu^t (6) (1g, 0.7 mM) with stirring at -20°C. The mixture was allowed to warm to room temperature with stirring for three and a half hours. TFA was evaporated and the residue triturated with ether, to give the title compound as a white solid (0.9g, 100%); m.p., 119 - 120°C; $[\alpha]_D^{20}$ -101.8° (c 0.8, CH_3OH); m/z 1350 (M+1, FAB); R_t 20.5 min.; (λ 278 nm).

Z-(Me)Thr-Nva-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OH (7a)

Z-(Me)Thr(Bu^t)-Nva-(3-11)-OBu^t (7) (0.52g, 0.40 mM) was treated with 90% TFA (5 cm^3) for two hours at room temperature and overnight at 0°C. Excess TFA was removed under vacuo and the residue chromatographed on Sephadex LH20 eluting with DMF. Evaporation of the appropriate fractions gave the title compound as a white solid (0.32g, 68%); m.p., 102 - 103°C; $[\alpha]_D^{24}$ - 105.7° (c 1.2, CH_3OH); δ_H (250 MHz, $CDCl_3$), 0.71 - 1.02 (39H, m, $-CH_3$ (Me)Leu, β-CH_3 (Me)Val, Val, CH_3 Nva), 1.11 (3H, d, CH_3 (Me)Thr), 1.14 - 1.26 (6H, d x d, CH_3 Ala, D-Ala), 1.27 - 1.30 (4H, m, β, γ, CH_2 Nva), 1.36 - 1.92 (12H, m, β-CH_2, γ-CH (Me)Leu), 1.93 - 2.29 (2H, m, β-CH Val, (Me)Val), 2.74 - 3.26 (21H, series of s, N-CH_3), 4.19 (1H, m, β-CH (Me)Thr), 4.29 - 4.39 (2H, d x d, CH_2 Sar), 4.39 - 5.14 (8H, m, α-CH), 5.14 (2H, s, CH_2Ph), 5.41 - 5.55 (2H, m, α-CH), 6.37 (1H, br.d., NH D-Ala), 7.18 (1H, br.s., NH Val), 7.34 (5H, s, ArH), 7.61 - 7.77 (1H, br.s., NH Ala), and 7.89 - 8.06 (1H, br.s., NH Nva); m/z 1300 (M+1, FAB).

H-(Me)Thr-Nva-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OH (7b)

Z-(Me)Thr-Nva-(3-11)-OH (7a) (300 mg, 0.19 mM) in CH_3OH (20 cm^3) was hydrogenated in the presence of 10% Pd/C (50 mg) for thirty-six hours under one atmosphere of pressure. The solution, after removal of the catalyst by filtration, was evaporated to give a solid, which was chromatographed on Sephadex G10 column eluting with water. The solid obtained after evaporation of water, was recrystallised from methanol/ether to give the title compound (260 mg, 88%); m.p., 122 - 124°C; $[\alpha]_D^{24}$ - 144.1° (c 1.3, CH_3OH); m/z 1166 ($\underline{M}^+$, DCI).

Z-(Me)Ser-Nva-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OH (8a)

Z-(Me)Ser(Bu^t)-Nva-(3-11)-OBu^t (8) (1.2g, 0.9 mM) was treated with 90% TFA (6 cm^3) at ambient temperature for two hours and at 0°C overnight. Removal of excess TFA, followed by silica gel chromatography using CH_2Cl_2/6% MeOH as eluents gave the title compound as a white solid (0.9g, 60%); m.p., 110 - 112°C; $[\alpha]_D^{24}$ - 134.9° (c 1.02, CH_3OH); R_t 10.6 min.; m/z 1286 ($\underline{M}^+$, EI).

H-(Me)Ser-Nva-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OH (8b)

Z-(Me)Ser-Nva-(3-11)-OH (8a) (0.8g, 0.6 mM) in CH_3OH (50 cm^3) was hydrogenolysed in the presence of 10% Pd/C for forty-eight hour at ambient temperature under one atmosphere of pressure. The catalyst was removed by filtration and the solvent was evaproated to give a solid, which was purified on a Sephadex G10 column eluting with water. Evaporation of the appropriate fractions afforded a solid which was crystallised from methanol/ether to give the title compound as a white solid (0.65g, 83%); m.p., 192 - 194°C; $[\alpha]_D^{24}$ - 101.6°; m/z 1153 (M+1, FAB).

Z-(Me)Thr-Nle-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OH (9a)

Z-(Me)Thr(Bu^t)-Nle-(3-11)-OBu^t (9) (0.53g, 0.40 mM) was treated with 90% TFA (5 cm^3)

at room temperature for two hours and overnight at 0°C. Excess TFA was removed under vacuo and the residue chromatographed on Sephadex LH20 column eluting with DMF. Evaporation of the appropriate fractions afforded the title compound as a white solid (0.31g, 60%); m.p., 103 - 105°C; $[\alpha]_D^{25}$ - 108.8° (c 1.1, CH_3OH); δ_H (250 MHz, $CDCl_3$), 0.70 - 1.03 (39H, m, β-CH_3 (Me)Val, Val, γ-CH_3 (Me)Leu, CH_3 Nle), 1.12 (3H, d, CH_3- (Me)Thr), 1.13 - 1.26 (6H, d x d, CH_3 Ala, D-Ala), 1.26 - 1.29 (6H, m, CH_2 Nle), 1.36 - 1.91 (12H, m, β-CH_2, γ-CH (Me)Leu), 1.92 - 2.02 (2H, m, β-CH Val, (Me)Val), 2.75 - 3.25 (21H, series of s, N-CH_3), 4.18 (1H, m, β-CH (Me)Thr), 4.28 - 4.38 (2H, br.d., CH_2 Sar), 4.38 - 5.16 (8H, m, α-CH), 5.15 (2H, s, CH_2-Ph), 5.20 - 5.53 (2H, m, α-CH), 6.38 - 7.14 (1H, m, NH D-Ala), 7.15 - 7.21 (1H, br.m., NH Val), 7.35 (5H, s, ArH), 7.60 - 7.76 (1H, br.d., NH Ala), and 7.89 - 8.06 (1H, br.d., NH Nle); m/z 1314 (M+1, FAB).

H-(Me)Thr-Nle-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OH (9b)

Z-(Me)Thr-Nle-(3-11)-OH (9a) (0.28g, 0.18 mM) in CH_3OH (20 cm^3) was hydrogenolysed in the presence of 10% Pd/C (50 mg) for forty-eight hours at one atmosphere of pressure. The solution, after removal of the catalyst by filtration, was evaporated to dryness and the residue chromatographed on a Sephadex G10 column, eluting with water. Evaporation of the appropriate fractions afforded the title compound which was recrystallised from methanol/ether (0.220g, 89%); m.p., 120 - 122°C; $[\alpha]_D^{24}$ - 161.6° (c 1.2, CH_3OH); m/z 1181 (M+1, FAB).

Z-(Me)Ser-Nle-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OH (10a)

Z-(Me)Ser(Bu^t)-Nle-(3-11) OBu^t (10) (1.41g, 1 mM) was treated with 90% TFA (5 cm^3) at ambient temperature for two hours and at 0°C overnight. Excess TFA was evaporated (under vacuo) and the residue purified on a silica gel column eluting with $CHCl_3$/5% MeOH. Evaporation of the appropriate fractions gave the title compound as a white foam, (0.91g, 75%); $[\alpha]_D^{24}$ - -106.3° (c 1.2, CH_3OH); m/z 1314; (M^+, DCI); R_t 11.4 min.

H-(Me)Ser-Nle-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OH (10b)

Z-(Me)Ser-Nle-(3-11)-OH (10a) (0.9g, 0.73 mM) in CH_3OH (50 cm^3) was hydrogenolysed in the presence of 10% Pd/C (0.1g) for forty hours at one atmosphere of pressure. The catalyst was removed by filtration and the solvent evaporated to give a solid, which was chromatographed on a Sephadex G10 column eluting with water. The residue obtained was solidified by trituration with ether and the triturated with ether/petroleum ether, to give the title compound as a white solid (0.75g, 81%); m.p., 78 - 79°C; $[\alpha]_D^{24}$ - 136.1° (c 1.0, CH_3OH); m/z (1167) (M+1, FAB).

Z-Hyp-Nle-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OH (11a)

Z-Hyp(Bu^t)-Nle-(3-11)-OBu^t (11) (2.8g, 1.97 mM) was treated with 90% TFA (5 cm^3) at ambient temperature for one hour and -5°C overnight. Excess TFA was removed under vacuo and the residue chromatographed on a silica gel column eluting with $CHCl_3$/5% MeOH. Evaporation of the appropriate fractions gave the title compound as a white solid (2.0g, 77%); m.p., 120 - 122°C; $[\alpha]_D^{24}$ - 141.8° (c 1.0, CH_3OH); δ_H (250 MHz, $CDCl_3$), 0.72 - 1.06 (39H, m, γ-CH_3 (Me)Leu, β-CH_3 (Me)Val, Val, CH_3 Nle), 1.15 - 1.25 (6H, d x d, CH_3Ala, D-Ala), 1.28 (6H,, m, CH_2 Nle), 1.36 - 1.80 (m, β-CH_2, γ-CH (Me)Leu), 1.85 - 2.26 (m, β-CH (Me)Val, Val, Hyp), 2.78 - 3.29 (18H, series of s, N-CH_3), 3.23 - 4.25 (3H, m, γ-CH, δ CH_2 Hyp), 4.32 (2H, d, CH_2 Sar), 4.37 - 5.10 (8H, m, α-CH), 5.11 (2H, s, Ph-CH_2), 5.43 - 5.52 (2H, m, α-CH), 6.40 (1H, br.s., NH D-Ala), 7.28 (5H, s, ArH), 8.07 - 8.39 (3H, br.m., -NH); R_t 11.4 min.; m/z 1312 (M+1, FAB).

H-Hyp-Nle-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OH (11b)

Z-Hyp-Nle-(3-11)-OH (11a) (2.62g, 2 mM) in CH_3OH (50 cm^3) was hydrogenolysed in the presence of 10% Pd/C catalyst (0.15g) for forty-eight hours. The catalyst was removed by filtration and the filtrate evaporated to give a solid, which was chromatographed on a Sephadex G10 column eluting with water. Evaporation of the appropriate fractions gave the title compound as a white solid (1.8g, 79%); m.p., 139 - 141°C; $[\alpha]_D^{23}$ - 127.8° (c 1.2, CH_3OH); m/z 1178 (M+1, FAB).

Z-(Me)Ser-Thr-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OH (12a)

90% TFA (5 cm^3) was added to Z-(Me)Ser(Bu^t)-Thr(Bu^t)-(3-11)-OBu^t (12) (0.9g, 0.6 mM) and the mixture stirred at room temperature for four hours. The residue, after evaporating TFA was applied directly 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 solid (0.4g, 50%); m.p., 119 - 120°C; $[\alpha]_D^{20}$ -146.6° (c 1.1, CH_3OH). Calculated for $C_{69}H_{109}N_{11}O_{16}\cdot H_2O$: C, 58.14; H, 8.41; N, 11.66. Found : C, 57.90; H, 8.33; N, 11.35%; m/z 1287 (M^+, DCI); R_t 8.1 min.

H-(Me)Ser-Thr-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val-OH (12b)

10% Pd/C catalyst (0.3g) was added to a solution of Z-(Me)Ser)-Thr-(3-11)-OH (12a) (300 mg, 0.23 mM) in CH_3OH (20 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 as a white solid (0.23g, 95%), m.p., 140 - 141°C; $[\alpha]_D^{20}$ 141° (c 0.9, CH_3OH); m/z 1153 (M^+, DCI).

Cyclosporin analogues

A. Cyclisations using Castro reagent

Cyclo-[(Me)Thr-Abu-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val] (13)

Dimethylamino pyridine (0.5g, 4.1 mM, 5 equiv.), followed by Castro reagent B.O.P. (1.5g, 3.4 mM, 4 equiv.) were added to a vigorously stirred solution of H-(Me)Thr-Abu-(3-11)-OH (3b) (0.9g, 0.8 mM, 1 equiv.) in CH_2Cl_2 (2.5L) at room temperature. After five days the solvent was evaporated and the residue applied directly on a silica gel column eluting with EtOAc/MeOH (97.3). Evaporation of the appropriate frctions gave the title compound as a white solid (0.09g, 10%); m.p., 165 - 167°C; $[\alpha]_D^{20}$ - 121° (c 1, MeOH); δ_H (250 MHz, $CDCl_3$, 2 confs.), 0.71 - 1.14 (39H, m, CH_3 Abu, Val, (Me)Leu, (Me)Val), 1.26 (3H, d, (Me)Thr-CH_3), 1.30 (6H, d, CH_3 L-Ala, D-Ala), 1.40 - 1.80 (10H, m, β-CH_2 Abu,

(Me)Leu), 1.82 - 2.30 (5H, m, β-CH Val, (Me)Val and γ-CH (Me)Leu), 2.70 - 3.46 (21H, series of s, N-CH_3), 3.14 - 3.20 (1H, d, α-CH Sar), 4.31 - 4.42 (2H, m, β-CH and OH of (Me)Thr), 4.61 - 4.72 (1H, m, α-CH L-Ala), 4.82 - 5.12 (5H, m, α-CH L-Ala, Abu, (Me)Val, Val and Sar), 5.51 (1H, d, α-CH, (Me)Thr), 5.71 - 5.92 (4H, m, α-CH, (Me)Leu), 6.90 (1H, d, NH D-Ala), 7.10 (1H, d, NH Val), 7.60 (1H, d, NH L-Ala), and 8.20 (1H, d, NH Abu); m/z 1134 (M+1, FAB); R_t 9.4 min.

Cyclo-[(Me)Ser-Abu-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu- (Me)Val] (14)

DMAP (0.27g, 2.2 mM, 4 equiv.), followed Castro reagent (0.78g, 1.76 mM, 4 equiv.) were added to a vigorously stirred solution of H-(Me)Ser-Abu-(3-11)-OH (4b) (0.5g, 0.44 mM, 1 equiv.) in CH_2Cl_2) (2.5L) at room temperature. After three days the solvent was evaporated and the residue applied directly on a silica gel column eluting with CH_2Cl_2:MeOH (95:5). Evaporation of the appropriate fractions gave the title compound as a white solid (0.15g, 15%); m.p., 212 - 214°C; $[\alpha]_D^{20}$ - 144° (c 1, MeOH); δ_H (250 MHz, $CDCl_3$, 1 conf.), 0.75 - 1.10 (39H, m, CH_3 of Val, (Me)Val, (Me)Leu, Abu), 1.15 - 1.35 (6H, d x d, CH_3 L-Ala, D-Ala), 1.55 - 1.50 (10H, br.m., β-CH_2 Abu, (Me)Leu), 1.85 - 2.55 (6H, β-CH (Me)Val, γ-CH (Me)Leu), 2.79 - 3.50 (21H, series of s, N-CH_3), 3.85 (2H, d, β-CH_2(Me)Ser), 4.45 - 4.55 (1H, m, α-CH Ala), 4.60 - 4.75 (5H, m, α-CH Sar, (Me)Val, Val (Me)Ser -OH), 4.79 - 4.85 (1H, m, α-CH D-Ala), 4.49 - 5.10 (1H, m, α-CH Abu), 5.15 - 5.28 (2H, d x d, α-CH (Me)Leu), 5.45 - 5.55 (1H, t, α-CH (Me)Ser), 5.60 - 5.75 (2H, d x d, α-CH, (Me)Leu), 7.15 (1H, d, N-H D-Ala), 7.35 (1H, d, NH Val), 7.95 (1H, d, NH Ala), and 8.40 (1H, d, NH Abu); m/z 1120 (M+1, FAB); R_t 11.4 min.

Cyclo[Hyp-Abu-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala(Me)Leu-(Me)Leu-(Me)Val] (15)

DMAP. (0.42g, 2.05 mM, 5 equiv.) followed by Castro reagent BOP. (1.23g, 1.64 mM, 4 equiv.) were added to a rapidly stirring solution of H-Hyp-Abu-(3-11)-OH (5b) (0.80g, 0.41 mM, 1 equiv.) in CH_2Cl_2 (2L) and the resulting mixture stirred at room temperature for four days. The solution was then concentrated to (100 cm^3) and chromatographed on silica gel eluting with EtOAc/MeOH (97:3). Evaporation of the appropriate fractions afforded the title compound as a white solid, (0.24g, 30%); m.p., 166 - 168°C; $[\alpha]_D^{20}$ - 240° (c 1, MeOH); δ_H (250 MHz, $CDCl_3$, 1 conf.), 0.81 - 1.01 (39H, m, CH_3 Abu, Val, (Me)Leu, (Me)Val), 1.27 (3H, d, CH_3 D-Ala), 1.36 (3H, d, CH_3 L-Ala), 1.39 - 1.80 (10H, m, β-CH_2 Abu, (Me)Leu), 1.82 - 2.38 (7H, m, β-CH Val and (Me)Val, β-CH_2 Hyp, and γ-CH (Me)Leu), 2.68 - 2.69 (3H, d, N-CH_3), 3.11 - 3.12 (1H, m, α-CH Sar), 3.25 - 3.30 (15H, series of s, N-CH_3), 3.84 - 3.89 (2H, d x d, α-CH Hyp), 2.84 - 3.89 (2H, d x d, δ-CH_2 Hyp), 4.04 - 4.20 (1H, m, γ-CH Hyp), 4.30 - 4.41 (1H, t, α-CH L-Ala), 4.44 (1H, br.s., -OH), 4.47 - 4.89 (5H, m, α-CH Val, (Me)Val, Sar, Abu and D-Ala), 4.93 - 5.09 (2H, m, α-CH, (Me)Leu), 5.13 - 5.31 (2H, d x d, α-CH (Me)Leu), 5.34 - 5.69 (1H, d x d, α-CH Hyp), 7.14 (1H, d, N-H D-Ala), 7.45 (1H, d, NH Val), 7.77 (1H, d, NH L-Ala), and 8.65 (1H, d, NH Abu); m/z 1133 (M+1, FAB); R_t 10.2 min.

Cyclo-[(Dab(Fmoc)-Abu-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val] (16)

DMAP (0.20g,1.65 mM, 5 equiv.), followed by Castro reagent (0.58g, 1.32 mM, 4 equiv.) were added to a rapidly stirring solution of H-Dab(Fmoc)-Abu- (3-11)-OH (6b) (0.45g, 0.33 mM, 1 equiv.) in CH_2Cl_2 (2L) and the mixture stirred at room temperature for three days. The solution was concentrated to (50 cm^3) and chromatographed initially on Sephadex LH20 eluting with DMF and then on silica gel eluting with EtOAc/MeOH 97:3%. Evaporation of the appropriate frctions afforded the title compound as a white solid (0.17g, 37%); m.p., 156 - 157°C; $[\alpha]_D^{20}$ - 190.2° (c 0.9, MeOH). Calculated for $C_{71}H_{112}N_{12}O_{13}\cdot 2H_2O$: C, 61.92; H, 8.43; N, 12.21. Found : C, 62.25; H, 8.39; N, 12.30%; δ_H (250 MHz, $CDCl_3$, 1 conf.), 0.78 - 1.12 (39H, m, CH_3 (Me)Leu, Val, (Me)Val, Abu), 1.33 - 1.37 (6H, d x d, CH_3 D-Ala , L-Ala), 1.40 - 1.72 (16H, m, β-CH_2 (Me)Leu, Abu, Dab and γ-CH (Me)Leu), 2.06 - 2.39 (2H, m, β-CH Val, (Me)Val), 2.77 - 3.44 (18H, series of s, N-CH_3), 4.21 - 4.49 (5H, m, γ-CH_2 Dab, α-CH Sar and L-Ala), 4.51 - 5.28 (5H, m, α-CH), 5.37 - 5.44 (2H, d x d, α-CH (Me)Leu), 5.64 - 5.68 (2H, m, α-CH, (Me)Leu), 6.27 - 6.37 (1H, m, Dab-NH), 6.56 (1H, d, Dab-NH), 7.31 (1H, d, NH D-Ala), 7.34 - 7.77 (9H, m, ArH and NH Val), 8.02 (1H, d, NH L-Ala), and 8.62 (1H, d, NH Abu); m/z 1341 (M+1, FAB); R_t 13.4 min.

Cyclo-[Dab-Abu-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val] (16a)

Cyclo-[Dab(Fmoc)-Abu-(3-11)] (16) (90 mg, 0.07 mM) was stirred in piperidine (1 cm^3) at room temperature for thirty minutes and then poured into water (20 cm^3). The piperidine-dibenzofulvene adduct was filtered and the filtrate evaporated to dryness. The residue was applied on Sephadex LH20 eluting with DMF; evaporation of the appropriate fractions afforded the title compound as a white solid (10 mg, 13%); m.p., 160 - 164°C; $[\alpha]_D^{20}$ -181° (c 1.1, MeOH); m/z 1118 (M+1, FAB); R_t 12.1 min.

Cyclo-[(Me)Thr-Nva-Sar-(Me)Leu-(Me)Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val] (17)

H-(Me)Thr-Nva-(3-11)-OH (7b) (250 mg, 0.21 mM) was added to Castro reagent (0.630 mg, 6 equiv.) in dichloromethane (750 ml) and DMAP (0.2g, 1.52 mM, 7 equiv.) added. The solution was stirred at room temperature for three days and evaporated. The oily residue was chromatographed on Sephadex LH20 eluting with DMF,and evaporation of the appropriate fractions afforded [$(Me)Thr^1$-Nva^2]-cyclosporin as pale yellow crystals (from ether/petroleum ether) (39 mg, 20%); m.p., 102 - 104°C; $[\alpha]_D^{24}$ - 147° (c 1.0, $CHCl_3$); m/z 1148 (M^+, FAB); R_t 11.0 min.

Cyclo-[(Me)Ser-Nva^2-Sar-(Me)Leu-(Me)Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val] (18)

Cyclisation was carried out by the Castro reagent at low concentration of the undecapeptide and at room temperature. H-(Me)Ser-Nva-(3-11)-OH (8b) (400 mg, 0.31 mM) was dissolved in CH_2Cl_2-THF (1:1) (80 ml). To this was added DMAP (0.20g, 2.17 mM, 7 equiv.), followed by the addition of the condensing agent [Bt-OP$(NMe_2)_3^+PF_6^-$] (0.822g, 1.86 mM, 6 equiv.). The solution was stirred at room temperature for three days and was concentrated by evaporation on a rotary evaporator to 20 ml, this solution was chromatographed on (i) a silica gel column eluting with CH_2Cl_2 + 5% MeOH, (ii) Sephadex LH20 eluting with DMF. Evaporation of the appropriate fraction afforded the title compound as white crystals (from

ether/petroleum ether) (72 mg, 20%); m.p., 112 - 114°C; $[\alpha]_D^{24}$ - 136°, (c 1.0, MeOH); m/z 1135 (M+1, FAB); R_t 11.6 min.

Cyclo-[(Me)Thr-Nle-Sar-(Me)Leu-(Me)Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val] (19)

H-(Me)Thr-Nle-(3-11)-OH (9b) (300 mg, 0.26 mM) was dissolved at ambient temperature in dichloromethane (800 ml). To this was added DMAP (0.21g, 1.82 mM, 7 equiv.), followed by Bt-OP(NMe_2)$_3^+PF_6^-$ (0.650g, 1.77 mM, 6 equiv.). The solution was stirred at room temperature for three days and solvent evaporated. The oily residue was subjected to chromatography on Sephadex LH20 eluting with DMF. Evaporation of the appropriate fractions afforded the title compound (29) as white crystals (from ether/petroleum ether) (46 mg, 14%); m.p., 94 - 95°C; $[\alpha]_D^{24}$ - 168° (c, 1.0, $CHCl_3$); m/z 1162 (M^+, FAB); R_t 11.2 min.

Cyclo-[(Me)Ser-Nle-Sar-(Me)Leu-(Me)Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val] (20)

H-(Me)Ser-Nle-(3-11)-OH (10b) (450 mg, 0.39 mM) was dissolved in THF-CH_2Cl_2 (1:1) (900 ml) at room temperature. To this was added DMAP (0.33g, 2.7 mM, 7 equiv.), followed by Bt-OP(NMe_2)$_3^+PF_6^-$ (0.975g, 2.2 mM, 6 equiv.). The solution was stirred at room temperature for three days and then the solvent was evaporated and the oily residue chromatographed twice; firstly on a silica gel column eluting with CH_2Cl_2 plus 5% MeOH and then on LH20 gel filtration column eluting with DMF. Evaporation of the appropriate fraction afforded cyclo[(Me)Ser-Nle-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)-Val] (20) as white crystals (from ether/petroleum ether) (67 mg, 16%; m.p., 108 - 110°C; $[\alpha]_D^{23}$ - 141° (c, 1.0, MeOH); m/z 1149 (M+1, FAB); R_t 11.4 min.

Cyclo[(Hyp-Nle-Sar-(Me)Leu-Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val] (21)

DMAP. (0.84g, 4.1 mM, 7 equiv.), followed by Castro reagent (2.61g, 3.48 mM, 6 equiv.) were added to a rapidly stirred solution of H-Hyp-Nle-(3-11)-OH (11b) (1.18g, 1 mM, 1 equiv.) in CH_2Cl_2 (2.5 ℓ), and the resulting mixture stirred at room temperature for three days. The solution was concentrated to (100 cm^3) and chromatographed on silica gel eluting with EtOAc/MeOH (97:3). Evaporation of the appropriate fractions gave a white crystalline solid (0.30g, 26%); m.p., 145°C; $[\alpha]_D^{23}$ - 152.4° (c 1.0, CH_3OH); δ_H (250 MHz, $CDCl_3$), 0.70 - 1.26 [36H, m, CH_3 (Me)Leu, Val, (Me)Val, Nle], 1.26 - 1.28 (3H, d, CH_3 D-Ala), 1.35 - 1.38 (3H, d, CH_3 Ala), 1.43 - 1.84 (12H, m, β,γ,δ- CH_2 Nle), 1.85 - 2.44 (8H, m, β-CH Val, (Me)Val, β-CH_2 Hyp, γ-CH (Me)Leu), 2.68 - 3.30 (18H, series of s, N-CH_3), 3.84 - 3.90 (2H, d x d, δ-CH, Hyp), 4.29 - 4.33 (1H, m, γ-CH, Hyp), 4.41 - 4.68 (1H, α-CH L-Ala), 4.70 (1H, br.s., -OH), 4.72 - 4.95 (4H, m, α-CH D-Ala, Val, (Me)Val, Sar), 4.96 - 5.16 (2H, m, α-CH, Hyp, Nle), 5.30 - 5.36 (2H, d x d, α-CH, (Me)Leu), 5.64 - 5.74 (2H, d x d, α-CH, (Me)Leu), 7.23 (1H, d, NH D-Ala), 7.77 (1H, d, NH Val), 8.10 (1H, d, NH L-Ala), and 8.68 (1H, d, NH Nle); m/z 1160 (M+1, FAB); R_t 10.8 min.

B. Cyclisation using propylphosphonic anhydride

H-Hyp-Nle-(3-11)-OH (11b) (0.843g, 0.72 mM) in dichloromethane (2L.) was cooled to -10°C. DMAP (0.62g, 5 mM, 7 equiv.) was added to the cooled solution followed by $(PrPO_2)_3$ (propylphosphonic anhydride) [(0.7 ml) of a 50% w/w solution in dichloromethane]. The solution was stirred for one hour at -10°C and four days at room temperature, then concentrated to about 20 ml by evaporation at ambient temperature. Immediate chromatography on a silica gel column using CH_2Cl_2 + 5% of MeOH without work-up gave four major fractions , the first being identified as the title compound (31) giving (60 mg, 7.3% yield) as white crystals (from ether/petroleum ether). All physical constants and spectroscopic data were in close agreement with the authentic sample obtained using the Castro reagent.

C. Cyclisation using diphenylphosphinyl chloride (Dpp.Cl)

H-Hyp-Nle-(3-11)-OH (11b) (0.580g, 0.5 mM) in dichloromethane (2 ℓ) was treated with DMAP (0.42g, 3 mM, 6 equiv.) at ambient temperature and DppCl (0.829g, 7 mM, 5 equiv.) in CH_2Cl_2 (5 ml) added. The mixture was stirred at ambient temperature for forty-eight hours, then the solvent was removed by evaporation and the residues chromatographed on silica gel using CH_2Cl_2 + 5% MeOH. Evaporation of the appropriate fractions afforded the title compound as white crystals from ether/petroleum ether (92 mg, 8% yield); m.p., 143 - 145°C; $[\alpha]_D^{23}$ - 150.2°, (c 1.0, MeOH). The spectroscopic data were closely similar to that obtained from material produced using the Castro reagent.

An attempt to repeat the procedure using activation at -20°C did not improve the yield.

Cyclo-[(Me)Ser-Thr-Sar-(Me)Leu-(Me)Val-(Me)Leu-Ala-D-Ala-(Me)Leu-(Me)Leu-(Me)Val] (22)

DMAP (0.10g, 0.85 mM, 5 equiv.) and Castro reagent (0.29g, 0.66 mM, 4 equiv.) were added to a solution of vigorously stirred H-(Me)Ser-Thr-(3-11)-OH (12b) (0.19g, 0.17 mM, 1 equiv.) in CH_2Cl_2 (1L), and the mixture stirred at room temperature for three days. 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 solid (9 mg, 5%); m.p., 144 - 146°C; $[\alpha]_D^{20}$ - 117° (c 1, MeOH), m/z 1136 (M+1, FAB); R_t 11.7 min.

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