53. Solid-Phase Total Synthesis of Cyclosporine Analogues

by **Soo Y. Ko**[1])* and **Roland M. Wenger***

Preclinical Research, *Sandoz Pharma Ltd.*, CH-4002 Basel

(10.I.97)

Syntheses of cyclosporine analogues are reported wherein the peptide couplings were achieved in solid phase. The *Wang* resin was used as the solid support, and the peptide couplings commenced with the residue 11 of the cyclosporine skeleton. The couplings proceeded in a stepwide manner up to the residue MeBmt[1], using symmetric anhydrides. The peptides were then cleaved off the resin, and the cyclization was achieved in solution using *Castro*'s reagent. The solid-phase synthesis described herein offers a very efficient method for the rapid synthesis of structurally diverse cyclosporine analogues in small quantities. The biological activities of the synthetic cyclosporine analogues were evaluated in two *in vitro* assays, including the IL-2 reporter gene assay and the cyclophilin binding assay. The structure-activity relationship is discussed.

1. Introduction. – Cyclosporine (CS), originally named Cyclosporin A, is an immunosuppressant known by the trade names *Sandimune*® and *Neoral*®, and is currently used for preventing allograft rejections and autoimmune diseases in humans [1]. It was isolated from the fungal species *Tolypocladium inflatum Gams* and its structure determined by chemical methods and X-ray crystallography [2]. The biological activity was discovered soon afterwards [3]. The chemical structure of CS is shown in the *Figure* and corresponds to cyclo(-MeBmt[1]-Abu[2]-Sar[3]-MeLeu[4]-Val[5]-MeLeu[6]-Ala[7]-D-Ala[8]-MeLeu[9]-MeLeu[10]-MeVal[11]-)[2]) undecapeptide.

The immunosuppressive activity of CS is believed to be based on the inhibition of the production of interleukin-2 (IL-2) at the transcription level [4]. However, the exact mode of action is still not completely understood, and it is only during the past several years that some aspects of how CS and other immunosuppressants interact with their respective receptors have come to light at the molecular levels [5].

CS binds tightly to its receptor, cyclophilin A (CYP). The structure of CS bound to its receptor CYP has only recently been revealed by NMR spectroscopy and by X-ray crystallography, and has been shown to be very different from the crystalline CS conformation or the one found in apolar solvents [6]. The resulting CYP-CS complex in turn binds to the Ca^{2+}-regulated protein phosphatase calcineurin. In this respect, the CYP-CS complex very much resembles another immunophilin-immunosuppressant, namely FKBP-FK506. Considering the structural diversity between CS and FK506 as well as between CYP and FKBP, it is remarkable that their modes of action are so similar.

---

[1]) Present address: *Sandoz* Institute for Medical Research, 5 Gower Place, London VC1E 6BN, U.K.

[2]) Abbreviations: MeBmt: (4*R*)-4-[(*E*)-but-2-enyl]-4,*N*-dimethyl-L-threonine; Abu: (2*S*)-2-aminobutanoic acid; Sar: sarcosine = *N*-methylglycine; MeLeu: *N*-methylleucine; Val: valine; Ala: alanine; MeVal: *N*-methylvaline; D-*cis*-Hyp. (2*R*,4*R*)-4-hydroxyproline (D-Pro (4-OH (*R*))); γAbu: 4-aninobutanoic acid; Fmoc: (9*H*-fluoren-9-ylmethoxy)carbonyl.

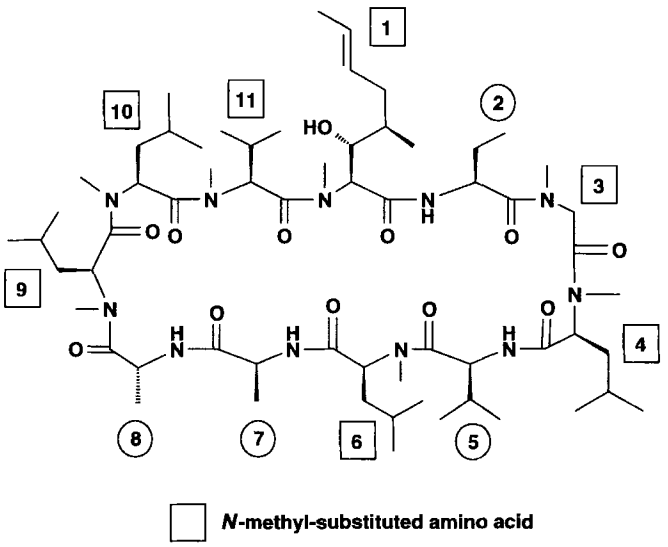

Figure. *Structure of Cyclosporin A* (CS)

In establishing the structure/immunosuppressive activity relationship, it is essential to have a large number of CS analogues with wide structural diversity. Many CS analogues have been obtained from natural sources. Thus, cyclosporines B–Z have been isolated from the same fungal source [7]. In addition, several CS metabolites have been identified [7]. These natural CS analogues, however, provide only limited variations from the parent CS structure. In order, for the medicinal-chemistry programme, to address the issues generated from the emerging knowledge of interactions of CS with its receptors cyclophilin and calcineurin, a wider variety of CS analogues is needed. These have to be prepared by chemical synthesis.

A very efficient way to prepare CS analogues is by employing natural cyclosporines as starting materials. One approach in this *partial* synthesis is to convert an amino-acid residue within CS to another in a regioselective reaction. A highlight of this approach is *Seebach's* very elegant enolate chemistry on CS, introducing various electrophiles at the Sar^3 α-position diastereoselectively [9]. A second approach involves a regioselective ring opening of a CS analogue, affording a secocyclosporin. Removal of the N-terminal amino-acid residue by *Edman* degradation is followed by a new residue insertion and re-cyclization in succession, leading to a new CS analogue. Several methods have been developed for the regioselective ring cleavages between residues 11–1 and 3–4 of CS as well as between residues 1–2 and 7–8 of [Thr^2] CS and [D-Ser^8] CS, respectively [10]. In addition, ring cleavages between residues 4–5 and 7–8 have been reported following regioselective thionations of the CS skeleton at residue 4 and 7, respectively [11].

As already alluded to, both these approaches depend on regioselective reactions of cyclic undecapeptides, still limiting the structural diversity of semisynthetic derivatives. Total synthesis is then the only way to attain the necessary structural diversity of CS analogues.

The first total synthesis of CS was reported in 1983, and total syntheses of several CS analogues followed [12]. In these early synthetic efforts, the peptide couplings were all performed in solution phase. With the frequent occurrence of sterically bulky amino acids in cyclosporines, including seven *N*-methyl-substituted amino acids out of eleven residues, the solid-phase peptide couplings had been thought to be very difficult. As a result, the total synthesis of CS analogues had been regarded to be very time-consuming and not a viable tool in a medicinal-chemistry programme as far as preparing a large number of structurally diverse CS analogues was concerned.

In parallel to the progress made in the area of immunopharmacology, we undertook a project to re-evaluate the feasibility of the solid-phase synthesis of CS analogues and to develop an efficient solid-phase synthetic strategy. The project eventually led to the rapid production of a variety of CS analogues in small quantities. A recent publication by *Rich* and coworkers [13] on their solid-phase synthetic efforts for cyclosporines has prompted us to disclose some of our results in this area now.

2. Results and Discussion. – 2.1. *Preliminary Studies and Strategy.* As our initial concern in the solid-phase synthesis of CS analogues had been the couplings of sterically bulky *N*-methyl-substituted amino acids, we first addressed this problem by screening various sets of coupling conditions. This and other related studies indicated that the use of symmetric anhydrides provided a quite reliable coupling method for a variety of (methylamino) acids. This attribute – that it works consistently well with many different amino acids – was thought to be crucial in this project as were searching for a *general* synthetic method for *structurally diverse* CS analogues. For this reason, the symmetric-anhydride coupling method was chosen for every coupling step to be performed on solid support. Having settled on the reagent for the coupling steps, we then addressed another very critical issue, namely the cyclization.

Perhaps a single most important element of synthetic strategy that one needs to consider when undertaking a total synthesis of CS is where to start the peptide couplings, *i.e.*, which two adjacent residues will be coupled at the end in a cyclization step. The answer to this question may be different if one does the peptide couplings in solid phase as opposed to in solution.

Most of the solution-phase syntheses of cyclosporines, *e.g.*, have been commenced at the residue L-Ala^7, and completed *via* cyclization between residues 7 and 8, sterically the least hindered pair within the cyclic undecapeptide skeleton. In solid-phase synthesis, on the other hand, a more crucial factor than contemplating which two residues will be most readily coupled in the cyclization step (which will be done in solution) is to consider which peptide bond will be most difficult (or almost impossible) to form in solid phase. The idea is that the most difficult amidation step would then be performed in solution in the final cyclization step. Therefore, the starting point for a solid-phase synthesis of cyclosporines will almost certainly be different from that for solution-phase syntheses.

In an attempt to locate the most difficult coupling step on solid support within the cyclosporine skeleton, preliminary studies were performed with a series of two adjacent amino-acid residues found in CS. Not unexpectedly, these studies revealed several difficult coupling steps, namely those between residues 10 and 11, 9 and 10, 6 and 5, and 11 and 1. Of these, the last pair coupling between residues MeVal^{11} and MeBmt^1 proved

particularly difficult: even after repeated treatments with Fmoc-MeVal[2]) symmetric anhydride on MeBmt bound to a solid support, the coupling was not successful. Therefore, it was decided that the solid-phase couplings would commence with the residue MeVal^{11} and proceed through residues 10, 9, .. up to residue 1; the final cyclization *in solution* would then be performed between residues MeVal^{11} and MeBmt^{1}. This strategy, of course, depends on a successful cyclization between these two bulky amino acids.

Using the linear undecapeptide 1,11-secocyclosporin, which was obtained from CS *via* ring opening between residues 11 and 1 [10], the cyclization reaction was studied under various reaction conditions. It was rewarding to observe that this cyclization was indeed possible in solution while the coupling between this pair of (methylamino) acids had failed in solid phase[3]). The best results were obtained when the cyclization was carried out using *Castro*'s reagent[4]) in high dilution [15]. The desired CS was produced after 5 days, accompanied by cyclosporin H (the D-MeVal^{11} analogues [12b] [7c]), which was separated by flash column chromatography (FC). Thus, we established the fundamentals of the solid-phase synthesis, *i.e.*, our choice of synthetic strategy and coupling method, and were now ready for the synthesis of cyclosporine analogues.

2.2. *Solid-Phase Synthesis of Cyclosporine Analogues.* The commercially available *Wang* resin[5]) was chosen as the solid support [16]. The first residue (residue 11, Fmoc-MeVal-OH for most of the analogues prepared in this project) was anchored using DCC/HOBt/DMAP in CH_2Cl_2 [17][6]). Use of 3 equiv. of the *N*-Fmoc-*N*-methylamino acid resulted in *ca.* 80% of anchoring on the OH group of the *Wang* resin. The remaining free OH groups on the resin were not blocked. After the Fmoc deprotection, peptide couplings proceeded using *N*-Fmoc-amino acid symmetric anhydrides up to the residue 2. The coupling reactions were performed in the presence of bromophenol blue indicator, which changed its color from blue to yellow as the amino terminus of the peptide chain on the resin was being consumed in the coupling reaction [18]. This indicator color change was reliable enough to enable us to obviate time-consuming HPLC monitoring of the coupling process. In general, three difficult couplings, between residues 11 and 10, 10 and 9, and 6 and 5, had to be repeated. The final residue 1 was coupled using Boc-MeBmt symmetric anhydride.

The Boc deprotection and cleavage of the peptide off the resin were conducted in a single operation using CF_3COOH. The linear undecapeptide 1,11-secocyclosporins were thus obtained in > 70% overall crude yield, and were shown to be > 85% pure by HPLC. The cyclizations were performed under the conditions already worked out in the preliminary studies using *Castro*'s reagent [15] and afforded the desired cyclosporine analogues, along with varying amounts of the D-amino acid epimeric by-products at the residue 11 (D-MeVal in most cases). Flash chromatographic purification yielded the final cyclosporine products. The CS analogues thus prepared are listed in *Table 1*. The structures of the synthetic products were confirmed by ^{1}H-NMR as well as MS.

---

[3]) *Galpin* and coworkers also reported cyclization between residues 11 and 1 of (1–11)secocyclosporines using *Castro*'s reagent. In their synthesis, a modified MeBmt was substituted for the residue 1 (see [14]).

[4]) *Castro*'s reagent: (1*H*-benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate.

[5]) *Wang* resin: 4-(benzyloxy)benzyl-alcohol resin.

[6]) Abbreviations: DCC: dicyclohexylcarbodiimide; HOBt. 1-hydroxy-1*H*-benzotriazole; DMAP: 4-(dimethylamino)pyridine.

Table 1. *Synthetic Cyclosporine Analogues*

Entry	Structural modifications[a])			Mol. wt.	^{1}H-NMR[b])	$[\alpha]_D$[c])
	residue positions	residue(s) in CS	residue(s) in analogue			
1	8	D-Ala	D-Pro	1227	6.83, 7.39, 7.45, 7.95, 8.08, 8.27 (6*d*, NH)[d])	−210.4 (*c* = 0.451)
2			D-*cis*-Hyp[2])	1243	2.73, 2.88, 3.11, 3.31, 3.32, 3.41, 3.50 (7*s*, MeN); 7.40, 7.59, 8.36 (3*d*, NH)[e])	−230.0 (*c* = 0.38)
3			D-Cys(^{t}Bu)	1289	1.3 (*s*, ^{t}Bu); 2.65–2.8 (*m*, 2H–C (*β*.8))	−210.9 (*c* = 0.354)
4			D-Asn	1244	2.48, 2.58 (*AB* of *ABX*, 2H–C (*β*.8)); 6.21, 7.26 (br. NH_2)	−230.2 (*c* = 0.295)
5			D-Gln	1258	2.23–2.28 (*m*, 2H–C (*γ*.8)); 4.85 (*dt*, H–C (*α*.8)); 6.45, 7.25 (br., NH_2)	−192.1 (*c* = 0.303)
6			Sar	1201	2.79, 2.80, 3.00, 3.05, 3.07, 3.17, 3.34, 3.37 (8*s*, MeN)[e])	−201.0 (*c* = 0.31)
7			D-MeAla	1215	2.72. 2.81, 2.97, 3.12, 3.16, 3.31, 3.40, 3.50 (8*s*, MeN); 7.40, 7.60, 8.37 (3*d*, NH)[e])	−193.2 (*c* = 0.448)
8	7	Ala	Val	1229	1.03, 1.06 (2*d*, Me_2C (*β*.7)); 4.45 (*dd*, H–C (*α*.7))	−189.3 (*c* = 1.03)
9			Ser	1218	3.6, 4.15 (*AB* of *ABX*, 2H–C (*β*.7)); 7.15 (2×), 7.4, 7.55 (4*d*, NH)	−183.6 (*c* = 1.03)
10			Thr	1231	1.6 (*d*, Me (*γ*.7)); 7.45 (*dd*, H–C (*α*.7)); 4.5 (*m*, H–C (*β*.7)) 7.05, 7.16, 7.17, 7.47 (4*d*, NH)	−173.7 (*c* = 1.06)
11			Gly	1187	3.55, 4.25 (*AB* of *ABX*, 2H–C (*α*.7))	−215.6 (*c* = 1.07)
12	5–6	Val[5]-MeLeu[6]	Leu[5]-Pro[6]	1185	3.3, 3.75 (*m*, 2H–C (*δ*.6)); 4.48 (*dd*, H–C (*α*.6)) 2.70, 2.72, 3.05, 3.10, 3.40, 3.56 (6*s*, MeN)	−186.7 (*c* = 0.474)
13	5	Val	Leu	1215	2.70, 2.72, 3.07, 3.10, 3.24, 3.40, 3.56 (7*s*, MeN); 7.04, 7.45, 7.57, 7.81 (4*d*, NH)	−212.6 (*c* = 0.38)
14			Thr	1203	1.26 (*d*, Me(*γ*.5)); 4.1 (*m*, H–C (*β*.5)); 4.35 (*d*, OH–C (*β*.5))	−230.2 (*c* = 0.60)
15			allo-Thr	1203	1.27, 1.30, 1.35 (3*d*, Me for residues 8, 5, and 7); 4.28–4.35 (*m*, H–C (*β*.5))	−210.5 (*c* = 0.295)
16	4	MeLeu	Pro	1171	3.9–4.0 (*m*, 2H–C (*δ*.4)); 2.69, 2.7, 3.14, 3.29, 3.35, 3.52 (6*s*, MeN)	−235.9 (*c* = 1.01)
17			MeVal	1187	2.71, 2.72 (3×), 2.79, 3.09, 3.10 (2×), 3.19 (2×); 3.35, 3.36, 3.39, 3.48 (14*s*, MeN)[d])	−226.1 (*c* = 0.398)
18			MeLeu-Leu[f])	1314	2.71, 2.72, 2.95, 3.05, 3.18, 3.40, 3.57 (7*s*, MeN)[e])	−188.2 (*c* = 0.428)
19	3	Sar	Gly	1187	2.71, 2.73, 3.14 (2×), 3.20, 3.47 (6*s*, MeN); 6.93, 7.14, 7.25, 7.60, 7.91 (5*d*, NH)[e])	−234.2 (*c* = 1.05)

Table 1 (cont.)

20			MeγAbu^{2})[g]	1229	2.68, 2.71, 2.77, 3.18, 3.20, 3.28, 3.49 (7*s*, MeN); 7.31, 7.89, 8.68, 8.86 (4*d*, NH)[e]	−176.7 (c=1.03)
21	2	Abu	MeAbu	1215	2.71, 2.74, 2.95, 3.02, 3.07, 3.09, 3.21, 3.58 (8*s*, MeN); 6.93, 6.99, 7.09 (3*d*, NH)	−241.1 (c=0.35)
22	2–3	Abu^{2}-Sar^{3}	MeAbu^{2}-D-MeAla^{3}	1229	2.70, 2.73, 3.03, 3.05, 3.08, 3.10, 3.18, 3.57 (8*s*, MeN); 6.03, 7.01, 7.03 (3*d*, NH)	−262.3 (c=0.36)
23	11	MeVal	MeThr	1203	2.60, 2.69, 2.95, 2.98, 3.11, 3.18, 3.44 (7*s*, MeN); 6.82, 7.21, 7.51, 7.72 (4*d*, NH)[e]	−164.7 (c=0.455)
24	9	MeLeu	Pro	1172	multiple conformations	−160.0 (c=0.553)

[a]) Variations from the structure of cyclosporine and their positions are shown.
[b]) In $CDCl_3$. Only those signals, characteristic of the structural variations or showing major differences from the ^{1}H-NMR of cyclosporine are listed. For the ^{1}H-NMR of cyclosporine, see [2c].
[c]) Taken in $CHCl_3$, at room temperature.
[d]) Mixture of two major conformations.
[e]) Major conformation.
[f]) Cyclic dodecapeptide.
[g]) A 35-membered ring structure.

2.3. *Biological Activities.* The biological activities of the synthetic CS analogues were evaluated in two *in vitro* assays: the IL-2 reporter gene assay, measuring immunosuppressive activity, and the binding affinity to CYP. The IL-2 reporter gene assay detects substances interfering with IL-2 gene activation along the T cell signalling pathway [19]. The CYP binding affinity was determined under competitive ELISA systems, using protein-conjugated ligand CS bound to a solid support and biotinylated CYP as specific recognition structure [20]. These assay results are listed in *Table 2*[7]).

2.4. *Structure-Activity Relationship. General.* The CYP binding domain of CS has been characterized using quantitative immunoassay [20], and more recently by NMR spectroscopy as well as X-ray analysis of the CYP-CS complex. CYP interacts mainly

Table 2. *Synthetic Cyclosporine Analogues and Their Biological Activities*[a])

Entry	Structural modifications[b])			IL-2	CYP Binding
	residue position	residue(s) in CS	residue(s) in analogue		
1	8	D-Ala	D-Pro	1	5
2			D-*cis*-Hyp[2])	1.5	6
3			D-Cys (^{t}Bu)	170	2.3
4			D-Asn	15	1
5			D-Gln	10	1
6			Sar	10	8
7			D-MeAla	1.6	2.5
8	7	Ala	Val	150	6
9			Ser	17	3
10			Thr	17	2.6
11			Gly	7	1.5
12	5–6	Val[5]-MeLeu[6]	Leu[5]-Pro[6]	2700	20
13	5	Val	Leu	1.3	1.5
14			Thr	3	5
15			allo-Thr	17	10
16	4	MeLeu	Pro	2700	> 20
17			MeVal	2500	0.54
18			MeLeu-Leu[c])	1500	> 91
19	3	Sar	Gly	14	6
20			MeγAbu[2])[d])	2400	>100
21	2	Abu	MeAbu	87	>100
22	2–3	Abu[2]-Sar[3]	MeAbu[2]-D-MeAla[3]	62	31
23	11	MeVal	MeThr	2400	>100
24	9	MeLeu	Pro	2500	>100

[a]) The values reported are relative IC_{50} to CS, *i.e.*, IC_{50}(analogue)/IC_{50}(CS).
[b]) Variations from the structure of CS at different positions are shown.
[c]) Cyclic dodecapeptide.
[d]) A 35-membered ring structure.

[7]) The values reported are relative IC_{50} to CS, *i.e.*, IC_{50}(analogue)/IC_{50}(CS).

with residues 1–3 and 9–11 of CS, which forms a continuous surface on one side of the molecule. No interaction of CYP with residues 4–8 of CS has been observed. The latter part (residues 4–8) is expected to bind to calcineurin and is called the effector domain of CS. It is regarded that the IL-2 assay [19], compared to the activity of CS, is an indirect measurement of the *combination* of the calcineurin binding affinity and CYP binding affinity, assuming that the synthetic analogue has the same penetration capability through the cell membrane as does the parent CS molecule.

Modifications of D-*Ala at Position 8.* Bulky residues such as *S*-(*tert*-butyl)-substituted D-Cys (D-Cys (^{t}Bu)), D-Asn, D-Gln at position 8 cause a significant loss in the activity of the derivatives in the IL-2 reporter gene assay (*Table 2, Entries 3–5)*, whereas the cyclic residues such as D-Pro and D-*cis*-Hyp[2]), have no negative effects on the immunosuppressive activity (*Entries 1* and *2*). These results are consistent with the fact that D-Ala[8] is a part of the CS effector domain. Consequently, derivation at position 8 does not affect the binding to CYP very much. An *N*-methyl group in position 8 ([Sar[8]] CS and [D-MeAla[8]] CS) is not beneficial for the immunosuppressive activity (*Entries 6* and *7*), although the negative effect is relative small for the latter case ([D-MeAla[8]] CS).

Modifications of Ala at Position 7. A large substituent such as the isopropyl group in [Val[7]]CS is not tolerated and causes at 150-fold decrease in immunosuppressive activity without a significant loss of CYP binding (*Entry 8*). Introduction of an OH group in position 7 (*e.g.*, as in [Ser[7]]CS, [Thr[7]]CS) is detrimental for the immunosuppressive activity, but not for the CYP binding (*Entries 9* and *10*). A glycine residue at position 7 diminishes the immunosuppressive activity (*Entry 11*), compared to the alanine residue in the parent CS. All these results indicate that the residue 7 is also crucial in the effector function of CS, but not for the CYP binding.

Modifications of MeLeu at Position 6. A Pro residue at position 6 as in [Leu[5], Pro[6]] CS is not accepted for the immunosuppressive activity (*Entry 12*).

Modifications of Val at Position 5. There is practically no difference between a Val and a Leu residue at position 5 (*Entry 13*). A Thr residue at position 5 is tolerated with a relatively small loss in immunosuppressive activity for the [Thr[5]]CS derivative (*Entry 14*). Interestingly, an allo-Thr residue is not so well tolerated at position 5, a 17-fold decrease in immunosuppressive activity being observed for [allo-Thr[5]]CS (*Entry 15*).

Modifications of MeLeu at Position 4. A Pro residue at position 4 destroys both immunosuppressive activity and the binding affinity to CYP (*Entry 16*). This is probably due to a drastic change in the conformation of this derivative. [MeVal][4]CS is an interesting case: its binding affinity to CYP is higher than that of CS, yet the immunosuppressive activity is lost completely (*Entry 17*) [21]. The CH_2 group of MeLeu residue at position 4 is apparently very critical in the interaction with calcineurin.

Modifications of Sar at Position 3. The *N*-methyl group of the Sar residue at position 3 is important for the binding to CYP and for the immunosuppressive activity, as shown by the reduced activity of [Gly[3]]CS (*Entry 19*). The overall CS backbone seems also critical for the immunosuppressive activity: incorporation of two more C-atoms in the ring, by the MeγAbu[2]) residue at position 3, results in a complete loss of CYP binding and, therefore, in no observable immunosuppressive activity (*Entry 20*, see also *Entry 18*).

Modifications of Abu at Position 2. The NH at position 2 of CS is important for the binding to CYP. No CYP binding and no immunosuppressive activity is observed for [MeAbu^2]CS and [MeAbu^2, D-MeAla^3]CS (*Entries 21* and *22*)[8]).

Modifications at Positions 11 and 9. Position 11 of CS is very critical for binding to CYP [12d]. Replacement of a Me group by an OH group at this position (MeThr^{11}) instead of MeVal^{11}) results in a complete loss of the CYP binding and a loss of immunosuppressive activity for this derivative (*Entry 23*). At position 9, MeLeu cannot be replaced by a Pro residue without a loss of immunosuppressive activity. CYP binding is also lost with this derivative (*Entry 24*).

Experimental Part

Materials. Wang resin was purchased from *Bachem. N*-Fmoc-*N*-methylamino acids were purchased (*Sygena*) or prepared following [23]. Boc-MeBmt-OH was obtained from CS *via* regioselective ring opening between residues 11 and 1, or 1 and 2, followed by selective scission of the MeBmt residue [10].

Anchoring of Fmoc-MeVal-OH on Wang *Resin.* To a soln. of Fmoc-MeVal-OH (4.04 g, 11.44 mmol), DCC[6]) (2.36 g, 11.44 mmol), HOBt[6]) (586 mg, 3.8 mmol) and DMAP[6]) (466 mg, 3.8 mmol) in CH_2Cl_2/dimethylformamide (DMF) 4:1 (*v*/*v*; 85 ml), *Wang* resin (0.76 mmol/g, 5.7 g, 3.8 mmol) was added. The mixture was shaken at 4° overnight. The resin was filtered, washed successively with DMF (3 ×), MeOH (3 ×), CH_2Cl_2 (2 ×), MeOH (2 ×), CH_2Cl_2 (2 ×), and MeOH (3 ×), and dried under vacuum: 6.77 g of Fmoc-MeVal-*Wang* resin, indicating *ca.* 80% anchoring. A small amount (9.0 mg) of the anchored resin was treated with 50% CF_3COOH in CH_2Cl_2 and the soln. analyzed by HPLC: comparison with authentic Fmoc-MeVal-OH indicated 77% substitution of the resin.

Solid-Phase Peptide Couplings: General Procedure. Starting from Fmoc-MeVal^{11}-*Wang* resin, Fmoc deprotection was performed with 20% piperidine in DMF (18 ml/mmol) for 10 min. The resin was filtered and washed with DMF (10 ×, each with 18 ml for 1 min). In the meantime, the next (residue 10) amino acid residue (*e.g.*, Fmoc-MeLeu-OH; 6 mol-equiv.) was treated with DCC (3 mol-equiv.) in CH_2Cl_2/DMF 3:1 (*v*/*v*; 18 ml/mmol) at 0° for 1 h. Resulting dicyclohexylurea was filtered off, and the filtrate containing Fmoc-amino acid symmetric anhydride (3 mol-equiv.) was added to the resin. An aliquot (24 µl) of bromophenol-blue soln. (0.04M in DMF) was added. The coupling was performed for 1.5 h at r.t. The resin was filtered and washed successively with DMF (10 × 18 ml, 1 min each), MeOH (5 × 18 ml, 1 min each), and DMF (5 × 18 ml, 1 min each). If necessary, recoupling was performed with further 3 mol-equiv. of symmetric anhydride. Fmoc Deprotection, washings, symmetric-anhydride couplings, and washings continued as described above. Finally, the residue 1 (MeBmt) was coupled using Boc-MeBmt symmetric anhydride. The resin was washed with DMF and MeOH as described above and then dried under vacuum.

Synthesis of [D-Pro^8]-Cyclosporine: Solid-Phase Peptide Couplings. Starting with Fmoc-MeVal-*Wang* resin (0.58 mmol/g, 1.73 g, 1 mmol), solid-phase peptide couplings proceeded following the *General Procedure* with Fmoc-MeLeu (residue 10, coupling repeated), then with Fmoc-MeLeu (residue 9, coupling repeated). The washed and dried resin weighed 1.943 g, indicating *ca.* 84% overall yield. The resin was divided in two parts, and the couplings continued with one half (0.97 g) using the following amino-acid anhydride derivatives successively: Fmoc-D-Pro (residue 8); Fmoc-Ala (residue 7); Fmoc-MeLeu (residue 6); Fmoc-Val (residue 5, coupling repeated); Fmoc-MeLeu (residue 4); Fmoc-Sar (residue 3); Fmoc-Abu (residue 2); Boc-MeBmt (residue 1). The washed and dried resin weighed 1.300 g. The weight gain of 0.33 g for the eight coupling steps reflected *ca.* 90% overall yield.

Boc-Deprotection and Cleavage off the Resin. The resin was treated with CF_3COOH in CH_2Cl_2 1:1 (*v*/*v*; 250 ml) at r.t. for 1 h. The resin was filtered off and washed with CH_2Cl_2 (500 ml). The combined filtrate was cooled in an ice bath and neutralized with 1N $NaHCO_3$ till pH 7. In total, *ca.* 1.6 l of the aq. $NaHCO_3$ soln. was needed. The aq. phase was extracted with CH_2Cl_2 (1 l) and the combined org. phase dried (Na_2SO_4) and evaporated to yield the crude linear 1,11-seco[D-Pro^8]cyclosporine as a solid (579 mg, *ca.* 93% crude yield). FAB-MS: 1246 (MH^+).

---

[8]) The introduction of a D-MeAla residue at position 3 of CS has a stabilizing effect on the bioactive CS conformation [22].

Cyclization to [D-Pro⁸]-Cyclosporine. The linear 1,11-seco[D-Pro⁸]cyclosporine (569 mg, *ca.* 0.45 mmol) was dissolved in CH_2Cl_2 (1.85 l). *Castro*'s reagent (634 mg, 1.37 mmol) and *N*-methylmorpholin (161 mg, 1.59 mmol) were added. The soln. was stirred at r.t. for 5 days, the solvent evaporated, and the residue purified by flash column chromatography (silica gel, 3% MeOH/AcOEt) to yield successively [D-Pro⁸, D-MeVal[11]]cyclosporine (141 mg, 25 %; $[\alpha]_D = -129.6$ ($c = 0.398$, $CHCl_3$)) and [D-Pro⁸]cyclosporine (236 mg, 42%; *Entry 1*, *Table 1*). $[\alpha]_D = -210.4$ ($c = 0.451$, $CHCl_3$). FAB-MS: 1228 (MH^+).

We gratefully acknowledge collaborations with our colleagues at *Sandoz* throughout this project: Drs. *F. Bobe*, *F. Cardinaux*, and *T. Petcher* for valuable discussions during the preliminary studies, Drs. *G. Zenke* and *V. Quesniaux* for performing *in vitro* assays, Dr. *C. Dalvit* and Mrs. *M. Ponelle* for the NMR analysis, and Mr. *J. Oetterli* for technical support.

REFERENCES

[1] Reviews: 'Progress in Allergy', Series Eds. K. Ishizaka, P. J. Lachmann, P. Kallós, and B. H. Waksman, Ed. J. F. Borel, Karger, Basel, 1986, Vol. 38; R. M. Wenger, 'Progress in the Chemistry of Organic Natural Products', Eds. W. Herz, H. Griesbach, G. W. Kirby, and C. Tamm, Springer-Verlag, Wien-New York, 1986, Vol. 50, p. 124; J. F. Borel, G. Baumann, I. Chapman, P. Donatsch, A. Fahr, E. A. Mueller, J. M. Vigouret, *Adv. Pharmacol.* **1996**, *50*, 115; J. F. Borel, Z. L. Kis, T. Beveridge, in 'The History of the Discovery and Development of Cyclosporine (*Sandimune*®): The Search for Anti-Inflammatory Drugs', Eds. V. J. Merluzzi and J. Adams, Birkhäuser, Boston, 1995, p. 27–63.

[2] A. Rüegger, M. Kuhn, H. Lichti, H. R. Loosli, R. Huguenin, C. Quiquerez, A. von Wartburg, *Helv. Chim. Acta* **1976**, *59*, 1075; T. J. Petcher, H. P. Weber, A. Rüegger, *ibid.* **1976**, *59*, 1480; H. Kessler, H. R. Loosli, H. Oschkinat, *ibid.* **1985**, *68*, 661; H. R. Loosli, H. Kessler, H. Oschkinat, H. P. Weber, T. J. Petcher, A. Widmer, *ibid.* **1985**, *68*, 682; H. Kessler, *ibid.* **1990**, *73*, 1818.

[3] M. Dreyfuss, E. Haerri, H. Hofmann, H. Kobel, W. Pache, H. Tscherter, *Eur. J. Appl. Microbiol.* **1976**, *3*, 125; H. Kobel, R. Traber, *ibid.* **1982**, *14*, 237.

[4] a) G. Baumann, G. Zenke, R. M. Wenger, P. Hiestand, V. Quesniaux, E. Anderson, M. H. Schreier, *J. Autoimmunity* **1992**, *5* (Suppl A), 67; b) J. Liu, *Trends Pharm. Si.* **1993**, *14*, 182.

[5] S. L. Schreiber, G. R. Crabtree, *Immunol. Today* **1992**, *13*, 136; S. L. Schreiber, M. W. Albers, E. J. Brown, *Acc. Chem. Res.* **1993**, *26*, 412.

[6] C. Weber, G. Wider, B. von Freiberg, R. Traber, W. Braun, H. Widmer, K. Wüthrich, *Biochemistry* **1991**, *30*, 6563; S. W. Fesik, R. T. Gampe, H. L. Eaton, G. Gemmercker, E. T. Olejniczak, P. Neri, D. A. Egan, R. Edalji, R. Simmer, R. Helfrich, J. Hochlowski, M. Jackson, *ibid.* **1991**, *30*, 6574; Y. Theriault, T. M. Logan, R. Meadows, L. Yu, E. T. Olejniczak, T. F. Holzman, R. L. Simmer, S. W. Fesik, *Nature (London)* **1993**, *261*, 88; G. Pflügel, J. Kallen, T. Schirmer, J. N. Jansonius, M. G. Zurini, M. D. Walkinshaw, *ibid.* **1993**, *361*, 91; V. Mikol, J. Kallen, G. Pflügl, M. D. Walkinshaw, *J. Mol. Biol.* **1993**, *234*, 1119; W. Braun, J. Kallen, V. Mikol, M. D. Walkinshaw, K. Wüthrich, *FASEB J.* **1985**, *9*, 63.

[7] R. Traber, M. Kuhn, A. Rüegger, H. Lichti, H. R. Loosli, A. von Wartburg, *Helv. Chim. Acta* **1977**, *60*, 1247; R. Traber, M. Kuhn, H. R. Loosli, W. Pache, A. von Wartburg, *ibid.* **1977**, *60*, 1568; R. Traber, H. R. Loosli, H. Hofmann, M. Kuhn, A. von Wartburg, *ibid.* **1982**, *65*, 1655; R. Traber, H. Hofmann, H. R. Loosli, M. Ponelle, A. von Wartburg, *ibid.* **1987**, *70*, 13.

[8] G. Maurer, H. R. Loosli, E. Schreier, B. Keller, *Drug Metab. Dispos.* **1984**, *12*, 120, R. M. Wenger, *Transplant. Proc.* **1990**, *22*, 1104; B. D. Kahn, *ibid.* **1990**, *22*, 1357; R. M. Wenger, *Chimia* **1992**, *46*, 314.

[9] D. Seebach, *Angew. Chem. Int. Ed.* **1988**, *27*, 1624; D. Seebach, A. K. Beck, H. G. Bossler, C. Gerber, S. Y. Ko, C. W. Murtiashaw, R. Naef, A. I. Shoda, A. Thaler, M. Krieger, R. Wenger, *Helv. Chim. Acta* **1993**, *76*, 1564.

[10] R. M. Wenger, in Peptides: 'Proceedings of the 24th Symposium of the European Peptide Society', Edinburgh, 8–13 September 1996, Mayflower Scientific Ltd., in press.

[11] M. K. Eberle, A.-M. Jutzi-Eme, F. Nuninger, *J. Org. Chem.* **1994**, *59*, 7249.

[12] R. M. Wenger, *Helv. Chim. Acta* **1983**, *66*, 2672; R. M. Wenger, *ibid.* **1984**, *67*, 502; R. M. Wenger, *Angew. Chem. Int. Ed.* **1985**, *24*, 77; R. M. Wenger, *Transplant. Proc.* **1986**, *18* (No. 6, Suppl. 5), 213; D. H. Rich, M. K. Dhaon, B. Dunlap, S. P. F. Miller, *J. Med. Chem.* **1986**, *29*, 978; W. J. Colucci, R. D. Tung, J. A. Petri, D. H. Rich, *J. Org. Chem.* **1990**, *55*, 2895.

[13] M. A. Yvonne, T. L. Thomas, G. R. Flentke, D. H. Rich, *J. Am. Chem. Soc.* **1995**, *117*, 7279.

[14] I. J. Galpin, A. Karim, A. Mohammed, A. Patel, *Tetrahedron Lett.* **1987**, *28*, 6517; I. J. Galpin, A. Mohammed, A. Patel, G. Priesley, *Tetrahedron* **1988**, *44*, 1763; I. J. Galpin, A. Mohammed, A. Patel, *ibid.* **1988**, *44*, 1773; *ibid.* **1988**, *44*, 1783.

[15] B. Castro, J. R. Dormoy, G. Evin, C. Selve, *Tetrahedron Lett.* **1975**, 1219; B. Castro, G. Evin, C. Selve, R. Seyer, *Synthesis* **1977**, 413; B. Castro, J. R. Dormoy, G. Evin, C. Selve, *J. Chem. Res. (S)* **1977**, 182.
[16] S. S. Wang, *J. Am. Chem. Soc.* **1973**, *95*, 1328.
[17] A. Grandas, X. Jorba, E. Giralt, E. Pedroso, *Int. J. Pept. Protein Res.* **1989**, *33*, 386.
[18] V. Krchňák, J. Vágner, P. Safár, M. Lebl, *Collect. Czech. Chem. Commun.* **1988**, *53*, 2542.
[19] P. S. Mattila, K. S. Ullman, S. Fiering, E. A. Emmel, M. McCutcheon, G. R. Crabtree, L. A. Herzenberg, *EMBO J.* **1990**, *9*, 4425; see also [4a].
[20] V. Quesniaux, M. H. Schreier, R. M. Wenger, P. C. Hiestand, M. W. Harding, M. H. V. van Regenmortel, *Eur. J. Immunol.* **1987**, *17*, 1359.
[21] G. Zenke, G. Baumann, R. M. Wenger, P. Hiestand, V. Quesniaux, E. Anderson, M. H. Schreier, *Ann. N.Y. Acad. Sci.* **1993**, 331.
[22] R. M. Wenger, J. France, G. Bovermann, L. Walliser, A. Widmer, H. Widmer, *FEBS Lett.* **1994**, *340*, 255.
[23] R. M. Freidinger, J. S. Hinkle, D. S. Perlow, B. H. Arison, *J. Org. Chem.* **1983**, *48*, 77.