Cyclosporins from *Mycelium sterilae* MS 2929

Marek Kuzma,[†] Petr Sedmera,*[,†] Alexandr Jegorov,[‡] and Vladimír Havlíček[†]

Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague, Czech Republic, and TAPI Galena, IVAX Pharmaceuticals, Research and Development, 370 05 České Budějovice, Czech Republic

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The structures of two new cyclosporins were elucidated by NMR and MS methods as *cyclo*[-MeBmt[1]-Abu[2]-Sar[3]-MeLeu[4]-Val[5]-MeLeu[6]-Ala[7]-D-Ala[8]-MeLeu[9]-MeNva[10]-MeVal[11]-] and *cyclo*[-MeBmt[1]-Abu[2]-Sar[3]-MeLeu[4]-Abu[5]-MeLeu[6]-Ala[7]-D-Ala[8]-MeLeu[9]-MeLeu[10]-MeVal[11]-].

The cyclosporins are a well-known family of cyclic peptides due to the use of their best known representative, cyclosporin A (**1**), as an immunosuppressant.[1] Cyclosporin A is used also for the treatment of various autoimmune diseases.[2−5] Some other biological activities of its derivatives, e.g., anti-HIV,[6,7] antiparasitic,[8] or multiple drug resistance modifying properties,[9] are currently under investigation. Besides their biological activity, cyclosporins are remarkable also by their ability to exist in numerous crystal forms.[10]

Cyclosporins are nonpolar, lipophilic cyclic undecapeptides of fungal origin. Because of their extraribosomal biosynthesis,[11] they contain several nonproteinogenic amino acids [e.g., (2*S*,3*R*,4*R*,6*E*)-3-hydroxy-4-methyl-2-methylamino-6-octenoic acid, MeBmt; aminobutyric acid, Abu; methylamino-octanoic acid; norvaline, Nva]. Thus far, about 32 natural cyclosporins have been reported.[12] One postsynthetic natural modification leading to peroxocyclosporins was also described.[13] In addition, 42 various cyclosporin analogues were synthetized *in vitro*; some of these were identical to compounds isolated from fungal cultures, and some others contained unnatural synthetic amino acids.[14−17] Many modified cyclosporins and cyclosporin derivatives were prepared, tested, and examined by various physicochemical methods.[18−23] Except for cyclosporin H [D-MeVal[11]]CsA,[24] all constituent amino acids in positions 1 to 7 and 9 to 11 belong to the L-series. The eighth position is occupied by D-alanine or, alternatively, by other D-amino acids incorporated *in vitro*. Five to seven residues in these compounds are *N*-methylated. The amino acid numbering starts with the largest, MeBmt (Figure 1), and Greek letters are used for atoms within the individual amino acids.

Various cyclosporin-producing fungi have apparently different spectra of analogues than the originally described *Tolypocladium inflatum*. Examples include cyclosporins [MeLeu[1]]CsA, [Leu[4]]CsA, [Ile[4]]CsA, [Leu[5]]CsA, and [Leu[9]]CsA by *T. terricola*,[25−27] [Ala[2], Val[11]]CsA by *Mycelium sterilae* MS 2929,[28] [Thr[2], Leu[5], Leu[10]]CsA by *Stachybotrys chartarum*,[29] [Thr[2], Leu[5], Ala[10]]CsA by *Acremonium luzulae* (Fuckel) W Gams,[30] and [Thr[2], Ile[5]]CsA by *Leptostroma* anamorph of *Hypoderma eucalyptii* Cooke and Harkn.[30] Cyclosporin analogues, e.g., SDZ 214-103 = [Thr[2], Leu[5], D-hydroxyisovaleric acid[8], Leu[10]]CsA, were produced also by the fungus *Cylindrotrichum oligospermum* (Corda), but by a different megasynthetase.[31]

Mother liquors from the large-scale fermentations of various producers represent a rich source of new cyclosporins.[35] In our case, a detailed HPLC examination of one fraction suspected to contain cyclosporin T showed the presence of three compounds, **2**−**4**. These compounds were isobaric and exhibited pseudomolecular peaks $[M + H]^+$ at *m*/*z* 1188.8 (ESI, Table 1) that were 14 mass units lower than cyclosporin A (**1**). Several known cyclosporins are possible candidates, e.g., CsB, CsE, CsL, CsP, CsT, CsU, CsW, [Leu[4]]CsA, or [Ile[4]]CsA. The product ion MS of protonated molecules of compounds **1**−**4** observed in a collision-induced dissociation (CID) experiment (Table 1) are relatively simple. Diagnostic are the *N*-terminal *b*-type ions formed by primary ring cleavage between amino acid residues 3−2, 1−11, and 6−5 (Figure 1).[32,33] The same masses of b_7^{3-2} ions in **1** and **2** indicate that the "missing" CH_2 is located in the 10th amino acid. The ^1H and ^{13}C NMR spectra indicated that this compound belongs to monodemethylcyclosporins (five *N*-H, six *N*-methyls) composed of Abu, 2 × Ala, Val, Leu, Sar, 3 × MeLeu, MeVal, and MeBmt (COSY, TOCSY). Therefore, it has the same amino acid composition as cyclosporin T. Comparison of ^{13}C NMR chemical shifts with those published[35] showed a good agreement. Nevertheless, the sequence established by HMBC and ROESY was found identical with CsT: *cyclo*[-MeBmt[1]-Abu[2]-Sar[3]-MeLeu[4]-Val[5]-MeLeu[6]-Ala[7]-D-Ala[8]-MeLeu[9]-Leu[10]-MeVal[11]-]; for ^1H NMR parameters see the Experimental Section.

The application of the same procedure to compound **3** also indicated a change at the 10th position. The examination of consecutive losses (the mass differences between the fragments within the same series) also clearly shows both intact parts and modified acids. However, these deductions remain ambiguous, as MS alone cannot differentiate between the replacement with a homological amino acid and *N*-demethylation. Seven *N*-methyls and four *N*-H's inferred from the NMR spectra place this compound in the "normal" series. The amino acid composition determined by COSY and TOCSY (Table 2) was Abu, 2 × Ala, Val, Sar, 3 × MeLeu, MeVal, MeBmt, and $-N(Me)CHCH_2CH_2CH_3$, (i.e., MeNva). The amino acid sequence was determined by HMBC (the couplings of *N*-H to carbonyls of the preceding amino acids, the couplings of *N*-Me to those carbonyls, and H-α and the carbonyl of the preceding amino acid, Table 2) and checked by ROESY (NOE between *N*-H and H-α or between *N*-methyl and H-α). A cross-peak between H-9α and H-10α indicates a *cis* peptidic bond between these amino acids. Thus, the sequence *cyclo*[-MeBmt[1]-Abu[2]-Sar[3]-MeLeu[4]-Val[5]-MeLeu[6]-Ala[7]-D-Ala[8]-MeLeu[9]-MeNva[10]-MeVal[11]-] was obtained.

Using a similar approach (identical masses of b_4^{1-11} ions in **1** and **4**), we determined the affected position as the fifth amino acid. This compound contains seven *N*-methyls and four *N*-H's so that it is also a member of the "normal" series. The amino acid composition determined by COSY and TOCSY (Table 3) was 2 × Abu, 2 × Ala, Sar, MeBmt, MeVal, 4 × MeLeu, i.e., indeed a new cyclosporin. Diagnostic peaks in HMBC 2-NH → 1-CO, 7-NH → 6-CO, 5-NH → 4-CO, 8-NH → 7-CO, 1-Me → 11-CO, 3-Me → 2-CO, 6-Me → 5-CO, 4-Me → 3-CO, 9-Me → 8-CO, 11-Me → 10-CO, and 10-Me → 9-CO lead unambiguously to the sequence *cyclo*[-MeBmt[1]-Abu[2]-Sar[3]-MeLeu[4]-Abu[5]-MeLeu[6]-Ala[7]-D-Ala[8]-MeLeu[9]-MeLeu[10]-MeVal[11]-]. The same conclusion was derived from

* Corresponding author. Phone: +420 296 442 646. Fax: +420 296 442 509. E-mail: sedmera@biomed.cas.cz.
[†] Institute of Microbiology.
[‡] R&D České Budějovice.

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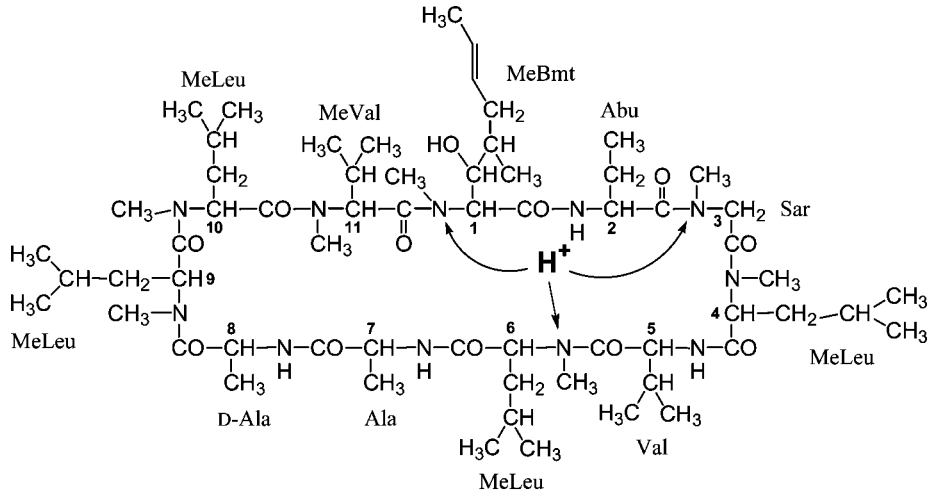

CsA *cyclo*[-MeBmt[1]-Abu[2]-Sar[3]-MeLeu[4]-Val[5]-MeLeu[6]-Ala[7]-D-Ala[8]-MeLeu[9]-MeLeu[10]-MeVal[11]-]

CsT (**2**) *cyclo*[-MeBmt[1]-Abu[2]-Sar[3]-MeLeu[4]-Val[5]-MeLeu[6]-Ala[7]-D-Ala[8]-MeLeu[9]-Leu[10]-MeVal[11]-]

3 *cyclo*[-MeBmt[1]-Abu[2]-Sar[3]-MeLeu[4]-Val[5]-MeLeu[6]-Ala[7]-D-Ala[8]-MeLeu[9]-MeNva[10]-MeVal[11]-]

4 *cyclo*[-MeBmt[1]-Abu[2]-Sar[3]-MeLeu[4]-Abu[5]-MeLeu[6]-Ala[7]-D-Ala[8]-MeLeu[9]-MeLeu[10]-MeVal[11]-]

Figure 1. Structure of cyclosporin A indicating amino acid numbering and sites of primary protonation. Protonation of amidic nitrogen atoms leads to amide bond cleavage.

Table 1. Product Ion Mass Spectra of Protonated Molecules of Compounds **1**−**4** Observed in the Collision-Induced Dissociation (CID) Experiments

	1		**2**		**3**		**4**	
	CsA		[Leu[10]]CsA		[MeNva[10]]CsA		[Abu[5]]CsA	
ion type	*m/z*	loss	*m/z*	loss	*m/z*	loss	*m/z*	loss
$[M + H]^+$	1202.8		1188.8		1188.8		1188.8	
$[M + Na]^+$	1224.8		1210.8		1210.8		1210.8	
b_9^{3-2}	934.7	268.2	920.7	268.1	920.8	268.0	920.5	268.3
b_8^{3-2}	821.6	113.1	807.5	113.2	807.6	113.2	807.4	113.0
b_7^{3-2}	694.5	127.1	694.5	113.0	694.5	113.1	680.2	127.1
b_6^{3-2}	567.4	127.1	567.4	127.1	567.4	127.1	553.2	127.0
b_5^{3-2}	496.3	71.1	496.3	71.1	496.3	71.1	482.2	71.0
b_4^{3-2}	425.3	71.0	425.3	71.0	425.3	71.0	411.0	71.2
$[b_{10}\text{-}18]^{1-11}$	1071.7	131.1	1057.9	130.9	1057.7	131.1	1057.6	131.2
$[b_9\text{-}18]^{1-11}$	944.7	127.0	944.7	113.2	944.7	113.0	930.5	127.1
$[b_8\text{-}18]^{1-11}$	817.6	127.1	817.6	127.1	817.6	127.1	803.4	127.1
$[b_6\text{-}18]^{1-11}$	675.5	142.1	675.5	142.1	675.5	142.1	661.3	142.1
$[b_5\text{-}18]^{1-11}$	548.4	127.1	548.3	127.2	548.3	127.2	534.1	127.2
$[b_4\text{-}18]^{1-11}$	449.3	99.1	449.3	99.0	449.3	99.0	449.1	85.0
b_6^{6-5}	637.5		623.5		623.5		637.2	
b_5^{6-5}	524.4	113.1	510.3	113.2	510.3	113.2	524.2	113.0
b_4^{6-5}	397.3	127.1	397.3	113.0	397.2	113.1	397.1	127.1

a ROESY experiment in which the following cross-peaks were observed: 2-NH vs H-1α, 7-NH vs H-6α, 5-NH vs H-4α, 8-NH vs H-7α; 1-Me vs H-11α, 3-Me vs H-2α, 6-Me vs H-5α, 4-Me vs H-3α_d, 9-Me vs H-8α, 11-Me vs H-10α.

The cross-peaks between H-9α and H-10α, commonly observed in the NOESY or ROESY spectra of cyclosporins, were found with **3** and **4**; that is, all peptidic bonds in these molecules are *trans,* except the 9−10 bond, which is *cis*. Such a cross-peak was absent in the spectra of **2**, but an H-3α vs H-4α was observed, indicating that the 3−4 peptidic bond is *cis*.

The naturally occurring cyclosporins differ from the CsA sequence in positions 1, 2, 4, 5, and 7.[31] Aminobutyric acid is widely distributed in cyclosporins, mostly in position 2, once its occurrence in position 4 (cyclosporin V) is known. Our new [Abu[5]]CsA (**4**) contains this amino acid as the fifth one (usually a nonmethylated position), and it is the third reported natural variation at this position ([Nva[5]]CsA = cyclosporin M[32] and [Leu[5]]CsA[26]), in addition to aIle, Ile, and cyclopropylglycine, which were incorporated at this position *in vitro*.[11]

Norvaline has been found several times in position 2 (cyclosporins G, M, N, O, X, Y) and once in position 5 (cyclosporin M);[32] however, it has never been found *N*-methylated. [MeNva[10]]CsA (**3**) containing MeNva in position 10 is the first representative of this type. Interestingly, variation in position 10 is rare also outside the genus *Tolypocladium*, and only nonmethylated Leu or Ala is reported.[29,30] The structures of both new cyclosporins further extend the limits of component combination possible in this family of natural compounds.

Experimental Section

General Experimental Procedures. Positive-ion ESI mass spectra were recorded on a Finnigan LCQ-DECA quadrupole ion trap mass spectrometer (Finnigan MAT, San Jose, CA) equipped with the Finnigan API-II ion source. The ionizer and ion transfer optics parameters of the ion trap were as follows: spray voltage 5500 V, capillary temperature

Table 2. NMR Data for Compound **3** (399.90 and 100.55 MHz, $CDCl_3$, 30 °C)

residue	atom[a]	δ_C	δ_H	HMBC
MeBmt	1-Me	33.99	3.519 (3 H, s)	1α,11-CO
	1α	59.08	5.471 (1 H, d, J = 6.1 Hz)	1-Me,1β,1-CO,11-CO
	1β	74.90	3.777 (1 H, dd, J = 6.9, 6.1 Hz)	
	1γ	36.06	1.625 (1 H, m)	1γ,1δ
	1γ-Me	17.03	0.698 (3 H, d, J = 6.2 Hz)	
	1δ	35.70	2.420 (1 H, m)	1η,1γ-Me
			1.646 (1 H, m)	
	1ε	129.78	5.362	(1 H, m)
	1η	126.14	5.338 (1 H, m)	1δ
	1ω	17.83	1.619 (3 H, dd, J = 4.5, 1.4 Hz)	1ε,1η
	1-CO	170.40		
Abu	2-NH		8.022 (1 H, d, J = 9.9 Hz)	1-CO
	2α	47.01	5.119 (1 H, ddd, J = 9.9,7.4, 7.4 Hz)	1-CO,2-CO
	2β	18.62	1.618 (1 H, m)	2α,2γ,2-CO
			1.274 (1 H, m)	2α,2γ,2-CO
	2γ	13.91	0.875 (3 H, t, J = 7.3 Hz)	2β
	2-CO	174.00		
Sar	3-Me	39.50	3.396 (3H, s)	2-CO,3α
	3α	50.31	4.717 (1 H, d, J = 13.9 Hz)	3-Me,3-CO
			3.186 (1 H, d, J = 13.9 Hz)	3-Me,3-CO
	3-CO	171.30		
MeLeu	4-Me	31.33	3.105 (3 H, s)	3-CO, 4α
	4α	55.52	5.335 (1 H, dd, J = 6.5, 4.2 Hz)	3-CO,4-Me,4-CO
	4β	36.06	1.999 (1 H, m)	
			1.594 (1 H, m)	
	4γ	24.90	1.431 (1 H, m)	
	4δ_u	21.17	0.875 (3 H, d, J = 6.5 Hz)	4α,4γ,4δ_d
	4δ_d	23.41	0.941 (3 H, d, J = 6.7 Hz)	4α,4γ,4δ_u
	4-CO	170.02		
Val	5-NH		7.451 (1 H, d, J = 8.4 Hz)	4-CO
	5α	55.41	4.680 (1 H, dd, J = 9.6, 8.4 Hz)	4-CO,5β,5-CO
	5β	31.22	2.452 (1 H, m)	5γ_d, 5γ_u
	5γ_u	18.32	0.888 (3 H, d, J = 6.9 Hz)	5α,5β,5γ_d
	5γ_d	19.86	1.076 (3 H, d, J = 6.5)	5α,5β,5γ_u
	5-CO	173.99		
MeLeu	6-Me	31.54	3.265 (3 H, s)	5-CO,6α
	6α	55.52	4.936 (1 H, dd, J = 10.0, 5.9 Hz)	5-CO,6-Me,6-CO
	6β	37.54	2.126 (1 H, m)	
			1.360 (1 H, m)	
	6γ	25.19	1.808 (1 H, m)	
	6δ_u	21.98	0.808 (3 H, d, J = 6.5 Hz)	6β,6γ,6δ_d
	6δ_d	23.54	0.950 (3 H, d, J = 6.5 Hz)	6β,6γ,6δ_u
	6-CO	171.66		
Ala	7-NH		7.720 (1 H, d, J = 7.6 Hz)	6-CO
	7α	48.41	4.533 (1 H, dq, J = 7.6,7.3 Hz)	6,CO,7β,7-CO
	7β	15.69	1.338 (3 H, d, J = 7.3 Hz)	7α,7-CO
	7-CO	171.15		
Ala	8-NH		7.210 (1 H, d, J = 8.1 Hz)	7-CO
	8α	45.09	4.814 (1 H, dq, J = 8.1, 6.9 Hz)	7-CO,8β,8-CO
	8β	18.10	1.260 (3 H, d, J = 6.9 Hz)	8α,8-CO
	8-CO	173.46		
MeLeu	9-Me	29.42	3.091 (3 H, s)	8-CO,9α
	9α	48.21	5.738 (1 H, dd, J = 10.9, 4.1 Hz)	8-CO,9-Me,9-CO
	9β	38.92	2.057 (1 H, m)	
			1.287 (1 H, m)	
	9γ	24.58	1.323 (1 H, m)	
	9δ_u	21.63	0.886 (3 H, d, J = 6.0 Hz)	9β,9γ,9δ_d
	9δ_d	23.54	0.952 (3 H, d, J = 6.2 Hz)	9β,9γ,9δ_u
	9-CO	170.58		
MeNva	10-Me	29.55	2.695 (3 H, s)	9-CO,10α
	10α	58.99	5.000 (1 H, dd, J = 7.0, 6.8 Hz)	9-CO,10-Me,10-CO
	10β	33.44	2.010 (1 H, m)	
			1.518 (1 H, m)	
	10γ	19.66	1.369 (1 H, m)	
			1.259 (1 H, m)	
	10δ	14.36	0.988 (3 H, t, J = 7.2 Hz)	10β,10γ
	10-CO	170.26		
MeVal	11-Me	29.90	2.692 (3 H, s)	1o-CO,11α
	11α	58.14	5.079 (1 H, d, J = 11.0 Hz)	10-CO, 11β,11γ_d, 11γ_u,11-Me,11-CO
	11β	29.15	2.142 (1 H, ddq, J = 11.0, 6.7, 6.5 Hz)	
	11γ_u	20.42	0.864 (3 H, d, J = 6.5 Hz)	11β,11γ_d
	11γ_d	18.62	1.035 (3 H, d, J = 6.7 Hz)	11β,11γ_u
	11-CO	173.77		

[a] Symbols d, u (downfield, upfield) were assigned according to proton resonances.

Table 3. NMR Data for Compound **4** (399.90 and 100.55 MHz, $CDCl_3$, 30 °C)

residue	atom[a]	δ_C[b]	δ_H	HMBC
MeBmt	1-Me	34.5	3.542 (3 H, s)	1α,1-CO,11-CO
	1α	59.3	5.556 (1 H, d, J = 5.1 Hz)	1-Me,11-CO
	1β	75.4	3.814 (1 H, dd, J = 8.9, 5.1 Hz)	
	1γ	36.8	1.601 (1 H, m)	1β
	1γ-Me	16.3	0.609 (3 H, d, J = 6.4 Hz)	1β,1γ,1δ
	1δ	36.7	2.554 (1 H, m)	1γ
			1.585 (1 H, m)	
	1ε	129.8	5.384 (1 H, dt, J = 15.1, 5.9 Hz)	
	1η	126.2	5.324 (1 H, dq, J = 15.1, 5.0 Hz)	
	1ω	17.8	1.630 (3 H, d, J = 5.0 Hz)	1δ,1ε,1η
	1-CO	170.6		
Abu	2-NH		7.857 (1 H, d, J = 9.9 Hz)	1-CO
	2α	48.8	5.029 (1 H, ddd, J = 9.9, 7.3, 7.3 Hz)	1-CO,2-CO
	2β	25.0	1.705 (1 H, ddq, J = 13.5,7.3,7.3 Hz)	2α,2γ,2-CO
			1.558(1 H, ddq, J = 13.5,7.3,7.3 Hz)	2α,2γ,2-CO
	2γ	9.9	0.845 (3 H, t, J = 7.3 Hz)	2β
	2-CO	174.2		
Sar	3-Me	39.7	3.395 (3 H, s)	2-CO,3α
	3α	50.4	4.769 (1H, d, J = 14.0 Hz)	3-Me,3-CO
			3.211 (1 H, d, J = 14.0 Hz)	3-Me,3-CO
	3-CO	171.6	-	
MeLeu	4-Me	31.0	3.110 (3 H, s)	3-CO,4α,4-CO
	4α	55.6	5.358 (1 H, dd, J = 8.0, 7.6 Hz)	4-Me,4-CO
	4β	36.0	2.003 (1 H, m)	
			1.451 (1 H, m)	
	4γ	24.6	1.291 (1 H, m)	
	4δ_u	21.1	0.875 (3H, d, J = 6.6 Hz)	4β,4γ,4δ_d
	4δ_d	23.4	0.945 (3 H, d, J = 6.7 Hz)	4β,4γ,4δ_u
	4-CO	169.9		
Abu	5-NH		7.336 (1 H, d, J = 8.4 Hz)	4-CO
	5α	51.9	4.712 (1 H, dd, J = 10.1,8.4,6.5 Hz)	5-CO
	5β	24.1	2.004 (1 H, m)	
			1.805 (1 H, m)	
	5γ	11.2	1.051 (3 H, d, J = 7.2 Hz)	5α,5β
	5-CO	173.9		
MeLeu	6-Me	31.1	3.249 (3 H, s)	5-CO,6α
	6α	55.6	4.900 (1 H, dd, J = 7.6, 7.6 Hz)	5-CO,6-Me, 6β,6-CO
	6β	38.1	1.991 (1 H, m)	6-CO
			1.712 (1 H, m)	
	6γ	25.8	1.273 (1 H, m)	
	6δ_u	21.8	0.882 (3 H, d, J = 6.4 Hz)	6β,6γ,6δ_d
	6δ_d	23.8	0.916 (3 H, d, J = 6.4 Hz)	6β,6γ,6δ_u
		172.2		
Ala	7-NH		7.622 (1 H, d, J = 8.2 Hz)	6-CO
	7α	48.3	4.629 (1 H, dq, J = 8.2, 7.3 Hz)	6-CO,7β,7-CO
	7β	15.9	1.353 (3 H, d, J = 7.3 Hz)	7α,7-CO
	7-CO	171.1		
Ala	8-NH		7.063 (1 H, d, J = 8.1 Hz)	7-CO
	8α	45.2	4.829 (1 H, dq, J = 8.1, 6.8 Hz)	7-CO,8β,8-CO
	8β	18.4	1.256 (3 H, d, J = 6.8 Hz)	8α,8-CO
	8-CO	173.4		
MeLeu	9-Me	29.4	3.071 (3 H, s)	8-CO,9α
	9α	48.3	5.721 (1 H, dd, J = 10.9, 4.5 Hz)	9-Me
	9β	39.0	2.093 (1 H, m)	
			1.282 (1 H, m)	9β,9γ,
	9γ	24.8	1.328 (1 H, m)	
	9δ_u	21.8	0.899 (3 H, d, J = 6.3 Hz)	9β,9γ,9δ_d
	9δ_d	25.5	0.965 (3 H, d, J = 6.3 Hz)	9β,9γ,9δ_u
	9-CO	170.0		10-Me
MeNva	10-Me	29.7	2.701 (3 H, s)	9-CO,10α
	10α	57.7	4.718 (1 H, dd, J = 11.0, 8.1 Hz)	10-Me,10-CO
	10β	40.8	2.135 (1 H, m)	
			1.200 (1 H, m)	10β,10γ,
	10γ	24.6	1.834 (1 H, m)	
	10δ_u	23.2	0.998 (3 H, d, J = 6.7 Hz)	10β,10γ,10δ_d
	10δ_d	23.7	1.089 (3 H, d, J = 6.6 Hz)	10β,10γ,10δ_u
	10-CO	169.9		
MeVal	11-Me	29.6	2.721 (3 H, s)	10-CO,11α
	11α	57.8	5.137 (1 H, d, J = 11.0 Hz)	10-CO,11-Me,11-CO
	11β	29.1	2.101 (1 H, m)	11α,11β,
	11γ_u	20.4	0.856 (3 H, d, J = 6.5 Hz)	11α,11β,11γ_d
	11γ_d	18.6	1.073 (3 H, d, J = 6.6 Hz)	11α,11β,11γ_u
	11-CO	173.7		

[a] Symbols d, u (downfield, upfield) were assigned according to proton resonances; [b] HMQC and HMBC readouts.

325 °C, capillary voltage 32 V, tube lens offset −10 V, octopole 1 offset −5.75 V, lens voltage −16 V, octopole 2 offset −9 V, octopole rf amplitude 450 V peak-to-peak (pp), and entrance lens voltage −40 V. A coaxial flow of nitrogen was used to stabilize the spray; helium was introduced at a pressure of 0.13 Pa to improve the trapping efficiency of the sample ions and to serve as the collision gas for the CID experiment. The spectra were scanned in the range m/z 50−2000, and the gating time was set to accumulate and trap 5×10^7 ions. The mass isolation window for precursor ion selection was set to 3 amu and centered to ^{12}C isotope of the pertinent ion. The relative activation amplitude was 35%, the activation time 30 ms. No broadband excitations were applied. NMR spectra were measured on a Varian Inova-400 spectrometer (399.89 and 100.55 MHz, respectively) in $CDCl_3$ at 303 K. Residual signal of $CDCl_3$ was used as an internal standard (δ_H 7.265, δ_C 77.00). ^1H NMR, ^{13}C NMR, APT, HOM2DJ, COSY, TOCSY, ROESY, HMQC, and HMBC spectra were measured using standard manufacturer's software (Varian Inc., Palo Alto, CA). Selective 1D-TOCSY was measured with a published sequence.[36] Chemical shifts are given in δ-scale [ppm], and coupling constants in Hz. Digital resolution allowed us to report chemical shifts of protons to three and coupling constants to one decimal place. Carbon chemical shift readouts from HMQC and HMBC are reported to one decimal place.

Fermentation and Isolation. The submerged cultivation of *Mycelium sterilae* MS 2929 has been described previously.[25] Extraction of mycelium with MeOH and chromatographic separation provided a crude mixture containing predominantly cyclosporins A, B, C, D, G, and F. [Abu^5]CsA (**4**) was found as a minor impurity in large-scale production batches of cyclosporin A and was purified by a combination of chromatography on silica gel (Merck) using EtOAc saturated with H_2O and reversed-phase chromatography on silica C-18 (Labio, Czech Republic) using an isocratic elution with a MeOH/H_2O (78:22 v/v) mixture and then with MeCN/MTBE/H_2O (65:7:28 v/v/v) on the same preparative RP-column. [MeNVa^{10}]CsA (**3**) was found in the cultivation supplemented with norvaline (1 g/L). It was isolated from the mycelium by extraction with MeOH, and the crude cyclosporine mixture was purified by a combination of chromatography on silica gel (Merck) with a DCM/MeOH (97:3 v/v) mixture and then by chromatography on silica C-18 (Labio, Czech Republic) using gradient elution with MeCN/H_2O. Both cyclosporins were obtained as amorphous solids (13.5 and 3.8 mg) directly by the evaporation of fractions from reversed-phase chromatography.

Compound **2**: ^1H NMR ($CDCl_3$) δ *MeBmt*^{1} 3.425 (1-Me, s), 5.330 (H-α, d; J = 3.5 Hz), 4.249 (H-β, dd; J = 10.6, 3.5 Hz), 1.481 (H-γ, m), 0.780 (1γ-Me, d; J = 6.7 Hz), 2.326 (H-δ_d, m), 2.027 (H-δ_u, m), 5.399 (H-ε, m), 5.492 (H-η, m), 1.644 (H-ω, dd; J = 6.0, 1.2 Hz); *Abu*^{2} 7.057 (2-NH, d; J = 6.4 Hz), 4.825 (H-α, ddd; J = 6.4, 6.4, 6.4 Hz), 1.775 (H-β, m), 0.924 (H-γ, t; J = 7.6 Hz); *Sar*^{3} 3.401 (3-Me, s), 4.834 (H-α_d, d; J = 14.2 Hz), 3.112 (H-α_u, d; J = 14.2 Hz); *MeLeu*^{4} 2.753 (4-Me, s), 4.688 (H-α, dd; J = 7.1, 7.0 Hz), 1.909 (H-β_d, m), 1.379 (H-β_u, m), 1.442 (H-γ, m), 0.903 (H-δ_d, d; J = 6.3 Hz), 0.898 (H-δ_u, d; J = 6.4 Hz); *Val*^{5} 8.111 (5-NH, d; J = 9.0 Hz), 4.562 (H-α, dd; J = 9.2, 9.0 Hz), 1.972 (H-β, m), 0.835 (H-γ_d, d; J = 6.7 Hz), 0.803 (H-γ_u, d; J = 6.6 Hz); *MeLeu*^{6} 3.188 (6-Me, s), 5.296 (H-α, dd; J = 9.5, 6.0 Hz), 1.904 (H-β_d, m), 1.734 (H-β_u, m), 1.609 (H-γ, m), 0.838 (H-δ_d, d; J = 6.6 Hz), 0.811 (H-δ_u, d; J = 6.6 Hz); *Ala*^{7} 8.078 (7-NH, d; J = 8.7 Hz), 4.538 (H-α, dq; J = 8.7, 6.9 Hz), 1.164 (H-β, d; J = 6.9 Hz); D-*Ala*^{8} 7.077 (8-NH, d; J = 9.1 Hz), 4.700 (H-α, dq; J = 9.1, 6.9 Hz), 1.332 (H-β, d; J = 6.9 Hz); *MeLeu*^{9} 3.077 (9-Me, s), 5.294 (H-α, dd; J = 11.3, 4.3 Hz), 1.962 (H-β_d, m), 1.589 (H-β_u, m), 1.427 (H-γ, m), 0.931 (H-δ_d, d; J = 6.6 Hz), 0.880 (H-δ_u, d; J = 6.5 Hz); *MeLeu*^{10} 6.958 (9-NH, d; J = 8.9 Hz), 5.021 (H-α, ddd; J = 11.2, 8.9, 3.0 Hz), 2.030 (H-β_d, m), 1.185 (H-β_u, m), 1.708 (H-γ, m), 0.997 (H-δ_d, d; J = 6.7 Hz), 0.980 (H-δ_u, d; J = 6.5 Hz); *MeVal*^{11} 3.045 (11-Me, s), 5.957 (H-α, d; J = 10.6 Hz), 2.280 (H-β, dqq; J = 10.6, 6.8, 6.5 Hz), 0.926 (H-γ_d, d; J = 6.5 Hz), 0.783 (H-γ_u, d; J = 6.8 Hz).

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