# 60. Synthesis of Cyclosporine[1])[2])

## Total Syntheses of 'Cyclosporin A' and 'Cyclosporin H', Two Fungal Metabolites Isolated from the Species *Tolypocladium Inflatum* GAMS

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*Summary*

The heptapeptide H-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl (**20**) was synthesized for coupling with the previously described cyclosporine tetrapeptide sequence Boc-D-Ala-MeLeu-MeLeu-MeVal-OH (**21**). The product of the coupling, the undecapeptide Boc-D-Ala-MeLeu-MeLeu-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl (**22**), was then deprotected and cyclized to cyclosporine (**1**).

The tetrapeptide diastereoisomer Boc-D-Ala-MeLeu-MeLeu-D-MeVal-OH (**23**) could also be used as a starting material to produce selectively the desired undecapeptide **22**. In this case, the *N*-methyl-D-valine unit, was selectively isomerized to the L-form by using the appropriate condensing agent. The diastereoisomeric undecapeptide Boc-D-Ala-MeLeu-MeLeu-D-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl (**24**) was also synthesized starting from **21** by using the mixed pivalic anhydride method to selectively invert the configuration of the *N*-methyl-L-valine. The structure of the undecapeptide **24** was confirmed by deprotection and cyclization to 'cyclosporin H', a natural product known to have the structure [D-MeVal[11]]cyclosporine (**2**).

**1. Introduction.** – 1.1. *General Remarks.* Cyclosporine[3]) originally named 'cyclosporin A' is a metabolite from the fungal species *Tolypocladium inflatum* GAMS [6]. Its structure was established by chemical degradation [3] and X-ray crystallographic analysis of an iodo derivative [7]. The structural assignments have later been confirmed by an X-ray crystallographic analysis of cyclosporine itself [8]. Its pharmacological and clinical action as a selective immunosuppressive drug has been widely documented [9].

[1]) Part III; for Part I and II, s. [1a] and [1b], respectively.

[2]) Part of the work has already been presented at the autumn session of the Swiss Chemical Society in Berne (October 15, 1983), for more details and previous presentations see [1c].

[3]) For practical reasons, it was previously proposed to define 'cyclosporin' as the cyclic undecapeptide with the structure of 'cyclosporin A' [2]. Now that the USAN name 'cyclosporine' has been accepted in the USA, this name will be adopted for the basic structure and other fungal metabolites of the same type. Cyclosporins B, C, D, and E published by *Rüegger et al.* [3] and *Traber et al.* [4] [5] then have, according to the peptide nomenclature, the following names: [Ala[2]]cyclosporine for cyclosporin B, [Thr[2]]cyclosporine for cyclosporin C, [Val[2]]cyclosporine for cyclosporin D and [Val[11]]cyclosporine for cyclosporin E.

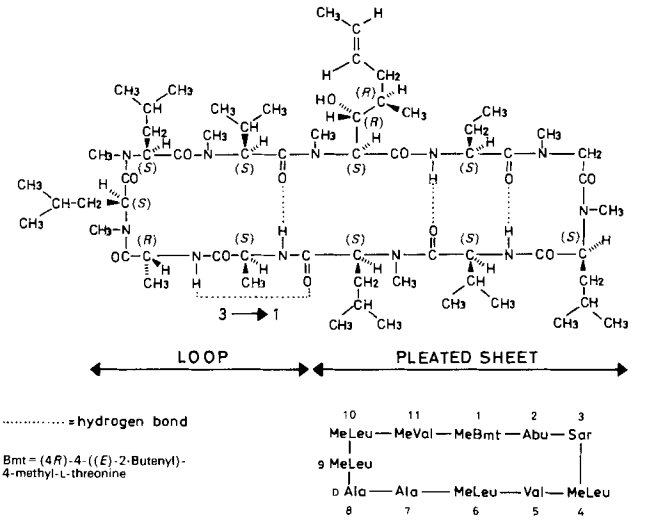



Fig. 1. *Schematic Representation of the Structure and Conformation of Cyclosporine* (**1**).

Cyclosporine *(Fig. 1)* is a homodetic cyclic undecapeptide, $C_{62}H_{111}N_{11}O_{12}$, mol. wt. 1202, which assumes a rather rigid conformation in the crystalline state [6][7] and in solution[4]). A large portion of the backbone, residues 1–6[5]), adopts an antiparallel $\beta$-pleated sheet conformation which contains three transannular H-bonds and is markedly twisted. The remaining residues 7–11 form an open loop. This loop contains the only D-residue in position 8 and the only *cis*-amide linkage between the two adjacent *N*-methylleucine residues 9 and 10. The remaining H-bond is of a 3→1 type, previously noted in a cyclic tetra- and pentapeptide [10], and which serves to hold the backbone in a folded L shape. Only four NH groups are available for H-bond formation since the remaining seven N-atoms are methylated. In cyclosporine, the amino acid in position 1[5]), (4*R*)-4-((*E*)-2-butenyl)-4, *N*-dimethyl-L-threonine (MeBmt)[6]) was first isolated following the stereospecific synthesis described in [1a]. The ten other amino acids are known aliphatic amino acids; they are L-2-aminobutyric acid in position 2, sarcosine in position 3, *N*-methyl-L-leucine in positions 4,6,9, and 10, L-valine in position 5, L-alanine in position 7, D-alanine in position 8, and *N*-methyl-L-valine in position 11. As the structure of cyclosporine was known and a synthesis of the new amino acid MeBmt available, the development of a total synthesis of cyclosporine (**1**) appeared attractive not only for its intrinsic worth, but also as an important tool for investigating the relationships between structure and biological activity[7]). It is important for

[4]) A $^1$H-NMR spectrum ($CDCl_3$) shows single resonances for each type of proton present in cyclosporine (**1**) demonstrating that one conformation exists in $CDCl_3$ (on the NMR time scale). This conformation in solution may be different from that in the crystalline state.

[5]) For the numbering of the amino acid sequence in cyclosporine see *Rüegger et al.* [3]. According to them, MeBmt is amino acid residue 1, Abu 2 and MeVal 11.

[6]) See *Footnotes 2* and *3* in [1a] and *Footnote 2* in [1b].

[7]) For a synthetic approach to the role of intermolecular and intramolecular forces in producing a functional conformation see [11].

further development in this field to determine the part of the molecule which is principally responsible for the immunosuppressive activity.

1.2. *Strategy Used for the Synthesis of Cyclosporine* (**1**). – The methods used for synthesizing cyclic peptides and the problems encountered during their syntheses were reviewed in detail by *Kopple* [12] and *Ovchinnikov et al.* [13] and authors cited therein. For the synthesis of cyclosporine (**1**), cyclization was chosen to be achieved at the peptide bond between the L-alanine in position 7 and the D-alanine in position 8 for five main reasons. *1*) The intrachain H-bonds between amide groups of the linear peptide, in this case, may operate so as to stabilize the open chain in folded conformations approximating the cyclic structure of cyclosporine and thus assist cyclization. The presence of sarcosine in the middle of the open peptide may facilitate chain folding of the same type as in cyclosporine *(Fig. 1)*, as described by *Venkatachalam* [14]. *2*) If the undecapeptide is stabilized in folded conformations similar to the cyclosporine conformation, then the *cis*-amide bond may be easily[8]) formed from the expected slightly more stable all-*trans*-peptide [15][16]. This is done by rotation around the bond between the two adjacent *N*-methylleucine residues before cyclization. *3*) The N-terminal and C-terminal residues (both alanines) small side chains, which may facilitate bringing the ends of the undecapeptide into close proximity and a suitable orientation for reaction. *4*) In many examples in the literature [12][17], a preference for ring formation over side reactions was noted for peptides containing N- or C-terminal amino acid residues with a D-configuration. *5*) The strategy of the synthesis was chosen specifically to preclude an *N*-methylamino acid at the N- and C-terminus of the undecapeptide, since bond formation between *N*-methylated amino acid residues presents more difficulties than for non-*N*-methylated derivatives [18–21][9]). Also this last reason, bond formation between the only consecutive pair of non-*N*-methylated amino acids in cyclosporine (**1**) appeared the logical choice for the cyclization step.

For the synthesis of the undecapeptide, a fragment-condensation technique introducing thhhamino acid MeBmt at the end of the synthesis was used. In this way, the

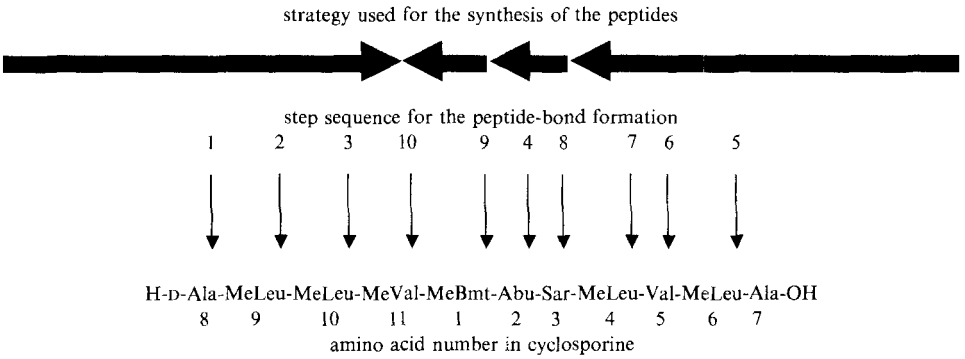



Fig. 2. *Strategy and Step Sequence Used for the Synthesis of the Peptides.*

[8]) In this case there is no H-bond stabilizing this peptide bond in the *trans*-arrangement.

[9]) See also cyclosporine synthesis part II [1b].

number of steps after the introduction of this amino acid was minimized. The peptide fragments were built up in the direction shown by the arrows in *Fig. 2* using the step sequence which is indicated numerically.

The amino groups of the amino acids and peptides being reacted were generally protected with a *tert*-butoxycarbonyl group (Boc) and the carboxyl groups with a benzyloxy group (benzyl ester; OBzl). The Boc-group was preferred to the Z-group for protection of the amino function for two reasons. Firstly, the Boc-group provides better protection against isomerization, as the electron density on the N-atom is slightly higher. As a negative consequence the coupling of the Boc-protected amino acids may be expected to be slower (see *Kovacs* [22] for a review of these racemization and coupling problems). Secondly, the undecapeptide contains the MeBmt amino acid residue, which has a double bond in its carbon chain. Removing a benzyl ester group by hydrolysis instead of hydrogenolysis ($Pd/H_2$) is expected to be easier than removing a Z-group by other methods. The benzyl ester was preferred to the methyl ester because its hydrolysis was expected to be easier[10]).

The heptapeptide H-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl was prepared essentially using the coupling procedures developed in [1b], and the sequence of coupling steps illustrated in *Fig. 2* was chosen so as to minimize the possibility of racemization (s. *Section 2*). Formation of the final amide linkage (bond 10) to produce the undecapeptide Boc-D-Ala-MeLeu-MeLeu-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl, was expected to be a difficult coupling due to steric hindrance, and some risk of racemization was anticipated [23][11]). In such couplings, the stereochemical outcome of the reaction will be governed by the rate of isomerization relative to coupling, the thermodynamic stability of the D- and L-forms of the activated carbonyl intermediate, and the relative rates with which each of the isomers of the activated carbonyl component reacts with the amine component. By using various coupling agents and experimental conditions, procedures were developed under which either the L- or the D-isomer of MeVal in the undecapeptide Boc-D-Ala-MeLeu-MeLeu-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl could be produced. This allowed the selective synthesis of undecapeptide intermediates for the synthesis of cyclosporine (**1**) [3] and [D-MeVal[11]]cyclosporine ( = cyclosporin H; **2**) [26] from the same starting materials. As will be described in detail in *Section 3*, the stereochemical outcome of the coupling reaction to form bond 10 depends only on the choice of condensing agent as both the starting DLLL- and DLLD-tetrapeptides produced the same results.

Cyclization to cyclosporine (**1**) was first attempted by the azide method developed by *Curtius* [27] and modified by *Honzl & Rudinger* [28] (see *Meienhofer* [29] for a review) for three reasons. The first is its reputation for producing minimal racemization, so that it is normally the method of choice, when cyclization cannot be carried out at glycine or proline residues. The second reason is the convenience of constructing a peptide chain from the benzyl ester of the C-terminal residue without prior removal of the benzyl ester group. The third is the possibility to separate activation and cycliza-

[10]) It was indeed possible to selectively hydrolyze the *N*-Boc-undecapeptide benzyl ester in presence of the *N*-Boc-undecapeptide methyl ester (1 equiv. of 0.2N NaOH in abs. EtOH (0.028M peptide) at −5°).

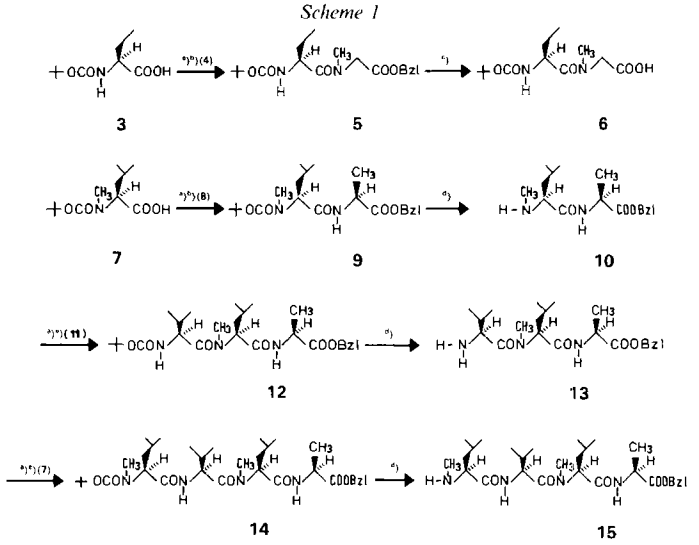
[11]) Recently, it has become apparent that certain difficult segment condensations gave high racemization figures [24] [25].

tion of the peptide. Diazotization of the hydrazide occurs under acidic conditions, and coupling does not occur until base is added to free the terminal amino group. The azide method produced cyclosporine (**1**) in low yield. The formation of side products is probably due to isomerization of *N*-methylamino acid residues under the strong acidic conditions required for diazotization. Cyclosporine (**1**) was obtained in better yield using a direct method of cyclization starting from the undecapeptide free at both ends. The reagents used for this purpose have been (1*H*-benzo[*d*][1,2,3]triazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (Bt-OP(NMe$_2$)$_3^+$PF$_6^-$) [30], propylphosphonic anhydride (Pr-PO$_2$)$_3$ [31] or the pentafluorophenol-dicyclohexylcarbodiimide complex [22]. These reagents are very convenient for use at room temperature, but the activation and cyclization steps cannot be separated.

The activation step, being bimolecular, requires a high concentration of reagent and peptide, while cyclization competes favorably with polymerization at a low concentration. Best results are obtained, if an excess of reagent is used, with the peptide in $10^{-3}$ to $10^{-4}$ M solution as already reported to be optimal for other cyclizations [12].

**2. Synthesis of the Heptapeptide H-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl**[12]) (**20**); *Table 1, Schemes 1* and *2*. – *The heptapeptide* **20** was synthesized by incorporating the amino acid MeBmt at the end of the synthesis *via* a coupling with the hexapeptide H-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl (**17**), which in turn was prepared by condensation of the peptide fragments Boc-Abu-Sar-OH (**6**) and H-MeLeu-Val-MeLeu-Ala-OBzl (**15**) (see *below*).

*Scheme 1*




a) 1.0 equiv. of pivaloyl chloride and 2.0 equiv. of *N*-methylmorpholine in $CHCl_3$.
b) Corresponding amino acid benzyl ester as base.
c) $H_2$/Pd/C/EtOH.
d) $CF_3COOH$.
e) Corresponding Boc-protected amino acid.

[12]) Abbreviations follow the IUPAC-IUB rules of the Commission on Biochemical Nomenclature [32a]; for full names see *Exper. Part.*

Table 1. *Synthesis of H-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl* (**20**). See *Schemes 1* and *2*.

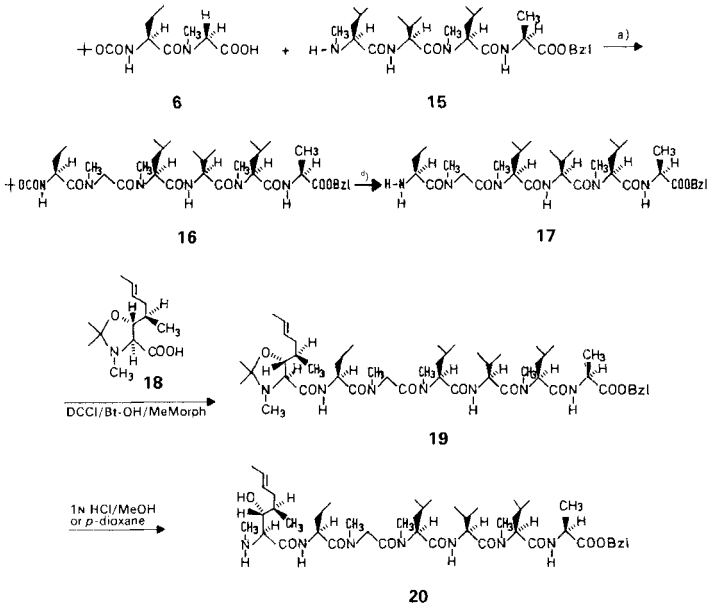
| Product Number | 1 MeBmt | 2 Abu | 3 Sar | 4 MeLeu | 5 Val | 6 MeLeu | 7 Ala | Yield [%] | $[\alpha]_D^{20}$ ($c$ = 1.0, $CHCl_3$) [°] |
|---|---|---|---|---|---|---|---|---|---|
| **3, 4** | Boc | OH H | OBzl | | | | | | |
| **5** | Boc | | OBzl | | | | | 87 | −4.2 |
| **6** | Boc | | OH | | | | | 97 | −5.3 |
| **7, 8** | >OH 100% | | | | | Boc OH H | OBzl | | |
| **9** |  | | | | | Boc | OBzl | 89 | −67.0 |
| **11, 10** | | | | | Boc OH | H | OBzl | 87 | −44.5 |
| **12** | | | | | Boc | | OBzl | 88 | −97.2 |
| **7, 13** | > OH | | | Boc OH | H | | OBzl | 97 | −102.0 |
| **14** | **18** | | | Boc | | | OBzl | 93 | −126.7 |
| **6, 15** | Boc | | OH H | | | | OBzl[a]) | 98 | −130.9 |
| **16** | Boc | | | | | | OBzl | 88 | −137.9 |
| **18, 17** | > OH | H | | | | | OBzl | 82 | −133.3 |
| **19** | > | | | | | | OBzl | 85 | −126.0 |
| **20** | H | | | | | | OBzl | 90 | −138.0 |

[a]) Crystalline.

The syntheses of the two peptides **6** and **15** are described in *Scheme 1*. *Table 1* summarizes the preparation of the heptapeptide **20** and gives the yield obtained for each step and the optical rotation measured for each product. In order to avoid racemization of *N*-methylamino acid residues, the stepwise elongation procedure from the carboxyl toward the amino end was used for the synthesis of the tetrapeptide **15**. Indeed, *McDermott & Benoiton* [32b] observed that *N*-methyl-L-leucyl residue in Z-Ala-MeLeu-OH partially racemized in a coupling reaction with an amine component by the DCCI/HOSu method even though no racemization of Z-Ala-Leu-OH occurred under the same conditions. They reported absence of racemization of alkyloxycarbonyl-*N*-methylamino acid during the course of coupling reactions.

For the preparation of the dipeptide Boc-Abu-Sar-OBzl (**5**) and the tetrapeptide Boc-MeLeu-Val-MeLeu-Ala-OBzl (**14**), the carboxyl groups of the corresponding Boc-protected amino acid were activated using the mixed pivalic anhydride method according to *Zaoral* [33] and modified by us [1b] in such a way that the anhydride formation with pivaloyl chloride occurs at −20 to −25°C in $CHCl_3$ during three to six hours in presence of two equiv. of *N*-methylmorpholine (MeMorph) before adding the amino acid or peptide esters to be coupled as free base. In the synthesis of these peptides, there is no coupling of consecutive *N*-methylated amino acids. This is reflected in the good yield obtained for each coupling: 87 and 89% for the dipeptides Boc-Abu-Sar-OBzl (**5**) and Boc-MeLeu-Ala-OBzl (**9**), respectively, 88% for the tripeptide Boc-Val-MeLeu-Ala-OBzl (**12**), and 93% for the tetrapeptide Boc-MeLeu-Val-MeLeu-Ala-OBzl (**14**). The benzyl protecting group of the dipeptide **5** was removed with $H_2$/Pd/C in EtOH at room temperature to give Boc-Abu-Sar-OH (**6**) in a yield of 97%. The Boc-protecting groups of the peptide intermediates **9**, **12** and **14** were removed with $CF_3COOH$ at −20°C, the acid neutralized with $NaHCO_3$, and the peptide bases H-MeLeu-Ala-OBzl (**10**), H-Val-MeLeu-Ala-OBzl (**13**), and H-MeLeu-Val-MeLeu-Ala-OBzl (**15**) isolated in 87, 97, and 98% yield, respectively, the tetrapeptide **15** being the only crystalline compound of this series.

*Scheme 2*




[a])–[d]) See *Scheme 1*.

The dipeptide Boc-Abu-Sar-OH (**6**) and tetrapeptide H-MeLeu-Val-MeLeu-Ala-OBzl (**15**) were coupled using the mixed pivalic anhydride method as described above in a yield of 88%. This peptide-fragment condensation was expected to succeed without racemization because of the C-terminal sarcosine[13]).

The amino acid MeBmt[14]) was incorporated as the derivative **18** having a five-membered ring protecting the hydroxy and the *N*-methylamino functions. This dimethyloxazolidine (or isopropylidene) protecting group was introduced quantitatively by refluxing the amino acid in acetone and has the advantage of avoiding epimerization of the amino acid MeBmt during peptide-bond formation. This means that the oxazolidine ring retains the thermodynamically more stable *trans*-configuration of substituents during carboxyl activation and peptide formation. On preparation, but before activation, the protected amino-acid derivative **18** was stabilized by addition of 1.1 equiv. of MeMorph. To prepare the protected heptapeptide **19** from **18** and the hexapeptide **17**, the dicyclohexylcarbodiimide (DCCI) coupling method was used in presence of *N*-hydroxybenzotriazol (Bt-OH) [34]. The desired heptapeptide **19** was obtained in a yield of 85%[15]). The observed *d* at 3.10 ppm in the $^1$H-NMR for the

[13]) The α-*C*-acylation mentioned by *Benoiton et al.* [32b], when sarcosyl is the coupling residue, was not observed.

[14]) For its preparation, see [1a].

[15]) In this case, the use of the mixed pivalic anhydride method (as described above and in [1b]) led to a 1:1 mixture of the desired heptapeptide **19** and the *N*-pivaloyl derivative of the starting hexapeptide **17**. The difficulty of this coupling is presumably due to the conformation of the hexapeptide in $CHCl_3$, which unselectively reacts with the mixed pivalic anhydride of **18**, while L-2-aminobutyric acid benzyl ester is coupled to it in high yield (unpublished results from our laboratory).

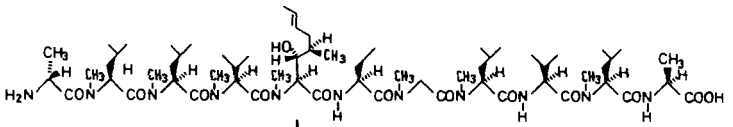
*a*-proton of the MeBmt residue confirms the expected *trans*-configuration of substituents on the oxazolidine ring of **19**[16]). The isopropylidene protecting group of the heptapeptide **19** is removed with 1 equiv. of 1N HCl/MeOH. After purification by chromatography, the heptapeptide H-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl (**20**) was obtained in 90% yield. The formation of 1 to 3% of a more polar heptapeptide methyl ester could not be avoided under this condition[17]).

Table 2. *Synthesis of Cyclosporine* (**1**) *by the Azide Method*

| Product Number | D-Ala | MeLeu | MeLeu | MeVal | MeBmt | Abu | Sar | MeLeu | Val | MeLeu | Ala | | Yield [%] | $[\alpha]_D^{20}$ (*c* = 1,0, $CHCl_3$) [°] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **21, 20** | Boc | | | OH | H | | | | | | | OBzl | | |
| **22** | Boc | | | | | | | | | | | OBzl | 73 | −182 |
| **25** | Boc | | | | | | | | | | | $NHNH_2$ | 86 | −173 |
| **26** | H | | | | | | | | | | | $NHNH_2$ | 95 | −189.2 |
| **1** | Cyclosporine | | | | | | | | | | | | 15 | −244.0 |

Table 3. *Synthesis of Cyclosporine* (**1**) *Using the Condensing Agent Bt-OP(NMe$_2$)$_3^+$PF$_6^-$*

| Product Number | D-Ala | MeLeu | MeLeu | MeVal | MeBmt | Abu | Sar | MeLeu | Val | MeLeu | Ala | | Yield [%] | $[\alpha]_D^{20}$ (*c* = 1,0, $CHCl_3$) [°] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **21, 20** | Boc | | | OH | H | | | | | | | OBzl | | |
| **22** | Boc | | | | | | | | | | | OBzl | 73 | −182.0 |
| **27** | Boc | | | | | | | | | | | OH | 70 | −173.4 |
| **28** | H | | | | | | | | | | | OH | 87 | −203.8 |



**28** → Bt-OP(NMe$_2$)$_3^+$PF$_6^-$, MeMorph/$CH_2Cl_2$/20°

MeLeu→MeVal→MeBmt→Abu→Sar

↑

**1** MeLeu 62 −244.5

↑

D-Ala←Ala←MeLeu←Val←MeLeu

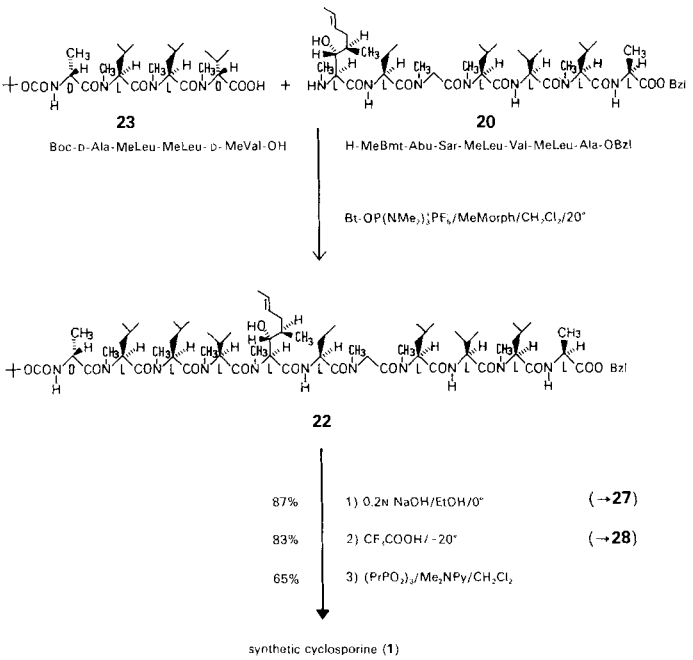
synthetic cyclosporine

[16]) The α-protons (H–C(4)) of the *cis*- and *trans*-forms of the corresponding methyl oxazolidine-4-carboxylate (*cf.* **18**, COOMe instead of COOH for the *trans*-form) are absorbing in $CDCl_3$ at 3.65 and 3.17 ppm, respectively (unpublished data from Dr. *Krieger* at *Sandoz*).

[17]) By using 1,4-dioxane instead of MeOH for the hydrolysis of **19** it was possible to exclude the formation of the heptapeptide methylester. The longer reaction time necessary in this case (3 days insted of 15 hours) makes this alternative disadvantageous (see *Exper. Part*).

**3. Synthesis of the Undecapeptide Boc-D-Ala-MeLeu-MeLeu-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl (22).** – For general remarks concerning the preparation of **22** see *Section 1.2*. For the coupling of the tetrapeptide Boc-D-Ala-MeLeu-

*Scheme 3*




MeLeu-MeVal-OH (**21**)[18]) and heptapeptide H-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl (**20**) (see top of *Table 2* and *3*) the use of the reagent Bt-OP$(NMe_2)_3^+$ $PF_6^-$, developed by *Castro et al.* [30], in presence of MeMorph is of considerable assistance for achieving this difficult linkage. The undecapeptide Boc-D-Ala-MeLeu-MeLeu-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl (**22**) is thus obtained at room temperature in a yield of 73%. Practically none of the undecapeptide isomer **24** *(Scheme 4)* was formed during this reaction, and unreacted heptapeptide **20** could be recovered. Starting from the isomeric tetrapeptide Boc-D-Ala-MeLeu-MeLeu-D-MeVal-OH (**23**)[18]) *(Scheme 3)* and using the same condensing agent Bt-OP$(NMe_2)_3^+PF_6^-$ under similar conditions for coupling with the heptapeptide **20** resulted in the formation of 47% of the undecapeptide **22**, which was isolated along with 46% of starting heptapeptide **20**. Again, practically no D-MeVal-containing undecapeptide isomer **24** was observed. This means that the condensing agent Bt-OP$(NMe_2)_3^+PF_6^-$

[18]) For its preparation, see [1b].

produces with **21** or **23** an activated tetrapeptide[19]), which stereospecifically[22]) reacts with the heptapeptide **20** to selectively produce **22** or that the amine component, the heptapeptide **20**, because of steric hindrance can only react with the activated tetrapeptide **21** containing *N*-methylvaline in the L-configuration. The structure of the undecapeptide **22** made from the tetrapeptide **23** was confirmed by deprotection and cyclization to cyclosporine (**1**; *Section 7*).

**4. Synthesis of the Undecapeptide Boc-D-Ala-MeLeu-MeLeu-D-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl (24).** – The synthesis of the undecapeptide **24** containing an *N*-methylvaline with a D-configuration was achieved starting from the tetrapeptide Boc-D-Ala-MeLeu-MeLeu-MeVal-OH (DLLL; **21**) with the aid of the mixed pivalic anhydride method described above to activate the carboxyl group *(Scheme 4)*. This also selectively inverted the configuration of the amino acid *N*-methylvaline, most probably by enolization[23]), before coupling with the heptapeptide H-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl (**20**). The asymmetric induction was only complete (no formation of **22**) on working at $-25\,^{\circ}C$. The D-MeVal-containing undecapeptide **24** which has an $[\alpha]_D$ of about 70° less negative than the L-MeVal-containing undecapeptide **22** and which is slightly less polar on TLC (silica gel; MeOH/$CHCl_3$) than **22**, was then isolated in a yield of 44%, and unreacted heptapeptide **20** was recovered (46%). Working at room temperature, a 4:1 mixture of undecapeptides **24** and **22** was obtained. This shows that the heptapeptide amine **20** reacts more easily at room temperature with the mixed anhydride of **21** than at low temperature, but that the rate of reaction with the corresponding anhydride of **23** is still faster. The results indicate that the activated species of **21** or **23** are different when working with the mixed pivalic anhydride method from those when using Bt-OP$(NMe_2)_3^+PF_6^-$ as condensing agent. This was not *a priori* true, because the asymmetric induction may depend on solvent polarity and temperature [35][24]).

**5. Synthesis of Cyclosporine (1) by the Azide Method** (*Table 2*[25]). – The benzyl group of the undecapeptide Boc-D-Ala-MeLeu-MeLeu-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl (**22**) was removed using hydrazine hydrate in dimethylformamide (DMF) at $-20\,^{\circ}C$, and the undecapeptide hydrazide **25** was isolated after evaporation followed by chromatography in a yield of 86%. The Boc-group of **25** was removed with $CF_3COOH$ at $-20\,^{\circ}C$ (→**26**; 95%). Cyclosporine (**1**) was then obtained

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[19]) The nature of this activated species has not yet been elucidated. It is probably different from an ester of –OHBt[20])[21]), it is no symmetric anhydride[20]) and no ketene[20]). A phosphorus derivative different from Bt-OP$(NMe_2)_3^+PF_6^-$ and hexamethylphosphoric triamide could not be detected by $^{31}P$-NMR spectroscopy. The reaction run in presence of methylamine instead of **20**, gave selectively and quantitatively Boc-D-Ala-MeLeu-MeLeu-MeVal-$NHCH_3$ ($[\alpha]_D^{20} = -212°$ ($c = 1.0$, $CHCl_3$)) or Boc-D-Ala-MeLeu-MeLeu-D-MeVal-$NHCH_3$ ($[\alpha]_D^{20} = -52°$ ($c = 1.0$, $CHCl_3$)) starting from **21** or **23**, respectively. The DCCI-method in presence of Bt-OH [34] resulted in no coupling reaction between **21** and **20** in $CH_2Cl_2$ at room temperature.

[20]) No IR absorption expected for an ester, ketene, or anhydride was observed.

[21]) When attempting to follow the activation in absence of **20**, Bt-OH crystallized from $CDCl_3$.

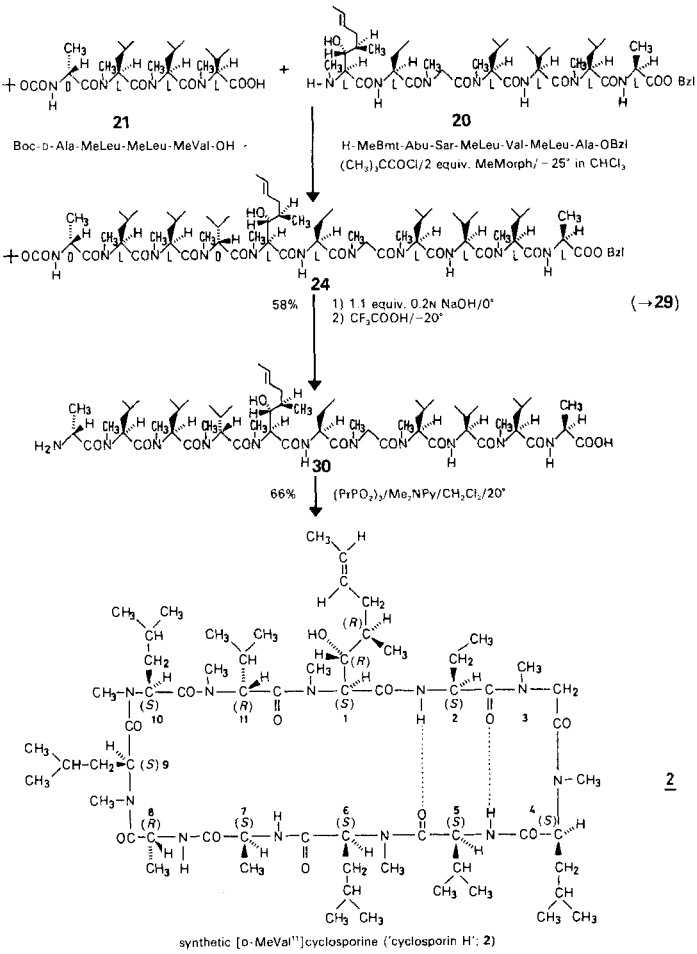
[22]) For the asymmetric induction on working with racemic 2,4-dialkyl-5(4*H*)-oxazolones, see *Benoiton et al.* [35].

[23]) For other examples of selective peptide formation with peptide fragments containing *N*-methylamino acid residues using the same technique, see [1b].

[24]) The Bt-OP$(NMe_2)_3^+PF_6^-$ reagent gives no coupling reaction with **21** and **20** when used at –20° in $CH_2Cl_2$ or $CHCl_3$.

[25]) For general remarks and literature, see *Section 2*.

*Scheme 4*




in 15% yield by cyclizing **26** first by the formation of the azide under acidic conditions (*tert*-butyl nitrite and 3 equiv. of HCl at – 20 °C in DMF) and then, following dilution of the azide solution (with DMF to a final concentration of 0.004M), by reaction of the protonated undecapeptide azide with diisopropylethylamine at – 20 °C. A better yield of **1** was achieved if the cyclization was effected with the Bt-OP$(NMe_2)_3^+PF_6^-$ reagent *(Section 6)*, the mixed phosphonic anhydride method *(Section 7)*, or the pentafluorophenol-DCCI complex *(Section 8)*.

**6. Synthesis of Cyclosporine (1) Using the Condensing Agent Bt-OP$(NMe_2)_3^+PF_6^-$[25] for the Cyclization of 28** *(Table 3)*. – The ester group of the undecapeptide Boc-D-Ala-MeLeu-MeLeu-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl (**22**) was removed by hydrolysis with NaOH at 0 °C. The corresponding undecapeptide acid **27** (70%) could be separated from unreacted starting material **22** (16%) by chromato-

graphy. The Boc group of **27** was removed with $CF_3COOH$ at $-20°C$ in a yield of 87%. Then, the unprotected H-D-Ala-MeLeu-MeLeu-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OH (**28**) was cyclized in $CH_2Cl_2$ (0.0123M) with 1 equiv. of Bt-OP$(NMe_2)_3^+PF_6^-$ [30] in the presence of MeMorph (two days at room temperature) to give crystalline cyclosporine (**1**), isolated in 30% yield. Working in more dilute solution ($10^{-3}$–$10^{-4}$M) with an excess of condensing agent the yield of **1** was ameliorated to 62%. This synthetic cyclosporine (**1**) was identical in all respects to the natural product [3], not only on comparison of all the usual physical and spectroscopic characteristics (IR, NMR, MS, m.p., mixed m.p., TLC and X-ray powder diffraction pattern), but also on comparison in several biological assays[26]).

**7. Synthesis of Cyclosporine (1) [Starting from D-Ala-MeLeu-MeLeu-D-MeVal-OH (23)] Using the Mixed Phosphonic Anhydride Method[25]) for the Cyclization of 28** *(Scheme 3)*. – The undecapeptide Boc-D-Ala-MeLeu-MeLeu-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl (**22**) prepared from Boc-D-Ala-MeLeu-MeLeu-D-MeVal-OH **23**[18]) (see *Section 3*) was deprotected by hydrolysis with NaOH in an ice box (→**27** (87%)) and by treatment with $CF_3COOH$ at low temperature (→**28** (83%) as already described in *Section 6*. A dilute solution (0.03M) of the unprotected undecapeptide **28** in $CH_2Cl_2$ was then treated with 1.5 equiv. of $(PrPO_2)_3$ [31] in the presence of 4-dimethylaminopyridine ($Me_2NPy$) [36] to effect cyclization at room temperature, and cyclosporine (**1**) was obtained in 35% yield . Working in more dilute ($10^{-3}$–$10^{-4}$M) solution with an excess of $(PrPO_2)_3$ enhanced the yield to 65%. This synthetic cyclosporine was identical in all respects with the natural cyclosporine [3]. This result not only firmly establishes the structure of **22** made from **23** *(Section 3)*, but also the dependence of asymmetric induction or isomerization on choice of the condensing agent in peptide synthesis when using the fragment-condensation technique[25])[27]).

**8. Synthesis of Cyclosporine (1) Using the Pentafluorophenol-DCCI Complex**. – The pentafluorophenol-DCCI complex described by *Kovacs* [22] is, at the present time among the best procedures for peptide synthesis. This method was applied to the cyclization of the undecapeptide **28** under the optimal dilution conditions found previously when using the Bt-OP$(NMe_2)_3^+PF_6^-$ reagent or the mixed phosphonic anhydride method *(Sections 6* and *7)*. A $10^{-3}$–$10^{-4}$M solution of **28** in $CH_2Cl_2$ was treated with 1.1 equiv. of DCCI in the presence of 2 equiv. of pentafluorophenol at room temperature to give cyclosporine (**1**) in 64% yield. The formation of **1** was slower than when using Bt-OP$(NMe_2)_3^+PF_6^-$ or $(PrPO_2)_3$. This can be attributed to the fact that cyclization was affected by the absence of base and by the presence of an excess of pentafluorophenol. From this single result it can be concluded that the method of *Kovacs* is very promising for the cyclization of peptides.

*Fig. 3* shows crystals of synthetic cyclosporine (**1**) obtained from diisopropyl ether.

**9. Synthesis of [D-MeVal[11]]Cyclosporine (2**; *Scheme 4*). – The undecapeptide Boc-D-Ala-MeLeu-MeLeu-D-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl (**24**) prepared starting from Boc-D-Ala-MeLeu-MeLeu-MeVal-OH (**21**; *Section 4*) was depro-

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[26]) These results will be published elsewhere.

[27]) For the preparation of the D-MeVal-containing undecapeptide isomer by use of the mixed pivalic anhydride method see *Section 4*.

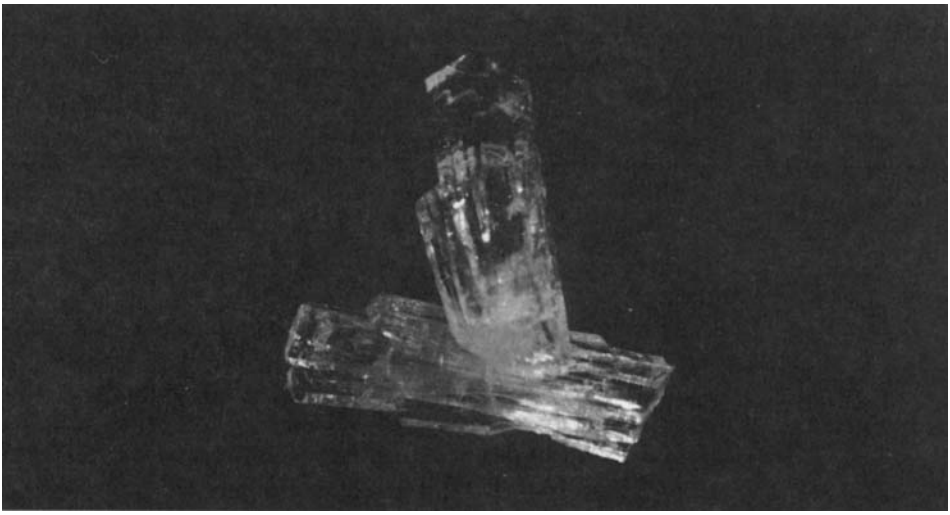

Fig. 3. *Crystals of the Synthetic Cyclosporine* (**1**). Crystallized from diisopropyl ether and photographed under red light by *R. Knoepfli*, Photo Dept. at *Sandoz*.

tected by hydrolysis with NaOH in an ice-box as described for **22** *(Section 6)*. The corresponding D-MeVal containing undecapeptide acid **29** (58%) could be separated from unreacted starting material **24** (25%) by chromatography. This undecapeptide **29** characterized by a relatively small negative $[\alpha]_D$ compared to its L-MeVal containing isomer **27** was further deprotected by treatment with $CF_3COOH$ at $-20°C$ to give H-D-Ala-MeLeu-MeLeu-D-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OH (**30**) in 77% yield. This unprotected peptide was cyclized using the mixed phosphonic anhydride method [31], as described in *Section 7* for **28**, to produce [D-MeVal[11]]cyclosporine (**2**) in a yield of 66%. This synthetic [D-MeVal[11]]cyclosporine was identical in all respects to the natural 'cyclosporin H' [26] by comparison of the physical and spectroscopic characteristics ($[\alpha]_D$, $^1H$- and $^{13}C$-NMR, m.p., mixed m.p., MS, and X-ray powder diffraction pattern).

This result demonstrates that the undecapeptide **24** has an *N*-methylvaline in the D-configuration and therefore confirms the asymmetric induction during its preparation using the mixed pivalic anhydride method previously described in *Section 4*.

**10. Conclusion.** – The successful total synthesis of cyclosporine (**1**) and [D-MeVal[11]]cyclosporine (**2**) using a fragment-condensation technique demonstrate that it is possible to synthesize peptides containing *N*-methylamino acids by this method with stereochemical control even under conditions recognized as most critical for isomerization. Moreover, it was possible to utilize the ready isomerization of *N*-methylamino acid derivatives and to synthesize an undecapeptide containing exclusively, at the position of carbonyl activation, the corresponding L-amino acid starting from a D-amino acid residue. Starting from an L-amino acid residue it was possible to synthesize an undecapeptide containing exclusively the corresponding D-amino acid by using the appropriate condensing agent.

The high yield obtained for the cyclization and the fact that the $^1$H-NMR spectrum of the undecapeptide **22** in $CDCl_3$ suggests the presence of mainly one conformation very similar to that of cyclosporine (**1**)[4])[28]) lead to the following supposition: the intrachain H-bonds of the activated linear undecapeptide can stabilize the open chain in folded conformations approximating the cyclic structure of cyclosporine (**1**) and thus assist cyclization. Using the fragment-condensation technique previously described it is now possible to synthesize very efficiently cyclosporine in 27.5% yield with respect to the amino acid MeBmt. Thus, due to the molecular weight increase during this synthesis, 1.6 g of cyclosporine can be produced starting from 1 g of the amino acid MeBmt.

The synthesis of cyclosporine (**1**) also opens the way to the preparation of analogs needed to attack the many unanswered problems concerning the structure-activity relationships of this drug.

We thank *Kurt Martin* and *Louis Walliser* for their capable technical assistance. We appreciate the valuable help by *H. R. Loosli, M. Ponelle,* and *T. Zardin* (NMR spectra), *W. Pfirter* (analysis), *C. Quiquerez* (MS), *O. Hurt* (CD), *H. Stocker* (for drawing the diagrams), *C. Weber* (for typing the manuscript), and *P. Meylan* (for the supply of larger quantities of the amino acid MeBmt). Special thanks to Dr. *T. Payne* for his assistance in improving the manuscript.

## Experimental Part

*General.* For remarks about instruments, starting materials, and peptide numbering see [1a][1b].

**1. *N*-(*tert*-Butoxycarbonyl)-L-2-aminobutyric Acid( = Boc-Abu-OH; 3), *N*-(*tert*-Butoxycarbonyl)-L-valine (11), L-Alanine Benzyl Ester (8), and Sarcosine Benzyl Ester (4).** – The *p*-toluenesulfonate of **8** was purchased from *Bachem, Feinchemikalien AG* (Bubendorf, Switzerland), and the *p*-toluenesulfonate of **4** from *Senn Chemicals* (Dielsdorf, Switzerland).

**2. *cyclo*[-((2*S*, 3*R*, 4*R*, 6*E*)-3-Hydroxy-4-methyl-2-methylamino-6-octenoyl)-L-2-aminobutyryl-sarcosyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl-L-alanyl-D-alanyl-*N*-methyl-L-leucyl-*N*-methyl-L-leucyl-*N*-methyl-L-valyl] ( = *cyclo*(-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-D-Ala-MeLeu-MeLeu-MeVal-), Cyclosporine[3]), or 'Cyclosporin A' [2][3]; 1).** – 2.1. *Procedure Involving an Azide (Table 2).* At −20°, 0.6 ml of 2M HCl/dioxane and 22.8 mg (0.32 mmol) of *tert*-butyl nitrite are added to 136 mg (0.11 mmol) of H-D-Ala-MeLeu-MeLeu-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-$NHNH_2$ (**26**) dissolved in 5 ml of DMF, and the mixture is stirred at − 20° for 45 min. A further 22.8 mg (0.22 mmol) of *tert*-butyl nitrite are then added, and the mixture is stirred for a further 2 h at − 20°, before being diluted with 20 ml of DMF. Then, 0.21 g (1.65 mmol) of ethyldiisopropylamine are added, and the mixture is stirred for 2 h at − 20°. The resulting solution is poured onto 100 ml of HCl at pH 3 and shaken twice with 200 ml of $Et_2O$. The $Et_2O$ phases are washed with 50 ml of $H_2O$, dried over $Na_2SO_4$, filtered, and evaporated. The 103 mg of residue are chromatographed on 50 g of silica gel using 2% MeOH/$CH_2Cl_2$ to yield 20 mg (15%) of **1** as a colourless oil, which is crystallized from acetone at −15°, m.p. 150°, $[\alpha]_D^{20} = -245°$ ($c$ = 1.0, $CHCl_3$). A mixed m.p. with natural 'cyclosporin A' gave no depression.

2.2. *Procedure Using the Condensing Agent Bt-OP(NMe$_2$)$_3^+$PF$_6^-$* [30] *and Starting from the Unprotected Undecapeptide* **28** *(Table 3).* At 20°, 0.08 ml (0.738 mmol) of *N*-methylmorpholine (MeMorph) and 326 mg (0.738 mmol) of (1*H*-benzo[*d*][1,2,3]triazol-1-yloxy)-tris-(dimethylamino)phosphonium hexafluorophosphate (Bt-OP(NMe$_2$)$_3^+$PF$_6^-$) are added to a solution of 900 mg (0.738 mmol) of H-D-Ala-MeLeu-MeLeu-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OH (**28**) in 60 ml of $CH_2Cl_2$ and the mixture is stirred for 2 days at 20°. The solution obtained is diluted with 200 ml of $CH_2Cl_2$ shaken with 50 ml of $H_2O$, the org. phase dried over $Na_2SO_4$, filtered, and evaporated. The amorphous residue is then chromatographed on 200 g of silica gel using

[28]) See *Exper. Part.*

4–30% MeOH/$CH_2Cl_2$: 266 mg (30%) of **1** that is crystallized from acetone/$Et_2O$ and purified by recrystallization from acetone at −15°; $[\alpha]_D^{20} = -244.5°$ ($c = 1.0$, $CHCl_3$), m.p. 150° and no depression on mixed m.p. with the naturally occurring 'cyclosporin A'. Identity with 'cyclosporin A' is also confirmed by $^1$H-NMR ($CDCl_3$) and by TLC on silica gel (5% MeOH/$CHCl_3$). Other reaction products, mostly more polar, were not characterized.

*Procedure Using the Condensing Agent Bt-OP(NMe$_2$)$_3^+$PF$_6^-$ in Excess and a Low Concentration of Undecapeptide* **28**. At r.t., 0.6 g (0.492 mmol) of **28** are dissolved in 2.0 l of $CH_2Cl_2$, 0.3 g (2.46 mmol; 5 equiv.) of 4-(dimethylamino)pyridine ($Me_2NPy$) and 0.87 g (1.968 mmol; 4 equiv.) of Bt-OP(NMe$_2$)$_3^+$PF$_6^-$ are added, and the solution is stirred with exclusion of moisture for 24 h. The solution obtained is concentrated to 100 ml and chromatographed without workup on 500 g of silica gel using 5% MeOH/$CH_2Cl_2$ to procedure 366 mg (62%) of **1**, $[\alpha]_D^{20} = -244.5°$ ($c = 1.0$, $CHCl_3$), identical in all respects with natural cyclosporine.

2.3. *Procedure Using the Mixed Propylphosphonic Anhydride Method* [31] *and the Undecapeptide* **28** *Made from Boc-*D*-Ala-MeLeu-MeLeu-*D*-MeVal-OH* (**23**) *(Scheme 3)*. To a solution of 151 mg (1.24 mmol; 3.75 equiv.) of $Me_2NPy$ in 10 ml of $CH_2Cl_2$, 405 mg (0.332 mmol) of **28** (made from **23**) are added. A solution of 53 mg (0.50 mmol; 1.5 equiv.) of $(PrPO_2)_3$ in 53 mg of $CH_2Cl_2$ is then added and the mixture stirred for 16 h at r.t. TLC control on silica gel using 5% MeOH/$CHCl_3$ showed no starting material remaining. The solution is then diluted with 200 ml of $CH_2Cl_2$ and shaken with 50 ml of sat. $NaHCO_3$ and 50 ml of $H_2O$. The org. phase is dried over $Na_2SO_4$, filtered, and evaporated. The residue is chromatographed on 150 g of silica gel using 5% MeOH/$CH_2Cl_2$ to yield 141 mg (35%) of **1**, which are crystallized twice from diisopropyl ether to yield 101 mg (25%) of **1**, m.p. 149–150°, $[\alpha]_D^{20} = -244.0°$ ($c = 1.0$, $CHCl_3$). Identity with the naturally occurring 'cyclosporin A' is also confirmed by $^1$H-NMR ($CDCl_3$) and by TLC on silica gel using 5% MeOH/$CHCl_3$. Other products are mostly more polar and were not characterized.

*Procedure Using the Condensing Agent $(PrPO_2)_3$ in Excess and Low Concentration of Undecapeptide* **28**. To a solution of 0.6 g (0.492 mmol) of **28** in 2 l of $CH_2Cl_2$ are added, with vigorous stirring, 0.3 g (2.46 mmol; 5 equiv.) of $Me_2NPy$ and 0.208 g (1.968 mmol; 4 equiv.) of $(PrPO_2)_3$ (0.32 ml of a 50% *w/w* solution in $CH_2Cl_2$). The clear colourless solution is stirred for a further 24 h at r.t. excluding moisture. It is then concentrated to 100 ml and chromatographed without workup on 500 g of silica gel using 5% MeOH/$CH_2Cl_2$ to give 383.5 mg (65%) of **1**, $[\alpha]_D^{20} = -244.5$ ($c = 1.0$, $CHCl_3$), identical in all respects with natural cyclosporine.

2.4. *Procedure Using a Pentafluorophenol-DCCI-Complex [22].* – To a solution of 0.6 g (0.492 mmol) of **28** in 2 l of $CH_2Cl_2$, 0.17 g (0.924 mmol; 0.10 ml) of pentafluorophenol and then 0.12 g (0.583 mmol) of DCCI are added with vigorous stirring, and stirring is continued for a further 24 h at r.t. with the exclusion of moisture. The resulting solution is concentrated to 200 ml, successively washed with 100 ml of 0.2N NaOH and 100 ml of $H_2O$, dried over $Na_2SO_4$, filtered, and evaporated. The residue is taken up in $Et_2O$, the undissolved dicyclohexylurea filtered off, and the filtrate evaporated. The residue is chromatographed on 500 g of silica gel using 5% MeOH/$CH_2Cl_2$ to yield 380 mg (64%) of **1**, $[\alpha]_D^{20} = -243.9°$ ($c = 1.0$, $CHCl_3$), identical in all respects with the natural cyclosporine. Synthetic cyclosporine (**1**): Powder diffraction diagram by the method of *Guinier & de Wolff* [37] (line distances in Å, lines of natural cyclosporine in brackets; *s* = strong, *m* = middle, *w* = weak; crystals of both samples from acetone): 13.1 *s* [13.0]; 11.5 *s* [11.3]; 10.3 *s* [10.3]; 9.8 *s* [9.6]; 9.5 *s* [9.4]; 8.9 *w* [8.7]; 8.3 *s* [8.2]; 7.1 *s* [7.0]; 6.3 *w* [6.2]; 6.2 *w* [6.1]; 5.95 *s* [5.84]; 5.63 *s* [5.6]; 5.32 *s* [5.4]; 5.25 *w* [5.25]; 4.9 *w* [4.9]; 4.85 *w* [4.8]; 4.6 *s* [4.7]; 4.56 *w* [4.55]; 4.5 *w* [4.45]; 4.3 *w* [4.35]; 4.19 *w* [4.25]; 4.05 *m* [4.1]; 3.89 *w* [3.9]; 3.8 *w* [3.8]; 3.78 *w* [3.73]; 3.69 *m* [3.66]; 3.5 *w* [3.6]; 3.49 *w* [3.49]; 3.35 [3.44]. $^1$H-NMR ($CDCl_3$, 360 MHz)[29])[5]): 0.71 (*d*, $J = 6$, 3H, $CH_3$–C(4$^1$)); 0.80–1.12 (*m*, 39H, $CH_3$–C(3$^2$), 2$CH_3$–C(4$^4$), 2$CH_3$–C(3$^5$), 2$CH_3$–C(4$^6$), 2$CH_3$–C(4$^9$), 2$CH_3$–C(4$^{10}$), 2$CH_3$–C(3$^{11}$)); 1.28 (*d*, $J = 6$, $CH_3$–C(2$^8$)); 1.38 (*d*, $J = 6$, 3H, $CH_2$–C(2$^7$)); 1.40, 1.70, 2.10 (3*m*, 17H, H–C(4$^1$), H–C(5$^1$), 2H–C(3$^2$), 2H–C(3$^4$), H–C(4$^4$), 2H–C(3$^6$), H–C(4$^6$), 2H–C(3$^9$), H–C(4$^9$), 2H–C(3$^{10}$), H–C(4$^{10}$), H–C(3$^{11}$)); 1.64 (*d*, $J = 3$, 3H, $CH_3$–C(7$^1$)); 2.43 (2*m*, 2H, H–C(5$^1$), H–C(3$^5$)); 2.69 (*s*, 3H, $CH_3$–N$^{10}$); 2.71 (*s*, 3H, $CH_3$–N$^{11}$); 3.11 (*s*, 3H, $CH_3$–N$^4$); 3.12 (*s*, 3H, $CH_3$–N$^9$); 3.27 (*s*, 3H, $CH_3$–N$^6$); 3.40 (*s*, 3H, $CH_3$–N$^3$); 3.51 (*s*, 3H, $CH_3$–N$^1$); 3.21, 4.74 (2*d*, $J = 15$, 2H, 2H–C(2$^3$)); 3.83 (br. *s*, 2H, H–C(3$^1$), HO–C(3$^1$)); 4.53 (*m*, 1H, H–C(2$^7$)); 4.65 (*t*, $J = 9$, 1H, H–C(2$^5$)); 4,83 (*m*, 1H, H–C(2$^8$)); 5.01 (*m*, 2H, H–C(2$^2$), H–C(2$^6$)); 5.10 (*m*, 1H, H–C(2$^{10}$)); 5.13 (*d*, $J = 12$, 1H, H–C(2$^{11}$)); 5.35 (*m*, 3H, H–C(6$^1$), H–C(7$^1$), H–C(2$^4$)); 5.50 (*d*, $J = 5$, 1H, H–C(2$^1$)); 5.71 (*dd*, $J = 12$, 4, 1H, H–C(2$^9$)); 7.19 (*d*, $J = 9$, 1H, H–N$^8$); 7.49 (*d*, $J = 9$, 1H, H–N$^5$); 7.70 (*d*, $J = 9$, 1H, H–N$^7$); 7.99 (*d*, $J = 10$, 1H, H–N$^2$). $^1$H-NMR ($CDCl_3$, in presence of $H_2O$; 360 MHz): same signals + 1.86 (*s*, $H_2O$); 3.83 (*m*, 1H, H–C(3$^1$)); 3.86 (*m*, 1H, HO–C(3$^1$)). A $^1$H-NMR of a mixture of synthetic and natural cyclosporine is identical in all respects to the $^1$H-NMR of the natural

[29]) Interpretation made with the help of double resonance and nuclear *Overhauser* effect measurements by *H. R. Loosli* (Physical Chemistry Department at *Sandoz*, Basle).

product. $^1$H-NMR ($CDCl_3$, 360 MHz, Plot 10 Hz/cm): signals between 1.57 and 1.60, which are typical for a $CH_3$ group attached to a *cis*-double bond are practically absent (less than 1%). MS (FD): 1202 ($M^+$), 1203 ($MH^+$), 1225 ($M + Na^+$). Anal. calc. for $C_{26}H_{111}N_{11}O_{12}$ (1202.635): C 61.9, H 9.3, N 12.8, O 16.0; found: C 61.8, H 9.4, N 12.6, O 15.9.

**3. [D-MeVal$^{11}$]Cyclosporine** (**2**; *Scheme 4*). – A solution of 170 mg (0.139 mmol) of H-D-Ala-MeLeu-MeLeu-D-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OH (**30**) in 3.5 ml of $CH_2Cl_2$ is added to a solution of 64 mg (0.523 mmol) of $Me_2NPy$ in 1 ml of $CH_2Cl_2$. Then, a solution of 22.2 mg (0.21 mmol) of $(PrPO_2)_3$ and 22.2 mg of $CH_2Cl_2$ is immediately added, the mixture stirred for 20 h at r.t., then diluted with 100 ml of $CH_2Cl_2$, and shaken with 50 ml of $H_2O$. The org. phase is dried over $Na_2SO_4$, filtered, and evaporated. The residue is chromatographed on silica gel using 5% MeOH/$CH_2Cl_2$ to yield 57 mg (34.1%) of **2** and 105 mg (62%) of **30**. The latter are converted to 53 mg (50%) of **2** using the same conditions as above, but with 2.44 equiv. (instead of 1.5 equiv.) of $(PrPO_2)_3$. The 110 mg (65.8%) of **2**, $[\alpha]_D^{20} = -166.5°$ ($c = 0.9$, $CHCl_3$) were crystallized from $Et_2O$ to yield 85 mg of pure **2** (51%), m.p. 161–163°, $[\alpha]_D^{20} = -175°$ ($c = 0.8$, $CHCl_3$). The mixed m.p. with natural 'cyclosporine H' [26] is not depressed. X-Ray powder diffraction pattern by the method of *Guinier & de Wolff* [37]: all lines are weak; the strongest lines, $d = 15.8$, 10.9, and 9.9 Å, are identical with the lines obtained from natural 'cyclosporin H'. $^1$H-NMR ((D)$_6$DMSO, 360 MHz, 180°; interpretation made in analogy to **29** and **27**): 0.79 (*d*, $J = 6$, 3H, $CH_3$–C(3$^{11}$)); 0.82–0.96 (*m*, 39H, $2CH_3$–C(4$^9$), $2CH_3$–C(4$^{10}$), $CH_3$–C(3$^{11}$), $CH_3$–C(4$^1$), $CH_3$–C(3$^2$), $2CH_3$–C(4$^4$), $2CH_3$–C(3$^5$), $2CH_3$–C(4$^6$)); 1.99 (*d*, $J = 6$, 6H, $CH_3$–C(2$^7$), $CH_3$–C(2$^8$)); 1.55, 1.72, 1.82 (3*m*, 16H, 2H–C(3$^9$), H–C(4$^9$), 2H–C(3$^{10}$), H–C(4$^{10}$), H–C(4$^1$), H–C(5$^1$), 2H–C(3$^2$), 2H–C(3$^4$), H–C(4$^4$), 2H–C(3$^6$), H–C(4$^6$)); 1.60 (*d*, $J = 3$, 3H, $CH_3$–C(7$^1$)); 2.10 (*m*, 1H, H–C(3$^5$)); 2.27 (*d*, $J = 15$, *m*, 2H, H–C(5$^1$), H–C(3$^{11}$)); 2.75, 2.83, 2.88 (6H), 2.92, 2.99, 3.12 (6*s*, total 21H, $CH_3$–N$^1$, $CH_3$–N$^3$, $CH_3$–N$^4$, $CH_3$–N$^6$, $CH_3$–N$^9$, $CH_3$–N$^{10}$, $CH_3$–N$^{11}$); 3.90 (*t*, $J = 6$, 2H, H–C(3$^1$), HO–C(3$^1$)); 4.15, 4.35 (2*d*, $J = 15$, 2H, 2H–C(2$^3$)); 4.30 (*m*, 1H, H–C(2$^{7\text{ or }8}$)); 4.62 (*t*, $J = 6$, 1H, H–C(2$^5$)); 4.68 (*m*, 1H, H–C(2$^2$)); 4.70 (*m*, 1H, H–C(2$^{8\text{ or }7}$)); 4.86 (*m*, 1H, H–C(2$^6$)); 5.02 (*m*, 2H, H–C(2$^1$), H–C(2$^4$)); 5.11 (*d*, $J = 9$, 1H, H–C(2$^{11}$)); 5.35, 5.41 (2*m*, 4H, H–C(2$^9$), H–C(2$^{10}$), H–C(6$^1$), H–C(7$^1$)); 6.88, 7.02, 7.10, 7.40 (4 br. *s*, 4H, H–N$^2$, H–N$^5$, H–N$^7$, H–N$^8$); identical with the $^1$H-NMR ((D)$_6$DMSO, +180°) of natural 'cyclosporin H'. $^1$H-NMR ($CDCl_3$, 360 MHz): complicated (8 or more conformers), but similar or identical to the $^1$H-NMR of 'cyclosporin H', see [26]. $^{13}$C-NMR ((D$_6$)DMSO, 90.5 Hz, +20°; high-temp. measurement is not possible because of the instability of **2**): complicated, but in all details identical with the $^{13}$C-NMR ((D$_6$)DMSO, similar conditions) of natural 'cyclosporin H'. MS (FD): 1202 ($M^+$), 1203 ($MH^+$).

**4. *N*-(*tert*-Butoxycarbonyl)-D-alanyl-*N*-methyl-L-leucyl-*N*-methyl-L-leucyl-*N*-methyl-L-valine (= Boc-D-Ala-MeLeu-MeLeu-MeVal-OH; 21) and *N*-(*tert*-Butoxycarbonyl)-D-alanyl-*N*-methyl-L-leucyl-*N*-methyl-L-leucyl-*N*-methyl-D-valine (= Boc-D-Ala-MeLeu-MeLeu-D-MeVal-OH; 23).** See [1b].

**5. ((2*S*, 3*R*, 4*R*, 6*E*)-3-Hydroxy-4-methyl-2-methylamino-6-octenoyl)-L-2-aminobutyryl-sarcosyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl-L-alanine Benzyl Ester (= H-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl; 20)** (*Schemes 1* and *2, Table 1*). – 5.1. N-(tert-*Butoxycarbonyl*)-L-*2-aminobutyryl-sarcosine Benzyl Ester* (= *Boc-Abu-Sar-OBzl*; **5**). To a solution of 16.65 g (77.1 mmol) of Boc-Abu-OH (**3**) in 500 ml of $CHCl_3$ precooled to – 20°, 10.4 ml (10.18 g; 84,8 mmol) of pivaloyl chloride and 15.4 g (162 mmol) of MeMorph are added, and the mixture is stirred for 3 h at – 20° under $N_2$. A solution of 16.6 g (92.5 mmol) of H-Sar-OBzl (**4**) in 500 ml of $CHCl_3$ is added and the mixture stirred for a further 15 h at – 20° under $N_2$. Then, the solution is warmed to r.t. and washed with 300 ml of 1N HCl, the aq. phase extracted with 200 ml of $CH_2Cl_2$ and the combined org. phase washed twice with 200 ml of sat. $K_2CO_3$. The aq. phases are extracted with 200 ml of $CH_2Cl_2$ and the combined org. phases dried over $K_2CO_3$, filtered, and concentrated. The residue (35 g) is chromatographed on 1 kg of silica gel using 1% MeOH/$CH_2Cl_2$ to yield 24.36 g (87)% of **5**, $[\alpha]_D^{20} = -4.2°$ ($c = 1.0$, $CHCl_3$). IR ($CH_2Cl_2$): 3400 (NH), 1740, 1700, 1645 (C=O). $^1$H-NMR ((D$_6$)DMSO, 360 MHz, 20°; 2 conformers): 0.76, 0.85 (2*t*, $J = 6$, 3H, 1:2, $CH_3$–C(3$^1$)); 1.37 (*s*, 9H, O*t*Bu); 1.47, 1.60 (2*m*, 2H, 2H–C(3$^1$); 2.84, 3.09 (2*s*, 3H, 1:2, $CH_3$–N$^2$); 3.97, 4.37 (2*d*, $J = 18$, 2H, 2H–C(2$^2$)); 4.10–4.43 (*m*, 1H, H–C(2$^1$)); 5.14, 5.18 (2*s*, 2H, 2:1, PhC*H*$_2$); 6.92, 7.03 (2*d*, $J = 9$, 1H, 2:1 H–N$^1$); 7.36 (*s*, 5H, *Ph*CH$_2$). $^1$H-NMR ((D)$_6$DMSO, 360 MHz, 150°): 0.83 (*t*, $J = 6$, 3H, $CH_3$–C(3$^1$)); 1.38 (*s*, 9H, O*t*Bu); 1.52, 1.66 (2*m*, 2H, 2H–C(3$^1$)); 3.03 (*s*, 3H, $CH_3$–N$^2$)); 4.1, 4.15 (2*d*, $J = 18$, 2H, 2H–C(2$^2$)); 4.35 (*m*, 1H, H–C(2$^1$)); 5.14 (*s*, 2H, PhC*H*$_2$); 5.94 (*s*, 1H, H–N$^1$); 7.33 (*s*, 5H, *Ph*CH$_2$. MS (FD): 364 ($M^+$). Anal. calc. for $C_{19}H_{28}N_2O_2$ (364.446): C 62.6, H 7.7, N 7.7, O 22.0; found: C 62.2, H 8.0, N 7.3, O 22.3.

5.2. N-(tert-*Butoxycarbonyl*)-L-*2-aminobutyryl-sarcosine* (= *Boc-Abu-Sar-OH*; **6**). A solution of 10.7 g (29.4 mmol) of **5** in 500 ml of abs. EtOH is hydrogenated for 2 h at r.t. using 2 g of 10% Pd/C. The suspension is filtered through talc, the filtrate evaporated, and the residue dried under high vacuum to yield 7.8 g (96.9%) of **6** as a colourless oil, $[\alpha]_D^{20} = -5.3°$ ($c = 1.0$, $CHCl_3$). IR ($CH_2Cl_2$): 3425*m*, 3300–2700*m*, 1735*s*, 1715*s*, 1660*s*,

*etc.* $^1$H-NMR ((D$_6$)DMSO, 360 MHz, 20°): 2 conformers. $^1$H-NMR ((D)$_6$DMSO, 360 MHz, 150°): 0.85 (*m*, 3H, $CH_3$–C(3$^1$)); 1.40 (*s*, 9H, O*t*Bu); 1.54, 1.69 (2*m*, 2H, 2H–C(3$^1$)); 3.0 (*s*, 3H, $CH_3$–N$^2$); 3.95 (br. *s*, 1H) and 4.09 (*d*, *J* = 18, 1H, together 2H–C(2$^2$)); 4.35 (*m*, 1H, H–C(2$^1$)); 5.90 (br. *s*, 1H, H–N$^1$); 12.5 (br. *s*, 1H, COOH). MS (LR): 274 (*M*$^+$), 218, 201, 175, 158, 145, 127, 118, 102, 96, 92, 72, 57, 44 *etc.* Anal. calc. for $C_{12}H_{22}N_2O_5$ (274.32): C 52.5, H 8.1, N 10.2, O 29.2; found: C 52.1, H 8.2, N 9.6, O 29.8.

5.3. N-(tert-*Butoxycarbonyl*)-N-*methyl*-L-*leucyl*-L-*alanine Benzyl Ester* (= *Boc-MeLeu-Ala-OBzl*; **9**). To a solution of 35 g (143 mmol) of Boc-MeLeu-OH (**7**) in 500 ml of $CHCl_3$ precooled to −20°, 19.2 ml (18.8 g; 157 mmol) of pivaloyl chloride and 31.5 ml (28.9 g; 286 mmol) of MeMorph are added, and the mixture is stirred for 3 h at −20° under $N_2$. The formation of the anhydride was followed by IR (IR ($CHCl_3$; after 3 h): 2940*s*, 2500*m*, 2450–2300*s*, 1810*s*, 1740*m*, 1690*s*, 1460*s*, 1390*s*, 1365*s*, 1320*m*, 1240–1200*m*, 1160*s*, 1040*s*, 1010*s*, 905*w*, 855*w*, 825*w*). Then 30.7 g (171 mmol) of H-Ala-OBzl (**8**) in 300 ml of $CHCl_3$ is added and the mixture stirred for further 15 h at −20° under $N_2$. IR ($CHCl_3$; before workup): 3400*w*, 2950*s*, 2540*m*, 2450–2300*s*, 1735*s*, 1685*s*, *etc.* solution obtained is worked up as in *Chap. 5.1* (twice 300 ml of $CH_2Cl_2$) and the residue (65 g) chromatographed on 2 kg of silica gel using 1% MeOH/$CH_2Cl_2$ to yield 51.8 g (89.2%) of **9** as a colourless oil, $[\alpha]_D^{20} = -67.0°$ (*c* = 1.0, $CHCl_3$). $^1$H-NMR ((D)$_6$DMSO, 360 MHz, 20°; 2 conformers); 0.88 (*d*, *J* = 6, 6H, 2$CH_3$–C(4$^1$)); 1.32 (*d*, *J* = 6, 3H, $CH_3$–C(2$^2$)); 1.39 (*s*, 9H, O*t*Bu); 1.30–1.50 (*m*, 1H, H–C(4$^1$)); 1.50 (*t*, *J* = 6, 2H–C(3$^1$)); 2.70 (*s*, 3H, $CH_3$–N$^1$); 4.33 (*m*, 1H, H–C(2$^2$)); 4.47, 4.68 (2*m*, 1H, H–C(2$^1$)); 5.08, 5.15 (2*d*, *J* = 12, 2H, *Ph*$CH_2$); 7.35 (*m*, 5H, *Ph*$CH_2$); 8.33 (*m*, 1H, H–N$^2$). MS (LR): 406 (*M*$^+$), 350, 333, 305, 291, 234, 200, 172, 144, 128, 100, 91, 57. Anal. calc. for $C_{22}H_{34}N_2O_5$ (406.527): C 65.0, H 8.4, N 6.9, O 19.7; found: C 65.1, H 8.4, N 6.4, O 19.9.

5.4. N-*Methyl*-L-*leucyl*-L-*alanine Benzyl Ester* (= *H-MeLeu-Ala-OBzl*; **10**). At −20°, 46.8 g (115 mmol) of **9** (precooled to −20°) are dissolved in 150 ml of $CF_3COOH$ (precooled to −20°) and stirred overnight at −20°. The cold mixture is poured onto ice/$H_2O$ containing 300 ml of sat. $NaHCO_3$, then 500 ml of $CH_2Cl_2$ are added, and the mixture is extracted. The aq. phasc is reextracted with 200 ml of $CH_2Cl_2$, the combincd org. phases dried over $Na_2SO_4$, filtered, and concentrated. The residue (38 g) is chromatographed on 2 kg of silica gel using 5% MeOH/$CH_2Cl_2$ to yield 30.6 g (87%) of **10** as a colourless oil, $[\alpha]_D^{20} = -44.5°$ (*c* = 1.0, $CHCl_3$). IR ($CHCl_3$): 3350*w*, 3000*m*, 1740*s*, 1660*s*, *etc.* $^1$H-NMR ((D)$_6$DMSO, 360 MHz, 20°): 0.80, 0.84 (2*d*, *J* = 6, 6H, 2$CH_3$–C(4$^1$)); 1.26 (*m*, 2H, 2H–C(3$^1$)); 1.31 (*d*, *J* = 6, 3H, $CH_3$–C(2$^2$)); 1.68 (*m*, 1H, H–C(4$^1$)); 1.80 (*s*, 1H, H–N$^1$); 2.16 (*s*, 3H, $CH_3$–N$^1$); 2.90 (*t*, *J* = 6, 1H, H–C(2$^1$)); 4.40 (*m*, 1H, H–C(2$^2$)); 5.10, 5.15 (2*d*, *J* = 12, 2H, PhC$H_2$); 7.36 (*m*, 5H, *Ph*$CH_2$); 8.29 (*d*, *J* = 6, 1H, H–N$^2$). $^1$H-NMR ($CDCl_3$, 90 MHz): 0.93, 0.96 (2*d*, *J* = 6, 6H, 2$CH_3$–C(4$^1$)); 1.42 (*d*, *J* = 7, 3H, $CH_3$–C(2$^2$)); 1.15–1.85 (*m*, 4H, 2H–C(3$^1$), H–C(4$^1$), H–N$^1$); 2.39 (*s*, 3H, $CH_3$–N$^1$); 3.0 (*m*, 1H, H–C(2$^1$)); 4.70 (*quint.* *J* = 8, 1H, H–C(2$^2$)); 5.20 (*s*, 2H, PhC$H_2$); 7.38 (*s*, 5H, *Ph*$CH_2$); 7.67 (*d*, *J* = 8, 1H, H–N$^2$). MS (LR): 306 (*M*$^+$), 291, 263, 249, 234, 199, 171, 142, 113, 100, 91, 57 *etc.* Anal. calc. for $C_{17}H_{26}N_2O_3$ (306.407): C 66.6, H 8.6, N 9.1, O 15.7; found: C 66.4, H 8.6, N 9.0, O 16.0.

5.5. N-(tert-*Butoxycarbonyl*)-L-*valyl*-N-*methyl*-L-*leucyl*-L-*alanine Benzyl Ester* (= *Boc-Val-MeLeu-Ala-OBzl*; **12**). At −20°, 12.9 ml (12.7 g; 106 mmol) of pivaloyl chloride and 23.3 ml (21.4 g; 212 mmol) of MeMorph are added to a solution of 23.0 g (106 mmol) of Boc-Val-OH (**11**) in 400 ml of $CHCl_3$ (precooled to −20°), and the mixture is stirred for 3h at −20° excluding moisture. Then, 27.23 g (89 mmol) of **10** in 100 ml of $CHCl_3$ is added dropwise and the mixture stirred for a further 18 h at −20°. The solution obtained is worked up as in *Chap. 5.1* (sat. $NaHCO_3$ instead of $K_2CO_3$, $Na_2SO_4$ instead of $K_2CO_3$) and the residue (52 g) chromatographed on 2 kg of silica gel with 1% MeOH/$CH_2Cl_2$ to yield 39.4 g (87.7%) of **12** as a colourless oil, $[\alpha]_D^{20} = -97.2°$ (*c* = 1.0, $CHCl_3$). CD (MeOH, *Uvasol*): $[\theta]_{229}^{25} = -28500$ (*w* = 22 nm). IR ($CHCl_3$): 3450*m*, 3320*w*, 1740*s*, 1715*s*, 1680*s*, 1640*s*. $^1$H-NMR ((D$_6$)DMSO, 360 MHz, 150°): 0.86 (*m*, 12H, 2$CH_3$–C(3$^1$), 2$CH_3$–C(4$^2$)); 1.31 (*d*, *J* = 6, 3H, $CH_3$–C(2$^3$)); 1.38 (*s*, 9H, O*t*Bu); 1.50 (*m*, 2H, 2H–C(3$^2$)); 1.69 (*m*, 1H, H–C(4$^2$)); 1.99 (*m*, 1H, H–C(3$^1$)); 2.92 (*s*, 3H, $CH_3$–N$^2$); 4.25 (*m*, 1H, H–C(2$^1$)); 4.39 (*m*, 1H, H–C(2$^3$)); 4.94 (br. *s*, 1H, H–C(2$^2$)); 5.10, 5.13 (2*d*, *J* = 12, 2H, PhC$H_2$); 5.90 (br. *s*, 1H, H–N$^1$); 7.32 (*m*, 5H, *Ph*$CH_2$) 7.45 (br. *s*, 1H, H–N$^3$). MS (LR): 505 (*M*$^+$), 440, 449, 432, 393, 345, 326, 307, 299, 277, 271, 253, 243, 225, 199, 184, 172, 156, 144, 128, 116, 100, 91, 72, 57. Anal. calc. for $C_{27}H_{43}N_3O_6$ (505.66): C 64.1, H 8.6, N 8.3, O 19.0; found: C 63.8, H 8.5, N 8.1, O 19.5.

5.6. L-*Valyl*-N-*methyl*-L-*leucyl*-L-*alanine Benzyl Ester* (= *H-Val-MeLeu-Ala-OBzl*; **13**). At −20°, 30.7 g (60.8 mmol) of **12** (precooled to −20°) are dissolved in 100 ml of $CF_3COOH$ (precooled to −20°) and stirred for 4 h at −20°. Workup and extraction of the cold mixture as in *Chap. 5.4* yield 23.8 g (96.7%) of **13** as a pale yellow oil, $[\alpha]_D^{20} = -102°$ (*c* = 1.0, $CHCl_3$). IR ($CHCl_3$): 3400*w*, *3345w*, 1740*m*, *1680s*, 1640*s*. $^1$H-NMR ($CDCl_3$, 90 MHz): 0.95 (*m*, 12H, 2$CH_3$–C(3$^1$) 2$CH_3$–C(4$^2$)); 1.34, 1.36 (2*d*, *J* = 7, 3H, $CH_3$–C(2$^3$)); 1.55 (*s*, 2H, 2H–N$^1$); 1.45–2.40 (*m*, 4H, 2H–C(3$^2$), H–C(4$^2$), H–C(3$^1$)); 2.78, 2.94 (2*s*, 3H, $CH_3$–N$^2$); 3.22 (*d*, *J* = 9) and 3.50 (*d*, *J* = 6, 1H, H–C(2$^1$)); 4.60 (*m*, 1H, H–C(2$^3$)); 5.20 (*m*, 3H, PhC$H_2$, H–C(2$^2$)); 6.63 (*d*, *J* = 7) and 9.38

(*d*, $J$ = 7, 1H, H–N$^3$); 7.36 (*s*, 5H, *Ph*CH$_2$). $^1$H-NMR ((D$_6$)DMSO, 360 MHz, 150°): 0.88 (*s*, 12H, 2CH$_3$–C(3$^1$), 2CH$_3$–(4$^2$)); 1.30 (*d*, $J$ = 6, 3H, CH$_3$–C(2$^3$)); 1.40–1.60 (*m*, 4H, 2H–C(3$^2$), 2H–N$^1$); 1.70 (*m*, 1H, H–C(4$^2$)); 1.82 (*m*, 1H, H–C(3$^1$)); 2.84 (*s*, 3H, CH$_3$–N$^2$); 3.39 (*d*, $J$ = 6, 1H, H–C(2$^1$)); 4.38 (*m*, 1H, H–C(2$^3$)); 4.90 (br. *s*, 1H, H–C(2$^2$)); 5.10, 5.12 (2*d*, $J$ = 12, 2H, PhC*H*$_2$); 7.33 (*s*, 5H, *Ph*CH$_2$); 7.45–7.85 (br. *s*, 1H, H–N$^3$). MS (LR): 405 ($M^+$), 390, 348, 334, 317, 306, 277, 234, 199, 184, 170, 141, 127, 100, 91, 72, 51, *etc.* Anal. calc. for $C_{22}H_{35}N_3O_4$ (405.538): C 65.2, H 8.7, N 10.4, O 15.8; found: C 64.8, H 8.7, N 10.2, O 16.0.

5.7. N-(tert-*Butoxycarbonyl*)-N-*methyl*-L-*leucyl*-L-*valyl*-N-*methyl*-L-*leucyl*-L-*alanine Benzyl Ester* (= *Boc-MeLeu-Val-MeLeu-Ala-OBzl*; **14**). At – 20°, 8.5 ml (8.3 g; 69.5 mmol) of pivaloyl chloride and 12.7 ml (11.7 g, 115.8 mmol) of MeMorph are added to a solution of 15.6 g (63.7 mmol) of Boc-MeLeu-OH (**7**) in 500 ml of $CHCl_3$ (precooled to – 20°), and the mixture is stirred at – 20° for 4 h. The formation of the anhydride is followed by IR and is complete after 4 h (IR ($CHCl_3$; after 3½ h): 1805*s*, 1740*w*, 1695*s*, 1040*s*, 1015*s*). Then, 23.45 g (57.9 mmol) of **13** in 100 ml of $CHCl_3$ are added, and the mixture is stirred for a further 15 h at – 20°, excluding moisture. The resulting solution (IR: practically no anhydride absorption at 1805, 1040, and 1015, but new amide peaks at 1640 and 1510), is worked up as in *Chap. 5.1* (sat. $NaHCO_3$ instead of $K_2CO_3$, $Na_2SO_4$ instead of $K_2CO_3$) and the residue (74 g) chromatographed on 2 kg of silica gel with 2% MeOH/$CH_2Cl_2$ to yield 34.2 g (94%) of **14** as a pale yellow oil, $[\alpha]_D^{20} = -126{,}7°$ ($c$ = 1.0, $CHCl_3$); IR ($CHCl_3$): 3400*m*, 3275*w*, 1740*s*, 1680*s*, 1640*s*. $^1$H-NMR ((D$_6$)DMSO, 360 MHz, 180°): 0.80–0.97 (6*d*, *J ca.* 6, 18H, 2CH$_3$–C(4$^1$), 2CH$_3$–C(3$^2$), 2CH$_3$–C(4$^3$)); 1.31 (*d*, $J$ = 6, 3H, CH$_3$–C(2$^4$)); 1.45 (*s*, 9H, O*t*Bu); 1.52, 1.65, 1.70 (3*m*, 6H, 2H–C(3$^1$), 2H–C(3$^3$), H–C(4$^1$), H–C(4$^3$)); 2.03 (*m*, 1H, H–C(3$^2$)); 2.73, 2.92 (2*s*, 6H, CH$_3$–N$^1$, CH$_3$–N$^3$); 4.40 (*m*, 1H, H–C(2$^4$)); 4.50 (*t*, $J$ = 6, 1H, H–C(2$^3$)); 4.62 (*t*, $J$ = 6, 1H, H–C(2$^2$); on addition of $D_2O$, *d* at 4.6, $J$ = 6); 4.95 (*t*, $J$ = 6, 1H, H–C(2$^1$)); 5.10, 5.15 (2*d*, $J$ = 12, 2H, PhC*H*$_2$); 6.84 (*d*, $J$ = 6, H–N$^{2\text{ or }4}$); 7.32 (*s*, 6H, *Ph*CH$_2$, H–N$^{4\text{ or }2}$). $^1$H-NMR ((D$_6$)DMSO, 360 MHz, 20°): 2 conformers. $^1$H-NMR ($CDCl_3$, 90 MHz, 1 main conformer): 0.80–1.0 (6*d*, $J$ = 6, 18H, 2CH$_3$–C(4$^1$), 2CH$_3$–C(3$^2$), 2CH$_3$–C(4$^3$)); 1.38, 1.42 (2*d*, $J$ = 7, 3H, 4:1, CH$_3$–C(2$^4$)); 1.50 (*s*, 9H, O*t*Bu); 1.1–1.90 (*m*, 6H, 2H–C(3$^1$), 2H–C(3$^3$), H–C(4$^1$), H–C(4$^3$)); 2.03 (*m*, 1H, H–C(3$^2$)); 2.78, 3.01, 2.74 (10%), 2.85 (10%) (4*s*, 6H, CH$_3$–N$^1$, CH$_3$–N$^3$); 4.40–4.92 (*m*, 3H, H–C(2$^2$), H–C(2$^3$), H–C(2$^4$)); 5.0–5.3 (*m*, 1H, H–C(2$^1$)); 5.2 (*s*, 2H, PhC*H*$_2$); 6.50, 6.78 (2*d*, $J$ = 8, 2H, H–N$^2$, H–N$^4$); 7.34 (*s*, 5H, *Ph*CH$_2$). MS (LR): 632 ($M^+$), 617, 576, 532, 489, 453, 426, 380, 354, 327, 307, 271, 253, 228, 211, 200, 184, 172, 150, 144, 128, 100, 91, 72, 57, *etc.* Anal. calc. for $C_{34}H_{56}N_4O_7$ (632.848): C 64.5, H 8.9, N 8.9, O 17.7; found: C 64.3, H 8.8, N 8.7, O 18.0.

5.8. N-*Methyl*-L-*leucyl*-L-*valyl*-N-*methyl*-L-*leucyl*-L-*alanine Benzyl Ester* (= *H-MeLeu-Val-MeLeu-Ala-OBzl*; **15**). At – 20°, 31.28 g (49.5 mmol) of **14** are precooled, dissolved in 150 ml of $CF_3COOH$ (precooled to – 20°), and stirred for 16 h at – 20°. The cold mixture is worked up as in *Chap. 5.4* and the residue crystallized from $Et_2O$/hexane to yield 25.8 g (98%) of **15**, m.p. 76–78°, $[\alpha]_D^{20} = -130.9°$ ($c$ = 1.0, $CHCl_3$). IR ($CHCl_3$): 3410*m*, 3350*m*, 1745*s*, 1680*s*, 1660*s*. $^1$H-NMR ((D$_6$)DMSO, 360 MHz, 150°): 0.80–0.95 (6*d*, *J ca.* 6, 18H, 2CH$_3$–C(4$^1$), 2CH$_3$–C(3$^2$), 2CH$_3$–C(4$^3$)); 1.30 (*d*, $J$ = 6, 3H, CH$_3$–C(2$^4$)); 1.38, 1.49 (2*m*, 4H, 2H–C(3$^1$), 2H–C(3$^3$)); 1.73 (*m*, 2H, H–C(4$^1$), H–C(4$^3$)); 2.09 (*m*, 1H, H–C(3$^2$)); 2.25 (*s*, 3H, CH$_3$–N$^1$); 2.50 (br. *s*, 1H, H–N$^1$); 2.93 (*s*, 3H, CH$_3$–N$^3$); 2.96 (*t*, $J$ = 6, 1H, H–C(2$^1$)); 4.40 (*m*, 1H, H–C(2$^4$)); 4.63 (*d*, $J$ = 6, H–C(2$^2$)); 4.97 (*t*, $J$ = 6, 1H, H–C(2$^3$)); 5.10, 5.15 (2*d*, $J$ = 9, 2H, PhC*H*$_2$); 7.20–7.40 (br. *s*, 2H, H–N$^2$, H–N$^4$); 7.32 (*s*, 5H, *Ph*CH$_2$. MS (LR): 532 ($M^+$), 489, 434, 390, 338, 305, 254, 211, 155, 100, 44, *etc.* Anal. calc. for $C_{29}H_{48}N_4O_5$ (532.73): C 65.4, H 9.1, N 10.5, O 15.0; found: C 65.2, H 8.8, N 10.4, O 15.0.

5.9. N-(tert-*Butoxycarbonyl*)-L-*2-aminobutyryl-sarcosyl*-N-*methyl*-L-*leucyl*-L-*valyl*-N-*methyl*-L-*leucyl*-L-*alanine Benzyl Ester* (= *Boc-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl*; **16**). To a solution of 6.9 g (25.2 mmol) of Boc-Abu-Sar-OH (**6**) in 250 ml of $CHCl_3$, 3.4 ml (3.3 g; 27.7 mmol) of pivaloyl chloride and 5.3 g (52.9 mmol) of MeMorph are added, and the mixture is stirred for 1 h at r.t. The formation of the anhydride was followed by (IR (after 45 min): 3400*w*, 2945*s*, 2800*w*, 2540*m*, 2400*s*, 1800*s*, 1720*w*, 1700*s*, 1640*s*, 1480*s*, 1395*s*, 1365*s*, 1240–1200*s*, 1060*s*, 1040*s*, 1005*s*, 950*w*, 830*w*). Then, 12.1 g (22.7 mmol) of **15** in 100 ml of $CHCl_3$ is added and the mixture stirred a further 15 h at r.t. under $N_2$ (no more anhydride absorption in the IR and no starting material on TLC). The solution is worked up as in *Chap. 5.1* (200 ml of 2N HCl, sat. $NaHCO_3$ instead of $K_2CO_3$, $Na_2SO_4$ instead $K_2CO_3$) and the residue (25 g) chromatographed on 2 kg of silica gel with 2% MeOH/$CH_2Cl_2$ to yield 15.75 g (88%) of **16** as a colourless oil, $[\alpha]_D^{20} = -137.9°$ ($c$ = 1.0, $CHCl_3$). CD (MeOH, *Uvasol*): $[\theta]_{226.5}^{23.5} = -80\,000$ ($w$ = 24 nm), $[\theta]_{194}^{23.5} = -21\,000$ ($w$ = 14 nm); $[\theta]_{204}^{23.5} = -12\,500$ (neg. min.). IR ($CHCl_3$): 3400*w*, 3300*w*, 1740*m*, 1710*m*, 1690*m*. $^1$H-NMR ((D)$_6$DMSO or $CDCl_3$, 360 MHz, 20°): at least 3 conformers. $^1$H-NMR ((D)$_6$DMSO, 360 MHz, 180°): 0.80 (*m*, 21H); 1.31 (*d*, $J$ = 6); 1.39 (*s*, 9H, O*t*Bu); 1.51, 1.68 (2*m*, 8H); 2.65 (*m*, 1H, H–C(3$^4$)); 2.88, 2.92, 2.99 (3*s*, 9H, 3 CONMe); 4.18, 4.30 (2*d*, $J$ = 15, 2H, 2H–C(2$^2$)); 4.38 (2*m*, 2H, H–C(2$^1$), H–C(2$^6$)); 4.57 (*dd*, $J$ = 9, 6, 1H, H–C(2$^4$)); 4.52, 4.98 (2 br. *s*, 2H, H–C(2$^3$), H–C(2$^5$)); 5.10, 5.15 (2*d*, $J$ = 9, 2H, PhC*H*$_2$); 5,85 (br. *s*, 1H,H–N$^1$); 7.25, 7.48 (2 br. *s*, 2H, H–N$^4$, H–N$^6$); 7.34 (*s*, 5H,

*Ph*$CH_2$). MS (LR): 788 ($M^+$), 732, 688, 645, 632, 609, 582, 536, 483, 452, 427, 395, 384, 328, 307, 298, 284, 257, 225, 201, 170, 157, 142, 100, 91, *etc.* Anal. calc. for $C_{41}H_{68}N_6O_9$ (789.034): C 62.4, H 8.7, N 10.7, O 18.2; found: C 62.3, H 8.4, N 10.5, O 18.6.

5.10. *L-2-Aminobutyryl-sarcosyl-*N*-methyl-*L*-leucyl-*L*-valyl-*N*-methyl-*L*-leucyl-*L*-alanine Benzyl Ester ( = H-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl;* **17***).* At $-20°$, 15.70 g (19.9 mmol) of **16** (precooled to $-20°$), are dissolved in 75 ml of $CF_3COOH$ (precooled to $-20°$) and stirred for 15 h at $-20°$. The cold mixture is worked up as in *Chap. 5.4* (200 ml of sat. $NaHCO_3$, twice 300 ml of $CH_2Cl_2$) and the residue chromatographed on 500 g of silica gel with 10% MeOH/$CH_2Cl_2$ to yield 11.17 g (81.5%) of **17** as colourless oil, $[\alpha]_D^{20} = -133.3°$ ($c = 1.0$, $CHCl_3$). CD (MeOH, *Uvasol*): $[\theta]_{226}^{23.5} = -81500$ ($w = 38$ nm); $[\theta]_{200}^{23.5} = +10500$ ($w = 10$ nm). IR ($CHCl_3$): 3650*w*, 3400*w*, 3280*m*, 1740*m*, 1680*s*, 1640*s*. $^1$H-NMR ($(D)_6$DMSO, 360 MHz, 180°): 0.89 (*m*, 21H, $CH_3$–C($3^1$), $2CH_3$–C($4^3$), $2CH_3$–C($3^4$), $2CH_3$–C($4^5$)); 1.32 (*d*, $J = 6$, 3H, $CH_3$–C($2^6$)); 1.42, 1.53, 1.72 (3*m*, 8H, 2H–C($3^1$), 2H–C($3^3$), H–C($4^3$), 2H–C($3^5$), H–C($4^5$)); 2.06 (*m*, 1H, H–C($3^4$)); 2.60 (br. *s*, 2H, 2H–$N^1$); 2.88, 2.91, 2.97 (3*s*, 9H, $CH_3$–$N^2$, $CH_3$–$N^3$, $CH_3$–$N^5$); 3.54 (*t*, $J = 6$, 1H, H–C($2^1$)); 4.19, 4.29 (2*d*, $J = 18$, 2H, 2H–C($2^2$)); 4.40 (*m*, 1H, H–C($2^6$)); 4.60 (*t*, $J = 6$, 1H, H–C($2^4$)); 4.82, 4.97 (2*t*, $J = 6$, 2H, H–C($2^3$), H–C($2^5$)); 5.11, 5.16 (2*d*, $J = 9$, 2H, Ph*C*$H_2$); 7.11, 7.32 (2 br. *s*, 2H, H–$N^4$, H–$N^6$); 7.32 (*s*, 5H, *Ph*$CH_2$). MS (LR): 688 ($M^+$), 670, 627, 614, 533, 492, 453, 383, 365, 354, 323, 307, 284, 266, 238, 222, 208, 180, 169, 148, 126, 100, 91, 72, *etc.* Anal. calc. for $C_{36}H_{60}N_6O_7$ (688.916): C 62.8, H 8.8, N 12.2, O 16.3; found: C 62.8, H 8.6, N 12.3, O 16.8.

5.11. *(4S,5R,1′R,3′E)-2,2,3-Trimethyl-5-(1′-methyl-3′-pentenyl)-4-oxazolidinecarboxylic Acid ( =* N,O*-isopropylidene-MeBmt-OH;* **18***).* A suspension of 201 mg (1 mmol) of (2*S*,3*R*,4*R*,6*E*)-3-hydroxy-4-methyl-2-methylamino-6-octenoic acid[6][14] in 80 ml of anh. acetone is heated under reflux for 24 h until a clear solution is obtained. The acetone is concentrated to 3 or 4 ml under vacuum and the remaining solution of **18** used directly for synthetics purposes without further purification. (If necessary this solution of **18** can be stabilized for storage by adding 1 equiv. of MeMorph). The MeMorph salt of **18** is also suitable for $^1$H-NMR measurements. $^1$H-NMR ($CDCl_3$, 360 MHz): 0.84 (*d*, $J = 6$, 3H, $CH_3$–C(1′)); 1.27, 1.42 (2*s*, 6H, $2CH_3$–C(2)); 1.65 (*d*, $J = 3$, 3H, $CH_3$–C(4′)); 1.75, 1.85, 2.25 (3*m*, 3H, H–C(1′), 2H–C(2′)); 2.40 (1*s*, 3H, $CH_3$–N (1 equiv. MeMorph)); 2.44 (1*s*, 3H, $CH_3$–N(3)); 2.62 (br. *s*, 4H, $2CH_2$–N (1 equiv. MeMorph)); 3.26 (*d*, $J = 6$, 1H, H–C(4)); 3.77 (*m*, 4H, $2CH_2$–O (1 equiv. MeMorph)); 3.98 (*t*, $J = 6$, 1H, H–C(5)); 5.44 (*m*, 2H, H–C(3′), H–C(4′)); 7.7 (br. *s*, 2H, $2HN^+$).

5.12. *((4S,5R,1′R,3′E)-2,2,3-Trimethyl-5-(1′-methyl-3′-pentenyl)-4-oxazolidincarbonyl)-L-2-aminobutyryl-sarcosyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl-L-alanine Benzyl Ester ( =* N,O*-Isopropylidene-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl;* **19***).* A solution of 1.5 g (6.22 mmol) of freshly prepared **18** in 5 ml acetone is diluted in 50 ml THF, and 0.7 g (6.93 mmol) of MeMorph are immediately added. Then, 1.67 g (12.4 mmol) of *N*-hydroxy-benzotriazole (Bt-OH) are dehydrated (by azeotropic distillation of $H_2O$ with two 50-ml portions of toluene) and added to the solution together with 4.28 g (6.22 mmol) of **17**. The resulting mixture is cooled to 0°, and 1.34 g (6.5 mmol) of DCCI are added. The mixture is allowed to warm to r.t., stirred for 15 h at 20°, diluted with 300 ml of $CH_2Cl_2$ and shaken with 200 ml of 1N $NaHCO_3$. The aq. phase is reextracted with 200 ml of $CH_2Cl_2$ and the combined org. phases dried over $Na_2SO_4$, filtered, and evaporated. The residue (7.8 g) is chromatographed on 500 g of silica gel using 3% MeOH/$CH_2Cl_2$ to yield 4.8 g (84.7%) of **19**, $[\alpha]_D^{20} = -126°$ ($c = 1.0$, $CHCl_3$). CD (MeOH, *Uvasol*): $[\theta]_{226}^{22} = -90600$ ($w = 26$ nm). IR ($CHCl_3$): 3400*w*, 3350*w*, 3275*w*, 2950*m*, 2925*m*, 2850*w*, 1740*m*, 1680*s*, 1640*s*, 1500*m*, 1480*m*, 1460*m*, 1400*w*, 1385*w*, 1365*w*, 1340*w*, 1300*w*, 1250*w*, 1200*m*, 1150*m*, 1090*w*, 940*w*, 900*w*. $^1$H-NMR ($(D)_6$DMSO, 360 MHz, 20°): many conformers. $^1$H-NMR ($(D_6)$DMSO, 360 MHz, 170°): 0.87 (*m*, 24H, $CH_3$–C($4^1$), $CH_3$–C($3^2$), $2CH_3$–C($4^4$), $2CH_3$–C($3^5$), $2CH_3$–C($4^6$)); 1.17 (*s*, 6H, O–C$(CH_3)_2$–N); 1.29 (*d*, $J = 6$, 3H, $CH_3$–C($2^7$)); 1.54, 1.70, 1.80 (3*m*, 11H, H–C($4^1$), 2H–C($5^1$), 2H–C($3^2$), 2H–C($3^4$), H–C($4^4$), 2H–C($3^6$), H–C($4^6$)); 1.60 (*s*, 3H, 3H–C($8^1$)); 2.04 (*m*, 1H, H–C($3^5$)); 2.26 (*s*, 3H, $CH_3$–$N^1$); 2.89, 2.93, 2.98 (3*s*, 9H, $CH_3$–$N^3$, $CH_3$–$N^4$, $CH_3$–$N^6$); 3.10 (*d*, $J = 6$, 1H, H–C($2^1$)); 3.69 (*dd*, $J = 6$, 15, 1H, H–C($3^1$)); 4.20, 4.32 (2*d*, $J = 18$, 2H, 2H–C($2^3$)); 4.40 (*m*, 1H, H–C($2^7$)); 4.60 (*dd*, $J = 6$, 15, 1H, H–C($2^5$)); 4.71 (*t*, $J = 6$, 1H, H–C($2^2$)); 4.81, 4.97 (2*t*, $J = 6$, H–C($2^4$), H–C($2^6$)); 5.11 (*s*, 2H, Ph*C*$H_2$); 5.40 (*m*, 2H, H–C($6^1$), H–C($7^1$)); 7.15, 7.30 (2 br. *s*, 2H, H–$N^2$, H–$N^5$); 7.56 (*d*, $J = 6$, 1H, H–$N^7$); 7.32 (*m*, 5H, *Ph*$CH_2$). MS (FD): 911 ($M^+$). Anal. calc. for $C_{49}H_{81}N_7O_9$ (912,233): C 64.5, H 9.0, N 10.7, O 15.8; found: C 64.3, H 8.9, N 10.8, O 16.0.

5.13. *((2S,3R,4R,6E)-3-Hydroxy-4-methyl-2-methylamino-6-octenoyl)-L-2-aminobutyryl-sarcosyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl-L-alanine Benzyl Ester ( = H-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl;* **20***).* 5.13.1. *In Methanol.* A solution of 1.5 g (1.6 mmol) of **19** in 16 ml of MeOH[30]) are stirred for 15 h at

[30]) The use of 1,4-dioxane instead of MeOH is only advantageous if traces (less than 5%) of heptapeptide methyl ester are to be excluded (see *5.13.2*).

r.t. in the presence of 1.6 ml of 1N HCl. The cleavage of the isopropylidene protecting group may be followed by TLC (silica gel, $CHCl_3$/MeOH 19:1). The acid in the reaction medium is neutralized with 1 g (12 mmol) of $NaHCO_3$ and the solvent evaporated completely taking care that the temp. does not rise above 30°. The residue is taken up in 10 ml of 5% MeOH/$CH_2Cl_2$ and chromatographed on 100 g of silica gel using 5% MeOH/$CH_2Cl_2$: 1.29 g (90%) of **20**[31]), $[\alpha]_D = -138.0°$ ($c = 1:0$, $CHCl_3$). CD (MeOH, *Uvasol*): $[\theta]_{226}^{26.5} = -85000$ ($w = 24$ nm). IR ($CH_2Cl_2$): 3400*w*, 3300*w*, 2950*m*, 2855*m*, 1740*m*, 1680–1620*s*, 1515*m*, 1500*m*, 1480*m*, 1460*m*, 1410*w*, 1300–1250*w*, 1200*w*, 1160*w*, 1100*w*, 1050*w*, 970*w*. $^1$H-NMR ((D)$_6$DMSO, 360 MHz, 180°): 0.83–0.98 (*m*, 24H, $CH_3$–C(4$^1$), $CH_3$–C(3$^2$), $2CH_3$–C(4$^4$), $2CH_3$–C(3$^5$), $2CH_3$–C(4$^6$)); 1.33 (*d*, $J = 7$, $CH_3$–C(2$^7$)); 1.56, 1.74, 1.85 (3*m*, 10H, H–C(5$^1$), H–C(4$^1$), 2H–C(3$^2$), 2H–C(3$^4$), H–C(4$^4$), 2H–C(3$^6$), H–C(4$^6$)); 1.60 (*d*, $J = 3$, 3H, $CH_3$–C(7$^1$)); 2.06 (*m*, 1H, H–C(3$^5$)); 2.28 (*m*, 1H, H–C(5$^1$)); 2.35 (*s*, 3H, $CH_3$–N$^1$); 2.40–2.88 (br. *s*, 2H, H–N$^1$, HO–C(3$^1$)); 2.89, 2.93, 2.99 (3*s*, 9H, $CH_3$–N$^3$, $CH_3$–N$^4$, $CH_3$–N$^6$); 2.96 (*d*, $J = 6$, 1H, H–C(2$^1$)); 3.43 (*t*, $J = 6$, 1H, H–C(3$^1$)); 4.20, 4.33 (2*d*, $J = 16$, 2H, 2H–C(2$^3$)); 4.42 (*m*, 1H, H–C(2$^7$)); 4.56, 4.59 (2*d*, $J = 8$, 1H, H–C(2$^5$)); 4.73, 4.81, 4.94 (3*t*, $J = 6$, 3H, H–C(2$^2$), H–C(2$^4$), H–C(2$^6$)); 5.10, 5.14 (2*d*, $J = 12$, 2H, PhC$H_2$); 5.43 (*m*, 2H, H–C(6$^1$), H–C(7$^1$)); 7.00, 7.19, 7.55 (3 br. *s*, 3H, H–N$^2$, H–N$^5$, H–N$^7$); 7.31 (*s*, 5H, *Ph*$CH_2$). $^1$H-NMR ($CDCl_3$, 360 MHz): 2 main conformers. MS (FD): 873 ($M$H$^+$). Anal. calc. for $C_{46}H_{77}N_7O_9$ (872.168): C 63.3, H 8.9, N 11.2, O 16.5; found: C 63.0, H 9.0, N 11.3, O 16.6.

5.13.2. *In Dioxane.* To a solution of 2.6 g (2.85 mmol) of **19** in 50 ml of 1,4-dioxane, 5 ml of $H_2O$ and 14.3 ml of 1N HCl are added. The resulting solution is stirred under $N_2$ for 3 days at r.t. neutralized with a small excess of solid $NaHCO_3$, and the 1,4-dioxane carefully evaporated below 40°. The residue is diluted with 50 ml of $H_2O$ and extracted 3 × with 200 ml of $CH_2Cl_2$. The org. phases are dried over $Na_2SO_4$, filtered, and evaporated. The residue (2.5 g) is chromatographed on 50 mg of silica gel using 3% MeOH/$CH_2Cl_2$ to yield 1.6 g (64%) of pure **20** $[\alpha]_D^{20} = -136.6°$ ($c = 1.0$, $CHCl_3$). MS and $^1$H-NMR: identical to those described in *5.13.1.*

**6. *N*-(*tert*-Butoxycarbonyl)-D-alanyl-*N*-methyl-L-leucyl-*N*-methyl-L-leucyl-*N*-methyl-L-valyl-((2*S*,3*R*,4*R*,6*E*)-3-hydroxy-4-methyl-2-methylamino-6-octenoyl)-L-2-aminobutyryl-sarcosyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl-L-alanine Benzyl Ester (= Boc-D-Ala-MeLeu-MeLeu-MeVal-MeB*m*t-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl; 22).**

6.1. *Starting from the* DLLL-*Tetrapeptide* **21** (see *Table 2* and *3* and *Exper. Part, Section 4*). At r.t., 278 mg (0.5 mmol) of Boc-D-Ala-MeLeu-MeLeu-MeVal-OH (**21**)[18]) followed by 479 mg (0.55 mmol) of **20** are dissolved in 10 ml of $CH_2Cl_2$. Then, 0.55 ml (50.5 mg; 0.5 mmol) of MeMorph in 1 ml of $CH_2Cl_2$ and 221 mg (0.5 mmol) of Bt-OP(NMe$_2$)$_3^+$ $PF_6^-$ are added to the solution, and the mixture is stirred for 22 h at r.t., the course of the reaction being followed to completion by TLC (silica gel, 5% MeOH/$CHCl_3$). The resulting solution is diluted with 200 ml of $CH_2Cl_2$, washed with 100 ml of $H_2O$ and the aq. phase extracted with 100 ml of $CH_2Cl_2$. The combined org. phases are dried over $Na_2SO_4$, filtered, and evaporated. The residue is chromatographed on 200 g of silica gel using 5% MeOH/$CH_2Cl_2$ to yield 513 mg (72.8%) of **22**, $[\alpha]_D^{20} = -182.0°$ ($c = 1.0$, $CHCl_3$) and 95 mg (20.0%) of **20**, $[\alpha]_D^{20} = -137.5°$ ($c = 1.0$, $CHCl_3$).

6.2. *Starting from the* DLLD-*Tetrapeptide* **23** (see *Scheme 3* and *Exper. Part, Section 4*). Using the above procedure, 556 mg (1.0 mmol) of Boc-D-Ala-MeLeu-MeLeu-D-MeVal-OH (**23**)[18]) are coupled with 871 mg (1.0 mmol) of **20** using 884 mg (2.0 mmol) of Bt-OP(NMe$_2$)$_3^+$ $PF_6^-$ to yield after 17 h and after chromatography on 200 g of silica gel 665 mg (47%) of pure **22**, $[\alpha]_D^{20} = -180.0°$ ($c = 1.0$, $CHCl_3$) and 400 mg (46%) of **20**, $[\alpha]_D^{20} = -137.5$ ($c = 1.0$, $CHCl_3$). None of the isomeric undecapeptide **24** could be isolated, and repeating the reaction gave the same result. $^1$H-NMR ((D$_6$)DMSO, 360 MHz,180°): identical to that of **22** made from **21** as described in *6.1*. Data of **22**: IR ($CH_2Cl_2$): 3420*w*, 3340*w*, 3280*w*, 2950*m*, 2860*w*, 1700*m*, 1680*m*, 1640*s*, 1540*w*, 1520*m*, 1470*m*, 1460*m*, 1410*w*, 1390*w*, 1370*w*, 1300–1240*w*, 1195*w*, 1160*w*, 1100*w*, 1050*w*, 970*w*, 845*w*. $^1$H-NMR ((D$_6$)DMSO, 360 MHz, 180°): 0.76, 0.83 (2*d*, $J = 6$, 6H, $2CH_3$–C(3$^4$)); 0.89 (*m*, 36H, $2CH_3$–C(4$^2$), $2CH_3$–C(4$^3$), $CH_3$–C(4$^5$), $CH_3$–C(3$^6$), $2CH_3$–C(4$^8$), $2CH_3$–C(3$^9$), $2CH_3$–C(4$^{10}$)); 1,20 (*d*, $J = 6$, 3H, $CH_3$–C(2$^1$)); 1.31 (*d*, $J = 6$, 3H, $CH_3$–C(2$^{11}$)); 1.38 (*s*, 9H, O*t*Bu); 1.60 (*s*, 3H, $CH_3$–C(7$^5$)); 1.54, 1.61, 1.80 (3*m*, 16H, 2H–C(3$^2$), H–C(4$^2$), 2H–C(3$^3$), H–C(4$^3$), H–C(4$^5$), H–C(5$^5$), 2H–C(3$^6$), 2H–C(3$^8$), H–C(4$^8$), 2H–C(3$^{10}$), H–C(4$^{10}$)); 2.05 (*m*, 1H, H–C(3$^9$)); 2.30 (*m*, 2H, H–C(5$^5$), H–C(3$^4$)); 2.89, 2.92, 2.99, 3.07 (4*s*, 21H, $CH_3$–N$^2$, $CH_3$–N$^3$, $CH_3$–N$^4$, $CH_3$–N$^5$, $CH_3$–N$^7$, $CH_3$–N$^8$, $CH_3$–N$^{10}$); 3.93 (*m*, 1H, H–C(3$^5$)); 4.07 (br. *s*, 1H, HO–C(3$^5$)); 4.19, 4.34 (2*d*, $J = 21$, 2H–C(2$^7$)); 4.42, 4,48 (2*m*, 2H, H–C(2$^1$), H–C(2$^{11}$)); 4.58, 4.61 (2*d*, $J = 9$, H–C(2$^9$)); 4.70 (br. *s*, 1H, H–C(2$^6$)); 4.80, 4.96 (2*t*, $J = 6$, H–C(2$^{2\text{ or }3}$), H–C(2$^{8\text{ or }10}$)); 5.01 (br. *s*, 1H, H–C(2$^5$)); 5.11 (*s*, 2H, PhC$H_2$); 5.15 (*d*, $J = 12$, H–C(2$^4$)); 5.41 (*m*, 4H, H–C(6$^5$), H–C(7$^5$), H–C(2$^{3\text{ or }2}$), H–C(2$^{10\text{ or }8}$)); 5.94 (br. *s*, 1H, H–N$^1$); 6.96, 7.11, 7.36 (3 br. *s*, 3H, H–N$^6$, H–N$^9$, H–N$^{11}$); 7.33 (*s*, 5H,

[31]) The last fraction of the chromatography (43 mg; 3%) containing mainly a heptapeptide methyl ester was discarded.

*Ph*$CH_2$). $^1$H-NMR ((D$_6$)DMSO, 360 MHz, 20°): more than 2 conformers. $^1$H-NMR ($CDCl_3$, 360 MHz, 20°; interpretation with the help of double resonance for the main conformer (more than 80%)): 0.53 (*d*, *J* = 6, 3H, $CH_3$–C($4^5$)); 0.79–1.05 (*m*, 39H, $2CH_3$–C($4^2$), $2CH_3$–C($4^3$), $2CH_3$–C($3^4$), $CH_3$–C($3^6$), $2CH_3$–C($4^8$), $2CH_3$–C($3^9$), $2CH_3$–C($4^{10}$)); 1.28, 1.30 (2*d*, *J* = 6, 6H, $CH_3$–C($2^1$), $CH_3$–C($2^{11}$)); 1.38 (*m*, 1H, H–C($4^5$)); 1.43 (*s*, 9H, O*t*Bu); 1.62 (*d*, *J* = 6, 3H, $CH_3$–C($7^5$)); 1.50–2.0 (*m*, 15H, 2H–C($3^2$), H–C($4^2$), 2H–C($3^3$), H–C($4^3$), H–C($5^5$), 2H–C($3^6$), 2H–C($3^8$), H–C($4^8$), 2H–C($3^{10}$), H–C($4^{10}$)); 2.25 (*m*, 1H, H–C($3^4$)); 2.30 (*m*, 1H, H–C($3^9$)); 2.59 (*m*, 1H, H–C($5^5$)); 3.01 (6H), 3.03, 3.09, 3.16, 3.32, 3.44 (6*s*, total 21H, $CH_3$–$N^2$, $CH_3$–$N^3$, $CH_3$–$N^4$, $CH_3$–$N^5$, $CH_3$–$N^7$, $CH_3$–$N^8$, $CH_3$–$N^9$); 3.58 (*d*, *J* = 15, 1H–C($2^7$)); 3.68 (*m*, 1H, H–C($3^5$)); 4.48 (*d*, *J* = 15, 1H–C($2^7$)); 4.50 (*m*, 1H, H–C($2^{11}$)); 4.62 (*m*, 1H, H–C($2^1$)); 4.70 (*t*, *J* = 6, 1H, H–C($2^{8\text{ or }10}$)); 4.90 (*t*, *J* = 6, 1H, H–C($2^9$)); 4.95 (*d*, *J* = 6, 1H, HO–C($3^5$)); 4.98 (*dd*, *J* = 12, 6, 1H, H–C($2^6$)); 5.08, 5.20 (2*d*, *J* = 12, 2H, Ph$CH_2$); 5.28 (*d*, *J* = 9, 1H, H–C($2^4$)); 5.35 (*d*, *J* = 6, 1H, H–$N^1$); 5.32, 5.40 (2*m*, 2H, H–C($2^2$), H–C($2^3$)); 5.51 (*m*, 3H, H–C($2^5$), H–C($6^5$), H–C($7^5$)); 7.10 (*d*, *J* = 9, 1H, H–$N^9$); 7.35 (*m*, 5H, *Ph*$CH_2$); 7.68 (*d*, *J* = 6, 1H, H–$N^{11}$); 7.86 (*d*, *J* = 9, 1H, H–$N^6$). MS (FD): 1411 ($MH^+$). Anal. cal. for $C_{74}H_{127}N_{11}O_{15}$ (1410.9): C 63.0, H 9.1, N 10.9, O 17.0; found: C 62.5, H 9.1, N 10.9, O 17.6.

**7. *N*-(*tert*-Butoxycarbonyl)-D-alanyl-*N*-methyl-L-leucyl-*N*-methyl-L-leucyl-*N*-methyl-L-valyl-((2*S*,3*R*,4*R*,6*E*)-3-hydroxy-4-methyl-2-methylamino-6-octenoyl)-L-2-aminobutyryl-sarcosyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl-L-alanine Hydrazide (= Boc-D-Ala-MeLeu-MeLeu-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-NHNH$_2$; 25).** At −20°, 0.83 g (0.59 mmol) of **22** are stirred for 5 h with 3 ml of $NH_2NH_2 \cdot H_2O$ in 3 ml of DMF. Then, the DMF and excess $NH_2NH_2 \cdot H_2O$ are evaporated at r.t. under high vacuum. The residue is dissolved in 250 ml of AcOEt and the solution shaken twice with 50 ml of sat. NaCl. The org. phase is dried over $Na_2SO_4$, filtered, and evaporated and the residue (0.79 g) chromatographed on 100 g of silica gel using 5% MeOH/$CH_2Cl_2$ to yield 0.67 g (86%) of **25**, $[a]_D^{20} = -173.0°$ (*c* = 1.0, $CHCl_3$). IR ($CH_2Cl_2$): 3425*w*, 3320*w*, 2950*m*, 2930*m*, 2875*m*, 1680*s*, 1640*m*. $^1$H-NMR ((D$_6$)DMSO, 360 MHz, 150°): 0.74, 0.80 (2*d*, *J* = 6, 6H, $2CH_3$–C($3^4$)); 0.89 (*m*, 36H, $2CH_3$–C($4^2$), $2CH_3$–C($4^3$), $CH_3$–C($4^5$), $CH_3$–C($3^6$), $2CH_3$–C($4^8$), $2CH_3$–C($3^9$), $2CH_3$–C($4^{10}$)); 1.19, 1.21 (2*d*, *J* = 6, 6H, $CH_3$–C($2^1$), $CH_3$–C($2^{11}$)); 1.39 (*s*, 9H, O*t*Bu); 1.60 (*s*, 3H, $CH_3$–C($7^5$)); 1.50, 1.70, 1.80 (3*m*, 16H, 2H–C($3^2$), H–C($4^2$), 2H–C($3^3$), H–C($4^3$), H–C($4^5$), H–C($5^5$), 2H–C($3^6$), 2H–C($3^8$), H–C($4^8$), 2H–C($3^{10}$), H–C($4^{10}$)); 2.07 (*m*, 1H, H–C($3^9$)); 2.29 (*m*, 2H, H–C($5^5$), H–C($3^4$)); 2.90, 2.99, 3.08 (3*s*, 21H, $CH_3$–$N^2$, $CH_3$–$N^3$, $CH_3$–$N^4$, $CH_3$–$N^5$, $CH_3$–$N^7$, $CH_3$–$N^8$, $CH_3$–$N^{10}$); 3.92 (*m*, 1H, H–C($3^5$)); 4.05 (br. *s*, 1H, HO–C($3^5$)); 4.18, 4.32 (2*d*, *J* = 15, 2H, 2H–C($2^7$)); 4.33 (*m*, 1H, H–C($2^{11}$)); 4.46 (*m*, 1H, H–C($2^1$)); 4.59 (*t*, *J* = 6, H–C($2^9$)); 4.70 (br. *s*, 1H, H–C($2^6$)); 4.81, 4.96 (2*t*, *J* = 6, H–C($2^{8\text{ or }2}$), H–C($2^{10\text{ or }3}$)); 5.03 (br. *s*, 1H, H–C($2^5$)); 5.12 (*d*, *J* = 12, H–C($2^4$)); 5.41 (*m*, 2H, H–C($6^5$), H–C($7^5$)); 5.38, 5.46 (2*t*, *J* = 6, 2H, H–C($2^{2\text{ or }8}$), H–C($2^{3\text{ or }10}$)); 6.04 (br. *s*, 1H, H–$N^1$); 7.03, 7.25 (2 br. *s*, 2 and 1H, H–$N^6$, H–$N^9$, H–$N^{11}$); 8.53 (br. *s*, 1H, CON*H*$NH_2$); 7.0–8.5 (br., 2H, CONHN$H_2$). MS (FD): 1335 ($MH^+$), 1123.

**8. D-Alanyl-*N*-methyl-L-leucyl-*N*-methyl-L-leucyl-*N*-methyl-L-valyl-((2*S*,3*R*,4*R*,6*E*)-3-hydroxy-4-methyl-2-methylamino-6-octenoyl)-L-2-aminobutyryl-sarcosyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl-L-alanine Hydrazide (= H-D-Ala-MeLeu-MeLeu-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-NHNH$_2$; 26).** At −20°, 0.73 g (0.55 mmol) of **25** are stirred for 5 h in 3 ml of $CF_3COOH$ under anh. conditions. Then, the solvent is evaporated at 0° (water-pump vacuum) and the residue dissolved in 200 ml of $CH_2Cl_2$ and shaken immediately with 100 ml of sat. $NaHCO_3$. The org. phase is dried over $Na_2SO_4$, filtered, and evaporated. The residue is chromatographed on 100 g of silica gel using 10% MeOH/$CH_2Cl_2$ to yield 0.64 g (94.3%) of **26** as a colourless oil, $[a]_D^{20} = -189.2°$ (*c* = 1.0, $CHCl_3$). IR ($CH_2Cl_2$): 3440*w*, 3305*m*, 2960*m*, 2875*m*, 1695*w*, 1680*w*, 1640*s*, 1535*m*, 1520*m*, 1490*m*. $^1$H-NMR ((D$_6$)DMSO, 360 MHz, 180°): 0.74 (*d*, *J* = 6, 3H, $CH_3$–C($3^4$)); 0.89 (br. *s*, 39H, $2CH_3$–C($4^2$), $2CH_3$–C($4^3$), $CH_3$–C($3^4$), $CH_3$–C($4^5$), $CH_3$–C($3^6$), $2CH_3$–C($4^8$), $2CH_3$–C($3^9$), $2CH_3$–C($4^{10}$)); 1.15, 1.22 (2*d*, *J* = 6, 6H, $CH_3$–C($2^1$), $CH_3$–C($2^{11}$)); 1.51, 1.70, 1.81 (3*m*, 16H, 2H–C($3^2$), H–C($4^2$), 2H–C($3^3$), H–C($4^3$), H–C($4^5$), H–C($5^5$), 2H–C($3^6$), 2H–C($3^8$), H–C($4^8$), 2H–C($3^{10}$), H–C($4^{10}$)); 1.60 (*s*, 3H, $CH_3$–C($7^5$)); 2.07 (*m*, 1H, H–C($3^9$)); 2.28 (*d*, *J* = 12, and *m*, H–C($5^5$) and H–C($3^4$), resp.); 2.48–2.82 (br., 2H, 2H–$N^1$); 2.87, 2.90, 2.91, 2.93 (6H), 2.98, 3.06 (6*s*, total 21H, $CH_3$–$N^2$, $CH_3$–$N^3$, $CH_3$–$N^4$, $CH_3$–$N^5$, $CH_3$–$N^7$, $CH_3$–$N^8$, $CH_3$–$N^{10}$); 3.85 (*m*, 1H, H–C($2^1$)); 3.92 (*m*, 1H, H–C($3^5$)); 4.11 (br. *s*, 1H, HO–C($3^5$)); 4.15, 4.35 (2*d*, *J* = 15, 2H, 2H–C($2^7$)); 4.36 (*m*, 1H, H–C($2^{11}$)); 4.61 (*t*, *J* = 6, H–C($2^9$)); 4.70 (br. *s*, 1H, H–C($2^6$)); 4.80, 4.95 (2*t*, *J* = 6, H–C($2^{8\text{ or }2}$), H–C($2^{10\text{ or }3}$)); 5.03 (br. *s*, 1H, H–C($2^5$)); 5.13 (*d*, *J* = 9, 1H, H–C($2^4$)); 5.41 (*m*, 4H, H–C($6^5$), H–C($7^5$), H–C($2^{2\text{ or }8}$), H–C($2^{3\text{ or }10}$)); 7.00, 7.17 (2 br. *s*, 4H, H–$N^6$, H–$N^9$, H–$N^{11}$, CON*H*$NH_2$); 7.3–8.5 (br., 2H, CONHN$H_2$). MS (FD): 1234 ($M^+$).

**9. *N*-(*tert*-Butoxycarbonyl)-D-alanyl-*N*-methyl-L-leucyl-*N*-methyl-L-leucyl-*N*-methyl-L-valyl-((2*S*,3*R*,4*R*,6*E*)-3-hydroxy-4-methyl-2-methylamino-6-octenoyl)-L-2-aminobutyryl-sarcosyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl-L-alanine (= Boc-D-Ala-MeLeu-MeLeu-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-**

**OH; 27).** – 9.1. *Starting from* **22** *Made from the* DLLL-*Tetrapeptide* **21** *(Table 3).* At 0°, 7.1 ml of 0.2N aq. NaOH are added to a solution of 2.0 g (1.42 mmol) of **22** in 50 ml of abs. EtOH (precooled), and the mixture is allowed to stand for 24 h at 0°. The clear colourless solution is adjusted to pH 5 with a few drops of conc. AcOH and fully evaporated under vacuum (water bath at 40°). The amorphous residue is shaken with 100 ml of $H_2O$ and twice with 200 ml of $CH_2Cl_2$. The org. phases are dried over $Na_2SO_4$, filtered, and evaporated to yield 1.9 g of a white foam. The latter is chromatographed on 400 g of silica gel using 10–30% MeOH/$CH_2Cl_2$ to yield 1.32 g (70.6%) of **27**, $[\alpha]_D^{20} = -173.4°$ ($c = 1.0$, $CHCl_3$) and 0.33 g (16.5%) of **22**, $[\alpha]_D^{20} = -182.1°$ ($c = 1.0$, $CHCl_3$).

9.2. *Starting from* **22** *Made from the* DLLD-*Tetrapeptide* **23** *(Scheme 3).* Using the procedure described above, 665 mg (0.472 mmol) of **22** (made from **23**) are converted to 539 mg (86.6%) of **27**, $[\alpha]_D^{20} = -178.4°$ ($c = 1.0$, $CHCl_3$). $^1$H-NMR ((D$_6$)DMSO, 360 MHz, 180°): identical with the $^1$H-NMR of **27** obtained in *9.1.* Data of **27**. $^1$H-NMR ((D$_6$)DMSO, 360 MHz, 180°): 0.75 (*d, J* = 6, 3H, $CH_3$–C($3^4$)); 0.80–0.98 (*m,* 39H, $2CH_3$–C($4^2$), $2CH_3$–C($4^3$), $CH_3$–C($3^4$), $CH_3$–C($4^5$), $CH_3$–C($3^6$), $2CH_3$–C($4^8$), $2CH_3$–C($3^9$), $2CH_3$–C($4^{10}$)); 1.18, 1.23 (2*d, J* = 6, 6H, $CH_3$–C($2^{11}$), $CH_3$–C($2^1$)); 1.37 (*s,* 9H, O*t*Bu); 1.55, 1.70, 1.82 (3*m,* 16H, 2H–C($3^2$), H–C($4^2$), 2H–C($3^3$), H–C($4^3$), H–C($4^5$), H–C($5^5$), 2H–C($3^6$), 2H–C($3^8$), H–C($4^8$), 2H–C($3^{10}$), H–C($4^{10}$)); 1.61 (*d, J* = 3, 3H, $CH_3$–C($7^5$)); 2.08 (*m,* 1H, H–C($3^9$)); 2.28 (*d, J* = 12, and *m,* 2H, H–C($5^5$) and H–C($3^4$), resp.); 2.88, 2.90, 2.91, 2.92 (6H), 2.98, 3.06 (6*s,* total 21H, $CH_3$–$N^2$, $CH_3$–$N^3$, $CH_3$–$N^4$, $CH_3$–$N^5$, $CH_3$–$N^7$, $CH_3$–$N^8$, $CH_3$–$N^{10}$); 3.92 (*t, J* = 6, 2H, H–C($3^5$), HO–C($3^5$)); 4.04 (*m,* 1H, H–C($2^{11}$)); 4.16, 4.36 (2*d, J* = 15, 2H–C($2^7$)); 4.46 (*m,* 1H, H–C($2^1$)); 4.60 (*t, J* = 6, 1H, H–C($2^9$)); 4.68 (*m,* 1H, H–C($2^6$)); 4.80 (*t, J* = 6, H–C($2^{8\text{ or }10}$)); 4.98 (*m,* 2H, H–C($2^{10\text{ or }8}$), H–C($2^5$)); 5.11 (*d, J* = 9, 1H, H–C($2^4$)); 5.38, 5.46 (2*t, J* = 6, H–C($2^2$) H–C($2^3$)); 5.41 (*m,* 2H, H–C($6^5$), H–C($7^5$)); 5.95 (br. *s,* 1H, H–$N^1$); 7.0 and 7.15 (2 br. *s,* 2 and 1H, H–$N^6$, H–$N^9$, H–$N^{11}$); 7.20–9.0 (br., 1H, COOH). Anal. calc. for $C_{67}H_{121}N_{11}O_{15}$ (1320.768): C 60.9, H 9.2, N 11.7, O 18.2; found: C 60.5, H 9.2, N 11.5, O 18.7.

**10. D-Alanyl-*N*-methyl-L-leucyl-*N*-methyl-L-leucyl-*N*-methyl-L-valyl-((2*S*,3*R*,4*R*,6*E*)-hydroxy-4-methyl-2-methylamino-6-octenoyl)-L-2-aminobutyryl-sarcosyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl-L-alanine (= H-D-Ala-MeLeu-MeLeu-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OH; 28).** – 10.1. *From* **27** *Made from the* DLLL-*Tetrapeptide* **21** *(Table 3).* To 1.2 g (0.91 mmol) of **27** at – 20°, 20 ml of $CF_3COOH$ precooled to – 20° are added with stirring. The clear and colourless solution is stirred for a further 1 h at – 20° and the solvent evaporated at – 20° (water-pump vacuum). The remaining oil is diluted with 200 ml of $CH_2Cl_2$ and shaken with 100 ml of sat. $NaHCO_3$. The org. phase is dried over $Na_2SO_4$, filtered, and evaporated to dryness. The white foam obtained is rubbed with 25 ml of $Et_2O$ and filtered through a sintered glass filter: 0.961 g (86.6%) of **28** as an amorphous white powder, $[\alpha]_D^{20} = -203.8°$ ($c = 1.0$, $CHCl_3$).

10.2. *From* **27** *Made from the* DLLD-*Tetrapeptide* **23** *(Scheme 3).* Using the procedure described above, 535 mg (0.405 mmol) of **27** (made from **23**) are converted to 410 mg (82.9%) of **28** obtained as a white powder, $[\alpha]_D^{20} = -201.2°$ ($c = 1.0$, $CHCl_3$); $^1$H-NMR ((D$_6$)DMSO, 360 MHz, 180°) and MS (FD): identical with that of **28** made from **21**, see *10.1.* Data of **28**: $^1$H-NMR ((D$_6$)DMSO, 360 MHz, 180°): 0.75 (*d, J* = 6, 3H, $CH_3$–C($3^4$)); 0.80–0.95 (*m,* 39H, $2CH_3$–C($4^2$), $2CH_3$–C($4^3$), $CH_3$–C($3^4$), $CH_3$–C($4^5$), $CH_3$–C($3^6$), $2CH_3$–C($4^8$), $2CH_3$–C($3^9$), $2CH_3$–C($4^{10}$)); 1.13 (*d, J* = 6, 3H, $CH_3$–C($2^1$)); 1.23 (*d, J* = 6, 3H, $CH_3$–C($2^{11}$)); 1.52, 1.70 (2*m,* 15H, 2H–C($3^2$), H–C($4^2$), 2H–C($3^3$), H–C($4^3$), H–C($4^5$), 2H–C($3^6$), 2H–C($3^8$), H–C($4^8$), 2H–C($3^{10}$), H–C($4^{10}$)); 1.60 (*d, J* = 3, 3H, $CH_3$–C($7^5$)); 1.82, 2.28 (2*m,* 2H, 2H–C($5^5$)); 2.08 (*m,* 1H,H–C($3^9$)); 2.28 (*m,* 1H, H–C($3^4$)); 2.88, 2.91 (6H), 2.93 (6H), 2.98, 3.06 (5*s,* total 21H, $CH_3$–$N^2$, $CH_3$–$N^3$, $CH_3$–$N^4$, $CH_3$–$N^5$, $CH_3$–$N^7$, $CH_3$–$N^8$, $CH_3$–$N^{10}$); 3.3–3.7 (br., 4H, 3H–$N^1$, HO–C($3^5$)); 3.76 (*m,* 1H, H–C($2^1$)); 3.93 (*t, J* = 6, 1H, H–C($3^5$)); 4.07 (*m,* 1H, H–C($2^{11}$)); 4.15, 4.35 (2*d, J* = 15, 2H, 2H–C($2^7$)); 4.61 (*dd, J* = 12, 6, 1H, H–C($2^9$)); 4.69 (*m,* 1H, H–C($2^6$)); 4.81 (*t, J* = 6, 1H, H–C($2^8$)); 5.0 (*m,* 2H, H–C($2^5$), H–C($2^{10}$)); 5.13 (*d, J* = 9, 1H, H–C($2^4$)); 5.38, 5.45 (2*t, J* = 6, 2H, H–C($2^2$), H–C($2^3$)); 5.42 (*m,* 2H, H–C($6^5$), H–C($6^7$)); 7.0, 7.15 (2 br. *s,* 2 and 1H, H–$N^6$, H–$N^9$, H–$N^{11}$). MS (FD): 1221 ($MH^+$). Anal calc. for $C_{62}H_{113}N_{11}O_{13}$ (1220.644): C 61.0, H 9.3, N 12.6, O 17.1; found: C 60.8, H 9.2, N 12.5, O 17.3.

**11. *N*(*tert*-Butoxycarbonyl)-D-alanyl-*N*-methyl-L-leucyl-*N*-methyl-L-leucyl-*N*-methyl-D-valyl-((2*S*,3*R*,4*R*,6*E*)-3-hydroxy-4-methyl-2-methylamino-6-octenoyl)-L-2-aminobutyryl-sarcosyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl-L-alanine Benzyl Ester (= Boc-D-Ala-MeLeu-MeLeu-D-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl; 24).** – *Starting from the* DLLL-*Tetrapeptide* **21** *and Selectively Epimerizing the* N-*Methyl*-L-*valine Residue.* To a solution of 556 mg (1.0 mmol) of Boc-D-Ala-MeLeu-MeLeu-MeVal-OH (**21**)[18] in 5 ml of $CHCl_3$, cooled to – 25°, are added 0.231 ml (212 mg; 2.1 mmol) of MeMorph and then 0.122 ml (120 mg; 1.0 mmol) of pivaloyl chloride. The mixture is stirred at – 25° for 6 h, and then 871 mg (1.0 mmol) of **20** are added as a white powder obtained from a $CH_2Cl_2$ solution by precipitation with petroleum ether. The mixture is stirred at – 25° for a further 4 days, then diluted with 100 ml of $CHCl_3$ and shaken with 50 ml of $H_2O$. The org. phase is dried over $Na_2SO_4$, filtered, and evaporated under vacuum. The residue is chromatographed on 200 g

of silica gel using 3% MeOH/$CH_2Cl_2$ to yield 820 mg of **24** contaminated with side products of lower mol. wt., which are removed by chromatography on 100 g of *Sephadex LH20* using 0.5% MeOH/$CH_2Cl_2$ to yield 616 mg (43.7%) of pure **24**, $[\alpha]_D^{20} = -113.0°$ ($c = 0.8$, $CHCl_3$). MS (FD): 1411 ($MH^+$). Undecapeptide **24** is hydrolyzed and characterized as the acid **29** (see *Chap. 12*). Beside **24**, 403 mg (46.2%) of **20**, $[\alpha]_D^{20} = -136.1°$ ($c = 1.0$, $CHCl_3$) are recovered from the column using 10% MeOH/$CH_2Cl_2$.

**12. *N*(*tert*-Butoxycarbonyl)-D-alanyl-*N*-methyl-L-leucyl-*N*-methyl-L-leucyl-*N*-methyl-D-valyl-((2*S*,3*R*,4*R*,6*E*)-3-hydroxy-4-methyl-2-methylamino-6-octenoyl)-L-2-aminobutyryl-sarcosyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl-L-alanine (= Boc-D-Ala-MeLeu-MeLeu-D-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OH; 29).** – At 0°, 2.0 ml of 0.2N aq. NaOH are added to a solution of 518 mg (0.367 mmol) of **24** in 10 ml of abs. EtOH (precooled), and the mixture is allowed to stand for 22 h at −7° (ice-box). The solution is then adjusted to pH 3 with 1N HCl, diluted with 50 ml of $H_2O$, and extracted twice with 100 ml of $CH_2Cl_2$. The org. phases are dried over $Na_2SO_4$, filtered, and evaporated. The residue is chromatographed on 100 g of silica gel using 15% MeOH/$CH_2Cl_2$ to yield 283 mg (59%) of **29**, $[\alpha]_D^{20} = -110.0°$ ($c = 1.0$, $CHCl_3$) as a white powder and 130 mg (25%) of **24**, $[\alpha]_D^{20} = -112.5°$($c = 1.0$, $CHCl_3$). Data of **29**: $^1$H-NMR ((D$_6$)DMSO, 360 MHz, 180°; different from that of **27**): 0.80 (*d*, $J = 6$, 3H, $CH_3$–C(3$^4$)), interpretation made in analogy to **27**); 0.85–1.0 (*m*, 39H, 2$CH_3$–C(4$^2$), 2$CH_3$–C(4$^3$), $CH_3$–(3$^4$), $CH_3$–C(4$^5$), $CH_3$–C(3$^6$), 2$CH_3$–C(4$^8$), 2$CH_3$–C(3$^9$), 2$CH_3$–C(4$^{10}$)); 1.20, 1.23 (2*d*, $J = 6$, 6H, $CH_3$–C(2$^1$), $CH_3$–C(2$^{11}$)); 1.39 (*s*, 9H, O*t*Bu); 1.55, 1.75, 1.82 (3*m*, 16H, 2H–C(3$^2$), H–C(4$^2$), 2H–C(3$^3$), H–C(4$^3$), H–C(4$^5$), H–C(5$^5$), 2H–C(3$^6$), 2H–C(3$^8$), H–C(4$^8$), 2H–C(3$^{10}$), H–C(4$^{10}$)); 1.61 (*d*, $J = 3$, 3H, $CH_3$–C(7$^5$)); 2.08 (*m*, 1H, H–C(3$^9$)); 2.31 (*m*, 2H, H–C(3$^4$), H–C(5$^5$)); 2.88, 2.89, 2.91, 2.93, 2.94, 3.00, 3.08 (7*s*, 21H, $CH_3$–N$^2$, $CH_3$–N$^3$, $CH_3$–N$^4$, $CH_3$–N$^5$, $CH_3$–N$^7$, $CH_3$–N$^8$, $CH_3$–N$^{10}$); 3.92 (*t*, $J = 6$, 2H, H–C(3$^5$), HO–C(3$^5$)); 3.98 (*m*, 1H, H–C(2$^{11}$)); 4.16, 4.37 (2*d*, $J = 15$, 2H–C(2$^7$)); 4.48 (*m*, 1H, H–C(2$^1$)); 4.62 (*t*, $J = 6$, 1H, H–C(2$^9$)); 4.70 (*m*, 1H, H–C(2$^6$)); 4.80 (*t*, $J = 6$, H–C(2$^{8\text{ or }10}$)); 4.95 (*t*, $J = 6$, 1H, H–C(2$^{10\text{ or }8}$)); 4.99 (*m*, 1H, H–C(2$^5$)); 5.08 (*d*, $J = 9$, 1H, H–C(2$^4$)); 5.39, 5.42 (2*t*, $J = 6$, H–C(2$^2$), H–C(2$^3$)); 5.41 (*m*, 2H, H–C(6$^5$), H–C(7$^5$)); 5.92 (br. *s*, 1H, H–N$^1$); 7.05, 7.12 (2 br. *s*, 2 and 1H, H–N$^6$, H–N$^9$, H–N$^{11}$); 7.30–8.50 (br., 1H, COOH). Anal. calc. for $C_{67}H_{121}N_{11}O_{15}$ (1320.768): C 60.9, H 9.2, N 11.7, O 18.2; found: C 60.6, H 9.2, N 11.6, O 18.6.

**13. D-Alanyl-*N*-methyl-L-leucyl-*N*-methyl-L-leucyl-*N*-methyl-D-valyl-((2*S*,3*R*,4*R*,6*E*)-3-hydroxy-4-methyl-2-methylamino-6-octenoyl)-L-2-aminobutyryl-sarcosyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl-L-alanine (= H-D-Ala-MeLeu-MeLeu-D-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OH; 30).** – At −20°, 260 mg (0.197 mmol) of **29** are added as a powder to 2.5 ml of $CF_3COOH$ (precooled) and stirred for 2 h at −20°. The solution is poured onto ice/$H_2O$ containing 20 ml of sat. $NaHCO_3$ and the mixture extracted twice with 100 ml of $CH_2Cl_2$. The org. phases are dried over $Na_2SO_4$, filtered, and evaporated under vacuum. The residue is chromatographed on *Sephadex LH20* using 0.5% MeOH/$CH_2Cl_2$ to yield 185 mg (77%) of **30** as white foam, $[\alpha]_D^{20} = -159.5°$ ($c = 0.9$, $CHCl_3$). $^1$H-NMR ((D$_6$)DMSO, 360 MHz, 180°): 0.81 (*d*, $J = 6$, 3H, $CH_3$–C(3$^4$)); 0.83–0.98 (*m*, 39H, 2$CH_3$–C(4$^2$), 2$CH_3$–C(4$^3$), $CH_3$–C(3$^4$), $CH_3$–C(4$^5$), $CH_3$–C(3$^6$), 2$CH_3$–C(4$^8$), 2$CH_3$–C(3$^9$), 2$CH_3$–C(4$^{10}$)); 1.30 (*d*, $J = 6$, 6H, $CH_3$–C(2$^1$), $CH_3$–C(2$^{11}$)); 1.55, 1.76, 1.85 (3*m*, 16H, 2H–C(3$^2$), H–C(4$^2$), 2H–C(3$^3$), H–C(4$^3$), H–C(4$^5$), H–C(5$^5$), 2H–C(3$^6$), 2H–C(3$^8$), H–C(4$^8$), 2H–C(3$^{10}$), H–C(4$^{10}$)); 1.61 (*d*, $J = 3$, 3H, $CH_3$–C(7$^5$)); 2.07 (*m*, 1H, H–C(3$^9$)); 2.30 (*m*, 2H, H–C(5$^5$), H–C(3$^4$)); 2.75 (br. *s*, 3H, 3H–N$^1$); 2.89, 2.90, 2.96, 3.00, 3.08 (5*s*, 21H, $CH_3$–N$^2$, $CH_3$–N$^3$, $CH_3$–N$^4$, $CH_3$–N$^5$, $CH_3$–N$^7$, $CH_3$–N$^8$, $CH_3$–N$^{10}$); 3.92 (*m*, 3H, H–C(2$^1$), H–C(3$^5$), HO–C(3$^5$)); 4.15, 4.36 (*d*, $J = 15$, 2H, 2H–C(2$^7$)); 4.28 (*m*, 1H, H–C(2$^{11}$)); 4.61 (*dd*, $J = 12$, 6, 1H, H–C(2$^9$)); 4.70 (*m*, 1H, H–C(2$^6$)); 4.81 (*t*, $J = 6$, 1H, H–C(2$^8$)); 4.95 (*m*, 2H, H–C(2$^5$), H–C(2$^{10}$)); 5.10 (*d*, $J = 9$, 1H, H–C(2$^4$)); 5.41 (*m*, 4H, H–C(2$^2$), H–C(2$^3$), H–C(6$^5$), H–C(7$^5$)); 7.05, 7.10 (2 br. *s*, 2 and 1H, H–N$^6$, H–N$^9$, H–N$^{11}$). MS (FD): 1221 ($MH^+$). Anal. calc. for $C_{26}H_{113}N_{11}O_{13}$ (1220.644): C 61.0, H 9.3, N 12.6, O 17.1; found: C 60.6, H 9.3, N 12.6, O 17.4.

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