Stereoselective Synthesis of [5-[4,4,4,4′,4′,4′-Hexafluoro-*N*-(2-hydroxyethoxy)-D-valine]]- and [5-[4,4,4,4′,4′,4′-Hexafluoro-*N*-(2-hydroxyethoxy)-L-valine]cyclosporin A¹)

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Addition of various amines to the 3,3-bis(trifluoromethyl)acrylamides **10a** and **10b** gave the tripeptides **11a**–**11f**, mostly as mixtures of epimers (*Scheme 3*). The crystalline tripeptide **11f$_2$** was found to be the N-terminal (2-hydroxyethoxy)-substituted (*R*,*S*,*S*)-ester $HOCH_2CH_2O$-D-Val(F_6)-MeLeu-Ala-O^{t}Bu by X-ray crystallography. The C-terminal-protected tripeptide **11f$_2$** was condensed with the N-terminus octapeptide **2b** to the depsipeptide **12a** which was thermally rearranged to the undecapeptide **13a** (*Scheme 4*). The condensation of the epimeric tripeptide **11f$_1$** with the octapeptide **2b** gave the undecapeptide **13b** directly. The undecapeptides **13a** and **13b** were fully deprotected and cyclized to the [5-[4,4,4,4′,4′,4′-hexafluoro-*N*-(2-hydroxyethoxy)-D-valine]]- and [5-[4,4,4,4′,4′,4′-hexafluoro-*N*-(2-hydroxyethoxy)-L-valine]]cyclosporins **14a** and **14b**, respectively (*Scheme 5*). Rate differences observed for the thermal rearrangements of **12a** to **13a** and of **12b** to **13b** are discussed.

Introduction. – Cyclosporin A (**1a**), the active ingredient of both *Sandimmune*® and *Neorale*®, is a powerful immunosuppressant embodying eleven amino acids in a cyclic array (for a comprehensive review, see [1]). Seven of the amino acids are methylated at the N-atom, one of these being *N*-methyl-L-valine (amino acid 11 = AA11). One L-valine (AA5) is found among the four other amino acids that form H-bridges to give cyclosporin A a distinct structure (*Fig. 1*).

Fluorine atoms, especially as trifluoromethyl (CF_3) groups [2][3], have been introduced into many positions of bioactive molecules, *e.g.*, angiotensin [3b,g]. These groups usually resist metabolic changes leading to the prospect of prolonging the bioavailability of such modified molecules. Due to profound changes in their polarities such polyfluorinated products may have enhanced lipophilicities and show novel chemical and physiological properties.

To the best of our knowledge, F-atoms have been introduced into cyclosporine A (**1a**) in two cases only. The 1η-fluorocyclosporin A (**1f**) was obtained by *Eberle* from **1a** *via* **1b** (the known intermediate for the preparation of the main metabolite (**1c**) of cyclosporine A [4]) and by the sequence **1b** → **1d** → **1e** → **1f** [5]. The preparation of [8-(β-fluoro-D-alanine)]cyclosporin (**1g**) was reported by *Patchett et al.* [6].

We described the synthesis of (−)-(4,4,4,4′,4′,4′-hexafluoro-D-valine (D-Val(F_6)) in high enantiomer excess by means of the addition of (+)-(*R*)-1-phenylethylamine (= (+)-(αR)-α-methylbenzenemethanamine) to the α-position of benzyl β,β-bis(tri-

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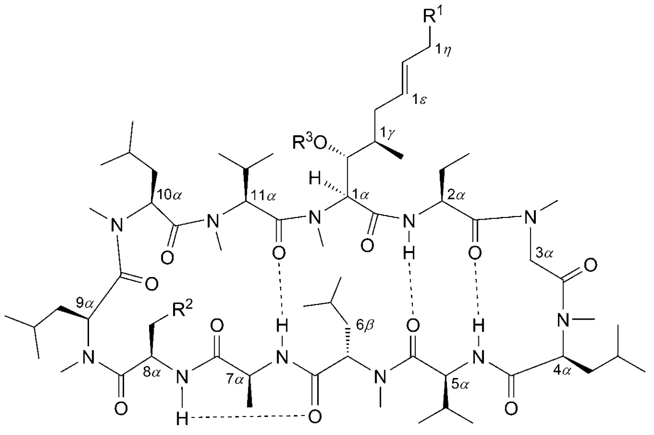
¹) This work was performed from 1998 to 2002.



	R^1	R^2	R^3
1a	H	H	H
1b	H	H	Ac
1c	OH	H	OH
1d	Br	H	Ac
1e	F	H	Ac
1f	F	H	H
1g	H	F	H

Fig. 1. *Cyclosporin* (**1a**) *and some derivatives*

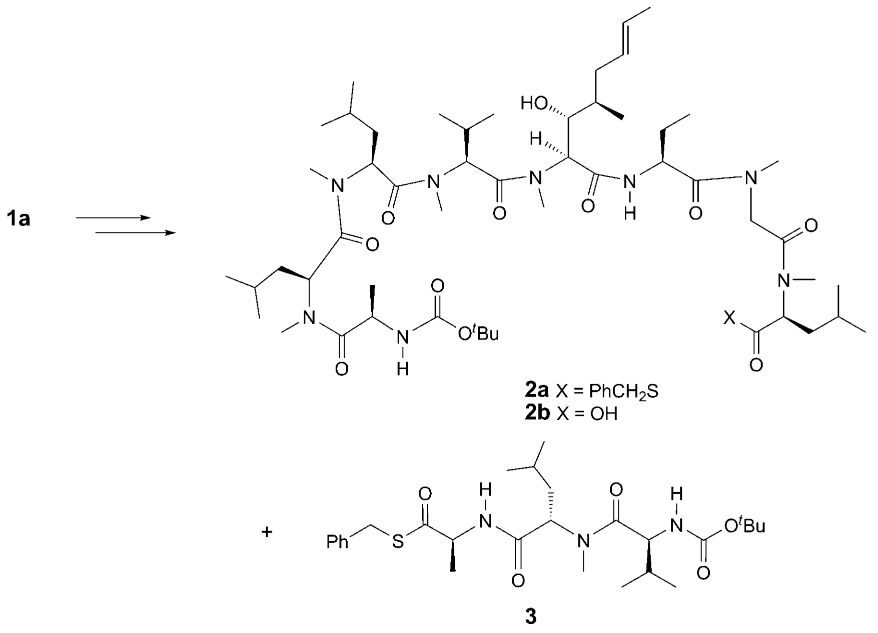
fluoromethyl)acrylate (= benzyl 4,4,4-trifluoro-3-(trifluoromethyl)but-2-enoate) as key step [7]. Similarly, (+)-4,4,4,4′,4′,4′-hexafluoro-L-valine (Val(F$_6$)) was prepared in high enantiomer excess (see below). The ready access to chiral hexafluorovaline is based on the observation by *Knunjants et al.* [3a] that β,β-bis(trifluoromethyl)acrylate reacts with NH$_3$ in the α-position of the C=C bond. Hexafluoroacetone was used as the starting material for our preparation of (*S*)-5,5,5,5′,5′,5′-hexafluoroleucine [8].

The amino acids with geminal CF$_3$ groups stimulated us to replace L-valine (AA5) in cylcosporin A (**1a**) by hexafluoro-L- and hexafluoro-D-valine. The feasibility of this plan is based on the observation [9] that the amide bonds of cyclosporin A can be cleaved selectively on the side of the N-atom of L-valine (between AA4 and AA5) or D-alanine (between AA7 and AA8) *via* thioamides and their corresponding thioamidates (pseudopeptide bonds) [9]. In the case of monothioamides, this leads to 4,5-secocyclosporin and 7,8-secocyclosporin, respectively. In the case of the bis-thioamide [$^4\Psi^5$, CS–NH; $^7\Psi^8$, CS–NH]cyclosporin, a concomitant cleavage of the corresponding bis-thioamidate leads to fragmentation of the cyclosporin producing the octapeptide **2a** and the tripeptide *S*-benzyl ester *N*-Boc-Val-MeLeu-Ala-SCH$_2$Ph (**3**; *Scheme 1*).

While *N*-methyl-L-leucine (AA6) is located in the center of the tripeptide fragment, L-valine (AA5) is strategically positioned at the N-terminus of the tripeptide **3** (*Scheme 1*). Thus, the problem of replacing L-valine (AA5) in cyclosporin A is reduced to the preparation of the tripeptide Val(F$_6$)-MeLeu-Ala as building block. [Val(F$_6$)^5]-cyclosporin would subsequently be prepared by two condensation steps between this tripeptide and the octapeptide **2b**, readily available from **2a** [9]. The protecting groups to be used during the synthesis depend on the specific strategy used for the cyclization of the linear undecapeptide to the [Val(F$_6$)^5]cyclosporin. When the cyclization is carried out between AA4 and AA5 (4/5 variant), then a benzyl ester of the tripeptide may be tolerated. However, when the cyclization is carried out between AA7 and AA8 (7/8 variant), then a *tert*-butyl ester of the tripeptide would be preferred.

The conventional ‘linear’ approach to the synthesis of the desired tripeptide would require the condensation of activated *N*-protected hexafluoro-L-valine with an ester

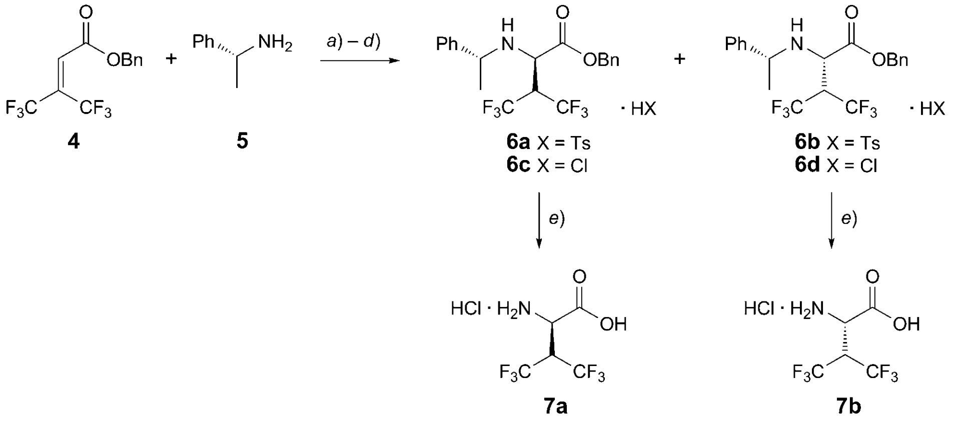
Scheme 1. *Cleavage Reaction of Cyclosporin A* (**1a**)



MeLeu-Ala-OR. In view of the rather high sensitivity of hexafluoro-D-valine benzyl ester towards Na_2CO_3, leading to rapid racemization [7], the use of activated esters of enantiomerically pure Val(F_6) was considered to give the tripeptide as a mixture of epimers. We, therefore, favored the preparation of the desired tripeptide from the N-terminal β,β-bis(trifluoromethyl)acrylamide of an ester Leu-Ala-OR[1] *via* addition of NH_3 or amines to the α-position of the C=C bond of the acrylamide moiety. This sequence is shorter by a few steps than the conventional linear approach. Furthermore, it was our hope that the chiral centers in MeLeu-Ala would enhance the chiral induction in the addition step. We considered such a selectivity to be more likely than the formation of a 1:1 mixture of diastereoisomers observed in the reaction of (1*R*)-1-phenylethylamine with benzyl β,β-bis(trifluoromethyl)acrylate [7].

Results and Discussion. – *(+)-4,4,4,4′,4′,4′-Hexafluoro-*L*-valine* (Val(F_6)). First, we prepared the hitherto unreported (+)-4,4,4,4′,4′,4′-hexafluoro-L-valine (**7b**) *via* separation of the diastereoisomers obtained from the addition of (+)-(1*R*)-1-phenylethylamine (**5**) to benzyl β,β-bis(trifluoromethyl)acrylate (**4**) [7] (*Scheme 2*). Treatment of this 1:1 mixture of diastereoisomeric adducts with TsOH in Et_2O led to **6a** and **6b** and subsequently to the rapid precipitation of the (*R*,*R*)-adduct **6a**. Hydrogenolysis of the corresponding HCl salt readily gave (−)-hexafluoro-D-valine **7a** in 38% yield and 98% ee. The (*S*,*R*)-diastereoisomer **6b** used for the preparation of (+)-hexafluoro-L-valine **7b** was obtained pure by repeated precipitation of **6a** in the presence of an excess of TsOH in Et_2O.

Scheme 2. *Synthesis of Hexafluorovalines* **7a** *and* **7b**



a) MeOH, 0° → r.t. *b*) TsOH, Et_2O. *c*) $NaHCO_3$. *d*) HCl, Et_2O. *e*) H_2, Pd/C.

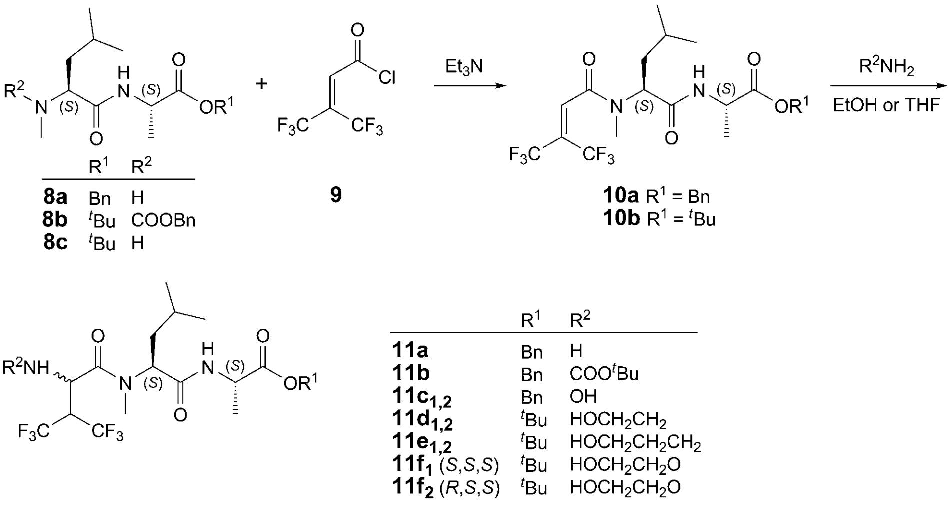
The oily (*S*,*R*)-diastereoisomer **6b** then led to the solid HCl salt **6d**, from which (+)-hexafluoro-L-valine **7b** was obtained in 29% yield and 98% ee. The stereoselective transformations of the readily available enantiomeric hexafluorovalines **7a** and **7b** as well as of the diastereoisomerically pure intermediates **6a** and **6b** are to be explored further. Obviously, (+)-hexafluoro-L-valine **7b** could be obtained more directly by starting with the addition of (−)-(1*S*)-1-phenylethylamine to **4** leading to the enantiomers of **6a** and **6b**. In this case, the TsOH addition salt of the enantiomer of **6b** should precipitate providing access to **7b** in fewer steps.

As mentioned above, the replacement of Me by CF_3 groups as, *e.g.*, in valine, should lead to a noticeable change in the chemical properties. Indeed, the lipophilicity of the hexafluorovaline anion, determined across the H_2O/nitrobenzene interface, is higher than that of the valine anion by *ca.* 8 kJ/mol [10][11].

Tripeptides. In an exploratory approach, the known dipeptide **8a** [12] was treated with 3,3-bis(trifluoromethyl)acryloyl chloride (=4,4,4-trifluoro-3-(trifluoromethyl)-but-2-enoyl chloride; **9**) [3a] to give the *N*-acylated dipeptide **10a** as a low-melting yellow solid (*Scheme 3*). The mass spectrum (MS) showed the expected molecular-ion peak at *m*/*z* 496. The ^{1}H-NMR spectrum of **10a** revealed the presence of a *s* at δ 7.12 assigned to the olefinic H-atom. The MeN group gave rise to a *s* at δ 2.90. Two *m* at δ 4.55 and 5.05 were assigned to the two H−C(α) of the dipeptide.

Treatment of **10a** with an EtOH solution of anhydrous NH_3 resulted in the formation of the tripeptide **11a** which was isolated as a single diastereoisomer in 37% yield (*Scheme 3*). The MS of **11a** showed the expected molecular-ion peak at *m*/*z* 513. Signals due to three H-atoms were detected between δ 4.15 and 5.15. These were assigned to the three H−C(α) supporting the formation of a tripeptide *via* addition of NH_3 to the α-position of the acryloyl moiety of **10a**. The *m* between δ 3.9 and 4.05 was assigned to the H-atom of the $(CF_3)_2CH$ group. The MeN group of MeLeu was observed as a *s* at δ 3.05. Furthermore, in the ^{13}C-NMR spectrum, three *d*s were

Scheme 3. *Synthesis of the Tripeptides* **11a**–**11f$_2$** *from the Dipeptides* **8a** *or* **8c**



	R^1	R^2
8a	Bn	H
8b	^{t}Bu	COOBn
8c	^{t}Bu	H

	R^1	R^2
11a	Bn	H
11b	Bn	COO^{t}Bu
11c$_{1,2}$	Bn	OH
11d$_{1,2}$	^{t}Bu	HOCH$_2$CH$_2$
11e$_{1,2}$	^{t}Bu	HOCH$_2$CH$_2$CH$_2$
11f$_1$ (*S*,*S*,*S*)	^{t}Bu	HOCH$_2$CH$_2$O
11f$_2$ (*R*,*S*,*S*)	^{t}Bu	HOCH$_2$CH$_2$O

observed between δ 49 and 55. These were assigned to the three N-substituted C(α)-atoms adding further support to the formation of the tripeptide **11a**. These results are in agreement with and represent the first example of an anti-*Michael* addition of NH$_3$ to a β,β-bis(trifluoromethyl)acrylamide moiety as in **10a** (see also [7]). The formation of a second diastereoisomer was not observed.

Reaction of the crude tripeptide **11a** with di(*tert*-butyl) dicarbonate [O(CO$_2$^{t}Bu)$_2$] gave the expected *N*-Boc-substituted product **11b** in 24% yield from **10a** as a crystalline product. This is supported by its MS, which showed a signal for [*M* + H]$^+$ at *m*/*z* 614. In the ^{1}H-NMR spectrum, the signals in the region between δ 4.5 and 5.35 were assigned to the three H–C(α) of the tripeptide **11b**. The H-atom of the (CF$_3$)$_2$CH group appeared as a *m* between δ 3.8 and 4.0. The ^{t}Bu and the MeN groups gave rise to *s*s at δ 1.41 and 3.30, respectively. Again the formation of a second diastereoisomer was not observed.

Next, the addition of NH$_2$OH to **10a** was investigated (*Scheme 3*). Two epimers were formed in a ratio of 1:6 and were isolated in pure form by column chromatography (silica gel). The less polar isomer **11c$_1$** was obtained as an oil, while the more polar major isomer **11c$_2$** was isolated in crystalline form. The MS of both isomers showed peaks for [*M*+H]$^+$ at *m*/*z* 530 with slight differences in their fragmentation patterns. In the ^{1}H-NMR spectrum of the less polar **11c$_1$**, the signals at δ 3.5, 3.7, and 5.10 are assigned to three H–C(α). For the more polar crystalline isomer **11c$_2$**, ^{1}H-NMR signals for two H–C(α) were observed between δ 4.45 and 4.60 and that of the third H–C(α) at δ 5.35. The configuration of the newly generated chiral centers of the isomers was not determined.

The addition of amines to the α-position of β,β-bis(trifluoromethyl)acrylic acid rather than to the β-position was first observed by *Knunjants et al.* [13]. Subsequently, *Eremeev et al.* explored the enantioselective addition of achiral amines to β,β-bis(trifluoromethyl)acrylamides containing a chiral amide [14]. In all cases, an α-addition to the β,β-bis(trifluoromethyl)acryloyl moiety with moderate de's of 20–40% was observed. As mentioned above, the addition of (1*R*)-1-phenylethylamine to benzyl β,β-bis(trifluoromethyl)acrylate to the α-position led to a 1:1 mixture of diastereoisomers, from which hexafluoro-D- and hexafluoro-L-valine were obtained.

This unique addition of nucleophiles to the α- rather than to the β-position of the acrylate is due to the presence of the two CF_3 groups in the β-position. Based on detailed computational results for the gas phase and a solvent with a formal dielectric constant of 7.0, this regioselectivity (anti-*Michael* addition) is interpreted in terms of a kinetically controlled addition of nucleophiles like cyanide in the α-position leading to an anion in the β-position, stabilized by the two adjacent CF_3 groups [15][16]. In addition, the partial positive charge and the coefficient of the LUMO are larger in the α-position of the β,β-bis(trifluoromethyl)acryloyl moiety. Whether the addition of the nucleophilic N-atom of the amine is synchronized with the protonation of the incipient anion (in MeOH as solvent) is an open question. We surmised that the depsipeptides **10a** and **10b**, in which the β,β-bis(trifluoromethyl)acryloyl moiety is attached to the dipeptide MeLeu-Val, would lead to a rather high diastereoselectivity in the α-addition of a primary amine. Apart from the addition of NH_3, all amines investigated gave the two diastereoisomers in a rather low ratio (**11c** 1:6, **11d** 3:4, **11e** 1:1, and **11f** 2:3), similar to those described by *Ermeleev et al.* mentioned above. In the case of **11f**, the two epimers were separated, and their configuration was established (see below).

Attempted Condensation of the Ester Val(F$_6$)-MeLeu-Ala-OCH$_2$Ph **11a** *with the Octapeptide* **2b**. With the tripeptide **11a** at hand, the formation of the amide bond between the octapeptide acid **2b** and the NH_2 group of Val(F$_6$) in **11a** was explored. Treatment of **2b** and **11a** with *N*-ethyl-*N'*-[3-(dimethylamino)propyl]carbodiimide (EDC), *N*,*N*-dimethylpyridin-4-amine (DMAP), and a catalytic amount of 1-hydroxy-7-aza-1*H*-benzotriazole (HOAt) over extended periods of time, up to 7 days, gave only trace amounts of the desired undecapeptide. This is in contrast with the observed acylation of the tripeptide **11a** with $(Boc)_2O$ to give the *N*-Boc-substituted tripeptide **11b** and also with the ready *N*-acylation of (−)-hexafluoro-D-valine **7a** with *Mosher's* acid chlorides, described earlier [7]. It may be argued that hexafluorovaline is about a thousand times weaker as a base than valine [7]. Furthermore, Val(F$_6$) might be sterically more hindered than Val itself, being one of the more hindered amino acids. In comparison to a Me group, the *van-der-Waals* radius of a CF_3 group is 35% larger, and the *van-der-Waals* volume is *ca.* 2.5 times as large [17]. Due to these considerations we did not try to optimize the peptide-forming conditions but decided to explore an alternative route. We surmised that an intramolecular peptide-forming reaction might be successful, where the intermolecular peptide-bond formation had failed. As an alternative reaction sequence, we visualized a 'lasso technique' involving α-additions to β,β-bis(trifluoromethyl)acrylamide derivatives **10a** and **10b** of (ω-hydroxyalkyl)amines with the functional groups being separated by two or three C-atoms. *O*-Acylation of the terminal OH group of the tripeptide with MeLeu in the *N*-protected octapeptide **2b** followed by an intramolecular O to N migration of the acyl group should generate the desired amide bond. Finally, ring closure would involve the intramolecular formation of the peptide bond between the least hindered alanines AA7 and AA8 producing [5-[hexafluoro-*N*-(oxyalkyl)-L-valine]]cyclosporins. Alternatively, 3-aminopropan-1-ol as the auxiliary may be replaced by the isosteric 2-(aminooxy)ethanol leaving the option for a reductive cleavage of the N–O bond either at the secocyclosporin or cyclosporin level. It should be noted that both termini of a 7,8-secocyclosporin would tolerate ^{t}Bu protecting groups, one as an ester and the other one as an *N*-Boc group. Both may be removed simultaneously prior to cyclization.

More Tripeptides as tert-*Butyl Esters.* For reasons mentioned above, we focused our attention on the preparation of tripeptides protected at the C-terminus (acid side) as *tert*-butyl esters. Thus, addition reactions of 2-aminoethanol, 3-aminopropan-1-ol, and 2-(aminooxy)ethanol with the *β*,*β*-bis(trifluoromethyl)acrylamide moiety of the dipeptide *tert*-butyl ester **10b** were investigated. The hitherto unknown dipeptide **8b** was prepared from commercial L-alanine *tert*-butyl ester hydrochloride and *N*-[(benzyloxy)carbonyl]-protected *N*-methyl-L-leucine [18] under standard peptide-forming conditions. Analytical data supported the structure assigned to **8b**. Removal of the (benzyloxy)carbonyl group *via* hydrogenation over Pd/C gave **8c**, which was treated immediately with **9** to give the *N*-acylated dipeptide **10b** in 98% yield. The MS of the product showed the expected $[M+H]^+$ peak at *m*/*z* 463. The fragment at *m*/*z* 407 may be due to the loss of 2-methylprop-1-ene. Both ^{1}H- and ^{13}C-NMR spectra are compatible with the assigned structure.

The *N*-alkylated tripetides **11d** and **11e** were prepared from **10b** *via* the addition of 2-aminoethanol and 3-aminopropanol, respectively. In both cases, mixtures of diastereoisomers were isolated, from which samples of the pure isomers were secured in both instances. The combined yield for the 2-aminoethanol addition products was 42% with an estimated ratio of 3 : 4 for the two diastereoisomers **11d$_1$** and **11d$_2$**. The less polar minor product **11d$_1$** was isolated as a liquid. According to its high-resolution MS, **11d$_1$** has a molecular-ion peak at *m*/*z* 524 ($[M+H]^+$). In the ^{1}H-NMR spectrum, sharp *s*s at δ 1.45 and 2.99 were assigned to the ^{t}Bu and the MeN group, respectively. Sharp signals corresponding to these groups were detected in the ^{13}C-NMR spectrum at δ 81.9 and 30.8, respectively. Three C-atoms assigned to the H–C(α) groups were observed between δ 56 and 48. The CH_2N and the CH_2O C-atoms gave rise to signals at δ 49.4 and 61.3, respectively. The more polar major isomer **11d$_2$** was isolated as a crystalline material also showing the $[M+H]^+$ peak at *m*/*z* 524. According to its ^{1}H-NMR spectrum, it may be concluded that **11d$_2$** is present as a single conformer with a *s* at δ 2.98 assigned to the MeN group. Other analytical data corresponded to those observed for the isomer **11e$_1$**.

The addition of the homologous 3-aminopropanol to **10b** resulted in the formation of the two epimers **11e$_1$** and **11e$_2$** in a 1 : 1 ratio obtained as liquids in a combined yield of 35%. In both cases, the MS showed the presence of the $[M+H]^+$ peak at *m*/*z* 538. In addition, both isomers were fully characterized by ^{1}H- and ^{13}C-NMR and IR spectra.

Finally, the reaction between 2-(aminooxy)ethanol [19] and **10b** led to a mixture of the two epimeric tripeptides **11f$_1$** and **11f$_2$** in an estimated ratio of 2 : 3. These were only partially separated by column chromatography in a combined yield of 92%. In the MS of the two isomers, the oily **11f$_1$** and the more polar, crystalline **11f$_2$** showed the expected $[M+H]^+$ peak at *m*/*z* 540.36 and 540.42, respectively. The less polar, liquid tripeptide **11f$_1$**, although pure according to TLC and HPLC, seems to be present in $CDCl_3$ solution in more than one conformation as determined by ^{1}H-NMR spectroscopy. The signals at δ 3.02 and 2.79 were assigned to the MeN group of this tripeptide and appeared in a ratio of 2 : 1. When the spectrum of **11f$_1$** was measured in CD_3OD

the signals were observed at δ 3.05 and 2.85 in a ratio of 10:1. In the ^{13}C-NMR spectrum of the tripeptide **11f$_1$**, a *t* detected at δ 61.0 was assigned to the CH_2OH C-atom based on a comparison with the spectra of the pure isomers of **11d$_1$**, **11d$_2$**, **11e$_1$**, and **11e$_2$**. The solid tripeptide **11f$_2$** showed a first-order ^{1}H-NMR spectrum in $CDCl_3$ indicating the presence of a single conformation on the NMR time scale. Interestingly, other esters of the tripeptides **11a**–**11e** described above all show ^{1}H-NMR spectra in $CDCl_3$ compatible with the presence of a single conformer for each tripeptide. The ^{13}C-NMR signal observed at δ 60.4 for the isomer **11f$_2$** was assigned to the CH_2OH C-atom.

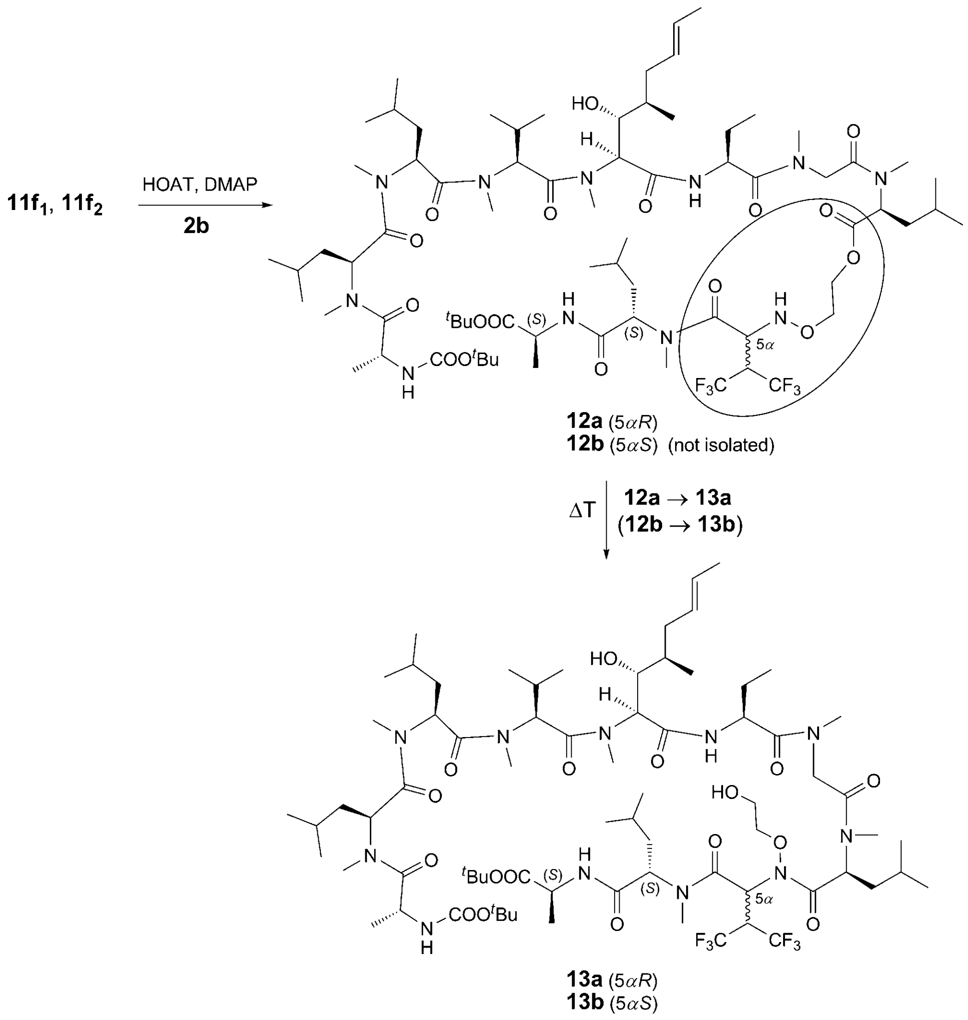
Most differences between the ^{13}C-NMR chemical shifts of the corresponding pairs of C-atoms for the diastereoisomers **11d$_1$**/**11d$_2$**, **11e$_1$**/**11e$_2$**, and **11f$_1$**/**11f$_2$** are small. Yet significant shift differences of 0.55, 0.50, and 0.45 ppm, respectively, were observed for the signals of the CH_2 groups of the *N*-methyl-L-leucine moiety (MeLeu). In all three cases, the CH_2 group associated with the less polar compound, *i.e.*, **11d$_1$**, **11e$_1$**, and **11f$_1$**, appeared at higher field. Based on these observed shift differences, the less polar isomers **11d$_1$** and **11e$_1$** were assigned the all-(*S*)-configuration. In retrospect, the observed chemical shift for the corresponding CH_2 at δ 36.5 for **11a**, obtained *via* the addition of NH_3 to **10a**, might suggest the (*R*)-configuration for the newly generated chiral center in **11a**. On the other hand, the observed chemical shift for the corresponding CH_2 groups at δ 35.93 for **11c$_2$**, obtained *via* the addition of NH_2OH to **10a**, would suggest the (*S*)-configuration for the newly generated chiral center in **11c$_2$**.

X-Ray Analysis of Tripeptide **11f$_2$**. As mentioned above, the more polar epimeric tripeptide **11f$_2$** was obtained as a crystalline product. A suitable crystal was grown from Et_2O/hexane and was subjected to a single-crystal X-ray-analysis [20]. The configuration was determined to be (*R*,*S*,*S*) for tripeptide **11f$_2$**. Surprisingly, and contrary to observations in solution (see NMR spectra), this diastereoisomer exists as a mixture of two different conformations in the solid state. As a consequence of the X-ray structure determination, the liquid, less polar tripeptide **11g$_1$** was assigned the (*S*,*S*,*S*) configuration.

Undecapeptides (*Secocyclosporins*). The depsipeptide **12a** was prepared from the *N*-Boc-protected octapeptide **2b** and the (*R*,*S*,*S*)-tripeptide **11f$_2$** (*Scheme 4*). This reaction was allowed to run for 10–14 days at room temperature under conventional peptide-forming conditions. The extended reaction times were due to the fact that the tripeptide **11f$_2$** and the condensation product **12a** could not be distinguished on TLC (*Scheme 4*). Thus, the presence of unreacted tripeptide had to be avoided. Chromatography of the crude product mixture gave a compound whose ESI-MS showed the expected $[M+H]^+$ peak at *m*/*z* 1545.21 for the condensation product **12a**. An interesting fragment peak was observed at *m*/*z* 351.25. Such fragments were conspicuously absent in either of the MS of **11f$_1$** and **11f$_2$**. In the ^{13}C-NMR spectrum of **12a**, the C-atoms of the hydroxyethoxy group were detected at δ 72.2 and 76.0, while the corresponding signals for the tripeptides **11f$_1$** and **11f$_2$** appeared at δ 61.0 and 75.0, and δ 60.5 and 75.4 ppm, respectively.

The doubly protected depsipeptide **12a** was heated to reflux in toluene during 60 h in which most of the starting material gave rise to a new, more polar product. According to spectral evidence, the depsipeptide had thermally rearranged to the protected

Scheme 4. *Condensation Reactions of* **11f$_1$** *and* **11f$_2$** *with* **2b** *and Formation of the Rearranged Products* **13a** *and* **13b**

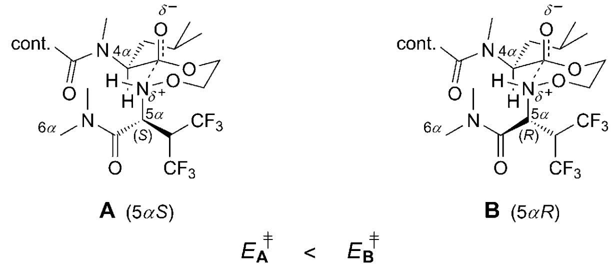


undecapeptide **13a**, a 7,8-secocyclosporin encompassing the modified amino acid *N*-2-hydroxyethoxy)-4,4,4,4′,4′,4′-L-hexafluoro-D-valine as AA5. The ESI-MS of this novel undecapeptide **13a** (calc. mass 1543.93) is distinguishable from the depsipeptide **12a** by its fragmentation pattern. The new product **13a** shows a peak at *m*/*z* 1544.74 for $[M+H]^+$. A peak found at *m*/*z* 1527.11 is most likely due to the loss of H_2O from AA1. A peak at *m*/*z* 1485.12 may be explained with the loss of OCH_2CH_2O from the molecular ion. Such a fragmentation is possible only for the peptide **13a** and not for the depsipeptide **12a**. It was especially gratifying to observe this fragment on the way to [D-Val(F$_6$)^5]cyclosporin, raising hope for a reductive cleavage of the incorporated auxiliary 2-(aminooxy)ethanol. Each of the three aforementioned peaks in the ESI-MS of **13a** showed three satellite peaks of $[M+22]^+$. This is due to to the replacement of a proton by Na^+. The absence of a peak at m/z 562 in the MS of **13a** further supports the notion of a fragment at m/z 539 and not the presence of an impurity in either **12a** or **13a**. In the ^{13}C-NMR spectrum of **13a**, the signals assigned to the C-atoms of the 2-hydroxyethoxy group were shifted upfield and were observed at δ 65.0 and 62.7, respectively.

The condensation between the *N*-Boc-octapeptide **2b** and the liquid diastereoisomeric (*S*,*S*,*S*)-tripeptide **11f₁** was carried out under similar conditions as described for the reaction of **11f₂** and **2b**. When a toluene solution of a sample, assumed to be the depsipeptide **12b**, was heated to reflux, no change was observed on TLC leading to the conclusion that the condensation product was the undecapeptide **13b**. The ESI-MS of **13b** showed the $[M+H]^+$ peak at m/z 1545.09 and a fragment peak at m/z 1527.08 due to the loss of H_2O, most likely from AA1. Both aforementioned peaks in the ESI-MS of **13b** showed the satellite peaks of $[M+22]^+$ corresponding to the Na^+ adducts. In the ^{13}C-NMR spectrum of **13b**, the signals assigned to the C-atoms of the 2-(hydroxyethoxy) group were observed at δ 72.2 and 62.8, respectively. This led to the conclusion that the rearrangement to **13b** had occurred under the conditions of the condensation of the tripeptide **11f₁** with the octapeptide **2b**.

Rearrangements. The difference between the depsipeptides **12a** and **12b** in their behavior under thermal conditions deserves some comment. Compound **12a** had to be heated to reflux in toluene for several days to achieve the conversion to the more polar peptide **13a**. The corresponding rearrangement from the depsipeptide **12b** to the undecapeptide **13b** apparently took place at room temperature. This may be explained considering the transition states **A** and **B** (*Fig. 2*) leading to the diastereoisomeric 3-oxy-tetrahydro-1,4,2-dioxazines, assumed intermediates for the O-to-N shift of the acyl groups. The two negatively polarized groups, the developing O-anion and the electronegative $(CF_3)_2CH$ group, achieve maximal separation if the two groups can be placed on opposite sides of the tetrahydro-1,4,2-dioxazine ring and assuming an *anti*periplanar conformation in the developing six-membered heterocyclic ring. For the case of the O-to-N acyl shift involving the hexafluoro-L-valine moiety (see **A**), fewer 1,3-interactions are found than in the case of the hexafluoro-D-valine moiety (see **B**), making the transition state in the former case more favorable.

*5-[Hexafluoro-*N*-(hydroxyethoxy)valine]]cyclosporins.* The linear undecapeptide **13a** was deprotected concomitantly at both termini by treatment with CF_3COOH and

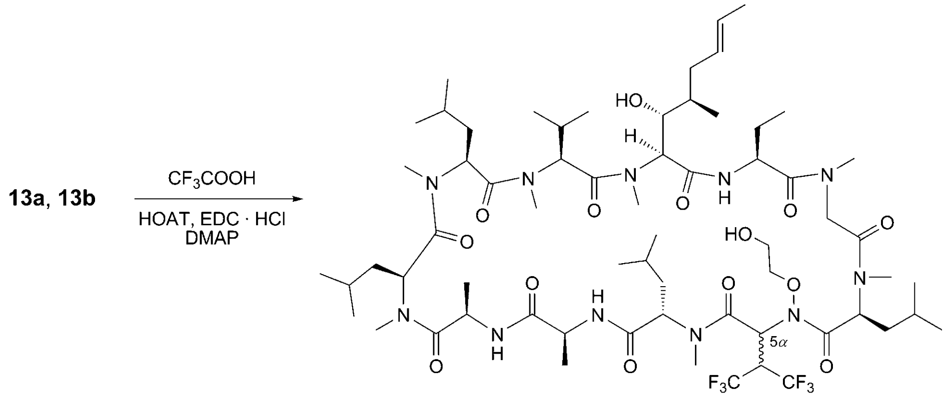


A (5αS) **B** (5αR)

Fig. 2. *Structural models* **A** *and* **B** *for the transition states of the rearrangements* **13a** → **14a** *and* **13b** → **14b**, *respectively*

subjected to ring closure under standard peptide-forming conditions but at high dilution. Following column chromatography (silica gel), the cyclic compound **14a** was obtained (*Scheme 5*). Its ESI-MS showed the expected peaks for $[M+H]^+$ at m/z 1370.85 and for $[M+Na]^+$ at m/z 1392.97. In the ^{13}C-NMR spectrum of **14a**, the signals assigned to the C-atoms of the 2-hydroxyethoxy substituent of AA5 were observed at δ 71.2 and 62.7, respectively.

Scheme 5. *Formation of the Derivatives of Cyclosporine* **14a** *and* **14b** *from* **13a** *and* **13b**



14a (5α*R*)
14b (5α*S*)

Likewise, deprotection of the termini (*N*-Boc and *tert*-butyl ester) of **13b** with CF_3COOH followed by the treatment with EDC gave the hexafluoro derivative **14b** of cyclosporin A. The ESI-MS showed the expected peak for $[M+H]^+$ at m/z 1370.81 and a peak at m/z 1374.86 ($[M+Na-H_2O]^+$). In the ^{13}C-NMR spectrum of **14b**, the signals assigned to the C-atoms of the 2-hydroxyethoxy substituent of AA5 were observed at δ 71.8 and 62.5, respectively.

The ^{1}H-NMR spectra of the novel cyclosporins **14a** and **14b** did not show the resolution usually associated with cyclosporins. This may be due to the fact that the amide H-atom of the hexafluorovaline residue (AA5) is replaced by a side chain (2-hydroxyethoxy) precluding the formation of a H-bridge to the C=O group of AA2 (*Fig. 1*).

Concluding Remarks. – The 4,4,4,4′,4′,4′-hexafluoro-*N*-(2-hydroxyethoxy)-D- or -L-valine was incorporated stereoselectively into cyclosporin A replacing the L-valine-residue (AA5) of the natural product **1a**. This was accomplished *via* the preparation of the tripeptides $HOCH_2CH_2O$-Val(F_6)-MeLeu-Ala-O^{t}Bu (**11f$_1$**, (*S,S,S*) and $HOCH_2$-CH_2O-D-Val(F_6)-MeLeu-Ala-O^{t}Bu **11f$_2$**, (*R,S,S*)) as building blocks. These epimeric compounds were prepared *via* the addition of 2-(aminooxy)ethanol to the acrylamide **10b**. The diastereoisomers **11f$_1$** and **11f$_2$** were condensed with the octapeptide **2b**. The depsipeptide **12a** was obtained from **11f$_2$** ((*R,S,S*)) and rearranged at 110° to the undecapeptide **13a**. The condensation of octapeptide **2b** with the tripeptide **11f$_1$**

((*S*,*S*,*S*)) produced the undecapeptide **13b** at room temperature. The peptides **13a** and **13b** were cyclized to the derivatives **14a** and **14b** of cyclosporin A, respectively.

The route *via* an intramolecular condensation was necessitated following our observation that an intermolecular condensation of **2b** with the secondary-amino group of the tripeptide **11a** did not produce the undecapeptide in reasonable yield. On the other hand, intramolecular amide-bond formations were successful employing a lasso technique *via* the depsipeptides **12a** and **12b** to produce the substituted secocyclosporins **13a** and **13b**, respectively. Successful intramolecular transformations where corresponding intermolecular reactions had failed, have been described previously in the literature [21]. As an example of the 'lasso technique' thioamides were used in the total synthesis of vitamin B_{12} to form enamines with the extrusion of the S-atom [22].

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Experimental Part

General. All reactions were performed under Ar or N_2. Chemicals were purchased from commercial suppliers and used without further purification. *N*-Ethyl-*N*′-[3-(dimethylamino)propyl]carbodiimide (EDC), *N*,*N*-dimethylpyridin-4-amine (DMAP), 1-hydroxy-1*H*-benzotriazole (HOBt), 1-hydroxy-1*H*-7-azabenzotriazole (HOAt), di(*tert*-butyl) dicarbonate ($(Boc)_2O$). Commercial solvents (except quality grade) were distilled prior to their use. TLC: silica-gel plates *SIL G/UV₂₅₄* (SiO_2; *Macherey & Nagel*). Column chromatography (CC): SiO_2 columns. NMR Spectra: *Bruker AC-300* (300 (^1H) and 75 MHz (^{13}C)) and *Bruker DRX-400* (376 (^{19}F)); in $CDCl_3$; δ in ppm rel. to Me_4Si ($\delta = 0$) for ^1H to $CDCl_3$ ($\delta = 77.0$) for ^{13}C, and to CCl_3F (external probe, $\delta = 0.0$) for ^{19}F-NMR, *J* in Hz; assignments tentative. EI- and ESI-MS (pos. mode): *Micromass-Autospec* and *Micromass-Platform* spectrometer, resp.; in *m*/*z* (rel. %).

N-*[(*tert*-Butoxy)carbonyl]*-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)oct-6-enoyl-(2*S*)-2-aminobutanoyl*-N-*methylglycyl*-N-*methyl*-L-*leucine* (**2b**). The side-chain *O*-acetyl-substituted *N*-Boc-octapeptide-SCH_2Ph (prepared from **1b**) [9] (6.0 g, 5.13 mmol) in DMF (60 ml) was stirred at r.t. overnight in the presence of 2N NaOH (40 ml, 80 mmol). Then, the mixture was neutralized with AcOH and concentrated under aspirator vacuum at 60–70°. The residue was dissolved in Et_2O and washed with 1N NaOH (5×50 ml) and then with brine ($2\times$). This was repeated four times. The combined org. phase was dried ($MgSO_4$) and concentrated: **2b** (2.3 g, 44%). ESI-MS: 1044.88 (35, $[M+Na]^+$), 1026.23 (48, $[M+Na-H_2O]^+$), 1022.75 (34, M^+), 1004.75 (38, $[M-H_2O]^+$).

*Benzyl (2*S*)-4,4,4-Trifluoro-2-{[(1*R*)-1-phenylethyl]amino}-3-(trifluoromethyl)butanoate 4-Methylbenzenesulfonate (=4,4,4,4′,4′,4′-Hexafluoro*-N-*[(1*R*)-1-phenylethyl]*-L-*valine Phenylmethyl Ester 4-Methylbenzenesulfonate (1:1)*; **6b**). A soln. of benzyl 4,4,4-trifluoro-3-(trifluoromethyl)but-2-enoate (**4**; 17.0 g, 57 mmol) in MeOH (50 ml) was cooled in an ice bath and treated with commercial (+)-(1*R*)-1-phenylethylamine (**5**); (6.9 g, 57 mmol) and kept at r.t. overnight. Then, $TsOH \cdot H_2O$ (10.8 g, 57 mmol) was added. The soln. was concentrated to *ca.* 10 ml, and Et_2O was added to precipitate a total of 13.5 g (22.8 mmol) of **6a** ((*R*,*R*)). The Et_2O soln. containing **6b** ((*S*,*R*)) was treated with sat. $NaHCO_3$ soln., dried (Na_2SO_4), and evaporated: 13.1 g of **6b** as an oil.

*Benzyl (2*S*)-4,4,4-Trifluoro-2-{[(1*R*)-1-phenylethyl]amino}-3-(trifluoromethyl)butanoate Hydrochloride (=4,4,4,4′,4′,4′-Hexafluoro*-N-*[(1*R*)-1-phenylethyl]*-L-*valine Phenylmethyl Ester Hydrochloride (1:1)*; **6d**). A soln. of **6b** (13.1 g, 30 mmol) in Et_2O was treated with an ice-cold soln. of HCl in Et_2O (100 ml) and the mixture concentrated. Fresh Et_2O (10 ml) was added and the HCl salt **6d** crystallized. After 2 h, the solid was filtered off to give 7.7 g (59%) of **6d**, containing 3% of **6c** (NMR).

Crystallizations from CH_2Cl_2/Et_2O and CH_2Cl_2/hexane gave a total of 3.98 g (30.9%) of pure **6d**. M.p. 133–135°. $[\alpha]_D = +13.20$ ($c = 1.09$, $CHCl_3$). ^{1}H-NMR: 1.98 (*d*, $J = 7$, Me); 4.22 (*s*, 1 H); 4.50–4.65 (*m*, 1 H); 4.80–5.0 (*dd*, CH_2); 5.45–5.65 (*m*, 1 H); 7.15–7.25 (*m*, 2 H); 7.30–7.47 (*m*, 6 H); 7.53–7.7 (*m*, 2 H); 8.0–9.5 (br., 1 H). ^{13}C-NMR: 164.9 (*s*); 134.7 (*s*); 133.0 (*s*); 130.0 (*d*); 129.3 (*d*); 129.1 (*d*); 129.0 (*d*); 128.9 (*d*); 128.6 (*d*); 69.7 (*t*); 62.4 (*d*); 54.5 (*d*); 48.2 (*m*, $J = 29.0$ Hz); 19.37 (*q*). ^{19}F-NMR: −62.93; −64.06. EI-MS: 419 (1), 418 (2), 404 (8), 328 (9), 284 (31), 268 (5), 120 (45), 105 (100), 91 (67), 77 (25). HR-MS: 419.13165 (M^+, $C_{20}H_{19}F_6NO_2^+$; calc. 419.131999).

*(+)-4,4,4,4′,4′,4′-Hexafluoro-*L*-valine Hydrochloride* (**7b** · HCl). A soln. of **6d** (4.0 g, 8.8 mmol) in MeOH (50 ml) was hydrogenated overnight in the presence of 5% Pd/C (1.5 g). The mixture was filtered through *Celite* and concentrated, and Et_2O was added (10 ml). The product **7b** · HCl solidified and was filtered to give 0.67 g of crystalline material. The residue of the filtrate was dissolved in H_2O and evaporated. This procedure was repeated and yielded an additional 1.38 g of **7b** · HCl: total yield 2.05 g (94%). M.p. 180–182°. $[\alpha]_D = +11.4$ ($c = 1.67$, H_2O). ^{1}H-NMR (CD_3OD): 4.22 (br. *s*, 1 H); 4.63 (*m*, 1 H). ESI-MS: 225 (0.2), 208 (0.1), 80 (100), 160 (8), 140 (6), 113 (18), 112 (10), 111 (5), 69 (30).

The *N*-acylation of **7b** · HCl with (−)-(*S*)-*Mosher*'s acid chloride was performed as described for the acylation of **7a** · HCl with (+)-(*R*)-*Mosher*'s acid chloride [8].

*[(Benzyloxy)carbonyl]-*N*-methyl-*L*-leucine-*L*-alanine* tert-*Butyl Ester* (**8b**). A soln. of commercial L-alanine *tert*-butyl ester hydrochloride (4.1 g, 22.7 mmol), EDC (5.0 g, 26.2 mmol), Z-MeLeu [14] (6.3 g, 22.6 mmol), and Et_3N (10 ml, 100 mmol) in CH_2Cl_2 (200 ml) was kept at r.t. for 2 h. The soln. was concentrated, and AcOEt (200 ml) was added. The mixture was washed with 0.5N HCl soln. (100 ml) and sat. Na_2CO_3 soln., dried ($MgSO_4$), and concentrated to give the crude product (9.58 g). This was filtered through SiO_2 with AcOEt/hexane 1:4 to give 7.56 g of pure **8b** (82.4%). TLC (AcOEt/hexane 1:3): single spot, R_f 0.62. $[\alpha]_D = -50.6$ ($c = 1.45$, $CHCl_3$). IR (film): 3332 (NH), 1736 (ester C=O), 1670 (amide C=O, urethane). ^{1}H-NMR: 0.75–0.95 (*m*, 2 Me); 1.26 (*d*, $J = 7$, Me); 1.42 (*s*, ^{t}Bu); 1.40–1.50 (br. *m*, CH); 1.62–1.70 (*m*, CH_2); 2.81 (*s*, MeN); 4.25–4.41 (*m*, 1 H−C(*α*)); 4.55–4.80 (*m*, 1 H−C(*α*)); 5.16 (*s*, CH_2O); 6.20–6.55 (br., NH); 7.25–7.41 (*m*, Ph). ^{13}C-NMR: 171.5, 170.2, 156.9 (3 C=O); 136.3 (arom. C); 128.2, 127.7, 127.4 (5 arom. CH); 81.4 (Me_3*C*); 67.2 (CH_2O); 56.5 (CH(*α*)); 48.3 (MeN); 36.4 (CH_2); 29.4 (CH(*α*)); 27.6 (*Me_3*C); 24.4 (CH); 22.9, 21.5, 17.9 (3 Me). EI-MS: 406 (0.2, M^+), 350 (0.6, $[M - 56]^+$, loss of $CH_2{=}CMe_2$), 333 (0.21, $[M - 73)]^+$, loss of C_4H_9O), 262 (0.25, $[M - 144]^+$, loss of Ala-O^{t}Bu), 234 (26, $[M - 172]^+$, loss of O=C-Ala-*O^{t}Bu*), 190 (49, [234−44], loss of CO_2), 91 (100, $C_6H_5CH_2^+$). HR-MS: 407.2541 ($[M + H]^+$, $C_{22}H_{35}N_2O_5^+$; calc. 407.2546).

N*-Methyl-*L*-leucine-(*S*)-alanine* tert-*Butyl Ester* (**8c**). A soln. of **8b** (7.3 g, 18 mmol) in MeOH (150 ml) was hydrogenated under normal pressure in the presence of 10% Pd/C (1 g) until the H_2 uptake stopped (*ca.* 325 ml, 15 mmol). The soln. was filtered through *Celite* and concentrated to give crude **8c** (4.10 g, 84%) which was used immediately for the next step. TLC (AcOEt/hexane 1:3): no starting **8b** left.

N*-Methyl-*N*-[4,4,4-trifluoro-1-oxo-3-(trifluoromethyl)but-2-en-1-yl]-*L*-leucine-*L*-alanine Benzyl Ester* (**10a**). A soln. of the dipeptide **8a** [9] (300 mg, 1 mmol) and Et_3N (2 g, 20 mmol) in CH_2Cl_2 (20 ml) was treated with 4,4,4-trifluoro-3-(trifluoromethyl)but-2-enoyl chloride (**9**; 450 mg, 2 mmol) [3a]. After 90 min at r.t., Et_2O (100 ml) was added, the org. phase washed with H_2O, dried (Na_2SO_4), and concentrated, and the residue subjected to CC (SiO_2, AcOEt/hexane 1:3): **10a** (480 mg, 96%). $[\alpha]_D = -52.8$ ($c = 2.38$, $CHCl_3$). ^{1}H-NMR: 0.92 (*d*, $J = 6.5$, Me); 0.96 (*d*, $J = 6.5$, Me); 1.45 (*d*, $J = 7.5$, Me); 1.25–1.55 (*m*, CH, CH_2); 2.90 (*s*, MeN); 4.50–4.65 (*m*, 1 H−C(*α*)); 5.05–5.10 (*m*, 1 H−C(*α*)); 5.11–5.21 (*dd*, CH_2O); 6.40–6.52 (*d*, NH); 7.12 (*s*, C=CH); 7.30–7.45 (*m*, Ph). MS: 496 (1, M^+), 477 (1.5, $[M - F]^+$), 440 (5, $[M - 56]^+$, loss of C_4H_8), 420 (5, $[440 - HF]^+$), 405 (1, $[M - \text{benzyl}]^+$, 318 (5, $[M - \text{Ala-OCH}_2\text{Ph}]^+$), 290 (11, $[318 - CO]^+$), 178 ($[\text{Ala-OCH}_2\text{Ph}]^+$), 91 (32, $C_7H_7^+$), 58 (100, $C_4H_{10}^+$).

N*-Methyl-*N*-[4,4,4-trifluoro-1-oxo-3-(trifluoromethyl)but-2-en-1-yl]-*L*-leucine-*L*-alanine* tert-*Butyl Ester* (**10b**). A soln. of **8c** (5.5 g, 20 mmol) and Et_3N (6 ml, 60 mmol) in CH_2Cl_2 (100 ml) was cooled in an ice/salt bath. A soln. of 4,4,4-trifluoro-3-(trifluoromethyl)but-2-enoyl chloride [3a] (**9**; 6.0 g, 26.5 mmol) in CH_2Cl_2 (60 ml) was added dropwise. After the addition was complete, the mixture was stirred at r.t. for 1 h, then a sat. K_2CO_3 soln. was added and the mixture extracted with CH_2Cl_2 and dried ($MgSO_4$). The solvent was evaporated and the crude product (11.5 g) filtered through SiO_2 first with

AcOEt/hexane 1:9 then with AcOEt/hexane 1:4: pure **10b** (9.13 g, 98%). Amber waxy substance. TLC (AcOEt/hexane 1:2): single spot, R_f 0.5. $[\alpha]_D = -83.0$ ($c = 1.675$, $CHCl_3$). IR (film): 3319 (NH), 1731 (ester C=O), 1632–1681 (amide C=O). ^{1}H-NMR: 0.87 (*d*, *J* = 6.7, 3 H); 0.91 (*d*, *J* = 6.6, 3 H); 1.28 (*d*, *J* = 7.0, 3 Me); 1.41 (*s*, ^{t}Bu); 1.35–1.50 (*m*, CH); 1.61–1.70 (*m*, CH_2); 2.86 (*s*, MeN); 4.28–4.35 (*m*, 1 H–C(α)); 5.10–5.30 (*m*, 1 H–C(α)); 6.50 (*d*, *J* = 7.35, NH); 7.08 (*s*, =CH). ^{13}C-NMR: 171.5, 168.5, 163.3 (3 C=O); 135.9 (=CH); 120.2 (*q*, *J* = 274, *C*F_3CH); 119.9 (*q*, *J* = 274, *C*F_3CH); 81.9 (Me_3*C*); 53.9 (CH(α)); 48.7 (MeN); 36.1 (CH_2); 31.2 (CH(α)); 27.8 (*Me*$_3$C); 24.6 (CH); 22.9 (Me); 21.8 (Me); 18.0 (Me). ^{19}F-NMR: –62.20; –64.23. EI-MS: 463.2 (20, $[M+H]^+$), 407.1 (60, $[M+H-56]^+$, loss of $CH_2{=}CMe_2$), 318.0 (90, $[M - \text{Ala-O}^t\text{Bu}]^+$), 290.0 (100, $[318 - F + H]^+$), 190.9 (6, $C_5HF_6O^+$), 162.9 (4, $C_4HF_6^+$), 144 (5, $[M-318]^+$). HR-MS: 463.2041 ($[M+H]^+$, $C_{19}H_{29}F_6N_2O_4^+$; calc. 463.2032).

*4,4,4,4′,4′,4′-Hexafluorovalyl-*N*-methyl-*L*-leucyl-*L*-alanine Benzyl Ester* (**11a**). A soln. of **10a** (1 g, 2 mmol) in EtOH (10 ml) was added to liq. NH_3 (20 ml). The mixture was allowed to warm to r.t. The solvent was evaporated, and the crude product was subjected to CC (SiO_2, AcOEt/hexane 1:3, then AcOEt/hexane 1:2 (→ **10a** (374 mg)), then AcOEt/hexane 1:1): **11a** (243 mg, 37% based on transformed **10b**) as a single diastereoisomer (tentatively (*R*,*S*,*S*)). ^{1}H-NMR: 0.85 (*d*, *J* = 7, 3 H); 0.92 (*d*, *J* = 7, 3 H); 0.95–1.00 (*m*, CH); 1.38 (*d*, *J* = 8, Me); 1.65–1.90 (*m*, NH_2, CH_2); 3.05 (*s*, MeN); 3.85–4.02 (*m*, CF_3CH); 4.15–4.22 (*d*, 1 H–C(α)); 4.50–4.65 (*m*, 1 H–C(α)); 5.10–5.15 (*d*, 1 H–C(α)); 5.16–5.20 (*dd*, CH_2O); 6.55–6.68 (*d*, NH); 7.31–7.42 (*m*, Ph). ^{13}C-NMR: 172.7, 172.0, 170.5 (3 C=O); 129.0 (CH); 121.3 (CH); 67.7 (CH_2O); 55.5, 49.4, 48.7 (3 CH(α)); 36.5 (CH_2); 30.9 (MeN); 25.2 (CH); 23.7, 21.8, 18.7 (3 Me). EI-MS: 513.3 (0.5, M^+), 457.2 (1.5, $[M - CH_2{=}CMe_2]^+$), 440 (0.5, $[457 - NH_3]^+$), 335.2 (5.5, $[M - \text{Bn} - \text{Ala}]^+$), 307.1 (60, $[335 - C{=}O]^+$), 278.1, $[335 - C_4H_9]^+$), 249.1 (42, $C_7H_7F_6N_2O^+$), 180.0 ($[249 - CF_3]^+$), 100.1 (100, $C_4H_8N_2O^+$), 91 (50, $C_7H_7^+$).

N*-[(*tert*-Butyl)carbonyl]-4,4,4,4′,4′,4′-hexafluorovalyl-*N*-methyl-*L*-leucyl-*L*-alanine Benzyl Ester* (**11b**). As described for **11a**, with **10a** (340 mg, 0.68 mmol) in THF (10 ml). The solvent was evaporated. Fresh THF (30 ml) was added together with $(Boc)_2O$ (1.0 g, 4.6 mmol). The mixture was kept at r.t. overnight. The solvent was evaporated and the residue subjected to CC (SiO_2, AcOEt/hexane 1:4): **11b** (100 mg, 24%; tentatively (*R*,*S*,*S*)). Crystals. ^{1}H-NMR: 0.86 (*d*, *J* = 6, 3 H); 0.94 (*d*, *J* = 6, 3 H); 1.37 (*d*, *J* = 7, Me); 1.41 (*s*, ^{t}Bu); 1.29–1.50 (*m*, 1 H); 1.60–1.78 (*m*, 2 H); 3.30 (*s*, MeN); 3.81–3.98 (*m*, CF_3CH); 4.49–4.62 (*m*, 1 H–C(α)); 5.02–5.11 (*m*, 1 H–C(α)); 5.15–5.20 (*dd*, CH_2O); 5.25–5.35 (*m*, 1 H–C(α)); 6.48–6.58 *m*, NH); 7.30–7.40 (*m*, Ph). EI-MS: 614.2 (15, $[M+H]^+$), 558.1 (14, $[614 - 56]^+$), 435.1 (18, $[M - \text{Bn} - \text{Ala}]^+$), 379.0 (100, $[435 - 56]^+$), 335.0 (10, $[379 - CO_2]^+$), 307.1 (11, $[\text{N} - \text{Boc} - \text{Val}(F_6) - \text{ketene}]^+$).

*4,4,4,4′,4′,4′-Hexafluoro-*N*-hydroxyvalyl-*N*-methyl-*L*-leucyl-*L*-alanine Benzyl Ester* (**11c₁**/**11c₂**). A soln. of **10a** (270 mg, 0.54 mmol) in CH_2Cl_2 (5 ml) was mixed with a soln. of NH_2OH in EtOH (1 ml, prepared from $NH_2OH \cdot H_2O$ (1 g) and AcONa (1 g)) and was kept at r.t. for 2 h. The mixture was diluted with AcOEt, washed with H_2O, dried (Na_2SO_4), and concentrated. The residue (300 mg) was subjected to CC (SiO_2, AcOEt/hexane 1:2): less polar, pure **11c₁** (20 mg) as an oil and pure **11c₂** (120 mg) as crystals.

Diasteroisomer **11c₁** (tentatively (*R*,*S*,*S*)): TLC: R_f 0.35 (AcOEt/hexane 1:2). ^{1}H-NMR: 0.90 (*d*, *J* = 6, 3 H); 0.94 (*d*, *J* = 6, 3 H); 1.34 (*d*, *J* = 7, 3 H); 1.60–1.80 (*m*, CH, CH_2); 3.06 (s, MeN); 3.65–3.80 (*m*, CF_3CH); 4.14–4.25 (*m*, NH); 4.38–4.8 (*m*, 2 H–C(α)); 5.05–5.21 (*m*, 1 H–C(α)); 5.14–5.20 (*dd*, CH_2O); 5.65–5.80 (br., OH); 6.45–6.60 (*d*, NH); 7.30–7.40 (*m*, Ph). EI-MS: 530.1 (22, $[M+H]^+$), 351.1 (100, $[M - \text{Ala-OCH}_2\text{Ph}]^+$), 323.1 (19, $[351 - CO]^+$).

Diastereoisomer **11c₂** (tentatively (*S*,*S*,*S*)): TLC: R_f 0.24 (AcOEt/hexane 1:2). M.p. 98–102°. $[\alpha]_D = -57.8$ ($c = 1.45$, $CHCl_3$). ^{1}H-NMR: 0.85–0.92 (*d*, 3 H); 0.94–0.99 (*d*, 3 H); 1.30–1.35 (*d*, Me); 1.18–1.45 (*m*, CH); 1.60–1.75 (*m*, 1 H); 1.78–1.95 (*m*, CH_2); 3.0 (*s*, MeN); 3.70–3.85 (*m*, CF_3CH); 4.45–4.60 (*m*, 2 H–C(α)); 5.15 (*s*, CH_2O); 5.30–5.40 (*m*, 1 H–C(α)); 5.81–5.91 (*d*, 1 H); 6.30–6.42 (*m*, 1 H, N*H*OH); 6.90–7.05 (*d*, CONH); 7.20–7.40 (*m*, Ph). ^{13}C-NMR: 17.8, 21.0, 23.3 (3 Me); 24.4 (CH); 30.6 (MeN); 35.93 (CH_2); 48.0 (*m*, CF_3*C*H); 48.4 (CH(α)); 55.7 (CH(α)); 56.2 (CH(α)); 67.2 (CH_2); 122.7 (*q*, *J* = 280, CF_3); 128.1 (CH); 128.4 (CH); 128.6 (CH); 135.3 (*s*); 169.7, 171.6, 172.5 (3 C=O). ^{19}F-NMR ($CDCl_3$): –0.013; –0.091. EI-MS: 530.2 (14, $[M+H]^+$), 393.1 (5, $[M - \text{BnOCOH}]^+$), 351.1 (100, $[M - \text{Bn} - \text{Ala}]^+$), 323.1 (32, $[351 - CO]^+$), 305.1 (13, $[\text{MeLeu} - \text{Val}(F_6)\text{Bn}]^+$), 196 (11, $(CF_3)_2CHNHOH^+$).

4,4,4,4′,4′,4′-Hexafluoro-N-*(2-hydroxyethyl)*-L- *and 4,4,4,4′,4′.4′-Hexafluoro*-N-*(2-hydroxyethyl)*-D-*valyl*-N-*methyl*-L-*leucine*-L-*alanine* tert-*Butyl Ester* (**11d$_1$** and **11d$_2$**, resp.). To a soln. of **10b** (924 mg, 2 mmol) in EtOH (50 ml) was added 2-aminoethanol (490 mg, 8 mmol) and kept at r.t. overnight. The solvent was evaporated, the residue dissolved in AcOEt, the soln. washed with H_2O dried (Na_2SO_4), and concentrated, and the residue (920 mg) separated by CC (SiO_2, hexane/AcOEt 2:1): less polar **11d$_1$** ((*S*,*S*,*S*); 120 mg) as an oil, a mixture of both isomers (150 mg), and more polar **11d$_2$** ((*R*,*S*,*S*); 160 mg) as crystals. Combined yield 42%.

(*S*,*S*,*S*)-Isomer **11d$_1$**: TLC (AcOEt/hexane 1:1): single spot, R_f 0.36. $[\alpha]_D = -72.1$ ($c = 1.56$, $CHCl_3$). IR (film): 3460, 3342, 1735, 1625 – 1675. ^{1}H-NMR: 0.86 (*d*, $J = 6.62$, 3 H); 0.92 (*d*, $J = 6.62$, 3 H); 1.24 (*d*, $J = 7.35$, Me); 1.41 (*s*, ^{t}Bu); 1.33 – 1.46 (*m*, CH); 1.58 – 1.76 (*m*, CH_2); 2.49 – 2.59 (*m*, 1 H of CH_2N); 2.75 – 2.85 (*m*, 1 H of CH_2N); 1.90 – 2.60 (br., NH, OH); 2.95 (*s*, MeN); 3.55 – 3.67 (*m*, CH_2O, CF_3CH); 4.05 (*d*, $J = 8.45$, 1 H–C(α)); 4.25 – 4.38 (*m*, 1 H–C(α)); 4.99 – 5.08 (*m*, 1 H–C(α)); 6.53 (*d*, $J = 7.35$, O=CNH). ^{13}C-NMR: 171.7, 171.7, 168.9 (3 C=O); 123.0 (*q*, $J = 281$, CF_3); 81.9 (Me_3*C*); 61.4 (CH_2O); 55.6 (CH(α)); 54.0 (CH(α)); 50.2 (*m*, $J = 26.2$, CF_3*C*H); 49.5 (CH_2N); 48.6 (CH(α)); 36.1 (CH_2); 30.9 (MeN); 27.9 (*Me*$_3$C); 24.9 (CH); 23.1 (Me); 21.8 (Me); 17.9 (Me). ^{19}F-NMR ($CDCl_3$): –61.74; –64.74. EI-MS: 524 (100, $[M+H]^+$), 468 (30, $[M-56]^+$, loss of CH_2=CMe_2), 379 (28, $[M-144]^+$, loss of Ala-O^{t}Bu), 351 (1, $[M-172]^+$, loss of O=C-Ala-O^{t}Bu), 243 (1, $[M-280]^+$, loss of $HOCH_2CH_2$-Val(F_6)-NHMe, 224 (53, $(CF_3)_2CHCHNHCH_2CH_2OH]^+$), 206 (12, $[224-F+H]^+$), 144 (4, [Ala-O^{t}Bu]$^+$), 128 (3, [CH_2=CHCOO^{t}Bu]$^+$). HR-MS: 524.256160 ($[M+H]^+$, $C_{21}H_{36}F_6N_3O_5^+$; calc. 524.255916).

(*R*,*S*,*S*)-Isomer **11d$_2$**: M.p. 86 – 87°. TLC (AcOEt/hexane 1:1): single spot, R_f 0.27. $[\alpha]_D = -52.5$ ($c = 1.57$, $CHCl_3$). IR (film): 3450, 3332, 1734, 1630 – 1685. ^{1}H-NMR: 0.82 (*d*, $J = 6.3$, 3 H); 0.89 (*d*, $J = 6.6$, 3 H); 1.28 (*d*, $J = 7.3$, Me); 1.40 (*s*, ^{t}Bu); 1.31 – 1.48 (*m*, CH); 1.61 – 1.69 (*m*, CH_2); 2.00 – 2.60 (br., NH, OH); 2.66 – 2.83 (*m*, CH_2N); 2.95 (*s*, MeN); 3.40 – 3.65 (*m*, CF_3CH, CH_2O); 4.15 (*d*, $J = 6.6$, 1 H–C(α)); 4.36 – 4.40 (*m*, 1 H–C(α)); 5.10 – 5.20 (*m*, 1 H–C(α)); 6.66 (*d*, $J = 7.36$, NH). ^{13}C-NMR: 172.3, 171.8, 169.8 (3 C=O); 123.1 (*q*, $J = 280$, CF_3); 82.5 (Me_3*C*); 61.9 (CH_2O); 55.3 (CH(α)); 54.2 (CH(α)); 50.1 (*m*, $J = 26.2$, CF_3*C*H); 49.9 (CH_2N); 48.8 (CH(α)); 36.6 (CH_2); 30.5 (MeN); 27.9 (*Me*$_3$C); 24.7 (CH); 23.2 (Me); 21.3 (Me); 18.4 (Me). ^{19}F-NMR ($CDCl_3$): –61.88; –64.91. EI-MS: 524 (100, $[M+H]^+$), 468 (35, $[M-56]^+$, loss of CH_2=CMe_2), 379 (33, $[M-144]^+$, loss of Ala-O^{t}Bu), 351 (4, $[M-172]^+$, loss of O=C-Ala-O^{t}Bu), 243 (4, $[M-280]^+$, loss of $HOCH_2CH$-Val(F_6)-NHMe); 224 (51, $(CF_3)_2CHCHNHCH_2CH_2OH^+$), 206 (14, $[224-F+H]^+$), 144 (7, [Ala-O^{t}Bu]$^+$), 128 (38, CH_2=CHCOO^{t}Bu$^+$). HR-MS: 524.2543 ($[M+H]^+$, $C_{21}H_{36}F_6N_3O_5^+$; calc. 524.2559).

4,4,4,4′,4′,4′-Hexafluoro-N-*(3-hydroxypropyl)*-L- *and 4,4,4,4′,4′,4′-Hexafluoro*-N-*(3-hydroxypropyl)*-D-*valyl*-N-*methyl*-L-*leucyl*-L-*alanine* tert-*Butyl Ester* (**11e$_1$** and **11e$_2$**, resp.). As described for **11d$_1$**/**11d$_2$**, with **10b** (700 mg, 1.5 mmol), EtOH (30 ml), and 3-aminopropan-1-ol (700 mg, 9 mmol). The crude product (1.3 g) was separated by CC (SiO_2, AcOEt/hexane 1:2): less polar **11e$_1$** ((*S*,*S*,*S*); 97 mg), a mixture of both isomers (98 mg), and more polar **11e$_2$** ((*R*,*S*,*S*); 85 mg) as oils. Combined yield 35%.

(*S*,*S*,*S*)-Isomer **11e$_1$**: TLC (AcOEt/hexane 1:1): single spot, R_f 0.30. $[\alpha]_D = -79.2$ ($c = 1.07$, $CHCl_3$). IR (film): 3334, 1735, 1639 – 1670, 1522. ^{1}H-NMR: 0.84 (*d*, $J = 6.3$, 3 H); 0.92 (*d*, $J = 6.6$, 3 H); 1.22 (*d*, $J = 7.0$, Me); 1.40 (*s*, ^{t}Bu); 1.36 – 1.45 (*m*, CH); 1.59 – 1.69 (*m*, 2 CH_2); 2.29 – 2.59 (br., OH); 2.44 – 2.55 (*m*, 1 H of CH_2N); 2.74 – 2.86 (*m*, 1 H of CH_2N); 2.94 (*s*, MeN); 3.51 – 3.66 (*m*, CF_3CH); 3.69 (*t*, $J = 5.9$, CH_2O); 3.99 (*d*, $J = 8.1$, 1 H–C(α)); 4.24 – 4.40 (*m*, 1 H–C(α)); 5.45 – 5.55 (*m*, 1 H–C(α)); 6.52 (*d*, $J = 7.4$, O=CNH). ^{13}C-NMR (CDC_3): 171.6, 168.8 (2 C=O); 81.9 (Me_3*C*); 62.1 (CH_2O); 54.9 (CH(α)); 54.2 (CH(α)); 50.1 (*m*, $J = 26$, CF_3*C*H); 48.6 (CH(α)); 46.4 (CH_2N); 36.1 (CH_2); 31.9 (CH_2); 30.5 (MeN); 27.9 (*Me*$_3$C); 24.9 (CH); 23.1 (Me); 21.8 (Me); 17.9 (Me). ^{19}F-NMR ($CDCl_3$): –61.89; –64.77. ESI-MS: 560.2538 ($[M+Na]^+$, $C_{22}H_{37}F_6N_3NaO_5^+$; calc. 560.2535), 538.2223 (100, M^+), 482.1693 (95, $[M-C_4H_8]^+$), 393.1564 (41, [M – Ala-O^{t}Bu]$^+$), 156.1052 (17, $C_8H_{14}NO_2^+$, MeLeu fragment), 128.1088 (62, $C_7H_{14}NO^+$, MeLeu fragment).

(*R*,*S*,*S*)-Isomer **11e$_2$**: TLC (AcOEt/hexane 1:1): single spot, R_f 0.18. $[\alpha]_D = -58.1$ ($c = 1.115$, $CHCl_3$). IR (film): 3331, 1732, 1632 – 1670, 1537. ^{1}H-NMR: 0.82 (*d*, $J = 6.3$, Me); 0.89 (*d*, $J = 6.6$, Me); 1.27 (*d*, $J = 7.0$, Me); 1.40 (*s*, ^{t}Bu); 1.35 – 1.40 (*m*, CH); 1.54 – 1.72 (*m*, 2 CH_2); 2.30 – 2.67 (br., OH); 2.46 – 2.62 (*m*, 1 H of CH_2N); 2.77 – 2.88 (*m*, 1 H of CH_2N); 2.99 (*s*, MeN); 3.47 – 3.62 (*m*, CF_3CH); 3.67 (*t*, $J = 5.5$, CH_2O); 4.04 (*d*, $J = 7.7$, 1 H–C(α)); 4.27 – 4.38 (*m*, 1 H–C(α)); 5.08 – 5.60 (*m*, 1 H–C(α)); 6.62 (*d*, $J = 7.0$, NH). ^{13}C-NMR: 171.8, 171.6, 169.8 (3 C=O); 122.9 (*q*, $J = 281$, CF_3); 82.2 (Me_3*C*); 61.9 (CH_2O);

55.2 (CH(α)); 54.1 (CH(α)); 50.1 (*m*, $J = 26.5$, CF_3*C*H); 48.7 (CH(α)); 46.3 (CH_2N); 36.7 (CH_2); 31.9 (CH_2); 30.6 (MeN); 27.9 (*Me*$_3$C); 24.7 (CH); 23.1 (Me); 21.4 (Me); 18.3 (Me). ^{19}F-NMR ($CDCl_3$): – 62.03; – 64.96. ESI-MS: 560.2643 ($[M + Na]^+$, $C_{22}H_{37}F_6N_3NaO_5^+$; calc. 560.2535), 538.2339 (100, M^+) 482.1798 (92, $[M - C_4H_8]^+$), 393.1633 (24, $[M - \text{Ala-O}^t\text{Bu}]^+$), 156.1066 (11, $C_8H_{14}NO_2^+$, MeLeu fragment), 128.1095 (35, $C_7H_{14}NO^+$, MeLeu fragment).

4,4,4,4′,4′.4′-Hexafluoro-N-*(2-hydroxyethoxy)*-L-*valyl- and 4,4,4,4′,4′,4′-Hexafluoro*-N-*(2-hydroxyethoxy)*-D-*valyl*-N-*methyl*-L-*leucine*-L-*alanine* tert-*Butyl ester* (**11f$_1$** and **11f$_2$**, resp.). As described for **11d$_1$/11d$_2$**, with **10b** (600 mg, 1.3 mmol), EtOH (20 ml) and 2-(aminooxy)ethanol [15] (300 mg, 3.9 mmol) at r.t. for 3 d. The crude product (858 mg) was separated by CC (SiO_2, AcOEt/hexane 1 : 2): less polar **11f$_1$** ((*S,S,S*); 141 mg, 20%) as an oil, a mixture of the diastereoisomers (284 mg, 41%), and more polar **11f$_2$** ((*R,S,S*); 217 mg, 31%) as crystals. Total yield 92%.

(*S,S,S*)-Isomer **11f$_1$**: TLC (AcOEt/hexane 1 : 1): single spot, R_f 0.37. $[\alpha]_D = -84.6$ ($c = 1.095$, $CHCl_3$); $[\alpha]_D = -87.7$ ($c = 1.12$, MeOH). IR (film): 3348, 1734, 1635 – 1670, 1517. ^{1}H-NMR ($CDCl_3$): 0.85 (*d*, $J = 6.5$, Me); 0.89 (*d*, $J = 6.5$, Me); 1.23 (*d*, $J = 7.0$, Me); 1.40 (*s*, ^{t}Bu); 1.40 – 1.50 (*m*, CH); 1.55 – 1.78 (*m*, CH_2); 1.90 – 2.28 (*m*, NH); 2.45 – 2.65, 3.27 – 3.41 (br., OH); 2.78, 3.00 (2*s*, ratio 1 : 2, MeN); 3.52 – 3.98 (*m*, $CHCF_3$, OCH_2CH_2O); 4.28 – 4.40 (*m*, 1 H–C(α)); 4.45 (*dd*, $J = 11.3, 9.5$) and 4.64 (*dd*, $J = 11.3, 10.0$, 1 H–C(α)); 4.83 (*dd*, $J = 10.5, 3.3$) and 4.96 (*dd*, $J = 9.3, 6.0$, 1 H–C(α)); 6.13 (*d*, $J = 11.5$) and 6.14 (*d*, $J = 11.5$, NH); 6.48 (*d*, $J = 7.5$) and 7.10 (*d*, $J = 8.0$, NH). ^{1}H-NMR (CD_3OD): 0.85 (*d*, $J = 6.3$, 3 H); 0.89 (*d*, $J = 6.3$, 3 H); 1.23 (*d*, $J = 7.4$, 3 H); 1.38 (*s*, ^{t}BuO); 1.43 – 1.75 (*m*, CH, CH_2); 2.85, 3.05 (2*s*, ratio 1 : 10, MeN); 3.50 – 3.75 (*m*, OCH_2CH_2O); 3.80 – 4.00 (*m*, CF_3CH); 4.10 – 4.23 (*dd*, 1 H–C(α)); 4.55 (*d*, $J = 7.5$, 1 H–C(α)); 5.05 – 5.15 (*dd*, 1 H–C(α)). ^{13}C-NMR ($CDCl_3$): 171.7 (C=O); 171.1 (C=O); 169.0 (C=O); 122.7 (*q*, $J = 280$, CF_3); 122.5 (*q*, $J = 280$, CF_3); 81.9 (Me$_3$*C*O); 75.0 (CH_2ON); 61.0 (CH_2OH); 59.7, 58.3 (CH(α)); 56.0, 55.0 (CH(α)); 48.5 (CH(α)); 48.3 (*m*, $J = 27$, CF_3*C*H); 36.2 (CH_2); 31.3, 29.9 (MeN); 27.8 (*Me*$_3$CO); 24.5 (CH); 23.0 (Me); 21.7 (Me); 17.9 (Me). ^{13}C-NMR (CD_3OD): 172.4 (C=O); 172.2 (C=O); 172.1 (C=O); 124.5 (*q*, $J = 283$, CF_3); 124.3 (*q*, $J = 283$, CF_3); 82.6, 79.4 (Me$_3$*C*); 76.1 (CH_2ON); 60.7 (CH_2OH); 56.5 (CH(α)); 56.4 (CH(α)); 50.1 (CH(α)); 49.2 (*m*, $J = 28$, CF_3*C*H); 38.3 (CH_2); 31.8 (CH); 28.2 (*Me*$_3$C); 25.7 (CH); 23.5 (Me); 22.3 (Me); 17.4 (Me). ^{19}F-NMR (CD_3OD): – 63.33 (minor); – 63.44, – 65.34 (10%), – 65.61 (90%). ESI-MS (MeOH): 601.32 (20, $[M + HF + NaF]^+$), 562.37 (73, $[M + Na]^+$), 540.36 (17, $[M + H]^+$), 484.23 (20, $[M + H - C_4H_8]^+$), 395.22 (100, $[M - 144]^+$, loss of Ala-O^{t}Bu), 367.31 (5, $[M - 172]^+$, loss of O=C-Ala-O^{t}Bu).

(*R,S,S*)-Isomer **11f$_2$**: TLC (AcOEt/hexane 1 : 1): single spot, R_f 0.30. M.p. 97 – 98° (from Et_2O/hexane). $[\alpha]_D = -28.0$ ($c = 1.095$, $CHCl_3$); $[\alpha]_D = -33.9$ ($c = 1.04$, MeOH). ^{1}H-NMR: 0.76 (*d*, $J = 6.2$, Me); 0.84 (*d*, $J = 6.6$, Me); 1.24 (*d*, $J = 7.0$, Me); 1.35 (*s*, ^{t}Bu); 1.52 – 1.72 (*m*, CH); 1.55 – 1.70 (*m*, 2 H); 2.98 (*s*, MeN); 3.20 – 3.55 (br., OH); 3.55 – 3.80 (*m*, OCH_2CH_2O, $CHCF_3$); 4.25 – 4.38 (*m*, 1 H–C(α)); 4.43 – 4.56 (*m*, 1 H–C(α)); 5.20 – 5.30 (*m*, 1 H–C(α)); 6.34 (*d*, $J = 11.7$, NH); 6.95 (*d*, $J = 7.4$, NH). ^{13}C-NMR: 171.9 (C=O); 171.8 (C=O); 169.8 (C=O); 122.67 (*q*, $J = 282$, CF_3); 122.5 (*q*, $J = 282$, CF_3); 81.9 (Me$_3$*C*O); 75.4 (CH_2ON); 60.5 (CH_2OH); 55.3 (CH(α)); 54.7 (CH(α)); 48.6 (CH(α)); 48.2 (*m*, $J = 27.5$, CF_3*C*H); 36.7 (CH_2); 30.7 (MeN); 27.7 (*Me*$_3$C); 24.3 (CH); 23.1 (Me); 21.0 (Me); 18.1 (Me). ^{19}F-NMR ($CDCl_3$): – 62.68, – 65.19. ESI-MS (MeOH): 562.31 (70, $[M + Na]^+$), 540.42 (40, $[M + H]^+$), 484.35 (20, $[M + H - C_4H_8]^+$), 395.22 (100, $[M - 144]^+$, loss of Ala-O^{t}Bu), 367.31 (10, $[M - 172]^+$, loss of O=C-Ala-O^{t}Bu). ESI-MS (neg. mode, MeCN/H_2O/NEt_3): 584.24 (25, $[M + CO_2?]^-$), 558.38 (70, $[M + F]^-$), 538.41 (15, $[M - H]^-$), 498.35 (20, $[M - H - 2\,HF]^-$), 478.39 (35, $[M - 1\,H - 3\,HF]^-$), 476.40 (95, $[478.39 - 2\,H]^-$), 436.40 (55, $[476.40 - 2\,HF]^-$), 416.31 (100, $[436.40 - HF]^-$), 359.32 (25, $C_{14}H_{19}F_6N_3O^-$).

The crystalline **11f$_2$** was dissolved in Et_2O (2 ml) and diluted with hexane (10 ml). The solvent was allowed to slowly evaporate. Residual solvent was removed from the crystals which were subjected to X-ray-analysis and found to be the (*R,S,S*)-isomer [20].

N-*(*tert-*Butoxy)carbonyl]*-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)oct-6-enoyl-(2*S*)-2-aminobutanoyl*-N-*methylglycyl*-N-*methyl*-L-*leucyloxy-Ψ(O–CH$_2$)-ethoxy-Ψ(O–NH)-4,4,4,4′,4′,4′-hexafluoro*-D-*valyl*-N-*methyl*-L-*leucyl*-L-*alanine* tert-*Butyl Ester* (**12a**). A mixture of *N*-Boc-octapeptide-OH **2b** (2.30 g, 2.17 mmol), tripeptide **11f$_2$** (650 mg, 1.18 mmol), EDC (600 mg, 3 mmol), DMAP (600 mg, 5 mmol), and a cat. amount of 1-hydroxy-7-aza-1*H*-benzotriazole (HOAt) in CH_2Cl_2 (70 ml) was kept at r.t. for 14 d. The mixture was filtered through SiO_2 (1 × 12 cm) with Cl_2Cl_2 and the filtrate concentrated: **12a** 1.725 g, 95%). TLC (AcOEt): 0.58. ^{1}H-NMR: 0.63 – 1.05 (*m*, 38 H); 1.19 – 1.34 (*m*, 10 H); 1.42 (*s*, ^{t}Bu); 1.45 (*s*, ^{t}Bu); 1.20 – 1.53 (*m*, 6 H); 1.56 – 1.82 (*m*, 12 H); 1.82 – 2.11 (*m*, 2 H); 2.21 – 2.45 (*m*, 2 H); 2.70 – 3.30 (*m*, 21 H); 3.50 – 4.12 (*m*, 3 H); 4.18 – 4.69 (*m*, 5 H); 4.75 – 5.02 (*m*, 1 H); 5.04 – 5.62 (*m*, 7 H); 6.12 – 7.00 (*m*, 3 H). ^{13}C-NMR: 15.6; 18.1; 18.2; 18.3; 18.4; 18.6; 19.8; 19.9; 21.6; 22.2; 22.8; 23.1; 23.6; 24.3; 24.9; 25.1; 27.2; 28.2; 28.6; 30.4; 30.5; 31.0; 31.1: 34.1; 36.4 (*t*); 36.6; 38.0; 38.4 (*t*); 40.2 (*t*); 43.6 (*t*); 46.9; 48.9; 51.5; 51.8; 54.8; 55.7; 62.8 (*t*); 62.8 (*t*); 69.3 (*t*); 72.2 (*t*); 128.8; 129.7; 137.2 (*s*); 155.2 (*s*); 168.3 (*s*); 168.8 (*s*); 170.8 (*s*); 171.5 (*s*); 171.7 (*s*); 173.1 (*s*); further signal 127.2. ^{19}F-NMR: – 62.45; – 64.72. ESI-MS: 1567.23 (35, $[M+Na]^+$), 1549.17 (65, $[M+Na-H_2O]^+$), 1545.11 (15, $[M+H]^+$), 1527.04 (20, $[M+H-H_2O]^+$), 562.21 (100), 539.27 (75).

Rearrangement of Depsipeptide (**12a**) *to* N$^{2.8}$*-[(*tert*-Butoxy)carbonyl]-[5-[4,4,4,4′,4′,4′-hexafluoro-*N*-(2-hydroxyethoxy)-*D*-valine]]-7,8-secocyclosporin* tert*-Butyl Ester* (= N*-(*tert*-Butoxy)carbonyl]-*D*-alanyl-*N*-methyl-*L*-leucyl-*N*-methyl-*L*-leucyl-*N*-methyl-*L*-valyl-(*2S,3R,4R,6E*)-3-hydroxy-4-methyl-2-(methylamino)oct-6-enoyl-(*2S*)-2-aminobutanoyl-*N*-methylglycyl-*N*-methyl-*L*-leucyl-4,4,4,4′,4′,4′-hexafluoro-*N*-(2-hydroxyethoxy)-*D*-valyl-*N*-methyl-*L*-leucyl-*L*-alanine* tert*-Butyl Ester*; **13a**). A soln. of the depsipeptide **12a** (20 mg, 0.02 mmol) in toluene (10 ml) was heated to reflux for 60 h. The solvent was evaporated. The more polar product was separated *via* prep. TLC (AcOEt). ^{1}H-NMR: 0.50 – 1.00 (*m*, 38 H); 1.42 (2*s*, 18 H); 1.15 – 2.20 (*m*, 34 H); 2.25 – 2.41 (*m*, 2 H); 2.68 – 3.30 (*m*, 21 H); 3.55 – 3.90 (*m*, 2 H); 4.05 – 5.00 (*m*, 8 H); 5.05 – 5.63 (*m*, 8 H). ^{13}C-NMR: 14.1; 15.2; 17.7; 17.8; 17.9; 18.0; 18.1; 18.2; 18.7; 18.9; 19.4; 19.5; 21.2; 21.7; 22.1; 22.3; 22.5; 22.8; 23.1; 23.9; 24.7; 24.8; 26.7; 27.0; 27.9; 28.2; 29.9; 30.0; 30.1; 30.4; 30.7; 36.7; 37.0 (*t*); 37.4; 37.9 (*t*); 39.8 (*t*); 46.5; 49.2 (*t*); 50.0; 51.1; 51.1; 51.4; 57.6; 62.6 (*t*); 63.4 (*t*); 64.2 (*t*); 65.0 (*t*); 77.2; 79.5; 127.6; 128.2; 155.2 (*s*); 170.8 (*s*); 171.6 (*s*); 172.0 (*s*); 173.1 (*s*); further signals at 127.1, 127.3, and 128.5. ^{19}F-NMR ($CDCl_3$): – 62.48; – 64.87. ESI-MS: 1566.92 (5, $[M+Na]^+$), 1549.16 (10, $[M+Na-H_2O]^+$), 1544.74 (8, $[M+H]^+$), 1527.11 (8, $[M+H-H_2O]^+$), 1507.11 ($C_{71}H_{123}F_6N_{11}NaO_{15}^+$), 1485.12 ($C_{71}H_{124}F_6N_{11}O_{15}^+$, loss of OCH_2CH_2O), 1143.90 (94, $C_{56}H_{98}N_9NaO_{14}^+$), 1121.72 (88, $C_{56}H_{99}N_9O_{14}^+$), 539.34 (100, $C_{21}H_{35}F_6N_3O_6^+$).

N$^{2.8}$*-[(*tert*-Butoxy)carbonyl][5-[4,4,4,4′,4′,4′-hexafluoro-*N*-(2-hydroxyethyoxy)-*L*-valine]]-7,8-secocyclosporin* tert*-Butyl Ester* (= N*-(*tert*-Butoxy)carbonyl]-*D*-alanyl-*N*-methyl-*L*-leucyl-*N*-methyl-*L*-leucyl-*N*-methyl-*L*-valyl-(*2S,3R,4R,6E*)-3-hydroxy-4-methyl-2-(methylamino)oct-6-enoyl-(*2S*)-2-aminobutanoyl-*N*-methylglycyl-*N*-methyl-*L*-leucyl-4,4,4,4′,4′,4′-hexafluoro-*N*-(2-hydroxyethoxy)-*D*-valyl-*N*-methyl-*L*-leucyl-*L*-alanine* tert*-Butyl Ester*; **13b**). A mixture of *N*-Boc-octapeptide-OH **2b** (4.1 g, 4 mmol), tripeptide **11f₁** (1.0 g, 1.9 mmol, EDC · HCl (1.1 g, 5.7 mmol), DMAP (2.0 g, 16.4 mmol), and a cat. amount of 1-hydroxy-7-aza-1*H*-benzotriazole (HOAt in CH_2Cl_2 (100 ml) was kept at r.t. for 14 d. The soln. was filtered through SiO_2 (1 × 15 cm): pure **13b** (2.05 g, 72%). TLC (AcOEt): 0.59. ^{1}H-NMR: 0.61 – 1.01 (*m*, 37 H); 1.39 (*s*, ^{t}Bu); 1.42 (s, ^{t}Bu); 1.15 – 1.62 (*m*, 19 H); 1.78 – 2.42 (*m*, 6 H); 2.67 – 3.26 (*m*, 21 H); 3.53 – 3.89 (*m*, 5 H); 3.92 – 4.10 (*m*, 1 H); 4.18 – 4.74 (*m*, 5 H); 4.83 – 4.99 (*m*, 1 H); 5.00 – 5.58 (*m*, 7 H); 6.07 – 6.97 (*m*, 3 H); 7.15 – 7.32 (*m*, 8 H). ^{13}C-NMR: 15.6; 18.1; 18.2; 18.3; 18.4; 18.6; 19.1; 19.3; 19.8; 19.9; 20.3; 21.6; 22.5; 22.7; 23.1; 23.2; 23.5; 24.3; 24.8; 25.1; 27.2; 28.2; 28.6; 30.4; 30.5; 30.7; 31.0; 31.1; 34.1; 36.4 (*t*); 36.6; 38.0; 38.3 (*t*); 40.2 (*t*); 43.5 (*t*); 46.9; 48.9; 51.5; 51.8; 54.8; 55.7; 62.8 (*t*); 62.9 (*t*); 69.3 (*t*); 72.2 (*t*); 77.6; 78.3; 128.8; 129.7; 137.7 (*s*); 155.6 (*s*); 168.6 (*s*); 168.8 (*s*); 169.3 (*s*); 171.3 (*s*); 171.4 (*s*); 171.9 (*s*); 172.1 (*s*); 172.2 (*s*); 172.3 (*s*); 172.6 (*s*); 173.6 (*s*); further signal at 127.7. ^{19}F-NMR($CDCl_3$): – 62.45; – 64.72. ESI-MS: 1567.08 (25, $[M+Na]^+$), 1549.01 (100, $[M+Na-H_2O]^+$), 1545.09 ($[M+H]^+$), 1527.08 (16, $[M-H_2O]^+$).

*[5-[4,4,4,4′,4′,4′-Hexafluoro-*N*-(2-hydroxyethoxy)-*D*-valine]]cyclosporin* (**14a**). The undecapeptide **13a** (105 mg, 0.07 mmol) was stirred at r.t. for 2 h in CF_3COOH (2 ml). The solvent was evaporated under aspirator vacuum, and sat. Na_2CO_3 soln. (10 ml) was added. The mixture was extracted with CH_2Cl_2 (2 × 25 ml) and the soln. dried ($MgSO_4$). A cat. amount of HOAt, EDC · HCl (382 mg, 2 mmol), and DMAP (366 mg, 3 mmol) were added to the soln. and stirred at r.t. for 5 d. The soln. was subjected to CC (SiO_2, AcOEt): **14a** (8 mg). TLC (AcOEt): R_f 0.56. ^{1}H-NMR: 0.60 – 1.00 (*m*, 33 H); 1.15 – 1.25 (*m*, 2 H); 1.35 – 2.15 (*m*, 26 H); 2.25 – 2.50 (*m*, 3 H); 2.52 – 3.33 (*m*, 20 H); 3.40 – 3.88 (*m*, 1 H); 4.20 – 4.40 (*m*, 5 H); 4.56 – 5.58 (*m*, 9 H); 6.55 – 7.70 (*m*, 5 H). ^{13}C-NMR: 14.2; 15.2; 15.9; 17.8; 17.9; 19.8; 21.3; 21.4; 21.7; 21.9; 22.0; 22.8; 23.1; 25.0; 28.6; 29.2; 29.2; 29.6; 29.9; 30.0; 30.5; 35.6; 36.4; 36.6; 36.7; 37.0; 37.1; 37.2; 37.3; 37.4 (*t*); 37.7; 37.8; 39.9; 40.3 (*t*); 42.0; 43.0; 46.6; 49.4; 49.9 (*t*); 50.1; 50.2; 51.1; 51.8; 54.62; 57.0; 58.6; 59.0

(*t*); 62.6, 63.0 (*t*); 64.3, 64.7 (*t*); 65.0, 65.4 (*t*); 70.7, 71.8 (*t*); 77.6, 86.2, 87.6 (*t*); 128.1; 128.8; 159.8; 168.4; 171.1; 171.3; 171.4; 171.7; further signals at 126.7, 126.8, 127.2, 127.4, 128.9, 128.9, and 129.0. ^{19}F-NMR ($CDCl_3$): −76.45. ESI-MS: 1392.97 (5, $[M+Na]^+$), 1370.85 (10, $[M+H]^+$), 1352.59 (5, $[M+H-H_2O]^+$), 1300.69 (8, $[M+H-CF_3]^+$), 1257.79 (20, $[M+H-CF_3-CH_2CH_2O]^+$), 1239.85 (100, $[M+H-CF_3-OCH_2CH_2OH]^+$).

*[5-[4,4,4,4′,4′,4′-Hexafluoro-*N*-(2-hydroxyethoxy)-*L*-valine]]cyclosporin* (**14b**). As described for **14a**, with undecapeptide **13b** (150 mg, 0.1 mmol), CF_3COOH (0.5 ml), and CH_2Cl_2 (5 ml) at r.t. overnight. Workup with sat. Na_2CO_3 soln. (10 ml) and CH_2Cl_2 (2 × 25 ml). Then with 1-hydroxy-7-aza-1*H*-benzotriazole (HOAt), EDC · HCl (380 mg, 2 mmol), and DMAP (250 mg, 2 mmol) at r.t. for 4 d. Most of the solvent was evaporated and the residue subjected to CC (SiO_2, 4% MeOH/Et_2O): **14b** (9 mg). TLC (AcOEt): R_f 0.4. ^{1}H-NMR: 0.57–1.08 (*m*, 35 H); 1.09–1.35 (*m*, 10 H); 1.35–1.50 (*m*, 6 H); 1.51–1.85 (*m*, 16 H); 1.86–2.45 (*m*, 5 H); 2.65–3.29 (*m*, 6 MeN); 3.31–4.60 (*m*, 8 H); 4.65–5.57 (*m*, 8 H); 6.02–7.02 (*m*, 4 H). ^{13}C-NMR: 18.2; 18.3; 18.4; 18.6; 18.7; 19.1; 19.3; 19.8; 19.9; 20.4; 21.5; 21.8; 22.1; 22.4; 23.6; 23.7; 23.9; 24.1; 24.3; 25.0; 25.0; 25.1; 25.3; 25.4; 25.5; 27.6; 30.2; 30.3; 30.4; 30.7; 30.8; 31.0; 31.1; 31.2; 31.6; 31.7; 32.0; 34.1; 35.7 (*t*); 37.6 (*t*); 38.0 (*t*); 45.7; 46.0; 49.2; 49.5; 49.9 (*t*); 50.1 (*t*); 50.6; 51.0; 52.7; 54.3; 55.5; 55.8; 56.8; 57.8; 62.9 (*t*); 72.2 (*t*); 77.6; 128.1; 128.5; 162.1; 168.1; 170.7; 170.8; 171.1; 171.6; 171.9; further signals at 125.9 and 127.8. ^{19}F-NMR ($CDCl_3$): −62.31; −64.79. ESI-MS: 1374.86 (10, $[M+Na-H_2O]^+$), 1370.81 (45, $[M+H]^+$), 1352.86 (55, $[M+H-H_2O]^+$), 688.02 (20, $[M+H+Na-H_2O)^{2+}$, 685.90 (100, $[M+2H]^{2+}$), 677.02 (55, $[M+2H-H_2O]^{2+}$).

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