270. Synthesis of Cyclosporine

Part II[1])

Synthesis of Boc-D-Ala-MeLeu-MeLeu-MeVal-OH[2]), a Part of the Peptide Sequence of Cyclosporine[3]), by Different Strategic Ways and Synthesis of its Isomers Boc-D-Ala-MeLeu-D-MeLeu-MeVal-OH, Boc-D-Ala-MeLeu-D-MeLeu-DMeVal-OH, and Boc-D-Ala-MeLeu-MeLeu-D-MeVal-OH as Reference Compounds

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Summary

Boc-D-Ala-MeLeu-MeLeu-MeVal-OH (DLLL) and its isomers DLDL, DLDD and DLLD were synthesized using several different strategic approaches and a modification of the mixed pivalic anhydride method for carboxyl activation. Alternatively, the *tert*-butoxycarbonyl (Boc) or benzyloxycarbonyl (Z) amino-protecting groups and the benzyloxy (OBzl) or *tert*-butoxy (*O*tBu) carboxyl-protecting groups were used to protect the reacting peptides. By monitoring the reaction temperature, it was possible to synthesize, starting from the tripeptide DLL as peptide model, either the tetrapeptide DLLL (−20°) or the tetrapeptide DLDL (+20°), selectively. Using ^{1}H-NMR spectroscopy to follow the mixed pivalic anhydride formation of the DLL- and DLD-tripeptides, it could be shown that anhydride formation is strongly dependent on the temperature. It is slow at −20° (several hours) and fast at +20° (20 to 40 min). The isomerization of the DLL-anhydride to the more stable DLD-anhydride can be reduced to a minimum by working at −20°, while this isomerization proceeds to near completion at +20°.

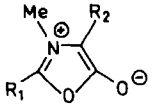
1. Introduction. – Cyclosporine [1c] [2] is an immunosuppressive cyclic undecapeptide containing seven *N*-methylamino acids. It belongs therefore to the group of *N*-methylaminoacid-containing natural products which include many peptide antibiotics [3–5], analogues of peptide hormones [6–8], and the insecticidal metabolite aspochracin [9] [10]. Current interest in *N*-methylamino-acid-containing antibiotics and the incorporation of *N*-methylated residues into biologically active peptides to improve

[1]) For part I, see [1a].

[2]) Abbreviations follow the IUPACIUB rules of the Commission on Biochemical Nomenclature [1b].

[3]) The name cyclosporine is used for the cyclic undecapeptide that has been named initially 'cyclosporin A', see [1c] [2]; meanwhile the following names are accepted in the following countries: USA, cyclosporine (USAN name), GB and France, ciclosporine and others, ciclosporin (INN name, WHO).

metabolic stability have prompted studies on the stereochemical outcome of coupling reactions of *N*-methylamino acids with the aim of finding conditions conducive to minimal racemization. *McDermott & Benoiton* [11] demonstrated the tendency of *N*-methylamino-acid derivatives to racemize rapidly during saponification and acidolysis. The same authors [12] studied the racemization of *N*-methylamino-acid residues during peptide-bond formation and made a comparison of the yields obtained by different methods for several model peptides. Epimerization occurred in varying degrees under all the coupling conditions studied. In all cases, the *N*-methylamino acids suffered more racemization than did the corresponding amino acid in the non-*N*-methylated analogues. *Davies & Mohammed* [13] with the aid of NMR-techniques came to the same conclusion studying the racemization in *N*-methylated amino-acid derivatives during peptide coupling in a model dipeptide system. They could as *McDermott & Benoiton* [12] confirm that the source of the racemization involving dicyclohexylcarbodiimide (DCCI) at room temperature is the oxazolonium intermediate **i**.



R^1 = phenyl or Z-NH-CH(CH_3)

R^2 = CH_3 or $(CH_3)_2CH$

i

The formation of **i** is temperature-dependent and its formation can be reduced at $-5°$ or even eliminated in presence of additives such as *N*-hydroxysuccinimide or *N*-hydroxybenzotriazole. The racemization which was still observed in the case of active esters, was then interpreted as being due to a direct ionization of the α-hydrogen atom of the ester species without the necessary formation of an oxazolonium intermediate **i**. These racemization studies [11–13] were made by coupling *N*-methylamino-acid derivatives with other non-*N*-methylated amino-acid derivatives. Bond formation between two *N*-methylated amino-acid derivatives presents more difficulties probably because of more steric hindrance and slower reaction. There are few examples in the literature describing peptide bond formation between two *N*-methylamino-acid derivatives. In the synthesis of triostin A analogues [4] [5] carboxyl-protected *N*-methylvaline derivatives were coupled with *N,S*-protected *N*-methylcysteine by the DCCI or the mixed carbonic anhydride methods [14] to give the desired compounds in moderate yields (50–70%). *Myokei et al.* [9] and *Chang et al.* [10] synthesized the dipeptide Z-MeVal-MeAla-OMe by the DCCI method in MeCN in a yield of 57%. Condensation of Z-MeVal-MeAla-OH with *N*-α-caprilyl-L-Orn-OMe using the DCCI technique gave in high yield a mixture of diastereoisomers. As expected, fragment condensation with a peptide containing an *N*-methylamino acid which must be activated for coupling is difficult, and so far no general procedure has been described.

It was clear to us that the structural parameters influencing racemization are quite complex, and the degree of racemization is often influenced by the structure of the amino acid, solvents, the nature of the group attached to the *N*-terminal end of the amino acid, the organic base, and coupling reagent used. Our aim in the present work is to describe how these problems could successfully be solved during the synthesis of Boc-D-Ala-MeLeu-MeLeu-MeVal-OH, making the peptide from the left to the right and thus using a strategy for its construction, which is very similar to the fragment condensation technique

used in peptide synthesis. This tetrapeptide will then be used as a building block in a total synthesis of cyclosporine [1c] [2].

To support the absolute configuration of Boc-D-Ala-MeLeu-MeLeu-MeVal-OH (DLLL), this tetrapeptide has been synthesized using different strategic approaches *(see Sect. 2.3)*. Also the synthesis of some of its possible isomers, the DLDL-, DLDD-, and DLLD-isomer, which could be formed specifically depending on the strategy used, is described either to identify unambigously the DLLL-isomer or to explain the epimerization processes occuring in some reactions.

2. General Remarks to the Synthesis of the Tetrapeptide Boc-D-Ala-MeLeu-MeLeu-MeVal-OH. – 2.1. *Choice of the Protecting Groups.* For the synthesis of Boc-D-Ala-MeLeu-MeLeu-MeVal-OH we used the classical *tert*-butoxycarbonyl (Boc) group as the aminoprotecting group and the no less classical benzyl ester group (OBzl) as carboxyl-protecting group.

At this point it should also be noted that as a precursor of cyclosporine the tetrapeptide D-Ala-MeLeu-MeLeu-MeVal-OH must be protected on its amino end in a way which can later be easily[4]) cleaved in the presence of a double bond on completion of the undecapeptide[5]) synthesis. If for any reason the benzyloxycarbonyl (Z) protecting group is used in the synthesis of the tetrapeptide, it will be necessary to remove it and replace it by the Boc or another suitable protecting group[4]) before proceeding with the cyclosporine synthesis. This was the case for Z-MeLeu-MeLeu-MeVal-OH *(Scheme 4)* as will be described later.

2.2. *Choice and Description of the Carboxyl-Activating Method.* The carboxyl groups for all the coupling reactions described in this paper were activated using a variation of the mixed pivalic anhydride method reported by *Zaoral* [17], and adapted for *N*-methyl-amino-acid derivatives by allowing slow anhydride formation in $CHCl_3$[6]) at $-20°$ with pivaloyl chloride (= trimethylacetyl chloride) in presence of 2 equivalents of a tertiary base such as *N*-methylmorpholine. In contrast to the well documented [18] mixed carbonic anhydrides, which rapidly epimerize in presence of excess of base, the epimerization of mixed pivalic anhydrides of *N*-methylamino-acid derivatives was reduced if 2 equivalents of base were used instead of 1 equivalent.

When Et_3N was used as base for the mixed pivalic anhydride couplings, instead of increasing the degree of epimerization as is normally observed [14], it had the opposite effect. *McDermott & Benoiton* [12] made the same observation in the case of a mixed carbonic anhydride coupling of an *N*-methylamino-acid derivative. In the case of mixed pivalic anhydrides of *N*-methylamino-acid derivatives reaction rates and epimerization of the activated amino acid were increased by acids and slowed down by strong base as it will be demonstrated later in *Table 2*.

The *N*-methylamino acid benzylester was added as base to the mixed pivalic anhydride solution and allowed to react at $-20°$ for as long as necessary for completion. Addition of the protected amino acid as base always gave cleaner coupling products and higher yields than the addition as hydrochloride or *p*-toluenesulfonic acid salt. This was the case even if more tertiary base was added at the same time.

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[4]) Other more problematic candidates for N-protection in this case are the (9-fluorenyl)methoxycarbonyl [15] and 2-(4-biphenylyl)-2-propanyloxycarbonyl [16] groups, which also are stable in presence of H_2/Pd/EtOH.

[5]) The choice of the cyclization step to cyclosporine and the strategy used for the synthesis of the undecapeptide is the subject of a forthcoming paper.

[6]) For convenience this solvent was used in our laboratory. Unsuccessful results using the mixed carbonic anhydride method in $CHCl_3$ and CH_2Cl_2 are known in the literature [14] [19] [20].

This can be rationalized by the higher degree of enolization of the mixed pivalic anhydride in presence of more acid or more salt, and therefore, in a more ionic environment. The steric hindrance and inductive depression of electrophilicity of the pivaloyl group direct the attack of the amino group almost exclusively to the carboxyl component. If pivaloyl derivatives of the amine component are obtained, it is generally due to the use of an excess of pivaloyl chloride or to incomplete anhydride formation and reaction of the amine with the unreacted pivaloyl chloride. It is seldom due to steric hindrance of the carboxyl or amine components.

Activation of the carboxyl group as an anhydride is necessary, since it yields a higher reactivity than most of the usual forms of activation used in peptide synthesis when applied to protected *N*-methylamino acids or *N*-protected peptidic *N*-methylamino-acid derivatives. This statement is specially true for very sterically hindered amino acids such as MeVal, MeIleu, MeBmt[7]) or even MeLeu.

Warnke & Young [7] also found independently that the mixed pivalic anhydride method [17] used for coupling *N*-methylamino-acid derivatives gave high yields and low epimerization rates. It should be noted that in their work only a mixed pivalic anhydride of *N*-Boc-*N*-methyl-phenylalanine is used as acylating component. The well documented [13] [21] stabilizing effect of the urethane Boc-group in this case permits reaction at room temperature to complete[8]) the formation of the mixed pivalic anhydride and for coupling without measurable increase of epimerization.

The reaction of pivaloyl chloride with the carboxyl group of *N*-methylaminoacid derivatives is very slow (hours) at low temperature ($-20°$) as it will be demonstrated later *(Table 3)* for the formation of the mixed pivalic anhydride of Boc-D-Ala-MeLeu-MeLeu-OH as a model. This slow reaction is due to the lack of reactivity of pivaloyl chloride with tertiary amines such as *N*-methylmorphine.

This difference in reactivity of pivaloyl chloride compared with that of alkyl chlorocarbonates[9]), which are known to react with tertiary amines to form alkoxycarbonylammonium ion complexes, which subsequently react with the carboxylates to give mixed carbonic anhydrides, suggests another mechanism for the production of mixed pivalic anhydrides. Support for the different reaction paths of pivaloyl chloride and isobutylchlorocarbonate was provided by IR spectroscopy of both reagents at room temperature in CH_2Cl_2 in presence of 2 equiv. of *N*-methylmorpholine. The former is highly stable and the latter reacts rapidly with the tertiary amine giving probably the ammonium complex mentioned above. This provokes a shift of the carboxyl absorption in the IR to longer wavelengths. This shift was not observed in the case of pivaloyl chloride even after 4 days at room temperature.

By contrast, mixed pivalic anhydrides of *N*-methylamino-acid derivatives seem to be more stable than their mixed carbonic anhydride analogs. At $-20°$ over a period of several hours, no decomposition *via* disproportionation (identical ^{1}H-NMR spectra of mixed pivalic anhydrides after 16 h at $-20°$ and after 20 min at room temperature) was observed *(Scheme 8, Table 3, Fig. 4)*. Further support for this difference in stability of both anhydrides was provided by the absence of peptide-bond formation in the case of the mixed carbonic anhydride while working under activation conditions which gave a good yield in the case of the mixed pivalic anhydride $4^1/_2$ h at $-20°$; *Table 2, Exper. IX* and *X*.

2.3 *Strategy Used for the Synthesis of the Peptides* (see *Fig. 1* and *Table 1)*. The peptides are built up in the direction shown by the horizontal arrows in *Fig. 1* using the step sequence, which is indicated numerically. Bonds 1, 2, and 3 were made first and the

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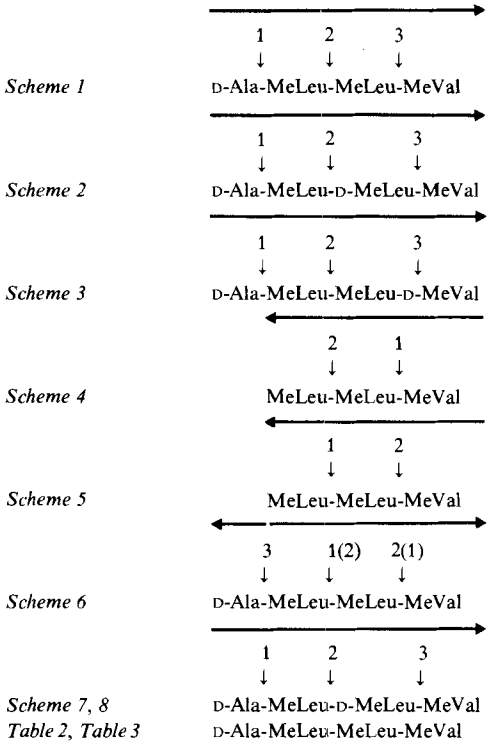
[7]) MeBmt = (4*R*)-4-[(2*E*)-2-butenyl]-4,*N*-dimethyl-L-threonine; for more details about this amino acid see [1a].

[8]) Pivaloyl chloride reacted under these coupling conditions faster with carboxyl groups than with *N*-methylamino groups of *N*-methylamino-acid derivatives *(Table 2, Exper. XI)*.

[9]) For introduction of the method, see [22–24]; precise reaction conditions in [14] [25] [26] and a recent review [18].

Fig. 1. *Strategy Used for the Synthesis of the Peptides (→) and Step Sequence for the Peptide Bond Formation (1,2,3)*



tetrapeptides Boc-D-Ala-MeLeu-MeLeu-MeVal-OBzl (**6**; *Scheme 1*), Boc-D-Ala-MeLeu-D-MeLeu-MeVal-OBzl (**10**; *Scheme 2*) and Boc-D-Ala-MeLeu-MeLeu-D-MeVal-OBzl (**12**; *Scheme 3*) so synthesized.

The tetrapeptide benzyl ester **6** could not be made directly starting from the right by making bond 3 first because of instantaneous formation of the diketopiperazine **21** *(Scheme 4)* from the dipeptide H-MeLeu-MeVal-OBzl. Spontaneous formation of a cyclic dipeptide by intramolecular aminolysis has already been reported in the case of *N*-methylamino-acid dipeptide analogs [5] [10]. A protecting group exchange and the use of the *tert*-butyl ester of *N*-methylvaline was sufficient to completely exclude the ring formation in the case of H-MeLeu-MeVal-O*t*Bu (**22**; *Scheme 4*). The dipeptide **22** was then used to make Z-MeLeu-MeLeu-MeVal-O*t*Bu (**23**)[10]) which was converted to the adequately protected H-MeLeu-MeLeu-MeVal-OBzl (**28**).

The same tripeptide was built up in the reverse direction making the MeLeu-MeLeu bond prior to the MeLeu-MeVal bond, using the desired Boc and benzyl ester protecting groups *(Scheme 5)*. This alternative for the synthesis of tripeptide **28** avoids the problem of the diketopiperazine formation and the laborious exchange of protecting groups in **23**

[10]) The tripeptide **23** was kindly synthesized as reference by Dr. *M. Krieger (Sandoz Ltd.*, Basle).

Table 1. *Different Strategic Approaches to the Synthesis of Boc-D-Ala-MeLeu-MeLeu-MeVal-OH (7) and Some of its Diastereomers Using the Mixed Pivalic Anhydride Coupling Method* [17]

Scheme	Product number (+added residue)	D-Ala	MeLeu	MeLeu	MeVal	Yield [%]	$[\alpha]_D^{20}$ [°]	*c* in $CHCl_3$	Side Products
1	**1**(+MeLeu-OBzl)	Boc–OH	H–OBzl						
1	**2**	Boc–	OBzl[a])			80	− 35.3	1.05	
1	**3**(+MeLeu-OBzl)	Boc–	OH[a])	H–OBzl		97	− 36.9	0.8	
1	**4**	Boc–		OBzl		71	−101.3	0.9	DDL
1,8	**5**(+MeVal-OBzl)	Boc–		OH[a])	H–OBzl	92	−112.6	0.85	
1,8	**6**	Boc–			OBzl	88	(−144.8)[b])	1.0	**10**(=DLDL)
1,8	**7**	Boc–			OH	78	−200.5	1.0	**11**(=DLDL)
	(17+)**18**		Z–OH	H–O*t*Bu					
4	**20**		Z–		O*t*Bu	59	−136.5	1.0	DL
4	(17+)**22**		Z–OH	H–	O*t*Bu	84	−118	1.0	DL
4	**23**		Z–		O*t*Bu	46	−165.5	1.0	DLL
4	**24**		Z–		OH	71	−184.5	1.0	
4	**25**		H–		OH[a])	85	−165.9	1.0	
4	**26**		Boc–		OH	74	−185.6	1.0	
4	**27**		Boc–		OBzl				
4	**28**		H–		OBzl[a])	38	−149.1	1.0	
	15(+MeLeu-OBzl)		Boc–OH	H–OBzl					
5	**29**		Boc–		OBzl	88	− 95.5	1.0	
5	**30**		Boc–		OH[a])	75	−120.6	1.0	
5	**27**		Boc–		OBzl	79	−129.8	1.0	LDL
5	(1+)**28**	Boc–OH	H–		OBzl[a])	60	−155.0	1.0	LDL
5	**26**		Boc–		OH	62	−185.3	1.0	
6	**6**	Boc–			OBzl	78	(−156.5)[b])	1.0	**10**(=DLDL)
6	**7**	Boc–			OH	86	−201.6	1.0	**11**(=DLDL)
		D-Ala	MeLeu	D-MeLeu	MeVal				
2	**3**(+D-MeLeu-OBzl)	Boc–	OH	H–OBzl					
2	**8**	Boc–		OBzl		75			DDD
2	**9**(+MeVal-OBzl)	Boc–		OH[a])	H–OBzl	74	− 60.3	0.82	DDD
2,8	**10**	Boc–			OBzl	47	(−83.9)[b])	0.84	**6**(=DLLL)
2,8	**11**	Boc–			OH	52	− 52.2	1.0	**7**(=DLLL)
		D-Ala	MeLeu	MeLeu	D-MeVal				
3	**5**(+D-MeVal-OBzl)	Boc–		OH	H–OBzl				
3	**12**	Boc–			OBzl	70	(−18.2)[b])	0.9	DLDD(43%)
3	**14**	Boc–			OH	54	−74.9	0.8	**13**(=DLDD)

[a]) Crystalline compound. [b]) Rotation of an impure compound.

(Scheme 4), which is necessary to obtain finally Boc-D-Ala-MeLeu-MeLeu-MeVal-OH (7)[11]). The synthesis of the latter from the tripeptide **28** is described in *Scheme 6*.

The fact that the same tetrapeptide Boc-D-Ala-MeLeu-MeLeu-MeVal-OH (7) has been obtained from three strategically different synthesis (123, 312 or 321 in *Fig. 1*) confirms that the stereochemistry has been successfully controlled during the *N*-methyl-amino-acid coupling in all three cases.

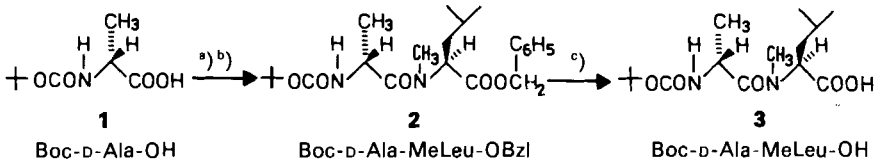
[11]) See also comments in *Sect. 2.1.*

Scheme 7 and *Table 2* describe the epimerization of the terminal *N*-methylleucine residue in Boc-D-Ala-MeLeu-MeLeu-OH (**5**) during peptide bond formation with *N*-methylvaline benzyl ester (**16**) and make a comparison of the yields obtained using different coupling conditions and methods for this model peptide.

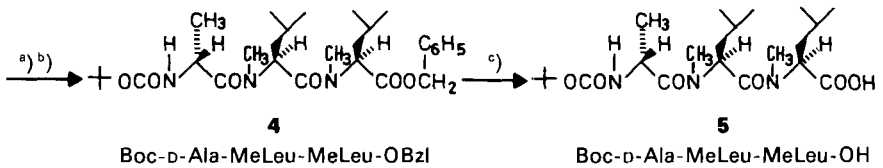
Scheme 8 and *Table 3* summarize the coupling results which are obtained for the tripeptides Boc-D-Ala-MeLeu-MeLeu-OH (**5**) and Boc-D-Ala-MeLeu-D-MeLeu-OH (**9**) as models, by coupling with *N*-methylvaline benzyl ester (**16**) using the modified mixed pivalic anhydride method and working at $+20°$ and $-20°$. *Figures 2–4* show some ^{1}H-NMR-spectra obtained during the activation of **5** and **9**. *Table 1* gives summary of all peptides synthesized with rotations and yields obtained for each of them. It also indicates which side products are formed during their synthesis.

3. Synthesis of Boc-D-Ala-MeLeu-MeLeu-MeVal-OH (7; *Scheme 1*). Boc-D-Ala-OH (**1**)[12]) is activated at $-20°$ using pivaloyl chloride in presence of two equiv. of *N*-methylmorpholine in $CHCl_3$ as described for *N*-methylamino acids *(see Sect. 2.2)* and reacted with MeLeu-OBzl[12]) as base to produce crystalline Boc-D-Ala-MeLeu-OBzl (**2**) in 80% yield. The benzyl protecting group is removed by hydrogenation at room temperature in alcohol and using Pd/C as catalyst. The dipeptide **3** is isolated as crystalline acid in

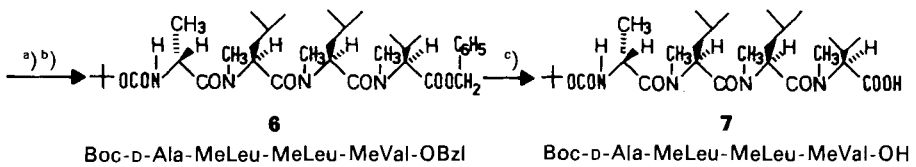
Scheme 1. *Synthesis of Boc-D-Ala-MeLeu-MeLeu-MeVal-OH* (7)



1 Boc-D-Ala-OH — **2** Boc-D-Ala-MeLeu-OBzl — **3** Boc-D-Ala-MeLeu-OH



4 Boc-D-Ala-MeLeu-MeLeu-OBzl — **5** Boc-D-Ala-MeLeu-MeLeu-OH



6 Boc-D-Ala-MeLeu-MeLeu-MeVal-OBzl — **7** Boc-D-Ala-MeLeu-MeLeu-MeVal-OH

[a]) 1.0 Equiv. of pivaloyl chloride and 2.0 equiv. of *N*-methylmorpholine in $CHCl_3$.
[b]) Corresponding *N*-methylamino acid benzyl ester as base.
[c]) H_2|Pd|C|EtOH.

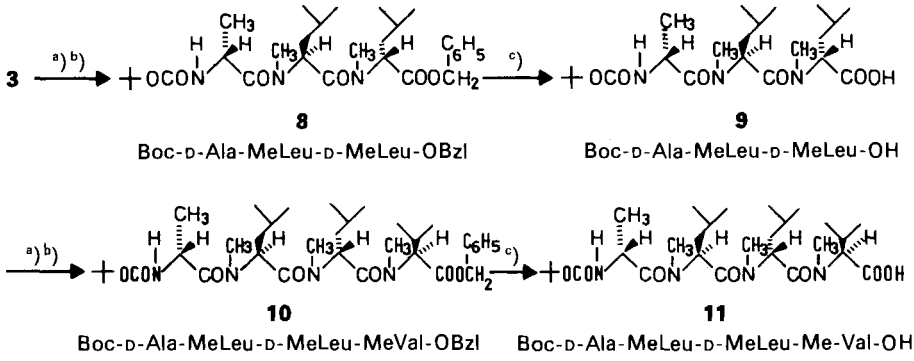
[12]) For starting materials see *Exper. Part.*

97% yield. This dipeptide **3** is activated similarly for 90 min at $-20°$ with pivaloyl chloride and *N*-methylmorpholine. MeLeu-OBzl[12]) is added at $-20°$. After 18 h at $-20°$, workup and chromatography Boc-D-Ala-MeLeu-MeLeu-OBzl (**4**) and Boc-D-Ala-D-MeLeu-MeLeu-OBzl are isolated in 71 and 5.4% yield, respectively. The tripeptide **4** is debenzylated as above and the product crystallized from hexane to give Boc-D-Ala-MeLeu-MeLeu-OH (**5**) in 92% yield. This tripeptide **5** is then activated for 2 h at $-20°$ to a mixed pivalic anhydride and reacted with MeVal-OBzl[12]) ($4^1/_2$ days, $-20°$, equimolar proportions of reactants). After standard workup, the desired protected tetrapeptide **6** is isolated as a 9:1 mixture of DLLL/DLDL-isomers **6** and **10** in 60% yield (30% of **5** is recovered). This isomeric ratio was determined by ^{1}H-NMR-spectroscopy in (D_6)dimethylsulfoxide ((D_6) DMSO) at 180°[13]) measuring the peak intensities of the H–C(2^4) protons[14]) of **6** and **10**. Working under similar conditions on a larger scale but doubling the time of activation (4 h), 88% of tetrapeptide **6** containing 10% of isomer **10** are isolated. This increase in yield demonstrates how important the activation procedure is. Other batches prepared later in our laboratory have shown that 5 h activation and 16 h reaction, both at $-20°$, and a small excess (1.2 equiv.) of *N*-methylvaline benzylester as base are optimal. The tetrapeptide isomer DLDL **10** cannot be separated from **6** by chromatography. Separation is achieved at the level of deprotected Boc-D-Ala-MeLeu-MeLeu-MeVal-OH (**7**) and its DLDL-isomer **11** (78% and 8% yield, resp. after chromatography).

This confirms that the epimerization during the coupling of *N*-methylamino-acid derivatives on a relatively large scale (19 g) can be held below 10% even by using equivalent proportions of reactants. By using 2 or 5 equiv. of MeVal-OBzl the epimerization can even be reduced to 6 of 4%, respectively *(see Sect. 9, Table 2, Exper. XII* and *XIII)*, which is very acceptable for such a difficult coupling reaction.

4. Synthesis of Boc-D-Ala-MeLeu-D-MeLeu-MeVal-OH (11; *Scheme 2*). – The tetrapeptide **11** is formed as the major product during the synthesis of **7** *(see Scheme 1)* when using the mixed pivalic anhydride method at room temperature. To study the formation of **11** and to firmly establish the structure of **7**, the synthesis of Boc-D-Ala-MeLeu-D-MeLeu-

Scheme 2. *Synthesis of Boc-D-Ala-MeLeu-D-MeLeu-MeVal-OH* (**11**)



a) b) c) See *Scheme 1*.

13) The ^{1}H-NMR-measurement technique in (D_6)DMSO at +180° was developed by *H. R. Loosli* of the physical department at *Sandoz*, Basle.

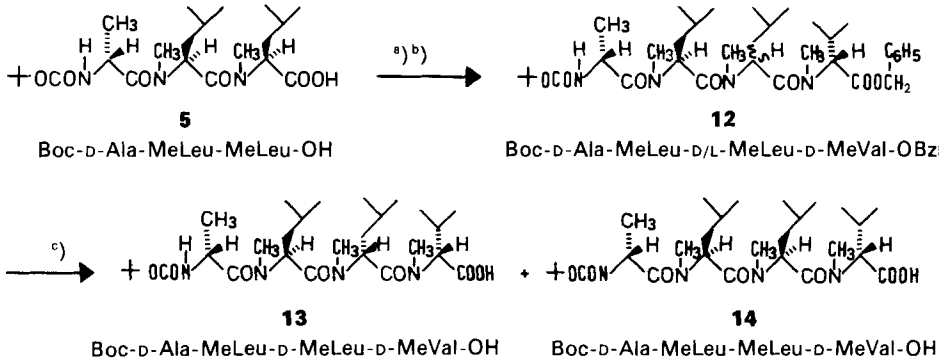
14) H–C(2^4) = Proton at C(2) (= C(α)) of MeVal in **6** and **10**; for peptide and protons numbering see *General* in *Exper. Part.*

OH (**9**) and Boc-D-Ala-MeLeu-D-MeLeu-MeVal-OH (**11**) became necessary. For this purpose Boc-D-Ala-MeLeu-OH (**3**) is condensed with MeLeu-OBzl[12]) using the mixed pivalic anhydride method. Boc-D-Ala-MeLeu-D-MeLeu-OBzl (**8**) contaminated with the DDD-isomer is isolated in 75% yield.

At the time this synthesis was carried out, we had not yet studied in detail the importance of temperature in the formation of a mixed pivalic anhydride or its influence on the epimerization. For this reason the reaction conditions chosen were as described in [17] or as described for mixed carbonic anhydrides [14] [25] [26], working at low temperature for the carboxyl activation and starting the coupling, then warming up to room temperature to complete the reaction. This explains the relatively high proportion of DDD-isomer obtained (*ca.* 20%).

Debenzylation of **8** and chromatography yield pure Boc-D-Ala-MeLeu-D-MeLeu-OH (**9**) as crystalline product in 74% yield. The mother liquors (25%) contaminated with **9** contain a more polar isomer, which is believed to be the expected DDD-isomer. The tripeptide **9** is activated with pivaloyl chloride in presence of 2 equiv. of *N*-methylmorpholine for 16 h at $-20°$ in $CDCl_3$, then reacted with MeVal-OBzl for 5 days at $-20°$. The formation of the anhydride and its reaction with the amine component are followed by ^{1}H-NMR (see below, *Sect. 10*, *Table 3*). A 4:1 by ^{1}H-NMR[13])[14]) mixture of diastereomers **10** and **6** is obtained in 47% yield. It should be noted here that the reaction conditions for **9** → **10**/**6** are not optimized in this experiment; they only allow a comparison with the experiment made with the DLL-isomer **5** (see *Sect. 10*, *Scheme 8*, *Table 3*). Finally, debenzylation of **10**/**6** yields a 7:3 mixture of diastereomers **11** and **7**, which, considering the small quantity of material used, confirms the proportion measured by ^{1}H-NMR. The isolation and the characterization of the DLDL-isomer **11** using this synthetic pathway and its identity with **11** described in *Sect. 3* firmly establish its structure.

Scheme 3. *Synthesis of Boc-D-Ala-MeLeu-MeLeu-D-MeVal-OH* (**14**)



a) b) c) See *Scheme 1*.

5. Synthesis of Boc-D-Ala-MeLeu-MeLeu-D-MeVal-OH (14; *Scheme 3*). – As the synthesis of Boc-D-Ala-MeLeu-D-MeLeu-OH (**9**) has been planned to compare its coupling with the one of its DLL-isomer **5**, the tetrapeptide Boc-D-Ala-MeLeu-MeLeu-D-MeVal-OH (**14**) is synthesized in order to compare its behaviour with the one of its DLLL-isomer **7** during the coupling reaction with the heptapeptide corresponding to the cyclosporine sequence 1 to 7[15])[16]). This peptide **14** can also be used later to perform the

[15]) This coupling with the heptapeptide will be described in a forthcoming paper (*cf. Footnote 5*).

[16]) For the numbering of the amino-acid sequence in cyclosporine, see *Rüegger et al.* [2].

synthesis of the natural cyclosporin H [27], which has a D-MeVal in position 11[16]). The DLDD-isomer **13** is isolated as an artefact of the synthesis of **14**. It nevertheless contributes to exclude its incorporation as an alternative for **7** or **14** during the condensation of **7** or **14** with the above-mentioned haptapeptide. Assuming this condensation would proceed, as described by *McDermott et al.* [12] or *Davies et al.* [13], with the intermediacy of **i** see *Sect. 1*, R^1 = Boc-D-Ala-MeLeu-NCH_3-CH[$(CH_3)_2CHCH_2$], $R^2 = (CH_3)_2CH$ as intermediate, then the formation of a DLDD-instead of a DLLL- or DLLD-peptide sequence would be quite possible as in case of an oxazolone.

Boc-D-Ala-MeLeu-MeLeu-OH (**5**) is activated as for the synthesis of **6** with pivaloyl chloride (2 equiv. of *N*-methylmorpholine, $-20°$, $3\frac{1}{2}$ h) and then reacted with D-MeVal-OBzl (6 days, $-20°$) to give a 3:2 mixture **12** of DLLD- and DLDD-isomers in 70% yield. After debenzylation of **12**, the DLDD- and DLLD-isomers **13** and **14** are separated and isolated as pure products in 40 and 54% yield, respectively.

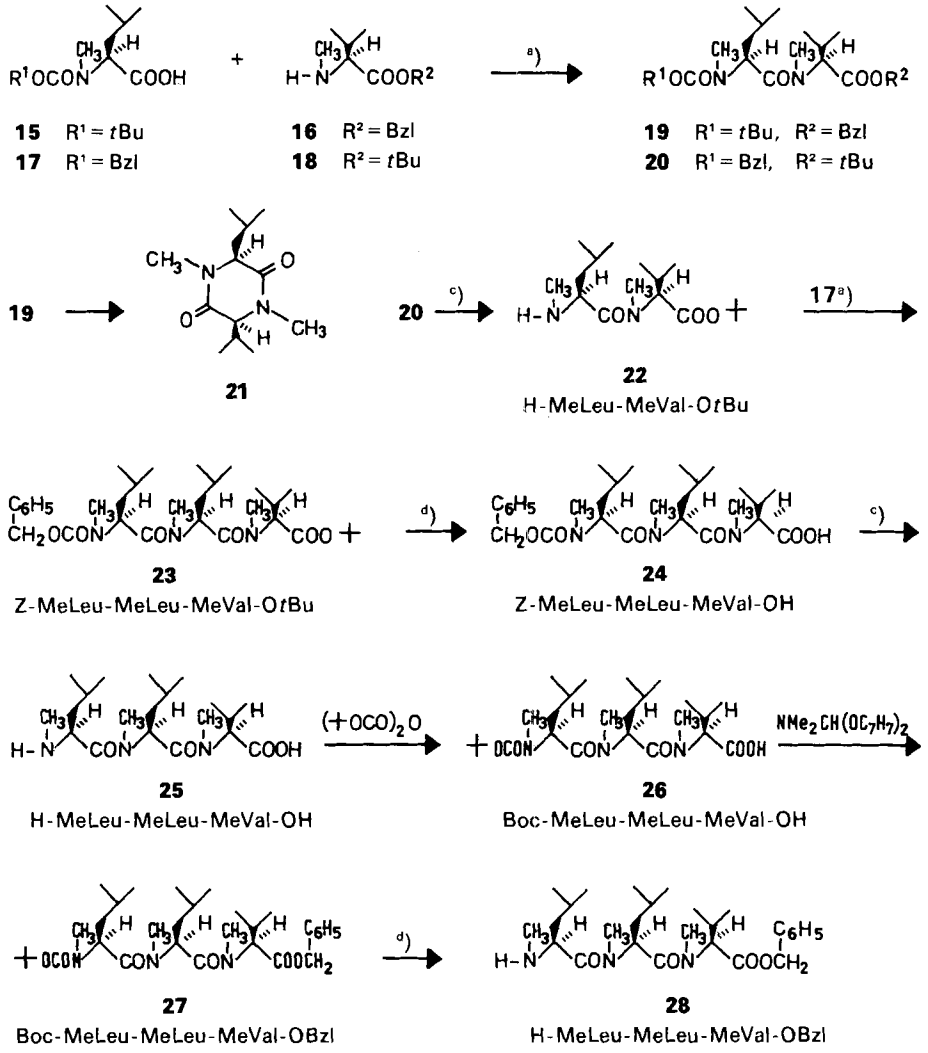
The reaction of D-MeVal-OBzl with the anhydride of **5** is relatively slow compared to the one of L-MeVal-OBzl with the same anhydride (→**6**). As described in *Sect. 10 (Table 3)* the *N*-methyl-L-amino acid seems to react faster with the DLL-anhydride **A** than with the DLD-anhydride **B**. In the case studied here the *N*-methyl-D-amino acid as expected reacts with more difficulty with the DLL-anhydride **A** than with the DLD-anhydride **B**. This could explain the relative high quantity of epimer formed during the reaction of activated **5** with D-MeVal-OBzl.

6. Synthesis of the Intermediate H-MeLeu-MeLeu-MeVal-OBzl (28), *Procedure A (Scheme 4)*. – An attempt to make the compound **28** *via* successive *N*-terminal additions of Boc-MeLeu-OH (**15**) to H-MeVal-OBzl (**16**)[12]) using the mixed pivalic anhydride method failed since after removing the Boc-group from the intermediate dipeptide Boc-MeLeu-MeVal-OBzl (**19**), the product cyclized to the diketopiperazine **21** when attempting the next coupling reaction at room temperature *(see Sect. 2.3)*. To avoid this cyclization the dipeptide H-MeLeu-MeVal-O*t*Bu (**22**) was synthesized starting from Z-MeLeu-OH (**17**) and H-MeVal-O*t*Bu (**18**)[12]) using the mixed pivalic anhydride for carboxyl activation (→**20**) and then H_2/Pd/C to remove the benzyloxycarbonyl(Z) protecting group. The second Z-MeLeu-OH (**17**) residue was then added to **22** using again the mixed pivalic anhydride method to give Z-MeLeu-MeLeu-MeVal-O*t*Bu (**23**).

It is interesting to note that for the formation of the MeLeu-MeVal dipeptides **19** and **20**, warming of the reaction mixture was necessary in both cases to promote coupling. This operation was done without greatly enhancing the epimerization of the mixed pivalic anhydride of the Boc or Z protected MeLeu derivatives **15** and **17**, respectively, demonstrating again the highly stabilizing effect of the urethane protecting groups.

Z-MeLeu-MeLeu-MeVal-O*t*Bu (**23**) is deprotected stepwise removing the *tert*-butyl group first by treatment with CF_3COOH at $-20°$ to give **24** in 71% yield. Then, with H_2/Pd/C crystalline H-MeLeu-MeLeu-MeVal-OH (**25**) is obtained in 85% yield. The tripeptide **25** is protected with the Boc group using di(*tert*-butyl) pyrocarbonate [28] [29] in presence of NaOH in dioxane to give Boc-MeLeu-MeLeu-MeVal-OH (**26**) in 74% yield. Identity of **26** with the tripeptide obtained by the reversed strategy *(see Sect. 7, Scheme 5)* has been confirmed by their identical physical data. Tripeptide **26** is benzylated using the method of *Brechbühler et al.* [30] by refluxing in benzene with *N,N*-dimethylformamide dibenzyl acetal to yield **27**. The Boc group of **27** is removed by treatment with CF_3COOH at $-20°$ to yield, after purification, crystalline H-MeLeu-MeLeu-MeVal-OBzl (**28**) in a yield of 38% over 2 steps. This product is in all aspects identical with the tripeptide obtained by the reversed strategy *(see Sect. 7, Scheme 5)*.

Scheme 4. *Synthesis of the Intermediate H-MeLeu-MeLeu-MeVal-OBzl* (**28**), *Procedure A*

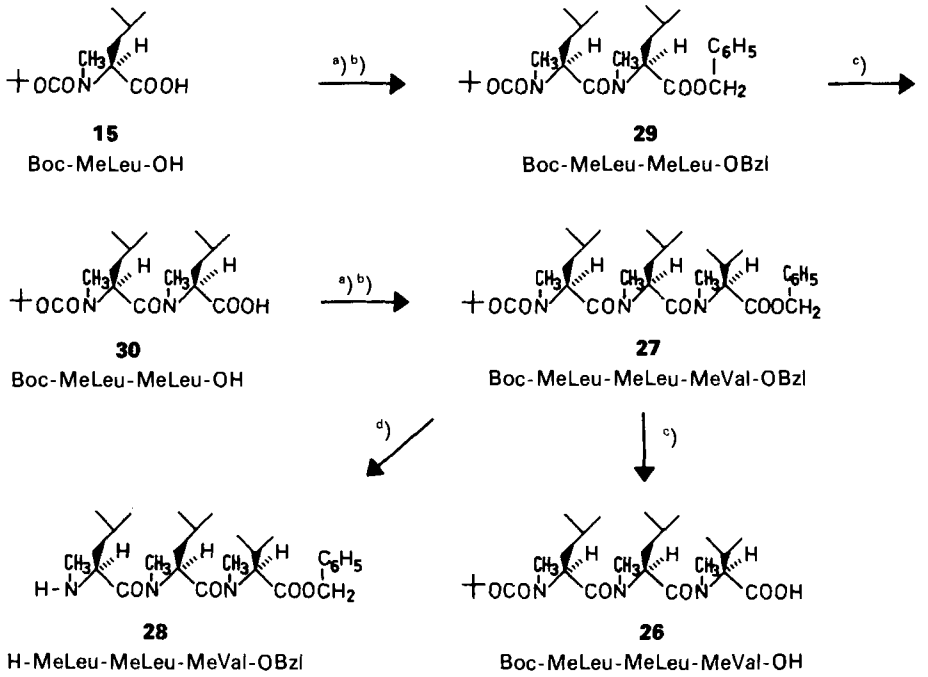


[a]) [c]) See *Scheme 1*. [d]) CF_3COOH.

The *Procedure A* for the synthesis of H-MeLeu-MeLeu-MeVal-OBzl (**28**) shows that it is possible to exchange the protecting groups of a tripeptide containing three *N*-methylamino acids, *e.g.* **23**, working under acidic and basic conditions which have been reported [11] to be critical for epimerization of *N*-methylamino-acid derivatives. The conditions used to exchange the protecting groups have not been optimized.

7. Synthesis of the Intermediate H-MeLeu-MeLeu-MeVal-OBzl (28), *Procedure B* (*Scheme 5*). – Boc-MeLeu-OH (**15**) and H-MeLeu-OBzl[12]) are first condensed using the mixed pivalic anhydride method as above (activating: 6 h, – 20°; coupling: 20 h, – 20°) to

Scheme 5. *Synthesis of the Intermediate H-MeLeu-MeLeu-MeVal-OBzl* (**28**), *Procedure B*



[a]) [b]) [c]) See *Scheme 1*. [d]) CF_3COOH.

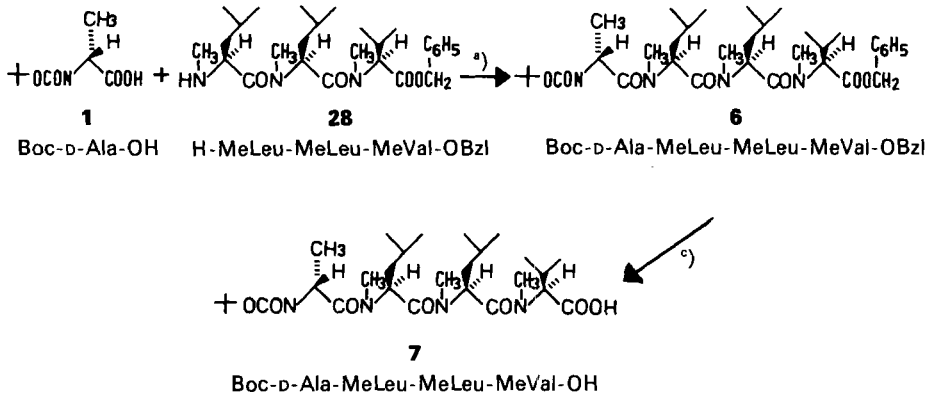
yield Boc-MeLeu-MeLeu-OBzl (**29**) in 88% yield. After debenzylation, Boc-MeLeu-MeLeu-OH (**30**) is obtained and activated using the same method as before. It is coupled with H-MeVal-OBzl (**16**)[12]) (3 days, $-20°$) to give in 79% yield Boc-MeLeu-MeLeu-MeVal-OBzl **27** which is contaminated with less than 5% of the LDL-isomer. (The latter isomer has been identified and quantified after treatment with CF_3COOH and coupling with **1** by the formation of Boc-D-Ala-MeLeu-D-MeLeu-MeVal-OBzl (**10**) and by the isolation after debenzylation of Boc-D-Ala-MeLeu-D-MeLeu-MeVal-OH (**11**) in 3.6% yield.) The N-protecting group of **27** is removed with CF_3COOH at $-20°$ to give pure crystalline H-MeLeu-MeLeu-MeVal-OBzl **28** in 60% yield. The tripeptide **27** is also debenzylated to give Boc-MeLeu-MeLeu-MeVal-OH (**26**). Compounds **28**, **27** and **26** are identical with the corresponding tripeptides obtained by *Procedure A (Sect. 6, Scheme 4)*.

For the synthesis of H-MeLeu-MeLeu-MeVal-OBzl (**28**), *Procedure B* is preferred to *Procedure A* because it not only solves the problem of the diketopiperazine formation, but also avoids the protecting group exchange which is necessary[17]) at the end of the synthesis using procedure A.

8. Synthesis of Boc-D-Ala-MeLeu-MeLeu-MeVal-OH (7) from the Intermediate H-MeLeu-MeLeu-MeVal-OBzl (28; *Scheme 6*). – To provide further evidence for the absolute configuration of the tetrapeptide **7** and to circumvent the possibility of epimerization of the dipeptide Boc-D-Ala-MeLeu-OH (**3**) during coupling (see *Sect. 3)*, an

[17]) See *Sect. 2.1*, choice of the protecting groups.

Scheme 6. *Synthesis of Boc-D-Ala-MeLeu-MeLeu-MeVal-OH* (7) *from H-MeLeu-MeLeu-MeVal-OBzl* (**28**)



1 Boc-D-Ala-OH

28 H-MeLeu-MeLeu-MeVal-OBzl

6 Boc-D-Ala-MeLeu-MeLeu-MeVal-OBzl

7 Boc-D-Ala-MeLeu-MeLeu-MeVal-OH

a)c) See *Scheme 1*.

approach to the synthesis of **7** using H-MeLeu-MeLeu-MeVal-OBzl (**28**; *Schemes 4* and *5)* was used. This approach incorporates both the 321 and 312 strategies *(Fig. 1)* simultaneously. Boc-D-Ala-OH (**1**) is activated at −20° with pivaloyl chloride and *N*-methylmorpholine and then reacted with **28** at − 20° for 17 h to give, after purification, Boc-D-Ala-MeLeu-MeLeu-MeVal-OBzl (**6**) in 78% yield. After debenzylation, **6** yields 86% of **7** and 3.6% of the by-product **11**, confirming that **27** had been prepared with less than 5% epimerization from the pivalic anhydride of Boc-MeLeu-MeLeu (**30**; *Scheme 5)*.

9. Variation of the Reaction Conditions during the Synthesis of Boc-D-Ala-MeLeu-MeLeu-MeVal-OH (7) and Boc-D-Ala-MeLeu-D-MeLeu-MeVal-OH (11) from Boc-D-Ala-MeLeu-MeLeu-OH (5; *Scheme 7, Table 2*). – A study of the coupling of Boc-D-Ala-MeLeu-MeLeu-OH (**5**) and H-MeVal-OBzl (**16**) with the aid of the mixed pivalic anhydride method under different conditions is summarized in *Table 2.*

Carboxyl activation and coupling reactions are made at different temperatures and by varying both the time and the base used. The tripeptide **5** is activated using 1 equiv. of pivaloyl chloride in presence of 2 equiv. of base (for comments on the quantity of base used see *Sect. 2.2*) in $^2/_3$ of the total volume of $CHCl_3$ used, $^1/_3$ being reserved for the addition of **16**. After the time necessary for the reaction, the mixture is worked up by diluting with H_2O, extracting with CH_2Cl_2, and chromatography on silica gel.

The obtained mixture of the protected isomeric tetrapeptides **6** and **10** is separated from recovered starting material **5** (yields in *Table 2*). The optical rotations of **6** and **10** measured in $CHCl_3$ differ by approximately 100° *(*see *Table 1)*. This difference permits to estimate the degree of epimerization during the coupling reaction by measuring the rotation of the mixture **6** and **10** obtained *(*see *Table 2*). Alternatively, having removed the protecting benzyl groups, the corresponding isomers **7** and **11** can be separated by TLC (silica gel, 5% MeOH/$CHCl_3$) or by column chromatography (silica gel, 10% MeOH/CH_2Cl_2) and their ratio be determined *(*see *Table 2)*.

If Et_3N is used as the base for the mixed pivalic anhydride preparation and coupling *(Table 2, Exper. I)*, the rate of the reaction is so slow that warming to 60° is necessary to get similar yields as with the use of *N*-methylmorpholine (MeMorph; *Exper. III*). This

Table 2. *Variation of the Activation and Coupling Conditions for the Reaction of Boc-D-Ala-MeLeu-MeLeu-OH(5) with H-MeVal-OBzl(16)*

Exper.	Activation Agent[a]) 1.0 equiv.	Anhydride Formation Temp. [°C]	Anhydride Formation Time [h]	Base[b]) 2 equiv.	Reaction Temp. [°C]	Reaction Time	**5** [mmol]	Solvent (total)	Yield **6/10+5** [%]	of **6/10** $[\alpha]_D^{20}$ [°]	*c* in $CHCl_3$	**7/11** from TLC or **6/10** calc. from [α]	**7/11** Isolated
I	+COCl	+20	2¼	Et_3N	+60	15 h	5	$CHCl_3$ (40 ml)	55+30	− 89	0.8	1:1	1.24:1.19
II	+COCl	−20	4	Et_3N	+20	8 d	5	$CHCl_3$ (40 ml)	57+26	−131	1.05	4:1	8.4:1.6
III	+COCl	−20	2¾	MeMorph	+20	15 h	5	$CHCl_3$ (30 ml)	60+25	− 85	1.05	1:2	–
IV	+COCl	−20	2½	MeMorph	−20	4½ d	15	$CHCl_3$ (100 ml)	50+30	−140	0.9	9:1	9:1
V	+COCl	−20	2½	MeMorph	−20	2½ d	5	$CHCl_3$ (40 ml)	48+30	−138	0.81	9:1	9:1
VI	+COCl	−10	4	MeMorph	−10	4½ d	7.5	$CHCl_3$ (50 ml)	49+30	−124	0.8	3:1	–
VII	+COCl	−20	3½	Py	−20	15 h	2.5	$CHCl_3$ (20 ml)	0+100	–	–	–	–
VIII	+COCl	−20	3½	Py	+20	33 h	2.5	$CHCl_3$ (20 ml)	55+25	− 63	0.8	1:4	1:4
IX	iBuOCOCl	−20	4½	MeMorph	−20	26 h	1	$CHCl_3$ (20 ml)	traces	–	–	–	–
X	+COCl	−20	4½	MeMorph	−20	26 h	1	$CHCl_3$ (20 ml)	56+30	−135	1.0	9:1	–
XI	+COCl	−20	0	MeMorph	−20	2 d	0.5	$CHCl_3$ (3 ml)	45[c])	−126	1.0	3:1	–
XII[d])	−COCl	−23	5	MeMorph	−22	2.5 d	0.5[d])	$CHCl_3$ (3 ml)	58+30	−151	1.0	19:1	94:6
XIII[e])	+COCl	−23	5	MeMorph	−22	2.5 d	0.5[e])	$CHCl_3$ (3 ml)	68	−155	1.0	19:1	96:4
XIV	DCCI/Bt-OH			–	+20	17 h	5	THF (120 ml)	0+100	–	–	–	–
XV	DCCI/Bt-OH[f])			–	+60	24 h	5	THF (120 ml)	other products	− 24	1.08	other products	–
XVI	Bt-OP$(NMe_2)_3^+$ PF_6^-[f])			Et_3N	+20	24 h	1	CH_2Cl_2 (20 ml)	55+25	−114	0.9	2:1	–
XVII	Bt-OP$(NMe_2)_3^+$ PF_6^-[f])			MeMorph	+20	22 h	1	CH_2Cl_2 (20 ml)	60+22	−122	0.77	3:1	–
XVIII	$(PrPO_2)_3$[f])			Me_2NPy	+20	5 d	2	CH_2Cl_2 (20 ml)	0+100	–	–	–	–
XIX	iBuO-H_2Qu-COOiBu[f])			–	+20	22 h	0.5	THF (5 ml)	45[g])	− 87	1.0	1:2	–
XX[h])	iBuO-H_2Qu-COOiBu[f])			–	+20	22 h	0.5	THF (5 ml)	13+30[i])	–	–	–	–
XXI[h])	iBuO-H_2Qu-COOiBu[f])			–	−20	24 h	0.5	$CHCl_3$ (10 ml)	traces[k])	–	–	–	–

[a]) DCCI = Dicyclohexylcarbodiimide, Bt-OH = *N*-hydroxybenzotriazol(=1*H*-benzo[*d*][1,2,3]triazol-1-ol), Bt-OP$(NMe_2)_3^+$ PF_6^- = (1*H*-benzo[*d*][1,2,3]triazol-1-yloxy)-tris(dimethylamino)phosphonium hexafluorophosphate, iBuO-H_2Qu-COOiBu = *N*-isobutoxycarbonyl-2-isobutoxy-1,2-dihydroquinoline (= isobutyl-2-isobutoxy-1,2-dihydroquinoline-1-carboxylate).

[b]) MeMorph = *N*-Methylmorpholine, Py = pyridine, Me_2NPy = *p*-(dimethylamino)pyridine.

[c]) 39% of *N*-methyl-*N*-pivaloyl-valine benzyl ester were also isolated.

[d]) Using 2 equiv. of H-MeVal-OBzl (base).

[e]) Using 5 equiv. of H-MeVal-OBzl (base).

[f]) 1.1 Equiv. of activation agent.

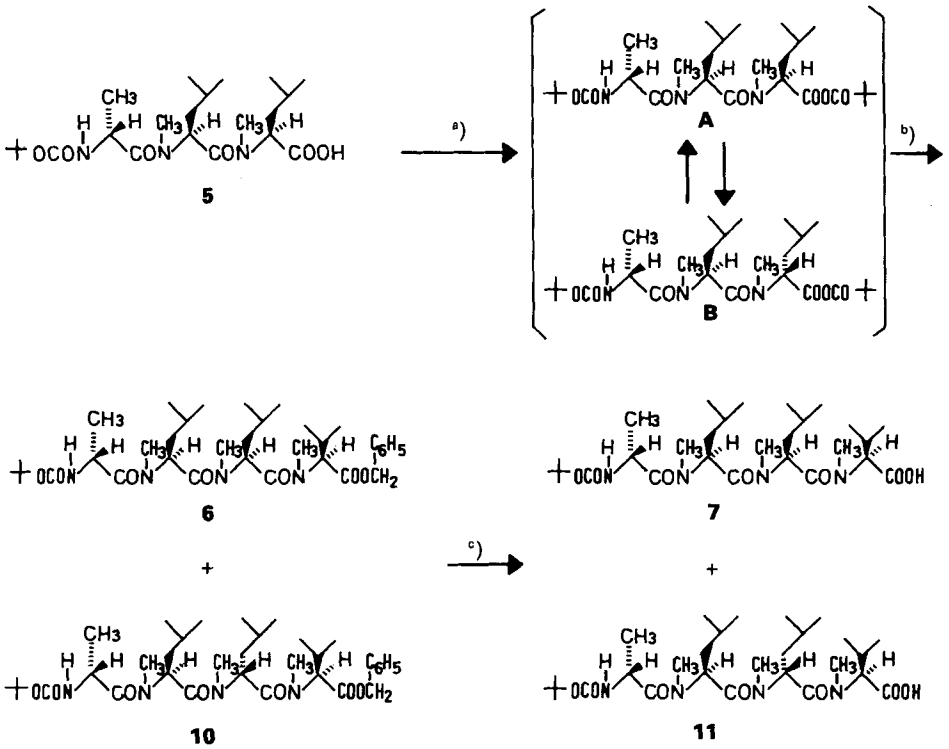
[g]) Besides 10% of iBuOOC-MeVal-OBzl (urethane).

[h]) **16** used as hydrochloride.

[i]) Besides 40% of iBuOOC-MeVal-OBzl (urethane).

[k]) No urethane formation (see *Footnote i*).

Scheme 7. *Synthesis of Boc-D-Ala-MeLeu-MeLeu-MeVal-OH* (7) *and Boc-D-Ala-MeLeu-D-MeLeu-MeVal-OH* (**11**) *from* **5**



a) b) c) See *Scheme 1*.

degree of coupling is accompanied by substantial epimerization due to the elevated temperature, and isolation of the product gives a 1:1 mixture of **6** and **10**. The epimerization could also be the result of the preparation of the anhydride at room temperature. Better results are obtained, when after activating at $-20°$, the mixture is left 8 days at room temperature to complete the reaction. In this case *(Exper. II)*, a 4:1 mixture of **6** and **10** is isolated. This means that even after a long reaction time in presence of a strong base such as Et_3N, much less epimerization is observed than with the weaker base *N*-methylmorpholine during 15 h reaction *(Exper. III)* under similar conditions.

Using *N*-methylmorpholine the reaction rate is also greatly enhanced. This is interpreted as the catalytic effect of HCl, which must dissociate more readily from amine salts under the *N*-methylmorpholine conditions and can protonate the amino-acid carbonyl group of the anhydride thereby favouring epimerization by an enolisation mechanism and also enhancing the reaction rate[18]). The problem is therefore to find a reaction temperature for which enolisation is minimized without significantly affecting the reaction rate.

[18]) For the accelerating effects of AcOH and other carboxylic acids on the reaction rate of active esters see the paper of *Schwyzer et al.* [31].

This can be achieved by working for 2 or 4 days at $-20°$ *(Exper. IV* and *V)*. Not more than 10% of **11** are isolated in both cases. The reaction temperature is critical since working at $-10°$ under similar conditions *(Exper. VI)* results in more epimerization as seen from the more positive rotation obtained ($[\alpha]_D^{20} = -124°$ *(Exper. VI) vs.* $-140°$ *(Exper. IV)*).

The choice of the base is also important. The use of a weaker base such as pyridine resulted in no reaction at $-20°$ *(Exper. VII)* or produced an elevated epimerization at room temperature as seen by the 1 : 4 mixture of **7** and **11**, isolated following debenzylation *(Exper. VIII)*.

Exper. IX and *X* demonstrate the difference existing between the mixed carbonic and mixed pivalic anhydride methods. The use of isobutyl chloroformate (for comments see *Sect. 2.2*) instead of pivaloyl chloride for activation at $-20°$ ($4\frac{1}{2}$ h) and attempting the coupling reaction at the same temperature (26 h) is completely unsuccessful. Under identical conditions, pivaloyl chloride produces a 9 : 1 mixture of **6** and **10** in 56% yield.

Exper. XI demonstrates that the reaction of pivaloyl chloride is very slow with the secondary amino group of H-MeVal-OBzl. This reaction is performed at $-20°$ without allowing time for carboxyl activation and working with equivalent proportions of reactants, adding pivaloyl chloride after having added H-MeVal-OBzl (**16**) as base. After reaction for 2 days, a 3 : 1 mixture of **6** and **10** is isolated in 45% yield. The formation of *N*-methyl-*N*-pivaloyl-valine benzyl ester in only 39% yield shows that pivaloyl chloride reacts faster with the carboxyl group of **5** than with the MeNH group of H-MeVal-OBzl under these conditions.

The *Exper. XII* and *XIII* show that the epimerization during the coupling reaction of **5** and **16** can be minimized by working with an excess of **16**. Under the experimental conditions used in these cases (5 h activation at $-23°$ and $2\frac{1}{2}$ days reaction at $-22°$, the epimerization was not more than 6 and 4% using 2 and 5 equiv. of H-MeVal-OBzl (**16**), respectively.

A comparison of the yields obtained by different coupling methods for the model peptide **5** is also made. The use of dicyclohexylcarbodiimide (DCCI) in presence of *N*-hydroxybenzotriazole (Bt-OH) at room temperature or at 60° in tetrahydrofuran (THF) gave none of the desired tetrapeptide *(Exper. XIV* and *XV)*. By working with (1*H*-benzo[*d*][1,2,3]triazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (Bt-OP$(NMe_2)_3^+$ PF_6^- [32] at room temperature in presence of Et_3N *(Exper. XVI)* or *N*-methylmorpholine *(Exper. XVII)* relative good yields on the tetrapeptides **6**/**10** were obtained, in a ratio of 2 : 1 and 3 : 1, respectively. Epimerization in this case seems to be base-catalyzed as more **10** is isolated by using Et_3N instead of *N*-methylmorpholine. The use of propylphosphonic anhydride $(PrPO_2)_3$ [33] in presence of *p*-(dimethylamino)pyridine (Me_2NPy) [34], a base reported to enhance[19]) peptide coupling, was unsuccessful in this case *(Exper. XVIII)*.

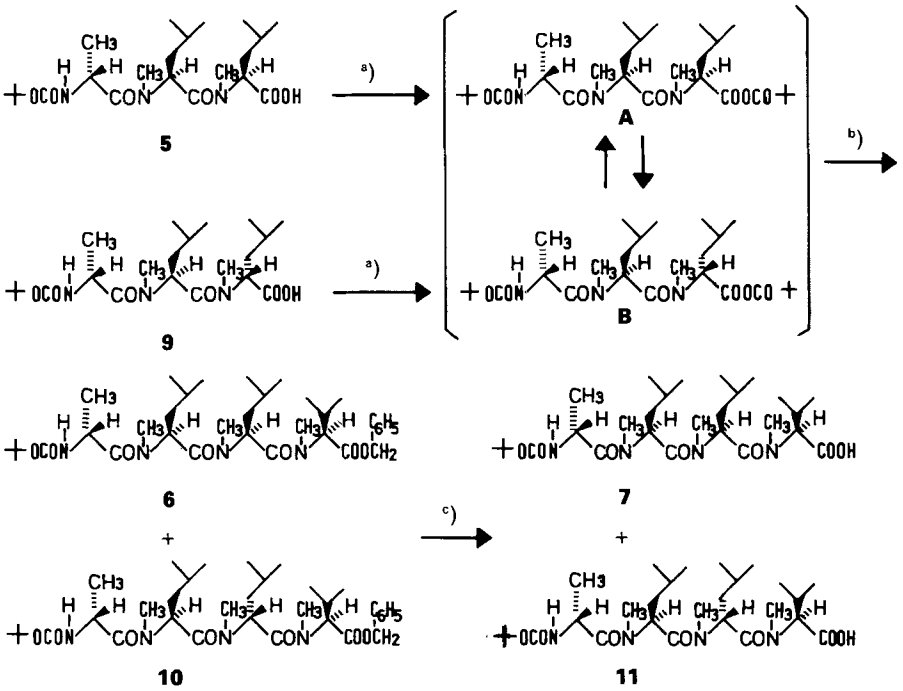
The use of *N*-isobutoxycarbonyl-2-isobutoxy-1,2-dihydroquinoline (iBuO-H_2Qu-COOiBu) [35] [36] in THF at room temperature for the coupling reaction *(Exper. XIX)* gives a 45% yield of a 1 : 1 mixture of **6** and **10**, besides 10% of the urethane iBu-OOC-MeVal-OBzl. The same condensing agent used with the hydrochloride of **16**

[19]) This effect of Me_2NPy was also observed in our laboratory, when used with $(PrPO_2)_3$ [33]. For more details see the forthcoming paper (*cf. Footnote 5*).

(Exper. XX) produces at room temperature this urethane as main product. When working at $-20°$ in $CHCl_3$ *(Exper. XXI)*[20] for 24 h no reaction occurs. iBuO-H_2Qu-COOiBu does not seem to be an adequate activating agent for the tripeptide **5**.

10. An ^{1}H-NMR Study of the Degree of Epimerization during the Synthesis of Boc-D-Ala-MeLeu-MeLeu-MeVal-OBzl (6) and Boc-D-Ala-MeLeu-D-MeLeu-MeVal-OBzl (10) from 5 and 9, respectively *(Scheme 8)*. – The results of a ^{1}H-NMR study of the

Scheme 8. *Synthesis of Boc-D-Ala-MeLeu-MeLeu-MeVal-OH* (**7**) *and Boc-D-Ala-MeLeu-D-MeLeu-MeVal-OH* (**11**)



[a]) [b]) [c]) See *Scheme 1*.

Table 3. *^{1}H-NMR Study of the Epimerization during the Synthesis of Boc-D-Ala-MeLeu-MeLeu-MeVal-OBzl* (**6**) *and Boc-D-Ala-MeLeu-D-MeLeu-MeVal-OBzl* (**10**) *from* **5** *and* **9**, *respectively*

Starting Material		Anhydride Formation		MeMorph equiv.	$CDCl_3$ in ml	Condensation Temp.	$CDCl_3$ (total)	Condensation Time	Yield[a]) (**6**/**10**)	of **6**/**10** $[\alpha]_D^{20}$	*c* in $CHCl_3$	**6**/**10** from ^{1}H-NMR	**7**/**11** isolated
	mmol	Temp. [°C]	Time [h]			[°C]	[ml]	(d = days)	[%]	[°]		[b])	
5	1	−20	16	2	15	−20	20	5 d	42	−136	0.92	82:18	82.5:17.5
5	1	+20	16	2	15	+20	20	29 h	31	−72.8	0.82	23:77	28:72
9	0.5	−20	16	2	7	−20	12	5 d	47	−83.9	0.84	23:77	32:68
9	0.3	+20	16	2	2	+20	5	4 d	30	−69.9	0.87	19:81	20:80

[a]) After workup with H_2O, extraction with CH_2Cl_2 and chromatography.
[b]) Signal of H-C(2^4) of **6** compared to the one of H-C(2^4) of **10**[14]).

[20]) The experiment was repeated under the same conditions using THF as solvent (one phase); no reaction occurred.

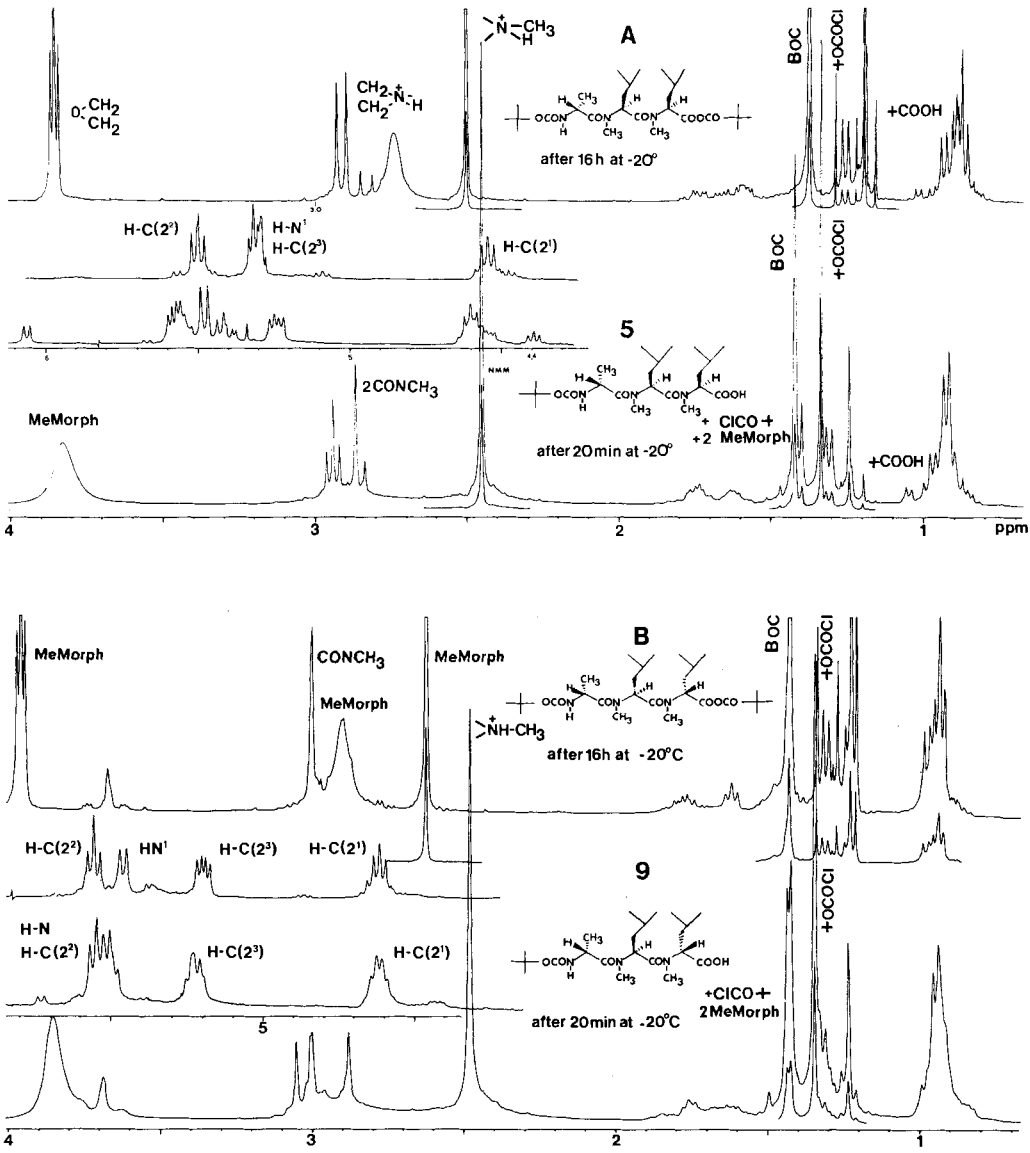


Fig. 2. *^{1}H-NMR Spectrum ($CDCl_3$, 360 MHz) of the Reaction Mixture from* **5***/Pivaloyl Chloride/*N*-Methylmorpholine (top) and* **9***/Pivaloyl Chloride/*N*-Methylmorpholine (bottom) at −20°*

coupling of Boc-D-Ala-MeLeu-MeLeu-OH (**5**) and Boc-D-Ala-MeLeu-D-MeLeu-OH (**9**) with H-MeVal-OBzl (**16**) using the mixed pivalic anhydride method at −20° and at +20° are summarized in *Table 3*. Activation of both **5** and **9** is carried out as usual but for 16 h in $CDCl_3$. The formation of the mixed anhydrides can be followed by ^{1}H-NMR spectros-

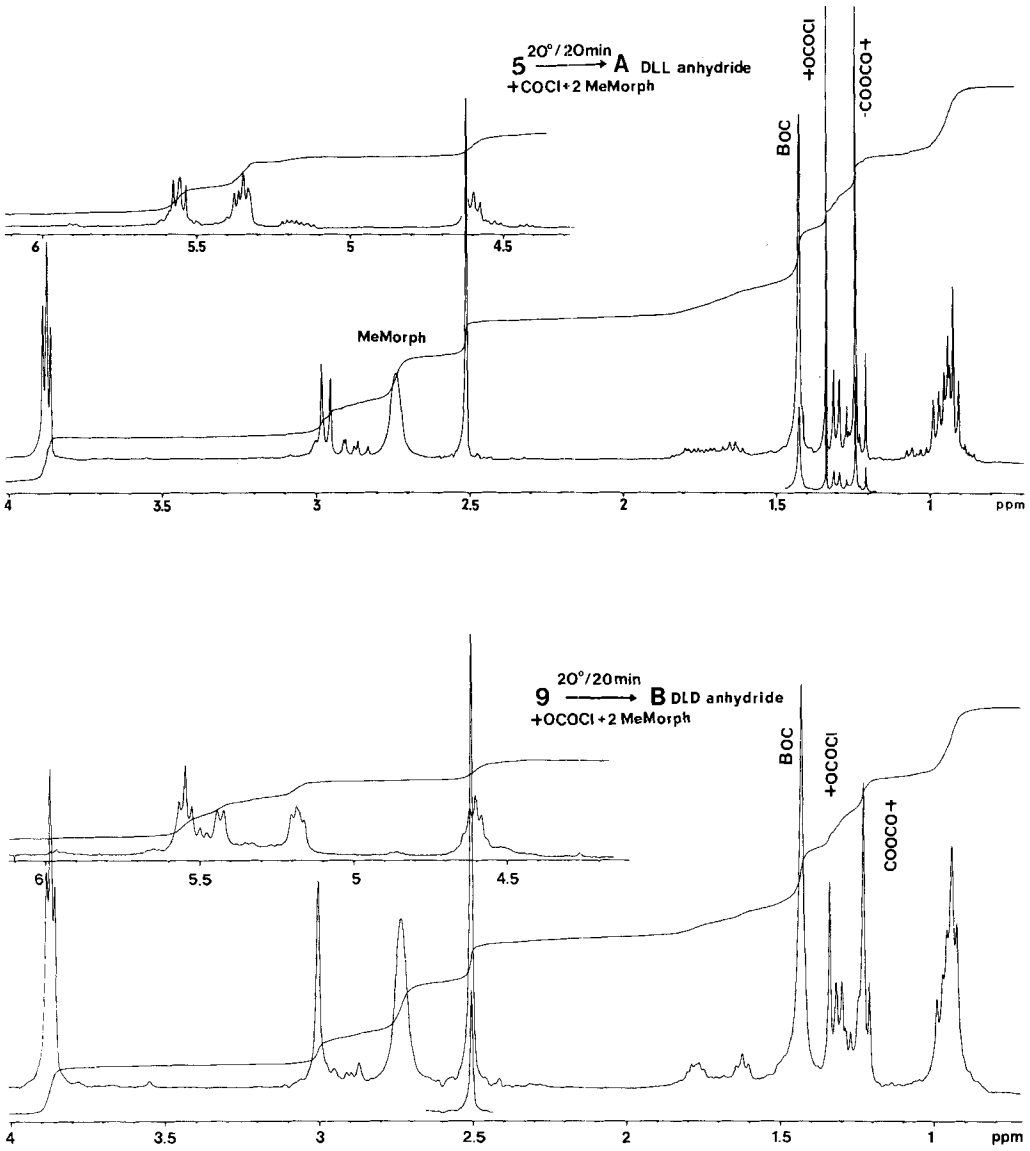


Fig. 3. *^{1}H-NMR Spectrum ($CDCl_3$, 360 MHz) of the Reaction Mixture from 5/Pivaloyl Chloride/N-Methylmorpholine (top) and 9/Pivaloyl Chloride/N-Methylmorpholine (bottom) at +20°*

copy. As shown in *Fig. 2*, after 20 min at $-20°$, the ^{1}H-NMR spectra show mainly unreacted **5** and **9** and after 16 h the corresponding anhydrides **A** and **B**. The anhydrides **A** and **B** are also obtained after 20 min reaction at $+20°$, as shown in *Fig. 3*. In *Fig. 4* the formation of the mixed pivalic anhydride **A** from **5** at $-20°$ (top) is shown as a function of

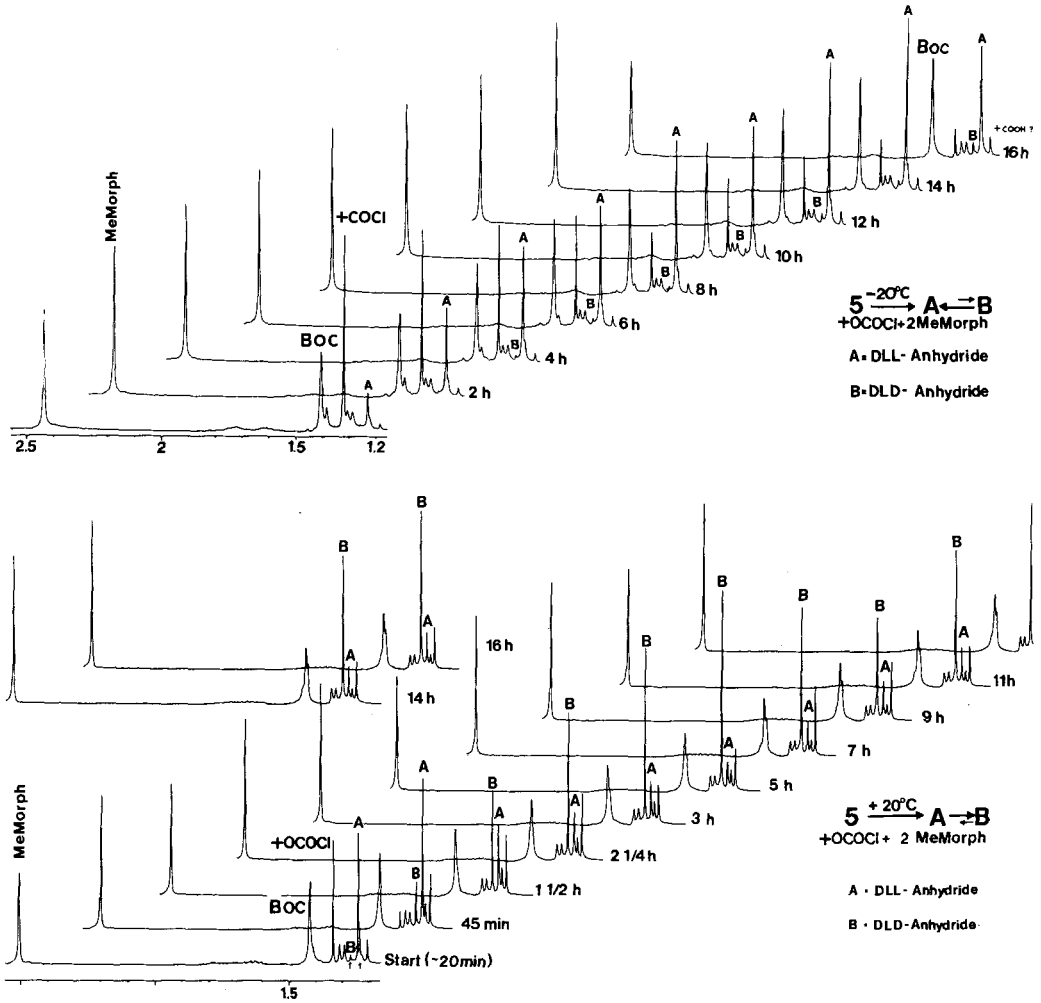


Fig. 4. *Formation of Anhydrides* **A** *and* **B** *from* **5**/*Pivaloyl Chloride*/N-*Methylmorpholine as a Function of Time.* ^{1}*H-NMR in* $CDCl_3$ *at 360 MHz, at* $-20°$ *(top) and* $+20°$ *(bottom).*

time. The disappearance of the peak corresponding to the *tert*-butyl group of the pivaloyl chloride and the appearance of the peak corresponding to the *tert*-butyl group of the anhydride **A** can be observed. After 16 h reaction, only a small quantity of the epimerized anhydride **B** is present. At $+20°$ (bottom), anhydride **A** is formed in 20–45 min and rapidly epimerized to anhydride **B**. After 90 min, more than 50% **B** is already formed, and after 5 h, **B** is the major product and equilibrium has been reached. The anhydride **B** is clearly more stable than anhydride **A** at $+20°$. As expected, the ^{1}H-NMR spectra obtained from **9** after 16 h activation at 20° corresponds to the one obtained from **5** at 20°. It shows that the same proportions of **B** and **A** (*ca.* 9 : 1) are obtained when approaching the position of equilibrium from the other side.

This ^{1}H-NMR study of the activation of **5** and **9** demonstrates that the mixed pivalic anhydride formation in $CDCl_3$ at $-20°$ is very slow. The formation of the mixed pivalic anhydride at $+20°$ is faster, but requires about 20–40 min for completion. On adding a solution of 1 equiv. of H-MeVal-OBzl (**16**) to these solutions of anhydrides and allowing these to react for 5 days at $-20°$ or one day at $+20°$, a proportion of tetrapeptides **6** and **10** similar to the proportion of the anhydride **A** and **B** observed at the respective temperatures is obtained. H-MeVal-OBzl (**16**) reacts at $-20°$ slightly faster with **A** than with **B**, and this explains why at $-20°$ a little more **7** is isolated from **9** than at 20°.

The proportions of the benzyl esters **6** and **10** are established by ^{1}H-NMR in (D6) DMSO at $+180°$, measuring the peak intensities of the *d* of H–C(2^4) of **10** and **6** at 4.55 and 4.61 ppm, respectively[13][14]). The product ratio is confirmed following debenzylation and isolation of **7** and **11**.

The conditions used for the experiments in $CDCl_3$ were not as optimal as those carried out in $CHCl_3$. The yields were lower and the rate of mixed anhydride formation was also slightly slower *(Table 2)*. However, these results contribute to the understanding of the reaction mechanism leading from **5** and **9** to the formation of **6** and **10**[21]). We believe that the reactive species which react with **16**, are the anhydrides **A** or **B** and that epimerization is only due to interconversion of **A** and **B** by enolisation. This interconversion is catalyzed more by acid than by base. The formation of an oxazolonium salt of type **i**, as observed on using DCCI *(Sect. 1)*, is probably not necessary for epimerization in the case of mixed pivalic anhydrides. The anhydrides **A** and **B** epimerize sufficiently rapidly themselves!

We thank *Kurt Martin* and *Louis Walliser* for their capable technical assistance. Thanks also are due to Dr. *H. Braunschweiger* and *F. Seemann* for the supply of larger quantities of *N*-methylamino acids and to Dr. *M. Krieger* for the synthesis of Z-MeLeu-MeLeu-MeVal-O*t*Bu as reference peptide. We appreciate the valuable help by *H. R. Loosli*, *M. Ponelle* and *T. Zardin* (NMR spectra), *W. Pfirter* (analysis), *C. Quiquerez* (mass spectra), *H. Stocker* (for the diagrams) and *C. Weber* (for typing the manuscript). It is a pleasure to acknowlege with sincere thanks Dr. *T. Payne* for his help in improving the manuscript.

Experimental Part

General. See [1a]. In addition: To simplify the description of the ^{1}H-NMR the following peptide numbering system has been adopted. Each peptide is numbered starting from the N-terminal end of the chain and attributing a number (superscript) to each amino-acid residue. Each amino-acid residue is numbered starting from the original carboxyl group. Thus, in H-D-Ala-MeLeu-MeLeu-MeVal-OH, H–C(2^1) is the proton at C(2)(= C(α)) of the D-alanine residue CH_3–C(3^4) is a Me group at C(3) of the valine residue, and CH_3-N^4 is the Me group on the N atom of the valine residue.

N-*(*tert*-Butoxycarbonyl)*-N-*methyl*-L-*leucine*, N-*benzyloxycarbonyl*-N-*methyl*-L-*leucine, and* N-*(*tert*-butoxycarbonyl)*-N-*methyl*-L-*valine* were purchased from *Bachem Feinchemikalien AG* (Bubendorf/Switzerland) or synthesized from the corresponding protected amino acids using the method of *Cheung & Benoiton* [37] for alkylation in THF with MeI in presence of NaH in excess at r.t. Samples used had the following physical constants. Boc-MeLeu-OH: m.p. 52°, $[\alpha]_D^{20} = -34.3°$ ($c = 0.8$, $CHCl_3$); Z-MeLeu-OH: m.p. 73–74°, $[\alpha]_D^{20} = -30.5°$ ($c = 1.0$, $CHCl_3$); Boc-MeVal-OH: m.p. 89°, $[\alpha]_D^{20} = -57.7°$ ($c = 1.0$, $CHCl_3$). For literature-values see [37] and ref. cit. therein.

[21]) To make sure that under the conditions used Boc-D-Ala-MeLeu-MeLeu-MeVal-OBzl (**6**) is not converted to Boc-D-Ala-MeLeu-DMeLeu-MeVal-OBzl (**10**), **6** (1 mmol; $[\alpha]_D^{20} = -170.1°$ ($c = 1.0$, $CHCl_3$, obtained pure by benzylation of **7** following the same procedure as for **26** → **27**) was added together with H-MeVal-OBzl (**16**, 1 mmol) to the mixed pivalic anhydride of phenylacetic acid (1 mmol) which was prepared under identical conditions as described for *Exper. X (Table 2)*. After 26 h, BzlCOMeVal-OBzl was isolated and the tetrapeptide **6** chromatographically recovered unchanged ($[\alpha]_D^{20} = -170.3°$ ($c = 1.0$, $CHCl_3$)).

N-*(*tert-*Butoxycarbonyl)*-N-*methyl*-D-*leucine and* N-*(*tert-*butoxycarbonyl)*-N-*methyl*-D-*valine* were synthesized from the corresponding protected amino acids using the method of *Cheung & Benoiton* [37] as above. Samples used had the following physical constants. Boc-D-MeLeu-OH: m.p. 53°, $[\alpha]_D^{20} = +33.5°$ ($c = 0.86$, $CHCl_3$); Boc-D-MeVal-OH: m.p. 88°, $[\alpha]_D^{20} = +58.0°$ ($c = 1.0$, $CHCl_3$).

N-*Methyl*-L-*leucine benzyl ester*, N-*methyl*-D-*leucine benzyl ester*, N-*methyl*-L-*valine benzyl ester, and* N-*methyl*-D-*valine benzyl ester* were synthesized from the corresponding Boc-amino acids in near quantitative yields using the method of *Brechbühler et al.* [30]. The Boc-amino acids were refluxed in benzene with *N,N*-dimethylformamide dibenzyl acetal for 2 h, the Boc protecting groups removed with CF_3COOH for 2 h at $-20°$ and the bases extracted at 0° with CH_2Cl_2 in presence of excess of sat. $NaHCO_3$. Samples used had the following rotations: H-MeLeu-OBzl: $[\alpha]_D^{20} = -6.09°$ ($c = 1.0$, $CHCl_3$); H-D-MeLeu-OBzl: $[\alpha]_D^{20} = +6.06°$ ($c = 0.9$, $CHCl_3$); H-MeVal-OBzl: $[\alpha]_D^{20} = -5.2°$ ($c = 1.18$, $CHCl_3$); H-D-MeVal-OBzl: $[\alpha]_D^{20} = +5.1°$ ($c = 1.0$, $CHCl_3$).

N-*Methyl*-L-*leucine benzyl ester hydrochloride*, N-*methyl*-D-*leucine benzyl ester hydrochloride*, N-*methyl*-L-*valine benzyl ester hydrochloride, and* N-*methyl*-D-*valine benzyl ester hydrochloride (stable forms)* were precipitated from Et_2O solutions of the corresponding amino acid ester bases by adding a solution of HCl – gas in Et_2O until pH 3 (paper indicator) was obtained. Samples used had the following physical constants, H-MeLeu-OBzl · HCl: m.p. 123–125°, $[\alpha]^{20} = +4.0°$ (589 nm), +12.0° (365 nm; $c = 0.95$, $CHCl_3$); H-D-MeLeu-OBzl · HCl: m.p. 124–126°, $[\alpha]^{20} = -3.9°$ (589 nm), −12.2° (365 nm; $c = 0.98$, $CHCl_3$); H-MeVal-OBzl · HCl: m.p. 151–153°, $[\alpha]^{20} = +1.0°$ (589 nm), +4.6° (365 nm; $c = 1.05$, $CHCl_3$); H-D-MeVal-OBzl · HCl: m.p. 150–153°, $[\alpha]^{20} = -1.0°$ (589 nm), −4.7° (365 nm; $c = 1.0$, $CHCl_3$).

N-*Benzyloxycarbonyl*-N-*methyl*-L-*valine* tert-*butyl ester* was synthesized from *N*-methylvaline and 2-methyl-1-propen in dioxane in presence of conc. H_2SO_4 according to the method of *McCloskey et al.* [38]. The sample used had the following physical constants (Dr. *M. Krieger*). B.p. 78°/16 Torr, $[\alpha]_D^{20} = +4.5°$ ($c = 1.0$, $CHCl_3$), stable as base.

1. Boc-D-Ala-MeLeu-MeLeu-MeVal-OH (7). – *Boc*-D-*Ala-MeLeu-OBzl* (**2**). A solution of 18.9 g (100 mmol) of Boc-D-Ala-OH (**1**; from *Bachem AG*) in 250 ml of $CHCl_3$ is cooled with stirring to $-20°$. Then, 23.1 ml (21.2 g, 210 mmol) of *N*-methylmorpholine and 12.2 ml (12.0 g, 100 mmol) of pivaloyl chloride are added. The mixture is stirred at $-20°$ for 2 h and anhydride formation followed by IR (IR of the $CHCl_3$ solution after 90 min: 3400*m*, 2900*s*, 2850*m*, 2780*m*, 2400*m*, 1810*s*, 1740*w*, 1700*s*, 1480*m*, 1450*s*, 1390*w*, 1360*s*, 1340*w*, 1280*m*, 1240 – 1200*m*, 1160*s*, 1140*m*, 1010*s*, 1080*w*, 1060*w*, 1030*s*, 1000*s*, 940*w*, 900*w*, 860*m*). Then, 23.5 g (100 mmol) of H-MeLeu-OBzl in 50 ml of $CHCl_3$ are added dropwise at $-20°$ within 5 min. Formation of **2** is followed by TLC and IR. After 19 h, no further anhydride can be detected. The resulting mixture is poured onto 300 ml of H_2O and extracted with 300 ml of $CHCl_3$. The org. phase is separated, washed with 200 ml of 0.5 N HCl, then with 100 ml of H_2O, dried over Na_2SO_4, filtered, and the solvent carefully evaporated below 40°. The residue obtained is crystallized from hexane to yield 32.5 g (79.9%) of **2**, $[\alpha]_D^{20} = -35.3°$ ($c = 1.05$, $CHCl_3$), m.p. 81°. IR (CH_2Cl_2): 3400*w*, 2925*w*, 2875*w*, 1740*m*, 1710*s*, 1650*m*, 1480*s*, 1415*w*, 1400*w*, 1375*w*, 1320*w*, 1220*w*, 1180*s*, 1135*m*, 1045*w*, 1050*m*, 1030*w*, 980*w*, 900*w*. ^{1}H-NMR ($CDCl_3$, 360 MHz, 6:1 mixture of conformers): 0.88, 0.94 (2*d*, $J = 6$, 6H, $2CH_3$–C(4^2); 1.20, 1.29 (2*d*, $J = 6$, 16:84, 3H, 3H–C(3^1)); 1.42 (*s*, 9H, O*t*Bu); 1.41 (*m*, 1H, H–C(4^2)); 1.75 (*m*, 2H, 2H–C(3^2)); 2.84, 2.95 (2*s*, 16:84, 3H, CH_3-N^2); 4.58, 4.65 (2*m*, 16:84, 1H, H–C(2^1)); 5.13 (*dd*, $J = 18, 12$, 2H, PhCH_2); 5.25 (*t*, $J = 6$, 1H, H–C(2^2)); 5.43, 5.57 (2*d*, $J = 9$, 16:84, 1H, H–N^1); 7.35 (*m*, 5H, *Ph*CH_2). ^{1}H-NMR ((D$_6$)DMSO, 360 MHz, 180°): 0.86, 0.88 (2*d*, $J = 6$, 6H, $2CH_3$–C(4^2)); 1.15 (*d*, $J = 6$, CH_3–C(2^1)); 1.35 (*s*, 9H, O*t*Bu); 1.55 (*m*, 1H, H–C(4^2)); 1.74 (*m*, 2H, 2H–C(3^2)); 2.90 (*s*, 3H, CH_3–N^2); 4.48 (*m*, 1H, H–C(2^1)); 4.89 (*t*, $J = 6$, H–C(2^2)); 5.12 (*s*, 2H, PhCH_2); 5.98 (br.*s*, 1H, H–N^1); 7.32 (*s*, 5H, *Ph*CH_2). ^{1}H-NMR ((D)$_6$DMSO, 360 MHz, 20°): 2 conformers. MS (LR): 406 (M^+), 391, 350, 333, 315, 294, 271, 256, 236, 215, 197, 171, 144, 91, 69 etc.

$C_{22}H_{32}N_2O_5$ (406.527) Calc. C 65.0 H 8.4 N 6.9 O 19.7% Found C 65.0 H 8.7 N 6.9 O 19.7%

Boc-D-*Ala-MeLeu-OH* (**3**). A solution of 32.45 g (79.92 mmol) of **2** in 800 ml of abs. EtOH containing 1.6 g of 10% Pd/C is treated with H_2 for 2 h at r.t. After uptake of the calculated quantity of H_2, the suspension is filtered through talc, the solvent evaporated, and the residue crystallized from hexane to yield 24.5 g (97%) of **3** with m.p. 152°, $[\alpha]_D^{20} = -36.9°$ ($c = 0.8$, $CHCl_3$). IR (CH_2Cl_2): 3410 – 2300 br., 1710*s*, 1650*s*, 1480*s*, 1410*m*, 1380*s*, 1235*m*, 1175*s*, 1085*w*, 1060*m*, 1030*w*, 850*w*. ^{1}H-NMR ((D$_6$)DMSO, 360 MHz, 20° or 150° (identical spectra)): 0.86, 0.92 (2*d*, $J = 6$, 6H, $2CH_3$–C(4^2)); 1.21 (*d*, $J = 6$, 3H, CH_3–C(2^1)); 1.39 (*s*, 9H, O*t*Bu); 1.55 (*m*, 1H, H–C(4^2)); 1.70 (*m*, 2H, 2H–C(3^2)); 2.90 (*s*, 3H, CH_3–N^2); 4.50 (*m*, 1H, H–C–(2^1)); 4.83 (br.*s*, 1H, H–C(2^2)); 5.98 (br.*s*, 1H, H–N^1); 6.5–8.0 (br., 1H, COOH).

$C_{15}H_{28}N_2O_5$ (316.401) Calc. C 56.9 H 8.9 N 8.9 O 25.3% Found C 56.8 H 8.9 N 8.9 O 25.1%

Boc-D-Ala-MeLeu-MeLeu-OBzl (**4**). To a solution of 24.5 g (77.5 mmol) of **3** in 200 ml of $CHCl_3$, 17.9 ml (16.4 g, 162.7 mmol) of *N*-methylmorpholine are added. Then, at $-20°$, 9.5 ml (9.32 g, 77.7 mmol) of pivaloyl chloride are added over 5 min, and the mixture is stirred for a further 90 min at $-20°$ (IR control; after 80 min: 3420*m*, 2950*s*, 2850*m*, 2800*m*, 2250*m*, 1820*s*, 1740*w*, 1700*s*, 1650*s*, 1500 – 1460*s*, 1420*m*, 1380*s*, 1285*m*, 1260 – 1210*m*, 1170*s*, 1120*s*, 1045*s*, 1010*s*, 940*w*, 905*m*, 860*m*). A solution of 18.2 g (77.5 mmol) of H-MeLeu-OBzl in 50 ml of $CHCl_3$ is added dropwise at $-20°$ within 5 min. After 18 h at $-20°$, no more anhydride is present (IR), and the solution obtained is poured onto 300 ml of H_2O and extracted with $CHCl_3$ (2 × 300 ml). The combined org. phases are dried over Na_2SO_4, filtered, and evaporated. The residue is chromatographed on 1 kg of silica gel using 2% $MeOH/CH_2Cl_2$ to yield 29.3 g (71% of **4**, $[\alpha]_D^{20} = -101.3°$ ($c = 0.9$, $CHCl_3$), and 2.2 g (5.4%) of Boc-D-Ala-D-MeLeu-MeLeu-OBzl, $[\alpha]_D^{20} = +4.35°$ ($c = 0.87$, $CHCl_3$). **4**: IR (CH_2Cl_2): 3400*w*, 2920*m*, 2860*m*, 1740*m*, 1700*s*, 1640*s*, 1470*s*, 1400*w*, 1375*m*, 1220*w*, 1170*m*, 1120*w*, 1080*w*, 1040*w*, 1020*w*, 950*w*. ^{1}H-NMR ($CDCl_3$): 2 conformers. ^{1}H-NMR ($(D)_6$DMSO, 20°): 3 conformers. ^{1}H-NMR ($(D)_6$DMSO, 150°, 360 MHz): 0.89 (*m*, 12H, $2CH_3$–C(2^2), $2CH_3$–C(2^3)); 1.17 (*d*, $J = 6$, 3H, CH_3–C(2^1)); 1.32–1.85 (*m*, 6H, 2H–C(2^2), 2H–C(2^3), H–C(4^2), H–C(4^3)); 1.38 (*s*, 9H, O*t*Bu); 2.83, 2.87 (2*s*, 6H, CH_3–N^2, CH_3–N^3); 4.44 (*m*, 1H, H–C(2^1)); 5.05 (br.*s*, 1H, H–C(2^2)); 5.11 (*s*, 2H, PhC*H*$_2$); 5.39 (*m*, 1H, H–C(2^3)); 6.05 (br.*s*, 1H, H–N^1); 7.33 (*m*, 5H, *Ph*CH_2). MS (LR): 533 (M^+), 518, 490, 477, 460, 389, 333, 299, 290, 271, 243, 225, 215, 191, 171, 154, 144, 116, 100, 91, 70, 57.

$C_{29}H_{47}N_3O_6$ (533.714) calc. C 65.3 H 8.9 N 7.9 O 18.0% Found C 65.1 H 8.9 N 7.9 O 18.6%

Boc-D-Ala-MeLeu-MeLeu-OH (**5**). A solution of 29.3 g (54.97 mmol) of **4** in 800 ml of abs. EtOH containing 1.5 g of 10% Pd/C is treated with H_2 for 2 h at r.t. After the uptake of the calculated amount of H_2, the suspension is filtered through talc, evaporated, and the residue crystallized from hexane to yield 22.4 g (92%) of **5**, $[\alpha]_D^{20} = -112.6°$ ($c = 0.85$, $CHCl_3$), m.p. 125–126°. IR (CH_2Cl_2): 3420 – 2450 (br), 1710*s*, 1640*s*, 1480*s*, 1405*m*, 1375*m*, 1240*w*, 1175*m*, 1130, 1100*w*, 1060*m*, 1022*w*, 920*w*, 850*w*. CD (CH_3OH (*Uvasol*), bisignate curve): $[\Theta]_{223.5}^{25} = -82{,}000$ ($w = 26$ nm), $[\Theta]_{197.5}^{25} = +67{,}000$ ($w = 14$ nm). ^{1}H-NMR ($(D)_6$DMSO, 20°, 360 MHz, only 1 conformer): 0.78–0.98 (*m*, 12H, $2CH_3$–C(2^2), $2CH_3$–C(4^3)); 1.12 (*d*, $J = 6$, 3H, CH_3–C(2^1)); 1.35 (*s*, 9H, O*t*Bu); 1.40–1.80 (*m*, 6H, 2H–C(3^2), 2H–C(3^3), H–C(4^2), H–C(4^3)); 2.77, 2.83 (2*s*, 6H, CH_3–N^2, CH_3–N^3); 4.41 (*m*, 1H, H–C(2^1)); 5.02 (*dd*, $J = 9$, 1H, H–C(2^2)); 5.90 (*t*, $J = 1$H, H–C(2^3)); 7.00 (br.*s*, 1H, H–N^1); 12.63 (br., 1H, COOH). MS (LR): 443 (M^+), 428, 399, 387, 370, 343, 299, 271, 243, 215, 197, 185, 171, 154, 128, 116, 100, 88, 69, 57.

$C_{22}H_{41}N_3O_6$ (443.588) Calc. C 59.6 H 9.3 N 9.5 O 21.6% Found C 59.0 H 9.6 N 9.2 O 21.7%

Boc-D-Ala-MeLeu-MeLeu-MeVal-OBzl (**6**). a) To a solution of 6.65 g (15.0 mmol) of **5** in 60 ml of $CHCl_3$, precooled to $-20°$, 3.58 ml (3.28 g, 31.5 mmol) of *N*-methylmorpholine and 1.86 ml (1.8 g, 15.2 mmol) of pivaloyl chloride are added. Conversion to the anhydride is completed after 2 h at $-20°$ (IR after 2 h: 3400*w*, 2940*s*, 2850*m*, 2800*w*, 2400*w*, 2200*w*, 1815*s*, 1780*w*, 1700*s*, 1640*s*, 1500 – 1460*s*, 1410*m*, 1370*s*, 1285*s*, 1240 – 1200*m*, 1170*s*, 1160*w*, 1120*s*, 1040*s*, 1010*s*, 900*w*, 860*m*). A solution of 3.32 g (15.0 mmol) of H-MeVal-OBzl in 50 ml of $CHCl_3$ is then added dropwise to the mixture at $-20°$. After 4 1/2 days (TLC and IR control), the solution is poured onto 200 ml of 1 *N* $NaHCO_3$ which was shaken mechanically, and then diluted with 300 ml of $CHCl_3$. After separation of the org. phase, the aq. phase is re-extracted with 100 ml of $CHCl_3$, the combined $CHCl_3$ phases dried over Na_2SO_4, filtered and evaporated. The residue is chromatographed on 600 g of silica gel using 2% $MeOH/CH_2Cl_2$ to yield 5.85 g (60%) of **6**, $[\alpha]_D^{20} = -143.7°$ ($c = 0.9$, $CHCl_3$) containing 10% of Boc-D-Ala-MeLeu-D-MeLeu-MeVal-OBzl (**10**), which can only be separated at the next step after removing the benzyl protecting group. By using 20% $MeOH/CH_2Cl_2$ as eluant, 1.97 g (29.6%) of **5**, $[\alpha]_D^{20} = -110°$ ($c = 0.9$, $CHCl_3$), m.p. 125°, is then isolated. **6**/**10**: IR (CH_2Cl_2): 3400*w*, 2920*m*, 2850*m*, 1730*m*, 1710*m*, 1640*s*, 1470*s*, 1400*w*, 1370*w*, 1230*w*, 1170*w*, 1125*w*, 1100*w*, 1050*w*, 1000*w*.

$C_{35}H_{58}N_4O_7$ (646.875) Calc. C 65.0 H 9.0 N 8.7 O 17.3% Found C 64.9 H 9.3 N 8.3 O 17.5%

b) On a larger scale, the reaction is carried out as follows: a solution of 18.98 g (42.8 mmol) of **5** in 170 ml of $CHCl_3$ is cooled to $-20°$, and 8.65 g (85.6 mmol) of *N*-methylmorpholine are added (temp. rises to $-12°$). The solution is cooled to $-20°$, then 5.14 g (42.8 mmol) of pivaloyl chloride are slowly added, and the mixture is stirred further at $-20°$ for 3 1/2 h (IR after 3 1/2 h: 1815*s*, 1780*w*). Then, 9.46 g (42.8 mmol) of H-MeVal-OBzl in 155 ml of $CHCl_3$ are added dropwise (for a while temp. rises up to $-15°$. The solution is re-cooled to $-20°$, stirred for 3 days at $-20°$, diluted with 500 ml of CH_2Cl_2, washed with 300 ml of H_2O, the aq. phase extracted with 300 ml of CH_2Cl_2, the combined org. phases dried over Na_2SO_4, filtered, and evaporated. The residue is chromatographed on 1.5 kg of silica gel using 1% $MeOH/CH_2Cl_2$ to yield 24.3 g (88%) of **6**, $[\alpha]_D^{20} = -144.8°$ ($c = 1.0$, $CHCl_3$), containing *ca.* 10% of DLDL-isomer **10**, which can only be separated after removing of the benzyl protecting group

(vide infra). ^{1}H-NMR ((D$_6$)DMSO, 360 MHz, 180°, **6**/**10** = 9:1): 0.80–0.90 (*m*, 15H, 2CH$_3$–C(4^2), 2CH$_3$–C(4^3), CH$_3$–C(3^4)); 0.98 (*d*, *J* = 6, 3H, CH$_3$–C(3^4)); 1.19 (*d*, *J* = 6, 3H, CH$_3$–C(2^1)); 1.40 (*s*, 9H, O*t*Bu); 1.47 (*m*, 4H, 2H–C(3^2), 2H–C(3^3)); 1.65 (*m*, 2H, H–C(4^2), H–C(4^3)); 2.25 (*m*, 1H, H–C(3^4)); 2.33, 2.36, 2.39 (10%) and 2.43 (3*s* + 1/10*s*, 9H, CH$_3$–N^2, CH$_3$–N^3, CH$_3$–N^4); 4.46 (*m*, 1H, H–C(2^1)); 4.56, 4.61 (*d*, *J* = 9, 1:9, 1H, H–C(2^4)); 5.13 (*dd*, *J* = 15, 24, 2H, PhCH$_2$); 5.36, 5.41 (2*t*, *J* = 6, 2H, H–C(2^2), H–C(2^3)); 5.76, 5.90 (2*s*, 1:9, 1H, H–N^1); 7.32 (*s*, 5H, *Ph*CH$_2$).

c) Starting from Boc-D-Ala-MeLeu-MeLeu-MeVal-OH (**7**): a solution of 1.11 g (2.0 mmol) of **7** ($[\alpha]_D^{25}$ = −200.5° (*c* = 1.0, CHCl$_3$) in 20 ml of benzene containing 0.81 g (0.78 ml, 1.5 equiv.) of *N*,*N*-dimethylformamide dibenzyl acetal is refluxed for 2 h and then evaporated. The residue is chromatographed on 75 g of *Sephadex LH 20* using 0.5% MeOH/CH$_2$Cl$_2$ to yield 1.06 g (82%) of pure **6** as an oil, $[\alpha]_D^{20}$ = −170.1° (*c* = 0.82, CHCl$_3$). ^{1}H-NMR ((D)$_6$DMSO, 360 MHz, 180°): 0.80–0.92 (*m*, 15H, 2CH$_3$–C(4^2), 2CH$_3$–C(4^3), CH$_3$–C(3^4)); 0.98 (*d*, *J* = 6, 3H, CH$_3$–C(2^1)); 1.39 (*s*, 9H, O*t*Bu); 1.47 (*m*, 4H, 2H–C(3^2), 2H–C(3^3)); 1.65 (*m*, 2H, H–C(4^2), H–C(4^3)); 2.25 (*m*, 1H, H–C(3^4)); 2.33, 2.36, 2.43 (3*s*, 9H, CH$_3$–N^2, CH$_3$–N^3, CH$_3$–N^4); 4.46 (*m*, 1H, HC(2^1)); 4.62 (*d*, *J* = 9, 1H, H–C(2^4)); 5.13 (*dd*, *J* = 15, 24, 2H, PhC*H*$_2$); 5.36, 5.41 (2*t*, *J* = 6, 2H, H–C(2^2), H–C(2^3)); 5.78 (*s*, 1H, H–N^1); 7.32 (*s*, 5H, *Ph*CH$_2$).

*Boc-D-Ala-MeLeu-MeLeu-MeVal-OH (**7**; Main Product) and Boc-D-Ala-MeLeu-D-MeLeu-MeVal-OH (**11**; Minor Isomer)*. A solution of 18.83 g (29.1 mmol) of **6** containing *ca.* 10% of DLDL-isomer **10** *(vide supra)* in 500 ml of abs. EtOH is treated with H$_2$ in presence of 1.0 g of 10% Pd/C for 2 h, at r.t. The suspension is filtered through talc and the filtrate evaporated. The residue is chromatographed on 800 g of silica gel using 10% MeOH/CH$_2$Cl$_2$ to yield 12.6 g (77.8%) of pure **7**, $[\alpha]_D^{20}$ = −200.5° (*c* = 1.0, CHCl$_3$, and 1.28 g (7.9%) of pure DLDL-isomer **11**, $[\alpha]_D^{20}$ = −55.2° (*c* = 1.0, CHCl$_3$). **7**: CD(CH$_3$OH *(Uvasol)*, bisignate curve): $[\Theta]_{226.5}^{25}$ = −180,000 (*w* = 26 nm), $[\Theta]_{197}^{25}$ = +153,000 (*w* = 16 nm). IR(CH$_2$Cl$_2$): 3410 – 2500 (br.), 1705 *m*, 1630 *s*, 1470 *m*, 1400 *w*, 1370 *w*, 1280 *w*, 1240 *w*, 1205 *w*, 1165 *m*, 1120 *w*, 1100 *w*, 1060 *w*, 1020 *w*, 850 *w*. ^{1}H-NMR ((D$_6$)DMSO, 180°, 360 MHz): 0.82, 0.99 (2*d*, *J* = 6, 6H, 2CH$_3$–C(3^4)); 0.86, 0.89 (2*d*, *J* = 6, 12H, 2CH$_3$–C(4^2), 2CH$_3$–C(4^3)); 1.19 (*d*, *J* = 6, 3H, CH$_3$–C(2^1)); 1.38 (*s*, 9H, O*t*Bu); 1.47 (*m*, 4H, 2H–C (3^2), 2H–C (3^3)); 1.68 (*m*, 2H, H–C(4^2), H–C (4^3)); 2.20 (*m*, 1H, H–C (3^4)); 2.84, 2.89, 2.94 (3*s*, 9H, CH$_3$–N^2; CH$_3$–N^3, CH$_3$–N^4); 4.42 (br.*s*, 1H, H–C(2^4)); 4.46 (*m*, 1H, H–C–(2^1)); 5.37, 5.44 (2*t*, *J* = 6, 2H, H–C (2^2), H–C (2^3)); 5.98 (br.*s*, 1H, H–N^1); 7.0–8.0 (br., 1H, COOH). ^{1}H-NMR (CDCl$_3$, 360 MHz, 2:3 mixture of 2 conformers): 0.87, 1.05, 0.97, 1.02 (4*d*, *J* = 6, 2:2:3:3, 6H, 2CH$_3$–C (3^4)); 0.90–1.00 (*m*, 12H, 2CH$_3$–C (4^2), 2CH$_3$–C (4^3)); 1.29 (*d*, *J* = 6, 3H, CH$_3$–C (2^1)); 1.39, 1.42 (2*s*, 6:4, 9H, O*t*Bu); 1.45, 1.60, 1.72, 1.83 (4*m*, 6H, 2H–C (3^2), 2H–C (3^3), H–C (4^2), H–C (4^3)); 2.29, 2.40 (2*m*, 6:4, 1H, H–C (3^4)); 2.80, 2.82, 2.96, 3.00, 3.04, 3.08 (6*s*, 9H, CH$_3$–N^2, CH$_3$–N^3, CH$_3$–N^4); 4.10, 4.33 (2*d*, *J* = 12, 6:4, 1H, H–C (2^4)); 4.64 (*m*, 1H, H–C (2^1)); 5.50 (*m*, 2H, H–C (2^2), H–C (2^3)); 5.91, 6.15 (2*d*, 4:6, *J* = 9, 1H, H–N^1); 6.50–7.10 (br., 1H, COOH). MS (LR): 556 (M^+), 541, 513, 500, 483, 456, 426, 412, 398, 387, 370, 352, 326, 299, 271, 243, 225, 215, 187, 182, 171, 142, 128, 100, 69 etc.

$C_{28}H_{52}N_4O_7$ (556.75) Calc. C 60.4 H 9.4 N 10.1 O 20.1% Found C 59.9 H 9.4 N 9.9 O 20.1%

11: CD(CH$_3$OH *(Uvasol)*, bisignate curve): $[\Theta]_{227}^{25}$ = −55,500 (*w* = 26 nm), $[\Theta]_{203.5}^{25}$ = +47,000 (*w* = 14 nm). ^{1}H-NMR ((D)$_6$DMSO, 360 MHz, 180°) (at r.t. 2 conformers): 0.80, 0.98 (2*d*, *J* = 6, 6H, 2CH$_3$–C(3^4)); 0.86, 0.89 (2*d*, *J* = 6, 12H, 2CH$_3$–C(4^2), 2CH$_3$–C(4^3)); 1.23 (*d*, *J* = 6, 3H, CH$_3$–C(2^1)); 1.40 (*s*, 9H, O*t*Bu); 1.50 (*m*, 4H, 2H–C(3^2), 2H–C(3^3)); 1.67 (*m*, 2H, H–C(4^2), H–C(4^3)); 2.19 (*m*, 1H, H–C(3^4)); 2.89, 2.90, 2.91 (3*s*, 9H, CH$_3$–N^2, CH$_3$–N^3, CH$_3$–N^4); 4.45 (*m*, 2H, H–C(2^1), H–C(2^4)); 5.37, 5.48 (2*t*, *J* = 6, 2H, H–C(2^2), H–C(2^3)); 5.86 (br.*s*, 1H, H–N^1); 11.5–12.0 (br., 1H, COOH). ^{1}H-NMR (CDCl$_3$, 360 MHz, 1 conformer): 0.88, 1.09 (2*d*, *J* = 6, 6H, 2CH$_3$–C(3^4)); 0.93, 0.96 (2*d*, *J* = 3, 12H, 2CH$_3$–C(4^2), 2CH$_3$–C(4^3)); 1.29 (*d*, *J* = 6, 3H, CH$_3$–C(2^1)); 1.41 (*s*, 9H, O*t*Bu); 1.43, 1.56 (2*m*, 4H, 2H–C(3^2), 2H–C(3^3)); 1.67, 1.75 (2*m*, 2H, H–C(4^2), H–C(4^3)); 2.21 (*m*, 1H, H–C(3^4)); 2.79, 2.87, 2.93 (3*s*, 9H, CH$_3$–N^2, CH$_3$–N^3, CH$_3$–N^4); 4.59 (*m*, 1H, H–C(2^1)); 4.90 (*d*, *J* = 9, 1H, H–C(2^4)); 5.26 (*d*, *J* = 6, 2H, H–C(2^2), H–C(2^3)); 9.50–10.50 (br., 1H, COOH). MS (LR): 557 (MH^+), 541, 513, 500, 488, 457, 426, 370, 352, 326, 299, 285, 259, 243, 225, 215, 207, 182, 171, 141, 128, 116, 100, 86, 69, 57 etc.

2. Boc-D-Ala-MeLeu-D-MeLeu-MeVal-OH (11). – *Boc-D-Ala-MeLeu-D-MeLeu-OBzl* (**8**). – To 1.51 g (4.78 mmol) of Boc-D-Ala-MeLeu-OH (**3**) in 15 ml of CHCl$_3$, 1.01 g (10.0 mmol) of *N*-methylmorpholine are added. This solution is immediately cooled to −20°, 0.57 g (4.78 mmol) of pivaloyl chloride are added, and the mixture is stirred for 75 min at −20°, (IR after 75 min: 1820 *s*). Then, a solution of 1.12 g (4.78 mmol) of H-D-MeLeu-OBzl in 10 ml of CHCl$_3$ is slowly added at −20° within 5 min. The mixture is stirred for 75 min at −20° and then for 16 h at r.t. The resulting solution is diluted with 300 ml of CH$_2$Cl$_2$ and washed with 200 ml of H$_2$O. The aq. phase is re-extracted with 100 ml of CH$_2$Cl$_2$, the combined org. phases dried over Na$_2$SO$_4$, filtered, and evaporated. The

residue is chromatographed on 500 g of silica gel using 2% MeOH/CH_2Cl_2: 1.9 g (75%) of **8** contaminated with some DDD-isomer. For further purification it is necessary to remove the benzyl protecting group *(vide infra)*.

Boc-D-*Ala*-*MeLeu*-D-*MeLeu*-*OH* (**9**). A solution of 1.5 g (2.81 mmol) of crude (**8**) in 50 ml of abs. EtOH containing 0.1 g of 10% Pd/C is treated with H_2 for 45 min at r. t. After uptake of the calculated amount of H_2 (64 ml/20°), the suspension is filtered through talc, the filtrate evaporated, and the residue crystallized twice from Et_2O-hexane to yield 0.91 g (73%) of pure **9**, m.p. 115–116°, $[\alpha]_D^{20} = -60.3°$ ($c = 0.82$, $CHCl_3$). The mother liquors (0.31 g/25%) contain a more polar isomer (TLC on silica gel using 10% MeOH/$CHCl_3$), which is believed to be the DDD-isomer. This material contaminated with about 50% of **9** has $[\alpha]_D^{20} = +39°$ ($c = 0.92$, $CHCl_3$). **9**: IR ($CHCl_3$): 3400*w*, 3100*w*, 2940*m*, 1710*s*, 1640*s*, 1500–1460*m*, 1410*m*, 1375*m*, 1260–1200*m*, 1170*m*, 1130*w*, 1100*w*, 1060*m*, 1030*w*, 900*w*, 860*w*. ^{1}H-NMR ($CDCl_3$, 360 MHz, only 1 conformer): 0.91, 0.96 (2*d*, $J = 3$, 12H, $2CH_3$–C(4^2), $2CH_3$–C(4^3)); 1.30 (*d*, $J = 6$, 3H, CH_3–C(2^1)): 1.42 (*s*, 9H, O*t*Bu); 1.55, 1.70, 1.77 (3*m*, 6H, 2H–C(3^2), 2H–C(3^3), H–C(4^2), H–C(4^3)); 2.84, 2.94 (2*s*, 6H, CH_3–N^2, CH_3–N^3); 4.69 (*m*, 1H, H–C(2^1)); 5.29 (*dd*, $J = 12, 6$, 1H, H–C(2^2) or H–C(2^3)); 5.49 (*t*, $J = 6$, 1H, H–C(2^3) or H–C(2^2)); 5.63 (*d*, $J = 9$, 1H, H–N^1); 8.0–9.1 (br. *s*, 1H, COOH). ^{1}H-NMR ($(D)_6$ DMSO, 360 MHz, 180°): 0.88, 0.91 (2*d*, $J = 3$, 12H, $2CH_3$–C(4^2), $2CH_3$–C(4^3)); 1.21 (*d*, $J = 6$, 3H, CH_3–C(2^1)); 1.39 (*s*, 9H, O*t*Bu); 1.48, 1.58, 1.65, 1.73 (4*m*, 6H, 2H–C(3^2), 2H–C(3^3), H–C(4^2), H–C(4^3)); 2.88, 2.91 (2*s*, 6H, CH_3–N^2, CH_3–N^3); 4.49 (*m*, 1H, H–C(2^1)); 4.86 (*t*, $J = 6$, 1H, H–C(2^2)); 5.37 (*t*, $J = 6$, 1H, H–C(2^3)); 5.82 (br. *s*, 1H, H–N^1); 7.0–8.5 (br., 1H, COOH). MS (LR): 443(M^+), 428, 399, 387, 370, 343, 326, 299, 269, 243, 225, 215, 197, 185, 171, 154, 144, 128, 116, 100, 69, 57.

Boc-D-*Ala*-*MeLeu*-D-*MeLeu*-*MeVal*-*OBzl* (**10**). A solution of 0.221 g (0.5 mmol) of **9** in 7 ml of $CDCl_3$ is cooled to −20°, 0.101 g (1.0 mmol) of *N*-methylmorpholine and 0.060 g (0.5 mmol) of pivaloyl chloride are added, and the anhydride formation is followed by ^{1}H-NMR and IR. After 16 h, 0.111 g (0.5 mmol) of H-MeVal-OBzl in 5 ml of $CDCl_3$ are added dropwise to the mixture at −20°. The formation of **10** and **6** is followed by ^{1}H-NMR. After 5 days, the solution is poured to 50 ml of H_2O, extracted with 200 and 100 ml of CH_2Cl_2 the combined org. phases dried over Na_2SO_4, filtered, and evaporated. The residue is chromatographed on 100 g of silica gel using 2% MeOH/CH_2Cl_2 to yield 152 mg (47% of **10** and **6** as a 77:23 mixture of diastereomers (from ^{1}H-NMR signals of H–C(2^4)), $[\alpha]_D^{20} = -83.9°$ ($c = 0.84$, $CHCl_3$). ^{1}H-NMR ((D_6) DMSO, 180°, **10**/**6** = 77:23): 0.78–0.90 (*m*, 15H, $2CH_3$–C(4^2), $2CH_3$–C(4^3), CH_3–C(3^4)); 0.92–1.03 (*m*, 3H, CH_3–C(3^4)); 1.20 (*d*, $J = 6$, 3H, CH_3–C(2^1)); 1.40 (*s*, 9H, O*t*Bu); 1.45, 1.65 (2*m*, 6H, 2H–C(3^2), 2H–C(3^3), H–C(4^2), H – C(4^3)); 2.28 (*m*, H–C(3^4)); 2.33, 2.36, 2.39, 2.43 (4*s*, 9H, CH_3–N^2, CH_3–N^3, CH_3–N^4); 4.46 (*m*, 1H, H–C(2^1)); 4.55, 4.61 (2*d*, $J = 9$, 77:23, 1H, H–C(2^4)); 5.13 (*m*, 2H, PhCH_2); 5.35, 5.42 (2*t*, $J = 6$, 2H, H–C(2^2), H–C(2^3)); 5.80, 5.92 (2*s*, 7:3, 1H, H–N^1); 7.31 (*s*, 5H, *Ph*CH_2). IR (CH_2Cl_2): 3400*w*, 2950–2800*m*, 1735*m*, 1700*m*, 1640*s*, 1500–1440*m*, 1400*m*, 1370*m*, 1240*w*, 1170*m*, 1130*w*, 1200*w*, 1060*w*, 1000*w*.

Boc-D-*Ala*-*MeLeu*-D-*MeLeu*-*MeVal*-*OH* (**11**) *from the Diastereomeric Mixture* **10**/**6** *(vide supra)*. Following the procedure described for **7**, 0.152 g (0.236 mmol) of **10**/**6** (77:23) are debenzylated and purified: 68 mg (52%) of **11**, $[\alpha]_D^{20} = -52.2°$ ($c = 1.0$, $CHCl_3$), and 32 mg (24%) of **7**, $[\alpha]_D^{20} = -196.3°$ ($c = 1.0$, $CHCl_3$). **11**: IR (CH_2Cl_2): 3400*m*, 2950*m*, 2850*m*, 2800–2400*w*, 1720(sh), 1700(sh), 1690(sh), 1680(sh), 1630*s*, 1480*m*, 1400*m*, 1370*m*, 1320*w*, 1280–1200(br.), 1160*m*, 1120*w*, 1100*w*, 1060*m*, 1020*w*, 860*w*. MS and NMR: identical to those described for **11** in *Sect. 1*. MS (HR): 557.3901 (MH^+, $C_{28}H_{53}N_4O_7$, calc. 557.3869), 556.3763 (M^+, $C_{28}H_{52}N_4O_7$, calc. 556.3836).

6 *and* **10** *from* **5** *and Isolation of the Products as* **11** *and* **7**, see *Table 3*. a) *Experiment Run at* −20°. The solution of 0.443 g (1.0 mmol) of **5** in 15 ml of $CDCl_3$ is cooled to −20°, 0.20 g (2.0 mmol) of *N*-methylmorpholine and 0.120 g (1 mmol) of pivaloyl chloride are added, and the anhydride formation is followed by ^{1}H-NMR and IR. After 16 h, 0.22 g (1.0 mmol) of H-MeVal-OBzl in 5 ml of $CDCl_3$ are added dropwise at −20°. The formation of **6** and **10** is followed by ^{1}H-NMR. After 5 days at −20°, the solution is worked up as for **10** *(vide supra)*: 272 mg (42%) of **6**/**10** (82:18); from ^{1}H-peaks of H–C(2^4), $[\alpha]_D^{20} = -136°$ ($c = 0.92$, $CHCl_3$).

Following the procedure described for **7**, 0.271 g (0.42 mmol) of **6**/**10** (82:18) are debenzylated and purified: 165 mg (71%) of **7**, $[\alpha]_D^{20} = -198.5°$ ($c = 1.0$, $CHCl_3$), 35 mg (15.0%) of **11**, $[\alpha]_D^{20} = -51°$ ($c = 1.0$, $CHCl_3$).

b) *Experiment Run at* +20°. As described above under *a*), but all at +20° and only 29 h instead of 5 days coupling time: 200 mg (31%) **6**/**10** (23:77); from ^{1}H-NMR peaks of H–C(2^4), $[\alpha]_D^{20} = -72.8°$ ($c = 0.82$, $CHCl_3$).

As under *a*), 0.194 g (0.30 mmol) of **6**/**10** (23:77) yield 42 mg (25%) of **7** $[\alpha]_D^{20} = -197°$ ($c = 1.0$, $CHCl_3$), and 108 mg (65%) of **11** $[\alpha]_D^{20} = -50°$ ($c = 1.0$, $CHCl_3$).

6 *and* **10** *from* **9** *and Isolation of the Products as* **7** *and* **11** (see *Table 3*). – *Reaction at* +20°. To a solution of 0.133 g (0.3 mmol) of **9** in 2 ml of $CDCl_3$ at +20°, 0.060 g (0.6 mmol) of *N*-methylmorpholine and 0.036 g (0.3 mmol) of pivaloyl chloride are added at +20°. The anhydride formation is followed by ^{1}H-NMR. After 16 h, 0.066 g (0.3 mmol) of H-MeVal-OBzl in 3 ml of $CDCl_3$ are added at +20°. The formation of **6** and **10** is followed by ^{1}H-

NMR. After 4 days at r. t., the solution is worked up as for **10** *(vide supra)*: 58.5 mg (30%) of **6**/**10** (19 : 81; from ^{1}H-NMR peaks of H–C(2^4)), $[\alpha]_D^{20} = -69.9°$ ($c = 0.87$, $CHCl_3$).

As under *a*) above, 58 mg (0.09 mmol) of **6**/**10** (19 : 81) yield 7.5 mg (15%) of **7**, $[\alpha]_D^{20} = -193°$ ($c = 0.75$, $CHCl_3$), and 30 mg (60%) of **11**, $[\alpha]_D^{20} = -49°$ ($c = 1.0$, $CHCl_3$).

3. Boc-D-Ala-MeLeu-MeLeu-D-MeVal-OH (14). – *Boc-*D*-Ala-MeLeu-*D/L*-MeLeu-*D*-MeVal-OBzl* (**12**). To a solution of 2.66 g (6.0 mmol) of **5** in 27 ml of $CHCl_3$ at $-20°$, 1.21 g (12.0 mmol) of *N*-methylmorpholine and 0.72 g (6.0 mmol) of pivaloyl chloride are successively added. The mixture is stirred for $3^1/_2$ h at $-20°$, then 1.33 g (6.0 mmol) of H-D-MeVal-OBzl in 5 ml of $CHCl_3$ are added dropwise at $-20°$. The mixture is stirred for 6 days at $-20°$, the solution diluted with 200 ml of CH_2Cl_2 and poured onto 200 ml of H_2O. The aq. phase is extracted with 100 ml of CH_2Cl_2, the collected org. phase dried over Na_2SO_4, filtered, and evaporated. The residue is chromatographed on 440 g of silica gel using 1% MeOH/CH_2Cl_2 to yield 2.72 g (70%) of **12** as a 3 : 2 mixture of DLLD- and DLDD-tetrapeptides, $[\alpha]_D^{20} = -18.2°$ ($c = 0.9$, $CHCl_3$). These isomers are separated after removing of the benzyl protecting group (*vide infra* **13** and **14**).

*Boc-*D*-Ala-MeLeu-*D*-MeLeu-*D*-MeVal-OH* (**13**) *and Boc-*D*-Ala-MeLeu-MeLeu-*D*-MeVal-OH* (**14**). A solution of 2.5 g (3.87 mmol) of **12** (see above) in 100 ml of abs. EtOH containing 0.2 g of 10% Pd/C is treated with H_2 for 75 min at r. t. The suspension is filtered through talc, the filtrate evaporated, and the residue chromatographed on 250 g of silica gel using 5% MeOH/CH_2Cl_2 to yield from the first fractions 0.86 g (40%) of **13**, $[\alpha]_D^{20} = +67.9°$ ($c = 0.94$, $CHCl_3$), and from later fractions 1.16 g (54%) of **14**, $[\alpha]_D^{20} = -74.9°$ ($c = 0.80$, $CHCl_3$). **13**: IR ($CHCl_3$): 3450–2400*w*, 2950*m*, 1740*m*, 1630*s*, 1500*m*, 1470*m*, 1410*m*, 1375*m*, 1280–1200*m*, 1165*m*, 1120*w*, 1065*m*, 1020*w*, 860*w*. ^{1}H-NMR ($CHCl_3$, 360 MHz): 0.90, 1.10 (2*d*, $J = 6$, 6H, $2CH_3$–C(3^4)); 0.95–1.05 (*m*, 12H, $2CH_3$–C(4^2), $2CH_3$–C(4^3)); 1.29 (*d*, $J = 6$, 3H, CH_3–C(2^1)); 1.41 (*s*, 9H, O*t*Bu); 1.42, 1.60 and 1.68, 1.82 (4*m*, 2 : 3 : 1, 6H 2H–C(3^2), 2H–C(3^3), H–C(4^2), H–C(4^3)); 2.27 (*m*, 1H, H–C(3^4)); 2.78, 2.98, 3.02 (3*s*, 9H, CH_3–N^2, CH_3–N^3, CH_3–N^4); 4.18 (*d*, $J = 9$, 1H, H–C(2^4)); 4.55 (*m*, 1H, H–C(2^1)); 5.25 (*d*, $J = 9$, 1H, H–N^1); 5.51, 5.71 (2*t*, $J = 6$, 2H, H–C(2^2), H–C(2^3)); 7.0–8.5 (br., 1H, COOH). ^{1}H-NMR ((D$_6$) DMSO, 360 MHz, 180°): 0.80, 1.00 (2*d*, $J = 6$, 6H, $2CH_3$–C(3^4)); 0.85, 0.91 (2*d*, $J = 3$, 12H, $2CH_3$–C(4^2), $2CH_3$–C(4^3)); 1.19 (*d*, $J = 6$, 3H, CH_3–C(2^1)); 1.39 (*s*, 9H, O*t*Bu); 1.50 (*m*, 4H, 2H–C(3^2), 2H–C(3^3)); 1.68 (*m*, 2H, H–C(4^2), H–C(4^3)); 2.19 (*m*, 1H, H–C(2^4)); 2.90, 2.92 (2*s*, 9H, CH_3–N^2, CH_3–N^3, CH_3–N^4); 4.47 (*m*, 1H, H–C(2^1)); 4.58 (*d*, $J = 12$, 1H, H–C(2^4)); 5.35, 5,40 (*m*, 2H, H–C(2^2), H–C(2^3)); 5.90 (br. *s*, 1H, H–N^1); 7.0–8.5 (br., 1H, COOH. MS (LR): 557 (MH^+), 541, 513, 483, 457, 426, 412, 370, 356, 352, 326, 299, 271, 243, 225, 197, 182, 171, 154, 140, 113, 100, 70, 57. MS (HR): 556.3787 (low intensity, M^+, $C_{28}H_{52}N_4O_7$, calc. 556.3836). **14**: IR ($CHCl_3$): 3400–2400*w*, 2950*m*, 1700*m*, 1630*s*, 1470*m*, 1400*m*, 1370*m*, 1280*m*, 1250–1200*m*, 1165*m*, 1120*w*, 1100*w*, 1060*w*, 860*w*. ^{1}H-NMR ($CDCl_3$, 360 MHz, 3 : 1 mixture of conformers): 0.85, 1.09 (2*d*, $J = 6$, 4.5H, 3/2CH_3–C(3^4)); 0.79, 1.00 (2*d*, $J = 6$, 1.5H, $^1/_2CH_3$–C(3^4)); 0.88–0.98 (*m*, 12H, $2CH_3$–C(4^2), $2CH_3$–C(4^3)); 1.30, 1.27 (2*d*, $J = 6$, 3 : 1, 3H, CH_3–C(2^1)); 1.40, 1.47 (2*s*, 3 : 1, 9H, O*t*Bu); 1.54, 1.70 (2*m*, 6H, 2H–C(3^2), 2H–C(3^3), H–C(4^2), H–C(4^3)); 2.30 (*m*, 1H, H–C(3^4)); 2.87, 2.89, 2.90, 2.92, 2.98, 3.00 (6*s*, 1 : 3 : 1 : 3 : 1 : 3, 9H, CH_3–N^2, CH_3–N^3, CH_3–N^4); 4.60 (*m*, 1H, H–C(2^1)); 4.73, 4.32 (2br. *s*, 3 : 1, 1H, H–C(2^4)); 5.52 (*dd*, $J = 15, 9$, 2H, H–C(2^2), H–C(2^3)); 5.77, 5.38 (2*d*, $J = 9$, 3 : 1, 1H, H–N^1); 7.0–8.2 (br., 1H, COOH). ^{1}H-NMR ((D)$_6$DMSO, 360 MHz, 180°): 0.79, 0.98 (2*d*, $J = 6$, 6H, $2CH_3$–C(3^4)); 0.87, 0.91 (2*d*, $J = 3$, 12H, $2CH_3$–C(4^2), $2CH_3$–C(4^3)); 1.19 (*d*, $J = 6$, 3H, CH_3–C(2^1)); 1.38 (*s*, 9H, O*t*Bu); 1.48, 1.63, 1.71 (3*m*, 6H, 2H–C(3^2), 2H–C(3^3), H–C(4^2), H–C(4^3)); 2.19 (*m*, 1H, H–C(3^4)); 2.84, 2.94 (2*s*, 2 : 1, 9H, CH_3–N^2, CH_3–N^3, CH_3–N^4); 4.47 (*m*, 2H, H–C(2^1), H–C(2^4)); 5.38 (*t*, $J = 6$, 1H, H–C(2^2)); 5.50 (br. *s*, 1H, H–C(2^3)); 5.98 (br. *s*, 1H, H–N^1); 6.5–8.0 (br., 1H, COOH). MS (LR): 556 (M^+), 541, 513, 500, 483, 467, 456, 426, 412, 398, 225, 197, 182, 171, 154, 128, 100, 70, 57. MS (HR): 556.3838 (M^+, $C_{28}H_{52}N_4O_7$, calc. 556.3836).

4. H-MeLeu-MeLeu-MeVal-OBzl (28), ***Procedure A.*** – *Boc-MeLeu-MeVal-OBzl* (**19**). To the solution of 0.515 g (2.1 mmol) of Boc-MeLeu-OH (**15**) in 20 ml of $CHCl_3$, 0.425 g (4.2 mmol, 0.58 ml) of Et_3N and 0.277 g (2.31 mmol, 0.28 ml) of pivaloyl chloride are added. The mixture is stirred for 30 min at r. t., the 0.56 g (2.53 mmol) of H-Val-OBzl (**16**) in 20 ml of $CHCl_3$ are added dropwise at r. t. The mixture is stirred for 2 h at 20° and then refluxed for $1^1/_2$ h. The mixture is cooled, diluted with 100 ml of $CHCl_3$, and washed with 50 ml of 1 N $NaHCO_3$. The aq. phase is re-extracted (3x) with 150-ml portions of $CHCl_3$, the combined $CHCl_3$ phase dried over Na_2SO_4, filtered, and evaporated. The residue is chromatographed on 100 g of silica gel using 1% MeOH/CH_2Cl_2 to yield 0.86 g (91.5%) of **19**, $[\alpha]_D^{20} = -140.6°$ ($c = 0.92$, $CHCl_3$). IR (CH_2Cl_2): 3050*w*, 2960*m*, 2940 (sh), 2870*w*, 1745*s*, 1700*s*, 1660*s*, 1480*m*, 1460*m*, 1400*m*, 1380*m*, 1330*m*, 1300–1250 (br.), 1200*m*, 1160*m*, 1080*w*, 1010*w*, 960*w*, 930*w*, 910*w*, 880*w*, 860*w*. ^{1}H-NMR (D$_6$ DMSO, 360 MHz, 150°): 0.83, 0.98 (2*d*, $J = 7$, 6H, $2CH_3$–C(3^2)); 0.88 (*d*, $J = 6$, 6H, $2CH_3$–C(4^1)); 1.41 (*s*, 9H, O*t*Bu); 1.45, 1.60 (2*m*, 3H, 2H–C(3^1), H–C(4^1)); 2.25 (*m*, 1H, H–C(3^2)); 2.63,

2.89 (2*s*, 6H, CH_3–N^1, CH_3–N^2); 4.62 (*d*, J = 9, 1H, H–C(2^2)); 4.93 (*d*, J = 7, 1H, H–C(2^1)); 5.13 (*dd*, J = 22, 9, 2H, PhCH_2); 7.31 (*s*, 5H, *Ph*CH_2). MS (LR): 448 (M^+) 375, 349, 333, 291, 212, 200, 170, 144, 128, 100, 91, 70, 52 *etc.*

Formation of cyclo(-MeLeu-MeVal-) **21** *When Attempting Coupling Reactions with H-MeLeu-MeVal-OBzl.* A solution of 9.0 g (20 mmol) of **19** in 40 ml (59.6 g, 523 mmol) of precooled (0°) CF_3COOH is stirred for 3 h at 0°. The CF_3COOH is evaporated under vacuum (water pumps) at 0°, the oily residue taken up in 300 ml of CH_2Cl_2, washed with 100 ml of sat. $NaHCO_3$, dried over Na_2SO_4, and evaporated (this last operation produced cyclization to **21**). H-MeLeu-MeVal-OBzl can be isolated as CH_2Cl_2 solution (IR: 1760*s*, 1660*s*). Attempts to condense the pivalic anhydride of Boc-MeLeu with H-MeLeu-MeVal-OBzl in CH_2Cl_2 at r. t. were unsuccessfull, only **21** could be isolated. The residue is chromatographed on 350 g of silica gel using 5% MeOH/CH_2Cl_2 to yield 4.50 g (93.8%) of **21**, $[\alpha]_D^{20}$ = +15.9° (c = 0.95, $CHCl_3$). IR (CH_2Cl_2): 1660*s*, no other C=O. ^{1}H-NMR ($CDCl_3$, 90 MHz): 0.95–1.20 (*m*, 12H, 2CH_3–C(4^1), 2CH_3–C(3^2)); 1.60, 1.80, 2.10 (3*m*, 4H, 2H–C(3^1), H–C(4^1), H–C(3^2)); 2.95, 3.0 (2*s*, 6H, CH_3–N^1, CH_3–N^2); 3.62 (*d*, J = 7, H–C(2^2)); 3.82 (*dd*, J = 13, 5, 1H, H–C(2^1)). MS (LR): 241 (M^+), 225, 197, 184, 169, 155, 141, 113, 100, 78, 52 *etc.*

Z-MeLeu-MeVal-OtBu (**20**)[22]. To a solution of 5.58 g (20 mmol) of Z-MeLeu-OH (**17**) in 50 ml of $CHCl_3$, 3.3 ml (30 mmol) of *N*-methylmorpholine are added, the solution is cooled to −20°, 2.45 ml (20 mmol) of pivaloyl chloride are added, and the mixture is stirred for 2 h at −20° (IR: 1820*s*, 1760*w*). Then 3.74 g (20 mmol) of H-MeVal-O*t*Bu · HCl and 3.3 ml (30 mmol) of *N*-methylmorpholine in 50 ml of $CHCl_3$ are slowly added at −20°. After 10 min, the solution is warmed to 20° and stirred at 20° for 30 min, then stirred for a further 2 h at 40°. The resulting solution is concentrated under vacuum, the residue dissolved in 400 ml of AcOEt, the solution washed successively with 100 ml of sat. $NaHCO_3$, 100 ml of 10% H_3PO_4, 100 ml of sat. $NaHCO_3$ and 100 ml of sat. NaCl. The org. phase is dried over $MgSO_4$ and evaporated. The residue (8.9 g) is chromatographed on 220 g of silica gel (0.04–0.063 mm) using 2% AcOEt/CH_2Cl_2 to yield 5.2 g (58.4%) of **20**, $[\alpha]_D^{20}$ = −136.5° (c = 1.0, $CHCl_3$). IR ($CHCl_3$): 2960*m*, 2940 (sh), 2880 (sh), 1720*s*, 1680*s*, 1640*s*, 1480–1430*m*, 1390*w*, 1365*m*, 1300*m*, 1200*w*, 1155*s*, 1130*m*, 980*w*. ^{1}H-NMR ((D$_6$)DMSO, 360 MHz, 120°): 0.75, 0.80, 0.90 (3*d*, J = 8, 1:2:1, 12H, 2CH_3–C(4^1), 2CH_3–C(3^2)); 1.35 (*s*, 9H, O*t*Bu); 1.55 (*m*, 3H, 2H–C(3^1), H–C(4^1)); 2.15 (*m*, 1H, H–C(3^2)); 2.75, 2.83 (2*s*, 6H, CH_3–N^1, CH_3–N^2); 4.36 (*d*, J = 10, 1H, H–C(2^2)); 4.98 (*t*, J = 7, 1H, H–C(2^1)); 5.08 (*s*, 2H, PhCH_2); 7.25 (*s*, *Ph*CH_2). ^{1}H-NMR ((D$_6$)DMSO, 360 MHz, 20°): more than 4 conformers.

H-MeLeu-MeVal-OtBu (**22**)[22]. A solution of 10 g (22.3 mmol) of **20** in 500 ml of abs. EtOH containing 3 g of 10% Pd/C is treated with H_2 for 30 min at r. t. The suspension is filtered through talc, the filtrate evaporated, the residue dissolved in 500 ml of 2.5% HCl, and the solution washed 3x with 200 ml of AcOEt. The aq. phase is neutralized to pH 9 with 5% NH_4OH. The suspension obtained is shaken 4x with 200 ml of Et_2O. The combined org. phases are washed with 200 ml of sat. NaCl, dried over $MgSO_4$, and evaporated: 5.9 g (84.2%) of light-yellow oily **22**, $[\alpha]_D^{20}$ = −118° (c = 1.0, $CHCl_3$). ^{1}H-NMR ((D$_6$)DMSO, 360 MHz, 120°): 0.79 , 0.86, 0.94 (3*d*, J = 8, 1:2:1, 12H, 2CH_3–C(4^1), 2CH_3–C(3^2)); 1.28 (*m*, 2H, 2H–C(3^1)); 1.35 (*s*, 9H, O*t*Bu); 1.73 (*m*, 1H, H–C(4^1)); 1.60–2.0 (br. *s*, 1H, H–N^1); 2.15 (*s*, 3H, CH_3–N^1); 2.18 (*m*, 1H, H–C(3^2)); 2.88 (*s*, 3H, CH_3–N^2); 3.40 (*dd*, J = 8, 6, H–C(2^1)); 4.46 (*d*, J = 10, H–C(2^2)). MS (FD): 314 (M^+, $C_{17}H_{34}N_2O_3$).

Z-MeLeu-MeLeu-MeVal-OtBu (**23**)[22]. To a solution of 5.26 g (18.8 mmol) of Z-MeLeu-OH (**15**) in 49 ml of $CHCl_3$, 2.1 ml (19 mmol) of *N*-methylmorpholine are added. The solution is cooled to −20°, 2.30 ml (18.8 mmol) of pivaloyl chloride are added, and after stirring for 2 h at −20°, 5.90 g (18.8 mmol) of **22** and 2.1 ml (19.0 mmol) of *N*-methylmorpholine in 49 ml of $CHCl_3$ are slowly added at −20° followed by further stirring at −20° for 1 h. The solution is allowed to warm to 20° and stirred for 3 h at r. t. The resulting solution is concentrated under vacuum, the oily yellow residue dissolved in 500 ml of AcOEt, the solution washed successively with 100 ml of sat. $NaHCO_3$, 100 ml of 10% H_3PO_4, 100 ml of sat. $NaHCO_3$, and 100 ml of sat. NaCl. The org. phase is dried over $MgSO_4$, filtered, and evaporated. The 10.13 g of residue are chromatographed on 400 g of silica gel (0.04–0.063 mm) using 1% MeOH/CH_2Cl_2 as eluant to yield 4.9 g (45%) of **23**, $[\alpha]_D^{20}$ = −165.5° (c = 1.0, $CHCl_3$). ^{1}H-NMR ((D$_6$)DMSO, 360 MHz, 120°): 0.78, 0.96 (2*d*, J = 6, 6H, 2CH_3–C(3^3)); 0.86 (*d*, J = 7, 12H, 2CH_3–C(4^1), 2CH_3–C(4^2)); 1.40 (*s*, 9H, O*t*Bu); 1.55 (*m*, 6H, 2H–C(3^1), 2H–C(3^2), H–C(4^1), H–C(4^2)); 2.15 (*m*, 1H, H–C(3^3)); 2.80, 2.85, 2.88 (3*s*, 9H, CH_3–N^1, CH_3–N^2, CH_3–N^3); 4.38 (*d*, J = 10, 1H, H–C(2^3)); 5.00 (*t*, J = 8, 1H, H–C(2^1) or H–C(2^2)); 5.40 (*t*, J = 7, 1H, H–C(2^2) or H–C(2^1)); 7.34 (*s*, 5H, *Ph*CH_2). MS (FD): 576 (M^+, $C_{32}H_{53}N_3O_6$).

Z-MeLeu-MeLeu-MeVal-OH (**24**). A solution of 5.0 g (8.68 mmol) of **23** in 30 ml of precooled (−20°) CF_3COOH is stirred for 4 h at −20°. The CF_3COOH is then removed under vacuum at 0° (water pump). The oily residue is diluted with 300 ml of CH_2Cl_2, the solution obtained washed with 200 ml of sat. $NaHCO_3$, the $NaHCO_3$ phase washed 2 × with 200 ml of CH_2Cl_2, the collected org. phases washed with 100 ml of sat. NaCl, dried with Na_2SO_4, filtered, and evaporated. The oily colorless residue is chromatographed on 250 g of silica gel using 5%

[22]) Prepared by Dr. *M. Krieger* at *Sandoz Ltd.*

MeOH/CH_2Cl_2 to yield 3.20 g (71%) of **24**, $[\alpha]_D^{20} = -184.3°$ ($c = 1.0$, $CHCl_3$). ^{1}H-NMR ((D$_6$) DMSO, 360 MHz, 120°): 0.75, 0.92 (2*d*, *J* = 7, 6H, 2CH_3–C(3^3)); 0.82 (*d*, *J* = 6, 12H, 2CH_3–C(4^1), 2CH_3–C(4^2)); 1.2–1.7 (*m*, 6H, 2H–C(3^1), 2H–C(3^2), H–C(4^1), H–C(4^2)); 2.15 (*m*, 1H, H–C(3^3)); 2.72, 2.73, 2.80 (3*s*, 9H, CH_3–N^1, CH_3–N^2, CH_3–N^3); 4.32 (*d*, *J* = 10, 1H, H–C(2^3)); 4.45 (*t*, *J* = 7, 1H, H–C(2^2) or H–C(2^1)); 5.05 (*s*, 2H, PhC*H*$_2$); 5.35 (*t*, *J* = 6, 1H, H–C(2^1) or H–C(2^2)); 7.29 (*s*, 5H, *Ph*CH_2); 7.5–8.5 (br., 1H, COOH). MS (FD): 520 (M^+, $C_{28}H_{49}N_3O_6$).

H-MeLeu-MeLeu-MeVal-OH (**25**). A solution of 6.45 g (12.4 mmol) of **24** in 500 ml of abs. EtOH containing 0.5 g of 10% Pd/C is treated for 3 h at 20°. The suspension obtained is filtered through talc, the filtrate evaporated, and the residue crystallized from Et_2O. 4.07 g (85%) of **25**, m.p. 192–193°, $[\alpha]_D^{20} = -165.9°$ ($c = 1.0$, $CHCl_3$). 1H-NMR ((D$_6$) DMSO, 90 MHz, 120°): 0.85 (*m*, 18H, 2CH_3–C(4^1), 2CH_3–C(4^2), 2CH_3–C(3^4)); 1.30, 1.60 (2*m*, 6H, 2H–C(3^1), 2H–C(3^2), H–C(4^1), H–C(4^2)); 2.15 (*m*, 1H, H–C(3^3)); 2.16 (*s*, 3H, CH_3–N^1); 2.82, 2.86 (2*s*, 1 : 2, 9H, CH_3–N^1, CH_3–N^2, CH_3–N^3); 3.40 (*t*, *J* = 7, 1H, H–C(2^1)); 4.45 (*d*, *J* = 10, 1H, H–C(2^3)); 5.50 (*t*, *J* = 6, 1H, H–C(2^1)); 6.5–8.0 (br., 1H, COOH). MS (FD): 386 (M^+, $C_{20}H_{39}N_3O_4$).

Boc-MeLeu-MeLeu-MeVal-OH (**26**). At r.t. 17.6 ml of 1N NaOH and 4.4 g (20.2 mmol) of di(*tert*-butyl)pyrocarbonate are successively added to a solution of 6.5 g (16.8 mmol) of **25** in 100 ml of dioxane and stirred for 15 h. The dioxane is then evaporated (temp. < 40°). The residue is diluted with 300 ml of AcOEt, acidified with 2N H_2SO_4 to pH 3 and extracted with 200 ml of H_2O. The aq. phase is extracted with a further 200 ml of AcOEt, the combined org. phases are dried over Na_2SO_4, filtered, and evaporated. The residue is chromatographed on 500 g of silica gel using 5% MeOH/CH_2Cl_2 to yield 6.0 g (73.5%) of **26**, $[\alpha]_D^{20} = -185.6°$ ($c = 1.0$, $CHCl_3$). This product is identical with **26** obtained by *Procedure B (vide infra)* IR ($CHCl_3$): 3200–2400*w*, 2950*m*, 1760 (sh), 1720 (sh), 1700 (sh), 1680 (sh), 1640*s*, 1580 (sh), 1480*m*, 1390*s*, 1370*s*, 1320*m*, 1290*w*, 1260–1200*w*, 1160*s*, 1130*m*, 1060*w*. ^{1}H-NMR ($CDCl_3$, 360 MHz): 4 conformers. ^{1}H-NMR ((D)$_6$ DMSO, 360 MHz, 180°): 0.82, 0.99 (2*d*, *J* = 6, 6H, 2CH_3–C(3^3)); 0.89 (br. *s*, 12H, 2CH_3–C(4^1), 2CH_3–C(4^2)); 1.42 (*s*, 9H, O*t*Bu); 1.45, 1.61, 1.70 (3*m*, 6H, 2H–C(3^1), 2H–C(3^2), H–C(4^1), H–C(4^2)); 2.20 (*m*, 1H, H–C(3^3)); 2.70 (*s*, 3H, CH_3–N^3); 2.90 (*s*, 6H, CH_3–N^1, CH_3–N^2); 4.43 (br. *s*, 1H, H–C(2^3)); 4.95 (*t*, *J* = 6, 1H, H–C(2^1)); 5.44 (*t*, *J* = 6, 1H, H–C(2^2)); 6.5–9.5 (br., 1H, COOH). MS (LR): 485 (M^+), 441, 412, 385, 355, 327, 299, 255, 228, 200, 172, 144, 100, 86, 57, 42 *etc.* MS (HR): 485.3452 (M^+, $C_{25}H_{47}N_3O_6$, calc. 485.3465).

Boc-MeLeu-MeLeu-MeVal-OBzl (**27**) *from* **26** *and its Conversion to H-MeLeu-MeLeu-MeVal-OBzl* (**28**). To a solution of 6.8 g (14.0 mmol) of **26** in 50 ml of benzene, 4.94 g (18.2 mmol) of *N,N*-dimethylformamide dibenzyl acetal are added. After 4 h of reflux, benzene and part of the unreacted *N,N*-dimethylformamide dibenzyl acetal are removed under vacuum on a rotary evaporator. The residue, 14.0 mmol (8.05 g) of crude **27** (containing some unreacted *N,N*-dimethylformamide dibenzyl acetal) is dissolved in 15 ml of CF_3COOH (pre-cooled to −5°) and stirred for 15 h at −5°. Then, CF_3COOH is removed under vacuum (water pump). The oily residue is dissolved in 200 ml of CH_2Cl_2 and washed with 100 ml of sat. $NaHCO_3$ and 100 ml of H_2O. The aq. phases are again extracted with 200 ml of CH_2Cl_2, the combined org. phases dried over Na_2SO_4, filtered through talc, and evaporated. The residue is dissolved in 300 ml of Et_2O and **28** precipitated as the hydrochloride with dry HCl gas. The precipitate is filtered off, dissolved in 300 ml of CH_2Cl_2, shaken with 200 ml of sat. $NaHCO_3$, the aq. phase again extracted with 200 ml of CH_2Cl_2, the combined org. phase dried over Na_2SO_4, filtered, and evaporated. The residue is chromatographed on 500 g of silica gel using 4% MeOH/CH_2Cl_2, then 10% MeOH/CH_2Cl_2 to yield 2.5 g (38% over 2 steps) of crystalline **28**, m.p 48–49°, $[\alpha]_D^{20} = -149.1°$ ($c = 1.0$, $CHCl_3$). IR (CH_2Cl_2): 3200*w*, 2959*m*, 2850*m*, 2800 (sh), 1730*m*, 1635*s*, 1460*m*, 1390*m*, 1365*w*, 1290–1250*m*, 1190*m*, 1130*m*, 1100*w*, 1080*w*, 1050*w*, 1000*m*, 900*w*, 880*w*, 840*w*. ^{1}H-NMR ($CDCl_3$, 360 MHz): 0.78, 0.99 (2*d*, *J* = 6, 6H, 2CH_3–C(3^3)); 0.88–0.98 (*m*, 12H, 2CH_3–C(4^1), 2CH_3–C(4^2)); 1,20, 1.32, 1.42, 1.66 (4*m*, 6H, 2H–C(3^1), 2H–C(3^2), H–C(4^1), H–C(4^2)); 1.85 (*m*, 1H, H–C(3^3)); 2.26 (*s*, 3H, CH_3–N^1); 2.94, 2.97 (2*s*, 6H, CH_3–N^2, CH_3–N^3); 3.40 (*dd*, *J* = 3, 9, 1H, H–C(2^1)); 4.91 (*d*, *J* = 9, 1H, H–C(2^3)); 5.16 (*dd*, *J* = 42, 12, 2H, Ph*CH*$_2$); 5.61 (*dd*, *J* = 6, 9, 1H, H–C(2^2)); 7.34 (*s*, 5H, *Ph*CH_2). ^{1}H-NMR ((D$_6$) DMSO, 360 MHz, 180°): 0.81, 0.98 (2*d*, *J* = 6, 6H, 2CH_3–C(3^3)); 0.85, 0.88 (2*d*, *J* = 3, 12H, 2CH_3–C(4^1), 2CH_3–C(4^2)); 1.32 (*m*, 2H), 1.50 (*m*, 3H), 1.65 (*m*, 1H, 2H–C(3^1), 2H–C(3^2), H–C(4^1), H–C(4^2)); 1.75 (*m*, 1H, H–C(3^3)); 2.21 (*s*, 3H, CH_3–N^1); 2.88, 2.90 (2*s*, 6H, CH_3–N^2, CH_3–N^3); 3.42 (*t*, *J* = 6, 1H, H–C(2^1)); 4.68 (*d*, *J* = 12, 1H, H–C(2^3)); 5.12 (*q*, *J* = 12, 2H, PhCH_2); 5.51 (*t*, *J* = 6, 1H, H–C(2^2)); 7.32 (*s*, 5H, *Ph*CH_2). MS (LR): 475 (M^+), 460, 441, 418, 376, 319, 293, 276, 238, 220, 197, 169, 156, 140, 128, 120, 100, 70, 51. 475.3445 (M^+, $C_{27}H_{45}N_3O_4$, calc. 475.3411).

5. H-MeLeu-MeLeu-MeVal-OBzl (28), ***Procedure B.*** – *Boc-MeLeu-MeLeu-OBzl* (**29**). To a solution of 15.0 g (61.22 mmol) of Boc-MeLeu-OH (**15**) in 120 ml of $CHCl_3$, cooled to −20°, 13.6 g (134.68 mmol) of *N*-methylmorpholine and 8.08 g (67.34 mmol) of pivaloyl chloride are added successively within 10 min, and the mixture is stirred for 6 h at −20°. Then, 14.38 g (61.22 mmol) of H-MeLeu-OBzl in 60 ml of $CHCl_3$ are added

dropwise at −20°, and the mixture is stirred for 20 h at −20°. The resulting mixture is shaken with 200 ml of sat. $NaHCO_3$, the org. phase separated, the aq. phase reextracted with 300 ml of $CHCl_3$, the combined org. phase washed with 100 ml of H_2O, dried over Na_2SO_4, filtered, and evaporated. The oily residue is chromatographed on 500 g of silica gel using 5% $MeOH/CH_2Cl_2$ to yield 24.9 g (88%) of **29**, $[\alpha]_D^{20} = -95.5°$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 2950*s*, 2850*m*, 1730*m*, 1680*s*, 1640*m*, 1480*m*, 1450*m*, 1390*m*, 1370*m*, 1320*w*, 1280*m*, 1160*m*, 1115*m*, 1040*s*, 1000*s*, 900*s*, 860*s*. ^{1}H-NMR ((D_6)DMSO or $CHCl_3$, 360 MHz, 20°): more than 4 conformers. ^{1}H-NMR ((D_6)DMSO, 360 MHz, 180°): 0.85–0.95 (*m*, 12H, $2CH_3$–C(4^1), $2CH_3$–C(4^2)); 1.40 (*s*, 9H, O*t*Bu); 1.45–1.82 (*m*, 6H, 2H–C(3^1), 2H–C(3^2), H–C(4^1), H–C(4^2)); 2.63, 2.88 (2*s*, 6H, CH_3–N^1, CH_3–N^2); 4.94, 5.01 (2*t*, $J = 6$, 2H, H–C(2^1), H–C(2^2); 5.12 (*s*, 2H, PhCH_2); 7.32 (*s*, 5H, *Ph*CH_2). MS (LR): 462 (M^+), 447, 419, 405, 389, 363, 347, 332, 319, 305, 290, 264, 236, 220, 200, 190, 172, 156, 144, 128, 100, 91, 65, 57 *etc.* MS (HR): 462.3114 (M^+, $C_{26}H_{42}N_2O_5$, calc. 462.3094).

Boc-MeLeu-MeLeu-OH (**30**). A solution of 24.9 g (53.9 mmol) of **29** in 500 ml of abs. EtOH containing 1.5 g of 10% Pd/C is treated by H_2 for 2h at r. t. The suspension is filtered through talc, the filtrate evaporated, and the residue crystallized from petroleum ether: 15.2 g (75%) of white crystalline **30**, $[\alpha]_D^{20} = -120.6°$ ($c = 1.0$, $CHCl_3$), m.p. 111–113°. IR ($CHCl_3$): 3100–2400*w*, 2950*m*, 1740*w* (sh), 1700*m* (sh), 1680*s* (sh), 1640*s*, 1480–1440*m*, 1395*s*, 1375*s*, 1320*m*, 1260–1200*m*, 1160*s*, 1140*m* (sh), 1100*w*, 960*w*, 880–840*w*. ^{1}H-NMR ((D_6)DMSO, 360 MHz, 180°): 0.91 (*s*, 12H, $2CH_3$–C(3^1), $2CH_3$–C(3^2)); 1.42 (*s*, 9H, O*t*Bu); 1.52, 1.66, 1.75 (3*m*, 6H, 2H–C(3^1), 2H–C(3^2), H–C(4^1), H–C(4^2)); 2.18, 2.89 (2*s*, 6H, CH_3–N^1, CH_3–N^2); 4.89 (br. *s*, 1H, H–C(2^2)); 4.98 (*dd*, $J = 6$, 9, 1H, H–C(2^1)); 5.2–7.0 (br., 1H, COOH). ^{1}H-NMR ($CDCl_3$, 360 MHz, 20°; 4 conformers (2:2:1:1)): 0.89–1.03 (*m*, 12H, $2CH_3$–C(4^1), $2CH_3$–C(4^2)); 1.41, 1.45, 1.49 (3*s*, 9H, O*t*Bu); 1.50 (*m*, 2H), 1.62 (*dd*, $J = 6$, 9, 2H), 1.76 (*t*, $J = 9$, 2H, 2H–C(3^1), 2H–C(3^2), H–C(4^1), H–C(4^2)); 2.59 and 2.68 (2*s*, 1H), 2.84 and 2.92 (2*s*, 1H), 2.72 and 2.97 (2*s*, 2H), 2.75 and 3.0 (2*s*, 2H, CH_3–N^1, CH_3–N^2); 4.64, 4.84, 4.99, 5.10 (4br. *s*, 0.66H, $^1/_3$H–C(2^1), $^1/_3$H–C(2^2)); 4.93 (*t*, $J = 6$, 0.33H), 5.14 (*t*, $J = 6$, 0.33H), 5.18 (*t*, $J = 9$, 0.33H), 5.28 (*t*, $J = 9$, 0.33H, $^2/_3$H–C(2^1), $^2/_3$H–C(2^2)); 8.0–9.5 (br., 1H, COOH). MS (FD): 373 (MH^+), 328, 258, 200. MS (HR): 372.2626 (M^+, $C_{19}H_{36}N_2O_5$, calc. 372.2625).

Boc-MeLeu-MeLeu-MeVal-OBzl (**27**). To the stirred solution of 15.90 g (42.7 mmol) of **30** in 120 ml of $CHCl_3$ at −20° are successively added 9.49 g (93.9 mmol) of *N*-methylmorpholine and 5.64 g (47.0 mmol) of pivaloyl chloride within 10 min. Stirring at −20° is continued for 5 h (IR control: 1820*s*, 1740*w*). Then, 9.44 g (42.7 mmol) of H-MeVal-OBzl in 100 ml of $CHCl_3$ are added dropwise within 10 min at −20°, and the mixture is stirred for 3 days at −20°. The solution is poured onto 200 ml of H_2O, diluted with 200 ml of CH_2Cl_2, the combined org. phase dried over Na_2SO_4, filtered, and evaporated. The oily residue is chromatographed on 500 g of silica gel using 3% $MeOH/CH_2Cl_2$ to yield 19.3 g (78.6%) of **27**, $[\alpha]_D^{20} = -129.8°$ ($c = 1.0$, $CHCl_3$). This product is contaminated with less than 10% of the LDL-isomer (this isomer is detected following the next step, the formation of **10**, and isolation after debenzylation of **11** (3.6%) as a minor by-product *(vide infra)*. **27**: IR ($CHCl_3$): 2950–2900*m*, 2850 (sh), 1730*w*, 1680*m*, 1640*s*, 1460*m*, 1390*m*, 1370*m*, 1320*w*, 1290*w*, 1240*w*, 1180 (sh), 1160*m*, 1130*m*, 1100*w*, 1060*w*, 1000*w*, 900*w*. ^{1}H-NMR ($CDCl_3$, 360 MHz, 2 conformers): 0.80, 1.0 (2*m*, 6H, $2CH_3$–C(3^3)); 0.90 (*m*, 12H, $2CH_3$–C(4^1), $2CH_3$–C(4^2)); 1.42, 1.45 (2*s*, 9H, O*t*Bu); 1.47, 1.58, 1.67 (3*m*, 6H, 2H–C(3^1), 2H–C(3^2), H–C(4^1), H–C(4^2)); 2.25 (*m*, 1H, H–C(3^3)); 2.72, 2.74, 2.90, 2.95, 2.99, 3.03 (6*s*, 9H, CH_3–N^1, CH_3–N^2, CH_3–N^3); 4.70–5.60 (*m*, 5H, PhCH_2, H–C(2^1), H–C(2^2), H–C(2^3)); 7.33 (*s*, 5H, *Ph*CH_2). ^{1}H-NMR ((D_6)DMSO, 360 MHz, 180°): 0.82, 0.98 (2*d*, $J = 6$, 6H, $2CH_3$–C(3^3)); 0.88–0.97 (*m*, 12H, $2CH_3$–C(4^1), $2CH_3$–C(4^2)); 1.42 (*s*, 9H, O*t*Bu); 1.43–1.72 (*m*, 6H, 2H–C(3^1), 2H–C(3^2), H–C(4^1), H–C(4^2)); 2.26 (*m*, 1H, H–C(3^3)); 2.70 (*s*, 3H, CH_3–N^3), 2.86, 2.87 (2*s*, 6H, CH_3–N^1, CH_3–N^2); 4.60 (*d*, $J = 12$, 1H, H–C(2^3)); 4.93, 5.42 (2*t*, $J = 6$, 2H, H–C(2^1), H–C(2^2)); 5.13 (*dd*, $J = 12$, 24, 2H, PhCH_2); 7.31 (*s*, 5H, *Ph*CH_2). MS (LR): 575 (M^+), 560, 519, 502, 386, 327, 313, 299, 276, 248, 228, 200, 184, 170, 144, 114, 100, 91, 85, 57 *etc.* MS (HR): (575.3955 (M^+, $C_{32}H_{53}N_3O_6$, calc. 575.3934).

H-MeLeu-MeLeu-MeVal-OBzl (**28**). A solution of 27 g (46.9 mmol) of **27** (prepared from **30**) in 40 ml of CH_2Cl_2 is added dropwise within 10 min to 40 ml of CF_3COOH, pre-cooled to −20°, and the solution is stirred for 16 h at −20°. Then, the solution is poured onto a mixture of 600 ml of CH_2Cl_2/sat. $NaHCO_3$ 1:1 and 500 g ice. The alcaline aq. phase is washed 3 × with 300 ml of CH_2Cl_2, the combined org. phase dried over Na_2SO_4, filtered, and evaporated. The 20 g (90%) of yellow residue are dissolved in 200 ml of Et_2O and HCl/Et_2O is added until pH 3 is reached. The precipitate of **28**·HCl is filtered off and shaken with 300 ml of CH_2Cl_2 and 200 ml of 5% K_2CO_3. The aq. phase is reextracted (3 ×) with 300 ml portions of CH_2Cl_2. The combined CH_2Cl_2 phases are dried over Na_2SO_4, filtered, evaporated, and dried under high vacuum: 13.2 g (59%) of **28**, $[\alpha]_D^{20} = -155°$ ($c = 1.0$, $CHCl_3$), m.p. 55–56° (neat crystallization), spectroscopically (IR, ^{1}H-NMR, MS) identical with **28** obtained by *Procedure A (vide supra)*. The acidic Et_2O mother liquor is neutralized with sat. $NaHCO_3$, the Et_2O phase washed with 30 ml of H_2O, dried over Na_2SO_4, filtered, and evaporated: 3.1 g (11.5%) of **27**.

Boc-MeLeu-MeLeu-MeVal-OH (**26**). A solution of 2.2 g (3.83 mmol) of **27** in 150 ml of abs. EtOH containing 0.3 g of 10% Pd/C is treated with H_2 for 3 h at r. t. The suspension is filtered through talc, the filtrate evaporated, and the residue chromatographed on 100 g of silica gel using 7.5% $MeOH/CH_2Cl_2$ to yield 1.16 g (62%) of pure **26**, $[\alpha]_D^{20} = -185.3°$ ($c = 1.0$, $CHCl_3$). IR, ^{1}H-NMR, MS: identical with those of **26** obtained by *Procedure A (vide supra)*. MS (HR): 485.3455 (M^+, $C_{25}H_{47}N_3O_{16}$, calc. 485.3465).

6. Boc-D-Ala-MeLeu-MeLeu-MeVal-OH (**7**) **from 28.** – *Boc-D-Ala-MeLeu-MeLeu-MeVal-OBzl* (**6**). To a solution of 0.38 g (2.0 mmol) of Boc-D-Ala-OH (**1**) in 40 ml of $CHCl_3$, 0.44 g (4.4 mmol) of *N*-methylmorpholine and 0.26 g (2.2 mmol) of pivaloyl chloride are added and stirred at $-20°$ for 2 h. Then, 0.95 g (2.0 mmol) of **28** in 4 ml of $CHCl_3$, cooled to $-20°$, are added. After 17 h stirring at $-20°$ (TLC and IR control), the solution is poured onto 200 ml of CH_2Cl_2 and shaken with 100 ml of sat. $NaHCO_3$. The aq. phase is re-extracted with 200 ml of CH_2Cl_2, the combined org. phase dried over Na_2SO_4, filtered, and evaporated. The residue is dissolved in 100 ml of Et_2O and the basic compounds are precipitated with HCl gas. The precipitate is filtered and the filtrate shaken with 100 ml of sat. $NaHCO_3$, dried over Na_2SO_4, and evaporated: 1.4 g of oily residue, $[\alpha]_D^{20} = -142°$ ($c = 1.0$, $CHCl_3$). This crude material is chromatographed on 60 g of silica gel using 2% $MeOH/CH_2Cl_2$ to yield 1.0 g (77.5%) of **6**, $[\alpha]_D^{20} = -156.5°$ ($c = 1.0$ $CHCl_3$). ^{1}H-NMR ((D_6)DMSO, 360 MHz, 180°): 0.80–0.90 (*m*, 15H, $2CH_3$–C(4^2), $2CH_3$–C(4^3), CH_3–C(3^4)); 0.98 (*d*, $J = 6$, 3H, CH_3–C(3^4)); 1.19 (*d*, $J = 6$, 3H, CH_3–C(2^1)); 1.40 (*s*, 9H, O*t*Bu); 1.48 (*m*, 4H, 2H–C(3^2), 2H–C(3^3)); 1.65 (*m*, 2H, H–C(4^2), H–C(3^2)); 2.25 (*m*, 1H, H–C(3^4)); 2.33, 2.36, 2.42 (3*s*, 9H, CH_3–N^2, CH_3–N^3, CH_3–N^4); 4.46 (*m*, 1H, H–C(2^1)); 4.62 (*d*, $J = 9$, 1H, H–C(2^4)); 5.13 (*dd*, $J = 15$, 24, 2H, $PhCH_2$); 5.35, 5.40 (2*t*, $J = 6$, 2H, H–C(2^2), H–C(2^3)); 5.90 (*s*, 1H, H–N^1); 7.32 (*s*, 5H, *Ph*CH_2).

Boc-D-Ala-MeLeu-MeLeu-MeVal-OH (**7**). A solution of 0.97 g (1.5 mmol) of **6**, prepared from **1** and **28**, in 150 ml of abs. EtOH is treated with H_2 in presence of 0.20 g of 10% Pd/C for 2 h at r. t. The suspension is filtered through talc and the filtrate evaporated. The residue is chromatographed on 100 g of silica gel using 6% $MeOH/CH_2Cl_2$ to yield 0.717 g (86%) of **7** $[\alpha]_D^{20} = -201.6°$ ($c = 1.0$, $CHCl_3$), and 0.030 g (3.6%) of **11**, $[\alpha]_D^{20} = -49°$ ($c = 0.95$, $CHCl_3$), identical in all respects (IR, ^{1}H-NMR, MS) to **7** and **11** prepared according to *Sect. 1* and *2*.

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