44. L-Phenylalanine Cyclohexylamide: A Simple and Convenient Auxiliary for the Synthesis of Optically Pure α,α-Disubstituted (*R*)- and (*S*)-Amino Acids

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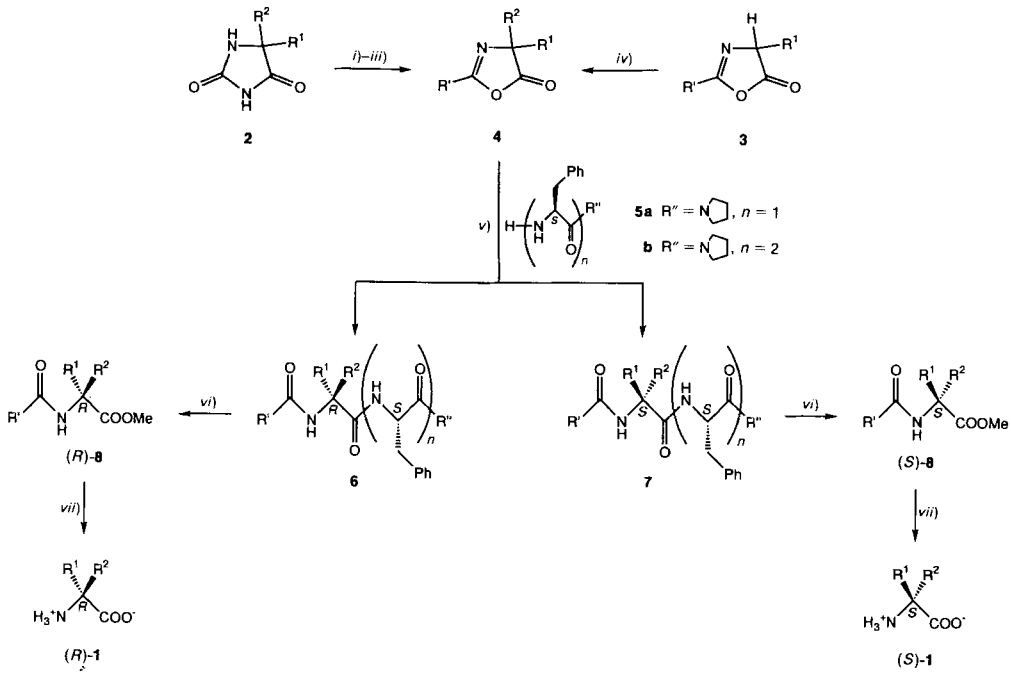
This work describes L-phenylalanine cyclohexylamide (**5c**) as a simple, cheap, and powerful chiral auxiliary for the synthesis of a series of optically pure α,α-disubstituted (*R*)- and (*S*)-amino acids of type **1**, such as (*R*)- and (*S*)-2-methyl-phenylalanine (**1a**), (*R*)- and (*S*)-2-methyl-2-phenylglycine (**1b**), and (*R*)- and (*S*)-2-methylvaline (**1c**; *Scheme 3*). These amino acids were efficiently transformed into the suitably protected and activated amino-acid building blocks (*R*)- and (*S*)-**12b** and (*R*)- and (*S*)-**12c** (*Scheme 4*) which are ready for incorporation into peptides by solution or solid-phase techniques. Based on the crystal structures of **6b**, **6c**, and **7a** belonging to the diastereoisomeric peptides series **6** and **7**, the absolute configurations of each member of the series were determined. β-Turn geometries of type II′ and I were observed for **6b** and **7a**, respectively, whereas **6c** crystallized in an extended conformation. The impacts of side-chain variation on conformation and crystal packing of these triamides are discussed.

1. Introduction. – Among the growing number of non-coded synthetic and naturally occurring amino acids, the open-chain and cyclic α,α-disubstituted amino acids of type **1** (*Scheme 1*) play an important role [1] [2] due to their inherent propensities to stabilize small peptides in rather well defined conformations, depending on the nature of the substituents R^1 and R^2 [3–6] (for further refs., see [6]). Especially the α-methylated α-amino acids of type **1** ((*R*)-**1**: R^2 = Me, $R^1 \neq$ H, Me; (*S*)-**1**: R^1 = Me, $R^2 \neq$ H, Me) have been the focus of many investigations as building blocks in the design of enzyme inhibitors [7] and due to their ability to stabilize 3_{10}- and α-helical as well as β-turn-type conformations in peptides [5].

Recently, we have shown [6] that a large variety of novel and interesting open-chain and cyclic α,α-disubstituted (*R*)- and (*S*)-amino acids could be synthesized in optically pure form using the strategy outlined in *Scheme 1*. Treatment of the 4,4-disubstituted 1,3-oxazol-5(4*H*)-ones **4** (which were obtained either from the hydantoins **2** *via* the classical *Bucherer-Bergs* reaction [8] or by α-alkylation of the 4-monosubstituted 2-phenyl-1,3-oxazol-5(4*H*)-ones **3** (R′ = Ph) [9–11]) with an optically pure amine **5** derived from L-phenylalanine, yielded the diastereoisomeric peptides **6** and **7**, which were separated by crystallization and/or flash chromatography (FC) [12] on SiO_2. Selective amide cleavage using trifluoromethanesulfonic acid (CF_3SO_3H) in MeOH gave the optically pure esters (*R*)- and (*S*)-**8**, which could be converted into the pure amino acids (*R*)- and (*S*)-**1** in good overall yields. We were able to show that the separation of the diastereoisomeric peptides **6** and **7** depended largely on the nature of R′ (Ph ≫ Me) and, even more importantly, on the amines **5a**, **b**.

[1]) Part of Ph. D. Thesis of *C.S.*, University of Zürich, 1993.

Scheme 1

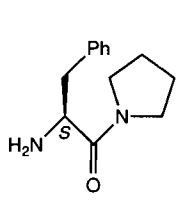


i) $Ba(OH)_2 \cdot 8\ H_2O$, Δ. *ii*) PhCOCl, NaOH. *iii*) *N,N'*-Dicyclohexylcarbodiimide (DCC), CH_2Cl_2. *iv*) NaH, R^2-X, DMF, 0°→r.t. *v*) 5, *N*-Methylpyrrolidin-2-one (NMP), 50–80°. *vi*) CF_3SO_3H, MeOH, 80°. *vii*) 25% aq. HCl soln./dioxane, 100°.

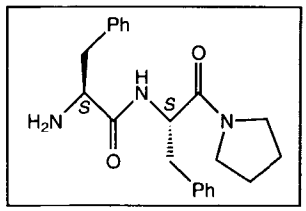
2. Design, Synthesis, and Properties of L-Phenylalanine Cyclohexylamide (5c). – In the course of an exploration of the different chromatographic behavior of the diastereoisomeric peptides of type **6** and **7** (*Scheme 1*), we obtained crystal structures of a pair of diastereoisomeric peptides, revealing a tight β-turn conformation [13] for the (*S,S*)-diastereoisomer of type **7**, but extended conformation for the corresponding (*R,S*)-diastereoisomer **6** [4] [14]. This finding prompted us to investigate a series of simple secondary amides of L-phenylalanine, *i.e.*, **5c–f**, which seemed well designed for a stabilization of a β-turn conformation in compounds of type **7**.

In a preliminary screening, we studied the separation properties of aminoamides **5c–f** when incorporated into amino-acid derivatives of type **6** and **7**, especially in cases where we previously observed poor or no separation [6]. It became evident that the L-phenylalanine cyclohexylamide (**5c**) showed the most promising properties in terms of separation, crystallizability, and solubility of the diastereoisomeric peptides of type **6** and **7**. In the present work, we demonstrate the power of the auxiliary **5c** in a synthesis of the three α-methylated amino acids (*R*)- and (*S*)-2-methyl-phenylalanine (**1a**), (*R*)- and (*S*)-2-methyl-2-phenylglycine (**1b**) and (*R*)- and (*S*)-2-methylvaline (**1c**). This procedure is particularly suited for a large-scale synthesis as documented in the *Exper. Part* for **1c**.

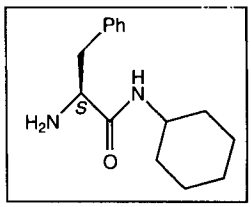
The four auxiliaries **5c–f** were obtained by standard methods (*Scheme 2*). Coupling of the commercially available (*tert*-butoxycarbonyl)-L-phenylalanine **9a** with the corre-



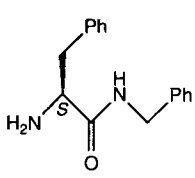
5a



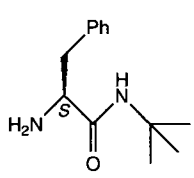
5b



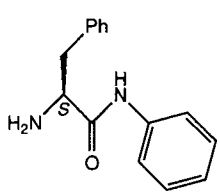
5c



5d



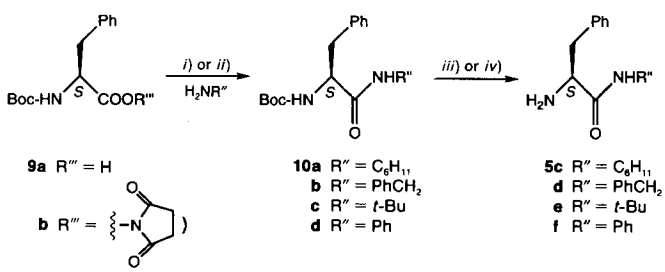
5e



5f

sponding amine using the mixed-anhydride coupling [15] [16] on large scale or the corresponding (*tert*-butoxycarbonyl)-L-phenylalanine succinimido ester **9b** (Boc-Phe-OSu) on small scale were the methods of choice for the synthesis of the intermediate amides **10a–d**. The cleavage of the Boc group was best performed using gaseous HCl in a mixture of AcOEt/THF [16] on a large scale or the standard CF_3COOH treatment in CH_2Cl_2 at 0° on small scale.

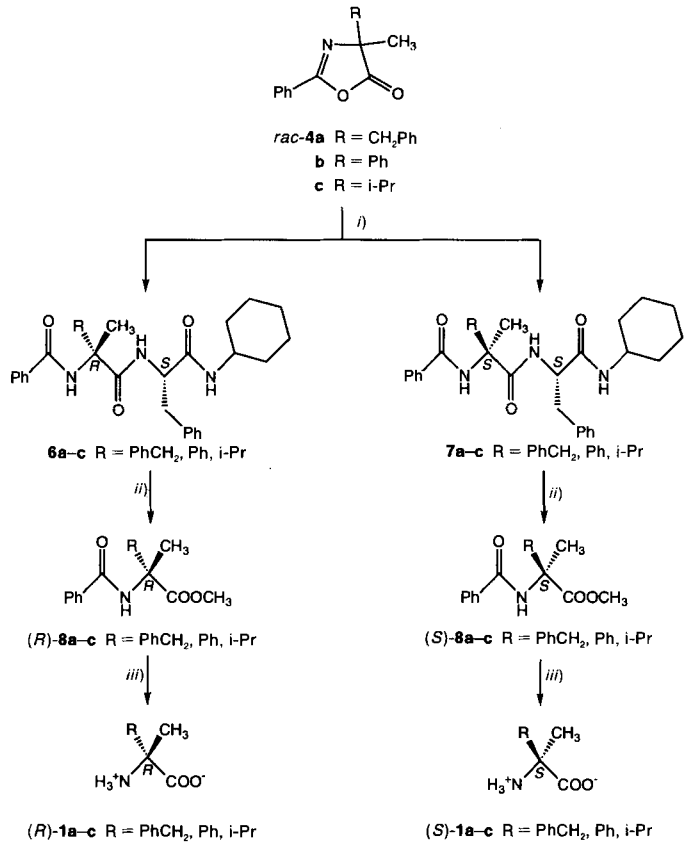
Scheme 2



i) *N*-Methylmorpholine, isobutyl chloroformate, H_2NR'' (*Method A*). *ii*) **9b**, H_2NR'', CH_2Cl_2 (*Method B*). *iii*) HCl (g), AcOEt/THF (*Method C*). *iv*) CF_3COOH, CH_2Cl_2, 0° (*Method D*).

3. Synthesis of Optically Pure (*R*)- and (*S*)-α-Amino-α-methyl Acids 1a–1c Using L-Phenylalanine Cyclohexylamide (5c). – The required 4,4-disubstituted 2-phenyl-1,3-oxazol-5(4*H*)-ones *rac*-**4a–c** were prepared using our α-alkylation procedure [11] in good overall yields. As outlined in *Scheme 3*, the 4,4-disubstituted 2-phenyl-1,3-oxazol-5(4*H*)-ones *rac*-**4a–c** were coupled to L-phenylalanine cyclohexylamide (**5c**; Phe–NHC_6H_{11}) to yield, after FC [12] and/or crystallization, the diastereoisomeric peptides **6a–c** and **7a–c** in pure form and good yields (*Table 1*).

Scheme 3



i) *N*-Methylpyrrolidin-2-one, Phe-NHC$_6$H$_{11}$ (**5c**), 50–80°. *ii*) CF_3SO_3H, MeOH, 80°. *iii*) 25% aq. HCl soln., dioxane, 100°.

Table 1. *Diastereoisomeric Peptides of Type* **6** *and* **7**

Oxazolone	R	Peptide[a])	Yield [%][b])	M.p. [°C]	$[\alpha]_D^{20}$ ($c = 0.2$)
rac-**4a**	$PhCH_2$	**7a** (*S*,*S*)	38	221–222	+2.0 (EtOH)
		6a (*R*,*S*)	39	198–199	+58.0 (EtOH)
rac-**4b**	Ph	**6b** (*R*,*S*)	48	142–144	−16.0 ($CHCl_3$)
		7b (*S*,*S*)	47	157–158	−24.5 ($CHCl_3$)
rac-**4c**	i-Pr	**6c** (*R*,*S*)	47	202.5–204	−70.0 ($CHCl_3$)
		7c (*S*,*S*)	46	141.5–143.5	−21.0 ($CHCl_3$)

[a]) The diastereoisomeric peptides **6** and **7** are listed according to their elution on SiO_2.
[b]) Yields based on isolated and recrystallized peptide.

The absolute configurations of the peptides **6** and **7** were unambiguously determined by the crystal structures of **6b**, **6c**, and **7a** (*Fig. 1*) based on the known (*S*)-configuration of L-phenylalanine (L-PheOH). Structural aspects will be discussed in more detail in *Chapt. 5*.

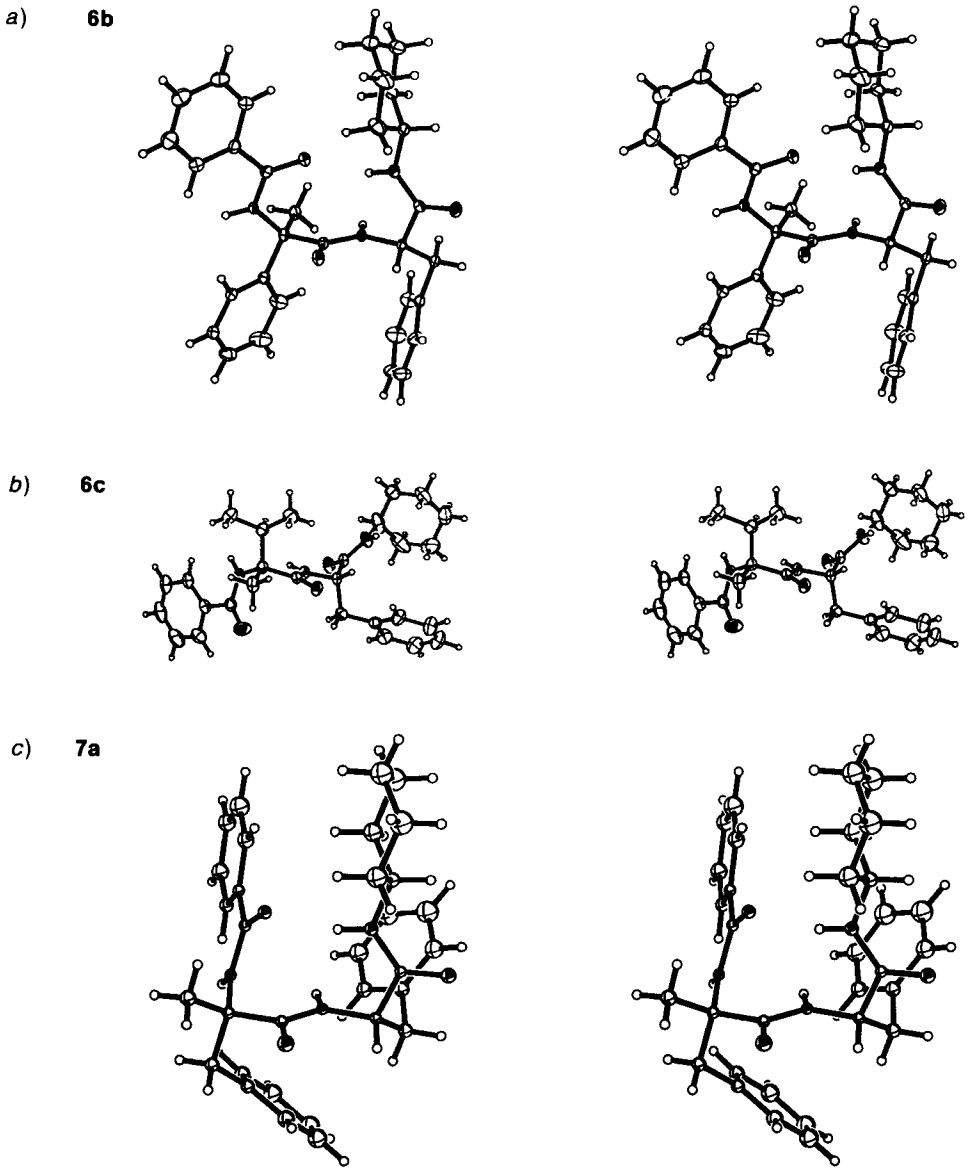


Fig. 1. *Stereoscopic projections of the X-ray structure of* a) *(*R*)-2-methyl-2-phenylglycine derivative* **6b** b) *2-methylvaline derivative* **6c**, *and* c) *2-methyl-phenylalanine derivative* **7a**

The diastereoisomers of types **6** and **7** were converted in the presence of CF_3SO_3H in MeOH at 80° [6] into the optically pure esters (*R*)- and (*S*)- **8a–c**, in excellent yields (*Table 2*). It is interesting to note that the chiral aminoamide **5c** could be recovered as the trifluoromethanesulfonate salt in optically pure form in yields greater than 90%, indicating that the selective amide cleavage is essentially quantitative. As previously shown [6], the selective amide cleavage is based on the fact that the formation of the intermediate

4,4-disubstituted (*R*)- and (*S*)-2-phenyl-1,3-oxazol-5(4*H*)-ones **4a–c** and the simultaneous liberation of L-phenylalanine cyclohexylamide trifluoromethanesulfonate salt (**5c**·CF_3SO_3H) is much faster than alternative amide cleavages. The transiently formed (*R*)- and (*S*)-**4a–c** were converted under the reaction conditions into the esters (*R*)- and (*S*)-**8a–c** (*cf. Scheme 3*).

Table 2. *Conversion of Peptides* **6** *and* **7** *into the Esters (*R*)- and (*S*)-***8a–c***

Peptide	R	Ester	Yield [%]	$[\alpha]_D^{20}$ ($CHCl_3$)
6a	$PhCH_2$	(*R*)-**8a**	95	−78.5 (*c* = 0.2)[a])
7a	$PhCH_2$	(*S*)-**8a**	96	+79.0 (*c* = 0.1)[a])
6b	Ph	(*R*)-**8b**	95	−22.0 (*c* = 0.2)
7b	Ph	(*S*)-**8b**	99	+23.5 (*c* = 0.2)
6c	i-Pr	(*R*)-**8c**	97	−20.5 (*c* = 0.2)
7c	i-Pr	(*S*)-**8c**	96	+21.5 (*c* = 0.2)

[a]) The + and − signs in [6] have been mixed up by mistake.

Finally, the optically pure esters (*R*)- and (*S*)-**8a–c** were hydrolyzed to the free amino acids (*R*)- and (*S*)-**1a–c** using 25% aqueous HCl solution in dioxan at 100°. The amino acids were purified by crystallization at pH 7 or chromatography on *Bio-Rad-50W-X8* cation-exchange resin (see *Exper. Part*).

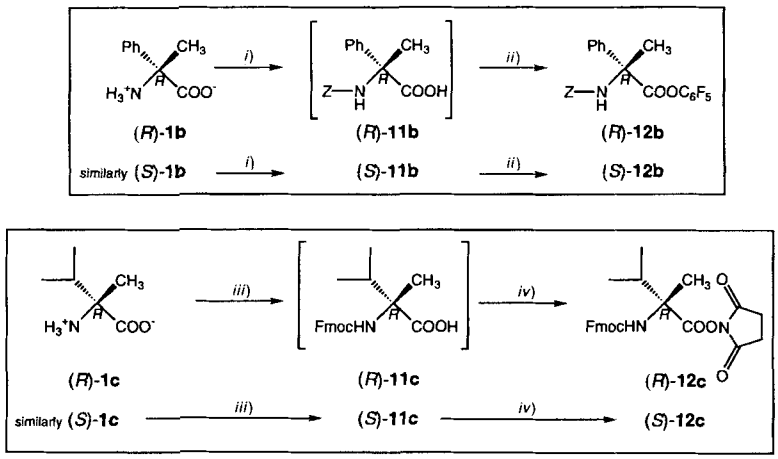
Table 3. *Hydrolysis of the Esters* **8** *to the Free Amino Acids* **1**

Ester	R	Amino acid	Yield [%]	M.p. [°C]	$[\alpha]_D^{20}$ (*c* = 0.2)
(*R*)-**8b**	Ph	(*R*)-**1b**	91	> 240	−54.0 (1N HCl)
(*S*)-**8b**	Ph	(*S*)-**1b**	91	> 243	+52.5 (1N HCl)
(*R*)-**8c**	i-Pr	(*R*)-**1c**	91	> 240	+4.5 (H_2O)
(*S*)-**8c**	i-Pr	(*S*)-**1c**	87	> 240	−4.5 (H_2O)

4. Conversion of (*R*)- and (*S*)-1b,c into Suitably Protected Activated Esters for Incorporation into Peptides. – To have suitably protected and activated amino-acid building blocks available for peptide synthesis, we tested several different types of protective groups for the amino group and activated esters for the 1-carboxy groups as shown in *Scheme 4*. The protection of the amino group with a Boc ((*tert*-butoxy)-carbonyl), Z (benzyloxycarbonyl), or Fmoc ((9*H*-fluoren-9-yl)methoxycarbonyl) group was best performed using the method of *Kricheldorf* [17] (*e.g.* (*R*)- and (*S*)-**1b,c** → (*R*)- and (*S*)-**11b,c**; *Scheme 4*), whereas the pentafluorophenyl esters, due to their excellent solubility and stability, were prepared in high yields and in optically and chemically pure form according to *U. Schmidt* and coworkers [18] (*e.g.* (*R*)- and (*S*)-**11b** → (*R*)- and (*S*)-**12b**; *Scheme 4*[2]). The best results were obtained by direct conversion of the intermediate acids (*R*)- and (*S*)-**11b,c** to the fully protected and activated esters (*R*)- and (*S*)-**12b,c**.

[2]) Also good results were obtained by the conversion of the acids (*R*)- and (*S*)-**11c** into the highly crystalline succinimido esters (*R*)- and (*S*)-**12c**.

Scheme 4



i) Me_3SiCl, CH_2Cl_2 or $CHCl_3$, reflux, then $(i\text{-}Pr)_2NEt$, Z-Cl. *ii*) *N*-Ethyl-*N'*-[3-(dimethylamino)propyl] carbodiimide (EDCl), pentafluorophenol, CH_2Cl_2. *iii*) Me_3SiCl, CH_2Cl_2 or $CHCl_3$, reflux, then $(i\text{-}Pr)_2NEt$, Fmoc-Cl. *iv*) DCC, *N*-Hydroxysuccinimide, DMF or CH_2Cl_2.

5. Determination of the Absolute Configurations of the α-Methylated α-Amino Acids 1a–c Based on the Crystal Structures of 6b, 6c, and 7a; Conformational Aspects in the Crystalline State. – The absolute configurations of the α-methylated amino-acid building blocks were determined by X-ray structure analyses of the *N*-benzoyl-protected Phe-NHC_6H_{11} derivatives **6b**, **6c**, and **7a**. This established the absolute configurations of the corresponding free amino-acid building blocks (*R*)-**1b**, (*R*)-**1c**, and (*S*)-**1a**, respectively. The technical data of these three crystal-structure determinations are given in *Table 4*, relevant geometrical data in *Table 5*.

The structures exhibit interesting aspects of peptide folding and packing. Peptide **6b** adopts a β-turn of type II' (*Fig. 2a*) with the two amino acids in the β-turn positions $(i + 1)$ and $(i + 2)$ and a transannular H-bond between the benzoyl C=O and cyclohexylamide NH groups. In this conformation, the Ph and $PhCH_2$ side chains are juxtaposed in the average plane of the β-turn. The N-terminal PhCO and the C-terminal $C_6H_{11}NH$ moieties extend in the opposite direction and are also juxtaposed in the average plane of the β-turn. With this pairwise arrangement of the hydrophobic groups, the molecules can pack in two-dimensional layers (*Fig. 2b*) exposing tightly packed arrays of Ph, $PhCH_2$, and cyclohexyl groups on either side, thus forming relatively smooth hydrophobic contact surfaces between adjacent layers. Note that except for the small indentations due to the shorter Ph side chains of the 2-methyl-2-phenylglycine units, the hydrophobic surfaces of the peptide layers are devoid of marked cavities or protrusions that could provide possibilities for tight side-chain interlocking. Within each layer, there are two orthogonal H-bond networks with all amide units doubly H-bonded. The first network is established by intermolecular H-bonds between the central peptide units. The second is formed by inter- and intramolecular H-bonds between the N- and C-terminal peptide units.

Table 4. *Experimental Conditions for the X-Ray Analysis of Compounds* **7a**, **6b**, *and* **6c**

	7a	**6b**	**6c**
Crystal data			
Empirical formula	$C_{32}H_{37}N_3O_3$	$C_{31}H_{35}N_3O_3$	$C_{28}H_{37}N_3O_3$
Color; habit	colorless, prismatic	colorless, prismatic	colorless, prismatic
Crystal size [mm]	0.15 × 0.25 × 0.9	0.15 × 0.25 × 0.4	unknown
Crystal system	orthorhombic	monoclinic	hexagonal
Space group	$P2_12_12_1$	$P2_1$	$P6_1$
Unit cell dimensions			
a [Å]	8.982 (2)	9.343 (4)	13.074 (2)
b [Å]	17.992 (3)	10.276 (4)	
c [Å]	18.231 (3)	14.590 (4)	26.579 (5)
α [°]			
β [°]		104.57 (3)	
γ [°]			
Volume [Å^3]	2946.0 (9)	1355.7 (8)	3934.5 (11)
Z	4	2	6
Formula weight	511.6	497.6	463.6
Density (calc.)	1.15	1.219	1.174
Absorption coefficient [mm^{-1}]	0.588	0.079	0.606
F(000)	1096	532	1500
Data collection			
Radiation	CuK_α	MoK_α	CuK_α
Temperature [K]	193	183	298
2θ Range [°]	0–112	0–56	0–113
Scan type	2θ-θ	ω	2θ-θ
Scan speed [°/min]	1.5–14.65	1.5–14.65	1.5–14.65
Scan range [ω]	0.7	0.6	1.0
Independent reflexions	2200	3488	1779
Observed reflexions	1822	2151	1674
Absorption correction	none	none	none
Solution and refinement			
Solution	direct methods	direct methods	direct methods
Data-to-parameter ratio	5.3:1	6.5:1	5.5:1
Final R index (obs. data)	4.55	4.73	4.10

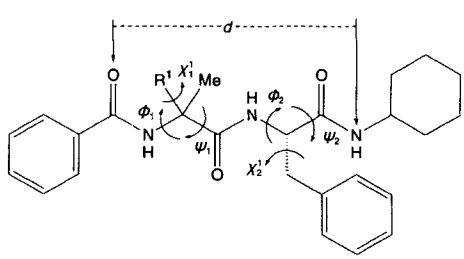


Table 5. *Turn Geometries of Triamides* **6b**, **6c**, *and* **7a** *in the Crystalline State.* Designation of torsional angles according to IUPAC-IUB recommendations [19].

	Angles [°]						d [Å]	Structural motif
	φ_1	ψ_1	φ_2	ψ_2	χ_1^1	χ_2^1		
6b	+49.9	−129.8	−90.3	+4.1	−23.8	−60.0	2.868	β-turn II′ ($\varepsilon\alpha$[20])
6c	+70.2	+39.7	−157.5	+117.4	+66.5	−168.8	–	extended ($\gamma\beta_E$[20])
7a	−59.5	−23.9	−90.0	−2.3	+63.5	+58.4	2.931	β-turn I ($\alpha\alpha$[20])

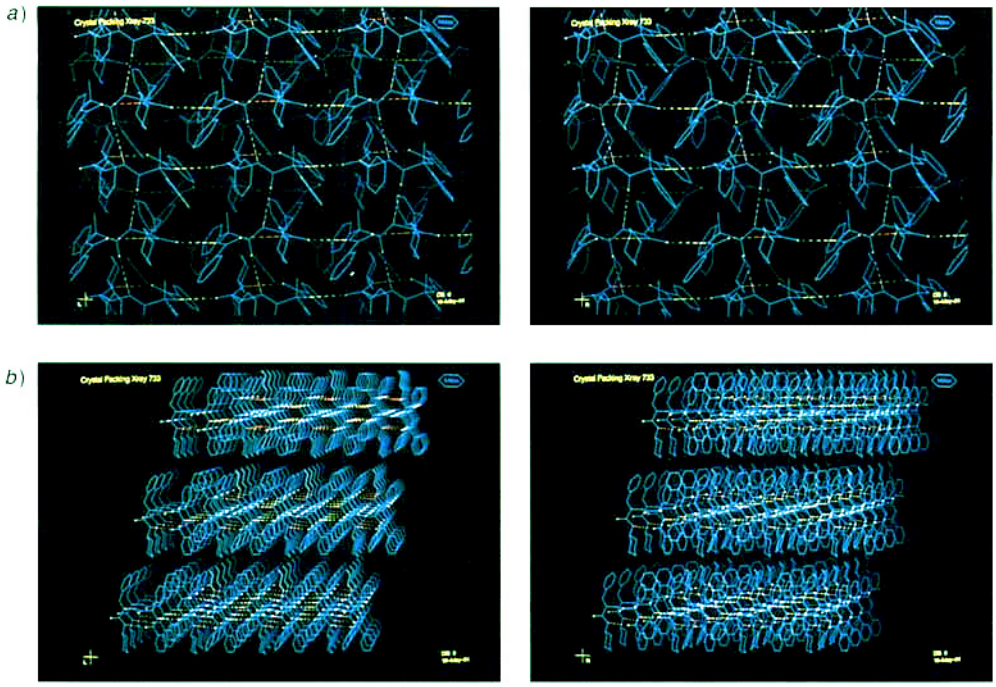


Fig. 2. *Stereoview of the crystal packing of* **6b**: a) *display of one two-dimensional layer of peptide molecules in the picture plane, with the two orthogonal H-bonding networks* (red, pink, and yellow dotted lines) *confined within the layer* (the Ph, $PhCH_2$, PhCO, and cyclohexyl units form tightly packed hydrophobic surfaces on the front and back side of the layer); b) *display of packed layers*

Going from **6b** to **6c**, the Ph group of the α-methylated amino acid is replaced by the somewhat smaller i-Pr group. This appears to be a minor overall structural change, particularly since the absolute (*R*,*S*)-configuration is maintained in both peptides. Accordingly, we might anticipate a similar peptide fold and crystal packing. By contrast, **6c** folds in an extended V-shaped conformation with the 2-methylvaline in a left-handed helical conformation, followed by Phe in an extended β-strand conformation (*Fig. 3*). There are no intramolecular H-bonds. The first two peptide NH groups are H-bonded to the C=O group of the 2-methylvaline unit of a neighboring peptide, whereas the third NH group interacts with the C=O group of the Phe unit of a neighboring peptide on the opposite side. The peptide molecules are stacked along a six-fold crew axis to form cylindrical piles with H-bonding occurring exclusively within the piles. The piles are tightly packed in a hexagonal array. Three adjacent piles are displayed in *Fig. 3b*. Their contiguous exposed surfaces contain aromatic and cyclohexyl residues that form smoothly ascending right-handed helical ribbons providing a complementary hydrophobic surface for the next incoming pile. Note that the tight packing of the piles forces the exposed cyclohexylamide units to adopt axial conformations. A survey in the *Cambridge Crystal Structure Database* [21] of well resolved crystal structures containing an unsubstituted cyclohexylamide unit uncovered 11 structures, 10 of which had this unit in the expected equatorial form, and only one [22] contained this unit in the energetically less

favorable axial conformation. Interestingly, this single case represents again a C-terminal cyclohexylamide derivative of a short peptide, and tight packing, this time between the cyclohexylamide units of neighboring molecules in an orthorhombic crystal lattice, appears to be the main reason for the unusual conformation. However, unlike **6c** this peptide folds as a β-turn.

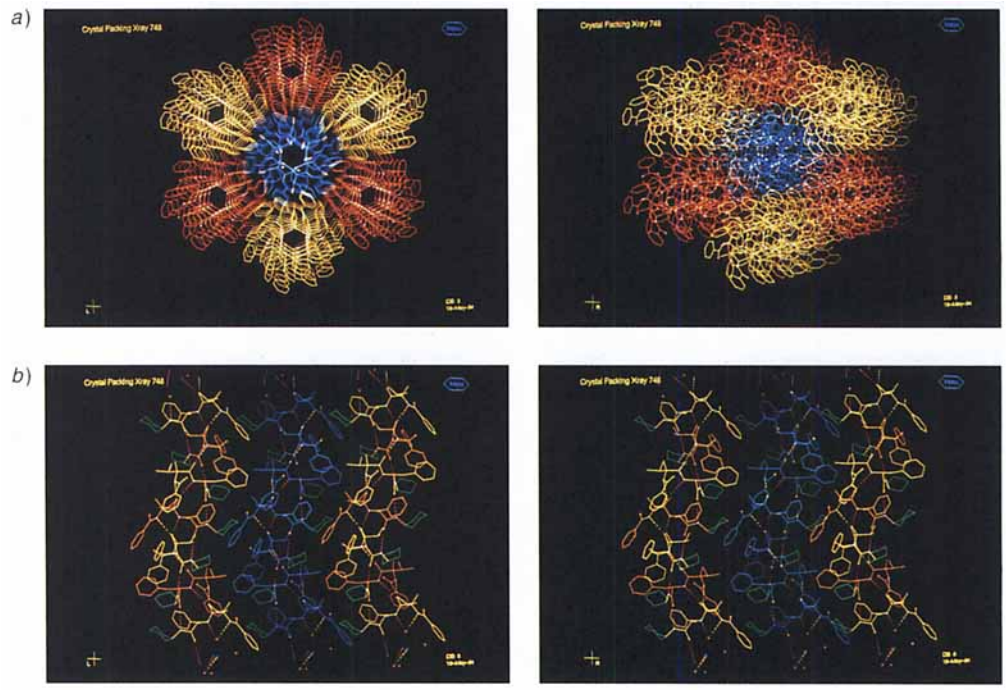


Fig. 3. *Stereoview of the crystal packing of* **6c**: a) *display of hexagonally packed piles of peptides* (blue, yellow, red); b) *side view of three juxtaposed peptide piles with alternate peptide molecules colored in yellow/orange and light/dark blue*. The cyclohexyl units are in green to highlight the six-fold screw axes describing the stacking of the peptides.

It is quite remarkable that the relatively modest structural difference between **6b** and **6c** of a Ph *vs.* an i-Pr group results in such a drastic conformational change from a β-turn to an extended peptide conformation and a change in packing from a layered structure with planar hydrophobic contact surfaces into a hexagonal packing with cylindrical hydrophobic contact surfaces. It appears that the minor packing defects noted above for the Ph groups in **6b** is aggravated by its replacement by the still smaller i-Pr moiety to an extent that a switch to a new peptide fold and packing occurs.

Turning to the crystal structure of **7a** (*Fig. 4*), we first note that each molecule folds as a β-turn of type I, not uncommon for an N- and C-terminus protected dipeptide formed by two L-amino acids. Furthermore, the transannular H-bonds of the β-turns are complemented by the intermolecular H-bonds between the N- and C-terminal peptide units. However, both $PhCH_2$ side chains of the Phe units adopt the less common *endo* conformation [23] with respect to the peptide backbone, leading to an intramolecular hydrophobic clustering of the three aromatic units and leaving the C-terminal cyclohexyl

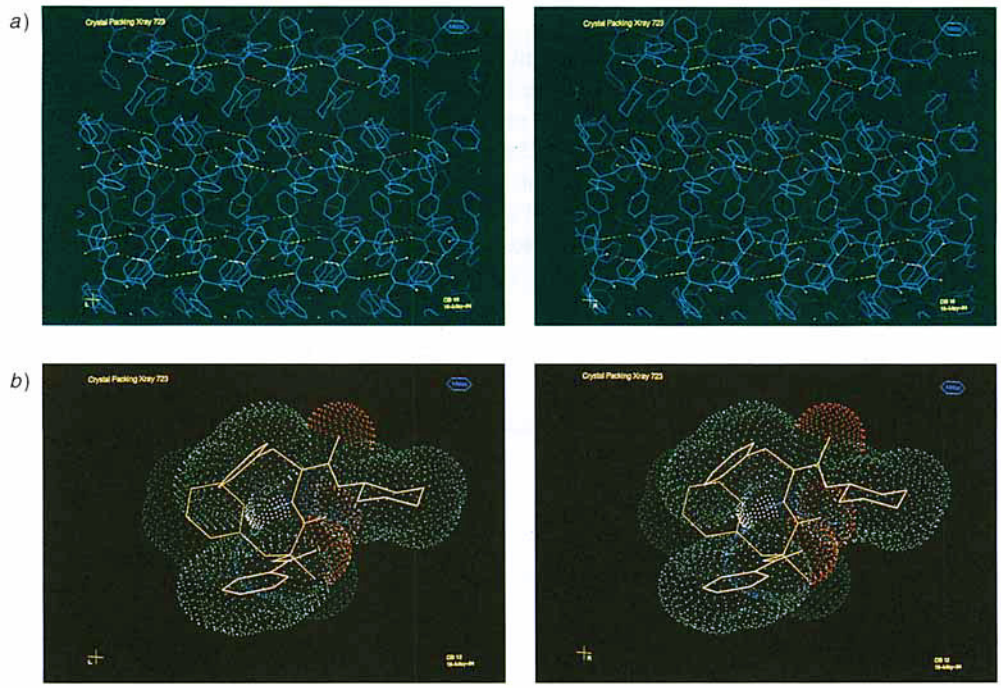


Fig. 4. *Stereoview of the crystal packing of* **7a**. a) *The peptide molecules are arranged in linear arrays with all intra- and intermolecular H-bonds* (red and yellow dotted lines) *confined within these arrays* (the linear arrays are packed through interlocking hydrophobic $PhCH_2$, PhCO, and cyclohexyl units as well as the central peptide C=O groups); b) *dotted* van der Waals *surface of one peptide molecule in the united-atom approximation* (the *van der Waals* surface of the isolated NH proton of the central peptide unit is displayed in white)

group exposed for intermolecular contacts. In this molecular conformation, the NH group of the central peptide unit is completely buried pointing towards the core of the cavity spanned by the three π-systems of the two $PhCH_2$ groups and the benzamido moiety. While π-type H-bonding between amide units and Ph groups have been noted for quite some time ([24] and ref. cit. therein), in which the NH bonds is oriented towards the center of the aromatic π-system, this type of interaction, in which the polar NH bonds lies at the edge of an essentially parallel to one or more π-electronic systems, appears not to have been described in the literature, but can occur implicity in the context of π-stacked unsaturated heteroatomic systems. If the arrangement in **7a** is energetically favorable, it could be characterized as a 'bifurcated' or even 'trifurcated π-type' H-bonding interaction by analogy to other more conventional H-bonding interactions. Conversely, however, it may also be that an inherently less favorable arrangement of the insulated peptide units is overcompensated by clustering of the bulky hydrophobic side chains, dictated by packing constraints of the crystal lattice and stabilized by extensive *van der Waals* contacts. Close examination of the crystal structure confirmed the absence of solvent molecules, but uncovered suboptimal packing of the molecules. In fact, the intramolecular cluster of aromatic moieties leaves two entry channels to the buried NH bonds (see *Fig. 6b*) which cannot be properly filled by side chains of neighboring molecules. However, these cavities, overlapping holes of *ca.* 15–25 $Å^3$, are too small to host a small solvent

molecule, such as H_2O, and would not allow a polar solvent molecule to engage in favorable interactions with its environment. Judging from the density of the crystal ($\rho = 1.15$ g/cm^3), the crystal packing is at the lower end of densities typically observed for organic crystals (*Fig. 5*), but clearly does not represent a particularly loosely packed structure. Indeed, crystals of smaller peptides typically exhibit densities in the range of 1.1–1.3 g/cm^3. A recent packing analysis of well resolved protein X-ray structures [25] uncovered cavities in virtually all proteins of more than 100 amino-acid residues. Cavities of volumes smaller than *ca.* 27 Å^3 are typically found without solvent molecules, and hydrophobic cavities are most abundantly encountered near the conformationally demanding side chains of Phe, Ile, or Leu. These consistent findings underline the potential of analyzing the crystal packing of small molecules, in particular peptides, for the understanding of the much more complex patterns of packing in globular proteins.

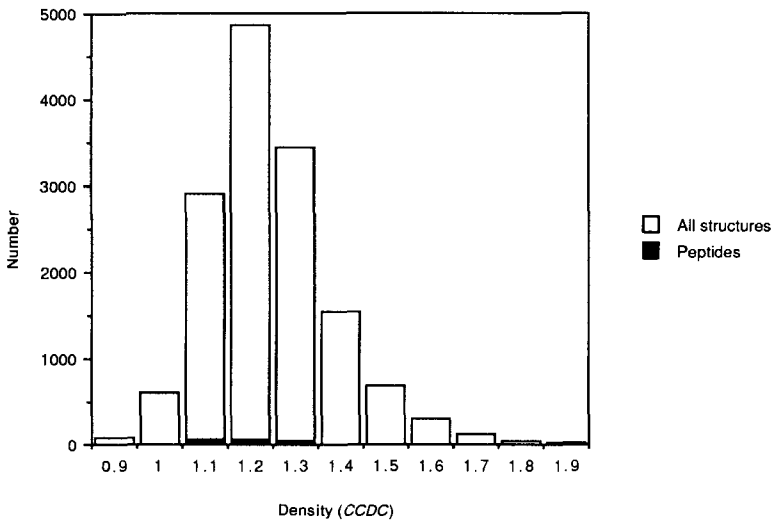


Fig. 5. *Histogram of crystal densities of organic compounds containing only C- and H-atoms, and optionally N- and O-atoms.* The densities are those given by the *Cambridge Crystal Structure Database* [21] for entries with atomic coordinates and no error flags; a few entries with densities < 0.9 g/cm^3 and > 2.0 g/cm^3 were eliminated for obvious errors in density calculations; all compounds containing a standard peptide backbone with at least three consecutive peptide units were classified as 'peptides', and their crystal-density distribution is displayed in black bars.

6. Conclusions. – Based on two crystal structures of a pair of diastereoisomeric peptides of types **6** and **7** (*Scheme 1*) [4] [14], we synthesized a series of simple secondary amides of L-phenylalanine, **5c–f**. From this series of chiral aminoamides, it turned out that L-phenylalanine cyclohexylamide (**5c**) showed excellent properties for the synthesis of optically pure disubstituted (*R*)- and (*S*)-amino acids of type **1** as outlined in *Scheme 3*. The chiral auxiliary **5c** compares very favorably with **5b** [6], which so far has been the best resolving agent in our approach [6]. In addition, **5c** is structurally simple, cheap, and can be easily synthesized on a large scale. This simple reagent opens ways to synthesize novel, structurally more complex α,α-disubstituted (*R*)- and (*S*)-amino acids of type **1** in optically pure form in a short and efficient manner. Due to their crystalline nature, the

resolved intermediate stereoisomers not only allow determination of the absolute configurations of the amino-acid building blocks by X-ray analysis, but also provide interesting insights into aspects of peptide conformation and crystal-packing interactions.

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Experimental Part

General. All reactions with air- or moisture-sensitive reactants were carried out in oven- or flame-dried glassware under a positive pressure of dry Ar. Reaction solvents and liquid reagents were purified by distillation shortly before use. CH_2Cl_2 was distilled from powdered CaH_2 and DMF over ninhydrin and kept over 4 Å molecular sieves. All other reactants were 'reagent grade' unless described otherwise. Anal. TLC: 2.5 × 10 cm precoated TLC plates, SiO_2 *60F-254*, layer thickness 0.25 mm (*E. Merck & Co.*, Darmstadt, Germany). Flash chromatography (FC): *E. Merck* SiO_2 *60* (230–400 mesh ASTM); according to [12]. M.p.: *Mel-Temp II* apparatus, *Laboratory Devices*, USA; uncorrected. IR Spectra: *Nicolet-7199-FT* spectrophotometer; solids in KBr pellets, liquids as thin films; characteristic bands in cm^{-1}. ^{1}H-NMR Spectra: *Bruker-AC-250* apparatus, at 250 MHz; $SiMe_4$ as internal standard; chemical shifts δ of signal centres and ranges in ppm, J in Hz. MS: FAB = fast-atom bombardment, ISP = ion spray.

General Methods. Method A. To a stirred mixture of 150.0 g (0.565 mol) of N^2-[(*tert*-butoxy)carbonyl]-L-phenylalanine (Boc-Phe-OH; **9a**) in AcOEt (1.5 l) cooled to −10° (CO_2/EtOH) were added *N*-methylmorpholine (62.3 ml, 0.565 mol) and isobutyl chloroformate (73.8 ml, 0.565 mol). The mixture was stirred for 30 min, then the corresponding amine (0.565 mol) was added at 0°. The mixture was allowed to come to r.t. and then stirred for 3 h. The suspension was poured onto ice (300 g), H_2O (0.5 l), the org. layer washed with cold 0.5N aq. HCl (2 × 0.5 l), sat. aq. $NaHCO_3$ soln. (2 × 0.5 l), sat. brine (2 × 0.5 l), dried ($MgSO_4$), and evaporated, and the residue purified and dried as indicated (→ **10**).

Method B. To a soln. of N^2-[(*tert*-butoxy)carbonyl]-L-phenylalanine succinimidoester (Boc-Phe-OSu; **9b**; 5.0 g, 13.8 mmol) in CH_2Cl_2 (30 ml) was added the corresponding amine (20.7 mmol) at 0°. The mixture was stirred for 1 h at 0°, slowly allowed to come to r.t., and poured onto ice (15 g)/0.5N aq. HCl (30 ml)/Et_2O (100 ml). The org. phase was washed with H_2O (2 × 30 ml) and sat. brine (2 × 30 ml), dried ($MgSO_4$), and evaporated. The residue was purified as indicated (→ **10**).

Method C. A mixture of 0.5 mol of amide **10** in THF (1 l) and AcOEt (1 l) was cooled to −30° (CO_2/EtOH) and gaseous HCl added till saturation occurred. The mixture was slowly warmed to r.t., stirred at r.t, and allowed to stay overnight under a steady stream of N_2 at r.t. The mixture was poured onto ice (200 g) and H_2O (300 ml) and the mixture brought to pH 8–9 by addition of sat. aq. K_2CO_3 soln. The aq. phase was extracted with AcOEt (2 × 750 ml), the combined org. phase washed with sat. brine (2 × 500 ml), dried ($MgSO_4$), and evaporated, and the residue purified as indicated (→ **5**).

Method D. To a soln. of 10.0 mmol of amide **10** in CH_2Cl_2 (30 ml) was added CF_3COOH (15 ml) at 0°. The mixture was stirred for 2 h at 0° and then evaporated. The residue was mixed with sat. aq. $NaHCO_3$ soln. (20 ml) and CH_2Cl_2 (50 ml), the aq. layer extracted with CH_2Cl_2 (2 × 30 ml), the combined org. phase dried ($MgSO_4$) and evaporated, and the residue purified as indicated (→ **5**).

Method E. A mixture of the 4,4-disubstituted 2-phenyl-1,3-oxazol-5(4*H*)-one of type **4** (15.0 mmol) and L-phenylalanine cyclohexylamide **5c** (4.80 g, 19.5 mmol) in *N*-methylpyrrolidin-2-one (NMP; 40 ml) was stirred under Ar for 24 h at 70°, cooled to r.t., and poured onto ice/H_2O (80 ml)/1N aq. HCl (80 ml)/AcOEt (150 ml). The org. layer was washed twice with H_2O (80 ml), the combined aq. phase extracted with AcOEt (50 ml), and the combined org. phase washed with sat. brine (100 ml), dried ($MgSO_4$), and evaporated. The residue was chromatographed on SiO_2 (1 kg), and the two diastereoisomeric peptides of type **6** and **7** were further purified as indicated.

Method F. To a stirred mixture of the diastereoisomeric peptides of type **6** or **7** (5.0 mmol) in dry MeOH (25 ml), in a pyrolysis tube, was added CF_3SO_3H (1.34 ml, 15.0 mmol) under Ar at 0°. The mixture was heated for 20 h at 80°, cooled to r.t., and evaporated. The residue was dried under reduced pressure, followed by addition of

CH_2Cl_2 (15 ml). The suspension was stirred for 30 min and filtered and the precipitate washed with CH_2Cl_2 (2 × 5 ml) and dried under reduced pressure to give 1.62–1.71 g (90–95%) of **5c**·CF_3SO_3H as a white solid, which can be reused (*Method D*). The combined filtrates were evaporated, and the residue was chromatographed (SiO_2 (180 g), AcOEt/hexane 1:2) to give the corresponding methyl esters of type **8** as indicated.

Method G. A mixture of the methyl ester of type **8** (5.0 mmol) in dioxane (10 ml) and 25% aq. HCl soln. (10 ml) was heated for 12 h at 100° in a pyrolysis tube, cooled to r.t., and poured onto H_2O/Et_2O 1:2 (30 ml). The aq. phase was washed with Et_2O (2 × 5 ml) and evaporated under reduced pressure. The residue was purified on a *Bio-Rad(50W-X8)* ion-exchange column (40 g; washed with H_2O until pH 7 and then eluted with 1% NH_4OH soln.) to yield, after drying in a desiccator over P_4O_{10} under reduced pressure, the amino acids of type **1** as white solids as indicated.

(R)-2-Methyl-2-phenylglycine ((*R*)-**1b**). From (*R*)-**8b** (1.19 g, 5.0 mmol) according to *Method G*: 750 mg (90.8%) of (*R*)-**1b**. M.p. (dec.) > 240°. $[\alpha]_D = -54.0$ (*c* = 0.2, 1N aq. HCl). IR (KBr): 3428*m* (br.), 3134*s* (br.), 3047*s* (br.), 2808*w*, 1630*m*, 1599*m*, 1499*w*, 1393*s*, 1359*m*, 1264*w*, 1230*w*, 697*w*. ^{1}H-NMR (250 MHz, DCl/D_2O): 7.65–7.5 (*m*, 5 arom. H); 5.17 (br. *s*, NH_3^+); 2.05 (*s*, Me). MS: 165 (< 1, M^+), 150 (2), 120 (100), 104 (10), 77 (10), 42 (54), 36 (54).

(S)-Isomer (S)-**1b**. From (*S*)-**9b** (1.0 g, 4.20 mmol) according to *Method G*: 630 mg (90.8%) of (*S*)-**1b**. M.p. (dec.) > 243°. $[\alpha]_D = +52.5$ (*c* = 0.2, 1N aq. HCl). MS, IR, and ^{1}H-NMR: in close agreement to those of (*R*)-**1b**.

(R)-2-Methylvaline ((*R*)-**1c**). From (*R*)-**8c** (1.67 g, 6.7 mmol) according to *Method G*: 800 mg (91%) of (*R*)-**1c**. M.p. (dec.) > 240°. $[\alpha]_D = +4.5$ (*c* = 0.2, H_2O). IR (KBr): 3425*w* (br.), 3157*m* (br.), 2972*m*, 2722*w*, 2539*w* (br.), 1609*s*, 1465*w*, 1405*s*, 1369*w*, 1317*m*, 1229*w*, 1160*w*, 1077*w*, 902*w*, 767*w*. ^{1}H-NMR (250 MHz, (D_6)DMSO): 7.25 (br. *s*, 3 NH); 1.92 (*sept.*, *J* = 6.9, Me_2CH); 1.19 (*s*, Me); 0.87, 0.86 (2*d*, *J* = 6.9, Me_2CH). MS: 132 (2, $[M + H]^+$), 88 (100), 86 (52), 70 (14), 69 (18), 43 (22), 42 (73), 41 (22).

(S)-Isomer (S)-**1c**. From (*S*)-**8c** (1.35 g, 5.41 mmol) according to *Method G*: 620 mg (87.4%) of (*S*)-**1c**. M.p. (dec.) > 240°. $[\alpha]_D = -4.5$ (*c* = 0.2, H_2O). IR, MS, and ^{1}H-NMR: in close agreement to those of (*R*)-**1c**.

L-*Phenylalanine Cyclohexylamide* (**5c**). From 254 g (0.733 mol) of **10a** according to *Method C*. Recrystallization from *t*-BuOMe and drying under reduced pressure gave 166.9 g (92.5%) of **5c**. White solid. M.p. 99–101°. $[\alpha]_D = -70.0$ (*c* = 0.2, $CHCl_3$). IR (KBr): 3297*s*, 3083*w*, 3028*w*, 2937*s*, 2853*m*, 1632*s*, 1546*s*, 1448*m*, 1389*w*, 1249*m*, 1092*w*, 746*m*, 701*s*. ^{1}H-NMR (250 MHz, $CDCl_3$): 7.45–7.2 (*m*, 5 arom. H); 7.15–6.95 (br. *m*, NH); 3.8–3.65 (*m*, NHC*H*(CH_2PH)); 3.65–3.5 (*m*, NHC*H*); 3.35–3.2, 2.8–2.65 (2*d*, *ABX*, NHCH(CH_2Ph)); 1.95–1.8 (*m*, 2 aliph. H); 1.8–1.05 (*m*, *ca.* 8 aliph. H). Anal. calc. for $C_{15}H_{22}N_2O$ (246.36): C 73.13, H 9.00, N 11.37; found: C 73.23, H 9.02, N 11.40.

From 4.50 g (13.0 mmol) of **10a** according to *Method D* and after recrystallization as described above: 3.05 g (95.2%) of **5c**. Spectral data: identical to those described above.

L-*Phenylalanine Benzylamide* (**5d**). From 5.0 g (14.1 mmol) of **10b** according to *Method D*. Recrystallization from *t*-BuOMe and drying gave 3.30 g (92%) of **5d**. White solid. M.p. 66–68°. $[\alpha]_D = -70.6$ (*c* = 0.5, $CHCl_3$). IR (KBr): 3413*w* (br.), 3302*m*, 1639*s*, 1605*w*, 1543*s*, 1453*m*, 1421*m*, 1257*w*, 1030*w*, 847*w*, 730*m*, 702*s*. ^{1}H-NMR (250 MHz, $CDCl_3$): 7.60 (br. *s*, NH); 7.45–7.1 (*m*, 10 arom. H); 4.55–4.3 (*m*, NHCH_2Ph); 3.75–3.55 (*m*, NHC*H*(CH_2Ph)); 3.4–3.25, 2.85–2.75 (2*m*, *ABX*, NHCH(CH_2Ph)); 1.33 (br. *s*, NH_2). MS: 254 (< 1, M^+), 163 (10), 120 (100), 91 (40).

L-*Phenylalanine* tert-*Butylamide* (**5e**). From 2.5 g (7.01 mmol) of **10c** according to *Method D*. Chromatography (SiO_2 (150 g), MeOH/$CHCl_3$ 1:4) and drying under reduced pressure gave 1.47 g (95%) of **5e**. Colorless oil. $[\alpha]_D = -68.0$ (*c* = 0.2, $CHCl_3$). IR (film): 3319*m* (br.), 3082*w*, 3028*w*, 2967*m*, 2927*m*, 1658*s*, 1602*w*, 1518*s*, 1453*m*, 1363*m*, 1227*m*, 741*m*, 701*m*. ^{1}H-NMR (250 MHz, $CDCl_3$): 7.4–7.2 (*m*, 5 arom. H); 7.02 (br. *s*, NH); 3.49 (*AMX*, $J_{AM} = 4.0$, $J_{AX} = 4.0$, $J_{AX} = 9.0$, NHC*H*(CH_2Ph)); 3.22 (*AMX*, $J_{MX} = 14,9$, NHCH(CH_2Ph)); 2.22 (*AMX*, NHCH(CH_2Ph)); 1.41 (br. *s*, NH_2); 1.34 (*s*, *t*-Bu).

L-*Phenylalanine Phenylamide* (**5f**). From 4.2 g (12.34 mmol) of **10d** according to *Method D*. Recrystallization from *t*-BuOMe gave 2.73 g (92%) of **5f**. White solid. M.p. 71.5–73.0°. $[\alpha]_D = -144.5$ (*c* = 0.2, $CHCl_3$). IR (KBr): 3384*m*, 3262*m*, 3058*w*, 3026*w*, 1664*s*, 1598*s*, 1517*s*, 1442*s*, 1315*w*, 883*w*, 747*m*, 695*m*. ^{1}H-NMR (250 MHz, $CDCl_3$): 9.45 (br. *s*, NH); 7.65–7.5 (*m*, 2 arom. H); 7.4–7.15 (*m*, 7 arom. H); 7.15–7.05 (*m*, 1 arom. H); 3.8–3.65 (*m*, NHC*H*(CH_2Ph)); 3.45–3.3, 2.85–2.7 (2*m*, *ABX*, NHCH(CH_2Ph)); 1.50 (br. *s*, NH_2). MS: 240 (1, M^+), 149 (11), 120 (100), 93 (20), 77 (10).

N^2-*[(S)-*N^2*-Benzoyl-2-methyl-phenylalanyl]-(S)-phenylalanine Cyclohexylamide* (**7a**) *and (R,S)-Isomer* **6a**. From *rac*-4-benzyl-4-methyl-2-phenyl-1,3-oxazol-5(4*H*)-one (**4a**; 1.12 g, 4.22 mmol) according to *Method E*. Chromatography (SiO_2 (250 g), i-PrOH/hexane 1:12→1:9) gave first, after recrystallization from AcOEt/hexane, 820 mg (38.0%) of **7a**. White solid. M.p. 221–222°. $[\alpha]_D = +2.0$ (*c* = 0.2, EtOH). IR (KBr): 3418*w*, 3296*w* (br.),

3062*w*, 3029*w*, 2983*m*, 2855*w*, 1683*s*, 1659*s*, 1633*s*, 1509*s*, 1370*w*, 745*w*, 703*m*. ^{1}H-NMR (250 MHz, (D$_6$)DMSO): 8.16 (br. *s*, NH); 7.95 (*d*, *J* = 8.0, NH); 7.9–7.8 (*m*, 2 arom. H); 7.66 (*d*, *J* = 8.0, NH); 7.6–7.45 (*m*, 3 arom. H); 7.3–7.05 (*m*, 8 arom. H); 6.85–6.7 (*m*, 2 arom. H); 4.4–4.25 (*m*, CHNH); 3.65–3.45 (br. *m*, CHNH); 3.4–3.25, 3.15–2.85 (2*m*, 2PhCH_2); 1.85–1.5 (*m*, aliph. H); 1.4–1.05 (*m*, 5 aliph. H); 1.23 (*s*, Me). ISP-MS: 534.4 (80, $[M + Na]^+$), 512.5 (100, $[M + H]^+$), 413.4 (20).

Crystallization from propane-1,3-diol afforded crystals of **7a** suitable for X-ray analysis (*cf. Table 4*; no solvent incorporated).

Further elution yielded, after crystallization from AcOEt/hexane, 850 mg (39.4%) of **6a**. M.p. 198–199°. $[\alpha]_D = +58.0$ (*c* = 0.2, EtOH). IR (KBr): 3420*w*, 3354*m*, 3317*m* (br.), 3061*w*, 2930*m*, 2852*w*, 1666*s*, 1647*s*, 1531*s*, 1495*m*, 1451*w*, 1310*w*, 751*w*, 707*m*. ^{1}H-NMR (250 MHz, (D$_6$)DMSO): 8.19 (br. *s*, NH); 7.95–7.85 (*m*, 1 NH, 2 arom. H); 7.6–7.4 (*m*, 1 NH, 3 arom. H); 7.25–7.1 (*m*, 8 arom. H); 6.95–6.85 (*m*, 2 arom. H); 4.6–4.45 (*m*, C*H*NH); 3.6–3.4 (br. *m*, C*H*NH); 3.41, 2.96 (2*d*, *AB*, J_{AB} = 13.5, C(Me)CH_2Ph); 3.3–3.15, 2.9–2.75 (2*m*, *ABX*, CH_2Ph); 1.85–1.5 (*m*, 5 aliph. H); 1.4–1.0 (*m*, 5 aliph. H); 1.02 (*s*, Me). ISP-MS: 534.5 (95, $[M + Na]^+$), 512.5 (100, $[M + H]^+$), 494.5 (10), 413.4 (25).

*N^2-[(*R*)-N^2-Benzoyl-2-methyl-2-phenylglycyl]-(*S*)-phenylalanine Cyclohexylamide* (**6b**) *and (*S,S*)-Isomer* **7b**. From *rac*-4-methyl-2,4-diphenyl-1,3-oxazol-5(4*H*)-one (**4b**; 800 mg, 3.18 mmol) according to *Method E*. Chromatography (SiO$_2$ (180 g), Et$_2$O→Et$_2$O/i-PrOH 99:1) gave first, after recrystallization from AcOEt/hexane and drying, 760 mg (48%) of **6b**. White solid. M.p. 142–144°. $[\alpha]_D = -16.0$ (*c* = 0.2, CHCl$_3$). IR (KBr): 3352*w*, 3339*m*, 3277*w*, 3060*w*, 3030*w*, 2930*m*, 2853*w*, 1651*s*, 1636*s*, 1515*s* (br.), 1447*s*, 1307*w*, 698*m*. ^{1}H-NMR (250 MHz, CDCl$_3$): 7.87 (br. *s*, NH); 7.85–7.75 (*m*, 2 arom. H); 7.6–7.25 (*m*, 8 arom. H); 7.2–7.1 (*m*, 2 arom. H); 7.0–6.9 (*m*, 2 arom. H); 6.29 (br. *d*, *J* = 8.1, NH); 6.53 (br. *d*, *J* = 8.1, NH); 4.6–4.45 (*m*, C*H*NH); 3.75–3.6 (*m*, C*H*NH); 3.1–2.95, 2.9–2.75 (2*m*, *ABX*, CH(CH_2Ph)); 2.05 (*s*, Me); 1.9–1.5 (*m*, 5 aliph. H); 1.45–0.85 (*m*, 5 aliph. H). ISP-MS: 520.4 (100, $M + Na]^+$), 498.4 (85, $[M + H]^+$), 399.3 (25).

Suitable crystals of **6b** for X-ray analysis could be grown from propane-1,3-diol without cocrystallization of this solvent (*cf. Table 4*).

Further elution yielded, after crystallization from AcOEt/hexane, 740 mg (46.8%) of **7b**. M.p. 157–158°. $[\alpha]_D = -24.5$ (*c* = 0.2, CHCl$_3$). IR (KBr): 3432*w*, 3316*m*, 3062*w*, 3028*w*, 2925*m*, 2854*w*, 1686*s*, 1641*s*, 1580*w*, 1544*m*, 1486*s*, 1448*m*, 1300*w*, 1239*w*, 1028*w*, 694*m*. ^{1}H-NMR (250 MHz, CDCl$_3$): 7.8–7.7 (*m*, 2 arom. H); 7.6–7.15 (*m*, 11 arom. H, NH); 7.15–7.05 (*m*, 2 arom. H); 6.15 (br. *d*, *J* = 8.1, NH); 6.03 (br. *d*, *J* = 8.1, NH); 4.7–4.55 (*m*, C*H*NH); 3.75–3.55 (*m*, C*H*NH); 3.2–3.0 (*m*, *ABX*, CH(CH_2Ph)); 1.92 (*s*, Me); 1.9–1.5 (*m*, 5 aliph. H); 1.4–0.9 (*m*, 5 aliph. H). ISP-MS: 520.4 (100, $[M + Na]^+$), 498.4 (79, $[M + H]^+$).

*N^2-[(*R*)-N^2-Benzoyl-2-methylvalyl]-(*S*)-phenylalanine Cyclohexylamide* (**6c**) *and (*S,S*)-Isomer* **7c**. From *rac*-4-isopropyl-4-methyl-2-phenyl-1,3-oxazol-5(4*H*)-one (**4c**; 3.5 g, 16.1 mmol) according to *Method E*. Chromatography (SiO$_2$ (1 kg), hexane/i-PrOH 10:1→7.1) gave first, after recystallization from Et$_2$O/hexane, 3.52 g (47.2%) of **6c**. White solid. M.p. 202.5–204.0°. $[\alpha]_D = -70.0$ (*c* = 0.2, CHCl$_3$). IR (KBr): 3322*m* (br.), 3062*w*, 3027*w*, 2933*m*, 2854*w*, 1667*s*, 1643*s* (br.), 1512*s*, 1450*w*, 1372*w*, 1286*w*, 1172*w*, 702*m*. ^{1}H-NMR (250 MHz, CDCl$_3$): 7.8–7.65 (*m*, 2 arom. H); 7.6–7.4 (*m*, 3 arom. H); 7.3–7.15 (*m*, 5 arom. H); 6.77 (br. *d*, *J* = 8.0, NH); 6.55–6.4 (br. *m*, 2 NH); 4.65–4.6 (*m*, NCH); 3.85–3.65 (*m*, NCH); 3.3–3.1 (*m*, PhCH_2); 2.35 (*sept.*, *J* = 6.9, Me$_2$C*H*); 2.0–1.5 (*m*, 6 aliph. H); 1.45 (*s*, Me); 1.45–1.0 (*m*, 4 aliph. H); 0.97, 0.67 (2*d*, *J* = 6.9, *Me*$_2$CH). ISP-MS: 486.5 (100, $[M + Na]^+$), 464.5 (43, $[M + H]^+$), 247.4 (70). Anal. calc. for C$_{28}$H$_{37}$N$_3$O$_3$ (463.62): C 72.54, H 8.04, N 9.06; found: C 72.27, H 8.34, N 9.11.

Crystallization from MeNO$_2$ yielded crystals of **6c** for X-ray structure determination (no solvent incorporated, *cf. Table 4*).

Further elution yielded 3.45 g (46.2%) of **7c**. M.p. 141.5–143°. $[\alpha]_D = -21.0$ (*c* = 0.2, CHCl$_3$). IR (KBr): 3422*w* (br.), 3311*m* (br.), 3062*w*, 3029*w*, 2933*m*, 2855*m*, 1645*s*, 1528*s*, 1487*m*, 1449*w*, 1287*w*, 1162*w*, 714*w*, 695*w*. ^{1}H-NMR (250 MHz, CDCl$_3$): 7.65–7.55 (*m*, 3 arom. H); 7.55–7.4 (*m*, 2 arom. H); 7.2–7.05 (*m*, 5 arom. H); 6.82 (br. *d*, *J* = 8.0, NH); 6.36 (br. *s*, NH); 6.28 (br. *d*, *J* = 8.2, NH); 4.75–4.65 (*m*, NCH); 3.85–3.65 (*m*, NCH); 3.2–3.05 (*m*, PhCH_2); 2.04 (*sept.*, *J* = 6.5, Me$_2$C*H*); 1.55–1.5 (*m*, 6 aliph. H); 1.55 (*s*, Me); 1.45–1.0 (*m*, *ca.* 4 aliph. H); 0.91, 0.81 (2*d*, *J* = 6.9, *Me*$_2$CH). ISP-MS: 486.5 (100, $[M + Na]^+$), 464.5 (50, $[M + H]^+$), 247.4 (75). Anal. calc. for C$_{28}$H$_{37}$N$_3$O$_3$ (463.62): C 72.54, H 8.04, N 9.06; found: C 72.31, H 8.25, N 9.22.

*Methyl (*R*)-N-Benzoyl-2-methyl-phenylalaninate* ((*R*)-**8a**). From **6a** (550 mg, 1.07 mmol) according to *Method F*. Recrystallization from AcOEt/hexane gave 305 mg (95.9%) of (*R*)-**8a**. M.p. 111–113°. $[\alpha]_D = +79.0$ (*c* = 0.1, CHCl$_3$). IR (KBr): 3361*s*, 3061*w*, 3035*w*, 3000*w*, 2945*w*, 1723*s*, 1654*s*, 1603*w*, 1531*s*, 1490*w*, 1375*w*, 1295*m*, 1271*m*, 1241*w*, 1117*s*, 723*m*. ^{1}H-NMR (250 MHz, CDCl$_3$): 7.65–7.5 (*m*, 2 arom. H); 7.55–7.35 (*m*, 3 arom. H); 7.25–7.15 (*m*, 3 arom. H); 7.1–7.0 (*m*, 2 arom. H); 6.81 (br. *s*, NH); 3.82 (*s*, MeO); 3.73, 3.29 (2*d*, *AB*, J_{AB} = 13.5, PhCH_2); 1.81 (*s*, Me). MS: 297 (1, $M^{+\cdot}$), 206 (22), 105 (100), 77 (31).

(S)-Isomer (S)-**8a**. From **7a** (700 mg, 1.37 mmol) according to *Method F*. Recrystallization from AcOEt/hexane gave 387 mg (95%) of (*S*)-**8a**. White solid. M.p. 111–113°. $[\alpha]_D = -78.5$ ($c = 0.2$, $CHCl_3$). IR, MS, and ^{1}H-NMR: in close agreement with those of (*R*)-**8a**.

Methyl (R)-N-*Benzoyl-2-methyl-2-phenylglycinate* ((*R*)-**8b**). From **6b** (320 mg, 0.63 mmol) according to *Method F*. Recrystallization from Et_2O/hexane gave 150 mg (99%) of (*R*)-**8b**. White solid. M.p. 114–115°. $[\alpha]_D = +23.5$ ($c = 0.2$, $CHCl_3$). IR (KBr): 3450*w* (br.), 3247*m* (br.), 3058*w*, 3000*w*, 2947*w*, 1736*s*, 1637*s*, 1600*w*, 1578*w*, 1315*m*, 1254*m*, 1122*m*, 917*w*, 722*m*, 691*m*. ^{1}H-NMR (250 MHz, $CDCl_3$): 7.9–7.8 (*m*, 2 arom. H); 7.6–7.3 (*m*, 8 arom. H, NH); 3.75 (*s*, MeO); 2.18 (*s*, Me). ISP-MS: 306.2 (100, $[M + Na]^+$), 284.2 (70, $[M + H]^+$).

(S)-Isomer (S)-**8b**. From **7b** (300 mg, 0.60 mmol) according to *Method F*. Recrystallization from Et_2O/hexane gave 136 mg (95%) of (*S*)-**8b**. White solid. M.p. 113.5–114.5°. $[\alpha]_D = -22.0$ ($c = 0.2$, $CHCl_3$). IR, MS, and ^{1}H-NMR: in close agreement with those of (*R*)-**8b**.

Methyl (R)-N-*Benzoyl-2-methylvalinate* ((*R*)-**8c**). From **6c** (3.2 g, 6.90 mmol) according to *Method F*. Drying under reduced pressure gave 1.68 g (97%) of (*R*)-**8c**. Colorless oil. $[\alpha]_D = -20.5$ ($c = 0.2$, $CHCl_3$). IR (film): 3346*w* (br.), 3061*w*, 2970*m*, 1738*s*, 1645*s*, 1589*m*, 1524*s*, 1374*m*, 1260*m*, 1144*m*, 1117*m*, 1090*w*, 692*m*. ^{1}H-NMR (250 MHz, $CDCl_3$): 7.80–7.75 (*m*, 2 arom. H); 7.55–7.4 (*m*, 3 arom. H); 6.52 (br. *s*, NH); 3.78 (*s*, MeO); 2.41 (*sept.*, $J = 6.9$, Me_2CH); 1.70 (*s*, Me); 1.06, 0.96 (2*d*, $J = 6.9$, Me_2CH). MS: 249 (< 1, $M^{+\cdot}$), 206 (12), 190 (12), 122 (10), 105 (100), 77 (38).

(S)-Isomer (S)-**8c**. From **7c** (3.0 g, 6.47 mmol) according to *Method F*. Drying under reduced pressure gave 1.55 g (96%) of (*S*)-**8c**. Colorless oil. $[\alpha]_D = +21.5$ ($c = 0.2$, $CHCl_3$). IR, MS, and ^{1}H-NMR: in close agreement with those of (*R*)-**8c**.

N^2-*[(*tert-*Butoxy)carbonyl]*-L-*phenylalanine Cyclohexylamide* (**10a**). From 145.2 g (0.547 mol) of **9a** according to *Method A*. Recrystallization from *t*-BuOMe and drying gave 128.0 g (67.5%) of **10a**. White solid. M.p. 143.0–143.5°. $[\alpha]_D = +5{,}0$ ($c = 0.2$, $CHCl_3$). IR (KBr): 3343*m*, 3315*m*, 3063*w*, 2933*m*, 2855*w*, 1689*s*, 1649*s*, 1539*s*, 1449*w*, 1365*w*, 1248*w*, 1171*m*, 699*w*. ^{1}H-NMR (250 MHz, $CDCl_3$): 7.35–7.15 (*m*, 5 arom. H); 5.41, 5.13 (2 br. *s*, 2 NH); 4.3–4.15 (*m*, NHC*H*(CH_2Ph)); 3.75–3.6 (*m*, C*H*NH); 3.15–3.05, 3.05–2.9 (2*m*, *ABX*, NHCH(CH_2Ph)); 1.9–1.5, 1.4–0.8 (2*m*, *ca.* 10 aliph. H); 1.42 (*s*, *t*-Bu). MS: 346 (< 1, $M^{+\cdot}$), 290 (4), 273 (4), 164 (2), 120 (56), 91 (15), 83 (15), 57 (100), 41 (28).

From 5.0 g (13.80 mmol) of **9b** according to *Method B*: 4.41 g (92.2%) of **10a**. M.p. 143–143.5°. Spectra: in close agreement with those described above.

N^2-*[(*tert-*Butoxy)carbonyl]*-L-*phenylalanine* tert-*Butylamide* (**10c**). From 5.0 g (13.80 mmol) of **9b** according to *Method B*. The residue was suspended in Et_2O/hexane overnight, filtered, and dried: 4.2 g (95%) of **10c**. White solid. M.p. 131–133°. $[\alpha]_D = +6.0$ ($c = 0.2$, $CHCl_3$). IR (KBr): 3312*m* (br.), 3064*w*, 2974*w*, 2931*w*, 1689*s*, 1657*s*, 1537*m* (br.), 1454*w*, 1250*w*, 1173*m*, 698*w*. ^{1}H-NMR (250 MHz, $CDCl_3$): 7.35–7.15 (*m*, 5 arom. H); 5.22 (br. *s*, 2NH); 4.2–4.05 (*m*, NHC*H*(CH_2Ph)); 3.2–3.05, 3.0–2.8 (2*m*, *ABX*, NHCH(CH_2Ph)); 1.43 (*s*, (*t*-Bu)N); 1.20 (*s*, *t*-Bu). FAB-MS: 343.5 (20, $[M + Na]^+$), 321.5 (35, $[M + H]^+$), 265.4 (50), 221.4 (100), 165.2 (35).

N^2-*[(*tert-*Butoxy)carbonyl]*-L-*phenylalanine Phenylamide* (**10d**). From 5.0 g (13.80 mmol) of **9b** according to *Method B*. Recrystallization from Et_2O/hexane afforded, after drying under reduced pressure, 4.38 g (93.2%) of **10d**. White solid. M.p. 126–128°. $[\alpha]_D = -20.5$ ($c = 0.2$, $CHCl_3$). IR (KBr): 3417*w* (br.), 3304*m*, 3114*w*, 3063*w*, 2979*w*, 2931*w*, 1691*m* (br.), 1666*s*, 1602*m*, 1546*m*, 1497*m*, 1443*m*, 1367*m*, 1249*m*, 1169*m*, 764*m*, 696*w*. ^{1}H-NMR (250 MHz, $CDCl_3$): 7.66 (br. *s*, NH); 7.4–7.2 (*m*, 9 arom. H); 7.15 7.05 (br. *m*, 1 arom. H); 5.12 (br. *s*, NH); 4.55–4.35 (*m*, NHC*H*(CH_2)Ph); 3.15 ('*d*', $J = 7.1$, NHCH(CH_2)Ph); 1.43 (*s*, *t*-Bu). ISP-MS: 363.0 (35, $[M + Na]^+$), 341.1 (35, $[M + H]^+$), 285.1 (70), 263.0 (25), 241.1 (100).

N^2-*[(*tert-*Butoxy)carbonyl]*-L-*phenylalanine Benzylamide* (**10b**). From 5.0 g (13.8 mmol) of **9b** according to *Method B*. Recrystallization from *t*-BuOMe gave, after drying under reduced pressure, 4.50 g (92%) of **10b**. White solid. M.p. 132–133°. $[\alpha]_D = +6.0$ ($c = 0.2$, $CHCl_3$). IR (KBr): 3420*w* (br.), 3334*s*, 3300*s*, 3063*w*, 3028*w*, 2981*w*, 1682*s*, 1658*s*, 1524*s*, 1454*w*, 1295*m*, 1241*m*, 1170*m*, 742*w*, 698*m*. ^{1}H-NMR (250 MHz, $CDCl_3$): 7.35–7.15 (*m*, 8 arom. H); 7.15–7.05 (*m*, 2 arom. H); 6.05 (br. *s*, NH); 5.04 (br. *s*, NH); 4.4–4.3 (*m*, NHC*H*(CH_2)Ph); 4.36 ('*d*', $J = 5.6$, NHCH_2Ph); 3.2–3.0 (*m*, NHCH(CH_2Ph)); 1.39 (*s*, *t*-Bu). MS: 354 (< 1, $M^{+\cdot}$), 298 (6), 281 (4), 164 (30), 120 (60), 91 (62), 57 (100).

Pentafluorophenyl (S)-N^2-*[(Benzyloxy)carbonyl]*-2-*methyl*-2-*phenylglycinate* ((*S*)-**12b**) via *(R)*-N^2-*[(Benzyloxy)carbonyl]*-2-*methyl*-2-*phenylglycine* ((*S*)-**11b**). To a stirred soln. of 517 mg (2.85 mmol) of (*S*)-**1b** in CH_2Cl_2 (12 ml) was added under Ar at 0° Me_3SiCl (0.91 ml, 7.14 mmol). The mixture was heated for 1 h at 50° and then cooled to 0°, 1.32 ml (7.70 mmol) of (i-Pr)$_2$NEt and 0.52 ml (3.71 mmol) of benzyl chloroformate were added, and the mixture was stirred for 17 h at r.t. and then poured onto ice/H_2O/AcOEt. The org. layer was washed with sat. brine (2 × 15 ml), dried ($MgSO_4$), and evaporated, and the residue chromatographed (SiO_2 (50 g), hexane/AcOEt 9:3:1) to yield, after drying under reduced pressure, 700 mg (82%) of (*S*)-**11b** as an amorphous solid, which was not

further purified. $[\alpha]_D = +38.5$ ($c = 0.2$, MeOH). To a stirred mixture of 650 mg (2.17 mmol) of (*S*)-**11b** and 520 mg (2.82 mmol) of pentafluorophenol in CH_2Cl_2 (10 ml) were added 541 mg (2.82 mmol) of *N*-ethyl-*N'*-[3-(dimethylamino)propyl]carbodiimide (EDCI)·HCl under Ar and ice-bath cooling in small portions. The mixture was stirred for 30 min at 0° and for 6 h at r.t. and then poured onto ice/H_2O/AcOEt. The org. layer was washed with sat. brine, dried ($MgSO_4$), and evaporated, and the residue chromatographed (SiO_2 (700 g), hexane/AcOEt 8:1) to yield, after drying under reduced pressure, 858 mg (85%) of (*S*)-**12b**. Slightly yellow oil. $[\alpha]_D = +19.0$ ($c = 0.2$, $CHCl_3$). IR (film): 3043*w*, 3335*w*, 3043*w*, 2945*w*, 1787*s*, 1717*s*, 1552*s*, 1451*m*, 1261*s*, 1214*m*, 1062*s*, 996*s*, 697*m*. ^{1}H-NMR (250 MHz, $CDCl_3$): 7.6–7.5 (*m*, 2 arom. H); 7.5–7.25 (*m*, 8 arom. H); 5.86 (br. *s*, NH); 5.15, 5.09 (2*d*, *AB*, $J_{AB} = 12.9$, PhCH_2O); 2.19 (*s*, Me). ISP-MS: 483.3 (100, $[M + NH_4]^+$), 466.3 (50, $[M + H]^+$), 405.2 (10).

*(R)-Isomer (R)-***12b**. From (*R*)-**1b** (576 mg, 3.18 mmol) according to the procedure described for (*S*)-**12b**: 1.11 g (75%) of (*R*)-**12b**. Colorless oil. $[\alpha]_D = -16.5$ ($c = 0.2$, $CHCl_3$). IR, MS, and ^{1}H-NMR: in close agreement with those of (*S*)-**12b**.

(S)-N^2-[(9H-Fluoren-9-yl)methoxycarbonyl]-2-methylvaline ((*S*)-**11c**). To a stirred suspension of (*S*)-**1c** (500 mg, 3.81 mmol) in CH_2Cl_2 (10 ml) was added under Ar at r.t. Me_3SiCl (1.20 ml, 9.53 mmol).The mixture was stirred for 1 h at 60° (→clear soln.) and then cooled to r.t. After addition of (i-Pr)$_2$NEt (1.43 ml, 8.4 mmol) and Fmoc-Cl (1.18 g, 4.57 mmol), the mixture was stirred for 2 h at 50°, cooled, and poured onto ice, sat. aq. $NaHCO_3$ soln. (15 ml), and Et_2O (20 ml). The aq. layer was extracted with Et_2O (2 × 10 ml), the combined org. phase extracted with sat. aq. $NaHCO_3$ soln., the combined aq. phase acidified carefully to pH 3 with 1N aq. HCl and extracted with Et_2O (3 × 15 ml). The combined org. fraction was dried ($MgSO_4$) and evaporated and the residue chromatographed (SiO_2 (120 g), AcOEt/hexane 1:1) to yield, after drying under reduced pressure, 960 mg (71.1%) of (*S*)-**11c**. White powder. $[\alpha]_D = +15.0$ ($c = 0.2$, $CHCl_3$). IR (KBr): 3414*w* (br.), 3067*w*, 3041*w*, 2970*w*, 1712*s*, 1507*m*, 1448*m*, 1341*w*, 1252*m*, 1107*w*, 1078*w*, 740*m*. ^{1}H-NMR (250 MHz, $CDCl_3$): 7.85–7.7 (*m*, 2 arom. H); 7.65–7.55 (*m*, 2 arom. H); 7.45–7.25 (*m*, 4 arom. H); 5.29 (br. *s*, NH); 4.55–4.3 (br. *m*, $CHCH_2$); 4.25–4.15 (*m*, $CHCH_2$); 2.4–2.15 (*m*, Me_2CH); 1.52 (*s*, Me); 1.05–0.8 (*m*, Me_2CH). MS: 196 (21), 178 (37), 166 (68), 165 (100), 115 (36), 87 (14), 43 (19), 42 (53), 41 (17).

Succinimido (R)-N^2-[(9H-Fluoren-9-yl)methoxycarbonyl]-2-methylvalinate ((*R*)-**12c**). To a stirred suspension of (*R*)-**1c** (500 mg, 3.81 mmol) in CH_2Cl_2 (10 ml) was added under Ar Me_3SiCl (1.2 ml, 9.53 mmol). The mixture was stirred for 1 h at 60° and then cooled to r.t. After addition of (i-Pr)$_2$NEt (1.43 ml, 8.40 mmol) and Fmoc-Cl (1.18 g, 4.57 mmol), the mixture was stirred for 2 h at 50°, cooled to r.t., and poured onto ice/0.1N aq. HCl (20 ml)/AcOEt (40 ml). The org. layer was extracted with sat. brine (25 ml), dried ($MgSO_4$), and evaporated. The residue was dried under reduced pressure overnight, dissolved in CH_2Cl_2 (10 ml), and treated under Ar with *N*-hydroxysuccinimide (658 mg, 5.72 mmol) and *N,N'*-dicyclohexylcarbodiimide (DCC; 865 mg, 4.19 mmol). The mixture was stirred for 18 h at r.t. and then filtered, the filter cake washed with CH_2Cl_2, the combined org. phase evaporated, and the residue chromatographed (SiO_2 (120 g), Et_2O/hexane 1:10→1:5) to yield, after drying under reduced pressure, 1.50 g (87.4%) of (*R*)-**12**. White solid. $[\alpha]_D = +10.5$ ($c = 0.2$, $CHCl_3$). IR (KBr): 3540*w*, 3405*w*, 3205*w* (br.), 3043*w*, 2964*w*, 1810*w*, 1783*m*, 1738*s*, 1661*w*, 1550*w*, 1281*m*, 1203*s*, 1055*m*, 763*w*, 736*w*. ^{1}H-NMR (250 MHz, $CDCl_3$): 7.8–7.7 (*m*, 2 arom. H); 7.65–7.55 (*m*, 2 arom. H); 7.45–7.25 (*m*, 4 arom. H); 5.20 (br. *s*, NH); 4.6–4.2 (*m*, $CHCH_2$); 2.80 (*s*, 4 aliph. H); 2.4–2.15 (br. *m*, Me_2CH); 1.67 (br. *s*, Me); 1.09, 1.06 (*d*, $J = 6.7$, Me_2CH). ISP-MS: 473.2 (45, $[M + Na]^+$), 468.6 (100, $[M + NH_4]^+$), 451.4 (35, $[M + H]^+$).

REFERENCES

[1] B. di Blasio, V. Pavone, A. Lombardi, C. Pedone, E. Benedetti, *Biopolymers* **1993,** *33*, 1037.

[2] B. V. V. Prasad, P. Balaram, *CRC Crit. Rev. Biochem.* **1984,** *16*, 307; I. L. Karle, P. Balaram, *ibid.* **1990,** *29*, 6747.

[3] P. Wipf, Ph. D. Thesis, University of Zürich, 1987.

[4] C. Spiegler, Ph. D. Thesis, University of Zürich, 1993.

[5] I. L. Karle, P. Balaram, *Biochemistry* **1990,** *29*, 6747; M. Mutter, *Angew. Chem.* **1985,** *97*, 639.

[6] D. Obrecht, C. Spiegler, P. Schönholzer, K. Müller, H. Heimgartner, F. Stierli, *Helv. Chim. Acta* **1992,** *75*, 1666.

[7] H. R. Almond, D. T. Manning, C. Niemann, *Biochemistry* **1962,** *1*, 233; M. Pankaskie, M. Abdel-Monem, *J. Med. Chem.* **1980,** *23*, 121.

[8] E. Ware, *Chem. Rev.* **1950,** *46*, 403; L. H. Goodson, I. L. Honigberg, J. J. Lehmann, W. H. Burton, *J. Org. Chem.* **1960,** *25*, 1920; S. N. Rastogi, J. S. Bindra, N. Anand, *Ind. J. Chem.* **1971,** 1175.

[9] B. Kübel, P. Gruber, H. Hurnaus, W. Steglich, *Chem. Ber.* **1979,** *112*, 128.

[10] D. Obrecht, H. Heimgartner, *Tetrahedron Lett.* **1984,** 1717.
[11] D. Obrecht, U. Bohdal, R. Ruffieux, K. Müller, *Helv. Chim. Acta* **1994,** *77,* 1423.
[12] W. C. Still, M. Kahn, A. Mitra, *J. Org. Chem.* **1978,** *43,* 2923.
[13] G. D. Rose, L. M. Gierasch, J. A. Smith, *Adv. Prot. Chem.* **1985,** *37,* 1–109.
[14] C. Spiegler, D. Obrecht, P. Schönholzer, J. J. Daly, K. Müller, in 3rd Naples Workshop on Bioactive Peptides, Capri, Italy, May 24–27, 1992, p. 34 (Poster C4).
[15] G. W. Anderson, J. F. Zimmermann, F. M. Callahan, *J. Am. Chem. Soc.* **1967,** *89,* 5012.
[16] S. F. Brady, R. M. Freidinger, W. J. Paleveda, C. D. Colton, C. F. Homnick, W. L. Wilter, P. Curley, R. F. Nutt, D. F. Veber, *J. Org. Chem.* **1987,** *52,* 764.
[17] H. Kricheldorf, *Liebigs Ann. Chem.* **1972,** *763,* 17; K. H. Altmann, E. Altmann, M. Mutter, *Helv. Chim. Acta* **1992,** *75,* 1198.
[18] U. Schmidt, H. Griesser, A. Lieberknecht, J. Hausler, *Angew. Chem. Int. Ed.* **1981,** *20,* 281; U. Schmidt, H. Griesser, A. Lieberknecht, J. Talbiersky, *ibid.* **1981,** *20,* 280; R. J. Heffner, J. Jiang, M. M. Joullie, *J. Am. Chem. Soc.* **1992,** *114,* 10181.
[19] IUPAC-IUB Commission on Biochemical Nomenclature, *Biochemistry* **1970,** *9,* 3471.
[20] C. M. Wilmot, J. M. Thornton, *J. Mol. Biol.* **1988,** *203,* 221; *Protein Eng.* **1990,** *3,* 479.
[21] Cambridge Structural Database, Version 5.06, October 1993.
[22] S. H. Gellman, D. R. Powell, J. M. Desper, *Tetrahedron Lett.* **1992,** *33,* 1963.
[23] H. Schrauber, F. Eisenhaber, P. Argos, *J. Mol. Biol.* **1993,** *230,* 592.
[24] J. B. O. Mitchell, C. L. Nandi, I. K. McDonald, J. M. Thornton, *J. Mol. Biol.* **1994,** *239,* 315.
[25] S. J. Hubbard, K.-H. Gross, P. Argos, *Protein Engineering* **1994,** *7,* 613.