97. Synthesis of Unnatural Lipophilic *N*-(9*H*-Fluoren-9-ylmethoxy)carbonyl-Substituted α-Amino Acids and Their Incorporation into Cyclic RGD-Peptides: A Structure-Activity Study

by **Marcus Koppitz**, **Martin Huenges**, **Rainer Gratias**, and **Horst Kessler***

Institut für Organische Chemie and Biochemie, Technische Universität München, Lichtenbergstr. 4, D-85747 Garching

and **Simon L. Goodman** and **Alfred Jonczyk**

Merck KGaA Preclinical Research, Frankfurter Str. 250, D-64271 Darmstadt

Dedicated to *Peter Welzel* on the occasion of his 60th birthday

(19.XII.96)

The $\alpha_v\beta_3$ integrin is implicated in human tumor metastasis and angiogenesis. It has been shown that structures of the sequence cyclo(-Arg^1-Gly^2-Asp^3-D-Phe^4-Xaa^5-) (**I**) and cyclo(-Arg^1-Gly^2-Asp^3-Phe^4-D-Xaa^5-) (**II**) bind with high affinity and the latter with high selectivity to this receptor. The residues Xaa and D-Xaa accept a broad variety of amino acids. Here, we report on the synthesis, activities, and conformational analysis of cyclic Arg-Gly-Asp (RGD) peptides containing lipophilic amino acids Xaa or D-Xaa in position 5. For **I**, these were (2*S*)-2-aminohexadecanoic acid (Ahd) and *N*-hexadecylglycine (Hd-Gly) and in **II**, D-Ahd and Hd-Gly, and, for control purposes, Ahd were incorporated (*Fig. 1*). The enantiomerically pure α-amino acids were obtained by non-enantioselective synthesis and subsequent enzymatic separation of isomers using acylase I (*Scheme*). Hd-Gly was prepared in a modified procedure according to *Stewart* from ethyl bromoacetate and hexadecylamine (*Scheme*). The synthesis and physicochemical properties of the corresponding (9*H*-fluoren-9-ylmethoxy)carbonyl (Fmoc) derivatives, compatible with solid-phase peptide synthesis, are described. Structure elucidation by NMR reveals that the lipid modification has no significant impact on the template structures when incorporated into them. For peptides **I** with Xaa = Ahd or Hd-Gly (**1** or **2**), a βII′/γ-turn-like arrangement with D-Phe in $i+1$ position of the β-turn is found. Peptides **II** with D-Xaa = D-Ahd or Hd-Gly (**3** or **4**) exhibit a βII′/γ-turn conformation with Gly in $i+1$ position of the β-turn, whereas **II** with Ahd instead of D-Xaa, *i.e.*, lacking a D-amino acid in position 4 or 5 (**5**), adopts no defined conformation. However, in assays of receptor specificity employing human $\alpha_v\beta_3$ integrin, the compounds exhibit IC_{50} values ranging from nanomolar to less than millimolar. These results indicate that although the arrangement of the pharmacophoric groups is preserved in the target compounds, the biological activity is highly dependent on spatial requirements of the lipid anchor in the receptor binding pocket. Obviously, only certain positions do not affect the binding.

1. Introduction[1]. – The development of a drug up to the stage of a marketable commodity often fails due to the insufficient bioavailability of the products. Although the substances may be highly active in isolated cell systems, the *in vivo* resorption of orally applied substances may be low and, therefore, they may be ruled out as potential drugs.

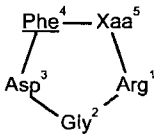
[1]) Abbreviations: Ade, (2*S*)-2-aminodecanoic acid; Ahd, (2*S*)-2-aminohexadecanoic acid; Fmoc, (9*H*-fluoren-9-ylmethoxy)carbonyl; HOBt, 1-hydroxy-1*H*-benzotriazole; HdGly, *N*-hexadecylglycine; Pam, {[4-(hydroxymethyl)phenyl]acetamido}methyl; Pbf, 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl; ROE, rotating-frame nuclear *Overhauser* effect; TBTU, *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate.

To improve their uptake, the strategy of lipoderivatization was introduced [1–3]. The conjugation of the active structures with lipophilic building blocks can significantly improve the pharmacokinetics by facilitating their passage through epithelial membranes, and may even be indispensable where the binding of the messenger molecule to a membrane-bound receptor is preceded by an interdigitation into cell membranes. To increase the bioavailability by lipoderivatization, many different modifications have been applied: conjugation with fatty acids [4]; incorporation of long-chain alcohols, Pam_3CysOH [5] or 1,2-dimyristoyl-3-mercaptoglycerol [6], and lipophilic amino acids [1]. The major advantage of lipophilic amino acids in comparison to many other modifications is the freedom of choice of the incorporation site when used for peptidic drugs. The bifunctionality of these building blocks imposes no restriction to the termini of the peptides and also allows the generation of cyclic structures.

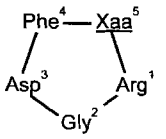
The interaction of cells with each other or with the extracellular matrix is controlled by adhesion phenomena involving integral cell surface adhesion receptors [7]. Integrins are one of the four major classes of surface receptors, and about one half of the known integrins bind to Arg-Gly-Asp as recognition sequence [8–10]. At least two subtypes of the integrin family, the fibrinogen and the vitronectin receptors, are involved in many pathological processes including osteoporosis, metastasis, and tumor-induced angiogenesis [11–14]. Therefore, a therapy involving blockade of integrin ligand-binding sites using synthetic compounds is conceivable. Recent results indicate that our designed cyclic peptide cyclo(-Arg^1-Gly^2-Asp^3-D-Phe^4-Val^5-) (**6**) containing the -Arg-Gly-Asp- moiety [15] suppresses tumor-induced angiogenesis in a chick chorioallantoic membrane (CAM) model [16] [17] and growth-factor-induced angiogenesis [18] and retinal neovascularisation in a hypoxic mouse model [19]. Therefore, therapy with cyclic Arg-Gly-Asp peptides appears promising. The binding affinity of the vitronectin receptor is very sensitive to the conformation of the Arg-Gly-Asp sequence. A spatial screening [20] of the presentation of the Arg-Gly-Asp sequence in cyclic hexa- and pentapeptides by variation of the flanking residues identified two structures with high activity for the vitronectin receptor [15]. Cyclo(-Arg^1-Gly^2-Asp^3-D-Phe^4-Xaa^5-) (**I**) and cyclo(-Arg^1-Gly^2-Asp^3-Phe^4-D-Xaa^5-) (**II**) both inhibit the receptor at nanomolar concentrations [21–24] (see *Fig. 1*). The amino acid in position 5 accepts a broad variety of hydrophilic and hydrophobic residues [24] and, consequently, represents an ideal position for the lipoderivatization of the peptides.

To investigate if highly active lipid-modified Arg-Gly-Asp peptides are accessible, we introduced two different unnatural amino acids into **I** and **II**. For the D-Phe-Xaa lead structure **I**, we incorporated (2*S*)-2-aminohexadecanoic acid (Ahd, → **1**) and the achiral amino acid *N*-hexadecylglycine (Hd-Gly, → **2**). For the Phe-D-Xaa template **II**, we incorporated D-Ahd (→ **3**) and Hd-Gly (→ **4**). In addition, an all-L-peptide **5** with Phe and Ahd was synthesized as control. In this study, we report on the synthesis of lipophilic *N*-(9*H*-fluoren-9-ylmethoxy)carbonyl(Fmoc)-substituted amino acids compatible with solid-phase peptide synthesis including their physicochemical properties, and the synthesis of lipid-modified cyclic Arg-Gly-Asp peptides, the biological activity of these peptides and, their structural analysis. A model for the structure-activity relationship is proposed.

2. Results. – 2.1. *Syntheses. (2*S*)- and (2*R*)-2-Aminodecanoic Acid* (Ade and D-Ade, resp.) *and (2*S*)- and (2*R*)-2-Aminohexadecanoic Acid* (Ahd and D-Ahd, resp.). The



I



II

Gly-Arg-Gly-Asp-Ser-Pro-Lys

III

1 Xaa = Ahd
2 Xaa = Hd-Gly
3 L-Xaa = L-Ahd
4 D-Xaa = Hd-Gly
5 D-Xaa = Ahd (L)
6 Xaa = Val [15]
7 Xaa = Lys
8 D-Xaa = D-Lys
9 D-Xaa = Val (L)
10 D-Xaa = D-Val

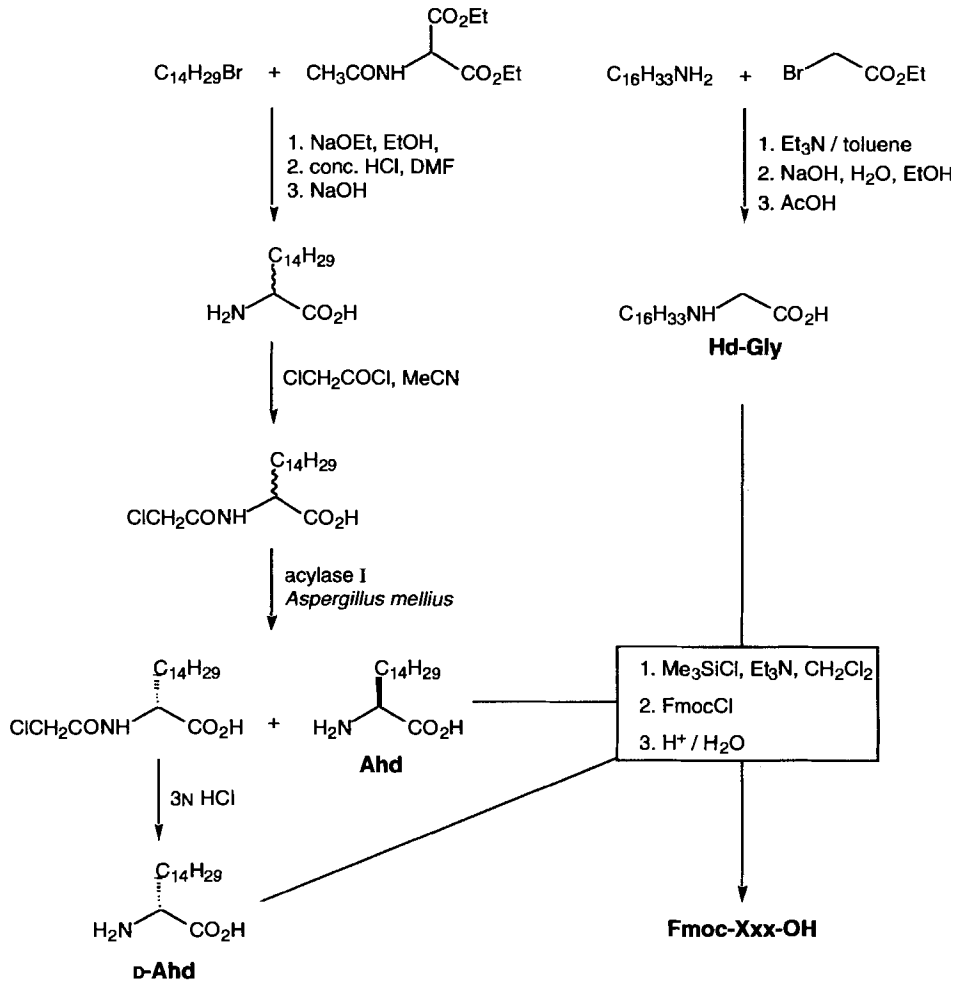
Fig. 1. *Designed target compounds, derived from lead structures c(RGDEX)* (**I**) *and c(RGDFX)* (**II**), *and standard* **III**. Hd-Gly is achiral and can substitute for L- and D-amino acids, underlined amino acids in **I** and **II** are in the D-configuration.

enantiomerically pure forms Ahd, D-Ahd, Ade, and D-Ade were obtained by non-enantioselective synthesis and subsequent separation of the racemate (*cf. Scheme*). Ade was not incorporated in the Arg-Gly-Asp peptides. According to a procedure of *Albertson*, the treatment of diethyl acetamidomalonate with 1-bromooctane or 1-bromotetradecane in the presence of NaOEt quantitatively yielded the corresponding alkyl dicarboxylates [25]. The subsequent hydrolysis and decarboxylation to the long-chain α-amino acids was performed by refluxing the crude diesters with conc. HCl solution in the presence of solubilizing DMF [26]. Neutralization with NaOH furnished the racemic amino acids. The separation of the enantiomers was performed enzymatically. Thus, the racemic amino acids were acetylated with chloroacetyl chloride in the absence of base and subsequently treated with acylase I [27]. Although the upper limit of the chain length for the enzymatic cleavage of aliphatic *N*-acetyl-L-amino acids is considered to be twelve C-atoms according to [28], we were able to obtain the enantiomerically pure Ahd (C_{16}) using acylase I from *Aspergillus mellius*, whereas acylase I from porcine kidney proved to be inactive. For quantitative yields, however, the reaction had to be performed in highly diluted solution, with extended reaction times (*ca.* 1 week) and with a large amount of the readily available and cheap enzyme. After filtration of the precipitated L-isomers Ahd and Ade, the D-isomers D-Ahd and D-Ade, respectively, were obtained by deacetylation with 3N HCl.

N-*Hexadecylglycine* (Hd-Gly). For the synthesis of Hd-Gly, we decided to react hexadecylamine with ethyl bromoacetate according to a procedure of *Stewart* [29], because this strategy promised reasonably yields without presenting the typical solubility problems (*cf. Scheme*). The reaction of ethyl bromoacetate with hexadecylamine was performed in a modified procedure in toluene under cooling with ice, which led to a significant increase in yield of the *N*-alkylated glycine ester (41 *vs.* 15%). Subsequent saponification with NaOH furnished the target compound with 95% yield ([29]: 74%).

Lipophilic Fmoc-amino Acids. Although a procedure for the preparation of the (*tert*-butoxy)carbonyl (Boc) derivatives of lipophilic amino acids has already been described, the corresponding Fmoc derivatives, which would allow the solid-phase synthesis of

Scheme. *Preparation of Lipophilic Fmoc-amino Acids.* The synthesis of Ade and D-Ade was carried out analogous to Ahd and D-Ahd using octyl bromide.



peptides according to the currently prevailing strategy, are still unknown. A major obstacle in the derivatization of these highly lipophilic, zwitterionic compounds is their very low solubility in any solvent. Nearly all reported strategies apply a one-step procedure using different acylating Fmoc reagents like chloride [30–33], azidoformate [34], tetrachloroethyl carbonate [35], norbornenedicarboximido-carbonate [36], and succinimidocarbonate [37] [38] to introduce the Fmoc moiety. However, the restriction to low temperatures (Fmoc lability) and the insufficient activity of these reagents make them unsuitable for lipophilic amino acids. We, therefore, used a two-step procedure with an intermediate bis-silylation [39] [40]. This procedure for the generation of protected amino acids was initially introduced by *Theodoropoulos* and coworkers for the trityl group [41] and later on developed as a general method to introduce protecting groups of the

urethane type. *Bolin* and coworkers were the first to apply this method to the synthesis of Fmoc-amino acids to prevent the formation of oligomers [42]. According to their procedure, we refluxed the lipophilic amino acids with 2 equiv. of Me_3SiCl and Et_3N in CH_2Cl_2, with an extended reaction time of 1 h to guarantee quantitative silylation. After cooling to room temperature, the products were obtained by reaction with Fmoc-Cl for 12 h and subsequent hydrolysis of the silyl ester (*cf. Scheme*). Extraction with CH_2Cl_2 and recrystallization from CH_2Cl_2/hexane furnished the very soluble Fmoc derivatives in 60–80% yields.

Lipophilic Cyclic Arg-Gly-Asp Peptides. The synthesis of the cyclic pentapeptides was carried out manually by the Fmoc-based [43] [44] *Merrifield* solid-phase method [45] [46] using a 2-chlorotrityl-chloride resin. After attachment of the first amino acid following the published procedure [47] (resin loading 0.9 mmol/g), the assembly of the peptides was continued by standard procedures using 2.5 equiv. of Fmoc-amino acid and TBTU/HOBt[1]) as activating system in 1-methylpyrolidin-2-one. Treatment with AcOH/CF_3CH_2OH in CH_2Cl_2 yielded the linear precursors without affecting the orthogonally cleavable *t*-Bu or Pbf[1]) side chain protecting groups of Asp or Arg, respectively. This allowed the subsequent cyclization with diphenylphosphoryl azide (DPPA) in presence of $NaHCO_3$ according to the solid-base strategy [48]. Deprotection with reagent K (see *Exper. Part*) for 2 h at room temperature yielded the crude cyclic compounds, which were finally purified by reversed-phase HPLC. The elution behavior of the lipid-modified compounds on HPLC proved to be very sensitive to the applied gradient, as the use of two flat gradients resulted in extreme peak broadening. Coupling and cyclization was successful in all cases, including the peptides with Hd-Gly with its sterically hindered secondary amino function, resulting in overall yields between 15 and 50% after HPLC.

2.2. *Biological Activities.* The tests were performed on microtiter plates coated with purified integrin receptors $\alpha_V\beta_3$ or $\alpha_{IIb}\beta_3$. The synthesized compounds were assayed by monitoring their ability to compete for binding of the soluble, biotinylated ligands vitronectin or fibrinogen. All measurements were performed using Gly-Arg-Gly-Asp-Ser-Pro-Lys (**III**) as external standard [24]. *Table 1* lists the observed biological activities as IC_{50} values as well as the relative IC_{50} values Q referring to the external standard ($Q = IC_{50}$(peptide)/IC_{50}(**III**)).

Although, with the exception of **5**, the compounds were designed according to the previously described lead structures, biological activities with IC_{50} values ranging from millimolar to nanomolar values were found. Except for **3**, a higher inhibitory activity on

Table 1. *Inhibition of Fibrinogen Binding to $\alpha_{IIb}\beta_3$ and Vitronectin Binding to $\alpha_V\beta_3$ by Lipid-Modified Arg-Gly-Asp-Peptides. $Q = IC_{50}$(peptide)/IC_{50}(**III**).*

	$\alpha_{IIb}\beta_3$ (Fb)		$\alpha_V\beta_3$ (Vn)	
	IC_{50} [nM]	Q	IC_{50} [nM]	Q
1	>10000	>10	3000	9.0
2	>10000	>10	>10000	>25
3	2000	2.0	500	1.5
4	2000	2.0	8.0	0.02
5	>10000	>10	>10000	>25
6	830	5	2.2	0.006

the $\alpha_V\beta_3$ receptor compared to $\alpha_{IIb}\beta_3$ is observed; this can be defined as the selectivity of cyclic pentapeptides for this receptor subtype. The weakest activity is found in the all-L-compound **5** lacking a structure-inducing amino acid in position 4 or 5. Both **1** and **3** containing a lipophilic α-amino acid exhibit IC_{50} values in the millimolar range. Although their affinity to $\alpha_V\beta_3$ still lies in the range of the linear reference peptide **III** which lacks any conformational control, they are *ca.* two orders of magnitude weaker than the cyclic lead peptides. A striking feature of **3** is its substantial loss of selectivity towards the $\alpha_V\beta_3$ receptor, since the activities for both $\alpha_{IIb}\beta_3$ and $\alpha_V\beta_3$ are similar to the linear reference peptide. In addition, the Phe-D-Ahd derivative **3** is slightly more active than the D-Phe-Ahd peptide **1** towards $\alpha_V\beta_3$, although peptides of the D-Phe-Xaa class normally have higher binding affinities than Phe-D-Xaa peptides [24]. This tendency is dramatically expressed by the *N*-alkylated compounds. Whereas D-Phe-HdGly peptide **2** is nearly inactive for both integrins, the Phe-HdGly compound **4** exhibits, with IC_{50} = 8 nM for the $\alpha_V\beta_3$ receptor, the highest activity and preserves its selectivity; its activity is within the range of the most active peptides found so far.

2.3. *Conformational Analysis.* The conformational behavior of cyclic peptides may be described according to the *Dunitz-Waser* concept [49–52] by substitution of a *trans* amide bond by a single bond. Hence, a cyclic pentapeptide corresponds conformationally to a cyclopentane. This means that four of the five C(α)-atoms are in one plane and one is above or below this plane (envelope conformation). Also, we expect high flexibility. The question of which C-atom is out of plane is determined by the chirality of the amino acids. Structure investigations using 2D-NMR and distance-geometry/ensemble techniques were performed as described in [53] [54]. Recent studies have demonstrated that all investigated compounds of the D-Phe-Xaa or the Phe-D-Xaa class can be divided in two different structural families [24]: for peptides of type **I** (-D-Phe^4-Xaa^5-) a βII′/γ-turn arrangement with D-Phe in $i+1$ position of the β-turn is preferred. For structures of type **II** (-Phe^4-D-Xaa^5-), in principle two different βII′/γ-turn arrangements are possible. Usually, the structure-inducing D-amino acid is forced into the center of the γ-turn, and Gly^2 is located in position $i+1$ of the β-turn. However, peptides with the β-branched amino acid D-Xaa = D-Val are exempt from this family and prefer a conformation with the D-amino acid in $i+1$ position of the β-turn.

Cyclo(-Arg^1-Gly^2-Asp^3-D-Phe^4-Ahd^5-) (**1**) *and Cyclo(-Arg^1-Gly^2-Asp^3-Phe^4-D-Ahd^5-)* (**3**). For **1** and **3** containing the lipophilic α-amino acid, the corresponding structural class can easily be assigned, and, therefore, the structure elucidation was confined to the comparison of all available data from NMR measurements with values obtained for a typical representative of the respective class. We choose cyclo(-Arg^1-Gly^2-Asp^3-D-Phe^4-Lys^5-) (**7**) and cyclo(-Arg^1-Gly^2-Asp^3-Phe^4-D-Lys^5-) (**8**) as extensively studied and closely related compounds. The data determined include chemical shifts, temperature gradients of the amine protons, homonuclear coupling constants, and interproton distances in DMSO using the respective 1D- and 2D-NMR experiments. Analysis of the NMR parameters provide clear evidence that the peptide conformation of **1** and **3** is identical to the lead structures cyclo(-Arg^1-Gly^2-Asp^3-D-Phe^4-Val^5-) (**6**) and cyclo(-Arg^1-Gly^2-Asp^3-Phe^4-D-Val^5-) (**10**), respectively. Comparison of **1** and **7** shows, *e.g.*, that the chemical shifts of their amide and H–C(α) resonances are very close (within 0.07 ppm) (*cf. Table 2*). Also, the pattern of temperature gradients of the amide protons and the coupling constants are very similar (*cf. Tables 3* and *4*). This is true even for

Table 2. *Comparison of NH and H–C(α) Chemical Shifts* [ppm] *of* **1**–**5** *with Those of Reference Peptides* **7**–**9**

	Peptide	Proton	Arg[1]	Gly[2]	Asp[3]	Phe[4] or D-Phe[4]	Xaa[5] or D-Xaa[5]
7	c(RGDFK)	HN	7.65	8.41	8.07	8.04	8.11
1	c(RGDFAhda)	HN	7.67	8.37	8.09	7.97	8.06
2	c(RGDF*N*-Hdg)	HN	7.34	8.02	7.91	7.98	
8	c(RGDFK)	HN	7.91	8.39	8.67	7.44	8.48
3	c(RGDFAhda)	HN	7.99	8.30	8.66	7.35	8.56
4	c(RGDF*N*-Hdg)	HN	7.61	8.67	8.58	7.53	
9	c(RGDFV)	HN	8.33	8.62	8.33	7.72	8.33
5	c(RGDFAhda)	HN	8.15	8.64	8.33	7.69	8.21
7	c(RGDFK)	H–C(α)	4.15	4.04, 3.22	4.63	4.42	3.96
1	c(RGDFAhda)	H–C(α)	4.17	4.03, 3.24	4.59	4.45	3.93
2	c(RGDF*N*-Hdg)	H–C(α)	4.14	4.02, 3.23	4.67	4.73	3.99, 3.35
8	c(RGDFK)	H–C(α)	4.26	3.75	3.52	4.30	4.10
3	c(RGDFAhda)	H–C(α)	4.28	3.76, 3.56	4.32	4.45	4.10
4	c(RGDF*N*-Hdg)	H–C(α)	4.29	3.74, 3.42	4.35	4.83	4.48, 2.96
9	c(RGDFV)	H–C(α)	4.19	3.96, 3.33	4.47	4.35	3.46
5	c(RGDFAhda)	H–C(α)	4.15	3.93, 3.35	4.42	4.34	3.77

Table 3. *Comparison of the Temperature Dependence of the Amide Protons of* **1**–**5** *with Those of Reference Peptides* **7**–**9**. Coefficients are given in parts per billion per K.

	Peptide	Arg[1]	Gly[2]	Asp[3]	Phe[4] or D-Phe[4]	Xaa[5] or D-Xaa[5]
7	c(RGDFK)	−1.3	−5.3	−4.2	−2.8	−4.0
1	c(RGDFAhda)	−1.7	−5.6	−5.1	−3.7	−4.3
2	c(RGDF*N*-Hdg)	−3.2	−1.6	−3.6	−4.4	
8	c(RGDFK)	−4.4	−4.4	−6.6	−0.9	−6.9
3	c(RGDFAhda)	−5.1	−4.5	−6.6	0.8	−6.6
4	c(RGDF*N*-Hdg)	−1.6	−5.2	−6.4	−1.6	
9	c(RGDFV)	−3.2	−5.5	−6.0	−1.4	−4.0
5	c(RGDFAhda)	−2.8	−4.3	−6.0	−2.0	−3.0

H–C(α)/H–C(β) coupling constants which are indicative for the side-chain orientation. In addition, an identical pattern of ROEs was found (*cf. Table 5*). Therefore, it is safe to conclude that **1** and **7** adopt an identical conformation, *i.e.*, a β II′/γ-turn arrangement with D-Phe[4] in *i*+*1* position of the β-turn with a certain degree of flexibility, mainly resulting from flipping motions of the Xaa[5]-Arg[1] amide bond [24] [55–57]. Likewise, a similar agreement of the NMR spectra of the Phe-D-Ahd compound **3** and the reference peptide **8** is observed (*cf. Tables 2–5*), suggesting a β II′/γ-turn arrangement with Gly[2] in *i*+*1* position of the β-turn.

Cyclo(-Arg[1]-Gly[2]-Asp[3]-Phe[4]-Ahd[5]-) (**5**). Peptide **5** does not contain a D-amino acid. Nonetheless, it has been shown that Gly can substitute for a D-amino acid, and, therefore, a preferred β II′/γ-turn conformation is conceivable. Our NMR studies revealed one conformation for **5** in DMSO on the NMR-shift time scale. However, NH/H–C(α) and H–C(α)/H–C(β) coupling constants of *ca.* 7 Hz and temperature coefficients in the range of −2 to −6 ppb/K suggest that the observed structure is a mixture of conforma-

Table 4. *Comparison of $^1H,^1H$ Vicinal Coupling Constants $^3J(HN,H-C(\alpha))$ and $^3J(H-C(\alpha)),H-C(\beta)$* [Hz] *with Those of Reference Peptides* **7**–**9**

	Peptide	Type[a])	Arg^1	Gly^2	Asp^3	Phe^4 or D-Phe^4	Xaa^5 or D-Xaa^5
7	c(RGDFK)	HN/H–C(α)	8.0	4.2, 7.6	8.6	7.2	7.6
1	c(RGDFAhda)	HN/H–C(α)	8.6	–[b])	8.0	7.1	7.7
2	c(RGDF*N*-Hdg)	HN/H–C(α)	7.5	4.8, 7.8	12.2	6.9	
8	c(RGDFK)	HN/H–C(α)	8.6	6.5, 5.0	–	7.6	7.6
3	c(RGDFAhda)	HN/H–C(α)	8.6	–[b])	7.5	7.5	7.4
4	c(RGDF*N*-Hdg)	HN/H–C(α)	9.7	7.4, 3.5	6.2	6.2	
9	c(RGDFV)	HN/H–C(α)	8.7	6.5, 5.3	8.0	8.0	7.9
5	c(RGDFAhda)	HN/H–C(α)	8.4	6.2, 4.8	7.8	8.4	7.8
7	c(RGDFK)	H–C(α)/H–C(β)	–		5.9, 8.4	5.9, 8.7	–
1	c(RGDFAhda)	H–C(α)/H–C(β)	9.1, 6.0		6.6, 8.6	6.1, 8.3	9.4, 5.0
2	c(RGDF*N*-Hdg)	H–C(α)/H–C(β)	6.6, 10.0		9.8, 7.0	7.7, 5.6	
8	c(RGDFK)	H–C(α)/H–C(β)	–		–[c])	5.5, 8.4	7.6
3	c(RGDFAhda)	H–C(α)/H–C(β)	8.3, 3.7		–[c])	4.6, 8.2	8.5, 5.3
4	c(RGDF*N*-Hdg)	H–C(α)/H–C(β)	4.2, 10.3		3.2	9.8, 6.2	
9	c(RGDFV)	H–C(α)/H–C(β)	–[c])		5.3/8.3	–[c])	8.5
5	c(RGDFAhda)	H–C(α)/H–C(β)	–[c])		–[c])	8.5, 8.3	9.5, 5.6

[a]) First value for high field signal, second value for low field signal.
[b]) Coupling constants not determined because of broad signals.
[c]) Signals overlapped, coupling constants not determined.

Table 5. *Comparison of NH/N*H *and NH/H–C(α) Distances* [ppm] *of* **1**–**5** *with Those of Reference Peptides* **7**–**9**. Experimental distances are calibrated for geminal $Arg^1H-C(\beta)$ to 178 pm.

Distance	**7**[a])	**1**[a])	**2**[a])	**8**[b])	**3**[b])	**4**	**9**	**5**
Arg^1NH/Gly^2NH	300	321	264	302	279	298		283
Arg^1NH/Phe^4NH		427		366	258			280
Arg^1NH/Xaa^5NH	232	256		345	336			
Gly^2NH/Asp^3NH	282	269						283
Asp^3NH/Phe^4NH				259	226	206	250	231
Phe^4NH/Xaa^5NH				333	343			246
$Arg^1NH/Arg^1H-C(\alpha)$	251	279	231	274	276	248	255	243
$Arg^1NH/Xaa^5H-C(\alpha)$	271	296	291	211	194	207		255
$Gly^2NH/Gly^2H-C(\alpha)$	270	299	254	240	265	294	310	283
$Gly^2NH/Gly^2H-C(\alpha)$	230	223	219	270	227	214	235	224
$Gly^2NH/Arg^1H-C(\alpha)$	234	228	217	237	217	204		
$Asp^3NH/Asp^3H-C(\alpha)$	266	277	249			240	289	219
$Asp^3NH/Gly^2H-C(\alpha)$	239	229	201	232	201	191		215
$Asp^3NH/Gly^2H-C(\alpha)$			349	331	397			467
$Phe^4NH/Phe^4H-C(\alpha)$	279	277	237	269	262		267	249
$Phe^4NH/Asp^3H-C(\alpha)$	226	230	208	344	298	299	285	262
$Phe^4NH/Gly^2H-C(\alpha)$				361	447	369	417	408
$Xaa^5NH/Xaa^5H-C(\alpha)$	276	281		278	290			228
$Xaa^5NH/Phe^4H-C(\alpha)$	212	205		214	198		240	230

[a]) D-Phe^4 instead of Phe^4 in the case of **1**, **2**, and **7**.
[b]) D-Xaa^5 instead of Xaa^5 in the case of **3** and **8**.

tions averaged on the NMR-shift time scale so that no further calculations were performed. Obviously, the potential of Gly to behave like a D-amino acid is not strong enough to induce one or two energetically low and stable conformations for the peptide backbone.

Cyclo(-Arg^1-Gly^2-Asp^3-D-Phe^4-HdGly^5-) (**2**) *and Cyclo(-Arg^1-Gly^2-Asp^3-Phe^4-HdGly^5-)* (**4**). Two conformations (ratio 90:10 in both cases) were observed for both **2** and **4**. Due to the strong Phe^4*H*–C(α)/HdGly^5*H*–C(1) and Phe^4*H*–C(α)/HdGly^5*H*–C(2) ROEs and the lack of Phe^4*H*–C(α)/HdGly^5*H*–C(α) ROEs, the dominant backbone conformation of both, **2** and **4**, is all-*trans*. On account of the low signal intensity and partial signal overlap, we could not completely characterize the minor populated conformations of **2** and **4**. For **4**, the spin systems of Phe^4 and HdGly^5 could be assigned in the minor populated conformation, and a strong Phe^4*H*–C(α)/HdGly^5*H*–C(α) ROE was found. This provides evidence for our assumption that the amide bond between D-Phe^4 and HdGly^5 is *cis* in the minor populated conformation. Probably, the amide bond between D-Phe^4 and HdGly^5 in **2** is also *cis*. Both peptides contain HdGly, which is, in principle, similar to Gly in terms of the side chains, but is not capable of forming amide H-bonds. These features make the adopted structures much less obvious, and we, therefore, performed a more detailed conformational analysis. The standard procedure (distance geometry followed by restrained molecular dynamics for the lowest-error distance-geometry structure in explicit solvent) yielded ambiguous results. For **4**, no convergence to distinct families within the best ten distance-geometry structures was achieved, and for **2**, only a convergence to an impossible structure was found (showing very close contacts between Arg^{1}NH/Phe^{4}NH and Gly^{2}NH/Asp^{3}NH which could not be detected in the NMR experiments). Even additional lower bound restraints, derived from missing ROEs did not improve the results. Hence, a combined distance geometry/ensemble approach, based on NMR derived ROE and ^3J data was used (see *Exper. Part*).

The results performed on ensembles of 500 structures indeed exhibit structural manifolds which could be clearly classified into conformational families (see below). Those families show the expected overall structure of cyclic pentapeptides containing one D-amino acid or only one Gly as structure inducing residue.

Cyclo(-Arg^1-Gly^2-Asp^3-D-Phe^4-HdGly^5-) (**2**) forms a similar β/γ-turn pattern with D-Phe^4 in *i*+*1* position of the β-turn as it was earlier observed for cyclo(-Arg^1-Gly^2-Asp^3-D-Phe^4-Xaa^5-) peptides [24]. For this class of cyclic pentapeptides, an inherent flexibility of the amide bonds (flipping or wobbling of whole amide-bond planes) was also found previously [24] [55]. For the HdGly-containing compound, an even higher flexibility is observed, which results in the additional structural families (*Fig. 2*). This might be induced by the Gly-like behavior of HdGly. Therefore, the main difference between the two most often observed families, *Family 1* and *Family 2*, is a flip of the *N*-alkylated amide bond next to HdGly, resulting in a rearrangement between βII′ (299 structures out of 500) and βI′ (100 structures out of 500). This might be due not only to the missing side chain in HdGly, which results in less steric hindrance for a flip, but also to the missing amide proton of this amide bond. This proton is often involved in an additional H-bond (γ-turn) to the carbonyl O-atom of Asp^3 thus stabilizing the amide bond in a βII′-like orientation [24]. Hence, the missing proton results in the flipping of the whole amide bond plane between D-Phe^4 and HdGly^5. But not only

this amide bond is more flexible: the amide bond between Asp[3] and D-Phe[4], normally involved in H-bonding, also shows a slightly increased flexibility. The γ-turn region exhibits the typical flexible behavior of cyclic pentapeptides, the so-called 'penta problem' [55]. This phenomenon represents the various positions of the amide bonds adjacent to Gly[2] in this peptide.

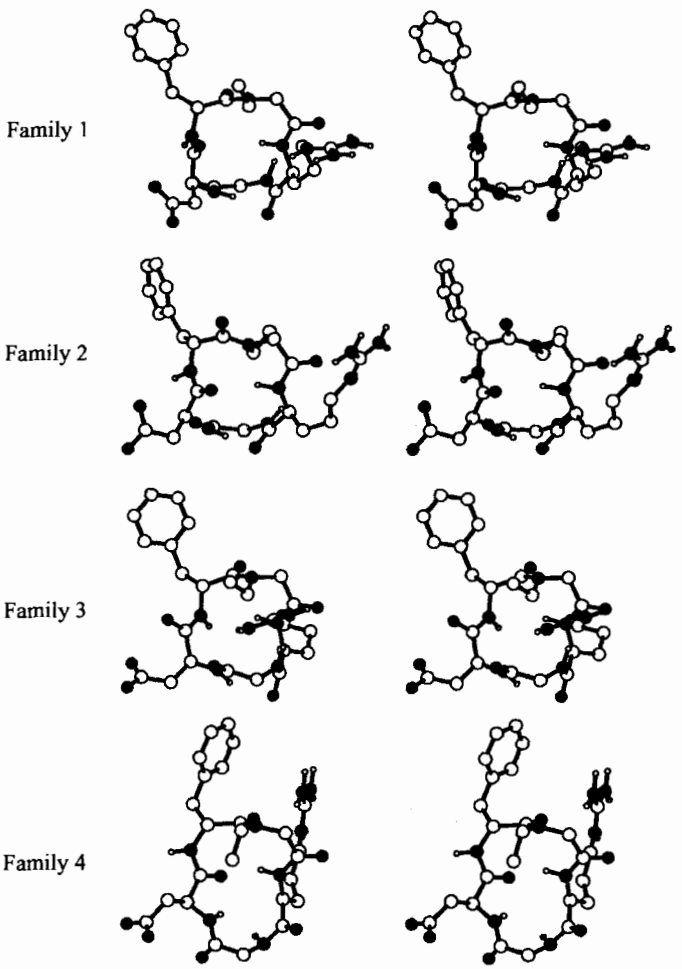


Fig. 2. *Schematic depiction of structure and flexibility of cyclo(-Arg[1]-Gly[2]-Asp[3]-D-Phe[4]-HdGly[5]-)* (**2**). Large circles show C (open), O (gray), and N (black) atoms. Polar H-atoms are indicated by small circles. The Hd alkyl chain is shortened to the first two C-atoms.

Cyclo(-Arg[1]-Gly[2]-Asp[3]-Phe[4]-HdGly[5]-) (**4**) has no structure-inducing D-amino-acid residue. Hence, Gly or the Gly-like HdGly could mimic the effect of a D-configurated residue. For peptides of the type cyclo(-Arg[1]-Gly[2]-Asp[3]-Xaa[4]-D-Yaa[5]-), a varying structure (either Gly[2] or D-Yaa[5] in $i+1$ of a β-turn), depending on the type of D-amino acid, was observed previously [24]. In our case, HdGly[5] indeed mimics the D-amino acid. The

overall structure can be summarized as a shifted, distorted β II'/γ-turn pattern with Gly^2 in $i + 1$-position of the β-turn and $HdGly^5$ in $i + 1$-position of the γ-turn. The γ-turn region shows the normal flexibility ($HdGly^5$ in $i + 1$ of a γ^i-turn in *Family 1*, in $i + 1$ of a γ-turn in *Family 2* and *3*, parallel orientation of the HdGly-adjacent amide bonds in *Family 4*; *Fig. 3*). In contrast to **2**, not the amide bond between $i + 1$ and $i + 2$ of the β-turn, but the Gly preceding amide bond between i and $i + 1$ seems to be more flexible. This flexibility allows varying positions for this amide bond. Hence, in *Family 1*, this amide bond shows a tendency to build a β II-turn with the D-amino acid simulating $HdGly^5$ in $i + 1$ and Gly^2 in $i + 2$.

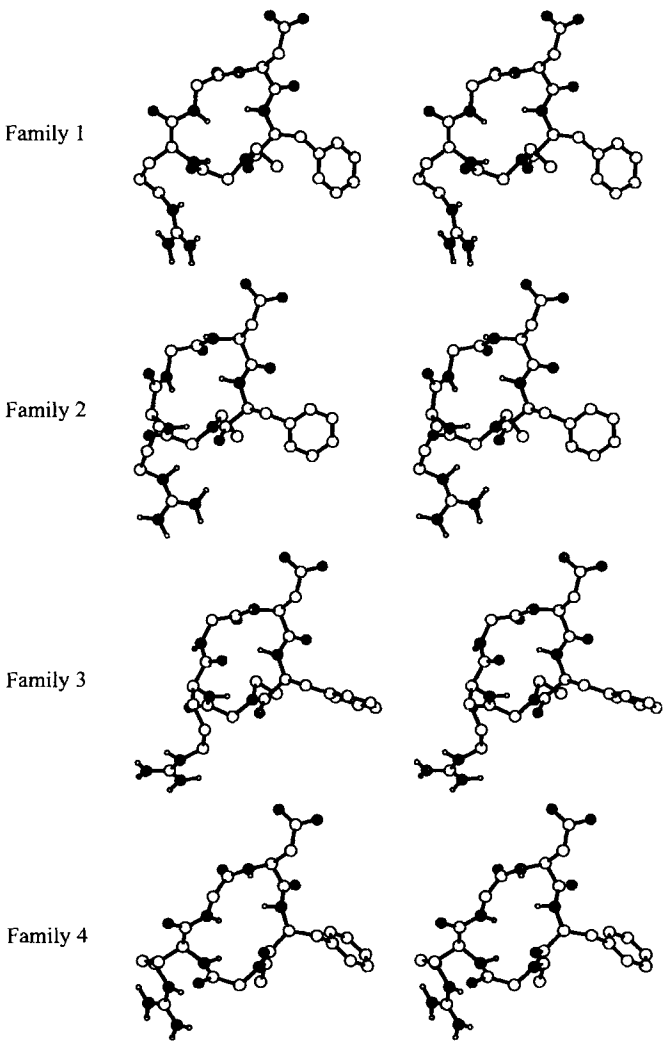


Fig. 3. *Schematic depiction of structure and flexibility of cyclo(-Arg¹-Gly²-Asp³-Phe⁴-HdGly⁵-)* (**4**). Large circles show C (open), O (gray), and N (black) atoms. Polar H-atoms are indicated by small circles. The Hd alkyl chain is shortened to the first two C-atoms.

Altogether, the only qualitatively interpretable populations of the ensemble calculations (**2**: 299:100:44:35; **4**: 189:122:119:60) suggest, that the D-amino-acid-containing compound **2** is more rigid than **4**. This might be due to a potential interchange of Gly^2 and $HdGly^5$ as pseudo-D-amino acid, which is not favored for the D-Phe-HdGly-peptide **2**. However, due to less available ROE data for peptide **4** compared with **2**, an artificial flexibility enhancement during the calculations cannot be completely ruled out, especially since in ensemble distance-geometry calculations, a simplified force field neglecting charges, *van-der-Waals* forces, and solvents is applied and, therefore, these calculations are higher dependent on the number of input ROEs.

3. Conclusion and Discussion. – In this study we demonstrate that enantiomerically pure long-chain α-amino acids with up to 16 C-atoms (Ade and Ahd) are accessible *via* non-stereoselective synthesis and subsequent separation of isomers using acylase I from *Aspergillus mellius*. The lipophilic *N*-hexadecylglycine (HdGly) could be obtained in reasonable yields using a modified approach of *Stewart* [29]. The extremely low reactivity of these compounds in procedures generating protected derivatives could be overcome by intermediate bis-silylation [39] [40]: the desired novel Fmoc derivatives were obtained in yields ranging from 60 to 80%.

The results of integrin binding assays demonstrated that the development of highly active lipid-modified Arg-Gly-Asp-peptides is possible. In general, the compounds exhibit selectivity for $\alpha_V\beta_3$ over $\alpha_{IIb}\beta_3$, which makes them potentially useful inhibitors of pathologies requiring vitronectin. NMR and distance-geometry/ensemble structure elucidation proves that the incorporation of lipidic amino acids does not effect the designed $\beta II'/\gamma$-template structures. It should be emphasized that this not only holds true for lipophilic α-amino acids, but also for *N*-alkylated glycines, which can serve as L- or D-amino acids in the respective template structures cyclo(-Arg^1-Gly^2-Asp^3-D-Phe^4-Xaa^5) (**I**) and cyclo(-Arg^1-Gly^2-Asp^3-Phe^4-D-Xaa^5) (**II**). Peptide **5** contains neither a D-amino acid nor a substitute like Gly in position 5 and is, therefore, excluded from these families. As a consequence, it exhibits the weakest activities. However, the IC_{50} data obtained for the remaining four peptides vary by at least four orders of magnitude, with values ranging from nanomolar to less than millimolar. In addition, the binding capacity of the usually higher active D-Phe-Xaa compounds **1** and **2** proves to be weaker than for the Phe-D-Xaa peptides **3** and **4**. We suspect that, when being in certain substituent positions, the long alkyl chain may impose sterical hindrances for the ligand binding in the receptor pocket, or its anchoring in cellular membranes prevents ligand docking to the integrin receptor. A spacer between the lipophilic anchor and the peptide would distinguish between these alternatives. Although in general preserved, **3** exhibits a significantly decreased selectivity for $\alpha_V\beta_3$, which might be due to different degrees of sterical hindrance for the two receptor subtypes, imposed by the lipophilic side chain, thereby counteracting the usual selectivity for $\alpha_V\beta_3$. A shift of the lipophilic side chain by one position from C(α) to N(α) obviously has an additional positive effect for receptor binding and might explain the big difference in activity of **3** and **4**. These findings are supplemented by the somewhat higher backbone flexibility of **4** compared to the other compounds. Although the template structure may be preserved at least roughly in the receptor-bound conformation, a thereby minor change in conformation at the binding site might place the long side chain in a sterically more favorable position. In conclusion,

the observed IC_{50} of 8 nM for **4** demonstrates that highly active lipid-modified Arg-Gly-Asp peptides can be designed. Future studies will have to focus on the bioavailability of such compounds in *in vivo* systems.

Experimental Part

General. Fmoc-amino acids were purchased from *Bachem* and *Senn* or prepared according to [38]. The 2-chlorotrityl-chloride resin was purchased from *CBL Patras*, TBTU from *Richelieu Biotechnologies* and DPPA from *Aldrich*. HOBt was synthesized according to [58]. TLC: aluminum sheets, silica gel *60 F254* (*Merck*) using the solvent systems BuOH/AcOH/H_2O 3:1:1 (*A*), $CHCl_3$/MeOH 9:1 (*B*), and $CHCl_3$/MeOH/AcOH 95:5:3 (*C*). M.p.: *Büchi 510* apparatus; uncorrected. Optical rotations: *Perkin-Elmer 241MC*. HPLC: *Beckmann 420*, *Waters 440* and *490E* controllers with *Nucleosil RP18*, 5-mm or 7-mm columns; elution with gradient mixtures of MeCN/0.1% CF_3COOH and H_2O/0.1% CF_3COOH. Peptide purification to > 98% homogeneity by prep. HPLC. MS: *Varian MAT 311A* for fast-atom-bombardment (FAB) and *Varian CH5* for chemical ionization (CI).

Assays for Peptide Activity. The procedure of the biological tests has been described in [24].

NMR Measurements. Bruker DMX600, AMX500, and *AC250* spectrometers, and processing on *Silicon Graphics* work stations with the UXNMR software package. The measurements were performed at 300 or 320 K using 4–15 mM solns. of the peptides in (D_6)DMSO (**1** and **3**) or in (D_6)DMSO in presence of 5% CF_3COOH (**2**, **4**, and **5**) to prevent aggregation. Assignment of resonances and determination of interproton distances was achieved by 2D-NMR techniques, *i.e.*, total correlation spectroscopy (TOCSY) [59–61], primitive exclusive correlated spectroscopy (P.E.COSY) [62–64], rotating-frame nuclear *Overhauser* and exchange spectroscopy (ROESY) [65–68] with a pulsed spinlock [69], heteronuclear multiple-quantum correlation (HMQC) [70–72], (HMQC-TOCSY) [73], and heteronuclear multibond correlation (HMBC) [74]. All chemical shifts are referenced to the (D_6)DMSO signal at 2.49 (^{1}H) and 39.5 ppm (^{13}C). Distance information for the structure investigation in DMSO were derived from ROESY spectra with mixing times of 200 ms. For correct conversion of measured ROESY integral volumes to distance constraints, the offset effect was taken into account using the isolated two-spin approximation (ISPA) [75]. ^3J(NH,H–C(α)) (using the method of [76]) and ^3J(H–C(α), H–C(β)) coupling constants were obtained from the P.E.COSY spectra. Temp. coefficients were determined between 300 and 340 K in steps of 10°.

Computational Methods. The distance-geometry and ensemble calculations were performed using a modified version [77] of the DISGEO program [78–81] following procedures similar to those described in [24] [55] [82]. With random metrization and embedding, 100 structures were calculated and subsequently optimized by distance- and angle-driven dynamics (DAAD) [77] using NOE-derived distance restraints and ^3J coupling restraints according to the *Karplus* equation [83–85] (see also [82]) (*Tables 6* and *7*). The ten structures with the lowest error were copied 50 times to produce ensembles of 500 structures for each peptide. The following DAAD calculations (20000 steps at high kinetic energy + 5000 steps at low kinetic energy) used, in analogy to [55], ensemble-averaged distance and ^3J restraints. The resulting ensembles were divided into families by clustering according to the ϕ/ψ-dihedral angles: first, a ϕ/ψ-map for each residue in the peptide was produced (*Fig. 4*). Subsequently, the residue which exhibited the most spread distribution was chosen to cluster according to the spots in the plot. For the resulting families an analogue procedure was repeated (ϕ/ψ-maps, clustering) until the average root mean square deviation (r.m.s.d.) of the backbone atoms within a family was smaller than 0.75 Å. **2**: *Family 1*: 299 members, average r.m.s.d. 0.38 Å; *Family 2*: 100 members, average r.m.s.d. 0.75 Å; *Family 3*: 44 members, average r.m.s.d. 0.12 Å; *Family 4*: 35 members, average r.m.s.d. 0.10 Å. **4**: *Family 1*: 189 members, average r.m.s.d. 0.51 Å; *Family 2*: 122 members, average r.m.s.d. 0.46 Å; *Family 3*: 119 members, average r.m.s.d. 0.54 Å; *Family 4*: 60 members, average r.m.s.d. 0.46 Å.

rac-*2-Aminodecanoic Acid* (DL-Ade) *and* rac-*2-Aminohexadecanoic Acid* (DL-Ahd). After dissolution of Na (330 mmol) in EtOH (250 ml), 450 mmol of octyl bromide (for Ade) or tetradecyl bromide (for Ahd) and diethyl acetamidomalonate (330 mmol) are added and refluxed for 24 h. After cooling to r.t., the mixture is poured on ice water (500 ml) and the precipitated diethyl (acetamido)alkylmalonate isolated by filtration. Subsequently, the crude white solid is refluxed with conc. HCl soln. (560 ml) and DMF (60 ml) for 48 h (*caution*: extreme foaming, especially for Ahd). After completion of the reaction, the suspension is poured in EtOH/H_2O 3:1 (300 ml) and neutralized with conc. NaOH soln. The precipitated wax-like, white product is filtered, washed with H_2O and dried *in vacuo*.

DL-Ade: Yield 51%. M.p. 273° (dec.). R_f 0.6 (*A*), 0 (*B*), 0 (*C*). DL-Ahd: Yield 65%. M.p. 235° (dec.). R_f 0.6 (*A*), 0 (*B*), 0 (*C*).

Table 6. *Coupling Constants* [Hz] *and Upper/Lower Limits* d_{up} *and* d_{low} *of Distances* [pm] *Used as Restraints for Distance-Geometry/Ensemble Calculations of* **2**

Coupling constants	^3J
Arg^{1}NH/Arg^{1}H–C(α)	7.5
Asp^{3}NH/Asp^{3}H–C(α)	12.2
Asp^{3}H–C(α)/Asp^{3}H$_{pro\text{-}R}$–C(β)	9.8
Asp^{3}H–C(α)/Asp^{3}H$_{pro\text{-}S}$–C(β)	7.0
D-Phe^{4}NH/D-Phe^{4}H–C(α)	6.9

Distances	d_{up}	d_{low}
Arg^{1}N*H*/Arg^1*H*–C(α)	254	208
Arg^{1}N*H*/Arg^1*H*–C(β)	388	268
Arg^{1}N*H*/Arg^1*H*–C(δ)	338	223
Arg^{1}N*H*/Gly^{2}N*H*	290	237
Arg^{1}N*H*/Gly^2*H*–C(α)	390	270
Arg^{1}N*H*/Asp^{3}N*H*	349	285
Arg^{1}N*H*/D-Phe^4*H*–C(α)	369	302
Arg^{1}N*H*/HdGly^5*H*–C(α)	354	228
Arg^{1}N*H*/HdGly^5*H*–C(1)	338	232
Arg^{1}N*H*/HdGly^5*H*–C(2)	427	286
Arg^{1}N*H*/HdGly^5*H*–C(3)	450	297
Arg^1*H*–N(ε)/Arg^1*H*–C(δ)	309	197
Arg^1*H*–C(α)/Arg^1*H*–C(β)	285	225
Arg^1*H*–C(α)/Arg^1*H*–C(γ)	335	220
Arg^1*H*–C(α)/Arg^1*H*–C(δ)	347	231
Arg^1*H*–C(α)/Gly^2*H*–C(α)	389	269
Arg^1*H*–C(β)/Arg^1*H*–C(δ)	359	224
Arg^1*H*–C(δ)/Arg^1*H*–C(δ)	298	187
Gly^{2}N*H*/Arg^1*H*–C(α)	239	195
Gly^{2}N*H*/Arg^1*H*–C(β)	338	273
Gly^{2}N*H*/Arg^1*H*–C(δ)	430	306
Gly^{2}N*H*/Gly^2*H*–C(α)	279	197
Asp^{3}N*H*/Arg^1*H*–C(α)	340	278
Asp^{3}N*H*/Arg^1*H*–C(β)	475	346
Asp^{3}N*H*/Gly^2*H*–C(α)	382	182
Asp^{3}N*H*/Asp^3*H*–C(α)	274	224
Asp^{3}N*H*/Asp^3*H*$_{pro\text{-}R}$–C(β)	292	239
Asp^{3}N*H*/Asp^3*H*$_{pro\text{-}S}$–C(β)	261	214
Asp^3*H*–C(α)/Gly^2*H*–C(α)	490	360
Asp^3*H*–C(α)/Asp^3*H*$_{pro\text{-}R}$–C(β)	295	241
Asp^3*H*–C(α)/Asp^3*H*$_{pro\text{-}S}$–C(β)	268	219
D-Phe^{4}N*H*/Asp^3*H*–C(α)	228	187
D-Phe^{4}N*H*/D-Phe^4*H*–C(α)	260	213
D-Phe^{4}N*H*/D-Phe^4*H*–C(β)	288	231
D-Phe^4*H*–C(α)/D-Phe^4*H*–C(β)	263	199
D-Phe^4*H*–C(α)/HdGly^5*H*–C(2)	340	251
D-Phe^4*H*–C(α)/HdGly^5*H*–C(3)	426	258
HdGly^5*H*–C(α)/HdGly^5*H*–C(1)	292	182
HdGly^5*H*–C(α)/HdGly^5*H*–C(2)	395	275
HdGly^5*H*–C(α)/HdGly^5*H*–C(3)	487	312
HdGly^5*H*–C(1)/HdGly^5*H*–C(2)	383	232
HdGly^5*H*–C(1)/HdGly^5*H*–C(3)	431	262

Table 7. *Coupling Constants* [Hz] *and Upper/Lower Limits* d_{up} *and* d_{low} *of Distances* [pm] *Used as Restraints for Distance-Geometry/Ensemble Calculations of* **4**

Coupling constants	^3J
$Arg^1NH/Arg^1H-C(\alpha)$	9.7
$Asp^3NH/Asp^3H-C(\alpha)$	6.2
$Phe^4NH/Phe^4H-C(\alpha)$	6.2

Distances	d_{up}	d_{low}
$Arg^1NH/Arg^1H-C(\alpha)$	273	223
$Arg^1NH/Arg^1H-C(\beta)$	309	208
$Arg^1NH/Arg^1H-C(\delta)$	389	270
Arg^1NH/Gly^2HN	327	268
$Arg^1NH/Phe^4H-C(\alpha)$	552	361
$Arg^1NH/HdGly^5H-C(\alpha)$	297	186
$Arg^1H-C(\alpha)/Arg^1H-C(\beta)$	280	220
$Arg^1H-C(\alpha)/Arg^1H-C(\delta)$	351	235
$Arg^1H-N(\varepsilon)/Arg^1H-C(\alpha)$	396	325
$Arg^1H-N(\varepsilon)/Arg^1H-C(\beta)$	353	217
$Arg^1H-N(\varepsilon)/Arg^1H-C(\delta)$	315	201
$Gly^2NH/Arg^1H-C(\alpha)$	225	183
$Gly^2NH/Arg^1H-C(\alpha)$	339	270
$Gly^2NH/Gly^2H-C(\alpha)$	325	192
$Asp^3NH/Gly^2H-C(\alpha)$	281	172
$Asp^3NH/Asp^3H-C(\beta)$	312	200
Asp^3NH/Phe^4NH	222	181
$Asp^3H-C(\alpha)/Asp^3H-C(\beta)$	330	216
$Phe^4NH/Gly^2H-C(\alpha)$	559	332
$Phe^4NH/Asp^3H-C(\alpha)$	298	255
$Phe^4NH/Asp^3H-C(\beta)$	389	269
$Phe^4NH/Phe^4H-C(\alpha)$	271	222
$Phe^4NH/Phe^4H-C(\beta)$	357	251
$Phe^4H-C(\alpha)/Phe^4H-C(\beta)$	356	176

rac-*2-(Chloracetylamino)decanoic Acid* ($ClCH_2CO$-DL-Ade) *and* rac-*2-(Chloroacetylamino)hexadecanoic Acid* ($ClCH_2CO$-DL-Ahd). To a suspension of DL-Ade or DL-Ahd (200 mmol) in MeCN abs. (200 ml) chloroacetyl chloride (210 mmol) is added and refluxed for 2 h. The yellow-brown soln. is evaporated, the residue dissolved in AcOEt, and the soln. filtered and evaporated. Recrystallization from AcOEt/hexane yields the pure products.

$ClCH_2CO$-DL-Ade: Yield 91%. R_f 0.9 (*A*), 0.2 (*B*), 0.4 (*C*). M.p. 88°. ^{1}H-NMR ((D_6)DMSO): 11.74 (br., COOH); 8.48 (*d*, NH); 4.17 (*m*, H–C(2)); 4.10 (*s*, $ClCH_2CO$); 1.66 (*m*, CH_2(3)); 1.26 (*s*, 12 H, CH_2(4) to CH_2(9)); 0.86 (*t*, Me(10)).

$ClCH_2CO$-DL-Ahd: Yield 65%. R_f 0.9 (*A*), 0.3 (*B*), 0.4 (*C*). M.p. 93°. ^{1}H-NMR ((D_6)DMSO): 8.43 (*d*, NH); 4.18 (*m*, H–C(2)); 4.10 (*s*, $ClCH_2CO$); 1.65 (*m*, CH_2(3)); 1.28–1.22 (*s*, 24 H, CH_2(4) to CH_2(15)); 0.85 (*t*, Me(10)). FAB-MS: 349 ($[M + H]^+$).

(S)-2-Aminodecanoic Acid (Ade) *and (S)-2-Aminohexadecanoic Acid* (Ahd). A suspension of $ClCH_2CO$-DL-Ahd (10.0 g, 28.6 mmol) in 5 l of H_2O is heated to 80° and adjusted to pH 7 by addition of 2N LiOH. After cooling to r.t., acylase I (1 g) from *Aspergillus mellius* is added and left to react. To redissolve precipitated unreacted starting compound and separate it from the product, the mixture is periodically heated to 80°, the warm soln. with the precipitated product centrifuged, and acylase I added until precipitation of the product is finished (5×, altogether 5 g of acylase I). For removal of potentially coprecipitated $ClCH_2CO$-D-Ahd, the white product is subsequently washed with hot H_2O and AcOEt and dried. Similarly, Ade is obtained, however, only 1 l H_2O and 1 g of acylase I are used.

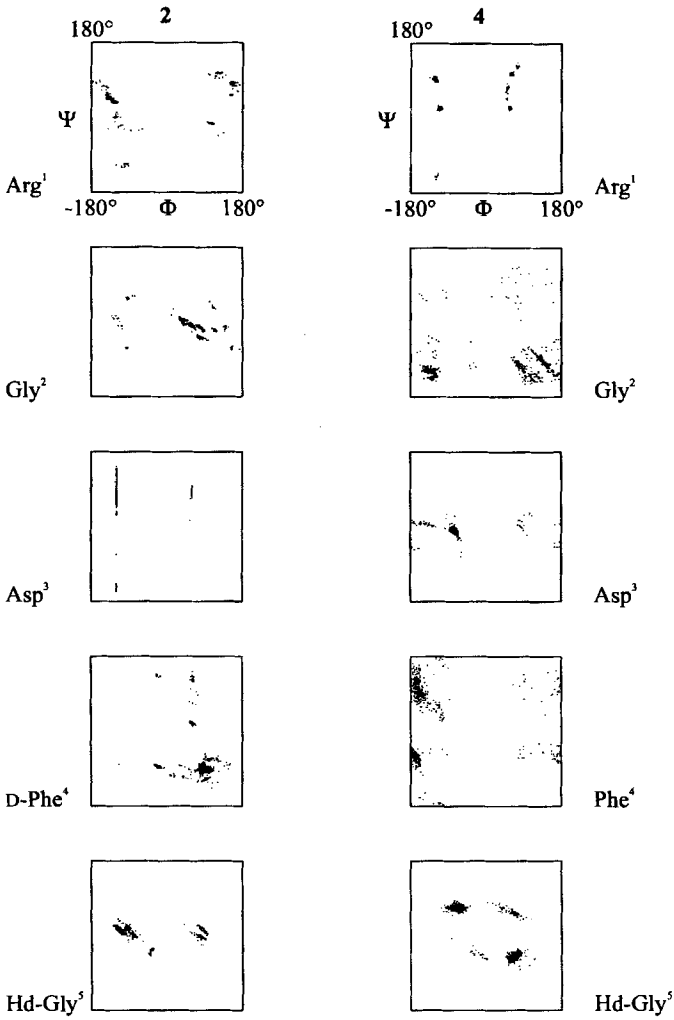


Fig. 4. *Comparison of the Φ/Ψ-plots of* **2** *and* **4**

Ade: Yield 63%. M.p. 278°. R_f 0.7 (*A*), 0.0 (*B*), 0.0 (*C*). $[\alpha]_D^{24} = +26.4°$ ($c = 0.5$, AcOH). FAB-MS: 188 ($[M + H]^+$).

Ahd: Yield 84% (12.0 mmol). M.p. 239°. ^{1}H-NMR ((D_6)DMSO): 8.38 (br., NH_2); 3.82 (*m*, H–C(2)); 1.72 (*m*, CH_2(3)); 1.28–1.22 (*s*, 24H, CH_2(4) to CH_2(15)); 0.85 (*t*, Me(16)). FAB-MS: 272 ($[M + H]^+$).

(R)-2-Aminodecanoic Acid (D-Ade) *and (R)-2-Aminohexadecanoic Acid* (D-Ahd). The filtrate containing $ClCH_2CO$-D-Ahd is acidified to pH 2 by addition of conc. HCl soln. The precipitated solid is filtered, washed with H_2O, and dried. For purification, the chloroacetyl compound is dissolved in AcOEt and the soln. filtered and evaporated. The residue is then suspended in 3N HCl (160 ml) and carefully refluxed for 8 h (strong foaming). After neutralization with solid KOH, the precipitated D-Ahd is filtered, washed with H_2O and AcOEt, and dried. Similarly, D-Ade is obtained.

D-*Ade*: Yield 70%. R_f 0.7 (*A*), 0.0 (*B*), 0.0 (*C*). M.p. 282°. $[\alpha]_D^{24} = -26.4$ ($c = 0.5$, AcOH). FAB-MS: 188 ($[M + H]^+$).

D-*Ahd*: Yield 83%. M.p. 241°. ^{1}H-NMR ((D$_6$)DMSO): 8.38 (br., NH$_2$); 3.82 (*m*, H–C(2)); 1.72 (*m*, CH$_2$(3)); 1.28–1.22 (*s*, 24H, CH$_2$(4) to CH$_2$(15)); 0.85 (*t*, Me(16)). FAB-MS: 272 ([*M* + H]$^+$).

N-*Hexadecylglycine Ethyl Ester* (HdGly-OEt). Within 1.5 h, a soln. of ethyl bromoacetate (290 mmol) in toluene (100 ml) is added dropwise under cooling with ice to a soln. of hexadecylamine (300 mmol) and Et$_3$N (300 mmol) in toluene (200 ml). After additional stirring for 48 h at r.t., the precipitated ammonium salt is filtered off and the solvent evaporated. Purification of the residue by fractionated destillation *in vacuo* yields Hd-Gly-OEt (41%). R_f 0.0 (*A*), 0.7 (*B*), 0.4 (*C*). B.p. 93°. ^{1}H-NMR (CDCl$_3$): 4.15 (*q*, CO$_2$C*H*$_2$Me); 3.36 (*s*, NHC*H*$_2$CO$_2$Et); 2.55 (*t*, (CH$_2$)$_{13}$C*H*$_2$CH$_2$); 1.23 (*t*, CO$_2$CH$_2$*Me*); 1.22 (*s*, 26H, Me(C*H*$_2$)$_{13}$CH$_2$); 0.84 (*t*, *Me*(CH$_2$)$_{13}$). EI-MS: 327 ([*M* + H]$^+$).

N-*Hexadecylglycine* (HdGly). For saponification, HdGly-OEt (122 mmol) in EtOH (300 ml) is treated with sat. aq. NaOH soln. (500 mmol) and refluxed for 1 h. After cooling to r.t., the precipitated amino-acid salt is filtered off and washed with EtOH. For transformation to the free acid, the residue is refluxed in AcOH (100 ml) and after cooling to r.t., precipitated with H$_2$O. Recrystallization from boiling EtOH (500 ml) yields Hd-Gly (95%). R_f 0.6 (*A*), 0.0 (*B*), 0.7 (*C*). M.p. 174°. EI-MS: 299 ([*M* + H]$^+$).

Lipophilic Fmoc-amino Acids. Lipophilic amino acid (10 mmol) and Me$_3$SiCl (20 mmol) are refluxed in abs CH$_2$Cl$_2$ (100 ml). After 15 min, Et$_3$N (20 mmol) is added and the mixture refluxed for another 60 min. After cooling to 0°, Fmoc-Cl (10 mmol) is added in one portion and the mixture is stirred overnight. After evaporation, the residue is stirred with 0.01M HCl (100 ml). Extraction with CH$_2$Cl$_2$ (3×), washing with H$_2$O (3×), drying (MgSO$_4$), and evaporation furnish the crude compounds. Recrystallization is performed from CH$_2$Cl$_2$/hexane.

Table 8. ^{1}*H-NMR Chemical Shifts* [ppm] *for* **1**–**5**

	Residue	NH	H–C(α)	H–C(β)	H–C(γ)	H–C(δ)	HN(ε)
1	Arg^1	7.67	4.17	1.71, 1.48	1.37	3.08	7.58
	Gly^2	8.37	4.03, 3.24				
	Asp^3	8.09	4.59	2.65, 2.39			
	D-Phe^4	7.97	4.45	2.94, 2.79			
	Ahd^5	8.06	3.93	1.52, 1.39			
2	Arg^1	7.34	4.14	1.74, 1.50	1.43	3.06	7.31
	Gly^2	8.02	4.02, 3.23				
	Asp^3	7.91	4.67	2.65, 2.35			
	D-Phe^4	7.98	4.73	2.91, 2.76			
	HdGly^5		3.99, 3.35	3.33, 2.91 [a]	1.21, 1.03 [b]		
3	Arg^1	7.99	4.28	1.69, 1.48	1.48	3.10	7.48
	Gly^2	8.30	3.76, 3.56				
	Asp^3	8.66	4.32	2.57			
	Phe^4	7.35	4.45	2.96, 2.83			
	D-Ahd^5	8.56	4.10	1.53, 1.40			
4	Arg^1	7.61	4.29	1.61, 1.46	1.52, 1.43	3.08	7.35
	Gly^2	8.67	3.74, 3.42				
	Asp^3	8.58	4.35	2.52			
	Phe^4	7.53	4.83	3.04, 2.69			
	HdGly^5		4.48, 2.96	3.05, 2.99 [a]	1.28 [b]		
5	Arg^1	8.15	4.15	1.69, 1.57	1.46	3.12	7.49
	Gly^2	8.64	3.93, 3.35				
	Asp^3	8.33	4.42	3.00, 2.96			
	Phe^4	7.69	4.34	3.00, 2.96			
	Ahd^5	8.21	3.77	1.79, 1.64			

[a] 2H–C(1) of HdGly^5.
[b] 2H–C(2) of HdGly^5.

(S)-2-{[(9H-Fluoren-9-ylmethoxy)carbonyl]amino}decanoid Acid (Fmoc-Ade): Yield 72%. M.p. 129°. $[\alpha]_D^{24} = -10.9$ ($c = 1$, DMF). $[\alpha]_D^{24} = +1.6$ ($c = 1$, CH_2Cl_2). ^{1}H-NMR ((D_6)DMSO): 12.51 (br., COOH); 7.95–7.26 (*m*, 8 arom. H); 7.62 (*d*, NH); 4.24 (*m*, H–C(2)); 4.24 (*m*, CH_2CH_2OCO); 3.93 (*m*, $CHCH_2OCO$); 1.64 (*m*, 2H–C(3)); 1.26 (*s*, 12H, CH_2(4) to CH_2(9)); 0.85 (*t*, Me(10)). CI-MS: 409 ($[M + H]^+$).

(S)-2-{[(9H-Fluoren-9-ylmethoxy)carbonyl]amino}hexadecanoid Acid (Fmoc-Ahd): Yield 45%. M.p. 98° $[\alpha]_D^{24} = -7.7$ ($c = 1$, DMF). $[\alpha]_D^{24} = +1.4$ ($c = 1$, CH_2Cl_2). ^{1}H-NMR ((D_6)DMSO): 12.51 (br., COOH); 7.95–7.26 (*m*, 8 arom. H); 7.62 (*d*, NH); 4.25 (*m*, H–C(2)); 4.25 (*m*, CH_2CH_2OCO); 3.93 (*m*, $CHCH_2OCO$); 1.64 (*m*, 2H–C(3)); 1.28–1.22 (*s*, 24H, CH_2(4) to CH_2(15)); 0.85 (*t*, Me(16)). CI-MS: 493 ($[M + H]^+$).

(R)-2-{[(9H-Fluoren-9-ylmethoxy)carbonyl]amino}hexadecanoid Acid (Fmoc-Ahd): Yield 59%. M.p. 99° $[\alpha]_D^{24} = +7.7$ ($c = 1$, DMF). $[\alpha]_D^{24} = -1.4$ ($c = 1$, CH_2Cl_2). ^{1}H-NMR ((D_6)DMSO): 12.51 (br., COOH); 7.95–7.26 (*m*, 8 arom. H); 7.62 (*d*, NH); 4.25 (*m*, H–C(2)); 4.25 (*m*, CH_2CH_2OCO); 3.93 (*m*, $CHCH_2OCO$); 1.64 (*m*, 2H–C(3)); 1.28–1.22 (*s*, 24H, CH_2(4) to CH_2(15)); 0.85 (*t*, Me(16)). CI-MS: 493 ($[M + H]^+$).

N-[(9H-Fluoren-9-ylmethoxy)carbonyl]-N-hexadecylglycine (Fmoc-HdGly): Yield 81%. M.p. 94°. ^{1}H-NMR ((D_6)DMSO, *cis/trans* 4:6): 7.93–7.26 (*m*, 8 arom. H); 7.62 (br., NH_2); 4.45, 4.25 (2*d*, $CHCH_2OCO$); 4.25 (*t*, $CHCH_2OCO$); 3.93, 3.80 (2*s*, H–C(α)); 3.21, 2.90 (2*t*, $(CH_2)_{14}CH_2NH$); 1.45 (*s*, $(CH_2)_{13}CH_2CH_2$); 1.23 (*s*, 24H, $MeCH_2(CH_2)_{12}$); 0.97 (*m*, $MeCH_2$); 0.84 (*t*, $Me(CH_2)_{13}$). CI-MS: 521 ($[M + H]^+$).

Synthesis of **1**–**5**. All linear precursor peptides were synthesized applying the Fmoc-based solid-phase method using a 2-chlorotrityl-chloride resin. Attachment of the first amino acid Fmoc-Gly-OH (for **1** and **2**) and Fmoc-Phe-OH (for **3**–**5**) was achieved using 1.0 equiv./g resin according the described procedure to give a final loading of 0.9 mmol/g resin. The peptides were assembled manually using 2.5 equiv. of Fmoc-Xaa-OH/TBTU/HOBt[1]) and Et(i-Pr)$_2$N for coupling in 1-methylpyrrolidin-2-one. Treatment of the resin with $AcOH/CF_3CH_2OH/CH_2Cl_2$

Table 9. *^{13}C-NMR Chemical Shifts* [ppm] *for* **1**–**5**

	Residue	CO	C(α)	C(β)	C(γ)	C(δ)
1	Arg[1]	171.1	51.5	28.1	24.8	40.1
	Gly[2]	169.3	43.0			
	Asp[3]	170.0	48.6	35.0	171.8	
	D-Phe[4]	170.5	54.0	37.1		
	Ahd[5]	172.1	54.4	31.1		
2	Arg[1]	[a])	53.3	29.6	26.3	41.4
	Gly[2]	[a])	44.7			
	Asp[3]	[a])	50.3	36.5		
	D-Phe[4]	[a])	51.9	36.6		
	HdGly[5]	[a])	52.4	50.6[b])	27.3[c])	
3	Arg[1]	171.9	51.4	28.2	24.7	39.9
	Gly[2]	168.9	42.6			
	Asp[3]	170.4	51.4	35.2		
	Phe[4]	170.5	53.6	37.1		
	D-Ahd[5]	171.4	52.2	28.2		
4	Arg[1]	173.4	53.0	30.2	26.3	41.3
	Gly[2]	169.9	44.5			
	Asp[3]	170.3	52.4	36.8		
	Phe[4]	170.6	51.7	39.1		
	HdGly[5]	169.7	51.3	49.0[b])	28.6[c])	
5	Arg[1]	173.7	52.3	28.9	25.5	40.4
	Gly[2]	170.6	43.6			
	Asp[3]	171.6	51.0	35.9		
	Phe[4]	171.8	56.2	36.8		
	Ahd[5]	173.8	56.8	30.6		

[a]) Could not be assigned unambiguously.
[b]) C(1) of HdGly[5].
[c]) C(2) of HdGly[5].

1:1:3 for 1 h and evaporation yielded the side-chain-protected peptide acids ready for cyclization without the need of an additional purification. Addition of DPPA (3 equiv.) and solid $NaHCO_3$ (5 equiv.) and stirring for 24 h gave the crude cyclic peptides after filtration, evaporation, and washing with H_2O. The removal of the *t*-Bu and Pbf groups was achieved by treatment with reagent K (85.5% TFA, 2.0% H_2O, 2.5% ethane-1,2-dithiol, 5% phenol, 5% thioanisole), subsequent precipitation, and washing with Et_2O. Purification of the peptides was performed by reversed-phase HPLC employing a gradient of 30–90% of MeCN/0.1% CF_3COOH in H_2O/0.1% CF_3COOH yielding 18% of **1**, 27% of **2**, 50% of **3**, 15% of **4**, and 23% of **5**. Complete assignment of all ^1H- and ^{13}C-NMR resonances of **1**–**5** is given in *Tables 8* and *9*, temp. coefficients and coupling constants of **1**–**5** are shown in *Tables 3* and *4*.

Financial support by the *Sonderforschungsbereich 369* and the *Fonds der Chemischen Industrie* is gratefully acknowledged. *M. K.* thanks the TU München for a fellowship. The authors thank Dr. *J. Winkler* for contributing the FAB mass spectra, *M. Kranawetter* and *B. Cordes* for HPLC separations, and *M. Koppe* for technical assistance.

REFERENCES

[1] I. Toth, R. A. Hughes, G. Dekany, A. M. Hillery, P. Ward, *Liebigs Ann. Chem.* **1994**, 685.
[2] I. Toth, M. Christodoulou, K. Bankowsky, N. Flinn, W. A. Gibbons, G. Godeau, E. Moczar, W. Hornebeck, *Int. J. Pharm.* **1995**, *125*, 117.
[3] H. Schild, K. Deres, K. H. Wiesmüller, G. Jung, H. G. Rammensee, *Eur. J. Immunol.* **1991**, *21*, 2649.
[4] F. Al-Obeidi, V. J. Hruby, N. Yaghoubi, M. M. Marwan, *J. Med. Chem.* **1992**, *35*, 118.
[5] J. Metzger, K.-H. Wiesmüller, R. Schaude, W. G. Besseler, G. Jung, *Int. J. Pept. Protein Res.* **1991**, *37*, 46.
[6] H. B. A. De Bont, J. H. van Boom, R. M. J. Liskamp, *Recl. Trav. Chim. Pays-Bas* **1992**, *111*, 222.
[7] C. H. Damsky, M. Bernfield, *Curr. Opin. Cell Biol.* **1990**, *2*, 813.
[8] S. M. Albelda, C. A. Buck, *FASEB J.* **1990**, *4*, 2668.
[9] D. Cox, T. Aoki, J. Seki, Y. Motoyama, K. Yoshida, *Med. Res. Rev.* **1994**, *14*, 195.
[10] D. A. Cheresh, R. P. Mecham, 'Integrins, Molecular and Biological Responses to the Extracellular Matrix', Academic Press, London, 1994.
[11] M. A. Horton, M. L. Taylor, T. R. Arnett, M. H. Helfrich, *Exp. Cell Res.* **1991**, *195*, 368.
[12] D. T. Denhardt, X. Guo, *FASEB J.* **1993**, *7*, 1475.
[13] M. Grano, P. Zigrino, S. Colucci, G. Zambonin, L. Trusolino, M. Serra, N. Baldini, A. Teti, P. C. Marchisio, A. Zambonin Zallone, *Exp. Cell Res.* **1994**, *212*, 209.
[14] R. J. Gould, *Perspectives in Drug Discovery and Design* **1994**, *1*, 537.
[15] M. Aumailly, M. Gurrath, G. Müller, J. Calvete, R. Timpl, H. Kessler, *FEBS Lett.* **1991**, *291*, 50.
[16] P. C. Brooks, R. A. F. Clark, D. A. Cheresh, *Science* **1994**, *264*, 569.
[17] P. C. Brooks, A. M. P. Montgomery, M. Rosenfeld, R. A. Reisfeld, T. Hu, G. Klier, D. A. Cheresh, *Cell* **1994**, *79*, 1157.
[18] M. Friedlander, P. C. Brooks, R. W. Shaffer, C. M. Kincaid, J. A. Varner, D. A. Cheresh, *Science* **1995**, *270*, 1500.
[19] H. P. Hammes, M. Brownlee, A. Jonczyk, A. Sutter, K. T. Preissner, *Nature Medicine* **1996**, *2*, 529.
[20] H. Kessler, B. Diefenbach, D. Finsinger, A. Geyer, M. Gurrath, S. L. Goodman, G. Hölzemann, R. Haubner, A. Jonczyk, G. Müller, E. Graf von Roedern, J. Wermuth, *Lett. Pept. Sci.* **1995**, *2*, 155.
[21] M. Gurrath, G. Müller, H. Kessler, M. Aumailly, R. Timpl, *Eur. J. Biochem.* **1992**, *210*, 911.
[22] G. Müller, M. Gurrath, H. Kessler, R. Timpl, *Angew. Chem. Int. Ed.* **1992**, *31*, 326.
[23] M. Pfaff, K. Tangemann, B. Müller, M. Gurrath, H. Kessler, R. Timpl, J. Engel, *J. Biol. Chem.* **1994**, *269*, 20233.
[24] R. Haubner, R. Gratias, B. Diefenbach, S. L. Goodman, A. Jonczyk, H. Kessler, *J. Am. Chem. Soc.* **1996**, *118*, 7461.
[25] N. F. Albertson, *J. Am. Chem. Soc.* **1946**, *68*, 450.
[26] W. A. Gibbons, R. A. Hughes, M. Charalambous, M. Christodoulou, A. Szeto, A. E. Aulabaugh, P. Mascagni, I. Toth, *Liebigs Ann. Chem.* **1990**, 1175.
[27] H. K. Chenault, J. Dahmer, G. M. Whitesides, *J. Am. Chem. Soc.* **1989**, *111*, 6354.
[28] S. M. Birnbaum, S.-C. J. Fu, J. P. Greenstein, *J. Am. Chem. Soc.* **1953**, *203*, 333.
[29] F. H. C. Stewart, *Austr. J. Chem.* **1961**, *14*, 654.
[30] L. A. Carpino, G. Y. Han, *J. Org. Chem.* **1972**, *37*, 3404.

[31] E. Atherton, H. Fox, D. Harkiss, C. J. Logan, R. C. Sheppard, B. J. Williams, *J. Chem. Soc., Chem. Commun.* **1978**, 537.
[32] C.-D. Chang, J. Meienhofer, *Int. J. Pept. Protein Res.* **1978**, *11*, 246.
[33] C.-D. Chang, *Int. J. Pept. Protein Res.* **1980**, *15*, 59.
[34] M. Tessier, F. Albericio, E. Pedroso, R. Eritja, E. Giralt, C. Granier, J. Van Rietschoten, *Int. J. Pept. Protein Res.* **1983**, *22*, 125.
[35] G. Barcelo, J.-P. Senet, G. Senney, J. Bensoam, A. Loffet, *Synthesis* **1986**, 627.
[36] P. Henklein, H.-V. Heyne, W. R. Halatsch, H. Niedrich, *Synthesis* **1987**, 166.
[37] L. Lapatsanis, G. Milias, K. Frouissios, M. Kolovos, *Synthesis* **1983**, 671.
[38] P. B. W. Ten Kortenaar, B. G. Van Diyk, M. Peeters, B. J. Raaben, P. J. H. Adam, G. I. Tesser, *Int. J. Pept. Protein Res.* **1986**, *27*, 398.
[39] H. R. Kricheldorf, *Liebigs Ann. Chem.* **1972**, *763*, 17.
[40] H. R. Kricheldorf, M. Fehrle, *Chem. Ber.* **1974**, *107*, 3533.
[41] K. Barlos, D. Papaioannou, D. Theodoropoulos, *J. Org. Chem.* **1982**, *47*, 1324.
[42] D. R. Bolin, I. L. Sytwu, F. Humiec, J. Meienhofer, *Int. J. Pept. Protein Res.* **1989**, *33*, 353.
[43] L. A. Carpino, *J. Am. Chem. Soc.* **1970**, *92*, 5748.
[44] G. B. Fields, R. L. Noble, *Int. J. Pept. Protein Res.* **1990**, *35*, 161.
[45] R. B. Merrifield, *J. Am. Chem. Soc.* **1963**, *85*, 2149.
[46] R. B. Merrifield, *Angew. Chem.* **1985**, *97*, 801.
[47] K. Barlos, O. Chatzi, D. Gatos, G. Stavropoulos, *Int. J. Pept. Prot. Res.* **1991**, *37*, 513.
[48] S. F. Brady, S. L. Varada, R. M. Freidinger, D. A. Schwenk, M. Mendlowski, F. W. Holly, D. F. Veber, *J. Org. Chem.* **1979**, *44*, 3101.
[49] J. D. Dunitz, J. Waser, *J. Am. Chem. Soc.* **1972**, *94*, 5645.
[50] H. Kessler, P. Krämer, G. Krack, *Isr. J. Chem.* **1980**, *20*, 180.
[51] H. Kessler, 'Conformational Mobility of the Backbone of Cyclic Tripeptides', in 'Stereodynamics of Molecular Systems', Ed. R. H. Sarma, Pergamon Press, Oxford–New York–Frankfurt–Paris, 1979, pp. 187.
[52] H. Kessler, R. Gratias, G. Hessler, M. Gurrath, G. Müller, *Pure Appl. Chem.* **1996**, *68*, 1201.
[53] H. Kessler, S. Seip, 'NMR of Peptides' in 'Two-Dimensional NMR Spectroscopy: Applications of Chemists and Biochemists', Eds. W. R. Croasmun and R. M. K. Carlson, VCH Publishers, New York, 1994, pp. 619.
[54] M. Eberstadt, G. Gemmecker, D. F. Mierke, H. Kessler, *Angew. Chem. Int. Ed.* **1995**, *34*, 1671.
[55] D. F. Mierke, M. Kurz, H. Kessler, *J. Am. Chem. Soc.* **1993**, *116*, 1042.
[56] H. Kessler, J. W. Bats, L. Lautz, A. Müller, *Liebigs Ann. Chem.* **1989**, 913.
[57] K. Romanowska, K. D. Kopple, *Int. J. Pept. Protein Res.* **1987**, *30*, 289.
[58] W. Koenig, R. Geiger, *Chem. Ber.* **1970**, *103*, 788.
[59] L. Braunschweiler, R. R. Ernst, *J. Magn. Reson.* **1983**, *53*, 521.
[60] A. Bax, R. A. Byrd, A. Aszalos, *J. Am. Chem. Soc.* **1984**, *106*, 7632.
[61] A. Bax, D. G. Davis, *J. Magn. Reson.* **1985**, *65*, 355.
[62] L. Müller, *J. Magn. Reson.* **1987**, *72*, 191.
[63] A. Bax, L. Lerner, *J. Magn. Reson.* **1988**, *79*, 429.
[64] D. Marion, A. Bax, *J. Magn. Reson.* **1988**, *80*, 528.
[65] A. A. Bothner-By, R. L. Stephens, J. Lee, D. C. Warren, R. W. Jeanloz, *J. Am. Chem. Soc.* **1984**, *106*, 811.
[66] A. Bax, D. G. Davis, *J. Magn. Reson.* **1985**, *63*, 207.
[67] D. Neuhaus, J. Keeler, *J. Magn. Reson.* **1986**, *68*, 568.
[68] C. Griesinger, R. R. Ernst, *J. Magn. Reson.* **1987**, *75*, 261.
[69] H. Kessler, C. Griesinger, R. Kerssebaum, K. Wagner, R. R. Ernst, *J. Am. Chem. Soc.* **1987**, *109*, 607.
[70] M. R. Bendall, D. T. Pegg, D. M. Dodrell, *J. Magn. Reson.* **1983**, *52*, 81.
[71] A. Bax, R. H. Griffey, B. L. Hawkins, *J. Am. Chem. Soc.* **1983**, *105*, 7188.
[72] A. Bax, R. H. Griffey, B. L. Hawkins, *J. Magn. Reson.* **1983**, *55*, 301.
[73] L. Lerner, A. Bax, *J. Magn. Reson.* **1986**, *69*, 375.
[74] A. Bax, M. T. Summers, *J. Am. Chem. Soc.* **1986**, *108*, 2093.
[75] D. Neuhaus, M. Williamson, 'The Nuclear Overhauser Effect in Structural and Conformational Analysis', VCH Publishers, New York, 1989.
[76] Y. Kim, J. H. Prestegard, *J. Magn. Reson.* **1989**, *84*, 9.
[77] D. F. Mierke, H. Kessler, *Biopolymers* **1993**, *33*, 1003.
[78] T. F. Havel, *Prog. Biophys. Molec. Biol.* **1991**, *56*, 43.

[79] G. M. Crippen, T. F. Havel, 'Distance Geometry and Molecular Conformation', Research Studies Press LTD., Somerset, England, John Wiley & Sons, New York, 1988.
[80] T. F. Havel, 'DISGEO, Quantum Chemistry Exchange Program', Exchange No. 507, Indiana University, 1986.
[81] T. F. Havel, K. Wüthrich, *Bull. Math. Bio.* **1984**, *46*, 673.
[82] R. Haubner, W. Schmitt, G. Hölzemann, S. L. Goodman, A. Jonczyk, H. Kessler, *J. Am. Chem. Soc.* **1996**, *118*, 7881.
[83] M. Karplus, *J. Chem. Phys.* **1959**, *30*, 11.
[84] M. Karplus, *J. Am. Chem. Soc.* **1963**, *85*, 2870.
[85] V. F. Bystrov, *Prog. Nucl. Magn. Reson. Spectrosc.* **1976**, *10*, 41.