α,α-Disubstituted Glycines Bearing a Large Hydrocarbon Ring: Peptide Self-Assembly through Hydrophobic Recognition

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Abstract: A method was developed for synthesizing α,α-disubstituted glycine residues bearing a large (more than 15-membered) hydrophobic ring. The ring-closing metathesis reactions of the dialkenylated malonate precursors proceed efficiently, particularly when long methylene chains tether both terminal olefin groups. Surprisingly, the amino groups of these α,α-disubstituted glycines are inert to conventional protective reactions (e.g., *N-tert*-butoxycarbonyl (Boc) protection: Boc_2O/4-dimethylaminopyridine (DMAP)/CH_2Cl_2; *N*-benzyloxycarbonyl (Z) protection: Z-Cl/DMAP/CH_2Cl_2). Curtius rearrangement of the carboxylic acid functionality of the malonate derivative after ring-closing metathesis leads to formation of an amine functionality and can be catalyzed by diphenylphosphoryl azide. However, only the intermediate isocyanates can be isolated, even in the presence of alcohols such as benzyl alcohol. The isocyanates obtained by Curtius rearrangement in an aprotic solvent (benzene) were isolated in high yields and treated with 9-fluorenylmethanol in a high-boiling-point solvent (toluene) under reflux to give the *N*-9-fluorenylmethoxycarbonyl (Fmoc)-protected aminomalonate derivatives in high yield. These hydrophobic amino acids can be incorporated into a peptide by Fmoc solid-phase peptide synthesis and the acid fluoride activation method. The stability of the monomeric α-helical structure of a 17-amino-acid peptide was enhanced by replacement of two alanine residues with two hydrophobic amino acid residues bearing a cyclic 18-membered ring. The results of sedimentation equilibrium studies suggested that the peptide assembles into hexamers in the presence of 100 mM NaCl.

Keywords: amino acids · peptides · protein design · ring-closing metathesis · self-assembly

Introduction

Hydrophobic amino acids play an essential role in the molecular architectures of proteins and peptides. These amino acids are commonly found in the transmembrane regions of membrane proteins and ion channels, embedded in lipid bilayers.[1] Farnesylation (15 carbon atoms) and geranylgeranylation (20 carbon atoms) of cysteine side chains are known to have dramatic effects on the hydrophobicity of proteins and have been proposed to be important for signal transduction.[2] Hydrophobic interaction also often plays a crucial role in protein folding and assembly in water. Thus, the control of molecular recognition of hydrophobic surfaces is regarded as one of the key elements in the rational design of artificial proteins and protein-interacting molecules with pharmaceutical potential.[3] Various unnatural amino acids with hydrophobic side chains have been explored as building blocks for peptides that provide novel hydrophobic cores.[4] Amino acids with Cα,α-disubstituted cycloaliphatic groups are intriguing because α,α-disubstitution constrains the conformation of a peptide chain and also causes changes in hydrophobicity.[5,6] A representative Cα,α-disubstituted glycine, α-aminoisobutyric acid (Aib), is known to induce a 3_{10}/

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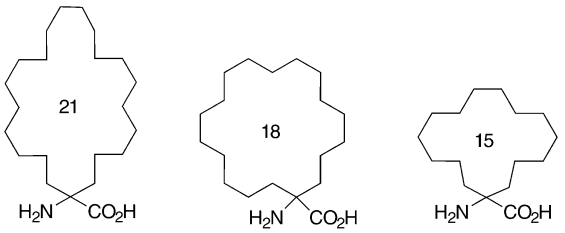
α-helical protein structure.[5] A series of systematic studies by Toniolo and co-workers has established that oligopeptides containing Cα,α-disubstituted cycloaliphatic groups show a preference for 3_{10}-helical and β-bend structures.[6] Their work has provided considerable information about the effects of amino acids with these side chains upon local secondary structures of the peptide molecule. However, nothing is yet known about amino acids bearing a larger hydrocarbon moiety (a 15-membered or larger ring) at the α,α-positions and the effects of such amino acids in longer peptides of 15–20 amino acids.

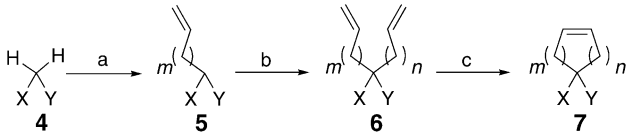
Herein, we describe a general approach to the synthesis of amino acids fused with a large saturated hydrocarbon ring at the α,α-positions (**1**, **2**, and **3**, Scheme 1).[6] Incorporation of two amino acid residues **2** bearing a cyclic 18-membered ring (referred to as C18) into an α-helical peptide resulted in the formation of a quaternary assembly through interactions of the hydrophobic core.[4]



Scheme 1. Macrocyclic α,α-disubstituted amino acids.

Results and Discussion

Synthesis of α,α-disubstituted glycines bearing a large hydrocarbon ring: α,α-Amino acids bearing 21-membered (**1**), 18-membered (**2**), 15-membered (**3**), and 14-membered rings, respectively, were synthesized efficiently through ring-closing metathesis reactions of the appropriate dialkenyl precursors (**6**), which were derived from malonate derivatives (Scheme 2).[7] Stepwise alkenylation of malonate derivatives **4** in the presence of dimsyl sodium (NaH/DMSO) to form the monoalkenylated intermediates **5** and then the dialkenylated precursors **6** was more efficient than direct double alkenylation, even when the same alkenyl bromide was used in both steps.[8] In most cases, the yield of the monoalkenylated product **5** was moderate to high, though the reaction is accompanied by formation of the corresponding dialkenylated compound as a minor product (**6** (except **6b**), see Table 1). The yields of the second alkenylation step are generally high and are insensitive to the chain length of the second alkenyl bromide (Table 2).



Scheme 2. General synthetic strategy for macrocyclization of malonate derivatives. a) CH_2=$CH(CH_2)_m$Br, NaH, DMSO, room temperature; b) CH_2=$CH(CH_2)_n$Br, NaH, DMSO, room temperature; c) [$(PCy_3)_2Cl_2$Ru=CHPh], CH_2Cl_2, reflux. DMSO = dimethyl sulfoxide.

The ring-closing metathesis reactions of the dialkenyl precursors **6** were carried out by treatment with Grubbs' ruthenium catalyst in dichloromethane (Table 3).[7] The yields of the desired cyclized products **7** were moderate to good, except for substrates containing a cyano group. The olefin products were obtained as mixtures of the *E* and *Z* forms; the ratio of these isomers was evaluated on the basis of the ^{13}C signals of the olefin moiety. The olefins were hydrogenated over Pd/C.

9-Fluorenylmethoxycarbonyl (Fmoc) solid-phase synthesis has become the most widely used method for peptide synthesis. Alternatively, the amino acid fluoride method can be used as a coupling system for the introduction of Aib into a peptide chain.[9] We used a combination of acid fluoride and *N*-Fmoc chemistry for the synthesis of peptides containing Cα,α-disubstituted cycloaliphatic amino acids. To prepare the Fmoc amino acid fluorides **12A–C**, we started from benzyl *tert*-butyl malonate **4c** (Scheme 3). The cyclic olefin **7** was prepared by means of ring-closing metathesis and hydrogenated, then reductive debenzylation of the ester gave the saturated half acids **8A–C**. Curtius rearrangement of the carboxylic acid functionalities of **8A–C** to amine functionalities was catalyzed by DPPA[10] but, unexpectedly, only the intermediate isocyanates **9A–C** could be isolated, even in the presence of alcohols such as benzyl alcohol.[11] We carried out Curtius rearrangement of **8A–C** in an aprotic solvent (benzene) and isolated the isocyanates **9A–C** in high yields. These isocyanates were treated with 9-fluorenylmethanol in the high-boiling-point solvent toluene under reflux to give the *N*-Fmoc-protected esters **10A–C** in high yield. These compounds was treated with TFA to give the acids **11A–C**, and then with DAST to give the corresponding acid fluorides **12A–C**.[9] Surprisingly, the amino groups of the related amine compounds **10** (R_2 = H, Et ester, derived from **7b**, **7j**, and **7g**) were inert to protective reactions (*N*-*tert*-butoxycarbonyl (Boc) protection: Boc_2O/4-dimethylaminopyridine (DMAP)/CH_2Cl_2; *N*-benzyloxycarbonyl (Z) protection: Z-Cl/DMAP/CH_2Cl_2).

Single-crystal X-ray diffraction structure analysis of the *N*-benzyl ethyl ester derivative of the 18-membered compound **2** (Figure 1) showed a flat and unfolded 18-membered ring.[12] The crystal structure of **2** shows a left-handed helical conformation (i.e. ϕ (+45.4°) and pseudo ψ(N1-C8-C26-O3) (+41.8°) are both positive). These backbone torsion angles ϕ and pseudo ψ are closer to the values expected for an α helix ($\phi = \pm 55°$, $\psi = \pm 45°$) than to those expected for a 3_{10} helix ($\phi = \pm 57°$, $\psi = \pm 30°$).[5b,6b,6p,6q] In addition, the bond angles indicate an asymmetric geometry for the Cα atom in the crystal structure: the bond angles τ(N1-C8-C9) (111.4°) and τ(N9-C8-C25) (112.3°) are greater than the ideal tetrahedral angle (109.45°), while the bond angles τ(C26-C8-C25) (107.9°) and τ(N1-C8-C26) (108.0°) are smaller than the tetrahedral value.

Table 1. Alkenylation of malonate derivatives with CH_2=$CH(CH_2)_m$Br.

Starting compound	m	n	X	Y	Alkenylation products Mono-	Yield [%]	Di-[a]	Yield [%]
4a	9	-	CO_2Me	CO_2Me	**5a**	64	**6a**	7
4b	9	-	CO_2Et	CO_2tBu	**5b**	82	**6b**	0
4c	9	-	CO_2Bn	CO_2tBu	**5c**	68	**6c**	13
4d	9	-	CN	CO_2Et	**5d**	33	**6d**	23
4e	9	-	CN	CN	**5e**	42	**6e**	14
4a	6	-	CO_2Me	CO_2Me	**5f**	65	**6f**	11
4b	6	-	CO_2Et	CO_2tBu	**5g**	79	**6g**	5
4c	6	-	CO_2Bn	CO_2tBu	**5h**	67	**6h**	9
4d	6	-	CN	CO_2Et	**5i**	51	**6i**	21

[a] Dialkenylation with CH_2=$CH(CH_2)_m$Br took place in a single step.

Table 2. Second alkenylation of monoalkenylated malonate derivatives with CH_2=$CH(CH_2)_n$Br.

Starting compound	m[a]	n	X	Y	Dialkenylation Product	Yield [%]
5a	9	9	CO_2Me	CO_2Me	**6a**	82
5b	9	9	CO_2Et	CO_2tBu	**6b**	95
5c	9	9	CO_2Bn	CO_2tBu	**6c**	84
5b	9	6	CO_2Et	CO_2tBu	**6j**	81
5d	9	2	CN	CO_2Et	**6k**	63
5e	9	2	CN	CN	**6l**	86
5f	6	6	CO_2Me	CO_2Me	**6f**	86
5g	6	6	CO_2Et	CO_2tBu	**6g**	87
5h	6	6	CO_2Bn	CO_2tBu	**6h**	64
5h	6	9	CO_2Bn	CO_2tBu	**6m**	86
5a	9	2	CO_2Me	CO_2Me	**6n**	79
5b	9	2	CO_2Et	CO_2tBu	**6o**	77

[a] The first alkenylation was carried out by treatment with CH_2=$CH(CH_2)_m$Br (see Table 1).

Table 3. Olefin metathesis of dialkenyl glycine derivatives.

Ring size	Compound	m	n	X	Y	Conditions T[a]	Time [h]	Product	Yield [%]	E/Z ratio[b]
21	**6a**	9	9	CO_2Me	CO_2Me	A	78	**7a**	64	85:15
21	**6b**	9	9	CO_2Et	CO_2tBu	B	26	**7b**	82	83:17
21	**6c**	9	9	CO_2Bn	CO_2tBu	B	12	**7c**	84	64:36
21	**6d**	9	9	CN	CO_2Et	A	163	**7d**	5	77:23
21	**6e**	9	9	CN	CN	A	118[c]	**7e**	23	79:21
18	**6j**	9	6	CO_2Et	CO_2tBu	B	12	**7j**	93	50:50
18	**6m**	6	9	CO_2Bn	CO_2tBu	B	20	**7m**	92	45:55
15	**6f**	6	6	CO_2Me	CO_2Me	A	24	**7f**	43	66:34
15	**6g**	6	6	CO_2Et	CO_2tBu	B	16	**7g**	62	49:51
15	**6h**	6	6	CO_2Bn	CO_2tBu	B	14	**7h**	72	50:50
15	**6i**	6	6	CN	CO_2Et	B	67	**7i**	8	nd
14	**6k**	9	2	CN	CO2Et	B	20	**7o**	5	nd
14	**6l**	9	2	CN	CN	A	50[d]	**7l**	0	-
14	**6n**	9	2	CO_2Me	CO_2Me	A	18[e]	**7n**	36	52:48
14	**6o**	9	2	CO_2Et	CO_2tBu	B	21	**7k**	32	nd

[a] A: room temperature; B: reflux. In both cases, the reactions were carried out in CH_2Cl_2. [b] Determined from the relative intensities of the peaks of the olefinic carbon atoms in the ^{13}C NMR spectra. [c] Additional heating at reflux for 9 h. [d] Additional heating at reflux for 7 h. [e] Additional heating at reflux for 118 h.

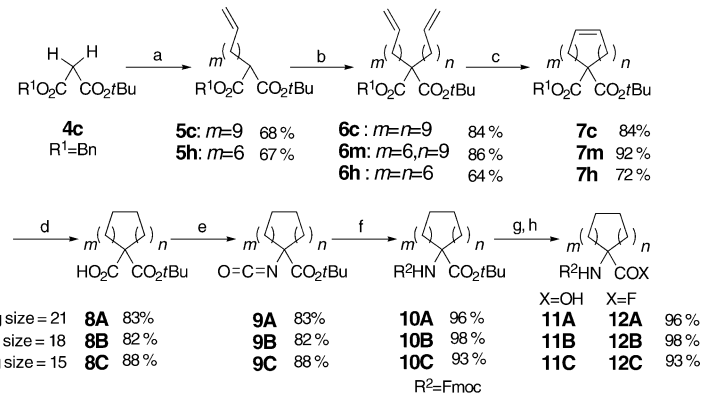
Helical peptides containing the amino acid bearing an 18-membered ring: The Fmoc solid-phase method has become the most widely used technique of peptide synthesis because of its easy handling.[13] Solution synthesis of oligopeptides containing α,α-disubstituted cycloaliphatic amino acids with medium-sized rings has been reported.[5,6] However, the applicability of the Fmoc solid-phase method to α,α-disubstituted glycines bearing a large cycloaliphatic ring has been little studied.

To examine the feasibility of solid-phase synthesis of amino acids bearing bulky hydrophobic side chains, which might cause considerable steric hindrance at the Cα-position, 17-amino-acid model peptides **II**–**IV** were designed (Figure 2). The design of these peptides was based on the sequence of the helical peptide **I**, which is composed of alanine, lysine, and glutamic acid residues and has been described by Marqusee and Baldwin.[14] Peptide **I** was shown to adopt a monomeric, α-helical structure by forming Glu^-–Lys^+ salt bridges between the side chains of these residues. We replaced the two alanine residues in peptide **I** (positions 3 and 10) with a C18 amino acid (peptide **II**) so that the C18 rings were both placed on the same side of the helix. We hypothesized that this arrangement might allow the peptide to form an assembly through the interaction of alternate large hydrophobic side chains with each other, as in a zipper motif (see Figure 3).

The energy-minimized structures of peptides **I** and **II** were obtained by using the OPLS-AA force field.[15] Two arrangements of the two 18-membered rings that produce stable conformations were found (Figure 3). Conformer A is more stable than conformer B by 11.2 kcal mol^{-1} (OPLS-AA force field and water as a solvent).

We examined the effect of the resulting hydrophobic cores of the peptide structures on helicity. For comparison, peptides with one C18 amino acid residue near the center (at position 10, peptide **III**) or near the N terminus (at position 3, peptide **IV**) were synthesized (Figure 2).

The peptide chains were constructed by Fmoc solid-phase peptide synthesis on Rink amide resin.[16] Although the steric hindrance at the Cα atom caused by the C18 ring might lead to inhibition of peptide chain elongation, a combination of Fmoc protection and acid fluoride activation allowed successful addition of the C18 amino acid and the next amino acids to the peptide chain.[9] A coupling system



Scheme 3. Synthesis of the Fmoc amino acid fluorides of macrocyclic α,α-disubstituted amino acids. a–c) As in Scheme 2; d) H_2, Pd/C, AcOEt; e) DPPA, Et_3N, benzene, reflux; f) 9-fluorenylmethanol, toluene, reflux; g) TFA, CH_2Cl_2, room temperature; h) DAST, CH_2Cl_2, room temperature. DAST = diethylaminosulfur trifluoride, DPPA = diphenylphosphoryl azide, TFA = trifluoroacetic acid.

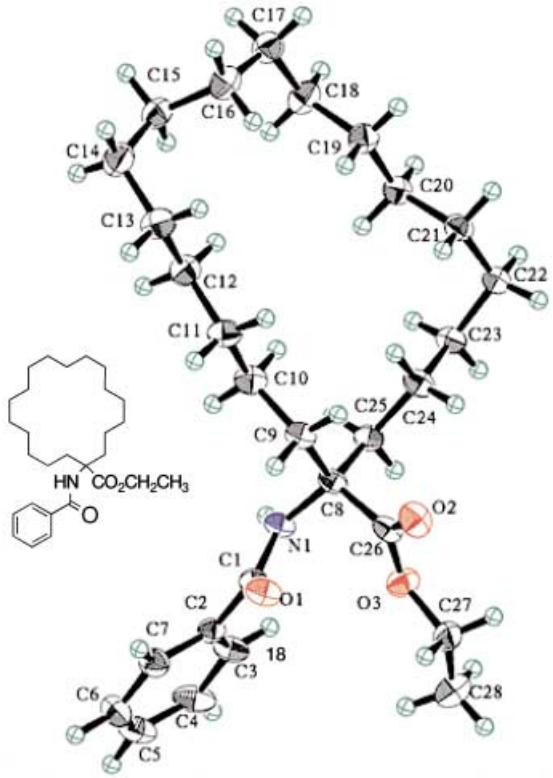


Figure 1. Single-crystal X-ray diffraction structure of the *N*-benzyl ethyl ester derivative of compound **2**, which contains an 18-membered ring.

Ac-AE[3]AAAKEAA[10]AKEAAAKA-NH_2 peptide **I** (native)

Ac-AE(**2**,C18)AAKEAA(**2**,C18)KEAAAKA-NH_2 peptide **II**

Ac-AEAAAKEAA(**2**,C18)KEAAAKA-NH_2 peptide **III**

Ac-AE(**2**,C18)AAKEAAAKEAAAKA-NH_2 peptide **IV**

(A=Ala, E=Glutamic acid, K=Lysine)

Figure 2. Amino acid sequences of peptides used in this study.

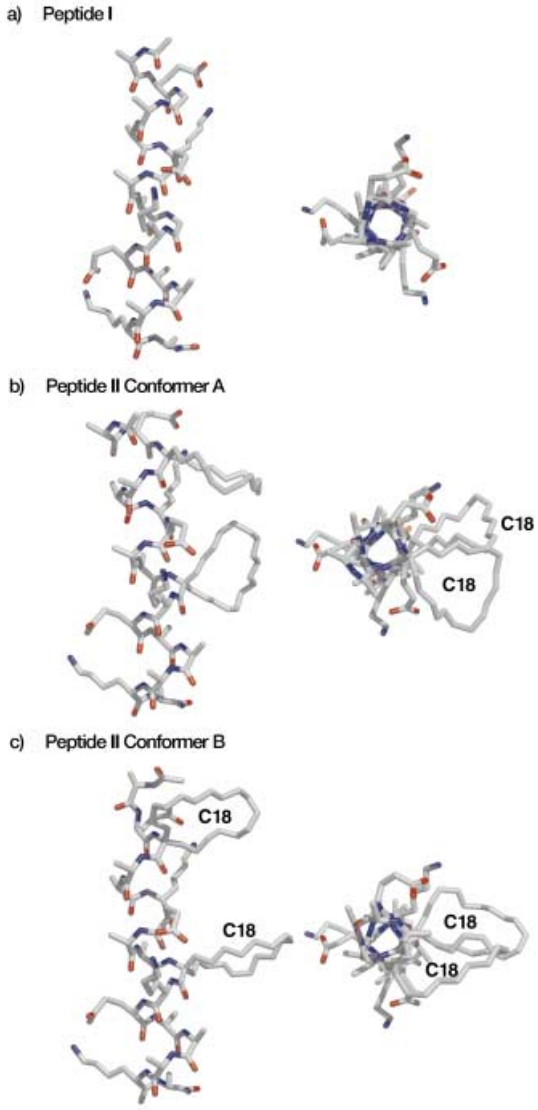


Figure 3. Energy-minimized α-helical structures of a) peptide **I** (original Baldwin peptide), b) conformer A of peptide **II**, c) confomer B of peptide **II**. Conformers A and B differ in the orientations of the two 18-membered rings. Conformer A is more stable than conformer B. Water was used as a solvent in the force field calculations.

involving benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP),[17] 1-hydroxybenzotriazole (HOBt), and 4-methylmorpholine (NMM) was used for the introduction of other amino acids to the peptide chain. The construction of the peptide was straightforward. No double coupling experiments were carried out for the introduction of amino acids in this experiment. The N terminus of the peptide chain was acetylated by treatment with acetic anhydride in the presence of NMM. The peptide resin was treated with trifluoroacetic acid/ethanedithiol (95:5) at 25 °C for 2 h and the sample was then purified by HPLC separation to give a pure peptide. The proposed structure of the product was supported by MALDI-TOF MS data. Total

yields of the peptides calculated on the basis of the amount of starting resin were 48 % (peptide **I**), 10 % (peptide **II**), 16 % (peptide **III**), and 32 % (peptide **IV**).[18] The retention times of the peptides in reversed-phase HPLC analyses in the same eluent provide information about the hydrophobicity of the peptides: those containing the C18 amino acid have a longer retention time than those without such a residue. Peptide **II** has the longest retention time and is therefore likely to be the most hydrophobic: 12.0 min (peptide **I**), 29.0 min (peptide **II**), 20.0 min (peptide **III**), 22.3 min (peptide **IV**).[19]

The CD spectra of peptides **I**–**IV** in the presence of 10 mM NaCl were all consistent with a helical structure (Figure 4a). The helical content of these peptides can be

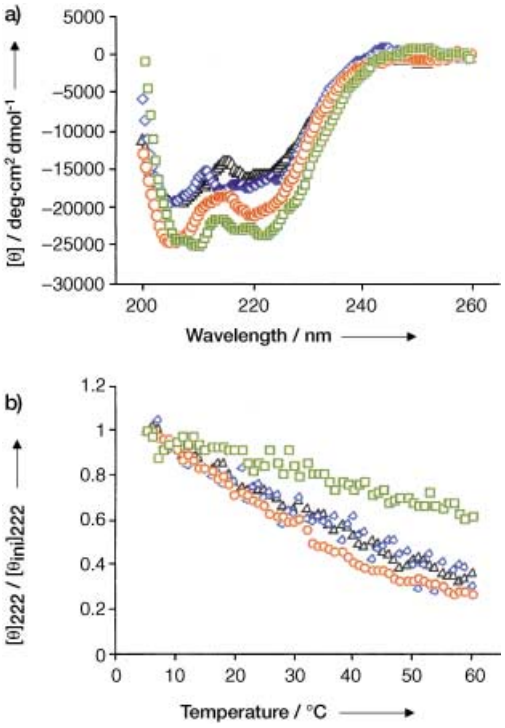


Figure 4. a) CD spectra of the peptides containing α,α-disubstituted glycine (**2**, C18) residue(s); b) the thermal stabilities of these peptides. Red circles, peptide **I** (20 μM); green squares, peptide **II** (16 μM); blue diamonds, peptide **III** (12 μM); black triangles, peptide **IV** (13 μM). All peptides were in a solution of 1 mM sodium citrate, 1 mM sodium borate, 1 mM sodium phosphate, 10 mM sodium chloride (pH 7.3). Spectra were taken at 20 °C (a), and with temperature elevation at 1 °C min^{-1} (b). The helical content of the peptides is compared in (b) in the form of the ratio $[\theta]_{222}/[\theta_{ini}]_{222}$, where $[\theta_{ini}]_{222}$ denotes molecular ellipticity at 222 nm and 5 °C.

evaluated from the intensity of the absorption at 222 nm. The $[\theta]_{222}$ values in order of magnitude are: peptide **II** (-2.37×10^4 deg cm^2 dmol^{-1}) > peptide **I** (-2.03×10^4 deg cm^2 dmol^{-1}) > peptide **III** (-1.65×10^4 deg cm^2 dmol^{-1}) ≈ peptide **IV** (-1.57×10^4 deg cm^2 dmol^{-1}). Peptide **II** showed significantly higher thermal stability than the other peptides (including the native peptide **I**; Figure 4b); although no significant cooperative transition between the folded and unfolded states was observed between 5 and 60 °C, the helical content of peptide **II** at 60 °C was 62 % of that observed at 5 °C, while the helicities of the other peptides (**I**, **III**, and **IV**) at 60 °C were approximately 30 % of those at 5 °C.

The helicity of peptide **II** is highly dependent on salt concentration (Figure 5a). The $[\theta]_{222}/[\theta]_{208}$ ratio calculated from the CD spectrum of peptide **II** in the presence of 100 mM NaCl was approximately 1.7 ($[\theta]_{222}$: -2.73×10^4 deg cm^2 dmol^{-1}; $[\theta]_{208}$: -1.64×10^4 deg cm^2 dmol^{-1}), while the corresponding value in the presence of 10 mM NaCl was 0.98 ($[\theta]_{222}$: -2.37×10^4 deg cm^2 dmol^{-1}; $[\theta]_{208}$: -2.42×10^4 deg cm^2 dmol^{-1}). This considerable change in $[\theta]_{222}/[\theta]_{208}$ ratio suggests that the peptide may have an aggregated structure in 100 mM NaCl.[20] In contrast, the $[\theta]_{222}/[\theta]_{208}$ ratio of peptide **I** in the presence of 100 mM NaCl was not significantly different from that with 10 mM NaCl (100 mM NaCl: $[\theta]_{222} = -1.85\times10^4$ deg cm^2 dmol^{-1}; $[\theta]_{208} = -1.65\times10^4$ deg cm^2 dmol^{-}; $[\theta]_{222}/[\theta]_{208} = 1.1$. 10 mM NaCl: $[\theta]_{222} = -2.03\times10^4$ deg cm^2 dmol^{-}; $[\theta]_{208} = -2.31\times10^4$ deg cm^2 dmol^{-}; $[\theta]_{222}/[\theta]_{208} = 0.90$; Figure 5b).

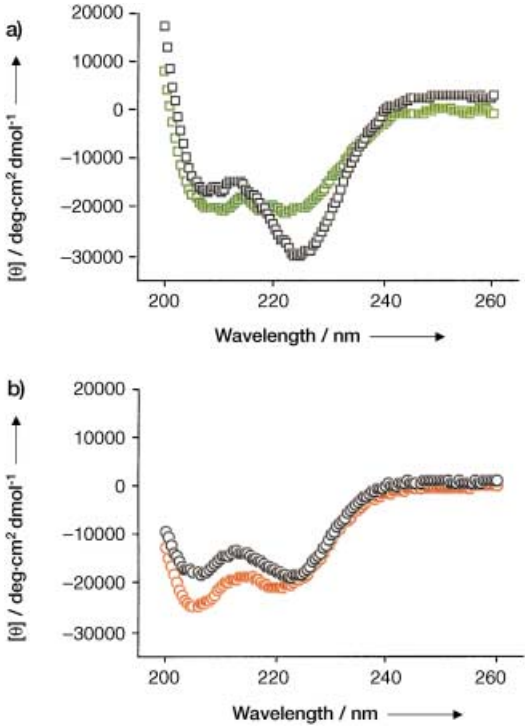


Figure 5. The effect of salt concentration on the CD spectra of a) peptide **II**, which contains α,α-disubstituted amino acid **2** (C18) and b) peptide **I** (does not contain the C18 residue). Spectra were measured with the peptides in solutions containing 100 mM sodium chloride (black) or 10 mM sodium chloride (colored) and in both cases 1 mM sodium citrate, 1 mM sodium borate, and 1 mM sodium phosphate (pH 7.3). The peptide concentration was 20 μM.

The assembly of peptide **II** was assessed by sedimentation equilibrium experiments at 40000 rpm (Figure 6a).[21,22] Nonlinear least-squares fitting to Equation (1) (see the Experimental Section) gave an apparent molecular weight (M_{app}) of $(1.13\pm0.056)\times10^4$, $(1.19\pm0.060)\times10^4$, $(1.27\pm0.064)\times10^4$, or $(1.24\pm0.062)\times10^4$ at peptide concentrations of 195, 220, 400, and 900 μM, respectively. The results obtained with a rotor speed of 30000 rpm gave similar values

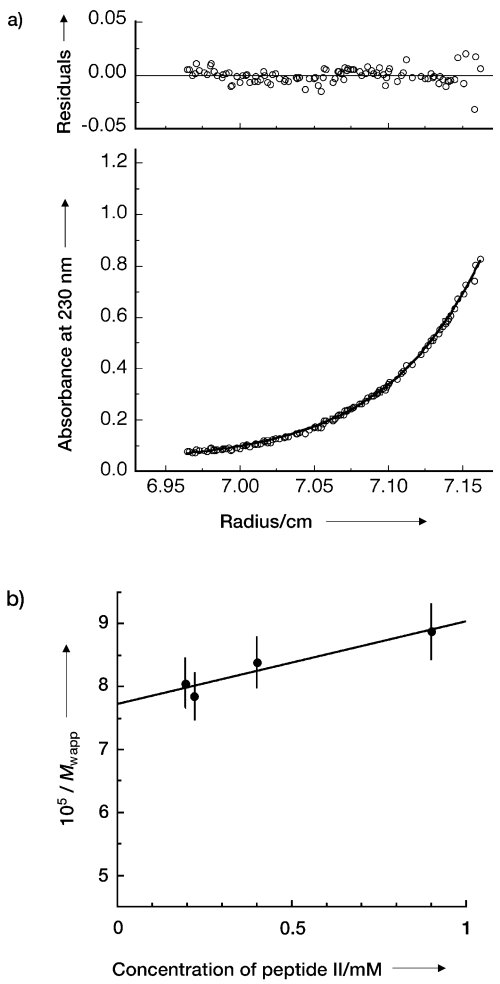


Figure 6. a) Sedimentation equilibrium analysis of peptide **II**. Peptide **II** (195 μM) was run at 40000 rpm at 20°C in 1 mM sodium citrate, 1 mM sodium borate, 1 mM sodium phosphate, and 10 mM sodium chloride (pH 7.3). b) Relationship between the peptide concentration and observed molecular weight (M_{app}). The mean molecular weight (M_w), which correponds to the reciprocal of the intercept on the ordinate at zero concentration, was calculated as $(1.30 \pm 0.065) \times 10^4$, which suggests that peptide **II** exists as a hexamer in the given solution.

of M_{app} within the range of experimental error (5%). The weight-average molecular weight for the peptide at infinite dilution (M_w) was calculated as $(1.30 \pm 0.065) \times 10^4$ (Figure 6 b), which is very close to the molecular weight of the hexamer (12348). The symmetrical residuals and the small 95% confidence intervals indicate the absence of the monomer and aggregates other than the hexamer in solution.

In conclusion, we have developed a novel approach for the preparation of α,α-disubstituted glycines bearing a large hydrophobic ring. These amino acids could be readily incorporated into a peptide chain by Fmoc solid-phase peptide synthesis and the acid fluoride activation method. A 17-amino-acid peptide containing two residues substituted with a C18 ring formed a stable helical structure, which suggests a potential helicogenic effect of these new amino acids. This result is consistent with the increased magnitude and thermal stability of helicity indicated by CD spectroscopy for this peptide. The 17-amino-acid peptide was also shown by sedimentation equilibrium analysis to aggregate to a hexamer. Although further study is necessary to elucidate the precise conformation of peptide **II**, it is clear that these α,α-disubstituted glycines with large hydrophobic rings will be useful as building blocks of peptide hyperstructure.

Experimental Section

General methods: All melting points were measured with a Yanaco Micro melting point apparatus and the uncorrected values are reported. Proton NMR spectra were measured on a JEOL Caliber-GX400 NMR spectrometer at 400 MHz with tetramethylsilane as an internal reference and $CDCl_3$ as the solvent, unless otherwise specified. High-resolution mass spectra (HRMS, EI^+) and FAB mass spectra (FAB^+) were recorded on a JEOL JMS-SX 102A spectrometer. Flash column chromatography was carried out on silica gel (silica gel 60, 40–63 μm, Merck). The combustion analyses were carried out in the microanalytical laboratory of the Graduate School of Pharmaceutical Sciences, The University of Tokyo. Matrix-assisted laser desorption ionization time-of-flight mass spectra (MALDI-TOF MS) were recorded on an Applied Biosystems Voyager-DE STR instrument. CD spectra were recorded on a Jasco J-600 spectrometer in a 2-mm cuvette. The full data for the synthetic materials are given in the Supporting Information.

Monoalkenylation of malonate derivatives

*Dimethyl monoundecenylmalonate (**5a**)*: A solution of dimethyl malonate (**4a**; 141.1 mg, 1.07 mmol) in DMSO (5 mL) was added dropwise to a solution of NaH (33.6 mg, 1.40 mmol, 1.3 equiv) in DMSO (4 mL) at room temperature. A solution of 11-bromo-1-undecene (315.3 mg, 1.35 mmol, 1.3 equiv) in DMSO (5 mL) was added and the mixture was stirred at room temperature for 4 h then added to ice-water and extracted with diethyl ether (4×30 mL). The organic phase was washed with brine and dried over Na_2SO_4. The residue (326.7 mg) obtained after evaporation of the solvent was flash chromatographed (silica gel; EtOAc/*n*hexane (1:18)) to give **6a** (dialkenyl product; 30.9 mg, yield: 7%; colorless oil) and **5a** (monoalkenyl product; 194.2 mg, yield: 64%; yellow oil).

5a: ^{1}H NMR: $\delta = 5.813$ (d, d, t, $J = 17.05$, 10.27, 6.78 Hz, 1H; CH_2=C*H*), 4.992 (d, d, d, $J = 17.05$, 3.67, 1.47 Hz, 1H; CH_2=CH-*cis*), 4.929 (m, 1H; CH_2=CH-*trans*), 3.738 (s, 6H; 2×$COOCH_3$), 3.359 (t, $J = 7.52$ Hz, 1H; CH), 2.063–2.009 (m, 2H; CH_2=CH–CH_2), 1.903–1.885 (m, 2H), 1.368–1.262 ppm (m, 14H).

6a: ^{1}H NMR: $\delta = 5.881$ (d, d, t, $J = 16.86$, 10.08, 6.60 Hz, 2H; C*H*=CH_2), 4.990 (d, d, d, $J = 17.05$, 3.67, 1.47 Hz, 2H; CH=CH_2-*cis*), 4.928 (m, 2H; CH=CH_2-*trans*), 3.704 (s, 6H; CO_2CH_3), 2.062–2.009 (m, 4H; CH_2=CH–CH_2), 1.877–1.834 (m, 4H), 1.367–1.117 ppm (m, 28H). HRMS: calcd for $C_{27}H_{48}O_4$: 436.3554; found: 436.3551.

Benzyl tert-*butyl undecenylmalonate (**5c**)*: Colorless oil (68% yield). ^{1}H NMR: $\delta = 7.355$–7.310 (m, 5H; Ph), 5.808 (d, d, t, $J = 17.04$, 10.08, 6.60 Hz, 1H; CH_2=C*H*), 5.163 (d, d, $J = 12.27$, 12.27 Hz, 2H; PhCH_2), 4.990 (d, d, d, $J = 17.04$, 3.11, 1.47 Hz, 1H; CH_2=CH-*cis*), 4.939–4.908 (m, 1H; CH_2=CH-*trans*), 3.267 (t, $J = 7.51$ Hz, 1H; CH), 2.034 (m, 2H; CH_2=CH–CH_2), 1.855 (m, 2H), 1.381 (s, 9H; *tert*-butyl), 1.348–1.241 ppm (m, total 14H).

6c (diundecanyl derivative): Colorless oil (13% yield). ^{1}H NMR: $\delta =$ 7.355–7.298 (m, 5H; Ph), 5.807 (d, d, t, $J = 16.85$, 10.08, 6.60 Hz, 2H; CH_2=C*H*), 5.137 (s, 2H; PhCH_2), 4.988 (d, d, d, $J = 17.04$, 3.66, 1.65 Hz, 2H; CH_2=CH-*trans*), 4.925 (m, 2H; CH_2=CH-*cis*), 2.034 (m, 4H; CH_2=CH–CH_2), 1.822 (m, 4H), 1.325 (s, 9H; *tert*-butyl), 1.381–1.241 ppm (m, total 28H).

Benzyl tert-*butyl octenylmalonate (**5h**)*: Colorless oil (67% yield). ^{1}H NMR: $\delta = 7.355$–7.309 (m, 5H; Ph), 5.792 (d, d, t, $J = 16.86$, 10.08, 6.59 Hz, 1H; CH_2=CH), 5.163 (d, d, $J = 12.27$, 12.27 Hz, 2H; PhCH_2), 4.983 (d, d, d, $J = 17.04$, 3.48, 1.47 Hz, 1H; CH_2=CH-*trans*), 4.23 (m, 1H; CH_2=CH-*cis*) 3.267 (t, $J = 7.51$ Hz, 1H; CH), 2.023 (m, 2H; CH_2=CH-CH_2), 1.857 (m, 2H), 1.382 (s, 9H; *tert*-butyl), 1.348–1.241 ppm (m, total 8H).

6h (dioctenyl derivative): Colorless oil (9% yield). ^{1}H NMR: $\delta = 7.348$–7.300 (m, 5H; Ph), 5.789 (d, d, t, $J = 17.04$, 10.26, 6.78 Hz, 2H; CH_2=C*H*), 5.137 (s, 2H; PhCH_2), 4.926 (d, d, d, $J = 17.04$, 3.66, 1.47 Hz, 2H; CH_2=

CH-*trans*), 4.922 (m, 2H; CH_2=CH-*cis*), 2.016 (m, 4H; CH_2=CHCH_2), 1.824 (br t, J = 8.43 Hz, 4H), 1.325 (s, 9H; *tert*-butyl), 1.354–1.115 ppm (m, total 16H).

Second alkenylation of malonate derivatives

*Dimethyl diundecenylmalonate (**6a**)*: A solution of dimethyl undecenylmalonate (**5a**; 187.3 mg, 0.66 mmol) in DMSO (2 mL) was added dropwise to a solution of NaH (29.6 mg, 1.2 mmol, 1.9 equiv) in DMSO (3 mL) at room temperature. A solution of 11-bromo-1-undecene (211.0 mg, 0.91 mmol, 1.4 equiv) in DMSO (1 mL) was added dropwise and the mixture was stirred for 5.5 h at room temperature. The whole mixture was poured into ice-water and extracted with diethyl ether. The organic layer was washed with brine, dried over Na_2SO_4, and the solvent was evaporated to give a residue (332.7 mg), which was flash chromatographed (silica gel; EtOAc/*n*hexane (1:18)) to give **6a** (237.3 mg, 82% yield, colorless oil).

Benzyl tert-*butyl diundecenylmalonate (**6c**)*: Colorless oil (84% yield).

Benzyl tert-*butyl dioctenylmalonate (**6h**)*: Colorless oil (64% yield).

Benzyl tert-*butyl octenylundecenylmalonate (**6m**)*: Colorless oil (86% yield). ^{1}H NMR: δ = 7.355–7.298 (m, 5H; Ph), 5.798 (m, 2H; CH_2=C*H*), 5.136 (s, 2H; $PhCH_2$), 4.982 (m, 2H; CH_2=CH-*trans*), 4.923 (m, 2H; CH_2=CH-*cis*), 2.035 (m, 4H; CH_2=CH–CH_2), 1.823 (m, 4H), 1.325 (s, 9H; *tert*-butyl), 1.398–1.092 ppm (m, total 22H).

Olefin ring-closing metathesis of dialkenyl glycine derivatives

*Methyl 1-(methoxycarbonyl)cyclohenicos-11-enecarboxylate (**7a**)*: A solution of diene **6a** (234.3 mg, 0.54 mmol) in dichloromethane (10 mL) was added over a period of 1 min to a solution of Grubbs' catalyst (benzylidene-bis(tricyclohexylphosphine)dichlororuthenium; 30.9 mg, 0.038 mmol, 7.0 mol%) in dichloromethane (50 mL) bubbled through with argon and the mixture was stirred under an Ar atmosphere at room temperature for 16.5 h. Since the reaction was incomplete, Grubbs' catalyst (21.6 mg, 0.025 mmol, 4.6 mol%) was added again and the whole mixture was stirred for 24 h. After evaporation of the solvent, the obtained residue (290.0 mg) was flash chromatographed (silica gel; EtOAc/*n*-hexane (1:22)) to give **7a** (140.4 mg, 64%). M.p.: 71.3–72.3 °C (colorless plates, recrystallized from methanol). ^{1}H NMR: δ = 5.330 (m, 2H; CH=CH), 3.700 (s, 6H; CO_2CH_3), 2.010 (m, 4H), 1.882 (m, 4H), 1.326–1.090 ppm (m, 28H). E/Z = 83/17 (determined from the ^{13}C NMR signals). Elemental analysis calcd (%) for $C_{25}H_{44}O_4$: C 73.48, H 10.85; found: C 73.25, H 10.87. MS (EI): 408 $[M]^+$.

tert-*Butyl 1-(benzyloxycarbonyl)cyclohenicos-11-enecarboxylate (**7c**)*: A solution of diene **6c** (2.5219 g, 4.55 mmol) in dichloromethane (250 mL) was added over a period of 30 min to a solution of Grubbs' catalyst (194.2 mg, 0.24 mmol, 6 mol%) in dichloromethane (750 mL) bubbled through with argon at room temperature. The mixture was heated at reflux with stirring under an Ar atmosphere for 12 h. After evaporation of the solvent, the obtained residue (290.0 mg) was flash chromatographed (silica gel; EtOAc/*n*hexane (1:30)) to give **7c** (2.0146 g, 84%, colorless oil), together with an unidentified dimeric product (228.1 mg, yield: 10%, pale yellow solid).

7c: ^{1}H NMR: δ = 7.342–7.289 (m, 5H; Ph), 5.326 (m, 2H; CH=CH), 5.137 (s, 2H; $PhCH_2$), 2.019 (m, 4H; CH_2–CH=CH–CH_2), 1.844 (m, 4H), 1.309 (s, 9H; *t*butyl) 1.339–1.078 ppm (m, total 32H). E/Z = 36/64 (determined from the ^{13}C NMR signals).

Dimeric product: ^{1}H NMR: δ = 7.343–7.297 (m, 10H; Ph), 5.382–5.328 (m, 4H; CH=CH), 5.136 (s, 4H; $PhCH_2$), 2.015–1.956 (m, 8H; CH_2–CH=CH–CH_2), 1.831 (m, 8H), 1.320 (s, 18H; *tert*-butyl), 1.436–1.082 ppm (m, total 64H).

tert-*Butyl 1-(benzoyloxycarbonyl)cyclopentadec-8-ene carboxylate (**7h**)*: A solution of diene **6h** (1.5216 g, 3.22 mmol) in dichloromethane (200 mL) was added over 20 min to a solution of Grubbs' catalyst (136.7 mg, 0.17 mmol, 5 mol%) in dichloromethane (500 mL) bubbled through with argon, at room temperature. The mixture was heated at reflux with stirring under an Ar atmosphere for 14 h. After evaporation of the solvent, the obtained residue was flash chromatographed (silica gel; EtOAc/*n*hexane (1:25→1:10) to give **7h** (1.0259 g, 72%, colorless oil), together with an undefined dimeric product (299.8 mg, 22% yield, brown oil).

7h: ^{1}H NMR: δ = 7.347–7.257 (m, 5H; Ph), 5.398–5.321 (m, 2H; CH=CH), 5.135–5.123 (s, 2H; $PhCH_2$), 2.027–1.974 (m, 4H; CH=CH–CH_2), 1.882–1.755 (m, 4H), 1.304–1.297 (s, 9H; *tert*-butyl), 1.422–1.052 ppm (m, total 16H). E/Z = 50/50 (determined from the ^{13}C NMR signals).

Dimeric product: ^{1}H NMR: δ = 7.343–7.289 (m, 10H; Ph), 5.352–5.320 (m, 4H; CH=CH), 5.136 (s, 4H; $PhCH_2$), 2.044–1.953 (m, 8H; CH_2–CH=CH–CH_2), 1.835 (m, 8H), 1.318 (s, 18H; *tert*-butyl), 1.495–1.084 ppm (m, total 32H).

tert-*Butyl 1-(benzoyloxycarbonyl)cyclooctadec-8-ene carboxylate (**7m**)*: A solution of diene **6m** (2.2836 g, 4.46 mmol) in dichloromethane (250 mL) was added over a period of 30 min to a solution of Grubbs' catalyst (196.8 mg, 0.24 mmol, 5 mol%) in dichloromethane (750 mL) bubbled through with argon, at room temperature. The mixture was heated at reflux with stirring under an Ar atmosphere for 20 h. After evaporation of the solvent, the obtained residue was flash chromatographed (silica gel; EtOAc/*n*hexane (1:30→1:20)) to give **7m** (1.9908 g, 92%, colorless oil), together with an undefined dimeric product (152.2 mg, 8% yield, brown oil).

7m: ^{1}H NMR: δ = 7.346–7.259 (m, 5H; Ph), 5.283 (m, 2H; CH=CH), 5.140 (s, 2H; $PhCH_2$), 2.033–1.981 (m, 4H; CH_2-CH=CH–CH_2), 1.883–1.822 (m, 4H), 1.310 (s, 9H; *tert*-butyl), 1.423–1.080 ppm (m, total 22H). E/Z = 45/55 (determined from the ^{13}C NMR signals).

Dimeric product: ^{1}H NMR: δ = 7.345–7.257 (m, 10H; Ph), 5.360–5.298 (m, 4H; CH=CH), 5.137 (s, 4H; $PhCH_2$-), 2.006–1.961 (m, 8H; CH_2-CH=CH–CH_2), 1.832 (m, 8H), 1.320 (s, 18H; *tert*-butyl), 1.495–1.084 ppm (m, total 44H).

Synthesis of acid fluorides 13A–C

*1-(*tert-*butoxycarbonyl)cyclohenicosane carboxylic acid (**8A**)*: A solution of *t*butyl 1-(benzyloxycarbonyl)cyclohenicos-11-enecarboxylate (**7c**; 2.0146 g, 3.83 mmol) in ethyl acetate (450 mL) was hydrogenated over 10% Pd/C (265.0 mg) at room temperature for 24 h. Filtration and evaporation of the solvent gave a residue (1.8042 g), which was flash chromatographed ($CHCl_3$/MeOH (40:1)) to give the saturated product **8A** (1.3996 g, 83% yield) as a colorless solid. M.p.: 89.2–91.3 °C. (colorless plates, recrystallized from *n*hexane). ^{1}H NMR: δ = 1.870 (m, 4H), 1.474 (s, 9H; *tert*-butyl), 1.290–1.126 ppm (m, total 36H). Elemental analysis calcd (%) for $C_{27}H_{50}O_4$: C 73.92, H 11.49; found: C 73.77, H 11.54.

*1-(*tert-*butoxycarbonyl)cyclooctadecane carboxylic acid (**8B**)*: A solution of *tert*-butyl 1-(benzyloxycarbonyl)cyclooctadec-8-enecarboxylate (**7m**; 1.9908 g, 4.11 mmol) in a mixture of ethyl acetate (250 mL) and methanol (50 mL) was hydrogenated over 10% Pd/C (268.8 mg) at room temperature for 24 h. Filtration and evaporation of the solvent gave a residue (1.8042 g), which was flash chromatographed ($CHCl_3$/MeOH (40:1)) to give the saturated product **8B** (1.3295 g, 82% yield) as a colorless solid. M.p.: 110.5–112.0 °C (colorless plates, recrystallized from *n*hexane). ^{1}H NMR: δ = 1.890 (m, 4H), 1.460 (s, 9H; *tert*-butyl), 1.401–1.192 ppm (m, total 30H). Elemental analysis calcd (%) for $C_{24}H_{44}O_4$: C 72.68, H 11.18; found: C 72.56, H 11.27.

*1-(*tert-*butoxycarbonyl)cyclopentadecane carboxylic acid (**8C**)*: A solution of *tert*-butyl 1-(benzyloxycarbonyl)cyclopentadec-8-enecarboxylate (**7h**; 1.0065 g, 2.28 mmol) in a mixture of ethyl acetate (300 mL) was hydrogenated over 10% Pd/C (130.8 mg) at room temperature for 28 h. Filtration and evaporation of the solvent gave a residue (812.0 mg), which was flash chromatographed ($CHCl_3$/MeOH (30:1)) to give the saturated product **8C** (712.7 mg, 88% yield) as a colorless solid. M.p.: 137.2–138.6 °C (colorless plates, recrystallized from *n*hexane). ^{1}H NMR: δ = 1.698 (m, 4H), 1.498 (s, 9H; *t*butyl), 1.477–1.242 ppm (m, total 24H). Elemental analysis calcd (%) for $C_{21}H_{38}O_4$: C 71.15, H 10.80; found: C 71.03, H 10.93.

tert-*Butyl 1-isocyanatocyclohenicosanecarboxylate (**9A**)*: A solution of 1-(*tert*-butoxycarbonyl)cyclohenicosane carboxylic acid (**8A**; 1.2402 g, 2.83 mmol), DPPA (1.0423 g, 3.79 mmol, 1.3 equiv), and triethylamine (293.1 mg, 2.90 mmol, 1.0 equiv) in benzene (15 mL) was heated at reflux under an argon atmosphere for 2 h. After evaporation of the solvent, the residue was flash chromatographed (silica gel; EtOAc/*n*hexane (1:25)) to give the product **9A** (1.1827 g, 96% yield) as a colorless oil. IR (neat): $\tilde{\nu}$ = 2250 cm^{-1} (N=C=O). ^{1}H NMR: δ = 1.761–1.608 (m, 4H), 1.497 (s, 9H; *tert*-butyl), 1.469–1.205 ppm (m, total 36H).

tert-*Butyl 1-isocyanatocyclooctadecanecarboxylate (**9B**)*: A solution of 1-(*tert*-butoxycarbonyl)cyclooctadecane carboxylic acid (**8B**; 1.2158 g, 3.07 mmol), DPPA (1.1067 g, 4.02 mmol, 1.3 equiv), and triethylamine

(319.8 mg, 3.17 mmol, 1.0 equiv) in benzene (15 mL) was heated at reflux under an argon atmosphere for 3 h. After evaporation of the solvent, the residue was flash chromatographed (silica gel; EtOAc/*n*hexane (1:25)) to give the product **9B** (1.1813 g, 98% yield) as a colorless oil. IR (neat): $\tilde{\nu}=2250\ cm^{-1}$ (N=C=O). ^1H NMR: $\delta=1.698$ (m, 4H), 1.498 (s, 9H; *tert*-butyl), 1.477–1.242 ppm (m, total 30H).

tert-*Butyl 1-isocyanatocyclopentadecanecarboxylate (**9C**)*: A solution of 1-(*tert*-butoxycarbonyl)cyclooctadecane carboxylic acid (**8C**; 641.0 mg, 1.81 mmol), DPPA (645.5 mg, 2.35 mmol, 1.3 equiv), and triethylamine (188.1 mg, 1.86 mmol, 1.0 equiv) in benzene (10 mL) was heated at reflux under an argon atmosphere for 2 h. After evaporation of the solvent, the residue was flash chromatographed (silica gel; EtOAc/*n*hexane (1:20)) to give the product **9C** (570.5 mg, 90% yield) as a colorless oil. IR (neat): $\tilde{\nu}=2250\ cm^{-1}$ (N=C=O). ^1H NMR: $\delta=1.819$–1.653 (m, 4H), 1.496 (s, 9H; *tert*-butyl), 1.420–1.317 ppm (m, total 24H).

tert-*Butyl 1-(Fmoc-amino)cyclohenicosanecarboxylate (**10A**)*: A solution of *tert*-butyl 1-isocyanatocyclohenicosanecarboxylate (**9A**; 1.1748 g, 2.70 mmol) and 9-fluorenylmethanol (689.3 mg, 3.51 mmol, 1.3 equiv) in dry toluene (10 mL) was heated at 130°C under an argon atmosphere for 42 h. Flash chromatography (EtOAc/*n*hexane (1:20)) of the mixture led to the recovery of **9A** (185.6 mg, 16%) and gave the *N*-Fmoc ester **10A** (1.4204 g, 73% yield) as a colorless oil. ^1H NMR: $\delta=7.757$ (d, $J=7.51$ Hz, 2H; H_4, H_5), 7.598 (d, $J=7.14$ Hz, 2H; H_1, H_8), 7.389 (d, d, $J=7.51$, 7.51 Hz, 2H; H_3, H_6), 7.303 (d, d, d, $J=7.51$, 7.51, 1.10 Hz, 2H; H_2, H_7), 5.568 (brs, 1H; NH), 4.344 (brd, $J=6.05$ Hz, 2H; H_{10}), 4.213 (t, $J=6.59$ Hz, 1H; H_9), 2.076 (brm, 2H), 1.817 (brm, 2H), 1.292 (s, 9H; *t*Bu), 1.509–1.088 ppm (m, total 36H).

tert-*Butyl 1-(Fmoc-amino)cyclooctadecanecarboxylate (**10B**)*: A solution of *tert*-butyl 1-isocyanatocyclooctadecanecarboxylate (**9B**; 1.1703 g, 2.98 mmol) and 9-fluorenylmethanol (757.9 mg, 3.86 mmol, 1.3 equiv) in dry toluene (3 mL) was heated at 130°C under an argon atmosphere for 12 h. The mixture was flash chromatographed (EtOAc/*n*hexane (1:12→1:10)) to give *N*-Fmoc ester **10B** (1.4454 g, 82% yield) as a colorless amorphous solid. ^1H NMR: $\delta=7.759$ (d, $J=7.51$ Hz, 2H; H_4, H_5), 7.598 (d, $J=7.51$ Hz, 2H; H_1, H_8), 7.392 (d,d, $J=7.33$, 7.33 Hz, 2H; H_3, H_6), 7.303 (d, d, d $J=7.51$, 7.51, 1.10 Hz, 2H; H_2, H_7), 5.217 (brs, 1H; NH), 4.353 (brm, 2H; H_{10}), 4.216 (t, $J=6.78$ Hz, 1H; H_9), 1.882 (m, 4H), 1.453 (s, 9H; *t*Bu), 1.310–1.242 ppm (m, total 30H).

tert-*Butyl 1-(Fmoc-amino)cyclopentadecanecarboxylate (**10C**)*: A solution of *tert*-butyl 1-isocyanatocyclopentadecanecarboxylate (**9C**; 561.2 mg, 1.60 mmol) and 9-fluorenylmethanol (413.4 mg, 2.11 mmol, 1.3 equiv) in dry toluene (2 mL) was heated at 130°C under an argon atmosphere for 20 h. The mixture was flash chromatographed (EtOAc/*n*hexane (1:8)) to give *N*-Fmoc ester **10C** (859.1 mg, 98% yield) as a colorless amorphous solid. ^1H NMR: $\delta=7.759$ (d, $J=7.51$ Hz, 2H; H_4, H_5), 7.598 (d, $J=7.51$ Hz, 2H; H_1, H_8), 7.393 (d, d, $J=7.33$, 7.33 Hz, 2H; H_3, H_6), 7.304 (d, d, d, $J=7.51$, 7.51, 1.28 Hz, 2H; H_2, H_7), 4.888 (brs, 1H; NH), 4.368 (brm, 2H; H_{10}), 4.220 (t, $J=6.76$ Hz, 1H; H_9), 1.889–1.739 (brm, 3H), 1.425 (s, 9H; *t*Bu), 1.366–1.241 ppm (m, total 25H).

*1-(Fmoc-amino)cyclohenicosanecarboxylic acid (**11A**)*: TFA (3 mL) was added to a solution of *tert*-butyl 1-(Fmoc-amino)cyclohenicosanecarboxylate (**10A**; 1.2376 g, 1.96 mmol) in dichloromethane (4 mL) at 0°C. The mixture was stirred at room temperature for 27 h then poured into ice-water (50 mL). The solution was extracted with dichloromethane (4×50 mL) and the organic layer was washed with brine and dried over Na_2SO_4. The residue (1.0358 g) obtained by evaporation of the solvent was flash chromatographed ($CHCl_3$/MeOH (40:1) to give the acid **11A** as a colorless solid. M.p.: 146.2–147.5°C (colorless needles, recrystallized from methanol). IR (KBr suspension): $\tilde{\nu}=1709\ cm^{-1}$ (C=O). ^1H NMR: $\delta=7.732$ (d, $J=7.51$ Hz, 2H; H_4, H_5), 7.581 (d, $J=7.33$ Hz, 2H; H_1, H_8), 7.396 (d, d, $J=7.14$, 7.14 Hz, 2H; H_3, H_6), 7.308 (d, d, d, $J=7.51$, 7.51, 1.10 Hz, 2H; H_2, H_7), 5.249 (brs, 1H; NH), 4.412 (brm, 2H; H_{10}), 4.214 (t, $J=6.41$ Hz, 1H; H_9), 2.007–1.888 (m, 4H), 1.371–1.285 ppm (m, total 36H). Elemental analysis calcd (%) for $C_{37}H_{53}NO_4$: C 77.18, H 9.28; N 2.43; found: C 77.05, H 9.43, N 2.41.

*1-(Fmoc-amino)cyclooctadecanecarboxylic acid (**11B**)*: TFA (2 mL) was added to a solution of *tert*-butyl 1-(Fmoc-amino)cyclooctadecanecarboxylate (**10B**; 409.4 mg, 0.757 mmol) in dichloromethane (2 mL) at 0°C. The mixture was stirred at room temperature for 27 h then poured into ice-water (50 mL). The solution was extracted with chloroform (4×50 mL) and the organic layer was washed with brine and dried over Na_2SO_4. The residue obtained by evaporation of the solvent was flash chromatographed ($CHCl_3$/MeOH (30:1)) to give the acid **11B** (361.8 mg, 98% yield) as a colorless solid. M.p.: 184.0–185.1°C (colorless needles, recrystallized from *n*hexane/CH_2Cl_2). IR (KBr suspension) $\tilde{\nu}=1723\ cm^{-1}$ (C=O). ^1H NMR: $\delta=7.762$ (d, $J=7.51$ Hz, 2H; H_4, H_5), 7.577 (d, $J=7.33$ Hz, 2H; H_1, H_8), 7.398 (d, d, $J=7.33$, 7.33 Hz, 2H; H_3, H_6), 7.310 (d, d, d, $J=7.51$, 7.51, 1.10 Hz, 2H; H_2, H_7), 5.006 (brs, 1H; NH), 4.442 (brm, 2H; H_{10}), 4.212 (t, $J=6.96$ Hz, 1H; H_9), 1.876 (m, 4H), 1.295–1.209 ppm (m, total 30H). Elemental analysis calcd (%) for $C_{34}H_{47}NO_4$: C 76.51, H 8.88, N 2.62; found: C 76.33, H 9.09, N 2.62.

*1-(Fmoc-amino)cyclopentadecanecarboxylic acid (**11C**)*: TFA (1 mL) was added to a solution of *tert*-butyl 1-(Fmoc-amino)cyclopentadecanecarboxylate (**10C**; 256.3 mg, 0.469 mmol) in dichloromethane (2 mL) at 0°C. The mixture was stirred at room temperature for 4 h then poured into ice-water (40 mL). The solution was extracted with chloroform (3×40 mL) and the organic layer was washed with brine and dried over Na_2SO_4. The residue obtained by evaporation of the solvent was flash chromatographed ($CHCl_3$/MeOH (30:1)) to give the acid **11C** (222.5 mg, 97% yield)) as a colorless solid. M.p.: 198.5–199.8°C (colorless needles, recrystallized from *n*hexane/CH_2Cl_2). ^1H NMR: $\delta=7.755$ (d, $J=7.51$ Hz, 2H; H_4, H_5), 7.573 (d, $J=7.14$ Hz, 2H; H_1, H_8), 7.392 (d, d, $J=7.33$, 7.33 Hz, 2H; H_3, H_6) 7.304 (d, d, d, $J=7.51$, 7.51, 1.28 Hz, 2H; H2, H7), 4.906 (brs, 1H; NH), 4.434 (brm, 2H; H_{10}), 4.209 (m, 1H; H_9), 1.820 (m, 3H), 1.344–1.295 ppm (m, total 25H). Elemental analysis calcd (%) for $C_{31}H_{41}NO_4$: C 75.73, H 8.41, N 2.85; found: C 75.54, H 8.45, N 2.82.

*1-(Fmoc-amino)cyclohenicosanecarboxylic acid fluoride (**12A**)*: A solution of DAST (133.7 mg, 0.829 mmol, 1.8 equiv) in dichloromethane (2 mL) was added to a solution of 1-(Fmoc-amino)cyclohenicosanecarboxylic acid (**11A**; 268.1 mg, 0.466 mmol) in dry dichloromethane (2 mL) at 0°C. The mixture was stirred at room temperature for 20 min then poured into ice-water (20 mL). The solution was extracted with dichloromethane (2×20 mL) and the organic layer washed with brine and dried over Na_2SO_4. The organic solvent was evaporated and the residue was flash chromatographed (EtOAc/*n*hexane (1:12)) to give colorless solid **12A** (228.5 mg, 85% yield). IR (KBr suspension): $\tilde{\nu}=1838\ cm^{-1}$ (C=O). ^1H NMR: $\delta=7.763$ (d, $J=7.51$ Hz, 2H; H_4, H_5), 7.568 (d, $J=7.69$ Hz, 2H; H_1, H_8), 7.402 (d, d, $J=7.51$, 7.51 Hz, 2H; H_3, H_6), 7.314 (d, d, d, $J=7.51$, 7.51, 1.10 Hz, 2H; H_2, H_7), 4.961 (brs, 1H; NH), 4.477 (brm, 2H; H_{10}), 4.212 (t, $J=6.41$ Hz, 1H; H_9), 1.880 (m, 4H), 1.374–1.241 ppm (m, total 36H).

*1-(Fmoc-amino)cyclooctadecanecarboxylic acid fluoride (**12B**)*: A solution of DAST (101.5 mg, 0.630 mmol, 1.3 equiv) in dichloromethane (1 mL) was added to a solution of 1-(Fmoc-amino)cyclooctadecanecarboxylic acid (**11B**; 250.9 mg, 0.471 mmol) in dry dichloromethane (1 mL) at 0°C. The mixture was stirred at room temperature for 45 min then poured into ice-water (20 mL). The solution was extracted with dichloromethane (2×20 mL) and the organic layer washed with brine and dried over Na_2SO_4. The organic solvent was evaporated and the residue was flash chromatographed (EtOAc/*n*hexane (1:12)) to give colorless solid **12B** (208.8 mg, 83% yield). IR (KBr suspension): $\tilde{\nu}=1838\ cm^{-1}$ (C=O). ^1H NMR: $\delta=7.762$ (d, $J=7.51$ Hz, 2H; H_4, H_5), 7.568 (d, $J=7.51$ Hz, 2H; H_1, H_8), 7.402 (d, d, $J=7.51$, 7.51 Hz, 2H; H_3, H_6), 7.314 (d, d, d, $J=7.33$, 7.33, 1.28 Hz, 2H; H_2, H_7), 4.878 (brs, 1H; NH), 4.500 (brm, 2H; H_{10}), 4.210 (t, $J=6.23$ Hz, 1H; H_9), 1.929–1.790 (m, 4H), 1.426–1.209 ppm (m, total 30H).

*1-(Fmoc-amino)cyclopentadecanecarboxylic acid fluoride (**12C**)*: A solution of DAST (96.1 mg, 0.596 mmol, 1.6 equiv) in dichloromethane (1 mL) was added to a solution of 1-(Fmoc-amino)cyclopentadecanecarboxylic acid (**11C**; 188.2 mg, 0.383 mmol) in dry dichloromethane (2 mL) at 0°C. The mixture was stirred at room temperature for 30 min then poured into ice-water (20 mL). The solution was extracted with dichloromethane (3×20 mL) and the organic layer was washed with brine and dried over Na_2SO_4. The organic solvent was evaporated and the residue was flash chromatographed (EtOAc/*n*hexane (1:9)) to give colorless solid **12C** (138.0 mg, 73% yield). IR (KBr suspension): $\tilde{\nu}=1839\ cm^{-1}$ (C=O). ^1H NMR: $\delta=7.755$ (d, $J=7.51$ Hz, 2H; H_4, H_5), 7.573 (d, $J=7.14$ Hz, 2H; H_1, H_8), 7.392 (d, d, $J=7.33$, 7.33 Hz, 2H; H_3, H_6), 7.304 (d, d, d, $J=7.51$, 7.51, 1.28 Hz, 2H; H_2, H_7), 4.906 (brs, 1H; NH), 4.434 (brm, 2H; H_{10}), 4.209 (m, 1H; H_9), 1.820 (m, 3H), 1.344–1.295 ppm (m, total 25H).

Curtius rearrangement of α,α-disubstituted monoethyl malonates[11]

*a) Monoethyl α,α-dipropylmalonate (**13a**; $R_1=R_2=$propyl in ref. [11])*: A solution of monoethyl α,α-dipropylmalonate (**13a**; 216.9 mg, 1.00 mmol), DPPA (284.4 mg, 1.03 mmol, 1.0 equiv), and triethylamine (112.5 mg, 1.11 mmol, 1.1 equiv) in benzene (5 mL) was heated under reflux in an argon atmosphere for 1.25 h. A solution of benzylalcohol (120.6 mg, 1.12 mmol, 1.1 equiv) in benzene (1 mL) was then added. The mixture was heated under reflux for 47 h. After evaporation of the solvent, the residue was dissolved in ethyl actetate (30 mL) and the organic layer was washed sequentially with 5% aqueous HCl, water, 2N aqueous $NaHCO_3$, and brine, then dried over Na_2SO_4. After evaporation of the solvent, the residue was flash chromatographed (silica gel; EtOAc/*n*hexane (1:12)) to give *N*-Z-dipropylglycine ethyl ester (**14a**; 219.2 mg, 68% yield) as a colorless oil. ^{1}H NMR: $\delta=7.353$ (m, 5H; Ph), 5.869 (s, 1H; NH), 5.071 (s, 2H; $PhCH_2$), 4.214 (q, $J=7.15$ Hz, 2H; $CO_2CH_2CH_3$), 2.292 (m, 2H; $CH_2CH_2CH_3$), 1.695 (m, 2H; $CH_2CH_2CH_3$), 1.293 (m, 2H; $CH_2CH_2CH_3$), 1.275 (t, $J=6.97$ Hz, 3H; $CO_2CH_2CH_3$), 1.009 (m, 2H; $CH_2CH_2CH_3$), 0.865 ppm (t, $J=7.33$ Hz, 6H; $CH_2CH_2CH_3$). HRMS: calcd for $C_{18}H_{27}NO_4$: 321.1941; found: 321.1934. A trace amount of the isocyanate **15a** was detected.

Preparation of peptides I–IV: Peptides were synthesized by the Fmoc solid-phase method[13] on Rink amide resin.[16] The Fmoc amino acid fluoride method[9] was employed to introduce the C18 amino acid and the successive amino acids to the peptide chain. The peptide resin was treated with 10 equivalents **12B** (for the introduction of C18), Fmoc-Ala-F (for the introduction of Ala in position 10 of peptides **II** and **III**), and Fmoc-Glu(O*t*Bu)-F (for the introduction of Glu in position 2 of peptides **II** and **IV**), respectively, in dimethylformamide (DMF) for 12 h. Other amino acids were introduced to the peptide resin by using a Shimadzu PSSM-8 synthesizer. The standard protocol with a PyBOP-HOBt-NMM coupling system was employed.[17] N-terminal acetylation was achieved by treatment with acetic anhydride and NMM (10 equiv each) in DMF at room temperature for 30 min. The obtained protected peptide resin was treated with TFA/1,2-ethanedithiol (95:5) at 20°C for 2 h. HPLC purification yielded the pure peptides. Total yields of the peptides calculated with respect to the initial amount of resin were as follows: peptide **I**, 48%; peptide **II**, 10%; peptide **III**, 16%; peptide **IV**, 32%. The fidelity of the products was ascertained by MALDI-TOF MS: $[M+H]^+$ found (calcd): peptide **I**: 1614.5 (1613.8); peptide **II**: 2060.1 (2059.6); peptide **III**: 1836.8 (1836.2); peptide **IV**: 1836.9 (1836.3).

Single-crystal X-ray diffraction structural analysis: A Bruker Smart 1000 CCD diffractometer employing graphite-monochromated $Mo_{K\alpha}$ radiation was used. The structure was solved by direct methods with the program SIR97 and was refined by full-matrix least-squares techniques. All calculations were performed with the teXsan crystal structure solution software package.

Analytical ultracentrifugation: Sedimentation equilibrium studies were performed in a Beckman–Coulter Optima XL-I analytical ultracentrifuge with a double-sector centerpiece and sapphire windows, at rotor speeds of 30000 and 40000 rpm and at 20°C. Absorbance scans were carried out at 230 nm in the radial step mode at 0.001-cm intervals and the data were collected as the average of 16 measurements at each radial distance. Equilibrium was considered to have been reached when replicate scans separated by 6 h were indistinguishable. The weight-average molecular weight (M_{app}) was estimated from Equation (1),[21] where r is the radius, c

$$M_{app} = 2RT/[(r_2^2-r_1^2)(1-\bar{\nu}\rho)\omega^2]\ln(c_2/c_1) \quad (1)$$

is the concentration of the peptide, $\bar{\nu}$ is the partial specific volume of the peptide, ρ is the density of the solvent, ω is the angular velocity of the rotor (in radians s^{-1}), R is the universal gas constant, T is the absolute temperature, and M_{app} is the apparent molecular weight.

The value of $\bar{\nu}$ was estimated from two sets of data obtained from sedimentation equilibrium experiments. We assumed that M_{app} does not depend on the density of the solution, in which case, Equation (2) applies.

$$M_{app} = 2RT/[(r_2'^2-r_1'^2)(1-\bar{\nu}\rho')\omega^2]\ln(c_2'/c_1') \quad (2)$$

By combining Equations (1) and (2), Equation (3) is produced.

$$(r_2'^2-r_1'^2)(1-\bar{\nu}\rho')\ln(c_2/c_1) = (r_2^2-r_1^2)(1-\bar{\nu}\rho)\ln(c_2'/c_1') \quad (3)$$

From the results of sedimentation equilibrium experiments in H_2O ($\rho=1.00$ g cm^{-3}) and in D_2O/H_2O (60:40; $\rho'=1.06$ g cm^{-3}), we determined $\bar{\nu}$ to be 0.760 cm^3 g^{-1}.

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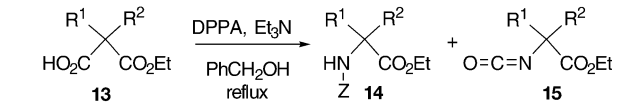
[11] This phenomenon is also seen with acyclic long aliphatic substituents at the α position. In the case of *n*-propyl disubstitution (**13a**), the intermediate isocyanate (**15a**) can react with benzyl alcohol to give the *N*-Z derivative (**14a**).



	R1	R2	Time [h]	[%]	[%]
a	*n*propyl	*n*propyl	47	68	trace
b	undecyl	undecyl	20	0	82
c	octyl	undecyl	20	0	82
d	21-membered ring		26	0	47
e	15-membered ring		15	0	68

[12] Crystallographic data: $C_{28}H_{45}NO_3$, $M_r = 443.67$, $0.48 \times 0.42 \times 0.15$ mm, monoclinic, space group $P2_1/n$, $a = 8.290(2)$, $b = 34.275(9)$, $c = 10.128(3)$ Å, $\beta = 113.102(4)°$, $V = 2647(1)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.113$ g cm^{-3}, $2\theta_{max} = 56.9°$, $T = 173$ K, $\mu(Mo_{K\alpha}) = 0.71$ cm^{-1}, $F(000) = 976.00$, Bruker Smart 1000 CCD diffractometer, ω–2θ scans, 2270 reflections measured, 5941 unique, 3747 with $I > 3.0\sigma(I)$, 290 variables, $R = 0.045$, $R_w = 0.057$, $S = 1.98$, $(\Delta/\sigma)_{max} = 0.291$, $\rho\Delta_{max} = 0.24$ eÅ^{-3}, $\Delta\rho_{min} = -0.19$ eÅ^{-3}.

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[18] Increased hydrophobicity of the peptides caused greater nonspecific adsorption onto the glass and plastic surfaces of the equipment used for their purification, which lowered the yield.

[19] Analysis conditions: column: Waters Symmetry 300 C18, 5 μm (4.6×150 mm); gradient: 10–90% B in A (A: H_2O containing 0.1% CF_3CO_2H; B: CH_3CN containing 0.1% CF_3CO_2H) over 40 min; flow: 1 mL min^{-1}; detection: 215 nm.

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