12. Experimental Section

12.1 Peptide Synthesis, Purification and Characterization

12.1.1 General Procedures, Reagents, and Instruments

Solvents, Chemicals, and Reagents

Water for HPLC and buffers was triple distilled or purified on a MilliPore device. Acetonitrile (HPLC grade) was purchased from Fluka or Acros Organics. TFA for HPLC was obtained from Merck (Uvasol® grade). Diethylether for peptide precipitation was dried over $CaCl_2$. All other solvents, chemicals, and reagents are declared in the sections of the experimental procedures in which they were used.

Peptide Synthesizers

Peptides were synthesized on (**A**) a semi-automatic peptide synthesizer ACT 90 (Advanced Chem Tech) in glass reactors with a volumetric capacity of 25mL, (**B**) an automatic peptide synthesizer ABI 431 A (Applied Biosystems) in 10mL Batc vessels, or (**C**) on an automatic peptide synthesizer Syro XP (MultiSynTech) in 10mL polypropylene reactors. Solvents for peptide synthesis (DMF and NMP) were commercially obtained "synthesis grade" or "p.a." and applied without further purification. Fully protected amino acids were purchased from Iris Biotech, NovaBiochem, or Fa. Gehardt if not declared otherwise.

Analytical HPLC

Analytical HPLC was performed on (**D**) a computer-controlled high-pressure-gradient LaChrom-HPLC-system (Merck-Hitachi), containing an Interface L-7000; two pumps L-7100 (one for each eluent); diode array detector L-7450; autosampler L-7200 with 100µL sample loop; solvent degasser L-7612; high-pressure gradient mixer; Rheodyne injection valve 7725i, 20µL sample loop; administration of the device, data recording and analysis was performed with the LaChrome Software version 4.0.

HPLC runs were performed with a flow rate of 1mL/min applying eluent gradients. Eluent A was 94.9% water, 5% ACN, 0.1% TFA, eluent B was 5% water, 94.9% ACN, 0.1% TFA. UV-detection occurred at λ_{abs} = 220nm for unlabelled peptides and

λ_{abs} = 320nm for Abz-labeled peptides. Samples were prepared by dilution with 94.9% water, 5% Acetonitrile and 0.1% TFA in standard autosampler vessels equipped with 100µL glass inlets.

The following reversed phase columns have been used: C4: Trentec C4 (300) Reprosil-Pur 5µm (250mm x 4.6mm); Vydac C4 (300)10µm (250mm x 4.6 mm); Vydac® Protein C4 10µM (250mm x 4..6mm). C8: Capcell SG300C8 5µM (250mm x 4.6mm); Phenomenex® Luna C8 (2) 5µM (250mm x 4.6mm); Phenomenex® Luna C8 (2) 10µM (250mm x 4.6mm).

Semi-preparative HPLC

Purification of peptides on a semi-preparative scale was performed on (**E**) a high pressure gradient HPLC-device (Knauer), containing HPLC programmer 50; two pumps 64 (one for each eluent); variable wavelength detector; plotter; injection valve 7125 and 1mL sample loop.

HPLC runs were performed with a flow rate of 5 mL/min applying eluent gradients. Eluent A was 94.9% water, 5% ACN, 0.1% TFA, eluent B was 5% water, 94.9% ACN, 0.1% TFA. UV-detection occurred at λ_{abs} = 220nm. Samples were prepared by dilution with 94.9% water, 5% Acetonitrile and 0.1% TFA in standard Eppendorf® or glass vessels. Peptides with poor solubility were treated with ultrasonication and/or diluted further with small amounts of ACN.

The following reversed phase columns have been used: Vydac Protein C4 10µm (250mm x 10mm).

Preparative HPLC

Purification of peptides in a preparative scale was performed on (**F**) a Computer-controlled high-pressure-gradient system LKB Bromma (Pharmacia), containing central processor LC2252; two pumps 2248 (one for each eluent); two wavelength UV-detector 2141; solvent degasser; motor valve PMV-7; high pressure gradient mixer; 1mL and 5mL sample loops or (**G**) a Knauer high-pressure-gradient system, containing interface Smartline Manager 5000 with solvent degasser; two pumps Smartline pump 1000 (one for each eluent); variable UV-detector 2500; injection valve; high-pressure-gradient mixer; 5mL sample loop.

HPLC runs were performed with a flow rate of 20mL/min applying eluent gradients. Eluent A was 94.9% water, 5% ACN, 0.1% TFA, eluent B was 5% water, 94.9% ACN, 0.1% TFA. UV-detection occurred at λ_{abs} = 220nm for unlabelled peptides and λ_{abs} = 320nm for Abz-labeled peptides. Samples were prepared by dilution with 94.9% water, 5% ACN and 0.1% TFA in standard Eppendorf® or 15mL Plastibrand® screw-cap centrifuge tubes. Peptides with poor solubility were treated with ultrasonication and/or diluted further with small amounts of ACN.

The following reversed phase columns have been used: Vydac Protein C4 10µm (250mm x 22mm) and Phenomenex® Luna C8 10µm (250mm x 21.2mm).

Mass Spectrometry

Different Instruments were used for the characterization of synthesized peptides: (**H**): MALDI-ToF-MS Voyager-DE™ RP (PerSeptive Biosystems) using a nitrogen laser (λ=337nm). (**I**): MALDI-ToF-MS Bruker Reflex III (Bruker Daltonics) with delayed extractions source, N_2 laser. (**J**): Agilent 6210 ESI-ToF-LC/MS spectrometer (Agilent Technologies Inc.) with direct infusion via a Harvard Apparatus 11 Plus syringe pump (Harvard Apparatus).

For MALDI-MS Peptides were co-crystallized with matrices 2,5-dihydroxybenzoic acid (Aldrich) or α-cyano-4-hydroxycinnamic acid (Sigma). Samples for ESI-MS were dissolved in 94.9% water, 5% ACN and 0.1% TFA or used directly after collection from analytical or preparative HPLC.

12.1.2 Solid Phase Peptide Synthesis

All peptides were synthesized from the C-terminal to the N-terminal end on solid support by applying the Fmoc/tert-butyl protecting group strategy. The following protected derivatives of standard canonical amino acids were used for the synthesis of all peptides:

Fmoc-L-Arg(Pbf)-OH (arginine), Fmoc-L-Glu(OtBu)-OH (glutamic acid), Fmoc-L-His(Trt)-OH (histidine), Fmoc-L-Leu-OH (leucine), Fmoc-L-Lys-OH (lysine), Fmoc-L-Ser(tBu)-OH (serine), Fmoc-Val-OH (valine). Fmoc-amino acids were purchased from Iris Biotech, NovaBiochem, or Fa. Gerhardt. Unless stated otherwise, the N-terminal end of all peptides was equipped with anthranilic acid to enable a photometric concentration determination. Therefore Abz(Boc)-OH purchased from Bachem has been used.

Peptide coupling reagents were obtained from Acros (DIPEA), Fluka (DBU) NovaBio-Chem, Iris Biotech (HOBt, TBTU) or Fa. Gerhardt (HOBt, TCTU, HCTU).

Resins

Fmoc-L-Leu-Wang resin which was obtained from Iris Biotech, NovaBiochem, or Fa. Gerhardt was utilized throughout. Different loadings ranging from 0.65 to 0.71mmol/g was used.

Coupling and Deprotection Procedures

Several aminium salts of benzotriazoles (i.e. TBTU, HCTU, TCTU) were applied for peptide coupling, while Fmoc-deprotection was carried out by sterically hindered bases such as piperidine and DBU. Depending on the peptide synthesizer, different coupling reagents and deprotection procedures were used. The detailed synthesis protocols are given in the following tables:

Table 12.1. *SPPS protocol of synthesizer (**A**) - automatic peptide synthesizer ACT90 (Advanced Chem Tech).*

Process	**Reagent**	**Time**
Fmoc deprotection	10mL 2%PIP, 2%DBU in DMF	3×10min.
+ Washing	1mL DMF	6×1min.
Coupling	3eq. Fmoc-Amino acid-OH, 3eq. HOBt, 3eq. TBTU in 10 mL DMF 6eq. DIPEA in 3mL DMF)	30min.
Washing	1mL DMF	3×1min.
Coupling	3eq. Fmoc-Amino acid-OH, 3eq. HOBt, 3eq. TBTU in 10 mL DMF 6eq. DIPEA in 3mL DMF)	30min.
Washing	1mL DMF	3×1min.

Table 12.2. *SPPS protocol of synthesizer (**B**) - automatic peptide synthesizer ABI 431 A (Applied Biosystems).*

Process	**Reagent**	**Time**
Fmoc deprotection	10mL 10%PIP, 10%DBU in DMF	3×10min.
+ Washing	2mL DMF	6×1min.
Coupling	3eq. Fmoc-Amino acid-OH, 3eq. HOBt, 3eq. TCTU in 10 mL DMF 6eq. DIPEA in 3mL DMF)	30min.
Washing	2mL DMF	5×1min.
Coupling	3eq. Fmoc-Amino acid-OH, 3eq. HOBt, 3eq. TCTU in 10 mL DMF 6eq. DIPEA in 3mL DMF)	30min.
Washing	2mL DMF	3×1min.

Table 12.3. *SPPS protocol of synthesizer (**C**) – automatic peptide synthesizer Syro XP (MultiSynTech).*

Process	**Reagent**	**Time**
Fmoc deprotection	2mL 40% PIP in DMF	2×10min.
+ Washing	2mL DMF	6×1min.
Coupling	4eq. Fmoc-Amino acid-OH + 4eq. HOBt in DMF (0.8mL) 4eq. TCTU or HCTU in DMF (1.2mL) 4eq. DIPEA in NMP (0.4mL)	30min.
Washing	2mL DMF	6×1min.
Coupling	4eq. Fmoc-Amino acid-OH + 4eq. HOBt in DMF (0.8mL), 4eq. TCTU or HCTU in DMF (1.2mL) 4eq. DIPEA in NMP (0.4mL)	30min.
Washing	2mL DMF	6×1min.

Cleavage from the Resin

After automatic or semi-automatic synthesis the resin was removed from the reactor and washed with 10mL dichloromethane for three times. The washed resin was shaken with 3mL cleavage solution (90% TFA, 10% TIS and 1% water) for 4h. Subsequently, the cleavage solution was filtered and the resin washed with 100% TFA keeping the total amount less than 4mL. For precipitation, up to 100mL ice-cold and dried diethylether was added to the combined mixture. The resulting suspension was stored overnight at -20ºC to ensure complete precipitation. Afterwards, the supernatant of the cold suspension was immediately removed by centrifugation. The precipitated raw peptide was washed with 15mL ice-cold diethylether and centrifuged. This washing procedure was repeated seven times and the peptides were kept in the air until completely dry.

12.1.3 Purification and Characterization

The dried raw peptides were purified by semi preparative and preparative HPLC using different 40 and 60 min gradients of eluents A (94.9% water, 5% ACN, 0.1% TFA) and B (5% water, 94.9% ACN, 0.1% TFA). Gradient and column were optimized individually for each peptide by using analytical HPLC. The following gradients have been applied:

(I) 0% B → 70% B in 30min, 70% B → 100% B in 2min, 100% B for 3min, 100% B → 0% B in 2min, 0% B for 3min

(II) 20% B for 15min, 20% B → 60% B in 30min, 60% B → 100% B in 5min, 100% B for 5min, 100% → 20% B in 5min

(III) 25% B for15min, 25% B → 70% B in 30min, 70% B → 100% B in 5min, 100 % B for 5min, 100% B → 25 % B in 5min.

After separation the purity of the collected fractions was determined by analytical HPLC using comparable gradients and columns. Fractions with sufficient purity were combined and ACN was removed by rotary evaporation. Lyophilization of the remaining aqueous solution yielded the pure peptide. Subsequently, the purity of the synthesized peptides was determined by analytical HPLC. The molecular weight of the obtained peptides was determined by MALDI-ToF or ESI-ToF MS.

12.1.4 Detailed Description of the Synthesized Peptides

VW01 *H_2N – LESKLKELESKLKELESKLKELESKL - COOH*

Synthesizer: **A**
Purification: Preparative HPLC System **F**
Vydac Protein C4 10µm (250mm x 22mm), gradient **(III)**
Formula: $C_{137}H_{243}N_{33}O_{45}$
Molecular weight: calculated: mono.: 3070.77Da avg.: 3072.59Da
determined: 3071.50Da at instrument **H**

VW02 *H - LKSELEKLKSELEKLKSELEKLKSEL - COOH*

Synthesizer: **A**
Purification: Preparative HPLC System **F**
Vydac Protein C4 10µm (250mm x 22mm), gradient **(III)**
Formula: $C_{137}H_{243}N_{33}O_{45}$
Molecular weight: calculated: mono.: 3070.77Da avg.: 3072.59Da
determined: 3071.20Da at instrument **H**

VW02 Abz *Abz - LKSELEKLKSELEKLKSELEKLKSEL - COOH*

Synthesizer: **C**
Purification: Preparative HPLC System **G**
Phenomenex® Luna C8 10µm (250mm x 21.2mm), gradient **(I)**
Formula: $C_{144}H_{248}N_{34}O_{46}$
Molecular weight: calculated: mono.: 3189.81Da avg.: 3191.71Da
determined: 3189.82Da at instrument **J**

VW03 *H_2N - LERKLEKLERKLEKLERKLEKLERKL - COOH*

Synthesizer: **A**
Purification: Preparative HPLC System **F** and **G**
F: Vydac Protein C4 10µm (250mm x 22mm), gradient **(III)**
G: Phenomenex® Luna C8 10µm (250mm x 21.2mm), gradient **(I)**
Formula: $C_{149}H_{271}N_{45}O_{41}$
Molecular weight: calculated: mono.: 3347.05Da avg.: 3349.02Da
determined: 3349.90Da at instrument **H**

VW04 *H_2N - LKEELKELKEELKELKEELKELKEEL - COOH*

Synthesizer: **B**
Purification: Preparative HPLC System **F**
Vydac Protein C4 10µm (250mm x 22mm), gradient **(III)**
Formula: $C_{145}H_{251}N_{33}O_{49}$
Molecular weight: calculated: mono.: 3238.82Da avg.: 3240.74Da
determined: 3241.6Da at instrument **H**

VW11 *H_2N - LEVELKVLSKSLVKLEVELKVLSKSL - COOH*

Synthesizer: **B**
Purification: Semi-preparative HPLC System **E**
Vydac Protein C4 10µm (250mm x 22mm), gradient **(III)**
Formula: $C_{135}H_{243}N_{31}O_{39}$
Molecular weight: calculated: mono.: 2922.8Da avg.: 2924.56Da
determined: 2925.17Da at instrument **H**

VW11 Abz *Abz - LEVELKVLSKSLVKLEVELKVLSKSL - COOH*

Synthesizer: **C**
Purification: Preparative HPLC System **G**
Phenomenex® Luna C8 10µm (250mm x 21.2mm), gradient **(III)**
Formula: $C_{142}H_{248}N_{32}O_{40}$
Molecular weight: calculated: mono.: 3041.84Da avg.: 3043.68Da
determined: 3044.07Da at instrument **H**

VW12 *H_2N - LKVELEVLSKSLVKLKVELEVLSKSL - COOH*

Synthesizer: **A**
Purification: Semi-preparative HPLC System **E**
Vydac Protein C4 10µm (250mm x 22mm), gradient **(III)**
Formula: $C_{135}H_{243}N_{31}O_{39}$
Molecular weight: calculated: mono.: 2922.80Da avg.: 2924.56Da
determined: 2923.02Da at instrument **H**

VW13 *H_2N - LKVELEVLSKSLVKLKSELEVLSKSL - COOH*

Synthesizer: **B**
Purification: Semi-preparative HPLC System **E**
Vydac Protein C4 10µm (250mm x 22mm), gradient **(III)**
Formula: $C_{133}H_{239}N_{31}O_{40}$
Molecular weight: calculated: mono.: 2910.76Da avg.: 2912.51Da
determined: 2912.19Da at instrument **H**

VW15 *Abz - LEVELKVLHKHLHHLEVELKVLSKSL - COOH*

Synthesizer: **B**
Purification: Preparative HPLC System **F**
Vydac Protein C4 10µm (250mm x 22mm), gradient **(II)**
Formula: $C_{142}H_{240}N_{38}O_{37}$
Molecular weight: calculated: mono.: 3069.81Da avg.: 3071.66Da
determined: 3071.96 Da at instrument **H**

VW16 *H_2N - LKVELEKLSKSLVVLKSELEKLSKSL - COOH*

Synthesizer: **B**
Purification: Semi-preparative HPLC System **E**
Vydac Protein C4 10µm (250mm x 22mm), gradient **(III)**
Formula: $C_{134}H_{242}N_{32}O_{40}$
Molecular weight: calculated: mono.: 2939.79Da avg.: 2941.55Da
determined: 2941.24Da at instrument **H**

VW17 *Abz - LKVELEKLKKELVVLKSELEKLKKEL - COOH*

Synthesizer: **C**
Purification: Preparative HPLC System **G**
Phenomenex® Luna C8 10µm (250mm x 21.2mm), gradient **(I)**
Formula: $C_{151}H_{265}N_{35}O_{41}$
Molecular weight: calculated: mono.: 3224.97Da avg.: 3226.93Da
determined: 3226.73Da at instrument **I**

VW18 *Abz - LKVELEKLKSELVVLKSELEKLKSEL - COOH*

Synthesizer: **C**
Purification: Preparative HPLC System **G**
Phenomenex® Luna C8 10µm (250mm x 21.2mm), gradient **(I)**
Formula: $C_{145}H_{251}N_{33}O_{43}$
Molecular weight: calculated: mono.: 3142.85Da avg.: 3144.74Da
determined: 3144.49Da at instrument **I**
3142.90Da at instrument **J**

VW19 *Abz - LKVELKELKKELVVLKSELKELKKEL - COOH*

Synthesizer: **C**
Purification: Preparative HPLC System **G**
Phenomenex® Luna C8 10µm (250mm x 21.2mm), gradient **(I)**
Formula: $C_{151}H_{265}N_{35}O_{41}$
Molecular weight: calculated: mono.: 3224.97Da avg.: 3226.93Da
determined: 3226.33Da at instrument **I**

VW23 *Abz - LKVELEVLHKHLEKLKSELVKLKKEL - COOH*

Synthesizer: **C**
Purification: Preparative HPLC System **G**
Phenomenex® Luna C8 10µm (250mm x 21.2mm), gradient **(I)**
Formula: $C_{152}H_{260}N_{38}O_{39}$
Molecular weight: calculated: mono.: 3241.95Da avg.: 3243.92Da
determined: 3243.23Da at instrument **I**

VW24	*Abz - LKVELEVLHKELHKLKSELVKLKKEL - COOH*	
Synthesizer:	**C**	
Purification:	Preparative HPLC System **G**	
	Phenomenex® Luna C8 10µm (250mm x 21.2mm), gradient **(I)**	
Formula:	$C_{152}H_{260}N_{38}O_{39}$	
Molecular weight:	calculated:	mono.: 3241.95Da avg.: 3243.92Da
	determined:	3243.19Da at instrument **I**

VW25	*Abz - LKVELEVLHSHLEKLKSELVKLKSEL - COOH*	
Synthesizer:	**C**	
Purification:	Preparative HPLC System **G**	
	Phenomenex® Luna C8 10µm (250mm x 21.2mm), gradient **(I)**	
Formula:	$C_{146}H_{246}N_{36}O_{41}$	
Molecular weight:	calculated:	mono.: 3159.83Da avg.: 3161.73Da
	determined:	3160.93Da at instrument **I**

VW26	*Abz - LKVELEVLHSELHKLKSELVKLKSEL - COOH*	
Synthesizer:	**C**	
Purification:	Preparative HPLC System **G**	
	Phenomenex® Luna C8 10µm (250mm x 21.2mm), gradient **(I)**	
Formula:	$C_{146}H_{246}N_{36}O_{41}$	
Molecular weight:	calculated:	mono.: 3159.82Da avg.: 3161.73Da
	determined:	3160.94Da at instrument **I**

VW28	*Abz - LKVELEVLKSELEKLKSELVKLKSEL - COOH*	
Synthesizer:	**C**	
Purification:	Preparative HPLC System **G**	
	Phenomenex® Luna C8 10µm (250mm x 21.2mm), gradient **(I)**	
Formula:	$C_{145}H_{251}N_{33}O_{43}$	
Molecular weight:	calculated:	mono.: 3142.85Da avg.: 3144.74Da
	determined:	3146.11Da at instrument **I**

VW29	*Abz-LKVELEKLKSELVVLHSHLEKLKSEL-OH*	
Synthesizer:	**C**	
Purification:	Preparative HPLC System **G**	
	Phenomenex® Luna C8 10µm (250mm x 21.2mm), gradient **(I)**	
Formula:	$C_{146}H_{246}N_{36}O_{41}$	
Molecular weight:	calculated:	mono.: 3159.83Da avg.: 3161.73Da
	determined:	3159.86Da at instrument **J**

VW30	*Abz-LKVELEKLKSELVVLHSELHKLKSEL-OH*

Synthesizer:	**C**		
Purification:	Preparative HPLC System **G**		
	Phenomenex® Luna C8 10µm (250mm x 21.2mm), gradient **(I)**		
Formula:	$C_{146}H_{246}N_{36}O_{41}$		
Molecular weight:	calculated:	mono.: 3159.83Da	avg.: 3161.73Da
	determined:	3159.86Da at instrument **J**	

12.2 Folding Studies

12.2.1 General Procedures and Instruments

Reaction Vessels

Unless stated otherwise, all experiments were carried out in standard 1.5 and 2.0mL Eppendorf®, Eppendorf® screw-top, or Eppendorf-type vessels obtained from different manufacturers. Larger amounts of peptide solution (>1.5mL) were prepared in 15mL Plastibrand® screw-cap centrifuge tubes purchased from VWR.

Buffer Solutions

Different buffers with a concentration of 10mM were applied to cover the pH range from 4.0 to 8.0.

pH 4.0 and 5.0	Acetate buffer AcOH and NaOAc (Acros Organics)
pH 7.4	Tris / HCl buffer HCl and trishydroxymethylaminomethane (Acros Organics)
pH 7.4 and 8.0	Phosphate buffer NaH_2PO_3 and Na_2HPO_4 (Fluka)

pH Determination and Adjustment

The pH of buffers and peptide solutions was determined by using a WTW pH526 pH meter equipped with an InLab® Micro electrode (Mettler Toledo). Commercially available buffers have been utilized for two-point calibration at pH 4.0 and 7.0. Concentrated solutions of the corresponding buffer components, i.e., AcOH, HCl, Tris, Na_2HPO_4, and NaH_2PO_4 in water were used to adjust peptide solutions to the given pH value.

12.2.2 Concentration Determination

The concentration of peptide stock solutions of VW11Abz and VW15 was determined by RP-HPLC with UV detection at λ_{abs} = 320nm (system D). Prior to analysis, a calibration curve using Abz-Gly-OH (synthesis grade, Bachem) dissolved in HPLC solvent A was recorded.

The stock solution concentrations of peptides VW17 and VW22 were determined by UV spectroscopy at λ_{abs} = 320nm using an Agilent 8453 DAD photometer and SUPRASIL quartz cell with a path length of 0.1cm. Prior to analysis, a calibration curve using Abz-Gly-OH (synthesis grade, Bachem) stock-solutions in the corresponding buffer at different concentrations was recorded.

The peptide concentration of the remaining Abz-labeled peptides was determined by UV spectroscopy at λ_{abs} = 320nm utilizing a Varian Cary 50 photometer (Varian Medical Systems) and 1.5mL PMMA cuvettes (Plastibrand®, VWR). Prior to analysis, a calibration curve using Abz-Gly-OH (synthesis grade, Bachem) stock-solutions in the corresponding buffer at different concentrations was recorded.

The concentration of unlabelled peptides was roughly estimated from the weighed amount using the molecular weight of peptide as TFA salt.

12.2.3 Circular Dichroism Spectroscopy

Instruments

The following circular dichroism instruments were used for the structural characterization of the synthesized peptides:

(**K**) Jasco J-715 spectrometer equipped with a Jasco PTC-348WI Pelletier temperature controller (Jasco GmbH). Administration of the device and data analysis was performed with the software J-700 for Windows. The following peptides were measured with this instrument: VW01, VW02, VW03, VW04, VW11, VW12, VW13, VW15, and VW16

(**L**) Jasco J-600 spectrometer (Jasco GmbH) equipped with a RCS-Lauda Thermo-Regulator (LAUDA Dr. R. Wobser GmbH & Co. KG). Administration of the device and data analysis was performed with the software J-700 for Windows. The following peptides were measured with this instrument: VW17, VW19, VW23, and VW24.

(**M**) Jasco J-810 spectropolarimeter (Jasco GmbH) equipped with a RCS-Lauda Thermo-Regulator (LAUDA Dr. R. Wobser GmbH & Co. KG). Administration of the device and data analysis was performed with the software, J-700 for Windows 95/NT. The following peptides were measured with this instrument: VW18, VW25, VW26, VW27, VW28, VW29, and VW30.

Parameters

All measured spectra were background corrected by subtraction of the corresponding buffer spectra which were obtained prior to analysis. The following parameters have been applied.

Start wavelength:	240nm	End wavelength:	190nm
Step resolution:	0.2 - 0.5nm	Scan speed:	50nm/min
Response:	1s	Band width:	1 - 2nm
Sensitivity:	50mdeg	Accumulation:	3 – 5scans
T	20°C		

Sample Preparation and Incubation

Samples of peptides VW01 to VW17 were prepared prior to measurement by dilution of a 1mM stock solution in the corresponding buffer. For time dependent measurements, previously diluted samples were stored and measured after the intended incubation time. The remaining peptides VW18 – VW30 were dissolved directly without using stock solutions. All samples were incubated at room temperature in the absence of external stimuli, e.g., shaking that might influence the amyloid formation process.

Metal ions were added using metal ion stock solutions containing 8mM $Cu(OAc)_2$ or $Zn(OAc)_2$ (ACS grade, Acros Organics) which prepared in pure triple distilled water to avoid binding of buffer components.

Cuvettes

Depending on the peptide concentration, quartz SUPRASIL cells with different path lengths were used. For concentrations below 200µM, ultra-micro cells with a path length of 0.1cm were applied. Higher peptide concentrations were measured in flap cover ultra-micro cells with 0.05 and 0.02cm path length.

Data Analysis

For all Abz-labeled peptides with concentration known from UV spectroscopy, CD values were converted into mean residue ellipticity *[θ]* using Microcal Origin (Version 6.0, Origin Lab Corporation) and the equation:

$$[\theta] = \frac{\theta 100 M}{c d N}$$

where θ is the ellipticity measured in degrees, M is the molecular weight, c is the peptide concentration in milligram per milliliter, and d is the optical path length of the cell in cm.

Ellipticity values for unlabelled peptides with concentrations estimated from the molar weight as TFA salt (indicated by c ~ instead of c =) are given as uncorrected θ values since it was impossible to determine the concentration accurately enough. Thus, the given CD intensities should not be overestimated and are not comparable with data from the literature. Nevertheless, the obtained spectra provide qualitative information on the peptide secondary structure.

12.2.4 Thermal Denaturation Experiments

Denaturation experiments were carried out at the J-715 spectrometer (instrument **K**) under the following conditions:

Wavelength	222nm		
T_{start}	20°C	T_{end}	90°C
Step Res.	0,1°C	Wait time	2s
Temp. Slope	3 - 5°C/min	Response	1s
Slit width	50µm	Band width	5nm
Sensitivity	20-100mdeg		

GndHCl (Fluka) was taken from 8M stock solutions in the corresponding 10mM buffer. Quartz SUPRASIL ultra-micro cells with a path length of 0.1cm were used throughout. For the calculation of melting points, θ_{222} at 0M GndHCl and 20°C has been defined as 100% folded, while the 0% folded value has been set as θ_{222} at 90°C and a GndHCl concentration where complete unfolding was obtained. Subsequently, the temperatures at which θ_{222} reached the virtual 50% folding value were determined from the spectra.

Thus, it is important to mention that the obtained melting points do not correspond to the curve inflection points.

12.2.5 ThT Assay

Samples for fluorescence staining experiments were prepared and incubated similarly to those for CD investigations. ThT obtained from Sigma Aldrich was used after purification by reversed-phase column chromatography. Fluorescence spectra were measured utilizing a luminescence spectrometer LS 50B (The Perkin Elmer Cooperation) and a 1.4mL fluorescence cuvette (10 x 2mm). Samples were prepared incubating 200µL peptide solution (100µM, 10mM phosphate buffer, pH 7.4, metal ions as denoted) with 200µL ThT (100µM, 10mM phosphate buffer, pH 7.4) for 30 minutes. Spectra were measured accumulating 10 scans from 470nm to 600nm with an excitation wavelength λ_{ex} = 450nm and background corrected by subtraction of the free dye's spectrum. The given aggregation traces were generated from the ThT fluorescence intensity at λ_{em} = 485nm recorded at several time points.

12.3 Electron Microscopy

All TEM measurements were carried out in collaboration with PD Dr. Christoph Böttcher and Dr. Hans von Berlepsch, Freie Universität Berlin, Research Center for Electron Microscopy, Fabeckstrasse 36a, 14195 Berlin, Germany.

Instrument

For all TEM measurement a Philips CM12 transmission electron microscope was used. Cryo-TEM samples were introduced using the Gatan cryo holder and stage (model 626, Gatan GmbH).

Sample Preparation

Samples for negative staining and cryo-TEM were dissolved directly in the corresponding buffer (10mM, pH 4.0: acetate buffer, pH 7.4: Tris / HCl buffer or phosphate buffer, metal ions as denoted) without using stock solutions. Subsequently, the pH was adjusted, the concentration determined by UV spectroscopy and the samples stored at room temperature. After the intended time of incubation samples were prepared for measurement.

Negative Staining TEM

Aliquots of 6µL of peptide solutions were absorbed for 1min to glow-discharged carbon-coated collodium films on 400-mesh copper grids. After blotting and negative staining with 1% phosphotungstic acid (PTA) the grids were air-dried. TEM micrographs were taken at primary magnification of 58300x using a defocus of 0.6µm.

Cryo-TEM

The samples for cryo-TEM were prepared at room temperature by placing a droplet (10µL) of the solution on a hydrophilized (60s plasma treatment at 8W using a BALTEC MED 020 device) perforated carbon filmed grid (Qantifoil). The grids were immediately vitrified in liquid ethane at its freezing point (-184°C) using a standard plunging device. The vitrified samples were transferred under liquid nitrogen into the electron microscope. Microscopy was carried out at -175°C sample temperature using the microscopes low dose protocol at primary magnification of 58300x. The defocus was chosen in all cases to be 1.5µm.

12.4 EPR Spectroscopy

EPR measurements were carried out in collaboration with Prof. Dr. Reinhardt Kirmse and Dipl. Chem. Jan Griebel, Universität Leipzig, Faculty for Chemistry and Mineralogy, Institute of Inorganic Chemistry, Johannisallee 29, 04103 Leipzig, Germany.

EPR spectra were recorded using a BRUKER ESP 300E X-band spectrometer (Bruker BioSpin Corp.) The samples (1mM peptide, 2mM $Cu(OAc)_2$, 10mM phosphate buffer pH 7.4) were prepared 2h before analysis and spiked with 10% glycerol to obtain non-crystalline ice. Measurements were carried out in 1mL Teflon tubes at 130K.

12.5 Gas Phase IR Spectroscopy

Gas phase IR spectroscopy was carried out in collaboration with Dr. Gert von Helden and Dipl. Phys. Peter Kupser, Fritz-Haber-Institut der Max-Planck-Gesellschaft, Faradayweg 4-6, 14195 Berlin, Germany, and Dr. Jos Oomens and Dr. Nick C. Polfer, FOM-Institute for Plasma Physics Rijnhuizen, Edisonbaan 14, NL-3439 MN Nieuwegein, The Netherlands.

Sample Preparation

In order to avoid higher order aggregation which might affect the ESI process, exclusively freshly prepared samples were used for the presented experiments. Therefore, the peptides were dissolved directly in NH_4OFor buffer (10mM, pH 4.5). The corresponding buffer components (diluted ammonia, formic acid) were purchased from Sigma Aldrich. The peptide concentration of 50µM was estimated from the molecular weight as TFA salt. The pH was adjusted to 4.5 using diluted formic acid solution. Subsequently, 40% MeOH (Acros Organics) were added.

Instrument Parameters

Measurements were carried out on a Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer installed at the free electron laser facility at FOM-Institute for Plasma Physics Rijnhuizen, Edisonbaan 14, NL-3439 MN Nieuwegein, The Netherlands. A detailed description of the instrument is described elsewhere.[272]

Since the used FT-ICR mass spectrometer is a laboratory-constructed instrument with restricted parameter stability and repeatability, the instrumental settings were optimized individually for each peptide solution. Consequently, only the most essential parameters of the FT-ICR mass spectrometer and the free electron laser FELIX are given in the following.

FT-ICR parameters

Flow rate	8µL/min		
ESI needle voltage	3.2kV	Cone voltage	100V
Drying gas temp.	60°C	Temp. source block	RT
Trapping voltage (Gert box)	2.8V	Hexapole accumulation	1s

FELIX parameters

Start wavelength	5.5µm	End wavelength	7.5µm
Step resolution	0.02µm	Irradiation time	2 – 3s
Attenuation	3dB	Averages at each λ	2