

SUPPORTING INFORMATION

Direct Solid-Phase Synthesis of the β -Amyloid (1–42) Peptide Using Controlled Microwave Heating

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General Experimental Details. All commercially available solvents and reagents were used without further purification. *N,N'*-Dimethylformamide (puriss. p.a., >99.8%) (DMF), 1-methyl-2-pyrrolidone (for peptide synthesis, >99.8%) (NMP), dichloromethane (reagent grade, >99.5%) (DCM), methanol (reagent grade, >99.5%), trifluoroacetic acid (reagent grade, >98%) (TFA), piperidine (reagent grade, >98%), dithiothreitol (99%) (DTT) and *N,N'*-diisopropylcarbodiimide (99%) (DIC) were purchased from standard sources. RAM ChemMatrix (substitution 0.50 mmol/g, Lot No. 07I06-13-05-029) was obtained from Martix Innovation (Canada). The Fmoc-amino acids and 1-hydroxybenzotriazole hydrate (HOBt) were purchased from Orpegen (Germany). For both microwave and conventionally heated experiments solid-phase reaction vessels (10 mL polypropylene syringe with frit and PTFE valve) were used. The comparison experiments at elevated temperature were performed in a PLS 4 × 6 manual solid-phase synthesizer. HPLC analyses were performed on Phenomenex Jupiter C4 column (250 mm × 4.6 mm, 5 μm) applying a linear gradient of 20–60% B in 15 min at 60 °C. The mobile phases were 0.1% TFA in H₂O (eluent A), and 0.1% TFA in MeCN (eluent B). The flow rate was 1 mL/min and the detection was at 215 nm. The crude products were purified on a semipreparative Phenomenex Jupiter C4 column (250 mm x 4.6 mm, 10 μm) applying a linear gradient of 30–100% B in 45 min. The flow rate was 4 mL/min and the detection was at 215 nm. The peptide masses were confirmed by MALDI-TOF MS. Amino acid analysis was performed on a standard amino acid analyzer equipped with an ion-exchange separation column, and postcolumn derivatization with ninhydrine was applied. Before analysis, samples were hydrolyzed with 6 N HCl in sealed and evacuated tubes at 110 °C for 24 h, 48 h and 72 h. ¹H NMR spectra were recorded in 0.5 mL d-TFA at 500 MHz.^{S2}

Racemization Analysis. The determination of enantiomeric peptide purity was performed by C.A.T. GmbH & Co. (Tübingen, Germany). The samples were hydrolyzed using deuterated solvents (6 N D₂O/DCl) and after derivatization with isopropanol and pentafluoropropionic anhydride, the enantiomeric purity of His was determined by GC-MS (see Table S2).^{S3}

Microwave Irradiation Experiments. The instrumental details regarding microwave irradiation and temperature measurements for coupling and deprotection protocols have been described in a previous publication.^{S1}

Synthesis of A β (1-42) Peptide Using Conventional Heating at 86 °C. A sample of 150 mg of RAM ChemMatrix resin (0.50 mmol/g) was transferred into a 10 mL bottom-filtration reaction vessel and was swollen in 4 mL of DCM for 30 min. The resin was then washed with NMP (2 \times 4 mL). In a separate vial the corresponding Fmoc-amino acid (0.375 mmol), DIC (0.375 mmol, 58 μ L) and HOBt (0.375 mmol, 61 mg) were combined in 1 mL NMP. The preactivated coupling cocktail was added to the resin after 2 min and the reaction mixture was heated at 86 °C for 11 min in the PLS 4 \times 6 synthesizer (single couplings). Incorporation of Fmoc-His(Trt)-OH residue was performed at room temperature, for detailed experimental conditions see below. The suspension was then washed with DMF, DCM (3 \times 4 mL each) and DMF (2 \times 4 mL). After this time, 2 mL of 30% piperidine in DMF was added to the resin. For removal of N $^{\alpha}$ -Fmoc group 30% piperidine in DMF containing 0.1 M HOBt was utilized after the 8 Ser residue to avoid potential aspartimide formation at 7 Asp. The reaction vessel was placed in the PLS 4 \times 6 synthesizer (86 °C) and heated with agitation at 86 °C for 1.5 min. The resin was then washed with 4 mL of DMF, and 2 mL of 30 % piperidine in DMF were subsequently added to the sample which was agitated in the PLS 4 \times 6 synthesizer for an additional 3.5 min at 86 °C. After the last deprotection step, the peptidyl resin was washed with DMF and DCM (5 \times 4 mL each) and dried under reduced pressure.

Incorporation of Fmoc-His(Trt)-OH residue. In a separate vial Fmoc-His(Trt)-OH (0.375 mmol, 232 mg), DIC (0.375 mmol, 58 μ L) and HOBt (0.375 mmol, 61 mg) were combined in 1 mL NMP. The preactivated coupling cocktail was added to the resin after 2 min, then the reaction mixture was placed in the PLS 4 \times 6 synthesizer and shaken for 1 h at room temperature. After the reaction time, the resin was washed with NMP (2 \times 4 mL) and the coupling step was repeated with freshly prepared coupling cocktail.

Final Cleavage. The peptide was cleaved from the solid support with a cleavage cocktail (10 mL) of TFA/DTT/water (95: 2.5: 2.5) at ambient temperature for 5 h. The resin was filtered and washed with a small amount of cleavage cocktail. The residual product was precipitated with ice-cold diethyl ether and the peptide was collected by filtration, dissolved in deionized water and lyophilized.

Table S1. Comparison of A β (1-42) Peptide Synthesized on RAM ChemMatrix Resin Using Conventionally Heated (86 °C) or Microwave (86 °C) Conditions.

A β (1-42) peptide

	CONV-SPPS 86 °C	CMW-SPPS 86 °C
Yield (%) ^a	78	76
Resin mass (mg) ^b	560	548

^aIsolated yield of the crude product. ^b Dried resin after the synthesis containing the protected peptide; theoretical mass: 650 mg.

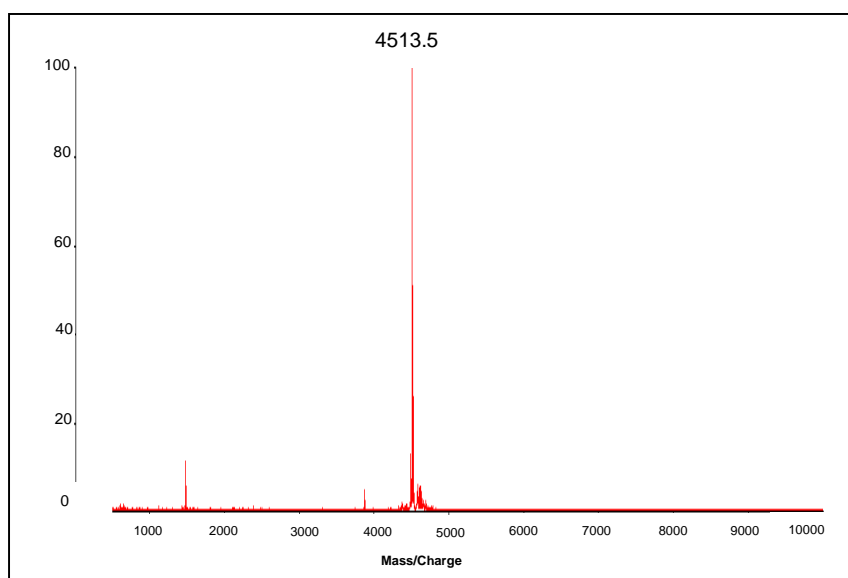


Figure S1. Identification of A β (1-42) peptide via MALDI-TOF MS. MS spectrum of A β (1-42) peptide synthesized on ChemMatrix resins on 0.075 mmol scale using microwave heating at 86 °C. MS spectra corresponding to the main peak (t_R = 4.14 min) $[M+H]^+ = 4513.5$ ($M_{av} = 4513.1$).

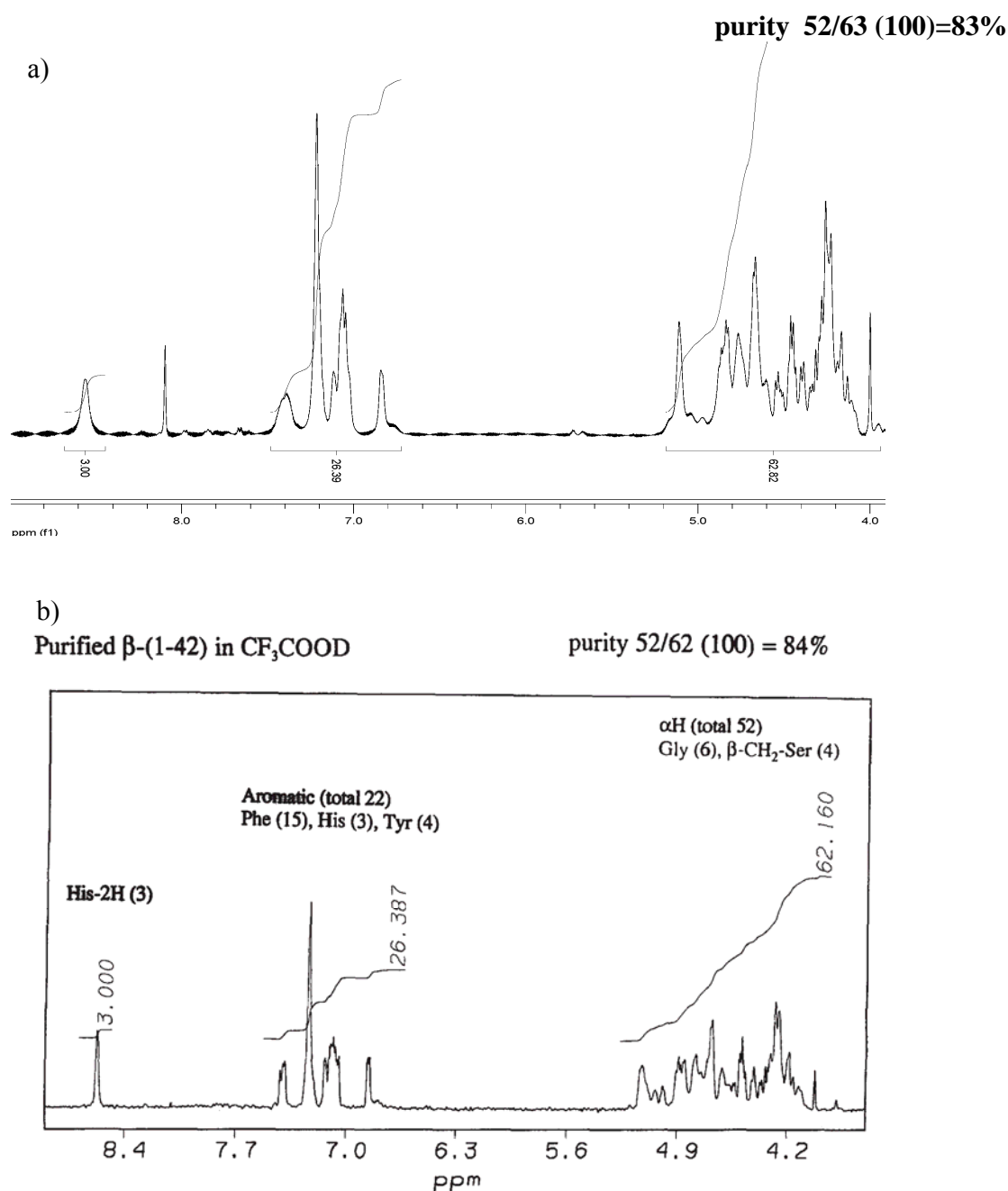


Figure S2. Comparison of (a) NMR Spectrum of the Crude A β (1-42) Peptide Synthesized Using Microwave Heating at 86 °C (b) Reference NMR Spectrum of Purified A β (1-42) Peptide from Zagorsky.^{S2} ^1H NMR spectra (500 MHz) of an (0.40 mM) in d-TFA solution (0.5 ml), which represents an expanded region of the spectrum. Integrals were scaled to the three His-2H protons at 8.56 ppm, and the total number of protons expected for pure A β (1-42) in the His-2H (8.56 ppm), aromatic (7.02-7.44 ppm), and the αH (3.87-5.25 ppm) regions are indicated in parenthesis. The percentage purity was based on the integral heights in the αH region (theoretical heights/ measured heights in the αH region). To ensure complete relaxation of all protons between scans, the pulse width was set to 45 ° rather than 90 °, and the total recycle delay was 8.2 sec.

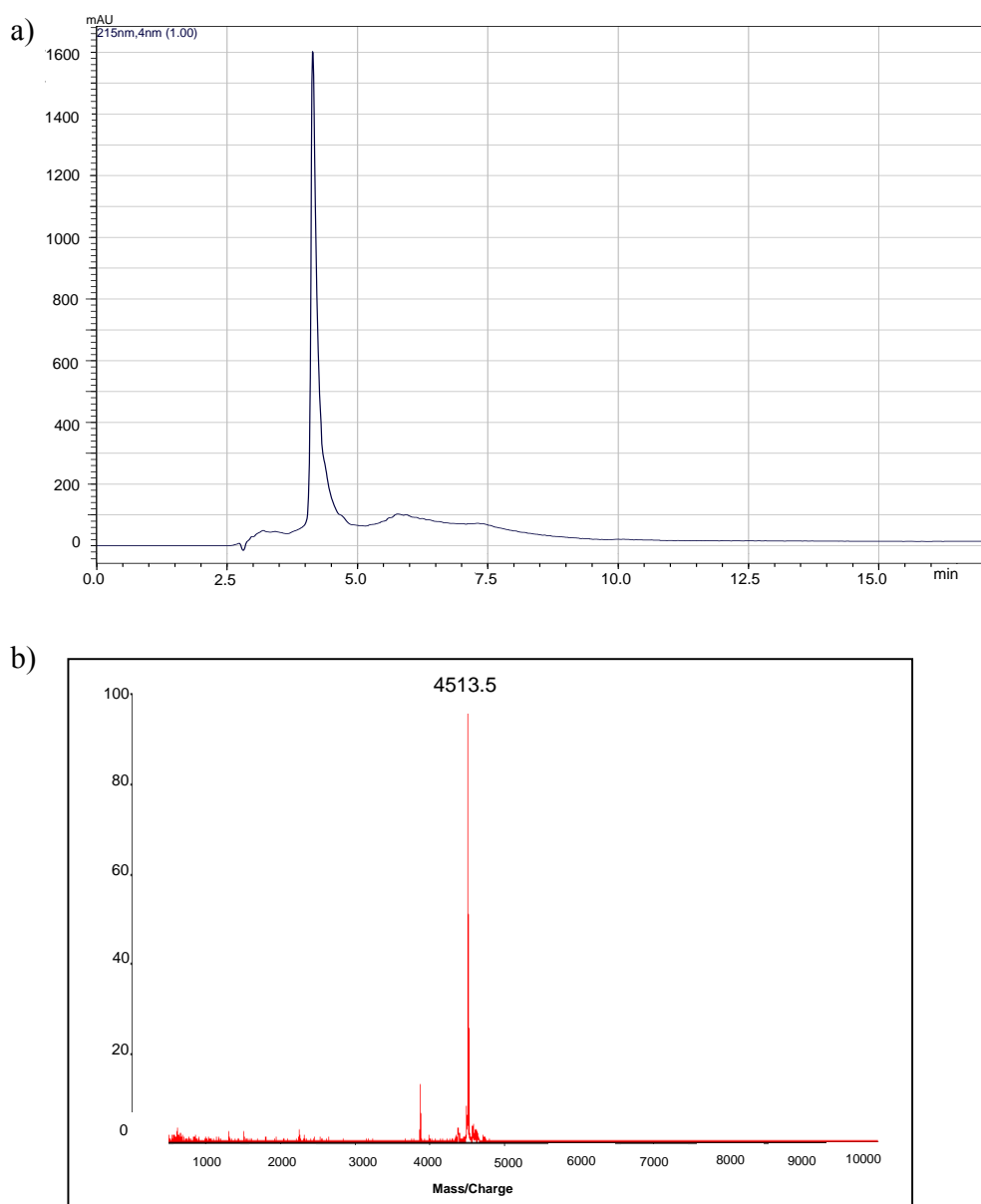


Figure S3. a) HPLC chromatogram of A β (1-42) peptide (analytical RP-HPLC peak area %, UV absorbance at 215 nm) synthesized on 0.075 mmol RAM ChemMatrix resin employing 5 equiv of Fmoc-amino acids at 86 °C coupling and deprotection temperature using conventional heating. b) MALDI-TOF mass spectra of the crude sample $[M+H]^+ = 4513.5$ ($M_{av} = 4513.1$).

Table S2. Amino Acid Analysis and Amino Acid Content of Crude A β (1-42) Peptide Synthesized on ChemMatrix Resin Using Conventionally Heated (86 °C) or Microwave (86 °C) Conditions.

Amino Acid[Calculated]	Amino Acid Analysis / Measured					
	MW-SPPS 86 °C			CONV-SPPS 86 °C		
	24h	48h	72h	24h	48h	72h
Asp [4]	4.08	3.97	3.67	4.00	3.88	3.90
Ser [2]	1.96	1.80	1.58	2.00	1.90	1.67
Glu [4]	4.68	4.49	4.46	4.50	4.39	4.39
Gly [6]	6.57	6.13	6.03	6.50	6.07	5.85
Ala [4]	4.75	4.79	4.72	4.83	4.68	4.86
Val [6]	5.36	5.91	6.03	5.33	6.00	6.19
Met [1]	0.98	0.67	1.05	1.00	0.88	0.91
Ile [3]	1.96	2.62	2.89	2.16	2.63	2.93
Leu [2]	2.04	2.17	1.84	2.25	2.12	2.43
Tyr [1]	0.85	0.75	0.79	0.83	0.73	0.70
Phe [3]	2.87	2.77	2.89	2.83	2.78	2.78
His [3]	2.79	2.77	2.63	2.75	2.78	2.78
Lys [2]	2.34	2.24	2.36	2.33	2.26	2.16
Arg [1]	0.77	0.90	1.05	0.67	0.92	0.70

Amino acid composition and the amino acid content were determined after the hydrolysis of crude A β (1-42) peptide using standard protocols. For this purpose, the peptides were hydrolyzed using 6 M HCl at 110 °C in evacuated and sealed tubes for 24 h, 48 h, 72 h. Using 24 h reaction time led to an incomplete hydrolysis of the crude peptide between Val and Ile residues, which are adjacent in the primary sequence. Complete hydrolysis could be achieved employing longer reaction time (48 h and 72 h), although slight decomposition of the sensitive amino acids (Ser, Met) was observed.

Amino Acid	Amino Acid Content											
	MW-SPPS 86 °C						CONV-SPPS 86 °C					
	$\mu\text{mol/mg}$ 24h	$\mu\text{g/mg}$ 24h	$\mu\text{mol/mg}$ 48h	$\mu\text{g/mg}$ 48h	$\mu\text{mol/mg}$ 72h	$\mu\text{g/mg}$ 72h	$\mu\text{mol/mg}$ 24h	$\mu\text{g/mg}$ 24h	$\mu\text{mol/mg}$ 48h	$\mu\text{g/mg}$ 48h	$\mu\text{mol/mg}$ 72h	$\mu\text{g/mg}$ 72h
Asp [4]	0,54	71,87	0,53	70,54	0,63	83,85	0,48	63,89	0,53	70,54	0,56	74,54
Ser [2]	0,26	27,32	0,24	25,22	0,26	27,32	0,24	25,22	0,26	27,32	0,24	25,22
Glu [4]	0,62	91,22	0,6	88,28	0,72	105,93	0,54	79,45	0,6	88,28	0,63	92,69
Gly [6]	0,87	65,31	0,82	61,56	1,03	77,32	0,78	58,55	0,83	62,31	0,84	63,06
Ala [4]	0,63	56,13	0,64	57,02	0,74	65,93	0,58	51,67	0,64	57,02	0,66	58,80
Val [6]	0,71	83,18	0,79	92,55	0,97	113,64	0,64	74,98	0,82	96,06	0,89	104,26
Met [1]	0,13	19,40	0,09	13,43	0,15	22,38	0,12	17,91	0,12	17,91	0,13	19,40
Ile [3]	0,26	34,10	0,35	45,91	0,48	62,96	0,26	34,10	0,36	47,22	0,42	55,09
Leu [2]	0,27	35,42	0,29	38,04	0,39	51,16	0,27	35,42	0,29	38,04	0,35	45,91
Tyr [1]	0,11	19,93	0,10	18,12	0,12	21,74	0,10	18,12	0,10	18,12	0,10	18,12
Phe [3]	0,38	62,77	0,37	61,12	0,46	75,99	0,34	56,16	0,38	62,77	0,4	66,08
His [3]	0,37	57,41	0,37	57,41	0,44	68,27	0,33	51,20	0,38	58,96	0,4	62,06
Lys [2]	0,31	45,32	0,3	43,86	0,38	55,55	0,28	40,93	0,31	45,32	0,31	45,32
Arg [1]	0,1	17,42	0,12	20,90	0,15	26,13	0,08	13,94	0,12	20,90	0,10	17,42
Amino Acid Content $\mu\text{g/mg}$		686,80		693,95		858,18		621,54		710,77		747,97
Amino Acid Content(%)		69		69		86		62		71		75

Table S3. Racemization Analysis of A β (1-42) Peptide Synthesized on RAM ChemMatrix Resin Using Conventionally Heated (86 °C) or Microwave (86 °C) Conditions.^{S3}

Amino acid	% D Enantiomer	
	CONV-SPPS 86°C	MW-SPPS 86 °C
Histidine	0.86	0.30

Fmoc-His(Trt)-OH residue were built into the sequence at room temperature in both cases.

***In vitro* Cytotoxicity of β -amyloid Peptide Synthesized under Microwave or Conventional Heating Conditions**

The adherent, SH-SY5Y human neuroblastoma cell line was cultured at 37 °C, 5% CO₂ in water-saturated atmosphere and grown in DMEM medium containing 10% fetal calf serum (FCS), 2 mM L-glutamine, 160 µg/mL gentamycin and 1 % nonessential amino acids (NEAA).^{S4,S5}

Cells were plated into 96-well plate with initial cell number of 1.5×10^4 per well. After 24 h incubation at 37°C, cells were treated with 50 µL β -amyloid peptide (DMEM medium containing 10% FCS) in 200 µL final volume containing 2.5% FCS. Cells were incubated with the two differently synthesized A β peptides at 1.69×10^{-2} to 1.10×10^2 µM concentration range for overnight. Control cells were treated with DMEM medium containing 2.5% FCS at 37°C for overnight. Four parallel measurements were performed in all cases.

After overnight incubation at 37 °C, the cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)-assay.^{S6,7} 45 µl MTT-solution (2 mg/ml) was added to each well. The respiratory chain^{S6} and other electron transport systems^{S8} reduce MTT and thereby form non-water-soluble violet formazan crystals within the cell.^{S9} The amount of these crystals can be determined spectrophotometrically and serves as an estimate for the number of mitochondria and hence the number of living cells in the well.^{S10} After 4 hrs of incubation cells were centrifuged for 5 min (1500 rpm) and supernatant was removed. The obtained formazan crystals were dissolved in 50 µL DMSO and optical density (OD) of the samples was measured at $\lambda = 540$ and 620 nm using ELISA Reader (iEMS Reader, Labsystems, Finland). OD₆₂₀ values was subtracted from OD₅₄₀ values. The percent of cytotoxicity was calculated using the following equation:

$$\text{Cytotoxicity (\%)} = [1 - (\text{OD}_{\text{treated}}/\text{OD}_{\text{control}})] \times 100;$$

where OD_{treated} and OD_{control} correspond to the optical densities of the treated and the control cells, respectively. In each case two independent experiments were carried out with 4–8 parallel measurements. The 50% inhibitory concentration (IC₅₀) values were determined from the dose-response curves. The curves were defined using Microcal™ Origin1 (version 6.0) software.

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