Supplementary Material (ESI) for Chemical Communications

Supporting information for:

Rational design of oligopeptide organizers for the peptide-guided formation of poly(ethylene oxide) nanofibers.

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Materials: *N*,*N*-Dimethylglycine (DMG, Aldrich, 97%); diisopropylethylamine (DIPEA; Acros, peptide grade), piperidine (Acros, peptide grade), trifluoracetic acid (TFA; Acros, peptide grade), 1-benzotriazoyloxytris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP, NovaBiochem) and 7-azabenzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorphosphate (PyAOP, Applied Biosystems, Darmstadt, Germany) have been applied as received. All other reagents were used as received from Aldrich.

Fmoc-amino acid derivatives (Fmoc-Thr(*t*Bu) OH, Fmoc-Gly OH, Fmoc-Val OH), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), *N*-methyl-2-pyrrolidone (NMP, 99.9+%, peptide synthesis grade) were used as received from IRIS Biotech GmbH, Germany. Dichloromethane (DCM; IRIS Biotech GmbH, peptide grade) was distilled from CaH_2 and *N*,*N*-dimethylformamide (DMF; Aldrich, 99+%) was distilled prior to use.

TentaGel® PEG Attached Peptide resin (TentaGel PAP) (loading: 0.24 mmol/g; M_n= 3200, PDI= 1.06 [GPC (THF, calibrated against linear PEO standards, PSS, Germany)] was purchased from Rapp, Polymere GmbH, Tuebingen, Germany.

Instrumentation: Mass spectrometry was performed on an electrospray time-of-flight (ESI-TOF) instrument (Shimadzu LCMS-QP8000α) equipped with the standard electrospray ion source operating in the positive mode (detector Gain: 1,6 kV) with a nozzle temperature of 150 °C and a voltage of 4.5 kV. The samples were dissolved in 50 µL of 0.1 vol.-% formic acid in methanol. Measurements were performed using an analyte concentration of 1 mg/mL at a flow rate of 0.4 mL/min.

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) measurements were performed on a Voyager-DE STR BioSpectrometry Workstation MALDI-TOF mass spectrometer (Perseptive Biosystems, Inc., Framingham, MA, USA). The samples were dissolved in 0.1% TFA in acetonitrile-water (1:1, v/v) at a concentration of

0.1 mg/mL. One μL of the analyte solution was mixed with 1 μL of alpha-cyano-4-hydroxycinnamic acid matrix solution consisting of 10 mg of matrix dissolved in 1 mL of 0.3% TFA in acetonitrile-water (1:1, v/v). From the resulting mixture 1 μL was applied to the sample plate. Samples were air-dried at ambient temperature (24°C). Measurements were performed at an acceleration voltage of 20 kV. Each spectrum obtained was the mean of 250 laser shots.

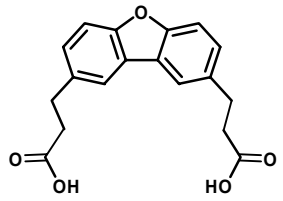
Nuclear magnetic resonance spectra (NMR) were recorded on a Bruker DPX-400 spectrometer at 400 MHz.

Circular dichroism UV spectra were recorded in solution on a Jasco 715 CD spectrometer using about 1 mg/mL sample concentration. Exact concentration was determined by UV spectroscopy via calibration against the 297 nm absorption of the carbazole derivative.

Transmission electron microscopy (TEM) was performed on a Zeiss EM 912Ω instrument at an acceleration voltage of 120kV. The samples were prepared as described previously.[1]

Atomic force microscopy (AFM) was performed on a NanoScope IIIa device (Veeco Instruments, Santa Barbara, CA) in tapping mode. Commercial silicon tips (Type NCR-W) were used with a tip radius <10 nm, employing a force constant of 42 N m^{-1} and a resonance frequency of 285 kHz. The image was recorded on a 10·10 μm e-scanner. The samples were spin-coated (4000 rpm) from solution (0.05-0.5 mg/mL) on freshly cleaved Mica substrates. Analyses of the aggregate dimension were carried out on a sample exhibiting separated structures by averaging over at least 30 values.

Kelly template [dibenzofuran-2,8-dipropanoic acid template[1] (I)]



Chemical structure of ***I***.

Synthesis of the template II

3,6-Dibromo-carbazole-9-acetic acid-ethyl ester ***IIa*** was synthesized from α-bromo ethyl acetate and 3,6-dibromo-9H-carbazole according to a procedure described previously.[2] [Yields (isolated): 84%]

^{1}H-NMR (400 MHz, $CDCl_3$), δ in ppm: 8.13 (s, 2H); 7.56 (d, 1H, J = 1.8 Hz); 7.54 (d, 1H, J = 1.8 Hz); 7.20 (d, 1 H, J = 8.6 Hz); 4.92 (s, 2H); 4.20 (q, 2H, J = 7.1 Hz); 1.21 (t, 3H, J = 7.1 Hz).

^{13}C-NMR (100 MHz, $CDCl_3$), δ in ppm: 167.7, 139.4, 129.3, 123.73, 123.3, 112.8, 110.0, 61.8, 44.7, 14.0.

*3,6-Bis(3-[*N-*[*tert-*butoxy carbonyl]-amino]propyl)-carbazol-9-acetic acid-ethyl ester* (***IIb***)

[*N-tert*-Boc protected 3,6-bis(3-aminopropyl)-carbazole-9-acetic acid-ethyl ester]

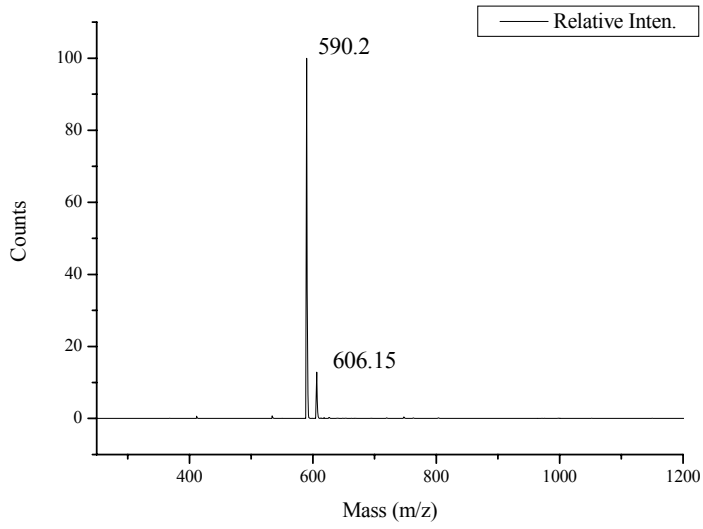
IIb was obtained by carrying out a Suzuki-Miyaura cross-coupling reaction of ***IIa*** and a hydroborated *N*-(*tert*-butoxy carbonyl)allylamine. The latter was accessed from *N-t*Boc allylamine and 9-borabicyclo[3.3.1]nonane in THF according to a procedure described previously.[3] The subsequent Suzuki-cross-coupling was carried out as a heterophase reaction in a mixture of toluene (350 mL) and aqueous KOH (102 mL, 1 molar). After dissolving of the hydroborated *N*-(*tert*-butoxy carbonyl)allylamine (40 mmol) and ***IIa***

(7 g, 17 mmol) the reaction mixture was carefully degassed by four freeze-pump-thaw cycles. After addition of the tetrakis(triphenylphosphine)platinum(0) catalyst (0.59 g, 0.51 mmol) and further degassing cycles the mixture was heated under reflux for 22 h. The reaction mixture was worked up by extraction with saturated $NaHCO_3$ solution (three times), followed by three times extraction with brine. The combined aqueous phases were extracted with chloroform and dried with sodium sulfate before distilling off the organic solvent under vacuum. The crude product was further purified by chromatography using silica (Silica 60, Fluka) as the stationary phase and hexane / ethyl acetate (4:1 v/v) eluent to give ***IIb*** in 45% yield.

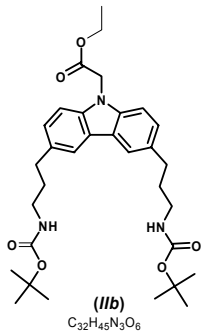
^{1}H-NMR (400 MHz, $CDCl_3$), δ in ppm: 7.84 (s, 2H) (NHBoc); 7.26 (s, 2H); 7.21 (s, 2H); 7.19 (s, 2H); 4.93 (s, 2H); 4.19 (q, 2H; J = 7.1 Hz); 3.18 (s, 4H); 2.80 (t, 4H; J = 7.7 Hz); 1.89 (m, 4H; J = 7.3 Hz); 1.44 (s, 27H); 1.22 (t, 3H; J = 7.1 Hz).

^{13}C-NMR (100 MHz, $CDCl_3$), δ in ppm: 168.5, 156.0, 139.4, 132.6, 126.4, 123.2, 119.7, 108.1, 79.0, 61.5, 44.8, 40.2, 33.0, 32.4, 27.4, 14.0.

Elemental analysis: C: 66.67% (th.: 67.70%); H: 7.85% (th.: 7.99%); N: 6.98% (th.: 7.40%); O: 16.95% (th.: 16.91%).



ESI-TOF-MS spectrum of ***IIb***.



Substance	m/z (th.)	m/z (exp.)
$[M]^+$	567.33	
$[M + Na]^+$	590.32	590.2
$[M + K]^+$	606.43	606.2

*3,6-Bis(3-[*N-*[*tert-*butoxy carbonyl]-amino]propyl)-carbazol-9-acetic acid* (***IIc***)

The final product ***IIc*** could be obtained by hydrolytic ester saponification of ***IIb***. Therefore ***IIb*** (2.0 g, 3.5 mmol) was dissolved in a mixture of aqueous KOH (14 mL, 1 mol/L) and THF (100 mL). The reaction mixture was refluxed for 23 h at 60° C. After the workup following a procedure described previously[4] ***IIc*** could be accessed in 99% yield.

^{1}H-NMR (400 MHz, DMSO-d_6), δ in ppm: 12.91 (s, 1H); 7.88 (s, 2H); 7.37 (d, 2H; J = 8.3 Hz); 7.20 (d, 2H; J = 8.6 Hz); 6.86 (t, 2H; J = 5.6 Hz); 5.11 (s, 2H); 2.95 (q, 4H, J = 6.3 Hz); 2.68 (t, 4H; J = 7.4 Hz); 1.73 (m, 4H, J = 7.1 Hz); 1.36 (s, 18H).

Synthesis of III

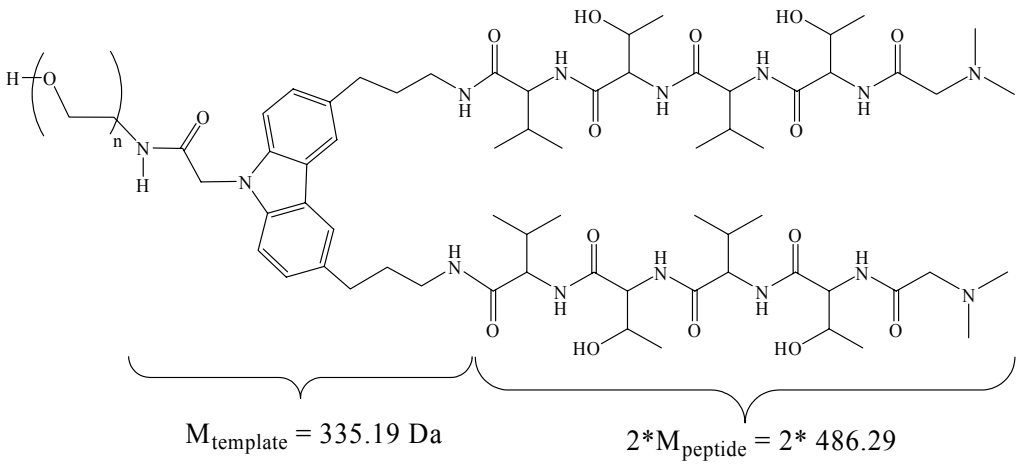
PEO-template-oligopeptide [(DMG-Thr-Val-Thr-Val)$_2$NH-Template-PEO$_{68}$]

The loading of the TentaGel® PAP resin (0.24 mmol/g; 0.1 mmol scale) with ***IIc*** was performed using standard solid-phase supported peptide synthesis protocols applying PyAOP/DIPEA/NMP chemistry.[5] Quantitative loading was achieved by repetitive coupling cycles exhibiting only small excess of ***II*** [amine functionalities on the support 0.8 eq.; ***IIc*** (1.0 eq.), PyAOP (1.2 eq.), DIPEA (2.0 eq.), NMP, 4 h coupling]. Double or triple couplings were necessary due to low excess and steric hindrance of the template. Quantitative loading was verified by a negative Kaiser test.[6] After removal of the *t*Boc protecting groups

[TFA/DCM (50 v/v%), 30 min.] the resin was loaded on an Applied Biosystems ABI 433a peptide synthesizer. Oligopeptide synthesis was performed in NMP as the solvent following standard ABI-Fastmoc double coupling protocols. Amino acid coupling was facilitated using HBTU/DIPEA. The synthesis was interrupted after each coupling cycle in order to verify full conversion with Kaiser tests. After final Fmoc-group removal [$(H_2N$-Thr-Val-Thr-Val$)_2$-Templ.-PEO)], amination of the terminal amine group was achieved with 10 equivalents excess of DMG using standard PyBOP/DIPEA protocols in DMF. The liberation of ***III*** was accomplished by 2-6 h treatment with the standard cleavage mixture [99% TFA, 1% trimethylsilylbromide (TMSBr)], followed by two washing cycles with TFA/DCM (10 v/v%). The oligopeptide was isolated by diethyl ether precipitation, centrifugation and dialysis (MWCO ≈ 1000 Da) followed by lyophilization from water. A yield of 45% of ***III*** was obtained without further purification.

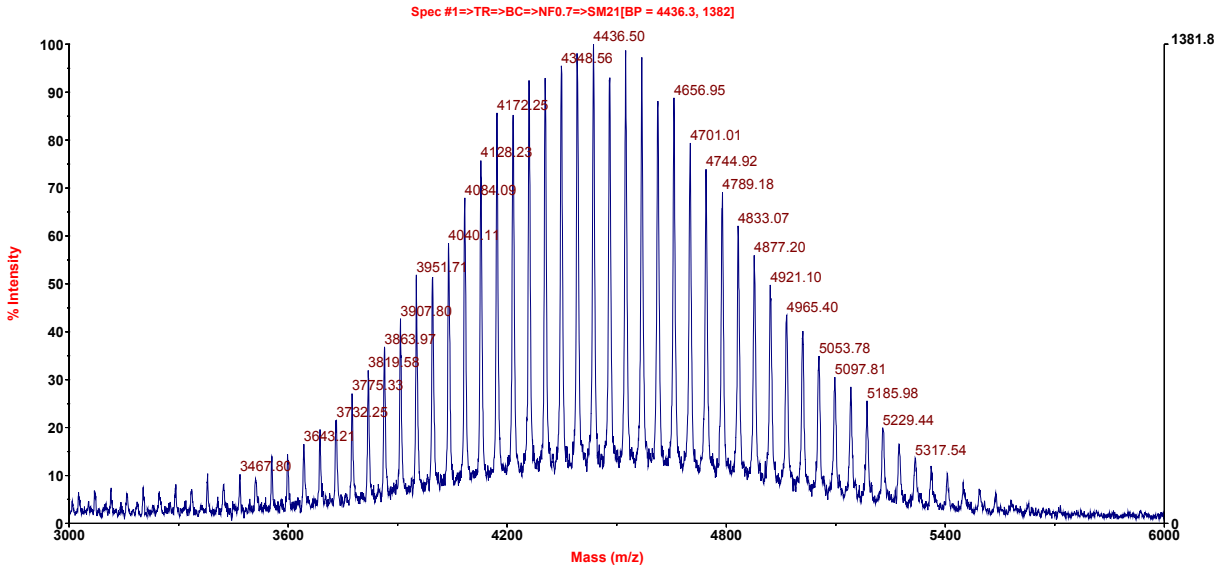
The MALDI-TOF-MS of ***III*** exhibits a single homologous series with a repetitive mass unit of about 44 Da, which could be assigned to the EO repetition unit of the PEO (M_{EO} = 44.03 Da).

Peak masses could be calculated by $M = M_{template}+2*M_{peptide}+n*M_{EO}+ M_{end\ groups}+M_{carrier\ ion.}$



Chemical structure of ***III***.

With fragment masses of $M_{template}$= 335.19 Da; $M_{peptide}$= 486.29 Da; M_{EO}= 44.03 Da and $2H^+$ the mass peaks could be assigned with an average reminder mass of about ±0.5 Da. E.g. the peak m/z = 4479.93 can be assigned to the target molecule with 72 repeat units of EO (n = 72) with a remainder mass of +0.5 Da.



*MALDI-TOF-MS of **III**.*

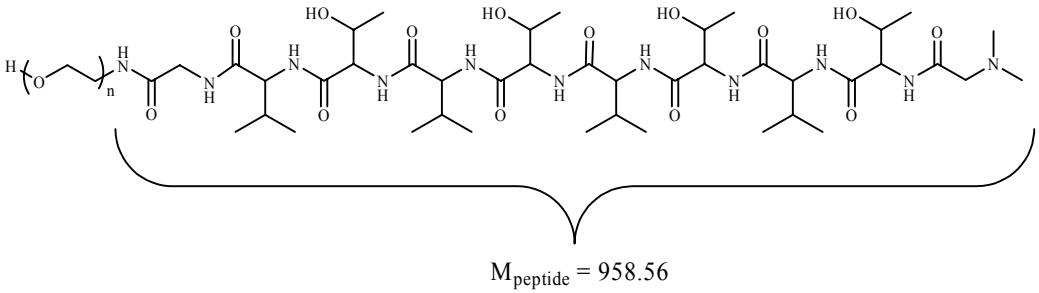
Synthesis of IV

PEO-oligopeptide (DMG-Thr-Val-Thr-Val-Thr-Val-Thr-Val-Gly-NH-block-PEO_{68})

The linear analog was synthesized following similar procedures described above.

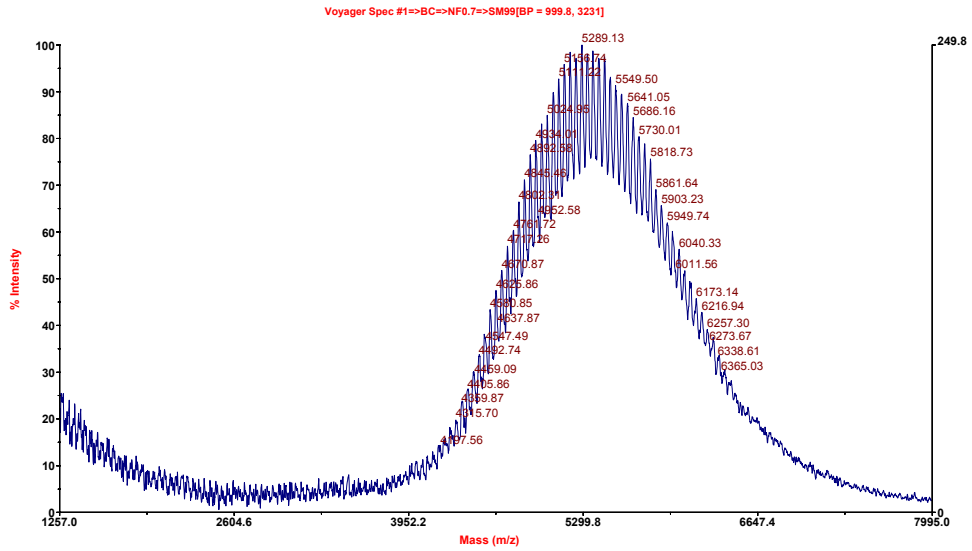
MALDI-TOF-MS spectra of the linear analogue ***IV***

The MALDI-TOF-MS of ***IV*** shows a reduced resolution in comparison to ***III***. However, an average repetitive mass unit of about 44 ± 4 Da could be determined assignable to the EO repetition unit.

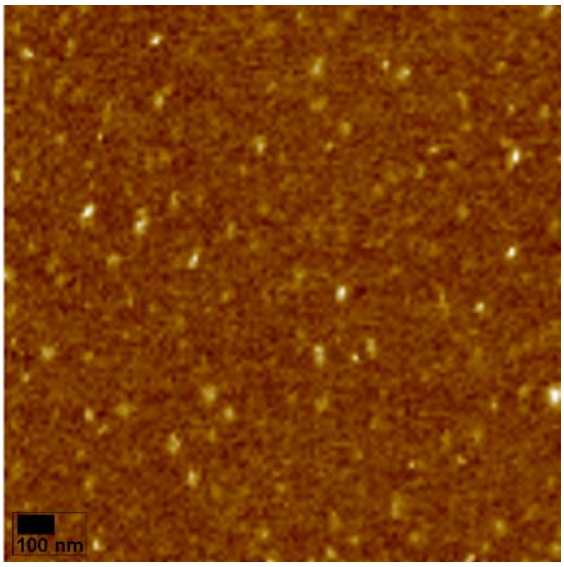


Chemical structure of ***IV***.

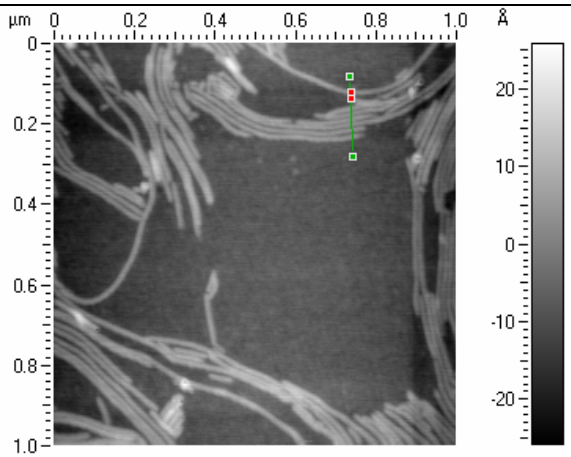
The mass peaks can be calculated according to $M = M_{peptide} + n*M_{EO} + M_{end\ groups} + M_{carrier\ ion}$ by taking the following fragment masses in account: $M_{peptide-H_2O}$ = 958.56 Da; M_{EO} = 44.03 Da; Na^+, K^+. Due to the low resolution an average remainder mass of ±2 Da can be found.

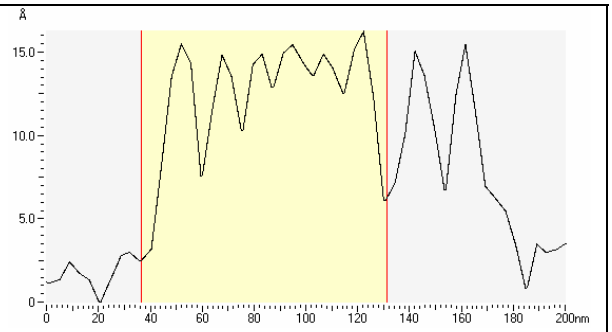


MALDI-TOF-MS spectra of the linear analogue ***IV***.



AFM micrograph of ***III*** (spin coating of a 0.1 mg/mL methanolic solution after the deaggregation procedure).





peak distances in nm (from left to right): 14.65, 13.10, 13.09, 13.01, 13.83 (evaluation of the average lateral distance took 52 distances in account)

AFM micrograph of ***III*** (left) (spin coated of an aq. solution (0.5 mg/mL), height image).

Height profile of a close parallel packing of fibers (right).

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