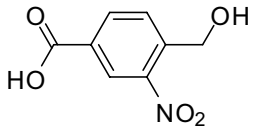
Experimental

Methods and materials. ^{1}H-NMR spectra were measured on a Varian Inova 400 MHz NMR spectrometer. MALDI-TOF spectra were measured on a Bruker Biflex II mass spectrometer, 2,5-dihydroxybenzoic acid (DHB) was used as matrix. Electrospray mass spectra were recorded on a Thermo LCQ or JEOL Accu-TOF. UV-Vis measurements were performed on a Varian Cary 50 UV-Vis spectrophotometer and all CD measurements were performed on a Jasco J-810 spectropolarimeter with Peltier temperature control. Transmission Electron Microscopy was performed on a JEOL 1010 transmission microscope. Peptides were synthesized on a Labortec SP4000 or a Labortec SP640 semi-automatic peptide synthesizer. The UV source was a Bluepoint 2 with a quartz UV guide (dr Hönle, 290-450 nm, 3 mW/cm^2). All reagents were obtained from common commercial sources and used as received.

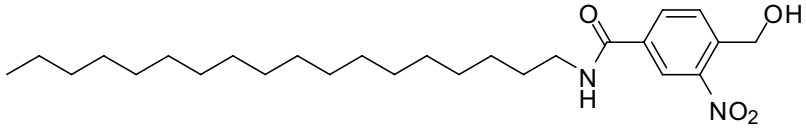
Synthesis

4-(hydroxymethyl)-3-nitrobenzoic acid



This compound was prepared according to a modified literature procedure.[1] 0.26 g (1.0 mmol) 3-nitro-4-bromomethylbenzoic acid and 0.53 g (5.0 mmol) Na_2CO_3 were dissolved in 4 mL H_2O and 4 mL acetone. The mixture was heated to reflux for 5 hours. Subsequently, the acetone was removed by evaporation. The residual solution was washed with diethyl ether and concentrated HCl was added until a precipitate was formed at pH 2. The resulting suspension was extracted with ethyl acetate while the pH was kept at 2. The combined organic layers were washed with water, dried over $MgSO_4$, filtered and concentrated to a brown solid. The product was purified by column chromatography on silica (eluent: dichloromethane, methanol, acetic acid (89/10/1, v/v/v)) (yield: 70%). R_f 0.26. The analytical data were in agreement with earlier reported data.[1, 2]

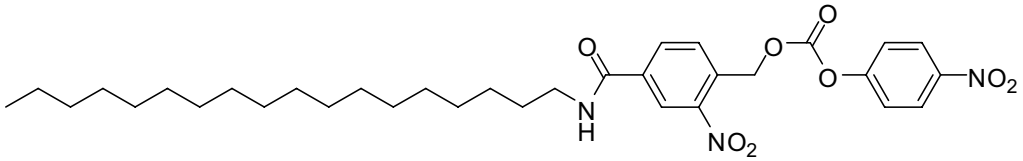
4-(hydroxymethyl)-3-nitro-N-octadecylbenzamide



0.20 g (1.0 mmol) 4-(hydroxymethyl)-3-nitrobenzoic acid and 0.27 g (1.0 mmol) $C_{18}H_{37}NH_2$ were dissolved in dichloromethane (2.5 mL), 0.44 g (1.0 mmol) benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphoniumhexafluorophosphate (BOP) and 345 µl (2.0 mmol) N,N-diisopropylethylamine were added and the mixture was stirred overnight while the pH was kept at 8. The reaction mixture was evaporated in vacuo and the product was dissolved in ethylacetate. The mixture was washed three times with 1M HCl and with water, 1M $NaHCO_3$, water and finally brine. The ethyl acetate was dried over $MgSO_4$ filtered and the solvent was evaporated in vacuo. The product was purified by column chromatography (eluent: dichloromethane, methanol, acetic acid (84/5/1, v/v/v)) to afford a 90% yield. R_f 0.29.

^{1}H-NMR (DMSO-d6) δ 8.70 1H (t), 8.58 1H (d), 8.25 1H (dd), 7.85 1H (d), , 5.60 1H (s), 4.80 2H (s), 3.25 2H (q), 1.5 2H (quint), 1.26 4H (m), 1.20 26H (s), 0.80 3H (t). ESI-Ion trap: Calcd. mw. for $C_{26}H_{45}N_2O_4$ = $[M+H]^+$: 449.34 , found: 449.36.

4-(octadecylcarbamoyl)-2-nitrobenzyl 4-nitrophenyl carbonate



0.37 g (1.00 mmol) 4-(hydroxymethyl)-3-nitro-N-octadecylbenzamide, 125 µl (1.50 mmol) pyridine and 0.10 g (1.05 mmol) 4-nitrophenyl chloroformate were dissolved in distilled THF and stirred at 0 °C for 2 hours under argon atmosphere. The solvent was evaporated in vacuo and the product was redissolved in dichloromethane. The mixture was washed with brine and 1M HCl, dried over Na_2SO_4 and the volatiles were evaporated in vacuo. Column chromatography (eluent: dichloromethane/methanol (97.5/2.5 v/v), afforded the product as a white solid in a 79% yield. R_f 0.54.
^{1}H-NMR (DMSO-d6) δ 8.80 1H (t), 8.58 1H (d), 8.30 2H (d), 8.25 1H (dd), 7.85 1H (d), 7.58 2H (d), 5.69 2H (s), 3.25 2H (q), 1.5 2H (quint), 1.26 4H (m), 1.20 26H (s), 0.80 3H (t). ^{13}C-NMR ($CDCL_3$) δ 14.3, 22.9, 27.2, 29.5, 29.6, 29.76, 29.81 29.9 (broad), 32.1, 40.8, 67.2, 121.9, 123.8, 125.6, 129.4, 132.8, 136.4, 145.8, 147.2, 152.2, 155.4, 164.7. ESI-TOF: Calcd. mw. for $C_{33}H_{47}N_3NaO_8$ = $[M+Na]^+$: 636.32608, found: 636.3217.

Fmoc-Lys-Thr-Val-Ile-Ile-Glu-NH-PEG-resin

The peptides were synthesized from a tentagel PAP resin (Rapp Polymere). The resin was swollen in N,N-dimethylformamide (DMF) for 30 minutes before use. The coupling reactions were carried out in DMF with three equivalents of Fmoc protected amino acid in the presence of N-hydroxybenzotriazole (HOBt; 3.6 equivalents) and diisopropylcarbodiimide (DIPCDI; 3.3 equivalents). The amide coupling was monitored for completion with a Kaiser Test.[3] The Fmoc protecting group was removed by treatment of the resin with a 20% solution of piperidine in DMF (v/v) (3 times 6 minutes). Between each step the resin was washed thoroughly with DMF. The completed peptide was washed with DMF, dichloromethane, isopropanol, dichloromethane and finally diethyl ether and subsequently dried in vacuo. The resin was divided in batches that were further functionalized.

Typical synthesis $H_{2n+1}C_nCO$-Lys-Thr-Val-Ile-Ile-Glu-NH-PEG-OH

Dry Fmoc-Lys-Thr-Val-Ile-Ile-Glu-NH-PEG-resin was swollen in DMF for 1 hour and subsequently treated with piperidine (20% in DMF v/v, three times 6 minutes). The resin was washed with DMF and dichloromethane. Three equivalents of the appropriate n-alkyl carboxylic acid were dissolved in dichloromethane; HOBt (3.6 equivalents, 1M in DMF) and neat DIPCDI (3.3 equivalents) were added and the resulting mixture was added to the resin. (For the acetyl group we employed 10 equivalents of acetic anhydride and 10 equivalents of pyridine instead) The suspension was agitated overnight. The resin was washed thoroughly with dichloromethane and methanol, finishing with dichloromethane. The final product was cleaved from the resin in 4 hours by treatment with TFA/water

(95/5 v/v). The resin was removed by filtration and the peptide was isolated by precipitation in diethyl ether.
A typical ^{1}H-NMR (DMSO-d6) is given for $H_{31}C_{15}CO$- Lys-Thr-Val-Ile-Ile-Glu-NH-PEG-OH **2f** : δ 12.1 1H (br s), 8.15 1H (d), 8.0 3H (m), 7.80 1H (t), 7.65 4H (m), 4.7 1H (s), 4.2 4H (m), 3.9 1H (q), 3.7 2H (t) 3.6 110H (s), 2.8 2H (t), 2.2 2H (q), 2.0 2H (m), 1.9 2H (d), 1.7 4H (m), 1.5 2H (m), 1.4 2H (m), 1.2 28H(s), 1.0 (q, 2H), 1.1 2H (d), 0.75 25H (m). A Maldi-TOF spectrum is shown on page S9.

***Synthesis of $H_{35}C_{17}$CO-linker- Lys-Thr-Val-Ile-Ile-Glu-NH-PEG-OH* 3**.
Fmoc-Lys-Thr-Val-Ile-Ile-Glu-NH-PEG-resin was swollen in DMF for 1 hour and subsequently treated with piperidine (20% in DMF v/v, three times 6 minutes). The resin was washed with DMF and dichloromethane. Two equivalents of 4-(octadecylcarbamoyl)-2-nitrobenzyl 4-nitrophenyl carbonate were dissolved in dry dichloromethane and three equivalents of N,N-diisopropylethylamine were added. The mixture was added to the resin and the reaction mixture was agitated overnight. The resin was washed using dichloromethane, DMF, dichloromethane, methanol and diethyl ether and dried in vacuo. The peptide was cleaved from the resin using a mixture of TFA, water, ethanedithiol and triisopropylsilane (92.5/2.5/2.5/2.5 by volume). The peptide was isolated by precipitation in diethylether.
^{1}H-NMR (DMSO-d6) (δ ppm): 12.1 1H (br s), 8.75 1H (t), 8.50 1H (d) 8.15 1H (d), 8.0 3H (m), 7.80 1H (t), 7.65 4H (m), 7.6 1H (d), 5.4 2H (s), 4.7 1H (s), 4.2 4H (m), 3.9 1H (q), 3.7 2H (t) 3.6 110H (s), 2.8 2H (t), 2.2 2H (q), 2.0 2H (m), 1.9 2H (d), 1.7 4H (m), 1.5 2H (m), 1.4 2H (m), 1.2 30H (s), 1.0 (q, 2H), 1.1 2H (d), 0.75 25H (m)

Fibril formation. This procedure was reported previously.[4] In short solutions of peptides with a concentration of 1 mg mL^{-1} were prepared in Mili-Q water, in siliconized Eppendorf tubes. The sample was filtered through a 0.2 μm filter prior to use. The samples were sonicated at 50 °C for 30 minutes. The peptide solutions were incubated for 5 days to allow for fibers to form.

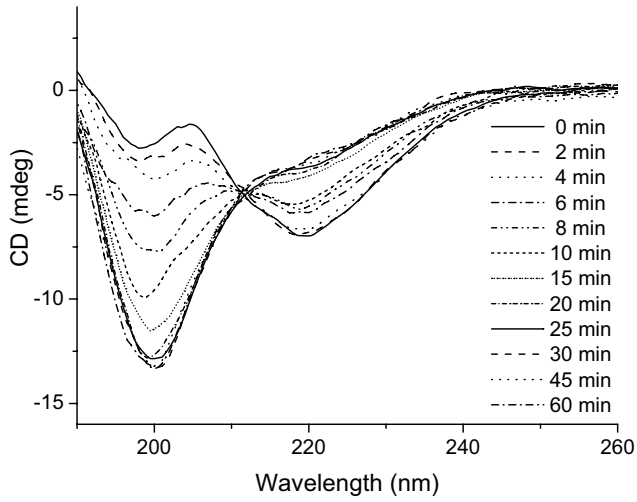
CD spectroscopy. Samples were measured at 25 °C in a 1 mm quartz cell. Temperature dependent CD measurements were performed in a temperature range from 10 to 90 °C. The temperature was decreased or increased at a speed of 3 °C per minute. The elipticity was followed at 222 nm at 0.5 nm intervals, while a full spectrum was measured every 10 °C.

TEM. A carbon-coated grid was floated on a drop of peptide solution for 5 minutes. The grid was blotted and dried in vacuo. The dried grids were platinum shadowed at an angle of approximately 45 degrees. The TEM images were obtained with a JEOL- JEM-1010, equipped with a CCD camera, at a 60 kV accelerating voltage.

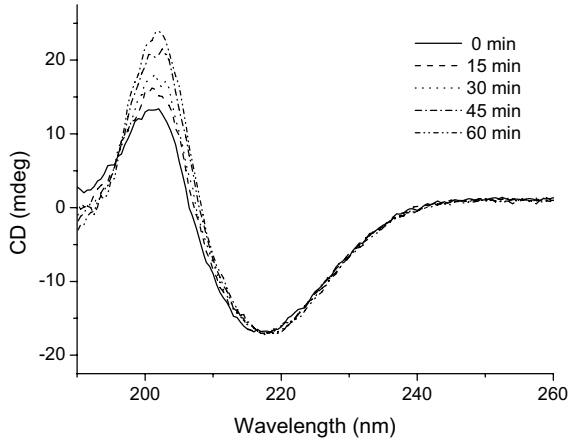
UV exposure. A solution of peptide in a 1 mm quartz cell was placed 5 cm below the UV source. The solution was exposed to the source between 1 and 15 minutes, determined by the timer-controlled shutter of the source. The sample was shaken and the CD spectrum was recorded. The procedure was repeated until the total exposure was 1 hour.

Subsequently a sample was taken and transferred to a TLC plate (chloroform, methanol, water 65/25/4, v/v/v, see page S7).

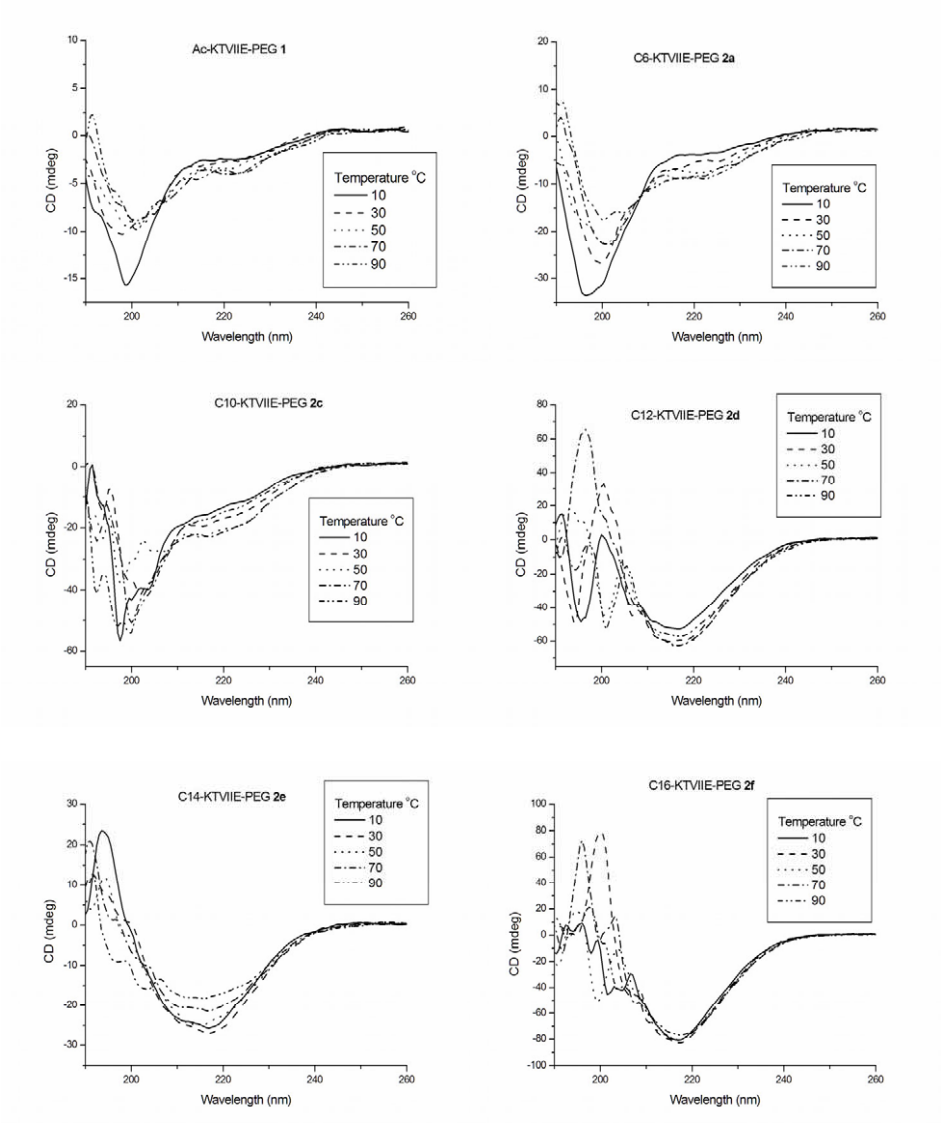
1 Additional CD spectra



1-1 Exposure of $H_{35}C_{17}CO$-linker-Lys-Thr-Val-Ile-Ile-Glu-NH-PEG-OH **3** to UV source.

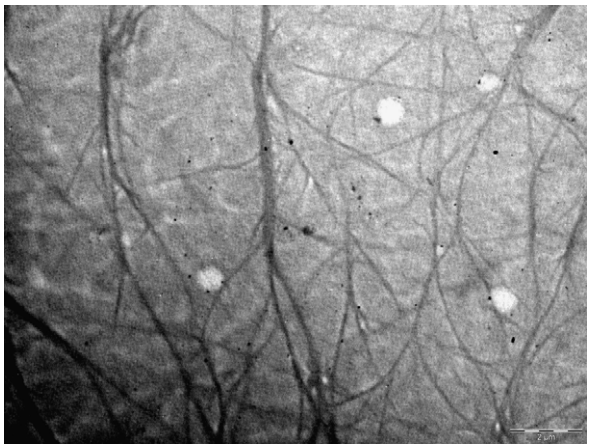


1-2 Exposure of $H_{31}C_{15}CO$- Lys-Thr-Val-Ile-Ile-Glu-NH-PEG-OH **2f** to UV source.

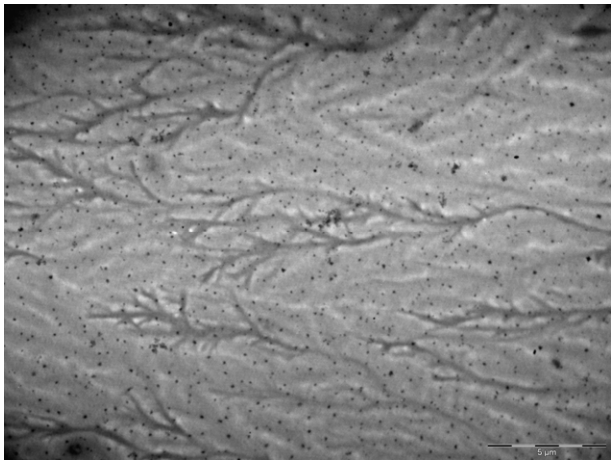


1-3 Temperature dependent CD spectra of Cn-KTVIIE-PEG **1, 2a** and **2c-f,**10-90°C

2 TEM images

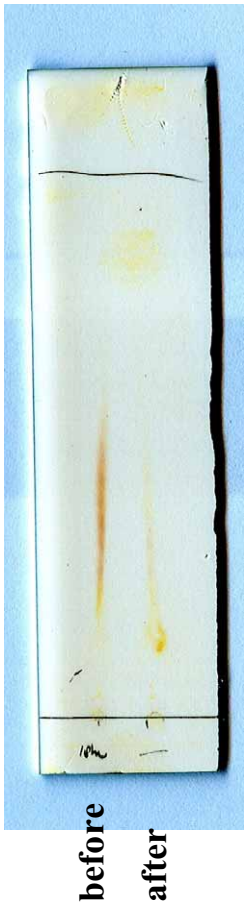


2-1 C16-KTVIIE-PEG **2f**



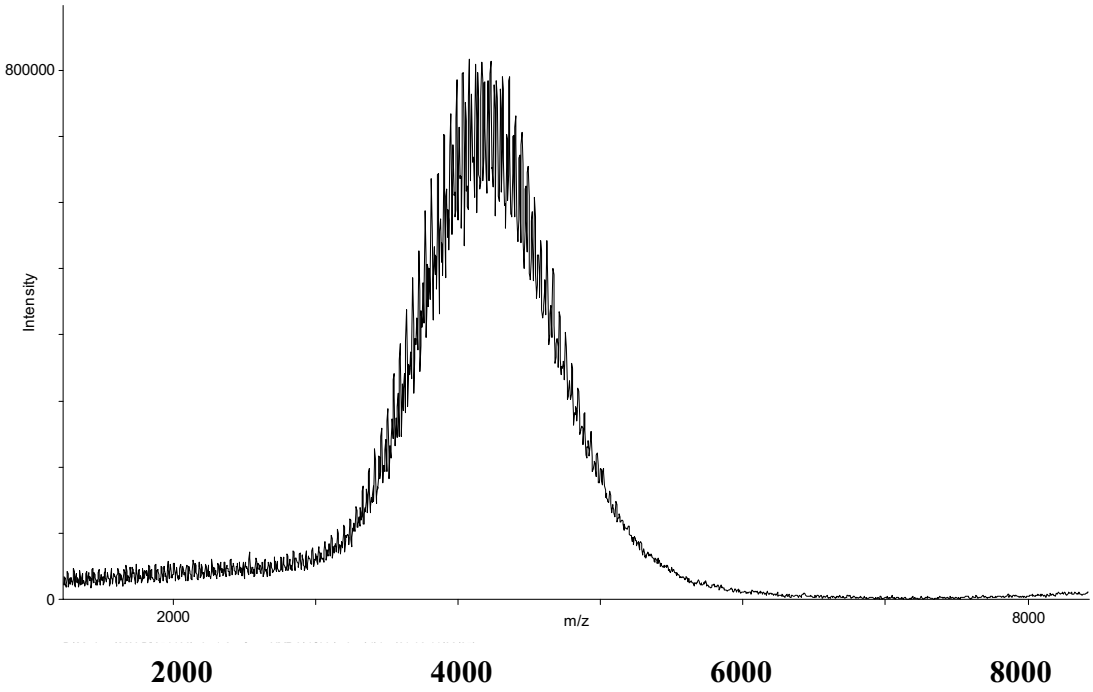
2-2 C18-linker-KTVIIE-PEG **3**

3 TLC



3-1 TLC of C18-linker-KTVIIE-PEG 3, before (left) and after (right) exposure to UV light, I_2 Stain**.**

4 Maldi-TOF



4-1 Maldi-TOF spectrum of C16-KTVIIE-PEG **2f**

References:
1. Eisenführ, A.; Arora, P.S.; Sengle, G.; Takaoka, L.R.; Nowick, J.S.; Famulok, M. *Bioorg. Med. Chem.*, **2003**, 11, 2, 235.
2. Nicolas, E.; Clemente, J.; Ferrer, T.; Albericio, F.; Giralt, E., *Tetrahedron,* **1997**, *53*, 3179.
3. Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Analytical biochemistry* **1970,** 34, 2, 595.
4. Meijer, J.T.; Roeters, M; Viola, V.; Löwik, D.W.P.M.; Vriend, G.; van Hest, J.C.M. *Langmuir*, **2007**, 23, 4, 2058