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Controlled disassembly of peptide amphiphile fibres‡

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Abstract: In this paper, the introduction of both a methionine residue and a nitrobenzyl derivative as a labile linker between the peptide part and the hydrophobic alkyl chain of a peptide amphiphile are presented. These modifications are shown not to inhibit the formation of structured assemblies that analogous peptide amphiphiles lacking the linkers are able to form. Moreover, the introduction of either labile linker allows removal of the peptide amphiphile's stabilizing hydrophobic moieties to initiate a controlled disassembly of fibre aggregates. This is achieved by either treatment with CNBr or UV irradiation, respectively. These disassembly mechanisms could be the starting point for methodology that allows further manipulation of self-assembled peptide amphiphile architectures. Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.

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Keywords: peptide amphiphiles; self-assembly; nano-fibres; caged peptides; switching assembly

INTRODUCTION

From the continual desire to scale down devices, a quest for well-defined architectures on a nano-sized scale has emerged, which can be generally divided in a top-down and a bottom-up approach [1]. For the latter, directed self-assembly has become an important tool to construct nano-structures and novel functional materials [2]. In this respect, peptides and peptide amphiphiles, specifically, are a very useful class of molecules that allow us to build self-assembled materials with both structural and functional control through the interplay between directional hydrogen bonds and hydrophobic interactions [3,4]. Therefore, interest in peptide amphiphile structures, particularly as a possible source for bioactive materials has increased recently [5–9]. Well-defined aggregates of peptides have, furthermore, been used to induce structure into materials, e.g. via the assembly of synthetic polymers [10–12].

However, in many cases, assembly is merely a spontaneous process that cannot be directed easily. In order to manipulate peptide assembly further, light [13–15] and chemical switches [16,17] have been developed in which subtle changes in hydrophobicity lead to an increased propensity for aggregation. Previously, we have shown the introduction of an *N*-terminal hydrophobic moiety induced fibre assembly of the otherwise soluble GANPNAAG peptide, which is derived from the CS protein of the Malaria parasite *Plasmodium Falciparum*, and has been suggested as a possible target in vaccination studies [18]. Only at elevated temperatures (up to 90 °C), the fibrous structures could be disassembled. Hence, subtle changes in the hydrophobicity have a profound effect on the self-assembly process.

While in most cases research has focussed on inducing assembly [13–17], here we report on a convenient methodology to actively disassemble peptide fibres [19] by manipulating the hydrophobic–hydrophilic balance by cleaving off the hydrophobic tail, shown schematically in Figure 1. Such controlled degradation processes would allow us to gain further control over the self-assembled structures, particularly with respect to the normally not controlled fibre length. For instance, in combination with alignment of the fibres, it should be possible to 'cut' the nano-fibres with UV light into pieces of highly defined length by using an appropriate grating as a mask or by means of an interference pattern that results in the disassembly of the irradiated areas [20].

This novel approach could therefore provide an additional tool in the development of new material with nano-scale architecture. Moreover, a controlled degradation of such fibres with a hydrophobic core and a biocompatible hydrophilic outer shell could conceivably be exploited as drug delivery vehicles with a controlled release mechanism. We have investigated

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two approaches to initiate fibre disassembly: the first involving a CNBr mediated hydrolysis, and the second a UV light-induced photolysis reaction.

RESULTS AND DISCUSSION

The peptides shown in Figure 2 were prepared on a Wang resin using a standard Fmoc-based protocol [21,22]. Compounds **1** and **2** were obtained after an acylation in the final step with palmitic acid as described earlier [18]. A photo-cleavable linker was introduced through treatment of the GANPNAAG peptide with *p*-nitrophenol carbonates **9a** and **9b** in the final solid-phase step in the preparation of peptides **3** and **4** respectively. The p-nitrophenol carbonate building blocks were synthesized from commercially available 3-nitro-4-bromomethylbenzoic acid, as shown in Scheme 1. Also the commercially available 2-nitroterephthalic acid-1-methyl ester was considered as a starting material for the preparation of benzylalcohol **7**. However, procedures to selectively reduce the methyl ester only resulted in products that were difficult to purify, and therefore, this route was not further explored. Alternatively, benzylbromide **6** was easily transformed into the corresponding alcohol **7** in an 88% yield [23]. The use of Benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (BOP) as a coupling reagent in the next step prevented any polymerization of **7** as might be expected when employing stronger acylating agents such as N,N′-dicyclohexyl-carbodiimide (DCC).

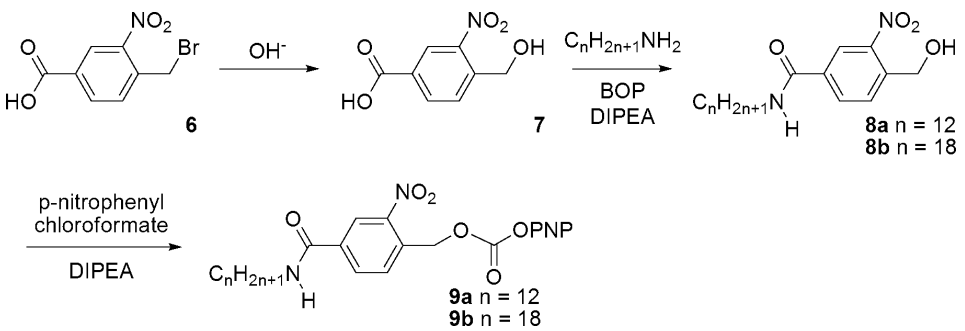
The two different chain lengths were chosen in advance because it was uncertain whether the nitrobenzyl moiety would affect the fibril formation. The combination of a C12 chain with the benzyl linker was estimated to be comparable in length with a C16 alkyl chain. The C18 analogue was expected to give more stable assemblies, due to its greater hydrophobicity. C12 and C18 compounds **8a** and **8b** were obtained in yields of at least 86% from carboxylic acid **7**. In a final step, 4-nitrophenyl chloroformate was added to benzyl alcohols **8** to afford building blocks **9a** and **9b** after column chromatography in an 80 and 79% yield, respectively.

Figure 1 Manipulating peptide assembly by changing the hydrophobic–hydrophilic balance. P1 = peptide, P2 = hydrophobic moiety.

First, it was established whether derivatives **2**–**4** all formed fibrillar structures like peptide amphiphile **1** does [18]. Assemblies of the peptide amphiphiles were prepared by hydration of the materials at a concentration of 0.20 mg/ml in Milli-Q water at 50 °C for 30 min, and subsequent sonication for 15 min at that temperature, which resulted in clear solutions which were allowed to cool to ambient temperature. These solutions were examined with circular dichroism (CD) spectroscopy [24] and transmission electron microscopy (TEM). All compounds displayed a very strong positive signal around 195 nm in their CD spectra, (See supporting information) which is caused by linear dichroism component due to partial alignment of the fibres in the sample cell and is strongly related to fibril formation with this type of amphiphiles, as was shown earlier for compound **1** and analogues thereof [18].

This was confirmed with TEM measurements which all revealed the presence of fibrillar structures, as is shown in Figure 4(a) for compound **3**. From these results, it can be concluded that the incorporation of neither linker had any adverse effect upon fibre formation. Moreover, the photo-cleavable nitrobenzyl-based linker added to the stability of the peptide fibres that were formed, because the same GANPNAAG peptide with only an *N*-terminal C12 dodecanoyl chain does not form fibrillar structures [18].

In a first approach, methionine was examined as a labile link between the GANPNAAG peptide and a hydrophobic palmitoyl tail (C_{16}-GP amphiphile **2**). Now, in order to induce cleavage of the hydrophobic moiety, formic acid and cyanogen bromide were added to the solution. Unfortunately, we were not able to monitor the cleavage process with CD spectroscopy hampered by the strong UV absorption of the CNBr and



Scheme 1 Synthesis of building blocks 9a and 9b.

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formic acid additives required for a reaction to occur. Nevertheless, mass spectrometry clearly gave evidence of the formation of free GANPNAAG in solution, in contrast to model compound **1** lacking the methionine, which was stable under these conditions. TEM pictures of a solution of **2** after CNBr treatment also showed the absence of any structures. Because we were not able to quantify the cleavage of the alkyl tail in time and correlate this directly to the fibre disassembly process, this method was not pursued any further, and reverted to an alternative approach.

In this second approach, we examined the response towards UV light of fibrils composed of photo-labile peptide amphiphiles **3** and **4**. For this, the fibril solutions were transferred to 1 mm quartz cells and irradiated with a strong UV source for a controlled time period, after which a CD spectrum was recorded. In Figure 3, it can clearly be seen that for C12 compound **3** the strong CD signal around 195 nm rapidly decreased upon irradiation. After 4 min the signal had completely transformed into that of a peptide with a random coil conformation, which is expected for the free GANPNAAG peptide in solution [25]. Therefore, it was concluded the fibril structures had been completely disassembled, which was corroborated by the fact that with TEM no structures could be found (Figure 4(b)). The decomposition of the peptide amphiphile was further evidenced by running a thin-layer chromatogram (TLC) of the irradiated solution after different periods of UV irradiation, as is shown in Figure 5. It can be seen that after 10 min all of the starting material had disappeared. The fact that all fibres had already disassembled after 4 min, as

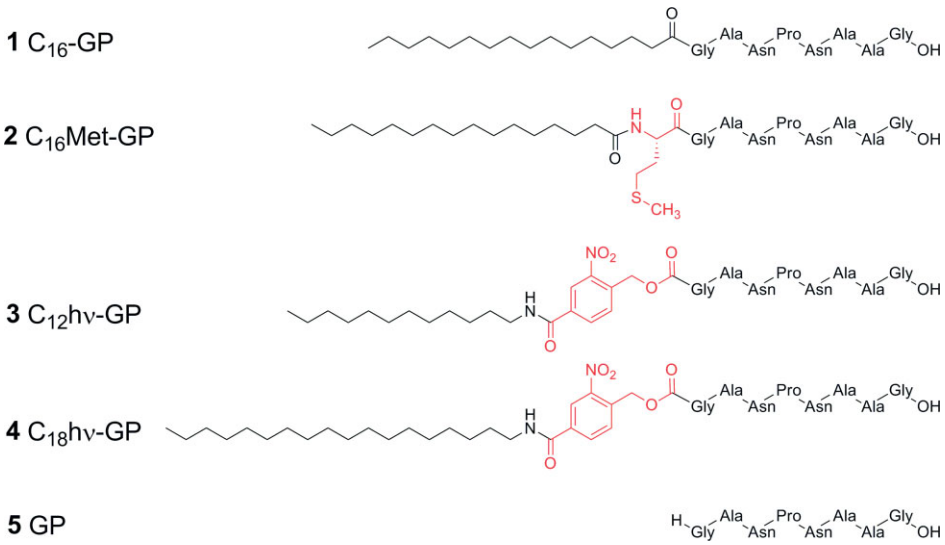

Figure 2 GANPNAAG-based peptides employed in this study.

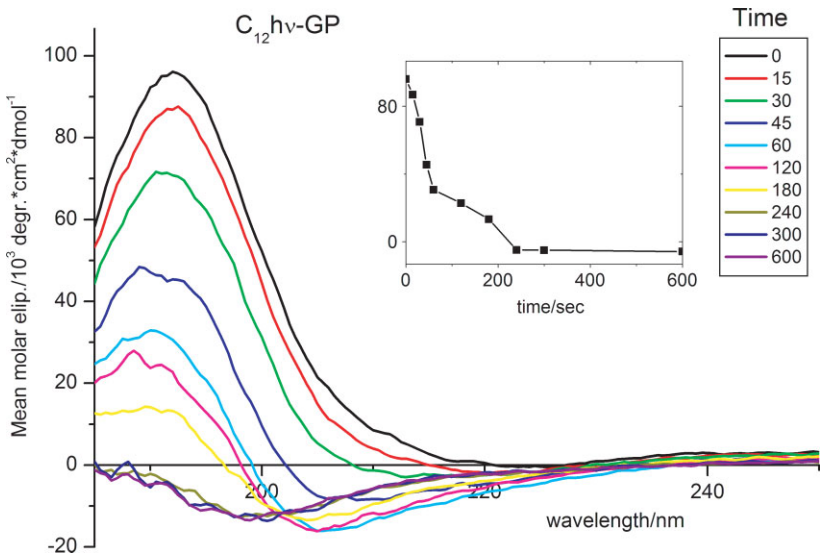

Figure 3 CD spectra of a $C_{12}h\nu$-GP (**3**) fibre solution treated with UV irradiation for different time periods (in seconds, irradiation time increases in the direction of the arrow). Inset: Mean molar ellipticity at 192 nm *versus* irradiation time.

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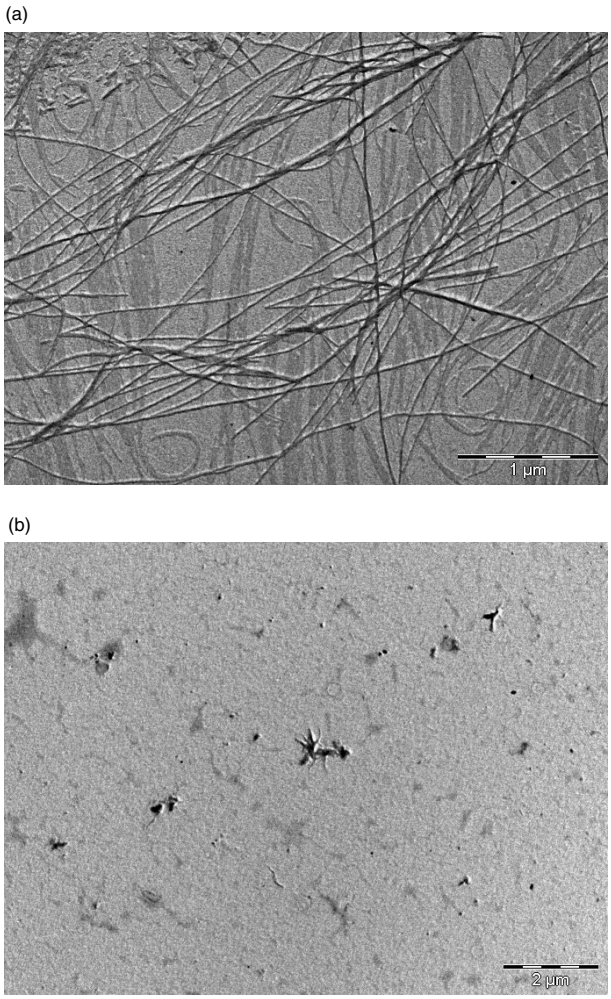

Figure 4 TEM images obtained from a $C_{12}h\nu$-GP (**3**) fibre solution before (a) and after (b) UV irradiation. Bars correspond to 1 and 2 μm respectively.

determined with CD, can be explained by assuming that at that time the peptide amphiphile concentration has been reduced below its critical aggregation concentration of 20 μM, approximately one-tenth of the starting concentration [18]. Additionally, the conversion of compound **3** into 'free' GANPNAAG **5** was verified by mass spectrometry (See supporting information). C18 peptide amphiphile **4** gave results comparable to peptide **3** (See supporting information). Finally, it was established that peptide **1**, lacking the photo-labile linker, was stable under the irradiation conditions described above (See supporting information); no changes were observed in the CD spectra or in the TEM pictures that were taken from the irradiated fibre solutions of **1.** These results indicate that the process of controlled disassembly of peptide amphiphile fibres can be successfully established using either a chemical (molecule **2**) or photochemical method, mediated by the photolabile linker in molecules **3** and **4**.

CONCLUSIONS

We have presented the introduction of both a methionine and a nitrobenzyl derivative as a labile linker between the peptide part and the hydrophobic alkyl chain of a peptide amphiphile. These modifications did not inhibit the formation of structured assemblies. Even more, the introduction of a photo-labile nitrobenzyl moiety added to the hydrophobic character of the peptide, reducing the required length of the *N*-terminal alkyl chain required for fibre formation. The introduction of labile linkers allowed removal of the peptide amphiphile's stabilizing hydrophobic moieties, which opened up the possibility of controlled disassembly of fibre aggregates. First, we demonstrated that amphiphiles with a methionine incorporated between the hydrophilic peptide and the hydrophobic *N*-terminal alkyl chain could be cleaved efficiently. Unfortunately, due to the cleavage conditions controlled fibre disassembly could not be established in a quantitative manner. Conversely, employing a 4-(hydroxymethyl)-3-nitrobenzoic acid based linker, controlled disassembly by irradiation with UV light could be accomplished. Currently, we are exploring the second strategy to further manipulate self-assembled peptide amphiphile architectures, by utilizing the cleavage mechanism to 'cut' aligned assemblies [20] with UV light into pieces of highly defined length.

EXPERIMENTAL

Wang resin was purchased from Bachem, amino acids from Novabiochem and all other chemicals were purchased from Baker, Fluka, Acros or Aldrich and were used as received. Mass spectra were recorded on a Thermo-Finnigan LCQ Advantage Max (ESI-Ion trap) or a JEOL AccuTOF JMS-T100CS (ESI-TOF). ^{1}H-NMR spectra were measured on a Varian Inova 400 MHz NMR spectrometer. CD measurements were performed on a Jasco J-810 spectropolarimeter with Peltier temperature control. TEM was performed on a JEOL 1010 transmission microscope. Peptides were synthesized on a

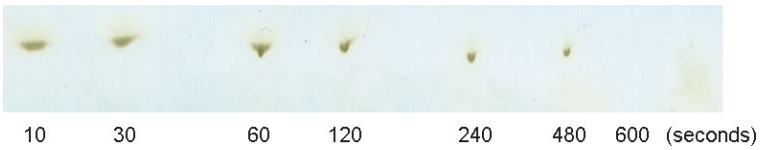

Figure 5 A TLC of samples of a $C_{12}h\nu$-GP (**3**) fibre solution treated with UV irradiation at different time intervals (in seconds).

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Labortec SP4000 or a Labortec SP640 semi-automatic peptide synthesizer.

General Procedure for Peptide Solid-Phase Synthesis

The peptide was prepared on a Wang resin using a standard Fmoc-based protocol [21,22]. Amino acids were coupled with 3.3 equiv *N*, *N*′-diisopropyl-carbodiimide (DIPCDI), 3.6 equiv 1-Hydroxybenzotriazole (HOBt) and 3 equiv Fmoc amino acid in DMF at room temperature. The couplings were carried out for at least 45 min and were monitored with a Kaiser test [26]. After every coupling the resin was washed with DMF and the Fmoc group was removed with 20% piperidine in DMF (3 times during 6 min) followed by a DMF wash. After the final *N*-terminal modification (see below) the resin was washed with DMF, dichloromethane (DCM), isopropyl alcohol, DCM and diethyl ether. The products were cleaved from the resin with TFA/water (95 : 5) for 2 h. The products were precipitated in diethyl ether, dissolved in acetic acid and lyophilized. The crude compounds had a purity of >90%.

$C_{16}H_{33}C(O)$-Gly-Ala-Asn-Pro-Asn-Ala-Ala-Gly-OH 1

Acylation with 3 equiv of palmitic acid dissolved in DCM was carried out with DIPCDI (3.3 eq.) and HOBt (3.6 eq.) as coupling agents. After cleavage and column chromotography (eluent: $CHCl_3/MeOH/H_2O$ 65 : 25 : 4) analytical data were found to be identical to that reported earlier [18].

R_f 0.21 (eluent: $CHCl_3/MeOH/H_2O$ 65 : 25 : 4 v/v). ^{1}H-NMR [DMSOd_6]: δ 0.85 (t, 3H), 1.12 (d, 3H), 1.20 (m, 30H), 1.48 (m, 2H), 1.85 (m, 3H), 2.18 (m, 3H), 2.40 (m, 2H), 2.55 (dd, 1H), 2.65 (dd, 1H), 3.65 (m, 3H), 3.75 (m, 3H), 4.20 (m, 4H), 4.40 (m, 1H), 4.73 (q, 1H), 6.92 (s, 1H), 7.10 (s, 1H), 7.20 (s, 1H), 7.52 (d, 1H), 7.64 (s, 1H), 7.72 (d, 1H), 7.88 (two d, 2H), 7.96 (broad s, 2H), 8.27 (d, 1H), 12.45 (broad s, 1H). ESI-TOF: Calcd. for $[C_{42}H_{72}N_{10}O_{12} + Na]^+$ 931.523, found 931.518.

$C_{16}H_{33}C(O)$-Met-Gly-Ala-Asn-Pro-Asn-Ala-Ala-Gly-OH 2

After the addition of the methionine residue the *N*-terminus of the peptide on the resin was acylated as decribed for compound **1**, which, after cleavage and purification by column chromotography (eluent: $CHCl_3/MeOH/H_2O$ 65 : 25 : 4), afforded amphiphile **2**.

R_f 0.22 (eluent: $CHCl_3/MeOH/H_2O$ 65 : 25 : 4 v/v). ^{1}H-NMR (DMSO-d_6) δ 0.82 (t, 3H), 1.15 (d, 3H), 1.21 (m, 30H), 1.44 (m, 2H), 1.80 (m, 5H), 2.00 (s, 3H), 2.09 (m, 3H), 2.38 (m, 2H), 2.61 (m, 2H), 3.50 (dd, 1H), 3.66 (m, 3H), 4.22 (m, 4H), 4.49 (m, 1H), 4.79 (q, 1H), 6.91 (s, 1H), 7.07 (s, 1H), 7.26 (s, 1H), 7.39 (d, 1H), 7.50 (s, 1H), 7.67 (s, 1H), 7.94 (d, 1H), 8.07 (m, 2H), 8.18 (t, 1H), 8.62 (d, 1H). ESI-TOF: Calcd. for $[C_{47}H_{81}N_{11}O_{13}S + Na]^-$ 1062.56206, found 1062.56337.

$C_{12}H_{25}C(O)$-hν linker-Gly-Ala-Asn-Pro-Asn-Ala-Ala-Gly-OH 3

After removal of the Fmoc grop (*vide infra*) the resin was washed with DCM, followed by the addition of 2 equiv of 4-(dodecylcarbamoyl)-2-nitrobenzyl 4-nitrophenyl carbonate **9a** dissolved in dry DCM and 3 equiv of *N*,*N*-diisopropylethylamine (DIPEA). The mixture was agitated overnight before the resin was subsequently washed with DCM, DMF, DCM, MeOH 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 and purified by column chromatography (eluent: $CHCl_3/MeOH/H_2O$ 65 : 25 : 4 v/v).

R_f 0.18 (eluent: $CHCl_3/MeOH/H_2O$ 65 : 25 : 4 v/v). ^{1}H-NMR (DMSO-d_6) δ 0.82 (t, 3H), 1.12 (d, 3H), 1.20 (m, 24H), 1.50 (m, 2H), 1.85 (m, 3H), 2.03 (m, CH_2 1H), 2.38 (m, 2H), 2.60 (m, 2H), 3.29 (m, 3H), 3.65 (m, 3H), 4.20 (m, 4H), 4.49 (m, 1H), 4.81 (q, 1H), 5.42 (dd, 2H), 6.90 (s, 1H), 7.07 (s, 1H), 7.26 (s, 1H), 7.37 (d, 1H), 7.65 (m, 2H), 7.76 (d, 1H), 7.94 (d, 1H), 8.21 (dd, 1H), 8.54 (dd, 1H), 8.72 (d, 1H), 8.81 (t, 1H). ESI-TOF: Calcd. for $[C_{47}H_{72}N_{12}O_{16} + Na]^+$ 1083.509, found 1083.506.

$C_{18}H_{37}C(O)$-hν linker-Gly-Ala-Asn-Pro-Asn-Ala-Ala-Gly-OH 4

This compound was prepared as described for peptide **3**.

R_f 0.22 (eluent: $CHCl_3/MeOH/H_2O$ 65 : 25 : 4 v/v). ^{1}H-NMR (DMSO-d_6) δ 0.82 (t, 3H), 1.17 (d, 3H), 1.20 (m, 36H), 1.50 (m, 2H), 1.79 (m, 3H), 2.01 (m, CH_2 1H), 2.34 (m, 2H), 2.60 (m, 2H), 3.30 (m, 3H), 3.65 (m, 3H), 4.20 (m, 4H), 4.53 (m, 1H), 4.84 (q, 1H), 5.42 (dd, 2H), 6.89 (s, 1H), 7.05 (s, 1H), 7.25 (s, 1H), 7.29 (d, 1H), 7.60 (s, 1H), 7.63 (t, 1H), 7.76 (d, 1H), 7.95 (d, 1H), 8.18 (d, 1H), 8.21 (dd, 1H), 8.54 (d, 1H), 8.81 (t, 1H), 8.90 (d, 1H). ESI-TOF: Calcd. for $[C_{53}H_{84}N_{12}O_{16} + Na]^+$ 1167.6026, found 1167.6028.

H-Gly-Ala-Asn-Pro-Asn-Ala-Ala-Gly-OH 5

The unmodified peptide **5** was obtained by direct cleavage (TFA/H_2O, 95 : 5 v/v, 2 h) from the resin after removal of the *N*-terminal Fmoc group. The crude material was used as a reference in mass spectrometry experiments. ESI-ion trap: Calcd. for $[C_{26}H_{42}N_{10}O_{11} - H]^-$ 669.3, found 669.4.

4-(hydroxymethyl)-3-nitrobenzoic acid 7

Compound **7** was prepared analogous to a literature procedure with a modified workup and purification [23]: 0.26 g (1.0 mmol) 3-nitro-4-bromomethylbenzoic acid **6** and 0.53 g (5.0 mmol) Na_2CO_3 were dissolved in 4 ml H_2O and 4 ml acetone. The mixture was placed under reflux for 5 h and then the acetone was evaporated *in vacuo*. The mixture was washed with diethyl ether (5 ml) and 2 M HCl was added until pH was ~2, at which point **7** became insoluble. The product was extracted with ethyl acetate (3 × 6 ml) while the pH was kept at 2. The organic layers were combined and washed with water (3 ml), dried over $MgSO_4$, filtered and concentrated to an orange solid (88% yield).

R_f 0.26 (eluent: 10% MeOH, 1% Acetic acid in DCM v/v). Analytical data were in accordance with that reported [27]. ^{1}H-NMR (DMSO-d_6): δ 4.86 (s, 2H, $C\underline{H}_2$–OH), 5.67(s, 1H, $CH_2O\underline{H}$), 7.95 (d, 1H, Ar–H), 8.25 (dd, 1H, Ar–H), 8.45 (d, 1H, Ar–H), 13.55 (s, 1H, $COO\underline{H}$).

4-(hydroxymethyl)-3-nitro-*N*-dodecylbenzamide 8a

To a solution of 1.50 g (7.61 mmol) acid **7** and 1.41 g (7.61 mmol) dodecyl amine in 15 ml DCM were added 3.37 g

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(7.61 mmol) BOP and 5.05 ml (15.2 mmol) DIPEA and this mixture was stirred overnight while keeping the pH at ~8. The reaction mixture was evaporated *in vacuo* and the residue was dissolved in ethyl acetate. The mixture was washed with 1 M HCl (3×), H_2O, 1 M $NaHCO_3$, H_2O and brine. The mixture was dried on $MgSO_4$ filtered and the solvent was evaporated *in vacuo*. The product was further purified by column chromatography (eluent: 7.5% MeOH and 1% acetic acid in DCM by volume) to afford 3.15 g of **8a** (78%).

R_f 0.37 (eluent: 7.5% MeOH and 1% acetic acid in DCM v/v). ^{1}H-NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.26 (m, 18H), 1.64 (qu, 2H), 3.48 (q, 2H), 5.05 (s, 2H), 6.26 (t, 1H), 7.88 (d, 1H), 8.06 (d, 1H), 8.44 (s, 1H).

4-(hydroxymethyl)-3-nitro-*N*-octadecylbenzamide 8b

This compound was prepared according to the procedure described for **8a** in a 90% yield. R_f 0.29 (eluent: 5% MeOH, 1% acetic acid in DCM v/v). ^{1}H-NMR (DMSO-d_6): δ 0.81 (t, 3H), 1.21 (m, 30H), 1.50 (qu, 2H), 3.22 (q, 2H), 4.81 (s, 2H), 5.60 (s, 1H), 7.89 (d, 1H), 8.20 (d, 1H), 8.48 (s, 1H), 8.69 (t, 1H).

4-(dodecylcarbamoyl)-2-nitrobenzyl 4-nitrophenyl carbonate 9a

A solution of 364 mg (1.00 mmol) **8a**, 250 µl (3.00 mmol) pyridine and 400 mg (2.10 mmol) 4-nitrophenyl chloroformate in 10 ml distilled tetrahydrofuran was stirred at 0 °C for 2 h under argon atmosphere. The solvent was evaporated *in vacuo* and the product was re-dissolved in DCM. This mixture was washed with brine and 1 M HCl, dried over Na_2SO_4 and evaporated *in vacuo*. Column chromatography (eluent: 2.5% MeOH in DCM v/v), afforded 0.42 g of white product **9a** (79%).

R_f 0.30 (eluent: 2,5% MeOH in DCM v/v). ^{1}H-NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.33 (m, 18H), 1.65 (qu, 2H), 3.49 (q, 2H), 5.76 (s, 2H), 6.53 (t, 1H), 7.42 (dt, 2H), 7.81 (d, 1H), 8.16 (dd, 1H), 8.29 (dt, 2H), δ 8.53 (d, 1H). ^{13}C-NMR ($CDCl_3$) δ 14.3, 22.9, 27.2, 29.5, 29.7, 29.8, 32.1, 40.8, 67.2, 121.9, 123.8, 125.6, 129.4, 132.8, 133.8, 136.4, 145.8, 147.2, 152.2, 155.4, 164.7. ESI-TOF: Calcd. for $[C_{27}H_{35}N_3O_8 + Na]^+$ 552.23210, found 552.23218.

4-(octadecylcarbamoyl)-2-nitrobenzyl 4-nitrophenyl carbonate 9b

This compound was prepared according to the procedure described for **9a** starting with 370 mg of alcohol **8b** to yield building block **9b** in a 79% yield.

R_f 0.54 (eluent: 2,5% MeOH in DCM v/v). ^{1}H-NMR (DMSO-d_6): δ 0.81 (t, 3H), 1.23 (m, 30H), 1.50 (qu, 2H), 3.22 (q, 2H), 5.70 (s, 2H), 7.58 (dt, 2H), 7.87 (d, 1H), 8.24 (dd, 1H), 8.32 (dt, 2H), 8.59 (d, 1H), 8.80 (t, 1H). ^{13}C-NMR ($CDCl_3$) δ 14.3, 22.9, 27.2, 29.5, 29.6, 29.76, 29.81 29.9, 32.1, 40.8, 67.2, 121.9, 123.8, 125.6, 129.4, 132.8, 133.8, 136.4, 145.8, 147.2, 152.2, 155.4, 164.7. ESI-TOF: Calcd. for $[C_{33}H_{47}N_3O_8 + Na]^+$ 636.326, found 636.322.

Fibril Formation

The peptide amphiphiles were suspended in Milli-Q water at a concentration of 0.20 mg/ml as reported previously [18]. The samples were heated at 50 °C for 30 min with occasional vortexing and subsequently sonicated for 15 min at that temperature. The clear peptide solutions were allowed to cool to ambient temperature before use.

Transmission Electron Microscopy

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

CNBr Teatment

To the fibril solutions, 50 mole equivalents of cyanogen bromide and 20–50 volume percent of formic acid were added. The mixtures were left to stand at ambient temperature from which, at regular intervals, samples were taken to be analysed by mass spectrocopy (ESI-ion trap).

UV Irradiation

A solution of peptide fibres in a 1 mm quartz cell was placed 10 cm below the output of a quartz light guide connected to a Bluepoint 2 UV source (Sadechaf UV). The exposure time was determined by the timer-controlled shutter of the source. For the TLC experiment at appropriate intervals a sample was taken and transferred to a TLC plate (eluent: $CHCl_3$/MeOH/H_2O 65 : 25 : 4 by volume).

Supplementary Material

Supplementary electronic material for this paper is available in Wiley InterScience at: http://www.interscience.wiley.com/jpages/1075-2617/suppmat/

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