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# Synthesis of Nanometer-sized Rod–Coil Block Copolymers

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*Abstract:* We present the solid-supported synthesis of a linear rigid rod-like tetradeca(*p*-benzamide) nanorod as well as a pentadeca(*p*-benzamide) nanorod carrying an amide *N*-hexyl side chain at the center of the rod (on the 8th amino acid of the molecule). These nanorods were conjugated with solubilizing poly(ethylene glycol) chains and their solution aggregation was investigated. Both rod–coil block copolymers form soluble aggregates even in polar aprotic solvents such as DMF and DMSO. We show that sequence-controlled nanometer-sized shape persistent aramide scaffolds can readily be prepared using solid-supported synthesis.

**Keywords:** Aramide · Block copolymers · Oligomers · Self-assembly · Solid-supported synthesis

Shape-persistent materials, in particular molecular rods with dimensions on the nanoscale are envisaged to be important scaffold materials for bottom-up approaches to nanomaterials.[1,2] Moreover, rod-like materials that are truly monodisperse and can be sequentially built with monomer sequence control are very attractive in this context.[3–17] Such materials would not only allow the shape-persistent construction of larger nanostructures but also allow the placement of functional groups at pre-determined positions within the rigid scaffold. Several synthetic strategies to rod-like molecules based on aramides have been described by us in the past: manual[18,19] and automated[20] solution syntheses as well as manual[3] and automated[21] solid-supported syntheses have been reported. For rod–coil copolymers based on well-defined oligo(*p*-benzamide)s (OPBA) and polydisperse poly(ethylene glycol) (PEG) we found that an oligomer length of seven repeat units was sufficient to induce strong aggregation in non-polar organic solvents such as chloroform or toluene.[4] A molecular mechanics model of an OPBA heptamer shows that the energetically most stable all-*trans* amide form has a C-to-N-terminal length of *ca.* 4.4 nm. In order to access larger nanostructures longer scaffolds than an OPBA heptamer would need to be synthetically accessible. Here we describe the solid-supported synthesis of an OPBA tetradecamer and a pentadecamer as well as their conjugation with poly(ethylene glycol) and investigation of block copolymer aggregation *via* transmission electron microscopy (TEM).

The solid-supported OPBA tetradecamer **1a** was synthesized on Wang resin (0.6 mmol $g^{-1}$) using the acid chloride of *N*-Fmoc (9-fluorenylmethyl chloroformate) and *N*-PMB (*p*-methoxybenzyl) protected 4-aminobenzoic acid according to a reported procedure.[4] Commercially available Wang resin was pre-functionalized with *N*-Fmoc-4-aminobenzoic acid which resulted in oligomers that did not carry the PMB protective group on the first phenyl ring (closest to the C-terminus, see Figs 1 and 2, top). The solid-supported synthesis of the tetradecamer **1a** (Fig. 1, top) was achieved in 68% overall yield over 14 amide coupling steps and 14 Fmoc deprotection steps (see Fmoc-cleavage elugram, Fig. 1, bottom). This corresponds to a yield of 97% per amide coupling. As shown previously[3,22–25] *N*-alkylated benzanilides adopt a *cis*-conformation (*E*) with respect to the phenyl rings leading to a coil-like oligomer chain. After acidic deprotection of the *N*-PMB groups the tetradecamer **1a** shown in Fig. 1, top, (shown as a linear *trans*-conformer for clarity) will adopt a straight linear rigid rod-like conformation (*trans*, *Z*). We have previously shown that copolymers of these rod-like oligomers form β-sheet-like aggregates in solution.[4,26,27]

The terminal amine of solid supported oligomer **1a** was further reacted with 4-pentynoic acid chloride to place a terminal alkyne on the *N*-terminus of **1a**. Poly(ethylene glycol)monomethyl ether mono azide (Mn *ca.* 5000 g $mol^{-1}$)[4] was subsequently reacted with the terminal alkyne of **1a** in analogy to a reported procedure.[4] Cleavage from the resin yielded the PEG-OPBA conjugate in its *N*-PMB protected form. This rod–coil precursor polymer (**1b**, see Scheme 1) is soluble in common organic solvents such as chloroform,

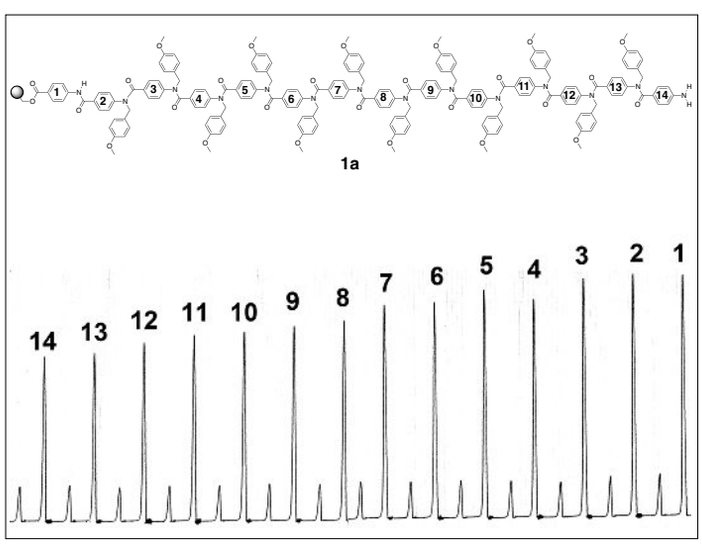


Fig. 1. Top: Chemical structure of solid supported tetradecamer **1a**. All but the first amide carry an acid labile *p*-methoxybenzyl protective group. Bottom: Fmoc-cleavage elugram quantifying the successful synthesis over altogether 28 synthetic steps (including amide coupling and Fmoc deprotection) in 68% overall yield. The numbering refers to the number of the amino acid coupled (see top).

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THF or DMSO and shows no aggregation when analyzed by chloroform gel permeation chromatography (GPC, see Fig. 3).

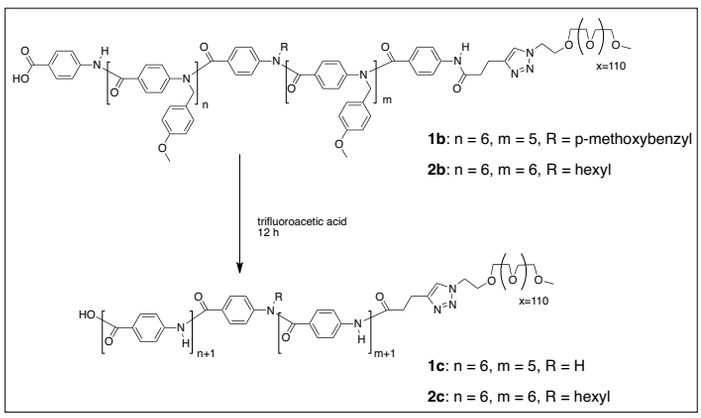
Solid-supported *p*-aminobenzoic acid oligomer **2a** (Fig. 2, top) was prepared in an analogous manner to **1a**.[3,4] However, the eighth aromatic amino acid (counted from the *C*-terminus) carried an *N*-hexyl side chain, which is stable under the acidic cleavage conditions for the PMB protective group (TFA:DCM = 1:1 vol/vol). As tertiary aromatic amides adopt a *cis*-conformation (*E*) with respect to the phenyl rings (*vide supra*), the eighth amide (counted from the *C*-terminus) should adopt a *cis* conformation (*E*) while all other amides should adopt *trans*-conformations (*Z*). We speculated that this might lead to a V-shaped molecular rod, which should exhibit different aggregation phenomena compared to its linear rigid rod counterpart **1a**.

Fig. 2, top, shows the molecular structure and the Fmoc-cleavage elugram (Fig. 2, bottom) for the *N*-protected 15mer heterosequence **2a**. In analogy to compound **1a**, **2a** was reacted with pentynoic acid chloride and subsequently PEG-monomethyl ether mono azide to yield the copolymer **2b**. The *N*-protected precursor polymer **2a** shows a similar organo-solubility as **1a** and no aggregation can be observed by GPC analysis in chloroform (Fig. 3).

Both block copolymers **1b** and **2b** were purified by preparative GPC after acidic cleavage from the solid support. We attribute the noticeable shift in the analytical GPC elugrams (Fig. 3) between **1b** and **2b** to a fractionation during preparative GPC purification. $^1$H-NMR analysis of block copolymers **1b** and **2b** confirmed the successful attachment of the oligomers to PEG. As the solid-supported synthesis was carried out with *p*-nitrobenzoyl chloride capping steps after the reaction of every monomer unit, only the target structures shown in Figs 1 and 2 carried the reactive terminal alkynes for PEG-azide conjugation. In other words, all lower oligomers were non-functionally terminated and separated by preparative GPC. Unfortunately, MALDI-ToF mass spectrometry analysis of the copolymers **1b** and **2b** was not successful.

Copolymers **1b** and **2b** were dissolved in TFA (100%) for 12 h at room temperature to cleave the PMB protective groups, as reported previously.[4] The immediate formation of a dark red color upon addition of TFA to **1b** and **2b** indicated the successful PMB deprotection reaction. After 12 h the polymers (**1c**, **2c**) were precipitated into dichloromethane and re-dissolved in DMSO:toluene (vol:vol = 1:1) under sonication. After *ca.* 15 min. a colorless solid of **1c** and **2c** started to precipitate from the solution. Optical microscopic investigation of the solids under crossed polarizers indicated the presence of anisotropic order. To further investigate the aggregation of **1c** and **2c** we dropcast freshly prepared DMSO/toluene solutions (**1c**, *vide supra*) or chloroform solution (**2c**) for transmission electron microscopic (TEM) investigation.

As can be seen in representative TEM images (Fig. 4, top and bottom), long rigid rods are observed for both rod–coil block copolymers **1c** and **2c**. The molecular formation mechanism for such OPBA-PEG rod–coil block copolymers was previously reported[3,26,28] and can be explained by a β-sheet-like aggregation of the OPBA blocks surrounded by a PEG corona. Within the rigid rod-like aggregates individual fibrils with widths of *ca.* 7 nm for both **1c** and **2c** can be seen. An observed width of 7 nm corresponds well to the width expected from a molecular mechanics model of the linear OPBA 14-mer **1c** (*ca.* 9 nm). However, this data does not support the assumption of a V-shaped geometry in case of block copolymer **2c** which should exhibit a distinctly shorter width in the TEM projection. We assume that both **1c** and **2c** adopt the linear all-*trans* form upon aggregation. We hypothesize that the energetic penalty for an unfavored *cis-trans* (*E* to *Z*) conformational change is overcompensated by additional intermolecular hydrogen bond formation between neighboring linear OPBA rods.




Scheme 1. *N*-Protected oligo(*p*-benzamide)-*block*-PEG copolymers **1b** and **2b** are *N*-deprotected under acidic conditions yielding nanorod–coil block copolymers **1c** and **2c**.

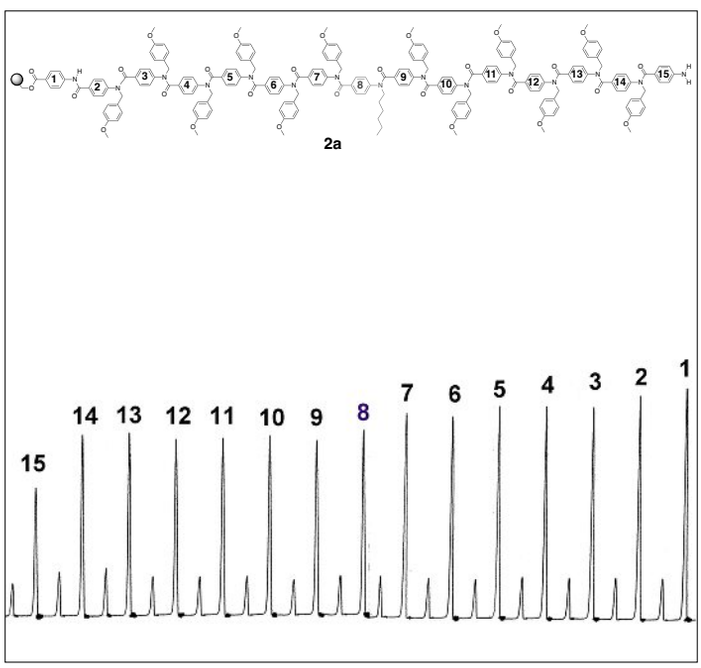



Fig. 2. Top: Chemical structure of solid supported pentadecamer **2a**. All but the first and the eighth amide carry an acid labile *p*-methoxybenzyl protective group. The eighth amide carries a hexyl group which cannot be removed under acidic conditions. Bottom: Fmoc-cleavage elugram quantifying the successful synthesis over altogether 30 synthetic steps (including amide coupling and Fmoc deprotection) in 54% overall yield. The numbering refers to the number of the amino acid coupled (see top).

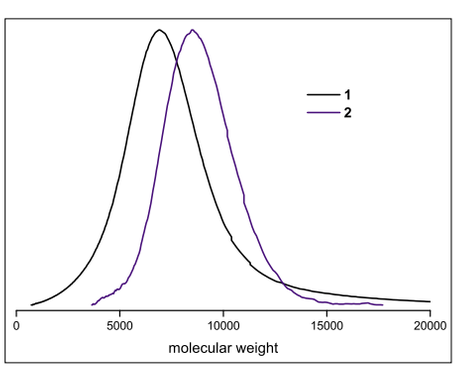



Fig. 3. GPC (chloroform) elugrams for *N*-protected OPBA-PEG block copolymers **1b** (black) and **2b** (blue).

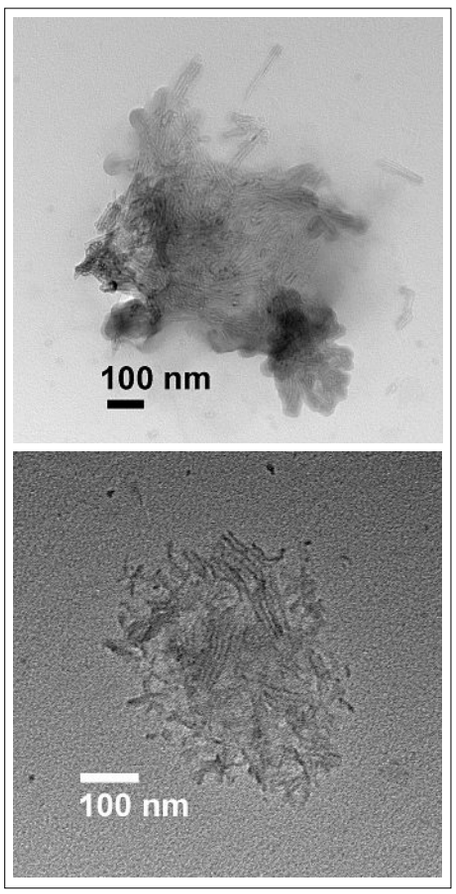



Fig. 4. Top: TEM image of OPBA-PEG block copolymer **1c** deposited from DMSO solution (0.5 mg $mL^{-1}$) onto a carbon-coated nickel TEM grid. Bottom: TEM image of OPBA-PEG block copolymer **2c** deposited from chloroform solution (0.5 mg $mL^{-1}$) onto a carbon-coated copper TEM grid.

## Conclusions

The work described here shows that the fast automated solid-supported synthesis of long chain hydrophobic oligoaramides is possible up to the pentadecamer in good yields. This approach furthermore allows the sequence-specific introduction of derivatives of *p*-aminobenzoic acid, here, *N*-hexyl-*N*-4'-methoxybenzyl-4-aminobenzoic acid. Introduction of an *N*-hexyl side chain at the central position (amino acid number eight counted from the C-terminus) of the pentadecamer was believed by us to alter the shape of the *N*-deprotected pentadecamer from all-linear to 'V-shaped' due to the *cis* (*E*) conformation of the *N*-hexylated amide. The pentadecamer and tetradecamer-PEG block copolymers precipitate from toluene/DMSO (1:1 vol:vol) solution upon *N*-deprotection. TEM investigation of the precipitate shows the formation of rod-like micelles. No evidence was found that the predicted non-linear geometry of the aramide oligomers existed in the solid state investigated by TEM. We believe that strong intermolecular hydrogen bond formation which is maximized in the linear oligomer overcompensates the energetic penalty for the *cis* (*E*) *N*-hexylated amide conformation.

## Experimental

Solvents of technical grade were purchased from Acros Organics. Solvents of p.a. quality were purchased from Fisher Scientific. All other reagents were purchased from either Acros Organics or Sigma-Aldrich and used without further purification. Deuterated solvents were purchased from Deutero GmbH. Dichloromethane was stored over calcium hydride and distilled freshly before use. NMP was provided by BASF SE Ludwigshafen, dried over $P_2O_5$ and freshly distilled prior to use.

$^1$H-NMR spectra were recorded at 300 MHz on a Bruker AC 300 spectrometer and the spectra referenced with respect to residual protonated solvent. Gel-permeation chromatography elugrams (GPC) were recorded in chloroform with an apparatus consisting of a Waters 717 plus autosampler, a TSP Spectra Series P 100 pump and a set of three GPC separation columns (PSS SDV columns (104/500/50 Å)). Signals were recorded with a TSP Spectra System UV 2000 (UV at 254 nm) and a Wyatt Optilab DSP (refractive index). The apparatus was calibrated with several polystyrene standards purchased from Polymer Standards Service (PSS).

Preparative GPC was carried out in DMF using a Knauer HPLC Pump 64, a Knauer Variable Wavelength Monitor (UV detector at 254 nm) and an MZ-GPC 250×30 mm SEC column (MZ-Gel SDplus, 10E3 Å, 10 µm) from MZ-Analysentechnik, Mainz, Germany.

TEM measurements were recorded on a Philips EM 420 transmission electron microscope with a $LaB_6$ cathode and an accelerating voltage of 120 kV. TEM-grids (carbon film on copper or nickel, 300 mesh) were purchased from Electron Microscopy Sciences, Hatfield, PA, USA.

The automated synthesis of oligo(*p*-benzamide)s was carried out on an Applied Biosystems model 431A peptide synthesizer with a modified synthesis protocol as described previously.[4]

Monomer and automated syntheses of shorter oligomers were reported previously.[4]

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