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Superstructures of Double Functionalized Host–Guest Acrylmonomers Containing Chiral Phenylalanine-*click*-cyclodextrin and Polymers

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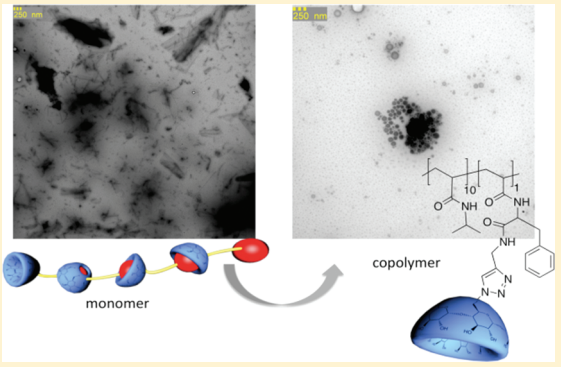
Supporting Information

ABSTRACT: We report the preparation of acrylic monomers D- or L-mono-(6-phenylalanine-acrylamido-6-deoxy)-β-cyclodextrin $\mathbf{3_D}/\mathbf{3_L}$ and their corresponding copolymers $\mathbf{4_D}/\mathbf{4_L}$ bearing NIPAAm and D- or L-phenylalanine as guest and β-cyclodextrin as host moieties. To implement the cyclodextrin resin (CD) into the monomer, microwave accelerated cycloaddition (click-reaction) was performed. For the new design of polymers having both host and guest species in the polymer side chain, inter- and intramolecular interactions could be observed. The resulting supramolecular structures were characterized by NMR, DLS, TEM, and LCST measurements.



INTRODUCTION

Supramolecular structures are ubiquitous in nature particularly in biological systems, the most common are DNA, microtubuli, or microfilaments which are built out of proteins.[1] Scientist have been inspired by nature and have investigated various systems mimicking biomolecules by synthetic supramolecular structures with interesting properties and functions.[2−6] Superstructures formed by hydrogen bonds were reported for the first time by Lehn et al.[7] Many contributions about the formation of supramolecular polymers formed by hydrogen bonding can be found in literature.[8−12] However there have been few works dealing with the self-assembly into supramolecular polymeric structures via host–guest interaction.[13,14] For example Harada et al. reported about the formation of supramolecular helical polymers, recently.[15] The self-assembly behavior of phenyl modified β-cyclodextrins has been investigated by Liu et al.[16]

Also self-assembly of gels through shape selective molecular recognition with CD for linear and cyclic guest molecules was investigated on a macroscopic scale very recently.[17] Furthermore there have been few works dealing with self-assembly of chiral compounds. For example a monomer end-capped with a cholesteryl group was threaded with CD to form a helical polymer.[18] Star polymers and polymer brushes were synthesized from amphiphilic chiral monomers and their self-assembly investigated.[19,20]

However there have been few works dealing with supramolecular structures based on host–guest interaction from chiral monomers.[21] Monomers based on macrocyclic hosts comprising both host and guest moiety offer a wide range of opportunities for new supramolecular materials and applications.[22] Next to calixarenes and cucurbiturils particularly cyclodextrins (CDs) are important macrocyclic hosts, because they are water-soluble, natural products suitable for medical applications.[23,24] Although cyclodextrins are frequently used for chiral separation of racemates in column chromatography, the phenomena of chiral recognition of synthetic polymers has not yet been extensively investigated.[25−29] Thus, we want to report about the preparation and properties of a new typ of polymerizable acrylic monomer containing chiral β-CD as host and phenylalanine as guest moiety in the same molecule. We chose CD as a host and phenylalanine as a guest compound because the aromatic moiety builds stable complexes with CD and phenylalanine is an important, chiral biomolecule.

MATERIALS AND EXPERIMENTAL SECTION

All reagents used were commercially available (Sigma-Aldrich, Acros Organics) and were used without further purification. β-cyclodextrin was obtained from Wacker Chemie GmbH, Burghausen, Germany, and used after drying overnight at a vacuum oil pump over P_4O_{10}. D- and L-Phenylalanine (98.5%) were purchased from Alfa Aesar GmbH & CoKG, Germany. Acryloylchloride (97%) and *N*-isopropylacrylamide (NIPAAm, 97%) were obtained from Sigma-Aldrich, Germany, and used as received. Azoisobutyronitrile (AIBN) (96%) and *N*,*N*-dimethylformamide (DMF) were purchased from Fluka, Germany. Dimethyl sulfoxide-d_6 (99.9 atom % D) and deuterium oxide, D_2O, were obtained from Deutero GmbH, Germany.

^{1}H NMR was performed using a Bruker Advance DRX 200 spectrometer operating at 200.13 or 500 MHz for protons using

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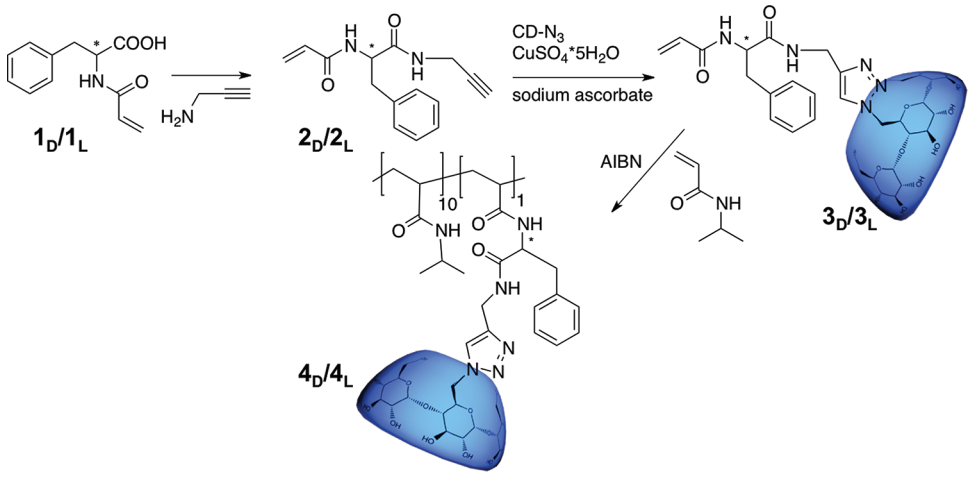
Scheme 1. Synthesis of Monomers 3_D, 3_L and Copolymers 4_D, 4_L



DMSO-d_6 or Deuteriumoxide 99.9% as solvents. The chemical shifts (δ) are given in ppm using the solvent peak as an internal standard. FT-IR spectra were recorded on a Nicolet 6700 FT-IR spectrometer equipped with an ATR unit. MALDI−TOF mass spectrometry (MALDI−TOF MS) was performed on a Bruker Ultraflex TOF time-of-flight mass spectrometer using a 337 nm nitogen laser. The samples were dissolved in acetonitrile/water and mixed with dithranol matrix. Molecular weights and molecular weight distributions were measured by size exclusion chromatography (SEC) using a Viscotek GPCmax VE2001 system that contained a column set with one Viscotek TSK guard column HHR-H 6.0 mm (ID) × 4 cm (L) and two Viscotek TSK GMHHR-M 7.8 mm (ID) × 30 cm (L) columns at 60 °C. *N,N*-Dimethylformamide (DMF, 0.1 M LiCl) was used as eluent at a flow rate of 1 mL × min^{-1}. A Viscotek VE 3500 RI detector and a Viscotek Viscometer model 250 were used. The system was calibrated with polystyrene standards with a molecular range from 580 D to 1 186 kD.

Turbidity experiments were performed on a Tepper cloud point photometer TP1. Relative transmission of a laser beam with a wavelength of 670 nm was recorded for each experiment. The measurements were performed at a temperature range between 5 and 70 °C and a heating rate of 1 °C min^{-1} using Hellma Suprasil precision cells 110 Q-S. Critical solution temperatures derived from these experiments were determined at 50% relative transmission. Dynamic Light Scattering (DLS) experiments were carried out with a Malvern Zetasizer Nano; ZS ZEN 3600 at a temperature of 20 °C. The particle size distribution is derived from a deconvolution of the measured intensity autocorrelation function of the sample by a General Purpose Methode (non-negative least squares) algorithm included in the DTS software. Each experiment was performed at least five times. Polarimetric measurements were performed at T = 20 °C in dimethyl sulfoxide or water (λ = 590 nm). Microwave-assisted synthesis was performed using a CEM Discover synthesis unit (monomode system). The temperature was measured by infrared detection with continuous feedback temperature control and maintained at a constant value by power modulation. Reactions were performed in closed vessels under controlled pressure. Transmission electron microscopy (TEM) images were recorded on a Zeiss EM902 A microscope at 80 kV.

- The *N*-acrylated aminoacid $\mathbf{1}_D/\mathbf{1}_L$ was prepared according to the method described before.[30]
- Mono(6-azido-6-desoxy)-β-CD was synthesized according to the method described before.[31]

D- or L-*N*-(1-Oxo-3-phenyl-1-(prop-2-yn-1-ylamino)propan-2-yl)acrylamide, $2_D/2_L$. The respective *N*-acrylated amino acids $\mathbf{1}_D/\mathbf{1}_L$ (10 mmol, 2.19 g) were solubilized in THF (100 mL) at room temperature. *N*-Methylmorpholine (1.01 g, 10 mmol) was then added to the amino acid solution, followed by further addition of isobutyl chloroformiate (10 mmol, 1.37 g). During the addition of the isobutyl chloroformiate, a white precipitate of *N*-methylmorpholine hydrochloride was formed. Propargylamine (10 mmol, 0.55 g) was then added to the reaction mixture. A slight formation of CO_2 could be observed. The mixture was further stirred for 1 h at room temperature. The hydrochloride salt was then filtered, and the clear solution evaporated to dryness. The product was further dried at a high vacuum pump to yield 1.3 g (47%) of white solid. Polarimetric measurement (DMSO) $\mathbf{2}_L$ α_D^{20} = 30°; $\mathbf{2}_D$ α_D^{20} = −28°. ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 8.60 (s, 1H), 8.43 (d, J = 8.7, 1H), 7.36 − 7.07 (m, 5H), 6.36 − 6.18 (m, 1H), 6.02 (dd, J = 2.4, 17.1, 1H), 5.57 (dd, J = 2.4, 10.0, 1H), 3.94−3.78 (m, 2H), 3.16 (t, J = 2.5, 1H), 3.00 (dd, J = 4.8, 13.7, 1H), 2.79 (dd, J = 9.7, 13.6, 2H). FT- IR (diamond) ν(cm^{-1}): 3273.3 (ν NH), 3069.6, 2961.5, 1645.7 (amide I), 1622.4 (C═C), 1547.2 (amide II), 1496.1 (Ar), 1436.2, 1382.4, 1325.0, 1241.4, 1224.6, 1192.7, 990.8. Anal. Calcd: C, 69.7; H, 5.43; N, 11.61. Found for $\mathbf{2}_L$: C, 69.7; H, 6.6; N, 10.6. Found for $\mathbf{2}_D$: C, 70.02; H, 6.4; N, 10.59.

D- or L-Mono(6-phenylalanine-acrylamido-6-deoxy)-β-cyclodextrin, $3_D/3_L$. We approached microwave-assisted cycloaddition by giving $\mathbf{2}_D/\mathbf{2}_L$ (0.2 g, 0.82 mmol) to a solution of mono-(6-azido-6-desoxy)-β-CD (1.42 g, 1.23 mmol) in 2 mL DMF in a pressure-resistant test tube. To the clear solution were added sodium ascorbate (25 mg, 0.1 mmol) and copper(II) sulfate pentahydrate (40 mg, 0.2 mmol). The tube was sealed and placed in the CEM monomode microwave and irradiated at 85 °C and 140 W for 60 min. After precipitating with acetone (50 mL) the products were collected by filtration to yield 1.5 g (80%) product. FT-IR (diamond) ν (cm^{-1}): 3356.4 (ν OH), 2927.2, 2102.7, 1652.1, 1497.1, 1437.5, 1385.8, 1254.9, 1082.1, 1029.1, 1003.0, 936.0, 863.9. MALDI TOF MS m/z = 1416 $[M + Na]^+$.

Copolymer $4_D/4_L$. A 0.5 g sample of $\mathbf{3}_D/\mathbf{3}_L$ (0.18 mmol) was solved with 0.24 g (1.8 mmol) *N*-Isopropylacrylamide in 10 mL DMF. The solution was flushed with argon for 15 min, and then 7.4 mg (1 wt %) AIBN in DMF was added to the solution. The mixture was further stirred at 80 °C for 24 h. The product was obtained by precipitation in diethylether and afterward purificated by dialysis in a MWCO 3500 and lyophilized to yield 0.6 g. ^{1}H NMR (200 MHz, D_2O): δ = 7.93−7.83 (m), 7.44−7.06 (m), 5.31−4.82 (m), 3.86 (s), 3.14−2.69 (m), 1.10 (s). FT-IR (diamond) ν (cm^{-1}): 3295.3 (OH), 2971.1, 2931.4, 1640.5 (amide I), 1536.8 (amide II), 1459.2 (Ar), 1387.1, 1367.1, 1240.5, 1153.7, 1130.7, 1080.7, 1032.0, 1004.3. $\mathbf{4}_L$ α_D^{20} = 14° $\mathbf{4}_D$ α_D^{20} = 18°, SEC measurement: $\mathbf{4}_D$, M_n = 45 000, P_D 1.3; $\mathbf{4}_L$, M_n = 42 000, P_D 1.3.

RESULTS AND DISCUSSION

Monomers *N*-acryloyl-D-phenylalanine ($\mathbf{1}_D$) and *N*-acryloyl-L-phenylalanine ($\mathbf{1}_L$) were prepared according to the method described before. Further modification of monomers **1** was

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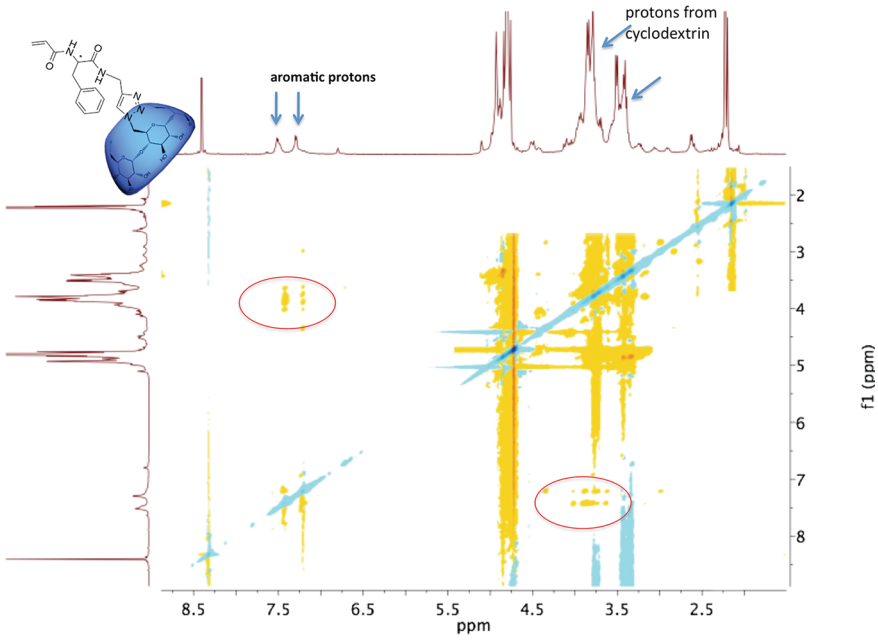

Figure 1. 2D ROESY NMR experiment of $\mathbf{3}_D$ showing the correlation between protons of the phenyl ring and protons of the inner cavity of CD.

carried out by condensation of the carboxylic group with propargylamine to obtain the corresponding alkin moiety *N*-(1-oxo-3-phenyl-1-(prop-2-yn-1-ylamino)propan-2-yl)acrylamide, $\mathbf{2}_D/\mathbf{2}_L$. The microwave accelerated click-reaction employing Cu(I) catalysis with sodium-ascorbate lead to the desired monomers mono-(6-phenylalanine-acrylamido-6-deoxy)-*β*-cyclodextrin, $\mathbf{3}_D$ and $\mathbf{3}_L$. To confirm that the monomers did not racemize significantly during the synthesis, polarimetric measurements were performed in dimethyl sulfoxide as solvent, $\mathbf{3}_D$ exhibited $\alpha_D^{20} = -28°$ and $\mathbf{3}_L$ $\alpha_D^{20} = +30°$, respectively. Copolymers $\mathbf{4}_D$ and $\mathbf{4}_L$ comprising *N*-isopropylacrylamide (NIPAAm), D-mono-(6-phenylalanine-acrylamido-6-deoxy)-*β*-cyclodextrin ($\mathbf{3}_D$) and L-mono(6-phenylalanine-acrylamido-6-deoxy)-*β*-cyclodextrin ($\mathbf{3}_L$), respectively, were obtained by free radical polymerization with AIBN as initiator (Scheme 1). The structures of all synthesized polymeric compounds have been characterized by spectroscopic methods, elemental analysis and polarimetric measurements. The molecular weights and molar weight distribution of polymers $\mathbf{4}_D$ and $\mathbf{4}_L$ were determined by size exclusion chromatography (SEC) showing similar results.

To confirm the formation of the proposed intermolecular interaction between the hydrophilic and hydrophobic moieties in case of the monomers $\mathbf{3}_D$ and $\mathbf{3}_L$ 2D ROESY-NMR-spectroscopy was carried out to show the expected correlation between the inner cavity protons of *β*-CD and the protons of the phenyl ring. Figure 1, as an example for a 2D ROESY NMR spectrum of $\mathbf{3}_D$, illustrates clearly interactions of protons from the phenyl group with protons of the CD cavity (for ROESY of $\mathbf{3}_L$, see Supporting Informations).

In addition the ^{1}H NMR spectra of the monomer exhibits clear peak shifts compared to the ^{1}H NMR spectra of *β*-CD which can be assigned to the complexation of the monomer $\mathbf{3}_D$ (Supporting Information).

The formation of large supramolecular structures formed by intramolecular interactions were found in DLS measurements of the monomers $\mathbf{3}_D$ and $\mathbf{3}_L$ exhibiting hydrodynamic diameters with a mean coil size of about 100 nm (Figure 2 as an example for $\mathbf{3}_D$). The structure can then be disassembled by addition of adamantylcarboxylate (ad-COO$^-$ K$^+$)in slight excess, which is a

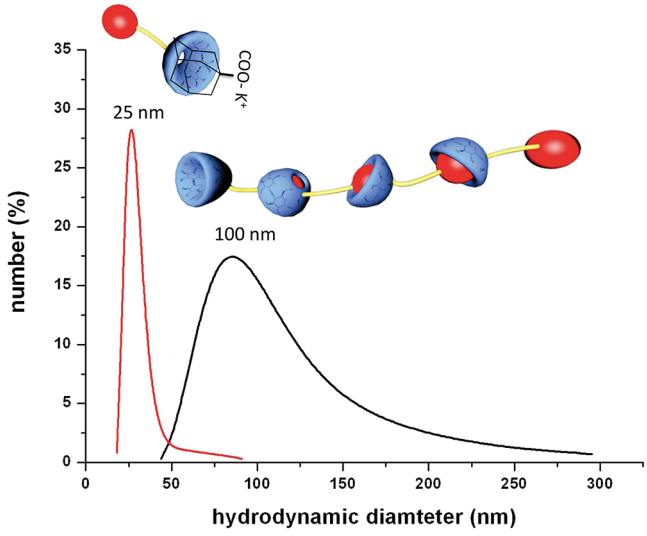

Figure 2. DLS measurement of monomers $\mathbf{3}_D$ (2 mg/mL) and $\mathbf{3}_D$ complexed with potassium adamantylcarboxylate (1 mg) and schematic illustration of complexes.

suitable competing guest molecule with a higher complex stability with *β*-CD than the phenylring of $\mathbf{3}_D$. After addition of ad-COO$^-$ K$^+$ to the aggregate of $\mathbf{3}_D$ the hydrodynamic diameter decreases as mentioned above down to 25 nm, as the *β*-CD moieties have the tendency to aggregate, the diameter does not further decrease even after addition of ad-COO$^-$ K$^+$ in great excess.

Furthermore, we were able to show that the supramolecular aggregates of **3** can also be disassembled by increasing the temperature of the solution and that the formation of the supramolecular structure can be monitored over time (Supporting Information).

In order to qualify the structure and nature of the supramolecular inclusion complexes, TEM (Transmission Electron Mikroscopy) was conducted. The microscope image (Figure 3) shows the formation of large linear structures for the

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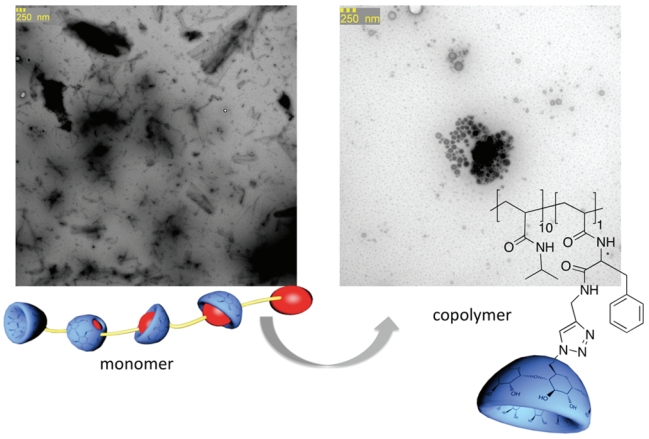

Figure 3. TEM measurement of 3_D exhibiting tube-like superstructures.

supramolecular structure formed from the monomers. In comparison the picture of the copolymers **4** exhibits round vesicles with a higher order than the aggregates of **3**. Both samples exhibit aggregation induced by the intermolecular host–guest interaction.

Furthermore, the solution properties of copolymers 4_D and 4_L were investigated. As expected, copolymers 4_D, 4_L (1:10) are soluble in cold water below the critical solution temperature (LCST). However, due to the presence of incorporated hydrophilic comonomers 3_D, 3_L a significant affect on the cloud point value of pure Poly-(NIPAAm) is shifted from 32 to 39.0 °C (3_L) and to 40.5 °C (3_D) respectively. The cloud point shift difference of 1.5 °C regarding the D- or L-enantiomer is a strong hint to different stereoinduced interactions (Figure 4).

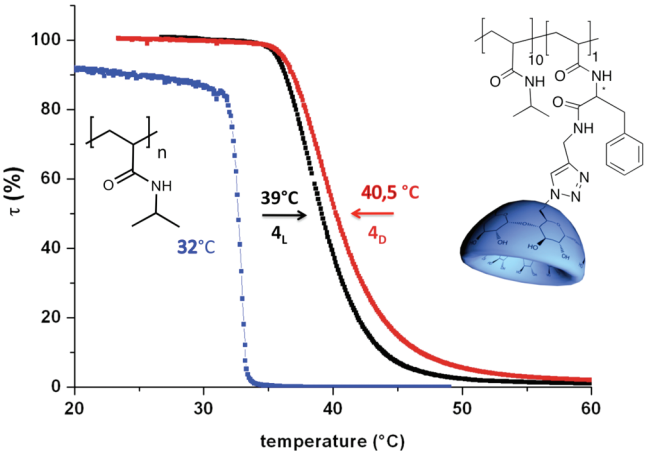

Figure 4. LCST measurements of 4_D and 4_L in comparison to Poly(NIPAAm) (10 mg/mL).

To confirm the supposed diastereomeric effects, DLS measurements of the copolymers were carried out. Surprisingly, in contrast to the small difference in cloud points copolymers exhibited strong differences of hydrodynamic diameters of 16 nm for the 4_L and 63 nm for the 4_D, respectively (Figure 5).

Since SEC measurements of 4_D and 4_L confirm nearly identical masses and mass distributions; the big difference of supramolecular aggregates in aqueous solution is a result of the phe chirality.

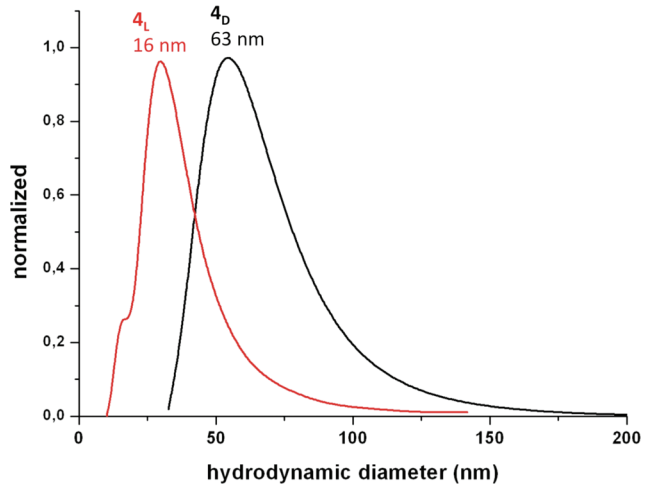

Figure 5. Hydrodynamic diameters of polymers 4_D and 4_L (1 mg/mL).

CONCLUSION

We have investigated the formation of a new cyclodextrin- and phenylalanine-based monomer and the resulting supramolecule. The intermolecular interaction was proven by 2D ROESY NMR experiments. The quality of the supramolecule was investigated via TEM and DLS, exhibiting large formations of 100 nm in diameter, which could be disassembled by addition of potassium adamantylcarboxylate. Thus we conclude that we have succeeded in synthesizing a new type of monomer bearing both host and guest moiety forming supramolecular structures. Furthermore we were able to show enantioselective recognition of copolymers containing D- or L-phenylalanine moieties by use of DLS and LCST measurement. The results clearly indicate that enantioselective recognition of the polymeric attached chiral amino acid takes place due to host–guest interaction with *β*-CD. The D-enantiomer of polymeric attached phe shows a much stronger increase in hydrodynamic diameter than the L-enantiomer due to CD-interaction.

ASSOCIATED CONTENT

Supporting Information

DLS measurements, ^{1}H NMR spectra, FT-IR spectra, and 2D ROESY NMR. This information is available free of charge via the Internet at http://pubs.acs.org/.

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Notes

The authors declare no competing financial interest.

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