5538 *Macromolecules* **2010**, *43*, 5538–5543
DOI: 10.1021/ma100812q

Macromolecules
ARTICLE

Poly(*N*-vinylpyrrolidone) Bearing Covalently Attached Cyclodextrin via Click-Chemistry: Synthesis, Characterization, and Complexation Behavior with Phenolphthalein

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Received April 16, 2010; Revised Manuscript Received June 2, 2010

ABSTRACT: We report about modification of poly(*N*-vinylpyrrolidone) (PVP) with monofunctional β-cyclodextrin (βCD) via click-chemistry. The modification was carried out by copper(I)-catalyzed microwave-assisted Huisgen-type cycloaddition of 3-propargyl-*N*-vinylpyrrolidone (**2a**) and mono(6-azido-6-deoxy)-β-cyclodextrin (**3**). We synthesized a copolymer (**5**) carrying CD moieties which has the same composition as the applied molar ratio of monomers. Complexation experiments of the obtained polymer (**5**) with phenolphthalein (PP) showed a lower binding constant compared to βCD caused by sterical effects of the polymer coil.

I. Introduction

Among the widely used hydrophilic polymers, poly(*N*-vinylpyrrolidone) (PVP) is a water-soluble, uncharged and nontoxic material with applications in various industrial fields as textile and pharmaceutical industries.[1−6] It is even approved by the FDA as food additive E1201. Its high proclivity for complexation of iodine and some drugs, e.g., of barbiturates, penicillin, or hormones,[2] makes it an excellent candidate for medical applications. It was used as blood plasma substitute during World War II. Still, it is used for prolonging the effectiveness of drugs and also for detoxication of organisms.[2] Currently, the ability of PVP derivatives as drug release/drug delivery systems is being investigated.[5−9]

Another outstanding candidate for medical and pharmaceutical applications is cyclodextrin (CD). It is well-known for its ability to act as a ring-shaped host for hydrophobic molecules[10] and thus also as drug carrier.[11] There are meanwhile several products available on the market containing CD inclusion complexes of poorly water-soluble drugs.[12] Previous studies reported about CD containing copolymers or networks based on acrylamide or (methyl) methacrylate. They are synthesized via polymer analogue reactions or copolymerization using different types of CD-derivatives and -monomers.[13−18] Up to now, polymers from *N*-vinylpyrrolidone (NVP) bearing covalently attached CD moieties have not been described in literature with exception of two copolymers from NVP and monoacrylamide CD and monomethacryl ethylamino CD which have been investigated very recently.[19,20]

Our current studies about modification of PVP[21] and CD encouraged us to synthesize a acrylate-free PVP system, which is modified with covalently attached CD moieties.

Furthermore, the inclusion of phenolphthalein (PP) as guest molecule into the NVP polymers was in focus of this work to evaluate the ability of attached CDs to act as host for suitable molecule fragments.

II. Experimental Section

A. Materials and Methods. β-Cyclodextrin was provided by Wacker-Chemie GmbH, Burghausen, Germany, and was used after drying overnight in oil-pump vacuum over phosphorus pentoxide. *N*-Vinylpyrrolidone, +99% was purchased from Sigma-Aldrich Chemicals, Germany, and was distilled before use. Propargyl bromide (80 wt % solution in toluene) was obtained from Aldrich Chemicals, Germany. Lithium diisopropylamide (LDA) (2 M solution in tetrahydrofuran/*n*-heptane/ethylbenzene) was purchased from Acros Organics, The Netherlands. Tetrahydrofuran (THF), extra dry over molecular sieves, water <50 ppm, stab., was purchased from Acros Organics. Copper(II) sulfate pentahydrate (puriss. p.a.; 99%) was obtained from Fluka and sodium L-(+)-ascorbate (99%) from AppliChem U.K. 2,2′-Azobis(isobutyronitrile) (AIBN) (98%) was purchased from Sigma-Aldrich, Germany and was used as received. Ethanol absolute was purchased from VWR and used as received. Buffer solution pH 10 was purchased from AppliChem, Germany. Chloroform-*d* 99.8% D, water <0.01%, was purchased from euriso-top, France. Dimethyl sulfoxide-d_6 99.9 atom % D was obtained from Deutero GmbH. Unless otherwise noted, all operations were performed under an inert atmosphere using dried glassware. The synthetic apparatus used was a two-necked flask, equipped with magnetic stirrer and nitrogen inlet. Dialysis membrane with a molecular weight cut off (MWCO) of 3.5 kDa was obtained from Spectra/Por, Germany.

Microwave reactions were performed on a Discover 1 (CEM), equipped with a 300 W power supply in self-tuning single mode design. Temperature is monitored by an infrared temperature sensor, reading vessel surface.

B. Measurements. ^{1}H NMR and ^{13}C NMR were performed using a Bruker Advance DRX 500 spectrometer at 500.13 MHz for ^{1}H and 125.77 MHz for ^{13}C spectra. The δ scale relative to tetramethylsilane was calibrated to the solvent value δ = 7.24 ppm for $CHCl_3$ and 2.51 ppm for DMSO-d_5. Copolymer compositions were determined by ^{1}H NMR.

Infrared (IR) spectra were recorded on a Nicolet 6700 FT-IR spectrometer equipped with a diamond single bounce ATR accessory at room temperature.

The structures of the monomers were verified by gas chromatography−mass spectrometry (GC/MS) using a Thermo Finnigan Trace DSQ (Dual-Stage Quadrupole). Ionization was carried out by electron ionization (EI).

MALDI−TOF−MS was performed on a Bruker Ultraflex TOF time-of-flight mass spectrometer using a 337 nm nitrogen

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 Published on Web 06/10/2010

laser. As matrix substance, 2,4-dihydroxybenzoic acid or dithranol were employed.

SEC−MALS experiments were made on a combined system comprising the following elements: refractive index detector Optilab rex (Wyatt Technologies, laser wavelength 658 nm), three angle light scattering detector miniDawn TREOS (Wyatt Technologies, laser wavelength 658 nm, detector angles at 43,5°, 90,0° and 136,5°), UV detector Waters 486 (Waters), column set of HEMAbio 300 and HEMAbio 100 (MZ-Analysentechnik), pump, degasser and autosampler (Agilent 1200, Agilent technologies). Eluent was ultrapure water at a flow rate of 1 mL/min. Molecular weight was calculated with Astra5 software from static light scattering data, using the Zimm model. As concentration source, the refractive index was used. Calibration of the system was performed by bovin serum albumin.

The hydrodynamic diameters of the monomer and copolymers were determined by dynamic light scattering (DLS) in backscattering mode on a Malvern Zetasizer Nano ZS ZEN3600 with a laser wavelength of 633 nm and a detection angle of 173°. Measured solutions contained 0.2−1 wt % substance in water and were performed in a glass cuvette with a layer thickness of 1 cm. Measurement results are calculated by the non-negative least-squares (NNLS) algorithm. Depending on measurement number- or volume-averaged diameters are used for characterization.

Determination of complexation constants was performed by UV−visible spectroscopy. The UV−visible-spectra of the samples were recorded on a Nicolet UV540 spectrometer in the range from 400 to 650 nm in a glass cuvette with a layer thickness of 1 cm. For the experiment six samples containing 2 mL of a 100 μM buffered PP solution at a pH of 10 and the same amount of buffered βCD or CD-polymer containing solutions of different concentrations (0 μM, 100 μM, 200 μM, 400 μM, 800 μM, 1600 μM) were prepared. The concentrations of the CD-polymer solutions relate to the CD ratio in the polymer determined by ^{1}H NMR. The measured absorbance of PP is reduced by the absorption of the pure polymer solution which has a light brown color and is plotted as reduced absorbance on the ordinate.

The complexation constant can be calculated from the spectroscopic data by Scott's equation, eq 1.

$$\frac{[\mathrm{CD}]}{\Delta A_{552\mathrm{nm}}} = \frac{[\mathrm{CD}]}{[\mathrm{PP}]\Delta\varepsilon} + \frac{1}{[\mathrm{PP}]\Delta\varepsilon \mathrm{K}} \quad (1)$$

[CD] and [PP] are the total molar concentrations of CD and PP, $\Delta A_{552\ \mathrm{nm}}$ is the change of absorbance at a wavelength of λ = 552 nm ($\Delta A = A_{\mathrm{PP}} - A_{\mathrm{CDxPP}}$) after addition of CD, K is the complexation constant and $\Delta\varepsilon$ is the difference between the molar absorption coefficients of free and complexed PP. This equation is valid for 1:1 complexes which are expected for PP.[22] From the regression equation of the resulting plot [CD]/$\Delta A_{552\ \mathrm{nm}}$ vs [CD] the complexation constant K (M^{-1}) can be calculated by correlation between slope and intercept (eq 2). The factor 1000 is applied for conversion from mM^{-1} to M^{-1}.

$$K = \frac{\mathrm{slope}}{\mathrm{intercept}} \times 1000 \quad (2)$$

An alternative for calculation of K is the mass action law of the complexation equilibrium. With known total concentrations of CD and PP and the concentration of the uncomplexed PP, determined via a PP calibration, K can be calculated by eq 3.

$$K = \frac{[\mathrm{PP}]_0 - [\mathrm{PP}]_\mathrm{f}}{[\mathrm{PP}]_\mathrm{f}([\mathrm{CD}]_0 - [\mathrm{PP}]_0 + [\mathrm{PP}]_\mathrm{f})} \quad (3)$$

$[\mathrm{PP}]_0$ and $[\mathrm{CD}]_0$: total concentration of components. $[\mathrm{PP}]_\mathrm{f}$ and $[\mathrm{CD}]_\mathrm{f}$: concentration of uncomplexed components. [CD·PP]: concentration of complex

Isothermal titrations were performed on a VP-ITC MicroCalorimeter from MicroCal (Northampton, MA) controlled by MicroCal's VP viewer 2000 ITC software. Titrations were performed with a 8.4 mM βCD and a CD-polymer solution of 8.4 mM CD equivalents into a solution of 0.84 mM or 0.56 mM PP in buffer respectively at a pH of 10 containing 0.5 vol % absolute ethanol. The concentrations of the CD-polymer solutions relate to the CD ratio in the polymer determined by ^{1}H NMR. During the experiment at 25 °C the host-solution was titrated with 20 injections at 3 μL followed by 47 injections at 5 μL into the sample cell with a spacing time of 240 s. The stirring speed was 300 min^{-1}. The heat of dilution was determined in a separate experiment and was subtracted from the binding isotherm. Because of the complex formation heat is released in direct proportion to the amount of binding and monitored over time. By integration of each peak, correction for the cell volume and sample concentration the binding isotherm is obtained. Data processing was done using Origin 7 with the MicroCal LLC ITC add-on. The obtained binding isotherm is fitted by a least-squares fit (LSF), which regards a number of independent binding sites. Indeed the complexation from cyclodextrin moieties attached to a polymer chain is not truly independent. However, the system was approximated by a "one binding site" model, as it otherwise turns out to be indissoluble.

C. Synthesis. *Synthesis of 3-Propargyl-N-vinylpyrrolidone* (***2a***). NVP (5.0 g, 45 mmol) dissolved in 20 mL of THF was added dropwise to 22.5 mL (90 mmol) of a dry 2.0 M THF solution of LDA at 0 °C. The resulting solution was stirred for 90 min at 0 °C. Propargyl bromide, 80 wt % solution in toluene, (6.7 g, 45 mmol) was dissolved in 15 mL of THF and added dropwise at 0 °C. After stirring overnight, the brown solution was mixed with water (40 mL). Dichloromethane (20 mL) was added and the aqueous layer was then extracted with further portions of dichloromethane (3 × 30 mL).

The combined organic phases were dried over anhydrous magnesium sulfate and the solvent was evaporated under reduced pressure. The crude product obtained was separated by column chromatography (light petroleum/ethyl acetate, 2:1) yielding 1.96 g (13.3 mmol, yield 30%, R_f 0.63) of monosubstituted product (**2a**) and 0.75 g (5.02 mmol, 12%, R_f 0.79) of disubstituted product (2b), both as pale yellow oils.

2a: ^{1}H NMR ($CDCl_3$): δ = 1.91 (t, ^3J(H,H) = 2.6 Hz, 1H, alkinyl CH_2CCH); 1.98 (dq, 1H, ^3J(H,H) = 9.1 Hz, 13.0 Hz, lactam CH_2CHCO); 2.28−2.46 (m, 2H, alkinyl $CHCH_2CCH$); 2.56−2.72 (m, 2H, lactam CH_2CH_2CH); 3.32−3.53 (m, 2H, lactam NCH_2CH_2); 4.33 (d, ^3J(H,H) = 16.0 Hz, 1H, vinyl $CHCH_2$); 4.39 (d, ^3J(H,H) = 9.1 Hz, 1H, vinyl $CHCH_2$); 7.03 (dd, ^3J(H,H) = 9.1 Hz, 16.0, 1H, vinyl $CHCH_2$) ppm.

^{13}C NMR ($CDCl_3$): δ = 20.64 (alkinyl $CHCH_2CCH$); 23.83 (lactam CH_2CH_2CH); 41.79 (lactam CH_2CHCO); 43.24 (lactam NCH_2CH_2); 70.44 (alkinyl CH_2CCH); 81.37 (alkinyl CH_2CCH); 95.16 (vinyl $CHCH_2$); 129.84 (vinyl $CHCH_2$) ppm.

FT-IR (diamond): ν = 2961 (m, CH), 2933 (w, CH), 2875 (w, CH), 1696 (ss, C=O), 1627 (ss, C=C), 1384 (s, CH_3) cm^{-1}.

GC/MS: m/z (%) = 140 (11), 139 (98) $[\mathrm{M}+\mathrm{H}]^+$, 138 (8) $[\mathrm{M}^+]$, 112 (12), 111 (100), 110 (11), 96 (14), 83 (30), 56 (58).

2b: ^{1}H NMR ($CDCl_3$): δ = 1.94 (t, ^3J(H,H) = 2.6 Hz, 2H, alkinyl CH_2CCH); 2.26 (m, 2H, lactam CH_2CHCO); 2.45 (m, 4H, alkinyl $CHCH_2CCH$); 3.46 (m, 2H, lactam CH_2CH_2CH); 4.33 (d, ^3J(H,H) = 16.0 Hz, 1H, vinyl $CHCH_2$); 4.39 (d, ^3J(H,H) = 9.1 Hz, 1H, vinyl $CHCH_2$); 7.04 (dd, ^3J(H,H) = 16.0, 9.1 Hz, 1H, vinyl $CHCH_2$) ppm.

^{13}C NMR ($CDCl_3$): δ = 21.46 (alkinyl $CHCH_2CCH$); 26.86 (alkinyl $CHCH_2CCH$); 34.38 (lactam CH_2CCO); 42.56 (lactam CH_2CH_2CH); 48.66 (lactam NCH_2CH_2); 71.35 (alkinyl CH_2CCH); 80.05 (alkinyl CH_2CCH); 95.75 (vinyl $CHCH_2$); 129.88 (vinyl $CHCH_2$), 171.58 (lactam CO) ppm.

FT-IR (diamond): ν = 2961 (m, CH), 2933 (w, CH), 2875 (w, CH), 1696 (ss, C=O), 1627 (ss, C=C), 1384 (s, CH_3) cm^{-1}.

GC/MS: m/z (%) = 187 (25) $[\mathrm{M}^+]$, 158 (30), 120 (100), 77 (70).

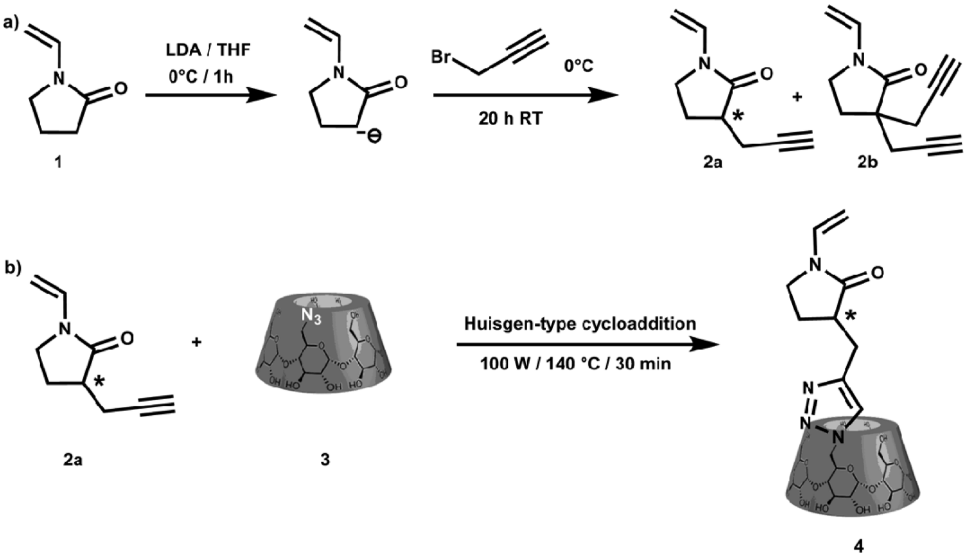

Figure 1. (a) Synthesis of mono (**2a**) and dipropinyl (**2b**) functionalized NVP; (b) microwave assisted Huisgen-type 1,3-dipolar cycloaddition of mono(6-azido-6-desoxy)-β-cyclodextrin (**3**) and 3-propargyl-*N*-vinylpyrrolidone (**2a**).

Synthesis of (*3-(6-Desoxy)-β-cyclodextrin-3H-1,2,3-triazole-4-yl)-N-vinylpyrrolidone* (***4***)

Mono(6-O-(p-tolylsulfonyl))-β-cyclodextrin. A 50.0 g (44.0 mmol) sample of βCD was dissolved in 500 mL of a 0.4 M aqueous solution of sodium hydroxide and cooled down to 0 °C. Subsequently, 35.0 g (184 mmol) of *p*-toluenesulfonyl chloride was added in small portions under intense stirring over 5 min to the solution. The resulting suspension was stirred for further 30 min below 5 °C and then filtered off quickly. The filtrate was neutralized with hydrochloric acid and stirred for 1 h. The resultant precipitate was filtered off, washed three times with water and dried overnight at 60 °C. For further drying the product was stored in a desiccator over phosphorus pentoxide. Yield: 24.7 g (19 mmol, 43%)

^{1}H NMR (DMSO-d_6): δ = 2.45 (3H, Ar−CH_3); 3.18−3.78 (42H, C-2,-3,-4,-5,-6); 4.79 (t, 6H, C-1); 5.74 (14H, C-2,-3 OH); 7.45 (d, 2H, Ar-*H*(meta)); 7.77 (d, 2H, Ar−*H*(ortho)) ppm.

FT-IR (diamond): ν = 3316 (OH), 2924 (CH), 2160 (CH), 2030, 1358 (S═O), 1152 (S−O), 1077 (OH), 1023 (CH), 945, 841, 756 (OH), 684 (CH) cm^{-1}.

MS (MALDI−TOF): m/z = 1311.4 [M + Na]$^+$.

Mono(6-azido-6-desoxy)-β-cyclodextrin (***3***). A 10.0 g (7.76 mmol) sample of mono(6-*O*-(*p*-tolylsulfonyl))-β-cyclodextrin was suspended in 100 mL of water and heated to 80 °C. Subsequently, 2.53 g (38.5 mmol) of sodium azide was added to the suspension and stirred for 4 h until the reaction mixture became transparent. The solution was precipitated in 600 mL of acetone and the white solid was filtered off and dried in a desiccator over phosphorus pentoxide yielding 9.37 g crude product. Recrystallization from water/acetone yields 6.0 g (5.2 mmol, 67%) of white powder.

^{1}H NMR (DMSO-d_6): δ = 3.32 (m, 14H, C-6); 3.60−3.84 (m, 28H, C-2, −3, −5); 4.44- 4.60 (m, 6H, C-6 OH); 4.85 (d, 6H,C-1); 4.92 (d, 1H, C-1); 5.73 (m, 14H, C-2,-3 OH) ppm.

FT-IR (diamond): ν = 3316 (OH), 2924 (CH), 2160 (CH), 2101 (N═N), 2033, 1364 (OH), 1152 (C−N) cm^{-1}.

MS (MALDI−TOF): m/z = 1182.4 [M + Na]$^+$.

(*3-(6-Desoxy)-β-cyclodextrin-3H-1,2,3-triazole-4-yl)-N-vinylpyrrolidone* (***4***). The reaction of 3-propargyl-*N*-vinylpyrrolidone (2a) (30 mg, 0.2 mmol) with mono(6-azido-6-desoxy)-β-cyclodextrin (3) (232 mL, 0.2 mmol) was carried out in 2 mL of DMF in the presence of Cu(I) generated in situ by the reduction of copper(II) sulfate (2.5 mg, 0.01 mmol) with sodium ascorbate (20 mg, 0.1 mmol) in a 10 mL microwave vessel. The vessel was placed in the CEM monomode microwave and irradiated at 140 °C and 100 W for 30 min. After precipitating the reaction mixture in 150 mL acetone, 200 mg (77%) product was isolated.

^{1}H NMR (DMSO-d_6): δ = 1.72−2.28 (m, 2H, 4); 2.7- 2.91 (m, 4H, 5, 6, b); 3.38 (12H, f′); 3.66 (24H, b′-e′, 3); 4.52 (10H, f′ OH, f, 1a/b); 4.86 (7H, a′, e); 5.05 (1H, a); 5.76 (14H, b/c OH); 6.98 (m, 1H, 2); 7.82 (d, 1H, 7) ppm (Figure 2a).

FT-IR (diamond): ν = 3330 (OH), 2927 (CH), 2104 (*N* = N), 1707 (C═O), 1658 (C═C), 1153(C−N), 1078 (OH), 1026 (CH), 945 (C═C), 754 (NH) cm^{-1}.

MS (MALDI−TOF): m/z = 1309.5 [M + H]$^+$, 1331.5 [M + Na]$^+$, 1347.5 [M + K]$^+$.

Synthesis of Poly[(*N-vinylpyrrolidone*)-*co*-(*3-(6-desoxy)-β-cyclodextrin-3H-1,2,3-triazole-4-yl)-N-vinylpyrrolidone*)] (***5***). NVP (0.2 g, 1.8 mmol) and monomer **4** (0.235 g, 0.18 mmol) were dissolved in 1.5 mL of DMF and flushed with nitrogen for 15 min. AIBN (3.25 mg, 0.02 mmol, 1 mol %) was added, and the solution was polymerized for 48 h at 70 °C. The solution was precipitated in diethyl ether. A light brown powder was obtained and dialyzed (MWCO = 3.5 kDa) for 4 days in water at room temperature yielding 132 mg (30%) of copolymer **5** after freeze-drying. The molecular weight was estimated to 16.850 g/mol for $\overline{M_n}$ by SEC. The PD was determined to 1.62.

^{1}H NMR (DMSO-d_6): δ = 1.00−2.50 (lactam CH_2, lactam C*H*, C-6); 2.78−4.00 (backbone C*H*, lactam CH_2, C-2, −3, −4, −5); 4.36−3.91 (7H, C-6 O*H*); 4.70−4.93 (7H, C-1); 5.51−6.00 (14H, C-2, -3 O*H*); 7.89 (1H, triazole N−*H*) ppm.

III. Results and Discussion

A. Preparation of Cyclodextrin-Functionalized PVP. To provide NVP polymers bearing covalently attached CD moieties two strategies were applied. The first approach was the free radical polymerization of 3-propargyl-*N*-vinylpyrrolidone (**2a**) to evaluate the click-type anchoring of CD-azide (**3**). But it turned out to be unsatisfactory in yield. We thus, prepared the monofunctional NVP-CD-derivative (**4**) from CD-azide (**3**) and monomer **2a** via click-reaction (Figure 1b). This new monomer **4** contains a vinyl group which has the same electronic properties as the double bond of NVP.

It is well investigated that NVP clearly differs in radical reactivity from many other common monomers. As a result, copolymerization with NVP undergoes a compositional drift during chain growth.[23] As expected, the functionalization of NVP in 3-position will not influence the reactivity of the vinyl group significantly.[7,21,24,25] Hence, only a small drift in

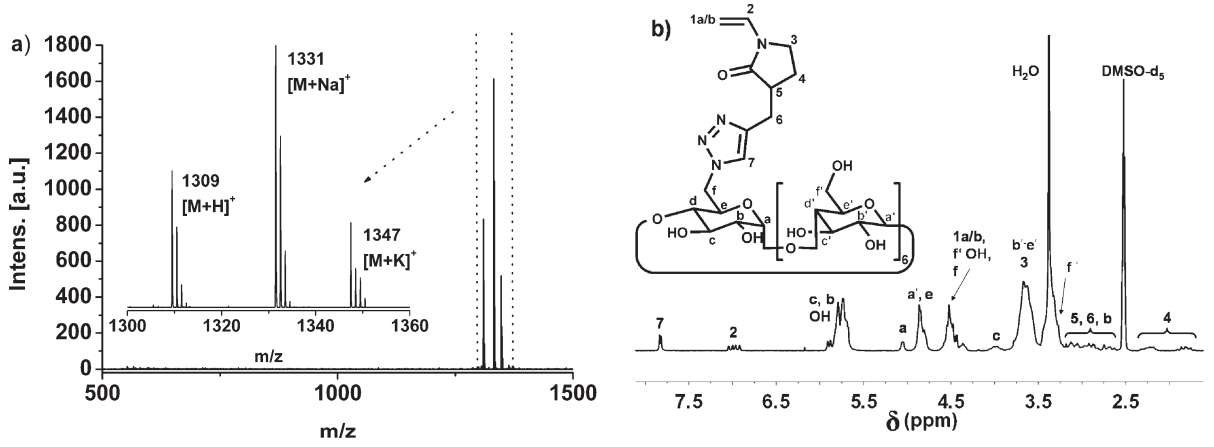

Figure 2. (a) MALDI−TOF mass spectrum of **4**; (b) ^{1}H NMR spectrum of **4**.

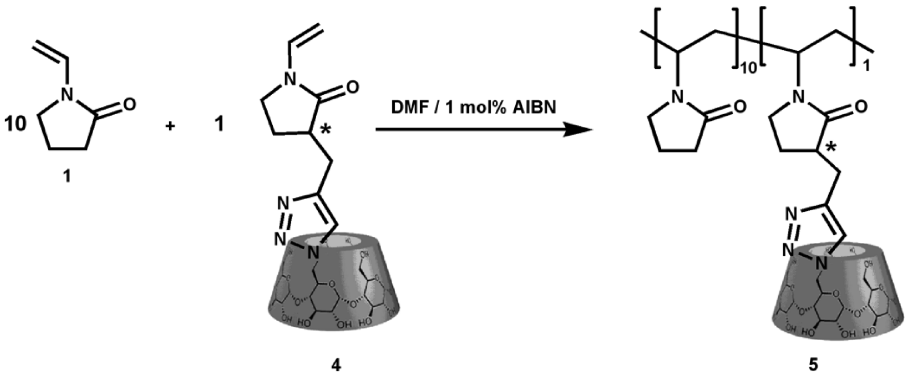

Figure 3. Free radical copolymerization of CD-monomer (**4**) and NVP (**1**).

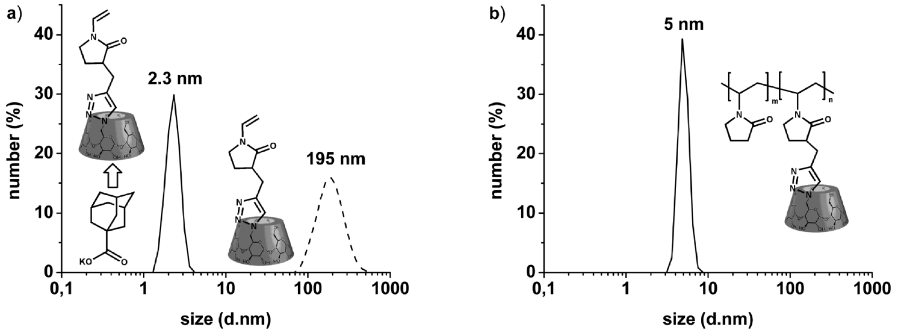

Figure 4. (a) Size distributions by number for pure CD-monomer **4** (---) and CD-monomer/Ad-COOK (−−) dissolved in water. (b) Size distribution by number for CD-polymer in water.

the composition of NVP and functionalized NVP is expected during copolymerization.[23,24] Only sterical effects may play a certain role.

We modified NVP with a propinyl group in 3-position in order to permit a Cu(I)-catalyzed microwave assisted Huisgen-type cycloaddition.[13] The propinyl component 3-propargyl-*N*-vinylpyrrolidone (**2a**) for the click-reaction was prepared in a common way[21] from NVP (**1**) via nucleophilic substitution in the 3-position (Figure 1a)). Bifunctional NVP (**2b**) is obtained as byproduct and can be separated by column chromatography. The monopropinyl modified NVP (**2a**) consists of a racemic mixture of two enantiomers which were not separated yet. In the following click-reaction (3-(6-desoxy)-β-cyclodextrin-3*H*-1,2,3-triazol-4-yl)-*N*-vinylpyrrolidone (**4**) is obtained (Figure 1b)). The structure and purity of product **4** is confirmed by ^{1}H NMR spectroscopy and MALDI−TOF mass spectrometry (Figure 2).

For copolymerization of NVP and monomer **4**, a free radical copolymerization with AIBN as initiator in DMF-solution was employed (Figure 3). However, the composition of copolymer **5** of 10:1 corresponds exactly to the applied molar ratio of monomers. The amount of CD in **5** was calculated from ^{1}H NMR data by comparing the signal from the single proton at the triazole ring (7.89 ppm) and the signals from the protons of the lactam ring in the region of 1−2.5 ppm. The molecular weight was found to be 16 850 g/mol ($\overline{M}_n$) and 27 250 g/mol ($\overline{M}_w$) with a PD of 1.62 and was calculated from static light scattering data obtained from SEC analyses.

The size distributions by number of copolymer **5** and CD-monomer **4** determined by DLS measurements in water are illustrated in Figure 4. A hydrodynamic diameter of about 5 nm for the copolymer coils (**5**) indicates the existence of a monodisperse solution (Figure 4a)). In contrast, an aqueous

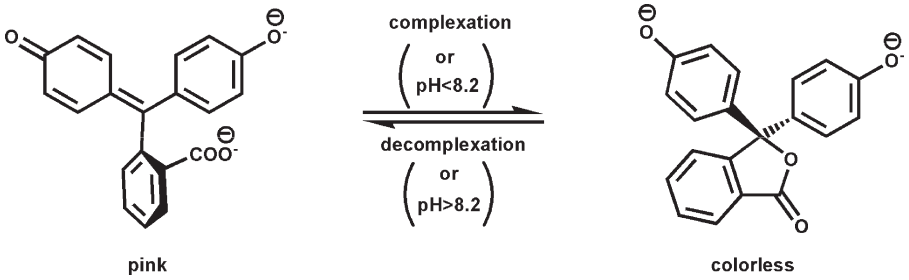

Figure 5. Equilibrium of intermolecular ring closure of PP influenced by pH and CD complexation.[22]

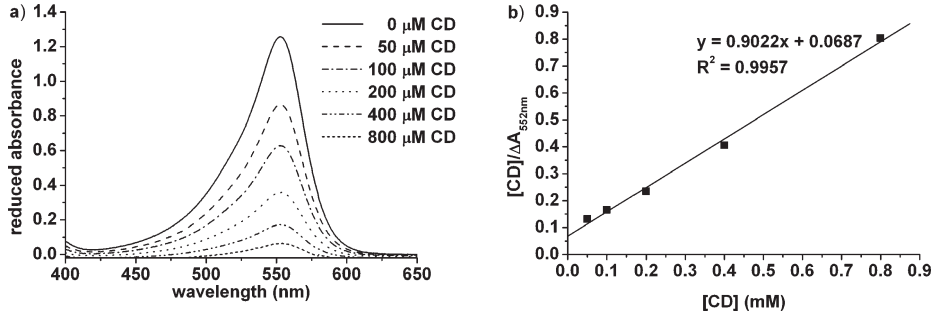

Figure 6. (a) UV−visible absorption spectra of bufferd 50 μM PP solutions (pH 10) with different concentrations of CD-containing polymer. (b) Scott's plot with regression equation.

Table 1. Binding Constant *K* of Copolymer 5 and Free βCD with PP Determined by UV−Vis Spectroscopy and ITC

	K_{UV-vis} Scott's equation (M^{-1})	K_{UV-vis} mass action law (M^{-1})	K_{ITC} (M^{-1})
CD-polymer 5	13 100	11 800	8040
βCD	18 000	24 000	30 000

Table 2. Thermodynamic Data Determined by ITC Experiments for CD-Polymer/PP and βCD/PP Systems

	n	*K* (M^{-1})	ΔH (kJ/mol)	$T\Delta S$ (kJ/mol)
CD-polymer **5**	1.21 ± 0.0068	8040 ± 220	−37.00 ± 1.17	−14.22
βCD	1.06 ± 0.0017	30 000 ± 500	−35.50 ± 0.29	−9.9

solution of the CD-monomer (**4**) contains large aggregates of about 200 nm. After the addition of adamantyl carboxylate (Ad-COOK) to this solution, the aggregates of **4** break up yielding a molecular dispersed solution of the CD-monomer/Ad-COOK complex (Figure 4b)). This behavior substantiates to self-aggregation of monomer **4** in aqueous solution.

B. Studies of Complexation Ability of CD Functionalized PVP (5) with Phenolphthalein. Complexation experiments with phenolphthalein (PP) were carried out to evaluate the host guest capability of PVP bound CD (5). A lower binding constant for polymer bound CD moieties in comparison to unbound βCD can be expected from sterical effects of the polymer coil and some intermolecular CD−CD interactions. Accordingly, lower binding constants for polymer bound CD were found compared to βCD (Table 1). The binding constants were determined by UV−visible (UV−vis) spectroscopy and isothermal titration calorimetry (ITC).

Determination of Complexation Constants by UV−Visible Spectroscopy. UV−vis spectroscopy is an indirect method to determine the binding constant *K* via change or shift of the absorption. The complex formation of CD and phenolphthalein can be quantified by UV−vis absorption as it is accompanied by a change of color from pink to colorless. Because of its characteristic absorption in the visible region at 552 nm the complexation/decomplexation equilibrium of PP with CD can be followed even with bare eyes.

Inclusion of PP into the CD cavity takes place only in case of a intramolecular ring closure which causes the interruption of the chromophoric system. This uncolored, less sterical demanding structure is normally formed at a pH lower than 8.2 (Figure 5).[22,26,27]

In Figure 6a, UV−vis-spectra of PP solutions with addition of different concentrations of CD-polymer are shown. As supposed, a significant decrease of absorbance with increasing CD concentration is observed. The linear dependence of the Scott's plot confirms the 1:1 complexation (Figure 6b)).[27]

An alternative for the calculation of *K* is the mass action law of the complexation equilibrium. Comparing the two mathematical methods a good accordance of the results is observed (Table 1). To get a conception in which range this results are comparative measurements with the model system βCD/PP were performed.

Determination of Complexation Constants by Isothermal Titration Calorimetry (ITC). Isothermal titration calorimetry is an immediate method for studying the complex formation by measurement of heat released or absorbed during a binding event. From the data set obtained, all binding parameters (binding constant *K*, reaction stoichiometry *n*, enthalpy ΔH, and entropy ΔS) can be determined in a single experiment.

The ITC data shown in Table 2 support the results of the UV−vis measurements: The binding constant of free βCD is higher than the one of polymer bound CD. These results confirm our expectations that complex formation might be influenced by attachment of CD to the polymer chain.[28] Observable, the absolute value of ΔH for polymer bound CD is slightly higher (1.5 kJ mol^{-1}) than ΔH of free βCD. This could be explained by additional attractive hydrophobic binding forces between the two phenyl rings of PP, sticking out the CD cavity, and the hydrophobic methylene groups of the lactam moieties or the polymer backbone. The observed unfavorable entropy contribution can be

explained by stronger binding forces which causes a loss of translational and rotational freedom.[29] Furthermore, the mobility of the host−guest complex in solution is limited by the attachment of the CD to the polymer. However, the average K value of 11 000 M^{-1} for polymer bound CD confirms that monovalent attached CD is still a very good host.

IV. Conclusion

We described the covalent attachment of βCD onto a propinyl-modified NVP accomplished by a Cu(I)-catalyzed Huisgen-type cycloaddition in high yield and purity. By copolymerization of this new monofunctional CD-monomer (**4**) with NVP the copolymer composition equates the applied molar ratio of monomers which allows the construction of a custom-made CD containing PVP. The CD moieties turned out to be still very suitable for complexation of hydrophobic guests like phenolphthalein.

We therefore present a new water-soluble polymer, which combines the binding properties of PVP with the inclusion capability of covalently attached CD. This polymer may find application as supramolecular carrier for, e.g., drugs, catalysts, or UV-absorbers.

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