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Cyclodextrin-Modified Polyesters from Lactones and from Bacteria: An Approach to New Drug Carrier Systems

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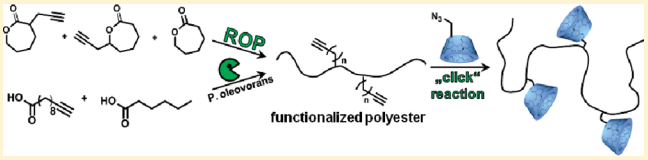
Supporting Information

ABSTRACT: Copper(I)-catalyzed cycloaddition of synthetic and bacterial copolyesters bearing pendant alkyne group with mono-(6-azido-6-desoxy)-β-cyclodextrin was carried out to synthesize β-CD-functionalized copolyester. The synthetic "clickable" copolyesters were obtained by the ring-opening copolymerization of the propargyl-modified lactones and ε-caprolactone. The bacterial copolyesters containing an alkyne group were biosynthesized from a mixture of 10-undecynoic acid and hexanoic acid by the Gram-negative bacteria *Pseudomonas oleovorans*. The modified products of the "click" reaction were characterized by FT-IR, ^{1}H NMR spectroscopy, and DSC. Furthermore, the host guest capability of covalently attached β-cyclodextrin moieties was proved by dynamic light scattering measurements.



INTRODUCTION

Aliphatic polyesters have deeply impacted biomedical and engineering fields, such as tissue scaffolding and therapeutic delivery.[1,2] Poly(lactide) (PLA), poly(glucolide) (PGA), poly-(ε-caprolactone), and their respective copolymers combine biocompatibility and biodegradability which make them leading candidates for resorbable implant materials, prosthetics, surgical sutures, vascular grafts, bone screws, and erodible polymers for drug delivery systems.[3−6] *Pseudomonas oleovorans* bacteria are able to produce poly(3-hydroxy alkanoate)s (PHAs) from suitable carbon sources with more than five carbon atoms in low nutrient media.[7] For example, PHAs from *n*-alkanes, *n*-alkanoic acids, *n*-alkynoic acids, and ω-phenoxy-substituted alkanoic acids have been produced and characterized.[8,9] The obtained PHAs are medium-chain-length (mcl) elastomers with a low degree of crystallinity and a low melting temperature.[9] In comparison to their chemically produced counterparts, PHAs from bacteria are chiral.[10−12]

An apparent drawback of standard aliphatic polyesters is the lack of functionalities on the polymer backbone which allow the tailoring of polyester properties for the specific applications or introduction of bioactive moieties. In recent years, "click chemistry" became a very important tool for the preparation of novel polymeric structures. Since the first report of Sharpless,[13] Cu(I)-catalyzed Huisgen-type[14,15] cycloaddition of azides and alkynes has been applied for synthesis of polymers with different architectures.[16] The "click" reaction is an attractive method for preparation of functionalized aliphatic polyesters due to the relatively mild reaction conditions and tolerance of the presence other functional groups. Emrick and co-workers[17−19] grafted polyesters with PEG, phosphorylcholine, and azide-functionalized camptothecin derivates via "click" reaction. Riva et al.[20,21] reported about the synthesis and "click" reactions of several alkynes onto azide-functionalized PCL. PHAs were modified by e.g. chlorination,[22] epoxidation,[23] grafting,[24] and cross-linking.[22,25] Langlois and co-workers modified oligomers from a PHA containing "clickable" end groups with azide-terminated PEG.[26]

Polymer analogous reactions of suitable polyesters with β-cyclodextrin (β-CD) derivatives lead to potential carrier systems for various substances. Cyclodextrins are cyclic oligomers of D-glucose units that are joined by 1,4-α-glucosidic linkages. CDs have the shape of a hollow cone and are able to include small hydrophobic, molecular guests into their cavity.[27] In this manner many hydrophobic substances become water-soluble, e.g., adamantane and its monosubstituted derivatives.[28] The pharmaceutical industry uses cyclodextrins to expand the bioavailability of various hydrophobic drugs.[29]

Up to now, linear biodegradable copolyesters bearing covalently attached cyclodextrin units have not been described in the literature. Thus, the aim of the present work is to describe linear polyesters bearing alkyne functionalities and their "click"-type modification with mono-(6-azido-6-desoxy)-β-cyclodextrin.

EXPERIMENTAL SECTION

Materials. β-Cyclodextrin was obtained from Wacker-Chemie GmbH (Burghausen, Germany) and used after drying overnight in a vacuum oil pump over P_4O_{10}. ε-Caprolactone (ε-CL) was purchased from Acros, dried over calcium hydride, distilled under reduced pressure,

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and stored over 0.4 nm molecular sieves under an argon atmosphere. Cyclohexanone was purchased from Aldrich, dried over magnesium sulfate, and distilled before use. Propargyl bromide (80 wt % solution in toluene) and lithium *N,N*-diisopropylamide solution (2.0 M in THF/heptanes/ethylbenzene) were purchased from Acros Organics and from Sigma-Aldrich, respectively. *m*-Chloroperbenzoic acid (70−75%) was purchased from Sigma-Aldrich and used as received. Adipic acid was obtained from Aldrich and dried overnight in a vacuum oil pump over P_4O_{10}. Sodium ascorbate (AppliChem), copper(II) sulfate (Carl Roth GmbH), *N,N′*-dicyclohexylcarbodiimide (AppliChem), and 2,2-diphenylethanamine (96%, Acros Organics) were used as received. 10-Undecynoic acid was purchased from Alfa Aesar in 96% purity. Hexanoic acid was obtained from Aldrich in 99.5% purity. Commercially available reagents and solvents were used without further purification.

In this study, stock cultures of *P. oleovorans* (ATCC 29347) were used. The strain was purchased from LGC Standards GmbH and maintained at −70 °C as glycerine culture (500 μL of glycerine, 500 μL of bacterial culture) after incubation. Cultivation of the microorganisms took place in following mineral medium: $(NH_4)_2HPO_4$ (1.1 g/L), K_2HPO_4 (5.8 g/L), KH_2PO_4 (3.7 g/L), $MgSO_4$ solution (0.1 mol/L, 10 mL), microelement solution (1 mL). The microelement solution was a 1 M HCl solution containing $FeSO_4 \cdot 7H_2O$ (2.78 g/L), $MnCl_2 \cdot 4H_2O$ (1.98 g/L), $CoSO_4 \cdot 7H_2O$ (2.81 g/L), $CaCl_2 \cdot 2H_2O$ (1.67 g/L), $CuCl_2 \cdot 2H_2O$ (0.17 g/L), $ZnSO_4 \cdot 7H_2O$ (0.29 g/L). The carbon source was added in a concentration 10 mmol/L. The cultivation was carried out in vigorously stirred flasks with baffles.

Measurements. ^{1}H NMR and ^{13}C NMR spectroscopies were performed using a Bruker Avance DRX 500 spectrometer at 500.13 MHz and at 125.77 MHz in DMSO-d_6 and $CDCl_3$ as solvent. Chemical shifts were referenced to the solvent value δ = 2.5 ppm for DMSO-d_6 and δ = 7.26 ppm for $CDCl_3$. IR measurements were performed using a FT-IR spectrometer (Nicolet 6700 FT-IR) equipped with an ATR unit. Molecular weight distributions were measured by size exclusion chromatography (SEC) using a HEMA-5 μm column set consisting of a precolumn of 4 nm and main column of 103, 102, and 10 nm. Tetrahydrofuran was used as eluent at a flow rate of 1 mL·min^{-1}. For online detection, a Waters 486 tunable absorbance detector (λ = 256 nm) and a Waters 410 differential refractometer were used. The system was calibrated with polystyrene standards with a molecular range from 580 Da to 1186 kDa. Differential scanning calorimetry (DSC) measurements were performed using a Mettler Toledo DSC 822 controler apparatus in a temperature range between −50 and 350 °C with a heating rate of 10 °C·min^{-1}. The melting point (T_m) values reported are taken from the third heating cycle. Dynamic light scattering measurements were carried out at 25 °C using Malvern Zetasizer Nano ZS90 instrumentation. The polymer concentration was ∼0.25 mg·mL^{-1}. The solutions were filtered through 0.45 μm Filtropur syringe filters prior to measurements. The particle size distribution was derived from a deconvolution of the measured intensity autocorrelation function of the sample by the general purpose mode algorithm included in the DTS software. Optical density (OD) measurements were carried out at λ = 660 nm using a Varian Cary 50 scan UV/vis spectrometer. Specific rotations were measured by Perkin-Elmer Polarimeter 341 at a wavelength of λ = 589 nm and a temperature of 20 °C. The specific rotation α_λ^T can be calculated by general equation

$$[\alpha]_\lambda^T = \frac{\alpha}{\beta d}$$

where T is temperature [K], λ the wavelength of irradiated light [nm], α the optical rotation [deg], β the concentration [mg/mL], and d the length of cell [dm].

*Synthesis of 2-Prop-2-ynylcyclohexanone (**1**).* A 250 mL round-bottom flask was purged with argon and cooled in a dry ice/acetone bath. A solution of lithium diisopropylamide (2.0 M in THF/heptane/ethylbenzene, 60 mL, 120 mmol) was added to the round-bottom flask and stirred for 30 min. An argon-purged solution of cyclohexanone (120 mmol) in 3 mL of THF was added dropwise to the LDA (lithium *N,N*-diisopropylamide) solution so that the temperature of solution did not exceed −70 °C. Propargyl bromide (120 mmol) in 10 mL of THF was also added dropwise by syringe and then stirred for an additional 60 min. The reaction mixture was then warmed up to room temperature and stirred overnight. The reaction was quenched with excess of aqueous ammonium chloride, washed twice with diethyl ether, dried over $MgSO_4$, and concentrated via a rotary evaporator. The following distillation under vacuum (T = 45 °C, p = 0.34 mbar) afforded **1** as a colorless liquid (56% yield). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 2.56 (ddd, J = 17.1 Hz, 4.6 Hz, 2.7 Hz, 1H), 2.48−2.42 (m, 1H), 2.39−2.34 (m, 2H), 2.30−2.23 (m 1H), 2.13 (ddd, J = 17.0 Hz, 8.4 Hz, 2.6 Hz, 1H), 2.07−2.03 (m, 1H), 1,92 (t, J = 2.6 Hz, 1H), 1.89−1.86 (m, 1H), 1.70−1.55 (m, 2H), 1.37 (ddd, J = 25.4 Hz, 12.7 Hz, 3.6 Hz, 1H). ^{13}C NMR (125 Hz, $CDCl_3$, ppm): δ 210.9, 82.7, 69.5, 49.6, 42.05, 33.3, 27.9, 25.2, 18.9. GC/MS: m/z (%) = 137 $[M + H]^+$, 136 $[M]^+$. FT-IR (diamond): ν = 3290 (w, C−H, −C≡CH), 2930 (m, CH), 2859 (m, C−H), 1703 (s, C═O), 1450, 1424, 1129 cm^{-1}.

*Synthesis of 3-/7-(Prop-2-ynyl)oxepan-2-one (**2a/b**).* 2-Prop-2-ynyl-cyclohexanone (60 mmol) was added to a solution of *m*-chloroperoxybenzoic acid (90 mmol) in 160 mL of methylene chloride. The reaction mixture was refluxed for 48 h. After cooling to room temperature and filtration, the solution was washed twice with aqueous sodium sulfite and with aqueous sodium hydrogen solution. Subsequent removal of the solvent under reduced pressure and distillation under oil pump vacuum yielded 80% the product as isomeric mixture (3- (70%) and 7-(prop-2-ynyl)oxepan-2-one (30%)). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 4.35−4.16 (m, 3H), 2.77−2.72 (m, 1H), 2.66−2.52 (m, 5H), 2.43 (ddd, J = 16.7 Hz, 8.1 Hz, 2.7 Hz, 1H), 2.31 (ddd, J = 17.1 Hz, 9.2 Hz, 2.6 Hz, 1H), 2.18−2.06 (m, 2H), 2.00−1.88 (m, 5H), 1.72−1.37 (m, 6H). ^{13}C NMR (125 Hz, $CDCl_3$, ppm): δ 176.03 (1A), 174.62 (1B), 82.40 (2A), 80.02 (2B), 77.68 (3B), 71.36 (4B), 70.21 (4A), 68.55 (5A), 42.05 (3A), 34.71 (5B), 33.58 (6B), 28.97 (6A), 28.86 (9A), 28.02 (7A), 27.84 (8B), 26.11 (7B), 22.93 (9B), 21.96 (8A). FT-IR (diamond): ν = 3277 (w, −C≡CH, C−H), 2933, 2859 (m, C−H), 1723 (s, C═O), 1443, 1289, 1174, 1051 cm^{-1}.

*Copolymerization of 3-/7-(Prop-2-ynyl)oxepan-2-one (**2a/b**), ε-CL, and Adipic Acid (**3**).* 3-/7-(Prop-2-ynyl)oxepan-2-one (3.2 mmol), ε-CL (22.9 mmol), and previously dried adipic acid (0.12 mmol) were weighted in a round-bottom flask. After homogenization, 0.5 mol % of $Sn(Oct)_2$ was added under an atmosphere of dry argon. The flask was immersed in oil bath at 130 °C for 24 h. The cold reaction product was dissolved in chloroform, precipitated twice in a cold mixture of diethyl ether/hexane (50:50), and dried in vacuum. The yield was 98%. ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 4.91 (m, 1H) 4.04 (t, J = 6.7 Hz, 2H), 2.44 (m, 2H), 2.28 (t, J = 7.5 Hz, 2H), 1.98 (s, 1H), 1.63 (m, 4H), 1.36 (m, 2H). Content of 3-/7-(prop-2-ynyl)oxepan-2-one (**2a** and **2b**) in copolymer 10 mol %. FT-IR (diamond): ν = 3309 cm^{-1}, 3283 cm^{-1} (m, −C≡CH, C−H), 3020, 2945, 2860 (m, C−H), 1726 (s, C═O), 1463 (m), 1157 (s, C−O), 753, 669 cm^{-1}. SEC (THF): $\bar{M}_n$ = 22 880, PD = 1.3. DSC: T_m = 44 °C, $T_{decomposition}$ = 313 °C.

*Reaction of Copolymer **3** with 2,2-Diphenylethanamine (**4**).* A solution of 1.034 g of copolymer and 88 mg of 2,2-diphenylethanamine (10-fold excess relating to end groups of polyester) in 4 mL of methylene chloride was cooled in a ice bath. After 46 mg of *N,N*-dicyclohexylcarbodiimide (5-fold excess relating to end groups of polyester) was added, the reaction mixture was warmed up to room temperature and then stirred for 24 h at 40 °C. The cold reaction product was filtered, precipitated twice in a cold methanol, and dried in a vacuum. The yield of copolyester **4** was 56%. ^{1}H NMR (500 MHz, DMSO-d_6, ppm): δ 7.27 (m, 8H), 7.16 (m, 2H), 4.81 (m, 1H), 4.71 (m, 1H), 3.98 (t, J = 6.4 Hz, 2H), 2.54−2.43 (m, 3H), 2.27 (t, J = 7.2 Hz, 2H), 1.54 (m, 4H), 1.29

(m, 2H). FT-IR (diamond): ν = 3271 (m, −C≡CH, C−H), 2940, 2863 (m, C−H), 1723 (s, C═O), 1366, 1292, 1241, 1164, 1087, 958 cm^{-1}. SEC (THF): $\bar{M}_n$ = 23 930. DSC: T_m = 42 °C, $T_{decomposition}$ = 320 °C.

Synthesis of Cyclodextrin Containing Copolymer ***6*** *via "Click" Reaction.* Mono-(6-azido-6-desoxy)-β-cyclodextrin (5-fold excess relating to the alkine group of polyester) was added to a solution of 233 mg of polyester **4** in 3.5 mL of DMF. To the clear solution were added 10 mol % sodium ascobate and 5 mol % copper(II) sulfate pentahydrate. The flask was immersed in oil bath at 95 °C for 24 h. The cold reaction product was precipitated in a mixture of acetone and distilled water (1:3) and dried in a vacuum. ^{1}H NMR (500 MHz, DMSO-d_6, ppm): δ 7.76−7.73 (m, 1H), 5.87−5.56 (m, 14H), 5.03−4.76 (m, 7H), 4.59−4.31 (m, 6H), 3.98 (t, J = 6.4 Hz, 2H), 3.64−3.56 (m, 28H), 2.27 (t, J = 7.1, 2H,), 1.53 (m, 4H), 1.27 (m, 2H). FT-IR (diamond): ν = 3364 (m, OH), 2933, 2863 cm^{-1} (m, C−H), 1726 cm^{-1} (s, C═O), 1649 (w, C═C), 1549, 1150, 1029, 849 cm^{-1}. DSC: T_m = 303 °C.

Biosynthesis of PHA. First a small-scale culture in 500 mL Erlenmeyer flasks was prepared with sodium octanoate as carbon source and used as inoculum. The main cultures were cultivated in 5 L flasks with 2 L medium. As carbon source, 0.83 g of 10-undecynoic acid and 1.74 g of hexanoic acid (totaling 10 mmol/L) were used, and 20 mL of inoculum was added. The flasks were stirred at 150 rpm. A temperature of 28 °C was maintained. The moment of maximum growth was verified by OD_{660} measurement (OD_{660} at maximum growth = 1.45). After 48 h the cells reached stationary phase and were harvested by centrifugation and lyophilized to yield 7.45 g dry cell mass. Extraction of the polymer was carried out in a Soxhlet extractor with chloroform as solvent for 6 h. The organic phase was concentrated to ca. 20 mL and precipitated in 250 mL of methanol. The PHA was gathered and dried *in vacuo* for 12 h. The extraction yielded 2.5 wt % polymer of the dry cell mass. ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 0.89 (t, J = 6 Hz, 3H), 1.23−1.73 (m, 14H), 1.93 (m, 1H), 2.17 (m, 2H), 2.51 (m, 4H), 5.16 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ = 14.03, 18.42, 18.54, 22.70, 24.28, 25.14, 28.20, 28.52, 28.75, 29.02, 31.70, 33.42, 33.97, 36.18, 39.23, 39.11, 68.50, 68.87, 70.80, 76.81, 77.23, 77.43, 77.65, 84.30, 84.76, 169.57, 169.63. FT-IR (diamond): ν = 3298 (m, −C≡CH, C−H), 2934 (m, C−H), 2863 (m, C−H), 2331 (w, −C≡CH, C≡C), 1731 (s, C═O). SEC (THF): $\bar{M}_n$ = 166 000, PD = 3.4. DSC: T_g = −24.7 °C.

Modification of PHA ***7*** *with Mono-(6-azido-6-desoxy)-β-cyclodextrin.* In a 10 mL pear-shaped flask 103.4 mg of PHA **7** (ca. 0.37 mmol related to triple bond) were dissolved in 4 mL of DMF under heating to 50 °C. Sodium ascorbate (6.6 mg, 0.03 mmol), copper(II) sulfate pentahydrate (4.3 mg, 0.02 mmol), and mono-(6-azido-6-desoxy)-β-cyclodextrin (567.0 mg, 0.49 mmol) were added to the solution. The solution was stirred at 90 °C (oil bath temperature) for 48 h. It was then cooled to RT and precipitated in aqueous NaCl solution (20%). The solid was separated by centrifugation. Afterward, it was diluted in DMSO and precipitated in distilled water. The reaction yielded 181.1 mg of clicked polymer (87.6% of theoretical yield). ^{1}H NMR (500 MHz, DMSO-d_6, ppm): δ 0.81 (t, J = 6 Hz, 3H), 1.10−1.78 (m, 24H), 2.09 (m, 2H), 2.27−2.73 (m, 9H) 3.09−3.41 (m, 14H), 3.41−3.79 (m, 28H), 4.35−4.55 (m, 6H), 4.65−4.86 (m, 7H), 4.94−5.14 (s, 3H), 5.54−5.85 (m, 14H), 7.68 (s, 1H). FT-IR (diamond): ν = 3329 (m, OH), 2932, 2864 (m, C−H), 2117 (w, C≡C), 1726 (s, C═O), 1650 (w, C═C), 1026 (vs, C−N) cm^{-1}, DSC: T_{g1} = −30.4 °C; T_{g2} = 153.5 °C.

RESULTS AND DISCUSSION

Preparation of Copolyester with Pendent Alkyne Group. The alkyne-functionalized lactones 3- and 7-(prop-2-ynyl)-oxepan-2-one (**2a**/**b**) were prepared in two steps. The first step was the nucleophilic substitution reaction of cyclohexanone with propargyl bromide to yield 2-prop-2-ynyl-cyclohexanone (**1**). Baeyer−Villiger oxidation of **1** with excess of *m*-chloroperoxybenzoic acid yielded the lactone mixture **2a**/**b** (Figure 1). Analysis by ^{13}C NMR has revealed that the reaction rendered an isomeric mixture of 70% 3- (**2b**) and 30% 7-(prop-2-ynyl)-oxepan-2-one (**2a**). For the ring-opening polymerization, the mixture of isomers was used without separation.

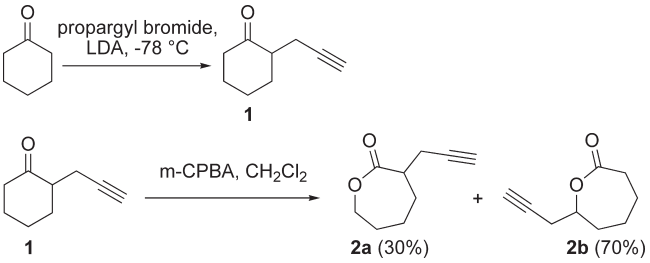

Figure 1. Synthesis of 3- and 7-(prop-2-ynyl)oxepan-2-one (**2a**/**b**) by Baeyer−Villiger oxidation of 2-prop-2-ynyl-cyclohexanone (1).

In our previous work the copolymerization of lactones **2a**/**b** with ε-caprolactone and the coupling of cyclodextrin azide afforded a supramolecular cross-linked polymer.[30] It was found that the hydrophobic cavity of the polyester attached cyclodextrin included the polyester chain as shown in Figure 2.

In respect of these former findings, the new approach of this work was to suppress the formation of pseudo-polyrotaxane by capping the copolyester chain with bulky end groups before the polyester will be functionalized by "click" reaction with β-CD-N_3.

The copolymerization of ε-caprolactone and lactone mixture **2a**/**b** was promoted by $Sn(Oct)_2$ in the presence of adipic acid as co-initiator and afforded a copolyester with terminal acid groups (Figure 3).

The obtained copolyester **3** was characterized by ^{1}H NMR, FT-IR, DSC, and SEC. The molar content of **2a**/**b** incorporated into the copolymers **3** was calculated by integration of the ^{1}H NMR spectral signals at δ = 1.97 ppm (C≡C*H* from monomers **2a**/**b**) and at δ = 4.9 ppm (OC*H*(CH_2C≡CH)) of the polymer backbone from **2b**) compared to the signal at δ = 4.03 ppm (OCH_2 of the polymer backbone from **2a** and ε-CL). The spectroscopically calculated incorporation ratio is about 90 mol % ε-CL and 10 mol % of **2a**/**b** and differs just a little from the feed ratio (88 mol % ε-CL:12 mol % of **2a**/**b**), indicating a nearly complete consumption of lactone **2a**/**b** during the polymerization. The thermal properties of **3** indicate that the obtained copolyester is a random copolymer. The signals from adipic acid protons C═OCH_2 and C═OCH_2(CH_2)$_2$ overlapped with the peaks at 2.28 ppm (C═OCH_2 of the polymer backbone from **2b** and ε-CL) and at 1.63 ppm (C═OCH_2(CH_2)CH_2(CH_2) of the polymer backbone from **2a**/**b** and ε-CL), respectively. Because of molar ratio of adipic acid to lactones (**2a**/**b** and ε-CL) of 1:217, the IR spectrum of **3** revealed only few weak stretches at 3309 and 3020 cm^{-1}, indicating the presence of acid end groups.

Synthesis of End-Capped Polyester 4 by Amidation with 2,2-Diphenylethanamine. As mentioned above, the amidation of acid end groups of the polyester **3** with 2,2-diphenylethanamine in the presence of DCC was carried out (Figure 3). We calculated the amount of acid groups in consideration of the number-average molecular weight $\bar{M}_n$ = 22 900 (determined by SEC) of the copolyester **3**. The 10-fold excess of 2,2-diphenylethanamine and 5-fold excess of DCC relating to end groups of polyester were used to ensure that all acid moieties would be reacted. The obtained reaction product was filtered and precipitated in cold methanol to remove the byproduct and the rest

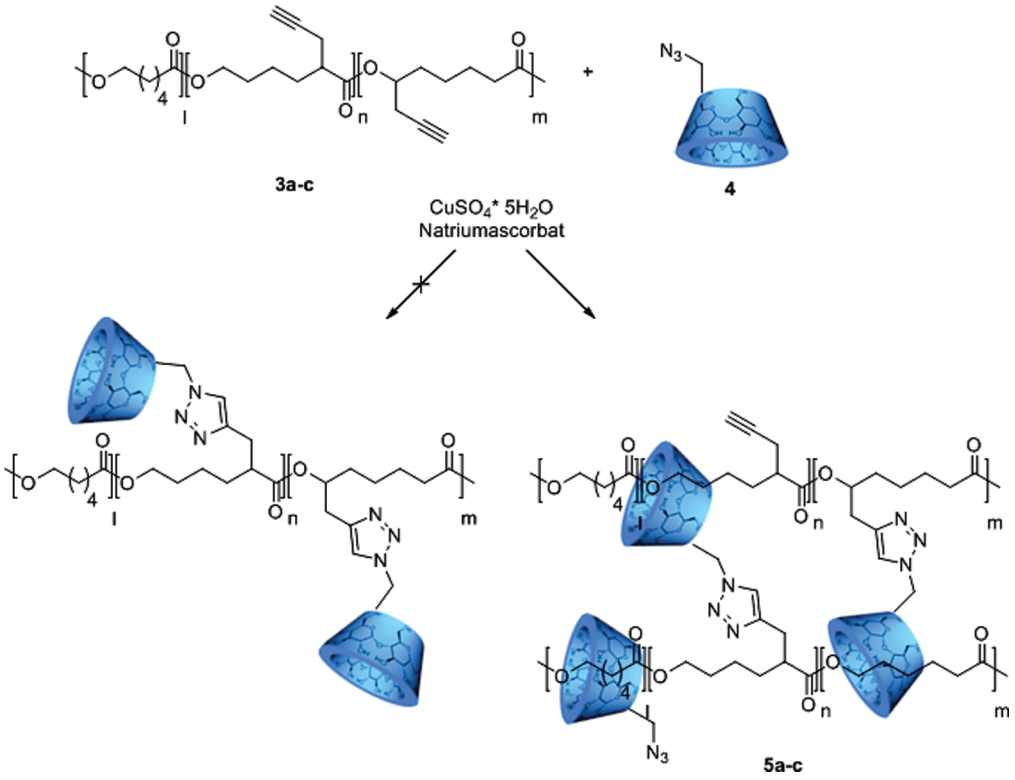

Figure 2. Reaction product of Huisgen-type 1,3-dipolar cycloaddition of propargyl-functionalized copolyester and mono(6-azido-6-desoxy)-β-cyclodextrin.[30]

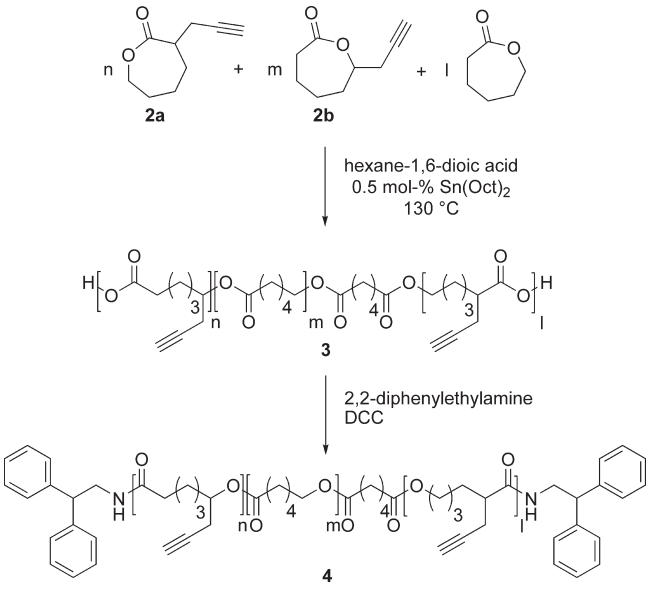

Figure 3. Synthesis of end-capped copolyester **4** by $Sn(Oct)_2$-initiated ROP of lactones **2a**/**b** and ε-caprolactone and following amidation.

of the educts. It was found that the average molar mass ($\bar{M}_n$) of copolyester was slightly increased after the amidation from 22 900 up to 23 900 according to SEC. The ^{1}H NMR spectrum of the end-capped polyester (measured in DMSO-d_6) showed the proton signals at 7.27 and 7.16 ppm ascribed to the both aromatic rings as well as the characteristic proton signals from the copolyester backbone at δ = 3.98, 2.27, 1.54, and 1.29 ppm. The amount of end groups was calculated by comparing the signals of

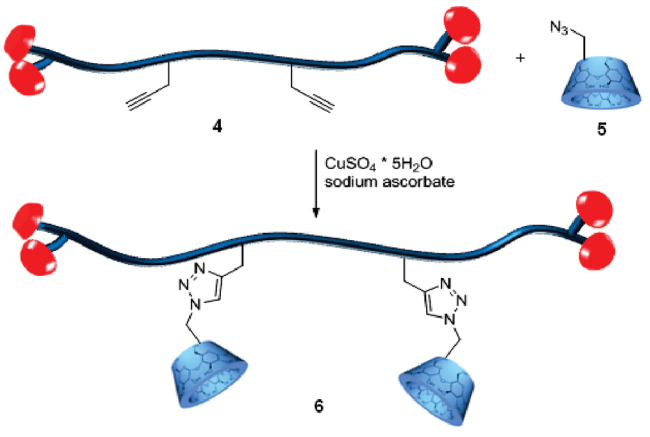

Figure 4. Schematic representation of synthesis of cyclodextrin-functionalized copolyester **6** by Huisgen-type 1,3-dipolar cycloaddition.

aromatic protons and signals at δ = 4.9 ppm ($OC(CH_2C{\equiv}CH)H$ of the polymer backbone from **2b**) and at δ = 4.03 ppm (OCH_2 of the polymer backbone from **2a** and ε-CL). The calculated ratio of end groups to **2a**/**b** and ε-CL is about 0.5 mol %:99.5 mol %. In consideration that the theoretical ratio calculated from the batch of **3** is 0.45 mol %:99.55 mol %, it could be assumed that the adipic acid was completely incorporated during the ring-opening polymerization.

Polyester-*graft*-β-CD Prepared by "Click" Chemistry. Mono-(6-azido-6-desoxy)-β-cyclodextrin (β-CD-N_3) **5** was synthesized according to a method described in the literature.[31] The "click" reaction of alkyne-functionalized copolyester **4** with mono-(6-azido-6-desoxy)-β-cyclodextrin (**5**) was performed in

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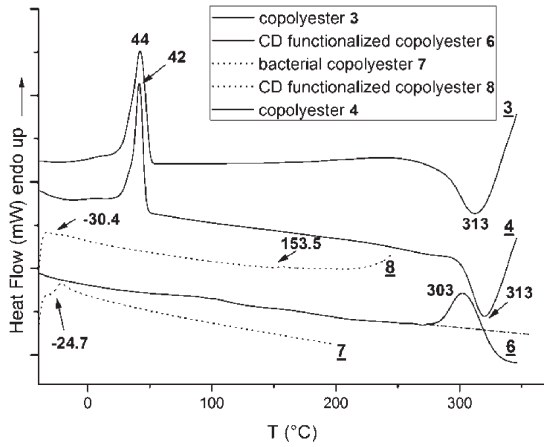

Figure 5. DSC thermogram of the precursor copolyester **3** (—), end-capped copolyester **4** (—), bacterial copolyester **7** (···), and CD-functionalized copolyester **6** (—) and **8** (···). The heating rate was 10 °C min^{-1}.

DMF as a solvent by the addition of copper(II) sulfate and sodium ascorbate as a catalyst system (Figure 4).

The isolated compound **6** was characterized by ^{1}H NMR and FT-IR spectroscopy. In contrast to the previously described product without bulky end groups (Figure 2), the product **6** was soluble in DMSO. The ^{1}H NMR spectrum confirmed the proposed structure of product **6**. The amount of CD in **6** calculated from ^{1}H NMR data by comparing the signal of the triazole proton (at 7.76 ppm) and the signals of polyester backbone protons in the region of 1.5−2.5 ppm was about 9 mol %. Considering the fact that the amount of acetylene groups of 10 mol % in copolyester **4** indicates that the "click" reaction takes place in a yield of at least 90%. Furthermore, the success of the functionalization of **4** with β-CD was also proven by the disappearance of the characteristic azide vibration at 2100 cm^{-1} for mono-(6-azido-6-desoxy)-β-cyclodextrin **5** in the FT-IR spectrum of the product **6**. Instead, a new peak was observed at 1647 cm^{-1} assigned to carbon−carbon double bond vibration, which proved the existence of the triazole ring in association with the vibration of carbon−nitrogen bond at 1027 cm^{-1}.

The thermal behavior of polymers **3**, **4**, and **6** was determined by DSC in the temperature range from −50 °C up to 350 °C (Figure 5). The DSC graph shows that the melting point of copolyester decreased from 44 °C down to 42 °C after the amidation with 2,2-diphenylethanamine. This suggests that already a small amount of the bulky end groups disrupt the formation of crystalline domains. Thus, the incorporation of β-CD by "click" reaction of polyester **4** changed significantly its thermal properties as expected. The DSC curve of **6** exhibits a melting point at 303 °C, which is closely followed by decomposition of the polymer. The reason for the high melting point could be the interchain formation of hydrogen bonds between OH groups of cyclodextrins.

Poly[(3-hydroxy-10-undecynoate)-*co*-(3-hydroxyhexanoate)] from *P. oleovorans*. *Pseudomonas oleovorans* was grown on a low nutrient medium and a 1:3 molar mixture of 10-undecynoic acid and hexanoic acid. After harvest of cells poly(3-hydroxy-10-undecynoate)-*co*-(3-hydroxyhexanoate) **7** has been obtained with an number-average weight of 166 × 10^3 g/mol via extraction with chloroform. The FT-IR spectrum of the copolyester **7** shows the characteristic C═O peak at 1731 cm^{-1}. At 2113 cm^{-1} a weak C≡C peak is noticeable. The ^{1}H NMR spectrum of **7** shows a triplet signal at δ = 0.85 ppm which originates from the methyl protons of the 3-hydroxyhexanoate part of the polymer. The signals at δ = 1.89 ppm and δ = 2.12 ppm can be assigned to the protons at the triple bond. The molar ratio of unsaturated to aliphatic building blocks has been determined by comparison of these peaks, and it was calculated to 1:0.6 (molar ratio). This means that about 65 mol % of the repeating units contain triple bonds. The difference between the initial molar ratio of 10-undecynoic acid and hexanoic acid (1:3) and the found molar ratio of the repeating units in the polymer can be explained by the bacterium preferring 10-undecynoic acid over hexanoic acid. As previously reported bacterial polyesters are copolymers even if only one carbon source has been fed.[7,9] Typically, in the β-oxidation cycle of bacterial metabolism degradation or elongation of side chains by two C units takes place.[11] Thus, PHA **7** is a complex copolymer, too. The ^{13}C NMR spectrum of the bacterial polyester shows signals of 3-hydroxy-10-undecynoate, 3-hydroxy-10-nonynoate, and 3-hydroxyhexanoate. The thermal properties of copolyester **7** indicate that it is a random copolymer. To simplify schemes and figures, all building blocks except 3-hydroxy-10-undecynoate and 3-hydroxyhexanoate have been omitted.

"Click" Reaction of Bacterial Poly(3-hydroxy-10-undecynoate-*co*-3-hydroxyhexanoate) 7 with Mono-(6-azido-6-desoxy)-β-cyclodextrin. The "click" reaction of copolyester **7** with mono-(6-azido-6-desoxy)-β-cyclodextrin (**5**) has been carried out according to Scheme 1. The obtained product **8** is soluble in DMSO, indicating linear structures without supramolecular cross-linking as observed in case of the smaller propargyl-modified poly(ε-caprolactone) (Figure 2). Thus, the longer side chains act as barrier groups comparable with the diphenyl end groups in polyester **4**. It can be admitted that, comparing the IR spectra of **5** and product **8**, the azide peak at 2100 cm^{-1} disappeared after reaction. Further, the signal of the carbon−nitrogen vibration appears at 1027 cm^{-1}. The IR spectrum indicates that the reaction was successful and proves that no remains of **5** are found in the product.

The ^{1}H NMR spectrum of the product **8** (Figure 6) shows the signal of the methyl protons of the saturated side chains. The conversion of the reaction has been determined by comparison of the peak at δ = 2.09 ppm and the peak at δ = 7.68 ppm which are assigned to the methylene group in proximity to the triple bond and the 1,2,3-triazole proton, respectively. The result is that 9.5 mol % cyclodextrin moieties are attached to the polymer.

The $[\alpha]_D^{20}$ values of mono-(6-azido-6-desoxy)-β-cyclodextrin **5** and polymers **7** and **8** were measured (Table 1). As expected, the calculated specific rotation of a pure mixture of the educts **5** and **7** differs significantly from the measured specific rotation of **8**.

The DSC curve of **8** in Figure 5 shows two glass transition temperatures at T_{g1} = −30.4 °C and at T_{g2} = 153.5 °C. Jasse and Akçatel showed that two or more glass transitions can occur in polyesteramides if H-bridge bonds are formed.[32] The reason for this behavior is an increase in stiffness of the polymer chain through H-bridge bonding. The modified polymer **8** has 9.5% of cyclodextrin attached to it. The cyclodextrin groups form interchain H-bridge bonds which are destroyed at T_{g2}.

Further, the host guest capability of β-CD-modified copolyesters **6** and **8** should be demonstrated by complexation with an appropriate guest. In this respect a preliminary experiment carried out showed that in DMSO sparingly soluble salts such

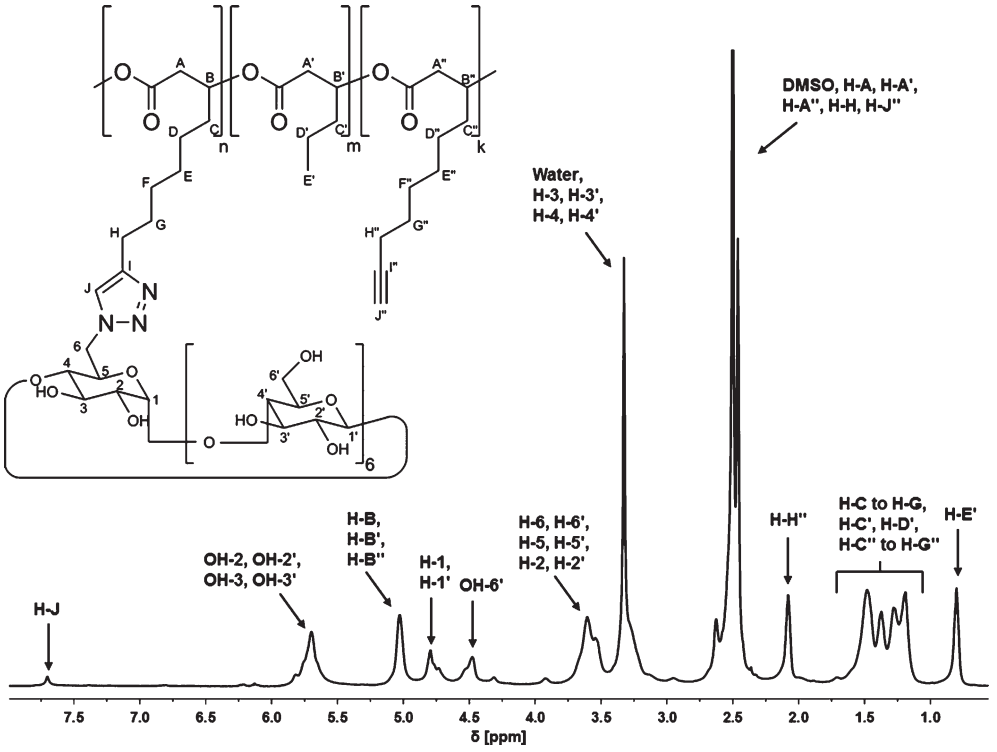

Figure 6. ^{1}H NMR (500 MHz) spectrum of β-CD-modified bacterial copolyester **8** in DMSO-d_6.

Scheme 1. Huisgen-Type "Click" Reaction of Poly[(3-hydroxy-10-undecynoate-)-*co*-(3-hydroxyhexanoate)] 7 with β-CD-N_3 (5)

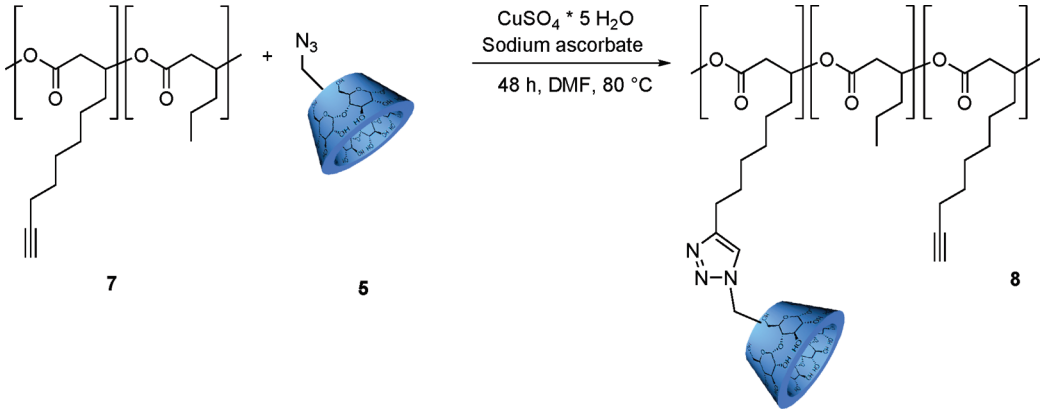

Table 1. Measurements of Optical Rotations of Substances Mono-(6-azido-6-desoxy)-β-cyclodextrin (5), Copolyester 7, and Copolyester 8

compound	specific rotation $[\alpha]_D^{20}$ [deg·L/dm·g][a]
5	132.4
7	2.8
8	47.3

[a] The error of the measurement is ±0.2.

as potassium adamantyl carboxylate were dissolved by the addition of solid β-CD. Thus, the size distributions of the polymers **6** and **8** were investigated by DLS in presence of adamantyl carboxylate (Figure 7). It was observed that the bacterial copolyester **8** possess a smaller hydrodynamic diameter than the synthetic copolyester **6**. In comparison to the copolyester **6**, the polyester **8** possesses long hydrophobic side chains that lead to a greater entanglement of the polyester molecules in a polar solvent such as DMSO.

By repeating the DLS measurement of polymers **6** and **8** in the presence of adamantyl carboxylate as guest, the hydrodynamic diameter increased substantially in both cases. This increase can be attributed to the electrostatic repulsion of negatively charged carboxylate groups leading to chain extension. As expected, the hydrodynamic diameter of bacterial copolyester **8** increased stronger (from 8.7 nm up to 227.8 nm) due to the greater molecular weight ($\bar{M}_n$ = 170 000) compared to copolyester **6** ($\bar{M}_n$ = 23 900), which hydrodynamic diameter increased from 12.9 nm up to 83.4 nm.

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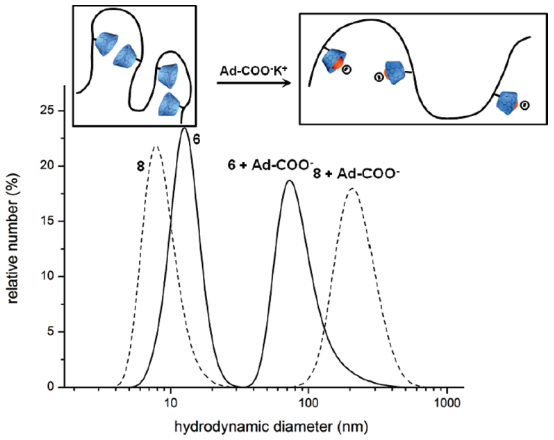

Figure 7. Size distributions by number of CD-modified polyester **6** (—) and **8** (---) in DMSO solution before and after the complexation with Ad-COOK (polymer concentration = 2.5 $g \cdot L^{-1}$, T = 25 °C).

The calculated theoretical length of the completely stretched copolyesters **6** and **8** is about 176 and 540 nm, respectively. Certainly, neither the synthetic nor the bacterial polyester exists as fully stretched chains in the solution. Nevertheless, the relative increase of the hydrodynamic diameters after the complexation is nearly the same for both polymers. This means that both hydrodynamic diameters of the copolyesters **6** and **8** increase by ~40% of their calculated length.

CONCLUSION

In summary, we described a new synthesis of β-CD-functionalized synthetic and bacterial polyester with biodegradable backbone. The synthesis of synthetic polyester relies on the ring-opening polymerization of unknown to literature alkyne-functionalized lactone followed by the Huisgen-type cycloaddition with mono-(6-azido-6-desoxy)-β-cyclodextrin. The end-capping of the copolyester chain with bulky groups inhibits the formation of pseudo-polyrotaxane and the consequent cross-linking. Furthermore, the bacterial copolyester bearing alkyne groups in the side chain was produced and also modified with β-cyclodextrin azide by the "click" reaction. NMR and FT-IR measurements confirmed the success of these reactions. The ability of covalently attached cyclodextrins to include guest molecules was proven by DLS measurements in the presence of admantyl carboxylate. Because of the ability of CD to complex hydrophobic substances, these polymers have a high potential for applications in supramolecular chemistry as a carrier for e.g. drugs or catalysts.

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ASSOCIATED CONTENT

Supporting Information. Figures 1−5. This material is available free of charge via the Internet at http://pubs.acs.org.

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