Lysine-Based Functional Blocked Isocyanates for the Preparation of Polyurethanes Provided with Pendant Side Groups

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ABSTRACT: This article describes a methodology to prepare polyurethanes (PUs), decorated with pendant (bio)functional side groups, by polymerizing (bio)functionalized blocked diisocyanates with polyols. Caprolactam blocked lysine diisocyanate methyl ester (BLDI-OMe) was prepared in high yields, by reacting the methyl ester of lysine with carbonyl biscaprolactam. In the absence of a catalyst, the polymerization of BLDI-OMe with polycaprolactone and polytetrahydrofuran resulted in strictly linear PUs due to the high selective reactivity of the blocked isocyanates (BIs). Although the ester appeared to be less reactive, we found hydrolyzing conditions for the ester, without affecting the BIs. The free acid groups were converted into a *N*-hydroxysuccinimide (NHS) activated ester, which was a versatile intermediate for further functionalization. After having demonstrated that model amines were able to substitute NHS without effecting the BIs groups, the same chemistry was used to couple biotin, giving a biotin functional caprolactam blocked lysine diisocyanate. The polymerization with polyols afforded the corresponding biotin-functional PUs. © 2015 Wiley Periodicals, Inc. J. Polym. Sci., Part A: Polym. Chem. **2015**, *53*, 2036–2049

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INTRODUCTION Polyurethanes (PUs) are extensively used for foams, coatings, paintings, cloths, insulators, biomedical implant and devices, due to the high elasticity, excellent biocompatibility, foaming ability and the wide range physical and chemical properties.[1–3] However, PUs decorated with (bio) active groups were still lacking. A vast amount of information on functional polyesters and polycarbonates has been reported,[4–7] but publications on functional PUs are still rare.

PUs are commonly prepared from diisocyanates and polyols. Isocyanates are toxic and unstable due to their ability to react with moisture, proteins,[8] they deactivate enzymes,[9–11] destroy cells,[12] and damage DNA.[13] Aliphatic diisocyanates are volatile and can easily invade into the body by inhalation.[14] Aromatic diisocyanates are less volatile, but their degradation products (aromatic diamines) are suspected to cause health issues as well. Diisocyanates are frequently reported to cause chemically induced occupational asthma[15] and deterioration of lung function after long-term repeated exposure.[16] Another limitation of isocyanates is that they do not allow the introduction of (pendant) functional groups, because of their high reactivity.[17]

An elegant solution to overcome all these problems is the use of blocked isocyanates (BIs).[18] In BIs, the reactivity of the isocyanates is temporarily reduced. Nonetheless, on heating with polydiols, they react in exactly the same way as unprotected diisocyanates, affording the same PUs, but only at a lower reaction rate. Due to the reduced reactivity and the absence of free isocyanate moieties these compounds are considered to be safe. Moreover, the reduced reactivity may allow chemical modifications and introduction of (bio)active groups. BIs are nowadays mainly used in industrial coating applications. As many as 90% of the references on BIs, found by Scifinder, are patents, which shows their industrial importance.[19,20] The most widely commercially used blocking agents are phenols, oximes, ε-caprolactam, and dibutyl malonate.[19,21]

The conventional route to prepare BIs is to react isocyanates directly with blocking groups, a method that obviously depends on the availability of the desired isocyanates. More

importantly, due to the high reactivity, functional isocyanates comprising active hydrogen moieties (—NH—, —OH, or —COOH) do not exist. As BIs are less reactive than isocyanates, they could possibly allow the introduction of functional groups and chemical modifications thereof. However, the common route to prepare BIs is starting from diisocyanates, which will obviously run into the same kind of limitations as encountered with isocyanates.

The methyl ester of lysine diisocyanate (LDI) is an excellent diisocyanate for making biocompatible PUs.[22–32] The degradation products of LDI-based PUs (lysine) are nontoxic, making these polymers attractive for biomedical applications, especially in soft tissue engineering. Lysine is a natural amino acid provided with two amino groups and a pendant acid group that could provide great potential for further functionalization. So far, functionalization of lysine diisocyanate has, to the best of our knowledge, not been done, because of the presence of the highly reactive isocyanate groups. To overcome these limitations, we studied an alternative methodology that circumvents the use of free isocyanates. Loontjens et al. had shown that primary amines react quantitatively with carbonyl biscaprolactam (CBC), forming caprolactam BIs.[33–36] Xiang and Asri et al. have reported the synthesis of hyperbranched polyurea starting from AB_2 monomers prepared from a triamine and CBC and got biocompatible amphiphilic hyperbranched polyurea micelles and antibacterial-coatings.[37–39] Here, we report the synthesis and characterization of a novel diisocyanate, caprolactam blocked lysine diisocyanates methyl ester (BLDI-OMe), and the corresponding linear and crosslinked PUs. By hydrolyzing the methyl ester selectively, while preserving the BIs, and by utilizing the acid moiety for the introductions of useful groups, various functionalized caprolactam blocked lysine diisocyanates have been prepared. The preparation of functionalized PUs, with *N*-hydroxysuccinimide (NHS) and biotin as pendant side groups, demonstrated the feasibility to incorporate pendant functional groups into the PUs by using functionalized BIs.

EXPERIMENTAL SECTION

Materials

CBC, ALLINCO®, was kindly obtained from DSM Innovation Center, (> 99 % pure according to HPLC) and used without purification. L-Lysine monohydrochloride, thionylchloride, calciumchloride dihydrate, hexyl amine, hexamethylenediamine, biotin, dibutyltin dilaurate (DBTDL, 95 %), 1-octanol, anhydrous magnesium sulfate, dicyclohexyl-carbodiimide (DCC), NHS, chloroform-d ($CDCl_3$-D, 99.8 atom % D) and dimethyl sulfoxide-d_6 (DMSO-d_6, 99.5 atom %D), poly(ethyleneglycol) ($M_n = 600$ Da) (PEG600), poly(ε-caprolactone) ($M_n = 530$ Da) (PCL530), polytetrahydrofuran ($M_n = 650$ Da) (PTHF650) were purchased from Sigma-Aldrich. Ethyl acetate, potassium hydroxide and anhydrous *N,N*-dimethylformamide (DMF), were purchased from Acros Organics. Methanol, acetone, chloroform, triethylamine (TEA), *n*-hexane, fuming hydrochloric acid 37%, tetrahydrofuran (THF), dichloromethane (DCM), were purchased from Lab-Scan Analytical Science. All compounds and solvents were used as obtained, unless stated otherwise.

Analysis

Column chromatography was performed on silica gel (Silica-P flash silica gel from Silicycle, size 40–63 μm). TLC was performed on silica gel 60/Kieselguhr F254. Components were visualized by UV, after staining, with a solution of a mixture of $KMnO_4$ (5 g) and K_2CO_3 (20 g) in H_2O (500 mL). High performance liquid chromatography (HPLC) was carried out on a Shimadzu LC-10ADVP HPLC equipped with a Shimadzu SPD-M10AVP diode array detector (nonchiral column, heptane/i-PrOH 95:5, gradient 30:70, 30 min, 0.5 mL/min, 254 nm). Element analysis (EA) was performed on a HEKAtech Gmbh Euro-EA CHNanalyzer. Mass spectra were recorded on a LTQ Orbitrap XL (ESI+). Proton and carbon nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) were recorded on a Varian AMX400 using $CDCl_3$ or DMSO-d_6 as solvent. Chemical shifts are reported in ppm with the solvent resonance as the internal standard ($CHCl_3$: δ 7.26 for ^{1}H, δ 77.0 for ^{13}C; DMSO-d_6: δ 3.3 and 2.5 for ^{1}H, δ 39.5 and 40.5 for ^{13}C). Data are reported as follows: chemical shifts, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet). Size exclusion chromatography (SEC) measurements were performed in dimethylformamide with 0.01M LiBr on a Viscotek GPC max equipped with model 302 TDA detectors, using two columns (Pl-gel 5μ 30 cm mixed-C from Polymer Laboratories). The data analysis was done using conventional calibration with polystyrene standards accompanied by in-house software. ATR-FTIR was done using broker IFS88 spectrometer equipped with a Golden Gate (Graseby specac) single reflection ATR accessory. Spectra resolution was 4 cm^{-1} and 32 scans were taken per spectrum. Differential Scanning Calorimetry (DSC) measurements were performed on a Perkin Elmer DSC 7 instrument. The samples with masses varying between 7 and 10 mg were heated from −60°C to +90°C with a rate of 10°C/min.

Synthesis of Caprolactam Blocked Lysine Diisocyanate Methylester

Synthesis of L-Lysine Methylester Dihydrochloride (1)

Lysine monohydrochloride (78.59 g, 0.43 mol) were suspended in 600 mL methanol, while being cooled at 0°C by using an ice bath. Thionyl chloride (45 mL, 0.62 mol) were added drop wise whilst maintaining the temperature below 20°C. The mixture was then refluxed for 18 h at 63°C under the nitrogen atmosphere. After the clear solution was cooled to room temperature within 4 h, the L-lysine methylester dihydrochloride crystals precipitated and were isolated by filtration and washed with acetone. The filtrate was concentrated to half of its original volume by evaporation of the solvent and cooled in ice to form an additional quantity of crystals. This fraction was filtered as well and washed with acetone and combined with the first fraction. The combined products were dried at 50°C for 48 h in a vacuum oven, yielding a white powder product. The purity was over 99%

based on HPLC, the yield 96.8%. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 1.38 to 1.61 (4H, m, $CH_2CH_2CH_2CH_2$), 1.84 (2H, m, CH_2CH), 2.74 (2H, m, NH_3ClCH_2), 3.75 (3H, s, OCH_3), 3.97(1H, t, CH), 8.29 (3H, s, CH_2NH_3Cl), 8.82 (3H, s, $CHNH_3Cl$)).

Synthesis of Caprolactam Blocked Lysine Diisocyanate Methylester (BLDI-OMe(2))

CBC (79.46 g, 0.32 mol) were dissolved in 150 mL chloroform at ambient temperature by stirring. To this solution L-lysine methylester dihydrochloride ((**1**), 36.63g, 0.16mol) were added. The resultant mixture was heated to 40°C, after which 45 mL (0.32mol) TEA were added. The resultant mixture was refluxed for 60 h in the nitrogen atmosphere. Next, the mixture was cooled to ambient temperature within 1 h, during which TEA hydrochloride crystals were formed. The crystals were removed by filtration. The filtrate was concentrated by evaporation to obtain viscous turbid orange oil liquid. This liquid was dissolved in 300 mL of a mixture of ethyl acetate and hexane (2:1, v:v). The resultant solution was extracted with 400 mL of a 0.5 M HCl aqueous solution, which contained 5% (w/v) $CaCl_2$ and 5% (w/v) NaCl. Thereafter with 400 mL 5% (w/v) $CaCl_2$ (aq), and subsequently with 400 mL 10%(w/v) Na_2CO_3 (aq). The organic solution was dried over anhydrous magnesium sulfate. After removing $MgSO_4$ by filtration, the solvent was removed by evaporation to yield a light yellow viscous oily substance in a yield of 97.5%.

Traces of CBC were removed by column chromatography with a mixture of hexane and THF (3:1, v:v). The compound was then dried in vacuum oven at 100°C for 4 days to remove THF, yielding a colorless viscous oily product. The purity was over 99.5% according to HPLC measurements, and the yield was over 80%. ^{1}H NMR(400 MHz, DMSO-d_6), δ: 1.37 to 1.84 (18H, m, $CH_2CH_2CH_2$ ring + $CHCH_2CH_2CH_2$), 2.66 (4H, m, CH_2CO), 3.22 (2H, m, $NHCH_2$), 3.69 (3H, s, OCH_3), 3.92 (4H, m, NCH_2), 4.42 (1H, q, CH), 9.21 (1H, t, CH_2NH), 9.66 (1H, d, CHNH). ^{13}C NMR (400 MHz, DMSO-d_6, δ): 179.94 ($NCOCH_2$, ring), 172.77 ($CHCOOCH_3$), 154.56 (NHCON), 52.53($COOCH_3$)). MS (ESI, *m/z*): $[M + H]^+$ calcd for $C_{21}H_{34}N_4O_6$, 439.25; found, 439.25. Anal. calcd for $C_{21}H_{34}N_4O_6$: C 57.25, H 7.81, N 12.78; found: C 57.49, H 7.89, N 12.60.

Model Reaction of BLDI-OMe with 1-Octanol (3)

A 100 mL glass flask was fitted with a reflux condenser, a nitrogen inlet and an outlet to a vacuum pump. After the flask was three times evacuated and refilled with nitrogen, to remove all oxygen, a solution of BLDI-OMe (2.0 g, 4.6 mmol) and 1-octanol (2.4 g, 18.4 mmol) in dry DMF (10 mL) was added and heated up to 125°C with or without 50 μL DBTDL (dibutyl tin dilaurate), while stirred for 72 h. The solvent and excess 1-octanol were removed by evaporation and then the residue was dissolved in 20 mL mixture of ethyl acetate and hexane (2:1, v:v). The resultant solution was washed with 100 mL 5% (m/v) $CaCl_2$ (aq), thereafter with 100 mL aqueous solution of Na_2CO_3 (10% w/v) and subsequently with 100 mL distilled water. The organic solution was dried over anhydrous magnesium sulfate. After removal of $MgSO_4$ by filtration, the solvent was removed by evaporation to yield a light yellow wax like product. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 0.81 (6H, m, CH_2CH_3), 1.23 to 1.62 (30H, m, alkane chain), 2.94 (2H, m, $NHCH_2$), 3.60 (3H, s, OCH_3), 3.88 to 3.93 (5H, m, $CH+CH_2CO$), 6.96 (1H, t, CH_2NH), 7.42 (1H, d, CHNH).

Polymerization of BLDI-OMe and PCL530

Optimizing the Ratios between PCL530 and BLDI-OMe

A 100-mL glass flask was fitted with a nitrogen inlet and an outlet to a vacuum pump. After the flask was three times evacuated and refilled with nitrogen, to remove all oxygen, a solution of BLDI-OMe (2.0 g, 4.6 mmol) and various amounts of PCL530 in the anhydrous DMF (10 mL) was added and heated to 125°C while stirring. After 72 h the polymer solutions were poured into the diethyl ether. The precipitate was separated by centrifugation and, after the supernatant was removed, the polymers were dried in a vacuum oven at 50°C for 48 h.

Polymerization Kinetics

A 100-mL glass flask was fitted with a nitrogen inlet and an outlet to a vacuum pump. After the flask was three times evacuated and refilled with nitrogen, to remove all oxygen, a solution of BLDI-OMe (2.0 g, 4.6 mmol) and PCL530 (2.38 g, 4.5 mmol) in the anhydrous DMF (10 mL) was added and heated up to 125°C while stirring, for several days. During the polymerization, after various time intervals, samples were taken and poured into the diethyl ether. The precipitate was separated by centrifugation and after the supernatant was removed the polymers were dried in a vacuum oven at 50°C for 48 h. The molecular weights were determined by SEC.

Bulk Polymerization of BLDI-OMe and PCL530 at Various Temperatures

A 100-mL glass flask was fitted with a nitrogen inlet and an outlet to a vacuum pump. After the flask was three times evacuated and refilled with nitrogen, to remove all oxygen, dry BLDI-OMe and PCL530 were added in a molar ratio of 1.025. The mixture was subsequently heated up to 125°C, 145, 160, 180°C, respectively, while stirring under vacuum, for various time till 24 h. Samples were taken and analyzed by SEC measurement directly, without purification by precipitation.

Bulk Polymerization of BLDI-OMe and PEG600

A 100-mL glass flask was fitted with a nitrogen inlet and an outlet to a vacuum pump. After the flask was three times evacuated and refilled with nitrogen, to remove all oxygen, dry BLDI-OMe and PEG600 were added in a 1:1 molar ratio. The mixture was heated up to 125°C while stirring under vacuum for 72 h. Samples were taken and analyzed by SEC measurement directly, without purification by precipitation.

Bulk Polymerization of BLDI-OMe and PTHF650

A 100-mL glass flask was fitted with a nitrogen inlet and an outlet to a vacuum pump. After the flask was three times

evacuated and refilled with nitrogen, to remove all oxygen, dry BLDI-OMe and PTHF650 were added in a 1:1 molar ratio. The mixture was heated up to 125°C while stirring under vacuum. After 48 h the temperature was raised further to 150°C for another 48 h. Samples were taken and analyzed by SEC measurement directly, without purification by precipitation.

Hydrolysis of BLDI-OMe to Caprolactam Blocked L-Lysine Diisocyanate Acid (BLDI-OH(4))

BLDI-OMe (10 g, 22.8 mmol) were suspended in 30 mL THF and 70 mL H_2O. The pH of this solution was adjust to 13 and maintained at this value for 5 h at room temperature by the continuous addition of an aqueous KOH solution (2mol/L). The reaction mixture was then acidified to a pH 4 with 4 M HCl, forming milk like suspension. The solution of the acid (BLDI-OH) was extracted by diethyl ether until there was no suspension formed when a new charge of acid was added. The resultant diethyl ether solution was dried over anhydrous $MgSO_4$. After removal of $MgSO_4$, the solvent was evaporated and the residue was dissolved in a mixture of chloroform and methanol (60:1). After flash chromatography with chloroform and methanol with ratio of 60:1, the elute was evaporated and the solid was died at 100°C for 7 days in the vacuum oven, giving 8 g (80%) free acid BLDI-OH. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.34 to 1.87 (18H, m, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$ring + $CHC\underline{H}_2C\underline{H}_2C\underline{H}_2$), 2.62 (4H, m, $C\underline{H}_2CO$), 3.18 (2H, m, $NHC\underline{H}_2$), 3.88 (4H, m, $NC\underline{H}_2$), 4.39 (1H, q, $C\underline{H}$), 9.21 (1H, t, $CH_2N\underline{H}$), 9.62 (1H, d, $CHN\underline{H}$), 10.25 (1H, s, $CO\underline{O}H$). ^{13}C-NMR (400 MHz, $CDCl_3$, δ): 179.9 ($N\underline{C}OCH_2$, ring), 174.5 ($CH\underline{C}OOH$), 154.5 ($NH\underline{C}ON$). MS (ESI, *m/z*): $[M + H]^+$ calcd for $C_{20}H_{32}N_4O_6$, 425.24; found, 425.24. Anal. calcd for $C_{20}H_{32}N_4O_6$: C 56.59, H 7.60, N 13.20; found: C 56.02, H 7.69, N 13.10.

Synthesis of BLDI-NHS(5) (Active Ester)

BLDI-OH (10 g, 23.6 mmol) were dissolved in 100 methylene chloride. To this solution dry and finely powdered NHS (2.74 g, 23.8 mmol, molar ratio 1.01 times of BLDI-OH) were added. The flask was cooled in an ice-water bath, after which dicyclohexyl carbodiimide (DCC, 4.88 g, 23.7 mmol) were added in a molar ratio of 1.005 times of BLDI-OH. The reaction mixture was stirred vigorously at 0°C for 1 h at room temperature, for 24 h. After the removal of precipitated dicyclohexyl urea by filtration, the crude product was extracted with distilled water. The resulting solution was dried over anhydrous $MgSO_4$. After removal of $MgSO_4$ by filtration, the product was further purified by flash chromatography with chloroform and methanol with ratio of 60:1. The pure product was collected to give 8.5 g (85%) BLDI-NHS. ^{1}H NMR (400 MHz, $CDCl_3$, δ: 1.42 to 2.04 (18H, m, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$ring + $CHC\underline{H}_2C\underline{H}_2C\underline{H}_2$), 2.67 to 2.82 (8H, m, $C\underline{H}_2CO$), 3.30 (2H, m, $NHC\underline{H}_2$), 3.98 (4H, m, $NC\underline{H}_2$), 4.80 (1H, q, $C\underline{H}$), 9.26 (1H, t, $CH_2N\underline{H}$), 9.81 (1H, d, $CHN\underline{H}$). MS (ESI, *m/z*): $[M + H]^+$ calcd for $C_{24}H_{35}N_5O_8$, 522.25; found, 522.25. Anal. calcd for $C_{24}H_{35}N_5O_8$: C 55.27, H 6.76, N 13.43; found: C 55.54, H 6.82, N 13.21.

Synthesis of BLDI-HA (6)

BLDI-NHS (10 g, 19.2 mmol) were dissolved in 100-mL methylene chloride. A large excess of hexylamine (6.4 mL, 48 mmol) was added. The reaction mixture was stirred at room temperature overnight. After removal of precipitated NHS salt by filtration, the solution was washed with 300 mL 5% (w/v) $NaHCO_3$ aqueous solution and with 300 mL brine (saturated NaCl aqueous solution). The resultant solution was dried over anhydrous $MgSO_4$ After removal of $MgSO_4$ by filtration and solvent by evaporation, the crude product was collected. Then after flash chromatography with chloroform and methanol with ratio of 60:1, the pure product was collected to give 9 g (90%) BLDI-HA. ^{1}H NMR (400 MHz, $CDCl_3$, δ: 0.83 (3H, m, $C\underline{H}_3$), 1.06 to 1.92 (26H, m, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$ring + chains), 2.68 (4H, t, $C\underline{H}_2CO$), 3.22 (4H, m, $NHC\underline{H}_2$), 3.94 (4H, m, $NC\underline{H}_2$), 4.23 (1H, q, $C\underline{H}$), 6.24 (1H, t, $N\underline{H}C_5H_{10}$), 9.24 (1H, t, $CH_2N\underline{H}$), 9.52 (1H, d, $CHN\underline{H}$). MS (ESI, *m/z*): $[M + H]^+$ calcd for $C_{26}H_{45}N_5O_5$, 508.35; found, 508.35. Anal. calcd for $C_{26}H_{45}N_5O_5$: C 61.51, H 8.93, N 13.80; found: C 62.06, H 9.16, N 13.54.

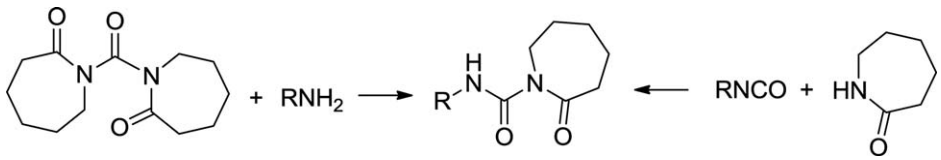
Synthesis of BLDI-NH-C6-Biotin

BLDI-NH-C_6-NH_2 (7)

BLDI-NHS (5 g, 9.6 mmol) were dissolved in 100 methylene chloride in a glass flask of 250 mL in a nitrogen atmosphere. A 10 times molar excess of hexamethylene diamine (11.2 g, 96 mmol) was added. The reaction mixture was stirred at room temperature overnight. After removal of precipitated NHS salt by filtration, the crude product was washed with 500 mL 5% (w/v) $NaHCO_3$ aqueous solution and subsequently with 300 mL brine (saturated NaCl aqueous solution). The resultant solution was dried over anhydrous $MgSO_4$. After filtering off $MgSO_4$ the solvent was evaporated in a rotavapor and further died at 45°C for 48 h in the vacuum oven. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.95-1.25 (28H, m, caprolactam ring $6C\underline{H}_2$ + alkyl chain $8C\underline{H}_2$), 2.68 (4H, m, ring $C\underline{H}_2CO$), 3.24 (4H, m, $NHC\underline{H}_2$), 3.95 (4H, m, caprolactam ring $NC\underline{H}_2$), 4.21 (1H, q, $C\underline{H}$), 6.45 (1H, t, $N\underline{H}C_6H_{12}$), 9.22 (1H, t, $CH_2N\underline{H}$), δ = 9.52 (1H, d, $CHN\underline{H}$).

Synthesis of BLDI-NH-C_6-Biotin (8)

N-hydroxysuccimide (1.32 g, 11.3 mmol) and dicyclohexylcarbodiimide (2.35 g, 11.4 mmol), were added to biotin (2.35 g, 9.6 mmol), dissolved in anhydrous 80 mL dimethylformammide (DMF), in a glass flask under nitrogen atmosphere. The reaction mixture was stirred for 24h at room temperature.[40] After dicyclohexyl urea was filtered off, BLDI-C_6-NH_2 in DMF (5.15 g, 9.5 mmol in 20 mL DMF) and TEA were added. After 4 h, the NHS salt was filtered off. The solution was concentrated by rotary evaporator and precipitated in diethyl ether. After flash chromatography with chloroform and methanol with ratio of 4:1, the elute solvent was evaporated and the solid was dried at 50°C for 2 days in the vacuum oven, giving 3 g (60%) biotinylated blocked lysine diisocyanate BLDI-NH-C_6-biotin.^{1}H-NMR (400 MHz, DMSO-d_6, δ): = 1.87–1.21 (32H, m, caprolactam ring $6C\underline{H}_2$ + alkyl chain $10C\underline{H}_2$), 2.18 and 2.02 (2H, $NHCOC\underline{H}_2$), 2.65 (4H, m, $C\underline{H}_2CON$), 2.78 and 2.56 (2H, m, biotin ring $SC\underline{H}_2$), 3.12-2.95



SCHEME 1 Formation of caprolactam blocked isocyanate starting from primary amines and CBC (left) or from isocyanates and caprolactam (right).

(7H, m, 3NHC$\underline{H}_2$ + SC$\underline{H}$), 3.86 (4H, m, NC$\underline{H}_2$), 4.12 (1H, q, biotin ring: CONHC$\underline{H}$CH), 4.28 (1H, q, OCC$\underline{H}$NHCO) 4.30 (1H, t, biotin ring: NHC$\underline{H}$CH$_2$), 6.34 (1H, d, biotin ring: CON$\underline{H}$CHCH$_2$), 6.40 (1H, d, biotin ring: CON$\underline{H}$CHCH), 7.74 (1H, t, CH$_2$N$\underline{H}$COCH$_2$), 8.06 (1H, t, CH$_2$N$\underline{H}$COCH), 9.10 (1H, t, CH$_2$N$\underline{H}$CO), 9.46 (1H, d, OCCHN$\underline{H}$CO). ESI-MS m/z: $(M+1)^+$ 751.06 $(M+Na)^+$ 773.04

Synthesis of NHS Functional PUs (PU-NHS)

A 100-mL glass flask was fitted with a nitrogen inlet and an outlet to a vacuum pump. After the flask was three times evacuated and refilled with nitrogen, to remove all oxygen, dry NHS activated ester of caprolactam blocked lysine diisocyanate (BLDI-NHS) was added in a molar ratio of 1.025 with respect to PCL530. BLDI-NHS was synthesized according to the method as described above. The mixture was subsequently heated up to 145°C, while stirring under vacuum for 4 h, and taking meanwhile samples.

Synthesis of Biotinylated PUs

A 10-mL glass flask was fitted with a nitrogen inlet and an outlet to a vacuum pump. After the flask was three times evacuated and refilled with nitrogen, to remove all oxygen, dry biotinylated monomers BLDI-NH-C_6-biotin was added in a roughly equal molar with respect to PCL530, respectively. The mixture was heated up to 160°C while stirring under vacuum for 24 h.

RESULTS AND DISCUSSION

Synthesis of Blocked Lysine Diisocyanates Methylester and Corresponding PUs

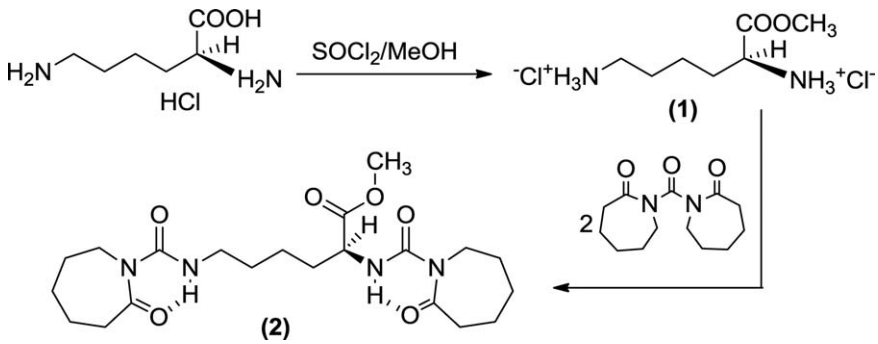
BIs are commonly prepared starting from isocyanates and blocking groups (Scheme 1). We found earlier an alternative method to make caprolactam BIs staring from primary amines and CBC.[35] The latter method makes the preparation of BIs independent of the availability of isocyanates.

The basic building blocks for all PUs are polyols and (blocked) di and triisocyanates, of which the latter are obtained from the corresponding di- and triamines and phosgene. In many biomedical applications, particularly in soft tissue scaffolds, degradable PUs are mandatory. It is understandable that, in that case, the degradation products should not be toxic. The precursor amines of the isocyanates are always one of the degradation products of PUs. Lysine is therefore a very promising diamine for biomedical applications because it is a natural diamine (amino acid) and comprises, besides two primary amino groups, a functionalizable acid group. The primary amino groups were, in principle, suitable for making BIs according to our novel method. The acid side group could be used to couple a variety of (bioactive) molecules.

To avoid possible side reactions with the acid group during the preparation of the blocked isocyanate moieties, according to our method, the acid groups were temporarily protected by converting them into the methyl esters (Scheme 2).

To prevent aminolysis of the ester moiety, the amino groups were protected by hydrochloric acid. In the commercial available lysine one of the amino groups was already blocked by HCl. It was furthermore anticipated that during the preparation of the acid chloride with thionyl chloride the liberated HCl would scavenge the second amino group of lysine. After protonation, amino groups become less reactive, which could prevent possible reactions with the methyl ester. The acid chloride reacted subsequently with the solvent, methanol, to form methyl ester, while liberating an additional amount of HCl. Indeed, due to the presence of HCl aminolysis of the methylester did not occur. After the clear solution was cooled to room temperature lysine methylester dihydrochloride crystals precipitated and were isolated by filtration. The white crystalline powder was collected in a yield of 96.8%. The purity of the crystals was over 99%, according to HPLC measurements.

Next, reactions of the lysine methyl ester with CBC were performed to convert the amino groups into the corresponding BIs. In a typical experiment a suspension of lysine methylester dihydrochloride in dry chloroform was added to a solution of CBC in chloroform (Scheme 2). Triethyl amine (TEA) was added to deprotonate the amino groups of lysine. After the solution was heated to the reflux temperature (61°C), the free primary amine groups of lysine started to substitute one of the caprolactam groups of CBC. The mixture was refluxed for 60 h in a nitrogen atmosphere. Longer reaction times were needed then found in previous studies because one of the amines, the α-amino group, was less reactive due to the electron withdrawing ability of the ester. It was found that after a reaction time of 60 h at 61°C and with a small excess CBC the conversion of both amino groups went to completion. Remarkably, no reactions took place between the amino groups and the methylester. Although the caprolactam ring of the formed blocked isocyanate, was substitutable as well by the amines, no such a reaction took place under the applied conditions. It was argued that the caprolactam BI was stabilized by intramolecular hydrogen bonding of the –



SCHEME 2 Synthesis of caprolactam blocked lysine methyl ester diisocyanate.

NH of the blocked isocyanate and the carbonyl group of caprolactam, as evidenced by ^{1}H NMR spectroscopy (NH peak at 9.21 and 9.66 ppm).

Besides the desired blocked isocyanate, caprolactam, and TEA hydrochloride were formed, of which the latter precipitated. Most of the TEA·HCl salt was removed by filtration. Caprolactam and residual TEA·HCl salt were removed by an aqueous extraction. The addition of $CaCl_2$, NaCl and HCl to the aqueous phase increased the solubility of caprolactam and decrease the solubility of blocked diisocyanate in water at same time. This method appeared to be a very suitable route to make BIs of the lysine methylester in high yields. The successful synthesis of the caprolactam blocked lysine diisocyanate methyl ester (BLDI-OMe) **(2)** was evidenced by ^{1}H NMR (Fig. 1). All signals were in accordance with the expected product. The signals of CBC (e.g., at 3.86ppm), the free amino groups and of caprolactam (e.g., at 2.4 ppm) were completely absent.

For sake of completeness, the ^{13}C NMR spectrum of the purified monomer was recorded as well. Although no quantitative analysis can be done, all the signals matched with the desired product. The signals of CBC (e.g., at 176 and 157 ppm) and of the amines had completely disappeared, whereas the signal of the released caprolactam (e.g., at 178 ppm) was almost invisible. Besides NMR techniques, the product was also analyzed by ESI-MS and elemental analysis, as well as by HPLC. Based on all these techniques it was concluded that the purity of product was over 99%. Importantly, the stability of the product appeared to be very high. Even, after storage for 3 years no changed was noticed by ^{1}H NMR. Compound **(2)** comprised two BIs and one methyl ester group. Our aim was to use the ester group to anchor bioactive moieties, and to use the BIs for making the corresponding bioactive PUs. Hence, it was essential for this work that both groups, the ester and the BIs, could be addressed independently.

Synthesis of PUs Based on the BLDI-OMe

Model Reaction of the Blocked Diisocyanate with 1-Octanol

The polyaddition reaction of polyols onto diisocyanates is the common route to make PUs. Yet, it is also possible to use

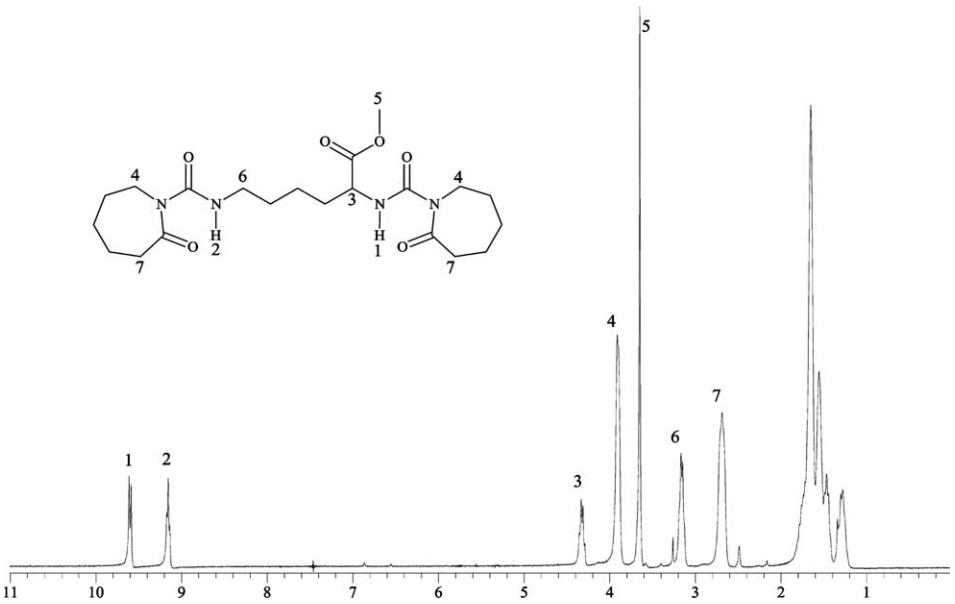
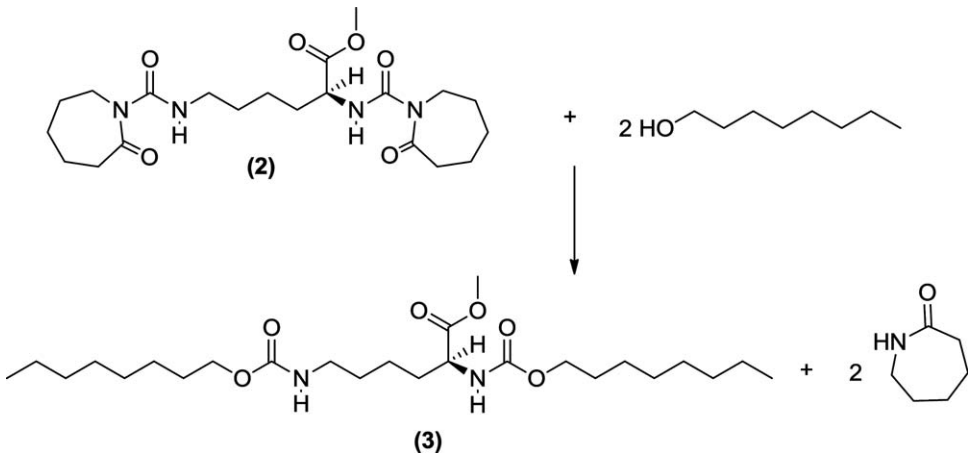


FIGURE 1 ^{1}H NMR spectrum of caprolactam blocked lysine diisocyanate methyl ester (**2**).



SCHEME 3 Model reaction of BLDI-OMe (**2**) and 1-octanol.

blocked diisocyanates, forming the same PUs. However, BIs are less reactive and higher temperatures are needed for the polymerization. The pendant ester functionality of **(2)** is in principle accessible for transesterification reaction, which could result in branched or even crosslinked products. Since there were no reports about using this new monomer, it was essential to find the conditions for maximizing the selectivity between blocked isocyanate groups and methyl ester groups. To study the selectivity of the reaction a monofunctional alcohol, 1-octanol, was chosen as model compound. The model reactions were studied in the presence as well in the absence of dibutyltin dilaurate (DBTDL), a common catalyst for the preparation of PUs. The progress of the reactions was followed by ^{1}H NMR. The deblocking of caprolactam blocked diisocyanate, in the presence of hydroxyl functional compounds, starts at about 125°C.[34,35] In order to suppress or even prevent the participation of the methyl ester group, a reaction temperature was kept as low as possible, that is, 125°C (Scheme 3).

During the reaction caprolactam was substituted by 1-octanol forming urethane linkages. The excess of octanol was removed by distillation and the formed caprolactam was easily removed by the aqueous washing steps. It was found that, in the presence of DBTDL, the BIs as well as the ester reacted to a large extent with octanol, that is, yielding branched products. In ^{1}H NMR analysis the sharp singlet of the methyl ester peak at 3.6 ppm was strongly reduced. As a result, the reaction of (**2**) with diols in the presence of DBTDL would lead to crosslinked products. Interestingly, the desired product was obtained in high yields when the reaction was performed without the tin catalyst (DBTDL). The ^{1}H NMR spectrum (Fig. 2) shows that the ester group of product was now completely preserved (3.6 ppm). The NH signals of the caprolactam blocked isocyanate moieties at 9.66 and 9.21 ppm, were absent after the reaction. Instead, two new NH signals appeared at 7.42 and 6.92 ppm, which were assigned to the hydrogen atoms of the urethane group. Importantly, the pendent methyl ester group survived 72 h at 125°C heating in the presence of an excess of 1-octanol, while the conversion of the BI groups of (**2**) was nearly 100%.

Solution Polymerization of BLDI-OMe and PCL530

Since the ester moiety of (**2**) did not react with the hydroxyl functional model compound, when no catalyst was used, linear PUs could be expected when polydiols were used. The most commonly used polydiols for synthesizing PUs are polyester and polyether diols. To demonstrate the feasibility of the concept, poly(ε-caprolactone)530 (PCL530) and poly(ethylene glycol)600 (PEG600) and polytetrahydrofuran650 (PTHF650) were selected as representative examples for the two classes of PUs. PCL comprising PUs are biocompatible, slowly hydrolysable and enzymatically degradable. The molar weight of common linear PUs has to be relatively high, to have a sufficient quantity of the crystalline hard phase to increase physical crosslinking, in order to achieve good mechanical properties. However, the crystalline residues, the remnants after degradation, caused inflammatory reactions *in vivo*.[41,42] Here, we chose purposely a low molecular weight PCL530 as soft segment and no chain extenders, forming amorphous PUs. Moreover, due to the asymmetrical structure of lysine diisocyanate it is difficult, maybe even impossible to obtain crystalline hard segments. The pendant ester side group offered the possibility to form in the presence of a tin catalyst covalent instead of physical crosslinks, which enabled to tune the mechanical properties of these amorphous polymers by the crosslink density.

Some initial polymerizations were conducted to establish the optimal ratio between the block isocyanate and PCL530. Since the number average molecular weight of PCL530 is not exactly 530 Da, the stoichiometric composition has to be found empirically.[43] Initially, the polymerizations were done by heating a solution of (**2**) and PCL530 in various molar ratios in anhydrous DMF at 125°C, which is above the deblocking temperature of caprolactam BIs. The polymerization scheme is depicted in Scheme 4.

Interestingly, all PUs appeared to be well soluble in DMF, even at room temperature, without any sign of gel formation,

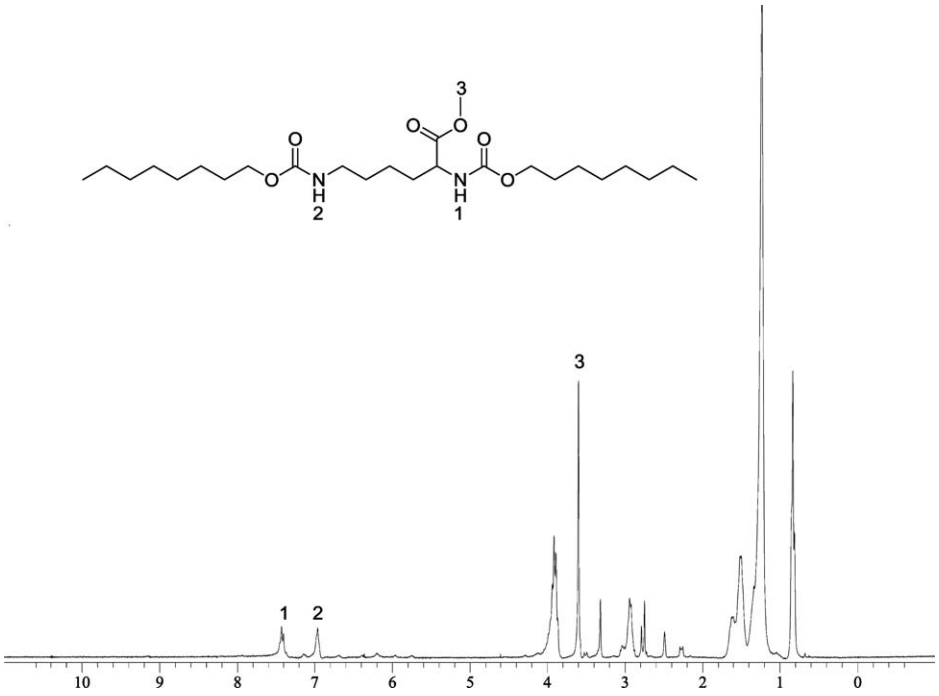
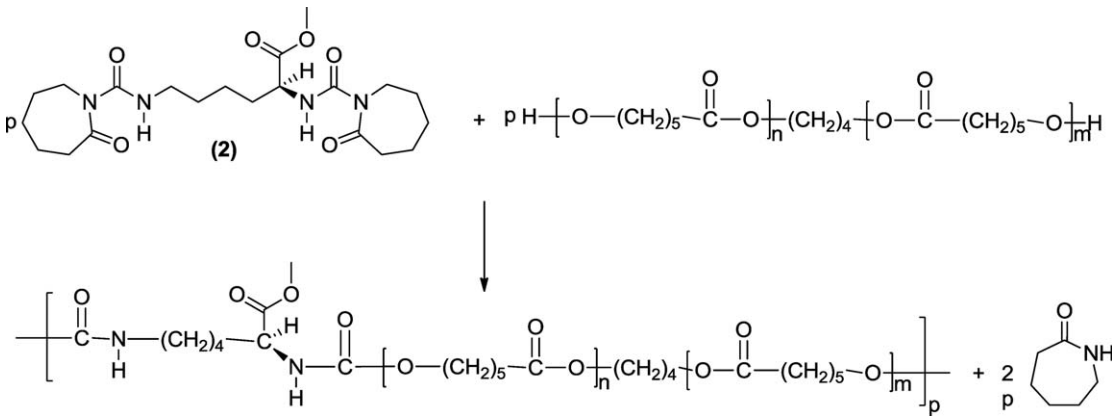


FIGURE 2 ^{1}H NMR spectrum of (**3**) obtained by the reaction of BLDI-OMe (**2**) and octanol, without using a catalyst.

indicating the absence of crosslink reactions after 72h at 125°C in the absence of a catalyst. The SEC analysis showed that the highest molecular weight was obtained at a molar ratio of 1.025 between (**2**) and PCL530. The polydispersity index (PDI) of all polymers was below 2, meaning that indeed no branching took place under those conditions. The molar ratio of 1.025 was also applied in the next series of polymerizations. After having established the optimal molar ratio, the polymerization kinetics of PUs from (**2**) and PCL530 were studied. From Figure 3 it can be seen that during the polymerization at 125°C until 192 h, the number average molecular weight (M_n) increased in time to about 10,000 Da. The polydispersity was low (< 2.0) until a polymerization time of 108 h ($M_n = 7300$ Da) and became somewhat larger (2.6) at the end of the polymerization (192 h), but was still narrow for condensation polymerizations that could potentially give branched or even crosslinked polymers.

In Figure 4, the ^{1}H NMR spectrum is given of the polyurethane with a M_n of 9700 Da and a PDI of 2.58. Importantly, the sharp singlet signal of methyl ester (COOC$\underline{H}_3$) at 3.5 ppm is nearly completely maintained, which confirms the model reactions (Scheme 3).

The solution polymerization (at 125°C), however, was very slow, which is practically not acceptable. Bulk polymerizations would increase the reaction rate and is, in addition, more common in commercial production units.



SCHEME 4 Synthesis of PUs from the blocked lysine diisocyanate (**2**) and poly(ε-caprolactone) (PCL), polymerized without catalyst.

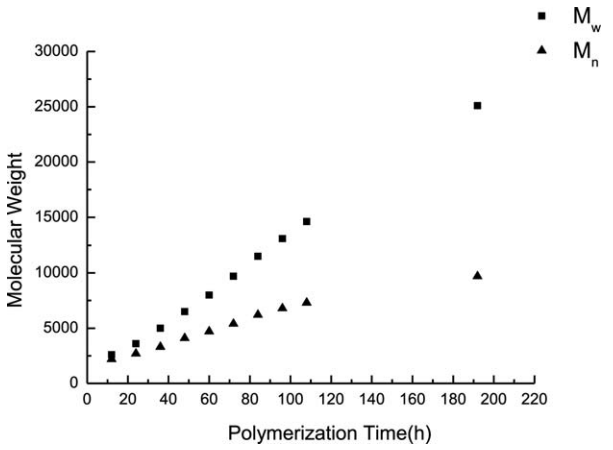


FIGURE 3 The relationship between molecular weights and reaction time of solution polymerization of PCL and (**2**) in DMF at 125°C in the absence of a catalyst.

Bulk Polymerization of (2) and PCL530

The polymerization was performed in bulk and at various temperatures (125, 145, 160, and 180°C). Besides a higher polymerization rate, the caprolactam formed during the reaction could perhaps advantageously evaporate because of the high temperature and vacuum conditions applied during the polymerization. As a result, a pure polymer would finally be obtained. The progress of the reaction, as monitored by ^{1}H NMR, showed that the caprolactam signals at 7.0 and 3.3 ppm were indeed absent.

As shown in Table 1, after 120 h reaction at 125°C, the M_n was already 18,000 Da in bulk conditions, while it was only 7000 Da in solution. Notice that the PDI is still only 2.1, which is very close to 2.0, as it should be for polycondensations without branching reactions. After a polymerization time of about 100 h the molecular weights still increased, although slowly. A number average molecular weight of 17,000 Da was obtained at 145°C after only 6 h reaction. Importantly, the PDI was still 1.9, indicating that no branching took place. A polymerization time of 6 h is a common reaction time in industrial processes. The number average molecular weight increased till 35,400 Da with PDI of 2.2 after 22 h at 145°C. The PDI of these polymers is still remarkably narrow. It is important to mention once more that strictly linear polymers are only obtained in the absence of a catalyst. The formation of only linear polymers was further supported by the presence of the sharp singlet of methyl ester ($COOC\underline{H}_3$) at 3.6 ppm. The methyl ester groups appeared to be amazingly stable even when heated for 24 h at 160°C (M_n = 53,600 Da, PDI = 2.93). ATR-FTIR measurement of the PUs revealed two distinctive vibration bands at 1672 and 1525 cm^{-1} that were not present in the PCL530 spectrum. These signals originated from urethane carbonyl stretching vibration (amide I) and NH deformation vibration (amide II).

TABLE 1 The Molecular Weights Determined by SEC of PUs Prepared from PCL530 and (**2**) in Bulk at Various Temperatures

T (°C)	Time (h)	M_n (Da)	M_w (Da)	PDI
125	48	5,900	8,700	1.47
125	120	18,600	39,000	2.10
145	6	17,400	33,000	1.91
145	22	35,400	80,100	2.26
160	4	13,000	22,400	1.73
160	24	56,300	165,000	2.93
180	4	36,800	333,500	9.06

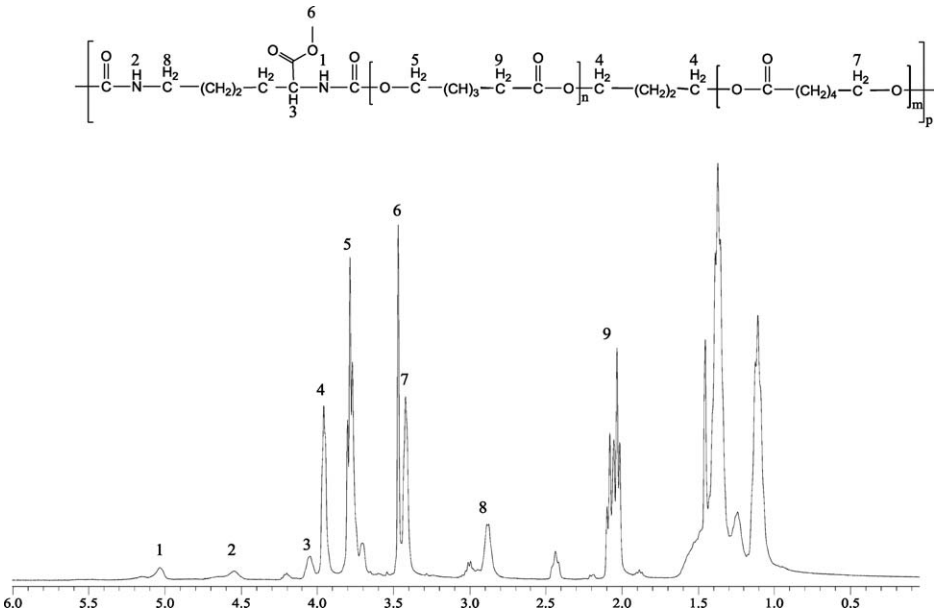


FIGURE 4 ^{1}H NMR spectrum of the polymer obtained from the solution polymerization of (**2**) and PCL530 with a M_n of 9700 Da and a PDI of 2.58.

TABLE 2 Glass Transition Temperatures (T_g) of PUs from PCL and (**2**), with Various Molecular Weights Which Were Prepared in Bulk

Sample(P#)	M_n (Da)	M_w (Da)	PDI	T_g (°C)
1 PUOMe-PCL530	4,300	6,500	1.5	−32.8
2 PUOMe-PCL530	9,900	19,200	1.9	−28.1
3 PUOMe-PCL530	23,900	48,500	2.0	−26.0
4 PUOMe-PCL530	36,900	90,100	2.44	−35.4
5 PUOMe-PCL530	52,500	169,800	3.24	−26.3

At 180°C, branching occurred, demonstrating that the hydroxyl group of PCL530 reacted with the methyl ester group, as evidenced by a broad PDI (9.06). These results revealed a very interesting feature that the polymerization temperature could be used as a tool for reacting selectively the hydroxyl groups of PCL either with only caprolactam BIs (CBIs) or with both, the CBIs and the pendant methyl ester. Up until 160°C the pendant methyl ester group remained intact, while at 180°C (**2**) behaved as a trifunctional monomer. This could be used to synthesize polyurethane networks which will be described in the future work.

Thermal Behavior of PUs Obtained from (2) and PCL530

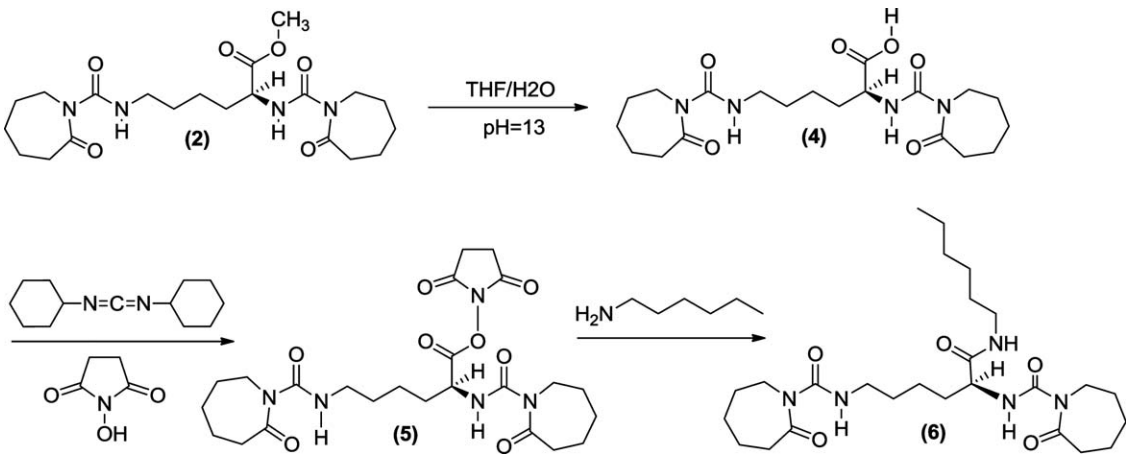
All PUs were transparent sticky wax like polymers, indicating that no crystallization took place. The lack of crystallinity was considered as an important advantage for biomedical applications to prevent inflammation reactions due to remnant crystal moieties, when the polymer degrades in the body. Hence, it was expected that the PUs would not have detectable melting temperatures. Possible melting temperatures and glass transition temperatures (T_gs) were determined by DSC. The samples were heated from −60 to 90°C, at heating rate of 10°C/min. Table 2 gives the glass transition temperatures (T_gs) of PUs with different molecular weights. None of the samples showed any indication of melting transitions. As expected, the T_g values were low (about −30°C), because of the flexible polyester spacer segment (PCL530). The T_g did not increase with increasing molecular weights of the PUs.

Bulk Polymerization of BLDI-OMe and PEG600 and PTHF650

PEG600 was selected as a representative example of polyether diols to synthesize PUs with (**2**). The polymerizations were performed at 125°C in bulk, under the same conditions as done with PCL. However, after a polymerization time of 72 h, an unexpected gelation took place, which was not found with PCL530, even at higher temperatures. The hydroxyl groups of PEG had apparently a higher reactivity toward the ester group than those of PCL's. One can speculate that hydroxyl groups of PEG are more acidic than the ones of PCL. The hydroxyl group in PEG is two methylene groups apart from the electron withdrawing β-oxygen atom. Moreover, the hydroxyl hydrogen of PEG can be activated by an intramolecular hydrogen bond with the next neighbour oxygen atom. The hydroxyl group in PCL is five methylene groups away from the ε-carbonyl group, resulting in a less acidic hydroxyl group. This deduction was supported by synthesis of PUs from (**2**) with another polyether (PTHF650), in which the oxygen atoms are four methylene units apart from the hydroxyl group. To compare the results with those of PEG600 the polymerization with PTHF650 was performed similarly in bulk at 125°C for 48 h and then at 150°C for another 48 h. Interestingly, the obtained polymer dissolved in the chloroform, meaning that no crosslinking took place. In a typical example, the polymer with a M_n of 10,000 Da had a PDI of 1.6, which indicated even the absence of any branching. It means that the methyl ester group did not react with the hydroxyl group of PTHF650. The formation of only linear polymers was further supported by the presence of the sharp singlet of methyl ester ($COOC\underline{H}_3$) at 3.6 ppm. Therefore, the unexpected crosslinking is specific for PEG. Although (**2**) does not give linear PUs with polyethylene glycol, these amorphous polymers (or mixtures of PEG and PCL) are perhaps even more useful for tissue engineering of biodegradable crosslinked polymeric materials as the crosslinking occurs without the use of a tin catalyst and under mild conditions.

Synthesis of Functionalized Blocked Lysine Diisocyanates

It was expected that an acid side group of (**2**) would be easier to functionalize than the stable methyl ester moiety.



SCHEME 5 Synthesis of functionalized blocked lysine diisocyanates.

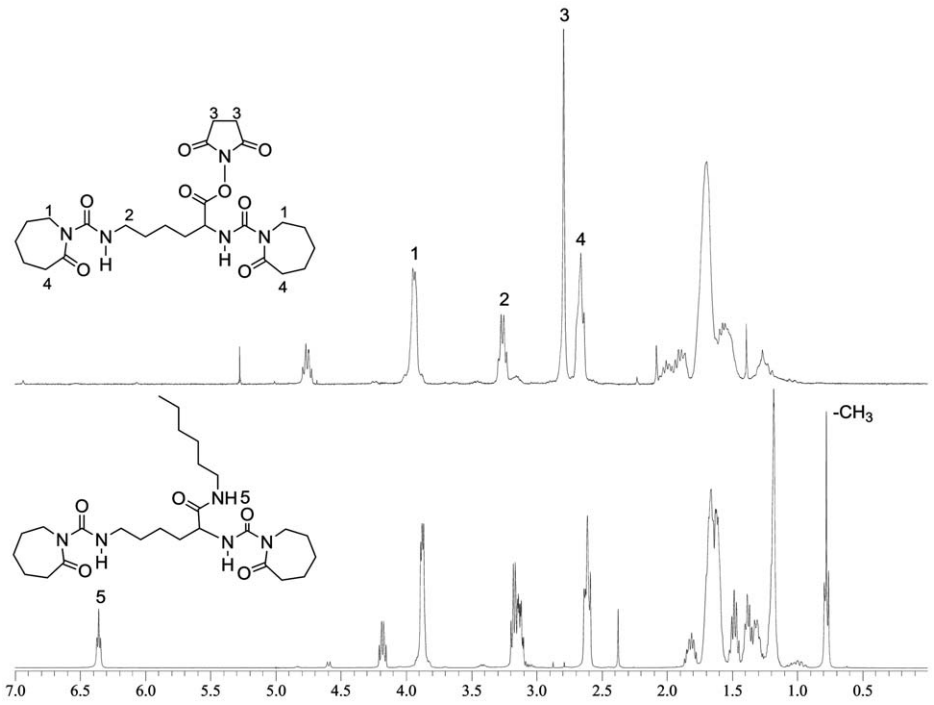


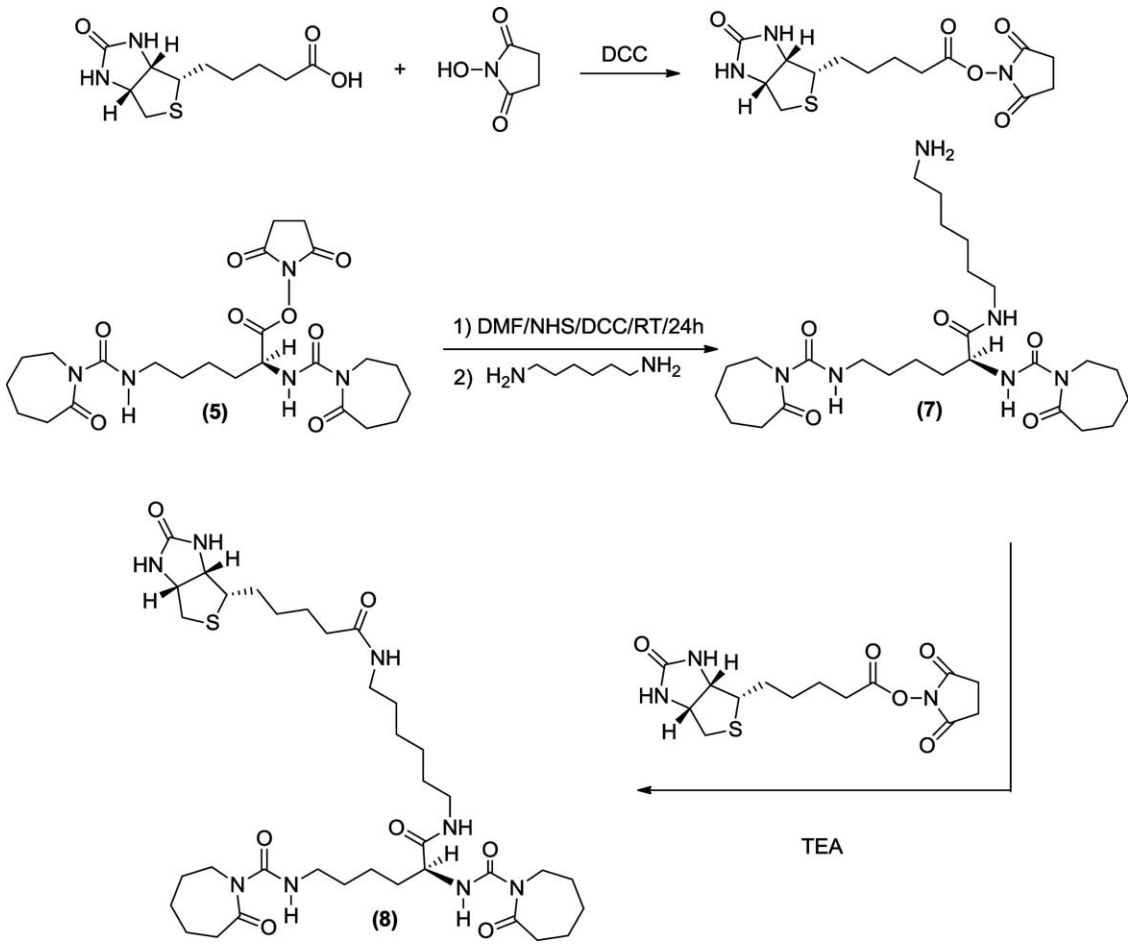
FIGURE 5 ^{1}H NMR spectra of series blocked diisocyanates: BLDI-NHS **(5)** and BLDI-HA **(6)**.

Hence, to enable the necessary derivatization of (**2**), reaction conditions had to be found to hydrolyze the methyl ester side group without hydrolyzing the blocked isocyanate groups. It was anticipated that it would be better to hydrolyze (and functionalize) the methylester before the polymerization, since the ester linkages of the polyester diols in the polyurethane backbone would be susceptible for hydrolysis as well. Interestingly, conditions were found at which the ester of **(2)** was complete hydrolyzed, without hydrolyzing the blocked diisocyanate groups (pH of 13 at room temperature; Scheme 5). The progress of the reaction was followed by the disappearance of CH_3 signal of methyl ester side chain (3.60 ppm), the appearance of free carboxylic acid signal (10.73 ppm) and the preservation of the blocked isocyanate groups (9.61 and 9.20 ppm). The reaction was complete after about 5 h. All ^{1}H NMR signals in the spectrum were in accordance with the expected product (**4**). ESI-MS and elemental analysis were supporting evidence that the desired product **(4)** was obtained.

The acid side group of (**4**) allowed attachment of a variety of (bioactive) compounds. To increase the general applicability, it seemed more appropriate to convert the acid into an activated ester,[44–46] which could more conveniently be converted into functional moieties under ambient conditions. As an appropriate option the acid moiety was converted into an NHS activated ester **(5)**. The reaction of (**4**) with NHS was activated by dicyclohexyl-carbodiimide (DCC) (Scheme 5). The disappearance of COOH signal and appearance of NHS signal at 10.25 and 2.82 ppm, respectively, were used to monitor the progress of the reaction. The reaction completed after 24 h at room temperature with high yields. All signals were in accordance with product (**5**). Other analysis method like ESI-MS and elemental analysis gave further evidence that the expected product was obtained. Although NHS active esters are reactive, they appeared to be stable enough to be stored under dry conditions for at least several months without any sign of deterioration.[40,47]

Hexylamine was used as a model compound to demonstrate the substitutability of NHS by amines, without destroying the BIs. Hexylamine substituted indeed at room temperature the NHS group completely, without affecting the blocked isocyanate groups, forming BLDI-HA **(6)** (Scheme 5). The disappearance of NHS signal (2.82 ppm) and appearance of amide (6.38 ppm) and CH_3 signals of hexylamine (0.8 ppm; Fig. 5) proved the progress of the reaction. ESI-MS and elemental analysis supported further that the desired product was obtained. The reaction was complete after about 4 h.

Biotin is an interesting functional group, as it offered a wide range of options to introduce bioactive components. Biotin interacts firmly with steptavidine, which in turn can accommodate many bioactive compounds.[48,49] To couple biotin onto (**5**) the activated ester group was first converted into an amino functionality. The amidation of (**5**), to prepare the amino functional BLDI (BLDI-NH-C_6-NH_2 (**7**)), was carried out with a 10-fold excess of hexamethylene diamine to reduce the possibility that both amino groups would react with (**5**). The disappearance of NHS signal (2.82 ppm) and appearance of amide signals (6.45 ppm) allowed following the progress of the reaction. The conversion of (**5**) into (**7**) was complete after about 12 h at room temperature. The excess of hexamethylene diamine was removed by an



SCHEME 6 Synthesis of biotin-NHS and biotinylated blocked lysine diisocyanates.

aqueous extraction. To facilitate the subsequent coupling to (**7**), the acid group of biotin was transferred into NHS-activated moiety. The coupling of NHS-activated biotin onto (**7**) was carried out in 12 h in DMF at room temperature. The crude product was isolated by precipitating in diethyl ether and purified by using column chromatography. Scheme 6 shows the synthetic route to prepare the biotinylated blocked lysine diisocyanate (**8**).

The ^{1}H NMR spectrum of (**8**; Fig. 6) shows the peaks at δ 6.40 and 6.34 ppm were very characteristic for biotin.[50] All other signals in the spectrum corresponded with the expected product and the formation of the desired product was further supported by the ESI-MS.

Synthesis of Functionalized PUs

PU-NHS

As aforementioned, (**2**) reacted with polydiols forming linear PUs, while preserving the methyl ester side group. The methyl ester appeared to be stable enough to prevent the formations of branching. Apparently, the ester next to the urethane moiety is amazingly stable. We therefore wondered if the reaction of polyester with the NHS-activated blocked isocyanate (**5**) could give linear polymers as well. To synthesize NHS functionalized PUs, **(5)** was heated at 145°C for 4 h in bulk with PCL530, as a representative polyester diol. The polymerization proceeded, as evidence by increase of viscosity and SEC measurements. The corresponding polymer was soluble in chloroform and had a M_n of 8700 Da and 2.4 in PDI. So, even the NHS-activated ester apparently survived largely the reaction conditions. This was further supported by the signals of the NHS-group, which was still present (2.82 ppm). Activated NHS ester groups of the polymer can in principle react with all kind of nucleophiles, which would be useful to attach for instance the N-terminus of peptides, for example RGD groups or proteins, forming a stable covalent amide bond. It could offer a platform to prepare a library of biofunctional PUs. The more details will be given in the next report.

PU-Biotin

Alternatively, functional PUs, for example, biotinylated PUs, could be obtained by functionalize the BI **(8)** (Scheme 6) before the polymerization with PCL530 (160°C for 6 h in bulk). Indeed, from the ^{1}H NMR analysis, the characteristic NH peaks of the biotin ring at 6.40 and 6.34 ppm were clear observed in biotinylated polymer. This was expected because of the stability of the amide linkage between biotin and blocked lysine disocyanate, and because of our experience

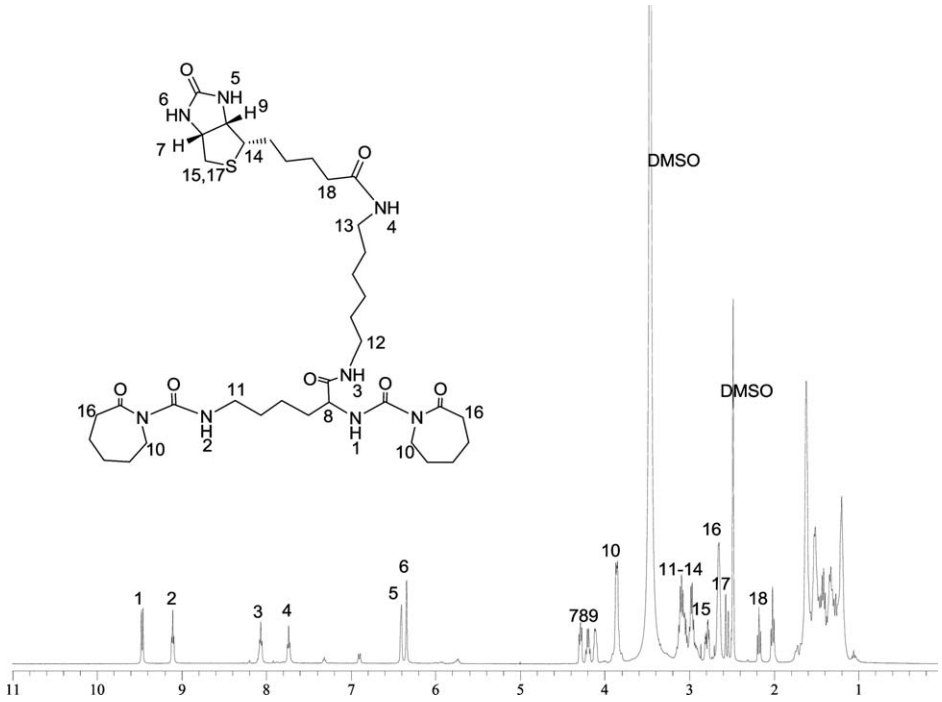


FIGURE 6 ^{1}H NMR spectrum of BLDI-C_6-biotin **(8)**.

that even the pendant methyl ester group of (**2**) survived during the polymerization with PCL. The molecular weight of biotinylated polyurethane was rather low (M_n of 3500 and PDI of 2.5), which could probably be ascribed to the low reactivity of the blocked isocyanate moiety on the α-position with respect to the electron withdrawing amide group. So far we investigated only the preparation of homopolymers with the functionalized BI to demonstrate the concept, although the biotin density was here by far overdone for practical use. Thus, PU copolymers with functionalized and nonfunctionalized BIs would be more beneficial, and it is expected that then higher molecular weights will be feasible. Future work will be focused on specific applications of biotinylated copolymers.

CONCLUSIONS

Caprolactam blocked lysine diisocyanate methyl ester (**2**) was successfully synthesized in high yields, by reacting the HCl-salt of the lysine methyl ester with CBC, in the presence of a base. A hydroxyl comprising model compound (1-octanol) reacted in the absence of a catalyst, in high yields, exclusively with the blocked isocyanate groups, without affecting the methyl ester. In the presence of a tin catalyst the methyl ester reacted as well, which could be utilized to make crosslinked PUs. Without catalyst PUs were successfully synthesized from (**2**) and PCL530, PTHF650 or PEG600. In solution as well as in bulk a typical step-growth polymerization with PCL was observed. Remarkably, the hydroxyl groups of PCL reacted in bulk even at 160°C still virtually exclusive with the blocked isocyanate groups without affecting the methylester group, yielding almost pure linear PUs. Even up to a M_n of 56,000 Da the PDI was not more than 2.9. Above 160°C the methyl ester reacted as well, yielding branched poly(ester-urethanes). In contrast, PEG600, reacted already at 125°C with the pendant ester group, resulting directly in amorphous poly(ether-urethane) networks. To introduce pendant biofunctional side groups the ester group was selective hydrolyzed without affecting the blocked isocyanate groups. The free acid group was converted into the NHS activated ester, which allowed further modification with amine functional compounds under mild conditions. This provided a general strategy to introduce a variety of (bio) molecules on polyurethane backbones. Biotinylated PUs and even NHS functional PUs were prepared to demonstrate the applicability of the methodology. This methodology makes it feasible to make linear as well as crosslinked amorphous PUs with bioactive moieties.

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