

Supporting Information

Direct Comparison of Solution and Solid Phase Synthesis of Sequence-Defined Macromolecules

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1 Experimental section

1.1. Materials:

The following chemicals were used as received from the following suppliers unless otherwise described. MeCN- d_3 \geq 99.8 %, DMSO- d_6 \geq 99.8 %, MeOH- d_4 \geq 99.8 % and CDCl₃ \geq 99.8 % were purchased from Euriso-top. Ethyl carbazate 97 %, Stearic acid 98 %, hydrochloric acid in 1,4-dioxane ca. 4 mol/L, isobutyraldehyde >98 %, propanal >98 %, and cyclohexanal >98 % were purchased from Tokyo Chemical Industry. Anhydrous ethyl acetate 99.8 %, 11-aminoundecanoic acid 97 %, thionyl chloride 99 %, trimethyl orthoformate 99 %, diisopropylamine (DIPEA) > 99.5 %, phosphorous (V) oxychloride 99 %, TLC silica gel F₂₅₄, cerium(IV)-sulfate 99 %, phosphomolybdic acid hydrate 99 %, sodium carbonate 98 %, sodium hydrogen carbonate > 95 %, sodium sulfate > 99 % (anhydrous), 2,3-dimethylbut-2-ene \geq 99.9 %, and 1,5,7-triazabicyclo[4.4.0]dec-5-ene 98 % (TBD) were purchased from Sigma-Aldrich. Hydrochloric acid 36 wt% was purchased from Chem Lab NV. *Trans,trans*-2,4-hexadien-1-ol (sorbic alcohol) was purchased from both Sigma-Aldrich (97 %) and Alfa Aesar (98 %). Silica gel 60 (0.040 - 0.063) was purchased from both Sigma-Aldrich and Rocc. Potassium Carbonate \geq 99 % and magnesium sulfate \geq 99 % were purchased from Carl Roth. Trifluoroacetic acid (TFA, peptide grade) and 2-chlorotriyl chloride resin (100-200 mesh, 1 % DVB, 1.6 mmol/g) were purchased from Iris Biotech GmbH. Diphenyl carbonate 99 % was purchased from Acros Organics. Acetonitrile (HPLC grade \geq 99.9 %), chloroform (HPLC grade \geq 99.9 %), and dichloromethane (DCM, HPLC grade \geq 99.9 %) were purchased from Sigma-Aldrich. Methanol (HPLC grade 99.8 %), triethylamine (99 %, anhydrous), dimethylformamide (DMF, 99.8 %, anhydrous), pyridine (99.5 %, anhydrous), and tetrahydrofuran (THF, 99.5 %, extra dry over molecular sieves) were purchased from Acros Organics. Ethanol and diethyl ether, both analytical reagent grade, were purchased from Fisher Scientific. Technical grade hexane and ethyl acetate, supplied by Univar, were used for purifications by column chromatography. All solvents were used without further purification, unless otherwise noted. Water, when used in the synthesis, was de-ionised. N₂O₄ gas was supplied by Gerling Holz & Co, Germany.

1.2. Characterisation:

NMR ^1H spectra were recorded at the Karlsruhe Institute of Technology (KIT, Germany) on a Bruker AVANCE DRX at 500 MHz and ^{13}C -NMR Attached Proton Test (APT) spectra were recorded at 125 MHz. CDCl_3 or CD_3OD were used as solvents. Chemical shifts are presented in parts per million (δ) relative to the resonance signal at 7.26 ppm (^1H , CDCl_3) and 77.16 ppm (^{13}C , CDCl_3) or 3.31 ppm (^1H , CD_3OD) and 49.00 ppm (^{13}C , CD_3OD), respectively.

NMR ^1H spectra were recorded at Ghent University (UGent, Belgium) on a Bruker Avance 300, a Bruker Avance 400, Bruker Avance 500 or a Bruker Avance II 700 and ^{13}C -NMR Attached Proton Test (APT) spectra were recorded at 100 MHz on a Bruker Avance 400. $\text{DMSO-}d_6$, CD_3OD , CDCl_3 or CD_3CN were used as solvents. Chemical shifts are presented in parts per million (δ) relative to the resonance signal at 2.50 ppm (^1H , $\text{DMSO-}d_6$) and 39.51 ppm (^{13}C , $\text{DMSO-}d_6$), 3.31 ppm (^1H , CD_3OD) and 49.00 ppm (^{13}C , CD_3OD), 7.26 ppm (^1H , CDCl_3) and 77.16 ppm (^{13}C , CDCl_3) or 1.94 ppm (^1H , CD_3CN) and 118.26 ppm (^{13}C , CD_3CN), respectively.

Coupling constants (J) are reported in Hertz (Hz). All measurements were recorded in a standard fashion at 25 °C unless otherwise stated. Full assignment of structures was aided by 2D NMR analysis (COSY, HSQC and HMBC).

Oligomers were characterized at Ghent University (UGent, Belgium) on a Waters **Size Exclusion Chromatography (SEC)** system equipped with a Waters 1515 isocratic pump, Waters 2410 refractive index detector (24 °C), Waters 717plus autosampler and a Waters 2487 dual λ absorbance UV detector and column oven. For separation, a three-column setup was used with one SDV 3 μm , 8 \times 50 mm precolumn and two SDV 3 μm , 1000 Å, 8 \times 300 mm columns supplied by PSS, Germany. Tetrahydrofuran (THF) stabilized with butylated hydroxytoluene (BHT, HPLC-SEC grade) supplied by Biosolve was used at a flow rate 1.0 mL min $^{-1}$. Calibration was carried out by three injections of a mixture of narrow polystyrene standards ranging from 162 to 38640 kDa.

Size Exclusion Chromatography (SEC) measurements were performed at the Karlsruhe Institute of Technology (KIT, Germany) on a SHIMADZU Size Exclusion Chromatography (SEC) system equipped with a SHIMADZU isocratic pump (LC-20AD), a SHIMADZU refractive index detector (24°C) (RID-20A), a SHIMADZU autosampler (SIL-20A) and a VARIAN column oven (510,

50°C). For separation, a three-column setup was used with one SDV 3 μm , 8×50 mm precolumn and two SDV 3 μm , 1000 Å, 3×300 mm columns supplied by PSS, Germany. Tetrahydrofuran (THF) stabilized with 250 ppm butylated hydroxytoluene (BHT, $\geq 99.9\%$) supplied by SIGMA-ALDRICH was used at a flow rate of 1.0 mL min⁻¹. Calibration was carried out by injection of eight narrow polymethylmethacrylate standards ranging from 102 to 58300 kDa.

Liquid Chromatography-Mass Spectrometry (LCMS) spectra were recorded on an Agilent technologies 1100 series LC/MSD system equipped with a diode array detector and single quad MS detector (VL) with an electrospray source (ESI-MS) for classic reversed phase LCMS and MS analysis. Analytic reversed phase HPLC (high-performance liquid chromatography) was performed with a Phenomenex Kmetex C18 (2) column with a solid core at 35 °C and a flow rate of 1.5 mL/min (5 μm , 150 x 4.6 mm) using a solvent gradient (0 \rightarrow 100 % acetonitrile in H₂O in 6 min) and the eluting compounds were detected via UV-detection ($\lambda = 214 \text{ nm}$).

Infrared spectra (IR) were recorded at the KIT on a Bruker Alpha-p instrument in a frequency range from 3998 to 374 cm⁻¹ applying KBr and Attenuated Total Reflection (ATR) technology or at UGent on a Perkin Elmer FTIR SPECTRUM 1000 spectrometer with ATR with a PIKE Miracle ATR unit.

High Resolution Mass Spectroscopy (HRMS) spectra were collected using an Agilent 6220 Accurate-Mass time-of-flight (TOF) equipped with a multimode ionisation (MMI) source.

Fast atom bombardment (FAB) mass spectra were recorded on a *Finnigan* MAT 95 instrument. The protonated molecule ion is expressed by the term: [(M+H)]⁺.

All **thin layer chromatography** experiments were performed on silica gel coated aluminium foil (silica gel 60 F₂₅₄, Sigma-Aldrich). Compounds were visualized by staining with Seebach-solution (mixture of phosphomolybdic acid hydrate, cerium(IV)-sulfate, sulfuric acid and water).

SEC-ESI-MS spectra were recorded on a Q Exactive (Orbitrap) mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) equipped with an HESI II probe. The instrument was calibrated in the m/z range 74–1822 using premixed calibration solutions (Thermo Scientific). A constant spray voltage of 4.6 kV, a dimensionless sheath gas of 8, and a dimensionless

auxiliary gas flow rate of 2 were applied. The capillary temperature and the S-lens RF level were set to 320 °C and 62.0, respectively. The Q Exactive was coupled to an UltiMate 3000 UHPLC System (Dionex, Sunnyvale, CA, USA) consisting of a pump (LPG 3400SD), autosampler (WPS 3000TSL), and a thermostated column department (TCC 3000SD). Separation was performed on two mixed bed size exclusion chromatography columns (Polymer Laboratories, Mesopore 250 × 4.6 mm, particle diameter 3 µm) with precolumn (Mesopore 50 × 4.6 mm) operating at 30 °C. THF at a flow rate of 0.30 mL·min⁻¹ was used as eluent. The mass spectrometer was coupled to the column in parallel to a RI-detector (RefractoMax520, ERC, Japan). 0.27 mL·min⁻¹ of the eluent were directed through the RI-detector and 30 µL·min⁻¹ infused into the electrospray source after post-column addition of a 100 µM solution of sodium iodide in methanol at 20 µL·min⁻¹ by a micro-flow HPLC syringe pump (Teledyne ISCO, Model 100DM). A 20 µL aliquot of a polymer solution with a concentration of 2 mg·mL⁻¹ was injected onto the HPLC system.

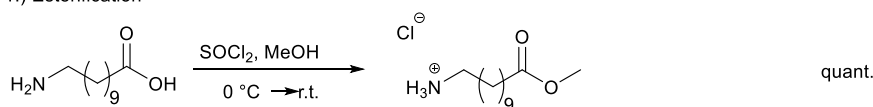
Orbitrap Electrospray-Ionisation Mass Spectrometry (ESI-MS) mass spectra were recorded on a Q Exactive (Orbitrap) mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) equipped with an atmospheric pressure ionisation source operating in the nebuliser assisted electrospray mode. The instrument was calibrated in the *m/z*-range 150-2000 using a standard containing caffeine, Met-Arg-Phe-Ala acetate (MRFA) and a mixture of fluorinated phosphazenes (Ultramark 1621, all from Sigma Aldrich). A constant spray voltage of 3.5 kV, a dimensionless sheath gas of 6, and a sweep gas flow rate of 2 were applied. The capillary voltage and the S-lens RF level were set to 68.0 V and 320°C, respectively.

Electron ionisation (EI) For the measurements that were performed with the electron ionisation (EI) method, an instrument by Finnigan, model MAT 90 (70 eV), was used with 3-nitrobenzyl alcohol (3-NBA) as matrix. For the interpretation of the spectra, molecular peaks [M]⁺, peaks of pseudo molecules [M+H]⁺ and characteristic fragment peaks are indicated with their mass to charge ratio (*m/z*) and their intensity in percent, relative to the most intense peak (100%).

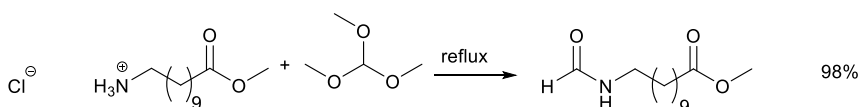
2 Linker molecule synthesis:

2.1 Synthesis of diene-isocyanide, L1:

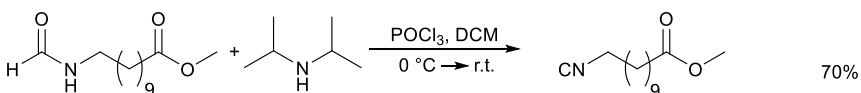
1.) Esterification



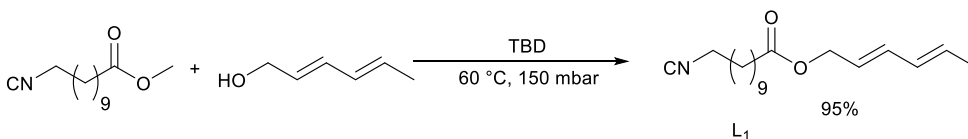
2.) N-formylation



3.) Dehydration



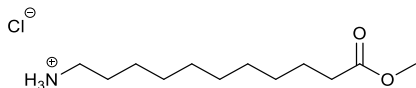
4.) Transesterification



Scheme S1: Four-step reaction scheme of the synthesis of linker molecule **L1**.

11-Methoxy-11-oxoundecan-1-ammonium chloride

Synthesised according to previously reported procedure.¹



15.0 g of 11-Aminoundecanoic acid (74.5 mmol, 1.0 eq.) was suspended in 150 mL methanol and the suspension was cooled in an ice bath to 0°C. Subsequently, 18.9 mL of thionyl chloride (31.0 g, 0.26 mol, 3.5 eq.) was added dropwise. After addition of the thionyl chloride, the solution was not cooled anymore and the reaction was stirred at room temperature overnight. The yellowish solution was then poured into 500 mL of cold diethyl ether and stored in the freezer for one hour. The product was filtered off and dried under high vacuum. 11-Methoxy-

11-oxoundecan-1-ammonium chloride was obtained as a white powder in a yield of 99 % (18.6 g). The crude product was used without further purification.

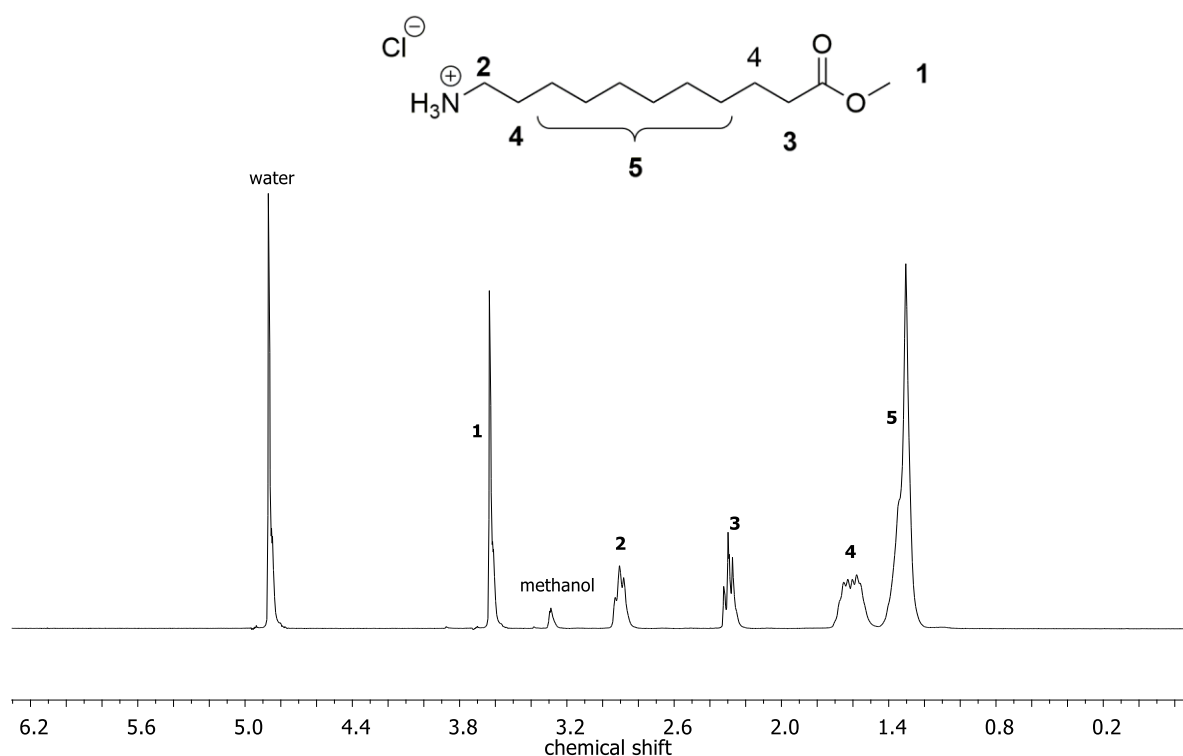
¹H-NMR: (300 MHz, CD₃OD) δ /ppm: 3.62 (s, 3H, OCH₃, ¹), 2.91 (t, *J* = 7.0 Hz, 2H, CH₂NH₃⁺, ²), 2.34 – 2.20 (t, *J* = 7.4 Hz, 2H, CH₂COOCH₃, ³), 1.75 – 1.47 (m, 4H, CH₂CH₂COOCH₃, CH₂CH₂NH₃⁺, ⁴), 1.45 – 1.15 (m, 12H, CH₂, ⁵).

¹³C NMR (101 MHz, CD₃OD) δ /ppm: 175.98, 51.95, 40.79, 34.78, 30.39, 30.37, 30.29, 30.15, 30.13, 28.55, 27.42, 25.99.

HRMS (EI) m/z: [M-H]⁺ calculated for [C₁₂H₂₆NO₂⁺]: 215.1885, found: 215.1885.

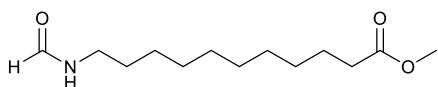
HRMS (EI) m/z: [M]⁺ calculated for [C₁₂H₂₆NO₂⁺]: 216.1964, found: 215.1964.

IR (ATR platinum diamond): ν [cm⁻¹] = 2919.6, 2849.0, 1722.7, 1609.3, 1561.6, 1510.5, 1468.4, 1444.0, 1419.7, 1375.8, 1361.1, 1334.4, 1306.4, 1277.0, 1245.3, 1210.8, 1174.4, 1114.6, 1097.5, 1042.5, 1001.3, 970.9, 938.3, 885.8, 791.9, 741.7, 723.4, 702.5, 593.8, 504.1, 450.0, 425.6.



Methyl 11-formamidoundecanoate

Synthesised according to previously reported procedure.¹



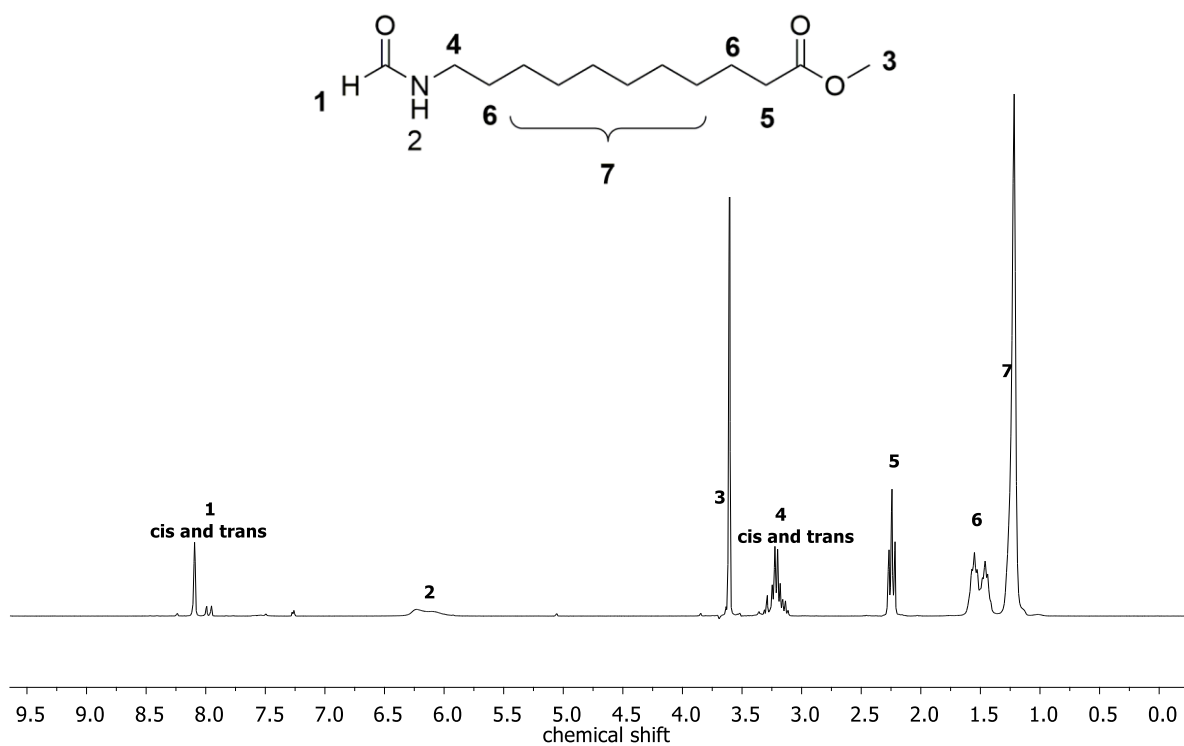
18.8 g of 11-methoxy-11-oxoundecan-1-aminium chloride (74.9 mmol, 1.0 eq.) was dissolved in 81 mL of trimethyl orthoformate (78.5 g, 0.74 mol, 10.0 eq.), heated to 100 °C and stirred under reflux for 12 hours. The solvent was evaporated under reduced pressure and 15.6 g (0.64 mol) of the product was obtained as a white solid in a yield of 98 %. The crude product was used without further purification.

¹H-NMR: (300 MHz, CDCl₃) δ /ppm: 8.19 – 7.87 (m, 1H, HCO, ¹), 6.37 – 5.88 (m, 1H, NH, ²), 3.61 (s, 3H, OCH₃, ³), 3.38 – 3.01 (m, 2H, CH₂, ⁴), 2.24 (t, *J* = 7.5 Hz, 2H, CH₂, ⁵), 1.69 – 1.36 (m, 4H, CH₂, ⁶), 1.22 (s, 12H, CH₂, ⁷).

¹³C NMR (101 MHz, CDCl₃) δ /ppm: 174.32, 155.78, 155.70, 155.62, 51.48, 41.69, 41.61, 41.52, 34.12, 29.32, 29.22, 29.14, 28.71, 26.35, 24.97.

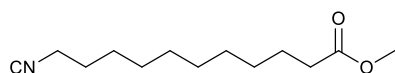
HRMS (EI) *m/z*: [M]⁺ calculated for [C₁₃H₂₅NO₃⁺]: 243.1834, found: 243.1835.

IR (ATR platinum diamond): ν [cm⁻¹] = 3260.2, 2915.9, 2849.2, 1734.8, 1682.9, 1638.4, 1536.6, 1463.7, 1435.9, 1379.1, 1334.7, 1301.2, 1268.8, 1226.8, 1204.6, 1169.6, 1113.3, 1058.1, 1001.9, 980.3, 905.0, 883.3, 738.2, 721.3, 518.8, 449.3.



Methyl 11-isocyanoundecanoate

Synthesised according to previously reported procedure.¹



12.8 g of Methyl 11-formamidoundecanoate was dissolved in 180 mL DCM and 22.3 mL of diisopropyl amine (16.1 g, 0.16 mol, 3.0 eq.) was added to the solution. The mixture was then cooled with an ice bath to 0 °C and 6.3 mL of phosphorus oxychloride (10.5 g, 68.8 mmol, 1.3 eq.) was added dropwise. After the addition, the ice bath was removed and the solution was allowed to warm up and was stirred for two hours at room temperature. The reaction was then quenched by addition of sodium carbonate solution (75 mL, 20 %) at 0 °C. The mixture was stirred for another 30 minutes at room temperature and subsequently, 80 mL of DCM and 80 mL of water were added. The phases were separated and the organic layer was washed with water (3 times 80 mL) and brine (80 mL). The combined organic layers were dried over sodium sulphate, the solvent was evaporated under reduced pressure and the crude product was purified by column chromatography (hexane:ethyl acetate 19:1 → 10:1). The product was obtained as a yellowish liquid in a yield of 70 % (8.4 g, 37.1 mmol)

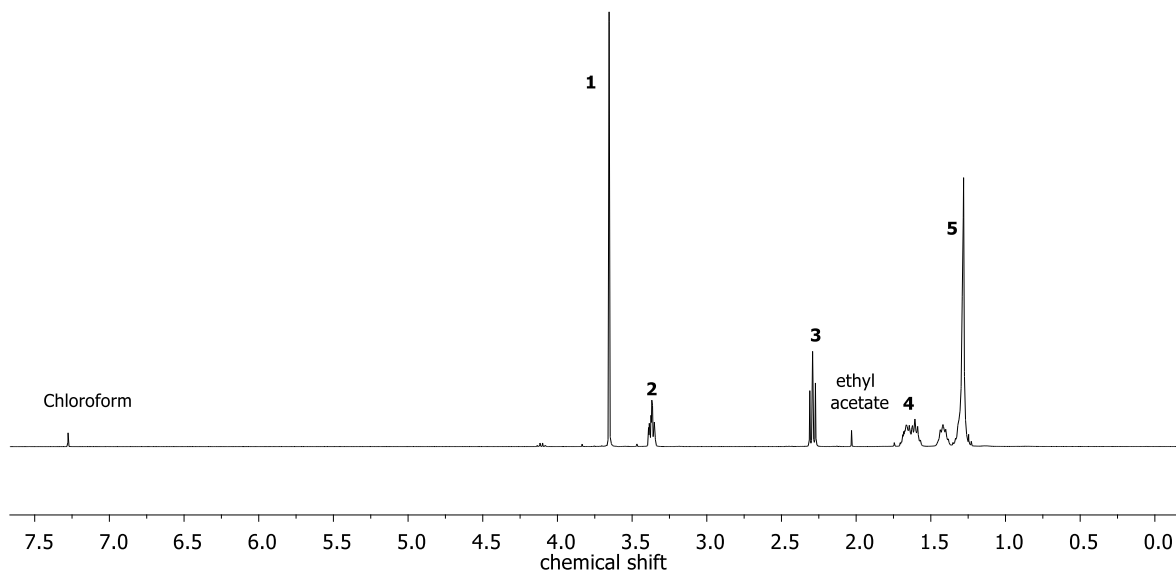
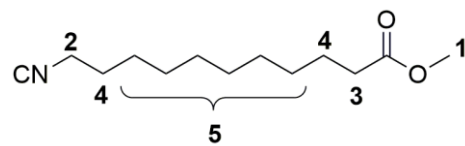
¹H-NMR: (400 MHz, CDCl₃) δ /ppm: 3.65 (s, 3H, OCH₃¹), 3.44 – 3.26 (m, 2H, CH₂²), 2.29 (t, J = 7.5 Hz, 2H, CH₂³), 1.77 – 1.56 (m, 4H, CH₂⁴), 1.48 – 1.18 (m, 12H, CH₂⁵).

¹³C NMR (75 MHz, CDCl₃) δ /ppm: 174.32, 155.70, 51.48, 41.69, 41.61, 41.52, 34.12, 29.32, 29.22, 29.14, 28.71, 26.35, 24.97.

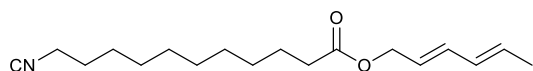
HRMS (EI) m/z [M]⁺ calculated for [C₁₃H₂₃NO₂⁺]: 225.1729, found: 225.1729.

IR (ATR platinum diamond): ν [cm⁻¹] = 2925.9, 2855.1, 2146.5 (isocyanide), 1735.6, 1435.9, 1353.1, 1194.8, 1170.0, 1104.5, 1010.8, 849.3, 722.4, 428.2.

R_f (hexane / ethyl acetate (5:1)) = 0.51.



(2E,4E)-hexa-2,4-diene-1-yl 11-isocyanoundecanoate



To a mixture of 8.3 g of methyl 11-isocyanoundecanoate (36.3 mmol, 1.0 eq.) and 7.2 g of sorbic alcohol (73.3 mmol, 2.0 eq.), 25.5 mg of TBD (1.8 mmol, 5.0 mol%) was added as a catalyst and the reaction mixture was heated to 60 °C under a reduced pressure of 175 mbar at the rotavapor for 1 hour. Subsequently, the pressure was further decreased to 6 mbar and the temperature was decreased to 50 °C for another two and a half hours. Methanol was distilled off during the reaction. The crude product was purified by column chromatography (hexane:ethyl acetate 19:1→15:1) and was obtained as a slightly yellow liquid in a yield of 95 % (10.1 g, 34.6 mmol).

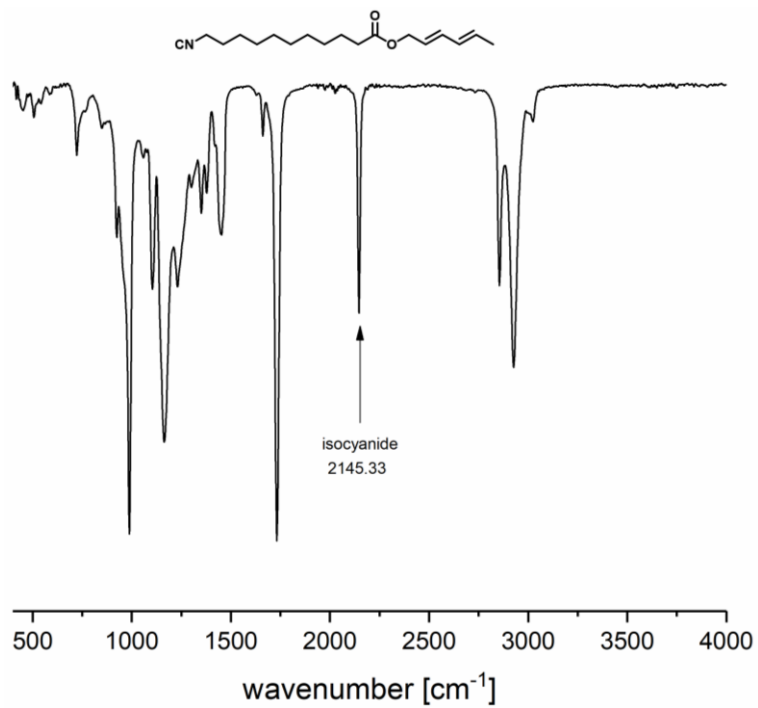
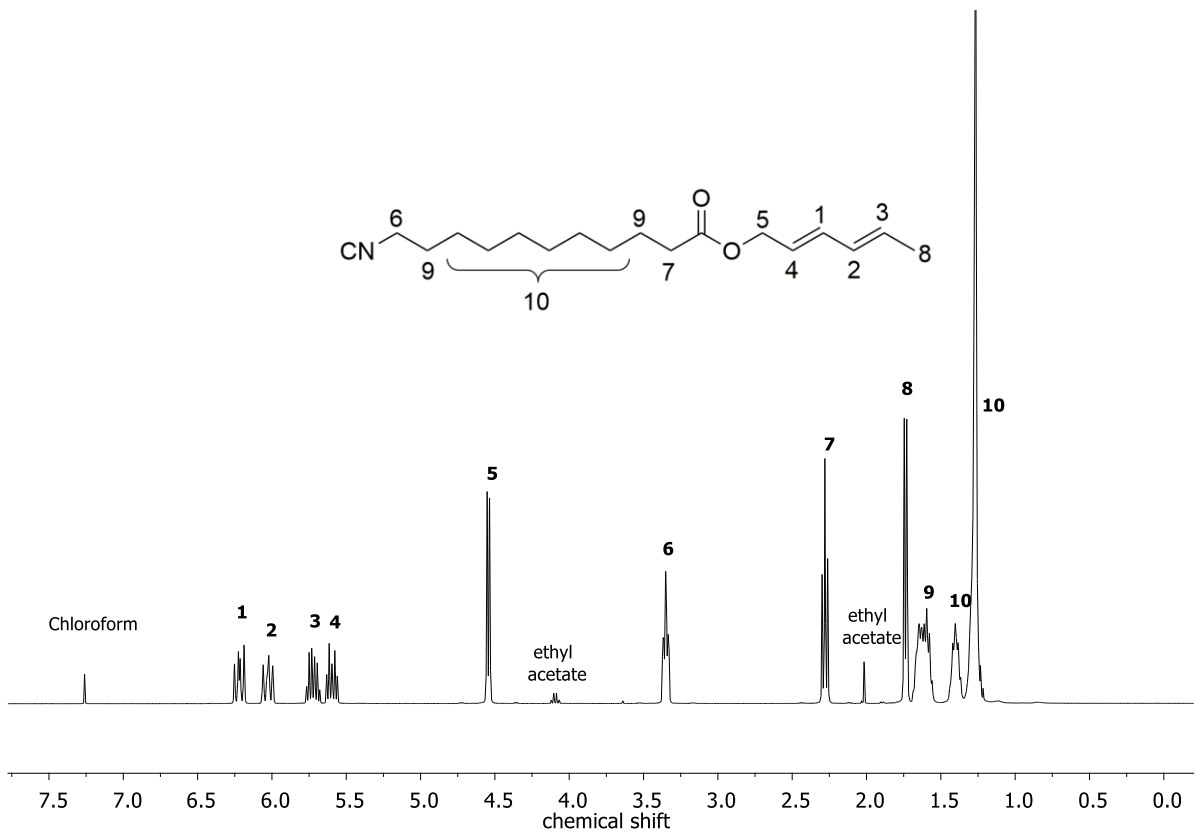
¹H-NMR: (400 MHz, CDCl₃) δ /ppm: 6.28 – 6.15 (m, 1H, HC=C, ¹), 6.08 – 5.97 (m, 1H, HC=C, ²), 5.79 – 5.65 (m, 1H, HC=C, ³), 5.65 – 5.53 (m, 1H, HC=C, ⁴), 4.54 (d, *J* = 6.6 Hz, 2H, COOCH₂, ⁵), 3.40 – 3.30 (m, 2H, CH₂, ⁶), 2.28 (t, *J* = 7.5 Hz, 2H, CH₂, ⁷), 1.74 (d, *J* = 6.7 Hz, 3H, CH₃, ⁸), 1.69 – 1.53 (m, 4H, CH₂, ⁹), 1.46 – 1.19 (m, 12H, CH₂, ¹⁰).

¹³C NMR (75 MHz, CDCl₃) δ /ppm: 173.57, 134.77, 131.15, 130.51, 123.91, 64.75, 41.66, 41.58, 41.49, 34.33, 29.30, 29.20, 29.13, 29.11, 28.70, 26.33, 24.94, 18.15.

HRMS (EI) *m/z* [*M*]⁺: calculated for [C₁₈H₂₉NO₂⁺]: 292.2198, found: 292.2198.

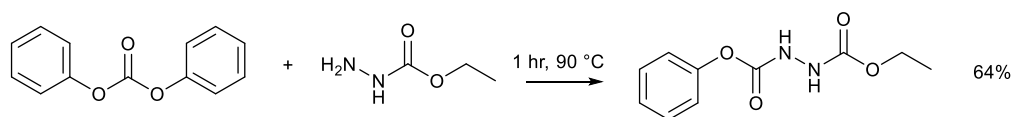
IR (ATR platinum diamond): *ν* [cm⁻¹] = 2926.4, 2855.1, 2146.4 (isocyanide), 1732.0, 1661.7, 1453.4, 1377.6, 1350.9, 1231.0, 1163.5, 1104.2, 988.0, 924.7, 722.8, 506.3.

R_f (hexane / ethyl acetate (5:1)) = 0.66.

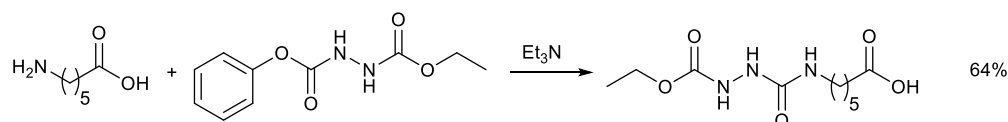


2.2 Synthesis of 1,2,4-triazoline-3,5-dione hexanoic acid (TAD-COOH), L2:

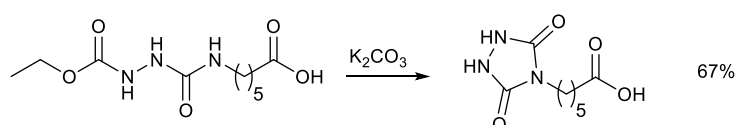
1.) Ethyl phenyl hydrazine-1,2-dicarboxylate synthesis



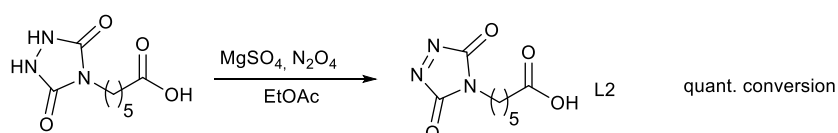
2.) Semicarbazide synthesis



3.) Cyclisation towards urazole



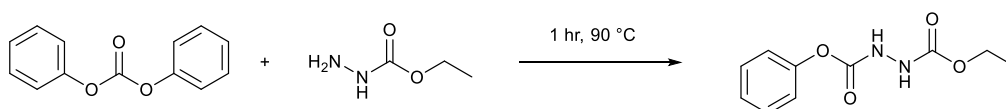
4.) Oxidation towards TAD



Scheme S2: Four-step reaction scheme of the synthesis of linker molecule, L2.

Synthesis of ethyl phenyl hydrazine-1,2-dicarboxylate

Synthesised according to previously reported procedure.²



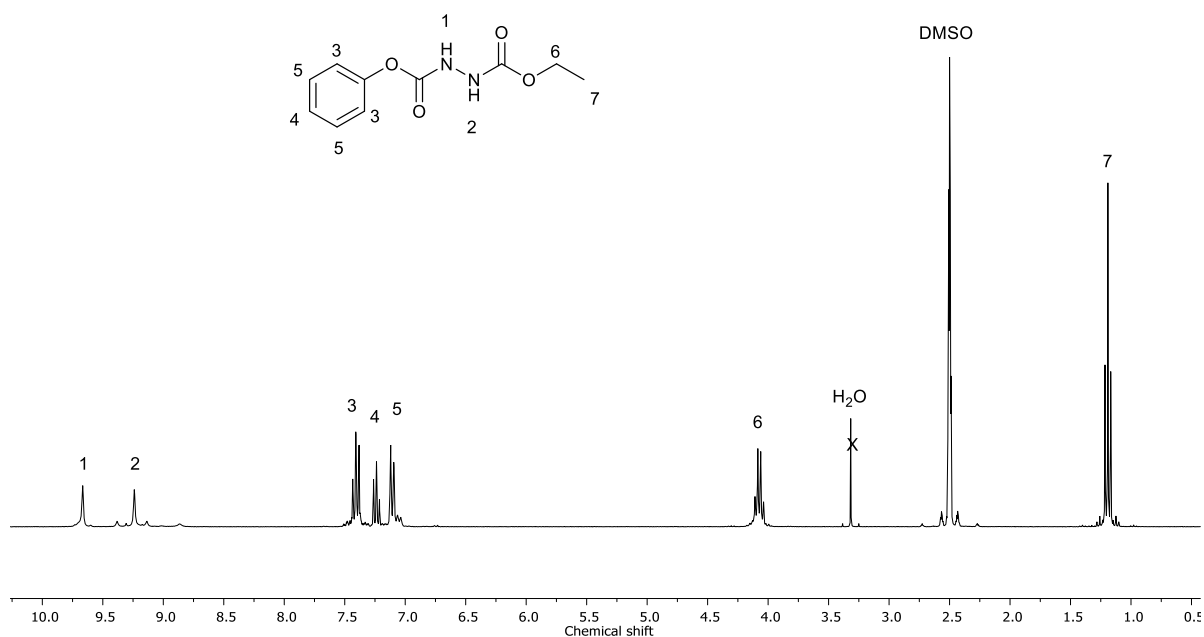
Diphenyl carbonate (60.02 g, 0.280 mol, 1.0 equiv.) and ethyl carbazate (58.37 g, 0.560 mol, 2.0 equiv.) were heated and stirred in bulk at 90 °C for 1 hour. The reaction was then precipitated into water (1.5 L) resulting in an emulsion. The precipitation was stirred fast for several hours and a white solid formed. The precipitation was filtered off and the precipitate was dried overnight under vacuum at 40 °C, resulting in a white, crystalline solid. Yield = 40.21 g, 64 %.

^1H NMR: (300 MHz, $\text{DMSO-}d_6$) δ /ppm: 9.67 (s, 1H, PhOC=ONH , ¹), 9.24 (s, 1H, $\text{NHC=OOCH}_2\text{CH}_3$, ²), 7.46-7.35 (m, 2H, Ph , ⁴), 7.30-7.21 (m, 1H, Ph , ⁵), 7.14-7.08 (m, 2H, Ph , ⁶), 4.07 (q, 2H, $J = 7.09, 7.09, \text{CH}_2\text{CH}_3$, ⁷), 1.19 (t, 3H, $J = 7.08, \text{CH}_2\text{CH}_3$, ⁸).

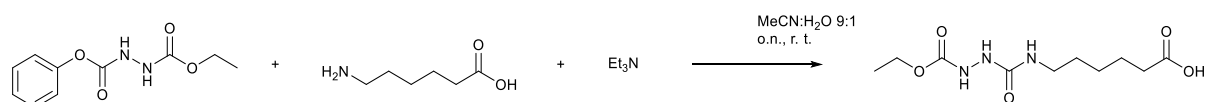
^{13}C NMR (APT, 100 MHz, $\text{DMSO-}d_6$) δ /ppm: 156.43, 154.86, 150.61, 129.48, 125.39, 121.47, 60.70, 14.52.

HRMS (ESI) m/z : $[\text{M} + \text{H}]^+$ for $[\text{C}_{10}\text{H}_{13}\text{N}_2\text{O}_4]^+$; calculated: 225.08698, found: 225.0872.

IR (ATR platinum diamond): ν [cm^{-1}] = 3225, 1742, 1699, 1519, 1489, 1224, 1189, 1160, 1094, 1045, 907, 792, 723, 688.



Synthesis of hexanoic acid semicarbazide



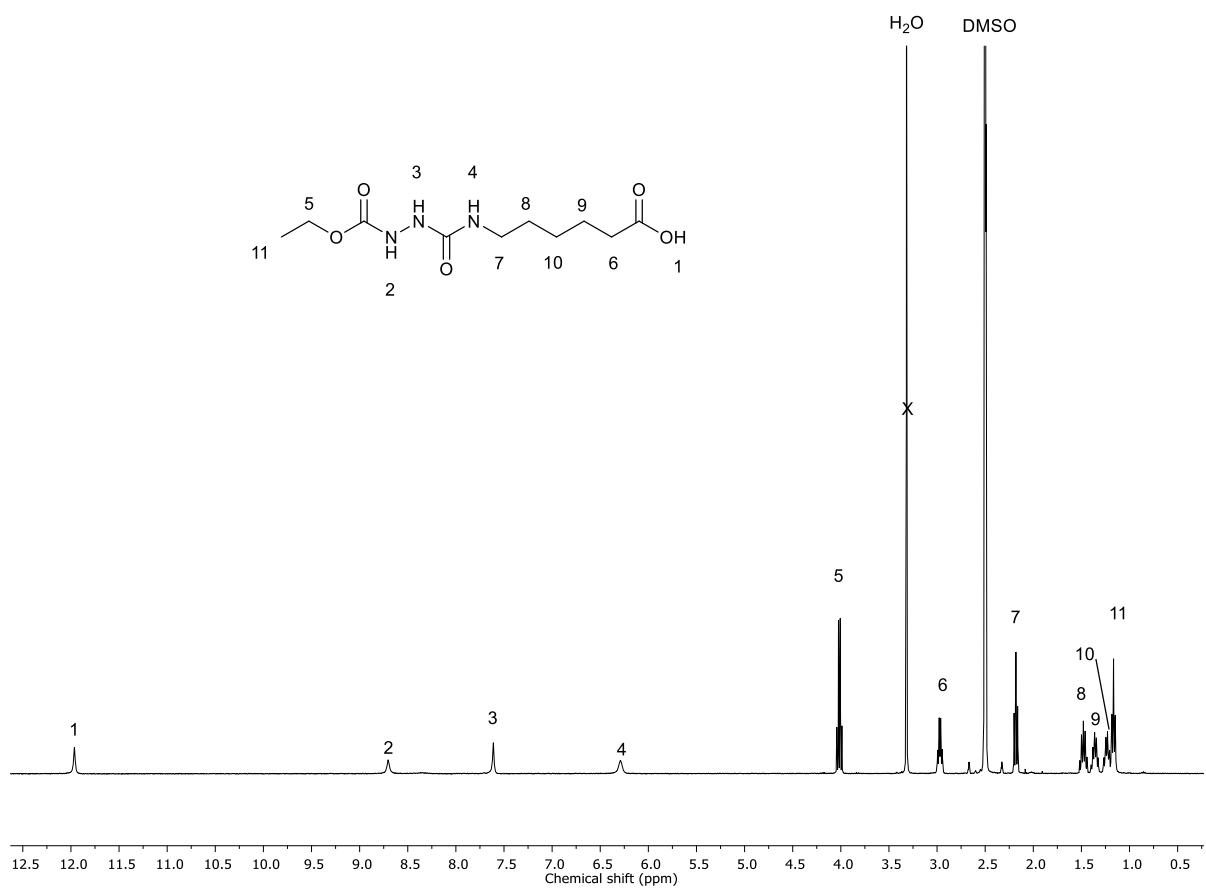
Aminocaproic acid (5.854 g, 44.6 mmol, 1.0 equiv.) and ethyl phenyl hydrazine dicarboxylate (10.0 g, 44.6 mmol, 1.0 equiv.) were dissolved in a 9:1 solution of acetonitrile:H₂O (150 mL). Triethylamine (12.4 mL, 89.2 mmol, 2.0 equiv.) was added and the reaction was stirred for at least 24 hours at room temperature. The acetonitrile was then removed *in vacuo* and then the residual water phase was diluted further with water (400 mL) and extracted three times with ethyl acetate to remove the phenol by-product from the reaction. The aqueous phase was then acidified to pH 1 with HCl in water (36 %) before removal of the water *in vacuo*. Water (20 mL) was then added and the product was stirred vigorously at room temperature overnight to extract any residual, unreacted aminocaproic acid. The reaction mixture was then filtered and the white precipitate dried overnight under vacuum at 40 °C. Yield = 7.40 g, 64 %.

¹H NMR (300 MHz, DMSO-*d*₆) δ /ppm: 11.96 (s, 1H, COOH_H, ¹), 8.70 (s, 1H, OC=ONH_H, ²), 7.61 (s, 1H, HNNHC=ONH, ³), 6.29 (s, 1H, HNNHC=ONH_H, ⁴), 4.02 (q, 2H, *J* = 7.08, 7.09, HNC=ONHCH₂, ⁵), 2.97 (q, 2H, *J* = 6.61, 6.65, CH₃CH₂O, ⁶), 2.18 (t, 2H, *J* = 7.38, CH₂COOH, ⁷), 1.48 (p, 2H, *J* = 7.40, 7.40, NHC=ONHCH₂CH₂, ⁸), 1.41-1.31 (m, 2H, CH₂CH₂COOH, ⁹), 1.28-1.20 (m, 2H, (CH₂)₂CH₂(CH₂)₂, ¹⁰), 1.17 (t, 3H, *J* = 7.09, CH₃, ¹¹).

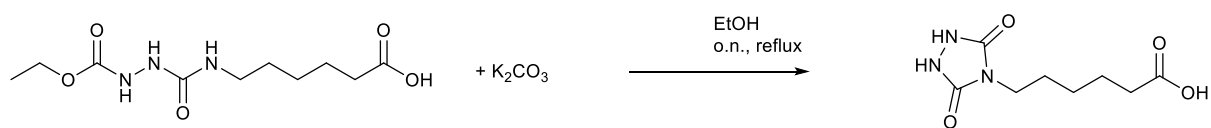
¹³C NMR (APT, 100 MHz, DMSO-*d*₆) δ /ppm: 174.44, 158.22, 156.89, 60.30, 38.96, 33.64, 29.58, 25.81, 24.25, 14.54.

HRMS (ESI) *m/z*: [M + H]⁺ for [C₁₀H₂₀N₃O₅⁺]; calculated: 262.1403, found: 262.1396.

IR (ATR platinum diamond): ν [cm⁻¹] = 3283, 2939, 1728, 1711, 1662, 1560, 1531, 1474, 1365, 1272, 1229, 1201, 1056, 898, 738, 648.



Ring closure of semicarbazide: Hexanoic acid urazole formation



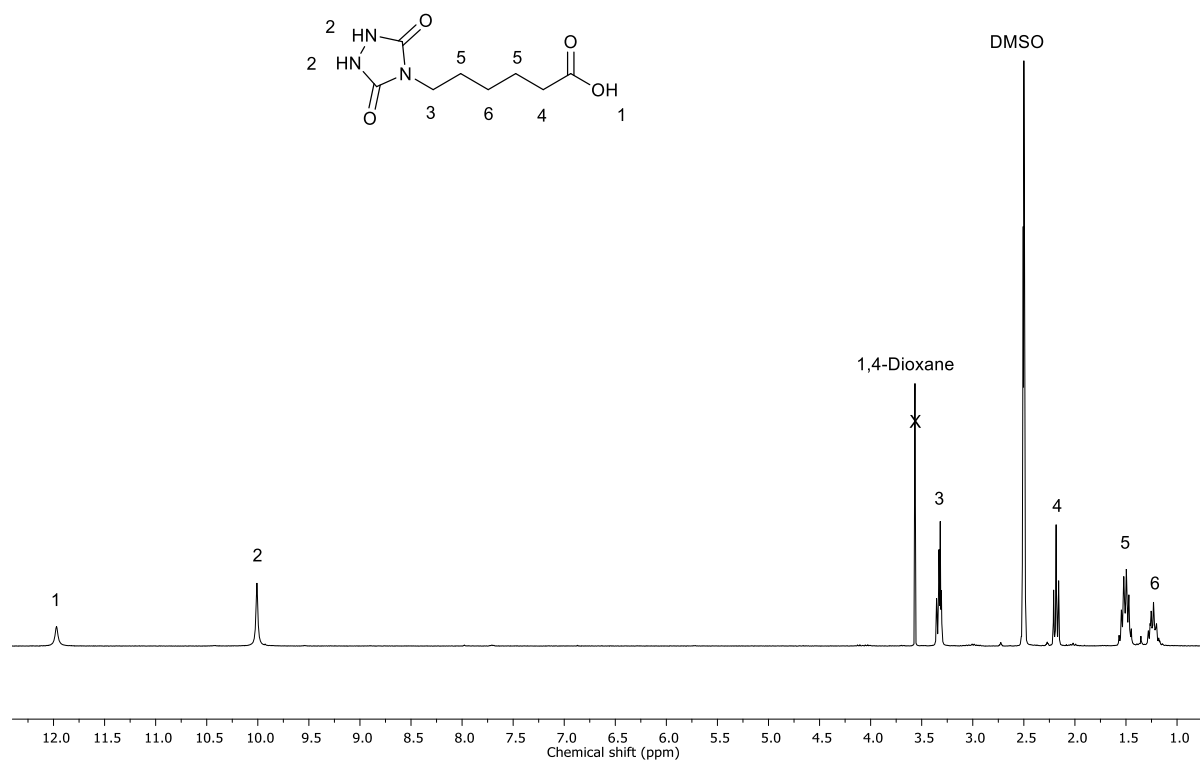
The semicarbazide (6.05 g, 23.2 mmol, 1.0 equiv.) was solubilised in ethanol (100 mL). K₂CO₃ (12.7 g, 91.9 mmol, 4.0 equiv.) was added and the reaction was stirred at reflux overnight. The reaction was then cooled to room temperature, filtered and the filtrate was evaporated *in vacuo* to complete dryness. The resulting solid was then solubilised in a minimum volume of 1,4-dioxane and then acidified at room temperature to pH = 1 with HCl in 1,4-dioxane. The precipitate was then filtered off and the solvent was then removed *in vacuo*, yielding a white, crystalline solid. Yield = 3.304 g, 67 %.

¹H NMR (400 MHz, DMSO-*d*₆) δ/ppm: 11.99 (s, 1H, COOH, ¹), 10.02 (s, 2H, HN-NH, ²), 3.39-3.30 (m, 2H, UrCH₂, ³), 2.18 (t, 2H, *J* = 7.33, CH₂COOH, ⁴), 1.50 (h, 4H, *J* = 6.07, 8.87, 7.47, CH₂CH₂CH₂CH₂CH₂, ⁵), 1.28-1.18 (m, 2H, (CH₂)₂CH₂(CH₂)₂, ⁶).

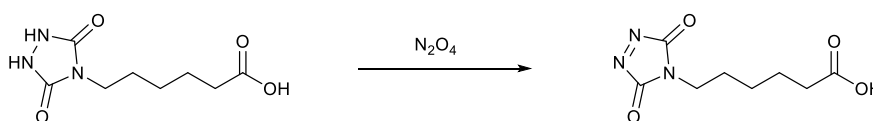
¹³C NMR (100 MHz, DMSO-*d*₆) δ/ppm: 174.38, 155.06, 37.75, 33.49, 27.27, 25.59, 24.02.

HRMS (ESI) *m/z*: [M + H]⁺ for [C₈H₁₄N₃O₄]⁺; calculated: 216.0984, found: 216.0981.

IR (ATR platinum diamond): ν [cm⁻¹] = 3166, 2931, 1668, 1474, 1415, 1348, 1225, 1190, 1116, 1030, 895, 849, 791, 767, 735, 639, 617.



Oxidation of urazole to corresponding 1,2,4-triazoline-3,5-dione



The urazole carboxylic acid (0.920 g, 4.27 mmol, 1.0 equiv.) was suspended in anhydrous ethyl acetate (100 mL). MgSO₄ (5.15 g, 42.8 mmol, 10 equiv.) was added. The reaction mixture was flushed for approximately 5 minutes with N₂O₄ gas, during which the white suspension became vividly pink in colour. The reaction mixture was then filtered and the solvent from the filtrate was removed *in vacuo*. The isolated TAD moiety was used stored under inert atmosphere at -20 °C because of the inherent high reactivity and instability of TAD compounds. ¹H NMR was used to determine the disappearance of the urazole proton resonances, to verify quantitative conversion, but the yield was not quantified.

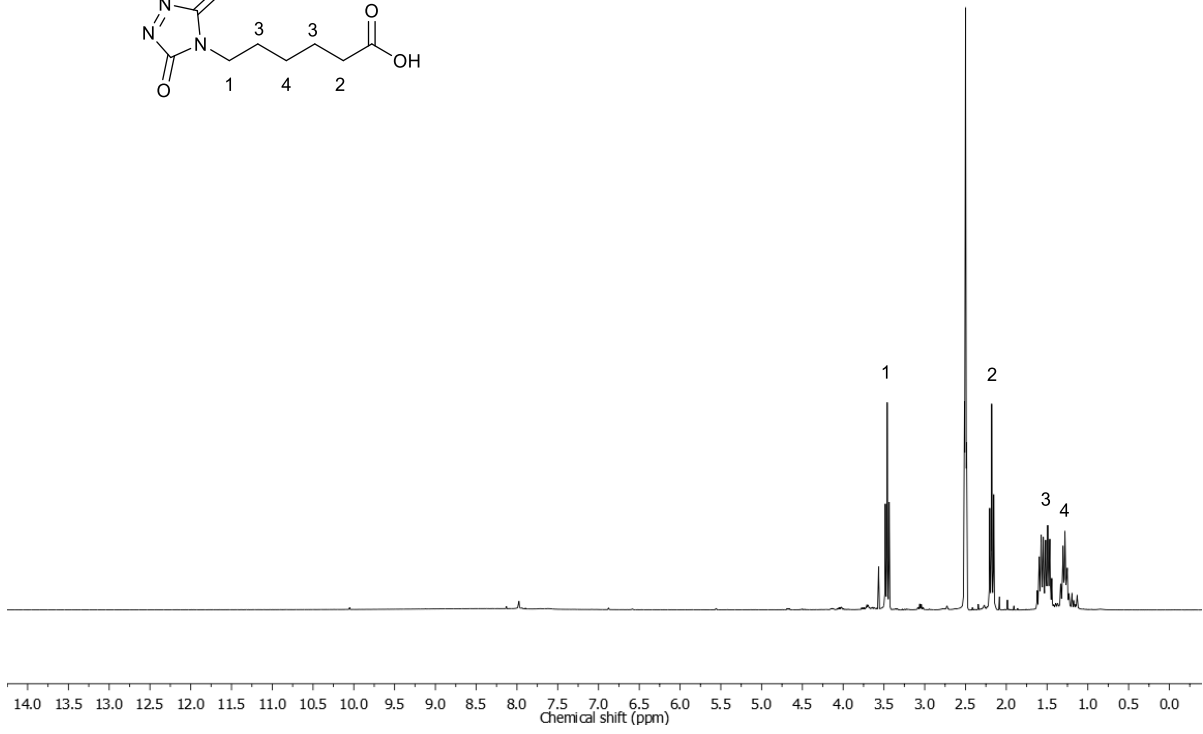
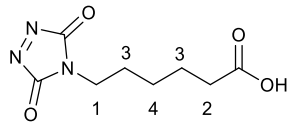
¹H NMR (300 MHz, DMSO-*d*₆) δ/ppm: 3.46 (t, 2H, *J* = 7.09, TADCH₂, ¹), 2.18 (t, 2H, *J* = 7.34, CH₂COOH, ²), 1.64-1.43 (m, 4H, CH₂CH₂CH₂CH₂CH₂, ³), 1.34-1.22 (m, 2H, (CH₂)₂CH₂(CH₂)₂, ⁴).

¹³C NMR (100 MHz, DMSO-*d*₆) δ/ppm: 174.34, 160.15, 40.50, 33.42, 26.39, 25.38, 23.89.

HRMS (ESI) *m/z*: [M + H]⁺ for [C₈H₁₂N₃O₄⁺]; calculated: 214.0822, found: 214.1879.

HRMS (ESI) *m/z*: [M + Na]⁺ for [C₈H₁₁N₃O₄]; calculated: 236.0642, found: 236.1720.

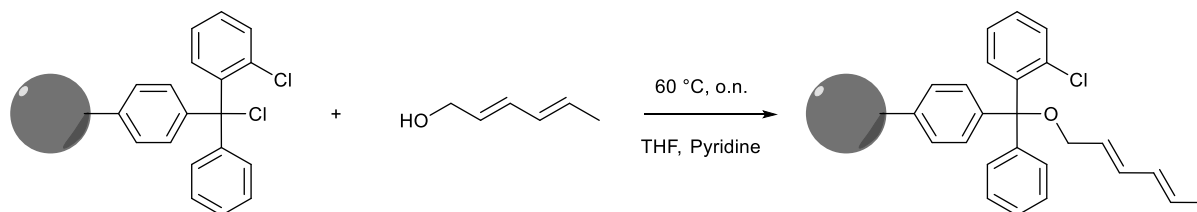
IR (ATR platinum diamond): ν [cm⁻¹] = 2934, 2357, 1742, 1698, 1525, 1393, 1336, 1270, 1212, 1176, 1137, 946, 853, 728, 677.



3 Solid-phase synthesis of sequence-defined oligomer

3.1 Loading of the 2-chlorotrityl chloride resin

Loading of the resin was achieved via an adapted version of a previously reported approach.³

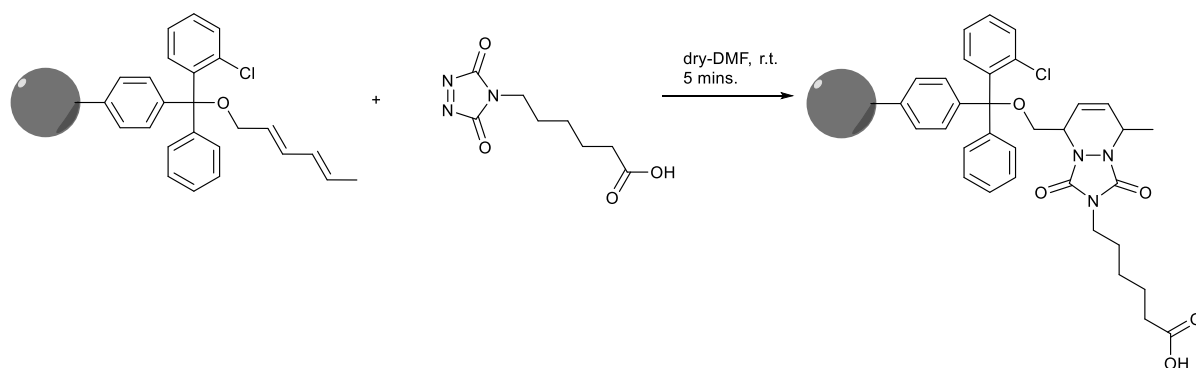


2-Chlorotrityl chloride resin (100-200 mesh, 1.6 mmol/g)(1.5 g, 1.0 equiv.) and sorbic alcohol (0.355 g, 1.5 equiv.) were dissolved in THF (15 mL) and pyridine (0.58 mL, 3.0 equiv.) under N₂ atmosphere and shaken vigorously at 60 °C overnight. The reaction was then filtered and shaken 3 times for 10 minutes with a 17:2:1 solution of DCM:MeOH:DIPEA (3 × 30 mL) to cap any unreacted sites, filtering after each 10 minute shake. The resin was then washed with DMF (3 × 30 mL), DCM (3 × 30 mL) and Et₂O (3 × 30 mL). The resin was then dried under vacuum at room temperature for 4 hours. The resin was then used directly for the first reaction step (*vide infra*).

3.2 Solid-phase synthesis protocol

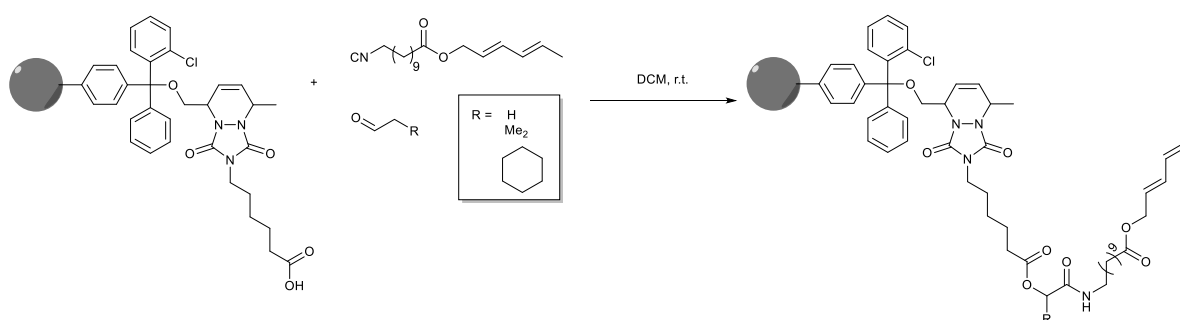
The synthesis was carried out via a two-step, iterative protocol. The protocol was repeated for up to 12 cycles, yielding a sequence-defined dodecamer. In addition to the dodecamer synthesised, a monomer, dimer, trimer and nonamer were also synthesised to allow full characterisation of the three components of the ABC sequence.

3.2.1 Step 1: TAD-COOH addition



The diene functionalised resin (50.0 mg, 1.0 equiv.) was swollen for at least 10 min. in anhydrous DMF (500 μ L). This was then filtered off. TAD-COOH (34 mg, 2.0 equiv.) solubilised in anhydrous DMF (500 μ L) was added and the reaction was shaken vigorously at room temperature for 5 minutes. The reaction was then filtered and the resin subsequently washed with DMF (\times 4), CHCl_3 (\times 4), MeOH (\times 4) and Et_2O (\times 4). The reaction was analysed by LCMS (see Figure S1). To do this, 2 mg of the reaction resin was removed and suspended for 5 minutes in a 1 % TFA solution (in DCM). This was then filtered, concentrated by evaporation and then diluted with acetonitrile.

3.2.2 Step 2: P-3CR



The resin from step 1 was swollen for at least 10 minutes in DCM (500 μ L). Then, the diene isocyanide linker molecule **L1** (0.350 g, 15 equiv.) and propanal, isobutyraldehyde or cyclohexanal (20 equiv.) were added. The reaction was shaken vigorously at room temperature for 30 minutes. After the 4th, 7th and 9th cycles, the reaction time was increased by a further 30 minutes as the reaction was slower as the chains lengthened (see IR monitoring

results). The reaction was then filtered and the resin subsequently washed with DMF (×4), CHCl₃ (×4), MeOH (×4) and Et₂O (×4). A 2 mg sample was again prepared for LCMS analysis after each cycle as described above up until the 8th cycle when the molecular weight of the oligomer was beyond the measurable limits of the instrument, thus thereafter no intermediate analysis via LCMS or HRMS was done.

This iterative, two-step protocol was repeated up to 12 times and was then capped as described below, resulting in a sequence-defined dodecamer. Yield = 14.4 mg, overall yield = 5 %.

3.2.3 Diene capping with Phenyl TAD

A separate monomer, dimer, trimer and dodecamer were synthesised and capped with phenyl TAD (synthesised according to previously reported work)⁴ to prevent further reaction of the conjugated double bond and to prevent partial hydrolysis of the conjugated ester bond during the mild cleavage conditions. The capping was done by first swelling the resin for at least 10 mins in anhydrous DMF (500 μL). This was then filtered off. Afterwards, PhTAD (5.0 equiv.) was dissolved in anhydrous DMF (500 μL) and added to the resin. The reaction was shaken vigorously at room temperature for 5 minutes. The reaction was then filtered, and the resin subsequently washed with DMF (×4), CHCl₃ (×4), MeOH (×4) and Et₂O (×4). The oligomer was cleaved entirely from the resin by suspension for 5 minutes in a 1 % TFA solution (in DCM). This was then filtered and concentrated by evaporation. The resulting colourless-yellow film was dried under vacuum at room temperature for several hours.

4 LCMS analysis of sequence-defined oligomers synthesised on solid-phase:

4.1 1st TAD-COOH Diels-Alder addition to the solid-phase resin:

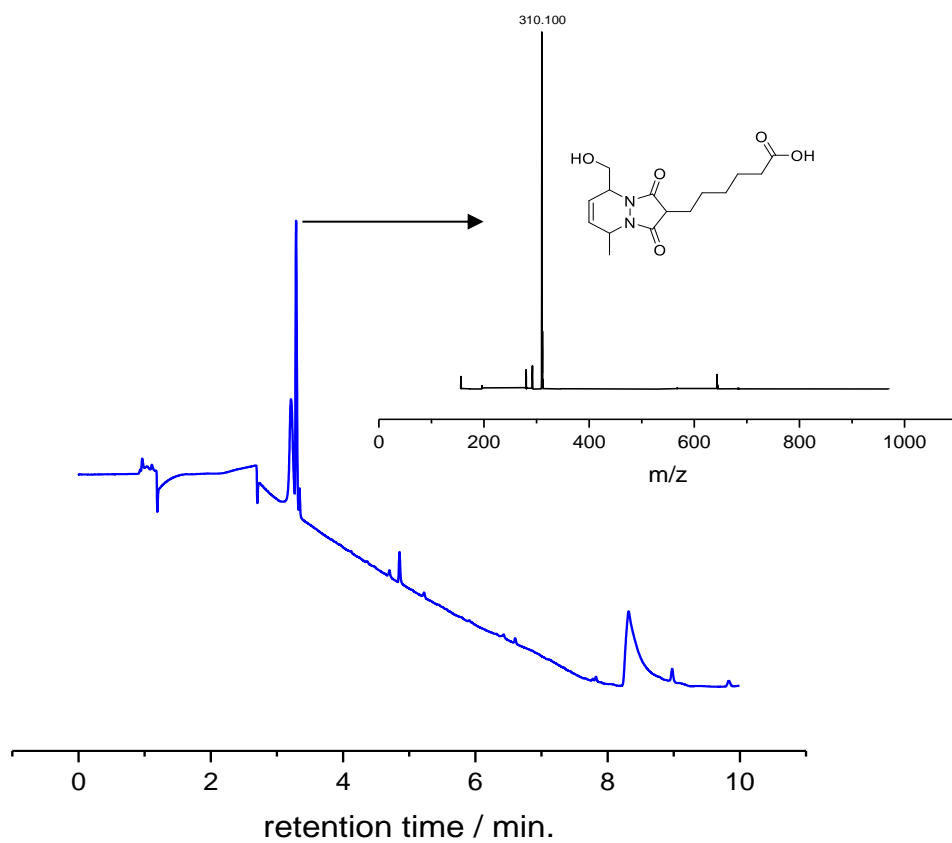


Figure S1: LCMS chromatogram ($\lambda = 214$ nm) of the adduct formed when TAD-COOH reacted with the diene-functionalised resin.

4.2 1st solid-phase P-3CR:

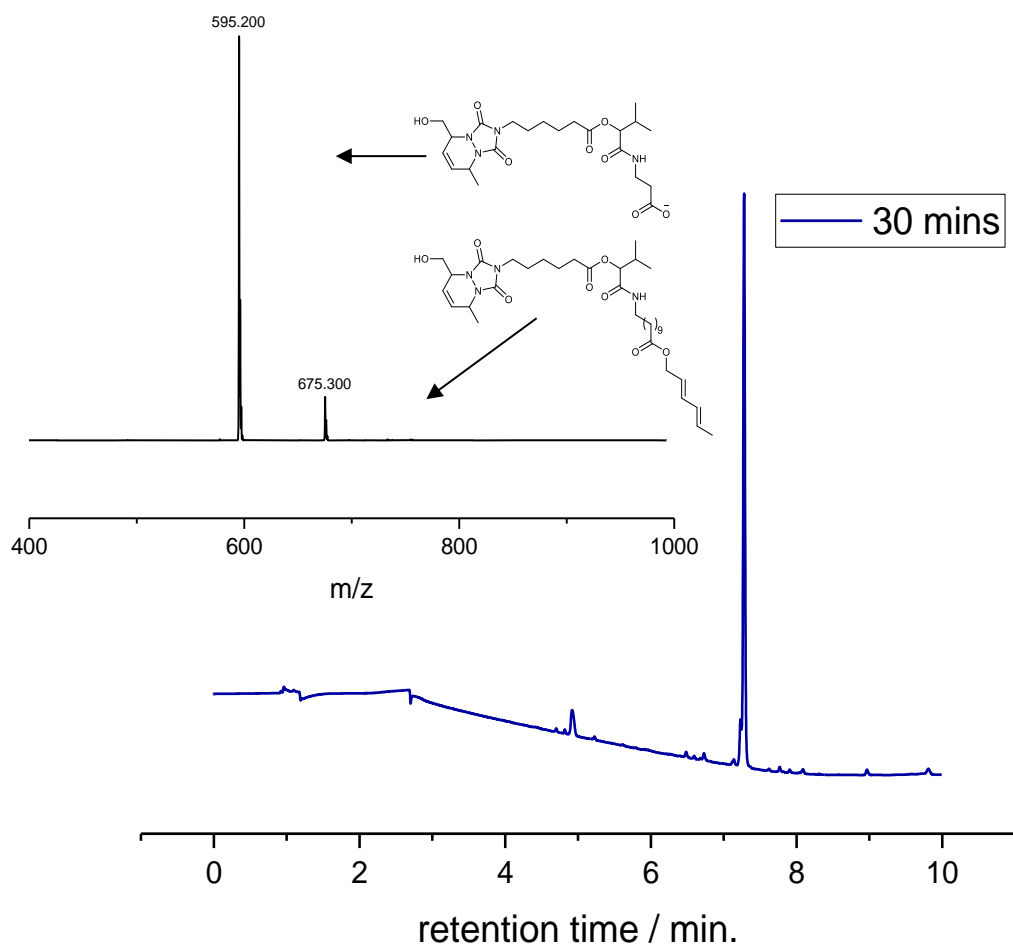


Figure S2: LCMS chromatogram ($\lambda = 214$ nm) of the 1st P-3CR on the solid-phase. The reaction is observed to be already complete after 30 minutes, with the only two peaks at 4.9 and 7.3 minutes both corresponding to the desired product. The ionisation of the molecule during LCMS accounts for the fragmentation pattern observed in the MS spectrum.

4.3 Stacked LCMS chromatograms showing the second up to the eighth iterative reaction cycle:

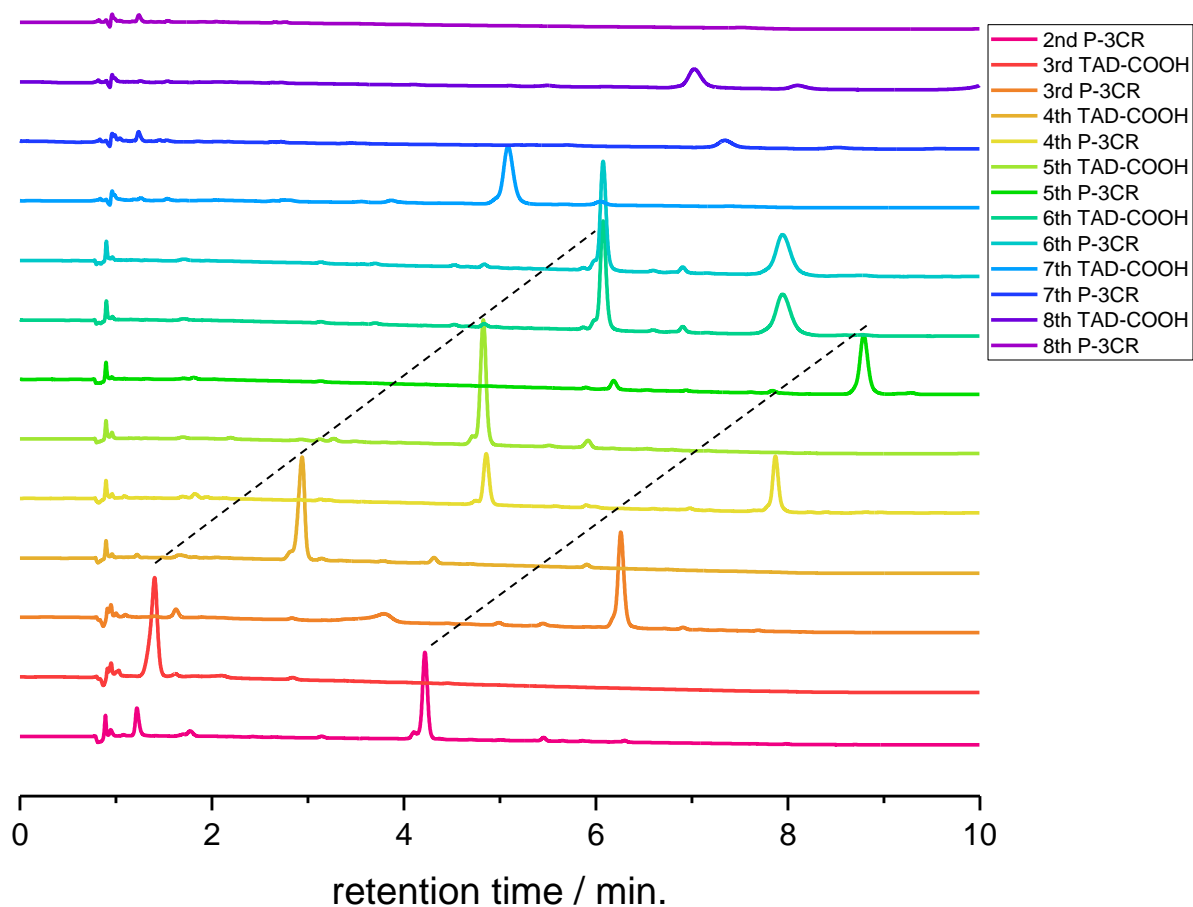


Figure S3: LCMS chromatograms ($\lambda = 214$ nm). A clear shift in the retention time between the carboxylic acid terminated chain and diene terminated chain can be seen. By following the same step in the cycle, one can also see a shift in retention time as the molecular weight increases. It should be noted that this does not continue in a linear fashion because from the 2nd to the 5th cycle, the solvent gradient (from acetonitrile to water) was 75-100 %, and from the 6th to the 8th cycle it was 90-100 %. The LCMS gives little information after the 7th TAD-COOH addition.

5 HRMS results of the solid-phase synthesised sequence-defined oligomers:

5.1 PhTAD capped monomer, dimer, trimer, nonamer and dodecamer:

| Sample name | m/z exp. | formula | m/z theo. | Δ m/z |
|-------------|-----------|--|-----------|--------------|
| Monomer | 872.4503 | [C ₄₄ H ₆₃ N ₇ O ₁₀ Na] ⁺ | 872.4529 | 0.0026 |
| Monomer | 850.4689 | [C ₄₄ H ₆₃ N ₇ O ₁₀ H] ⁺ | 850.4709 | 0.0020 |
| Dimer | 1429.8302 | [C ₇₃ H ₁₀₉ N ₆ O ₇ NH ₄] ⁺ | 1429.8341 | 0.0038 |
| Trimer | 2051.1721 | [C ₁₀₆ H ₁₆₁ N ₁₅ O ₂₄ Na] ⁺ | 2051.1731 | 0.0010 |
| Trimer | 1037.0803 | [C ₁₀₆ H ₁₆₁ N ₁₅ O ₂₄ Na ₂] ²⁺ | 1037.0812 | 0.0009 |
| Trimer | 2029.1907 | [C ₁₀₆ H ₁₆₁ N ₁₅ O ₂₄ H] ⁺ | 2029.1912 | 0.0005 |
| Trimer | 1015.0980 | [C ₁₀₆ H ₁₆₁ N ₁₅ O ₂₄ H ₂] ²⁺ | 1015.0992 | 0.0012 |
| Nonamer | 2704.6421 | [C ₂₈₂ H ₄₄₈ N ₃₆ O ₆₄ Na ₂] ²⁺ | 2704.6346 | 0.0075 |
| Nonamer | 1810.7606 | [C ₂₈₂ H ₄₄₈ N ₃₆ O ₆₄ Na ₃] ³⁺ | 1810.7528 | 0.0078 |
| Nonamer | 1363.8164 | [C ₂₈₂ H ₄₄₈ N ₃₆ O ₆₄ Na ₄] ⁴⁺ | 1363.8119 | 0.0045 |
| Nonamer | 1095.6492 | [C ₂₈₂ H ₄₄₈ N ₃₆ O ₆₄ Na ₅] ⁵⁺ | 1095.6475 | 0.0017 |
| Nonamer | 2682.6045 | [C ₂₈₂ H ₄₄₈ N ₃₆ O ₆₄ H ₂] ²⁺ | 2682.6527 | 0.0482 |
| Dodecamer | 3669.7145 | [C ₃₈₂ H ₅₉₉ N ₅₁ O ₈₇ Na ₂] ²⁺ | 3669.6900 | 0.0245 |
| Dodecamer | 2454.1257 | [C ₃₈₂ H ₅₉₉ N ₅₁ O ₈₇ Na ₃] ³⁺ | 2454.1231 | 0.0026 |
| Dodecamer | 1846.3446 | [C ₃₈₂ H ₅₉₉ N ₅₁ O ₈₇ Na ₄] ⁴⁺ | 1846.3396 | 0.0050 |
| Dodecamer | 1481.6702 | [C ₃₈₂ H ₅₉₉ N ₅₁ O ₈₇ Na ₅] ⁵⁺ | 1481.6695 | 0.0007 |
| Dodecamer | 1238.5566 | [C ₃₈₂ H ₅₉₉ N ₅₁ O ₈₇ Na ₆] ⁶⁺ | 1238.5561 | 0.0005 |
| Dodecamer | 1064.9032 | [C ₃₈₂ H ₅₉₉ N ₅₁ O ₈₇ Na ₇] ⁷⁺ | 1064.9037 | 0.0005 |
| Dodecamer | 2432.1402 | [C ₃₈₂ H ₅₉₉ N ₅₁ O ₈₇ H ₃] ³⁺ | 2432.1411 | 0.0009 |
| Dodecamer | 1824.3595 | [C ₃₈₂ H ₅₉₉ N ₅₁ O ₈₇ H ₄] ⁴⁺ | 1824.1411 | 0.0018 |
| Dodecamer | 1459.6874 | [C ₃₈₂ H ₅₉₉ N ₅₁ O ₈₇ H ₅] ⁵⁺ | 1459.6876 | 0.0004 |
| Dodecamer | 1216.5735 | [C ₃₈₂ H ₅₉₉ N ₅₁ O ₈₇ H ₆] ⁶⁺ | 1216.5742 | 0.0007 |
| Dodecamer | 1043.0632 | [C ₃₈₂ H ₅₉₉ N ₅₁ O ₈₇ H ₇] ⁷⁺ | 1042.9218 | 0.1414 |

5.2 Nonamer isotopic pattern and SEC-ESI-MS results (solid-phase, before optimisation):

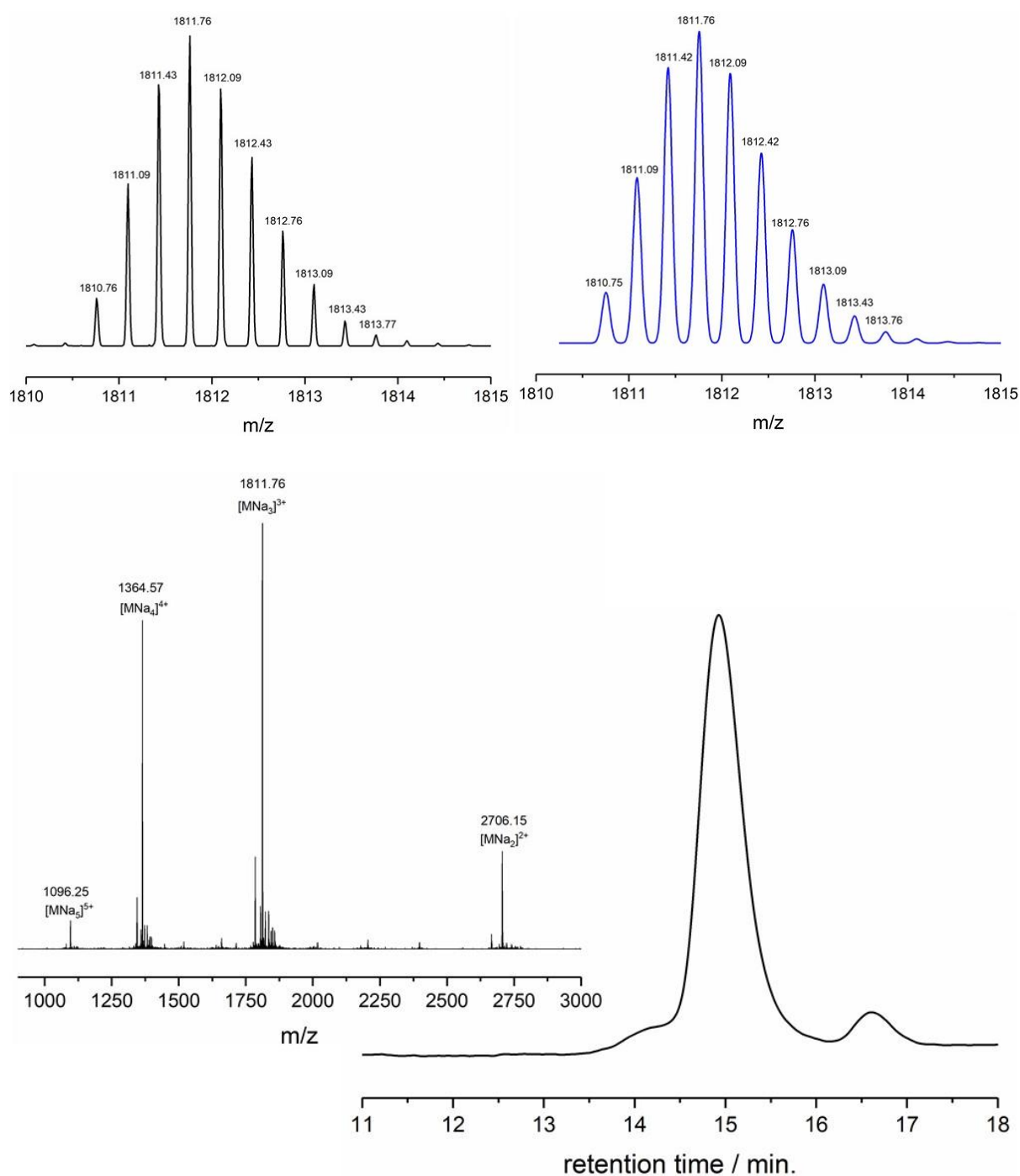


Figure S4: SEC-ESI-MS chromatogram and corresponding mass spectrum as well as isotopic pattern at 14.90 min retention time of the nonamer synthesised on solid phase.

5.3 Dodecamer isotopic pattern and SEC-ESI-MS results (solid phase, before optimisation):

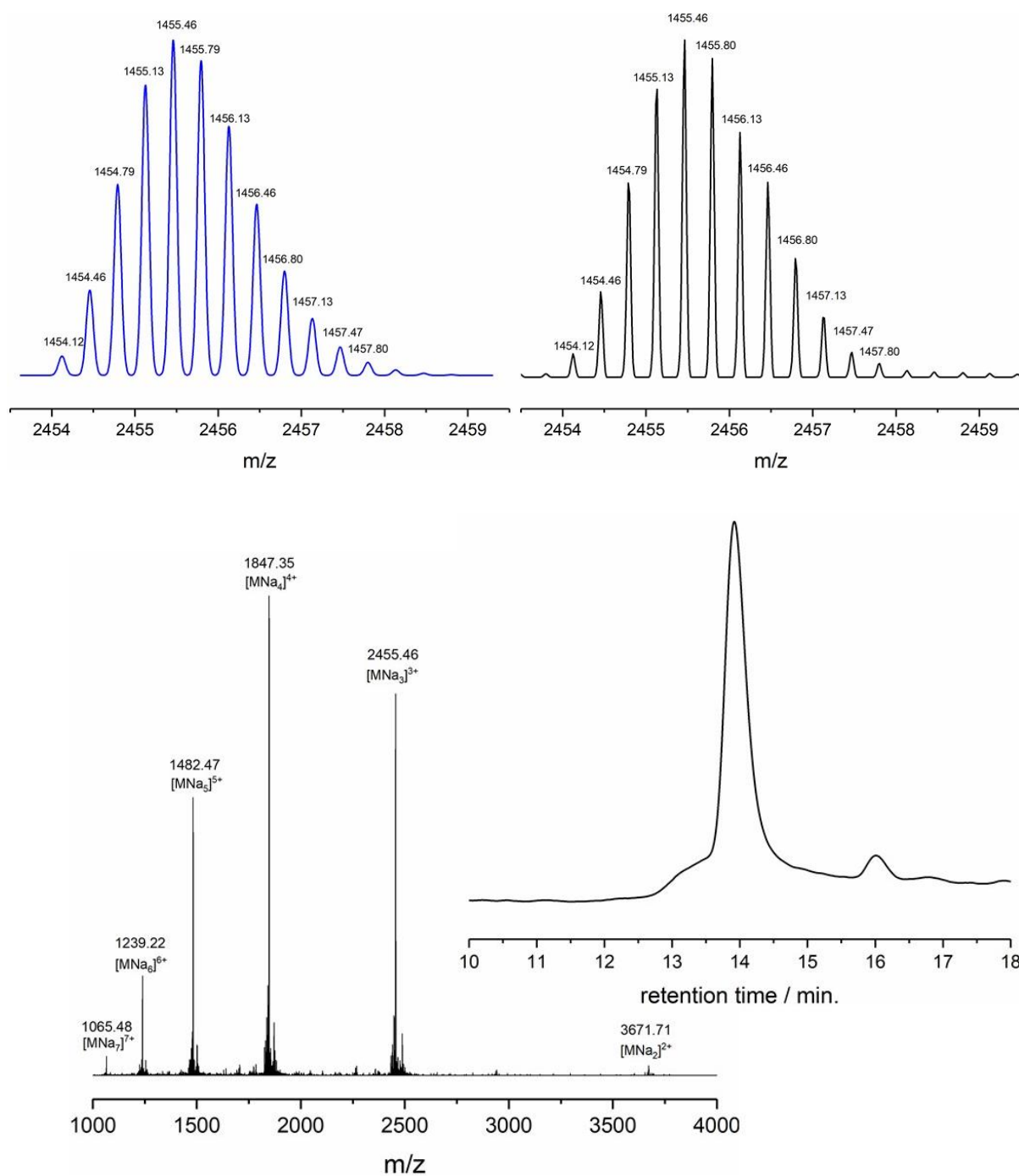


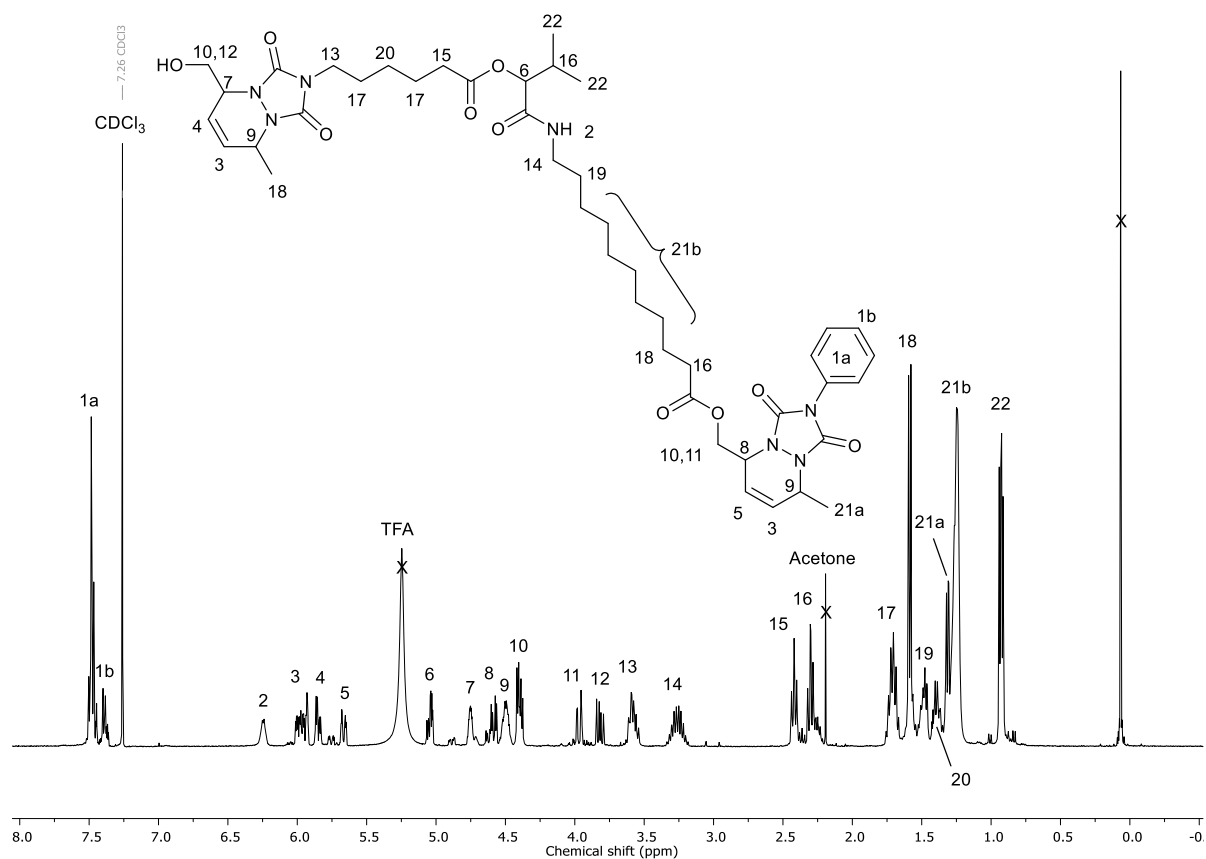
Figure S5: SEC-ESI-MS chromatogram and corresponding mass spectrum as well as isotopic pattern at 13.91 min retention time of the dodecamer synthesised on solid phase.

5.4 HRMS results of growing oligomer (solid phase, 2nd-9th cycle):

| Sample name | m/z exp. | formula | m/z theo. | Δ m/z |
|-----------------------------------|-----------|--|-----------|--------------|
| 2 nd TAD COOH addition | 888.5066 | [C ₄₄ H ₇₀ N ₇ O ₁₂] ⁺ | 888.5070 | 0.0004 |
| 2 nd P-3CR | 1237.7671 | [C ₆₅ H ₁₀₅ N ₈ O ₁₅] ⁺ | 1237.7694 | 0.0023 |
| 3 rd TAD COOH addition | 1450.8398 | [C ₇₃ H ₁₁₆ N ₁₁ O ₁₉] ⁺ | 1450.8444 | 0.0046 |
| 3 rd TAD COOH addition | 1467.8684 | [C ₇₃ H ₁₁₅ N ₁₁ O ₁₉ NH ₄] ⁺ | 1467.8709 | 0.0025 |
| 3 rd P-3CR | 1854.1453 | [C ₉₈ H ₁₅₇ N ₁₂ O ₂₂] ⁺ | 1854.1530 | 0.0023 |
| 4 th TAD COOH addition | 2084.2503 | [C ₁₀₆ H ₁₆₇ N ₁₅ O ₂₆ NH ₄] ⁺ | 2084.2545 | 0.0042 |
| 4 th P-3CR | 2447.5242 | [C ₁₂₈ H ₂₀₄ N ₁₆ O ₂₉ NH ₄] ⁺ | 2447.5318 | 0.0076 |
| 5 th TAD COOH addition | 2665.5801 | [C ₁₃₆ H ₂₁₅ N ₁₉ O ₃₃ Na] ⁺ | 2665.5622 | 0.0179 |
| 5 th P-3CR | 3014.8362 | [C ₁₅₇ H ₂₅₀ N ₂₀ O ₃₆ Na] ⁺ | 3014.8239 | 0.0123 |
| 6 th TAD COOH addition | 3227.9143 | [C ₁₆₅ H ₂₆₁ N ₂₃ O ₄₀ Na] ⁺ | 3227.8880 | 0.0263 |
| 6 th TAD COOH addition | 1625.4449 | [C ₁₆₅ H ₂₆₁ N ₂₃ O ₄₀ Na ₂] ²⁺ | 1625.4440 | 0.0009 |
| 6 th TAD COOH addition | 1091.2903 | [C ₁₆₅ H ₂₆₁ N ₂₃ O ₄₀ Na ₃] ³⁺ | 1091.2924 | 0.0021 |
| 6 th P-3CR | 1827.1003 | [C ₁₉₀ H ₃₀₂ N ₂₄ O ₄₃ Na ₂] ²⁺ | 1827.0984 | 0.0019 |
| 6 th P-3CR | 1225.7278 | [C ₁₉₀ H ₃₀₂ N ₂₄ O ₄₃ Na ₃] ³⁺ | 1225.7286 | 0.0008 |
| 7 th TAD COOH addition | 3844.3025 | [C ₁₉₈ H ₃₁₃ N ₂₇ O ₄₇ Na] ⁺ | 3844.2716 | 0.0309 |
| 7 th TAD COOH addition | 1933.6581 | [C ₁₉₈ H ₃₁₃ N ₂₇ O ₄₇ Na ₂] ²⁺ | 1933.6358 | 0.0223 |
| 7 th TAD COOH addition | 1296.7524 | [C ₁₉₈ H ₃₁₃ N ₂₇ O ₄₇ Na ₃] ³⁺ | 1296.7536 | 0.0012 |
| 7 th P-3CR | 2115.2764 | [C ₂₂₀ H ₃₅₀ N ₂₈ O ₅₀ Na ₂] ²⁺ | 2115.2745 | 0.0019 |
| 8 th TAD COOH addition | 2221.8201 | [C ₂₂₈ H ₃₆₁ N ₃₁ O ₅₄ Na ₂] ²⁺ | 2221.8120 | 0.0081 |
| 8 th P-3CR | 2396.4470 | [C ₂₄₉ H ₃₉₆ N ₃₂ O ₅₇ Na ₂] ²⁺ | 2396.4428 | 0.0042 |
| 8 th P-3CR | 1605.2920 | [C ₂₄₉ H ₃₉₆ N ₃₂ O ₅₇ Na ₃] ³⁺ | 1605.2916 | 0.0004 |
| 9 th P-3CR | 2704.6414 | [C ₂₈₂ H ₄₄₈ N ₃₆ O ₆₄ Na ₂] ²⁺ | 2704.6346 | 0.0068 |
| 9 th P-3CR | 1810.7607 | [C ₂₈₂ H ₄₄₈ N ₃₆ O ₆₄ Na ₃] ³⁺ | 1810.7518 | 0.0089 |
| 9 th P-3CR | 1363.8149 | [C ₂₈₂ H ₄₄₈ N ₃₆ O ₆₄ Na ₄] ⁴⁺ | 1363.8119 | 0.0030 |
| 9 th P-3CR | 1095.6473 | [C ₂₈₂ H ₄₄₈ N ₃₆ O ₆₄ Na ₅] ⁵⁺ | 1095.6474 | 0.0001 |

6 NMR spectra of solid-phase synthesised sequence-defined oligomers:

6.1 PhTAD capped monomer:



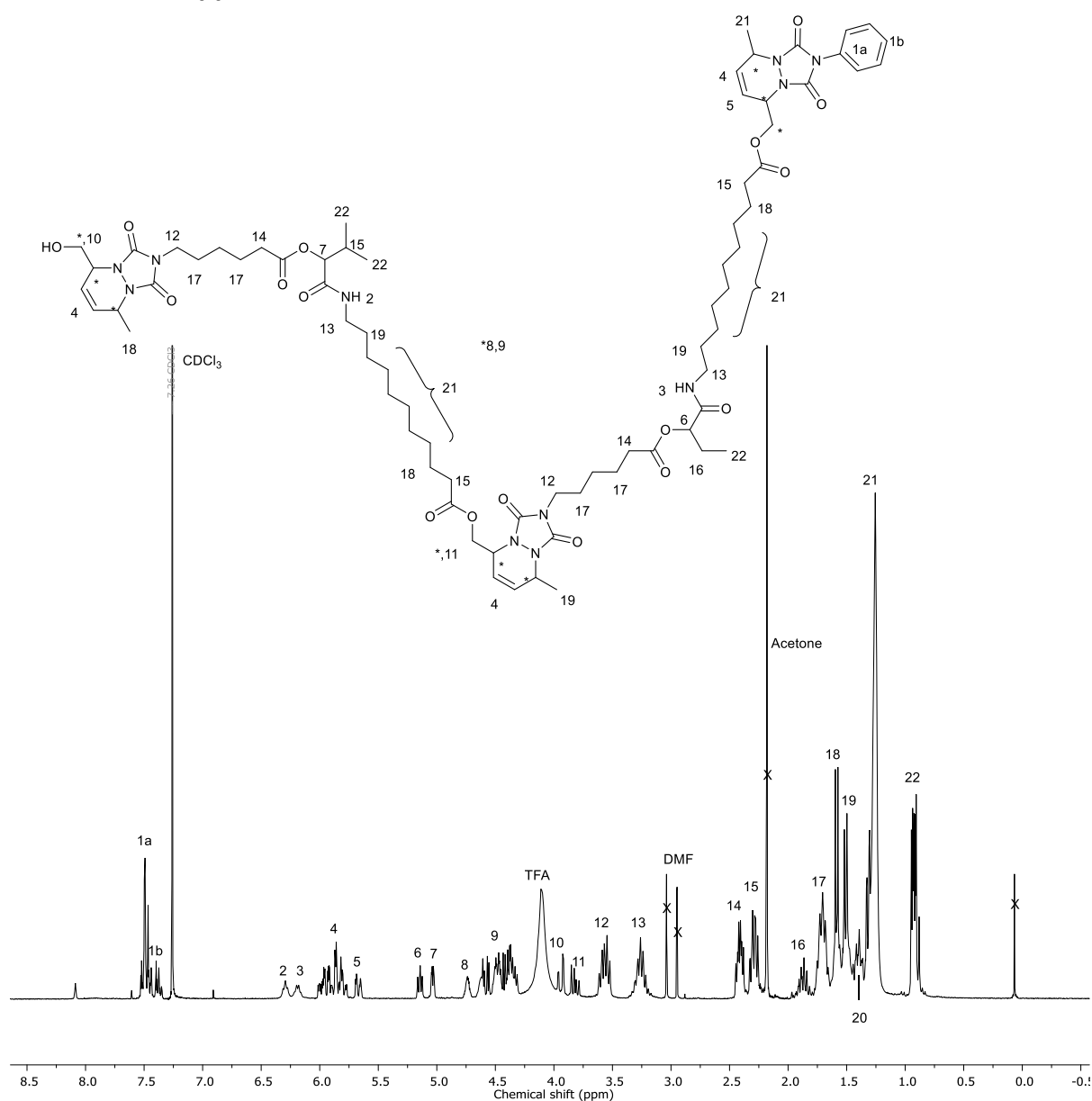
¹H NMR (300 MHz, CDCl₃) δ /ppm: 7.58-7.44 (m, 4H, ^{1a}), 7.43-7.35 (m, 1H, ^{1b}), 6.24 (s, 1H, ²), 6.02-5.92 (m, 2H, ³), 5.88-5.82 (m, 1H, ⁴), 5.70-5.64 (m, 1H, ⁵), 5.03 (dd, 1H, $J = 1.65, 4.76$, ⁶), 4.75 (s, 1H, ⁷), 4.66-4.55 (m, 1H, ⁸), 4.49 (s, 2H, ⁹), 4.40 (dd, 2H, $J = 5.13, 11.60$, ¹⁰), 3.94 (d, 1H, $J = 9.94$, ¹¹), 3.82 (dd, 1H, $J = 7.51, 12.39$, ¹²), 3.65-3.53 (m, 2H, ¹³), 3.41-3.13 (m, 2H, ¹⁴), 2.42 (t, 2H, $J = 7.45$, ¹⁵), 2.33-2.21 (m, 3H, ¹⁶), 1.78-1.65 (m, 4H, ¹⁷), 1.59 (d, 5H, $J = 6.59$, ¹⁸), 1.53-1.45 (m, 2H, ¹⁹), 1.45-1.35 (m, 2H, ²⁰), 1.35-1.16 (m, 14H, ^{21a,b}), 0.93 (dd, 5H, $J = 4.21, 6.77$, ²²).

¹³C NMR (APT, 100MHz, CDCl₃) δ /ppm: 173.80, 172.47, 170.90, 158.85, 158.44, 155.67, 1525.60, 151.56, 131.02, 129.89, 29.34, 129.06, 128.58, 126.00, 121.17, 120.90, 77.96, 65.77, 64.90, 62.70, 59.11, 53.14, 51.33, 49.65, 39.81, 39.34, 34.12, 33.93, 30.71, 29.49, 29.40, 29.17, 27.53, 26.87, 26.00, 24.78, 24.31, 19.69, 18.79, 17.95, 1711.

HRMS (ESI)

| m/z exp. | formula | m/z theo. | Δ m/z |
|----------|--|-----------|--------------|
| 872.4503 | $[\text{C}_{44}\text{H}_{63}\text{N}_7\text{O}_{10}\text{Na}]^+$ | 872.4529 | 0.0026 |
| 850.4689 | $[\text{C}_{44}\text{H}_{63}\text{N}_7\text{O}_{10}\text{H}]^+$ | 850.4709 | 0.0020 |

6.2 PhTAD capped dimer:



^1H NMR (300 MHz, CDCl_3) δ /ppm: 7.56-7.44 (m, 4H, 1^{a}), 7.42-7.33 (m, 1H, 1^{b}), 6.30 (t, 1H, $J = 5.90, 5.63, 2^{\text{}}$), 6.19 (d, 2H, $J = 5.59, 3^{\text{}}$), 6.04-5.75 (m, 5H, $4^{\text{}}$), 5.71-5.64 (m, 1H, $5^{\text{}}$), 5.14 (dd, 1H, $J = 5.02, 6.47, 6^{\text{}}$), 5.03 (dd, 1H, $J = 1.69, 4.69, 7^{\text{}}$), 4.78-4.29 (m, 10H, $8,9^{\text{}}$), 3.94 (dd, 1H, $J = 2.33,$

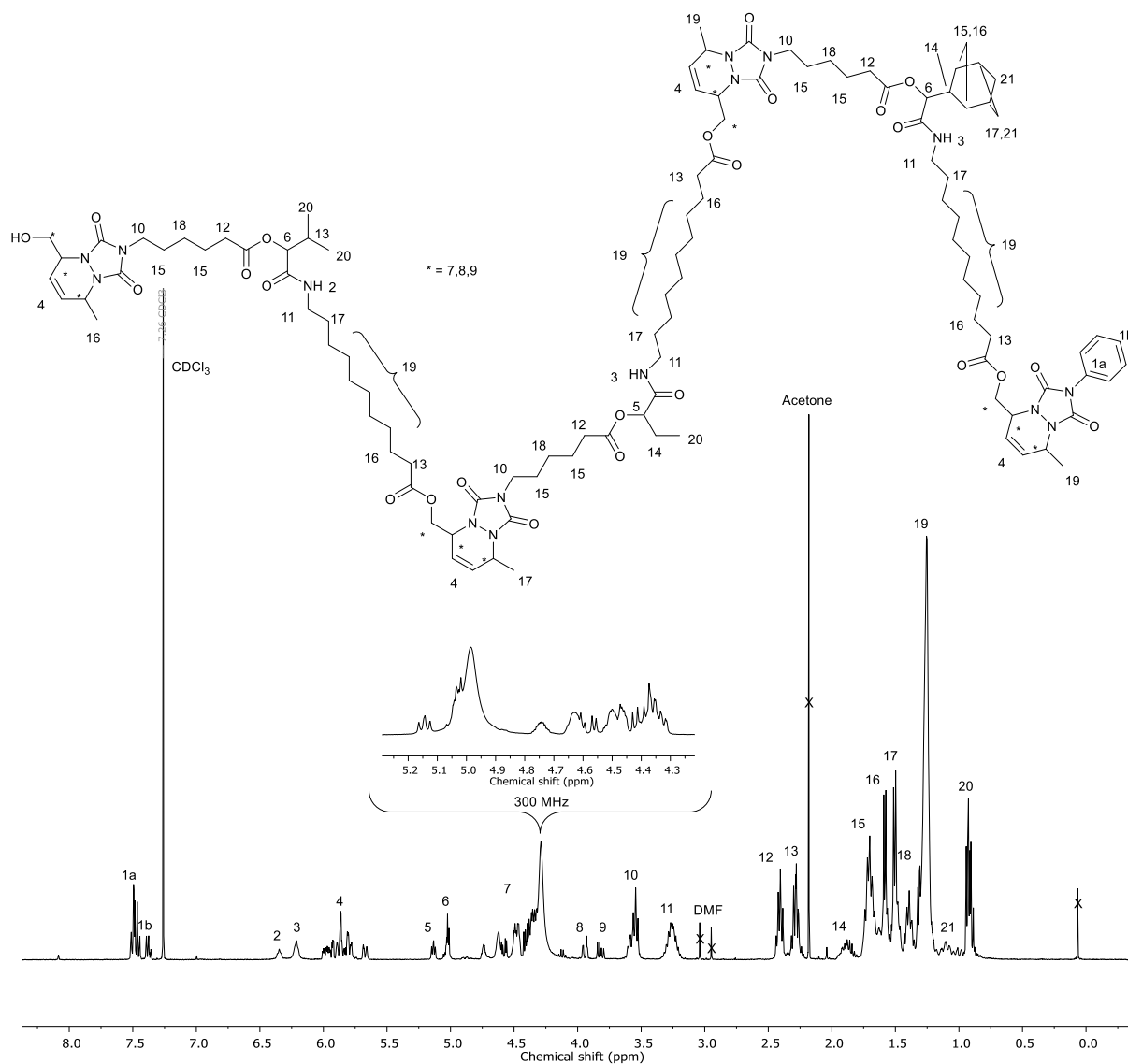
12.40, ¹⁰), 3.82 (ddd, 1H, *J* = 0.70, 7.27, 12.37, ¹¹), 3.63-3.50 (m, 4H, ¹²), 3.36-3.17 (m, 4H, ¹³), 2.47-2.36 (m, 4H, ¹⁴), 2.29 (ddd, 5H, *J* = 5.03, 6.99, 8.18, ¹⁵), 1.95-1.80 (m, 2H, ¹⁶), 1.70 (p, 8H, *J* = 7.11, 6.94, ¹⁷), 1.59 (d, 7H, *J* = 6.59, ¹⁸), 1.55-1.46 (m, 7H, ¹⁹), 1.46-1.36 (m, 4H, ²⁰), 1.36-1.11 (m, 27H, ²¹), 0.99-0.87 (m, 9H, ²²).

¹³C NMR (APT, 100MHz, CDCl₃) δ /ppm: 173.64, 173.56, 170.61, 170.21, 129.96, 129.29, 129.00, 128.41, 125.88, 121.53, 121.11, 121.05, 78.07, 74.83, 65.11, 62.74, 62.68, 59.35, 59.30, 53.16, 52.93, 51.31, 51.08, 49.65, 39.63, 39.26, 38.99, 34.13, 34.10, 31.08, 30.70, 29.57, 29.46, 29.34, 29.30, 29.23, 29.20, 27.67, 27.57, 26.94, 26.94, 26.11, 26.06, 25.25, 24.81, 24.39, 19.64, 19.51, 18.85, 17.96, 17.94, 17.14.

HRMS (ESI)

| m/z exp. | formula | m/z theo. | Δ m/z |
|-----------|--|-----------|--------|
| 1429.8302 | [C ₇₃ H ₁₀₉ N ₆ O ₇ NH ₄] ⁺ | 1429.8341 | 0.0038 |

6.3 PhTAD capped trimer:



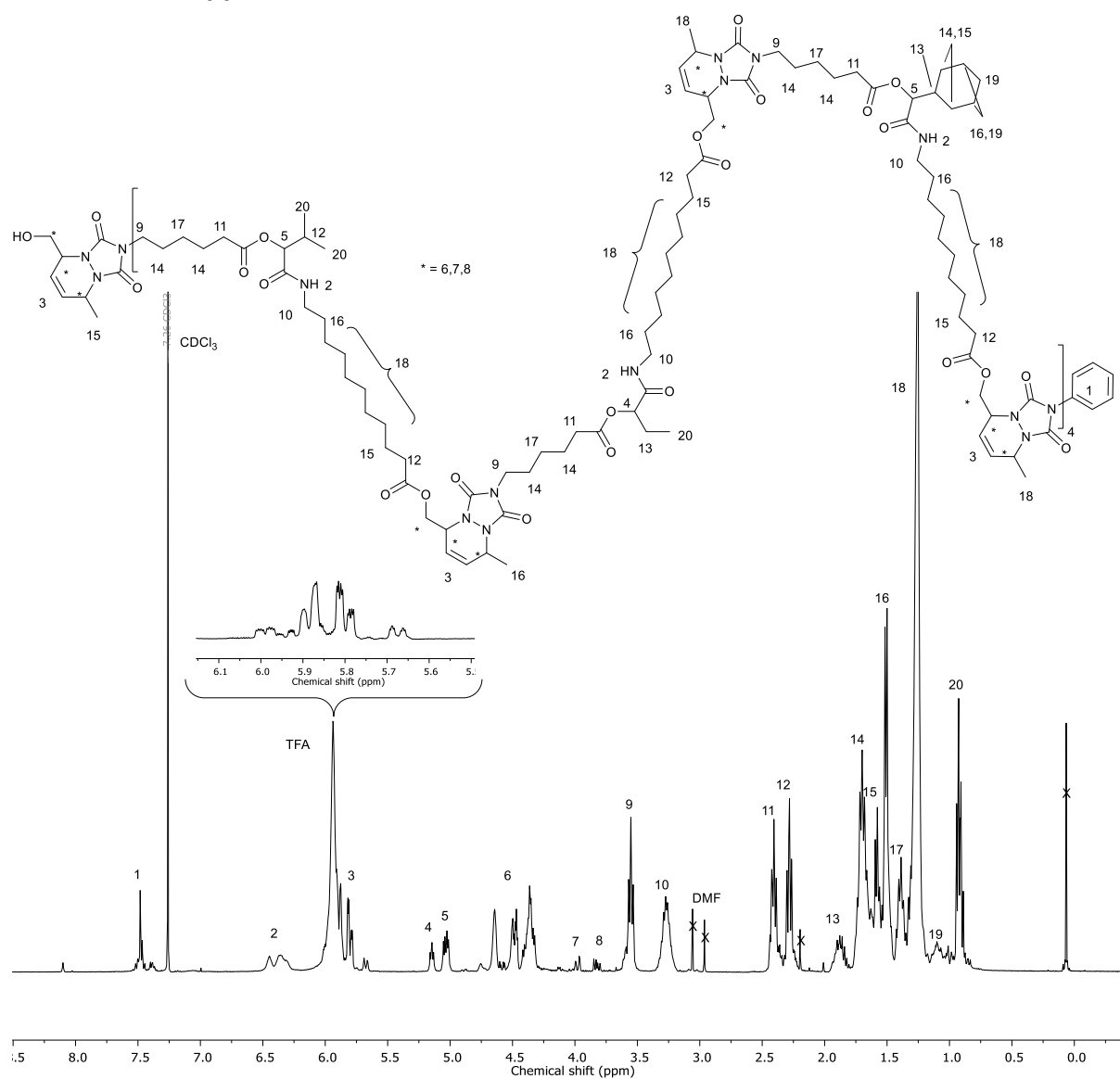
^1H NMR (400 MHz, CDCl_3) δ /ppm: 7.54-7.43 (m, 4H, 1^{a}), 7.41-7.34 (m, 1H, 1^{b}), 6.35 (s, 1H, 2), 6.21 (s, 2H, 3), 6.03-5.61 (m, 8H, 4), 5.18-5.09 (m, 1H, 5), 5.09-4.99 (m, 1H, 6), 4.77-4.17 (m, 14H, 7), 3.93 (dd, 1H, $J = 2.31, 9.76, 2.05$, 8), 3.84 (dd, 1H, $J = 6.80, 4.88, 7.32$, 9), 3.64-3.49 (m, 6H, 10), 3.38-3.16 (m, 6H, 11), 2.40 (t, 6H, 12), 2.37-2.22 (m, 7H, 13), 1.99-1.81 (m, 3H, 14), 1.80-1.65 (m, 14H, 15), 1.58 (d, 11H, $J = 6.59$, 16), 1.51 (d, 11H, $J = 6.59$, 17), 1.43-1.34 (m, 6H, 18), 1.34-1.17 (m, 42H, 19), 1.14-1.00 (m, 4H, 21), 1.00-0.78 (m, 9H, 20).

^{13}C NMR (APT, 100MHz, CDCl_3) δ /ppm: 173.59, 172.39, 170.88, 170.45, 170.42, 158.83, 158.41, 152.75, 129.93, 129.87, 129.29, 129.02, 128.45, 125.91, 121.42, 121.06, 121.00, 78.02, 77.64, 74.77, 65.02, 62.75, 62.68, 59.26, 59.22, 53.15, 52.92, 51.31, 51.09, 39.70, 39.67, 39.27, 39.02, 34.12, 34.10, 34.04, 33.99, 30.70, 29.84, 29.54, 29.47, 29.45, 29.43, 29.35, 29.33, 29.31, 29.27, 29.22, 29.19, 27.67, 26.92, 26.13, 26.09, 26.04, 24.80, 24.40, 24.37, 19.65, 19.50, 18.83.

HRMS (ESI)

| Sample name | m/z exp. | formula | m/z theo. | Δ m/z |
|-------------|-----------|--|-----------|--------------|
| Trimer | 2051.1721 | $[\text{C}_{106}\text{H}_{161}\text{N}_{15}\text{O}_{24}\text{Na}]^+$ | 2051.1731 | 0.0010 |
| Trimer | 1037.0803 | $[\text{C}_{106}\text{H}_{161}\text{N}_{15}\text{O}_{24}\text{Na}_2]^{2+}$ | 1037.0812 | 0.0009 |
| Trimer | 2029.1907 | $[\text{C}_{106}\text{H}_{161}\text{N}_{15}\text{O}_{24}\text{H}]^+$ | 2029.1912 | 0.0005 |
| Trimer | 1015.0980 | $[\text{C}_{106}\text{H}_{161}\text{N}_{15}\text{O}_{24}\text{H}_2]^{2+}$ | 1015.0992 | 0.0012 |

6.4 PhTAD capped dodecamer:



¹H NMR (400 MHz, CDCl₃) δ /ppm: 7.55-7.35 (m, 5H, ¹), 6.50-6.27 (m, 12H, ²), 6.07-5.63 (s, 26H, ³), 5.18-5.10 (m, 4H, ⁴), 5.08-4.98 (m, 8H, ⁵), 4.80-4.24 (m, 50H, ⁶), 3.97 (dd, 1H, *J* = 2.06, 10.44, 1.76, ⁷), 3.85 (dd, 1H, *J* = 7.23, 4.57, 7.73, ⁸), 3.55 (t, 24H, *J* = 7.26, ⁹), 3.34-3.18 (m, 24H, ¹⁰), 2.48-2.33 (m, 24H, ¹¹), 2.33-1.21 (m, 25H, ¹²), 1.97-1.78 (m, 12H, ¹³), 1.78-1.61 (m, 56H, ¹⁴), 1.58 (t, 35H, *J* = 6.90, ¹⁵), 1.51 (d, 44H, *J* = 6.47, ¹⁶), 1.45-1.35 (m, 24H, ¹⁷), 1.35-1.17 (m, 168H, ¹⁸), 1.16-0.97 (m, 16H, ¹⁹), 0.96-0.82 (m, 36H, ²⁰).

¹³C NMR (APT, 100MHz, CDCl₃) δ /ppm: 173.73, 172.49, 172.29, 171.45, 171.07, 171.00, 158.93, 158.52, 153.82, 152.70, 129.87, 129.76, 129.34, 129.07, 128.61, 126.01, 121.13, 120.93, 116.35, 113.51, 77.84, 74.67, 64.85, 62.77, 53.14, 52.92, 51.08, 40.12, 39.85, 39.09, 34.09, 33.96, 30.72, 29.54, 29.45, 29.42, 29.33, 29.25, 29.21, 27.63, 27.50, 26.90, 26.09, 26.05, 26.02, 25.90, 25.26, 24.79, 24.34, 19.70, 19.55, 18.79, 17.08.

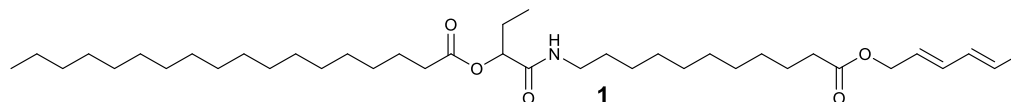
IR (ATR platinum diamond): ν [cm⁻¹] = 3355, 2930, 2856, 1740, 1692, 1555, 1461, 1427, 1372, 1209, 1154, 891, 805, 780, 766, 693, 646.

HRMS (ESI)

| Sample name | m/z exp. | formula | m/z theo. | Δ m/z |
|-------------|-----------|--|-----------|--------|
| Dodecamer | 3669.7145 | [C ₃₈₂ H ₅₉₉ N ₅₁ O ₈₇ Na ₂] ²⁺ | 3669.6900 | 0.0245 |
| Dodecamer | 2454.1257 | [C ₃₈₂ H ₅₉₉ N ₅₁ O ₈₇ Na ₃] ³⁺ | 2454.1231 | 0.0026 |
| Dodecamer | 1846.3446 | [C ₃₈₂ H ₅₉₉ N ₅₁ O ₈₇ Na ₄] ⁴⁺ | 1846.3396 | 0.0050 |
| Dodecamer | 1481.6702 | [C ₃₈₂ H ₅₉₉ N ₅₁ O ₈₇ Na ₅] ⁵⁺ | 1481.6695 | 0.0007 |
| Dodecamer | 1238.5566 | [C ₃₈₂ H ₅₉₉ N ₅₁ O ₈₇ Na ₆] ⁶⁺ | 1238.5561 | 0.0005 |
| Dodecamer | 1064.9032 | [C ₃₈₂ H ₅₉₉ N ₅₁ O ₈₇ Na ₇] ⁷⁺ | 1064.9037 | 0.0005 |
| Dodecamer | 2432.1402 | [C ₃₈₂ H ₅₉₉ N ₅₁ O ₈₇ H ₃] ³⁺ | 2432.1411 | 0.0009 |
| Dodecamer | 1824.3595 | [C ₃₈₂ H ₅₉₉ N ₅₁ O ₈₇ H ₄] ⁴⁺ | 1824.1411 | 0.0018 |
| Dodecamer | 1459.6874 | [C ₃₈₂ H ₅₉₉ N ₅₁ O ₈₇ H ₅] ⁵⁺ | 1459.6876 | 0.0004 |
| Dodecamer | 1216.5735 | [C ₃₈₂ H ₅₉₉ N ₅₁ O ₈₇ H ₆] ⁶⁺ | 1216.5742 | 0.0007 |
| Dodecamer | 1043.0632 | [C ₃₈₂ H ₅₉₉ N ₅₁ O ₈₇ H ₇] ⁷⁺ | 1042.9218 | 0.1414 |

7 Solution-phase synthesis of sequence-defined oligomer:

1st Passerini reaction



0.20 g of Stearic acid (0.70 mmol, 1.0 eq.) was dissolved in 2.0 mL dichloromethane (DCM) (0.35 M). 100 μ L Propanal (82.0 mg, 1.40 mmol, 2.0 eq.) and 0.31 g of linker molecule **L1** (1.05 mmol, 1.5 eq.) were added and the mixture was stirred at room temperature for 12 hours. Subsequently, the solvent was removed under reduced pressure and the crude product was purified by column chromatography (hexane/ethyl acetate 15:1 \rightarrow ethyl acetate) to afford product **1** as a white solid in a yield of 97 % (433 mg, 0.68 mmol). Furthermore, the excess of the monomer was recovered (67 mg, 0.23 eq.) and could be reused.

¹H NMR (400 MHz, CDCl₃) δ /ppm: 6.28 – 6.19 (m, 1H, ¹), 6.11 – 5.96 (m, 2H, ¹), 5.80 – 5.68 (m, 1H, ¹), 5.68 – 5.53 (m, 1H, ¹), 5.15 (dd, $J = 6.6, 4.9$ Hz, 1H, ²), 4.57 (d, $J = 6.6$ Hz, 2H, ³), 3.36 – 3.15 (m, 2H, ⁴), 2.39 (t, $J = 7.5$ Hz, 2H, ⁵), 2.30 (t, $J = 7.6$ Hz, 2H, ⁵), 2.01 – 1.81 (m, 2H, ⁶), 1.81 – 1.72 (m, 3H, ⁷), 1.72 – 1.55 (m, 6H, ⁸), 1.54 – 1.17 (m, 40H, ⁹), 1.00 – 0.75 (m, 6H, ¹⁰).

¹³C NMR (101MHz, CDCl₃) δ /ppm: 172.59, 169.81, 77.36, 74.94, 39.34, 34.50, 32.07, 29.84, 29.80, 29.75, 29.61, 29.51, 29.42, 29.36, 29.29, 26.97, 25.22, 25.15, 22.84, 18.26, 14.26, 9.14.

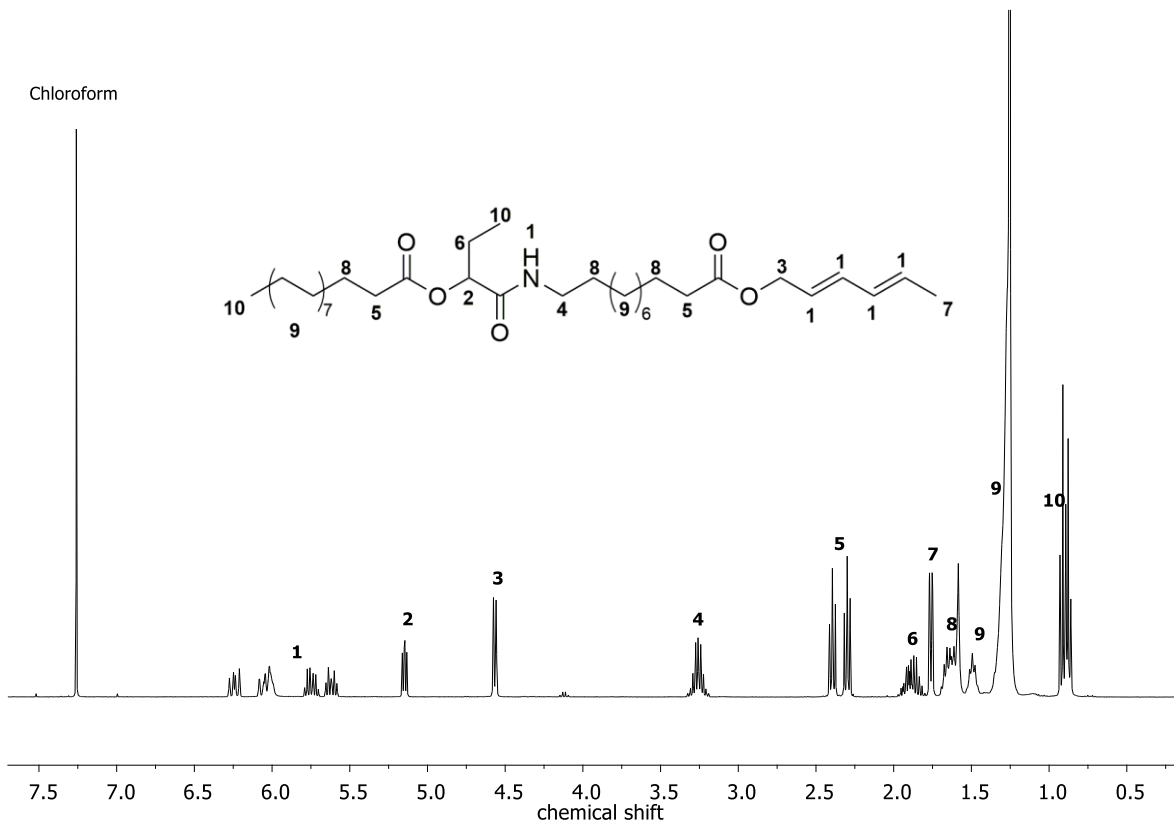
ESI-MS:

| m/z exp. | formula | m/z theo. | Δ m/z |
|----------|---|-----------|--------------|
| 656.5205 | [C ₃₉ H ₇₁ NO ₅ Na] ⁺ | 656.5224 | 0.0019 |
| 634.5405 | [C ₃₉ H ₇₁ NO ₅ H] ⁺ | 634.5392 | 0.0013 |

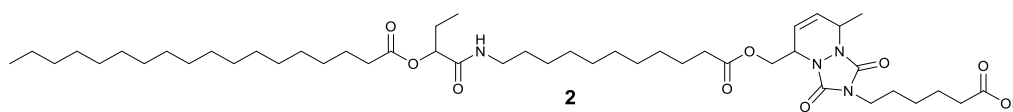
IR (ATR platinum diamond): ν [cm⁻¹] = 3255.6, 3090.6, 2916.9, 2849.8, 1733.2, 1653.1, 1549.0, 1466.4, 1380.3, 1292.6, 1270.4, 1254.7, 1240.4, 1210.1, 1188.6, 1160.3, 1105.0, 987.4, 923.2, 721.5, 699.2, 511.4, 440.2, 396.0.

R_f: (hexane / ethyl acetate (4:1)) = 0.56.

Chloroform

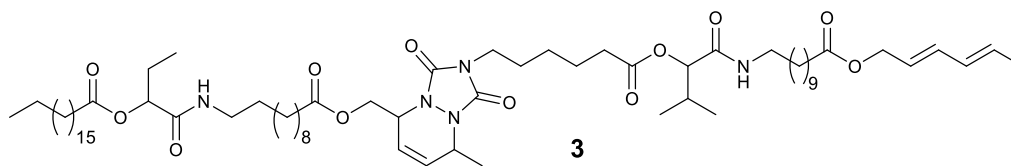


1st TAD-Diels Alder reaction



0.402 g of **1** (0.634 mmol, 1.0 eq.) was dissolved in 3 mL dry ethyl acetate and 0.150 g of linker molecule **L2** (0.697 mmol, dissolved in 1 mL dry ethyl acetate) was added dropwise to the solution, until it stayed pink, indicating that **1** was fully converted. 20 μ L of 2,3-dimethylbut-2-ene (17 mg, 0.20 mmol) was added to the reaction mixture in order to quench the excess of the TAD compound **L2**. The solvent was evaporated under reduced pressure and the crude product **2** was used without further purification or analysis.

2nd Passerini reaction



0.516 g of **2** (0.609 mmol, 1.0 eq.) was dissolved in 3 mL DCM. Subsequently, 0.11 mL of isobutyraldehyde (87 g, 1.218 mmol, 2.0 eq.) and 0.266 g of **L1** (0.914 mmol, 1.5 eq.) were added. The reaction was stirred for 12 hours at room temperature and the crude product was purified by column chromatography (hexane:ethyl acetate 10:1→1:2). Product **3** was obtained as a yellowish, highly viscous oil in a yield of 88 % (648 mg, 0.535 mmol, overall yield over two reaction steps).

¹H NMR (500 MHz, CDCl₃) δ /ppm: 6.46 – 5.43 (m, 8H, ¹), 5.19 – 5.09 (m, 1H, ²), 5.06 – 4.94 (m, 1H, ³), 4.60 (s, 1H, ⁴), 4.55 (d, *J* = 6.6 Hz, 2H, ⁵), 4.50 – 4.43 (m, 1H, ⁶), 4.34 (m, 2H, ⁷), 3.59 – 3.48 (m, 2H, ⁸), 3.33 – 3.14 (m, 4H, ⁹), 2.47 – 2.34 (m, 4H, ¹⁰), 2.34 – 2.20 (m, 5H, ¹¹), 1.88 (m, 2H, ¹²), 1.65 (m, 14H, ¹³), 1.50 (d, *J* = 6.4 Hz, 6H, ¹⁴), 1.43 – 0.97 (m, 54H, ¹⁵), 0.96 – 0.81 (m, 12H, ¹⁶).

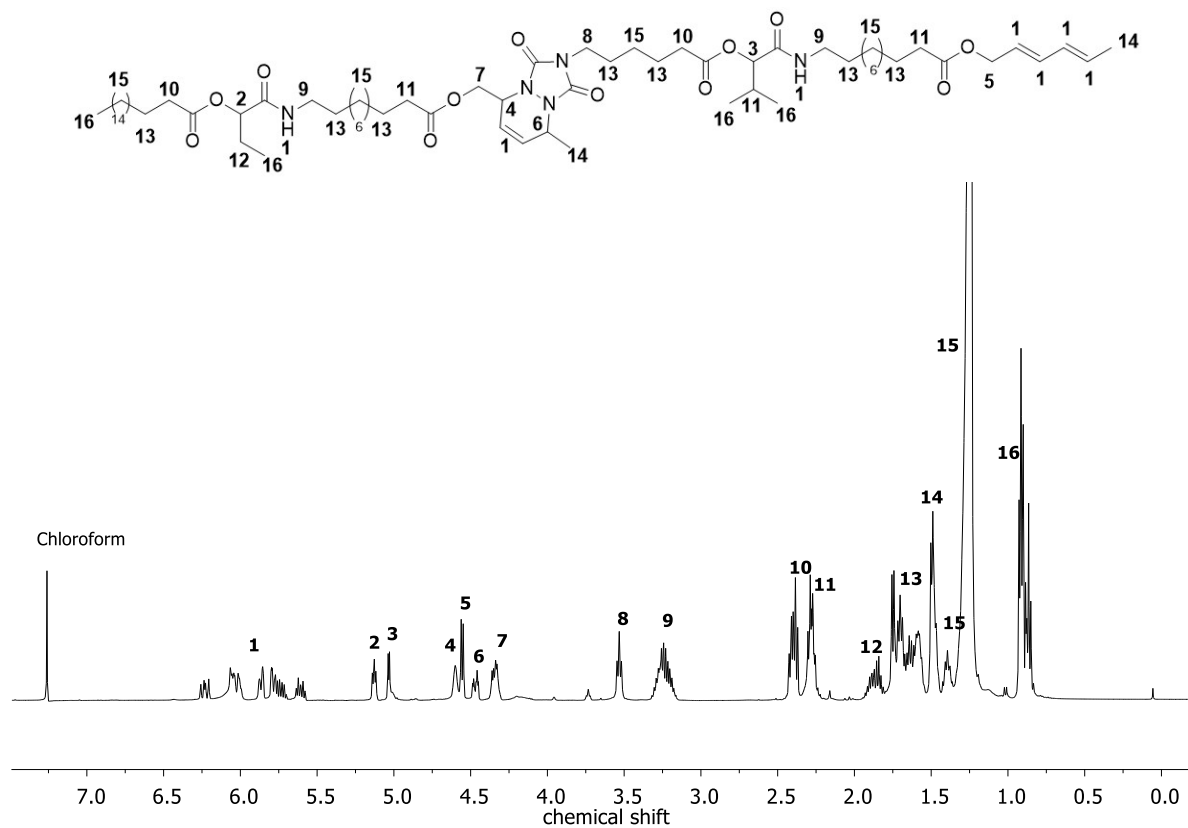
¹³C NMR (126 MHz, CDCl₃) δ /ppm: 173.77, 173.45, 172.58, 172.32, 169.78, 169.34, 153.97, 153.93, 152.78, 152.76, 134.90, 131.32, 130.56, 129.95, 129.93, 123.95, 121.18, 121.17, 78.09, 74.88, 68.09, 64.88, 62.70, 52.91, 52.89, 51.07, 51.04, 39.31, 39.20, 38.92, 34.45, 34.21, 34.12, 34.08, 32.04, 30.67, 29.81, 29.79, 29.77, 29.72, 29.70, 29.58, 29.48, 29.39, 29.35, 29.33, 29.25, 29.23, 27.70, 26.98, 26.94, 26.17, 25.72, 25.20, 25.11, 25.05, 24.99, 24.80, 24.49, 22.81, 19.44, 19.42, 19.30, 18.90, 18.26, 17.08, 15.51, 14.25, 9.13.

ESI-MS:

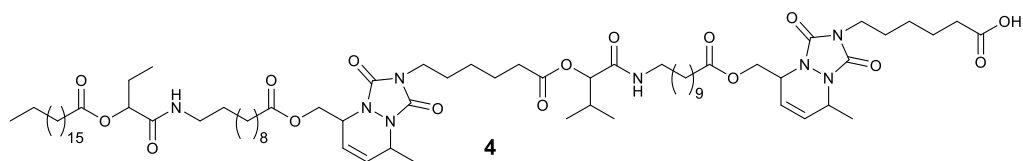
| m/z exp. | formula | m/z theo. | Δ m/z |
|-----------|--|-----------|--------------|
| 1232.8729 | $[\text{C}_{69}\text{H}_{119}\text{N}_5\text{O}_{12}\text{Na}]^+$ | 1232.8747 | 0.0018 |
| 627.4334 | $[\text{C}_{69}\text{H}_{119}\text{N}_5\text{O}_{12}\text{Na}_2]^{2+}$ | 627.9320 | 0.49 |
| 426.2844 | $[\text{C}_{69}\text{H}_{119}\text{N}_5\text{O}_{12}\text{Na}_3]^{3+}$ | 426.2844 | 0.00 |
| 1210.8928 | $[\text{C}_{69}\text{H}_{119}\text{N}_5\text{O}_{12}\text{H}]^+$ | 1210.8928 | 0.00 |
| 605.4518 | $[\text{C}_{69}\text{H}_{119}\text{N}_5\text{O}_{12}\text{H}_2]^{2+}$ | 605.9500 | 0.49 |

IR (ATR platinum diamond): ν [cm^{-1}] = 3306.1, 2918.1, 2850.7, 1738.9, 1705.0, 1652.0, 1534.5, 1455.0, 1421.5, 1376.8, 1235.2, 1161.7, 1111.7, 988.9, 765.6, 721.7, 413.1.

R_f : (hexane / ethyl acetate (1:1)) = 0.42.

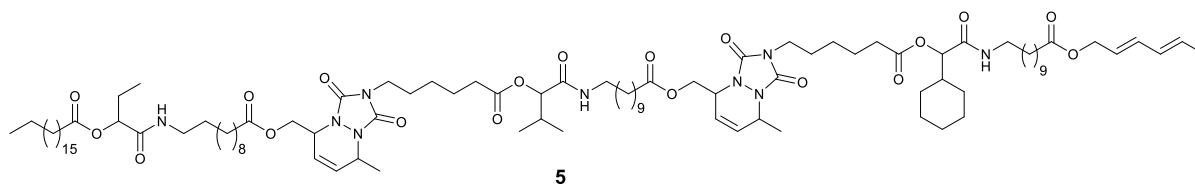


2nd TAD-Diels Alder reaction



0.568 g of **3** (0.469 mmol, 1.0 eq.) was dissolved in 3 mL dry ethyl acetate and 0.110 g of linker molecule **L2** (0.516 mmol, 1.1 eq., dissolved in 1 mL dry ethyl acetate) was added dropwise to the solution, until it stayed pink, indicating that **3** was fully converted. 20 μ L of 2,3-dimethylbut-2-ene (17 mg, 0.20 mmol) was added to the reaction mixture in order to quench the excess of the TAD compound **L2**. The solvent was evaporated under reduced pressure and the crude product **4** was used without further purification or analysis.

3rd Passerini reaction



0.647 g of **4** (0.454 mmol, 1.0 eq.) was dissolved in 3 mL DCM. Subsequently, 0.11 mL of cyclohexanal (0.102 g, 0.908 mmol, 2.0 eq.) and 0.198 g of **L1** (0.681 mmol, 1.5 eq.) were added. The reaction was stirred for 12 hours at room temperature and the crude product was purified by column chromatography (hexane:ethyl acetate 5:1 → 1:3). Product **5** was obtained as a yellowish, highly viscous oil in a yield of 73 % (591 mg, 0.323 mmol, overall yield over two reaction steps).

¹H NMR (500 MHz, CDCl₃) δ /ppm: 6.28 – 5.55 (m, 11H, ¹), 5.14 – 5.09 (m, 1H, ²), 5.02 (m, 2H, ³), 4.60 (s, 2H, ⁴), 4.55 (d, *J* = 6.6 Hz, 2H, ⁵), 4.51 – 4.41 (m, 2H, ⁶), 4.441 – 4.29 (m, 4H, ⁷), 3.53 (t, *J* = 7.2 Hz, 4H, ⁸), 3.32 – 3.15 (m, 6H, ⁹), 2.45 – 2.34 (m, 6H, ¹⁰), 2.33 – 2.23 (m, 7H, ¹¹), 1.98 – 1.80 (m, 3H, ¹²), 1.78 – 1.55 (m, 22H, ¹³), 1.49 (d, *J* = 6.5 Hz, 9H, ¹⁴), 1.42 – 0.98 (m, 78H, ¹⁵), 0.95 – 0.79 (m, 12H, ¹⁶).

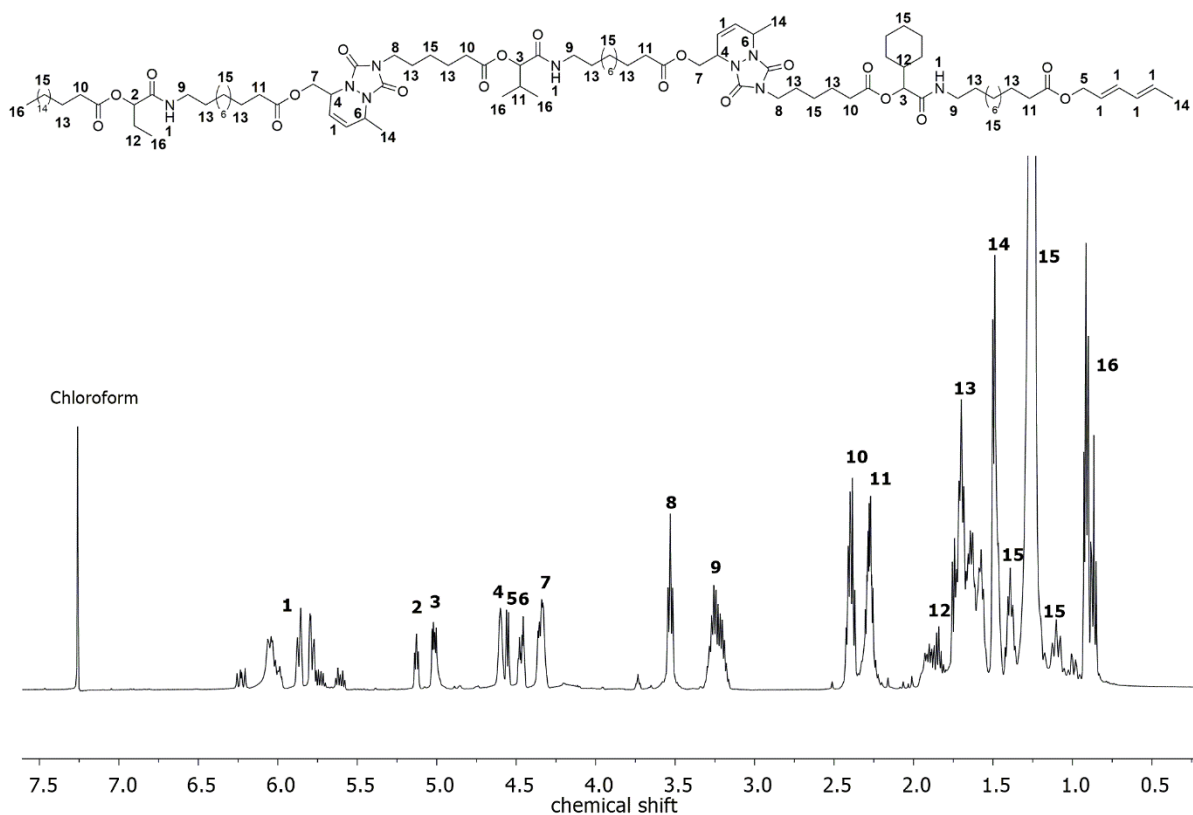
¹³C NMR (126 MHz, CDCl₃) δ /ppm: 173.77, 173.45, 172.59, 172.35, 172.31, 169.78, 169.36, 169.29, 153.95, 153.91, 152.79, 152.77, 134.90, 131.32, 130.56, 129.92, 123.95, 121.19, 78.09, 77.80, 74.88, 68.10, 64.88, 62.70, 52.92, 51.05, 51.02, 40.12, 39.31, 38.93, 34.45, 34.12, 34.08, 32.04, 30.67, 29.81, 29.79, 29.77, 29.72, 29.70, 29.58, 29.48, 29.39, 29.36, 29.33, 29.25, 29.23, 27.71, 27.40, 26.98, 26.94, 26.18, 26.10, 25.99, 25.73, 25.20, 25.11, 25.06, 24.80, 24.66, 24.49, 24.47, 22.81, 19.42, 18.90, 18.27, 17.10, 14.25, 9.13.

ESI-MS:

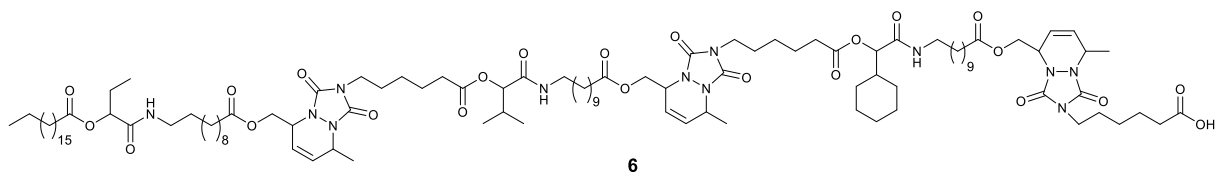
| m/z exp. | formula | m/z theo. | Δ m/z |
|-----------|---|-----------|--------------|
| 1849.2576 | $[\text{C}_{102}\text{H}_{171}\text{N}_9\text{O}_{19}\text{Na}]^+$ | 1849.2583 | 0.00070 |
| 936.1214 | $[\text{C}_{102}\text{H}_{171}\text{N}_9\text{O}_{19}\text{Na}_2]^{2+}$ | 936.1238 | 0.0024 |
| 1827.2785 | $[\text{C}_{102}\text{H}_{171}\text{N}_9\text{O}_{19}\text{H}]^+$ | 1827.2764 | 0.0021 |
| 914.1408 | $[\text{C}_{102}\text{H}_{171}\text{N}_9\text{O}_{19}\text{H}_2]^{2+}$ | 914.1418 | 0.0010 |

IR (ATR platinum diamond): ν [cm^{-1}] = 3309.2, 2923.6, 2852.8, 1736.9, 1703.5, 1533.2, 1453.3, 1421.6, 1376.0, 1236.1, 1158.1, 1114.2, 989.1, 765.6, 721.9.

R_f : (hexane / ethyl acetate (1:2)) = 0.28.

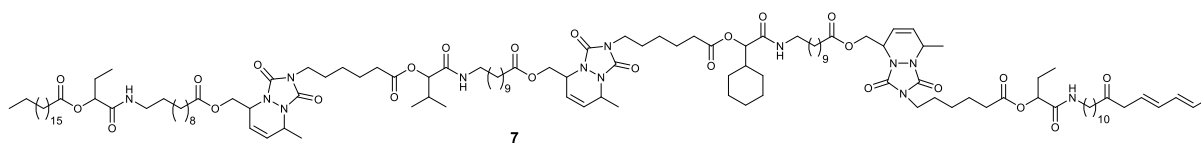


3rd TAD-Diels Alder reaction



0.545 g of **5** (0.298 mmol, 1.0 eq.) was dissolved in 3 mL dry ethyl acetate and 73 mg of linker molecule **L2** (0.343 mmol, 1.1 eq., dissolved in 2 mL dry ethyl acetate) was added dropwise to the solution, until it stayed pink, indicating that **5** was fully converted. 20 μ L of 2,3-dimethylbut-2-ene (17 mg, 0.20 mmol) was added to the reaction mixture in order to quench the excess of the TAD compound **L2**. The solvent was evaporated under reduced pressure and the crude product **6** was used without further purification or analysis.

4th Passerini reaction



0.571 g of **6** (0.282 mmol, 1.0 eq.) was dissolved in 2 mL DCM. Subsequently, 0.11 mL of propanal (0.098 g, 1.69 mmol, 6.0 eq.) and 0.370 g of **L1** (0.681 mmol, 4.5 eq.) were added. The reaction was stirred for 18 hours at room temperature and the crude product was purified by column chromatography (hexane:ethyl acetate 2:1→1:6). Product **7** was obtained as a yellowish, highly viscous oil in a yield of 82 % (472 mg, 0.231 mmol, overall yield over two reaction steps).

¹H NMR (500 MHz, CDCl₃) δ /ppm: 6.35 – 5.51 (m, 14H, ¹), 5.18 – 5.06 (m, 2H, ²), 5.06 – 4.95 (m, 2H, ³), 4.60 (s, 3H, ⁴), 4.55 (d, *J* = 6.6 Hz, 2H, ⁵), 4.51 – 4.43 (m, 3H, ⁶), 4.39 – 4.29 (m, 6H, ⁷), 3.58 – 3.44 (m, 6H, ⁸), 3.32 – 3.14 (m, 8H, ⁹), 2.48 – 2.35 (m, 8H, ¹⁰), 2.34 – 2.19 (m, 9, ¹¹), 1.98 – 1.80 (m, 5H, ¹²), 1.79 – 1.53 (m, 30H, ¹³), 1.49 (d, *J* = 6.6 Hz, 12H, ¹⁴), 1.42 – 0.95 (m, 92H, ¹⁵), 0.94 – 0.83 (m, 15H, ¹⁶).

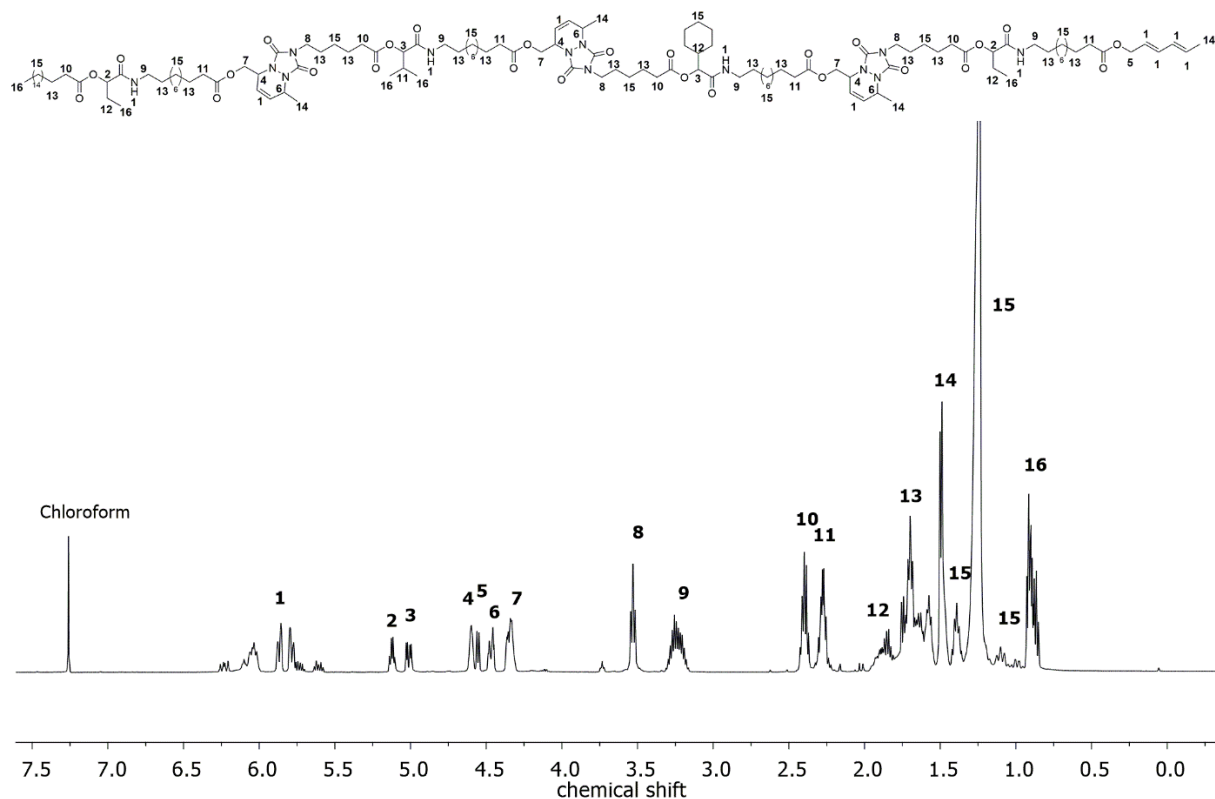
¹³C NMR (126 MHz, CDCl₃) δ /ppm: 173.78, 173.46, 172.59, 172.35, 172.18, 169.78, 169.70, 169.36, 169.30, 153.95, 153.91, 152.79, 134.90, 131.32, 130.56, 129.92, 123.95, 121.19, 78.10, 77.80, 74.98, 74.88, 68.09, 64.88, 62.70, 52.92, 51.05, 51.02, 40.12, 39.35, 39.31, 38.93, 34.45, 34.12, 34.08, 32.04, 30.67, 29.81, 29.79, 29.77, 29.72, 29.70, 29.58, 29.48, 29.39, 29.36, 29.33, 29.23, 27.71, 27.42, 26.98, 26.96, 26.94, 26.17, 26.13, 26.10, 25.99, 25.73, 25.20, 25.11, 25.05, 24.80, 24.49, 24.47, 24.43, 22.81, 19.42, 18.90, 18.27, 17.10, 14.25, 9.13, 9.12.

ESI-MS:

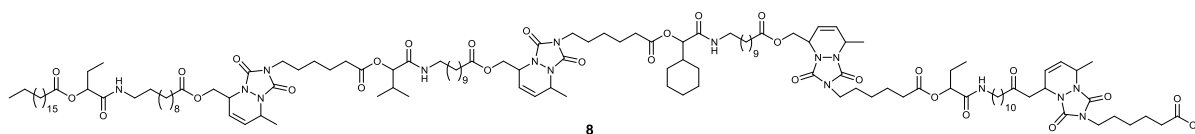
| m/z exp. | formula | m/z theo. | Δ m/z |
|-----------|--|-----------|--------------|
| 2411.5938 | $[\text{C}_{131}\text{H}_{217}\text{N}_{13}\text{O}_{26}\text{Na}]^+$ | 2411.5950 | 0.00012 |
| 1217.2901 | $[\text{C}_{131}\text{H}_{217}\text{N}_{13}\text{O}_{26}\text{Na}_2]^{2+}$ | 1217.2921 | 0.0020 |
| 819.0242 | $[\text{C}_{131}\text{H}_{217}\text{N}_{13}\text{O}_{26}\text{Na}_3]^{3+}$ | 819.1911 | 0.16 |
| 2389.6139 | $[\text{C}_{131}\text{H}_{217}\text{N}_{13}\text{O}_{26}\text{H}]^+$ | 2389.6131 | 0.00080 |
| 1195.3119 | $[\text{C}_{131}\text{H}_{217}\text{N}_{13}\text{O}_{26}\text{H}_2]^{2+}$ | 1195.3102 | 0.0017 |
| 797.0047 | $[\text{C}_{131}\text{H}_{217}\text{N}_{13}\text{O}_{26}\text{H}_3]^{3+}$ | 797.2092 | 0.20 |

IR (ATR platinum diamond): ν [cm^{-1}] = 3306.6, 2924.1, 1853.1, 1736.9, 1702.6, 1533.6, 1453.6, 1421.6, 1376.7, 1237.0, 1157.8, 1114.9, 989.2, 765.7, 722.3.

R_f: (hexane / ethyl acetate (1:6)) = 0.68.

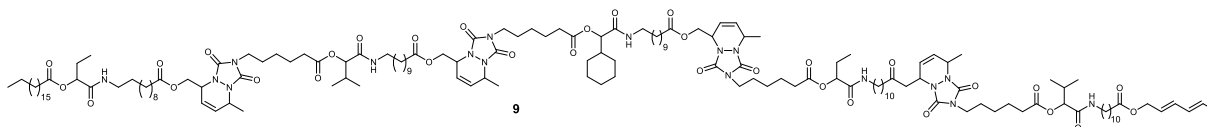


4th TAD-Diels Alder reaction



0.419 g of **7** (0.175 mmol, 1.0 eq.) was dissolved in 3 mL dry ethyl acetate and 43 mg of linker molecule **L2** (0.202 mmol, 1.1 eq. dissolved in 2 mL dry ethyl acetate) was added dropwise to the solution, until it stayed pink, indicating that **7** was fully converted. 20 μ L of 2,3-dimethylbut-2-ene (17 mg, 0.20 mmol) was added to the reaction mixture in order to quench the excess of the TAD compound **L2**. The solvent was evaporated under reduced pressure and the crude product **8** was used without further purification or analysis.

5th Passerini reaction



0.455 g of **8** (0.175 mmol, 1.0 eq.) was dissolved in 2 mL DCM. Subsequently, 0.095 mL of isobutyraldehyde (0.075 g, 1.05 mmol, 6.0 eq.) and 0.229 g of **L1** (0.787 mmol, 4.5 eq.) were added. The reaction was stirred for 24 hours at room temperature and the crude product was purified by column chromatography (hexane:ethyl acetate 3:2→EtOAc). Product **9** was obtained as a yellowish, highly viscous oil in a yield of 94 % (486 mg, 0.164 mmol, overall yield over two reaction steps).

¹H NMR (500 MHz, CDCl₃) δ /ppm: 6.33 – 5.53 (m, 17H, ¹), 5.17 – 5.08 (m, 2H, ²), 5.06 – 4.97 (m, 3H, ³), 4.60 (s, 4H, ⁴), 4.56 (d, *J* = 6.6 Hz, 2H, ⁵), 4.51 – 4.41 (m, 4H, ⁶), 4.40 – 4.29 (m, 8H, ⁷), 3.60 – 3.46 (m, 8H, ⁸), 3.36 – 3.13 (m, 10H, ⁹), 2.45 – 2.34 (m, 10H, ¹⁰), 2.32 – 2.19 (m, 12H, ¹¹), 1.98 – 1.83 (m, 5H, ¹²), 1.80 – 1.53 (m, 38H, ¹³), 1.50 (d, *J* = 6.5 Hz, 15H, ¹⁴), 1.42 – 0.97 (m, 106H, ¹⁵), 0.95 – 0.80 (m, 21H, ¹⁶).

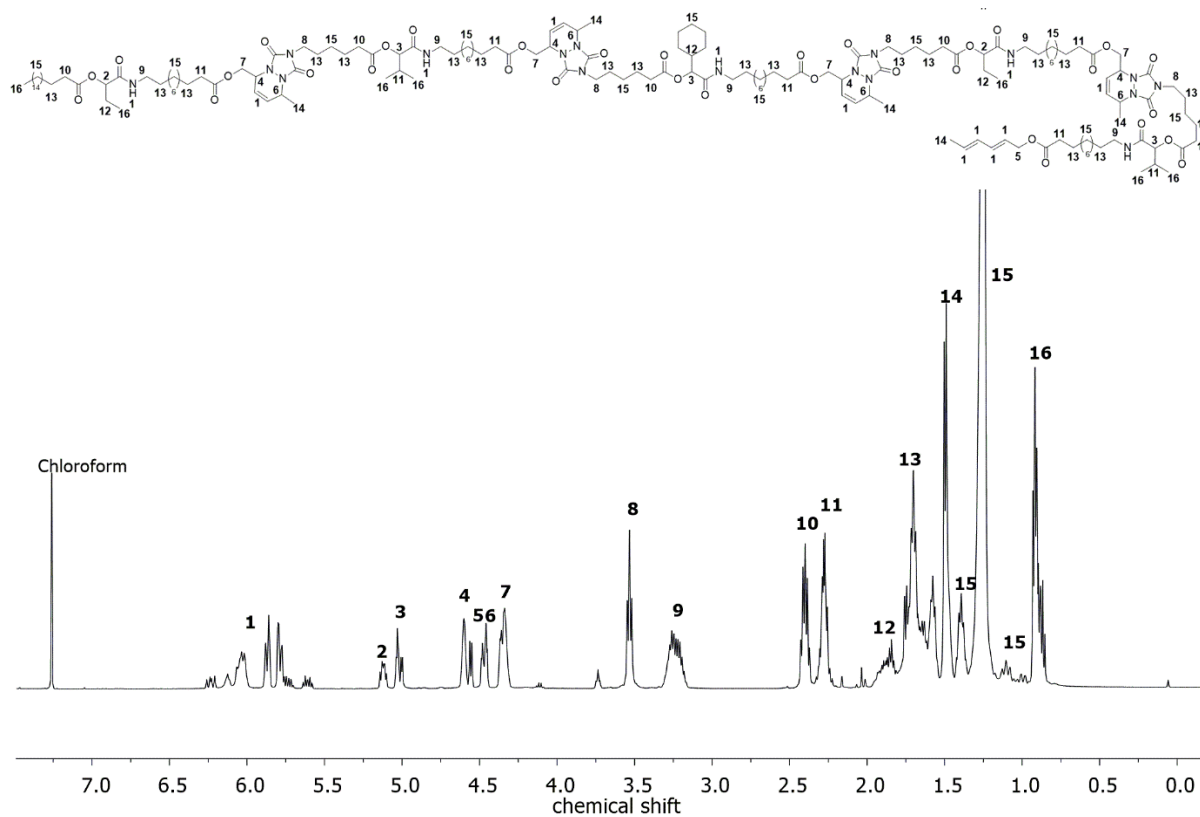
¹³C NMR (126 MHz, CDCl₃) δ /ppm: 173.78, 173.46, 172.59, 172.35, 172.21, 169.78, 169.72, 169.36, 169.31, 153.95, 153.92, 152.80, 134.90, 131.32, 130.56, 129.92, 123.96, 121.20, 78.10, 77.81, 74.98, 74.88, 68.10, 64.89, 62.71, 52.93, 51.05, 51.03, 40.12, 39.36, 39.32, 38.93, 34.46, 34.13, 34.09, 32.05, 30.68, 29.82, 29.80, 29.78, 29.73, 29.71, 29.59, 29.49, 29.39, 29.36, 29.34, 29.24, 27.71, 27.43, 26.99, 26.97, 26.95, 26.18, 26.15, 26.10, 26.00, 25.73, 25.23, 25.20, 25.12, 25.06, 24.81, 24.49, 24.44, 22.82, 19.42, 18.91, 18.27, 17.11, 14.26, 9.14.

ESI-MS:

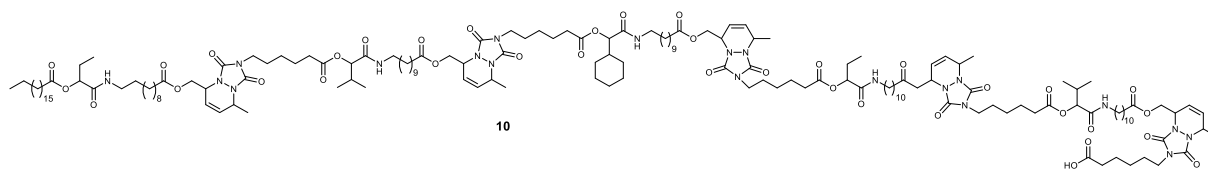
| m/z exp. | formula | m/z theo. | Δ m/z |
|-----------|---|-----------|--------------|
| 2987.9553 | $[C_{161}H_{265}N_{17}O_{33}Na]^+$ | 2987.9473 | 0.00080 |
| 1505.4646 | $[C_{161}H_{265}N_{17}O_{33}Na_2]^{2+}$ | 1505.4683 | 0.0037 |
| 1011.3071 | $[C_{161}H_{265}N_{17}O_{33}Na_3]^{3+}$ | 1011.3086 | 0.0015 |
| 2965.9715 | $[C_{161}H_{265}N_{17}O_{33}H]^+$ | 2965.9654 | 0.0061 |
| 1483.4880 | $[C_{161}H_{265}N_{17}O_{33}H_2]^{2+}$ | 1483.4863 | 0.0017 |
| 989.3254 | $[C_{161}H_{265}N_{17}O_{33}H_3]^{3+}$ | 989.3266 | 0.0012 |

IR (ATR platinum diamond): ν [cm^{-1}] = 3337.5, 2924.7, 2853.4, 1736.8, 1702.1, 1533.1, 1453.8, 1421.8, 1376.0, 1237.2, 1157.7, 1115.7, 990.1, 765.5, 722.3, 537.0.

R_f: (ethyl acetate) = 0.50.

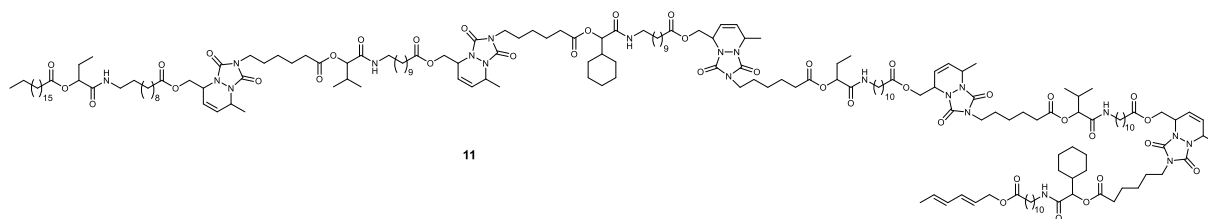


5th TAD-Diels Alder reaction



0.471 g of **9** (0.159 mmol, 1.0 eq.) was dissolved in 3 mL dry ethyl acetate and 39 mg of linker molecule **L2** (0.343 mmol, 1.1 eq. dissolved in 2 mL dry ethyl acetate) was added dropwise to the solution, until it stayed pink, indicating that **9** was fully converted. 20 μ L of 2,3-dimethylbut-2-ene (17 mg, 0.20 mmol) was added to the reaction mixture in order to quench the excess of the TAD compound **L2**. The solvent was evaporated under reduced pressure and the crude product **10** was used without further purification or analysis.

6th Passerini reaction



0.505 g of **10** (0.159 mmol, 1.0 eq.) was dissolved in 2 mL DCM. Subsequently, 0.12 mL of cyclohexanal (0.107 g, 0.954 mmol, 6.0 eq.) and 0.209 g of **L1** (0.702 mmol, 4.5 eq.) were added. The reaction was stirred for 24 hours at room temperature and the crude product was purified by column chromatography (hexane:ethyl acetate 2:1→EtOAc). Product **11** was obtained as a yellowish, highly viscous oil in a yield of 70 % (400 mg, 0.112 mmol, overall yield over two reaction steps).

¹H NMR (500 MHz, CDCl₃) δ /ppm: 6.29 – 5.50 (m, 20H, ¹), 5.11 – 4.99 (m, 2H, ²), 4.99 – 4.90 (m, 4H, ³), 4.54 (s, 5H, ⁴), 4.50 (d, *J* = 6.6 Hz, 2H, ⁵), 4.45 – 4.35 (m, 5H, ⁶), 4.33 – 4.24 (m, 10H, ⁷), 3.55 – 3.39 (m, 10H, ⁸), 3.28 – 3.08 (m, 12H, ⁹), 2.41 – 2.30 (m, 12H, ¹⁰), 2.28 – 2.12 (m, 14H, ¹¹), 1.94 – 1.75 (m, 6H, ¹²), 1.73 – 1.46 (m, 46H, ¹³), 1.44 (d, *J* = 6.5 Hz, 18H, ¹⁴), 1.41 – 0.90 (m, 130H, ¹⁵), 0.89 – 0.72 (m, 21H, ¹⁶).

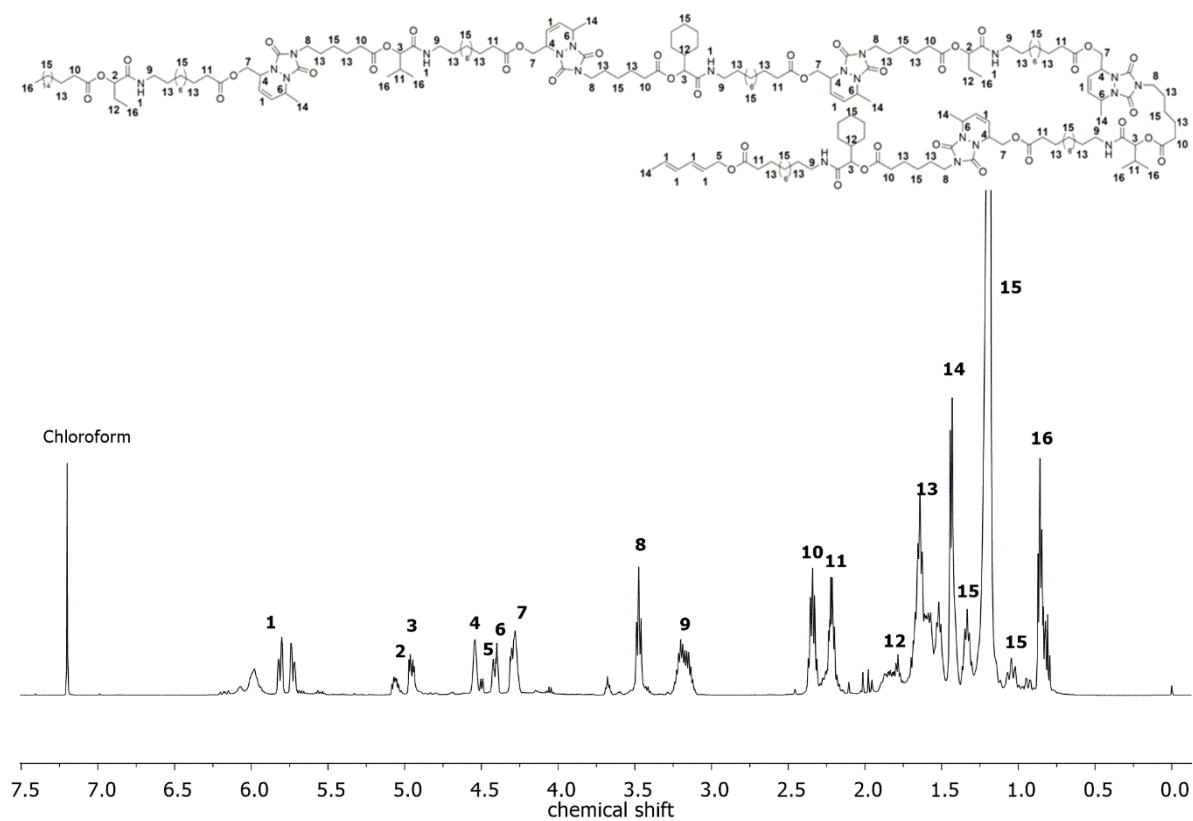
¹³C NMR (126 MHz, CDCl₃) δ /ppm: 173.47, 172.60, 172.36, 169.80, 169.38, 169.32, 153.92, 153.89, 152.80, 134.91, 131.33, 130.57, 129.92, 123.96, 121.20, 78.10, 77.81, 77.41, 77.16, 76.91, 74.98, 74.88, 68.10, 64.89, 62.71, 52.93, 51.03, 40.12, 39.32, 38.94, 34.46, 34.13, 34.09, 32.05, 30.68, 29.82, 29.78, 29.73, 29.59, 29.49, 29.40, 29.37, 29.34, 29.24, 27.72, 27.43, 26.99, 26.97, 26.95, 26.18, 26.15, 26.10, 26.00, 25.73, 25.23, 25.20, 25.12, 25.06, 24.81, 24.49, 24.48, 24.44, 22.82, 19.43, 18.91, 18.28, 17.11, 14.26, 9.14.

ESI-MS:

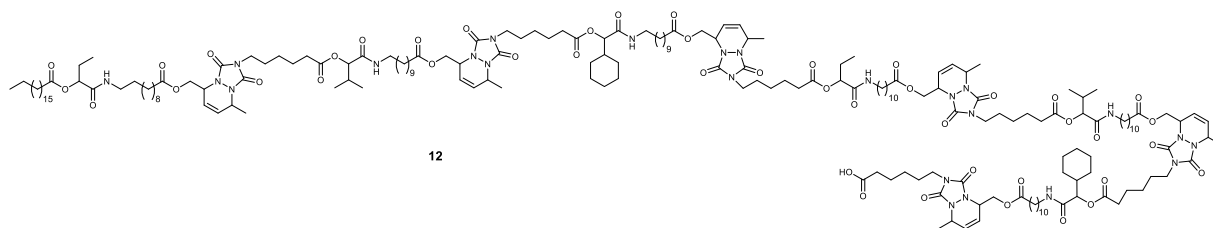
| m/z exp. | formula | m/z theo. | Δ m/z |
|-----------|--|-----------|--------------|
| 3604.3611 | $[\text{C}_{194}\text{H}_{317}\text{N}_{21}\text{O}_{40}\text{Na}]^+$ | 3604.3309 | 0.0291 |
| 1813.6621 | $[\text{C}_{194}\text{H}_{317}\text{N}_{21}\text{O}_{40}\text{Na}_2]^{2+}$ | 1813.6601 | 0.0020 |
| 1216.7808 | $[\text{C}_{194}\text{H}_{317}\text{N}_{21}\text{O}_{40}\text{Na}_3]^{3+}$ | 1216.7698 | 0.011 |
| 3582.3633 | $[\text{C}_{194}\text{H}_{317}\text{N}_{21}\text{O}_{40}\text{H}]^+$ | 3582.3490 | 0.014 |
| 1791.6817 | $[\text{C}_{194}\text{H}_{317}\text{N}_{21}\text{O}_{40}\text{H}_2]^{2+}$ | 1791.6781 | 0.0036 |
| 1194.7871 | $[\text{C}_{194}\text{H}_{317}\text{N}_{21}\text{O}_{40}\text{H}_3]^{3+}$ | 1194.7878 | 0.0007 |

IR (ATR platinum diamond): ν [cm^{-1}] = 3325.9, 2924.7, 2853.3, 1736.9, 1701.7, 1534.3, 1453.5, 1421.7, 1376.3, 1237.1, 1157.7, 1115.7, 765.4, 722.2, 537.1.

R_f: (ethyl acetate) = 0.68.

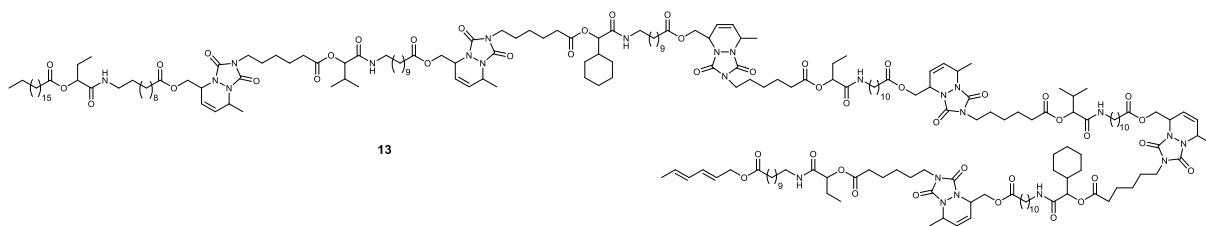


6th TAD-Diels Alder reaction



0.360 g of **11** (0.100 mmol, 1.0 eq.) was dissolved in 3 mL dry ethyl acetate and 25 mg of linker molecule **L2** (0.115 mmol, 1.15 eq. dissolved in 2 mL dry ethyl acetate) was added dropwise to the solution, until it stayed pink, indicating that **11** was fully converted. 20 μ L of 2,3-dimethylbut-2-ene (17 mg, 0.20 mmol) was added to the reaction mixture in order to quench the excess of the TAD compound **L2**. The solvent was evaporated under reduced pressure and the crude product **12** was used without further purification or analysis.

7th Passerini reaction



0.379 g of **12** (0.10 mmol, 1.0 eq.) was dissolved in 2 mL DCM. Subsequently, 43 μ L of propanal (0.035 g, 0.60 mmol, 6.0 eq.) and 0.131 g of **L1** (0.45 mmol, 4.5 eq.) were added. The reaction was stirred for 24 hours at room temperature and the crude product was purified by column chromatography (hexane:ethyl acetate 1:2 \rightarrow EtOAc). Product **13** was obtained as a yellowish, highly viscous oil in a yield of 75 % (277 mg, 0.067 mmol, overall yield over two reaction steps).

¹H-NMR: (500 MHz, CDCl₃) δ /ppm: 6.28 – 5.51 (m, 23H, ¹), 5.17 – 5.08 (m, 3H, ²), 5.07 – 4.97 (m, 4H, ³), 4.60 (s, 6H, ⁴), 4.56 (d, $J = 6.6$ Hz, 2H, ⁵), 4.51 – 4.43 (m, 6H, ⁶), 4.40 – 4.29 (m, 12H, ⁷), 3.64 – 3.46 (m, 12H, ⁸), 3.36 – 3.11 (m, 14H, ⁹), 2.49 – 2.36 (m, 14H, ¹⁰), 2.33 – 2.19 (m, 16H, ¹¹), 1.97 – 1.81 (m, 8H, ¹²), 1.80 – 1.55 (m, 54H, ¹³), 1.50 (d, $J = 6.5$ Hz, 21H, ¹⁴), 1.43 – 0.95 (m, 144H, ¹⁵), 0.94 – 0.74 (m, 24H, ¹⁶).

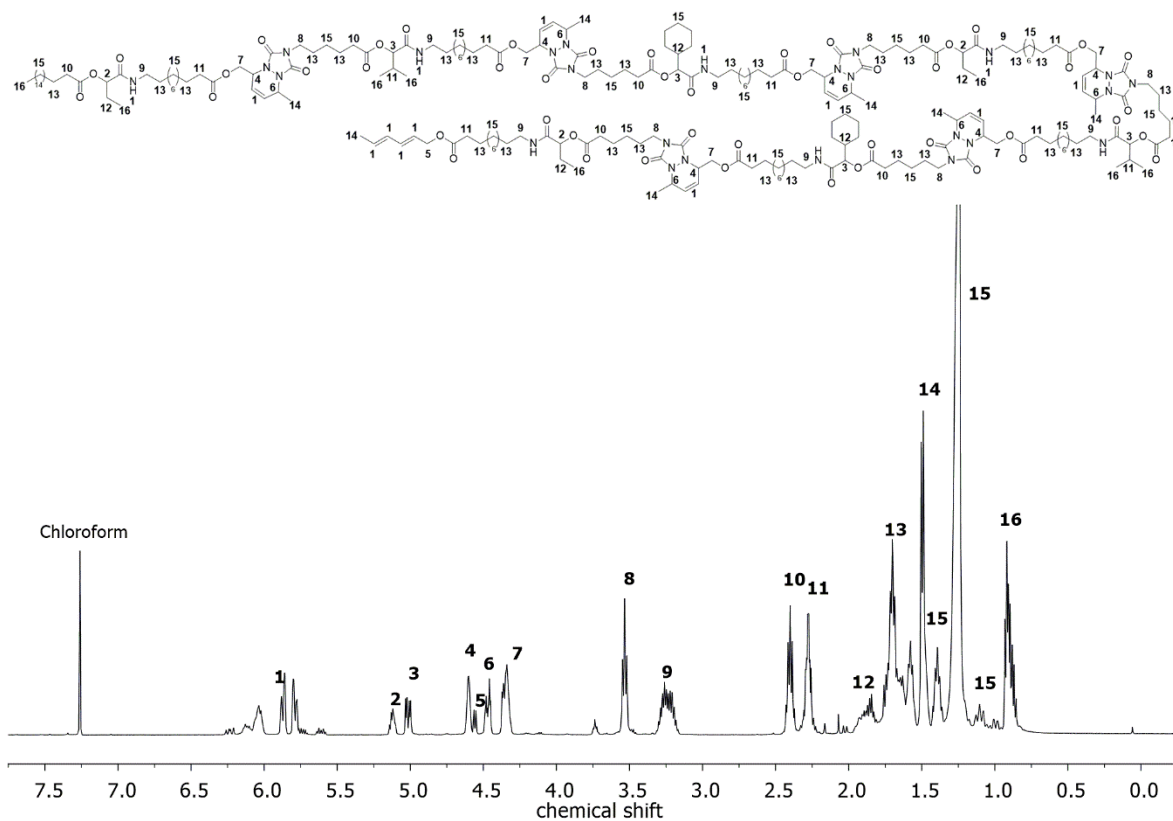
¹³C NMR (126 MHz, CDCl₃) δ /ppm: 173.79, 173.47, 172.60, 172.36, 172.22, 172.19, 169.79, 169.73, 169.37, 169.32, 153.93, 153.89, 152.82, 152.80, 134.91, 131.33, 130.57, 129.92, 123.96, 121.20, 78.10, 77.81, 74.98, 74.88, 68.10, 64.89, 62.71, 52.93, 51.03, 51.00, 40.12, 39.36, 39.31, 38.93, 34.46, 34.13, 34.09, 32.05, 30.67, 29.90, 29.82, 29.78, 29.73, 29.59, 29.49, 29.39, 29.36, 29.34, 29.24, 27.72, 27.43, 26.99, 26.96, 26.18, 26.14, 26.10, 26.00, 25.73, 25.22, 25.20, 25.12, 25.06, 24.81, 24.49, 24.48, 24.44, 22.82, 19.42, 18.91, 18.27, 17.11, 14.26, 9.14.

ESI-MS:

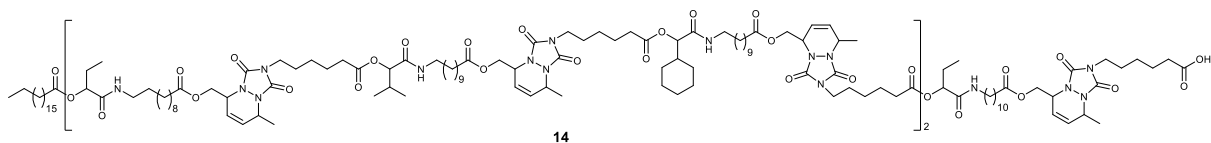
| m/z exp. | formula | m/z theo. | Δ m/z |
|-----------|--|-----------|--------------|
| 2094.8369 | $[\text{C}_{223}\text{H}_{363}\text{N}_{25}\text{O}_{47}\text{Na}_2]^{2+}$ | 2094.8284 | 0.0085 |
| 1404.2249 | $[\text{C}_{223}\text{H}_{363}\text{N}_{25}\text{O}_{47}\text{Na}_3]^{3+}$ | 1404.2153 | 0.0096 |
| 1058.9108 | $[\text{C}_{223}\text{H}_{363}\text{N}_{25}\text{O}_{47}\text{Na}_4]^{4+}$ | 1058.9088 | 0.0020 |
| 4144.7088 | $[\text{C}_{223}\text{H}_{363}\text{N}_{25}\text{O}_{47}\text{H}]^+$ | 4144.6856 | 0.014 |
| 2072.8495 | $[\text{C}_{223}\text{H}_{363}\text{N}_{25}\text{O}_{47}\text{H}_2]^{2+}$ | 2072.8464 | 0.0232 |
| 1382.2363 | $[\text{C}_{223}\text{H}_{363}\text{N}_{25}\text{O}_{47}\text{H}_3]^{3+}$ | 1382.2334 | 0.0029 |
| 1036.9266 | $[\text{C}_{223}\text{H}_{363}\text{N}_{25}\text{O}_{47}\text{H}_4]^{4+}$ | 1036.9269 | 0.0003 |

IR (ATR platinum diamond): ν [cm^{-1}] = 3336.0, 2924.9, 2853.6, 1736.9, 1701.1, 1534.2, 1453.7, 1422.0, 1377.1, 1237.4, 1115.8, 989.9, 765.5, 722.2, 637.6, 538.2.

R_f : (ethyl acetate) = 0.37.

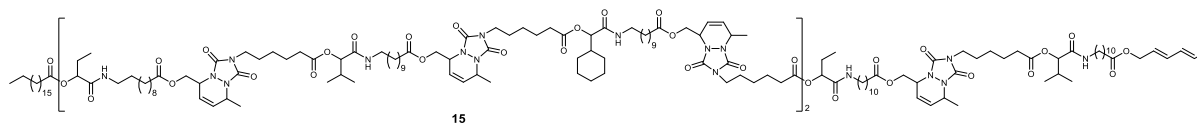


7th TAD-Diels Alder reaction



0.236 g of **13** (0.056 mmol, 1.0 eq.) was dissolved in 3 mL dry ethyl acetate and 14 mg of linker molecule **L2** (0.066 mmol, 1.15 eq. dissolved in 2 mL dry ethyl acetate) was added dropwise to the solution, until it stayed pink, indicating that **13** was fully converted. 20 μ L of 2,3-dimethylbut-2-ene (17 mg, 0.20 mmol) was added to the reaction mixture in order to quench the excess of the TAD compound **L2**. The solvent was evaporated under reduced pressure and the crude product **14** was used without further purification or analysis.

8th Passerini reaction



0.249 g of **14** (0.057 mmol, 1.0 eq.) was dissolved in 2 mL DCM. Subsequently, 31 μ L of isobutyraldehyde (25 mg, 0.342 mmol, 6.0 eq.) and 0.075 g of **L1** (0.260 mmol, 4.5 eq.) were added. The reaction was stirred for 24 hours at room temperature and the crude product was purified by column chromatography (hexane:ethyl acetate 1:2 \rightarrow EtOAc+6 % MeOH). Product **14** was obtained as a yellowish, highly viscous oil in a yield of 94 % (253 mg, 0.053 mmol, overall yield over two reaction steps).

¹H NMR (500 MHz, CDCl₃) δ /ppm 6.30 – 5.52 (m, 26H, ¹), 5.15 – 5.07 (m, 3H, ²), 5.04 – 4.94 (m, 5H, ³), 4.59 (s, 7H, ⁴), 4.55 (d, $J = 6.6$ Hz, 2H, ⁵), 4.49 – 4.43 (m, 7H, ⁶), 4.39 – 4.29 (m, 14H, ⁷), 3.58 – 3.48 (m, 14H, ⁸), 3.33 – 3.13 (m, 16H, ⁹), 2.47 – 2.35 (m, 16H, ¹⁰), 2.33 – 2.20 (m, 19H, ¹¹), 1.99 – 1.79 (m, 8H, ¹²), 1.77 – 1.53 (m, 62H, ¹³), 1.49 (d, $J = 6.6$ Hz, 24H, ¹⁴), 1.45 – 0.94 (m, 158H, ¹⁵), 0.94 – 0.80 (m, 30H, ¹⁶).

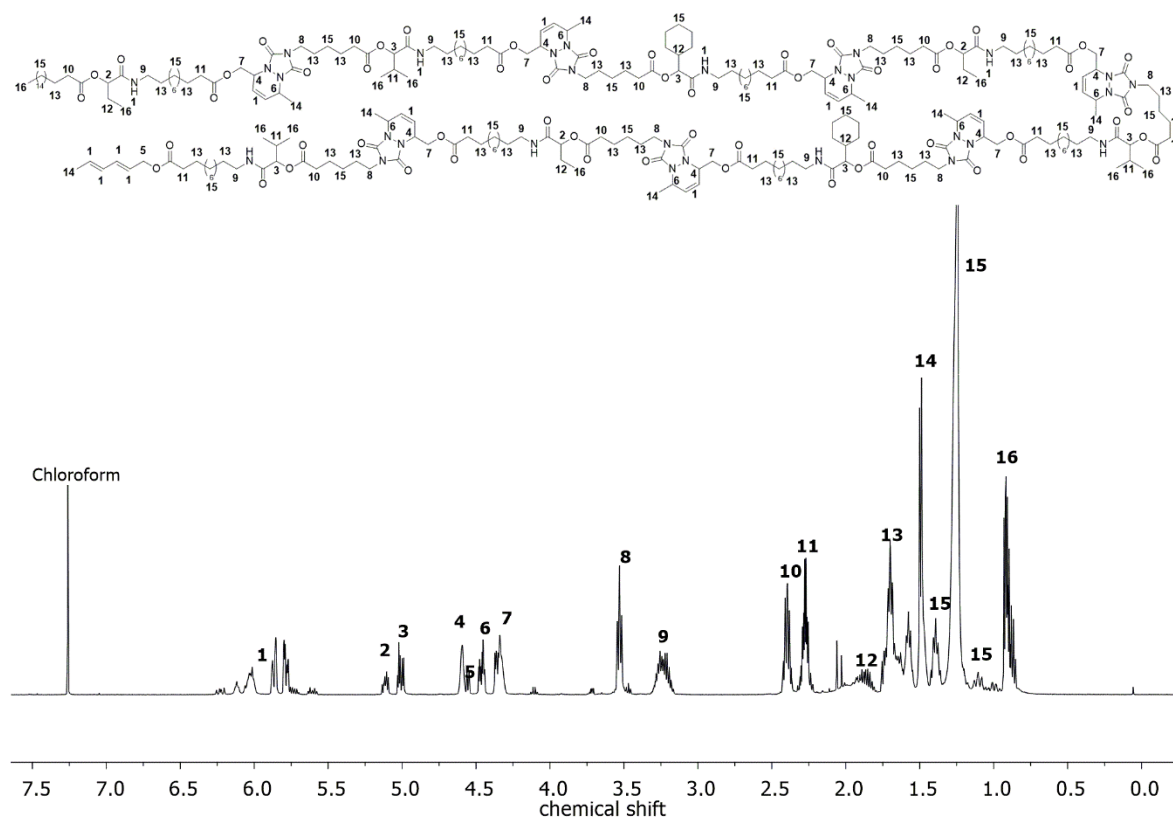
¹³C NMR (126 MHz, CDCl₃) δ /ppm: 173.73, 173.42, 172.57, 172.32, 172.19, 169.78, 169.72, 169.35, 169.30, 153.94, 153.91, 152.82, 152.81, 134.86, 131.25, 130.60, 129.93, 124.00, 121.24, 78.14, 77.84, 77.73, 75.01, 74.91, 64.85, 62.72, 52.96, 51.04, 51.02, 40.14, 39.36, 39.33, 38.94, 34.46, 34.14, 34.09, 32.04, 30.68, 29.80, 29.79, 29.73, 29.58, 29.48, 29.38, 29.35, 29.33, 29.24, 27.70, 27.48, 26.98, 26.96, 26.19, 26.15, 26.11, 26.01, 25.23, 25.20, 25.12, 25.06, 24.82, 24.50, 24.48, 24.45, 22.80, 19.41, 18.89, 18.22, 17.13, 14.23, 9.13.

ESI-MS:

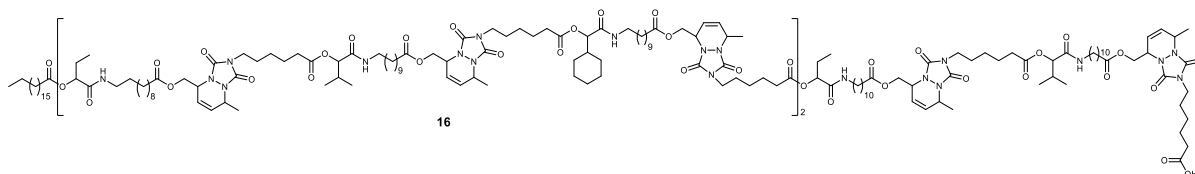
| m/z exp. | formula | m/z theo. | Δ m/z |
|-----------|--|-----------|--------------|
| 2383.0051 | $[\text{C}_{253}\text{H}_{411}\text{N}_{29}\text{O}_{54}\text{Na}_2]^{2+}$ | 2383.0045 | 0.0006 |
| 1596.3312 | $[\text{C}_{253}\text{H}_{411}\text{N}_{29}\text{O}_{54}\text{Na}_3]^{3+}$ | 1596.3328 | 0.0016 |
| 1202.9986 | $[\text{C}_{253}\text{H}_{411}\text{N}_{29}\text{O}_{54}\text{Na}_4]^{4+}$ | 1202.9969 | 0.0017 |
| 2361.0244 | $[\text{C}_{253}\text{H}_{411}\text{N}_{29}\text{O}_{54}\text{H}_2]^{2+}$ | 2361.0226 | 0.0018 |
| 1574.3540 | $[\text{C}_{253}\text{H}_{411}\text{N}_{29}\text{O}_{54}\text{H}_3]^{3+}$ | 1574.3508 | 0.0032 |
| 1181.2661 | $[\text{C}_{253}\text{H}_{411}\text{N}_{29}\text{O}_{54}\text{H}_4]^{4+}$ | 1181.0149 | 0.2512 |

IR (ATR platinum diamond): ν [cm^{-1}] = 3337.4, 2925.3, 2853.6, 1736.9, 1700.8, 1534.2, 1453.8, 1422.0, 1376.9, 1237.4, 1157.6, 1116.2, 765.5, 722.2, 537.2.

R_f: (ethyl acetate) = 0.125.

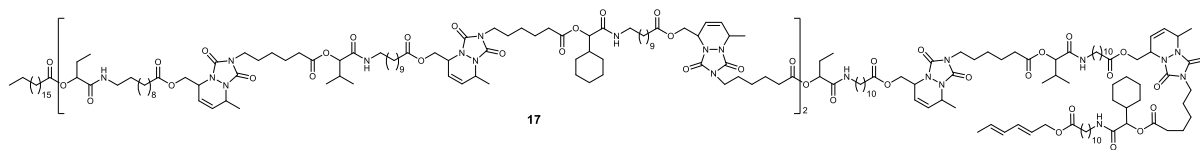


8th TAD-Diels Alder reaction



0.202 g of **15** (0.043 mmol, 1.0 eq.) was dissolved in 3 mL dry ethyl acetate and 11 mg of linker molecule **L2** (0.049 mmol, 1.15 eq. dissolved in 2 mL dry ethyl acetate) was added dropwise to the solution, until it stayed pink, indicating that **15** was fully converted. 20 μ L of 2,3-dimethylbut-2-ene (17 mg, 0.20 mmol) was added to the reaction mixture in order to quench the excess of the TAD compound **L2**. The solvent was evaporated under reduced pressure and the crude product **16** was used without further purification or analysis.

9th Passerini reaction



0.211 g of **16** (0.043 mmol, 1.0 eq.) was dissolved in 2 mL DCM. Subsequently, 0.32 μ L of cyclohexanal (29 mg, 0.257 mmol, 6.0 eq.) and 0.56 g of **L1** (0.193 mmol, 4.5 eq.) were added. The reaction was stirred for 24 hours at room temperature and the crude product was purified by column chromatography (hexane : ethyl acetate 1:2.5 \rightarrow EtOAc+10 % MeOH). Product **17** was obtained as a yellowish, highly viscous oil in a yield of 78 % (179 mg, 0.0335 mmol, overall yield over two reaction steps).

¹H NMR (500 MHz, CDCl₃) δ /ppm: 6.27 – 5.53 (m, 29H, ¹), 5.14 – 5.06 (m, 3H, ²), 5.03 – 4.95 (m, 6H, ³), 4.59 (s, 8H, ⁴), 4.55 (d, J = 6.6 Hz, 2H, ⁵), 4.48 – 4.41 (m, 8H, ⁶), 4.38 – 4.27 (m, 16H, ⁷), 3.59 – 3.45 (m, 16H, ⁸), 3.30 – 3.13 (m, 18H, ⁹), 2.46 – 2.34 (m, 18H, ¹⁰), 2.32 – 2.18 (m, 21H, ¹¹), 1.97 – 1.78 (m, 9H, ¹²), 1.77 – 1.52 (m, 70H, ¹³), 1.49 (d, J = 6.6 Hz, 27H, ¹⁴), 1.42 – 0.94 (m, 182H, ¹⁵), 0.94 – 0.76 (m, 30H, ¹⁶).

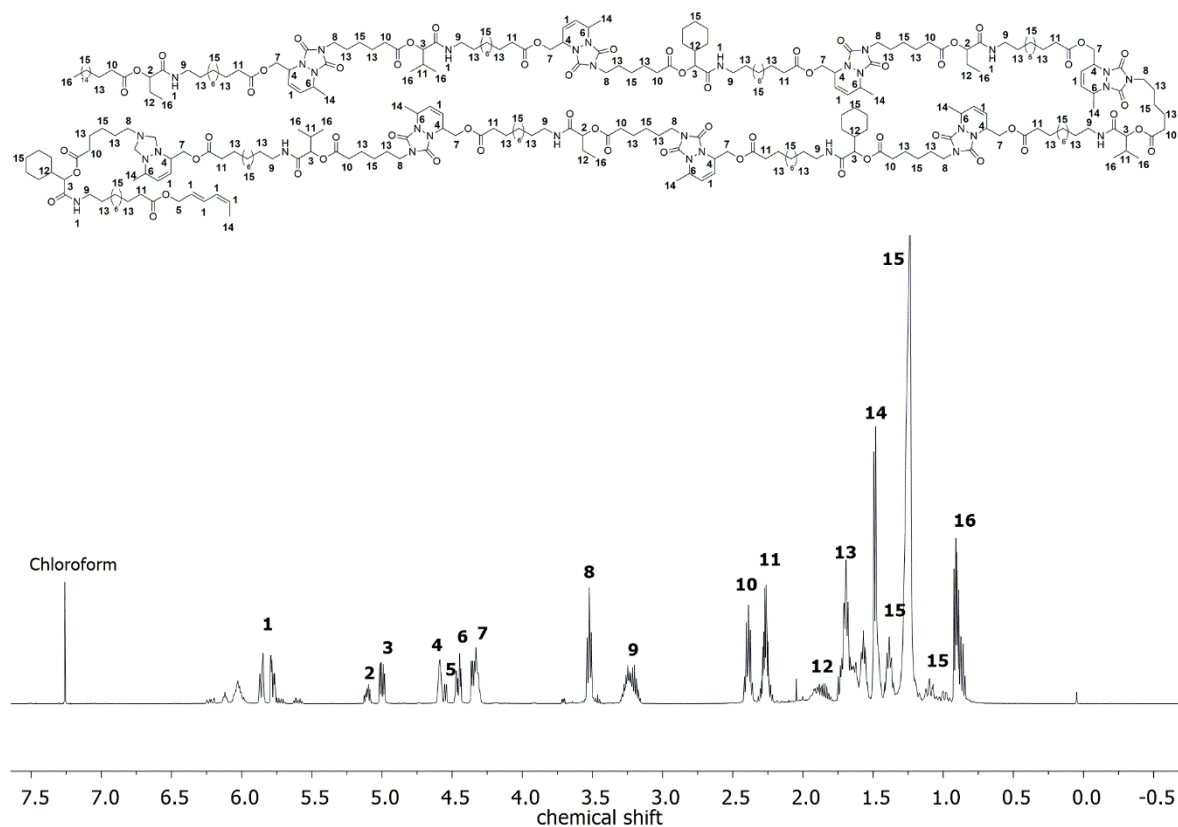
¹³C NMR (126 MHz, CDCl₃) δ /ppm: 173.40, 172.55, 172.31, 172.18, 169.75, 169.69, 169.33, 169.27, 153.91, 153.88, 152.78, 152.77, 134.85, 131.25, 130.56, 129.90, 123.96, 121.20, 78.09, 77.79, 74.97, 74.87, 64.83, 62.69, 52.93, 52.91, 51.02, 50.99, 40.11, 39.33, 39.29, 38.91, 34.43, 34.11, 34.06, 32.01, 30.65, 29.78, 29.73, 29.71, 29.55, 29.45, 29.35, 29.32, 29.31, 29.21, 27.68, 27.43, 26.96, 26.93, 26.16, 26.12, 26.08, 25.98, 25.20, 25.18, 25.09, 25.03, 24.78, 24.47, 24.45, 24.42, 22.78, 19.39, 18.87, 18.22, 17.10, 14.21, 9.11.

ESI-MS:

| m/z exp. | formula | m/z theo. | Δ m/z |
|-----------|--|-----------|--------------|
| 2691.2043 | $[\text{C}_{286}\text{H}_{463}\text{N}_{33}\text{O}_{61}\text{Na}_2]^{2+}$ | 2691.1963 | 0.0080 |
| 1801.7965 | $[\text{C}_{286}\text{H}_{463}\text{N}_{33}\text{O}_{61}\text{Na}_3]^{3+}$ | 1801.7940 | 0.0025 |
| 1357.0934 | $[\text{C}_{286}\text{H}_{463}\text{N}_{33}\text{O}_{61}\text{Na}_4]^{4+}$ | 1357.0928 | 0.0006 |
| 2669.2178 | $[\text{C}_{286}\text{H}_{463}\text{N}_{33}\text{O}_{61}\text{H}_2]^{2+}$ | 2669.2144 | 0.0034 |
| 1779.8157 | $[\text{C}_{286}\text{H}_{463}\text{N}_{33}\text{O}_{61}\text{H}_3]^{3+}$ | 1779.8120 | 0.0037 |
| 1335.1125 | $[\text{C}_{286}\text{H}_{463}\text{N}_{33}\text{O}_{61}\text{H}_4]^{4+}$ | 1335.1108 | 0.0017 |
| 1068.2902 | $[\text{C}_{286}\text{H}_{463}\text{N}_{33}\text{O}_{61}\text{H}_5]^{5+}$ | 1068.2901 | 0.0001 |

IR (ATR platinum diamond): ν [cm^{-1}] = 3336.7, 2925.2, 2853.6, 1737.1, 1700.5, 1533.9, 1453.6, 1422.0, 1377.3, 1237.0, 1157.5, 1116.1, 765.5, 722.3, 537.1, 423.1.

R_f: (ethyl acetate) = 0.19.



8 SEC-ESI-MS analysis of the sequence-defined nonamer synthesised in solution

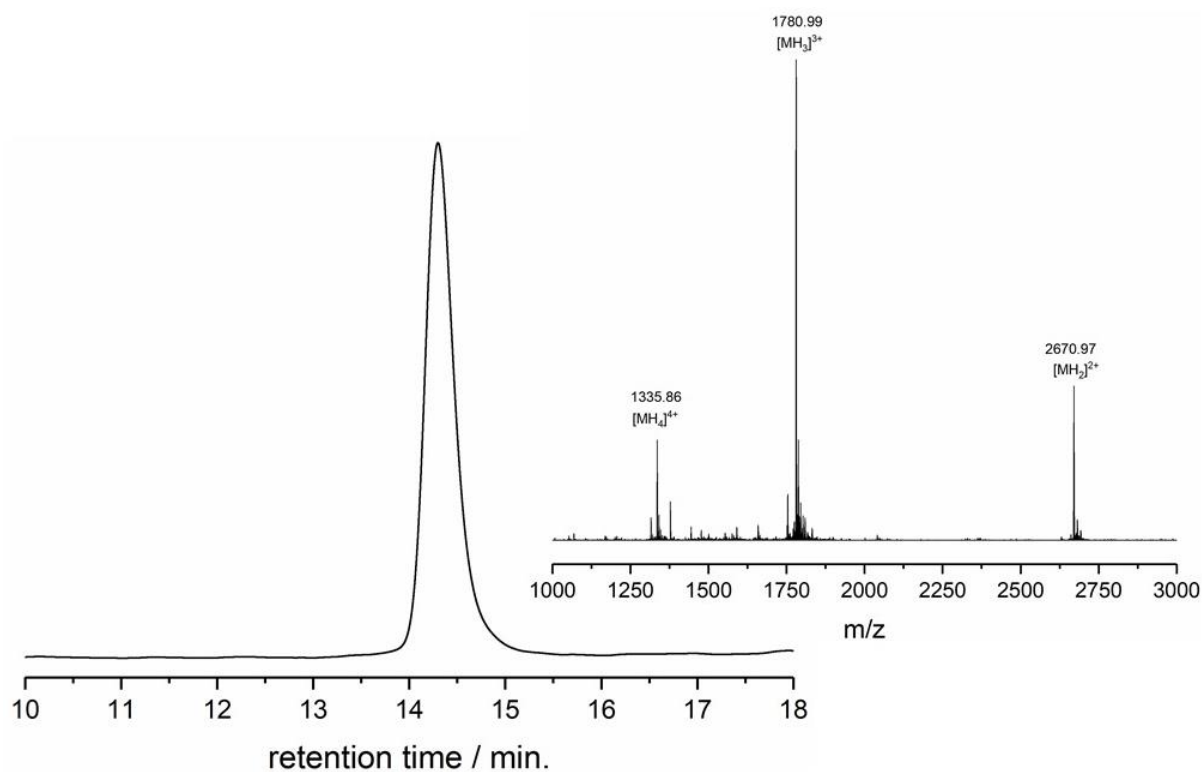


Figure S6: SEC chromatogram of the sequence-defined nonamer prepared in solution and the corresponding ESI-MS spectrum at a retention time of 14.30 minutes. The doubly- (2670.97 m/z), triply- (1780.99 m/z) and quadruply (1335.86 m/z) charged cations were clearly observed.

9 Analysis of isotope pattern of sequence-defined nonamer (solution phase):

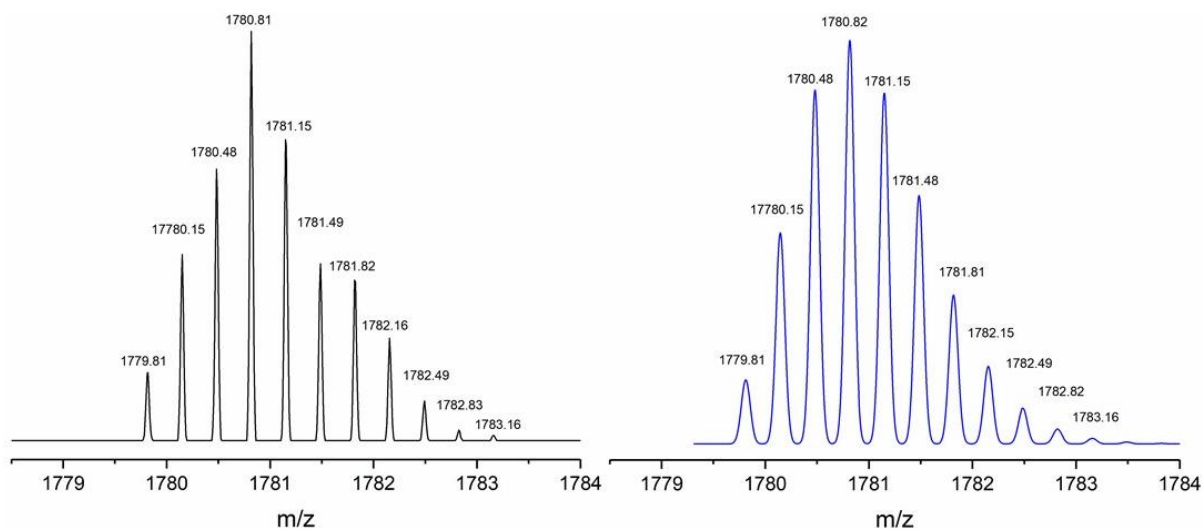


Figure S7: Left (black): The measured ESI-MS spectrum is shown. Right (blue): The isotope pattern was theoretically calculated with the program *mmass*. The measured isotope pattern is in very good agreement with the theoretical one for the assumed chemical formula.

10 Kinetic evaluation of the P-3CR via online IR:

10.1 Online IR Investigation of the reaction kinetics of the first P-3CR using 3 different aldehydes

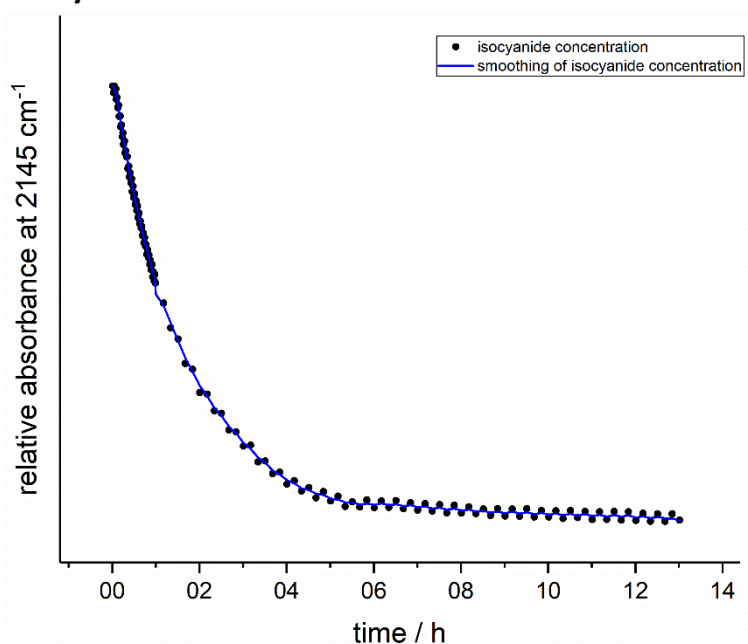


Figure S8: Online IR analysis of the P-3CR with stearic acid, linker molecule L1 and propanal depicting the decrease of the isocyanide peak at 2145.33 cm⁻¹ with time. Reaching the plateau indicates full conversion, since L1 was used in excess. The reaction is complete after 5h20min.

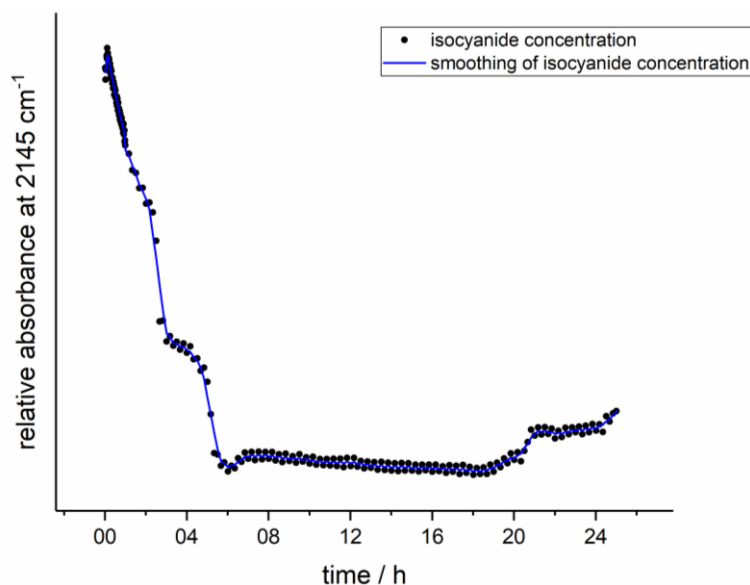


Figure S9: Online IR analysis of the P-3CR with stearic acid, linker molecule L1 and isobutyraldehyde depicting the decrease of the isocyanide peak at 2145.33 cm⁻¹ with time. Reaching the plateau indicates full conversion, since L1 was used in excess. The reaction is complete after 6h00min.

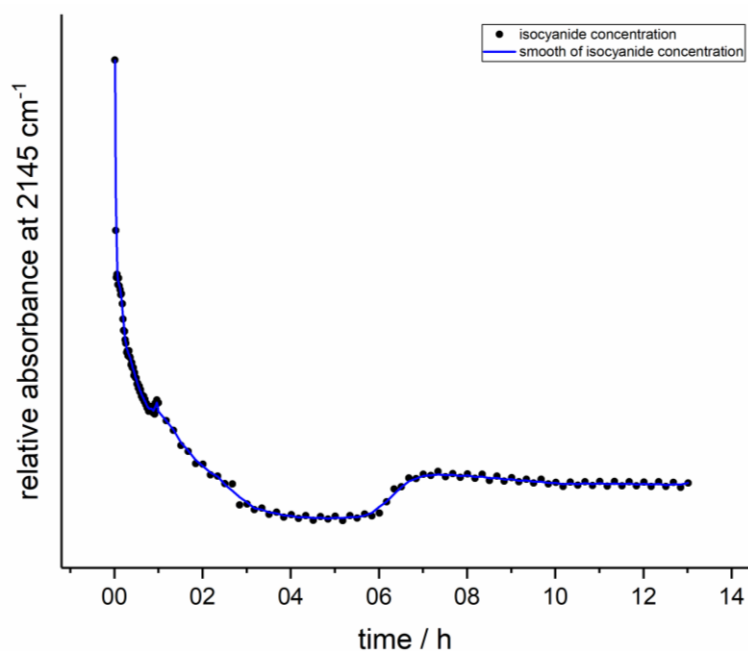


Figure S10: Online IR analysis of the P-3CR with stearic acid, linker molecule L1 and cyclohexylaldehyde depicting the decrease of the isocyanide peak at 2145.33 cm^{-1} with time. Reaching the plateau indicates full conversion, since L1 was used in excess. The reaction is complete after 4h30min.

10.2 Kinetic evaluation of the P-3CR after the fifth reaction cycle

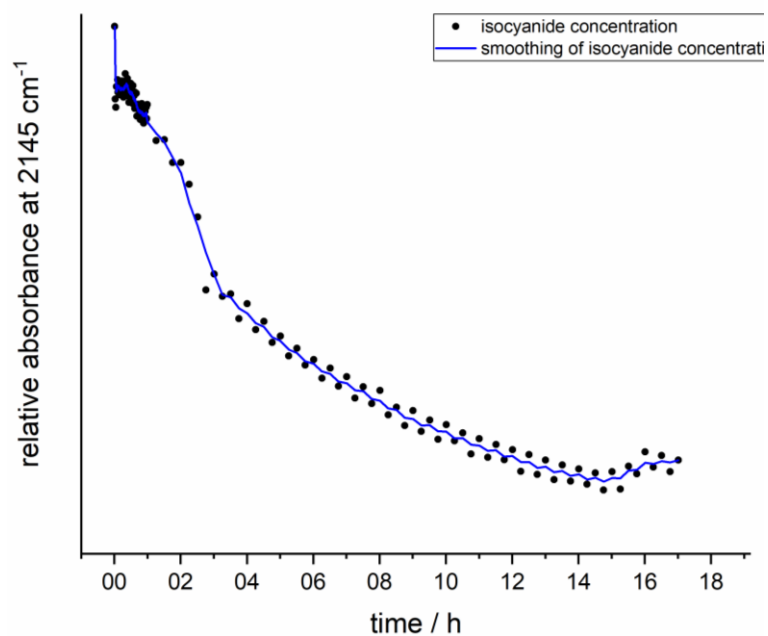


Figure S11: Online IR analysis of the P-3CR during the fifth reaction cycle depicting the decrease of isocyanide peak at 2145.33 cm^{-1} with time. Reaching the plateau indicates full conversion, since L1 was used in excess. The reaction is complete after 14h45min.

11 References:

- 1 S. Oelmann and M. A. R. Meier, *RSC Adv.*, 2017, **7**, 45195–45199.
- 2 L. Vlaminck, B. Van de Voorde and F. E. Du Prez, *Green Chem.*, 2017, **19**, 5659–5664.
- 3 S. Martens, J. Van Den Begin, A. Madder, F. E. Du Prez and P. Espeel, *J. Am. Chem. Soc.*, 2016, **138**, 14182–14185.
- 4 O. Türünç, S. Billiet, K. De Bruycker, S. Ouardad, J. Winne and F. E. Du Prez, *Eur. Polym. J.*, 2015, **65**, 286–297.