Supporting information

Stereocontrolled, multi-functional sequence-defined oligomers through automated synthesis

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1 Manual solid-phase synthesis

Figure S1: Reactions are performed in a disposable solid-phase reactor (left), equipped with a frit. This contains the resin and reagents, after reaction the frit allows a straightforward removal of the reagents and washing of the resin. The reactor is put on a laboratory shaker (right) to ensure efficient mixing.

2 Manipulation of the solid support – automated synthesis protocol

2.1 Layout of the liquid handling robot (Intavis MultiPep RSI)

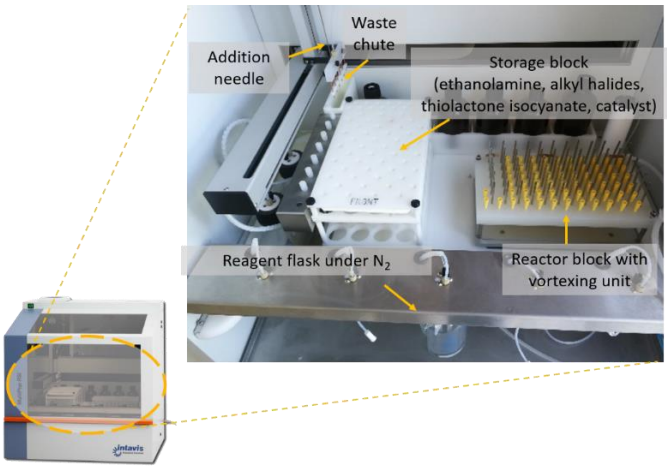

Figure S2: Automated synthesis was performed on a solid-phase peptide synthesiser, equipped with a vertexing unit and reactor block with 72 position for 5 mL reactor columns. During the synthesis the reactor columns are always open to the air. Reagent solution are stored in a storage block that is sealed with a septum sheet to minimise evaporation of volatiles.

2.2 Reagent stock solutions and solvents

- Solution 1 [EA]: ethanolamine (3 mol/l) and dimethylphenylphosphine (0.15 mol/L) in dimethylformamide
- Solution 2 [TLa-NCO]: α-isocyanato-γ-thiolactone (1.5 mol/L) in dry chloroform
- Solution 3 [catalyst]: zirconium(IV) acetylacetonate (0.15 mol/L) in dry $CHCl_3$
- Other solutions: alkyl bromide (3 mol/l) in dimethylformamide

Dimethylformamide for washing were directly taken from a 2.5 L solvent bottle by the machine. Other washing solvents (*e.g.* chloroform and methanol) were stored in a reagent flask under N_2.

2.3 Synthetic protocol - instructions for liquid handling robot

100 mg of thiolactone-functionalised rink-amide resin with a loading of 0.5 mmol/g was used for the automated synthesis. For the amine-thiol-bromo coupling 667 µL of the (alkyl) bromide solution was taken, which corresponds to the addition of 40 equivalents. Next, 334 µL of solution 1 was taken corresponding to the addition of 20 eq ethanolamine and 1 eq DMPP. For the chain extension 934 µL of solution 2 was added, corresponding to 28 eq α-isocyanato-γ-thiolactone. Next, 187 µL of solution 3 was added, corresponding to 0.56 eq of zirconium(IV) acetylacetonate.

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********** method ******************************

 Prepare

1       RinseNeedle          2000 / 2500 ul
2       WashColumns          2000 µl, Reservoir->Peptides*,  2x
3       Extract              60 s
        ...

 Cycle                       1. -> 10. (count= 10)

5       Coupling             667, Peptides
6       Aliquot              334 µl, EA->Peptides
7       WaitShaker           01 : 00 : 01 = 1 h
8       Extract              60 s
9       Coupling             667, Peptides
10      Aliquot              334 µl, EA->Peptides
11      WaitShaker           01 : 00 : 01 = 1 h
12      WashColumns          2000 µl, Reservoir->Peptides*,  2x
13      WashColumns          2000 µl, MeOH->Peptides*,  2x
14      WashColumns          2000 µl, CHCl3->Peptides*,  3x
15      Extract              60 s
16      Aliquot              934 µl, tl-iso->Peptides
17      Aliquot              187 µl, catalyst->Peptides
18      WaitShaker           01 : 00 : 00 = 1 h
19      Aliquot              500 µl, Reservoir->Peptides
20      WaitShaker           00 : 01 : 01 = 1 min
21      WashColumns          2000 µl, Reservoir->Peptides*,  2x
22      WashColumns          2000 µl, CHCl3->Peptides*,  3x
23      Extract              30 s
24      Aliquot              934 µl, tl-iso->Peptides
25      Aliquot              187 µl, catalyst->Peptides
26      WaitShaker           01 : 00 : 00 = 1 h
27      Aliquot              500 µl, Reservoir->Peptides
28      WaitShaker           00 : 01 : 01 = 1 min
29      WashColumns          2000 µl, Reservoir->Peptides*,  2x
30      WashColumns          1500 µl, MeOH->Peptides*,  2x
31      WashColumns          2000 µl, CHCl3->Peptides*,  3x
32      WashColumns          1000 µl, Reservoir->Peptides*,  2x
33      Extract              30 s
        ...

 Final

34      Extract              300 s
```

Swelling of the resin (2x 5 min)

Amine-thiol-ene coupling (1/2)

Amine-thiol-ene coupling (2/2)

Washing of the resin

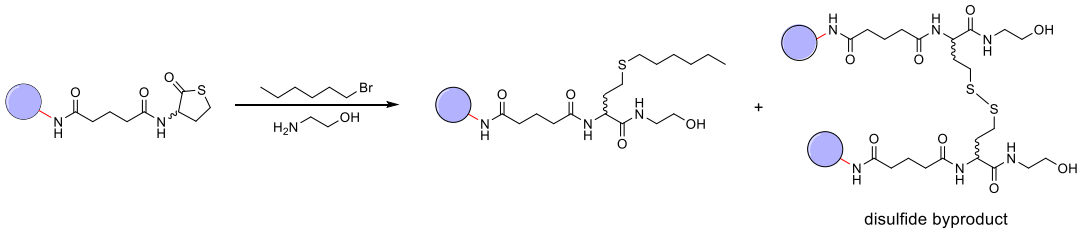
Chain extension with TLa-NCO (1/2)
At the end 500 µl is added to aid the solubilisation of the formed urea byproduct.

Chain extension with TLa-NCO

Washing of the resin

This protocol was directly exported from the Intavis MultiPep RSI software**.** Additional explanation was added.

3 Solvent screening amine-thiol-bromo coupling



Scheme S1. One-pot amine-thiol-bromo coupling

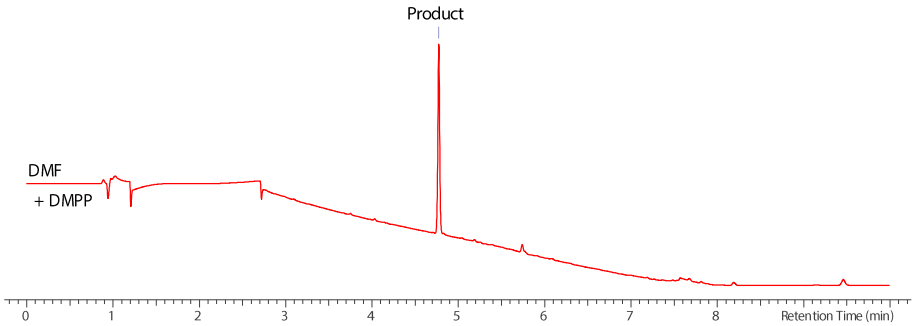
A Rink amide resin with an immobilised thiolactone moiety was first swollen in chloroform, an excellent solvent for the used resin. After 5 minutes, ethanolamine (15 eq) and 1-bromohexane (20 eq) were added. Following an extended reaction time (12 h), the final product was cleaved from the resin and analysed by liquid chromatography mass spectrometry (LCMS, **Figure S3**). This analysis indicated that besides the desired product, also a major fraction of disulfide by-product was formed.

Repeating this reaction in different solvents revealed that the amount of a disulfide was drastically affected by the used solvent (see supporting information for detailed procedures). In dipolar aprotic solvents such as tetrahydrofuran (THF), ethyl acetate or dimethylformamide (DMF) a strong decrease of the amount of disulfide side-product was observed (**Table 1**). When sodium iodide (NaI) was added as a nucleophilic catalyst, a further decrease could be observed. However, it proved to be impossible to completely eliminate the formation of this undesired coupling product with this method. Nevertheless, as already suggested in a previous study,[1,2] the addition of dimethylphenylphosphine (DMPP) and a drop of water to the reaction mixture permitted to eliminate the disulfide by-product.

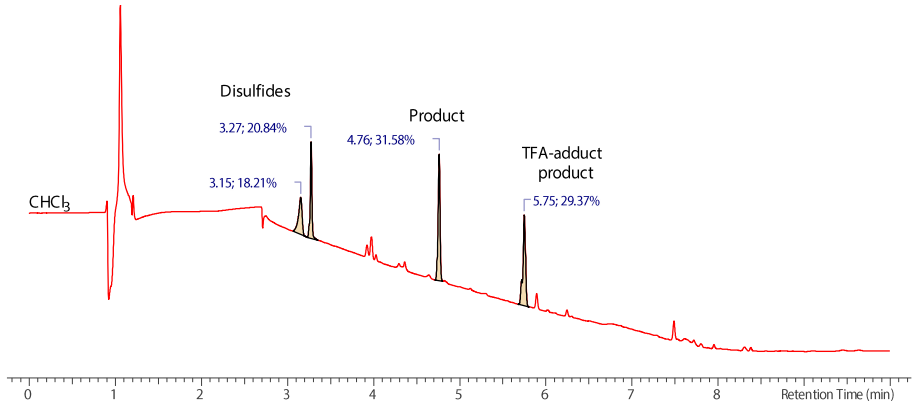
Table S1: Comparison of different solvents on the formation of disulfides in the model reaction (Scheme S1). [a] Relative fractions calculated from the liquid chromatography traces. [b] 25 mol% of NaI relative to 1-bromohexane. [c] Addition of 2 eq. of DMPP and an equal volume of water.

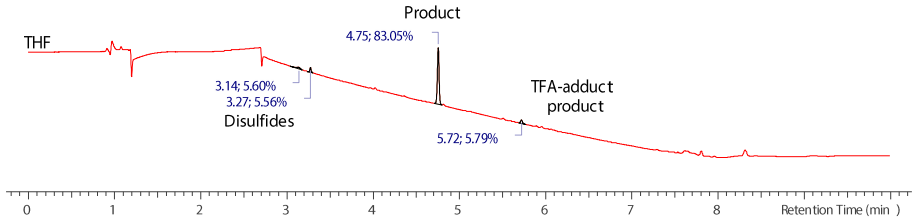
Solvent	Boiling point [°C]	Fraction disulfide[a] [%]	Fraction product[a] [%]
Chloroform	61.2	39	61
THF	66	11	89
Ethyl acetate	77.1	14	86
DMF	153	16	84
DMF / NaI[b]	153	10	90
DMF / DMPP[c]	153	0%	> 99%

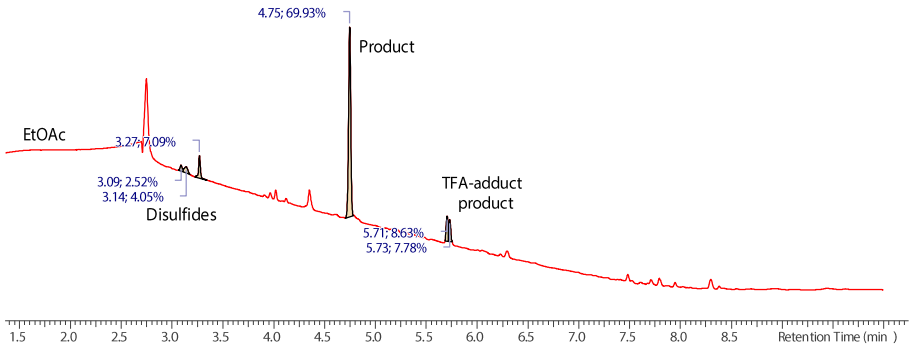
Detailed experimental procedure: Rink-amide resin (100 mg, 0.4 mmol/g), functionalised with a thiolactone moiety, was put into a solid phase reactor. 1 ml of the solvent was added and the resin was allowed to swell for 5 minutes. Next, 1-bromohexane (112 µL, 0.8 mmol, 20 eq) and ethanolamine (36 µl, 0.6 mmol, 15 eq) were added. After shaking overnight, the reagent mixture was removed and the resin was washed with dimethylformamide (4x), methanol (4x), chloroform (4x) and diethyl ether (4x). For the analyses, a sample of ~2 mg resin was taken and cleaved. The products were isolated and analysed by LCMS.

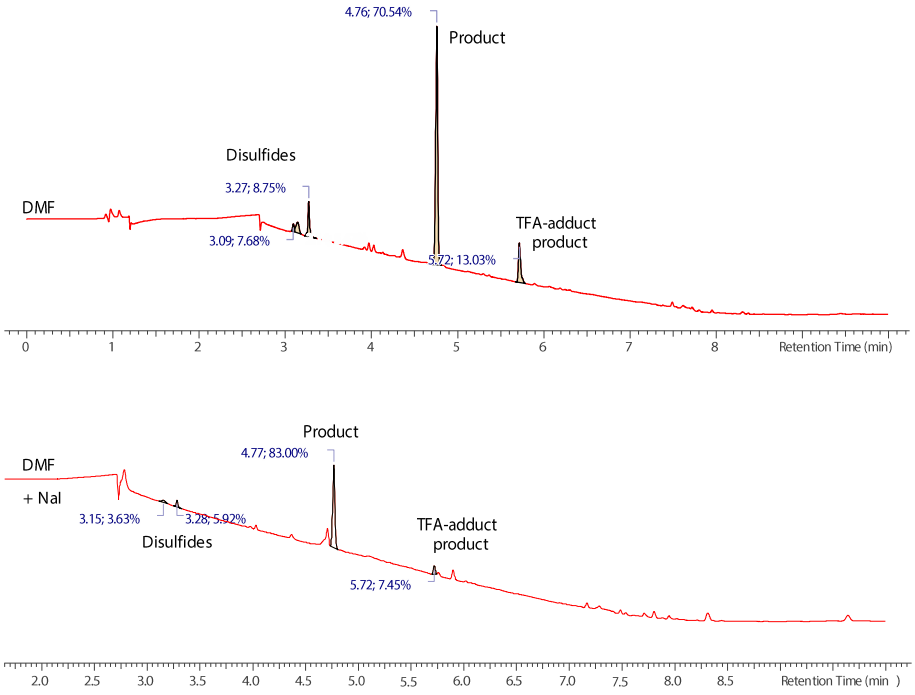


2 eq. dimethylphenylphosphine (DMPP) and a drop of water was added.









25 mol% (relative to 1-bromohexane) was added as nucleophilic catalyst.

Figure S3: HPLC traces of the model reaction (Scheme S1) in different solvents.

4 Kinetics of amine-thiol-bromo coupling

Kinetics of the amine-thiol-bromo coupling were studied by making use of the liquid handling robot. To this end, 1-bromooctane was used as alkyl halide. The reaction time was varied from 5 to 90 minutes, the samples after cleavage from the resin were analysed by LCMS. In addition, the reaction was also performed twice for 15 and 30 minutes whereby fresh reagents were added.

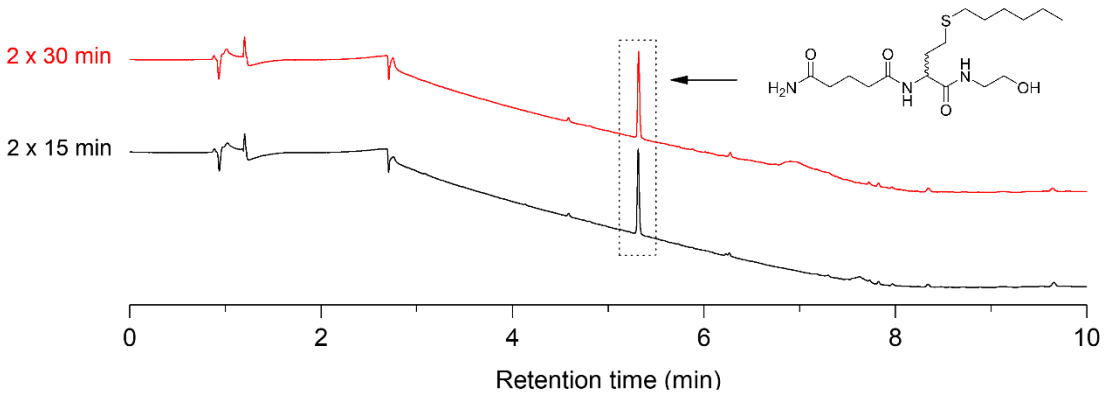


Figure S4: LC-ESI-MS analysis of the amine-thiol-bromo coupling performed twice for 15 and 30 minutes.

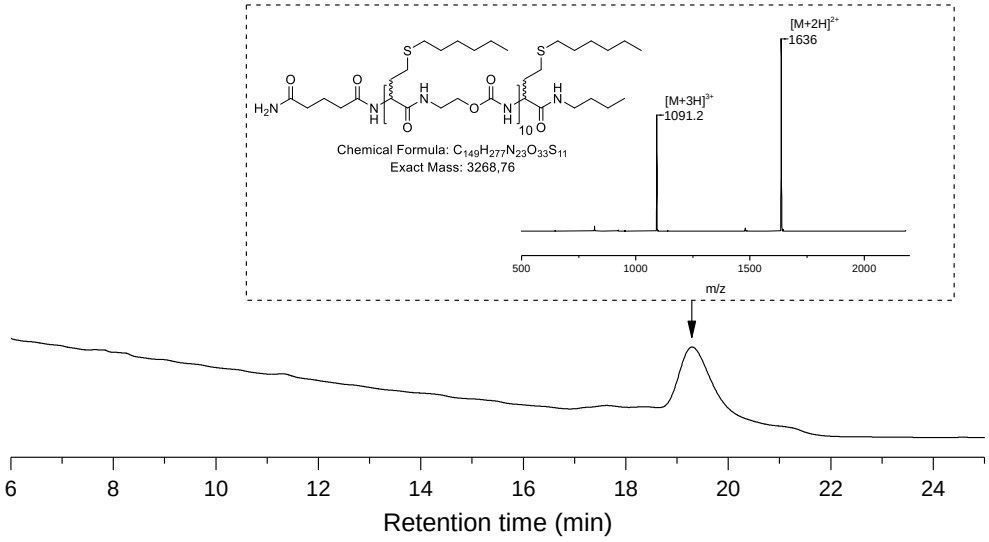


Figure S5: LC-ESI-MS analysis of *at*-undeca-C6.

5 SEC analysis during the synthesis of an octadecamer

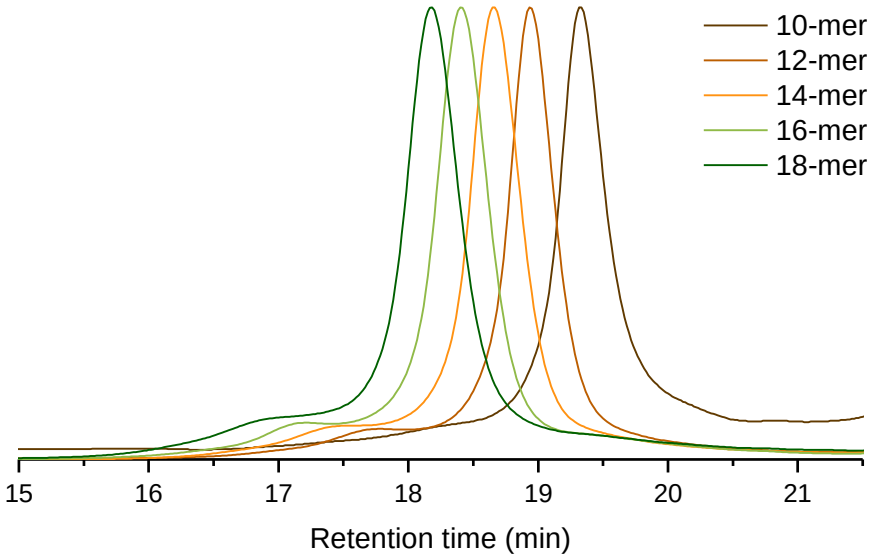
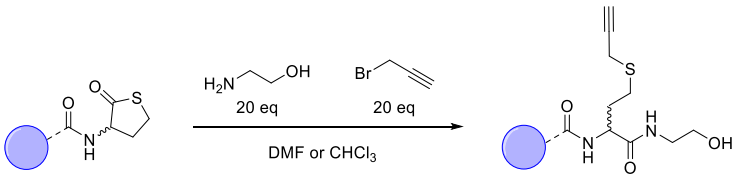


Figure S6: Size exclusion chromatography (SEC) traces of oligomers samples during the synthesis of an octadecamer in a single continuous run after the 10, 12, 14, 16 and 18th reaction cycle.

6 Post-synthesis modification



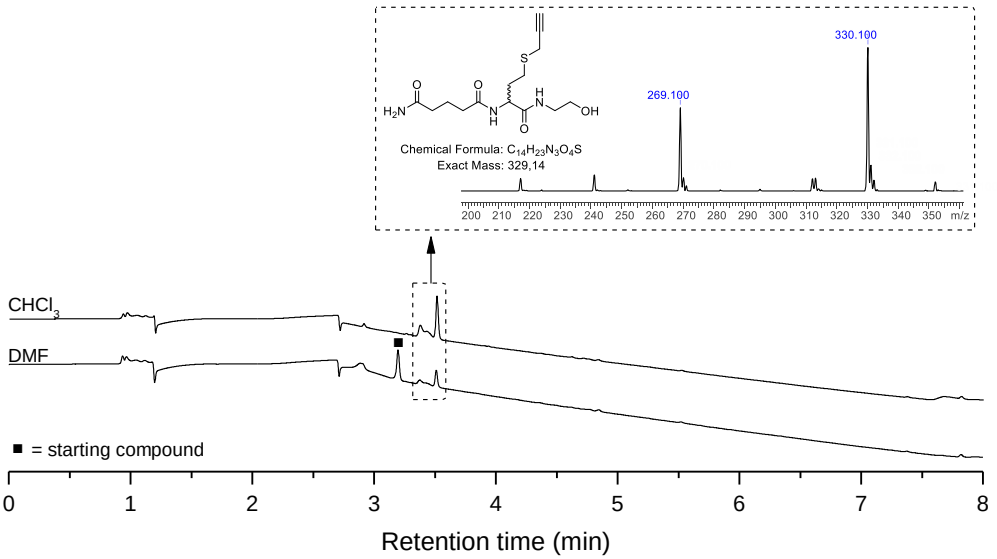


Figure S7: LC-ESI-MS analysis of the test reaction with propargyl bromide in chloroform and dimethylformamide. In chloroform only the product peak could be identified, while in dimethylformamide both the starting compound and product are present.

6.1 CuAAC with *1-azidodecane*

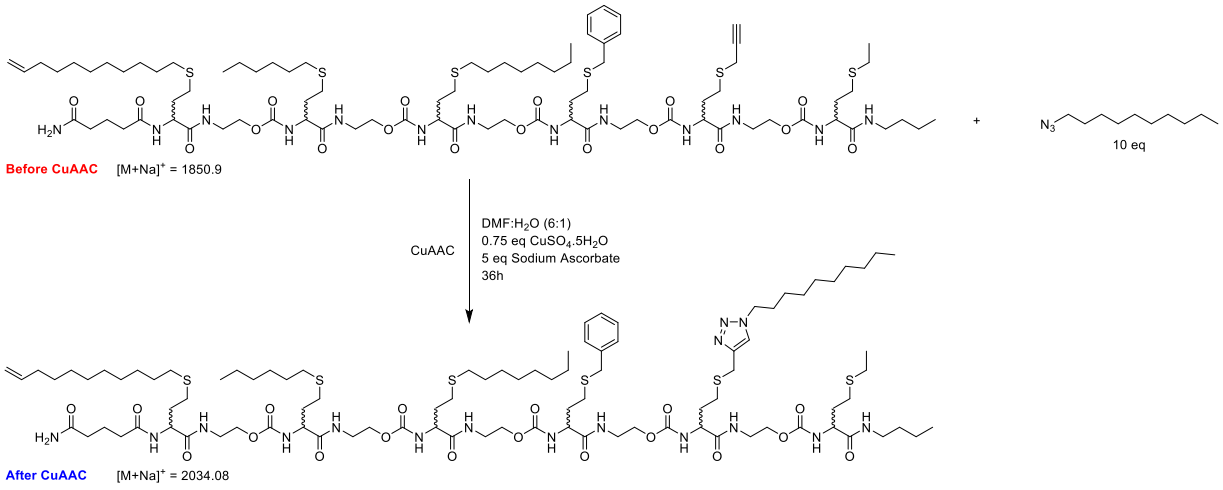


Figure S8a: On resin CuAAC reaction with *1-azidodecane*.

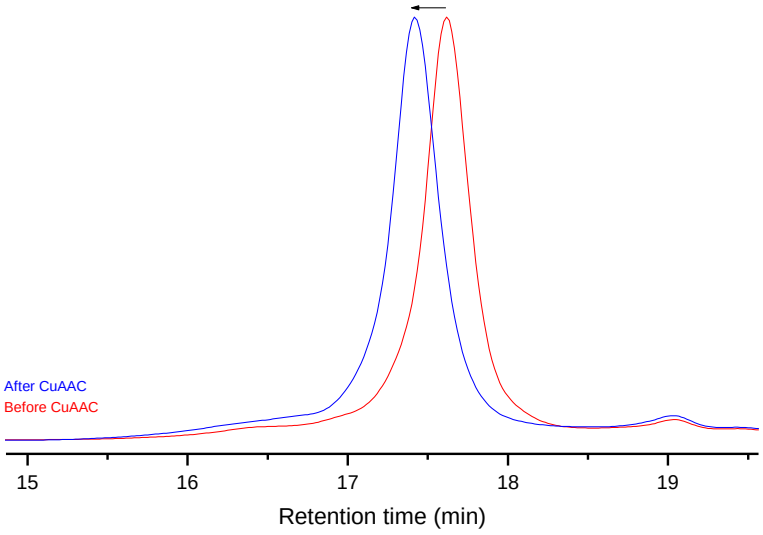


Figure S8b: GPC analysis of on resin CuAAC reaction with *1-azidodecane*.

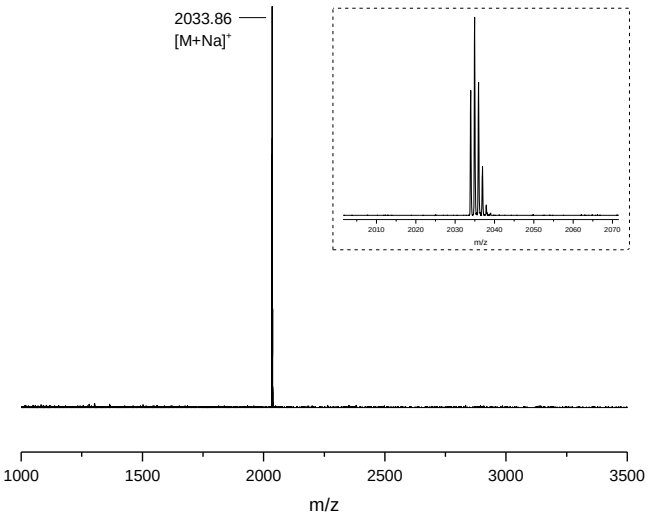


Figure S8c: MALDI analysis of on resin CuAAC reaction with *1-azidodecane*.

6.2 CuAAC with *11-azido-3,6,9-trioxaundecan-1-amine*

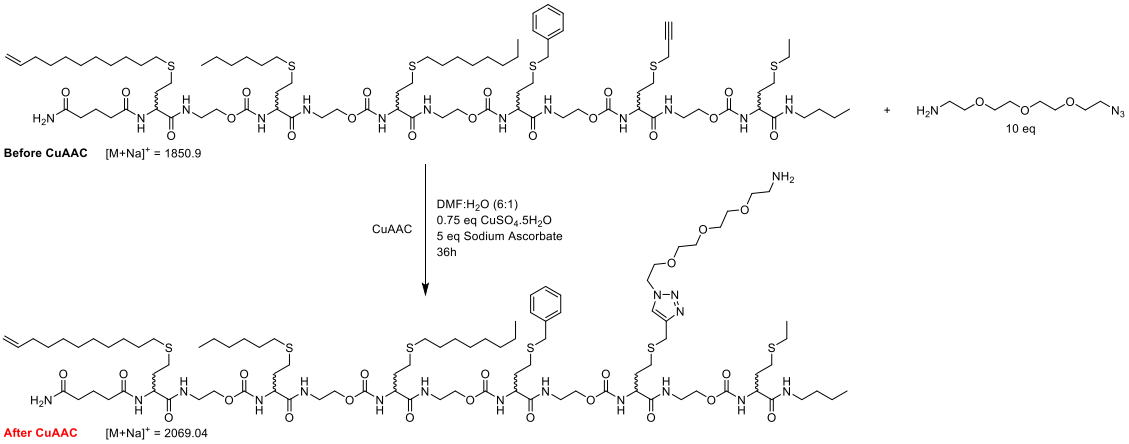


Figure S9a: On resin CuAAC reaction with *11-azido-3,6,9-trioxaundecan-1-amine*.

LCMS indicates the presence of a diazide in the commercial compound.

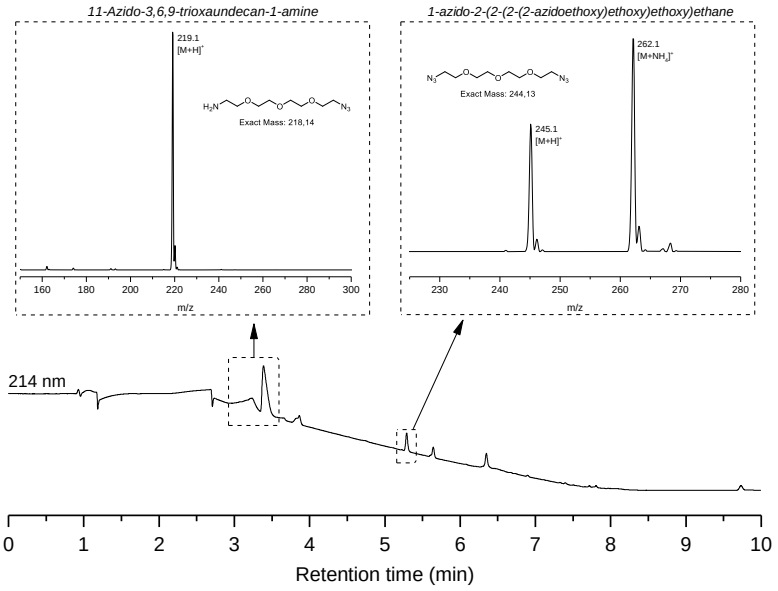


Figure S9b: LCMS analysis of *11-azido-3,6,9-trioxaundecan-1-amine*.

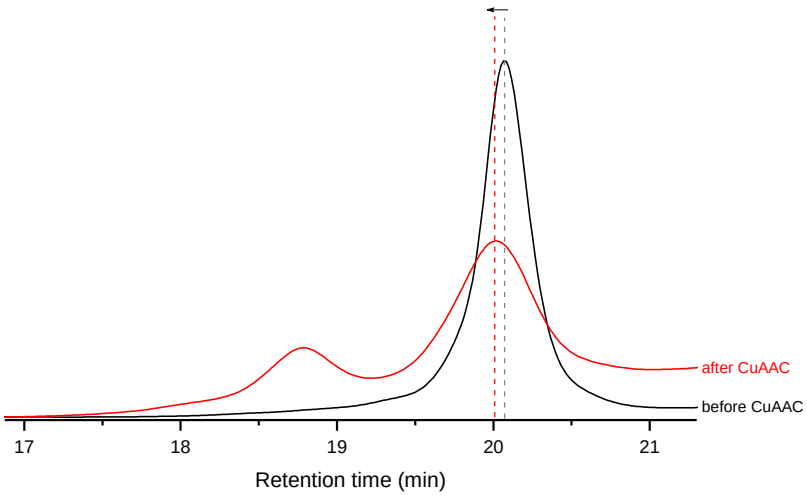


Figure S9c: SEC analysis of CuAAC reaction with *11*-azido-*3,6,9-trioxaundecan-1-amine*.

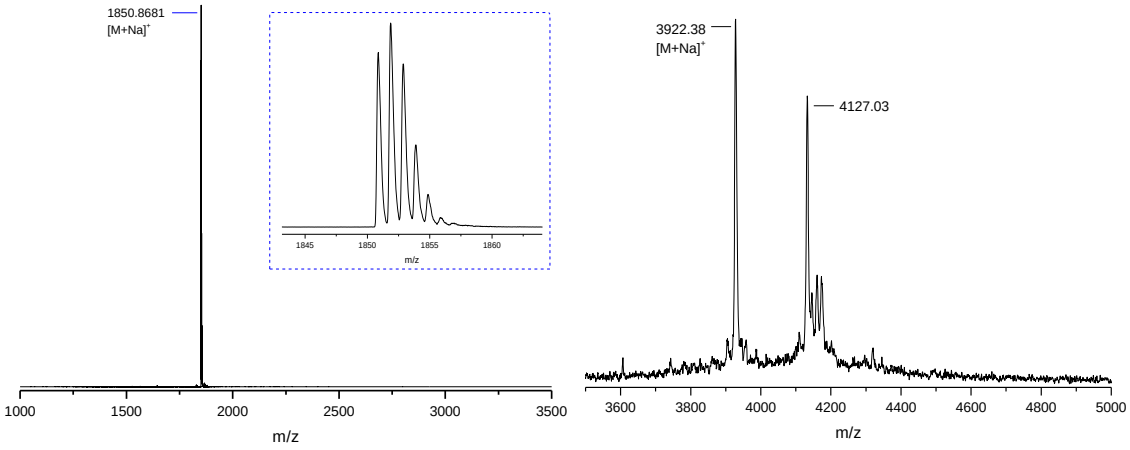


Figure S9d: MALDI spectrum after CuAAC reaction with *11-azido-3,6,9-trioxaundecan-1-amine* measured in reflective mode (left) and linear mode (right).

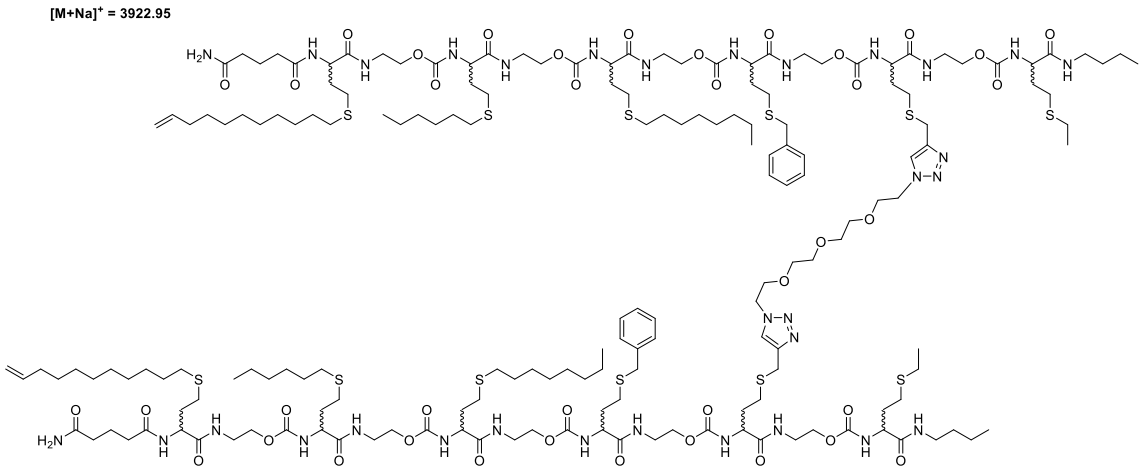


Figure S9e: High molecular weight structure formed by the CuAAC coupling of two hexamers with the diazide impurity present in *11-azido-3,6,9-trioxaundecan-1-amine*.

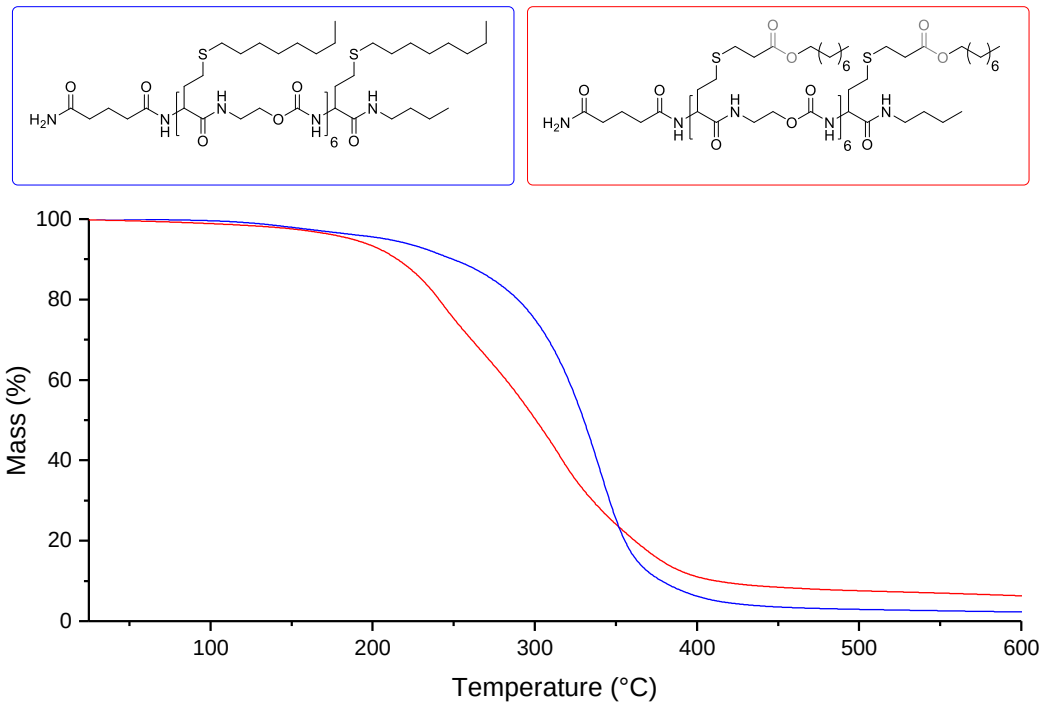
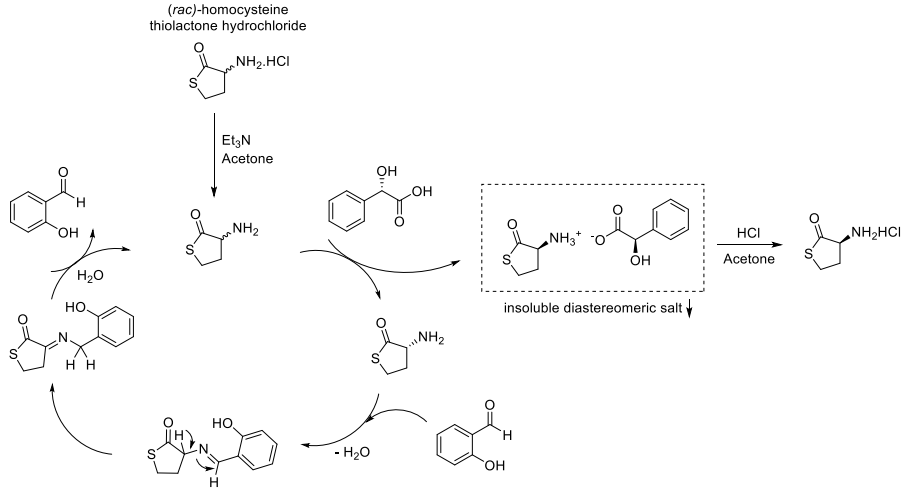


Figure S10: Thermogravimetric analysis of an oligomer synthesised *via* the protocol developed in this work (blue) and an oligomers with ester groups in the side-chain, synthesised using a previously reported protocol.[3]

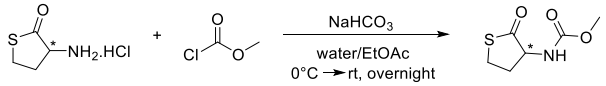
7 Chiral resolution of thiolactone derivatives

7.1 Chiral resolution of (*rac*)-homocysteine thiolactone hydrochloride



Scheme S2. Scheme of the chiral resolution procedure, procedure is included in the experimental part.[4]

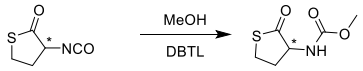
7.2 Synthesis of (*rac*)-, (*R*)- and (*S*)-methyl(homocysteine thiolactone) carbamate (Method A)



Scheme S3. Synthesis of (*rac*)-, (*R*)- and (*S*)-methyl(homocysteine thiolactone) carbamate [5]

Respectively (*rac*)-, (*R*)- *or* (*S*)-homocysteine thiolactone hydrochloride (0.5 g , 1.3 mmol, 1 eq) was solubilised in a water/ethyl acetate mixture (1:1) and cooled down in an ice bath. Next, sodium bicarbonate (0.24 g, 2.86 mmol, 2.2 eq) was added and stirred for 15 minutes. Then, methyl chloroformate (110 µl, 1.43 mmol, 1.1 eq) was added dropwise with a syringe and the reaction was stirred overnight at room temperature. After acidification with HCl (36 wt% in water) to pH 1, the mixture was transferred to a separation funnel and additional water was added. The aqueous phase was extracted with ethyl acetate (3x) and the organic fractions were collected and dried with magnesium sulphate. After removal of the solvent and drying in a vacuum oven, the final product was obtained. **^{1}H NMR (300 MHz, $CDCl_3$):** δ(ppm) = 5.21 (br, 1H), 4.33 (m, 1H), 3.71 (s, 3H), 3.29 (m, 2H), 2.87 (m, 1H), 2.00 (m, 1H)

7.3 Synthesis of (*rac*)-, (*R*)- and (*S*)-methyl(homocysteine thiolactone) carbamate (Method B)



Scheme S4. Synthesis of (*rac*)-, (*R*)- and (*S*)-methyl(homocysteine thiolactone) carbamate (Method B)

Respectively (*rac*)-, (*R*)- or (*S*)-α-isocyanato-γ-thiolactone (200 mg, 1.4 mmol) was solubilised in 10 mL methanol and a drop of dibutyltin dilaurate (DBTL) was added. The reaction was stirred overnight and subsequently concentrated *in vacuo*, yielding methyl(homocysteine thiolactone)carbamate. Quantitative yields were obtained. **^{1}H NMR (300 MHz, $CDCl_3$):** δ(ppm) = 5.21 (br, 1H), 4.33 (m, 1H), 3.71 (s, 3H), 3.29 (m, 2H), 2.87 (m, 1H), 2.00 (m, 1H).

8 Study of the stereo-controlled oligomers

Table S2: Difference in solubility of the stereocontrolled oligomer iso$_{(R)}$-nona-C8 and the non-controlled, atactic oligomer at-nona-C8. Solution of ~0.5 mg/ml were made and heated to reflux temperature in order to solubilise the oligomers. [a] precipitate started forming again when the sample was cooled down to room temperature.

Solvent	**Dielectric constant**	**Stereocontrolled oligomer** *at*-nona-C8	**Non-stereocontrolled oligomer** *iso*(*R*)-nona-C8
Methylcyclohexane	2.0	×	Not investigated
Decalin	2.1	×	Not investigated
1,4-dioxane	2.3	✓	✓
Tetrahydrofuran	7.6	✓	✓
Dichloromethane	8.9	±[a]	✓
1,2-dichloroethane	10.3	✓	✓
Isopropanol	17.9	×	✓
Ethanol	24.5	×	✓
Methanol	32.7	×	✓
N,N-dimethylformamide	36.7	✓	✓
Acetonitrile	37.5	×	✓
Water	80.0	×	×

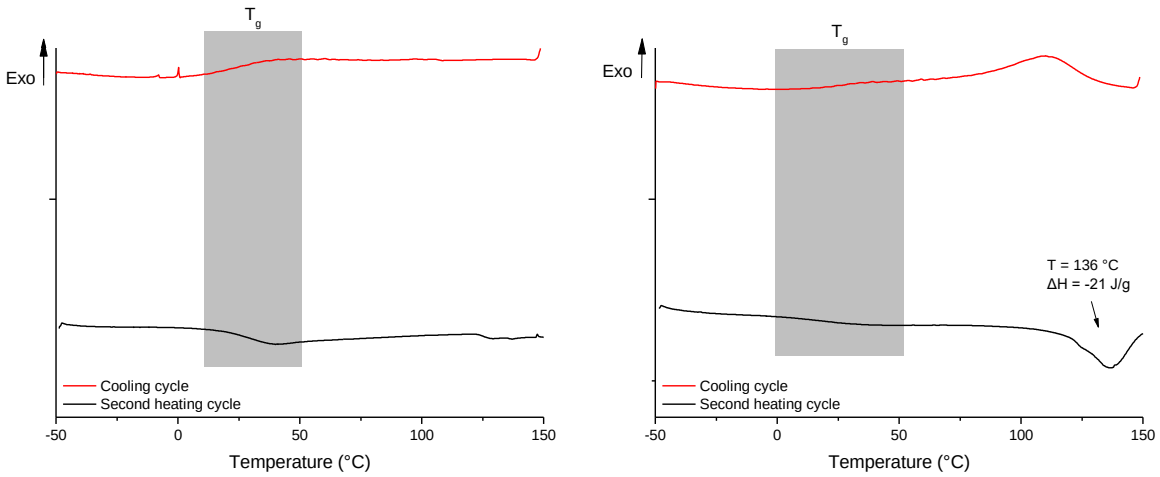


Figure S11: DSC-analysis of *at*-nona-C8 (left) and the stereo-controlled oligomer *iso*(*R*)-nona-C8 (right).

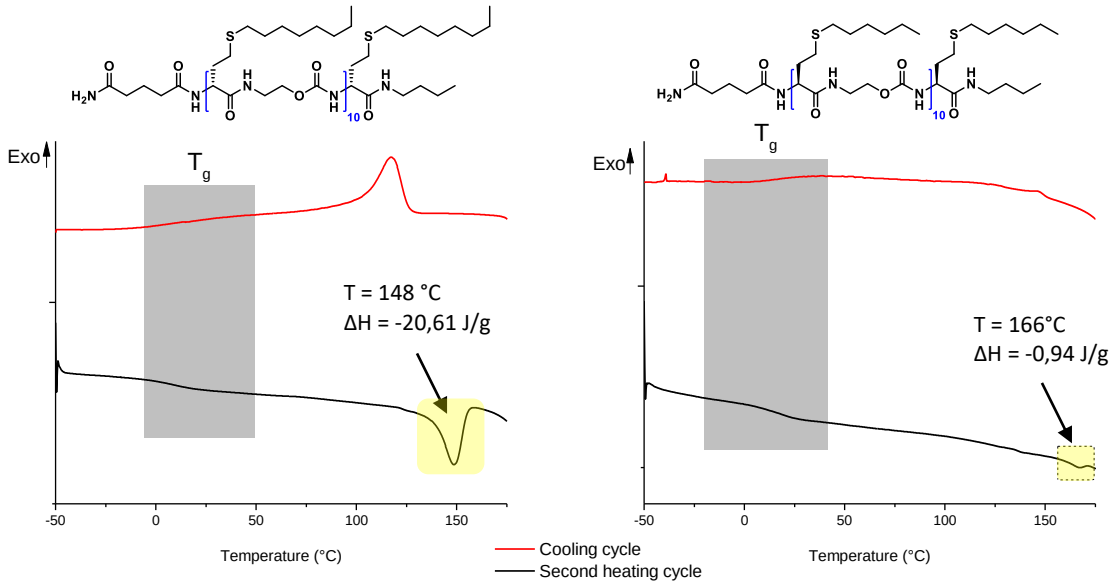


Figure S12. DSC-analysis of *iso*(*R*)-undeca-C8 (left) and the stereo-controlled oligomer *iso*(*S*)-undeca-C6 (right).

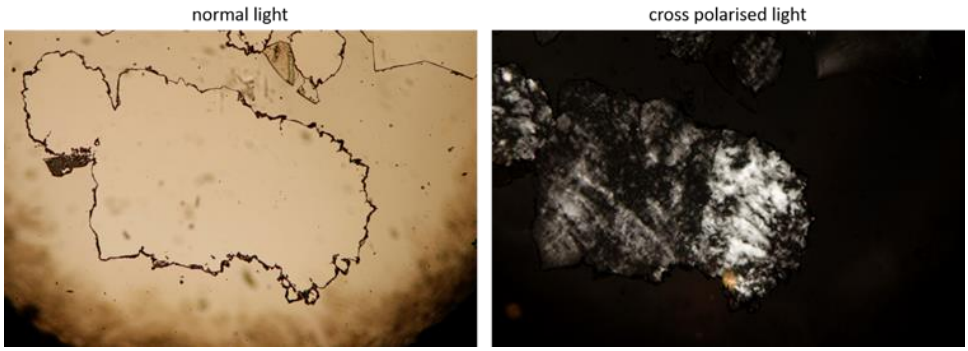


Figure S13: Optical microscopy images (20x magnification) of *iso*(*R*)-undeca-C8 under normal (left) and cross polarised light (right).

9 Bibliography

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