Supporting Information:

Papain-catalysed mechanochemical synthesis of oligopeptides by milling and twin-screw extrusion; application in the Juliá-Colonna enantioselective epoxidation

Karen J. Ardila-Fierro,[a] Deborah E. Crawford,[b] Andrea Körner,[c] Stuart L. James,[b]

Carsten Bolm,[a] and José G. Hernández*[a]

a) Institute of Organic Chemistry, RWTH Aachen University, Landoltweg 1, D-52074 Aachen, Germany.
b) School of Chemistry and Chemical Engineering, Queen's University Belfast, David Keir Building, Stranmillis Road, Belfast, BT9 5AG, UK.
c) DWI – Leibniz Institut for Interactive Materials e.V., Forckenbeckstr. 50, 52074, Aachen, Germany.

jose.hernandez@oc.rwth-aachen.de

CONTENTS

1. General information

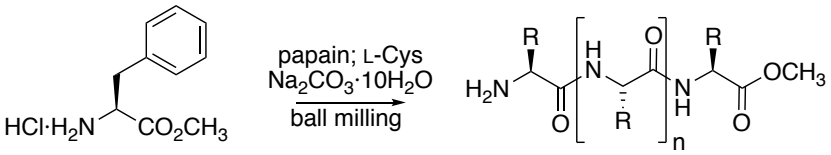
All NMR spectra were recorded on a VNMRS 400 or a VNMRS 600 spectrometer operating at 400 or 600 MHz for ^{1}H NMR and 100 or 150 MHz for ^{13}C NMR. Proton chemical shifts are reported in parts per million on the δ scale and are calibrated using the residual non-deuterated solvent signal as an internal reference (DMSO-d_6: δ = 2.50 ppm; $CDCl_3$: δ = 7.26 ppm). Carbon chemical shifts are reported in part per million on the δ scale and are calibrated using the residual non-deuterated solvent signal as an internal reference ($CDCl_3$: δ = 77.1 ppm). Mass spectra were acquired on a Finnigan SSQ7000 (EI, 70 eV) spectrometer. Analytical HPLC measurements for the determination of the enantiomer ratios were performed on an Agilent 1100 and with a chiral stationary phase (254 mm in length, 4.6 mm in internal diameter) (CHIRALPAK® IA; n-heptane:EtOH 80:20; 0.8 mL/min or n-heptane:2-propanol 96:4; 0.8 mL/min; 20 °C; 254 nm. IR-spectra were recorded on a Perkin Elmer 100 FT/IR spectrometer. TGA analysis was done in a Mettler Toledo DSC/TGA 1 Star, ran in air using aluminium pans with a heating rate of 20 degrees per minute.

MALDI-ToF MS Analysis: The peptide samples were dissolved in trifluoroacetic acid (TFA) to a concentration of 5 mg/mL. A matrix solution of α-cyano-4-hydroxycinnamic acid (CCA) with a concentration of 20 mg/mL in TA80 (80:20, ACN: 0.1% TFA in water) was prepared and sample and matrix solution were mixed in a ratio of 1:1. 1 μL of the sample/matrix solution was prepared on the target plate (ground steel target, Bruker) and dried. Mass spectra were acquired using a 1 kHz Laser Bruker UTX MALDI-ToF mass spectrometer (Bruker, Bremen, Germany) with pulsed ion extraction. The spectra were recorded in the reflector mode with a repetition rate of 1000 Hz signals from positive ions; 3000 spectra were added to a sum spectrum. While recording the sample stage moved in a random fashion on a sample spot allowing 500 laser shots per position. The calibration was carried out externally using a mixture of peptides (Peptide Calibration Standard mono, #8222570 from Bruker Daltonics, Bremen, Germany) prepared on a near neighbour spot. The spectra were baseline subtracted (Top hat algorithm) and mass peak annotation was obtained with the SNAP detection algorithm (width 0.5 at 80% height).
The characterisation of the molecular weight distributions, determination of polydispersity and mixture deconvolution were performed with the Flex Analysis ™ software (Bruker Daltonics, Bremen) and the Polymerix™ software tool from Sierra Analytics, Modesto, CA 95356 USA.

Chemicals were purchased from commercial suppliers and used without further purification. Papain from *Carica papaya* powder [≥3U/mg] was purchased from Sigma-Aldrich.

Oligomerisation reactions by milling were carried out in a RETSCH MM400 mixer mill using 10 mL milling vessels made of ZrO_2 with one milling ball of 10 mm in diameter of the same material or in a FRITSCH planetary micro mill model "Pulverisette 7 classic line", using 45 mL milling vessels made of ZrO_2 with five milling balls of the same material (10 mm in diameter) as milling media. Epoxidation experiments by milling were conducted in the planetary ball mill using 12 mL ZrO_2 milling vessels loaded with 20 milling balls of 5 mm in diameter. Extrusion experiments were carried out using a Three-Tec corotating twin-screw extruder, 40/1 L/D, 360L stainless steel with six heating zones.

2. General procedure for the mechanochemical papain catalysed oligomerisation of 1a by ball milling



1a; Phe

2a; R = CH_2Ph

a) Mixer mill

A mixture of **1a** (49.8 mg, 0.231mmol), papain powder (20 mg), L-Cys (7.7 mg, 0.064 mmol) and $Na_2CO_3 \cdot 10H_2O$ (66.1 mg, 0.231 mmol) was milled for 2 h in a mixer-mill at 25 Hz, using a 10 mL ZrO_2 milling jar with one ZrO_2 ball of 10 mm in diameter. After the milling was stopped, the reaction mixture was filtered with water (50 mL). To facilitate the drying of the peptide, the residue can be washed with diethyl ether (20 mL). However, this step can be omitted without affecting the yield of the reaction. Next, the residue was dried at 60 °C overnight to afford the product **2a**. Control experiments in the absence of the mechanical milling, followed by the standard filtration procedure did not afford **2a**. Thus, ruling out the oligomerisation of **1a** during the workup.

b) Planetary ball mill

A mixture of **1a** (500 mg, 2.318 mmol), papain powder (200 mg), L-Cys (77.81 mg, 0.642 mmol) and $Na_2CO_3 \cdot 10H_2O$ (663.34 mg, 2.318 mmol) was milled for 2 h in a planetary ball mill at 600 rpm, using a 45 mL ZrO_2 milling jar with five ZrO_2 balls of 10 mm in diameter. After the milling was stopped, the reaction mixture was filtered with water (100 mL). To facilitate the drying of the peptide, the residue can be washed with diethyl ether (50 mL). However, this step can be omitted without affecting the yield of the reaction. Next, the residue was dried at 60 °C overnight to afford the product **2a**.

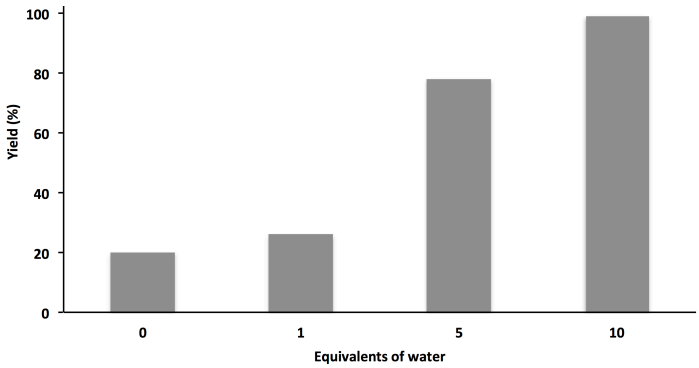


Figure S1. Effect of the crystalline water contained in the base on the yield of the oligomerisation of **1a** in the planetary ball mill at 600 rpm for 2 h. Control over the amount of crystalline water added to the reaction was achieved by using Na_2CO_3, $Na_2CO_3 \cdot H_2O$, $Na_2CO_3 \cdot 10H_2O$ or mixtures of them.

3. General procedure for the mechanochemical papain catalysed reaction of 1a in the twin-extruder

A mixture of **1a** (10 g, 0.046 mol), papain powder (4.0 g), L-Cys (1.55 g, 0.012 mol) and $Na_2CO_3 \cdot 10H_2O$ (13.26 g, 0.046 mol) was initially mixed by hand and fed into the volumetric twin-screw hopper. The reagents were introduced into the screw extruder at 30 rpm with the extruder barrel heated at 50 °C. The material started exiting the barrel after 45 min as a molten mixture. After the process was stopped, the reaction mixture was filtered with water (500 mL). To facilitate the drying of the peptide, the residue can be washed with diethyl ether (200 mL). However, this step can be omitted without affecting the yield of the reaction. Next, the residue was dried at 60 °C overnight to afford the product **2a**.

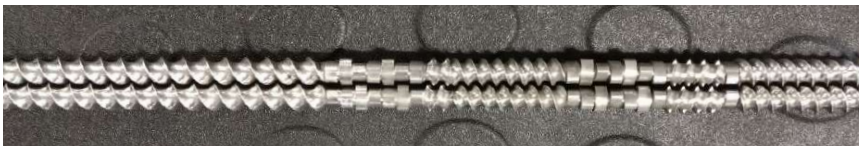
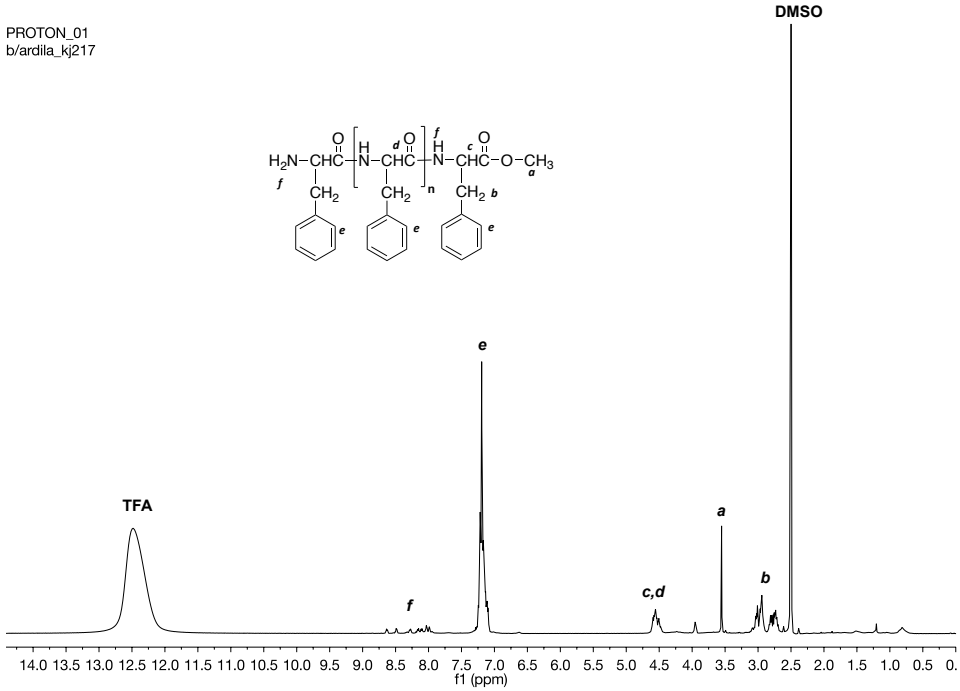


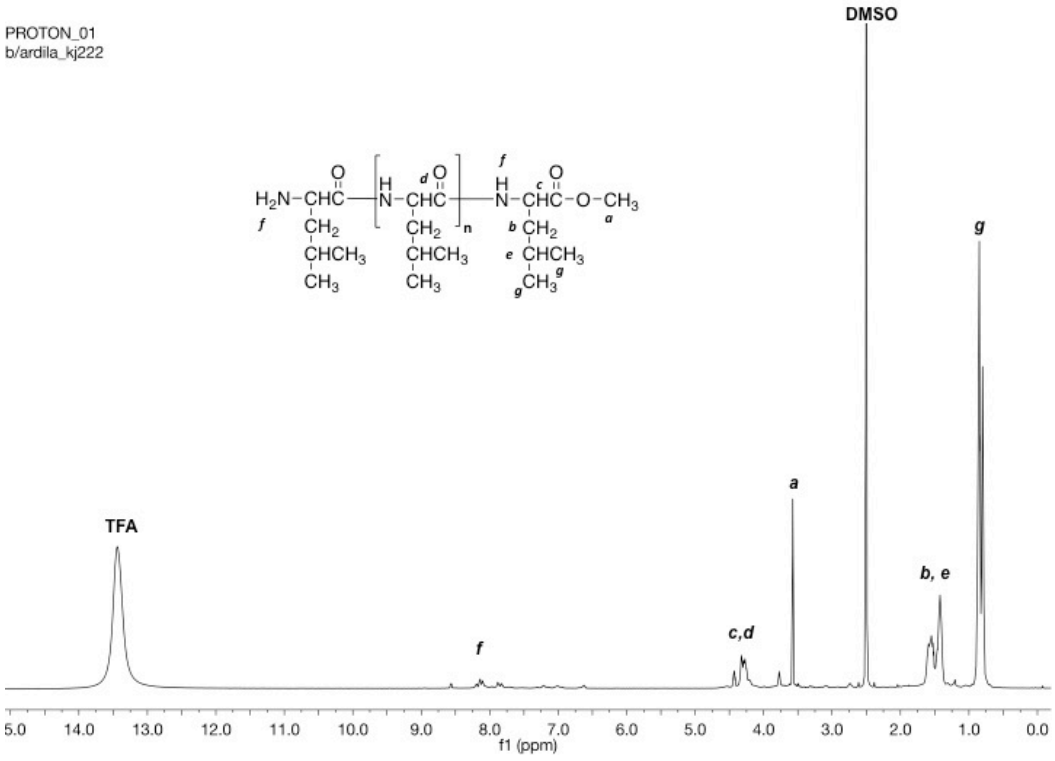
Figure S2. Steel twin-screw set configuration employed in the oligomerisation of the amino acids (12 mm in diameter, 40 cm in length).

4. Representative NMR spectra of 2a-d and 4a-c

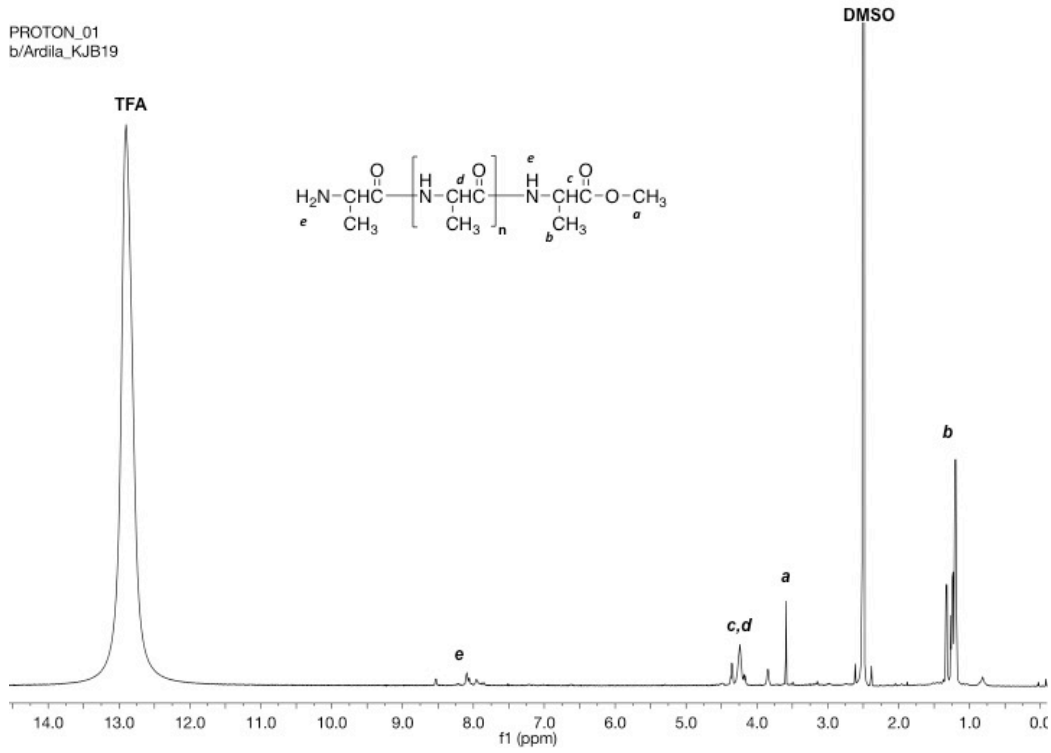
^{1}H NMR spectrum of oligo-Phe (2a) made by ball milling



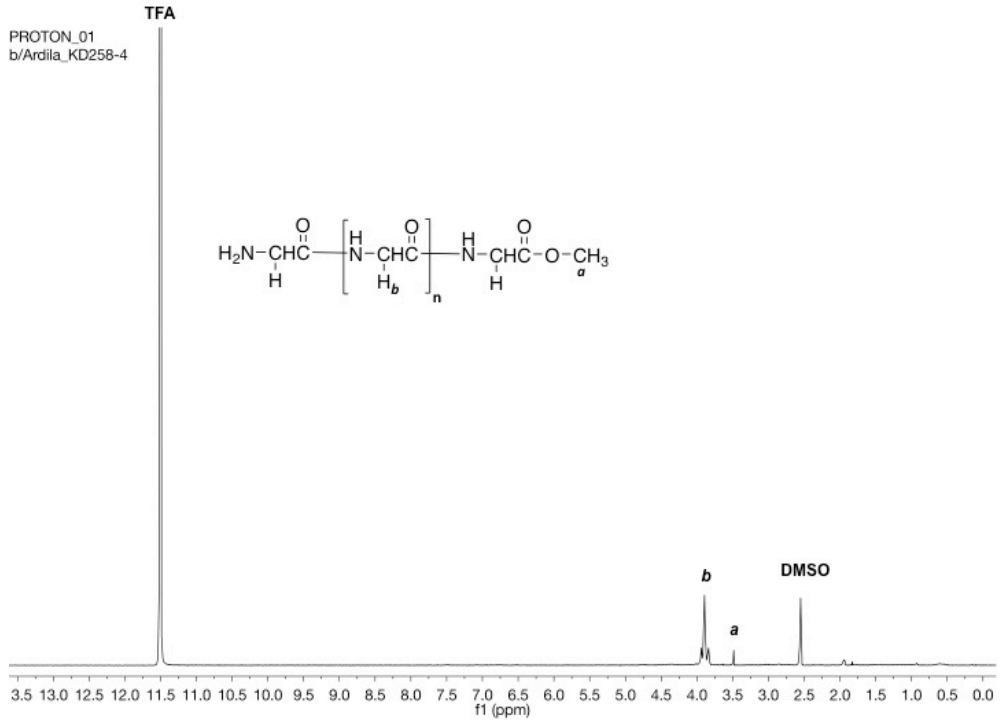
^{1}H NMR spectrum of oligo-Leu (2b) made by ball milling



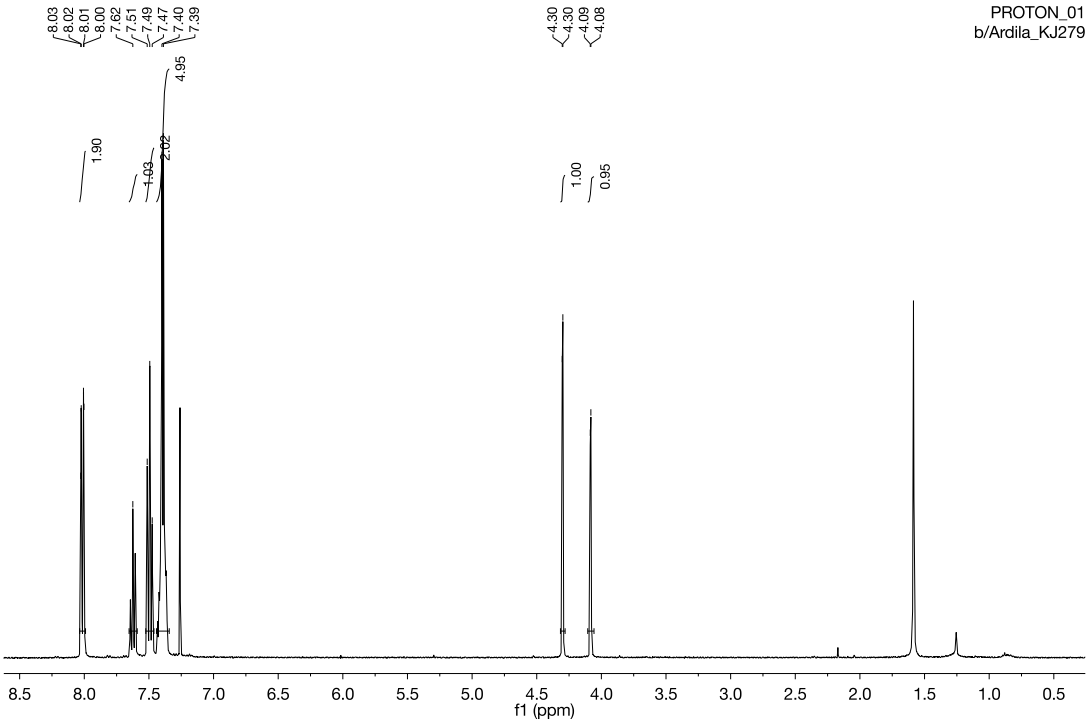
^{1}H NMR spectrum of oligo-Ala (2c) made by extrusion



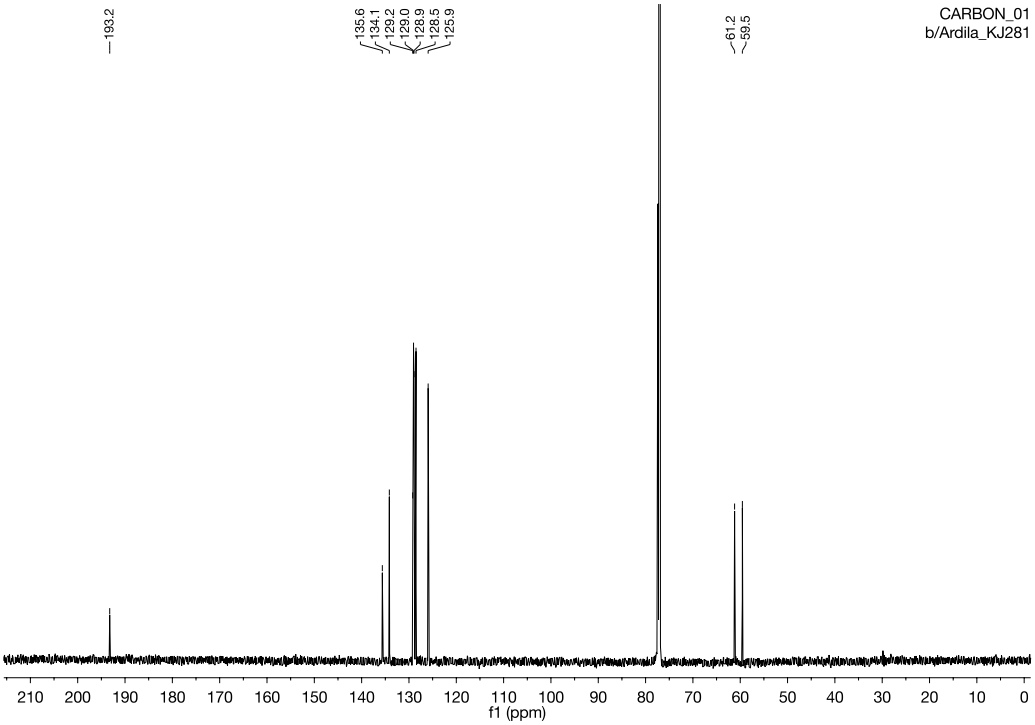
^{1}H NMR NMR spectrum of oligo-Gly (2d) made by ball milling



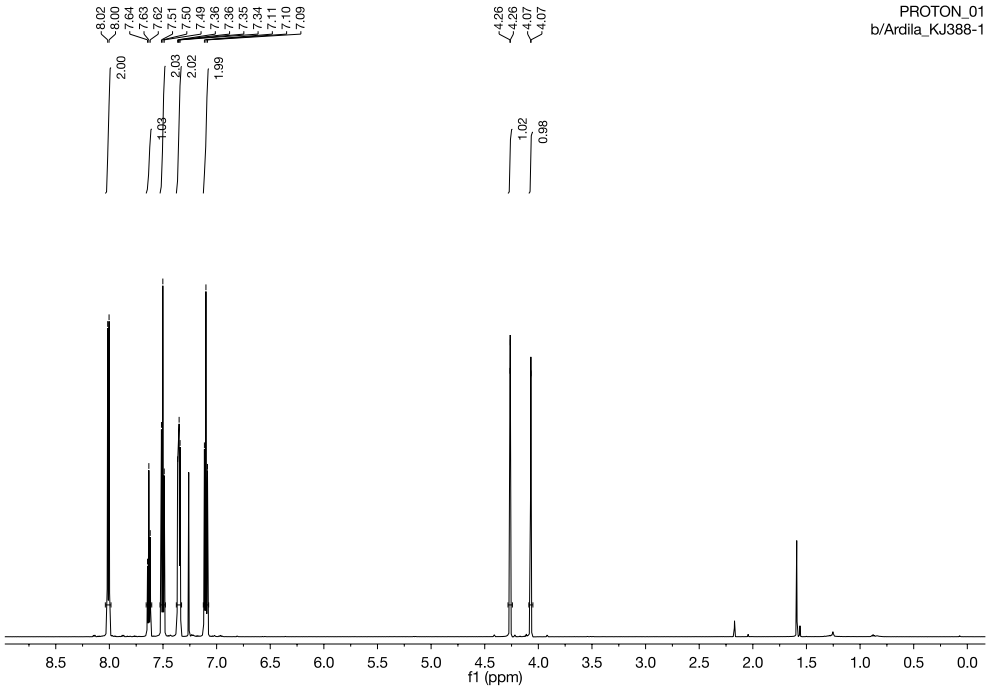
^{1}H NMR spectrum of epoxide 4a



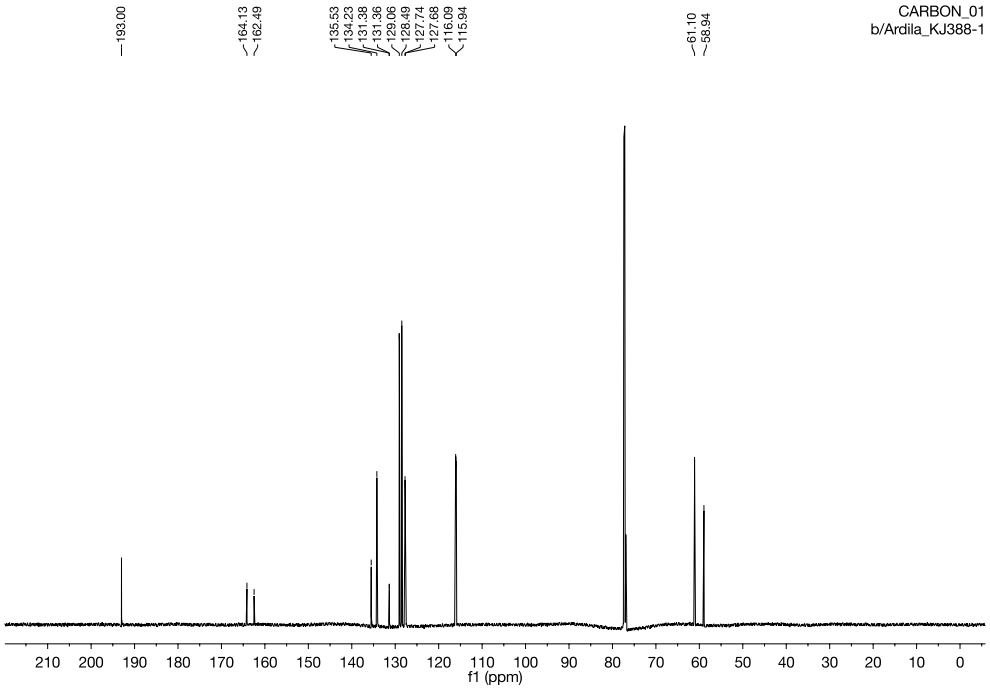
^{13}C NMR spectrum of epoxide 4a



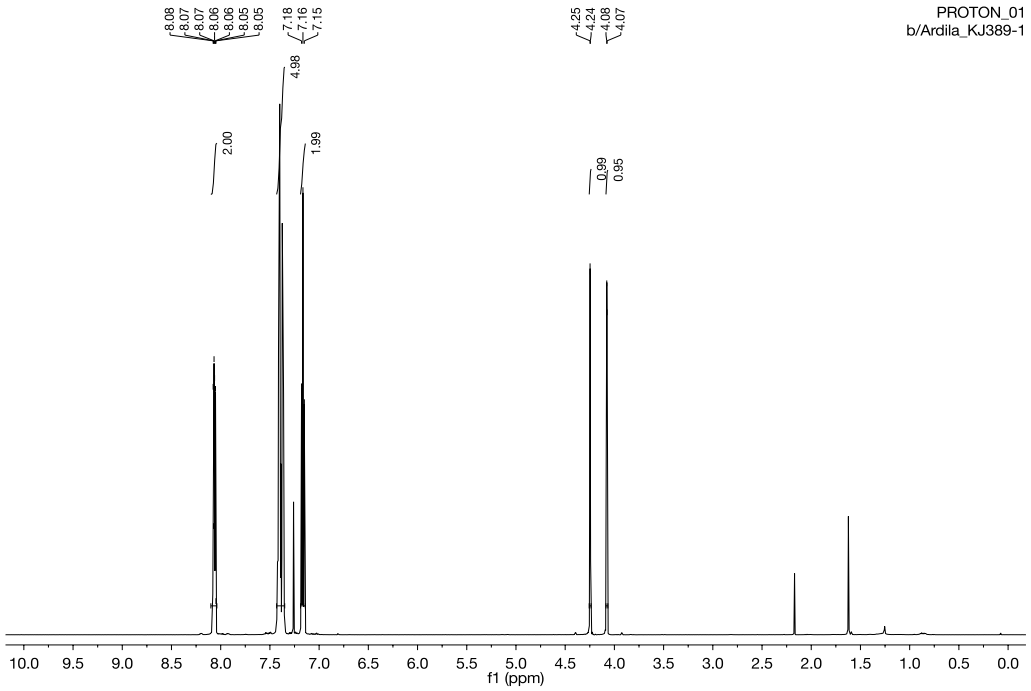
^{1}H NMR spectrum of epoxide 4b



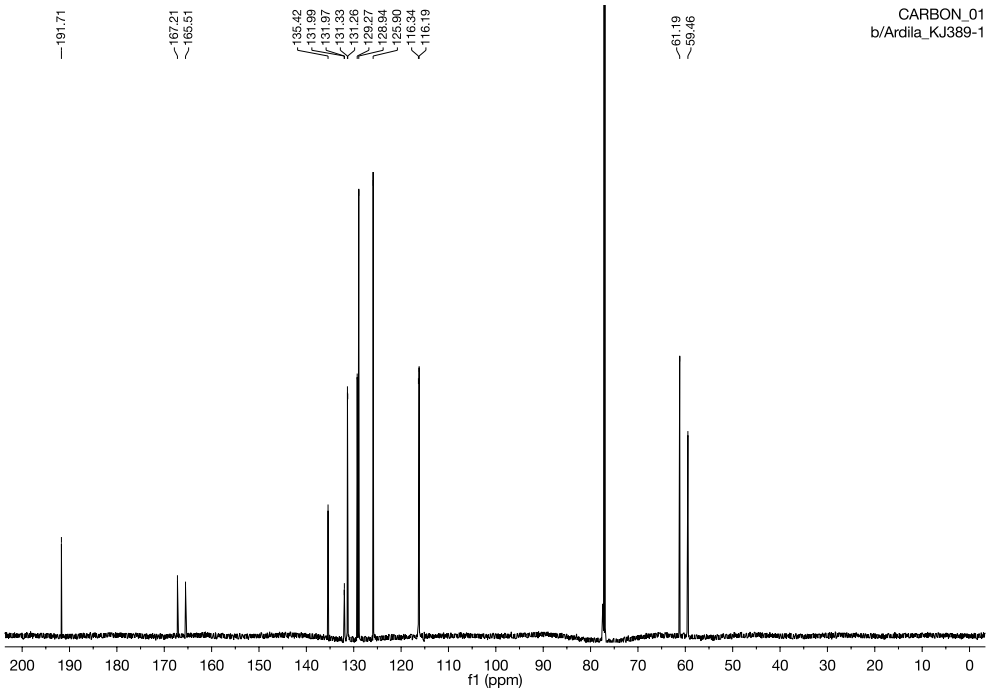
^{13}C NMR spectrum of epoxide 4b



^{1}H NMR spectrum of epoxide 4c

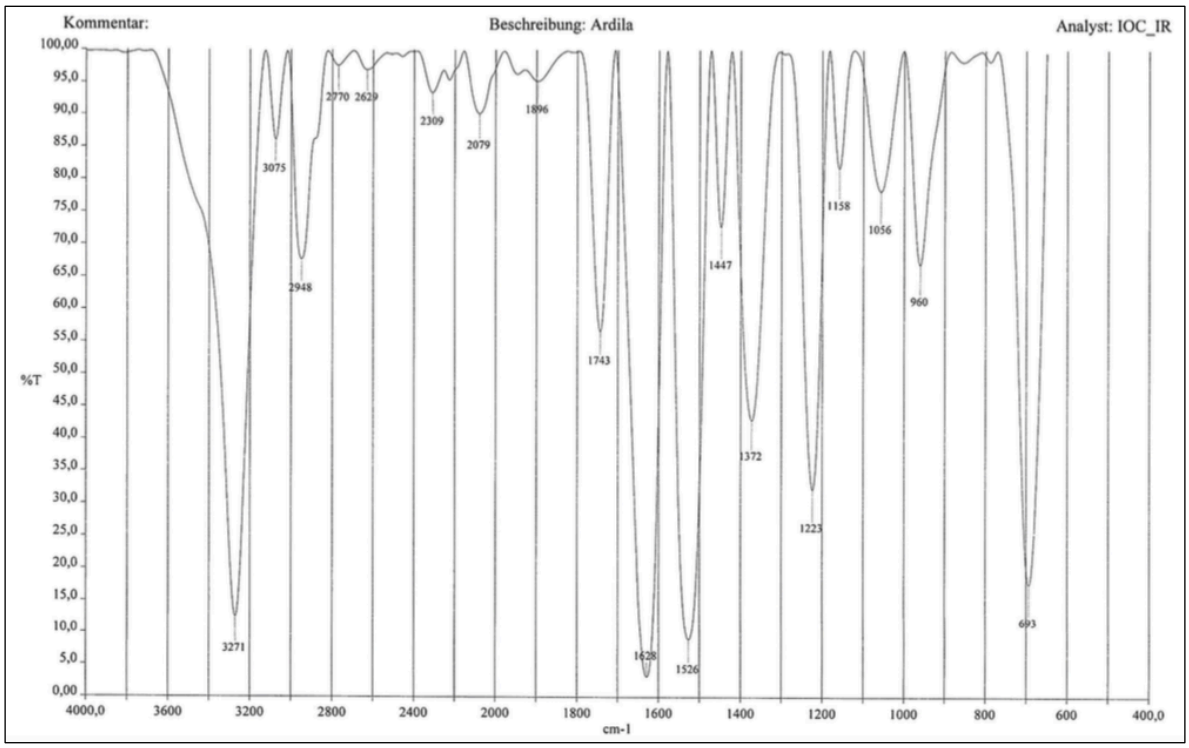


^{13}C NMR spectrum of epoxide 4c

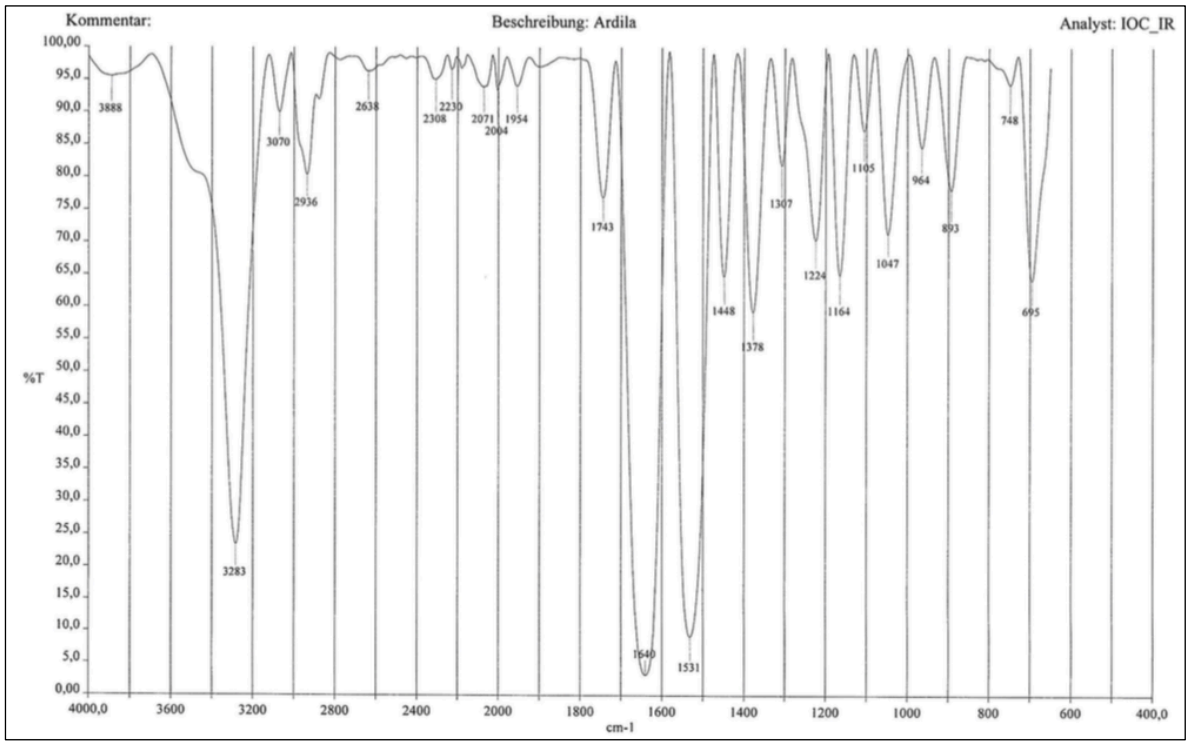


5. Representative IR spectra of 2a-d

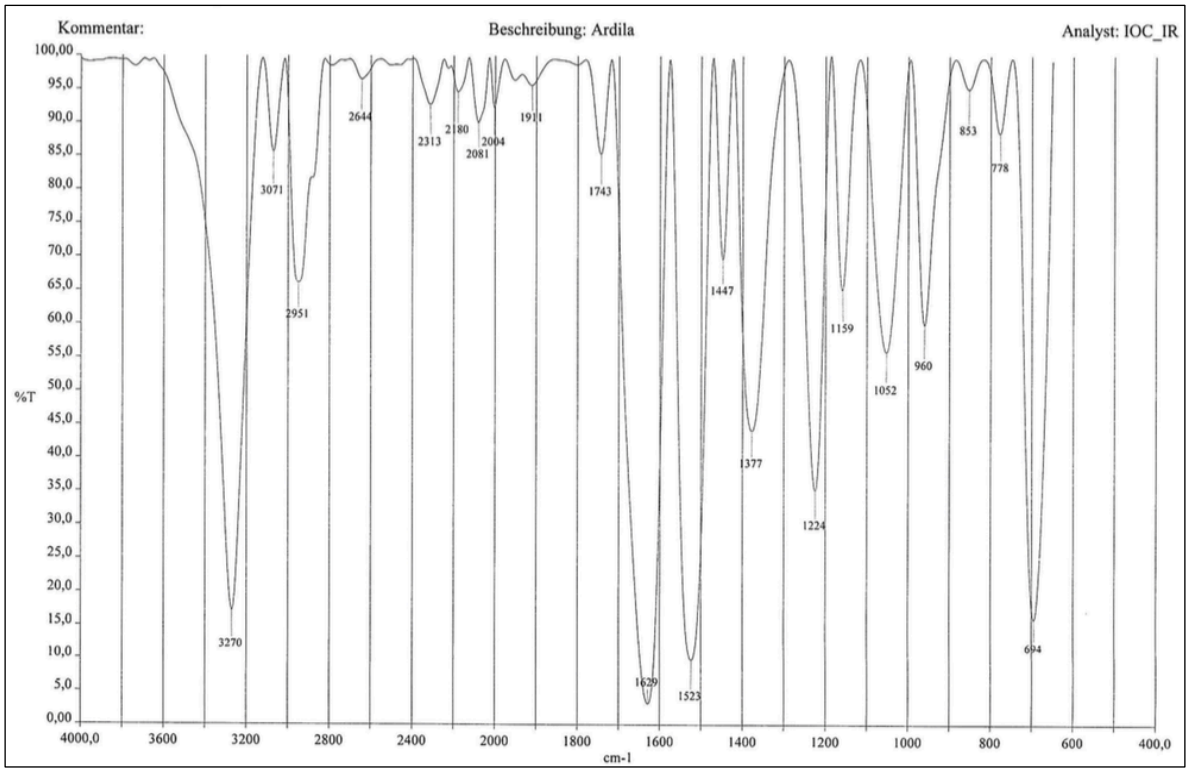
IR spectrum of oligo-Phe (2a)



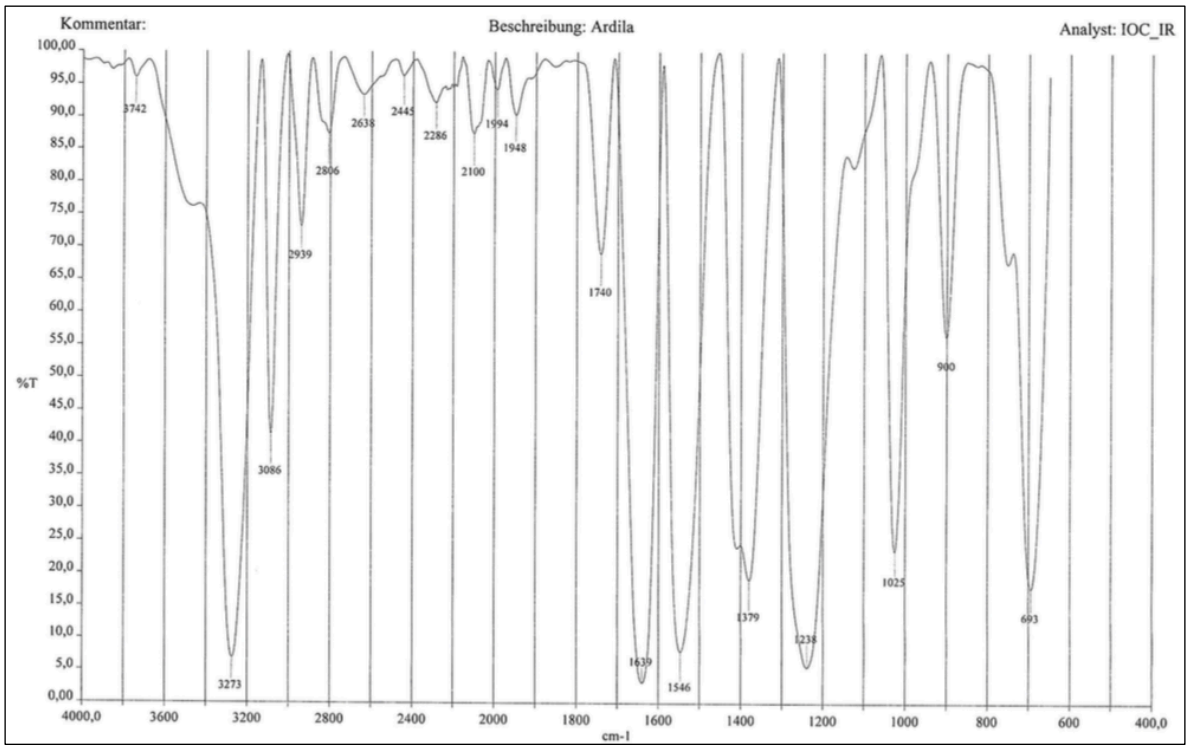
IR Spectrum of oligo-Leu (2b)



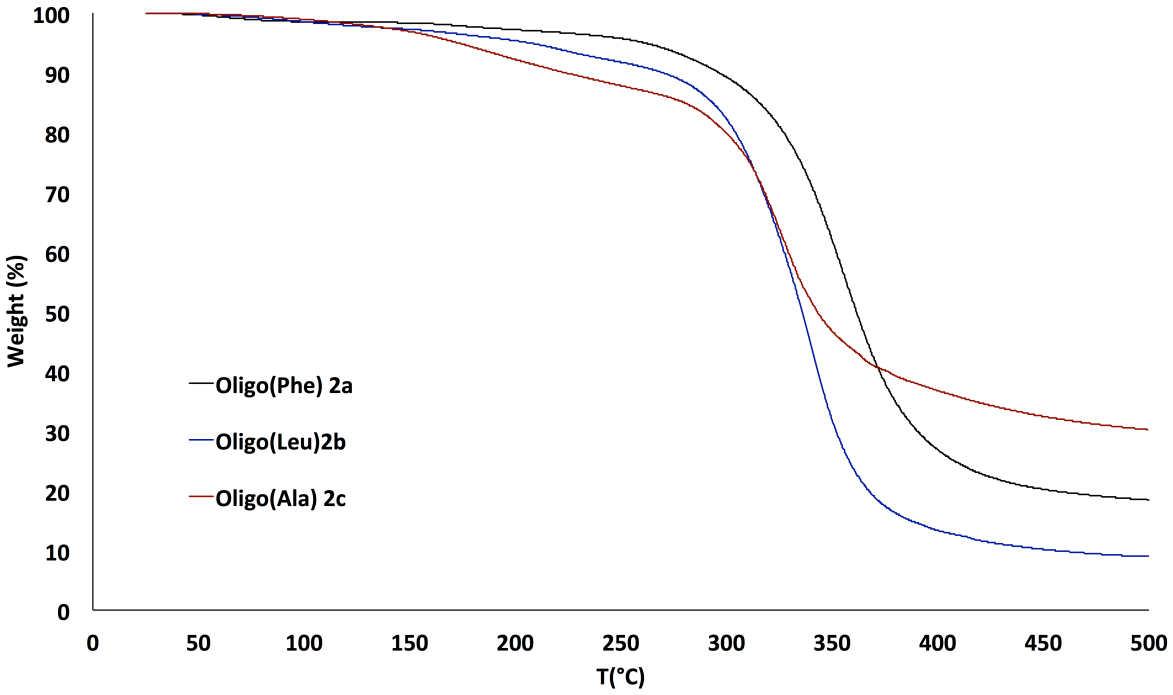
IR Spectrum of oligo-Ala (2c)



IR Spectrum of oligo-Gly (2d)

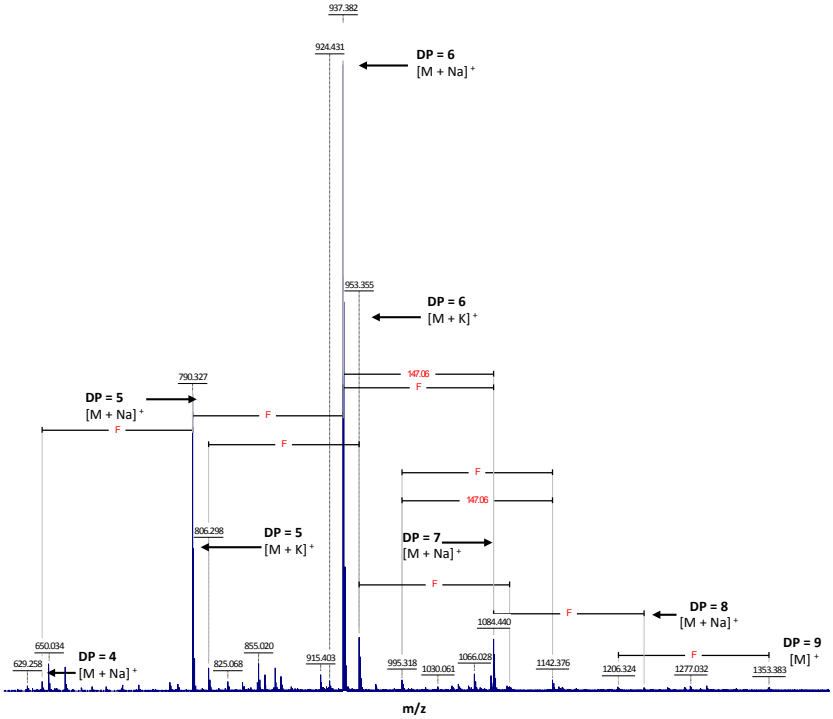


6. Thermogravimetric analysis (TGA) of the oligopeptides 2a-c made by twin-screw extrusion

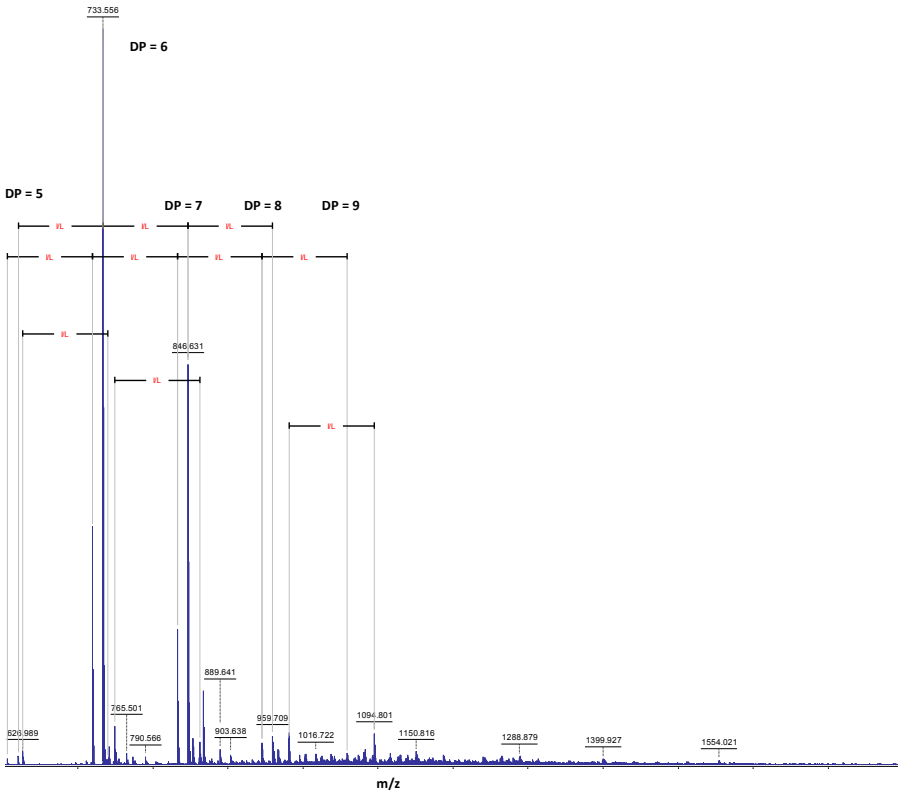


7. Representative MALDI-ToF MS spectra and analysis

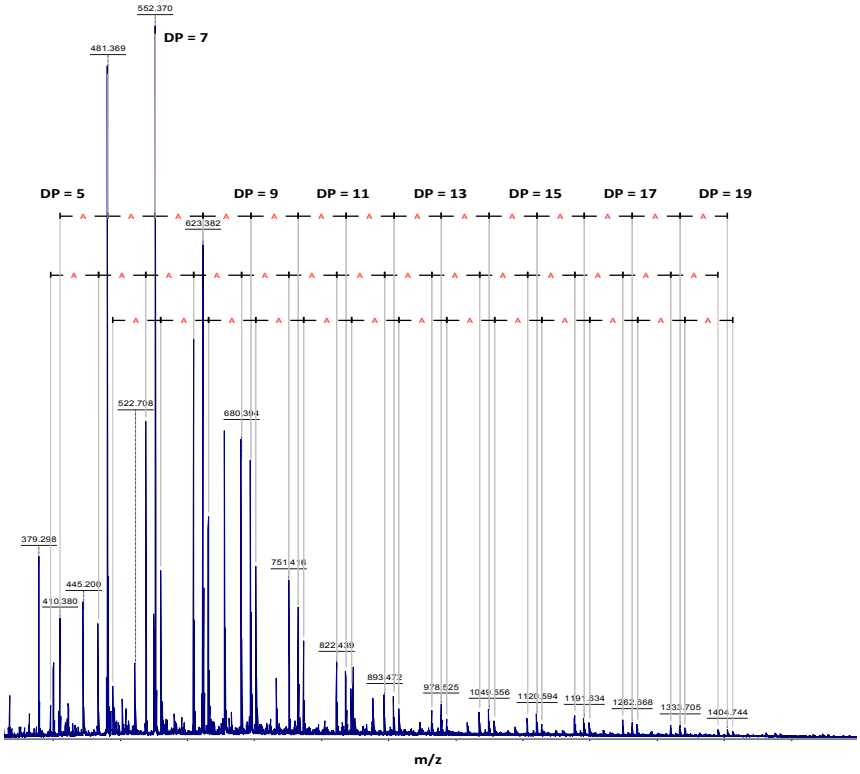
MALDI-ToF MS mass spectrum of the oligo-Phe-OMe **2a** made in a mixer mill



MALDI-TOF MS mass spectrum of the oligo-Leu-OMe **2b** made by extrusion



MALDI-ToF MS mass spectrum of the oligo-Ala-OMe **2c** made in a planetary ball mill



MALDI-ToF MS mass spectrum of the oligo-Gly-OMe **2d** made in a planetary ball mill

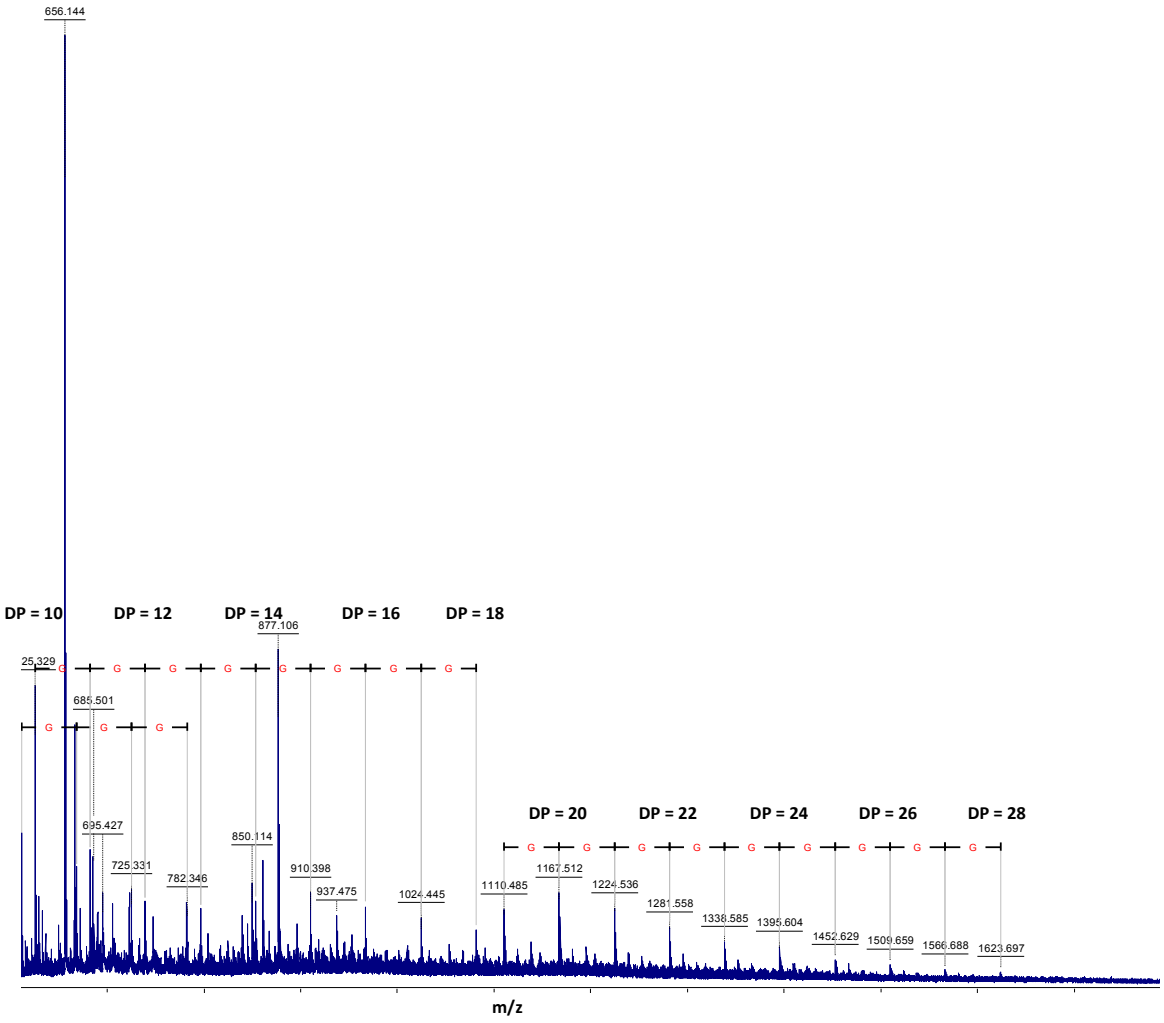
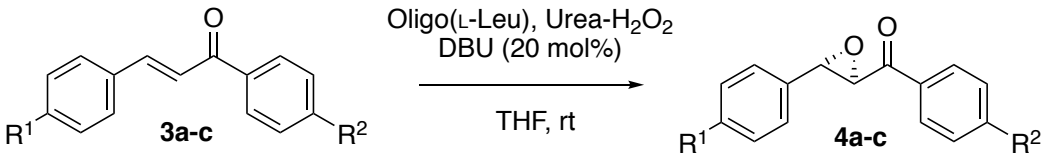


Table S1: Homopolymer results summary for the oligomers of Phe, Leu, Ala, and Gly (Polymerix, Sierra Analytics) by MALDI ToF MS; DP av. NMR: average DP as determined by ^{1}H NMR spectroscopy

Oligo Phe-OMe **2a**							
Series Label	Mn	PD	DPn	DP av. NMR			
Total/Average	1054,793	1,1	7,0	8			
S1	992,268	1,1	6,5				
S2	1270,237	1,2	8,4				
S5	1110,05	1,2	7,4				
Series Label	Alpha End Group	Repeat	Omega End Group	Charge State	Adduct	Loss	Formula
S1	H	C_9H_9NO	CH_3O	1	Na		H $[C_9H_9NO]$n CH_3O + Na
S2	H	C_9H_9NO	CH_3O	1	K		H $[C_9H_9NO]$n CH_3O + K
S5	H	C_9H_9NO	OH	1	Na		H $[C_9H_9NO]$n OH + Na
Oligo Leu-OMe **2b**							
Series Label	Mn	PD	DPn	DP av. NMR			
Total/Average	1172,238	1,2	10,1	8,5			
S1	1017,162	1,2	8,7				
S2	1416,739	1,2	12,2				
S5	1321,055	1,2	11,5				
Series Label	Alpha End Group	Repeat	Omega End Group	Charge State	Adduct	Loss	Formula
S1	H	$NHCHCOCH_2CHCH_3CH_3$	CH_3O	1	Na		H $[NHCHCOCH_2CHCH_3CH_3]$n CH_3O + Na
S2	H	$NHCHCOCH_2CHCH_3CH_3$	CH_3O	1	K		H $[NHCHCOCH_2CHCH_3CH_3]$n CH_3O + K
S5	H	$NHCHCOCH_2CHCH_3CH_3$	OH	1	Na		H $[NHCHCOCH_2CHCH_3CH_3]$n OH + Na
Oligo Ala-OMe **2c**							
Series Label	Mn	PD	DPn	DP av. NMR			
Total/Average	702,163	1,2	9,5	15			
S1	672,478	1,2	9,0				
S4	746,658	1,2	10,3				
Series Label	Alpha End Group	Repeat	Omega End Group	Charge State	Adduct	Loss	Formula

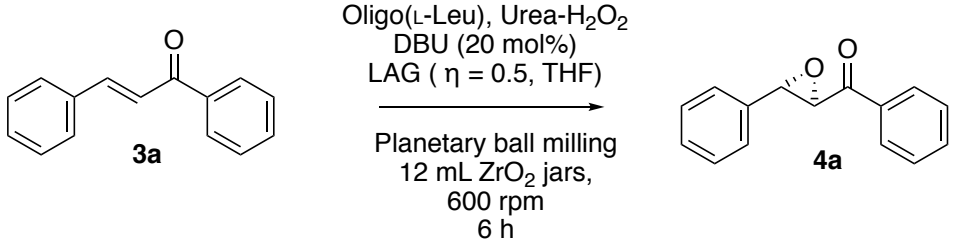
S1	H	C_3H_5NO	CH_3O	1	Na		H [C_3H_5NO]n CH_3O + Na
S4	H	C_3H_5NO	OH	1	Na		H [C_3H_5NO]n OH + Na
Oligo Gly-OMe **2d**							
Series Label	Mn	PD	DPn	DP av. NMR			
Total/Average	1277,494	1,2	21,9	26			
S1	1253,551	1,2	21,4				
S3	1304,891	1,2	22,3				
S5	1275,923	1,2	22,1				
Series Label	Alpha End Group	Repeat	Omega End Group	Charge State	Adduct	Loss	Formula
S1	H	$NHCH_2CO$	CH_3O	1	Na		H [$NHCH_2CO$]n CH_3O + Na
S3	H	$NHCH_2CO$	CH_3O	1	K		H [$NHCH_2CO$]n CH_3O + K
S5	H	$NHCH_2CO$	OH	1	Na		H [$NHCH_2CO$]n OH + Na
Oligo Leu-OMe **2b**							
Series Label	Mn	PD	DPn	DP av. NMR			
Total/Average	1064,74	1,2	9,2	8,2			
S1	893,705	1,2	7,6				
S2	1371,252	1,2	11,8				
S5	1085,477	1,3	9,4				
S6	1560,971	1,2	13,6				
Series Label	Alpha End Group	Repeat	Omega End Group	Charge State	Adduct	Loss	Formula
S1	H	$NHCHCOCH_2CHCH_3CH_3$	CH_3O	1	Na		H [$NHCHCOCH_2CHCH_3CH_3$]n CH_3O + Na
S2	H	$NHCHCOCH_2CHCH_3CH_3$	CH_3O	1	K		H [$NHCHCOCH_2CHCH_3CH_3$]n CH_3O + K
S5	H	$NHCHCOCH_2CHCH_3CH_3$	OH	1	Na		H [$NHCHCOCH_2CHCH_3CH_3$]n OH + Na
S6	H	$NHCHCOCH_2CHCH_3CH_3$	OH	1	Na	H_2O	H [$NHCHCOCH_2CHCH_3CH_3$]n OH + Na - H_2O

8. Procedure for the Juliá-Colonna epoxidation in solution



To a suspension of the mechanochemically made oligo-L-leucine catalyst[1] (75 mg) in THF (2 mL), chalcone derivatives (**3a-c**) (0.24 mmol), DBU (7.31 mg, 20 mol%), and urea-H_2O_2 (27.10 mg, 0.28 mmol) were added. The reaction mixture was stirred at room temperature for 48 h; a further amount of urea-H_2O_2 (27.10 mg, 0.28 mmol) was added to the reaction each day in order to achieve full conversion. After the reaction was complete, the oligo-L-leucine was removed by suction filtration, and washed with ethyl acetate (20 mL). The filtrate was concentrated and the products were purified by column chromatography (silica gel, n-pentane:ethyl acetate) to afford epoxides **4a-c** as a while-off solids.

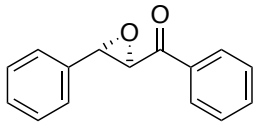
9. Procedure for the Juliá-Colonna epoxidation in a ball mill



Chalcone (**3a**) (50.00 mg, 0.24 mmol), mechanochemically made oligo-L-leucine[1] (75 mg), DBU (7.31 mg, 20 mol%), urea-H_2O_2 (27.10 mg, 0.28 mmol) and THF (71 μL) were placed into a 12 mL ZrO_2 milling vessel with 20 ZrO_2 balls of 5 mm in diameter. The reaction mixture was milled for 6 h at 600 rpm (6 x 60 min; 10 min break). After the milling was stopped, the content of the milling vessel was transferred into a sintered funnel. Using a minimal amount of ethyl acetate the milling vessels were washed and the oligo-L-leucine was removed by suction filtration. The filtrate was concentrated and the product was purified by column chromatography (silica gel, n-pentane:ethyl acetate 100:1) to afford epoxide **4a** as a white-off solid.

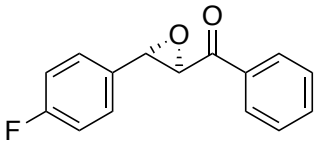
10. Characterisation of products 4a-c

(2*R*,3*S*)-Epoxy-1,3-diphenylpropan-1-one (4a)[2]



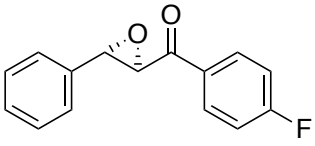
Epoxide **4a** was obtained as a white solid (75% yield, section 8); ν_{max}/cm^{-1} 2922, 1683, 1590, 1230. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 8.03–8.00 (m, 2H), 7.62 (tt, *J* = 7.8, 1.3 Hz, 1H), 7.50 (dd, *J* = 8.2, 7.3 Hz, 2H), 7.44–7.37 (m, 5H), 4.30 (d, *J* = 1.9 Hz, 1H), 4.08 (d, *J* = 1.8 Hz, 1H). **^{13}C{^{1}H} NMR** (150 MHz, $CDCl_3$) δ 193.2, 135.6, 134.1, 129.2, 129.0, 128.9, 128.5, 125.9, 61.2, 59.5. **MS (EI, 70 eV)** *m/z* (%) = 224 (38) [M^+], 105 (100), 77 (12).

(2*R*,3*S*)-Epoxy-3-(4-fluorophenyl)-1-phenylpropan-1-one (4b)[3]



Epoxide **4b** was obtained as a white solid (83% yield, section 8); ν_{max}/cm^{-1} 2924, 1685, 1598, 1218. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 8.01(d, *J* = 7.8, 2H), 7.66–7.60 (m, 1H), 7.52–7.48 (m, 2H), 7.35 (dd, *J* = 5.35, 3.18 Hz 2H), 7.12–7.08 (m, 2H), 4.26 (brs, 1H), 4.07 (brs, 1H). **^{13}C{^{1}H} NMR** (150 MHz, $CDCl_3$) δ 193.0, 163.3 (d, ^1J (C,F) = 244.9 Hz), 135.5, 134.2, 131.3 (d, ^4J (C,F) = 2.9 Hz), 129.0, 128.4, 127.7 (d, ^3J (C,F) = 8.6 Hz), 116.0 (d, ^2J (C,F) = 21.8 Hz), 61.1, 58.4. **MS (EI, 70 eV)** *m/z* (%) = 242 (24) [M^+], 105 (100), 77 (35).

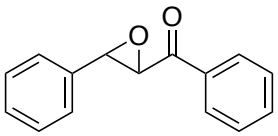
(2*R*,3*S*)-2,3-Epoxy-3-phenyl-1-(4-fluorophenyl)-1-propan-1-one (4c)[3]



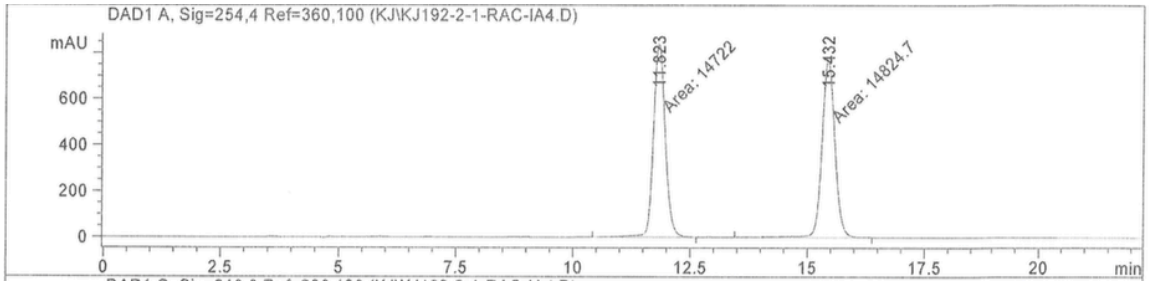
Epoxide **4c** was obtained as a white solid (78% yield, section 8); ν_{max}/cm^{-1} 3066, 1677, 1596, 1231. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 8.09–8.04(m, 2H), 7.43–7.35 (m, 5H), 7.19–7.14 (m, 2H), 4.25 (d, *J* = 1.65, Hz 1H), 4.08 (d, *J* = 1.61, 1H). **^{13}C{^{1}H} NMR** (150 MHz, $CDCl_3$) δ 191.7, 166.3 (d, ^1J (C,F) = 257.9 Hz), 135.4, 131.9 (d, ^4J (C,F) = 3.1 Hz), 131.2 (d, ^3J (C,F) = 9.1 Hz), 129.2, 128.9, 125.9, 116.2 (d, ^2J (C,F) = 22.4 Hz), 61.1, 59.4. **MS (EI, 70 eV)** *m/z* (%) = 242 (25) [M^+], 123 (100), 95 (31).

11. Enantiomeric ratios of 4a-c determined by chiral stationary phase high performance liquid chromatography (CSP-HPLC)

CHIRALPAK® IA; n-heptane:EtOH 80:20, 0.8 mL/min

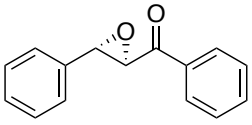


rac-**4a**
(2*R*,3*S*)-**4a** 11.823 min
(2*S*,3*R*)-**4a** 15.432 min

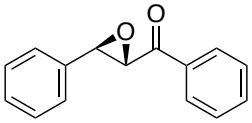


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.823	MM	0.2918	1.47220e4	840.91040	49.8262
2	15.432	MM	0.3208	1.48247e4	770.17126	50.1738

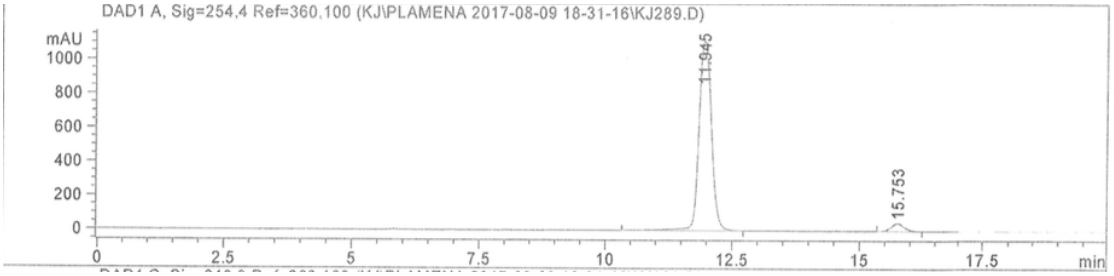
Product obtained from the enantioselective epoxidation in solution



(2*R*,3*S*)-**4a**
95.86%

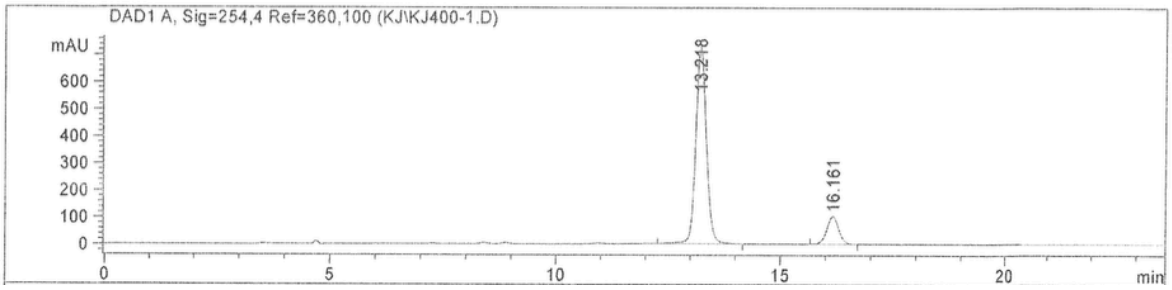


(2*S*,3*R*)-**4a**
4.13%



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.945	BB	0.2698	1.90185e4	1110.71631	95.8690
2	15.753	BB	0.2742	819.51349	45.91649	4.1310

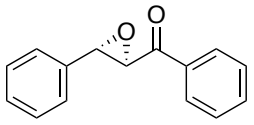
Product obtained from the enantioselective epoxidation in solution (second cycle)



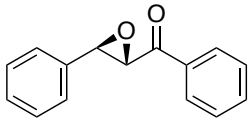
Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.218	BB	0.2515	1.19208e4	726.39105	86.3688
2	16.161	BB	0.2811	1881.39441	102.99103	13.6312

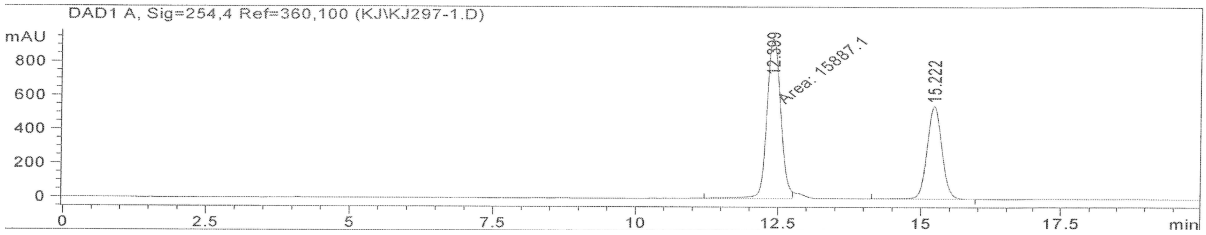
Product obtained from the enantioselective epoxidation by ball milling.



(2*R*,3*S*)-**4a**
61.64%

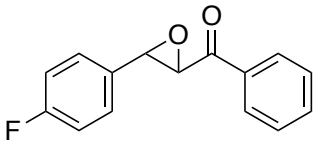


(2*S*,3*R*)-**4a**
38.35%



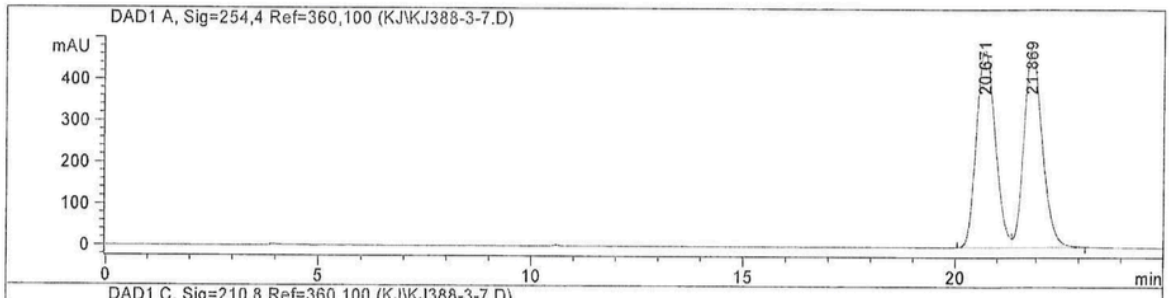
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.399	MF	0.2811	1.58871e4	941.96722	61.6402
2	15.222	BB	0.2793	9886.83887	545.77020	38.3598

The absolute configuration of (2*R*,3*S*)-**4** and (2*S*,3*R*)-**4** was confirmed by comparing their experimental retention times to reported literature.[4] Moreover, the epoxidation of **3** was conducted using poly-L-Leu made by ring-opening polymerization of L-Leu-NCA. This experiment gave a mixture of (2*R*,3*S*)-**4** (major) and (2*S*,3*R*)-**4** (minor) with identical retention times compared to the values obtained utilising the mechanochemically made oligo-L-Leu **2b**.



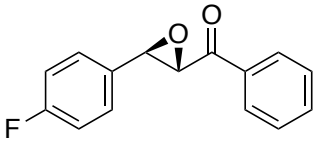
rac-**4b**

CHIRALPAK® IA; n-heptane:2-propanol 96:4, 0.8 mL/min

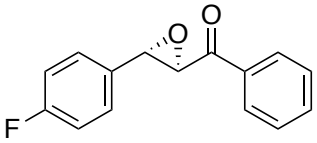


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	20.671	BV	0.4955	1.51270e4	475.05618	49.6364
2	21.869	VB	0.4962	1.53486e4	476.00363	50.3636

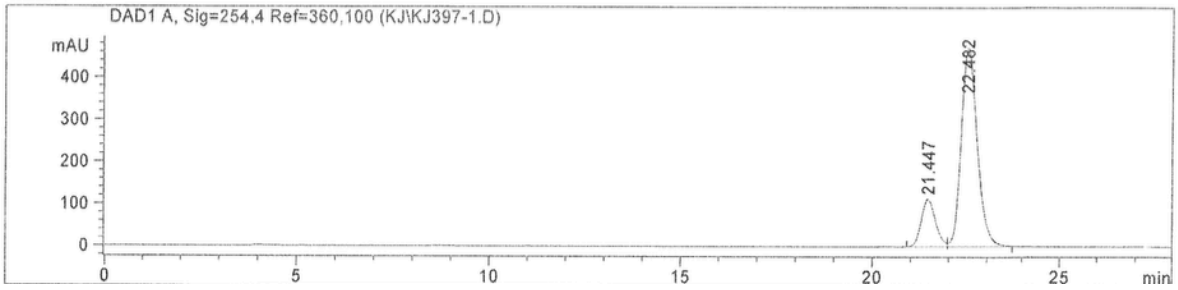
Product obtained from the enantioselective epoxidation in solution



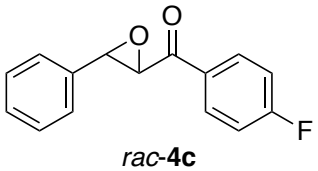
(2*S*,3*R*)-**4b**
17.6%



(2*R*,3*S*)-**4b**
82.4%

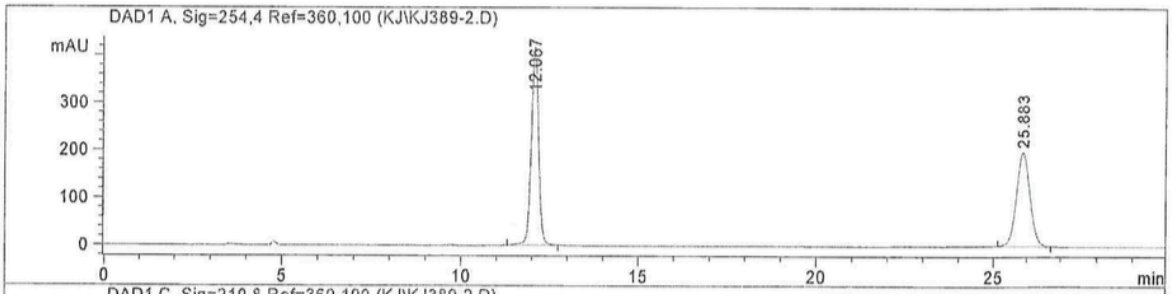


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	21.447	BV	0.3978	2942.41846	113.01099	17.5950
2	22.482	VB	0.4470	1.37806e4	471.44492	82.4050



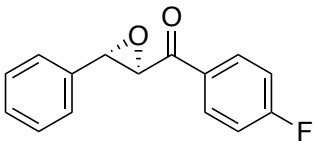
rac-**4c**

CHIRALPAK® IA; n-heptane:EtOH 80:20, 0.8 mL/min

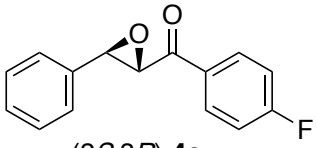


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.067	BB	0.2199	6007.69678	417.35529	51.1795
2	25.883	BB	0.4492	5730.78906	197.09204	48.8205

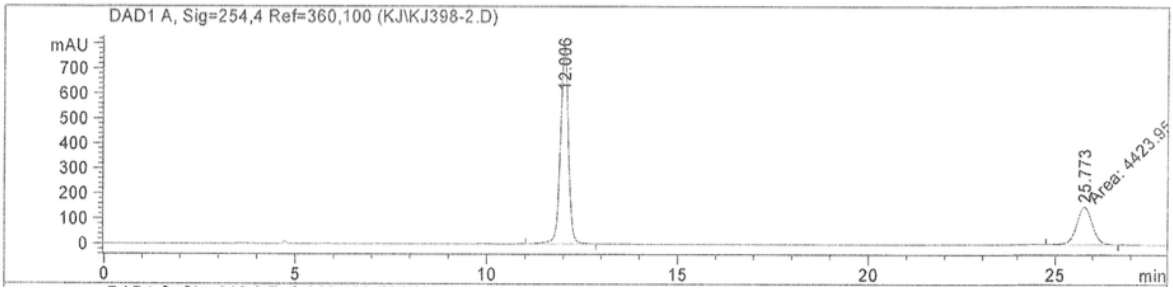
Product obtained from the enantioselective epoxidation in solution



(2*R*,3*S*)-**4c**
72.4%



(2*S*,3*R*)-**4c**
27.6%

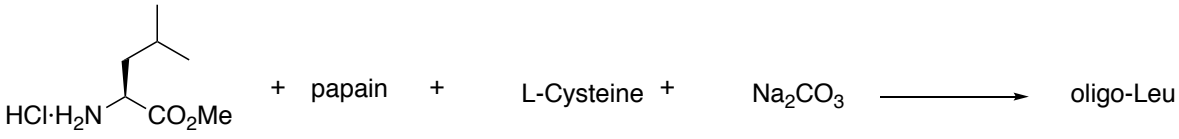


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.006	BB	0.2255	1.16185e4	790.48889	72.4235
2	25.773	MM	0.4935	4423.95117	149.41365	27.5765

12. E-Factor calculations for the synthesis of oligo-Leu

a) Planetary ball mill

Reaction and quantities. (Crystalline water coming from the $Na_2CO_3 \cdot 10H_2O$ and water used for filtration of the oligo-Leu was not considered as a waste in the calculation).

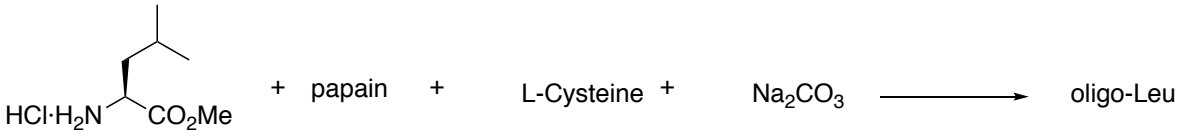


0.5 g | 0.23 g | 0.09 g | 0.29 g | 0.26 g

-**Total amount of reactants**: 0.5 g + 0.23 g + 0.09 g + 0.29 g = 1.11 g
-**Amount of final product**: 0.26 g
-**Amount of waste**: (1.11 – 0.26) g = 0.85 g
-E-Factor = Amount of waste/Amount of product = 0.85 g/0.26 g = 3.26

b) Extruder

Reaction and quantities. (Crystalline water coming from the $Na_2CO_3 \cdot 10H_2O$ and water used for filtration of the oligo-Leu was not considered as a waste in the calculation).



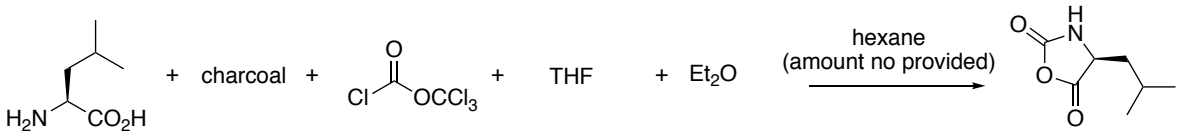
16.77 g | 8.0 g | 3.10 g | 9.78 g | 6.80 g

-**Total amount of reactants**: 16.77 g + 8.0 g + 3.10 g + 9.78 g = 37.65 g
-**Amount of final product**: 6.80 g
-**Amount of waste**: (37.65 – 6.80) g = 30.85 g
-E-Factor = Amount of waste/Amount of product = 30.85 g/6.80 g = 4.53

c) Solution

Reaction and quantities

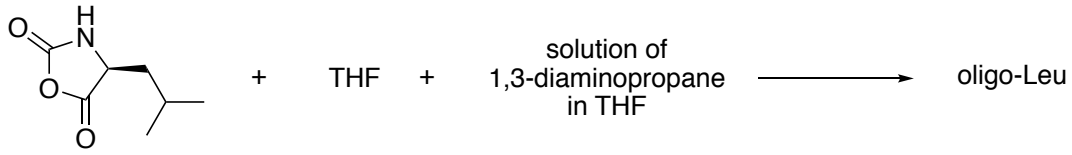
Part 1. Synthesis of Leu-NCA. The amounts are based on a protocol reported in the literature.[5]



4.0 g | 0.08 g | 4.62 g | 71.12 g | 213.9 g | 3.5 g

-**Total amount of reactants**: 4.0 g + 0.08 g + 4.62 g + 71.12 g + 213.9 g = 293.7 g
-**Amount of final product**: 3.5 g
-**Amount of waste**: (293.7 – 3.5) g = 290.2 g
-E-Factor = Amount of waste/Amount of product = 290.2 g/3.5 g = 82.91

Part 2. ROP of Leu-NCA. The amounts correspond to an experimental attempt to prepare oligo-Leu based on a protocol reported in the literature.[5]



0.20 g | 2.66 g | 0.045 g | 0.16 g

-**Total amount of reactants**: 0.2 g + 2.66 g + 0.045 g = 2.90 g
-**Amount of final product**: 0.16 g
-**Amount of waste**: (2.90 – 0.16) g = 2.74 g
-E-Factor = Amount of waste/Amount of product = 2.74 g/0.16 g = 17.12

-Total E-Factor = 82.91 + 17.12 = 100.0

13. References

1) The oligo(Leu) catalyst requires activation before use. For a detailed methodology, see: S. Baars, K.-H. Drauz, H-P. Krimmer, S.M. Roberts, J. Sander, J. Skidmore and G. Zanardi, *Org. Process Res. Dev.*, 2003, **7**, 509–513.
2) M. Xiang, X. Ni, X. Yi, A. Zheng, W. Wang, M. He, J. Xiong, T. Liu, Y. Ma, P. Zhu and T. Tang, *ChemCatChem,* 2015, **7**, 521–525.
3) C. Zeng, D. Yuan, B. Zhao and Y. Yao, *Org. Lett.,* 2015, **17**, 2242–2245.
4) J. Ye, Y. Wang, R. Liu, Q. Zhang, J. Chen and X. Liang, *Chem. Comm.,* 2003, 2714–2715.
5) C. Bérubé, X. Barbeau, P. Lagüe and N. Voyer, *Chem. Commun.,* 2017, **53**, 5099–5102.