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Influence of metal softness on the metal-organic catalyzed polymerization of morpholin-2,5-diones to oligodepsipeptides

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ABSTRACT

Synthetic access to oligodepsipeptides (ODP), polymers with high potential in biomedicine, is given by the ring-opening polymerization (ROP) of morpholine-2,5-diones (MDs). Classically, the ROP of MDs is mostly conducted by coordination-insertion polymerization using metal-organics as a catalyst e.g. tin(II) di(2-ethyl hexanoate) ($Sn(Oct)_2$). This ROP has been shown to be significantly more difficult to conduct than the corresponding ROP of dilactide, which was related to different electronic properties of the monomers and potential steric crowding. Here, we investigated the ROP of 3-(*S*)-sec-butylmorpholine-2,5-dione (BMD) by varying the catalyst's hardness, comparing $Sn(Oct)_2$ with the ethoxides of indium, magnesium, aluminium and iron(III), as well as with iron(II) acetate. The ROP of BMD with [$Sn(Oct)_2$] in bulk at 135 °C for 24 h gave ODP with a number-average molecular weight (M_n) = 4.5 kDa. $Mg(OEt)_2$ gave the best results among the other investigated metal ethoxides with ODP of M_n = 4 kDa and a conversion ratio of 57 mol%. On the other hand, high

polymerization temperature was needed (160 °C) in the case of $In(OEt)_3$, which resulted in partial degradation, while $Al(OEt)_3$ and $Fe(OEt)_3$ did not result in polymerization. Very effective for the ROP of the studied MD proofed to be $Fe(OAc)_2$, giving OBMD with a M_n = 5.8 kDa, a polydispersity of 1.1, a conversion ratio of 86 mol%, and no racemization. This catalyst likewise performed well in the polymerization of Ser- and Tyr-based MDs. Fe(II) is softer than Sn(II) and may support the ROP by promoting the alkoxide transfer step of the polymerization, while supressing the formation of unreactive coordination complexes. In contrast, the metal alkoxides investigated were harder than Fe(II) or Sn(II), but had low steric demand. The results suggest that the hardness of the central atom is the key property in the polymerization, while steric considerations are of lower importance. In addition, a synthesis of MDs with protected side chains in improved yields was introduced. This was achieved by in situ formation of an alkyl iodide that is very effective in the ring closing reaction.

1. Introduction

Synthetic biomaterials have been intensively studied during the last few decades for potential applications in biomedicine, including tissue- and organ-engineering, gene therapy, controlled drug delivery, and regenerative medicine [1-3]. For such purposes, materials with specific properties and functions such as degradation time, mechanical properties, presence of specific chemical groups, stimuli responsivity, and biocompatibility are required [4-7]. Oligodepsipeptides (ODP), i.e. alternating *co*-oligomers of α-amino and α-hydroxy acids, have been introduced to provide biomaterials with freely available functional groups and degradability without acidification of the environment [8]. The amino acid subunits within the ODP structure offer a synthetic access to introduce a large variety of pendent groups on the polymer backbone. Additionally, the amine groups formed simultaneously to carboxylic acid groups during the hydrolysis of ODPs act as internal buffer system [9]. However, the use of

oligodepsipeptides is still somewhat limited due to the challenges in synthesizing their monomers, the morpholine-2,5-diones (MDs), as well as in the ring-opening polymerization (ROP) of the MDs.

Three synthetic routes to MDs have been reported: Cyclization of (1) *N*-(α-haloacyl)-α-amino acid in diluted solution in presence of a base as acid scavenger; (2) *N*-(α-hydroxyacyl)-α-amino acids or *N*-(α-hydroxyacyl)-α-amino acids esters under diluted conditions in presence of *p*-toluenesulfonic acid or methanesulfonic acid as a catalyst; or (3) *O*-(α-aminoacyl)-α-hydroxycarboxylic acids in presence of a base [10]. The final yield for MDs is in all cases low to mild [11], with the first route giving the highest yield, thus being applied to prepare the more expensive MDs with protected functional groups such as (3*S*)-3-[(Benzyloxycarbonyl)methyl]morpholine- 2,5-dione [12], while MDs based on aliphatic amino acids were synthesized according to the second route e.g. (*S*)-3-((*S*)-*sec*-butyl)morpholine-2,5-dione (BMD) [10]. Further increase of the yield of MDs is required for a broader access to ODPs, and may be improved by the introduction of a better leaving group than the commonly applied bromides or chlorides. This should be performed in situ to avoid adding a full synthetic step to the synthesis.

ODPs have been mainly synthesized by ROP of MDs using either metal-organic compounds or enzymes as catalysts [13, 14]. In general, metal-organic catalysts used for ROP of cyclic ester as well as MDs catalyze this reaction by a coordination-insertion mechanism, where the carbonyl oxygen first coordinates with the metal centre. This is followed by a nucleophilic attack of alkoxide ligand on the carbonyl carbon (Scheme 1).

Morpholine-2,5-diones with a bulky substituent are less reactive than *L,L*-dilactides in the ROP so that generally ODPs are received in low yields and with limited molar mass. The low reactivity in the ring-opening polymerization could be partially related to the steric hindrance [4]. Further differences in reactivity compared to *L,L*-dilactide could be related to (i) possible

coordination of the MD to a metal complex in a non-productive way via the oxygen of the amide group (Scheme 1b, VIII) and (ii) participation of the amido N-H group in a reaction leading to stable chelating ligands which are kinetically inert for further ring-opening reaction (Scheme 1b, VII) [15]. Additionally, chelation of the metal via the amide nitrogen after ring-opening will lead to an un- or less reactive species (Scheme 1b, VI). The HSAB principle, which can be used to rationalize the behaviour of complex formation and chemical reactivity [16], suggests that a softer catalyst than the generally used $Sn(Oct)_2$ may be beneficial for the ROP of MDs, as the unproductive coordination may be reduced, and the transfer of the nucleophile from the metal centre to the carbonyl carbon may be increased by decreasing the affinity of the *O*- and *N*-coordination of the metal catalyst. This is in line with the findings of copolymerizing ε-caprolactone and 2-oxepane-1,5-dione, where oxophilic catalysts showed lower activity due to unproductive coordination to the ketone [17]. While the HSAB principle has been successfully used to explain chemical reactions, it should be noted that its application may be difficult due to several competing influences [18], which in this particular case includes the steric hindrance.

To explore whether the metal catalyst hardness and/or the steric hindrance influences its reactivity towards the ROP of MDs, we investigated here $Sn(Oct)_2$, $Fe(OAc)_2$, $Fe(OEt)_3$, $In(OEt)_3$, $Al(OEt)_3$, and $Mg(OEt)_2$ in the ring-opening of (*S*)-3-sec-butyl-morpholine-2,5-dione (BMD), comparing yield, conversion ratio, number average molecular weight formed, the polydispersity, as well as retention of stereochemistry. The most promising of the new catalysts, $Fe(OAc)_2$ was furthermore compared to $Sn(Oct)_2$ regarding the reaction kinetics, formation of side products, and in the polymerization of MDs with bulky substituents, (*S*)-3-((benzyloxy)methyl)morpholine-2,5-dione (BnSerMD) and (*S*)-3-(benzyloxy)benzyl)-morpholine-2,5-dione (BnTyrMD). In addition, it is shown that the yield of MDs can be increased by in situ activating the ring closure.

2. Experimental section

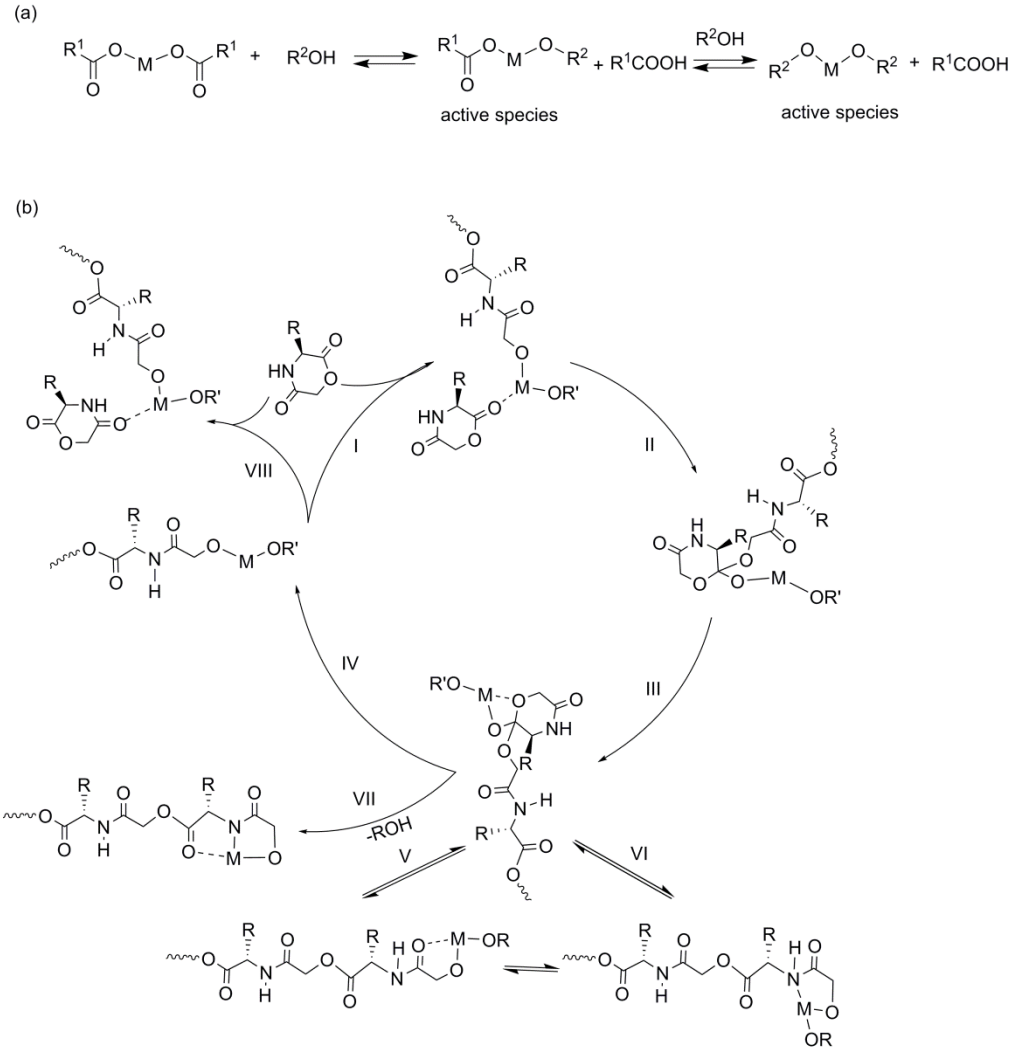
2.1. Materials

Potassium iodide 99.5%, iron(II) acetate ≥99.99%, Magnesium ethoxide 98%, trimethylamine ≥99.5%, 1,8-octanediol 98%, dimethylformamide 99.8%, sodium hydroxide 95%, magnesium sulfate anhydrous ≥97 %, hydrochloric acid 37%, 1,4 dioxane (anhydrous) 99.8%, and aluminum ethoxide 97% were purchased from Sigma-Aldrich and used as received. *O*-Benzyl-L-serine 99%, bromoacetyl bromide, iron(III) ethoxide 99.6% and indium(III) ethoxide 99.9% were obtained from Alfa Aesar and were used as received. *O*-Benzyl-L-tyrosine was bought from Iris biotech GMBH. Chloroform 99% and diethyl ether ≥99.5% were purchased from Roth.

2.2 General Instrumentation

^{1}H and ^{13}C NMR spectra were recorded at room temperature on a DRX 500 Avance spectrometer (500 MHz, Bruker, Rheinstetten, Germany; software Topspin version 1.3) using deuterated dimethylsulfoxid (DMSO-d_6), or deuterated chloroform ($CDCl_3$) as solvents.

Mass spectra were measured on an ultrafleXtreme MALDI-ToF spectrometer (Bruker, Bremen, Germany). Trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB) was used as matrix.



Scheme 1 General ROP mechanism of morpholine-2,5-diones using metal-organic catalysts (a) initiation, (b) propagation. (I) Coordination of metal center with the carbonyl oxygen of the growing chain, (II) nucleophilic attack, (III) rearrangement, (IV) ring-opening. A termination of the reaction may occur by (V) coordination of metal center with the carbonyl oxygen of the growing chain, (VI) coordination of metal center with the nitrogen of amide bond, and (VII) elimination the hydrogen of NH amide and chelating the metal.

The polydispersity of depsipeptide oligomers was determined on a multidetector GPC, which consisted of a GRAM VS1 precolumn (40 mm x 4.6 mm), a GRAM 30Å 5091312 and a GRAM 1000Å 71111 column (both 250 mm x 4.6 mm) (all PSS, Mainz, Germany), a CO-200 column oven (W.O. electronics, Langenzersdorf, Austria), an isocratic pump 980, an automatic injector 851-AS, a LG 980-02 ternary gradient unit, a multiwave length detector MD-910, a RI detector RI-930 (all Jasco, Gross-Umstadt, Germany), a differential viscometer

(WGE Dr. Bures, Dallgow-Doeberitz, Germany), a Wyatt miniDawn Tristar light scattering detector (Wyatt Technology Corporation, Santa Barbara, USA), a degasser ERC-3315 (Ercatech, Berne, Switzerland), and dimethylformamide (0.4 wt% toluene as internal standard, 35 °C, 1.0 mL.min^{-1}) as eluent by universal calibration with polystyrene standards using WINGPC 6.2 (PSS) software.

2.3 **Syntheses**

2.3.1. Synthesis of β-*O*-Benzyl *N*-(bromoacetyl)-L-serine (**3a**) [19]:

15 g (0.077 mmol) *O*-benzyl-L-serine was added to 180 ml of 1,4-dioxane/water (1/1, v/v) and 3.2 g (1.05 eq) of sodium hydroxide (NaOH) in a 500 ml three neck glass reactor equipped with a vertical stirrer. The mixture was cooled to 4 °C. Then, 12.1 ml (0.13 mmol) of bromoacetyl bromide dissolved in 30 ml anhydrous 1,4 dioxane and 6.77 g (0.17 mmol) of NaOH dissolved in 30 ml water were simultaneously added dropwise to the mixture within 2 h while the pH was kept ≥ 9. The solution was warmed to room temperature. The solution was acidified using hydrochloric acid, extracted then three times with diethyl ether. The combined organic layers were then washed with brine three times followed by drying the organic layer over magnesium sulfate. The solvent was removed under vacuum to yield a dark brown oily raw product. The raw product was purified by column chromatography using a mixture of chloroform /methanol/acetic acid (10:0.5:0.1) as eluent. The solvents were removed by rotary evaporator and the final product was further dried at 60 °C under vacuum for two days to yield light yellow crystals (12.85 g, 53 %), melting point: 142 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ= 9.03 – 8.38 (bs, 1H, COO*H*), 7.37 – 7.23 (m, 6H, *Ph* and N*H*), 4.78 – 4.66 (m, 1H, C*H*CO), 4.63 – 4.45 (m, 2H, CH_2Ph), 4.01 – 3.84, 3.80 – 3.58 (2m, 3H, 1H, OCH_2, CH_2Br) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 173.7 (*C*OOH), 166.2 (*C*ONH), 137.1,

128.6, 128.1, 127.8 (*Ph*), 73.47 *C*H_2Ph), 68.80 (CH*C*H_2O), 53.14 (*C*HNH), 28.51 (*C*H_2Br) ppm.

2.3.2. β-*O*-benzyl- *N*(bromoacetyl)-L-tyrosine **3b**

15 g (0.055 mol) of *O*-Benzyl-L-tyrosine and 2.3 g (1.05 eq) of NaOH were added to 500 ml three neck flask containing a mixture of 1,4-dioxane/water (1/1, v/v). The solution was cooled down using an ice bath to a temperature of 4 °C. Then, a solution of 11.5 ml (0.137 mol) of bromoacetyl bromide dissolved in 20 ml of anhydrous 1,4 dioxane and a solution of 8.8 g of NaOH dissolved in 20 ml water were simultaneously added dropwise to the flask within 2 h. The product was isolated by filtration and purified by recrystallization to give white crystals (8.22 g, 48%). Melting point 163-165 °C; ^{1}H NMR (500 MHz, MeOD): δ = 7.46 – 7.27 (m, 6H, *Ph*CH_2O, N*H*CO), 7.19 – 7.11 (m, 2H, O*Ph*), 6.94 – 6.89 (m, 2H, O*Ph*), 5.06 – 5.02 (s, 2H, C*H*$_2$O), 4.67 – 4.58 (m, 1H, C*H*CO), 3.90 – 3.76 (m, 2H, C*H*$_2$Br), 3.19 – 3.12, 3.00 – 2.92 (2m, 1H, 1H, C*H*$_2$CH) ppm. ^{13}C NMR (126 MHz, MeOD): δ = 173.1 (*C*OOH), 168 (NH*C*O), 158.2, 137.8, 130.4, 129.2, 128.4, 127.8, 127.5, 114.9 (2*Ph*), 70 (*C*H_2O), 54.6 (*C*HCO), 36.4 (*C*H_2CH), 27.4 (*C*H_2Br) ppm.

2.3.3. Synthesis of (*S*)-3-((benzyloxy)methyl)morpholine-2,5-dione (BnSerMD) **5a**

5.5 ml of triethylamine was added to 250 ml DMF in a 500 ml three neck round bottom flask equipped with a reflux condenser. The solution was warmed to 60 °C. Then, 2.5 g (7.9 mmol) of β-*O*-Benzyl *N*-(bromoacetyl)-L-serine dissolved in 40 ml of DMF was added to the solution under nitrogen using a syringe pump within 8h. The resulting solution was stirred at 60 °C for further 16 h. DMF was removed then with a rotary evaporator. The resulting oily product was dissolved in chloroform and washed three times with saturated sodium bicarbonate solution followed by washing with distilled water. The organic layer was then dried over magnesium sulfate and concentrated under vacuum. The final product was purified

by recrystallization from chloroform to yield white crystals (0.87 g, 47%).^{1}H NMR (500 MHz, DMSO): δ = 8.6 – 8.5 (s, 1H, CON*H*), 7.4 – 7.2 (m, 5H, *Ph*), 4.7 – 4.6 (m, 2H, COCH_2), 4.6 – 4.5 (s, 2H, CH_2Ph), 4.4 – 4.3 (q, ^{3}J = 2.4, 1H, C*H*CO), 3.9 – 3.8 (dd, ^{3}J = 9.8, 3.1 Hz, 1H, OCH_2CH) and 3.7 – 3.6 (dd, ^{3}J = 9.8, 2.8 Hz, 1H, OCH_2CH) ppm; ^{13}C NMR (126 MHz, DMSO): δ = 167.63 (*C*OOH), 166.13 (*C*ONH), 138.5, 129.20, 128.49, 128.23 (*Ph*), 73.39 (*C*H_2Ph), 72.10 (*C*H_2CO), 68.33 (CH*C*H_2), 54.74 (NH*C*H) ppm. $[\alpha]^{20}_{489\,nm}$ = 8.2.

For the activation of the ring closure reaction by potassium iodide, 1.6 g (9.5 mmol) of KI was dissolved in the precursor solution in DMF, which was then added dropwise into the DMF/triethylamine solution under nitrogen at 60 °C.

2.3.4. Synthesis (S)-3-(4-(benzyloxy)benzyl)morpholine-2,5-dione (BnTyrMD) **5b**

1.3 g (4.8 mmol) of β-*O*-benzyl- *N*(bromoacetyl)-L-tyrosine was dissolved in 35 ml of DMF and was dropwise added to 160 ml of DMF containing 4.25 ml of trimethylamine under nitrogen at 60 °C. The total reaction time was 24h. The purification proceeded as described for 5a. The final product consists of white crystals (192 mg, 18.6%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.37 – 7.21 (m, 5H, *Ph*CH_2), 7.10 – 7.00, 6.92 – 6.84 (2m, 2H, 2H, *Ph*O), 6.77 – 6.68 (s, 1H, N*H*), 5.03 – 4.95 (s, 2H, CH_2O), 4.41 – 4.32 (m, 2H, CH_2CO), 3.80 – 3.73 (d, ^{3}J = 16.3 Hz, 1H, C*H*NH), 3.14 – 3.00 (d, *J* = 5.3 Hz, 2H, CH_2CH) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 166.7 (*C*OOH), 166.1 (*C*ONH), 159 , 137, 131.2, 129, 128.5, 127.9, 126.5, 116.1 (2*Ph*), 70.5(PhCH_2O), 67.38 (*C*H_2OCO), 53.8 (*C*HNH), 39.5 (PhCH_2CH) ppm. $[\alpha]^{20}_{489\,nm}$ = 18.4

2.3.5. Synthesis of oligo((S)-3-((S)-sec-butyl)morpholine-2,5-dione) (OBMD) **7a**:

3.9 ml (1.1 mmol) of freshly prepared 0.3 M solution of $Sn(Oct)_2$ in anhydrous THF was added in 50 ml oven dried Schlenk tube. The tube was sealed with rubber septum and a vacuum of about 0.005 mbar was applied for 20 min in order to remove the solvent. This was followed by addition of 0.88 g (6 mmol) of 1,8 octane diol and 30 g (0.175 mol) of BMD. After several evacuation-refill cycles, the tube was placed in a preheated oil bath at 135 °C. The purification was performed by precipitation from chloroform solution in diethyl ether. This step was repeated three times. The final product was dried under vacuum to yield a light yellow polymer (22.3 g, 74%). ^{1}H NMR (500 MHz, DMSO): δ = 8.33 – 8.13 (m, 23H, N*H*), 7.64 – 7.57 (d, 2H, N*H* $COCH_2OH$), 5.48 – 5.40 (t, 2H, O*H*), 4.63 – 4.37 (m, 45 H, $COCH_2O$), 4.31 – 4.21 (m, 22 H, COC*H*NH), 4.20 – 4.12 (m, 2H, 2 COC*H*NH end group), 4.04 – 3.87 (m, 4H, 2 CH_2CH_2O initiator), 3.82 – 3.73 (m, 4H, CH_2OH), 1.85 – 1.63 (m, 24H, $CHCH_2CH_3$), 1.52 – 1.43 (q, 4H, 2 $CH_2CH_2CH_2O$), 1.44 – 1.32, 1.13 – 1.01 (m, 27H, 28 H, CH_2CH_3), 1.24 – 1.16 (m, 5H, 2 $CH_2CH_2CH_2CH_2O$), 0.90 – 0.61 (m, 166H, CH_2CH_3, $CHCH_3$). ^{13}C NMR (126 MHz, $CDCl_3$): δ = 170.7 (O*C*OCH), 166.5 (NH*C*OCH_2), 62.2 ($COCH_2O$), 55.9 (CO*C*HNH), 36.5 ($CHCHCH_3$), 24.4 ($CHCH_2CH_3$), 15.1 ($CHCH_3$), 11.6 (CH_2CH_3) ppm.

The synthesis procedure for the ROP of BMD using other catalysts was carried out in a similar fashion except that all other catalysts were added as solids. The results are summarized in Table 2.

2.3.6. Synthesis of oligo((S)-3-((benzyloxy)methyl)morpholine-2,5-dione) OBnSerMD **7b**

38 µl of (11 µmol) of 0.3 M of solution of $Sn(Oct)_2$ in THF was added to 10 ml Schlenk tube. After removing the solvent, 0.4 g (1.7 mmol) of BnSerMD and 8.5 mg (0.06 mmol) of 1,8 octane diol were added. The tube was placed in an oil bath preheated to 150 °C for 15 min,

then placed in an oil bath preheated to 135 °C for 24 h. The product was dissolved in chloroform and was precipitated in diethyl ether three times followed by drying under vacuum for 24 h. The final product was a light brown powder (0.12 g, 30%). ^{1}H NMR (500 MHz, DMSO): δ = 8.75 – 8.25 (m, 13H, N*H*), 7.91 – 7.79 (m, 2H, 2 N*H*COCH$_2$OH), 7.46 – 6.95 (m, 93H, *Ph*), 5.63 – 5.53 (m, 2H, 2 O*H*), 4.84 – 4.23 (m, 83H, OC*H*$_2$Ph, COC*H*NH, COC*H*$_2$O), 4.06 – 3.85 (m, 4H, 2 CH$_2$C*H*$_2$O), 3.84 – 3.39 (m, 24H, CHC*H*$_2$O), 1.48 – 1.32 (m, 4H, 2 CH$_2$C*H*$_2$CH$_2$O), 1.19 – 1.00 (m, 8H, C*H*$_2$C*H*$_2$CH$_2$CH$_2$O) ppm.

^{13}C NMR (126 MHz, CDCl$_3$): δ = 172.8 (*C*OCH$_2$OH), 170 (CH$_2$CH$_2$O*C*O), 167.3 (O*C*OCH), 165.1 (NH*C*OCH$_2$) 138.7-128.4 (CH$_2$*Ph*), 73 (O*C*H$_2$Ph), 69.8 (CH*C*H$_2$O), 65.5 (CH$_2$*C*H$_2$O), 62 (CO*C*H$_2$CO), 60.3 (CO*C*H$_2$OH), 53 (CO*C*HNH), 29.3, 28.9 and 26 (2 OCH$_2$CH$_2$CH$_2$CH$_2$) ppm.

The synthesis procedure for the ROP of BnSerMD using Fe(OAc)$_2$ was carried out in a similar way except that the catalyst was added as a solid. The results are summarized in Table 2.

2.3.7. Synthesis of oligo((S)-3-(4-(benzyloxy)benzyl)morpholine-2,5-dione) OBnTyrMD **7c**

8 µl (2 µmol) of 0.3 M of Sn(Oct)$_2$ solution in THF was added to 10 ml Schlenk tube. The solvent was removed by applying vacuum for 20 min. This was followed by addition 0.1 g (0.3 mmol) of BnTyrMD and 1.6 mg (0.01 mmol) of 1,8 octanediol. After several evacuation-refilling cycles, the tube was placed in an oil bath preheated to 165 °C for 20 min, then in an oil bath at 135 °C for 24 h. The purification was carried out by precipitation in diethyl ether three times. The polymer was collected and dried for 24h to yield a light brown polymer (47 mg, 47 %). ^{1}H NMR (500 MHz, DMSO): δ = 8.56 – 8.27 (m, 16H, N*H*), 7.87 – 7.75 (m, 2H, N*H* COCH$_2$OH), 7.41 – 7.14, 7.13 – 6.97, 6.88 – 6.66 (m, 124H, 47H, 48H, 2 *Ph*), 5.54 –

5.42 (dt, 2H, 2 O*H*), 5.02 – 4.80 (m, 47H, OC*H*$_2$Ph), 4.61 – 4.25 (m, 53H, COC*H*NH, COCH$_2$O), 3.93 – 3.76 (m, 4H, CH$_2$C*H*$_2$O), 3.74 – 3.63 (m, 4H,2 COC*H*$_2$OH), 3.05 – 2.62 (m, 42H, CHC*H*$_2$Ph), 1.41 – 1.25 (m, 4H, 2 CH$_2$C*H*$_2$CH$_2$O), 1.16 – 1.01 (m, 8H, 2 C*H*$_2$C*H*$_2$CH$_2$CH$_2$O) ppm.

^{13}C NMR (126 MHz, $CDCl_3$): δ = 171.4 (O*C*OCH), 167.4 (NH*C*OCH$_2$), 157-115 (CH$_2$*Ph*OCH$_2$*Ph*), 70 (O*C*H$_2$Ph), 65.4 (CH$_2$*C*H$_2$O), 63 (CO*C*H$_2$OCO), 61.9 (CO*C*H$_2$OH), 54.3 (CO*C*HNH), 36.4(CH*C*H$_2$Ph) 29.3, 28.8 and 26 (2 OCH$_2$CH$_2$CH$_2$CH$_2$) ppm.

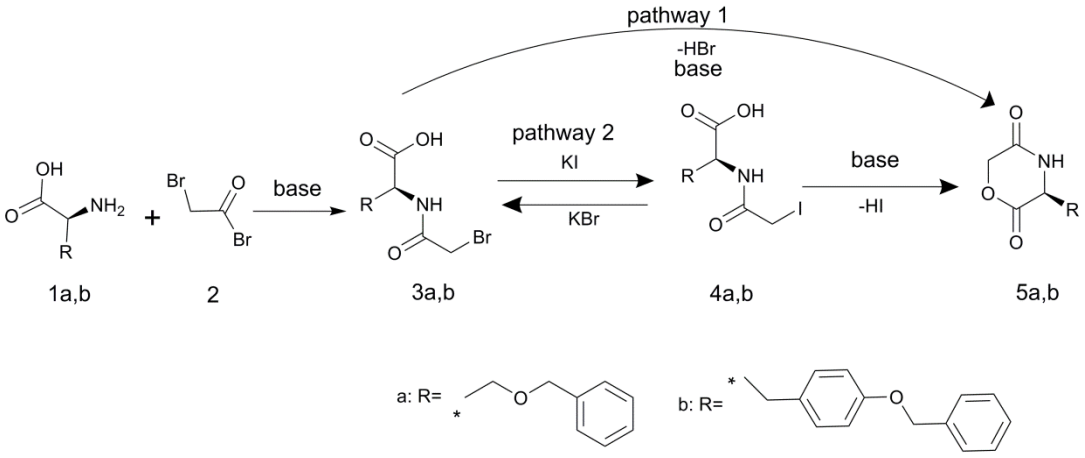
The synthesis procedure for the ROP of BnTyrMD using $Fe(OAc)_2$ was carried out in a similar way, except that the catalyst was added as a solid. The results are summarized in (Table 2)

3. Results and discussion

3.1. Synthesis of MDs

The synthesis of oligodepsipeptides comprises the formation of MDs and their subsequent oligomerization. Typically, MDs with protecting groups are prepared in a two-step synthesis. First, an α-halo substituted acylhalide is reacted with an amino acid to form an *N*-(α-haloacetyl)-α-amino acid. This is then followed by the ring closuring reaction of the resulting

precursor in presence of a base, e.g. trimethylamine, as an acid scavenger



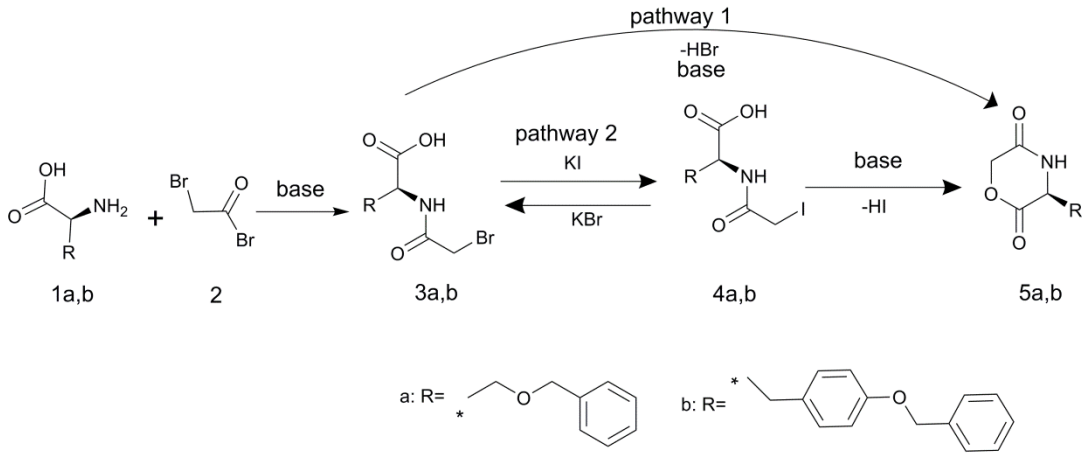
Scheme 2). Bromide or chloride derivatives have been used in the literature as haloacetyl halogenide in the first synthesis step as iodides tend to be unstable. The possibility of catalyzing nucleophilic substitution reactions by adding a species acting first as good nucleophile and then as good leaving group is a well-known synthetic strategy.[20] Here, it is demonstrated that the iodide derivative of the precursor can be produced in situ by addition of KI ion during the cyclization reaction.

An equilibrium state between compounds **3a,b** and **4a,b** occurred directly upon addition of KI. This equilibrium was proven in ^{13}C-NMR spectra of the precursor solution before and after addition KI (Fig.1). In a complete conversion of 3a to 4a, the peaks g and q would completely disappear, which is not the case. Instead, a mixture of the compounds 3a and 4a with a constant ratio is formed, which corresponds to the constant ratio of the peaks g and q compared to k and l in the ^{1}H NMR spectra.

Two precursors based on the *O*-Bn-protected amino acids serine (Ser) and tyrosine (Tyr) were chosen to study the effect of adding KI to the ring closure reaction. It should be further mentioned that pure precursors were used for the comparison. In the amidation reaction bromoacetic acid is formed as a side product. Presence of this compound during the cyclization reaction might causes decrease of the final yield by a condensation reaction of

bromoacetic acid with the precursor. Presence of KI in the reaction solution increased the final yield in both cases by about 10 mol%, from 45±2 % to 54±2 for BnSerMD, and from 19% to 30% for BnTyrMD.

It has been reported previously that a trans configuration of the CO-NH bond lowers the yield of the cyclization reaction.[21] It can, therefore, be speculated that the Tyr precursor adopts a higher percentage of trans configuration than the Ser derivative. It could be anticipated that the equilibrium of the cis/trans isomerization may be shifted towards the cis configuration at higher temperature.



Scheme 2 Synthetic route to prepare BnSerMD and BnTyrMD**.**

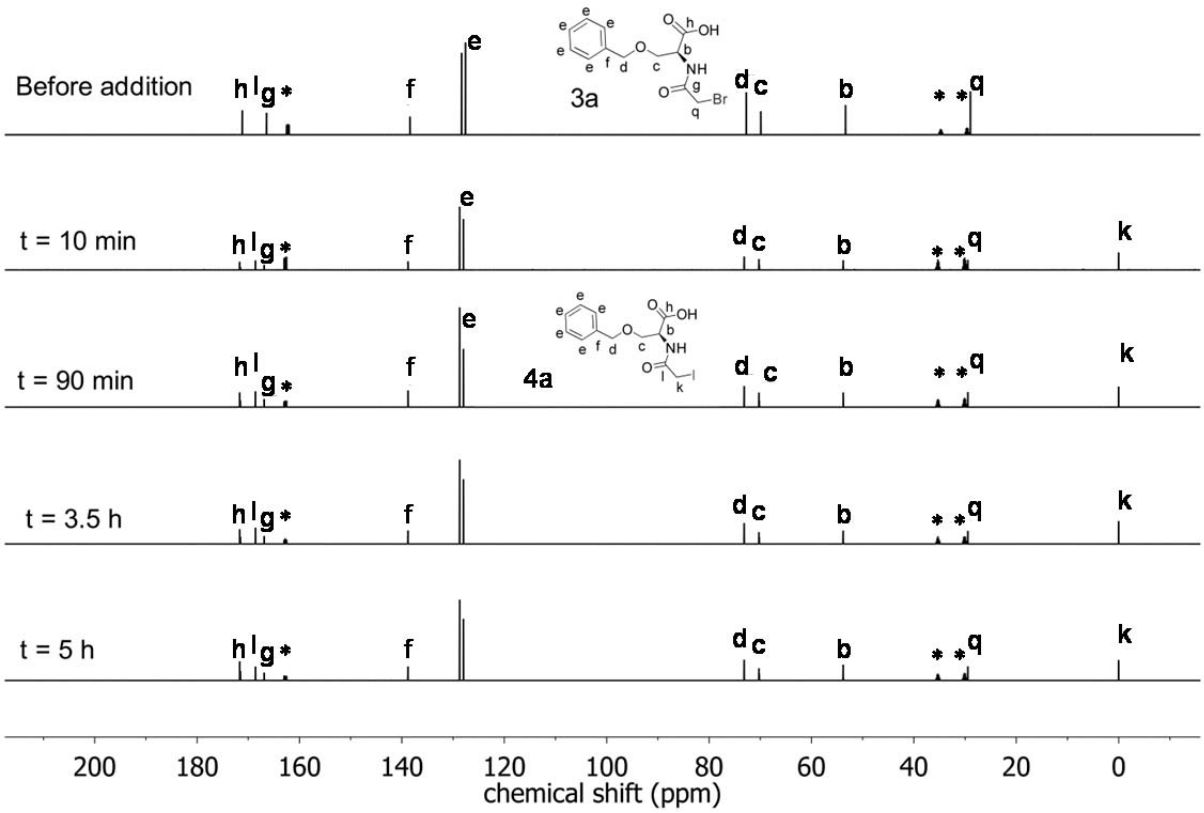
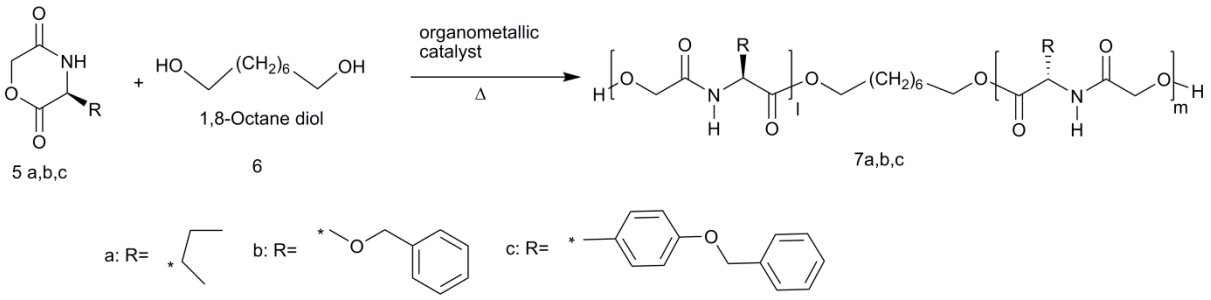


Fig. 1. ^{13}C NMR of β-*O*-Benzyl *N*-(bromoacetyl)-L-serine before and after addition of KI. After the addition, the spectrum is representing a mixture of compounds 3a and 4a. * indicates signals from the solvent, *N*,*N*-dimethylformamide.

However, carrying out the ring closing reactions at temperatures >60 °C led to undesirable side reaction, which lowered the final yield significantly.

3.2. Synthesis of ODP applying different catalysts

The established procedure for the ODP diols is the ROP of MD in the melt under addition of a diol as initiator and a metal-organic catalyst (Scheme 3).



Scheme 3 Synthesis of ODP–diol by ROP of MD using a metal organic catalyst, and a diol as initiator.

The metal-organic catalyst is a Lewis acid and catalyzes the polymerization reaction *via* a coordination-insertion polymerization mechanism (Scheme 1). While the softness of the central metal will influence monomer coordination and efficacy of alkoxide transfer, other aspects should likewise be considered. The analysis of the mechanism suggested that the polymerization will be furthermore affected by the bulkiness of the ligands R^1COO^- as well as the bulkiness and nucleophilicity of alkoxides R^2O^-, radius and acidity of the central metal and length of the metal alkoxide bond. Thus, the effect of these factors on the ROP of MDs was studied by using metal-organic catalysts of various central metals and ligands using BMD as monomer (Table 1). The results revealed that $Fe(OAc)_2$ was the most suitable catalyst for the ROP of BMD, with optimal conditions at 135 °C giving polymer of higher M_n and smaller polydispersity index (M_w/M_n) than $Sn(Oct)_2$, which is the most utilized catalyst for the ROP of MDs.

In fact, Fe^{2+} is a softer Lewis acid than Sn^{2+} [22]. According to the hard and soft acids and bases (HSAB) theory, Fe^{2+} can form weaker metal-oxygen bond compared to Sn^{2+}, as alkoxides are classified as a hard base, which facilitates the nucleophilic attack of the alkoxide to the carbonyl bond of MDs in the case of Fe^{2+} (Scheme 1b, I). Additionally, the chelate of Fe^{2+} with the carbonyl of the amide bond, which leads to non-active species, will be weaker

or less likely than for the harder Sn^{2+}. Similar effects have been reported previously by comparing the ROP of dilactides using magnesium as harder Lewis acid and zinc as a softer Lewis acid catalyst.[23] Furthermore, the acetate ligand of $Fe(OAc)_2$ is less bulky than octoate in the case of $Sn(Oct)_2$. Therefore, the expected steric hindrance during the reaction with the initiator forming the active catalyst species as well as during the polymerization process caused by ligands in the case of the single substituted Acyl-M-octolate will be smaller for $Fe(OAc)_2$ than for $Sn(Oct)_2$.

It has been reported that $Fe(OAc)_2$ required high temperatures (≥ 180 °C) for the polymerization of *L,L*-dilactide, which is close to the decomposition temperature of $Fe(OAc)_2$ [24]. In fact, in the case of ROP of BMD the reaction proceeded smoothly at 135 °C, while at higher temperatures OBMD with smaller molar mass and smaller yield compared to the one synthesized at 135 °C were formed. Additionally, ^{1}H-NMR spectra of the OBMD synthesized at higher temperatures indicated 16-20 mol% racemization at the stereocentres. The specific optical rotation of oligomers is not only influenced by the optical purity of the monomers, but also from the oligomer conformation in solution and therefore here is not a suitable method for the determination of the degree of racemization. The reason for the efficiency of $Fe(OAc)_2$ at 135 °C could be a higher solubility of this catalyst in the melt of MD than in the melt of *L,L*-dilactide. In order to clarify whether $Fe(OAc)_2$ non-productively binds to the amide function, we conducted a series of experiments polymerizing *D,D*-dilactide with $Fe(OAc)_2$ adding increasing amounts of the non-polymerizable amide ethylacetamide. It was expected that the rate of polymerization would decrease if Fe(II) coordinates to the amide. This was in fact not the case, supporting the hypothesis of this work that the hardness of the metal catalyst is of importance for the coordination and, hence, the polymerization.

Table 1

ROP of BMD using different metal organic metallic catalysts at various temperatures for 24h.

Catalyst	Temperature (°C)	Initiator/monomer ratio	Conversion[a] (mol%)	$M_{n,\ NMR}$ (g mol^{-1})	M_w/M_n	Yield (%)
$Sn(Oct)_2$ [c]	135	1/29	88	4500	1.19	74
$Fe(OAc)_2$ [b,c]	135	1/29	86	5800	1.13	54
$Fe(OAc)_2$ [b,c]	160	1/29	86	4500	1.18	36
$In(OEt)_3$ [c]	160	1/59	60	3100	---[d]	10
$Mg(OEt)_2$ [c]	160	1/29	57	4000	1.17	30
$Al(OEt)_3$ [c]	135	1/59	--[e]	--[e]	--[e]	--[e]
$Fe(OEt)_3$ [c]	135	1/59	--[e]	--[e]	--[e]	--[e]

[a] calculated by ^{1}H-NMR.

[b] 1,10-decanediol was used here as initiator, since its higher boiling point than of 1,8-octanediol was required for the experiments at high temperature.

[c] catalyst/monomer ratio was 1/149.

[d] was not measured since there was not enough material.

[e] no reaction occurred.

Metal alkoxides of indium, magnesium, aluminum and iron(III) have shown a high reactivity towards the ROP of cyclic esters.[25-28] Additionally, it has been reported that for iron(III) alkoxides increasing the bulkiness of the ligands decreases the resulting molar mass [24]. Thus, in all cases metal ethoxides were investigated for comparison in activity. $Al(OEt)_3$ and $Fe(OEt)_3$ did not result in polymerization, putatively due to a lack of solubility in the monomer melt. $In(OEt)_3$ and $Mg(OEt)_2$ catalyzed the ROP of BMD, but showed less reactivity than $Fe(OAc)_2$ and $Sn(Oct)_2$, which was reflected by the lower conversion ratio and lower yield. Additionally, a temperature of 160 °C was needed for both catalysts to promote the polymerization reaction which could be the reason for the low yield as high temperature can promote the degradation of the polymer chains. It is worth noting that the all metal-

alkoxides studied here are harder Lewis acids than $Sn(Oct)_2$. Although their steric hindrance was low, their catalytic activity was lower than of the Sn(II) and Fe(II) species studied. This may suggest that properties of the central atom are of high relevance for the catalytic activity. In fact, only few metal-organic Lewis acids are handable under the required reaction conditions and are as soft as Sn(II) and Fe(II).

The most promising new catalyst, $Fe(OAc)_2$, was compared to the standard $Sn(Oct)_2$ in terms of polymerization kinetics and side reactions. $Fe(OAc)_2$ showed a little slower polymerization rate compared to $Sn(Oct)_2$, which corresponds to the observed slower monomer conversion ratio and slower increasing of M_n over time (Fig. 2 (a) and (b)). (Fig. 2 (b)) shows also that the maximum conversion to be reached is similar for both catalysts, with a conversion ratio of about 80 mol% after 3-5 h. The molar mass and conversion rate are linearly correlated, indicating a living chain growth mechanism, as is typical for ROP.

The M_n of OBMD prepared with $Sn(Oct)_2$ decreased after reaching a maximum conversion at ~5h (Fig. 2a). MALDI analysis showed that this catalyst is active in transesterification after the maximum polymerization was reached. Among the products identified in the MALDI were e.g cyclic structures (species 2), products from transesterification and transamidation (species 4, 6 and 8), as well as OBMD with 2-ethylhexanoate end groups (species 5 and 7) (Fig. 3 (a) and (e)). These adducts were almost suppressed by carrying out the polymerization reaction just for 3.5 hours (Fig. 3 (b), Table 2). An effect of reaction time on side reaction was also seen for $Fe(OAc)_2$ (Fig. 3 (c) and (d); Table 2). Intramolecular transamidation reaction in polydepsipeptide formation has been reported for the case of ROP of 3,6-dimethyl-2,5-morpholinedione using amino-alkoxy-bis(phenolate) as a catalyst [29]. Such reaction leads to the formation of two species, a linear ODP with an amine end group and cyclic polymer chain with an odd number of subunits, of which only the former was found in our experiments. In fact, reaction of growing chains with 2-ethylhexanoate leads to chains unreactive in

polymerization. Thus, the reaction time for the ROP should be closely monitored. Formation of oligomers ending with the carboxylate ligand were not seen in the spectrum of OBMD formed by using $Fe(OAc)_2$ as a catalyst (Fig. 3 (c) and (d)). Actually, the polymerization temperature for OBM is higher than the boiling point of acetic acid, which may result in evaporation of the acetic acid formed during the polymerization. This could also contribute to the higher activity of $Fe(OAc)_2$ compared to $Sn(Oct)_2$, as the evaporation shifts the equilibrium state (Scheme 1a) to the right. Table 2 shows that decreasing the amount of $Fe(OAc)_2$ dramatically lowered the resulting M_n and yield.

Further investigation of activity of $Fe(OAc)_2$ towards ROP of different MDs was carried out by using BnSerMD and BnTyrMD as monomers for the ROP with a reaction time of 24 h. Shorter polymerization time was, however, carried out for both monomers when $Sn(Oct)_2$ was used, as its kinetic with BMD showed decreasing of final M_n when polymerization proceeded for more than ~5 h.

The results demonstrated better suitability of $Fe(OAc)_2$ than of $Sn(Oct)_2$ for the ROP of BzSerMD reflected by obtaining ODP with higher molar mass and in higher yield.

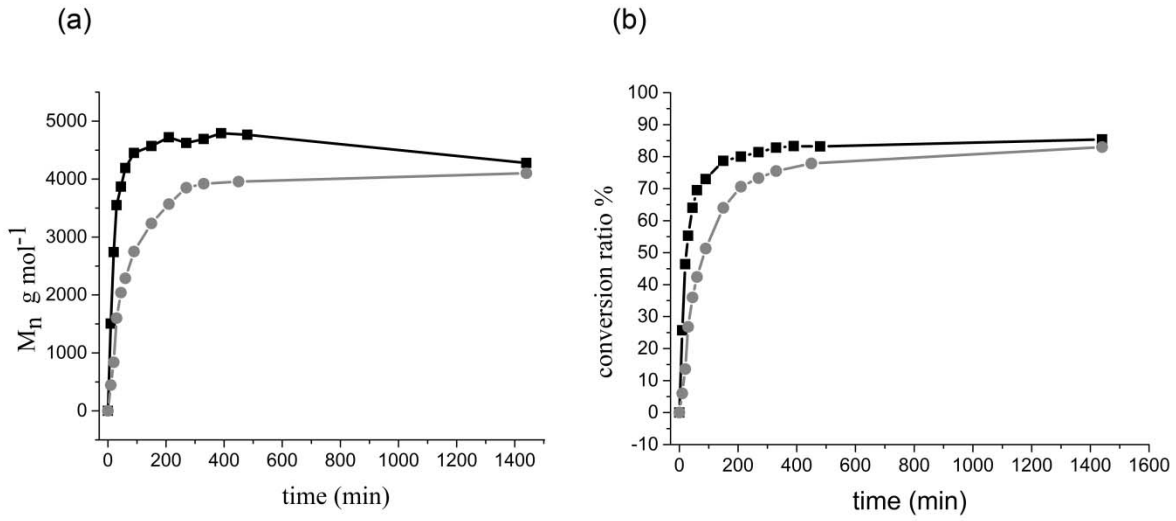
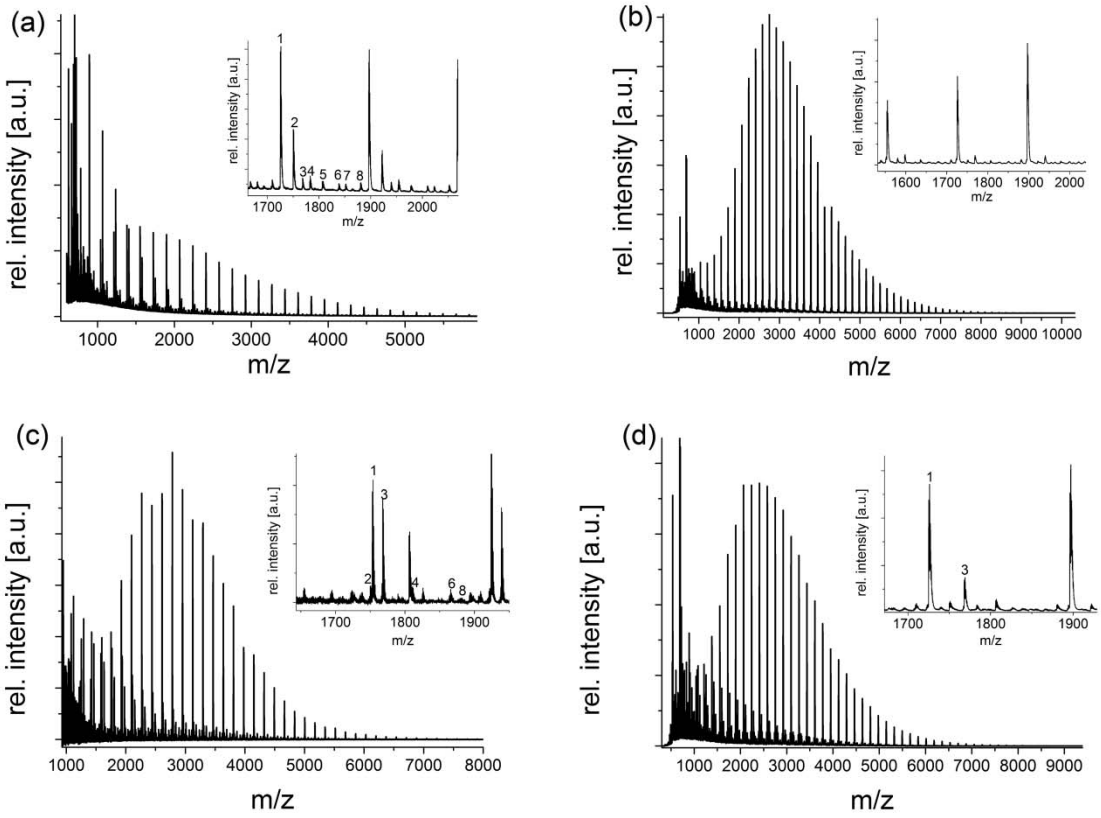


Fig. 2. (a) Plot of M_n versus time during the ROP of BMD using either $Sn(Oct)_2$ (black) or $Fe(OAc)_2$ (gray line) as a catalyst at 135 °C. (b) Plot of monomer conversion ratio versus time during the ROP of BMD using either $Sn(Oct)_2$ (black line) or $Fe(OAc)_2$ (grey line) as a catalyst at 135 °C. The lines are added as guide to the eye. The conversion ratio was calculated by comparing the integrals of the CH peak of the monomer at 4.04 – 3.97 ppm with the CH peak of the polymer at 4.40 – 4.20 ppm, while M_n was calculated here by comparison the integrals of the CH peak at 4.40 – 4.20 ppm with the CH_2 peak of the end group at 3.82 – 3.73 ppm before purification of the polymer. The error for M_n and the conversion rate is estimated to be ~ 5%.



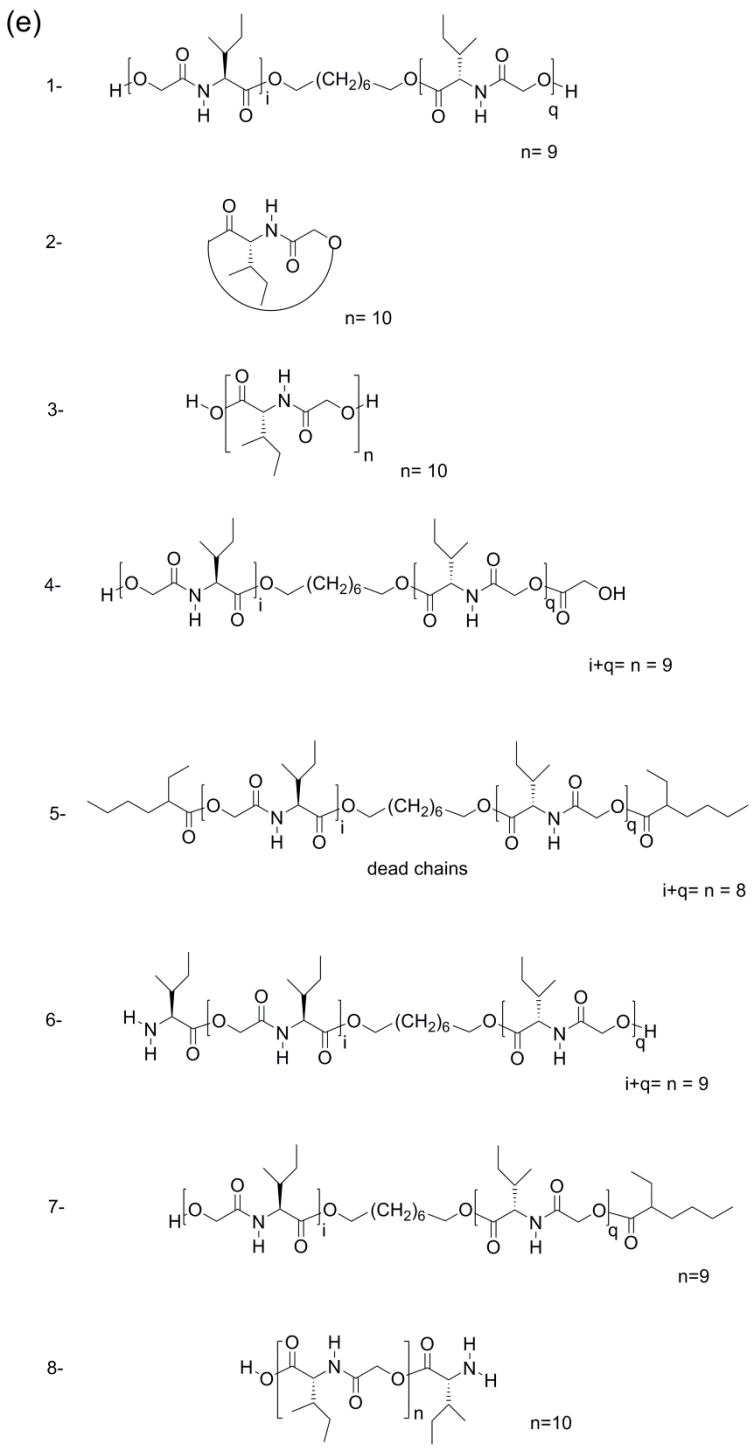


Fig. 3. MALDI spectra of OBMD-diol synthesized at 135 °C using $Sn(Oct)_2$ as catalyst for (a) 24 h, (b) 3.5h or using $Fe(OAc)_2$ for (c) 24 h (d) 5.5h, (e) Structure of species shown in MALDI analysis.

ODP with smaller molar mass, however, were observed when $Fe(OAc)_2$ was used for the ROP of BzTyrMD compared to $Sn(Oct)_2$ with the same reaction conditions. This could be

attributed to a potential steric hindrance by the bulky substituent on the amino acid substructure. In fact, increasing the bulkiness of substituent will hinder the coordination and insertion process of both MD and growing chains during the polymerization. The effect of substituent bulkiness, however, decreases if the metal center has a large radius since the species, which are attached or approach the metal atom will have more space on its surface. This could explain the higher reactivity of $Sn(Oct)_2$ towards ROP BzTyrMD, as Sn^{2+} has larger ionic radius compared to Fe^{2+}.

4. Conclusions

The efficacy of the ring closing reaction of *N*-(α-bromoacetyl)-α-amino acids could be improved by the nucleophilic catalysis with iodide. The iodide acts first as nucleophile exchanging the bromide, and subsequently as good leaving group for the carboxylate. The ring closing in both cases is temperature sensitive. Comparing different metal alkoxides and carboxylates in the ROP of MDs, the softer Fe(II) and Sn(II) catalysts outperformed the harder, however potentially less sterically hindered, In(III) and Mg(II) catalysts. This suggests that in fact the coordination and alkoxide transfer are of paramount importance for the catalytic cycle, while steric consideration only influences the overall efficacy of the ROP. A potential benefit of iron catalyst for the ROP is the lower toxicity of iron compared to tin, which is of relevance for application of ODP in pharmaceutical engineering. This aspect, as well as the application of iron catalysis to oligodepsipeptide synthesis will be subject to further study.

Acknowledgments

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Table 2. ROP of BMD, BnSerMD and BnTyrMD using either $Sn(Oct)_2$ or $Fe(OAc)_2$ as catalyst at different reaction times.

Monomer[a]	catalyst	Temperature (°C)	Time (h)	Conversion (mole%)	$M_{n,\,NMR}$ (g mol^{-1})	M_w/M_n	Yield (%)
BMD[b]	$Sn(Oct)_2$	135	3.5	84	4500	1.09	86
BMD[b]	$Fe(OAc)_2$	135	5.5	74	5600	1.14	73
BMD[c]	$Fe(OAc)_2$	135	24	48	1800	1.13	24
BMD[d]	$Fe(OAc)_2$	135	24	23	800	1.36	14
BnSerMD[b]	$Sn(Oct)_2$	135	24	--[e]	4750	1.45	30
BnSerMD[b]	$Sn(Oct)_2$	135	5	--[e]	4700	1.26	37
BnSerMD[b]	$Fe(OAc)_2$	135	24	--[e]	7800	1.45	60
BnTyrMD[b]	$Sn(Oct)_2$	150	24	78	7500	1.28	47
BnTyrMD[b]	$Sn(Oct)_2$	150	5	77	8040	1.27	64
BnTyrMD[b]	$Fe(OAc)_2$	150	24	79	6000	1.41	43

[a] 1,8-octane diol was used as initiator for all experiments with initiator/ monomer ratio 1/29.

[b] -Catalyst/monomer ratio was 1/149.

[c] Catalyst/monomer ratio was 1/500.

[d] Catalyst/monomer ratio was 1/1000.

[e] Determination of the conversion rate from ^{1}H NMR spectra was not possible due to signal overlapping.

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