Matrix-Assisted Laser Desorption/Ionization Mass Spectrometric Study of the Oligomers Formed from Lactic Acid and Diphenylmethane Diisocyanate

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ABSTRACT: The reaction between lactic acid and diphenylmethane diisocyanate was studied by ^{1}H NMR, ^{13}C NMR, and matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy. The structure of the oligomers formed was also investigated and supported by using the postsource decay method. Analysis of the products revealed that, besides linear chain copolymer formation, branching and homooligomerization of lactic acid also took place. A mechanism is suggested for the cooligomerization processes.

Introduction

Because of the practical and environmental utilities, the biodegradable and biocompatible macromolecules have recently gained growing interest, and one of the most important and dynamically developing group of these compounds is the urethane-type polymers.[1]

There are only a few publications in the literature that discuss the synthesis of biodegradable polyurethanes from polylactic acid (PLA),[1−5] and the common feature of the reported procedures is the use of hexamethylene diisocyanate (HDI) and toluene diisocyanate (TDI) under relatively drastic reaction conditions, such as high temperature and long reaction time. However, the chain structure of these polymers is not discussed, and it is apparent that neither well-adaptable procedures nor the structures of such biodegradable and biocompatible polyurethanes produced from PLA and diisocyanates are reported.

Lactic acid (LA) and polylactic acid carry two functional groups (hydroxyl and carboxyl) with different reactivities toward the isocyanate. The hydroxyl group is about 1 order of magnitude more reactive than the carboxyl group.[6] As a primary goal, we decided to investigate the reaction products formed from LA and diphenylmethane diisocyanate (MDI) and also to find out the structures of the produced cooligomers in order to establish the mechanism of the cooligomerization under relatively mild reaction conditions and short reaction time.

Matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy (MALDI-TOF MS), a relatively new advanced technique for the analysis of large, nonvolatile molecules,[8,9] has gained great importance in the characterization of synthetic polymers and copolymers.[10−16] Also, with the use of the postsource decay (PSD) method[17] the microstructure of the copolymers with masses up to 3000 Da can be determined.[18] Analysis of MDI prepolymer mixtures and adducts were achieved by MALDI-TOF MS and field-desorption mass spectroscopy.[19−21]

Analysis of the polymers and/or oligomers with relatively low molecular weights by MALDI-TOF MS and MALDI-TOF MS/PSD allowed to get a deeper insight into the structure of these products. To our best knowledge, no other article has been published so far on this subject.

Experimental Section

Materials. 4,4′-Diphenylmethane diisocyanate and tin octoate were provided by BorsodChem Rt, Hungary. Lactic acid was purchased from Sigma. Toluene was distilled and stored over sodium metal before use.

Reaction of LA with MDI. The reaction of LA with MDI was performed in a two-necked flask equipped with a reflux condenser, a calcium chloride guard tube, and a magnetic stirrer. The flask was heated with an external oil bath.

2 g (0.022 mol) of LA and 2.7 g (0.011 mol) of MDI were dissolved in 10 mL of warm toluene, and 1×10^{-4} mol of tin octoate catalyst was added to the solution. The temperature was kept at 110 °C for 10 min, and then the reactions were terminated by adding 1 mL of methanol or ethanol to the reaction mixtures. The product was filtered off and extracted several times with CH_2Cl_2 to remove the unreacted LA and MDI, and then it was dried at ambient temperature.

Yield: 80−85%. The products are white powders, which are soluble in *N*,*N*-dimethylformamide.

Oligomerization of MDI. In the previously described apparatus 1 g (0.004 mol) of MDI was dissolved in 10 mL of warm toluene, and 1×10^{-4} mol of tin octoate catalyst was added to the solution. The temperature was kept at 110 °C for 3 h, and the reactions were terminated by the addition of methanol (1 mL). The product was filtered off and dried.

Yield: 15−20%. The product is a white powder, partly soluble in tetrahydrofuran and completely in *N*,*N*-dimethylformamide.

Instrumental Analysis. *NMR*. NMR measurements were carried out with a Bruker DRX-500 spectrometer at 300 K temperature in $CDCl_3$ solution, and TMS was used as reference for chemical shift scale. Typical ^{1}H and ^{13}C 90° pulses were 10 and 16 μs, respectively. Homonuclear correlations were obtained in a double quantum filtered COSY experiment while a gradient enhanced HSQC experiment was run for carbon−proton correlations. Quantitative 1D ^{13}C NMR spectra

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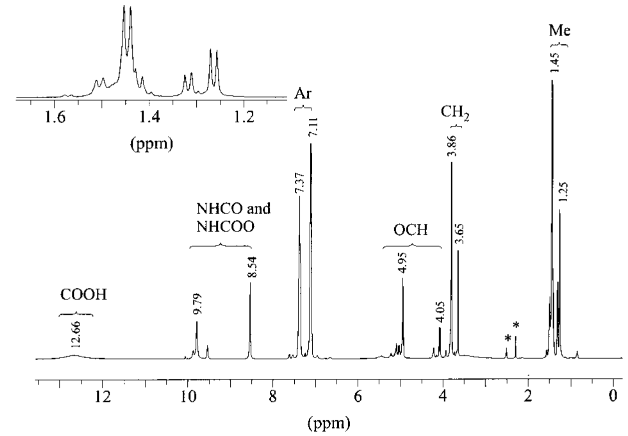


Figure 1. ^{1}H NMR spectrum of the products of the MDI−LA reaction (* solvent impurities).

were measured with the inverse gated decoupling method using 4 s waiting time without decoupling and a 55° carbon read pulse.

MALDI-TOF MS. MALDI-TOF measurements were performed with a Bruker BIFLEX III mass spectrometer. In all cases 19 kV acceleration voltage was used with pulsed ion extraction (PIE). The positive ions were detected in the reflectron mode (20 kV). A nitrogen laser (337 nm, 3 ns pulse width, 10^6−10^7 W/cm^2) operating at 4 Hz was used to produce laser desorption, and 50−60 shots were summed. The spectra were externally calibrated with poly(ethylene glycol) (PEG) standard ($M_n = 1450$ g/mol, MWD = 1.02) with linear calibration. Samples were prepared with DHB (2,5-dihydroxybenzoic acid) matrix. The analyte solution (5 mg/mL) was mixed in 10:1 v/v ratio (matrix:analyte). In the case of the MDI oligomers, dithranol (20 mg/mL) was used as matrix instead of DHB because of better resolutions, and LiCl (5 mg/mL) was added to the matrix/analyte solution to enhance cationization. The analyte solution (5 mg/mL) was mixed in 50:5:1 v/v ratio (matrix:analyte:LiCl). The solvent was *N*,*N*-dimethylformamide. A volume of 0.5−1 μL of these solutions was deposited onto the sample plate (stainless steel) and allowed to air-dry or accelerated by hot air stream. The differences between the measured and the calculated masses were within 0.1 Da in each case.

MALDI-TOF MS/PSD. All of the PSD spectra were recorded by selection of the ion to be studied, using the pulser allowing ±10 Da window for selection. In each segment the reflectron voltage was decreased in 14−16 steps, and 100−120 laser shots were summed. The segments were pasted and calibrated using an XMASS 5.0 software from Bruker. The PSD was calibrated using the fragmentation pattern of Adrenocorticotropic Hormone (ACTH).

Results and Discussion

NMR Experiments. The reaction between LA and MDI was carried out in 2:1 molar ratio, and the products were investigated by ^{1}H NMR and ^{13}C NMR spectroscopy. The ^{1}H NMR spectrum of the reaction products with the assignments is shown in Figure 1.

The presence of urethane (NHCOO) and amide protons (NHCO) (8.5−10.1 ppm) indicates that the reaction between MDI and LA took place, and based on the ^{1}H NMR spectrum (see Figure 1), the average number ($n_{\mathrm{LA/MDI}}$) of lactic acid reacted per MDI units can be calculated by eq 1.

$$n_{\mathrm{LA/MDI}} = \frac{I_{\mathrm{m}} N_{\mathrm{Ar}}}{I_{\mathrm{Ar}} N_{\mathrm{m}}} \tag{1}$$

where I_{m} and I_{Ar} are the integral values of the methyl (Me) and aromatic (Ar) signals, respectively, and N_{m} and N_{Ar} are the number of Me ($N_{\mathrm{m}} = 3$) and Ar protons ($N_{\mathrm{Ar}} = 8$) per unit, respectively. In our experiments an $n_{\mathrm{LA/MDI}} = 2.2$ value was obtained, which is close to the initial ratio of LA/MDI. The proposed general structure of the MDI−LA cooligomer is shown in Scheme 1.

In the ^{1}H NMR spectrum the most characteristic part is the region of methyl doublets, and the 2D COSY spectrum (Figure 2) proves that all of these Me groups are coupled to OCH groups to exhibit a quartet structure.

The two most intensive Me doublets (1.45 and 1.25 ppm) and methine quartets (4.95 and 4.05 ppm) display two independent spin systems. Figure 3 shows the ^{13}C NMR spectrum of the reaction products.

In the ^{13}C NMR spectrum the major Me lines appear at 17.36 and 20.88 ppm, and the methines are at 68.79 and 66.23 ppm. The signals of the CH$_2$ group are found at 40.51 ppm, signals of aromatic CH-s at 138.09−135.29, 129.33, and 118.78 ppm, and carbamoylic carbons are found at 153.23 and 153.01 ppm, while the carboxylic group is detected at 176.79 ppm and the lactate carbonyl at 173.11 ppm. On the basis of the NMR investigations, it is concluded that the reaction product is a mixture containing the cooligomers with similar ratio of MDI−LA units, but their microstructures are different.

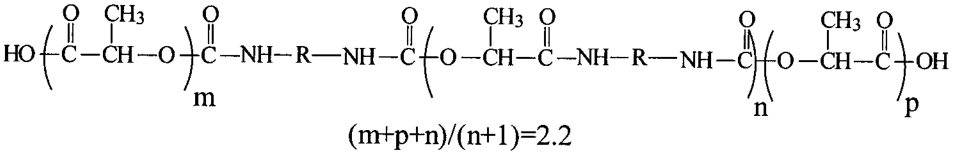
To find out the microstructures of the cooligomers formed, MALDI-TOF MS and MALDI-TOF−PSD MS/MS experiments were performed.

MALDI-TOF MS Experiments. Independently, the reactions were quenched with methanol and ethanol. Practically the same peaks are present in the MALDI-TOF MS spectra in both cases, indicating that the oligomers did not contain an isocyanate end group in significant amount before methanol or ethanol was added to the reaction mixture. If LA reacts via its carboxyl group, decarboxylation may take place,[6] i.e., $M_{\mathrm{etanol}} = M_{\mathrm{lactic\ acid}} - M_{\mathrm{CO_2}} = 46$ Da (Scheme 2).

The MALDI-TOF MS spectrum of the products of the MDI−LA reaction terminated by methanol is shown in Figure 4. In Figure 4, the mass of the sodium cationized peaks $[\mathrm{M + Na}]^+$ is presented. Besides these peaks the protonated $[\mathrm{M + H^+}]$, the potassium cationized $[\mathrm{M + K}]^+$, and the $[\mathrm{M + 2Na - H}]^+$ peaks also appear. The $[\mathrm{M + H}]^+$ and $[\mathrm{M + 2Na - H}]^+$ peaks are characteristic of the carboxylic acids. The detection of compounds with masses lower than 300−400 Da is obscured by the presence of the matrix and matrix-cluster ions.

The peak at 453 Da corresponds to LA−MDI−LA, indicating that the hydroxyl group of LA has reacted with MDI (to form two urethane bonds). Peaks at 409

Scheme 1. Proposed General Structure of the MDI−LA Cooligomer



$$\mathrm{HO}\left(\overset{\mathrm{O}}{\overset{\|}{\mathrm{C}}}-\overset{\mathrm{CH_3}}{\overset{|}{\mathrm{CH}}}-\mathrm{O}\right)_{m}\overset{\mathrm{O}}{\overset{\|}{\mathrm{C}}}-\mathrm{NH}-\mathrm{R}-\mathrm{NH}-\overset{\mathrm{O}}{\overset{\|}{\mathrm{C}}}\left(\mathrm{O}-\overset{\mathrm{CH_3}}{\overset{|}{\mathrm{CH}}}-\overset{\mathrm{O}}{\overset{\|}{\mathrm{C}}}-\mathrm{NH}-\mathrm{R}-\mathrm{NH}-\overset{\mathrm{O}}{\overset{\|}{\mathrm{C}}}\right)_{n}\left(\mathrm{O}-\overset{\mathrm{CH_3}}{\overset{|}{\mathrm{CH}}}-\overset{\mathrm{O}}{\overset{\|}{\mathrm{C}}}\right)_{p}\mathrm{OH}$$

$(m+p+n)/(n+1)=2.2$

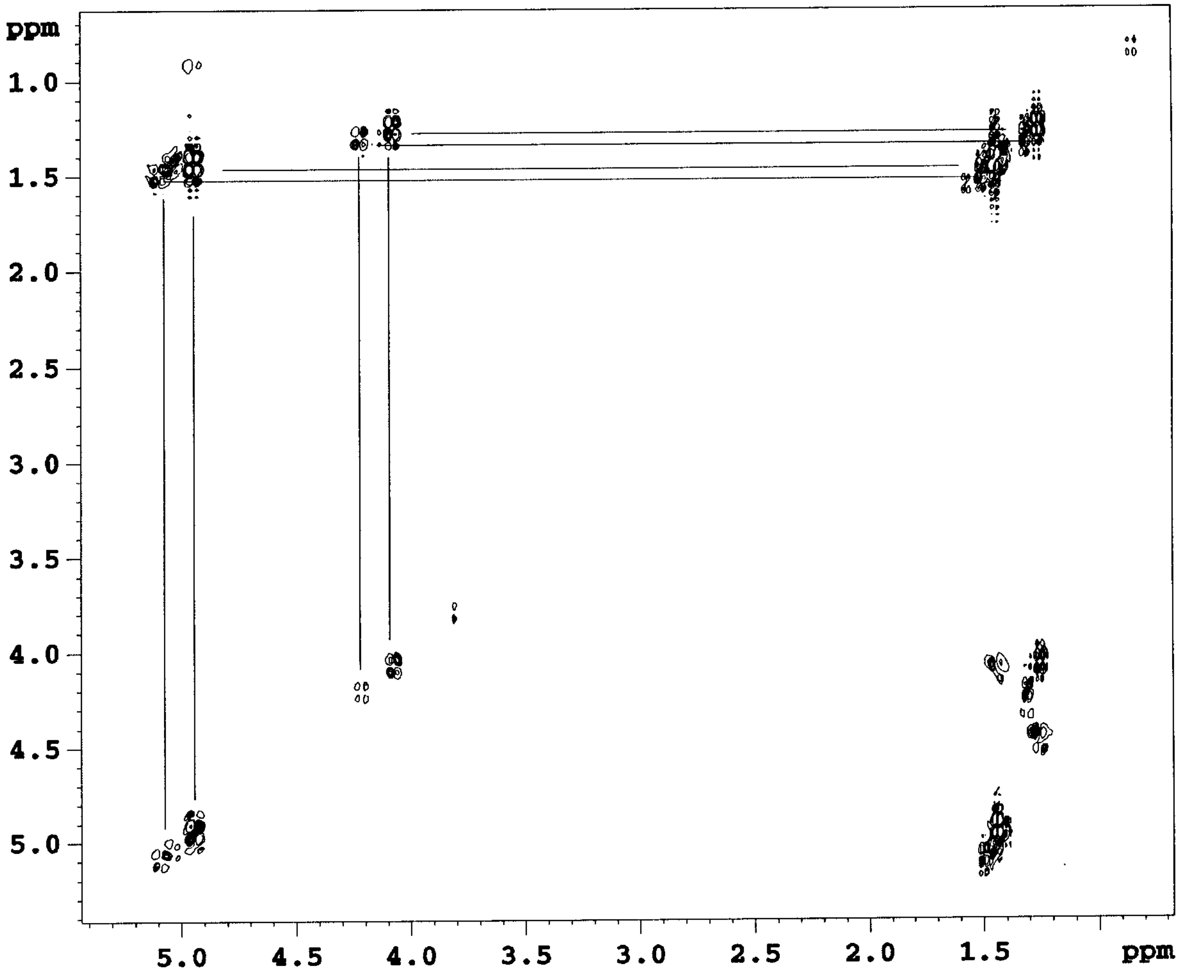


Figure 2. ^{1}H DQF-COSY spectrum of the products of the MDI−LA reaction.

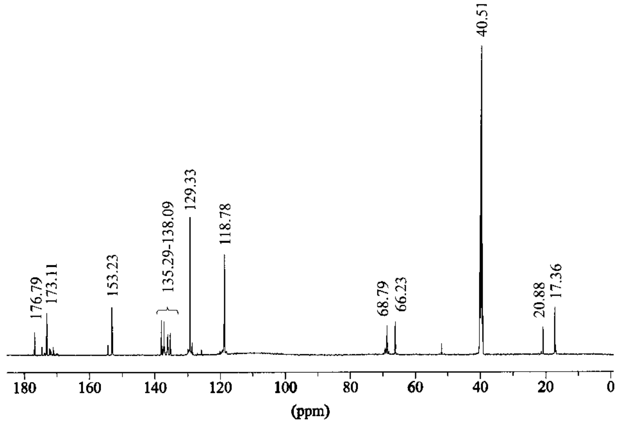
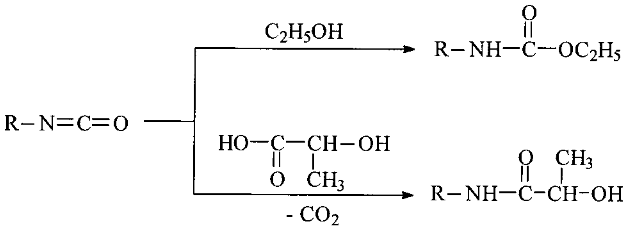


Figure 3. ^{13}C NMR spectrum of the products of the MDI−LA reaction.

Scheme 2. Structure of the Isocyanate End Group Terminated by Methanol and Lactic Acid Reacted with Its Carboxyl Group



Da with low intensity can be recognized, which correspond to the mass of $M_{\text{LA-MDI-LA}} - M_{\text{CO}_2}$; i.e., the carboxyl group of one of the two lactic acids has reacted (forming urethane and amide bonds), and the peaks at 749, 1045, and 1341 Da demonstrate that both groups of LA have reacted. The intensity of the peak at 1341 Da (not shown) is very low but significant. It is also apparent that when the carboxyl group is involved, the reaction is accompanied by decarboxylation. Insertion of dilactic acid into the chain can also be recognized (peaks at 525, 597 and 821); i.e., self-esterification of LA also takes place. The mass of this series can be expressed as

$$M' = 453 + 296n_1 + 72n_2 \qquad (2)$$

where 296 Da is the mass of MDI + LA−CO$_2$, indicating that CO$_2$ elimination also takes place when the carboxyl group of lactic acid is reacted with MDI, 72 Da is the mass of the repeating unit of oligolactic acid, and n_1 and n_2 are the number of the repeating units. In addition, another series of peaks at 677, 901, and 973 Da could also be recognized as shown in Figure 4, which does not fit to the series defined by eq 2, and can be formulated by eq 3

$$M' = 453 + 224n_1 + 72n_2 \qquad (3)$$

The repeating unit of mass is 224 Da, which can be

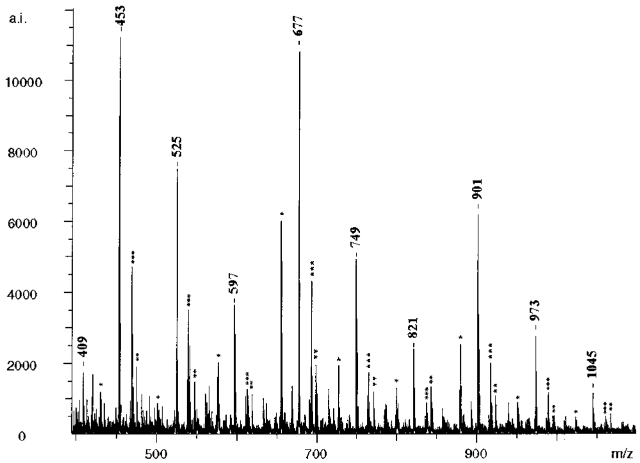


Figure 4. MALDI-TOF MS spectrum of the products of the MDI−LA reaction. The peaks where the mass is presented correspond to the M + Na$^+$ adducts. (*), (**), and (***) represent the [M + H]$^+$, [M + 2Na − H]$^+$, and [M + K]$^+$ adducts, respectively (matrix: DHB, no additional cationization agent was added to the sample).

Table 1. Calculated Mass (*M′*) of the Sodiated Copolymer Series with *M′* = 453 + 296*n*$_1$ + 72*n*$_2$ and *M′* = 453 + 224*n*$_1$ + 72*n*$_2$

$M' = 453 + 296n_1 + 72n_2$			$M' = 453 + 224n_1 + 72n_2$		
n_1	n_2	M'	n_1	n_2	M'
0	1	525	1	0	677
0	2	597	1	1	749
1	0	749	1	2	821
1	1	821	2	0	901
2	0	1045	2	1	973

attributed to the mass of MDI + H_2O − CO_2; i.e., one end group of MDI (isocyanate) is transformed to an amine function, and this is a usual way when isocyanates react with water,[6,7] which is probably present from the self-esterification of lactic acid.

By comparing eq 2 with eq 3, it unfortunately gives the mass of the 296 Da repeating unit, i.e., the same as it does eq 2 (for example, when $n_1 = n_2 = 1$ in eq 3). Therefore, some isomeric peaks should also be considered. As examples, Table 1 shows some calculated masses by eqs 2 and 3.

Table 1 shows several peaks (749 and 821 Da) of the same mass belonging to different series. To support the possible structures of the oligomers formed, PSD experiments were carried out by selecting some sodiated precursor ion peaks ([M + Na$^+$]) for PSD. The PSD spectra of the selected cooligomers are expected to occur by the cleavage of the C−N and C−O bonds.

The PSD spectrum of the precursor ion of 453 Da mass shows the decrease of mass by 72, 90, 116, and 133 Da, which indicates a symmetrical LA-MDI-LA structure, and it was found that all of the fragment ions retain the sodium cation. The PSD spectrum of the precursor ion of 525 Da mass reveals the decrease of mass by 72, 90, 116, and 133 Da and further mass decrease by 144 and 162 Da, which indicates that the oligomers must contain a dilactic acid unit as well. Consequently, it was concluded that the structure of the oligomer with a precursor ion mass of 525 Da is LA-MDI-LA-LA. (The PSD spectra of the precursor ions of 453 and 525 Da together with the assignments are available in the Supporting Information.)

The PSD spectrum of the precursor ion of 749 Da is shown in Figure 5.

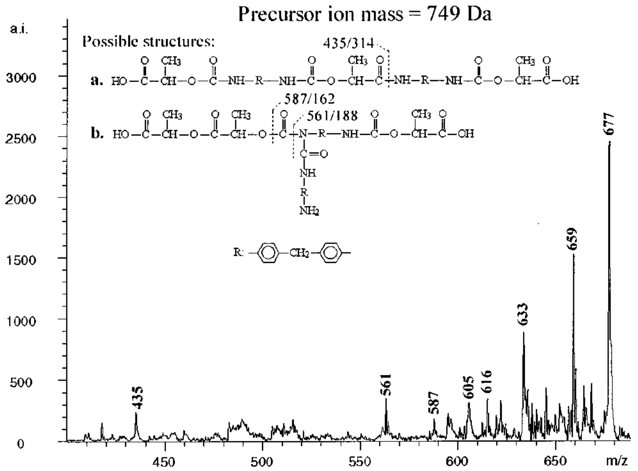


Figure 5. MALDI-TOF MS/PSD spectrum of the selected sodiated precursor ion with mass of 749 Da. The proposed structures of the oligomers are shown in the figure (matrix: DHB, no additional cationization agent was added to the sample).

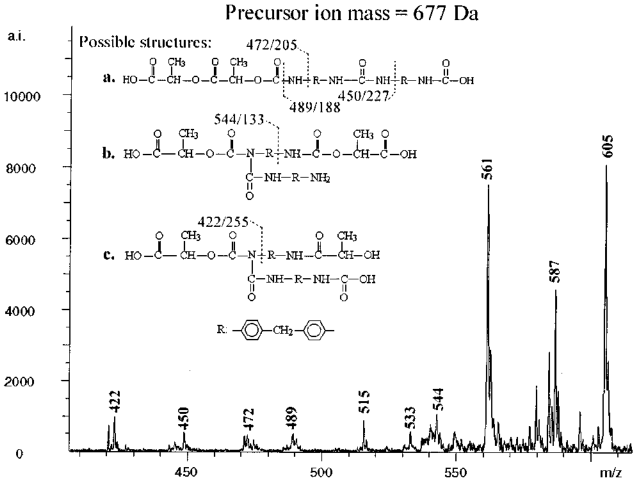
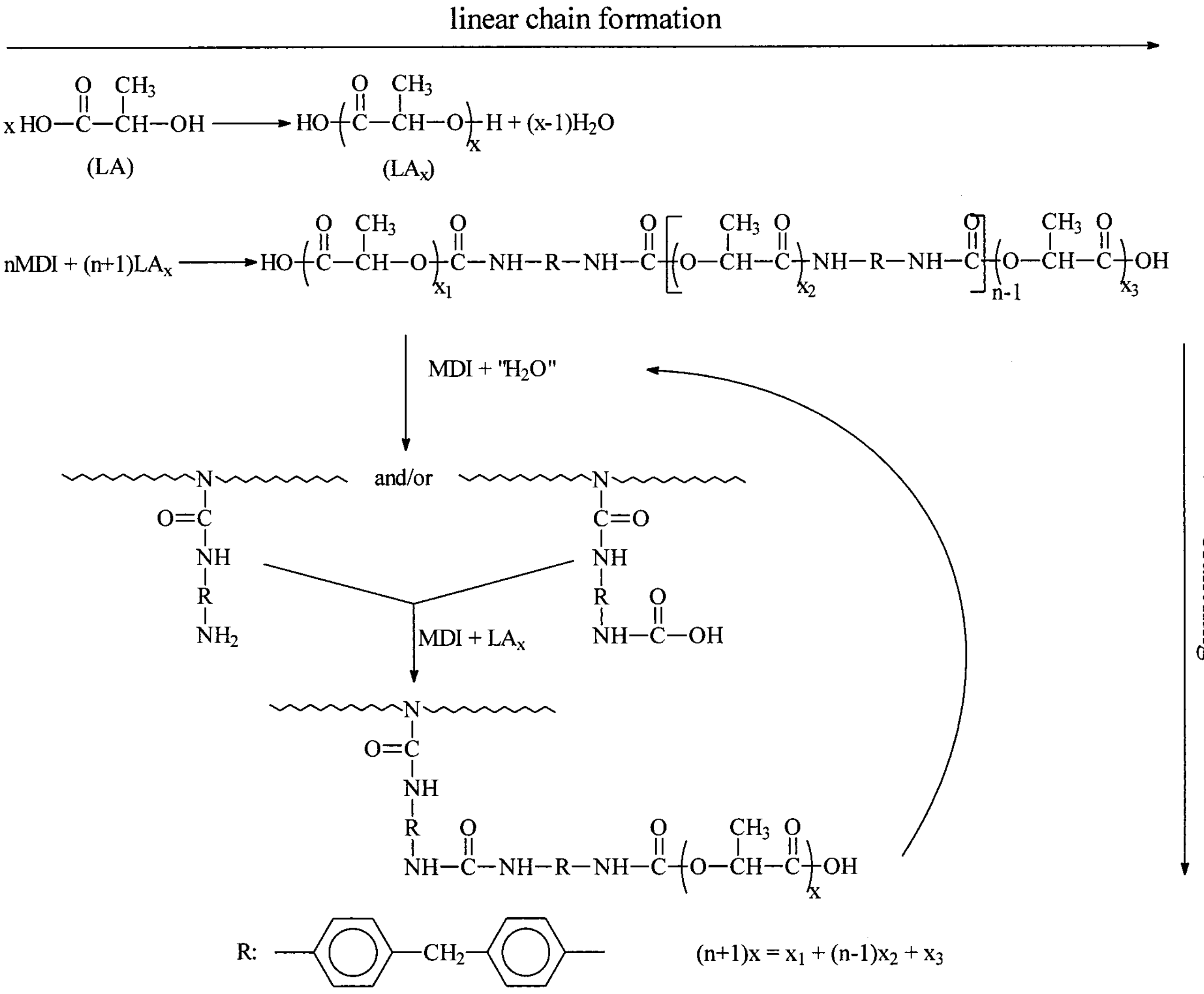


Figure 6. MALDI-TOF MS/PSD spectrum of the selected sodiated precursor ion with mass of 677 Da. The proposed structures of the oligomers are shown in the figure (matrix: DHB, no additional cationization agent was added to the sample).

The peaks at *m/z* 616 and 435 are characteristic of structure **a** (see Figure 5). This structure corresponds to the series of $M' = 453 + 296n_1 + 72n_2$ (with $n_1 = 1$ and $n_2 = 0$). However, as shown in Figure 5, the loss of mass by 144, 162, and 188 Da (at *m/z* 605, 587, and 561, respectively) also appears. As previously mentioned, this mass decrease is attributed to the dilactic acid units; therefore, an oligomer with structure **b** should also be present ($M' = 453 + 224n_1 + 72n_2$; $n_1 = 1$ and $n_2 = 1$). This means that the peak at 749 Da corresponds to a mixture of the oligomers with structures **a** and **b** shown in Figure 5. The PSD spectrum with a precursor ion mass of 677 Da is shown in Figure 6.

The appearance of the peak at *m/z* 544 revealed a chain end containing a single LA unit, and on the basis of the precursor ion mass, structure **b** (Figure 6) can be proposed. Under the PSD conditions the mass decreases by 144, 162, 188, and 205 Da (at *m/z* 533, 515, 489, and 472, respectively) indicate a dilactic chain end, and according to this finding, structure **a** (Figure 6) is proposed. On the basis of the appearance of the peaks at *m/z* 450 and 422, structures **b** and **c** (Figure 6) should

Scheme 3. Proposed Reaction Mechanism for the Reaction of LA with MDI (Experimental Conditions: See Experimental Section)

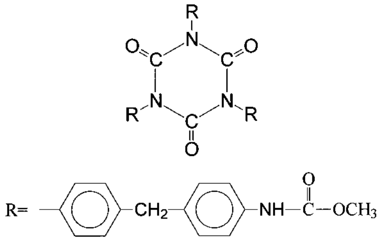


also be considered. This implies that the peak corresponds to the mixture of at least three different oligomers. However, considering that the oligomers with carbamoic acid structures are not stable, their concentration should be small;[6,7] thus, it is concluded that the peak at 677 Da is mostly due to the oligomer with structure **b**. PSD experiments with the oligomers of higher masses failed because of their low intensity. On the basis of these structural findings, we came to the conclusion that the oligomerization process took place as shown in Scheme 3.

As shown in Scheme 3, linear chains are formed from LA and MDI. Simultaneously, self-esterification of LA takes place yielding LA homooligomers, which can also build into the linear chain. The linear chain may react with additional MDI molecules. In the presence of water the free isocyanate group of MDI is transformed into amino and/or carbamoic acid functional groups, which can readily react with MDI and LA and/or LA homooligomers to yield branched oligomers.

Oligomerization of MDI. It is well-known that isocyanate-type compounds oligomerize in the presence of a catalyst.[6] Therefore, in parallel experiments the oligomerization of MDI was investigated under the same experimental conditions employed for the reaction between MDI and LA using tin octoate catalyst. It was found that no significant oligomerization of MDI took place within 10 min, but after 3 h reaction time mainly trimerization of MDI was observed. The oligomerization reaction was quenched with methanol. The MALDI-TOF MS results support the formation of an MDI trimer; i.e., a significant $[M + Li]^+$ peak at *m*/*z* 853.24 is present (the calculated monoisotopic mass for the $[C_{48}H_{42}O_9N_6 + Li]^+$ adduct is 853.31 Da), and the structure of the trimer is shown in Scheme 4.

Scheme 4. Structure of the Trimer Formed from MDI



The amount of the product was lower than that of the MDI−LA reaction with respect to the conversion, indicating that trimerization or oligomerization of MDI is not significant relative to the MDI−LA reaction.

Conclusion

MALDI-TOF MS combined with PSD provides extremely valuable information on the structures of the individual oligomers, which can be found in the very complex mixtures formed in the MDI–LA reaction. Formation of lactic acid oligomers, built into the chain, could also be observed by MALDI-TOF MS. Thus, MALDI-TOF MS is considered as a very powerful tool for the characterization of such complex mixtures.

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Supporting Information Available: The PSD spectra of the sodiated precursor ions of 453 and 525 Da together with the identified structures. This material is available free of charge via the Internet at http://pubs.acs.org.

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