Analysis of Macrocyclic Polystyrenes. 2. Mass Spectrometric Investigations

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ABSTRACT: Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry is shown to be a new and powerful technique for the analysis of macrocyclic polystyrenes and their linear precursors. The oligomer distributions for samples of different molar masses are obtained and the end groups of the linear precursors are identified. The chemical structure of the cyclization products is determined and it is shown that in the MALDI process the labile benzylic chlorine substituent at the macrocycle is abstracted, to yield a double bond. It is shown that MALDI mass spectrometry alone does not provide full information on the composition of the reaction products. The chemical structure of the macrocycles and the linear byproducts can be determined by chromatographic separation and subsequent MALDI analysis.

Introduction

Organic macrocycles are of considerable interest for a number of applications in biological and chemical processes; however, their synthesis is complicated and requires very specific and generally complex procedures. Most of the strategies developed to cyclize linear polymer chains involve the end-to-end ring closure of a living α,ω-dicarbanionic polystyrene by coupling the active ends with a difunctional nucleophile under highly diluted conditions. However, using this strategy, intermolecular coupling is encountered to a certain extent, resulting in the formation of undesired linear polycondensates.[1−3]

Owing to the difficulties with the cyclization of α,ω-dicarbanionic oligomers, a new strategy for the synthesis of macrocyclic polystyrenes has been developed by Deffieux et al.[4−6] It consists of a single-step, pseudo-unimolecular cyclization process of a linear α-diethyl acetal-ω-styrenylpolystyrene of controlled molar mass. This cyclizing precursor is synthesized by living anionic polymerization. The analysis of the cyclization products by conventional NMR and gel permeation chromatography (GPC) techniques indicates that cyclization occurred; a quantitative determination of the relative amount of cyclic oligomers, however, is not possible. In a previous publication, a HPLC technique was developed that enables the cyclization products to be separated into the residual linear precursor fraction, a fraction of noncyclized linear oligomers, and the cyclic fraction. Quantification of the different fractions has been carried out using internal calibration, and the cyclization yield has been determined.[7] A first information on the chemical structure of the cyclization products was obtained by a relatively new technique of mass spectrometry, matrix-assisted laser desorption/ionization (MALDI).

This new and most promising method for the ionization of large molecules and the analysis according to their molar mass and functionality was introduced recently by Karas and Hillenkamp.[8−10] Compared to other mass spectrometric techniques, the accessible mass range has been extended considerably, and the technique is fast and instrumentally very simple. Moreover, relatively inexpensive commercial instrumentation has become accessible. In principle, the sample to be investigated and a matrix solution are mixed in such a ratio that matrix separation of the sample molecules is achieved. After drying, a laser pulse is directed onto the solid matrix to photoexcite the matrix material. This excitation causes the matrix to explode, resulting in the expulsion and soft ionization of the sample molecules. Once the analyte is ionized, it is accelerated and analyzed in a time-of-flight (TOF) mass spectrometer. As a result, the analyte is separated according to the molar mass of its components, and in the case of heterogeneous polymers, additional information on chemical composition may be obtained. In a number of papers it was shown that polymers may be analyzed up to relative molar masses of about 200.000 Da.[11,12] In particular, work on the analysis of polystyrene has been published in a number of papers.[13−15] Recently, it was shown by two of the authors that functionally heterogeneous polymers can be analyzed with respect to the degree of polymerization and the type of functional groups.[16−18]

The present paper is aimed at completing our work on the analysis of polystyrene macrocycles by discussing some of the MALDI results.

Experimental Section

Synthesis of Macrocyclic Polystyrenes. The synthesis of the samples under investigation is described in detail in a previous paper.[5] In brief, 3-lithiopropionaldehyde, prepared from 3-chloropropionaldehyde and lithium, was used as the initiator in the anionic polymerization of styrene. The resulting acetal-terminated living poly(styryllithium) was reacted successively with diphenylethylene and *p*-(chloromethyl) styrene to form the linear α-diethyl acetal-ω-styrenylpolystyrene. The chain cyclization by end-to-end ring closure was then performed by transforming the acetal end group into a iodo ether end group followed by the addition of $SnCl_4$ for promoting the cyclization reaction. Macrocyclic polystyrenes were obtained in high yield in the molar mass range up to about 20.000 g/mol. The samples under investigation, including the linear precursors and the cyclic reaction products, are summarized in Table 1.

MALDI-MS. These investigations were conducted on a Kratos Kompact MALDI 3. A pulsed nitrogen laser producing

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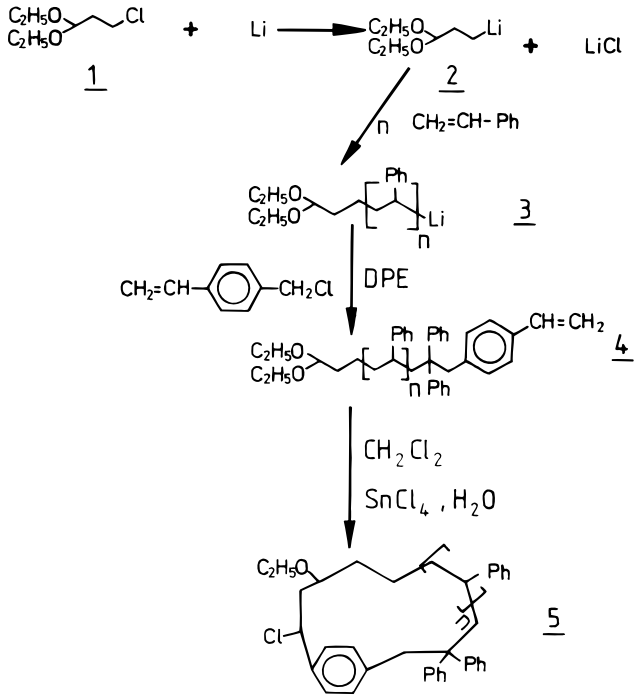
Table 1. Molar Masses[a] of the Linear Precursors and the Cyclization Products, Determined by GPC

code	sample	M_w	M_n
1 L	PS 1900 L	1860	1780
1 C	PS 1900 C	1530	1450
3 L	PS 2200 L	2160	2060
3 C	PS 2200 C	1720	1630
4 L	PS 3300 L	3310	3200
4 C	PS 3300 C	2640	2550

[a] Molar mass in g/mol.

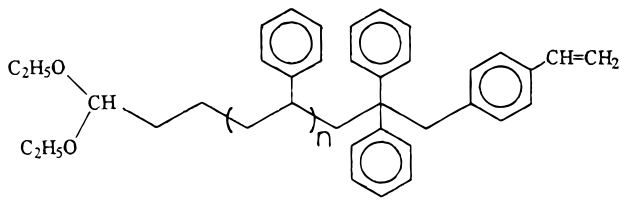
Scheme 1. General Pathway for the Synthesis of the Cyclic Polystyrenes



a wavelength of 337 nm was used for laser desorption/ionization. A TOF mass spectrometer with a 20 kV accelaration voltage was used to obtain the mass spectra. The samples were dissolved in tetrahydrofuran and mixed with the matrix dithranol (1,8,9-trihydroxyanthracene) or nitrophenyl octyl ether. For promoting the formation of $M + Ag^+$ molecular ions, small amounts of silver trifluoroacetate were added to the solution. After drying of the mixture of the sample and the matrix on the sample holder, the measurements were carried out using the following conditions: polarity, positive; flight path, reflection; mass, high (20 kV accelaration voltage); 100−200 shots per sample.

Results and Discussion

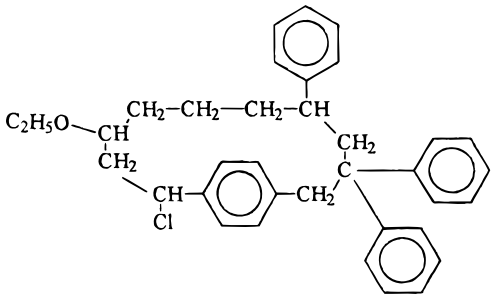
As was already outlined in the first part of this publication, the cyclization reaction is assumed to correspond to the mechanism given in Scheme 1.[7] Accordingly, the linear precursor for the cyclization reaction is a α-diethyl acetal-ω-styrenyl polystyrene of the following structure



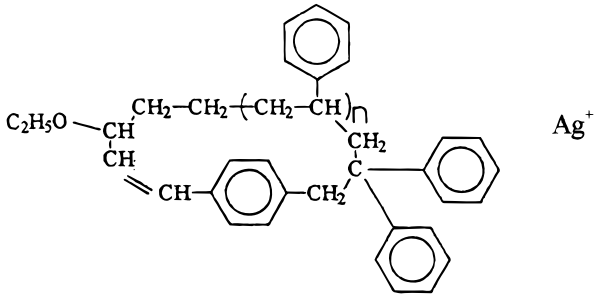
The MALDI spectrum of a low molar mass sample of linear precursor is presented in Figure 1. Dithranol was used as the matrix and small amounts of silver trifluoroacetate were added to promote ionization resulting in the formation of $M + Ag^+$ molecular ions, as has been proposed by several authors.[19,20] The spectrum shows a number of peaks having a peak-to-peak mass increment of 104 g/mol. This mass increment exactly equals the mass of the repeat unit in the linear precursor. Accordingly, each peak represents one oligomer of a homologous series. The peaks are due to intact $M + Ag^+$ molecular ions, their mass numbers equal $108 + M_c + 104n$, 108 being the atomic mass of Ag^+ (this mass has been taken since the silver isotopes of 107 and 109 are poorly resolved; the exact mass number is 107.8682), M_c being the mass of the end group, and n being the degree of polymerization. Knowing M_c and n of each oligomer, the corresponding mass number can be calculated for $M + Ag^+$ and compared to the experimental data.

For the proposed chemical structure of the linear precursor the mass numbers are calculated by $M + Ag^+ = 536 + 104n$. The calculated and experimental mass numbers are in excellent agreement (see Table 2), indicating clearly that the mass spectrum corresponds to the proposed chemical structure. As can be seen, the spectrum shows only one oligomer series. This confirms the high purity of the sample, which was assumed from the chromatographic investigations, outlined in the first part of this publication.[7]

The linear precursor is subjected to a cyclization reaction, resulting in a cyclization product, the MALDI spectrum of which is given in Figure 2. Similar to Figure 1, an oligomer distribution with peak-to-peak mass increments of 104 Da is obtained (peak series 1). This peak series is accompanied by two minor peak series 2 and 3 having the same mass increment. Initially it was assumed that in the cyclization reaction the following cyclic structure is formed:



However, the observed mass numbers do not fit this structure. It was assumed that during the preparation or the UV irradiation in the MALDI process the chlorine is abstracted and macroions of the following structure are formed



(2)

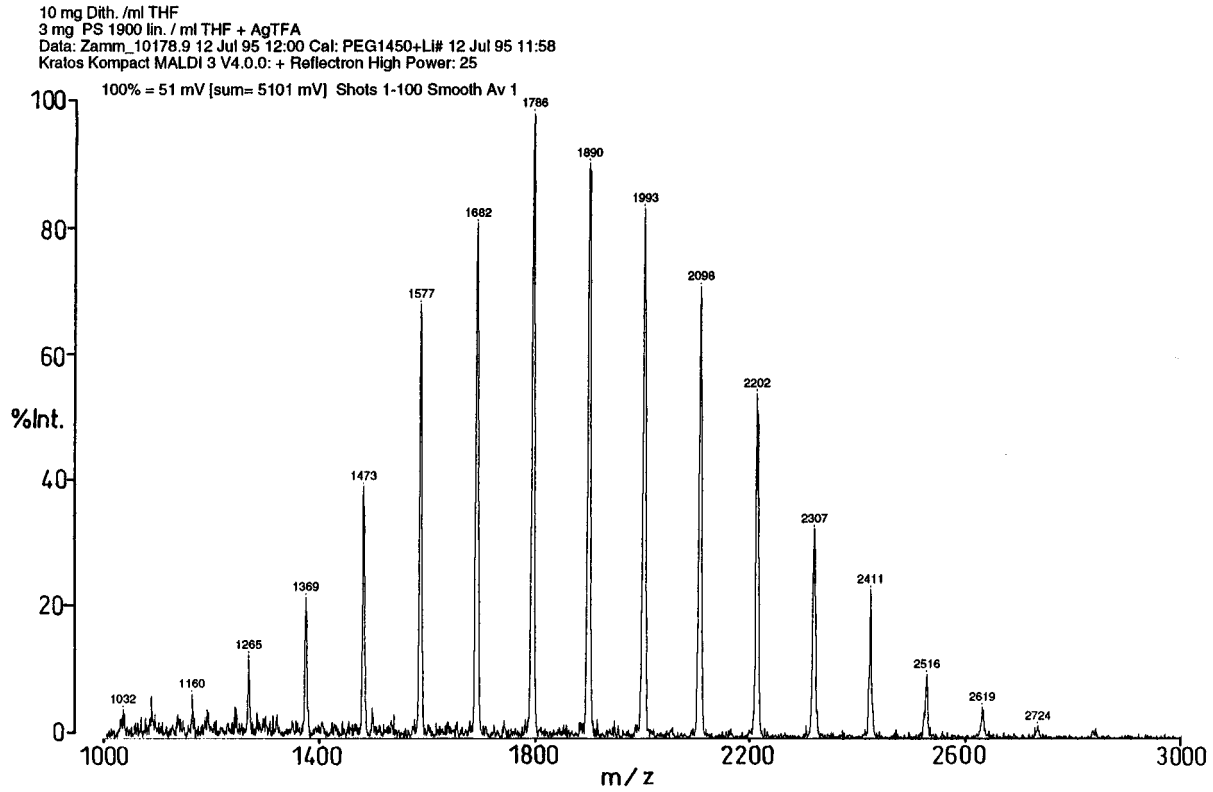
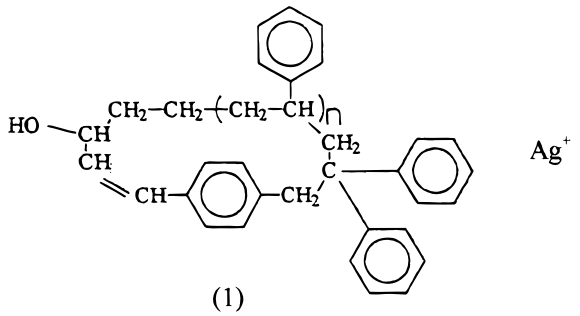


Figure 1. MALDI spectrum of sample PS 1900 L (matrix, dithranol).

Table 2. Calculated and Measured Mass Numbers (*m*/*z*) of the Linear Precursor PS 1900 L ($M + Ag^+ = 536 + 104n$)

	$M + Ag^+$ (g/mol)			$M + Ag^+$ (g/mol)	
n	calcd	expl	*n*	calcd	expl
6	1160.9	1160	14	1994.1	1993
7	1265.1	1265	15	2098.3	2098
8	1369.2	1369	16	2202.4	2202
9	1473.4	1473	17	2306.6	2307
10	1577.5	1577	18	2410.7	2411
11	1681.7	1682	19	2514.9	2516
12	1785.8	1786	20	2619.0	2619
13	1890.0	1890	21	2723.2	2724

which perfectly fits the peak series 2 ($M + Ag^+ = 491 + 104n$). The major peak series 1 has a mass difference of −28 Da versus series 2, which was found to correspond to the structure



(1)

$M+Ag^+ = 463 + 104n$

This structure can formed via the intermediate formation of a α-formyl-terminated precursor from the α-acetal compound and subsequent cyclization. Finally, peak series 3 exhibits a mass difference of −18 Da versus series 1, presumably due to the abstraction of the hydroxy group in the MALDI process. In conclusion, the MALDI spectrum of the cyclization product shows only peaks of cyclic oligomers. The very small fraction of residual linears, which is present in the cyclization product, does not manifest itself in the spectrum.

Assuming that the relative intensities of the peaks in one oligomer series are equivalent to concentration, the molar mass distribution for the linear and the cyclic sample are calculated. As is expected from the reaction mechanism, the maxima and the widths of both distributions are perfectly similar. The difference in the average values results from the transformation of the acetal group in the cyclization reaction, lowering the mass of each oligomer by 73 g/mol.

	m_p	n_p	M_w	M_n	M_w/M_n
P51900L	1786	12	1790	1750	1.02
P51900C	1712	12	1720	1670	1.03

(molar masses in g/mol)

The mass ratio of the oligomer series 1 and 2 in PS 1900 C cannot be determined directly from the MALDI spectrum. Since peak intensity is a function of ionization probability and, therefore, chemical structure, different structures may yield peaks of different intensity. In addition, peak intensity is affected by the matrix. This can be shown easily by running a MALDI experiment of PS 1900 C in a different matrix. Figure 3 represents the spectrum of PS 1900 C using o-nitrophenyl octyl ether as the matrix. In this case series 2 appears much more intense than peak series 1, while in Figure 2 peak series 1 is more intense. In addition, a significant shift toward lower masses is observed for both peak series. While the maximum population for both peak series is expected to appear around 1900 Da, it appears at about 1600 Da for peak series 2 and at about 1100 Da for peak series 1. This is a strong indication of the fact that matrix effects and/or fragmentation must not be neglected as has been shown in a recent paper.[21]

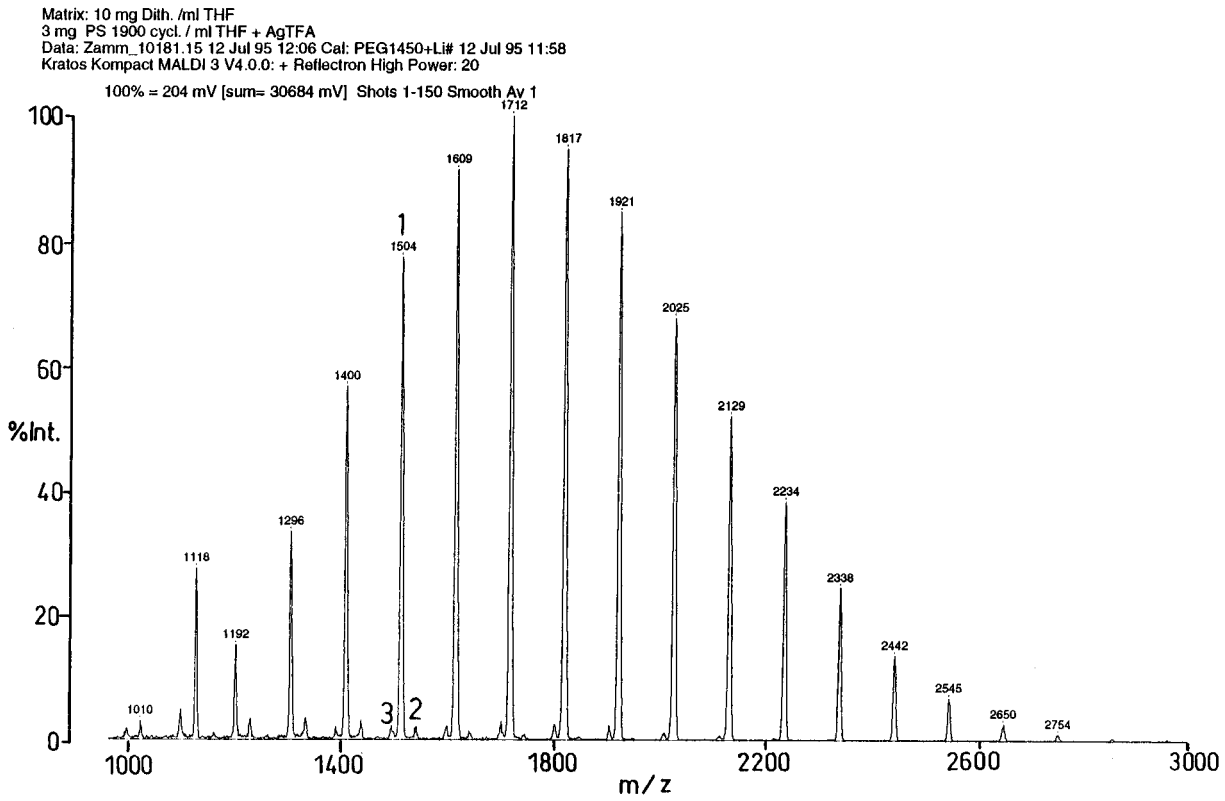


Figure 2. MALDI spectrum of sample PS 1900 C (matrix, dithranol).

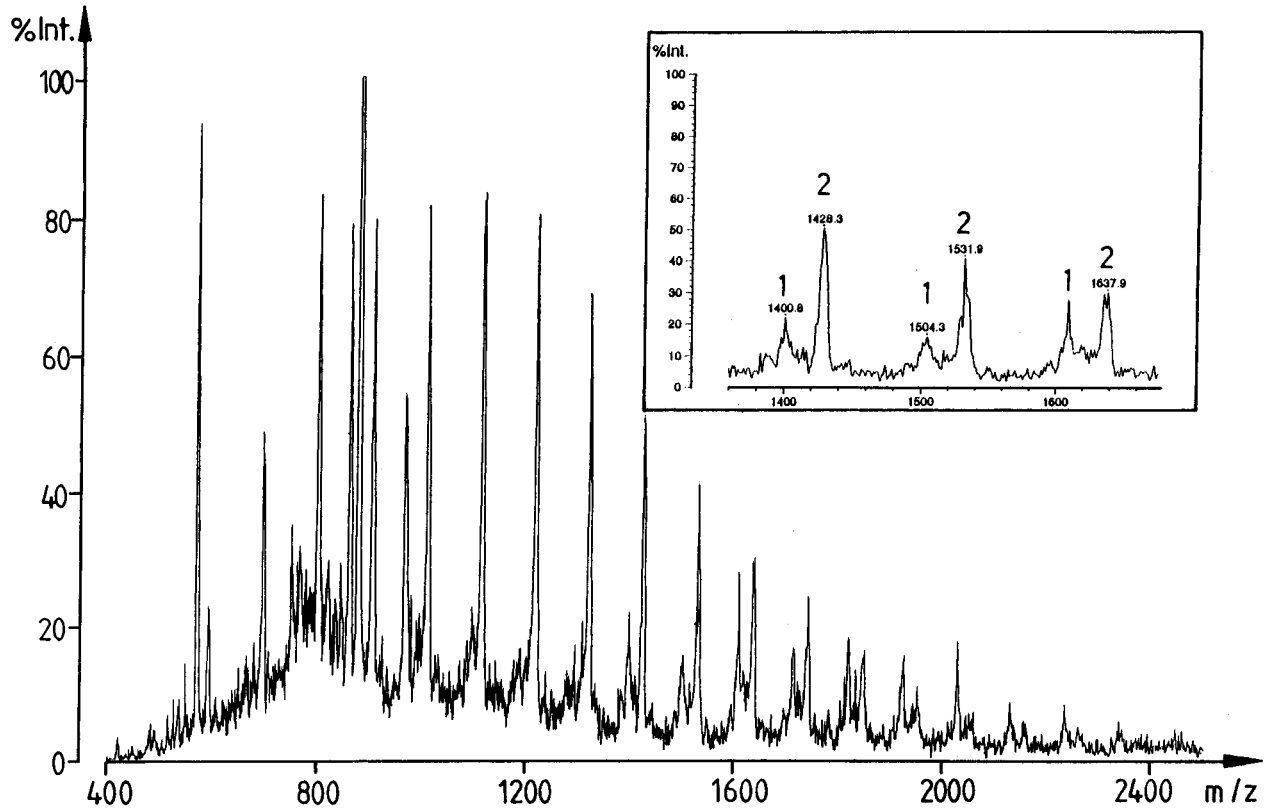


Figure 3. MALDI spectrum of sample PS 1900 C (matrix, *o*-nitrophenyl octyl ether).

The cyclization reaction of PS 2200 L occurs also under favorable conditions. The MALDI spectrum of the linear precursor shows one oligomer series, indicating high purity of the sample. The corresponding cyclization product exhibits the major peak series 1 with minor peaks of series 2 and 3 (see Figure 4). The calculated molar mass distributions for PS 2200 L and PS 2200 C are in good agreement, as can be seen in Table 3.

Compared to the rather pure linear precursors PS 1900 L and PS 2200 L, the sample PS 3300 L looks more heterogeneous (see Figure 5). In addition to the expected peak series of α-diethyl acetal-ω-styrenylpolystyrene in the mass range of 2000−4200 Da (●), mass

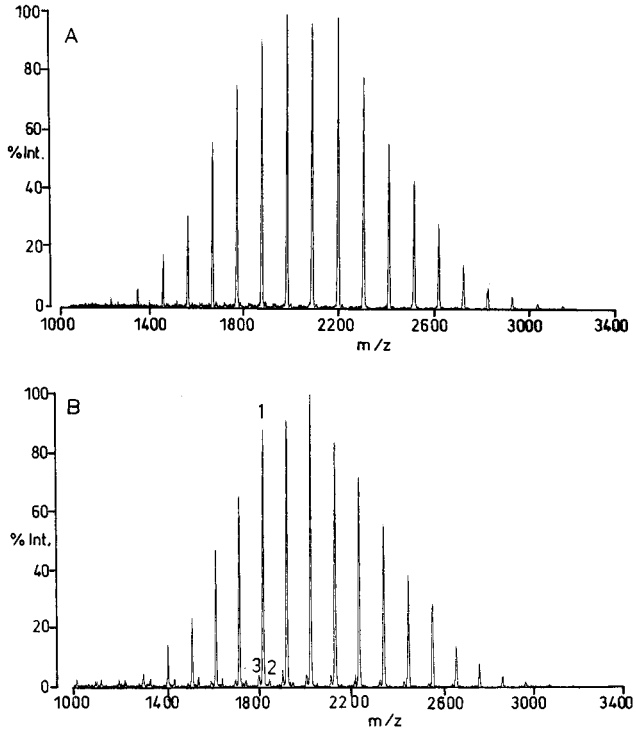


Figure 4. MALDI spectra of samples PS 2200 L (A) and PS 2200 C (B) (matrix, dithranol).

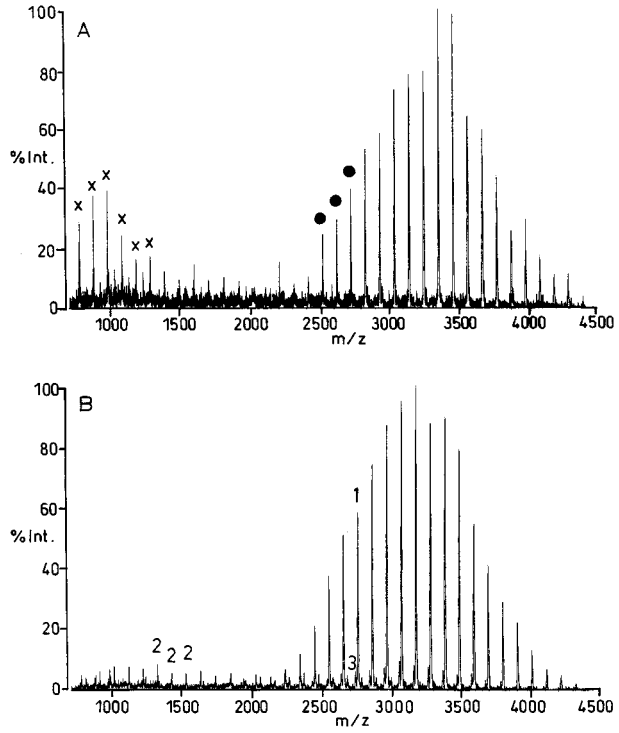


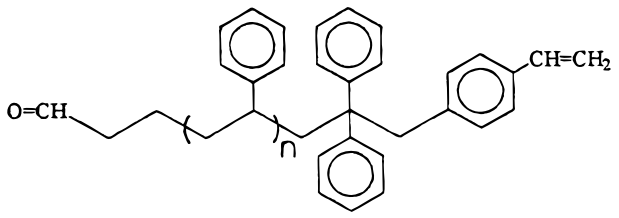
Figure 5. MALDI spectra of samples PS 3300 L (A) and PS 3300 C (B) (matrix, dithranol).

Table 3. Molar Mass[a] Distributions of Different Oligomer Series of Linear Precursors and Cyclization Products, Determined by MALDI-MS

	m_p	n_p	M_w	M_n	M_w/M_n
PS 2200 L	2096	15	2010	1960	1.03
PS 2200 C	2027	15	1960	1910	1.03
PS 2200 L (●)	3350	27	3230	3190	1.01
PS 2200 L (x)			630	580	1.09
PS 2200 C (1)	3173	26	3100	3060	1.01
PS 2200 C (3)			3050	3000	1.02

[a] Molar mass in g/mol.

peaks at 981, 1085, 1189, 1293 (etc.) Da indicate that a second polystyrene-based oligomer series is present in the sample. This oligomer series exhibits a mass difference of −74 Da versus the major series. It is well-known that acetals are rather labile chemical structures. They can readily undergo a transformation into the corresponding aldehyde. The mass difference between the acetal and the aldehyde is exactly 74 Da. It is, therefore, assumed that the oligomer series x is due to α-formyl-ω-styrenyl oligomers.



(x)

Surprisingly, the calculated molar mass of this oligomer series is much lower than the major fraction (see Table 3). The reaction product resulting from the cyclization of PS 3300 L shows the known peak series 1−3, series 1 being the major product. Its molar mass distribution agrees well with the molar mass distribution of the linear precursor; however, the molar mass

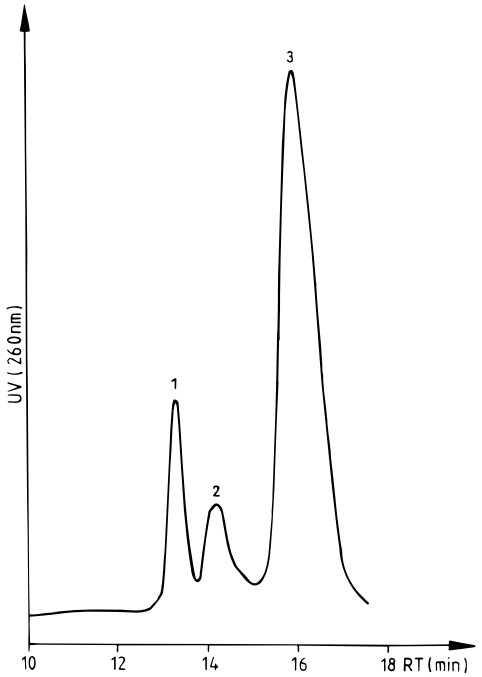
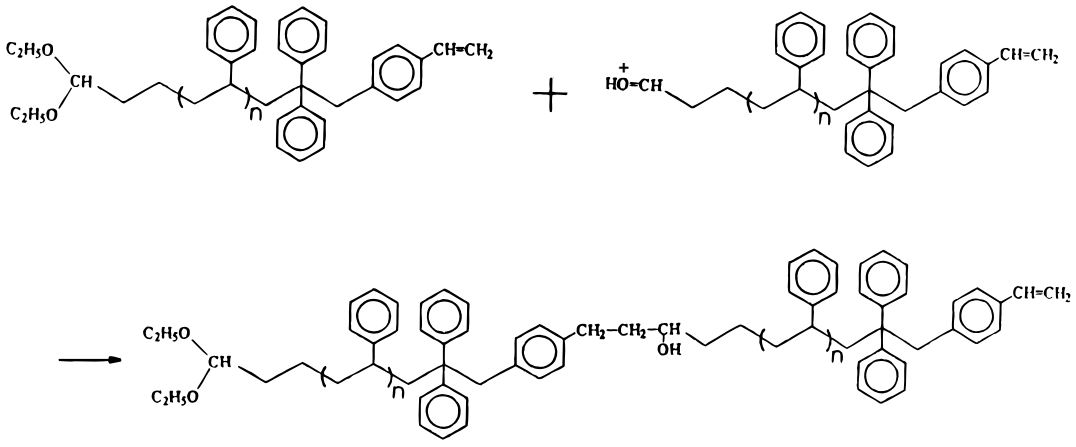


Figure 6. Chromatogram of sample PS 3300 C, indicating separation into three fractions, (stationary phase, YMC silica gel; eluent, THF−hexane 45:55 vol %).

range of series 2 seems to be gradually lower than that of series 1 and 3. The lower molar mass range of the minor fractions x and 2 in Figure 5A,B do not agree with the proposed reaction mechanism. From the reaction mechanism it must be assumed that the population maxima for all oligomer series are in the same mass range. However, Figure 3 indicates that mass shifts can occur due to matrix effects or fragmentation. Therefore, it was assumed that an artifact must be considered and the measured peak intensities and mass ranges of x and 2 do not reflect the real situation. Fragmentation could have occurred to a certain extent, which was found to

Scheme 2



$M+Ag^+ = 999 + 104\ n$

n	M (calc.)	M (exp.)
17	2769.6	2770
18	2873.7	2874
19	2977.9	2977

become important for higher molar mass samples. This assumption is additionally supported by the fact that the GPC experiments of PS 3300 L did not indicate any lower molar mass fraction.

Unfortunately, the spectrum of the cyclization product does not give information on whether the formyl-terminated oligomers x did cyclize similar to the acetal-terminated species (●), because the oligomer masses of series x and the cyclic oligomers of series 1 are similar. However, in model experiments it was shown that formyl-terminated oligomers may cyclize.[7]

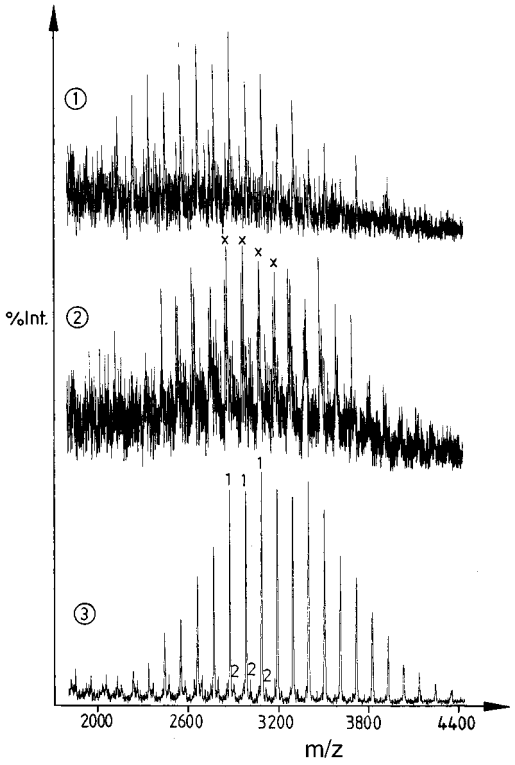


Figure 7. MALDI spectra of fractions 1−3 after chromatographic separation of sample PS 3300 C (matrix, dithranol).

As has been shown, MALDI experiments alone do not yield full information on the composition of the cyclization products. In particular, the residual linears do not manifest themselves in the spectrum. Therefore, a preparative chromatographic separation of sample PS 3300 C was carried out to isolate the linear fractions and to analyze them by MALDI-MS. The chromatogram of sample PS 3300 C shows the separation into three fractions (see Figure 6), fractions 1 and 2 assumingly being residual linears and fraction 3 being the cyclic reaction product. This is confirmed by the MALDI spectra of the fractions given in Figure 7. Fraction 3 evidently contains the oligomer series 1 and 3, while fraction 2 shows a number of different peak series. The major peak series is due to the formyl-terminated oligomers (x), as was assumed previously from the chromatographic behavior. The other peak series are assumed to belong to "desactivated" linears; their chemical structure, however, could not be derived from the peak masses. Unexpectedly, fraction 1 shows a peak series at 2770, 2874, 2977 (etc.) Da, which does not agree with the acetal-terminated linear precursor. If one assumes, however, that condensation reactions could occur, the reaction in Scheme 2 would be possible.

The resulting structure of the reaction products agrees perfectly with the observed mass peaks. In addition, the end groups of these oligomers are similar to the end groups of the linear precursor, and therefore, the chromatographic behavior can be assumed to be similar. Accordingly, the addition products should coelute with the acetal-terminated linear precursor in peak 1.

References and Notes

(1) Roovers, J.; Toporowski, P. M. *Macromolecules* **1983**, *16*, 843.
(2) Geiser, D.; Höcker, H. *Macromolecules* **1980**, *13*, 653.
(3) Hogen-Esch, T. E.; Sundararajan, J.; Toreki, W. *Macromol. Chem. Macromol. Symp.* **1991**, *47*, 23.
(4) Deffieux, A.; Schappacher, M.; Rique-Lurbet, L. *Polymer* **1994**, *35*, 4562.
(5) Rique-Lurbet, L.; Schappacher, M.; Deffieux, A. *Macromolecules* **1994**, *27*, 6318.

(6) Deffieux, A.; Schappacher, M.; Rique-Lurbet, L. *Macromol. Symp.* **1995**, *95*, 103.
(7) Pasch, H.; Deffieux, A.; Henze, I.; Schappacher, M.; Rique-Lurbet, L. *Macromolecules* **1996**, in press.
(8) Karas, M.; Hillenkamp, F. *Anal. Chem.* **1988**, *60*, 2299.
(9) Beavis, R. C.; Chait, B. T. *Rapid Commun. Mass Spectrom.* **1989**, *3*, 233.
(10) Hillenkamp, F.; Karas, M.; Beavis, R. C.; Chait, B. T. *Anal. Chem.* **1991**, *63*, 1193A.
(11) Bahr, U.; Deppe, A.; Karas, M.; Hillenkamp, F.; Giessmann, U. *Anal. Chem.* **1992**, *64*, 2866.
(12) Danis, P. O.; Karr, D. E.; Mayer, F.; Holle, A.; Watson, C. H. *Org. Mass Spectrom.* **1992**, *27*, 843.
(13) Kahr, M. S.; Wilkins, C. L. *J. Am. Soc. Mass Spectrom.* **1993**, *4*, 453.
(14) Montaudo, G.; Montaudo, M. S.; Puglisi, C.; Samperi, F. *Rapid Commun. Mass Spectrom.* **1994**, *8*, 1011.
(15) Danis, P. O.; Karr, D. E.; Xiong, Y.; Owens, K. G. *Rapid Commun. Mass Spectrom.* **1996**, *10*, 862.
(16) Pasch, H.; Unvericht, R. *Angew. Makromol. Chem.* **1993**, *212*, 191.
(17) Pasch, H.; Gores, F. *Polymer* **1995**, *36*, 1999.
(18) Braun, D.; Ghahary, R.; Pasch, H. *Polymer* **1996**, *37*, 777.
(19) Llenes, C. F.; O'Malley, R. M. *Rapid Commun. Mass Spectrom.* **1992**, *6*, 564.
(20) Dean, P. A.; O'Malley, R. M. *Rapid Commun. Mass Spectrom.* **1993**, *7*, 53.
(21) Lehrle, R. S.; Sarson, D. S. *Polymer Degrad. Stab.* **1996**, *51*, 197.

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