Analysis of Macrocyclic Polystyrenes. 1. Liquid Chromatographic Investigations

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ABSTRACT: Macrocyclic polystyrenes prepared by a single-step, pseudo-unimolecular cyclization of a linear α-(diethoxyethyl)-ω-styrenylpolystyrene can effectively be analyzed by liquid chromatography at the critical point of adsorption. Using silica gel as the stationary phase and THF−hexane as the eluent, the macrocyclic oligomers are separated from their linear precursors and other nonfunctional linear oligomers. The quantitative determination of the cyclization yield can be carried out via appropriate detector calibration for the linears and cyclics. Additional information on the chemical structure of the linears and cyclics is obtained by matrix-assisted laser desorption/ionization (MALDI) mass spectrometry. In conclusion, a possible cyclization mechanism is given including an interpretation of the MALDI behavior of the samples.

Introduction

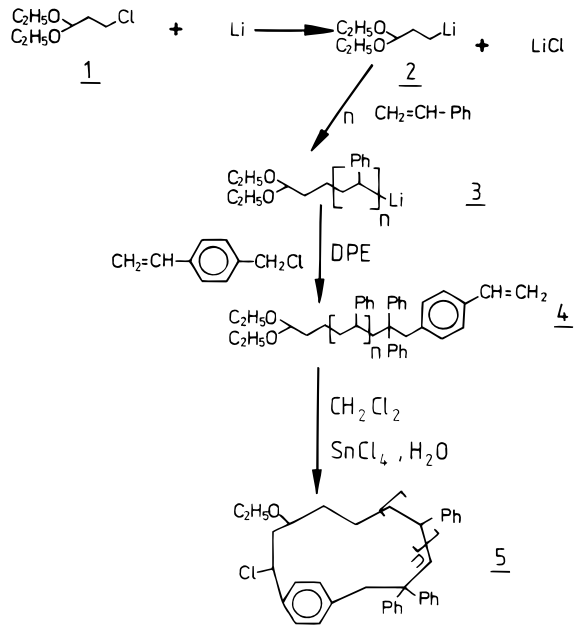
The very specific role of natural and synthetic organic macrocycles in many biological and chemical processes has attracted considerable efforts toward the design and synthesis of new organic molecules with cyclic structures. The remarkable properties of calixarenes and cyclodextrins have been the driving force for the rapid development of this research area. The controlled synthesis and the characterization of ring-shaped macromolecules represent a significant challenge to the organic chemist; in particular vinyl type macrocyclic polymers must be prepared using very specific and generally complex synthesis procedures.

Most of the strategies developed to cyclize polystyrene 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-(diethoxyethyl)-ω-styrenylpolystyrene of controlled molar mass. This cyclizing precursor has been synthesized by living anionic polymerization according to the reaction pathway given in Scheme 1.

The analysis of the resulting macrocycles was partially possible by nuclear magnetic resonance (NMR) and gel permeation chromatography (GPC). NMR clearly showed the disappearance of the end groups; however, the structure of the macrocycles could not be fully identified. The cyclic polymer structure was assumed to correspond to derivate **5** (Scheme 1), the chlorine atom resulting from abstraction of a halogen from the Lewis acid catalyst. The molar mass distribution of the cyclization products was determined by GPC.

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



The cyclics exhibit a lower hydrodynamic volume than the linear precursors; a complete separation into a linear and a cyclic fraction, however, could not be achieved. Accordingly, the yield of cyclized polystyrene was calculated on a basis of the relative areas attributed to cyclic polystyrene and of the crude product.

One of the prominent tasks of polymer analysis is the exact determination of the chemical structure of the reaction products. In particular, the ratio of linear precursor to cyclic product is important information for the optimization of the preparation process. Therefore, the present contribution is dedicated to the development of an appropriate separation method using interaction chromatography. In a forthcoming paper the mass spectrometric analysis of the products will be discussed.

Experimental Section

Synthesis of Macrocyclic Polystyrenes. The synthesis of the samples under investigation is described in detail in a

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Table 1. Molar Masses of the Linear Precursors and the Cyclization Products

code	sample	10^{-3} M_w	10^{-3} M_n
1L	PS 1900 L	1.86	1.78
1C	PS 1900 C	1.53	1.45
2L	PS 2000 L		2.00
2C	PS 2000 C		2.00
3L	PS 2000 L	2.16	2.06
3C	PS 2200 C	1.72	1.63
4L	PS 3300 L	3.31	3.20
4C	PS 3300 C	2.64	2.55
5L	PS 6250 L		6.25
5C	PS 6250 C		6.25
6L	PS 8000 L	8.62	8.25
6C	PS 8000 C	6.62	6.42
7L	PS 15000 L	14.80	14.40
7C	PS 15000 C	11.90	11.50
8L	PS 25000 L	24.00	23.70
8C	PS 25000 C	23.10	21.70
9L	PS 2200(a)		
10L	PS 2200(b)		
9C	PS 2200(c)		
10C	PS 2200(d)		

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-(diethoxyethyl)-ω-styrenylpolystyrene. The chain cyclization by end-to-end ring closure was then performed by transforming the acetal end group into a cationic end group by slow addition of the linear polymer in solution into a large volume of methylene chloride containing $SnCl_4$ for promoting the cyclization reaction. Macrocyclic polystyrenes were obtained in high yield in the molar mass range up to about 20 kg/mol. The samples under investigation, including the linear precursors and the cyclic reaction products, are summarized in Table 1.

GPC Experiments. The GPC experiments were performed on a Waters modular GPC system using five 300 × 8 mm i.d. Ultrastyragel columns (1000 Å, 2 × 500 Å, 2 × 100 Å) and tetrahydrofuran as the mobile phase. The system was calibrated using narrow distributed polystyrene calibration standards.

Chromatography at the Critical Point of Adsorption. These measurements were conducted on a modular HPLC system, comprising a Waters model 510 pump, a Waters DRI detector 410, a Knauer UV/vis filter photometer, a Rheodyne six-port injection valve, and a Waters column oven. The column was either Eurogel RP-100, 5 μm average particle size, 100 Å average pore size, 250 × 4 mm i.d. or YMC silica gel of the same particle and pore size. The eluent was tetrahydrofuran−hexane; the flow rate was 0.5 mL/min unless otherwise specified. Aliquots (20 μL) of 0.5−1 wt % polymer solutions were injected.

Results and Discussion

For first information on the molar mass distribution of the linear precursors and the cyclization products, a GPC analysis was performed; see Table 1. The chromatograms for two samples of different molar masses are shown in Figure 1. As can be seen in both cases, the linear precursors elute earlier than the cyclics due to their larger hydrodynamic volume. The difference in the elution volumes of the two peaks, however, is too small to determine the residual linear fraction in the cyclization product. A small peak at lower elution volume indicates a fraction of higher molar mass in the cyclization product. This fraction could be the result of condensation reactions. To summarize, GPC can be used to determine the molar mass distribution of the linear precursors and the cyclization products. The separation of the cyclics and the linears is not possible

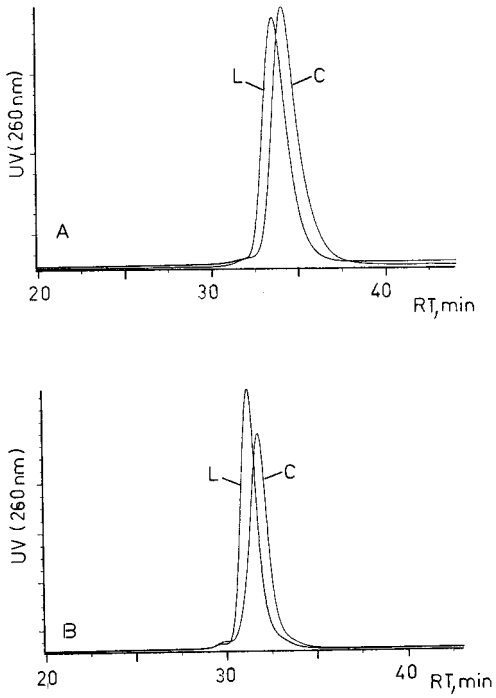


Figure 1. GPC chromatograms of cyclic polystyrenes (C) and their linear precursors (L): PS 3300 L and PS 3300 C (A); PS 8000 L and PS 8000 C (B). Stationary phase: Ultrastyragel. Eluent: THF.

by GPC, and therefore, the molar mass distribution of the cyclics and the cyclization yield cannot be accurately determined.

Liquid chromatography at the critical point of adsorption is a useful new method for the determination of different types of molecular heterogeneity in polymers. Operating at the transition point of size exclusion and adsorption modes of liquid chromatography, this method is capable of separating polymers according to their functionality. At the critical point of adsorption the polymer chain behaves like an invisible part of the macromolecule and only the heterogeneities (functional groups, different architectures) manifest themselves chromatographically.[7−10] It has been shown in previous investigations that chromatography at the critical point of adsorption is also useful for separating cyclic oligomers from their corresponding linear analogs.[11,12] Therefore, this technique shall be used for the separation of linear and cyclic polystyrenes.

The critical point of adsorption for polystyrene was determined on different stationary phases. In a first set of experiments a polymer-based Eurogel RP-100, consisting of cross-linked polystyrene of an average pore size of 100 Å, were tested. The eluent comprised mixtures of tetrahydrofuran (THF) and *n*-hexane. For the determination of the critical point of adsorption a number of calibration standards of different molar masses must be measured in eluents of different composition. Figure 2 shows the changes in the elution behavior of polystyrene calibration standards as a function of the composition of the eluent. At high concentrations of THF in the eluent (>40% by volume), the retention time decreases as the molar mass of the sample increases. Accordingly, retention corresponds to the size exclusion mode. The adsorption mode is operating at THF concentrations <33% by volume in the eluent, where the retention time of the samples increases with increasing molar mass. At an eluent composition of THF−hexane 34.6:65.4% by volume, the

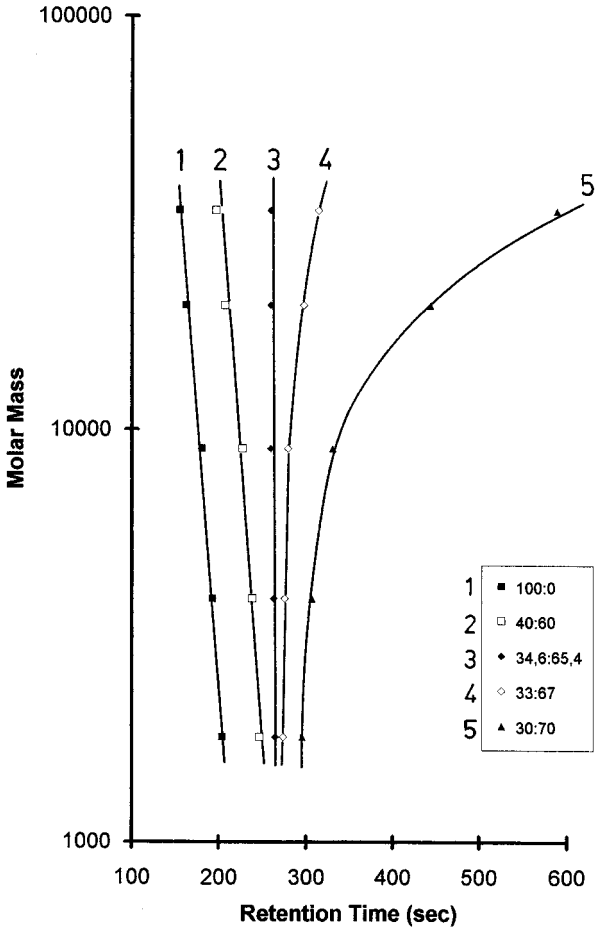


Figure 2. Critical diagram of molar mass vs retention time for polystyrene. Stationary phase: Eurogel P-RP-100. Eluent: THF−hexane.

retention time does not change with molar mass, and at this "critical point of adsorption" separation is accomplished exclusively with respect to the chemical or topological heterogeneity regardless of molar mass.

The chromatographic analysis of the linear precursors (L) and the cyclization products (C) is carried out now under chromatographic conditions, corresponding to the critical point of polystyrene. The critical behavior is active for L as well as for C, as can be seen from Figure 3 for two samples. Both linear samples elute at about 290 s, whereas both cyclics elute about 30 s later at about 325 s. The cyclics exhibit a shoulder at the lower retention time part of the elution peaks, indicating the presence of residual linears. Chromatograms of mixtures of the cyclic and linear samples (mass ratio 3:1 C:L) measured with a RI detector are given in Figure 4 (upper part). These chromatograms show a clear separation into C and L, the ratio of the peak areas corresponds to concentration, indicating that C and L have a similar response in the refractive index detector. A different picture is obtained when a UV detector at a wavelength of 280 nm is used; see Figure 4 (lower part). The peak of the linear component at a retention time of about 270 s is much more intense, than expected from its concentration. Obviously, the extinction coefficient of the linears is much higher due to the vinyl end group. This on the other hand gives the opportunity to determine very small amounts of residual linears in the cyclization products. Unfortunately, the retention time difference between the linear and cyclic fractions of about 30 s is comparable to the width of the individual

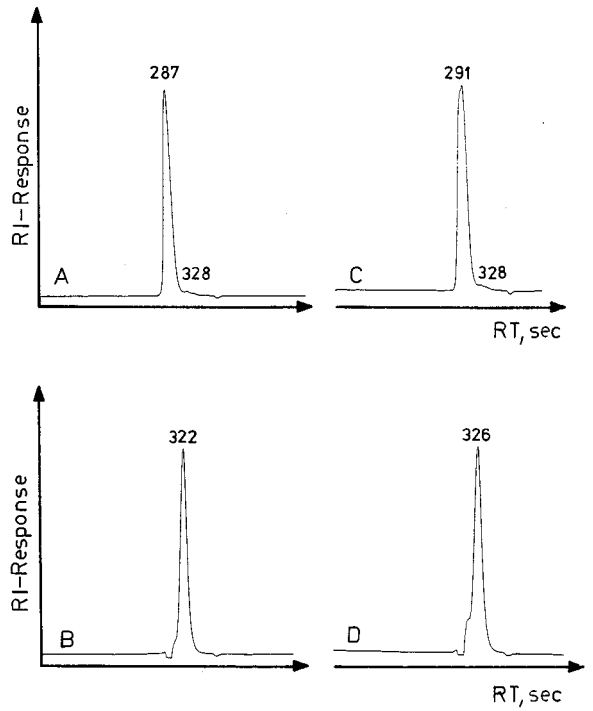


Figure 3. Critical chromatograms of samples PS 3300 L (A), PS 3300 C (B), PS 8000 L (C), and PS 8000 C (D). Stationary phase: Eurogel P-RP-100. Eluent: THF−hexane 34.6:65.4% by volume.

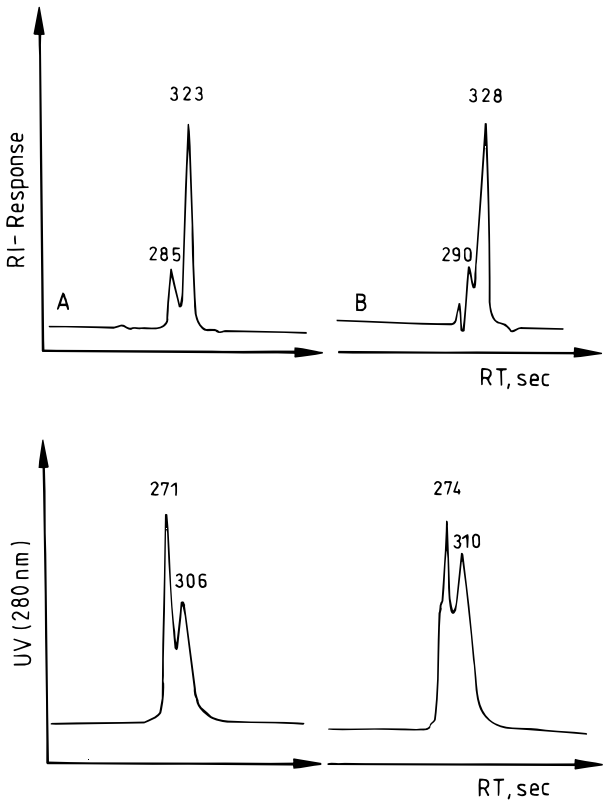


Figure 4. Critical chromatograms of mixtures of PS 3300 L/PS 3300 C (A) and PS 8000 L/PS 8000 C (B) in a mass ratio of 1:3. Chromatographic conditions: see Figure 3. Detector: RI or UV (280 nm).

peaks. Therefore, separation is far from ideal and needs to be improved for proper quantification.

In order to increase the specific interactions of the polar end groups in the linears with the stationary phase, in a second set of experiments a more polar

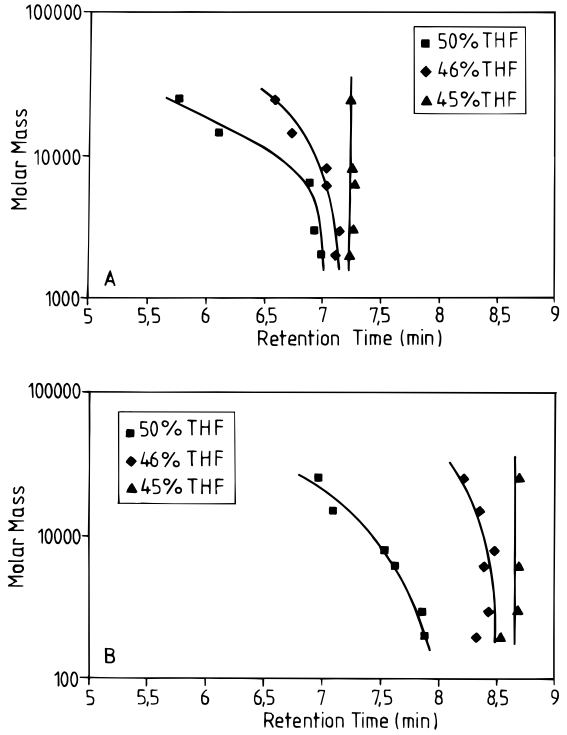


Figure 5. Critical diagrams of molar mass vs retention time for linear (A) and cyclic (B) polystyrene. Stationary phase: YMC silica gel. Eluent: THF−hexane.

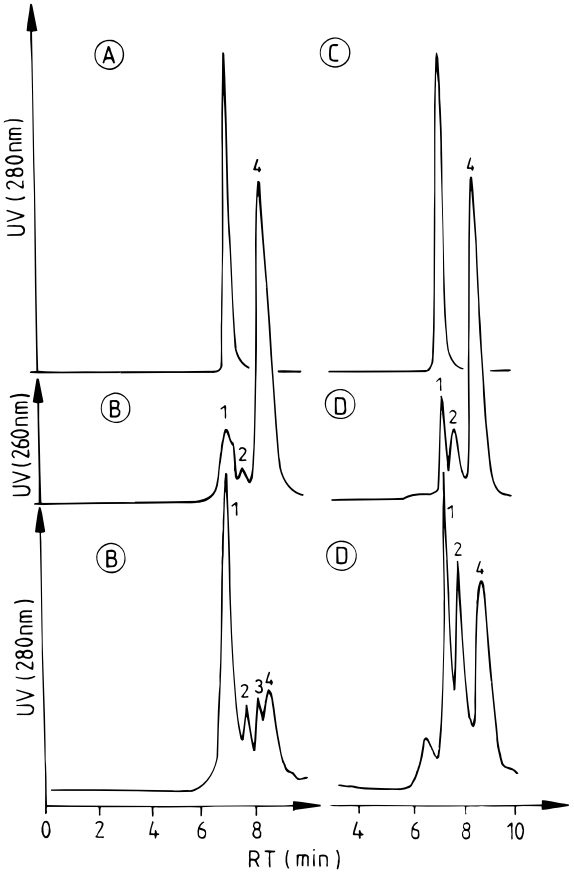


Figure 6. Critical chromatograms of samples PS 2000 L (A), PS 2000 C (B), PS 3300 L (C), and PS 3300 C (D). Stationary phase: YMC silica gel. Eluent: THF−hexane 45:55% by volume.

stationary phase was used. Instead of the nonpolar polymer-based stationary phase, the separations were carried out on bare silica gel of the same average particle and pore sizes. As the eluent, similar to the previous experiments, THF−hexane was used. In addition to the samples of 3300 and 8000 g/mol, used in the first set of experiments, a whole selection of C and L in the molar mass range 2000−25 000 g/mol were prepared. Using these samples, it was possible to determine the critical eluent composition separately for the linears and the cyclics.

The elution behavior of a series of linear precursors and cyclization products is shown in Figure 5A,B. Similar to the previous experiments, a nice transition from the size exclusion separation mode at high THF concentrations to the critical mode at lower THF concentrations in the eluent is observed. As is predicted by the theory, for both linears and cyclics the critical point of adsorption corresponds to an eluent composition of THF−hexane 45:55% by volume. The linears elute at about 435 s compared to 522 s for the cyclics. Accordingly, the retention time difference between L and C is about 90 s, 3 times as much as in the first set of experiments (in both cases the flow rate is 0.5 mL/min). Since the peak width remains at about 30 s, a remarkable increase in selectivity is obtained.

Using these chromatographic conditions, the available cyclization products are investigated; see Figure 6 for two low molar mass samples. The chromatograms in the upper part represent the linear precursors, detected with a UV detector at 280 nm. For both the PS 2000 L and PS 3300 L only one single peak is obtained, indicating the high purity of these samples. The cyclization reaction products are clearly heterogeneous in composition. The chromatograms of PS 2000 C and PS 3300 C resulting from UV detection at 260 nm show one major peak and two or three minor peaks at lower retention times. The UV traces at 260 nm result mainly from the absorption characteristics of the styrene repeat units, and therefore, it can be assumed that the UV response of the linear and cyclic fractions are of the same magnitude. Accordingly, the peak intensities can be assumed to reflect the concentration profile. The most intense peak (peak 4) obviously corresponds to the cyclic polystyrene, whereas the smaller peaks appear in the same retention time range as the linear precursor. In particular, the retention time of peak 1 equals the retention time of the linears. Peaks 2 and 3 are assumed to result from linear oligomers with end groups other than those causing peak 1. These could be formed as a result of an end group modification during the cyclization reaction, leading to nonreactive linears. This interpretation is in agreement with the shape of the chromatograms, recorded at a detector wavelength of 280 nm. At this wavelength oligomers with a vinyl end group, i.e., the linear precursors, exhibit a very high UV response, while linear oligomers without this end group or cyclics exhibit a lower UV response. Accordingly, peak 4 (corresponding to the cyclics) is lower in intensity compared to peak 1, which was assigned to the vinyl-terminated linear precursor. Peaks 2 and 3 also exhibit an increased UV response compared to the cyclics and, therefore must have a vinyl end group. Consequently, the chemical transformation to nonreactive linears must occur at the diethyl acetal end group.

The investigation of the higher molar mass samples reveals that the linear precursors in this case are of much lower purity; see Figure 7. In addition to the expected peak 1 a more or less pronounced second peak appears, having a retention time similar to that of peak 2 in Figure 6. It is assumed that the synthesis of the

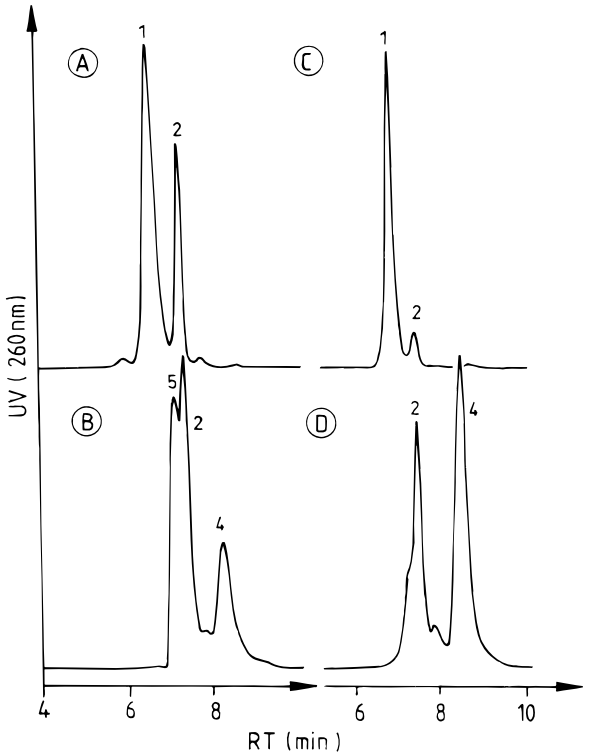


Figure 7. Critical chromatograms of samples PS 25000 L (A), PS 25000 C (B), PS 15000 L (C), and PS 15000 C (D). Stationary phase: YMC silica gel. Eluent: THF−hexane 45:55% by volume.

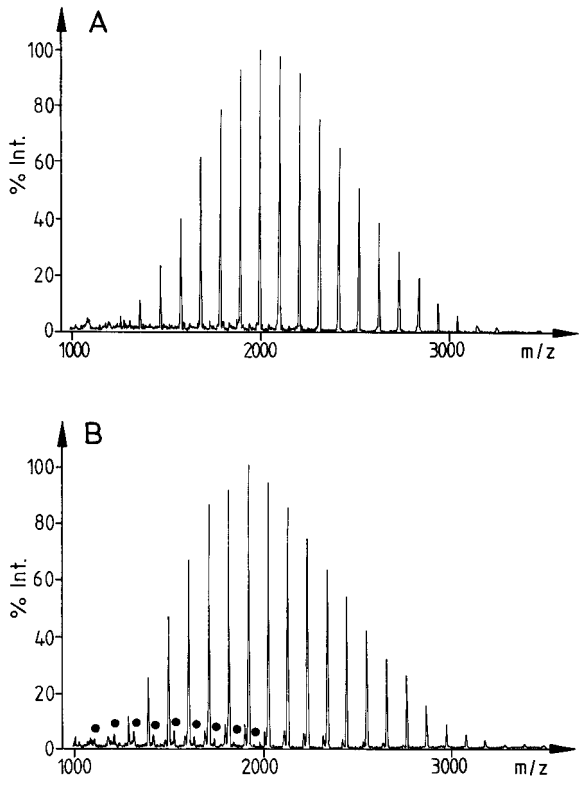
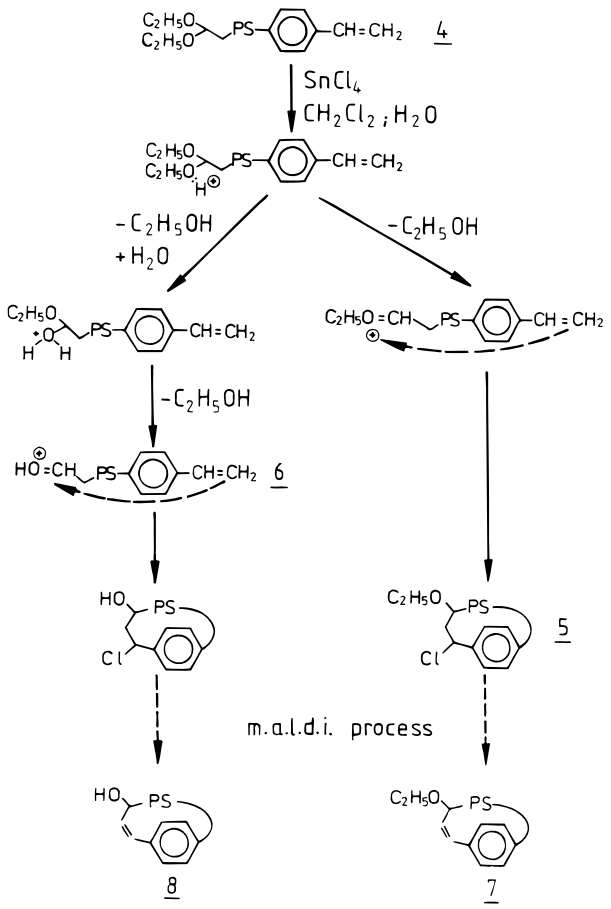


Figure 8. MALDI spectra of samples PS 2000 L (A) and PS 2000 C (B).

higher molar mass linears is accompanied by the formation of a significant amount of nonfunctional species. Accordingly, these nonreactive oligomers are found also in the cyclization products. The relative peak areas of the cyclic polystyrenes (peak 4) suggest that the cyclization yield in this case is rather low. In the case of PS 25000 C only a small peak for the cyclics is

Scheme 2. Possible Cyclization Mechanisms and Related Structures

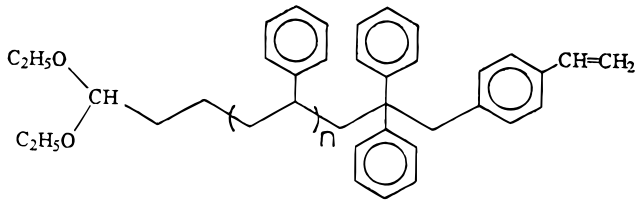


obtained; in addition to the nonreacted oligomers in peak 2, an additional fraction of linears is found at a retention time of 7.1 min (peak 5).

For a first insight into the oligomer distributions and the chemical structure of L and C, the samples PS 2000 L and PS 2000 C were investigated by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). Dithranol was used as the matrix, for cationization of the oligomers silver trifluoroacetate was added to the sample solution. A detailed description of the MALDI experiments will be given in a forthcoming publication.[13] The MALDI spectra of PS 2000 L and C are presented in Figure 8A,B. Each peak in the spectra represents a styrene oligomer, cationized by the attachment of a silver cation ($M + Ag^+$). The peaks are characterized by a mass increment of 104 Da from one peak to the next; this mass increment exactly equals the mass of the repeat unit in polystyrene.

The peaks in the mass range 1000−3500 Da of Figure 8A correspond to one oligomer series. The observed peak masses are in full agreement with the calculated masses for the proposed structure of the linear precursor **4**; see Scheme 1.

The MALDI spectrum of the cyclization product PS 2000 C is shown in Figure 8B, revealing two different oligomer series. Unfortunately, neither of them fits the expected structure **5**; see Scheme 1. Since this structure is assumed to contain a benzylic chlorine atom, it was expected that the spectrum would show peaks for different isotopes (as was the case in other applications). However, an isotopic substructure is not found and it is suspected that during the laser irradiation of the sample in the MALDI process the labile benzylic chlo-

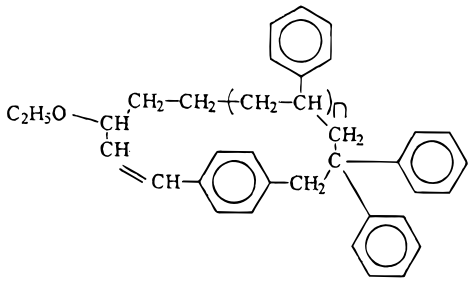


4

$M+Ag^+ = 536 + 104\ n$

n	M (calc.)	M (exp.)
10	1579 g/mol	1580 g/mol
11	1683 g/mol	1684 g/mol
12	1787 g/mol	1788 glmol

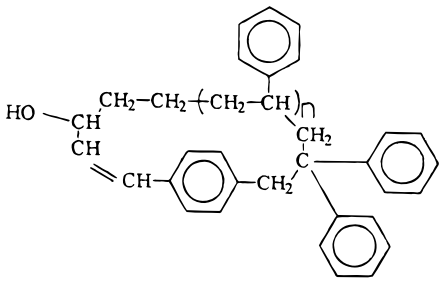
rine is abstracted. Indeed, the calculated masses for chlorine-free macrocycles correspond to the observed masses of the oligomer series **7** (●).



7

M+Ag+ = 491 + 104 n

The major oligomer series has a mass difference of −28 Da toward oligomer series ●. This oligomer series could correspond to the following chemical structure.



8

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

The question is now if **8** is formed in the cyclization reaction or during the MALDI experiment. A possible reaction pathway would be the intermediate formation of an α-formyl-terminated precursor **6** by hydrolysis of **4** during its storage, or more likely, during the cyclization procedure. **6** could then cyclize to form a hydroxy-substituted cyclic structure (Scheme 2).

In order to prove the possibility of a cyclization of **6** to **8**, the following experiment was carried out: An acetal-terminated linear precursor **4** (sample PS 2200-(a), molar mass 2200 g/mol) is transformed into the corresponding formyl-terminated precursor **6** (sample

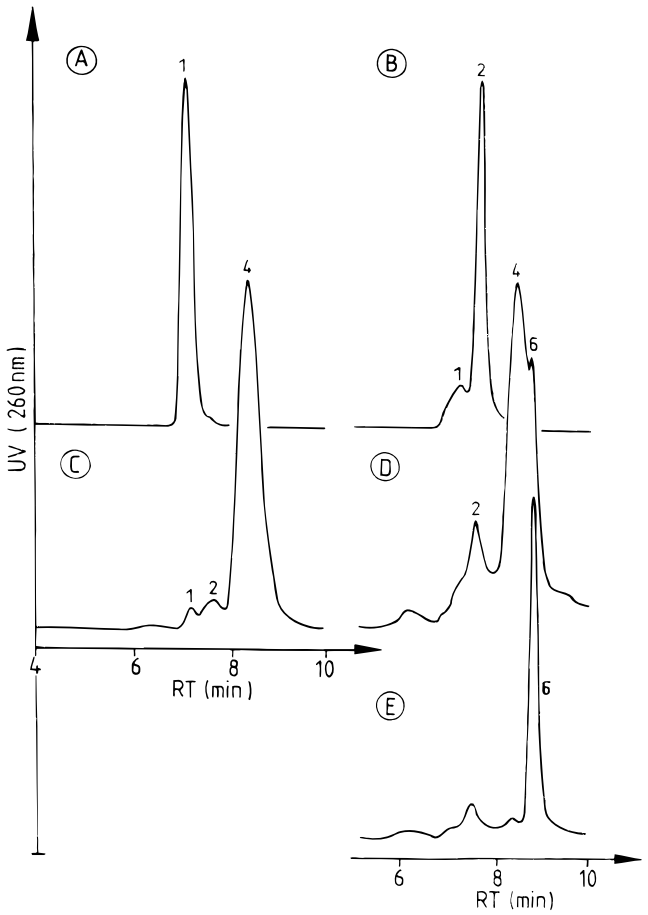


Figure 9. Critical chromatograms of samples PS 2200(a) (A), PS 2200(b) (B), PS 2200(c) (C), and PS 2200(d) (D). Chromatographic conditions: see Figure 6. Detector wavelength: 260 nm (A−D) or 280 nm (E).

PS 2000(b)) by treatment on acidic resin in order to derivatize the acetal into the aldehyde group. In separate reactions **4** and **6** were cyclized and the resulting reaction products were investigated by liquid chromatography and MALDI-MS. The chromatograms, given in Figure 9A−E, show that the deacetalization of **4** results in the formation of **6** in high yield (peak 2 in Figure 9B); residual nonreacted **4** is detected as well (peak 1 in Figure 9B). After the cyclization of **4** and **6**, the reaction products give very similar chromatograms (samples PS 2200(c) and PS 2200(d)). In particular, the major fraction elutes at the same retention time (peak 4 in Figure 9C,D); small amounts of linear precursors **4** and **6** (peaks 1 and 2, respectively) are also present in the reaction mixture, as was shown previously. The significant difference between the cyclization products of **4** and **6** is that the cyclization of **6** results in the formation of an additional product, which appears as peak 6 in Figure 9D. The UV response of the product at 280 nm is very high (see Figure 9E) and can, therefore, be attributed to either a nonfunctional linear fraction or a macrocyclic fraction with increased UV activity, possibly of structure **7** or **8**. The MALDI spectra fully support the results of the chromatographic separations.[13] The spectra of the cyclization products are very similar and reveal peak series of **7** and **8**, similar to Figure 8B.

To summarize, the chromatographic investigations and the MALDI experiments are in close agreement and suggest the reaction mechanism in Scheme 2. For the determination of the cyclization yield, the amounts of the residual precursor and the cyclic fraction must be

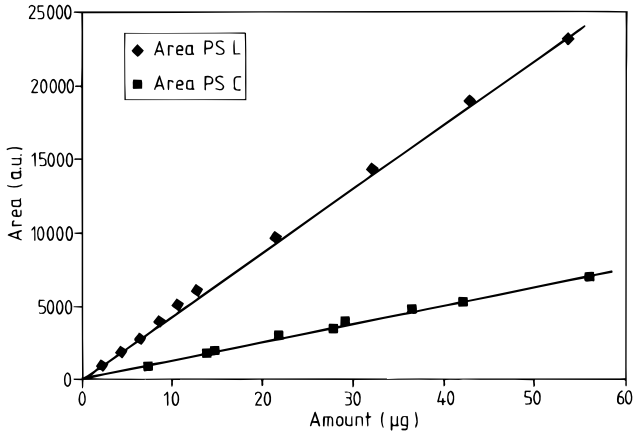


Figure 10. Calibration curves of peak area vs amount for linear (PS L) and cyclic polystyrene (PS C) at a UV detector wavelength of 260 nm.

determined from the chromatograms. Since the UV responses of the linears and the cyclics are quite different for different detector wavelengths, concentration is not directly related to the area of the peaks. An internal calibration with samples of L and C is carried out at a detector wavelength of 260 nm. The corresponding calibration curves are shown in Figure 10. Using these calibration curves, the relative amounts of linear and cyclic species can be determined. Referring to Figures 6 and 7, peak 1 represents the residual acetal-terminated linears **4**, whereas peak 2 is assumed to represent linears **6** and other nonfunctional linears. The response factors of peaks 1 and 2 are assumed to be similar. Peak 4 represents the cyclic reaction product and is quantified using the corresponding calibration curve.

The relative amounts of the different species in the cyclization products are summarized in Table 2. In agreement with previous findings,[4−6] the cyclization yield is 90−95%. However, the higher molar mass samples 7C and 8C exhibit a cyclization yield of only 80 or 57%, respectively.

Table 2. Quantitative Composition of the Cyclization Products, in % by Weight

sample	peak 1 $(EtO)_2CH-CH=CH_2$	peak 2 $OCH-CH=CH_2$	peak 4 cyclics
PS 1900 C	2.2	4.6	93.2
PS 2000 C	6.5	1.5	92.0
PS 2200 C	6.7	1.4	91.9
PS 3000 C	4.2	4.7	91.0
PS 3300 C	3.2	1.9	94.9
PS 6250 C	9.7		90.3
PS 8000 C	5.6	3.7	90.6
PS 15000 C	16.4	2.7	80.9
PS 25000 C	40.5	1.9	57.6

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