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Toward a More Accurate Structural Determination of High Molecular Weight Polyrotaxanes Based on Cyclodextrins by MALDI−TOF MS

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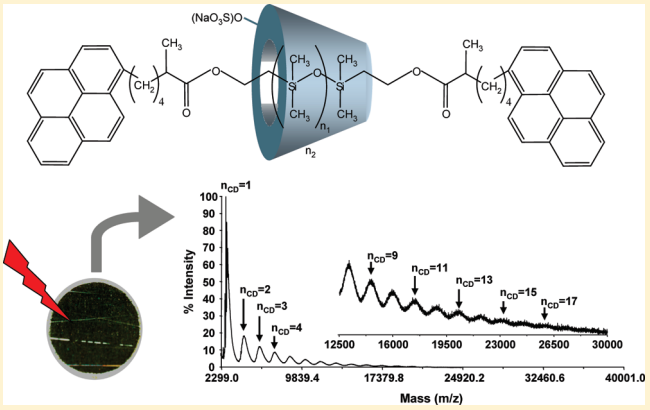
Supporting Information

ABSTRACT: High molecular weight polyrotaxanes are usually characterized by nuclear magnetic resonance and size exclusion chromatography but rarely by mass spectrometry. This article reports effort to detect high molecular weight structures of cyclodextrin based polyrotaxanes (CD based PRs) by matrix-assisted laser desorption ionization mass spectrometry (MALDI−TOF MS). A particular attention was paid to verify the composition of the polyrotaxanes by determination of the number of macrocycles borne by the polyrotaxane and the polyrotaxanes synthetic route. Various matrices including crystalline or ionic liquid ones were screened to optimize the detection. Dimethylformamide as solvent widely used in supramolecular chemistry, but unusual in mass spectrometry, was successfully employed during all analyses. To prove the ability of the optimized MALDI−TOF MS conditions, three different polyrotaxane structures varying by polymer nature, i.e., poly(ethylene oxide) or polydimethylsiloxane and cyclodextrins type i.e. α or γ with or without modifications were evaluated. Finally, we have concluded that 1,1,3,3-tetramethylguanidinium salts of both 2-(4-hydroxyphenylazo)benzoic acid and 2′,4′,6′-trihydroxyacetophenone, are the most reliable and efficient matrices. These ones afforded the possibility to detect masses of 24804 and 26447 g·mol^{-1}, corresponding to poly[17]- and poly[18]rotaxanes, respectively.



INTRODUCTION

Pseudopolyrotaxanes (PPRs) and polyrotaxanes (PRs) are physical complexes of cyclic molecules threaded on a polymer chain.[1−4] Contrary to PPRs, PRs are end-capped at both ends with bulky groups to prevent dethreading. Basically, purification/separation steps of PPRs/PRs were performed by size exclusion chromatography (SEC) and next suitable analytical techniques[5−17] were used to provide consistent data. Among the various employed techniques, the most popular are $^{1}H/^{13}C$ NMR,[5−13] mass spectrometry (MS),[6,9,11,14] and also UV/fluorescence spectroscopies,[6,7,9,11,15] which do not give information on the fine structure, i.e., exact composition of high molecular weight part of the sample. Nevertheless, in supramolecular chemistry, MS is often dramatically underestimated, presumably due to the difficulty of transferring intact self-assembled architectures from solution to gas phase.[16] Since their introduction, the two soft ionization methods, electrospray (ESI)[18,19] and matrix-assisted laser desorption/ionization (MALDI) in mass spectrometry[20,21] has been an enormous impact on the analysis of biopolymers but also synthetic polymers.[22,23] The majority of PRs exhibits masses below ≈4500 g·mol^{-1} and as regards to rather low instrumentation cost, are easily amenable to ESI, resulting in its preferential use.[6,13,16] Nevertheless, ESI generates multicharged species which greatly complicate the interpretation of spectra, particularly when complex mixtures and/or high masses are analyzed. Indeed, MALDI hyphenated to a time-of-flight analyzer (MALDI−TOF) has become the technique of choice for polymer analysis because of the simplicity of the mass spectra which show mainly single-charged ions and a theoretically unlimited mass range. However, polymer analysis such as polydimethylsiloxane (PDMS)[24,25] and others was often complicated by the coexistence of several distributions, and the difficulty to select an effective analyte(s)/matrix(ces)/solvent(s) combination. Several factors in sample preparation have been proved to affect MALDI results,[26−29] and after optimization of instrumental parameters, the choice of the matrix(ces) and solvent(s) remains the most delicate task for a successful detection. Since their introduction, cyclodextrins (CDs) are undoubtedly among the most used host during PRs synthesis.[30−32] The most common reported matrices for analyzing CD based PRs in

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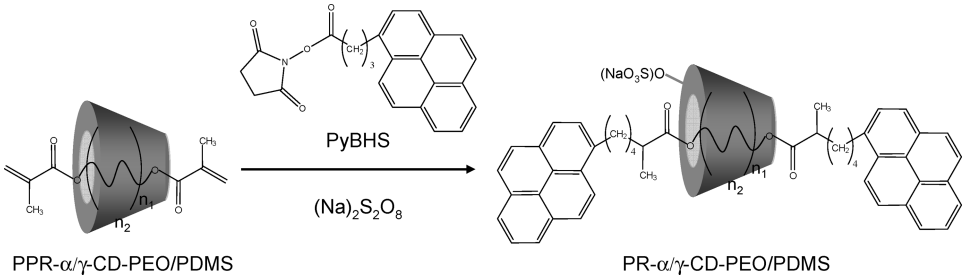
Scheme 1. General Process Used for the Synthesis of the Polyrotaxanes of This Study[a]



[a] n_1 is the degree of polymerization of the polymer and n_2 is the number of threaded cyclodextrins.

MALDI are α-cyano-4-hydroxycinnamic acid,[12] 2,5-dihydroxybenzoic acid,[15] and sinapinic acid.[5] *trans*-3-Indoleacrylic acid,[17] 2′,4′,6′-trihydroxyacetophenone,[9] 2,5-dihydroxybenzoic acid,[10] and dithranol[17] were also successfully applied to detection of PPRs/PRs without CD. Nevertheless, it is well-known that these solid matrices rely on cocrystallization with analyte(s), leading to heterogeneity which results in poor shot to shot reproducibility. In consequence, detection limits can be decreased and signal corresponding to high masses lost. Additionally, the use of competing media such as protic matrices or solvent can break weak bonds resulting in degradation of complexes.

The recently introduced ionic liquid matrices (ILMs)[33] have demonstrated their potentiality as soft matrices. These new compounds are salts composed of crystalline matrices mixed with organic bases.[34] Ionic liquid matrices generate a tremendous interest because they allow an easier dissolution of the analyte, a homogeneous spotting of the matrix−analyte mixture and a high vacuum resistance. These matrices were particularly useful to avoid, or at least decrease, labile group losses and have been successfully applied to peptides,[35−38] proteins,[33,39−41] (poly)-saccharides,[35,39−43] DNA oligomers,[44] or also (phospho)lipids.[35,37]

To the best of our knowledge, few papers state of the use of ILMs in the synthetic polymer analysis by MALDI MS, mainly concerning PEG,[33,35] or biodegradable compounds[45] and as an evidence none study has been reported in PRs analysis. Up to now, there are only few reports in the literature regarding the successful detection of high molecular weight (e.g., >4000 $g \cdot mol^{-1}$) CD based PRs by MALDI−TOF.[5,9,11,12,15] Therefore, we have attempted to partially fill the gap. In the study herein, we report effort to detect high molecular weight CD based PRs using MALDI−TOF MS. A particular attention has been paid to obtain information about the architectures, such as number of cyclodextrins threaded and thereby eventual modifications, but also to verify the polyrotaxane synthetic route.

EXPERIMENTAL SECTION

Materials. 2-(4-hydroxyphenylazo)benzoic acid (HABA), α-cyano-4-hydroxycinnamic acid (CHCA), sinapinic acid (SA), 2′,4′,6′-trihydroxyacetophenone (THAP), 2,5-dihydroxybenzoic acid (DHB), dithranol (DIT), *trans*-3-indoleacrylic acid (IAA), *all-trans*-retinoic acid (RA), butylamine (BUT), aniline (AN), 1,1,3,3-tetramethylguanidine (TMG), *N*,*N*-diisopropylethylamine (DEA), trimethylsilylimidazole (TMSI), sodium persulfate ($(Na_2)S_2O_8$), and 1-pyrene butyric acid *N*-hydroxy succinimide ester (PyBHS) were purchased from Sigma-Aldrich Co. (Saint Quentin Fallavier, France). Dimethylformamide (DMF), diethyl oxide (Et_2O), and chloroform ($CHCl_3$) was provided from Carlo Erba-SDS (Val de Reuil, France). α,ω-dimethacrylate poly(ethylene oxide) ($\overline{M_n}$=1000 $g \cdot mol^{-1}$, I_p = 1.1, $\bar{f}$ = 2) was purchased from Polysciences and its purity was controlled by NMR, SEC, ESI MS and MALDI−TOF MS. α-cyclodextrin was kindly supplied by Wacker. γ-cyclodextrin and α,ω-dimethacrylate polydimethylsiloxane were supplied by Menicon society (Japan).

Synthesis of the Cyclodextrin-Based Polyrotaxanes. PR-αCD-PEO and PR-αCDSi-PEO are synthesized as described in a precedent paper.[46] PR-γCD-PDMS synthetic route will be detailed in a subsequent paper.[47] The synthesis of polyrotaxanes (PRs) from polypseudorotaxanes (PPRs) via radical coupling was performed according to a general process summarized in Scheme 1. Structures of the three studied polyrotaxanes are reported in Figure 1.

Preparation of the Crystalline and Ionic Liquid Matrices. All solid matrices were made at 20 mg/mL in DMF, which is among the most employed solvents used during synthesis of supramolecular structure. Ionic liquid matrices used in this study were prepared as described elsewhere.[42] Briefly, preparation of ILMs was made as follows: all matrices, except IAA and RA, were mixed with either BUT, AN, or TMG at a molar ratio of 1:2 in methanol. Other ILMs were made by mixing an equimolar amount of solid matrix and amino compound in methanol. The solution was then sonicated for 15 min at 40 °C. After removal of methanol by centrifugal evaporation in a SpeedVac for 3 h at room temperature, ILMs were left in vacuum overnight. Final solutions were then prepared at a concentration of 90 mg/mL in DMF for use as a matrix. Once prepared, DMF solutions of ILMs could be stored at 4 °C up to 1 week and were used without further purification. The exact composition and molecular weights of the polyrotaxanes are not known and data obtained by SEC and NMR were not accurate. Consequently, molar solutions could not be prepared.

Preparation of the Sample. Extract issues from synthesis were available in low amounts, e.g., between 0.76 and 1.4 mg and were next solubilized in DMF at a final concentration of 76 mg/mL. Samples for MALDI-MS analysis concentration were prepared by mixing 0.5 to 1 μL of sample and an identical volume of ionic liquid matrix. Sequential dilutions of samples were directly achieved in matrix from 1:1 to 1:80 (v:v analyte:matrix) to find the optimal analyte to matrix ratio. Then, 1 μL of the mixture was deposited on a mirror polished stainless steel MALDI target and allowed to dry at room temperature and atmospheric pressure during five and 20 minutes for ILMs and solid matrices, respectively. According to sample dilution, amounts of purified extracts from 34 to 0.48 μg per spot were analyzed.

Mass Spectrometry. MALDI−TOF MS experiments were performed using a Perseptive Biosystems Voyager-DE Pro STR MALDI−TOF mass spectrometer (Applied Biosystems/MDS Sciex, Foster City, CA). This instrument was equipped with a nitrogen UV laser (λ = 337 nm) pulsed at a 20 Hz frequency. The mass spectrometer was operated in both the positive and negative ion reflector mode with an accelerating potential of ±20 kV and a grid percentage equal to 70%.

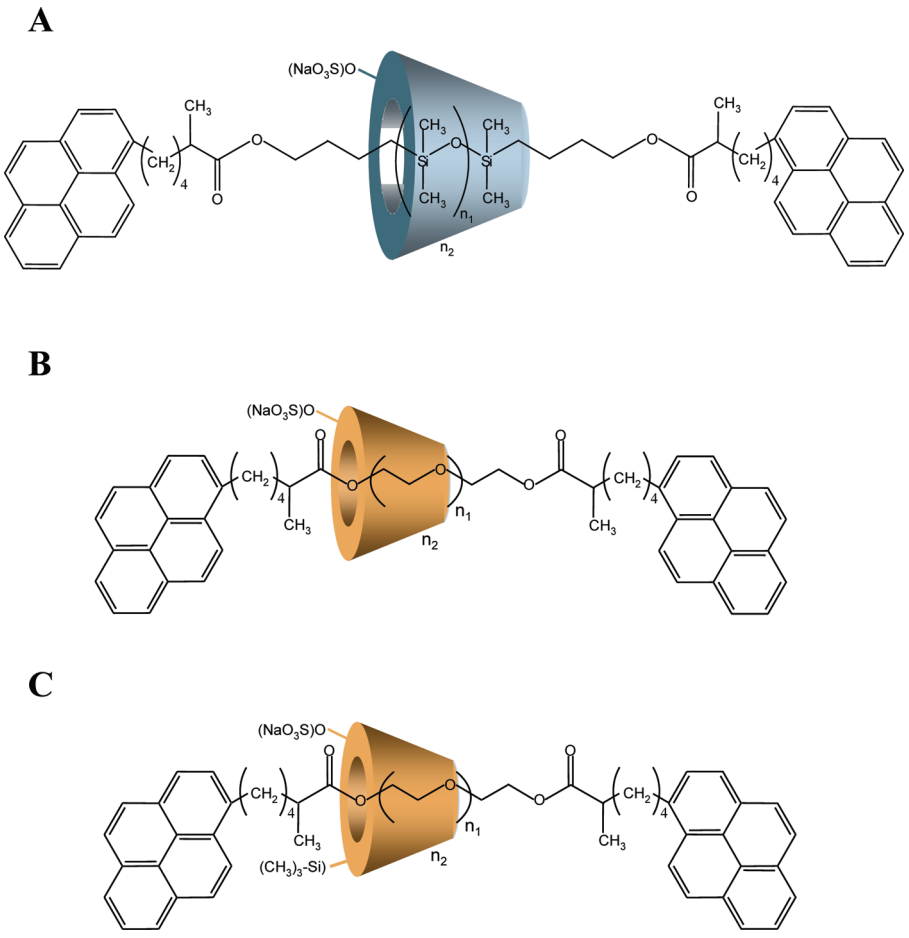


Figure 1. Structures of the cyclodextrin based polyrotaxanes used in this study. Sulfated γ-cyclodextrin with α,ω-dimethacrylate polydimethylsiloxane functionalized with dipyrenyl derivatives (PR- γCD-PDMS) (A), α,ω dipyrenyl poly(ethylene oxide) (PR-αCD-PEO) (B), partially silylated α-cyclodextrin with α,ω-dipyrenyl poly(ethylene oxide) (PR-αCDSi-PEO) (C). n_1 is the degree of polymerization of the polymer and n_2 is the number of cyclodextrins threaded.

Mass spectra were recorded with the laser intensity set just above the ionization threshold (2800 in arbitrary units, on our instrument) to avoid fragmentation and labile group losses, to maximize the resolution (pulse width 3 ns) and to result in a strong analyte signal with minimal matrix interference. Time delay between laser pulse and ion extraction was set to 450 ns. A set of parameters in the linear mode was also tested consisting of an accelerating potential of $\pm$25 kV, a grid percentage of 93%, an extraction delay of 800 ns and a laser power adjusted to 3200 in arbitrary units. Typically, mass spectra were obtained by accumulation of 200 and 800−1000 laser shots in reflector and linear mode, respectively; and processed using Data Explorer 4.0 software (Applied Biosystems).

RESULTS AND DISCUSSION

1. Assessment of the Host Composition. The first synthetic step consisted in transforming the as-received, unmodified γCDs into a modified γCDs by adding a sodium sulfate group. We recorded the MALDI spectra of the reaction products, but we do not report them. In fact, the cited host is used to produce PRs and the purification does not have 100% efficiency and thus, free CDs are present in the sample. Figure 2 reports the MALDI spectra of the PRs. Signals due to PRs are systematically absent. All peaks are due to free CDs. From experience of our laboratory, CHCA was used as matrix and an analyte to matrix ratio equal to 1:1 (v:v) was tested. As regard to the sample studied, it was unrealistic to determine an analyte to matrix molar ratio, since extracts contained a mixture of supramolecular complexes but also a minor presence of free CDs in the sample according to the SEC purification and characterization. Thus, only matrix to analyte ratio with weight or volume data can be considerated rigorously here, to evaluate the efficiency of the excess of matrix on the quality of spectra. These residual CDs were probably coeluted with the PR-γCD-PDMS as described elsewhere for other guests with PRs.[17]

Identification of free modified CDs showed a snapshot of the structure of CDs used during the PRs synthesis. Indeed, in positive ionization mode γCDs were mainly detected under sodiated forms at m/z 1319.41, m/z 1421.35 and in minor occurrence at m/z 1523.23 and m/z 1625.13 corresponding to the unmodified, monosulfated, disulfated and trisulfated forms, respectively (Figure 2A). The mass increment equal to 101.9 mass units was presumably due to the reactivity of CDs hydroxyl groups with sodium persulfate during radical synthesis yielding to a SO_3Na attachment. Analysis in negative detection mode exhibited a very good response, confirming presence of γCDs with 1, 2, or 3 sulfates under desodiated form with peaks at m/z 1375.37, 1477.30, and 1579.21, respectively (Figure 2B).

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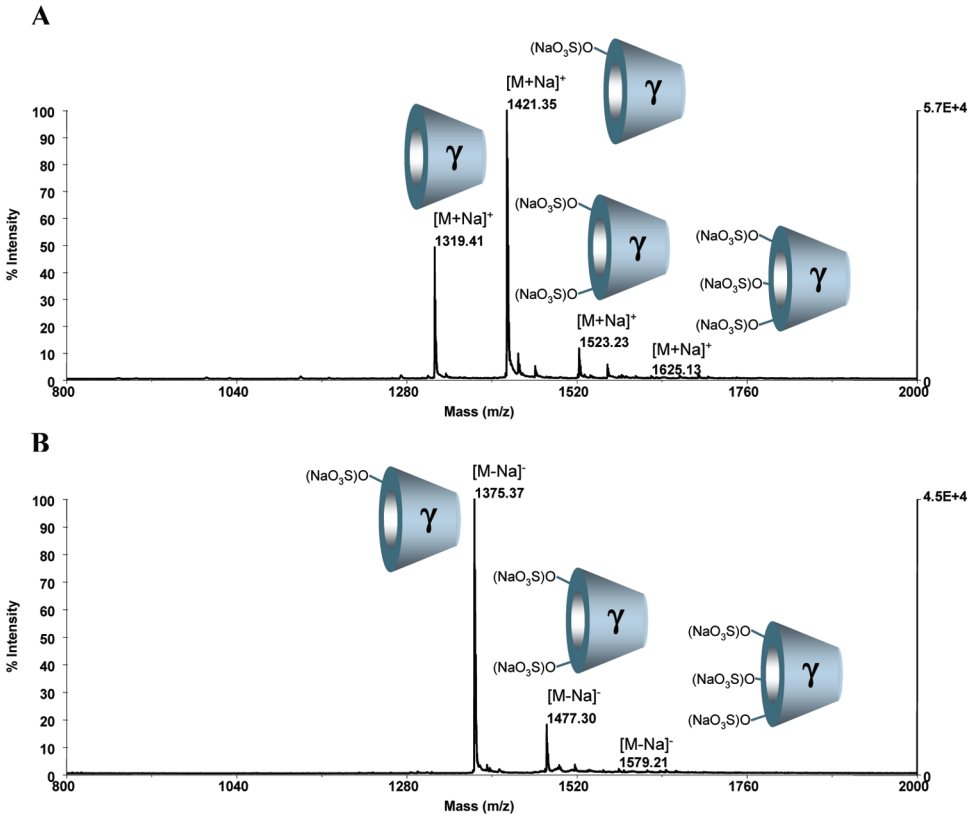

Figure 2. Positive (A) and negative (B) ion reflector MALDI−TOF mass spectra of sulfated γCDSO$_3$ sample with CHCA at 20 mg/mL in DMF at 1:1 (sample:matrix v:v).

2. Characterization of Polyrotaxanes with Standard Crystalline Matrices. Eight crystalline matrices were evaluated, five usually used for biomolecules analysis, e.g., SA, CHCA, DHB, HABA, and THAP, and three frequently employed during synthetic polymer studies, e.g., DIT, IAA, and RA. When 0.5 μL of matrix was mixed with an equal volume of sample, the resulting analyte to matrix ratio was estimated to be 4:1 with ≈38 μg and 10 μg of material, respectively. Taking into account that native free γCDs own the lowest mass which can be observed in the sample with 1297.12 g·mol^{-1}, and RA the highest mass of the tested matrices with 300.43 g·mol^{-1}, the minimum analyte to matrix molar ratio was about 1:1. Moreover, considering that high molecular weight PRs were presumably major components of the sample as confirmed by SEC (data not shown), the molar analyte to matrix ratio was expected to be largely >1. The building block of the modified PDMS was constituted of propylpyrene grafted on methacrylate at both extremities. Indeed, considering two siloxane units, the theoretical average mass of the resulting structure is equal to 875.2912 g·mol^{-1} (Figure 1A). The increase of the degree of polymerization ($D\overline{P}_n$) is attempted to be characterized by sequential mass increments of about 74.15 mass units corresponding to a siloxane as repetitive unit. The results obtained for PR-γCD-PDMS with the crystalline matrices at analyte to matrix ratio of ≈1:1 and matrix excess of 20, 40, and 80 fold are summarized in Table S1 in the Supporting Information.

At the light of the results, no signal corresponding to analytes was recorded for PR-γCD-PDMS in either positive or negative mode when DIT or RA were used, even in large excess (data not shown). The matrices SA, CHCA, and HABA did not provide reliable signals corresponding to poly[3]rotaxanes or higher in both negative and positive modes, through the dilution range studied. DHB led to detection of poly[9]rotaxanes both in negative and positive mode. Absence of satisfying results with usually used matrices for polymer analysis could reveal the importance of CDs in the successful detection of PRs. Results obtained with THAP were slightly better than with DHB. THAP contains weakly acidic hydroxyl functions and is therefore preferentially used for acid/labile molecules.[48] Indeed, differences mainly hold in its efficiency to improve signal intensity in both detection modes of structures exhibiting additional 1 or 2 CDs. IAA provided the most reliable signal allowing to detect ions at m/z 18950 and m/z 20361 in positive and negative detection modes, respectively. Theses masses were attributed to poly[13]- and poly[14]rotaxanes (Figure 3, parts A and B and Table S1 in the Supporting Information).

These impressive results were obtained with an analyte to matrix ratio of 1:80. Further excess of matrix (1:160 v:v) did not significantly improve the quality and the fullness of information on the resulting spectrum (data not shown). pK_a represents the solution phase acidity of the protonated matrix, and could be an explanation of its effectiveness. Except for RA and DIT with pK_a of 4.79 and 7.15,[49] IAA and THAP have the highest value equal to 4.59 and 7.76,[49] respectively. These values reflect a weakly acidic and slightly basic nature as compare to the 1.17−3.57 range encountered for all other studied matrices.[49]

With regards to most matrices, no general rule concerning the effect of sample dilution in matrices can be pointed out. Interestingly, differences in efficiency were noteworthy between matrices for a given analyte to matrix molar ratio, and also

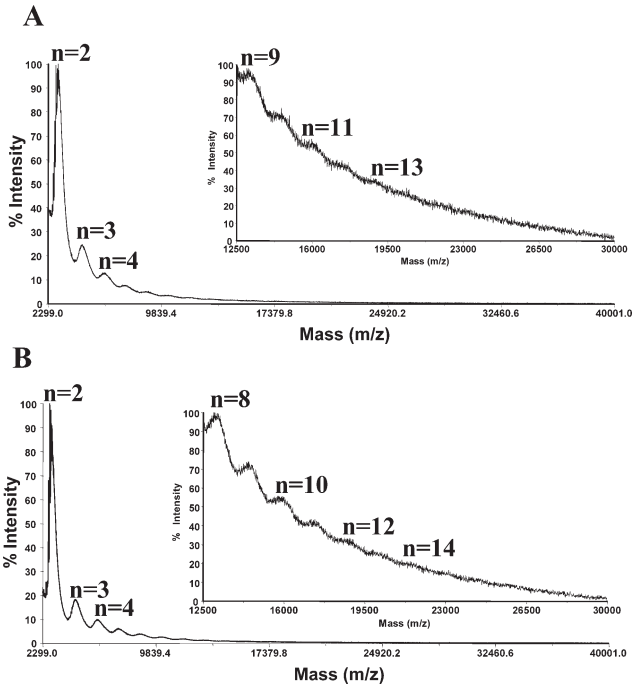

Figure 3. Positive (A) and negative (B) ion linear MALDI−TOF mass spectra of PR-γCD-PDMS polyrotaxane sample with IAA at 20 mg/mL in DMF. The sample was analyzed at 1:80 (sample:matrix v:v). Number *n* is the sum of the numbers of CDs and polymer chain in the polyrotaxane structure. Insets show the envelops in higher mass range.

between analyte to matrix ratio for a given matrix (see Table S1 and Figures S1 and S2 in Supporting Information). According to the sulfo groups grafted on CDs, the detection was more sensitive in negative than in positive mode. Envelops were readily observed in negative mode, presumably because this mode acted as a noise filter. Moreover, sulfation supports the solubility in polar solvents such as DMF. As polymers and CDs were mainly detected under sodiated form, it was presumed that CD based PRs were preferentially present with the same adduct. Consequently to the dilution effect, a better embedment of analyte by the increase of matrix molecules yielded to an improvement of signal intensity and to higher m/z detected. The relative enhancement of spot homogeneity may be at the origin of the diminished suppression effect of lower masses toward higher ones. In the most diluted samples, matrix ions are abundant, but analytes being diluted, a lower discrimination effect occurred toward high masses. As a general rule, the signal intensities of poly[n+1]rotaxanes were frequently the half of poly-[n]rotaxanes, except for poly[2]rotaxanes to poly[3]rotaxanes where we remarked a signal reduction by a factor of about 4. Presumably, these differences were a direct consequence of the mass discrimination effect toward the lower masses widely observed during MALDI analysis. This effect could be also accentuated by a lower desorption of high molecular weight PRs bearing a higher number of CDs, and which becomes more compact. This fact impaired the cation attachment and then leads to less efficient ionization processes.

Some criteria were used to evaluate the effectiveness of the tested matrix compounds: (i) the detection of signal corresponding to a PR structure (ii) the highest mass detectable and its corresponding intensity (iii) the relative resolution between the different mass distribution (iv) the range of sample dilutions which can be detected. Finally, according to the aforementioned criteria, matrices can be sorted as a function of their effectiveness in the following order: IAA > THAP > DHB > SA ≫ CHCA ≫ HABA ≫ RA, DIT. MALDI matrices are classified as "hot" or "cold" when their proton affinity (PA) is low or high, respectively. Basically, cold matrices lead to the best results. In this study, there seems to be no clear correlation between PA and the analyte signal produced. Indeed, reported values of PA were 893.88, 893.04, 855.78, 875.88, 841.55, 949.98, and 885.51 kJ·mol^{-1} for IAA, THAP, DHB, SA, CHCA, HABA, and DIT, respectively.[50] It was therefore concluded that a systematic study of matrix effect is useful, because the choice of the matrix plays a paramount role in a successful detection of PRs.

3. Estimation of Mass Distribution. Traditionally, polymer materials are analyzed by different techniques to access to the molecular weight information such as weight-average molecular weight ($\overline{M_w}$) and the number-average molecular weight ($\overline{M_n}$). In our samples which contain many PR populations, both NMR and SEC analysis did not permit to obtain accurate data. Because of these limitations, MALDI−TOF MS appears as a valuable solution to a more accurate determination of $\overline{M_w}$ and $\overline{M_n}$ values for a given polydispersity.

With regard to the polymer polydispersity and the variable modification of the CDs (1 to 3 sulfo groups), the low resolution of the linear mode did not permit to observe multiple species constituting a given distribution. In addition, distributions were complicated by the existence, even at a minor state, of multiple recombinations of radicals to give PRs which resulted from radical synthetic routes.[46] Taking into account that a given CD was unimolecular with mainly one sulfate group, the number of different mass distributions was certainly due to an increased number of threaded γCDs. Average masses were matched with theoretical structures considering the number of polymer units (n_1; Figure 1A) and the number of monosulfated γCDs (n_2; Figure 1A). In addition, the measured mass difference between the beginning and the end of a given distribution allowed to estimate the corresponding $\overline{DP_n}$ range. To summarize, the calculated mass $m(n)$ of a n given peak from the molecular weight distribution is a linear function of both number of polymer repeated units n_1 and the number of monosulfated γCDs n_2

$$m(n) = m_{dimer} + n_1 \times 74.15 + n_2 \times 1399.1708 + / - m_{cation} \quad (1)$$

where m_{dimer} is the mass of the α,ω-dipyrenyl polymer already including 2 siloxane units and m_{cation} is the mass of the alkali cation (generally 22.98 for Na^+) and the corresponding sign depends on the detection mode used, e.g., negative or positive. For example, the second distribution detected in negative detection mode, with IAA used as matrix and a 1:80 analyte to matrix ratio (Figure 4) afforded deciphering of some structural information such as (i) the number of γCDs threaded corresponding to the number of distribution, here 2 (ii) according to eq 1, the peak identified at m/z 4911.28 in Figure 4 was associated with a desodiated form $[M - Na]^-$ with m_{dimer} = 875.2912 Da, n_1 = 16, and n_2 = 2. For instance, the peak identified at m/z 4883.28 corresponding to the deducted weight-average molecular weight ($\overline{M_w}$) was assigned to a 19-mer with 2 γCDSO$_3$ threaded along the polymer. This mass was in good agreement with the calculated value of 4911.21 g·mol^{-1}. (iii) From eq 1, the peak

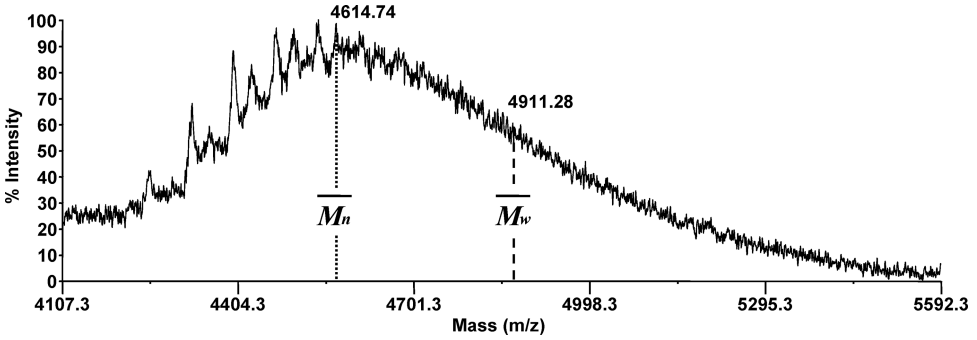

Figure 4. Negative ion linear MALDI−TOF mass spectrum of the second distribution observed during analysis of PR-γCD-PDMS sample with IAA at 20 mg/mL in DMF. The sample was analyzed at 1:80 ratio (sample:matrix v:v). $\overline{M}_n$ and $\overline{M}_w$ were the number-average molecular weight and the weight-average molecular weight, respectively.

identified at m/z 4614.74 was attributed to the number-average molecular weight ($\overline{M}_n$) with 2 γCDSO$_3$ threaded on a polymeric backbone constituted of 14 siloxane units. This mass matched very well with the theoretical value of 4614.64 g·mol^{-1}. (iv) The $\overline{DP}_n$ range from m/z 4107.3 to m/z 5592.3, corresponding to a net mass difference of 1485, which after divided by 74.15 led to about 20. These last data highlighted that detected PRs of the second distribution contained 9 to 29 siloxane units.

Obtained masses were consistent with the maximum number of γCDs which can be threaded on a PDMS chain, e.g., two macrocyles per three siloxane units.[3] Unfortunately, spectra acquired in solid matrices, have been obtained with a time-consuming procedure, requiring a whole spot interrogation. Furthermore, a very low resolution and low signal-to-noise ratios (S/N) were observed between each distributions for m/z higher than m/z 9000, even when using the matrix IAA. Interestingly, taking into account the low resolution, the highest detected masses obtained with IAA and other matrices exhibited rather good mass accuracy, reaching 2−14 Da and 11−35 Da in positive and negative mode, respectively. Nevertheless, doping with salts such as NaCl, KCl, or LiCl, well-known to promote cation attachment in polymer analysis, induced signal extinction in the 1−50 mM range (data not shown).

4. Use of Ionic Liquid Matrices To Improve Detection of High Molecular Weight Polyrotaxanes. Ionic liquid matrices (ILMs) are ideally suited to overcome drawbacks of crystalline matrices, because no acid is added and no crystallization is needed. To improve resolution between the distributions, S/N and then access to higher m/z than in solid matrices, 24 ILMs were tested. A great number of ILMs can theoretically be made, by varying both the solid matrices and basic counterions. Nevertheless, our study was restricted to the use of the six solid matrices which generated a signal in the previous section. These matrices were associated with four different amino compounds already used in other studies: an aromatic amine (aniline; AN), an alkyl amine (butylamine, BUT), a secondary amine (1,1,3,3-tetramethylguanidine, TMG) and a tertiary amine (*N*,*N*-diisopropylethylamine; DEA). According to the available proton site of a given matrix, either 1 or 2 equiv(s) of amine were added to constitute ILMs. In this way, solid matrices and/or amine structure could restrain the ILM choice. Indeed, IAA contains only one carboxylic acid as protonating site and then only one equivalent of amine can be used. In a similar way, it has been demonstrated that a sterically hindered base such as DEA does not remove the phenolic proton.[39] According to the studied

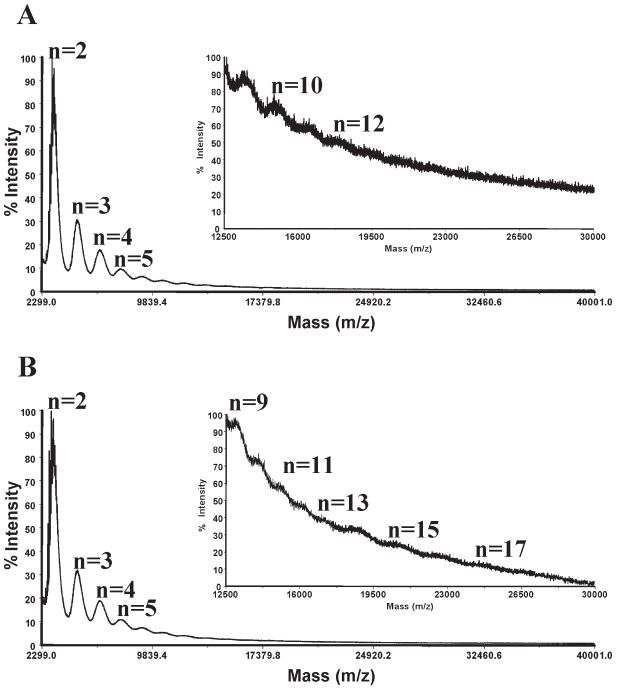

Figure 5. Positive (A) and negative (B) ion linear MALDI−TOF mass spectra of PR-γCD-PDMS polyrotaxane sample with HABA-TMG$_2$ at 90 mg/mL in DMF. The sample was analyzed at 1:20 ratio (sample: matrix v:v). Insets show the envelops in higher mass range. The number n is the sum of the numbers of CDs and polymer chain in the polyrotaxane structure.

matrices, only 1 equiv of DEA was added. Here, a mixture made with 0.5 μL of sample and an equal volume of original ILMs, yielded an analyte to matrix mass ratio of ≈1.2 and so an analyte to matrix molar ratio >1. Indeed, decreasing the ratio could greatly improve the detection of high molecular weight species either for ILM which has already allowed detection and even for those where no signal was recorded at a ratio of 1. To check this issue, further dilutions of sample were directly achieved in the ILMs by a factor of 20, 40, and 80. Both the number of CDs threaded and the polymer size were not totally defined following the methodology of the previous section. Results are summarized in Table S2 in Supporting Information.

Among all ILMs studied, it clearly appeared that HABA-TMG$_2$ and THAP-TMG$_2$ yielded the best results in terms of

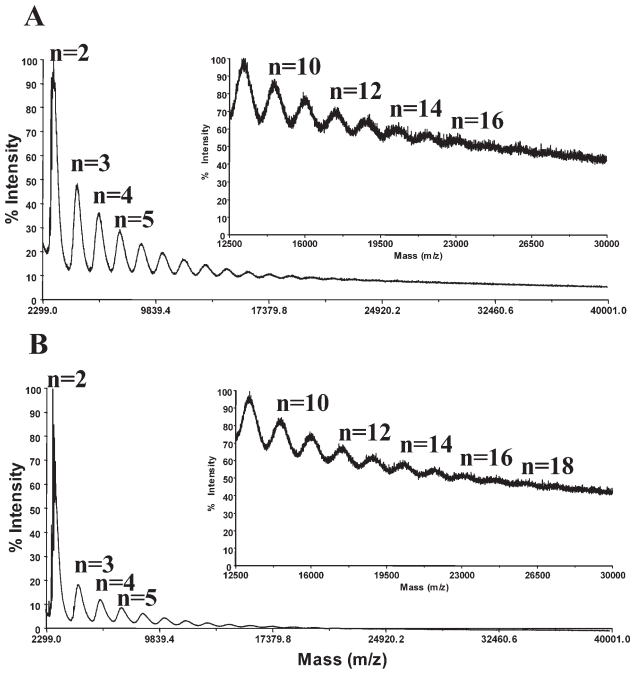

Figure 6. Positive (A) and negative (B) ion linear MALDI−TOF mass spectra of PR-γCD-PDMS polyrotaxane sample with THAP-TMG_2 at 90 mg/mL in DMF. The sample was analyzed at 1:20 ratio (sample: matrix v:v). Insets show the envelops in higher mass range. Number n is the sum of the numbers of CDs and polymer chain in the polyrotaxane structure.

both highest detected masses and signal intensity. Hence, according to our samples, HABA-TMG_2, afforded detection of poly[5]- to poly[14]rotaxanes, addressing a highest mass detected of about 20869 Da in positive mode (Figure 5A and Table S2). Likewise, negative mode analysis led to the successful determination of poly[5]- to poly[17]rotaxanes with masses reaching 24804 Da (Figure 5B and Table S2).

Finally, THAP-TMG_2, which has already demonstrated its potentiality for analysis of glycopeptides and glycans,[51] gave the best results with detection of masses in the range 7529−26447 Da range corresponding to poly[5]- until poly[18]rotaxanes in positive mode (Figure 6A), and between 3380 and 26426 Da range identifying poly[2]- to poly[18]rotaxanes in negative mode (Figure 6B). The best results with both HABA-TMG_2 and THAP-TMG_2 were obtained with an optimal analyte to matrix ratio of 1:20.

Examples of spectra corresponding to other dilutions are given in Figures S3 and S4 in the Supporting Information. Taking into consideration the criteria to classify matrices, the order of ILMs efficiency was as follows: THAP-TMG_2 ≥ HABA-TMG_2. A complete sorting of studied ILMs is given in Supporting Information. Likewise, mass accuracy obtained with ILMs was superior to those of conventional matrices, reaching the 2−8 Da range for both THAP-TMG_2 and HABA-TMG_2, even at m/z superior to 20000. The assumption forth put over the efficiency of TMG holds in its guanidine moiety that provides the highest PA value, and consequently acts as the strongest base. Furthermore, the guanidinium ion resulting from protonation of TMG, can also induce strong π-cations interactions with the pyrenyl groups at the extremities of PRs. These interactions can greatly support the desorption processes, during the transfer from solid/liquid to gaseous phase. It can be quoted out that, except for IAA and THAP, effectiveness of TMG based ILMs seemed not to be correlated with PA of solid matrix counterpart. Nevertheless, the basic properties of these ILMs could greatly help in the ionization step, i.e. deprotonation of the analyte by enhancing proton transfer from the analyte to the matrix. In this respect, for a classical matrix, deprotonation of the analyte would be more difficult since the matrix only shows acidic properties. As the molecular weight and the polydispersity of samples increase, MALDI−TOF determination becomes less reliable since it cannot be ascertained that the entire molecular weight distribution travels equally through the spectrometer tube. Basically, high molecular weight species require higher laser power for the desorption/ionization processes than low molecular weight structures. Moreover, literature reported that laser fluence must be slightly higher during analysis with ILMs than conventional matrices. Here, application of higher laser energy did not favor better detection, and the main consequence was an overall higher noise.

The complex interplay between acid−base and analyte is influenced by factors like PA, gas phase acidity/basicity, initial ion velocity after desorption and questions about the charge state of the analytes found in matrices. Armstrong et al.[39] have judiciously highlighted that usually effective cations must have some properties such as pK_a >11 and PA > 930 kJ·mol^{-1}. The studied amino compounds exhibit pK_a equal to 15.20, 10.98, 10.69, and 4.61 for TMG, DEA, BUT, and AN respectively.[49] The corresponding PA are 1031.6, 994.3, 921.5, and 882.5 kJ mol^{-1}, respectively.[52] The addition of strong bases, such as amino compounds, considerably increases the global PA of the resulting ILM. It means that ILMs systematically present higher PA than an acidic matrix alone. This characteristic induces a stronger proton capture, which can lead to a high cation (Na^+, K^+, ...) availability for analytes such as CDs and polymers. This is one of the possible reasons of the high efficiency of HABA, which has been previously demonstrated to promote cations attachment and thus the probable stabilization of sulfate groups during MALDI MS analysis.[42,53] Finally, for most ILMs, the general trend indicated that maximal signal intensity and highest m/z were obtained with ratios of 1:20 and/or 1:40. Further dilutions usually led to a signal equal or slightly superior to a 1:1 ratio. Nevertheless, sometimes, high sample dilution generated signal extinction. Homogeneous and transparent well-defined drops were easily observed after spotting, for dilution couples generating signal equal or higher to poly[3]rotaxanes in positive mode or poly[4]rotaxanes in negative mode. Interestingly, only half of laser shots used for solid matrices was required to obtain reliable signal with ILMs.

The apparent good dissolution between sample and efficient ILMs was confirmed by the smooth liquid aspect and the absence of residual aggregate on the MALDI plate. The ILM could afford a high number of sequential shots, demonstrating a very attractive property for complex mixture analysis. Indeed, after laser irradiation, the part of desorbed matrix is replaced by a new one depicting a strong regenerating capability. This feature was due to both simultaneous higher viscosity and higher thickness as compared to the dried droplet preparation during solid matrix deposition. Viscosity of ILMs was further increased by the use of DMF as solvent. It is a strongly polar and aprotic solvent acting as a powerful solubilizing agent. Furthermore, it presents higher vapor pressure at 25 °C (0.439 kPa) than more volatile solvents such as methanol (16.9 kPa) or ethanol (7.87 kPa) commonly used for ILMs, allowing to keep the liquid property for a longer time. Moreover, the boiling point of DMF (153 °C) is by far

Table 1. Recapitulative Results for PR-αCD-PEO and PR-αCDSi-PEO in THAP-TMG_2 and HABA-TMG_2 and an Analyte to Matrix Ratio of 1:80 v:v[a]

		detection mode					
		positive			negative		
sample	matrix	expt $\overline{M}_n$	theor $\overline{M}_n$	[n] rotaxanes ($\overline{DP}_n$; CDs)	expt $\overline{M}_n$	theor $\overline{M}_n$	[n] rotaxanes ($\overline{DP}_n$; CDs)
PR-αCD-PEO	THAP-TMG_2	8172 (1724)	8172	7 (24; 6)	8896 (761)	8993	8 (16; 7)
	HABA-TMG_2	11482 (250)	11485	10 (26; 9)	9968 (204)	9968	9 (16; 8)
PR-αCDSi-PEO	THAP-TMG_2	5036 (1324)	5036	4 (25; 3)	9028 (458)	9025	8 (19; 7)
	HABA-TMG_2	9292 (469)	9291	8 (25; 7)	9966 (514)	9968	9 (16; 8)

[a] The detected (expt $\overline{M}_n$) and calculated (theor $\overline{M}_n$) highest number average molecular weight both in positive and negative detection modes depending of matrix. Number in round brackets represents the signal intensity. Number in square brackets [n] is the sum of the numbers of CDs and polymer chain in the polyrotaxane structure. $\overline{DP}_n$ is the degree of polymerization i.e. the number of polymeric units. CDs indicate the number of cyclodextrins threaded. ND: no polyrotaxane structures detected.

higher than alcohol ones. All of these features procured an increased vacuum stability to the ILMs. According to the sample nature, the types of interactions provided by the solvent govern the choice to ensure both the solubilizing of analytes and the molecular cohesion of the ILM.[54] DMF was particularly interesting because it can both form hydrogen bonds like alcohols and also polarize other molecules. For comparison purpose, DMF exhibits a dipolar moment of 3.82 D at 25 °C, instead of 1.70 and 1.69 D, for methanol and ethanol, respectively. A hypothesis could be formulated concerning the solubility of PR-γCD-PDMS which could be supported by $\pi-\pi$ interactions between pyrenyl extremities and aromatic moieties of matrices and also π-dipole interactions with DMF.

5. Usefulness of Ionic Liquid Matrices with Other CD-Based Polyrotaxanes. After evaluation of several solid and ionic liquid matrices, it clearly appeared that tetramethylguanidinium salts of THAP and HABA provided the most promising results. To further investigate their versatility, a second series of experiments addressed the detection of two other challenging CD based PRs. They were constituted of a poly(ethylene oxide) backbone with αCDs threaded along the chain (PR-αCD-PEO) (Figure 1B) and with partially silylated αCDs (PR-αCDSi-PEO) (Figure 1C), which can form fewer hydrogen bonds. After several dilution assays, it was observed that a dilution at 1:80 v:v as analyte to matrix ratio was optimal for the two PRs. Interestingly, the spectrum acquired in negative detection presented a better definition between distributions than those obtained in positive detection mode. The highest detected masses were not systematically correlated to this fact (Figures S5 and S6 in Supporting Information). Furthermore, significant differences were noteworthy according to the kind of tetratramethylguanidinium based matrices employed (Table 1).

Indeed, it appeared that analysis of PR-αCD-PEO in THAP-TMG_2 permitted to detect masses reaching m/z 8172 and 8896 in positive and negative mode, respectively. These values were attributed to poly[7]rotaxanes and poly[8]rotaxanes (Figures S5-A and S5-C). Contrary to PR-γCD-PDMS, better results were noteworthy with HABA-TMG_2 allowing to detect masses of 11482 and 9968 Da, corresponding to poly[10]rotaxanes and poly[9]rotaxanes (Figures S5-B and S5-D). According to the SEC results, the PR-αCD-PEO (run 5)[46] contains only 30% of free CDs (Table S3). This result is in agreement with the detection of the poly[10]rotaxanes by MALDI−TOF MS.

Concerning PR-αCDSi-PEO, all spectra were scattered by several peaks over and between the distributions. Presumably, these ions resulted from various modifications of CDs such as sulfation and silylation, as confirmed by thorough examination of the low mass range i.e. m/z 950−1700 acquired in reflectron mode (data not shown). At first appearance, as well as without silylation, the several CDs modifications disrupted the polyrotaxanes cohesion leading hard to discern populations. In spite of these hindrances, highest masses of 5036 and 9028 Da were revealed with THAP-TMG_2, which corresponded to poly[7]rotaxanes and poly[8]rotaxanes (Figures S6-A and S6-C). At the same time, as for PR-αCD-PEO, HABA-TMG_2 outperformed THAP-TMG_2 allowing to detect masses of 9292 and 9966 Da, corresponding to poly[8]- and poly[9]rotaxanes (Figures S6-B and S6-D). Although there were differences between the matrices, all masses were consistent with the maximum number of αCDs which can be threaded on a PEO chain e.g. One CD per 2 ethylene oxide units.[3]

On the other hand, it appears that the dethreading is lower with high molecular weights probably due to a higher molecular cohesion. In fact, according to the quantification by ^{1}H NMR, PR-αCDSi-PEO (run 15)[46] seemed to contain only 3 persilylated CDs (Table S4). Furthermore, small angle neutron scattering analysis revealed random coil behavior, which is presumably due to a low amount of CDs borne by the polymer (Figure S7). This result was an additional evidence of the presence of PRs with chains bearing the maximum of CDs as highlighted by MALDI−TOF MS. In spite of the fact that MALDI−TOF MS is not a quantitative method, the approach afforded analysis of high molecular weight PRs, even present in a minor state in the sample. This fact is in agreement with the dethreading kinetics. The dethreading is longer when the polymer chain length increases.

Finally, it surprisingly appeared that the results in terms of resolution and highest mass detected with these two αCD-PEO-based PR strongly differed. Indeed, the qualities of spectrum is clearly depending on judicious choices of both MS analysis mode (positive or negative) and the appropriate ILM (THAP-TMG_2 versus HABA-TMG_2). However, a noteworthy accuracy was recovered for the two ILMs, with only 0−3 Da for both THAP-TMG_2 and HABA-TMG_2 and even independent of the considerated αCD-PEO-based PRs.

CONCLUSIONS

Polyrotaxanes were known to be extremely difficult to analyze by MALDI MS, due to their size and structure fragility. At the light of results, it seemed that numerous ester linkages

constituting the studied polyrotaxanes resist to the MALDI process allowing to keep their structures intact. Nevertheless, it is not possible to completely exclude a partial decomposition. The results obtained clearly showed that conventional (crystalline) matrices bias the calculation/estimation of polyrotaxanes structures toward low masses. Ionic liquids matrices represent an alternative choice, because they ensure a better dispersion of the analyte in a liquid solution, and thus partially compensate for the discrimination effect. Dimethylformamide, an unusual solvent in mass spectrometry, was successfully used for the analysis of supramolecular architectures such as CD based PRs. Their polar and aprotic properties demonstrated a universal character to simultaneously solubilize analytes, matrix and counterions, but also in strengthening the basic property of the ILMs. With regard to the various ILMs tested, it appeared that tetramethylguanidinium salts of moderately acid or aprotic matrices are most dedicated to the detection of high molecular weight cyclodextrin based polyrotaxanes. These matrices have permitted to detect, for the first time, structures up to 26400 $g \cdot mol^{-1}$. Another good point holds in the high versatility of THAP-TMG_2 and HABA-TMG_2 toward a variety of cyclodextrins and polymers. In summary, MALDI MS is a powerful tool to detect and study supramolecular architectures with a successful detection obviously depending on both host and guest features.

■ ASSOCIATED CONTENT

Supporting Information. Characterization of polyrotaxanes with standard crystalline matrices and use of ionic liquid matrices to improve detection of high molecular weight polyrotaxanes. This material is available free of charge via the Internet at http://pubs.acs.org.

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