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Powerfully Solvating Matrices for MALDI-TOF Spectroscopy of Aromatic Polyesters

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ABSTRACT: The starting premise of this work was that matrices containing powerfully solvating groups would enhance the quality of MALDI-TOF spectra of condensation polymers. The solvating groups in these matrices would pry the polymer aggregates apart and make it easy for individual ionized polymer molecules to "fly". Ten polyfluorinated azobenzenes and stilbenes were synthesized. The laser desorption ionization spectra of the pure fluorinated matrices show the formation of radical cations ($M^{\bullet+}$) and anions ($M^{\bullet-}$) in addition to the more conventional $[M + H]^+$ and $[M-H]^-$ ions. Dimerization followed by loss of fluorine is also observed. The effectiveness of these compounds as MALDI matrices was tested with four aromatic polyesters: poly(3-ethyleneoxybenzoate) (3EOB), Vectra, poly(1,4-dimethylenecyclohexane terephthalate) (CHDT), and poly(ethylene terephthalate) (PET). The azobenzene-containing matrices were much better than their stilbene analogues and gave good quality positive ion spectra, better than those obtained with the conventional matrix dithranol. With the best matrix, 4,4′-dihydroxyoctafluoroazobenzene, ions up to *m*/*z* 12 000 were detected for the CHDT and PET polymers.

Introduction

Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry has been successfully used for a wide variety of polymers since the appearance of the paper by Hillenkamp and co-workers.[1] Excellent reviews are available[2−4] and provide further insight into this growing and important field of research. Some of these studies have concentrated on solvent effects[5] while insoluble polymers have been analyzed by using the solvent-less technique.[6−9] Polyesters such as poly(bisphenol A carbonate)[10] and oligomeric poly(ethylene terephthalate), PET,[11,12] have been successfully analyzed by MALDI-TOF. These polyesters contain saturated (sp^3) carbons, and this has a strong effect on solubility.

In contrast to biopolymers, for which only a few well-established matrices are used, the matrices currently used in MALDI-TOF analyses of polymers embody a wide variety of chemical structures identified on more or less empirical grounds; they include dithranol (DTH), 2-(4-hydroxyphenylazo)benzoic acid (HABA), and its methyl ester (MHABA). Of course, one cannot argue with the success of these and several other matrices with a great variety of synthetic polymers! However, the rational design of new MALDI matrices has not yet become a routine protocol, and MALDI-TOF analyses of aromatic polyesters with the conventional matrices are still a challenge. As a contribution to this matter, we postulate that the matrix which solvates the polymer to the greatest extent will give optimal MALDI spectra. Such a matrix will pry apart the condensed polymer and enable the individual molecule to "fly". This article examines the application of this concept to several commercially important polyesters, such as poly(3-ethyleneoxybenzoate), 3EOB, Vectra, poly(1,4-dimethylenecyclohexane terephthalate), CHDT, and poly(ethylene terephthalate), PET.

Polyesters of varying structure differ greatly in solubility.[13] Aliphatic polyesters are soluble in various solvents, while at the other extreme all-para, all-aromatic polyesters such as Vectra are only difficultly soluble. The main factor determining solubility is hydrogen bonding between the carbonyl group lone pair electrons and a solvent containing a pseudo-acidic hydrogen, the most powerful of which is pentafluorophenol.

Poly(ethylene terephthalate) is an enormously important commercial aromatic polyester. Accordingly, it has been the subject of several MALDI-TOF studies, both of the unmodified polymer[11,12] and of chemically[14] and thermally[15] modified polymer. As to the former, only low molecular weight oligomers have given pure MALDI spectra.

Another important polyester, Vectra A950, is a copolymer of 4-hydroxybenzoic acid (HBA) and 6-hydroxynaphthoic acid (HNA) and an important high-technology polymer used in many applications. We have previously attempted to analyze Vectra samples by MALDI-TOF spectroscopy. Even for low molecular weight ("truncated") Vectra, only very low molecular weight ions were detected by MALDI-TOF by using conventional matrices such as dithranol and HABA, even though we knew the polymer to be of higher molecular weight.[16] In addition, the complexity of the ion formation was indicated by the detection of four series of ions, including acylium ions that are very likely truncation products. To shed further light on this matter, we recently synthesized a well-defined set of alternating oligomers of HBA with HNA and determined their MALDI-TOF spectra using conventional matrices (DTH, HABA, and MHABA).[17] Again, complex sets of ions were observed as well as sequence randomization.

As mentioned, the question arose whether incompatibility of the difficultly soluble polymer with the matrices might be at least partly responsible for the observed complicated spectra. An early discussion of the important role solubility plays in the quality of a MALDI-TOF spectrum demonstrated improved spectrum quality when using a single organic solvent.[18]

Because pentafluorophenol appears to be the most powerful solvent by virtue of its highly pseudo-acidic hydrogens, we selected this structure for incorporation into our matrices. Our concept is most closely related to that of Hoteling et al.[19,20] The designed matrices should also contain azobenzene or stilbene moieties to impart the necessary UV-absorbing property

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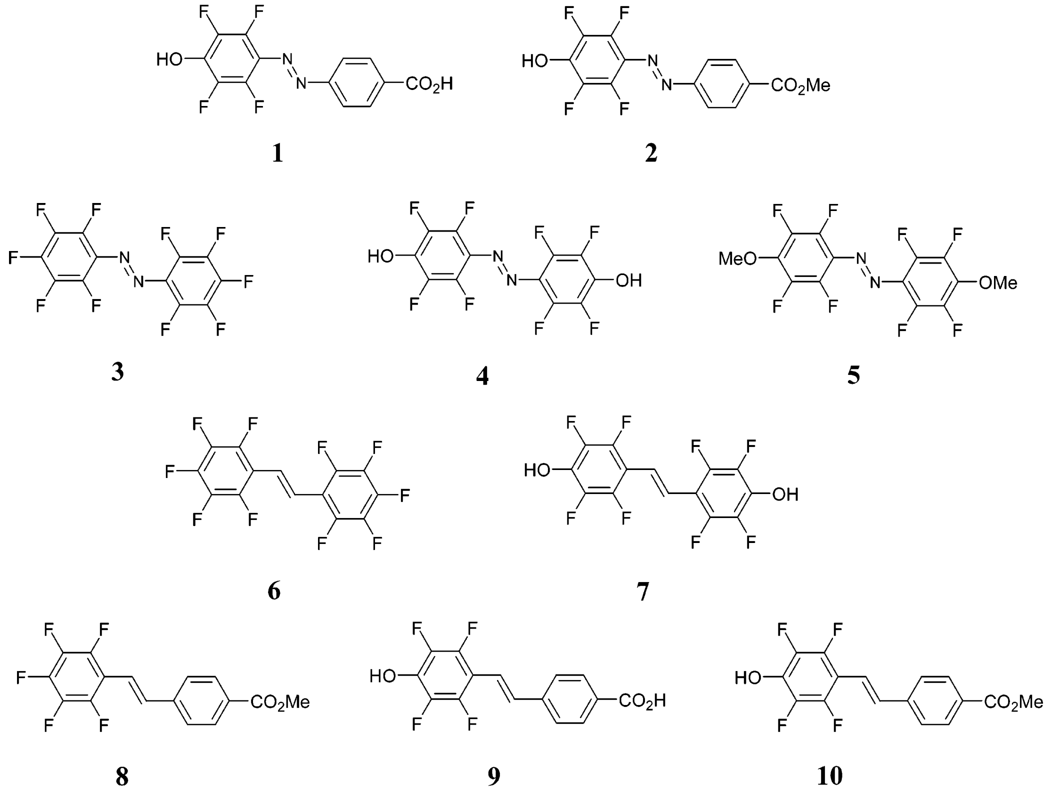
Scheme 1. Perfluoroazobenzene and -stilbene Derivatives Proposed as Possible Matrices



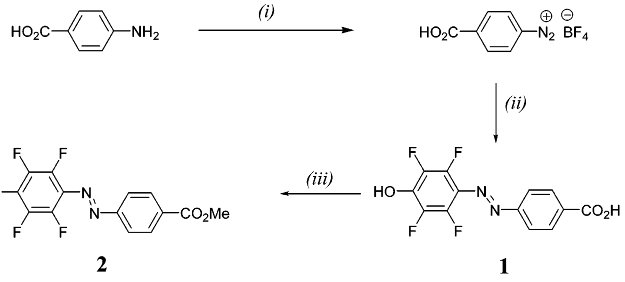
to the matrix. On the basis of these two factors, we designed polyfluoroazobenzene and -stilbene derivatives as potential matrices, and these are shown in Scheme 1; the laser desorption ionization (LDI) spectra of the matrices and the MALDI-TOF spectra of four aromatic polyesters obtained with these matrices will be discussed.

Finally, we note that MALDI matrices can be classified as "cold" or "hot" matrices on the basis of their proton affinity (PA) values.[21] Even though the PAs of our newly synthesized matrices are not known, their relative performance for polymer ionization can be compared to conventional matrices. One important issue is mass discrimination: "hotter" matrices can truncate polymers to alter mass distribution. The degree of truncation, of course, also depends on the applied laser power that is, again, related to the matrix ionization efficiency. Although our present studies do not allow us to provide detailed description of ion formation by using our tailor-made matrices, the results presented here are encouraging us to conduct further studies to reveal details of ion formation mechanisms.

Results and Discussion

Synthesis of Azobenzene- and Stilbene-Based MALDI Matrices. The synthesis of azobenzenes **1** and **2** is depicted in Scheme 2. The diazotization of 4-aminobenzoic acid was performed in anhydrous THF containing excess boron trifluoride etherate complex.[22] The 4-carboxybenzenediazonium tetrafluoroborate precipitated while *tert*-butyl nitrite was added and was filtered off. Addition of this diazonium salt to potassium tetrafluorophenoxide, prepared in situ by treatment of tetrafluorophenol with potassium carbonate in sulfolane, afforded 4-(2′,3′,5′,6′-tetrafluoro-4′-hydroxyphenylazo)benzoic acid (**1**) in 80% yield. This acid was converted into its methyl ester **2** in 91% yield.

Scheme 2. Synthesis of Azobenzene Matrices 1 and 2[a]

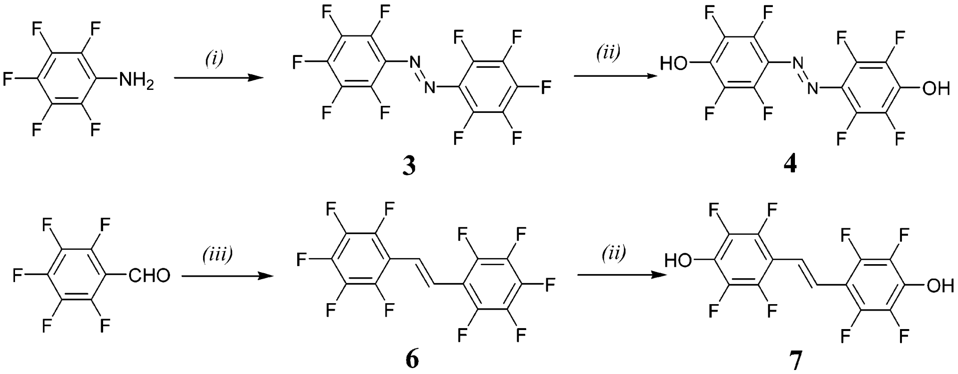


[a] Reagents and conditions: (*i*) $BF_3{\cdot}Et_2O$/*t*-BuONO, THF, −15 to −5 °C; (ii) 2,3,5,6-tetrafluorophenol/K_2CO_3, sulfolane, 80 °C, 1 h; (iii) TsOH, MeOH, reflux, 18 h.

Decafluoroazobenzene was a useful precursor in the synthesis of several hydroxyfluoroazobenzenes which were evaluated as inhibitors of steroid biosynthesis.[23] The oxidation of pentafluoroaniline with aqueous sodium hypochlorite gave the corresponding decafluoroazobenzene (**3**)[24] (Scheme 3), but in only 24% yield; the oxidation of fluoroanilines by inorganic oxidants in aqueous solution is complicated by their poor solubility. The addition of an organic solvent immiscible with the aqueous phase, such as dichloromethane or ethyl acetate, slows the reaction further, since the fluoroaniline is sequestered in the organic phase. However, the oxidation of pentafluoroaniline under phase transfer conditions[25] gave decafluoroazobenzene (**3**) in the previously reported yield.[24] Hydrolysis of **3** by aqueous potassium hydroxide and tetrabutylammonium bisulfate under phase transfer conditions using toluene as solvent gave the corresponding bis(tetra-*n*-butylammonium) salt, which was directly treated with sulfuric acid to give 4,4′-dihydroxyoctafluoroazobenzene (**4**) in 60% yield. Etherification was achieved under basic conditions (K_2CO_3) using methyl iodide to give 4,4′-dimethoxyoctafluoroazobenzene (**5**) in nearly quantitative yield.

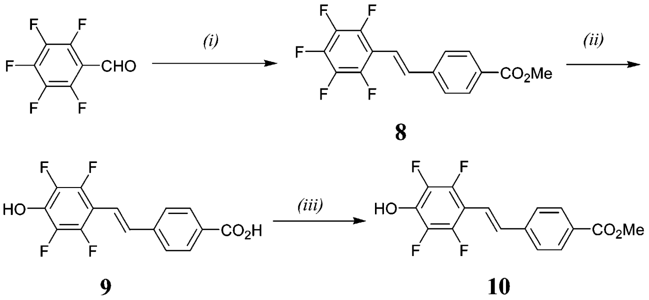
trans-Decafluorostilbene (**6**) was obtained in 70% yield by McMurry coupling of pentafluorobenzaldehyde using low-valent titanium.[26] Subsequent conversion to the bis-phenol **7** proceeded

Scheme 3. Synthesis of Matrices 3, 4, 6, and 7[a]



[a] Reagents and conditions: (*i*) NaOCl, H_2O, rt, 18 h; (ii) KOH, *n*-Bu_4NHSO_4, toluene/H_2O, rt, 1.5 h then H_2SO_4; (iii) $TiCl_4$/Zn, THF, 0 °C, C_6F_5CHO, 0 °C to reflux, 2 h.

Scheme 4. Synthesis of Matrices 8−10[a]



[a] Reagents and conditions: (i) methyl 4-(diethoxyphosphorylmethyl)benzoate/*t*-BuOK, THF, 0 °C to rt, 2 h; (ii) KOH, *n*-Bu_4NHSO_4, toluene/H_2O, rt, 1.5 h then H_2SO_4; (iii) TsOH, MeOH, reflux, 18 h.

in a similar manner as for **3** with aqueous potassium hydroxide under phase transfer conditions.

For the synthesis of the remaining matrices, methyl 4-(diethoxyphosphorylmethyl)benzoate was prepared by a Michaelis−Arbuzov reaction of commercially available methyl 4-(bromomethyl)benzoate and triethyl phosphite.[27] Wittig−Horner olefination of the phosphonate with pentafluorobenzaldehyde afforded methyl 4-(2-pentafluorophenylvinyl)benzoate (**8**) in 75% yield (Scheme 4). The expected *trans* configuration of **8** was confirmed by the 16.8 Hz coupling constant between the vinyl protons in its ^1H NMR spectrum. Hydrolysis of **8** by aqueous KOH gave the hydroxy acid **9** in 56% yield. Conversion to its methyl ester **10** with MeOH/TsOH proceeded in 90% yield.

Laser Desorption Ionization Spectra of Matrices 1−10. The behavior of these new matrices under laser desorption ionization conditions was first investigated. Figure 1 shows the positive (a) and negative (b) laser desorption ionization (LDI) spectra of matrix **3**. This is a perfluorinated azobenzene derivative matrix, containing no hydrogens, so the appearance of radical cations ($M^{\bullet+}$) and anions ($M^{\bullet-}$) is not surprising. One might assume that these radical ions can initiate electron transfers to form radical ions of the analytes. The appearance of peaks three mass units lower than the molecular weight (*m*/*z* 359) can be interpreted as resulting from an O/F exchange process which might occur on the MALDI plate either before or during ionization. The ion at *m*/*z* 167 in the negative ion spectrum is assigned as $C_6F_5^-$, the appearance of which is not surprising since the C−N bond should be weak and the $C_6F_5^-$ ion stable. Dimerizations associated with losses of F_2 (*m*/*z* 686), C_6F_6 (*m*/*z* 538), and C_6F_6N (*m*/*z* 524) are also observed in the negative ion spectrum. The appearance of positively charged ions at *m*/*z* 525, 600, and 628 is not well understood, but we tentatively assign them as $C_{24}F_{11}N_2^{\bullet+}$, $C_{24}H_2F_{14}ON_2^{\bullet+}$, and $C_{24}H_2F_{14}ON_4^{\bullet+}$, respectively. They are presumably products of dimerization followed by fragmentation. It is worth noting here that the negative ion LDI spectrum of the decafluorinated stilbene derivative **6** (spectrum not shown) is more complex containing negatively charged ions that might be associated with the presence of oxygen, e.g., ions at *m*/*z* 164 ($C_6F_4O^-$), *m*/*z* 165 ($C_6F_4OH^-$), and *m*/*z* 357 ($C_{14}H_2F_9O^-$). The $[M-H]^-$ ion is also detected at *m*/*z* 359 because the only source of hydrogens is the stilbene −CH═CH− moiety. This is especially interesting because the $[M-H]^-$ anion is not always detected in the negative ion LDI spectrum of the other stilbene analogues (see below).

Figure 2 shows the positive (a) and negative (b) laser desorption ionization spectrum of matrix **4**. Both spectra are relatively simple. In the negative ion LDI spectrum of **4** the appearance of the $[M-H]^-$ (*m*/*z* 357) and $[2(M-H) + Na]^-$ (*m*/*z* 737) ions can easily be rationalized on the basis of the presence of two phenolic hydroxyl groups. Note that at higher laser power three additional peaks with much lower intensities are also present, and they can be assigned as sodiated/potassiated ions of doubly (negatively) charged trimers (not visible in Figure 2b). In the positive ion spectrum (Figure 2a) the protonated (*m*/*z* 359) and sodiated molecules (*m*/*z* 381) are present. The origin of the low-intensity ion at *m*/*z* 598 is more difficult to interpret, but it is presumably related to a dimerization followed by subsequent losses of fluorine, and a tentative formula of $C_{24}H_4F_{12}NO_4$ is suggested.

Another good example of a radical anion formation is the $M^{\bullet-}$ ion (*m*/*z* 328) observed for the stilbene analogue, matrix **8** (Figure 3a). This ion is accompanied by an anion at *m*/*z* 325 presumably related to an F/O exhange ($C_{16}H_9OF_4^-$). Similarly to the negative ion LDI spectrum of **4**, shown in Figure 2b, the $[M-H]^-$ ion (*m*/*z* 327) is a dominant ion in the spectrum of another phenol azobenzene analogue **2** (Figure 3b). Sodiated and potassiated dimerization and trimerization are also detected together with a small intensity ion at *m*/*z* 164 ($C_6F_4O^-$).

Overall, the laser desorption ionization spectra of our synthesized matrices indicate the formation of both even and odd electron cations and anions as well as dimerizations associated with and/or related to losses of fluorines. The formation of radical ions can be rationalized on the basis of the highly aromatic character of these matrices. As expected, the relative intensities of the ions depend strongly on the laser power with a general trend of observing more dimerization and trimerization with higher laser power.

MALDI-TOF Spectra of Aromatic Polyesters Obtained with the Synthesized Matrices. *Poly(3-ethyleneoxybenzoate) (3EOB).* The well-soluble poly(3-ethyleneoxybenzoate), 3EOB,

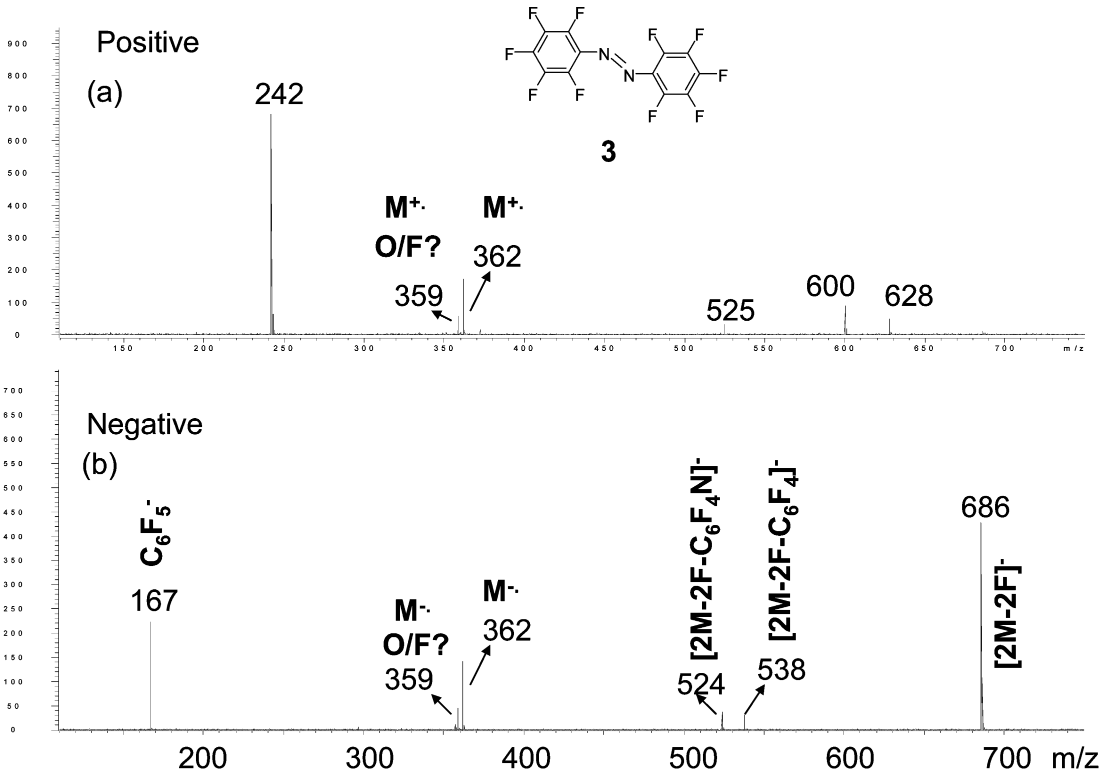


Figure 1. (a) Positive and (b) negative laser desorption ionization (LDI) spectra of matrix **3**.

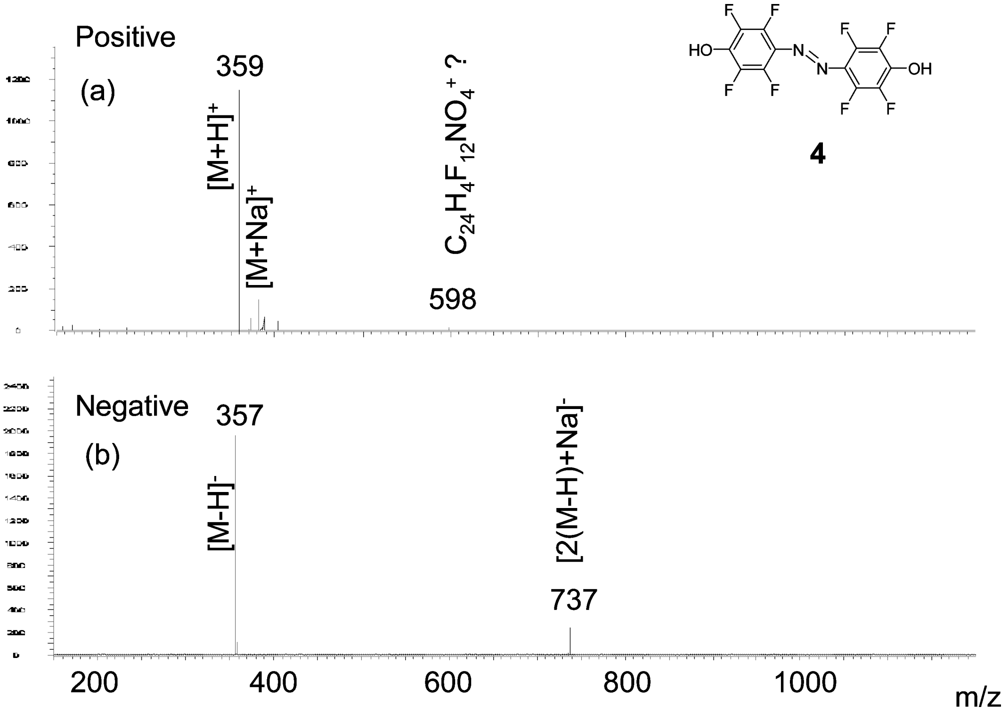


Figure 2. (a) Positive and (b) negative laser desorption ionization (LDI) spectra of matrix **4**.

was used to carry out a comparative study of the ionization efficiencies of azobenzene- and stilbene-contaning matrices. The main goal of using this polymer was to compare the performance of these matrices when polymer solubility is not an issue. The 3EOB polymer was prepared by condensation of 3-hydroxybenzoic acid with ethylene carbonate in the presence of stannous octanoate, a reaction we are continuing to investigate. This polymer has been briefly mentioned only in the patent literature.

Two representative spectra are shown in Figure 4 to demonstrate that, in general, there is a striking difference between the spectra obtained with azobenzene matrices (**1**−**5**) and stilbene matrices (**6**−**10**): azobenzene matrices give much more intense signals, i.e., better ionization efficiencies (Figure 4a) than the stilbene derivatives (Figure 4b). Among the azobenzene matrices, the presence of a phenolic OH or carboxyl group helps; the order of ionization efficiency is **4** ≥ **2** > **1** > **5** > **3**. The perfluorinated azobenzene matrix **3** does not perform well, presumably due to the lack of phenolic groups.

With the "good" matrix **4**, the reflectron detection technique was used to detect ions with higher resolution and mass accuracy (Figure 5). The "triplet" series of ions were unambiguously

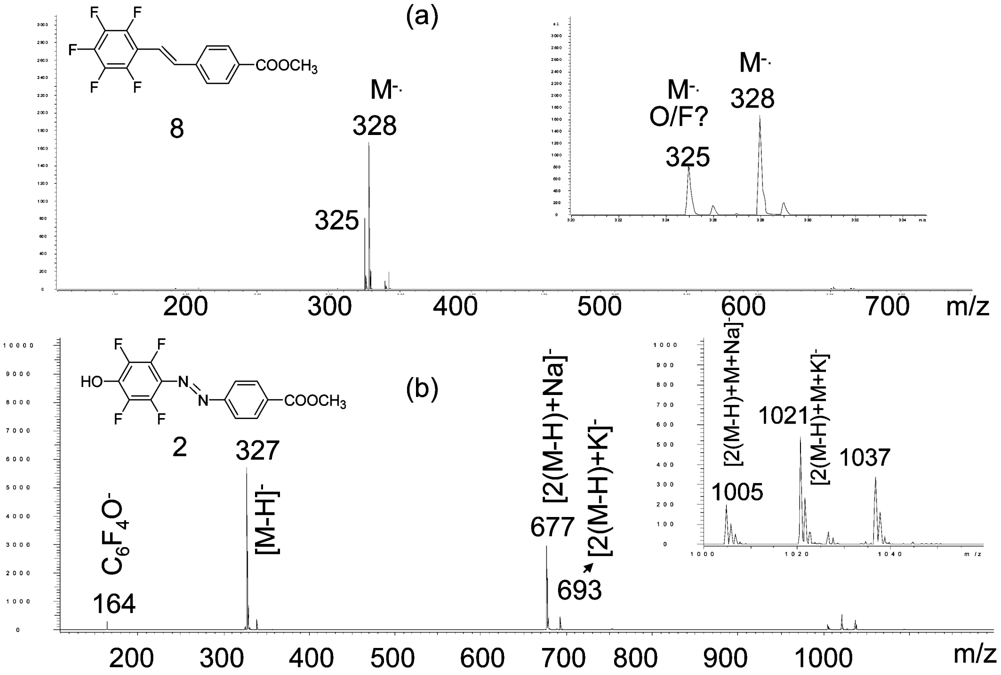


Figure 3. Negative laser desorption ionization spectra of (a) matrix **8** and (b) matrix **2**.

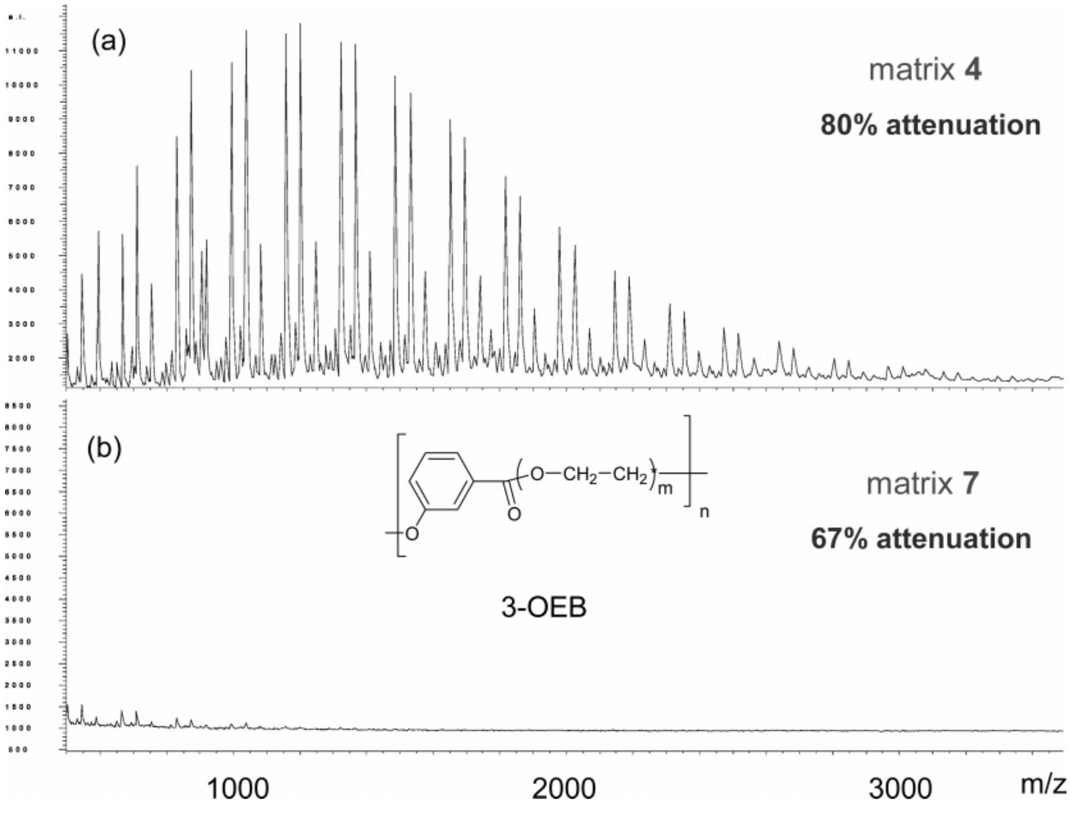


Figure 4. MALDI-TOF spectra of the polymer 3-EOB obtained with (a) matrix **4** and (b) matrix **8** (linear mode).

assigned as related to different numbers of the $-OCH_2CH_2-$ units (see differences of 44 Da indicated in the inset of Figure 5). A dominant series of ions is clearly separated by 164 Da, which corresponds to the repeating unit $-OC_6H_4(CO)OCH_2$-CH_2-. The main series of ions can be assigned as potassiated ions with the elements of water as the end groups, accompanied by the corresponding sodiated ions at *m*/*z* values less by 16 mass units (inset in Figure 5). For example, the ions at *m*/*z* 1205 and 1189 can be assigned as potassiated (K^+) and sodiated (Na^+) $H-(OC_6H_4(CO)OCH_2CH_2)_7-OH$, respectively. The most likely structure for the *m*/*z* 1161 ions lacks the CH_2CH_2O unit at the "right end" of the *m*/*z* 1205 structure as indicated above, and the *m*/*z* 1249 ions have an additional CH_2CH_2O unit at the "left end".

Vectra—Low and High Molecular Weight Polymers. For truncated (low molecular weight) Vectra polymer samples that we previously investigated,[16] matrix **4** was found to be the best matrix. The quality of spectra obtained with matrix **4** is comparable to those obtained by the "conventional" matrix dithranol (DTH) described in ref 1 (spectrum not shown). Even though the use of matrix **4** did not lead to *significantly* better quality spectra than DTH and HABA for Vectra and truncated

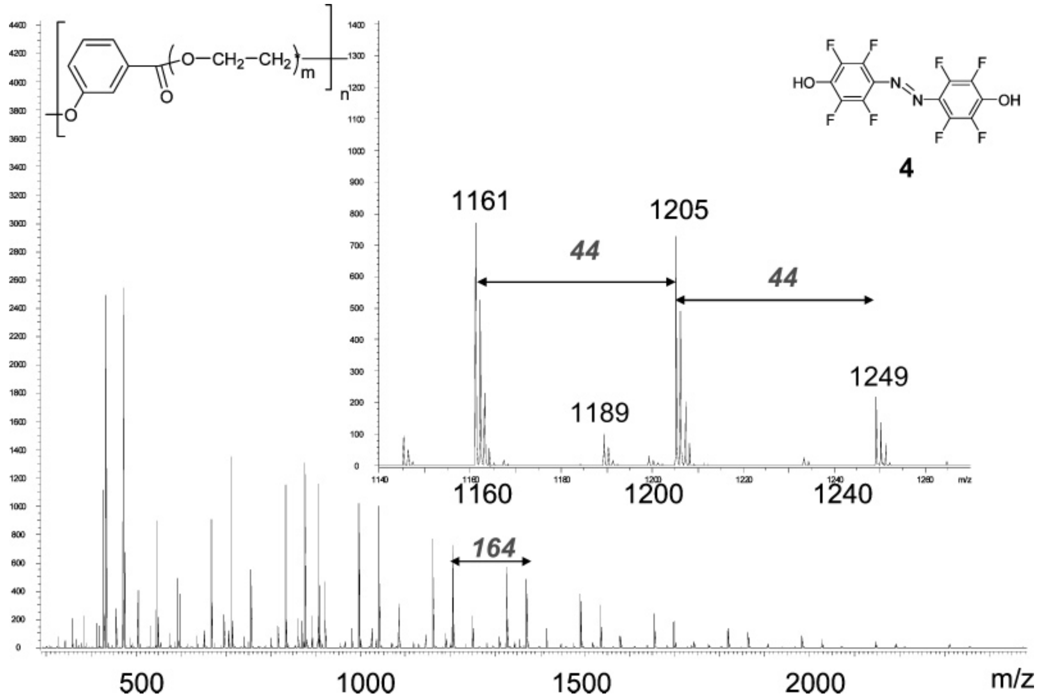


Figure 5. MALDI-TOF spectrum of the polymer 3-EOB obtained with matrix **4** (reflectron mode).

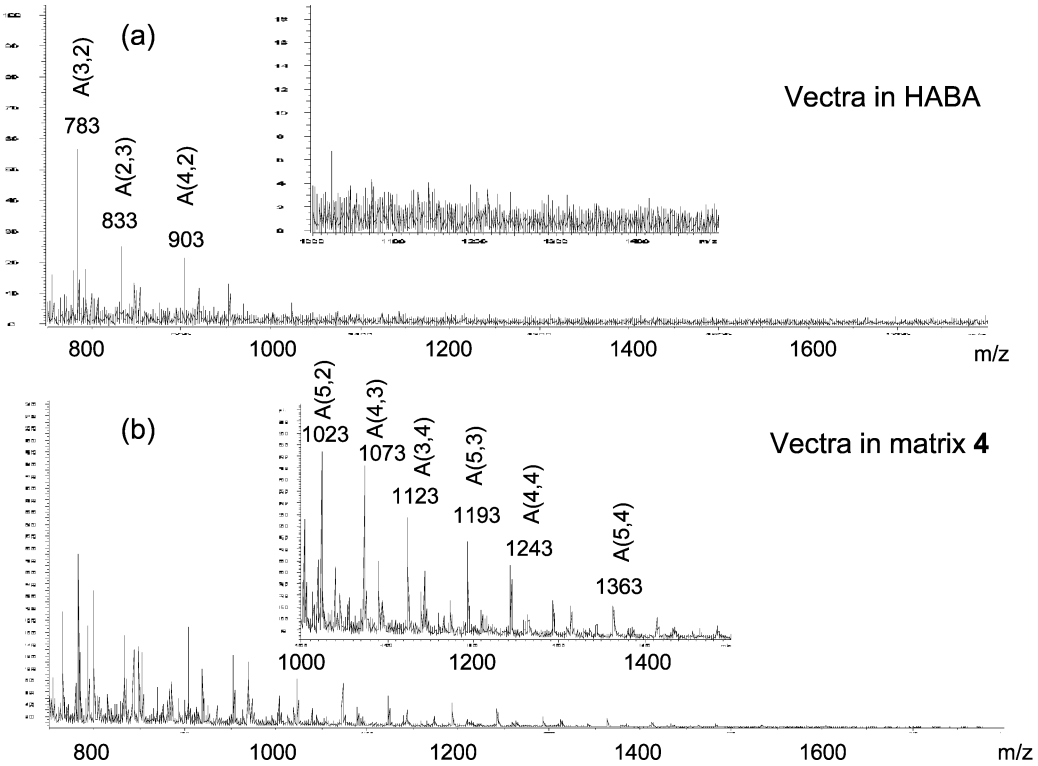


Figure 6. LDI-TOF spectra of a high molecular weight Vectra polymer obtained with (a) HABA and (b) matrix **4**. A(*i*,*j*) denotes ions Ac[B_i+N_j]-$OHNa^+$ (series A in ref 1).

Vectra polymers, more reliable and reproducible results were obtained with matrix **4**. Equally good spectra were obtained with matrix **2** (spectrum not shown). Similarly to 3EOB (see above), stilbene derivatives gave very poor quality spectra (not shown).

Matrix **4** allowed us to record a reasonable MALDI-TOF spectrum for a high molecular weight Vectra, a spectrum which is comparable to that obtained with HABA (compare parts b and a of Figure 6). In both cases, the main series of ions found are Ac[B_i+N_j]$OHNa^+$. These ions were denoted as A(*i*,*j*) type ions in ref 16, and the same notation is used here for consistency (*i* and *j* denote the number of benzoic (B) and naphthoic (N) acid residues). The doubly sodiated species are more pronounced in the spectrum with HABA than with matrix **4**.

In keeping with our concept, we used our most powerful solvents to obtain the MALDI-TOF analysis sample. These included pentafluorophenol, 2,3,4,6-tetrafluororphenol, and 3,5-trifluoromethylphenol. We might anticipate even greater MALDI ionization improvements for polymers easier to solvate than Vectra.

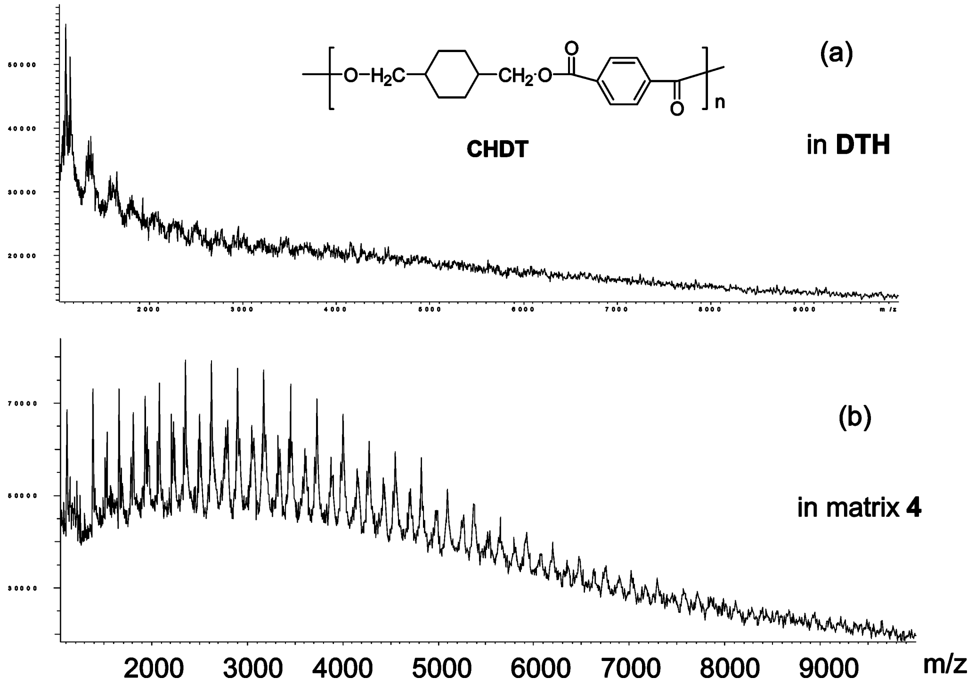


Figure 7. MALDI-TOF spectra of a CHDT polymer obtained with (a) dithranol (DTH) and (b) matrix **4** (linear mode).

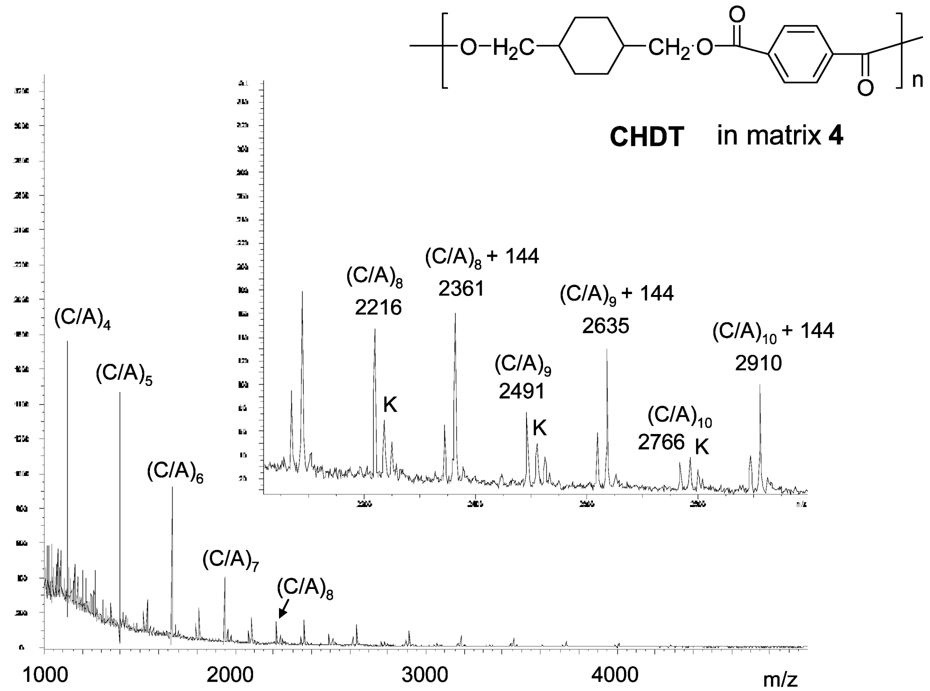


Figure 8. MALDI-TOF spectra of a CHDT polymer obtained with matrix **4** (reflectron mode). $(C/A)_n$ denotes either cyclic ions or open chain acylium ions, while $(C/A)_n + 144$ denotes linear ions with one more cyclohexyl unit with its methyleneoxy end groups (formal addition of $-OCH_2-C_6H_{10}-CH_2O-$).

Poly(1,4-dimethylenecyclohexane terephthalate) (*CHDT*). The MALDI-TOF spectra of CHDT obtained with DTH and **4** are shown in parts a and b of Figure 7, respectively. In both cases, 3,5-bis(trifluoromethyl)phenol was used as the solvent for the polymer. Ions up to 10 000 Da appear in the MALDI-TOF spectrum obtained with **4**, while DTH did not result in useful data. Similar results were obtained using 2,3,5,6-tetrafluorophenol, although ions only up to ∼8000 Da were detected with **4** (spectrum not shown). Using the reflectron detection technique, we were able to assign the two main ion series: one can be assigned as either a sodiated cyclic-CHDT series or the isomeric acyclic acylium ions (series C/A in Figure 8); this series is dominant at lower *m*/*z* values (<2400 u). The other series is more dominant at higher *m*/*z* values (>2400 u) and can be assigned as a sodiated acyclic series that is higher by 144 Da than the (C/A) series. These ions apparently have diol units at both ends (CA + 144 series in Figure 8).

(Polyethylene terephthalate) (PET) Polymers. The MALDI-TOF spectrum of PET obtained with matrix **4** is shown in Figure 9. In the linear mode (Figure 9), ions up to about *m*/*z* 12 000 were detected, much higher than previously observed. This is a dramatic example of the usefulness of matrix **4**. The best results were obtained with the solvent 2,3,5,6-tetrafluorophenol. Again, the reflectron mode provides better mass accuracy, and we assign the main series of peaks as the sodiated cyclic polymer and/or the isomeric acylium cation (e.g., the 10-mer with a

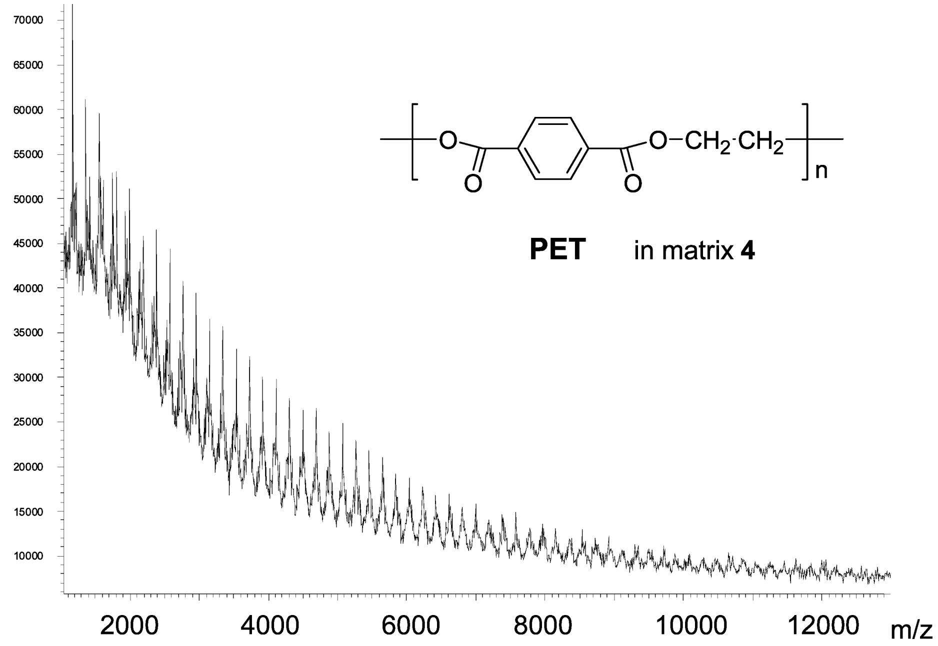


Figure 9. MALDI-TOF spectra of a polymer PET with matrix **4** using the linear mode.

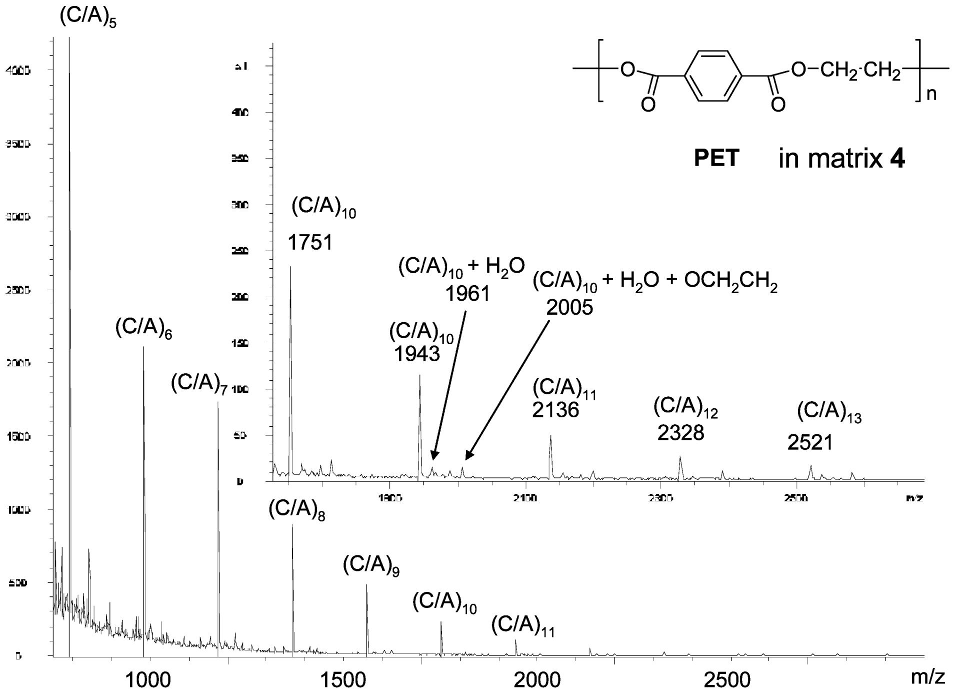


Figure 10. MALDI-TOF spectra of a polymer PET with matrix **4** using the reflectron mode. $(C/A)_n$ denotes either cyclic ions or open acylium ions, while $(C/A)_n + H_2O + OCH_2CH_2$ denotes linear ions with one more ethylene oxide unit and a water end group.

sodium ion gives a peak at *m/z* 1943, $(C/A)_{10}$ in Figure 10). The second strongest series (at 62 mass units higher, e.g., *m/z* 2005 for the 10-mer) is most likely related to a linear polymer with an additional OCH_2CH_2 unit and H_2O end group. This assignment is supported by the presence of another series that is 18 mass unit higher than the cyclic/acylium (C/A) series (e.g., *m/z* 1961 for $C_{10} + H_2O$).

Conclusions

Our results lend support to our original hypothesis, namely, that high-quality MALDI-TOF spectra of intractable polymers can be obtained using matrices containing powerfully solvating groups. These groups pry the aggregated polymer chains apart and make it easier for the individual ionized molecules to fly. We demonstrated the power of this concept with aromatic polyesters, but it should apply to other intractable polymers as well.

Additional studies are required to further investigate the ionization mechanism including the role of the cationization agents, such as alkali ions and/or silver cation. The formation of sodiated and potassiated molecules under our present conditions is dominant; thus, we assume that the "prying apart" mechanism is coupled with cationization in the MALDI plume. Direct electron transfer giving the radical cations or anions of the investigated polymers was also anticipated on the basis of the laser desorption ionization of the pure matrices, but related ions were not detected in the MALDI-TOF spectra. These mechanistic studies are currently in progress in our laboratories.

Experimental Section

Equipment and Instrumentation. Melting points were recorded on an Electrothermal capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AM-250 (250 MHz) instrument. ^{19}F NMR spectra were recorded on a Varian Unity-300 (282 MHz) instrument. ^{1}H NMR chemical shifts are reported in units of ppm relative to tetramethylsilane, and ^{19}F NMR chemical shifts are reported in units of ppm relative to hexafluorobenzene. The solvents are indicated in parentheses before the chemical shift values.

All materials and solvents were reagent grade and used as received from Aldrich without further purification unless otherwise indicated. Tetrahydrofuran was dried by distillation over sodium/benzophenone. Vectra was obtained from Aldrich. Other polymer samples were obtained as indicated in the Acknowledgements or synthesized.

4-Carboxybenzenediazonium Tetrafluoroborate.[22] To a stirred solution of boron trifluoride etherate (2.13 g, 15 mmol) cooled at −15 °C was added a solution of 4-aminobenzoic acid (1.37 g, 10 mmol) in 20 mL of dry THF. After the addition, a solid amine−BF_3 complex had formed, and additional THF (10 mL) was added to produce a homogeneous solution. Then, a THF solution (10 mL) of *tert*-butyl nitrite (1.24 g, 12 mmol) was added dropwise to the vigorously stirred reaction solution over a 15 min period. Following complete addition, the temperature of the reaction solution was maintained at −15 °C for 10−15 min and then slowly warmed to 5 °C to allow complete precipitation. Pentane (100 mL) was then added to the reaction mixture, and the solid was suction-filtered and washed with cold ether. Recrystallization from a mixture of acetonitrile and ether (1:3) gave diazonium salt (2.05 g, 86%). ^{1}H NMR (CD_3CN, δ): 8.92 (d, 2H, $^3J = 8.7$ Hz, $H_{2,6}$), 8.51 (d, 2H, $^3J = 8.7$ Hz, $H_{3,5}$).

Methyl 4-(Diethoxyphosphorylmethyl)benzoate.[27] A mixture of methyl 4-(bromomethyl)benzoate (12.5 g, 54.5 mmol) and triethyl phosphite (20 mL, 110 mmol) was stirred at 160 °C under an argon atmosphere for 2 h. The excess triethyl phosphite was removed by distillation in vacuum. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 95:5) to afford the phosphonate in 95% yield as colorless oil. ^{1}H NMR ($CDCl_3$, δ): 7.97 (d, 2H,$^3J = 8.4$ Hz, C_6H_4), 7.36 (dd, 2H,$^3J = 8.4$ Hz, and $^4J_{PH} = 2.5$ Hz, C_6H_4), 4.01 (m, 4H, OCH_2CH_3), 3.89 (s, 3H, OCH_3), 3.18 (d, 2H, $J_{PH} = 22.1$ Hz, CH_2P), 1.23 (t, 6H, $^3J = 8.2$ Hz, OCH_2CH_3).

4-(2′,3′,5′,6′-Tetrafluoro-4′-hydroxyphenylazo)benzoic Acid (1). To a solution of 2,3,5,6-tetrafluorophenol (1.58 g, 9.5 mmol) in 20 mL of sulfolane was added potassium carbonate (1.382 g, 10 mmol), and the reaction mixture was stirred at room temperature for 35 min. Then, 4-carboxybenzenediazonium tetrafluoroborate (2 g, 8.5 mmol) was added in portions to the phenoxide solution over 5 min, and the mixture was heated at 80 °C for 1 h. After cooling, HCl (6 M, 50 mL) was added, and the solid was filtered and washed several times with water and water/methanol (1:1). Recrystallization from THF/hexane gave **1** (2.13 g, 80%) as a red-orange solid, mp 264 °C. ^{1}H NMR (DMSO-d_6, δ): 8.22 (d, 2H, $^3J = 8.6$ Hz, $H_{2,6}$), 7.97 (d, 2H, $^3J = 8.6$ Hz, $H_{3,5}$). ^{19}F NMR (300 MHz, DMSO-d_6, δ): −151.8 (F-2,6), −164.05 (F3,5). MS: 315 MH^+.

Methyl 4-(2′,3′,5′,6′-Tetrafluoro-4′-hydroxyphenylazo)benzoate (2). 4-(Tetrafluoro-4′-hydroxyphenylazo)benzoic acid (1.57 g, 5 mmol) and TsOH (ca. 50 mg) were stirred at reflux for 18 h in anhydrous methanol (75 mL). After evaporation of methanol in vacuo, the residue was dissolved in CH_2Cl_2. The resulting organic solution was washed with water, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/EtOAc = 95:5) to give **2** (1.49 g, 91%) as an orange solid, mp 202 °C. ^{1}H NMR (DMSO-d_6, δ): 8.14 (d, 2H, $^3J = 8.6$ Hz, $H_{2,6}$), 7.91 (d, 2H, $^3J = 8.6$ Hz, $H_{3,5}$), 3.89 (s, 3H, $COOCH_3$). ^{19}F NMR (300 MHz, DMSO-d_6, δ): −151.8 (F-2,6), −164.05 (F3,5). MS: 327.1 [$(M-H)^-$, 100%].

Decafluoroazobenzene (3).[24] A suspension of pentafluoroaniline (5 g, 27.3 mmol) in 150 mL of a 10.25% NaOCl solution was stirred vigorously at room temperature overnight. The mixture was extracted with ether (3 × 100 mL), and the combined extracts were washed five times with water, dried over $MgSO_4$, and concentrated to give a red-orange solid. Recrystallization from ethanol gave **3** (1.2 g, 24%) as a red-orange crystals, mp 142 °C. ^{19}F NMR (300 MHz, $CDCl_3$, δ): −153.38 to −153−67 (m, 3F), −166.29 to −166.49 (m, 2F). MS: 362 ($M^{\bullet+}$, 54%), 195 ($C_6F_5N_2^+$, 47%), 167 ($C_6F_5^+$, 100%).

4,4′-Dihydroxyoctafluoroazobenzene (4).[23] To a stirred solution of decafluoroazobenzene (1 g, 2.76 mmol) in toluene (15 mL) was added a solution of KOH (7.5 g) in H_2O (15 mL) followed by *n*-Bu_4NHSO_4 (1.87 g, 5.52 mmol). The reaction mixture was stirred at room temperature for 1.5 h. Ether (100 mL) was added, followed by 2 M H_2SO_4 (60 mL). The organic phase was separated and again washed with 2 M H_2SO_4 and then with water (2 × 60 mL) and dried over $MgSO_4$. After evaporation of the solvents, the residue was recrystallized from EtOH/H_2O = 1:2 to give **4** (0.6 g, 60%) as an orange powder, mp 224 °C. ^{19}F NMR (300 MHz, DMSO-d_6, δ): −152.13 (F-2,6), −162.83 (F-3,5). MS: 358 ($M^{\bullet+}$, 54%).

4,4′-Dimethoxyoctafluoroazobenzene (5). A solution of **4** (0.1 g, 280 μmol) in *N*,*N*-dimethylacetamide (5 mL) was cooled at 0 °C under argon, and potassium carbonate (116 mg, 840 μmol) was added. Excess methyl iodide (5 mL) was added, and the reaction mixture was stirred overnight at room temperature, poured into water (10 mL), and extracted with CH_2Cl_2 (3 × 15 mL). The combined extracts were washed with water and brine and dried over Na_2SO_4. After removal of solvents, the residue was purified by column chromatography (silica gel, CH_2Cl_2/gradient methanol, 5%) to afford **5** (102 mg, 95%) as orange-red crystals, mp 217 °C. ^{1}H NMR ($CDCl_3$, δ): 3.75 (s, 6H, OCH_3). ^{19}F NMR (300 MHz, DMSO-d_6, δ): −153.29 (F-2,6), −164.10 (F-3,5).

Decafluorostilbene (6). To a stirred suspension of $TiCl_4$ (1.2 mL, 11 mmol) in dry THF (prepared by slow addition of $TiCl_4$ to THF (50 mL) under Ar at 0 °C), was added zinc (1.50 g, 23 mmol), and the mixture was stirred at 0 °C for 1 h. A solution of pentafluorobenzaldehyde (1.96 g, 10 mmol) in THF (15 mL) was added dropwise over 10 min, and the reaction mixture was heated at reflux for 2 h. After cooling, water (100 mL) was added and the mixture extracted with CH_2Cl_2 (2 × 100 mL). The combined extracts were washed with water and brine, dried over $MgSO_4$, and evaporated in vacuo. The residue was purified by column chromatography on silica gel, eluting with petroleum ether, to give **6** (1.26 g, 70%) as a white solid, mp 172 °C. ^{1}H NMR ($CDCl_3$, δ): 7.09 (s, 2H, $CH{=}CH$). ^{19}F NMR (300 MHz, DMSO-d_6, δ): −147.83 to −147.93 (m, 2F), −157.8 (t, 1F, $J = 21$ Hz), −165.9 to −166.1 (m, 2F).

4,4′-Dihydroxyoctafluorostilbene (7). The same procedure as described for **4**, starting from decafluorostilbene (**6**, 1.08 g, 3 mmol), KOH (8 g), and *n*-Bu_4NHSO_4 (2.03 g, 6 mmol). After workup, the residue was recrystallized from methanol to give **7** (0.62 g, 58%) as a light yellow solid, mp 128−130 °C. ^{1}H NMR ($CDCl_3$, δ): 7.08 (s, 2H, $CH{=}CH$). ^{19}F NMR (300 MHz, DMSO-d_6, δ): −143.6 to −148.8 (m, 2F), −162.3 to −163.3 (m, 2F).

Methyl 4-(β-Pentafluorophenylvinyl)benzoate (8). To a stirred solution of methyl 4-(diethoxyphosphorylmethyl)benzoate (3.43 g, 12 mmol) in dry THF (80 mL) under Ar was added *t*-BuOK (1.58 g, 15 mmol) in several portions at 0 °C. The mixture was stirred at 0 °C for 30 min, a THF solution (20 mL) of pentafluorobenzaldehyde (1.96 g, 10 mmol) was slowly added, and the mixture was stirred for 2 h at room temperature. After evaporation of THF, the residue was dissolved in CH_2Cl_2. The solution was washed with water, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2) to give **8** (2.46 g, 75%) as a white solid, mp 148−150 °C. ^{1}H NMR ($CDCl_3$, δ): 8.04 (d, 2H, $^3J = 8.3$ Hz, $H_{3,5}$), 7.58 (d, 2H, $^3J = 8.3$ Hz, $H_{2,6}$), 7.43 (d, 1H, $^3J = 16.8$ Hz, $CH{=}$), 7.12 (d, 1H, $^3J = 16.8$ Hz, $CH{=}$), 3.93 (s, 3H, $COOCH_3$). ^{19}F NMR (300 MHz, $CDCl_3$, δ): −142.6 to −144.2 (m, 3F), −156.9 to −159.1 (m, 1F), −163. 3 to −163.4 (m, 1F). MS: 327.1 [$(M-H)^-$, 100%].

4-[β-(2′,3′,5′,6′-Tetrafluoro-4′-hydroxyphenyl)vinyl]benzoic Acid (9). The same procedure as described for **4**, starting from 0.99 g (3 mmol) of methyl 4-(2-pentafluorophenylvinyl)benzoate (**8**), KOH (4 g), and *n*-Bu_4NHSO_4 (1.02 g, 3 mmol). After workup, the residue was recrystallized from methanol to give **9** (0.53 g, 56%) as a white solid, mp 186 °C. ^{1}H NMR (DMSO-d_6, δ): 8.12 (d, 2H, $^3J = 8.3$ Hz, $H_{3,5}$), 7.63 (d, 2H, $^3J = 8.3$ Hz, $H_{2,6}$), 7.46 (d, 1H, $^3J = 16.8$ Hz, C*H*=), 7.2 (d, 1H, $^3J = 16.8$ Hz, C*H*=). ^{19}F NMR (300 MHz, DMSO-d_6, δ): -144.06 to -144.2 (m, F-2,3), -157.3 to -158.3 (m, F-5,6). MS: 312 ($M^{\bullet+}$, 24%).

Methyl 4-[β-(2′,3′,5′,6′-Tetrafluoro-4′-hydroxyphenyl)vinyl]-benzoate (10). **9** (0.35 g, 1.12 mmol) and TsOH (ca. 25 mg) were stirred at reflux for 6 h in anhydrous methanol (45 mL). After evaporation of methanol under reduced pressure, the residue was dissolved in CH_2Cl_2. The resulting organic solution was washed with water, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/EtOAc = 95:5) to give **10** (0.33 g, 90%) as a white solid, mp 110−111 °C. ^{1}H NMR (DMSO-d_6, δ): 8.01 (d, 2H, $^3J = 8.3$ Hz, $H_{3,5}$), 7.54 (d, 2H, $^3J = 8.3$ Hz, $H_{2,6}$), 7.38 (d, 1H, $^3J = 16.8$ Hz, C*H*=), 7.11 (d, 1H, $^3J = 16.8$ Hz, C*H*=), 3.92 (s, 3H, COOC*H*$_3$). ^{19}F NMR (300 MHz, DMSO-d_6, δ): -144.08 to -144.22 (m, F-2,3), -157.35 to -158.37 (m, F-5,6). MS: 325.2 [$(M-H)^-$, 100%].

Poly(3-ethyleneoxybenzoate) (3EOB). In a 25 mL round-bottom flask were placed 3-hydroxybenzoic acid (5.6 g, 41 mmol), ethylene carbonate (3.52 g, 40 mmol), stannous octanoate (0.1 mL), and a magnetic stirring bar. The flask was fitted with a distillation head with two thermometers and a receiver and placed in a thermally stable oil bath (Syltherm 800). The exit port led to a flask containing saturated aqueous barium hydroxide solution. Heating was performed for 50 min total. At 100 °C the mixture was homogeneous, and at 200 °C heavy precipitation of barium carbonates demonstrated CO_2 evolution. At 260 °C bath temperature, a lower head temperature of 208 °C showed that ethylene glycol was refluxing. At 285 °C bath temperature this ceased, and polymerization was completed at 300 °C for 15 min. The flask cooled to a viscous, fiber-forming mass. Chilling led to glassy pale brown polymer (4.50 g), which was ground up and partitioned between 5% aqueous potassium bicarbonate and ether. Only slight CO_2 evolution occurred. The mixture was filtered and dried to give 3EOB (2.36 g, 36%).

MALDI-TOF Measurements. All the matrices were tested by laser desorption ionization (LDI). For LDI studies we used both THF solutions of the matrices and direct deposition of solid matrices on the MALDI plate. The aromatic polyesters Vectra, poly(ethylene terephthalate) (PET), 1,4-dimethylenecyclohexane terephthalate (CHDT), and poly(3-ethyleneoxybenzoate) (3EOB) were studied using our synthesized MALDI matrices. Solvents investigated included 2,3,5,6-tetrafluorophenol (TFF), pentafluorophenol (PFF), hexafluoroisopropanol (HFIP), trifluoroacetic acid (TFA), and bis-(3,5-trifluoromethyl)phenol (BTFF). The matrices were dissolved in tetrahydrofuran (THF) in a concentration range of about 0.1 M. LDI and MALDI-TOF experiments were carried out on a Bruker Reflex III instrument with a N_2 laser (337 nm). For the polymer studies, the matrix solution was spotted first followed by spiking the matrix with the polymer solutions. Both the linear and reflectron ion detection techniques were applied. For systematic studies on the polymer 3EOB, the laser attenuation was kept at 80% and 67% for azobenzene and stilbene matrices, respectively.

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References and Notes

(1) Bahr, U.; Deppe, A.; Karna, M.; Hilenkamp, F. Mass Spectrometry of Synthetic Polymers by UV-Matrix-Assisted Laser Desorption/Ionization. *Anal. Chem.* **1992**, *64*, 2866−2869.

(2) Nielen, M. W. F. MALDI Time-of-Flight Mass Spectrometry of Synthetic Polymers. *Mass Spectrom. Rev.* **1999**, *18*, 309−344.

(3) *Mass Spectrometry of Polymers;* Montaudo, G., Lattimer, R. P., Eds.; CRC Press: Boca Raton, FL, 2002.

(4) Montaudo, G.; Samperi, F.; Montaudo, M. S. Characterization of Synthetic Polymers by MALDI-MS. *Prog. Polym. Sci.* **2006**, *31*, 277−357.

(5) Yalcin, T.; Day, Y.; Li, L. Matrix-assisted Laser desorption/ionization time-of-flight mass spectrometry for polymer analysis: solvent effect in sample preparation. *J. Am. Soc. Mass Spectrom.* **1998**, *9*, 1303−1310.

(6) Skelton, R.; Dubois, F.; Zenobi, R. M. MALDI Sample Preparation Method Suitable for Insoluble Polymers. *Anal. Chem.* **2000**, *72*, 1707−1710.

(7) Zenobi, R.; Breudke, K.; Knochenmuss, K.; Lehmann, E.; Steriensoh, E. Fundamental and Application of Maldi Mass Spectrometry. *Adv. Mass Spectrom.* **2001**, *15*, 143−149.

(8) Gies, A. P.; Nonidez, W. K.; Anthanstein, M.; Cook, R. C.; Mays, J. W. *Rapid Commun. Mass Spectrom.* **2002**, *16*, 1903−1910.

(9) Trimpin, S.; Rouhanipour, A.; Az, R.; Raeder, H. J.; Muellen, K. New Aspects: Matrix-Assisted Laser Desorption/Ionization Time-of-flight Mass Spectrometry; a Universal Solvent-free Sample Preparation. *Rapid Commun. Mass Spectrom.* **2001**, *15*, 1364−1373.

(10) Kricheldorf, H. R.; Schwartz, G. Cyclic Polymers by Kinetically Controlled Step-Growth Polymerization. *Macromol. Rapid Commun.* **2004**, *24*, 359−381.

(11) Jackson, A. T.; Yates, H. T.; MacDonald, W. A.; Scrivens, J. H.; Crtichley, G.; Brown, J.; Deery, M. J.; Jonniga, K. B.; Brookes, C. Time-Lag Focusing and Cation Attachment in the Analysis of Synthetic Polymers by Matrix Assisted Laser Desorption/Ionization-Time-of-Flight Mass Spectrometry. *J. Am. Soc. Mass Spectrom.* **1997**, *8*, 132−139.

(12) Glidden, J.; Wyttenbach, T.; Batka, J. J.; Weis, P.; Jackson, A. T.; Scrivens, J. H.; Bowers, M. T. (Poly Ethylene Terephthalate) Oligomers Cationized by Alkali Ions: Structures Energetics and Their Effect on Mass Spectrum and the Matrix-Assisted Laser Desorption/Ionization Process. *J. Am. Soc. Mass Spectrom.* **1999**, *10*, 883−895.

(13) Scheirs, J. In *Chemistry and Technology of Polyesters and Copolyesters*; Scheirs, J., Long Muellen, T. E., Eds.; Wiley: New York, 2002.

(14) Blais, J. C.; Tessier, M.; Bolbach, G.; Remaud, B.; Rozes, L.; Guittard, J.; Brunot, A.; Marechal, E.; Tabet, J. C. Matrix-Assisted Laser Desorption Ionization Time of Time-of-Flight Mass Spectrometry of Synthetic Polyesters. *Int. J. Mass Spectrom. Ion Processes* **1995**, *144*, 131−8.

(15) Ciolacu, F. C. L.; Choudhury, N. R.; Dutta, N.; Voelcker, N. H. MALDI-TOF MS and DIOS-MS Investigation of the Degradation and Discoloration of Poly(ethylene terephthalate). *Macromolecules* **2006**, *39*, 7872−7881.

(16) Somogyi, A.; Bojkova, N.; Padias, A. B.; Hall, H. K., Jr. Analysis of All-Aromatic Polyesters by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry. *Macromolecules* **2005**, *38*, 4067−4071.

(17) Elandaloussi, E.-H.; Somogyi, A.; Padias, A. B.; Bates, R. B.; Hall, H. K., Jr. Rearrangements of Comonomer Units of Well-Defined Vectra Oligomers during MALDI-TOF Mass Spectral Measurements. *Macromolecules* **2006**, *39*, 6913−6923.

(18) Danis, P. A.; Karr, D. E. A Facile Sample Preparation for the Analysis of Synthetic Organic Polymers by Matrix-assisted Laser Desorption/Ionization. *Org. Mass Spectrom.* **1993**, *28*, 923−925.

(19) Hoteling, A. J.; Erb, W. J.; Tyson, R. J.; Owen, K. G. Exploring the Importance of the Relative Solubility of Matrix and Analyte in Maldi Sample Preparation Using HPLC. *Anal. Chem.* **2004**, *76*, 5157−5164.

(20) Hoteling, A. J.; Mourey, T. H.; Owens, K. G. Importance of Solubility in the Sample Preparation of Poly(Ethylene Terephthalate) for Maldi-TOF MS. *Anal. Chem.* **2005**, *77*, 750−756.

(21) Mirza, S. P.; Raju, N. P.; Vairamani, M. Estimation of the Proton Affinity Values of Fifteen Matrix-Assisted Laser Desorption/Ionization Matrices under Electrospray Ionization Conditions Using the Kinetic Method. *J. Am. Soc. Mass Spectrom.* **2004**, *15*, 431−435.

(22) Doyle, M. P.; Bryker, W. J. Alkyl Nitrite-Metal halide Deamination Reaction. 6. Direct Synthesis of Arenediazonium Tetrafluoroborate

Salts From Aromatic Amines, Tert-Butyl Nitrite, And Boron Trifluoride Etherate In Anhydrous Media. *J. Org. Chem.* **1979**, *44*, 1572−4.

(23) Jarman, M.; Barrie, S. E.; Deadman, J. J.; Houghton, J.; McCague, R.; Rowlands, M. G. Hydroxyperfluoroazobenzenes: Novel Inhibitors of Enzymes of Androgen Biosynthesis. *J. Med. Chem.* **1990**, *33*, 2452.

(24) Burdon, J. C.; Morton, J.; Thomas, D. F. Aromatic Polyfluoro Compounds Part XXIII: Polyfluoroazo, -azoxy and -hydrazo-benzene. *J. Chem. Soc.* **1965**, 2621.

(25) Deadman, J. J.; Jarman, M.; McCague, R. A Benzoquinone Diimine from the Oxidation of Pentafluoroaniline by Hypochlorite. *J. Chem. Soc., Perkin Trans. 2* **1989**, 971.

(26) (a) McMurry, J. E. Carbonyl-Coupling Reactions Using Low-Valent Titanium. *Chem. Rev.* **1989**, *89*, 1513. (b) Furstner, A.; Bogdanovic, B. New Developments in the Chemistry of Low-Valent Titanium. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2443.

(27) Duranti, E. N. A Multifunctional Photorefractive Material. *Synth. Commun.* **1999**, *29*, 4201.

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