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Application of Stored Waveform Ion Modulation 2D-FTICR MS/MS to the Analysis of Complex Mixtures

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Component identification of complex mixtures, whether they are from polymeric formulations or combinatorial synthesis, by conventional MS/MS techniques generally requires component separation by chromatography or mass spectrometry. An automated means of acquiring simultaneous MS/MS data from a complex mixture without prior separation is obtained from stored waveform ion modulation (SWIM) two-dimensional FTICR MS/MS. The technique applies a series of SWIFT excitation waveforms whose frequency domain magnitude spectrum is a sinusoid increasing in frequency from one waveform to the next. The controlled dissociation of the precursor ions produces an associated modulation of the product ion abundances. Fourier transformation of these abundances reveals the encoded modulation frequency from which connectivities of precursor and product ions are observed. The final result is total assignment of product ions for each precursor ion in a mixture from one automated experiment. We demonstrated the applicability of SWIM 2D-FTICR MS/MS to two diverse samples of industrial importance. We characterized structured polyester oligomers and products derived from combinatorial synthesis. Fragmentation pathways identified in standard serial ion isolation MS/MS experiments were observed for trimethylolpropane/methyl hexahydrophthalic anhydride. A 20-component sample derived from combinatorial synthesis was fragmented, and the template ion along with another key fragment ion was identified for each of the 20 components.

The performance of commercially available products is naturally predicated upon the molecular structure of the materials whether it is for organic coatings or medicinal agents. The exact molecular structure of these compounds is important for structure−property/activity relationships. Ionization techniques such as electrospray, matrix-assisted laser desorption, direct laser desorption, and atmospheric pressure chemical ionization afford molecular ions from which a molecular formula can be derived. The experimentally obtained molecular formula coupled with a knowledge of the chemistry allows one to postulate structures for unknowns and verify chemistries of targeted compounds. Further, product/precursor ion profiles obtained by MS/MS experiments provide data for evaluation of the proposed structures. The following paragraphs describe the importance of the exact molecular structure in the automotive and pharmaceutical industries, respectively.

Due to environmental pressure, there is a continuing need to reduce volatile organic compound emissions in automotive finishes, which cure under ambient conditions, while still affording outstanding performance characteristics such as resistance to environmental etching. This concern is particularly true in a typical small auto body paint/repair shop. Further, these shops prefer to dry and cure such coatings as fast as possible. One such approach involves using a low molecular weight polyester linear or branched cycloaliphatic moiety-containing oligomer.[1] These hydroxyl functional oligomers formulated at nearly 100% solids can be rapidly cross-linked with isocyanates, therefore satisfying the needs of the body shop and government mandates regarding emissions. As we move to lower molecular weight building blocks, the exact structure, for example, functionality, is more critical to ensuring the targeted properties than for the higher molecular weight predecessors. Single-stage soft ionization mass spectrometry provides structural information such as repeat unit, end groups, and chain length. Tandem mass spectrometry corroborates structure assignments and therefore can also be used in support of patents. Due to the complexity of these samples, most often chromatography is coupled to mass spectrometry to reduce the number of components entering the mass spectrometer at any given time.[2]

In the pharmaceutical industry, drug discovery of lead compounds has been expedited by innovations in combinatorial synthesis providing structurally diverse libraries of a multitude of compounds in a short time.[3−7] As such, structure−activity relationships can be probed quickly to find a lead compound

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whose structure can be fine-tuned by the synthetic chemist. The implementation of advanced analytical techniques such as high-performance liquid chromatography/mass spectrometry with electrospray ionization provide for the identification and characterization of these complex mixtures in serial fashion, i.e., one component at a time, if they can be chromatographically separated.[8−10] A multicomponent analytical technique parallel to drug discovery by combinatorial synthesis instead of the one component at a time strategy would be beneficial.

Component identification of complex mixtures, whether they are from polymeric formulations or combinatorial synthesis, by conventional MS/MS techniques generally requires component separation by chromatography or mass spectrometry. The non-destructive method of ion detection along with the trapped ion capabilities of Fourier transform ion cyclotron resonance mass spectrometry (FTICR MS/MS) provides a unique way in which to interrogate ions. An automated means of acquiring simultaneous MS/MS data is obtained from three unique two-dimensional FTICR MS/MS experimental schemes. All components of a mixture are fragmented simultaneously, and the resultant product ions are subsequently linked to their precursor ion. In 1987, Pfandler et al.[11] demonstrated a method by which two identical excitation pulses separated by an incrementally time-varying delay caused precursor ions to obtain varying cyclotron radii and hence caused differing degrees of fragmentation. After multiple mass spectra were acquired, a second Fourier transform revealed connectivities of product−precursor ions. The method showed proof of concept but required inconveniently short increments. McLafferty et al.[12] and Williams et al.[13] demonstrated the use of a Hadamard transform FTICR MS/MS experiment yielding product−precursor ion connectivities. Unfortunately, implementation of this approach required prior knowledge of the mixture under investigation to generate the proper Hadamard masks. Stored waveform ion modulation (SWIM) 2D-FTICR MS/MS[14] used in this work does not suffer from these limitations. The technique applies a series of SWIFT excitation waveforms whose frequency domain magnitude spectrum is a sinusoid increasing in frequency from one waveform to the next. The controlled dissociation of the precursor ions produces an associated modulation of the product ion abundances. Fourier transformation of these abundances reveals the encoded modulation frequency from which connectivities of precursor and product ions are observed. The final result is total assignment of product ions for each precursor ion in a mixture from one automated experiment.

Initially, SWIM 2D-FTICR MS/MS was demonstrated on two chemical systems: acetone monomer-proton-bound dimer interconversion and proton transfer between pyrrolidine and 3-methylpyridine.[14] Subsequent work demonstrated simultaneous CAD MS/MS applicability to a bradykinin/angiotensin mixture ionized by matrix-assisted laser desorption[15] and an ion−molecule reaction study of uranium and thorium with tribenzocyclotriyne.[16] SWIM 2D-FTICR MS/MS was originally proposed for complex mixture analysis.[14] In this paper, we implement the technique to simultaneously fragment and characterize reactive oligomers,[1] which are precursors to urethane coatings,[17,18] and multicomponent samples derived from combinatorial synthesis.[19] The chemical systems discussed in the following examples represent simple introductory mixtures with diversity in mass and clean reactions with relatively few side products.

EXPERIMENTAL SECTION

Samples. (1) Reactive Oligomer Intermediate. The intermediate was prepared from reaction of trimethylolpropane (TMP; Perstorp Polyols, Inc., Toledo, OH) with methyl hexahydrophthalic anhydride (MHHPA; Milliken Chemical, Spartanburg, SC). See ref 1 for specific information regarding the synthesis. The commercial MHHPA is a 70:30 (w/w) mixture of methylated to nonmethylated HHPA. As such, the reaction produces a mixture of compounds differing in mass by 14 Da consisting of three major components (Scheme 1).

(2) Twenty-Component Sample Derived from Combinatorial Synthesis. Forty samples containing 20 components each were prepared in-house, in which two moieties, X and Y, were varied on the template molecule as follows (Scheme 2).[20] The benzimidazole aldehyde was reductively aminated with 20 different amines (X). After 2.5 days, the reactions were combined, filtered, and concentrated. The 20-component mixtures were distributed into 40 wells. To each well was added a unique aldehyde (Y). Each well represents 20 different X's and a specific Y, thus yielding 800 potential products. The aldehyde shown in Scheme 2 was used for this study.

Instrumentation. (1) 3-T Finnigan NewStar FTMS. The conventional one-dimensional MS/MS data for the polymer cross-linker were obtained from direct CO_2 laser desorption FTMS with potassium ion doping.[21] The 2D-SWIM experiments were performed on the Finnigan instrument because of the proprietary SWIFT software and hardware necessary to generate and execute the SWIFT waveforms. The second-generation Finnigan electrospray source was used to generate the ions. Samples were delivered via direct infusion with a syringe pump at 1−2 μL/min. The capillary voltage was set to 3 keV for all experiments. Nitrogen was used as a sheath gas and argon as the ion capture and collision gas.

(2) 3-T Bruker BioApex FTMS. Direct infusion at 1 μL/min to the Analytica electrospray source of the BioApex FTMS was

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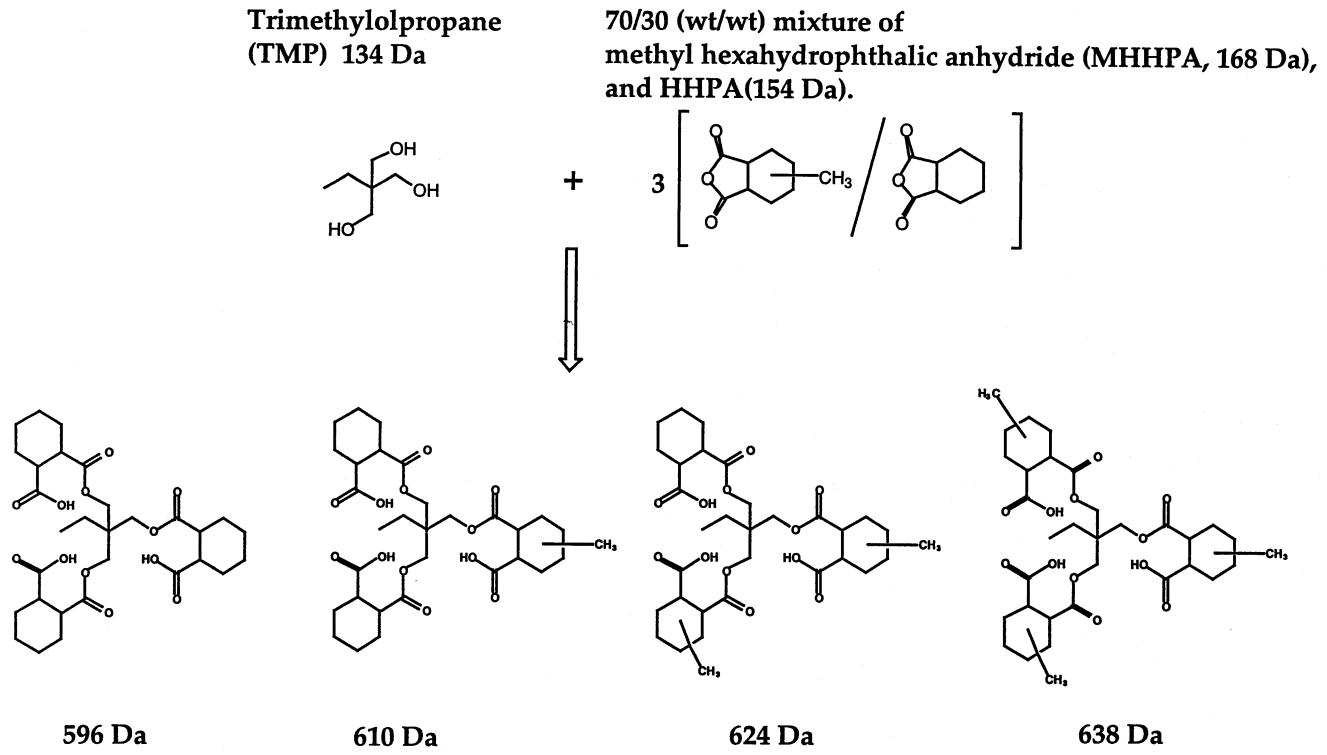
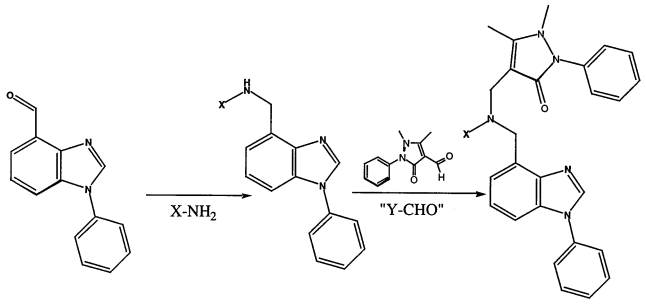
Scheme 1. Synthesis of the Reactive Oligomer Intermediate



Scheme 2. Synthesis of the Benzimidazole Library



used to acquire the conventional one-dimensional MS/MS data for each component of the combinatorial sample. Nitrogen was used as the electrospray sheath gas and the collision gas.

Experiments. (1) Conventional MS and MS/MS of the Reactive Oligomer Intermediate (Sample 1). The intermediate was dissolved in tetrahydrofuran (THF) to yield ~10% (w/v) concentration. One hundred microliters of solution was deposited on a cellulose substrate disk (Grade 474 filter paper, 28306-380, VWR, Media PA) followed by 50 μL of a saturated KI solution in THF as a source of potassium. The sample was dried at 100 °C for 15 min prior to introduction to the high-vacuum system of the Finnigan NewStar FTMS. A more detailed description of sample preparation is given in ref 22. Sample analysis is initiated by laser desorption of the material and potassium salts, which undergo ion/molecule reactions to produce cationized molecules, all of which were singly charged monopotassiated. For specifics of the cationization process, consult ref 23. Collisionally activated dissociation (CAD) is accomplished through sustained off-resonance irradiation (SORI)[24] at a frequency offset of −1800 Hz from the unperturbed cyclotron frequency of the precursor ion for 1 s with argon gas admitted via a pulse valve assembly to a pressure of 7 $\times$ 10^{-7} Torr. After a 5-s delay, the ion population is sampled by use of linear sweep excitation and broadband detection.[21]

(2) SWIM 2D-FTICR MS/MS of the Reactive Oligomer Intermediate. The intermediate was dissolved in THF to a concentration of ~50 μg/mL; sodium iodide was added to the THF solution to a concentration of 15 μg/mL (100 μM). A total of 128 stored waveform ion modulation SWIFT excitation waveforms were generated so that the precursor ions' cyclotron frequencies were covered in the higher modulation portion of the SWIM waveform. The only information needed prior to analysis is the precursor ion frequency bandwidth to thus ensure the precursor frequencies fall within the modulation range of the SWIM waveform. The SWIM modulation occurred from approximately m/z 600 to 940 and the precursor ions of interest were in the range of m/z 600−650. Generation of new SWIM waveforms is not necessary between samples as long as the ions to be interrogated fall within the mass range of the SWIM waveforms. The SWIM waveforms were generated using code written in-house with PV-WAVE (Visual Numerics, Inc., Houston, TX).

A graphical representation of the SWIM 2D-FTICR MS/MS experimental pulse sequence is given in Scheme 3. The radio frequency irradiation inducing fragmentation is modified from the conventional FTICR MS/MS pulse sequence, in that SORI is replaced by the SWIM excitation waveforms and the ion isolation pulse is removed.

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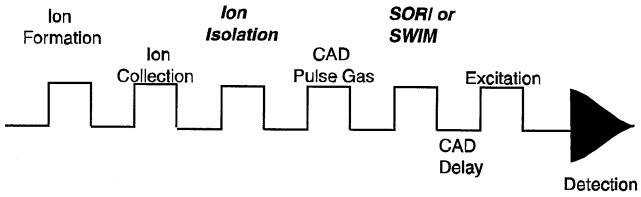
Scheme 3. Conventional and 2D-SWIM Pulse Sequence



The automated SWIM experiment proceeds as follows. After ES ionization and ion capture,[2] a pulse of collision gas is admitted into the ICR cell to a pressure of ~8 × 10^{-7} Torr. The SWIM excitation pulse is triggered. After a 3-s delay for CAD and removal of collision gas, the resultant ion population is sampled by use of linear sweep excitation and broadband detection. Each free ion decay (FID) represents the sum of four transients of 16 384 data points. All conditions are held constant for the next experiment except that *the next SWIM excitation pulse* is used, which consists of an additional half period sine wave modulation. The half-period sine wave increase provides the second-dimension encoding of the precursor and product ions.[14] After that FID is collected, the next SWIM waveform is used until all FIDs generated from unique SWIM excitation waveforms are collected. The 128 FID data sets of the Finnigan Odyssey files were extracted from the Odyssey file format, converted to Bruker NMR data format, and imported to a Bruker (Billerica, MA) NMR data station for 2D-FT data processing.

(3) Conventional MS and MS/MS of Combinatorial Components (Sample 2). The sample containing 20 components derived from combinatorial chemistry was presented in acetonitrile/water/acetic acid (70:30:0.1, % v/v) at 0.1 mg/mL total material and subsequently diluted by a factor of 50 yielding a final concentration of ~200 nmol L^{-1} per component.

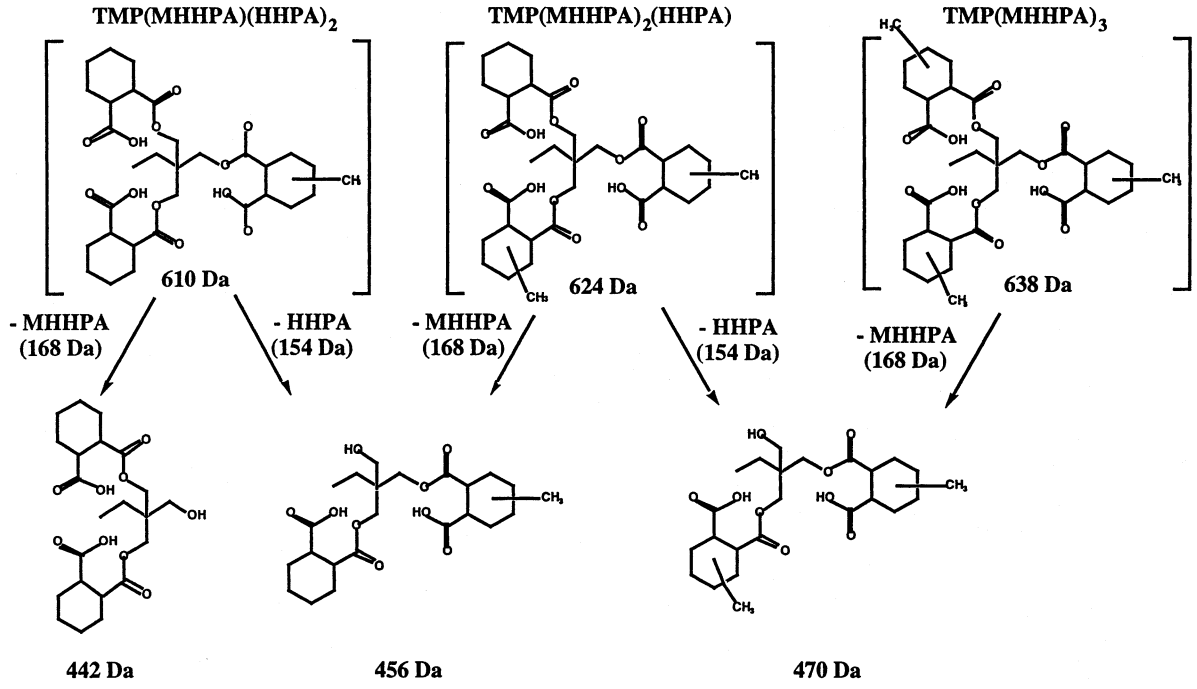
One microliter of solution was consumed for each electrospray experiment. CAD MS/MS data were obtained by use of SORI (−300 Hz offset) with nitrogen collision gas on the BioApex FTMS.[19]

(4) SWIM 2D-FTICR MS/MS of Combinatorial Components. With the increased sample complexity of the 20-component mixture, each FID obtained for this sample mixture represents the sum of eight transients of 16 384 data points to increase signal magnitude and reduce signal fluctuations. To maintain experimental time similar to that of the reactive oligomer intermediates, only 64 SWIM waveforms were used and hence 64 FIDs collected. All other experimental conditions are similar to that of experiment 2, SWIM 2D-FTICR MS/MS of the reactive oligomer intermediate.

RESULTS AND DISCUSSION

Conventional MS and MS/MS of the Reactive Oligomer Intermediate. To obtain structural information of molecules by use of mass spectrometry requires fragmentation of the molecule by some form of collisional process or high-energy ionization. If the sample is pure, then an in-source fragmentation process such as electron ionization or electrospray nozzle/skimmer dissociation may be used. However, in the event that the sample is a mixture, as are samples normally submitted for analysis, some means of separating/isolating the compounds are required such as chromatography or ion isolation. As an example for a simple mixture, the trifunctional reactive oligomer intermediate provides three major precursor ions, $[M + K]^+$, from m/z 649 $[610 + K]^+$ to 677 $[638 + K]^+$ with spacings of 14 Da because the HHPA reactant is a 70:30 (w/w) mixture of methylated to nonmethylated HHPA (see Schemes 1 and 4 and ref 21 for the MS and MS/MS data). MS/MS data are obtained by isolating each molecular ion in the ICR cell through rf ejection of all other ions. Subsequently, the isolated molecular ion is rf-excited and allowed to undergo collisions with argon gas for a few seconds. The remaining molecular ions and product ions are then excited and detected to reveal structural characteristics of the molecule. Each molecular ion for which structural information is needed must be individually isolated in turn and collisionally activated.

Scheme 4. Fragmentation Products of the Reactive Oligomer Intermediates



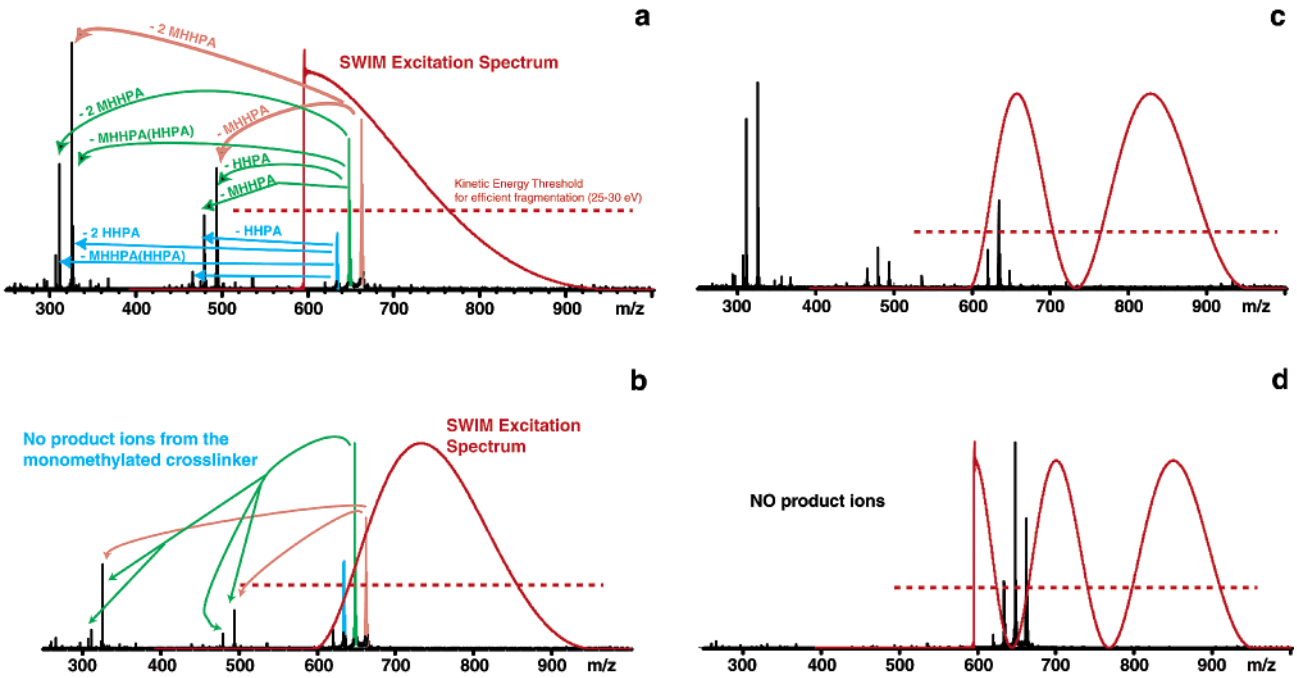


Figure 1. MS/MS data obtained from the application of SWIM excitation waveforms 1, 2, 4, and 5, respectively. The SWIM waveform is superimposed on each associated mass spectrum.

The CAD MS/MS mass spectrum of the monomethylated trifunctional acid cross-linker, m/z 649 $[M + K]^+$, yields two ions at m/z 481 $[442 + K]^+$ and 495 $[456 + K]^+$, representing the loss of MHHPA (168 Da) and HHPA (154 Da), respectively. In turn, the rf-isolated dimethylated compound at m/z 663 $[624 + K]^+$ provides ions at m/z 495 $[456 + K]^+$ and 509 $[470 + K]^+$, representing the loss of MHHPA and HHPA, respectively in the predicted ratio of 2:1. Finally, isolation and fragmentation of the trimethylated compound at m/z 677 yields as expected only one fragment ion of m/z 509 $[470 + K]^+$, the loss of MHHPA.

Under this methodology, fragmenting all three components simultaneously would not provide much useful information. Identical fragment ions exist between certain molecular ions (see Scheme 4). More importantly, it is ambiguous as to which molecular ion produced what fragment ions. Tagging ions by use of radiolabeling or isotopic labeling of starting materials would prove useless as these tags are associated with each final product and as such would not assist in differentiating product ion/precursor ion connectivities in the simultaneous fragmentation of this mixture.

Basics of SWIM Two-Dimensional FTMS MS/MS. An extrinsic methodology of tagging each molecular ion is provided by SWIM 2D-FTMS, thus allowing for the simultaneous fragmentation and characterization of all components in a mixture. The basic experiment is the same as that for conventional CAD MS/MS given above. The ion isolation step is removed and the SORI excitation is changed to a series of SWIFT excitation waveforms that have a unique sine wave encoded power spectrum.

In each experiment utilizing a specific SWIM waveform, ions of different mass are excited to different ICR orbital radii. As ions are excited to larger orbital radii, there is an associated increase in both the number of collisions and the collision energy, thereby providing an increase in the number of fragment ions. By varying the ion velocity (ICR orbital radii) in a systematic manner (modulation) for each molecular ion, the precursor ion population and the associated fragment ions will also vary systematically but 180° out of phase. In other words as the ion velocity (radii) increases, more fragment ions are produced and less molecular ions are observed; as one signal increases the other decreases. The series of SWIM encoded waveforms tag each molecular ion with a unique modulation frequency allowing one to deconvolute the encoded information by use of a second Fourier transformation. The frequency tag provides a means to elucidate which product ions originate from which precursors in the simultaneous CAD MS/MS analysis of a mixture.

Figure 1a shows the first SWIM encoded SWIFT excitation spectrum superimposed on the subsequent MS/MS mass spectrum obtained from the reactive oligomer intermediate mixture. In this case, all of the precursor ions are excited to ICR orbital radii in which sufficient fragmentation occurs. The dashed line of Figure 1a represents the kinetic energy threshold at which fragmentation is observed, which we estimate is 25−30 eV lab frame precursor ion translational energy. Fragment ions are observed in the m/z 480 and 320 ranges. The data are representative of simultaneously fragmenting all molecular ions in a standard CAD experiment. It is readily apparent that little useful information is provided by just this single MS/MS spectrum of the components in this mixture.

Figure 1b shows the MS/MS spectrum obtained when the second SWIM waveform is used to induce fragmentation on all components of the mixture. The SWIM excitation waveform power profile has increased by a half-period of a sine wave over that used in Figure 1a. No product ions are observed from the monomethylated cross-linker because these precursor ions have not reached the ICR orbital radii/kinetic energy necessary to fragment. In contrast, the trimethylated cross-linker has undergone sufficient fragmentation as its magnitude is below that of the dimethylated cross-linker. Note the selectivity in fragmentation

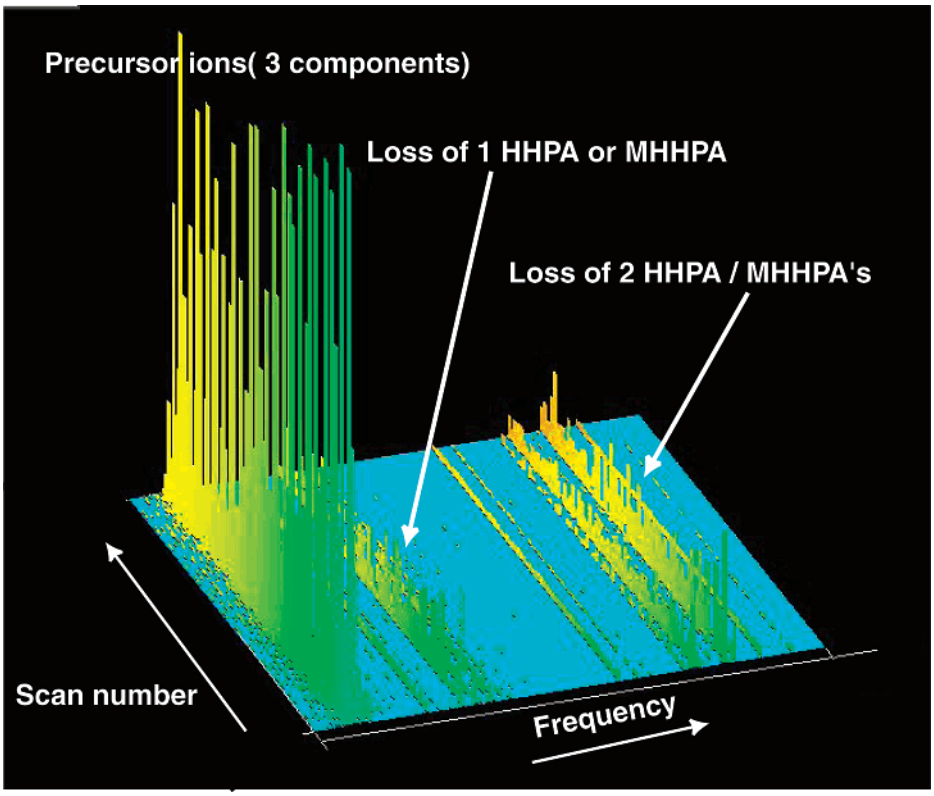


Figure 2. Representative series of the data obtained from the consecutive application of the SWIM waveforms. Modulation of the product and precursor ion abundances is observed.

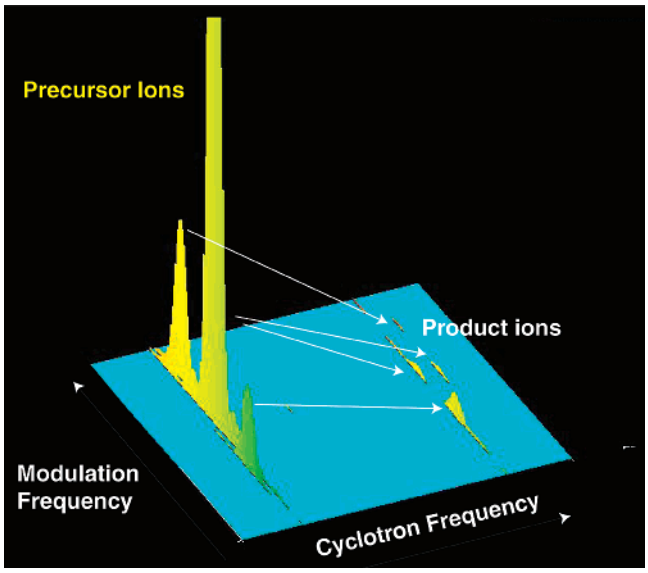


Figure 3. Power plot of the SWIM two-dimensional Fourier transform mass spectrum of the reactive oligomer intermediate.

extent provided by the SWIM excitation for these precursor species differing by 14 Da. Figure 1c shows the fourth SWIM excitation with two full periods used in this SWIFT rf-excitation pulse. Data set three is not shown for brevity. Almost all of the trimethylated cross-linker has fragmented as the rf-excitation energy imparted by the SWIM waveform is near its maximum at the cyclotron frequency of this molecular ion. Finally, Figure 1d shows that no product ions are observed for the fifth SWIM modulation waveform as all four of the molecular ions lie in a nodal region of the waveform. No energy was deposited into the molecular ions; thus fragment ions are not observed. Note that the mass domain displays of the SWIM excitation profiles are not equivalent in periodicity as the waveforms are encoded/generated in the frequency domain, and ICR frequency is inversely related to m/z.

Subsequent SWIM modulations are applied to the molecular ions in series. Each SWIM waveform profile increases by a half-period of a sine wave providing the extrinsically applied frequency tag.[14] FIDs are collected until all 128 SWIM excitation waveforms have been used.

Second-Dimension Fourier Transformation. A representative series of the SWIM MS/MS spectra acquired from the oligomer mixture are shown in Figure 2. Although it is readily apparent that the abundances of the molecular and fragment ions are systematically changing, it is not apparent which fragment ions are derived from which molecular ions. However, if we take the magnitude of a given data point, or m/z value, from each spectrum, then that new data set can be interpreted as an individual transient or "time" varying signal. Subsequent Fourier transformation of each of these "transients" in turn provides the second dimension. As an example, taking the magnitude value of the dimethylated molecular ion from each spectrum and Fourier transforming that data series will provide a frequency spectrum with a single observed frequency, that of the modulation frequency encoded by the SWIM waveform for the molecular ion. Fourier transformation of the data series representing the magnitude of the fragment ion at m/z 479 $[456 + Na]^+$ (representing one MHHPA and one HHPA remaining on the molecule) would show two signals at the modulation frequency of the mono- and the

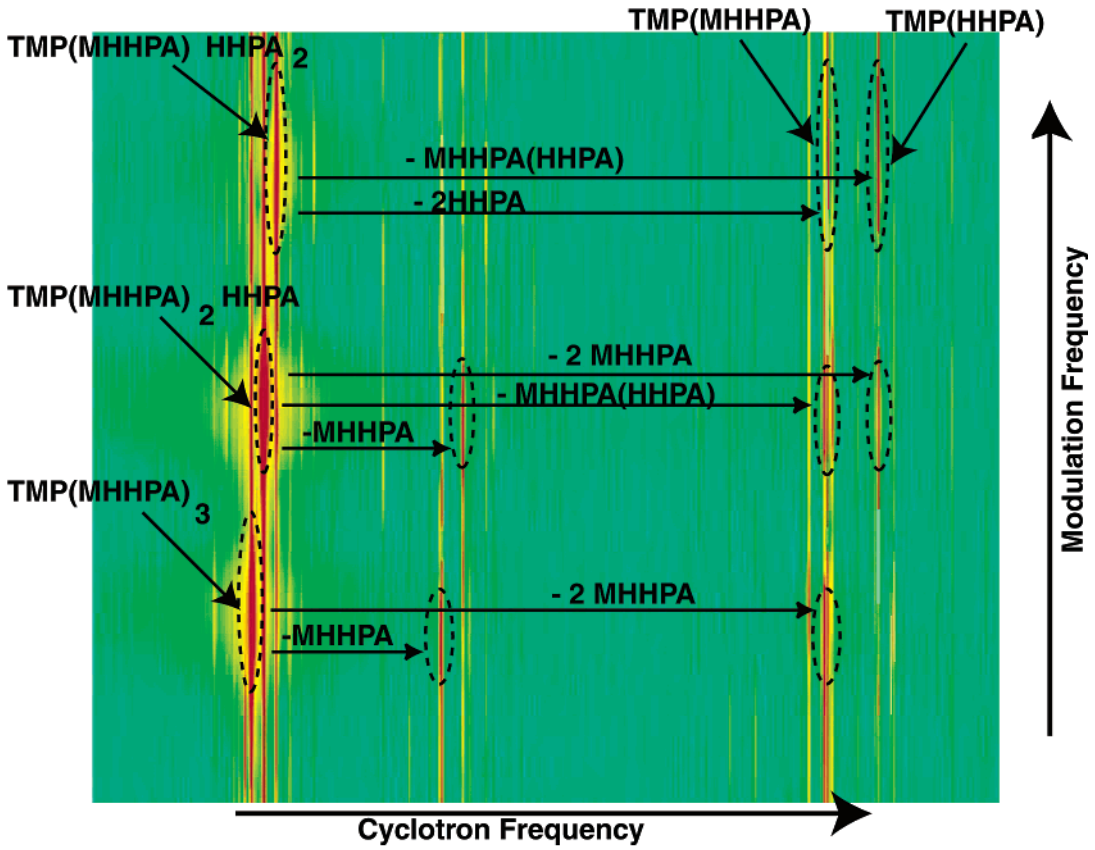


Figure 4. Contour plot of the data shown in Figure 3. Horizontal projections along a given modulation frequency show connectivities between product ions and their precursor ions. For example, the precursor ion, $TMP(MHHPA)_2$ HHPA, fragments to three product ions, loss of one MHHPA, two MHHPA, and loss of one MHHPA and HHPA.

dimethylated cross-linker (see Scheme 4) thereby proving that the ion at m/z 479 is related to these and only these two molecular ions.

As with 2D-NMR experiments, the data may be displayed either as a magnitude/power spectrum (Figure 3) or as a contour spectrum (Figure 4). Any product ion derived from a modulated precursor ion is observed as an off-diagonal signal at the same modulation frequency of the precursor. In other words, horizontal projections show connectivity between ions. Figure 4 shows a magnified region of the full 2D-SWIM data set and hence the diagonal line for precursor ions appears more vertical in this expansion. Proceeding from the lower left of the contour spectrum, Figure 4, a horizontal projection to the right from the molecular ion of $TMP(MHHPA)_3$, m/z 661 $[638 + Na]^+$, shows two strong intensities, one at the cyclotron frequency of the fragment ion, $TMP(MHHPA)_2$, a loss of one MHHPA. Second, further to the right is another signal for the loss of two MHHPA moieties producing the ion TMP(MHHPA). As we proceed up to the next molecular ion, $TMP(MHHPA)_2$HHPA, horizontal projections show three signals representative of losses of the following: one MHHPA; one MHHPA and one HHPA; and finally two MHHPA.

From this one automated experiment, the conventional mass spectrum of the sample is observed along the diagonal while all MS/MS data are observed as horizontal projections for each molecular ion. In addition, two other data types normally attributed to scanning two analyzer instruments such as sectors or triple quadrupoles are now available from the two-dimensional FTICR MS/MS experiment. Precursor ion analyses or scans, in which ions yielding a particular product ion are investigated, are observed in Figure 4. Precursor ions yielding the product ion, TMP-(MHHPA), are found by looking for signals at the cyclotron frequency of the product ion and then taking horizontal projections from those signals to the diagonal line of the precursors. Also, common neutral losses are observed by drawing two lines parallel to the precursor ion main diagonal line (in m/z vs m/z display mode) separated by the appropriate mass difference, such as 168 Da, MHHPA. Neutral losses are observed when two signals separated by the difference are observed along the same horizontal line.

It is important to note that a stable ion source delivering approximately the same number of precursor ions to the ICR cell is required to reduce total ion population variation. This variation in initial ion population introduces noise in the second FT dimension, termed T1 noise, which is observed as the vertical tracts emanating from each signal of Figure 4. For an excellent discussion of noise analysis, refer to *Int. J. Mass Spectrom.* **2001**, *210/211*, 101−111. Our first attempt at 2D-SWIM analysis of these oligomers using laser desorption was unsuccessful due to the greater than 50% shot-to-shot variation in initial ion population. Electrospray ionization provided a stable source of ions for these experiments.

SWIM 2D-FTICR MS/MS of a Sample Derived from Combinatorial Synthesis. The power of SWIM 2D-FTICR MS/MS becomes more apparent as sample complexity increases in the number of components as for those samples derived from combinatorial synthesis. Furthermore, two-dimensional FT/FTICR

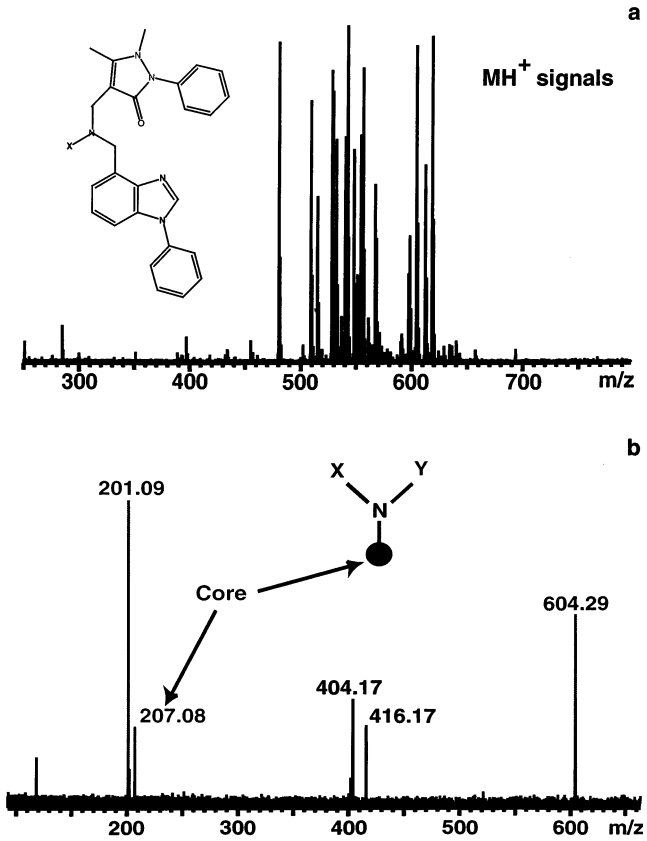


Figure 5. Standard CAD MS/MS data (b) of a component isolated from a combinatorial library (a). The core or template is observed at *m*/*z* 207.08 and the variant moiety, Y, that was held constant in this well is observed at *m*/*z* 201.09.

MS/MS relies on a linear relationship between SWIM excitation amplitude and product ion relative abundance. The structural similarities between these compounds should lead to similar fragmentation pathways and energetics thus making the characterization of these compounds amenable to the 2D SWIM approach. Figure 5a shows the one-dimensional mass spectrum of the 20-component benzimidazole variants synthesized by combinatorial methodologies.[19] As previously mentioned, to characterize each of these components requires either chromatographic separation or ion isolation. Figure 5b shows the MS/MS data for one of the desired components (*m*/*z* 604) rf-isolated from the mixture. The core or template product ion is observed at *m*/*z* 207. The Y moiety is observed at *m*/*z* 201; see Scheme 2. The MS/MS data for all 20 components of this specific well show product ions at *m*/*z* 207 and 201 (not shown for brevity).[19]

Figure 6a shows the mass spectrum obtained from the application of the second SWIM waveform. The core fragment ion is observed at *m*/*z* 207 along with a range of fragment ions in the *m*/*z* 350−420 region. Figure 6b shows the next mass spectrum collected in which SWIM waveform 3 is used containing 1.5 periods. Comparing these two data sets, it is apparent that the SWIM waveforms are fragmenting the precursor ions selectively. The upper mass region of molecular ions from *m*/*z* 580 to 620 has been fragmented whereas the lower mass region of molecular ions below *m*/*z* 580 has not obtained sufficient kinetic energy to fragment.

Finally, Figure 7 shows the SWIM 2D-FTICR MS/MS contour plot for the combinatorial sample. Each precursor ion (along the red diagonal line) shows an associated signal at the cyclotron frequency of *m*/*z* 207, the core benzimidazole moiety, thus illustrating that these components were derived from this specific template in combinatorial synthesis. Also observed are signals at *m*/*z* 201, the Y moiety. Product ions in the *m*/*z* 400 range produced weak off-diagonal signals below the contour level shown in Figure 7. In general, for this chemical system, four product

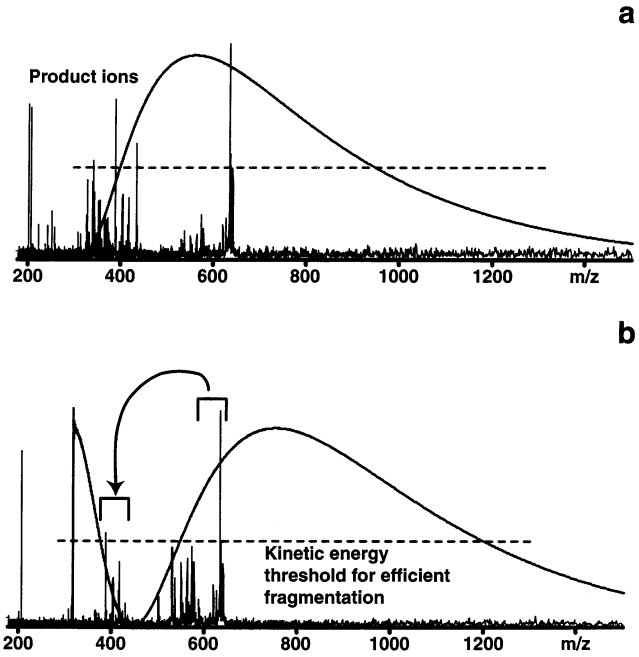


Figure 6. SWIM waveforms 2 and 3 superimposed on the associated mass spectrum from the 20-component sample.

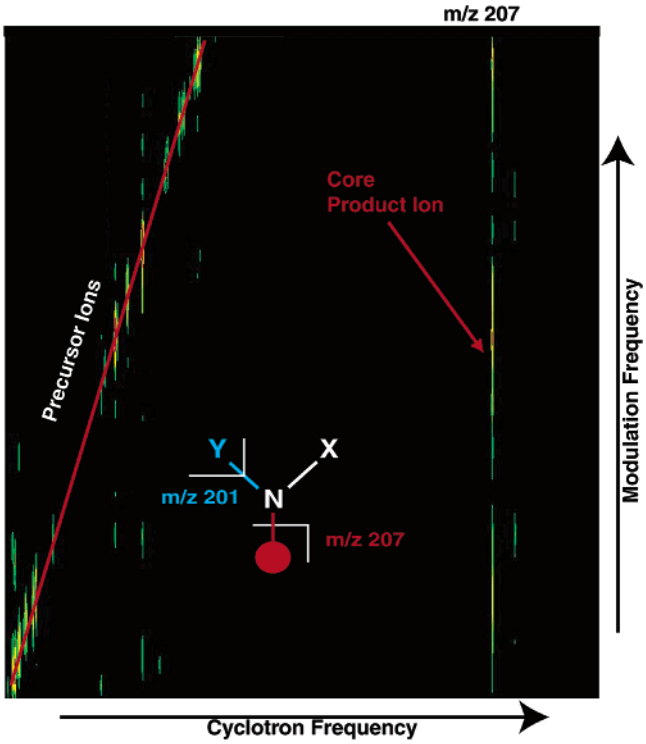


Figure 7. SWIM 2D-FTICR MS/MS contour plot for the 20-component sample derived from combinatorial synthesis. The original mass spectrum is observed along the red diagonal line. Each precursor ion fragments to the product ions at *m*/*z* 207 and 201.

ions are derived from each precursor ion collection. If the precursor ion abundance prior to dissociation is assigned an arbitrary value of 100, then the SWIM modulation of this molecular ion packet would run from 0 to 100 peak to peak for its sine wave in the second dimension. If the product ion distribution produces equal abundance for the four product ions, then their signals would only run from 0 to $100/4 = 25$ peak to peak. Thus, the magnitude for each product ion in the 2D display would be one-fourth of the parent ion. Additionally, each parent ion of this combinatorial mixture produces two identical product ions, m/z 207 and 201. Even though the second-dimension sinusoidal signal for the product ion is complex, the magnitude of product ions m/z 201 and 207 produced from the 20 components would vary from 0 to $(100/4) \times 20 = 500$, which brings the signals of these two fragment ions above that of the noise level provided by any instability in the ion source. The magnitude of all product ions can be increased by coadding additional transients, i.e., 16 instead of 8 transients collected per SWIM waveform.

CONCLUSIONS

SWIM 2D-FTICR MS/MS data acquisition was successfully applied to two diverse samples of industrial importance. Simultaneous CAD of such mixtures would produce a highly complex data set from which little useful information can be gleaned. The ability to encode a frequency marker on precursor ions and their products provides a simple means to correlate fragmentation pathways in a complex mixture and is automated.

The TMP/MHHPA system containing three components provided a good test case for the technique. The fragmentation pathways identified in the conventional MS/MS data were observed in the SWIM 2D acquisition. An excitation threshold can be approximated from the SWIM excitation waveforms below which no product ions are observed. Although the information is useful for determining energetics for collisionally activated dissociation, the net effect for SWIM 2D-FTICR MS/MS is a signal decrease in the second dimension, because the data in the second dimension degrade from a sinusoidal wave to a clipped sinusoid, which introduces harmonics. An offset added to the SWIM waveforms, and linearly varying ion kinetic energy by use of stored modulation of kinetic energy (SMOKE) instead of ion radius (velocity), would reduce the harmonic content as previously suggested.[25] Second-dimension resolution is improved by increasing the number of data points (mass spectra) collected. As would be expected, increased resolving power is realized with an increase in magnetic field strength as reported by Marshall et al.[26] The digital resolution of the SWIM waveforms also plays a role in resolving closely spaced masses. If two ions close in mass experience the same second-dimension radius modulation, then they will each have the same second-dimension frequency tag. For the chemical systems described here, the digital resolution of the SWIM waveforms was sufficient. Guidelines for SWIM waveform creation to ensure second-dimension resolution will be addressed in future work. From the 2D data obtained, we correlated all product ions with their respective precursor ions. The SWIM 2D technique works well for these types of oligomers.

The SWIM 2D data set for the combinatorial sample showed the expected product ion relating to the core moiety. The additional product ions observed in Figure 6 were weakly observed in the 2D contour plot. The presence of the m/z 207 product ion can be used to identify the compounds as originating from the combinatorial library. Ideally, the technique could be used as a screening method for mixtures to identify components derived from combinatorial synthesis. The structural similarity of these molecules yields similar fragmentation pathways and energy requirements thus being highly amenable to SWIM 2D-FTICR MS/MS. The technique is currently being developed as a strategy for the specific identification and structure elucidation of multicomponent systems. This strategy will be combined with those established criteria for component identification.[19]

The multiplex advantage obtained by standard FTMS data acquisition for precursor ions, in that all ions are measured simultaneously, is now realized by SWIM 2D-FTICR MS/MS for product ions in the second dimension. We are currently investigating other polyesters and combinatorial libraries by this automated technique.

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