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Chromatographic peak deconvolution of constitutional isomers by multiple-reaction-monitoring mass spectrometry☆

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ABSTRACT

Highly efficient and sophisticated separation techniques are available to analyze complex compound mixtures with superior sensitivities and selectivities often enhanced by a 2nd dimension, e.g. a separation technique or spectroscopic and spectrometric techniques. For enantioselective separations numerous chiral stationary phases (CSPs) exist to cover a broad range of chiral compounds. Despite these advances enantioselective separations can become very challenging for mixtures of stereolabile constitutional isomers, because the on-column interconversion can lead to completely overlapping peak profiles. Typically, multidimensional separation techniques, e.g. multidimensional GC (MDGC), using an achiral 1st separation dimension and transferring selected analytes to a chiral 2nd separation are the method of choice to approach such problems. However, this procedure is very time consuming and only predefined sections of peaks can be transferred by column switching to the second dimension. Here we demonstrate for stereolabile 1,2-dialkylated diaziridines a technique to experimentally deconvolute overlapping gas chromatographic elution profiles of constitutional isomers based on multiple-reaction-monitoring MS (MRM-MS). The here presented technique takes advantage of different fragmentation probabilities and pathways to isolate the elution profile of configurational isomers.

1. Introduction

In the last decades numerous highly efficient chiral selectors and chiral stationary phases (CSPs) were developed to achieve enantioselective separations in GC [1–3], HPLC [4–7], SFC, CE [8–12] and CEC [13,14]. These advances have boosted many areas in chemistry and beyond, e.g. screening of enantioselective reactions, enantioselective catalysts—the most recent example is the development of organocatalysis [15–18], which is in most cases associated with enantioselective transformations, structure elucidation of natural products and the development of enantiomerically pure drugs only to mention a few. At the same time technical advances have reached a very high level, which allow to analyze complex compound mixtures with superior sensitivities often enhanced by a 2nd dimension, e.g. a separation technique or spectroscopic and spectrometric techniques, and speed which allows to perform high-throughput screening assays with separation techniques, e.g. high-throughput multiplexing chromatography. Other highly interesting areas are the investigation of the dynamics of stereolabile compounds by enantioselective dynamic chromatography and electrophoresis [19–23], and the investigation of reaction kinetics [24,25] by combining chemical synthesis and separations in (enantioselective) on-column reaction chromatography [26,27]. In particular catalytically active stationary phases open the opportunity to investigate numerous reactions simultaneously and under the exactly same reaction conditions, because educt libraries can be used. This results in extraordinary high throughputs [28] to determine reaction kinetics, which allows us to determine activation parameters and to elucidate reaction mechanisms [26,27]. However, on-column conversions of several compounds at the same time can lead to quite complex peak profiles [29]. It is important to note that these processes have to be independent from each other, because competing reactions lead to undefined reaction kinetics. In the present publication such a challenging example is presented for mixtures of stereolabile constitutional isomers of 1,2-dialkylated diaziridines [30]. 1,2-Dialkylated diaziridines have two stereogenic nitrogen atoms, which are subject to interconversion.

Compounds with stereogenic nitrogen atoms are an intriguing class of compounds which inspired many scientists [31] for more than one century. In 1890, Werner [32] transferred the concept of tetrahedral tetravalent carbon of van't Hoff and Le Bel to trivalent nitrogen. Attempts made by Meisenheimer et al. to isolate chiral tertiary amines of the type *NRR′R″* suggested that rapid pyramidal inversion leads to optical inactivity [33]. In 1944, Prelog and Wieland [34] recognized the inherent chirality of Tröger's base [35,36] due to the two stereogenic nitrogen atoms related by C_2 symmetry. In 1967, Mannschreck et al. [37] reported that nitro-

☆ Dedicated to Prof. Volker Schurig on the occasion of his 70th birthday.

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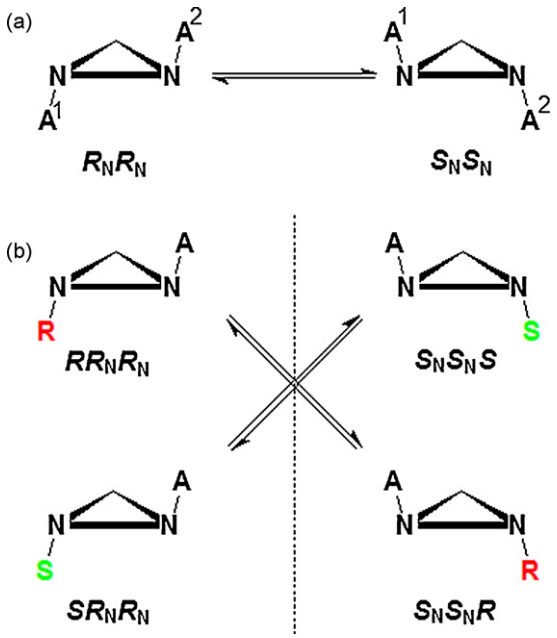

Fig. 1. (a) Enantiomerization ($A^1 = A^2$) and (b) epimerization (*R*, *S* denotes a chiral substituent, e.g. *sec.*-butyl group), of 1,2-disubstituted diaziridines. In case of two chiral substituents, e.g. two *sec.*-butyl groups there are four epimers and two interconverting enantiomers (1-(*R*)-*sec.*-butyl-2-(*S*)-*sec.*-butyl-(R_N,R_N)-diaziridine and formally 1-(*S*)-*sec.*-butyl-2-(*R*)-*sec.*-butyl-(S_N,S_N)-diaziridine, which is in correct nomenclature 1-(*R*)-*sec.*-butyl-2-(*S*)-*sec.*-butyl-(S_N,S_N)-diaziridine).

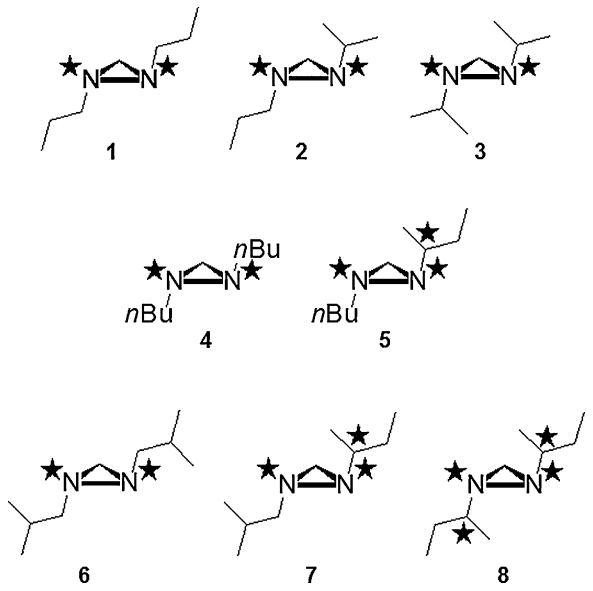

Fig. 2. Structures of the here investigated diaziridines: 1,2-di-*n*-propyldiaziridine **1**, 1-isopropyl-2-*n*-propyldiaziridine **2**, 1,2-diisopropyldiaziridine **3**, 1,2-di-*n*-butyldiaziridine **4**, 1-*n*-butyl-2-*sec.*-butyldiaziridine **5**, 1,2-diisobutyldiaziridine **6**, 1-*sec.*-butyl-2-isobutyldiaziridine **7**, and 1,2-di-*sec.*-butyldiaziridine **8**.

gen inversion in diaziridines is hindered in the NMR time scale and determined later interconversion barriers by dynamic NMR (DNMR) [38–40]. Two strategies have been successfully applied by Brois [41], Eschenmoser and co-worker [42], Lehn and Wagner [43] and Kostyanovsky et al. [44] to achieve the isolation of stereoisomers with stereogenic nitrogen [45,46]: (i) Introduction of lone-pair containing substituents (–OR, Cl, F) at the stereogenic nitrogen atom [47–50], and (ii) incorporation of stereogenic nitrogen into a constrained (three-membered) ring [51].

In monocyclic diaziridines the adjacent alkyl substituents are in *trans* [52–54]. Interconversion takes place by a double interconversion process at the chirotopic nitrogen atoms. An enantiomerization process takes place, if the substituents A^1 and A^2 are equal (cf. Fig. 1a). In the case that there is one chiral substituent the diaziridines will epimerize (cf. Fig. 1b); *R*, *S* denotes a chiral substituent, e.g. *sec.*-butyl group. In case of two chiral substituents, e.g. two *sec.*-butyl groups there are four interconverting epimers and two interconverting enantiomers (1-(*R*)-*sec.*-butyl-2-(*S*)-*sec.*-butyl-(R_N,R_N)-diaziridine and formally 1-(*S*)-*sec.*-butyl-2-(*R*)-*sec.*-butyl-(S_N,S_N)-diaziridine, which is in correct nomenclature 1-(*R*)-*sec.*-butyl-2-(*S*)-*sec.*-butyl-(S_N,S_N)-diaziridine).

These (mixed) diaziridines (cf. Fig. 2) are obtained from a statistical synthesis according to a modified procedure [30] described in literature [55]. Their enantiomeric and epimeric pairs show excellent separation factors on Chirasil-β-Dex [56–58] in GC. However, the on-column interconversion [59] of these stereolabile compounds leads to completely overlapping peak profiles of the constitutional stereoisomers. For the determination of kinetic parameters by enantioselective dynamic gas chromatography a complete separation is necessary to perform peak shape analysis by iterative computer simulation [35,60–74] or direct calculation utilizing the approximation function [75,76] or the unified equation [77–84]. To achieve a complete separation of the constitutional isomers and the stereoisomers multidimensional separation techniques, e.g. multidimensional GC (MDGC) [85,86], using an achiral 1st separation dimension and transferring selected analytes to a chiral 2nd separation are the method of choice. However, this procedure is very time consuming and only predefined sections of peaks can be transferred by column switching to the second dimension. This constitutes that the advantage of using a library of compounds to investigate their dynamics under exactly the same conditions and therefore achieving a high-throughput is given up on the expense of a complete separation. Two-dimensional GC (GC × GC) can overcome these drawbacks. It has been demonstrated that elution profiles of interconverting stereoisomers can be temporarily resolved and evaluated [87–89]. Another strategy is the application of peak deconvolution procedures [90–97] to unfold the single trace of each constitutional isomer. In literature there are several approaches [98,99] described to use for example Gaussian functions [100,101] or to use chiroptical detectors [102] to separate overlapping peaks into individual peaks. However, such strategies are difficult to apply to the chromatogram of interconverting isomers, because the plateau formed by the interconverting isomers can be described by an array of Gaussian functions.

Here we demonstrate for stereolabile 1,2-dialkylated diaziridines a technique to experimentally deconvolute overlapping gas chromatographic elution profiles of constitutional isomers based on multiple-reaction-monitoring MS (MRM-MS) [103–105]. The here presented method takes advantage of different fragmentation probabilities and pathways to isolate the elution profile of configurational isomers.

2. Experimental

2.1. Materials

n-Propylamine, isopropylamine, *n*-butylamine, *sec.*-butylamine, (*S*)-*sec.*-butylamine, isobutylamine, formaldehyde, sodium hydroxide and sodium hypochlorite were purchased from Fluka (Taufkirchen, Germany). Diethylether was distilled from sodium suspension for purification. Electron impact mass spectra (EI-MS, ion source temperature 225 °C) were recorded on a Thermo GC quadrupole—ion trap mass spectrometer Trace GC PolarisQ (Thermo, San Jose, CA, USA) using the Xcalibur software package (Thermo, San Jose, CA, USA).

2.2. Synthesis

The mixed diaziridines were prepared according to a modified method [30] by Ohme et al. [55]. 125 mmol of the alkylamines (mixture 1: *n*-propylamine/isopropylamine; mixture 2: *n*-butylamine/*sec.*-butylamine; mixture 3: isobutylamine/*sec.*-butylamine) and 50 ml 5N NaOH are combined in a 250 ml flask equipped with a magnetic stirrer and cooled to 0 °C. Formaldehyde solution (36%, w/w, 12.5 ml) is slowly added. After 1 h, 75 ml sodium hypochlorite solution (13%, w/w) is added dropwise at 0 °C and after complete addition, the mixture is allowed to warm up to r.t. The mixture is stirred at r.t. for 12 h, then after 1 h waiting for phase separation the organic layer is separated and washed with diluted sodium thiosulfate solution and water. After drying over KOH and filtration the crude diaziridine mixture is distilled under reduced pressure. Solutions of these mixtures in diethylether were used without further separation.

For the stereochemical assignment of the *sec.*-butyl substituted derivatives, the same synthesis procedure was performed using (*S*)-*sec.*-butylamine.

2.2.1. 1,2-Di-n-propyldiaziridine **1**

Separation factor $\alpha = 1.04$ on Chirasil-β-Dex at 100 °C and 50 kPa inlet pressure.

MS *m*/*z* (relative intensity) 128.07 (M^+, 0.63), 100.14 (2.85), 99.1 (47), 86.13 (1.6), 85.12 (8.42), 72.11 (7.12), 71.07 (28.12), 70.08 (8.08), 68.13 (1.3), 58.09 (7.32), 57.04 (100), 56.09 (6.3), 55.14 (0.72), 54.1 (1.75), 51.98 (1.36).

2.2.2. 1-Isopropyl-2-n-propyldiaziridine **2**

Separation factor $\alpha = 1.18$ on Chirasil-β-Dex at 100 °C and 50 kPa inlet pressure.

MS *m*/*z* (relative intensity) 128.1 (M^+, 0.79), 113.13 (27.84), 99.12 (24.02), 86.13 (3.2), 85.09 (30.5), 72.12 (8.34), 71.09 (43.95), 70.12 (6.64), 59.1 (2.21), 58.08 (18.7), 57.06 (100), 56.08 (30.64), 55.12 (2.2), 54.11 (3.25), 51.98 (2.15).

2.2.3. 1,2-Diisopropyldiaziridine **3**

Separation factor $\alpha = 1.20$ on Chirasil-β-Dex at 100 °C and 50 kPa inlet pressure.

MS *m*/*z* (relative intensity) 128.07 (M^+, 1.39), 114.14 (4.69), 113.1 (61.44), 86.08 (1.06), 85.14 (1.75), 82.14 (2.14), 72.12 (5.64), 71.07 (100), 70.14 (2.75), 69.14 (1.36), 58.11 (11.54), 57.12 (2.07), 56.08 (32.15), 55.11 (1.21), 54.1 (2.55).

2.2.4. 1,2-Di-n-butyldiaziridine **4**

Separation factor $\alpha = 1.04$ on Chirasil-β-Dex at 100 °C and 50 kPa inlet pressure.

MS *m*/*z* (relative intensity) 156.1 (M^+, 1), 155.1 (1), 141.17 (1.47), 114.12 (1.76), 113.09 (18.16), 99.11 (10.81), 87.13 (1.05), 86.09 (15.97), 85.07 (5.57), 84.08 (23.08), 82.09 (4.56), 80.11 (1.51), 72.08 (9.68), 71.05 (100), 70.09 (12.41), 69.09 (1.12), 68.11 (1.63), 67.11 (1.32), 59.12 (1.14), 58.09 (3.36), 57.06 (30.67), 56.07 (9.09), 55.07 (4.09), 53.09 (1.16), 45.08 (1.69), 44.04 (39.39), 43.03 (9.98), 41.9 (30.75).

2.2.5. 1-n-Butyl-2-sec.-butyldiaziridine **5**

Separation factors on Chirasil-β-Dex at 100 °C and 50 kPa inlet pressure: 1-*n*-butyl-2-(*R*)-*sec.*-butyldiaziridine $\alpha = 1.15$, 1-*n*-butyl-2-(*S*)-*sec.*-butyldiaziridiner $\alpha = 1.24$.

MS *m*/*z* (relative intensity) 156.1 (M^+, 0.85), 155.1 (0.9), 141.13 (4.52), 128.13 (1.78), 127.09 (19.8), 113.09 (8.05), 112.12 (1.7), 100.13 (2.36), 99.08 (28.7), 87.13 (2.03), 86.08 (35.09), 85.09 (100), 84.13 (18.99), 82.15 (3.49), 72.15 (14.09), 71.13 (16.02), 70.14 (17.39), 69.13 (1.65), 68.13 (1.51), 58.12 (13.38), 57.09 (92), 56.12 (36.26), 55.13 (5.37), 54.13 (1.54), 45.15 (1.84), 44.09 (31.96), 43.08 (8.7), 41.98 (37.09).

2.2.6. 1,2-Diisobutyldiaziridine **6**

Separation factor $\alpha = 1.16$ on Chirasil-β-Dex at 100 °C and 50 kPa inlet pressure.

MS *m*/*z* (relative intensity) 156.06 (M^+, 1.31), 114.09 (2.86), 113.05 (35.82), 86.09 (11.36), 84.09 (5.34), 72.08 (7.85), 71.05 (5.45), 70.08 (3.72), 58.07 (4.99), 57.02 (100), 56.07 (4.1), 55.07 (3.54), 44.05 (5.36), 43.03 (3.83), 41.9 (18.51).

2.2.7. 1-sec.-Butyl-2-isobutyldiaziridine **7**

Separation factors on Chirasil-β-Dex at 100 °C and 50 kPa inlet pressure: 1-(*R*)-*sec.*-Butyl-2-isobutyldiaziridine $\alpha = 1.16$, 1-(*S*)-*sec.*-butyl-2-isobutyldiaziridine $\alpha = 1.33$.

MS *m*/*z* (relative intensity) 156.07 (M^+, 1.23), 141.12 (4.64), 128.12 (2.67), 127.08 (29.67), 114.12 (1.58), 113.07 (19.39), 100.13 (1.11), 99.09 (7.92), 87.14 (1.24), 86.1 (20.47), 85.08 (15.69), 84.1 (6.42), 82.13 (1.98), 72.11 (13.42), 71.07 (51.41), 70.1 (6.54), 68.13 (1.1), 58.1 (8.09), 57.06 (100), 56.1 (23.66), 55.1 (4.3), 54.11 (1.2), 45.12 (1.11), 44.07 (13.38), 43.06 (5.17), 41.95 (29.77).

2.2.8. 1,2-Di-sec.-butyldiaziridine **8**

Separation factors on Chirasil-β-Dex at 100 °C and 50 kPa inlet pressure: 1,2-(*R*,*R*)-di-*sec.*-butyldiaziridine $\alpha = 1.17$, 1,2-(*S*,*S*)-di-*sec.*-butyldiaziridine $\alpha = 1.46$, 1,2-(*R*,*S*)-di-*sec.*-butyldiaziridine $\alpha = 1.30$.

MS *m*/*z* (relative intensity) 156.08 (1.31), 141.11 (6.07), 128.1 (4.11), 127.08 (46.02), 100.08 (0.95), 99.09 (6.68), 87.12 (1.08), 86.09 (17.1), 85.08 (20.37), 84.1 (3.66), 72.08 (11.62), 71.06 (100), 70.09 (8.77), 69.09 (1.78), 68.11 (2.2), 58.1 (7.36), 57.08 (19.14), 56.06 (45.5), 55.06 (4.26), 54.08 (2.11), 53.09 (1.32), 45.06 (1.73), 44.02 (18.96), 43.03 (5.41), 41.92 (25.69), 40.85 (63.41).

2.3. Enantioselective GC–MS and GC–MRM-MS

Separation of the stereoisomers was performed on a Thermo Trace PolarisQ GC-MS equipped with an autosampler/split injector (250 °C) and a flame-ionisation detector (250 °C). For the stereoisomeric separations a fused silica column coated with Chirasil-β-Dex [56–58] (25 m × 0.25 mm i.d., 0.5 μm film thickness) was employed. All experiments were carried out under isothermal conditions at 120 °C and an inlet pressure of 40 kPa using He as inert carrier gas.

For the selection of suitable precursor ions GC–EI-MS chromatograms and mass traces in the full scan mode were recorded. MS/MS experiments with precursor ions showing high abundance were conducted and characteristic transitions were selected.

The multiple-reaction-monitoring MS (MRM-MS) experiments were performed by isolation of several precursor ions with an isolation width of ±1 Da. Then for each *m*/*z* ratio of the precursor ions an excitation voltage of 5 V and He as collision gas at a flow rate of 0.3 ml/min was applied to the ion trap. After 15 ms of excitation the ions were again selected for defined *m*/*z* ratios to monitor the selected transitions.

2.4. Data deconvolution

Data deconvolution was performed using a program written in Delphi (Embarcadero Technologies, San Francisco, USA), which reduces the data size of the raw data files obtained by the Xcalibur software (San Jose, CA, USA) and allows a 3D representation of the single MS and MRM traces.

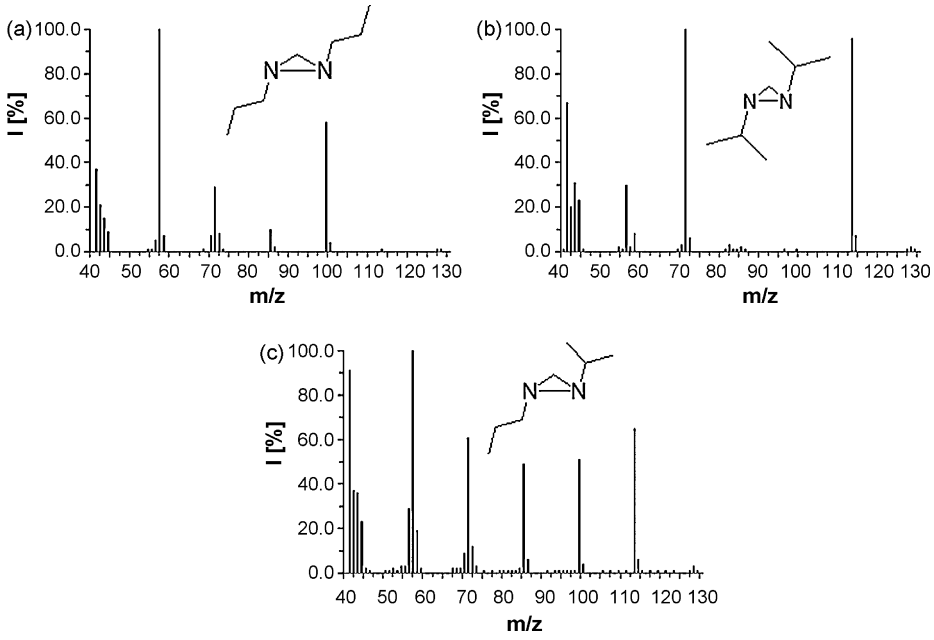

Fig. 3. EI-MS spectra of constitutional isomers: (a) 1,2-di-*n*-propyldiaziridine **1**, (b) 1,2-diisopropyldiaziridine **3**, and (c) 1-isopropyl-2-*n*-propyldiaziridine **2**.

3. Results and discussion

Three mixtures of 1,2-disubstituted diaziridines have been synthesized by statistical syntheses using equimolar amounts of two alkylamines, which are condensed with formaldehyde to the aminal and then the oxidative ring closure is achieved by adding sodium hypochlorite. After vacuum distillation the following three mixtures were obtained: (i) 1,2-di-*n*-propyldiaziridine **1**, 1-isopropyl-2-*n*-propyldiaziridine **2**, and 1,2-diisopropyldiaziridine **3**, (ii) 1,2-di-*n*-butyldiaziridine **4** and 1-*n*-butyl-2-*sec.*-butyldiaziridine **5**, and (iii) 1,2-diisobutyldiaziridine **6**, 1-*sec.*-butyl-2-isobutyldiaziridine **7**, and 1,2-di-*sec.*-butyldiaziridine **8** (cf. Fig. 2). It is important to note that for mixture (ii) only two constitutional isomers are obtained, which can be explained by the fact that *n*-butylamine is more reactive than *sec.*-butylamine and therefore exclusively **4** and **5** are obtained.

These mixtures show increasing stereochemical complexity. Mixture (i) is composed of only 3 constitutional isomers, which can be separated in enantiomeric pairs, giving a total number of 6 stereoisomers. For mixture (ii) an enantiomeric pair for **4** and 2 epimeric pairs for **5** can be observed giving in total 6 stereoisomers. Mixture (iii) consists of an enantiomeric pair for **6**, 2 epimeric pairs for **7** and an enantiomeric pair and 2 epimeric pairs for **8**, giving 12 stereoisomers.

To demonstrate the principle of GC-MRM-MS for the separation of constitutional isomers, we first focus on the simplest mixture (i). In Fig. 3 the EI-MS spectra of the three constitutional isomers 1,2-di-*n*-propyldiaziridine **1** (Fig. 3a), 1,2-diisopropyldiaziridine **3** (Fig. 3b), and 1-isopropyl-2-*n*-propyldiaziridine **2** (Fig. 3c) are depicted. The MS spectra of 1,2-di-*n*-propyldiaziridine **1** (Fig. 3a), 1,2-diisopropyldiaziridine **3** (Fig. 3b) are very different in their peak intensities for the fragments *m*/*z* 56, 71, 85, 99, and 113 Da. For 1-isopropyl-2-*n*-propyldiaziridine **2** (Fig. 3c) all peaks are present which are present in diaziridines **1** and **3**. This constitutes that the application of SIM-MS would not improve the separation, because there is always an overlap with one of the other diaziridines.

Therefore it is important to understand the fragmentation pathways of the single constitutional isomers in greater detail. For this purpose fragment ions with *m*/*z* ratios of 56, 71, 85, 99, and 113 Da were selected and subjected to further MS/MS experiments to elucidate the fragmentation pathway under more controlled conditions. The fragment ions are selected by an ion trap mass selector, and then they are excited by application of an excitation voltage to induce fragmentation by collision with He inside the ion trap. Here we found that the fragments with *m*/*z* ratios of 99 and 113 Da underwent transitions to characteristic fragment ions, which can be unambiguously assigned to a certain constitutional isomer (cf. Fig. 4).

In 1-isopropyl-2-*n*-propyldiaziridine **2** there is an isopropyl and an *n*-propyl substituent present, which leads to both characteristic pathways via the fragments with 99 and 113 Da. But by controlled fragmentation the characteristic fragment with *m*/*z* 83 Da can be obtained. From these results we chose the following transitions for the enantioselective GC–MRM-MS experiments: *m*/*z* 99 Da → 57 Da, *m*/*z* 113 Da → 71 Da, and *m*/*z* 113 Da → 83 Da. For instrumental reasons the selection and detection windows were

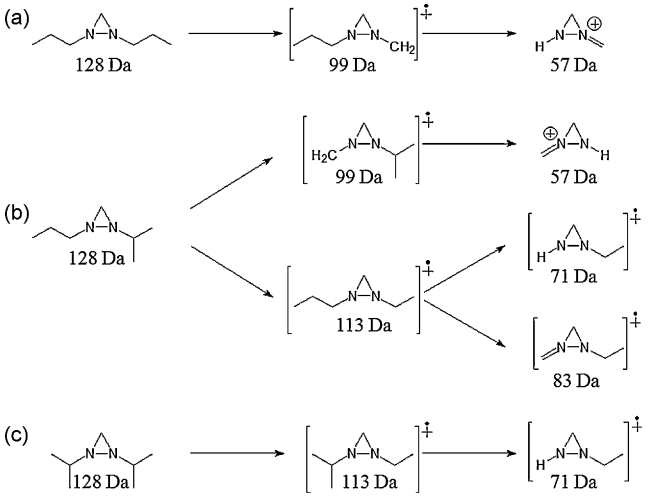

Fig. 4. Selected fragmentation pathways and transitions used for GC-MRM-MS measurements of (a) 1,2-di-*n*-propyldiaziridine **1**, (b) 1-isopropyl-2-*n*-propyldiaziridine **2**, and (c) 1,2-diisopropyldiaziridine **3**.

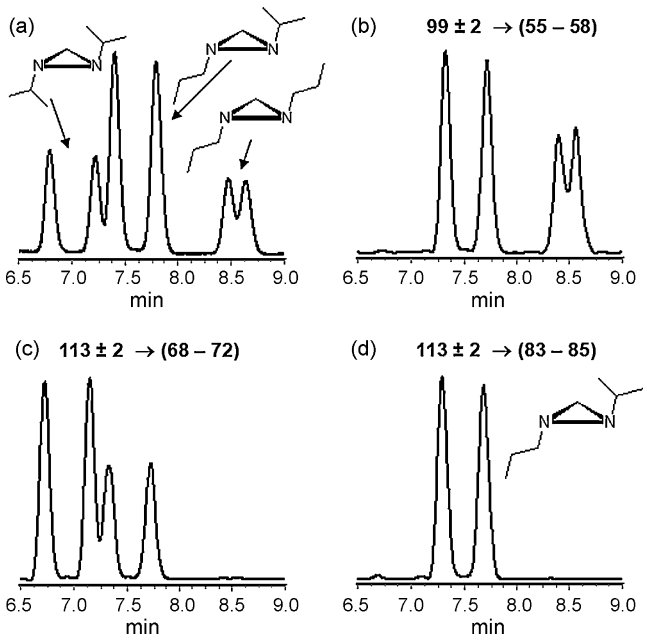

Fig. 5. Total ion current chromatograms of the enantiomer separation of 1,2-diisopropyldiaziridine, 1-isopropyl-2-propyldiaziridine and 1,2-dipropyldiaziridine obtained by GC–MS and GC–MRM-MS (25 m Chirasil-β-Dex, i.d. 250 μm, film thickness 500 nm, isothermal at 120 °C, 40 kPa He). The transitions in the MRM-MS experiments were obtained by collisionally induced fragmentation of the respective parent ions: (a) MS trace 40-200 Da, (b) MS/MS trace of the transition 99 ± 2 Da → 55–58 Da, (c) MS/MS trace of the transition 113 ± 2 Da → 68–72 Da, and (d) MS/MS trace of the transition 113 ± 2 Da → 83–85 Da.

increased by ±2 Da or were adjusted to detection windows given by instrumental limitations.

The advantage of MRM-MS is that it is not necessary to perform the separation several times to obtain certain mass traces, but the selected experiments can be executed in a single run. However, it is recommended to limit the number of selected fragment ions, because the overall time resolution will be limited by the number of MS/MS experiments. In Fig. 5a the chromatogram of the three constitutional isomers with the well separated enantiomers is depicted. Here, in this simple case it is obvious which enantiomeric pairs belong together, however, the advantage of MRM-MS becomes obvious for the chromatograms shown in Fig. 5b–d. The MS/MS traces of the transitions 99 ± 2 Da → 55–58 Da, 113 ± 2 Da → 68–72 Da, and 113 ± 2 Da → 83–85 Da demonstrate clearly that the constitutional isomers can be well separated compared to the conventional chromatogram. In particular at elevated temperatures to perform dynamic studies of the interconverting stereoisomers this constitutes a valuable experimental tool without theoretical estimation of probable peak forms.

To demonstrate the general applicability of this protocol we applied the same experimental steps to the other two more complex mixtures of diaziridines: (i) chromatographic separation and interpretation of the MS spectra obtained by a full scan, (ii) selection of fragment ions with high intensities, (iii) study of the fragmentation pathways of the selected fragment ions by MS/MS, and (iv) selection of suitable transitions for GC–MRM-MS experiments.

In Fig. 6a the chromatogram of 1,2-di-*n*-butyldiaziridine **4** and 1-*n*-butyl-2-*sec*.-butyldiaziridine **5** is shown, which does not immediately allow assignment of stereochemical relationship (cf. Fig. 6). Here the MS/MS traces of the transitions 71 ± 2 Da → 40–43 Da and 85 ± 2 Da → 54–58 Da give cleaner chromatograms. For 1,2-di-*n*-butyldiaziridine it is possible to extract

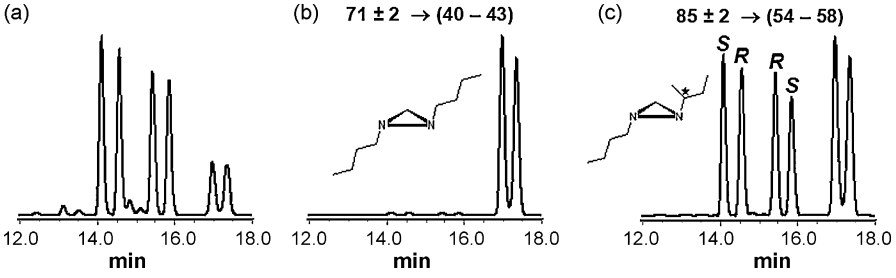

Fig. 6. Total ion current chromatograms of the separation of the enantiomers of 1,2-dibutyldiaziridine, of the epimeric pairs of 1-butyl-2-*sec*.-butyldiaziridine and the epimeric pairs and enantiomers of 1,2-di-*sec*.-butyldiaziridine (only minor component) obtained by GC–MS and GC–MRM-MS (25 m Chirasil-β-Dex, i.d. 250 μm, film thickness 500 nm, isothermal at 120 °C, 40 kPa He). The transitions in the MRM-MS experiments were obtained by collisionally induced fragmentation of the respective parent ions: (a) MS trace 40–200 Da, (b) MS/MS trace of the transition 71 ± 2 Da → 40–43 Da, and (c) MS/MS trace of the transition 85 ± 2 Da → 54–58 Da.

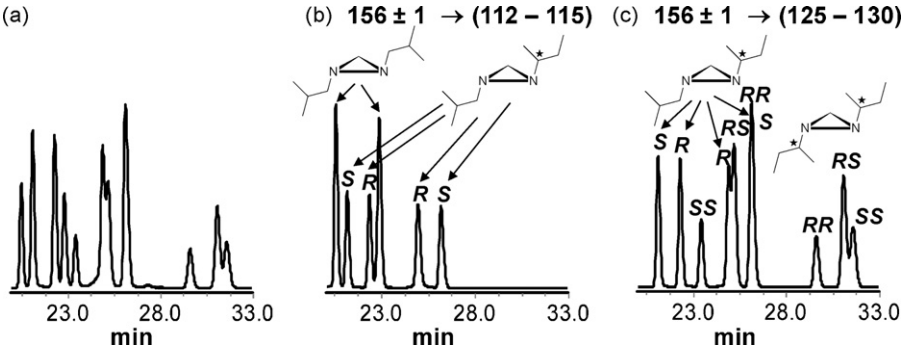

Fig. 7. Total ion current chromatograms of the separation of the enantiomers of 1,2-diisobutyldiaziridine, the epimeric pairs of 1-*sec*.-butyl-2-isobutyldiaziridine and the epimeric pairs and enantiomers of 1,2-di-*sec*.-butyldiaziridine obtained by GC–MS and GC–MRM-MS (25 m Chirasil-β-Dex, i.d. 250 μm, film thickness 500 nm, isothermal at 120 °C, 40 kPa He). The transitions in the MRM-MS experiments were obtained by collisionally induced fragmentation of the respective parent ions: (a) MS trace 40–150 Da, (b) MS/MS trace of the transition 156 ± 1 Da → 112–115 Da, and (c) MS/MS trace of the transition 156 ± 1 Da → 125–130 Da.

almost exclusively the two stereoisomers. By synthesis of 1-*n*-butyl-2-(*S*)-*sec.*-butyldiaziridine (*S*)-5 using (*S*)-*sec.*-butylamine the stereochemical assignment of the *sec.*-butyl substitutents could be achieved. Another advantage of MRM-MS is, that peaks of impurities are removed and do not influence the peak shape analysis of the obtained chromatograms, which could lead to wrong kinetic data.

The chromatogram of mixture (iii) containing 1,2-diisobutyldiaziridine **6**, 1-*sec.*-butyl-2-isobutyldiaziridine **7**, and 1,2-di-*sec.*-butyldiaziridine **8** shows only 11 peaks instead of the expected 12 stereoisomers and it is obvious that interpretation of the elution order can easily lead to misinterpretation of the results (cf. Fig. 7a).

Despite the fact that the $M^{+\bullet}$ peaks in the MS spectra showed only very low intensities (around 1% of the base peak), these ions were selected for further MS/MS experiments because more characteristic transitions were obtained. In Fig. 7b and c two transitions 156 ± 1 Da → 112–115 Da and 156 ± 1 Da → 125–130 Da are depicted. The interpretation of these results taking the stereochemistry and peak intensities into account makes the assignment of the enantiomeric and epimeric pairs very easy. The enantiomeric pairs of 1,2-diisobutyldiaziridine **6** and the epimeric pairs of 1-*sec.*-butyl-2-isobutyldiaziridine **7** can be assigned according to Fig. 7b. Here again (*S*)-*sec.*-butylamine was used to synthesize mixture (iii) exclusively with (*S*)-configuration of the *sec.*-butyl substituents to assign the elution order. 1,2-(*R*,*S*)-di-*sec.*-butyldiaziridine (*R*,*S*)-8 was identified by taking into account, that from the statistical synthesis of 1,2-(*R*,*S*)-di-*sec.*-butyldiaziridine **8** a ratio of 1:1:2 for (*R*,*R*)-8:(*S*,*S*)-8:(*R*,*S*)-8 is obtained.

Applying the here described procedure to separate the constitutional isomers by enantioselective GC–MRM-MS it is straightforward to determine the separation factors on Chirasil-β-Dex (100 °C and 50 kPa inlet pressure) to estimate the influence of the bulkiness and configuration of the substituents on the separation factor α: 1,2-di-*n*-propyldiaziridine **1** $\alpha = 1.04$, 1-isopropyl-2-*n*-propyldiaziridine **2** $\alpha = 1.18$, 1,2-diisopropyldiaziridine **3** $\alpha = 1.20$, 1,2-di-*n*-butyldiaziridine **4** $\alpha = 1.04$, 1-*n*-butyl-2-(*R*)-*sec.*-butyldiaziridine (*R*)-5 $\alpha = 1.15$, 1-*n*-butyl-2-(*S*)-*sec.*-butyldiaziridine (*S*)-5 $\alpha = 1.24$, 1,2-diisobutyldiaziridine **6** $\alpha = 1.16$, 1-(*R*)-*sec.*-butyl-2-isobutyldiaziridine (*R*)-7 $\alpha = 1.16$, 1-(*S*)-*sec.*-butyl-2-isobutyldiaziridine (*S*)-7 $\alpha = 1.33$, 1,2-(*R*,*R*)-di-*sec.*-butyldiaziridine (*R*,*R*)-8 $\alpha = 1.17$, 1,2-(*S*,*S*)-di-*sec.*-butyldiaziridine (*S*,*S*)-8 $\alpha = 1.46$, and 1,2-(*R*,*S*)-di-*sec.*-butyldiaziridine (*R*,*S*)-8 $\alpha = 1.30$. From these data it is obvious that bulkier substituents and in the case of *sec.*-butyl substituents the (*S*)-isomer yield higher separation factors α.

4. Conclusions

The here presented technique based on enantioselective GC–MRM-MS constitutes a valuable tool to obtain chromatograms of separated constitutional isomers even in complex mixtures of several stereoisomers. Furthermore the correct assignment of the elution order is facilitated. The most significant result is, that the elution profiles of overlapping constitutional isomers can be experimentally deconvoluted, which allows to perform temperature dependent measurements for the determination of kinetic data of these interconverting stereoisomers. The advantage of using reaction mixtures from statistical or combinatorial synthesis libraries without further separation to determine kinetic data can be fully exploited. This increases the overall sample throughput and more important, delivers comparable data under the very same reaction or interconversion conditions. Another advantage, which can be envisaged, is, that MRM-MS can be easily used to absolutely quantify analytes by introduction of characteristic groups into the target molecules, which is of importance to determine thermodynamic equilibrium constants of interconverting diastereoisomers, which might show different detection responses.

The here presented approach is not limited to GC analyses but can be also applied to liquid and electrophoretic separation techniques coupled with mass spectrometry. The only requirement is the possibility to perform MS/MS or even MS^n experiments, which can be done with an ion trap or triple–quadrupole instrument.

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