The effect of gas-phase reactions on the quantitation of cyclic hydrazone libraries by electrospray ionization (ESI) mass spectrometry†

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Using mass spectrometry coupled with LC analysis we report evidence of diastereomer dependent fragmentation and oligomerization reactions in the ionization of acyl-hydrazone-based libraries of cyclic oligomers. These effects can significantly affect the accuracy of MS-based quantitations, but also provide a venue for examining ionization effects in dynamic combinatorial libraries (DCLs).

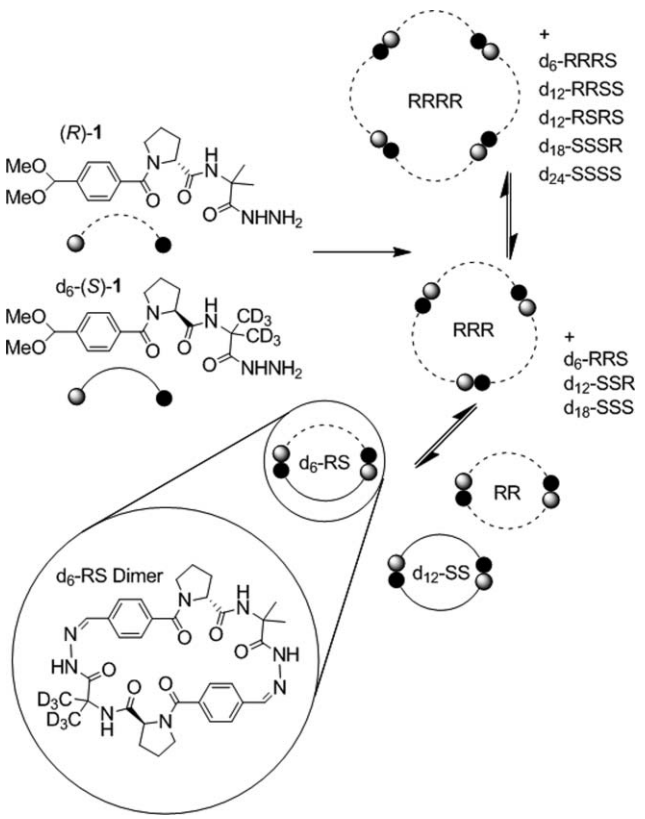
Introduction

Dynamic combinatorial chemistry (DCC) is an efficient method often used to search for new molecular receptors.[1] Reversible bonds between monomers are utilized to establish the dynamic qualities of the libraries, which allow the library speciation to respond to an added template.[2] A qualitative interpretation of a library's binding profile is occasionally sufficient to understand the recognition/amplification trends, but quantitative characterization is necessary when the distribution or number of components is significant. In dynamic combinatorial libraries (DCLs) containing more than one monomer, amplified components can be difficult to identify because of incomplete chromatographic resolution or molecular weight degeneracies.[3] Separation can become even more challenging when there is stereochemical diversity in the library.

As part of an effort to discover new receptors capable of enantiorecognition, our group has been exploring the use of racemic libraries as a means for testing stereochemically complex mixtures. The guiding notion in these works was that one could discover enantioselective receptors from racemic libraries if the analyte was enantiomerically pure and methods were available to detect the enantio-imbalances in the receptor that would result from an enantioselective host–guest interaction and resultant enantiomeric amplification. To measure these perturbations, a laser polarimeter (LP) detector was used to detect the chiroptical changes in the initially racemic library. Since this approach generates a signal (optical enrichment) from an otherwise null background (racemic, optically inactive), LP enables one to discriminate between simple amplification and enantiomer-selective amplification.

Results

In one such application, LP was used to detect an enantio-imbalance in a (−)-adenosine amplified dimer.[4] The library was additionally characterized by UPLC-MS on a pseudo-enantiomeric library wherein one enantiomer was deuterated to provide a unique mass signature for each library member.[5] As shown in Scheme 1, this pseudo-racemic library system provided a stereochemically complex library, wherein a slight change in retention time of the library was noted for the H- and D-labelled compounds (Fig. 1). The slight retention differential of the D-labelled species was observable in both the UV and LP traces.



Scheme 1 The combined enantiomers of compound **1** ((***R***)-**1**, (***S***)-**1**-d_6) form a pseudo-racemic DCL composed of stereochemically diverse dimers, trimers, and tetramers. All library members are in thermodynamic equilibrium.

All the cyclic species were readily separable by the size of the cyclomer, but the stereoisomers tended to overlap in HPLC,

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† Electronic supplementary information (ESI) available: Details of the HPLC-LP and UPLC-MS analyses, and selected mass ion chromatograms for the dimers, trimers and tetramers in the DCL. See DOI: 10.1039/b808490d

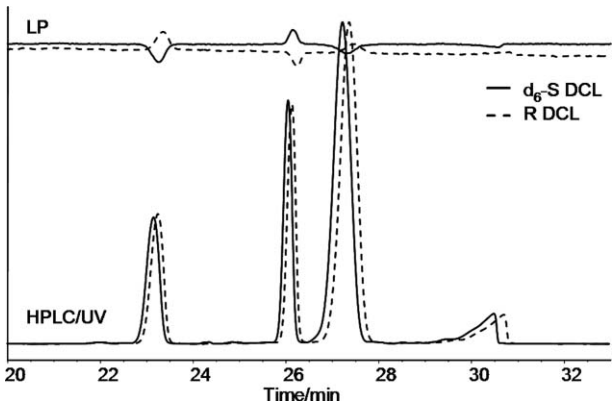


Fig. 1 HPLC chromatogram of the DCL derived from **(*R*)-1** (dotted) and **(*S*)-1-d_6** (solid) along with their respective LP traces. The deuterated enantiomer elutes slightly before the protonated enantiomer.

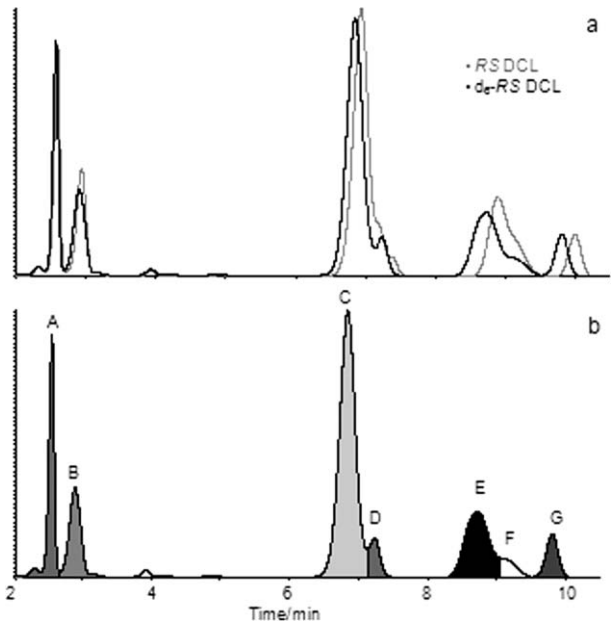


Fig. 2 (a) UPLC-UV scan demonstrating the slightly shorter elution times of the deuterated stereoisomers. (b) The trace of the pseudo-racemic DCL is divided into regions to designate the species prevalent under each peak: A (*RS*), B (*RR*, *SS*), C (*RRS*, *SSR*, *SSS*), D (*RRR*), E (*RRRS*, *SSSR*, *SSSS*), F (*RRRR*), G (*RRSS*, *RSRS*).

though UPLC provided better separation. Fig. 2a compares the UPLC-MS chromatograms of racemic and pseudo-racemic DCLs to illustrate the improved resolution of d_6-*RS* deuterated libraries. The first peak (A) of Fig. 2b was pure hetero-dimer (d_6-*RS*), while B housed the *RR* and d_{12}-*SS* homo-dimers. No diastereomeric resolution was observed in the racemic trimer (C), but with D-labelling, *RRR* was observable as a shoulder (D). Regions E, F and G contained the tetramers, with d_6-*RRRS* and d_{18}-*SSSR* being the principal constituents of E; d_{24}-*SSSS* homo-tetramer was also present and tailed into F. The meso-tetramers, d_{12}-*RRSS* and/or d_{12}-*RSRS*, were separated from the chiral isomers (G).

When templated with (−)-cytidine, the speciation shifted in response, and UPLC-MS was utilized to quantify the resulting diastereomer imbalances.[6] Careful examination of the data, however, indicated that the total ion chromatogram (TIC) incorrectly measures the absolute isomer ratios.

The UPLC-MS software enabled the extraction of an ion chromatogram for a single mass range, which displayed where the selected mass appeared in the chromatogram. Since each isomer in the pseudo-racemic library had a unique mass signature (except for *RSRS* and *RRSS*), this approach should have enabled an isomer selected chromatogram. These "selected range" ion chromatograms were obtained for each of the stereoisomers of the dimer, trimer and tetramer. Under ideal conditions, one would expect to only observe each species once.

Unexpectedly, however, several of the species were seemingly eluting in multiple locations in the chromatogram. Fig. 3 and 4 outline for dimers and tetramers, respectively, how some stereoisomer scans were complicated by more than the single expected peak. This was especially true for the tetramers and to a lesser extent, the dimers. Little trimer was observed in the other eluting species, indicating that it was not a common reaction product (see ESI† for a representative figure).

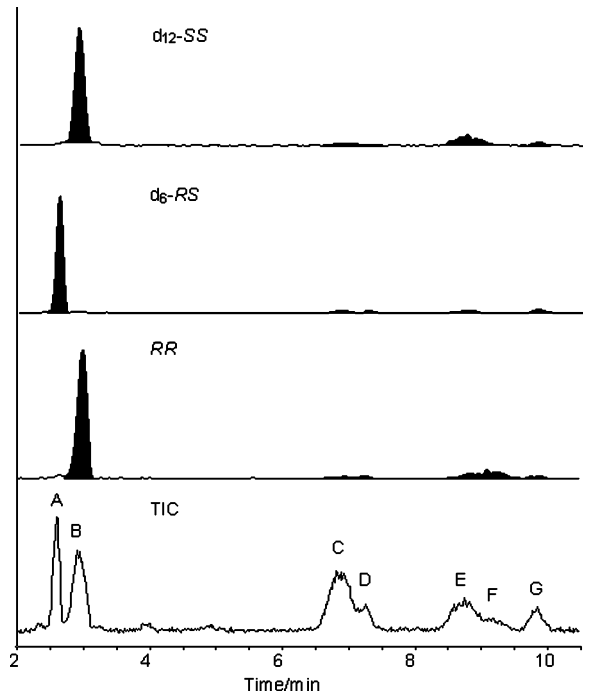


Fig. 3 Dimer selected chromatograms, selected for d_{12}-*SS*, d_6-*SR*, and *RR*, followed by a total ion chromatogram. Small amounts of dimer appear in both the trimer (C, D) and tetramer (E, F, G) regions.

Discussion

Observing a peak in the chromatogram at a location other than its natural retention time indicated that it was generated as the eluting species reacted in the ionization chamber. Examining such "selected range" ion chromatograms for the DCL members therefore provides insight into the reactions taking place between the ionization process and detection.

The dimer selected ion chromatograms (Fig. 3) revealed that trimers and tetramers were each able to fragment into dimers, and not unexpectedly, it was more favorable for tetramers to do

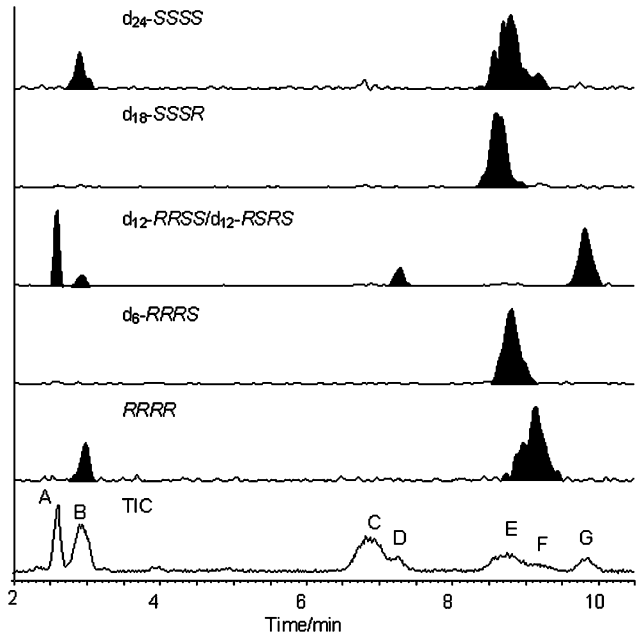
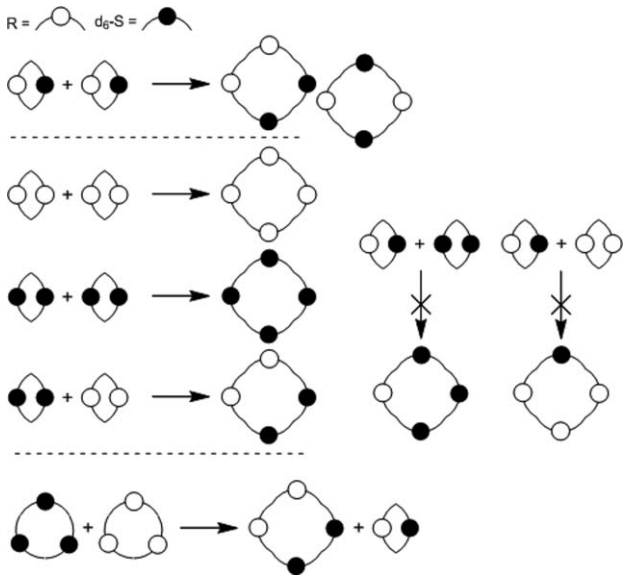


Fig. 4 Selected range ion chromatograms for the indicated ions. Peak labels in the TIC correspond to the regions discussed in Fig. 2 and the text.



Scheme 2 Empirical selection rules extracted from the isomer-selected ion chromatograms. Cases where cross-reactions do not occur result from the non-overlapping elution times of the oligomers.

so. We rationalize this by noting that a trimer would need to unimolecularly generate a stoichiometric quantity of monomer to make a dimer. Tetramers on the other hand could simply fragment into two dimers without generating a high energy product.[7] Bimolecular reactions are also possible and this could be a source of the dimer, for example trimer + trimer = dimer + tetramer, *etc*.

Rational selection rules for these fragmentations could be generated by examining the stereochemical relationship between the oligomer source and the resultant products (Scheme 2). For example, in the d_{24}-*SSSS* scan (Fig. 4), one notes that there is no peak in the *RS* region, indicating that this dimer does not form the homo-tetramer. This observation was explained by noting that the primary products of a bimolecular *RS* dimerization could never generate a homochiral oligomer. Similarly, little of the *RRRS* or *SSSR* structures were generated in either of the dimer regions since these products would require the cross reaction of a homo- and hetero-chiral dimer. Since these dimers don't co-elute they would never be in the ionization chamber at the same instant, and thus the cross product does not form.

The *meso*-tetramer scan, however, demonstrated that it could be synthesized by a number of reaction pathways. It was highly favored to form in the *RS* stream where dimerization could yield either form of the *meso* compound depending on the regioselectivity. It was also observed in the homochiral region, where the cross product of *RR* and *SS* more likely generated the *SSRR* stereoisomer. The *meso*-tetramer was also generated in a narrow region of the trimer scan, specifically where the *RRR* trimer eluted, and not elsewhere. We presume a cross reaction with an *SSS* tail into this peak, and co-generation of a dimer.

Conclusions

The default mode of quantifying the results of an LC-MS is to examine the relevant mass region from a total ion chromatogram (TIC), which is a sum of every mass spectrum obtained during the chromatography. When each eluent has a unique mass and no chemistry occurs, this approach should be accurate. However, even if each species has a unique mass but reaction chemistry is possible, then erroneous quantitations may occur due to post-separation cross-contamination. In other words, the ratio of diastereomers in the dimer and tetramer quantified from the TIC, is distorted by reactions in the ionization chamber that convert some tetramer to dimer and some dimer to tetramer. Since the stereochemistry of these reactions is controlled by the source ions (Scheme 2), and their presence or absence is dictated by the elution (or co-elution) profile of the LC, *a broad variety of outcomes are possible*! Vexing is the notion that these effects will also depend on the resolution, or lack thereof, in the LC component of the analysis.

Our experience with the above system has shown that the most reliable method for accurately quantifying the diastereomer ratios is to sum the mass spectra for only the chromatographic peak in question. This ensures that no contributions to this ion are made by the reaction chemistry of compounds eluting elsewhere, which occurs if one utilizes the TIC for the measurement. Of course, if incomplete resolution occurs in the peak in question, then one must be cognizant of the potential for reaction chemistry between the co-eluting compounds. We suspect that inter- and intramolecular reactions between co-eluting species[8] may be widespread in hydrazone-based libraries.

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Notes and References

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7 Under hydrating conditions one could conceivably generate the aldehyde form of the monomer, which would obviate the need for a high energy intermediate.

8 Practically, these unwanted side reactions could not be completely avoided in the hydrazone DCL analysis. Voltages (capillary and cone) and desolvation gas flow rate in the MS chromatogram conditions were each found to affect the magnitude of these reactions. Similarly sensitive were the LC mobile phase, the nature of the library species, and whether sodium or potassium adducts were observable. Optimization of the above conditions can minimize the fragmentation and oligomerization side reactions, with the latter being additionally sensitive to concentration.