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Quantitative Chiral Analysis of Sugars by Electrospray Ionization Tandem Mass Spectrometry Using Modified Amino Acids as Chiral Reference Compounds

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Rapid quantitative enantiomeric analysis of mannose, glucose, galactose, and ribose is achieved using electrospray ionization and cluster ion dissociation with data analysis by the kinetic method. Several modified amino acids (*N*-Ac-L-Phe, *N*-benzoyl-L-Phe, *N*-*t*-Boc-L-Phe, *N*-Ac-L-Pro, *N*-*t*-Boc- L-Pro, *N*-Fmoc-L-Pro, *N*-Ac-L-Tyr, *O*-Me-L-Tyr) and four transition divalent metal cations (Co^{2+}, Cu^{2+}, Ni^{2+}, and Zn^{2+}) were tested to select the best system for chiral recognition and quantitation of each sugar. Quantitative determinations of the enantiomeric compositions of sugar solutions were achieved using either multiple- or two-point calibration curves; differences between the actual and experimental values were <2% enantiomeric excess (ee).

Significant progress has been made during the past few years on chiral identification and quantification[1−3] based exclusively on mass spectrometry. It is possible to classify the mass spectrometry methods used for chiral recognition and quantification into four types:

(1) Host−guest diasteromeric adducts are generated using a chiral guest and analyzed in a single-stage mass spectrometer in the first type of experiment. One of the enantiomeric hosts is isotopically labeled, and thus, the corresponding mixture of diasteromeric adducts can be mass-resolved. Chemical ionization,[4,5] fast atom bombardment (FAB),[6−8] matrix-assisted laser desorption/ionization (MALDI),[9] and electrospray ionization (ESI)[10] mass spectrometry have all been used in this type of experiment.

(2) Chiral recognition in the second type of experiment is based on ion/molecule reactions.[11] Diasteromeric adducts, generated by inserting the analyte into a chiral host molecule, are mass-selected and allowed to react with a neutral reagent that need not be chiral. Chiral distinction is possible, since the rates of guest exchange depend on the chirality of the enantiomeric guest.[12−15]

(3) A third group of methods for chiral recognition is based on collision-induced dissociation (CID) of diasteromeric adducts in a tandem mass spectrometry (MS/MS) experiment.[16−19]

(4) A fourth approach uses tandem mass spectrometry and the kinetic method to quantify chiral effects.[20−22] This methodology has been successfully applied to quantitative analysis of amino acids,[23,24] α-hydroxyacids[25] and some drugs.[26,27] It is the subject of the present study.

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The kinetic method is an approximate procedure that is normally applied to make relative thermochemical determinations on the basis of the rates of competitive dissociations of mass-selected cluster ions.[28−31] The relative rates of unimolecular dissociations of a mass-selected cluster ion, A···H^+···B, eq 1, are measured

$$AH^+ + B \leftarrow A\cdots H^+\cdots B \rightarrow A + BH^+ \qquad (1)$$

A simplified theoretical treatment based on the kinetics of unimolecular dissociation, then leads to the standard form of the kinetic method,[32−34] eq 2.

$$\ln([AH^+]/[BH^+]) = \Delta GB/RT_{eff} \qquad (2)$$

where $[AH^+]$ and $[BH^+]$ are the abundances of the two protonated bases, ΔGB is the difference in the gas-phase basicities of compounds A and B, R is the ideal gas constant and T_{eff} is the effective temperature[35,36] of the activated dimeric ion, A···H^+···B. When applying this simplified equation, it is assumed that the entropy requirements for the competitive dissociation channels are equal, the activation energies for the reverse reactions are insignificant, and that there are no other isomeric forms of the activated clusters. The method is sensitive to small differences in thermochemical values and is also applicable to nonvolatile and polar compounds, including peptides and other biomolecules, that are difficult to analyze by other methods.

Carbohydrates serve as energy stores and metabolic intermediates and are linked to many proteins and lipids in compounds of great biological importance. Polysaccharides are structural elements in the cell walls of bacteria and plants as well as in the exoskeletons of arthropods. In addition, ribose and deoxyribose form part of the structural framework of RNA and DNA. Recent studies have revealed that carbohydrate groups on cell surfaces play key roles in cell−cell recognition. For example, fertilization begins with the binding of a sperm cell to a specific oligosaccharide on the surface of the egg. In addition, carbohydrates are increasingly known as information-rich molecules of significance in cell development and repair.[37] For the chiral analysis of sugars, chromatographic methods are usually employed.[38] The present study describes the application of the kinetic method to rapid enantiomeric quantitation of simple sugars using, as examples, mannose, glucose, galactose, and ribose.

EXPERIMENTAL SECTION

Sugars and modified amino acids were purchased from Sigma-Aldrich and were used without purification. All experiments were performed using a commercial LCQ ion trap mass spectrometer (Thermo Finnigan, San Jose, CA), equipped with an ESI source and operated in the positive ion mode. Spectra shown represent the average of ∼50 scans, each requiring 0.2 s. Samples were infused into the ESI source via a syringe pump at a flow rate of 3−5 μL min^{-1}. Typical ESI conditions were as follows: heated capillary temperature, 150 °C; sheath gas (N_2) flow rate, 30 units (roughly 0.75 L/min); spray voltage, 5.00 kV; capillary voltage, 5 V; tube lens offset voltage, 20 V; and syringe pump, 5−10 μL/min. In the full scan MS^2 and MS^3 operating modes, the parent ion of interest was first isolated by applying an appropriate waveform across the end cap electrodes of the ion trap to resonantly eject all trapped ions except those ions of the m/z ratio of interest. The isolated ions were then subjected to a supplementary ac signal to resonantly excite them and so cause collision-induced dissociation (CID). The collision energy was set to a value at which product ions were produced in intermediate relative abundance. The CID conditions were optimized for each analyte. Mass/charge ratios (m/z) are reported using the Thomson unit (1 Th = 1 atomic mass per unit positive charge).[39] Aqueous methanol solutions that were used contained a mixture of sugar (S_D or S_L, 2×10^{-4} mol/L), a reference compound (chiral modified amino acid, ref*, 2×10^{-4} mol/L), and a transition metal ion (M = Co^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, 1×10^{-4} mol/L).

RESULTS AND DISCUSSION

The singly charged trimeric ions $[M(ref^*)_2(S) - H]^+$ were formed in high abundance in the mass spectra (not shown) of electrosprayed solutions containing the enantiomerically pure sugar (D or L), a chiral reference compound (ref*), and the salt of a metal (M). In addition to the trimeric cluster of interest, several other species, such as the protonated sugar, its proton-bound dimer, protonated reference compounds, proton-bound dimers of the reference compound, sodiated species, and other metal complexes, such as $[M(ref)_n - H^+]$ and $[M(sugar)_n - H^+]$ were also observed in these mass spectra. The trimeric ions, $[M(ref^*)_2(S) - H]^+$, were mass-selected and fragmented by collision-induced dissociation in a quadrupole ion trap to form the pair of dimeric ions $[M(S_D)(ref^*) - H]^+$ or $[M(S_L)(ref^*) - H]^+$ and $[M(ref^*)_2 - H]^+$. The energy diagram in Scheme 1 shows the competitive dissociations of the trimeric complexes $[M(ref^*)_2(S_D) - H]^+$ and $[M(ref^*)_2(S_L) - H]^+$ to form these dimeric fragments.

In this diagram, the difference in energy between the diastereomeric ions $[M(ref^*)(S_D) - H]^+$ and $[M(ref^*)(S_L) - H]^+$ results in differences in their rates of formation and, hence, in the relative abundance ratios (R_D or R_L) for the D and L isomers,

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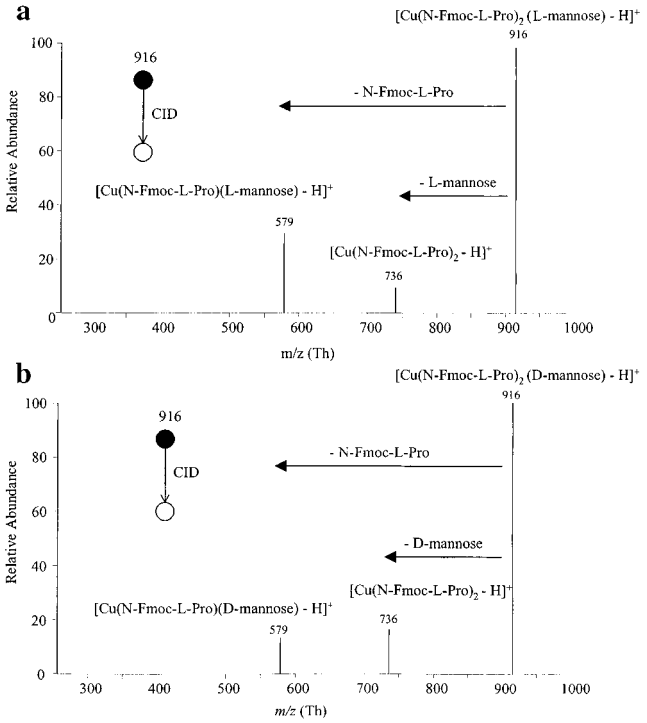
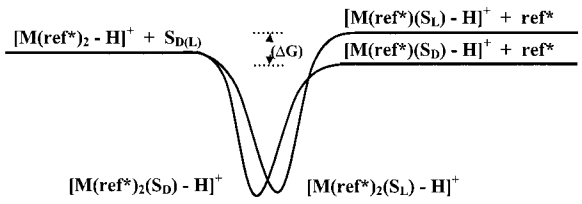


Figure 1. MS/MS product ion spectra of (a) [Cu(L-mannose)(L−*N*-Fmoc-L-Pro)$_2$ − H]$^+$. (b) [Cu(D-mannose)(*N*-Fmoc-L-Pro)$_2$ − H]$^+$. The CID activation level is chosen as 10%, corresponding to ∼250 mV AC.

Scheme 1



defined in eqs 3 and 4, respectively.

$$R_D = [M(S_D)(ref^*) - H]^+/[M(ref^*)_2 - H]^+ \quad (3)$$

$$R_L = [M(S_L)(ref^*) - H]^+/[M(ref^*)_2 - H]^+ \quad (4)$$

A typical application of this methodology is shown in Figure 1, where chiral distinction between D- and L-mannose is achieved using Cu^{2+} as the metal cation and *N*-Fmoc-L-Pro as the chiral reference compound. There is a large difference between the ratios R_D and R_L, and this is due to the difference in stability of the diastereomeric ions [Cu(D-mannose)(*N*-Fmoc-L-Pro) − H]$^+$ and [Cu(L-mannose)(*N*-Fmoc-L-Pro) − H]$^+$, both measured relative to the same reference ion [M(ref*)$_2$ − H]$^+$ but in different experiments.

The ratio of the individual ratios, R_D to R_L, is defined as R_{chiral} (eq 5) and indicates the level of chiral discrimination achievable in a particular experiment.

$$R_{chiral} = R_D/R_L \quad (5)$$

Clearly, when $R_{chiral} = 1$, there is no chiral discrimination, which denotes that this specific arrangement of metal and chiral reference is not able to create effective enantiomer distinction under these specific conditions. Therefore, the best systems to be selected are those that can provide R_{chiral} as far as possible from unity, provided accurate abundance ratios can still be measured.

The relationship between the abundance ratio R ([M(ref*)(S) − H]$^+$]/M(ref *)$_2$ − H]$^+$) and the enantiomeric excess (ee) of the chiral sugar can be obtained from the equation for the kinetic method (eq 2).[24,26] The natural logarithm of the ratio R is proportional to the difference in free energy [$\Delta(\Delta G)$] for formation of the two diasteromeric ions [M(S_D)(ref*) − H]$^+$ and [M(S_L)-(ref*) − H]$^+$ (Scheme 1). The relationship can be written as

$$\ln(R) = \Delta(\Delta G)/RT_{eff} \quad (6)$$

where T_{eff} is the *average* temperature of the two activated complexes for the two competitive reactions. When the sugar sample is made up of the pure D or L enantiomers, $\Delta(\Delta G)$ becomes $\Delta(\Delta G)_D$ or $\Delta(\Delta G)_L$. For an enantiomeric mixture with an excess of enantiomer D, for example, one can write

$$\Delta(\Delta G) = [\Delta(\Delta G_D)(1 + ee)/2] + [\Delta(\Delta G_L)(1 - ee)/2]$$

$$= [\Delta(\Delta G_D) + \Delta(\Delta G_L)]/2 + [\Delta(\Delta G_D) - \Delta(\Delta G_L)]^*ee/2 \quad (7)$$

Moreover, the combination of eqs 6 and 7 furnishes an expression describing a linear relationship between the enantiomeric excess (ee) and ln(R), as shown in eq 8.

$$\ln(R) = [\Delta(\Delta G_D) + \Delta(\Delta G_L)]/2RT_{eff} + [\Delta(\Delta G_D) - \Delta(\Delta G_L)]^*ee/2RT_{eff} \quad (8)$$

Modified amino acids (*N*-Ac-L-Phe, *N*-benzoyl-L-Phe, *N*-*t*-Boc-L-Phe, *N*-Ac-L-Pro, *N*-*t*-Boc-L-Pro, *N*-Fmoc-L-Pro, *N*-Ac-L-Tyr, and *O*-Me-L-Tyr) were chosen as reference compounds, since (a) they can be easily obtained in enantiomerically pure form; (b) their large substituents should facilitate distinction between the two diasteromeric dimer ions and (c) in previous studies, some of these compounds proved to be useful as reference compounds in chiral analysis.[25−27] The cations Co^{2+}, Cu^{2+}, Ni^{2+}, and Zn^{2+} were selected as the central metal ions owing to their well-known ability to form complexes with oxygen and nitrogen-containing compounds as well as their use in previous successful applications of the kinetic method to chiral analysis of amines and other compounds.[24,26] The results of the experiments on chiral recognition of mannose, glucose, galactose, and ribose using the chiral reference compounds and the metal cations are summarized in Table 1. The best systems for chiral analysis of each sugar are shown in bold in this Table. In three of the four systems, Cu^{2+} is the best cation, as it often is in other classes of analyte.[24,26]

Using the selected systems, linear relationships of ln(R) versus the molar fraction of D-mannose (Figure 2), D-glucose (Figure 3), D-galactose (Figure 4), and D-ribose (not shown, $R^2 = 0.9994$) were observed, in each case with excellent correlation coefficients. Such linear correlations are intrinsic to the kinetic method, as shown above. Using the appropriate calibration curve, the enantiomeric composition of prepared sugar solutions was determined, and

Table 1. Chiral Selectivity Factor (R_{chiral}) Using Different Metal Cations and Various Modified Amino Acids as Reference Compounds

system		chiral selectivity factor (R_{chiral})[a]			
reference	metal	mannose	glucose	galactose	ribose
N-Ac-L-Phe	Co^{2+}				0.94
	Cu^{2+}		0.81	0.90	1.68[b]
	Ni^{2+}	1.31			
	Zn^{2+}	0.83	0.87	0.79	
N-benzoyl-L-Phe	Co^{2+}		0.95	0.92	0.95
	Cu^{2+}	0.39		0.90	1.00
	Ni^{2+}				0.93
	Zn^{2+}	0.93		0.87	
N-*t*-Boc-L-Phe	Co^{2+}				
	Cu^{2+}	1.40	1.19		
	Ni^{2+}				
	Zn^{2+}	1.25	1.14		
N-Ac-L-Pro	Co^{2+}	1.30	1.23		
	Cu^{2+}				
	Ni^{2+}				
	Zn^{2+}	1.47	2.17		
N-*t*-Boc- L-Pro	Co^{2+}	0.63	0.67	0.67	0.95
	Cu^{2+}		0.48		0.90
	Ni^{2+}	0.86			1.08
	Zn^{2+}	0.55		0.62	
N-Fmoc-L-Pro	Co^{2+}	0.59	0.55	1.88	
	Cu^{2+}	0.26[b]	0.44[b]	0.66	
	Ni^{2+}	0.57	0.64	1.48	0.93
	Zn^{2+}			2.10	
N-Ac-L-Tyr	Co^{2+}		0.86		1.01
	Cu^{2+}	1.37	0.78	1.26	1.72
	Ni^{2+}				
	Zn^{2+}	0.94	0.84	0.68	
O-Me-L-Tyr	Co^{2+}	1.64	0.80		1.69
	Cu^{2+}				
	Ni^{2+}	2.89		0.55[b]	
	Zn^{2+}	1.38		0.70	

[a] R_{chiral} is defined in eq 5. CID activation level is optimized for each experiment and then kept constant for the measurement of each enantiomer. [b] Best values for R_{chiral} obtained for each sugar.

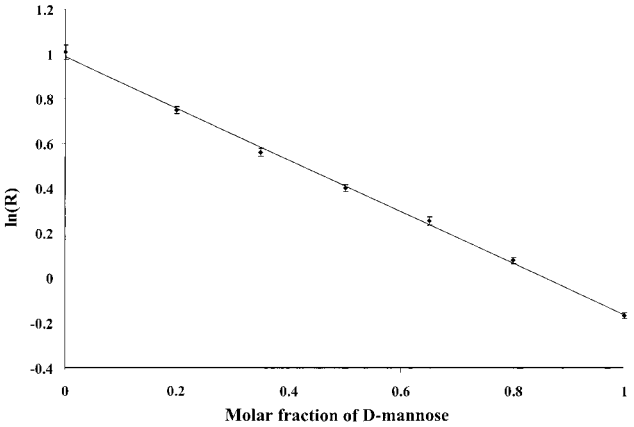


Figure 2. Calibration curve for chiral analysis of mannose using Cu^{2+} as the metal cation and *N*-Fmoc-L-Pro as the chiral reference compound. The chiral selectivity factor (R_{chiral}) is 0.26. The correlation coefficient (R^2) is 0.9985. Error bars represent 95% confidence level.

excellent results were obtained, as illustrated by the data of Table 2. Importantly, it was also verified that the relative concentrations of sugar vs reference did not affect the chiral recognition in these systems.[40] For example, the concentration ratios between the mannose and *N*-Fmoc-L-Pro were changed from 3:1 to 1:3. However, the ratios R_D and R_L remained practically constant over this interval, with measured values of 0.83 ± 0.02 and 3.19 ± 0.06,

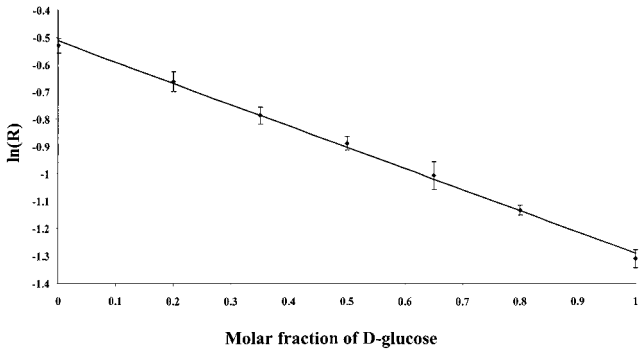


Figure 3. Calibration curve for chiral analysis of glucose using Cu^{2+} as the metal cation and *N*-Fmoc-L-Pro as the chiral reference compound. The chiral selectivity factor (R_{chiral}) is 0.44. The correlation coefficient (R^2) is 0.9974. Error bars represent 95% confidence level.

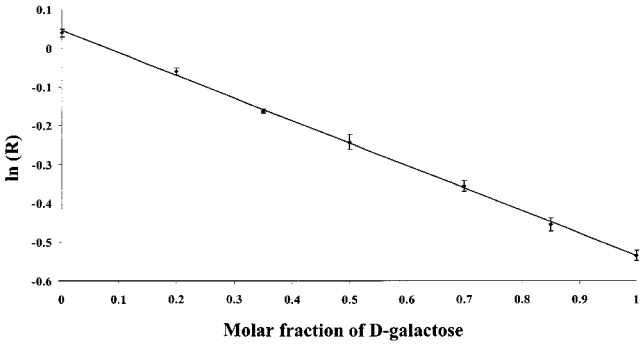


Figure 4. Calibration curve for chiral analysis of galactose using Ni^{2+} as metal cation and *O*-Me-L-Tyr as the chiral reference compound. The chiral selectivity factor (R_{chiral}) is 0.55. The correlation coefficient (R^2) is 0.999. Error bars represent 95% confidence level.

Table 2. Actual and Experimental Values for the Enantiomeric Composition of Some Sugar Solutions

	enantiomeric excess of D-sugar (% ee)		
sugar	actual	experimental[a]	difference[c]
mannose	−52	-51.6 ± 0.5[b]	−0.4
mannose	−92	-94 ± 3[b]	2
glucose	66	68 ± 2[b]	2
galactose	70	75 ± 6[b]	5
galactose	40	40 ± 5[b]	0
ribose	76	76 ± 3[b]	0

[a] Values obtained from the respective calibration curves using the best systems described in Table 1. [b] Average of five measurements. The interval represents 95% of confidence.

respectively. This is a necessary result for successful quantitative analysis of unknown samples.

Once the linearity of the semilog plots had been established, a two-point calibration curve allowed a faster but still reliable method of quantitative analysis. In the case of mannose, the calibration line was built using the two pure enantiomers and the chemical system (*N*-Fmoc-L-Pro, Cu^{2+}) highlighted in Table 1. By the use of this two-point calibration curve, the enantiomeric composition of several mannose samples was determined. Excellent results were observed, with consistently good agreement between the actual and the measured values, as shown in Table 3.

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Table 3. Enantiomeric Composition of Some Mannose Solutions Determined Using a Two-Point Calibration Curve

enantiomeric excess of D-sugar, %		
actual	experimental[a]	difference (% ee)
80	82 ± 2[b]	2
60	58 ± 2[b]	−2
−80	−78 ± 2[b]	−2
−60	−64 ± 2[b]	4

[a] Values obtained from a two-point calibration curve using the best systems described in Table 1. [b] Given as 95% of confidence.

The present study has described a new method for rapid enantiomeric determination of sugars. The measurements are simple and rapid, and they require very small amounts of sample for analysis. It can be envisioned that the present method could be advantageously applied to in situ analysis of extraterrestrial samples obtained from meteorites[41] or at the surface of other planets. The presence of nonracemic sugars (or other biomolecules) would be of great interest in connection with current ideas on the extraterrestrial origin of biological molecules and of homochirality.[42] Further extension of this method, which represents a general mass spectrometric method for gas-phase chiral analysis, to the study of other important compounds of natural occurrence, including terpenoids and alkaloids, is in progress. One limitation of the present method is its restriction to the quantification of mixtures containing only one enantiomeric pair. Attempts to extend the method to the analysis of mixtures of samples containing additional components, including isomeric sugars, are underway.

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