



SC9- Joensuu, 19-22 April 2022

# Isomer discrimination in FT-ICR MS and hyphenated techniques



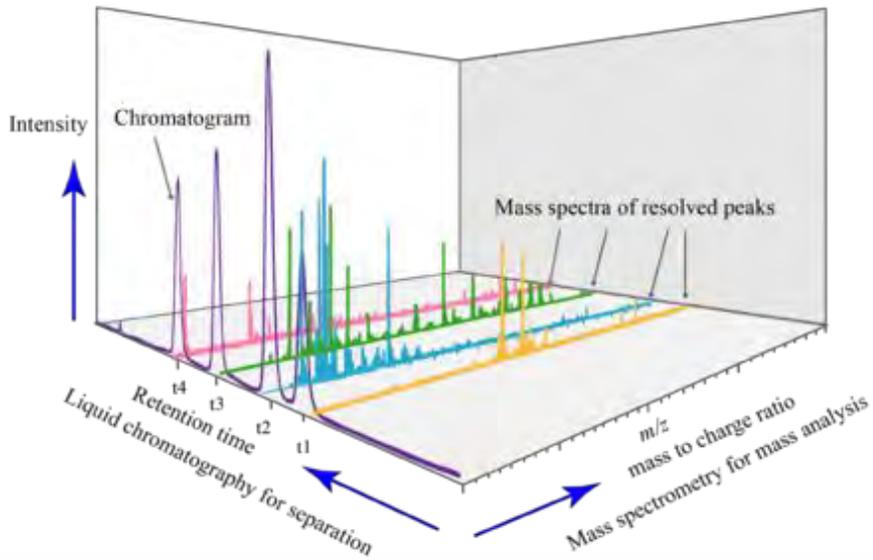
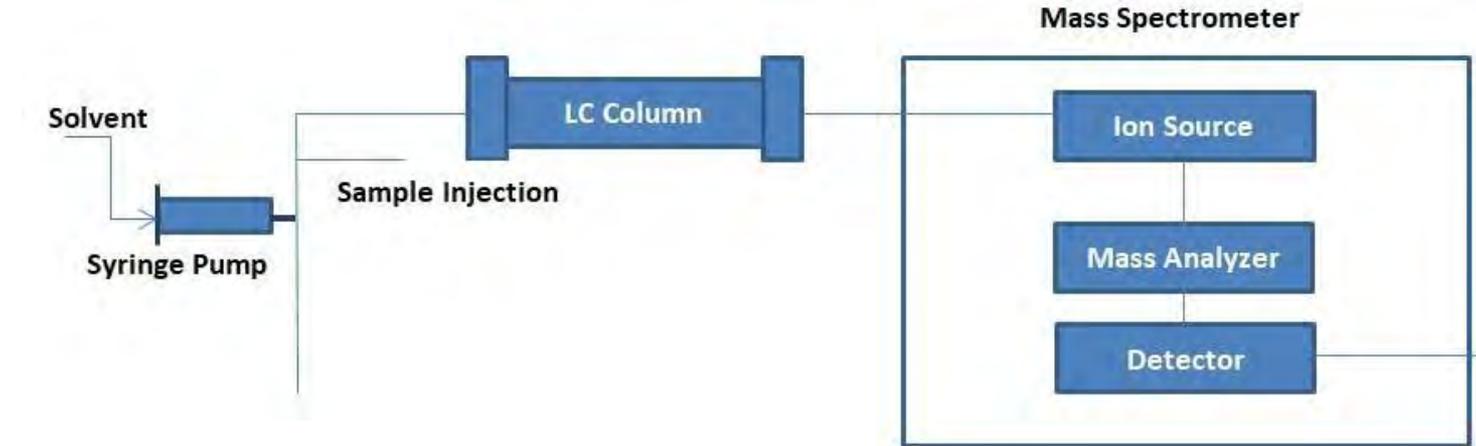
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# LC-MS/MS



# Approaches complementary for isomer differentiation by FT-ICR MS

- IMRs
- IRMPD spectroscopy
- Ion Mobility Spectrometry



# Ion Molecule Reactions



# Ion Molecule Reactions (IMRs)

## Where to perform IMR:

- Chemical ionization ion source
- Atmospheric pressure ion source
- rf-only quadrupole or a triple quadrupole
- Ion-trapping instruments: linear quadrupole ion-trap and FT-ICR (the most versatile MS)

## In FT-ICR:

- low pressure measurements ( $10^{-5}$ - $10^{-8}$  torr)
- reagent introduction with variable leak valves and/or pulsed valves
- time and energy control of reactions
- mass selection of reactant and product ions
- structural characterization by CID, ECD, IRMPD
- multistep  $MS^n$  sequences
- high resolution, high mass accuracy mode of operation



# IMR by FT-ICR

## Pros

- sensitivity
- specificity
- speed
- efficiency
- numerous reaction-based strategies
- no extensive purification/sample preparation
- (indirect) structural information

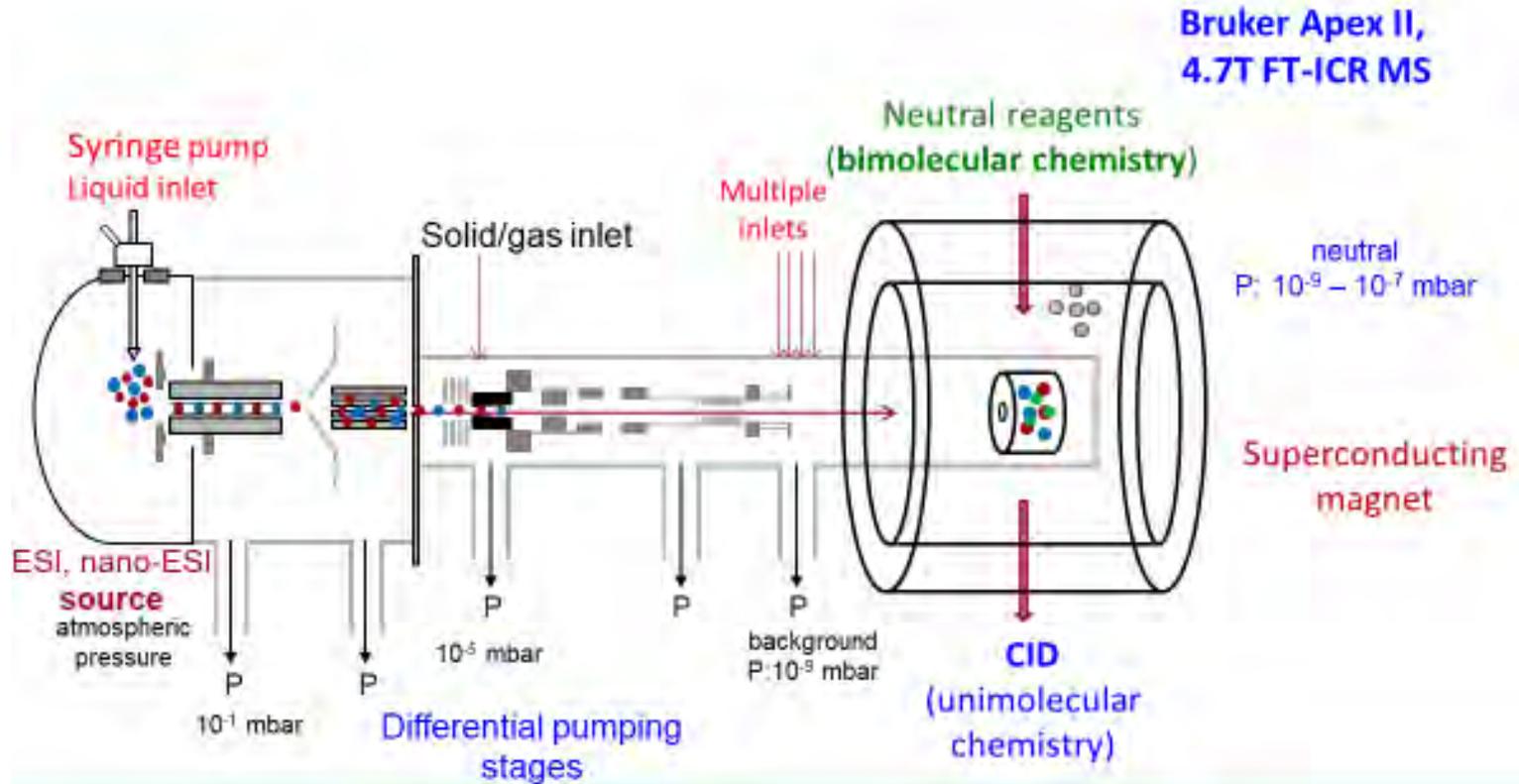
## Cons

- inferred information on neutral products
- volatile neutrals (b.p. < 200 °C)

- Structure (isomer differentiation)
- Reactivity (intrinsic)
- Reaction Mechanisms
- Isolation and characterization of elusive intermediates



# Ion Molecule Reactions (IMRs)



# Reaction Rates



$$-\frac{dR(t)}{dt} = k n R(t) \quad \text{bimolecular reaction}$$

$n$  = number density of neutral N

In a conventional bimolecular process the number density of neutral reactant would decrease with time. Here, it does not.

$$I(t) = I_0 e^{-nkt} \quad \text{pseudo-first order reaction}$$

$$\ln \frac{I(t)}{I_0} = -nkt \quad k = k_{exp}$$



**The total signal intensity is used to normalize the data and avoid errors from slight variations in the number of ions.**

**The signal intensity of  $I_{(t)}$  can be monitored as a function of time and the rate constants for the disappearance of reactant ions and the appearance of product ions are obtained.**



Typically, the reproducibility of  $k_{\text{exp}}$  values is within 10%;  
while the error in the absolute rate constants is estimated to  
be  $\pm 30\%$ .

It is mainly due to uncertainty in pressure measurements.



The efficiency ( $\Phi$ ) of an ion molecule reaction can be determined by comparing the experimental rate constant ( $k_{exp}$ ) with a theoretical estimate of the capture rate constant as percentages of the collision rate constant ( $k_{coll}$ ).

$$\Phi = \frac{k_{exp}}{k_{coll}} \quad \text{measure of reaction probability per collision} \\ \text{(number of events that bring to reaction)}$$

Many exothermic reactions exhibit unit reaction probability at room T;  
others proceed with reaction efficiency much less than unity.



## ADO theory

ADO: averaged dipole orientation

$$k_{\text{ADO}} = \frac{2\pi q}{\sqrt{\mu}} \left\{ \sqrt{\alpha} + C\mu_{\text{D}} \left( \frac{2}{\pi k_{\text{B}} T} \right)^{1/2} \right\}$$

the first term is the Langevin contribution

**Interaction:** ion- dipole

$\mu$  is the reduced mass;  $\mu_{\text{D}}$  is the permanent dipole;  
 $C$  is a correction factor depending on  $\mu_{\text{D}} / \alpha^{1/2}$  ;  
 $k_{\text{B}}$  is Boltzmann's constant

The dipole orientation is not considered

**$k_{\text{ADO}}$  predicts accurate Proton Transfer rate constants**



## Types of Ion-Molecule Reactions

- **Electron-Transfer**
- **Proton transfer**
- **H-atom/ O-atom transfer**
- **H/D exchange**
- **Functional-group selective**
- **Nucleophilic displacement**
- **Radiative association**



# Proton-Transfer Reactions



base A of unknown GB (PA)

base B of known GB (PA)

by using several reference bases B, the GB (PA) of A can be determined

## Bracketing method: kinetics



- measurement of  $k_{\text{exp}}$
- presence of gaseous B

## Equilibrium method: equilibrium



- measurement of  $K_{\text{eq}}$
- presence of gaseous A and B

$$K_{\text{eq}} = \frac{[\text{BH}]^+ [\text{A}]}{[\text{AH}]^+ [\text{B}]} \quad \ln K_{\text{eq}} = \frac{-\Delta G^\circ}{RT}$$



# Chiral differentiation in host-guest complexes

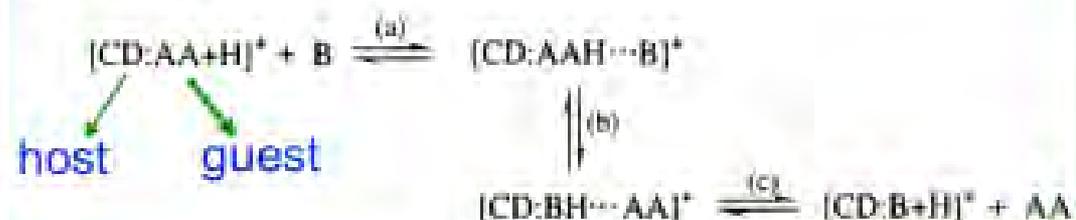
*J. Am. Chem. Soc.* 1998, 120, 7387-7388

## Gas-Phase Chiral Differentiation of Amino Acid Guests in Cyclodextrin Hosts

Javier Ramirez, Fei He, and Carlito B. Lebrilla\*

chiral differentiation of AAs is of immediate analytical importance

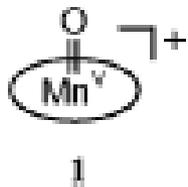
### Scheme 1



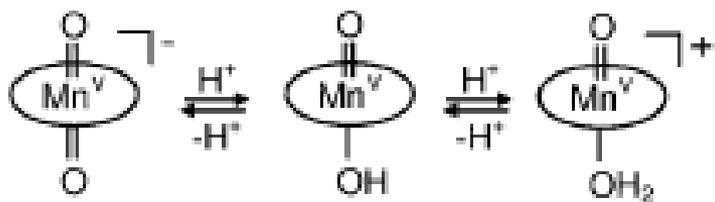
- complexes of protonated  $\beta$ -cyclodextrin-amino acid (Ala; Val; Phe) react with n-propylamine by **exchanging the AA guest of cyclodextrin host** for alkylamine;
- the exchange rates are found to differ according to the chirality of the AA
- Valine is the most reactive and shows the greatest selectivity:  $k_L/k_D = 1.6$  (Alanine);  $k_L/k_D = 3.1$  (Valine);  $k_L/k_D = 0.8$  (Phenylalanine).
- **the differences may be related to the way the AA is included into the host cavity.**



# OAT by Mn(V)-Oxo porphyrin complex



Mn<sup>V</sup>-oxo-porphyrin complexes prepared in aqueous solution:  
diamagnetic d<sup>2</sup> low-spin ground state



high pH

low pH

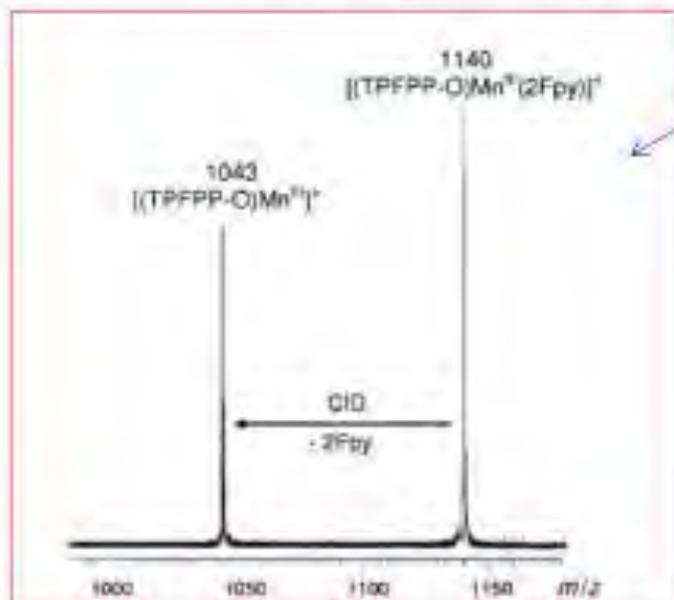
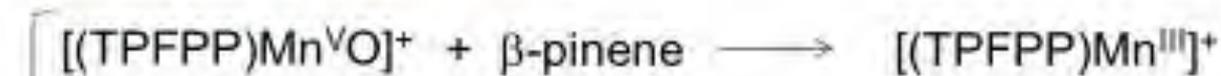
The OAT reactivity in water is found to be strongly pH-dependent: prototropic equilibria



L = propene, (E)-2-butene, (Z)-2-butene, styrene, cyclohexene, 1,3,5-CHT, (+)-camphene, indene,  $\beta$ -pinene, (R)-(+)-limonene. OAT reactivity increases with the olefin's IE value



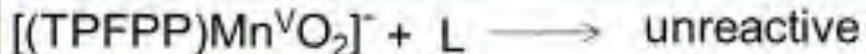
## Gas-phase titration



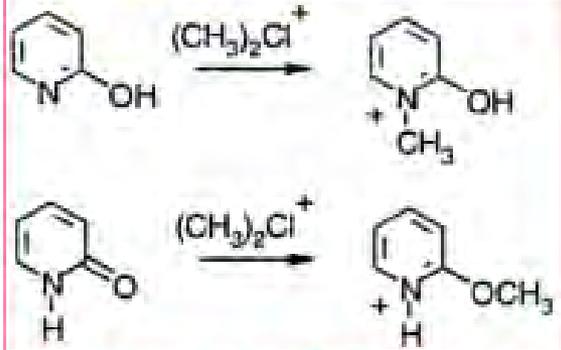
CID

Table 1. Kinetic data for the reaction of  $[(TPFPP)Mn^V O]^+$  (**1**) with selected olefins in the gas-phase.<sup>[a]</sup>

Olefin (IE) <sup>[b]</sup>	$k_{\text{exp}}$ <sup>[c,d]</sup>	$\phi$ <sup>[e]</sup>
propene (9.73)	0.0020	0.021
3,3-dimethyl-1-butene (9.45)	0.041	0.43
( <i>E</i> )-2-butene (9.10)	0.072	0.78
( <i>Z</i> )-2-butene (9.11)	0.75	8.1
allylbenzene (7.8–8.7)	0.070	0.76
styrene (8.46)	0.63	6.8
cyclohexene (8.95)	0.75	7.7
1,4-cyclohexadiene (8.8)	0.69	7.8
1-propene, 2-methoxy (8.64) <sup>[f]</sup>	1.7	16
1,3,5-cycloheptatriene (8.30)	1.9	21
(+)-camphene ( $\leq 8.86$ )	2.6	28
indene (8.14) <sup>[g]</sup>	1.2	12
(1 <i>S</i> )-(-)- $\alpha$ -pinene (8.07)	9.0	100
$\beta$ -pinene (n.a.)	9.3	100
( <i>R</i> )-(+)-limonene (8.3)	9.2	100



# Identification of functional groups in organic compounds

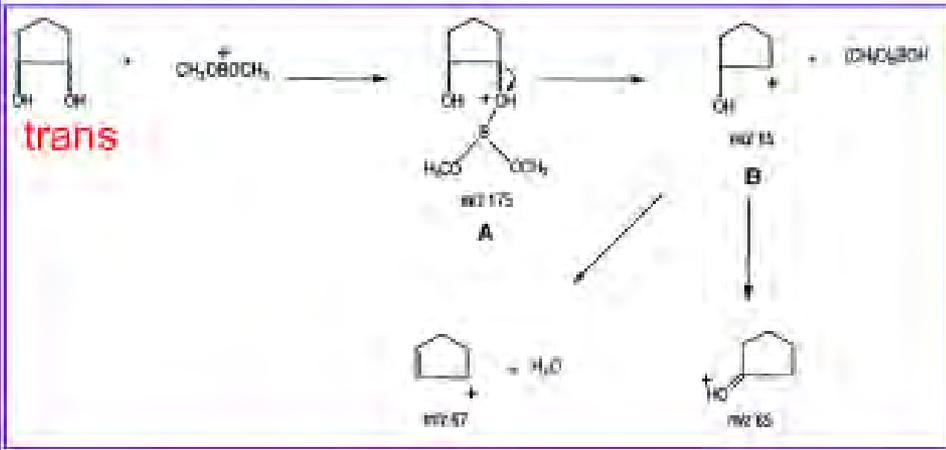
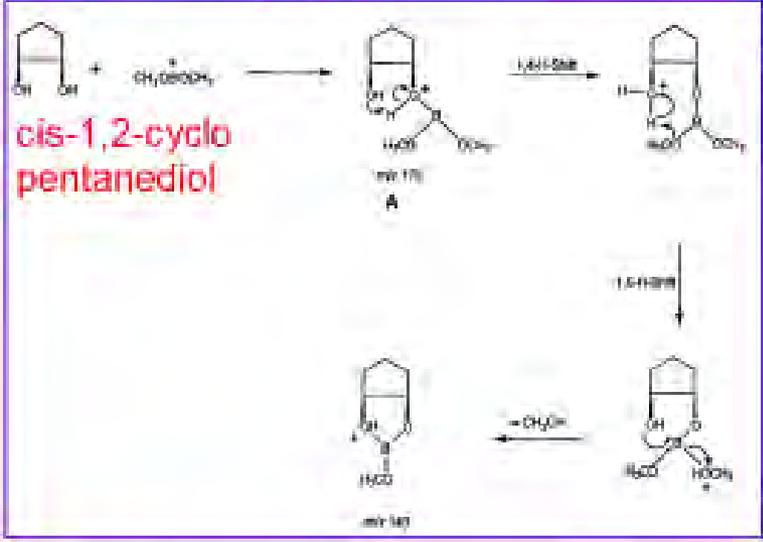


O'Hair, Eur. J. Mass Spectrom. 1995

Identification via CID of independently synthesized products

ELSEVIER International Journal of Mass Spectrometry and Ion Physics 141 (1995) 239–248  
 MIMS Mass Spectrometry and Ion Microscopy

Differentiation of stereoisomeric diols by using  $\text{CH}_3\text{OB}^+\text{OCH}_3$  in a small Fourier transform ion cyclotron resonance mass spectrometer  
 D.T. Leck<sup>a</sup>, T.D. Ranatunga<sup>a</sup>, R.L. Smith<sup>a</sup>, T. Partanen<sup>b</sup>, P. Vainonaho<sup>b,c</sup>, H.I. Kenttämää<sup>b,c</sup>



# Structural assignment of $C_5H_{11}Si^+$ adducts: effect of different pressures

International Journal of Mass Spectrometry 334 (2013) 58–66



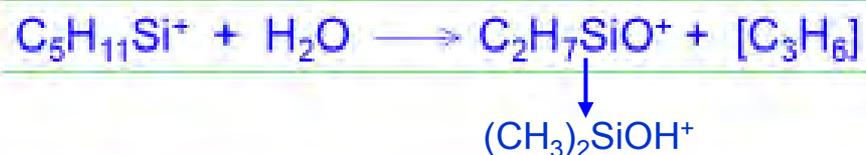
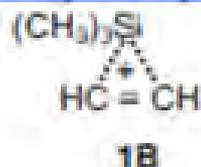
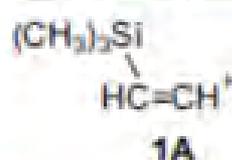
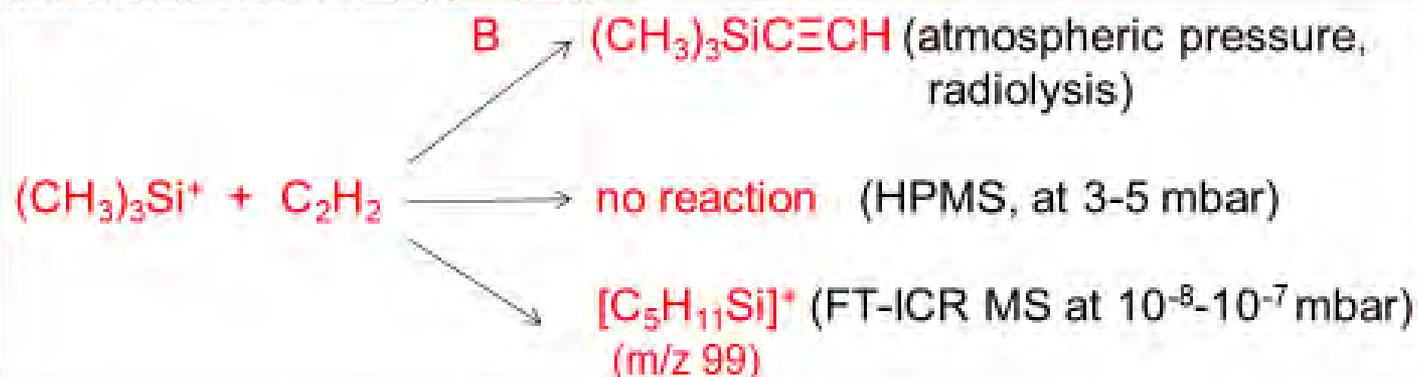
Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: [www.elsevier.com/locate/ijms](http://www.elsevier.com/locate/ijms)

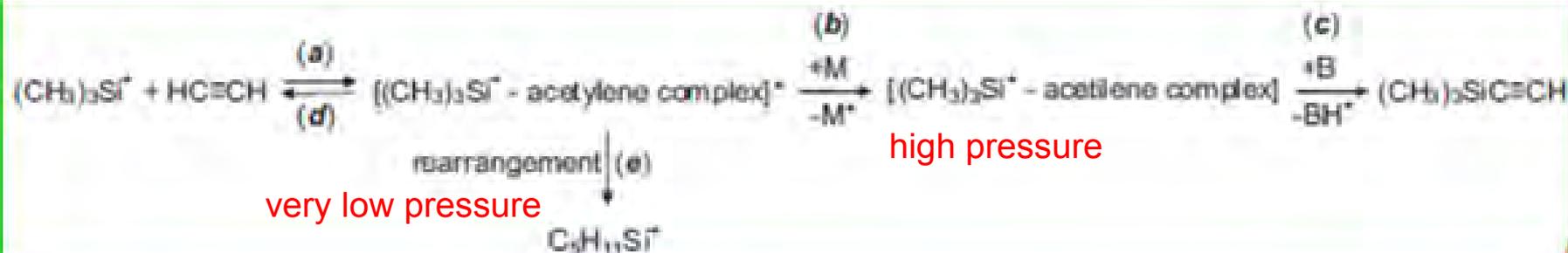
## Isomeric $C_5H_{11}Si^+$ ions from the trimethylsilylation of acetylene: An experimental and theoretical study

Hans-Ulrich Siehl<sup>a,\*</sup>, Sandra Brixner<sup>a</sup>, Cecilia Coletti<sup>b</sup>, Nazzareno Re<sup>b</sup>, Barbara Chiavarino<sup>c</sup>,  
Maria Elisa Crestoni<sup>c</sup>, Alberto De Petris<sup>d</sup>, Simonetta Fornarini<sup>c,\*,e</sup>



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S. Fornarini et al., I.J.M.S. 2013

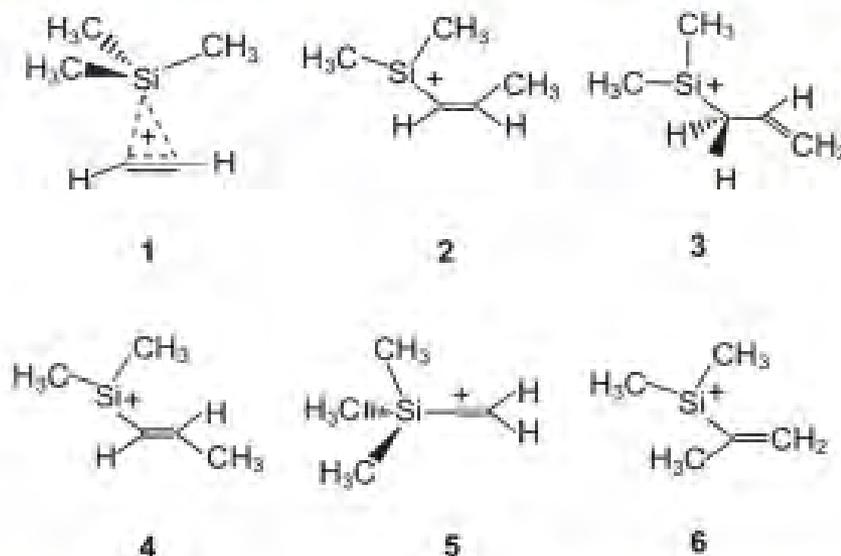


IMR with appropriate neutrals as diagnostic tools to test ion structures

model ions formed from  $\text{Cl-C}_3\text{H}_6$  of synthesized  $(\text{CH}_3)_3\text{Si}$ -substituted propenes

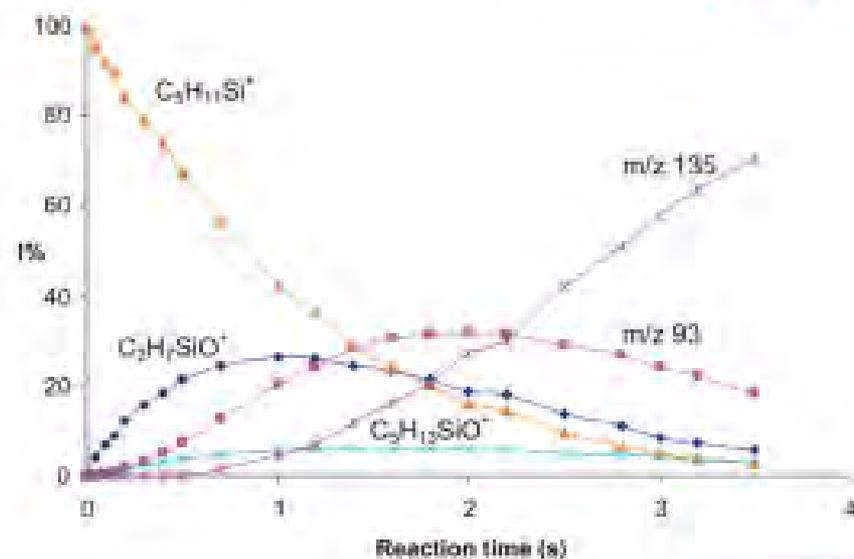
potentially diagnostic reagents:

$\text{H}_2\text{O}$   
*i*- $\text{C}_3\text{H}_7\text{OH}$   
*t*- $\text{C}_4\text{H}_9\text{OCH}_3$





$\phi$  not suitable to discriminate different  $\text{C}_5\text{H}_{11}\text{Si}^+$



$\phi$  not suitable to discriminate different  $\text{C}_5\text{H}_{11}\text{Si}^+$  ;

the branching ratios  $\text{C}_2\text{H}_7\text{SiO}^+/\text{C}_5\text{H}_{13}\text{SiO}^+ = 4.3 - 21$   
are more informative

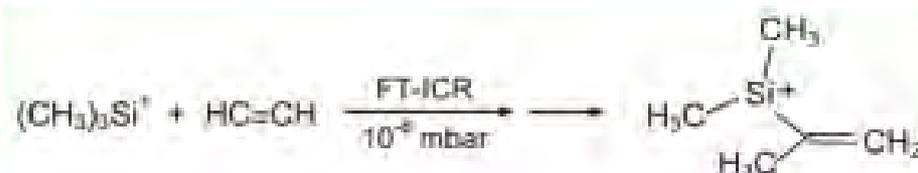


**Table 2**Product ratios for the gas phase reaction of  $C_5H_{11}Si^+$  ions with ROR' nucleophiles.

Precursor of reactant ion <sup>a</sup>	ROR'	$P_1/P_2^b$
(Z)-1-(Trimethylsilyl)propene (2)	<i>i</i> -C <sub>3</sub> H <sub>7</sub> OH	$8.5 \pm 1.7^c$
(E)-1-(Trimethylsilyl)propene (4)	<i>i</i> -C <sub>3</sub> H <sub>7</sub> OH	$11 \pm 1.8^c$
2-(Trimethylsilyl)propene (6)	<i>i</i> -C <sub>3</sub> H <sub>7</sub> OH	$20 \pm 5^c$
Allyltrimethylsilane (3)	<i>i</i> -C <sub>3</sub> H <sub>7</sub> OH	$4.3 \pm 0.5^c$
<b>S</b>	<i>i</i> -C <sub>3</sub> H <sub>7</sub> OH	$21 \pm 2^c$
(Z)-1-(Trimethylsilyl)propene (2)	<i>t</i> -C <sub>4</sub> H <sub>9</sub> OCH <sub>3</sub>	$2.6 \pm 0.3^d$
(E)-1-(Trimethylsilyl)propene (4)	<i>t</i> -C <sub>4</sub> H <sub>9</sub> OCH <sub>3</sub>	$2.5 \pm 0.3^d$
2-(Trimethylsilyl)propene (6)	<i>t</i> -C <sub>4</sub> H <sub>9</sub> OCH <sub>3</sub>	$4.6 \pm 0.7^d$
Allyltrimethylsilane (3)	<i>t</i> -C <sub>4</sub> H <sub>9</sub> OCH <sub>3</sub>	$24 \pm 5^d$
<b>S</b>	<i>t</i> -C <sub>4</sub> H <sub>9</sub> OCH <sub>3</sub>	$4.1 \pm 0.7^d$

 $P_1 = C_2H_7SiO^+$  and  $P_2 = C_5H_{13}SiO^+$  $P_1 = C_3H_9SiO^+$  and  $P_2 = C_6H_{15}SiO^+$ 

⚡ not suitable to discriminate different  $C_5H_{11}Si^+$ ;  
 the branching ratio  $C_3H_9SiO^+/C_6H_{15}SiO^+ = 2.5 - 24$  are more informative

m/z 89.0961 ( $C_5H_{13}O^+$ )m/z 89.0417 ( $C_3H_9SiO^+$ )**2-propenyl-dimethylsilyl cation**

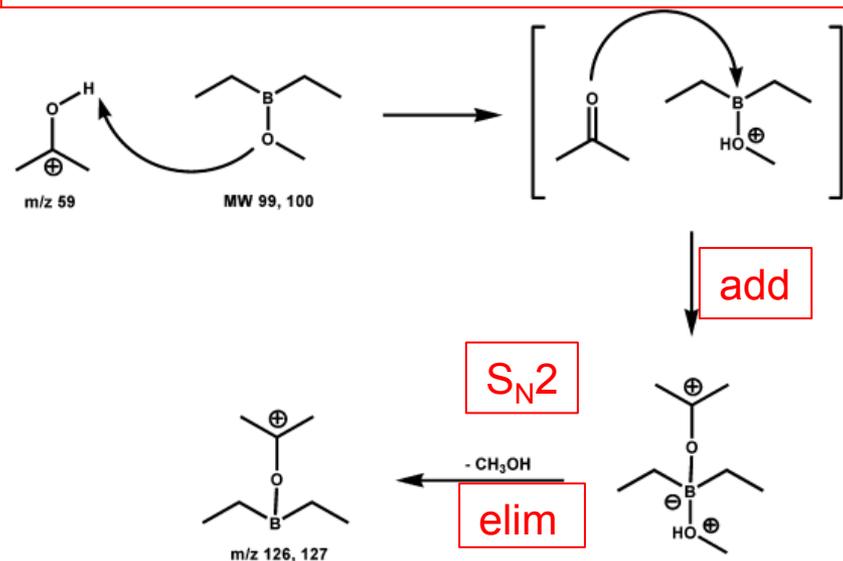
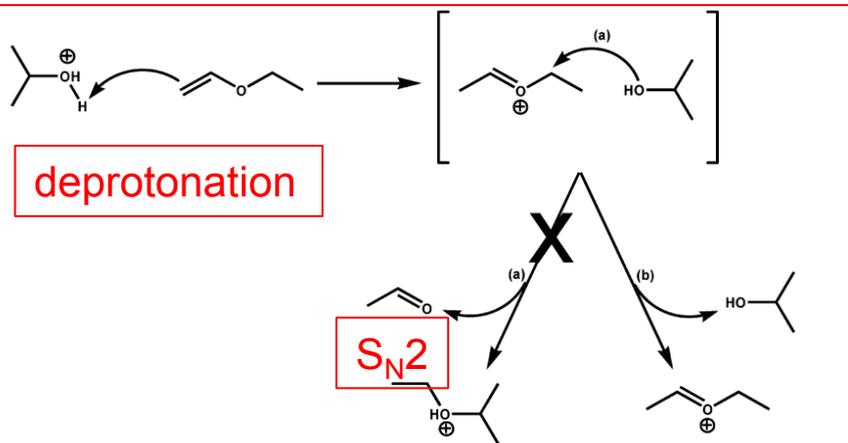
# Functional-group selective IMRs

*Anal. Chem.* 2004, 76, 964–976

## Ion–Molecule Reactions for Mass Spectrometric Identification of Functional Groups in Protonated Oxygen-Containing Monofunctional Compounds

Michael A. Watkins,<sup>†</sup> Jason M. Price,<sup>†,‡</sup> Brian E. Winger,<sup>§</sup> and Hilka I. Kenttämä<sup>\*,†</sup>

- 1) ethyl vinyl ether
- 2) diethylmethoxyborane



-11.6 kcal mol<sup>-1</sup>

-20.5 kcal mol<sup>-1</sup>

**diethylmethoxyborane** reacts with protonated monofunctional oxygen-containing analytes (**alcohols, ketones, aldehydes, esters, ethers, carboxylic acids, amides**) by deprotonation followed by substitution of methanol: **provides structure elucidation for unknown mixture components**



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H. I. Kenttämä, *Anal. Chem.* 2004; M. N. Eberlin,  
*J. Mass Spectrom.* 2006; H. I. Kenttämä, *Mass Spectrom. Rev.* 2021

# Functional-group selective IMR

## Research Article



Received: 25 January 2014

Revised: 27 February 2014

Accepted: 27 February 2014

Published online in Wiley Online Library

*Rapid Commun. Mass Spectrom.* 2014, 28, 1107–1116  
(wileyonlinelibrary.com) DOI: 10.1002/rcm.6884

### Probing the exposure of the phosphate group in modified amino acids and peptides by ion-molecule reactions with triethoxyborane in Fourier transform ion cyclotron resonance mass spectrometry

Francesco Lanucara<sup>1,2\*</sup>, Simonetta Fornarini<sup>3</sup>, Claire E. Eyers<sup>2</sup> and Maria Elisa Crestoni<sup>3</sup>



**Scheme 1.** Addition-elimination reaction of phosphorylated amino acids and peptides with alkoxyboranes B(OR)<sub>3</sub>.

Potential to measure the effect of local environment, the exposure and accessibility of a phosphate moiety on the surface of a biomolecule and to distinguish positional phosphorylated peptide isomers

The reaction efficiency allows to explore the accessibility of phosphate groups in biomolecules



**Making Mass Spectrometry See the Light:**

**Infrared Multiple Photon Dissociation spectroscopy**



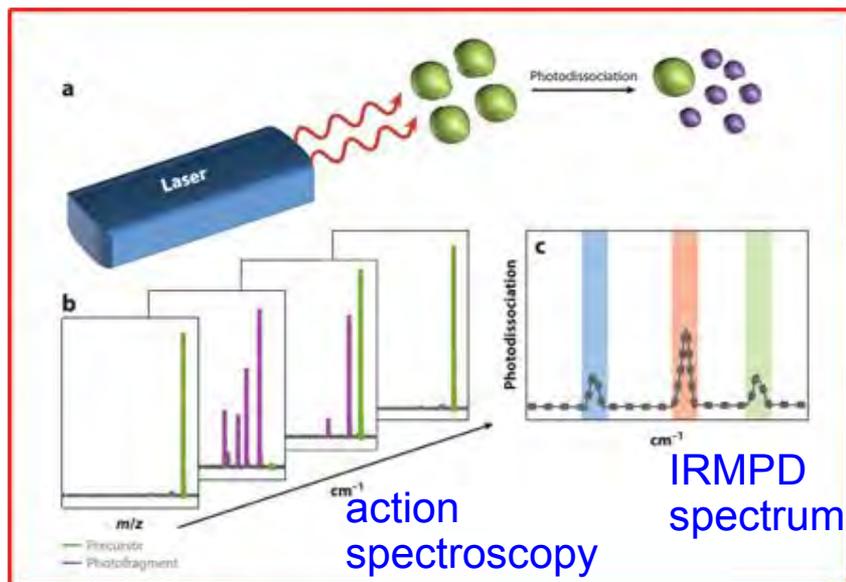
# Biomolecular vibrations

**Table 1.2** Vibrational ranges for common biomolecular chemical moieties

Description	Frequency/cm <sup>-1</sup>
P-OH stretch/P-OH wag	920–1,080
Amide NH bending	1,475–1,525
Amide CO stretch	1,675–1,725
Carboxylic acid CO stretch	1,725–1,775
Symmetric NH <sub>2</sub> stretch	3,350–3,400
Asymmetric NH <sub>2</sub> stretch	3,400–3,450
Amide NH stretch	3,300–3,500
Indole/Imidazole NH stretch	3,480–3,520
Carboxylic acid OH stretch	3,540–3,600
Alcohol OH stretch	3,600–3,675
Phosphate OH stretch	3,650–3,700



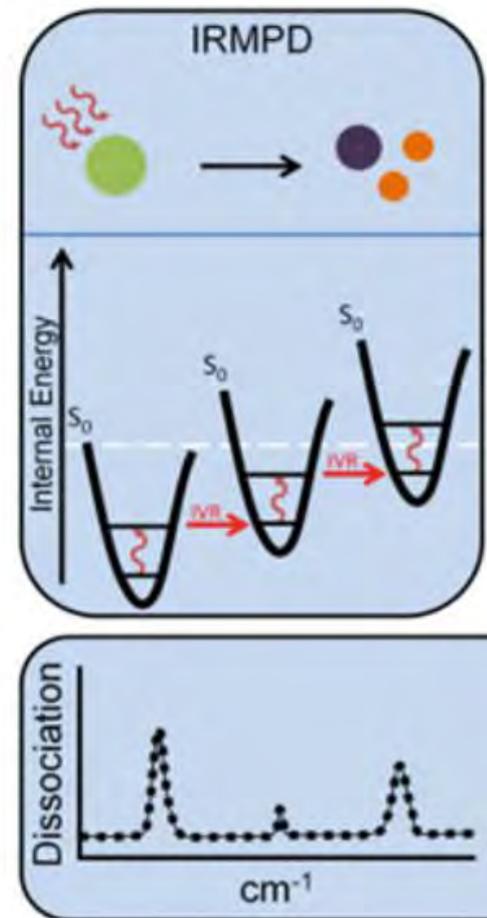
# Infrared Multiple Photon Dissociation (IRMPD) : an action spectroscopy



$m/z$  selection

action  
spectroscopy

IRMPD  
spectrum



# Infrared action spectroscopy technique

- fingerprint ( $700\text{-}2000\text{ cm}^{-1}$ ) at CLIO-FEL (Orsay)
- CH, NH, OH stretching range ( $2800\text{-}3800\text{ cm}^{-1}$ ) at Dip. CTF



@ CLIO-FEL (Orsay)



@ Dip. CTF (Sapienza University)

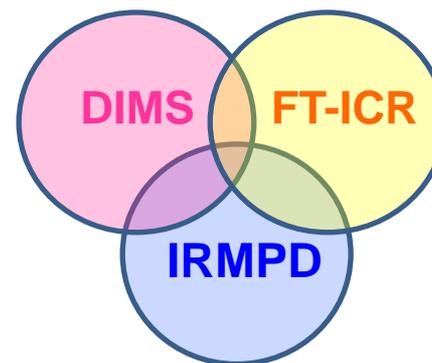
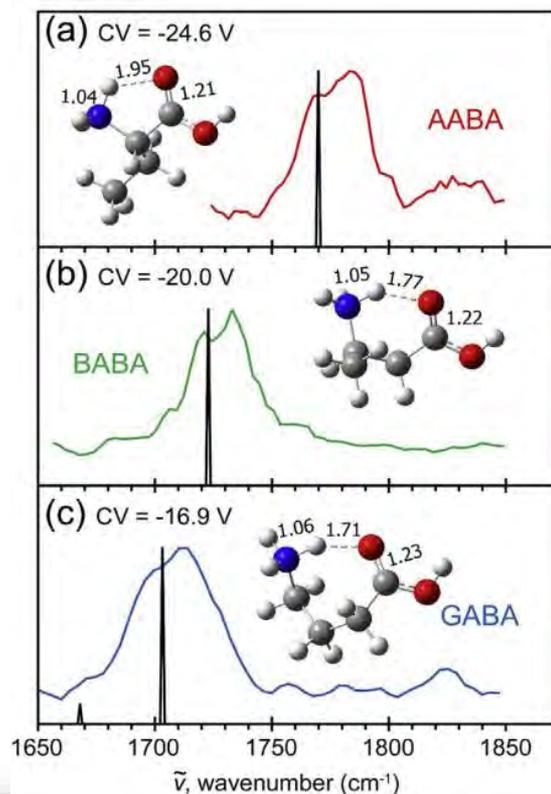




## Infrared isomer-specific fragmentation for the identification of aminobutyric acid isomers separated by differential mobility spectrometry

Yali Wang, Eskander Alhajji, Bernard Rieul, Francis Berthias, Philippe Maître\*

Laboratoire de Chimie Physique (UMR8000), Université Paris-Sud, CNRS, Université Paris Saclay, 91405, Orsay, France



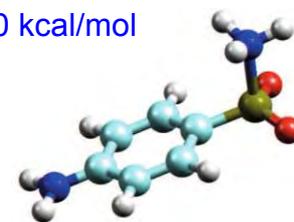
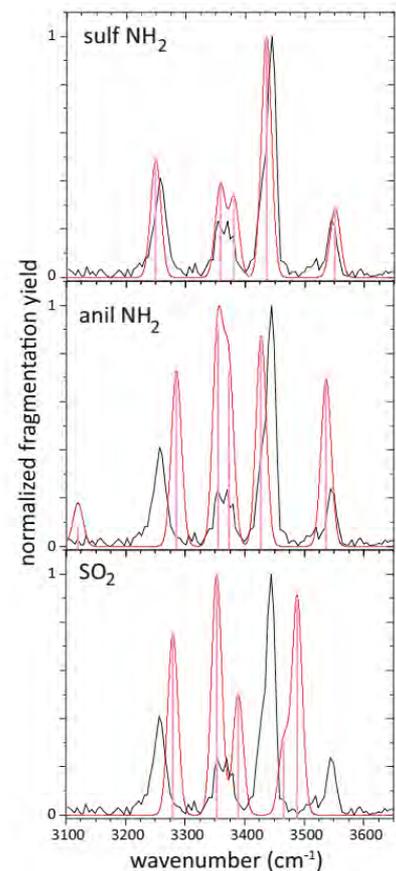
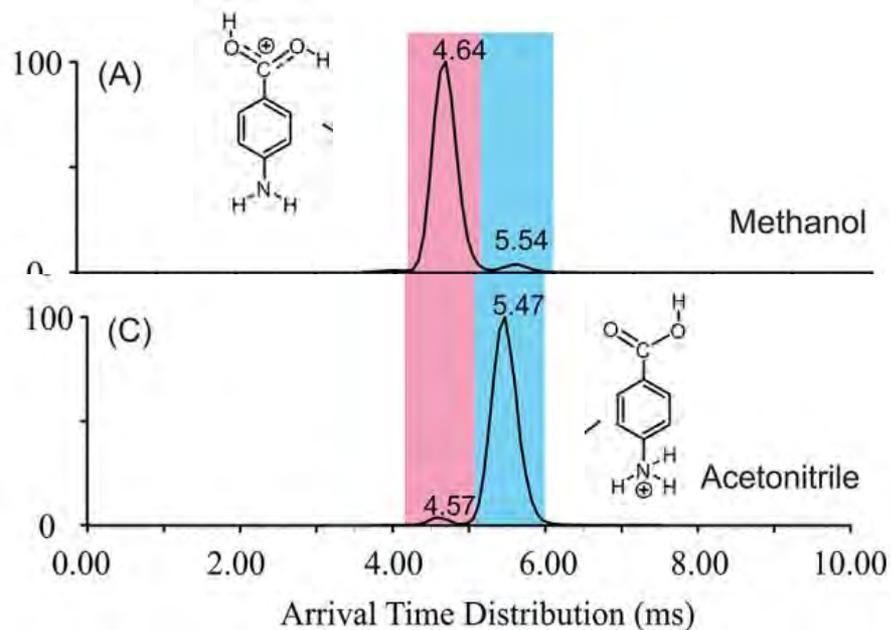
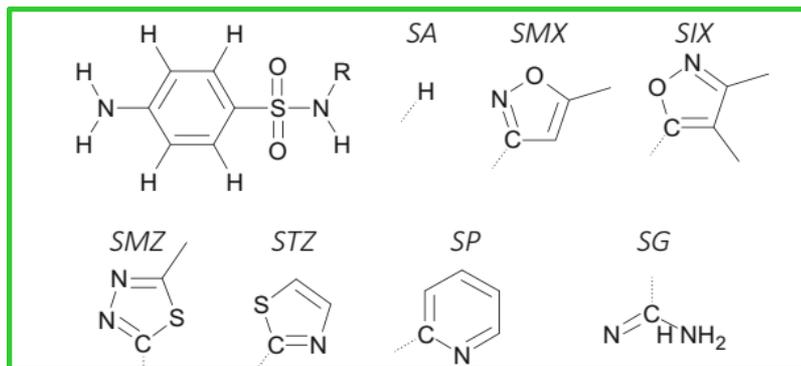
three orthogonal techniques

Isomer-selective fragmentation of each isomer (AABA, BABA, and GABA) at a selected wavelength allows for their structural characterization



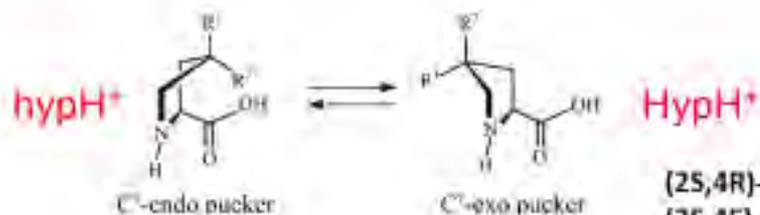
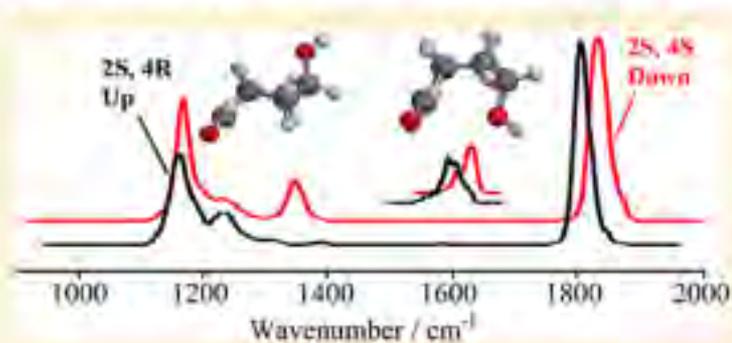
# Preferred protonation site of a series of sulfa drugs in the gas phase revealed by IR spectroscopy

Thomas Uhlemann, Giel Berden, and Jos Oomens



## Discrimination of 4-Hydroxyproline Diastereomers by Vibrational Spectroscopy of the Gaseous Protonated Species

*J. Phys. Chem. B* 2012, 116, 8771–8779

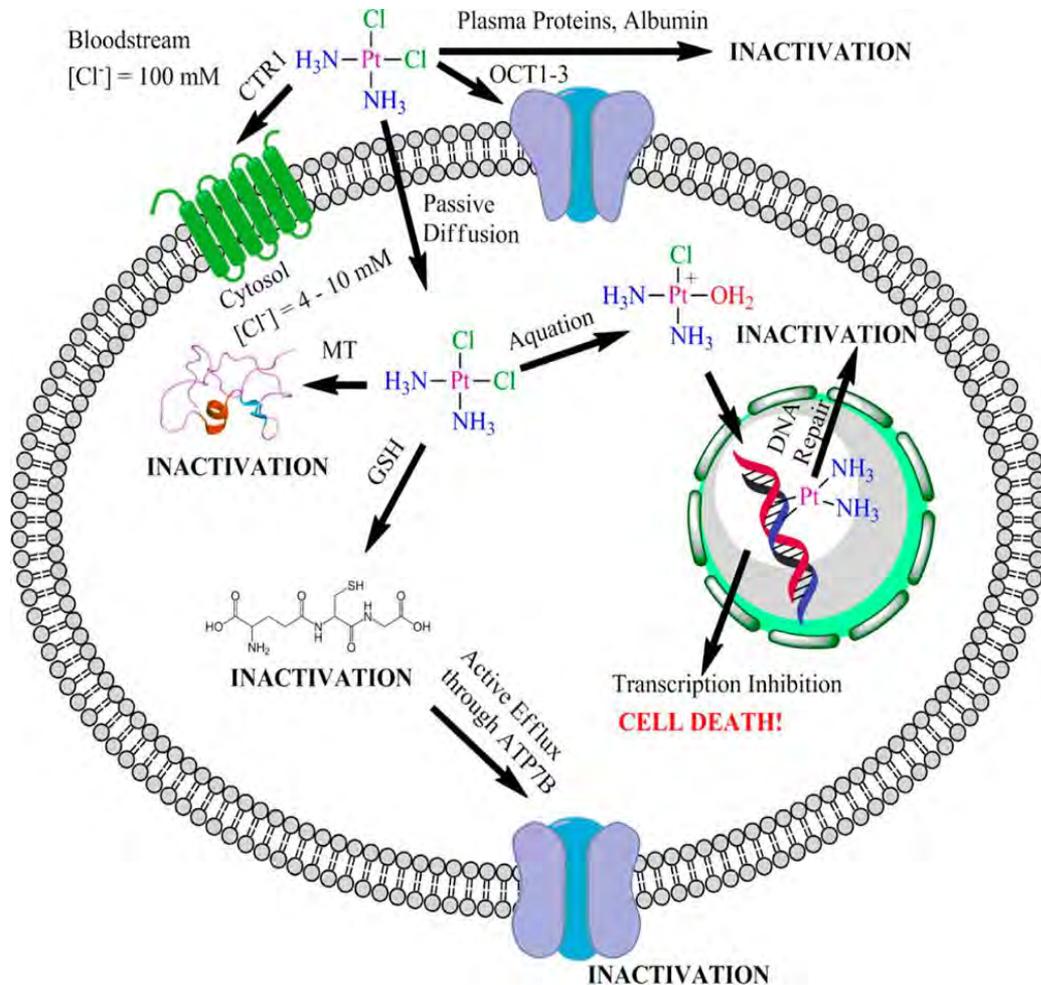
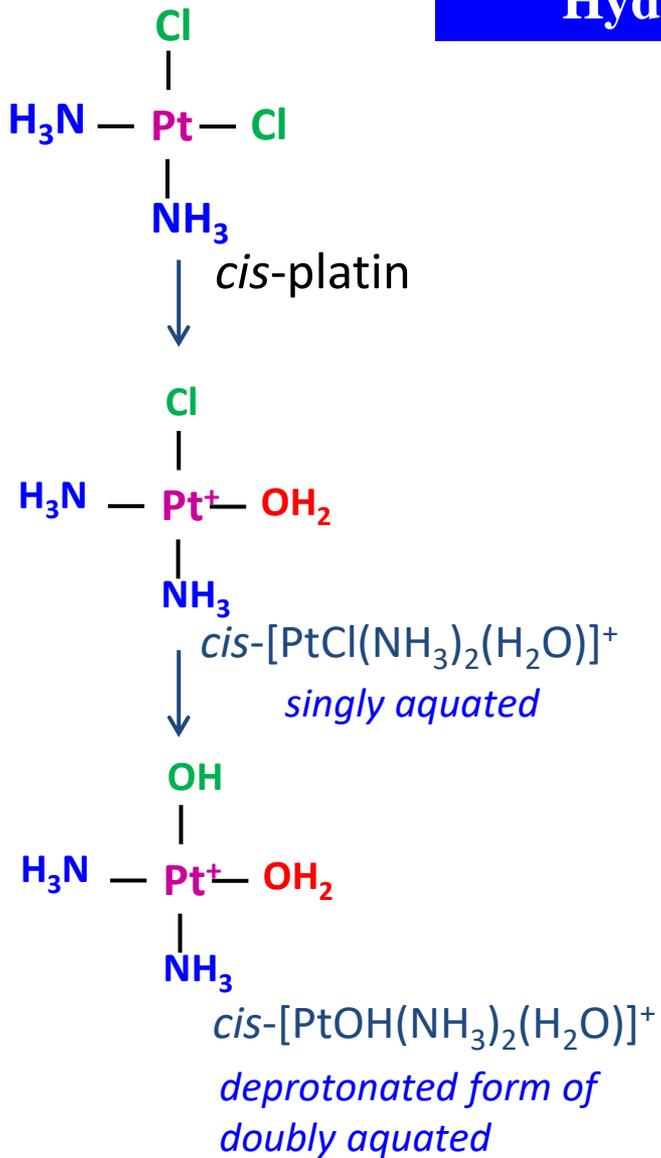


(2S,4R)-4-hydroxyproline = HypH<sup>+</sup>

(2S,4S)-4-hydroxyproline = hypH<sup>+</sup>



# Hydrolysis of cis and transplatin



in solution it is not easy to separate the influence of the nature of the incoming and leaving ligands



# Chemistry of a platinum-based anticancer drug

## Vibrational Signatures of the Naked Aqua Complexes from Platinum(II) Anticancer Drugs

*J. Phys. Chem. Lett.* 2013, 4, 3631–3635

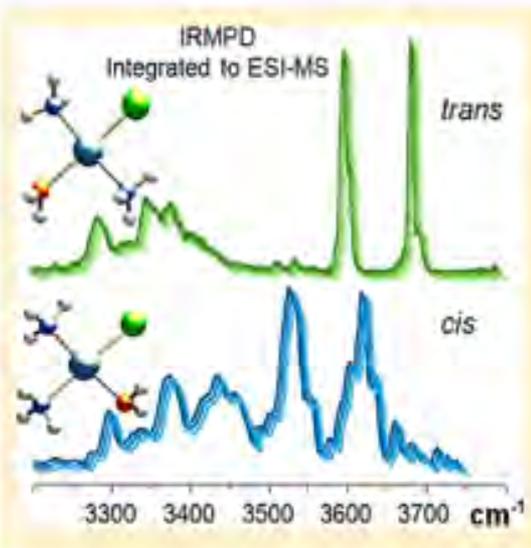
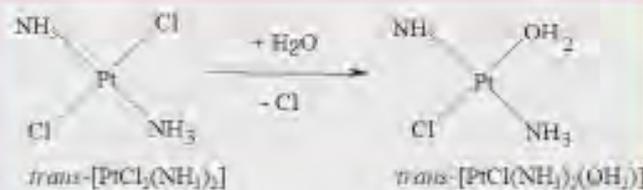
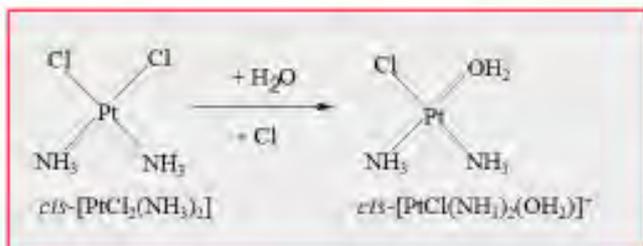
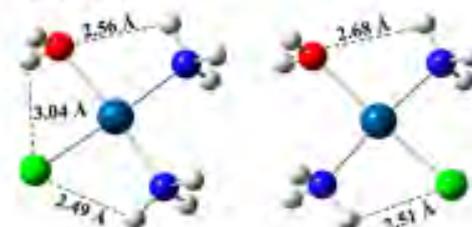
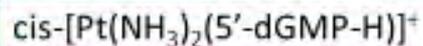
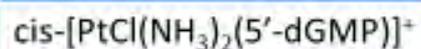
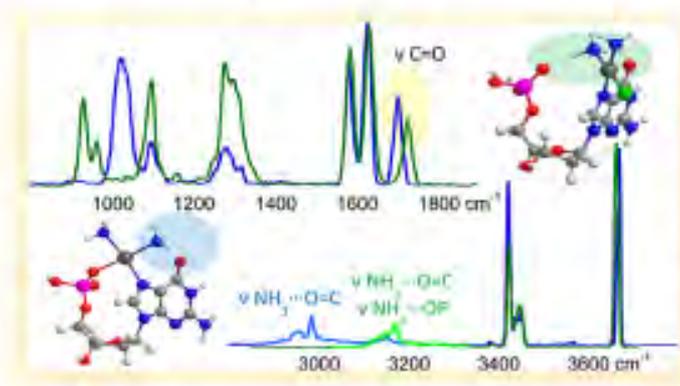


Chart 1. Structures of *cis*-[PtCl(NH<sub>3</sub>)<sub>2</sub>(H<sub>2</sub>O)]<sup>+</sup> and *trans*-[PtCl(NH<sub>3</sub>)<sub>2</sub>(H<sub>2</sub>O)]<sup>+</sup>



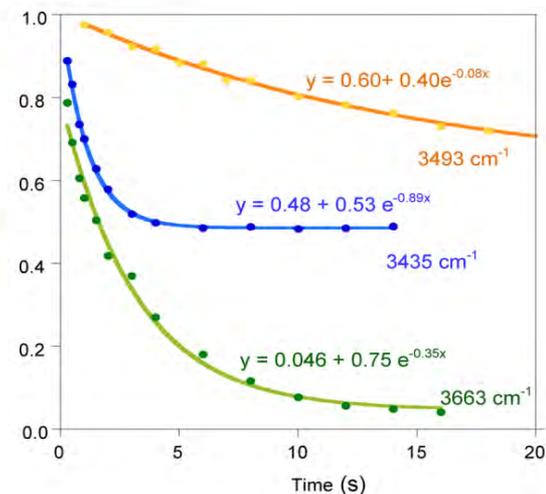
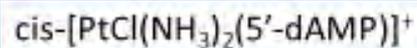
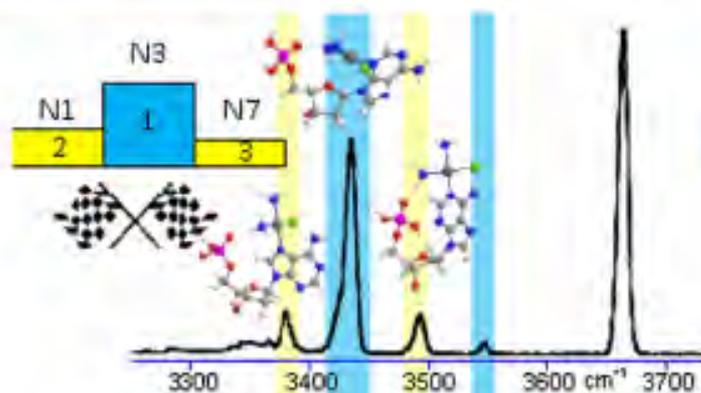
## Interaction of Cisplatin with 5'-dGMP: A Combined IRMPD and Theoretical Study

*Inorg. Chem.* 2015, 54, 3513–3522



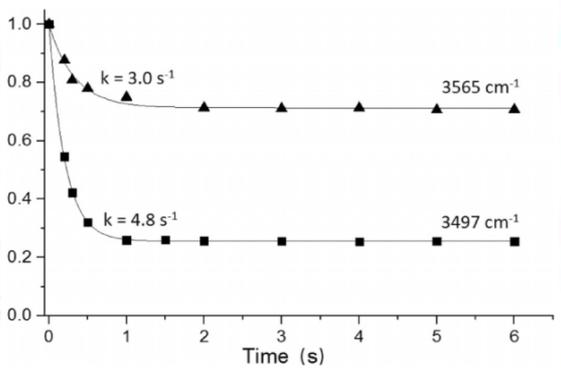
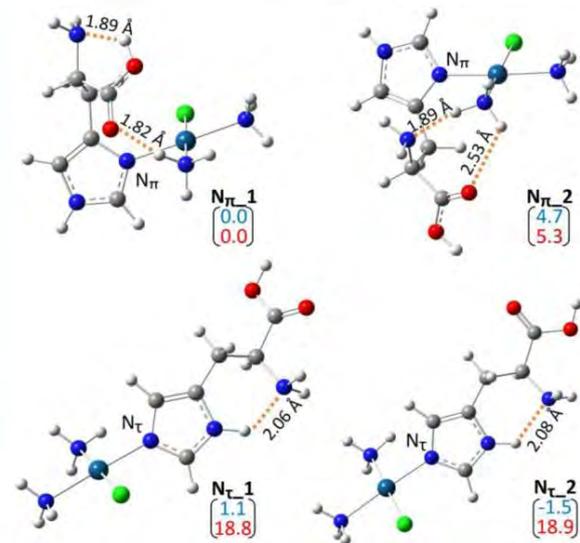
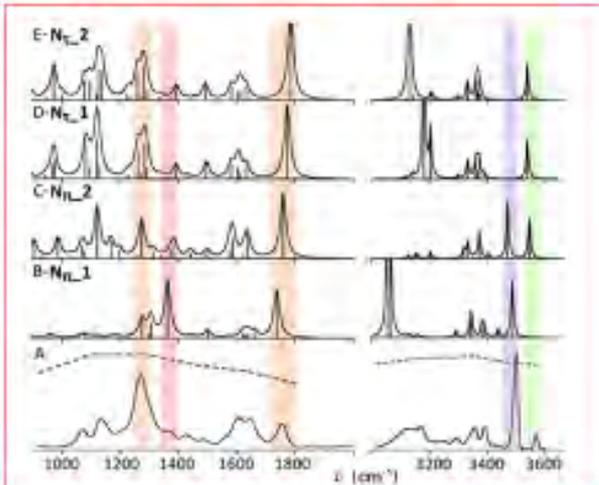
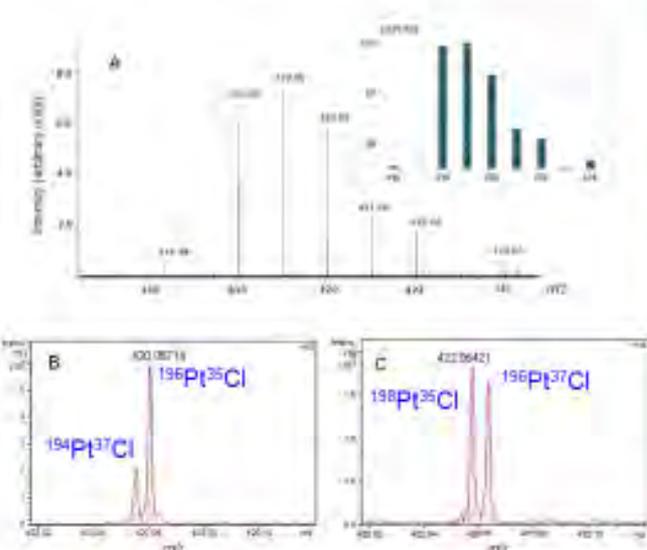
## Undervalued N3 Coordination Revealed in the Cisplatin Complex with 2'-Deoxyadenosine-5'-monophosphate by a Combined IRMPD and Theoretical Study

*Inorg. Chem.*, 2017, 56, pp 8793–8801



# Cisplatin Primary Complex with L-Histidine Target Revealed by IR Multiple Photon Dissociation (IRMPD) Spectroscopy

ChemPhysChem 2017, 18, 318–325

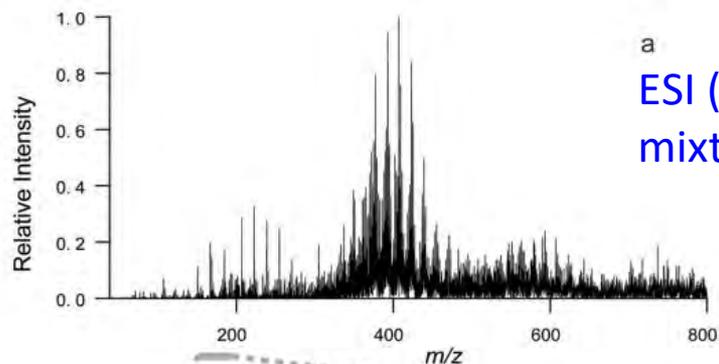


**N<sub>π</sub> conformers: 25% (unreactive)**  
**N<sub>π\_1</sub> : 45%**  
**N<sub>π\_2</sub> : 30%**

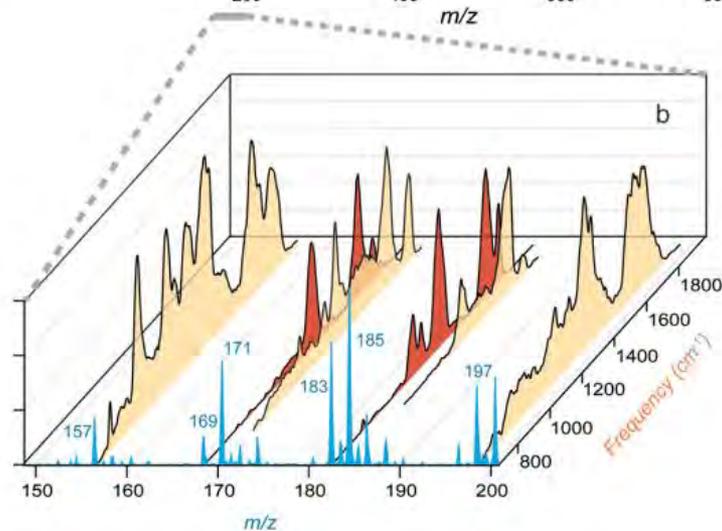
## Infrared Ion Spectroscopy of Environmental Organic Mixtures: Probing the Composition of $\alpha$ -Pinene Secondary Organic Aerosol

Emma Q. Walhout,<sup>†</sup> Shelby E. Dorn,<sup>§</sup> Jonathan Martens,<sup>‡</sup> Giel Berden,<sup>‡</sup> Jos Oomens,<sup>‡,||</sup>  
Paul H.-Y. Cheong,<sup>\*,§</sup> Jesse H. Kroll,<sup>⊥</sup> and Rachel E. O'Brien<sup>\*,†,⊥</sup>

Organic Aerosol

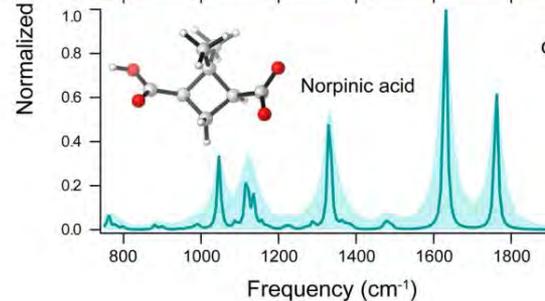
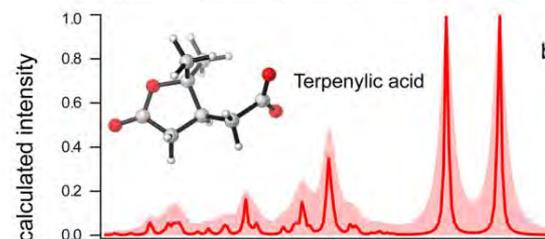
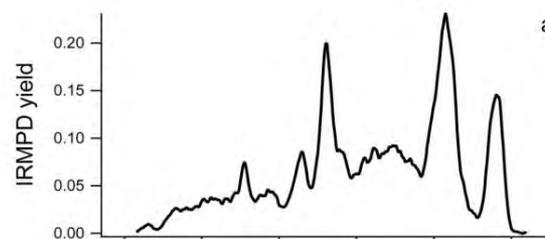
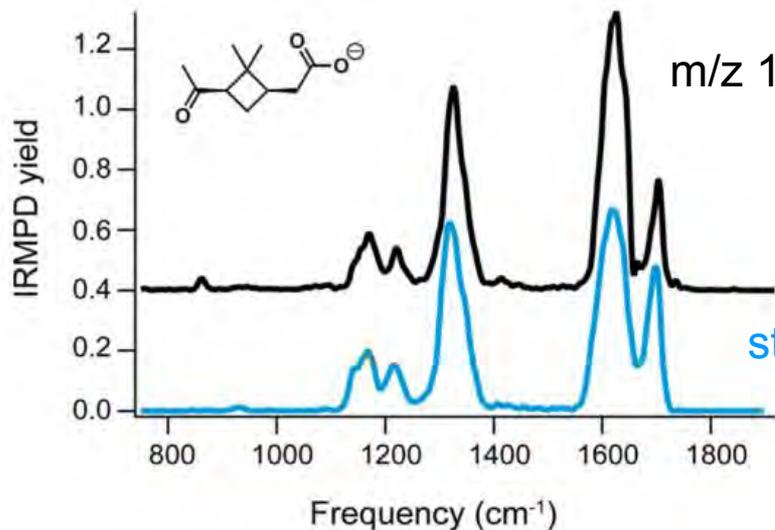


ESI (-) mass spectrum of  $\alpha$ -pinene ozonolysis mixture



fingerprint region ( $600\text{--}1900\text{ cm}^{-1}$ ) @FEL

# Good spectral matches between stds and unknowns



computed

computed



# Molecular Basis for the Remarkably Different Gas-Phase Behavior of Deprotonated Thyroid Hormones Triiodothyronine (T3) and Reverse Triiodothyronine (rT3): A Clue for Their Discrimination?

Davide Corinti,\* Barbara Chiavarino, Mattia Spano, Aura Tintaru, Simonetta Fornarini, and Maria Elisa Crestoni\*



Cite This: *Anal. Chem.* 2021, 93, 14869–14877



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Scheme 1. Schematic Representation of Tetraiodothyronine (T4), Triiodothyronine (T3), and Reverse Triiodothyronine (rT3)

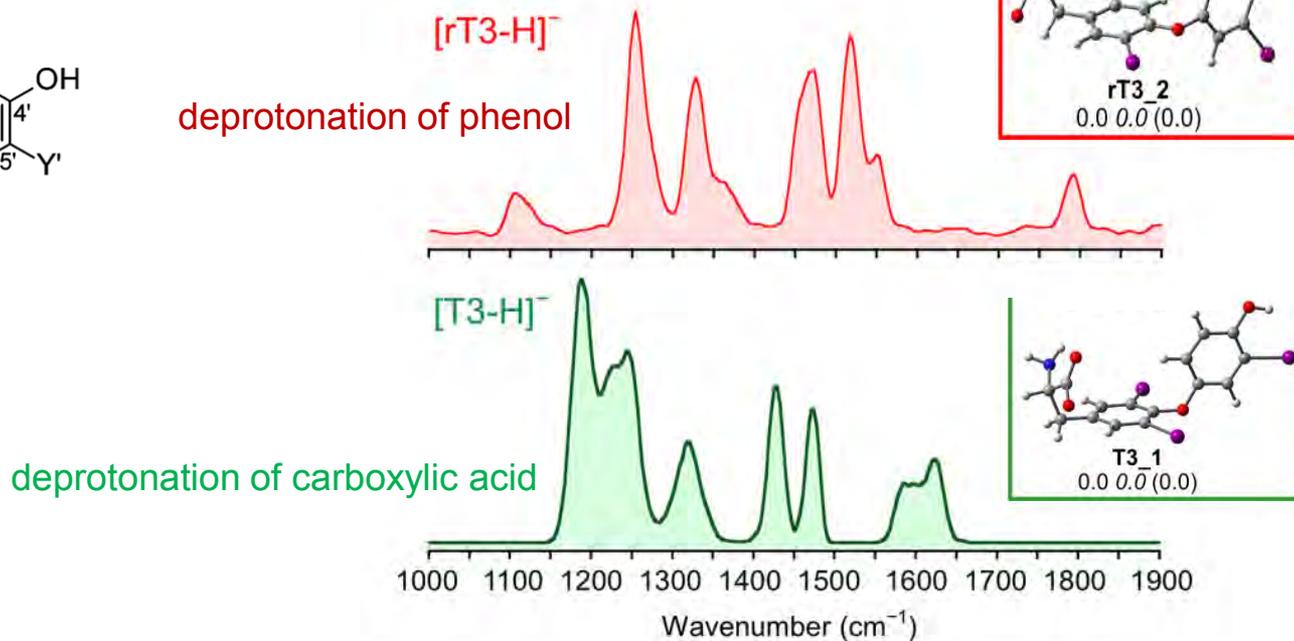
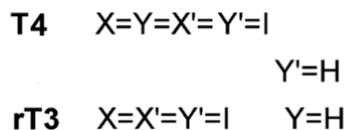
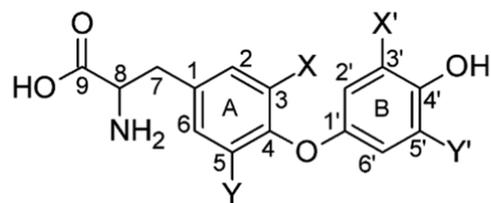
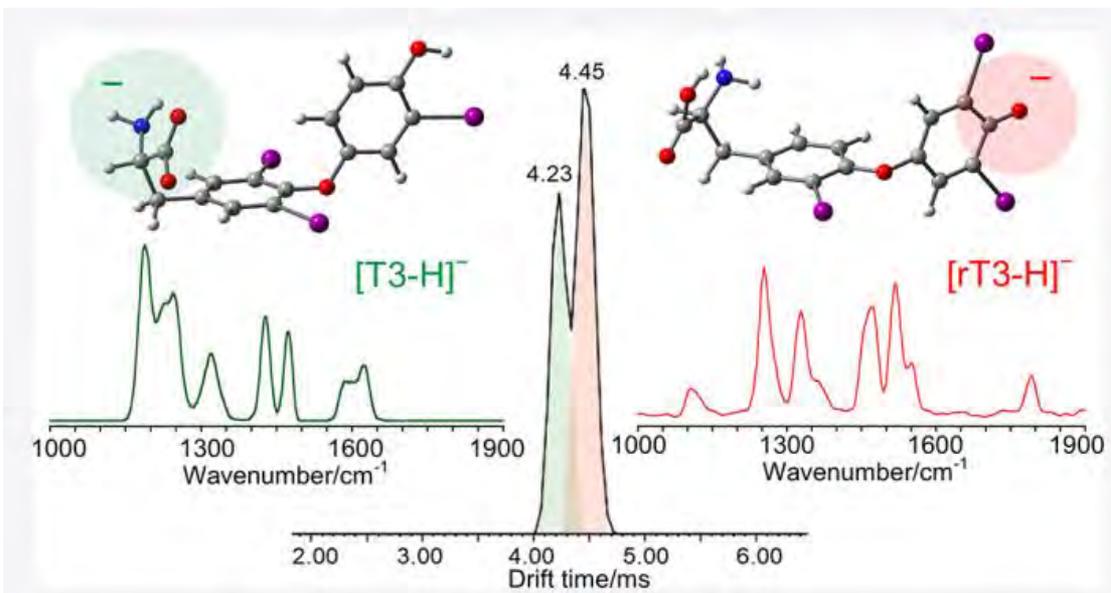


Figure 1. IRMPD spectra of [T3-H]<sup>-</sup> and [rT3-H]<sup>-</sup> in the fingerprint range.





### Theoretical calculations (B3LYP/D3)

**T3**  $\Delta G_{\text{acid}}(\text{T3}) = 1345 \text{ kJ mol}^{-1}$

**rT3**  $\Delta G_{\text{acid}}(\text{rT3}) = 1326 \text{ kJ mol}^{-1}$

acetylacetone ( $1409 \text{ kJ mol}^{-1}$ )

TFA ( $1328 \text{ kJ mol}^{-1}$ )

**Table 2. Kinetic Data for the Proton Transfer Reaction  $[\text{X-H}]^- + \text{TFA} \rightarrow \text{X} + [\text{TFA-H}]^-$**

X	$k_{\text{exp}}^{\text{a}}$	Eff (%) <sup>b,c</sup>
T3	2.53	22
rT3	1.12	10

<sup>a</sup>Second-order rate constant in units of  $10^{-10} \text{ cm}^3 \text{ s}^{-1}$  at 298 K, estimated error:  $\pm 30\%$ . <sup>b</sup>Eff =  $k_{\text{exp}}/k_{\text{ADO}} \times 100$ , where  $k_{\text{ADO}}$  is  $1.14 \times 10^{-9}$ .<sup>55,56</sup> <sup>c</sup>Proton transfer reaction is accompanied by TFA addition, yielding  $[(\text{r})\text{T3} + \text{TFA-H}]^-$  (Eff (%) = 7 and 4 for X = T3 and rT3, respectively).



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Thank you for the kind attention



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