



SC9- Joensuu, 19-22 April 2022

Isomer discrimination in FT-ICR MS and hyphenated techniques



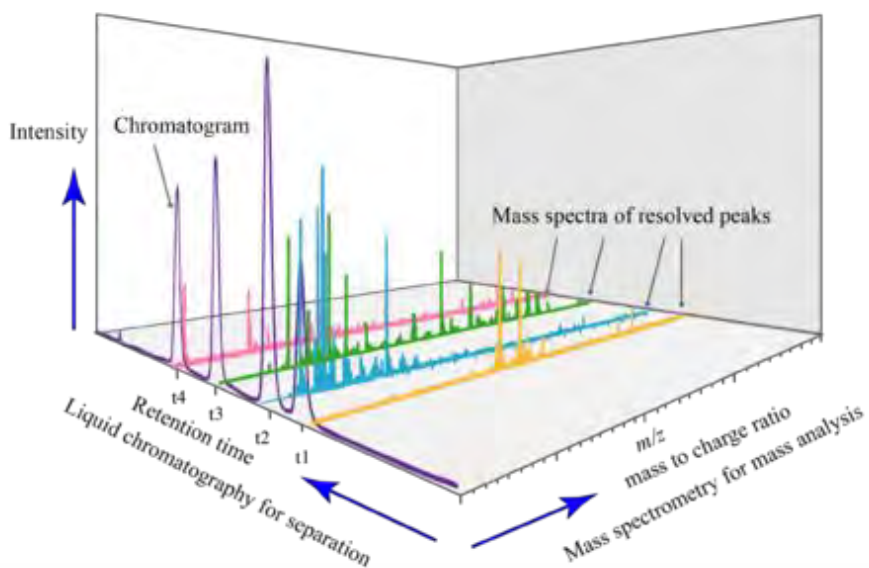
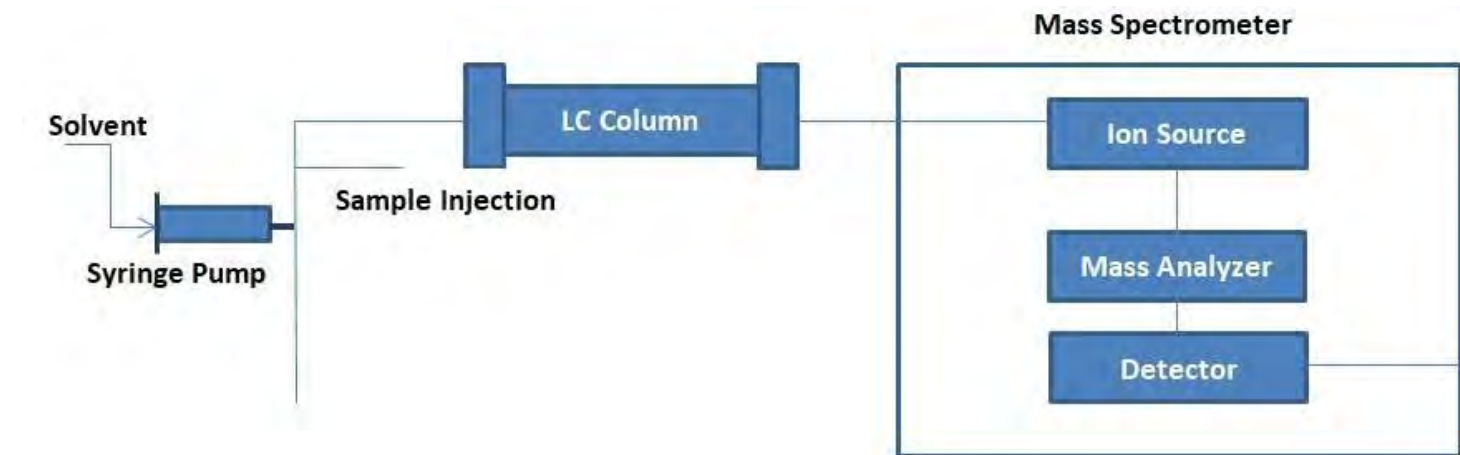
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UNIVERSITÀ DI ROMA

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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 of 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

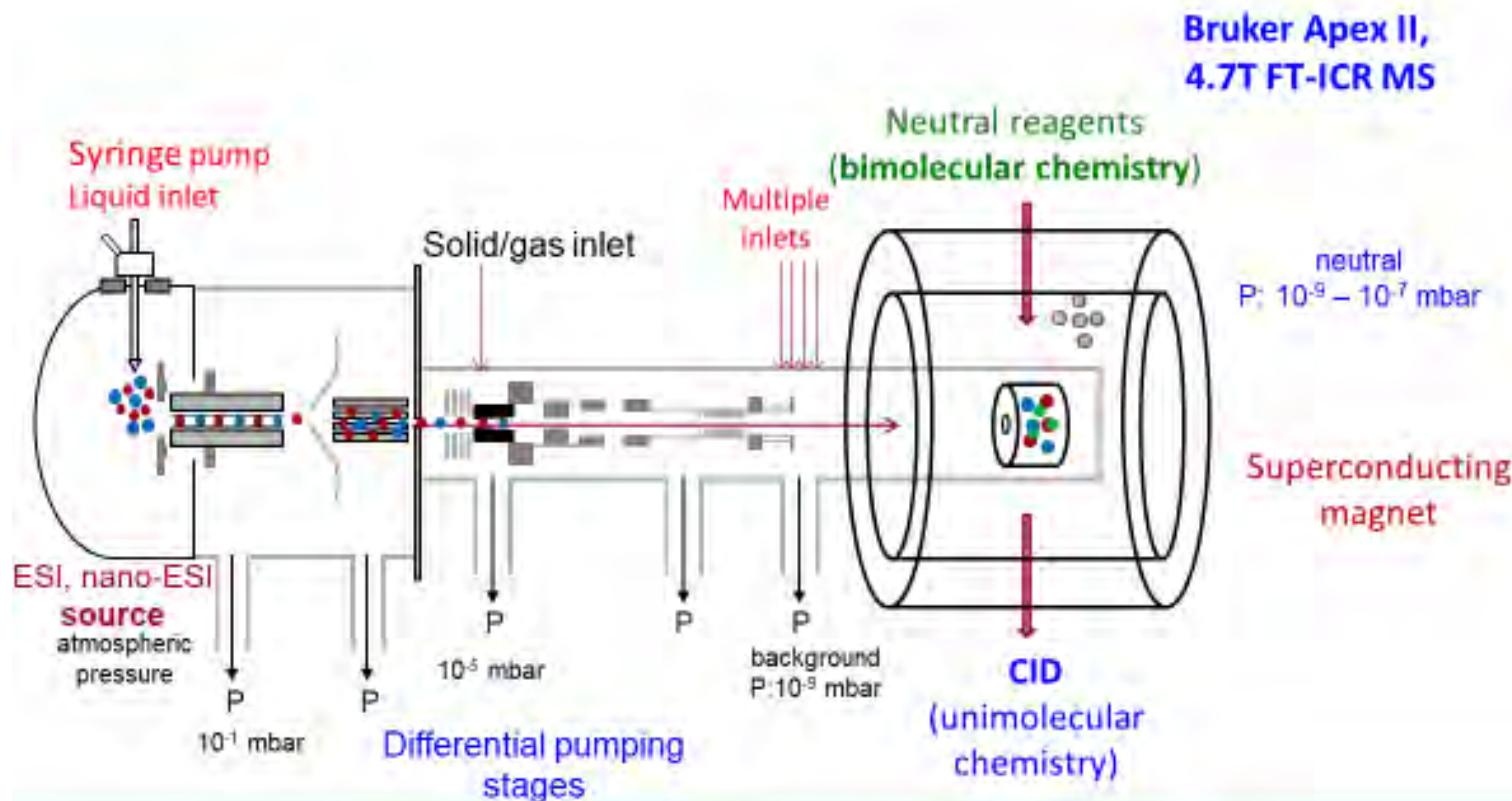
Cons

- 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.

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

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



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_{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 (Φ) 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

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

the first term is the Langevin contribution

Interaction: ion- dipole

μ is the reduced mass; μ_D is the permanent dipole;

C is a correction factor depending on $\mu_D / \alpha^{1/2}$;

k_B is Boltzmann's constant

The dipole orientation is not considered

k_{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_{exp}
- presence of gaseous B

Equilibrium method: equilibrium



- measurement of K_{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

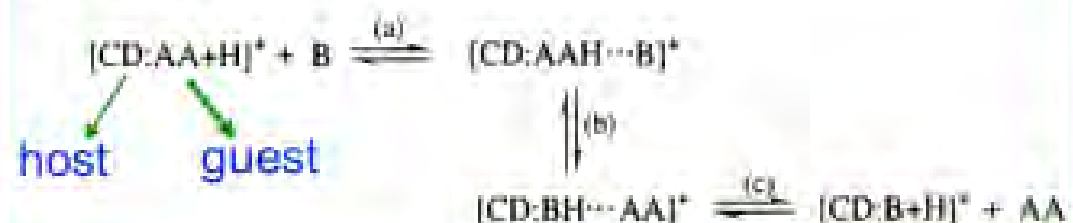
J. Am. Chem. Soc. 1998, 120, 7387-7388

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

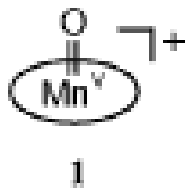
Scheme 1



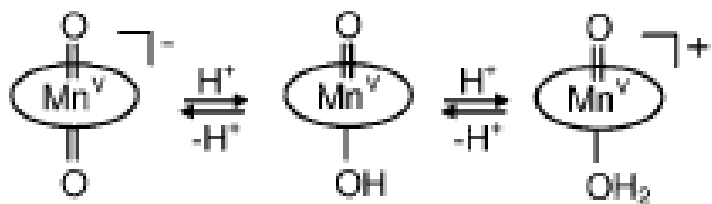
- complexes of protonated β -cyclodextrin-amino acid (Ala; Val; Phe) react with n-propylamine by **exchanging the AA guest of cyclodextrin host** for alkylamine;
- the exchange rates are found to differ according to the chirality of the AA
- Valine is the most reactive and shows the greatest selectivity: $k_L/k_D = 1.6$ (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



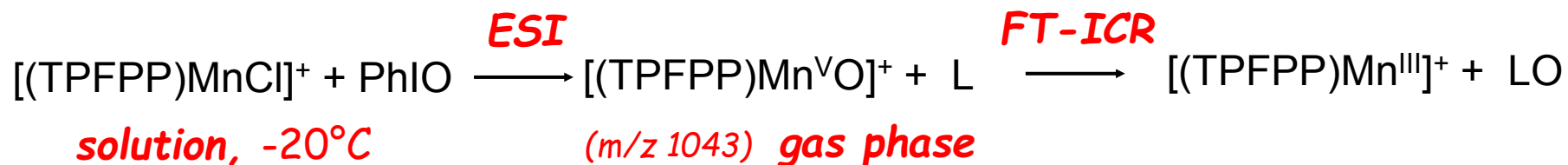
Mn^V-oxo-porphyrin complexes prepared in aqueous solution:
diamagnetic d² low-spin ground state



high pH

low pH

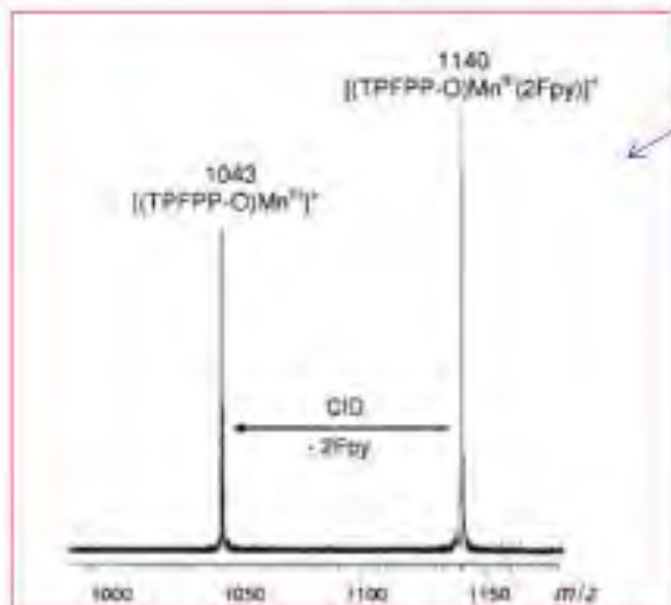
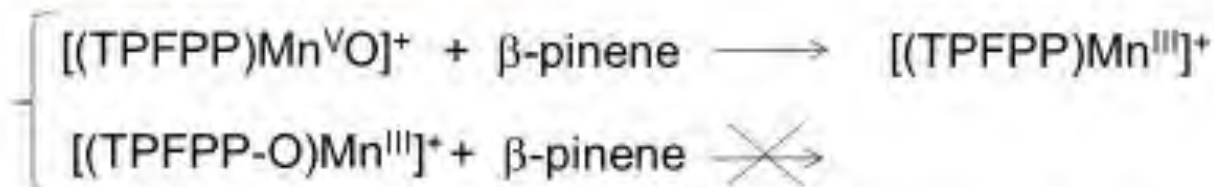
The OAT reactivity in water is found to be strongly pH-dependent: prototropic equilibria



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



Gas-phase titration



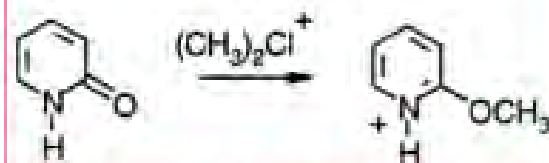
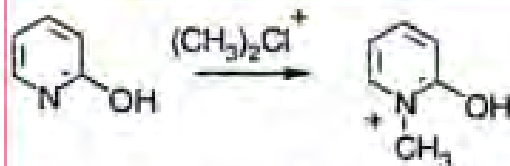
CID

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

Olefin (IE) ^[b]	k_{exp} ^[c,d]	Φ ^[e]
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) ^[f]	1.7	16
1,3,5-cycloheptatriene (8.30)	1.9	21
(+)-camphene (≤ 8.86)	2.6	28
indene (8.14) ^[g]	1.2	12
(1 <i>S</i>)-(-)- α -pinene (8.07)	9.0	100
β -pinene (n.a.)	9.3	100
(<i>R</i>)-(+)-limonene (8.3)	9.2	100



Identification of functional groups in organic compounds



Identification via CID of independently synthesized products

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



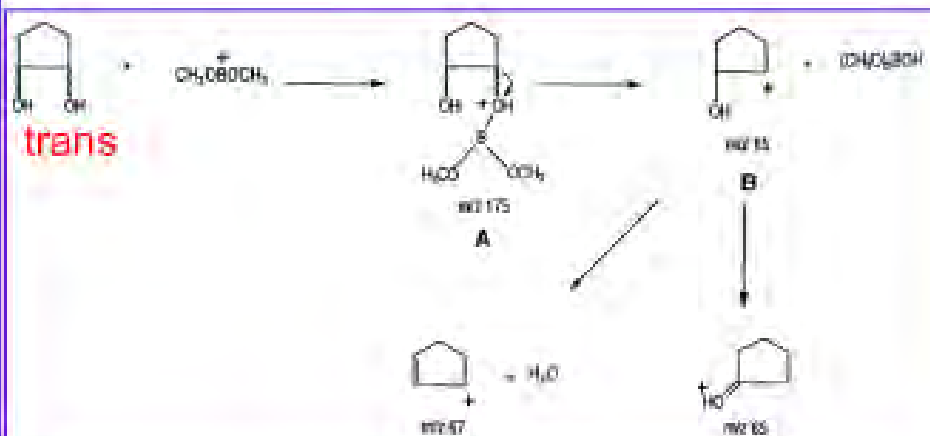
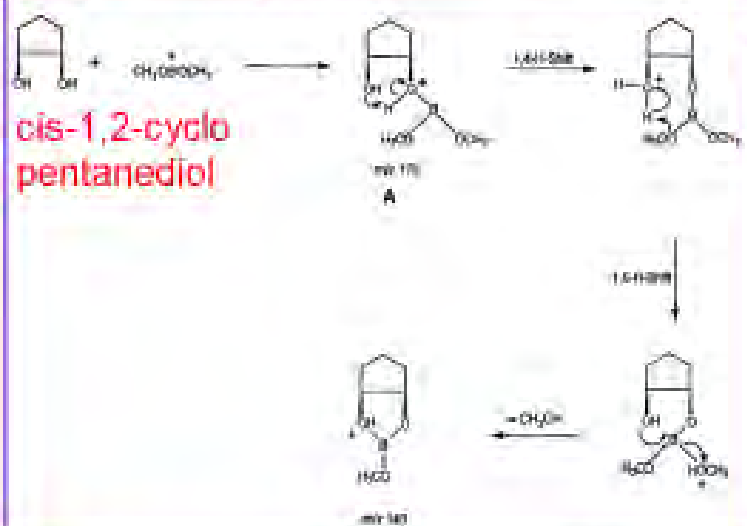
ELSEVIER

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



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^a, T.D. Ranatunga^a, R.L. Smith^a, T. Partanen^b, P. Vainitalo^{b,*},
H.I. Kenttämää^{a,*}



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O'Hair, Eur. J. Mass Spectrom. 1995;
H. I. Kenttämää, Int. J. Mass Spectrom. Ion Proc. 1995

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

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



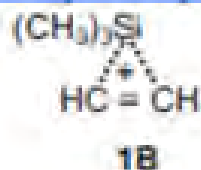
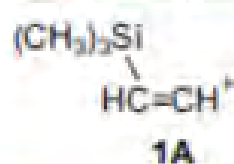
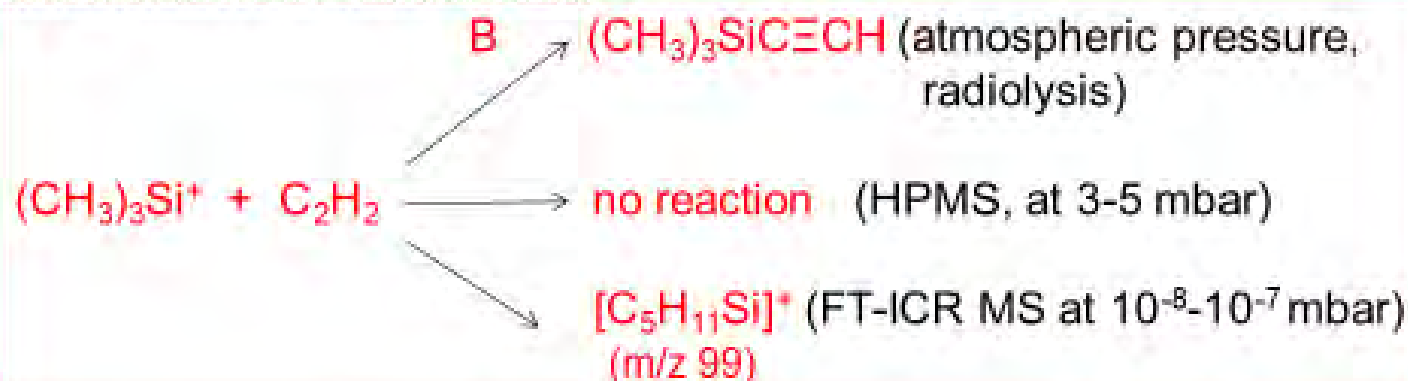
Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

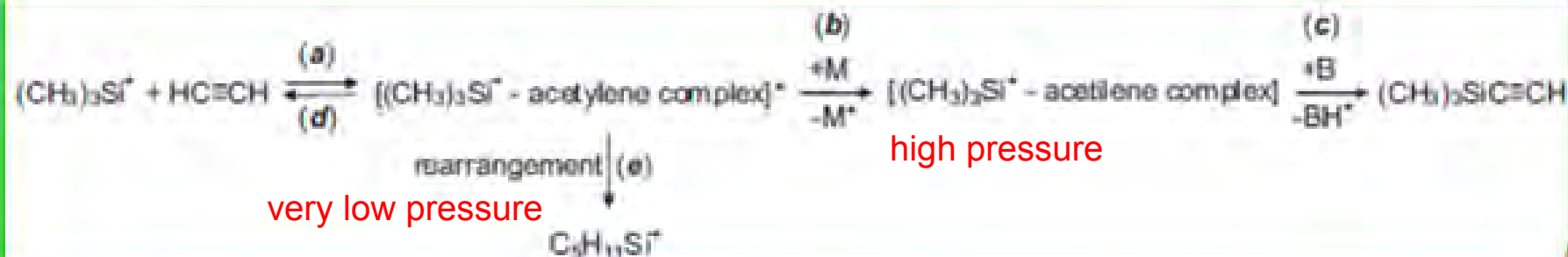
Isomeric $C_5H_{11}Si^+$ ions from the trimethylsilylation of acetylene: An experimental and theoretical study

Hans-Ulrich Siehl^{a,*}, Sandra Brixner^a, Cecilia Coletti^b, Nazzareno Re^b, Barbara Chiavarino^c,
Maria Elisa Crestoni^c, Alberto De Petris^d, Simonetta Fornarini^{c,e,*}



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

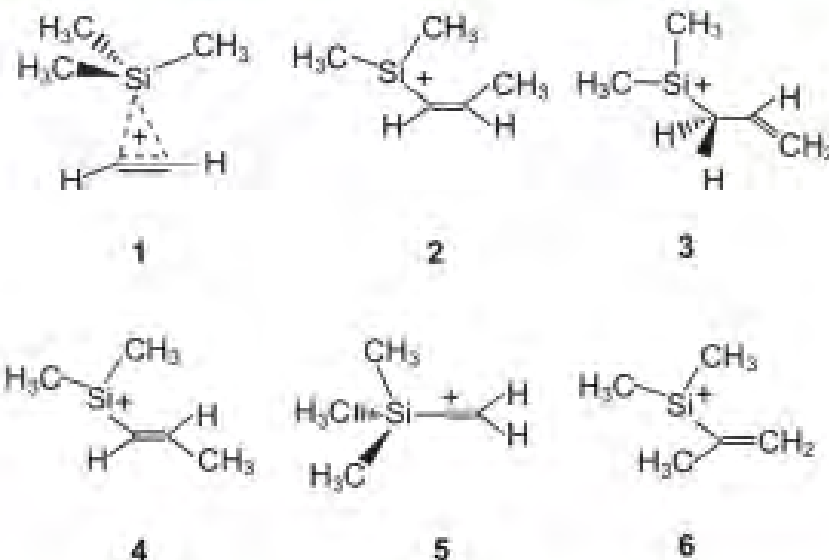


IMR with appropriate neutrals as diagnostic tools to test ion structures

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

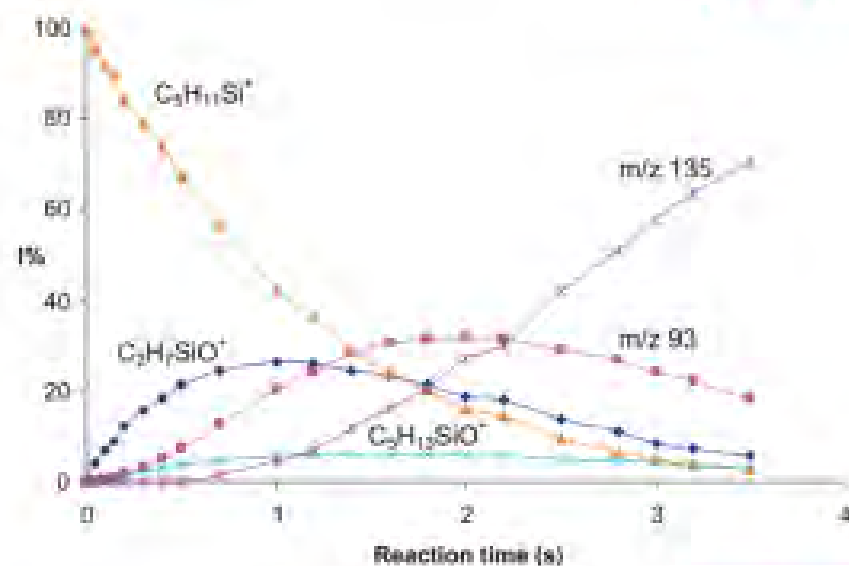
potentially diagnostic reagents:

H_2O
 $i\text{-C}_3\text{H}_7\text{OH}$
 $t\text{-C}_4\text{H}_9\text{OCH}_3$





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



ϕ 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

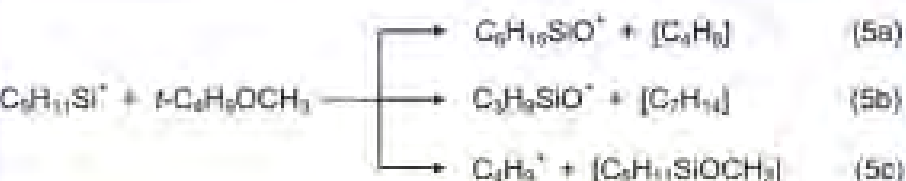
Table 2

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

Precursor of reactant ion ^a	ROR ⁺	P_1/P_2^b
(Z)-1-(Trimethylsilyl)propene (2)	<i>i</i> -C ₃ H ₇ OH	8.5 ± 1.7^c
(E)-1-(Trimethylsilyl)propene (4)	<i>i</i> -C ₃ H ₇ OH	11 ± 1.8^c
2-(Trimethylsilyl)propene (6)	<i>i</i> -C ₃ H ₇ OH	20 ± 5^c
Allyltrimethylsilane (3)	<i>i</i> -C ₃ H ₇ OH	4.3 ± 0.5^c
S	<i>i</i> -C ₃ H ₇ OH	21 ± 2^c
(Z)-1-(Trimethylsilyl)propene (2)	<i>t</i> -C ₄ H ₉ OCH ₃	2.6 ± 0.3^d
(E)-1-(Trimethylsilyl)propene (4)	<i>t</i> -C ₄ H ₉ OCH ₃	2.5 ± 0.3^d
2-(Trimethylsilyl)propene (6)	<i>t</i> -C ₄ H ₉ OCH ₃	4.6 ± 0.7^d
Allyltrimethylsilane (3)	<i>t</i> -C ₄ H ₉ OCH ₃	24 ± 5^d
S	<i>t</i> -C ₄ H ₉ OCH ₃	4.1 ± 0.7^d

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

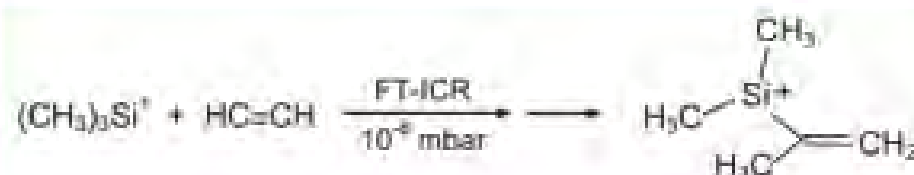
$P_1 = C_3H_9SiO^+$ and $P_2 = C_6H_{15}SiO^+$



⚡ 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 ($C_3H_9SiO^+$)



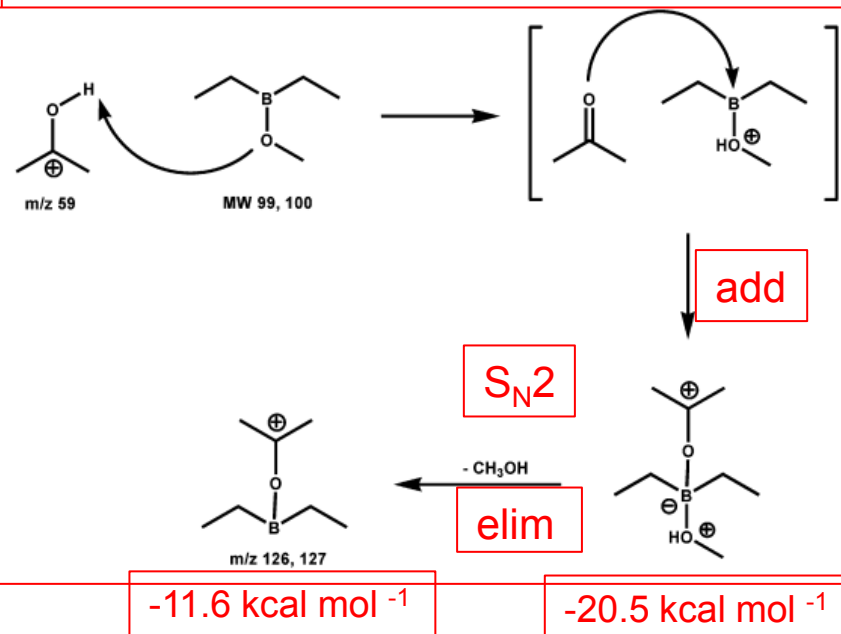
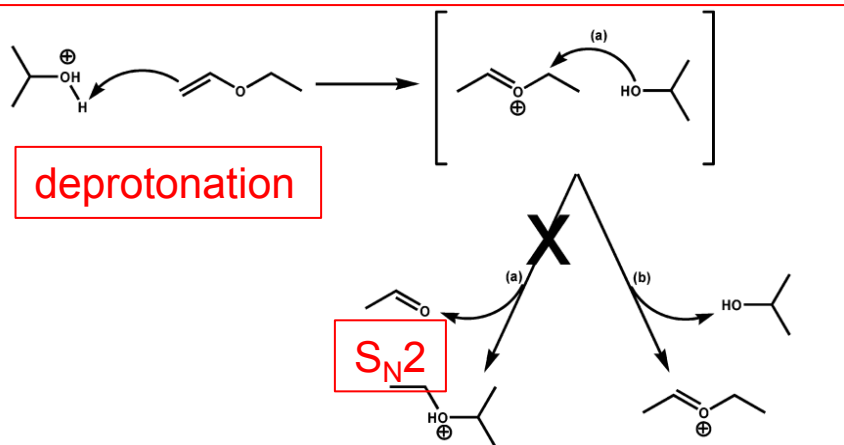
2-propenyl-dimethylsilyl cation

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,[†] Jason M. Price,^{†,‡} Brian E. Winger,[§] and Hilkka I. Kenttämää^{*,†}



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

Functional-group selective IMR

Research Article



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Accepted: 27 February 2014

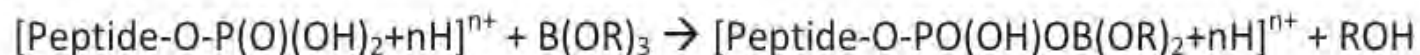
Published online in Wiley Online Library

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^{1,2*}, Simonetta Fornarini³, Claire E. Eyers² and Maria Elisa Crestoni³



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

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



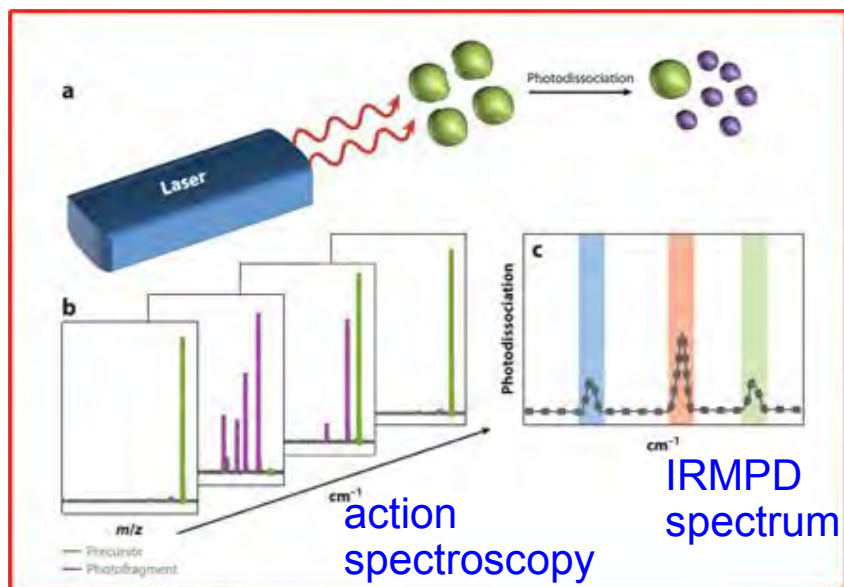
Biomolecular vibrations

Table 1.2 Vibrational ranges for common biomolecular chemical moieties

Description	Frequency/cm ⁻¹
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 ₂ stretch	3,350–3,400
Asymmetric NH ₂ 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



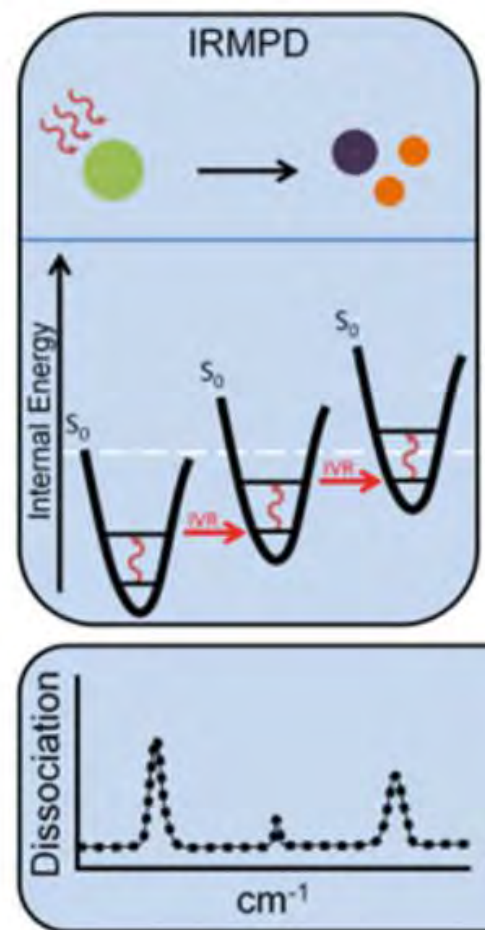
Infrared Multiple Photon Dissociation (IRMPD) : an action spectroscopy



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)

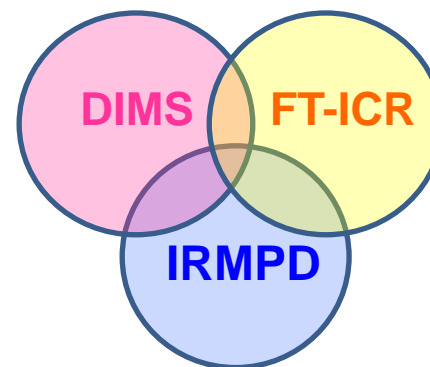
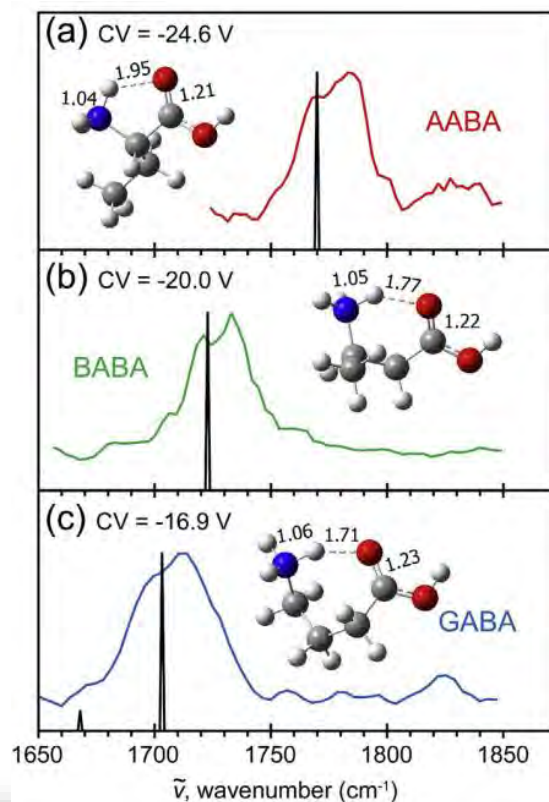




Infrared isomer-specific fragmentation for the identification of aminobutyric acid isomers separated by differential mobility spectrometry

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



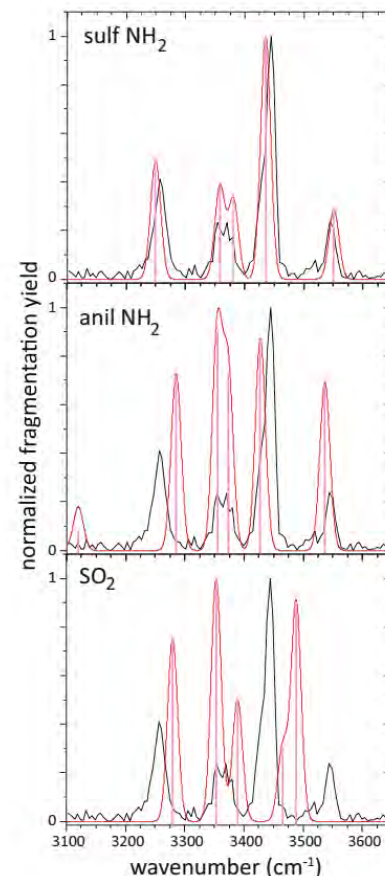
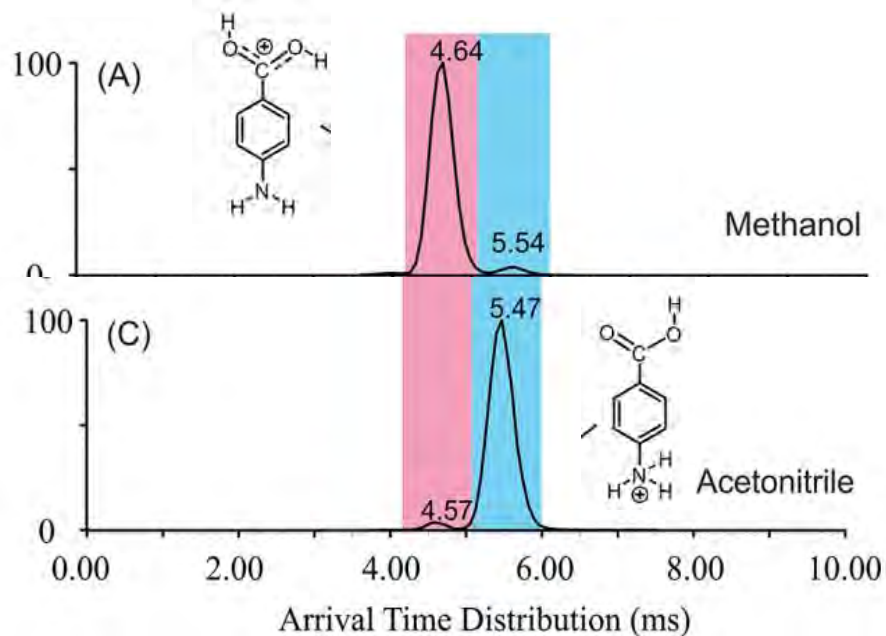
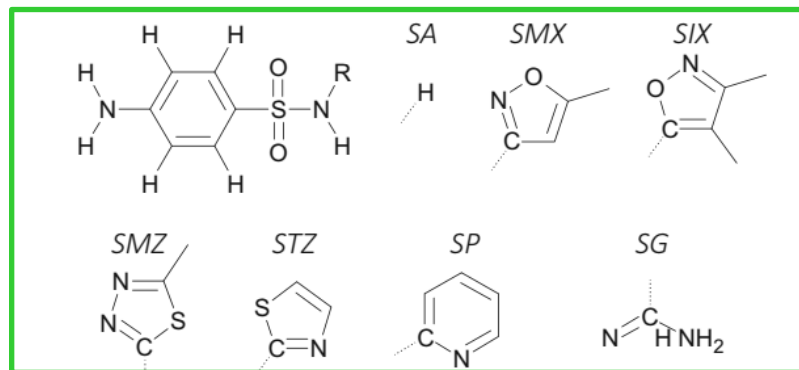
three orthogonal techniques

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

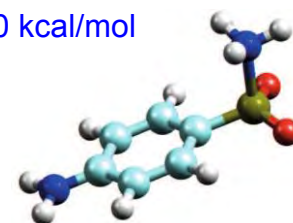
Thomas Uhlemann, Giel Berden, and Jos Oomens



0.0 kcal/mol

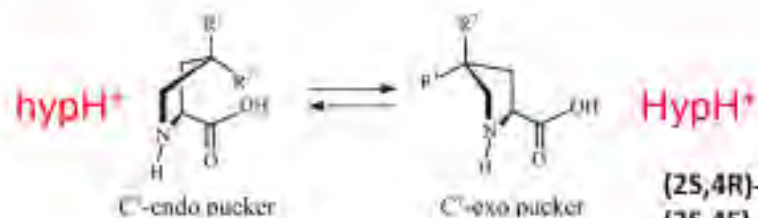
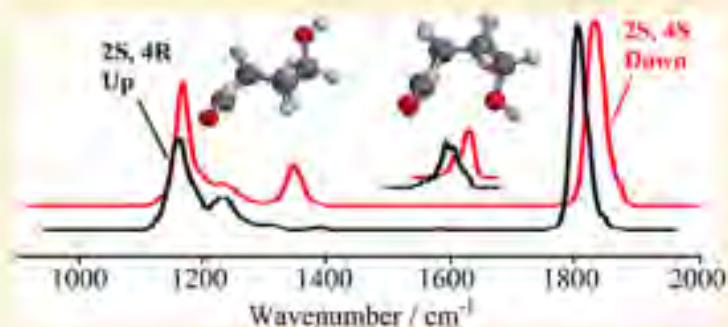
6.3 kcal/mol

4.0 kcal/mol



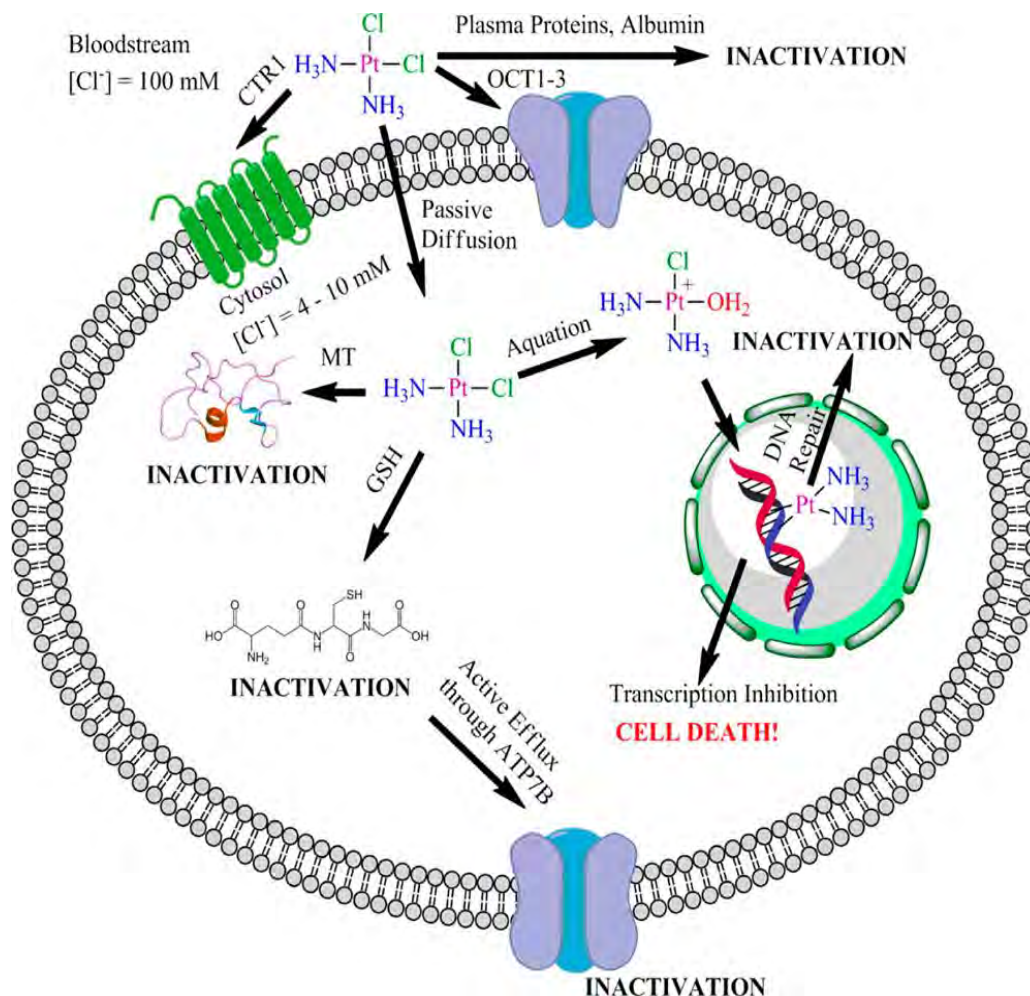
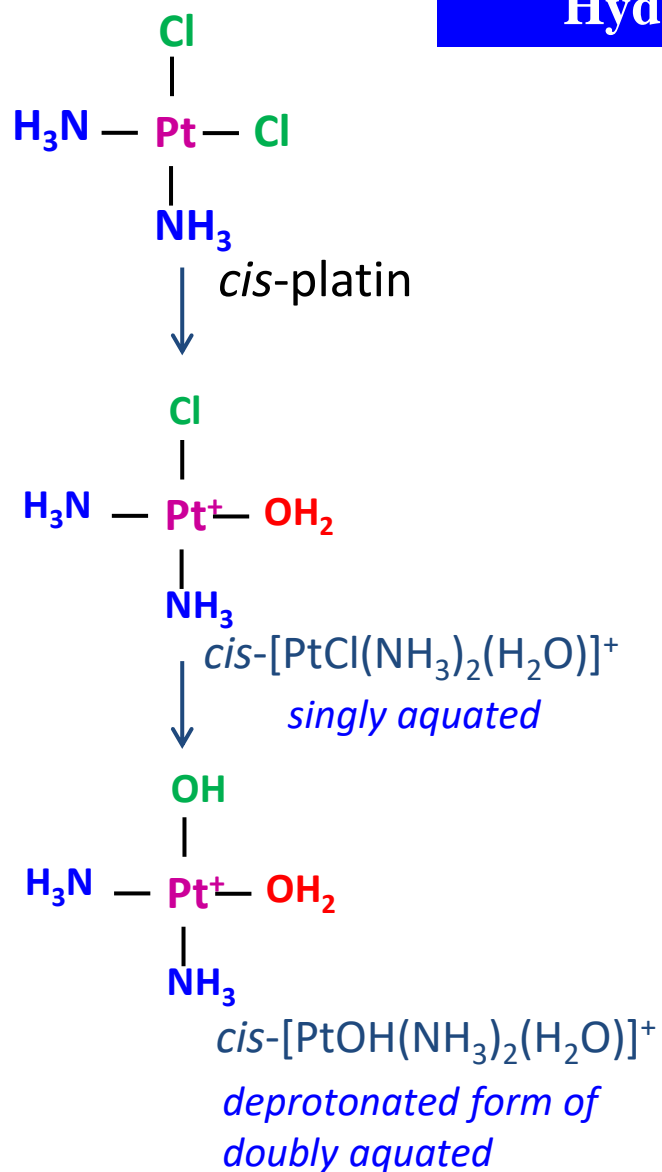
Discrimination of 4-Hydroxyproline Diastereomers by Vibrational Spectroscopy of the Gaseous Protonated Species

J. Phys. Chem. B 2012, 116, 8771–8779



(2S,4R)-4-hydroxyproline = Hyp
(2S,4S)-4-hydroxyproline = hyp

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

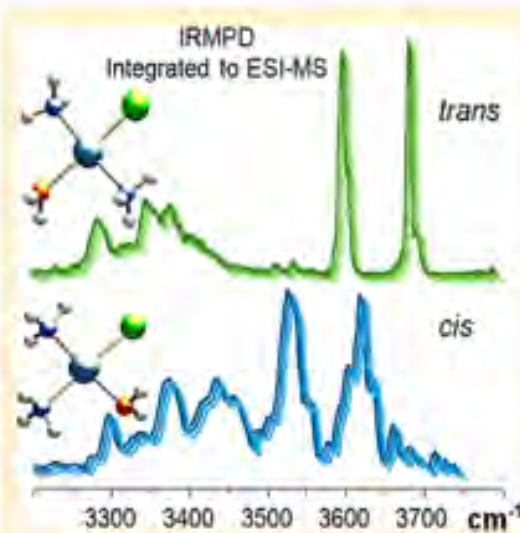
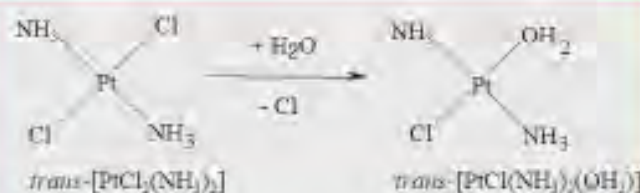
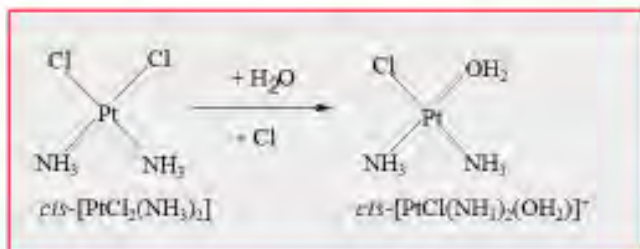
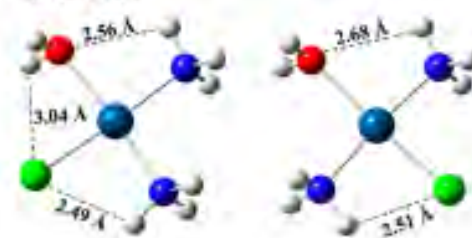
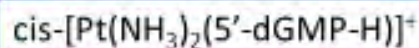
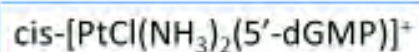
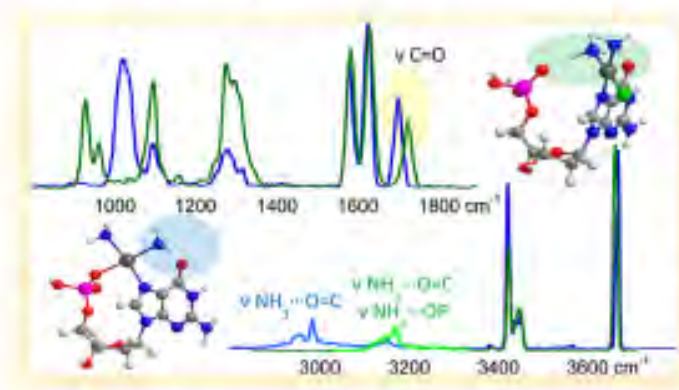


Chart 1. Structures of *cis*-[PtCl(NH₃)₂(H₂O)]⁺ and *trans*-[PtCl(NH₃)₂(H₂O)]⁺



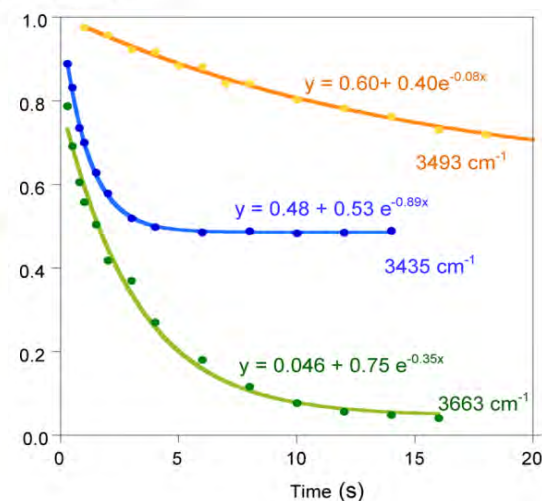
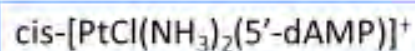
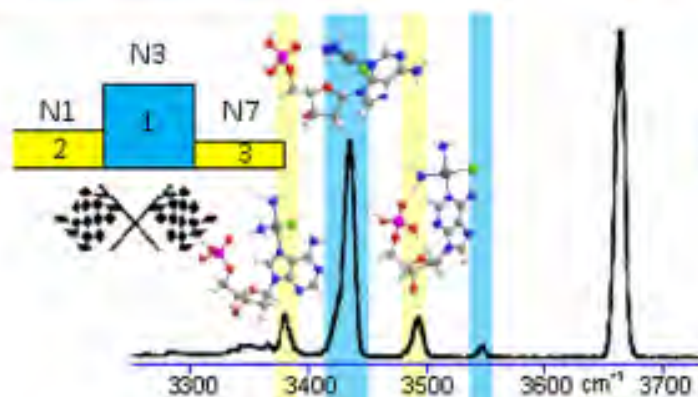
Interaction of Cisplatin with 5'-dGMP: A Combined IRMPD and Theoretical Study

Inorg. Chem. 2015, 54, 3513–3522



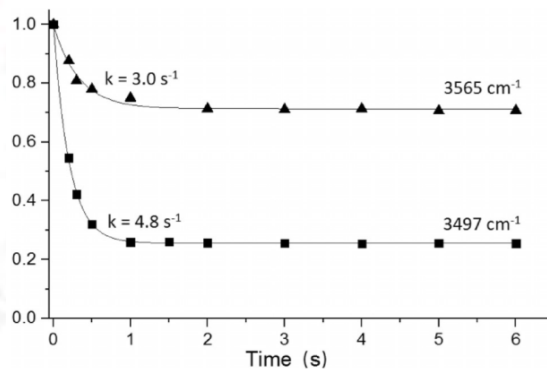
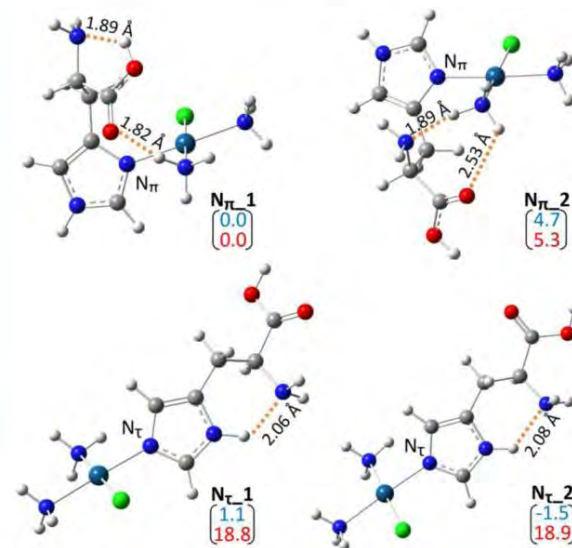
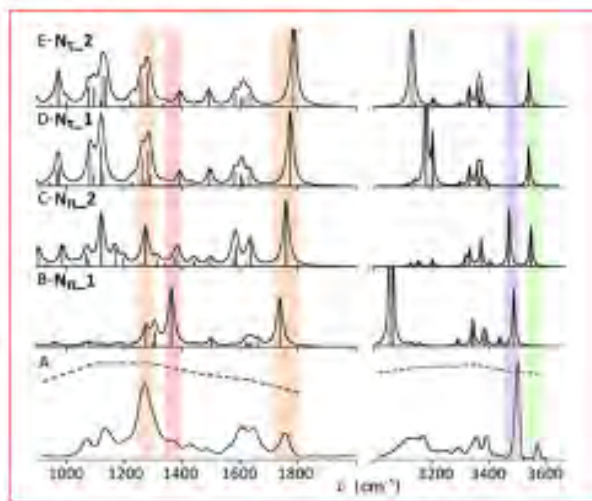
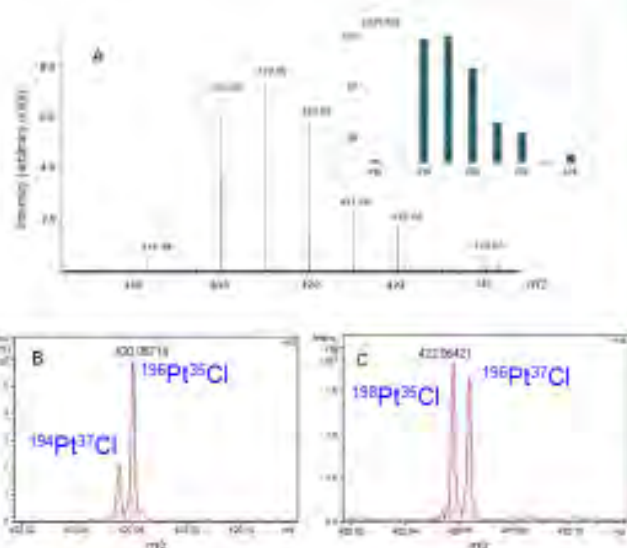
Undervalued N3 Coordination Revealed in the Cisplatin Complex with 2'-Deoxyadenosine-5'-monophosphate by a Combined IRMPD and Theoretical Study

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



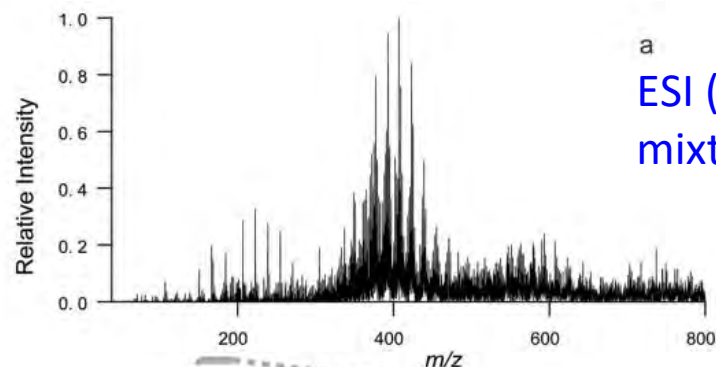
N_τ conformers: 25% (unreactive)
N_π_1 : 45%
N_π_2 : 30%



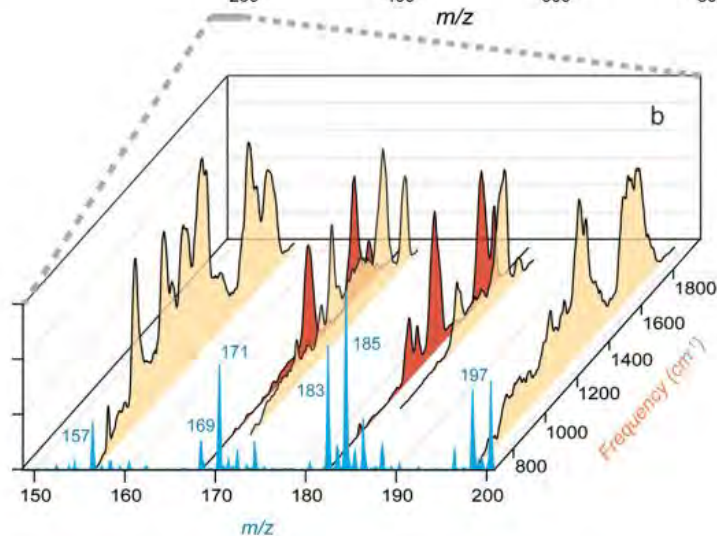
Infrared Ion Spectroscopy of Environmental Organic Mixtures: Probing the Composition of α -Pinene Secondary Organic Aerosol

Emma Q. Walhout,[†] Shelby E. Dorn,[§] Jonathan Martens,[‡] Giel Berden,[‡] Jos Oomens,^{‡,||}
Paul H.-Y. Cheong,^{*,§} Jesse H. Kroll,[⊥] and Rachel E. O'Brien^{*,†,⊥}

Organic Aerosol

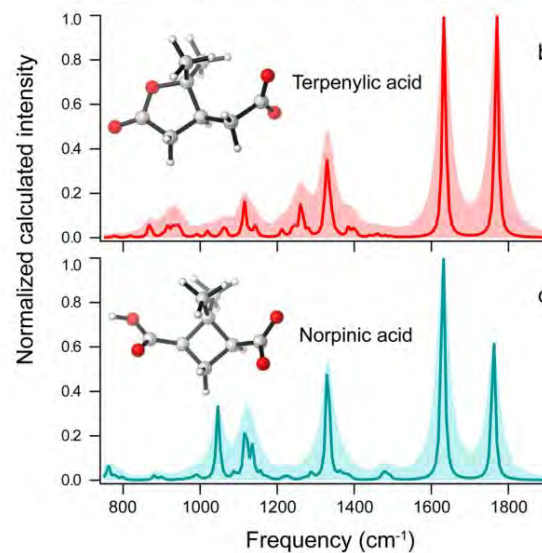
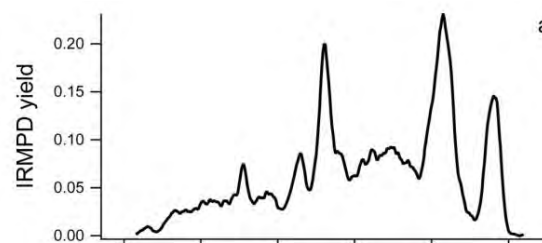
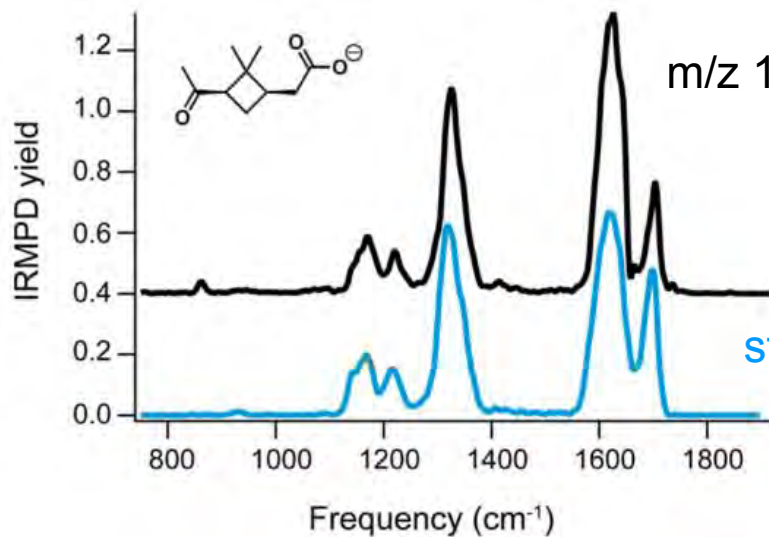


ESI (-) mass spectrum of α -pinene ozonolysis mixture



fingerprint region (600-1900 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*

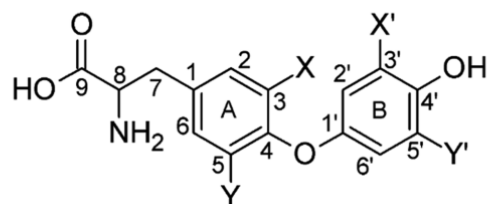


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Scheme 1. Schematic Representation of Tetraiodothyronine (T4), Triiodothyronine (T3), and Reverse Triiodothyronine (rT3)



T4 X=Y=X'=Y'=I

Y'=H

rT3 X=X'=Y'=I Y=H

deprotonation of carboxylic acid

[rT3-H]⁻
deprotonation of phenol

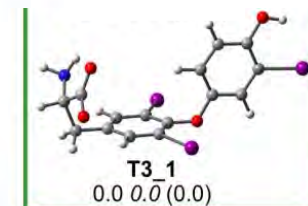
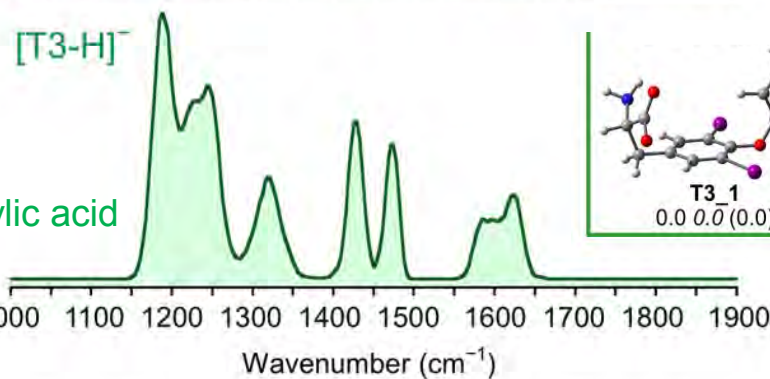
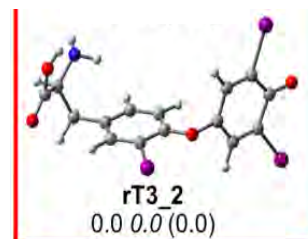
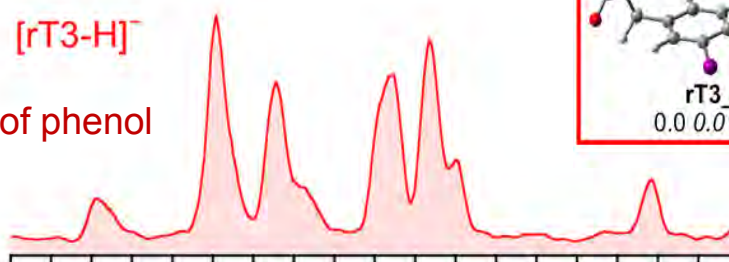
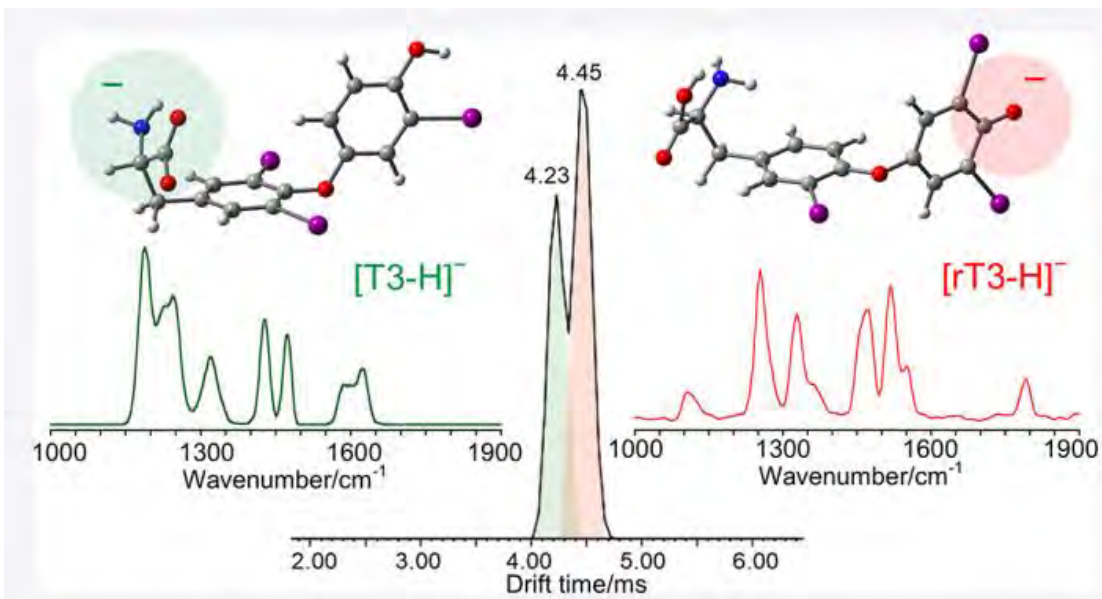


Figure 1. IRMPD spectra of [T3-H]⁻ and [rT3-H]⁻ in the fingerprint range.





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 kJ mol^{-1})

TFA (1328 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_{exp}^a	Eff (%) ^{b,c}
T3	2.53	22
rT3	1.12	10

^aSecond-order rate constant in units of $10^{-10} \text{ cm}^3 \text{ s}^{-1}$ at 298 K, estimated error: $\pm 30\%$. ^bEff = $k_{\text{exp}}/k_{\text{ADO}} \times 100$, where k_{ADO} is 1.14×10^{-9} .^{55,56} ^cProton 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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