Fall 2014

The Kinetic Method: Determination of Proton Affinity of Proline by Cis and Trans Hydroxylation

Vinod Kanchi
Governors State University

THE KINETIC METHOD: DETERMINATION OF PROTON AFFINITY OF PROLINE BY *CIS* AND *TRANS* HYDROXYLATION

VINOD KANCHI

[redacted]

GOVERNORS STATE UNIVERSITY

Dr. JOONG- WON SHIN

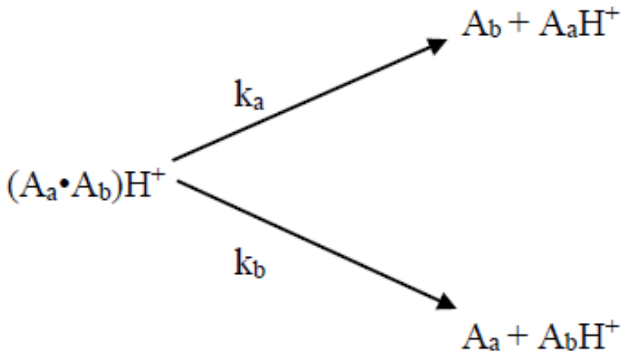
Abstract:

Unknown Proton affinity (PA) of the amino acids were determined by comparing them with known PA of the reference standards. Initially we mixed 2 amino acids together [unknown (PA) and known (PA)] and introduce into mass spectrometer using direct infusion. After injecting we observe the peaks. The protonated dimer peak of known PA and unknown PA samples were isolated and fragmented. Once they were fragmented protonated dimer will produce two peaks corresponding to amino acid with unknown PA and amino acids with known PA. Based on the ratio of these amino acids we determine the unknown PA of an amino acid. Proline, *trans*-4-hydroxyproline and c*is*-4-hydroxyproline were used as amino acids with unknown PA and tryptophan, asparagine, tyrosine, methionine and phenylalanine as reference bases.

Introduction:

The use of mass spectrometry in analysis of bio molecules is one of the most common ways to determine the properties of a molecule. For instance PA of an amino acid can be found using a reference standard amino acid with known PA. This method is known as kinetic method and was developed by Cooks. Kinetic method is one of the best methods to determine the PA of a molecule. PA is related to the rate at which dissociation of a protonated dimer occurs to get protonated, thus rate constant of an amino acid in a protonated dimer will dictate its PA [1].

In a protonated dimer of an amino acid A_a and A_b with rate constant k_a and k_b; and PA PA_a and PA_b respectively, where A_a is an amino acid with unknown PA and A_b is an amino acid with known PA. [2]



Based on the amount of A_aH^+ and A_bH^+ formed will determine the PA of the unknown. Plotting the $\ln(I_a/I_b)$ vs known proton affinities of the reference bases we calculate the unknown PA of the amino acid by using the following equation. We can calculate the effective temperature using the slope and PA by using Y intercept. I_a and I_b in the equation 2 are the intensities of amino acid with unknown PA and reference standard respectively. [2]

$$\ln\frac{k_a}{k_b} = -\frac{PA_a}{RT} + \frac{PA_b}{RT} \quad \text{.....} \textbf{\textit{Equation 1}}$$

$$\ln\frac{k_a}{k_b} \approx \ln\frac{I_a}{I_b} \quad \text{.....} \textbf{\textit{Equation 2}}$$

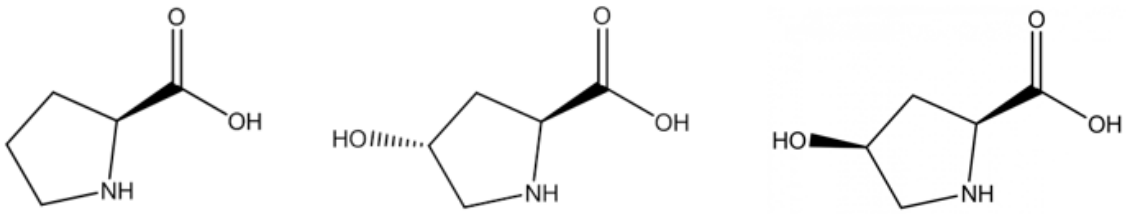


***Fig 1*:** Structure of amino acids with unknown PA. [4]

Experimental:

Solvent: 80% water, 20% methanol, and 1% acetic acid. Different percentage volumes of water and methanol were tried and above solvent mixture was most suitable in dissolving all the amino acid which were used in the experiment.

Instrumental conditions:

All the experiments were conducted using Agilent technologies LC/MS Trap XCT.

Nebulizer: 40 psi

Dry gas: 5 L/min

Temperature: 300^{o}C

MS2 was conducted at the same fragmentation voltage amplitude for all the protonated dimer solutions at 0.37V.

Proline, *trans*-4-hydroxyproline and *cis*-4-hydroxyproline were used as unknown amino acids and tyrosine, tryptophan, asparagine, methionine and phenylalanine were used as known reference amino acids. Amino acids with known PA values were mixed with amino acids with unknown PA values in equal concentrations to form protonated dimers with each other. Proline was mixed with tryptophan, asparagine, methionine and phenylalanine individually with the end concentration of 50mM.

Cis-4-hydroxyproline and *trans*-4-hydroxyproline were mixed with tyrosine, tryptophan and asparagine individually. **Note:** Preparation of aqueous tyrosine mixture was difficult since it is partially soluble in water so 1 ml of concentrated HCl solution was added.

All the 50mM solutions were analyzed using electrospray tandem mass spectrometer by direct infusion using a syringe with a flow rate 0.3 ml/hour

Once all the parameters were set the peak corresponding the protonated dimer of the two amino acid solution injected was identified, isolated and fragmented (MS2). The fragmentation was done at 0.37V voltage amplitude.

The data was exported to excel to get the accurate intensity values and natural log of ratios for the 2 amino acids was calculated. A graph was plotted between the natural log of ratios of intensities $\ln(I_a/I_b)$ vs PA values of the reference amino acids. Based on the slope and the x-intercept values effective temperature and PA value respectively were calculated.

Results:

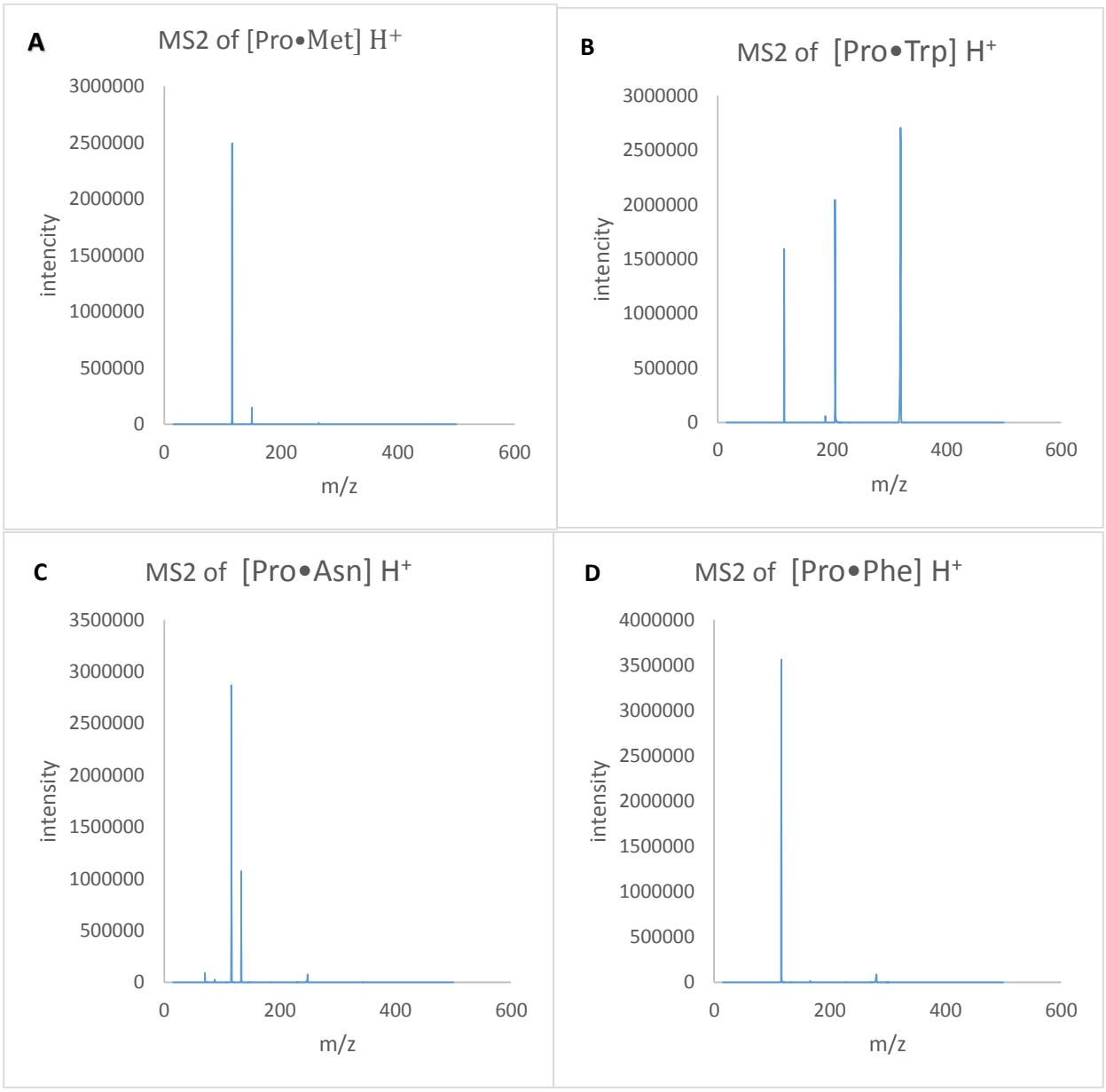


Fig A: Ms2 of [Pro•Met] H^+; **Fig B**: Ms2 of [Pro•Trp] H^+; **Fig C**: Ms2 of [Pro•Asn] H^+; **Fig D**: Ms2 of [Pro•Phe] H^+ at fragmentation voltage of 0.37V.

Reference amino acids	PA (kJ • mol^{-1})	ln(I_a/I_b)
phenylalanine	919.7	5.001
methionine	927.4	2.82103
asparagine	933.3	0.985994
tryptophan	936.3	-0.248

Table 1: Natural log of ratios of intensities [ln(I_a/I_b)] vs PA values of the reference amino acids.[2]

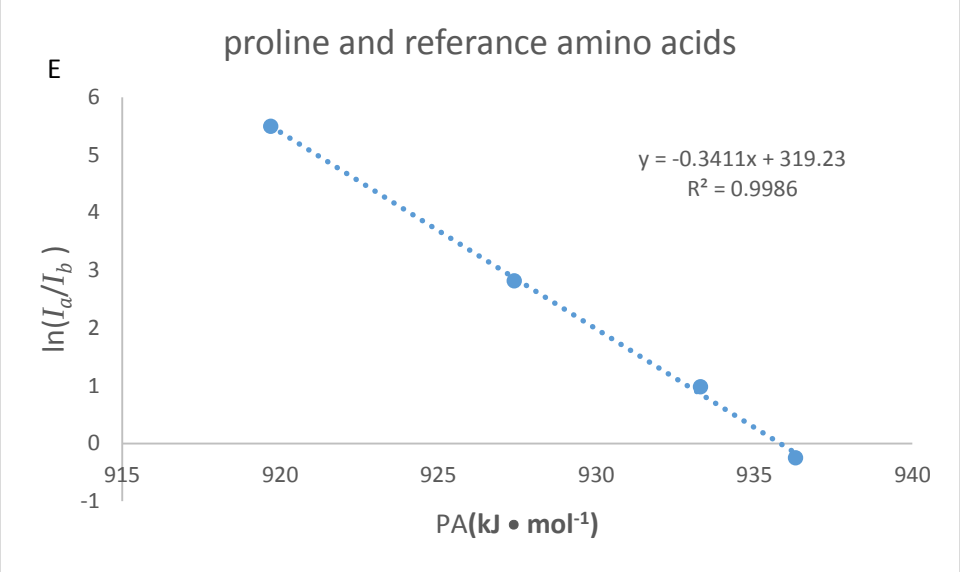


Figure E: the figure E is the graph plotted between the natural log of ratios of intensities [ln(I_a/I_b)] vs PA values of the reference amino acids.

From the equation in the graph we can find the proton affinity of proline using the formula below [2]:

$$\ln\frac{k_a}{k_b} = -\frac{PA_a}{RT} + \frac{PA_b}{RT} \text{.....} \textit{Equation 1}$$

$$\ln\frac{k_a}{k_b} \approx \ln\frac{I_a}{I_b} \text{.....} \textit{Equation 2}$$

$$y = -0.3411x + 319.23$$

$$-0.3411x = -\frac{PA_a}{RT} \text{ (eq: 1)}$$

$$-0.3411 = -\frac{1}{RT}$$

$$T_{eff} = \frac{1}{0.008314(\text{kJ}\bullet\ \text{K}^{-1}\bullet\text{mol}^{-1}) \times -0.3411(\text{kJ}\bullet\text{mol}^{-1})}$$

$$T_{eff} = 352.62\ \text{K}$$

Proton affinity of proline:

$$y = -0.3411x + 319.23$$

$$y = 0$$

$$0.3411x = 319.23$$

$$x = \frac{319.23}{0.3411}\ kJ \bullet mol^{-1}$$

$$x = 935.88\ kJ \bullet mol^{-1}$$

In the similar way the unknown proton affinities of *cis* and *trans*-4-hydroxyproline was found using the known proton affinities of reference amino acids.

Reference amino acid	**PA (kJ • mol^{-1})**
Tryptophan	936.3
Asparagine	933.3
Methionine	927.4
Phenylalanine	919.7
Tyrosine	921.6

Table 2: reference amino acids used and their PA values [2].

set no	**Proline**		***trans*-4-hydroxyproline**		***cis*-4-hydroxyproline**	
	PA (kJ • mol^{-1})	**Effective temp (K)**	**PA (kJ • mol^{-1})**	**Effective temp (K)**	**PA (kJ • mol^{-1})**	**Effective temp (K)**
1	935.9	352.6	939.9	436.7	938.4	375.1
2	935.7	339.9	938.3	391.7	939.4	440.9
3	935.4	323.9	938.1	369.3	939.9	461.7
4	935.8	369.8	939.7	476.5	940.0	475.2
mean	**935.7**	**346.6**	**938.9**	**418.6**	**939.4**	**438.2**
Standard deviation	**0.2**	**19.4**	**0.9**	**47.7**	**0.7**	**44.4**
Standard error	**0.1**	**9.7**	**0.5**	**23.9**	**0.6**	**22.2**

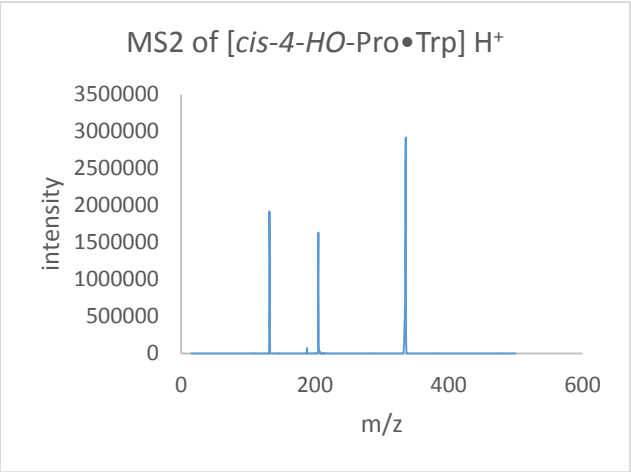
Table 3: PA and effective temperatures of proline, *trans*-4-hydroxyproline and *cis*-4-hydroxyproline

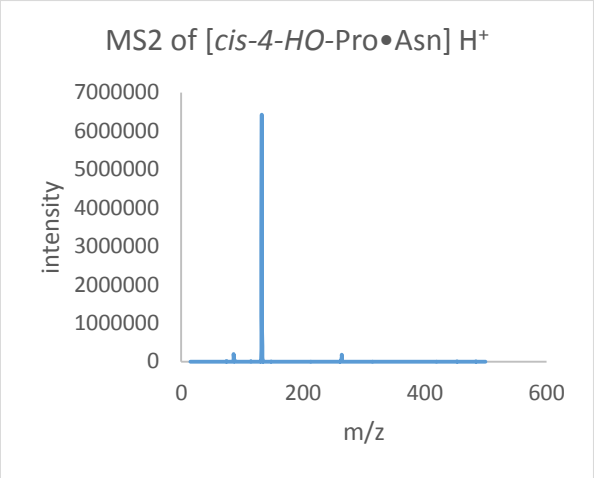
The experiments were repeated 4 time and the results were reproducible. We obtained a standard deviation of 0.2 kJ • mol^{-1}, 0.9 kJ • mol^{-1}, 0.7 kJ • mol^{-1} for proline, *trans*-4-hydroxyproline and *cis*-4-hydroxyproline respectively to its mean.

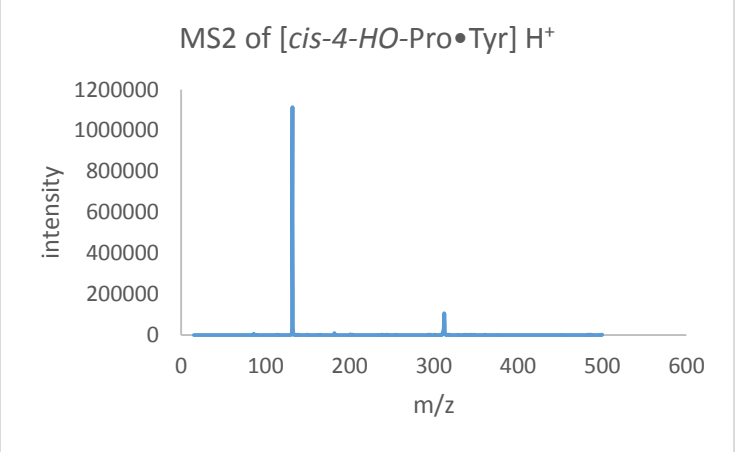
Conclusion:

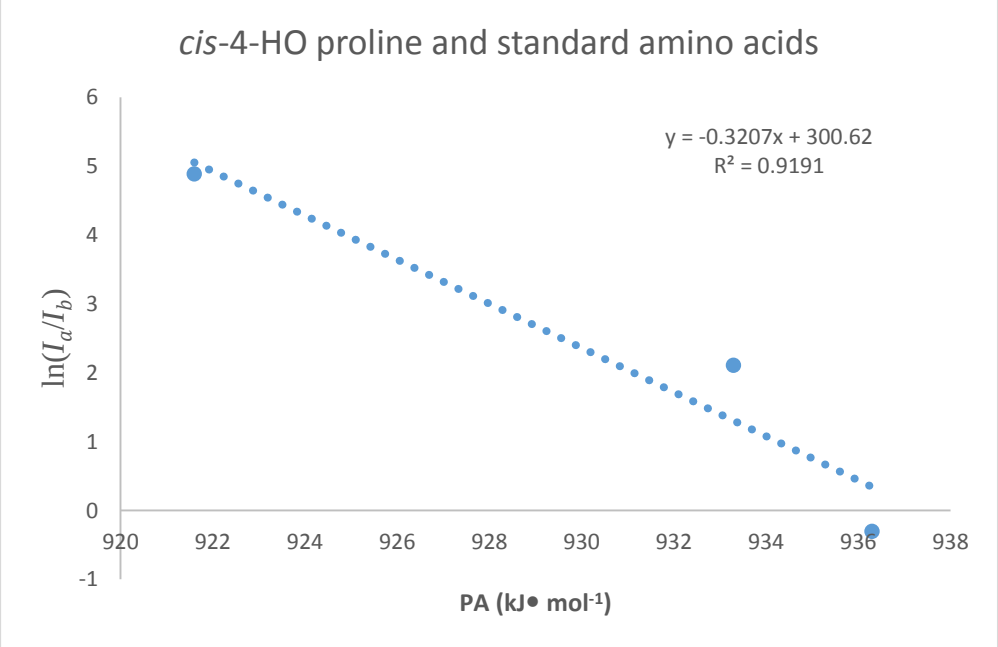
The proton affinity of proline is very similar to the proton affinity obtained from a previous work [2]. There is a significant difference in PA upon addition of hydroxyl group to proline. The PA of *cis* and *trans*-4-hydroxyproline possibly increases because of the hydrogen bonding facilitated by the presence of hydroxyl group. Although there is an increase in PA from proline to hydroxyproline; there is no difference in PA among *cis* and *trans*-4-hydroxyproline as shown in the calculation from Equation1 and results obtained in Table3. This result is in contradiction to that of a previous work [3].

Cis-4-hydroxy proline:

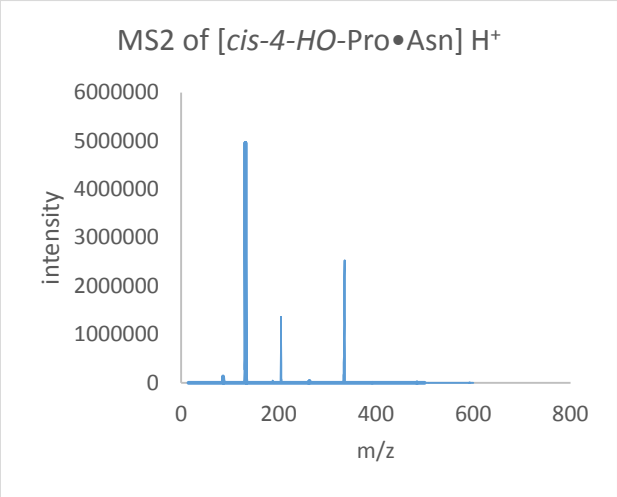


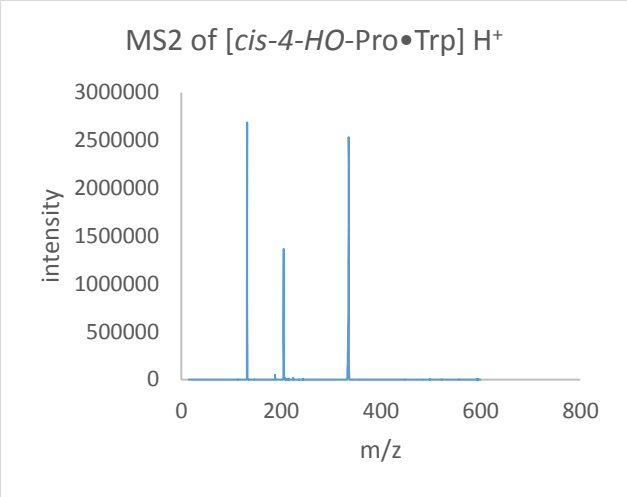


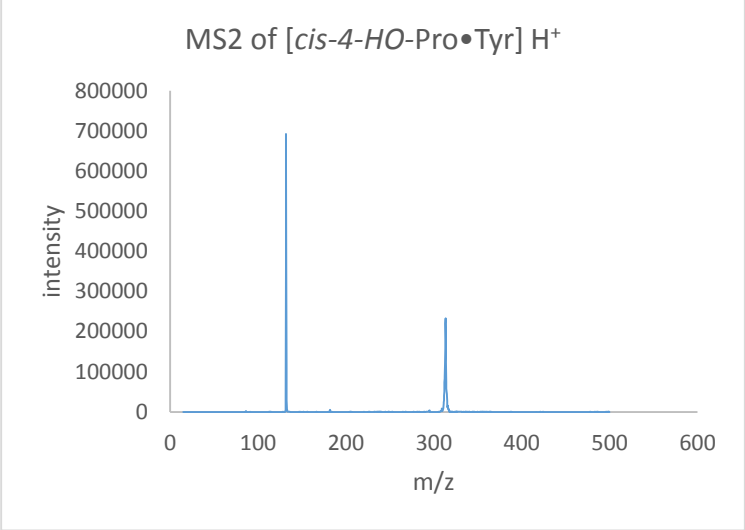


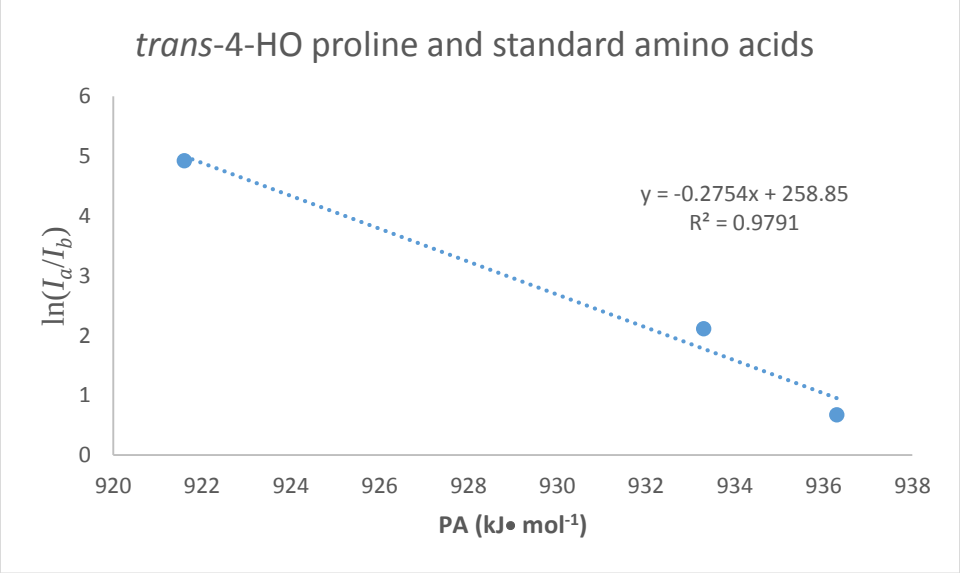


***Trans*-4-hydroxy proline:**









Reference:

[1] S. Mezzache, C. Afonso, C. Pepe, P. Karoyan, F. Fournier, J.-C. Tabet, Rapid Commun. Mass Spectrom. 17 1626 (2003).

[2] C. Afonso, F. Modeste, P.Breton, F. Fournier and J.-C. Tebet, Eur.J. Mass Spectrom. 6, 443-449 (2000).

[3] S. Mezzache, C. Pepe, P. Karoyan, F. Fournier, J.-C. Tabet, Rapid Commun. Mass Spectrom. 19 2279-2283 (2005).

[4] http://store.p212121.com