# NMR SPECTROSCOPY USING A CHIRAL LANTHANIDE SHIFT REAGENT TO DETERMINE THE ENANTIOMERIC PURITY OF NICOTINE AND THE GEOMETRIC PARAMETERS OF LANTHANIDE-NICOTINE COMPLEX

THANH THI THU THUY[1], THAI DUY DUONG[2], PHAM NGOC KHANH[3],
DANG VU LUONG[1], NGUYEN TIEN TAI[1]

[1]*Institute of Chemistry, Vietnam Academy of Science and Technology (VAST)*

[2]*Da Lat University*

[3]*Institute of Natural Product Chemistry, VAST*

## ABSTRACT

*In this paper, we reported a simple method for determining the enantiomeric purity of nicotine extracted from tobacco leaves by $^{13}$C-NMR spectroscopy using a chiral shift reagent Ytterbium(III) tris[3-(trifluoropropylhydroxy-methylene)-l-camphorate] (Yb(tfc)$_3$). The results showed that the studied native nicotine contained 98% S-Nicotine and 2% R-Nicotine. The geometric parameters of Yb(tfc)$_3$-Nicotine complex were calculated by means of well-known McConnell-Robertson equation.*

## I - INTRODUCTION

Nicotine is a highly addictive alkaloid. The primary commercial source of nicotine is extracted from dried tobacco leaves. As a water and lipid soluble substance, nicotine is easy absorbed via respiratory tissues, skin and the gastrointestinal tract. The tar contained in cigarettes increases smoker's risk of lung cancer, emphysema, and bronchial disorders [1]. Like many other natural products such as amino acids, nucleosides, carbohydrates and phospholipids, nicotine consists of enantiomeric molecules with different properties. Natural nicotine is often found in form of S-isomer. It is not the R-nicotine but rather the S-nicotine that increases the locomotors activity [2, 3]. This explains S-enantiomer is the addictive agent in tobacco. Therefore, determining the enantiomeric composition of nicotine is very important to estimate its toxicity.

The use of NMR spectroscopy in the presence of Lanthanide Shift Reagents (LSRs) for direct determination of enantiomeric purity has been studied so far. The aim of our research is to apply this simple method for estimating enantiomeric purity of some selected natural chiral products. In this work, we have performed studies on a natural nicotine sample extracted from tobacco leaves using $^{13}$C-NMR with a chiral lanthanide shift reagent Yb(tfc)$_3$. This method can be applied for the determination of enantiomeric purity of nicotine in some other products such as chewing gums, skin absorption patches, inhalators, nasal sprays…The geometric parameters of the complex consisting of Yb(tfc)$_3$ and nicotine were also calculated for better understanding the influence of lanthanide shift reagents on nicotine's structure.

## II - EXPERIMENTAL

### 1. Material

Nicotine extracted from tobacco leaves was kindly provided by our colleagues at Institute of Chemistry, VAST.

Lanthanide shift reagent (LSR) Ytterbium(III) tris[3-(trifluoropropylhydroxy-methylene)-*l*-camphorate] is obtained from Aldrich.

### 2. NMR experiment

NMR spectra were recorded on a Bruker AVANCE 500 spectrometer. $CDCl_3$ was used as a solvent and TMS was used as an internal standard. For quantitative estimation, it is important to prepare substrate and reagent solutions. In this experiment, the substrate concentration was kept constant and reagent concentration varied. Nicotine sample was dissolved in $CDCl_3$ then an accurately weighted amount of $Yb(tfc)_3$ was added. More details of sample preparation for NMR experiment can be found in our previous paper [4].

## III - RESULT AND DISSCUSION

The chemical formula of nicotine is $C_{10}H_{14}N_2$. The structures of 2 enantiomers are presented in figure 1.

The chemical structure of nicotine was confirmed by ^{1}H, ^{13}C-NMR. Chemical shift assignments are listed in table 1.
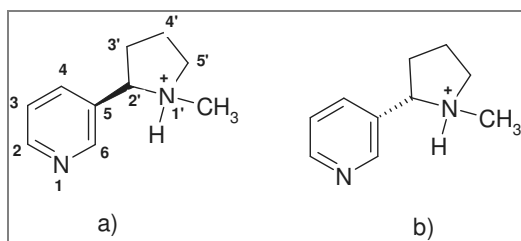


*Figure 1*: Chemical structure of nicotine a) R-enantiomer, b) S-enantiomer

Lanthanide shift reagent (LSR) used in this work is Ytterbium(III) tris[3-(trifluoro-propylhydroxymethylene)-l-camphorate] known as $Yb(tfc)_3$.

*Table 1*: Chemical shift (ppm) assignments of ^{1}H and ^{13}C NMR spectra

| Position | [1]H | [13]C |
|---|---|---|
| 2 | 8.5 | 148.2 |
| 3 | 7.2 | 123.1 |
| 4 | 7.7 | 138.4 |
| 5 | | 134.4 |
| 6 | 8.6 | 149.1 |
| 2' | 3.1 | 68.4 |
| 3' | 2.2 and 2.0 | 34.9 |
| 4' | 1.9 and 1.8 | 22.3 |
| 5' | 3.2 and 2.3 | 56.6 |
| $CH_3$ | 2.2 | 40.0 |

By adding $Yb(tfc)_3$ into the solution of nicotine, a complex of these components is formed. Because $Yb(tfc)_3$ is a weak Lewis acid, it tends to coordinate with a Lewis base with rich electron density. In nicotine molecule, there are two nitrogen atoms, which can coordinate with the LSR. Comparing the base strength of two nitrogen centers, the nitrogen in pyridin ring has higher electron density so $Yb(tfc)_3$ coordinates to this nitrogen.

By adding $Yb(tfc)_3$, the proton signals in ^{1}H-NMR spectra of nicotine were strongly broadened. So, the quantitative analysis of the impurity was very difficult.

In order to calculate an enantiomer excess, ^{13}C-NMR was used in the presence of $Yb(tfc)_3$. Several samples of $Yb(tfc)_3$ and nicotine with different molar ratios were prepared in order to find out the optimal condition to get a best separation of enantiomeric signals. The result showed that the more $Yb(tfc)_3$/nicotine molar ratio increases, the more signals shift to downfield (Figure 2). It can be explained that LSRs act like an additional magnetic field in the sample and dramatically change the chemical shifts of the signals. In the case of $Yb(tfc)_3$,

since Yb is a paramagnetic metal, it has a tendency to shift the signals to downfield.

Using the Yb(tfc)$_3$/Nicotine molar ratio of 0.3 : 1.0, we obtained the best separation of 2 nicotine enantiomers in NMR spectra, by which all signals of aliphatic enantiomeric carbons were separated and assigned (figures 2&3). Determination of enantiomeric ratio was performed at the C2' (chiral center) resonance and this ratio can be calculated by integration values of the signals of C2'(+) and (-).

The result showed the enantiomeric ratio was 1:0.02 approximately. The optical purity or the enantiomeric excess (ee%) of a sample can be determined as follows:

*Optical purity = % enantiomeric excess = % enantiomer1 - % enantiomer2.*

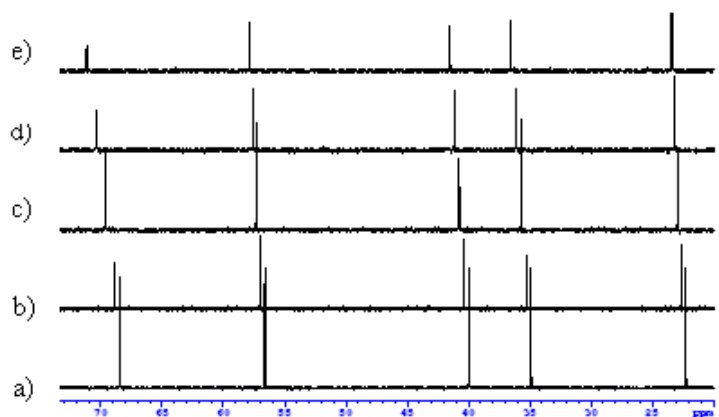So, the optical purity of studied native S-nicotine is 96%.



*Figure 2*: $^{13}$C NMR spectra (aliphatic carbon region)
a) without Yb(tfc)$_3$, b) Yb(tfc)$_3$/Nicotine molar ratio 0.1 : 1.0, c) 0.2: 1.0, d) 0.3 : 1.0, e) 0.35: 1.0
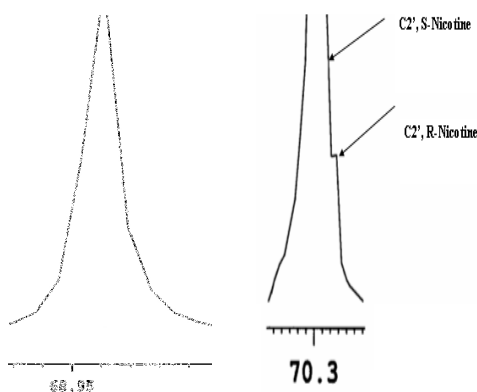


*Figure 3*: Expanded areas of C2' signal without Yb(tfc)$_3$ and with Yb(tfc)$_3$/nicotine molar ratio 0.3:1.0

The fact that chemical shifts of carbon atoms of nicotine is proportional to the ratio between Yb(tfc)$_3$ and nicotine as shown in figure 4 and some geometric parameters of Yb(tfc)3-Nicotine complex can be estimated using these curves. From the slope of each line (figure 4b, an example for C2'), spatial distance (paramagnetic distance) between Yb ion (paramagnetic center) and appropriate carbon atom on the complex can be calculated by well-known McConnell-Robertson equation

$$LIS = k\frac{(3\cos^2\theta - 1)}{r^3} \qquad (1)$$

where LIS is lanthanide induce-shift defined by difference of chemical shifts with and without lanthanide shift reagent, $k$ is a constant depended on lanthanide-substrate complex, $r$ is distance between the lanthanide ion and the

nuclear being observed, and $\theta$ is angle between the principle magnetic dipolar axis of the complex and the distance vector as defined in Figure 5 [5, 6].

In the case of chiral carbon C2', the equation is y = 0.64x + 67.62 (2), with the value k = 1.38 for Yb-pyridine complex [7] and LIS value obtained from the NMR spectra. By combining (1) and (2), the spatial distance $r$ and the angle $\theta$ were calculated as 1.27 Å and 46.2° respectively.

Following the same manner, $r$ and $\theta$ values were estimated for all aliphatic carbon atoms and the result are summarized in table 2.
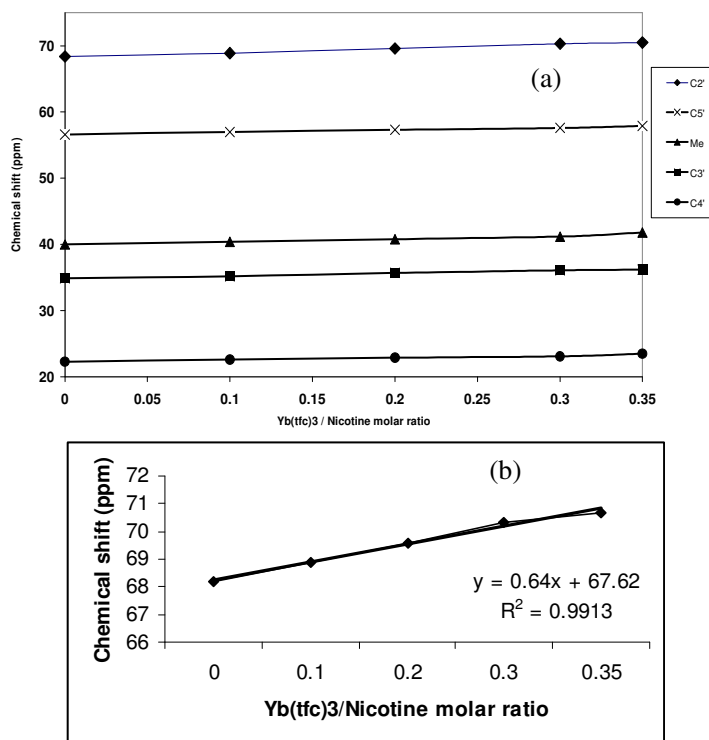


Figure 4: Dependence of chemical shifts of 5 aliphatic carbons (a) and
C2' (b) on molar ratio between Yb(tfc)₃ and nicotine

Table 2: Geometric parameters of Yb(tfc)3-Nicotine complex

| Carbon | $r$, Å | $\Theta, o$ |
|--------|--------|------|
| 2' | 1.27 | 46.2 |
| 3' | 1.52 | 47.3 |
| 4' | 1.65 | 48.4 |
| 5' | 1.66 | 39.2 |
| CH₃ | 1.46 | 50.3 |

The Yb(tfc)3-Nicotine complex is shown in figure 5.
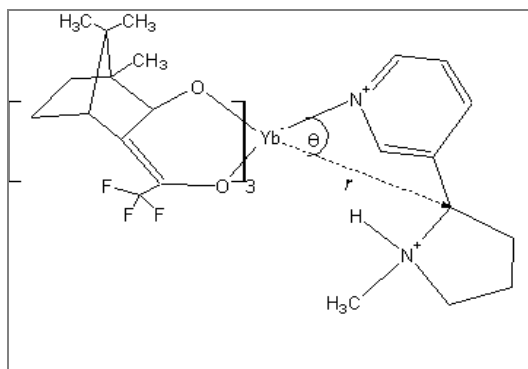


Figure 5: Yb(tfc)₃ – Nicotine complex

656

## IV - CONCLUSION

The enantiomeric purity of studied native nicotine can be quickly and reliably determined by NMR method with reagent Yb(tfc)$_3$. The results showed that $^{13}$C-NMR method can detect few percents of the minor enantiomer of nicotine. For studied sample, S-nicotine was found to be 96% enantiomerically pure. Formation of Yb(tfc)$_3$-Nicotine complex in the shift reagent experiments was proposed and its geometric parameters were calculated.

Our results of using NMR spectroscopy in the presence of LSRs on determining not only the enantiomeric excess of some natural products (nicotine and linalool [4]) but also the geometric parameters of LSR-substance complex would be a good beginning for further investigation in this field.

*Corresponding author*: **Thanh Thi Thu Thuy**
Institute of Chemistry, VAST
18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam.

## REFERENCES

1. http://toxipedia.org/conf/display/toxipedia/Nicotine

2. Andrew J Grottick et al. Neuropsychopharmacology, Reversal of a Vigilance Decrement in the Aged Rat by Subtype-Selective Nicotinic Ligands, **28**, 880 - 887 (2003).

3. Joshua T. Ayers et al. The AAPS Journal, **7** (3) Article 75, (2005).

4. Thanh Thi Thu Thuy, Pham Ngoc Khanh, Dang Vu Luong. Journal of Science and Technology **45** (3A) (2007) 63-78.

5. http://web.mit.edu/5.32/www/Appendix_2_Chiral_GC_03.pdf.

6. E. P. Serebryakov et al, Russian Chemical Bulletin, **52** (3), 734 - 739 (2003).

7. Lanthanide shift reagents in stereochemical analysis, edited by Terence C. Morrill, VCH publisher (1986).