

Variable-temperature NMR studies of 2-(pyridin-2-yl)-1H-benzo[d]imidazole

Anchi Yeh^{1*}, Chi-Yu Shih¹, Lieh-Li Lin¹, Shung-Jim Yang² and Cheng-Tung Chang³

¹Department of Chemical Engineering, Chengshiu University, Kaohsiung, Taiwan, R.O.C. ²Department of Chemical Engineering, Vanung University, Chung-Li, Taiwan, R.O.C. ³Department of Products, Taiwan Textile Research Institute, Taipei, Taiwan, R.O.C

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Abstract Method of variable-temperature of ¹H-NMR as well as ¹³C-NMR can be used to determine the location of coalescence temperature on the NMR spectrum. To solve the Gutowsky-Holm equation, the activation rotation-energy can be computed. Hence, for the synthesized compound of 2-(pyridin-2-yl)-1H-benzo[d]imidazole, we observed the coalescence temperature of H₉ and H₁₀ located at 283K on ¹H-NMR spectrum but the calculated activation rotation-energy was 12.69kcal/mole. Meanwhile, the coalescence temperature of H₈ and H₁₁ was at 303K on ¹H-NMR spectrum but the activation rotation-energy was 15.20kcal/mole. Additionally, the coalescence temperature of C₉ and C₁₀ on ¹³C-NMR spectrum was also at 303K as well as the calculated activation rotation-energy was 14.4kcal/mole. [Life Science Journal. 2009; 6(4): 1– 4] (ISSN: 1097 – 8135)

Key words: NMR spectrum, Gutowsky-Holm equation

1. Introduction

Natural anti-cancer compounds, for instance, berberine, cryptolepine and camptothecin are basically poly-ring structured. Neidle et al. (2003) reported the mechanism of the anti-cancer for some of poly-ring compounds may have the stability if combining with DNA, such as Imidazole ring. Compounds with bisbenzimidazole group can be bonded to DNA's minor groove [1-5]. The temperature and the rotation energy of the molecules can be observed through Variable-temperature NMR [6]. For molecule planar structure, when it does not free to rotate at the temperature below the bearing range of DNA, the structure can be easier bonding to DNA. This report demonstrates the temperature and energy of 2-(pyridin-2-yl)-1H-benzo[d]imidazole in rotation via

using variable temperature of ¹H-NMR and ¹³C-NMR.

2. Material and Methods

Picolinic acid (20 mmol, 3.4634 g), 2,3-diaminonaphthalene (20 mmol, 3.1640 g), and polyphosphoric acid (PPA, 20ml) were added to an empty flask. The mixture was heated at 200°C for 4 hours. After cooling to room temperature, de-ionized water (500ml) shall be added with stirring. The solid was collected by suction filtration and purified by column chromatography with ethyl acetate/hexane (1:1). Colorless crystal of 2-(pyridin-2-yl)-1H-benzo[d]imidazole was obtained in 80% yield. All NMR works were done by using 500 MHz and DMF-d⁷.

*Correspondence: acyeh@csu.ecu.tw, Dr. Yeh, Anchi

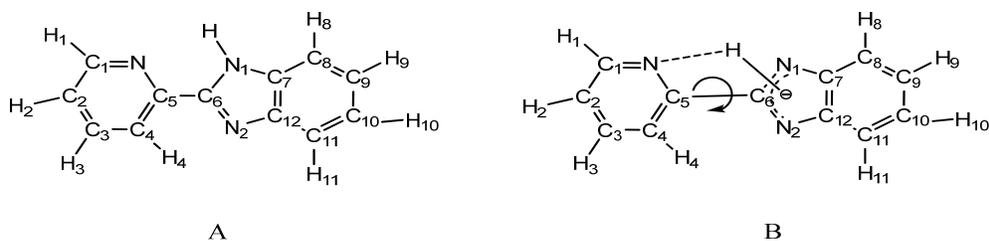


Figure 1. (A) Structure of 2-(pyridin-2-yl)-1H-benzo[d]imidazole, (B) Rotation of 2-(pyridin-2-yl)-1H-benzo[d]imidazole

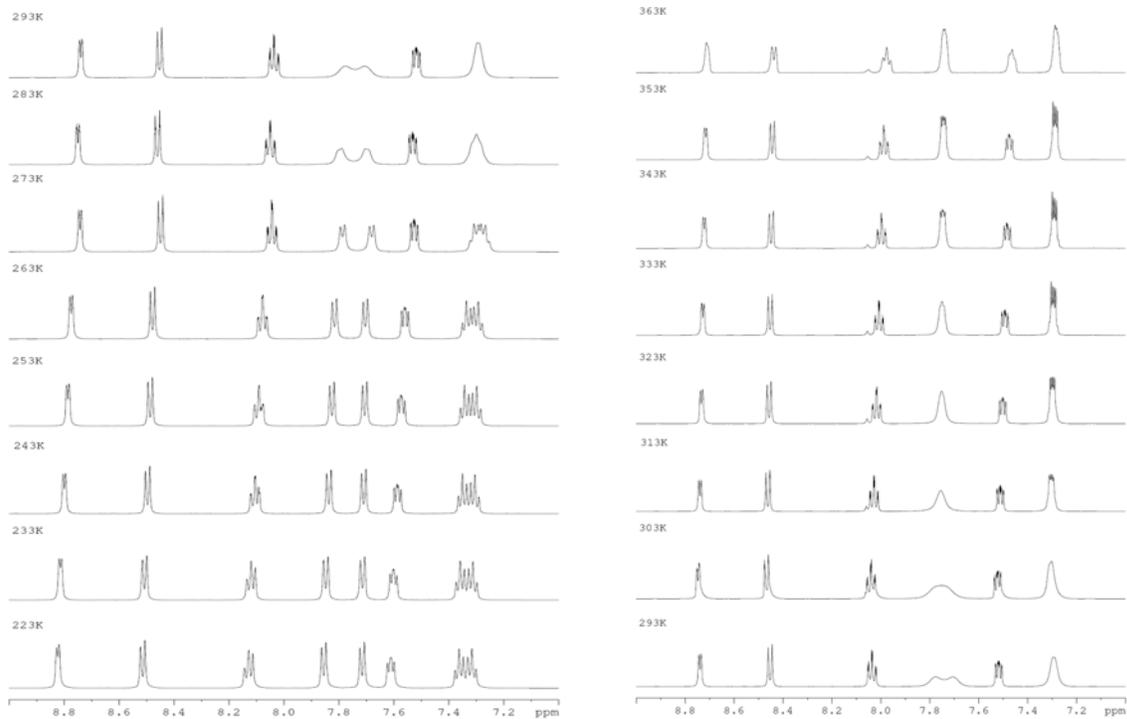


Figure 2. Variable-temperature ^1H -NMR of BIP. (Left) low temperature, (Right) high

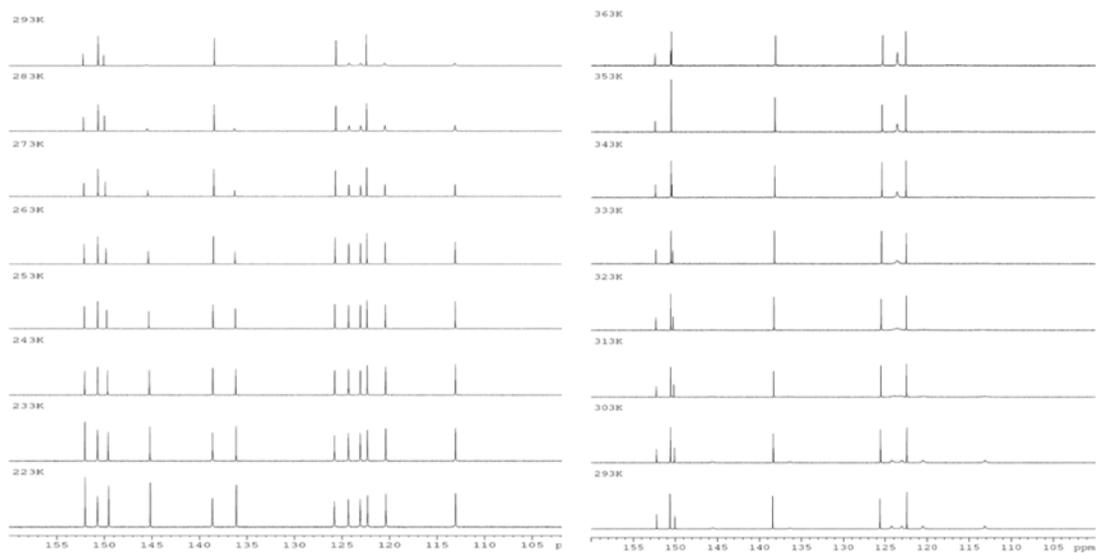


Figure 3. Variable-temperature ^{13}C -NMR of BIP. (Left) low temperature, (Right) high temperature

3. Results and Discussion

3-1 ¹H-NMR spectrum analysis

The C₅-C₆ bond is a single bond of 2-(pyridin-2-yl)-1H-benzo[d]imidazole. At the low temperature, single bond would not free rotate but compose stable conformational molecule (Figure 1). In Figure 2 (in left), it depicts that the characteristic of variable-temperature of ¹H-NMR at low temperature. When the temperature below 263K, 2-(pyridin-2-yl)-1H-benzo[d]imidazole was a stable conformational molecule. The spectra of ¹H-NMR is listed as follows:

8.82ppm (H₁,d), 8.51ppm (H₄,d), 8.11ppm (H₃,dd), 7.86 ppm (H₁₁,d), 7.71ppm (H₈,d), 7.60ppm, (H₂,dd), 7.38 ppm, (H₁₀,dd), 7.31ppm (H₉,dd).

In Benzylimidazole, H₁₁ and H₁₀ were located at down field but H₈ and H₉ at up field for N₁ is an electron-donating amine group and N₂ is electron-withdrawing imine group. So H₈ and H₉ have relative high electron density compares to H₁₁ and H₁₀ which located at down field.

As the temperature rises to 283K, the single bond of C₅-C₆ starts to rotate slowly. The activation rotation-energy is strong enough to make the chemical-shift of H₉ and H₁₀ being overlapped and leads to a phenomenon of broad (Figure 2). According to Gutowsky-Holm

$$\left(\Delta G = 0.00457T_c \left(9.97 + \log \left(\frac{T_c}{\Delta \delta} \right) \right) \right), \text{ coalescence}$$

temperature (T_c) [7] is the combination temperature of 2 peaks where $\Delta \delta$ is the difference between the two peaks as the chemical-shift. The coalescence temperature of H₉ and H₁₀ is located at 283K. Its activation rotation-energy is 12.69 kcal/mole. However, at this temperature, H₈ and H₁₁ can not be distinguished.

As the temperature rises to 303K, the speed of

rotation starts to increase. It makes the chemical-shift of H₈ and H₁₁ being overlapped. (As Figure 2 (Right) is high variable temperature of ¹H-NMR). Here the activation energy of rotation is 15.2kcal/mole. So 303K should be the coalescence temperature of H₈ and H₁₁. H₉₋₁₀ and H₈₋₁₁ intensity would be enhanced as the temperature goes up. The H of pyridine group may not even be affected into change on benzylimidazole group at 353K. When the temperature goes up to 363K, the broad are appeared of pyridine group. The observation results should be affected on the fast rotation of Benzylimidazole group. Because of the limited NMR machine, we are only to be able to work up to 363K.

3-2 ¹³C-NMR spectrum analysis

A stable conformational molecule has been formed at low temperature. Figure 3 (Left) shows the ¹³C-NMR spectrum at low variable temperature. Temperature control is from 223K to 293K. When the temperature is 223K, the spectrum of ¹³C-NMR is as follows:
151.9ppm(C₅), 150.5ppm(C₁), 149.2ppm(C₆),
144.9ppm(C₁₂), 138.3ppm(C₃), 135.8ppm(C₇),
125.5ppm(C₄), 124.0 ppm(C₁₀), 122.8 ppm(C₉),
122.0ppm(C₂), 120.1 ppm(C₁₁), 112.8ppm(C₈).

As in Figure 3 (Left), C₁₁ and C₁₀ are in down field but C₈ and C₉ are in up field. As the temperature rises from 223K to 293K, the intensity of C (C₈, C₁₁, C₉, C₁₀, C₇, C₁₂, C₆) is reducing in benzylimidazole group. However, the reducing rate of C₆ is relatively less than other locations of C. The effect from rotation to the C₆ is thus smaller because it is the pivot point of rotation. From the spectrum of high variable temperature of ¹³C-NMR (As figure 3 Right), we could know the coalescence temperature is located at 313K of benzylimidazole group. By solving Gutowsky- Holm equation, rotation of C₉ and C₁₀ in benzylimidazole group is about 14.4kcal/mole. Moreover, the intensity peaks of C₈, C₁₁, C₇, C₁₂ start getting broad at 293K, and the peak

disappeared at 313K. Even when the temperature goes up to 363K, the peaks of C₈, C₁₁, C₇, C₁₂ are all invisible. The chemical-shift of C₈, C₁₁, C₇, C₁₂ apart too far to be combined into a new peak at 363K. In contrary, the chemical-shift of C₉, C₁₀ is less apart from each other. Therefore, a new peak has been combined at 313K and its intensity is increased by raising temperature. The chemical-shift of C₆ would be moved to downfield when the density of peak was affected by raising temperature. The chemical-shift of C₆ is the same as C₁ at 353K, but being more downfield at 363K. The N of pyridine and H of imidazole ring would be made strong interaction with each other which may cause part of N carrying with positive charges. However, the electron density of C₆ increases for the part of N₁-C₆-N₂ carrying with negative

charge and causing chemical-shift of C₆ at up field. But the rotation rate goes up as the temperature rising and some parts of negative charges fell over slowly on N₁ and N₂. C₆ carrying with negative charge can turn into quaternary-C from tertiary-C. Meanwhile, the chemical-shift of C₆ would have moved toward downfield.

4. Conclusions

The 2-(pyridin-2-yl)-1H-benzo[d]imidazole was synthesized and we found the lowest coalescence temperature under 283K and activation rotation-energy was 12.69kcal/mole. The investigation turns out that the compounds 2-(pyridin-2-yl)-1H-benzo[d]imidazole potentially at 283K can be easy bonded with DNA.

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