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Note

Direct observation of the unstable intermediates in radical addition reaction by using an interfacing microchip combined with an NMR

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Direct observation of the unstable intermediate in the radical addition reaction of the oxime ether 1 mediated by triethylborane (Et_3B) is described using ^1H and ^{11}B micro channeled cell for synthesis monitoring (MICCS), which was recently developed as an interfacing microchip for NMR. It was possible that the signal of the intermediate was observed as a result of using MICCS technique with a standard NMR instrument. This result supports the structure of the intermediate analyzed by diffusion-ordered spectroscopy (DOSY) NMR method in a previous paper. The procedure of micro channeled cell for synthesis monitoring-nuclear magnetic resonance (MICCS-NMR) was much easier than that of DOSY method. It was proven that it could be applied to the reaction in an anhydrous condition. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS: Nuclear magnetic resonance; MIcro channeled cell for synthesis monitoring; ^{11}B; intermediate; oxime ether; Et_3B; radical reaction

INTRODUCTION

Nuclear magnetic resonance (NMR) spectroscopy is used as a routine method to determine the chemical structure of organic compounds.[1] Some researchers have requirements to observe the progress of chemical reactions of organic compounds. Analysis of reaction intermediates plays an important role to elucidate the progress of chemical reactions. As for a number of reactions, the structure analysis by NMR is difficult because most of the reaction intermediates are unstable.

We have reported the elucidation of the intermediate structure in the radical reaction of the oxime ether **1** mediated by triethylborane (Et_3B) using 2D- and 3D-DOSY method.[2] In this study, the intermediate was separately observed in the reaction mixture. However, the preparation of reagent solutions was complex. The reagent solutions were directly mixed in an NMR sample tube under argon gas.

The direct mixing of chemical reagents in an NMR sample tube was carried out to observe the progress of chemical reactions and the unstable intermediates. In using this procedure, however the delay time between the reagents introduction and the NMR measurement probably causes problems in observing the short-term reaction progress and the short-lived intermediates.

McGarrity *et al.* have reported rapid-injection NMR methodology to observe the reactive intermediates in chemical syntheses.[3,4] The observation of reactive intermediates with half-lives of around 100 ms at a concentration level below 10^{-1} mol/l was possible with this methodology, and it might be effective for these kinds of applications. A demerit of this injection system was that it requires specially assembled equipment on the NMR instrument. This hampered normal organic chemists to easily adopt the methodology.

Kakuta *et al.* have reported a study regarding changes in protein conformation using the combined technique with a static mixer and a microcoil-based microfluidic NMR probe.[5] This technique has the advantage to detect small volume of samples. However, it is necessary to use a specially assembled NMR probe for this study.

We have developed a new microchip, which was able to monitor a reaction process by combination with a standard NMR instrument.[6] This device was named MICCS. MICCS-NMR has several merits in the field of organic chemistry.

1. The mixing parts and the detection part are very close.
2. Three reagent solutions can be introduced at same time.

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3. The mixing ratios of reagent solutions and the reaction temperature were easily controlled by adjusting the flow rates of reagent delivery system and probe temperature.
4. When the reaction conditions were stable, the reaction mixture of stable state was continuously delivered from the mixing part to the detection part.

According to these merits, this technique has the following advantages over other methods.

1. Detection of short-lived intermediates is possible.
2. Two-step chemical reactions can be observed.
3. Examination of the reaction conditions is very easy by a real-time monitoring with NMR.
4. Long-time integration to observe small amount of products and the unstable intermediates would be possible.

From these characteristics, the direct observation of the reaction progress, which is the changes in the raw materials, unstable intermediates and products, would be possible by the measurement of the nucleus that has short relaxation time (e.g. ^{11}B). In this paper, ^{1}H and ^{11}B MICCS-NMR were used to observe the intermediate in this reaction to evaluate the applicable fields of MICCS-NMR technology. The results were compared to those of DOSY method of the previous paper.[2]

EXPERIMENTAL

^{1}H and ^{11}B MICCS-NMR were carried out using a JEOL JNM-ECA-500 (Tokyo, Japan) equipped with a TH5FG probe. WET[7] was used for solvent suppression in ^{1}H MICCS-NMR and the number of scans were 16, The parameters for ^{1}H measurement were as follows: The 90° pulse width was 13.5 µs, repetition time was about 10.5 s (relaxation delay was 5.0 s, acquisition time was about 5.5 s). For ^{11}B MICCS-NMR, single pulse with ^{1}H decoupling was used and number of scans were 32. The parameter of the 90° pulse width was 20 µs, of the repetition time was about 1.0 s (relaxation delay was about 0.5 s, acquisition time was about 0.5 s). Chemical solutions were delivered into micro-channels using syringe pumps (MD1001 with the BS-MD1200, Bioanalytical Systems Inc., West Lafayette, IN). Syringes and MICCS were coupled with fused-silica capillary tubings (150 µm ID × 375 µm OD) purchased from GL Science (Tokyo, Japan).

Acetone-d_6, purchased from Sigma-Aldrich Inc. (St Louis, MO), was used as an external lock. It was injected into the gap between the outside wall of MICCS and the inside wall of the 5-mm O.D. sample tube (MICCS was inserted into the sample tube.). CH_2Cl_2 and methanol were purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). CH_2Cl_2 was dehydrated grade. The design of flow route of MICCS and the schematic diagram of MICCS-NMR are shown in Fig. 1.

CH_2Cl_2 solutions of oxime ether **1** (1 mol/l) and triethylborane (**2**) (1 mol/l) were introduced into the two inlet ports of MICCS by syringe pumps. Both solutions were mixed at the 1st Y-shaped channel in Fig. 1, and a radical addition reaction occurred. CH_2Cl_2 solution of methanol was introduced into the 3rd inlet port and was mixed with reaction mixture at the 2nd Y-shaped channel to quench the reaction. The reaction temperature was not controlled (room temperature), because temperature control is not necessary in this reaction.[8] The flow rates of the above three solutions were changed while consecutive measurements were taken as shown in Table 1. The flow-rate ratio of **1**/**2** was consecutively changed from 10/0.5 to 1.0/5.0 µl/min. At the time, when flow-rate ratio of **1**/**2** is 5.0/5.0 µl/min, the intensity of the intermediate

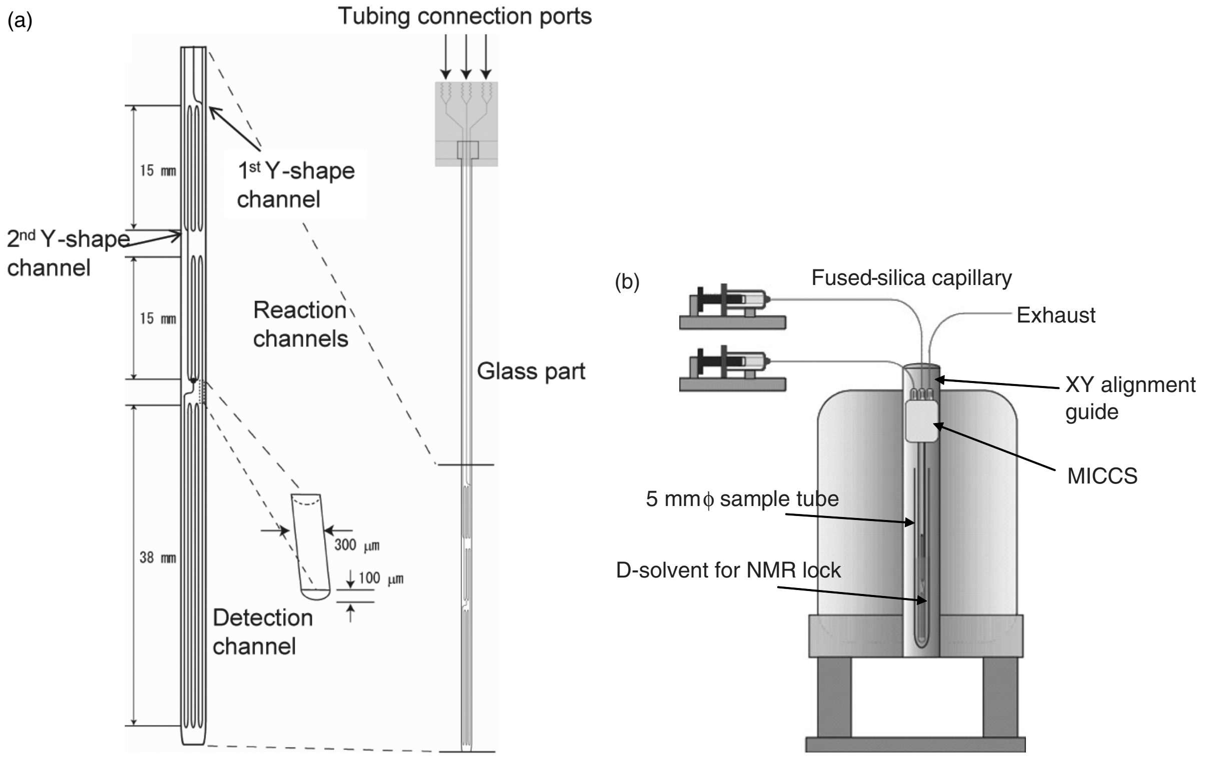

Figure 1. (a) The design of flow route of MICCS and (b) the schematic diagram of MICCS-NMR.

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was expected to be maximum. After that, by adding CH_2Cl_2 solution of methanol, it was expected that the intermediate would change to the final product.

On the basis of our prior work, the pathway of this reaction was proposed in Scheme 1. Structure of the intermediate **4** was proposed by 2D- and 3D-DOSY method as described in the literature.[2]

RESULT AND DISCUSSION

A 2D-display of the ^{1}H-NMR spectra is shown in Fig. 2. Sliced spectrum at the flow rates of **1** and **2** of 5 μl/ min each and the expected structure of the intermediate **4** are shown in Fig. 3. Table 2 shows the assignment of the ^{1}H signal for the structure **4**. The spectrum shown in Fig. 3 is consistent with the structure of the intermediate for **4**, as reported in the literature.[2] Three characteristic signals of **4** were observed at δ 1.8, δ 4.0 and δ 4.5 ppm. In Fig. 2, the spectra of **4**, which contain these three signals, were clearly observed at the spectra # 3–7. And then, by adding methanol, these three signals disappeared and different signals at δ 1.4 and δ 4.5 ppm were observed at the spectra # 8–11. These spectra were consistent with the structure of the final product **3**. We could obtain not only the structural information of **4** but also that of **3** at single analysis. Therefore, the reaction progress, which is changes in the raw materials, the unstable intermediates andthe products, was observed by using MICCS-NMR technique. Complex signals at δ 0.5 − 1.0 ppm corresponded to the ethyl group bonded to boron element. Since MICCS-NMR gives high-resolution spectra as described in the literature,[6] the signals of ethyl group of the raw material **2** and the intermediate **4** could be distinguished.

Table 1. The flow rates of the three reagent solutions in ^{1}H NMR experiment

Spectrum no.	Oxime ether 1 (μl/ min)	2 (μl/ min)	CH_3OH (μl/ min)
1	10.0	0.5	0.0
2	9.0	1.0	0.0
3	8.0	2.0	0.0
4	7.0	3.0	0.0
5	6.0	4.0	0.0
6	5.0	5.0	0.0
7	5.0	5.0	0.5
8	4.5	4.5	1.0
9	4.0	4.0	2.0
10	3.5	3.5	3.5
11	2.0	4.0	2.0
12	1.0	5.0	5.0

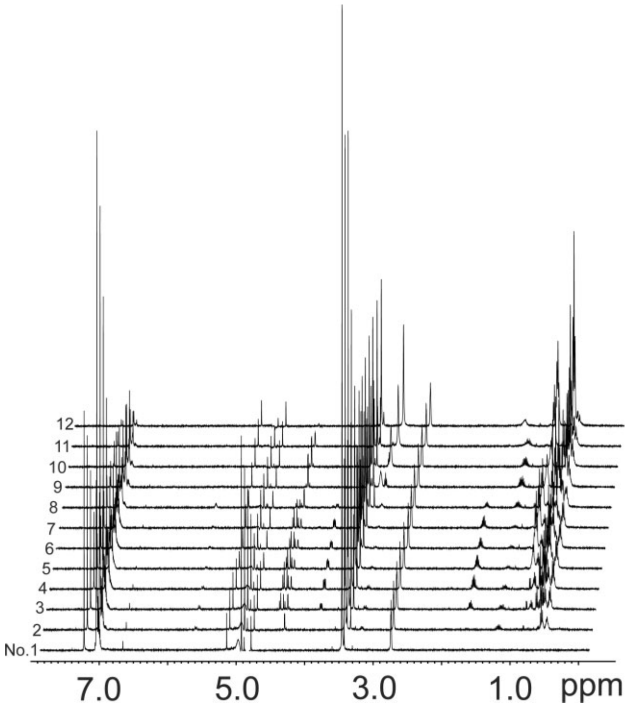
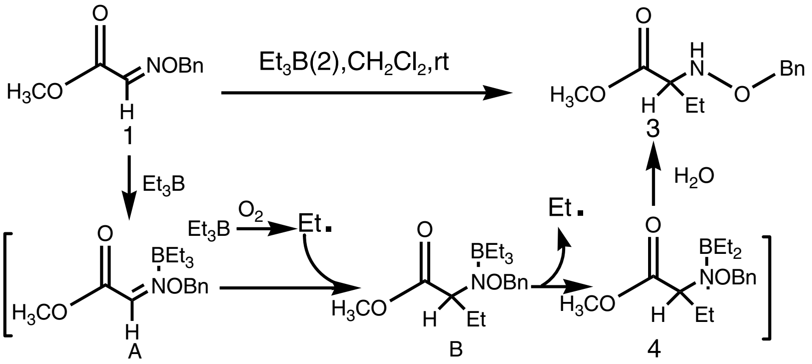


Figure 2. 2D-display of the ^{1}H-NMR spectra.

Table 2. The ^{1}H-NMR assignment of the intermediate **4**

No.	ppm
1	3.97(1H,dd,$J = 8.9,6.1$Hz)
2	1.79(2H, m)
3	0.72(3H)
1′	4.51(1H,d,$J = 9.8$) and 4.57(1H,d,$J = 9.8$)
1″	0.93(3H) and 0.76(3H)
2″	0.80(3H) and 0.72(3H)
OCH_3	3.58(3H,s)
Phenyl	7.12–7.18(5H)



Scheme 1. Proposed the mechanism on the radical reaction of Et_3B and oxime ether 1.

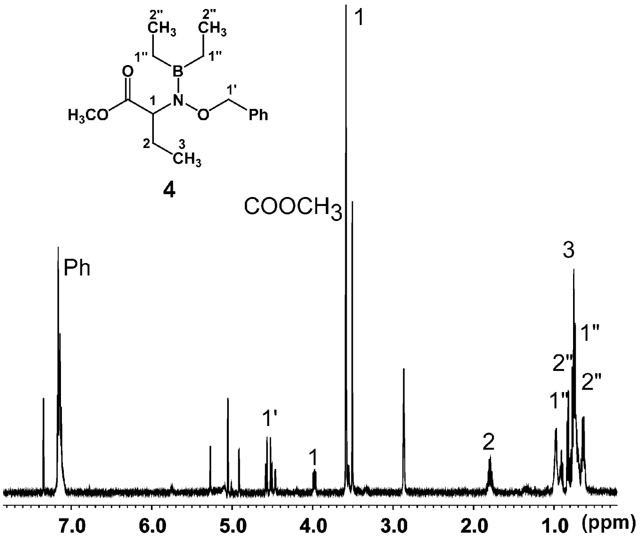


Figure 3. Sliced ^{1}H-NMR spectrum of the intermediate 4.

A 2D-display of the ^{11}B-NMR spectra was shown in Fig. 4. The total flow rate of the two solutions was 4.0 μl/ min. The flow-rate ratio of the two solutions was changed during measurement (v (**1**)/v (**2**); 4/0, 3/1, 2.5/1.5, 2.0/2.0, 1.5/2.5, 1/3 and 0/4). Methanol was not used in this experiment. The intensity of the signals at δ 86.6 ppm assigned to triethylborane (**2**) was increased with increasing the flow rate. The intensity of the signals at δ 53.9 ppm associated to **4** was maximum at the flow rates of 2 μl/ min each for **1** and **2**. This chemical shift was also consistent with the structure of **4**. The signals at δ 60.0 ppm would be assigned to the decomposed compound of **2**. Because of the merits of MICCS-NMR (# 1 and # 4) as described above, ^{11}B-NMR analysis was possible in this study. On the other hand, ^{11}B-NMR could not analyze in the DOSY method, since ^{11}B nucleus has short relaxation time.

The results using MICCS method were obtained much easier than DOSY method. In DOSY method, the reagent solutions were carefully prepared and mixed in an NMR sample tube under argon gas to avoid the decomposition of the intermediate. On the other hand, in MICCS method, the reagent solutions were prepared under the atmospheric condition. And the required time for MICCS-NMR measurement was shorter than that for DOSY. This device can be used under the anhydrous condition in the organic reaction. Moreover, the structural information of the intermediate could be obtained even in the multiple nuclear NMR.

CONCLUSION

The direct observation of the unstable intermediate in radical addition reaction was succeeded using a ^{1}H- and ^{11}B MICCS-NMR. These results support the structure **4** for the intermediate elucidated by 2D- and 3D-DOSY method as described in previous paper. The reagent preparation of MICCS-NMR was much easier compared to that of DOSY. MICCS-NMR could be expected to be a commercially available tool to analyze the reaction intermediates and the reaction progress in a chemical reaction.

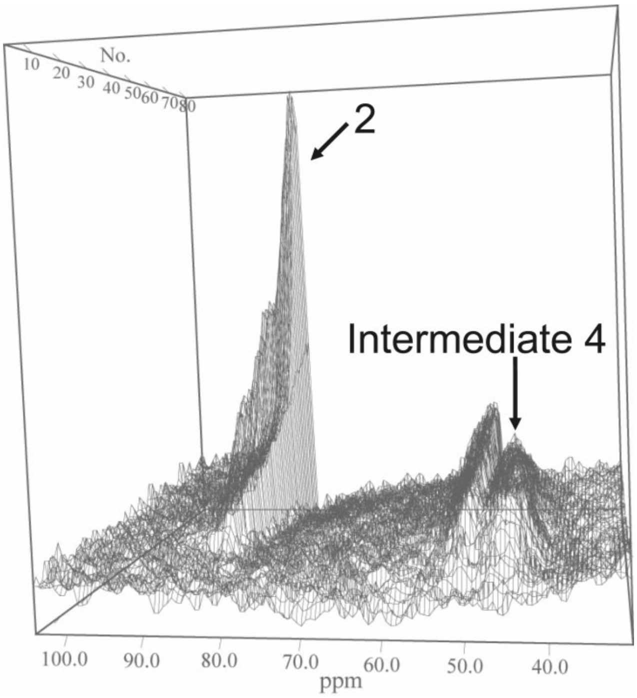


Figure 4. 2D-display of the ^{11}B-NMR spectra.

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