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Mechanism for the direct synthesis of tryptophan from indole and serine: a useful NMR technique for the detection of a reactive intermediate in the reaction mixture

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The reaction mechanism for the biomimetic synthesis of tryptophan from indole and serine in the presence of Ac_2O in AcOH was investigated. Although the time-course ^{1}H-NMR spectra of the reaction of 5-methoxyindole with *N*-acetylserine were measured in the presence of $(CD_3CO)_2O$ in CD_3CO_2D, the reactive intermediate could not be detected. This reaction was conducted without 5-methoxyindole in order to elucidate the reactive intermediate, but the intermediate could not be isolated from the reaction mixture. Since the intermediate would be expected to have a very short life time, and therefore be very difficult to detect by conventional analytical methods, the structure of the intermediate was elucidated using a 2D-NMR technique, diffusion-ordered spectroscopy (DOSY). Two intermediates were detected and confirmed to be 2-methyl-4-methyleneoxazol-5(4H)-one and 2-methyl-4-hydroxymethyloxazol-5(4H)-one. The present results demonstrated that DOSY is a powerful tool for the detection of unstable intermediates.

Supporting information may be found in the online version of this article.

Keywords: NMR; tryptophan; synthesis; indole; serine; biosynthesis; mechanism; DOSY

Introduction

Tryptophan and its analogs have interesting biological activities. Numerous synthetic methods for obtaining racemic and optically active tryptophan derivatives have been reported, and the development of efficient synthetic methods is of interest to many synthetic chemists. Of these methods, biomimetic-type syntheses are very attractive because one-step synthesis from serine and indole is not only practical but also mechanistically interesting. In this approach, an S_N2-type reaction occurs directly at the C_3-position of the indole with an inactivated hydroxyl group attached at the carbon atom of L-serine. Although there have been several reports[1] of the direct synthesis of tryptophan from indole and serine or their analogs, the yields were unsatisfactory (3%), so this approach is not practical.

During the course of our investigation of the total synthesis of ergot alkaloids, we developed[2] a two-step synthesis of optically pure (***S***)-4-bromotryptophan (**4**) from 4-bromoindole (**1**) and *dl*-serine (**2**) involving the kinetic resolution of acylase (Scheme 1). The one-step formation of *N*-acetyl-4-bromotryptophan (**3**) was the first example of the practical biomimetic synthesis of tryptophan. Yamada's group[3] applied this method to the synthesis of optically pure 7-bromotryptophan. Recently, Sanderson *et al*. also reported[4] the synthesis of optically active tryptophan analogs having various substituents on the indole ring. However, the drawback of this reaction is the formation of racemers even if optically active L-serine is used. Therefore, in order to develop an asymmetric synthesis strategy, a precise understanding of the mechanism is indispensable.

We previously proposed[2] a plausible mechanism, shown in Scheme 2. An α,β-unsaturated azlactone, 2-methyl-4-methyleneoxazol-5(4H)-one (**9**) is a key intermediate which acts as a reactive Michael acceptor towards 4-bomroindole (**1**). The intermediate (**9**) formed from serine azlactone (**7**) or 2-acetamideacrylate (**8**) *via* *N*-acetylserine (**6**). It was reported[5] that the reaction of **8** with indole gave *N*-acetyltryptophane in 55% yield. In contrast, Sanderson proposed[4] another intermediate, cyclic oxonium ion (**11**), which is attacked by indole in an S_N2-type reaction.

Herein we report a detailed investigation of the mechanism of this reaction using a new 2D-NMR technique, diffusion-ordered spectroscopy (DOSY). DOSY is a useful method for obtaining structural information on unstable intermediates in the reaction mixture.

Results and Discussion

The reactions shown in Scheme 2 proceed through *N*-acetylserine (**6**) as an initial intermediate. We selected the reaction of

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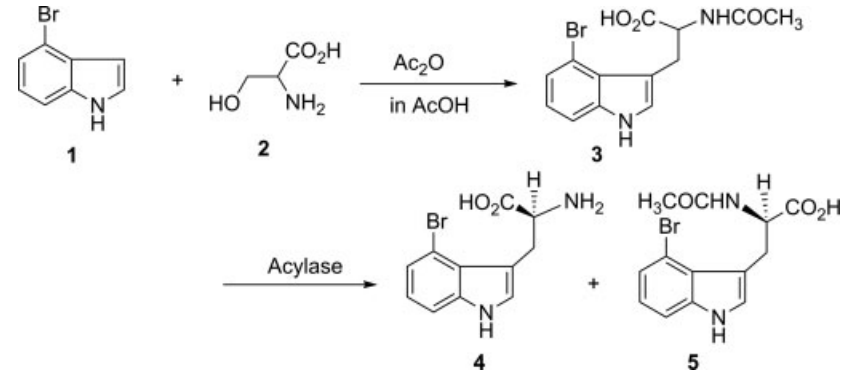
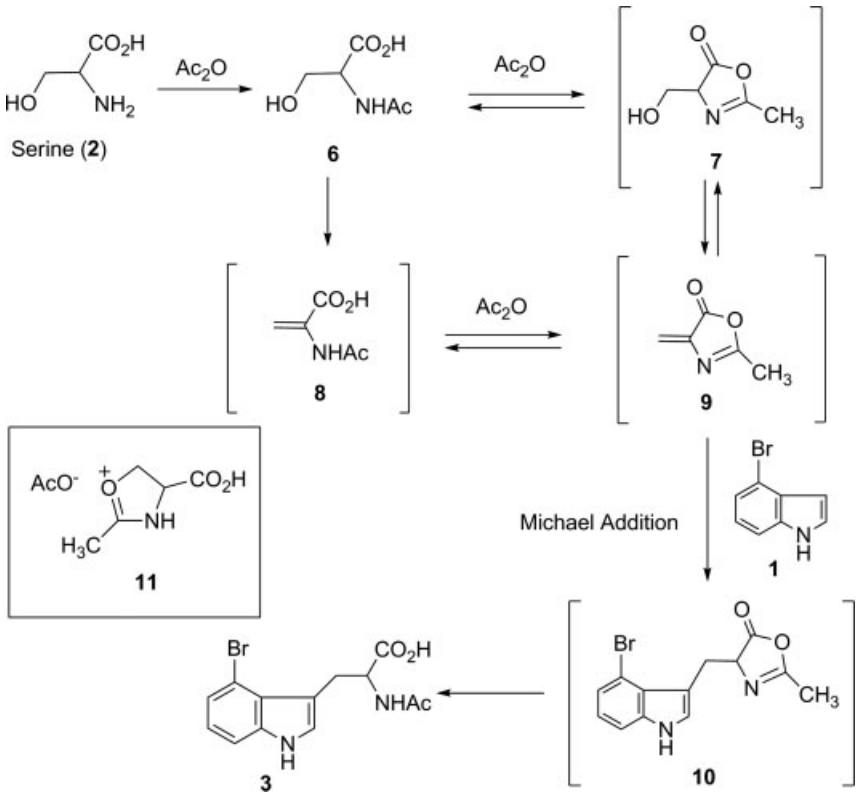
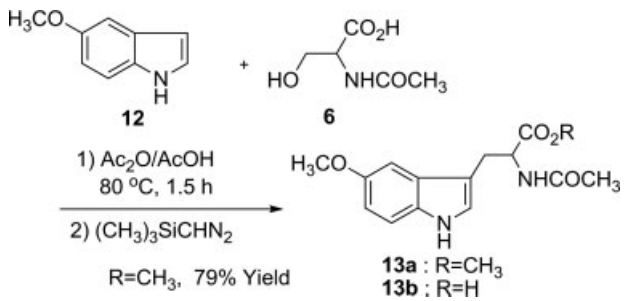


Scheme 1. Two-step synthesis of optically active 4-bromotryptophan.



Scheme 2. Plausible mechanism for the formation of *N*-acetyltryptophan (**3**).



Scheme 3. Reaction of 5-methoxyindole (**12**) with *N*-acetylserine (**6**).

6 with 5-methoxyindole (**12**) as a model reaction for easy assignment of the aromatic part of the ^{1}H-NMR spectrum. The reaction proceeded smoothly to give the product, *N*-acetyl-5-methoxytryptophan methyl ester (**13a**), in 79% yield after esterification with $(CH_3)_3SiCHN_2$ (Scheme 3).

The time-course ^{1}H-NMR spectra of this reaction were measured in the presence of $(CD_3CO)_2O$ in CD_3CO_2D. The spectra are shown in Fig. 1. As the reaction proceeds, the peak due to **12** at δ 7.15 ppm (peak a) disappears, while the peaks due to the methylene proton of **13b** (peaks b) become prominent (Fig. 1A–D). Although a pair of double doublets (peaks c, δ 3.8 and 4.1 ppm) arising from the methylene proton of **6** remain after 15 min, by which time starting material **12** has almost disappeared (Fig. 1C), those peaks disappear after 60 min, and unknown new peaks at δ 6 and δ 6.4 ppm (peaks d) appear (Fig. 1D).

Since these new peaks should be due to the reaction of **6** with Ac_2O, the reaction of **6** with Ac_2O-d_6 was carried out and NMR spectra were measured (Fig. 2A–C). Although the same two sets of peaks (peaks d) appeared in the reaction mixture, these

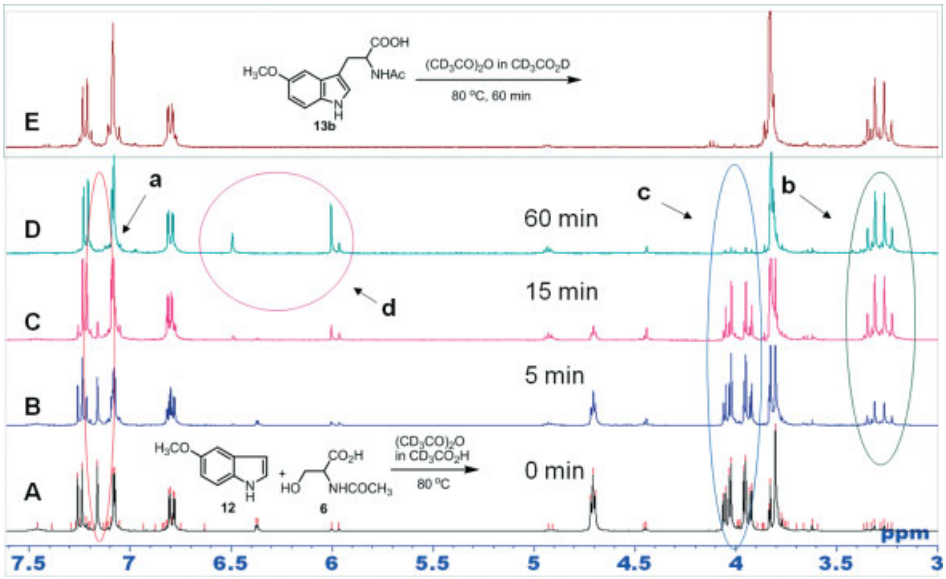


Figure 1. ^{1}H-NMR spectrum of the reaction of 5-methoxyindole (**12**) with 2.0 equiv. of *dl*-serine (**6**) at 80 °C in the presence of $(CD_3CO)_2O$ in CD_3CO_2D, obtained using a 400-MHz NMR. (A) 0 min, (B) 5 min, (C) 15 min, (D) 60 min and (E) 5-methoxytryptophan (**13b**) with Ac_2O-d_6 in CD_3CO_2D at 80 °C after 60 min.

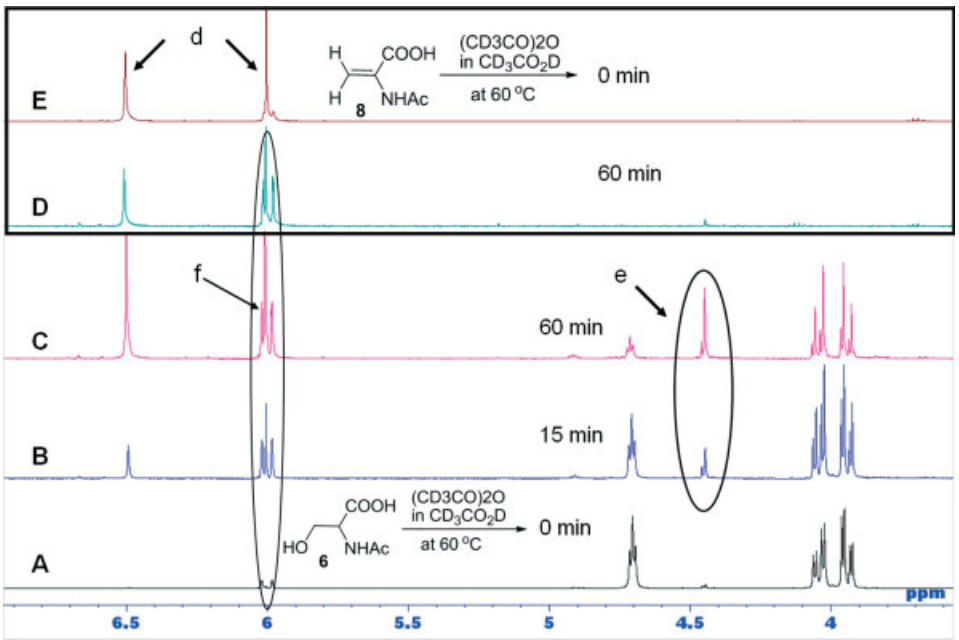


Figure 2. ^{1}H-NMR spectra of the reaction of *N*-acetylserine (**6**) (A–C) and 2-acetamidoacrylic acid (**8**) (D and E) with $(CD_3CO)_2O$ in CD_3CO_2H at 60 °C, obtained using a JEOL ECP-400 (400 MHz).

peaks were assigned to the olefinic protons of 2-acetamidoacrylic acid (**8**) (Fig. 2E), which is known[6] to form under these reaction conditions. We also observed the formation of **8** by heating of serine (**2**) or *N*-acetylserine (**6**) with Ac_2O in AcOH followed by direct evaporation of the solvent and Ac_2O. However, two new sets of peaks were observed at δ 4.5 ppm (peak e) and δ 6.0 ppm (peaks f) during the reaction of **6** with **12** (Fig. 2B and C). Peaks f were also observed in the reaction of **8** with Ac_2O-d_6 after 60 min (Fig. 2D). We speculated that peaks e and f arose from the reactive intermediates.

The above speculation was strongly supported by the results of DOSY, which is a useful 2D NMR method for spectrum separation[7] based on the different diffusion coefficients of compounds. The DOSY spectrum and the slice data are shown in Figs 3 and 4, respectively. The slice spectrum in Fig. 4A shows the presence of an unstable intermediate and the slice spectrum in Fig. 4C shows a mixture of **6** and another unstable intermediate. These unknown intermediates were speculated to be oxazolone **7** and **9,** derived from **6** (Scheme 1), as shown in Fig. 4A and C.

The structures of these intermediates were further confirmed on the basis of various 2D NMR spectra. The ^{1}H- and ^{13}C spectra of the reaction mixture after 60 min are shown in expanded form, the range from δ 3.5 to 6.6 ppm, in Fig. 5. In the ^{1}H-detected pulse field gradient multiple quantum coherence (PFG-HMQC) spectrum

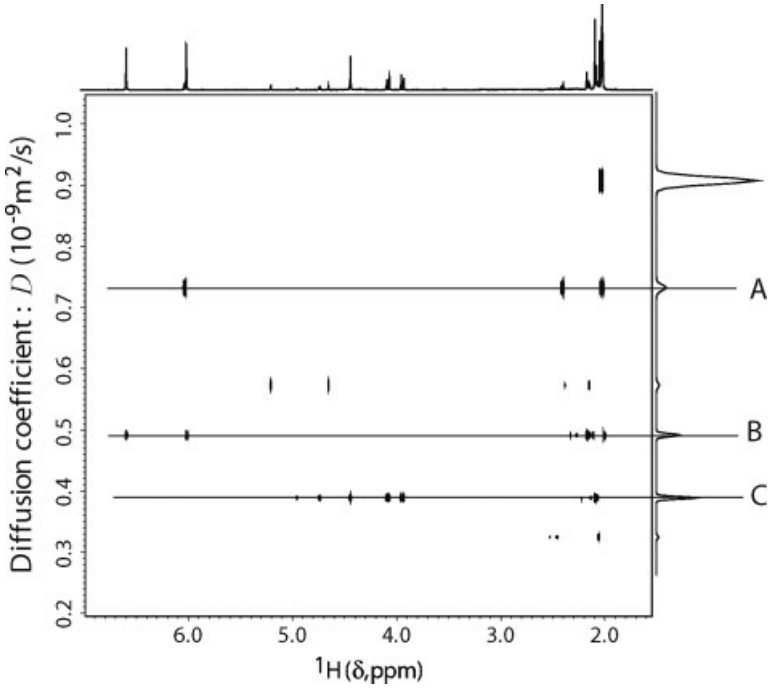


Figure 3. ^{1}H-DOSY spectrum of the reaction of *N*-acetylserine with Ac_2O-d_6 obtained using a JEOL ECX-400 (400 MHz).

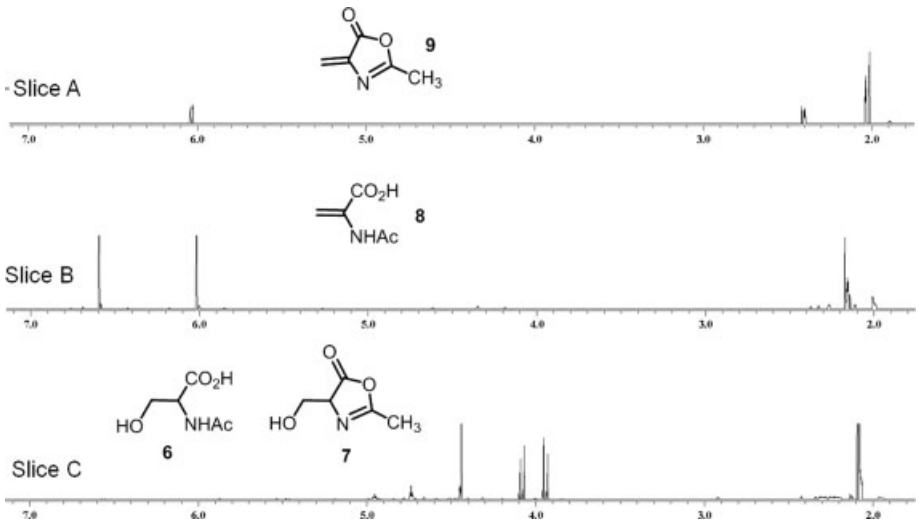


Figure 4. Slice spectra of Fig. 3.

for ^{1}H–^{13}C correlations, cross peaks were observed between H_c and C_c for compound **7** and H_g and C_g for compound **9**, and in the detected pulse field gradient multiple-bond heteronuclear multiple bond coherence (PFG-HMBC) spectrum, cross peaks were observed between H_c and C_d for compound **7** and H_g and C_h for compound **9**. [The data are shown in the supporting information.] All of the peaks for compounds **6**, **7**, **8** and **9** could be rationally assigned, as shown in Fig. 5.

In addition, ^{1}H–^{15}N PFG-HMBC in Fig. 6 strongly supported the above estimation, i.e. the spectrum showed cross peaks between ^{15}N signals at δ 370 and 495 ppm and ^{1}H methylene signals of **7** and olefinic protons of **9**, in addition to the cross peaks of **6** and **9**.

Although all spectral data showed that our proposed mechanism is rational, Sanderson[4] proposed a different mechanism (Scheme 4). They concluded that the cationic species (**11**) derived from *N*,*O*-diacetylserine (**14**) is the key intermediate which is reactive in nucleophilic substitution reactions, and that **11** equilibrates to *N*-acetyldehydroalanine (**8**). They also mentioned that *N*, *O*-diacetylserine (**14**) was obtained as a major product from the reaction of **2** and Ac_2O, without the formation of **8**. In contrast, in our NMR experiments, there is no evidence for the formation of **14** or intermediate **11**: there are no signals other than those due to **6**, **7**, **8** and **9** in the reaction mixture of **6** with Ac_2O-d_6 in AcOH-d_4, even after 60 min, as shown in Fig. 2C. Furthermore, the olefinic protons of **8** gradually increased in intensity with time (Fig. 2A–2C), and the broad triplet at δ 4.7 ppm, which was the asymmetric proton of *N*-acetylserine (**6**), was gradually decreased by the exchange of deuterium proton through oxazolone (**7**). This result clearly indicates that **8** is a major by-product and that *N*, *O*-diacetylserine (**14**) is not formed in the reaction mixture.

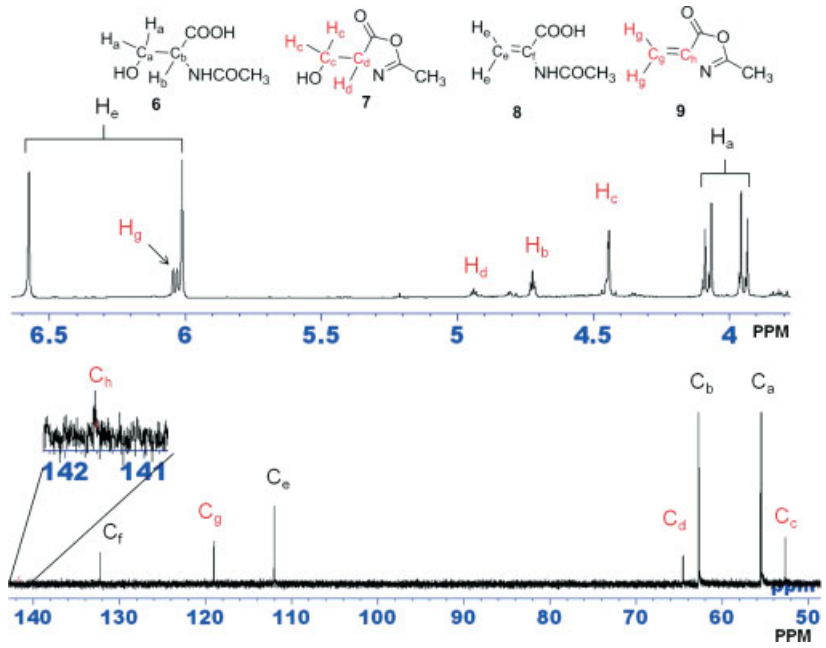


Figure 5. Assignment of ^{1}H- and ^{13}C-NMR spectra based on two-dimensional spectra (H–H DQFCOSY, H–C PFG-HMQC and PFG-HMBC) obtained using a JEOL ECP-500 (500 MHz).

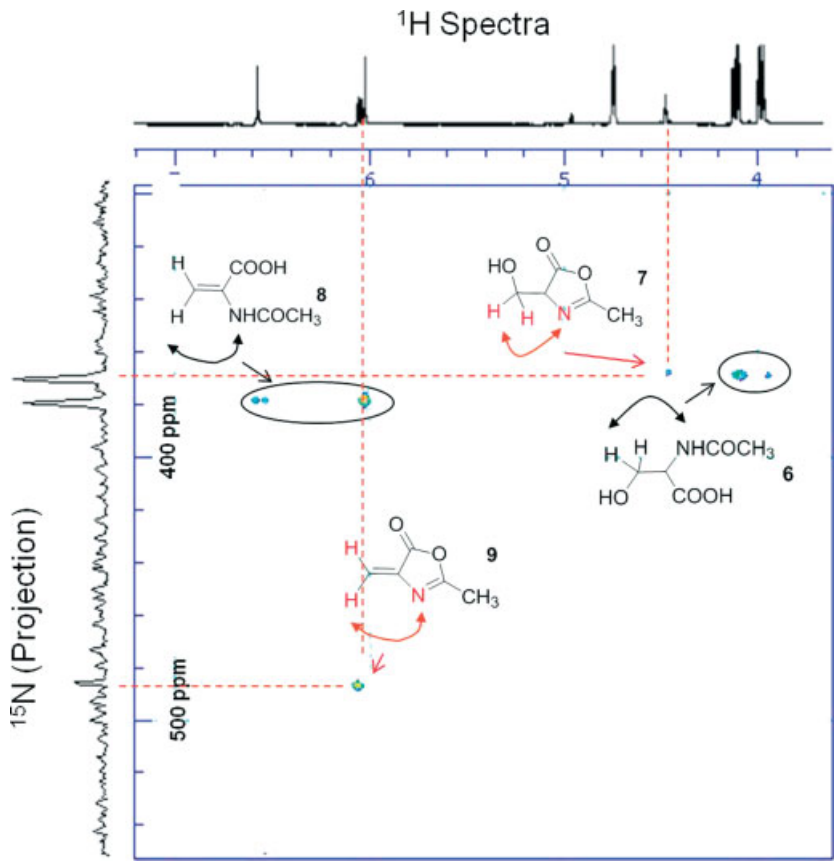
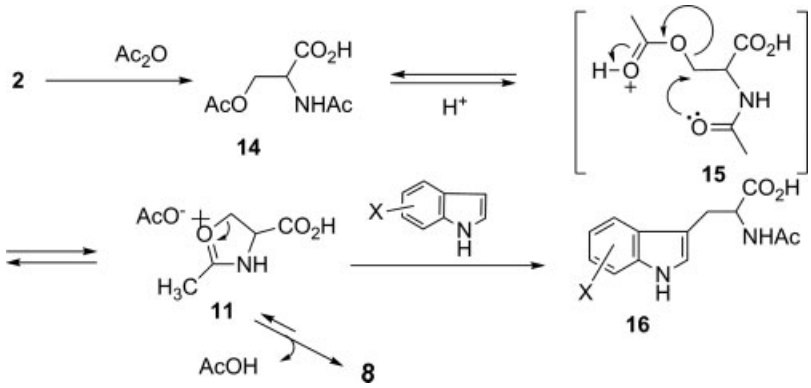


Figure 6. ^{1}H–^{15}N HMBC spectrum. Reference for ^{15}N is $CH_3C^{15}N$ at 249 ppm. Obtained using a JEOL ECP-500 (500 MHz).

Conclusion

Using a new 2D-NMR technique, DOSY and other NMR measurement techniques for the reaction of serine with acetic anhydride, we confirmed that the intermediate is impossible to isolate. The present results shows that NMR measurement, especially DOSY, is a powerful tool for the detection and structure elucidation of unstable intermediates in the reaction mixture. The



Scheme 4. Proposed mechanism by Sanderson[4].

asymmetric Michael addition might be a guiding principle for the present reaction in order to obtain optically active tryptophan derivatives. We are now applying this reaction to asymmetric syntheses based on this mechanistic principle.

Experimental

Synthesis of 5-methoxytryptophan metyl ester (13b) with *N*-acetylserine (6)

A mixture of **12** (150 mg, 1.0 mmol), *dl*-*N*-acetylserine (*dl*-**6**) (308 mg, 2.1 mmol), and Ac_2O (0.4 ml, 4.0 mmol) in AcOH (3.6 ml) was heated at 80 °C for 1.5 h under argon. The mixture was basified with 30% KOH in an ice bath and washed with organic solvent (AcOEt : benzene = 1 : 1). The aqueous layer was acidified with *conc*. HCl and extracted 3 times with AcOEt. The combined organic layers were washed with H_2O and brine and dried over $MgSO_4$. After evaporation of solvent and azeotropic removal of AcOH with benzene, the resulting brown solid (337 mg) was dissolved in AcOEt (4 ml) and MeOH (2 ml). To this solution, 2.0 M trimethylsilyldiazomethane ($TMSCHN_2$, 2.7 ml, 5.5 mmol) in hexane was added and kept for 30 min at room temperature. Then, AcOH was added to quench the reaction and the solution was diluted with water. The aqueous layer was extracted 3 times with AcOEt and the combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine and dried over $MgSO_4$. After solvent evaporation, the resulting brown oil was subjected to silica gel chromatography (4 : 1 benzene : acetone) to give 231 mg of **13b** (79% yield) as a colorless amorphous. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.97 (s, 3H), 3.29 (m, 2H), 3.71 (s, 3H), 3.85 (s, 3H), 4.95 (m, 1H), 5.99 (br d, J = 8.0 Hz, 1H), 6.85 (dd, J = 8.8, 2.4 Hz, 1H), 6.95 (d, J = 2.4 Hz, 1H), 6.98 (d, J = 2.4 Hz, 1H), 7.25 (d, J = 8.8 Hz, 1H), 8.01 (br s, 1H). IR ν_{max} (KBr) 3404, 1739, 1655 cm^{-1} EI–MS m/z 290 (M^+, 70%), 160 (base peak). Anal. calcd for $C_{15}H_{18}N_2O_4$ (290.31) : C, 62.06; H, 6.25; N, 9.95. Found: C, 61.99; H, 6.53; N, 9.39.

NMR experiment for the reaction of 5-methoxyindole with *N*-acetylserine

A mixture of *N*-acetylserine (*dl*-**6**) (43 mg, 0.29 mmol) and Ac_2O-d_6 (0.088 ml, 96 mg, 0.30 mmol) in d_6-AcOH (0.7 ml) was heated in an NMR tube at 80 °C for the times indicated in Fig. 1A–D. ^{1}H-NMR spectra were measured at 80 °C on a JEOL JNM-ECP-400 NMR spectrometer and were recorded in δ units, parts per million (ppm). The chemical shifts were measured relative to tetramethylsilane.

NMR experiment for the reaction of *N*-acetylserine with Ac_2O-d_6

A mixture of *N*-acetylserine (*dl*-**6**) (62 mg, 0.43 mmol) and Ac_2O-d_6 (0.127 ml, 138 mg, 0.43 mmol) in Ac_2O-d_6 (0.7 ml) was heated in an NMR tube at 80 °C for 1.5 h under argon. ^{1}H and ^{13}C NMR spectra were measured on a JEOL ECP-500 NMR equipped with TH5ATFG2 probe at 27°. The diffusion times were optimized for every experiment. The gradient strengths used in this study were between 3[T/M] − 0.3[T/M], and the 90° pulse of ^{1}H is 12.7 µs.

^{1}H-observed field gradient (FG) double-quantum filtered correlation spectroscopy (FG-DQFCOSY) was used for the measurement of absolute values. The double pulsed-field-gradient-spin-echo (DPFGSE) sequence was used as the excitation sculpting technique.[8] The effect was to selectively invert protons attached to carbon-12 while leaving intact protons bound to carbon-13 nuclei. For the DPFGSE experiments a recycle delay of 2 s was employed, operating at 500 MHz and 80 °C using a deuterium lock system. Filled rectangles are 90° pulses; open rectangles are 180° pulses that were conventional 180° pulses on the proton channel and composite 90°x, 240°y, 90°x inversion pulses on the ^{13}C channel.

The BIRD elements are labeled by the phase of the central proton 180° pulse. All FG pulses were 1 ms followed by a recovery delay of 200 µs.

On the basis of the ^{13}C–^{1}H correlations obtained from the DPFGSE HMQC spectra, we assigned the ^{13}C chemical shifts corresponding to the ^{1}H chemical shifts. A long-range heteronuclear correlation was observed in the ^{1}H spectra obtained by the FG-HMBC measurements. Quaternary carbon atoms were assigned on the basis of the long-range correlation.

Two-dimensional ^{1}H-observed DPFGSE HMQC and HMBC spectra permitted distinction between ^{1}H and ^{13}C ($^1J_{H-C}$) by restricting the observational frequency range.

^{1}H–^{15}N HMBC spectra can lead to significantly better results as shown in Fig. 6. $CH_3C^{15}N$ at 249 ppm was used as a reference, and the spectra were obtained on a JEOL ECP-500.

DOSY measurements

^{1}H-DOSY[9] spectra were measured on a JEOL JNM-ECA-500 equipped with a TH5FG probe-head with an actively shielded Z-gradient coil. ^{1}H-DOSY used the bipolar pulse pairs stimulated echo-longitudinal eddy current delay (BPPSTE-LED)[10] pulse sequence at 30 °C. The gradient amplitude (g) was changed from 1 to 30 G cm^{-1} in 32 steps. Measurement conditions for

^{1}H-DOSY were diffusion time 0.3 s, FG pulse width 1.2 ms and eddy current delay 50 ms. We used the SPLMOD algorithm for DOSY processing supported by JEOL (DELTA version 4.34). In the supporting information, we showed the DOSY spectra and slice data which were treated with Levenberg-Marquardt method as one of the most reliable DOSY algolism.

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Supporting information

Supporting information may be found in the online version of this article.

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