

# Reaction of Monosodium Glutamate in Water using a Closed Vessel Reactor: Improvement of the Organic Chemistry Experiment in Cambodian High School by Using Cambodian Materials. Part 3.

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## Abstract

In this study, a high concentrated reactions of disodium glutamate in water at 180 °C using closed vessel by intramolecular condensation gave pyroglutamate in quantitative yield. When this reaction was carried out using AJI-NO-MOTO®, coloring was observed. To investigate the coloring mechanism, 1D and 2D NMR spectra of the reaction mixtures were measured and the products were identified.

## I. Introduction

Our group had been developed experimental procedures for conducting 4-methylbenzene-1-sulfonic acid-catalyzed esterification of benzoic acid with dodecan-1-ol in the absence of solvent as the continuation of our studies on creating procedures which can be applicable to Cambodian high school.<sup>1,2</sup> Then, our demonstration of olive oil, tapioca starch, and iodine in a plastic bottle using Cambodian materials were reported.<sup>3,4</sup> The demonstration could benefit chemistry teachers who have difficulty in purchasing and accessing reagents in a difficult situation of the novel coronavirus epidemic and the school closure in Cambodia. It is necessary to create experimental procedures which can be made full or perfect in Cambodia.

The author had traveled to Cambodia in November 2018, August and December 2019 to conduct surveys of chemical experiment facilities in high schools, demonstrations of experiments, and classes using molecular models. Also, I searched for something that could be used for organic

chemistry experiments at local supermarkets. There, I noticed that AJI-NO-MOTO®<sup>6</sup> was on sale. In addition, seasonings containing monosodium glutamate as the main component such as Rachachuros<sup>7</sup> and Thaichuros<sup>8</sup> were sold. AJI-NO-MOTO® and its similar products are available everywhere at low cost. Therefore, I decided to investigate an organic reaction using monosodium glutamate, which is easily available in Cambodia, as a starting material.

Also, because our group<sup>3,4</sup> had been used olive oil, I carried out the reaction of olive oil with monosodium glutamate in order to obtain weakly acidic surfactant sodium *N*-acylglutamate **5**.<sup>9</sup> Olive oil is a fatty acid ester of glycerol, and the amino group of the amino acid can attack the carbonyl group of the ester to form an amide as shown in Figure 2.

A closed vessel reactor was used for the reaction in water as solvent at higher than 100 °C as a model of a pressure cooker or a food pouch. The reaction mixtures were analyzed for structure determination by measurements of nuclear magnetic resonance (NMR) spectra.

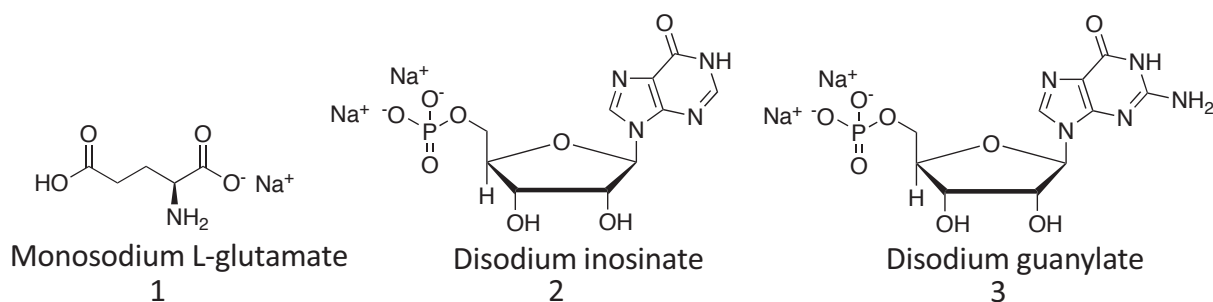


Figure 1. Structure of monosodium L-glutamate (**1**), disodium inosinate (**2**), and disodium guanylate (**3**).

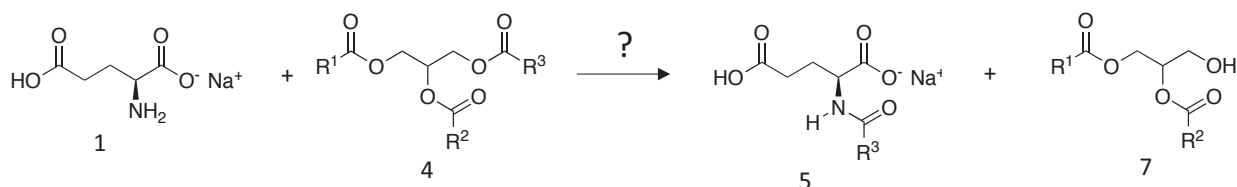


Figure 2. Proposed reaction of olive oil **4** with monosodium glutamate (**1**) to give a weakly acidic surfactant (sodium *N*-acylglutamate) **5**.

## II. Results and discussion

### 1. Reaction of AJI-NO-MOTO® in the presence of olive oil and water

A mixture of AJI-NO-MOTO® (0.338 g, 1.8 mmol) in water (1.0 mL) and olive oil (0.5 mL) was placed in a glass vial with a silicone cap. The vial with stirring bar was then placed in a closed vessel reactor Monowave 50 for 1 h at 120 °C. The organic layer was dissolved with CDCl<sub>3</sub> and the aqueous layer was dissolved with D<sub>2</sub>O. The solutions were monitored by measurements of <sup>1</sup>H and <sup>13</sup>C NMR spectra. The CDCl<sub>3</sub> solution showed the presence of recovered olive oil. The D<sub>2</sub>O solution showed the presence of new peaks. HMBC spectrum (D<sub>2</sub>O) of the reaction mixture (Table 1, entry 1) is shown in Figure 3. The red line shows a peak of a new product in 1D spectrum, the red cycle show a correlation of that between <sup>1</sup>H and <sup>13</sup>C in 2D spectrum. The structure of the product was determined by mainly HMBC spectrum as shown in Figure 4. The cyclic structure **8** was supported from <sup>3</sup>J<sub>H,C</sub> correlation from CH peak (4.03 ppm) to amide carbonyl group (181.71 ppm). Sodium L-pyrroglutamate **8** was formed by an intramolecular condensation reaction of **1** at carboxy group and amino group. The formation of **8** was reported in literatures.<sup>10–13</sup>

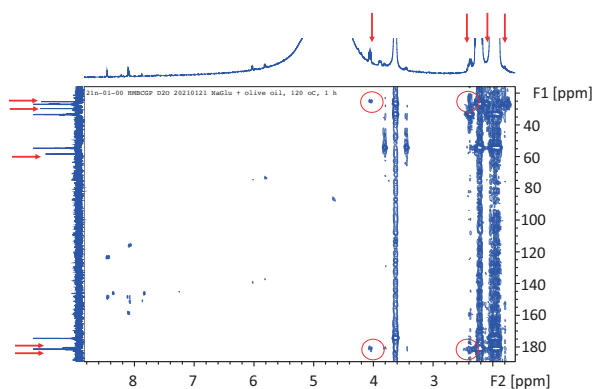


Figure 3. HMBC spectrum (D<sub>2</sub>O) of the reaction mixture (Table 1, entry 1) of AJI-NO-MOTO® in the presence of olive oil and water.

The relative yield of **8** and recovery of monosodium glutamate (**1**) were determined by integration of CH peaks at 4.03 ppm and 3.64 ppm as shown in table 1 (entry 1). I was interested in this reaction in water, so I decided to further

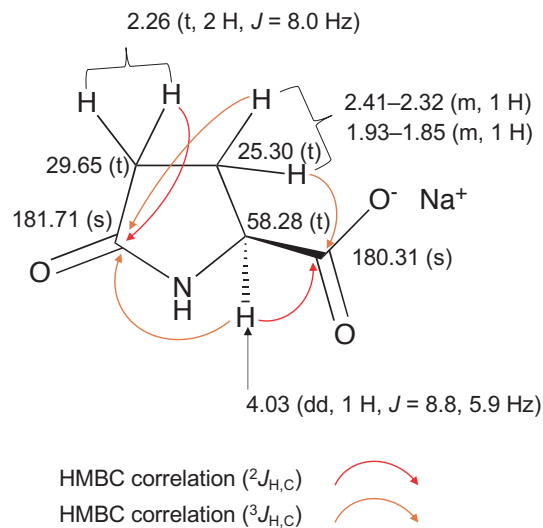


Figure 4. Assignments of <sup>1</sup>H and <sup>13</sup>C NMR spectra (D<sub>2</sub>O) for **8** in the reaction mixture (Table 1, entry 11).

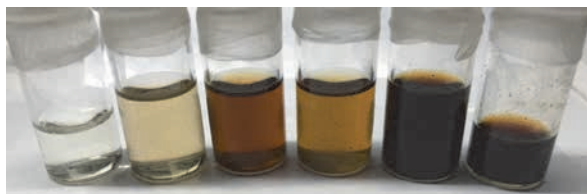
investigate the reaction conditions. Next, when the reaction temperature was set to 150 °C, the yield of **8** increased to 38% (Table 1, entry 2). At the same temperature in the absence of olive oil, **8** was obtained in good yield (Table 1, entry 3). These results indicated that there was no need to add olive oil to the reaction. Also, the formation of compound **5** could not be detected by NMR measurement of the solution of the reaction mixture in D<sub>2</sub>O or CDCl<sub>3</sub>.

### 2. Reaction of AJI-NO-MOTO® in water

In order to decrease the starting material **1**, the reaction temperature was elevated within the maximum pressure (2.0 MPa) for the apparatus. When the reaction temperature was set to 180 °C, the pressure increased to 1.3 MPa at the initial stage of the reaction, and then became constant at 1.0 MPa. Therefore, the maximum temperature was 180 °C and it was impossible to increase the amount of the solvent. The results are shown in Table 1, Entries 3–5. The reaction mixture had a burnt smell of Chinese food or the smell of seasoned Chinese noodles fried in vegetable oil. As the reaction temperature increased, the reaction mixture turned dark brown. In order to investigate the cause of this color change, the reactions of the reagent monosodium glutamate were investigated.

### 3. Reaction of monosodium glutamate in water

Monosodium L-glutamate monohydrate (**1**) was purchased from Nacalai Tesque (Kyoto, Japan). The reactions were carried out in as shown in Table 1, Entries 6, 7 to give **8** in excellent yields, and also under high concentrated reaction conditions (Entry 8). The reaction mixture became pale brown and darker as the molar concentration of compound **1** increased as shown in Figure 5 a) and b). In an experiment with the same amount of substance of AJI-NO-MOTO® (Table 1, Entry 9), the colors of the reaction mixture showed a large difference with Entry 8 as shown in Figure 5 b) and c). AJI-NO-MOTO® contains 97.5% monosodium glutamate, 1.25% disodium inosinate, and 1.25% disodium guanylate.<sup>5</sup> To find out the cause of the color difference, investigation for the reaction of **1** in the presence of disodium inosinate (**2**), which was cheaper and more affordable.



a) Entry 7 b) Entry 8 c) Entry 9 d) Entry 10 e) Entry 11 f) Entry 12

Figure 5. Color comparison of the final reaction mixtures under the reaction conditions as shown in Table 1.

### 4. Reaction of sodium glutamate in the presence of disodium inosinate in water

Disodium inosinate (**2**) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). The reactions were carried out in as shown in Table 1, Entries 10 and 11 to give **8** in excellent yields. The pictures for the reaction mixtures of Entries 10 and 11 are shown in Figure 5 d) and e). To clarify the effect of the presence of monosodium glutamate (**1**), the reaction of **2** in H<sub>2</sub>O was carried out as shown in Table 1, entry 12. These results showed that the higher the concentration of disodium inosinate (**2**), the darker the color of the reaction mixture. From the comparison of the experimental results of Entries 9 and 10 in Table 1, it was presumed that disodium guanylate (**3**) also had the same effect as **2**.

<sup>1</sup>H NMR spectra of AJI-NO-MOTO®, the reaction mixture of Entries 9, 10, and 11 in Table 1 in D<sub>2</sub>O are shown in Figure 6. Compared with the spectrum of AJI-NO-MOTO®, products with different chemical shifts at the nucleobase portion (8.4–7.1 ppm) and the anomeric position (6.5–5 ppm) of the sugar were formed during the reaction. Therefore, we decided to determine the structural assignment of compounds **2** and **3** in AJI-NO-MOTO® or of the compounds in the reaction mixtures using 2D NMR.

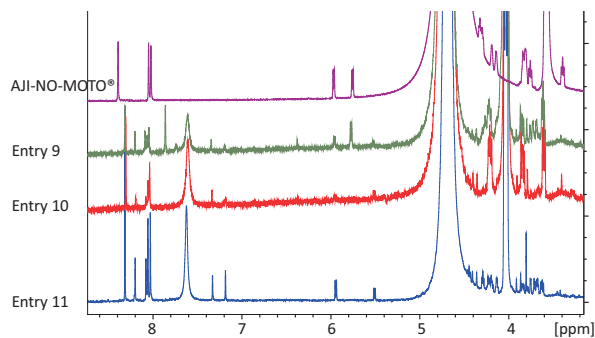


Figure 6. <sup>1</sup>H spectra (D<sub>2</sub>O) of the reaction mixtures.

At first, assignments of <sup>1</sup>H and <sup>13</sup>C NMR spectra of commercially available reagent **2** in D<sub>2</sub>O was carried out as shown Figure 7. The important correlations of purine bases in the HMBC spectrum are shown below. H-2 at 7.92 ppm had two <sup>3</sup>J<sub>H,C</sub> correlations with C-4 at 148.66 ppm and with C-6 at 158.51 ppm. H-8 at 8.36 ppm had two <sup>3</sup>J<sub>H,C</sub> correlations with C-4 and with C-5 at 124.16 ppm, and one <sup>4</sup>J<sub>H,C</sub> correlations with C-6. Anomeric H-1' at 5.76 ppm had two <sup>3</sup>J<sub>H,C</sub> correlations with C-4 and C-8. In <sup>13</sup>C NMR, <sup>13</sup>C-<sup>31</sup>P couplings with phosphate group were observed at 84.68 ppm (C-4') and 63.52 ppm (C-5').

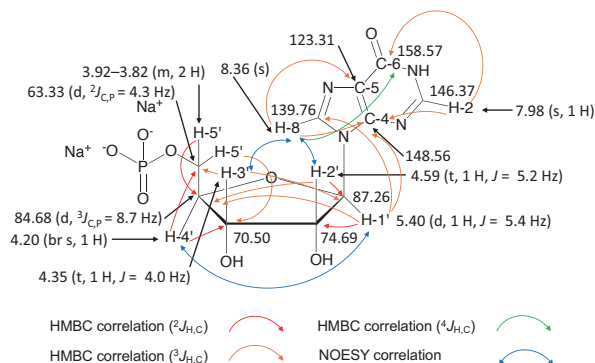


Figure 7. Assignments of <sup>1</sup>H and <sup>13</sup>C NMR spectra (D<sub>2</sub>O) for **2** with using 2D spectra.

Next, assignments of <sup>1</sup>H and <sup>13</sup>C NMR spectra of **2** in D<sub>2</sub>O solution of commercially available AJI-NO-MOTO® were carried out as shown in Figure 8. In this case, several correlations were not observed due to large signal in the presence of large amounts of monosodium glutamate (**1**).

In addition, assignments of <sup>1</sup>H and <sup>13</sup>C NMR spectra of disodium guanylate (**3**) in D<sub>2</sub>O solution of commercially available AJI-NO-MOTO® were carried out as shown in Figure 9. The results of these two assignments later helped determine whether phosphate group was connected to 5'-position of D-ribose in the case of Entry 11.

Since two anomeric signals of D-ribose derivatives (5.94 and 5.51 ppm in <sup>1</sup>H NMR) were observed in the reaction mixture of entry 11, existence of two types for *N*-glycosides was estimated. The compounds **9** (H-1': 5.94 ppm) and **10**

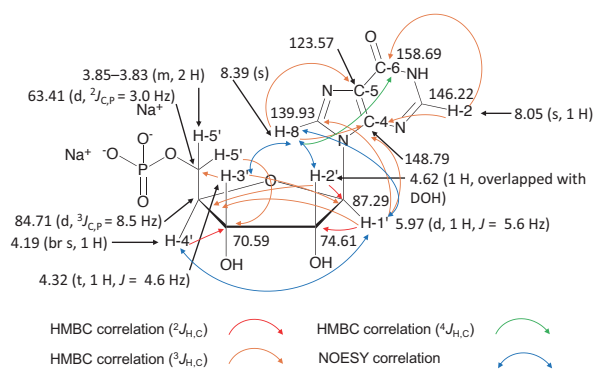


Figure 8. Assignments of  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra ( $\text{D}_2\text{O}$ ) for **2** in a solution of AJI-NO-MOTO<sup>®</sup> with using 2D spectra.

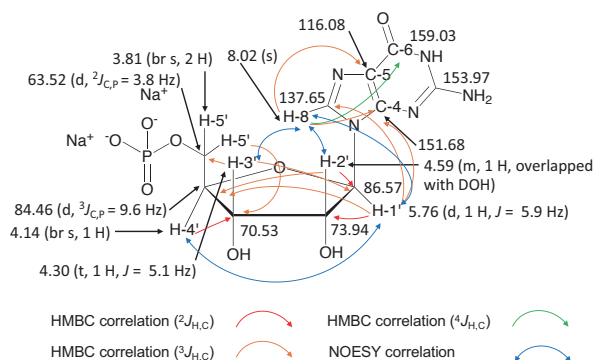


Figure 9. Assignments of  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra ( $\text{D}_2\text{O}$ ) for **3** in the solution of AJI-NO-MOTO<sup>®</sup> with using 2D spectra.

(H-1': 5.51 ppm) had a hypoxanthine unit and a ribose unit and showed chemical shifts very close to those of compound **2** and correlations in the two-dimensional spectra as shown in Figures 10 and 11. The chemical shifts were likely to shift because compound **8** in the solution was present in a proportion of 99% or more. Therefore, pure compound **2** was added to the reaction mixture of entry 11 and then the NMR spectra were measured. As a result, both showed different signals from compound **2**. These compounds did not show any  $^{13}\text{C}$ - $^{31}\text{P}$  coupling with phosphate group in  $^{13}\text{C}$  NMR spectra. Furthermore, in compound **10**, there was no hydrogen at the 2-position of the purine ring. Therefore, it was clarified that the hypoxanthine moiety was hydrolyzed and converted to the xanthine moiety. These products were identified as known inosine (**9**)<sup>14</sup> and xanthosine (**10**).<sup>15</sup>

As a result of further inspection for the reaction mixture of entry 11, hypoxanthine (**12**) and xanthine (**13**) were also formed. The ratio of these products was **9**:**10**:**12**:**13** = 13:8:27:52. In the absence of the compound **1** under the same reaction conditions (Entry 12), the ratio was **9**:**10**:**12**:**13** = 2:3:30:65. It seemed that the decomposition of *N*-glycosides were slightly suppressed by compound **1**. Under these reaction conditions, uric acid (**14**) might also be produced, but it could not be detected by proton NMR due to exchange

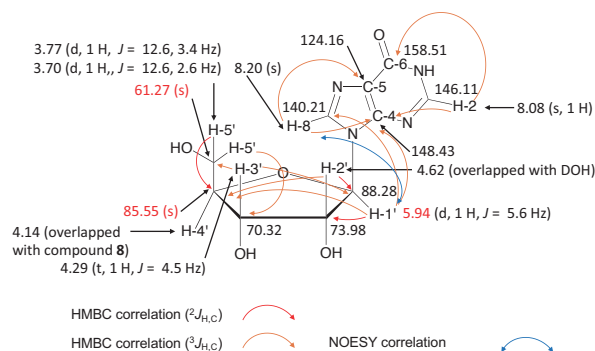


Figure 10. Assignments of  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra ( $\text{D}_2\text{O}$ ) for inosine (**9**) in the reaction mixture of Table 1, Entry 11 with using 2D spectra.

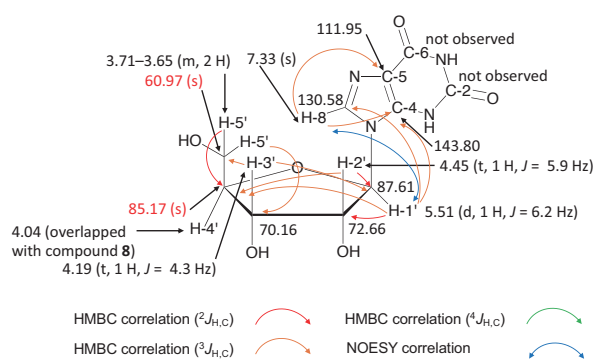


Figure 11. Assignments of  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra ( $\text{D}_2\text{O}$ ) for xanthosine (**10**) in the reaction mixture of Table 1, Entry 11 with using 2D spectra.

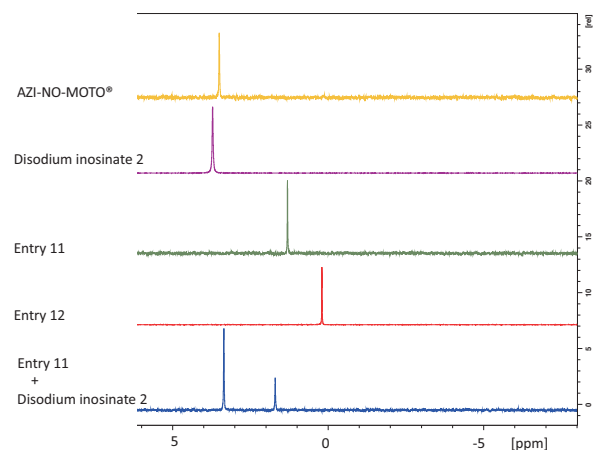


Figure 12. Comparison of  $^{31}\text{P}$  NMR spectra ( $\text{D}_2\text{O}$ ).

with  $\text{D}_2\text{O}$ .

Comparison of  $^{31}\text{P}$  NMR spectra in  $\text{D}_2\text{O}$  is shown in Figure 12. Peaks at higher chemical shift (3–4 ppm) were due to 5'-phosphates and at lower chemical shift (2–0 ppm) were due to inorganic phosphates. A plausible formation pathway of the products **9**–**14** by hydrolysis is shown in Figure 13. First, the phosphate ester was hydrolyzed, and then the hypoxanthine moiety was hydrolyzed at the same time as the *N*-glycoside moiety.

Since ribose was produced by this reaction, it was

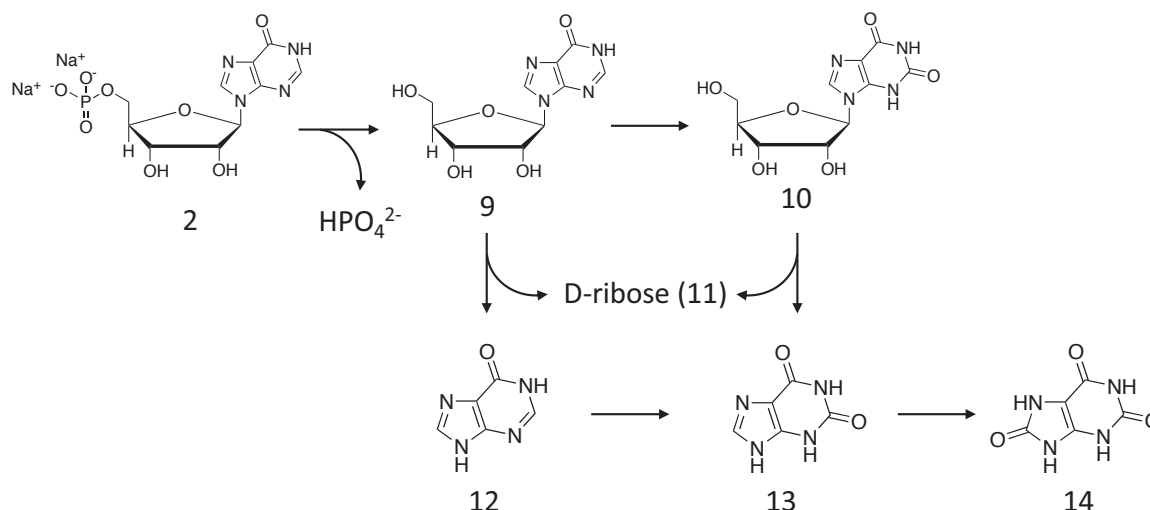


Figure 13. An plausible formation pathway of the products 9–14.

presumed that ribose was colored by further Maillard reaction<sup>16</sup> with compound 1. Therefore, if a commercially available Ajinomoto is used in this reaction, it causes coloring.

### III. Conclusion

In this way, I demonstrated that a high concentrated reactions of disodium glutamate in water at 180 °C using closed vessel by intramolecular condensation gave pyroglutamate in quantitative yield. When this reaction was carried out using AJI-NO-MOTO®, coloring was observed. The coloring mechanism was presumably Maillard reaction of ribose, which was formed by hydrolysis of disodium inosinate, with monosodium glutamate.

If the reaction will be improved to carried out by using a pressure cooker, a food pouch, or a microwave oven, it may be possible to perform this reaction at a high school in Cambodia in the future. And also, our group examined the reaction on a large scale. When the reaction mixture was reacted with benzyl bromide, an ester could be synthesized, which could be used as a plasticizer for an eraser. These will be reported in the future.

## IV. Experimental section

### 1. General

All reagents and solvents were of reagents grade quality and purchased commercially. Structure of synthetic compounds were confirmed by <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded with a Bruker AVANCE III instrument operating at 400.13 and 100.62 MHz, respectively. Chemical shifts were referenced to TMS in CDCl<sub>3</sub> as internal standard. Structures of the

reaction products were confirmed by <sup>1</sup>H NMR, <sup>13</sup>C NMR, and two-dimensional NMR (COSY, HSQC, HMBC, and NOESY) spectroscopy. Monosodium L-glutamate monohydrate was purchased from Nacalai Tesque (Kyoto, Japan).

### 2. Reaction of AJI-NO-MOTO® in a mixture of olive oil and water

A mixture of AJI-NO-MOTO® (0.338 g, 1.8 mmol) in water (1.0 mL) and olive oil (0.5 mL) was placed in a glass vial with a silicone cap. The vial with stirring bar was then placed in a Monowave 50 for 1 h at 120 or 150 °C. After had been allowed to reach room temperature, the organic layer was dissolved with CDCl<sub>3</sub> and the aqueous layer was dissolved with D<sub>2</sub>O. The solutions were monitored by measurements of <sup>1</sup>H and <sup>13</sup>C NMR spectra.

### 3. Reaction of AJI-NO-MOTO® in water

A mixture of AJI-NO-MOTO® (0.338 g, 1.8 mmol) in water (1.0 mL) was placed in a glass vial with a silicone cap. The vial with stirring bar was then placed in a Monowave 50 for 1 h at 150, 150, or 180 °C. After had been allowed to reach room temperature, the aqueous layer was dissolved with D<sub>2</sub>O. The solution was monitored by measurements of <sup>1</sup>H and <sup>13</sup>C NMR spectra.

### 4. Reaction of monosodium glutamate in water

A mixture of monosodium L-glutamate monohydrate (0.170, 0.338, or 1.691 g, 0.908, 1.80, or 9.04 mmol) in water (1.0 mL) was placed in a glass vial with a silicone cap. The vial with stirring bar was then placed in a Monowave 50 for 1 h at 180 °C. After had been allowed to reach room temperature, the aqueous layer was dissolved with D<sub>2</sub>O. The solution was monitored by measurements of <sup>1</sup>H and <sup>13</sup>C NMR spectra.



Table 1. Optimization of reaction conditions.

Entry	AJI-NO-MOTO® (mmol)	1 (mmol)	2 (mmol)	Water / mL	Olive oil /mL	Temp. (°C)	Yield of 8 (%)	Recovery of 1 (%)
1	1.8	–	–	1.0	0.50	120	3.2	96.8
2	1.8	–	–	1.0	0.50	150	38.0	62.0
3	1.8	–	–	1.0	–	150	84.4	15.6
4	1.8	–	–	1.0	–	170	91.0	9.0
5	1.8	–	–	1.0	–	180	99.3	0.7
6	–	0.91	–	1.0	–	180	99.0	1.0
7	–	1.80	–	1.0	–	180	99.0	1.0
8	–	9.04	–	1.0	–	180	98.9	1.1
9	9.0	–	–	1.0	–	180	99.1	0.9
10	–	8.92	0.05	1.0	–	180	99.0	1.0
11	–	9.04	0.50	1.0	–	180	99.4	0.6
12	–	–	0.50	1.0	–	180	-	-

### 5. Reaction of monosodium glutamate in the presence of disodium inosinate in water

A mixture of monosodium L-glutamate monohydrate (1.670 or 1.691 g, 8.924 or 9.036 mmol) and disodium inosinate (21 mg or 0.196 g, 0.05 or 0.50 mmol) in water (1.0 mL) was placed in a glass vial with a silicone cap. The vial with stirring bar was then placed in a Monowave 50 for 1 h at 180 °C. After had been allowed to reach room temperature, the aqueous layer was dissolved with D<sub>2</sub>O. The solution was monitored by measurements of <sup>1</sup>H and <sup>13</sup>C NMR spectra.

#### Acknowledgment

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