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Synthesis of L-Glutamic Acid 5- and 1-n-Butyl Ester As Promoters of Sediment Formation in Raw Soy Sauce

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Recently we first isolated Promoter of sediment formation at 60℃ and purified from raw soy sauce by heating. The structure of promoter was identified as L-glutamic acid n-butyl ester. Reported here, new selective synthetic method of L-glutamic acid 5-butyl ester and L-glutamic acid 1-butyl ester was achieved by selectively using two benzyl reagents.

Key Words: Selective Synthesis, Promoters of Sediment Formation, L-Glutamic Acid n-Butyl Ester, Soy Sauce

1. INTRODUCTION

One of the traditional fermented seasonings used in Japan is Soy sauce, and in the final process of soy sauce brewing the most important step is *Hiire* (heat treatment). At temperatures of 70 to 85℃, raw soy sauce (filtrate of the fermented mash) is pasteurized, and stored in a tank, in order to stop the microbial and enzymatic reaction and to stabilize the flavor and color. During Hiire, the sediment gradually formed. But it is difficult to filter off this sediment. It causes a loss of approximately 10% of the total volume in the brewery that the sediment from soy sauce is removed after pasteurization. This decreases the yield and the commercial value of the sauce. The main component of this sediment is protein. However sediment formation does not follow the protein heat-denaturation curve, a phenomenon that has been reported to be caused both by the activity of thermostable neutral protease (1-3) and by the hydrophobicity of alkaline protease (4,5). Promoter of sediment formation at 60℃ was first isolated and purified from raw soy sauce by heating (6). The structure of promoter was identified as L-glutamic acid n-butyl ester. This ester was interested in possibility of

coagulation of proteins and promoter of sediment formation for the practical production of soy sauce. Generally esters (1 and 2) were synthesized from L-Glutamic Acid and n-butanol by using mineral acid (7). But this method gave low yield and no selectivity. Therefor we planned new selective synthetic method of L-glutamic acid 5-alkyl ester and 1-alkyl ester to search for a more active compound and to study the structure-activity analyses (Fig.1). This new synthetic method was using two different benzyl reagents for selective synthesis of two types of esters. In the case of ester **1**, benzyl bromide was used. Otherwise in the case of ester **2**, benzyl alcohol was used. According to this plan, selective synthesis of L-glutamic acid 5-butyl ester **1** and 1-butyl ester **2** was initially tried out. And in this paper we reported that the selective synthesis of these esters was succeeded.

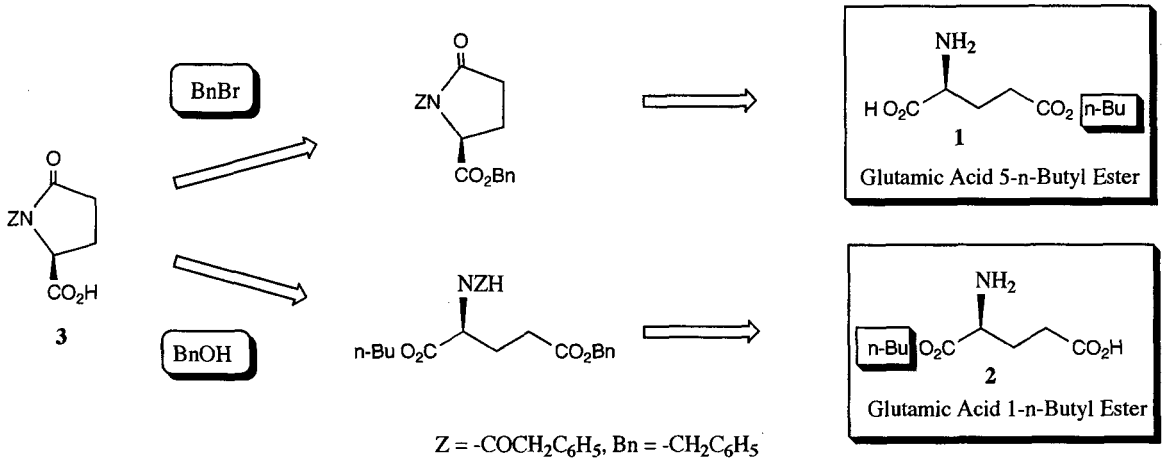

Figure 1. Synthetic Plan of Glutamic Acid 5-n-Butyl Ester **1** and Glutamic Acid 1-n-Butyl Ester **2**

2. PROCEDURE

2-1. Following the synthetic plan we begun to prepare 1-benzyl ester **4** and 5-n-buthyl ester **5** from commercially available N-carbobenzyloxycarbonyl L-pyroglutamic acid **3** under Danishefsky-Hirama condition (9). Esterification of acid **3** with benzyl bromide in the presence of Et_3N at room temperature gave 1-benzyl ester **4**, which was converted to free acid at the end of the synthetic plan, in 90% yield and amide ring cleavage of ester **4** was achieved by n-butanol and Et_3N at 100℃ in 82% successfully. Butanol was used for 5-butyl ester (Fig. 2).

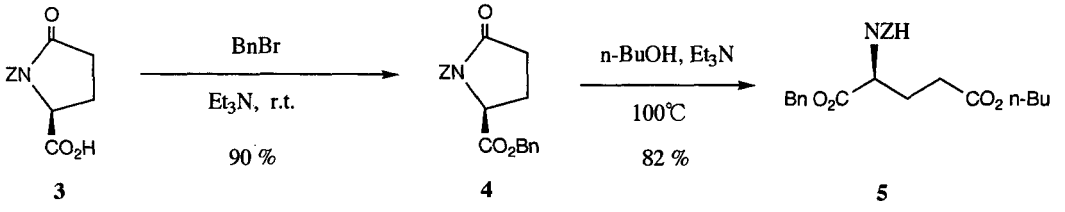

Figure 2. Synthesis of **5** from N-Butoxycarbonyl L-Pyroglutamic Acid **3**

Deprotection of ester **5** by hydrogenation to 5-butyl ester **1** was troublesome. Catalytic amount of Pd/C, Pt/C and $Pd(OH)_2$, served to remain benzyl protecting groups and yield ester **1** very low. Interestingly increase of Pt/C from catalytic amount to 1 equivalent produced ester **1** in 89% yield (Fig. 3).

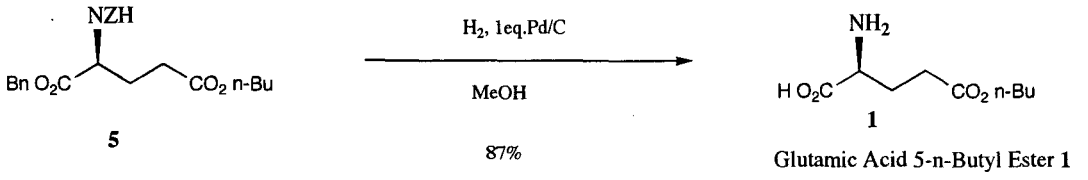

Figure 3. Synthesis of L-Pyroglutamic Acid 5-Butyl Acid **1**

As the synthesis of L-glutamic acid 5-butyl ester **1** was achieved, we took a step forward to the synthesis of 1-butyl ester **2**. Different from the synthesis of 5-butyl ester, starting material **3** was first alkylated by n-butyl bromide in 88% yield. And then amide ring was cleavaged by benzyl alcohol in 72% yield (Fig. 4).

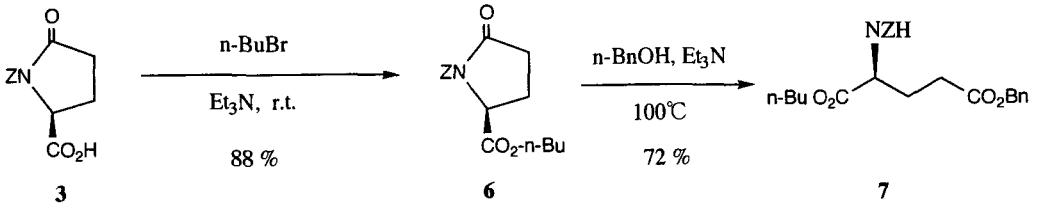

Figure 4. Synthesis of **7** from N-Butoxycarbonyl L-Pyroglutamic Acid **3**

The last stage was the removal of two protecting groups by hydrogenation. Following the amount of catalysis and the condition of above mention, we successfully got L-pyroglutamic acid 1-n-butyl ester **2** in 92% yield (Fig.5).

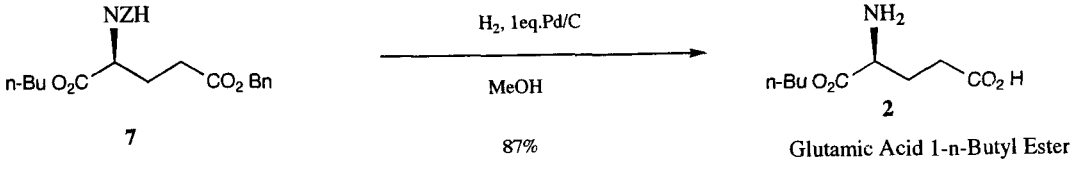

Figure 5. Synthesis of L-Pyroglutamic Acid 1-Butyl Acid **2**

2-2. Experimental Method

In each reaction a hot plate stirrer was used for heating or stirring or for performing both operations under nitrogen atmosphere, in the case of hydrogenation, hydrogen atmosphere. Syringe techniques were applied for transfer of air-sensitive reagents and dry solvents. Basic condition reactions were quenched by saturated aqueous solution of NH_4Cl. Products were extracted with dichloro metane and concentrated on a rotary evaporator and purified by flash column chromatography or thin-layer chromatography on silicagel. The chemical structure of product was determined by 1H nuclear magnetic resonance (NMR) spectra (JEOL GX 270) and Infrared (IR) spectra (BIO-RAD FTS-60A/896).

3. RESULT AND DISCUSSION

Selective synthesis of L-glutamic acid 5-butyl ester **1** and 1-butyl ester **2** was completed (Fig. 6) and yields of each steps were satisfied (Table Ⅰ and Table Ⅱ).

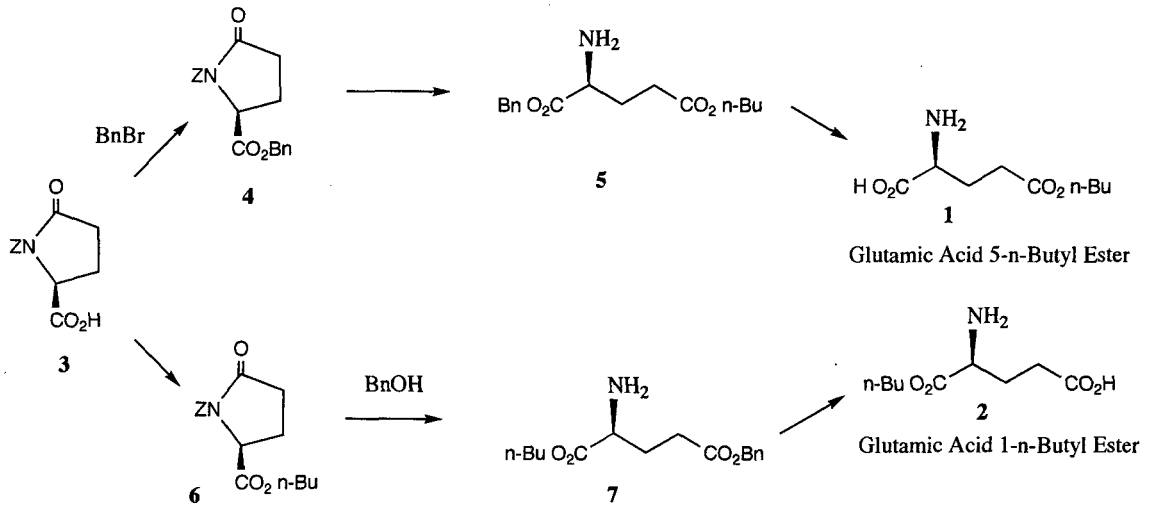

Figure 6. Selective Synthetic Method of Glutamic Acid 5-n-Butyl Ester **1** and Glutamic Acid 1-n-Butyl Ester **2**

Table I . Synthesis of L-glutamic acid 5-butyl ester

Substrate	Yield
4	98% (from 3)
5	81% (from 4)
6	87% (from 5)

Table I . Synthesis of L-glutamic acid 1-butyl ester

Substrate	Yield
7	88% (from 3)
8	72% (from 7)
9	92% (from 8)

In these synthetic procedure, two benzyl reagents, benzyl bromide and n-benzyl alcohol, were used selectively. On the one hand benzyl bromide was used for protecting of 1-carboxylia acid on L-glutamic acid 5-butyl ester **1**, and on the other n-benzyl alcohol was used for cleavage of amide ring and protecting 5-carboxylic acid on L-glutamic acid 1-butyl ester **2**. Both of cases benzyl groups were removed and derived to free carboxylic acids of synthetic target **1** and **2**. Now sediment-forming activity of these ester **1** and **2** is being examined.

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