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Process Research and Development of L-Alanyl-L-glutamine, a Component of Parenteral Nutrition

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Abstract:

A large-scale manufacturing method of L-alanyl-L-glutamine used for a component of parenteral nutrition has been studied. The method consisted of a reaction of D-2-chloro- or D-2-bromopropionic acid with thionyl chloride and Schotten−Baumann reaction with L-glutamine followed by ammonolysis reaction. The intermediate D-2-chloropropionyl-L-glutamine was found to be more stable than its bromo analogue. In the ammonolysis reaction, the former intermediate needed a higher reaction temperature, but the by-products produced had little effect on the quality of the final product. The structures of the by-products were conjectured mainly by mass spectrometry and they were removed by anion resin treatment and recrystallization.

Introduction

Recent studies have suggested important metabolic roles for L-glutamine and required possible sources of L-glutamine for parenteral nutrition.[1] However, L-glutamine is not included in currently available amino acid solutions.[2] The solubility of L-glutamine in water is decreased, and its stability in water is also low. In addition, L-glutamine may decompose into toxic products, pyroglutamic acid and ammonia.[1] Therefore, peptides containing L-glutamine have taken the place of L-glutamine. L-Alanyl-L-glutamine (**1**; AlaGln) has higher solubility and higher stability than L-glutamine and is used as the infusion component.[1,2]

For the production of AlaGln, there are several known methods, and the well-known methods are as follows: (1) methods of using a protecting group, for example, the method which comprises condensing an *N*-benzyloxycarbonyl-L-alanine (Cbz-Ala) with protected L-glutamine in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC) and removing the protecting group from the intermediate,[3,4] the method which comprises condensing Cbz-Ala with protected γ-methyl L-glutamate in the presence of DCC, removing the protecting group from the intermediate compound, and further reacting the deprotected product with ammonia,[5] and the method which reacts an active ester of Cbz-Ala with nonprotected L-glutamine and removes the protecting group from the intermediate compound;[6] (2) the method of producing AlaGln via *N*-carboxyl anhydride;[7] and (3) the method using D-2-bromopropionyl chloride as a starting compound via an intermediate compound, D-2-bromopropionyl-L-glutamine.[8]

The methods (1) using a protecting group need the step of removing the protecting group from the intermediate compound, and the operation for the step is complicated. Therefore, the methods (1) yield AlaGln at higher cost. The method (2) uses *N*-carboxyl anhydride of L-alanine without involving a protecting group. However, significant by-products are produced, for example, tripeptides, and the yield of the intended product is low. In addition, it is difficult to purify the intended product. In the method (3), since an acid chloride that has a high reactivity towards water is added to an aqueous solution of L-glutamine, for example, in the reaction of D-2-bromopropionyl chloride,[8] the method suffers from partial hydrolysis of the acid chloride. Therefore, the method (3) yields by-products, and the yield of the intended product is low. Since the produced D-2-bromopropionyl-L-glutamine is purified by extraction with organic solvent, the yield of the product is low. In addition, in the method (3), since the ammonolysis of D-2-bromopropionyl-L-glutamine is carried out at an elevated temperature, by-products are considerably enhanced, and the optical purity of the produced AlaGln is often low.

Results and Discussion

Among the methods described above for manufacturing AlaGln, the authors selected the method via D-2-halopropionyl-L-glutamine as the best for a large-scale production from the viewpoint of cost and procedures and started the process development study (Scheme 1).

In the previous papers,[8] the reaction of D-2-bromopropionyl chloride, which was prepared from D-2-bromopropionic acid (**2a**) and thionyl chloride and used without isolation, with L-glutamine was carried out in water. There were problems associated with the decomposition of the acid chloride as well as the difficulty of the product isolation.

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(1) Abumrad, N. N.; Morse, E. L.; Lochs, H.; Williams, P. E.; Adibi, S. A. *Am. J. Pysiol*. **1989**, *257*, E228 and references therein.

(2) Yoshida, S.; Shirouzu, Y.; Yoshizumi, T.; Ishibashi, N.; Noake, T.; Kaibara, A.; Yamasaki, K.; Shirouzu, K. *JJPEN* **1996**, *18*, 381.

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Scheme 1

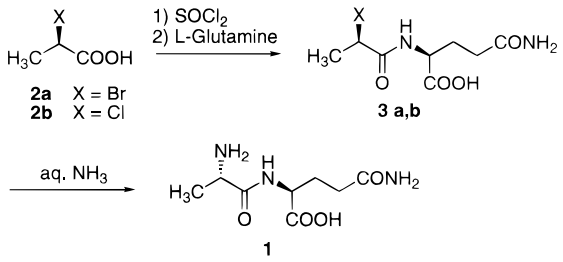

Table 1. **Production of 3a and 3b**[a]

substrate[b] (scale: g)	product	yield[c] (%)	purity[d] (%)	diastereomeric excess (% de)
2a (11.5 g)	**3a**	72.4	98.1	97.1
2a (880 g)	**3a**	70.0	97.5	96.6
2b (600 g)	**3b**	81.7	99.8	99.7

[a] Reactions were carried out via acid chloride, see Experimental Section. [b] Optical purity **2a**: 87.4 % ee, **2b**: 98.8 % ee. [c] Isolated yields from **2a** and **2b**. [d] HPLC area % of the isolated **3a** and **3b**.

These problems seemed to be overcome under the Schotten−Baumann procedures. The reaction of D-2-bromopropionyl chloride, prepared from **2a**, and L-glutamine proceeded fast in a two-phase condition of toluene−water with alkali. The produced D-2-bromopropionyl-L-glutamine (**3a**) crystallized from the water layer after acidifying. However, on a large scale, the produced D-2-bromopropionyl-L-glutamine (**3a**) showed lower diastereomeric excess[9] and contained a larger amount of impurities than on a small scale (Table 1), presumably due to the instability of D-2-bromopropionyl-L-glutamine (**3a**). Therefore, the authors modified the method of using D-2-bromopropionyl-L-glutamine (**3a**) to its chloro analogue, D-2-chloropropionyl-L-glutamine (**3b**), because the chloro substituent was more stable than the bromo substituent. The starting material, D-2-chloropropionic acid (**2b**), is obtained by a method similar to the bromo compound, that is, synthesis from D-alanine,[10] L-lactate,[11] or enzymatic resolution of DL-chloropropionic acid or its ester.[12]

The stability of bromo and chloro intermediates **3a**,**b** in each condition of pH 10 (reaction), pH 6 (neutralization), pH 2 (crystallization) is shown in Figures 1−5. The diastereomeric excess of D-2-bromopropionyl-L-glutamine (**3a**) remains almost unchanged at pH 10 and 6 at 30 °C; yet, at 50 °C it decreased at each pH (Figures 1 and 2). The content, which was measured by HPLC method, decreased at pH 10 and 6 at 50 °C (Figure 4). At pH 2, the

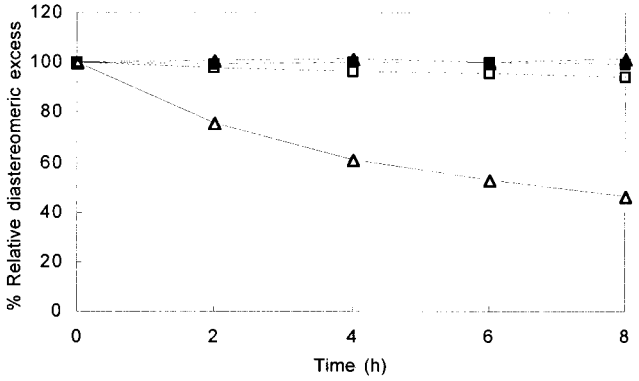

Figure 1. **Stability of 3a and 3b: diastereomeric excess of 3a and 3b at pH 10. Temperature, 50 °C and 30 °C. Key to symbols: △, 3a, 50 °C; □, 3a, 30 °C; ▲, 3b, 50 °C; ■, 3b, 30 °C.**

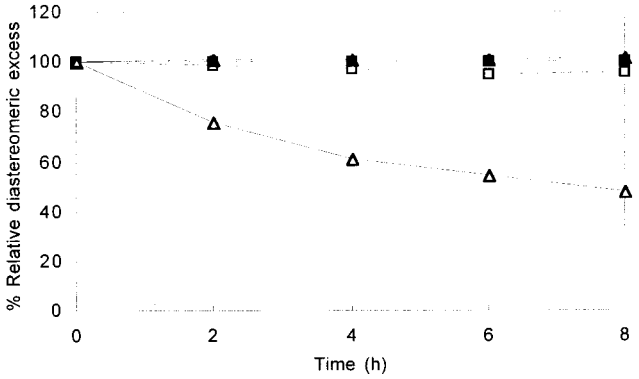

Figure 2. **Stability of 3a and 3b: diastereomeric excess of 3a and 3b at pH 6. Temperature, 50 °C and 30 °C. Key to symbols: △, 3a, 50 °C; □, 3a, 30 °C; ▲, 3b, 50 °C; ■, 3b, 30 °C.**

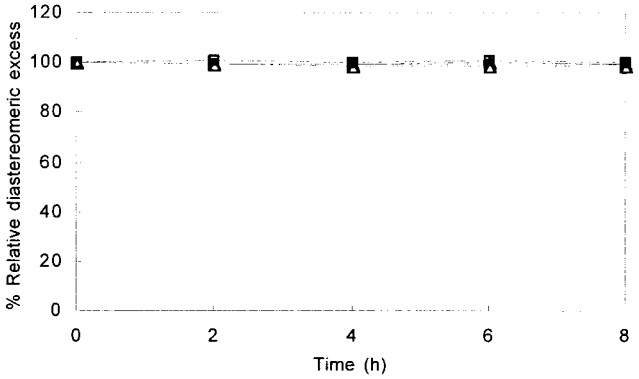

Figure 3. **Stability of 3a and 3b: diastereomeric excess of 3a and 3b at pH 2. Temperature, 50 °C and 30 °C. Key to symbols: △, 3a, 50 °C; □, 3a, 30 °C; ▲, 3b, 50 °C; ■, 3b, 30 °C.**

diastereomeric excess of D-2-bromopropionyl-L-glutamine (**3a**) did not change even at 50 °C (Figure 3), and the content slightly decreased (Figure 4). The stability at pH 2 was probably due to insolubility of the crystals.[13] These results were also observed when using the real reaction solution adjusted to each pH (data are not shown). On the other hand, D-2-chloropropionyl-L-glutamine (**3b**) did not show any decrease both of the diastereomeric excess (Figures 1−3) and the content (Figure 5) at each pH and temperature. Therefore, in the manufacture of AlaGln, the use of D-2-

(9) Pure L-isomer of glutamine was used in the reaction, and at this reaction step the racemization to D-glutamine or the amount of intermediates containing D-glutamine (D-2-bromopropionyl-D-glutamine, L-2-bromopropionyl-D-glutamine) were not checked. At the final step, D-glutamine-containing peptides (L-alanlyl-D-glutamine and D-alanyl-D-glutamine) were monitored by HPLC; however, they were not detected either in the reaction mixture or in the final product.[18] Therefore, the optical purity was represented by diastereomeric excess % (% de) of D-2-bromopropionyl-L-glutamine to L-2-bromopropionyl-L-glutamine. In the production of D-2-chloropropionyl-L-glutamine, the same representation on the optical purity is also used.

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(11) Gerrard, W.; Richmond, M. J. *J. Chem. Soc*. **1945**, 853.
(12) Kircher, G.; Scollar, M. P.; Kribanov, A. M. *J. Am. Chem. Soc*. **1985,** *107*, 7072.

(13) In these experiments, at pH 2 the substrates were not soluble in water, and the data were obtained by using the undissolved heterogeneous solution.

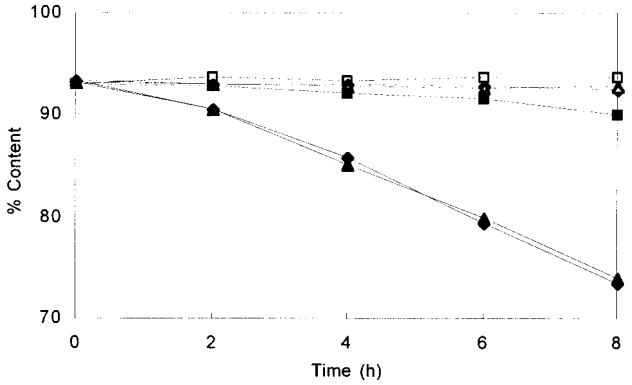


Figure 4. **Stability of 3a: content of 3a at 30 °C and 50 °C. pH, 2, 6 and 10. Key to symbols: □, 30 °C, pH 2; △, 30 °C, pH 6; ◇, 30 °C, pH 10; ■, 50 °C, pH 2; ▲, 50 °C, pH 6; ◆, 50 °C, pH 10.**

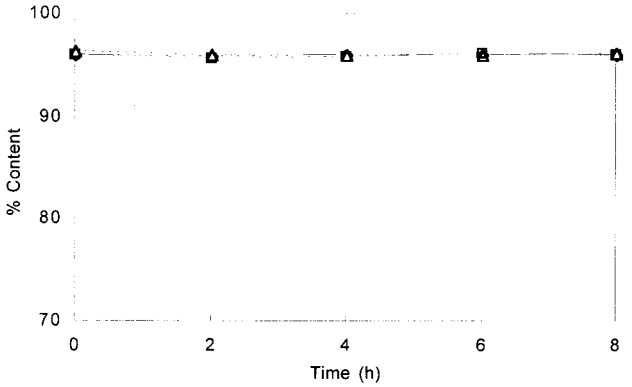


Figure 5. **Stability of 3b: content of 3b at 50 °C. pH, 2, 6 and 10. Key to symbols: □, pH 2; △, pH 6; ◇, pH 10.**

chloropropionyl-L-glutamine (**3b**) was expected to avoid the fatal decomposition in the intermediate stage. Fortunately, the starting material D-2-chloropropionic acid (**2b**) was obtained with higher optical purity than its bromo analogue **2a** (Table 1). Although in the crystallization step the undesired diastereomers remained in the mother liquor, the yield and diastereomeric excess of D-2-halopropionyl-L-glutamine **3a**,**b** depended on the optical purity of the starting acids **2a**,**b**. The result of the production of D-2-chloropropionyl-L-glutamine (**3b**) on a large scale is shown in Table 1.

In the next step, the ammonolysis reaction, the reactivity of D-2-bromopropionyl-L-glutamine (**3a**) and its chloro analogue **3b** were compared. The reaction using D-2-bromopropionyl-L-glutamine (**3a**) and aqueous ammonia was reported to proceed at an elevated temperature of about 80 °C.[8] However, it was found that the reaction progressed fast at 40 °C and slowly even at room temperature. The reaction of D-2-chloropropionyl-L-glutamine (**3b**) needed a higher temperature (60 °C), and the diastereomeric excess of the obtained AlaGln was slightly lower than that of the case of using D-2-bromopropionyl-L-glutamine (**3a**) (Table 2).

On the other hand, impurities in the reaction mixture with D-2-chloropropionyl-L-glutamine (**3b**) were greater, presumably due to the higher reaction temperature. Indeed, in the test of stability with the isolated AlaGln, the diastereomeric excess of AlaGln in aqueous ammonia did not change at the

Table 2. **Ammonolysis of 3a and 3b[a]**

substrate diastereomeric excess (% de)	conditions		AlaGln		
	temp (°C)	time (h)	yield[b] (%)	purity[c] (%)	diastereomeric excess (% de)
3a (97.2)	20	20	78.1	99.0	98.9
3a (96.6)	40	6	78.7	99.2	98.6
3b (96.9)	50	15	71.2	97.5	97.4
3b (96.9)	60	9	72.9	98.1	97.5

[a] Other reaction conditions: 28% aq. NH_3 38 mol. equiv to **3a** and **3b**; 40−100 g of subatrates were used. [b] Isolated yields. [c] HPLC area % of the isolated AlaGln; before purification.

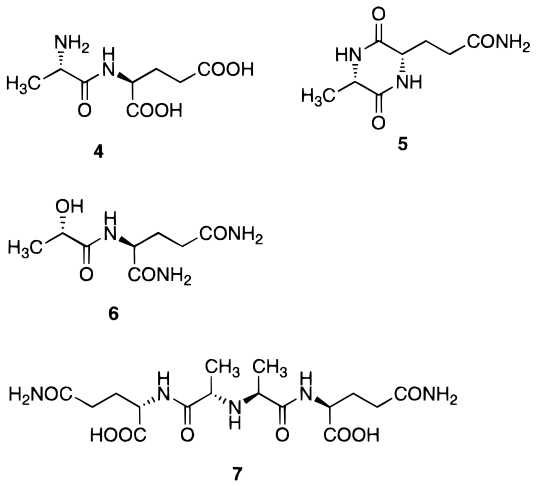


Figure 6. **Structures of by-products.**

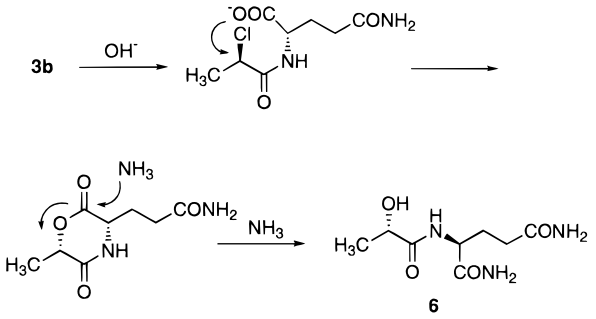
reaction temperature (60 °C); however, the content of AlaGln decreased, and several decomposed products appeared. The chemical structures of the two major decomposition products were inferred from LC-MS analysis as L-alanyl-L-glutamic acid (**4**) and a diketopiperazine **5** derived from AlaGln, respectively (Figure 6). The former was identified by HPLC analysis with the purchased standard sample. The diketopiperazine formation by the thermal decomposition of the dipeptide was well-known.[14] Therefore, for confirming the structure of the compound **5** AlaGln was heated in the differential scanning calorimeter (DSC) apparatus.[15] The thermally decomposed product by being heated until 230 °C was obtained by washing the DSC cell with water and the thus obtained compound was correlated with the diketopiperazine **5** by HPLC analysis.

In the ammonolysis reaction of D-2-chloropropionyl-L-glutamine (**3b**), another by-product was observed, which was almost undetected in the reaction of D-2-bromopropionyl-L-glutamine (**3a**). This compound was isolated by HPLC separation, and the chemical structure was proposed to be a hydrolyzed compound **6** from the mass spectrum. The

(14) Kopple, K. D.; Ghazarian, H. G. *J. Org. Chem.* **1968**, *33*, 862.

(15) Thermogravimetry and differential thermal analysis of AlaGln showed an endothermic peak at 229 °C with loss of 1 mol of water and then gradual decomposition with loss of weight. (TG-DTA conditions; MAC Science TG-DTA 2000, sample amount: ~5 mg, heating rate: 10 °C/min) The endothermic peak was supposed to be the formation of the diketopiperazine. The same thermal analysis pattern was observed with L-alanyl-L-leucine (endothermic peak at 215 °C) and glycyl-L-glutamine (at 216 °C).

Scheme 2



structure and the stereochemistry were confirmed by comparing with the synthetic product from L-2-acetoxypropionic acid. While the intermediate was not detected, it was suspected that the hydroxy compound **6** was produced by the intramolecular substitution reaction of the carboxylate anion affording a cyclization intermediate and followed by ammonolysis. Therefore, it finally retained the inverse stereochemistry at the OH-substituted position (Scheme 2).

In the ammonolysis reaction, the method using D-2-bromopropionyl-L-glutamine (**3a**) seems superior than the chloro analogue regarding the yield and gentle reaction condition. However, from the viewpoint of the ease of obtaining the precursor compound mentioned in the previous synthetic step and of cost performance,[16] the authors selected the method of using D-2-chloropropionyl-L-glutamine (**3b**) as the more profitable method for manufacturing AlaGln and optimized the ammonolysis procedures. Typically, the reaction was carried out under heating D-2-chloropropionyl-L-glutamine (**3b**) in 28% aqueous ammonia of 38 equiv based on D-2-chloropropionyl-L-glutamine (**3b**) in a pressure vessel and continued until the amount of D-2-chloropropionyl-L-glutamine (**3b**) decreased to 3% with HPLC monitoring. First, the reaction temperature was surveyed. The reaction at 60 °C finished in 9 h, and at 50 °C it took 15 h. The produced AlaGln was isolated after concentration of the reaction mixture followed by acidification and crystallization from aqueous methanol. The diastereomeric excess and the amount of by-products in the reaction mixture were almost the same at 60 °C and 50 °C, and the results of the reaction were shown in Table 2. In the reaction at 60 °C, the amount of ammonia was decreased gradually from 30 equiv to 5 equiv. The relation between the amount of ammonia and the yield and the diastereomeric excess of the isolated AlaGln are indicated in Figure 7.

While the reaction with 5 equiv of ammonia was slow and did not finish within 15 h, the amount of ammonia was found to be adequate with 10 equiv. The diastereomeric excess of the isolated AlaGln was unchanged in each experiment even at 5 equiv of ammonia. In that case, the unreacted D-2-chloropropionyl-L-glutamine (**3b**) was removed in the crystallization step, and the quality of the isolated AlaGln was excellent; however, the yield was too

(16) When D-2-bromo and D-2-chloropropionic acid (**2a**,**b**) are prepared from L-lactate in the well-known method, the cost depends on $SOBr_2$ and $SOCl_2$. The former is more expensive than the latter. (cf. $SOBr_2$: ¥17,000/100 g, $SOCl_2$: ¥14,900/100 mL (163 g) from the Aldrich catalog, 1998−1999, Japan).

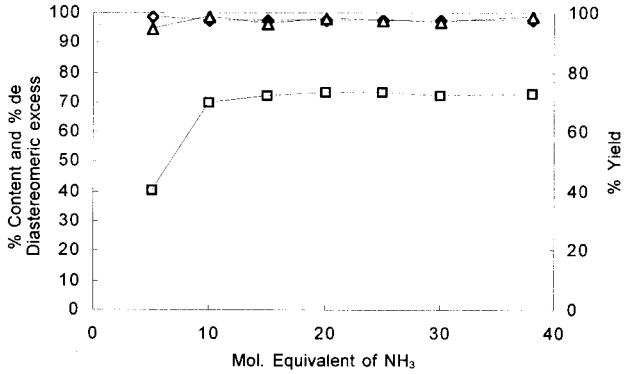


Figure 7. **Relation between the amount of ammonia and production of AlaGln: Reaction temperature, 60 °C. Key to symbols: □, yield; △, content; ◇, diastereomeric excess.**

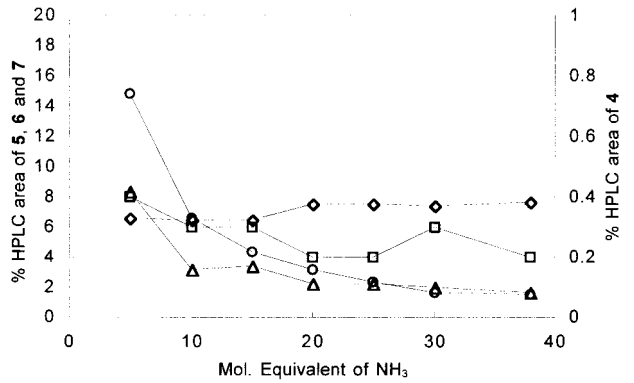


Figure 8. **Relation between the amount of ammonia and by-products 4, 5, 6, and 7 in the reaction mixture. Reaction temperature, 60 °C. Key to symbols: □, 4; △, 5; ◇, 6; ○, 7.**

low (40%). The amounts of impurities in the reaction mixture increased as the amount of ammonia decreased (Figure 8). Two by-products significantly increased as the quantity of ammonia decreased. One of these by-products had already been identified as compound **5**. The other was inferred from LC-MS to be a dimeric product **7**[17](Figure 6). The quantity of two further by-products, **4** and **6**, hardly changed. The compound **4** was observed at no more than 0.4% (HPLC area) even in the reaction with 5 equiv of ammonia.

While most of the by-products produced in the reaction were removed by crystallization, a small portion of dimeric compound **7** as well as another by-product **4** still remained in the crystals. Those compounds have an acidic character; therefore, a process of passing through an anion resin was adapted as a purification step combined with recrystallization. Although the kind of the anion resin was not surveyed, a common anion resin, WA-30 (Mitsubishi Chemical, Co), gave a good result (Table 3; details are described in Experimental Section). As a final step, an activated charcoal treatment of the eluent through the resin after being neutralized and a crystallization from ethanol were adopted from the consideration of decolorization and removal of undesir-

(17) To support the suspected structure of the dimeric compound **7**, the following confirmation was carried out: when AlaGln and D-2-chloropropionyl-L-glutamine (**3b**) was heated with sodium carbonate in water at 100 °C, the product had the same retention time of HPLC analysis and showed the same MS spectrum with the compound **7**. Therefore, the compound **7** was supposed to be derived from the reaction of the produced AlaGln and another molecular of **3b** under ammonia-deficient reaction conditions.

Table 3. **Purification of AlaGln**

	HPLC area (%)[b]					
	1	**4**	**5**	**6**	**7**	isomer
before purification	97.73	0.12	0.02	0.06	0.12	1.28
resin eluent[a]	98.53	N.D.	0.01	0.05	N.D.	1.32
recrystall.	99.80	N.D.	N.D.	N.D.	N.D.	0.19

[a] Collected fractionsts of resin treatment, see Experimental Section. [b] N.D.: Not Detected (less than 0.01 %).

able biological contamination such as endotoxins. The use of ethanol was also adopted from a wider allowance of the residual solvent than methanol.

Summary

Process research and development of AlaGln have been performed for large-scale manufacture. From the viewpoint of the stability and the cost, the method via D-2-chloropropionyl-L-glutamine (**3b**) was adopted. In the following ammonolysis reaction, the structures of the by-products were inferred mainly from mass spectrometry, and a method removing these compounds by passing through the anion resin was developed. From the studies described here, a manufacturing method of AlaGln used for a component of parenteral nutrition has been accomplished.

Experimental Section

General. ^{1}H NMR spectra were recorded at 300 MHz on a Bruker AC-300 spectrometer, and signals are given in ppm using TMS as an internal standard. IR spectra were recorded on a Shimadzu FTIR-4300 spectrophotometer. Optical rotation was measured with Jasco P-1020 polarimeter. HRMS were recorded on a Micromass LCT or a JEOL LMS SX-102 mass spectrometer. LC-MS spectra were recorded on Micromass Qauatro mass spectrometer. D-2-Bromopropionic acid (**2a**; (*R*)-2-bromopropionic acid) was purchased from Osaka Synthetic Chemical Laboratories Inc. and D-2-chloropropionic acid (**2b**; (*R*)-2-chloropropionic acid) was purchased from Nippon Fine Chemical Co., Ltd. For HPLC analysis of the diastereomeric excess,[9] L-alanyl-D-glutamine and D-alanyl-L-glutamine were purchased from Bachem AG, and D-alanyl-D-glutamine was synthesized.[8] Other reagents and solvents are of commercial quality.

HPLC analyses: Purity and optical purity of **3a**: column, YMC-Pack ODS-AQ313 (YMC), eluent: 0.01 mol/L KH_2PO_4; detection, UV 210 nm; retention times, **3a**: 31.0 min, isomer (L-2-bromopropionyl-L-glutamine): 37.5 min. Purity of **3b**: column, Shim-Pack CLC-ODS (Shimadzu); eluent, aqueous solution of 0.01 mol/L KH_2PO_4 and 0.01 mol/L sodium octanesulfonic acid adjusted to pH 2.5 by H_3PO_4: MeOH (100:1); detection, UV 210 nm; retention time, **3b**: 6.3 min. Optical purity of **3b**: column, YMC-Pack ODS-AQ313; eluent 0.01 mol/L KH_2PO_4; retention times, **3b**: 25.1 min, isomer (L-2-chloropropionyl-L-glutamine): 27.9 min. Purity and diastereomeric excess of AlaGln(**1**):[18] column, TSK gel ODS-120 T (Tosoh); eluent, aqueous solution of 0.01 mol/L KH_2PO_4 and 0.01 mol/L sodium octanesulfonic acid adjusted to pH 2.5 by H_3PO_4:MeOH (100:1); detection, UV 210 nm; retention times, **1**: 15.0 min, isomer (D-alanyl-L-glutamine): 18.4 min, **4**: 26.3 min, **5**: 6.0 min, **6**: 4.0 min, **7**: 34.0 min.

LC-MS analyses: HPLC conditions: column, Lichrosorb-NH_2 (GL Science); eluent, 0.05 mol/L $AcONH_4$ and MeCN (35:65), detection UV 210 nm, retention times, **4**: 44.7 min, **5**: 3.3 min, **7**: 51.0 min. MS conditions: ion mode, ESI positive; scan range, *m*/*z* 30−1000; source temp. 120 °C. Found (*m*/*z*) L-alanyl-L-glutamic acid (**4**) $C_8H_{14}N_2O_5$: 219 $(M + H)^+$, (2*S*,5*S*)-2-(2-carbamoylethyl)-5-methylpiperazine-3,6-dione (**5**) $C_8H_{13}N_3O_3$: 200 $(M + H)^+$, (2*S*,4*S*)-3-aza-2,4-dimethyl-1,5-pentanedioyl-di-L-glutamine (**7**) $C_{16}H_{27}N_5O_8$: 418 $(M + H)^+$.

Synthesis of D-2-Chloropropionyl-L-glutamine (3b). To D-2-chloropropionic acid (600 g, 5.53 mol; 98.8% ee), was added $SOCl_2$ (724 g, 6.08 mol) at 65 °C (*Caution: the scrubber should be completely prepared for removing poisonous gas*!) and heated further for 1 h at 85 °C. The reaction mixture was cooled to room temperature to give 745 g of the oily product. The mixture (722 g) was diluted with toluene (400 mL) to make a toluene solution of D-2-chloropropionyl chloride (5.36 mol). A mixture of L-glutamine (784 g, 5.36 mol), H_2O (300 mL), and toluene (150 mL) was cooled to 0−5 °C and stirred, and then to the cooled solution was added 5 mol/L aqueous NaOH (1000 mL). To the solution, were added concurrently a toluene solution of D-2-chloropropionyl chloride described above and 5 mol/L aqueous NaOH below 10 °C while maintaining the pH of the reaction solution at pH 10. The reaction mixture was stirred further 1 h at 10 °C. To the mixture, was added concentrated HCl (30 mL) at room temperature to adjust the pH to 6. Then, toluene was removed by extraction. To the water layer, was added concentrated HCl (410 mL) at room temperature to adjust the pH to 2. The precipitated crystals were filtered and dried in vacuo to afford **3b**: 1038 g (4.38 mol; diastereomeric excess 99.7% de; yield 81.7% from D-2-chloropropionic acid); mp 153 °C dec (recrystallized twice from H_2O); $[\alpha]^{20}_D$ −9.9 ° (*c* = 5, H_2O); ^{1}H NMR (DMSO-d_6) δ = 1.54 (3H, d, *J* = 6.6 Hz, *CH*$_3$), 1.70−2.10 (2H, m, *CH*$_2$CH$_2$CONH$_2$), 2.14 (2H, t, *J* = 7.1 Hz, CH$_2$*CH*$_2$CONH$_2$), 4.13−4.23 (1H, m, *CH*NH−), 4.59 (1H, q, *J* = 6.7 Hz, *CH*Cl), 6.82 (1H, s, CO*NH*), 7.37 (1H, s, CO*NH*), 8.60 (1H, d, *J* = 7.7 Hz, −*NH*−); IR (KBr) υ = 1738, 1662 cm^{-1}; HRESIMS calcd for $C_8H_{13}{}^{35}ClN_2O_4$ *m*/*z* 235.0486 $(M - H)^+$, found 235.0494.

Synthesis of L-Alanyl-L-glutamine (1). D-2-Chloropropionyl-L-glutamine (**3b**) (60.0 g, 0.24 mol; 96.9% de) and 28% aqueous NH_3 (600 mL) were put into a 1 L glass autoclave with mechanical stirring at room temperature. The resulting solution was allowed to stand at 60 °C for 8 h. The reaction mixture was cooled to room temperature and concentrated to ∼150 mL in vacuo, and then MeOH was added dropwise to the residue at room temperature. The

(18) The optical purity of AlaGln(**1**) was calculated as diastereomeric excess % (% de) of **1** to D-alanyl-L-glutamine from the HPLC analysis described in the Experimental Section. Other isomers were confirmed not to be contained in the final product by the following HLPC conditions: column, Crown-Pack CR (+) and/or (−) (Daicel); eluent, 0.1% aqueous $HClO_4$ (pH 2); detection, UV 210 nm.

precipitated crystals were filtered and dried in vacuo to afford 35.4 g (diastereomeric excess 97.6% de; yield 69.0%) of a crude product. A solution of the crude product (50.0 g) in H_2O (100 mL) was passed through an anion resin column packed with WA-30 (50 mL), and H_2O was charged continuously (SV = 1). Fractions containing AlaGln were collected (~250 mL) and concentrated in vacuo until 100 mL. The solution was treated with activated carbon (0.5 g) and filtered. The filtrate was heated to 50 °C, and EtOH (50 mL) was added dropwise to the solution at 50 °C. The solution was gradually cooled to room temperature, and the precipitated crystals were taken out by filtration and dried in vacuo to afford the purified L-alanyl-L-glutamine (**1**): 42.5 g; mp 217 °C dec (lit.[5] 215−216 °C dec); $[\alpha]^{20}_D$ −3.4 ° (*c* = 10, 1 mol/L HCl).

Synthesis of L-2-Hydroxypropionyl-L-glutamic acid Diamide (6). To a solution of L-2-acetoxypropionic acid (1.0 g, 7.6 mmol) in THF (30 mL) was added 5 mL of THF solution of DCC (1.6 g, 7.6 mmol) at 0 °C and then a solution of L-glutamic acid diamide (1,1 g, 7.6 mmol) and Et_3N (0.8 g, 7.6 mmol) in water (5 mL) was added to the cooled solution. The mixture was stirred at 0 °C for 3 h, and the precipitated white solid was filtered. To the filtrate was added 28% aqueous ammonia, and the mixture was stirred at 25 °C for 2 h, and then the reaction mixture was concentrated to half volume in vacuo. The concentrated solution was passed through an anion resin column packed with WA-30 (30 mL), and the fractions containing the desired compound were collected. The collected fractions were passed through a cation resin column packed with SK-1B (30 mL), and the fractions were combined and concentrated in vacuo to afford a white solid of **6**: 0.95 g (57.3%); $[\alpha]^{20}_D$ −7.9 ° (*c* = 1.25, H_2O); ^{1}H NMR (DMSO-d_6) δ = 1.28 (d, *J* = 6.8 Hz, 3H,*CH*$_3$), 1.80−2.00 (m, 2H, *CH*$_2$CH$_2$CONH$_2$), 2.14 (t, *J* = 7.6 Hz, 2H, CH$_2$*CH*$_2$CONH$_2$), 4.06 (q, *J* = 6.6 Hz, 1H, *CH*OH), 4.28−4.32 (m, 1H, *CH*NH−), 6.81 (s, 1H, CO*NH*), 7.20 (s, 1H, CO*NH*), 7.41 (s, 1H, CO*NH*), 7.54 (s, 1H, CO*NH*), 7.77 (d, *J* = 8.0 Hz, 1H, −*NH*−); IR (KBr) υ = 3400, 1665 cm^{-1}. A portion of this solid was purified further by HPLC separation for identification: HRFABMS calcd for $C_8H_{15}N_3O_4$ *m*/*z* 218.1141 $(M + H)^+$, found 218.1115.

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