# Synthesis of N-Substituted Glucosamine Derivatives and Their Angiotensin I Converting Enzyme Inhibitory Activity.

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**Summary**

Inhibitory activity of glucosamine derivatives against angiotensin I converting enzyme (ACE) was investigated. Many N-substituted glucosamine derivatives were synthesized *via* 1,3,4,6-tetra-O-acetyl-glucosamine hydrochloride. Among them, N-(phenylalanyl-alanyl-prolyl)-glucosamine was found to be the most effective for ACE inhibition, and the half inhibitory concentrations ($IC_{50}$) was estimated as 300$\mu$M. Furthermore, N-(3, 7-dimethyl-2(E/Z), 6-hexadien-carbonyl)-glucosamine and N-(p-dimethylamino benzoyl)-glucosamine were found to be effective for the inhibition of ACE, and their $IC_{50}$ values were estimated as 5 mM and 8 mM, respectively.

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**Key words :** glucosamine, Angiotensin I converting enzyme, inhibitor.

## Introduction

Recently, carbohydrate conjugates with amino acids or peptides have received considerable attention because of their unique characteristics and physiological activities.[1)-5)] $\alpha$-D-Glucosamine (GlcN), a kind of aminosugar, has been found in natural glucoconjugates and is well known to be the component of chitin or mucin. Furthermore, GlcN was proposed to be important moiety for the exhibition of physiological activities of the conjugates and many derivatives of it have been synthesized[1),6)-7)]. In addition, the inhibitory activity of GlcN on the angiotensin I converting enzyme (ACE [EC 3.4.15.1] has been reported[8)]. In the course of developing new artificial sweeteners using GlcN derivatives[9)], we found that some of the derivatives have the potent ACE inhibitory activity. The inhibitors of ACE is expected to decrease the blood pressure and to be drugs for antihypertension. So in the present study, many derivatives were synthesized by introducing carboxylic acids into the amino group of GlcN and their ACE inhibitory activities were estimated. The synthetic routes of N-substituted GlcN derivatives had

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been reported by Rocci *et al.*[7], but their direct condensation method produced many by-products. Therefore, a new synthetic route of N-substituted GlcN derivatives *via* 1,3,4,6-tetra-O-acetyl-GlcN was performed.

## Experiments

**General procedures.** ACE (0.25 U) from rabbit lung was purchased from Sigma Chemical Co. Hippuryl-histidyl-leucine (Hip-His-Leu) and t-butoxycarbonyl amino acids were obtained from Peptide Institute Inc. GlcN hydrochloride, fatty acids, aromatic carboxylic acids and other synthetic reagents were purchased from Wako Pure Chemical Co.

The purity of the products and intermediates obtained in the present study was confirmed by HPLC analysis using TOSOH SC8010 HPLC system. Thin layer chromatography (TLC) was carried out on Merck Silica Layer 60 with the solvent system, chloroform : methanol : acetic acid (85 : 15 : 3 v/v). The chemical structure of the products and intermediates were examined by IR, $^{1}H$ NMR and $^{13}C$ NMR analysis. At the measurements of melting points for the identification of products and others, all obtained values are uncorrected.

**Estimation of ACE inhibitory activity.** The ACE inhibitory activities of all the products and intermediates were assayed *in vitro* by a modification of the method of Cushman and Cheung[10]. ACE inhibitor, Hip-His-Leu (5 mM) and NaCl (300 mM) were dissolved in 100 mM sodium borate buffer (pH 8.3) and incubated with 2.5 mU of ACE at 37°C for 2 hours. After the reaction of ACE, the concentration of separated hippuric acid was detected by HPLC. The concentration of ACE inhibitor required to inhibit 50% of ACE activity was defined as the $IC_{50}$ value.

**Preparation of N-substituted GlcN derivatives.** All the experiments were conducted

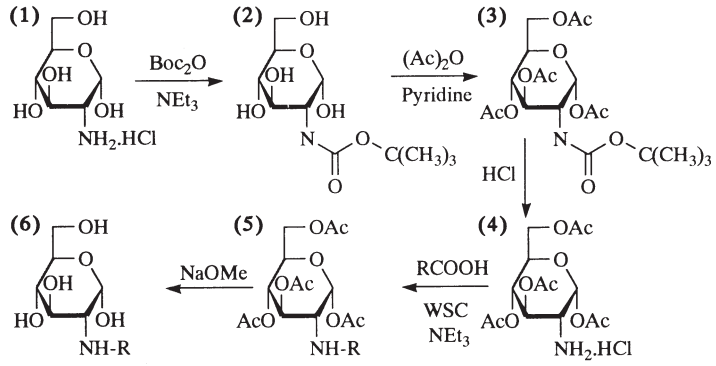



**Fig. 1 Synthetic routes of N-substituted GlcN derivatives**

$Boc_2O$ : Di-t-butyldicarbonate, $Ac_2O$ : Acetic anhydride, Ac : Acetyl
WSC : Water soluble carbodiimide, $NEt_3$ : Triethylamine, Me : Methyl

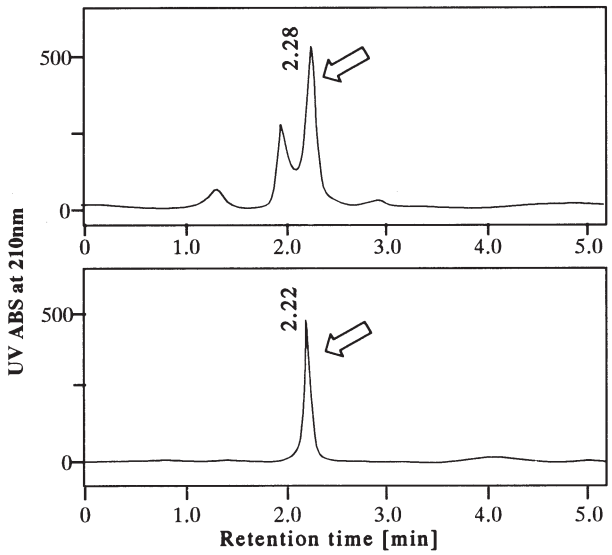



**Fig. 2 Results of HPLC analysis of N-phenylalanyl-GlcN. HCl synthesized with direct condensation method[7] (upper) and *via* 1,3,4,6-tetra-O-acetyl-GlcN (lower).**
HPLC: TOSOH TSKgel-ODS120T 150mm×4.8mm
10 % $CH_3CN$/Water, 1 ml/min, 40°C.

at room temperature, unless noted otherwise. Figure 1 shows our new strategy for the synthesis of N-substituted GlcN *via* 1,3,4,6-tetra-O-acetyl-GlcN. According to the method of Pozdnev[11], N-t-butoxycarbonyl-GlcN**(2)** was synthesized. After the substitution of hydroxyl groups with acetyl groups; **(3)**, treatment with 4M HCl in dioxane yielded 1,3,4,6-tetra-O-acetyl-GlcN hydrochloride**(4)**. After the introduction of a carboxylic acid by using water soluble carbodiimide, the intermediate**(5)** was treated with 1M sodium methoxide in methanol solution to produce the proposed compound**(6)**. Results of identification by HPLC analysis were demonstrated in Figure 2.

**Synthesis of N-phenylalanyl-GlcN hydrochloride.**

**(a) Preparation of N-t-butoxycarbonyl-GlcN*;2*.** GlcN hydrochloride (4.31 g) and triethylamine (2.0g) were dissolved in 50 ml of 50% aqueous dioxane solution. Di-t-butyldicarbonate (12.0g) was added to the solution and stirred over night. To the reaction mixture, 200 ml of ethyl acetate was added and stirred for 30 min. After the filtration, the precipitate was gathered and washed with diethyl ether; yield=10.44g (93.5%), M. p. 110-111°C, $[\alpha]^{22°}$(methanol, C=1)+61.4°, Rf=0.20.

**(b) Preparation of 1,3,4,6-tetra-O-acetyl-GlcN hydrochloride *;4*.** Compound ***2*** (2.79g) was dissolved in pyridine (25 ml) and acetic anhydride (10.2g) was added, and the solution was stirred over night. The reaction mixture was evaporated *in vacuo* and the residue was dissolved in ethyl acetate, and the solution was washed with 4% $NaHCO_3$, 10% citric

**Table 1. $^1$H NMR chemical shifts ($\delta$, ppm) of compound *2*–*7*.**

| Compound*1 | Sugar ring | | | | | | Introduced moiety | |
|---|---|---|---|---|---|---|---|---|
| | H-1 | H-2 | H-3 | H-4 | H-5 | H-6′ | | |
| ***2***[a] | 4.96t | 3.11m | 3.1-3.6m | 3.60m | 4.41t | 4.62dd 4.84dd | **Boc** | 1.38s,6.34d |
| ***3***[b] | 6.17d | 4.00m | 5.26m | 5.16m | 4.27m | 4.10m 4.30m | **Boc** | 1.41s,4.70d |
| | | | | | | | **Ac** | 2.04,2.06,2.11,2.19;s |
| ***4***[c] | 6.36d | 4.00dd | 5.27t | 5.16t | 4.30m | 4.14d 4.37d | **Ac** | 2.10,2.14,2.23,2.25;s |
| ***5***[b] | 6.17d | 4.00m | 5.29t | 5.19t | 4.44dt | 4.03dd 4.25dd | **Boc** | 1.43s,6.45d |
| | | | | | | | **Ac** | 2.03,2.04,2.09,2.20;s |
| | | | | | | | **Phe** | 3.10m,4.60q,7.10,7.30dd |
| ***6***[c] | 5.25m | 3.40m | 3.4-3.5m | 3.50m | 3.80t | 3.70m 3.80m | **Boc** | 1.42s,6.45d |
| | | | | | | | **Phe** | 3.13m,4.40q,7.30m |
| ***7***[c] | 5.91d | 3.17m | 3.2-3.6m | 3.69m | 4.66t | 5.03d 5.03d | **Phe** | 3.08m,4.25q,7.20s |

*1 : These numbers correspounds to the compounds shown in the text.
a : In $(CD_3)_2SO$(from $Me_4Si$), b : $CDCl_3$(from $Me_4Si$),
c : $D_2O$(from Sodium 2, 2-dimethyl-2-silapentane-5-salfonate).

**Table 2. $^{13}$C NMR chemical shifts ($\delta$, ppm) of compounds *2*–*7*.**

| No.*1 | Sugar ring | | | | | | Introduced moiety*2 | |
|---|---|---|---|---|---|---|---|---|
| | C-1 | C-2 | C-3 | C-4 | C-5 | C-6 | | |
| ***2***[a] | 90.7 | 55.7 | 71.9 | 70.2 | 70.9 | 61.1 | **Boc**– | 155.3,77.5;$C_4$/28.2;$C_1$ |
| ***3***[b] | 91.1 | 52.2 | 71.0 | 67.8 | 69.7 | 61.6 | **Boc**– | 154.9,80.4;$C_4$/28.2;$C_1$ |
| | | | | | | | **Ac** | 171.1,170.7,169.2,168.7;$C_4$/20.9,20.7,20.6;$C_1$ |
| ***4***[c] | 91.5 | 53.3 | 71.9 | 71.1 | 69.2 | 64.2 | **Ac** | 176.2,175.5,175.2,174.0;$C_4$/22.8,22.8,22.7, 22.6;$C_1$ |
| ***5***[b] | 90.4 | 50.9 | 70.5 | 67.6 | 69.7 | 61.6 | **Ac** | 171.4,170.7,169.2,168.7;$C_4$/20.9,20.7,20.6, |
| | | | | | | | **Boc**–**Phe**– | 20.5;$C_1$ 171.7,155.0,136.2,72.1;$C_4$/28.0,54.3,125.9,127.7, 129.3;$C_{3,1}$/37.7;$C_2$ |
| ***6***[c] | 97.1 | 53.5 | 77.9 | 72.9 | 73.4 | 63.6 | **Boc**–**Phe**– | 171.6,151.3,136.0,71.9;$C_4$/28.1,54.0,126.1,127.5, 129.1;$C_{3,1}$/37.5;$C_2$ |
| ***7***[c] | 95.0 | 54.8 | 75.3 | 69.7 | 69.7 | 60.2 | **Phe**– | 168.1,136.1;$C_4$/51.8,125.1,127.5,129.3;$C_{3,1}$/ 36.2;$C_2$ |

*1 : These numbers correspounds to the compounds shown in the text.
*2 : $C_1$,primary carbon ; $C_2$,secondary carbone ; $C_3$,tertiary carbon ; $C_4$,quaternary carbon.
a : In $(CD_3)_2SO$(from $Me_4Si$), b : $CDCl_3$(from $Me_4Si$),
c : $D_2O$(from Sodium 2, 2-dimethyl-2-silapentane-5-salfonate).

acid and saturated NaCl solutions, successively. After the removal of moisture by anhydrous $Na_2SO_4$, the solution was evaporated *in vacuo* ; compound ***3***. To the residue, 4M HCl in dioxane (50 ml) was added and the resultant solution was left for 2 hours. Diethyl ether (100 ml) was added to the solution and the precipitate was filtrated, compound ***4***; 2.32g (66.2%), M. p. 150–152(dec.), $[\alpha]^{21°}$($H_2O$, C=1)+136.5°. Rf=0.76.

**Table 3. Physical Constants of N-substituted-GlcN derivatives.**

| Introduced moiety | Yield (%) | M. p. (°C) | Rf |
|---|---|---|---|
| Propionic acid | 38 | 145-150(dec.) | 0.06 |
| n-Butyric acid | 23 | 142-145(dec.) | 0.08 |
| iso-Butyric acid | 24 | 145-150(dec.) | 0.10 |
| n-Valeric acid | 23 | 138-140(dec.) | 0.16 |
| iso-Valelic acid | 23 | 143-140(dec.) | 0.12 |
| 2-Methyl-butyric acid | 21 | 147-148(dec.) | 0.40 |
| n-Caproic acid | 23 | 135-137(dec.) | 0.18 |
| 3,7-Dimethyl-2,6-octadienoic acid | 10 | 120-121(dec.) | 0.42*1 |
| Benzoic acid | 40 | 148-150(dec.) | 0.38 |
| Phenylacetic acid | 40 | 118-120(dec.) | 0.16 |
| Hydrocinnamic acid | 46 | 145-150(dec.) | 0.20 |
| Cinnamic acid | 36 | 108-110(dec.) | 0.10 |
| Cuminic acid | 46 | 122-123(dec.) | 0.20 |
| Phenoxyacetic acid | 43 | 108-110(dec.) | 0.22 |
| p-t-Butylbenzoic acid | 45 | 106-108 | 0.03 |
| p-Methoxybenzoic acid | 39 | 94- 96 | 0.02 |
| p-Dimethylaminobenzoic acid | 6 | 116-118 | 0.00 |
| Benzyloxycarbonyl-Phe-Ala-Pro | 20 | 86- 90 | 0.02 |
| Benzyloxycarbonyl-Phe-Ala | 36 | 118-121 | 0.06 |
| Benzyloxycarbonyl-Phe | 38 | 108-110 | 0.26 |
| Benzyloxycarbonyl-Ala-Pro | 38 | 95- 96 | 0.14 |
| Benzyloxycarbonyl-Ala | 54 | Hygroscopic*2 | 0.16 |
| Benzyloxycarbonyl-Pro | 36 | 109-111 | 0.20 |
| Phe-Ala-Pro | 40 | 120-126 | 0.04 |
| Phe-Ala | 74 | 167-168 | 0.14 |
| Phe | 60 | 145-146 | 0.18 |
| Ala-Pro | 25 | Hygroscopic*2 | 0.10 |
| Ala | 31 | Hygroscopic | 0.20 |
| Pro | 10 | Hygroscopic | 0.18 |

*1: 3-E/Z mixture.
Only one spot of the product was detected on TLC plate.

*2: These products had no constant melting points due to the hygroscopic properties.

**(c) Preparation of N-phenylalanyl-GlcN hydrochloride *;7*.** To the solution of compound ***4*** (0.38g), N-t-butoxycarbonyl-phenylalanine (0.26g), triethylamine (0.1g) and water soluble carbodiimide hydrochloride (0.20g) were added and the mixture was stirred at 0°C for 2 hours and over night at room temperature. The reaction mixture was washed with 4% $NaHCO_3$ and 10% citric acid solutions, and distilled water, successively. Then the solution was dried over the anhydrous $Na_2SO_4$. The residue ; (compound ***5***) obtained

by evaporation *in vacuo* was dissolved in methanol (10 ml) and 4 M $CH_3ONa$ in methanol (0.1 ml) was added. The mixture was stirred for 1 hour at room temperature, then added amberlite cation exchanger resin CG-50, (0.1g), stirred for 1 hour and evaporated after the removal of resin by filtration. To the residue ; (compound ***6***), 4 M HCl in dioxane (10 ml) was added and the resultant solution was left for 1 hour. The reaction mixture was evaporated and the residue was triturated with diethyl ether to give product ***7*** ; 0.29g (90%), M. p. 150-151°C (dec.), $[\alpha]^{18°}$($H_2O$, c=1)−2.3°. Rf=0.18.

**Analysis data.** NMR analysis data on compound ***2***-***7*** are summarized in Tables 1 and 2, and physical constants of all products obtained are listed in Table 3.

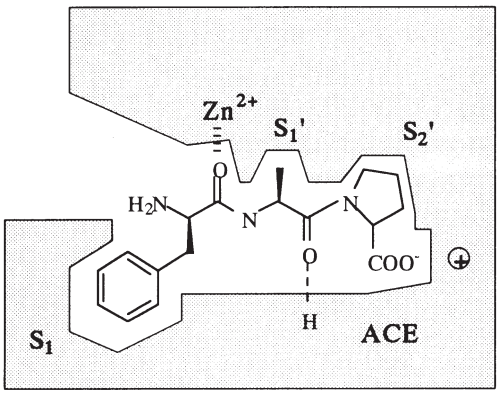
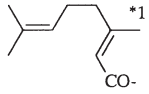



**Fig. 3 Interaction between ACE and Phe-Ala-Pro.**

**Table 4. ACE inhibitory activity of N-substituted GlcN(1).**
**[Fatty acids]**

| Introduced moiety | ACE inhibitory activity (%) | | | |
|---|---|---|---|---|
| | 200 | 20 | 1 | $IC_{50}$[mM] |
| H- | 57 | 30 | 4 | 137 |
| $CH_3CO$- | 37 | 3 | 0 | 483 |
| $CH_3CH_2CO$- | 78 | 44 | 0 | 31 |
| $CH_3CH_2CH_2CO$- | 43 | 10 | 0 | 322 |
| $(CH_3)_2CHCO$- | 43 | 19 | 0 | 411 |
| $CH_3CH_2CH_2CH_2CO$- | 32 | 9 | 0 | >500 |
| $(CH_3)_2CHCH_2CO$- | 27 | 8 | 0 | >500 |
| $CH_3CH_2(CH_3)CHCO$- | 57 | 38 | 12 | 75 |
| $CH_3CH_2CH_2CH_2CH_2CO$- | 10 | 0 | 0 | >500 |
|  | 100 | 99 | 2 | 5 |
| HCl.GlcN$(Ac)_4$ | 97 | 57 | 0 | 15 |



*1 : E/Z mixture.

## Results and Discussion

**Effects of the chain length of N-substituted moiety.** The interaction of an inhibitor and ACE has been investigated by many workers[12]. Figure 3 illustrates the suggested

**Table 5. ACE inhibitory activity of N-substituted GlcN(2).**
**[Aromatic carboxylic acids]**

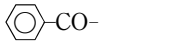
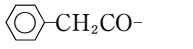
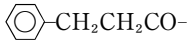
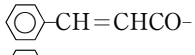
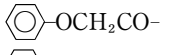
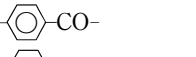
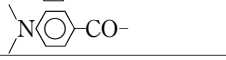
| Introduced moiety | ACE inhibitory activity (%) | | | |
|---|---|---|---|---|
| | 200 | 20 | 1 (mM) | $IC_{50}$ [mM] |
|  | 40 | 24 | 4 | 335 |
|  | 66 | 0 | 0 | 122 |
|  | 27 | 3 | 0 | >1000 |
|  | 22 | 3 | 0 | >1000 |
|  | 66 | 0 | 0 | 124 |
|  | 26 | 11 | 0 | >1000 |
|  | 66 | 9 | 0 | 107 |
|  | 100 | 67 | 10 | 8 |

**Table 6. ACE inhibitory activity of N-substituted GlcN(3).**
**[Peptides]**

| Introduced moiety | ACE inhibitory activity (%) | | |
|---|---|---|---|
| | 1.0 | 0.1(mM) | $IC_{50}$ [mM] |
| Benzyloxycarbonyl-Phe-Ala-Pro | 52 | 19 | 0.8 |
| Benzyloxycarbonyl-Phe-Ala | 8 | 0 | |
| Benzyloxycarbonyl-Phe | 13 | 10 | |
| Benzyloxycarbonyl- Ala-Pro | 25 | 10 | |
| Benzyloxycarbonyl- Ala | 15 | 2 | |
| Benzyloxycarbonyl- Pro | -1 | 0 | |
| Phe-Ala-Pro | 84 | 15 | 0.3 |
| Phe-Ala | 22 | 0 | |
| Phe | 10 | 1 | |
| Ala-Pro | 11 | 0 | |
| Ala | 13 | 0 | |
| ***cf.*** Pro | 0 | 0 | |
| Benzyloxycarbonyl-Phe-Ala-Pro | | 84 | 0.0074 |
| H-Phe-Ala-Pro | | 86 | 0.0014 |
| Benzyloxycarbonyl-Phe-Ala | | 0 | |

interaction between ACE and phenylalanyl-alanyl-proline (Phe-Ala-Pro)[13]. In the active center of ACE, S1' site was proposed to interact with alanine residue. Therefore, fatty acids were introduced into GlcN in the present study. Results of ACE inhibition assay are summarized in Table 4. From the results, all the N-substituted GlcN derivatives influenced the activity of ACE, particularly, N-(3,7-dimethyl-2E/Z,6-hexadien-carbonyl)-GlcN inhibited ACE, and the $IC_{50}$ value was estimated 5 mM. As the $IC_{50}$ values of GlcN itself has been reported to be 140 mM, the present N-substituted GlcN derivatives improved ACE inhibitory activities. No relation was observed between the chain length of the introduced moiety and ACE inhibitory activity.

**Effects of aromatic ring.** As illustrated in Figure 3, aromatic ring is suggested to interact with S1 site of ACE. So the aromatic derivatives were synthesized, and their inhibitory activities were estimated. The results of assay are summarized in Table 5. From the result, N-(p-dimethylaminobenzoyl)-GlcN inhibited the activity of ACE and the $IC_{50}$ value was estimated 8 mM. No relation was observed between the structure of the introduced aromatic moiety and the ACE inhibitory activity.

**Effects of amino acids or peptides.** Some amino acids and peptides consisting of Phe, Ala and Pro were introduced into GlcN, and the results of ACE inhibitory assays are summarized in Table 6. The ACE inhibitory activity increased remarkably by the introduction of these amino acids and peptides into GlcN. Among the derivatives, N-(phenylalanyl-alanyl-prolyl)-GlcN inhibited the activity of ACE and the $IC_{50}$ value was estimated 300 $\mu$M. Introduction of Phe-Ala-Pro into GlcN potentiated obviously the inhibitory activity of glucosamine. However, Phe-Ala-Pro itself has much more potent inhibitory activity than either of them, and then, the binding with the glucosamine reduced its activity largely. Some complex relations between the ACE active center, the peptide and glucosamine are suggested.

## Conclusion

From the present results, some informations about the inhibition from of GlcN against the activity of ACE were obtained. Introduction of the particular moiety into amino group of GlcN, which was suggested to be interact with S1 or S1', S2' site of ACE, increased the inhibitory activity of GlcN to the mM order of $IC_{50}$ values. As the ACE inhibitor derived from protein enzymatic hydrolysates exerts its activity at $\mu$M order[14], the GlcN derivatives obtained here may be still insufficient for a practical use. The proposed mechanism[10),12)] concerning the hydrogen bond between ACE active site and inhibitor suggests that the inhibition also may be done *via* non covalent bond between GlcN derivatives and ACE active site in the case of the present study.

## Reference


1) Horvat S., Horvat J., Varga-defterdarovic L., Pavelic K., Cheung N. N. and Schller P. W., Methionine-enkephalin related glyco conjugates., *Int. J. Peptide Protein Res.,* **41**, 399-404(1993).

2) Tamura M., Shoji M., Nakatsuka T., Kinomura M., Okai H. and Fukui S., Methyl 2,3-di-O-(L-aminobutyryl)-α-D-glucopyranoside, a new sweet substance. and taste of related compounds of neutral amino acids and D-Glucose derivatives., *Agric. Biol. Chem.,* **49**(9), 2579-2586(1985).

3) Tamura M., Miyoshi T., Mori N., Kinomura K., Kawaguchi M., Ishibashi N. and Okai H., Mechanism for the bitter tasting potency of peptides using O-aminoacyl sugars as model compounds., *Agric. Biol. Chem.,* **54**(6), 1401-1409(1990).

4) Tamura M., Nakamura K., Kinomura K. and Okai H., Reletionship between taste and structure of O-aminoacyl sugars countering basic amino acids., *Biosci. Biotech. Biochem.,* **57**(1), 20-23(1993).

5) Ladesic B., Kantoci D., Meider H. and Hadzija O., Complexes of Fe(3) with amino sugars and small glycopeptides., *J. Inorg. Biochem.,* **48**, 55-62(1992).

6) Otvos L. and Kraicsovits F., Nitrogen participation in the deacylation of D-glucosamine and α-Chymotrypsin derivatives. Explanation of the stereo specificity of Acyl-α-Chymotrypsin hydrolysis., *Tetrahedron,* **48**(23). 5009-5014(1992).

7) Rocchi R., Biondi L., Cavaggion F., Filira F., Gobbo M., Dagan S. and Frindkin M., Synthesis and biological activity of tuftsin and rigin derivatives containing monosaccharides or monosaccharide derivatives., *Int. J. Peptide Res.,* **29**, 262-275(1987).

8) Schonherr E., Jones G. A. and Slakey L. L., Gastric and salivary mucins inhibit angiotensin-converting enzyme, *Biochem. J.,* **286**, 425-433(1992).

9) Kayahara H., Kawakami A., Okutani Y., Nakanishi U. and Tadasa K., Synthesis of Perillartine analogies and evaluation of taste., *J. Fac. Agric. Shinshu Univ.,* **28**, 35-44(1991).

10) Cushman D. W. and Cheung H.S., Spectrophotometric assay and properties of the Angiotensin-converting enzyme of rabbit lung., *Biochem. Pharmacol.,* **20**, 1637-1648(1971).

11) Giannis A. and Henk T., Synthese chiraler Bausteine aus D-Glucosamin-Hydrochlorid., *Liebigs. Ann. Chem.,* 789-793(1991). (**Pref**. (9), Pozdnev V. F., *Khim. Prir. Soedin.,* 408. (1980). [*Chem. Abstr.,* 93, 186700b].).

12) Cushman D. W, Cheung H. S., Sabo E. F. and Ondetti M. A., Design of potent competitive inhibitors of Angiotensin-converting enzyme. Carboxyalkanoyl and mercaptoalkanoyl amino acids., *Biochemistry,* **16**, 5484-5491(1977).

13) Maruyama S., Mitachi H., Tanaka H., Tomizuka N. and Suzuki H., Studies on the active site and antihypertensive activity of Angiotensin I converting enzyme inhibitors derived from Casein., *Agric. Biol. Chem.,* **51**(6), 1581-1586(1987).

14) Kawakami A., Kayahara H., Synthesis of Leu-Lys-Tyr derivatives and their interaction with Angiotensin I converting enzyme, *J. Jap. Soc. Nut. Food Sci.,* **46**(5), 425-428(1993).

# Ｎ置換グルコサミン誘導体の合成と それらのアンジオテンシンＩ変換酵素阻害能

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グルコサミン（GlcN）誘導体によるアンジオテンシンＩ変換酵素（ACE）阻害能について研究した。1,3,4,6-tetra-O-acetyl-GlcN 塩酸塩を中間体として多数のＮ置換グルコサミン誘導体を合成した。

その結果，N-(phenylalnyl-alanyl-prolyl)-GlcN が最も強い阻害能を示し，その50％活性阻害濃度 $IC_{50}$ 値は 300$\mu$M と計算された。さらに，N-(3,7-dimethyl-2(E/Z),6-hexadien-carbonyl)-GlcN，及び N-(p-dimethylaminobenzoyl)-GlcN に比較的強い ACE 阻害能の発現が認められ，その $IC_{50}$ 値はそれぞれ5mM 及び8mM と計算された。

キーワード：グルコサミン，アンジオテンシンＩ変換酵素，阻害剤

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