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Synthesis of *N*-Tripeptidyl-D-glucosamine by the Stepwise Reaction of *N*-Carboxy α-Amino Acid Anhydride[1)]

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N-Peptidyl-D-glucosamines were synthesized by the stepwise reaction of *N*-carboxy α-amino acid anhydrides (4-alkyl-2,5-oxazolidinediones) with D-glucosamine hydrochloride in the presence of equimolar sodium methoxide in a mixture of acetonitrile and methanol, or in methanol, at a low temperature. Polymerization reaction of *N*-carboxy α-amino acid anhydrides (NCAs) did not occur at −50 °C. The mechanism of the reaction was then studied.

Proteins and peptides containing sugar residues, glycoproteins and glycopeptides, are widely distributed in nature, and have many bioactive properties.[2)] Therefore, the syntnesis of models of glycoproteins and glycopeptides are important. In the carbohydrate-peptide linkage, the covalent linkage between the amino sugar-amino acid bonds, have been considered to be of two types. One is a peptide linkage which is formed between the amino residue of glucosamine and the carboxyl residue of amino acid. This bond is generally synthesized by the coupling reaction of an amino group of glucosamine with a carboxyl residue of an amino acid, the amino residue of which is protected, and following removal of protection.[3-8)] However, many glycopeptides are unstable to acids or bases. They are decomposed with changing color by both acids or bases. It is therefore important to avoid the deprotecting reaction in strong acidic or basic media.

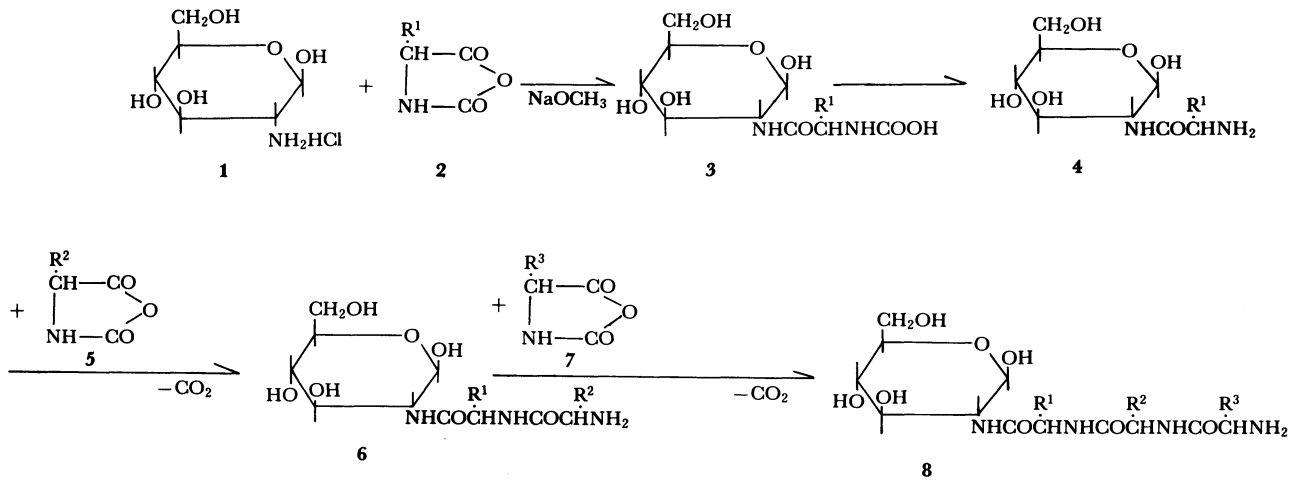
We attempted the synthesis of several *N*-peptidyl-D-glucosamines by the stepwise reaction of *N*-carboxy α-amino acid anhydrides (NCAs) with D-glucosamine having free hydroxyl groups in the mixed solvent of acetonitrile and methanol, or in methanol.

The mechanism of polymerization of NCAs have been studied extensively.[9)] In comparison, there is little information on the stepwise reaction of an α-amino or hydroxyl residue with NCA. Bailey reports the synthesis of dipeptides by the stepwise reaction of NCAs with an amino acid ethyl ester in chloroform at −40 °C.[10)] This reaction does not occur for any amino acid ester except glycine ethyl ester in chloroform because the polymerization reaction of NCAs occur.[11)]

In a previous paper, we reported that the stepwise synthesis of oligopeptides with regular sequence by the method of controlled reaction of NCAs in an acetonitrile water heterogeneous system.[12,13)] In another previous study, we reported the steric hindrance of the stepwise reaction of NCAs with the α-amino acid esters.[14)] These stepwise syntheses of oligopeptides involves no, or limited removal of protecting groups. Further, these reactions did not lead to racemization.[15)]

These studies suggest that NCAs can be utilized for the amino acylation of amino residues of many organic compounds.

When L-alanine was used in the reaction with D-glucosamine, polymerization of the NCA proceeded at −40 °C, but did not proceeded at −50 °C. However the stepwise reaction of L-alanine NCA (**2**; $R^1=CH_3$) with D-glucosamine (**1**) did proceed at −55 °C. It was considered that the steric hindrance of the side chains of both NCA and the compound having α-amino residue determine whether polymerization of NCA or the stepwise reaction proceeds at −20 °C.[11)] As the polymerization of L-alanine NCA proceeded at −40 °C, the side chain of D-glucosamine must be more bulky than that of L-alanine NCA. The fact that the stepwise reaction of L-alanine NCA with D-glucosamine proceeded indicate that the steric hindrance of the side chains of both L-alanine NCA and the α-amino residue of the compound do not affect the reaction at −55 °C. The decarboxylation of the carbamic acid (**3**→**4**) must not occur below −54 °C., since carbamic acids are stable at a low temperature. NCAs are



initiated with alcohols to produce dipeptides, polypeptides, and small amounts of amino acid esters.[9] However, the stepwise reaction of NCA with D-glucosamine does proceed in methanol at −55 °C. This indicates that the rate of reaction of NCA with the amino residue of D-glucosamine is faster than that of NCA with the hydroxyl group of methanol.

N-Dipeptidyl-D-glucosamine (**6**) was synthesized by addition of an acetonitrile solution of NCA (**5**) to the solution obtained by filtration of the reaction mixture of *N*-aminoacyl-D-glucosamine (**4**), followed by the same procedure as described above (**4**+**5**→**6**). *N*-Tripeptidyl-D-glucosamine (**8**) was synthesized by addition of the third NCA (**7**) with *N*-dipeptidyl-D-glucosamine (**6**) by the same synthetic procedure of *N*-dipeptidyl-D-glucosamine (**6**+**7**→**8**).

Experimental

D-Glucosamine hydrochloride and amino acids used were commercial products. NCAs were prepared as reported previously.[16]

NMR spectra were obtained on a Brucker WH-270 spectrometer, the chemical shifts are given in δ values (ppm) from sodium 2,2-dimethyl-2-silapentane-5-sulfonate.

N-L-*Alanyl*-D-*glucosamine* (*4*; $R^1=CH_3$). D-Glucosamine hydrochloride (1.5 g, 23.3 mmol) was dissolved in methanol solution (92 cm³) of sodium methoxide (1.3 g, 24 mmol). The solution of L-alanine NCA (2; $R^1=CH_3$, 2.7 g, 23 mmol) in acetonitrile (92 cm³) was then added, and stirred at −50 °C for 120 h. After the reaction, the mixture was raised to room temperature and filtered to remove a small amount of precipitate, and distilled *in vacuo* to remove the solvent. The residual product was dissolved in ethanol and water (90 : 10 v/v). The solution was filtered, and dried *in vacuo* to obtain pure *N*-L-alanyl-D-glucosamine: yield 6.2 g (80%); $[\alpha]_D^{22}$ −8.3° (*c* 0.012 CH_3OH); NMR[17] (0.04 g/ml D_2O) δ=1.36 (t Ala C<u>H</u>$_3$), 3.29 and 3.73 (m glucosamine OH), 4.4 (Ala CH), 7.23 (m CON<u>H</u> and N<u>H</u>$_2$). Found: C, 43.30; H, 6.88; N, 10.55%. Calcd for $C_9H_{18}O_6N_2$: C, 43.20; H, 6.88; N, 11.20%.

N-(L-*Alanyl*-L-*valyl*)-D-*glucosamine* (*6*; $R^1=(CH_3)_2CH$, $R^2=CH_3$). D-Glucosamine hydrochloride (**1**, 5 g, 23.3 mmol) was dissolved in a methanol solution (92 cm³) of sodium methoxide (1.3 g, 24 mmol). The solution of L-valine NCA (**2**; $R^1=(CH_3)_2CH$, 3.4 g, 24 mmol) in acetonitrile solution (92 cm³) was then added, and stirred for 120 h at −50 °C. After the reaction, the mixture was raised to room temperature and filtered to remove a small amount of precipitate. The filtrate was cooled to −50 °C, the solution of L-alanine NCA (2.6 g, 23 mmol) in acetonitrile (52 cm³) and methanol (52 cm³) was then added, and allowed to react as described above. The reaction mixture was raised to room temperature, filtered, and distilled *in vacuo* to obtain the crude product. The product was dissolved in the minimum amount of methanol, filtered, diluted with a half volume of diethyl ether, and filtered again. The solution part was distilled *in vacuo* to obtain crude *N*-(L-alanyl-L-valyl)-D-glucosamine. The crude product thus obtained was purified by columm chromatography, (Sephadex G-10, 2.5×45 cm). Elution with ethanol and water (1 : 1 v/v) separated contaminent, sodium chloride, and pure *N*-(L-alanyl-L-valyl)-D-glucosamine: yield 2.0 g (26%); $[\alpha]_D^{22}$ −22.7° (*c* 0.016 CH_3OH); NMR (2 mg/cm³ DMSO-d_6) δ=0.88 (q Val (C<u>H</u>$_3$)$_2$), 1.28 (t Ala C<u>H</u>$_3$), 1.59 (Val βC<u>H</u>), 3.38, 3.63, and 3.86 (glucosamine OH), 4.23 (Ala and Val αC<u>H</u>), 7.94, 8.02, 8.12, and 8.95 (N<u>H</u>CO and N<u>H</u>$_2$). Found: C, 48.89; H, 8.12; N, 12.12%. Calcd for $C_{14}H_{27}O_7H_3$: C, 48.14; H, 7.74; N, 12.02%.

N-(*γ-Methyl*-L-*α-glutamyl*-L-*phenylalanyl*-L-*alanyl*)-D-*glucosamine* (*8*; $R^1=CH_3$, $R^2=CH_2C_6H_5$, $R^3=CH_2CH_2COOCH_3$). D-Glucosamine hydrochloride (4.3 g, 20 mmol) was dissolved in a methanol solution (86 cm³) of sodium methoxide (1.1 g, 20 mmol). The solution was cooled to −50 °C. The solution of L-alanine NCA (2.5 g, 21 mmol) in acetonitrile (50 cm³) previously cooled to −50 °C was added to the solution of glucosamine, and allowed to react for 70 h at −50 °C, and subsequently for 70 h at −20 °C. After the reaction the resulting mixture was warmed to room temperature, and filtered to remove the precipitate. The filtrate was cooled to −20 °C. Crystals of L-phenylalanine NCA (3.1 g, 16.2 mmol) were added, and allowed to react for 70 h at −20 °C. After the reaction the system was warmed and filtered to separate the precipitate. The clear solution was cooled again to −20 °C, added to the solution of γ-methyl-L-glutamate NCA (3.3 g, 17.6 mmol) in acetonitrile (35 cm³), and allowed to react as above. After the reaction the resulting mixture was warmed, filtered, and distilled *in vacuo* to obtain the crude product (7.5 g, 71% yield). The crude product was purified as *N*-(L-valyl-L-alanyl)-D-glucosamine. Pure product: $[\alpha]_D^{22}$ −12.7° (*c* 0.0063 CH_3OH); NMR (2 mg/cm³ DMSO-d_6) δ=1.29 (Ala C<u>H</u>$_3$), 1.86 (Glu C<u>H</u>$_2$), 3.03 (Phe C<u>H</u>$_2$), 3.39 and 3.61 (glucosamine OH), 3.62 (Glu COOC<u>H</u>$_3$), 3.64 (glucosamine O<u>H</u>), 4.30 (Ala and Glu αCH), 7.25 (Phe C_6H_5), 7.75 and 8.59 (m CON<u>H</u> and N<u>H</u>$_2$). Found: C, 53.75; H, 5.96; N, 9.84%. Calcd for $C_{24}H_{36}O_{10}N_4$: C, 53.33; H, 6.67; N, 10.37%.

The other *N*-tripeptidyl-D-glucosamines were synthesized by the reactions of D-glucosamine (**1**) with the first NCA (**2**), following the second NCA (**5**), and the third NCA (**7**) under almost similar conditions to the tripeptidyl-D-glucosamine described above (Eq. 1).

N-(L-*Alanyl*-L-*leucyl*-L-*alanyl*)-D-*glucosamine* (*8*; $R^1=CH_3$, $R^2=(CH_3)_2CHCH_2$, $R^3=CH_3$). The pure product was obtained in a 40% yield: $[\alpha]_D^{22}$ −33.1° (*c* 0.0136 CH_3OH); NMR (DMSO-d_6 2 mg/cm³) δ=0.85, 0.87, and 0.90 (Leu (C<u>H</u>$_3$)$_2$), 1.22 (Ala C<u>H</u>$_3$), 1.47 and 1.59 (Leu βC<u>H</u>$_2$ and γC<u>H</u>), 3.38 and 3.63 (glucosamine O<u>H</u>), 4.29 (Ala αC<u>H</u>), 4.39 (Leu αC<u>H</u>), 7.93, 8.12, 8.22, and 8.29 (CON<u>H</u> and N<u>H</u>$_2$). Found: C, 48.78; H, 7.95; N, 12.21%. Calcd for $C_{18}H_{34}O_8N_4$: C, 49.76; H, 7.80; N, 12.12%.

N-(L-*Leucyl*-L-*leucyl*-L-*phenylalanyl*)-D-*glucosamine* (*8*; $R^1=CH_2C_6H_5$, $R^2=(CH_3)_2CHCH_2$, $R^3=(CH_3)_2CHCH_2$). This pure product was obtained in a 35% yield: $[\alpha]_D^{22}$ −22.5° (*c* 0.0142 CH_3OH): NMR (DMSO-d_6 2 mg/cm³) δ=0.92 (t Leu (C<u>H</u>$_3$)$_2$), 1.44 and 1.59 (Leu βCH_2 and γC<u>H</u>), 2.98 (Phe βCH_2), 3.24, 3.30, 3.36, and 3.39 (glucosamine O<u>H</u>), 4.39 (Leu αC<u>H</u>), 4.51 (Phe αC<u>H</u>), 7.24 (Phe C_6<u>H</u>$_5$), 7.81, 8.08, 8.22, and 8.33 (CON<u>H</u> and N<u>H</u>$_2$). Found: C, 59.83; H, 8.00; N, 10.11%. Calcd for $C_{27}H_{44}O_8N_4$: C, 58.70; H, 7.94; N, 10.37%.

N-(L-*Leucyl*-L-*phenylalanyl*-L-*leucyl*)-D-*glucosamine* (*8*; $R^1=(CH_3)_2CHCH_2$, $R^2=CH_2C_6H_5$, $R^3=(CH_3)_2CHCH_2$). The pure product was obtained in a 43% yield. NMR peaks were similar to those of *N*-(L-leucyl-L-leucyl-L-phenylalanyl)-D-glucosamine. $[\alpha]_D^{22}$ −21.6° (*c* 0.0176 CH_3OH). Found: C, 58.70; H, 7.72; N, 10.19%. Calcd for $C_{27}H_{44}O_8N_4$: C, 58.70; H, 7.94; N, 10.37%.

N-(*β-Benzyl*-L-*α-aspartyl*-*β-benzyl*-L-*α-aspartyl*-*β-benzyl*-L-*α-aspartyl*)-D-*glucosamine* (*8*; $R^1=R^2=R^3=CH_2COOCH_2C_6H_5$). Elution of the chromatograph (Sephadex G-10, 2.5×45 cm) was carried out with methanol and water (1 : 1 v/v). The pure product was obtained in a 36% yield. NMR (DMSO-d_6 2 mg/cm³) δ=2.66 and 2.77 (Asp βC<u>H</u>$_2$), 3.22 and 3.59 (glucosamine O<u>H</u>), 4.35 (Asp αC<u>H</u>), 5.13 (Asp Bz C<u>H</u>$_2$), 7.39 (Asp Bz C_6<u>H</u>$_5$), 8.19 (CON<u>H</u> and N<u>H</u>$_2$). Found: C, 57.99; H, 5.49; N, 7.05%. Calcd for $C_{39}H_{46}O_{10}N_4$: C, 58.94; H, 5.70; N, 7.05%. $[\alpha]_D^{22}$ −7.35° (*c* 0.0136 CH_3OH)

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