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Studies on D-Glucosamine Derivatives. XII.*1 The Synthesis of *N*-Acetyl-3-*O*- and -4-*O*-aminoacyl-D-glucosamines

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As a part of the investigation of the stabilities of the esters between *N*-acetyl-D-glucosamine and various amino acids, *N*-acetyl-3-*O*- and 4-*O*-aminoacyl-D-glucosamines were synthesized. Benzyl *N*-acetyl-4, 6-*O*-benzylidene-α- and -β-D-glucosaminide, and benzyl *N*-acetyl-3-*O*-benzyl-6-*O*-trityl-α- and -β-D-glucosaminide were condensed with *N*-benzyloxycarbonyl-amino acids by the dicyclohexylcarbodiimide method, followed by the step-by-step removal of the protecting groups. The α-Glycosidic benzyl group, however, could not be removed by the catalytic hydrogenolysis in the presence of oxalic acid, probably because of a steric hindrance.

In a previous report[1] of this series, 1, 2-dideoxy-1-glycylamino-2-acetamido-D-glucosamines have been synthesized. Such a *N*-acyl-glycosylamine linkage between sugars and amino acids has already been ascertained in glycoproteins[2] and in the process of the biological nucleotide synthesis[3].

However, the corresponding ester linkage[4] has been scarcely found, probably because of its instability. In order to examine the stabilities of the ester linkage between sugars and amino acids, we have synthesized the simple ester compounds between *N*-acetyl-D-glucosamine and various amino acids, of which the selective synthesis of *N*-acetyl-6-*O*-aminoacyl-D-glucosamines was reported in the previous paper.

In the present paper, the synthesis of 3-*O*- and 4-*O*-aminoacyl derivatives of *N*-acetyl-D-glucosamine will be described.

Results

The Synthesis of *N*-Acetyl-3-*O*-aminoacyl-D-glucosamines.—Kochetkov et al.[5] isolated 3-*O*-(*N*-benzyloxycabonyl*2-glycyl)-D-glucose as a by-product in the synthesis of 6-*O*-(*N*-Z-glycyl)-D-glucose from D-glucose and *N*-Z-glycine, while the homologues were synthesized by Muramatsu[6] by the condensation of 1, 2 ; 5, 6-di-*O*-isopropylidene-D-glucofuranose with *N*-Z-amino acids.

For the purpose of introducing the aminoacyl groups into the C-3 hydroxyl group of *N*-acetyl-D-glucosamine, we prepared benzyl *N*-acetyl-4, 6-*O*-benzylidene-α-[7] and -β-D-glucosaminide (A-I and B-I)*3 as starting materials.

The compound B-I was newly prepared by the benzylidenation of benzyl *N*-acetyl-β-D-glucosaminide, which had been isolated from the corresponding α, β-mixture[8] by the fractional crystallization of its acetate.

First of all, A-I was condensed with an equimolar amount of *N*-Z-amino acids (a: *N*-Z-glycine, b: *N*, *N'*-di-Z-L-lysine) in pyridine by dicyclohexylcarbodiimide (DCC) to give benzyl *N*-acetyl-3-*O*-(*N*-Z-aminoacyl)-4, 6-*O*-benzylidene-α-D-glucosaminides (A-II) in 72—84% yields. In the case of B-I, which is much less soluble than A-I in pyridine, such a condensation procedure as A-I gave the corresponding product in a low yield, accompanied by the ureide. In order to avoid the formation of the ureide,[9] a large quantity of *N*-Z-amino-acids (a, b, c; β-benzyl-*N*-Z-L-aspartate) were preliminary converted to the corresponding acid anhydrides by DCC in dioxane. After the dioxane had been removed, a solution of B-I in pyridine was added to the concentrated residue without isolating the acid anhydrides, and the mixed solution was allowed to stand at room temperature

*1 Part XI: *J. Chem. Soc. Japan, Pure Chem. Sect.* (*Nippon Kagaku Zasshi*), **85**, 511 (1964).

1) J. Yoshimura and H. Hashimoto, *J. Chem. Soc. Japan, Pure Chem. Sect.* (*Nippon Kagaku Zasshi*), **85**, 239 (1964).

2) I. Yamashina and M. Makino, *J. Biol. Chem.*, **51**, 359 (1962); V. Bogdanov, E. Kaverzneva and A. Andereyeva, *Biochim. Biopys. Acta*, **65**, 168 (1962); T. H. Jr. Plummer and C. H. W. Hirs, *J. Biol. Chem.*, **239**, 2530 (1964); K. Izumi, M. Makino and I. Yamashina, *J. Biochem.*, **51**, 365 (1962).

3) G. R. Greenberg, *J. Biol. Chem.*, **190**, 611 (1951).

4) A. Gottschalk and W. H. Murphy, *Biochem. Biophys. Acta*, **46**, 81 (1961).

5) N. K. Kochetkov, V. A. Derevitskaya, L. M. Likhosherstov, N. V. Molodtsov and S. G. Kara-Murza, *Tetrahedron*, **18**, 273 (1962).

*2 The benzyloxycarbonyl group is abbreviated as Z.

*3 The prefixes A and B are used for the α- and β-anomers respectively.

6) N. Muramatsu, *J. Chem. Soc. Japan, Pure Chem. Sect.* (*Nippon Kagaku Zasshi*), **84**, 861 (1963).

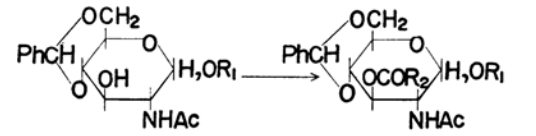
7) R. Kuhn and H. H. Baer, *Ann.*, **611**, 236 (1958).

8) J. Yoshimura, M. Funabashi, S. Ishige and T. Sato, *J. Chem. Soc. Japan, Pure Chem. Sect.* (*Nippon Kagaku Zasshi*), **85**, 142 (1964).

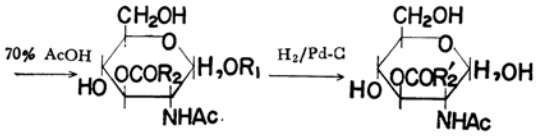
9) I. Muramatsu, ibid., **82**, 83 (1961).

for 24 hours. In this way benzyl *N*-acetyl-3-*O*-(*N*-Z-aminoacyl)-4, 6-*O*-benzylidene-β-D-glucosaminides (B-II) were obtained in 60—70% yields. The infrared spectra of A-II and B-II showed no absorption band of hydroxyl (at ca. 3500 cm^{-1}), but it did show absorption bands of ester carbonyl (at 1730—1750 cm^{-1}) and urethane carbonyl (at 1690—1710 cm^{-1}).

The next step, involving the hydrolysis of the benzylidene group of A-II and B-II with 70% acetic acid, gave benzyl *N*-acetyl-3-*O*-(*N*-Z-aminoacyl)-α- and -β-D-glucosaminides (A-III and B-III) in 60—75% yields.



A-I, B-I A-II, B-II



A-III, B-III IV

$R_1 = -CH_2C_6H_5$ a) $R_2 = -CH_2NHZ$

$Ph = -C_6H_5$ b) $R_2 = -CH(CH_2)_4NHZ$ (NHZ)

$Ac = -COCH_3$

$Z = -COOCH_2C_6H_5$ c) $R_2 = -CHCH_2COOCH_2C_6H_5$ (NHZ)

a) $R'_2 = -CH_2NH_2 \cdot 1/2(COOH)_2 \cdot 2H_2O$

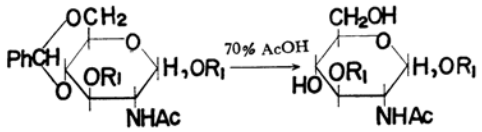
b) $R'_2 = -CH(CH_2)_4NH_2 \cdot (COOH)_2 3H_2O$ (NH_2)

c) $R'_2 = -CHCH_2COOH \cdot 2H_2O$ (NH_2)

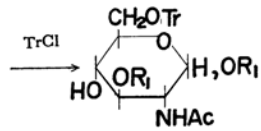
The hydrogenolysis of benzyl and benzyloxycarbonyl groups in A-III and B-III with palladium-charcoal (10%) gave 3-*O*-aminoacyl derivatives (IV) in ca. a 30% yield in the case of B-III, but the α-glycoside benzyl group of A-III could not be eliminated. This fact may be explained in terms of the steric hindrance of the C-2 *cis* acetamide group in the α-configuration, as was found in the case of phenyl 2, 3, 4, 6-tetra-*O*-acetyl-α- and -β-D-glucoside.[10]

The constants of the series of new compounds up to A, B-III and IV are shown in Tables I and II respectively.

The Synthesis of *N*-Acetyl-4-*O*-aminoacyl-D-glucosamines.—As a starting material to prepare 4-*O*-aminoacyl derivatives, benzyl *N*-acetyl-3-*O*-benzyl-6-*O*-trityl-α- and -β-D-glucosaminide (A-VII and B-VII) were synthesized in the following way.



A-V, B-V A-VI, B-VI

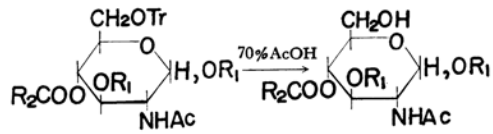


A-VII, B-VII

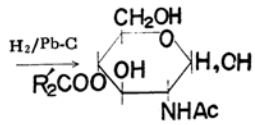
$Tr = -C(C_6H_5)_3$

Benzyl *N*-acetyl-3-*O*-benzyl-4, 6-*O*-benzylidene-α- and -β-D-glucosaminide (A-IV and B-IV) were obtained in 74—80% yields by reacting A-I or B-I with benzylchloride in dimethylformamide in the presence of potassium hydroxide respectively. When the benzylation of B-I was effected in dimethylsulfoxide with sodium hydride, the tribenzylated product (probably the *N*-benzylated derivative) was isolated as a by-product.

For the removal of the benzylidene group, A-V and B-V were then hydrolyzed in 70% acetic acid by refluxing them for an hour; this gave benzyl *N*-acetyl-3-*O*-benzyl-α- and -β-D-glucosaminide (A-VI and B-VI) in 72—85% yields. Finally, A-VII and B-VII were obtained by the triphenylmethylation of A-VI and B-VI in the usual method.



A-VIII, B-VIII A-IX, B-IX



X

a) $R_2 = -CH_2NHZ$

b) $R_2 = -CH(CH_2)_4NHZ$ (NHZ)

a) $R'_2 = -CH_2NH_2 \cdot 1/2(COOH)_2 \cdot 2H_2O$

b) $R'_2 = -CH(CH_2)_4NH_2 \cdot (COOH)_2 \cdot 3H_2O$ (NH_2)

The condensation of A-VII and B-VI with an equimolar amount of *N*-Z-amino acids (a and b) was carried out in a small amount of pyridine using DCC to give benzyl *N*-acetyl-3-*O*-benzyl-4-*O*-(*N*-Z-aminoacyl)-6-*O*-trityl-α- and -β-D-glucosaminides (A-VIII and B-VIII).

The partial hydrolysis of A-VIII and B-VIII with 70% acetic acid at 100°C gave benzyl *N*-

10) C. E. Ballou, S. Roseman and K. P. Link, *J. Am. Chem. Soc.*, **73**, 1140 (1951).

Table I. Benzyl *N*-acetyl-3-*O*-(*N*-Z-aminoacyl)-D-glucosaminide derivatives

Compound	Molecular formula (Mol. wt.)	M.p., °C	$[\alpha]_{578}^{15}$	(*c*, solvent)	Found (Calcd.) C, %	H, %	N, %
A-IIa	$C_{32}H_{34}O_9N_2$ (590)	203—204	+77	(0.51, P*)	64.90 (65.07)	5.93 (5.80)	4.90 (4.74)
B-IIa	$C_{32}H_{34}O_9N_2$ (590)	250(decomp.)	−81	(0.50, P)	64.80 (65.07)	5.75 (5.80)	4.84 (4.74)
A-IIb	$C_{44}H_{49}O_{11}N_3$ (795)	195	+33	(0.51, P)	66.22 (66.40)	6.52 (6.21)	5.44 (5.28)
B-IIb	$C_{44}H_{49}O_{11}N_3$ (795)	208—210	−78	(0.50, P)	66.36 (66.40)	6.07 (6.21)	5.54 (5.28)
B-IIc	$C_{41}H_{42}O_{11}N_2$ (738)	185—186	−88	(0.50, P)	66.42 (66.65)	5.96 (5.73)	3.73 (3.80)
A-IIIa	$C_{25}H_{30}O_9N_2$ (502)	81—83	+79	(5.54, M**)	59.60 (59.75)	6.08 (6.02)	5.71 (5.58)
B-IIIa	$C_{25}H_{30}O_9N_2$ (502)	151—152	−30	(0.20, M)	58.95 (59.75)	6.28 (6.02)	5.48 (5.58)
A-IIIb	$C_{37}H_{45}O_{11}N_3$ (707)	151	+71	(0.51, M)	62.49 (62.76)	6.61 (6.40)	6.11 (5.93)
B-IIIb	$C_{37}H_{45}O_{11}N_3$ (707)	145	−23	(0.20, M)	62.29 (62.76)	6.46 (6.40)	6.17 (5.93)
B-IIIc	$C_{34}H_{38}O_{11}N_2$ (650)	158—159	−59	(0.22, M)	62.93 (62.74)	6.13 (5.89)	4.36 (4.30)

P*=Pyridine, M**=Methanol

Table II. *N*-Acetyl-3- and 4-*O*-aminoacyl-D-glucosamines

Compound	Molecular formula	R_f	Found (Calcd.) C, %	H, %	N, %
IVa	$C_{10}H_{18}O_7N_2 \cdot 1/2(COOH)_2 \cdot 2H_2O$	0.19	37.77 (36.80)	6.42 (6.40)	7.40 (7.80)
IVb	$C_{14}H_{27}O_7N_3 \cdot (COOH)_2 \cdot 3H_2O$	0.10	38.64 (38.94)	6.79 (7.15)	8.37 (8.52)
IVc	$C_{12}H_{20}O_9N_2 \cdot 2H_2O$	0.15	39.32 (38.71)	6.35 (6.49)	7.22 (7.53)
Xa	$C_{10}H_{18}O_7N_2 \cdot 1/2(COOH)_2 \cdot H_2O$	0.16	37.31 (36.80)	5.92 (6.40)	7.88 (7.80)
Xb	$C_{14}H_{27}O_7N_3 \cdot (COOH)_2 \cdot 3H_2O$	0.12	39.95 (38.94)	7.35 (7.15)	8.24 (8.52)

Table III. Benzyl *N*-acetyl-3-*O*-benzyl-4-*O*-(*N*-Z-aminoacyl)-D-glucosaminide derivatives

Compound	Molecular formula (Mol. wt.)	M.p., °C	$[\alpha]_{578}^{15}$	(*c*, methanol)	Found (Calcd.) C, %	H, %	N, %
A-VIIIa	$C_{51}H_{50}O_9N_2$ (834)	84—86	+77	0.54	73.48 (73.36)	6.06 (6.04)	3.61 (3.36)
B-VIIIa	$C_{51}H_{50}O_9N_2$ (834)	123—125	−6.5	0.47	73.68 (73.36)	6.24 (6.04)	3.50 (3.36)
A-VIIIb	$C_{63}H_{65}O_{11}N_3$ (1039)	82—84	+49	0.51	72.69 (72.74)	6.30 (6.30)	4.33 (4.04)
B-VIIIb	$C_{63}H_{65}O_{11}N_3$ (1039)	syrup	−6.9	0.44	72.54 (72.74)	6.60 (6.30)	4.22 (4.04)
A-IXa	$C_{32}H_{36}O_9N_2$ (592)	151—153	+107	0.50	64.70 (64.65)	6.07 (6.12)	4.97 (4.73)
B-IXa	$C_{32}H_{36}O_9N_2$ (592)	186—188	−15	0.20	64.54 (64.65)	6.03 (6.12)	4.90 (4.73)
A-IXb	$C_{44}H_{51}O_{11}N_3$ (797)	152	+49	0.51	66.11 (66.23)	6.58 (6.44)	5.33 (5.27)
B-IXb	$C_{44}H_{51}O_{11}N_3$ (797)	140	−16	0.40	66.22 (66.23)	6.66 (6.44)	5.48 (5.27)

acetyl-3-*O*-benzyl-4-*O*-(*N*-Z-aminoacyl)-α- and -β-D-glucosaminides (A-IX and B-IX) in 70—80% yields.

The glycosidic α-benzyl group of A-IX could not be removed by catalytic hydrogenolysis in the presence of oxalic acid, as has been shown in the case of A-III.

The protecting groups of B-VIII could be successfully eliminated, and 4-*O*-aminoacyl derivatives (X) could be isolated as oxalates.

The constants of the *N*-Z-aminoacyl compound of this series and X are shown in Tables III and II respectively.

Experimental

Melting points were uncorrected. Optical rotations were measured at 578 mμ with a Carl-Zeis Polarimeter. Paper chromatography was carried out by the ascending technique on Toyo Roshi No. 50 filter paper, using an upper layer of *n*-buthanol, acetic acid and water (4 : 1 : 5) as a developer, and a 0.1% ninhydrin solution in *n*-butanol as a spray reagent.

The Preparation of Benzyl *N*-Acetyl-β-D-glucosaminide.—Benzyl *N*-acetyl-α, β-D-glucosaminide (50 g.; ca. an equimolar mixture), prepared by the convenient glycosidation method[7] of *N*-acetyl-D-glucosamine, was dissolved in dry pyridine (100 ml.) and acetic anhydride (70 ml.), and then allowed to stand overnight at room temperature. The reaction mixture was then poured into a large amount of ice water. The precipitated crystal was filtered, washed several times with water, and dried in vacuo. The fractional crystallization of the crude product (70 g.) was repeated several times from methanol to give benzyl *N*-acetyl-3, 4, 6-tri-*O*-acetyl-β-D-glucosaminide (20 g.), m. p. 163—164°C (Lit.,[11] m. p. 165—167°C). The corresponding α-

11) R. Kuhn and W. Kirschenlohr, *Ber.*, **86**, 1331 (1953).

anomer (30 g.) was obtained from the mother liquor, m. p. 108°C, $[\alpha]_D^{20}$ +120° (*c* 1.0, methanol).

Found: C, 57.87; H, 6.21; N, 3.32. Calcd. for $C_{21}H_{27}O_9N$(437): C, 57.66; H, 6.22; N, 3.22%.

A more effective separation of the α, β-anomer is attained by a previous separation of the mixture by the decantation of the ether suspension, in which the β-anomer is much lighter than the α-anomer.

The β-acetate was then deacetylated with CH_3ONa to give benzyl *N*-acetyl-β-D-glucosaminide in a 85% yield. M. p. 203—204°C (Lit.,[11] m. p. 205—206°C).

Benzyl *N*-Acetyl-4, 6-*O*-benzyliden-β-D-glucosaminide (B-I).—A mixture of benzyl *N*-acetyl-β-D-glucosaminide (25 g.), freshly fused and powdered $ZnCl_2$ (25 g.), and benzaldehyde (90 ml.) was shaken at 60°C for an hour. The homogeneous reaction mixture was then agitated with petroleum ether.

The resulting crystalline mass was filtered, and washed several times with petroleum ether, with water, and then with a mixture of methanol and petroleum ether.

The dried crude product (36 g.) was recrystallized from methanol (800 ml.) and pyridine (200 ml.); yield, 26 g. m. p. 251—252°C (decomp.).

A small part was recrystallized twice from methanol. M. p. 260—261°C (decomp.) $[\alpha]_{578}^{20}$ −40° (*c* 0.50, pyridine).

Found: C, 65.87; H, 6.48; N, 3.56. Calcd. for $C_{22}H_{25}O_6N$: C, 66.15; H, 6.31; N, 3.51%.

This compound was converted to the corresponding 3-*O*-acetate by the usual acetylation method. M. p. 258—259°C (decomp.), $[\alpha]_{578}^{20}$ −56° (*c* 0.30, pyridine).

Found: C, 65.24; H, 5.98; N, 3.41. Calcd. for $C_{24}H_{27}O_7N$ (441): C, 65.29; H, 6.16; N, 3.17%.

Benzyl *N*-Acetyl-3-*O*-(*N*-benzyloxycarbonyl-aminoacyl)-4, 6-*O*-benzylidene-α-D-glucosaminides (A-II).—0.015 mol. of *N*-Z-amino acids (a; *N*-Z-glycine, b; *N*, *N'*-di-Z-L-lysine) and DCC (0.018 mol.) were added to a solution of benzyl *N*-acetyl-4, 6-*O*-benzylidene-α-D-glucosaminide (0.012 mol.) in pyridine (50 ml.), and the mixture was allowed to stand in a refrigerator overnight. The precipitate of dicyclohexylurea was filtered off, and the filtrate was concentrated under reduced pressure. The gelatinous residue was washed several times with ether and then dried in vacuo.

The crude product was recrystallized from ethanol and a small amount of pyridine; yield, 72—79%.

Benzyl *N*-Acetyl-3-*O*-(*N*-benzyloxycarbonyl-aminoacyl)-4, 6-*O*-benzylidene-β-D-glucosaminides (B-II).—0.03 mol. of *N*-Z-amino acids (a, b, c; β-benzyl *N*-Z-L-aspartate) and DCC (0.036 mol.) were dissolved in dry dioxane (40 ml.) and stirred at room temperature for 2 hr. The precipitate was filtered and washed twice with dry dioxane. The combined dioxane was evaporated in vacuo, a cold pyridine solution (160 ml.) of B-I was then added to the resulting residue, and the mixture was kept at room temperature overnight.

After the pyridine had been completely removed, the gelatinous residue was washed several times with ether or ethyl acetate. The semi-crystalline product was recrystallized from ethanol and pyridine (1 : 1); yield, 60—75%.

Benzyl *N*-Acetyl-3-*O*-(*N*-benzyloxycarbonyl-aminoacyl)-α- and -β-D-glucosaminides (A-III and B-III).—A-II or B-II (0.005 mol.) was suspended in 70% acetic acid (100 ml.) and heated on a boiling-water bath for 30 min. with occasional stirring. After the solution had been concentrated in vacuo, the gelatinous residue was then washed once with ether and recrystallized from ethanol, or from ethanol and ether; yield, 68—78%.

The Hydrogenolysis of Benzyl *N*-Acetyl-3-*O*-(*N*-benzyloxycarbonyl-glycyl)-α-D-glucosaminide (A-IIIa).—Three grams of A-IIIa and 0.45 g. of oxalic acid were dissolved in 50 ml. of methanol, and then 2 g. of Pd-C (5%) was added. The mixture was shaken at room temperature with hydrogen for about 5 hr. After the catalyst had been removed, the filtrate was concentrated in vacuo, and ethanol was added. The precipitate was filtered, washed with ethanol and dried in vacuo. The white amorphous powder (1.2 g.), which had a molecular formula consistent with $C_{17}H_{24}O_7N_2 \cdot 1/2(COOH)_2 \cdot 2H_2O$, did not reduce Fehling's solution.

Found: C, 48.24; H, 6.29; N, 5.63. Calcd.: C, 48.12; H, 6.50; N, 6.23%.

***N*-Acetyl-3-*O*-aminoacyl-D-glucosamines (IV).**—0.002 mol. of B-III and oxalic acid (0.003—0.004 mol.) were dissolved in 80% methanol (15—20 ml.), and 10% Pd-C (1.0—1.5 g.) was added. The mixture was then shaken at room temperature with hydrogen until the absorption had substantially ceased (20—48 hr.).

After the catalyst had been removed, ether was added to the filtrate and centrifugation was carried out to collect the precipitate. For the purification, the precipitated oil or solid was dissolved in several drops of water, reprecipitated with ethanol, centrifugated, and dried in vacuo. The amorphous hygroscopic powder, which reduced Fehling's solution and which gave a single ninhydrin positive spot in paper chromatography and paper electrophoresis, was obtained in ca. a 30% yield.

Benzyl *N*-Acetyl-3-*O*-benzyl-4, 6-*O*-benzylidene-α- and -β-D-glucosaminide (A-V and B-V).—(a) Into a mixture of 5 g. of A-I or B-I in dimethylformamide (100—150 ml.) and potassium hydroxide (3.5 g.), benzyl chloride (7.8 g.) was added drop-by-drop over a 15—20 min. period with vigorous stirring at 70—75°C. The reaction conditions were then maintained for a further 30 min. In the case of the compound A-I, the reaction solution was then poured into a large amount of ice water. The precipitate was filtered, washed several times with water and methanol, and dried in vacuo. The crude product (5.1 g.) was recrystallized from hot pyridine-water (100 ml.+50 ml.). Yield, 5 g. (80%).

In the case of B-I, the reaction solution was allowed to stand at room temperature. The crystalline product was filtered, washed several times with methanol and recrystallized from pyridine (70 ml.) and DMF (20 ml.); yield, 4.5 g. (74%).

A-V: m. p. 264—265°C (decomp.), $[\alpha]_{578}^{13}$ +85° (*c* 0.50, pyridine).

Found: C, 70.79; H, 6.79; N, 3.11%.

B-V: m. p. 266—267°C (decomp.), $[\alpha]_{578}^{17}$ −75° (*c* 0.20, dimethylformamide).

Found: C, 71.62; H, 6.31; N, 2.97. Calcd. for $C_{29}H_{31}O_6N$ (399): C, 71.74; H, 6.38; N, 2.86%.

(b) Compound B-I (10 g.) was dissolved in dimethylsulfoxide (150 ml.) at 60°C and NaH (2.5 g.) was gradually added. Into this opaque solution, benzylchloride (16 g.) was added drop-by-drop over a 10 min. period with stirring and with keeping the

temperature at 70—80°C. The reaction solution was then poured into a large amount of ice water, and the precipitate was filtered, washed several times with water, and then with methanol.

The crude product was recrystallized from pyridine (20 ml.) and DMF (40 ml.); yield, 7.0 g. (57%).

From methanol washings, a tribenzylated product (1 g.) which showed no infrared absorption band (1550 cm^{-1}) of the secondary amido, was obtained.

M. p. 116—118°C, $[\alpha]_{578}^{15}$ −29° (*c* 0.56, pyridine).

Found: C, 74.81; H, 6.21; N, 2.51. Calcd. for $C_{36}H_{37}O_6N$ (579): C, 74.59; H, 6.43; N, 2.42%.

Benzyl *N*-Acetyl-3-*O*-benzyl-α- and -β-D-glucosaminide (A-VI and B-VI).—A-V or B-V (14 g.) was suspended in 400—500 ml. of 70% acetic acid and refluxed for an hour. The reaction solution was then concentrated under reduced pressure to a crystalline residue. The product was washed with ether and recrystallized from ethanol; yield, 72—85%.

A-VI: m. p. 178—179°C, $[\alpha]_{578}^{13}$ +165° (*c* 0.51, methanol).

Found: C, 65.69; H, 7.06; N, 3.71%.

B-VI: m. p. 169—170°C, $[\alpha]_{578}^{15}$ −20° (*c* 0.50, ethanol).

Found: C, 65.39; H, 6.81; N, 3.46. Calcd. for $C_{22}H_{27}O_6N$: C, 65.82; H, 6.78; N, 3.49%.

Benzyl *N*-Acetyl-3-*O*-benzyl-6-*O*-trityl-α- and -β-D-glucosaminide (A-VII and B-VII).—A-VI or B-VI (4 g.) and tritylchloride (3.3 g.) were dissolved in pyridine (30 ml.) and heated on a boiling-water bath for 2 hr. The reaction solution was then poured into ice water, and the gummy precipitate was extracted with methylene chloride, washed with water, and dried over sodium sulfate.

After the methylene chloride had been removed, the resulting syrup was crystallized from ethanol-petroleum ether. Yield, 70—72%.

A-VII: m. p. 169°C, $[\alpha]_{578}^{13}$ +85° (*c* 0.50, methanol).

Found: C, 76.38; H, 6.52; N, 2.39%.

B-VII: m. p. 143—145°C, $[\alpha]_{578}^{18}$ −19° (*c* 0.27, methanol).

Found: C, 76.16; H, 6.53; N, 2.14. Calcd. for $C_{41}H_{41}O_6N$ (643): C, 76.49; H, 6.42; N, 2.18%.

Benzyl *N*-Acetyl-3-*O*-benzyl-4-*O*-(*N*-benzyloxycarbonyl-aminoacyl)-6-*O*-trityl-α- and -β-D-glucosaminides. (A-VIII and B-VIII).—A-VII or B-VII (0.003 mol.), *N*-Z-amino acids (a or b, 0.003 mol.) and DCC (0.0036 mol.) were dissolved in dry pyridine (10 ml.) and then allowed to stand overnight in a refrigerator. The precipitate was filtered and washed with toluene. The filtrate and washings were concentrated in vacuo to a syrup. The product was dissolved in a small amount of ethanol, and treated with active carbon, and an equal amount of ether was added. Finally petroleum ether was added until a turbidity had appeared. Yield, 70—73%.

Benzyl *N*-Acetyl-3-*O*-benzyl-4-*O*-(*N*-benzyloxycarbonyl-aminoacyl)-α- and -β-D-glucosaminides (A-IX and B-IX).—A-VIII or B-VIII (0.005 mol.) was suspended in 70% acetic acid (30—50 ml.) and heated on a boiling-water bath while being stirred until it had dissolved completely. The reaction solution was then cooled with ice water to precipitate triphenyl carbinol, this was filtered off, and the filtate was concentrated in vacuo. The gelatinous residue was recrystallized from ethanol or ethanol and ether. Yield, 70—80%.

***N*-Acetyl-4-*O*-aminoacyl-D-glucosamines (X).**—B-IX (0.0012 mol.) and oxalic acid (0.0018—0.0036 mol.) were dissolved in 80% methanol (20 ml.), and 10% Pd-C (1.0—1.5 g.) was added. The reaction mixture was then shaken at room temperature with hydrogen until absorption had substantially ceased (20—24 hr.). After the catalyst had then been removed, a large amount of ether was added to the filtrate and centrifugation was carried out to collect the precipitate. The supernatant was removed, and the precipitate was dissolved in several drops of water, reprecipitated with ethanol, and centrifugated. The ethanol was decanted, and the resulting residue was dried in vacuo. The amorphous hygroscopic powder, which reduced Fehling's solution and which gave single ninhydrin-positive spot in paper chromatography and paper electrophoresis, was obtained. Yield, 39% (Xa), 51% (Xb).

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