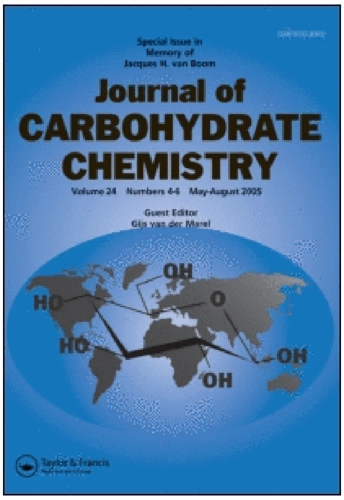

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Syntheses of Methyl 2,3-di-O-Glycyl-α-D-glucopyranoside and 4,6-di-O-Glycyl-2,3-di-O-methyl-α-D-glucopyranoside, and Removal of Aminoacyl Groups from Sugar Moieties

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SYNTHESES OF METHYL 2,3-DI-O-GLYCYL-α-D-GLUCOPYRANOSIDE AND 4,6-DI-O-GLYCYL-2,3-DI-O-METHYL-α-D-GLUCOPYRANOSIDE, AND REMOVAL OF AMINOACYL GROUPS FROM SUGAR MOIETIES

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ABSTRACT

To confirm the potential usefulness of amino acid residues as protecting groups for sugar hydroxyls, methyl 2,3-di-O-glycyl-α-D-glucopyranoside (5) and methyl 4,6-di-O-glycyl-2,3-di-O-methyl-α-D-glucopyranoside (7) were synthesized as reference compounds. Conditions were then established for the removal of these aminoacyl groups from the sugar molecules. The reference compounds were easily prepared by condensation of methyl α-D-glucopyranoside derivatives with N-protected glycine in the presence of dicyclohexylcarbodiimide (DCC). The aminoacyl groups were removed by alkaline treatment, as were conventional acyl groups and also with ease by enzymatic hydrolysis using Pronase E. Conventional ester and ether protecting groups are not removed by such enzymatic treatment. Removal of aminoacyl group from sugar moieties on a practical scale is also described.

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INTRODUCTION

From work now in progress in this laboratory on the synthesis of O-aminoacyl sugars, we found that the aminoacyl group can serve as a useful protecting group for sugar hydroxyls.[1] Methyl 4,6-O-benzylidene-α-D-glucopyranoside (1) and methyl 2,3-di-O-methyl-α-D-glucopyranoside (2) were used as the sugar models for this study. To the free hydroxyl groups at C-2 and C-3 positions of compound 1 or at C-4 and C-6 positions of compound 2 respectively, a series of aminoacyl groups, [NH_2-$(CH_2)_n$-CO-], were introduced.

In the present paper, we describe the preparation of these compounds, and removal of O-aminoacyl groups from sugar moieties.

RESULTS AND DISCUSSION

Cleavage of acyl groups from O-acylsugars in alkaline solution.

In the preliminary experimants, we introduced several acyl groups (from formyl to n-capryl) onto C-2 and C-3 hydroxyls of compound 1, and the relative rates of saponification were studied by hydrolysis in aqueous sodium hydroxide. Di-O-acyl derivatives were synthesized by a conventional method using the corresponding acyl chloride.[2] Saponification was carried out in aqueous sodium hydroxide with a 1.2/1 mol-equivalent of alkali to the acyl group. The hydrolysis was monitored by thin-layer chromatography, the relative rates being based on the time required for complete hydrolysis compared to acetate as 100 (Table I). An increase in the number of methylene groups is accompained by a decrease in the relative rates from formate to propionate. The rates were almost the same over the acyl range of n-butyrate to n-caprate. Essentially, amino acids correspond to carboxylic acids having some sub-

Table I

Relative Rates of Hydrolysis of Methyl 2,3-di-O-acyl-α-D-glucopyranosides by Saponification*

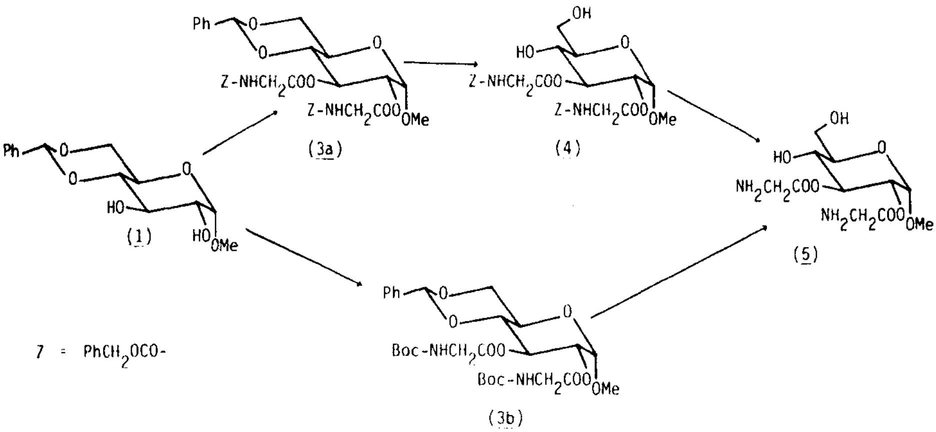
Acyl	Relative Rate**
HCO-	390
CH_3CO-	100
CH_3CH_2CO-	65
$CH_3CH_2CH_2CO$-	40
$CH_3CH_2CH_2CH_2CO$-	35
$CH_3CH_2CH_2CH_2CH_2CO$-	30
$CH_3CH_2CH_2CH_2CH_2CH_2CO$-	20
$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CO$-	20

* Hydrolysis was carried out in a sodium hydroxide solution including 1.2 mol-equivalent of alkali per each acyl group.
** Relative to the value of acetyl=100.

stituent groups, so they should serve as a hydroxyl protecting group.

Synthesis of methyl 2,3-di-O-glycyl-α-D-glucopyranoside (5) and methyl 4,6-di-O-glycyl-2,3-di-O methyl-α-D-glucopyranoside (7)

The synthetic procedures for the title compounds are summerized in Scheme I. N-(Benzyloxycarbonyl)-glycine and compound 1 were coupled in a mixed solution of carbon tetrachloride and pyridine in the presence of DCC. A 2,3-di-O-[N-(benzyloxycarbonyl)glycyl] derivative (3a) was obtained and the benzylidene group of 3a selectively removed with 90 % acetic acid. The oily product obtained (4) was hydrogenated in the presence of palladium black to give methyl 2,3-di-O-glycyl-α-D-glucopyranoside (5). Di-O-[N-(t-butoxy-carbonyl)glycyl] derivative (3b) was obtained easily from N-(t-butoxycarbonyl)glycine and 1. It was con-

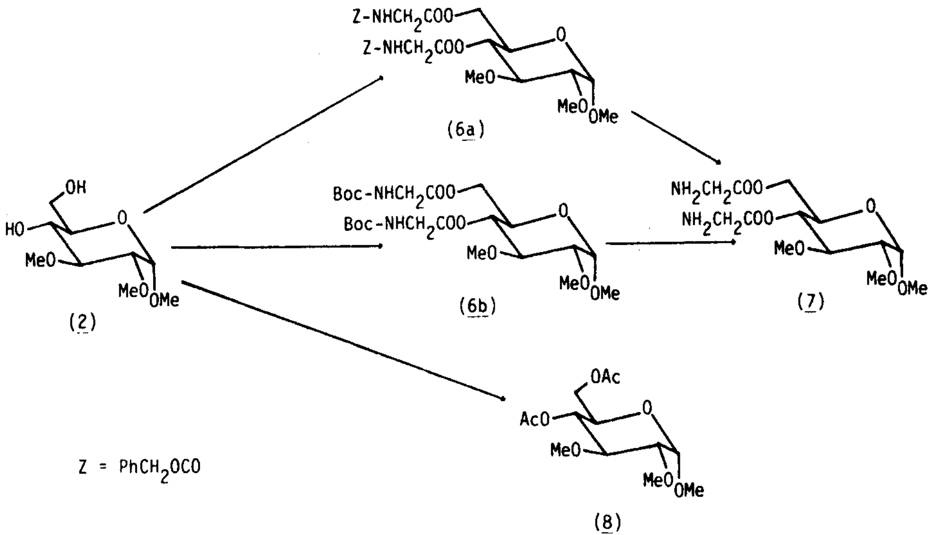


SCHEME I

verted to 5 by the action of hydrogen chloride in dioxane. The synthetic routes of methyl 4,6-di-O-glycyl-2,3-di-O-methyl-α-D-glucopyranoside (7) are shown in Scheme II. A 4,6-di-O-[N-(benzyloxycarbonyl)-glycyl] derivative (6a) was prepared from N-(benzyl-oxycarbonyl)glycine and 2 in good yield by a procedure analogous to that described for 3a. Catalytic hydrogenation of 6a in acidic solution gave 7. This compound was also prepared via 4,6-di-O-[N-(t-butoxy-carbonyl)glycyl] derivative (6b) which was obtained from N-(t-butoxycarbonyl)glycine and 2 by the same method as has been described for the preparation of 3b. The purity of the aminoacyl sugars and their intermediates were confirmed by TLC and elemental analysis.

Removal of O-aminoacyl group from sugar moieties by saponification.

The O-aminoacyl sugar derivatives were hydrolyzed in a dilute alkaline solution including a 1.2 mol equivalent of base per ester bond. These reaction conditions were based on these generally adopted for



SCHEME II

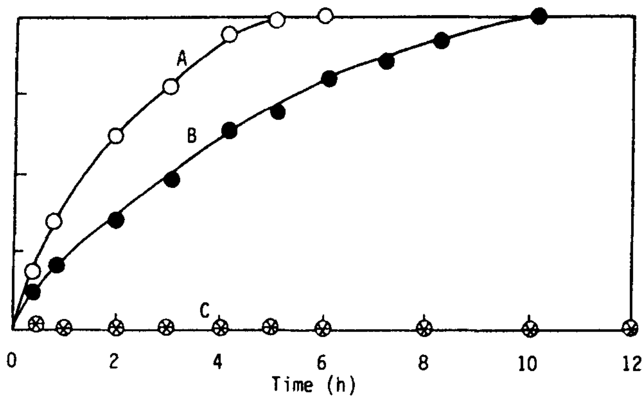


Fig. 1. Hydrolysis of aminoacyl Sugars with Pronase E
A; methyl 2,3-di-O-glycyl-α-D-glucopyranoside (5)
B; methyl 4,6-di-O-glycyl-2,3-di-O-methyl-α-D-glucopyranoside (7)
C; methyl 4,6-di-O-acetyl-2,3-di-O-methyl-α-D-glucopyranoside (8)

removal of C-terminal protecting esters in peptide synthesis. The relative rates of hydrolysis calculated with the di-O-acetyl derivative being shown as 100, are listed in Table II. The aminoacyl groups were completely removed within 3 h. These rates though slower than those for acetate hydrolysis are still considered as being in a practical range. A summary of the hydrolysis procedure on a 1.00 g scale is shown in Fig. 2.

In order to examine the stability of these acylated sugars to acidic conditions, acylated sugars were allowed to stand at room temperature in 2 M-HCl containing 10 mol-equivalent of acid per ester bond for 24 h. Absolutely no deacylation occurred.

Enzymatic degradation of O-aminoacyl sugars.

It is well known that Pronase E hydrolyzes amino acid ester and peptide ester bonds as well as amide bonds. Thus we undertook to remove the O-aminoacyl group enzymatically from protected sugars. Compound 5 (100 mg) was dissolved in 0.5 M Tris-HCl buffer, and incubated by a Pronase E solution at 37°C. Hydrolysis was monitored by TLC and an amino acid analyzer. The same procedure was applied to 7. In both cases, the O-aminoacyl groups was easily removed with the aid of enzyme, and without decomposition of the sugar moieties. We also treated methyl 4,6-di-O-acetyl-2,3-di-O-methyl-α-D-glucopyranoside (8) with Pronase E. However, Pronase E did not hydrolyze any acetate. The time course of hydrolysis is shown in Fig. 1.

On a more practical scale, 2,3-di-O-glycyl derivative (1.00 g, 3.3 mmol) was hydrolyzed with Pronase E according to the conditions generally adopted for hydrolysis of synthetic substrates.[3] Purification (Fig. 2-c) gave methyl α-D-glucopyranoside in 82 % yield. This compound exhibited the same spectral data and physical constants as the compound obtained by saponification.

Table II

Times to Complete Hydrolysis of Acyl Sugars

OH position	Acyl	Time (min)	Relative rate
2,3-di-O-	acetyl	40	100
	glycyl	70	57
	γ-aminobutyryl*	120	33
	succinyl*	80	50
	glutaryl*	100	40
4,6-di-O-	acetyl	60	67
	glycyl	120	33
	γ-aminobutyryl*	140	29
	succinyl*	135	30
	glutaryl*	150	27

* In order to compare with glycyl, synthesized conventionally.

a) methyl 2,3-di-O-glycyl-α-D-glucopyranoside 1.00 g (3.3 mmol)

↓ NaOH

methyl α-D-glucopyranoside + glycine + NaOH

↓ Amberlite CG-120 (H^+ form)
column size 1.2ϕ × 50 cm

methyl α-D-glucopyranoside 0.58 g (3.0 mmol, 90 %)

b) methyl 4,6-di-O-glycyl-2,3-di-O-methyl-α-D-glucopyranoside 1.00 g (2.5 mmol)

↓ 1) NaOH
2) Amberlite CG-120 (H^+ form)

methyl 2,3-di-O-methyl-α-D-glucopyranoside 0.57 g (2.2 mmol, 88 %)

c) methyl 2,3-di-O-glycyl-α-D-glucopyranoside 1.00 g (3.3 mmol)

↓ 1) Pronase E
2) carcoal
3) ion-exchange chromatography

methyl α-D-glucopyranoside 0.53 g (2.7 mmol, 82 %)

Fig. 2. Procedure of Sugar Recovery on a 1.0 g Scale

Table III

Comparison of the Deblocking Conditions for Glycyl and Other Protecting Groups

Protecting group	deblocking			
	OH^-	H^+	H_2/Pd	Enzyme
acetyl	++	±	-	-
benzyl	-	-	++	-
benzylidene	-	++	+	-
trityl	-	++	-	-
glycyl	++	-	-	++

+; labile, -; stable

A comparison of the methods for deblocking the aminoacyl and conventional protecting groups is presented in Table III. Results indicate that the aminoacyl group can serve as a protecting group of carbohydrate hydroxyl groups and offers the advantage of being removed enzymatically. The aminoacyl groups have ionic functions and so offer other techniques for the purification of protected sugar derivatives.

EXPERIMENTAL

General methods. Melting points were uncorrected. Optical rotations were determined for solutions in a 0.1 dm tube with a UNION model PM-101 polarimeter. IR spectra were recorded with a JASCO model IRA-1 spectrophotometer. TLC was performed on 5 cm plates coated with Silica Gel G (MERCK). The destance of solvent travel was 4.5 cm and the zones were detected by spraying the chromatograms with 0.1 M H_2SO_4 or ninhydrin. For blocked amino group detection, spraying was done with 25 % HBr/AcOH (w/w) and then ninhydrin. Column chromatography of silica gel was performed on Kieselgel type 60 (70-230 mesh, MERCK), the ratio of the column to its length was 4:125 and the flow rate was 1-2 mL/min. Quantitative analysis of amino acid residues were performed with a HITACHI model KLA-5 amino acid analyzer. Evaporations were conducted *in vacuo*. Samples for elemental analysis were dried at 60°C over phosphorus pentaoxide *in vacuo*.

Starting materials. Methyl 4,6-*O*-benzylidene-α-*D*-glucopyranoside (**1**) and methyl 2,3-di-*O*-methyl-α-*D*-glucopyranoside (**2**) were synthesized according to references.[4,5] Their homogeneity was ascertained by comparison of spectral data and physical constants from authentic samples. Amino acid derivatives were prepared in the usual manner using benzyloxycarbonyl chloride[6] or 2-*t*-butoxycarbonylimino-2-phenylacetonitrile.[7]

Methyl 2,3-di-O-acyl-α-D-glucopyranoside.[2] To a solution of **1** (1.0 mmol) in pyridine, 3.0 mmol of each acyl chloride $H(CH_2)_nCOCl$ was added. The reaction mixture was stirred at -15°C for 1 h then at 25°C for 12 h. Reaction mixture was chromatographed on silica gel using the solvent system benzene-acetone 9:1 (v/v), and the main fractions were combined and evaporated. The residual oil was treated with 60 % aqueous acetic acid solution (100 mL) at 90°C for 2 h. Title compounds were produced after treatment of silica gel column chromatography.

Methyl 4,6-O-benzylidene-2,3-di-O-[N-(benzyloxycarbonyl)glycyl]-α-D-glucopyranoside (3a). A suspension of *N*-(benzyloxycarbonyl)glycine (1.26 g) and DCC (1.28 g) in cabon tetrachloride (10 mL) was stirred at 0°C for 20 min. A solution of **1** (0.57 g) in pyridine (5 mL) was added and stirring was continued for 24 h at room temperature. The resulting *N,N'*-dicyclohexylurea was filtered off and the filtrate was evaporated to dryness. The residual oil was dissolved in ethyl acetate and washed with 4 % sodium hydrogen carbonate and water successively. The organic layer was dried (Na_2SO_4), evaporated, and crystallized with ether-petroleum ether: 1.10 g (82 %), m.p. 69-70°C, $[\alpha]_D^{25}$ +17° (c 1.0, chloroform), Rf 0.83 (chloroform-methanol 9:1 v/v); IR data, ν_{max}^{KBr} 1650, 1550 (amide), 760, 700 (phenyl), 1700, 1980 (ester) and 3300 cm^{-1} (NH).

Anal. Calcd for $C_{34}H_{36}O_{12}N_2$: C, 61.52; H, 5.72; N, 4.19. Found: C, 61.44; H, 5.80; N, 4.22.

Methyl 2,3-di-O-[N-(benzyloxycarbonyl)glycyl]-α-D-glucopyranoside (4). Compound 3a (0.67 g) was dissolved in dioxane (5 mL), then 90 % acetic acid (100 mL) was added and kept at 75°C for 2.5 h. The clear solution was evaporated and the residue was dried by several codistillations with benzene to give oily residue wich did not crystallize: 0.47 g (80 %), $[\alpha]_D^{25}$ +36° (c 1.0, chloroform), Rf 0.58 (chloroform-methanol 9:1 v/v); IR data, ν_{max}^{nujol} 1650, 1545 (amide), 755, 700 (phenyl), 1700, 1780 (ester) and 3350 cm^{-1} (broad OH and NH).

Anal. Calcd for $C_{27}H_{32}O_{12}N_2$: C, 56.24; H, 5.59; N, 4.86. Found: C, 55.99; H, 5.67; N, 4.97.

Methyl 2,3-di-O-glycyl-α-D-glucopyranoside (5). Compound 4 (0.29 g) was dissolved in methanol (2 mL) containing 2 mL of acetic acid. Palladium black (about 2 g) was added to a solution and a gentle stream of hydrogen bubbled through the stirred solution. After 4 h, the catalyst was filtered off and the filtrate was evaporated. The residue was dissolved in a small portion of acetone, addetion of hydrogen chloride in dioxane (2.4 eq.) gave hygroscopic crystals: 0.15 g (76 %), $[\alpha]_D^{25}$ 71° (c 1.0, methanol), Rf 0.67 (n-butanol-acetic acid-pyridine-water 4:1:1:2 v/v); IR data, ν_{max}^{nujol} 1690, 1760 (ester), 3090 (NH_3^+) and 3400 cm^{-1} (NH).

Anal. Calcd for $C_{11}H_{22}O_8N_2Cl_2$: C, 34.70; H, 5.84; N, 7.36. Found: C, 34.82; H, 5.78; N, 7.42.

Methyl 4,6-O-benzylidene-2,3-di-O-[N-(t-butoxycarbonyl)glycyl]-α-D-glucopyranoside (3b). Compound 3b was prepared according to the procedure for 3a but employing Boc-Gly-OH (1.05 g): 1.03 g (86 %), m.p. 88-90°C, $[\alpha]_D^{25}$ +21° (c 1.0, chloroform), Rf 0.72 (chloroform-methanol 9:1 v/v); IR data, ν_{max}^{KBr} 1665, 1550 (amide), 1720, 1700 (ester), 740, 690 (phenyl) and 3280 cm^{-1} (NH).

Anal. Calcd for $C_{28}H_{40}O_{12}N_2$: C, 56.05; H, 6.28; N, 4.61. Found: C, 55.98; H, 6.71; N, 4.66.

Compound 5 from 3b. 4M-HCl in dioxane (5 mL) was added to 3b (0.60 g) and the solution was allowed to stand at room temperature for 1.5 h. Hygroscopic crystals of 5 were obtained, (0.28 g, 70 %).

Methyl 4,6-O-[N-(benzyloxycarbonyl)glycyl]-2,3-di-O-methyl-α-D-glucopyranoside (6a). A similar procedure to that used for the synthesis of 3a, except compound 2 (0.45 g) was the starting material instead of 1. Powder form of 6a was obtained: 0.87 g (71 %), m.p. 85-87°C, $[\alpha]_D^{25}$ +53° (c 1.0, chloroform), Rf 0.80 (chloroform-methanol 9:1 v/v); IR data, ν_{max}^{KBr} 1655, 1545 (amide), 730, 690 (phenyl), 1700, 1760 (ester) and 3380 cm^{-1} (NH).

Anal. Calcd for $C_{29}H_{36}O_{12}N_2$: C, 56.95; H, 6.20; N, 4.55. Found: C, 57.28; H, 5.96; N, 4.60.

Methyl 4,6-di-O-[N-(t-butoxycarbonyl)glycyl]-2,3-di-O-methyl-α-D-glucopyransodie (6b). The procedure is employed for 3b but beginning with 2 (0.45 g). Needles of 6b were obtained, 0.95 g (88 %), m.p. 69-71°C, $[\alpha]_D^{25}$ +48° (c 1.0, chloroform), Rf 0.64 (chloroform-methanol 9:1 v/v); IR data, ν_{max}^{KBr} 1670, 1565 (amide), 1740, 1695 (ester) and 3385 cm^{-1} (NH).

Anal. Calcd for $C_{23}H_{40}O_{12}N_2$: C, 51.36; H, 8.02; N, 5.12. Found: C, 51.48; H, 7.51; N, 5.22.

Methyl 4,6-di-O-glycyl-2,3-di-O-methyl-α-D-glucopyranoside (7). To a solution of 6a (0.61 g) in acetic acid-methanol 1:1 (v/v) solution, palladium black (1 g) was added as catalyst and hydrogen bubbled through the stirred solution for 5 h. The catalyst was filtered off and the filtrate was evaporated to dryness. The residual oil was dissolved in ethanol (5 mL) and 4M-HCl in dioxane (0.5 mL) was added to the solution. Compound 7 was obtained as needles, 0.32 g (61 %), m.p. 67-68°C, $[\alpha]_D^{25}$ +66° (c 1.0, methanol), Rf 0.67 (n-butanol-acetic acid-pyridine-water 4:1:1:2 v/v); IR data, ν_{max}^{KBr} 1730, 1690 (ester) and 3030 cm^{-1} (NH_3^+).

Anal. Calcd for $C_{13}H_{26}O_8N_2Cl_2$: C, 38.15; H, 6.40; N, 6.85. Found: C, 38.22; H, 6.31; N, 6.80.

Compound 7 from 6b. To a solution of 6b (0.58 g) in dioxane (2 mL), 4 M-HCl in dioxane (5 mL) was added and the solution was allowed to stand at room temperature for 2 h. Compound 7 crystallized from solution, was filtered, and was recrystallized from acetone, 0.40 g (66 %).

Methyl 4,6-di-O-acetyl-2,3-di-O-methyl-α-D-glucopyranoside (8) To a solution of compound 2 (0.20 g) in pyridine (0.5 mL), acetic anhydride (0.2 mL) was added. The reaction mixture was heated at 80°C for 3 h, and poured into water (20 mL). The solution was extracted with chloroform, and the organic layer was washed with water and dried (Na_2SO_4). A colorless oil (0.26 g, 85 %) was obtained after evaporation, Rf 0.32 (chloroform-methanol 9:1 v/v); IR data, ν_{max}^{nujol} 1750 cm^{-1} (OAc).

Anal. Calcd for $C_{13}H_{22}O_8$: C, 50.97; H, 7.24. Found: C, 50.66; H, 7.20.

Hydrolysis of acyl sugars.

(a) saponification: To a solution of a sample (0.2 mmol) of each aminoacyl sugar and N-methylmorpholine (0.022 mL) in water (1 mL), 1 M-NaOH (0.48 mL) was added. The reaction mixtures were kept at room temperature. The hydrolysis was followed by TLC, the times required for the disappearance of the starting aminoacyl sugars being listed in Table I and Table II.

(b) acid hydrolysis: A sample (0.2 mmol) of each aminoacyl sugar dissolved in 2 M-HCl (4.00 mL) was kept at room temperature. The hydrolysis was monitored by TLC.

(c) enzymatic hydrolysis: A sample (100 mg) of either 5 or 7 was dissolved in 0.5 M Tris-HCl buffer (pH 8.0, 4.00 mL) containing 1.0 M $CaCl_2$ (0.4 mL). 1.0 % Pronase E solution (0.2 mL) was added to the solution and diluted to 20 mL with water. The hydrolysis was followed by TLC and the times required for complete hydrolysis were measured.

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