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Strong competitive inhibition of porcine pancreatic alpha-amylase by aminodeoxy derivatives of maltose and maltotriose *

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ABSTRACT

The syntheses are described of 6-amino-6-deoxymaltose (**2**), the 6-amino-6-deoxy (**4**), 6′-amino-6′-deoxy (**6**), and 6″-amino-6″-deoxy (**8**) derivatives of maltotriose, and the methyl α- (**10**) and β-glycoside (**12**) and the 1-deoxy derivative (**16**) of **4**. The K_i values (μM) of these competitive inhibitors of porcine pancreatic alpha-amylase were: **2**, 88; **4**, 1.9; **6**, 2.0; **8**, 175; **10**, 360; **12**, 9000; **16**, 7600 (cf. 1800 for maltotriose and 3000 for methyl α-maltotrioside). The low values for **4** and **6** reflect reinforcement of the normal binding by ionic attraction and, possibly, interaction of the reducing end groups with the protein.

INTRODUCTION

Numerous carbohydrate-type amines are competitive inhibitors of glycanases and glycoside hydrolases, usually with low inhibition constants due to interaction of the cationic ligands with anionic functional groups in the active site[1]. This view is based on kinetic studies with glycosylamines[2], glycosamines[3], and glycosides aminated in the aglycon[4] and their corresponding glycoside hydrolases. We now report on the synthesis and interaction with alpha-amylase of several derivatives of maltose and maltotriose, with amino groups variously at positions 6, 6′, and 6″.

RESULTS AND DISCUSSIONS

Syntheses.—Incubation of 6-azido-6-deoxy-D-glucose[5] with cyclomaltohexaose (α-cyclodextrin, αCD) and CGT-ase[6] gave 6-azido-6-deoxymaltose (**1**) and 6-azido-

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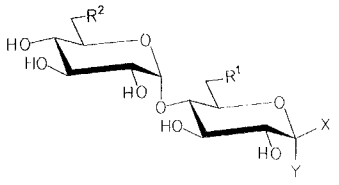


1 R^1 = N_3, R^2 = OH, X, Y = H, OH

2 R^1 = NH_2, R^2 = OH, X, Y = H, OH

6-deoxymaltotriose (**3**) which were separated by chromatography on Biogel P2. Hydrogenation of **1** gave 6-amino-6-deoxymaltose (**2**), and of **3** gave 6-amino-6-deoxymaltotriose (**4**). Likewise, 6′-azido-6′-deoxymaltose[7] was converted into 6′-azido-6′-deoxymaltotriose (**5**) and thence into 6′-amino-6′-deoxymaltotriose (**6**).

Acetolysis[8] of 1,6-anhydro-6″-azido-6″-deoxy-β-maltotriose octa-acetate[8] and *O*-deacetylation of the product gave 6″-azido-6″-deoxymaltotriose (**7**), hydrogenation of which gave 6″-amino-6″-deoxymaltotriose (**8**).

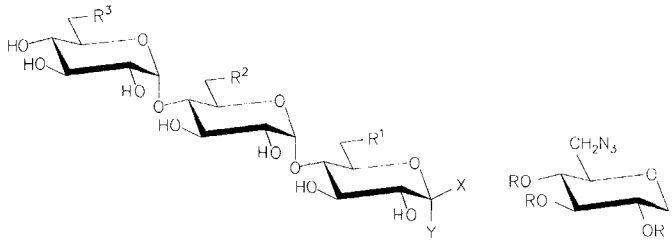
Incubation of methyl 6-azido-6-deoxy-α-D-glucopyranoside[9] with αCD and CGT-ase gave methyl 6-azido-6-deoxy-α-maltotrioside (**9**), hydrogenation of which gave methyl 6-amino-6-deoxy-α-maltotrioside (**10**). Likewise, methyl 6-azido-6-deoxy-β-D-glucopyranoside[10] was converted into methyl 6-azido-6-deoxy-β-maltotrioside (**11**) and thence into methyl 6-amino-6-deoxy-β-maltotrioside (**12**).

Azide displacement of 2,3,4-tri-*O*-acetyl-1,5-anhydro-6-*O*-tosyl-D-glucitol[11] gave the 6-azido-6-deoxy derivative **13**, *O*-deacetylation of which afforded 1,5-anhydro-6-azido-6-deoxy-D-glucitol (**14**). Incubation of **14** with αCD and CGT-ase gave 1,5-anhydro-6-azido-6-deoxy-4-*O*-α-maltosyl-D-glucitol (**15**), hydrogenation of which yielded 6-amino-1,5-anhydro-6-deoxy-4-*O*-α-maltosyl-D-glucitol (**16**).

The derivatives **2**, **4**, **6**, and **8** could not be isolated directly or as salts, and the decomposition encountered was probably a browning reaction[12].

Binding studies.—The inhibition constants (K_i values) for 6-amino-6-deoxymaltose (**2**, 88 μM), 6-amino-6-deoxymaltotriose (**4**, 1.9 μM), 6′-amino-6′-deoxymaltotriose (**6**, 2.0 μM), and 6″-amino-6″-deoxymaltotriose (**8**, 175 μM) were comparable to those of the most efficient competitive inhibitors of alpha-amylase known (e.g., acarbose[13], 10–18 μM) and much lower than that (1.8 mM) for maltotriose[14]. The sequence of K_i values **8** > **2** ≫ **4** and **6** is strong support for the five-subsite model of alpha-amylase proposed by Robyt and French[15].

For the neutral azides (**3**, **5**, and **7**) of maltotriose, which correspond to the amines **4**, **6**, and **8**, respectively, the K_i values (**3**, 1.7; **5**, 16; **7**, 17 mM; the azide **1** had no significant affinity) differed by only one order of magnitude, which means that only normal binding involving hydrogen bonds and van der Waals effects are responsible for the inhibition. It is probably also irrelevant which of the three adjacent subsites, ABC, BCD, or CDE, are occupied. When an amino group is introduced into the ligand, the situation changes dramatically and the binding of



3 $R^1 = N_3$, $R^2 = R^3 = OH$, X,Y = H, OH

4 $R^1 = NH_2$, $R^2 = R^3 = OH$, X,Y = H, OH

5 $R^1 = R^3 = OH$, $R^2 = N_3$, X,Y = H, OH

6 $R^1 = R^3 = OH$, $R^2 = NH_2$, X,Y = H, OH

7 $R^1 = R^2 = OH$, $R^3 = N_3$, X,Y = H, OH

8 $R^1 = R^2 = OH$, $R^3 = NH_2$, X,Y = H, OH

9 $R^1 = N_3$, $R^2 = R^3 = OH$, X = H, Y = OMe

10 $R^1 = NH_2$, $R^2 = R^3 = OH$, X = H, Y = OMe

11 $R^1 = N_3$, $R^2 = R^3 = OH$, X = OMe, Y = H

12 $R^1 = NH_2$, $R^2 = R^3 = OH$, X = OMe, Y = H

15 $R^1 = N_3$, $R^2 = R^3 = OH$, X = Y = H

16 $R^1 = NH_2$, $R^2 = R^3 = OH$, X = Y = H

13 R = Ac

14 R = H

the monosaccharide units (i.e., occupation of three subsites) is reinforced in **4** and **6** by ionic attraction (Fig. 1). However, **8** has to pay for ionic interaction by losing one binding subsite.

On conversion of 6-amino-6-deoxymaltotriose (**4**) into its methyl α-glycoside (**10**), the K_i value increases from 1.9 μM to 0.36 mM (cf. 3.0 mM for methyl α-maltotrioside[14]) and further to 7.6 mM for the 1-deoxy derivative **16** and 9.0 mM for the methyl β-glycoside **12**.

Thus, three factors appear to be responsible for the tight binding of 6-amino-6-deoxymaltotriose (**4**) and 6′-amino-6′-deoxymaltotriose (**6**) to alpha-amylase, associated with the maltotriosyl unit, the ionic interactions, and the reducing end group. The role of the last named factor is obscure because a significant effect is found only in the aminated compounds with a factor of almost 200 between the K_i values of **4** and its methyl α-glycoside **10**, whereas those of maltotriose and its methyl α-glycoside are almost equal. The possibility of the formation of a Schiff's base between the basic reducing maltotriose derivatives and a free amino group in the binding site is being investigated.

EXPERIMENTAL

General methods.—Optical rotations were measured with a Polartronic I (Schmidt and Haensch), IR spectra with a Perkin–Elmer 1320 spectrophotometer,

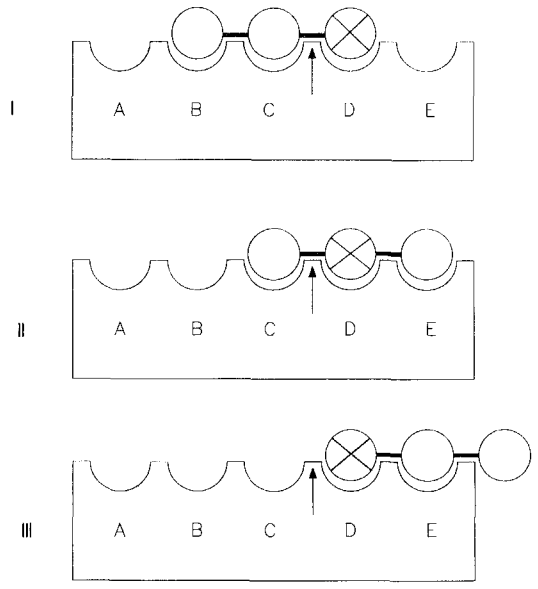


Fig. 1. Schematic presentation of the binding modes for the five subsites (A–E) of porcine pancreatic alpha-amylase: I, 6-amino-6-deoxymaltotriose (**4**); II, 6′-amino-6′-deoxymaltotriose (**6**); III, 6″-amino-6″-deoxymaltotriose (**8**); ○, D-Glc*p* residue; ⊗, D-Glc6N residue; the arrow indicates the catalytic site. Binding is arranged to give optimal ionic interaction of the amino group of the ligand and the carboxyl group in the active site of the enzyme (subsite D).

and pH values with a WTW Typ E 50 pH 0–14 pH meter. All reactions were monitored by TLC on Silica Gel 60 F_{254} (Merck). Column (140 × 2.5 cm) chromatography on Biogel P2 (−400 mesh, Bio-Rad) was performed at 40°C by elution at 100 mL/h with distilled and degassed water. Kinetic data were obtained with an Eppendorf photometer (405 nm), with a transformation unit and a Siemens Kompensograph X-T C-1011. ^{1}H NMR spectra (250 MHz) were recorded with a Bruker WM 250 spectrometer for solutions in $CDCl_3$ (Internal Me_4Si). Melting points are uncorrected.

Enzymes.—CGT-ase [(1 → 4)-α-D-glucan 4-α-D-glucanotransferase, cyclising, EC 2.4.1.19, 760 U/mL] from *Bacillus macerans* was a gift from Boehringer Mannheim, alpha-amylase [(1 → 4)-α-D-glucan glucanohydrolase, EC 3.2.1.1, 1260 U/mg] from porcine pancreas and beta-amylase [(1 → 4)-α-D-glucan maltohydrolase, EC 3.2.1.2, 845 U/mg] from *Ipomoea batatas* were purchased from Boehringer Mannheim, and α-D-glucosidase (maltase, EC 3.2.1.20, Type I) from yeast was purchased from Sigma.

Calculation of the concentration by titration of the amino groups.—To well-stirred aqueous solutions of **2** (0.18 mmol, 20 mL, pH 10.2), **4** (0.113 mmol, 20 mL, pH 10.01), and **6** (0.072 mmol, 25 mL, pH 9.8) was added 0.1 M HCl dropwise to pH 5.5: **2**, 1.75 mL, 0.175 mmol; **4**, 1.1 mL, 0.11 mmol; and **6**, 0.65 mL, 0.065 mmol.

Determination of the inhibition constants (K_i).—*p*-Nitrophenyl α-maltotrioside (Boehringer Mannheim) was used as substrate (0.2–5.4 mM, K_M 2.1 mM) in 50

mM triethanolamine–triethanolamine · HCl buffer (pH 7.0, 10 mM $CaCl_2$) at 30°C. Inhibitors were used in the following concentrations: **2**, 32–127 μM; **3**, 1–10 mM; **4**, 0.4–4.5 μM; **5**, 1–12 mM; **6**, 0.5–5 μM; **7**, 0.3–2 mM; **8**, 40–300 μM; **10**, 0.127–0.538 mM; **12**, 1.53–9.2 mM; **14**, 1.62–8.7 mM. Each assay involved 15 U/mL of alpha-amylase.

6-Azido-6-deoxymaltose (**1**) *and 6-azido-6-deoxymaltotriose* (**3**).—A solution of 6-azido-6-deoxy-D-glucose[5] (700 mg, 3.6 mmol) and cyclomaltohexaose (α-cyclodextrin, αCD; 700 mg, 0.72 mmol) in distilled water (3 mL) was incubated with CGT-ase (60 μL, 46 U) at room temperature. After 2 h, when TLC showed that equilibrium had been reached, the enzyme was inactivated (95°C, 5 min), and acetic acid (50 μL) and beta-amylase (50 U) were added. The solution was kept for 30 min, the enzyme was denaturated (5 min, 95°C), and the solution was diluted with distilled water (20 mL) and subjected to chromatography on Biogel P2. The appropriate fractions were combined and freeze-dried to give **1** (131 mg, 0.36 mmol) and **3** (120 mg, 0.3 mmol). Compound **1** had R_F 0.39 (7:2:1 EtOAc–MeOH–H_2O), $[\alpha]_D$ +151° (*c* 0.75, H_2O); ν_{max}^{KBr} 2120 cm^{-1} (N_3). *Anal.* Calcd for $C_{12}H_{21}N_3O_{10} \cdot 1.5H_2O$: C, 36.55; H, 6.13; N, 10.65. Found: C, 35.96; H, 6.05; N, 10.23.

Compound **3** had R_F 0.48 (4:2:1 EtOAc–MeOH–H_2O), $[\alpha]_D$ +115° (*c* 1, H_2O); ν_{max}^{KBr} 2120 cm^{-1} (N_3). *Anal.* Calcd for $C_{18}H_{31}N_3O_{15} \cdot H_2O$: C, 39.49; H, 6.08; N, 7.68. Found: C, 40.34; H, 6.01; N, 7.25.

6-Amino-6-deoxymaltose (**2**).—A solution of **1** (39 mg, 0.1 mmol) in distilled water (3 mL) was hydrogenated, as described for **8**, to yield **2** as an aqueous solution; R_F 0.27 (30:20:15 $CHCl_3$–MeOH–NH_3), $[\alpha]_D$ +59° (*c* 0.75, H_2O).

6-Amino-6-deoxymaltotriose (**4**).—A solution of **3** (45 mg, 0.086 mmol) in distilled water (3 mL) was hydrogenated, as described for **8**, to yield **4** as an aqueous solution; R_F 0.12 (20:25:12 $CHCl_3$–MeOH–NH_3), $[\alpha]_D$ +57° (*c* 0.64, H_2O).

6′-Azido-6′-deoxymaltotriose (**5**).—A solution of 6′-azido-6′-deoxymaltose[7] (600 mg, 1.6 mmol) and αCD (600 mg, 0.61 mmol) in distilled water (3 mL) was treated with CGT-ase, as described for **3**, to yield, after freeze-drying, **5** (180 mg, 0.34 mmol), R_F 0.47 (4:2:1 EtOAc–MeOH–H_2O), $[\alpha]_D$ +167° (*c* 1.1, H_2O); ν_{max}^{KBr} 2130 cm^{-1} (N_3). *Anal.* Calcd for $C_{18}H_{31}N_3O_{15} \cdot H_2O$: C, 39.49; H, 6.08; N, 7.68. Found: C, 39.58; H, 5.94; N, 7.34.

6′-Amino-6′-deoxymaltotriose (**6**).—A solution of **5** (81 mg, 0.15 mmol) in distilled water (4 mL) was hydrogenated, as described for **8**, to yield **6** as an aqueous solution; R_F 0.12 (20:25:12 $CHCl_3$–MeOH–NH_3); $[\alpha]_D$ +90° (*c* 0.66, H_2O).

6″-Azido-6″-deoxymaltotriose (**7**).—Acetolysis[8] of 2,3,2′,3′,6′,2″,3″,4″-octa-*O*-acetyl-1,6-anhydro-6″-azido-6″-deoxy-β-maltotriose[8] (1.2 g, 1.4 mmol) gave a product, to a solution of which in dry MeOH (15 mL) was added methanolic M NaOMe (0.5 mL). The mixture was stirred overnight, then deionised by passage through a column (5.0 × 2.0 cm) of ICN silica gel (32–63, 60A) by washing with dry MeOH. The eluate was concentrated in vacuo, and a solution of the resulting slightly

yellow oil in water (25 mL) was subjected to chromatography on Biogel P2 to yield, after freeze-drying, **7** (505 mg, 68%), R_F 0.48 (4:2:1 EtOAc–MeOH–H_2O), $[\alpha]_D$ +123° (*c* 1, H_2O); ν_{max}^{KBr} 2120 cm^{-1} (N_3). *Anal.* Calcd for $C_{18}H_{31}N_3O_{15}$: C, 40.83; H, 5.90; N, 7.94. Found: C, 39.65; H, 5.89; N, 7.86.

6"-Amino-6"-deoxymaltotriose (**8**).—A solution of **7** (136 mg, 0.26 mmol) in distilled water (5 mL) was stirred vigorously under H_2 in the presence of 10% Pd/C (40 mg) for 2 h, when TLC indicated the absence of **7**. The mixture was filtered to give **8** as an aqueous solution; R_F 0.12 (20:25:12 $CHCl_3$–MeOH–NH_3); $[\alpha]_D$ +78° (*c* 0.67, H_2O).

Methyl 6-azido-6-deoxy-α-maltotrioside (**9**).—A solution of methyl 6-azido-6-deoxy-α-D-glucopyranoside[9] (1.5 g, 6.8 mmol) and αCD (1.5 g, 1.5 mmol) in distilled water (10 mL) was treated with CGT-ase, as described for **3**, to yield, after freeze-drying, **9** (315 mg, 0.58 mmol), R_F 0.3 (7:2:1 EtOAc–MeOH–H_2O), $[\alpha]_D$ +186° (*c* 2.0, H_2O); ν_{max}^{KBr} 2095 cm^{-1} (N_3). *Anal.* Calcd for $C_{19}H_{33}N_3O_{15}$: C, 41.99; H, 6.12; N, 7.73. Found: C, 41.36; H, 6.20; N, 7.27.

Methyl 6-amino-6-deoxy-α-maltotrioside hydrochloride (**10**).—A solution of **9** (51 mg, 0.09 mmol) in MeOH (4 mL) was stirred vigorously under H_2 for 2.5 h in the presence of 10% Pd/C (25 mg). TLC then indicated the absence of **9**. The solution was filtered, methanolic HCl (0.911 mL, 0.1 M) was added, and the solvent was evaporated to yield **10** (51.2 mg, 0.089 mmol) as a colorless syrup, R_F 0.22 (30:20:15 $CHCl_3$–MeOH–NH_3), $[\alpha]_D$ +167° (*c* 1.08, H_2O). *Anal.* Calcd for $C_{19}H_{35}NO_{15} \cdot HCl$: C, 41.19; H, 6.55; N, 2.53. Found: C, 39.91; H, 6.29; N, 2.49.

Methyl 6-azido-6-deoxy-β-maltotrioside (**11**).—A solution of methyl 6-azido-6-deoxy-β-D-glucopyranoside[10] (1.5 g, 6.8 mmol) and αCD (1.5 g, 1.5 mmol) in distilled water (10 mL) was treated with CGT-ase, as described for **3**, to yield, after freeze-drying, **11** (323 mg, 0.59 mmol), R_F 0.29 (7:2:1 EtOAc–MeOH–H_2O), $[\alpha]_D$ +125° (*c* 0.76, H_2O); ν_{max}^{KBr} 2100 cm^{-1} (N_3). *Anal.* Calcd for $C_{19}H_{33}N_3O_{15}$: C, 41.99; H, 6.12; N, 7.73. Found: C, 41.44; H, 6.15; N, 7.69.

Methyl 6-amino-6-deoxy-β-maltotrioside hydrochloride (**12**).—A solution of **11** (95 mg, 0.17 mmol) in MeOH (4 mL) was hydrogenated, as described for **10**, to yield **12** (94.8 mg, 0.168 mmol) as a colorless syrup, R_F 0.22 (30:20:15 $CHCl_3$–MeOH–NH_3), $[\alpha]_D$ +105° (*c* 1.06, H_2O). *Anal.* Calcd for $C_{19}H_{35}NO_{15} \cdot HCl$: C, 41.19; H, 6.55; N, 2.53. Found: C, 39.82; H, 6.93; N, 2.39.

2,3,4-Tri-O-acetyl-1,5-anhydro-6-azido-6-deoxy-D-glucitol (**13**).—To a solution of 2,3,4-tri-*O*-acetyl-1,5-anhydro-6-*O*-tosyl-D-glucitol[11] (4.8 g, 10.8 mmol) in DMF (15 mL) was added NaN_3 (1.85 g, 30 mmol). The solution was kept at 80°C for 2 h, then diluted with water (100 mL), and extracted with ether (3 × 50 mL). The combined extracts were washed with water, dried ($MgSO_4$), and concentrated. Flash-column chromatography (1:3 EtOAc–cyclohexane) of the residue yielded **13** (2.9 g, 85%), R_F 0.5 (1:1 EtOAc–cyclohexane), mp 75°C (from MeOH), $[\alpha]_D$ +81° (*c* 1.18, MeOH); λ_{max}^{KBr} 2100 cm^{-1} (N_3). ^1H NMR data: δ 5.2 (t, 1 H, $J_{4,5}$ 9.8 Hz, H-4), 5.01 (ddd, 1 H, $J_{2,3}$ 9.7 Hz, H-2), 4.96 (t, 1 H, $J_{3,4}$ 9.8 Hz, H-3), 4.18 (dd, $J_{1eq,1ax}$ 11.3 Hz, $J_{1eq,2}$ 6 Hz, H-1*eq*), 3.57 (ddd, 1 H, $J_{5,6a}$ 5.7 Hz, $J_{5,6b}$ 3.8 Hz, H-5),

3.32 (dd, 1 H, $J_{1ax,2}$ 9.8 Hz, H-1*ax*), 3.3 (m, 2 H, H-6a,6b), 2.02 (s, 3 H, OAc), 2.03 (s, 3 H, OAc), 2.04 (s, 3 H, OAc). *Anal.* Calcd for $C_{12}H_{17}N_3O_7$: C, 45.72; H, 5.43; N, 13.33. Found: C, 45.40; H, 5.33; N, 13.20.

*1,5-Anhydro-6-azido-6-deoxy-*D*-glucitol* (**14**).—To a solution of **13** (2.9 g, 9.2 mmol) in MeOH (10 mL) was added methanolic M NaOMe (100 μL). The solution was kept at room temperature overnight, then filtered through a short column of silica gel, and concentrated to yield **14** (1.68 g, 96%), R_F 0.49 (17:2:1 EtOAc–MeOH–H_2O), mp 107°C (from MeOH), $[\alpha]_D$ +58° (*c* 1.61, H_2O); ν_{max}^{KBr} 2110 cm^{-1} (N_3). *Anal.* Calcd for $C_6H_{11}N_3O_4$: C, 38.10; H, 5.86; N, 22.20. Found: C, 37.93; H, 5.72; N, 22.08.

*1,5-Anhydro-6-azido-6-deoxy-4-*O*-α-maltosyl-*D*-glucitol* (**15**).—A solution of **14** (1.5 g, 7.9 mmol) and αCD (1.8 g, 1.8 mmol) in distilled water (10 mL) was treated as described for **3**, to yield, after freeze-drying, **15** (303 mg, 0.59 mmol), R_F 0.31 (7:2:1 EtOAc–MeOH–H_2O), $[\alpha]_D$ +161° (*c* 0.76, H_2O); ν_{max}^{KBr} 2095 cm^{-1} (N_3). *Anal.* Calcd for $C_{18}H_{31}N_3O_{14}$: C, 42.10; H, 6.09; N, 8.18. Found: C, 41.63; H, 5.95; N, 7.99.

*6-Amino-1,5-anhydro-6-deoxy-4-*O*-α-maltosyl-*D*-glucitol hydrochloride* (**16**).—A solution of **15** (92 mg, 0.18 mmol) in MeOH (4 mL) was hydrogenated, as described for **10**, to yield **16** (91.8 mg, 0.175 mmol), R_F 0.24 (30:20:15 $CHCl_3$–MeOH–NH_3), $[\alpha]_D$ +105° (*c* 1.06, H_2O). *Anal.* Calcd for $C_{18}H_{31}NO_{14} \cdot HCl$: C, 41.24; H, 6.54; N, 2.67. Found: C, 39.77; H, 6.38; N, 2.34.

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