Aminosugars. XXVI. Synthesis of Amido-bonded Disaccharides Containing Hexosaminuronic Acids[1]

Juji YOSHIMURA, Hiroaki ANDO, Tadahide SATO, Shigeru TSUCHIDA,* and Hironobu HASHIMOTO

Laboratory of Chemistry for Natural Products, Faculty of Science, Tokyo Institute of Technology, Meguro-ku, Tokyo 152

(Received February 7, 1976)

Synthesis and properties of amido-bonded disaccharides containing hexosaminuronic acids are described. The amino group of D-gluco- and D-mannosamine derivatives was acylated with D-gluco- and D-mannosaminuronic acid derivatives by the dicyclohexylcarbodiimide method, and the protecting groups of a few products were removed. Some of them showed the additive patterns of components in their PMR spectra.

2-Acetamido-2-deoxy-D-glucuronic acid[2-4] and -D-mannuronic acid[5] have been found as a component of several bacterial cell-walls. These hexosaminuronic acids also arouse interest in their polyfunctional character. For examples, several antibiotics such as blasticidine S,[6] gougerotin,[7] and polyoxin C[8] include an amido bond between a certain hexosaminuronic acid and a peptide. Although amido-bonded disaccharides have not been found in nature up to date, we synthesized some of them containing usual aminosugars and uronic acids, and analyzed their PMR spectra as the additive pattern of component sugars in a previous paper.[9]

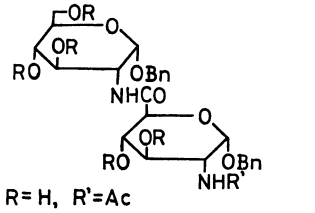
In this paper, similar disaccharides between hexosaminuronic acids and aminosugars were synthesized, and their properties were examined.

Results and Discussion

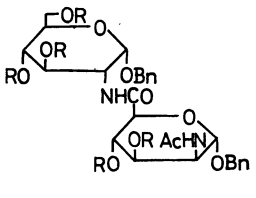
In this study, benzyl 2-amino-2-deoxy-α-D-glucopyranoside (**1**),[10] benzyl 2-amino-4,6-*O*-benzylidene-2-deoxy-α-D-mannopyranoside (**2**)[11] and its de-*O*-benzylidenated derivative (**3**)[9] were used as aminosugars, and benzyl 2-acetamido- (**4**)[12] and 2-benzyloxycarbonylamido-2-deoxy-α-D-glucopyranosiduronic acid (**5**)[13] benzyl 2-acetamido-2-deoxy-α-D-mannopyranosiduronic acid (**6**)[11] as hexosaminuronic acids.

Condensation of **1** and **4** or **5** in pyridine by the dicyclohexylcarbodiimide (DCC) method for five days gave precipitates of a mixture of the product and dicyclohexylurea (DCU), from which benzyl 2-(benzyl 2-acetamido- (**7**) or 2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranosiduronyl) amino-2-deoxy-α-D-glucopyranoside (**8**) was obtained by fractional crystallization both in 71% yield. These compounds having the D-*gluco*-type unit are not so soluble even in water and alcohols, and have higher melting points compared with that including the D-*manno*-type unit. The structure of **7** was confirmed by the conversion into the corresponding penta-*O*-acetate (**9**). Similar condensation of **1** and **6**, and separation of the product and DCU by a silica gel column chromatography gave benzyl 2-(benzyl 2-acetamido-2-deoxy-α-D-mannopyranosiduronyl)amino-2-deoxy-α-D-glucopyranoside (**10**) in 64% yield. Acetylation of **10** gave the corresponding penta-*O*-acetate (**11**) in 80% yield.

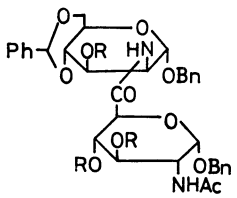
In the case of **2** and **4**, and also **3** and **6**, the corresponding disaccharides were separated in a pure state only as the tri-*O*-acetate (**13**) and penta-*O*-acetate (**15**), respectively. Treatment of **13** in ethanol with a tiny amount of metallic sodium gave benzyl 2-(benzyl



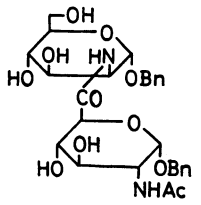
7 R=H, R'=Ac
8 R=H, R'=benzyloxycarbonyl
9 R=R'=Ac



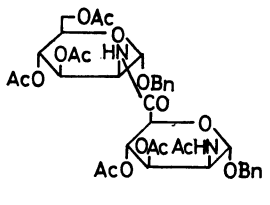
10 R=H
11 R=Ac



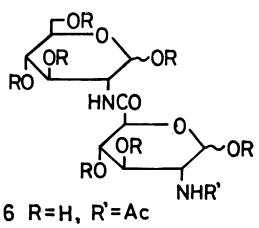
12 R=H
13 R=Ac



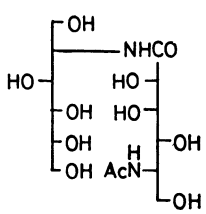
14



15



16 R=H, R'=Ac
17 R=H, R'=H·HCl
18 R=R'=Ac



19

* Present address: Arai High School, Koizumo Arai-shi 944.

Table 1. The additivity of NMR signals in amido-bonded disaccharides observed in partial ring-protons[a,b]

	H_1	H_2	H_3	H_4
9	5.07; d, $J_{1,2}$=3.6		5.75; t, $J_{3,4}$= 9.9	5.52; t, $J_{4,5}$= 9.2
	5.32; d, $J_{1,2}$=3.6			
	(5.17; d, $J_{1,2}$=3.4)	(*ca.* 4.81)	(5.73; t, $J_{3,4}$= 9.8)	(5.34; t, $J_{4,5}$= 9.2)
	[5.27; d, $J_{1,2}$=3.6]	[*ca.* 4.93]	[5.82; t, $J_{3,4}$=12.0]	[5.72; t, $J_{4,5}$= 9.0]
15	4.79; d, $J_{1,2}$=*ca.* 1.5	$J_{2,3}$=4.0	5.34; q, $J_{3,4}$=10.0	5.14; t, $J_{4,5}$=*ca.* 10.0
	4.88; d, $J_{1,2}$=*ca.* 1.0			
	(4.80; d, $J_{1,2}$=*ca.* 1.0	(4.62; q, $J_{2,3}$=4.5)	(5.34; q, $J_{3,4}$=10.0)	(5.12; t, $J_{4,5}$= 9.3)[9]
	[4.93; d, $J_{1,2}$=2.0]	[4.70; $J_{2,3}$=4.5]	[5.40; q, $J_{3,4}$=10.0]	[5.25; q, $J_{4,5}$=10.0][11]
18	6.13; d, $J_{1,2}$=3.8	*ca.* 4.45; m	5.28; t, $J_{3,4}$= 9.8	5.13; t, $J_{4,5}$= 8.5
	(6.13; d, $J_{1,2}$=3.6)	(*ca.* 4.48; m)	(5.20; t, $J_{3,4}$= 9.8)	(5.16; t, $J_{4,5}$= 9.0)

a) NMR data (chemical shift, splitting and coupling constant) in parentheses and brackets show those of component *O*-acetylated aminosugar and uronic acid moiety, respectively. b) Compound **9** and its component sugars were measured in pyridine-d_5 and others in chloroform-d_1.

2-acetamido-2-deoxy-α-D-glucopyranosiduronyl)amino-4,6-*O*-benzylidene-2-deoxy-α-D-mannopyranoside (**12**). Partial hydrolysis of **12** with 60% acetic acid gave the corresponding de-*O*-benzylidenated product (**14**).

On the other hand, hydrogenation of **7** and **8** in the presence of palladium–charcoal gave the corresponding free sugars (**16** and **17**) having fairly high melting points in 80 and 66% yields, respectively. Further hydrogenation of **16** with excess sodium borohydride gave quantitatively the corresponding crystalline alditol (**19**). Acetylation of **16** in pyridine with acetic anhydride unexpectedly gave the unchanged materials, but acid-catalyzed acetylation with perchloric acid–acetic anhydride gave successfully the corresponding hepta-*O*-acetate (**18**). The glycosidic configurations of **18** were determined to be α, α from the coupling constants, $J_{1,2}$=3.8 Hz (Fig. 1) and specific rotation ($[\alpha]_D^{23}$ +115°). For the synthesis of amido-bonded trisaccharides, condensation of **17** with **5** by the DCC method was tried several times, but only unsuccessful results were obtained, partly due to the insolubility of **17**.

As was pointed out in the previous paper, PMR spectra of amido-bonded sugars were supposed to show the additive patterns of the component monosaccharides.

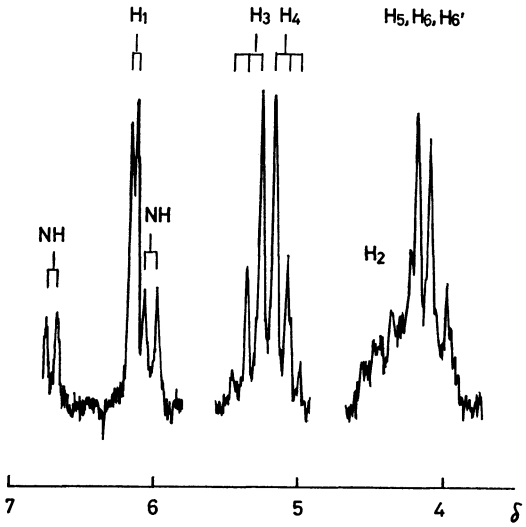


Fig. 1. Ring-proton signals in the NMR spectrum of **18** (in $CDCl_3$ at 100 MHz).

However, the analysis of spectra of pyranose-pyranose type compounds mentioned above were rather difficult due to superimposed signals. The simplest signal pattern in the spectrum of **18** indicated that H_1, H_3, and H_4 protons in both pyranose-rings are almost in the same magnetic circumstance. As was shown in Table 1, the similar additivity was scarcely observed in the spectra of **9** and **15** having the same configuration in both pyranose-rings.

Experimental

The melting points were determined on a sulfuric acid bath and uncorrected. Optical rotations were measured in a 0.5-dm tube with a Carl Zeiss photoelectric polarimeter. NMR spectra were recorded at 100 MHz with a JNM-4H-100 spectrometer. IR spectra were recorded with a Hitachi Model EPI-G2 spectrophotometer. Chemical shifts and coupling constants were recorded in δ and Hz units and IR frequencies in cm^{-1}.

Benzyl 2-(Benzyl 2-acetamido-2-deoxy-α-D-glucopyranosiduronyl)-amino-2-deoxy-α-D-glucopyranoside (*7*). To a solution of benzyl 2-amino-2-deoxy-α-D-glucopyranoside (**1**) hydrochloride(14.0 g, 45.8 mmol) and benzyl 2-acetamido-2-deoxy-α-D-glucopyranosiduronic acid (**4**) (15.0 g, 46.1 mmol) in dry pyridine (140 ml) containing triethylamine (4.6 g, 46 mmol) was added DCC (10 g, 50 mmol), and the resulting solution was stirred at room temperature for 5 days. The gelatinous materials deposited were filtered. The amorphous solid obtained by addition of acetic acid (5%, 10 ml) to the above filtrate was also gathered. Both solids were dissolved in DMF and insoluble DCU was filtered off. The residues obtained by evaporation of the filtrate were crystallized twice from methanol–pyridine. Yield, 18.8 g (71%); mp 283—284 °C (decomp); $[\alpha]_D^{23}$ +156° (*c* 1.0, DMF); IR: 1650 and 1550 (amide) cm^{-1}.

Found: C, 57.96; H, 6.20; N, 4.81%. Calcd for $C_{28}H_{36}N_2O_{11}$: C, 58.32; H, 6.29; N, 4.86%.

Acetylation of **7** by the usual manner, and crystallization of the product from ethanol gave the corresponding penta-*O*-acetate (**9**) in 80% yield. Mp 201—202 °C; $[\alpha]_D^{23}$ +66° (*c* 1.0, pyridine); IR: 1745 (ester), 1685, 1660, 1525 (amide) cm^{-1}.

Found: C, 57.73; H, 5.79; N, 3.52%. Calcd for $C_{38}H_{46}N_2O_{16}$: C, 58.01; H, 5.89; N, 3.56%.

Benzyl 2-(Benzyl 2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranosiduronyl)amino-2-deoxy-α-D-glucopyranoside (*8*). Condensation of hydrochloride of **1** (11 g, 36 mmol) and benzyl 2-

benzyloxycarbonylamino-2-deoxy-α-D-glucopyranosiduronic acid (**5**) (15 g, 36 mmol) in a similar manner as **7**, and recrystallization of the product from pyridine–methanol gave **8** in 71 % (17 g) yield. Mp 272—272.5 °C (decomp); $[\alpha]_D^{23}$ +170° (*c* 1.0, pyridine); IR: 1650, 1525 (amide) cm^{-1}.

Found: C, 60.85; H, 6.02; N, 4.29%. Calcd for $C_{34}H_{40}N_2O_{11}$: C, 61.07; H, 6.03; N, 4.19%.

Benzyl 2-(Benzyl 2-acetamido-2-deoxy-α-D-mannopyranosiduronyl)-amino-2-deoxy-α-D-glucopyranoside (**10**). A solution of hydrochloride of **1** (2.2 g, 7.2 mmol), benzyl 2-acetamido-2-deoxy-α-D-mannopyranosiduronic acid (**6**) (2.4 g, 7.3 mmol) and DCC (1.5 g, 7.3 mmol) in pyridine (6 ml) and triethylamine (1.2 ml) was stirred for 5 days at room temperature, and DCU deposited was filtered off. Evaporation of the filtrate gave a syrup which was fractionally separated on a silica gel column (benzene–methanol=10 : 1) to give the crystalline product which was recrystallized three times from ethanol. Yield, 2.7 g (65%), mp 223—223.5 °C; $[\alpha]_D^{23}$ +142° (*c* 0.57, pyridine), IR: 1655, 1555, 1520 (amide) cm^{-1}.

Found: C, 58.21; H, 6.24; N, 5.07%. Calcd for $C_{28}H_{36}N_2O_{11}$: C, 58.32; H, 6.29; N, 4.86%.

Base-catalyzed acetylation of **10** in a usual manner gave the corresponding penta-*O*-acetate (**11**) in 80% yield, which was purified from ethanol–petroleum ether. Amorphous white solid sintered at 85 °C and melted at 124—125 °C. $[\alpha]_D^{23}$ +54° (*c* 0.92, pyridine); IR: 1740 (ester), 1680, 1510 (amide) cm^{-1}.

Found: C, 57.84; H, 5.95; N, 3.43%. Calcd for $C_{38}H_{46}N_2O_{16}$: C, 58.01; H, 5.89; N, 3.56%.

Benzyl 2-(Benzyl 2-acetamido-3,4-di-O-acetyl-2-deoxy-α-D-glucopyranosiduronyl)amino-3-O-acetyl-4,6-O-benzylidene-2-deoxy-α-D-mannopyranoside (**13**). A solution of benzyl 2-amino-4,6-*O*-benzylidene-2-deoxy-α-D-mannopyranoside (**2**) (1.5 g, 4.2 mmol), **4** (1.4 g, 4.3 mmol), and DCC (1.0 g, 4.85 mmol) in pyridine (20 ml) was stirred for 3 days at room temperature, filtered, and then the filtrate was evaporated to give a syrup (2.6 g). To a solution of this syrup in pyridine (4 ml) was added acetic anhydride (1.5 g), and after 2 h, the reaction mixture was poured into ice–water. The solid mass deposited was collected, dried, and then recrystallized from large amount of ethanol to give **13** in 69% yield. Mp 252—253 °C; $[\alpha]_D^{23}$ +90.5° (*c* 1.1, pyridine); IR: 1680, 1560 (amide) cm^{-1}.

Found: C, 62.01; H, 5.65; N, 3.76%. Calcd for $C_{41}H_{46}N_2O_{14}$: C, 62.27; H, 5.86; N, 3.54%.

De-*O*-acetylation of **13** (2.0 g, 2.5 mmol) in absolute ethanol (600 ml) with a trace amount of metallic sodium gave a syrup which was crystallized from methanol to give **12** in 72% (1.2 g) yield. Mp 153—155 °C; $[\alpha]_D^{23}$ +97.8° (*c* 0.64, pyridine); IR: 1650, 1525 (amide) cm^{-1}.

Found: C, 63.01; H, 6.22; N, 4.35%. Calcd for $C_{35}H_{40}N_2O_{11}$: C, 63.24; H, 6.07; N, 4.21%.

Benzyl 2-(Benzyl 2-acetamido-2-deoxy-α-D-glucopyranosiduronyl)-amino-2-deoxy-α-D-mannopyranoside (**14**). A solution of **12** (0.50 g, 0.75 mmol) in 60% acetic acid (20 ml) was heated for 1 h at 80 °C, and then evaporated to give a hard syrup which was crystallized from methanol. Yield, 0.33 g (76%); mp 138—140 °C; $[\alpha]_D^{23}$ +107° (*c* 0.19, DMF); IR: 1680, 1560 (amide) cm^{-1}.

Found: C, 58.01; H, 6.42; N, 4.76%. Calcd for $C_{28}H_{36}N_2O_{11}$: C, 58.32; H, 6.29; N, 4.86%.

Benzyl 2-(benzyl 2-acetamido-3,4-di-O-acetyl-2-deoxy-α-D-mannopyranosiduronyl)amino-3,4,6-tri-O-acetyl-2-deoxy-α-D-mannopyranoside (**15**). Benzyl 2-amino-2-deoxy-α-D-mannopyranoside (**3**) (0.37 g, 1.2 mmol) and **6** (0.40 g, 1.2 mmol) were condensed in a similar manner as above, and the syrupy product was acetylated to give syrupy penta-*O*-acetate (1.0 g) which was purified on a silica gel column (benzene–methanol=10: 1). Yield, 0.71 g (75%); $[\alpha]_D^{23}$ +72.8° (*c* 0.60, pyridine); IR, 1750 (ester), 1680, 1670, and 1545 (amide) cm^{-1}.

Found: C, 57.72; H, 5.81; N, 3.32%. Calcd for $C_{38}H_{46}N_2O_{16}$: C, 58.01; H, 5.89; N, 3.58%.

2-(2-Acetamido-2-deoxy-D-glucopyranuronyl)amino-2-deoxy-D-glucopyranose (**16**). A suspension of **7** (5.0 g, 8.7 mmol) in methanol (100 ml) adjusted to pH 2 with hydrochloric acid was hydrogenated in the presence of palladium–charcoal (10%, 10 g), and then filtered. Concentration of the filtrate gave no products, but, extraction of the filtered mass several times with hot water gave white solid which was recrystallized from hot water. Yield, 2.8 g (80%); mp 261—262 °C (decomp); IR: 1650, 1620, 1570, and 1540 (amide) cm^{-1}.

Found: C, 42.25; H, 6.31; N, 7.23%. Calcd for $C_{14}H_{24}N_2O_{11}$: C, 42.42; H, 6.10; N, 7.07%.

2-(2-Amino-2-deoxy-D-glucopyranuronyl)amino-2-deoxy-D-glucopyranose Hydrochloride (**17**). A suspension of **8** (12.0 g, 17.9 mmol) in water containing equimolar amount of hydrochloric acid was hydrogenated in the presence of palladium–charcoal (10%, 10 g), filtered, and then evaporated to give a white mass which was recrystallized from water–ethanol. Yield 4.6 g (66 %); mp 230—231 °C (decomp); $[\alpha]_D^{23}$ +80° +62.4° (*c* 1.0, H_2O, 72 h); IR: 1640, 1510 (amide), 1560 ($NH_2 \cdot HCl$) cm^{-1}.

Found C, 36.40; H, 6.01; N, 6.79; Cl, 9.09%. Calcd for $C_{12}H_{22}N_2O_{10} \cdot HCl$: C, 36.88; H, 5.93; N, 7.17; Cl, 9.07%.

2-(2-Acetamido-1,3,4-tri-O-acetyl-2-deoxy-α-D-glucopyranuronyl)amino-1,3,4,6-tetra-O-acetyl-2-deoxy-α-D-glucopyranose (**18**). To an ice-cooled suspension of **16** (0.60 g, 1.5 mmol) in acetic anhydride (15 ml) was added several drops of perchloric acid under stirring, and the resulting solution was kept at room temperature for 30 min, poured into ice–water, and then extracted with chloroform. The extract was washed successively with water, aqueous sodium hydrogencarbonate and water, dried and then evaporated to give crystals which were recrystallized from ethanol. Yield, 0.63 g (60%); mp 190—191 °C $[\alpha]_D^{23}$ +115° (*c* 1.0, $CHCl_3$).

Found: C, 48.92; H, 5.69; N, 4.06%. Calcd for $C_{28}H_{38}N_2O_{18}$: C, 48.70; H, 5.55; N, 4.06%.

2-(5-Acetamido-5-deoxy-L-gulonyl)amino-2-deoxy-D-glucitol (**19**). To an aqueous solution of **16** (0.30 g, 0.76 mmol) was added portionwise sodium borohydride (0.10 g, 3 mmol) with stirring, the resulting solution was stirred at room temperature for 2 h, and then neutralized with Amberlite 120 (H^+ form). The neutral solution was evaporated, and a methanol solution of the residue was evaporated repeatedly to remove boric acid. The product thus obtained was crystallized from methanol–ether. Yield 0.30 g (99%); mp 83—84 °C; $[\alpha]_D^{23}$ −21° (*c* 1.0, H_2O); IR, 1660, 1640, and 1550 (amide).

Found: C, 41.59; H, 7.06; N, 6.87%. Calcd for $C_{14}H_{28}N_2O_{11}$: C, 42.00; H, 7.05; N, 7.00%.

The authors are indebted to the members of the Laboratory of Organic Analysis for microanalysis and to Mr. H. Matsumoto for NMR measurements.

References

1) Part XXV, J. Yoshimura, M. Iwakawa, and Y. Ogura *Bull. Chem. Soc. Jpn.*, **49**, 2506 (1976).
2) W. R. Clark and J. Mclaughlin, *J. Biol. Chem.*, **230**, 81 (1958).
3) A. R. Williamson and S. Zamenhof, *J. Biol. Chem.*, **238**, 2255 (1963).
4) S. Hanessian and T. H. Haskell, *J. Biol. Chem.*, **239**, 2758 (1964).
5) S. Hase and Y. Matsushima, *J. Biochem.*, **69**, 559 (1971).
6) S. Takeuchi and H. Yonehara, *J. Antibiot. (Tokyo)*, **11**, 1 (1958).

7) T. Kanzaki and A. Miyake, *J. Antibiot. (Tokyo)*, **15**, 93 (1962).
8) K. Isono and S. Suzuki, *Tetrahedron Lett.*, **203** (1968).
9) J. Yoshimura, Y. Motoki, M. Ikeda, N. Oda, and H. Hashimoto, *Nippon Kagaku Kaishi*, **1975**, 1958.
10) J. Yoshimura, K. Fujimori, Y. Sugiyama, and H. Ando., *Bull. Chem. Soc. Jpn.*, **44**, 3131 (1971).
11) J. Yoshimura, H. Sakai, N. Oda, H. Hashimoto, *Bull. Chem. Soc. Jpn.*, **45**, 2027 (1972).
12) J. Yoshimura, T. Sato, and H. Ando, *Bull. Chem. Soc. Jpn.*, **42**, 2352 (1965).
13) H. Weidmann and H. K. Zimmerman, *Ann. Chem.*, **639**, 198 (1961); K. Heyns and H. Paulsen, *Chem. Ber.*, **88**, 188 (1955).
14) J. Yoshimura, H. Hashimoto, and H. Ando, *Carbohyd. Res.*, **4**, 435 (1967); **5**, 82 (1967).
15) H. Weidmann and H. K. Zimmerman, *Ann. Chem.*, **684**, 226 (1965).