References and Notes

1) Part IV (a); part III, M. Suzuki and Y. Sasaki, *Chem. Pharm. Bull.*, **27**, 1797 (1979).
2) Job plots was achieved so that the sum of the concentrations comes to 0.07 M. When molar ratios of γ-cdx to orange II are from 0.5 to 1.5, the above phenomenon appears.
3) The microscopic texture was viewed using the micro melting measurement apparatus with the polaroid plate ($\times$60).
4) A. Johansson and T. Drakenberg, *Mol. Cryst. Liq. Cryst.*, **14**, 23 (1971).
5) P.J. Black, K.D. Lawson, and T.J. Frautt, *Mol. Cryst. Liq. Cryst.*, **7**, 201 (1969).
6) M. Suzuki and Y. Sasaki, *Chem. Pharm. Bull.*, **27**, 609 (1979).
7) In preparation.
8) M. Suzuki and Y. Sasaki, *Chem. Pharm. Bull.*, **27**, 1343 (1979).

Faculty of Pharmaceutical Sciences,
Osaka University,
133-1, Yamada-Kami, Suita, Osaka, Japan

Miyoko Suzuki*
Yoshio Sasaki

Received October 30, 1980

[Chem. Pharm. Bull. 29(2) 587—590 (1981)]

Chemical Modification of Lactose. XVI.[1] Synthesis of Lacto-N-neohexaose

Reaction of 1,6-anhydro-2,2′,3,4′-tetra-O-benzyl-β-lactose (1 mol eq.) with the acetylated oxazoline of N-acetyllactosamine (5 mol eq.) gave the corresponding 1,6-anhydro-β-tetrasaccharide (**3**, 24.5%) and hexasaccharides (**8**, 53.5%). The protecting groups of **3** and **8** were removed by the following series of reactions to provide 6′-N-acetyllactosaminyllactose (**7**) and lacto-N-neohexaose (**12**), respectively: debenzylation followed by acetylation, acetolysis, and de-O-acetylation. ^{13}C-NMR spectral data for 1,6-anhydro-β-derivatives of **7** and **12** are presented.

Keywords——synthesis; human milk oligosaccharide; lacto-N-neohexaose; oxazoline glycosidation method; 6′-N-acetyllactosaminyllactose; 1,6-anhydro-β-tetrasaccharide; 1,6-anhydro-β-hexasaccharide; ^{13}C-NMR

The occurrence and the structure of lacto-N-neohexaose (**12**) in human milk were reported by Kobata and Ginsburg,[2] and the existence of more complex oligosaccharides having **12** as a partial structure has been described.[3] We now report a synthesis of **12** together with 6′-N-acetyllactosaminyllactose (**7**) as a by-product.

A mixture of 1,6-anhydro-2,2′,3,4′-tetra-O-benzyl-β-lactose (**1**)[4] (1 mol eq.) and the acetylated oxazoline of N-acetyllactosamine (**2**)[5] (3 mol eq.) in dry 1,2-dichloroethane containing 0.01 M anhyd. p-toluenesulfonic acid was stirred at 60—65° for 48 hr under nitrogen. After 48 hr, more **2** (2 mol eq.) was added and stirring was continued for further 24 hr. The mixture was neutralized and concentrated to dryness: TLC showed two spots. By column chromatography on Kieselgel 60 (Merck, 70—230 mesh) with $CHCl_3$–ether–MeOH (7: 7: 1, v/v), the products were separated into tetra- and hexasaccharide fractions. The former was re-chromatographed with $CHCl_3$–acetone (3: 1) to isolate the protected tetrasaccharide (**3**, 24.5%) as amorphous powder, $[\alpha]_D^{21}$ −10.8° ($CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.84, 1.98, 2.01, 2.06, 2.16 (21H, all s, OAc$\times$6, NAc), 5.51 (1H, s, H-1, β-Glc), 5.65 (1H, d, exchangeable with D_2O, $J_{NH,2'}$=8.5 Hz, NH), 7.20—7.44 (20H, m, aromatic protons). Hydrogenolytic debenzyl-

ation of **3**, followed by acetylation, gave the dodecaacetate (**4**, 92.4%) as amorphous powder, $[\alpha]_D^{20}$ −27.8° ($CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.95, 1.96, 2.05, 2.12, 2.13 (36H, all s, OAc×11, NAc), 5.46 (1H, s, H-1, β-Glc), 6.28 (1H, d, exchangeable with D_2O, $J_{NH,2''}$=8.5 Hz, NH). De-O-acetylation of **4** yielded the 1,6-anhydro-β-tetrasaccharide (**5**, 73.5%), crystallizable from MeOH as needles, mp 197—199°, $[\alpha]_D^{23}$ −34.8° (H_2O). ^{1}H-NMR (D_2O): 2.51 (3H, s, NAc), 4.55 (1H, d, $J_{1',2'}$=8 Hz, H-1′, β-Gal), 4.90 (1H, d, $J_{1''',2'''}$=7 Hz, H-1‴, β-Gal), 4.98 (1H, d, $J_{1'',2''}$=6 Hz, H-1″, β-GlcNAc), 5.90 (1H, s, H-1, β-Glc). The signals of anomeric protons were assigned by comparison with the found values for 1,6-anhydro-β-lactose (**13**, 5.30 ppm, s, H-1; 4.48 ppm, d, $J_{1',2'}$=8 Hz, H-1′) and methyl β-N-acetyllactosaminide (**15**, 4.89 ppm, d, $J_{1,2}=J_{1',2'}$=8 Hz, H-1 and H-1′).

The aforementioned hexasaccharide fraction was re-chromatographed with $CHCl_3$–EtOH (19:1) to isolate the protected hexasaccharide (**8**, 53.5%) as an amorphous powder, $[\alpha]_D^{21}$ −13.8° ($CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.53, 1.83, 1.99, 2.06, 2.09, 2.16 (42H, all s, OAc×12, NAc×2), 5.88 (1H, d, exchangeable with D_2O, $J_{NH,2''}$ or $_{2''''}$=8 Hz, NH), 7.24—7.44 (20H, m, aromatic protons). The octadecaacetate (**9**) and 1,6-anhydro-β-hexasaccharide (**10**) were prepared from **8** and **9**, respectively, by the procedures similar to those described in the tetrasaccharide series. **9**: amorphous powder, $[\alpha]_D^{25}$ −11.1° ($CHCl_3$), 88.6% yield. ^{1}H-NMR ($CDCl_3$): 1.94, 1.98, 2.06, 2.12, 2.15 (54H, all s, OAc×16, NAc×2), 5.48 (1H, s, H-1, β-Glc), 5.77 (1H, d, exchangeable with D_2O, $J_{NH,2''}$ or $_{2''''}$=8 Hz, NH), 6.41 (1H, d, exchangeable with D_2O, $J_{NH,2''''}$ or $_{2''}$=8 Hz, NH). **10**: white powder, $[\alpha]_D^{22}$ −26.3° (H_2O), 72% yield. ^{1}H-NMR (D_2O): 2.50, 2.53 (6H, each s, NAc×2), 5.91 (1H, s, H-1, β-Glc).

The completely proton-decoupled ^{13}C-NMR of **5** and **10** were measured in D_2O at room temperature with **13** and **15** as reference compounds. The results are summarized in Table I. The signals for the corresponding carbon atoms in **15** and N-acetyllactosaminyl residue of **5** showed similar chemical shifts, but the resonance of C-6′ of **5** (69.9 ppm) was deshielded by 7.6 ppm, as compared with that for C-6′ of **13** (62.3 ppm). Similarly, the resonances for C-6′ (70.2 ppm) and C-3′ (82.8 ppm) of **10** were deshielded by 7.9 and 9.1 ppm, as compared with those for C-6′ (62.3 ppm) and C-3′ (73.7 ppm) of **13**, respectively.

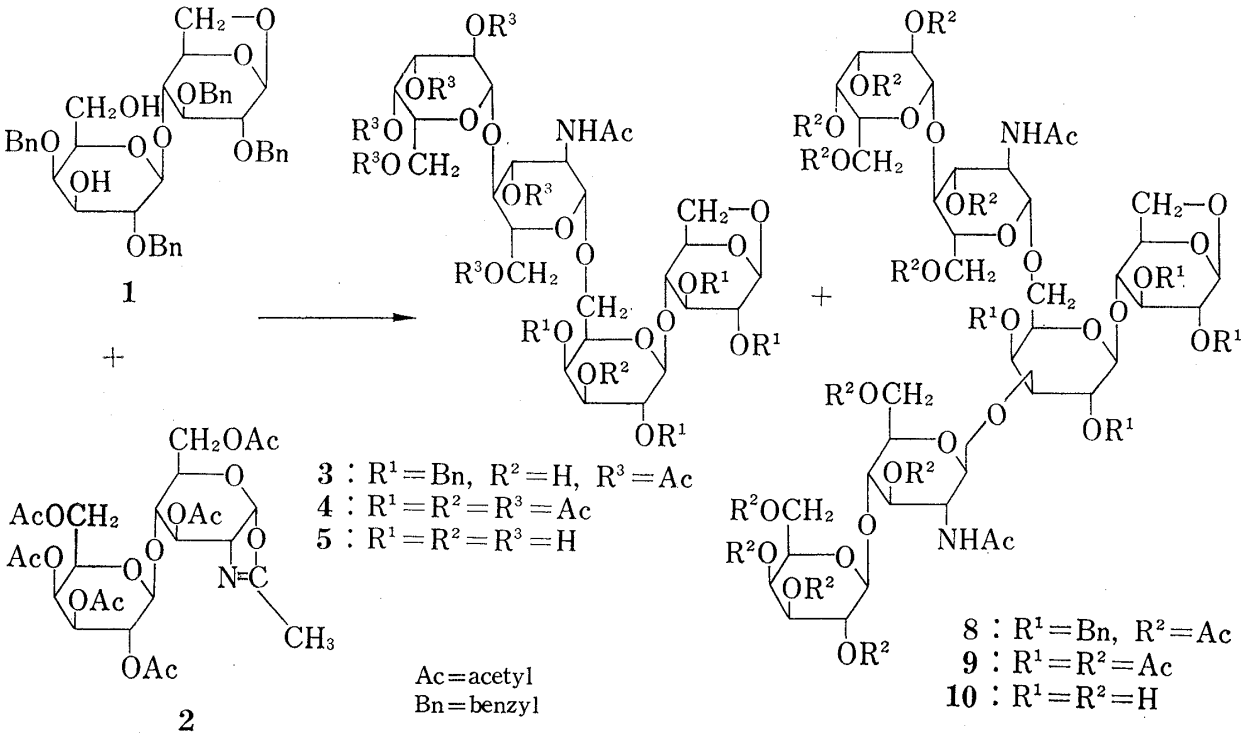


Chart 1

13: Galβ 1→4 Glucosan
B A

15: Galβ 1→4 Methyl β-GlcNAc
B A

5: Galβ 1→4 GlcNAcβ 1→6 Galβ 1→4 Glucosan
D C B A

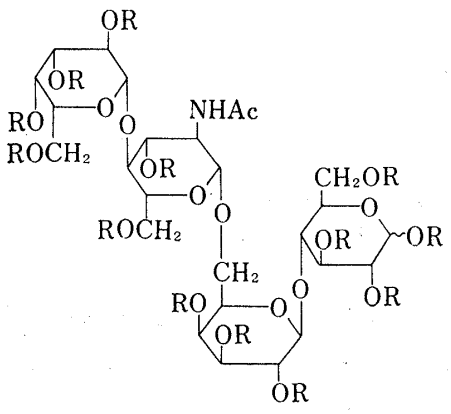
10: Galβ 1→4 GlcNAcβ 1→6, 3 Galβ 1→4 Glucosan
Galβ 1→4 GlcNAcβ 1→ B A
F E

Glucosan = 1,6-anhydro-β-D-glucopyranose

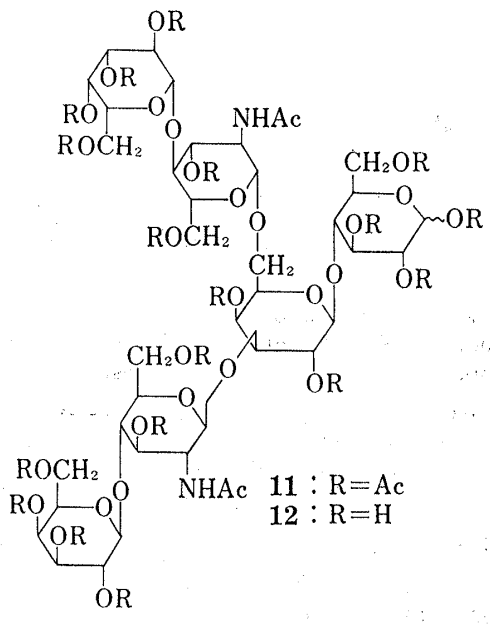
TABLE I. ^{13}C Chemical Shifts, δ(ppm) from TMS

		C-1	C-2	C-3	C-4	C-5	C-6	$NCOCH_3$	$NCOCH_3$	OMe
13[a]	A	102.6	71.2	72.7	78.9	75.3	66.3			
	B	103.3	71.9	73.7	69.9	76.5	62.3			
15[b]	A	103.0	56.2	73.7	79.8	75.9	61.3	23.4	175.8	58.3
	B	104.1	72.2	73.7	69.8	76.5	62.2			
5[c]	A	102.5	70.9	72.5	78.7	75.1	66.2			
	B	103.1	71.7	73.7	69.7	74.8	69.9			
	C	102.3	56.2	73.5	79.6	75.9	61.2	23.4	175.8	
	D	104.0	72.1	73.5	69.7	76.5	62.2			
10[d]	A	102.5	70.8	72.5	78.5	75.0	66.1			
	B	103.0	70.8	82.8	69.7	74.7	70.2			
	C	102.5	56.2	73.3[e]	79.6	75.9	61.2	23.4	175.7	
	D	104.0	72.1	73.7[e]	69.7	76.5	62.2			
	E	104.0	56.4	73.7[e]	79.5	75.8	61.2	23.5	176.0	
	F	104.0	72.1	73.7[e]	69.7	76.5	62.2			

a) O-β-D-Galactopyranosyl-(1→4)-1,6-anhydro-β-D-glucopyranose.
b) Methyl O-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside.
c) O-β-D-Galactopyranosyl-(1→4)-O-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-O-β-D-galactopyranosyl-(1→4)-1,6-anhydro-β-D-glucopyranose.
d) O-β-D-Galactopyranosyl-(1→4)-O-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[O-β-D-galactopyranosyl-(1→4)-O-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)]-O-β-D-galactopyranosyl-(1→4)-1,6-anhydro-β-D-glucopyranose.
e) Assignments may be reversed.



6 : R=Ac
7 : R=H



11 : R=Ac
12 : R=H

Chart 2

The 1,6-anhydro-β-rings of **4** and **9** were cleaved with an acetolysis mixture (H_2SO_4–Ac_2O–AcOH, 1: 70: 30, v/v) to give the tetrasaccharide tetradecaacetate (**6**) and hexasaccharide eicosaacetate (**11**), respectively, as anomeric mixtures containing α-anomer predominantly. **6**: amorphous powder, $[\alpha]_D^{22}$ +7.2° ($CHCl_3$), 94.3% yield. ^{1}H-NMR ($CDCl_3$): 1.97, 1.99, 2.03, 2.08, 2.16, 2.19 (42H, all s, OAc×13, NAc), 5.81 (*ca.* 0.3H, d, $J_{1,2}$=8 Hz, H-1, β-Glc), 6.29 (1H, br. s, exchangeable with D_2O, NH), 6.37 (*ca.* 0.7H, d, $J_{1,2}$=3.5 Hz, H-1, α-Glc). **11**: amorphous powder, $[\alpha]_D^{22}$ +12.7° ($CHCl_3$), 93.9% yield. ^{1}H-NMR ($CDCl_3$): 1.93, 1.98, 2.07, 2.16 (60H, all s, OAc×18, NAc×2), 5.62 (1H, d, exchangeable with D_2O, $J_{NH,2''}$ or $_{2''''}$=8 Hz, NH), 6.30 (<1H, d, $J_{1,2}$=3.5 Hz, H-1, α-Glc), 6.40 (1H, d, exchangeable with D_2O, $J_{NH,2''''}$ or $_{2''}$=8 Hz, NH).

De-O-acetylation of **6** and **11** with methanolic MeONa gave **7** (73.5% yield) as a white powder, $[\alpha]_D^{19}$ +11.8° (H_2O) [lit.[6] mp 185—187°, $[\alpha]_D$ +8° (H_2O)], and **12** (80% yield), crystallizable from aq. EtOH as grains, mp 223—225°, $[\alpha]_D^{21}$ +9.1° (no mutarotation, H_2O), respectively.

The data of elemental analysis of all these compounds were in good agreement with the theoretical values.

References and Notes

1) Part XV: T. Takamura, T. Chiba, and S. Tejima, *Chem. Pharm. Bull.*, accepted.
2) A. Kobata and V. Ginsburg, *Arch. Biochem. Biophys.*, **150**, 273 (1972).
3) A. Kobata, K. Yamashita, and Y. Tachibana, "Methods in Enzymology," Vol. 50, ed. by V. Ginsburg, Academic Press, New York, San Francisco, and London, 1978, pp. 216—226.
4) Part XIV: T. Takamura, T. Chiba, and S. Tejima, *Chem. Pharm. Bull.*, in press.
5) R. Kaifu and T. Osawa, *Carbohydr. Res.*, **52**, 179 (1976).
6) S.E. Zurabyan, V.A. Markin, V.V. Pimenova, B.V. Rozynov, V.L. Sadovskaya, and A. Ya. Khorlin, *Bioorg. Khim.*, **4**, 928 (1978).

Faculty of Pharmaceutical Sciences,
Nagoya City University, Tanabe-dori,
Mizuho-ku, Nagoya, 467, Japan

Tsukasa Takamura
Taku Chiba
Setsuzo Tejima*

Received November 29, 1980

[Chem. Pharm. Bull. 29(2) 590—593 (1981)]

Identification of a Reactive Metabolite of the Mutagen, 2-Amino-3-methylimidazolo[4,5-*f*]quinoline

A reactive major metabolite of the mutagen, 2-amino-3-methylimidazolo[4,5-*f*]-quinoline (IQ), by rat liver microsomes was 2-hydroxyamino-3-methylimidazolo[4,5-*f*]-quinoline (N–OH–IQ). The synthesis and reaction with DNA of N–OH–IQ were discussed.

Keywords——mutagen; 2-amino-3-methylimidazolo[4,5-*f*]quinoline; IQ; 2-hydroxyamino-3-methylimidazolo[4,5-*f*]quinoline; metabolic activation; microsomes; hydroxylamine; hydroxyaminoimidazole; carcinogen; DNA modification

Recent studies showed that pyrolysis products of proteins and amino acids contain strong mutagens, and active compounds were isolated and their structures were determined.[1] Among these compounds, 3-amino-5H-pyrido[4,3-*b*]indoles (Trp-P)[1a] from a pyrolysate of tryptophan and 2-aminodipyrido[1,2-*a*: 3′,2′-*d*]imidazoles (Glu-P)[1b] from a pyrolysate of