References and Notes

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 (x 60).
7) In preparation.

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Received October 30, 1980

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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-acetyllactosaminylactosyl (7) and lacto-N-neohexaose (12), respectively: debenzylation followed by acetylation, acetylation, and de-O-acetylation. 13C-NMR spectral data for 1,6-anhydro-β-derivatives of 7 and 12 are presented.

Keywords—synthesis; human milk oligosaccharide; lacto-N-neohexaose; oxazoline glycosylation method; 6’-N-acetyllactosaminylactosyl; 1,6-anhydro-β-tetrasaccharide; 1,6-anhydro-β-hexasaccharide; 13C-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-acetyllactosaminylactosyl (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°C 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 CHCl₃–ether–MeOH (7:7:1, v/v), the products were separated into tetra- and hexasaccharide fractions. The former was re-chromatographed with CHCl₃–acetic (3:1) to isolate the protected tetrasaccharide (3, 24.5%) as amorphous powder, [α]D_20° = -10.8° (CHCl₃). 1H-NMR (CDCl₃): 1.84, 1.98, 2.01, 2.06, 2.16 (2H, all s, OAc × 6, NAc), 5.51 (1H, s, H-1, β-Glc), 6.55 (1H, d, exchangeable with D₂O, J_H,α'=8.5 Hz, NH), 7.20—7.44 (20H, m, aromatic protons). Hydrogenolytic debenzyli-
ation of 3, followed by acetylation, gave the dodecaacetate (4, 92.4%) as an amorphous powder, $[\alpha]_D^{25} = -27.8^\circ$ (CHCl$_3$). $^1$H-NMR (CDCl$_3$): 1.95, 1.96, 2.05, 2.12, 2.13 (3H, all s, OAc$x_{11}$, NAc), 5.46 (1H, s, H-1, $\beta$-Glc), 6.28 (1H, d, exchangeable with D$_2$O, $J_{NH,2'''} = 8.5$ Hz, NH). De-O-acetylation of 4 yielded the 1,6-anhydro-$\beta$-tetrasaccharide (5, 73.5%), crystallizable from MeOH as needles, mp 197–199$^\circ$, $[\alpha]_D^{25} = -38.8^\circ$ (H$_2$O). $^1$H-NMR (D$_2$O): 2.51 (3H, s, NAc), 4.55 (1H, d, $J_{1',2'} = 8$ Hz, H-1', $\beta$-Gal), 4.90 (1H, d, $J_{1''',2'''} = 7$ Hz, H-1''', $\beta$-Gal), 4.98 (1H, d, $J_{1''',2'''} = 6$ Hz, H-1'', $\beta$-GlcNAc), 5.90 (1H, s, H-1, $\beta$-Glc). The signals of anomic protons were assigned by comparison with the found values for 1,6-anhydro-$\beta$-lactose (13, 5.30 ppm, s, H-1; 4.48 ppm, d, $J_{1',2'} = 8$ Hz, H-1') and methyl $\beta$-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, $[\alpha]_D^{25} = -13.8^\circ$ (CHCl$_3$). $^1$H-NMR (CDCl$_3$): 1.53, 1.83, 1.99, 2.06, 2.09, 2.16 (42H, all s, OAc$x_{12}$, NAc$x_2$), 5.88 (1H, d, exchangeable with D$_2$O, $J_{NH,2'''}$ or $J_{2''',3'''} = 8$ Hz, NH), 7.24–7.44 (20H, m, aromatic protons). The octadecaacetate (9) and 1,6-anhydro-$\beta$-hexasaccharide (10) were prepared from 8 and 9, respectively, by the procedures similar to those described in the tetrasaccharide series. 9: amorphous powder, $[\alpha]_D^{25} = -11.1^\circ$ (CHCl$_3$), 88.6% yield. $^1$H-NMR (CDCl$_3$): 1.94, 1.98, 2.06, 2.12, 2.15 (54H, all s, OAc$x_{16}$, NAc$x_2$), 5.48 (1H, s, H-1, $\beta$-Glc), 5.77 (1H, d, exchangeable with D$_2$O, $J_{NH,2'''}$ or $J_{2''',3'''} = 8$ Hz, NH), 6.41 (1H, d, exchangeable with D$_2$O, $J_{NH,2'''}$ or $J_{2''',3'''} = 8$ Hz, NH). 10: white powder, $[\alpha]_D^{25} = -26.3^\circ$ (H$_2$O), 72% yield. $^1$H-NMR (D$_2$O): 2.50, 2.53 (6H, each s, NAc$x_2$), 5.91 (1H, s, H-1, $\beta$-Glc).

The completely proton-decoupled $^{13}$C-NMR of 5 and 10 were measured in D$_2$O 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.
Glucosan = 1,6-anhydro-β-D-glucopyranose

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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.
e) Assignments may be reversed.

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#### Chart 2

- **6**: R = Ac
- **7**: R = H
- **11**: R = Ac
- **12**: R = H
The 1,6-anhydro-β-rings of 4 and 9 were cleaved with an acetylation mixture (H₂SO₄-Ac₂O-AcOH, 1:70:30, v/v/v) to give the tetrasaccharide tetradecacetate (6) and hexasaccharide eicosacetate (11), respectively, as anomic mixtures containing α-anomer predominantly. 6: amorphous powder, [α]₂⁰° +7.2° (CHCl₃), 94.3% yield. ¹H-NMR (CDCl₃): 1.97, 1.99, 2.03, 2.08, 2.16, 2.19 (4H, all s, OAc x 13, NAc), 5.81 (ca. 0.3H, d, J₁,₂ = 8 Hz, H-1, β-Glc), 6.29 (1H, br. s, exchangeable with D₂O, NH), 6.37 (ca. 0.7H, d, J₁,₂ = 3.5 Hz, H-1, α-Glc). 11: amorphous powder, [α]₂⁰° +12.7° (CHCl₃), 93.9% yield. ¹H-NMR (CDCl₃): 1.93, 1.98, 2.07, 2.16 (60H, all s, OAc x 18, NAc x 2), 5.62 (1H, d, exchangeable with D₂O, JₙH₂ₙ₂° or q'' = 8 Hz, NH), 6.30 (<1H, d, J₁,₂ = 3.5 Hz, H-1, α-Glc), 6.40 (1H, d, exchangeable with D₂O, JₙH₂ₙ₂° or q'' = 8 Hz, NH).

De-O-acetylation of 6 and 11 with methanolic MeONa gave 7 (73.5% yield) as a white powder, [α]₂⁰° +11.8° (H₂O) [lit.], mp 185—187°, [α]₂° +8° (H₂O)], and 12 (80% yield), crystallizable from aq. EtOH as grains, mp 223—225°, [α]₂⁰° +9.1° (no mutarotation, H₂O), respectively.

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

References and Notes


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Received November 29, 1980

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

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

Keywords—mutagen; 2-amino-3-methylimidazo[4,5-f]quinoline; IQ; 2-hydroxyamino-3-methylimidazo[4,5-f]quinoline; metabolic activation; microsomes; hydroxylamine; hydroxymimidazole; 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. Among these compounds, 3-amino-5H-pyrido[4,3-b]indoles (Trp-P) from a pyrolysate of tryptophan and 2-aminodipyrido[1,2-a:3',2'-d]imidazoles (Glu-P) from a pyrolysate of