Carbohydrate Research, 243 (1993) 385–391
Elsevier Science Publishers B.V., Amsterdam

Note

A convenient synthesis of *N*-acetyllactosamine-linked oligosaccharides from phenyl 3,6,2′,3′,4′,6′-hexa-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-lactopyranoside *

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(Received December 2nd, 1991; accepted April 1st, 1992)

The *N*-acetyllactosamine unit [β-D-Gal*p*-(1 → 4)-β-D-Glc*p*NAc] is found in the oligosaccharide structure of many N- and O-linked glycoproteins and is a recognized component of the oligosaccharides of milk[2]. It is a basic unit for the antigenic determinants of a number of human blood group activities[3] and for the ABH type 2 determinants[4]. Interest in the chemical synthesis of *N*-acetyllactosamine-containing oligosaccharides has greatly increased as these molecules can be effectively employed in the study of exo- and endo-glycosidases, glycosyltransferases, and lectins. They can also play an important role as synthetic antigens[5].

In recent years, considerable progress has been made in the development of new catalysts and the utilization of different glycosylating agents towards a more effective and efficient oligosaccharide synthesis. We hereby report an improved preparation of *N*-acetyllactosamine-linked oligosaccharides.

Synthesis of 2-acetamido-2-deoxy-β-D-glucopyranosyl-linked oligosaccharides has involved the use of oxazolines[6], the bromides[7] and chlorides[8] of 2-deoxy-2-phthalimido sugars, and more recently the trichloroacetimidate[9], fluoride[10], and phenylthio[11] derivatives as glycosyl donors. Lemieux and Burzynska[12] utilized 3,6,2′,3′,4′,6′-hexa-*O*-acetyl-2-deoxy-2-phthalimido-β-D-lactosyl chloride, prepared from D-lactal hexaacetate, as a glycosyl donor for the synthesis of lactosamine-containing oligosaccharides. Our present approach involves the preparation and use of phenyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**2**) as an effective glycosylating reagent for the desired compounds. Compound **2** was obtained by treatment of

* Synthetic Studies in Carbohydrates, Part LXXXV. For Part LXXXIV, see ref. 1. This investigation was supported by Grant No. DMB 87-15954 awarded by the National Science Foundation and, in part, by Grant No. CH 419 from the American Cancer Society.

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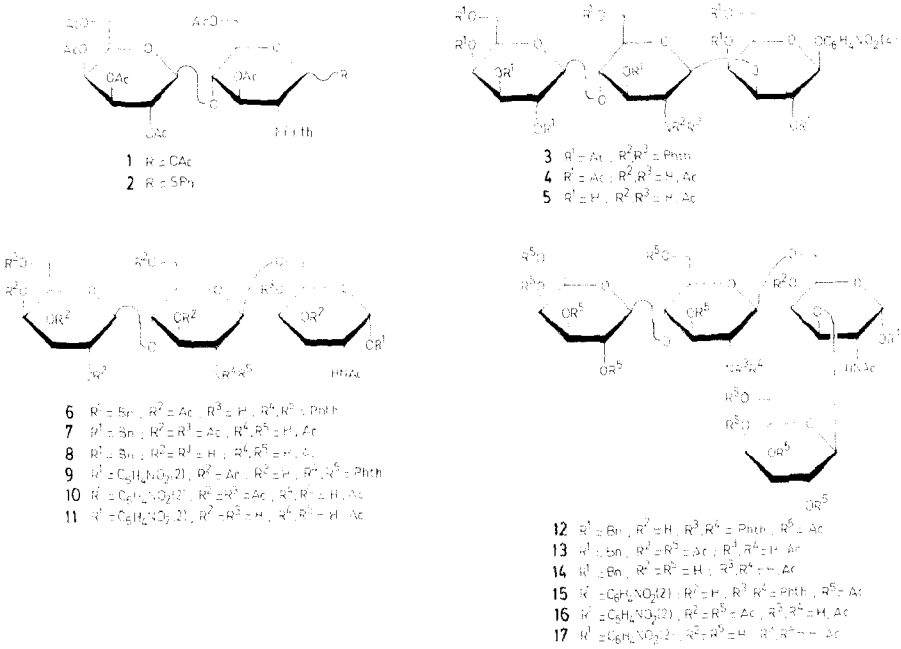

Scheme 1.

O-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1 → 4)-1,3,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-α,β-D-glucopyranose (**1**)[13] with (phenylthio)trimethylsilane and trimethylsilyl triflate in 77% yield after silica gel column chromatography.

Glycosylation of 4-nitrophenyl 2,4,6-tri-*O*-acetyl-β-D-galactopyranoside[14] with **2** in dichloromethane in the presence of *N*-iodosuccinimide–triflic acid[15], followed by the removal of the phthalimido group and *N*,*O*-acetylation of the crude material **3**, afforded the fully acetylated compound **4** in 54% yield after silica gel chromatography. The ^{1}H NMR spectrum of **4** is in accord with the structure assigned. Zemplén transesterification of **4** furnished the known trisaccharide[16] **5** in 65% yield. A similar *N*-iodosuccinimide–triflic acid-catalyzed glycosylation of benzyl or 2-nitrophenyl 2-acetamido-3-*O*-acetyl-2-deoxy-α-D-galactopyranoside[17] and benzyl or 2-nitrophenyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 3)-2-acetamido-2-deoxy-α-D-galactopyranoside[18] with glycosyl donor **2** and processing in a manner analogous to that described for **3** (to give **5**) afforded compounds **8**, **11**, **14**, and **17**, respectively, in good yield. Their structural assignment was confirmed by ^{13}C NMR spectroscopy and FABMS (see Experimental section). These compounds can be employed as synthetic or artificial antigens after reduction of the nitro group and subsequent coupling of the resulting amino group to a protein. Such compounds are also expected to be useful in specificity studies of an antibody raised against a related synthetic antigen that we are currently investigating.

TABLE I

Proposed ^{13}C NMR assignments [a]

Residue or group	Compound	C-1	C-2	C-3	C-4	C-5	C-6	NAc
β-D-Gal*p*-(1 → 4)	**8**	105.63	72.42	75.21	72.01	77.52	63.76	
β-D-Glc*p*NAc-(1 → 6)		104.30	57.84	75.28	81.33	78.11	62.85	25.01
α-D-Gal*p*NAcOBn		98.95	52.63	71.26	71.30	73.72	70.22	24.64
β-D-Gal*p*-(1 → 4)	**11**	105.71	73.77	75.34	71.37	77.60	63.82	
β-D-Glc*p*NAc-(1 → 6)		103.95	57.73	75.34	81.36	78.18	62.97	24.89
α-D-Gal*p*NAcOC$_6$H$_4$NO$_2$(2)		99.88	52.47	70.97	71.37	73.86	70.06	24.75
β-D-Gal*p*-(1 → 4)	**14**	105.74	72.55	75.34	72.26	77.60	63.83	
β-D-Glc*p*NAc-(1 → 6)		104.32	57.92	75.34	81.41	79.80	62.92	24.07
β-D-Gal*p*-(1 → 3)		107.46	73.46	75.34	72.42	77.81	63.83	
α-D-Gal*p*NAcOBn		99.18	51.39	78.19	71.69	73.80	71.40	24.75
β-D-Gal*p*-(1 → 4)	**17**	105.73	73.54	75.41	71.44	77.63	63.83	
β-D-Glc*p*NAc-(1 → 6)		104.00	57.73	75.36	81.37	79.62	63.00	24.89
β-D-Gal*p*-(1 → 3)		107.46	73.77	75.41	71.82	77.89	63.83	
α-D-Gal*p*NAcOC$_6$H$_4$NO$_2$(2)		99.95	51.19	78.21	71.39	73.95	71.24	24.81

[a] For solutions in D_2O with Me_4Si as the external standard.

^{13}C NMR assignments.—In the ^{13}C NMR spectrum of **2**, the resonance for C-1 was observed at δ 82.86 which accounts for the anomeric β-D configuration of the 2-deoxy-2-phthalimidoglucopyranose unit. In the ^{13}C NMR spectra of compounds **8**, **11**, **14**, and **17**, a resonance for C-1 of 2-acetamido-2-deoxy-β-D-glucopyranose was observed at δ 103.95–104.32, a clear indication of β-D configuration for the newly introduced D-GlcNAc unit in these compounds (Table I). Similarly, the resonance for C-6 of the 2-acetamido-2-deoxy-α-D-galactopyranose group displayed a downfield shift (δ 70.06–71.40) in these compounds, confirming the site of glycosylation.

EXPERIMENTAL

General methods.—These were exactly the same as described earlier[11].

Phenyl O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-3,6-di-O-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**2**).—To a stirred solution of *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-1,3,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-α,β-D-glucopyranose (**1**, 10 g) in CH_2Cl_2 (200 mL) was added (phenylthio)trimethylsilane (25 mL) and trimethylsilyl triflate (10 mL). Stirring was continued for 72 h at room temperature. After neutralization with Et_3N, the mixture was diluted with $CHCl_3$, washed with water, dried, and concentrated. The residue was applied to a column of silica gel and eluted with a solvent gradient consisting of 30–40% EtOAc in hexane (500 mL) to give **2** as an amorphous solid (8.2 g, 77%); $[\alpha]_D$ +30° (*c* 1.1, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 101.02 (C-1′), 82.86 (C-1), 77.32 (C-4), and 53.92 (C-2). Anal. Calcd for $C_{38}H_{41}NO_{17}S$: C, 55.94; H, 5.02; N, 1.72. Found: C, 55.69; H, 4.94; N, 1.74.

General procedure for glycosidation.—A solution of **2** (1.2 mmol), acceptor sugar (1 mmol), and *N*-iodosuccinimide (2.5 mmol) in CH_2Cl_2 (40 mL) was stirred for 0.5 h with 4A molecular sieves (6.0 g) under an Ar atmosphere at ~0°C. Then a dilute solution of trifluoromethanesulfonic acid (0.2 mL in 20 mL CH_2Cl_2) was added dropwise. Stirring was continued at the same temperature for another 1 h and the acid was neutralized with a few drops of Et_3N. The mixture was filtered through Celite, the solids were thoroughly washed with $CHCl_3$ and the filtrate and washings were combined, successively washed with water, satd $NaHCO_3$ solution, 10% $Na_2S_2O_3$ solution, dried, and concentrated in vacuo.

4-Nitrophenyl O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-O-(2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1 → 3)-2,4,6-tri-O-acetyl-β-D-galactopyranoside (**4**).—Glycosylation of 4-nitrophenyl 2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (0.32 g) with **2** afforded compound **3** which was treated with 0.02 M NaOMe in MeOH (30 mL) for 4 h at room temperature. The base was neutralized with Amberlite IR-120 (H^+) cation-exchange resin, the resin suspension was filtered off, and the filtrate concentrated to give a solid. This was heated under reflux for 16 h in a mixture of EtOH (70 mL) and hydrazine hydrate (1.5 mL). The solvent was evaporated to give a residue which was dissolved in pyridine

(40 mL) and Ac_2O (20 mL) and stirred overnight at room temperature. Pyridine and Ac_2O were removed under reduced pressure. The residue was applied to a column of silica gel and eluted with a solvent gradient consisting of 30–40% acetone in $CHCl_3$ (300 mL) to give **4** (0.4 g, 54.4%); $[\alpha]_D$ +19.5° (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.12 (d, $J \sim 9$ Hz, 2 H, arom.), 6.97 (d, $J \sim 9$ Hz, 2 H, arom.), and 2.12–1.83 (cluster of s, 30 H, OAc and NAc). Anal. Calcd for $C_{44}H_{56}N_2O_{27}$: C, 50.57; H, 5.40; N, 2.68. Found: C, 50.42; H, 5.67; N, 2.53.

4-Nitrophenyl O-β-D-*galactopyranosyl-(1 → 4)*-O-*(2-acetamido-2-deoxy-β-*D-*glucopyranosyl)-(1 → 3)-β-*D-*galactopyranoside* (**5**).—Compound **4** (0.12 g) in 0.025 M methanolic NaOMe (20 mL) was stirred at room temperature for 16 h. The base was neutralized by the addition of a few drops of glacial AcOH, and the solid material filtered off and thoroughly washed with EtOH. The solid was dissolved in water and treated with Amberlite IR-120 (H^+) cation-exchange resin. Filtration and lyophilization afforded known compound **5** (0.05 g, 65%); $[\alpha]_D$ −23.5° (*c* 0.4, H_2O) which corresponds to the value reported[16].

Benzyl O-*(2,3,4,6-tetra*-O-*acetyl-β-*D-*galactopyranosyl)-(1 → 4)*-O-*(3,6-di*-O-*acetyl-2-deoxy-2-phthalimido-β-*D-*glucopyranosyl)-(1 → 6)-2-acetamido-3*-O-*acetyl-2-deoxy-α-*D-*galactopyranoside* (**6**).—Glycosidation with benzyl 2-acetamido-3-*O*-acetyl-2-deoxy-α-D-galactopyranoside (0.35 g) afforded **6** (0.51 g, 65%) after silica gel column chromatography (25–30% acetone in $CHCl_3$; 250 mL); $[\alpha]_D$ +48.5° (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.83–7.60 (m, 2 H, arom.), 7.33–7.03 (m, 7 H, arom.), and 2.08–1.61 (cluster of s, 24 H, OAc and NAc). Anal. Calcd for $C_{49}H_{58}N_2O_{24}$: C, 55.57; H, 5.52; N, 2.65. Found: C, 55.62; H, 5.35; N, 2.62.

Benzyl O-*(2,3,4,6-tetra*-O-*acetyl-β-*D-*galactopyranosyl)-(1 → 4)*-O-*(2-acetamido-3,6-di*-O-*acetyl-2-deoxy-β-*D-*glucopyranosyl)-(1 → 6)-2-acetamido-3,4-di*-O-*acetyl-2-deoxy-α-*D-*galactopyranoside* (**7**).—Compound **6** was treated exactly as described for the preparation of **4** (from **3**) to give **7** (0.35 g, 73%) after silica gel column chromatography (solvent gradient consisting of 30–40% acetone in $CHCl_3$; 300 mL); $[\alpha]_D$ +31° (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.33 (s, 5 H, arom.) and 2.20–1.77 (cluster of s, 30 H, OAc and NAc). Anal. Calcd for $C_{45}H_{60}N_2O_{24}$: C, 53.35; H, 5.97; N, 2.77. Found: C, 53.13; H, 6.05; N, 2.66.

Benzyl O-β-D-*galactopyranosyl-(1 → 4)*-O-*(2-acetamido-2-deoxy-β-*D-*glucopyranosyl)-(1 → 6)-2-acetamido-2-deoxy-α-*D-*galactopyranoside* (**8**).—Compound **7** (0.3 g) was *O*-deacetylated exactly the same as described for the preparation of **5** (from **4**) to give compound **8** (0.19 g, 95%); $[\alpha]_D$ +67.5° (*c* 0.7, H_2O); MS: *m/z* 677.4 $[M + 1]^+$ and 675.1 $[M - 1]^-$; For ^{13}C NMR data, see Table I. Anal. Calcd for $C_{29}H_{44}N_2O_{16}$: C, 51.47; H, 6.55; N, 4.14. Found: C, 51.18; H, 6.65; N, 3.92.

2-Nitrophenyl O-*(2,3,4,6-tetra*-O-*acetyl-β-*D-*galactopyranosyl)-(1 → 4)*-O-*(3,6-di*-O-*acetyl-2-deoxy-2-phthalimido-β-*D-*glucopyranosyl)-(1 → 6)-2-acetamido-3*-O-*acetyl-2-deoxy-α-*D-*galactopyranoside* (**9**).—Glycosidation of 2-nitrophenyl 2-acetamido-3-*O*-acetyl-2-deoxy-α-D-galactopyranoside (0.7 g) gave compound **9** (1.1 g, 69%) after silica gel column chromatography (solvent gradient consisting of 20–30% acetone in $CHCl_3$; 200 mL); $[\alpha]_D$ +12.5° (*c* 0.6, $CHCl_3$); ^1H NMR

($CDCl_3$): δ 7.93–7.23 (m, 8 H, arom.) and 2.20–1.77 (cluster of s, 24 H, OAc and NAc). Anal. Calcd for $C_{48}H_{55}N_3O_{26}$: C, 52.89; H, 5.09; N, 3.85. Found: C, 52.99; H, 4.85; N, 3.87.

2-Nitrophenyl O-β-D-*galactopyranosyl-(1 → 4)*-O-*(2-acetamido-2-deoxy-β-*D-*glucopyranosyl)-(1 → 6)-2-acetamido-2-deoxy-α-*D-*galactopyranoside* (**11**).—This compound was obtained by the same reaction sequence as described for the preparation of **8** (from **6**), amorphous (0.07 g, 69%); $[\alpha]_D$ +77° (*c* 0.4, H_2O); MS: *m/z* 708.4 $[M + 1]^+$ and 706.7 $[M - 1]^-$; For ^{13}C NMR data, see Table 1. Anal. Calcd for $C_{28}H_{41}N_3O_{18}$: C, 47.52; H, 5.84; N, 5.94. Found: C, 47.75; H, 5.80; N, 5.80.

Benzyl O-*(2,3,4,6-tetra-*O-*acetyl-β-*D-*galactopyranosyl)-(1 → 4)*-O-*(2-acetamido-3,6-di-*O-*acetyl-2-deoxy-β-*D-*glucopyranosyl)-(1 → 6)*-O-*[(2,3,4,6-tetra-*O-*acetyl-β-*D-*galactopyranosyl)-(1 → 3)]-2-acetamido-4-*O-*acetyl-2-deoxy-α-*D-*galactopyranoside* (**13**).—Glycosidation of benzyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 3)-2-acetamido-2-deoxy-α-D-galactopyranoside (0.51 g), followed by removal of phthalimido group and *N,O*-acetylation with pyridine–Ac_2O, as described for the preparation of **4**, afforded compound **13** (0.65 g, 63%) after silica gel column chromatography (3% MeOH in $CHCl_3$; 200 mL); $[\alpha]_D$ +29° (*c* 1.3, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.32 (s, 5 H, arom.) and 2.13–1.77 (cluster of s, 39 H, OAc and NAc). Anal. Calcd for $C_{57}H_{76}N_2O_{32}$: C, 52.61; H, 5.89; N, 2.15. Found: C, 52.76; H, 5.73; N, 2.17.

Benzyl O-β-D-*galactopyranosyl-(1 → 4)*-O-*(2-acetamido-2-deoxy-β-*D-*glucopyranosyl)-(1 → 6)*-O-*[β-*D-*galactopyranosyl)-(1 → 3)]-2-acetamido-2-deoxy-α-*D-*galactopyranoside* (**14**).—*O*-Deacetylation of **13** (0.4 g) gave compound **14** (0.2 g, 78%), amorphous; $[\alpha]_D$ +55° (*c* 0.5, H_2O); MS: *m/z* 839.5 $[M + 1]^+$ and 837.2 $[M - 1]^-$; for ^{13}C NMR data, see Table I. Anal. Calcd for $C_{35}H_{54}N_2O_{21}$: C, 50.11; H, 6.49; N, 3.34. Found: C, 50.00; H, 6.40; N, 3.18.

2-Nitrophenyl O-*(2,3,4,6-tetra-*O-*acetyl-β-*D-*galactopyranosyl)-(1 → 4)*-O-*(2-acetamido-3,6-di-*O-*acetyl-2-deoxy-β-*D-*glucopyranosyl)-(1 → 6)*-O-*[2,3,4,6-tetra-*O-*acetyl-β-*D-*galactopyranosyl)-(1 → 3)]-2-acetamido-4-*O-*acetyl-2-deoxy-α-*D-*galactopyranoside* (**16**).—Glycosidation of 2-nitrophenyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 3)-2-acetamido-2-deoxy-α-D-galactopyranoside (0.08 g), followed by the sequence of steps used for the preparation of **4**, afforded compound **16** (0.11 g, 69%) after silica gel column chromatography (5% MeOH in $CHCl_3$; 200 mL); $[\alpha]_D$ +10° (*c* 0.3, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.91–7.22 (m, 4 H, arom.) and 2.18–1.76 (cluster of s, 39 H, OAc and NAc). Anal. Calcd for $C_{56}H_{73}N_3O_{34}$: C, 50.49; H, 5.52; N, 3.15. Found: C, 50.72; H, 5.45; N, 3.20.

2-Nitrophenyl O-β-D-*galactopyranosyl-(1 → 4)*-O-*(2-acetamido-2-deoxy-β-*D-*glucopyranosyl)-(1 → 6)*-O-*[β-*D-*galactopyranosyl-(1 → 3)]-2-acetamido-2-deoxy-α-*D-*galactopyranoside* (**17**).—*O*-Deacetylation of compound **16** (0.09 g) with 0.025 methanolic NaOMe afforded compound **17** (0.035 g, 60%); $[\alpha]_D$ +62° (*c* 0.3, H_2O); for ^{13}C NMR data, see Table I. Anal. Calcd for $C_{34}H_{51}N_3O_{23} \cdot H_2O$: C, 45.99; H, 6.02; N, 4.73. Found; C, 45.80; H, 6.29; N, 4.64.

ACKNOWLEDGMENTS

The authors are grateful to Mr. Walter Tabaczynski and Mr. Brock DeLappe for recording the ^{13}C NMR and mass spectra.

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