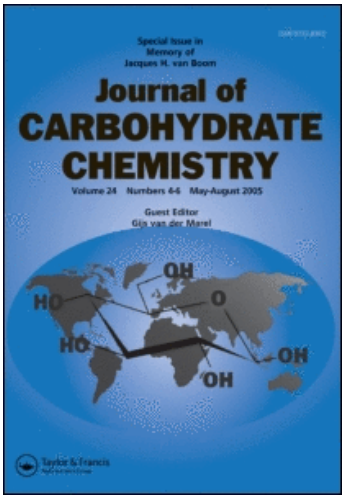

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EXPEDIENT CONVERSION OF LACTOSE INTO VERSATILE DERIVATIVES OF LACTOSAMINE AND β-D-GALACTOSYL-(1→4)-D-MANNOSAMINE

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ABSTRACT

A practical, nine-step protocol is described for the preparation of synthetically useful *N*-acetyllactosamine (LacNAc) derivatives as well as LacNAc itself from lactose using the benzoylated oxime of lactos-2-ulosyl bromide **2** as the key intermediate. All steps are performed with simple reagents, do not require chromatography, are large-scale adaptable and allow overall yields of 30%.

INTRODUCTION

Oligosaccharides containing *N*-acetyllactosamine (LacNAc) as a structural component constitute antennary sugar chains of complex and hybrid types of *N*-glycoproteins,[1] tumor-associated glycolipids,[2] and cell adhesion molecules.[3] As these are involved in intercellular recognition,[4] they are of particular biological importance.

Various chemical syntheses of LacNAc have been developed over the years, either by assembling the disaccharide from its monosaccharide components, i.e., employing suitably protected galactosyl donors and *N*-acetylglucosamine acceptors[5] or, alternatively, by its

elaboration from lactose.[6] Neither approach has proved to be very practical in preparative terms, thereby making the large scale acquisition of LacNAc cumbersome.

Of the enzymatic procedures elaborated for the preparation of LacNAc,[7] the one recently reported by Sakai et al.,[7d] who utilized the galactose transfer from lactose to *N*-acetylglucosamine by a commercially available β-galactosidase from *Bacillus circulans*, appears to have greatly improved its accessibility; it allows isolation of 5 g of LacNAc from 20 g of lactose after a comparatively simple chromatographic separation.[7d] However, the present prohibitively high price[8] for LacNAc, strongly impedes its use for subsequent chemical operations.

With respect to straightforward, practical syntheses of LacNAc-containing oligosaccharides of biological relevance, there is a clear need for further efficient procedures for the preparation of LacNAc, and an array of suitably blocked derivatives with which to directly enter into oligosaccharide assemblies. As a consequence, we have chosen to probe the easily accessible oxime of lactos-2-ulosyl bromide **2** derived from lactose for the generation of synthetically useful LacNAc derivatives, and here describe a variety of these via reaction sequences that are readily large scale adaptable.

RESULTS AND DISCUSSION

The hydroxylactal ester **1**, readily accessible from lactose in a high yield (76%) 3-step sequence,[9] was utilized as a basic intermediate for the transformation of lactose into LacNAc derivatives, and afforded the key compound, the benzoylated oxime of lactos-2-ulosyl bromide **2** (75% overall yield) after hydroxyaminolysis,[10] *O*-benzoylation, and photobromination.[9,11]

Previous studies on the glycosidation of **2** as well as the reduction of the respective oximes of α- and β-glycosiduloses have provided ample evidence that the α-glycosides are selectively converted into α-D-glucosaminides, whilst the β-glycosides are led to β-D-mannosaminides.[9,12] Hence, generation of versatile LacNAc derivatives would conceivably result from α-selective replacement of the anomeric bromide in **2** by acyloxy or suitable (i.e. readily removable) alkoxy groups, whilst their β-anomer would lead to β-D-Gal-(1→4)-D-ManNAc analogues upon oxime reduction. Accordingly, the stereoselectivity of the glycosidation of **2** was studied in detail, using carboxylate (acetate and benzoate) salts and *p*-methoxybenzyl alcohol as an easily removable glycosidic group.

In evaluating the stereoselectivity in displacement reactions of **2** (Table 1) several features are noteworthy. Reaction of **2** with sodium acetate or sodium benzoate in ethers, e.g., 1,4-dioxane or THF for 2 d at ambient temperature in the presence of

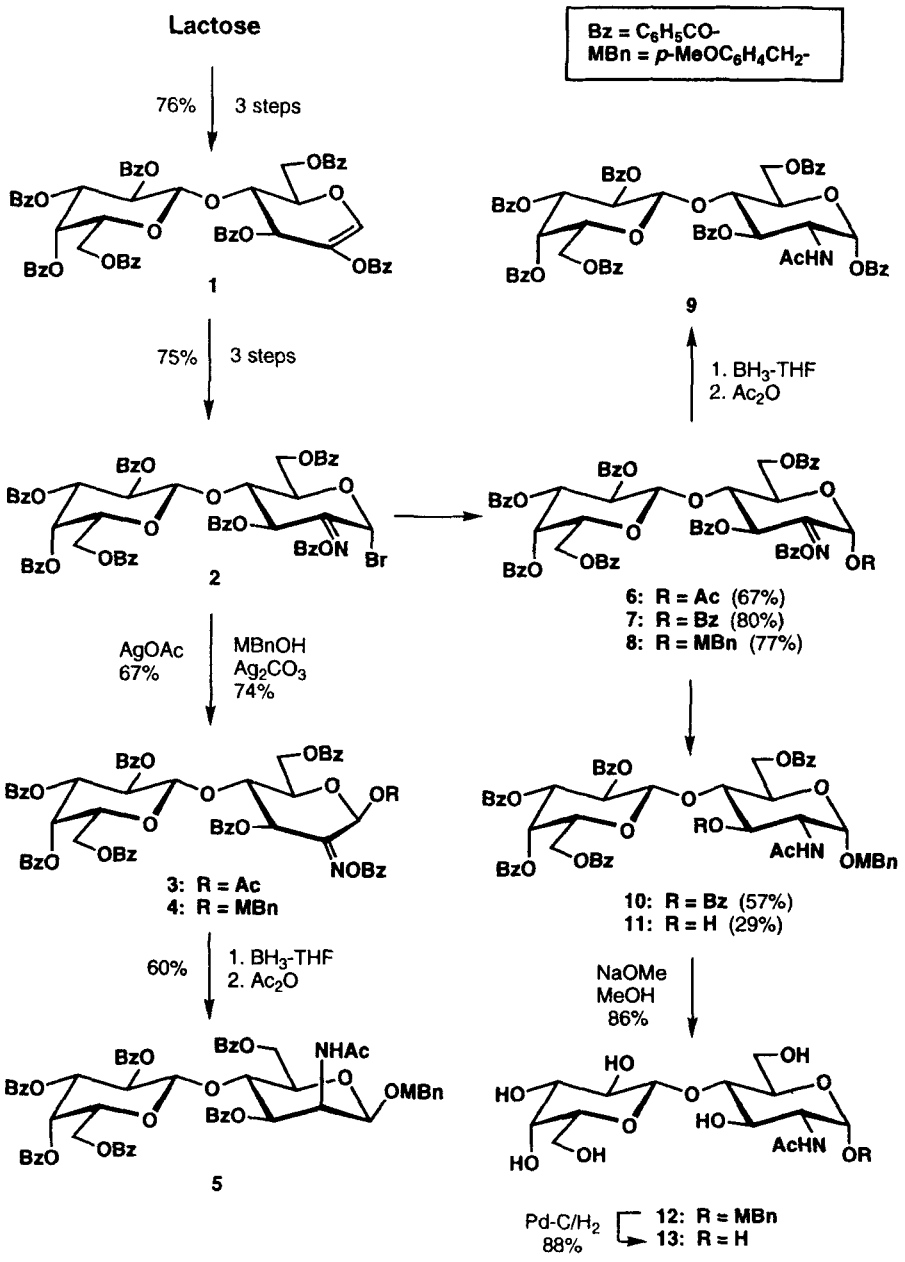

Table 1. Displacement of Bromide **2** by Acyloxy and *p*-Methoxybenzyl Groups

Run	Reagents	Solvents	Products Yields (%)[a]	Major Product	α : β[b]
1	AcONa	DMF	68	**3**	2 : 3
2	AcONa	MeCN	73	**3**,**6**	1 : 1
3	AcONa	$MeNO_2$	73	**6**	3 : 2
4	AcONa	Me_2CO	70	**3**,**6**	1 : 1
5	AcONa	$(CH_2Cl)_2$	c	—	—
6	AcONa	THF	71	**6**	10 : 1
7	AcONa	dioxane	74	**6**	10 : 1
8	AcONa	$(CH_2OMe)_2$	71	**6**	6 : 1
9	AcOAg	$(CH_2OMe)_2$	70	**3**	1 : 20
10	$(AcO)_2Hg$	$(CH_2OMe)_2$	71	**6**	2 : 1
11	BzONa	dioxane	88	**7**	10 : 1
12[d]	MBnOH[e]	dioxane	82	**8**	15 : 1
13[f]	MBnOH	CH_2Cl_2	78	**4**	1 : 20

a. These data designate yields of the anomeric mixture of the products. b. Anomeric ratios were estimated on the basis of ^1H NMR. c. A 94% of the educt was recovered. d. Promoted by *s*-collidine-iodine. e. *p*-methoxybenzyl alcohol. f. Promoted by silver carbonate-iodine in the presence of MS-3A.

dessicant (MS 3A) provides the respective α-acylated disaccharides **6** and **7** predominantly in yields of 65-80% (runs 6,7, and 11), where α/β selectivity is 10 : 1. This course may be rationalized on the reasonable assumption that solvents like dioxane or THF promote double inversion at the anomeric center via intermediate β-alkoxonium ion formation.[12,13] Changing the counter ion from sodium to silver (run 9) has a major effect on the stereoselectivity, which is now reversed (20 : 1 in favor of the β-anomer **3**) probably due to a high proportion of direct S_N2 displacement of the bromine by the silver acetate, giving **3** in 67% yield. Similarly, *p*-methoxybenzyl glycosides were smoothly obtained such that solvent participating glycosidation of **2** in dioxane led to the α-glycoside **8** in 82% yield (run 12), whilst silver carbonate-promoted glycosidation afforded the β-glycoside **4** in 78% yield (run 13).

Preparative utilization of these results was first demonstrated for the stereoselective conversion of the β-glycoside **4** into the β-D-Gal-(1→4)-β-D-ManNAc derivative **5**.

Thus, the hydride reduction of **4** with borane-tetrahydrofuran (BH_3·THF) complex gave, upon *N*-acetylation, the disaccharide **5** in 60% yield.

On the other hand, MBn α-glycoside **8** was subjected to hydroboration with excess BH_3·THF complex followed by *N*-acetylation to afford mainly MBn α-LacNAc derivatives **10** and **11** in 57 and 29% yields, respectively, after chromatographic separation. The structure of **11** was assigned to the 3-OH free analogue of **10** on the basis of its ^1H NMR spectra (see experimental), and by conversion to **10** by *O*-benzoylation. A very small amount of a by-product isolated (2.3% yield) from the reduced mixture proved to be the disaccharide containing α-ManNAc derivative. Hence, the total yield of LacNAc derivatives was estimated to be 86%, with the *gluco / manno* selectivity for the reduction of **8** being 37 : 1.

For preparative purposes, this high stereoselectivity should allow the one-pot synthesis of LacNAc glycoside **12** from **8** without isolation of the intermediates **10** and **11**. Thus, exposing the crude reaction mixture from **8** to 0.1 M sodium methoxide in methanol solution afforded **12** in 73% yield based on **8**. Subsequent catalytic hydrogenolysis of **12** provided an 88% yield of *N*-acetyllactosamine **13** which was unequivocally identified on the basis of optical rotation[7d] as well as ^1H and ^{13}C NMR data.

Configurational Assignments : In the series of 2-oximinoglycoses **3**,**6**, and **7** as well as glycosides **4** and **8**, lacking a C-2 proton, $J_{3,4}$ values are quite useful for assignment of the anomeric configuration, i.e., the α-anomers **6**,**7**, and **8** showed sizable $J_{3,4}$ couplings of about 9 Hz, clearly revealing a $^4C_1$ conformation of the pyranoid ring. In contrast, the respective β-anomers **3** and **4** exhibited smaller coupling constants of 3 - 4 Hz, indicating a conformation substantially distorted towards the twist-boat form. This conformational peculiarity appeared to be caused by steric repulsion between 2-benzoyloxyimino group and anomeric substituent, which has been previously observed for this type of compound.[9,14,15] Alternatively, $J_{C1,H1}$ values gave useful information of their anomeric configurations, i. e., the α-anomer **8** and the respective β-anomer **4** exhibited values of 177 and 171 Hz, respectively (ca. 10 Hz larger than the normal glucopyranosides having around 170 and 160 Hz for the respective anomers[16]), which may be characteristic for 2-(benzoyloxyimino)glycosides.

Configuration of the amino sugar moieties of **9**, **10**, and **11** obtained from **7** as described for **8** → **10**, could be readily assigned to be *gluco* from their $J_{2,3}$ values of 11.0, 10.7, and 10.0 Hz, respectively, whilst that of **5** was assigned as *manno* from its $J_{2,3}$ value of 4.0 Hz. The corresponding anomeric configurations were determined by $J_{C1,H1}$ values, ca. 170 Hz for α-anomers **10** and **11**, and 160 Hz for β-anomer **5**.

In conclusion, we have developed an effective, practical, novel reaction sequence for the acquisition of a variety of synthetically useful LacNAc and β-D-Gal-(1→4)-β-D-

ManNAc derivatives from lactose. The protocol is readily adaptable to large scale preparations and, in no phase, requires recourse to chromatography.

EXPERIMENTAL

Melting points are reported uncorrected: Büchi SMP-20, Bock Monoscop, and Yamato MP-1. Spectral measurements: Perkin Elmer 141 and Jasco DIP-180 (rotations); Bruker WM 300, and Varian XL-400 (^{1}H and ^{13}C NMR); JMS D-100 (MS) instruments. FAB-MS were measured with a matrix (PEG 400 or *m*-NBA) in acetone. TLC: Merck Silica Gel F_{254} plastic sheets were used to monitor the reactions and to ascertain the purity of the products; solvent systems are given individually and were the same for TLC and column chromatography. Spots were visualized by UV light (254 nm) or by charring with 10% aqueous H_2SO_4. Column chromatography: Merck Silica Gel 60 (70-230 mesh).

1-*O*-Acetyl-3,6-di-*O*-benzoyl-2-(benzoyloxyimino)-4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-β-D-*arabino*-hexopyranose (3). A mixture of oximinoglycosyl bromide **2**[9] (102 mg, 0.09 mmol) and AgOAc (30 mg, 0.18 mmol) in dry 1,2-dimethoxyethane (2 mL) was stirred in the dark at room temperature for 40 h, followed by dilution with CH_2Cl_2, washing with water, drying (Na_2SO_4) and concentration to dryness. The syrupy residue was purified by elution from a silica gel column with 10:1 toluene - EtOAc to give 3 (71 mg, 70%) containing trace of the α-anomer (α:β = 1:20 by ^{1}H NMR): mp 102-104 °C (Et_2O - pentane); $[\alpha]_D^{20}$ +45.6 ° (*c* 0.25, $CHCl_3$); ^{1}H NMR (100 MHz, $CDCl_3$) δ 2.06 (s, 3H, OAc), 5.18 (d, 1H, H-1'), 5.63 (dd, 1H, H-3'), 5.87 (dd, 1H, H-2'), 6.02 (broad d, 1H, H-4'), 6.43 (d, 1H, H-3), 7.2-8.2 (aromatic H and H-1), 3.9-4.7 (other protons); $J_{3,4}$ = 3.5, $J_{1',2'}$ = 8, $J_{2',3'}$ = 10.5, $J_{3',4'}$ = 3.5, $J_{4',5'}$ = 1 Hz.

Anal. Calcd for $C_{63}H_{51}NO_{19}$ (1126.1): C, 67.20; H, 4.56; N, 1.24. Found: C, 66.93; H, 4.41; N, 1.24.

***p*-Methoxybenzyl 3,6-Di-*O*-benzoyl-2-(benzoyloxyimino)-4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-β-D-*arabino*-hexopyranoside (4).** Oximinoglycosyl bromide **2**[9] (114 mg, 0.1 mmol) was added to a mixture of *p*-methoxybenzyl alcohol (70 mg, 0.5 mmol), Ag_2CO_3 (84 mg, 0.3 mmol), and I_2 (26 mg, 0.1 mmol) in dry CH_2Cl_2 (2 mL) containing powdered molecular sieves 3A (100 mg). The mixture was stirred in the dark at room temperature for 2 d. Dilution with dichloromethane (20 mL), filtration through a pad of Celite, successive washing of the filtrate with 5% $NaHCO_3$ (20 mL) and water (3 x 20 mL), drying (Na_2SO_4), and concentration to dryness gave a residue which was eluted from a silica gel column with 8:1 toluene - EtOAc

to afford **4** (94 mg, 78%) as a white powder (α:β = 1:20, ^{1}H NMR): mp 102-103 °C (Et_2O - pentane); $[\alpha]_D^{20}$ +22 ° (*c* 0.5, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.72 (s, 3H, OCH_3), 4.0 (m, 1H, H-5), 4.35-4.52 (m, 5H, H-6,5',6'), 4.58 (dd, 1H, H-4), 4.71 and 4.81 (each d, 1H, $C\underline{H}_2Ph$), 5.14 (d, 1H, H-1'), 5.60 (dd, 1H, H-3'), 5.81 (dd, 1H, H-2'), 5.92 (d, 1H, H-1), 5.97 (s, 1H, H-4'), 6.36 (d, 1H, H-3), 6.72 (d, 2H, *m*-H of MBn); $J_{3,4}$ = 4.0, $J_{4,5}$ = 7.0, $J_{1',2'}$ = 8.0, $J_{2',3'}$ = 11.3, $J_{3',4'}$ = 3.5, $J_{4',5'}$ = 1.0 Hz; ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.1 (OCH_3), 61.1 (C-6'), 63.8 (C-6), 67.9 (C-4'), 69.8 (C-2'), 69.9 ($\underline{C}H_2Ph$), 70.3 (C-3), 71.6 (C-3'), 71.8 (C-5'), 72.9 (C-5), 77.2 (C-4), 91.3 (C-1), 102.3 (C-1'), 113.8 (*m*-C of MBn), 157.3 (C-2); $J_{C1,H1}$ = 171, $J_{C1',H1'}$ = 159 Hz; MS (FD) *m/z* 1204 $(M)^+$.

Anal. Calcd for $C_{69}H_{57}NO_{19}$ (1204.2): C, 68.82; H, 4.77; N, 1.16. Found: C, 68.73; H, 4.87; N, 1.13.

***p*-Methoxybenzyl 2-Acetamido-3,6-di-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-2-deoxy-β-D-mannopyranoside (5).** A solution of the β-glycoside **4** (120 mg, 0.1 mmol) in dry THF (1.2 mL) was treated at – 10 °C with a 1 M solution of BH_3·THF complex (1.2 mL) at - 10 °C under an atmosphere of N_2. The mixture was stirred for 0.5 h and then allowed to attain room temperature. After stirring for 2 h, excess reagent was quenched at 0 °C with MeOH (2 mL) , followed by addition of Ac_2O (1 mL). After stirring for 1 h at ambient temperature, the mixture was passed through Amberlite IR-45 resin (3 g) and washed with MeOH. The eluants and washings were concentrated in vacuo to a syrup, which was eluted from a silica gel column with toluene - EtOAc (1:1→1:2). The major fraction was concentrated and crystallized from 1:2:4 EtOAc - Et_2O - pentane to provide 68 mg (60%) of **5** as a colorless powder: mp 111-113 °C; $[\alpha]_D^{20}$ +31.7 ° (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.91 (s, 3H, NAc), 3.80 (s, 3H, OMe), 3.70-3.91 (m, 3H, H-5',6'), 3.75 (m, 1H, H-5), 4.14 (dd, 1H, H-4), 4.46 and 4.70 (each d, 1H, $C\underline{H}_2Ph$), 4.50 and 4.61 (each dd, 1H, H-6), 4.70 (d, 1H, H-1), 4.82 (td, 1H, H-2), 4.95 (d, 1H, H-1'), 5.41 (dd, 1H, H-3), 5.49 (dd, 1H, H-3'), 5.71 (dd, 1H, H-2'), 5.74 (d, 1H, NH), 5.82 (d, 1H, H-4'), 6.84 (d, 2H, *m*-H of MBn); $J_{1,2}$ = 1.8, $J_{2,3}$ = 4.0, $J_{2,NH}$ = 8.5, $J_{3,4}$ = $J_{4,5}$ = 8.5, $J_{5,6a}$ = 2.5, $J_{5,6b}$ = 5.3, $J_{6a,6b}$ = 12.0, $J_{1',2'}$ = 7.8, $J_{2',3'}$ = 10.5, $J_{3',4'}$ = 3.5, $J_{4',5'}$ = 0.5, $J_{5',6'a}$ = 9.5, $J_{6'a,6'b}$ = 15 Hz; ^{13}C NMR (100 MHz, $CDCl_3$) δ 23.25 ($CO\underline{C}H_3$), 50.14 (C-2), 55.28 (OMe), 61.08 (C-6'), 62.80 (C-6), 67.46 (C-4'), 69.97 (C-2'), 70.06 ($\underline{C}H_2Ph$), 71.26 (C-5'), 71.56 (C-3'), 72.39 (C-3), 73.27 (C-5), 74.38 (C-4), 96.50 (C-1), 101.47 (C-1'), 113.97 (*m*-C of MBn), 170.19 (NHCO); $J_{C1,H1}$ = $J_{C1',H1'}$ = 160 Hz; MS (FAB) *m/z* 1128 $(M)^+$.

Anal. Calcd for $C_{64}H_{57}NO_{18}$·1/2 H_2O (1128.2): C, 67.60; H, 5.14; N, 1.23. Found: C, 67.48; H, 5.10; N, 1.25.

1-*O*-Acetyl-3,6-di-*O*-benzoyl-2-(benzoyloxyimino)-4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-α-D-*arabino*-hexopyranose (6). A mixture of oximinoglycosyl bromide **2**[9] (1.2 g, 1.05 mmol) and NaOAc (258 mg, 3.15 mmol) in dry dioxane (15 mL) was stirred at room temperature for 2 d, followed by dilution with CH_2Cl_2 (50 mL), washing with water (3 x 50 mL), drying (Na_2SO_4), and concentration to dryness. The residue was eluted from a silica gel column with 10:1 toluene - EtOAc to give 0.88 g (74%) of **6**, contaminated with about 5% of the β-anomer (^1H NMR): mp 118-120 °C (Et_2O - pentane); $[\alpha]_D^{19}$ +83.2 ° (*c* 0.5, $CHCl_3$); ^1H NMR (100 MHz, $CDCl_3$) δ 2.19 (s, 3H, OAc), 5.06 (d, 1H, H-1'), 5.48 (dd, 1H, H-3'), 5.7-5.9 (m, 2H, H-2',4'), 6.44 (d, 1H, H-3), 7.1-8.3 (aromatic H and H-1), 3.9-4.7 (other protons); $J_{3,4}$ = 9, $J_{1',2'}$ = 8, $J_{2',3'}$ = 11, $J_{3',4'}$ = 3.5 Hz.

Anal. Calcd for $C_{63}H_{51}NO_{19}$ (1126.1): C, 67.20; H, 4.56; N, 1.24. Found: C, 67.13; H, 4.57; N, 1.07.

1,3,6-Tri-*O*-benzoyl-2-(benzoyloxyimino)-4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-α-D-*arabino*-hexopyranose (7). A mixture of oximinoglycosyl bromide **2**[9] (1.0 g, 0.872 mmol) and NaOBz (0.503 g, 3.49 mmol) in dry dioxane (15 mL) was stirred at room temperature for 2 d, and then processed as described for **6**. Crystallization from Et_2O - pentane provided 0.91 g (88%) of **7**, containing about 5% of the β-anomer (^1H NMR): mp 117-119 °C; $[\alpha]_D^{20}$ +104 ° (*c* 0.5, $CHCl_3$); ^1H NMR (300 MHz, CDCl) δ 3.99 (dd, 1H, H-5'), 4.08 and 4.14 (each dd, 1H, H-6'), 4.41 (dd, 1H, H-5), 4.44 and 4.59 (each dd, 1H, H-6), 4.63 (dd, 1H, H-4), 5.08 (d, 1H, H-1'), 5.47 (dd, 1H, H-3'), 5.79 (dd, 1H, H-2'), 5.84 (d, 1H, H-4'), 6.53 (d, 1H, H-3), 7.71 (s, 1H, H-1); $J_{3,4} = J_{4,5}$ = 8.5, $J_{1',2'}$ = 8.0, $J_{2',3'}$ = 10.2, $J_{3',4'}$ = 3.3, $J_{4',5'}$ = 1.0 Hz; ^{13}C NMR (75 MHz, $CDCl_3$) δ 61.01 (C-6'), 61.75 (C-6), 67.48 (C-4'), 69.84 (C-2'), 70.43 (C-3), 71.29 (C-5), 71.54 (C-5'), 71.71 (C-3'), 76.82 (C-4), 84.62 (C-1), 101.6 (C-1'), 155.43 (C-2), 162.30-165.58 (8 x CO); MS (FAB) *m/z* 1211 $(M + Na)^+$.

Anal. Calcd for $C_{68}H_{53}NO_{19}$ (1188.2): C, 68.74; H, 4.50; N, 1.18. Found: C, 68.48; H, 4.47; N, 1.17.

***p*-Methoxybenzyl 3,6-Di-*O*-benzoyl-2-(benzoyloxyimino)-4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-α-D-*arabino*-hexopyranoside (8).** A mixture of *p*-methoxybenzyl alcohol (2.59 g, 18.7 mmol), powdered molecular sieves 3A (1.8 g), and I_2 (0.95 g, 3.74 mmol) in dry dioxane (40 mL) was stirred in the dark at room temperature for 1 h. To the mixture were added oximinoglycosyl bromide **2**[9] (4.29 g, 3.74 mmol) and *s*-collidine (0.54 mL, 4.11 mmol), and stirring was continued for 2 d. The mixture was diluted with CH_2Cl_2 (200 mL) and filtered through a pad of Celite. The filtrate was washed with aqueous 0.1 M $Na_2S_2O_3$ (250 mL), water (250 mL), 5%

$NaHCO_3$ (250 mL), and water (3 x 250 mL). After drying (Na_2SO_4), the solvent was removed to give **8** as a syrup, sufficiently pure for performing the reduction (i.e. → **10**, cf. below). For analytical purposes, the syrupy **8** was purified by elution from a silica gel column with 6:1 toluene - EtOAc to afford 3.70 g (82%) of **8** as a white powder (α:β = 15:1, ^1H NMR): mp 100-102 °C (EtOAc - Et_2O - pentane); $[\alpha]_D^{24}$ +107 ° (*c* 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 3.74 (s, 3H, OCH_3), 3.8-4.2 (m, 3H, H-5',6'), 4.4-4.7 (m, 3H, H-5,6), 4.51 (dd, 1H, H-4), 4.59 and 4.77 (each d, 1H, $C\underline{H}_2Ph$), 5.04 (d, 1H, H-1'), 5.48 (dd, 1H, H-3'), 5.78 (dd, 1H, H-2'), 5.82 (d, 1H, H-4'), 6.04 (s, 1H, H-1), 6.45 (d, 1H, H-3), 6.84 (d, 2H, *m*-H of MBn); $J_{3,4}$ = 9.0, $J_{1',2'}$ = 8.0, $J_{2',3'}$ = 10.5, $J_{3',4'}$ = 3.5 Hz; ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.1 (OCH_3), 60.8 (C-6'), 62.0 (C-6), 67.3 (C-4'), 68.5 ($\underline{C}H_2Ph$), 68.9 (C-5), 69.9 (C-2'), 70.4 (C-3), 71.4 (C-5'), 71.6 (C-3'), 77.6 (C-4), 88.6 (C-1), 101.2 (C-1'), 114.0 (*m*-C of MBn), 156.8 (C-2); $J_{C1,H1}$ = 177, $J_{C1',H1'}$ = 162 Hz; MS (FAB) *m/z* 1204 (M^+).

Anal. Calcd for $C_{69}H_{57}NO_{19}$ (1204.2): C, 68.82; H, 4.77; N, 1.16. Found: C, 68.54; H, 4.75; N, 1.18.

2-Acetamido-1,3,6-tri-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-2-deoxy-α-D-glucopyranose (Hepta-*O*-benzoyl-*N*-acetyl-lactosamine) (9). A solution of α-benzoate **7** (150 mg, 0.126 mmol) in THF (1.5 mL) was treated with a 1 M solution of BH_3·THF complex in THF (1.52 mL) as described for **4** → **5**. *N*-Acetylation, followed by elution from a silica gel column with toluene - EtOAc (1:1→1:2, gradient) afforded 75.6 mg (54%) of **9** as an amorphous solid: mp 130-133 °C (Et_2O - pentane); $[\alpha]_D^{20}$ +88.6 ° (*c* 0.5, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 1.84 (s, 3H, NAc), 3.85 (dd, 1H, H-5'), 3.98 (m, 2H, H-6'), 4.14 (td, 1H, H-5), 4.39 (dd, 1H, H-4), 4.49 (d, 2H, H-6), 4.77 (td, 1H, H-2), 4.95 (d, 1H, H-1'), 5.39 (dd, 1H, H-3'), 5.72 (dd, 1H, H-2'), 5.77 (dd, 1H, H-4'), 5.79 (dd, 1H, H-3), 5.84 (d, 1H, NH), 6.48 (d, 1H, H-1); $J_{1,2}$ = 3.5, $J_{2,3}$ = 11.0, $J_{2,NH}$ = 9.0, $J_{3,4}$ = 9.3, $J_{4,5}$ = 10.0, $J_{5,6}$ = 2.5, $J_{1',2'}$ = 8.0, $J_{2',3'}$ = 10.3, $J_{3',4'}$ = 3.5, $J_{4',5'}$ = 1.0, $J_{5',6'}$ = 6.7 Hz; ^{13}C NMR (100 MHz, $CDCl_3$) δ 23.06 ($CO\underline{C}H_3$), 51.67 (C-2), 61.02 (C-6'), 61.75 (C-6), 67.43 (C-4'), 69.88 (C-2'), 71.14 (C-5), 71.43 (C-5'), 71.48 (C-3), 71.87 (C-3'), 75.19 (C-4), 91.27 (C-1), 101.20 (C-1'); MS (FAB) *m/z* 1134 $(M + Na)^+$, 1112 $(M + H)^+$.

Anal. Calcd for $C_{63}H_{53}NO_{18}·H_2O$ (1112.1): C, 66.96; H, 4.91; N, 1.24. Found: C, 66.97; H, 4.74; N, 1.29.

The next eluting, minor fractions from the column were characterized by ^1H NMR as the heptabenzoates of β-D-Gal-(1→4)-α-D-ManNAc and β-D-Gal-(1→4)-β-D-ManNAc (8 mg each, about 5% yield), and conceivably, *O*→*N* -benzoyl-migrated lactosamine by-product (7 mg).

***Borane Reduction of Lactosuloside Oxime 8*: *p*-Methoxybenzyl 2-Acetamido-3,6-di-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-2-deoxy-α-D-glucopyranoside (10) and its 3-*O*-Debenzoylated Analogue (11).** A solution of **8** (241 mg, 0.2 mmol) in THF (2 mL) was treated with a 1M solution of BH_3·THF complex (2.4 mL) as described for **4**→**5**. Quenching with MeOH, *N*-acetylation with Ac_2O (1 mL) and processing as described for **5** afforded 220 mg of a syrup which was purified by elution from a silica gel column with $CHCl_3$ - EtOAc (2:1→1:2 gradient). The first fraction gave 129 mg (57.1%) of **10**: mp 102-104 °C (EtOAc - Et_2O - pentane); $[\alpha]_D^{25}$ +84.2 ° (*c* 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 1.80 (s, 3H, NAc), 3.80 (s, 3H, OMe), 3.82 (m, 1H, H-5'), 3.90 (m, 2H, H-6'), 4.08 (m, 1H, H-5), 4.21 (dd, 1H, H-4), 4.4-4.6 (m, 2H, H-6), 4.45 (m, 1H, H-2), 4.42 and 4.65 (each d, 1H, C$\underline{H}_2$Ph), 4.91 (d, 1H, H-1), 4.92 (d, 1H, H-1'), 5.39 (dd, 1H, H-3'), 5.65 (dd, 1H, H-3), 5.70 (dd, 1H, H-2'), 5.75 (d, 1H, H-4'), 5.78 (d, 1H, NH), 6.87 (d, 2H, *m*-H of MBn); $J_{1,2}$ = 4.0, $J_{2,3}$ = 10.7, $J_{2,NH}$ = 10.0, $J_{3,4}$ = 9.0, $J_{4,5}$ = 9.5, $J_{1',2'}$ = 7.7, $J_{2',3'}$ = 10.3, $J_{3',4'}$ = 3.5, $J_{4',5'}$ = 0.5 Hz; ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.1 (CO$\underline{C}H_3$), 52.0 (C-2), 55.3 (OMe), 60.9 (C-6'), 62.2 (C-6), 67.4 (C-4'), 68.9 (C-5), 69.6 (CHPh), 69.9 (C-2'), 71.3 (C-5'), 71.8 (C-3'), 71.9 (C-3), 76.1 (C-4), 96.1 (C-1), 101.2 (C-1'), 114.0 (*m*-C of MBn); $J_{C1,H1}$ = 171, $J_{C1',H1'}$ = 160 Hz; MS (FAB) *m/z* 1129 $(M + H)^+$.

Anal. Calcd for $C_{64}H_{57}NO_{18}$·1/2 H_2O (1128.2): C, 67.60; H, 5.14; N, 1.23. Found: C, 67.50; H, 5.05; N, 1.18.

The second fraction gave 59 mg (29%) of pentabenzoate **11**: mp 99-101 °C (EtOAc - Et_2O - pentane); $[\alpha]_D^{20}$ +146.1 ° (*c* 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 1.89 (s, 3H, NAc), 3.81 (s, 3H, OMe), 3.8-4.0 (m, 4H, H-3,4,5,5'), 4.1-4.5 (m, 3H, H-6,6'), 4.24 (td, 1H, H-2), 4.36 and 4.59 (each d, 1H, C$\underline{H}_2$Ph), 4.62 (dd, 1H, H-6), 4.87 (d, 1H, H-1), 5.06 (d, 1H, H-1'), 5.57 (d, 1H, NH), 5.59 (dd, 1H, H-3'), 5.90 (dd, 1H, H-2'), 5.97 (d, 1H, H-4'), 6.88 (d, 2H, *m*-H of MBn); $J_{1,2}$ = 3.5, $J_{2,3}$ = 10.0, $J_{2,NH}$ = 9.0, $J_{1',2'}$ = 8.0, $J_{2',3'}$ = 10.5, $J_{3',4'}$ = 3.5, $J_{4',5'}$ < 0.5 Hz; ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.3 (CO$\underline{C}H_3$), 52.4 (C-2), 55.3 (OMe), 62.2 (C-6'), 62.9 (C-6), 68.0 (C-4',5'), 69.5 (C-2'), 69.6 (C$\underline{H}_2$Ph), 71.0 (C-5), 71.5 (C-3'), 72.4 (C-3), 82.8 (C-4), 96.4 (C-1), 102.3 (C-1'), 114.0 (*m*-C of MBn), 170.0 (NHCO); MS (FAB) *m/z* 1024 $(M)^+$.

Anal. Calcd for $C_{57}H_{53}NO_{17}$·1/2H_2O (1024.0): C, 66.27; H, 5.27; N, 1.36. Found: C, 66.30; H, 5.20; N, 1.09.

The third fraction gave a residue (5.2 mg, 2.3%), which on the basis of its NMR data was identified as *p*-methoxybenzyl 2-acetamido-3,6-di-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-2-deoxy-α-D-mannopyranoside: $[\alpha]_D^{20}$ +79.4 ° (*c* 0.2, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 1.90 (s, 3H, NAc), 3.79 (s, 3H, OMe), 3.83 and

4.13 (each dd, 1H, H-6'), 3.91 (dd, 1H, H-5'), 4.05-4.13 (m, 2H, H-4,5), 4.43 and 4.63 (each d, 1H, C$\underline{H}_2$Ph), 4.47 and 4.53 (each dd, 1H, H-6), 4.73 (d, 1H, H-1), 4.76 (td, 1H, H-2), 4.96 (d, 1H, H-1'), 5.48 (d, 1H, H-3'), 5.57 (d, 1H, NH), 5.72 (dd, 1H, H-2'), 5.79 (d, 1H, H-3), 5.80 (d, 1H, H-4'), 6.85 (d, 2H, *m*-H of MBn); $J_{1,2}$ = 1.5, $J_{2,3}$ = 4.5, $J_{2,NH}$ = 9.0, $J_{3,4}$ = 8.5, $J_{5,6a}$ = 4.0, $J_{5,6b}$ = 1.5, $J_{6a,6b}$ = 12.0, $J_{1',2'}$ = 8.0, $J_{2',3'}$ = 10.3, $J_{3',4'}$ = 3.5, $J_{4',5'}$ = 2.0, $J_{5',6'a}$ = 8.0, $J_{5',6'b}$ = 5.0, $J_{6'a,6'b}$ = 10.5 Hz; ^{13}C NMR (100 MHz, $CDCl_3$) δ 23.26 (CO$\underline{C}H_3$), 50.58 (C-2), 60.85 (C-6'), 62.57 (C-6), 67.39 (C-4'), 68.98 ($\underline{C}H_2$Ph), 69.21 (C-5), 70.00 (C-2'), 70.36 (C-3), 71.14 (C-5'), 71.67 (C-3'), 74.68 (C-4), 97.28 (C-1), 101.52 (C-1'), 113.98 (*m*-C of MBn), 169.47 (NHCO); $J_{C1,H1}$ = 169, $J_{C1',H1'}$ = 161 Hz; MS (FAB) *m/z* 1128 $(M)^+$.

***p*-Methoxybenzyl 2-Acetamido-4-*O*-(β-D-galactopyranosyl)-2-deoxy-α-D-glucopyranoside (12) from Oximinolactos-2-uloside 8.** A solution of **8** (2.4 g, 2 mmol) in THF (24 mL) was treated at – 10 °C with a 1 M solution of BH_3·THF complex (24 mL) under an atmosphere of N_2. The mixture was stirred at – 10 °C for 0.5 h and then at room temperature for 2 h. After quenching with MeOH (15 mL), *N*-acetylation was followed by stirring with Ac_2O (5 mL) at ambient temperature for 1 h. The mixture was passed through Amberlite IR-45 (OH^-) resin and washed with MeOH. Concentration of the combined eluant and washings gave a syrup (2.1 g), which was subjected to de-*O*-benzoylation with NaOMe (590 mg, 11 mmol) in MeOH (100 mL) for 20 h at 25 °C. Neutralization with dry Dowex 50W X8 (H^+) resin, filtration, concentration to dryness, and decantation of the residue from CH_2Cl_2 gave **12** (730 mg, 73% based on **8**) as a colorless powder. An analytical sample of **12** was obtained by elution from a silica gel column with 1:1 $CHCl_3$ - MeOH. Crystallization from MeOH - Et_2O gave **12** as colorless crystals: mp 247-248 °C; $[\alpha]_D^{20}$ +119.4 ° (*c* 1.0, H_2O); ^{1}H NMR (300 MHz, D_2O) δ 1.83 (s, 3H, NAc), 3.43 (dd, 1H, H-2'), 3.56 (dd, 1H, H-3'), 3.6-3.7 (m, 4H, H-4,5',6), 3.73 (s, 3H, OMe), 3.75-3.80 (m, 5H, H-2,3,5,6'), 3.81 (d, 1H, H-4'), 4.36 (d, 1H, H-1'), 4.38 and 4.58 (each d, 1H, C$\underline{H}_2$Ph), 4.81 (d, 1H, H-1), 6.91 and 7.26 (each d, 2H, aromatic H); $J_{1,2}$ = 2.5, $J_{1',2'}$ = 7.8, $J_{2',3'}$ = 10.5, $J_{3',4'}$ = 3.5, $J_{4',5'}$ < 0.5 Hz; ^{13}C NMR (75 MHz, D_2O) δ 22.53 (CO$\underline{C}H_3$), 54.09 (C-2), 56.15 (OMe), 60.62 and 61.77 (C-6,6'), 69.31 (C-4'), 70.20 (C-3), 71.53 (C-5), 71.75 (C-2'), 73.31 (C-3'), 76.11 (C-5'), 79.51 (C-4), 96.08 (C-1), 103.68 (C-1'), 114.88 (*m*-C of MBn), 130.32 (*ipso*-C of MBn), 131.15 (*o*-C of MBn), 159.62 (*p*-C of MBn), 174.95 (NHCO); MS (FD/20 mA) *m/z* 526 $(M + Na)^+$, 504 $(M + H)^+$.

Anal. Calcd for $C_{22}H_{33}NO_{12}$·1/2 H_2O (503.5): C, 51.56; H, 6.69; N, 2.73. Found: C, 51.33; H, 6.46; N, 2.49.

2-Acetamido-4-*O*-(β-D-galactopyranosyl)-2-deoxy-α-D-glucopyranose (*N*-Acetyllactosamine) (13). A solution of **12** (730 mg, 1.45 mmol) in MeOH -

water (100 mL, 1:1) was hydrogenated in the presence of 10% Pd-C (200 mg) under an atmosphere of H_2 (2.0×10^5 Pa) for 3 d. The mixture was filtered through a pad of Celite and concentrated. Purification by filtration through a short column of silica gel with 1:1 $CHCl_3$ - MeOH gave **13** (486 mg, 88%) as a colorless, chromatographically homogeneous solid; HPLC [Dionex, 12 cm; 1:1 NaOH - 1M NaOAc]: > 99% purity. Crystallization from MeOH gave **13** as a microcrystalline powder: mp 150-160 °C (dec.); $[\alpha]_D^{20}$ +28.1 ° (3 min) → +24.8 ° (3 h) (*c* 0.98, water) [lit.[7d] $[\alpha]_D^{25}$ + 27.0 ° (*c* 1.0, water)] ; ^{1}H NMR (300 MHz, in D_2O) δ 1.83 (s, 3H, N-$COCH_3$), 4.39 (d, 1H, H-1'), 4.65 (d, 1H, H-1β), 5.12 (d, 1H, H-1α); $J_{1\alpha,2}$ = 1.0, $J_{1\beta,2}$ = 7.2, $J_{1',2'}$ = 7.9 Hz; ^{13}C NMR (75 MHz, D_2O) δ 22.79 (CO$\underline{C}H_3$-α), 24.17 (CO$\underline{C}H_3$-β), 54.61 (C-2α), 57.11 (C-2β), 60.86 (C-6α), 61.00 (C-6β), 61.92 (C-6'), 69.45 (C-4'), 71.16 (C-3α), 70.18 (C-3β), 71.87 (C-2'), 73.32 (C-5β), 73.42 (C-3'), 75.74 (C-5α), 76.25 (C-5'), 79.32 (C-4β), 79.73 (C-4α), 91.42 (C-1α), 95.74 (C-1β), 103.82 (C-1'), 175.35 (NH$\underline{C}$O-α), 175.62 (NH$\underline{C}$O-β); α:β = ca. 2:1. These data agreed well with those by Sakai et al.[7d] except for the observation that their signal positions are consistently higher by 2 ppm; MS (FAB) *m/z* 406 $(M + Na)^+$.

Anal. Calcd for $C_{14}H_{25}NO_{11}\cdot H_2O$ (383.1): C, 41.90; H, 6.78; N, 3.49. Found: C, 41.87; H, 6.69; N, 3.45.

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