Pergamon

Bioorganic & Medicinal Chemistry, Vol. 4, No. 5, pp 683–692, 1996

0968-0896/96 $15.00 + 0.00

S0968-0896(96)00064-8

Towards Oligosaccharide Libraries: A Study of the Random Galactosylation of Unprotected *N*-Acetylglucosamine

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Abstract—A single reaction of an unprotected β-D-GlcNAc glycoside with tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate in dioxane, catalyzed by BF_3-etherate, was shown to yield all six possible Gal-GlcNAc disaccharides. This result is surprising not only because significant amounts of α-linked disaccharides were formed, despite the presence of a participating group at O-2 of the glycosyl donor, but also because glycosylation of the primary OH-6 is not the dominant reaction. These results suggest 'random-glycosylation' to be a valid strategy for the rapid production of oligosaccharide libraries. Copyright © 1996 Elsevier Science Ltd

Introduction

The use of combinatorial chemistry for the synthesis of large numbers of molecules, either as individual compounds or as mixtures, is considered to be one of the frontiers of organic chemistry applied to drug discovery. The early successes were in the fields of peptide and oligonucleotide libraries where the well established chemistry of amide-bond and phosphodiester-bond formation led to rapid progress. Subsequently, other types of high yielding chemical reactions were applied to the combinatorial chemistry of small organic molecules both in solutions and on the solid phase.[1]

Despite the recognized biological significance[2] of cell-surface oligosaccharides as targets for bacterial, toxin, and viral attachment, and in mammalian cell–cell adhesion, approaches to the chemical assembly of oligosaccharide libraries from the monomer units has barely been explored.[3,4] This is undoubtedly because of the serious logistical difficulties that are sure to be encountered in a 'systematic' approach to this problem. Namely, all of the sugars have either three or four hydroxyl groups which may undergo glycosylation and, therefore, also branching. In addition, a new stereocenter is formed on glycosylation which can result in either an α- or β-glycosidic linkages which are usually axial and equatorial, respectively.

In mammalian oligosaccharide biosynthesis, there are nine common building blocks in use (D-Glc, D-Gal, D-Man, L-Fuc, D-Xyl, D-GlcNAc, D-GalNAc, Neu-5-Ac) all of which are present in their pyranose forms and all of which have either three or four hydroxyl-groups that can undergo glycosylation. These may be assembled to form 119,736 reducing trisaccharides and over 18 million tetrasaccharides,[5] typical sizes for biologically active ligands. Many more sugar building blocks are used by microorganisms and plants. As shown in Figure 1, the systematic approach to assembling such libraries would be to prepare the orthogonally O-protected monomer building blocks and then coupling these using established or emerging glycosyla-

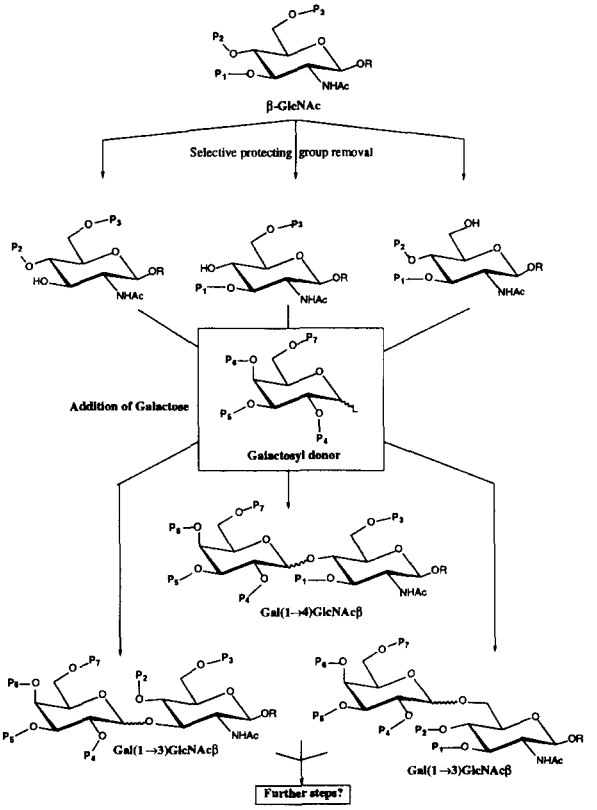


Figure 1. A hypothetical sequence using orthogonally protected monosaccharide building blocks in the process of assembling an oligosaccharide library. Protecting groups P1–P7 must be removable independently.

tion methods.[6] If trisaccharides were the target size for a library, this approach would require the use of seven or eight orthogonal OH-protecting groups, a number that is not available. In addition, since each sugar must be capable of being added first or second (or later) in the sequence, at least two sets of independently protected monomer building blocks must be prepared. Even if the required set of orthogonally protected building blocks were available, however, this approach would still fall prey to the unpredictability of currently available glycosylation chemistry that is extremely sensitive to the nature and position of protecting groups, both with respect to yield and to the anomeric configuration of the product.

In view of the difficulties summarized above for the preparation of discrete oligosaccharides of known structure, we have elected to examine 'random-glycosylation'[4] for the production of oligosaccharide *mixtures* as an alternative strategy. In the successful application of this approach, completely unprotected sugars would be glycosylated to provide a mixture of glycosides wherein all of the possible products would be present. As in all cases where mixtures are produced, the ability to rapidly screen these mixtures for activity and then to 'deconvolute ' the mixture [i.e., identify the active component(s)] will be the key to their utility.

There have until recently[3,4,7] been no systematic studies on the glycosylation of completely unprotected oligosaccharides. In fact, efficient use has been made of partially protected oligosaccharides for the production of single compounds in schemes that rely on introduction of bulky protecting groups to sterically hinder adjacent hydroxyl groups.[8] In the present work, we investigate whether conditions could be developed for the random addition of galactose to N-acetylglucosamine units to yield all six possible Gal-GlcNAc sequences (**1–6**, Fig. 2) of which the β(1→3)- and β(1→4)-linked disaccharides (**1** and **2**) are common units of glycoproteins and glycolipids.

To facilitate analysis of random glycosylation reactions, we synthesized all six possible products (**1–6**) so that reference standards would be available to simplify the determination of product distributions. This was accomplished using standard techniques of oligosaccharide synthesis as outlined in Schemes 1 and 2. The strategy involved the synthesis of the three protected βGlcNAc derivatives **10**, **12**, and **15** having OH-3, OH-4, and OH-6, respectively, free for galactosylation. Acetobromogalactose (**16**),[9] with a participating acetyl group at O-2, was used to produce the β-glycosides while the tetra-O-benzyl bromide **17**[10] afforded the α-linked disaccharides. For the synthesis of the blocked disaccharide **23**, the inital product had to be de-O-acetylated, purified by chromatography, and then re-O-acetylated to provide pure material. Reactions were not optimized since the objective was simply the production of samples of the six authentic standards. A hydrophobic aglycone was included in these standards to facilitate the separation of the final disaccharide mixtures from reaction by-products, arising from donor degradation, on C-18 chromatography supports.[4,11] In addition, a removable aromatic chromophore[12] (the *p*-methoxyphenyl group) was included in the aglycone to allow detection and quantitation by UV-absorbance in HPLC. The standards could be separated on a PAC-column permitting direct analysis of mixtures by HPLC. The separation achieved is shown in Figure 3 where a typical chromatogram obtained for a random glycosylation run is shown.

For the galactosylation reactions we initially examined 2,3,4,6-tetra-O-benzyl-galactopyranosyl imidate (**28**)[13] and dibenzylphosphite (**29**)[14] as donors (for structures see Table 1), as was previously used for random α-fucosylation.[4] Under these conditions, as expected,

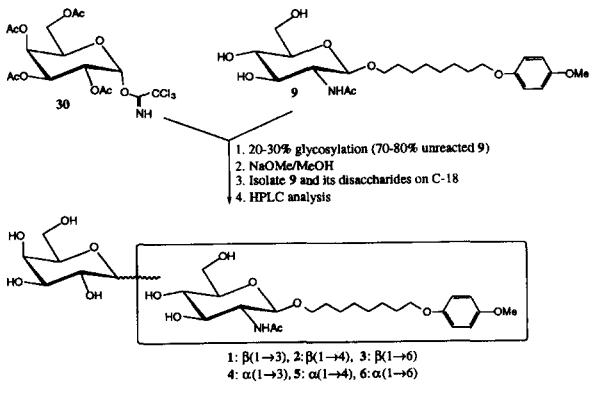
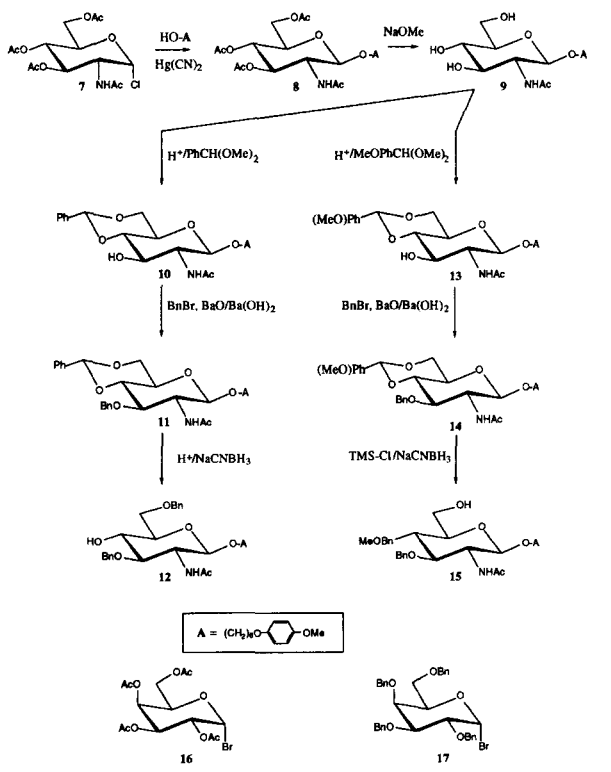
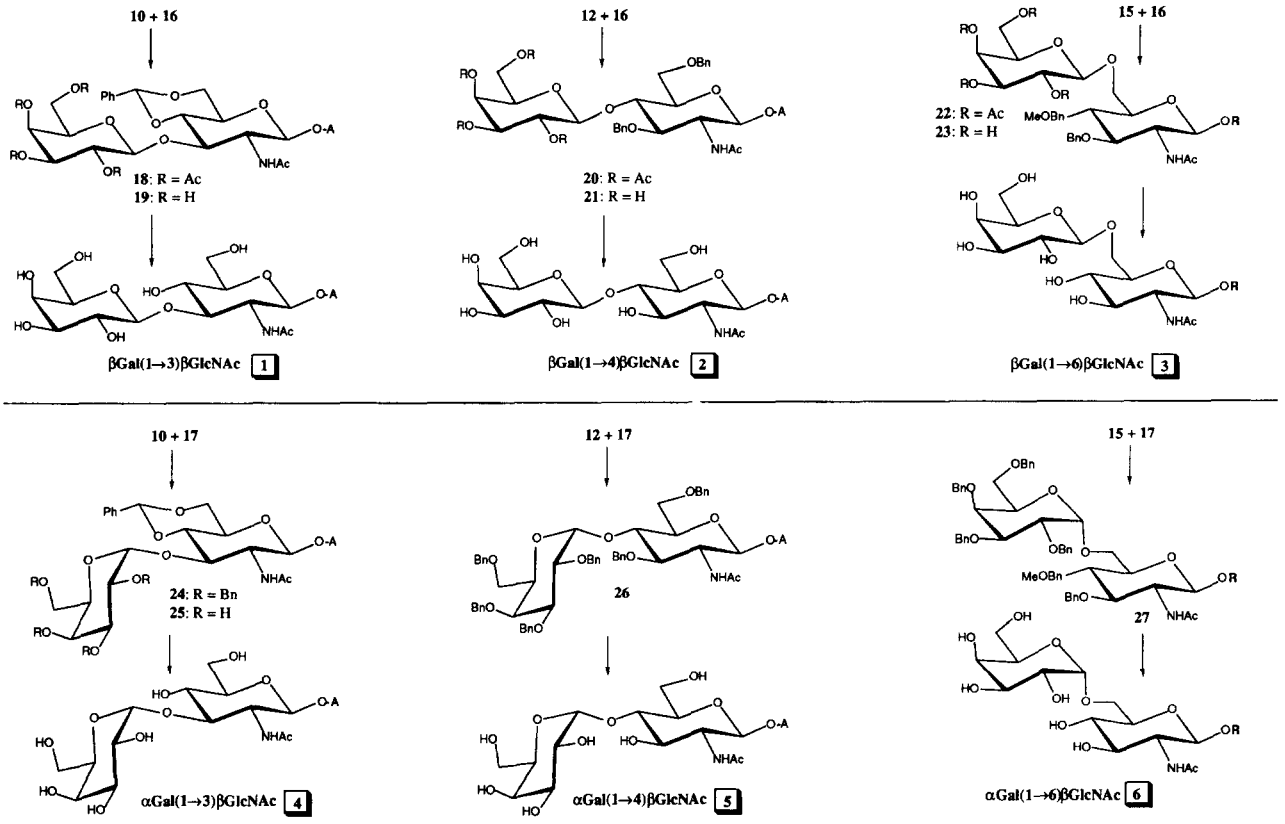


Figure 2. A typical random galactosylation sequence corresponding to entries 3–6 in Table 1.



Scheme 1.



Scheme 2.

the β(1→3) and β(1→4) linked disaccharides were formed in only minor amounts (Table 1, entries 1 and 2). To increase the proportion of the β-anomers, we, therefore, examined the use of the peracetylated galactopyranosyl imidate (**30**)[13] as the donor. Reactions were poor in DMF, but in dioxane a clean mixture of products was obtained. Table 1 summarizes the results of varying the temperature and other parameters of the reaction. Two equivalents of donor were required to give a conversion of 20–30% of **9** to disaccharides **1–6**. Only traces of trisaccharides were suggested by peaks eluting when the column was washed with more polar solvent mixtures, but the identity of these peaks was not investigated.

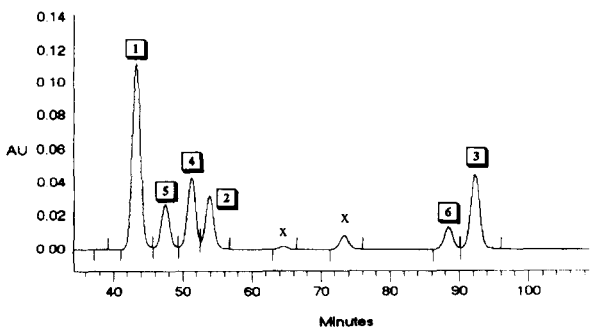
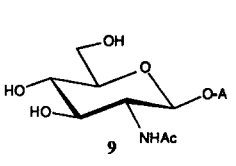


Figure 3. HPLC separation of disaccharides **1–6** produced by random glycosylation of **9** using **30**, as described in Figure 2 and Table 1, entry 5. The elution order of the disaccharides was established by co-injection of synthetic reference standards. 'X' denoted non-carbohydrate UV (292 nm) absorbing contaminants.

Using imidate **30** as the donor (Fig. 3) at either 20° or 50 °C (entries 4 and 5), all six possible disaccharides were formed, the α(1→6) anomer (**6**) being the least favored. This was also the case when phosphite **31**[15] was used as the donor (entry 7). It is surprising that so much α-anomer formed in a reaction that yields almost exclusively β-glycosides when using partially-protected acceptors in non-polar solvents. In addition, the product of galactosylation of the primary OH-6, intuitively the least hindered and most reactive alcohol, did not dominate the reaction products, indeed the β(1→3) disaccharide **1** was the major product. The possibility that the β(1→6) product **3** might have formed rapidly but then been selectively destroyed was eliminated by determining that the product ratios did not change significantly when the reaction was run for 10, 30 min, 3 or 14 h even though very little product had formed in the shorter reaction times (data not shown). The possibility that co-eluting UV-absorbing non-carbohydrate impurities might have cause an overestimation of the amount of β(1→3) disaccharide **1** was eliminated for the reaction products of the experiment summarized in entry 5 where **1** is clearly the major product (42%). The first four peaks of the chromatogram (Fig. 2) were collected as a single fraction, as were the last two peaks, and the relative intensities of the anomeric signals in the ^{1}H NMR spectra of these fractions, assigned by comparison with the authentic standards,

Table 1. Effect of donor and reaction conditions on the distribution of products obtained by galactosylation of GlcNAc-derivatives **9**.[a]



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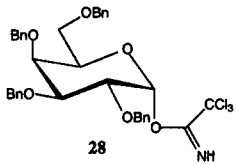

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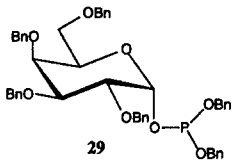

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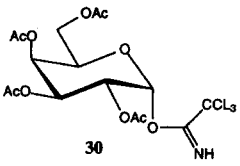
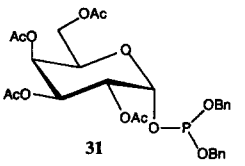


30



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Entry	Donor	Promoter[b]	Solvent	Temp	Production distriibution 1 (β1→3)	2 (β1→4)	3 (β1→6)	4 (α1→3)	5 (α1→4)	6 (α1→6)
1	**28**	BF_3–Et_2O	DMF	20°	~2	<2	30	15	17	35
2	**29**	TMS–OTf	DMF	−20°	<2	<2	15	23	11	51
3	**30**	BF_3–Et_2O	Dioxane	0°	32	7	22	22	10	5
4	**30**	BF_3–Et_2O	Dioxane	20°	30	11	20	22	11	6
5	**30**	BF_3–Et_2O	Dioxane	50°	42	10	19	13	10	5
6	**30**	BF_3–Et_2O	Dioxane	80°	45	13	2	24	12	2
7	**31**	TMS–OTf	Dioxane	20°	16	26	36	6	8	7

[a]The yields were determined by HPLC using co-injection of reference standards for structural verification.
[b]Either 0.1 or 0.2 equiv were used for reaction times of ca. 14 h.

independently confirmed the results of Table 1 (within 5% error). Replicates of runs yielded product distributions within 10% of each other.

The results in Table 1 show that the anomeric configuration of the products of random galactosylation can be biased towards the α-glycosides by use of **28** or **29** as the donors, or all of the products can be obtained in a single run using **30** or **31** as donors. While all disaccharides are clearly present in significant amount in the product, they are not formed in equal amount and it is hoped that further studies on galactosyl and other donors will lead to an improvement in the product distributions. However, since biological ligands are generally only two to four sugars in size, only one to three chemical glycosylation reactions in sequence need to be combined to yield a potentially large useful oligosaccharide library. In fact, even the mixtures produced in entries 1 and 2 (Table 1) have been successfully used[3] to measure the activity of a fucosyltransferase enzyme present in human milk that acts only on **3** and **4**, present in this mixture in under 5%.

Experimental

General methods

TLC was performed on silica gel 60-F_{254} (Merck) with detection by quenching of fluorescence, and by charring with H_2SO_4. Unless otherwise noted, CC was performed on silica gel 60 (Merck, 40–63 mm) or Iatrobeads (Iatron Laboratories). Optical rotations were measured with a Perkin-Elmer 241 polarimeter at 22±2°. Melting points were measured with a Fisher-Johns melting point apparatus. ^{1}H NMR spectra were recorded at 300 MHz (Bruker AM-300), 360 MHz (Bruker AM-360), or 500 MHz (Varian UNITY 500) on solution in $CDCl_3$ (internal TMS, δ 0), or D_2O (internal acetone, δ 2.225). ^{13}C NMR spectra were recorded at 75 or 90 MHz on the same instruments in $CDCl_3$ (internal TMS, δ 0) or D_2O (internal dioxane, d 67.4). Only partial NMR data are reported, the other data were in accord with the proposed structures. The assignments of ^{13}C resonances are tentative. Fast atom bombardment-mass spectra (FABMS) were obtained on a Kratos AEIMS9 instrument by the departmental microanalytical laboratory. Elemental analyses were carried out on a Caro Erba EA1108. Methanol was dried by distillation from Mg, and toluene from CaH_2.

8-*p*-Methoxyphenoxyloctyl 2-acetamido-3,4,6-tri-*O*-acetyl-β-D-glucopyranoside (8). Mercuric cyanide (15.9 g, 63 mmol) and anhydrous $CaSO_4$ (25 g) were added to a solution of 8-*p*-methoxyphenoxyloctanol (11 g, 43 mmol) in dry toluene (200 mL). The mixture was protected from moisture while stirring for 1 h (rt) prior to addition of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (**7**, 12 g, 32.8 mmol). The mixture was stirred for 4 days, then CH_2Cl_2 was added, solids were removed by filtration, and the filtrate was sequentially washed with 10% aq NaCl, satd aq $NaHCO_3$ and H_2O (2×). After drying ($MgSO_4$) and evaporation, the product crystallized from hexane:Et_2O (1:5) (14.5 g). Another 2.8 g was obtained after chromatography on silica gel column (toluene:EtOAc 1:1) for a combined yield of 91%. R_f 0.16 (toluene:EtOAc 1:1); mp 99 °C; $[\alpha]_D$ −9.1 (*c* 1.2; $CHCl_3$); ^{1}H NMR (360 MHz, $CDCl_3$): δ 5.78 (d, 1 H, $J_{NH,2}$ = 8.8 Hz, NH), 5.31 (dd, 1 H, H-3), 5.08 (dd, 1 H, H-4), 4.69 (d, $J_{1,2}$ = 8.3 Hz, H-1), 3.89 (s, 3 H, OCH_3), 3.48 (m, 1 H, H-2), 2.08, 2.03, 2.02 (3 s, 9 H, 3 $COCH_3$), and 1.93 (s, 3 H, $NHCOCH_3$); ^{13}C NMR (300 MHz, $CDCl_3$): δ 170.84, 170.72, 170.16, 169.42 (3 $\underline{C}OCH_3$, $NH\underline{C}OCH_3$), 100.67 (C-1), 72.39, 71.73, 68.81 (C-3, C-4, C-5), 69.85, 68.52 (2 OCH_2), 62.23 (C-6), 55.74 (C-2), 54.88 ($O\underline{C}H_3$), 29.39, 29.39, 29.29, 29.21, 25.97, 25.76, 23.27

($NHCOCH_3$), 20.73, 20.68, 20.62 (3 $COCH_3$); Anal. calcd for $C_{29}H_{43}NO_{11}$: C, 59.89; H, 7.40; N, 2.41. Found: C, 59.59; H, 7.63; N, 2.43.

8-*p*-Methoxyphenoxyloctyl 2-acetamido-2-deoxy-β-D-glucopyranoside (9). A solution of **8** (10 g, 17 mmol) in dry MeOH (150 mL) containing NaOMe (100 mg) was stirred at rt for 24 h, then neutralized with Dowex 50 (H^+) and filtered. The solvent was evapd to leave **9** as a white powder. R_f 0.15 (toluene:EtOH, 5:1); mp 155 °C; $[\alpha]_D$ −17.0 (*c* 1.2 MeOH); ^1H NMR (360 MHz, $CDCl_3$): δ 4.37 (d, 1 H, $J_{1,2}$ = 8.4 Hz, H-1), 3.72 (s, 3 H, OCH_3), 3.25 (m, 1 H, H-2), 1.95 (s, 3 H, $NHCOCH_3$).

8 -*p*-Methoxyphenoxyloctyl 2 -acetamido- 4,6 -*O*-benzylidene-2-deoxy-β-D-glucopyranoside (10). To a soln of **9** (3.47 g, 7.62 mmol) in dry DMF (10 mL) was added *p*-toluenesulfonic acid (125 mg) and α,α-dimethoxytoluene (1.75 mL) and the mixture was heated at 60 °C under the vacuum of a water aspirator. After 3 h, Et_3N (3 drops) was added and the solvent evapd. The residue was purified by silica gel CC (CH_2Cl_2:EtOAc:MeOH 5:5:1) to give **10** (4 g, 94%) which was crystallized from CH_2Cl_2–Et_2O. R_f 0.6 ($CHCl_3$:MeOH 10:1); mp 224 °C; $[\alpha]_D$ −47.1 (*c* 1.0; $CHCl_3$); ^1H NMR (360 MHz, $CDCl_3$): δ 5.82 (d, 1 H, $J_{NH,2}$ = 5.2 Hz, NH), 5.53 (s, 1 H, benzylidene), 4.69 (d, 1 H, $J_{1,2}$ = 8.3 Hz, H-1), 4.15 (dd, 1 H, $J_{3,4}$ = 9.3; $J_{4,5}$ = 9.2 Hz, H-4), 3.76 (s, 3 H, OCH_3), 3.44 (m, 1 H, H-2), 2.03 ($NHCOCH_3$); ^{13}C NMR (300 MHz, CD_3OD): δ 172.03 ($NHCOCH_3$), 101.29, 101.19 (C-1 and benzylidene), 81.21, 70.79, 65.83 (C-3, C-4, C-5), 69.58, 68.26 (2 OCH_2), 68.17 (C-6), 56.70 (C-2), 55.12 (OCH_3), 29.02, 29.02, 28.86, 28.78, 25.51, 25.31, 22.05 ($NHCOCH_3$); Anal. calcd for $C_{30}H_{41}NO_8$: C, 66.29; H, 7.55; N, 2.58. Found: C, 66.09; H, 7.56, N, 2.60.

8-*p*-3-Methoxyphenoxyloctyl 2-acetamido-4,6-*O*-benzylidene-3-*O*-benzyl-2-deoxy-β-D-glucopyranoside (11). Barium oxide (3.92 g, 25.6 mmol), barium hydroxide octahydrate (1.08 g, 3.42 mmol), and benzyl bromide (0.8 mL) were added to a soln of **10** (2.1 g, 3.86 mmol) in dry DMF (50 mL) and the resulting mixture stirred at rt for 12 h, then partitioned between CH_2Cl_2 and ice water. The organic layer was washed with 20% HOAc, then aq $NaHCO_3$, dried (Na_2SO_4), and concd. Chromatography of the residue on silica gel (CH_2Cl_2:EtOAc, 3:1) yielded **11** as a crystalline compound (2.2 g, 91%). R_f 0.59 (toluene:EtOAc, 2:1); mp 195 °C; $[\alpha]_D$ −1.1 (*c* 1.2 $CHCl_3$); ^1H NMR (360 MHz, $CDCl_3$): δ 5.57 (s, 1 H, benzylidene), 5.51 (d, 1 H, $J_{NH,2}$ = 7.5 Hz, NH), 5.00 (d, 1 H, $J_{1,2}$ = 8.3 Hz, H-1), 3.76 (s, 3 H, OCH_3), 3.66 (dd, 1 H, $J_{3,4}$ = 9.2, $J_{2,3}$ 9.2 Hz, H-3), 3.22 (m, 1 H, H-2), 1.87 (s, 3 H, $NHCOCH_3$); ^{13}C NMR (300 MHz, $CDCl_3$): δ 170.88 ($NHCOCH_3$), 101.13, 100.53 (C-1 and benzylidene), 82.54, 76.76, 65.84 (C-3, C-4, C-5), 57.18 (C-2), 74.27, 69.99 (2 OCH_2), 68.62 (OCH_2Ph), 55.66 (OCH_3), 29.38, 29.23, 29.13, 29.13, 25.87, 25.68, 23.07 ($NHCOCH_3$); Anal. calcd for $C_{37}H_{47}NO_8$: C, 70.14; H, 7.42; N, 2.21. Found: C, 70.18; H, 7.28; N, 2.13.

8-*p*-Methoxyphenoxyloctyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (12). To a soln of **11** (1 g, 1.5 mmol) and sodium cyanoborohydride (0.85 g, 13.5 mmol) in dry THF (40 mL) at 0 °C containing powdered 3 Å molecular sieves (1 g) and a crystal of methyl orange indicator, was added dropwise satd HCl in Et_2O until the solution became acidic (color change from yellow to pink). Further addition was continued very slowly during 2 days until TLC indicated the complete disappearance of starting compound. Et_3N (0.5 mL) was then added and the mixture was filtered through celite and washed with CH_2Cl_2. The filtrates were washed with satd aq $NaHCO_3$ and H_2O, then concd. The residue was purified by silica gel CC (toluene:EtOAc, 1:1) to give **12** as a white solid (0.66 g (70%). R_f 0.4 (toluene:EtOAc, 1:1); mp 107 °C; $[\alpha]_D$ −1.3 (*c* 1.0; $CHCl_3$); ^1H NMR (360 MHz, $CDCl_3$): δ 5.79 (d, 1 H, $J_{NH,2}$ 7.8 Hz, NH), 4.70 (d, 1 H, $J_{1,2}$ = 8.8 Hz, H-1), 3.88 (dd, 1 H, $J_{2,3}$ = 7.0 Hz, $J_{3,4}$ = 9.4 Hz, H-3), 3.65 (s, 3 H, OCH_3), 3.53 (ddd, 1 H, $J_{3,4}$ = 9.4 Hz, $J_{4,5}$ = 9.0 Hz, $J_{OH,4}$ = 2.9 Hz, H-4), 3.25 (m, 1 H, H-2), 3.04 (b, 1H, OH),1.78 (s, 3 H, $NHCOCH_3$); ^{13}C NMR (300 MHz, $CDCl_3$): δ 100.04 (C-1), 80.62, 73.86, 73.06 (C-3, C-4 C-5), 74.11 and 73.6 (OCH_2), 69.62, 70.61 (2 OCH_2Ph), 56.74 (C-2), 55.70 (OCH_3), 170.50 ($NHCOCH_3$), 23.46 ($NHCOCH_3$), 29.46, 29.46, 29.31, 29.21, 25.94, 25.79; Anal. calcd for $C_{37}H_{49}NO_8$: C, 69.92; H, 7.71; N, 2.2. Found: C, 70.15; H, 7.82; N, 2.28.

8-*p*-Methoxyphenoxyloctyl 2-acetamido-4,6-*O*-(*p*-methoxy-benzylidene)-2-deoxy-β-D-glucopyranoside (13). A soln of **9** (3 g, 6.59 mmol), *p*-methoxybenzaldehyde dimethyl acetal (1 mL), and *p*-toluenesulfonic acid (150 mg) in dry DMF (10 mL) was stirred under the vacuum of a water aspirator at 60 °C for 3 h. Et_3N (three drops) was then added, the solvent was evapd and the residue purified by silica gel chromatography (CH_2Cl_2:EtOAc, 1:1) to provide 3.3 g of crystalline product (87%). R_f 0.53 ($CHCl_3$:MeOH, 10:1); mp 227 °C; $[\alpha]_D$ -17.4 (*c* 1.0 $CHCl_3$); ^1H NMR (360 MHz, $CDCl_3$): δ 5.74 (d, 1 H, NH), 5.51 (s, 1 H, benzylidene), 4.72 (d, 1 H, $J_{1,2}$ = 8.3 Hz, H-1), 4.16 (dd, 1 H, $J_{3,4}$ = 9.1, $J_{4,5}$ = 9.3 Hz, H-4), 3.79, 3.76 (2 s, 6 H, OCH_3), 3.40 (m, 1 H, H-2), 2.05 (s, 3H, $NHCOCH_3$); ^{13}C NMR (300 MHz, $CDCl_3$): δ 172.04 ($NHCOCH_3$),103.07, 100.72 (C-1, and benzylidene), 75.67, 74.32, 70.53 (C-3, C-4, C-5), 69,18, 68.22 (2 OCH_2O), 61.25 (C-6), 55.76 (C-2), 54.98, 54.87 (2 OCH_3), 21.86, 28.96, 28.96, 28.81, 28.78, 25.46, 25.32, 21.86 ($NHCOCH_3$); Anal. calcd for $C_{31}H_{43}NO_9$: C, 64.92, H, 7.50; N, 2.44. Found: C, 64.78, H, 7.72, N, 2.44.

8-*p*-Methoxyphenoxyloctyl 2-acetamido-3-*O*-benzyl-4,6-(*p*-methoxybenzylidene)-2-deoxy-β-D-glucopyranoside (14). A soln of **13** (3.3 g, 5.76 mmol) in dry DMF (30 mL) was stirred for 12 h at rt in the presence of barium oxide (5.4 g), barium hydroxide octahydrate (1.5 g), and benzyl bromide (1.2 mL). The mixture was diluted with CH_2Cl_2, washed with ice-cold aq 20% HOAc, aq $NaHCO_3$, then H_2O, dried (Na_2SO_4), and

concd. The residue was purified by chromatography on silica gel (toluene:EtOAc 2:1), to give **14** (3.3 g, 87%). R_f 0.54 (toluene:EtOAc, 2:1); mp 207 °C; $[\alpha]_D$ −4.5 (*c* 0.7; $CHCl_3$); ^1H NMR (360 MHz, $CDCl_3$): δ 5.53 (s, 1 H, benzylidene), 5.50 (d, 1 H, NH), 4.99 (d, 1 H, J = 8.3 Hz, H-1), 3.81, 3.76 (2 s, 6 H, 2 OCH_3), 3.21 (m, 1 H, H-2), 1.93 (s, 3 H, $NHCOCH_3$); ^{13}C NMR (300 MHz, $CDCl_3$): δ 170.70 ($NHCOCH_3$), 100.84, 100.66, (C-1, and benzylidene), 82.27, 76.98, 65.73 (C-3, C-4, C-5), 74.03, 71.86 (2 OCH_2), 68.50 (OCH_2Ph), 62.46 (C-6), 55.92 (C-2), 55.32, 54.97 (2 OCH_3), 29.32, 29.32, 29.05, 29.05, 25.79, 25.58, 23.10 ($NHCOCH_3$); Anal. calcd for $C_{38}H_{49}NO_9$: C, 68.77; H, 7.39; N, 2.11. Found: C, 68.87; H, 7.44; N, 2.15.

8-*p*-Methoxyphenoxyloctyl 2-acetamido-3-*O*-benzyl-4-*O*-(*p*-methoxybenzyl)-2-deoxy-β-D-glucopyranoside (15). At 0 °C, a soln of trimethylsilyl chloride (9 mmol) in CH_3CN (6 mL) was added dropwise to a stirring mixture containing **14** (1 g, 1.5 mmol), sodium cyanoborohydride (0.6 g, 9 mmol), and powdered 3 Å molecular sieve (1 g) in CH_3CN:toluene (5:1, 30 mL). The mixture was stirred for 24 h at rt, then filtered through Celite and poured into ice-cold satd aq $NaHCO_3$. The aq phase was extracted with CH_2Cl_2 and extracts were washed with satd aq $NaHCO_3$, dried ($MgSO_4$), filtered, and concentrated. The residue was purified by chromatography on silica gel (toluene:EtOAc, 3:1) to yield **15** (0.35 g, 35%) as a white solid. R_f 0.32 (toluene:EtOAc, 1:1); mp 168 °C; $[\alpha]_D$ −0.4 (*c* 1.0; $CHCl_3$); ^1H NMR (360 MHz, $CDCl_3$): δ 5.39 (d, 1 H, $J_{NH,2}$ = 7.8 Hz, NH), 4.73 (d, 1 H, $J_{1,2}$ = 8.8 Hz, H-1), 3.72, 3.69 (2 s, 6 H, 2 OCH_3), 3.25 (m, 1 H, H-2), 1.77 (s, 3 H, $NHCOCH_3$); ^{13}C NMR (300 MHz, $CDCl_3$): δ 171.03 ($NHCOCH_3$), 100.79 (C-1), 81.39, 77.96, 75.16 (C-3, C-4, and C-5), 74.71, 74.39 (2 OCH_2), 70.04, 68.67 (2 OCH_2Ph), 61.62 (C-6), 56.02 (C-2), 55.70, 55.19 (2 OCH_3), 29.41, 29.41, 29.22, 29.22, 25.87, 25.67, 23.28 ($NHCOCH_3$); Anal. calcd for $C_{38}H_{51}NO_9$: C, 68.57; H, 7.67; N, 2.1. Found: C, 68.22; H, 7.87; N; 2.2.

8-*p*-Methoxyphenoxyloctyl 2-acetamido-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (18). A soln of bromide **16** (480 mg, 1.17 mmol) in toluene:CH_3NO_2 (1:1) (5 mL) was added to a stirred mixture of **10** (500 mg, 0.78 mmol), mercuric cyanide (800 mg), and powdered $CaSO_4$ (1 g) in the same solvent mixture (30 mL). After stirring for 12 h at 40 °C, the mixture was diluted with CH_2Cl_2 (200 mL), washed sequentially with aq NaCl, aq $NaHCO_3$ and H_2O, then concd. The residue was purified on a silica gel (toluene:EtOAc, 1:1) to yield **18** as a white solid (530 mg, 78%). R_f 0.6 (toluene:acetone, 3:1); $[\alpha]_D$ −2.0 (*c* 4.8 $CHCl_3$); ^1H NMR (360 MHz, $CDCl_3$): δ 5.8 (d, 1 H, J = 8.5 Hz, NH), 5,5 (s, 1 H, benzylidene), 5.17 (d, 1 H, J 8.7 Hz, H-1), 4.73 (d, 1 H, J = 8.7 Hz, H-1′), 3.75 (s, 3 H, OCH_3), 3.0 (m, 1 H, H-2), 1.98, 1.97, 1.99, 1,99, 2.10 (4 $OCOCH_3$ and $NHCOCH_3$); ^{13}C NMR (300 MHz, $CDCl_3$): δ 169.50 ($NHCOCH_3$), 100.15, 99.33, 101.62 (C-1, C-1′, and benzylidene), 80.88, 71.10, 70.59, 69.45, 66.91, 65.86 (C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 68.63, 61.09 (C-6, C-6′), 58.68 (C-2), 70.31, 69.48 (2 OCH_2), 29.52, 29.38, 29.34, 29.26, 26.29, 25.83, 23.20 ($NHCOCH_3$); Anal. calcd for $C_{44}H_{59}NO_{17}$: C, 60.48; H, 6.76; N, 1.60. Found: C, 60.234; H, 6.74; N, 1.65.

8-*p*-Methoxyphenoxyloctyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(β-D-galactopyranosyl)-β-D-glucopyranoside (19). Compound **18** (100 mg, 0.12 mmol) was dissolved in dry MeOH (5 mL) containing a trace of NaOMe. After stirring overnight (rt) the solution was neutralized with Dowex-50W (H^+), the resin was removed by filtration and the solvent was evapd to yield **9** as a syrup (78 mg). R_f 0.6 (CH_2Cl_2:acetone 1:2); $[\alpha]_D$ −2.3 (*c* 5.0 MeOH); ^1H NMR (360 MHz, CD_3OD–$CDCl_3$): δ 5.56 (s, 1 H, benzylidene), 4.51 (d, 1 H, J = 9.3 Hz, H-1), 4.32 (d, 1 H, J = 7.6 Hz, H-1′), 3.70 (s, 3 H, OCH_3), 1.90 (s, 3 H, $NHCOCH_3$); ^{13}C NMR (300 MHz, $CDCl_3$–CD_3OD): δ 172.22 ($NHCOCH_3$), 102.64, 101.47, 101.47 (C-1, C-1′, and benzylidene), 79.66, 76.39, 74.92, 73.02, 70.42, 70.02, 66.03 (C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 70.02, 68.70 (2 OCH_2), 68.56 (C-6), 61.40 (C-6′), 55.79 (C-2), 55.56 (OCH_3), 29.13, 29.13, 29.05, 29.05, 25.78, 25.54, 22.70 ($NHCOCH_3$); FAB-MS: *m/z* 706 $[M+H]^+$, *m/z* 728 $[M+Na]^+$.

8-*p*-Methoxyphenoxyloctyl 2-acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-β-D-glucopyranoside (1). Compound **19** (50 mg, 0.07 mmol) was dissolved in HOAc:H_2O (1:1) (5 mL) and stirred at 80 °C for 3 h. Co-evaporation with toluene left a residue which was purified on a silica gel ($CHCl_3$:MeOH, 4:1) to provide the disaccharide as white powder (35 mg, 80%). R_f 0.6 ($CHCl_3$:CH_3OH, 4:1); $[\alpha]_D$ −8.5 (0.5; CH_3OH); ^1H NMR (360 MHz, DMSO-d_6): δ 4.39 (d, 1 H, J = 8.3 Hz, H-1), 4.10 (d, 1 H, J = 8.5 Hz, H-1′), 3.65 (s, 3 H, OCH_3), 1.78 (s, 3 H, $NHCOCH_3$); ^{13}C NMR (300 MHz, DMSO-d_6): δ 171.23 ($NHCOCH_3$), 104.44, 100.98 (C-1, C-1′), 84.83, 76.69, 76.09, 73.29, 70.97, 69.28, 68.45 (C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 69.13, 68.67 (2 OCH_2), 61.27, 61.04 (C-6, C-6′), 55.77 (OCH_3), 54.77 (C-2), 29.48, 29.48, 29.30, 29.24, 29.48, 26.01, 25.87, 23.18 ($NHCOCH_3$). FAB-MS: *m/z* 618 $[M+H]^+$, *m/z* 640 $[M+Na]^+$.

8-*p*-Methoxyphenoxyloctyl2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranoside (21). A soln of bromide **16** (540 mg, 1.3 mmol) in dry CH_2Cl_2 (1 mL) was added to a soln of **12** (400 mg, 0.63 mmol), silver trifluoromethanesulfonate (300 mg, 1.36 mmol), and tetramethylurea (0.16 mL, 1.3 mmol) in dry CH_2Cl_2 (30 mL) and powdered 4 Å molecular sieves (2 g) at −20 °C under Ar, and the mixture was stirred for 78 h (rt) in the dark. The mixture was then filtered through a pad of Celite and the filtrate was washed with aq sodium thiosulfate, satd aq $NaHCO_3$, and H_2O prior to drying and evapn. The residual syrup was chromatographed on a silica gel (toluene:EtOAc 1:1) to give a fraction containing disaccharide **20**. Dry MeOH containing NaOMe (3 mL) was added and after 2 h at rt, the soln was neutralized by addition of Dowex

50 (H^+) resin, filtered and concd. The residue purified on silica gel (CH_2Cl_2:EtOAc 1:1) to give the disaccharide **21** as a syrup (370 mg, 74%). R_f 0.5 (CH_2Cl_2:acetone, 1:1); $[\alpha]_D$ 0.66 (*c* 2.0 in $CHCl_3$); ^1H NMR (360 MHz, CD_3OD): δ 4.45 (d, 1 H, J = 8.7 Hz, H-1), 4.38 (d, 1 H, J = 7.7 Hz, H-1'), 3.71 (s, 3 H, OCH_3), 3.45 (m, 1 H, H-2), 1.90 (s, 3 H, $NHCOCH_3$); ^{13}C NMR (300 MHz, CD_3OD): δ 173.09 ($NHCOCH_3$), 104.27, 102.63 (C-1, C-1'), 82.44, 77.53, 77.47, 76.38, 74.89, 73.05, 70.43 (C-3, C-4, C-5, C-2', C-3', C-4', C-5'), 76.47, 74.89 (2 OCH_2), 70.61, 69.61 (2 OCH_2Ph), 69.44 (C-6), 62.99 (C-6'), 56.45 (C-2), 56.12 (OCH_3), 30.64, 30.64, 30.52, 30.38, 27.13, 27.03, 23.04 ($NHCOCH_3$); Anal. calcd for $C_{43}H_{59}NO_{13}$: C, 64.74; H, 7.40; N, 1.76. Found: C, 64.51; H, 7.53; N, 1.71.

8-*p*-Methoxyphenoxyloctyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (20). Compound **21** (100 mg, 0.125 mmol) was dissolved in Ac_2O:pyridine (1:1, 5 mL) at 0 °C and stirred at rt for 1 h. Cold aq $NaHCO_3$ was then added and the mixture was extracted with CH_2Cl_2, dried ($MgSO_4$), and concd. The residue was purified on silica gel (toluene:EtOAc, 1:1) to give **20** as syrup (115 mg, 95%). R_f 0.5 (toluene:acetone 2:1); $[\alpha]_D$ −1.45 (*c* 2.0; $CHCl_3$); ^1H NMR (360 MHz, $CDCl_3$): δ 5.89 (d, 1 H, $J_{NH,1}$ = 8.4 Hz, NH), 4.65 (d, 1 H, J = 9.4 Hz, H-1), 4.50 (d, 1 H, J = 8.7 Hz, H-1'), 3.75 (s, 3 H, OCH_3), 3.40 (m, 1 H, H-2), 2.10, 2.04, 2.02, 2.0, (4 $OCOCH_3$), 1.95 (s, 3 H, $NHCOCH_3$); ^{13}C NMR (300 MHz, $CDCl_3$): δ 169.85 ($NHCOCH_{33}$), 99.91, 99.84 (C-1, C-1'), 76.82, 75.50, 74.28, 70.67, 70.67, 69.33, 66.90 (C-3, C-4, C-5, C-2', C-3', C-4', C-5'), 73.59, 73.22 (2 OCH_2), 69.33, 68.87 (2 OCH_2Ph), 68.67 (C-6), 60.85 (C-6'), 55.39 (OCH_3), 53.39 (C-2), 29.53, 29.53, 29.39, 29.35, 26.03, 25.97, 170.22, 170.02, 170.02, 170.22 (4 $OCOCH_3$), 20.84, 20.84, 20.64, 20.57 (4 $OCOCH_3$), 23.37 ($NHCOCH_3$); Anal. calcd for $C_{51}H_{67}NO_{17}$: C, 63.42; H, 6.94; N, 1.45. Found: C, 63.45; H, 6.98; N, 1.48.

8-*p*-Methoxyphenoxyloctyl 2-acetamido-2-deoxy-4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranoside (2). Compound **21** (100 mg, 0.125 mmol) was dissolved in dry MeOH (5 mL) containing Pd/C (5%, 100 mg) and stirred under an atmospheric pressure of H_2 at rt for 24 h. Filtration of the catalyst followed by evaporation left a glass which was purified on a Iatrobeads ($CHCl_3$:MeOH 4:1) to yield **3** as a white powder (57 mg, 75%). R_f 0.6 ($CHCl_3$:MeOH, 3:1); $[\alpha]_D$ −0.47 (*c* 4.2; DMSO); ^1H NMR (360 MHz, DMSO-d_6): δ 7.72 (d, 1 H, J = 8.2 Hz, NH), 4.58 (d, 1 H, J = 8.4, H-1), 4.28 (d, 1 H, J = 7.6 Hz, H-1'), 3.68 (s, 3 H, OCH_3), 1.76 (s, 3 H, $NHCOCH_3$); ^{13}C NMR (300 MHz, pyridine-d_5): δ 170.18 ($NHCOCH_3$), 105.99, 102.03 (C-1, C-1'), 83.35, 77.26, 76.49, 75.19, 73.69, 72.52, 70.05 (C-3, C-4, C-5, C-2', C-3', C-4', C-5'), 69.39, 68.63 (2 OCH_2), 62.30, 61.98 (C-6, C-6'), 57.09 (C-2), 55.59 (OCH_3), 30.03, 29.67, 29.60, 29.60, 26.27, 26.21, 23.60 ($NHCOCH_3$); FAB-MS: *m/z* 618 $[M+H]^+$, *m/z* 640 $[M+Na]^+$.

8-*p*-Methoxyphenoxyloctyl 2-acetamido-3-*O*-benzyl-2-deoxy-6-*O*-(β-D-galactopyranosyl)-4-*O*-(*p*-methoxybenzyl)-β-D-glucopyranoside (23). A mixture of **15** (500 mg, 0.75 mmol), bromide **16** (460 mg, 1.2 mmol), tetramethylurea (0.155 mL, 1.3 mmol), silver triflate (260 mg, 1.2 mmol), and 4 Å powdered molecular sieve (2 g) in dry CH_2Cl_2 (30 mL) was stirred in the dark at −20 °C for 4 h, then at room temperature for 30 h. The mixture was diluted with CH_2Cl_2, filtered, washed with aq sodium thiosulfate, H_2O, dried (Na_2SO_4) and concd. Purification of the residue on silica gel (toluene:EtOAc, 1:1) yielded a fraction containing **22**. This fraction was deacetylated in NaOMe–MeOH (15 mL) at rt for 2 h, neutralized with Dowex 50(H^+) resin, filtered, and concd. The residue was purified on a silica gel (CH_2Cl_2:EtOAc, 1:1) to yield recovered acceptor **15** (200 mg) and disaccharide **23** (260 mg, 69% based on consumed **15**) as a syrup. R_f 0.55 (CH_2Cl_2:acetone, 1:2); $[\alpha]_D$ −1.4 (*c* 5.0; CH_3OH:$CHCl_3$ 1:1); ^1H NMR (360 MHz, CD_3OD): δ 4.41 (d, 1 H, $J_{1,2}$ = 8.34 Hz, 1-H), 4.20 (d, 1 H, $J_{1',2'}$ 7.67 Hz, H-1'), 3.77, 3.72 (2 s, 6 H, 2 OCH_3),1.86 (s, 3 H, $NHCOCH_3$); ^{13}C NMR (300 MHz, CD_3OD–$CDCl_3$): δ 171.28 ($NHCOCH_3$), 103.57, 100.55 (C-1, C-1'), 80.05, 74.58, 74.10, 72.99, 70.53, 68.32, 74.58 (C-3, C-4, C-5, C-2', C-3', C-4', C-5'), 74.29, 73.91 (2 OCH_2), 68.00, 68.14 (2 OCH_2Ph), 60.65 (C-6 and C-6'), 55.07, 54.86 (2 OCH_3), 54.37 (C-2), 28.88, 28.74, 28.74, 28.74, 25.38, 25.25, 21.81 ($NHCOCH_3$); FAB-MS: *m/z* 828 $[M+H]^+$, *m/z* 850 $[M+Na]^+$.

8-*p*-Methoxyphenoxyloctyl 2-acetamido-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-3-*O*-benzyl-2-deoxy-4-*O*-(*p*-methoxybenzyl)-β-D-glucopyranoside (22). Compound **23** (50 mg, 0.06 mmol) was dissolved in Ac_2O:pyridine (1:1) at 0 °C, then stirred at rt for 2 h. The mixture was added to cold aq $NaHCO_3$, extracted with CH_2Cl_2, dried ($MgSO_4$), and concd. The residue was purified on silica gel (toluene:acetone, 1:1) to yield **22** as a syrup (51 mg, 83%). R_f 0.75 (toluene:acetone, 2:1); $[\alpha]_D$ −2.9 (*c*; 3.0 $CHCl_3$); ^1H NMR (360 MHz, $CDCl_3$): δ 5.73 (d, 1 H, $J_{NH,1}$ = 8 Hz, NH), 4.75 (d, 1 H, J = 9.5 Hz, H-1), 4.61 (d, 1 H, J = 8.0 Hz, H-1'), 3.78, 3.70 (2s, 6 H, 2 OCH_3), 1.89 (s, 3 H, $NHCOCH_3$); ^{13}C NMR (300 MHz, $CDCl_3$): δ 169.18 ($NHCOCH_3$), 101.28, 99.70 (C-1, C-1'), 80.27, 78.52, 74.90, 71.08, 70.70, 68.88, 67.10 (C-3, C-4, C-5, C-2', C-3', C-4', C-5'), 68.65, 61.28 (C-6, C-6'), 69.64, 68.65 (OCH_2Ph and OCH_2PhOMe), 74.64 and 74.23 (OCH_2), 68.52 (C-6), 61.28 (C-6'), 57.10 (C-2), 55.76, 55.32 (2 OCH_3), 29.71, 29.50, 29.38, 29.38, 26.01, 25.94, 23,52 ($NHCOCH_3$); Anal. calcd for $C_{52}H_{69}NO_{18}$; C, 62.71; H, 6.93; N, 1.40. Found: C, 62.45, H, 6.836, N, 1.657.

8-*p*-Methoxyphenoxyloctyl 2-acetamido-2-deoxy-6-*O*-(β-D-galactopyranosyl)-β-D-glucopyranoside (3). A soln of **23** (300 mg, 0.36 mmol) in dry MeOH (50 mL) containing 10% Pd/C (300 mg) was stirred under an atmosphere of H_2 for 24 h at rt. The catalyst was removed on Celite, which was washed with MeOH, EtOH, and EtOH:CH_2Cl_2 (1:1). The combined filtrates and washings were concd and the residue was

purified on silica gel ($CHCl_3$:MeOH, 3:1) to provide **5** as a white powder (155 mg, 70%). R_f 0.6 ($CHCl_3$:MeOH, 4:1); $[\alpha]_D$ −0.94 (*c* 1.2 MeOH); ^1H NMR (360 MHz, CD_3OD–D_2O): δ 4.46 (d, 1 H, J = 8.3 Hz, H-1), 4.21 (d, 1 H, J = 9.2 Hz, H-1′), 3.74 (s, 3 H, OCH_3), 1.98 (s, 3 H, $NHCOCH_3$); ^{13}C NMR (300 MHz, pyridine-d_5): δ 170.64 ($NHCOCH_3$), 105.85, 102.19 (C-1, C-1′), 77.21, 76.94, 76.43, 75.28, 72.56, 72.44, 70.27 (C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 69.99, 69.42 (2 OCH_2), 68.66 (C-6), 62.35 (C-6′), 57.63 (C-2), 55.59 (OCH_3), 30.00, 29.63, 29.56, 29.54, 26.22, 26.22, 23.49 ($NHCOCH_3$); FAB-MS: *m/z* 618 $[M+H]^+$, *m/z* 640 $[M+Na]^+$

8-*p*-Methoxyphenoxyloctyl 2-acetamido-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (24). A suspension of **10** (500 mg, 0.92 mmol), silver carbonate (2 g, 7.2 mmol), silver triflate (0.24 g, 1.09 mmol), and $CaSO_4$ (2 g) in dry CH_2Cl_2 (30 mL) was stirred at −25 °C under Ar for 2 h. Then bromide **17** (2 g) in CH_2Cl_2 (3 mL) was added dropwise, the mixture was stirred in the dark for 3 h at −25 °C then at rt for 12 h, filtered through Celite, washed with aq sodium thiosulfate, aq $NaHCO_3$, and H_2O, then dried ($MgSO_4$), and concd. The residue was chromatographed on silica gel (hexane:EtOAc 3:1) to yield unreacted **10** (110 mg) and a crude disaccharide fraction that was acetylated (Ac_2O:pyridine, 1:1, 2 mL) and the resulting mixture was purified again on silica gel using the same solvent system. Disaccharide **24** was obtained as a white solid (574 mg, 74% based on consumed **10**). R_f 0.53 (toluene:acetone, 2:1); $[\alpha]_D$ 0.97 (*c* 1.0; $CHCl_3$); ^1H NMR (360 MHz, $CDCl_3$): δ 5.75 (d, 1 H, $J_{NH,1}$ = 9.0 Hz, NH), 5.5 (d, 1 H, $J_{1',2'}$ = 3.9 Hz, H-1′), 5.3 (s, 1 H, benzylidene), 4.7 (d, 1 H, $J_{1,2}$ = 8.9 Hz, H-1), 3.7 (s, 3 H, OCH_3), 1.75 (s, 3 H, $NHCOCH_3$); ^{13}C NMR (300 MHz, $CDCl_3$): δ 170.01 ($NHCOCH_3$), 102.48, 101.67 (C-1, and benzylidene), 96.82 (C-1′), 82.34, 78.58, 75.57, 75.10, 74.16, 69.87, 65.97 (C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 73.62, 70.92 69.87, 68.55, (4 OCH_2Ph), 74.26, 73.62, (2 OCH_2), 71.22, 68.55 (C-6, C-6′), 55.66 (OCH_3), 54.49 (C-2), 29.62, 29.36, 29.32, 29.29, 25.95, 25.69, 23.02 ($NHCOCH_3$); Anal. calcd for $C_{64}H_{75}NO_{13}$: C, 72.11; H, 7.04; N, 1.31. Found: C, 71.782; H, 7.15; N, 1.374.

8-*p*-Methoxyphenoxyloctyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(α-D-galactopyranosyl)-β-D-glucopyranoside (25). Compound **24** (300 mg, 0.28 mmol) was hydrogenated in dry MeOH (5 mL) in the presence of 10% Pd/C (300 mg) for 12 h at rt. Filtration and evapn of the solvent yielded the title compound as a syrup (180 mg, 90%). R_f: 0.4 (toluene:acetone, 1:3); $[\alpha]_D$ +1.48 (*c* 4.1; MeOH); ^1H NMR (360 MHz, $CDCl_3$–CD_3OD): δ 5.60 (s, 1 H, benzylidene), 5.41 (d, 1 H, $J_{1',2'}$ = 3.7 Hz, H-1), 4.50 (d, 1 H, $J_{1,2'}$ = 8.2 Hz, H-1), 3.75 (s, 3 H, OCH_3), 3.47 (m, 1 H, H-2), 1.98 (s, 3 H, $NHCOCH_3$); ^{13}C NMR (300 MHz, pyridine-d_5): δ 171.03 ($NHCOCH_3$), 102.84, 101.45 (C-1, C-1′), 101.34 (benzylidene), 83.47, 76.72, 72.88, 71.89, 70.96, 69.38, 66.67 (C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 70.07, 68.95 (2 OCH_2), 69.38, 62.56 (C-6, C-6′), 56.14 (C-2), 55.90 (OCH_3), 30.28, 29.97, 29.91, 29.82, 26.59, 26.50, 23.99 ($NHCOCH_3$); Anal. calcd for $C_{36}H_{51}NO_{13}$: C, 61.28; H, 7.23; N, 1.98. Found: C, 60.999; H, 7.174; N, 1.848.

8-*p*-Methoxyphenoxyloctyl 2-acetamido-2-deoxy-3-*O*-(α-D-galactopyranosyl)-β-D-glucopyranoside (4). Compound **25** (100 mg, 0.14 mmol) was heated to 80 °C in Ac_2O:H_2O (1:1) for 1 h. Repeated co-evaporation with toluene was followed by purification on Iatrobeads (CH_2Cl_2:EtOH, 1:1) to give **2** as a white solid (72 mg, 83%). R_f 0.6 ($CHCl_3$:MeOH, 3:1); $[\alpha]_D$ +4.69 (*c* 5.6; MeOH); ^1H NMR (360 MHz, CD_3OD): δ 5.11 (d, 1 H, $J_{1',2'}$ = 3.9 Hz, H-1′), 4.36 (d, 1 H, $J_{NH,1}$ = 8.3 Hz, H-1), 3.69 (s, 3 H, OCH_3), 3.30 (m, 1 H, H-2), 1.93 (s, 3 H, $NHCOCH_3$); ^{13}C NMR (300 MHz, pyridine-d_5): δ 170.83 ($NHCOCH_3$), 103.69, 102.27 (C-1 and C-1′), 86.12, 77.80, 73.05, 72.03, 71.42, 71.21, 70.86, 69.37 (C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 69.37, 68.59 (2 OCH_2), 62.43, 62.31 (C-6, C-6′), 55.79 (C-2), 55.55 (OCH_3), 29.98, 29.61, 29.55, 29.55, 26.23, 26.18, 23.74 ($NHCOCH_3$); FAB-MS: *m/z* 618 $[M+H]^+$, *m/z* 640 $[M+Na]^+$.

8-*p*-Methoxyphenoxyloctyl 2-acetamido-3,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-2-deoxy-β-D-glucopyranoside (26). A suspension of **12** (500 mg, 0.78 mmol), silver carbonate (2.4 g, 8.73 mmol), and Drierite (2 g) in dry CH_2Cl_2 (20 mL) was stirred at −25 °C under argon in the dark for 2 h. Then bromide **17** (2 g) in CH_2Cl_2 (5 mL) and silver triflate (0.24 g, 1.09 mmol) were added. The mixture was stirred at −25 °C for 4 h, then at rt for 24 h. CH_2Cl_2 was added, the mixture was filtered and the filtrate washed with aq sodium thiosulfate, H_2O, dried ($MgSO_4$), and concd. The residue was chromatographed on silica gel (toluene:EtOAc 3:1) to give a disaccharide-containing fraction and unreacted **12** (145 mg). The disaccharide fraction was acetylated (Ac_2O:pyrisdine, 1:1, 1 mL) and further chromatographed (toluene:EtOAc, 5:1). Compound **26** was obtained as a syrup (505 mg, 78%). R_f 0.6 (toluene:acetone, 5:1); $[\alpha]_D$ +2.4 (*c*; 1.2 $CHCl_3$); ^1H NMR (360 MHz, $CDCl_3$): δ 6.81 (d, 1 H, $J_{NH,2}$ = 7.9 Hz, N*H*), 4.85 (d, 1 H, $J_{1',2'}$ = 3.8 Hz, H-1′), 4.63 (d, 1 H, $J_{1,2}$ = 8.5 Hz, H-1), 3.69 (s, 3 H, OCH_3), 1.56 (s, 3 H, $NHCOCH_3$); ^{13}C NMR (300 MHz, $CDCl_3$): δ 169.86 ($NHCOCH_3$), 99.45, 97.48 (C-1, C-1′), 79.00, 76.04, 74.94, 74.89, 74.69, 72.74, 70.10 (C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 74.33, 74.22, 73.43, 73.01, 71.65, 71.08, 70.59, 69.14, 68.67, 68.41 (6 CH_2Ph, 2 OCH_2, C-6, and C-6′), 55.76 (OCH_3), 49.29 (C-2), 29.57, 29.57, 29.41, 29.41, 26.05, 26.05, 22.80 ($NHCOCH_3$); Anal. calcd for $C_{71}H_{83}NO_{13}$: C, 73.64; H, 7.17; N, 1.21. Found: C, 73.32; H, 7.01; N, 1.27.

8-*p*-Methoxyphenoxyloctyl 2-acetamido-2-deoxy-4-*O*-(α-D-galactopyranosyl)-β-D-glucopyranoside (5). Compound **26** (800 mg, 0.69 mmol) was dissolved in dry MeOH (15 mL) containing 5% Pd/C (800 mg) and

hydrogenated at rt for 30 h. TLC examination showed the presence of a single spot (R_f 0.3 in $CHCl_3$:MeOH, 5:1). The catalyst was removed by filtration on Celite, which was washed with several portions of hot EtOH, the filtrates were concd and the residue purified on silica gel (CH_2Cl_2:EtOH 1:1) to provide **4** as white solid (225 mg, 53%). R_f 0.6 ($CHCl_3$:MeOH, 3:1); $[\alpha]_D$ −9.47 (*c* 4.6; DMSO); ^{1}H NMR (360 MHz, CD_3OD): δ 5.20 (d, 1 H, $J_{1',2'} = 3.9$ Hz, H-1'), 4.40 (d, 1 H, $J_{1,2} = 7.8$ Hz, H-1), 3.70 (s, 3 H, OCH_3), 1.95 (s, 3 H, $NHCOCH_3$); ^{13}C NMR (300 MHz, pyridine-d_5): δ 103.49, 102.16 (C-1, C-1'), 82.02, 76.88, 75.37, 73.63, 71.60, 71.50, 71.03 (C-3, C-4, C-5, C-2', C-3', C-4', C-5'), 69.36, 68.59 (2 OCH_2), 62.69, 62.12 (C-6, C-6'), 57.17 (C-2), 55.56 (OCH_3), 30.08, 30.00, 29.63, 29.57, 26.24, 26.19, 170.51 ($NHCOCH_3$), 23.52 ($NHCOCH_3$); FAB-MS: *m/z* 618 $[M+H]^+$, *m/z* 640 $[M+Na]^+$.

8-*p*-Methoxyphenoxyloctyl 2-acetamido-3-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-2-deoxy-4-*O*-(*p*-methoxybenzyl)-β-D-glucopyranoside (27). A suspension of **15** (500 mg, 0.75 mmol), silver carbonate (2 g, 7.2 mmol), silver triflate (240 mg, 1.09 mmol), and $CaSO_4$ (2 g) in dry CH_2Cl_2 (30 mL) was stirred at −25 °C under argon, then **17** (2 g) in CH_2Cl_2 was added dropwise. The mixture was stirred in the dark for 3 h at −25 °C, then at rt for 12 h, filtered through Celite, washed with aq sodium thiosulfate, aq $NaHCO_3$, and H_2O. After drying ($MgSO_4$), the solvent was evapd and the residue was purified on silica gel (toluene:EtOAc, 3:1). Unreacted **17** (170 mg) was recovered along with an impure disaccharide fraction which was acetylated as described above. Disaccharide **27** was obtained after chromatography on silica gel (toluene:EtOAc, 5:1) as a white solid (440 mg, 74.7%). R_f 0.55 (toluene:acetone, 5:1); $[\alpha]_D$ +3.12 (*c* 5.9 in $CHCl_3$); ^{1}H NMR (360 MHz, $CDCl_3$): δ 6.85 (d, 1 H, $J_{NH,2} = 9.0$ Hz, N*H*), 4.93 (d, 1 H, $J_{1',2'} = 3.71$ Hz, H-1'), 4.68 (d, 1 H, $J = 9.0$ Hz, H-1), 3.76 (s, 3 H, OCH_3), 3.73 (s, 3 H, OCH_3), 1.63 (s, 3 H, $NHCOCH_3$); ^{13}C NMR (300 MHz, $CDCl_3$): δ 169.90 ($NHCOCH_3$), 99.49, 97.48 (C-1, C-1'), 79.02, 76.05, 75.00, 74.92, 74.71, 70.11, 71.66 (C-3, C-4, C-5, C-2', C-3', C-4', C-5'), 74.41, 74.20, 73.44, 72.70, 71.09, 70.22, 70.11, 69.17, 68.70, 68.43 (6 OCH_2Ph, 2 OCH_2, C-6, C-6'), 55.79, 55.24 (2 OCH_3), 49.35 (C-2), 29.60, 29.60, 29.60, 29.44, 29.09, 29.09, 22.83 ($NHCOCH_3$); Anal. calcd for $C_{72}H_{85}NO_{14}$: C, 72,78; H, 7.25; N, 1.28. Found: C, 72.496; H, 7.238; N, 1.28.

8-*p*-Methoxyphenoxyloctyl 2-acetamido-2-deoxy-6-*O*-(α-D-galactopyranosyl)-β-D-glucopyranoside (6). A soln of **27** (750 mg, 0.63 mmol) in dry MeOH (40 mL) containing 10% Pd/C (750 mg) was hydrogenated at rt for 42 h. After filtration and concn, the residue was purified on Iatrobeads (CH_2Cl_2:EtOH 1:1) to provide **6** as a syrup (280 mg, 72%). R_f 0.55 ($CHCl_3$:CH_3OH, 3:1); $[\alpha]_D$ +3.68 (*c* 1.9; MeOH); ^{1}H NMR (360 MHz, CD_3OD): δ 4.87 (d, 1 H, $J_{1',2'} = 1.2$ Hz, H-1'), 4.42 (d, 1 H, $J_{1,2} = 8.4$ Hz, H-1), 3.72 (s, 3 H, OCH_3), 3.40 (m, 1 H, H-2), 1.96 (s, 3 H, $NHCOCH_3$); ^{13}C NMR (300 MHz, CD_3OD): δ 173.62 ($NHCOCH_3$), 102.95, 100.07 (C-1, C-1'), 76.71, 76.23, 75.96, 74.93, 73.46, 72.53, 72.08 (C-3, C-4, C-5, C-2', C-3', C-4', C-5'), 70.72, 69.61 (2 OCH_2), 67.10 (C-6), 62.50 (C-6'), 57.42 (C-2), 56.13 (OCH_3), 30.64, 30.64, 30.51, 30.51, 27.13, 27.03, 23.09 ($NHCOCH_3$); FAB-MS: *m/z* 618 $(M+H^+)$, *m/z* 640 $[M+Na]^+$.

General procedure for random galactosylation

Glycoside **9** (360 mg) was dissolved in the solvent indicated in Table 1 and the donor (2 equiv) was added. The temperature was adjusted to the indicated temperature and the promoter (0.1 or 0.2 equiv) in CH_2Cl_2 (0.05 mL) was added. After the indicated time, 3 drops of triethylamine were added to quench the reaction, which was then concentrated to dryness. In the case of entries 1 and 2, the residue was dissolved in MeOH and hydrogenated over 10% Pd/C for 16 h. After filtration and evaporation, the residue was dissolved in H_2O and loaded on C-18 column (5–10 mL), which was eluted with water to remove material not attached to the hydrophobic aglycone. Elution with methanol gave the unreacted acceptor and disaccharide mixture for HPLC analysis. In the case of entries 3–7, the hydrogenation step was replaced by a treatment with NaOMe–MeOH to effect de-O-acetylation. The reaction mixture was then neutralized with Dowex 50 (H^+), the resin was removed by filtration and the MeOH evapd prior to the C-18 column. In the case of entry 1, the reaction was performed in the presence of $CaSO_4$ (150 mg). In the case of entry 2, the reaction was performed in the presence of 4A molecular sieves (300 mg).

^{1}H NMR spectroscopy of oligosaccharide mixtures was performed at 500 MHz in CD_3OD at 45 °C. Under these conditions, signals for the anomeric protons are: **1** (δ 4.48, $J = 8.1$ Hz) and (δ 4.25, $J = 7.6$ Hz); **2** (δ 4.38, $J = 7.6$ Hz, 4.37, $J = 7.6$ Hz); **3** (δ 4.40, $J = 8.4$ Hz) and (δ 4.32, $J = 7.0$ Hz); **4** (δ 5.15, $J = 3.8$ Hz) and (δ 4.43, $J = 8.4$ Hz); **5** (δ 5.20, $J = 4.0$ Hz) and (δ 4.41, $J = 8.2$ Hz); **6** (δ 4.87, $J = 3.2$ Hz) and (δ 4.43, $J = 8.5$ Hz). All of the glycosides used in the present work displayed a sharp four-proton singlet near 6.8 ppm whether in $CDCl_3$, DMSO-d_6 or CD_3OD.

HPLC analysis of oligosaccharide mixtures was performed on a Dionex HPLC system with UV-monitoring at 92 nm. A Waters partisphere PAC column (4.6 × 250 mm) was used with CH_3CN:H_2O (94:6) as eluent at a flow rate of 1 mL/min.

Acknowledgements

The authors thank Dr. Beat Ernst for a gift of *p*-methoxyphenoxyloctanol and Dr. Albin Otter for recording the 500 MHz NMR spectra of the disaccharide mixtures. This work was supported by Ciba–CANADA.

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(*Received in U.S.A.* 12 *September* 1995; *accepted* 14 *December* 1995)