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Spacer-modified trisaccharide glycosides that mimic the biantennary Asn-linked oligosaccharide acceptor of (1 → 4)-β-D-galactosyltransferase and can be used as competitive inhibitors and for irreversible deactivation

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ABSTRACT

The biantennary spacer-modified trisaccharide glycoside methyl 3,6-di-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyloxyethyl)-α-D-mannopyranoside (**5**) was synthesised and used together with several 2-acylamino-2-deoxy-D-glucose derivatives in competition experiments with β-D-galactosyltransferase. Compound **5** was an acceptor substrate (K_M 0.18 mM) comparable to the biantennary core heptasaccharide of glycoproteins (K_M 0.13 mM). Replacing the *N*-acetyl group by other *N*-acyl groups did not alter the kinetic parameters significantly. When the *N*-acyl group was iodoacetyl, the compound was an irreversible inhibitor.

INTRODUCTION

We have described[1] syntheses and applications of spacer-modified disaccharides as acceptors and photoaffinity reagents for the binding site of bovine (1 → 4)-β-D-galactosyltransferase.

Spacer-modified oligosaccharides, i.e., molecules where parts of the native oligosaccharide have been replaced by flexible spacers, can be recognised by the corresponding receptor proteins[2] and can be used as competitive inhibitors of natural-ligand binding. The spacer can accommodate photolabile or chemically reactive groups that can react irreversibly at the receptor-binding site. Following the concept[3] of "shorthand synthesis", two mannose residues in the terminal pentasaccharide moiety of the heptasaccharide **1** have been replaced by equivalent flexible spacers. The resulting spacer-modified trisaccharide glycoside **4** should be an asymmetrical acceptor for possible branch-specific galactosylation as demon-

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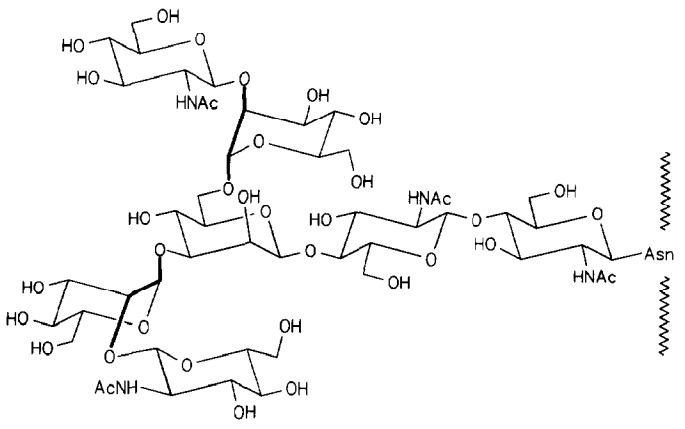

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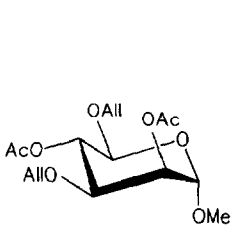
strated for a biantennary oligosaccharide[4] and its corresponding Asn-linked glycoprotein[5]. Thus, it may be possible to label the acceptor-binding site of (1 → 4)-β-D-galactosyltransferase preferentially with one branch of a suitable spacer-modified oligosaccharide.

RESULTS AND DISCUSSION

Syntheses.—Whereas the central 3,6-linked Man in **1** determines the branch specificity of galactosylation, the flanking 3- and 6-linked Man form equivalent bridges and so differentiate only the two terminal GlcNAc groups. It is assumed that the 3- and 6-linked Man can be replaced by C_2 bridging segments corresponding to C-1,2 (emphasised in **1**) without loss of regioselectivity as an acceptor for galactosylation. Starting with methyl α-D-mannopyranoside, the 3- and 6-positions were *O*-allylated with allyl bromide via the 3,6-distannylated intermediate[6]. After acetylation of HO-2,4 (→ **2**), the allyl groups were ozonolysed and the product was reduced with sodium borohydride to yield the diol **3**. Silver triflate-catalysed glycosylation of **3** with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide[7] gave **4**. Removal of the acetyl and phthalimido groups from **4** with butylamine followed by *N*-acylation with various acid anhydrides gave the spacer-modified oligosaccharide glycosides **5**–**9**. For purposes of comparison, the 2-acylamino-2-deoxy-D-glucose derivatives **10**–**12**[8] were also prepared. Enzymic galactosylation of **5** gave two isomeric mono-*O*-β-D-galactopyranosyl derivatives (**13a,b**), which were not separated, and also the di-*O*-β-D-galactosylated derivative **14**.

Galactosylation experiments.—Each of the mono- and oligo-saccharide derivatives could be galactosylated by UDP-Gal and bovine (1 → 4)-β-D-galactosyltransferase (Table I), although it has been claimed that changing the *N*-acetyl group



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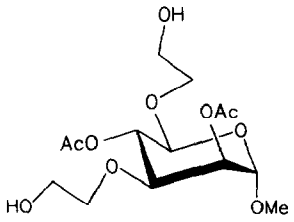

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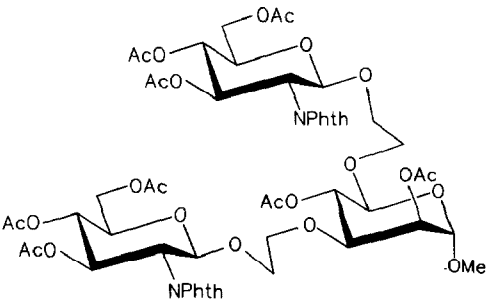

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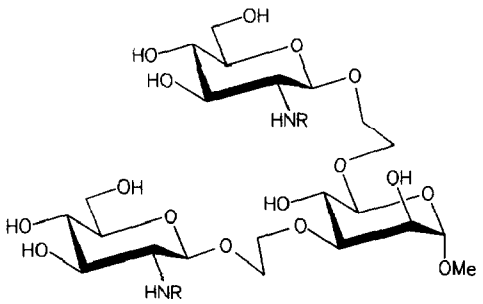
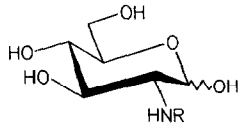
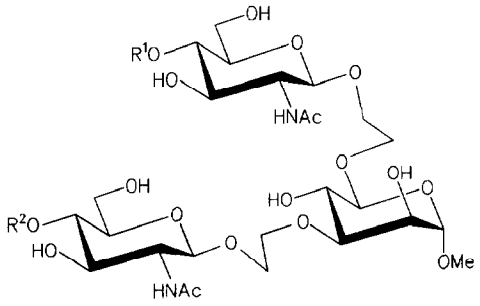


5 R = Ac
6 R = $COCH_2I$
7 R = COPr
8 R = COBu
9 R = COPen



10 R = COPr
11 R = COBu
12 R = COPen



13a R^1 = H, R^2 = β-D-Gal*p*

13b R^1 = β-D-Gal*p*, R^2 = H

14 R^1 = R^2 = β-D-Gal*p*

would prevent galactosylation[9]. The acceptor properties of the *N*-acetyl derivative **5** (K_M 0.18 mM) are similar to those of a glycoprotein (K_M 0.13 mM)[5] or a glycopeptide (K_M 0.25 mM)[10], but significantly different from those of GlcNAc (K_M 8.3 mM)[11] and of a monogalactosylated oligosaccharide (K_M 2.0 mM)[10].

The first GlcNAc in **5** reacts much faster than the second. In a competition experiment that involved equivalent amounts of **5** and the monogalactosylated product **13a,b**, the former was galactosylated approximately four times faster than the latter (Fig. 1). This result agrees well with previous findings[1,4,5,10,12].

Exchanging the NAc groups in **5** for other acyl groups (**7–9**) affected the rates of galactosylation only slightly, as found in competition experiments (Table I). Only a species with a very different structure, such as the spacer-modified disaccharide 1,10-di-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-azi-1,10-decanediol (**15**), showed a significant rate enhancement, which may be caused by its increased

TABLE I

Rates of galactosylation relative to that of **5** [a]

Acceptor	Relative rates [a]
5	1
13a,b	0.26
7	0.8
8	0.85
9	1.43
GlcNAc	0.06
15	11

[a] Determined in competition experiments.

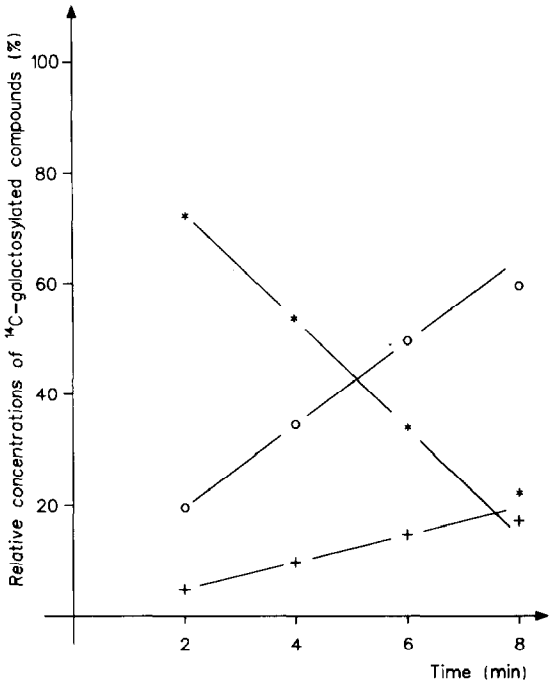
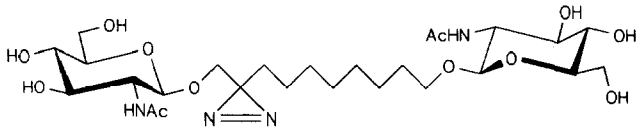


Fig. 1. Competition for galactosylation by UDP-[^{14}C]Gal and galactosyltransferase between equivalent amounts of **13a,b** (+) and **5** (○) at 0.5 mM (*, UDP-[^{14}C]Gal).

hydrophobic interaction with the enzyme. Also, the slightly higher rate of galactosylation for the *N*-hexanoyl derivative **9** can be explained by this effect. The sensitivity of galactosyltransferase in this respect is well known[9].



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When (1 → 4)-β-D-galactosyltransferase was preincubated with the *N*-iodoacetyl derivative **6**, the enzyme was deactivated irreversibly (Fig. 2). Deactivation was prevented in the presence of the competing acceptor **15**. Therefore, it is assumed that **6** and **15** occupy the same binding site of the enzyme. Kinetic investigation also with (1 → 4)-β-D-galactosyltransferases of various origins[4,5,10,12] indicated the existence of a separate cooperative binding site for the second GlcNAc moiety. Studies are in progress to clarify this point.

EXPERIMENTAL

General methods.—All reactions were monitored by TLC on Silica Gel 60 F_{254} (Merck). Radioactivity on TLC plates was scanned with a Berthold TLC-Linear-

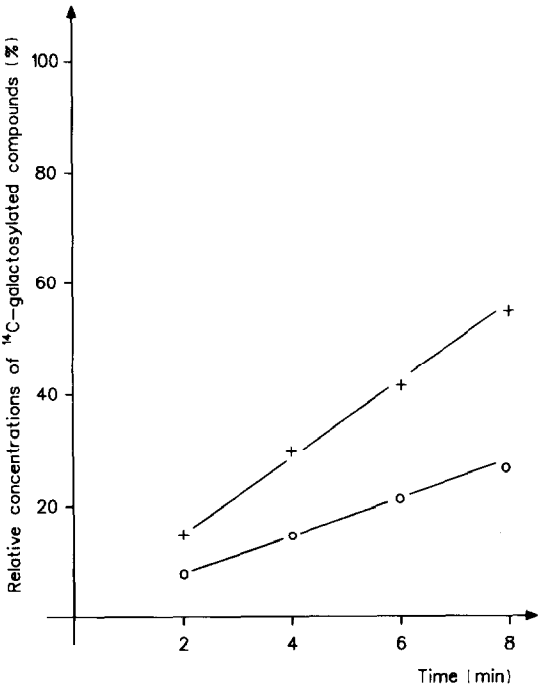

Fig. 2. Irreversible deactivation of galactosyltransferase by **6**: O, enzyme was preincubated with **6** for 60 min, then UDP-[^{14}C]Gal and **5** were added; +, enzyme was protected by **15** during 60-min preincubation with **6**. The reference reaction (enzyme and **5** were stored for 60 min, then UDP-[^{14}C]Gal was added) gave the same results as (+).

Analyser LB 282. Flash-column chromatography[13] was performed on Silica 32–63, 60A (ICN Biomedicals). Melting points were measured with a Büchi melting-point apparatus and are uncorrected. Optical rotations were obtained with a Schmidt & Haensch Polartronic I polarimeter. ^{1}H NMR spectra were recorded with Bruker WM 250 and 400 spectrometers for solutions in $CDCl_3$ and CD_3OD (internal Me_4Si). Acetylation was effected[14] with pyridine–Ac_2O. Ozonolyses were carried out with a Fischer ozone generator 500 M. Elemental analyses were obtained with a Perkin–Elmer 240 analyser. Size-exclusion chromatography was performed on Bio-Gel P-2 (−400 mesh, Bio-Rad).

Materials.—UDP-[^{14}C]Gal (300 mCi/mmol, Amersham-Buchler), UDP-Gal (Fluka), UDP-Glc (Sigma), β-D-galactosyltransferase [UDP-galactose: *N*-acetylglucosamine 4-β-D-galactosyltransferase; EC 2.4.1.22] from bovine milk (4 U/mg, Sigma), and UDP-galactose 4-epimerase (EC 5.1.3.2) from galactose-adapted yeast (10 U/mg, Sigma) were used.

Methyl 2,4-di-O-acetyl-3,6-di-O-allyl-α-D-mannopyranoside (**2**).—A solution of methyl 3,6-di-*O*-allyl-α-D-mannopyranoside[6] (10.0 g, 36.45 mmol) in pyridine (300 mL) and Ac_2O (150 mL) was left overnight, then concentrated in vacuo, and toluene (2 × 50 mL) was evaporated from the residue. Flash-column chromatogra-

phy (1:5 cyclohexane–EtOAc) then yielded **2** (11.2 g, 86%), isolated as a colourless syrup; $[\alpha]_D^{23}$ +38° (*c* 1, $CHCl_3$); R_F (1:3 cyclohexane–EtOAc) 0.23. ^1H NMR data (250 MHz, $CDCl_3$): δ 5.85 (m, 2 H, allyl), 5.32–5.10 (m, 6 H, H-2,4 and allyl), 4.71 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 4.14–3.76 (m, 6 H, H-3,5 and allyl), 3.54 (2 s, 2 H, H-6a,6b), 3.40 (s, 3 H, OMe), 2.14 (s, 3 H, Ac), 2.07 (s, 3 H, Ac).

Anal. Calcd for $C_{17}H_{26}O_8$: C, 56.97; H, 7.31. Found: C, 56.74; H, 7.27.

*Methyl 2,4-di-*O*-acetyl-3,6-di-*O*-hydroxyethyl-α-*D*-mannopyranoside* (**3**).—Ozone (30 L/h O_2, 10 mmol O_3/h) was bubbled through a solution of **2** (10.0 g, 27.9 mmol) in MeOH (300 mL) and molecular sieves (5 g, 4A) at −78° until a blue colour persisted (~3 h). The excess of O_3 was removed with a stream of O_2, $NaBH_4$ (3.17 g, 83.7 mmol) was added portionwise, and the solution was allowed to attain room temperature, then concentrated to dryness in vacuo. Flash-column chromatography (1:3 cyclohexane–EtOAc) of the residue gave **3**, isolated as a colourless syrup (8.7 g, 85%); R_F 0.4 (2:1 cyclohexane–EtOAc), $[\alpha]_D^{23}$ +31.5° (*c* 1, MeOH). ^1H NMR data (250 MHz, $CDCl_3$): δ 5.30 (dd, 1 H, H-2), 5.26 (t, 1 H, H-4), 4.73 (d, 1 H, H-1), 3.81 (ddd, 1 H, H-5), 3.80 (dd, 1 H, H-3), 3.75–3.49 (m, 10 H, H-6a,6b and spacer), 2.83 (s, 1 H, OH), 2.70 (s, 1 H, OH), 2.16 (s, 3 H, Ac), 2.11 (s, 3 H, Ac); $J_{1,2}$ 1.8, $J_{2,3}$ 3.3, $J_{3,4}$ 9.9, $J_{4,5}$ 9.9 Hz.

Anal. Calcd for $C_{15}H_{26}O_{10}$: C, 49.18; H, 7.15. Found: C, 49.35; H, 7.41.

*Methyl 2,4-di-*O*-acetyl-3,6-di-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyloxyethyl)-α-*D*-mannopyranoside* (**4**).—A solution of silver triflate (5 g, 19.46 mmol), *sym*-collidine (2.6 mL, 19.46 mmol), and **3** (3.54 g, 8.85 mmol) in CH_2Cl_2 (100 mL) was stirred at room temperature for 1 h with powdered molecular sieves (3 g, 4A), then cooled to −20°. A solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide[7] (9.7 g, 19.46 mmol) in CH_2Cl_2 (150 mL) was added during 30 min, and the mixture was allowed to attain 25°, then stirred for 8 h, filtered through Celite, and concentrated in vacuo. Flash-column chromatography (1:2 cyclohexane–EtOAc) of the residue and crystallisation from ether yielded **4** (8.4 g, 76%); R_F 0.18 (1:2 cyclohexane–EtOAc); mp 140°; $[\alpha]_D^{23}$ +33° (*c* 1, $CHCl_3$). ^1H NMR data (250 MHz, $CDCl_3$): δ 7.80 (m, 8 H, Phth), 5.78 (dd, 1 H, Glc H-3), 5.75 (dd, 1 H, Glc H-3), 5.44 (d, 1 H, Glc H-1), 5.42 (d, 1 H, Glc H-1), 5.18 (t, 1 H, Glc H-4), 5.17 (t, 1 H, Glc H-4), 5.14 (dd, 1 H, Man H-2), 4.78 (t, 1 H, Man H-4), 4.52 (d, 1 H, Man H-1), 4.39–4.27 (m, 4 H, Glc H-2,2,6a,6a), 5.17 (dt, 2 H, Glc H-6b,6b), 3.93–3.82 (m, 4 H, Glc H-5,5, Man H-5,6b), 3.68 (dd, 2 H, Man H-4,6a), 3.60–3.45 (m, 6 H, spacer), 3.33–3.10 (m, 2 H, spacer), 3.25 (s, 3 H, OMe), 2.15–1.87 (4 s, 24 H, 8 Ac); Glc, $J_{1,2}$ 8.25, $J_{2,3}$ 10.5, $J_{3,4}$ 10.0, $J_{4,5}$ 10.0, $J_{5,6a}$ 3.9, $J_{5,6b}$ 2.4, $J_{6a,6b}$ 12.6 Hz; Man, $J_{1,2}$ 1.65, $J_{2,3}$ 3.3, $J_{3,4}$ 9.75, $J_{4,5}$ 9.75, $J_{5,6a}$ 3.3, $J_{5,6b}$ 1.9, $J_{6a,6b}$ 9.75 Hz.

Anal. Calcd for $C_{55}H_{64}N_2O_{18}$: C, 55.00; H, 5.37; N, 2.33. Found: C, 55.05; H, 5.62; N, 2.67.

*Methyl 3,6-di-*O*-(2-acetamido-2-deoxy-β-*D*-glucopyranosyloxyethyl)-α-*D*-mannopyranoside* (**5**).—A solution of **4** (1.2 g, 0.88 mmol) in $BuNH_2$–EtOH (2:1, 50 mL) was boiled under reflux overnight, then concentrated in vacuo. A solution of

the residue in H_2O (100 mL) was washed with EtOAc (2 × 30 mL), then passed through a column of Dowex 1X2 (HO^-) resin (100–200 mesh, 60 mL), and freeze-dried. A solution of the resulting amorphous amine in MeOH (50 mL) was treated with Ac_2O (269 mg, 2.64 mmol) and pyridine (0.5 mL) for 1 h, then concentrated in vacuo. Flash-column chromatography (5:2:1 EtOAc–MeOH–H_2O) of the residue gave the product, a solution of which in H_2O (20 mL) was freeze-dried to yield colourless amorphous **5** (400 mg, 66%); R_F 0.31 (3:2:1 EtOAc–MeOH–H_2O); $[\alpha]_D^{23}$ −2° (*c* 1, H_2O). ^1H NMR data (400 MHz, CD_3OD): δ 4.63 (d, 1 H, $J_{1,2}$ 1.8 Hz, Man H-1), 4.51 (2 d, 2 H, $J_{1,2}$ 8.7 Hz, Glc H-1,1), 3.29 (s, 3 H, OMe), 1.92 (2 s, 6 H, 2 Ac).

Anal. Calcd for $C_{27}H_{28}N_2O_{18}$: C, 47.09; H, 7.02; N, 4.03. Found: C, 47.07; H, 7.04; N, 4.03.

*Methyl 3,6-di-*O*-(2-deoxy-2-iodoacetamido-β-*D*-glucopyranosyloxyethyl)-α-*D*-mannopyranoside* (**6**).—Treatment of **4** (1 g, 0.73 mmol), as described above, and acylation of the amine with iodoacetic anhydride (775 mg, 2.19 mmol) gave amorphous **6** (360 mg, 52%); R_F 0.45 (4:2:1 EtOAc–MeOH–H_2O); $[\alpha]_D^{23}$ +1° (*c* 1, H_2O). ^1H NMR data (400 MHz, CD_3OD): δ 4.78 (d, 1 H, $J_{1,2}$ 1.8 Hz, Man H-1), 4.61 (t, 2 H, $J_{1,2}$ 8.7 Hz, Glc H-1,1), 3.41 (s, 3 H, OMe), 2.06 (s, 4 H, 2 $COCH_2I$).

Anal. Calcd for $C_{27}H_{46}I_2N_2O_{18}$: C, 34.48; H, 4.93; N, 2.98. Found: C, 34.50; H, 4.89; N, 3.00.

*Methyl 3,6-di-*O*-(2-butyramido-2-deoxy-β-*D*-glucopyranosyloxyethyl)-α-*D*-mannopyranoside* (**7**).—Treatment of **4** (1 g, 0.73 mmol), as described above, and acylation of the amine with butyric anhydride (346 mg, 2.19 mmol) gave amorphous **7** (355 mg, 65%); R_F 0.39 (4:2:1 EtOAc–MeOH–H_2O); $[\alpha]_D^{23}$ −4° (*c* 1, H_2O). ^1H NMR data (400 MHz, CD_3OD): δ 4.78 (d, 1 H, $J_{1,2}$ 1.8 Hz, Man H-1), 4.61 (2 d, 2 H, $J_{1,2}$ 8.7 Hz, Glc H-1,1), 3.41 (s, 3 H, OMe), 2.26 (2 t, 4 H, PrCO), 1.62 (m, 4 H, PrCO), 0.92 (2 t, 6 H, PrCO).

Anal. Calcd for $C_{31}H_{56}N_2O_{18}$: C, 49.99; H, 7.58; N, 3.76. Found: C, 49.53; H, 7.47; N, 3.76.

*Methyl 3,6-di-*O*-(2-deoxy-2-valeramido-β-*D*-glucopyranosyloxyethyl)-α-*D*-mannopyranoside* (**8**).—Treatment of **4** (1 g, 0.73 mmol), as described above, and acylation of the amine with valeric anhydride (408 mg, 2.19 mmol) gave amorphous **8** (340 mg, 58%); R_F 0.49 (4:2:1 EtOAc–MeOH–H_2O); $[\alpha]_D^{23}$ −1° (*c* 1, H_2O). ^1H NMR data (400 MHz, CD_3OD): δ 4.76 (d, 1 H, $J_{1,2}$ 1.8 Hz, Man H-1), 4.61 (2 d, 2 H, $J_{1,2}$ 8.7 Hz, Glc H-1,1), 3.39 (s, 3 H, OMe), 2.29 (m, 4 H, BuCO), 1.59 (m, 4 H, BuCO), 1.37 (m, 4 H, BuCO), 0.92 (2 t, 6 H, BuCO).

Anal. Calcd for $C_{33}H_{60}N_2O_{18}$: C, 51.29; H, 7.83; N, 3.62. Found: C, 50.98; H, 7.81; N, 3.60.

*Methyl 3,6-di-O-(2-deoxy-2-hexanoylamino-β-*D*-glucopyranosyloxyethyl)-α-*D*-mannopyranoside* (**9**).—Treatment of **4** (1 g, 0.73 mmol), as described above, and acylation of the amine with hexanoic anhydride (469 mg, 2.19 mmol) gave amorphous **9** (345 mg, 59%); R_F 0.55 (4:2:1 EtOAc–MeOH–H_2O); $[\alpha]_D^{23}$ ~0° (*c* 1, H_2O). ^1H NMR data (400 MHz, CD_3OD): δ 4.72 (d, 1 H, $J_{1,2}$ 1.8 Hz, Man H-1),

4.61 (2 d, 2 H, $J_{1,2}$ 8.7 Hz, Glc H-1,1), 3.39 (s, 3 H, OMe), 2.25 (m, 4 H, PenCO), 1.65 (m, 4 H, PenCO), 1.36 (m, 8 H, PenCO), 0.93 (2 t, 6 H, PenCO).

Anal. Calcd for $C_{35}H_{64}N_2O_{18}$: C, 52.49; H, 8.05; N, 3.50. Found: C, 52.20; H, 8.06; N, 3.72.

2-Deoxy-2-valeramido-α-D-glucopyranose (**11**).—2-Amino-2-deoxy-D-glucose hydrochloride (5 g, 23.2 mmol) was added to methanolic 0.1 M NaOMe (232 mL). The mixture was stirred for 5 min, and pyridine (1 mL) and then valeric anhydride (6.5 g, 34.8 mmol) were added. The clear solution became turbid within 10 min. After complete crystallisation (4°, 1 h), the product was collected and recrystallised (EtOH) to yield **11** (5.2 g, 85%); R_F 0.61 (4:2:1 EtOAc–MeOH–H_2O); mp 198°; $[\alpha]_D^{23}$ +32° (equil.; *c* 1, H_2O). ^1H NMR data (250 MHz, $CDCl_3$) of the α,β-tetraacetate: δ 6.19 (d, 0.5 H, H-1α), 5.70 (d, 0.5 H, H-1β), 5.57 (d, 1 H, NH), 5.31–5.08 (m, 2 H, H-3,4), 4.49 (m, 1 H, H-2), 4.27 (dd, 1 H, H-6a), 4.13 (dd, 1 H, H-6b), 4.01 (ddd, 0.5 H, H-5α), 3.81 (ddd, 0.5 H, H-5β), 2.22–1.92 (5 s, 14 H, 4 Ac and BuCO), 1.55 (m, 2 H, BuCO), 1.38 (m, 2 H, BuCO), 0.88 (t, 3 H, BuCO); $J_{1,2\alpha}$ 3.45, $J_{1,2\beta}$ 8.85, $J_{2,NH}$ 9.3, $J_{4,5}$ 9.75, $J_{5,6a}$ 4.0, $J_{5,6b}$ 2.4 Hz.

Anal. Calcd for $C_{11}H_{21}NO_6$: C, 50.18; H, 8.04; N, 5.32. Found: C, 49.99; H, 7.95; N, 5.27.

Galactosylation by UDP-[^{14}C]Gal and β-D-galactosyltransferase from bovine milk.—The standard incubation mixtures contained 0.05 M Tris-HCl (pH 7.4, plus 10 mM $MnCl_2$, 150 mM NaCl, and 0.4% of Triton X-100), 0.2 mM UDP-Gal, UDP-[^{14}C]Gal (0.25 μCi/mmol) {for radioactive assays}, mM acceptor (total), and 0.25 μg (1 mU) of enzyme in a total volume of 60 μL at 37°. For weak acceptors, the concentration was increased appropriately (up to 20-fold) in order to enhance the accuracy. Galactosylation was monitored by TLC (radioactivity was detected by scanning) and the R_F values (4:2:1 EtOAc–MeOH–H_2O) are given in Table II.

Preparative syntheses of methyl-6-O-(2-acetamido-2-deoxy-β-D-glucopyranosyloxyethyl)-3-O-[O-β-D-galactopyranosyl-(1 → 4)-(2-acetamido-2-deoxy-β-D-glucopyranosyloxyethyl)-α-D-mannopyranoside (**13a**), *methyl-3-O-(2-acetamido-2-deoxy-β-D-glucopyranosyloxyethyl)-6-O-[O-β-D-galactopyranosyl-(1 → 4)-(2-acetamido-2-deoxy-β-D-glucopyranosyloxyethyl)]-α-D-mannopyranoside* (**13b**), *and methyl 3,6-di-O-[O-β-D-galactopyranosyl-(1 → 4)-(2-acetamido-2-deoxy-β-D-glucopyranosyloxyethyl)]-α-D-mannopyranoside* (**14**).—A mixture of **5** (20.7 mg, 30 μmol), UDP-glucose (36.6 mg, 60 μmol), UDP-galactose-4-epimerase (0.1 mg, 1 U), and galactosyltransferase (0.067 mg, 0.25 U) was incubated at 37° in the aforementioned buffer (1 mL

TABLE II

The R_F values of acceptors **5** and **7–12**

	Acceptor						
	5	**7**	**8**	**9**	**10**[8]	**11**	**12**[8]
Mono-*O*-galactosylated	0.14	0.26	0.35	0.41	0.41	0.47	0.50
Di-*O*-galactosylated	0.08	0.18	0.23	0.28			

total volume). After 48 h, UDP and UDP-galactose were removed using Dowex 1X2 (Cl^-) resin (100–200 mesh, 5 mL) with H_2O as the eluent. The eluate, which contained the galactosylated products and **5**, was concentrated under reduced pressure to ~0.5 mL, applied to a column (2.6 × 85 cm) of Bio-Gel P-2, and eluted with H_2O at 8 mL/h. The appropriate fractions were freeze-dried to yield **13a,b** (9.7 mg, 38%), **14** (7.3 mg, 24%), and **5** (3.7 mg, 18%).

Irreversible deactivation of β-D-galactosyltransferase by **6**.—Galactosyltransferase (0.25 μg, 1 mU) was incubated with **6** (0.75 μg, 0.8 nmol) in the aforementioned buffer (40 μL) at 37°. After 1 h, a solution of **5** (0.055 mg, 0.08 μmol) and UDP-[^{14}C]Gal (9.76 μg, 0.016 μmol, 0.25 μCi/mmol) in buffer (40 μL) were added and the rate of galactosylation was monitored by TLC. In a parallel experiment, the competitive inhibitor **15** (0.048 mg, 0.08 μmol) was added to the enzyme together with **6**. After 1 h, galactosylation was started by adding UDP-[^{14}C]Gal and monitored as described above.

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REFERENCES

1 S.-C. Áts, J. Lehmann, and S. Petry, *Carbohydr. Res.*, 233 (1992) 125–139.
2 J. Lehmann and L. Ziser, *Carbohydr. Res.*, 205 (1990) 93–103; C.P.J. Glaudemans, J. Lehmann, and M. Scheuring, *Angew. Chem. Int. Ed. Engl.*, 28 (1989) 1669–1670; S. Jegge and J. Lehmann, *Carbohydr. Res.*, 133 (1984) 247–254.
3 Y.C. Lee, *Ciba Found. Symp.*, 145 (1989) 80–95.
4 W.M. Blanken, A. van Vliet, and D.H. van den Eijnden, *J. Biol. Chem.*, 259 (1984) 15131–15135.
5 M.R. Paquet, S. Narasimhan, H. Schachter, and M.A. Moscarello, *J. Biol. Chem.*, (1984) 4716–4721.
6 T. Ogawa, K. Katano, K. Sasajima, and M. Matsui, *Tetrahedron*, 37 (1981) 2779–2786.
7 R.U. Lemieux, T. Takeda, and B.Y. Chung, *ACS Symp. Ser.*, 39 (1976) 90–115.
8 Y. Inouye, K. Onodera, S. Kitaoka, and H. Shigehiro, *J. Am. Chem. Soc.*, 78 (1956) 4722–4724.
9 C.R. Geren, S.C. Magee, and K.E. Ebner, *Arch. Biochem. Biophys.*, 172 (1976) 149–155.
10 A.K. Rao and J. Mendecino, *Biochemistry*, 17 (1978) 5632–5637.
11 F.L. Schanbacher and K.E. Ebner, *J. Biol. Chem.*, 245 (1970) 5057–5061.
12 S. Fujii, T. Nishiura, A. Nishikawa, R. Miura and N. Taniguchi, *J. Biol. Chem.*, 265 (1990) 6009–6018.
13 M.C. Still, M. Khan, and A. Mitra, *J. Org. Chem.*, 43 (1978) 2923–2925.
14 M.L. Wolfrom and A. Thomson, *Methods Carbohydr. Chem.*, 2 (1963) 211–215.