Carbohydrate Research, 204 (1990) 141–144
Elsevier Science Publishers B.V., Amsterdam

Note

Spacer-modified oligosaccharides as potential affinity reagents for glycosyltransferases: the preparation and enzymic galactosylation of 1,10-bis(2-acetamido-2-deoxy-β-D-glucopyranosyloxy)decane*

Jochen Lehmann† and Stefan Petry
Institut für Organische Chemie und Biochemie der Universität Freiburg, Albertstraße 21, D-7800 Freiburg i.Br. (F.R.G.)

(Received September 11th, 1989; accepted for publication, November 9th, 1989)

Many glycoconjugates of eukaryotic cells, glycolipids, and glycoproteins have specific and important biological functions[1]. In order to fulfil these functions, the assembly of the determinant carbohydrate portions must be specific, reproducible, and regulated. The systems that control reproducible biosyntheses of complex oligosaccharides rely on the high specificity of glycosylating enzymes[2]. Glycosyltransferases are specific with respect to both the donor and the acceptor, and irreversible inhibitors, such as those used to block glycoside hydrolases[3] through covalent modification of the active site, are not yet known. The main obstacle is the high specificity of the glycosyltransferases. Groups for the covalent modification of active sites must be attached to the ligand in such a way that they do not prevent specific binding. For example, the first spacer-modified "reducing" disaccharide consisted of two α-D-glucose residues (1→4)-linked by an acyclic 6-membered spacer and competitively inhibited porcine pancreatic alpha-amylase[4].

We now describe the synthesis of a "non-reducing" spacer-modified disaccharide, which mimics part of the biantennary core structure of *N*-linked glycoconjugates (Fig. 1) where the central three mannose residues are replaced by an extended aliphatic spacer. A spacer that connects both monosaccharide moieties "head on" must be 10 atoms long (cf. Fig. 1) and be conformationally flexible so that photolabile or chemically reactive groups can be attached for affinity labelling[5]. Also, the "handle" that replaces the chitobiosyl residue can be attached to the spacer at positions 5 or 7 (ref. 5).

In order to explore the above type of spacer-modified disaccharide, the synthesis of a prototype has been explored.

* Presented at EUROCARB V, the Vth European Carbohydrate Symposium, Prague, Czechoslovakia, August 21–25, 1989.
† Author for correspondence.

0008-6215/90/$ 03.50

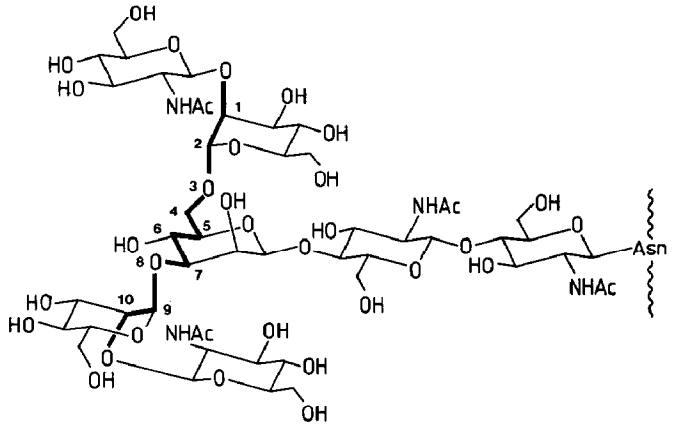
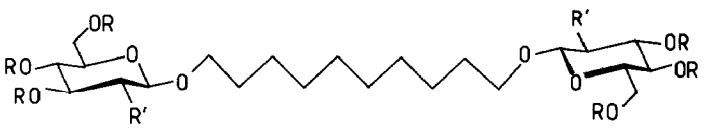


Fig. 1. The biantennary core structure of *N*-linked glycoconjugates. The segment represented by the numbered, solid line is mimicked by the 10-membered spacer in compound 3.

2-deoxy-2-phthalimido-β-D-glucopyranosyloxy)decane (**1**). After deblocking, *O*- and *N*-acetylation (→**2**), and removal of the *O*-acetyl groups, 1,10-bis(2-acetamido-2-deoxy-β-D-glucopyranosyloxy)decane (**3**) was obtained crystalline. Compound **3** was a good acceptor for the lactose synthetase system and, with UDP-D-galactose, yielded both the mono- (R_F 0.26; 7:2:1 EtOAc–MeOH–H_2O) and di-galactosylated (R_F 0.14) compounds, as could be demonstrated by t.l.c. When treated with *E. coli* β-D-galactosidase, each of these products yielded galactose (R_F 0.23) and **3** (R_F 0.28).

Compound **3** could also be galactosylated by using ^{14}C-UDP-D-galactose and the microsomal fraction of liver cells that is known to contain a specific membrane-bound galactosyltransferase[8].



1 R = Ac, R′ = NPhth
2 R = Ac, R′ = NHAc
3 R = H, R′ = NHAc

EXPERIMENTAL

General. — Melting points are uncorrected. Optical rotations were measured with a Perkin–Elmer 141 polarimeter. All reactions were monitored by t.l.c. on Silica Gel F_{254} (Merck). Column chromatography by the flash method[9] was performed on ICN-silica 32-63 (ICN Biomedicals). ^{1}H-N.m.r. spectra were recorded with a Bruker WM 250 spectrometer at 250 MHz for solutions in $CDCl_3$ (internal Me_4Si). Mass spectra were recorded with a Finnigan MAT-312 spectrometer.

1,10-Bis(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyloxy)decane (**1**). — To a solution of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose[6] (5 g, 10.47 mmol) in CH_2Cl_2 (100 mL) was added $SnCl_4$ (1.18 mL, 10 mmol) under anhydrous conditions. The mixture was stirred for 15 min at room temperature, 1,10-decanediol (0.61 g, 3.5 mmol) was added, and stirring was continued for 4 h. The mixture was poured into ice–water (250 mL) with stirring, the organic layer was separated, the aqueous layer was extracted with CH_2Cl_2 (5 x 60 mL), and the organic layers were combined, neutralised with saturated aqueous $NaHCO_3$ (200 mL), dried (Na_2SO_4), and concentrated to yield an oil that crystallised from EtOH to give **1** (2 g). Flash chromatography (1:1 cyclohexane–EtOAc) of the material in the mother liquor gave more **1** (0.5 g; total yield, 2.5 g, 71%), m.p. 145–146°, $[\alpha]_D^{20}$ +19.5° (*c* 1.1, dichloromethane); R_F 0.2. ^{1}H-N.m.r. data: δ 0.75 (m, 2 H, aliphatic CH_2), 0.95 (m, 10 H, 5 aliphatic CH_2), 1.4 (m, 4 H, 2 aliphatic CH_2), 1.88 (s, 6 H, 2 OAc), 2.06 (s, 6 H, 2 OAc), 2.13 (s, 6 H, 2 OAc), 3.35 (m, 2 H, OCH_2), 3.77–3.9 (m, 4 H, $J_{4,5}$ 10.5, $J_{5,6}$ 4.5, $J_{5,6'}$ 3 Hz, OCH_2 and 2 H-5), 4.18 (dd, 2 H, $J_{6,6'}$ 13.5 Hz, 2 H-6), 4.35 (m, 4 H, $J_{1,2}$ 8.7, $J_{2,3}$ 10.5 Hz, 2 H-2,6), 5.2 (dd, 2 H, $J_{3,4}$ 9 Hz, 2 H-4), 5.36 (d, 2 H, 2 H-1), 5.8 (dd, 2 H, 2 H-3), 7.74 (m, 8 H, aryl), and 7.86 (m, 8 H, aryl).

Anal. Calc. for $C_{50}H_{60}N_2O_{20}$: C, 59.40; H, 6.14; N, 2.77. Found: C, 59.39; H, 5.80; N, 2.72.

1,10-Bis(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyloxy)decane (**2**). — To a suspension of **1** (2 g, 1.98 mmol) in EtOH (20 mL) was added $H_2NNH_2 \cdot H_2O$ (4 mL, 82 mmol), and the mixture was boiled under reflux. The reaction was monitored by t.l.c. After 2–4 h, only one product could be detected by t.l.c. (R_F 0.15, 7:2:1 EtOAc–MeOH–H_2O). The white precipitate was collected and washed with MeOH (100 mL), and the combined filtrate and washings were concentrated. The resulting yellow oil was treated with pyridine (30 mL) and Ac_2O (10 mL) for 5 h, the mixture was poured into ice–water (150 mL) and extracted with CH_2Cl_2 (5 × 50 mL), and the combined extracts were washed with 0.1M HCl (3 × 20 mL) to remove phthalimidohydrazide, then with water (100 mL) and aqueous 3% $NaHCO_3$ (100 mL), dried (Na_2SO_4), and concentrated. The residue (1.6 g, 96%) was homogeneous by t.l.c. (R_F 0.7, 7:2:1 EtOAc–MeOH–H_2O) but failed to crystallise. A part of this product was purified by flash chromatography (1:5 cyclohexane–EtOAc). ^{1}H-N.m.r. data: δ 1.27 (m, 12 H, 6 aliphatic CH_2), 1.56 (m, 4 H, 2 aliphatic CH_2), 1.95 (s, 6 H, 2 OAc), 2.04 (m, 12 H, 2 NAc, 2 OAc), 2.10 (s, 6 H, 2 OAc), 3.48 (m, 2 H, OCH_2), 3.71 (m, 2 H, $J_{4,5}$ 10, $J_{5,6}$ 4.5, $J_{5,6'}$ 3 Hz, 2 H-5), 3.84 (m, 4 H, $J_{1,2}$ 8, $J_{2,3}$ 9, $J_{NH,2}$ 9 Hz, 2 H-2, OCH_2), 4.13 (m, 2 H, $J_{6,6'}$ 12 Hz, 2 H-6'), 4.28 (dd, 2 H, 2 H-6), 4.71 (d, 2 H, H-1), 5.06 (dd, 2 H, $J_{3,4}$ 10 Hz, 2 H-4), 5.32 (dd, 2 H, 2 H-3), 5.97 (d, 2 H, 2 *H*NAc).

1,10-Bis(2-acetamido-2-deoxy-β-D-glucopyranosyloxy)decane (**3**). — To a suspension of **2** (1.5 g, 1.8 mmol) in MeOH was added methanolic M NaOMe (0.5 mL). After 15 min, crystals appeared and, after 3 h, t.l.c. (R_F 0.28, 7:2:1 EtOAc–MeOH–H_2O) showed the reaction to be complete. The mixture was neutralised with aqueous 30% AcOH, and the crystals were collected and recrystallised from MeOH (50 mL) to give **3** (734 mg, 84%), m.p. 234–235°, $[\alpha]_D^{20}$ −30° (*c* 1, methyl sulfoxide). Mass spectrum (c.i.

NH_3, 70 eV): *m/z* 581 (15%) $[M + 1]^+$, 539 (3.6%) $[M - 41]^+$, 378 (40%) $[M + 1 + H - C_8H_{14}O_5N]^+$, 204 (100%) $[C_8H_{14}O_5N]^+$.

Anal. Calc. for $C_{26}H_{48}N_2O_{12}$: C, 53.79; H, 8.28; N, 4.83. Found: C, 53.55; H, 8.27; N, 4.70.

Galactosylation of **3** *by the galactosyltransferase from bovine milk.* — To a suspension of **3** (5.8 mg, 10 μmol) in Tris-HCl (pH 7.4; 1 mL, 50 μmol) were added $MnCl_2 \cdot 4H_2O$ (7.9 mg, 40 μmol) and UDP-D-galactose (12 mg, 20 μmol). The reaction was started by the addition of enzyme (EC 2.4.1.22; 1 mg, 1–2 units, Sigma) at 37° and monitored by t.l.c. (7:2:1 EtOAc–MeOH–H_2O). After 20 min, no **3** could be detected, and mono- (R_F 0.26) and di-galactosylated products (R_F 0.14) were present. The compounds were isolated by flash chromatography and each was a substrate for *E. coli* β-D-galactosidase.

ACKNOWLEDGMENTS

Professor D. Horton is thanked for valuable discussion, and the Bundesminister für Forschung und Technologie (0319050 A8) for financial support.

REFERENCES

1 J. Montreuil, *Adv. Carbohydr. Chem. Biochem.*, 37 (1980) 157–223.
2 R. W. Stoddart, *The Biosynthesis of Polysaccharides*, Croom Helm, Kent, 1984, pp. 286.
3 G. Legler, *Hoppe-Seyler's Z. Physiol. Chem.*, 349 (1968) 767–774; M. Brockhaus and J. Lehmann, *FEBS Lett.*, 62 (1976) 154–156; M. L. Sinnott and P. J. Smith, *J. Chem. Soc., Chem. Commun.*, (1976) 223–224; J. Lehmann and B. Schwesinger, *Chem. Ber.*, 111 (1978) 3961–3964.
4 S. Jegge and J. Lehmann, *Carbohydr. Res.*, 133 (1984) 247–254.
5 C. Ats, J. Lehmann, and S. Petry, unpublished work.
6 R. U. Lemieux, T. Takeda, and B. Y. Chung, *ACS Symp. Ser.*, 39 (1976) 90.
7 S. Hanessian and J. Banoub, *Carbohydr. Res.*, 59 (1977) 261–267.
8 C. Bauer, W. Reutter, and P. Röthig, personal communication.
9 W. C. Still, M. Khan, and A. Mitra, *J. Org. Chem.*, 43 (1978) 2923–2925.