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Note

Enzymic synthesis of oligosaccharides on an α-chymotrypsin-sensitive polymer. *O*-(β-D-Galactopyranosyl)-(1→4)-*O*-(β-D-glucopyranosyl)-(1→4)-D-glucopyranose

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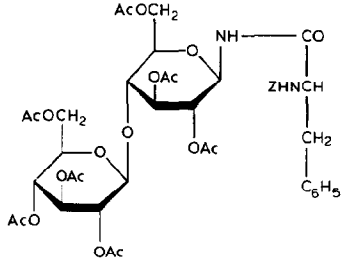
Previous work from this laboratory has dealt with enzymic synthesis of oligosaccharides on light-sensitive polymer supports[1,2]. The present paper describes an alternative approach based on literature data concerning low-molecular-weight, acyl-protecting groups that may be removed with enzymes[3–5]. Thus, an acceptor saccharide was converted into a glycosylamine and linked to a water-soluble



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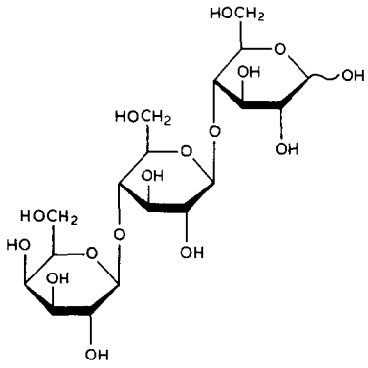

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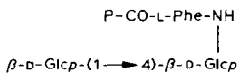

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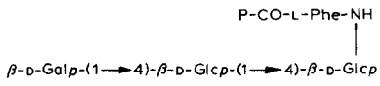

4

Z = benzyloxycarbonyl

P = poly(acrylamide)

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polymer *via* an amide bond with L-phenylalanine (a 1-*N*-L-phenylalanyl linkage), presumably[6] an α-chymotrypsin sensitive bond. Oligosaccharide products of interest could then be obtained by sequential glycosyltransferase reactions followed by α-chymotrypsin digestion.

Compound **1**, synthesized by a carbodiimide condensation of 2,3,6-tri-*O*-acetyl-4-*O*-(2,3.4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranosylamine[7,8] and *N*-benzyloxycarbonyl-L-phenylalanine, was hydrogenolyzed, affording (without isolation) a free amino derivative. The latter compound was treated with a soluble polyacrylamide (M_r ~6000) containing active esters, and subsequently a soluble carbodiimide was also added to increase attachment. Following evaporation to dryness, the mixture was treated with sodium methoxide to deacetylate the product, and ultrafiltration afforded polymer **3**. The *cellobio* residues on this polymer served as receptors for a D-galactosyltransferase reaction using labeled UDP-Gal as the D-galactosyl donor[1]. Finally, the polymeric product **4**, purified by ultrafiltration, was subjected to α-chymotrypsin digestion, thus releasing the free trisaccharide product **2**.

It is perhaps significant to note that oligosaccharides possessing *N*-glycosylamide linkage to polymers and having the ability to serve as substrates for glycosyltransferase reactions bear a close similarity to many glycoproteins[9]. Moreover, in the present study, a release of free oligosaccharides was achieved with a proteolytic enzyme. However, the yields of this synthetic route (condensations and release) are not as high as with light-sensitive polymers[2].

EXPERIMENTAL

General. — Materials, methods, and equipment were as described[1]. α-Chymotrypsin (EC 3.4.21.1) from bovine pancreas (3 times crystallized) was a product of Sigma Chemical Co.

*2,3,6-Tri-*O*-acetyl-1-*N*-(*N*-benzyloxycarbonyl-*L*-phenylalanyl)-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)-β-*D*-glucopyranosylamine* (**1**). — 2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranosylamine[7,8] (0.82 g, 1.3 mmol), *N*-benzyloxycarbonyl-L-phenylalanine (0.39 g, 1.3 mmol), and *N,N*-dicyclohexylcarbodiimide (0.9 g) were dissolved in dichloromethane (200 mL) and stirred overnight at room temperature. The precipitate was removed by filtration, the solution evaporated, and the residue applied to a silica gel column (25 g, 0.9 cm diam.) and eluted with 1:2 ethyl acetate–chloroform (3.2-mL fractions). Compound **1** emerged in fractions 19–27, (0.8 g, 67%) and crystallized from ethyl acetate–petroleum ether, m.p. 223–224°, $[\alpha]_D^{25}$ −32.2 ±0.6° (*c* 0.97, chloroform); ν_{max}^{KBr} 3340 (NH), 1750 (CO, ester), 1700 (CO, urethane), and 1520 cm^{-1} (NH); ^{1}H-n.m.r. (80 MHz): δ 7.32 and 7.22 (arom.), 5.08 (s over m, $C_6H_5CH_2$), 2.13 (s, 3 H, $OCOCH_3$), 2.09 (s, 3 H, $OCOCH_3$), 2.03 (s, 3 H, $OCOCH_3$), 2.01 (s, 6 H, 2 $OCOCH_3$), 1.98 (s, 3 H, $OCOCH_3$), and 1.92 (s, 3 H, $OCOCH_3$).

Anal. Calc. for $C_{43}H_{52}N_2O_{20}$: C, 56.32; H, 5.71; N, 3.05. Found: C, 56.20; H, 5.68; N, 3.35.

4-O-β-D-Glucopyranosyl-1-N-[(N-P-oxycarbonyl)-L-phenylalanyl]-β-D-glucopyranosylamine (**3**). — Compound **1** (0.229 g, 250 μmol) was hydrogenolyzed (310 KPa, 20 h at room temperature) in methanol (100 mL) in the presence of 10% palladium-on-charcoal. The catalyst was removed by filtration, the solution was evaporated, and the residue dissolved with stirring at 40° (water-bath temperature) in a *N,N*-dimethylformamide (2.5 mL)–0.3M *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer (1.5 mL, pH 7.5.). Poly(acrylamide)–poly(*N*-acryloxysuccinimide)[10] (PAN, 660 μmol of active ester/g, 0.55 g) was added and stirring continued at 40° for 2 h. 3-(3-Dimethylaminopropyl)-1-ethylcarbodiimide hydrochloride (EDCD, 60 mg) was added and stirring continued overnight at room temperature. The mixture was evaporated *in vacuo* and the residue stirred overnight in 0.1M methanolic sodium methoxide at 4°. The mixture was made neutral with acetic acid, evaporated, and extracted with water (80 mL). The extract was filtered through Celite, and the filtrate dialyzed by use of Diaflo YM2 ultrafiltration membranes (Amicon, Lexington, MA 02173) and lyophilized to yield polymer **3** (0.43 g). Polymer **3** (20 mg) was incubated with α-chymotrypsin (1 mg) in 3mM Tris buffer (0.66 mL, pH 8.0) containing 45mM KCl and 15mM $CaCl_2$ for 22 h at 30°. The mixture was dialyzed (Diaflo YM2), and the nondializable residue in the dialysis cell was collected and lyophilized. The contents of cellobiose of the polymer (71 μmol/g) and of cellobiose remaining on the polymer following release (21 μmol/g; yield 31%) were determined by the phenol–H_2SO_4 test[11].

4-O-(4-O-β-D-Galactopyranosyl-β-D-glucopyranosyl)-[1-N-(N-P-oxycarbonyl)-L-phenylalanyl]-β-D-glucopyranosylamine (**4**). — Polymer **3** (100 mg), UDP-Gal (4 mg) labeled by UDP-D-[U-^{14}C]galactose (196 000 c.p.m./mg), α-lactalbumin (3 mg), and D-galactosyltransferase (1 unit, EC 2.4.1.22) from bovine milk in 25mM sodium cacodylate buffer (3 mL, pH 7.0 containing 3mM $MnCl_2$ and 0.1% mercaptoethanol) were incubated for 21 h at 37°. The product (**4**) was purified by extensive dialysis (Diaflo YM2) until only very little radioactivity (5 × that of blank) emerged in the eluates. The polymer (**4**; 260 000 c.p.m./g, 2.2 μmol Gal/g) was collected after lyophilization.

O-β-D-Galactopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→4)-D-glucopyranose (**2**). — Polymer **4** (85 mg) was incubated with α-chymotrypsin (3 mg) in 3mM Tris buffer (2 mL, pH 8.0) containing 45mM KCl and 15mM $CaCl_2$ for 22 h at 30°. The mixture was dialyzed (Diaflo YM2), and the dialyzate collected and lyophilized. The residue was dissolved in water (3 mL), the pH adjusted to 4.8 with 0.2M acetic acid, and after 4 h at room temperature, the solution was passed through Amberlite IR-120 cation-exchange resin (H^+) and lyophilized. The residue contained 29.5% of the radioactivity (6620 c.p.m.) and 25.6% of the radioactivity remained on the α-chymotrypsin-treated polymer. The product (**2**) was identified by descending paper chromatography on a Whatman 3 MM paper in 25:6:25 (v/v) 1-butanol–acetic acid–water (upper phase). The radioactivity of 1-cm segments of

the paper was counted[1] and compared to that of an authentic sample[12]. Standards were detected with silver nitrate[13].

Compound **2** (1300 c.p.m.) was dissolved in acetic anhydride (0.15 mL)–pyridine (0.3 mL) and the mixture was kept overnight at room temperature. Ice was added, the mixture evaporated *in vacuo,* and the residue extracted with chloroform. The extract was examined by t.l.c. in 1:1 chloroform–ethyl acetate. The acetylation product migrated as an authentic sample[12] of **2** acetylated in the same manner.

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