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Polymers having (1→4)- and (1→6)-linked α-D-glucopyranosyl groups as acceptors in the glycogen synthase reaction*,†

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ABSTRACT

Insoluble, light-sensitive polymers linked to maltose, maltotriose, a glycogen-branch point trisaccharide, and panose were synthesized and served in a comparative study as acceptors in the glycogen synthase (UDP-D-glucose:glycogen 4-α-D-glucosyltransferase, EC 2.4.1.11) reaction. The highest transfer rate was observed with the *maltotrio* polymer. Extending the acceptor linearly with (1→4)-linked α-D-glucopyranosyl residues improved the transfer, whereas (1→6)-linked α-D-glucopyranosyl branches decreased it.

INTRODUCTION

A previous investigation at this laboratory employing polymer-bound oligosaccharides as acceptors dealt with two basic aspects of glycogen synthase: (a) The demonstration of *de novo* synthesis, and (b) the study of primer requirements and the determination of the extent of transfer[1].

Transfer yields in glycosyltransferase and transglycosylation reactions were not high enough with soluble acceptor-polymers, an observation that prompted an attempt to introduce polymers having longer spacers in order to improve accessibility of saccharide acceptors to glycosylation reactions[2]. This proved to be the case for galactosyltransferase[2] but did not seem to be the case for the bulkier glycogen synthase. It was, however, considered that even low yields can be useful in the comparative study of transfer efficiencies. Thus, we report herein the use of acceptors posessing both (1→4)- and (1→6)-α-D-glucopyranosyl linkages attached to insoluble polymers to relate the effect of 1→6 substitution on acceptor efficiency.

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RESULTS AND DISCUSSION

The synthesis of three 4-carboxymethyl-2-nitrobenzyl glycosides out of the four employed in this study for the preparation of acceptor polymers has been described[1,3]. In the case of the maltotrioside **1**, however, the method previously used gave poor yields. Therefore, a different bromination and glycosidation method was used and by applying silver triflate as a catalyst, the β-glycoside was obtained in 28% yield. This yield was limited owing to the formation of the acetylated aglycon **3** as a byproduct. Similar transesterfication is a known side-reaction where silver triflate is used as a catalyst, presumably the result of the formation of an 1,2-orthoester intermediate[4]. An analogous synthesis starting from *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→4)-1,2,3,6-tetra-*O*-acetyl-α,β-D-glucopyranose[5] yielded (28%) of the corresponding *β-pano* derivative **4**, as indicated by ^{1}H-n.m.r. spectrometric and analytical data. The ester protecting groups of the 4-carboxymethyl-2-nitrobenzyl aglycon of maltose, maltotriose (**1**), the glycogen branching-point trisaccharide, and compound **4** were removed (**1** and **4** giving **2** and **5**, respectively), and the resulting carboxylic acid derivatives were condensed with aminohexyl-substituted poly(acrylamide) gel beads (H_2N-Ⓟ) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCD) to yield **6**, **7**, **10**, and **12**, respectively.

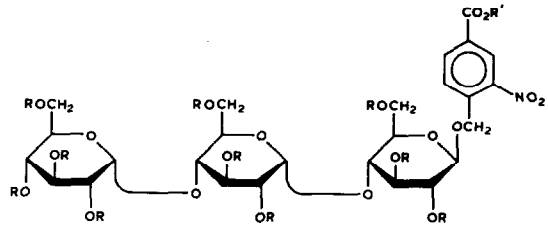
Polymers **6**, **7**, **10**, and **12** served as acceptors in the glycogen synthase reaction to give polymers **8**, **9**, **11**, and **13**, respectively (see Scheme 1). Owing to their polymeric handles, these were readily isolated and purified. Since ^{14}C-labeled UDP-D-glucose was used in the experiments, the incorporation of D-glucose was accurately determined from the bound radioactivity (Table I). As observed in the past[1], an improved transfer was observed when the *maltotrio* polymer **7** was compared to the *malto* polymer **6**, as well as an improvement in the yields when the byproducts were removed by ultrafiltration and repeated incubation (Table I, Experiment 2). Moreover, the transfer to saccharide acceptors possessing (1→6)-α-D-glucopyranosyl in addition to (1→4)-α-D-glucopyranosyl linkages (**10** and **12**) was considerably lower than to those possessing only (1→4)-α-D-glucopyranosyl linkages.

TABLE I

Glycogen synthase-catalyzed incorporation[a] of D-glucose into acceptor polymers

Acceptor	*Experiment 1*		*Experiment 2*	
	μmol/g	*Transfer (%)*	*μmol/g*	*Transfer (%)*
6	1.58	0.63		
7	0.58	0.87	5.2	7.76
10	0.11	0.16	0.17	0.24
12	0.28	0.20	0.96	0.68

[a] Details of incubation conditions are described in the Experimental section.



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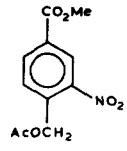
2 R = R′ = H



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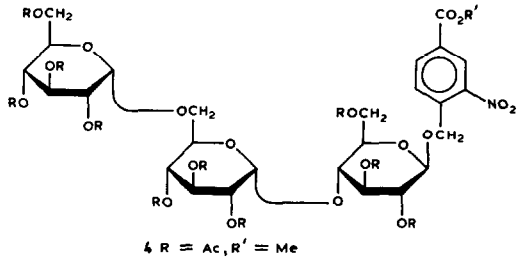
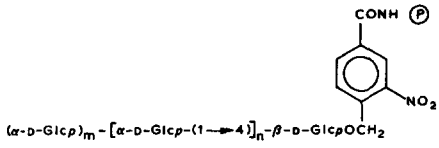
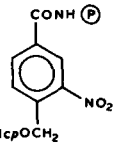


4 R = Ac, R′ = Me
5 R = R′ = H



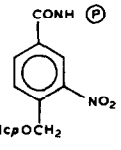
$(\alpha\text{-D-Glc}p)_m\text{-}[\alpha\text{-D-Glc}p\text{-}(1\rightarrow4)]_n\text{-}\beta\text{-D-Glc}p\text{OCH}_2$

6 n = 1, m = 0
7 n = 2, m = 0
8 n = 1, m > 0
9 n = 2, m > 0



$(\alpha\text{-D-Glc}p)_m\text{-}\alpha\text{-D-Glc}p\text{-}(1\rightarrow4)\text{-}[(\alpha\text{-D-Glc}p)_p\text{-}\alpha\text{-D-Glc}p\text{-}(1\rightarrow6)]\text{-}\beta\text{-D-Glc}p\text{OCH}_2$

10 p = 0, m = 0
11 p ≥ 0, m ≥ 0

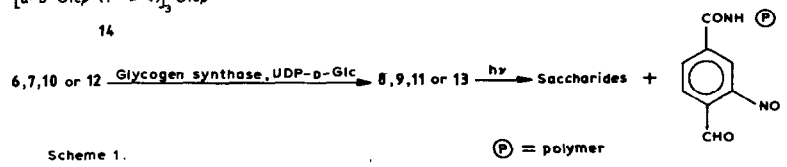


$(\alpha\text{-D-Glc}p)_m\text{-}\alpha\text{-D-Glc}p\text{-}(1\rightarrow6)\text{-}\alpha\text{-D-Glc}p\text{-}(1\rightarrow4)\text{-}\beta\text{-D-Glc}p\text{OCH}_2$

12 m = 0
13 m > 0

$[\alpha\text{-D-Glc}p\text{-}(1\rightarrow4)]_3\text{-Glc}p$

14



6,7,10 or 12 —Glycogen synthase, UDP-D-Glc→ 8,9,11 or 13 —hν→ Saccharides +

Ⓟ = polymer

Scheme 1.

Although panose presents just one possible acceptor-site to glycogen synthase, in contrast to two for the branching-point trisaccharide, when polymers **10** and **12** were compared, panose proved to be a better acceptor than the branching-point trisaccharide. This might be, at least in part, a consequence of steric effects, as a panose acceptor is a chainlike saccharide that may adopt a linear conformation and extend further from the polymer backbone (in **12**), as compared to the branching-point trisaccharide (in **10**). Molecular models showed that the difference in length is nearly one sugar unit when the panose model is in a *gg* conformation[6] at the α-D-(1→6)- linkage. Although a complete assignment of the ^{1}H-n.m.r. resonances for **5** was not possible, the H-5 and H-6 resonances could be assigned unequivocally for each sugar ring; the predominance of *gg*-rotamers in all C-5–C-6 linkages of **5** was suggested by the ^{1}H-n.m.r. coupling constants of $J_{5,6(S)}$ 2.1–2.3 and $J_{5,6(R)}$ 4.9–5.5 Hz. This conformation is in contrast to that in the crystalline form, which was found to be *gt* at the (1→6) linkage[7]. In summary the observed acceptor-efficiencies in the glycogen synthase reaction were **7**>**6**>>**12**>**10** (Table I).

Irradiation (followed by lyophilization, paper chromatography, and counting of the radioactivity) of products of Experiment 2 revealed that whereas 92% of the radioactivity was released from polymer **11** (a product of low-transfer yield), polymers **9** and **13** released no more than 16 and 12% of the bound radioactivity, respectively, and only 3 and 2% of the counts were in the maltotetraose region, and the rest corresponded to higher mol.wt. oligosaccharides (see Scheme 1) (*cf.* ref. 1). The material obtained from the irradiation of polymer **9** and corresponding, in paper chromatography, to maltotetraose (**14**) was partially characterized earlier[1], and methylation analysis, described herein, established its structure as maltotetraose.

A current proposal for the biosynthesis of glycogen starts with glycogenin, which self glucosylates (UDP-D-glucose serving as the donor) to form an acceptor for glycogen synthase, thus providing a maltosaccharide chain (refs. 8 and 9, and refs. cited therein). The last suggestion that bound maltodextrins are necessary is compatible with our finding in the study of acceptors on carrier polymers, *i.e.*, sequential addition of (1→4)-α-D-glucopyranosyl groups to the acceptor results in an improved transfer (and incorporation of D-glucose) with glycogen synthase[1,10]. Furthermore, comparative results presented herein suggest that (1→6)-α-D-glucopyranose units in the acceptor are detrimental to the transfer catalyzed by glycogen synthase. This implies that metabolically, partially degraded glycogen, where (1→6)-α-D-glucopyranose units appear at or next to the nonreducing end, is initially a lesser acceptor of glycogen synthase until additional (1→4)-α-D-glucopyranosyl groups are linked.

Two polymers (**9** and **13**) that are transfer products of the glycogen synthase reaction carry a high proportion of incorporated D-glucose units that are not released by irradiation but can possibly be released by amyloglucosidase (*cf.* refs. 1 and 10). One possible explanation is that the photochemical cleavage proceeded, as usual, almost to completion but that the higher mol. wt. product remains adsorbed to the polymer beads.

Nevertheless, the use of polymers as acceptors, in a study of acceptors' specificity, provides the opportunity of facile isolation of products in the presence of acceptors

(glycogen, glycogenin) donors, byproducts, salts, etc.; subsequently the products can be subjected to structural analysis.

EXPERIMENTAL

General — Melting points were recorded with a Büchi 510 apparatus and optical rotations measured with a Bendix polarimeter. ^{1}H-N.m.r. spectra were recorded with a Bruker WH 300 or a Bruker AM 500 instrument at 300 and 500 MHz, respectively. All reactions were carried out under protection from light and, if not involving polymers, monitored by t.l.c. on Silica Gel 60 F_{254} sheets (E. Merck, D-6600 Darmstadt, Germany). Compounds were detected by viewing under u.v. and by staining with 10% H_2SO_4 in ethanol. Glycogen synthase (UDP-D-glucose: glycogen (1→4)-α-D-glucosyltransferase, EC 2.4.1.11, from rabbit muscle) and chemicals were obtained from Sigma Chemical Co. (St. Louis, MO 63178, U.S.A.) unless otherwise mentioned. UDP-D-[U-^{14}C]glucose was purchased from the Radiochemical Centre (Amersham, Bucks, HP75LL, U.K.). Additional methods and equipment were as described earlier[11]. Acceptor saccharide linked to a carrier polymer was quantitatively determined by the phenol–H_2SO_4 test[12].

4-Carboxymethyl-2-nitrobenzyl O-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-(1→4)-O-(2,3,6-tri-O-acetyl-α-D-glucopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**1**). — Hydrogen bromide was bubbled through a solution of *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→4)-1,2,3,6-tetra-*O*-acetyl-β-D-glucopyranose[13,14] (6.8 g, 7.0 mmol) in dry dichloromethane (50 mL) for 1.5 h. The solution was then diluted with dry toluene (20 mL), concentrated, and the residual solvent two times coevaporated with toluene. The remaining bromide was dissolved in dry dichloromethane (20 mL), the solution stirred with molecular sieve (4A) for 30 min, and subsequently added to a previously prepared mixture of methyl 4-hydroxymethyl-3-nitrobenzoate[11] (1.05 g, 5.0 mmol) in benzene (22 mL), nitromethane (10 mL, stirred with molecular sieve 4A for 2 h), and silver triflate (1.8 g, 7.0 mmol) at 0°. The mixture was stirred overnight while warming up to room temperature, and then diluted with dichloromethane, filtered over Celite, washed with a $NaHCO_3$–$Na_2S_2O_3$ solution and water, dried (Na_2SO_4), and concentrated. The residue was chromatographed on silica with 2:1 (v/v) ethyl acetate–petroleum ether. The acetylated alcohol, methyl 4-acetoxymethyl-3-nitrobenzoate **3** (690 mg, 58%), was eluted first, m.p. 77°; ^{1}H-n.m.r. (300 MHz, $CDCl_3$): δ 2.19 (s, 3 H, OAc), 3.98 (s, 3 H, OMe), 5.56 (s, 2 H, C_6H_3–CH_2), 7.37 (d, 1 H, $J_{3,5}$ 1.7 Hz, H-3), 7.70 (d, 1 H, $J_{5,6}$ 8.1 Hz, H-6), and 8.29 (dd, 1 H, H-5).

Anal. Calc. for $C_{11}H_{11}NO_6$: C, 52.18; H, 4.37; N, 5.53. Found: C, 52.50; H, 4.35; N, 5.29.

Compound **1** was eluted next (1.58 g, 28%), m.p. 87–89°, $[\alpha]_D^{20}$ + 68° (*c* 0.5, chloroform); ^{1}H-n.m.r. (500 MHz, C_6D_6): δ 1.67, 1.68 1.81, 1.83, 1.84, 1.89, 1.90, 1.91, 1.91, and 1.96 (each s, 3 H each, 10 OAc), 2.94 (ddd dt, 1 H, $J_{4,5}$ 9.3, $J_{5,6a}$ 3.0, $J_{5,6b}$ 4 Hz, H-5), 3.40 (s, 3 H, OMe), 3.85 (dd t, 1 H, $J_{3,4}$ 9.0, $J_{4,5}$ 9.3 Hz, H-4), 4.00 (dd, 1 H, 1 H, $J_{6a,6b}$ 12.5

Hz, H-6a), 4.04 (dd t, 1 H, $J_{3',4'}$ 9.0, $J_{4',5'}$ 9.7 Hz, H-4'), 4.12 (ddd dt, 1 H, $J_{5',6a'}$ 3.0, $J_{5',6a'}$ 3.2 Hz, H-5'), 4.23 (dd, 1 H, H-6b),4.29 (ddd dt, *1 H*, $J_{4'',5''}$ 10.2, $J_{5'',6a''}$ 3.5, $J_{5'',6b''}$ 2.5 Hz, H-5''), 4.35 (dd, $J_{6a',6b'}$ 12.4 Hz, H-6a'), 4.40 (dd, 1 H, $J_{6a'',6b''}$ 12.4 Hz, H-6b''), 4.49 (dd, 1 H, H-6a''), 4.66 (dd, 1 H, H-6b'), 4.80 and 4.98 (each d, 1 H each, *J* 15.0 Hz C_6H_3-CH_2),4.90 (dd, 1 H, $J_{1',2'}$ 4.0, $J_{2',3'}$ 10.3 Hz, H-2'), 5.10 (dd, 1 H, $J_{1'',2''}$ 4.0, $J_{2'',3''}$ 10.6 Hz, H-2''), 5.14 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.4 Hz, H-2), 5.37 (d, 1 H, H-1'), 5.41 (d, 1 H, H-1), 5.42 (dd t, 1 H, H-3), 5.43 (dd t, 1 H, $J_{3'',4''}$ 9.6 Hz, H-4''), 5.67 (d, 1 H, H-1''), 5.84 (dd, 1 H, H-3''), 5.86 (dd, 1 H, H-3'), 7.38 (d, 1 H, $J_{5''',6a'''}$ 8.2 Hz, H-6'''), 7.93 (dd, 1 H, $J_{3''',5'''}$ 1.7 Hz, H-5'''), and 8.54 (d, 1 H, H-3'''). Figures indicated without, with one or two primes refer to carbohydrate protons; figures indicated with three primes refer to aromatic protons.

Anal. Calc. for $C_{47}H_{59}NO_{30}$: C, 50.49; H, 5.32; N, 1.25; Found: C, 50.77; H, 5.35; N, 1.02.

4-Carboxymethyl-2-nitrobenzyl O-*(2,3,4,6-tetra*-O-*acetyl*-α-D-*glucopyranosyl)-(1→6)*-O-*(2,3,4-tri*-O-*acetyl*-α-D-*glucopyranosyl)-(1→4)-2,3,6-tri*-O-*acetyl*-β-D-*glucopyranoside* (**4**). — Hydrogen bromide was bubbled through a solution of *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→4)-1,2,3,6-tetra-*O*-acetyl-α,β-D-glucopyranose[4] in dichloromethane (10 mL) for 45 min. The yellow solution was then diluted with toluene, concentrated, and the residual solvent coevaporated two times with toluene. The foamy residue, dissolved in dichloromethane (5 mL), was stirred with molecular sieve (4A) for 30 min, and then added to a previously prepared and cooled (0°) mixture of methyl 4-hydroxymethyl-3-nitrobenzoate (220 mg, 1.04 mmol) in dichloromethane (4 mL, stirred with molecular sieve 4A) for 2 h) and silver triflate (225 mg, 1.0 mmol). Stirring was continued overnight at room temperature and the mixture was worked up as described for the preparation of **1**. The residue was chromatographed on silica with 3:2 (v/v) ethyl acetate–petroleum ether to yield **4** (180 mg, 28%), amorphous, $[\alpha]_D^{18}$ + 49.5° (*c* 0.8, dichloromethane); ^{1}H-n.m.r. (500 MHz, $CDCl_3$): δ 2.00, 2.00, 2.03, 2.05, 2.05, 2.08, 2.09, 2.12, 2.13, 2.13 (each s, 3H each, 10 OAc), 3.48(dd, 1 H, $J_{5',6b'}$ 2.2, $J_{6a',6b'}$ 11.3 Hz, H-6b'), 3.72 (ddd, 1 H, $J_{5',6a'}$ 4.8 Hz, H-6a'), 3.76 (ddd, 1 H, $J_{4,5}$ 9.8, $J_{5,6a}$ 4.2, $J_{5,6b}$ 2.6 Hz, H-5), 3.97 (s, 3 H, OMe), 3.97 (dd t, 1 H, $J_{3,4}$ 9.6Hz, H-4), 4.02 (ddd, 1 H, $J_{4',5'}$ 10.4 Hz, H-5'), 4.05 (ddd, 1 H, $J_{4'',5''}$ 10.4, $J_{5'',6a''}$ 4.4, $J_{5'',6b''}$ 2.6 Hz, H-5''), 4.09 (dd, 1 H, $J_{6a'',6b''}$ 12.4 Hz, H-6b''), 4.26 (dd, 1 H, H-6a'') 4.29 (dd, 1H, $J_{6a,6b}$ 12.1 Hz, H-6a), 4.50 (dd, 1 H, H-6b), 4.73 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.77 (dd, 1 H, $J_{1',2'}$ 4.0, $J_{2',3'}$ 10.6 Hz, H-2'), 4.85 (dd, 1 H, $J_{1'',2''}$ 3.6 $J_{2'',3''}$ 10.2 H-2'), 5.02 (dd, 1 H, $J_{2,3}$ 10.5 Hz, H-2), 5.02 (dd t, 1 H, $J_{3',4'}$ 9.2 Hz, H-4'), 5.04 (dd t, 1 H, $J_{3'',4''}$ 9.8 Hz, H-4''), 5.15 (d, 1 H, H-1''), 5.13 and 5.33 (each d, each 1H, *J* 15.6 Hz, C_6H_3-CH_2), 5.34 (dd t, 1 H, H-3'), 5.35 (d, 1 H, H-1'), 5.36 (dd t, 1 H, H-3), 5.44 (dd t, 1 H, H-3''), 7.82 (d, 1 H, $J_{5''',6'''}$ 8.1 Hz, H-6'''), 8.27 (dd, 1 H, $J_{3''',5'''}$ 1.8 Hz, H-5'''), and 8.71 (d, 1 H, H-3'''). Figures indicated without, with one or two primes refer to carbohydrate protons; figures indicated with three primes refer to aromatic protons.

Anal. Calc. for $C_{47}H_{59}NO_{30}$: C, 50,49; H, 5.32; N, 1.25. Found: C, 50.64; H, 5.29; N, 1.34.

4-Carboxy-2-nitrobenzyl O-α-D-*glucopyranosyl-(1→4)*-O-α-D-*glucopyranosyl-(1→4)*-β-D-*glucopyranoside* (**2**) — Compound **1** (1.54 g, 1.3 mmol) was treated with

0.1M sodium methoxide (13 mL) in methanol (40 mL) and dichloromethane (3 mL) for 30 min. After neutralization with cation-exchange resin (Amberlite IR-120, H^+) and filtration, the solution was concentrated. The resulting, clear syrup was suspended in water (20 mL) and treated with 1.5M NaOH (20mL) for 10 to 30 min. The base was neutralized again with cation-exchange resin, the suspension filtered, and the solution concentrated to yield **2** (771 mg, 82%), amorphous, $[\alpha]_D^{18}$ + 76° (*c* 0.64, water); ^{1}H-n.m.r. (500 MHz, D_2O): δ 3.28 (dd t, 1 H, $J_{3'',4''}$ 9.8, $J_{4'',5''}$ 10.0 Hz, H-4''), 3.29 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.8 Hz, H-2), 3.44 (ddd, 1 H, $J_{4,5}$ 9.6, $J_{5,6a}$ 5.4, $J_{5,6b}$ 2.0 Hz, H-5), 3.46 (dd, 1 H, $J_{1'',2''}$ 4.0, $J_{2'',3''}$ 10.0 Hz, H-2''^{a}), 3.47 Hz (dd, 1 H, $J_{1',2'}$ 3.9, $J_{2',3'}$ 10.0 Hz, H-2'^{a}), 3.50 (m, 1 H, H-5'^{b}), 3.50 (dd t, 1 H, $J_{3',4'}$ 9.4, $J_{4',5'}$ 9.0 Hz, H-4''^{c}), 3.55 (dd t, 1 H, $J_{3,4}$ 9.5 Hz, H-4^c), 3.58 (ddd, 1 H, $J_{5'',6a''}$ 5.0, $J_{5'',6b''}$ 2.0 Hz, H-5''^{b}), 3.62 (dd, 1 H, $J_{5',6a'}$ 4.8, $J_{6a',6b'}$ 12.0 Hz, H-6a'^{d}), 3.63 (m, 1 H, $J_{6a,6b}$ 12.0 Hz, H-6a^d), 3.63 (dd t, 1 H, H-3^e), 3.68 (dd, 1 H, $J_{6a'',6b''}$ 12.0 Hz, H-6a''), 3.70 (dd t, 1 H, H-3'^{e}), 3.70 (dd, 1 H, $J_{5',6b'}$ 2.2 Hz, H-6b'^{d}), 3.71 (dd, 1 H, H-6b''^{d}), 3.76 (dd, 1 H, H-6b^d), 3.81 (dd t 1 H, H-3''^{e}), 4.43 (d, 1 H, H-1), 5.06 and 5.16 (each d, each 1 H, *J* 15.0 Hz, C_6H_3-CH_2), 5.25 (d, 1 H, H-1''), 5.25 (d, 1 H, H-1'), 7.78 (d, 1 H, $J_{5''',6'''}$ 8.0 Hz, H-6'''), 8.08 (dd, 1 H, $J_{3''',5'''}$ 1.7 Hz, H-5'''), and 8.44 (d, 1 H, H-3'''). Figures indicated without, with one or two primes refer to carbohydrate protons; figures indicated with three primes refer to aromatic protons; the attributions of signals marked by superscripts, a, b, c, d, and e, may be inverted.

Anal. Calc. $C_{26}H_{37}NO_{20}\cdot H_2O$: C, 44.51; H, 5.60; N, 1.99. Found: C, 44.08; H, 5.45; N, 2.03.

4-Carboxy-2-nitrobenzyl O-α-D-*glucopyranosyl-(1→6)*-O-α-D-*glucopyranosyl-(1→4)*-β-D-*glucopyranoside* (**5**). — Compound **4** (143 mg, 0.13 mmol) in methanol (9 mL) was treated with 0.1M sodium methoxide (2 mL) in methanol for 30 min. The base was neutralized (Amberlite IR-120, H^+), and, after filtration, the solution was concentrated. The resulting syrup was dissolved in water (3 mL) and treated with M NaOH (3 mL) for 20 min. Neutralization, filtration, and evaporation to dryness yielded **5** (72 mg, 81%); amorphous, $[\alpha]_D^{18}$ + 36.5° (*c* 0.58, water); ^{1}H-n.m.r. (500 MHz, D_2O): δ 3.34 (dd, H-1, $J_{1,2}$ 8.0, $J_{2,3}$ 9.6 Hz, H-2), 3.34 (dd t, 1 H, $J_{3'',4''}$ 9.0, $J_{4'',5''}$ 9.6 Hz, H-4''^{a}), 3.41 (dd t, 1 H, $J_{3',4'}$ 9.0, $J_{4',5'}$ 9.6 Hz, H-4'^{a}), 3.48 (dd, 1 H, $J_{1',2'}$ 3.6, $J_{2',3'}$ 9.6 Hz, H-2'^{b}), 3.50 (m, 1H, H-5), 3.51 (dd, 1 H, $J_{1'',2''}$ 3.8, $J_{2'',3''}$ 9.9 Hz, H-2''^{b}), 3.57 (dd t, 1 H, $J_{3,4}$ 9.2 Hz, H-3), 3.60 (dd t, 1 H, $J_{4,5}$ 9.2 Hz, H-4^a), 3.63 (m, 1 H,H-5''^{c}), 3.66 (m, 2 H, H-3',3''), 3.66 (dd, 1 H, $J_{5',6a'}$ 2.3, $J_{6a',6b'}$ 11.2 Hz, H-6a'^{d}), 3.68 (dd, 1 H, $J_{5'',6b''}$ 5.0, $J_{6a'',6b''}$ 12.0 Hz, H-6b''^{d}) 3.70 (dd, 1 H, $J_{5,6b}$ 5.5, $J_{6a,6b}$ 12.2 Hz, H-6b^d), 3.76 (dd, 1 H, $J_{5'',6a''}$ 2.1 Hz, H-6a''^{d}), 3.82 (ddd, 1 H, $J_{5',6b'}$ 4.8 Hz, H-5'), 3.85 (dd, 1 H, $J_{5,6a}$ 2.1 Hz, H-6a^d) 3.89 (dd, 1 H, H-6b'^{d}), 4.49 (d, 1 H, H-1), 4.87 (d, 1 H, H-1') 5.11 and 5.22 (each d, 1 H each, *J* 15.0 Hz, C_6H_3-CH_2), 5.31 (d, 1 H, H-1''), 7.86 (d,1 H, $J_{5''',6'''}$ 8.1 Hz, H-6'''), 8.17 (dd, 1 H, $J_{3''',5'''}$ 1.7 Hz, H-5'''), and 8.52 (d, 1 H, H-3'''). Figures indicated without, with one or two primes refer to carbohydrate protons; figures indicated with three primes refer to aromatic protons; the attributions of signals marked by superscripts a, b, c, and d, may be inverted.

Anal. Calc. for $C_{26}H_{37}NO_{20}$: C, 45.68; H, 5.45; N, 2.05. Found: C, 45.89; H, 5.34; N, 1.99.

Acceptor polymers. — Aminohexyl-substituted poly(acrylamide) gel beads (H_2N-

Ⓟ, 0.3 mequiv. NH_2/g) were treated as described for the preparation of 2-nitro-4-(*N*-Ⓟ-carboximido)benzyl α-D-glucopyranoside[2]. 4-Carboxy-2-nitrobenzyl *O*-α-D-glucopyranosyl-(1→4)-β-D-glucopyranoside[1,2] (0.18 mmol), EDCD (1.06 mmol), and polymer (300 mg) gave 2-nitro-4-(*N*-Ⓟ-carboxamido)benzyl *O*-α-D-glucopyranosyl-(1→4)-β-D-glucopyranoside (**6**; 200 mg) which released 250 μmol of maltose/g upon irradiation. Compound **2** (1.1 mmol), EDCD (6.2 mmol), and polymer (2.0 g) gave 2-nitro-4-(*N*-Ⓟ-carboxamido)benzyl *O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-β-D-glucopyranoside (**7**; 1.84 g), which released 67 μmol of maltotriose/g upon irradiation. 4-Carboxymethyl-2-nitrobenzyl O-α-D-glucopyranosyl-(1→4)-[*O*-α-D-glucopyranosyl)-(1→6)]-β-D-glucopyranoside[3] (0.42 mmol), EDCD (2.3 mmol), and polymer (1.4 g) gave 2-nitro-4-(*N*-Ⓟ-carboxamido)benzyl *O*-α-D-glucopyranosyl-(1→4)-[*O*-α-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside (**10**, 1.42 g), which released 70 μmol of trisaccharide/g. Compound **5** (0.12 mmol), EDCD (0.7 mmol), and polymer (300 mg) gave 2-nitro-4-(*N*-Ⓟ-carboxamido)benzyl *O*-α-D-glucopyranosyl-(1→6)-*O*-α-D-glucopyranosyl-(1→4)-β-D-glucopyranoside (**12**, 290 mg), which released 142 μmol of panose/g.

Glycogen synthase reaction. — The incubation conditions were based on those of Salsas and Larner[15], and on our previous work[1]. In Experiment 1, the polymer (5 μmol of sugar acceptor) was preincubated for 20 min, with stirring at 30°, in a mixture (2.0 mL) containing UDP-D-[U-^{14}C]glucose (6.5 μmol, 144 670 d.p.m./μmol), 50mM tris-(hydroxymethyl)aminomethane hydrochloride (Tris·HCl; pH 7.8), 5mM ethylenedinitrilo(tetracetic acid) (EDTA, 5mM 1,4-dithio-DL-threitol, 10mM D-glucose 6-phosphate, and 2mM Na_2SO_4. Glycogen synthase (0.5 unit in 0.6 mL) was added and the incubation was continued for 18 h at 30°. The polymer was isolated by filtration, washed extensively with 5% aqueous NaCl and water (until no radioactivity was released in the washings), and lyophilized.

In Experiment 2, the incubation was carried out as in Experiment 1, but in ultrafiltration cells equipped with Diaflo PM-10 membranes (Amicon, Lexington, MA 02173, U.S.A.). After 6 and 12 h of incubation, most of the solution was removed by filtration and the remainder washed with 50mM Tris·HCl buffer, pH 7.8. The incubation mixture and glycogen synthase were added and the incubation was continued for a total of 18 h.

Release of saccharides. — The polymer was suspended in water (100 mg/20 mL) and irradiated (20 h) in an RPR-100 apparatus (Rayonet, the Southern New England Ultraviolet Company, Hamden, CT 06514, U.S.A.) with RPR 3500Å lamps. The treated polymer was removed by filtration and washed with warm water, the filtrate and the washings were lyophilized, and the residue was subjected to descending paper chromatography (Whatman No. 3MM paper; 3:5:1:3 pyridine–butanol–benzene–water, upper phase; the maltodextrin markers were stained by $AgNO_3$[16]).

Methylation analysis. — Compound **14**, isolated after irradiation of **9** from the region in paper chromatography corresponding to maltotetraose, was subjected to methylation analysis[17] to give two peaks (R_T 4.66 and 7.35 min, on a 530μ methylsilicone

semicapillary column, Hewlett–Packard; temp., 150°; flow, 10 mL of N_2/min) which comigrated with 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl- and 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-glucitol, respectively (retention time for 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methyl-D-glucitol, 7.98 min).

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