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ENZYMIC SYNTHESIS OF OLIGOSACCHARIDES ON A POLYMER SUPPORT. LIGHT-SENSITIVE, SUBSTITUTED POLYACRYLAMIDE BEADS*

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ABSTRACT

Two light-sensitive, *gluco* and *cellobio* polymers, were synthesized by formation of an amide bond between 4-carboxy-2-nitrobenzyl β-D-glucopyranoside or 4-carboxy-2-nitrobenzyl 4-*O*-β-D-glucopyranosyl-β-D-glucopyranoside, respectively, and aminoethyl-substituted, polyacrylamide-gel beads. Subsequently, the polymers were used as acceptors of glycosyltransferase (D-galactosyltransferase, EC 2.4.1.22) and transglycosylase (lysozyme, EC 3.4.1.17) activity. As 2-nitrobenzyl derivatives, the polymers did undergo intramolecular oxidation-reduction upon irradiation (>320 nm), followed by the release of the free, reducing oligosaccharides.

INTRODUCTION

Oligosaccharide synthesis on polymer supports, although not yet competitive with classical solution chemistry, has attracted considerable interest in recent years[1,2]. In our initial contribution to the field[2], we discussed the advantages and disadvantages of this synthetic approach. The argument presented at the time holds even now, the major difficulties in synthesis on polymer supports being (a) incomplete glycosylation reactions; (b) scarcity of reagents suitable as building blocks, particularly when stereochemical control of the glycosidic bond synthesized is desired, which results in a severely limited number of synthetic structures attainable; and (c) necessity to introduce improved protecting groups.

In the current work, we attempted to overcome the difficulties mentioned under (b) and (c) by a combined, organic-enzymic approach. A similar synthesis of a substituted disaccharide has been described[3]. Our goal was to synthesize free oligosaccharides with a suitable polymer. Such a support has to be stable under reaction conditions, provide for suitable accessability to substrates and enzymes in

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aqueous solution, and possess a strong link to an acceptor saccharide, but eventually permitting the release of an intact, free oligosaccharide.

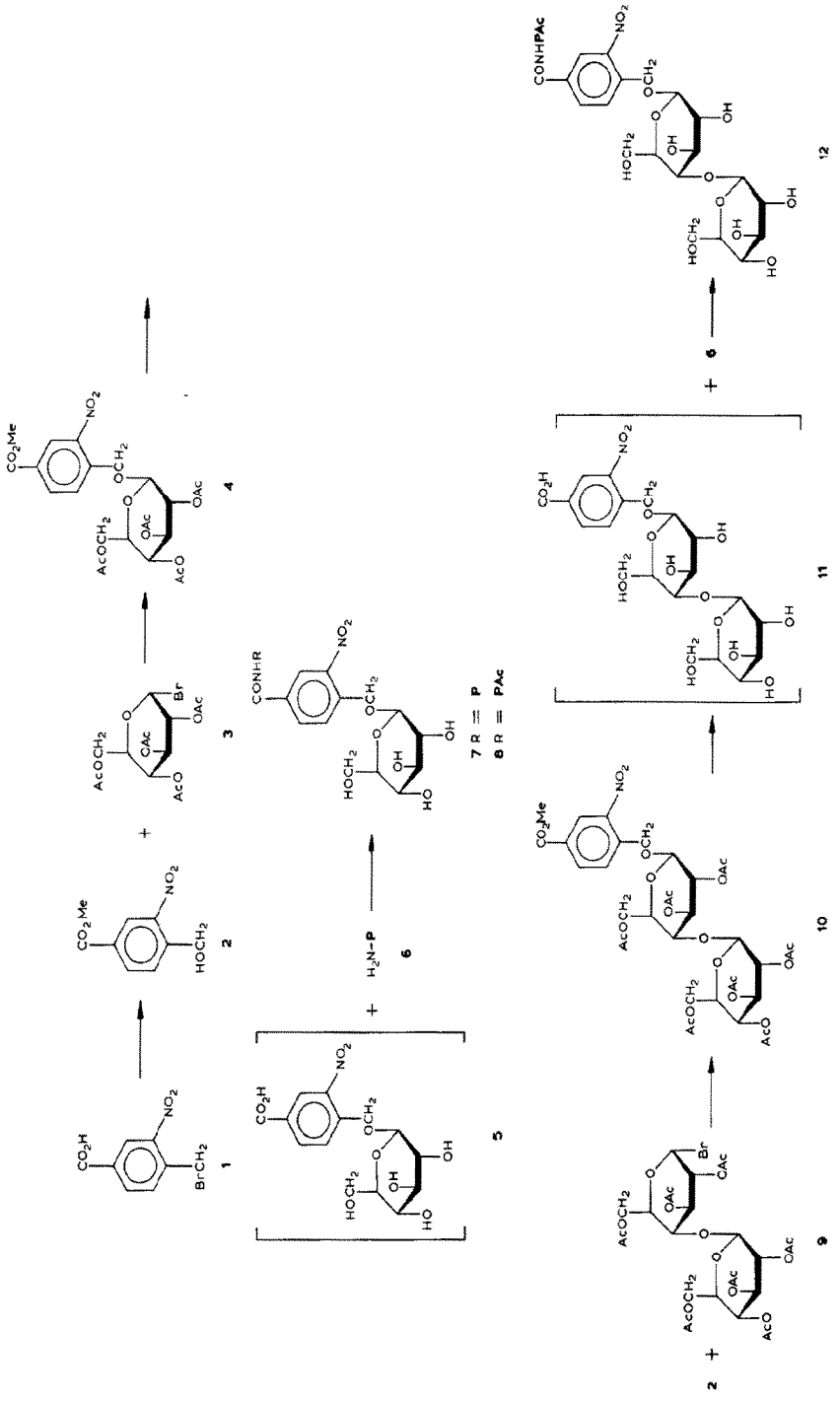
RESULTS AND DISCUSSION

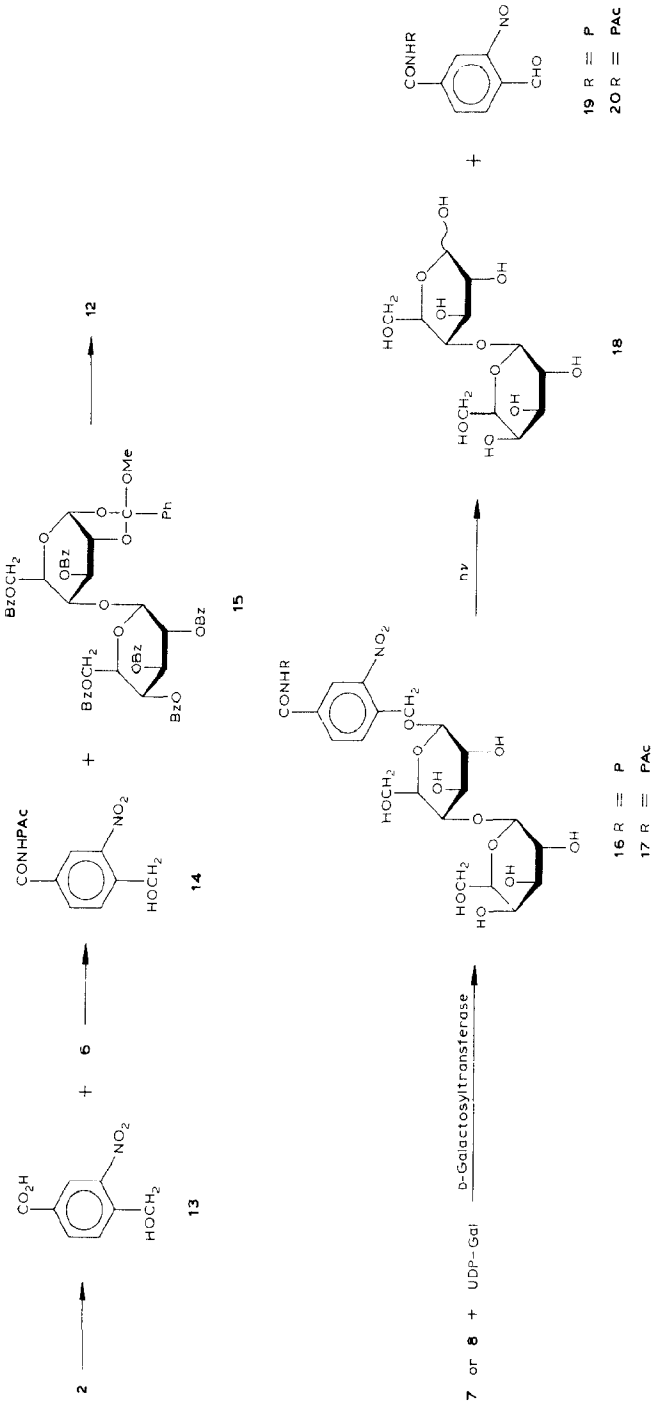
Two glycosides, 4-carboxymethyl-2-nitrobenzyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (**4**) and 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranoside (**10**), were synthesized by a Koenigs–Knorr condensation of methyl 4-hydroxymethyl-3-nitrobenzoate (**2**) and the corresponding glycosyl bromides **3** and **9**. Both compounds, pure according to t.l.c., are the β-D anomers, as evidenced by low optical rotations and $J_{1,2}$ values in the ^{1}H-n.m.r. spectra ($J_{1,2}$ 7.919 for **4**, and 7.877 for **10**). Comparison of the ^{1}H-n.m.r. spectrum of **10** with that of **4** suggests that the signals at δ 4.055, 4.374, and 4.648 correspond to the glycosyl residue linked to the aromatic aglycon, and the signals at δ 3.813, 4.128, 4.521, 4.522, and 4.931 correspond to the other residue. Removal of the protecting groups from **4** and **10** gave **5** and **11**, respectively, not isolated and having a free carboxylic group suitable for the formation of a stable amide bond to aminoethyl substituted polyacrylamide gel beads **P**-NH_2 (**6**).

Linking the unprotected glycoside **5** to the polymer **6**, in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCD) as the condensing agent, proceeded, however, in modest yield, and in even lower yield in the case of the bulklier, unprotected glycoside **11**. The polymers synthesized in this fashion were used either as such (**7**) or *N*-acetylated (**8** and **12**). A second procedure for the preparation of a *cellobio* polymer (**12**) was the initial attachment of **13** to **6**, followed by glycosylation with the orthoester **15** and debenzoylation. At present, the first procedure is preferred on the basis of yields of attachment.

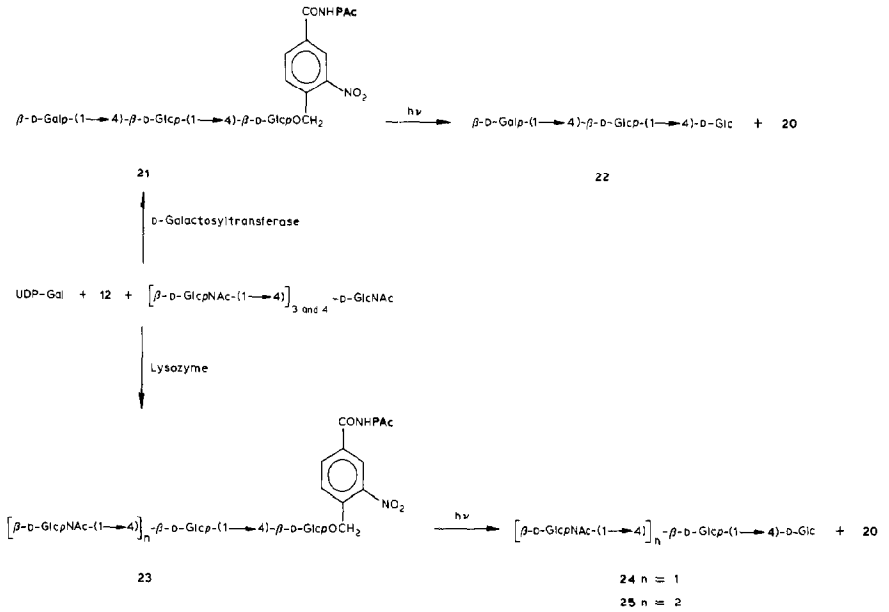
The *gluco* (**7** or **8**) and the *cellobio* (**12**) polymers served as acceptors, in D-galactosyltransferase reactions with labeled UDP-α-Gal as the D-galactosyl donor under the conditions of Nunez and Barker[3], to give the oligosaccharide-polymers **16**, **17**, and **21**, respectively. The polymer **6** devoid of glycosyl residue did not serve as acceptor in this reaction. The disaccharide-linked polymer **12** served also as acceptor, in transglycosylation catalyzed by lysozyme with chitin oligosaccharides as donors, to give polymer **23**.

All 2-nitrobenzyl derivatives (**1**, **2**, **4**, **7**, **8**, **10**, **12**, **13**, **14**, **16**, **17**, **21**, and **23**) are light sensitive, and those having a saccharide residue linked by an *O*-glycosyl bond (**4**, **7**, **8**, **10**, **12**, **16**, **17**, **21**, and **23**) were expected to release the saccharide residue upon irradiation[2,4,5]. A plausible mechanism for the release involves intramolecular oxidation–reduction and formation of a hemiacetal intermediate that splits into a reducing sugar and a nitrosoaldehyde. As judged by the release of radioactivity upon irradiation of ^{3}H-labeled **16**, **17**, and ^{14}C-labeled **21**, the release was quantitative. Irradiation of polymers **16** and **17** gave lactose, and the nitrosoaldehyde polymers **19** and **20** possessing a typical aldehyde absorbance at 1710 cm^{-1} (Fig. 1A and 1B). Irradiation of **21** released *O*-β-D-galactopyranosyl-(1→4)-*O*-β-




2
13
+ 6
14
+
15
12
7 or 8 + UDP-Gal
β-Galactosyltransferase
16 R = P
17 R = PAc
hν
18
+
19 R = P
20 R = PAc

D-glucopyranosyl-(1→4)-D-glucopyranose (**22**) and polymer **20** (Fig. 1C). Irradiation of **23** released polymer **20** and oligosaccharides containing both 2-acetamido-2-deoxy-D-glucose and D-glucose; two of these, *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-*O*-β-D-glucopyranosyl-(1→4)-D-glucopyranose (**24**) and *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→4)-D-glucopyranose (**25**) were isolated following paper chromatography in solvent systems *C* and *D*, in 4.1% and 4.7% yield (transfer, irradiation, and extraction), respectively. The structural assignment of the synthesized oligosaccharides was based on chromatographic data, including authentic markers for **22** (ref. 6), **24**, and **25** (ref. 7).



The low accessability of amino functions in **6**, already at the stage of chemical attachment, was disappointing, providing only modest proportions of acceptor for the D-galactoyltransferase or transglycosylation reaction, a step that is impeded also by steric restrictions of the polymer. The transglycosylation reaction proceeded with a somewhat higher efficiency than the D-galactosyltransferase reactions.

The attachment of acceptor saccharides to a polymer *via* light-sensitive glycoside bonds has the advantage of a convenient cleavage, unaffected by steric factors, of free, reducing oligosaccharides from the polymer. Routinely, at the end of synthesis, the products are cleaved from the polymer by irradiation and purified by chromatography. At the present state of development, the method is applicable to reactions having very low transfer efficiencies, and may serve in the study of the ac-

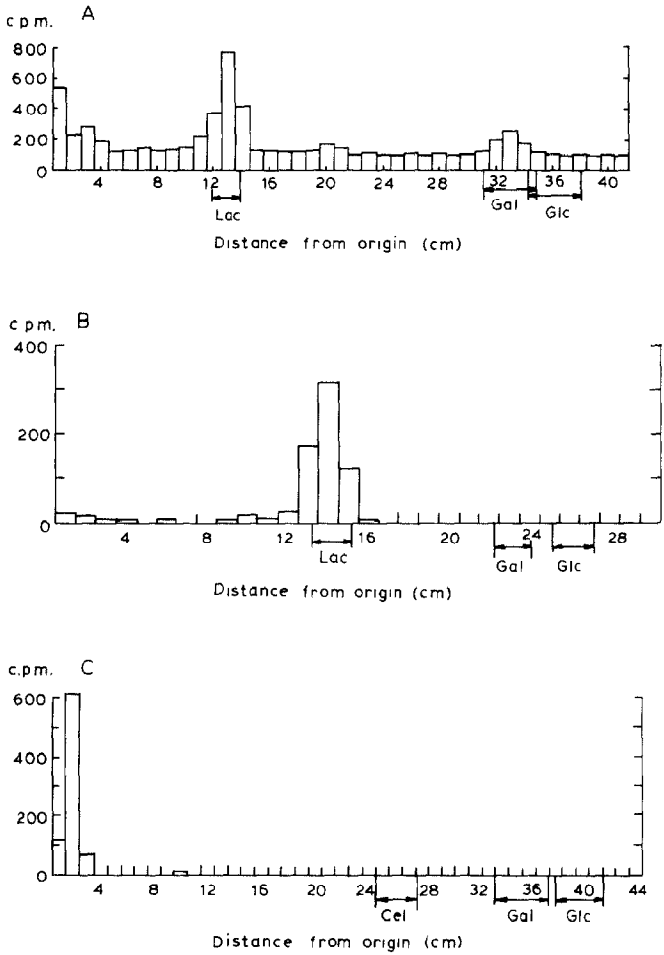


Fig. 1. Chromatographic separation of radioactive products released by irradiation of galactosylated polymers: (A) products released by the irradiation of polymer **16** (91 mg, solvent system *A*); (B) products released by the irradiation of polymer **17** (112 mg, solvent system *B*); and (C) products released by the irradiation of polymer **21** (284 mg, solvent system *B*); the peak was identified in a separate chromatography as **22**. Abbreviations: Lac, lactose; Cel, cellobiose.

ceptor specificity of enzymes, particularly in cases where the separation of products from reactants is very difficult.

EXPERIMENTAL

General. — Melting points were recorded with a Büchi 510 apparatus. Optical rotations were determined with a Bendix polarimeter. ^{1}H-N.m.r. spectra were recorded, for solutions in (^{2}H)chloroform, with a Varian Model FT80a (80 MHz) or a Bruker WH-270 (270 MHz) instrument; i.r. spectra, for potassium bromide

discs, with a Perkin–Elmer Model 237 spectrophotometer; u.v. spectra with a Beckman DB instrument; and colorimetric measurements with a Zeiss PMQ II instrument. Photolysis was carried out in a RPR-100 apparatus (Rayonet, The Southern New England Ultraviolet Company, Hamden, CT 06514) with RPR 3500A lamps in Pyrex glassware. Cross-linked polyacrylamide gel beads, Bio-Gel P-300 (100–200 mesh, wet), were obtained from Bio-Rad Laboratories (Richmond, CA 94804), and enzymes and chemicals from Sigma Chemical Co. (St. Louis, MO 63178) and Aldrich Chemical Co. (Milwaukee, WI 53201, U.S.A.). The enzymes used were: D-galactosyltransferase (EC 2.4.1.22) from bovine milk, and lysozyme (three times crystallized, EC 3.2.1.17) from chicken egg-white. UDP-D-[U-^{14}C]galactose, and UDP-D-[6-^{3}H]galactose were purchased from the Radiochemical Centre (Amersham, Bucks, HP75LL, U.K.). Scintillation counting was performed on a mixture (5 mL) containing toluene (676 mL), Triton X-100 (363 mL), 1,4-bis(5-phenyl-2-oxazolyl)benzene (200 mg), and 2,5-diphenyloxazole (5 mg); paper chromatograms were cut into 1-cm segments and wet with water (0.5 mL) prior to counting. Nitro derivatives described in this work are light-sensitive and routinely kept in the dark. Polymers were washed, handled, and titrated according to Inman[8].

Chromatographies. — Descending paper chromatography was performed on Whatman No. 1 paper for qualitative work, and on Whatman No. 3MM paper, previously washed with water and methanol, for preparative work. The paper was developed descending with (*A*) 4:1:1 1-butanol–ethanol–water, (*B*) 3:5:1:3 pyridine–1-butanol–benzene–water (upper phase), (*C*) 2:1:2 ethyl acetate–pyridine–water (upper phase), and (*D*) 25:6:25 1-butanol–acetic acid–water (upper phase), all solvents v/v. The sugars were detected with the silver nitrate[9] or aniline hydrogenphthalate[10] reagents, and oligosaccharides containing 2-acetamido-2-deoxy-D-glucose also with sodium hydroxide[11]. T.l.c. was performed on precoated Silica gel $60F_{254}$ sheets (E. Merck D-6600 Darmstadt, F.R.G.), and compounds were detected by viewing under u.v. light or by spraying with sulfuric acid. Column chromatography was performed on Silica gel 60 (0.063–0.2 mm, Merck).

Methyl 4-hydroxymethyl-3-nitrobenzoate (**2**). — 4-Bromomethyl-3-nitrobenzoic acid[12] (7 g) and anhydrous sodium acetate (7 g) were suspended in acetic acid (12 mL). The mixture was boiled under reflux, with stirring and under calcium chloride protection, for 24 h, and then was cooled to room temperature. A solution of M hydrogen chloride in methanol (50 mL) was added, and the reflux was resumed overnight. The solution was evaporated *in vacuo* to dryness (50° bath), water was added, and the resulting solution was extracted with ether (5 × 120 mL). The combined ether extracts were washed with cold M sodium hydrogencarbonate (3 × 100 mL) and water, treated with active charcaol, and dried (sodium sulfate). Compound **2** crystallized from ether–petroleum ether (2.7 g, 48%); m.p. 79°; $\lambda_{max}^{CHCl_3}$ 244 (ε 7348), 260 sh (6420), and 300 nm sh (1690); ν_{max}^{KBr} 3330–3240 (OH), 1730 (CO), and 1540 cm^{-1} (NO_2); ^{1}H-n.m.r. (80 MHz): δ 3.98 (s, 3 H, $COCH_3$),

5.08 (s, 2 H, benzylic CH_2), 7.91 and 8.33 (ABdd, 2 H, J 8.0 Hz, aromatic, of which the lower field d, is further split J 1.6 Hz), and 8.72 (d, H 1, $J \simeq 1.6$ Hz, aromatic).

Anal. Calc. for $C_9H_9NO_5$: C, 51.17; H, 4.30; N, 6.63. Found: C, 51.29; H, 4.39; N, 6.47.

*4-Carboxymethyl-2-nitrobenzyl 2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranoside* (**4**). — This compound was prepared, in analogy to a literature procedure[4], with the following amounts: 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide[13] (5 g, 12.1 mmol), compound **2** (2.2 g, 10.4 mmol), calcium sulfate (1 g), calcium carbonate (2 g), nitromethane (40 mL), and silver perchlorate (2 g). Following filtration through a Celite filter and evaporation, the residue was applied to a silica gel column (300 g, 3 cm in diameter) which was successively eluted with the following mixtures of ethyl acetate–petroleum ether (v/v): 1:9 (1 L), 4:21 (0.5 L), 1:4 (0.5 L), 1:3 (1 L), 3:7 (2.5 L) affording unreacted **2** (0.3 g), and 7:13 (1 L) giving **4**. The separation was monitored by t.l.c. in 3:7 (v/v) ethyl acetate–petroleum ether. Compound **4** was crystallized from ethyl acetate–petroleum ether (2.32 g, 48%); m.p. 112–113°, $[\alpha]_D^{25}$ −24.6 ±1.9° (*c* 0.22, chloroform); $\lambda_{max}^{CHCl_3}$ 244 (ε 8641), and 260 nm sh (6853); ν_{max}^{KBr} 1750–1730 (CO), and 1530 cm^{-1} (NO_2); ^{1}H-n.m.r. (270 MHz): δ 2.030 (s, 3 H, $OCOH_3$), 2.041 (s, 3 H, $OCOH_3$), 2.068 (s, 3 H, $OCOCH_3$), 2.087 (s, 3 H, $OCOCH_3$), 3.772 (o, 1 H, $J_{4,5}$ 9.898, $J_{5,6}$ 4.619, $J_{5,6'}$ 2.309 Hz, H-5), 3.981 (s, 3 H, OCH_3), 4.147 (q, 1 H, $J_{6,6'}$ 12.537 Hz, H-6'), 4.304 (q, 1 H, H-6), 4.705 (d, 1 H, $J_{1,2}$ 7.919 Hz, H-1), 5.062–5.358 (m, 5 H, H-2,-3,-4, benzylic CH_2) including 5.156 (q, 1 H, $J_{2,3}$ 9.238 Hz, H-2), 7.843 (d, 1 H, $J_{A,B}$ 8.248 Hz, aromatic A), 8.297 (q, $J_{B,X}$ 1.649 Hz, aromatic B), and 8.737 (d, aromatic X). Interpretation of the spectrum was supported by double irradiation experiments: irradiation at δ 3.77 caused the collapse of the quartet at δ 4.147 to a doublet ($J \simeq 12.5$ Hz), the quartet at δ 4.304 to a doublet ($J \simeq 12.5$ Hz), and a change at δ ~ 5.13; irradiation at δ 5.18 brought about the change of the octet at δ 3.772 to a narrow multiplet, and the collapse of the doublet at δ 4.705 to a singlet; irradiation at δ 4.7 caused the collapse of the quartet at δ 5.156 to a doublet ($J \simeq 9$ Hz); irradiation at δ 4.1 changed the octet at δ 3.772 to a quartet ($J \simeq 6$, $J \simeq 9$ Hz) and affected the quartet at δ 4.304; and irradiation at δ 4.3 affected the nearby quartet (δ 4.147) and caused the collapse of the octet at δ 3.772 to a quartet ($J \simeq 9$, $J \simeq 2$ Hz).

Anal. Calc. for $C_{23}H_{27}NO_{14}$: C, 51.02; H, 5.03; N, 2.59. Found: C, 51.10; H, 5.00; N, 2.62.

*4-Carboxymethyl-2-nitrobenzyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)-β-*D*-glucopyranoside* (**10**). — This compound was prepared and purified as described for compound **4**, but starting from 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-D-glucopyranosyl bromide[14] (7.4 g, 10.4 mmol). Following filtration through a Celite filter and evaporation, the residue was applied to a silica gel column (100 g, 2 cm in diameter), eluted with 1:1 (v/v) ethyl acetate–petroleum ether (10-mL fractions). Unreacted **2** was recovered

from fractions 15–26 (0.35 g), and **10** (4.23 g, 58%) was eluted on fractions 37–70 following detection by t.l.c. (3:7, v/v, ethyl acetate–petroleum ether). Compound **10** was recrystallized from ethyl acetate–petroleum ether as needles (3.29 g), m.p. 150°, $[\alpha]_D^{27}$ −11.7 ±1.1° (*c* 0.26, chloroform); $\lambda_{max}^{CHCl_3}$ 244 (ε 9727) and 260 nm sh (7473); ν_{max}^{KBr} 1750–1730 (CO) and 1535 cm^{-1} (NO_2); ^{1}H-n.m.r. (270 MHz): δ 1.986 (s, 3 H, $OCOCH_3$), 2.012 (s, 3 H, $OCOCH_3$), 2.038 (s, 6 H, 2 $OCOCH_3$), 2.058 (s, 3 H, $OCOCH_3$), 2.094 (s, 3 H, $OCOCH_3$), 2.119 (s, 3 H, $OCOCH_3$), 3.626–3.697 (m, 2 H, H-5a, -5b), 3.813 (apparent t, 1 H, $J_{3,4}$ 9.17, $J_{4,5}$ 9.696 Hz, H-4), 3.977 (s, 3 H, OCH_3), 4.055 (q, 1 H, $J_{5a,6'a}$ 1.99, $J_{6a,6'a}$ 12.687 Hz, H-6'a), 4.128 (q, 1 H, $J_{5b,6b}$ 5.487, $J_{6b,6'b}$ 12.001 Hz, H-6b), 4.374 (q, 1 H, $J_{5a,6a}$ 4.457 Hz, H-6a), 4.521 (d, 1 H, $J_{1c,2c}$ 8.6 Hz, H-1c), 4.522 (q, 1 H, $J_{5b,6'b}$ 1.7 Hz, H-6'b), 4.648 (d, 1 H, $J_{1d,2d}$ 7.877 Hz, H-1d), 4.931 (t, 1 H, $J_{2c,3c}$ 8.7 Hz, H-2c), 5.056–5.302 (m, 6 H), 7.806 (d, 1 H, J_{AB} 8.230 Hz, aromatic A), 8.274 (q, 1 H, J_{BX} 1.714 Hz, aromatic B), and 8.721 (d, aromatic X). Partial correlation (designated by the same lettering) of saccharide ring hydrogens was the result of double irradiation experiments: irradiation at δ 3.65 caused the collapse of the triplet at δ 3.813 to a doublet ($J \simeq 9.17$ Hz), the quartets at δ 4.055, 4.128, 4.374, and 4.522 to doublets ($J \simeq$ 12.7, 12.0, 12.7, and 12.0 Hz, respectively), and the change of pattern at δ ~5.09; irradiation at δ 5.1 transformed the multiplet at δ 3.636–3.697 to a considerably narrower one, the doublet at δ 4.648 collapsed to a singlet, and the multiplets between δ 5.12–5.30 were affected; irradiation at δ 3.81 caused the narrowing of the multiplets at δ 3.626–3.692 and a change in the multiplets at δ ~5.22; irradiation at δ 4.05 changed the multiplet at δ 3.626–3.697 and caused the collapse of the quartet at δ 4.374 to a narrow doublet ($J_{4,5} \simeq 4.5$ Hz); irradiation at δ 4.1 changed the multiplet at δ 3.626–3.697 and affected the quartet at δ 4.522; irradiation at δ 4.37 caused a change in the multiplet at δ 3.626–3.697 and the collapse of the quartet at δ 4.055 to a doublet ($J \simeq 2.0$ Hz); irradiation at δ 4.52 changed the multiplet at δ 3.626–3.697 and caused the collapse of the triplet at δ 4.931 to a doublet ($J \simeq 8.7$ Hz); irradiation at δ 4.64 caused a change in pattern at δ ~5.04; and irradiation at δ 4.93 caused the collapse of the doublet at δ 4.521 to a singlet and affected the spectrum at δ ~5.18–5.12.

Anal. Calc. for $C_{35}H_{43}NO_{22}$: C, 50.66; H, 5.22. Found: C, 50.90; H, 5.33.

4-Hydroxymethyl-3-nitrobenzoic acid (**13**). — Compound **2** (200) mg was stirred in 6M sodium hydroxide (20 mL) for 1 h at room temperature. The mixture was washed with ether (10 mL), acidified (6M hydrochloric acid), and extracted with ethyl acetate. The extract was evaporated and the residue crystallized from water as slightly yellowish needles (186 mg, quantitative yield), m.p. 174–175°; ν_{max}^{KBr} 3600–3200 (OH), 1670 (CO), and 1530 cm^{-1} (NO_2); ^{1}H-n.m.r. [80 MHz, ($^2H_6$)dimethyl-sulfoxide]: δ 4.90 (s, 2 H, benzylic CH_2), 7.98 and 8.28 (ABdd, 2 H, *J* 8.1 Hz, aromatic, of which the lower field d is further split, *J* 1.5 Hz), and 8.48 (d, 1 H, *J* 1.5 Hz, aromatic).

Anal. Calc. for $C_8H_7NO_5$: C, 48.74; H, 3.58. Found: C, 48.69; H, 3.66.

2-Nitro-4-[N-(**P** *and* **PAc**)*-carboxamido*]*benzyl β-*D*-glucopyranoside* (**7** *and*

8). — Compound **4** (383 mg, 0.7 mmol) was dissolved in methanolic 0.1M sodium methoxide (1.7 mL) and kept overnight at 4°. The solvent and additional methanol were evaporated *in vacuo* at room temperature. The residue was dissolved in 6M sodium hydroxide (6.7 mL) and, after 1 h at room temperature, the solution was made neutral with 6M hydrochloric acid. This solution containing the unblocked glycoside was used for condensation with aminoethyl-substituted polyacrylamide gel[8,15] **P**-NH_2 (**6**) (0.96 g, 0.37 meq. NH_2/g, preswollen in water, 47 mL). *N,N*-Dimethylformamide (25 mL) was added, the mixture kept at room temperature, the pH adjusted to 4.7, and EDCD (6 portions, each 200 mg in 1.2 mL of water)[16] was added at 2-h intervals. Stirring was continued overnight, the polymer was filtered off, washed with 2:1, v/v, *N,N*-dimethylformamide–water (until no absorbance at 300 nm was observed), and then M hydrochloric acid, water, 0.2M sodium carbonate, and water, and finally lyophilized. Upon irradiation, polymer **7** released 36 μeq. of Glc/g (9.7% yield, attachment and release).

Polymer **7** (810 mg) was stirred in water (40 mL) and, after swelling, sodium hydrogencarbonate (810 mg) was added. The mixture was cooled to 4°, acetic anhydride (0.53 mL) added, and the stirring continued overnight. Polymer **8** was isolated and washed as described for polymer **7**; it contained 80 μeq. of NH_2/g and released, upon irradiation, 37 μeq. of Glc/g; ν_{max}^{KBr} 3550–3250 (OH) and 1690–1620 cm^{-1} (CO).

2-Nitro-4-(N-**PAc**-*carboxamido*)*benzyl 4*-O-β-D-*glucopyranosyl*-β-D-*glucopyranoside* (**12**). — (*a*). This compound was prepared as described for compound **8** but starting from the *cellobio* derivative **10**, and **6** containing 3.9 meq. of NH_2/g. Polymer **12** released 11 μeq. of cellobiose/g upon irradiation (0.3% yield; 1.6% yield for a polymer containing 0.37 meq. of NH_2/g) and contained 0.2 meq. of NH_2/g; ν_{max}^{KBr} 3500–3200 (OH) and 1680–1620 cm^{-1} (CO).

(*b*). Polymer **14** (1 g; 0.2 meq. of bound compound **13**/g, found by difference in NH_2 titration) and 3,6-di-*O*-benzoyl-1,2-*O*-(1-methoxylbenzylidene)-4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-α-D-glucopyranose[17] (**15**) (542 mg, 0.5 mmol) in chlorobenzene (50 mL) was stirred under reflux and a nitrogen atmosphere. Part of the solvent (25 mL) was distilled off, 2,6-dimethylpyridinium perchlorate (0.2 mL, 0.2M in 1,2-dichloroethane) injected into the reaction mixture, and the treatment continued for 16 h. The mixture was brought to room temperature, and the polymer was collected by filtration, washed successively with chlorobenzene, chlorobenzene–*N,N*-dimethylformamide, *N,N*-dimethylformamide, *N,N*-dimethylformamide–methanol, and methanol. The polymer was suspended in sodium methoxide (15 mL, 0.1M in methanol) and stirred overnight at 4°. Filtration, and washing with methanol and water afforded polymer **12** releasing 5.1 μeq. of cellobiose/g upon irradiation.

4-Hydroxymethyl-3-nitrobenzamido-**PAc** (**14**). — Compound **13** (588 mg, 4 mmol) was added to **6** (0.37 meq. of NH_2/g; 5.44 g) previously swollen in 1:1 (v/v) water–*N,N*-dimethylformamide (600 mL). The pH of the stirred mixture was brought to 4.7, EDCD (4 portions of 0.5 g each) added at 2-h intervals, and stirring

continued overnight. Titration indicated the presence of 0.17 meq. of NH_2/g. Free amino groups were blocked and the polymer was washed as described for polymer **8**. Titration indicated the presence of 0.02 meq. of NH_2/g.

2-Nitro-4-[N-(**P** *and* **PAc**)*-carboxamido*]*benzyl 4-*O-*β*-D-*galactopyranosyl-β*-D-*glucopyranoside* (**16** *and* **17**). — (*a*). Polymer **7** (630 mg, 36 μeq. of Glc/g), UDP-Gal (40 mg) and UDP-D-[6-^{3}H]galactose (624 000 c.p.m./mg), galactosyltransferase (5 units), and α-lactalbumin (20 mg) in sodium cacodylate buffer (30 mL, pH 7.0, 0.25mM containing 3mM $MnCl_2$ and 0.1% mercaptoethanol) were incubated with stirring for 3 days at 37°. Polymer **16** was collected by filtration and washed extensively with water until no radioactivity was observed in eluates.

(*b*). Polymer **8** (630 mg, 37 μeq. of Glc/g), UDP-Gal (80 mg) and UDP-D-[U-^{14}C]galactose (68 600 c.p.m./mg), galactosyltransferase (8 units), and α-lactalbumin (20 mg) were incubated in the buffer (22.4 mL) just described for 2 days and washed as described in (*a*) to give polymer **17**.

2-Nitro-4-(N-**PAc**-*carboxamido*)*benzyl* O-*β*-D-*galactopyranosyl-*(*1→4*)-O-*β*-D-*glucopyranosyl-*(*1→4*)-O-*β*-D-*glucopyranoside* (**21**). — Polymer **12** (300 mg, 11 μeq. of cellobiose/g), UDP-Gal (40 mg) and UDP-D-[U-^{14}C]galactose (48 000 c.p.m./mg), galactosyltransferase (4 units), and α-lactalbumin (10 mg) were incubated in buffer (80 mL) for 2 days and washed as described for compound **16**.

Transfer of chitin oligosaccharides to polymer **12**. — Polymer **12** (1 g, 67 μeq. of cellobiose/g) was suspended in 0.1M ammonium acetate buffer (15 mL, pH 5.25). A 1:1 mixture of tetra- and penta-*N*-acetylchitopentaose[18] (309 mg), and lysozyme (0.9 mL, 3 mg/mL) was added and the mixture stirred for 48 h at 37°. The product, polymer **23**, was collected by filtration or centrifugation following repeated washing with warm water and centrifugation.

Estimation of the amount of sugar released from the polymer by irradiation. — The polymer (10–15 mg) was suspended with stirring in water (1 mL) in a closed test-tube, and irradiated for 20 h. The suspension was filtered, when applicable, and D-glucose[19], or in other cases total sugar[20], was determined in the filtrate. No increase in saccharide release was observed after 10 h. In experiments with radioactive material, the irradiation resulted in a quantitative release of radioactivity from the polymer.

Release of saccharide. — Polymer **16**, **17**, **21**, or **23** was suspended, by stirring at a concentration of 1 g/L, in water in a closed cylinder and irradiated for 20 h. The irradiated polymer **19** or **20** [ν_{max}^{KBr} 3550–3150 (OH), 1710 (CHO), and 1680–1610 cm^{-1} (CO)] was filtered off, the filtrate was lyophilized, and the products were examined by and isolated from preparative paper chromatography. Based on the number of c.p.m. of the starting polymers **16** and **17**, the yield for isolated lactose (**18**; galactosyltransferase addition, irradiation, and chromatography) was 0.06 and 0.36%, respectively. Similarly, starting from polymer **21**, the yield of product **22** was 0.87%.

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REFERENCES

1 J. M. J. FRÉCHET, in P. HODGE AND D. C. SHERRINGTON (Eds.), *Polymer-supported Reactions in Organic Synthesis*, Wiley, 1980, pp. 407–434.
2 U. ZEHAVI AND A. PATCHORNIK, *J. Am. Chem Soc.*, 95 (1973) 5673–5677.
3 H. A. NUNEZ AND R. BARKER, *Biochemistry*, 19 (1980) 489–495.
4 U. ZEHAVI, B. AMIT, AND A. PATCHORNIK, *J. Org. Chem.*, 37 (1972) 2281–2285.
5 U. ZEHAVI AND A. PATCHORNIK, *J. Org. Chem.*, 37 (1972) 2285–2288.
6 F. L. SCHANBACHER AND K. E. EBNER, *J. Biol. Chem.*, 245 (1970) 5057–5061.
7 U. ZEHAVI, J. J. POLLOCK, V. I. TEICHBERG, AND N. SHARON, *Nature (London)*, 219 (1968) 1152–1154.
8 J. K. INMAN, *Methods Enzymol.*, 34 (1972) 30–58.
9 N. SHARON AND R. W. JEANLOZ, *J. Biol. Chem.*, 235 (1960) 1–5.
10 S. M. PARTRIDGE, *Nature (London)*, 164 (1949) 443.
11 N. SHARON AND S. SEIFTER, *J. Biol. Chem.*, 239 (1964) PC 2398.
12 D. H. RICH AND S. K. GURWARA, *J. Am. Chem. Soc.*, 97 (1975) 1575–1579.
13 C. E. REDEMANN AND C. NIEMANN, *Org. Synth.*, 22 (1942) 1–4.
14 E. FISCHER AND G. ZEMPLÉN, *Ber.*, 43 (1910) 2536–2543.
15 J. K. INMAN AND H. M. DINTZIS, *Biochemistry*, 8 (1969) 4074–4082.
16 P. CUATRECASAS, *J. Biol. Chem.*, 245 (1970) 3059–3065.
17 N. K. KOCHETKOV, A. YA KHORLIN, A. F. BOCHKOV, L. B. DEMUSHKINA, AND I. O. ZOLOTUKHIN, *Zh. Obshch. Khim.*, 37 (1967) 1272–1277; *J. Gen. Chem. USSR (Eng. Transl.)*, 37 (1967) 1205–1209.
18 U. ZEHAVI AND R. W. JEANLOZ, *Biochem. Prep.*, 13 (1971) 14–18.
19 E. RAABO AND T. C. TERKILDSEN, *Scand. J. Clin. Lab. Invest.*, 12 (1960) 402–407.
20 M. K. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS, AND F. SMITH, *Anal. Chem.*, 28 (1956) 350–356.