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Synthesis of chromogenic substrates of α-amylases on a cyclodextrin basis [1]

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Abstract

One-pot acetylation and subsequent partial acetolysis of α-, β- and γ-cyclodextrins resulted in crystalline peracetylated malto-hexaose, -heptaose, and -octaose, respectively. Prolonged acetolysis of β-cyclodextrin gave a mixture of acetylated maltooligosaccharides, from which peracetylated malto-triose, -tetraose, and -pentaose were isolated. The acetylated oligosaccharides were converted into α-acetobromo derivatives, and then transformed into 4-nitrophenyl and 2-chloro-4-nitrophenyl β-glycosides. From the 4-nitrophenyl glycosides 4,6-*O*-benzylidene derivatives were prepared, which were used together with the free glycosides as substrates of porcine pancreatic α-amylase. © 1997 Elsevier Science Ltd.

Keywords: Acetolysis; Cyclodextrins; Maltooligosaccharide derivatives; α-Amylase substrate

1. Introduction

Higher maltooligosaccharides are valuable synthetic intermediates and often used as substrates of different α-amylases [1–12]. However, their chemical synthesis is rather tedious and cannot be used for preparative purposes. α-, β-, and γ-cyclodextrins (CD's), prepared on industrial scale, contain six, seven and eight α-(1 → 4)-bonded glucopyranosyl units, respectively. Although the conversion of these cyclic oligosaccharides into linear maltooligosaccharides either by enzymatic or acid hydrolysis [13–20] seems to be obvious, all of these efforts have failed, because under the conditions used the linear dextrins are more sensitive than their cyclic counterparts, i.e. the rate-determining step of these hydrolytic procedures is the opening of the cyclodextrin rings [21]. However, a Japanese patent reported the maleic acid-catalysed hydrolysis of β-cyclodextrin in a yield of 60% [22].

The H_2SO_4-catalysed acetolysis of peracetylated α-, β- and γ-cyclodextrins was patented and reported in 1988 by us [23] and in 1995 by N. Sakairi and coworkers [24,25]. Under the applied conditions about 80% of the peracetylated cyclodextrins were converted into acetylated maltohexaose, -heptaose, and -octaose. In the case of β-cyclodextrin, the unchanged acetylated starting compound could be re-

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[1] Dedicated to Professor Dr. Hans Paulsen on the occasion of his 75th birthday.

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moved selectively by crystallization in form of its toluene complex. The overall yield of the acetylated maltooligomers was about 70%, and all of the three linear dextrins were isolated in α-anomeric form.

2. Results and discussion

The aim of our research was the preparation of suitable substrates for the measurement of the activity of human pancreatic α-amylase in biological fluids. Instead of the generally used methods for the preparation of acetylated cyclodextrins, the cyclodextrins were acetylated in acetic anhydride in the presence of various acids (H_2SO_4, HCl, $HClO_4$) and Lewis acids ($ZnCl_2$, $AlCl_3$, $FeCl_3$). $FeCl_3 \cdot 6H_2O$ turned out to be the most suitable catalyst. β-Cyclodextrin **2** was suspended in acetic anhydride, and 0.25 equivalent of $FeCl_3.6H_2O$ was added to the suspension with cooling. The suspension became a homogenous solution on stirring, and after 2.5 h at 35–40 °C the acetylation was complete. The temperature of the reaction mixture was raised to 70 °C for 3.5 h. During this time the acetolysis was nearly complete; the amount of the peracetylated β-cyclodextrin was below 15%; the amount of the acetylated matoheptaose reached 45–50%, but all of the other hydrolysis products were also present. The syrupy product, obtained after usual workup, was crystallized from EtOH to give **5** with a 95% purity (the ratio of the α and β anomers was 4.3:1), and after an additional two recrystallizations the purity of the compound **5** was 99.0% (the ratio of the α and β anomers was 9:1) and the overall yield was 22%. The same procedure was applied for the acetolysis of α-**1** and γ-cyclodextrin **3** to give the crystalline peracetylated maltohexaose **4** and the peracetylated maltooctaose **6**, respectively (Fig. 1).

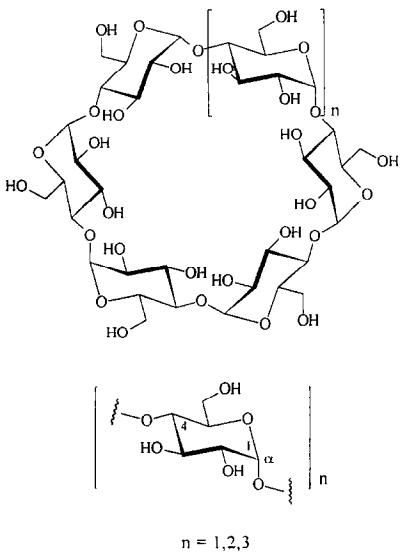


Fig. 1. Cyclodextrins (CD's): **1**: $n = 1$, α-CD; **2**: $n = 2$, β-CD; **3**: $n = 3$, γ-CD.

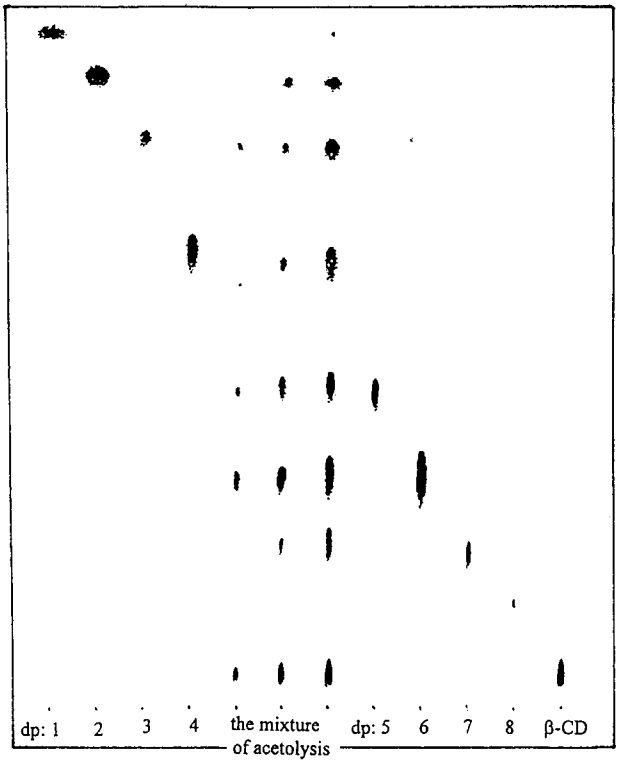


Fig. 2. Separation of peracetylated maltooligosaccharides (DP 1–8) and peracetylated β-CD on TLC, 86:14 hexane–ethyl-acetate. Three samples from the acetolysis mixture in different concentration after 5 h reaction time.

In order to prepare lower maltooligomers (maltotriose, -tetraose, and -pentaose) the time of the acetolysis of the cheap β-cyclodextrin peracetate was extended to 5 h. For following the product-composition of the acetolysis a suitable TLC (Fig. 2) and an HPLC technique (Hewlett Packard, diol column, see Fig. 3) were elaborated. The desired peracetylated malto-triose **7**, -tetraose **8**, and -pentaose **9** were separated by column chromatography. The degree of polymerization of the complete series of the peracetylated maltooligomers was determined by counting the methyl signals of the acetyl region (1.8–2.3 ppm) in the ^1H NMR spectra [26] (Fig. 4).

Compounds **4–9** were treated with HBr–CH_3COOH in dry dichloromethane and the α-acetobromo sugars **10–15** were isolated in crystalline form, and their anomeric purity was verified by measuring the $J_{1,2}$ values. The bromosugars **10–15**

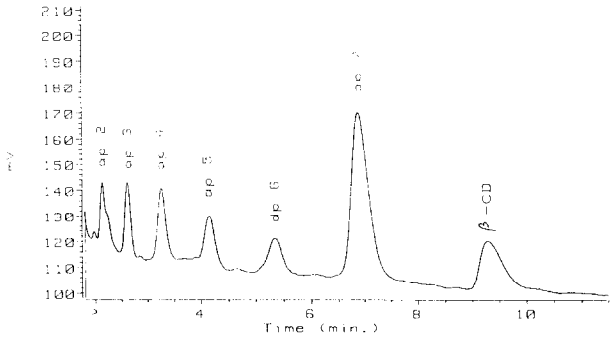


Fig. 3. Separation of peracetylated maltooligosaccharides (DP 2–7) obtained from the acetolysis of peracetylated β-CD by HPLC. It was carried out on APS column using 1:1 hexane–ethyl-acetate.

were used to glycosylate 4-nitro- (NP) and 2-chloro-4-nitrophenol (CNP) to furnish the peracetylated 4-nitrophenyl β-maltooligomers **16–21** and the peracetylated 2-chloro-4-nitrophenyl β-maltooligomers **22–27**. Removal of the acetyl groups of the glycosides **16–21** resulted in the chromogenic 4-nitrophenyl β-maltooligomer glycosides **28–33** and the 2-chloro-4-nitrophenyl β-maltooligomer glycosides **34–39**. The β-anomeric configuration in the case of the NP-glycosides **28–33** and CNP-maltotrioside **37** was confirmed by measuring the $J_{1,2}$ coupling constants of the 'reducing end' which were found to be 8.5–9 Hz. In the case of other CNP-glycosides **34–36** and **38**, **39** the $J_{1,2}$ coupling constants of the 'reducing end' could not be measured because the anomeric proton at the 'reducing end' is overlapped with other anomeric protons in the region of 5.3–5.5 ppm. (See Fig. 5.)

Both series were used as substrates of porcine pancreatic α-amylase. The 4-nitrophenyl β-maltooligomers were treated with α,α-dimethoxytoluene in the presence of *p*-toluenesulphonic acid to prepare their 4,6-*O*-benzylidene acetals [27] **40–45**, whose structure was proved by the detection of the presence of the acetalic hydrogen in the region of 5.6–5.7 ppm.

These modified α-amylase substrates showed high stability towards different α-glycosidases, and their use provided a valuable information about the topology of the catalytic site, as well as the cleavage pattern of the porcine pancreatic α-amylase [12].

3. Experimental

General methods.—Optical rotations were measured at rt with a Perkin–Elmer 241 automatic polarimeter. The ^1H (200, 500 MHz) and ^{13}C NMR (50.3 MHz) spectra were recorded with a Bruker WP-200 SY and a Bruker DRX-500 spectrometer (internal Me_4Si). Melting points were determined on a Kofler apparatus and are uncorrected. TLC was performed on Kieselgel 60 F_{254} (Merck) with detection by spraying with aq 50% H_2SO_4 followed by heating. Column chromatography was performed on Kieselgel 60 (Merck 63–200 mesh). A Hewlett–Packard 1090 series II Liquid Chromatograph equipped with a refractive index detector, an automatic sampler and a ChemStation was used for separation. The separation was made on an APS 5 μm (0.46 × 20) column using various hexane–ethyl-acetate mixtures.

General procedures.—Preparation of peracetylated maltooligomers from cyclodextrins (**4**,**5**,**6**). $FeCl_3 \cdot 6H_2O$ (2 g, 7.4 mmol) was suspended in Ac_2O (100 mL, 1.06 mol), cyclodextrin (α-, β- or γ-CD) (30 mmol) was added in small portions under cooling (< 40 °C), and the mixture was stirred vigorously for 2.5 h. Then the reaction temperature was raised to 70 °C and the mixture was stirred for another 3.5 h. The mixture was poured into water (2 L), the resulting crystalline product was filtered off, washed with water, dried and crystallized three times from EtOH to obtain **4**, **5** and **6** in 20%, 22% and 23.5% yield, respectively.

Preparation of peracetylated maltooligomers from β-CD (**7**,**8**,**9**).—To a stirred suspension of $FeCl_3 \cdot 6H_2O$ (2 g, 7.4 mmol) in Ac_2O (100 mL, 1.06 mol) was added β-CD **2** (34 g, 30 mmol) under cooling in small portions. After 2.5 h, the reaction temperature was raised to 70°C and the mixture was stirred for another 5 h. The reaction mixture was poured into water, the resulting product was dissolved in CH_2Cl_2, washed with water, dried, concentrated and the crude product was chromatographed on silica gel with 9:1 → 8:2 CH_2Cl_2–acetone to obtain **7**, **8** and **9**.

Preparation of α-acetobromo maltooligomers (**10–15**).—To a solution of the maltooligomer peracetate (1 mmol) in dry CH_2Cl_2 (5 mL) was added HBr–CH_3COOH (3 mL) at 0 °C. After 3.5 h, the mixture was diluted with CH_2Cl_2 (50 mL), washed subsequently with ice-water (20 mL), sat aq $NaHCO_3$ (20 mL), ice-water (3 × 10 mL) until neutralization and concentrated to obtain **10–15** in 75–96% yields.

Preparation of peracetylated 4-nitrophenyl and 2-chloro-4-nitrophenyl β-maltooligomers (**16–27**).—To a stirred solution of the α-bromo-maltooligomers **10–15** (1 mmol) in dry pyridine (4 mL) were added 4-nitrophenol or 2-chloro-4-nitrophenol (1.12 mmol)

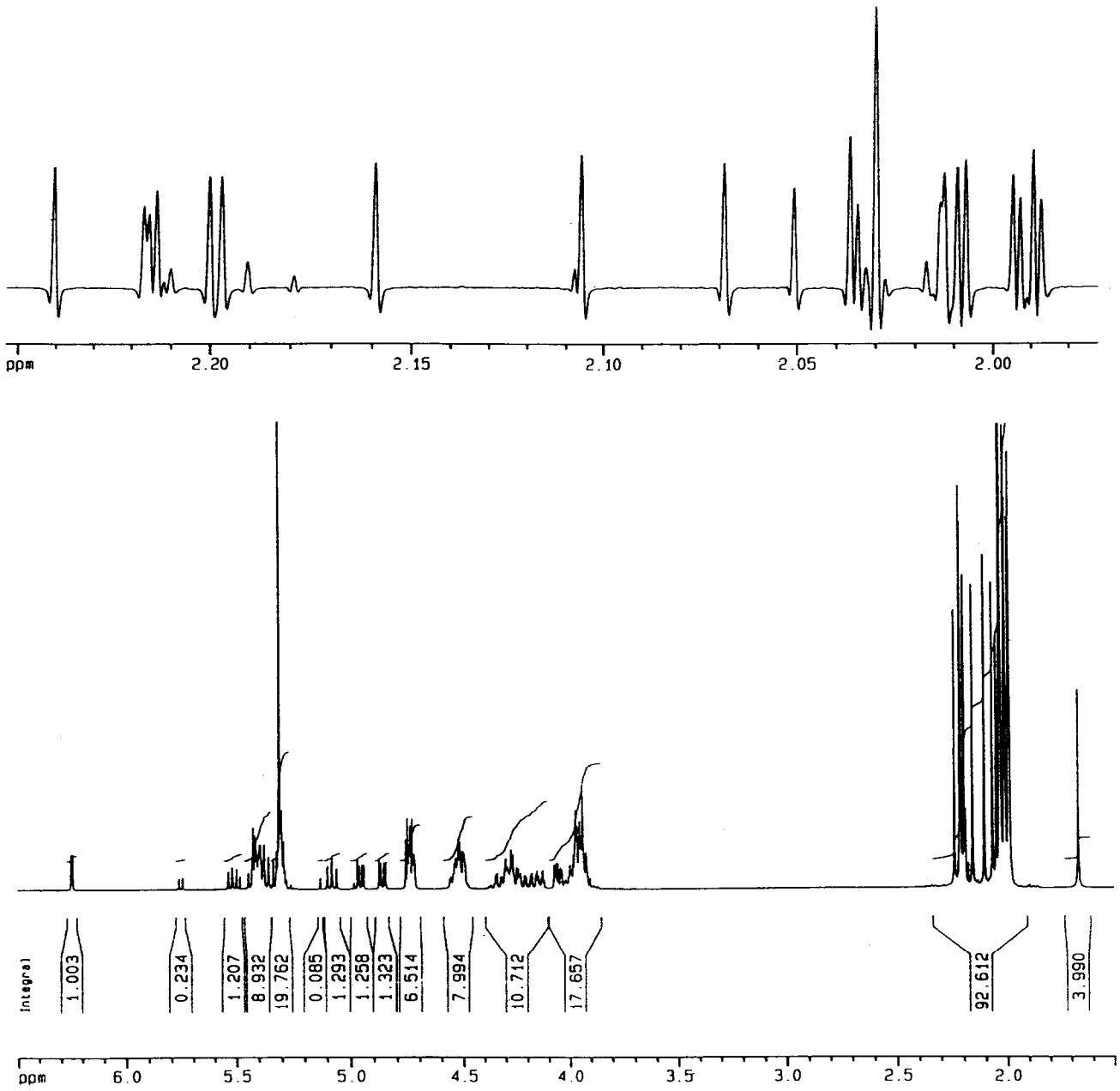
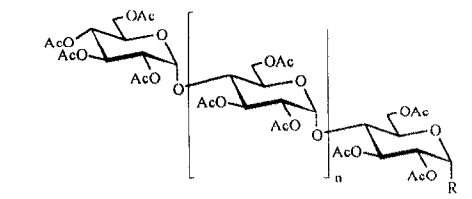


Fig. 4. ^{1}H NMR spectrum and expanded region of the acetyl–methyl signals of tricosa-*O*-acetyl-α-maltoheptaose, (^{1}H NMR, 500 MHz, $CDCl_3$).

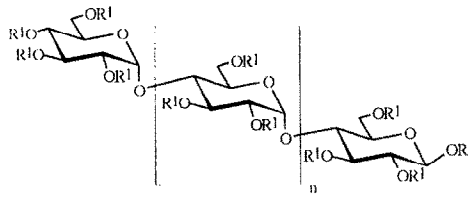
and dried Ag_2CO_3 (1.16 mmol). The reaction mixture was stirred vigorously in the dark for 2–3 h at rt and then concentrated to dryness. The solid residue was diluted with CH_2Cl_2 and filtered. The filtrate was washed with aq 5% NaOH, water, dried, concentrated and purified by column chromatography to obtain **16–27** in 50–65% yields.

Preparation of 4-nitrophenyl β-maltooligomer glycosides (**28–33**) and 2-chloro-4-nitrophenyl β-maltooligomer glycosides (**34–39**).—To a solution of the peracetylated maltooligomers **16–27** (1 g) in MeOH (30 mL) was added a catalytic amount of $NaOCH_3$ (pH ≅ 8), and the mixture was stirred for 24 h at rt. After neutralization with Amberlite IR 120 (H^+) resin the mixture was filtered and evaporated. The resulting foam was dissolved in a small amount of water, after treatment with decolorizing carbon it was filtered through the SM-113, 0.1 μm membrane filter and than it was lyophilised to obtain **28–39** in 80–90% yield.

Preparation of 4-nitrophenyl 4,6-O-benzylidene-β-maltooligomers (**40–45**).—To a solution of each of

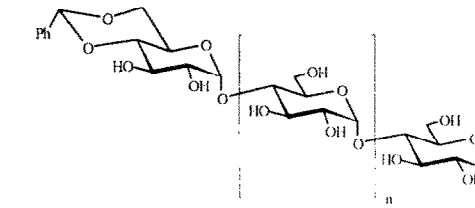


	n	R
4	4	OAc
5	5	OAc
6	6	OAc
7	1	OAc
8	2	OAc
9	3	OAc
10	4	Br
11	5	Br
12	6	Br
13	1	Br
14	2	Br
15	3	Br



	n	R	R¹
16	4	NP	Ac
17	5	NP	Ac
18	6	NP	Ac
19	1	NP	Ac
20	2	NP	Ac
21	3	NP	Ac
22	4	CNP	Ac
23	5	CNP	Ac
24	6	CNP	Ac
25	1	CNP	Ac
26	2	CNP	Ac
27	3	CNP	Ac

	n	R	R¹
28	4	NP	H
29	5	NP	H
30	6	NP	H
31	1	NP	H
32	2	NP	H
33	3	NP	H
34	4	CNP	H
35	5	CNP	H
36	6	CNP	H
37	1	CNP	H
38	2	CNP	H
39	3	CNP	H



	n
40	4
41	5
42	6
43	1
44	2
45	3

Fig. 5.

the 4-nitrophenyl β-maltooligomer glycosides **28–33** (4 mmol) in *N*,*N*-dimethylformamide (20 mL) was added α,α-dimethoxytoluene (3 equiv) and *p*-toluenesulphonic acid (0.3 g) and the mixture was stirred for 3 h at 50 °C. After evaporation of the solvent in vacuo, the mixture was diluted with CH_2Cl_2 and washed with aq. $NaHCO_3$ and then with water until neutralization, dried, concentrated and the crude product was purified by column chromatography with 75:50:12 CH_2Cl_2–MeOH–H_2O to obtain **40–45** in 45–55% yield.

Eicosa-O-acetyl-α-maltohexaose (**4**).—mp 122–125 °C; $[\alpha]_D$ +131.6° (*c* 1.01, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$): δ 6.25 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 1.90–2.30 (cluster of s, 20 OAc). Anal. Calcd for $C_{76}H_{102}O_{51}$: C, 49.84; H, 5.61. Found: C, 49.63; H, 5.57.

Tricosa - O - acetyl - α - maltoheptaose (**5**).—mp 136–138 °C; $[\alpha]_D$ +148.5° (*c* 1.5, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 6.25 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 1.90–2.30 (cluster of s, 22 OAc) 1.6 (s, 3 H, anomer OAc). Anal. Calcd for $C_{88}H_{118}O_{59}$: C, 49.86; H, 5.61. Found: C, 49.70; H, 5.58.

Hexacosa - O - acetyl - α - maltooctaose (**6**).—mp 138–140°C; $[\alpha]_D$ +137.6° (*c* 0.73, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$): δ 6.25 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 1.90–2.30 (cluster of s, 26 OAc). Anal. Calcd for $C_{100}H_{134}O_{67}$: C, 49.88; H, 5.61. Found: C, 49.69; H, 5.57.

Undeca-O-acetyl-α-maltotriose (**7**).—mp 84–87

°C; $[\alpha]_D$ +112.6° (*c* 0.57, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$): δ 6.25 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 1.90–2.30 (cluster of s, 11 OAc). Anal. Calcd for $C_{40}H_{54}O_{27}$: C, 49.69; H, 5.63. Found: C, 49.50; H, 5.58.

Tetradeca-O-acetyl-α-maltotetraose (**8**).—mp 102–106 °C; $[\alpha]_D$ +119.3° (*c* 0.39, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$): δ 6.25 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 1.90–2.30 (cluster of s, 14 OAc). Anal. Calcd for $C_{52}H_{70}O_{35}$: C, 49.76; H, 5.62. Found: C, 49.92; H, 5.67.

Heptadeca-O-acetyl-α-maltopentaose (**9**).—mp 118–120 °C; $[\alpha]_D$ +118.3° (*c* 0.31, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$): δ 6.25 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 1.90–2.30 (cluster of s, 17 OAc). Anal. Calcd for $C_{64}H_{86}O_{43}$: C, 49.81; H, 5.62. Found: C, 49.60; H, 5.56.

Nonadeca-O-acetyl-α-maltohexaosyl bromide (**10**).—mp 98–100 °C; $[\alpha]_D$ +146.8° (*c* 0.92, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$): δ 6.50 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 1.90–2.30 (cluster of s, 19 OAc). Anal. Calcd for $C_{74}H_{99}BrO_{49}$: C, 47.98; H, 5.39; Br, 4.31. Found: C, 47.78; H, 5.32.

Docosa-O-acetyl-α-maltoheptaosyl bromide (**11**).—mp 95–97 °C; $[\alpha]_D$ +160.8° (*c* 0.95, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$): δ 6.50 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 1.90–2.30 (cluster of s, 22 OAc). Anal. Calcd for $C_{86}H_{115}BrO_{57}$: C, 48.25; H, 5.41; Br, 3.73. Found: C, 48.04; H, 5.35.

Pentacosa-O-acetyl-α-maltooctaosyl bromide (**12**).—mp 102–104 °C; $[\alpha]_D$ +142.6° (*c* 0.21, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$): δ 6.50 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 1.90–2.30 (cluster of s, 25 OAc). Anal. Calcd for $C_{98}H_{131}BrO_{65}$: C, 48.46; H, 5.44; Br, 3.29. Found: C, 48.63; H, 5.38.

Deca-O-acetyl-α-maltotriosyl bromide (**13**).—mp 80–82 °C; $[\alpha]_D$ +135.2° (*c* 0.92, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$): δ 6.50 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 1.90–2.30 (cluster of s, 10 OAc). Anal. Calcd for $C_{38}H_{51}BrO_{25}$: C, 46.21; H, 5.20; Br, 8.09. Found: C, 46.02; H, 5.25.

Trideca-O-acetyl-α-maltotetraosyl bromide (**14**).—mp 88–90 °C; $[\alpha]_D$ +143.8° (*c* 0.96, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$): δ 6.50 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 1.90–2.30 (cluster of s, 13 OAc). Anal. Calcd for $C_{50}H_{67}BrO_{33}$: C, 47.07; H, 5.29; Br, 6.26. Found: C, 46.85; H, 5.22.

Hexadeca-O-acetyl-α-maltopentaosyl bromide (**15**).—mp 92–94 °C; $[\alpha]_D$ +145.4° (*c* 0.91, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$): δ 6.50 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 1.90–2.30 (cluster of s, 16 OAc). Anal. Calcd for $C_{62}H_{83}BrO_{41}$: C, 47.61; H, 5.35; Br, 5.11. Found: C, 47.40; H, 5.28.

p-Nitrophenyl O-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-(1 → 4)-tetrakis-[O-(2,3,6-tri-O-acetyl-α-D-glucopyranosyl)-(1 → 4)]-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**16**).—mp 126–128 °C; $[\alpha]_D$ +104.6° (*c* 0.93, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$): δ 8.25 (d, 2 H, Ph), 7.10 (d, 2 H, Ph), 2.20–1.80 (cluster of s, 19 OAc). Anal. Calcd for $C_{80}H_{103}NO_{52}$: C, 50.29; H, 5.43; N, 0.73. Found: C, 50.10; H, 5.38.

p-Nitrophenyl O-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-(1 → 4)-pentakis-[O-(2,3,6-tri-O-acetyl-α-D-glucopyranosyl)-(1 → 4)]-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**17**).—mp 132–134 °C; $[\alpha]_D$ +110.3° (*c* 0.91, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$): δ 8.25 (d, 2 H, Ph), 7.10 (d, 2 H, Ph), 2.20–1.80 (cluster of s, 22 OAc). Anal. Calcd for $C_{92}H_{119}NO_{60}$: C, 50.25; H, 5.45; N, 0.64. Found: C, 50.06; H, 5.40.

p-Nitrophenyl O-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-(1 → 4)-hexakis-[O-(2,3,6-tri-O-acetyl-α-D-glucopyranosyl)-(1 → 4)]-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**18**).—mp 136–138 °C; $[\alpha]_D$ +120.1° (*c* 0.73, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$): δ 8.25 (d, 2 H, Ph), 7.10 (d, 2 H, Ph), 2.20–1.80 (cluster of s, 25 OAc). Anal. Calcd for $C_{104}H_{135}NO_{68}$: C, 50.22; H, 5.47; N, 0.56. Found: C, 50.00; H, 5.42.

p-Nitrophenyl 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 (**19**).—mp 122–124 °C; $[\alpha]_D$ +61.3° (*c* 0.92, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$): δ 8.35 (d, 2 H, Ph), 7.05 (d, 2 H, Ph), 2.20–1.90 (cluster of s, 10 OAc). Anal. Calcd for $C_{44}H_{55}NO_{28}$: C, 50.53; H, 5.30; N, 1.34. Found: C, 50.75; H, 5.35.

p-Nitrophenyl O-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-(1 → 4)-bis-[O-(2,3,6-tri-O-acetyl-α-D-glucopyranosyl)-(1 → 4)]-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**20**).—mp 118–120 °C; $[\alpha]_D$ +76.9° (*c* 1.12, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$): δ 8.25 (d, 2 H, Ph), 7.10 (d, 2 H, Ph), 2.20–1.90 (cluster of s, 13 OAc). Anal. Calcd for $C_{56}H_{71}NO_{36}$: C, 50.41; H, 5.36; N, 1.05. Found: C, 50.20; H, 5.40.

p-Nitrophenyl O-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-(1 → 4)-tris-[O-(2,3,6-tri-O-acetyl-α-D-glucopyranosyl)-(1 → 4)]-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**21**).—mp 120–123 °C; $[\alpha]_D$ +95.6° (*c* 1.1, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$): δ 8.25 (d, 2 H, Ph), 7.10 (d, 2 H, Ph), 2.20–1.80 (cluster of s, 16 OAc). Anal. Calcd for $C_{68}H_{87}NO_{44}$: C, 50.34; H, 5.40; N, 0.86. Found: C, 50.10; H, 5.47.

2-Chloro-4-nitrophenyl O-*(2,3,4,6-tetra*-O-*acetyl-α*-D-*glucopyranosyl)-(1 → 4)-tetrakis-[*O-*(2,3,6-tri*-O-*acetyl-α*-D-*glucopyranosyl)-(1 → 4)]-2,3,6-tri*-O-*acetyl-β*-D-*glucopyranoside* (**22**).—mp 126–128 °C; $[\alpha]_D$ +81.5° (*c* 0.36, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$): δ 8.30 (s, 1 H, Ph), 8.15 (d, 1 H, Ph), 7.30 (d, 1 H, Ph), 2.30–1.90 (cluster of s, 19 OAc). Anal. Calcd for $C_{80}H_{102}ClNO_{52}$: C, 49.40; H, 5.29; Cl, 1.82; N, 0.72. Found: C, 49.18; H, 5.34.

2-Chloro-4-nitrophenyl O-*(2,3,4,6-tetra*-O-*acetyl-α*-D-*glucopyranosyl)-(1 → 4)-pentakis-[*O-*(2,3,6-tri*-O-*acetyl-α*-D-*glucopyranosyl)-(1 → 4)]-2,3,6-tri*-O-*acetyl-β*-D-*glucopyranoside* (**23**).—mp 140–142 °C; $[\alpha]_D$ +105.1° (*c* 0.15, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$): δ 8.30 (s, 1 H, Ph), 8.15 (d, 1 H, Ph), 7.30 (d, 1 H, Ph), 2.30–1.90 (cluster of s, 22 OAc). Anal. Calcd for $C_{92}H_{118}ClNO_{60}$: C, 49.48; H, 5.33; Cl, 1.59; N, 0.63. Found: C, 49.70; H, 5.27.

2-Chloro-4-nitrophenyl O-*(2,3,4,6-tetra*-O-*acetyl-α*-D-*glucopyranosyl)-(1 → 4)-hexakis-[*O-*(2,3,6-tri*-O-*acetyl-α*-D-*glucopyranosyl)-(1 → 4)]-2,3,6-tri*-O-*acetyl-β*-D-*glucopyranoside* (**24**).—mp 141–143 °C; $[\alpha]_D$ +107.6° (*c* 0.99, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$): δ 8.30 (s, 1 H, Ph), 8.15 (d, 1 H, Ph), 7.30 (d, 1 H, Ph), 2.30–1.90 (cluster of s, 25 OAc). Anal. Calcd for $C_{104}H_{134}ClNO_{68}$: C, 49.54; H, 5.36; Cl, 1.41; N, 0.56. Found: C, 49.31; H, 5.42.

2-Chloro-4-nitrophenyl 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* (**25**).—mp 104–106 °C; $[\alpha]_D$ +47.6° (*c* 1.05, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$): δ 8.30 (s, 1 H, Ph), 8.15 (d, 1 H, Ph), 7.25 (d, 1 H, Ph), 2.30–1.90 (cluster of s, 10 OAc). Anal. Calcd for $C_{44}H_{54}ClNO_{28}$: C, 48.92; H, 5.04; Cl, 3.28; N, 1.30. Found: C, 48.70; H, 5.11.

2-Chloro-4-nitrophenyl O-*(2,3,4,6-tetra*-O-*acetyl-α*-D-*glucopyranosyl)-(1 → 4)-bis-[*O-*(2,3,6-tri*-O-*acetyl-α*-D-*glucopyranosyl)-(1 → 4)]-2,3,6-tri*-O-*acetyl-β*-D-*glucopyranoside* (**26**).—mp 112–115 °C; $[\alpha]_D$ +73.2° (*c* 1.07, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$): δ 8.30 (s, 1 H, Ph), 8.15 (d, 1 H, Ph), 7.25 (d, 1 H, Ph), 2.30–1.90 (cluster of s, 13 OAc). Anal. Calcd for $C_{56}H_{70}ClNO_{36}$: C, 49.15; H, 5.16; Cl, 2.59; N, 1.02. Found: C, 49.30; H, 5.10.

2-Chloro-4-nitrophenyl O-*(2,3,4,6-tetra*-O-*acetyl-α*-D-*glucopyranosyl)-(1 → 4)-tris-[*O-*(2,3,6-tri*-O-*acetyl-α*-D-*glucopyranosyl)-(1 → 4)]-2,3,6-tri*-O-*acetyl-β*-D-*glucopyranoside* (**27**).—mp 113–116 °C; $[\alpha]_D$ +77.9° (*c* 1.06, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$): δ 8.30 (s, 1 H, Ph), 8.15 (d, 1 H, Ph), 7.30 (d, 1 H, Ph), 2.30–1.90 (cluster of s, 16 OAc). Anal. Calcd for $C_{68}H_{86}ClNO_{44}$: C, 49.29; H, 5.23; Cl, 2.14; N, 0.85. Found: C, 49.06; H, 5.29.

p-Nitrophenyl O-*(α*-D-*glucopyranosyl)-(1 → 4)-tetrakis-[*O-*α*-D-*glucopyranosyl-(1 → 4)]-β*-D-*glucopyranoside* (**28**).—$[\alpha]_D$ +92.7° (*c* 1.1, H_2O); ^1H NMR (200 MHz, D_2O): δ 8.30 (d, 2 H, Ph), 7.20 (d, 2 H, Ph), 5.30 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), ^{13}C NMR (50.3 MHz, D_2O): δ 162.53, 143.18 ($aromatic_q$), 126.84, 117.28 (aromatic), 100.56, 100.13, (anomeric carbons), 61.22 (C-6 carbons). Anal. Calcd for $C_{42}H_{65}NO_{33}$: C, 45.37; H, 5.89; N, 1.26. Found: C, 45.15; H, 5.84.

p-Nitrophenyl O-*(α*-D-*glucopyranosyl)-(1 → 4)-pentakis-[*O-*α*-D-*glucopyranosyl-(1 → 4)]-β*-D-*glucopyranoside* (**29**).—$[\alpha]_D$ +112.1° (*c* 0.6, H_2O); ^1H NMR (200 MHz, D_2O): δ 8.30 (d, 2 H, Ph), 7.20 (d, 2 H, Ph), 5.28 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), ^{13}C NMR (50.3 MHz, D_2O): δ 162.62, 143.21 ($aromatic_q$), 126.89, 117.35 (aromatic), 100.62, 100.20, (anomeric carbons), 61.20 (C-6 carbons). Anal. Calcd for $C_{48}H_{75}NO_{38}$: C, 45.25; H, 5.93; N, 1.10. Found: C, 45.02; H, 6.01.

p-Nitrophenyl O-*(α*-D-*glucopyranosyl)-(1 → 4)-hexakis-[*O-*α*-D-*glucopyranosyl-(1 → 4)]-β*-D-*glucopyranoside* (**30**).—$[\alpha]_D$ +116.7° (*c* 0.14, H_2O); ^1H NMR (200 MHz, D_2O): δ 8.30 (d, 2 H, Ph), 7.20 (d, 2 H, Ph), 5.30 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1). Anal. Calcd for $C_{54}H_{85}NO_{43}$: C, 45.16; H, 5.97; N, 0.98. Found: C, 44.94; H, 5.90.

p-Nitrophenyl O-*(α*-D-*glucopyranosyl)-(1 → 4)*-O-*α*-D-*glucopyranosyl-(1 → 4)-β*-D-*glucopyranoside* (**31**).—$[\alpha]_D$ +50.6° (*c* 1.01, H_2O); ^1H NMR (200 MHz, D_2O): δ 8.25 (d, 2 H, Ph), 7.20 (d, 2 H, Ph), 5.30 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), ^{13}C NMR (50.3 MHz, D_2O): δ 162.47, 143.21 ($aromatic_q$), 126.85, 117.23 (aromatic), 100.62, 100.39, 100.09 (anomeric carbons), 77.74, 77.53 (C-4 carbons, except for the non-reducing end), 61.25 (C-6 carbons). Anal. Calcd for $C_{24}H_{35}NO_{18}$: C, 46.08; H, 5.64; N, 2.24. Found: C, 46.30; H, 5.57.

p-Nitrophenyl O-*(α*-D-*glucopyranosyl)-(1 → 4)-bis-[*O-*α*-D-*glucopyranosyl-(1 → 4)]-β*-D-*glucopyranoside* (**32**).—$[\alpha]_D$ +67.16° (*c* 1.0, H_2O); ^1H NMR (200 MHz, D_2O): δ 8.25 (d, 2 H, Ph), 7.20 (d, 2 H, Ph), 5.30 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), ^{13}C NMR (50.3 MHz, D_2O): δ 162.58, 143.14 ($aromatic_q$), 126.84, 117.31 (aromatic), 100.72, 100.21, (anomeric carbons), 78.24, 77.95, 77.71 (C-4 carbons, except for the non-reducing end), 61.29 (C-6 carbons). Anal. Calcd for $C_{30}H_{45}NO_{23}$: C, 45.75; H, 5.76; N, 1.78. Found: C, 45.55; H, 5.83.

p-Nitrophenyl O-*(α*-D-*glucopyranosyl)-(1 → 4)-tris-*

[O-α-D-glucopyranosyl-(1 → 4)]-β-D-glucopyranoside (**33**).—$[\alpha]_D$ +79.6° (*c* 0.9, H_2O); ^1H NMR (200 MHz, D_2O): δ 8.30 (d, 2 H, Ph), 7.20 (d, 2 H, Ph), 5.30 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), ^{13}C NMR (50.3 MHz, D_2O): δ 162.61, 143.22 (aromatic$_q$), 126.88, 117.35 (aromatic), 100.71, 100.22, (anomeric carbons), 61.27 (C-6 carbons). Anal. Calcd for $C_{36}H_{55}NO_{28}$: C, 45.52; H, 5.84; N, 1.47. Found: C, 45.30; H, 5.92.

2-Chloro-4-nitrophenyl O-(α-D-glucopyranosyl)-(1 → 4)-tetrakis-[O-α-D-glucopyranosyl-(1 → 4)]-β-D-glucopyranoside (**34**).—$[\alpha]_D$ +100.5° (*c* 0.6, H_2O); ^1H NMR (200 MHz, D_2O): δ 8.40 (s, 1 H, Ph), 8.20 (d, 1 H, Ph), 7.35 (d, 1 H, Ph), 5.30–5.50 (bs, 6 H, anomeric protons). Anal. Calcd for $C_{42}H_{64}ClNO_{33}$: C, 44.00; H, 5.63; Cl, 3.09; N, 1.22. Found: C, 44.21; H, 5.55.

2-Chloro-4-nitrophenyl O-(α-D-glucopyranosyl)-(1 → 4)-pentakis-[O-α-D-glucopyranosyl-(1 → 4)]-β-D-glucopyranoside (**35**).—$[\alpha]_D$ +105.7° (*c* 0.6, H_2O); ^1H NMR (200 MHz, D_2O): δ 8.40 (s, 1 H, Ph), 8.20 (d, 1 H, Ph), 7.40 (d, 1 H, Ph), 5.30–5.50 (bs, 7 H, anomeric protons). Anal. Calcd for $C_{48}H_{74}ClNO_{38}$: C, 44.06; H, 5.70; Cl, 2.71; N, 1.07. Found: C, 44.25; H, 5.76.

2-Chloro-4-nitrophenyl O-(α-D-glucopyranosyl)-(1 → 4)-hexakis-[O-α-D-glucopyranosyl-(1 → 4)]-β-D-glucopyranoside (**36**).—$[\alpha]_D$ +117.5° (*c* 0.6, H_2O); ^1H NMR (200 MHz, D_2O): δ 8.40 (s, 1 H, Ph), 8.20 (d, 1 H, Ph), 7.40 (d, 1 H, Ph), 5.30–5.50 (bs, 8 H, anomeric protons). Anal. Calcd for $C_{54}H_{84}ClNO_{43}$: C, 44.10; H, 5.76; Cl, 2.41; N, 0.95. Found: C, 43.88; H, 5.83.

2-Chloro-4-nitrophenyl O-(α-D-glucopyranosyl)-(1 → 4)-O-α-D-glucopyranosyl-(1 → 4)-β-D-glucopyranoside (**37**).—$[\alpha]_D$ +49.5° (*c* 0.5, H_2O); ^1H NMR (200 MHz, D_2O): δ 8.40 (s, 1 H, Ph), 8.20 (d, 1 H, Ph), 7.35 (d, 1 H, Ph), 5.40–5.50 (2 d, 2 H, anomeric protons), 5.35 (d, 1 H, $J_{1,2}$ 9 Hz, H-1). Anal. Calcd for $C_{24}H_{34}ClNO_{18}$: C, 43.68; H, 5.19; Cl, 5.37; N, 2.12. Found: C, 43.95; H, 5.13.

2-Chloro-4-nitrophenyl O-(α-D-glucopyranosyl)-(1 → 4)-bis-[O-α-D-glucopyranosyl-(1 → 4)]-β-D-glucopyranoside (**38**).—$[\alpha]_D$ +70.5° (*c* 0.36, H_2O); ^1H NMR (200 MHz, D_2O): δ 8.40 (s, 1 H, Ph), 8.20 (d, 1 H, Ph), 7.35 (d, 1 H, Ph), 5.35–5.50 (bs, 4 H, anomeric protons). Anal. Calcd for $C_{30}H_{44}ClNO_{23}$: C, 43.83; H, 5.39; Cl, 4.31; N, 1.70. Found: C, 44.06; H, 5.39.

2-Chloro-4-nitrophenyl O-(α-D-glucopyranosyl)-(1 → 4)-tris-[O-α-D-glucopyranosyl-(1 → 4)]-β-D-glucopyranoside (**39**).—$[\alpha]_D$ +81.0° (*c* 0.43, H_2O); ^1H NMR (200 MHz, D_2O): δ 8.40 (s, 1 H, Ph), 8.20 (d, 1 H, Ph), 7.35 (d, 1 H, Ph), 5.30–5.50 (bs, 5 H, anomeric protons). Anal. Calcd for $C_{36}H_{54}ClNO_{28}$: C, 43.93; H, 5.53; Cl, 3.60; N, 1.42. Found: C, 43.68; H, 5.59.

p-Nitrophenyl O-(4, 6-O-benzylidene-α-D-glucopyranosyl)-(1 → 4)-tetrakis-[O-α-D-glucopyranosyl-(1 → 4)]-β-D-glucopyranoside (**40**).—$[\alpha]_D$ +64.4° (*c* 0.54, H_2O); ^1H NMR (200 MHz, D_2O): δ 8.25 (d, 2 H, Ph), 7.60–7.30 (m, 5 H, Ph), 7.20 (d, 2 H, Ph), 5.70 (s, 1 H, PhC*H*). Anal. Calcd for $C_{49}H_{69}NO_{33}$: C, 49.04; H, 5.80; N, 1.17. Found: C, 49.26; H, 5.70.

p-Nitrophenyl O-(4, 6-O-benzylidene-α-D-glucopyranosyl)-(1 → 4)-pentakis-[O-α-D-glucopyranosyl-(1 → 4)]-β-D-glucopyranoside (**41**).—$[\alpha]_D$ +86.3° (*c* 0.96, H_2O); ^1H NMR (200 MHz, D_2O): δ 8.25 (d, 2 H, Ph), 7.50–7.30 (m, 5 H, Ph), 7.25 (d, 2 H, Ph), 5.60 (s, 1 H, PhC*H*). Anal. Calcd for $C_{55}H_{79}NO_{38}$: C, 48.49; H, 5.85; N, 1.03. Found: C, 48.27; H, 5.93.

p-Nitrophenyl O-(4, 6-O-benzylidene-α-D-glucopyranosyl)-(1 → 4)-hexakis-[O-α-D-glucopyranosyl-(1 → 4)]-β-D-glucopyranoside (**42**).—$[\alpha]_D$ +89.5° (*c* 0.9, H_2O); ^1H NMR (200 MHz, D_2O): δ 8.30 (d, 2 H, Ph), 7.60–7.40 (m, 5 H, Ph), 7.25 (d, 2 H, Ph), 5.75 (s, 1 H, PhC*H*), 5.30 (d, 1 H, $J_{1,2}$ 9 Hz, H-1). Anal. Calcd for $C_{61}H_{89}NO_{43}$: C, 48.06; H, 5.88; N, 0.92. Found: C, 48.29; H, 5.80.

p-Nitrophenyl O-(4, 6-O-benzylidene-α-D-glucopyranosyl)-(1 → 4)-O-α-D-glucopyranosyl-(1 → 4)-β-D-glucopyranoside (**43**).—$[\alpha]_D$ +43.5° (*c* 0.7, acetone); ^1H NMR (200 MHz, CD_3OD): δ 8.25 (d, 2 H, Ph), 7.60–7.30 (m, 5 H, Ph), 7.25 (d, 2 H, Ph), 5.60 (s, 1 H, PhC*H*). Anal. Calcd for $C_{31}H_{39}NO_{18}$: C, 52.16; H, 5.51; N, 1.96. Found: C, 52.36; H, 5.57.

p-Nitrophenyl O-(4, 6-O-benzylidene-α-D-glucopyranosyl)-(1 → 4)-bis-[O-α-D-glucopyranosyl-(1 → 4)]-β-D-glucopyranoside (**44**).—$[\alpha]_D$ +37.6° (*c* 0.15, H_2O)); ^1H NMR (200 MHz, CD_3OD, D_2O): δ 8.20 (d, 2 H, Ph), 7.50–7.30 (m, 5 H, Ph), 7.25 (d, 2 H, Ph), 5.60 (s, 1 H, PhC*H*). Anal. Calcd for $C_{37}H_{49}NO_{23}$: C, 50.74; H, 5.64; N, 1.60. Found: C, 50.53; H, 5.70.

p-Nitrophenyl O-(4, 6-O-benzylidene-α-D-glucopyranosyl)-(1 → 4)-tris-[O-α-D-glucopyranosyl-(1 → 4)]-β-D-glucopyranoside (**45**).—$[\alpha]_D$ +41.3° (*c* 0.9, H_2O); ^1H NMR (200 MHz, D_2O): δ 8.20 (d, 2 H, Ph), 7.60–7.30 (m, 5 H, Ph), 7.10 (d, 2 H, Ph),

5.75 (s, 1 H, PhC*H*), 5.10 (d, 1 H, $J_{1,2}$ 9.0 Hz, H-1). Anal. Calcd for $C_{43}H_{59}NO_{28}$: C, 49.76; H, 5.73; N, 1.35. Found: C, 49.99; H, 5.65.

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