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# Syntheses of modified 2-chloro-4-nitrophenyl β-maltopentaosides as useful substrates for assay of human alpha amylase

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## ABSTRACT

Twenty-three novel 2-chloro-4-nitrophenyl β-D-maltopentaosides modified at the $6^5$ and/or $4^5$ position were synthesized as substrates for human alpha amylase. Two human alpha amylases hydrolyzed $6^5$-deoxy-$6^5$-, $6^5$-*O*-, and $4^5$,$6^5$-di-*O*-substituted derivatives at essentially a single D-glucosidic linkage, but $4^5$,$6^5$-*O*-bridged and $4^5$-*O*-substituted derivatives were hydrolyzed at two or more linkages. The amylases displayed smaller $K_m$ values for the compounds having hydrophobic modifications. In these derivatives, 2-chloro-4-nitrophenyl *O*-(6-bromo-6-deoxy-α-D-glucopyranosyl)-(1 → 4)-tris[*O*-α-D-glucopyranosyl-(1 → 4)]-β-D-glucopyranoside (**10**), 2-chloro-4-nitrophenyl *O*-(6-azido-6-deoxy-α-D-glucopyranosyl)-(1 → 4)-tris[*O*-α-D-glucopyranosyl-(1 → 4)]-β-D-glucopyranoside (**19**), and 2-chloro-4-nitrophenyl *O*-[6-*O*-(*N*-isopropyl)carbamoyl-α-D-glucopyranosyl]-(1 → 4)-tris[*O*-α-D-glucopyranosyl-(1 → 4)]-β-D-glucopyranoside (**30**), which were rapidly hydrolyzed by the two amylases at a limited position at an approximately equal rate, were shown to be very useful blocked-type substrates for assay of human alpha amylase.

## INTRODUCTION

Determination of the catalytic concentration of alpha amylase (EC 3.2.1.1) in human serum and urine is a diagnostic aid in various diseases[1]. Where acute pancreatitis is suspected this is especially the most frequently employed test[2]. It is also well known that there are two amylases in human body fluids, salivary alpha amylase (HSA) and pancreatic alpha amylase (HPA). A number of methods for the determination of the total activity of the two amylases have been developed, based on different principles and using various substrates[3,4].

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In order to block partial hydrolysis of the substrate when using alpha glucosidase or glucoamylase as a coupled enzyme, several groups have recently reported methods[5], which employed 4-nitrophenyl maltooligosides modified at their terminal (nonreducing-end) D-glucosyl group as the substrates. However, the influence of various modifications at a terminal D-glucosyl group on the hydrolysis, using a variety of systematically synthesized substrates, has not yet been reported. Additionally, some of the reported substrates[5] were hydrolyzed by two amylases to give many products in a different manner, resulting in non-stoichiometric measurements; for others, disadvantages in the convenience of the methods were pointed out[6]. It is, therefore, highly desirable that a suitable substrate be developed having good solubility in water, high affinity, rapid and equal reaction rate with each of the two amylases, and cleavage at a limited position.

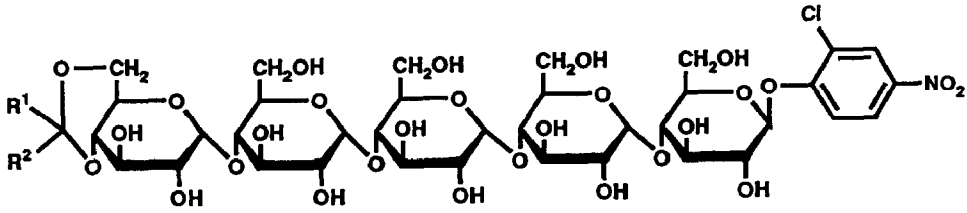
The object of the present work was to investigate systematically the effects of modifications at a terminal D-glucosyl group of maltooligosaccharides on the hydrolysis by alpha amylase and to find a useful substrate for the alpha amylase assay.[7] In this report we describe the syntheses of twenty-three modified 2-chloro-4-nitrophenyl β-maltopentaosides and interesting modes of action of two amylases on the derivatives.

## RESULTS AND DISCUSSION

*Synthesis.*—Maltopentaose was selected as the maltooligosyl moiety of the substrates synthesized because it reportedly has a positional selectivity and high reactivity in its hydrolysis by alpha amylase[8]. 2-Chloro-4-nitrophenol was selected as the aglycon of the substrates because of its higher sensitivity ($\epsilon$ 16 100) and more stable coloration than 4-nitrophenol[9] at pH 7, which is the optimum reaction pH for human alpha amylase[10].

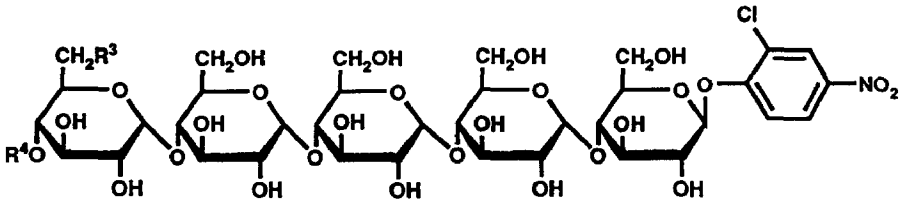
Condensation of 2-chloro-4-nitrophenyl β-maltopentaoside[11] (**1**) with tetramethyl orthocarbonate, trimethyl orthoacetate, and triethyl orthoacetate in *N,N*-dimethylformamide (DMF) in the presence of Amberlyst-15E gave 2-chloro-4-nitrophenyl $4^5,6^5$-*O*-(1,1-dimethoxy)methylidene-β-maltopentaoside (**2**, yield 67%), $4^5,6^5$-*O*-(1-methoxy)ethylidene-β-maltopentaoside (**3**, yield 20%), and $4^5,6^5$-*O*-(1-ethoxy)ethylidene-β-maltopentaoside (**4**, yield 32%), respectively. Compounds **2–4** which have alkoxy groups in a methylidene bridge were designed to increase water solubility. Reaction of benzaldehyde dimethyl acetal and acetone dimethyl acetal with non-blocked maltopentaoside **1** in DMF in the presence of *p*-toluenesulfonic acid gave the $4^5,6^5$-*O*-benzylidene derivative **5** (yield 68%) and $4^5,6^5$-*O*-isopropylidene derivative **6** (yield 32%), respectively. Since these two alkylidene derivatives (**5** and **6**) have five glucosyl residues and the β-2-chloro-4-nitrophenyl structure, we expected that alpha amylases would hydrolyze them more rapidly and at a more limited position than similar substrates previously reported[5], which have seven glucosyl residues and the α-4-nitrophenyl glucoside structure.

The $6^5$-deoxy-$6^5$-substituted derivatives were designed to investigate the influ-



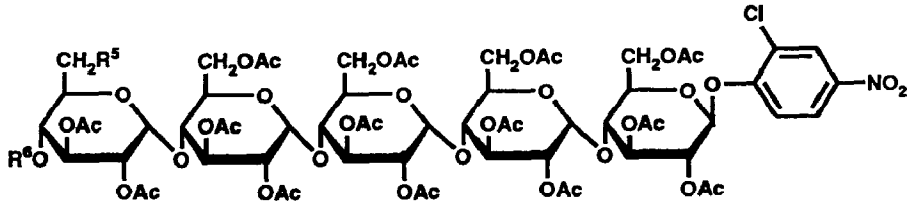
| | $R^1$ | $R^2$ |
|---|---|---|
| 2 | OMe | OMe |
| 3 | OMe | Me |
| 4 | OEt | Me |
| 5 | Ph | H |
| 6 | Me | Me |

ence of molecular size and the effects of the absence of a $6^5$-O atom in similar structures on hydrolysis by alpha amylase. These derivatives were prepared as follows. First, the benzylidene derivative **5** was acetylated conventionally to afford **7** in 88% yield. The $6^5$-bromide **8** was given then obtained in 87% yield by oxidative bromination of **7** with *N*-bromosuccinimide in $CCl_4$–1,1,2,2-tetrachloroethane. Treatment of **8** with LiCl in *N'*,*N'*,*N'*-hexamethylphosphoric triamide (HMPA) gave the $6^5$-chloride **9** in 97% yield. *O*-Deacetylation of **8** and **9** with $K_2CO_3$ in MeOH gave 2-chloro-4-nitrophenyl $6^5$-bromo-$6^5$-deoxy-β-maltopentaoside **10** (yield 73%), and $6^5$-chloro-$6^5$-deoxy-β-maltopentaoside **11** (yield 71%), respectively. Acetylation of **2** and subsequent treatment with aq AcOH removed the dimethoxymethylidene group to afford $4^5$,$6^5$-diol **12** in 66% yield from **2**. Selective *p*-toluenesulfonylation of **12** with 15 equiv of reagent in pyridine at room temperature and subsequent acetylation gave the $6^5$-*O*-*p*-toluenesulfonyl derivative **13** in 46% yield from **12**. Compound **13** was then treated with NaI in butanone and with $NaN_3$ in dimethyl sulfoxide to furnish the $6^5$-iodide **14** (yield 95%) and $6^5$-azide **15** (yield 95%), respectively. In order to obtain $6^5$-deoxy-$6^5$-fluoro derivative, we used the *tert*-butyldimethylsilyl (TBDMS) group for the protection of $6^5$-OH and benzoyl group for the protection of $4^5$-OH, as follows. Reaction of **12** and TBDMS-Cl using imidazole as catalyst in DMF effected selective silylation to afford the $6^5$-*O*-TBDMS derivative, and conventional benzoylation then gave the $4^5$-*O*-benzoyl-$6^5$-*O*-TBDMS derivative. Selective removal of the TBDMS group by aq AcOH then afforded the $6^5$-ol **16** (yield 56%, from **12**), which was treated with diethylaminosulfur trifluoride (DAST) to furnish the $6^5$-fluoride **17** (yield 89%). Attempts to obtain the $6^5$-fluoride by direct fluorination of **12** with DAST, without protection of the OH group, were unsuccessful because $4^5$-OH was also fluorinated under these conditions. As with **8** and **9**, compounds **14**, **15**, and **17** were *O*-deacetylated to afford 2-chloro-4-nitrophenyl $6^5$-deoxy-$6^5$-iodo-β-maltopentaoside **18** (yield 59%), $6^5$-azido-$6^5$-deoxy-β-maltopentaoside **19** (yield 72%), and $6^5$-deoxy-$6^5$-fluoro-β-maltopentaoside **20** (yield 58%), respectively.




| | $R^3$ | $R^4$ |
|---|---|---|
| **1** | OH | H |
| **10** | Br | H |
| **11** | Cl | H |
| **18** | I | H |
| **19** | $N_3$ | H |
| **20** | F | H |
| **28** | OCONHPh | H |
| **29** | OCONH$^t$Bu | H |
| **30** | OCONH$^i$Pr | H |
| **31** | OCONH$^i$Pr | CONH$^i$Pr |
| **32** | OH | CONH$^i$Pr |
| **33** | OCONHEt | CONHEt |
| **42** | $OCH_2OMe$ | H |
| **43** | $OCH_2OCH_2CH_2OMe$ | H |
| **44** | $OCH_2OCH_2Ph$ | H |
| **45** | $OCH_2OMe$ | $CH_2OMe$ |
| **46** | $OCH_2OCH_2CH_2OMe$ | $CH_2OCH_2CH_2OMe$ |
| **47** | $OCH_2OCH_2Ph$ | $CH_2OCH_2Ph$ |
| **48** | OH | $CH_2OMe$ |

The $6^5$-*O*-and/or $4^5$-*O*-(*N*-alkyl)carbamoyl derivatives were prepared to investigate the presence of the *O*-carbamoyl group, the effects of modification of the position, and the influence on the hydrolysis of the alkyl chain length in similar structures. Condensation of **12** with phenyl and *tert*-butyl isocyanate selectively gave the $6^5$-*O*-(*N*-phenyl)carbamate **21** (yield 84%), and $6^5$-*O*-(*N*-*tert*-butyl)carbamate **22** (yield 86%), respectively. We did not attempt to prepare the $4^5$,$6^5$-di-*O*-carbamoyl derivatives of **21** and **22**, because their *O*-deacetylated derivatives seemed to have low water solubility. Reaction of isopropyl isocyanate with **12** gave the $6^5$-*O*-(*N*-isopropyl)carbamate **23** (yield 99%) at 70°C, whereas boiling under reflux gave the $4^5$,$6^5$-di-*O*-(*N*-isopropyl)carbamate **24** (yield 78%). In order to obtain the $4^5$-*O*-carbamoyl derivative, we used the TBDMS group for protection of $6^5$-OH in the same manner as with **16**. Reaction of **12** with TBDMS-Cl afforded the $6^5$-*O*-TBDMS derivative, and subsequent reaction with isopropyl isocyanate gave the $6^5$-*O*-TBDMS-$4^5$-*O*-(*N*-isopropyl)carbamate **25** (yield 61% from **12**). Compound **25** underwent removal of the TBDMS protecting group by aq AcOH to afford the $4^5$-*O*-(*N*-isopropyl)carbamoyl-$6^5$-ol **26** (yield 93%). The short alkyl chain of ethyl isocyanate exerted no positional selectivity in the




| | $R^5$ | $R^6$ |
|---|---|---|
| **7** | –OCHPh– | |
| **8** | Br | Bz |
| **9** | Cl | Bz |
| **12** | OH | H |
| **13** | OTs | Ac |
| **14** | I | Ac |
| **15** | $N_3$ | Ac |
| **16** | OH | Bz |
| **17** | F | Bz |
| **21** | OCONHPh | H |
| **22** | OCONH$^t$Bu | H |
| **23** | OCONH$^i$Pr | H |
| **24** | OCONH$^i$Pr | CONH$^i$Pr |
| **25** | OTBDMS | CONH$^i$Pr |
| **26** | OH | CONH$^i$Pr |
| **27** | OCONHEt | CONHEt |
| **34** | $OCH_2OMe$ | H |
| **35** | $OCH_2OCH_2CH_2Me$ | H |
| **36** | $OCH_2OCH_2Ph$ | H |
| **37** | $OCH_2OMe$ | $CH_2OMe$ |
| **38** | $OCH_2OCH_2CH_2OMe$ | $CH_2OCH_2CH_2OMe$ |
| **39** | $OCH_2OCH_2OPh$ | $CH_2OCH_2OPh$ |
| **40** | OTBDMS | $CH_2OMe$ |
| **41** | OH | $CH_2OMe$ |

condensation at 60°C and a mixture of $6^5$-*O*- and $4^5$-*O*-carbamoyl derivatives was obtained. The product ratio in the mixture was 9:1 (integral values in $^1$H NMR, data not shown). Attempts to separate individual carbamoyl derivatives from the mixture, directly or after *O*-deacetylation, were unsuccessful for closely similar structures. When the condensation was carried out under reflux conditions, the $4^5$,$6^5$-di-*O*-(*N*-ethyl)carbamate **27** was obtained in 84% yield. On the other hand, attempts to condense **12** with methyl isocyanate were unsuccessful because of the low reactivity of the reagent. As with the other acetyl derivatives, compounds **21**–**24**, **26**, and **27** were *O*-deacetylated to afford 2-chloro-4-nitrophenyl $6^5$-*O*-(*N*-phenyl)carbamoyl-β-maltopentaoside **28** (yield 59%), $6^5$-*O*-(*N*-*tert*-butyl)carbamoyl-β-maltopentaoside **29** (yield 62%), $6^5$-*O*-(*N*-isopropyl)carbamoyl-β-maltopentaoside **30** (yield 66%), $4^5$,$6^5$-di-*O*-(*N*-isopropyl)carbamoyl-β-maltopentaoside **31**, (yield 83%), 4-*O*-(*N*-isopropyl)carbamoyl-β-maltopentaoside **32** (yield 89%), and $4^5$,$6^5$-di-*O*-(*N*-ethyl)carbamoyl-β-maltopentaoside **33** (yield 54%), respectively.

The $6^5$-*O*- and/or $4^5$-*O*-(1-alkoxy)methyl derivatives were prepared to investigate the influence of the *O*-(alkoxy)methyl structure when present in the substrates, and were expected to have high water solubility. Reaction of **12** with methoxymethyl (MOM) chloride, 2-methoxyethoxymethyl (MEM) chloride, and benzyloxymethyl (BOM) chloride gave the $6^5$-*O*-MOM derivative **34** (yield 90%), the $6^5$-*O*-MEM derivatives **35** (yield 89%), and the $6^5$-*O*-BOM derivative **36** (yield 76%), respectively. These selective $6^5$-*O*-monosubstitutions were accomplished by using 5 equiv of alkoxymethyl chloride and *N*-ethyldiisopropylamine in boiling dichloromethane, while the $4^5$,$6^5$-di-*O*-substitutions were performed using 12 equiv of the reagents in refluxing $CH_3CN$ to give the $4^5$,$6^5$-di-*O*-MOM derivative **37** (yield 87%), the $4^5$,$6^5$-di-*O*-MEM derivative **38** (yield 74%), and the $4^5$,$6^5$-di-*O*-BOM derivative **39** (yield 61%), respectively. The $4^5$-*O*-MOM derivative **41** was prepared in 43% yield from **12** by a method similar to that used for **26** via the $6^5$-TBDMS derivative **40**. As with other acetyl derivatives, compounds **34–39** and **41** were *O*-deacetylated to afford 2-chloro-4-nitrophenyl $6^5$-*O*-MOM-β-maltopentaoside **42** (yield 56%), $6^5$-*O*-MEM-β-maltopentaoside **43** (yield 56%), $6^5$-*O*-BOM-β-maltopentaoside **44** (yield 81%), $4^5$,$6^5$-di-*O*-MOM-β-maltopentaoside **45** (yield 79%), $4^5$,$6^5$-di-*O*-MEM-β-maltopentaoside **46** (yield 69%), $4^5$,$6^5$-di-*O*-BOM-β-maltopentaoside **47** (yield 70%), and $4^5$-*O*-MOM-β-maltopentaoside **48** (yield 93%), respectively.

*Confirmation of structures of the maltopentaosides.*—Structures of the compounds synthesized as substrates for alpha amylase were established by spectral data and elemental analyses as described in the experimental section. The $^1H$ NMR spectra of all compounds showed a signal (1 H, doublet) at δ 5.2–5.3 assigned to the H-1a * proton and showing a large coupling constants ($J_{1a,2a}$ 7.0–7.5 Hz), and a characteristic signal pattern for the 2-chloro-4-nitrophenyl group in the aromatic region indicating the presence of a β-2-chloro-4-nitrophenyl glucoside structure. Additionally, the $^1H$ NMR spectra also showed signals (4 H, each a doublet) at δ 5.0–5.2 assigned to the H-1b–1e * protons and which displayed small coupling constants ($J_{1b-e,2b-e}$ 3.0–4.5 Hz), indicating the presence of four α-glucosidic bonds. These results designated that all compounds had a β-2-chloro-4-nitrophenyl maltopentaoside structure.

*Kinetic parameters and patterns of action of two human alpha amylases on the modified maltopentaosides.*—Compound **47** was barely soluble in water, but the other twenty-two maltopentaosides were tested to determine kinetic parameters and patterns of action of HPA and HSA. In the evaluation of kinetic parameters, each sample was incubated with each of the two amylases in the presence of coupled enzymes in phosphate buffer (pH 7.0) at 37°C as described in the experimental section. The Michaelis constant ($K_m$) and the relative rate of hydrolysis of maltopentaosides were examined by incubation, and the amount of

* The designations a-e indicate the glucose residue, from the aglycon end to the reducing end.

TABLE I

Kinetic parameters of the action of two human alpha amylases on substrate modified maltopentaosides

| Compd | $K_m$ (mM) | | Relative rate of hydrolysys | | |
|---|---|---|---|---|---|
| | HPA | HSA | HPA | HSA | $V_{HPA}/V_{HSA}$ |
| **1** | 0.29 | 0.37 | 1.00 [a] | 1.00 [a] | 1.00 |
| **2** | 0.31 | 0.29 | 0.75 | 0.99 | 0.75 |
| **3** | 0.27 | 0.29 | 0.93 | 1.09 | 0.86 |
| **4** | 0.34 | 0.38 | 0.61 | 0.72 | 0.85 |
| **5** | 0.19 | 0.25 | 0.37 | 0.45 | 0.84 |
| **6** | 0.31 | 0.33 | 0.34 | 0.40 | 0.83 |
| **10** | 0.11 | 0.14 | 1.17 | 1.15 | 0.98 |
| **11** | 0.15 | 0.17 | 1.12 | 1.31 | 0.85 |
| **18** | 0.10 | 0.16 | 1.32 | 1.28 | 1.03 |
| **19** | 0.17 | 0.28 | 0.97 | 0.97 | 1.00 |
| **20** | 0.046 | 0.086 | 0.25 | 0.52 | 0.47 |
| **28** | 0.10 | 0.12 | 0.88 | 0.80 | 1.11 |
| **29** | 0.15 | 0.15 | 1.09 | 0.95 | 1.14 |
| **30** | 0.14 | 0.17 | 1.12 | 1.18 | 0.95 |
| **31** | 0.12 | 0.14 | 0.95 | 1.04 | 0.91 |
| **32** | 0.15 | 0.22 | 0.95 | 0.94 | 1.01 |
| **33** | 0.11 | 0.15 | 1.10 | 1.21 | 0.92 |
| **42** | 0.36 | 0.43 | 1.18 | 1.05 | 1.12 |
| **43** | 0.45 | 0.63 | 1.05 | 0.90 | 1.17 |
| **44** | 0.17 | 0.17 | 1.34 | 1.17 | 1.14 |
| **45** | 0.23 | 0.31 | 0.92 | 0.98 | 0.94 |
| **46** | 0.43 | 0.59 | 0.89 | 0.93 | 0.96 |
| **48** | 0.19 | 0.25 | 1.00 | 1.24 | 0.81 |

[a] The relative rates of hydrolysis assume the value for **1** as unity.

products was measured by increments of absorbance per min at 400 nm. The $K_m$ values were calculated by the method of least squares with the use of Lineweaver–Burk plots. In the study of patterns of action, each sample was incubated with each of the two amylases in the absence of coupled enzymes and aliquots were analyzed by HPLC to determine amounts of the hydrolyzed products. These results are shown in Tables I and II.

The active sites of depolymerases and especially of endoglycanases such as human alpha amylase are considered to consist of tandem subsites geometrically complementary to several glucose residues[12]. As a matter of convenience, it is assumed that the active site of the enzyme consists of nine subsites per glucosyl moiety (G) numbered $S_1$–$S_9$ from the reducing end, and that the glucosidic bonds of the substrates are split between $S_5$ and $S_6$, as shown Scheme 1. Therefore, the binding modes of the substrates to the active sites on the course of hydrolysis may be estimated from the action patterns of the enzymes on the substrates.

Table I indicates that the more hydrophobic are the modifications made at the $6^5$-position, the smaller are the $K_m$ values for the amylases; that is, the enzymes apparently have affinity for maltopentaosides having a hydrophobic group at the 6-position of their nonreducing terminal glucosyl moiety. As shown in Scheme 1,

TABLE II

Patterns of the action of two human alpha amylases on substrate modified maltopentaosides [a]

| Compd | Ratio of products with HPA | | | | Ratio of products with HSA | | | |
|---|---|---|---|---|---|---|---|---|
| | $G_4$-CNP | $G_3$-CNP | $G_2$-CNP | $G_2$-CNP | $G_4$-CNP | $G_3$-CNP | $G_2$-CNP | $G_1$-CNP |
| **1** | 0.08 | 0.08 | 0.81 | 0.03 | 0.04 | 0.11 | 0.83 | 0.02 |
| **2** | | 0.59 | 0.36 | 0.05 | | 0.69 | 0.27 | 0.04 |
| **3** | 0.01 | 0.67 | 0.30 | 0.02 | 0.01 | 0.72 | 0.25 | 0.01 |
| **4** | 0.06 | 0.29 | 0.61 | 0.04 | 0.12 | 0.38 | 0.49 | 0.01 |
| **5** | 0.01 | 0.80 | 0.18 | 0.01 | | 0.86 | 0.13 | 0.01 |
| **6** | 0.01 | 0.44 | 0.55 | | 0.01 | 0.55 | 0.44 | |
| **10** | | | 0.98 | 0.02 | | | 0.99 | 0.01 |
| **11** | | 0.02 | 0.98 | | | 0.01 | 0.99 | |
| **18** | | | 0.98 | 0.02 | | | 0.99 | 0.01 |
| **19** | | 0.02 | 0.98 | | | | 1.00 | |
| **20** | | 0.01 | 0.96 | 0.03 | | 0.01 | 0.97 | 0.02 |
| **28** | | 0.01 | 0.95 | 0.04 | | | 0.99 | 0.01 |
| **29** | | 0.01 | 0.94 | 0.05 | | | 0.97 | 0.03 |
| **30** | | | 0.97 | 0.03 | | | 0.99 | 0.01 |
| **31** | | | 0.99 | 0.01 | | | 0.99 | 0.01 |
| **32** | | 0.15 | 0.82 | 0.03 | | 0.17 | 0.81 | 0.02 |
| **33** | | | 0.98 | 0.02 | | | 0.98 | 0.02 |
| **42** | | | 0.95 | 0.05 | | | 0.97 | 0.03 |
| **43** | | | 0.95 | 0.05 | | | 0.96 | 0.04 |
| **44** | | | 0.99 | 0.01 | | | 0.99 | 0.01 |
| **45** | | | 0.97 | 0.03 | | | 0.97 | 0.03 |
| **46** | | | 0.97 | 0.03 | | | 0.98 | 0.02 |
| **48** | | 0.38 | 0.60 | 0.02 | | 0.54 | 0.45 | 0.01 |

[a] Abbreviations: $G_4$-CNP, 2-chloro-4-nitrophenyl β-D-maltotetraoside; $G_3$-CNP, 2-chloro-4-nitrophenyl β-D-maltotrioside; $G_2$-CNP, 2-chloro-4-nitrophenyl β-D-maltoside; and $G_1$-CNP, 2-chloro-4-nitrophenyl β-D-glucopyranoside.

the two human alpha amylases hydrolyzed $6^5$-substituted derivatives (containing $6^5$-deoxy-$6^5$-, $6^5$-mono-*O*-, and $4^5$,$6^5$-di-*O*-substituted derivatives) at essentially a sole D-glucosidic linkage to produce only 2-chloro-4-nitrophenyl β-D-maltoside, whereas $4^5$-mono-*O*-substituted derivatives were hydrolyzed at two or more linkages to give maltooligosachharides of various lengths. These results suggest that as reported by Omichi et al.[13], there are similar hydrophobic residues on $S_8$ in the active site of each amylase and the residue is located close to the 6-position of the nonreducing end of maltopentaosides when the ES-complex is formed. In the case of $4^5$,$6^5$-*O*-bridged derivatives, the hydrophobic part is altered to the extent that the terminal glucosyl moiety is considered not to be able to selectively bind with $S_8$ in the active site. Interestingly, it was also found that the smaller is the size of modifications made, the lower is the ratio of the relative rate for the two amylases ($V_{HPA}/V_{HSA}$0), as shown in Table I. Modified maltopentaosides having a halogen at the $6^5$-position clearly indicate the tendency ($V_{HPA}/V_{HSA}$: **18 > 10 > 11 > 20**). This result suggests that the hydrophobic residues in the active site of the two amylases have somewhat different characters. These findings, which were obtained

in our study using many systematically synthesized substrates, provide significant data to advance studies on the active-site structure in alpha-amylases and assay of the enzyme.

Furthermore, the foregoing results establish that compounds **10**, **19**, and **30**, out of twenty-two compounds are very useful substrates for assay of human alpha-amylase since they were hydrolyzed rapidly by the two amylases at a limited position at an approximately equal rate. Tests for their clinical application, lasting stability in a buffer with coupled enzymes, precision, linearity, interference, and so on are in progress.

## EXPERIMENTAL

*Reagents and materials.*—All chemicals were of reagent grade unless otherwise noted. Alpha amylases (from human pancreatic juice and saliva) were obtained from International Reagents Corp., Kobe (Japan). α-D-Glucosidase (from yeast) and β-D-glucosidase (from sweet almond) were obtained from Toyobo Co., Ltd., Osaka (Japan).

*Apparatus.*—All melting points were determined on Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were determined with a Jasco DIP-360 digital polarimeter at 25°C. IR spectra were taken with a Jasco A-202 spectrometer. $^1$H NMR spectra were taken at 199.5 MHz and $^{13}$C NMR spectra were taken at 50.10 MHz with a Jeol JNM-FX200 spectrometer with $Me_4Si$ as an internal standard. Spectra were recorded in solutions of 10:1 (v/v) $Me_2SO$-$d_6$–$D_2O$ unless stated otherwise. High-performance liquid chromatography (HPLC) was performed on a (*A*) Cosmosil $C_{18}$ column (4.6 mm i.d. × 150 mm) or (*B*) TSK gel Amide-80 column (4.6 mm i.d. × 250 mm) with a flow rate of 1.0 mL/min using a Jasco pump (880-PU) and a UV (280 nm) detector (Jasco UVIDEC-100-V), at room temperature. Visible spectra (400 nm) were recorded with a Hitachi M-80 spectrometer. Column chromatography was performed on Merck Kiesel Gel 60 ($SiO_2$, 230–400 mesh) and YMC-GEL ODS-AQ (120-S50, from Yamamura Chemical Laboratories Co., Ltd., Japan).

*2-Chloro-4-nitrophenyl O-[4,6-O-(1,1-dimethoxy)methylidene-α-D-glucopyranosyl)-(1 → 4)-tris[O-α-D-glucopyranosyl-(1 → 4)]-β-D-glucopyranoside* (**2**).—Tetramethyl orthocarbonate (1.50 mL, 11.3 mmol) and Amberlyst 15E (750 mg) were added to a stirred solution of 2-chloro-4-nitrophenyl β-maltopentaoside[11] (**1**, 1.50 g, 1.52 mmol) in DMF (7.5 mL), and the mixture was kept for 4 h at 35°C. Then, the solution was slowly dropped into 100 mM phosphate buffer (pH 7.0, 2.0 L) under ice-cooling. The resulting mixture was evaporated in vacuo to leave a syrupy residue, which was chromatographed on ODS gel with 3:7 (v/v) $CH_3CN$–$H_2O$ to give pale-yellow, amorphous **2** (1.07 g, 1.01 mmol, yield 66.5%); $[\alpha]_D$ +86.7° (*c* 0.50, 50 mM phosphate buffer, pH 7.0); $\nu_{max}$ 3420 (OH), 2940 (CH, aliph.), 1588, 1490 (arom.) 1524, 1352 ($NO_2$) cm$^{-1}$; $^1$H NMR: δ 3.25–3.90 (m, 30

| Compd | Active site | | | | | | | | | Frequency | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | $S_9$ | $S_8$ | $S_7$ | $S_6$ | $S_5$ | $S_4$ | $S_3$ | $S_2$ | $S_1$ | HPA | HSA |
| **1** | | | | G- | -G- | G- | G- | G- | CNP | 0.08 | 0.04 |
| | | | G- | G- | -G- | G- | G- | CNP | | 0.08 | 0.11 |
| | | G- | G- | G- | -G- | G- | CNP | | | 0.81 | 0.83 |
| | G- | G- | G- | G- | -G- | CNP | | | | 0.03 | 0.02 |
| **2** | | | BG- | G- | -G- | G- | G- | CNP | | 0.59 | 0.69 |
| | | BG- | G- | G- | -G- | G- | CNP | | | 0.36 | 0.27 |
| | BG- | G- | G- | G- | -G- | CNP | | | | 0.05 | 0.04 |
| **3** | | | | BG- | -G- | G- | G- | G- | CNP | 0.01 | 0.01 |
| | | | BG- | G- | -G- | G- | G- | CNP | | 0.67 | 0.72 |
| | | BG- | G- | G- | -G- | G- | CNP | | | 0.30 | 0.25 |
| | BG- | G- | G- | Ge | -G- | CNP | | | | 0.02 | 0.01 |
| **4** | | | | BG- | -G- | G- | G- | G- | CNP | 0.06 | 0.12 |
| | | | BG- | G- | -G- | G- | G- | CNP | | 0.29 | 0.38 |
| | | BG- | G- | G- | -G- | G- | CNP | | | 0.61 | 0.49 |
| | BG- | G- | G- | G- | -G- | CNP | | | | 0.04 | 0.01 |
| **5** | | | | BG- | -G- | G- | G- | G- | CNP | 0.01 | 0 |
| | | | BG- | G- | -G- | G- | G- | CNP | | 0.80 | 0.86 |
| | | BG- | G- | G- | -G- | G- | CNP | | | 0.18 | 0.13 |
| | BG- | G- | G- | G- | -G- | CNP | | | | 0.01 | 0.01 |
| **6** | | | | BG- | -G- | G- | G- | G- | CNP | 0.01 | 0.01 |
| | | | BG- | G- | -G- | G- | G- | CNP | | 0.44 | 0.55 |
| | | BG- | G- | G- | -G- | G- | CNP | | | 0.55 | 0.44 |
| **10** | | BG- | G- | G- | -G- | G- | CNP | | | 0.98 | 0.99 |
| | BG- | G- | G- | G- | -G- | CNP | | | | 0.02 | 0.01 |
| **11** | | | BG- | G- | -G- | G- | G- | CNP | | 0.02 | 0.01 |
| | | BG- | G- | G- | -G- | G- | CNP | | | 0.98 | 0.99 |
| **18** | | BG- | G- | G- | -G- | G- | CNP | | | 0.98 | 0.99 |
| | BG- | G- | G- | G- | -G- | CNP | | | | 0.02 | 0.01 |
| **19** | | | BG- | G- | -G- | G- | G- | CNP | | 0.02 | 0 |
| | | BG- | G- | G- | -G- | G- | CNP | | | 0.98 | 1.00 |
| **20** | | | | BG- | -G- | G- | G- | G- | CNP | 0.01 | 0.02 |
| | | | BG- | G- | -G- | G- | G- | CNP | | 0.96 | 0.97 |
| | | BG- | G- | G- | -G- | G- | CNP | | | 0.03 | 0.02 |
| **28** | | | | BG- | G- | -G- | G- | G- | CNP | 0.01 | 0 |
| | | BG- | G- | G- | -G- | G- | CNP | | | 0.95 | 0.99 |
| | BG- | G- | G- | G- | -G- | CNP | | | | 0.04 | 0.01 |
| **29** | | | | BG- | G- | -G- | G- | G- | CNP | 0.0 | 0 |
| | | BG- | G- | G- | -G- | G- | CNP | | | 0.94 | 0.97 |
| | BG- | G- | G- | G- | -G- | CNP | | | | 0.05 | 0.03 |
| **30** | | BG- | G- | G- | -G- | G- | CNP | | | 0.97 | 0.99 |
| | BG- | G- | G- | G- | -G- | CNP | | | | 0.03 | 0.01 |
| **31** | | BG- | G- | G- | -G- | G- | CNP | | | 0.99 | 0.99 |
| | BG- | G- | G- | G- | -G- | CNP | | | | 0.01 | 0.01 |

| Compd | Active site | | | | | | | | | Frequency | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | $S_9$ | $S_8$ | $S_7$ | $S_6$ | $S_5$ | $S_4$ | $S_3$ | $S_2$ | $S_1$ | HPA | HSA |
| **32** | | | BG- | G- | -G- | G- | G- | CNP | | 0.15 | 0.17 |
| | | BG- | G- | G- | -G- | G- | CNP | | | 0.82 | 0.81 |
| | BG- | G- | G- | G- | -G- | CNP | | | | 0.03 | 0.02 |
| **33** | | BG- | G- | G- | -G- | G- | CNP | | | 0.98 | 0.98 |
| | BG- | G- | G- | G- | -G- | CNP | | | | 0.02 | 0.02 |
| **42** | | BG- | G- | G- | -G- | G- | CNP | | | 0.95 | 0.97 |
| | BG- | G- | G- | G- | -G- | CNP | | | | 0.05 | 0.03 |
| **43** | | BG- | G- | G- | -G- | G- | CNP | | | 0.95 | 0.96 |
| | BG- | G- | G- | G- | -G- | CNP | | | | 0.05 | 0.04 |
| **44** | | BG- | G- | G- | -G- | G- | CNP | | | 0.99 | 0.99 |
| | BG- | G- | G- | G- | -G- | CNP | | | | 0.01 | 0.01 |
| **45** | | BG- | G- | G- | -G- | G- | CNP | | | 0.99 | 0.99 |
| | BG- | G- | G- | G- | -G- | CNP | | | | 0.01 | 0.01 |
| **46** | | BG- | G- | G- | -G- | G- | CNP | | | 0.97 | 0.98 |
| | BG- | G- | G- | G- | -G- | CNP | | | | 0.03 | 0.02 |
| **48** | | | BG- | G- | -G- | G- | G- | CNP | | 0.38 | 0.54 |
| | | BG- | G- | G- | -G- | G- | CNP | | | 0.60 | 0.45 |
| | BG- | G- | G- | G- | -G- | CNP | | | | 0.02 | 0.01 |

Scheme 1. Schematic representation of substrate binding to subsites of two human alpha amylases on the substrates. Abbreviations: BG, modified glucosyl moiety; G, glucosyl moiety; CNP, β-2-chloro-4-nitrophenyl moiety

H, H-2a-e–6a-e), 3.23 and 3.30 (s, each 3 H, OMe), 5.04 (d, 2 H, *J* 3.2 Hz, H-1), 5.10 (d, 1 H, *J* 3.7 Hz, H-1), 5.12 (d, 1 H, *J* 3.4 Hz, H-1), 5.27 (d, 1 H, *J* 7.6 Hz, H-1a), 7.47 (d, 1 H, *J* 9.3 Hz, H-6 of 2-chloro-4-nitrophenyl = CNP), 8.19 (dd, 1 H, *J* 9.3 Hz and 2.7 Hz, H-5 of CNP), and 8.31 (d, 1 H, *J* 2.7 Hz, H-3 of CNP); $^{13}C$ NMR: δ 50.9 and 51.8 (2 OMe), and 118.6 (O-*C*$(OMe)_2$-O); $t_R$ (column: (*B*), eluent: 3:1 (v/v) $CH_3CN$–$H_2O$): 5.6 min. Anal. Calcd for $C_{39}H_{58}ClNO_{30} \cdot 2H_2O$: C, 42.88; H, 5.72; N, 1.28. Found: C, 42.66; H, 5.61; N, 1.34.

*2-Chloro-4-nitrophenyl O-[4,6-O-(1-methoxy)ethylidene-α-D-glucopyranosyl]-(1 → 4)-tris[O-α-D-glucopyranosyl-(→ 4)]-β-D-glucopyranoside* (**3**).—Condensation of trimethyl orthoacetate (1.50 mL, 11.8 mmol) with **1** (1.50 g, 1.52 mmol), as described for **2**, gave pale-yellow, amorphous **3** (315 mg, 0.303 mmol, yield 19.9%); $[\alpha]_D$ +91.3° (*c* 0.51, 50 mM phosphate buffer, pH 7.0); $\nu_{max}$ 3410 (OH), 2930 (CH, aliph.), 1586, 1488 (arom.), 1524, 1350 ($NO_2$) $cm^{-1}$; $^1H$ NMR: δ 1.37 (s, 3 H, Me), 3.23 (s, 3 H, OMe), 5.05 (d, 2 H, *J* 3.7 Hz, H-1), 5.09 (d, 1 H, *J* 3.7 Hz, H-1), 5.12 (d, 1 H, *J* 4.4 Hz, H-1), 5.26 (d, 1 H, *J* 7.6 Hz, H-1a), 7.47 (d, 1 H, *J* 9.3 Hz, H-6 of CNP), 8.18 (dd, 1 H, *J* 9.3 Hz and 2.7 Hz, H-5 of CNP), and 8.31 (d, 1 H, *J* 2.7 Hz, H-3 of CNP); $^{13}C$ NMR: δ 22.8 (Me), 51.3 (OMe), and 113.2 (O-*C*(OMe)Me-O); $t_R$

(column: (*B*), eluent: 3:1 (v/v) $CH_3CN$–$H_2O$): 6.0 min. Anal. Calcd for $C_{39}H_{58}ClNO_{29}$: C, 45.03; H, 5.62; N, 1.35. Found: C, 44.89; H, 5.51; N, 1.15.

*2-Chloro-4-nitrophenyl O-[4,6-O-(1-ethoxy)ethylidene-α-D-glucopyranosyl]-(1 → 4)-tris[O-α-D-glucopyranosyl-(1 → 4)-β-D-glucopyranoside* (**4**).—Condensation of triethyl orthoacetate (1.50 mL, 8.18 mmol) with **1** (1.50 g, 1.52 mmol), as described for **2**, gave pale-yellow, amorphous **4** (509 mg, 0.483 mmol, yield 31.6%); $[\alpha]_D$ +85.2° (*c* 0.42, MeOH); $\nu_{max}$ 3400 (OH), 2930 (CH, aliph.), 1584, 1486 (arom.), 1520, 1350 ($NO_2$) $cm^{-1}$; $^1H$ NMR: δ 1.16 (t, 3 H, *J* 6.9 Hz, $OCH_2CH_3$), 1.38 (s, 3 H, Me), 5.03 (d, 2 H, *J* 3.7 Hz, H-1), 5.08 (d, 1 H, *J* 2.9 Hz, H-1), 5.10 (d, 1 H, *J* 3.2 Hz, H-1), 5.27 (d, 1 H, *J* 7.6 Hz, H-1a), 7.47 (d, 1 H, *J* 9.1 Hz, H-6 of CNP), 8.18 (dd, 1 H, *J* 9.1 Hz and 2.7 Hz, H-5 of CNP, and 8.31 (d, 1 H, *J* 2.7 Hz, H-3 of CNP); $^{13}C$ NMR: δ 15.9 ($OCH_2CH_3$), 23.4 (O-C(OEt)$CH_3$-O), 58.8 ($OCH_2CH_3$), and 112.4 (O-C(OEt)Me-O); $t_R$ (column: (*B*), eluent: 3:1 (v/v) $CH_3CN$–$H_2O$): 4.8 min. Anal. Calcd for $C_{40}H_{60}ClNO_{29}$: C, 45.57; H, 5.74; N, 1.33. Found: C, 45.13; H, 5.89; N, 1.28.

*2-Chloro-4-nitrophenyl O-(4,6-O-benzylidene-α-D-glucopyranosyl)-(1 → 4)-tris[O-α-Dglucopyranosyl-(1 → 4)]-β-D-glucopyranoside* (**5**).—Benzaldehyde dimethyl acetal (11.4 mL, 76.0 mmol) and *p*-toluenesulfonic acid monohydrate (2.25 g, 11.4 mmol) were added to a stirred solution of **1** (15.0 g, 15.2 mmol) in DMF (225 mL), and the mixture was kept for 4 h at 50°C. Then, the solution was slowly dropped into ice–water (1.0 L) with stirring. The resulting mixture was neutralized by adding a satd aq $Na_2CO_3$ solution slowly with stirring under ice-cooling, and washed three times with 300 mL of $CH_2Cl_2$. The aqueous layer was evaporated in vacuo to leave a syrupy residue, which was chromatographed on ODS gel with 2:3 (v/v) EtOH–$H_2O$ to give **5** as a pale-yellow powder (11.2 g, 10.4 mmol, yield 68.3%); mp 196–200°C (from MeOH–$^i$PrOH); $[\alpha]_D$ +64.0° (*c* 0.25, 1,4-dioxane); $\nu_{max}$ 3410 (OH), 2940 (CH, aliph.), 1586, 1486 (arom.), 1520, 1350 ($NO_2$) $cm^{-1}$; $^1H$ NMR ($D_2O$): δ 5.05 (d, 2 H, *J* 3.4 Hz, H-1), 5.12 (d, 1 H, *J* 4.4 Hz, H-1), 5.13 (d, 1 H, *J* 4.4 Hz, H-1), 5.25 (d, 1 H, *J* 7.6 Hz, H-1a), 5.56 (s, 1 H, O-C*H*(Ph)-O), 7.35 (d, 1 H, *J* 9.0 Hz, H-6 of CNP), 7.55 (br s, 5 H, Ph), 8.14 (dd, 1 H, *J* 9.0 and 2.7 Hz, H-5 of CNP), and 8.29 (d, 1 H, *J* 2.7 Hz, H-3 of CNP); $^{13}C$ NMR: δ 125.8, 126.8, 128.4, and 138.1 (Ph), and 129.2 (O-*C*H(Ph)-O); $t_R$ (column: (*B*), eluent: 3:1 (v/v) $CH_3CN$-$H_2O$): 4.8 min. Anal. Calcd for $C_{43}H_{58}ClNO_{28} \cdot 1.6H_2O$: C, 46.90; H, 5.60; N, 1.27. Found: C, 46.46; H, 5.40; N, 1.30.

*2-Chloro-4-nitrophenyl O-(4,6-O-isopropylidene-α-D-glucopyranosyl]-(1 → 4)-tris[O-α-D-glucopranosyl-(1 → 4)]-β-D-glucopyranoside* (**6**).—Condensation of acetone dimethyl acetal (1.87 mL, 15.3 mmol) with **1** (3.00 g, 3.05 mmol), as described for **5**, gave pale-yellow, amorphous **6** (1.01 g, 0.973 mmol, yield 31.9%); $[\alpha]_D$ +79.8° (*c* 0.50, MeOH); $\nu_{max}$ 3410 (OH), 2940 (CH, aliph.), 1586, 1486 (arom.), 1520, 1348 ($NO_2$) $cm^{-1}$; $^1H$ NMR: δ 1.31 and 1.43 (s, each 3 H, 2 Me), 5.05 (d, 2 H, *J* 3.7 Hz, H-1), 5.08 (d, 1 H, *J* 4.9 Hz, H-1), 5.12 (d, 1 H, *J* 3.7 Hz, H-1), 5.26 (d, 1 H, *J* 7.8 Hz, H-1a), 7.47 (d, 1 H, *J* 9.3 Hz, H-6 of CNP), 8.18 (dd, 1 H, *J* 9.3 and 2.7 Hz, H-5 of CNP), and 8.30 (d, 1 H, 2.7 Hz, H-3 of CNP); $^{13}C$ NMR: δ 19.5

and 29.4 (O-C*Me*$_2$-O), and 99.4 (O-*C*Me$_2$-O); $t_R$ (column: (*B*), eluent: 3:1 (v/v) $CH_3CN$-$H_2O$): 6.7 min. Anal. Calcd for $C_{39}H_{58}ClNO_{28}$: C, 45.73; H, 5.71; N, 1.37. Found: C, 45.53; H, 5.69; N, 1.28.

*2-Chloro-4-nitrophenyl* O-*(2,3-di-*O*-acetyl-4,6-*O*-benzylidene-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* (**7**).—Acetic anhydride (100 mL, 1.06 mol) was added to a stirred solution of **5** (13.3 g, 12.4 mmol) in pyridine (200 mL), and the mixture was kept at room temperature for 2 days. The solution was then evaporated in vacuo to leave a syrupy residue, which was chromatographed on $SiO_2$ gel with 1:49 (v/v) MeOH–$CH_2Cl_2$ to give **7** (18.1 g, 10.9 mmol, yield 87.9%); mp 130–135°C (prisms, from EtOH–$Et_2O$); $[\alpha]_D$ +84.0° (*c* 0.25, 1,4-dioxane); $\nu_{max}$ 1756 (C=O) $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 2.00–2.21 (cluster of s, 42 H, 14 OAc), and 7.26–7.41 (m, 6 H, H-6 of CNP and O-C(*Ph*)H-O); $t_R$ (column: (*A*), eluent: 3:1 (v/v) $CH_3CN$–$H_2O$): 7.7 min. Anal. Calcd for $C_{71}H_{86}ClNO_{42}$: C, 51.34; H, 5.22; N, 0.84. Found: C, 51.56; H, 5.24; N, 1.00.

*2-Chloro-4-nitrophenyl* O-*(2,3-di-*O*-acetyl-4-*O*-benzoyl-6-bromo-6-deoxy-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* (**8**).—Barium carbonate (14.8 g, 75.0 mmol) was added to a stirred solution of compound **7** (2.99 g, 1.80 mmol) in $CCl_4$ (120 mL) and 1,1,2,2-tetrachloroethane (120 mL), and the resulting mixture was heated under reflux with stirring. Then, *N*-bromosuccinimide (427 mg, 2.40 mmol) was added to the refluxing solution and boiling was continued for 1 h. The mixture was cooled and filtered through a pad of Celite. The insoluble material was washed with $CH_2Cl_2$. The filtrate and washings were evaporated in vacuo to leave a syrupy residue, which was chromatographed on $SiO_2$ gel with 25:1:99 (v/v/v) EtOAc–MeOH–$CH_2Cl_2$ to give **8** (2.71 g, 1.56 mmol, yield 86.5%); mp 121.5–123.5°C (from $Et_2O$); $[\alpha]_D$ +75.4° (*c* 0.50, 1,4-dioxane); $^1H$ NMR ($CDCl_3$): δ 3.41 (dd, 1 H, *J* 12.0 and 5.5 Hz, H-6e$_a$), 3.51 (dd, 1 H, *J* 12.0 and 2.3 Hz, H-6e$_b$), 7.46 (t, 2 H, *J* 7.5 Hz, H-3, 5 of Bz), 7.60 (t, 1 H, *J* 7.5 Hz, H-4 of Bz), and 8.00 (d, 2 H, *J* 7.5 Hz, H-2, 6 of Bz); $t_R$ (column: (*A*), eluent: 7:3 (v/v) $CH_3CN$–$H_2O$): 16.3 min. Anal. Calcd for $C_{71}H_{85}BrClNO_{42}$: C, 49.02; H, 4.92; N, 0.81. Found: C, 48.97; H, 4.90; N, 0.72.

*2-Chloro-4-nitrophenyl* O-*(2,3-di-*O*-acetyl-4-*O*-benzoyl-6-chloro-6-deoxy-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* (**9**).—Lithium chloride (1.98 g, 46.8 mmol) was added to a stirred solution of **8** (2.71 g, 1.56 mmol) in HMPA (100 mL) and the resulting mixture was heated for 3 h at 70°C. Toluene (500 mL) was added to the solution, and the mixture was washed with water, dried ($Na_2SO_4$), and evaporated in vacuo to leave a syrupy residue, which was chromatographed on $SiO_2$ gel with 25:1:99 (v/v/v) EtOAc–MeOH–$CH_2Cl_2$ to give **9** (2.43 g, 1.50 mmol, yield 96.5%); mp 109–111°C (from $Et_2O$); $[\alpha]_D$ +70.2° (*c* 0.52, 1,4-dioxane); $^1H$ NMR ($CDCl_3$): δ 3.60–3.63 (ABX, 2 H, H-6e); $t_R$ (column: (*A*), eluent: 7:3 (v/v)

$CH_3CN$–$H_2O$): 15.3 min. Anal. Calcd for $C_{71}H_{85}Cl_2NO_{42}$: C, 50.30; H, 5.05; N, 0.83. Found: C. 50.07; H, 5.19; N, 0.74.

*2-Chloro-4-nitrophenyl O-(6-bromo-6-deoxy-α-D-glucopyranosyl)-(1 → 4)-tris[O-(α-D-glucopyranosyl)-(1 → 4)]-β-D-glucopyranoside* (**10**).—Potassium carbonate (133 mg, 0.964 mmol) was added to a suspension of **8** (1.52 g, 0.874 mmol) in MeOH (150 mL) with stirring, and the mixture was kept for 5 h at room temperature. Then 100 mM phosphate buffer (pH 6.5, 200 mL) was added to the solution, half of the solvent was evaporated in vacuo, and a solution of the residue in water was obtained. Gel-column chromatography on ODS ($H_2O$ → 5% → 10% → 20% $CH_3CN$, stepwise) of the solution gave pale-yellow, amorphous **10** (665 mg, 0.636 mmol, yield 72.8%), $[\alpha]_D$ +85° (*c* 0.50, $H_2O$); $\nu_{max}$ 3400 (OH), 2930 (CH, aliph.), 1584, 1486 (arom.), 1520, 1350 ($NO_2$) $cm^{-1}$; $^1H$ NMR: δ 5.05 (d, 2 H, *J* 3.7 Hz, H-1), 5.12 (d, 2 H, *J* 3.7 Hz, H-1), 5.26 (d, 1 H, *J* 7.8 Hz, H-1a), 7.47 (d, 1 H, *J* 9.3 Hz, H-6 of CNP), 8.19 (dd, 1 H, *J* 9.3 and 2.7 Hz, H-5 of CNP), and 8.30 (d, 1 H, *J* 2.7 Hz, H-3 of CNP); $^{13}C$ NMR: δ 35.4 ($CH_2Br$); $t_R$ (column: (*B*), eluent: (v/v) $CH_3CN$–$H_2O$): 6.3 min. Anal. Calcd for $C_{36}H_{53}BrClNO_{27}$: C, 40.49; H, 5.20; N, 1.31. Found: C, 40.21; H, 5.16; N, 1.34.

*2-Chloro-4-nitrophenyl O-(6-chloro-6-deoxy-α-D-glucopyranosyl)-(1 → 4)-tris[O-(α-D-glucopyranosyl)-(1 → 4)]-β-D-glucopyranoside* (**11**).—*O*-deacetylation of **9** (2.22 g, 1.31 mmol) with $K_2CO_3$, as described for **10**, gave pale-yellow, amorphous **11** (938 mg, 0.936 mmol, yield 71.4%); $[\alpha]_D$ +92.4° (*c* 0.51, $H_2O$); $\nu_{max}$ 3420 (OH), 2920 (CH, aliph.), 1586, 1484 (arom.), 1520, 1348 ($NO_2$) $cm^{-1}$; $^1H$ NMR: δ 5.02 (d, 2 H, *J* 3.7 Hz, H-1), 5.09 (d, 1 H, *J* 3.7 Hz, H-1), 5.11 (d, 1 H, *J* 4.4 Hz, H-1), 5.26 (d, 1 H, *J* 7.6 Hz, H-1a), 7.47 (d, 1 H, *J* 9.3 Hz, H-6 of CNP), 8.19 (dd, 1 H, *J* 9.3 and 2.7 Hz, H-5 of CNP), and 8.30 (d, 1 H, *J* 2.7 Hz, H-3 of CNP); $^{13}C$ NMR: δ 45.5 ($CH_2Cl$); $t_R$ (column: (*B*), eluent: 3:1 (v/v) $CH_3CN$–$H_2O$): 7.1 min. Anal. Calcd for $C_{36}H_{53}Cl_2NO_{27}$: C, 43.12; H, 5.33; N, 1.40. Found: C, 43.22; H, 5.28; N, 1.42.

*2-Chloro-4-nitrophenyl O-(2,3-di-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* (**12**).—Acetylation of **2** (12.0 g, 8.52 mmol) as described for **7**, gave a syrupy residue containing the acetyl derivative, which was dissolved in AcOH (400 mL). Water (100 mL) was added to the solution and the mixture was stirred at 30°C for 2 days. Dichloromethane (2.0 L) was added and the mixture was washed with water, dried ($Na_2SO_4$), and evaporated in vacuo to leave a syrupy residue, which was chromatographed on $SiO_2$ gel with 66:2.5:33 (v/v/v) EtOAc–MeOH–$CH_2Cl_2$ to give **12** (8.86 g, 5.63 mmol, yield 66.1%); mp 126–130°C (from $Et_2O$); $[\alpha]_D$ +88.0° (*c* 0.25, 1,4-dioxane); $\nu_{max}$ 3480 (OH), 1752 (C=O) $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 1.91–2.12 (cluster of s, 42 H, 14 OAc); $t_R$ (column: (*A*), eluent: 7:3 (v/v) $CH_3CN$–$H_2O$): 4.2 min. Anal. Calcd for $C_{64}H_{82}ClNO_{42} \cdot 0.5H_2O$: C, 48.60; H, 5.29; N, 0.89. Found: C, 48.47; H, 5.24; N, 0.96.

*2-Chloro-4-nitrophenyl O-(2,3,4-tri-O-acetyl-6-O-tolylsulfonyl-α-D-glucopyranosyl)-(1 → 4)-tris[O-(2,3,6-tri-O-acetyl-α-D-glucopyranosyl)-(1 → 4)]-2,3,6-tri-O-ace-*

*tyl-β-D-glucopyranoside* (**13**).—*p*-Toluenesulfonyl chloride (21.1 g, 110 mmol) was added to a solution of **12** (11.6 g, 7.38 mmol) in pyridine (300 mL), and the mixture was stirred for 5 h at room temperature. Then $Ac_2O$ (150 mL) was added to the solution and the solution was stirred for additional 15 h at room temperature. The mixture was evaporated in vacuo to leave a syrupy residue, which was chromatographed on $SiO_2$ gel with 40:1:100 (v/v/v) EtOAc–MeOH–$CH_2Cl_2$ to give **15** (5.79 g, 3.27 mmol, yield 44.3%); mp 116.5–118°C (from $Et_2O$); $[\alpha]_D$ +92.6° (*c* 0.69, 1,4-dioxane); $^1H$ NMR ($CDCl_3$): δ 2.45 (s, 3H, Ph-C$H_3$) and 7.35 and 7.78 (2 d, each 2 H, *J* 8.2 Hz, Ph of Ts); $t_R$ (column: (*A*), eluent: 3:1 (v/v) $CH_3CN$–$H_2O$): 6.7 min. Anal. Calcd for $C_{73}H_{90}ClNO_{45}S$: C, 49.56; H, 5.13; N, 0.79. Found: C, 49.40; H, 5.09; N, 0.83.

*2-Chloro-4-nitrophenyl O-(2,3,4-tri-O-acetyl-6-deoxy-6-iodo-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* (**14**).—Treatment of NaI (5.08 g, 33.9 mmol) with **13** (2.00 g, 1.13 mmol) in butanone (120 mL), as described for **9**, gave **14** (1.84 g, 1.07 mmol, yield 94.7%); mp 127–129°C (from $Et_2O$); $[\alpha]_D$ +91.0° (*c* 0.63, 1,4-dioxane); $^1H$ NMR ($CDCl_3$): δ 3.13 (dd, 1 H, *J* 11.2 and 6.2 Hz, H-6e$_a$), 3.28 (dd, 1 H, *J* 11.2 and 1.5 Hz, H-6e$_b$), and 3.68 (ddd, 1 H, *J* 8.8, 6.2, and 1.5 Hz, H-5e); $t_R$ (column: (*A*), eluent: 3:1 (v/v) $CH_3CN$–$H_2O$): 6.0 min. Anal. Calcd for $C_{66}H_{83}ClNO_{42}$: C, 45.96; H, 4.85; N, 0.81. Found: C, 45.87; H, 4.84; N, 0.68.

*2-Chloro-4-nitrophenyl O-(2,3,4-tri-O-acetyl-6-azido-6-deoxy-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* (**15**).—Treatment of $NaN_3$ (1.23 g, 18.9 mmol) with **13** (2.25 g, 1.27 mmol), as described for **14**, gave **15** (2.05 g, 1.21 mmol, yield 95.3%); mp 121–123°C (from $Et_2O$); $[\alpha]_D$ +82.2° (*c* 0.51, 1,4-dioxane); $\nu_{max}$ 2106 ($N_3$) $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 3.31–3.33 (ABX, 2 H, H-6e); $t_R$ (column: (*A*), eluent: 7:3 (v/v) $CH_3CN$-$H_2O$): 17.6 min. Anal. Calcd for $C_{66}H_{83}ClN_4O_{42}$: C, 48.34; H, 5.10; N, 3.42. Found: C, 48.22; H, 5.15; N, 3.45.

*2-Chloro-4-nitrophenyl O-(2,3-di-O-acetyl-4-O-benzoyl-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* (**16**).—*tert*-Butylchlorodimethylsilane (1.84 g, 12.2 mmol) was added to a solution of **12** (4.72 g, 3.00 mmol) in DMF (90 mL) containing imidazole (2.59 g, 24.0 mmol) and the mixture was stirred for 8 h at room temperature. Toluene (1.0 L) was added and the mixture was washed with water, dried ($Na_2SO_4$), and evaporated in vacuo. The syrupy residue was dissolved in pyridine (100 mL), benzoyl chloride (3.50 mL, 30.1 mmol) was added, and the mixture was stirred for 30 h at room temperature. The mixture was then evaporated in vacuo to leave a syrup containing the $4^5$-*O*-benzoyl-$6^5$-*O*-TBDMS derivative, which was dissolved in AcOH (300 mL), water (75 mL) was added, and the mixture was stirred for 6 h at 45°C. Dichlormethane (500 mL) was added to the solution, and the mixture was washed with water, dried ($Na_2SO_4$), and evaporated in vacuo to leave a syrup, which was chromatographed on $SiO_2$ gel with 50:1:99 (v/v/v) EtOAc–MeOH–$CH_2Cl_2$ to give **16** (2.80 g, 1.67 mmol, yield 55.7% from **12**); mp 127–129°C (from

$Et_2O$); $[\alpha]_D$ +84.6° (*c* 0.78, 1,4-dioxane); $\nu_{max}$ 3350 (OH) $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 1.91–2.20 (cluster of s, 42 H, 14 OAc), 7.46 (t, 2 H, *J* 7.6 Hz, H-3, 5 of Bz), 7.61 (t, 1 H, *J* 7.6 Hz, H-4 of Bz), and 7.99 (d, 2 H, *J* 7.6 Hz, H-2, 6 of Bz); $t_R$ (column: (*A*), eluent: 7:3 (v/v) $CH_3CN$–$H_2O$): 8.6 min. Anal. Calcd for $C_{71}H_{86}ClNO_{43}$: C, 50.85; H, 5.17; N, 0.84. Found: C, 50.67; H, 5.24; N, 0.93.

*2-Chloro-4-nitrophenyl* O-*(2,3-di-*O*-acetyl-4-*O*-benzoyl-6-deoxy-6-fluoro-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* (**17**).—Diethylaminosulfur trifluoride (DAST, 1.38 mL, 10.5 mmol) in diglyme (20 mL) was added dropwise to the solution of **16** (2.67 g, 1.60 mmol) in diglyme (55 mL) at −20°C and the mixture was stirred for 1 h at −20°C. After additional stirring for 16 h at room temperature, MeOH (10 mL) was added to the mixture to decompose excess DAST. Toluene (500 mL) was added and the mixture was washed with 5% aq $NaHCO_3$ and water, dried ($Na_2SO_4$), and evaporated in vacuo to a syrup, which was chromatographed on $SiO_2$ gel with 20:1:99 (v/v/v) EtOAc–MeOH–$CH_2Cl_2$ to give **17** (2.37 g, 1.41 mmol, yield 88.1%); mp 123–125°C (from $Et_2O$); $[\alpha]_D$ +82.4° (*c* 0.58, 1,4-dioxane) $t_R$ (column: (*A*), eluent: 7:3 (v/v) $CH_3CN$–$H_2O$): 14.9 min. Anal. Calcd for $C_{71}H_{85}ClFNO_{42}$: C, 50.79; H, 5.10; N, 0.83. Found: C, 50.88; H, 5.06; N, 0.64.

*2-Chloro-4-nitrophenyl* O-*(6-deoxy-6-iodo-α-*D*-glucopyranosyl)-(1 → 4)-tris[*O*-(α-*D*-glucopyranosyl)-(1 → 4)]-β-*D*-glucopyranoside* (**18**).—*O*-Deacetylation of **14** (1.45 g, 0.841 mmol) with $K_2CO_3$, as described for **10**, gave pale-yellow, amorphous **18** (546 mg, 0.499 mmol, yield 59.3%); $[\alpha]_D$ +80.0° (*c* 0.51, $H_2O$); $\nu_{max}$ 3400 (OH), 2930 (CH, aliph.), 1584, 1484 (arom.), 1518, 1328 ($NO_2$) $cm^{-1}$; $^1H$ NMR: δ 5.05 (d, 2 H, *J* 3.7 Hz, H-1), 5.12 (d, 2 H, *J* 3.9 Hz, H-1), 5.26 (d, 1 H, *J* 7.6 Hz, H-1a), 7.47 (d, 1 H, *J* 9.3 Hz, H-6 of CNP), 8.19 (dd, 1 H, *J* 9.3 and 2.7 Hz, H-5 of CNP), and 8.31 (d, 1 H, *J* 2.7 Hz, H-3 of CNP); $^{13}C$ NMR: δ 10.4 ($CH_2I$); $t_R$ (column: (*B*), eluent: 3:1 (v/v) $CH_3CN$–$H_2O$): 6.8 min. Anal. Calcd for $C_{36}H_{53}ClINO_{27}$: C, 39.52; H, 4.88; N, 1.28. Found: C, 39.60; H, 4.74; N, 1.18.

*2-Chloro-4-nitrophenyl* O-*(6-azido-6-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-tris[*O*-(α-*D*-glucopyranosyl)-(1 → 4)]-β-*D*-glucopyranoside* (**19**).—*O*-Deacetylation of **15** (2.05 g, 1.21 mmol) with $K_2CO_3$, as described for **10**, gave pale-yellow, amorphous **19** (876 mg, 0.868 mmol, yield 71.7%); $[\alpha]_D$ +92.4° (*c* 0.52, $H_2O$); $\nu_{max}$ 3410 (OH), 2930 (CH, aliph.), 2110 ($N_3$), 1584, 1484 (arom.), 1520, 1348 ($NO_2$), 1274, 1150, 1078, and 1024 (C-O) $cm^{-1}$; $^1H$ NMR: δ 3.05–3.90 (m, 30 H, H-2a-e–6a-e), 5.05 (d, 2 H, *J* 3.7 Hz, H-1), 5.10 (d, 2 H, *J* 3.7 Hz, H-1), 5.26 (d, 1 H, *J* 7.6 Hz, H-1a), 7.47 (d, 1 H, *J* 9.3 Hz, H-6 of CNP), 8.19 (dd, 1 H, *J* 9.3 and 2.7 Hz, H-5 of CNP), and 8.29 (d, 1 H, *J* 2.7 Hz, H-3 of CNP); $^{13}C$ NMR: δ 52.5 ($CH_2N_3$); $t_R$ (column: (*B*), eluent: 3:1 (v/v) $CH_3CN$–$H_2O$): 6.4 min. Anal. Calcd for $C_{36}H_{53}ClN_4O_{27}\cdot 1.5H_2O$: C, 41.72; H, 5.45; N, 5.41. Found: C, 41.77; H, 5.35; N, 5.43.

*2-Chloro-4-nitrophenyl* O-*(6-deoxy-6-fluoro-α-*D*-glucopyranosyl)-(1 → 4)-tris[*O*-(α-*D*-glucopyranosyl)-(1 → 4)]-β-*D*-glucopyranoside* (**20**).—*O*-Deacetylation of **17** (2.28 g, 1.36 mmol) with $K_2CO_3$, as described for **10**, gave pale-yellow, amorphous **20** (774 mg, 0.785 mmol, yield 57.7%); $[\alpha]_D$ +97.3° (*c* 0.53, $H_2O$); $\nu_{max}$ 3400 (OH),

2930 (CH, aliph.), 1638, 1586, 1486 (arom.), 1518, 1350 ($NO_2$) $cm^{-1}$; $^1H$ NMR $\delta$ 4.55 (2 H, br d, $J$ 47.9 Hz, H-6e), 5.06 (d, 2 H, $J$ 3.4 Hz, H-1), 5.10 (d, 1 H, $J$ 3.7 Hz, H-1), 5.13 (d, 1 H, $J$ 3.9 Hz, H-1), 5.25 (d, 1 H, $J$ 7.6 Hz, H-1a), 7.47 (d, 1 H, $J$ 9.3 Hz, H-6 of CNP), 8.19 (dd, 1 H, $J$ 9.3 and 2.7 Hz, H-5 of CNP), and 8.31 (d, 1 H, $J$ 2.7 Hz, H-3 of CNP); $^{13}C$ NMR: $\delta$ 83.0 (d, $J$ 169.7 Hz, $CH_2F$); $t_R$ (column: (*B*), eluent: 3:1 (v/v) $CH_3CN$–$H_2O$): 6.6 min. Anal. Calcd for $C_{36}H_{53}ClFNO_{27}$: C, 43.84; H, 5.42; N, 1.42. Found: C, 43.59; H, 5.62; N, 1.44.

*2-Chloro-4-nitrophenyl O-[2,3-di-O-acetyl-6-O-(N-phenyl)carbamoyl-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**).—Phenyl isocyanate (3.30 mL, 30.5 mmol) was added to a solution of **12** (2.90 g, 1.84 mmol) in pyridine (300 mL) containing 4A molecular sieves (6.0 g). The mixture was stirred for 5 h at room temperature, and inorganic material was collected on a layer of Celite and washed with $CH_2Cl_2$. The combined filtrate and washings were evaporated in vacuo to leave a syrupy residue, which was chromatographed on $SiO_2$ gel with 100:1:200 (v/v/v) EtOAc–MeOH–$CH_2Cl_2$ to give **21** (2.61 g, 1.54 mmol, yield 83.7%); mp 123–125°C (from $Et_2O$); $[\alpha]_D$ +81.5° (*c* 0.42, 1,4-dioxane); $^1H$ NMR ($CDCl_3$): $\delta$ 4.61 (br s, 1 H, OH), 7.06 (t, 1 H, $J$ 7.3 Hz, COPh), and 7.30 and 7.46 (2 t, each 2 H, $J$ 7.3 Hz, COPh); $t_R$ (column: (*A*), eluent: 3:1 (v/v) $CH_3CN$–$H_2O$): 7.4 min. Anal. Calcd for $C_{71}H_{87}ClN_2O_{43}$: C, 50.40; H, 5.18; N, 1.66. Found: C, 50.40; H, 5.23; N, 1.65.

*2-Chloro-4-nitrophenyl O-[2,3-di-O-acetyl-6-O-(N-tert-butyl)carbamoyl-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* (**22**).—Condensation of *tert*-butyl isocayanate (5.5 mL, 48.2 mmol) with **12** (2.50 g, 1.59 mmol) for 4 h at 70°C, as described for **21**, gave **22** (2.28 g, 1.36 mmol, yield 85.5%); mp 124–126°C (from $Et_2O$); $[\alpha]_D$ +77.4° (*c* 0.50, 1,4-dioxane); $\nu_{max}$ 3370 (OH) $cm^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ 1.32 (s, 9 H, $^tBu$) and 3.43 (br t, 1 H, $J$ 9.7 Hz, H-4e); $t_R$ (column: (*A*), eluent: 3:1 (v/v) $CH_3Cn$–$H_2O$): 8.3 min. Anal. Calcd for $C_{69}H_{91}ClN_2O_{43}$: C, 49.57; H, 5.49; N, 1.68. Found: C, 49.50; H, 5.64; N, 1.78.

*2-Chloro-4-nitrophenyl O-[2,3-di-O-acetyl-6-O-(N-isopropyl)carbamoyl-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* (**23**).—Condensation of isopropyl isocyanate (4.7 mL, 47.8 mmol) with **12** (2.50 g, 1.59 mmol) for 4 h at 70°C, as described for **21**, gave **23** (2.62 g, 1.58 mmol, yield 99.4%); mp 114–116°C (dec) (from $Et_2O$); $[\alpha]_D$ +73.9° (*c* 0.50, 1,4-dioxane); $\nu_{max}$ 3360 (OH) $cm^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ 1.14 and 1.17 (2 d, each 3 H, $J$ 6.4 and 6.8 Hz, 2 Me of $^iPr$) and 3.45 (br t, 1 H, $J$ 9.6 Hz, H-4e); $t_R$ (column: (*A*), eluent: 3:1 (v/v) $CH_3CN$–$H_2O$): 6.5 min. Anal. Calcd for $C_{68}H_{89}ClN_2O_{43}$: C, 49.26; H, 5.41; N, 2.14. Found: C, 49.68; H, 5.55; N, 2.11.

*2-Chloro-4-nitrophenyl O-[2,3-di-O-acetyl-4,6-di-O-(N-isopropyl)carbamoyl-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* (**24**).—Condensation of isopropyl isocyanate (11.3 mL, 115 mmol) with **23** (6.33 g, 3.82 mmol) for 16 h at 90°C, as described for **21**, gave **24** (5.16 g, 2.96 mmol, yield 77.5%); mp 130–131°C (dec) (from $Et_2O$); $[\alpha]_D$

+81.6° (*c* 0.50, 1,4-dioxane); $^1$H NMR ($CDCl_3$): δ 1.12, 1.13, 1.15, and 1.16 (4 d, each 3 H, *J* 6.8, 5.6, 6.6, and 6.4 Hz, 4 Me of $^i$Pr); $t_R$ (column: (*A*), eluent: 3:1 (v/v) $CH_3CN$–$H_2O$): 9.0 min. Anal. Calcd for $C_{72}H_{97}ClN_3O_{44}$: C, 49.59; H, 5.61; N, 2.41. Found: C, 49.78; H, 5.55; N, 2.31.

*2-Chloro-4-nitrophenyl* O-*[2,3-di*-O-*acetyl-4*-O-(N-*isopropyl)carbamoyl-6*-O-(tert-*butyldimethyl)silyl-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* (**25**).—Reaction of **12** (1.83 g, 1.16 mmol) with TBDMS-Cl was carried out as described for **16** to leave a syrupy residue. Condensation of isopropyl isocyanate (5.0 mL, 50.9 mmol) with the residue for 5 h at 85°C, as described for **21**, gave **25** (1.27 g, 0.717 mmol, yield 61.4% from **12**); mp 118–120°C (from $Et_2O$); $[\alpha]_D$ +87.3° (*c* 0.53, 1,4-dioxane); $^1$H NMR ($CDCl_3$): δ 0.04 and 0.06 (2 s, each 3 H, 2 Si-Me), 0.90 (s, 9 H, 3 Me of $^t$Bu) and 1.12 (d 6 H, *J* 6.6 Hz, 2 Me of $^i$Pr); $t_R$ (column: (*A*), eluent: 4:1 (v/v) $CH_3CN$–$H_2O$): 16.4 min. Anal. Calcd for $C_{74}H_{103}ClN_2O_{43}Si$: C, 50.15; H, 5.86; N, 1.58. Found: C, 49.98; H, 5.92; N, 1.49.

*2-Chloro-4-nitrophenyl* O-*[2,3-di*-O-*acetyl-4*-O-(N-*isopropyl)carbamoyl-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* (**26**).—As for the *O*-debenzylidenation of **7**, *O*-desilylation of **25** (1.17 g, 0.661 mmol) with aq AcOH was carried out to afford **26** (1.02 g, 0.616 mmol, yield 93.2%); mp 121–123°C (from $Et_2O$); $[\alpha]_D$ +90.0° (*c* 0.53, 1,4-dioxane); $\nu_{max}$ 3470 (OH) cm$^{-1}$; $^1$H NMR ($CDCl_3$): δ 1.14 (d, 6 H, 2 Me or $^i$Pr) and 3.05–3.15 (m, 2 H, H-6e); $t_R$ (column: (*A*), eluent: 3:1 (v/v) $CH_3CN$–$H_2O$): 6.5 min. Anal. Calcd for $C_{68}H_{89}ClN_2O_{43}$: C, 49.26; H, 5.41; N, 2.14. Found: C, 49.08; H, 5.57; N, 2.14.

*2-Chloro-4-nitrophenyl* O-*[2,3-di*-O-*acetyl-4,6-di*-O-(N-*ethyl)carbamoyl-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**).—Condensation of ethyl isocyanate (6.3 mL, 79.6 mmol) with **12** (2.50 g, 1.59 mmol) for 8 h at 90°C, as described for **21**, gave **27** (2.28 g, 1.33 mmol, yield 83.6%); mp 83.5–85.5°C (dec) (from $Et_2O$); $[\alpha]_D$ +79.8° (*c* 0.58, 1,4-dioxane); $^1$H NMR ($CDCl_3$): δ 1.15 and 1.18 (2 t, each 3 H, *J* 7.3 and 7.6 Hz, 2 $NCH_2CH_3$), 3.29 and 3.32 (2 q, each 2 H, *J* 7.3 and 7.6 Hz, 2 $NCH_2CH_3$), and 7.05 (br s, 2 H, NH); $t_R$ (column: (*A*), eluent: 3:1 (v/v) $CH_3CN$–$H_2O$): 6.5 min. Anal. Calcd for $C_{70}H_{93}ClN_3O_{44}$: C, 49.00 H, 5.46; N, 2.45. Found: C, 48.77; H, 5.50; N, 2.69.

*2-Chloro-4-nitrophenyl* O-*[6*-O-(N-*phenyl)carbamoyl-α*-D-*glucopyranosyl]-(1 → 4)-tris[*O-(*α*-D-*glucopyranosyl)-(1 → 4)-β*-D-*glucopyranoside* (**28**).—*O*-Deacetylation of **21** (2.61 g, 1.54 mmol) with $K_2CO_3$, as described for **10**, gave pale-yellow, amorphous **28** (996 mg, 0.902 mmol, yield 58.6%); $[\alpha]_D$ +68.2° (*c* 0.50, MeOH); $\nu_{max}$ 3410 (OH), 2930 (CH, aliph.), 1716 (C=O), 1600, 1486 (arom.), 1522, 1350 ($NO_2$) cm$^{-1}$; $^1$H NMR: δ 5.06 (br s, 3 H, H-1), 5.11 (d, 1 H, *J* 3.9 Hz, H-1), 5.26 (d, 1 H, *J* 7.8 Hz, H-1a), 6.98 (t, 1 H, *J* 7.3 Hz, COPh), 7.26 and 7.45 (2 d, each 2 H, *J* 7.3 Hz, COPh), 7.47 (d, 1 H, *J* 9.0 Hz, H-6 of CNP), 8.18 (dd, 1 H, *J* 9.0 Hz and 2.7 Hz, H-5 of CNP), and 8.31 (d, 1 H, *J* 2.7 Hz, H-3 of CNP); $^{13}$C NMR: δ

64.6 ($CH_2OCONH$–), 119.3, 129.5, and 139.8 (CONH*Ph*), and 154.4 (*C*ONHPh); $t_R$ (column: (*B*), eluent: 3:1 (v/v) $CH_3CN$–$H_2O$): 4.8 min. Anal. Calcd for $C_{43}H_{62}ClN_2O_{29} \cdot 1.5H_2O$: C, 45.69; H, 5.53; N, 2.48. Found: C, 45.67; H, 5.39; N, 2.54.

*2-Chloro-4-nitrophenyl O-[6-O-(*N*-tert-butyl)carbamoyl-α-*D*-glucopyranosyl]-(1 → 4)-tris[O-(α-*D*-glucopyranosyl)-(1 → 4)]-β-*D*-glucopyranoside* (**29**).—*O*-Deacetylation of **22** (2.22 g, 1.32 mmol) with $K_2CO_3$, as described for **10**, gave pale-yellow, amorphous **29** (889 mg, 0.821 mmol, yield 62.2%); $[\alpha]_D$ +79.3° (*c* 0.50, MeOH); $\nu_{max}$ 3430 (OH), 2940 (CH, aliph.), 1704 (C=O), 1586, 1486 (arom.), 1522, 1350 ($NO_2$) $cm^{-1}$; $^1H$ NMR: δ 1.23 (s, 9 H, $^t$Bu), 3.95 (dd, 1 H, *J* 10.5 Hz and 6.1 Hz, H-6e$_a$), 4.18 (br d, 1 H, *J* 10.5 Hz, H-6e$_b$), 4.99 (d, 1 H, *J* 3.7 Hz, H-1), 5.05 (d, 2 H, *J* 3.7 Hz, H-1), 5.11 (d, 1 H, *J* 3.7 Hz, H-1), 5.25 (d, 1 H, *J* 7.3 Hz, H-1a), 7.47 (d, 1 H, *J* 9.2 Hz, H-6 of CNP), 8.17 (dd, 1 H, *J* 9.2 and 2.7 Hz, H-5 of CNP), and 8.29 (d, 1 H, *J* 2.7 Hz, H-3 of CNP); $^{13}C$ NMR: δ 29.6 (C*Me*$_3$), 50.3 (*C*Me$_3$), 63.9 ($CH_2OCONH$–), and 155.6 (*C*ONH$^t$Bu); $t_R$ (column: (*B*), eluent: 3:1 (v/v) $CH_3CN$–$H_2O$): 6.1 min. Anal. Calcd for $C_{41}H_{63}ClN_2O_{29}$: C, 45.45; H, 5.86; N, 2.59. Found: C, 45.69; H, 5.77; N, 2.56.

*2-Chloro-4-nitrophenyl O-[6-O-(*N*-isopropyl)carbamoyl-α-*D*-glucopyranosyl]-(1 → 4)-tris[O-(α-*D*-glucopyranosyl)-(1 → 4)-β-*D*-glucopyranoside* (**30**).—*O*-Deacetylation of **23** (2.62 g, 1.58 mmol) with $K_2CO_3$, as described for **10**, gave pale-yellow, amorphous **30** (1.08 g, 1.04 mmol, yield 65.7%); $[\alpha]_D$ +81.2° (*c* 0.50, MeOH); $\nu_{max}$ 3400 (OH), 2930 (CH, aliph.), 1696 (C=O), 1584, 1486 (arom.), 1522, 1350 ($NO_2$), 1274, 1150, 1078, and 1036 (C–O) $cm^{-1}$; $^1H$ NMR: δ 1.06 (d, 6 H, *J* 6.6 Hz, 2 Me of $^i$Pr), 4.00 (dd, 1 H, *J* 11.5 and 5.9 Hz, H-6e$_a$), 4.19 (d, 1 H, *J* 11.5 Hz, H-6e$_b$), 5.01 (d, 1 H, *J* 5.4 Hz, H-1), 5.04 (d, 2 H, *J* 3.7 Hz, H-1), 5.10 (d, 1 H, *J* 3.7 Hz, H-1), 5.26 (d, 1 H, *J* 7.6 Hz, H-1a), 7.47 (d, 1 H, *J* 9.2 Hz, H-6 of CNP), 8.18 (dd, 1 H, *J* 9.2 and 2.7 Hz, H-5 of CNP), and 8.29 (d, 1 H, *J* 2.7 Hz, H-3 of CNP); $^{13}C$ NMR: δ 23.4 (CH*Me*$_2$), 43.3 (*C*HMe$_2$), 64.3 ($CH_2OCONH$–), and 156.3 (*C*ONH$^i$Pr); $t_R$ (column: (*B*), eluent: 3:1 (v/v) $CH_3CN$–$H_2O$): 7.3 min. Anal. Calcd for $C_{40}H_{61}ClN_2O_{29} \cdot 1.5H_2O$: C, 43.82; H, 5.88; N, 2.56. Found: C, 43.68; H, 5.72; N, 2.56.

*2-Chloro-4-nitrophenyl O-[4,6-di-O-(*N*-isopropyl)carbamoyl-α-*D*-glucopyranosyl]-(1 → 4)-tris[O-(α-*D*-glucopyranosyl)-(1 → 4)]-β-*D*-glucopyranoside* (**31**).—*O*-Deacetylation of **24** (1.63 g, 0.937 mmol) with $K_2CO_3$, as described for **10**, gave pale-yellow, amorphous **31** (897 mg, 0.776 mmol, yield 82.8%); $[\alpha]_D$ +87.4° (*c* 0.50, $H_2O$); $\nu_{max}$ 3400 (OH), 2940 (CH, aliph.), 1702 (C=O), 1584, 1486 (arom.), 1522, 1348 ($NO_2$) $cm^{-1}$; $^1H$ NMR: δ 1.05 (d, 12 H, *J* 6.4 Hz, 4 Me of $^i$Pr), 5.02 (d, 2 H, *J* 3.2 Hz, H-1), 5.10 (d, 2 H, *J* 3.4 Hz, H-1), 5.27 (d, 1 H, *J* 7.3 Hz, H-1a), 7.46 (d, 1 H, *J* 9.0 Hz, H-6 of CNP), 8.18 (dd, 1 H, *J* 9.0 and 2.7 Hz, H-5 of CNP), and 8.29 (d, 1 H, *J* 2.7 Hz, H-3 of CNP); $^{13}C$ NMR: δ 23.0 (CH*Me*$_2$), 42.9 (*C*HMe$_2$), 63.5 ($CH_2OCONH$–), 72.9 (*C*HOCONH–), and 155.5 and 155.7 (*C*ONH$^i$Pr); $t_R$ (column: (*B*), eluent: 3:1 (v/v) $CH_3CN$–$H_2O$): 4.2 min. Anal. Calcd for $C_{44}H_{68}ClN_3O_{30}$: C, 45.77; H, 5.94; N, 3.64. Found: C, 45.81; H, 5.98; N, 3.49.

*2-Chloro-4-nitrophenyl O-[4-O-(N-isopropyl)carbamoyl-α-D-glucopyranosyl]-(1 → 4)-tris[O-(α-D-glucopyranosyl)-(1 → 4)-β-D-glucopyranoside* (**32**).—*O*-Deacetylation of **26** (970 mg, 0.585 mmol) with $K_2CO_3$, as described for **10**, gave pale-yellow, amorphous **32** (554, mg, 0.518 mmol, yield 88.6%); $[\alpha]_D$ +77.7° (*c* 0.50, MeOH); $\nu_{max}$ 3420 (OH), 2950 (CH, aliph.), 1700 (C=O), 1588, 1490 (arom.), 1524, 1352 ($NO_2$) $cm^{-1}$; $^1H$ NMR: δ 1.05 (d, 6 H, *J* 6.6 Hz, 2 Me of $^i$Pr), 4.35 (t, 1 H, *J* 9.3 Hz, H-4e), 5.06 (d, 2 H, *J* 3.7 Hz, H-1), 5.13 (d, 2 H, *J* 3.7 Hz, H-1), 5.26 (d, 1 H, *J* 7.6 Hz, H-1a), 7.46 (d, 1 H, *J* 9.3 Hz, H-6 of CNP), 8.19 (dd, 1 H, *J* 9.3 and 2.7 Hz, H-5 of CNP), and 8.31 (d, 1 H, *J* 2.7 Hz, H-3 of CNP); $^{13}C$ NMR: δ 22.9 ($CHMe_2$), 42.9 ($CHMe_2$), 78.5 ($CH_2OCONH$–), 155.8 ($CONH^iPr$); $t_R$ (column: (*B*), eluent: 3:1 (v/v) $CH_3CN$–$H_2O$): 7.3 min. Anal. Calcd for $C_{40}H_{61}ClN_2O_{29}$: C, 44.93; H, 5.75; N, 2.62. Found: C, 44.78; H, 5.84; N, 2.58.

*2-Chloro-4-nitrophenyl O-[4,6-di-O-(N-ethyl)carbamoyl-α-D-glucopyranosyl]-(1 → 4)-tris-[O-(α-D-glucopyranosyl)-(1 → 4)]-β-D-glucopyranoside* (**33**).—*O*-Deacetylation of **27** (2.28 g, 1.33 mmol) with $K_2CO_3$, as described for **10**, gave pale-yellow, amorphous **33** (803 mg, 0.713 mmol, yield 53.6%); $[\alpha]_D$ +80.0° (*c* 0.54, $H_2O$); $\nu_{max}$ 3390 (OH), 2950 (CH, aliph.), 1710 (C=O), 1586, 1486 (arom.), 1514, 1350 ($NO_2$) $cm^{-1}$; $^1H$ NMR: δ 1.01 (d, 6 H, *J* 7.2 Hz, 2 $NCH_2CH_3$), 3.02 (q, 4 H, *J* 7.2 Hz, 2 $NCH_2CH_3$), 5.06 (d, 2 H, *J* 3.7 Hz, H-1), 5.12 (d, 2 H, *J* 3.4 Hz, H-1), 5.26 (d, 1 H, *J* 7.8 Hz, H-1a), 7.46 (d, 1 H, *J* 9.3 Hz, H-6 of CNP), 8.19 (dd, 1 H, *J* 9.3 and 2.7 Hz, H-5 of CNP), and 8.31 (d, 1 H, *J* 2.7 Hz, H-3 of CNP): $^{13}C$ NMR: δ 15.4 ($NCH_2CH_3$), 35.6 ($NCH_2CH_3$), 63.5 ($CH_2OCONH$–), 79.8 ($CHOCONH$–); and 156.1 and 156.3 ($CONHEt$); $t_R$ (column: (*B*), eluent: 3:1 (v/v) $CH_3CN$–$H_2O$): 6.1 min. Anal. Calcd for $C_{42}H_{55}ClN_3O_{30}$: C, 44.74; H, 5.81; N, 3.73. Found: C, 44.66; H, 5.98; N, 3.89.

*2-Chloro-4-nitrophenyl O-[2,3-di-O-acetyl-6-O-(methoxy)methyl-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* (**34**).—Chlormethyl methyl ether (MOM-Cl, 402 mg, 5.00 mmol) and *N*-ethyldiisopropylamine (0.869 mL, 5.00 mmol) were added to a solution of **12** (1.57 g, 1.00 mmol) in $CH_2Cl_2$ (30 mL), and the mixture was refluxed for 2 h with stirring. Then the mixture was evaporated in vacuo to leave a syrupy residue, which was chromatographed on $SiO_2$ gel with 1:100 (v/v) MeOH–$CH_2Cl_2$ to give **34** (1.46 g, 0.902 mmol, yield 90.2%); mp 113–115°C (from $Et_2O$); $[\alpha]_D$ +91.7° (*c* 0.54, 1,4-dioxane); $\nu_{max}$ 3490 (OH) $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 2.99 (br s, 1 H, H-4e) and 3.38 (s, 3 H, OMe); $t_R$ (column: (*A*), eluent: 3:1 (v/v) $CH_3CN$–$H_2O$): 9.4 min. Anal. Calcd for $C_{66}H_{86}ClNO_{43} \cdot 2H_2O$: C, 47.96; H, 5.49; N, 0.85. Found: C, 47.68; H, 5.20; N, 0.81.

*2-Chloro-4-nitrophenyl O-[2,3-di-O-acetyl-6-O-(2-methoxyethoxy)methyl-α-D-glucopyranosyl]-(1 → 4)-tris[O-(2,3,6-tri-O-acetyl-α-D-glucopryanosyl)-(1 → 4)]-2,3,6-tri-O-acetyl-β-D-glucopyranoside* (**35**).—Reaction of **12** (942 mg, 0.600 mmol) with MEM-Cl (375 mg, 3.0 mmol), as described for **34**, gave **35** (880 mg, 0.530 mmol, yield 88.5%); mp 110–112°C (from $Et_2O$); $[\alpha]_D$ +91.6° (*c* 0.49, 1,4-dioxane); $\nu_{max}$ 3470 (OH) $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 3.36–3.45 (m, 1 H, H-4e) and 3.38 (s, 3 H,

OMe); $t_R$ (column: (*A*), eluent: 3:1 (v/v) $CH_3CN$–$H_2O$): 9.3 min. Anal. Calcd for $C_{68}H_{90}ClNO_{44} \cdot H_2O$: C, 48.65; H, 5.52; N, 0.83. Found: C, 48.51; H, 5.41; N, 0.78.

*2-Chloro-4-nitrophenyl O-[2,3-di-O-acetyl-6-O-(benzyloxy)methyl-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* (**36**).—Reaction of **12** (1.54 g, 1.00 mmol) with BOM-Cl (783 mg, 5.0 mmol), as described for **34**, gave **36** (1.28 g, 0.758 mmol, yield 75.9%); mp 103–105°C (from $Et_2O$); $[\alpha]_D$ +90.8° (*c* 0.52, 1,4-dioxane); $\nu_{max}$ 3470 (OH) $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 2.90 (br s, 1 H, H-4e) and 7.26–7.35 (m, 6 H, $OCH_2Ph$ and H-6 or CNP); $t_R$ (column: (*A*), eluent: 3:1 (v/v) $CH_3CN$–$H_2O$): Anal. Calcd for $C_{72}H_{90}ClNO_{43} \cdot 2.3H_2O$: C, 49.84; H, 5.50; N, 0.81. Found: C, 49.69; H, 5.24; N, 0.82.

*2-Chloro-4-nitrophenyl O-[2,3-di-O-acetyl-4,6-di-O-acetyl-4,6-di-O-(methoxy)methyl-α-D-glucopyranosyl]-(1 → 4)-tris[O-(2,3,6-tri-O-acetyl-α-D-gluco-pyranosyl)-(1 → 4)]-2,3,6-tri-O-acetyl-β-D-glucopyranoside* (**37**).—MOM-Cl (966 mg, 12.0 mmol) was added to a solution of **12** (1.88 g, 1.20 mmol) in $CH_3CN$ (30 mL), and the mixture was refluxed for 3 h with stirring. Then the mixture was evaporated in vacuo to leave a syrupy residue, which was chromatographed on $SiO_2$ gel with 1:200 (v/v) MeOH–$CH_2Cl_2$ to give **37** (1.72 g, 1.04 mmol, yield 86.6%); mp 110–113°C (from $Et_2O$); $[\alpha]_D$ +87.4° (*c* 0.47, 1,4-dioxane); $^1H$ NMR ($CDCl_3$): δ 3.33 and 3.38 (2 s, each 3 H, 2 OMe); $t_R$ (column: (*A*), eluent: 3:1 (v/v) $CH_3CN$–$H_2O$): 12.4 min. Anal. Calcd for $C_{68}H_{90}ClNO_{44} \cdot 2H_2O$: C, 48.13; H, 5.58; N, 0.83. Found: C, 47.89; H, 5.23; N, 0.75.

*2-Chloro-4-nitrophenyl O-[2,3-di-O-acetyl-4,6-di-O-(2-methoxyethoxy)methyl-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* (**38**).—Reaction of **12** (942 mg, 0.600 mmol) with MEM-Cl (750 mg, 6.0 mmol), as described for **37**, gave **38** (780 mg, 0.446 mmol, yield 74.4%); mp 91–93°C (from $Et_2O$); $[\alpha]_D$ +89.6° (*c* 0.56, 1,4-dioxane); $^1H$ NMR ($CDCl_3$): δ 3.36 and 3.39 (2 s, each 3 H, 2 OMe); $t_R$ (column: (*A*), eluent: 3:1 (v/v) $CH_3CN$–$H_2O$): 11.5 min. Anal. Calcd for $C_{72}H_{98}ClNO_{46}$: C, 49.45; H, 5.65; N, 0.80. Found: C, 49.25; H, 5.68; N, 0.78.

*2-Chloro-4-nitrophenyl O-[2,3-di-O-acetyl-4,6-di-O-(benzyloxy)methyl-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* (**39**).—Reaction of **12** (1.88 g, 1.20 mmol) with BOM-Cl (1.88 g, 12.0 mmol), as described for **37**, gave **39** (1.33 g, 0.734 mmol, yield 61.2%); mp 92–95°C (from $Et_2O$); $[\alpha]_D$ +86.7° (*c* 0.47, 1,4-dioxane); $^1H$ NMR ($CDCl_3$): δ 7.27–7.32 (m, 11 H, 2 $OCH_2Ph$ and H-6 of CNP); $t_R$ (column: (*A*), eluent: 3:1 (v/v); $CH_3CN$–$H_2O$): 36.7 min. Anal. Calcd for $C_{80}H_{98}ClNO_{44} \cdot H_2O$: C, 52.48; H, 5.50; N, 0.76. Found: C, 52.54; H, 5.37; N, 0.77.

*2-Chloro-4-nitrophenyl O-[2,3-di-O-acetyl-6-O-(*tert*-butyldimethyl)silyl-4-O-(methoxy)methyl-α-D-glucopyranosyl]-(1 → 4)-tris[O-(2,3,6-tri-O-acetyl-α-D-glucopy-ranosyl)-(1 → 4)]-2,3,6-tri-O-acetyl-β-D-glucopyranoside* (**40**).—Reaction **12** (3.16 g, 2.01 mmol) with TBDMS-Cl as described for **25**, and following reaction with methoxymethyl chloride as described for **37** gave **40** (1.72 g, 0.994 mmol, yield

49.7% from **12**); mp 113–115°C (from $Et_2O$); $[\alpha]_D$ +88.7° (*c* 0.50, 1,4-dioxane); $^1H$ NMR ($CDCl_3$): δ 0.07 (s, 6 H, 2 Si-Me), 0.90 (s, 9 H, 3 Me of $^t$Bu), and 3.31 (s, 3 H, OMe); $t_R$ (column: (*A*), eluent: 3:1 (v/v) $CH_3CN$–$H_2O$): 20.2 min. Anal. Calcd for $C_{72}H_{100}ClNO_{43}Si$: C, 49.96; H, 5.82; N, 0.81. Found: C, 49.67; H, 5.90; N, 0.87.

*2-Chloro-4-nitrophenyl* O-*[2,3-di-*O*-acetyl-4-*O*-(methoxy)methyl-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* (**41**).—*O*-Desilylation of **40** (1.61 g, 0.931 mmol) with aq AcOH as described for **26** gave **41** (1.29 g, 0.798 mmol, yield 85.7%); mp 118–120°C (from $Et_2O$); $[\alpha]_D$ +89.7° (*c* 0.51, 1,4-dioxane); $\nu_{max}$ 3500 (OH) $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 3.37 (s, 3 H, OMe); $t_R$ (column: (*A*), eluent: 3:1 (v/v) $CH_3CN$–$H_2O$): 9.4 min. Anal. Calcd for $C_{66}H_{86}ClNO_{43}$: C, 49.03; H, 5.36; N, 0.87. Found: C, 48.89; H, 5.42; N, 0.86.

*2-Chloro-4-nitrophenyl* O-*[6-*O*-(methoxy)methyl-α-*D*-glucopyranosyl)-(1 → 4)-tris[*O*-(α-*D*-glucopyranosyl)-(1 → 4)]-β-*D*-glucopyranoside* (**42**).—*O*-Deacetylation of **34** (880 mg, 0.544 mmol) with $K_2CO_3$, as described for **10**, gave pale-yellow, amorphous **42** (316 mg, 0.307 mmol, yield 56.4%); $[\alpha]_D$ +89.0° (*c* 0.51, MeOH); $\nu_{max}$ 3420 (OH), 2930 (CH, aliph.), 1588, 1490 (arom.), 1524, 1350 ($NO_2$) $cm^{-1}$; $^1H$ NMR: δ 3.26 (s, 3 H, OMe), 4.56 (s, 2 H, $OCH_2O$), 5.02 (d, 1 H, *J* 3.9 Hz, H-1), 5.04 (d, 2 H, *J* 3.7 Hz, H-1), 5.11 (d, 1 H, *J* 3.7 Hz, H-1), 5.26 (d, 1 H, *J* 7.6 Hz, H-1a), 7.47 (d, 1 H, *J* 9.3 Hz, H-6 of CNP), 8.19 (dd, 1 H, *J* 9.3 Hz and 2.8 Hz, H-5 of CNP), and 8.31 (d, 1 H, *J* 2.8 Hz, H-3 of CNP); $^{13}C$ NMR: δ 55.8 (OMe) and 97.2 ($OCH_2O$); $t_R$ (column: (*B*), eluent: 3:1 (v/v) $CH_3CN$–$H_2O$): 8.8 min. Anal. Calcd for $C_{38}H_{58}ClNO_{29} \cdot 2H_2O$: C, 42.88; H, 5.87; N, 1.32. Found: C, 42.80; H, 5.66; N, 1.24.

*2-Chloro-4-nitrophenyl* O-*[6-*O*-(2-methoxyethoxy)methyl-α-*D*-glucopyranosyl]-(1 → 4)-tris[*O*-(α-*D*-glucopyranosyl)-(1 → 4)]-β-*D*-glucopyranoside* (**43**).—*O*-Deacetylation of **35** (880 mg, 0.530 mmol) with $K_2CO_3$, as described for **10**, gave pale-yellow, amorphous **43** (316 mg, 0.295 mmol, yield 55.6%); $[\alpha]_D$ +84.6° (*c* 0.51, MeOH); $\nu_{max}$ 3430 (OH), 2930 (CH, aliph.), 1584, 1486 (arom.), 1522, 1350 ($NO_2$) $cm^{-1}$; $^1H$ NMR: δ 3.26 (s, 3 H, OMe), 4.63 (s, 2 H, $OCH_2O$), 5.01 (d, 1 H, *J* 3.9 Hz, H-1), 5.03 (d, 2 H, *J* 4.4 Hz, H-1), 5.10 (d, 1 H, *J* 3.9 Hz, H-1), 5.27 (d, 1 H, *J* 7.3 Hz, H-1a), 7.47 (d, 1 H, *J* 9.2 Hz, H-6 of CNP), 8.19 (dd, 1 H, *J* 9.2 and 2.7 Hz, H-5 of CNP) and 8.30 (d, 1 H, *J* 2.7 Hz, H-3 of CNP); $^{13}C$ NMR: δ 59.0 (OMe), 67.2 and 67.8 ($OCH_2CH_2O$), and 96.0 ($OCH_2O$); $t_R$ (column: (*B*), eluent: 3:1 (v/v) $CH_3CN$–$H_2O$): 4.8 min. Anal. Calcd for $C_{40}H_{62}ClNO_{30} \cdot 2H_2O$: C, 43.35; H, 6.00; N, 1.26. Found: C, 43.00 H, 5.75; N, 1.28.

*2-Chloro-4-nitrophenyl* O-*[6-*O*-(benzyloxy)methyl-α-*D*-glucopyranosyl]-(1 → 4)-tris[*O*-(α-*D*-glucopyranosyl)-(1 → 4)]-β-*D*-glucopyranoside* (**44**).—*O*-Deacetylation of **36** (1.28 g, 0.756 mmol) with $K_2CO_3$, as described for **10**, gave pale-yellow, amorphous **44** (675 mg, 0.611 mmol, yield 80.8%); $[\alpha]_D$ +80.7° (*c* 0.50, MeOH); $\nu_{max}$ 3400 (OH), 2930 (CH, aliph.), 1584, 1488 (arom.), 1522, 1350 ($NO_2$) $cm^{-1}$; $^1H$ NMR: δ 4.55 (s, 2 H, $OCH_2O$), 4.72 (s, 2 H, $OCH_2Ph$), 5.04 (d, 3 H, *J* 3.7 Hz,

H-1), 5.11 (d, 1 H, *J* 3.4 Hz, H-1), 5.26 (d, 1 H, *J* 7.6 Hz, H-1a), 7.20–7.40 (m, 5 H, $CH_2Ph$), 7.47 (d, 1 H, *J* 9.3 Hz, H-6 of CNP), 8.19 (dd, 1 H, *J* 9.3 and 2.9 Hz, H-5 of CNP), and 8.30 (d, 1 H, *J* 2.9 Hz, H-3 of CNP); $^{13}C$ NMR: δ 69.6 ($CH_2Ph$), 95.4 ($OCH_2O$), 128.6, 128.9, 129.4, and 139.1 ($CH_2Ph$); $t_R$ (column: (*B*), eluent: 3:1 (v/v) $CH_3CN$–$H_2O$): 5.2 min. Anal. Calcd for $C_{44}H_{62}ClNO_{29} \cdot 2H_2O$: C, 46.34; H, 5.83; N, 1.23. Found: C, 45.86; H, 5.54; N, 1.24.

*2-Chloro-4-nitrophenyl O-[4,6-di-O-(methoxy)methyl-α-D-glucopyranosyl]-(1 → 4)-tris[O-(α-D-glucopryanosyl)-(1 → 4)]-β-D-glucopyranoside* (**45**).—*O*-Deacetylation of **37** (1.52 g, 0.915 mmol) with $K_2CO_3$, as described for **10**, gave pale-yellow, amorphous **45** (773 mg, 0.721 mmol, yield 78.8%); $[\alpha]_D$ +92.8° (*c* 0.50, MeOH); $\nu_{max}$ 3420 (OH), 2930 (CH, aliph.), 1586, 1488 (arom.), 1524, 1350 ($NO_2$) $cm^{-1}$; $^1H$ NMR: δ 3.27 and 3.31 (2 s, each 3 H, 2 OMe), 4.58 (s, 2 H, 6-$OCH_2O$), 4.67 and 4.82 (2 d, each 1 H, *J* 6.4 Hz, 4-$OCH_2O$), 5.05 (d, 2 H, *J* 3.7 Hz, H-1), 5.07 (d, 1 H, *J* 3.4 Hz, H-1), 5.12 (d, 1 H, *J* 3.7 Hz, H-1), 5.25 (d, 1 H, *J* 7.6 Hz, H-1a), 7.47 (d, 1 H, *J* 9.2 Hz, H-6 of CNP), 8.18 (dd, 1 H, *J* 9.2 and 2.7 Hz, H-5 of CNP), and 8.31 (d, 1 H, *J* 2.7 Hz, H-3 of CNP); $^{13}C$ NMR: δ 55.9 and 56.2 (2 OMe), 97.3 and 98.3 (2 $OCH_2O$); $t_R$ (column: (*B*), eluent: 3:1 (v/v) $CH_3CN$–$H_2O$): 5.9 min. Anal. Calcd for $C_{40}H_{62}ClNO_{30} \cdot 1.6H_2O$: C, 43.58; H, 5.97; N, 1.27. Found: C, 43.31; H, 5.71; N, 1.24.

*2-Chloro-4-nitrophenyl O-[4,6-di-O-(2-methoxyethoxy)methyl-α-D-glucopyranosyl]-(1 → 4)-tris[O-(α-D-glucopyranosyl)-(1 → 4)]-β-D-glucopyranoside* (**46**).—*O*-Deacetylation of **38** (780 mg, 0.446 mmol) with $K_2CO_3$, as described for **10**, gave pale-yellow, amorphous **46** (359 mg, 0.309 mmol, yield 69.3%); $[\alpha]_D$ +81.1° (*c* 0.50, MeOH); $\nu_{max}$ 3430 (OH), 2930 (CH, aliph.), 1584, 1486 (arom.), 1522, 1348 ($NO_2$) (C–O) $cm^{-1}$; $^1H$ NMR: δ 3.25 and 3.26 (2 s, each 3 H, 2 OMe), 4.64 (s, 2 H, 6-$OCH_2O$), 4.73 and 4.86 (2 d, each 1 H, *J* 6.4 Hz, 4-$OCH_2O$), 5.03 (d, 2 H, *J* 3.2 Hz, H-1), 5.05 (d, 1 H, *J* 2.9 Hz, H-1), 5.10 (d, 1 H, *J* 3.7 Hz, H-1), 5.27 (d, 1 H, *J* 7.6 Hz, H-1a), 7.47 (d, 1 H, *J* 9.2 Hz, H-6 of CNP), 8.19 (dd, 1 H, *J* 9.2 and 2.7 Hz, H-5 of CNP), and 8.31 (d, 1 H, *J* 2.7 Hz, H-3 of CNP); $^{13}C$ NMR: δ 58.2 and 58.3 (2 OMe), 66.6, 66.8, and 67.3 (2 $OCH_2CH_2O$), 95.3 and 96.2 (2 $OCH_2O$); $t_R$ (column: (*B*), eluent: 3:1 (v/v) $CH_3CN$–$H_2O$): 5.8 min. Anal. Calcd for $C_{44}H_{70}ClNO_{32} \cdot 2.5H_2O$: C, 43.84; H, 6.27; N, 1.16. Found: C, 43.66; H, 6.03; N, 1.14.

*2-Chloro-4-nitrophenyl O-[4,6-di-O-(benzyloxy)methyl-α-D-glucopyranosyl]-(1 → 4)-tris[O-(α-D-glucopyranosyl)-(1 → 4)]-β-D-glucopyranoside* (**47**).—*O*-Deacetylation of **39** (1.33 g, 0.734 mmol) with $K_2CO_3$, as described for **10**, gave pale-yellow, amorphous **47** (630 mg, 0.514 mmol, yield 70.0%); $[\alpha]_D$ +92.8° (*c* 0.51, 1:1, (v/v) 1,4-dioxane–$H_2O$); $\nu_{max}$ 3420 (OH), 2930 (CH, aliph.), 1584, 1490 (arom.), 1520, 1350 ($NO_2$) $cm^{-1}$; $^1H$ NMR: δ 4.53 and 4.60 (2 s, each 2 H, $OCH_2Ph$), 4.70 (s, 2 H, 6-$OCH_2O$), 4.84 and 4.96 (2 d, each 1 H, *J* 6.6 Hz, 4-$OCH_2O$), 5.05 (d, 2 H, *J* 3.7 Hz, H-1), 5.09 (d, 1 H, *J* 4.2 Hz, H-1), 5.13 (d, 1 H, *J* 3.7 Hz, H-1), 5.25 (d, 1 H, *J* 7.6 Hz, H-1a), 7.30 (br s, 10 H, 2 $CH_2Ph$), 7.46 (d, 1 H, *J* 9.3 Hz, H-6 of CNP), 8.16 (dd, 1 H, *J* 9.3 and 2.7 Hz, H-5 of CNP), and 8.30 (d, 1 H, *J* 2.7 Hz, H-3 of

CNP); $^{13}C$ NMR: $\delta$ 70.0 and 70.7 (2 $CH_2Ph$), 95.7 and 96.6 (2 $OCH_2O$), 128.7, 128.8, 128.9, 129.5, 139.1 and 139.2 ($CH_2Ph$); $t_R$ (column: (*B*), eluent: 3:1 (v/v) $CH_3CN$–$H_2O$): 4.0 min. Anal. Calcd for $C_{52}H_{70}ClNO_{30} \cdot 2.5H_2O$: C, 49.19; H, 5.95; N, 1.10. Found: C, 48.96; H, 5.76; N, 1.11.

*2-Chloro-4-nitrophenyl O-[4-O-(methoxy)methyl-α-D-glucopyranosyl]-(1 → 4)-tris[O-(α-D-glucopyranosyl)-(1 → 4)]-β-D-glucopyranoside* (**48**).—*O*-Deacetylation of **41** (1.22 g, 0.755 mmol) with $K_2CO_3$, as described for **10**, gave pale-yellow, amorphous **48** (719 mg, 0.699 mmol, yield 92.6%); $[\alpha]_D$ +93.8° (*c* 0.50, MeOH); $\nu_{max}$ 3410 (OH), 2940 (CH, aliph.), 1584, 1486 (arom.), 1518, 1350 ($NO_2$) $cm^{-1}$; $^1H$ NMR: $\delta$ 3.31 (s, 3 H, OMe), 4.67 and 4.81 (2 d, each 1 H, *J* 6.3 Hz, $OCH_2O$), 5.05 (d, 2 H, *J* 4.2 Hz, H-1), 5.07 (d, 1 H, *J* 4.2 Hz, H-1), 5.12 (d, 1 H, *J* 3.7 Hz, H-1), 5.26 (d, 1 H, *J* 7.3 Hz, H-1a), 7.47 (d, 1 H, *J* 9.3 Hz, H-6 of CNP), 8.19 (dd, 1 H, *J* 9.3 and 2.8 Hz, H-5 of CNP), and 8.31 (d, 1 H, *J* 2.8 Hz, H-3 of CNP); $^{13}C$ NMR: $\delta$ 56.0 (OMe) and 97.6 ($OCH_2O$); $t_R$ (column: (*B*), eluent: 3:1 (v/v) $CH_3CN$–$H_2O$): 8.8 min. Anal. Calcd for $C_{38}H_{58}ClNO_{29} \cdot 2H_2O$: C, 42.88; H, 5.87; N, 1.32. Found: C, 42.69; H, 5.77; N, 1.30.

*Michaelis constants* ($K_m$).—A solution of coupled enzymes (110 U/mL α-D-glucosidase and 13 U/mL β-D-glucosidase, 1.0 mL) in 50 mM phosphate buffer (pH 7.0, containing 40 mM NaCl and 2.0 mM $MgCl_2$) was added to a solution of HPA or HSA in water (150 U/mL, 0.25 mL), and the enzymic solution was incubated at 37°C for 1 min. Then a solution of twenty-two substrates (each 2.0 mL) in the same buffer was added to the solution of enzyme and the mixture was incubated. After 2 min, the reaction was monitored by the increase in absorbance at 400 nm for 2 min. For the blank, water was added instead of the substrate solution. The $K_m$ values of hydrolysis of the substrates were calculated by the method of least squares with the use of Lineweaver–Burk plots as shown in Table I.

*Action patterns.*—A solution of twenty-two substrates (each ~ 8-fold the concentration of the $K_m$ value, 2.0 mL) in the same buffer was added to a solution of to a HPA or HSA in $H_2O$ (150 U/mL, 0.25 mL), and the mixture was incubated for 15 min at 37°C. Then 0.1 mL of the mixture was added to 0.9 mL of $CH_3CN$ to stop the reaction. A sample (5 μL) was analyzed by HPLC. Patterns of action of the substrates are summarized in Table II.

*Relative rate of hydrolysis.*—A solution of coupled enzymes (1.0 mL) as just described was added to a solution of HPA or HSA in $H_2O$ (each 0.25 mL), and the enzyme solution was incubated for 1 min at 37°C. Then, using a solution of twenty-two substrates and **1** (each ~ 8-fold the concentration of the $K_m$ value, 2.0 mL), the increase in absorbance at 400 nm was determined by the foregoing method. From the amounts of *A* per min obtained the relative rates of hydrolysis shown in Table I were determined.

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