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Further Syntheses Employing Phosphorylase

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Abstract—Maltopentaose was immobilized on silica gel and used as a recyclable primer in the reaction of glycogen phosphorylase with D-glucal. An improved method to obtain 2-deoxy-α-D-arabino-hexopyranosyl phosphate (**4**) by using this catalyst as well as the specific synthesis of low molecular weight, water-soluble 2-deoxy-maltooligosaccharides (**12**, **13**, **14** and **15**) are described. Further investigations with modified phosphorylase substrates showed that mannosyl phosphate (**16**) can be slowly transferred to the primer maltotetraose. α-1,4-Mannosyl-maltotetraose (**17**) and its degradation product α-1,4-mannosyl-maltose (**18**) were identified. © 1997 Elsevier Science Ltd.

Introduction

Glycogen phosphorylase (EC 2.4.1.1.) is the well-known enzyme responsible for the formation or degradation of α(1,4)-glucans. Belonging to the non-Leloir transferases, phosphorylase requires an activated substrate such as the glucosyl phosphate ester (α-Glc1P). With this substrate a glucan chain, the so-called primer $(Glc)_n$, can be elongated with release of phosphate. The commercially available glucosyl phosphate is produced from starch in the reverse reaction:

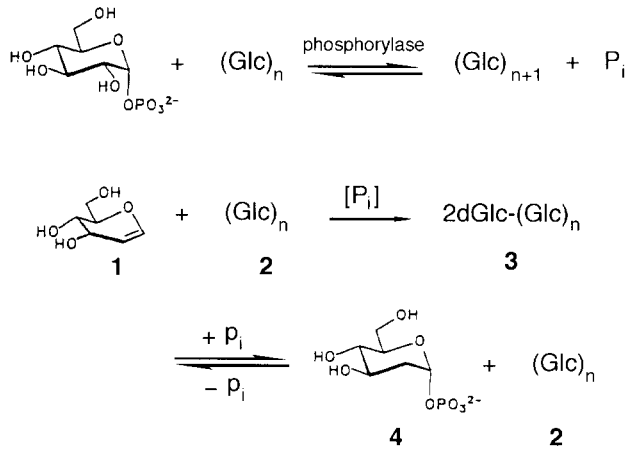
$$\alpha\text{-D-Glc-1-P} + (\alpha\text{-1,4-Glc})_n \rightleftharpoons (\alpha\text{-1,4-Glc})_{n+1} + P_i$$

Phosphorylases from various sources are regulated in different ways. Whereas the skeletal muscle enzyme can be interconverted from an inactive phosphorylase *b* to the active *a*-form by protein phosphorylation, the potato phosphorylase occurs only in one active form. Additionally, all phosphorylases underlie multiple allosteric regulations.[1]

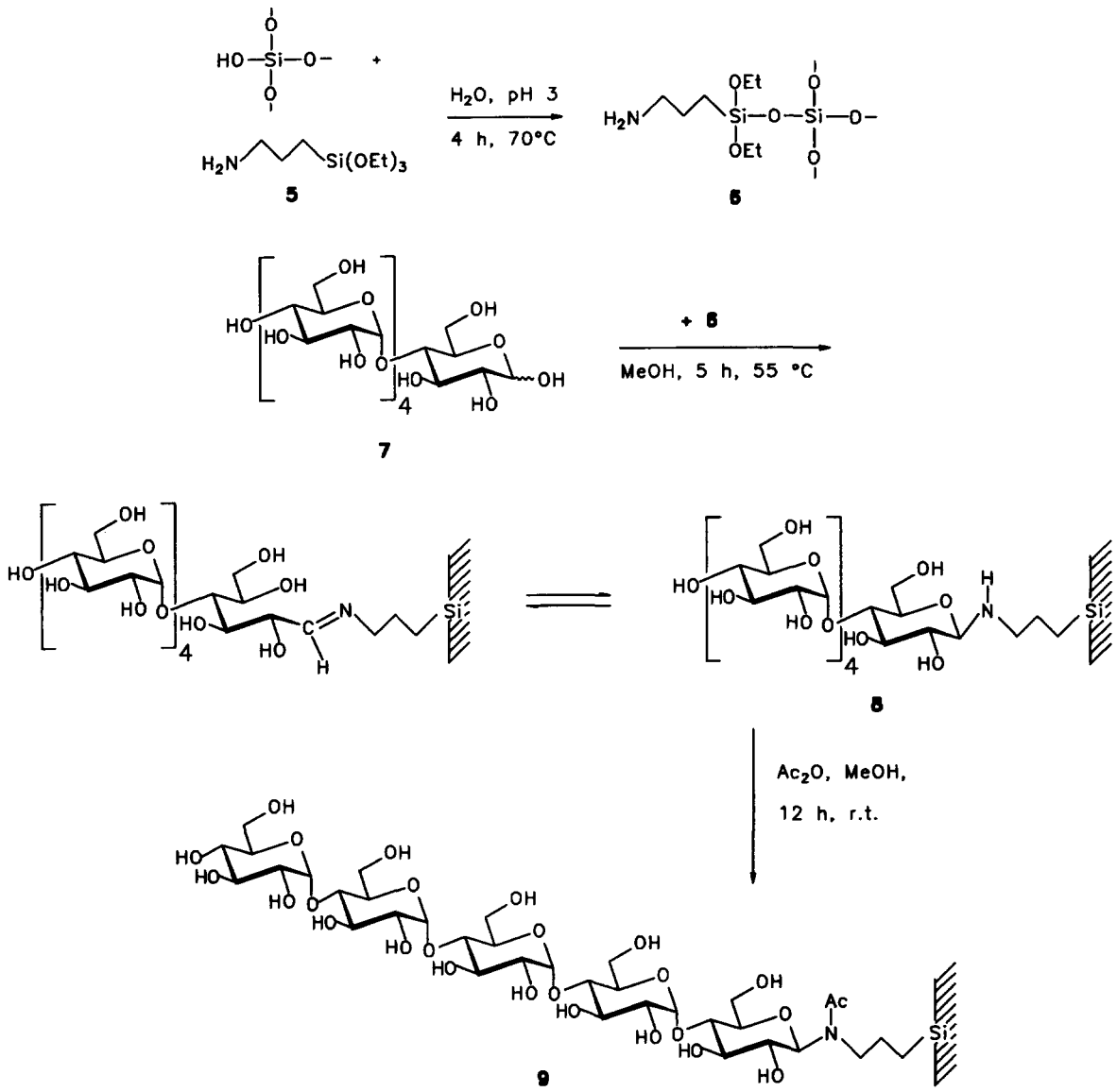
In vivo phosphorylase mainly plays a role in the degradation reaction, by which stored energy is mobilized as glucosyl phosphate. However, in vitro the enzyme can also be used for preparative syntheses under appropriate reaction conditions. Pfannemüller et al. described the preparation of star- and comb-shaped glucan polymers employing the enzyme from potatoes,[2] and enzymatic grafting of amylose on polysiloxanes was recently reported.[3] The substrate for glycogen phosphorylase could be obtained in a one-pot reaction by cleavage of sucrose with sucrose phosphorylase.[4]

Instead of glucosyl phosphate the unsaturated D-glucal (**1**) can act as a substrate for phosphorylase in the presence of inorganic phosphate.[5] Thus, unnatural maltooligosaccharides containing 2-deoxyglucose with an average dp (degree of polymerization) of 20 were synthesized on a preparative scale.[6] However, these compounds are only soluble in DMSO or in alkali. For an application in subsequent enzyme reactions water-soluble analogues would be desirable. One aim of this work was the synthesis of soluble oligomers with a higher deoxyglucose content that could be submitted to degradation by amylolytic enzymes. In the phosphorolytic direction 2-deoxy-α-D-arabino-hexopyranosyl phosphate (**4**) was produced from glucal by the same reaction.[6] Glycosyl phosphates are important intermediates en route to nucleotide sugars increasingly used in enzymatic glycosylations. The synthesis of specifically deoxygenated oligosaccharides represents a common approach to study carbohydrate–protein interactions.

Withers et al. synthesized 2-deoxyglucosyl phosphate (**4**) enzymetically in a two-step process.[7] With phosphorylase, compound **4** is obtained in a one-pot reaction, although the formation starting from **1** takes place in two subsequent steps. First, a 2-deoxyglucosyl unit is transferred to the glucan primer that is catalysed by inorganic phosphate. In the second step, 2-deoxy-



Scheme 1.



Scheme 2.

glucose is released by phosphorolysis to yield **4** and in the overall reaction the primer remains unchanged. A direct formation of the glycosyl phosphate can be excluded, since no reaction is observed in the absence of a primer.

Generally, maltotetraose is applied as a primer since this proved to be the minimum chain length for the phosphorylase primer in the synthetic direction. Further degradation of this substrate occurs only at a very low rate and thus the undesirable formation of glucosyl phosphate is prevented.[8] However, since pure maltotetraose is rather expensive (Boehringer: 220 DM/g) it was of interest to recycle this substrate efficiently.

Immobilized carbohydrates are often used in affinity chromatography for the purification of proteins, whereas enzymes bound to different matrices can serve as catalysts in batchwise or continuous production. In this special case the carbohydrate primer can be considered as the catalyst. An immobilized maltooligosaccharide is easily recovered from the incubation mixture and can be employed again in new reaction cycles. Additionally, the reaction of phosphorylase with other unnatural substrates was studied and α-D-mannosyl phosphate turned out to be transferred, though very slowly, to maltotetraose.

Results

Preparation of 2-deoxyglucosyl phosphate with an immobilized primer

The binding of enzymes can be accomplished on inorganic support materials such as porous glasses or silica gel. After coupling of an aminopropyl spacer to the gel the protein is immobilized via amide linkages.[9]

Such an inorganic matrix is available at a low price in comparison with other commercial polymers. This method was combined with the procedure of Blomberg et al.[10] who described the binding of reducing oligosaccharides by a glycosylamide linkage to a polymer carrying amino groups.

Silica gel was substituted with aminopropyl groups by aqueous silanization with γ-aminopropyltriethoxysilane (**5**) and subsequent binding of a maltooligosaccharide leads to the labile glycosyl amine **8**, which was stabilized as the glycosyl amide **9** by treatment with acetic anhydride. Although this resulted in a slightly finer powdered gel material, its function as a carrier was not impaired.

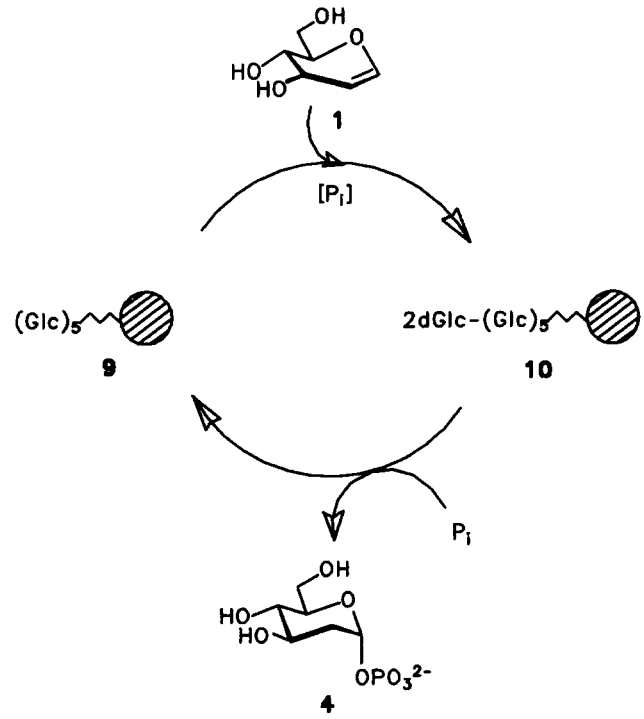
Maltopentaose (**7**) was used instead of the tetraose in order to compensate the blocking of the reducing end by the glycosidic linkage. The pentasaccharide was also used by Niemann et al. when the primer was modified to a *p*-nitrophenyl glycoside.[11] The degree of substitution on the gel was not determined directly, but with regard to the amount of re-isolated maltopentaose it can be assumed that 93% of the material was bound to the silica gel, that is 49 μmol/g gel.

On incubation of phosphorylase with glucal, inorganic phosphate and the immobilized maltopentaose, 2-deoxyglucosyl phosphate was formed rapidly. The equilibrium of the reaction can be shifted to the product side with high concentrations of phosphate, but when a fivefold excess was applied the purification of the product turned out to be rather laborious. Therefore not more than 1.5 equiv of phosphate referred to glucal were employed in the reactions.

The reaction time has to be adjusted carefully in a way that high yields of 2-deoxyglucosyl phosphate are obtained but the formation of 2-deoxy-maltooligomers is prevented. Since the product is also a substrate for the reverse reaction, the elongation of the primer will prevail at a certain concentration. At a degree of polymerization higher than about 12 the oligosaccharides become insoluble in water. This would render the immobilized primer useless for further reaction cycles. For that reason not too long reaction times and a dilute incubation mixture are favourable.

After the reaction the gel was removed by filtration, and could be used repeatedly for new incubations. The filtrate was freed from enzyme by ultrafiltration and worked up by ion exchange and size exclusion chromatography.

Thus with 70 U phosphorylase and 2 mmol glucal a yield of 52% of compound **4** was obtained. This is lower than in experiments with free maltotetraose in which up to 65% were isolated after 3–4 days,[6] but with the immobilized primer the incubation time is reduced to two days and the relatively high costs for maltotetraose can be saved.



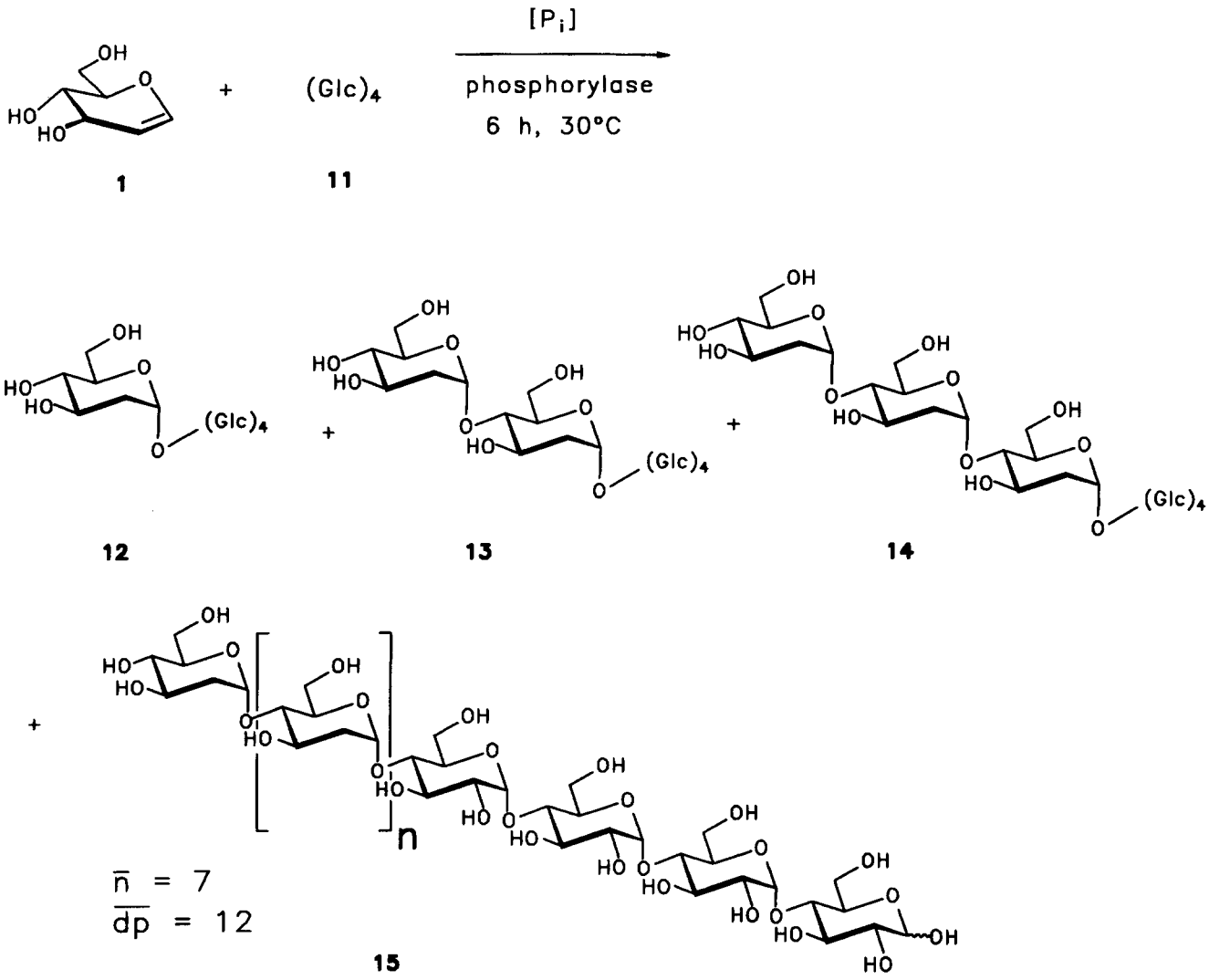
Scheme 3.

Synthesis of low molecular weight 2-deoxy-maltooligosaccharides

Previously, the synthesis of maltooligosaccharides containing 2-deoxyglucose with an average dp of 20 has been described.[6] In this study only water-soluble analogues were synthesized and the smaller oligosaccharides in this series were obtained separately on a preparative scale. So far there is no primer available consisting of deoxyglucose, therefore, the oligosaccharides will contain at least four normal glucose units at the reducing end.

Glucal was applied in considerable excess in order to shift the equilibrium to the product side with a glucal:primer ratio of 15:1. The distribution of the chain length varied with the incubation time which was adjusted to obtain soluble products with the highest possible dp. An average dp of 12 seems to be the upper limit for solubility in water. Oligosaccharides of higher chain lengths precipitated from aqueous solutions more or less rapidly, depending on temperature and concentration, probably due to retrogradation as it is also observed with linear amylose molecules.

After an incubation time of 6 h with 23 U phosphorylase and only 0.05 equiv of phosphate only the formation of soluble products was observed. The penta-, hexa- and heptasaccharides **12**, **13** and **14** could be separated completely by size exclusion chromatography and were isolated in 17, 12 and 8% yield, respectively, referred to **11**. Additionally, a fraction of higher molecular weight (**15**) with an average dp of 12 was obtained in 33% yield. Thus altogether 70% of the maltotetraose was transformed into 2-deoxy-maltooligosaccharides.



Scheme 4.

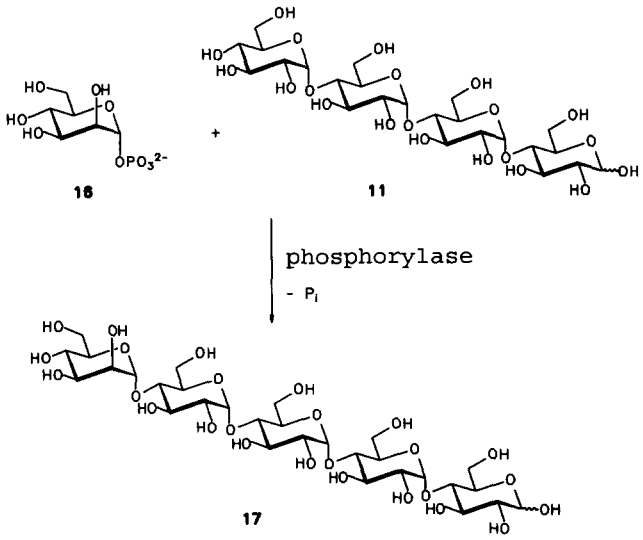
The dp of the products could be determined by integration of the ^{1}H NMR signals in D_2O as it was already done in DMSO-d_6 with the 2-deoxy-maltooligosaccharides of higher molecular weight.[6] The oligomers of dp 5–7 showed better resolved spectra and in addition to the anomeric protons and the signals of axial and equatorial H-2 further peaks could be assigned unequivocally by comparison of the homologous series and with the aid of two-dimensional NMR spectroscopy.

The four different anomeric protons of 2-deoxyglucose, of the inner glucose units and the glucose at the reducing end in α- and β-configuration appear at 5.45, 5.37, 5.20 and 4.61 ppm, respectively. H-3 of the 2-deoxyglucosyl residues is observed at 4.00 ppm, except H-3 of the terminal sugar residue, which overlaps with other signals. At 3.35 ppm the terminal H-4 appears, and the peak at 3.22 was assigned to H-2 of the β-glucosyl residue at the reducing end. Furthermore, the H-2 signals of the deoxyglucose at about 2.2 and 1.7 ppm can be used as references for the integration.

Mannosyl phosphate as a substrate for phosphorylase

After 2-deoxy-glucosyl phosphate was demonstrated to be quite a good substrate for phosphorylase, mannosyl phosphate (**16**) was also tested. Although the equatorial hydroxyl group is not essential for the enzyme, the 2-epimer compound **16** is expected to have a low affinity to the enzyme due to steric hindrance by the axial hydroxy group. In previous investigations no transfer of mannosyl residues from **16** was observed.[12] However, it could now be shown that **16** is in fact a substrate for phosphorylase, though a rather poor one requiring long incubation times.

The incubation mixture of the reaction with **16** was worked up in the usual way after seven days. After such an extended reaction time disproportionation of compound **11** occurred, which was not observed in other experiments. However, in addition to a series of maltooligosaccharides, mannose-containing products were found. Twelve mg of a pentasaccharide fraction were obtained by size exclusion chromatography, that



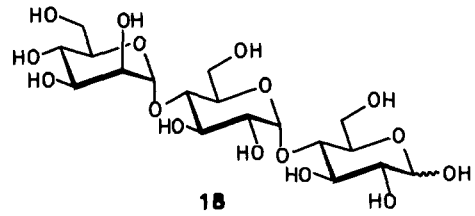
Scheme 5.

consisted of maltopentaose and 4⁴-*O*-(α-D-mannosyl)-maltotetraose **17**. This mixture could not yet be separated completely by the available techniques. The mannosyl-maltotetraose was identified in the mixture by ^{1}H NMR spectroscopy. At 5.25 ppm a new H-1 signal appears with a coupling constant of 1.7 Hz, and the new peak at 4.01 ppm could be assigned to H-2 of the mannosyl residue ($J_{1,2}$ = 1.7, $J_{2,3}$ = 3.5 Hz) by means of two-dimensional spectroscopy. The position of the mannosyl residue at the non-reducing end of maltotetraose can be assigned due to the lower intensity of the terminal H-4 signal in comparison to the integral of the α- and β-H-1 at the reducing end. Whereas in a pure maltooligosaccharide the ratio of the integrals [α- + β-H-1]:[H-4$^{\omega}$] is 1:1, in this case the ratio is 1:0.6, implying that the pentasaccharide fraction contains about 60% **7** and 40% **17**.

The reaction was carried out with different samples of potato phosphorylase and was also confirmed with a commercial phosphorylase *a* from rabbit muscle. Therefore, it can be excluded that the transfer of a mannosyl unit was caused by another than the phosphorylase activity.

From an experiment with a phosphorylase preparation, that had been purified by dialysis only, the trisaccharide 4²-*O*-(α-D-mannosyl)-maltose **18** was obtained. Obviously there was still an amylase activity present in this enzyme sample and pentasaccharide **17** was cleaved to yield **18**. This compound was isolated and its NMR spectrum contributed to the structure elucidation of the mannose-containing products.

In this spectrum there is no H-4 signal of the terminal glucose residue, characteristic for maltooligosaccharides observed and this confirms the position of the mannose at the non-reducing end. The glycosidic protons of mannose and glucose were found to resonate at 5.25 and 5.37 ppm, respectively. The α- and β-H-1 of



Scheme 6.

the reducing end could be seen at 5.19 and 4.61 ppm, respectively.

The coupling constant $J_{1,2}$ = 1.7 as well as $J_{2,3}$ = 3.5 Hz indicate that the mannosyl unit is α-linked. Because of the equatorial position of H-2 in the mannose it is sometimes difficult to determine the configuration from the ^{1}H–^{1}H coupling constants. However, slightly smaller couplings are reported in the literature for β-mannosides ($J_{\beta1,2}$=0.5–1.0, $J_{\beta2,3} \approx$ 3.0 Hz) than for α-mannosides ($J_{\alpha1,2} \approx$ 1.5, $J_{\alpha2,3} \approx$ 3.5 Hz).[13] In this case, by comparison with the values in the literature, it is clear that the mannose is α-linked.

Discussion

The substrate specificity of phosphorylase has been investigated by inhibition studies with several glucose 1-phosphate analogues,[14] and a series of deoxy- and deoxy-fluoro-glucosyl phosphates have been tested as substrates.[15] Thus Withers et al. determined the influence of each single hydroxy group on the binding at the catalytic site and the contribution of the respective hydrogen bonds. Any modification of the glucose 1-phosphate diminished the substrate properties severely. OH-3 and OH-6 have the strongest interactions with the enzyme, whereas deletion of the hydrogen bonds at OH-1 or OH-2 resulted in a minor decrease of affinity.[16]

Although mannosyl phosphate is only a poor substrate and yields of the product were rather low, it was now demonstrated for the first time that this compound can be transferred at all by phosphorylase. Whereas experiments with various D-glucal derivatives modified at the 3- or 6-position as substrates for phosphorylase were not successful,[17] the result with mannose 1-phosphate underlines the higher catalytic flexibility of the enzyme with regard to the position 2.

On incubation of **11** without any glycosyl phosphate for seven days a small amount of disproportionation products of the tetraose was formed even with a highly purified enzyme. This can be explained by phosphorolytic degradation of **11**, yielding the trimer and glucose 1-phosphate, which is then transferred to another tetraose molecule. Although maltotetraose is known to be the smallest substrate that is accepted by phosphorylase, there is still a slight activity with maltotriose.[8] This reaction can be observed in the absence of other substrates and after a long incubation

time, whereas no disproportionation was found after 6 h (i.e. under conditions used for preparation of 2-deoxymaltooligosaccharides).

With maltotetraose [**11**, $(Glc)_4$] as a primer the first product in the reaction with glucal is the 2^5-deoxy-maltopentaose (**12**), which was described before,[18] but no yields reported. This oligomer and also the next higher analogues $2^5,2^6$-dideoxy-maltohexaose (**13**) and $2^5,2^6,2^7$-trideoxy-maltoheptaose (**14**) are interesting candidates for crystallization experiments. Saenger et al. succeeded in preparing crystals of a *p*-nitrophenyl maltohexaoside barium triiodide complex that could be investigated by X-ray diffraction analysis.[19] A similar investigation of these deoxygenated derivatives could shed some light on the influence of hydrogen bonds between the 2- and 3-hydroxy groups for the conformation of maltooligosaccharides (cf. ref 20).

Experimental

General

All incubations were carried out in a buffer solution with 20 mM Tris, 20 mM sodium acetate and 0.02% NaN_3 at pH 6.9. For ultrafiltration a stirred cell (Amicon 8050) with a membrane with 10 kD cut-off was used; the anion exchange resin was DEAE-Fractogel 650 S in a 3×35 cm column, for size exclusion chromatography a column (Fractogel TSK HW-40 S, 2×90 cm) was supplied with peristaltic pump and R_i-detector. Product fractions were analysed additionally by TLC with CH_3CN:ethyl acetate:propanol:water 85:20:50:50 for oligosaccharides or 2-propanol:acetone:1 M lactic acid 2:2:1 for glycosyl phosphates and then freeze dried.

Enzymes

Potato phosphorylase was isolated by ammonium sulphate precipitation according to the method of Pfannemüller et al.[2] and the crude enzyme was dialysed for three days against buffer solution containing 20 mM Tris, 20 mM NaCl, 15 mM EDTA and 0.02% NaN_3 (pH 6.9). Additional purification was achieved by chromatography on an anion exchanger, equilibrated with Tris buffer, and elution with a gradient of 0–0.3 M NaCl in buffer solution. Active fractions were detected by determination of phosphate liberated in the synthetic reaction with the assay method of Fiske and Subbarow.[21] Phosphorylase *a* from rabbit skeletal muscle was obtained from Sigma.

2-Deoxy-α-D-arabino-hexopyranosyl phosphate (4). D-Glucal (**1**) (300 mg, 2.0 mmol), KH_2PO_4 (408 mg, 3.0 mmol) and maltopentaose substituted silica gel (**9**) (1.8 g wet gel) in buffer solution (30 mL) were shaken gently with potato phosphorylase (3 mL, 70 U) at 30 °C for two days. The enzyme was removed by ultrafiltration and the filtrate was subjected to an anion exchange column. Unreacted **1** (138 mg, 46%) was eluted with water, and after elution with a gradient of $NaHCO_3$ (0–0.2 M) the product fraction was lyophilized and desalted by gel chromatography to obtain 306 mg **4** (52%). The spectroscopic data were in accord with the product obtained using the free primer.[5]

Aminopropyl silica gel (6). In a 100 mL plastic flask silica gel Florisil (5 g, 150–250 μm, 60–100 mesh ASTM) in distilled water (90 mL) and γ-aminopropyl-triethoxysilane (**5**) (12 mL, 77 mmol) were adjusted to pH 3 with 2 M HCl and shaken gently for 4 h at 70 °C. The gel was filtered off, washed with water (100 mL) and dried overnight at 115 °C.

Immobilized maltopentaose (9). Dry aminopropyl silica gel (**6**) (1.6 g) was suspended in anhyd methanol (10 mL), maltopentaose (**7**, 70 mg, 0.08 mmol) was added, the mixture was shaken at 55 °C for 5 h and the gel was filtered off. From the filtrate 4.6 mg of **7** were recovered. The gel was suspended again in anhyd methanol (8 mL), acetic anhydride (2 mL) added, and shaken at room temperature for 12 h. After filtration the residue was washed with water (50 mL).

Low molecular weight 2-deoxy-maltooligosaccharides. D-Glucal (**1**, 300 mg, 2.0 mmol), maltotetraose (**11**, 82 mg 0.12 mmol), and KH_2PO_4 (14 mg, 0.10 mmol) in buffer solution (15 mL) were incubated with potato phosphorylase (1 mL, 23 U) for 6 h at 30 °C. After ultrafiltration the solution was subjected to anion exchange chromatography. Neutral oligosaccharides were eluted with water, freeze dried, and separated by size exclusion chromatography. Unreacted **1** was recovered, yields are based on **11**.

2^5-Deoxy-maltopentaose (12). 16.8 mg (20.7 μmol, 17%); $[\alpha]_D^{20}$ +123.4 (*c* 0.7, H_2O); ^1H NMR (D_2O) δ 5.47 (d, 1H, H-1^5), 5.38 (m, 3H, H-1^2, H-1^3, H-1^4), 5.20 (d, 0.4H, α-H-1), 4.61 (d, 0.6H, β-H-1), 3.35 (t, 1H, H-4^5), 3.22 (dd, 0.6H, β-H-2), 2.21 (dd, 1H, H-2^5_{eq}), 1.70 (ddd ≈ dt, 1H, H-2^5_{ax}); $J_{\alpha1,2} = 4.1$, $J_{\beta1,2} = 8.2$, $J_{\beta2,3} = 9.1$, $J_{3(5),4} = J_{4(5),5} = 9.4$, $J_{1,2ax} = 4.1$, $J_{2ax,2eq} = 13.2$, $J_{2eq,3} = 5.1$, $J_{2ax,3} \approx 13.2$ Hz.

$2^5,2^6$-Dideoxy-maltohexaose (13). 13.3 mg (13.9 μmol, 12%); $[\alpha]_D^{20}$ +128.5 (*c* 0.8, H_2O); ^1H NMR (D_2O) δ 5.47 (m, 2H, H-1^5, H-1^6), 5.38 (m, 3H, H-1^2, H-1^3, H-1^4), 5.20 (d, 0.4H, α-H-1), 4.61 (d, 0.6H, β-H-1), 4.02 (ddd, 1H, H-3^5), 3.35 (t, 1H, H-4^6), 3.24 (dd, 0.6H, β-H-2), 2.23 (dd, 1H, H-2^5_{eq}), 2.19 (dd, 1H, H-2^6_{eq}), 1.79 (ddd ≈ dt, 1H, H-2^6_{ax}), 1.72 (ddd ≈ dt, 1H, H-2^5_{ax}); $J_{\alpha1,2} = 3.5$, $J_{\beta1,2} = 8.1$, $J_{\beta2,3} = 9.6$, $J_{1(5),2ax} = 4.1$, $J_{2ax(5),2eq} = 13.2$, $J_{2ax(5),3} = 11.7$, $J_{2eq(5),3} = 4.6$, $J_{3(5),4} = 8.1$, $J_{1(6),2ax} = 4.1$, $J_{2ax(6),2eq} = 12.4$, $J_{2ax(6),3} = 11.7$, $J_{3(6),4} = 9.4$, $J_{4(6),5} = 9.4$ Hz.

$2^5,2^6,2^7$-Trideoxy-maltoheptaose (14). 10 mg (9.1 μmol, 8%); $[\alpha]_{578}^{20}$ +146.7 (*c* 0.14, H_2O); ^1H NMR (D_2O) δ 5.45 (m, 3H, dGlc-H-1), 5.37 (m, 3H, Glc-H-1), 5.20 (d, 0.4H, α-H-1), 4.61 (d, 0.6H, β-H-1), 4.00 (m, 2H, H-3^5, H-3^6), 3.34 (t, 1H, H-4^7), 3.22 (dd, 0.6H, β-H-2), 2.12-2.25 (m, 3H, H-2_{eq}), 1.65–1.83 (m, 3H, H-2_{ax}); $J_{\alpha1,2} = 4.1$,

$J_{\beta1,2}$ = 8.2, $J_{\beta2,3}$ = 9.1, $J_{3(7),4}$ = $J_{4(7),5}$ = 9.4, $J_{1(5),2ax}$ = 3.6, $J_{2ax,2eq} \approx 13.0$, $J_{2eq,3}$ = 4.1 Hz.

(15). 71.3 mg (38.8 μmol, 33%); ^{1}H NMR (D_2O) δ 5.46 (m, 8H, dGlc-H-1), 5.37 (m, 3H, Glc-H-1), 5.20 (d, 0.4H, α-H-1), 4.61 (d, 0.6H, β-H-1), 4.00 (m, 7H, H-3^5 to H-3$^{\omega-1}$), 3.34 (t, 1H, H-4$^\omega$), 3.23 (dd, 0.6H, β-H-2), 2.11–2.23 (m, 8H, H-2$_{eq}$), 1.65–1.83 (m, 8H, H-2$_{ax}$); $J_{\alpha1,2}$ = 4.1, $J_{\beta1,2}$ = 8.2, $J_{\beta2,3}$ = 9.1, $J_{3(\omega),4}$ = $J_{4(\omega),5}$ = 9.4 Hz.

4^4-*O*-(-D-Mannosyl)-maltotetraose (17). α-D-Mannopyranosyl phosphate (**16**, 140 mg, 0.46 mmol) and **11** (20 mg, 0.03 mmol) in buffer solution (20 mL) were incubated with potato phosphorylase (4 mL, 92 U) or with phosphorylase *a* from rabbit muscle (50 U) for seven days at 30 °C. After ultrafiltration and anion exchange chromatography the oligosaccharides were separated by size exclusion chromatography and freeze dried. In the pentasaccharide fraction (12 mg) maltopentaose (**7**) and **17** were obtained in a ratio of 3:2 according to the NMR integrals, corresponding to a yield of **17** of 4.8 mg (2%).

[7 + 17]. ^{1}H NMR (D_2O) δ 5.36 (m, 9H, Glc-H-1), 5.25 (d, 1H, Man-H-1), 5.19 (d, 1.2H, α-H-1), 4.61 (d, 1.3H, β-H-1), 4.02 (dd, 1H, Man-H-2), 3.38 (dd ≈ t, 1.5H, H-4^5), 3.24 (dd, 1.3H, β-H-2); $J_{1,2(Man)}$ = 1.7, $J_{2,3(Man)}$ = 3.5, $J_{\alpha1,2(Glc)}$ = 3.6, $J_{\beta1,2(Glc)}$ = 8.1, $J_{\beta2,3(Glc)}$ = 9.6, $J_{3,4(5)}$ = $J_{4,5}$ = 9.6 Hz.

4^2-*O*-(-D-Mannosyl)-maltose (18). Compound **16** (230 mg, 0.76 mmol) and **11** (38 mg, 0.06 mmol) in buffer solution (15 mL) were incubated for four days with a sample of potato phosphorylase (4 mL, 68 U) that had been purified by dialysis but not by additional anion exchange chromatography. The isolation was carried out as described before and 5.0 mg (10 μmol, 1.3%) **18** were obtained. $[\alpha]_D^{20}$ +116 (*c* 0.20, H_2O); ^{1}H NMR (D_2O) δ 5.37 (d, 1H, H-1′), 5.25 (d, 1H, H-1″), 5.19 (d, 0.4H, α-H-1), 4.61 (d, 0.6H, β-H-1), 4.02 (m, 1H, H-2″), 3.23 (dd, 0.6H, β-H-2); $J_{\alpha1,2}$ = 3.6, $J_{\beta1,2}$ = 8.1, $J_{\beta2,3}$ = 9.2, $J_{1',2'}$ = 4.1, $J_{1'',2''}$ = 1.7 Hz.

Acknowledgements

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