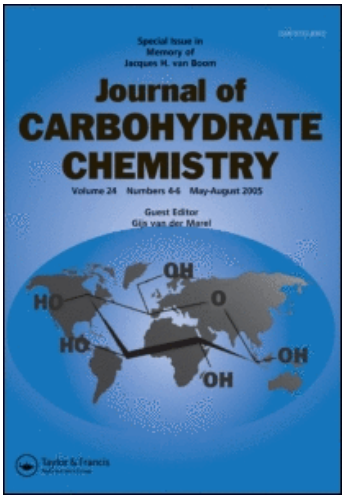


Journal of Carbohydrate Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

Synthesis of Sucros-6-yl D-Glucos-2-Yl Phosphate via the Hydrogenphosphonate Approach

Mats Lindberg[a]; Thomas Norberg[a]; Stefan Oscarson[a]
[a] Department of Organic Chemistry, Stockholm University, STOCKHOLM, SWEDEN

To cite this Article Lindberg, Mats , Norberg, Thomas and Oscarson, Stefan(1992) 'Synthesis of Sucros-6-yl D-Glucos-2-Yl Phosphate via the Hydrogenphosphonate Approach', Journal of Carbohydrate Chemistry, 11: 3, 243 — 253
To link to this Article: DOI: 10.1080/07328309208017991
URL: http://dx.doi.org/10.1080/07328309208017991

J. CARBOHYDRATE CHEMISTRY, 11(3), 243-253 (1992)

SYNTHESIS OF SUCROS-6-YL D-GLUCOS-2-YL PHOSPHATE VIA THE HYDROGENPHOSPHONATE APPROACH.

Mats Lindberg, Thomas Norberg, and Stefan Oscarson

Department of Organic Chemistry, Stockholm University,
S-106 91 STOCKHOLM, SWEDEN

Received May 23, 1991 - Final form December 30, 1991

ABSTRACT

The synthesis of a biologically active phosphodiester, sucros-6-yl D-glucos-2-yl phosphate, corresponding to the tentative structure first proposed for agrocinopine C, is described. The key step in the synthesis is the coupling between a protected glucos-2-yl hydrogenphosphonate and a sucrose derivative unprotected at the 6 position. The synthetic compound proved to be nonidentical to the native material, thereby initiating a renewed structure elucidation.

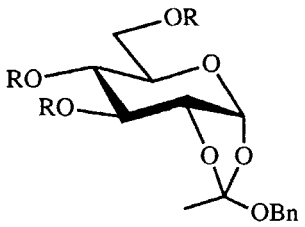
INTRODUCTION

Agrocinopine C belongs to a class of compounds occurring exclusively in plant crown gall tumors. These tumors are induced by transfer of a DNA-segment (T-DNA) from a plasmid (Ti-plasmid) in *Agrobacterium tumefaciens* bacteria. The T-DNA is incorporated into the plant cell genome and codes for the production of opines as well as uncontrolled cell growth. Outside the transferable region, the bacterial plasmid also contains genes for opine catabolism, thus enabling the bacteria to utilize opines as nutrient

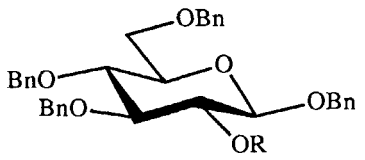
sources. Several different opines, associated with different strains of the bacteria, have been described.[1,2] Among these, there are four carbohydrate phosphodiesters, the agrocinopines A to D. Protected derivatives of agrocinopine A and B have been synthesized,[3] and we have previously reported the total synthesis of agrocinopine A.[4] In this paper we report the total synthesis of a glucose sucrose phosphodiester, suggested to be the structure of agrocinopine C.[5]

RESULTS AND DISCUSSION

Benzyl 3,4,6-tri-*O*-benzyl-β-D-glucopyranoside (**4**) was synthesized in five steps from tetra-*O*-acetyl-α-D-glucopyranosyl bromide.[6] The glucosyl bromide was dissolved in collidine and treated with benzyl alcohol and tetra-*n*-butylammonium bromide[7] to give the *exo*-isomer of **1** in 66% yield. Deacylation with methanolic sodium methoxide and subsequent benzylation with sodium hydride and benzyl bromide in DMF gave **2** (83%). Mercury(II) bromide-catalyzed rearrangement[8] with benzyl alcohol in refluxing nitromethane gave 87% of **3**, which was deacylated to give **4** (92%). Treatment of this alcohol with phosphorus triimidazolide in acetonitrile at 0 °C with subsequent hydrolysis[9] gave benzyl 3,4,6-tri-*O*-benzyl-β-D-glucopyranosid-2-yl triethylammonium hydrogenphosphonate (**5**) in 89% yield.



1 R=Ac
2 R=Bn

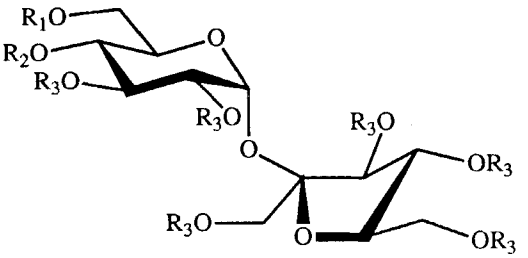


3 R=Ac
4 R=H
5 R=P(O)(H)O⁻

Sucrose was treated with benzaldehyde dimethyl acetal in DMF and a catalytic amount of *p*-toluenesulfonic acid[10] to give a complex mixture of products. To this mixture an excess of sodium hydride and benzyl bromide was added. After purification on a silica gel column 2,3,1',3',4',6'-hexa-*O*-benzyl-4,6-*O*-benzylidenesucrose (**6**) was isolated in 38% yield. A reductive opening of the dioxane ring to give the heptabenzyl compound **7** with 6-OH free was attempted. Lithium aluminum hydride/aluminum chloride in

diethyl ether-dichloromethane[11] and the method using borane trimethylamine/aluminum chloride in toluene[12] were tried. Both methods gave good regioselectivity to the desired isomer but also by-products and poor reproducibility with yields varying between 20 and 50%.

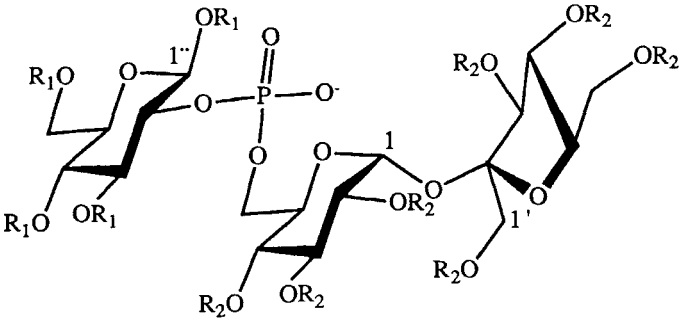
Condensation of **5** and **7** in pyridine using pivaloyl chloride[13,14] as coupling agent followed by *in situ* oxidation[15] with iodine-water proceeded smoothly to give the phosphodiester **9**. However, the deprotection of this compound proved to be difficult. The triethylammonium salt was hydrogenolyzed over Pd/C at 400 kPa for 7 days to give only minor amounts of the unprotected phosphodiester together with a mixture of partially benzylated compounds and products from hydrolysis of the phosphodiester linkage. The sodium salt was less resistant to hydrogenolysis but also gave a complex mixture of products.



6 R_1,R_2=Bzd, R_3=Bn
7 R_1=H, R_2=R_3=Bn
8 R_1=H, R_2=R_3=Ac

Since this route to sucros-6-yl D-glucos-2-yl phosphate involved both difficult preparations of compounds **6** and **7** with consecutive column chromatography of complex product mixtures, and severe problems with the deprotection of **9**, a search for a more convenient approach was made. Our attention was drawn to work by Otake[16] in which he reports the syntheses of two heptaacetylsucroses with 6-OH and 6'-OH free, respectively, *via* a tritylation-acetylation-detritylation procedure. This proved indeed to be a less time-consuming way of getting a sucrose derivative suited for coupling to the H-phosphonate monoester **5**. In the procedure by Otake the isomeric monotritylsucroses were separated prior to the acetylation. We found it as easy to carry out the acetylation *in situ* followed by chromatographic separation of the two isomers. Detritylation of the 6-*O*-trityl compound in aqueous acetic acid then gave **8**. The structure of **8** was ascertained by homonuclear decoupling ^{1}H NMR experiments showing H-4 of the glucose moiety as a double doublet at 4.98 ppm.

Condensation of **5** with **8** followed by oxidation, in the same way as with **5** and **7**, gave the protected phosphodiester **10** in 80 % yield. Debenzylation of **10** was achieved by hydrogenolysis in ethanol over Pd/C for 6 hours. Subsequent deacetylation with methanolic sodium methoxide gave, after anion exchange chromatography on DEAE-Sephadex and gel filtration, **11** in 41% yield as an anomeric mixture. ^{1}H NMR values, determined by 2D NMR experiments for all synthetic compounds, are given in Tables 1 and 2.



9 R_1=R_2=Bn
10 R_1=Bn, R_2=Ac
11 R_1=R_2=H

However, the ^{13}C NMR spectrum recorded for the synthetic product was not identical to the one obtained from the native material. A sample of the synthetic product was submitted to Dr. Max E. Tate, Waite Agricultural Institute, Adelaide, for comparison with the natural agrocinopine C. Direct comparison of the natural and synthetic products indicated that both samples exhibited similar biological activity in their effect of agrocin 84 uptake, but were clearly distinguishable in their degradative behaviour. This led to a correction of the locus of the phosphate diester linkage originally proposed in the unpublished University of Adelaide, 1983 B. Ag. Sci. Hons. thesis data of Ms. A. Savage, from position C-6 to position C-2 of the sucrose moiety.

EXPERIMENTAL

General methods. Concentrations were performed at 1-2 kPa at <40 °C (bath). Melting points are corrected. Optical rotations were recorded at room temperature for solutions in chloroform, unless otherwise stated, using a Perkin-Elmer 241 polarimeter. NMR spectra were recorded for solutions in $CDCl_3$ (internal Me_4Si) unless otherwise stated, using a JEOL

Table 1. ^{1}H NMR chemical shifts and $^{3}J_{H,H}$ coupling constants for ring hydrogens of glucose derivatives.

	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b
1	5.69	4.33	5.21	4.91	3.96	[4.20]	
	5.3	2.9	2.8	9.6	1.0	n.d.	-
2	5.75	4.40	3.88	3.70	3.80	3.65	3.67
	5.0	n.d.	n.d.	n.d.	n.d.	n.d.	-
3	4.41	5.08	3.63	3.72	3.48	3.72	3.77
	8.0	9.2	9.0	9.2	2.2/4.2	11.0	-
4	4.36	[3.53 - 3.67]			3.48	[3.67 - 3.80]	
	7.6	n.d.	n.d.	n.d	n.d	n.d	-
5	4.50	4.27	3.70	3.60	3.47	3.69	3.74
	7.4	8.7	8.8	9.3	2.3/4.4	10.9	-

Table 2. ^{1}H NMR chemical shifts and $^{3}J_{H,H}$ coupling constants for ring hydrogens of sucrose derivatives.

	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b	H-1'a	H-1'b	H-3'	H-4'	H-5'	H-6'a	H-6'b	H-1''	H-2''	H-3''	H-4''	H-5''	H-6a''	H-6b''
6	5.71	3.45	3.97	3.55	4.13	n.d.	n.d.	3.66	3.53	4.45	4.21	4.08	3.62	3.67							
	3.7	9.4	9.4	9.4	n.d.	n.d.	-	11.8	-	7.5	7.7	n.d.	n.d.	-							
7	5.77	3.36	3.91	3.41	4.00	3.47	3.56	3.65	3.53	4.43	4.26	4.02	3.63	3.70							
	3.7	9.6	9.2	10.0	2.4/4.9	11.6	-	10.8	-	7.7	7.8	3.4/5.2	10.6	-							
8[a]	5.72	4.84	5.48	4.98	4.12	[3.77-3.57]		[4.4-4.1]		[5.0-4.9]		[4.4-4.1]									
	3.6	10.4	9.7	10.3	n.d.	n.d.	-	n.d.	-	n.d.	n.d.	n.d.	n.d.	-							
9	5.51	4.74	5.40	5.07	4.26	[4.08]		[4.10]		5.46	5.33	4.15	4.29	4.45	4.43	4.35	3.66	3.54	3.40	[3.62]	
	3.7	10.3	9.6	9.8	n.d.	n.d.		n.d.		6.2.	6.2.	n.d.	n.d.	-	7.8	7.8	9.0	9.4	n.d.	n.d.	
10[b]	5.42	3.59	3.77	3.51	3.98	[4.14]		[3.41]		4.20	4.07	3.88	n.d.	n.d.	5.41	3.98	3.84	3.48	n.d.	n.d.	n.d.

a. Determined by one-dimensional experiments only.

b. Chemical shifts are those for the dominating isomer with α-configuration at the glucose anomer. Coupling constants could not be determined due to the complexity of the spectrum.

JNM-GSX 270 instrument or a Bruker AM 500 instrument. TLC was performed on Silica Gel F_{254} (Merck) with detection by UV light when applicable or by charring with sulfuric acid. Column chromatography was performed on Matrex™ Silica Gel 0.035-0.070 mm (Amicon Corp.)

3,4,6-Tri-*O*-acetyl-1,2-*O*-(1-benzyloxyethylidene)-α-D-glucopyranoside (1). 2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide[6] (8.22 g, 20 mmol) was dissolved in *sym*-collidine (20 mL) and benzyl alcohol (2.1 mL, 20 mmol) was added. The mixture was warmed to 50 °C, and tetra-*n*-butylammonium bromide (2 g, 6.2 mmol) was added. After 15 h, chloroform (50 mL) was added and the diluted reaction mixture was washed with 2M aqueous HCl, saturated aqueous sodium hydrogencarbonate and water. The organic phase was dried with sodium sulfate, filtered and concentrated. Purification on a silica gel column (toluene-ethyl acetate 4:1) gave **1** (5.82 g, 66%), $[\alpha]_D$ +20° (*c* 1.1, pyridine).

Anal. Calcd for $C_{21}H_{26}O_{10}$: C, 57.5; H, 6.0. Found: C, 57.4; H, 6.0.

^{13}C NMR:δ 20.7, 21.0, 63.1, 65.8, 67.1, 68.2, 70.1, 73.2, 97.0, 121.4, 127.5-128.4, 137.5, 169.1, 169.9, 170.6.

3,4,6-Tri-*O*-benzyl-1,2-*O*-(1-benzyloxyethylidene)-α-D-glucopyranoside (2). Compound **1** (1.02 g, 2.3 mmol) was dissolved in methanol (15 mL) and 0.1 M methanolic sodium methoxide (0.15 mL) was added. When TLC (toluene-ethyl acetate 2:1, ethyl acetate-methanol 4:1) indicated complete reaction, DMF (15 mL) was added. The methanol was evaporated and benzyl bromide (1.75 mL, 14.7 mmol) was added. This solution was then added dropwise, with stirring, to a suspension of sodium hydride (0.64 g, 14.7 mmol, 55% in mineral oil, washed twice with dry hexane) in DMF (5 mL). After 2 h, excess reagent was destroyed with methanol, and the reaction mixture was partitioned between water and toluene. The aqueous phase was extracted twice with toluene and the combined organic phases were dried with sodium sulfate, filtered and concentrated. Purification of the residue on a silica gel column (toluene-ethyl acetate 12:1, 1% pyridine) afforded **2** (1.12 g, 83%), $[\alpha]_D$ +14° (*c* 0.5, pyridine).

Anal. Calcd for $C_{36}H_{38}O_7$: C, 74.2; H, 6.6. Found: C, 74.2; H, 6.7.

^{13}C NMR:δ 22.1, 65.6, 69.3, 70.6, 72.0, 73.0, 73.5, 75.1, 75.8, 78.7, 98.0, 121.4, 127.5-128.6, 137.8-138.2.

Benzyl 2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranoside (3). Compound **2** (1.12 g, 1.9 mmol) was dissolved in nitromethane (20 mL). Benzyl alcohol (0.4 mL, 3.9 mmol) and mercury(II) bromide (0.23 g, 0.64 mmol) were added at room temperature. The reaction mixture was refluxed for 30 min. After quenching with pyridine and evaporation of the

solvent the product was purified on a silica gel column (toluene-ethyl acetate 9:1) to give **3** (0.97 g, 87%). Recrystallization from toluene-hexane gave crystals with m.p. 64-66 °C, $[\alpha]_D$ -24° (*c* 1.1, chloroform).

Anal. Calcd for $C_{36}H_{38}O_7$: C, 74.2; H, 6.6. Found: C, 74.1; H, 6.6.

^{13}C NMR:δ 21.0, 68.8, 70.4, 73.2, 73.6, 75.2, 75.2, 75.3, 78.1, 83.1, 99.8, 127.7-128.6, 137.5-138.3, 169.7.

Benzyl 3,4,6-Tri-*O*-benzyl-β-D-glucopyranoside (4). Compound **3** (0.83 g) was dissolved in dichloromethane/methanol (1:1, 9 mL) and 0.1 M methanolic sodium methoxide (1 mL) was added. When TLC (toluene-ethyl acetate 6:1) indicated complete reaction, the solution was neutralized with Dowex-50 (H^+) and concentrated to give crystalline **4** (0.71 g, 92%). Recrystallization from ethyl acetate-hexane gave crystals with mp 87-89 °C, $[\alpha]_D$ -24° (*c* 1.0, chloroform). Lit.[17] mp 87-88 °C, $[\alpha]_D$ -25° (*c* 1.2 chloroform).

^{13}C NMR:δ 69.0, 71.2, 73.6, 74.9, 75.2, 75.2, 75.3, 77.6, 84.6, 101.8, 127.8-128.6, 137.3-138.7.

(Benzyl 3,4,6-Tri-*O*-benzyl-β-D-glucopyranosid-2-yl) Triethylammonium Hydrogenphosphonate (5). To an ice cold solution of imidazole (1.94 g, 28 mmol) in acetonitrile (50 mL) was added PCl_3 (0.75 mL, 8.6 mmol) and shortly thereafter triethylamine (4.2 mL, 30 mmol). The mixture was stirred for 15 min, whereafter a solution of **4** (1.08 g, 2 mmol) in acetonitrile (50 mL) was added dropwise during 30 min. The reaction mixture was left for 2 h at room temperature, then water (5 mL) was added and the stirring continued for 30 minutes. The reaction mixture was concentrated, and then co-concentrated with pyridine-triethylamine (4:1, 25 mL). The residue was partitioned between chloroform and 1 M aqueous triethylammonium hydrogen-carbonate. The aqueous phase was washed three times with chloroform, the combined organic phases were dried over sodium sulfate and concentrated. The product was purified on a silica gel column (ethyl acetate-methanol 9:1) to give **5** (1.29 g, 91%), $[\alpha]_D$ -20° (*c* 2.0, chloroform).

Elemental analysis was performed on a sample of the free acid obtained by passing a sample of **5** through a Dowex 50(H^+) column. Evaporation of the solvent methanol gave a crystalline material. Recrystallization from methanol gave mp 144-146 °C, $[\alpha]_D$ -24° (*c* 1.0, chloroform).

Anal. Calcd for $C_{34}H_{37}O_8P$: C, 67.5; H, 6.2. Found: C, 67.5; H, 6.2.

^{13}C NMR:δ 68.6, 70.9, 73.5, 75.0, 75.2, 75.5, 77.7, 78.0($J_{C,P}$ 6.5 Hz), 83.3($J_{C,P}$ 4.1 Hz), 99.6($J_{C,P}$ 2.3 Hz), 127.6-128.5, 136.7-138.1.

2,3,1',3',4',6'-Hexa-*O*-benzyl-4,6-*O*-benzylidenesucrose (6). Sucrose (2.5 g, 7.3 mmol) was dissolved in *N,N*-dimethylformamide (100 mL). Benzaldehyde dimethylacetal (2.2 mL, 15 mmol) and *p*-toluenesulfonic acid (0.2 g, 1 mmol) were added. After 12 h, the reaction mixture was concentrated to approximately half the volume. Benzyl bromide (10.4 mL, 87 mmol) was added, and the mixture was then added dropwise to a stirred and cooled (ice) suspension of sodium hydride (3.9 g, 89 mmol, 55% in mineral oil, pre-washed twice with hexane) in *N,N*-dimethylformamide (10 mL). After completed addition, the reaction was left with stirring for 2 h at room temperature. Excess reagent was destroyed by addition of methanol, and the reaction mixture was then partitioned between water and toluene. The aqueous phase was washed twice with toluene, the combined organic phases were washed with water, dried over sodium sulfate and concentrated. Purification by repeated silica gel column chromatography (toluene-ethyl acetate 15:1) gave **6** (2.7 g, 38 %), $[\alpha]_D$ +36° (*c* 0.72, chloroform).

Anal. Calcd for $C_{61}H_{62}O_{11}$: C, 75.4; H, 6.4. Found: C, 75.8; H, 6.6.

^{13}C NMR:δ 62.8, 69.0, 70.7, 71.6, 72.6, 72.8, 73.1, 73.3, 73.5, 75.2, 78.6, 79.3, 79.5, 81.4, 82.2, 83.6, 90.5, 101.2, 104.6, 126.2-128.9, 137.8-138.3.

2,3,4,1',3',4',6',-Hepta-*O*-benzylsucrose (7). With lithium aluminum hydride/aluminum chloride: Compound **6** was dissolved in diethyl ether-dichloromethane (1:1, 10 mL/mmol). Lithium aluminum hydride (4 equivalents) was added and the mixture was warmed to reflux. Aluminum chloride (4 equivalents) in diethyl ether (5 mL/mmol **6**) was added dropwise to the boiling reaction mixture. When TLC (toluene-ethyl acetate 6:1) indicated that all starting material was consumed, the temperature was lowered to room temperature and excess reagent was destroyed by addition of ethyl acetate. Water was added to precipitate aluminum salts, the reaction mixture was filtered through Celite and then partitioned between diethyl ether and water. The aqueous phase was extracted twice with ether, the combined ethereal solutions were dried over sodium sulfate and concentrated.

With trimethylamine borane/aluminum chloride: To a solution of **6** in toluene (40 mL/mmol) was added 4Å molecular sieves, borane trimethylamine (4 equivalents) and aluminum chloride (4 equivalents) with stirring at room temperature. When TLC (toluene-ethyl acetate 6:1) showed nearly complete removal of the acetal compound, the reaction mixture was treated with Dowex 50(H^+) resin, filtered, concentrated, and co-concentrated from methanol three times.

The residues were subjected to silica gel column chromatography (toluene-ethyl acetate 9:1) to give **7** in yields between 20 and 50% as a syrup, $[\alpha]_D$ +46° (*c* 1.1, chloroform).

Anal. Calcd for $C_{61}H_{64}O_{11}$: C, 75.3; H, 6.6. Found: C, 75.7; H, 6.9.

^{13}C NMR:δ 62.0, 70.1, 71.3, 71.8, 72.1, 72.6, 73.0, 73.2, 73.5, 74.9, 75.5, 77.8, 79.1, 79.9, 80.8, 81.8, 83.4, 89.1, 104.4, 127.5-128.5, 138.0-138.3.

2,3,4,1',3',4',6'-Hepta-*O*-acetylsucrose[16] (8). To a solution of sucrose (6.8 g, 20 mmol) in dry pyridine (200 mL) was added trityl chloride (6.7 g, 24 mmol) in small portions under 4 h. The reaction mixture was left for 5 days, whereafter acetic anhydride (20 mL, 210 mmol) was added. When the reaction was complete (TLC, toluene-ethyl acetate 2:1), the solution was concentrated to a heavy syrup and then co-concentrated twice from toluene. Column chromatography (toluene-ethyl acetate 2:1) afforded 2,3,4,1',3',4',6'-hepta-*O*-acetyl-6-*O*-tritylsucrose which was detritylated in 60% aqueous acetic acid at 50 °C for 20 minutes to give **8** in the same yield as previously reported.

^{13}C NMR:δ 20.7, 20.7, 61.5, 63.1, 63.6, 68.9, 69.4, 70.5, 71.2, 74.8, 75.7, 78.9, 89.8, 103.9, 170.0, 170.1, 170.2, 170.3, 170.8.

(Benzyl 3,4,6-Tri-*O*-benzyl-β-D-glucopyranosid-2-yl) 2,3,4,1',3',4',6'-Hepta-*O*-acetylsucros-6-yl Sodium Phosphate (10). Compounds **5** (0.253 g, 0.36 mmol) and **8** (0.228 g, 0.36 mmol) were dissolved in dry pyridine, co-concentrated with dry pyridine (4 mL) two times, then dissolved in dry pyridine (4 mL) and cooled in ice. Pivaloyl chloride (0.108 mL, 0.90 mmol) was added and the mixture was stirred for 15 min. A solution of iodine (0.182 g, 0.72 mmol) in pyridine-water (96:4, 4 mL) was added and stirring was continued for 15 min. The reaction mixture was then partitioned between chloroform and saturated aqueous sodium bisulphite solution. The organic phase was washed with 2 M aqueous sulphuric acid, saturated aqueous sodium hydrogencarbonate and water, dried over sodium sulfate and concentrated. The residue was purified on a silica gel column (chloroform-methanol 19:1). The eluate was concentrated and the residue taken up in chloroform-methanol 1:1 and passed through a Dowex-50(Na^+) column to give **10** (0.361 g, 80 %), $[\alpha]_D$ +21° (*c* 1.1, chloroform).

Anal. Calcd for $C_{60}H_{70}NaO_{26}P$: C, 57.1; H, 5.6. Found: C, 57.2; H, 5.6.

^{13}C NMR:δ 20.7, 20.8, 62.9, 63.9, 64.4, 68.8, 68.9, 69.5, 69.9, 70.4, 70.8, 73.4, 74.8, 75.3, 75.5, 75.6, 76.8, 76.9, 77.5, 79.2, 84.7, 89.8, 100.6, 103.9, 127.7-128.6, 137.2-138.2, 169.9-171.3.

Sucrose-6-yl D-Glucos-2-yl Phosphate, Sodium Salt (11). Compound **10** (78 mg) was dissolved in ethanol (8 mL) and hydrogenolyzed over palladium on charcoal at 400 kPa for 6 h. The catalyst was filtered off and the solution was concentrated. The residue was dissolved in methanolic sodium methoxide (5 mL, 10 mM). When TLC (ethyl acetate-acetic acid-methanol-water 5:3:3:2) showed complete reaction the solution was neutralized with Dowex-50(H^+). The ion exchange resin was filtered off and the solution was concentrated to dryness. The residue was taken up in water and subjected to a Sephadex-DEAE column and eluted with a sodium chloride gradient (0-0.4 M). All fractions containing **11** were pooled and lyophilized. Gel-permeation chromatography on a Bio-Gel P-2 column finally afforded pure **11** (15.3 mg, 41 %), $[\alpha]_D$ +52° (*c* 0.9, equil., water).

Anal. Calcd for $C_{18}H_{32}NaO_{19}P \cdot H_2O$: C 34.6; H 5.5. Found: C 34.7; H 5.5.

^{13}C NMR: (D_2O, internal acetone δ=31) δ 61.3, 61.4, 62.1, 63.4, 65.2 ($J_{C,P}$=5.5 Hz), 69.8, 69.9, 70.2, 71.4, 72.0, 72.2, 72.3, 72.3, 72.4, 73.1, 74.9, 76.0, 76.1, 76.3, 76.3, 76.6, 77.1, 79.5, 79.6, 82.1, 91.6, 92.7, 92.8, 95.8 ($J_{C,P}$=4.6 Hz), 104.4, 104.4.

ACKNOWLEDGEMENTS

We thank The Swedish National Board for Technical Development and The Swedish Natural Science Research Council for financial support and Prof. Per J. Garegg for his kind interest.

REFERENCES

1. J. Tempé and A. Goldmann in *Molecular Biology of Plant Tumors*, G. Kahl and J. S. Schell, Eds.; Academic Press: New York, 1982, p 428.
2. J. G. Ellis and P. J. Murphy, *Mol. Gen. Genet.*, **181**, 36 (1981).
3. M. Franzkowiak and J. Thiem, *Liebigs Ann. Chem.*, 1065 (1987).
4. M. Lindberg and T. Norberg, *J. Carbohydr. Chem.*, **7**, 749 (1988).
5. M. E. Tate, personal communication.
6. R. U. Lemieux, *Methods Carbohydr. Chem.*, **2**, 221 (1963).
7. R. U. Lemieux and A. R. Morgan, *Can. J. Chem.*, **43**, 2199 (1965).
8. P. J. Garegg and I. Kvarnström, *Acta Chem. Scand.*, **B30**, 655 (1976).
9. P. J. Garegg, T. Regberg, J. Stawinski, and R. Strömberg, *Chemica Scripta*, **26**, 59 (1986); *Chem. Abstr.*, **105**:153486z (1986).

10. R. Khan, K. S. Mufti, and M. R. Jenner, *Carbohydr. Res.*, **65**, 109 (1978).
11. A. Lipták, I. Jodál, and P. Nánási, *Carbohydr. Res.*, **44**, 1 (1975).
12. M. Ek, P. J. Garegg, H. Hultberg, and S. Oscarson, *J. Carbohydr. Chem.*, **2**, 305 (1983).
13. B. C. Froehler and M. D. Matteucci, *Tetrahedron Lett.*, **27**, 469 (1986).
14. P. J. Garegg, I. Lindh, T. Regberg, J. Stawinski, R. Strömberg, and C. Henrichson, *Tetrahedron Lett.*, **27**, 4051 (1986).
15. P. J. Garegg, T. Regberg, J. Stawinski, and R. Strömberg, *J. Chem. Soc., Perkin Trans. 1.*, 1269 (1987).
16. T. Otake, *Bull. Chem. Soc. Jpn.*, **43**, 3199 (1970).
17. K. Takeo and Y. Suzuki, *Carbohydr. Res.*, **162**, 95 (1987).