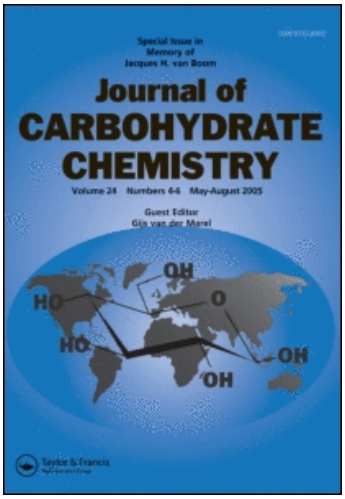

Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

Phosphodiester-Bridged Saccharide Structures

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To cite this Article Thiem, Joachim and Franzkowiak, Monika(1989) 'Phosphodiester-Bridged Saccharide Structures', Journal of Carbohydrate Chemistry, 8: 1, 1 — 28
To link to this Article: DOI: 10.1080/07328308908047989
URL: http://dx.doi.org/10.1080/07328308908047989

J. CARBOHYDRATE CHEMISTRY, 8(1), 1-28 (1989)

REVIEW ARTICLE

PHOSPHODIESTER-BRIDGED SACCHARIDE STRUCTURES

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TABLE OF CONTENTS

I. INTRODUCTION

Among the most important naturally occuring phosphodiester structures are the nucleic acids (DNA and RNA), as well as the family of phospholipids. The phosphodiester bridges between the individual nucleotide units give rise to formation of long-chain polymers with molecular weights of some hundreds of millions, in which the complete genetic information of life processes is accumulated. The phospholipids, on the other hand comprise phosphodiesters which link a sphingosine or acylated glycerine derivative with a nitrogen

base. Owing to their amphiphilic properties they are also of particular biological significance. In aqueous media they promote the formation of ordered structures like lipid double layers in biological membranes.

In contrast, there is less information about the significance and the properties of phosphodiester structures which link saccharides exclusively. In particular in the recent times increasing interest has been focused on the detailed properties of phosphodiesters in various areas with respect to their particular chemical properties. Two different types of phosphate linkages in saccharides were observed. In one of them the chemically exposed anomeric center of s sugar unit is linked via the phosphate to a primary or secondary hydroxy function of another sugar unit. In the second linkage the phosphate function connects two hydroxy groups of the two sugar units.

II. General Procedures for the Synthesis of Phosphodiester Linkages in Natural Compounds

The development of various approches to the syntheses of natural products primarily results from the chemistry of deoxyribonucleic acids. The classical phosphodiester method by Khorana et al.[1] was followed by the phosphotriester process,[2] and the more recent phosphite method.[3] Further, the oxyphosphorane procedure of Ramirez et al.[4] represents a strategically impressive, however, chemically comparatively difficult approach for the synthesis of phosphodiesters.

Following the classical methods, blocked phosphoester dichlorides are used for the phosphorylation and intermediate activation by formation of the corresponding tri- or tetrazolide or imidazolide derivatives. The phosphite variation employs the more reactive phosphite ester dichlorides, and after the esterification a terminal oxidative step leads to the phosphates. In the oxyphosphorane method the synthetic sequence is based on the preparatively less accessible cyclic enediol phosphoryl derivative (CEP method).

III. Anomerically-Bridged Phosphodiester Saccharide Structures

Phosphodiester structures which bridge the anomeric center of one sugar unit with a hydroxy group of another sugar unit are primarily found at the polysaccharide terminus of glycoproteins. For instance, in lysosomal enzymes phosphorylated sugar units, generally mannose-6-phosphates, usually occur. By their interaction with membrane-bound mannose-6-phosphate receptors the translocation of these enzymes into the lysosomes is effected.[5,6] The formation of such a phosphomannopyranosyl binding site of lysosomal enzymes is effected with GlcNAc-P-transferase which transfers GlcNAc-1-phosphate to mannose. After subsequent cleavage of GlNAc with GlcNAc-phosphodiesterase[7,8] the mannose-6-phosphate is formed which binds to the receptor.

In this context Matta et al.[9] synthesized methyl 6-(ammonium-2-acetamido-2-deoxy-α-D-glucopyranosylphosphate)-α-D-mannopyranoside (**6**). This was used as a reference compound for an enzyme assay for UDP-*N*-acetyl-glucosamine-1-phosphotransferase with methyl α-D-mannopyranoside as the acceptor substrate.

The DCC-mediated esterification of the peracetylated and α-phosphorylated glucosamine **2** obtained from **1** with methyl 2,3-*O*-isopropylidene-α-D-mannopyranoside (**3**) gave after 3 days the protected compound **4** in 28% yield. Deblocking procedures gave the derivative **5** and, after salt formation, **6**. In the latter compound the phosphodiester bond shows considerable hydrolytic stability. Cleavage was effected into GlcNAc and methyl 6-*O*-phosphoryl-α-D-mannopyranoside only after incubation in 10 mM HCl at 100°C for 30 min.

Similar phosphodiester structures with α-D-mannopyranosyl phosphate units are also known and syntheses of 1→4- and 1→6-phosphate-linked mannoses were reported by Cawley et al.[10] Compounds of this type were isolated from extracellular phosphomannans of yeast (*Hansenula holstii*[11]) as well as from cell walls of *Saccharomyces cerivisiae*[12,13] and *Kloeckera brevis*.[14]

The peracetylated α-D-mannopyranosyl phosphate **8a** obtained from **8b** was condensed with both the regioselectively

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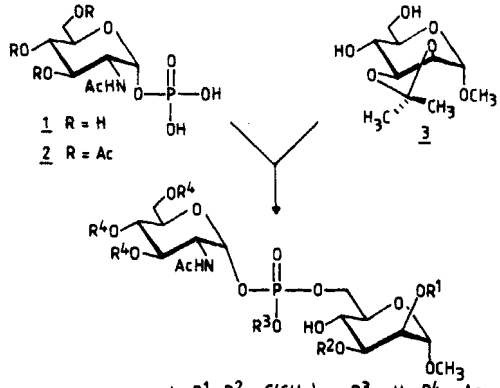
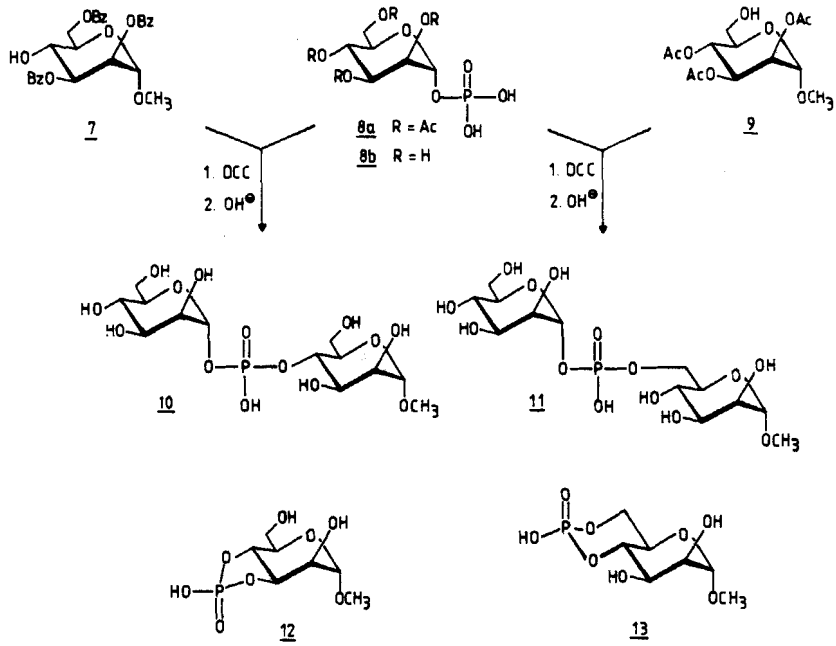


1 R = H
2 R = Ac

4 $R^1+R^2 = C(CH_3)_2$, R^3 = H, R^4 = Ac
5 $R^1 = R^2 = R^3 = R^4$ = H
6 $R^1 = R^2 = R^4$ = H, $R^3 = NH_4$

SCHEME 2



8a R = Ac
8b R = H

1. DCC
2. $OH^{\ominus}$

unblocked methyl α-D-mannopyranosides 7 or 9. Using DCC in anhydrous pyridine and a subsequent deacylation step afforded the phosphodiesters 10 and 11, respectively, which were characterized as cyclohexylammonium salts.

During the isolation procedure and the work-up of these esters a phosphate migration via cyclic phosphates in alkaline or acid media could not be excluded. Thus, a reliable assignment of the original phosphate position was problematic. Therefore, both the regioisomeric phosphodiesters 10 and 11 were hydrolyzed at 100°C in 2N HCl or in 0,5 N NaOH and the hydrolysis products analyzed. Owing to their structure and that of the cyclic phosphate derivatives 12 and 13 a phosphate migration from *O*-4 to *O*-6 could be ruled out.[10]

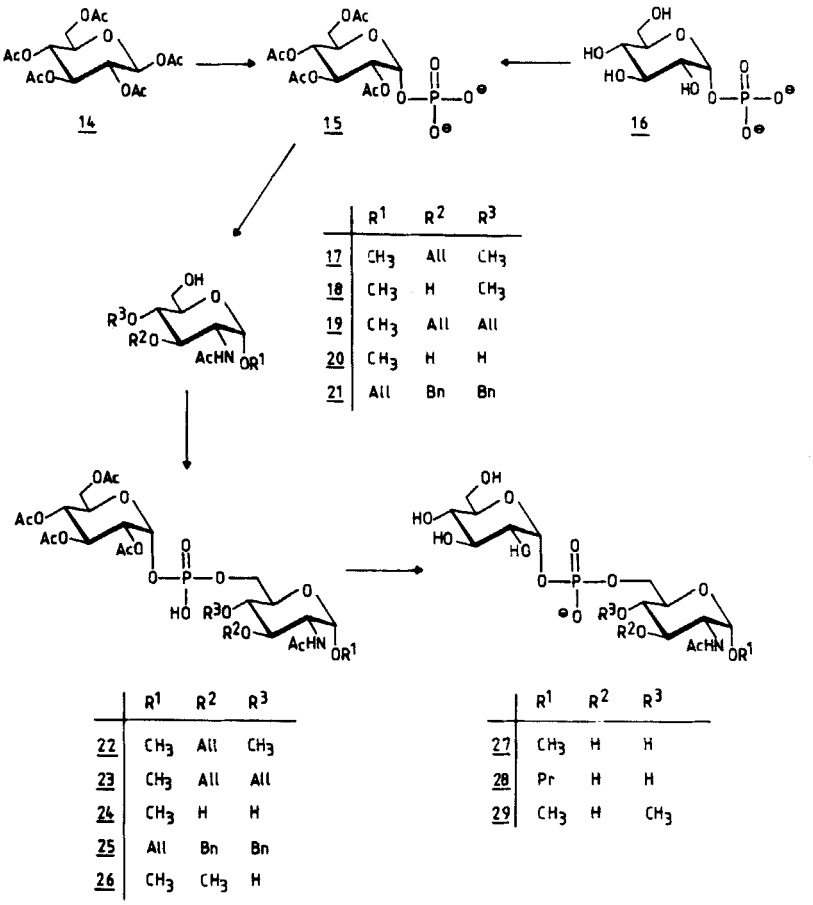
Following structure analysis of the bacterial cell walls of *Micrococcus lysodeiktious* it was proposed that 2-acetamido-2-deoxy-D-glucose residues may adopt the same function as *N*-acetyl muramic acid 6-phosphate and link the antigenic polysaccharides with the peptidoglycan chains via a phosphodiester bond.[15-17] Therefore, the diester components 27-29 with the phosphate linkage between the anomeric center of glucose and the 6-position of *N*-acetylglucosamine were prepared as model compounds.[18]

The preparation of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl phosphate (15) from penta-*O*-acetyl-β-D-glucopyranose (14) with crystalline phosphoric acid is somewhat complex and proceeds with only 20% yield. In contrast the Cori ester 16 can be easily acetylated to give 15 in 61% yield.

All the various glycosides 17 to 21 could be condensed with 15 in anhydrous pyridine by use of triisopropylbenzene sulfonic acid chloride for 48 hours at room temperature to give the corresponding diesters 22 to 26 in 40-50% yield. Deblocking procedures for 23 and 24, for 25, and for 22 lead to the glycosides 27, 28, and 29, respectively, which were thoroughly characterized.

Syntheses of similar phosphate-bridged derivatives like 38 and 46 were described by Ogawa et al.[19] following the phosphite procedure of Letsinger et al.[3] Treatment of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose (30) with PCl_3 at -78°C to the dichlorophosphite intermediate 31, further

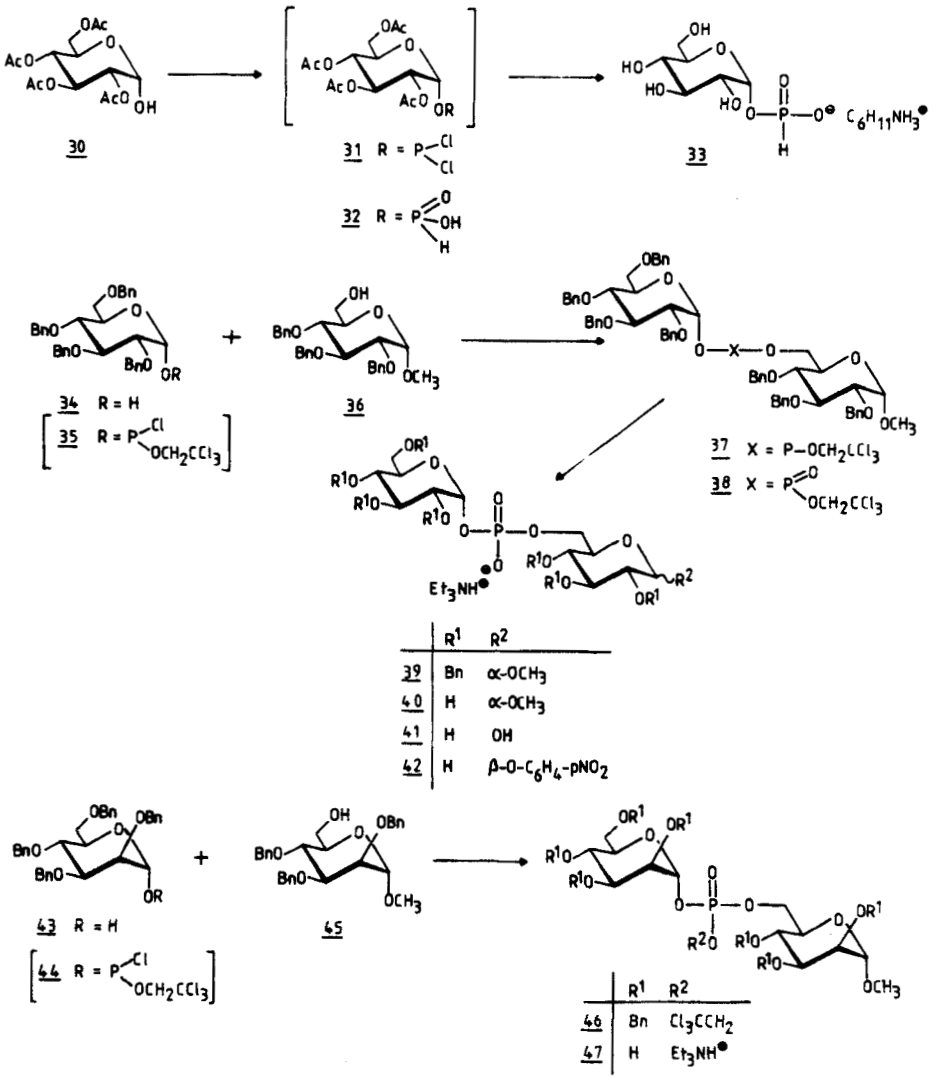
SCHEME 3



hydrolysis to the phosphite 32 and final Zemplén deacetylation gave the unblocked α-D-glycopyranosyl phosphite stabilized as its cyclohexylammonium salt 33 in 40% overall yield.

By reaction of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose (34) with 2,2,2-trichloroethyl phosphodichloridite at -78°C in tetrahydrofuran and in the presence of diisopropylethylamine the phosphitylated derivative 35 was formed *in situ*. Its treatment with methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (36) lead to the phosphorous acid diester 37. Following oxidation with O_2-AIBN[20] for 14 hours the

SCHEME 4



blocked triester derivative **38** was obtained as a 1:1 diastereomeric mixture. The trichloroethyl ester group was cleaved using Zn-Cu in the presence of 2,4-pentanedione and triethylamine to give **39**. Subsequently, the benzyl groups were removed by Birch reduction leading to methyl α-D-glucopyranoside-6-yl-(α-D-glucopyranosyl)phosphate (**40**) in 16% overall yield. In **40** as well as the precursor **38** the NMR data show $^{4}J_{H-2,P}$ = 3.2 and 2.4 Hz, respectively, which is

in accord with a *trans*-antiperiplanar arrangement of H-2 and the phosphorus atom.

At the same time Kochetkov et al.[21] synthesized the 1→P→6-linked diglucosyl phosphate **42**. 2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl phosphate (**15**) was condensed with 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose and gave, after deacetylation, the completely unblocked derivative **41**. Similarly, condensation of **15** with *p*-nitrophenyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside and subsequent Zemplén deacylation afforded the glycoside **42**. For these structures extensive ^{13}C-NMR evidence was presented.

The same approach as in the *gluco* series was applied by Ogawa et al.[19] to the mannose derivatives. Thus, the benzylated mannopyranose **43** was activated to the phosphite derivative **44** which was esterified using the mannoside **45** with an unblocked 6-position to give, after oxidation, the 1→P→6-linked dimannosyl phosphate **46**. Its deprotection led to the final product **47**. The ^{1}H- and ^{13}C-NMR data gave evidence for the structural assignment. In contrast to the *gluco* epimers no $^{4}J_{H-2,P}$ longe range coupling was observed for **46**.

Koro et al. believe that oligosaccharide structures are responsible for the localisation of cell surface glycoproteins within the cell.[22,23] Retinal ligatin, a membrane glycoprotein, links oligosaccharide chains of the high mannose type which are terminated by a phosphodiester bound D-glucose or D-galactose. Thus Hindsgaul et al.[24] report syntheses of phosphate-bridged α-Gal-1→P→6-α-Man (**51**) and α-Glc-1→P→6-α-Man (**50**, **52**) structures which are supposed to be ligatin receptors.

The syntheses follow principally the approach of Cawley et al.[10] for the preparation of phosphate-bridged mannoses **10** and **11**. First the peracetylated α-D-glycopyranosyl phosphates in the *gluco* and the *galacto* series, **15** and **48**, were prepared according to McDonald's method.[25] Condensation of these phosphates with the 6-hydroxy mannoside **49** in the presence of DCC was less effective because extended reaction times (18 hours) were needed and only modest yields (20-30%) were obtained. Much better results could be achieved with

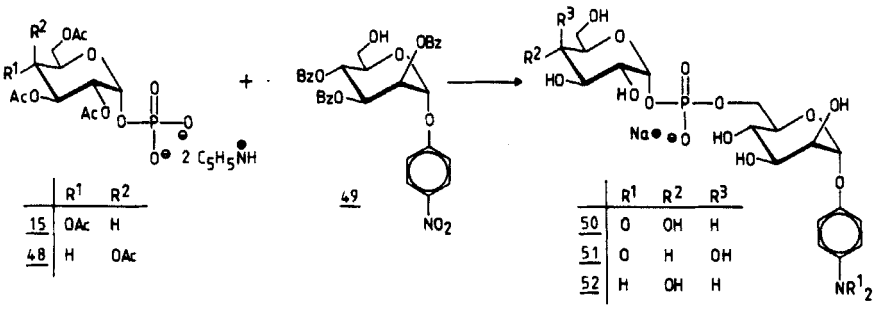
triisopropylbenzenesulfonic acid 3-nitro-triazolide (TPSNT) in place of DCC i.e. shorter reaction times (7 hours) and enhanced yields (70%). Deacetylation with triethylamine in aqueous methanol did not induce phosphate migration or hydrolysis but gave the unblocked species **50** and **51**. By reduction of the former the *p*-amino-phenyl glycoside 52 was obtained.

$^{1}H,^{31}P$- and $^{13}C,^{31}P$-NMR coupling constants were determined which gave evidence of the phosphate bridging and their conformational properties. From the coupling constants $J_{H\text{-}6a,P}$ and $J_{H\text{-}6b,P} \approx 5\text{-}6$ Hz the expected equilibrium of the conformers was observed, with 80-85% on the side of the *anti*-periplanar arrangement of the phosphorus atom and C-5 of mannose. In the glucose residue of **50** the NMR data give evidence of a dihedral angle of 40° between P and H-1. Furthermore, from the large long-range coupling $^{4}J_{P,H\text{-}2} = 2.8$ Hz a coplanar W arrangement of H2-C2-C1-O-P was assigned, which is accord with an *anti*-periplanar relation of P and C-2. These findings support the authors supposition that the phosphodiester linkage is stretched in aqueous medium and allows the easily accessible terminal group to function as a recognition marker.

A novel group of phosphorylating agents **53b-d**,[26] and **54e**[27] which allow a cleavage of the phosphate protecting group under mild alkaline conditions was applied by van Boom et al.[28] to the synthesis of glycosyl phosphates.

Treatment of 2,3,4-tri-*O*-benzyl-*α*-L-fucopyranose (**54**) with the phosphitylating agent **53b** led to the relatively stable pure fucosyl phosphite **55** which was activated with 1H-tetrazole (40-75% yield), and this condensed with the mannose derivative **56** to give the phosphite ester **57**. After oxidation with *t*-butyl hydroperoxide to **58** the *β*-cyanoethyl group was cleaved with ammonia-saturated methanol. Final hydrogenolysis led to the unblocked fucosyl-1→P→6-mannose compound **59**. Similarly, the fucose derivative **54** could be activated with 1H-triazole followed by condensation with **55** to give the triester phosphite **60**. Oxidation to **61** and cleavage of the phosphate as well as sugar blocking groups afforded the anomerically phosphate-bridged 1→P→1-bisfucose derivative **62**.

SCHEME 5

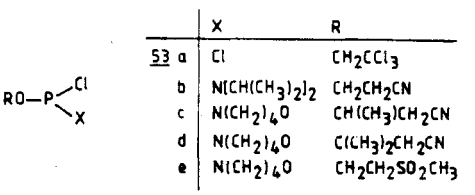


	R^1	R^2
15	OAc	H
48	H	OAc

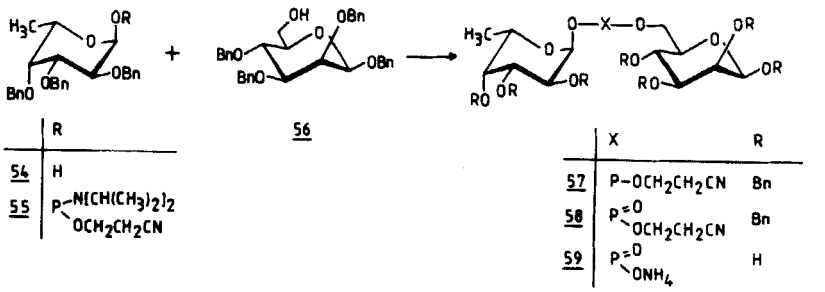
49

	R^1	R^2	R^3
50	O	OH	H
51	O	H	OH
52	H	OH	H

SCHEME 6



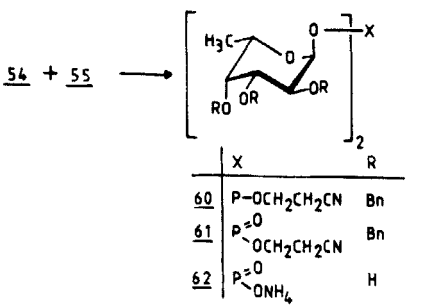
	X	R
53 a	Cl	CH_2CCl_3
b	$N[CH(CH_3)_2]_2$	CH_2CH_2CN
c	$N(CH_2)_4O$	$CH(CH_3)CH_2CN$
d	$N(CH_2)_4O$	$C(CH_3)_2CH_2CN$
e	$N(CH_2)_4O$	$CH_2CH_2SO_2CH_3$



	R
54	H
55	$P(-N[CH(CH_3)_2]_2)(-OCH_2CH_2CN)$

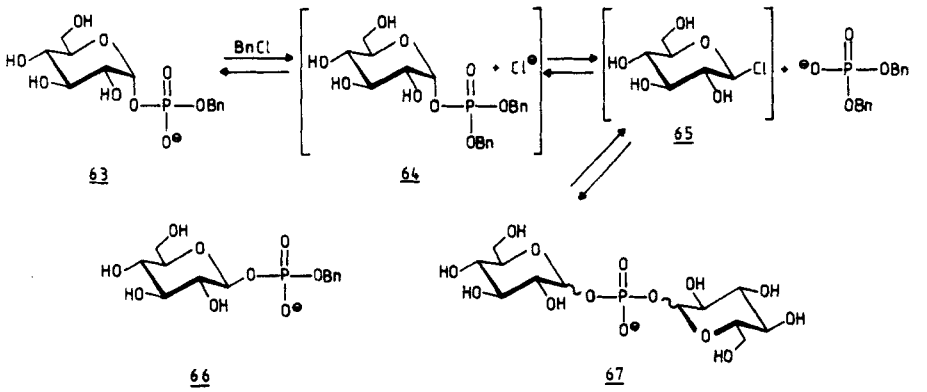
56

	X	R
57	$P(-OCH_2CH_2CN)$	Bn
58	$P(=O)(-OCH_2CH_2CN)$	Bn
59	$P(=O)(-ONH_4)$	H



	X	R
60	$P(-OCH_2CH_2CN)$	Bn
61	$P(=O)(-OCH_2CH_2CN)$	Bn
62	$P(=O)(-ONH_4)$	H

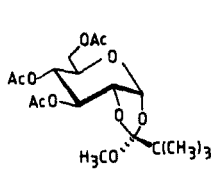
SCHEME 7



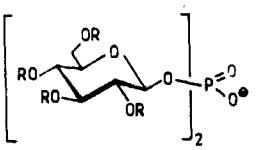
Some surfactants based on carbohydrates were investigated by van Bekkum et al.,[29] for example the reaction of α-D-glucose-1-phosphate with a fatty acid alcohol or an alkyl-aryl alcohol to give long chain phosphates. Tetramethyl ammonium salts of mono as well as dialkyl phosphates easily reacted with alkyl halides to give phosphotriesters. Similarly, the diesters could be obtained by addition of one molar equivalent of alkyl halide to the corresponding monoalkyl phosphate in acetonitrile as solvent.[30] By application of this procedure the *bis*-(tetrabutylammonium) salt of α-D-glucopyranose-1-phosphate with benzyl chloride or bromide in acetonitrile afforded α-D-glucopyranosyl benzylphosphate (**63**) in 80-90% yield.[29] Surprisingly, addition of excess benzyl chloride did not lead to the corresponding triester derivative **64**. Rather, a series of reactions occured which gave dibenzyl phosphate, α- and β-D-glucopyranosyl benzyl phosphate (**63** and **66**), and a mixture of the three isomeric anomerically phosphate-bridged glucoses **67** (α,α; α,β; β,β). The formation of these compounds was assumed to occur via a dynamic equilibrium of phosphodi- and triesters, of which the phosphotriester **64** and the glucosyl chloride derivative **65** are probably intermediates.

More practical syntheses of β-D-glucopyranose-1-phosphate (**72**)[31,32] are of interest. Starting with 3,4,5-tri-*O*-

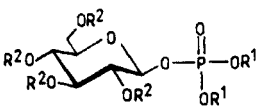
SCHEME 8



68



	R
69	Ac
70	H



	R^1	R^2
71	H	Ac
72	H	H
73	$C_6H_{11}NH_3$	H

acetyl-1,2-*O*-(1-*endo*-methoxy-2,2-dimethylpropylidene)-α-D-glucopyranose (68), phosphorylation using anhydrous phosphoric acid in anhydrous oxolane in the presence of lithium hydroxide gave exclusively the desired compound 72.[33] However, following neutralization of the reaction mixture with ammonium hydroxide, only the peracetylated phosphodiester derivative 69 (22% yield) was obtained. Its formation is thought to be dependent on the presence of phosphoric anhydride in the neutralization step. This was further substantiated by experiments which showed that an excess of orthoester 68 and the absence of phosphorus pentaoxide led to a mixture of mono- and diesters. Subsequent to neutralization with ammonia no further diester formation was observed. Both diesters 69 and 70 hydrolyze under reflux in acid medium within 10 min. At room temperature 70 begins to hydrolyze after 3.5 h, and the acetylated form remains stabile for 12 h.

The preparation of 70 requires no phosphate protecting groups. Subsequent deacetylation gave 72 and the cyclohexylammonium salt 73. Application of this procedure to the formation of β-L-fucopyranosyl phosphate was accomplished similarly.[34]

IV. Non Anomerically-Bridged Phosphodiester Saccharide Structures

There is comparatively little information about the occurrence and significance of saccharide phosphodiesters in

which the phosphate group links two sugars via the normal hydroxy functions. Biochemical processes which could account for their formation in the organism are not at hand, thus it is not surprising that such structures were not observed hitherto, e.g. in the polysaccaride portions of glycoproteins.

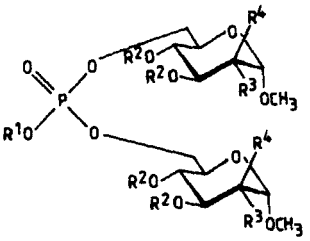
However, early reports pointed to the fact that phosphorylated starches like potato and rice starch contained minor amounts of phosphodiester linkages. These were concluded to feature primarily 2→P→6, 3→P→6, and 6→P→6 linkages. Attractive rheological properties of phosphorylated starches furthered their industrial preparation starting from corn starch for example which does not contain phosphate groups. Treatment of native starches with phosphorus oxy-chloride or sodium trimetaphosphate gives rise to the preparation of such phosphate-modified starches. Owing in particular to their phosphodiester structures these phosphorylated starches possess properties which are of primary importance for the food industry with respect to the production of canned and fast food products.[35-37]

The patent literature reported a recent synthesis of 6→P→6-phosphate-bridged methyl α-D-glycopyranosyl units in the *gluco* (**76**) and the *manno* series (**77**).[38] Following phosphitylation of both the corresponding methyl 2,3,4-tri-*O*-benzyl-α-D-glycopyranosides with 2,2,2-trichlorethyl phosphorous dichloride in tetrahydrofuran at -78°C and subsequent oxidation using iodine, the phosphotriester derivatives **74** and **75** were obtained, the deblocking of which led to the unprotected species **76** and **77**, respectively.

The approach applied here is identical to the one described in Scheme 4.[19] Chuanzhong et al.[39] also used the phosphite method to prepare several 6→P→6-, and 6→P→3-phosphate linked saccharides. Starting with methoxy phosphorous dichloride they obtained phosphites which were oxidized again with iodine. This led to the completely blocked phosphotriesters derivatives **78-80**.

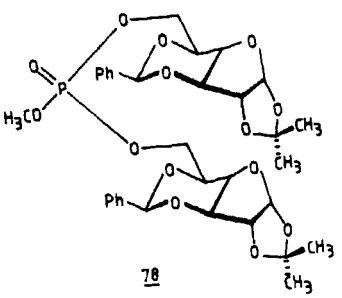
There is considerable interest in specific macroscopic effects of phosphate-modified starches with respect to molecular structural features, as well as to their digestibility. This induced extensive synthetic studies for the preparation

SCHEME 9



	R^1	R^2	R^3	R^4
74	Cl_3CCH_2	Bn	OBn	H
75	Cl_3CCH_2	Bn	H	OBn
76	H	H	OH	H
77	H	H	H	OH

SCHEME 10



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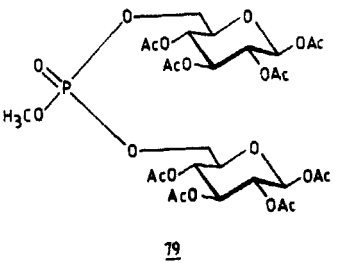

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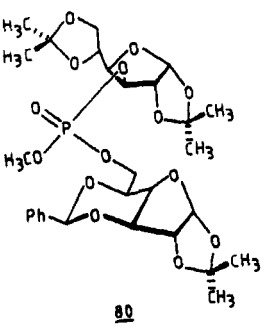

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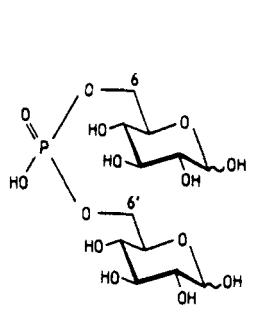
SCHEME 11



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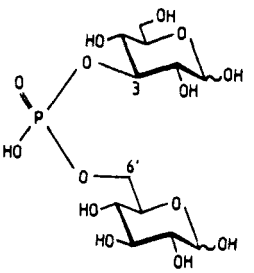

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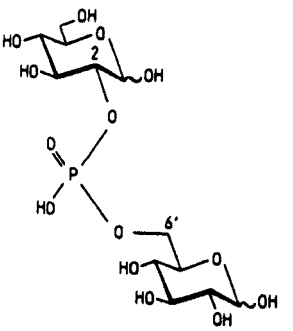


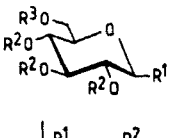
83

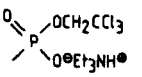
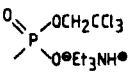
of the possible model structures of 6→P→6-, 6→P→3-, and 6→P→2-phosphate-bridged glucose units **81**-**83**.[40]

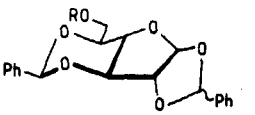
The approach applied followed a modified phosphotriester procedure. First, a number of different selectively blocked glucose derivatives **84**, **86**, **88**, **90**, **92**, **94**, **96**, **98**, and **100**, were treated with 2,2,2-trichloroethyl phosphodichloridate or 2-chlorophenyl phosphodichloridate and following activation with 1,2,4-triazole[41] the monophosphates **85**, **87**, **89**, **91**, **93**, **95**, **97**, **99**, **101**, and **102** were obtained and characterized. For the second condensation step 1-mesitylenesulfonic acid [1H]-3-nitrotriazole (MSNT)[42] was used as the promoter and by treatment with the 6-hydroxy glucose derivatives **84**, **86**, and **88** the phosphotriesters **103**, **106**, **107**, **109**, **111**, **113**, **115**, **116**, and **117** were synthesized. Generally these reactions proceeded completely in approximately 1 hour in yields typically of 60-80% without optimization. The unsymmetical 6→P→2- and 6→P→3-phosphate-bridged triesters gave 1:1 diastereomeric mixtures as expected.

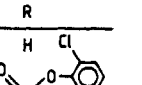
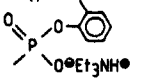
The phosphate protecting groups were cleaved with zinc/triisopropylbenzene sulfonic acid[43] or pyridine-2-carbaldoxime/tetramethylguanidine[44] and this led to the diester salts **104**, **105**, **108**, **110**, **112**, **114**, and **118**. Finally, Zemplén deacetylation as well as hydrogenolysis led to the phosphodiesters **81**-**83** as their sodium salts. Both, ^{1}H- and ^{13}C-NMR spectra gave evidence that the unblocked

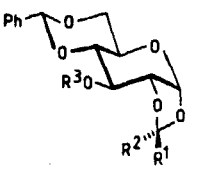
SCHEME 12

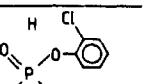
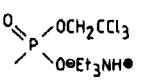
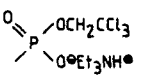


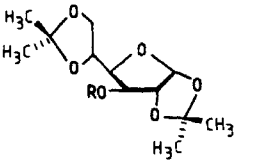
	R^1	R^2	R^3
84	β-OAc	Ac	H
85	β-OAc	Ac	
86	α-OBn	Bn	H
87	α-OBn	Bn	

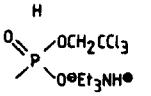


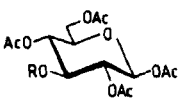
	R
88	
89	

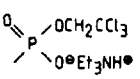


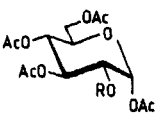
	R^1	R^2	R^3
90	Ph,H		H 
91	Ph,H		
92	Ph	H	H
93	Ph	H	
94	H	Ph	H
95	H	Ph	

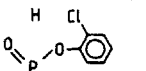
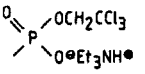


	R
96	H
97	

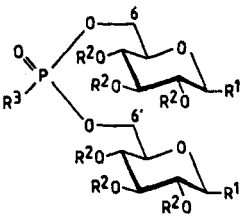


	R
98	H
99	

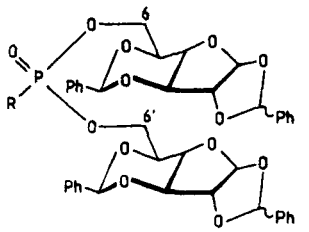


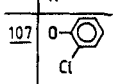
	R
100	H 
101	
102	

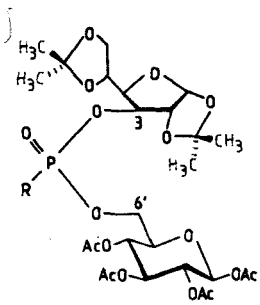
SCHEME 13



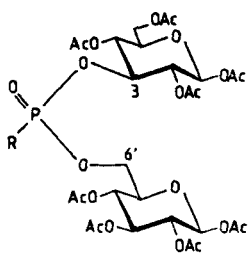
	R^1	R^2	R^3
103	β-OAc	Ac	OCH_2CCl_3
104	β-OAc	Ac	$O^{\ominus}Et_3NH^{\oplus}$
105	α,β-OAc	Ac	$O^{\ominus}Na^{\oplus}$
106	α-OBn	Bn	OCH_2CCl_3



	R
107	
108	$O^{\ominus}Na^{\oplus}$



	R
109	OCH_2CCl_3
110	$O^{\ominus}Et_3NH^{\oplus}$

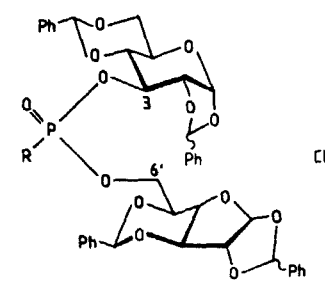


	R
111	OCH_2CCl_3
112	$O^{\ominus}Et_3NH^{\oplus}$

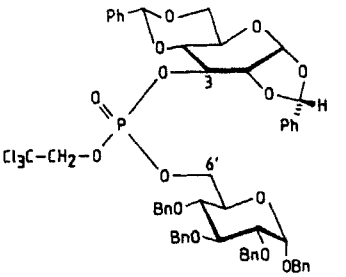
6→P→3 and 6→P→2-bridged species **82** and **83** each occur as a mixture of four different anomers e.g. 6α→P→3α, 6α→P→3β, 6β→P→3α, and 6β→P→3β.

A novel phosphorylation approach proved effective in the preparation of the symmetrical diester salt **104**. In a one-pot-procedure the tetraacetate **84** and methyl phosphorodichloridate in pyridine gave the salt **104** in 60% yield. Intermediate and the phosphorylating agent in this reaction is the *N*-methyl pyridium salt of the dichlorophosphate.[45] Thus this method does not require specific phosphate blocking and deblocking procedures.

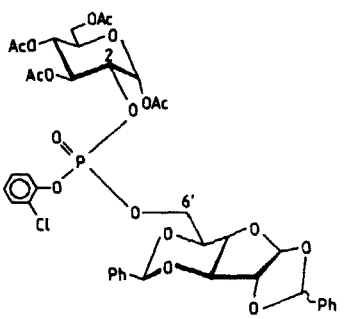
SCHEME 14



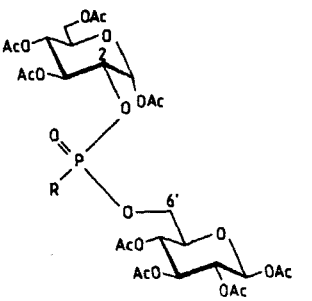
	R
113	O-(2-Cl-C6H4)
114	O⊖Na⊕



115

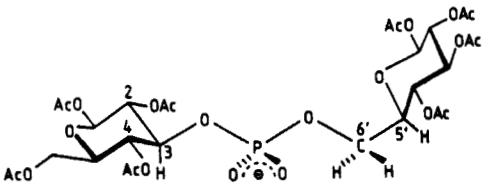


116

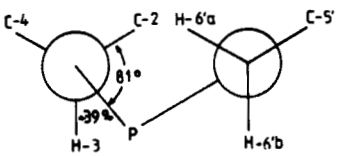


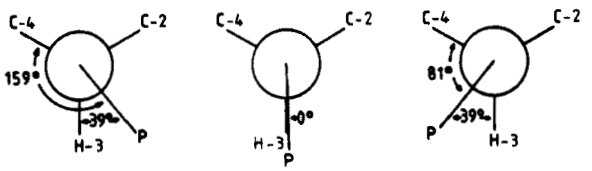
	R
117	OCH_2CCl_3
118	$O⊖Et_3NH⊕$

From ^{31}P-, ^{1}H-, and ^{13}C-NMR studies and in particular the $J_{^{13}C,^{31}P}$ coupling constants the spatial arrangement around the phosphate bridge was elucidated in the per-acetylated derivatives **104**, **112**, and **118**. In the symmetrical isomer **104** as well as the 6-phosphorylated glucose residue in **112** and **118** *anti*-periplanar arrangements of C-5 and the phosphorus atom were observed. Furthermore, compound **118** showed a preferred conformation with almost *anti*-periplanar positions of C-3 and phosphorus. However, in **112**, for the relative conformation of C-2 and phosphorus three rotational isomers I-III were taken into account in the time average.

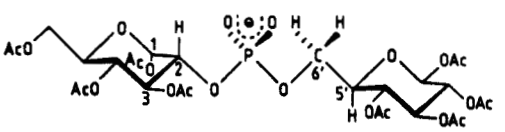


112





I II III



118

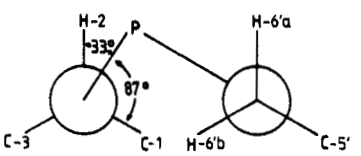
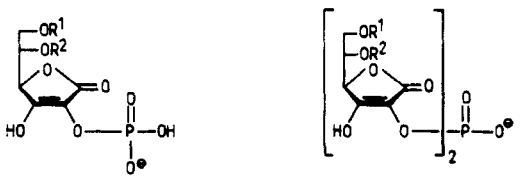


FIGURE 1

SCHEME 15



	R^1	R^2	
119	$H_3C-C-CH_3$		121
120	H	H	122

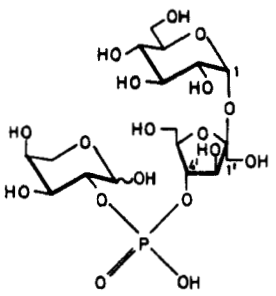
Studying the synthesis of L-ascorbate-2-phosphate **120** as a potential form of a redox stable vitamine C Seib et al.[46] also obtained the bis-(L-ascorbate-2)-phosphate **122**.

Phosphorylating of 5,6-*O*-isopropylidene-L-ascorbate with $POCl_3$ at 0-5°C in pyridine at pH 12-13 gave preferentially the monoester phosphate **119** and only 3% of **121**. Without pyridine and at pH 8-13 the yield of **121** rose to 32%. The same applies to the preparation of the unblocked derivatives **120** and **122** directly from L-ascorbate. These findings correspond to those of Behrmann et al.[33] (cf. Scheme 8).

A completely different class of compounds is realized in the unusual phosphodiesters agrocinipine A and B, **123** and **124**. These belong to a group of opines and represent enzyme products, of tumor inducing plasmides (T_i plasmide) of plant grown gall tumors which are caused by the pathogenic plasmide of certain agrobacteria. The structure elucidation was performed by enzymatic degradation experiments as well as ^{13}C NMR studies.[47] These were confirmed recently by 2D-^1H-NMR studies.[48]

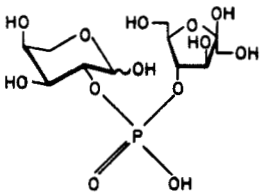
The unusual significance of these compounds goes along with their hydrolysis properties which differ from that of the normally occurring anomerically phosphate bridged sugar units. The genes for the catabolic turnover of the opines are also localized on the T_i plasmide. Holsters et al.[49] isolated a gene which cleaves both agrocinopine A and B. This obviously codes for a phosphodiesterase which can hy

SCHEME 16



Agrocinopin A

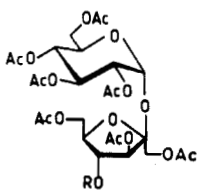
123

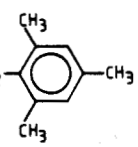


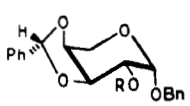
Agrocinopin B

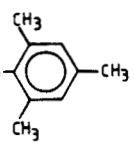
124

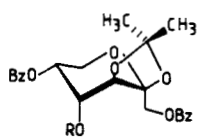
SCHEME 17



	R
125	H
126	$Cl_3CCH_2OPO_2^{\ominus}Et_3NH^{\oplus}$
127	

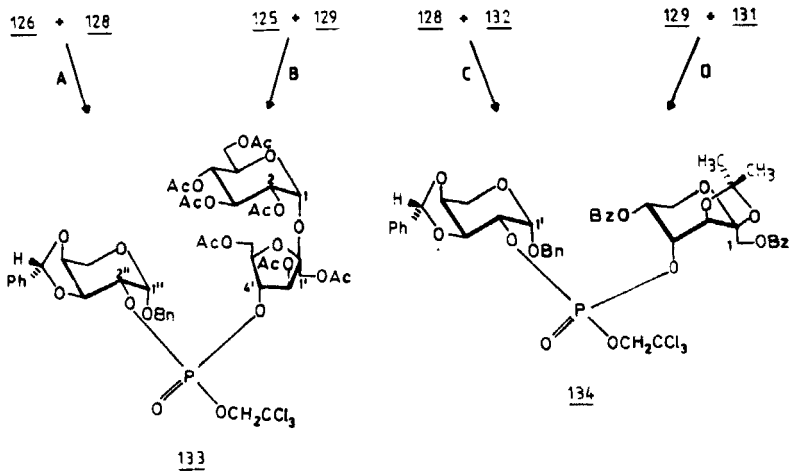


	R
128	H
129	$Cl_3CCH_2OPO_2^{\ominus}Et_3NH^{\oplus}$
130	



	R
131	H
132	$Cl_3CCH_2OPO_2^{\ominus}Et_3NH^{\oplus}$

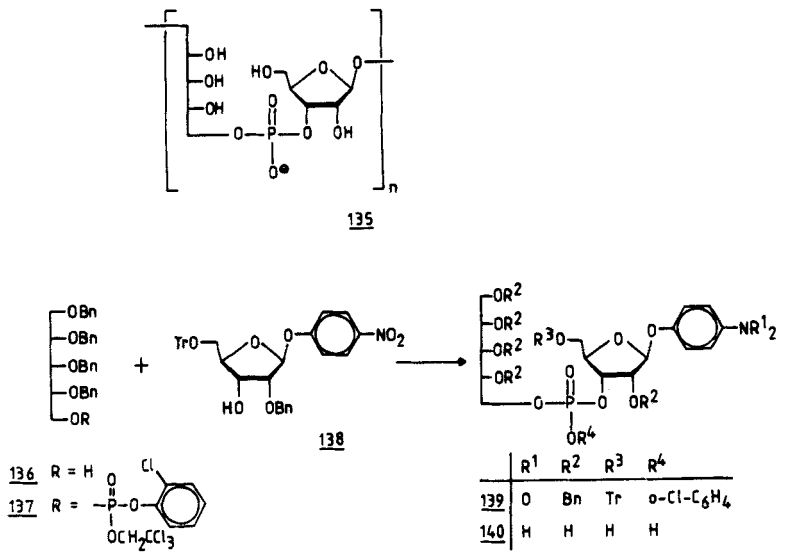
SCHEME 18



drolyze both phosphoester linkages. However, neither snake venom phosphodiesterase nor phosphodiesterase from bovine brain was effective.

Both compounds were synthesized recently[50] following the previously applied modified triester procedure.[40] The sucrose heptaacetate 125[51] was prepared and transformed with 2,2,2-trichlorethyl phosphodichloridate and 1,2,4-triazole in pyridine into the monophosphate **126**. Similarly, the L-arabinopyranoside **128** gave the phosphate **129**, and the fructopyranose derivative **131** the phosphate **132**. In the second condensation step a considerable excess of MSNT and longer reaction times (24-36 hours) were required owing to the less reactive secondary alcohol functions. This goes along with the formation of *O*-sulfonated products like **127** and **130** and consequently partial turnovers and average yields of 20-50% for **133** and **134**. In either case both ways (as depicted in Scheme 18) gave comparable yields, however, condensation of the sucrose phosphate **126** with **128** as hydroxy component gave **133** as a single diastereomer. In all the other entries **133** and **134** were obtained as the 1:1 diastereomeric mixtures as expected.

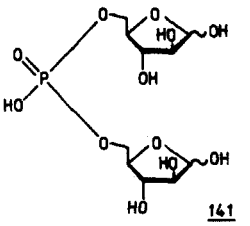
SCHEME 19



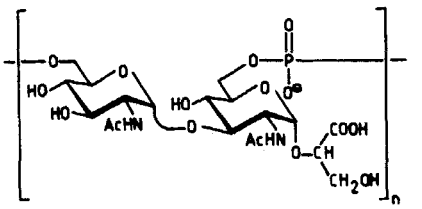
The *Haemophilus influenza* type b bacterium which is responsible for meningitis in children produces a capsular polysaccharide antigen with the structure **135**.[52,53] The repeating unit β-D-ribofuranoside-3-(D-ribit-5-yl-phosphate) was prepared by Garegg et al.[54] in the form of the *p*-aminophenyl glycoside **140** which allows a coupling to proteins.[55]

By phosphorylation of 1,2,3,4-tetra-*O*-benzyl-D-ribitol (**132**) with 2,2,2-trichloroethyl 2-chlorophenyl phosphochloridate the triester **137** was obtained quantitavely as a diasteromeric mixture. Following cleavage of the trichloroethyl group using zinc, condensation with the ribofuranoside derivative **138** was performed employing 3-nitro-1-(2,4,6-triisopropylbenzene sulfonic acid)-1,2,4-triazol as promoter. This gave the sugar triester **139** in 88% yield as a diastereomeric mixture. Deblocking of the 2-chlorophenyl group with pyridine-2-carbaldoxime/*N,N,N,N*-tetramethylguanidine and subsequent hydrogenolysis transferred the *p*-nitrophenyl glycoside **139** into the *p*-aminophenyl β-D-ribofuranoside-3-(D-ribit-5-yl-phosphate) (**140**) in 38% yield.

SCHEME 20



141



142

Quite recently further structures linking two primary sugar hydroxy groups were reported. Part of a certain glycoprotein from the multicellular green algae *Volvox carteri* was shown to be a bis sugar phosphodiester assigned as the 5,5'-phosphate-bridged D-arabinofuranose **141**. This structural unit is thought to be integrated into larger entities and possibly to link different carbohydrate chains.[56]

The polysaccharide antigen from *Peptostreptococcus anaerobius* was demonstrated to be the polymer **142** which consists of two α,1→3-linked *N*-acetylglucosamine units the reducing end of which is α-glycosylated with the 2-position of glyceric acid. It also carries a 6-phosphate group which spans the ester linkage to the 6-position of the following non-reducing GlcNAc residue.[57]

Further information about the biochemical pathways responsible for formation and degradation of phosphate-bridged saccharides via non activated hydroxy groups will be certainly of considerable interest and will undoubtedly arise from future research efforts.

V. Acknowledgement

This work was supported by the *Arbeitsgemeinschaft Industrieller Forschungsvereinigungen* (AIF) through means of the *Bundesministerium für Wirtschaft* (BMWi) and the *Forschungskreis der Ernährungsindustrie* (FEI) which is gratefully acknowledged.

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