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Review

Natural phosphoglycans containing glycosyl phosphate units: structural diversity and chemical synthesis

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Dedicated to the memory of Professor Nikolay K. Kochetkov

Abstract—An anomeric phosphodiester linkage formed by a glycosyl phosphate unit and a hydroxyl group of another monosaccharide is found in many glycopolymers of the outer membrane in bacteria (e.g., capsular polysaccharides and lipopolysaccharides), yeasts and protozoa. The polymers (phosphoglycans) composed of glycosyl phosphate (or oligoglycosyl phosphate) repeating units could be chemically classified as poly(glycosyl phosphates). Their importance as immunologically active components of the cell wall and/or capsule of numerous microorganisms upholds the need to develop routes for the chemical preparation of these biopolymers. In this paper, we (1) present a review of the primary structures (known to date) of natural phosphoglycans from various sources, which contain glycosyl phosphate units, and (2) discuss different approaches and recent achievements in the synthesis of glycosyl phosphosaccharides and poly(glycosyl phosphates).

Keywords: Polysaccharides; Phosphoglycans; Anomeric phosphodiesters; Structure; Synthesis

Contents

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1. Introduction

A glycosidic linkage between monosaccharides is a common type of the inter-monomeric linkage in carbohydrate-containing polymers. Studies over the past 40 years have shown that a different type of intersaccharide linkage is also widespread: a phosphodiester linkage formed by a glycosyl phosphate unit and a hydroxyl group of another monosaccharide. This type of linkage is found in many polymers (commonly named 'polysaccharides', but should be properly termed 'phosphoglycans') of the cell wall or capsule of bacteria and yeasts, as well as in the extracellular and surface phosphoglycans of protozoan parasites (*Leishmania*). It is also present in glycan chains of some glycoproteins in animals. In many cases, the glycosyl phosphate units participating in the formation of phosphodiester linkages are constituents of the antigenic determinants defining the immunological specificity.

Natural phosphoglycans composed of glycosyl phosphate (or oligoglycosyl phosphate) repeating units could be chemically classified as poly(glycosyl phosphates). Their importance as immunologically active components of the cell wall and/or capsule of numerous pathogenic microorganisms upholds the need to develop potential routes for the chemical preparation of these biopolymers. Thus, the synthesis of glycosyl phosphosaccharides, that is, anomeric phosphodiesters in which phosphorus is linked to one monosaccharide through the hemiacetal hydroxyl group at C-1 and to another through an alcoholic hydroxyl group, becomes a primary task. Chemical formation of glycosyl phosphosaccharides is complicated as both the correct stereochemistry at C-1 and the lability of anomeric phosphodiester linkages must be taken into consideration.

Historically, the first attempts at the preparation of glycosyl phosphosaccharides were made using the phosphodiester and the phosphorous (phosphite) triester methods. The *glycosyl hydrogenphosphonate method*, which was developed in the late 1980s, seemed to provide the most efficient means of constructing phosphodiester linkages of this type. The mechanism of the glycosyl H-phosphonate condensation was studied by ^{31}P NMR spectroscopy and a wide range of glycosyl phosphosaccharides containing (1→2)-, (1→3)-, (1→4)- and (1→6)-phosphodiester linkages were synthesised. Further, the method was applied to the synthesis of phosphoglycans of biological origin. As a result, long-chain fragments of biopolymers containing several glycosyl phosphate units were prepared using both stepwise and blockwise approaches. Polycondensation of a partially protected disaccharide H-phosphonate monomer was used to prepare the first synthetic poly(glycosyl phosphates) representing the phosphoglycan component of the surface lipophosphoglycan from *Leishmania donovani*. Finally, polymer-supported syntheses of *Leishmania* phosphoglycans were developed using mono- and di-saccharide H-phosphonate derivatives for the chain elongation.

To date, two review papers describing the chemical synthesis of phosphodiester-linked carbohydrate structures (excluding oligonucleotides, which are not a subject of the present paper as well) were published: first, by Thiem and Franzkowiak in 1989[1] and, more recently, by Hansson and Oscarson in 2000.[2] In addition, the first chemical syntheses of phosphoglycans from *Leishmania* were reviewed by Gigg and Gigg in their paper[3] dedicated to the synthesis of glycosylphosphatidylinositol anchors. Here, we discuss various methodologies and some specific features for the chemical preparation of glycosyl phosphosaccharides and synthetic poly(glycosyl phosphates). In the first part of this paper, we present the primary structures (known to date) of natural phosphoglycans from various sources, which contain glycosyl phosphate units, and briefly discuss their biological functions.

2. Occurence in Nature, primary structure and biological functions of phosphoglycans containing glycosyl phosphate units

2.1. Capsular and O-specific antigens of Gram-negative bacteria

Surface polysaccharides are characteristic components of the outer membrane of bacteria, constituting a barrier

between the bacterial cells and the surrounding environment. Capsule polysaccharides (CPS) and cell-wall lipopolysaccharides (LPS) are major components[4] of the outer membrane of Gram-negative bacteria. These complex membrane glycopolymers are believed to be indispensable for normal maintenance, growth and reproduction of bacterial cells.[5] Generally, bacterial polysaccharides have a regular structure where oligosaccharide units are repeated along the polysaccharide chain. The repeating units may be linear or branched and contain up to about eight monosaccharide residues.[4c]

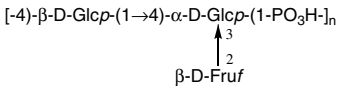
The CPS (which are usually negatively charged[4b,4c]) function as the primary serotype-specific antigens in many bacteria. It has been known for some time[4b,4c,6] that poly(glycosyl phosphates) are present amongst the polymers forming a capsular antigen (K-antigen) of some strains of *Escherichia coli*, *Haemophilus influenzae*, *Actinobacillus pleuropneumoniae*, *Pasteurella haemolitica* and *Neisseria meningitidis* (the structures are given in Charts 1–3). *E. coli* K11, K51 and K52 cause urinary tract or extra-intestinal infections in humans,[7–9] while *H. influenzae* type 'c' and type 'f' are non-pathogenic strains.[10–14] *A. pleuropneumoniae* causes contagious pleuropneumonia in pigs.[15] *P. haemolitica* organism is responsible for pneumonia and septicaemia in sheep and cattle.[16] *N. meningitides* A is a well-known causative agent of meningitis in humans.[17–19]

Cell-wall LPS of Gram-negative bacteria consist of a hydrophobic (and membrane associated) lipid A domain, a non-repeating core oligosaccharide and a distal O-specific polysaccharide built up from repeating units.[4a,4c,29–31] Lipid A is known as a mediator of endotoxic activities[29b] and the O-specific polysaccharide (also termed the somatic antigen, O-antigen or O-chain) exerts the predominant serological specificity of the microorganism.[4a,31] The O-chains of LPS may be either neutral or negatively charged.[4c]

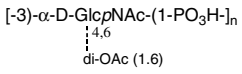
The presence of phosphodiester-linked oligosaccharide repeating units in O-specific antigens of some strains of *E. coli*,[32] *Shigella boydii*, *Proteus* spp., *Citrobacter rodentium* and *Hafnia alvei* (the structures are given in Charts 4–6) was discovered during the past 10 years. Shiga toxin-producing *E. coli* O172 strain was isolated from a case of human hemorrhagic colitis,[33] while the enteroinvasive *E. coli* O173 causes diarrhoea.[34] *S. boydii* type 13 is related distantly to other *Shigella* forms, but its O-antigen gene cluster has high levels of sequence similarity with *Vibrio cholerae* gene clusters.[35] Gram-negative bacteria of the genus *Proteus* are human facultative pathogens,[36–38] which frequently cause wound and urinary-tract infections that can result in severe complications (bacteremia, pyelonephritis). *H. alvei* is an enterobacterial opportunistic pathogen that causes typical nosocomial infections, including enteric, urinary and respiratory tract disorders.[39–41]

There are some invasive bacteria (e.g., *Neisseria* and *Haemophilus*) whose lipopolysaccharides do not contain the O-specific chain and are then called lipo-oligosaccharides. Their core oligosaccharides are substituted with a few monosaccharide residues without a regular pattern.[4c] The lack of the O-chain is typical also for the so called rough strains (or rough mutants, R-mutants) of bacteria.[46] The structure of the lipo-oligosaccharide of the

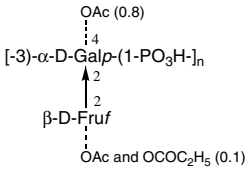
Capsular antigens of *Escherichia coli*



E. coli K11 [7]

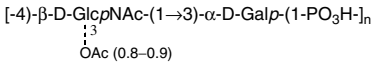


E. coli K51 [8]

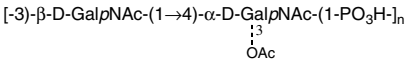


E. coli K52 [9]

Capsular antigens of *Haemophilus influenzae*



H. influenzae type "c" [10, 12, 14]



H. influenzae type "f" [11, 13, 14]

Chart 1. See Refs. 7–14.

Capsular antigens of *Actinobacillus (Haemophilus) pleuropneumoniae*

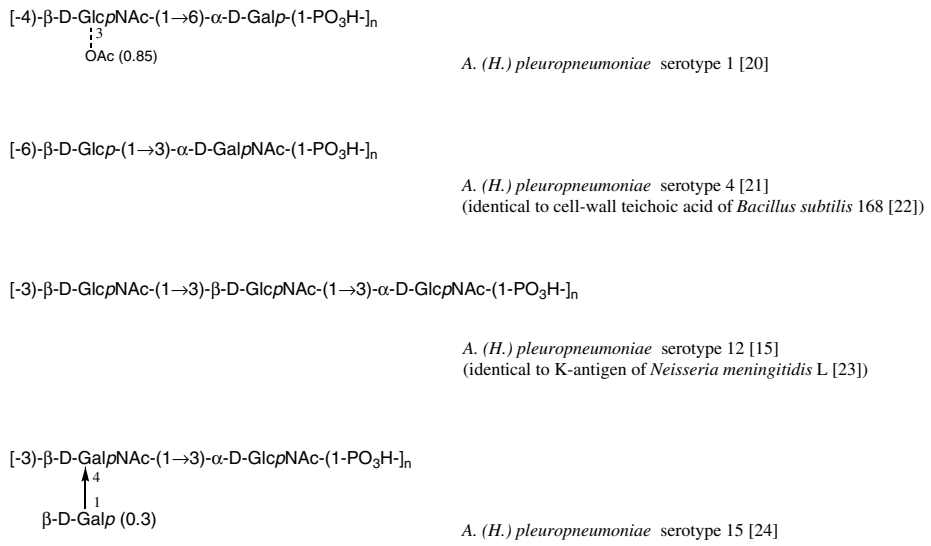
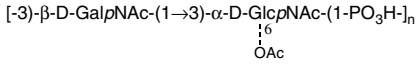


Chart 2. See Refs. 15 and 20–24.

Capsular antigen of *Pasteurella haemolitica*



P. haemolitica serotype A7 [16]

Capsular antigens of *Neisseria meningitidis*

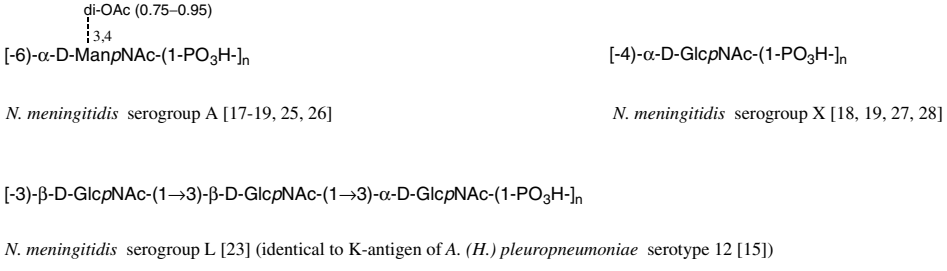


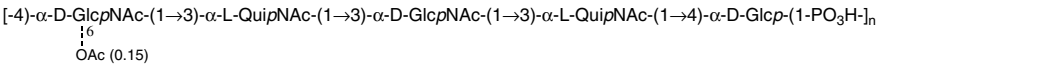
Chart 3. See Refs. 15–19, 23 and 25–28.

marine bacterium *Arenibacter certesii* strain KMM 3941^T was elucidated (Chart 7) and shown to contain an unusual α-D-galactosyluronic acid phosphate unit in the side chain of the core region.[48] The lipo-oligosaccharides of two strains of *Campylobacter lari* (*Campylobacter* spp. are leading causes of human enteritis) were isolated (the structures are not shown here) and found[47a,47b] to be associated with water-soluble extracellular polysaccharides of a poly(glycosyl phosphate) structure containing very rare pentose and heptose constituents (Chart 7).

The surface polysaccharide [a poly(glycosyl phosphate)] from *Shewanella putrefaciens* strain S29 (Chart 8), which was assigned[49] to neither type of antigen, structurally looks more similar to some O-antigenic structures. But it could also be an extracellular polysaccharide associated with LPS or lipo-oligosaccharide of the bacterium.

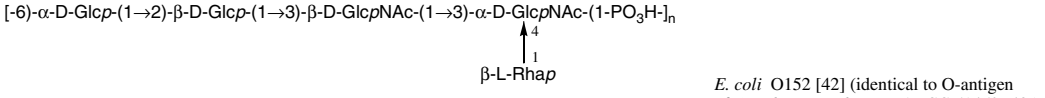
Porphyromonas gingivalis strain W50 (*P. gingivalis* is an important aetiologic agent in human periodontal diseases) produces a cell-surface anionic polysaccharide (Chart 8), which is distinct from both the LPS and the

O-Specific antigen of *Shigella boydii*

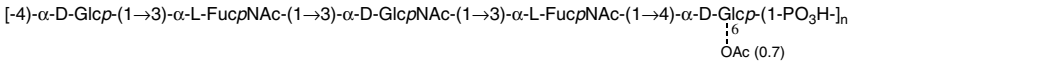


S. boydii type 13 [35]

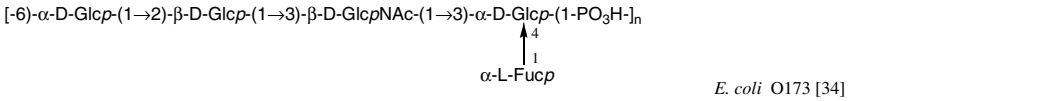
O-Specific antigens of *Escherichia coli*



E. coli O152 [42] (identical to O-antigen of *Citrobacter rodentium* ATCC 51459 [43])



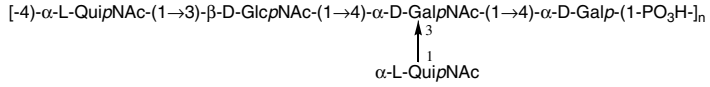
E. coli O172 [33]



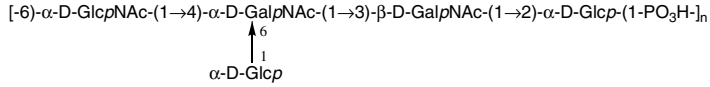
E. coli O173 [34]

Chart 4. See Refs. 33–35, 42 and 43.

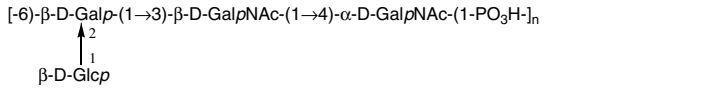
O-Specific antigens of *Proteus*



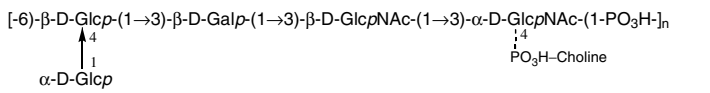
P. vulgaris O1 (OX19) [36]



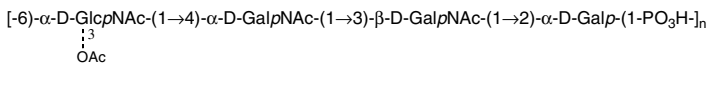
P. vulgaris O21 [37]



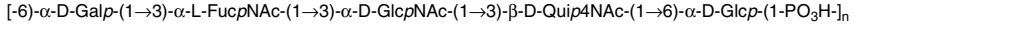
P. vulgaris O34 [44]



P. mirabilis O18 [38]



P. mirabilis O48 [37]

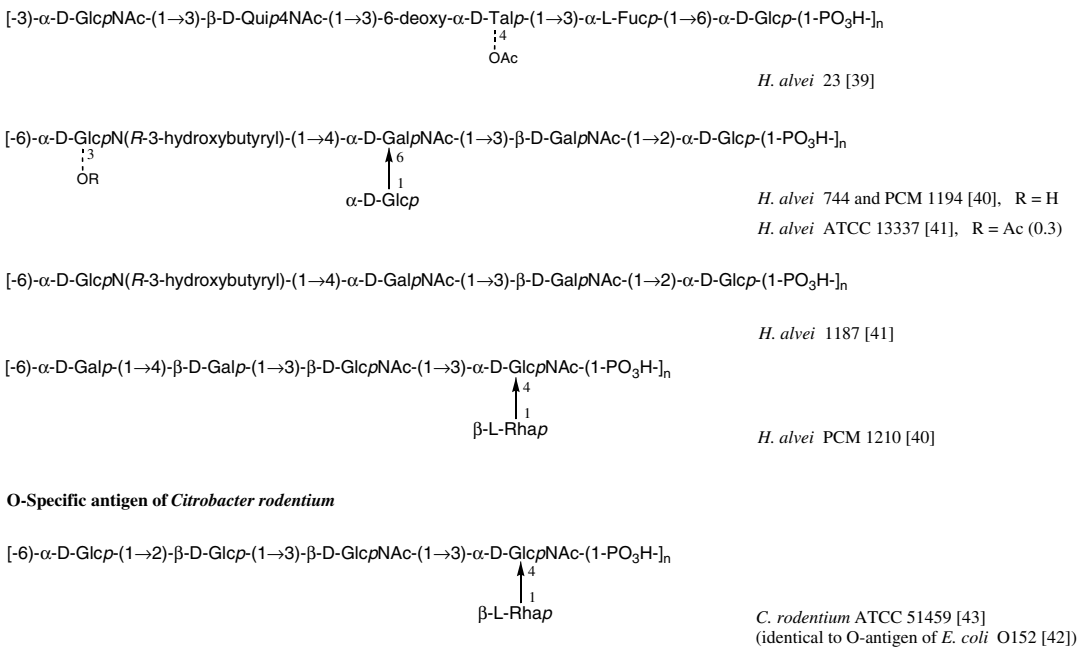


P. penneri 60 (classified into a new *Proteus* serogroup O70) [45])

Chart 5. See Refs. 36–38, 44 and 45.

serotype capsule polysaccharide of the strain. It has the structure of a branched phosphomannan, resembling the phosphomannans of yeasts (see Section 2.3).[50] The structure is different from poly(glycosyl phosphates) since it contains a mannobiosyl phosphate unit in the side chain linked to the linear mannan backbone.

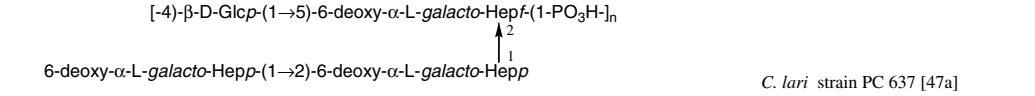
O-Specific antigens of *Hafnia alvei*

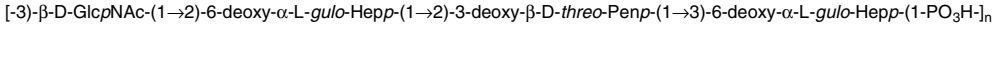


O-Specific antigen of *Citrobacter rodentium*

Chart 6. See Refs. 39–43.

Extracellular polysaccharides associated with lipo-oligosaccharides of *Campylobacter lari*





Lipo-oligosaccharide of *Arenibacter certesii*

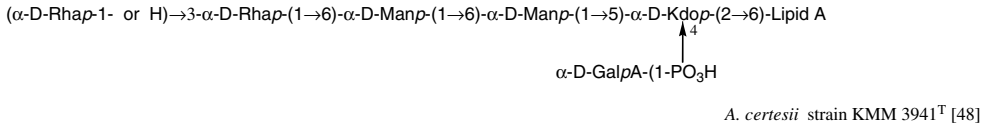


Chart 7. See Refs. 47 and 48.

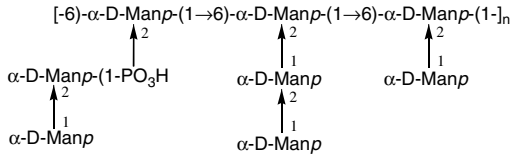
Brief comparative analysis of the structures listed above shows that K-antigenic poly(glycosyl phosphates) have relatively short (from mono- to tri-saccharide phosphate) repeating units, while O-antigenic phosphoglycans are made up from either tetra- or penta-saccharide phosphate blocks. The O-antigenic polymers have a more diverse carbohydrate composition (14 distinct monosaccharides without counting diverse anomeric configurations) than the K-antigens (six distinct sugars). There are some rare monosaccharides (6-deoxy-D-talopyranose, 2-acetamido-2,6-dideoxy-L-glucopyranose, 4-acetamido-4,6-dideoxy-D-glucopyranose and 2-deoxy-2-[(*R*)-3-hydroxybutyramido]-D-glucopyranose) found in O-antigenic phosphoglycans. The extracellular phosphoglycans from *C. lari* have the most unusual monosaccharide composition. They are built up from mainly 6-deoxy-L-heptose residues (both pyranoses and furanoses) and contain three sugars not previously found

Water-soluble surface polysaccharide from *Shewanella putrefaciens*

[-4)-α-L-Qui*p*NAc-(1→3)-α-D-Glc*p*NAc-(1→3)-β-D-Qui*p*4NAc-(1→3)-α-D-Gal*p*-(1-PO_3H-]$_n$

S. putrefaciens strain S29 [49]

Cell-surface anionic polysaccharide from *Porphyromonas gingivalis*



P. gingivalis strain W50 [50]

Chart 8. See Refs. 49 and 50.

in polysaccharides: 6-deoxy-L-*galacto*-heptofuranose, 6-deoxy-L-*gulo*-heptopyranose and 3-deoxy-D-*threo*-pentopyranose. Most of the poly(glycosyl phosphate) structures from Gram-negative bacteria have branched repeating units with a monosaccharide or ester residue in the side chain.

2.2. Cell-wall and capsular glycopolymers of Gram-positive bacteria

The cell walls of most Gram-positive bacteria are composed of anionic glycopolymer(s) and peptidoglycan, which are normally covalently linked. The glycopolymers (which account for 10–60% of the cell-wall weight[51]) are usually a group-specific antigen and a type-specific antigen(s). The group-specific antigen (or common antigen) is always present and occurs in identical form in closely related bacteria. The structure of the type-specific antigen may vary among different strains of the same bacterial species.[52] In some organisms, the latter glycopolymer takes the form of a capsule and functions as a capsular antigen (traditionally named as a 'capsule polysaccharide').

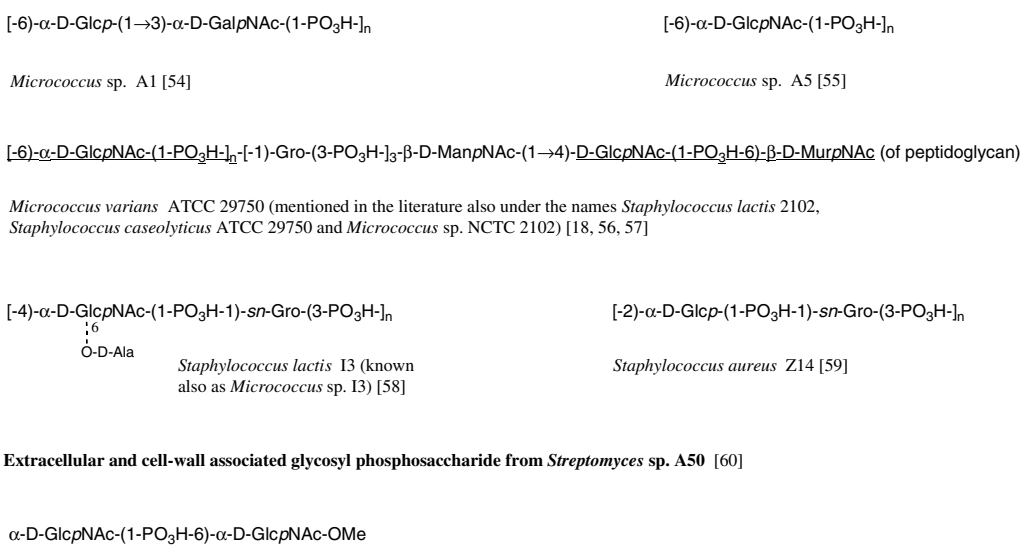
There are three major types of anionic glycopolymers found in Gram-positive bacteria (for a recent review see Ref. 51), which are teichoic acids, teichuronic acids (the non-phosphorylated polymers) and sugar-phosphate polymers. The teichoic acid group includes poly(glycerol phosphates), poly(erythritol phosphates), poly(ribitol phosphates), poly(arabinitol phosphates) and poly(mannitol phosphates) with a phosphodiester linkage mainly between primary hydroxyl groups, while secondary hydroxyls are unsubstituted or glycosylated. There are also more complex poly(glycosylpolyol phosphate) teichoic acids. None of these polymers contain glycosyl phosphate units, therefore their structures are not discussed here. The sugar-phosphate polymers, that is, poly(glycosyl phosphates), were found in the cell walls of micrococci and staphylococci (Chart 9), bacilli and actinomycetes (Chart 10), streptococci (Charts 12 and 13) and in the capsules of pneumococci (Charts 11 and 12).[6,51,53]

Micrococcus and *Staphylococcus* species are among the common microflora in mammals; *S. aureus* is known as a major pathogen. Cell walls of *Micrococcus* sp. A5 and *M. varians* ATCC 29750 contain identical poly(α-D-Glc*p*NAc phosphate) polymers (Chart 9).[55,56] For the latter strain, the structure of the linker region between the phosphoglycan and peptidoglycan was elucidated[57] and seemed to consist of a glycosyl phosphosaccharide unit D-GlcNAc-(1-PO_3H-6)-β-D-MurNAc (where *N*-acetyl-D-muramic acid is a part of the peptidoglycan). The same unit was found to be a part of the linker region in the cell wall of *Bacillus pumilus* AHU 1650 (Chart 10).[61] *S. lactis* I3[58] and *S. aureus* Z14[59] synthesise unusual cell-wall phosphoglycans (Chart 9), where the glycosyl phosphate and glycerol phosphate fragments alternate along the regular phosphoglycan chain.

A non-polymeric glycosyl phosphosaccharide structure, which is identical to phosphodiester fragments of anionic glycopolymers from *Micrococcus* sp. A5 and *M. varians* ATCC 29750, was detected in the extracellular culture broth and in the cell walls of *Streptomyces* sp. A50 (Chart 9).[60] The biological functions of this small molecule remain unknown.

Bacillus and *Clostridium* organisms occur widely in soil, water and mud. Some bacilli and clostridia synthesise cell-wall surface layer (S-layer) glycoproteins.[63] In *Clostridium symbiosum* HB25, the carbohydrate part of the glycoprotein is represented by a poly(glycosyl phosphate) with a tetrasaccharide phosphate repeating unit (Chart 10). Relatively simple phosphoglycan structures, containing di- and tri-saccharide phosphate repeating units, are found in the cell wall of actinomicetes (Chart 10), including *Actinoplanes*,[64] *Streptomyces*,[65] *Micromonospora*[66] (all three occur in soil, aquatic habitats and plant litter) and *Brevibacterium*[67] (which occurs in certain cheeses) species.

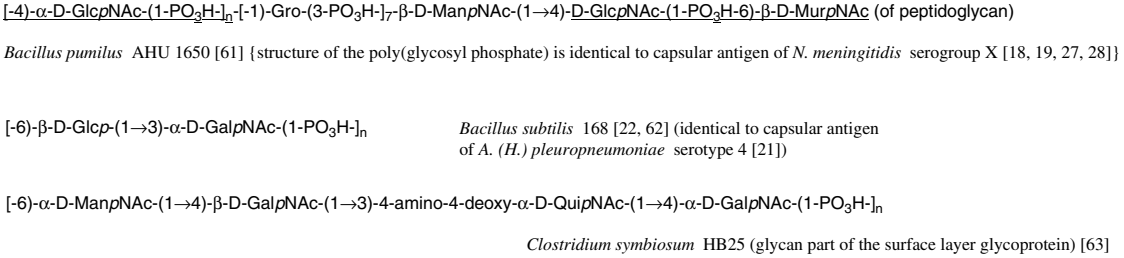
Cell-wall anionic polymers of *Micrococcus* and *Staphylococcus*



Extracellular and cell-wall associated glycosyl phosphosaccharide from *Streptomyces* sp. A50 [60]

Chart 9. See Refs. 18 and 54–60.

Cell-wall anionic polymers of *Bacillus* and *Clostridium*



Cell-wall anionic polymers of *Actinoplanes*, *Streptomyces*, *Micromonospora* and *Brevibacterium*

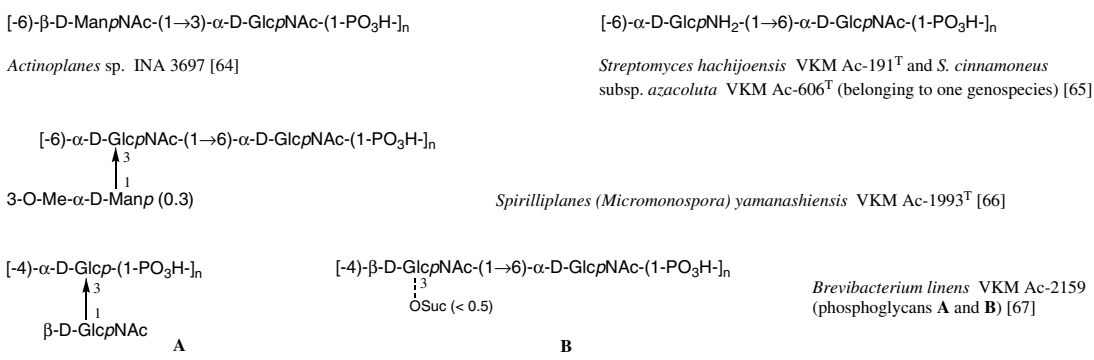


Chart 10. See Refs. 18, 19, 21, 22 and 61–67.

Streptococcus pneumoniae (the pneumococcus) is the most common etiological agent of bacterial pneumonia in humans throughout the world, resulting in high morbidity and mortality. All strains of pathogenic pneumococci are equipped with a layer of capsular glycopolymer, which was shown to be essential for virulence as it protects the bacterium from the non-specific immune defences of the host during infection.[81] In total, 90 type-specific capsular pneumococcal antigens (possessing distinct chemical structures; data given for 1999) that show serological reactivity are recognised.[72,82] Eight of these have poly(glycosyl phosphate)

Capsular antigens of *Streptococcus pneumoniae*

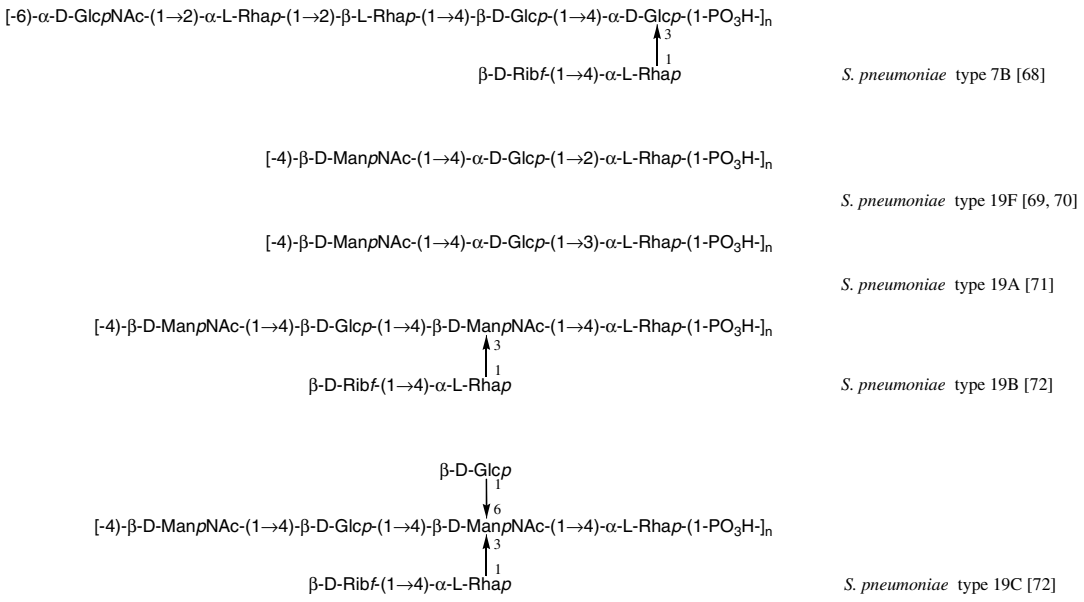
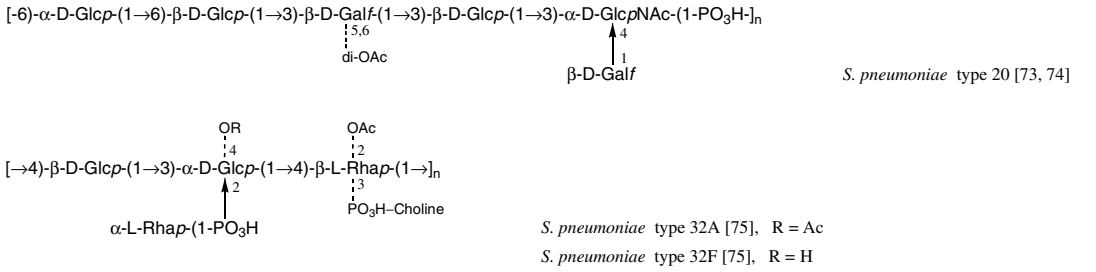


Chart 11. See Refs. 68–72.

Capsular antigens of *Streptococcus pneumoniae* (continued)



Cell-wall anionic polymers of *Streptococcus*

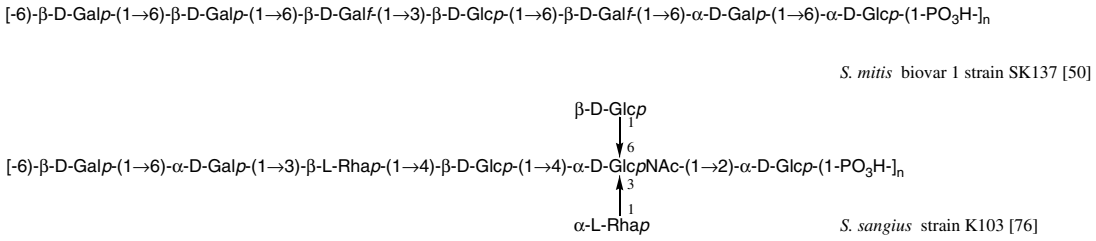


Chart 12. See Refs. 50 and 73–76.

structures (Charts 11 and 12),[68–75] including the most virulent *S. pneumoniae* types 19F and 19A (both capsule polymers are included in the 23-valent pneumococcal vaccine currently in use in North America). There are striking structural similarities among the pneumococcal capsules 19F,[69,70] 19A,[71] 19B and 19C.[72] All four of them possess an identical fragment α-L-Rha*p*-(1-PO_3H-4)-β-D-Man*p*NAc. The 19F and 19A structures differ only in the substitution position of the α-L-Rha*p* residue. The 19C polymer differs from the 19B polymer by the presence of an additional β-D-Glc*p* side chain. These structural homologies could provide a molecular basis

for the known strong serological cross-reactivity[72,81] within the group 19 *S. pneumoniae*.

Strains of *Streptococcus mitis*, *S. sangius*, *S. gordonii* and *S. oralis*, which occur in the human mouth, are also capable of synthesising complex poly(glycosyl phosphate) polymers (Charts 12 and 13). These structures[50,76–80] (often containing the β-D-Gal*f* residue) have some similarity to certain capsular antigens of pneumococci (e.g., *S. pneumoniae* type 20[73,74]), but the polymers are tightly associated with the cell wall and serve as receptors for lectins contained in pathogenic actinomicetes that colonise the tooth surface in co-aggregation with streptococci.[83,84]

A slime-forming *Lactococcus lactis* subsp. *cremoris* (previously known as *Streptococcus cremoris*) SBT 0495 that occurs in dairy products produces an extracellular phosphoglycan, which seems to be a part of the slime material.[85,86] This phosphoglycan (Chart 13) has a polysaccharide backbone and contains a glycosyl phosphate unit in the side chain.

By comparing the structures of the phosphoglycans from Gram-positive bacteria, one may conclude that cell-wall polymers from micrococci, staphylococci, bacilli and actinomicetes (Charts 9 and 10) have short and mostly linear mono-, di- and (in one case) tri-saccharide phosphate repeating units, while *C. symbiosum* HB25 synthesises a linear poly(tetrasaccharide phosphate). The cell-wall polymer of *Staphylococcus lactis* I3 bears D-alanine ester as a side chain. This substituent was found previously as a component of many teichoic and teichuronic acids.[51] The structure of the polymer itself (as well as that from *S. aureus* Z14, Chart 9) could be described as a hybrid of a poly(glycosyl phosphate) structure and a poly(glycerol phosphate) teichoic acid.

In capsular antigens of *S. pneumoniae* and in cell-wall polymers of other streptococci, the length of the building blocks varies from tri- to octa-saccharide phosphate and the molecules are mostly branched. Their side chains consist of mono- or di-saccharide units, ester residues or a glycosyl phosphate unit, as in the capsular antigens of *S. pneumoniae* types 32A and 32F (Chart 12). The two last structures, as well as the phosphoglycan from *L. lactis* subsp. *cremoris* SBT 0495 (Chart 13), differ from the poly(glycosyl phosphates) in that they contain a glycosyl phosphate unit in the side chain rather than in the main chain. There are a few rare sugars found in anionic glycopolymers, notably 2-acetamido-4-amino-2,4,6-trideoxy-D-glucopyranose (*N*-acetyl-D-bacillosamine in *C. symbiosum* HB25), D-ribofuranose (rare in polysaccharides) and D-galactofuranose; both of the latter sugars are present in streptococcal phosphoglycans.

Quite remarkably, D-mannose, commonly found in the phosphoglycans of yeasts and protozoan parasites (see Sections 2.3 and 2.4), has rarely been found as a component of bacterial phosphoglycans containing glycosyl phosphate residues. The only reported structure is the cell-surface phosphomannan from the Gram-negative bacterium *P. gingivalis*.[50]

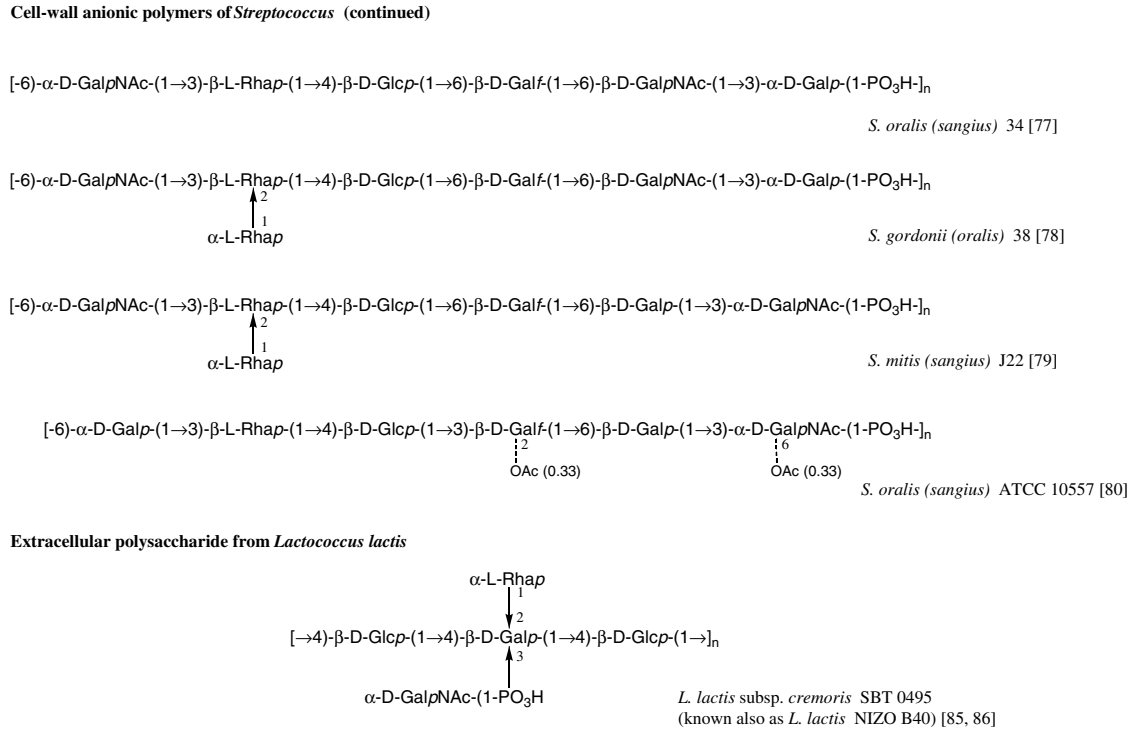


Chart 13. See Refs. 77–80, 85 and 86.

2.3. Cell-wall and extracellular phosphoglycans of yeasts

Mannans and phosphomannans (branched polymers of D-mannose) comprise 30–50% of the yeast cell wall or form an extracellular slime. Most of the phosphomannans have an α-(1→6)-linked backbone and side chains of various lengths with α-(1→2), α-(1→3) and, sometimes, β-(1→2) glycosidic linkages.[87–89] The α-D-Man*p*-(1-PO_3H-6)-α-D-Man*p* phosphodiester units are present in the side chains of the majority of the phosphomannans, but in *Hansenula capsulata* NRRL Y-1842 (see below) they form the backbone of the polymer instead.[90] Both α-D-Glc*p* 1-phosphate and α-D-Glc*p*NAc 1-phosphate residues are rarely found. Cell-wall phosphomannans often determine the antigenic specificity of the cells[95] and may provide surface specificity utilised in cell interaction during yeast mating.[94] Phosphomannans of the extracellular slime provide adhesive properties for yeast cells.[87–89]

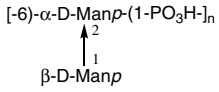
In contrast to bacterial phosphoglycans, only two examples were reported where yeast phosphomannans possess regular poly(glycosyl phosphate) structures (Chart 14). *H. capsulata* NRRL Y-1842 synthesises an extracellular poly(α-D-Man*p* phosphate) polymer bearing β-D-Man*p* side chains.[90] The yeast *Pichia* (*Hansenula*) *holstii* NRRL Y-2448 produces a viscous, highly branched extracellular phosphomannan consisting of a regular hexasaccharide-repeated core **A** and linear oligomannosyl phosphate structures X, which account for about 90% of the phosphomannan weight and form a part of the side-chain substituents.[91–93] Most of the structures X are composed of the poly(pentamannosyl phosphate) polymer **B** containing at least 10 repeating units ($n \sim 10$). In other side chains, the polymer **B** is replaced by the tetramannnosyl phosphate **C** and, to a much lesser extent, by hexa- (**D**), tri- (**E**) and di-saccharide (**F**) phosphates.

The structures of a few more phosphoglycans of yeasts are given in Chart 15. *Kloeckera brevis*[94,95] and *Saccharomices cerevisiae*[96,97] produce structurally similar branched cell-wall phosphomannans containing about 10 mol % of phosphate. A trisaccharide phosphodiester fragment, α-D-Man*p*-(1-PO_3H-6)-[α-D-Man*p*-(1→2)]-α-D-Man*p*, present in the side chain was shown to be the immunodominant group for the cell surface.[95] The yeast *Pichia pastoris* (widely used for the preparation of recombinant proteins) synthesises a cell-wall phosphomannan (11 mol % of phosphate) containing the glycosyl phosphosaccharide fragments α-D-Glc*p*-(1-PO_3H-6)-α-D-Man*p* and α-D-Glc*p*NAc-(1-PO_3H-6)-α-D-Man*p* in the side chains.[98] The ratio of D-Man:D-Glc:D-GlcNAc was found to be 30:3:(0.3–1). The phosphodiester fragment α-D-Glc*p*-(1-PO_3H-6)-α-D-Man*p* is also found in the cell-wall phosphomannan isolated from *Hansenula polymorpha*.[99] Both main and side chains of the polymer (primary structure is not depicted) contain α-(1→6)- and α-(1→2)-linked D-Man*p* residues. All polymer phosphates were present in the form of phosphodiester and the α-D-Glc*p* 1-phosphate unit (5.5 mol %) was, in turn, shown to be an important immunochemical determinant.

The extracellular phosphogalactomannan of *Candida mucifera* CCY 29-170-1 (14 mol % of phosphate) has a standard structure (Chart 15) with α-D-Man*p*-(1-PO_3H-6)-α-D-Man*p* fragments in the side chains.[100] Additional β-D-Gal*p* residues (12 mol %) occupy

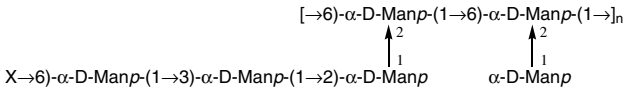
Extracellular phosphomannans of yeasts

Hansenula capsulata NRRL Y-1842 [90]



Pichia (Hansenula) holstii NRRL Y-2448 [91-93]

A



B [-6)-α-D-Man*p*-(1→3)-α-D-Man*p*-(1→3)-α-D-Man*p*-(1→3)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1-PO_3H-]$_n$

C α-D-Man*p*-(1→3)-α-D-Man*p*-(1→3)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1-PO_3H-

D α-D-Man*p*-(1→3)-α-D-Man*p*-(1→3)-α-D-Man*p*-(1→3)-α-D-Man*p*-(1→3)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1-PO_3H-

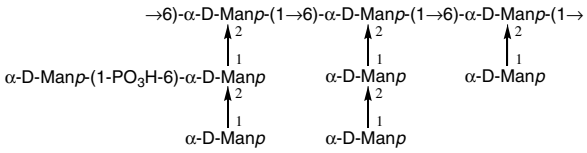
E α-D-Man*p*-(1→3)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1-PO_3H-

F α-D-Man*p*-(1→2)-α-D-Man*p*-(1-PO_3H-

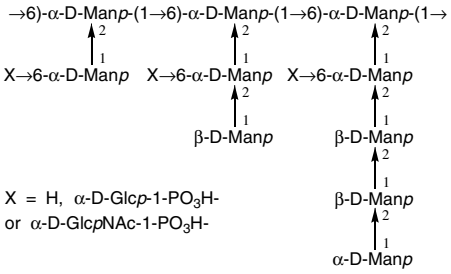
} = X

Chart 14. See Refs. 90–93.

Cell-wall phosphomannans of yeasts

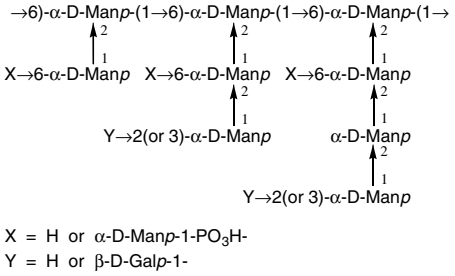


Kloeckera brevis [94, 95] and *Saccharomyces cerevisiae* [96, 97]



Pichia pastoris [98]

Extracellular phosphogalactomannan of *Candida mucifera* CCY 29-170-1 [100]



Extracellular phosphogalactan of *Sporobolomyces* spp NRRL Y-6493 and NRRL Y-6502 [101]

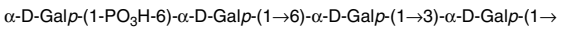


Chart 15. See Refs. 94–98, 100 and 101.

terminal positions on some D-mannose side chains. The structures of the extracellular phosphogalactans of *Sporobolomyces* spp. NRRL Y-6493 and NRRL Y-6502 (10 mol % of phosphate, 90 mol % of D-Gal) were shown[101] to contain polymer phosphate exclusively in the form of α-D-Gal*p*-(1-PO_3H-6)-α-D-Gal*p* phosphodiester units at the non-reducing end (only a partial structure of the polymer was presented in the paper).

2.4. Cell-surface and secreted (extracellular) phosphoglycans of *Leishmania* protozoan parasites

Leishmania are a genus of sandfly transmitted protozoan parasites that cause a spectrum of debilitating and often fatal diseases in humans throughout the tropics and subtropics. During their life cycle, which involves a promastigote stage(s) in the midgut of their insect vector (the sandfly) and an amastigote stage in the phagolysosomes of the mammalian macrophage, *Leishmania* survive and proliferate in highly hostile environments. Their survival strategies involve the formation of an elaborate and dense cell-surface glycocalyx composed of diverse stage-specific glycoconjugates that form a protective barrier.[102,103] Some of these macromolecules (including various phosphoglycans) were shown to be essential for virulence of the parasite.[104,105]

Lipophosphoglycan (LPG) is a predominant cell-surface glycoconjugate of *Leishmania* promastigotes. It contains a poly(glycosyl phosphate) structure consisting of galactosylmannnosyl phosphate repeating units (Chart 16), where the nature of the X and Y substituents varies according to the species. *L. donovani*[102,106] synthesises a linear phosphoglycan, whereas those of *L. mexicana*,[107,108] *L. major*[109–111] and *L. aethiopica* have branched structures bearing non-stoichiometric mono- and/or di-saccharide components (X) linked at *O*-3 of the β-D-Gal*p* residue. In *L. major*, the composition of the side chains varies and depends on the developmental stage of the parasite. In the LPG produced by *L. aethiopica*,[112] there are also some additional α-D-Man*p* residues (Y) linked to D-mannose residues in the main chain. All LPG molecules are capped with a mannobiosyl phosphate unit at the non-reducing terminus and contain a glycan core linked to an inositolphospholipid anchor[102] at the reducing end of the chain.

In addition to cell-surface LPG, *Leishmania* promastigotes produce a number of secreted glycoproteins, where a peptide core is modified (glycosylated) with LPG-type phosphoglycans.[114,115] *L. donovani* secretes an acid phosphatase (sAP) in which the peptide chains are heavily glycosylated on C-terminal serine/threonine domains. The glycans (in fact, phosphoglycans) consist of the same disaccharide phosphate repeating units found in the corresponding LPG (Chart 16) and are phosphodiester-linked to select serine residues.[103] All species of *Leishmania* promastigotes synthesise secreted, filamentous proteophosphoglycan (fPPG), which forms a highly viscous gel surrounding the parasite cells. Compositionally, fPPG consists of 95% phosphoglycans in a species-specific manner (Chart 16). The poly(glycosyl

Phosphoglycan region of lipo- and proteo-phosphoglycans of *Leishmania*

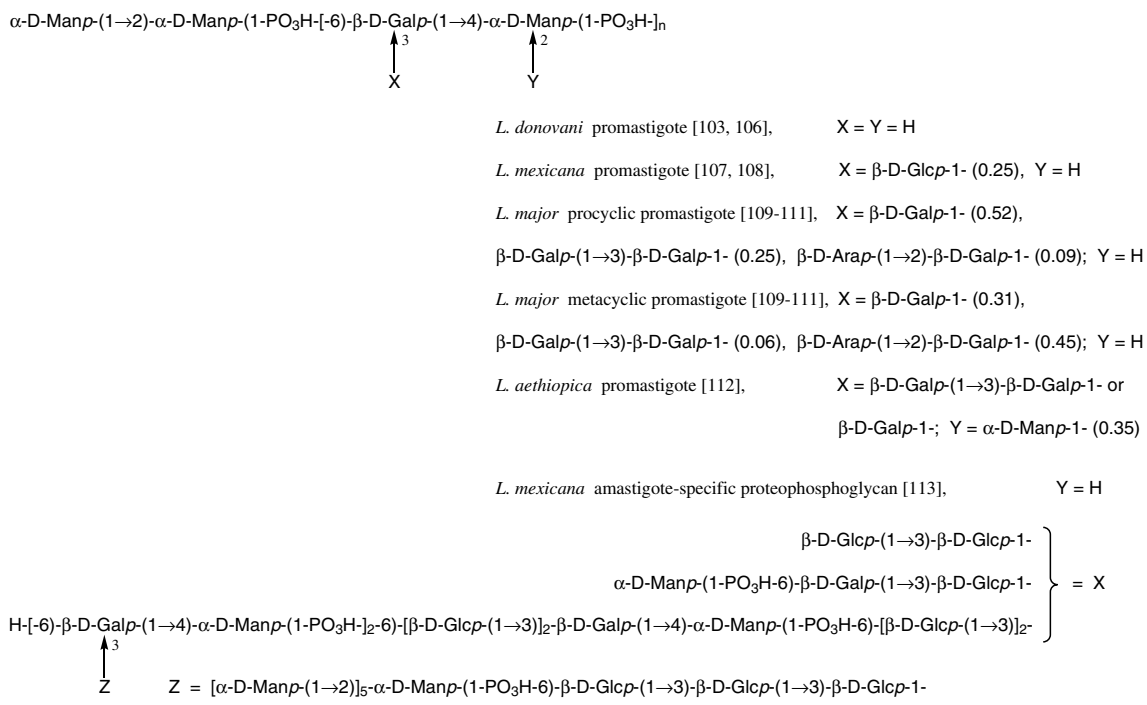


Chart 16. See Refs. 103 and 106–113.

phosphate) chains are attached to serine residues of the peptide component *via* phosphodiester groups.

Hydrophilic phosphoglycans have been found in culture supernatants of *Leishmania* promastigotes and have been structurally characterised for *L. major*,[116] *L. mexicana*[117] and *L. donovani*.[118] They are essentially the corresponding LPG molecules without the glycan core-inositolphospholipid region.

L. mexicana amastigotes synthesise large amounts of a macromolecular amastigote-specific proteophosphoglycan (aPPG) and secrete it (in mg/ml concentrations!) into the phagolysosome of the mammalian macrophage.[113] Some phosphoglycan chains are similar to those found in *L. mexicana* LPG, but the majority represent novel stage-specific highly branched structures (Chart 16), including (1→3)-linked glucobiose and glucotriose moieties and long phosphorylated side chains. The phosphoglycans are attached to serine residues of the protein backbone, most likely through the phosphate groups. The aPPG is believed to activate the complement system *via* the mannose-binding pathway. It may also contribute to the binding of *Leishmania* to host cells and play a role in modulation of the biology of the infected macrophage.[103]

2.5. Glycosyl phosphosaccharide fragments of glycan chains in animal glycoproteins

Two glycosyl phosphosaccharide structures were identified in the glycoproteins of animals. The phosphodiester α-D-Glc*p*-(1-PO_3H-6)-α-D-Man*p* was found to be a terminal fragment of high-mannose type oligosaccharide chains in some plasma membrane glycoproteins (e.g., retinal ligatin in chicken[119,120] and a 62-kDa cytoplasmic glycoprotein in rat liver).[121] Allegedly, the glycoproteins function as baseplates for recognition and attachment of peripheral glycoproteins to the cell surface by binding with glycan chains terminating in α-D-Glc*p* 1-phosphate.

The phosphodiester α-D-Glc*p*NAc-(1-PO_3H-6)-α-D-Man*p* was found to be a component of a number of lysosomal enzymes (hydrolases) and intermediates in the biosynthesis in mammals of glycoproteins containing a D-mannose 6-phosphate residue, which works as a marker for the translocation of these enzymes to lysosomes.[122–124] The D-Man*p* 6-phosphate marker is formed by the concerted action of two enzymes: α-D-Glc*p*NAc-phosphate transferase[125,126] (which transfers α-D-Glc*p*NAc 1-phosphate *en block* from UDP-Glc*p*NAc to a D-Man*p* acceptor) and α-D-Glc*p*NAc phosphodiesterase[127,128] (working *via* an α-*N*-acetylglucosaminidase mechanism[129] and releasing the phosphoric monoester). The uncovering of D-Man*p* 6-phosphate residues appeared to be critical for the selective transport of the hydrolases to lysosomes.[130]

2.6. Analysis of glycosyl phosphosaccharide structures revealed in natural phosphoglycans

Not surprisingly, phosphoglycans isolated from bacterial cell walls and capsules have highly diverse

monosaccharide representation. Similar diversity is typical for bacterial glycopolymers/polysaccharides in general.[3,6,29–31] On analysing the glycosyl phosphate units (as a part of the glycosyl phosphosaccharide fragment), α-D-Glc*p*NAc 1-phosphate appears to be the most widely represented as it was found in 21 out of the 64 bacterial phosphoglycans (in total) listed above (Sections 2.1 and 2.2). α-D-Glc*p* 1-phosphate has 15 entries, followed by α-D-Gal*p*NAc 1-phosphate (nine entries), α-D-Gal*p* 1-phosphate (eight entries) and α-L-Rha*p* 1-phosphate (six entries). Amongst the phosphosaccharide units (i.e., sugars phosphorylated at either position 2, 3, 4 or 6), α-D-Glc*p*NAc 6-phosphate (seven entries), α-D-Glc*p* 6-phosphate (five entries), α-D-Glc*p*NAc 4-phosphate (four entries) and β-D-Man*p*NAc 4-phosphate (four entries) seem to be represented more than any others. Two glycosyl phosphosaccharide structures that appear most often in bacterial phosphoglycans are α-D-Glc*p*NAc-(1-PO_3H-6)-α-D-Glc*p*NAc and α-L-Rha*p*-(1-PO_3H-4)-β-D-Man*p*NAc (4 entries each), closely followed by α-D-Glc*p*NAc-(1-PO_3H-6)-α-D-Glc*p* and α-D-Glc*p*-(1-PO_3H-6)-α-D-Glc*p*N[(*R*)-3-hydroxybutyryl] (3 entries each).

The same sort of analysis performed for yeast phosphoglycans (eight structures in total; Section 2.3) revealed α-D-Man*p*-(1-PO_3H-6)-α-D-Man*p* as the most frequently found glycosyl phosphosaccharide unit, whereas in the phosphoglycans from *Leishmania* parasites (five basic structures in total; Section 2.4) it was α-D-Man*p*-(1-PO_3H-6)-β-D-Gal*p*.

It is strikingly evident that the overwhelming majority of glycosyl phosphate units found in natural phosphoglycans from various sources has an α-D- or α-L-hexopyranose configuration. This includes the 1-phosphates of α-D-glucopyranose, α-D-galactopyranose, α-D-mannopyranose, 2-acetamido-2-deoxy-α-D-glucopyranose, 2-acetamido-2-deoxy-α-D-galactopyranose, 2-acetamido-2-deoxy-α-D-mannopyranose, α-L-rhamnopyranose and 6-deoxy-α-L-*gulo*-heptopyranose. In all of these structures (including the last one[47b]), the phosphate group occupies an axial position at C1, and is favoured by the anomeric effect.[131]

To date, only one glycofuranosyl phosphate structure [6-deoxy-α-L-*galacto*-heptofuranose 1-phosphate in the extracellular poly(glycosyl phosphate) from *C. lari* strain PC 637][47a] was reported in the phosphoglycans. There are also only two reports wherein β-D-hexopyranosyl phosphate units were identified in the glycopolymers. β-D-Glucopyranose 1-phosphate was shown to be a side-chain terminal unit in the cell-wall phosphoglycan (containing D-Glc, D-Gal, D-GalNAc and L-Rha) of group D *Streptococcus*, strain N.[132] Similarly, 2-acetamido-2-deoxy-β-D-glucopyranose 1-phosphate was found as a side-chain immunodeterminant in the cell-wall phosphoglycan (containing D-GlcNAc, D-Gal, D-GalNAc and L-Rha) isolated from group L *Streptococcus*, strain D 167A.[133] In both cases, however, complete elucidation of the phosphoglycan structures was not achieved. No NMR data of the glycosyl phosphate units were reported, but anomeric configurations were assigned using enzymatic, chemical and immunochemical methods instead.

3. Chemical synthesis of glycosyl phosphosaccharides

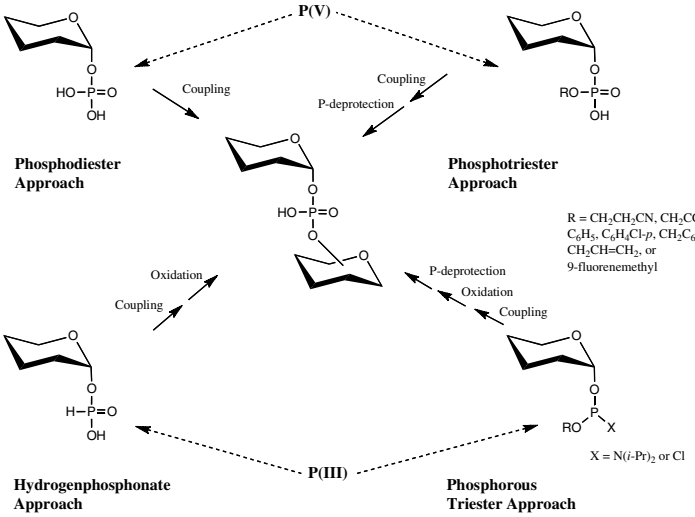
To date, major efforts have been directed towards the preparation of glycosyl phosphosaccharide structures with α-D- or α-L-hexopyranosyl phosphate constituents. There are reports on the synthesis of fragments of natural phosphoglycans and model phosphodiesters containing the 1-phosphates of α-D-mannopyranose, α-D-glucopyranose, α-D-galactopyranose, 2-acetamido-2-deoxy-α-D-glucopyranose, 2-acetamido-2-deoxy-α-D-mannopyranose, 2-acetamido-2-deoxy-α-D-galactopyranose, α-D-rhamnopyranose, α-L-rhamnopyranose and α-L-fucopyranose.

Five basic synthetic methodologies have been used for the preparation of glycosyl phosphosaccharides, embracing phosphodiester, phosphotriester, phosphorous (or phosphite) triester (including phosphoramidite and phosphorochloridite versions) and hydrogenphosphonate (H-phosphonate) approaches and another based on the glycosylation reaction. The first four methods (Scheme 1) were originally designed for nucleotide chemistry and involve the condensation of various P-containing glycosyl components (electrophiles) with alcohol monosaccharide derivatives (nucleophiles). The last one (see Section 3.4) is a solely carbohydrate method as it involves the reaction of a glycosyl donor (electrophile) with a P-containing glycosyl acceptor (nucleophile).

The phosphodiester approach was developed for nucleotide chemistry in the mid-1950s by Khorana's group[134,135] and dominated the field for 20 years.[136] The phosphotriester[137] and H-phosphonate[138] methods were pioneered by Todd's group at the same time. These methods were re-introduced later in more effective modifications by Narang and co-workers[139] and Catlin and Cramer[140] (phosphotriester) in 1973 and Garegg et al.,[141–143] Stawinski and Strömberg[144] and Froehler and Matteucci[145] (H-phosphonate) in the mid-1980s. In 1981, Beaucage and Caruthers[146] and McBride and Caruthers[147] discovered the phosphoramidite method, which became widespread and popular because of its efficiency and simplicity; it was subsequently developed for the automated synthesis of oligonucleotides.[148]

3.1. Phosphoric diester and phosphoric triester methods [P(V) chemistry]

3.1.1. Phosphodiester method. This is the simplest method in which a protected glycosyl phosphate is coupled with a monohydroxyl (or, in some cases, a dihydr-

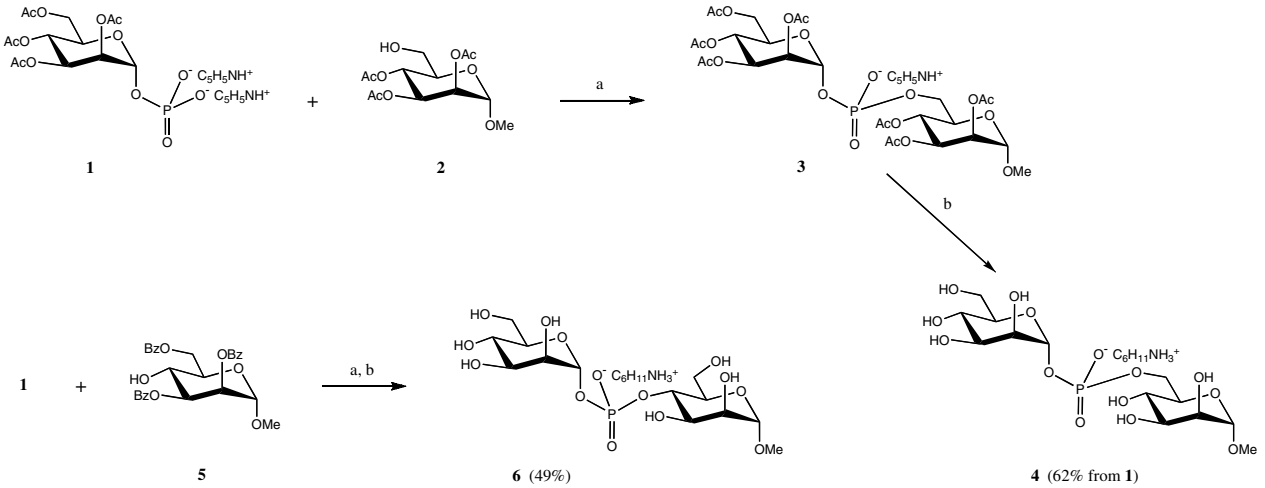


Scheme 1.

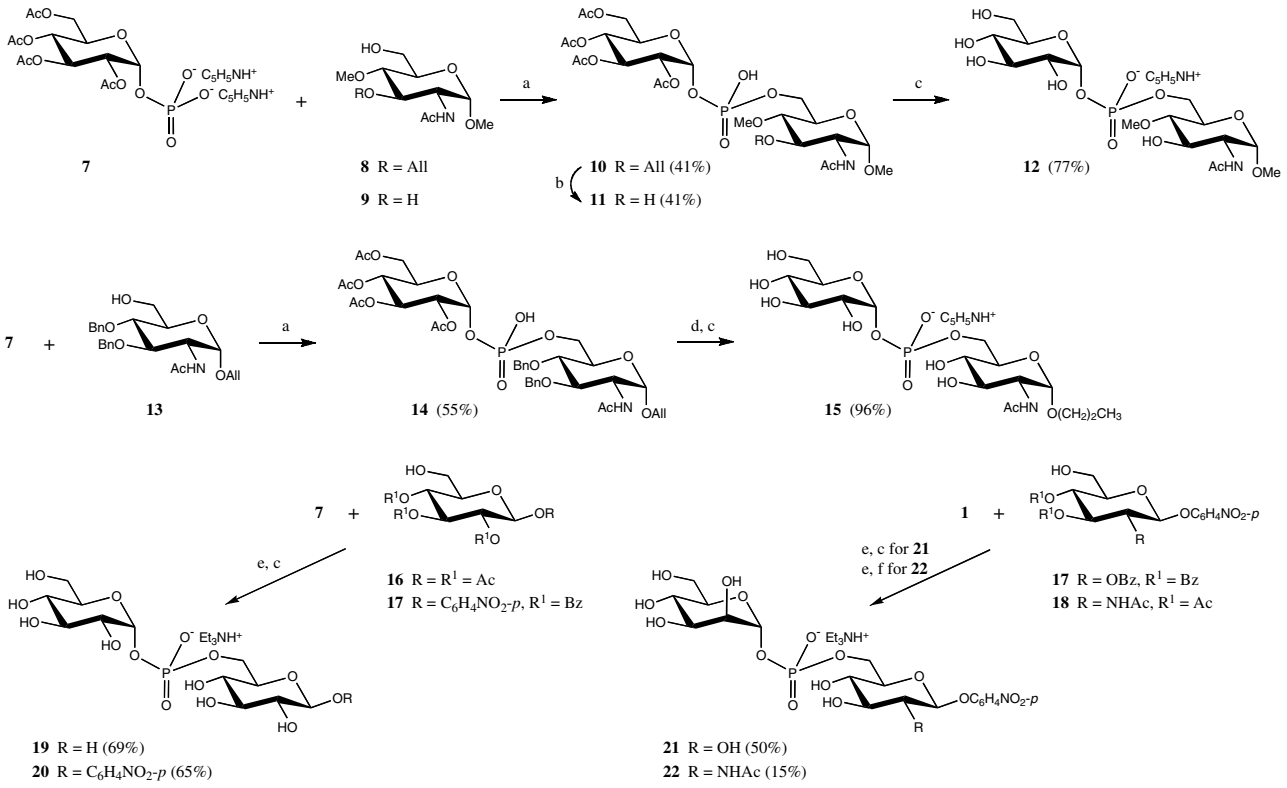
oxyl) carbohydrate derivative in the presence of a condensing reagent. The condensing reagent used was either dicyclohexylcarbodiimide (DCC), 2,4,6-triisopropylbenzenesulfonyl chloride (TPS-Cl), or 3-nitro-1-(2,4,6-triisopropylbenzenesulfonyl)-1,2,4-triazole (TPS-NT).

The first chemical synthesis of glycosyl phosphosaccharides was described in 1971 by Cawley and Letters.[149] The reaction between the acetylated mannosyl phosphate **1** and the 6-OH derivative **2** in pyridine in the presence of DCC (Scheme 2) provided the protected phosphodiester **3**, which was deacetylated without purification by adding aq NaOH. The (1→6)-linked mannosyl phosphomannoside **4** was isolated by precipitation in 62% yield. The (1→4)-linked mannosyl phosphodiester **6** (49%) was synthesised from phosphate **1** and the 4-OH compound **5** in a similar manner. It was reported that a large excess of DCC (5–10 equiv) is essential to minimise side reactions and to obtain the best results in the condensation step. This work was performed in the research laboratory of the world-renowned beer manufacturer Guinness & Co. (Dublin). The unusual source of the publication could be explained by the fact that the (1→6)-linked phosphodiester **4** has a clear brewing-related origin: it represents the terminal immunodominant fragment of the cell-wall phosphomannans from the yeasts *S. cerevisiae* and *K. brevis* (Section 2.3). The phosphodiester was found also as an internal fragment of the extracellular phosphomannans from the yeasts *Hansenula holstii* Y-2448 and *H. capsulata* Y-1842 (Section 2.3).

A few years later, Jeanloz and co-workers[150] reported the preparation of the *N*-acetyl-D-glucosamine 6-(α-D-glucopyranosyl phosphate) glycosides **12** and **15** (Scheme 3). The condensation of the acetylated glucosyl phosphate **7** with the 6-OH derivatives **8** or **13** in the presence of TPS-Cl gave the protected (1→6)-linked phosphodiesters **10** (41%) and **14** (55%), respectively, which were isolated by preparative TLC on silica gel. Cleavage of the 3-*O*-allyl protecting group from **10**



Scheme 2. Reagents and conditions: (a) DCC (9 equiv), pyridine, 37 °C; (b) (i) aq NaOH, pyridine, pH 11; (ii) AG 50W (H^+); (iii) $C_6H_{11}NH_2$.



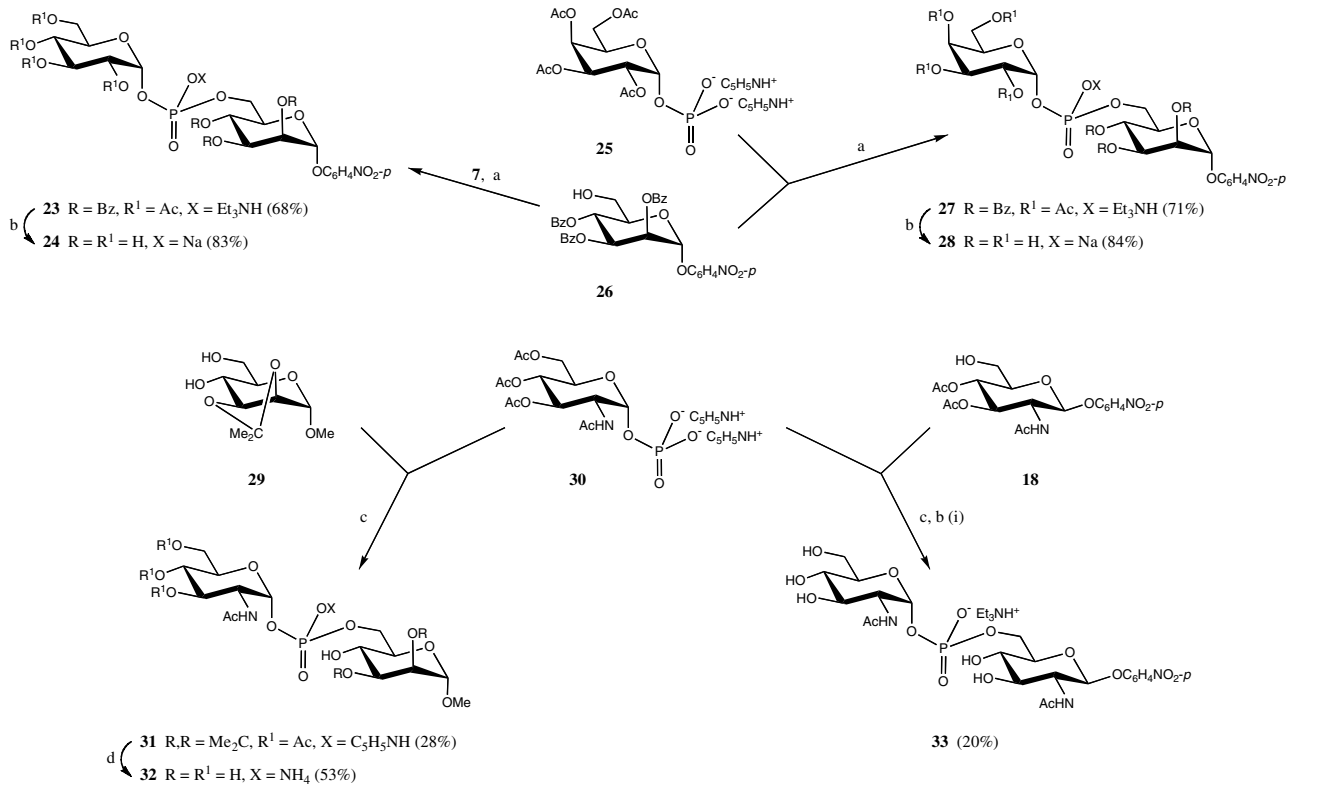
Scheme 3. Reagents and conditions: (a) TPS-Cl, pyridine; (b) (i) $(Ph_3P)_3RhCl$, aq ethanol, 77 °C; (ii) $HgCl_2$, aq acetone; (c) (i) NaOMe, MeOH; (ii) AG 50W or Dowex 50W ($C_5H_5NH^+$); (d) H_2, Pd/C, MeOH; (e) DCC (8 equiv), pyridine; (f) Et_3N–MeOH–water (1:3:1).

afforded the phosphodiester **11** (41%). It was also prepared by direct coupling of glycosyl phosphate **7** and the 3,6-diol derivative **9**, which proceeded regioselectively in 41% yield. Deacetylation of compound **11** with methanolic NaOMe provided phosphodiester **12** (77%). Glycosyl phosphosaccharide **15** was prepared from **14** by consecutive debenzylation (H_2, Pd/C) and deacetylation in 96% yield. Glycosyl phosphates **1** and **7** were also used by Shibaev's group[151,152] for the synthesis of the (1→6)-linked phosphodiesters **19**–**22**. The glycophosphorylation of the 6-OH derivatives **16**–**18** with phosphates **1** or **7** in the presence of DCC (as it is listed on Scheme 3), followed by deacylation, gave the required compounds **19**–**21** in 50–69% yield (isolated by ion-exchange chromatography) and phosphodiester **22** in a meagre 15% yield.

The phosphodiester methodology was applied to the chemical syntheses of some biologically important glycosyl phosphosaccharides. The phosphodiester α-D-Glc*p*-(1-PO_3H-6)-α-D-Man*p* **24** is found as a terminal fragment of high-mannose type oligosaccharide chains of some plasma membrane glycoproteins in animals and is allegedly involved in recognition/localisation processes (Section 2.5). It also represents the immunochemical determinant of the phosphomannan from the yeast *H. polymorpha* 52–251 (Section 2.3). Compound **24** was synthesised[153] by condensation of derivatives **7** and **26** in the presence of TPS-NT (→**23**; Scheme 4), followed by deacylation with Et_3N in aq methanol. Phosphodisaccharide **28**, the galactosyl phosphate analogue of **24**, was synthesised from compounds **25** and **26** in a similar manner. The yields for both condensation and deprotection steps were quite high: the protected phosphodiesters **23** (68%) and **27** (71%) were isolated by standard flash-column chromatography on SiO_2, while the deprotected compounds **24** (83%) and **28** (84%) were isolated by gel-permeation chromatography using a Bio-Gel P-2 column.

The phosphodiester α-D-Glc*p*NAc-(1-PO_3H-6)-α-D-Man*p* **32**, a component of a number of lysosomal enzymes and intermediates in the biosynthesis of glycoproteins (in mammals) containing a D-mannose 6-phosphate residue (Section 2.5), was prepared[154] from *N*-acetyl-D-glucosamine 1-phosphate **30** and diol **29** by means of their coupling in the presence of DCC [which produced regioselectively the (1→6)-linked derivative **31** only], followed by deprotection. Similarly, glycosyl phosphate **30** and the 6-OH derivative **18** were used for the synthesis of phosphodiester **33**,[155] which represents an internal fragment of the cell-wall polymer of the Gram-positive bacterium *Micrococcus varians* ATCC 29750 (Section 2.2).

To summarise, the phosphodiester approach was historically the first method used for the successful synthe-



Scheme 4. Reagents and conditions: (a) (i) TPS-NT, pyridine, 37 °C; (ii) Dowex 50W (H^+); (iii) Et_3N; (b) (i) Et_3N–MeOH–water (1:2:1); (ii) Dowex 50W (Na^+); (c) DCC, pyridine; (d) (i) aq TFA, $CHCl_3$; (ii) NH_3, MeOH.

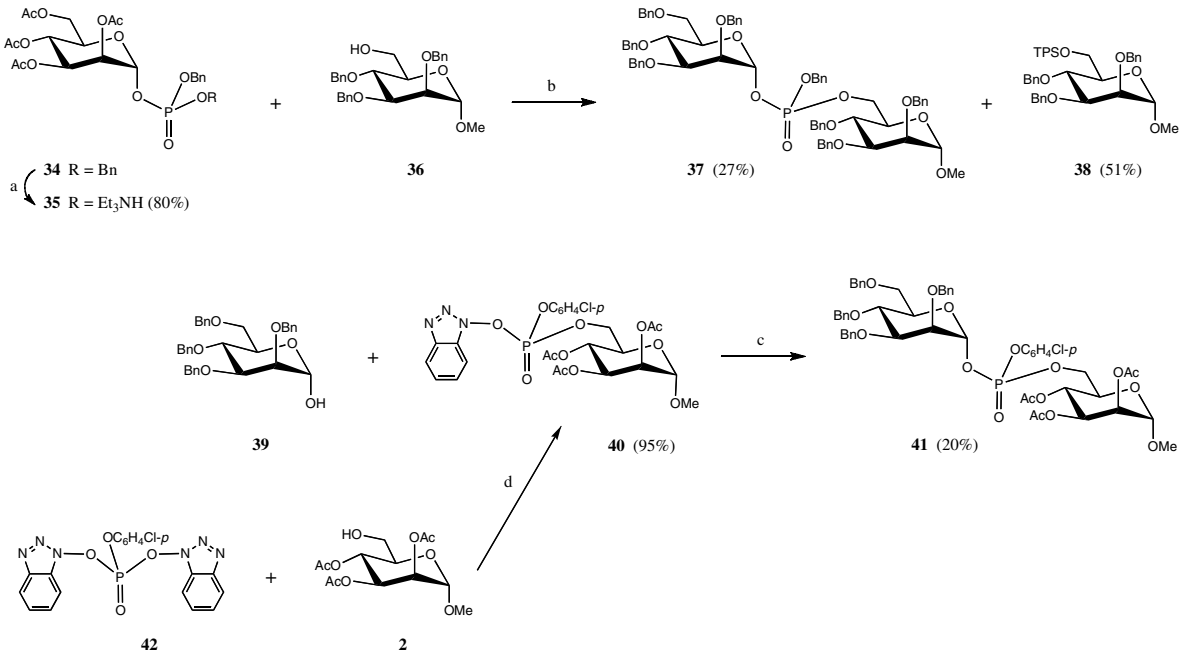
sis of glycosyl phosphosaccharides. Most of the prepared compounds contained (1→6)-phosphodiester linkages [with one exception: the (1→4)-linked diester **6**]. The yields of final products (calculated on the condensation and deprotection steps) ranged from 69% to 15% for the esters of α-D-Man*p* 1-phosphate, α-D-Glc*p* 1-phosphate and α-D-Gal*p* 1-phosphate, and seemed to be invariably low for the derivatives of α-D-Glc*p*NAc 1-phosphates **32** (15%) and **33** (20%). The coupling reactions in the presence of DCC or TPS-Cl proceeded slowly and took from 2 to 10 days to complete. By using TPS-NT, the condensation time was reduced to 7 h. The protected glycosyl phosphates **1**, **7**, **25** and **30** were prepared either using glycosylation reactions (see Section 3.4) or by acetylation of commercially available glycosyl phosphates.

3.1.2. Phosphotriester method. The phosphotriester approach varies from the phosphodiester by the presence of an extra P-protecting group on the glycosyl phosphate component, which should be prepared prior to its coupling with a monohydroxyl sugar derivative. The use of this method in nucleotide chemistry resulted in significant increases in the yields for the condensation step and paved the way for the first solid-phase synthesis of oligodeoxyribonucleotides.[156] By contrast, the phosphotriester approach to glycosyl phosphosaccharides appeared to be less effective and was limited to the synthesis[157] of two P-protected glycosyl phosphodiesters **37** and **41** (Scheme 5). The first synthesis used coupling of benzyl 2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl phosphate **35** with the partly protected mannoside **36** in the presence of TPS-Cl and *N*-methylimidazole (a nucleophilic catalyst). The yield of phosphotriester **37** was low (27%; as a diastereoisomeric mixture) and the 6-*O*-(2,4,6-triisopropylbenzenesulfonyl)-D-mannoside derivative **38** (51%) was isolated as the main (but undesired) product. The P-protected glycosyl phosphate **35**, in turn, was made from the corresponding glycosyl trichloroacetimidate *via* its transformation to the glycosyl dibenzyl phosphate **34**,[158] followed by P-monodeprotection with NaI (anionic debenzylation).

The second synthesis utilised the hemiacetal derivative **39** for the coupling with the activated benzotriazolyl ester of the mannoside 6-(*p*-chlorophenyl)-phosphate **40** (synthesised by the reaction of the alcohol derivative **2** with the phosphorylating reagent **42**[159]), but again produced the desired phosphotriester **41** in poor yield (20%; as a diastereomeric mixture).

3.2. Phosphorous triester (phosphite triester) methods [P(III) chemistry]

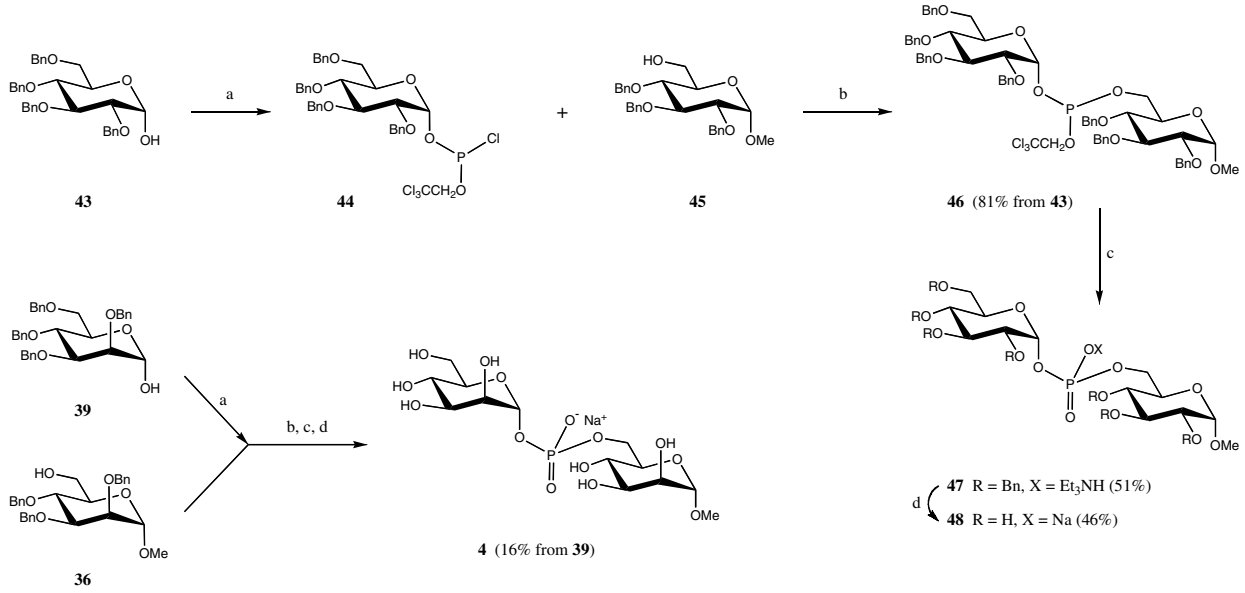
3.2.1. Phosphorochloridite method. The first use of P(III) compounds to form glycosyl phosphites and glycosyl phosphosaccharides was reported by Ogawa and



Scheme 5. Reagents and conditions: (a) (i) NaI, acetone, 60 °C; (ii) Amberlite IR-120 (Et_3NH^+); (b) TPS-Cl, *N*-methylimidazole, pyridine; (c) *N*-methylimidazole, pyridine, 37 °C; (d) pyridine, THF, 37 °C.

Seta.[160] The preparation of glycosyl phosphites (H-phosphonates) from 2,3,4,6-protected hemiacetal derivatives was performed using PCl_3 as the phosphitylating reagent, followed by hydrolysis. This paper also demonstrates the application of 2,2,2-trichloroethyl phosphorodichloridite, as shown in Scheme 6. The reagent was used firstly to phosphitylate glucose hemiacetal **43**, thus forming glycosyl phosphorochloridite **44**, which then provided phosphorous triester **46** (81%) by its reaction with the monosaccharide derivative **45**.

Triester **46** was then oxidised (O_2, AIBN) and P-deprotected, using a Zn–Cu couple in the presence of 2,4-pentanedione, to produce the phosphodiester **47** (51%). Subsequent debenzylation (Birch reduction) gave methyl α-D-glucopyranoside 6-(α-D-glucopyranosyl phosphate) **48** (46%; 19% based on **43**). By employing



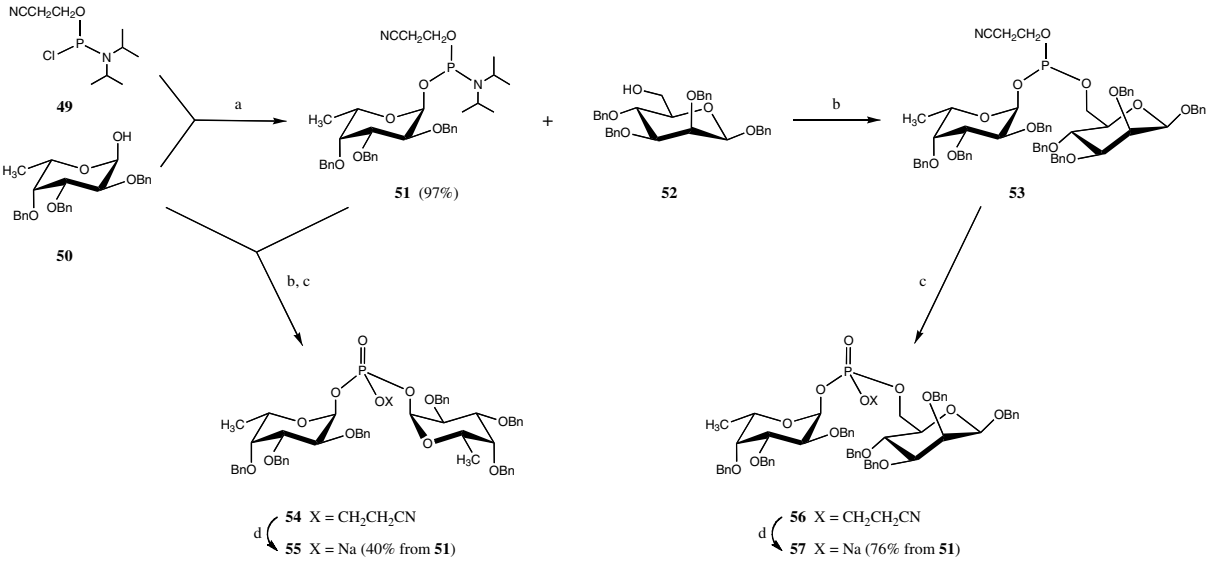
Scheme 6. Reagents and conditions: (a) $CCl_3CH_2OPCl_2$, $(i\text{-Pr})_2NEt$, THF, −78 °C; (b) $(i\text{-Pr})_2NEt$, THF, −78 °C; (c) (i) O_2, AIBN, benzene, 70 °C; (ii) Zn–Cu, 2,4-pentanedione, Et_3N; (d) Na, NH_3, EtOH.

2,3,4,6-tetra-*O*-benzyl-α-D-mannose **39** and methyl 2,3,4-tri-*O*-benzyl-α-D-mannoside **36**, the same reaction sequence afforded methyl α-D-mannopyranoside 6-(α-D-mannopyranosyl phosphate) **4** in 16% overall yield.

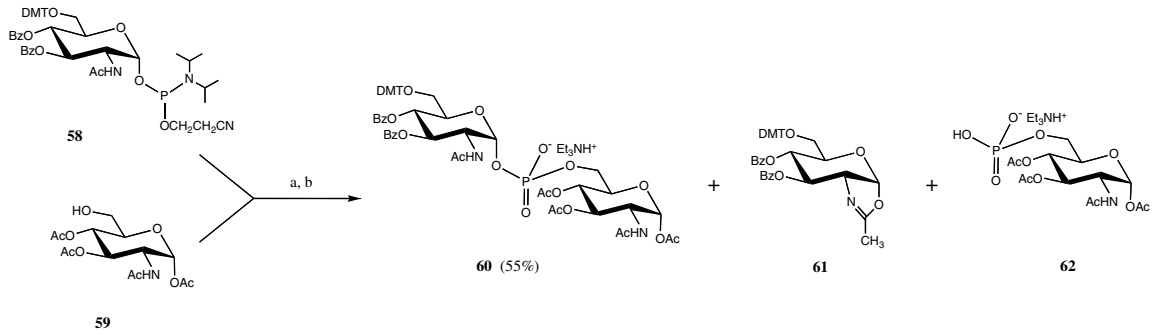
3.2.2. Phosphoramidite method. The use of P(III) compounds was continued and developed with the introduction of the phosphoramidite method by van Boom's group.[161] They used 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite **49** to form glycosyl phosphoramidite **51** from L-fucose hemiacetal derivative **50** in 97% yield (Scheme 7). The phosphoramidite was then converted (nearly completely; monitored by ^{31}P NMR) to phosphorous triester **53** by reaction with mannoside **52** in the presence of 1*H*-tetrazole. Compound **53** was subsequently oxidised (*tert*-BuOOH; →**56**, as a diastereoisomeric mixture) and P-deprotected (NH_3) producing the (1→6)-linked phosphodiester **57** in 76% yield (based on compound **51**). The (1→1)-linked phosphodiester **55** (40%) was also made by the same method. Highly efficient formation of phosphotriester **54** (made by coupling compounds **51** and **50**, followed by oxidation) was monitored by ^{31}P NMR, but ammonolysis of the cyanoethyl group from triester **54** was accompanied by partial cleavage of the glycosyl phosphate linkage.

Additional problems surfaced in the application of the phosphoramidite method to derivatives of *N*-acetyl-D-glucosamine.[162] It was found that the synthesis of phosphodiester **60** (Scheme 8) by coupling glycosyl phosphoramidite **58** and compound **59**, followed by oxidation and P-deprotection (Et_3N), occurred in only 55% yield. The moderate yield was explained by partial cleavage of the glycosyl phosphate linkage in the intermediate phosphotriester that might have been facilitated by 2-acetamido group participation,[163] leading to the formation of the oxazoline derivative **61** and 6-phosphate **62**.[162]

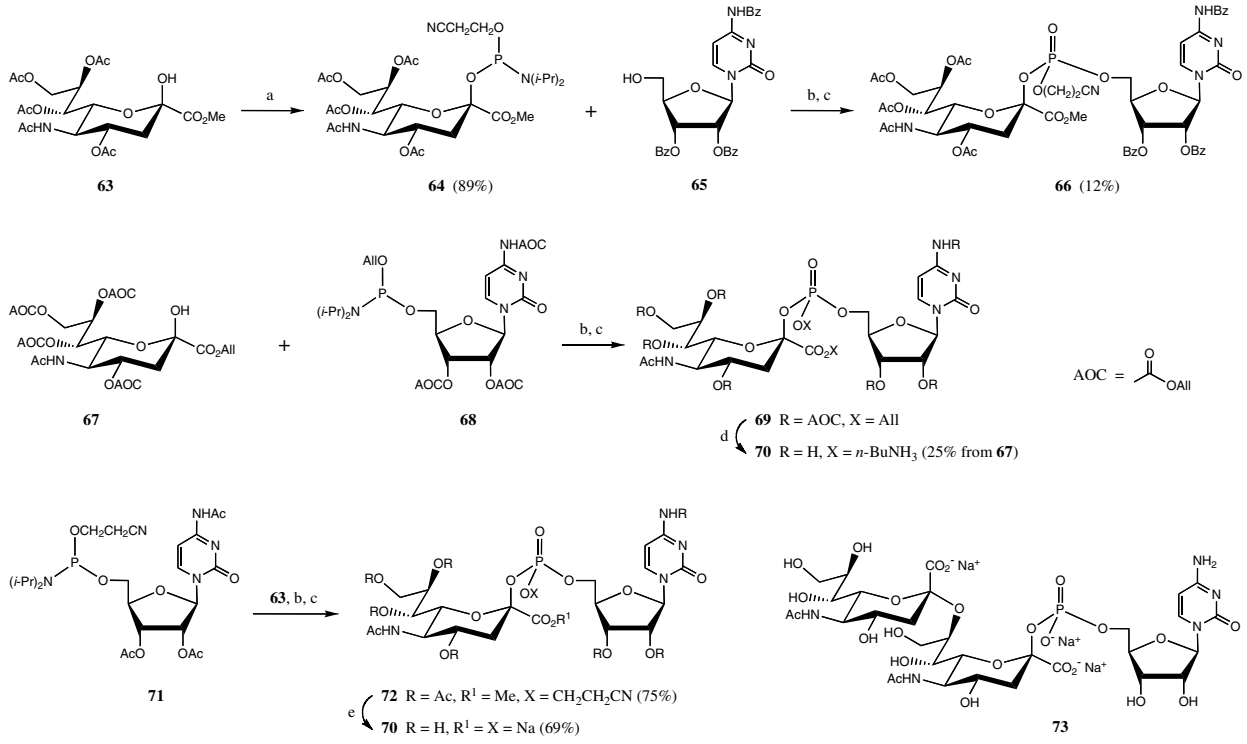
The phosphoramidite method was applied to chemical syntheses of the biologically important nucleotide sugar cytidine-5′-monophospho-*N*-acetylneuraminic acid (CMP-Neu5Ac) **70** (Scheme 9). First, Wong and coworkers[164] reported the preparation of the sialyl



Scheme 7. Reagents and conditions: (a) (*i*-Pr)$_2$NEt, DCM; (b) 1*H*-tetrazole, CH_3CN; (c) *tert*-butylhydroperoxide; (d) NH_3, MeOH.



Scheme 8. Reagents and conditions: (a) 1*H*-tetrazole, CH_3CN; (b) *tert*-butylhydroperoxide, Et_3N.



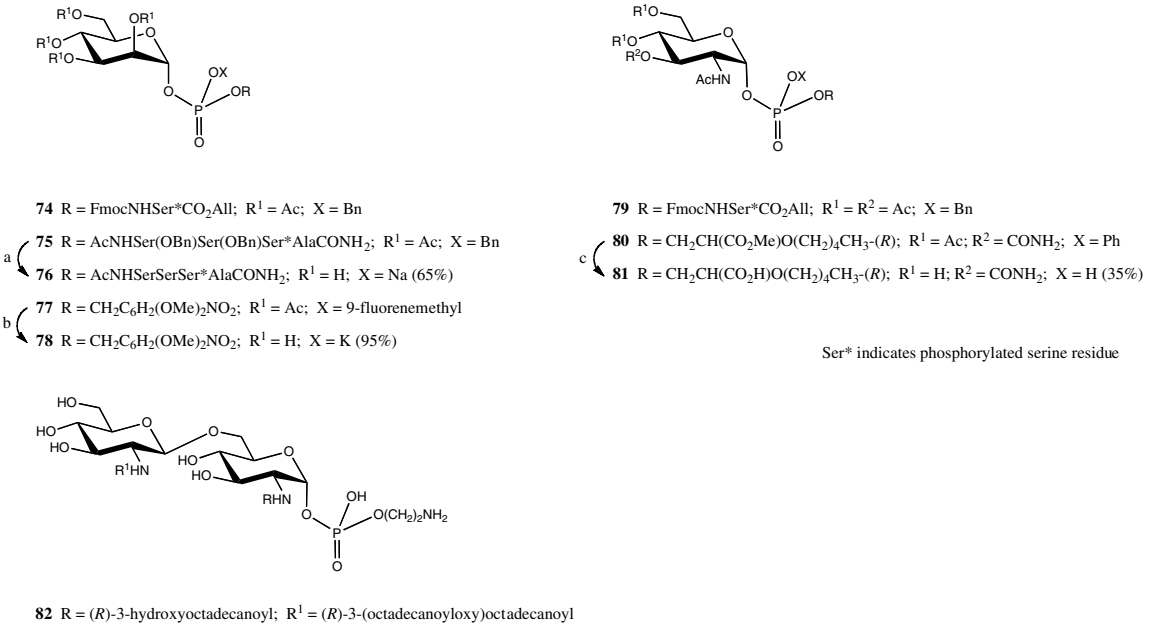
Scheme 9. Reagents and conditions: (a) 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite, $(i\text{-Pr})_2NEt$, DCM; (b) 1*H*-tetrazole, CH_3CN, −40 °C; (c) *tert*-butylhydroperoxide; (d) (i) $n\text{-BuNH}_3{}^+HCOO^-$; (ii) PPh_3, $(PPh_3)_4Pd$, THF; (e) (i) DBU, THF; (ii) NaOMe, MeOH, H_2O.

phosphoramidite derivative **64** (89%) from the hemiacetal derivative **63** and 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite. The phosphoramidite was coupled with tri-*O*,*N*-benzoylcytidine **65**, followed by oxidation under standard conditions, to give the O,N-protected phosphotriester **66** in a meagre 12% yield (as a diastereoisomeric mixture). Similar reaction of the cytidine 5′-phosphoramidite derivative **68** with the allyloxycarbonyl protected sialic acid hemiacetal **67** appeared to be more promising.[165] After coupling and oxidation, phosphotriester **69** was deprotected without purification by in situ addition of PPh_3 and $(PPh_3)_4Pd$ to provide CMP-Neu5Ac **70** in 25% yield (based on **67**).

The most successful procedure was published by Kajihara et al.[166] Condensation of the sialic acid hemiacetal **63** and the tri-*O*,*N*-acetylcytidine 5′-phosphoramidite **71** in the presence of 1*H*-tetrazole, followed by oxidation (*tert*-BuOOH), gave the protected phosphotriester **72** (75%, as a diastereoisomeric mixture). Subsequent P-deprotection (DBU) and treatment with NaOMe in aq methanol afforded CMP-Neu5Ac disodium salt **70** in 69% yield (52% based on **63**). The same synthetic route was used effectively for the synthesis of the CMP-Neu5Ac-dimer **73**[166] and for the preparation of a set of bisubstrate-type sialyltransferase inhibitors[167] containing CMP-Neu5Ac as a fragment of the molecular structure.

The phosphoramidite method was used also for the preparation of glycosyl phosphodiesters where the alcohol moiety was of a non-carbohydrate nature (Scheme 10). Coupling of the corresponding glycosyl phosphoramidite derivative with the corresponding alcohol, followed by oxidation (as described above for the preparation of compounds **54** and **56**), provided phosphotriesters **74** (73%),[168] **75** (65%),[168] **79** (41%)[168] and **80** (24%).[169] Triester **77** (61%) was made in a similar manner from 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranose and nitroveratryl 9-fluorenemethyl *N*,*N*-diisopropylphosphoramidite.[170] All the phosphotriesters were formed as diastereoisomeric mixtures. It should be noted that the yields for mannosyl phosphates **74**, **75** and **77** were markedly higher than those for derivatives of *N*-acetyl-D-glucosamine **79** and **80**. As the phosphodiester moiety linked to the anomeric carbon atom is known to be a 'good' leaving group (i.e., possesses high leaving-group ability),[163,171–173] the data reflect the higher stability of the glycosyl phosphate linkage in phosphotriesters of neutral hexopyranoses compared to similar compounds of 2-acetamido-2-deoxyhexoses, where the linkage could be weakened by the 2-acetamido group participation (see Scheme 8).

Compound **75** was then converted into mannosyl phosphopeptide **76** (65%; this structure represents a fragment of *Leishmania* proteophosphoglycan) by successive P-deprotection (NaI), debenzylation (H_2, Pd/C) and deacetylation ($NH_2NH_2{\cdot}H_2O$).[168] Phosphotriester **77** was P-deprotected with pyrrolidine, followed by deacetylation (aq KOH), to form diester **78** (95%).[170]



Scheme 10. Reagents and conditions: (a) (i) NaI, DMF; (ii) H_2, Pd/C, MeOH; (iii) $NH_2N_2{\cdot}H_2O$, MeOH; (b) (i) pyrrolidine, DCM; (ii) aq. KOH, pH 13; (c) (i) H_2, PtO_2, ethyl acetate; (ii) aq NaOH, MeOH, 0 °C; (iii) NH_3, MeOH.

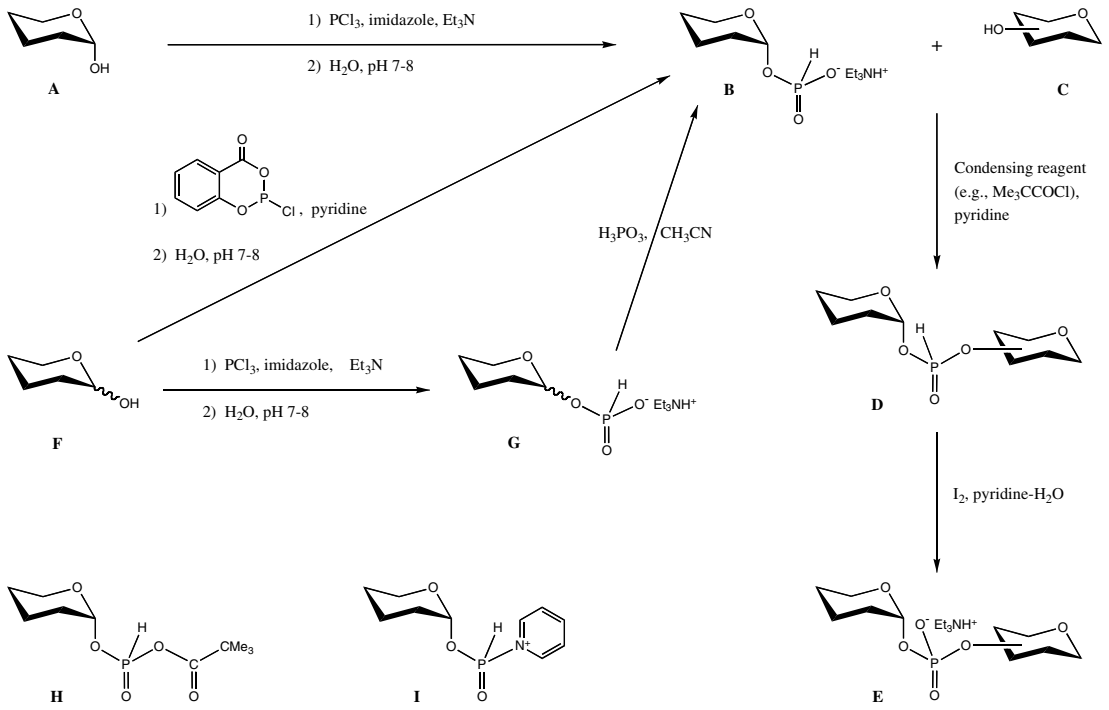
Deprotection of triester **80** (H_2/PtO_2 followed by aq NaOH and then NH_3/MeOH) gave glycosyl phosphoglycerate **81** (35%).[169] The *N*,*N*′-lipidated disaccharide 2-aminoethyl phosphate **82** (representing the structure of *Helicobacter pylori* lipid A) was synthesised by the reaction of an appropriately protected (with benzyl-type O-protecting groups) disaccharide hemiacetal and 2-(benzyloxycarbonylamino)ethyl benzyl *N*,*N*-diisopropylphosphoramidite in the presence of 1*H*-tetrazole, followed by oxidation (*m*-chloroperbenzoic acid) and deprotection (H_2, $Pd(OH)_2/C$), in 25% yield (based on the hemiacetal).[174]

3.3. Hydrogenphosphonate method [P(III) chemistry]

The glycosyl hydrogenphosphonate (H-phosphonate) method (Scheme 11) is one where a glycosyl H-phosphonate derivative **B** is coupled with a monohydroxyl component **C** in the presence of a condensing reagent to produce a phosphorous (H-phosphonic) diester **D**, which then easily forms the targeted glycosyl phosphodiester **E** on oxidation. Trimethylacetyl chloride is the most frequently used condensing reagent (see references in Scheme 12), but adamantane-1-carbonyl chloride,[175] diphenyl phosphorochloridate,[176] bis-(2-oxooxazolidin-3-yl)-phosphinic chloride[175] and TPS-NT[176] have also been employed. The oxidation reaction is normally performed in situ using iodine in aqueous pyridine. Glycosyl H-phosphonate **B** can be effectively prepared from the corresponding α-hemiacetal derivative **A** using either tri-imidazolylphosphine (made from PCl_3, imidazole and Et_3N),[141b,175–177] 2-chloro-1,3,2-benzodioxaphosphorin-4-one (commonly named salicylchlorophosphite)[162,178] or diphenyl phosphite[179,180] for phosphonylation followed by hydrolysis.

It has been reported that the α,β-hemiacetal derivative **F** can also be used to form pure α-glycosyl H-phosphonate **B** on reaction with either salicylchlorophosphite in pyridine[181] or H_3PO_3 in the presence of 2-chloro-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphorinane,[182,183] suggesting that the reactivity of the α-hemiacetal constituent towards these reagents is markedly higher than that of the β-hemiacetal. Alternatively, α,β-glycosyl H-phosphonate **G** (prepared from hemiacetal **F** using standard techniques) can be converted into α-glycosyl H-phosphonate **B** on treatment with H_3PO_3 in acetonitrile.[184–186]

The first examples utilising the H-phosphonate approach for the synthesis of glycosyl phosphosaccharides were described by van Boom's group[162,178] and by one of us (A.V.N.)[187] in the mid-1980s. This method (the overall results are summarised in Scheme 12; see Refs. 162,176–178,184 and 187–198) appeared to be highly efficient in the preparation of protected (1→6)-linked phosphodiesters of *N*-acetyl-α-D-glucosamine **60** (entry 2) and α-D-mannopyranoses **89** and **91** (entries 3 and 4), providing 78–87% yields over the condensation–oxidation steps. It should be noted that compound **60** was synthesised by the phosphoramidite method (Scheme 8) in 55% yield only. The phosphodiester α-D-Man*p*-(1-PO_3H-6)-α-D-Man*p* **4** (entry 3), which was formed from **89** upon standard deprotection, was also prepared in much better overall yield than by the phosphodiester (Scheme 2) and phosphorochloridite (Scheme 6) methods.



Scheme 11.

The H-phosphonate method is also highly effective in the synthesis of protected (1→2)-linked (**94** and **105**), (1→3)-linked (**96**) and (1→4)-linked (**98**, **100**, **102** and **109**) phosphodiesters (entries 5–11), which were prepared in 67–87% yield. After convenient deprotection, glycosyl phosphosaccharides **4**, **92**, **95**, **97**, **6**, **103**, **106** and **110** (each containing an α-D-mannosyl phosphate unit; entries 3–7 and 9–11) were isolated in high yields using gel-permeation or ion-exchange chromatography.

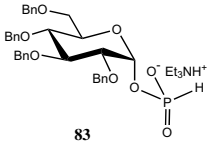
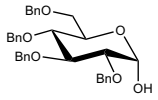
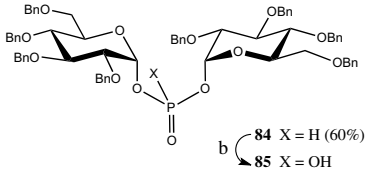
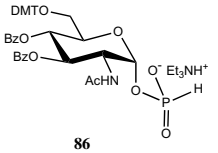
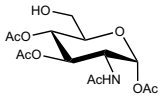
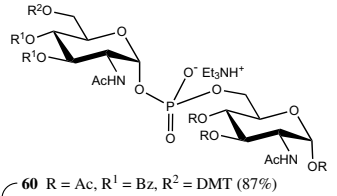
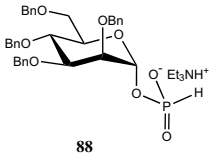
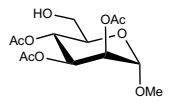
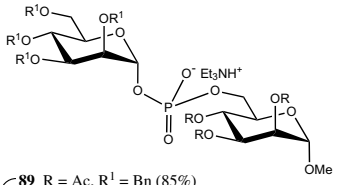
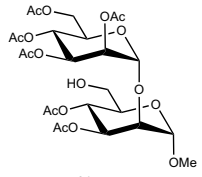
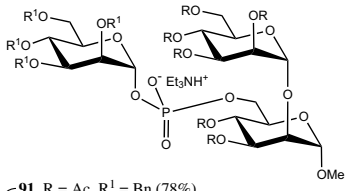
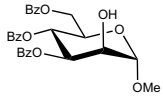
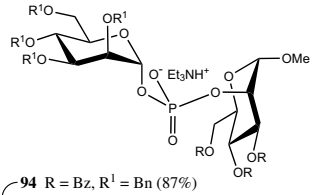
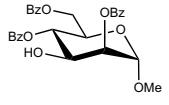
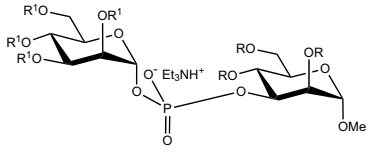
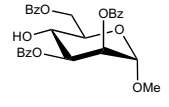
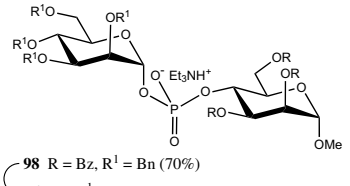
Similarly, the method provided good results in the synthesis of (1→1)- and (1→6)-linked phosphodiesters containing α-D-glucosyl phosphate units (entries 1 and 12–14) and (1→6)- and (1→3)-linked phosphodiesters of α-D-galactopyranose (entries 15 and 16). In the last two examples, glycosyl phosphosaccharide fragments **119** and **122** of the yeast phosphogalactan from *Sporobolomyces* (Section 2.3) and of the bacterial capsule phosphoglycan from *E. coli* K52 (Section 2.1), respectively, were prepared.

It was mentioned earlier that both the phosphodiester and the phosphoramidite methods did not work particularly well in the synthesis of glycosyl phosphosaccharides and glycosyl phosphodiesters containing *N*-acetyl-α-D-glucosamine 1-phosphate units (Schemes 4, 8 and 10). This problem seems to be overcome by applying the glycosyl H-phosphonate methodology. The preparation of various phosphodiesters containing glycosyl phosphates of *N*-acetyl-α-D-glucosamine (entries 2 and 17–21) as well as (1→6)-linked phosphodiesters containing *N*-acetyl-α-D-mannosamine 1-phosphate units (entries 22 and 23) has been achieved. It is noteworthy that biologically important glycosyl phosphosaccharide fragments of the cell-wall phosphoglycan from *M. varians* ATCC 29750 (Section 2.2) and bacterial capsule glycopolymers (Section 2.1) from *E. coli* K51, *N. meningitidis* serogroup X and *N. meningitidis* serogroup A (compounds **87**, **132**, **135** and **141**, respectively; entries 2, 20, 21 and 23) were successfully synthesised.

Furthermore, the first chemical syntheses of more complex fragments of phosphoglycans built up from oligosaccharide phosphate repeating units (see Section 4) were reported.

Glycosyl H-phosphonate derivatives were shown to be useful synthetic tools for the synthesis of structural (isosteric and isoelectronic) analogues of glycosyl phosphodiesters modified at the phosphate moiety.[197,198] Condensation of disaccharide H-phosphonate **142** and dec-9-en-1-ol, followed by oxidation with sulfur powder (instead of iodine), provided the protected thiophosphodiester **143** (entry 24), which was then debenzoylated to form thiophosphate **144**. The glycosyl boranophosphate derivatives **147** and **149** (entries 25 and 26) were synthesised *via* coupling of glycosyl H-phosphonate **145** with the corresponding alcohol (**146** or **148**), silylation of the H-phosphonic diester formed with bis(trimethylsilyl)trifluoracetamide, followed in turn by boronation with a borane complex and hydrolysis.

To summarise, the H-phosphonate method appears to be the most efficient for the preparation of glycosyl phosphosaccharides linked through either primary or secondary hydroxyl groups and containing monosaccharides of diverse composition. The main favourable

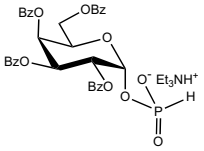
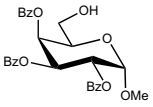
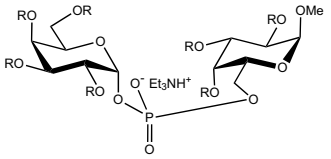
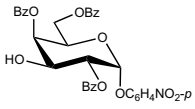
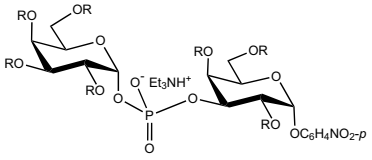
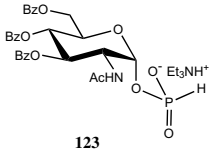
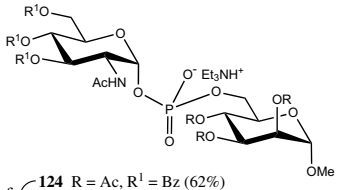
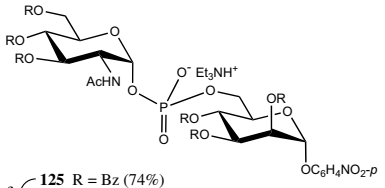
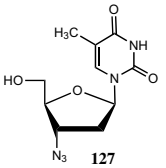
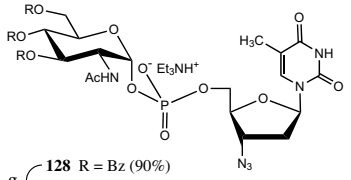
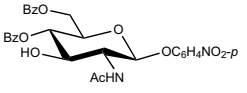
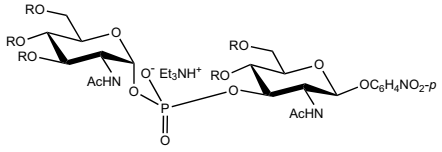
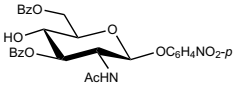
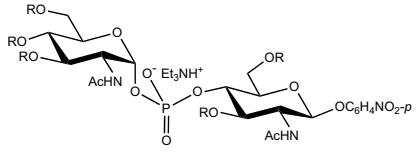
Entry	Glycosyl H-phosphonate	Monohydroxyl derivative	Reaction conditions	Reaction product	Reference
1.	 **83**	 **43**	a	 b → **84** X = H (60%); **85** X = OH	[178]
2.	 **86**	 **59**	c, b	 d → **60** R = Ac, R^1 = Bz, R^2 = DMT (87%); **87** R = R^1 = R^2 = H	[162]
3.	 **88**	 **2**	c, b	 e, f → **89** R = Ac, R^1 = Bn (85%); **4** R = R^1 = H (93%)	[176, 187]
4.	**88**	 **90**	c, b	 e, f → **91** R = Ac, R^1 = Bn (78%); **92** R = R^1 = H (94%)	[176, 188]
5.	**88**	 **93**	c, b	 e, g → **94** R = Bz, R^1 = Bn (87%); **95** R = R^1 = H (58%)	[189, 190, 177]
6.	**88**	 **95**	c, b	 e, g → **96** R = Bz, R^1 = Bn (77%); **97** R = R^1 = H (77%)	[189, 190, 177]
7.	**88**	 **5**	c, b	 e, g → **98** R = Bz, R^1 = Bn (70%); **6** R = R^1 = H (83%)	[189, 190, 177]

Scheme 12. Reagents and conditions: (a) Me_3CCOCl, Et_3N, CH_3CN; (b) I_2, pyridine–water (95:5); (c) Me_3CCOCl, pyridine; (d) (i) NH_3, MeOH; (ii) aq CH_3COOH; (e) H_2, Pd/C, MeOH–THF; (f) Et_3N–MeOH–water (1:2:1); (g) 0.05 M NaOMe in MeOH (or in MeOH–1,4-dioxane); (h) Na, NH_3 liq., THF, −33 °C; (j) S_8, toluene–pyridine; (k) (i) $CF_3CON(SiMe_3)_2$, THF; (ii) BH_3·2-chloropyridine; (iii) 1 M aq Et_3NHHCO_3.

Entry	Glycosyl H-phosphonate	Monohydroxyl derivative	Reaction conditions	Reaction product	Reference
8.	**88**	**99**	c, b	**100** (72%)	[191, 177]
9.	**101**	**99**	c, b	g → **102** R = Bz (69%); **103** R = H (75%)	[191, 177]
10.	**101**	**104**	c, b	g → **105** R = Bz (67%); **106** R = H (88%)	[191, 177]
11.	**107** R = $PhCO(CH_2)_2CO$	**108**	c, b	g → **109** R = Ac, R^1 = $PhCO(CH_2)_2CO$ (70%); **110** R = R^1 = H (83%)	[191]
12.	**111**	**2**	c, b	f → **112** R = Ac, R^1 = Bz (62%); **113** R = R^1 = H (97%)	[192, 193]
13.	**111**	**26**	c, b	f → **114** R = Bz (73%); **24** R = H (86%)	[192, 193]
14.	**83**	**36**	c, b	h → **115** R = Bn (82%); **113** R = H (78%)	[180]

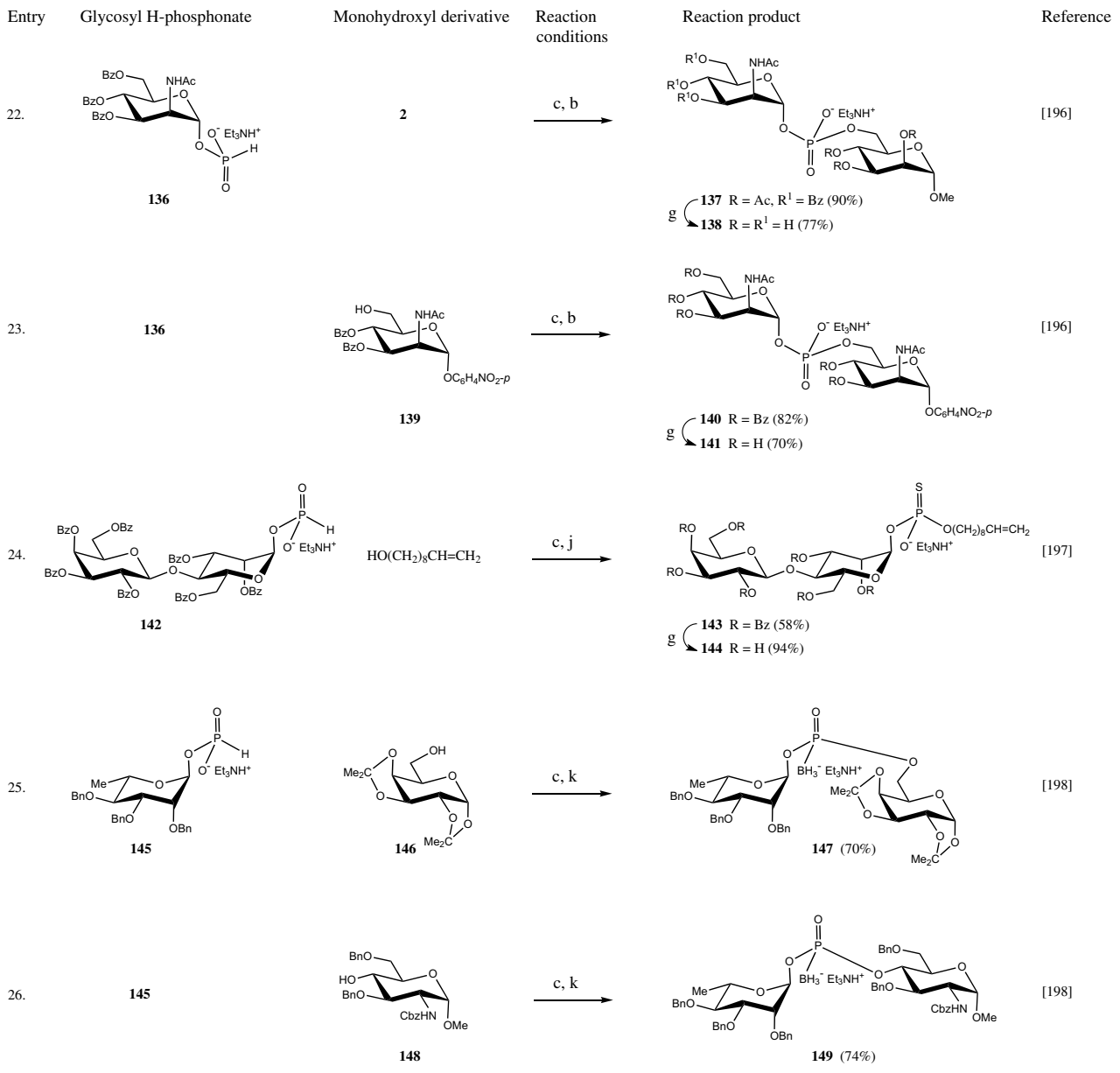
Scheme 12. (*continued*)

characteristics of the method are high effectiveness and high reaction rate for all of the three steps involved: that is, the preparation of a glycosyl H-phosphonate derivative (requires 20–40 min to achieve depending on the

Entry	Glycosyl H-phosphonate	Monohydroxyl derivative	Reaction conditions	Reaction product	Reference
15.	 **116**	 **117**	c, b	 g → **118** R = Bz (80%), **119** R = H (80%)	[184]
16.	**116**	 **120**	c, b	 g → **121** R = Bz (91%), **122** R = H (68%)	[184]
17.	 **123**	**2**	c, b	 f → **124** R = Ac, R^1 = Bz (62%), **32** R = R^1 = H (93%)	[192, 193]
18.	**123**	**26**	c, b	 f → **125** R = Bz (74%), **126** R = H (68%)	[192, 193]
19.	**123**	 **127**	c, b	 g → **128** R = Bz (90%), **129** R = H (81%)	[194]
20.	**123**	 **130**	c, b	 g → **131** R = Bz (72%), **132** R = H (79%)	[195, 193]
21.	**123**	 **133**	c, b	 g → **134** R = Bz (71%), **135** R = H (71%)	[195, 193]

Scheme 12. (*continued*)

reagent used[162,175–181,188–196]), its condensation with a monohydroxyl component (completed within 2–5 min as demonstrated[162,176] when the reaction was monitored by ^{31}P NMR) and subsequent oxidation of the



Scheme 12. (*continued*)

H-phosphonic diester (needs 5–10 min for completion, also indicated[176] by ^{31}P NMR). The latter two steps are normally performed as a 'one-pot' procedure.

The H-phosphonate approach also has an advantage over the phosphorous triester methods (Section 3.2) in that it does not require a P-protecting group. When P-protection is present (see examples in Schemes 6–8), it forms phosphorous diester and phosphoric diester (after oxidation) moieties, which both possess high leaving-group ability while linked to the anomeric carbon atom.[163,199] This usually reduces the stability of glycosyl phosphite and glycosyl phosphate linkages in the corresponding triester intermediates (compared to diesters **D** and **E**, Scheme 11), thus decreasing the yields of final products. The labile nature of anomeric dialkyl phosphite and dialkyl phosphate derivatives (which is due to a tendency to form a stabilised glycosyl carbocation when activated) was demonstrated by examples where they were used as glycosyl donors (see also Section 3.4) in the glycosylation reaction.[171–173,199]

The reaction of glycosyl H-phosphonates (**B**; Scheme 11) with trimethylacetyl chloride and alcohols (**C**) was studied[200] by ^{31}P NMR spectroscopy and it was demonstrated that the mixed anhydride **H** is the main reactive intermediate leading to the formation of H-phosphonic diester **D**, which is due to be further oxidised to phosphodiester **E**. It was also observed, however, that prolonged delay (>24 h) of the oxidation step can lead to partial transformation of **D** into C-phosphonate and phosphorous triester derivatives, thus reducing the yield

of **E**. In accord with data[201] describing the mechanism of the H-phosphonate condensation in pyridine, the solvent can participate in the reaction as a nucleophilic catalyst to assist in the transformation of **H** into **D** *via* the activated pyridinium intermediate **I**.

3.4. The glycosylation reaction

The glycosylation reaction, which involves substitution of a suitable leaving group at the anomeric centre with a phosphoric acid derivative, was used extensively for the preparation of 1,2-*cis* and 1,2-*trans* glycosyl phosphates.[202] There are reports on the reaction of glycosyl bromides with various salts (silver,[203] tetrabutylammonium[204] or triethylammonium[205,206]) of dibenzyl phosphoric acid or its tributylstannyl ester.[207] Acetylated glycosyl nitrates form 1-phosphates on reaction with caesium dibenzyl phosphate or caesium diphenyl phosphate.[208] Dibenzyl phosphoric acid itself reacted smoothly with Brigl's anhydride (3,4,6-tri-*O*-acetyl-1,2-anhydro-α-D-glucopyranose),[209] 1,2-oxazolines[210,211] or 1,2-orthoesters[212] to provide glycosyl phosphates without adding any catalyst. Glycosyl trichloroacetimidates are capable of glycosylating various phosphoric acid mono- or di-esters in a similar manner,[158,213,214] whereas the reaction of dibenzyl hydrogen phosphate with 1-thioglycosides required activation with *N*-iodosuccinimide.[215] The yields of glycosyl phosphates varied from moderate to good, while the stereochemical outcome of the reaction depended on the protecting-group pattern, the stereochemistry of the glycosyl donor (for trichloroacetimidates) and the reaction conditions.

There are just a few examples of the synthesis of glycosyl phosphosaccharides using the glycosylation methodology. An efficient and practical synthesis of CMP-Neu5Ac **70** (Scheme 13) was performed[216] starting from sialic acid hemiacetal **63** *via* the formation of sialyl diethyl phosphite **150**[217] as a glycosyl donor. The subsequent reaction with tri-*O*,*N*-acetylcytidine 5′-phosphate **151** (no catalyst needed) proceeded stereoselectively and gave β-linked phosphodiester **152** (50%), which was converted into crystalline CMP-Neu5Ac (68%) on standard deprotection.

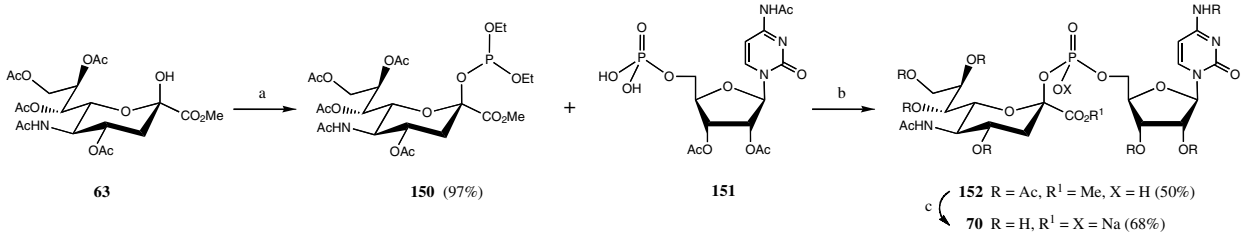
A combination of the glycosylation and the H-phosphonate methods was developed in an attempt to prepare phosphodiesters α-D-Gal*p*-(1-PO_3H-4)-3-*O*-Ac-β-D-Glc*p*NAc and 3-*O*-Ac-α-D-Gal*p*NAc-(1-PO_3H-3)-β-D-Gal*p*NAc,[218,219] which represent repeating units of capsular phosphoglycan of *H. influenzae* type 'c' and 'f', respectively (Section 2.1). The monosaccharide H-phosphonate derivative **157** (Scheme 14) was synthesised and used as a glycosyl acceptor in reactions with various glycosyl donors **153**–**156** (for conditions and yields see Table 1). The glycosylation product (the corresponding H-phosphonic diester) was oxidised (in situ) with I_2 in aq pyridine to provide phosphodiester **158** as a mixture of α- and β-glycosyl phosphates (which were not separated). The yields ranged from 55% to 76%. The glycosylation with β-trichloroacetimidate **153** seemed to be non-selective and gave an excess of the β-anomer (α/β = 1:2), while the best α-selectivity (α/β = 9:1) was achieved in the reaction of α-glycosyl chloride **155** in the presence of AgOTf.

Similarly, phosphodiester **162** (an α,β-mixture) was produced from the H-phosphonate derivative **161** using either thioglycoside **159** or glycosyl bromide **160** as glycosyl donors. The DMTST-assisted reaction with the thioglycoside gave an excellent α/β-ratio (15:1) and high yield (74%) of the product. Tetrasaccharide phosphodiester **165**, representing a protected dimeric structure of the type 'c' capsular phosphoglycan, was synthesised using biosyl chloride **163** as a donor and disaccharide H-phosphonate **164** as an acceptor. The glycosylation in the presence of AgOTf, followed by oxidation, afforded phosphodiester **165** in 67% yield as a pure α-glycosyl phosphate.[219]

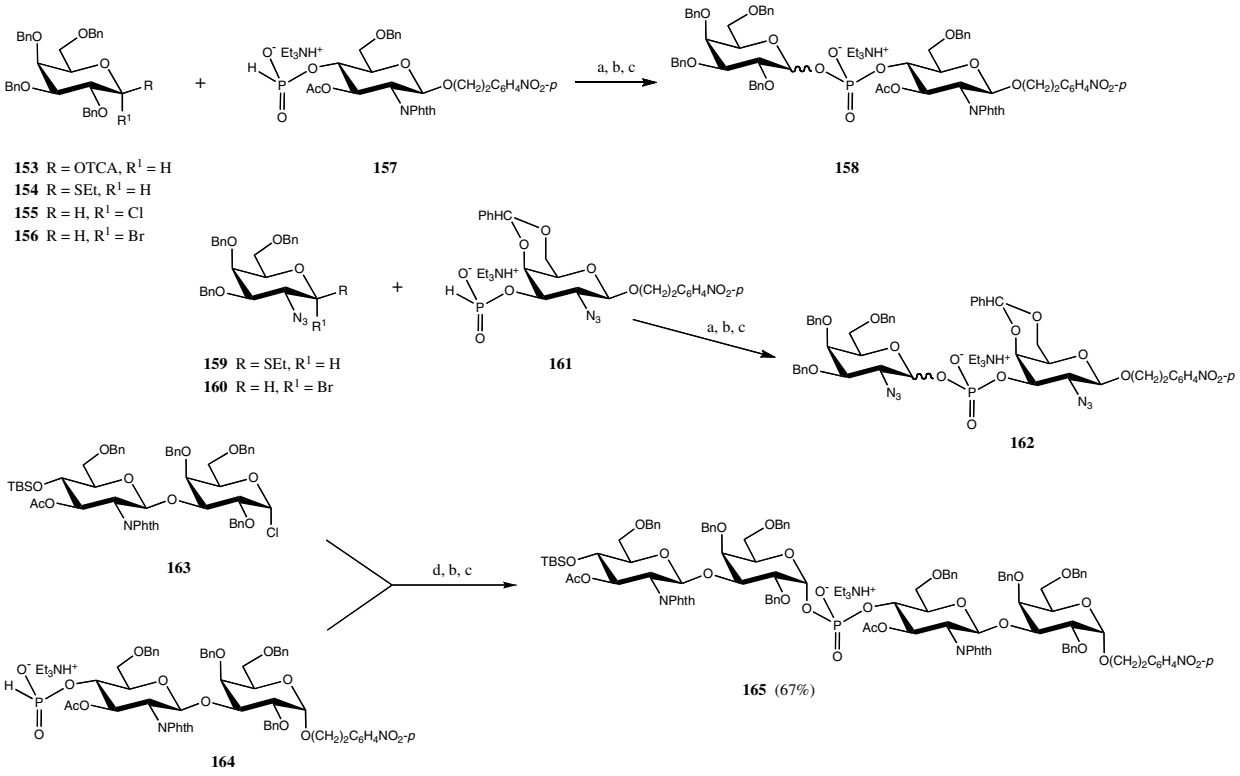
4. Chemical synthesis of natural phosphoglycans using glycosyl H-phosphonate chemistry

4.1. Syntheses of complex phosphoglycan fragments containing one phosphodiester bridge

The glycosyl H-phosphonate approach appears to be the method of choice for the efficient and reliable construction of various phosphodiester linkages in glycosyl phosphosaccharides of diverse monosaccharide composition. The method was further developed for the synthesis of poly(glycosyl phosphates) (see Sections 4.2



Scheme 13. Reagents and conditions: (a) $(EtO)_2PCl$, $(i\text{-}Pr)_2NEt$, CH_3CN; (b) CH_3CN, THF, DMF, −15 °C; (c) NaOMe, MeOH, then H_2O.



Scheme 14. Reagents and conditions: (a) promoter, DCM; (b) Et_3N; (c) I_2, aq pyridine, −40 °C; (d) AgOTf, DCM.

Table 1. Conditions and yields in the preparation of compounds **158** and **162**

Donor	Acceptor	Promoter	α/β-ratio	Yield of the phosphodiester
153	**157**	TMSOTf	1:2	76% of **158**
154	**157**	DMTST	5:1	68% of **158**
155	**157**	AgOTf	9:1	55% of **158**
156	**157**	Et_4NBr	2:1	60% of **158**
159	**161**	DMTST	15:1	74% of **162**
160	**161**	AgOTf	3:1	76% of **162**

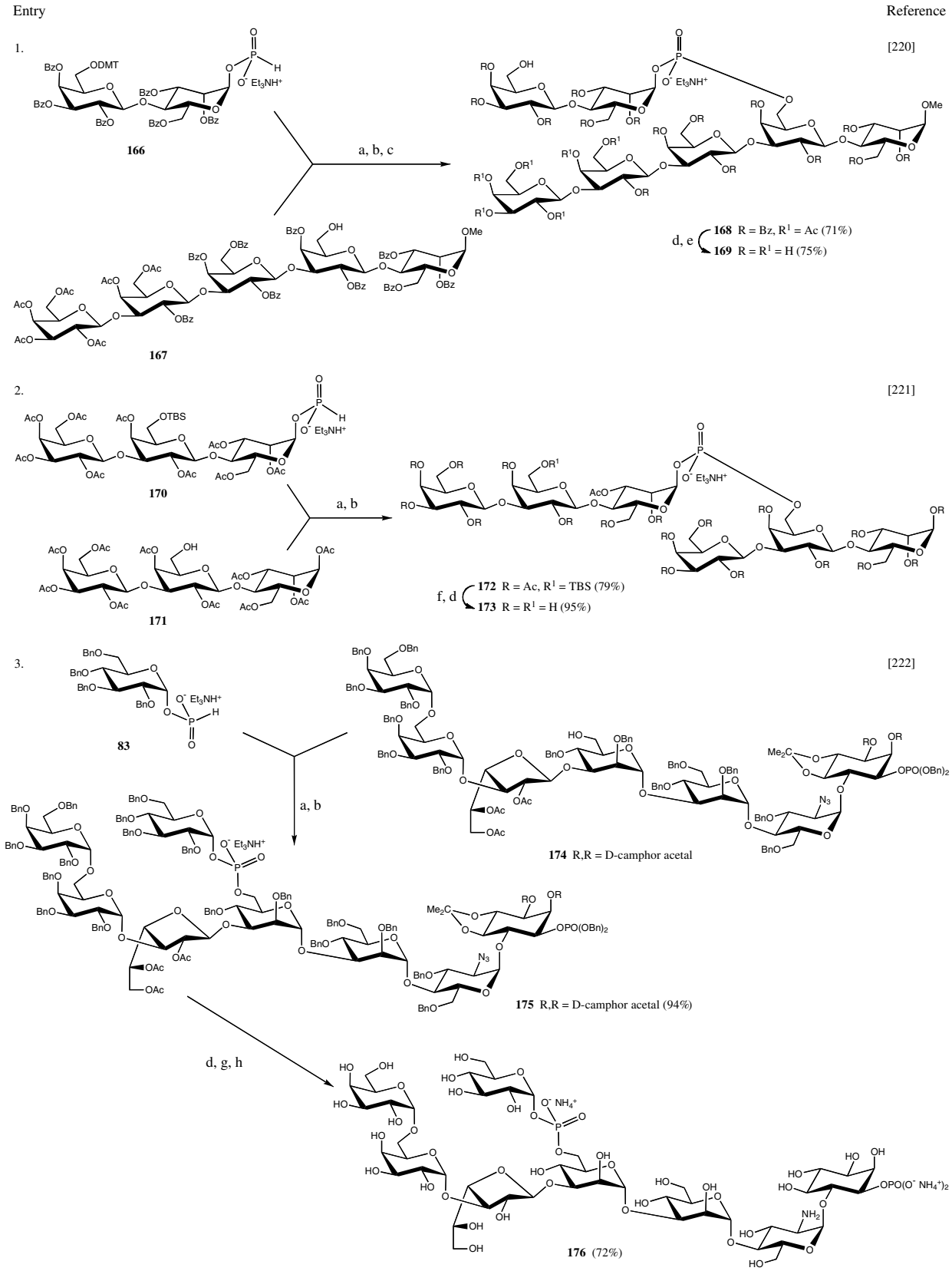
and 4.3) and applied to the preparation of more complex fragments of phosphoglycans built up from oligosaccharide phosphate repeating units (Scheme 15).

There are reports on the preparation of branched molecules, notably heptasaccharide monophosphate **169** (entry 1) and hexasaccharide monophosphate **173** (entry 2), representing side-chain containing fragments of the surface lipophosphoglycan (LPG) from *Leishmania major* parasite (Section 2.4). The first compound was synthesised by coupling the disaccharide H-phosphonate derivative **166** and the pentasaccharide monohydroxyl compound **167** followed by oxidation (→**168**, 71%) and standard deacylation.[220] Hexasaccharide phosphodiester **173** was prepared from the trisaccharide derivatives **170** and **171** in a similar manner.[221]

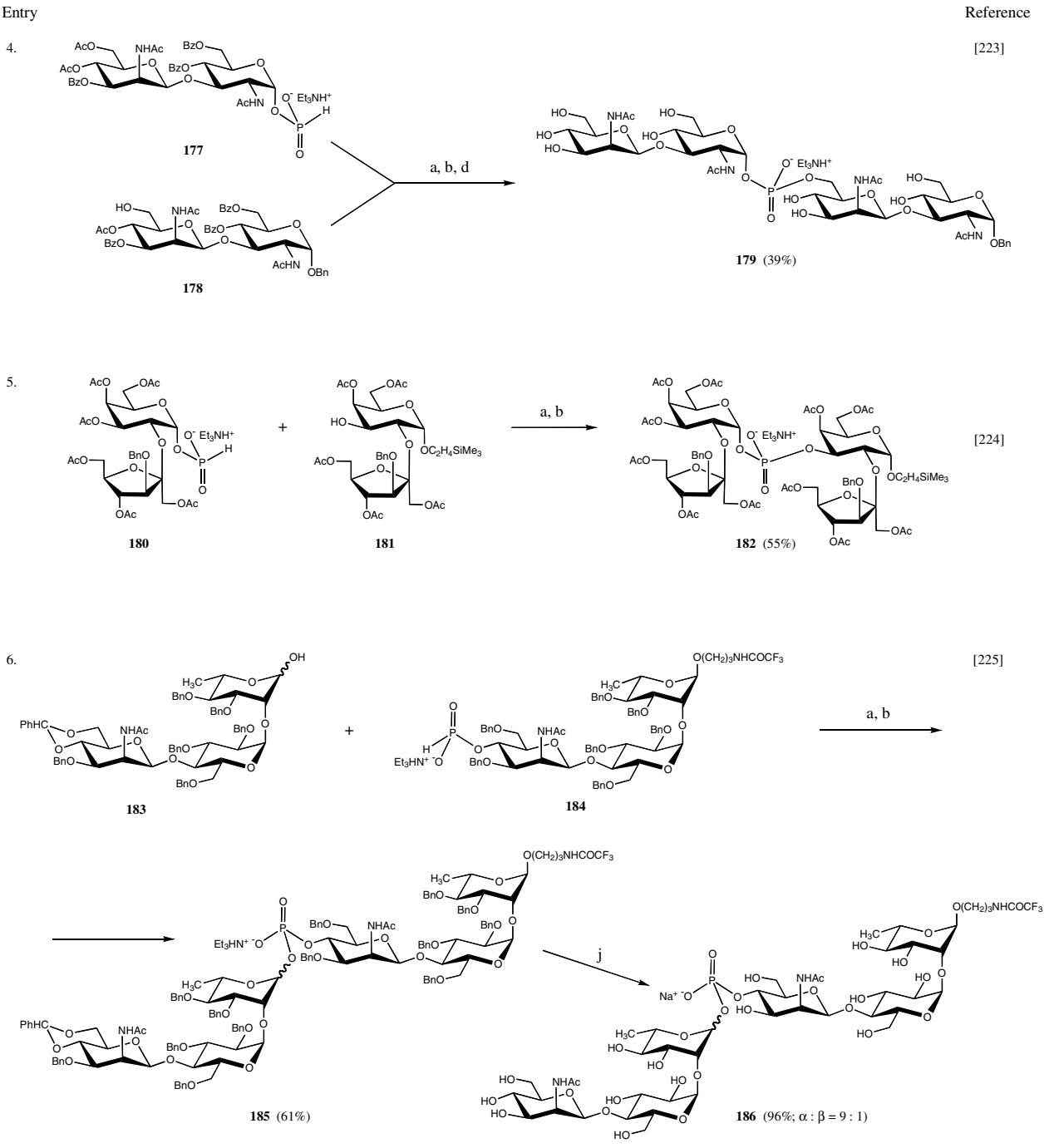
The first synthesis of the complex glycan-phosphoinositol core component of *Leishmania* LPG, notably compound **176** (entry 3) containing an α-D-glucopyranosyl phosphate unit in the side chain, was achieved through the condensation of the α-D-glucopyranosyl H-phosphonate derivative **83** and the partially protected hexasaccharide-inositol block **174**, followed by oxidation (→**175**, 94%) and deprotection.[222]

Tetrasaccharide monophosphate **179** (entry 4), a fragment of the cell-wall glycopolymer of *Actinoplanes* sp. INA 3697 (Section 2.2), was synthesised by condensation of disaccharide H-phosphonate **177** and the monohydroxyl derivative **178**, followed by oxidation and standard deprotection.[223] The protected tetrasaccharide monophosphate derivative **182** (entry 5), representing a fragment of the capsular phosphoglycan from *E. coli* K52 (Section 2.1) that contains branching D-fructose residues, was prepared from the disaccharide blocks **180** and **181**.[224]

The synthesis of a hexasaccharide monophosphate fragment of *S. pneumoniae* 19F capsular phosphoglycan (Section 2.2) was attempted starting from the trisaccharide hemiacetal derivative **183** (entry 6).[225] Since efforts to convert **183** into the corresponding glycosyl H-phosphonate resulted in an inseparable mixture of anomeric H-phosphonates (α/β = 7:3), it was decided to make the H-phosphonate component **184** from the corresponding trisaccharide monohydroxyl derivative. Coupling of hemiacetal **183** and H-phosphonate **184**, followed by oxidation (→**185**, 61%) and deprotection, provided



Scheme 15. Reagents and conditions: (a) Me_3CCOCl, pyridine; (b) I_2, pyridine–water (95:5); (c) TFA–DCM (1:99), 0 °C, then aq work-up; (d) NaOMe, MeOH; (e) 1% NaOH in aq MeOH; (f) 48% aq HF–CH_3CN (5:95), 0 °C; (g) Na, NH_3 liq., THF, −33 °C; (h) 0.1 M aq HCl; (j) H_2, $Pd(OH)_2$/C, aq MeOH, then Dowex 50 (Na^+).

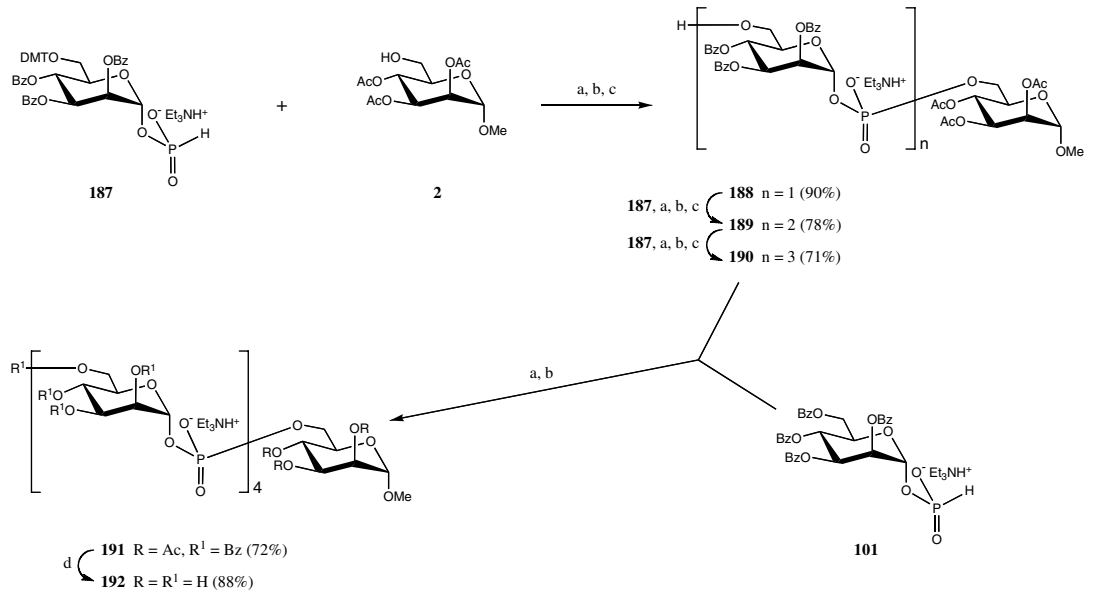


Scheme 15. (*continued*)

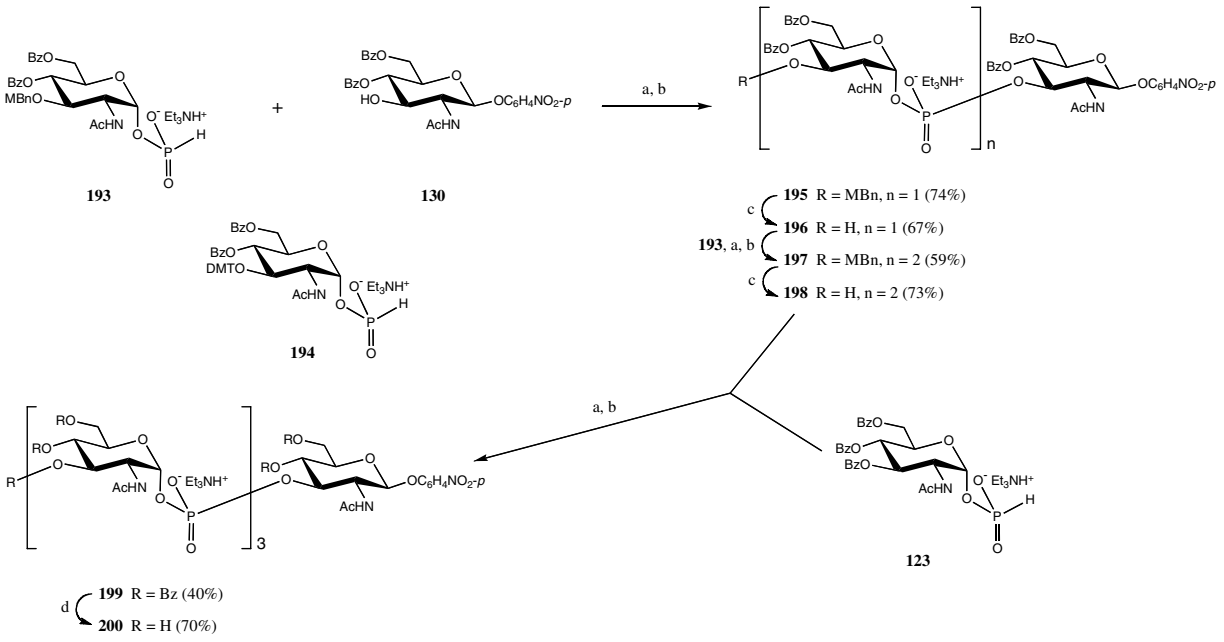
hexasaccharide phosphodiester **186**, which contained 90% of the required α-L-rhamnopyranosyl phosphoric diester and a small proportion of its β-anomer.

4.2. Syntheses of poly(glycosyl phosphates) using stepwise chain elongation

4.2.1. Bacterial and yeast surface structures built up from monosaccharide phosphate repeating units. The first syntheses of poly(glycosyl phosphates) were reported by the Moscow group (Nikolaev et al.) in the early 1990s.[226–229] They synthesised pentamannosyl tetraphosphate **192** (Scheme 16) and tetra(*N*-acetyl-D-glucosaminyl) triphosphate **200** (Scheme 17), which are fragments of the exophosphomannan from *H. capsulata* Y-1842 (Section 2.3) and of the capsular antigen from *E. coli* K51 (Section 2.1), respectively. The (1→6)-linked phosphoglycan **192** was prepared from the suitably protected mannosyl H-phosphonate derivatives **187** and **101** (for the consecutive introduction of the mannosyl phos-



Scheme 16. Reagents and conditions: (a) Me_3CCOCl, pyridine; (b) I_2, pyridine–water (95:5); (c) TFA–DCM (1:99), 0 °C, then aq work-up; (d) 0.1 M NaOMe in MeOH–1,4-dioxane, then anion-exchange chromatography.



Scheme 17. Reagents and conditions: (a) Me_3CCOCl, pyridine; (b) I_2, pyridine–water (95:5); (c) cerium(IV) ammonium nitrate, aq CH_3CN; (d) 0.05 M NaOMe in MeOH–1,4-dioxane, then anion-exchange chromatography.

phate units) and the monohydroxyl derivative **2**.[226,227,229] The 6-*O*-dimethoxytrityl (DMT) was chosen as a temporary protecting group, since it could be removed in very mild acidic conditions (1% TFA in DCM, 0 °C, 1–2 min) without interfering with the glycosyl phosphate linkages.

The chain-elongation cycle for the synthesis of the protected pentamannosyl tetraphosphate **191** involved the coupling of a glycosyl H-phosphonate derivative with a hydroxyl acceptor, followed by oxidation of the resulting H-phosphonic diester to the phosphoric diester prior to removal of the DMT-protecting group. The oxidation step performed during each elongation cycle is essential, since the higher stability of glycosyl phosphoric diesters permits selective deprotection and chromatographic isolation of the products, whereas the same operations with the glycosyl H-phosphonic diesters led to significant degradation.[176,227,228]

The yields of the protected oligo(mannosyl phosphates) **188**–**191** appeared to be consistent (71–90%). Standard deacylation of **191** led to the targeted phosphoglycan **192** (88%), which was isolated by anion-exchange chromatography. The overall yield of **192** was 31%, based on the first hydroxyl acceptor **2**.

The (1→3)-linked phosphoglycan **200** (Scheme 17) was prepared from the suitably protected glycosyl H-phosphonate derivatives **193** and **123** and the monohydroxyl derivative **130** using the same chain-elongation strategy;[228,229] note that 3-*O*-*p*-methoxybenzyl worked well as a temporary protecting group. The coupling of **193** and **130**, followed by oxidation (→**195**, 74%) and demethoxybenzylation (with cerium(IV) ammonium nitrate), produced phosphodiester **196** (67%). Two more consecutive chain extensions resulted in the protected oligomer **199**, which furnished the phosphoglycan **200** on standard debenzoylation.

The authors first tried to make use of the 3-*O*-DMT-protected H-phosphonate derivative **194**, but the 3-*O*-DMT group seemed to be incompatible with the oxidation conditions. After condensation of **194** and **130** (which resulted in the complete formation of the corresponding H-phosphonic diester, as indicated by ^{31}P NMR), followed by standard oxidation (I_2 in aq pyridine) and detritylation, the expected phosphodiester derivative **196** was isolated in only 18% yield and was accompanied by pyrophosphate and phosphate monoester by-products. Any attempts to use different oxidation reagents [e.g., a mixture of *tert*-BuOOH–(*tert*-BuO)$_2$ (8:2) or a solution of *tert*-BuOOH in toluene] failed to improve the outcome of the reaction.[230]

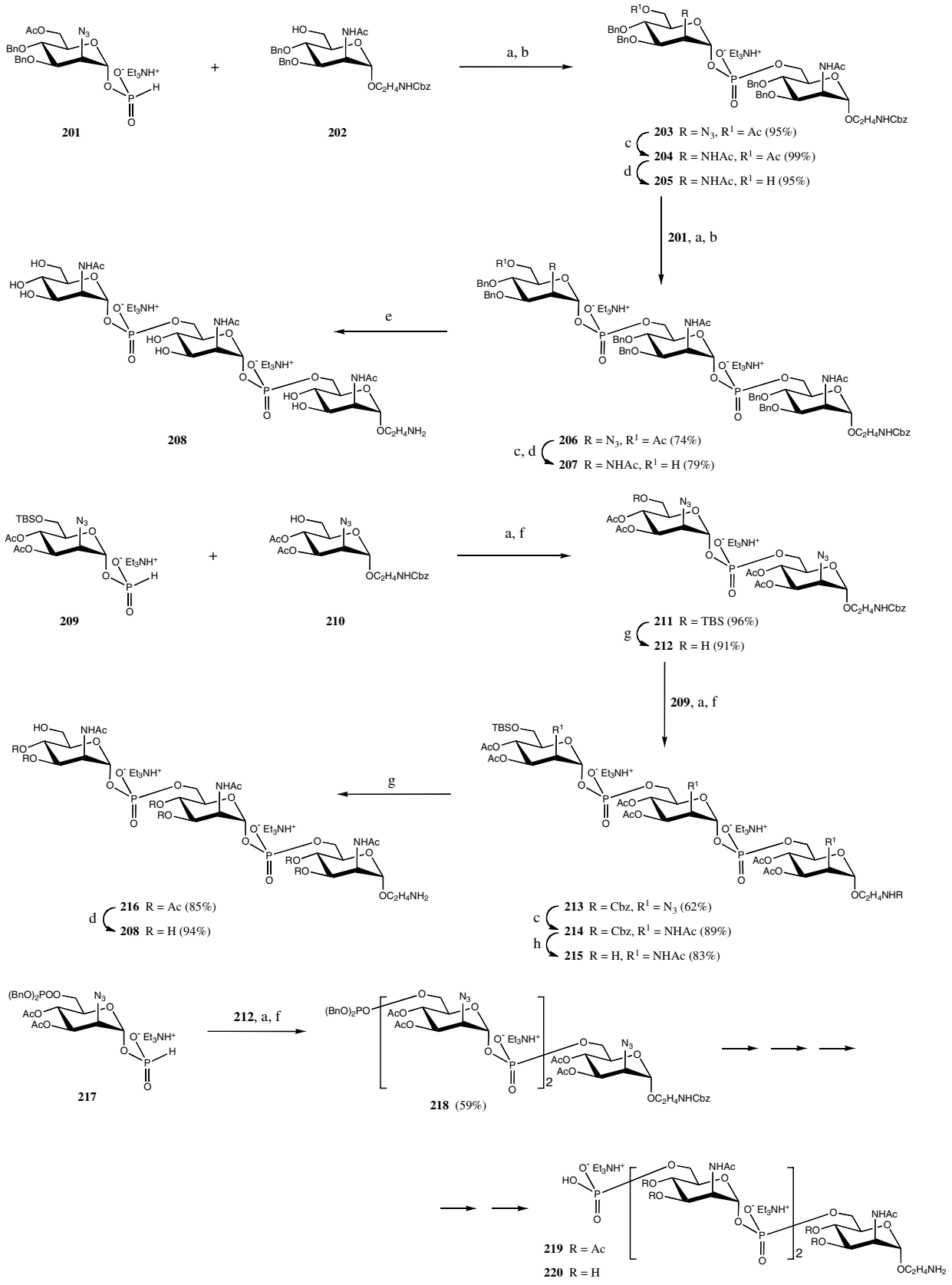
The (1→6)-linked tri(*N*-acetyl-D-mannosaminyl) diphosphate **208** (Scheme 18), representing a fragment of the capsular antigen from *N. meningitidis* serogroup A (Section 2.1), was synthesised by Pozsgay and coworkers[231] from the 2-azido-2-deoxy-D-mannosyl H-phosphonate derivative **201** and the monohydroxyl derivative **202**, using benzyl ethers for permanent O-protection and 6-*O*-acetyl as a temporary protecting group. Two consecutive chain extensions provided the protected dimeric (**203**) and trimeric (**206**) fragments in high yields. The 2-azido group was reduced, followed in turn by N-acetylation, in each of these derivatives prior to removal of the *O*-acetyl protecting group. However, further chain elongation of trimer **207** with H-phosphonate **201** failed to produce the expected tetrameric fragment. The authors assumed that this failure might be related to degradation of the phosphodiester linkage(s) in the *N*-acetyl-D-mannosamine environment during the oxidation step. Catalytic hydrogenolysis of **207** afforded phosphoglycan **208**, which then was conjugated (though the 2-aminoethyl spacer) to human serum albumin using a novel cycloaddition methodology, thereby providing a potential synthetic anti-meningitis vaccine. A glycoconjugate with a 'shortened' glycan chain was made from the dimeric derivative **205** in a similar manner.

The preparation of the same phosphoglycan **208**, starting from the building blocks **209** and **210**, was reported recently by the Stockholm group (Oscarson and coworkers).[232] By operating a different protecting-group pattern (*O*-acetates for permanent protection and 6-TBS ether for temporary protection), the authors were able to synthesise phosphoglycan **216** containing a 3,4-diacetate grouping in each *N*-acetyl-D-mannosamine unit. This structure is closer to the native capsular antigen (see Chart 3), where 3- and 4-OH are acetylated to an extent of about 37.5–47.5% each (i.e., 0.75–0.95 equiv of *O*-acetate in total). The synthesis of the 6″-*O*-phosphorylated phosphoglycan structures **219** and **220** has been also performed by making use of the 6-*O*-phosphorylated H-phosphonate derivative **217** for the second chain elongation.

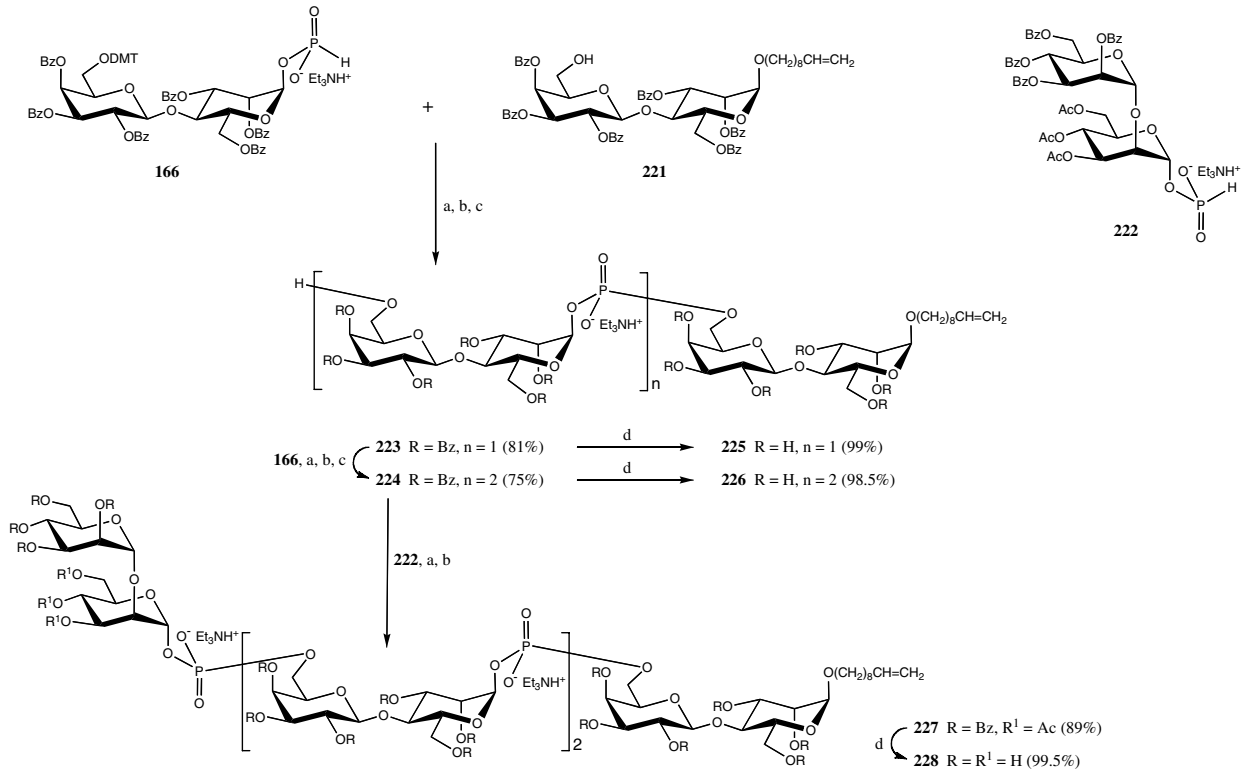
4.2.2. *Leishmania* phosphoglycans. Stepwise chain elongation was successfully implemented for the synthesis of a set of *Leishmania* (Section 2.4) phosphoglycans by the Dundee group (Nikolaev et al.). First, the *L. donovani* phosphoglycans **225**, **226** and **228** [built up from disaccharide phosphate repeating units in (1→6)-phosphodiester linkage] were synthesised (Scheme 19) using the disaccharide H-phosphonate derivatives **166** and **222** and the monohydroxyl block **221**.[175,233] The 6′-DMT ether was chosen again as a temporary protecting group, while benzoates and acetates served for permanent O-protection. This time, the H-phosphonate condensations were performed in the presence of adamantane-1-carbonyl chloride. The protected phosphoglycan derivatives **223**, **224** and **227** were isolated after each chain-elongation cycle in 75–89% yield. Final O-deacylation afforded the targeted compounds quantitatively. Phosphoglycans **225**, **226** and **228** contain a dec-9-enyl aglycon moiety that permits the preparation of neoglycoconjugates by consecutive ozonolysis of the double bond and coupling to a protein carrier by reductive amination.[234–236]

The branched phosphoglycan from *Leishmania mexicana* **233**, containing β-D-glucose in the side chain (Scheme 20), was synthesised by making use of disaccharide and trisaccharide H-phosphonates **166** and **230**, respectively.[237] Chain elongation was carried out with dec-9-en-1-ol as the first monohydroxyl acceptor. After final O-debenzoylation of **232** (which required a higher concentration of methanolic NaOMe than for *L. donovani* phosphoglycans, seemingly because the 2-benzoic ester of the 'middle' D-Gal*p* unit is more stable than the others), phosphosaccharide **233** was isolated by anion-exchange chromatography.

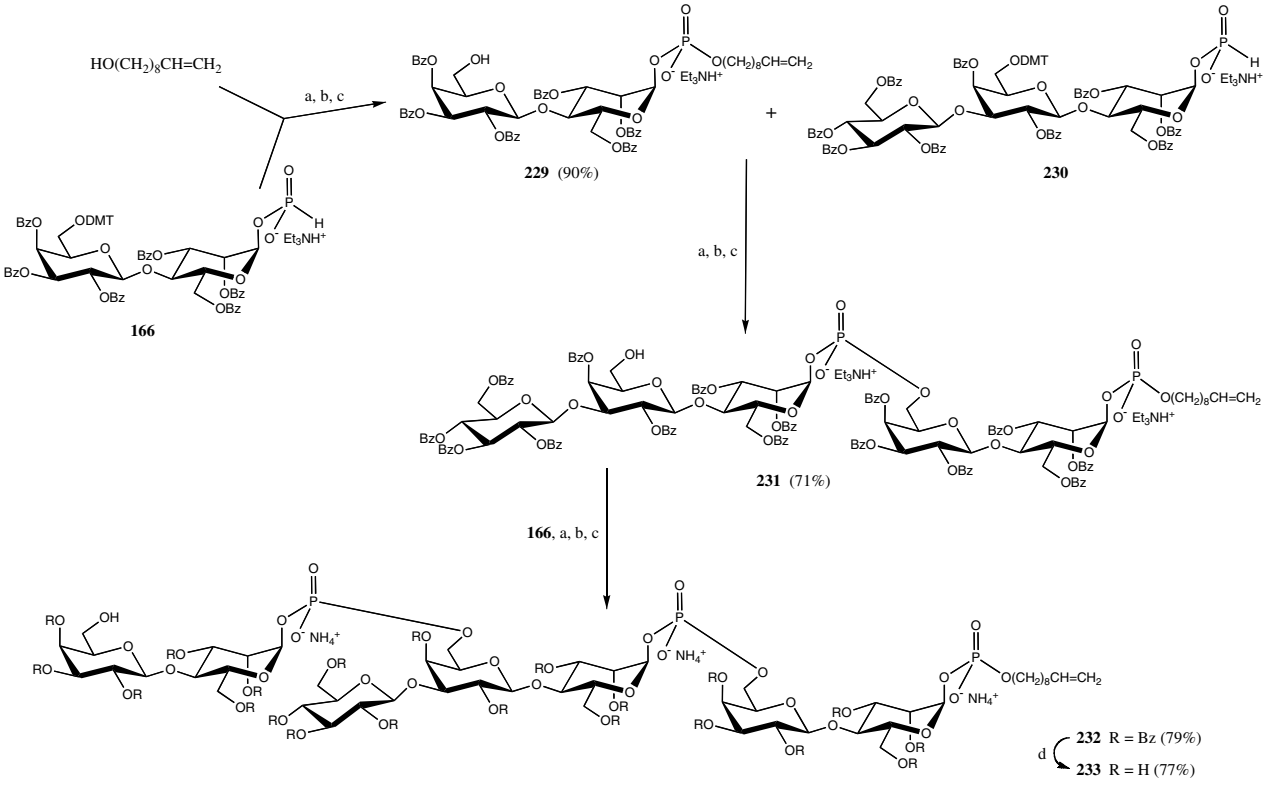
Phosphoglycan **233** was then coupled to a protein carrier (recombinant tetanus toxin fragment C) employing the ozonolysis/reductive amination technique.[234,235] The *L. mexicana*-TetC glycoconjugate (a novel synthetic



Scheme 18. Reagents and conditions: (a) Me_3CCOCl, pyridine; (b) I_2, pyridine–water (95:1), 0 °C; (c) (i) $NaBH_4$, $NiCl_2{\cdot}6H_2O$, MeOH; (ii) Ac_2O, MeOH, 0 °C; (d) NaOMe, MeOH; (e) H_2, 10% Pd/C, 1 M aq $Et_3N{\cdot}HOAc$ (pH 7), ethanol, then gel-permeation chromatography; (f) I_2, pyridine–water (98:2), −40 °C; (g) $Et_3N{\cdot}3HF$, THF; (h) H_2, Pd/C, MeOH, Amberlite IR-45 (OH^-).



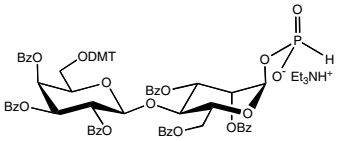
Scheme 19. Reagents and conditions: (a) adamantane-1-carbonyl chloride, pyridine; (b) I_2, pyridine–water (95:5); (c) TFA–DCM (1:99), 0 °C, then aq work-up; (d) 0.05 M NaOMe in MeOH.



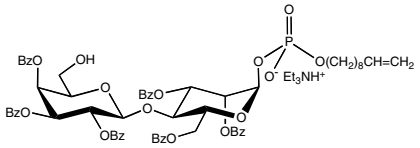
Scheme 20. Reagents and conditions: (a) adamantane-1-carbonyl chloride, pyridine; (b) I_2, pyridine–water (95:5); (c) TFA–DCM (1:99), 0 °C, then aq work-up; (d) 0.25 M NaOMe in MeOH, then anion-exchange chromatography.

glycovaccine) was shown to be protective in mice against the bite of *Leishmania*-infected sand flies.[236]

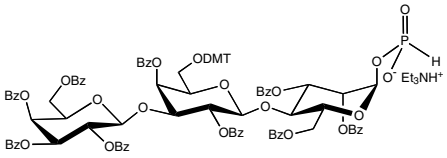
The branched phosphoglycans **236**–**238**[238,239] and **239**–**241**[240–242] from *L. major* (Scheme 21), containing disaccharide, trisaccharide and tetrasaccharide phosphate repeating units, were prepared in a similar fashion using the H-phosphonate building blocks **166**, **234** and **235** and the monohydroxyl derivative **229**. The synthesis of decaglycosyl triphosphate **241**,[241,242] which is the largest molecule of the set, is shown in Scheme 22.



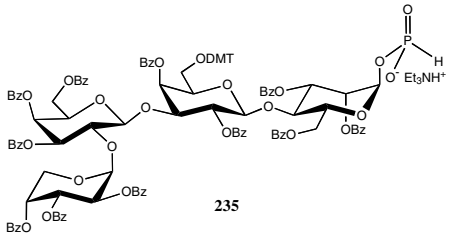
166



229



234



235

β-D-Gal*p*-(1→4)-α-D-Man*p*-(1-PO_3H-6)-β-D-Gal*p*-(1→4)-α-D-Man*p*-(1-PO_3H-6)-β-D-Gal*p*-(1→4)-α-D-Man*p*-1-PO_3H-O-$[CH_2]_8CH{=}CH_2$ **236**

β-D-Gal*p*-(3,1)-

β-D-Gal*p*-(1→4)-α-D-Man*p*-(1-PO_3H-6)-β-D-Gal*p*-(1→4)-α-D-Man*p*-(1-PO_3H-6)-β-D-Gal*p*-(1→4)-α-D-Man*p*-1-PO_3H-O-$[CH_2]_8CH{=}CH_2$ **237**

β-D-Gal*p*-(3,1)-

β-D-Gal*p*-(1→4)-α-D-Man*p*-(1-PO_3H-6)-β-D-Gal*p*-(1→4)-α-D-Man*p*-(1-PO_3H-6)-β-D-Gal*p*-(1→4)-α-D-Man*p*-1-PO_3H-O-$[CH_2]_8CH{=}CH_2$ **238**

β-D-Gal*p*-(3,1)- β-D-Gal*p*-(3,1)-

β-D-Gal*p*-(1→4)-α-D-Man*p*-(1-PO_3H-6)-β-D-Gal*p*-(1→4)-α-D-Man*p*-(1-PO_3H-6)-β-D-Gal*p*-(1→4)-α-D-Man*p*-1-PO_3H-O-$[CH_2]_8CH{=}CH_2$ **239**

β-D-Gal*p*-(3,1)- β-D-Gal*p*-(3,1)-

β-D-Gal*p*-(2,1)-

β-D-Ara*p*

β-D-Gal*p*-(1→4)-α-D-Man*p*-(1-PO_3H-6)-β-D-Gal*p*-(1→4)-α-D-Man*p*-(1-PO_3H-6)-β-D-Gal*p*-(1→4)-α-D-Man*p*-1-PO_3H-O-$[CH_2]_8CH{=}CH_2$ **240**

β-D-Gal*p*-(3,1)- β-D-Gal*p*-(3,1)-

β-D-Gal*p*-(2,1)-

β-D-Ara*p*

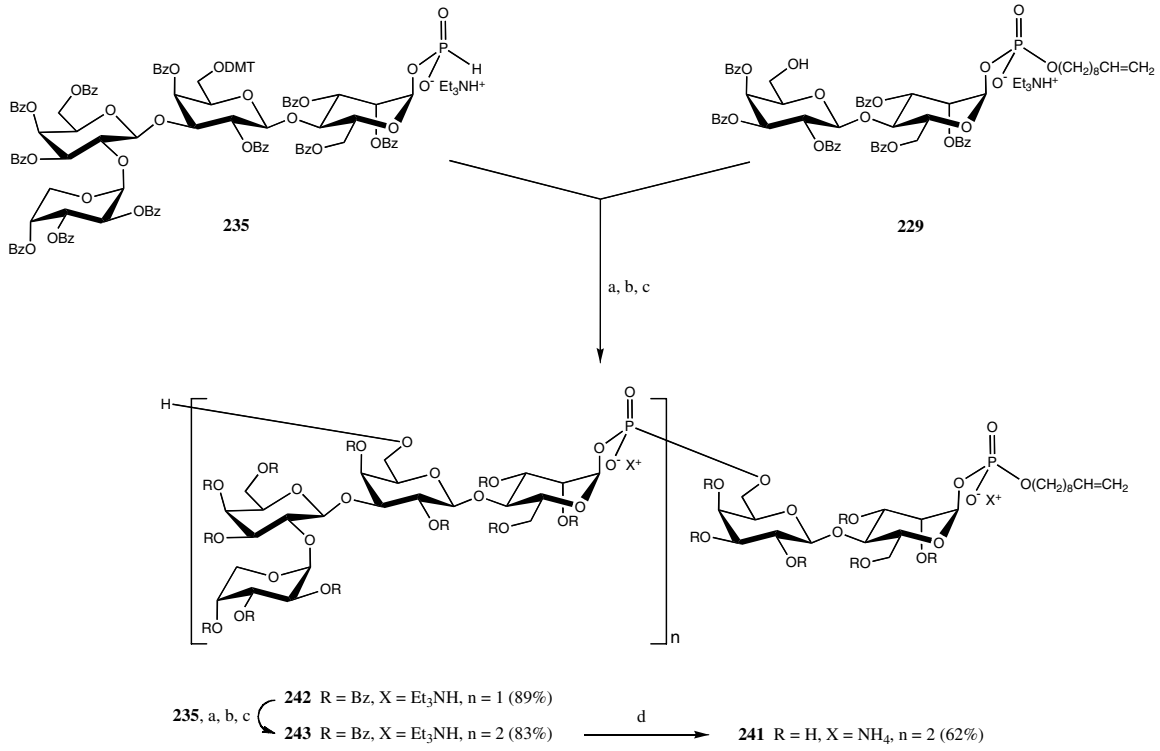
β-D-Gal*p*-(1→4)-α-D-Man*p*-(1-PO_3H-6)-β-D-Gal*p*-(1→4)-α-D-Man*p*-(1-PO_3H-6)-β-D-Gal*p*-(1→4)-α-D-Man*p*-1-PO_3H-O-$[CH_2]_8CH{=}CH_2$ **241**

β-D-Gal*p*-(3,1)- β-D-Gal*p*-(3,1)-

β-D-Gal*p*-(2,1)- β-D-Gal*p*-(2,1)-

β-D-Ara*p* β-D-Ara*p*

Scheme 21.



Scheme 22. Reagents and conditions: (a) Me_3CCOCl, pyridine; (b) I_2, pyridine–water (95:5); (c) TFA–DCM (1:99), 0 °C, then aq work-up; (d) 0.25 M NaOMe in MeOH, then anion-exchange chromatography.

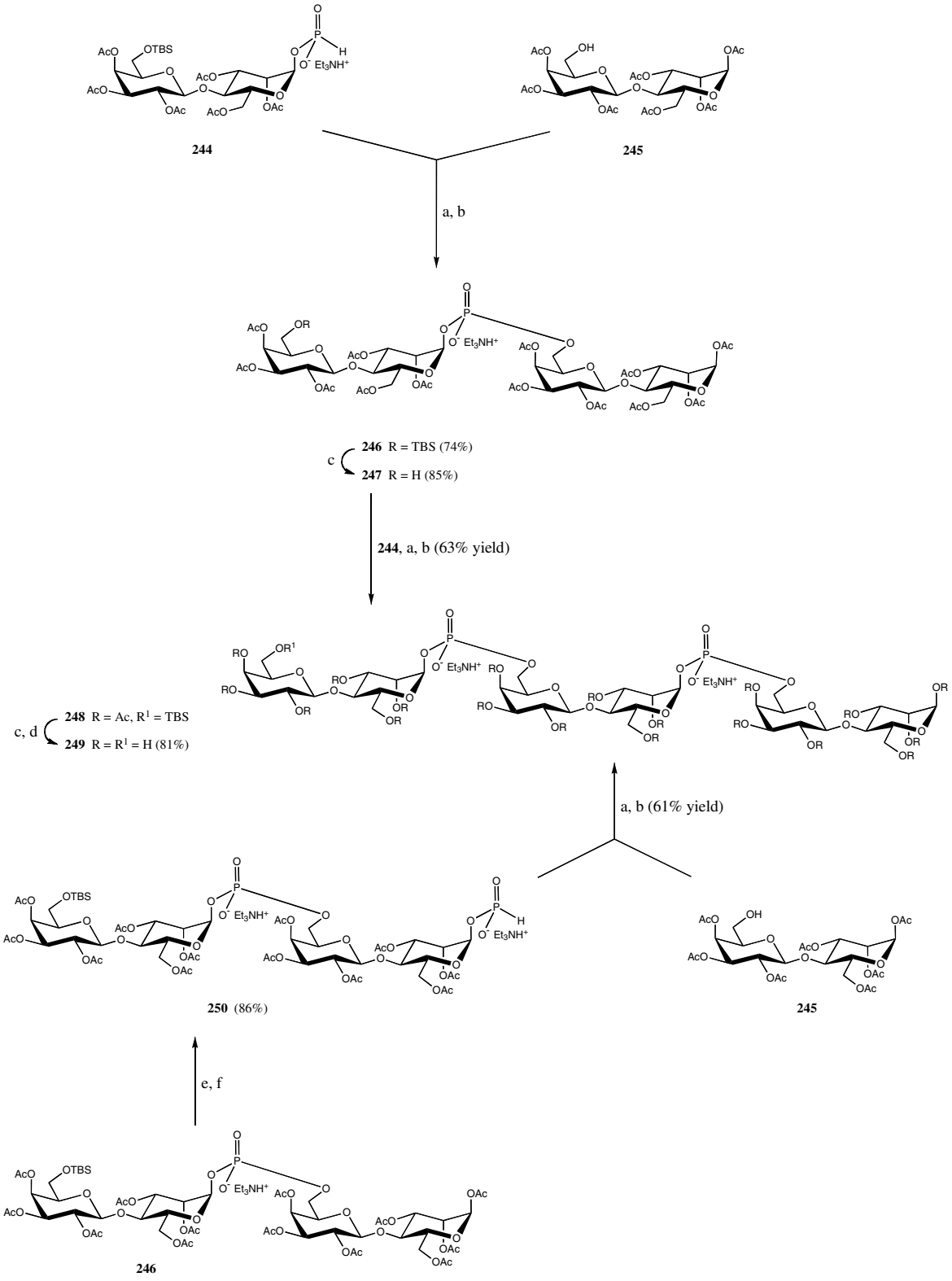
The preparation of the *L. donovani* hexaglycosyl triphosphate structure **249** (which is similar to phosphoglycan **226**, but lacking the dec-9-enyl moiety) was reported by the New Delhi group (Ruhela and Vishwakarma).[243,244] They operated a different protecting-group pattern (Scheme 23): acetic esters for permanent O-protection and the 6′-TBS ether for temporary protection. The first chain elongation (**244**+**245**) afforded the protected tetrasaccharide phosphate derivative **246** (74%), which then was converted into both the monohydroxyl derivative **247** (85%, through desilylation) and the H-phosphonate derivative **250**[†] (86%, through consecutive anomeric deprotection and H-phosphonylation). Thus, the protected phosphoglycan **248** was synthesised by performing a chain extension in two ways: starting from either the reducing termini (i.e., **245**→**246**→**247**→**248**) or the non-reducing termini (i.e., **244**→**246**→**250**→**248**) of the molecule. Standard two-step deprotection provided the targeted phosphoglycan **249** (81%).

4.2.3. Capsular antigens of *H. influenzae* and *S. pneumoniae*. Phosphoglycans **257** (Scheme 24) and **265** (Scheme 25) representing fragments of the capsular antigens from *H. influenzae* type ‘c’ and ‘f’ (Section 2.1), respectively, were synthesised by the Stockholm group.[245] The phosphoglycans are composed of disaccharide phosphate repeating units linked by either (1→4)- (for type ‘c’) or (1→3)- (for type ‘f’) phosphodiester linkages. The type ‘c’ phosphoglycan **257**, which contains 3-*O*-Ac-β-D-Glc*p*NAc and α-D-Gal*p* residues, was synthesised (Scheme 24) using the disaccharide H-phosphonate derivative **251** and the monohydroxyl block **252**. The 3′-TBS ether was chosen as a temporary protecting group, while the *O*-benzyl groups served for permanent protection. This selection of protecting groups allowed the authors to retain the 3-*O*-acetyl group on the Glc*p*NAc residue after the final deprotection.

The condensation of **251** and **252** followed by oxidation gave the protected tetrasaccharide phosphate **253** in an acceptable 71% yield. After desilylation with $Et_3N{\cdot}3HF$, product **254** was coupled with H-phosphonate **251** to produce the hexasaccharide diphosphate block **255** in 36% yield. Total deprotection of **255**, involving desilylation followed by hydrogenolysis over palladium catalyst, provided the type ‘c’ phosphoglycan **257** (77%), which was isolated by gel-permeation chromatography. Hydrogenolysis of **254** gave the shortened phosphoglycan **256**.

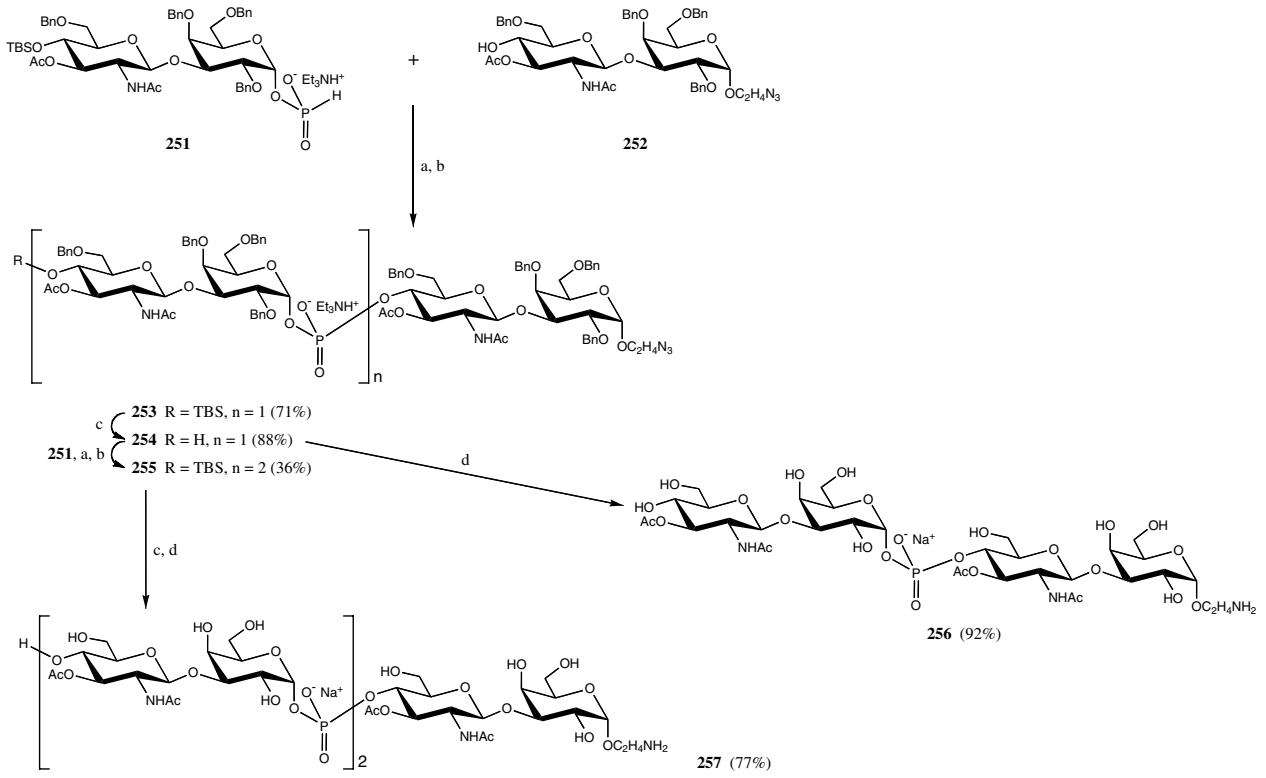
The type ‘f’ phosphoglycan **265** (Scheme 25), containing β-D-Gal*p*NAc and 3-*O*-Ac-α-D-Gal*p*NAc residues, was synthesised in a similar manner from the disaccha-

[†] Phosphodiester-containing glycosyl H-phosphonate structures of this type were described earlier by the Dundee group[247] and used for the blockwise synthesis of poly(glycosyl phosphates) (see Section 4.3.2).

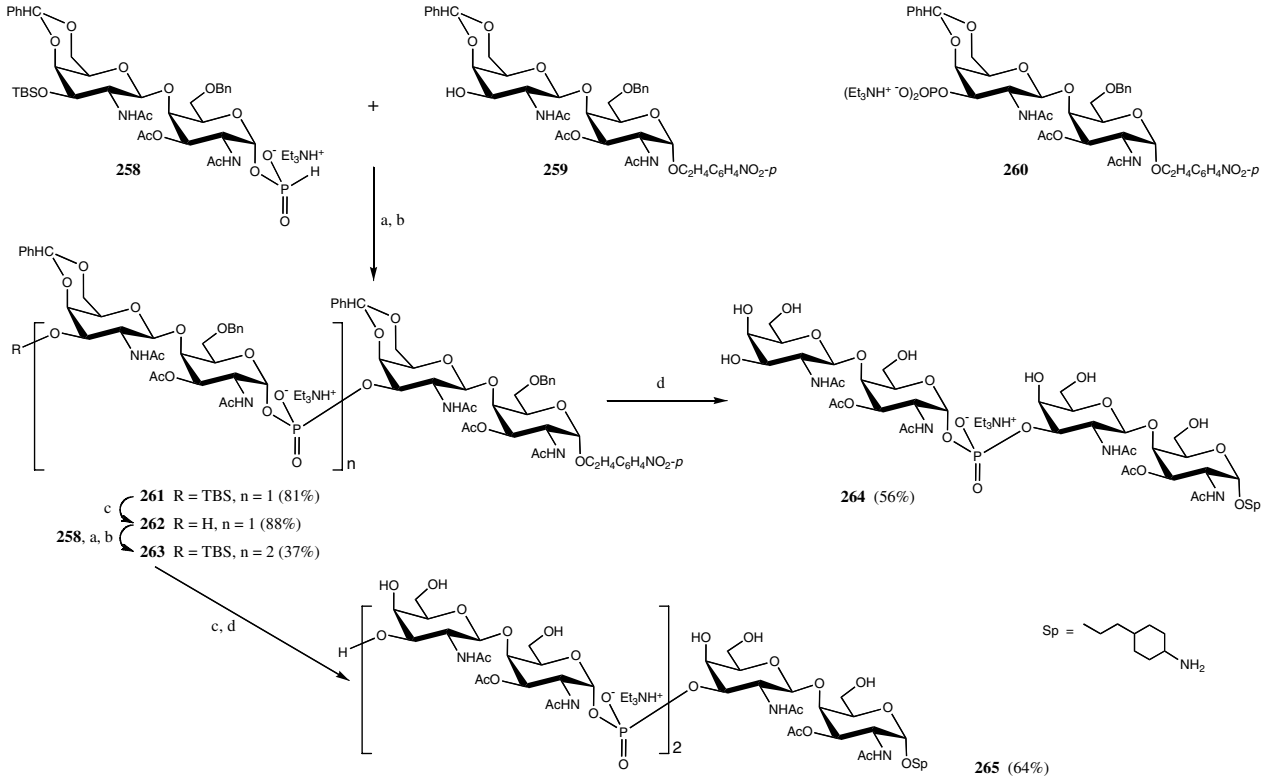


Scheme 23. Reagents and conditions: (a) Me_3CCOCl, pyridine; (b) I_2, pyridine–water (95:5); (c) 48% aq HF–CH_3CN (5:95), 0 °C; (d) 0.56 M NaOMe in MeOH; (e) Me_2NH, MeCN; (f) (i) tri-imidazolylphosphine, MeCN; (ii) 1 M aq Et_3NHHCO_3 (pH 7).

ride H-phosphonate **258** and the monohydroxyl compound **259**. The selected pattern of protecting groups was compatible with retaining the 3-*O*-acetyl group on the Gal*p*NAc residue during the final deprotection. As happened in the previous synthesis, the first coupling (**258**+**259**) afforded phosphodiester **261** in high yield



Scheme 24. Reagents and conditions: (a) Me_3CCOCl, pyridine; (b) I_2, pyridine–water (95:5), −40 °C; (c) $Et_3N{\cdot}3HF$, THF; (d) H_2, Pd/C, 50% aq acetic acid–ethanol (1:1) then gel-permeation chromatography.



Scheme 25. Reagents and conditions: (a) Me_3CCOCl, pyridine; (b) I_2, pyridine–water (95:5), −40 °C; (c) $Et_3N{\cdot}3HF$, THF; (d) H_2, Pd/C, 50% aq acetic acid–ethanol (1:1) then gel-permeation chromatography.

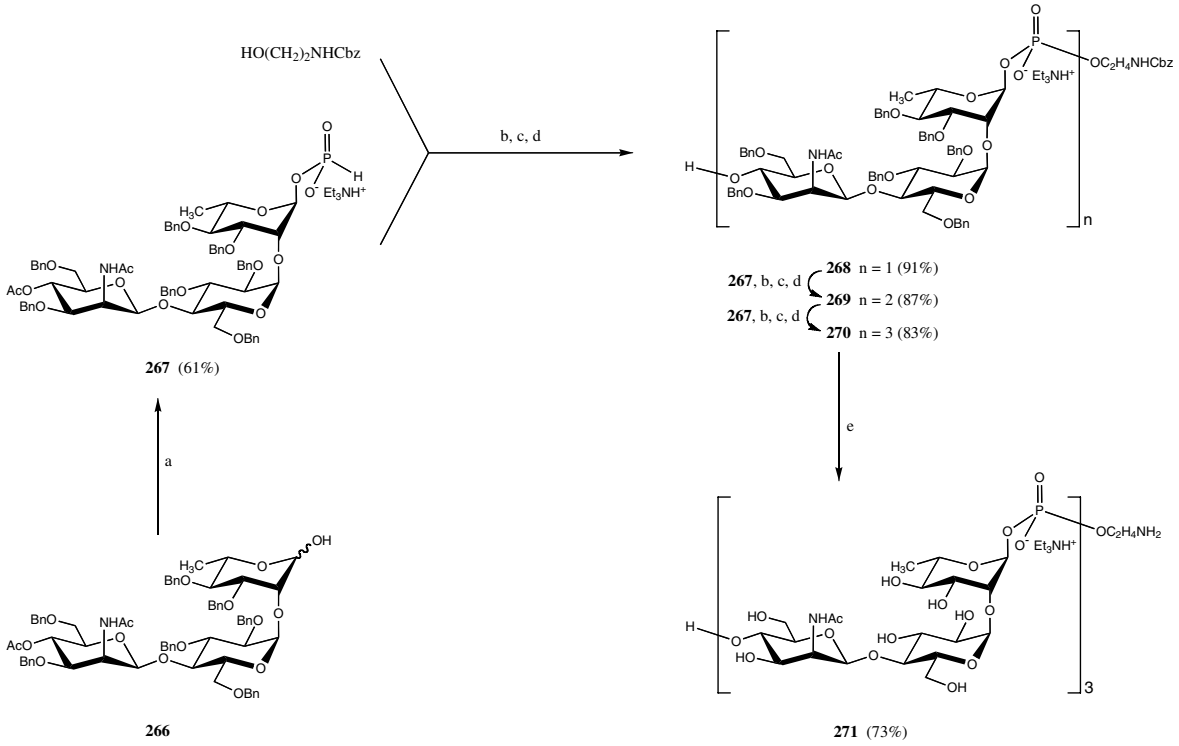
(81%), but at the second coupling (**258**+**262**) the yield of the product (**263**) dropped to 37%. Phosphomonoester **260** (40%), resulting from cleavage of the phosphodiester bond in the monohydroxyl acceptor **262**, was isolated as well. The authors suggest that it was mainly the oxidation conditions that caused decomposition. Desilylation of **263**, followed by hydrogenolysis, provided the deprotected phosphoglycan **265** (64%).

Nilsson and Norberg[182] reported the preparation of a nonaglycosyl triphosphate fragment (Scheme 26) from *S. pneumoniae* 19F capsular antigen (Section 2.2). Phosphoglycan **271**, containing trisaccharide phosphate repeating units joined by (1→4)-phosphodiester linkages, was designed for further coupling (through the 2-aminoethyl spacer) to a protein carrier to produce a potential synthetic anti-pneumococcal vaccine. The trisaccharide H-phosphonate derivative **267** with an α-L-Rha*p* configuration was made by treating hemiacetal **266** (an α,β-mixture) with H_3PO_3 in the presence of a condensing reagent (2-chloro-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphorinane[183]). Benzyl ethers were used for permanent O-protection and the 4-*O*-acetyl was used as a temporary protecting group.

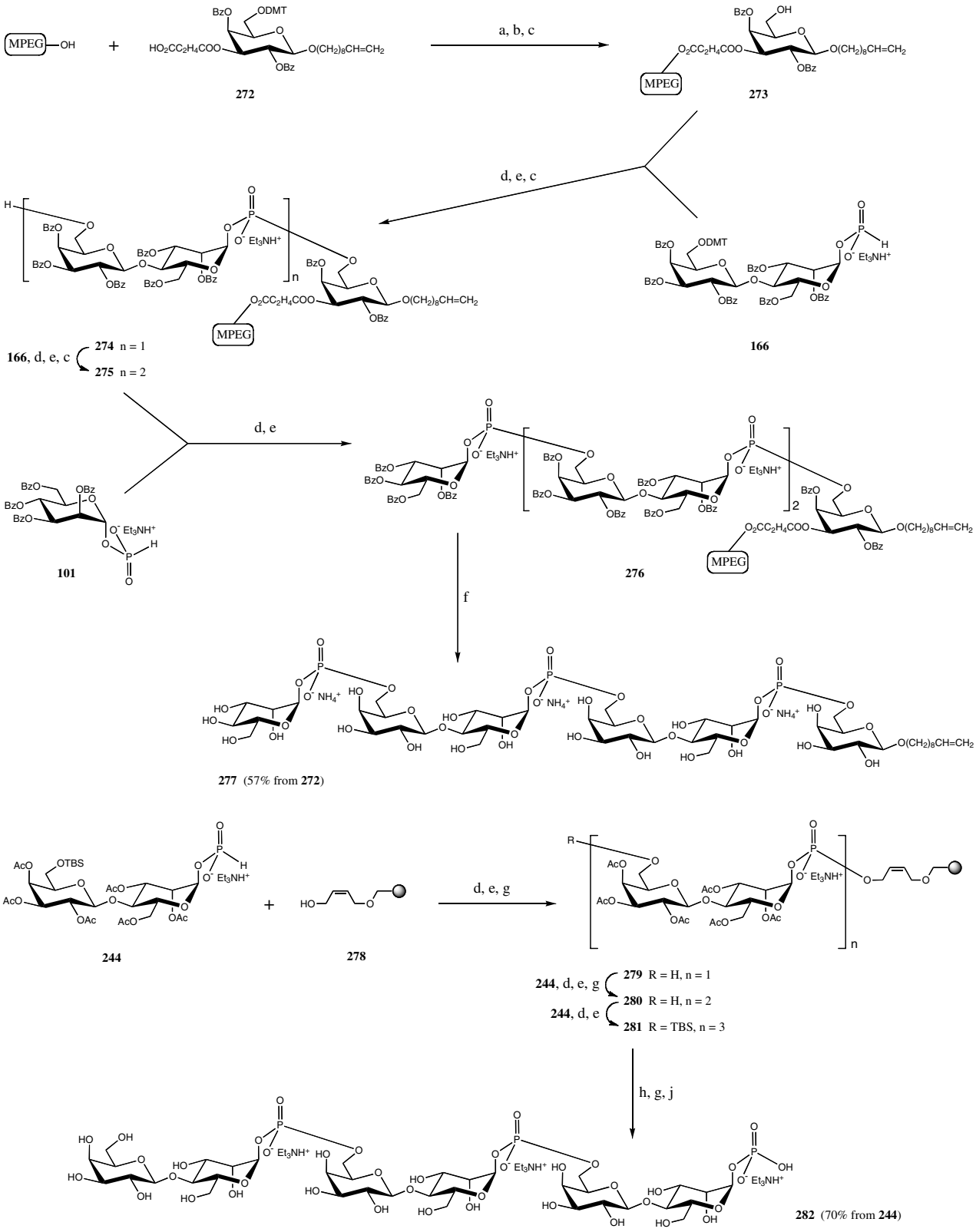
Chain extension was performed using *N*-Cbz-aminoethanol as the first monohydroxyl acceptor. In contrast to the syntheses of *H. influenzae* phosphoglycans, where the coupling yields were reduced as the chain grew, the yields of the protected phosphoglycans **268**–**270** isolated after each chain-elongation cycle (including condensation, oxidation and deacetylation) were in the 83–91% range. Hydrogenolysis of **270** gave the deprotected phosphoglycan **271** in 73% yield.

4.3. Alternative synthetic approaches to poly(glycosyl phosphates)

4.3.1. Polymer-supported syntheses. Stepwise chain elongation in combination with the H-phosphonate condensation protocol was developed further for polymer-supported syntheses of poly(glycosyl phosphates). The Dundee group reported[246] the preparation of the *L. donovani* phosphoglycan structure **277** (Scheme 27) using the glycosyl H-phosphonates **166** and **101** for consecutive chain elongations, the 6-*O*-DMT-protected β-D-galactoside derivative **272** as a masked hydroxyl acceptor and monomethyl polyethylene glycol (MPEG; $M = 5000$) as a polymer support. The advantage of MPEG is that it allows for both solid-phase and solution-phase techniques: the MPEG-bound products are soluble in most organic solvents and water; however, they can be precipitated (quantitatively) from ether or cold ethanol greatly simplifying their purification. The succinic ester linker was used for binding galactoside **272** to the polymer in the presence of 1-(2-mesitylenesulfonyl)-3-nitro-1,2,4-triazole (MS-NT) in 91% yield. Successive acetylation of any residual OH-groups



Scheme 26. Reagents and conditions: (a) (i) H_3PO_3, 2-chloro-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphorinane, pyridine; (ii) 1 M aq Et_3NHHCO_3 (pH 7); (b) Me_3CCOCl, pyridine; (c) I_2, water; (d) 0.2 M NaOMe in MeOH; (e) H_2, Pd/C, 50% aq acetic acid–ethanol (1:1) then gel-permeation chromatography.



Scheme 27. Reagents and conditions: (a) MS-NT, 1-methylimidazole, DCM; (b) Ac_2O, pyridine; (c) TFA–DCM (1:99), 0 °C, then Et_3N–MeOH; (d) Me_3CCOCl, pyridine; (e) I_2, pyridine–water (95:5); (f) 0.05 M NaOMe in MeOH, then anion-exchange chromatography; (g) 48% aq HF–CH_3CN (5:95), 0 °C; (h) $(Ph_3P)_3RhCl$, 0.01 M aq HCl–propan-1-ol–toluene (0.08:1:2); (j) Et_3N–MeOH–water (1:5:2).

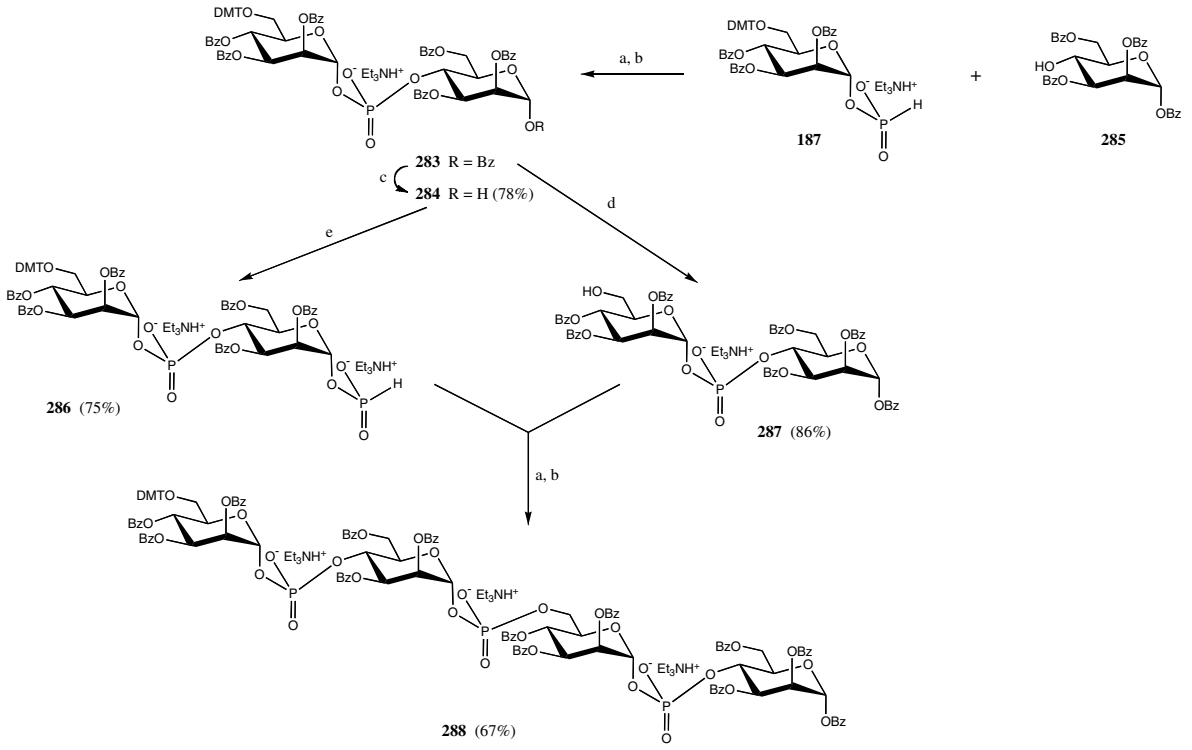
and acidic detritylation afforded the MPEG-bound hydroxyl acceptor **273**. Standard chain-elongation cycle engaging the disaccharide H-phosphonate derivative **166** (i.e., condensation, oxidation and detritylation)

was applied twice to provide polymer **275**. H-phosphonate **101** was then used to terminate the chain extension (→**276**). After total deprotection with methanolic NaOMe, which also cleaved the product from MPEG, hexaglycosyl triphosphate **277** (57% from **272**, corresponding to an average of 95% per step) was isolated by anion-exchange chromatography.

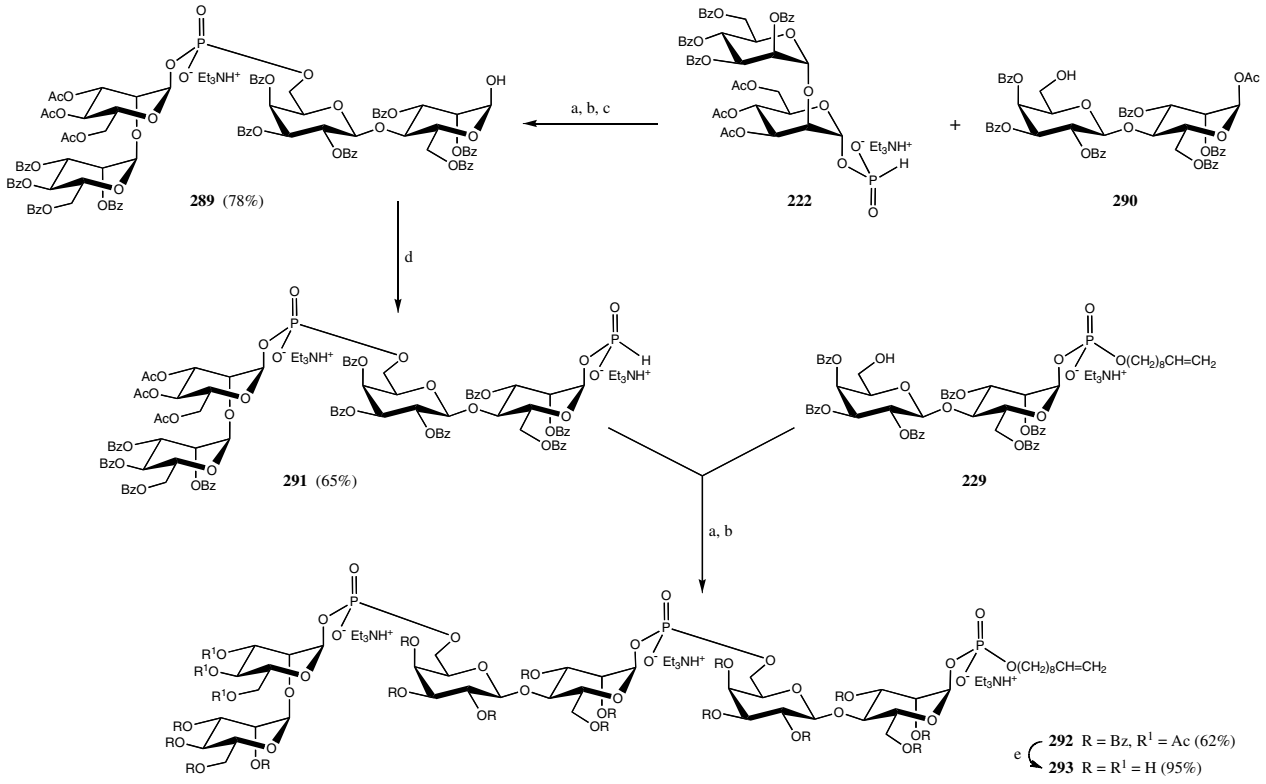
A solid-phase synthesis of the *L. donovani* phosphoglycan **282** (Scheme 27) was performed by the New Delhi group[244] by making use of the linker functionalised Merrifield resin **278** and the disaccharide H-phosphonate **244**. The iterative chain-elongation cycle was carried out three times to give the polymer-bound phosphoglycan **281**. The coupling efficiency of each cycle was estimated to be over 90%. Cleavage of the phosphoglycan from the resin with Wilkinson's catalyst, followed by desilylation (aq HF in CH_3CN) and deacetylation, provided the targeted hexasaccharide triphosphate **282** in 70% yield (based on **244**).

4.3.2. Blockwise chain elongation. A blockwise chain elongation strategy, involving the H-phosphonate condensation of two phosphodiester blocks, was evolved by the Dundee group.[247] First the model tetramannosyl triphosphate **288** with alternate (1→4)- and (1→6)-phosphodiester linkages (Scheme 28) was synthesised starting from the monosaccharide H-phosphonate and the monosaccharide monohydroxyl derivatives **187** and **285**, respectively. Their condensation and subsequent oxidation resulted in phosphodiester **283**, which was then converted into both the monohydroxyl phosphodiester block **287** (by acidic detritylation, 86%) and the hemiacetal phosphodiester block **284** (by anomeric debenzoylation with Me_2NH, 78%). The latter formed the glycosyl H-phosphonate phosphodiester block **286** (75%) on reaction with tri-imidazolylphosphine. The coupling of **286** and **287** gave the protected phosphomannan **288** (67%), which could be used for further blockwise chain elongation to form a linear octamannosyl heptaphosphate. A block synthesis would be particularly advantageous in the preparation of regular phosphoglycans as fewer coupling steps are required than in stepwise elongation of the chain.

Hexaglycosyl triphosphate **293** (Scheme 29), representing a structure at the non-reducing end of *L. donovani* phosphoglycan, was also prepared using the blockwise approach. The hemiacetal phosphodiester block **289** was formed (78%) by the condensation of disaccharide H-phosphonate **222** and the monohydroxyl derivative **290** followed by oxidation and anomeric deacetylation. It was then converted into the glycosyl H-phosphonate phosphodiester block **291** (65%) by standard H-phosphonylation. Further coupling with the earlier described[237] monohydroxyl phosphodiester block **229** (see Scheme 20), followed by oxidation (→**292**) and total deprotection, afforded the targeted phosphoglycan **293**.



Scheme 28. Reagents and conditions: (a) Me_3CCOCl, pyridine; (b) I_2, pyridine–water (95:5); (c) Me_2NH, MeCN–THF–toluene; (d) TFA-DCM (1:99), 0 °C, then aq work-up; (d) (i) tri-imidazolylphosphine, MeCN–pyridine; (ii) 1 M aq Et_3NHHCO_3 (pH 7).



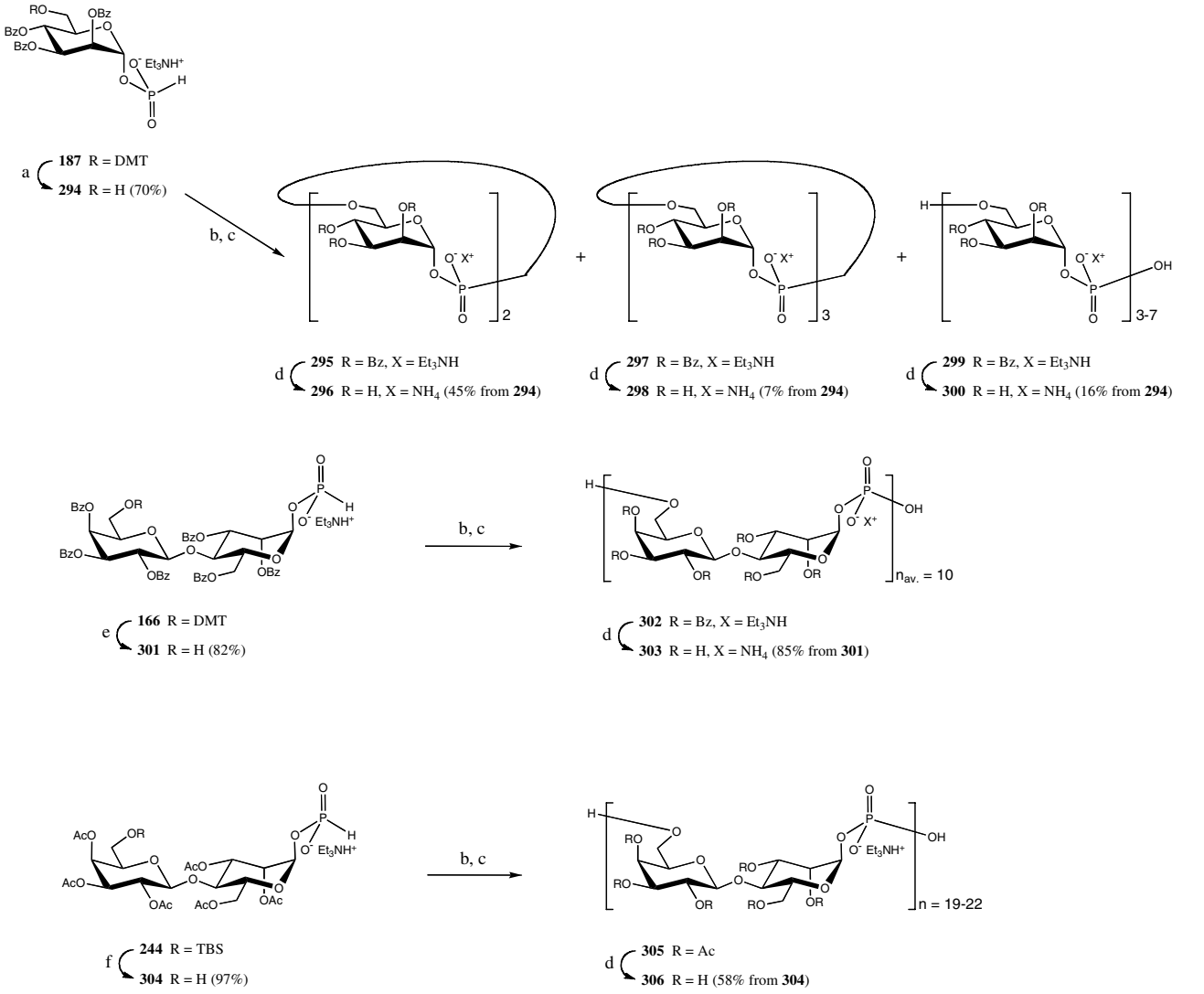
Scheme 29. Reagents and conditions: (a) adamantane-1-carbonyl chloride, pyridine; (b) I_2, pyridine–water (95:5); (c) Me_2NH, MeCN–THF; (d) (i) tri-imidazolylphosphine, MeCN; (ii) 1 M aq Et_3NHHCO_3 (pH 7); (e) 0.05 M NaOMe in MeOH.

4.3.3. The polycondensation approach. The high efficiency and high reaction rate of the H-phosphonate condensation reaction, which was demonstrated by various authors when the reaction was invigilated by ^{31}P NMR,[162,176,245] prompted researchers to investigate the polycondensation reaction of a partially protected H-phosphonate monomer as an approach to poly(glycosyl phosphates). Thus, trimethylacetyl chloride mediated polycondensation of the mannosyl H-phosphonate derivative **294** (Scheme 30) was first attempted by the Moscow group.[248,249] After oxidising the reaction intermediates with iodine, the three major fractions isolated by flash chromatography on SiO_2 were subjected to conventional debenzoylation. Unexpectedly, the polycondensation was found to give the cyclic (1→6)-linked di(α-D-mannosyl phosphate) **296** (45%) as the main product, as well as small proportions of the cyclic trimer **298** (7%) and the linear tri-hepta(α-D-mannosyl phosphates) **300** (16%).

A polycondensation of the bifunctional monomer **301** (performed by the Dundee group[250]) appeared to be more successful. Compound **301**, in turn, was prepared by mild acidic detritylation of the H-phosphonate derivative **166**. A high concentration (1 M) of the monomer was used in order to avoid the formation of cyclic products. The reaction of **301** with Me_3CCOCl in a pyridine–Et_3N mixture was monitored by ^{31}P NMR, which indicated the smooth formation of the corresponding H-phosphonic diester intermediate (δ_P 7.5, $J_{P,H} = 720$ Hz). Subsequent oxidation provided the protected polymer **302** (δ_P −2.81, major signal), which was isolated by precipitation from ethanol. The major product (85%) isolated after deprotection of **302** was the linear poly(glycosyl phosphate) **303**, representing the phosphoglycan part of the LPG from *L. donovani*, with an average degree of polymerisation 10. The range of apparent chain length was estimated as $n = 4$–25.

The preparation of the *L. donovani* phosphoglycan **306**, which differs from **303** in the chain length, was reported by the New Delhi group.[244] Polycondensation of the bifunctional monomer **304**, containing *O*-acetyl (instead of *O*-benzoyl) protecting groups, and subsequent oxidation seems to favour the formation of longer polymer chains. After deacetylation of the reaction product (**305**), polymer **306** with a degree of polymerisation $n = 19$–22 was isolated in 58% yield.

The H-phosphonate polycondensation methodology has been used recently by Verez-Bencomo et al.[251] for the preparation of the first synthetic glycoconjugate vaccine, namely an anti-meningitis vaccine against *H. influenzae* type 'b' in humans. The results of clinical testing in the target population demonstrated that this synthetic glycovaccine containing fully synthetic phosphosaccharides is as effective as its natural counterpart. The vaccine was registered in Cuba in 2003 and is now part of the nation immunisation programme.



Scheme 30. Reagents and conditions: (a) CCl_2HCO_2H–DCM (4:96), 0 °C, then aq work-up; (b) Me_3CCOCl, pyridine–Et_3N (9:1); (c) I_2, pyridine–water (95:5); (d) 0.1 M NaOMe in MeOH–1,4-dioxane–$CHCl_3$, then anion-exchange chromatography; (e) TFA–DCM (1:100), −12 °C then aq work-up; (f) AcOH–THF–water (3:1:1).

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