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The chemical synthesis of *Leishmania donovani* phosphoglycan via polycondensation of a glycobiosyl hydrogenphosphonate monomer

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Abstract

A polycondensation of 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl)-α-D-mannopyranosyl hydrogenphosphonate in the presence of trimethylacetyl chloride has been used to synthesize a linear poly[β-D-galactopyranosyl-(1 → 4)-α-D-mannopyranosyl phosphate] representing the phosphoglycan part of the lipophosphoglycan from *Leishmania donovani*.

Keywords: Synthesis; Phosphoglycan; *Leishmania*; Glycosyl H-phosphonate

1. Introduction

In recent papers [1,2], a highly efficient method for the synthesis of long-chain fragments of natural poly(glycosyl phosphates) via glycosyl hydrogenphosphonates has been described. These phosphoglycans, which are surface antigens of numerous microorganisms [3] and parasitic protozoa [4], are composed of mono- or oligo-saccharide repeating units connected by phosphoric diester bridges between the hemiacetal and alcoholic hydroxyl groups of the neighbouring units. A series of oligo(glycosyl phosphate) fragments of the yeast and bacteria phosphoglycans [1] and of the lipophosphoglycan (LPG) from the protozoan parasite *Leishmania donovani* [2] has been prepared

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using a stepwise strategy for the elongation of the chain. The polycondensation of partially protected glycosyl hydrogenphosphonates in the presence of a condensing reagent could be an alternative approach to synthetic poly(glycosyl phosphates). However, the only published example [5], a polycondensation of 2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl hydrogenphosphonate, was found to give cyclic (1 → 6)-linked di(α-D-mannosyl phosphate) as the main product (45%) and only a minor fraction (16%) of linear (1 → 6)-linked tri–hepta(α-D-mannosyl phosphates). We now report the application of the polycondensation reaction for the preparation of the phosphoglycan part of the LPG from *Leishmania donovani* consisting of (1 → 6)-linked β-D-galactopyranosyl-(1 → 4)-α-D-mannopyranosyl phosphate repeating units [4].

α-D-Man*p*-(1 → 2)-α-D-Man*p*-(1 → P → 6)-[β-D-Gal*p*-(1 → 4)-α-D-Man*p*-(1 → P → 6)]$_n$-GPI anchor

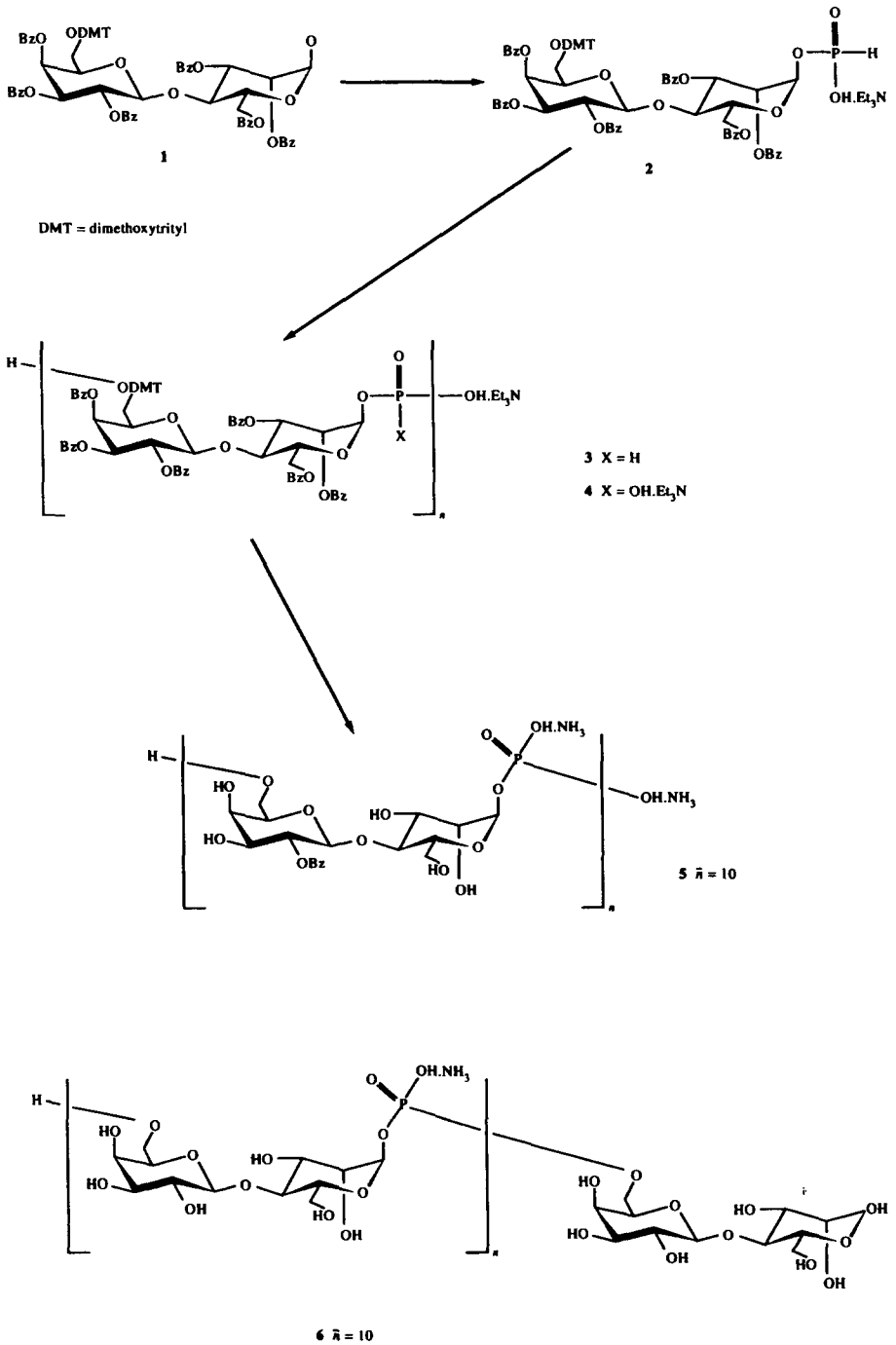
LPG *Leishmania donovani*

2. Results and discussion

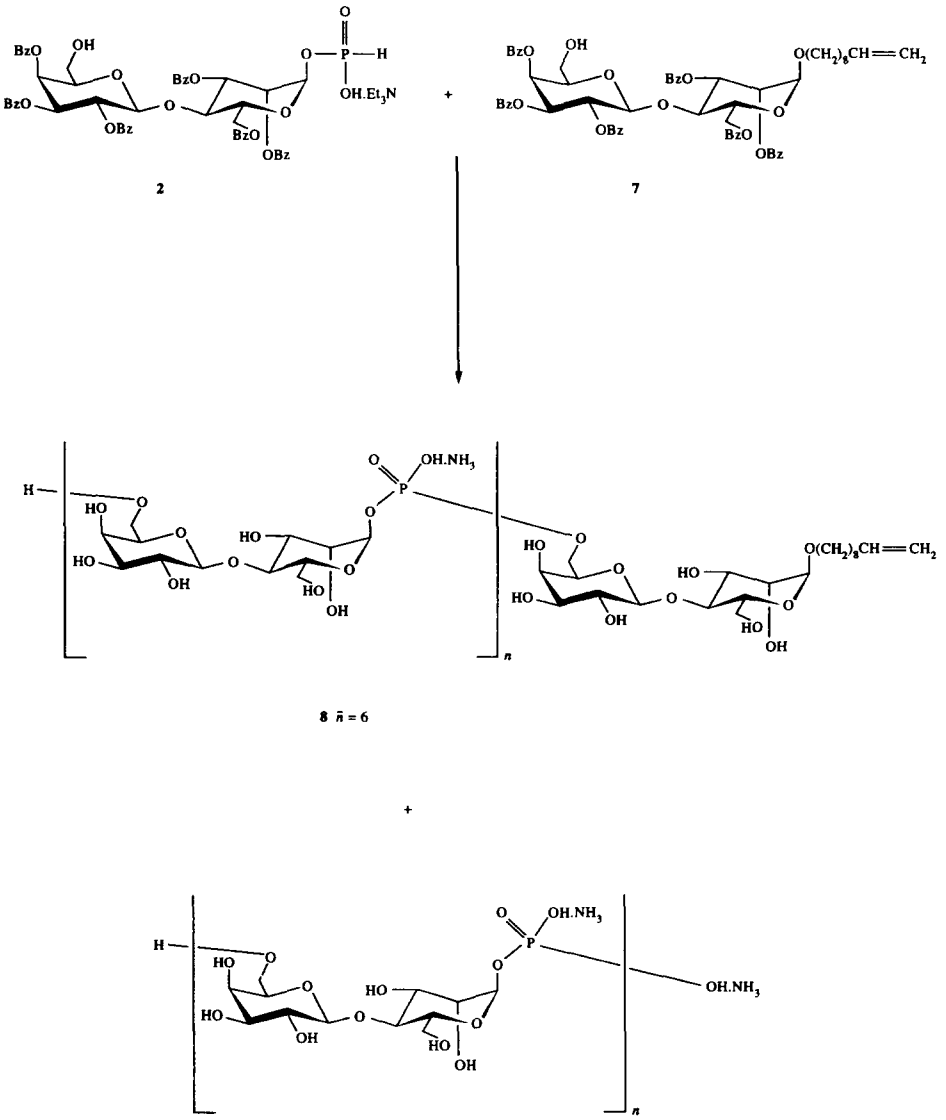
Our approach is based on the use of 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl)-α-D-mannopyranosyl hydrogenphosphonate (**2**) as a bifunctional monomer for the synthesis of the linear phosphoglycans **5** and **8** (Schemes 1 and 2). The monomer **2** was prepared from the recently described [2]α-hemiacetal derivative **1** by successive phosphitylation with tri-imidazolylphosphine and mild dedimethoxytritylation with 1% CF_3CO_2H in dichloromethane in an overall yield of 82%. Signals characteristic of the hydrogenphosphonate group [δ_P 0.14, δ_{HP} 7.0 ($^1J_{H,P}$ = 638 Hz), $\delta_{H\text{-}1}$ 5.71 ($^3J_{H,H}$ = 2.0, $^3J_{H,P}$ = 8.6 Hz), $\delta_{C\text{-}1}$ 92.59 ($^2J_{C,P}$ = 3.6 Hz), $\delta_{C\text{-}2}$ 70.92 ($^3J_{C,P}$ = 7.2 Hz)] were present in the ^{31}P, ^1H, and ^{13}C NMR spectra. The main signal in the ES(−) mass spectrum corresponded to the pseudomolecular ion for the derivative **2**: *m/z* 1028.8 $[M - Et_3NH]^-$.

The polycondensation of the hydrogenphosphonate **2** was accomplished in 10:1 pyridine–triethylamine in the presence of trimethylacetyl chloride (2.5 equiv). Quite a high concentration of the monomer (1 M instead of 0.1 M in [5]) was used to avoid the formation of cyclic products. The signal of **2** was absent and the major signal characteristic of hydrogenphosphonic diesters **3** [δ_P 7.5 ($^1J_{P,H}$ 720 Hz)] was present in the ^{31}P NMR spectrum of the reaction mixture after 50 min. After 2 h, the mixture (which resembled a viscous glue) was treated with iodine in aqueous pyridine to oxidize the hydrogenphosphonic diesters to phosphoric diesters, as shown by the appearance of the new spectrum with the main signal at δ_P −1.86. The benzoylated phosphoglycan **4** was isolated as a powder after working up and precipitation from ethanol. In accordance with the proposed structure, the ^{31}P NMR spectrum of **4** contained the major signal for phosphoric diester (δ_P −2.81) and the minor signal for phosphoric monoester (δ_P 0.29) with the ratio of the integral intensities of ca. 12:1.

The deprotection of **4** with 0.1 M NaOMe in methanol–dioxane–chloroform led to a polymeric product, which was eluted with the void volume on a column of Bio-Gel P-4 in water [6] and was fractionated by ion-exchange chromatography on Fractogel TSK DEAE-650 (HCO_3^- form) by elution with 0–0.5 M aqueous NH_4HCO_3. The major



Scheme 1.



Scheme 2.

fraction (85%), which was eluted with 0.22–0.41 M NH_4HCO_3, was shown to be the linear (1 → 6)-linked poly(galactosylmannosyl phosphate) **5** with the average degree of polymerization $\bar{n} = 10$. The minor fraction eluted earlier (10%) was shown to have a linear structure of the shorter oligo(glycobiosyl phosphate) **6** ($\bar{n} = 3$–4), which did not contain a phosphomonoester residue at the reducing end of the chain. The NMR data,

GC-MS analysis, and gel filtration on a column of Sephadex G-50 were used to prove the structure and to determine the degree of polymerization for **5** and **6**.

In accordance with the structure **5**, the ^{13}C NMR spectrum contained the main series of 12 signals corresponding to the β-D-galactopyranosyl-(1 → 4)-α-D-mannopyranosyl units (1 → 6)-linked through phosphate residues (see Experimental section). The position of the phosphodiester linkages was confirmed by the C-1 (δ 97.15 br) and C-2 (δ 71.16, $J_{C,P}$ ~ 8 Hz) signals of the mannose and C-5 (δ 75.07, $J_{C,P}$ ~ 7 Hz) and C-6 (δ 65.84 br) of the galactose residues, which were shifted as a result of the α-and β-effects of phosphorylation and coupled with P (or broadened). The α configuration of the mannosyl phosphate fragments followed from the positions of the mannose C-3 (δ 70.07) and C-5 (δ 73.67) resonances. The latter were close to the chemical shifts of C-3 and C-5 of α-D-mannopyranosyl phosphate [7] taking into account the influence of the β-D-galactopyranosyl substituent at position 4. A few minor signals of the terminal nonphosphorylated β-D-galactopyranosyl group (δ_{C-1} 104.22, δ_{C-5} 76.73, δ_{C-6} 62.51) were also identified. In the ^{31}P NMR spectrum of **5**, the major signal for phosphoric diester (δ −1.30) and the minor signal (δ 1.20) corresponding to a mannosyl phosphate terminal residue were present with a ratio of ca. 10 : 1. The characteristic H-1 signals of β-D-galactopyranosyl (δ 4.47, $J_{1,2}$ = 7.8 Hz) and α-D-mannopyranosyl phosphate (δ 5.44, $J_{1,P}$ = 6.8 Hz) units were present in the ^1H NMR spectrum of **5** (in a ratio of 1 : 1). The spectrum of **5** treated with alkaline phosphatase, to cleave the phosphoric monoester residues, showed additional minor signals of the reducing α-D-mannose (δ_{H-1} 5.20) and β-D-mannose (δ_{H-1} 4.95) residues with the ratio of the integral intensities of mannosyl phosphate H-1 to mannose H-1 of ca. 9.2 : 1. Taken together, these data indicate that **5** has $\bar{n} = 10$.

The ^{13}C NMR spectrum of the oligomer **6** was similar to that of **5** except that a set of signals corresponding to the terminal reducing mannose residue and nonphosphorylated galactosyl residue was present in addition to the major series corresponding to the internal β-D-galactopyranosyl and α-D-mannopyranosyl phosphate units (see Experimental section). The only characteristic signal (δ −1.23) in the ^{31}P NMR spectrum and the H-1 resonances of the α-D-mannopyranosyl phosphate (δ 5.45, $J_{1,P}$ = 7.0 Hz) and the reducing α,β-D-mannose (δ 5.19 and 4.92) residues in the ^1H NMR spectrum confirmed the presence of the phosphoric diester bridges and the absence of a terminal phosphomonoester group in **6**. From the ^1H NMR spectrum, which did not change after digestion of **6** with the alkaline phosphatase, the average chain length was found to be $\bar{n} = 4$.

GC-MS analysis of Me_3Si-derivatives, after treatment of **5** and **6** with alkaline phosphatase followed by reduction with $NaBH_4$ and methanolysis (0.5 M HCl/MeOH) [6], indicated the presence of mannose and mannitol in the proportions corresponding to $\bar{n} = 10$ for **5** and $\bar{n} = 3.5$ for **6**. No mannitol was revealed after similar analysis of **5** without alkaline phosphatase digestion.

An independent confirmation of the molecular masses of **5** and **6** was obtained by gel filtration on a calibrated column of Sephadex G-50 eluted with 0.5 M NH_4HCO_3. The calibration diagram indicating linear dependence of the elution volume on the logarithm of molecular mass (Fig. 1) was prepared using the synthetic oligo(glycosyl phosphates) **10–13** [2,8] and polythymidylic acid $d(pT)_{12-18}$ as standards. The range of apparent

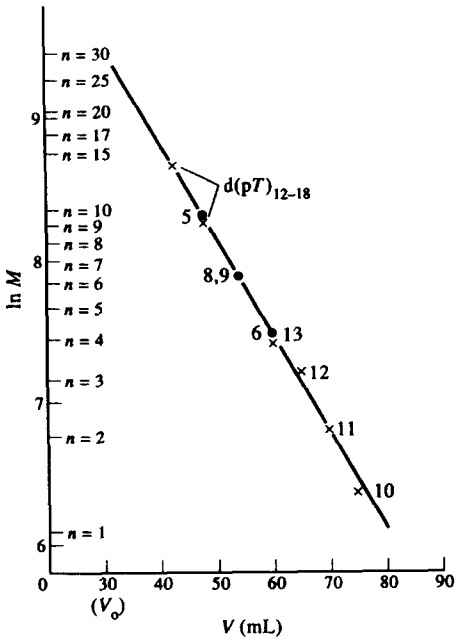


Fig. 1. Determination of the average degree of polymerization for **5**, **6**, **8**, and **9** by gel filtration. The Sephadex G-50 column (see Experimental section) was calibrated with compounds **10**–**13** and with polythymidylic acid $d(pT)_{12-18}$, as indicated (x). The graph shows the relationship of ln M versus elution volume. The calculated values of ln M corresponding to different degrees of polymerization (n) of structure **5** are shown on the left. The measured elution volumes of compounds **5**, **6**, **8**, and **9** are indicated on the diagram (●).

chain length was estimated as $n = 4$–25 for **5** and $n = 2$–7 for **6**, but the maxima of the peaks corresponded to the same average "$\bar{n}$" values as determined by the NMR and GC-MS analyses.

$$[\text{Man}p(\alpha 1 \rightarrow 2)\text{Man}p(\alpha)\text{-}PO_4NH_4]_l\text{-}[6\text{Gal}p(\beta 1 \rightarrow 4)\text{Man}p(\alpha)\text{-}PO_4NH_4]_m\text{-}[6\text{Gal}p(\beta 1 \rightarrow 4)\text{Man}p(\alpha)\text{O}]_n\text{-R}$$

$$R = (CH_2)_8CH{=}CH_2$$

10 $l = n = 0$, $m = 1$
11 $l = 0$, $m = n = 1$
12 $l = 0$, $m = 2$, $n = 1$
13 $l = n = 1$, $m = 2$

The phosphoglycan **8** containing a 9-decenyl moiety at the reducing end of the chain was prepared by the copolycondensation of the monomer **2** and the monohydroxylic 9-decenyl bioside **7** (0.1 equiv) [2]. After debenzoylation of the reaction products, the phosphoglycan **8** ($\bar{n} = 6$) was isolated in a yield of 60% by ion-exchange chromatography (see above). A later eluted fraction (25%) was shown to be the linear (1 → 6)-linked poly(galactosylmannosyl phosphate) **9** ($\bar{n} = 6$–7), which did not contain a terminal 9-decenyl bioside unit.

Phosphoglycan **8** migrated in TLC (see Experimental section) slower than octaglycosyl triphosphate **13**, presumably because of the longer chain. The ^{1}H and ^{13}C NMR spectra of **8** contained both the main sets of signals corresponding to the β-D-galactopyranosyl-(1 → 4)-α-D-mannopyranosyl phosphate backbone (see Experimental section) and the signals of the 9-decenyl α-D-mannopyranoside fragment [Man: δ_{H-1} 4.87, δ_{C-1} 100.54, δ_{C-2} 70.81, δ_{C-3} 70.64, δ_{C-4} 78.65, δ_{C-5} 72.39; $-(CH_2)-$: δ_H 1.30, 2.05, 3.40, δ_C 26.67, 29.38, 34.46; $CH_2=CH-$: δ_H 4.96 ($^{3}J_{H,H}$ = 10 Hz), 5.04 ($^{3}J_{H,H}$ = 18 Hz), 5.92 (m), δ_C 115.28, 138.97]. The presence of the phosphoric diester groups was confirmed by the single resonance (δ − 1.40) in the ^{31}P NMR spectrum. The average $\bar{n}$ = 6 was determined from the ratio of the integral intensities of α-D-mannopyranosyl phosphate H-1 (δ 5.43) and the =CH– (δ 5.92) of the decenyl group in the ^{1}H NMR spectrum. This was confirmed by gel filtration on the calibrated column of Sephadex G-50 (see Fig. 1). The structure of the phosphoglycan **9** was confirmed by similar methods.

To summarize, the first chemical synthesis of a natural phosphoglycan, a poly(glycobiosyl phosphate) structure, has been achieved using the polycondensation of a glycosyl hydrogenphosphonate derivative.

3. Experimental

Optical rotations were measured with a Perkin–Elmer 141 polarimeter. GC-MS analysis was performed on a Hewlett–Packard 5890 MSD system with a 30 m × 0.25 mm Econocap SE-54 bonded phase column (Alltech) using electron impact (70 eV). The negative ion electro-spray mass spectrum (ES-MS) was recorded with a VG Quattro system (VG Biotech, UK). NMR spectra (^{1}H at 200 and 500 MHz, ^{13}C at 50.3 and 125 MHz, and ^{31}P at 81 MHz) were recorded with Bruker AM-200 and AM-500 spectrometers for solutions in $CDCl_3$, C_5D_5N, or D_2O. Chemical shifts (δ in parts per million) are given relative to those for Me_4Si (for ^{1}H and ^{13}C) and external aqueous 85% H_3PO_4 (for ^{31}P). TLC was performed on Alugram Sil G/UV_{254} (Macherey-Nagel, Duren) using (**A**) 85 : 15 $CHCl_3$–MeOH, (**B**) 10 : 10 : 3 $CHCl_3$–MeOH–water, and (**C**) 8 : 2 2-PrOH–water with detection by charring with 15 : 85 : 5 H_2SO_4–water–EtOH. Ion-exchange chromatography was accomplished on a column (1.5 × 30 cm) of Fractogel TSK DEAE-650 (S) (HCO_3^- form) (Merck) by elution at 1 mL min^{-1} (fraction volume, 5 mL) with a linear gradient of NH_4HCO_3 (0–0.5 M) in water (for **5** and **6**) or in 9 : 1 water–EtOH (for **8** and **9**). Gel filtration was accomplished on a column (1.5 × 50 cm; void volume, 30 mL; bed volume, 88 mL) of Sephadex G-50 (F) by elution at 1 mL min^{-1} (fraction volume, 1 mL) with 0.5 M NH_4HCO_3 (pH 9), using phenol–H_2SO_4 for detection. The column was calibrated with diglycosyl monophosphate **10** [8] (FW 558, elution volume V_e 75 mL), tetraglycosyl monophosphate **11** [2] (FW 902, V_e 70 mL), hexaglycosyl diphosphate **12** [2] (FW 1323, V_e 65 mL), octaglycosyl triphosphate **13** [2] (FW 1745, V_e 60 mL), and polythymidylic acid $d(pT)_{12-18}$ ammonium salt (Sigma) (FW 3855–5783, V_e 43–48 mL). Solutions were concentrated in vacuo at < 40°C.

Triethylammonium 2,3,6-tri-O-benzoyl-4-O-(2,3,4-tri-O-benzoyl-β-D-galactopyranosyl)-α-D-mannopyranosyl hydrogenphosphonate (**2**).—To a stirred solution of imidazole (623 mg, 9.15 mmol) in MeCN (16 mL) at 0°C was added PCl_3 (0.24 mL, 2.75 mmol) followed by Et_3N (1.34 mL, 9.62 mmol). Stirring was continued for 15 min, and a solution of **1** [2] (864 mg, 0.68 mmol) in MeCN (16 mL) was added dropwise during 30 min at 0°C. The mixture was stirred for 10–15 min at 20°C and quenched with 1 M Et_3NHHCO_3 (TEAB) (pH 8, 4.6 mL). After 15 min, $CHCl_3$ (200 mL) was added, and the organic layer was washed successively with ice–water (2 × 80 mL) and cold 0.5 M TEAB (2 × 80 mL), dried by filtration through cotton wool, and concentrated. The residue was dissolved in CH_2Cl_2(50 mL), and CF_3CO_2H (0.5 mL) was added at −12°C under stirring. After 2 min, the solution was washed successively with ice-cold satd aq $NaHCO_3$ (2 × 30 mL) and 0.5 M TEAB (2 × 30 mL), dried by filtration through cotton wool, and concentrated. Column chromatography of the residue on Kieselgel 60 (CH_2Cl_2 – MeOH–triethylamine, 2–9% of MeOH, 1% of Et_3N) gave **2** (635 mg, 82%) as a solid; $[\alpha]_D^{15}$ + 67° (*c* 1, $CHCl_3$); R_f 0.38 (solvent **A**). ES-MS (negative ion mode): *m/z* 1028.8 $[M - Et_3NH]^-$. NMR data ($CDCl_3$): ^1H, δ 1.25 (t, 9 H, 3 CH_3CH_2), 2.93 (q, 6 H, 3 CH_3CH_2), 3.09 (dd, 1 H, $J_{6a',6b'}$ = 12.0 Hz, H-6a'), 3.24 (dd, 1 H, H-6b'), 3.60 (t, 1 H, $J_{5',6a'} = J_{5',6b'}$ = 6.8 Hz, H-5'), 4.45 (ddd, 1 H, $J_{5,6a}$ = 3.1 Hz, H-5), 4.50 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.1 Hz, H-4), 4.55 (dd, 1 H, $J_{6a,6b}$ = 12.5 Hz, H-6a), 4.65 (dd, 1 H, $J_{5,6b}$ = 1.7 Hz, H-6b), 4.92 (d, 1 H, $J_{1',2'}$ = 7.8 Hz, H-1'), 5.37 (dd, 1 H, $J_{2',3'}$ = 10.3 Hz, H-3'), 5.60 (d, 1 H, $J_{3',4'}$ = 3.4 Hz, H-4'), 5.65 (dd, 1 H, $J_{1,2}$ = 2.0 Hz, H-2), 5.71 (dd, 1 H, $J_{1,P}$ = 8.6 Hz, H-1), 5.73 (dd, 1 H, H-2'), 5.94 (dd, 1 H, $J_{2,3}$ = 3.4 Hz, H-3), 7.0 (d, 1 H, $J_{H,P}$ = 637.8 Hz, HP), 7.10–8.05 (m, C_6H_5); ^{13}C, δ 9.13 and 45.58 (CH_3CH_2), 59.94 (C-6'), 62.40 (C-6), 68.37 (C-4'), 69.78 (C-3), 70.10 (C-2' + C-5), 70.92 (d, $J_{C,P}$ = 7.2 Hz, C-2), 71.80 (C-3'), 73.04 (C-4), 74.11 (C-5'), 92.59 (d, $J_{C,P}$ = 3.6 Hz, C-1), 100.54 (C-1'), 128.18–129.95 and 132.98–133.57 (C_6H_5), 164.96–166.33 (COO); ^{31}P (in C_5D_5N): δ 0.14.

Polycondensation of the monomer **2**.—Compound **2** (535 mg, 0.473 mmol) was dried by evaporation of pyridine (3 × 3 mL) therefrom. The residue was dissolved in 10 : 1 pyridine–triethylamine (0.50 mL), and trimethylacetyl chloride (0.087 mL, 0.706 mmol) was added under magnetic stirring. Another portion of Me_3CCOCl (0.058 mL, 0.471 mmol) was added in 45 min. After 2 h, the mixture became very viscous, and a freshly prepared solution of iodine (480 mg, 1.89 mmol) in 19 : 1 pyridine–water (3 mL) was added under stirring with Vortex (to dissolve the "glue"). After 30 min, $CHCl_3$ was added, and the organic layer was successively washed with cold 1 M $Na_2S_2O_3$ and 0.5 M TEAB, dried with $MgSO_4$, and concentrated to dryness. The residue (insoluble in MeOH, EtOH, ether, and toluene) was dissolved in $CHCl_3$ (2 mL), and cold EtOH (500 mL) was added to the solution under magnetic stirring. The mixture was kept overnight at 0°C, and the precipitate was collected and dried in vacuo to give crude **4** (~ 500 mg) as a powder; $[\alpha]_D^{24}$ + 52.5° (*c* 1, $CHCl_3$). ^{31}P NMR data ($CDCl_3$): δ −2.81, 0.29 (~ 12 : 1).

Phosphoglycans **5** *and* **6**.—To a solution of **4** (125 mg) in MeOH (5 mL), 1,4-dioxane (5 mL), and $CHCl_3$ (3 mL) was added 4.6 M NaOMe in MeOH (0.25 mL). The mixture was kept for 5 h at 20°C and for 16 h at 1°C, then diluted with MeOH, deionized with Dowex 50W-X4 (H^+) resin, filtered, immediately neutralized with Et_3N,

and concentrated to dryness, and water was evaporated from the residue (5 × 5 mL). Ion-exchange chromatography of the residue gave **5** (45 mg, 85%) as a solid; eluted with 0.22–0.41 M NH_4HCO_3; $[\alpha]_D^{20}$ − 18° (*c* 0.2, H_2O); $R_f \sim 0$ (solvents **B** and **C**); gel chromatography on Sephadex G-50: V_e 34–60 mL, peaks maximum–48 mL. NMR data (D_2O): ^1H, δ (inter alia) 3.57 (dd, $J_{2,3} = 8.8$ Hz, H-2, Gal), 3.70 (dd, $J_{3,4} = 3.0$ Hz, H-3, Gal), 3.99 (m, H-4, Gal), 4.07 (m, H-2, Man), 4.47 (d, $J_{1,2} = 7.8$ Hz, H-1, Gal), 5.44 (dd, $J_{1,P} = 6.8$ Hz, H-1, Man); ^{13}C, δ 61.56 (C-6, Man), 62.50 (minor, C-6, Gal terminal), 65.84 (br, C-6, Gal), 69.46 (C-4, Gal), 70.07 (C-3, Man), 71.16 (d, $J_{C,P} \sim 8$ Hz, C-2, Man), 72.13 (C-2, Gal), 73.67 (C-3, Gal + C-5, Man), 75.07 (d, $J_{C,P} \sim 7$ Hz, C-5, Gal), 76.73 (minor, C-5, Gal terminal), 78.21 (C-4, Man), 97.15 (br, C-1, Man), 104.22 (minor, C-1, Gal terminal), 104.58 (C-1, Gal); ^{31}P, δ −1.30, 1.20 (10 : 1).

Earlier eluted (0.16–0.21 M NH_4HCO_3) was oligomer **6** (6 mg, 10%), solid; $[\alpha]_D^{20}$ −20° (*c* 0.2, H_2O); $R_f \sim 0$ (solvents **B** and **C**); gel chromatography on Sephadex G-50: V_e 52–70 mL, peaks maximum–60 mL. NMR data (D_2O): ^1H, δ (inter alia) 3.57 (dd, $J_{2,3} = 10.0$, H-2, Gal), 3.69 (dd, $J_{3,4} = 3.0$ Hz, H-3, Gal), 3.99 (m, H-4, Gal), 4.06 (m, H-2, Man), 4.48 (d, $J_{1,2} = 8.0$ Hz, H-1, Gal), 4.92 (minor, H-1, Man(β) terminal), 5.19 (minor, H-1, Man(α) terminal), 5.45 (dd, $J_{1,P} = 7.0$ Hz, H-1, Man); ^{13}C, δ 61.67 (br, C-6, Man), 62.52 (minor, C-6, Gal terminal), 65.85 (br d, $J_{C,P} \sim 4$ Hz, C-6, Gal), 69.57 (C-4, Gal), 70.05 (C-3, Man), 70.30 (minor, C-4, Gal terminal), 70.90 (minor, C-2, Man terminal), 71.24 (br d, $J_{C,P} \sim 7$ Hz, C-2, Man), 72.02 (minor, C-5, Man terminal), 72.24 (C-2, Gal), 72.44 (minor, C-2, Gal terminal), 73.77 (C-3, Gal + C-5, Man), 73.96 (minor, C-3, Gal terminal), 75.08 (d, $J_{C,P} \sim 8$ Hz, C-5, Gal), 76.77 (minor, C-5, Gal terminal), 77.27 (minor, C-4, Man glycosylated by Gal terminal), 78.41 (C-4, Man), 95.11 (minor, C-1, Man terminal), 97.29 (br d, $J_{C,P} \sim 4$ Hz, C-1, Man), 104.39 (minor, C-1, Gal terminal), 104.70 (C-1, Gal); ^{31}P, δ −1.23.

Copolycondensation of **2** *and* **7**: *phosphoglycans* **8** *and* **9**.—A mixture of **2** (105 mg, 0.093 mmol) and **7** (10 mg, 0.009 mmol) was dried by evaporation of pyridine (3 × 2 mL) therefrom. The residue was dissolved in 10 : 1 pyridine–Et_3N (0.10 mL), trimethylacetyl chloride (0.027 mL, 0.221 mmol) was added, the mixture was stirred for 2 h at 20°C, and a freshly prepared solution of iodine in 19 : 1 pyridine–water (2 mL) was added. After 30 min, $CHCl_3$ was added, and the organic layer was successively washed with cold 1 M $Na_2S_2O_3$ and cold 0.5 M TEAB, dried by filtration through cotton wool, and concentrated to dryness. The residue was treated with 0.08 M NaOMe in 1 : 1 MeOH–1,4-dioxane (20 mL) for 16 h at 15°C, then diluted with MeOH, deionized with Dowex 50W-X4 (H^+) resin, filtered, neutralized with Et_3N, and concentrated, and water was evaporated from the residue (5 × 5 mL). Ion-exchange chromatography of the residue gave **8** (26 mg, 60%) as a solid; eluted with 0.15–0.26 M NH_4HCO_3; $[\alpha]_D^{20}$ + 16.2° (*c* 0.5, H_2O); R_f 0–0.20 (solvent **B**), 0–0.12 (solvent **C**); gel chromatography on Sephadex G-50: V_e 40–65 mL, peaks maximum–54 mL. NMR data (D_2O): ^1H, δ (inter alia) 1.30, 2.05, and 3.40 (minor, $-CH_2-$), 3.60 (dd, H-2, Gal), 3.70 (dd, H-3, Gal), 3.95 (m, H-4, Gal), 4.08 (m, H-2, Man), 4.43 (br d, $J_{1,2} = 8.0$ Hz, H-1, Gal), 4.87 (minor, H-1, Man terminal), 4.96 and 5.04 (minor, 2 d, $^3J_{H,H} = 10$ Hz and 18 Hz, $H_2C=$), 5.43 (br dd, $J_{1,P} = 7.6$ Hz, H-1, Man), 5.92 (minor, m, $-HC=$); ^{13}C, δ 26.67, 29.38, and 34.46 (minor, $-CH_2-$), 61.51 (br, C-6, Man), 62.24 (minor, C-6, Gal terminal), 65.57 (br, C-6, Gal), 69.38 (C-4, Gal + minor, $-OCH_2-$), 69.85 (C-3, Man),

70.0 (minor, C-4, Gal terminal), 70.64 (minor, C-3, Man terminal), 70.81 (minor, C-2, Man terminal), 71.19 (br, C-2, Man), 72.05 (C-2, Gal), 72.17 (minor, C-2, Gal terminal), 72.39 (minor, C-5, Man terminal), 73.63 (C-3, Gal + C-5, Man), 73.78 (minor, C-3, Gal terminal), 74.95 (br, C-5, Gal), 76.52 (minor, C-5, Gal terminal), 77.24 (minor, C-4, Man glycosylated by Gal terminal), 78.11 (br, C-4, Man), 78.65 (minor, C-4, Man terminal), 97.04 (br, C-1, Man), 100.54 (minor, C-1, Man terminal), 104.20 (minor, C-1, Gal terminal), 104.45 (br, C-1, Gal), 115.28 (minor, $CH_2=$), 138.97 (minor, –CH=); ^{31}P, δ – 1.40.

Later eluted (0.27–0.38 M NH_4HCO_3) was phosphoglycan **9** (10 mg, 25%), solid; $[\alpha]_D^{20}$ – 17° (*c* 0.2, H_2O); $R_f \sim 0$ (solvents **B** and **C**); gel chromatography on Sephadex G-50: V_e 40–66 mL, peaks maximum–54 mL. NMR data (D_2O): ^{13}C, δ 61.78 (C-6, Man), 62.53 (minor, C-6, Gal terminal), 65.83 (br d, $J_{C,P} \sim 4$ Hz, C-6, Gal), 69.55 (C-4, Gal), 70.04 (br C-3, Man), 71.32 (br d, $J_{C,P} = 7.4$ Hz, C-2, Man), 72.25 (C-2, Gal), 72.40 (minor, C-2, Gal terminal), 73.72 (C-3, Gal + C-5, Man), 73.90 (minor, C-3, Gal terminal), 75.07 (br d, $J_{C,P} = 7.4$ Hz, C-5, Gal), 76.76 (minor, C-5, Gal terminal), 77.23 (minor, C-4, Man glycosylated by Gal terminal), 78.24 (C-4, Man), 97.27 (br d, $J_{C,P} = 3.7$ Hz, C-1, Man), 104.43 (minor, C-1, Gal terminal), 104.69 (C-1, Gal); ^{31}P, δ – 1.45, 1.38 (6.5 : 1); the ^1H NMR spectrum was similar to the spectrum of **5**.

Alkaline phosphatase digestion.—A solution of the phosphoglycan (0.5–1 mg of **5**, **6**, or **9**) in 0.01 M NH_4HCO_3 (1 mL) was treated with bovine intestinal alkaline phosphatase (10 units; Sigma P 8647) for 16 h at 37°C. Methanol (1 mL) was added, and the mixture was evaporated to dryness. The residue was dissolved in D_2O to record ^1H NMR spectra or reduced with $NaBH_4$ followed by methanolysis and GC-MS analysis.

Reduction, methanolysis, and composition analysis.—A sample of the phosphoglycan digested with the alkaline phosphatase was treated with $NaBH_4$ (1 mg) in 0.1 M NH_4OH (0.3 mL) for 1 h at 20°C, then diluted with water, deionized with Dowex 50W-X4 (H^+) resin, and concentrated, and methanol was evaporated from the residue (3 × 2 mL). Methanolysis of the residue with 0.5 M HCl in MeOH (4 h, 85°C) followed by trimethylsilylation and GC-MS composition analysis of the products were accomplished in accordance with ref. [6].

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