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Rapid assembly of phosphate-bridged tetra-mannose by ionic liquid-supported oligosaccharide synthesis

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ABSTRACT

An efficient ionic liquid-supported oligosaccharide synthesis (ILSOS) strategy was described for the synthesis of linear oligo-phosphomannan. A new cleavable benzyl carbamate-type IL supporter containing 5-aminopentanyl linker was designed as an acceptor IL tag to facilitate this synthesis. The chain elongation on IL tag was achieved by H-phosphonate chemistry, including condensation with α-mannosyl H-phosphonate, *in situ* oxidation reaction and subsequent deprotection. After four cycles, linear α-(1 → 6)-tetra-mannan phosphate was obtained with a total yield of 52.7% within 45 h. The IL tagged product exhibited a tunable solubility in polar and non-polar solvent systems that facilitate a chromatography-free purification in the assembly process. The IL tag could be easily removed after hydrogenolysis treatment after the final step, to afford an amine terminated linker at the reducing end of phosphoglycan for further conjugation with a carrier protein. This methodology offered an efficient and chromatography-free approach for the synthesis of phosphoglycan.

1. Introduction

Phosphoglycans are natural polysaccharides composed of multiple glycosyl phosphate repeating units. One distinct feature of this class of carbohydrates is that the glycosidic linkage is a phosphodiester formed by a glycosyl phosphate and a hydroxyl group from another monosaccharide [1]. Phosphoglycans are widely found on the cell wall, capsule or surface of bacterial, yeast and protozoa [2–4]. In many pathogenic microorganisms, phosphoglycans are important virulence factors that can be used as an antigenic determinant to develop glycoconjugate vaccines for preventing microbial infections [5,6]. The biological importance of phosphoglycans, such as those derived from the pathogenic strain of *Haemophilus influenzae* type b (Hib) and *Neisseria meningitides* serogroup A and X, have stimulated many efforts to develop chemical methods to synthesize homogenous phosphoglycan fragments for immunological studies [7–9]. For instance, step-wise or block-wise chain elongation reaction in the solution phase is a traditional chemistry to prepare phosphoglycans [10,11]. However, time-consuming and tedious chromatography purification was required after each step in this approach. To simplify the purification process, supported carbohydrate synthesis strategies were investigated for rapid assembly of phosphoglycans, where solid [12]and soluble [13] polymers as well as fluorinated tag [14] were used as supporters. Polymer-supported methods allow the easy isolation and purification of products at the final step by a simple filtration, but usually large accesses of reagents are required in the reaction process. In addition, the relatively low loading capacity of polymer was another serious limitation. Alternatively, light fluorous tag [15] was developed as a supporter to construct wall teichoic acid fragment, a phosphoglycan drived from *Enterococcus faecalis*, in multi-milligram scale. However, fluorous solid phase extraction was required in each step to separate fluorous tagged products. Nevertheless, developing a robust and cost-effective approach for the synthesis of phosphoglycan is still a demanding challenge.

Ionic liquids (ILs) are a class of salts, typically composed of an organic cation and an inorganic or organic anion. In the past decades, applications of ILs in synthetic chemistry, as solvents, catalysts and reagents, have been intensively explored due to their unique physical and chemical properties [16,17]. In carbohydrate synthesis, the use of versatile of ILs as recyclable solvents and promoters in glycosylation was well documented because of its favorable kinetic and thermodynamic behaviors [18,19]. Furthermore, ILs displayed a tunable solubility in polar and non-polar solvents, which made ILs be an excellent carrier in carbohydrate synthesis. Accordingly, strategies based on ILs supported oligocarbohydrate synthesis (ILSOS) were developed successfully. For

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example, ester-linker IL support was applied to synthesize homolinear α-(1 → 6) linked tetra- and octa-mannoside [20]. Ionic Catch and Release Oligosaccharide Synthesis (ICROS) is an effective strategy capable of synthesizing β-(1 → 6) and β-(1 → 2)-linked oligosaccharides library [21,22]. Li's group demonstrated that benzyl carbamate-type IL tag installed at the anomeric position of glycosylation acceptor could be used to synthesize an α-linked nonamannoside [23]. Recently, the power of ILSOS strategy was impressively proved by the synthesis of a complex hetero-branched pentasaccharide on a 10 g scale [24]. However, up to now, the application of ILSOS strategy for the synthesis of challenging phosphoglycan has never been reported.

Here, we describe an ILs support approach for the synthesis of phosphoglycan. A (1 → 6) phosphodiester α-linked tetra-mannose was used as a model, which is a terminal immuno-dominant phosphomannan fragment derived from yeasts *S. cerevisiae* and *K. brevis* [25,26]. In this synthesis, a new benzyl carbamate-type IL support containing 5-aminopentanyl linker was designed and installed to the reducing end of phosphoglycan, which exhibited a tunable solubility in a polar and non-polar solvent system that facilitates a chromatography-free purification in the assembly process. The IL tag could be easily removed by a convenient hydrogenation condition in the final step while the linker was kept at the reducing end of phosphoglycan, allowing for further conjugation with a carrier protein for immunological studies.

2. Results and discussion

The target phosphomannan, α-(1 → 6) phosphodiester tetra-mannose **1,** and retrosynthetic analysis are presented in Fig. 1. IL tagged and full protected tetra-saccharide was designed as **2,** which was synthesized by key intermediate **3** and **4**. To assemble this mannosyl phosphosaccharide, H-phosphate chemistry was chosen to form the phosphodiester linkage, which enables the condensation of glycosyl H-phosphonate with primary alcohol in monosaccharide and subsequent oxidation in a one-pot procedure. In addition, mannosyl H-phosphonate **3** is quite stable compared with phosphoramidite method and α pure mannosyl H-phosphonate could be prepared from the corresponding α, β-mannose hemiacetal derivative conventionally. Benzyl carbamate-type IL tag **4** was designed to contain a 5-aminopentanyl linker and served as an acceptor type tag.

Our synthesis started with preparing building blocks **3** as shown in Scheme 1, where the triphenylmethyl (Trt) group is utilized as a temporary protecting group for chain extension while the other three hydroxyl groups in mannose were protected by the acetyl group. D-mannose reacted with triphenylmethyl chloride in the presence of a catalytic amount of 4-dimethylaminopyridine and then followed by acetylation to give peracetylated compound **6**. After selective removal of the anomeric acetyl group in **6**, hemiacetal **7** was afforded in a yield of 84% over three steps by only one final silica gel chromatographic purification. The preparation of α pure mannosyl H-phosphonate was achieved by a modified two-step procedure. Firstly, hemiacetal **7** was treated with diphenyl phosphite in the presence of pyridine to produce a mixture of mannosyl-H-phosphonate derivative(α:β = 1:1). Then, the α/β-mannosyl H-phosphonate was further treated with excess phosphorous acid in anhydrous acetonitrile for 2 days to obtain α-mannosyl H-phosphonate **3** (α:β = 8:1) as a major product in good yield, which was separable in silica gel column. The coupling constant of $J_{C1\text{-}H1}$ (174.0 Hz) in mannosyl H-phosphonate **3** unambiguously confirmed that α-anomeric phosphate was formed [27].

Synthesis of benzyl carbamate-type IL tag **4** was shown in Scheme 2. Briefly, **9** was synthesized following a reported procedure [23], which further reacted with bis-(4-nitrophenyl) carbonate to give **10** in a yield of 92%. Upon treatment with 5-amino-1-pentanol, **10** was transformed to **4** in excellent yield, where the hydroxyl group will be used to couple with glycosyl H-phosphate as an acceptor tag. The design of N-benzyloxycarbonyl group and linker in IL tag **4** allowed a mild hydrogenation condition to remove the IL tag in the global deprotection step (see Scheme 3).

With building blocks in hand, IL tag **4** was firstly coupled with α-mannosyl H-phosphonate **3** in the presence of pivaloyl chloride. The condensation was completed smoothly in 5 h and *in situ* oxidation was performed by adding iodine in moist pyridine. The crude IL tagged product **11–1** was obtained by regular work-up procedure, such as dichloromethane extraction and aqueous wash-up. The IL supported product **11–1** was further purified by a convenient "dissolution-precipitation" procedure that was performed by dissolving crude product in a small volume of MeOH (about 2 mL/g) and then precipitated the IL tagged product by the addition of 6.0–8.0 equiv. volume of ether. The isolated product **11–1** was treated with 5%TFA/DCM to remove trityl protecting group and the resulting IL-tagged product **11–2** was isolated by the same "dissolution-precipitation" procedure, which was characterized by ^{1}H-, ^{13}C, ^{31}P NMR and MS spectrum. The recovery yield of **11–2** is 95% over two steps and the purity was 98% as analyzed by HPLC, indicating the "dissolution-precipitation" protocol is a highly efficient method to separate IL tagged product.

Next, we attempted to assembly α-(1 → 6) phosphodiester linked oligomannose using the above established protocol. IL-tagged

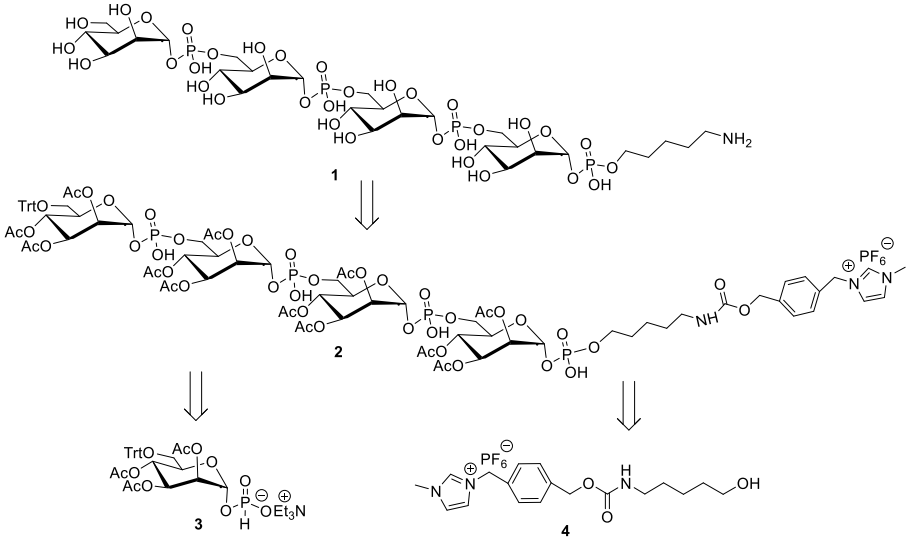
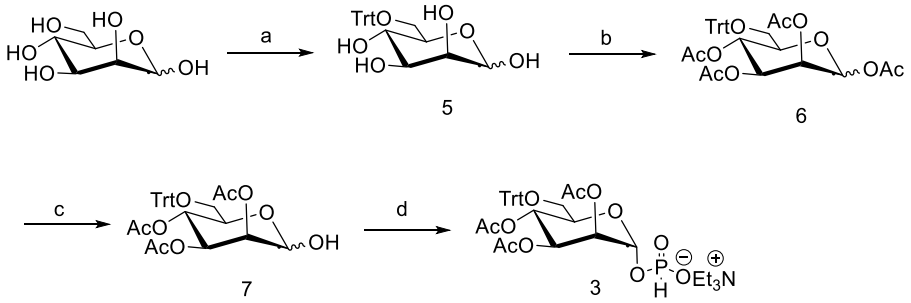
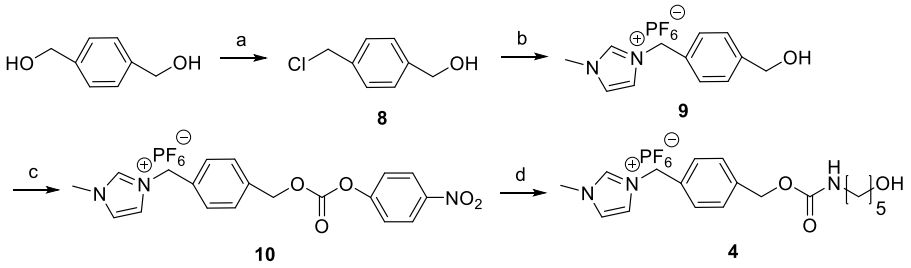


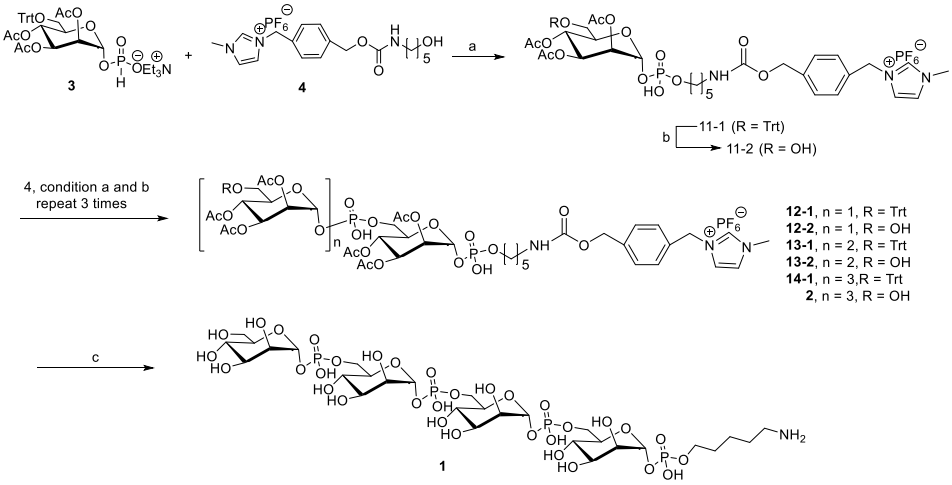
Fig. 1. Retrosynthetic analysis of phosphoglycan **1** and the design of intermediates.



Scheme 1. Reagent and conditions: (a) TrtCl, DMAP, pyridine; (b) Ac2O, pyridine; (c) (NH4)2CO3, MeOH:THF = 2:1, 87% in 3 steps; (d) (1) diphenyl phosphite, pyridine, room temperature, 2 h, TEA:H2O = 1:1, 1 h; (2) H3PO3, MeCN, room temperature, 4 d, 65% in 2 steps.



Scheme 2. Reagent and conditions: (a) HCl, toluene; (b) N-methylimidazole, KPF_6, MeCN, reflux, overnight, 85% in 2 steps; (c) bis(4-nitrophenyl)carbonate, pyridine, MeCN, 92%; (d) 5-amino-1-pentanol, MeCN, 95%.



Scheme 3. The assembly of α-(1 → 6)-oligomannan phosphate tetra-saccharide. Reagent and conditions: (a) PivCl, Py, then I_2, Py:H_2O = 9:1; (b) 5%TFA/CH_2Cl_2, 95% in 2 steps; (c) MeONa, MeOH, then 10% $Pd(OH)_2$/C, H_2, 82%.

monosaccharide **11**–**2** was used as an acceptor to further couple with mannosyl H-phosphonate **3** under the same condition. The chain elongation process was repeated three times. In each cycle, a similar IL tag aided purification procedure was applied. Thus, di-, tri- and tetrasaccharide were obtained efficiently, respectively. The results were summarized in Table 1. Completing one cycle of carbohydrate chain elongation totally took approximately 8 h, including one coupling reaction, one deprotection reaction and two purification operations. The IL tag aided isolation yield in each cycle is readily maintained ranging from 89% to 95%. The isolated mono-, di-, tri- and tetraphosphomannan, naming 11-2, 12-2, 13-2 and 2, were fully characterized by ^{1}H-, ^{13}C, ^{31}P NMR and HR-MS spectrum. And the purity of these compounds was determined by HPLC analysis as well, which was 94, 96 and 87%, respectively (Fig. 2 and Table 1**, entry 2–4**). Finally, the final global deprotection of **2** was achieved efficiently by the Zemplén

Table 1
Rapid synthesis of target tetrasaccharide.

Entry	Operation[a]	Product	Time[b](min)	Yield(%)	Purity(%)
1	A,B,C,B	11–2	360 + 30+60 + 30	95	98
2	A,B,C,B	12–2	360 + 30+60 + 30	95	94
3	A,B,C,B	13–2	360 + 30+90 + 30	89	96
4	A,B,C,B	2	360 + 30+90 + 30	89	87

[a] A: condensation and *in situ* oxidation. B: IL tag aided dissolution-precipitation purification. C: removal of trityl group.

[b] Typical time of condensation and oxidation is about 6 h; typical time of centrifugal purification is 30 min. The removing trityl protection group is about 1 h.

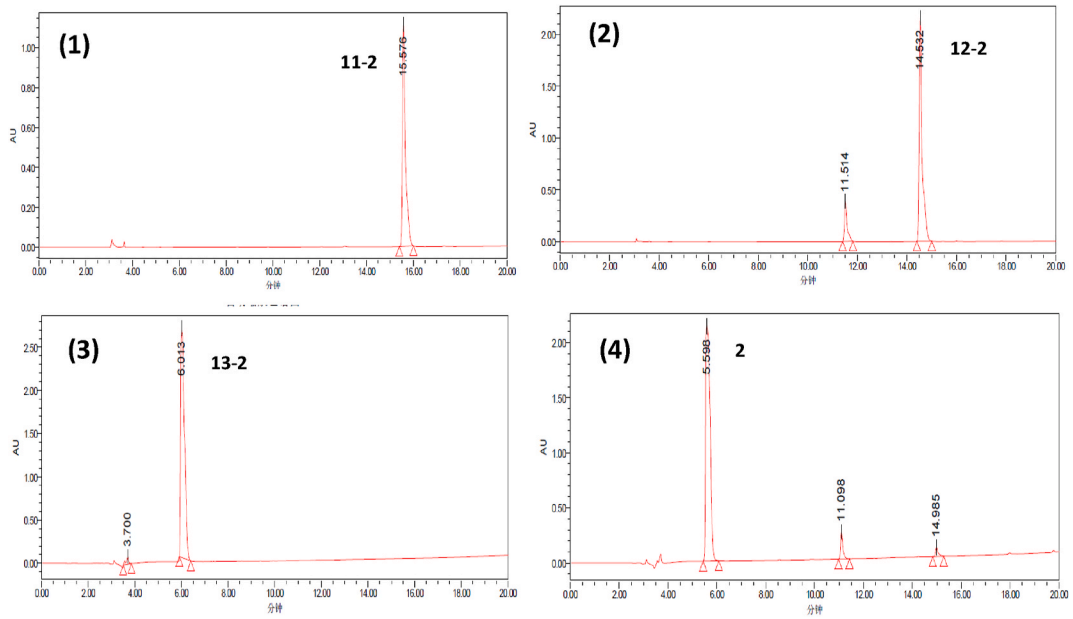


Fig. 2. Determination the purity of IL-tagged product by HPLC analysis. HPLC conditions: gradient elution (MeCN(0.1% TFA): H2O (0.1% TFA) = 10 : 90–90 : 10 in 20 min; (1) retention time of compound 11-2: 15.57 min; purity 98%; (2) retention time of compound 12-2: 14.53 min; purity 94%; (3) retention time of 13-2: 6.01 min; purity 96%; (4) retention time of 2: 5.60 min; purity 87%.

transesterification reaction to remove the acetyl protecting group and subsequent hydrogenation to remove the benzyl carbamate-type IL tag. The crude product was further desalted by a Sephadex LH-20 column to give **1** in an 82% yield after lyophilization. Its structure was fully characterized by ^{1}H-, ^{13}C-, ^{31}P- NMR and MS spectrum.

3. Conclusion

In summary, an efficient ionic liquid-supported oligosaccharide synthesis (ILSOS) strategy was described for the synthesis of linear oligophosphomannan. A new cleavable benzyl carbamate-type IL tag containing 5-aminopentanyl linker was designed as a receptor type of IL tag to facilitate this synthesis. The assembly α-(1 → 6) phosphodiester linked oligomannose on IL tag was achieved by H-phosphonate chemistry, including condensation with α-mannosyl H-phosphonate, *in situ* oxidation reaction and subsequent deprotection. The IL tag product was separated conveniently by "dissolution-precipitation" procedure without the need of chromatography purification. After four cycles, linear α-(1 → 6)-tetra-mannan phosphate was obtained with a total yield of 52.7% and good purity. The acetyl protecting group and IL tag in the final product was deprotected in two steps by Zemplén transesterification reaction and subsequent hydrogenation condition, respectively, to afford α-(1 → 6) phosphodiester linked tetra-mannose containing a 5-aminopentanyl linker. This methodology will provide an efficient approach to rapidly synthesize other phosphoglycans with biological importance for bacterial vaccine studies.

4. Experimental

4.1. General methods

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded in deuterated solvents on a Bruker Avance III 400 MHz spectrometer. Chemical shifts are reported in parts per million (ppm, δ) relative to tetramethylsilane (0.00) for d-chloroform, or the residual protic solvent peak for other solvents. ^{1}H NMR splitting patterns with observed first order coupling are designated as singlet (s), doublet (d), triplet (t), or quartet (q). Carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on a Bruker Avance III 400 MHz spectrometer. Mass spectra (MS) were obtained using electrospray ionization (ESI). Analytical thin layer chromatography (TLC) was carried out on TLC plates pre-coated with silica gel (250 μm layer thickness). Visualization was accomplished using either a UV lamp or sulfuric acid alcohol (5 mL H_2SO_4, 95 mL MeOH). Flash column chromatography was performed on silica gel (300–400 mesh). Solvent mixtures used for TLC and flash column chromatography are reported in v/v ratios. Commercially available reagents and solvents were used without further purification.

4.1.1. General ILSOS separation procedure

After filtration and concentration of the reaction mixture, the residue was dissolved in a small volume of MeOH (about 2 mL/g), followed by the addition of 6–8 equiv. volume of ether under shaking until no more visible precipitate was generated. The precipitate was collected by centrifugation (7000 rpm, 2 min, 3 times) to obtain IL tagged product as a white powder.

4.1.2. General HPLC conditions

HPLC analyses were performed on a Waters 2695 infinite analyzer with a Welch Topsil CX-C18 column (5 μm, 4.6 × 250 mm), detection wavelength of 254 nm, mobile phase of MeCN/H_2O (0.1% TFA) and a constant flow rate of 1 mL min^{-1}.

4.1.3. 6-O-(triphenylmethyl)-2,3,4-triacetate-D-mannopyranose(7)

D-mannose (3 g, 16.7 mmol) and DMAP (0.18 g, 1.7 mmol) was dissolved in anhydrous pyridine (30 mL) and triphenylmethyl chloride (7.0 g, 25 mmol) was added. The mixture was stirred at 80 °C for 18 h. After cooling down to 0 °C, 8.5 mL of Ac_2O was added, and the solution was stirred for overnight. The mixture was poured into ice-cold water and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to give compound 6. To a solution of the compound 6 (10 g, 16.9 mmol) in MeOH: THF(60 mL; 2 : 1 v/v) was added ammonium carbonate (3.3 g, 33.8 mmol) at room temperature. The solution was allowed to stir at room temperature for 4 h. The solvents were removed under reduced pressure. The residue was diluted with CH_2Cl_2 (100 mL), washed with satd. H_2O (100 mL), the combined organic phase was collected and dried over Na_2SO_4. After the solvent was removed, the crude product was purified by SiO_2 column chromatography eluting with CH_2Cl_2–CH_3OH (10 : 1) to give pure compound **7**

[28](7.6 g, 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (d, J = 7.3 Hz, 6H), 7.21 (t, J = 7.4 Hz, 6H), 7.18–7.11 (m, 4H), 5.31 (s, 1H), 5.29 (s, 1H), 5.18 (d, J = 2.3 Hz, 1H), 5.16 (s, 1H), 4.11–4.06 (m, 1H), 4.06–4.00 (m, 1H), 3.59 (s, 1H), 3.57 (d, J = 3.7 Hz, 1H), 3.19 (dd, J = 10.4, 2.2 Hz, 1H), 3.05 (dd, J = 10.4, 4.8 Hz, 1H), 2.35 (t, J = 7.2 Hz, 1H), 2.10 (s, 3H), 1.96 (s, 1H), 1.90 (s, 3H), 1.67 (s, 3H), 1.35 (s, 1H), 1.17 (t, J = 7.1 Hz, 2H), 0.89 (d, J = 6.7 Hz, 1H), 0.84–0.75 (m, 2H); MS (ESI) *m/z*: Calcd. for $C_{31}H_{31}O_9$, 547.20, found 548.10 $[M+H]^+$

4.1.4. 6-O-(triphenylmethyl)-,2,3,4-triacetate-α-D-mannopyranosyl hydrogenphosphonate triethylammonium salt (3)

To a solution of the hemiacetal derivative **7** (7.6 g, 13.8 mmol) in pyridine (50 mL) was added diphenyl phosphite (22.7 mL, 96.6 mmol) at room temperature and it was allowed to stir at room temperature for 3 h. The reaction mixture was cooled to 0 °C, diluted with TEA: H_2O (40 mL; 1 : 1 v/v) and it was stirred further for 30 min. The solvents were removed under reduced pressure and co-evaporated with toluene (2 × 50 mL). The residue was diluted with CH_2Cl_2 (100 mL), washed with satd. $NaHCO_3$ (100 mL), the combined organic phase dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by SiO_2 column chromatography eluting with CH_2Cl_2–CH_3OH–TEA (95 : 5: 1) to furnish anomeric mixture of phosphonate derivative as pale yellow syrup (8.3 g, 98%). To a solution of the obtained phosphonate derivative in anhydrous CH_3CN (50 mL) was added phosphorous acid (2.3 g, 27.6 mmol) and the solution was allowed to stir at room temperature for 4 days. The reaction was quenched by adding triethylamine (5 mL) at 0 °C, concentrated under reduced pressure. The residue was diluted with CH_2Cl_2 (100 mL), washed with satd. $NaHCO_3$ (100 mL), the combined organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by SiO_2 column chromatography eluting with CH_2Cl_2–CH_3OH–TEA (90 : 10: 1) to give compound **3** (6.3 g, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (s, 0.5H), 7.38 (d, J = 7.4 Hz, 6H), 7.20 (t, J = 7.5 Hz, 7H), 7.13 (t, J = 7.2 Hz, 4H), 6.22 (s, 0.5H), 5.60 (dd, J = 8.8, 1.8 Hz, 1H), 5.38–5.28 (m, 2H), 4.19–4.08 (m, 1H), 3.47 (q, J = 7.3 Hz, 1H), 3.16 (dd, J = 10.3, 2.3 Hz, 1H), 3.01 (dd, J = 10.3, 4.6 Hz, 1H), 2.86 (q, J = 7.3 Hz, 10H), 2.10 (s, 3H), 1.88 (s, 3H), 1.64 (s, 3H), 1.35 (t, J = 7.3 Hz, 1H), 1.19 (t, J = 7.3 Hz, 15H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.10, 109.08, 168.24, 142.80, 127.78, 126.71, 125.87, 92.01, 91.97, 85.39, 76.39, 76.08, 75.76, 69.98, 68.61, 65.39, 61.18, 52.44, 51.78, 44.77, 19.90, 19.75, 19.58, 8.29, 6.86; ^{31}P NMR (162 MHz, $CDCl_3$) δ 0.01; HRMS (ESI) *m/z*: Calcd. for $C_{31}H_{31}O_{11}P$, 612.1760, found 612.1740 $[M+H]^+$.

4.1.5. 3-[4-benzyl (4-nitrophenyl) carbonate]-1-methylimidazolium hexafluorophosphate (10)

Compound 8 (3 g, 19.1 mmol) was dissolved in dry CH_3CN (30 mL) under argon, and it was added N-methylimidazole (3.2 mL, 38.2 mmol) and KPF_6 (3.5 g, 19.1 mmol). The reaction was refluxed overnight at 80 °C with stirring, and TLC showed complete conversion. The reaction mixture was cooled to room temperature, filtered, and concentrated under vacuum. The residue was dissolved in CH_3CN (1 mL) and precipitated with diethyl ether (10 mL) to obtain ILs support 9 (3.4 g, 88.0%) as a colorless oil: To a solution of 9 in dry MeCN (30 mL) was added bis(4-nitrophenyl)carbonate (8.7 g, 28.6 mmol) and pyridine(1 mL). The reaction was stirring for 8 h and monitored by TLC. The reaction mixture was purified *via* the general ILSOS separation procedure, giving a yellow syrup as 10 (5.7 g, 92%).^{1}H NMR (400 MHz, DMSO) δ 9.21 (s, 1H), 8.32 (d, J = 8.1 Hz, 2H), 7.75 (d, J = 28.1 Hz, 2H), 7.57 (d, J = 8.1 Hz, 2H), 7.50 (dd, J = 24.7, 7.7 Hz, 4H), 5.45 (s, 2H), 5.33 (s, 2H), 3.86 (s, 3H), 2.50 (s, 2H); ^{13}C NMR (101 MHz, DMSO) δ 155.71, 152.39, 145.69, 137.22, 135.79, 129.81, 129.44, 129.01, 125.90, 124.52, 123.04, 122.84, 70.30, 52.03, 40.64, 40.43, 40.22, 40.01, 39.80, 39.59, 39.38, 36.36; HRMS (ESI) *m/z*: Calcd. for $C_{19}H_{18}N_3O_5$, 514.0967, found 514.0940 $[M+H]^+$.

4.1.6. 4-((1-methylimidazol-3-yl)methyl)benzyl-(5-hydroxypentyl) carbamate hexafluorophosphate salt (4)

To a solution of compound 10 (5.7 g, 17.1 mmol) in dry MeCN (40 mL) was added 5-amino-1-pentanol (2.4 mL, 25.65 mmol) in ice bath. The reaction was kept for 4 h with TLC showing the absence of substrate. The reaction mixture was warmed to room temperature, and purified *via* the general ILSOS separation procedure, giving a yellowish syrup as **2** (4.9 g, 95%).^{1}H NMR (400 MHz, MeOD) δ 7.44 (d, J = 5.8 Hz, 2H), 7.31 (s, 4H), 5.28 (s, 2H), 4.98 (s, 2H), 3.80 (s, 3H), 3.43 (t, 2H), 3.20 (s, 1H), 3.00 (t, 2H), 1.42 (m, 4H), 1.26 (m, 2H). ^{13}C NMR (101 MHz, MeOD) δ 157.35, 138.60, 133.33, 128.46, 128.07, 123.81, 122.15, 65.19, 61.42, 52.35, 48.26, 48.05, 47.84, 47.63, 47.41, 47.20, 46.99, 40.38, 35.11, 31.84, 29.27, 22.70. HRMS (ESI) *m/z*: Calcd. for $C_{18}H_{26}N_3O_3$, 332.2008, found 332.2050 $[M\text{-}PF_6]^+$.

4.1.7. 4-((1-methylimidazol-3-yl)methyl)benzyl (5-2,3,4-triacetate-α-D-mannopyranosyl hydrogen phosphonate)pentyl)carbamate, hexafluorophosphate salt (11-2)

A mixture of compound 4 (1 g, 3.01 mmol) and H-phosphonate derivative 3 (3.67 g, 6.02 mmol) were co-evaporated with anhydrous pyridine under vacuum for two times. The mixture was dissolved in anhydrous pyridine (30 mL) and pivaloyl chloride (0.73 mL, 6.02 mmol) was added dropwisely at room temperature over 10 min under argon. The reaction mixture was stirred further for 5 h. The reaction mixture was cooled to 0 °C and was added a solution of I_2 (1.53 g, 6.02 mmol) in pyridine: water (10 mL; 9:1, v/v) over 15 min. The cooling was stopped and the reaction mixture was stirred further for 1 h. The residue was diluted with CH_2Cl_2 (100 mL), washed with saturated solution of $Na_2S_2O_3{\cdot}5H_2O$ (50 mL). The combined organic layers dried over Na_2SO_4 and the solvent was removed. The reaction mixture was purified via the general ILSOS separation procedure to obtain **11**–**1**. Then residue **11**–**1** was dissolved in 5%TFA/DCM (30 mL) and the solution was allowed to stir at room temperature for 1 h. The solvent removed under reduced pressure and the reaction mixture was purified via the general ILSOS separation procedure, giving a yellowish syrup as **11**–**2** (2.0 g, 95%). ^{1}H NMR (400 MHz, MeOD) δ 8.90 (s, 1H), 7.48 (d, J = 13.9 Hz, 2H), 7.33 (s, 4H), 5.46 (d, J = 7.1 Hz, 1H), 5.39 (s, 1H), 5.32 (s, 2H), 5.21 (d, J = 9.2 Hz, 2H), 4.98 (s, 1H), 3.83 (m, 3H), 3.6–3.4 (m, 2H), 3.20 (s, 3H), 3.02 (t, J = 4.2 Hz, 2H), 2.02 (s, 3H), 1.94 (s, 3H), 1.86 (s, 3H), 1.58–1.56 (m, 2H), 1.44–1.41 (m, 2H), 1.33–1.22 (m, 2H); ^{13}C NMR (101 MHz, MeOD) δ 170.22, 170.19, 157.26, 145.83, 138.80, 136.67,]136.64, 133.39, 128.43, 128.02, 127.34, 127.04, 123.90, 122.33, 71.97, 69.27, 65.85, 65.08, 60.55, 52.40, 40.27, 35.19, 31.66, 29.88, 29.33, 29.01, 22.64, 22.32, 19.26, 19.19, 13.01.^{31}P NMR (162 MHz, MeOD) δ −3.26, −135.41, −139.80, −144.19, −148.58, −152.97. HRMS (ESI) *m/z*: Calcd. for $C_{30}H_{42}N_3O_{14}P$, 699.2399, found 699.2377 $[M\text{-}PF_6]^+$.

4.1.8. 4-((1-methylimidazol-3-yl)methyl)benzyl (5-2,3,4-triacetate-α-D-mannopyranosyl hydrophosphate)pentyl)carbamate-(1 → 6)-2,3,4-triacetate-α-D-mannopyranosyl hydrophosphate hexafluorophosphate salt (12-2)

A mixture of compound **11**–**2** (1 g, 1.43 mmol) and H-phosphonate derivative **3** (1.75 g, 1.88 mmol) were co-evaporated with anhydrous pyridine under vacuum for two times. The mixture was dissolved in anhydrous pyridine (20 mL) and pivaloyl chloride (0.35 mL, 1.88 mmol) was added drop wise at room temperature over 10 min under argon. The reaction mixture was stirred further for 5 h. The reaction mixture was cooled to 0 °C and to the cooled reaction mixture was added a solution of I_2 (0.72 g, 1.88 mmol) in Py:H_2O (3 mL; 9:1 v/v) over 15 min. The cooling was stopped and the reaction mixture was stirred further for 1 h. The residue was diluted with CH_2Cl_2 (80 mL), washed with saturated solution of $Na_2S_2O_3{\cdot}5H_2O$ (40 mL). the combined organic layers dried over Na_2SO_4 and the solvent removed under reduced pressure. The reaction mixture was purified via the General ILSOS separation procedure. The residue was dissolved in 5%TFA/CH_2Cl_2 (15 mL) and the solution was allowed to stir at room temperature for 1 h. The solvent removed

under reduced pressure. and the reaction mixture was purified via the general ILSOS separation procedure, giving a yellowish syrup as 12-2 (1.45 g, 95%).^{1}H NMR (400 MHz, MeOD) δ 7.59 (d, J = 16.7 Hz, 2H), 7.41 (d, J = 8.2 Hz, 4H), 5.47 (d, J = 10.5 Hz, 1H), 5.42 (s, 3H), 5.38–5.26 (m, 3H), 5.10 (d, J = 14.8 Hz, 2H), 4.65–4.00 (m, 6H), 3.92 (m, 3H), 3.90–3.82 (m, 2H), 3.70–3.44 (m, 4H), 2.24–1.85 (m, 18H), 1.62 (m, 2H), 1.50 (m, 2H), 1.40 (m, 2H). ^{13}C NMR (101 MHz, MeOD) δ 177.86, 170.24, 157.27, 147.45, 144.01, 143.23, 133.52, 130.30, 130.09, 128.49, 127.97, 127.63, 127.45, 124.27, 123.88, 122.25, 93.57, 71.90, 71.48, 71.17, 69.25, 65.97, 65.57, 65.13, 63.14, 60.57, 52.37, 52.10, 48.47, 46.23, 40.32, 35.24, 30.05, 29.07, 26.72, 26.14, 22.71, 21.91, 19.40, 19.32, 19.25, 19.23, 19.20.^{31}P NMR (162 MHz, MeOD) δ −2.37, −135.86, −140.23, −144.60, −148.96, −153.33. HRMS (ESI) m/z: Calcd. for $C_{42}H_{58}N_3O_{25}P_2$, 1066.2829, found 1066.2806 $[M\text{-}PF_6]^+$.

4.1.9. 4-((1-methylimidazol-3-yl)methyl)benzyl (5-2,3,4-triacetate-α-D-mannopyranosyl hydrophosphate)pentyl)carbamate-(1 → 6)-2,3,4-triacetate-α-D-mannopyranosyl hydrophosphate-(1 → 6)-2,3,4-triacetate-α-D-mannopyranosyl hydrophosphate, hexafluorophosphate salt (13-2)

A mixture of compound **12–2** (0.7 g, 0.65 mmol) and a-H-phosphonate derivative **3** (0.8 g, 1.3 mmol) were co-evaporated with anhydrous pyridine under vacuum for two times. The mixture was dissolved in anhydrous pyridine (10 mL) and pivaloyl chloride (0.15 mL, 1.3 mmol) was added to it drop wise at room temperature over 10 min under argon. The reaction mixture was stirred further for 5 h. The reaction mixture was cooled to 0 °C and to the cooled reaction mixture was added a solution of I_2 (0.33 g, 1.3 mmol) in pyridine: water (1 mL; 9:1 v/v) over 15 min. The cooling was stopped and the reaction mixture was stirred further for 1 h. The residue was diluted with CH_2Cl_2 (50 mL), washed with satd. saturated solution of $Na_2S_2O_3{\cdot}5H_2O$ (25 mL). The combined organic layers dried over Na_2SO_4 and the solvent removed under reduced pressure. The reaction mixture was purified via the General ILSOS separation procedure. The residue was dissolved in 5%TFA/DCM (10 mL) and the solution was allowed to stir at room temperature for 1.5 h. The solvent removed under reduced pressure and the reaction mixture was purified via the General ILSOS separation procedure, giving **13–2** as a yellowish syrup (0.84 g, 89%). ^{1}H NMR (400 MHz, MeOD) δ 8.54 (s, 1H), 7.59 (d, J = 18.6 Hz, 3H), 7.42 (s, 4H), 5.47 (d, J = 9.4 Hz, 1H), 5.45 (m, 1H), 5.41 (s, 2H), 5.32 (t, J = 13.3 Hz, 5H), 5.08 (s, 2H), 4.55 (m, 4H), 4.25 (t, J = 7.6 Hz, 3H), 4.11 (d, J = 8.8 Hz, 2H), 3.92 (m, 4H), 3.90–3.78 (m, 4H), 3.71–3.41 (m, 5H), 2.20–1.87 (m, 28H), 1.62 (m, 2H), 1.51 (m, 2H), 1.40 (m, 2H),. ^{13}C NMR (101 MHz, MeOD) δ 171.00, 170.10, 157.27, 138.81, 130.58, 130.31, 130.10, 128.46, 128.23, 127.56, 126.82, 126.39, 123.85, 122.27, 93.61, 69.75, 69.68, 65.61, 65.06, 62.02, 55.97, 52.38, 48.03, 47.81, 48.55, 47.60, 47.49, 47.39, 47.17, 46.96, 40.27, 35.19, 30.07, 29.31, 29.03, 26.13, 25.82, 22.70, 19.28, 19.23, 19.16.^{31}P NMR (162 MHz, MeOD) δ −1.06,-2.39,-135.85,-140.22,144.58,-148.95,-153.32. HRMS (ESI) m/z: Calcd. for $C_{54}H_{74}N_3O_{36}P_3$, 1433.3259, found 1433.3209 $[M\text{-}PF_6]^+$.

4.1.10. 4-((1-methylimidazol-3-yl)methyl)benzyl(5-2,3,4-triacetate-α-D-mannopyranosylhydrophosphate)pentyl)carbamate-(1 → 6)-2,3,4-triacetate-α-D-mannopyra-nosylhydrophosphate-(1 → 6)-2,3,4-triacetate-α-D-mannopyranosylhydrophosph-ate-(1 → 6)-2,3,4-triacetate-α-D-mannopyranosylhydrophosphate hexafluorophosphate salt (2)

A mixture of compound **13–2** (0.4 g, 0.28 mmol) and H-phosphonate derivative **3** (0.34 g, 0.56 mmol) were co-evaporated with anhydrous pyridine under vacuum for two times. The mixture was dissolved in anhydrous pyridine (8 mL) and pivaloyl chloride (0.07 mL, 0.56 mmol) was added dropwisely at room temperature over 10 min under argon. The reaction mixture was stirred further for 5 h. The reaction mixture was cooled to 0 °C and a solution of I_2 (0.14 g, 0.56 mmol) in Py:H_2O (1 mL; 9:1 v/v) was added over 15 min. The cooling was stopped and the reaction mixture was stirred further for 1 h. The residue was diluted with CH_2Cl_2 (50 mL), washed with satd. saturated solution of $Na_2S_2O_3{\cdot}5H_2O$ (25 mL). The combined organic layers dried over Na_2SO_4 and the solvent was removed under reduced pressure. The reaction mixture was purified *via* the general ILSOS separation procedure. The residue was dissolved in 5%TFA/CH_2Cl_2 (8 mL) and the solution was allowed to stir at room temperature for 1.5 h. The solvent was removed under reduced pressure and the reaction mixture was purified *via* the general ILSOS separation procedure, giving **2** as yellowish syrup (0.4 g, 80%). ^{1}H NMR (400 MHz, MeOD) δ8.55 (s, 1H), 7.59 (d, J = 17.8 Hz, 2H), 7.43 (s, 4H), 5.48–5.25 (m, 8H), 5.08 (s, 2H), 4.59 (d, J = 23.7 Hz, 4H), 4.29–4.17 (m, 3H), 4.12 (d, J = 15.9 Hz, 2H), 3.91 (d, J = 13.9 Hz, 6H), 3.63 (d, J = 11.1 Hz, 2H), 3.49 (dd, J = 13.2, 6.2 Hz, 2H), 3.12 (t, J = 5.2 Hz, 2H), 2.24–1.92 (m, 36H), 1.69–1.55 (m, 2H), 1.55–1.45 (m, 2H), 1.43–1.32 (m, 2H). ^{13}C NMR (101 MHz, MeOD) δ 177.23, 170.27, 128.57, 127.97, 127.83, 127.07, 127.07, 126.62, 71.87, 69.87, 48.58, 48.37, 48.15, 47.94, 47.73, 47.51, 47.30, 47.09, 39.08, 38.87, 38.66, 38.45, 38.24, 38.03, 37.82, 29.37, 21.98, 19.69, 19.59, 19.52.^{31}P NMR (162 MHz, MeOD) δ-0.44, −1.64, −136.32, −140.69, −145.07, −149.44, −153.32. HRMS (ESI) m/z: Calcd. for $C_{66}H_{90}N_3O_{47}P_4$, 1800.3690, found 1800.3693 $[M\text{-}PF_6]^+$.

4.1.11. 6-Aminopentyl-O-α-D-mannopyranosylphosphate-(1 → 6)-α-D-mannopyranosylphosphate-(1 → 6)-α-D-mannopyranosylphosphate-(1 → 6)-α-D-mannopyranoside (1)

The IL support **2** (0.2 g, 0.11 mmol) was dissolved in MeOH (5 mL), a solution of NaOMe (0.1 M in MeOH) was added, and the mixture was stirred for 1 h with TLC showing completion of de-acetylation. AcOH was dropped to neutralize MeONa. The reaction mixture was filtered and concentrated under reduced pressure to give the deacetylated product. To the solution of the deacetylated product in MeOH: Formic acid (5 mL; 1:1 v/v) was added 10% $Pd(OH)_2$/C (10 mg) under inert atmosphere at room temperature. The reaction mixture was allowed to stir under H_2 (4 atm) at room temperature for 9 h. The reaction mixture was filtered through a Celite bed and the filtering bed was washed with H_2O (3 × 30 mL). The filtrate was concentrated under reduced pressure and was dissolved in H_2O and eluted through a column filled with Sephadex LH-20. The eluate was concentrated to afford the target compound **1** (0.1 g, 82%) as white powder. ^{1}H NMR (400 MHz, D_2O) δ5.33 (d, J = 5.7 Hz, 3H), 5.06 (s, 1H), 3.94–3.51 (m, 27H), 3.06 (t, J = 5.0 Hz, 1H), 1.54 (dd, J = 12.7, 6.9 Hz, 2H), 1.44 (t, J = 14.0 Hz, 2H), 1.28 (dd, J = 7.1, 4.8 Hz, 2H); ^{13}C NMR (101 MHz, D_2O) δ 182.35, 96.16, 96.10, 95.15, 95.11, 73.88, 73.79, 73.00, 70.63, 70.55, 70.47, 70.10, 69.96, 69.92, 67.02, 66.46, 61.20, 60.85, 53.15, 53.09, 46.73, 39.33, 38.39, 29.50, 28.13, 26.59, 24.80, 22.35, 20.24; ^{31}P NMR (162 MHz, D_2O) δ −2.16, −2.28. HRMS (ESI) m/z: Calcd. for $C_{29}H_{53}NO_{33}P_4$, 1090.1348, found 1090.1366 $[M+Na]^+$.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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