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Solid-Phase Synthesis of Fluorinated Analogues of Glycosyl 1-Phosphate Repeating Structures from *Leishmania* using the Phosphoramidite Method

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Bacterial and protozoan sugar chains contain glycosyl 1-phosphate repeating structures; these repeating structures have been studied for vaccine development. The fluorinated analogues of [β-Gal-(1→4)-α-Man-(1→6)-P-]$_n$, which are glycosyl 1-phosphate repeating structures found in *Leishmania*, were synthesised using the solid-phase phosphoramidite method. This method has been less extensively studied for the synthesis of glycosyl 1-phosphate units than *H*-phosphonate chemistry. A stepwise synthesis of a compound containing five such repeating units has been conducted using the phosphoramidite method herein, which is the longest glycosyl 1-phosphate structures to be chemically constructed in a stepwise manner.

1. Introduction

Monosaccharides such as glucose, mannose, and galactose and oligo and polysaccharides are well-known biomolecules. These compounds play a wide range of biological roles, from an energy source to recognition between cell surfaces.[1] The sugar units in oligo and polysaccharides are linked by means of glycosidic bonds. Condensation between anomeric hydroxy groups and hydroxy groups in sugars leads to the formation of these bonds. In contrast, many bacterial and protozoan sugar chains contain phosphodiester linkages between an anomeric carbon and a carbon from another sugar.[2] Compounds containing the repeating structures of these glycosyl 1-phosphate units are known as phosphoglycans. For example, α-D-mannopyranosyl 1-phosphate repeating structures ([α-Man-(1→6)-P-]$_n$) are the constituents of *Hansenula capsulata* Y-1842 exophosphomannan,[3] and α-D-galactopyranosyl 1-phosphate repeating structures ([α-Gal-(1→3)-P-]$_n$) were found in capsular antigens of *E. coli*.[4] The phosphoglycans from *Leishmania* contain disaccharide 1-phosphate repeating units ([β-Gal-(1→4)-α-Man-(1→6)-P-]$_n$, Figure 1 a), and many such oligosaccharide 1-phospahte repeating structures have also been reported.[2,5] These glycosyl 1-phosphate repeating structures are known to play important roles in immunological responses and infections;[6] in addition, some phosphoglycans have been studied for vaccine development.[7]

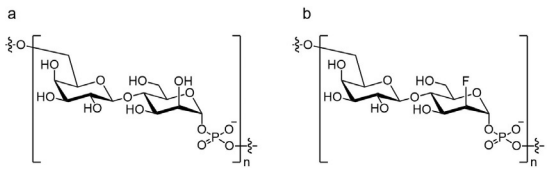

Figure 1. a) Structure of disaccharide 1-phosphate repeating units found in *Leishmania*. b) The fluorinated analogues of the repeating units studied herein.

The chemical synthesis of phosphoglycans is essential for the elucidation of their functions. Using this method, these compounds can be supplied in both large quantity and homogenous quality for their application in vaccines and therapeutics. Owing to the chemical instability and high polarity of glycosyl 1-phosphates and their derivatives, constructing their structures and synthetic intermediates is challenging. Due to these difficulties, the synthesis of glycosyl 1-phosphate repeating structures is limited in the number of repeating units possible. Vishwakarma et al. performed the chemical synthesis of compounds containing [β-Gal-(1→4)-α-Man-(1→6)-P-]$_{19–22}$, which are the longest reported chemically synthesised glycosyl-1 phosphate repeating structures, to the best of our knowl-

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edge.[8] They followed a polycondensation approach using a *H*-phosphonate building block; however, the length of the repeating structures could not be precisely regulated using this approach. In this regard, performing a stepwise synthesis using adequate building blocks could be advantageous. The examples of glycosyl 1-phosphate repeating structures which have been synthesised in this manner are limited to be relatively short oligomers. Vishwakarma et al. and Nikolaev et al. synthesised the leishmanial [β-Gal-(1→4)-α-Man-(1→6)-P-]$_3$ using the *H*-phosphonate method.[8,9] Nikolaev et al. have also reported the synthesis of a variety of other phospophoglycans such as compounds containing [α-Man-(1→6)-P-]$_4$ and [α-GlcNAc-(1→3)-P-]$_3$.[2,3] The synthesis of compounds containing [β-GlcNAc-(1→3)-α-Gal-(1→4)-P-]$_2$ and [β-GlcNAc-(1→3)-α-GalNAc-(1→4)-P-]$_2$ was reported by Oscarson et al.[10] In addition, Lay et al. and Chhikara et al. respectively used the *H*-phosphonate chemistry to construct [α-GlcNAc-(1→4)-P-]$_3$ and [α-ManNAc-(1→6)-P-]$_3$ structures.[11] These studies suggest that the current limitation for the length of the glycosyl 1-phosphate repeating structures seem to be 2–4 repeating structures obtained by *H*-phosphonate chemistry. In contrast, chemical synthesis of oligodeoxyribonucleotides (ODNs) and oligoribonucleotides (ORNs) have been significantly developed over a few decades. Despite containing multiple phosphodiester linkages, ODNs with sizes of more than 20mers can now be easily synthesised and are commercially available. Nowadays, ODNs and ORNs are synthesised using the solid-phase phosphoramidite method rather than the *H*-phosphonate method.[12] High efficiency of condensation reaction and low polarity of phosphotriester intermediates, which are particularly important in the synthesis of long oligomers, can be achieved using the phosphoramidite method.[13] However, this method has not been actively studied for the synthesis of phosphoglycans compared to the oligonucleotide synthesis. As for the phosphoramidite method, the most crucial difference between oligonucleotides and glycosyl 1-phosphate repeating structures is the stability of synthetic intermediates. Glycosyl 1-phosphate derivatives, in contrast to ODNs and ORNs, can be easily degraded through elimination of phosphorous-containing groups to generate oxocarbenium cation.[14] Although the phosphoramidite chemistry has been applied to the synthesis of glycosyl 1-phosphate structures,[15] long oligomers cannot be synthesised using this method. In particular, the phosphotriester linkages are one of the most labile functionalities in the intermediates.[16] In the standard phosphoramidite method used for DNA and RNA synthesis, trifluoroacetic and dichloroacetic acids are commonly used for the removal of trityl type protecting groups. However, glycosyl 1-phosphotriesters are unstable under acidic conditions because the protonation of phosphotriester oxygen atoms is expected to accelerate the elimination of the phosphotriester group from the sugar.

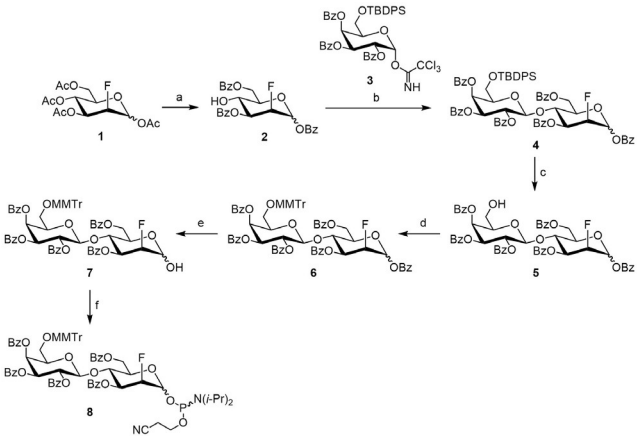
One of the general strategies for resolving the instability of natural compounds or their synthetic intermediates is the use of their analogues.[17–19] Although some of these modifications might have been effective for stabilisation of glycosyl 1-phosphate derivatives, the number of repeat units has currently been limited to three.[17a]

We previously reported 2-deoxy-2-fluoromannopyranosyl 1-phosphate derivatives as analogue molecules of [α-Man-1-P-] structure.[14b] A fluorine atom is electronically equivalent to a hydroxy group and its van der Waals radius is between hydrogen and oxygen atoms.[20] The usefulness of fluorinated sugar analogues has been demonstrated by 2-deoxy-2-(^{18}F)fluoro-D-glucose, which has been used for positron emission tomography.[21] Some studies have shown that fluorinated sugars serve as a glycosidase substrate or inhibitor.[22] Therefore, fluorinated sugar analogues have potential for sugar-based therapeutics and biological tools. In our previous report, we demonstrated the stability under acidic conditions of both mannosyl 1-phosphotriesters and mannosyl 1-phosphodiesters could significantly increase by a fluorine atom in place of 2-OH.[14b] The fluorine atom was expected to stabilise these compounds by preventing the formation of oxocarbenium cation due to the strong electron withdrawing property. Chemically stable analogues of mannosyl 1-phosphodiesters are expected to be useful as therapeutics in regarding to their handling and/or drug duration. Here, we hypothesised a phosphoramidite solid-phase synthesis could be applicable to the synthesis of sugar analogues containing 2-deoxy-2-fluoro-glycosyl 1-phosphate structures. In this study, we aimed to synthesise the fluorinated analogues of leishmainal glycosyl 1-phophate repeating structures (Figure 1 b, [β-Gal-(1→4)-α-ManF-(1→6)-P-]$_n$).

2. Results and Discussion

2.1. Synthesis of the Disaccharide Building Block

A disaccharide phosphoramidite was synthesised as shown in Scheme 1. Compound **1** was prepared according to procedures in the literature.[23] After deacetylation of compound **1**, treatment with approximately three equivalents of benzoyl chloride (BzCl) afforded a protected fluorosugar bearing 4-OH **2**. Compound **2** was glycosylated with **3**[24] to afford disaccharide **4**. The *tert*-butyldiphenylsilyl (TBDPS) group of **4** was removed by



Scheme 1. Synthesis of disaccharide phosphoramite **8**. Reagents and conditions: a) NaOMe, MeOH, RT (i); BzCl, pyridine, $<$M-$>$40 °C, 60 %, 2 steps (ii); b) **3**, TMSOTf, CH_2Cl_2, RT, 77 %; c) TBAF, AcOH, THF, 0 °C to RT, 92 %; d) MMTrCl, pyridine, RT, 20 h, 91 %; e) $MeNH_2$, THF-MeOH, $<$M-$>$30 °C, 83 %; f) 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite, 82 %, α:β = 97:3.

treatment with tetramethylammonium fluoride (TBAF)[25] followed by the protection of the newly generated hydroxy group by a 4-methoxytrityl (MMTr) group to afford **6**. The anomeric benzoyl group in the **6** was selectively removed by treatment with methylamine to afford compound **7**,[26] then phosphoramidite building block **8**, with anomeric ratio of α:β = 97:3 was obtained by phosphitylation of **7** in 82% yield. The anomeric isomers were partially resolved, and compound **8** with anomeric ratios of α:β = 76:24 and 99:1 were used for the following studies. It is noted that there were four diastereomers derived from both the anomeric carbon atom in the mannopyranose and the phosphorous atom, and their complete separation was difficult.

2.2. Solid-Phase Synthesis of Compounds Containing Disaccharide 1-Phosphate Repeating Units

We studied the solid-phase synthesis using phosphoramidite unit **8** based on a standard synthetic procedure as shown in Scheme 2.[13] Sugar elongation cycle contains mainly three steps. The first step is the condensation reaction using phosphoramidite **8**, in the presence of 4,5-dicyanoimidazole (DCI). The second step is oxidation of the generated phosphite into phosphotriester using (+)-(8,8-dichlorocamphorylsulfonyl)oxaziridine (DCSO),[27] and the third is detritylation with trifluoracetic acid (TFA) to re-generate 6-OH for the next condensation. After repeating of this cycle, removal of benzoyl groups and cyanoethyl groups and release of compounds containing glycosyl 1-phosphate repeating units can be simultaneously conducted in the "deprotection and release" step. We chose a controlled pore glass (CPG), which was commonly used for phosphoramidite DNA synthesis. A hydroquinone spacer was introduced to CPG to enable us to quantitatively analyse the compounds by UV absorption (see the Supporting Information).

We first conducted the synthesis of compounds containing the two repeating units, [β-Gal-(1→4)-α-ManF-(1→6)-P-]$_2$, according to Scheme 2 using **8** with anomeric ratio of 99:1 (α:β), and reaction conditions of the deprotection and release were optimised. As shown in Figure 2, four-hour treatment with methylamine was enough for removal of the protecting groups and release of compounds from the solid support. From MS analysis, however, there were multiple compounds that were suggested to have the same molecular weight as compound **10**. The results suggested that stereo- and/or regioisomers were generated during solid-phase synthesis. One of the most likely origins of the peaks was the generation of stereoisomers because we used excess amounts of **8** containing a small amount of the β anomer. To study stereoisomer generation, we synthesised compounds containing one repeating unit **9** and two repeating units **10** by the same procedure using compound **8** with two different anomeric ratios, one is α:β = 99:1 (Figure 3a, 3b) and the other is 76:24 (Figure 3c, 3d). Comparing Figure 3a with Figure 3c, both of which illustrate the synthesis of disaccharide **9**, we found that there are two peaks derived from disaccharides. It was clear that the later peak was significantly larger in Figure 3c than that in Figure 3a. These results suggested the later peak was derived from the β-isomer (i.e. [β-Gal-(1→4)-β-ManF-(1→6)-P-]). Figure 3b and 3d illustrate the synthesis of the tetrasaccharide **10**. Four peaks were observed derived from tetrasaccharides on HPLC profiles and the main peak should be derived from compounds containing the [β-Gal-(1→4)-α-ManF-(1→6)-P-]$_2$ structure. Other three peaks were expected to be of other three isomers, that is, compounds containing [β-Gal-(1→4)-α-ManF-(1→6)-P-β-Gal-(1→4)-β-ManF-(1→6)-P-], [β-Gal-(1→4)-β-ManF-(1→6)-P-β-Gal-(1→4)-α-ManF-(1→6)-P-], and [β-Gal-(1→4)-β-ManF-(1→6)-P-β-Gal-(1→4)-β-ManF-(1→6)-P-] structures, and these peak areas are similar to that of **10**. These results clearly showed that the anomeric purities of mannosyl 1-phosphate products were lower than that of **8**, which suggested higher reactivity of the β-isomer of **8** (**8**β). To confirm the dif-

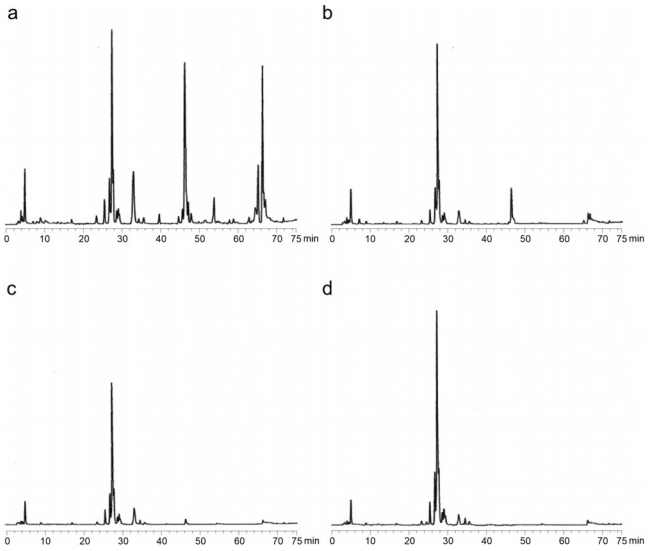
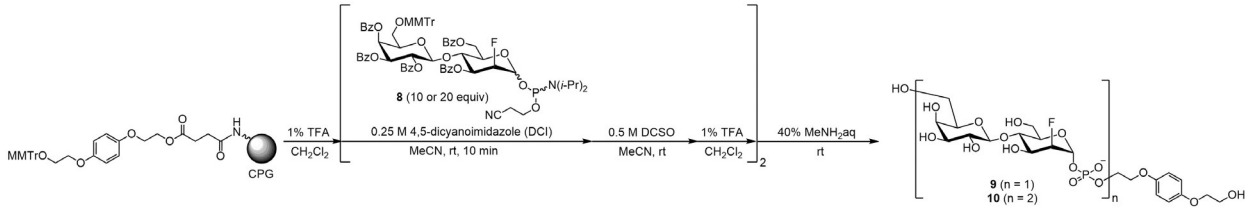


Figure 2. Reverse-phase HPLC (RP-HPLC) profiles at 286 nm of crude products after the solid-phase synthesis according to Scheme 2 (n = 2) after a) 15 min, b) 30 min, c) 1 h, and d) 4 h treatment with methylamine. 10 equiv. of **8** (α:β = 99:1) were used in each case. RP-HPLC was performed with a linear gradient of 0–20% acetonitrile for an initial 60 min, and then 20–100% acetonitrile (between 60–65 min) in 0.1 M triethylammonium acetate buffer, and finally acetonitrile (between 65–75 min) at 30 °C.



Scheme 2. Solid-phase synthesis of compounds containing glycosyl 1-phosphate repeating units by a phosphoramidite method.

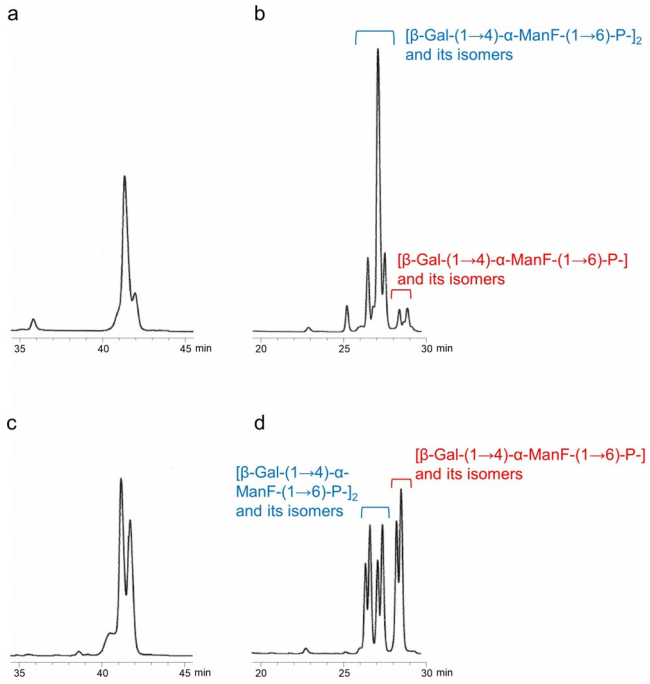
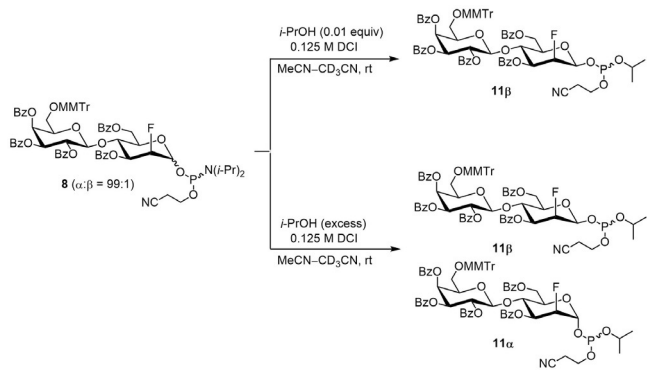


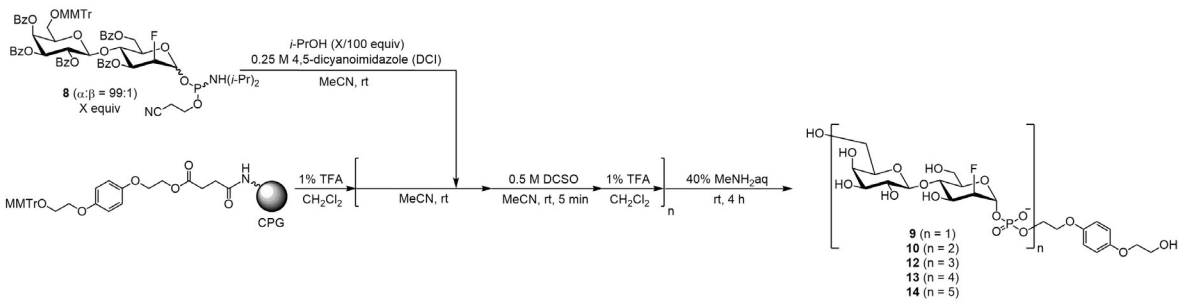
Figure 3. RP-HPLC profiles at 286 nm of crude products after the solid-phase synthesis according to Scheme 2 after 4 h treatment with methylamine. a) **8** (α:β = 99:1, 10 equiv) was used for the synthesis of compounds containing the one repeating unit ($n = 1$). b) **8** (α:β = 99:1, 10 equiv) was used for the synthesis of compounds containing the two repeating units ($n = 2$). c) **8** (α:β = 76:24, 10 equiv) was used for the synthesis of compounds containing the one repeating unit ($n = 1$). d) **8** (α:β = 76:24, 20 equiv) was used for the synthesis of compounds containing the two repeating units ($n = 2$). RP-HPLC was performed with a linear gradient of 0–8.7 % acetonitrile for 52 min [in (a) and (c)] or 0–16 % acetonitrile for 48 min [in (b) and (d)] in 0.1 M triethylammonium acetate buffer at 30 °C. Whole HPLC profiles are provided in the Supporting Information.



Scheme 3. Condensation reaction of **8** with isopropyl alcohol to form phosphite **11**. The reaction was monitored by ^{31}P NMR spectroscopy.

ference in reactivity of two isomers, condensation reaction was conducted in solution and monitored by ^{31}P NMR (see Supporting Information). In the experiments in Scheme 3, compound **8** with anomer ratio with 99:1 (α:β) was reacted with a quantity of isopropyl alcohol equivalent to the amount of the β-isomer. After the reaction, only β-mannosyl 1-phosphite triester **11β** was observed as glycosyl 1-phosphite derivatives. On the other hand, when compound **8** was reacted with excess amount of isopropyl alcohol, **11α** was primarily observed. These results also supported the higher reactivity of **8**β.

This difference in reactivity was troublesome as considerable β-isomers were produced even when using **8** with high anomeric purity as shown in Figure 3a and 3b. Therefore, we devised an improved strategy for the solid-phase synthesis to solve these problems, and the overall procedures are shown in Scheme 4. The only difference between Scheme 4 and the conventional method (Scheme 2) is that phosphoramidite **8** had initially been activated by DCI and reacted with a small amount of isopropyl alcohol to consume **8β** completely, before addition to the reaction vessel containing solid support, in the new strategy. The HPLC profiles in the synthesis of compounds containing the two repeating units **10** by this pre-activation method was shown in Figure 4b. In the HPLC profile no peak derived from stereoisomers was observed, which contrasts with the result from the conventional method in Figure 4a. Therefore, we finally synthesised compounds containing the one to five repeating units according to Scheme 4 and calculated the HPLC yields from the peak area derived from the hydroquinone spacer. It was clear from optimisation of reaction conditions that the use of 20 equivalents of **8** was better for each condensation reaction than using 10 equivalents. In these conditions, compounds containing one repeating unit of **9** and two repeating units of **10** could be synthesised with HPLC yields of 98 % and 93 %, respectively (Figure 5a, 5b; Table 1). As these yields were high enough to study for the synthesis of longer oligomers containing three to five repeating units, these longer oligomers were also synthesised by the present method. As shown in Figure 5c and Figure 5d, it was demonstrated that compound containing the three and four repeating units **12** and **13** could be synthesised with high HPLC yields, 95 % and 91 %, respectively, with trace amounts of



Scheme 4. Modified solid-phase synthesis containing a pre-activation step for phosphoramidite **8**.

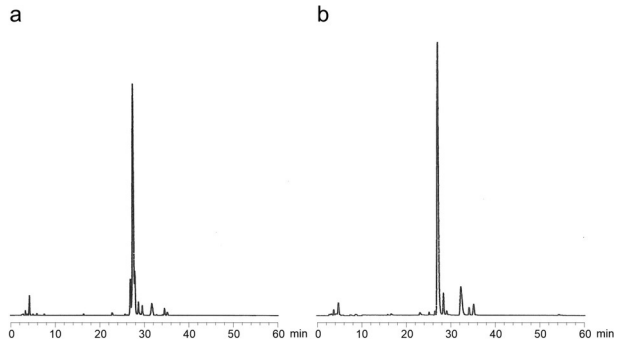


Figure 4. RP-HPLC profiles at 286 nm of crude products after the solid-phase synthesis ($n=2$). a) The synthesis was conducted according to Scheme 2 except for condensation time of 20 min using **8** (α:β = 99:1). b) The synthesis was conducted according to Scheme 3 using 10 equiv of **8** (α:β = 99:1). RP-HPLC was performed with a linear gradient of 0–20% acetonitrile for 60 min in 0.1 M triethylammonium acetate buffer at 30 °C.

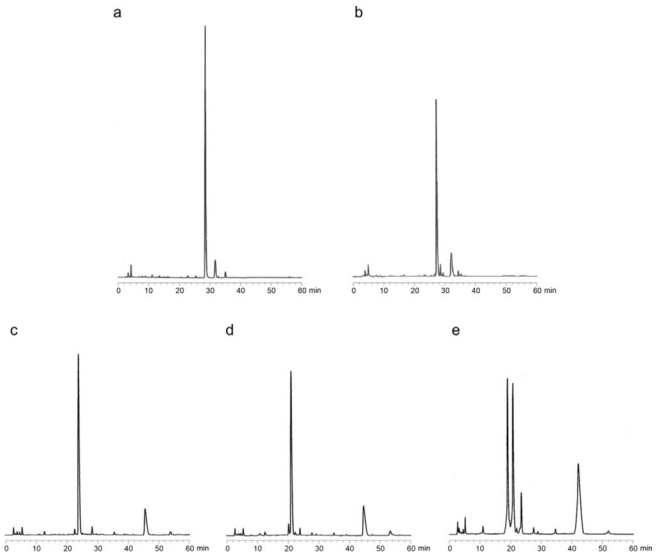


Figure 5. RP-HPLC profiles at 286 nm of crude products after the solid-phase synthesis ($n=1$–5) using **8** (α:β = 99:1) according to Scheme 3. Condensation conditions and HPLC yields are on Table 1. RP-HPLC was performed with a linear gradient of 0–20% acetonitrile for 60 min in 0.1 M triethylammonium acetate buffer (pH 7) at 30 °C in (a) and (b). a) $n=1$. Compound **9** was eluted at 28.4 min. b) $n=2$. Compound **10** was eluted at 27.0 min. In each case, *N*-methylbenzamide was eluted at 32 min. RP-HPLC was and with a linear gradient of 0–10% acetonitrile for 60 min in 0.1 M ammonium acetate buffer (pH 7) at 30 °C in (c), (d), and (e). c) $n=3$. Compound **12** was eluted at 23.6 min. d) $n=4$. Compound **13** was eluted at 20.8 min. e) $n=5$. Compound **14** was eluted at 18.1 min. In each case, *N*-methylbenzamide was eluted at 41–45 min.

shorter oligomers. Compared with these oligomers, the synthesis of **14** containing five repeating units were slightly difficult. As shown in Figure 5e, it was clear that the condensation of octasaccharide was less efficient, even using 40 equiv. of **8**, than those of other shorter oligosaccharides. Although the HPLC yields of the compounds containing the five repeating units **14** were 39%, significantly lower than those of shorter oligomers, this is the first example of the synthesis of compounds containing five glycosyl 1-phosphate repeating units except for a polycondensation method to the best of our knowledge. All the compounds were isolated by HPLC and identified by MS spectrometry (Table 1). The solid-phase phosphoramidite method could construct four and five repeating units of [β-Gal-(1→4)-α-ManF-(1→6)-P-] with the pre-activation process in this study. The results showed the potency of the phosphoramidite method for the synthesis of compounds containing acid-stable 2-deoxy-2-fluoro-mannopyranosyl 1-phosphate repeating units.

3. Conclusions

We synthesised compounds containing [β-Gal-(1→4)-α-ManF-(1→6)-P-]$_5$, the longest glycosyl 1-phosphate repeating units that have been chemically synthesised in a stepwise manner, to the best of our knowledge. We accomplished the synthesis based on a solid-phase phosphoramidite method, and a problem concerning the high reactivity of the β isomer of the phosphoramidite was overcome by the consumption of the isomer before solid-phase reaction with a pre-activation step. Investigation for the synthesis of further longer oligomers and enzymatic activities of the synthesised analogues are now in progress.

Experimental Section

General Procedure

All solution-phase reactions were conducted under an argon atmosphere. ^{1}H NMR spectra were obtained at 300 MHz on a JEOL AI300 spectrometer and at 400 MHz on a JEOL Lambda 400 spectrometer with tetramethylsilane (TMS) as an internal standard (δ 0.0) in $CDCl_3$. ^{31}P NMR spectra were obtained at 162.0 MHz on a JEOL Lambda 400 spectrometer with 85% H_3PO_4 (δ 0.0) as an external standard. ^{19}F NMR spectra were obtained at 376.3 MHz on a JEOL Lambda 400 spectrometer. Mass spectra were recorded on a JMS-700 spectrometer (JEOL) with trifluoroacetic acid as an external standard (δ−76.5). Mass spectra were recorded on a 910-MS

Table 1. Reaction conditions and results of the synthesis of compounds containing [β-Gal-(1→4)-α-ManF-(1→6)-P-]$_n$ structure ($n=1$–5) according to Scheme 3.

Entry	Product	Condensation conditions 8 (α:β = 99:1) [equiv]	Time [min]	ESI-MS Calcd (*m/z*)		Found	HPLC yield [%]
1	9 ($n=1$)	20	60	$[C_{22}H_{33}FO_{16}P]^-$	603.1496	603.1496	98
2	10 ($n=2$)	20	20	$[C_{34}H_{53}F_2O_{28}P_2]^{2-}$	504.1050	504.1050	94
3	12 ($n=3$)	20	20	$[C_{46}H_{73}F_3O_{40}P_3]^-$	1415.2849	1415.2843	95
4	13 ($n=4$)	20	20	$[C_{58}H_{93}F_4O_{52}P_4]^-$	1825.3525	1825.3513	91
5	14 ($n=5$)	40	20	$[C_{70}H_{112}F_5O_{64}P_5]^2$	1113.2064	1113.2053	39

FTMS system (Varian) or a Voyager System 4327 (Applied Biosystems). Analytical TLC was performed on Merck Kieselgel 60-F254 plates. Silica gel column chromatography was carried out using silica gel 60 N (63–210 μm or 40–50 μm) as a neutral silica gel, and Chromatorex NH-DM1020 as an NH silica gel. Gel filtration chromatography was conducted using Sephadex® LH-20. RP-HPLC was carried out using a μBondasphere 5 μm C18, 100 Å, 3.9 mm × 150 mm (Waters). Organic solvents were purified and dried according to the appropriate procedures. Anomeric ratio was calculated from relative peak areas in ^{19}F NMR and ^{31}P NMR.

Standard Procedure for Solid-Phase Synthesis with Pre-Activating Step

The CPG bearing MMTr group via hydroquinone spacer (23.8 or 58.4 μmol g^{-1}) was used for the solid-phase synthesis. Manual solid-phase synthesis of compounds glycosyl 1-phosphate repeating units was conducted using a small glass filter with a stopper at the top and stopcock at the bottom as a reaction vessel, and all reactions were conducted under argon atmosphere. Before synthesis, solvents were dried over molecular sieves. Solutions of 4,5-dicyanoimidazole (DCI) and (+)-(8,8-dichlorocamphorylsulfonyl)oxaziridine (DCSO) in acetonitrile were also dried over molecular sieves, respectively. Disaccharide phosphoramidite **8** was dried under reduced pressure. CPG bearing MMTr group via a hydroquinone spacer (23.8 μmol g^{-1}, 21.0 mg, see SI) was placed into the reaction vessel. The resin was treated with 1% TFA in dichloromethane (1 mL, 15 sec × 3).

After the MMTr-deprotection, the resin was washed with dichloromethane (1 mL × 4) and acetonitrile (1 mL × 3) and then dried over reduced pressure. A pre-activated solution that contains disaccharide phosphoramidite **8** (20 μmol, α:β = 98:2), DCI (75 μmol), and 2-propanol (0.2 μmol) in acetonitrile (150 μL), was added to the resin. The mixture was mixed by a vortex oscillator for 20 min. Then the resin was washed with acetonitrile (1 mL × 3) and then dried under reduced pressure. A solution of DCSO (0.5 M) in acetonitrile (150 μL) was added to the resin. After 5 min, the resin was washed with acetonitrile (1 mL × 3) and dichloromethane (1 mL × 3). The resin was treated with 1% TFA in dichloromethane (1 mL, 15 sec × 4). The procedural steps in this paragraph were repeated 1–5 times.

Then the resin was washed with dichloromethane (1 mL × 3) and transferred to an eggplant flask. The resin was treated with 40% aqueous solution of methylamine (4 mL) for 4 h. The resulting solution was filtered and collected, and freeze-dried. Finally, the crude product was analysed by RP-HPLC.

Compound 2

Compound **1** (5.45 g, 15.6 mmol) was dissolved in methanol (156 mL). Sodium methoxide (84.1 mg, 1.56 mmol) added to the solution and it was stirred for 1 h. The reaction was quenched by addition of Dowex 50W X8 (H^+ form) and the mixture was filtered. The filtrate was dried over Na_2SO_4, filtered, and concentrated. The crude product containing 2-deoxy-2-fluoro-D-mannopyranse was coevaporated with pyridine, and then dissolved in pyridine (200 mL). The solution was stirred and cooled to −40 °C and the diluted solution of benzoyl chloride in pyridine (5.4 mL BzCl in 111 mL pyridine) was added dropwise to the cooled solution over 10 min. After 2 h, additional benzoyl chloride (0.5 mL) was added and the mixture was stirred for 3 h. The reaction was quenched by addition of methanol, and then the mixture was concentrated. The mixture was dissolved in dichloromethane and washed with water. The water layer was back-extracted with dichloromethane. The organic layers were combined and washed with brine, and then dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography (hexane-ethyl acetate (3:1, v/v)) to afford pure **2** as colourless solid (4.64 g, 9.38 mmol, 60%, α:β = 98:2).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.14–8.08 (6H, m), 7.68–7.44 (9H, m), 6.60 (1H, dd, J = 1.8, 6,6 Hz, H-1), 5.68–5.54 (1H, m, H-3), 5.14–4.90 (2H, m, H-2, H-5), 4.50 (1H, dd, J = 1.8, 12.4 Hz, H-6a), 4.33–4.16 (2H, m, H-4, H-6b), 3.31 (1H, d, J = 4.1 Hz, 4-OH). ^{19}F NMR δ -(203.9–204.2) (α), -(220.1–220.4) (β). MALDI-TOF MS Calcd ms for $C_{27}H_{23}FNaO_8^+$ $[M + Na]^+$ 517.13 Found 517.13.

Compound 4

Glycosyl donor **3** (9.85 g, 11.3 mmol) and glycosyl acceptor **2** (4.64 g, 9.38 mmol) were coevaporated with toluene and dissolved in dichloromethane (188 mL). Trimethylsilyl trifluoromethanesulfonate (0.51 mL) was added dropwise to the solution over 5 min and it was stirred for 40 min. The reaction was quenched by addition of triethylamine (10 mL) and the solution was washed with saturated aqueous solutions of $NaHCO_3$. The water layers were combined and back-extracted with dichloromethane. The organic layers were combined and then dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography (neutral silica gel, hexane-ethyl acetate (5:1, v/v)) to afford pure **4** as colourless foam (8.70 g, 7.20 mmol, 77%, α:β = 93:7).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.10–7.94 (6H, m), 7.84–7.72 (4H, m), 7.65–7.20 (25H, m), 7.08 (1H, t, J = 7.4 Hz), 6.99 (2H, t, J = 7.6 Hz), 6.86 (2H, t, J = 7.7 Hz), 6.53 (1H, dd, J = 1.9, 6.6 Hz, H-1 (= $H1_{Man}$)), 5.92 (1H, d, J = 3.2 Hz, H-4′ (= $H\text{-}4_{Gal}$)), 5.74–5.61 (2H, m, H-3, H-2′), 5.48–5.42 (1H, m, H-3′), 5.04 (1H, td, J = 2.2, 48.9 Hz, H-2), 4.87 (1H, d, J = 7.8 Hz, H-1′), 4.58–4.42 (3H, m), 4.10 (1H, J = 9.8 Hz), 3.77 (1H, dd, J = 5.9, 8.5 Hz), 3.56 (1H, dd, J = 5.6, 10.0 Hz), 3.36 (1H, t, J = 9.4 Hz). 0.94 (9H, s). ^{19}F NMR ($CDCl_3$, 376 MHz) δ -(203.9–204.2) (α), -(220.0–220.4) (β). MALDI-TOF MS Calcd ms for $C_{70}H_{63}FNaO_{16}Si^+$ $[M + Na]^+$ 1229.37 Found 1229.19.

Compound 5

Disaccharide **4** (7.23 g, 6.0 mmol) was dissolved in tetrahydrofuran (113 mL) and acetic acid (0.41 mL) was added. A solution of tetrabutylammonium fluoride trihydrate (1.89 g) in tetrahydrofuran (6 mL), which was preliminarily dried over molecular sieves, was added to the disaccharide solution after cooling to 0 °C. The mixture was warmed to RT and stirred for 4.5 h and then the reaction was quenched by addition of a saturated aqueous solution of $NaHCO_3$. The mixture was diluted with toluene and washed with saturated aqueous solutions of $NaHCO_3$. The water layers were back-extracted with toluene. The organic layers were combined and then dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography (neutral silica gel, hexane-ethyl acetate (3:1 to 1:1, v/v)) to afford pure **5** as colourless foam (5.37 g, 5.54 mmol, 92%, α:β > 99:1).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.11–7.93 (10H, m), 7.78–7.19 (20H, m), 6.55 (1H, dd, J = 2.1, 6.9 Hz, H-1), 5.86–5.75 (2H, m, H-3, H-2′), 5.63 (1H, d, J = 3.2 Hz, H-4′), 5.41 (1H, dd, J = 3.3, 10.4 Hz, H-3′), 5.06 (1H, td, 2.5, 48.5 Hz, H-2), 4.96 (1H, d, J = 8.0 Hz, H-1′), 4.59–4.46 (3H, m, H4), 4.18 (1H, d, J = 9.6 Hz), 3.65 (1H, t, J = 6.9 Hz) 3.28–3.20 (1H, m), 3.08–2.99 (1H, m), 2.39 (1H, t, J = 6.9 Hz, OH).

^{19}F NMR ($CDCl_3$, 376 MHz) δ -(202.9–203.2) (α), -(218.5–218.9) (β, trace). MALDI-TOF MS Calcd ms for $C_{54}H_{45}FNaO_{16}^+$ $[M + Na]^+$ 991.26 Found 991.02.

Compound 6

Compound **5** (1.45 g, 1.5 mmol) was coevaporated with pyridine and dissolved in pyridine (15 mL). 4-Methoxytrityl chloride (1.39 g, 4.5 mmol) was added to the solution and it was stirred for 20 h. Additional 4-methoxytrityl chloride (1.39 g, 4.5 mmol) was added and the solution was stirred for 7 h. The reaction was quenched by addition of methanol and the mixture was concentrated. The crude product was diluted with dichloromethane and washed with saturated aqueous solutions of $NaHCO_3$. The water layers were combined and back-extracted with dichloromethane. The organic layers were combined, and then dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography (neutral silica gel, hexane-ethyl acetate (3.5:1 to 3:1, v/v)) to afford pure **6** as colourless foam (1.70 g, 1.37 mmol, 91 %, α:β = 96:4).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.10–7.93 (8 H, m), 7.76–7.07 (32 H, m), 6.90 (2 H, t, *J* = 7.7 Hz), 6.63 (2 H, d, *J* = 7.8 Hz), 6.53 (1 H, dd, *J* = 2.1, 6.8 Hz, H-1), 5.85 (1 H, d, *J* = 2.9 Hz, H-4′), 5.73–5.58 (2 H, m, H-3, H-2′), 5.39 (1 H, dd, *J* = 3.1, 10.3 Hz, H-3′), 5.11 (1 H, d, *J* = 48.7 Hz, H-2), 4.84 (1 H, d, *J* = 8.0 Hz, H-1′), 4.70–4.42 (3 H, m, H-4), 4.10 (1 H, d, *J* = 9.5 Hz), 3.74–3.66 (4 H, m), 3.41–3.34 (1 H, m), 3.21 (1 H, t, *J* = 8.6 Hz). ^{19}F NMR ($CDCl_3$, 376 MHz) δ -(204.0–204.3) (α), -(219.9–220.4) (β). MALDI-TOF MS Calcd ms for $C_{74}H_{61}FNaO_{17}^+$ $[M + Na]^+$ 1263.38 Found 1263.15.

Compound 7

Disaccharide **6** (1.61 g, 1.30 mmol) was dissolved in tetrahydrofuran (8 mL) and cooled to −30 °C. The mixed solution of tetrahydrofuran (5.0 mL) and 40 wt % methylamine in methanol (1.46 mL) was added dropwise to the disaccharide solution. After stirring for 5 h, the mixture was diluted with cold toluene and then concentrated. The crude product was purified by silica gel column chromatography (neutral silica gel, hexane-ethyl acetate (2:1 to 1:1, v/v)) and then gel filtration chromatography (neutral silica gel, dichloromethane-methanol (1:1, v/v)) to afford pure **7** as colourless foam (1.22 g, 1.08 mmol, 83 %, α:β = 93:7).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.06–7.90 (6 H, m), 7.75–7.67 (4 H, m), 7.53 (2 H, t, *J* = 7.5 Hz), 7.56–7.07 (23 H), 6.92 (2 H, t, *J* = 7.5 Hz), 6.63 (2 H, d, *J* = 7.8 Hz), 5.85 (1 H, d, 2.9 Hz, H-4′), 5.66–5.36 (4 H, m, H-1, H-3, H-2′, H-3′), 4.97 (1 H, d, *J* = 49.8 Hz, H-2), 4.82 (1 H, d, *J* = 8.1 Hz, H-1′), 4.54–4.44 (3 H, m, H-4), 4.20 (1 H, d, *J* = 9.5 Hz), 3.72–3.64 (4 H, m, OMe, H-5′), 3.40–3.33 (1 H, m, 6-H′a), 3.19 (1 H, t, *J* = 8.6 Hz, 6-H′b), 2.76 (1 H, t, *J* = 3.3 Hz, OH). ^{19}F NMR ($CDCl_3$, 376 MHz) δ -(204.9–205.2) (α), -(223.8–224.1) (β). MALDI-TOF MS Calcd ms for $C_{67}H_{57}FNaO_{16}^+$ $[M + Na]^+$ 1159.35 Found 1159.11.

Compound 8

Disaccharide **7** (1.16 g, 1.0 mmol) was coevaporated with toluene and dissolved in toluene (10 mL). The solution was cooled to 0 °C and *N*,*N*-diisopropylethylamine (514 μL, 3.0 mmol) was added. 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (335 μL, 1.5 mmol) was added and the solution was warmed to room temperature. After stirring for 4 h, additional 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (112 μL, 0.5 mmol) was added. After 1 h, the mixture was diluted with dichloromethane and washed with saturated aqueous solutions of $NaHCO_3$. The water layers were combined and back-extracted with dichloromethane. The organic layers were combined, and then dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography (NH-silica gel, hexane-ethyl acetate (2:1, v/v)) to afford pure **8** as colourless foam (1.03 g, 0.81 mmol, 81 %, α:β = 97:3).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.09–7.92 (6 H, m), 7.78–7.65 (4 H, m), 7.59–7.05 (25 H, m), 6.95–6.85 (2 H, m), 6.61 (2 H, d, *J* = 8.8 Hz), 5.90 (1 H, s, H-4′), 5.66–5.38 (4 H, m, H-1, H-3, H-2′, H-3′), 5.03–4.74 (2 H, m, H-2, H-1′), 4.60–4.37 (3 H, m, H-4, H-5, H-6a), 4.11–3.98 (1 H, m), 3.92–3.50 (8 H, m), 3.43–3.32 (1 H, m), 3.19 (1 H, q, *J* = 9.2 Hz), 2.63, 2.38 (2 H, t, t, *J* = 6.2, 6.4 Hz, OCH_2CH_2CN, diastereomers). 1.23–1.04 (12 H, m). ^{19}F NMR ($CDCl_3$, 376 MHz) -(202.3–202.6), -(203.1–203.4) (α, diastereomers), -(219.7–220.1), -(220.2–220.5) (β, diastereomers) ^{31}P NMR ($CDCl_3$, 162 MHz) δ 154.4 (β), 152.6 (α), 151.5 (β), 149.5 (α). MALDI-TOF MS Calcd ms for $C_{76}H_{74}FN_2NaO_{17}^+$ $[M + Na]^+$ 1359.46 Found 1359.28.

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