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Synthesis and properties of 2-deoxy-2-fluoromannosyl phosphate derivatives

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ABSTRACT

2-Deoxy-2-fluoromannosyl phosphotriester and phosphodiester derivatives were synthesized and they were demonstrated to be more stable than the 2-OH compounds under acidic conditions. 2-Fluoro substituted analog of α-mannopyranosyl 1-phosphodiester **20** was found to be significantly stable at pH 1, where 2-OH compound **21** was gradually hydrolyzed. In addition, 2-F analogs of α-mannopyranosyl 1-phosphotriesters also was found to be stable under both Brønsted and Lewis acidic conditions. These results strongly suggested that the 2-F substitution is useful for both the synthesis of mannosyl phosphodiester derivatives and improvement of their chemical stability.

1. Introduction

The α-glycosyl phosphate unit, which has a phosphodiester linkage between the anomeric carbon in one sugar and a carbon atom in another sugar, is known as a constituent of capsular polysaccharides (CPS) in pathogenic bacteria such as *Neisseria meningitidis* and *Streptococcus pneumoniae*,[1] and the glycocalyx of parasitic protozoans such as *Leishmania* and *Trypanosoma*.[2] These units are considered to be important components in biological phenomena including immunological responses and infection.[3] Furthermore, there have been some reports in which some phosphoglycans have shown vaccine activity, and vaccines based on them have been developed.[4]

Therefore, stable provision of pure compounds containing the α-glycosyl phosphate structure is useful for both the elucidation of their functions and the development of α-glycosyl phosphate-based vaccines and drugs. However, this remains a challenge for both biologists and chemists, because sugar chains in living systems have structural heterogeneity and are present in very small quantities; furthermore, their structures and their synthetic intermediates are chemically unstable in some cases.[5] Therefore, chemical modification has been spotlighted as a method for the stable supply of pure compounds containing the α-glycosyl phosphate structure.[6] In this study, we focus on 2-deoxy-2-fluoromannopyranosyl 1-phosphate derivatives as glycosyl phosphate analogs.

Derivatives of α-mannopyranosyl 1-phosphate can be seen in the glycocalyx of *Leishmania*[1a] and *Hansenula capsulata* Y-1842 exophosphomannan,[7] and there are some derivatives containing α-2-*N*-acetylmannosamine 1-phosphate units in CPS in *Neisseria meningitidis* (Fig. 1).[1a] In this study, we chose the Man-α-(1 → 6)-*P*-Man structure as a synthetic target in the model compounds, poly-(mannopyranosyl 1-phosphates), in *Hansenula capsulata* Y-1842 exophosphomannan.

A fluorine atom is electronically equivalent to a hydroxyl group. Indeed, 2-deoxy-2-(^{18}F)fluoro-D-glucose is widely used as a glucose analog for positron emission tomography (PET),[8] and fluorinated sugars have been reported as sugar analogs that serve as substrates or inactivators for glycosidases, glycosyltransferases, and other enzymes.[9] Furthermore, a fluorine atom shows strong electron withdrawing properties, and it can be expected to be advantageous for the stabilization of α-glycosyl phosphate structures and their synthetic intermediates. Some glycosyl 1-P structures and their synthetic intermediates are degraded mainly through elimination of the phosphorous containing groups at the anomeric position and generation of the glycosyl oxocarbenium ion.[5,10] A fluorine atom at the 2-position of a pyranose can destabilize such a cation and make α-glycosyl phosphate structures and their intermediates harder to chemically degrade.

Therefore, we firstly aimed to synthesize mannopyranosyl 1-

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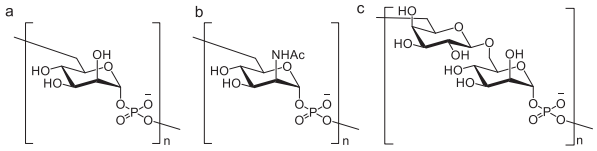


Fig. 1. α-mannopyranosyl 1-phosphate repeating units seen in (a) *Hansenula capsulata* Y-1842 exophosphomannan (b) CPS from *Neisseria meningitidis* (c) *Leishmania* glycocalyx.

diethylphosphate and 2-deoxy-2-fluoromannopyranosyl 1-diethylphosphate derivatives as analogs of the synthetic intermediates using the phosphoramidite method, which is one of the most commonly used synthetic methods of synthesizing α-glycosyl phosphate derivatives.[1a] Secondly, we synthesized α-glycosyl phosphate derivatives containing an α-Man-(1-PO_4^--6)-Man structure. Furthermore, the chemical stabilities of mannopyranosyl and 2-deoxy-2-fluoromannopyranosyl derivatives were compared.

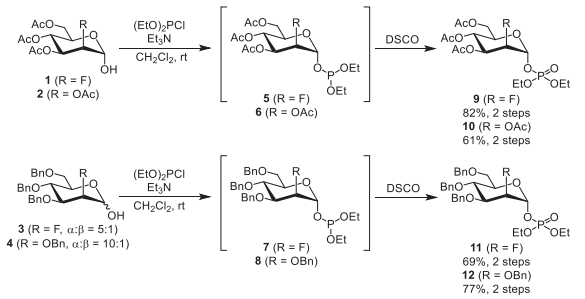
2. Results and discussion

2.1. Synthesis of phosphotriester analogs

Compounds **1**–**4** were known compounds and their synthetic procedures are described in the literature.[11] The anomeric hydroxyl group of each compound was phosphitylated using diethyl chlorophosphite to afford compounds **5**–**8**. All compounds were subsequently oxidized with (+)-(8,8-dichlorocamphorylsulfonyl) oxaziridine (DCSO) and then mannosyl α-1-diethylphosphate derivatives **9**–**12** were obtained in good yields (82%, 61%, 69%, 77% respectively).[12] Incidentally, the β anomer was not observed in these reactions except in the synthesis of compound **12**, in which the β anomer was isolated in 21% yield (Scheme 1).

2.2. Stability of mannosyl phosphotriesters under acidic conditions

A glycosyl phosphotriester structure is an intermediate in the synthesis of glycosyl phosphate derivatives in the phosphoramidite method. One of the most serious concerns regarding these reactions is the instability of the phosphotriester intermediates under acidic conditions, such as in deprotection. Therefore, the stability of mannosyl phosphotriesters **9**–**12** in dichloromethane containing 3% (v/v) dichloroacetic acid (DCA), which is prescribed for the removal of trityl type protecting groups,[13] was evaluated (Table 1, left column). In these experiments, the reaction time was 24 h, and the unreacted phosphotriester was recovered by silica gel column chromatography. Most large quantities of 2-F and 2-OAc compounds **9**–**11** remained intact under the conditions and 69–79% of them were recovered. On the other hand, 2-OBn compound **12** was slowly degraded (almost completely disappeared in 24 h by TLC monitoring, see SI) and nothing was recovered after the reaction. The major degraded products were hydrolyzed product **4** and anomeric dichloro acetate. By comparing the results for **9**–**11** and **12**, it is clearly demonstrated that electron withdrawing 2-F and 2-OAc substituents stabilized the mannosyl phosphotriesters in the presence of dichloroacetic acid.



Scheme 1. Synthesis of mannopyranosyl 1-diethylphosphate and 2-deoxy-2-fluoromannopyranosyl 1-diethylphosphate derivatives.

Next, the stability of these compounds under Lewis acidic conditions was also investigated (Table 1, right column). In these experiments, dichloromethane containing 0.1 M of $BF_3{\cdot}Et_2O$ was used to achieve Lewis acidic conditions. Under the conditions, the 2-*O*-benzyl mannosyl phosphotriester **12** was completely degraded in 1 h (see SI). Furthermore, 2-*O*-acetyl one **10** was slowly degraded, and nothing was recovered after 24 h of exposure to the acidic solution. On the other hand, 2-fluoro mannosyl phosphotriesters **9** and **11** were observed to be intact even after 24 h, and 83% and 71% of **9** and **11**, respectively, were recovered. From these results, it was shown that introducing a fluoro group instead of an OAc or OBn groups at the 2-position of mannopyranose is much effective for stabilization of mannosyl phosphotriesters under both Brønsted and Lewis acidic conditions.

2.3. Synthesis of mannosyl phosphates

Next, we synthesized compounds containing the Man-α-(1 → 6)-*P*-Man structure as analogs of *Hansenula capsulata* Y-1842 exophosphomannan. Firstly, mannose derivatives containing a hydroxyl group on their anomeric carbon atom, **13** and **14**, were prepared. Compound **13** was synthesized from a known acetyl-protected compound (see SI),[11a] and **14** was prepared by a procedure described in the literature.[14] Compounds **13** and **14** were phosphitylated using 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite to afford **15** and **16** in 86% and 73% yields, respectively. The 6-hydroxy compound of thiomannoside **17** was prepared by slight modification of a procedure described in the literature.[15] In the condensation reaction of phosphoramidite monomer **16** with alcohol **17**, the reaction did not proceed efficiently and over 80% of unreacted **17** was recovered when 1-*H*-tetrazole was used as an acidic activator. These condensation reactions proceeded well when 5-ethylthio-1-*H*-tetrazole (ETT) was used as a more acidic activator,[16] and subsequently oxidation using *tert*-butyl hydroperoxide[17] afforded Man-α-(1 → 6)-*P*-Man precursors **18** and **19** in good yield as shown in Scheme 2. The cyanoethyl groups on compounds **18** and **19** were removed with triethylamine and the benzoyl groups were subsequently removed in the presence of ammonia to afford the ammonium salts of the Man-α-(1 → 6)-*P*-Man derivatives. These compounds were purified by reversed phase HPLC (RP-HPLC) and were finally transformed to the sodium salts on ion-exchange resin to afford compounds **20** and **21** (41% and 26% yields, respectively) (see Scheme 3).

2.4. Stability of Man-α-(1 → 6)-P-Man structures under acidic conditions

To evaluate the properties of α-2-deoxy-2-fluoromannosyl 1-phosphate **20** and α-mannosyl 1-phosphate **21**, these compounds were exposed to aqueous solutions at pH 7.0, 5.0, and 1.0 and the degradation was monitored by RP-HPLC. At pH 7.0 and 5.0, no degradation was observed with compounds **20** and **21**. On the other hand, degradation of 2-OH compound **21** was observed at pH 1.0 at 37 °C and it was almost completely decomposed after 48 h (Fig. 2). In contrast, 2-F derivative **20** was found to be completely stable under the same acidic conditions. These results clearly indicated that the 2-F substituent stabilized not only mannosyl