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Mono, di and tri-mannopyranosyl phosphates as mannose-1-phosphate prodrugs for potential CDG-Ia therapy

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Abstract—An efficient and convergent method for the synthesis of mannose-1-phosphate prodrugs is described as a potential therapy for congenital disorders of glycosylation-Ia (CDG-Ia). The key feature of the proposed approach is the silver assisted nucleophilic substitution of 2,3,4,6-tetra-*O*-protected-α-D-mannopyranosyl bromides with various silver phosphate salts to afford mono, di, and tri-mannopyranosyl phosphates. A preliminary biological evaluation of the synthesized phosphate prodrugs has been carried out.

The Congenital Disorders of Glycosylation[1] (CDG) belong to a recently recognized group of inherited multisystem metabolic disorders characterized by the abnormal glycosylation of a number of serum glycoproteins.[2] The most common clinical case associates an encephalomyopathy[3] and a peripheral neuropathy.[4] Among the CDG, CDG-Ia[5] and CDG-Ib[6] are the most frequently encountered forms and are related to a deficiency in the production of GDP-mannose (guanosine-diphosphate-mannose). GDP-mannose is generated from mannose-6-phosphate (Fig. 1) which is derived from either glucose, fructose or mannose metabolism. Mannose-6-phosphate can be generated by transformation of fructose-6-phosphate by phosphomannose isomerase (PMI), or by phosphorylation of the free intracellular mannose by an hexokinase. The mannose-6-phosphate (M6P) is then isomerised by the action of phosphomannose mutase (PMM) into mannose-1-phosphate (M1P). This one is subsequently converted to GDP-mannose by the action of guanosine-monophosphate transferase.

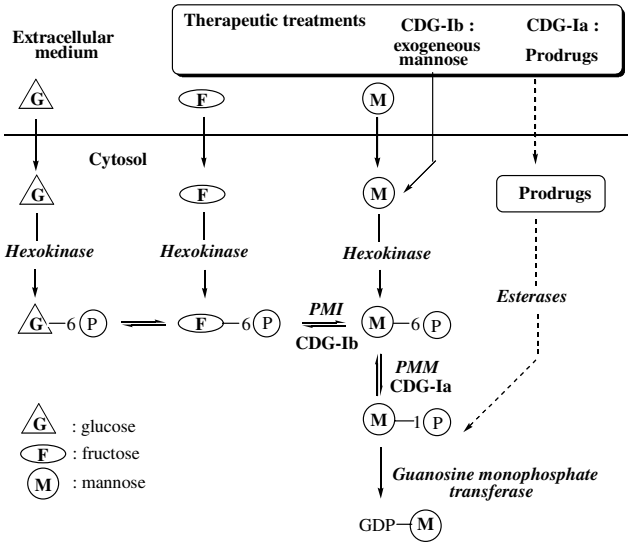

Figure 1. GDP-mannose biosynthesis.

Enzymatic deficiencies of PMM and PMI are, respectively, responsible for CDG-Ia and Ib. CDG-Ib patients

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can be treated by oral supplementation with free mannose in order to offset the lack of M6P.[7]

However, there is currently no treatment for patients suffering from CDG-Ia which accounts for 70% of type I observed cases. Due to good recovery in patients affected with CDG-Ib obtained by supplementation with mannose, one could speculate treating CDG-Ia patients with M1P supplementation. However, due to its high polarity limiting passive diffusion through membranes and its relative instability in blood, it seems more appropriate to use prodrugs[8] of M1P. Such drugs should be neutral lipophilic phosphotriesters.

The goal of this work was to synthesize various phosphoester prodrugs containing at least one *O*-protected-1-mannosyl moiety (Fig. 2).

Indeed, expected behaviour from such prodrugs would be: (i) an easy diffusion from extracellular medium to cytosol due to its high lipophilicity, (ii) release of M1P in cytosol by esterases and (iii) possible transphosphorylation catalyzed by the guanosine-monophosphate transferase to directly produce GDP-mannose.

In a recent work, Marquadt et al. described the synthesis of protected derivatives of mono-1-mannopyranosyl phosphate.[9] Their strategy involved reaction between a conveniently 2,3,4,6-*O*-protected mannose as a nucleophile with either a dibenzyl di-*iso*-propylphosphoramidite or chloridophosphites as an electrophile, followed by subsequent oxidation at the phosphorus atom. Alternatively, the preparation of bis-pivaloyloxymethyl (POM) peracetylated M1P was also described in better yield by nucleophilic substitution of chlorophosphoryl-bisPOM by peracetylated mannose.[10] Our work focused on a complementary and more convergent approach which involves a silver assisted nucleophilic substitution of a conveniently *O*-protected 1-mannopyranosyl bromide, as an electrophile, by an anionic phosphate ester.

According to that general approach, the synthesis of mono, di, and tri-α-D-mannopyranosyl phosphates is straightforward. It has to be pointed out that, among these M1P prodrugs, the symmetrical trimannopyranosyl phosphate would have the advantage of leading to M1P and consequently, to GDP-mannose, whatever the phosphorus–oxygen bond to be disconnected. So, we herein report an efficient and practical method for the large-scale synthesis of mono, di, and tri-α-D-mannopyranosyl phosphates from commercially available D-mannose.

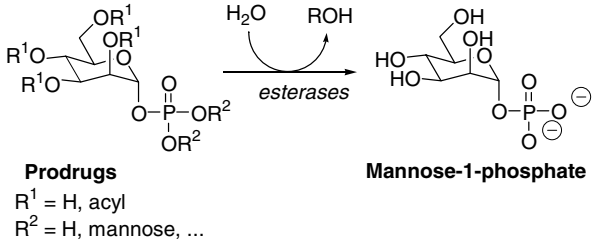
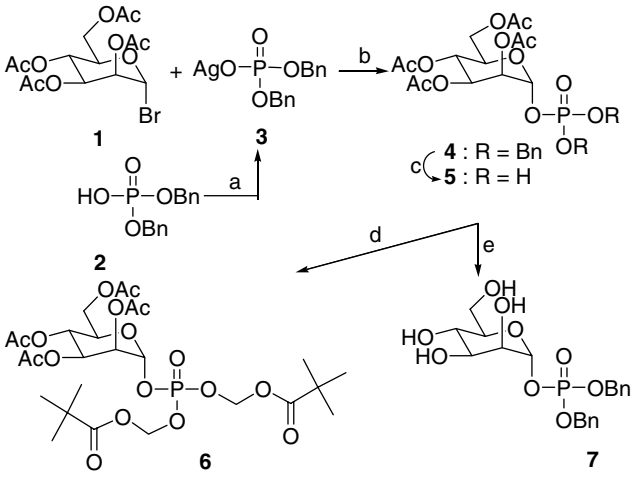


Figure 2. Structure of the targeted prodrugs.

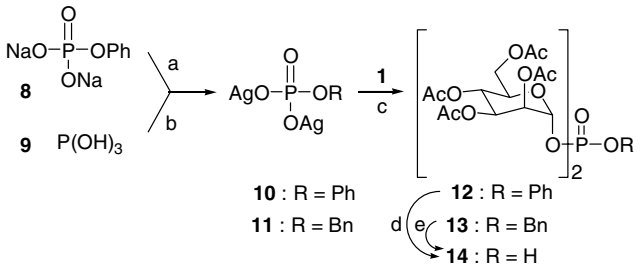


Scheme 1. Reagents and conditions: (a) Na_2CO_3, H_2O then $AgNO_3$, H_2O, rt, 77%; (b) toluene, MS 4 Å, rt, 18 h, 94%; (c) H_2, Pd/C 10%, 2 h, 100%; (d) Ag_2CO_3, CH_3CN, chloromethyl pivalate (7 equiv), 24 h, 52%; (e) MeONa, MeOH, then Amberlite® resin IR120 H^+.

The synthesis of mono-α-D-mannosyl phosphate prodrugs is outlined in Scheme 1 from 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide[11] **1** and silver dibenzyl phosphate **3**. The peracetylated α-D-mannosyl bromide **1** was easily obtained by acetylation of mannose (Ac_2O, DMAP, Et_3N, CH_2Cl_2, rt) followed by bromination (HBr-AcOH)[12] in 86% overall yield. The phosphate **3** resulted from treatment of the commercially available dibenzyl phosphate **2** with sodium carbonate and precipitation with silver nitrate (77% yield).[13] The silver assisted nucleophilic substitution of bromomannose **1** by silver dibenzyl phosphate **3** was carried out in toluene in the presence of an excess of molecular sieves 4 Å and efficiently led to the dibenzyl peracetylated α-D-mannopyranosyl phosphate **4**.[14] Indeed, this compound was isolated in 94% yield as a single α-anomer. Hydrogenolysis of both benzyl esters with dihydrogen in the presence of 10% Pd/C quantitatively led to the phosphate **5** which could be then converted into its POM derivative[9,10] **6** (52% yield). The acetyl and POM[15] protecting groups are expected to facilitate cell penetration and to be further cleaved into M1P by intracellular esterases and phosphoesterases. Alternatively, methanolysis of the acetate protecting groups of **4** led to the phosphate **7** as a substrate for further *O*-chemoselective protection of the primary alcohol function.

We next turned to the preparation of di-α-D-mannopyranosyl phosphate prodrugs according to this strategy (Scheme 2). It involved the disilver phenyl phosphate **10** which could be prepared by silver nitrate treatment of the commercially available disodium phenylphosphate **8**. Its condensation with the peracetylated mannopyranosyl bromide **1** in toluene formed the corresponding phenyl mannopyranosyl phosphate **12** in 78% yield. Then, hydrogenolysis of phenyl moiety in the presence of PtO_2 in methanol gave the phosphate **14** in moderate yield (27%).

To improve the yield of the hydrogenolysis reaction, we observed that it was more efficient to use the monoben-

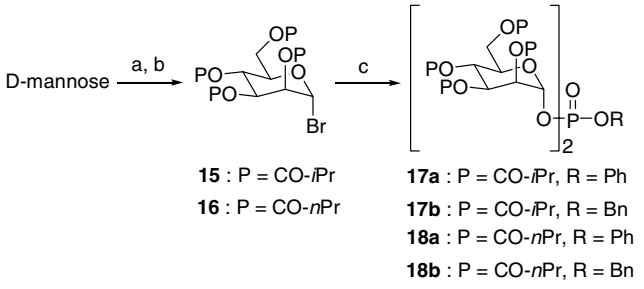


Scheme 2. Reagents and conditions: (a) $AgNO_3$, H_2O, rt, 91%; (b) i—BnOH, Et_3N, I_2, ii—$C_6H_{11}NH_2$ excess, resin 50X8-100 Na^+ form then $AgNO_3$, H_2O, rt, 62%; (c) toluene, MS 4Å, 18 h, rt, 78 and 70% for **12** and **13**, respectively; (d) H_2 (1 atm), PtO_2 10%, 2 h, 27%; (e) H_2, Pd/C 10%, THF–MeOH, 60%.

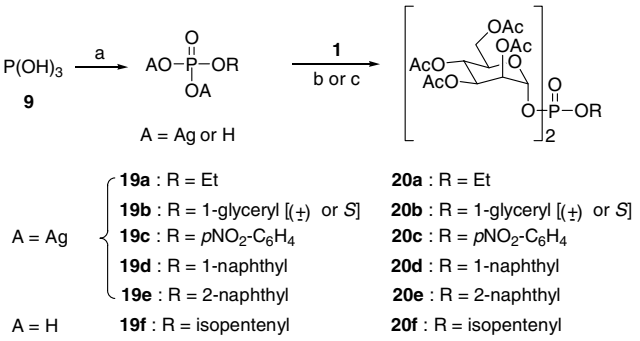
zyl phosphate analogue **13**. Its preparation required condensation of the corresponding disilver benzyl phosphate **11**. This reagent was prepared by treatment of phosphorous acid with a solution of benzyl alcohol and triethylamine containing iodine.[16] Precipitation as its dicyclohexyl ammonium salt, followed by cation exchange (Dowex® resin 50X8-100 Na^+ form), afforded the disilver benzylphosphate **11** which was isolated by silver nitrate treatment as previously. Then, the nucleophilic substitution of the peracetylated 1-mannopyranosyl bromide **1** as above yielded the benzyl dimannopyranosyl phosphate **13** (70%). As expected, subsequent hydrogenolysis in the presence of 10% Pd/C afforded the deprotected phosphate **14** in good yield.

Interestingly, this strategy could be extended, on one hand, to other *O*-protected α-D-mannosyl bromides which can be easily prepared from D-mannose (Scheme 3).

Thus, treatment of D-mannose with *i*- or *n*-butyric anhydride (DMAP, Et_3N, CH_2Cl_2, rt) followed by bromination (HBr, AcOH) gave the corresponding mannosyl bromide **15** or **16** in 61% and 70% overall yield, respectively. Then, nucleophilic substitution was carried out, as above, with disilver phosphate compound **10** or **11** yielding, respectively, the lipophilic phenyl or benzyl di-mannosyl phosphate **17a**,**b** or **18a**,**b**. On the other hand, diversity could be introduced on the phosphate reagent, since alkyl, alkenyl, benzyl or aryl phosphate



Scheme 3. Reagents and conditions: (a) $(i\text{-PrCO})_2O$ or $(n\text{-PrCO})_2O$, DMAP, Et_3N, CH_2Cl_2, rt; (b) HBr, AcOH, 61% and 70% overall yield from D-mannose; (c) **10** or **11**, toluene, MS 4 Å, 18 h, rt.

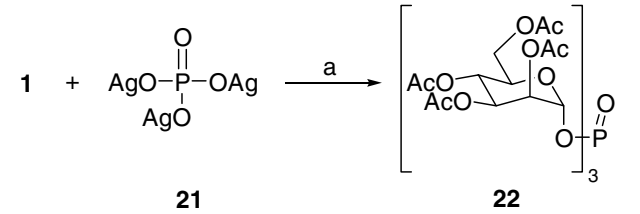


Scheme 4. Reagents and conditions: (a) i—EtOH for **19a** (or isopentenol for **19f**), Et_3N, I_2, ii—$C_6H_{11}NH_2$ excess, resin 50X8-100 Na^+ form then $AgNO_3$, H_2O, rt; (b) toluene, MS 4 Å, 18 h, rt; (c) Ag_2CO_3, CH_2Cl_2, MS 4 Å, 18 h, rt.

could be used as nucleophile towards the peracetylated α-D-mannosyl bromide (Scheme 4). Thus, treatment of phosphorous acid with ethanol in the same conditions as for the preparation of the benzyl phosphate **11** supplied the corresponding disilver phosphate **19a** in 90% yield. In addition, glyceryl, *p*-nitrophenyl, 1-naphthyl and 2-naphthyl phosphates, commercially available as their dicyclohexyl ammonium, disodium and monosodium salts, respectively, were turned into their corresponding disilver phosphates **19b–e** in quantitative overall yield. Nucleophilic substitution of the mannopyranosyl bromide **1** in toluene with derivatives **19a–e** led to the expected dimannopyranosyl phosphates **20a–e** in good to excellent yield. In the specific case of isopentenyl moiety (3-methyl-but-3-en-1-yl), the suitable conditions involved direct condensation of the diacid **19f**, in the presence of a stoichiometric amount of silver carbonate, affording **20f** in moderate yield (25%). Intermediate **19f** was readily obtained from the corresponding dicyclohexyl ammonium precursor through H^+ resin treatment. Among these derivatives, the *p*-nitrophenyl and naphthyl phosphate esters have the advantage of exhibiting a chromophore which facilitates the kinetic studies during the hydrolysis into MIP; while, the isopentenyl and glyceryl phosphate esters provide leaving groups expected to be non-toxic.

Finely, the same strategy was also suitable to obtain the tri-α-D-mannosyl phosphate **22**[17] (Scheme 5).

Thus, treatment of the commercially available trisilver phosphate **21** with peracetylated α-D-mannosyl bromide **1** afforded the prodrug **22** in 31% yield.



Scheme 5. Reagents and conditions: (a) toluene, MS 4 Å, 3 weeks, rt, 31%.

A preliminary biological evaluation of the synthesized di-mannosyl phosphate prodrugs **12**, **14**, **17a**, **18b** and **20d** has been carried out. These prodrugs have been screened, on the one hand, for their toxicity, and on the other, for their ability to interfere with 2[^{3}H]mannose incorporation into cellular glycoconjugates. In fact, if mannose 1-phosphate can be generated from the prodrugs intracellularly, we would expect it to compete with 2[^{3}H]mannose 1-phosphate for entry into the glycoprotein biosynthetic pathway.[18,19] Lymphoblast cells derived from both a control subject and a CDG-Ia patient were preincubated for 30 min with prodrugs (300 μM) in 500 μL glucose-free RPMI 1640 medium supplemented with 0.5 mM glucose and 10% dialysed foetal calf serum prior to addition of 50 μCi 2[^{3}H]mannose. The incubations were continued for a further 30 min before extracting cellular glycoconjugates as previously described. [18] Results revealed that **12** (26%), **17a** (78%), **18b** (71%) and **20d** (56%) inhibited 2[^{3}H]mannose incorporation into glycoconjugates. The toxicity of prodrugs was evaluated by measuring the release of cellular lactate dehydrogenase into the cell culture medium after treating the cells with 300 μM prodrugs for 16 h, and in no case was the toxicity greater than 25% higher than that observed with the drug carrier methanol alone. The mechanism of action of the di-mannosyl phosphate prodrugs is presently under investigation.

In summary, we have described the synthesis of various mannose-1-phosphate prodrugs by nucleophilic substitution of 2,3,4,6-tetra-*O*-protected-α-D-mannopyranosyl bromides with silver phosphate salts. The versatility of this convergent and efficient strategy has been demonstrated by the synthesis of mono, di and tri-α-D-mannopyranosyl phosphate prodrugs for which the phosphate and carbohydrate protecting groups can be chosen to adjust the pharmacomodulation in the correction of the hypoglycosylation pattern. According to the described method, the synthesis of a family of M1P prodrugs leading to various nontoxic leaving groups is under progress. The preliminary in vitro biological evaluation of the synthesized prodrugs showed that di-mannosyl prodrugs are promising membrane-permeant derivatives of mannose-1-phosphate. Further work concerning such prodrugs is currently in progress.

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