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Synthesis of bis-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-L-serinyl phosphate, as a prodrug of mannose-1-phosphate

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Abstract—An efficient synthesis of di-mannosyl serinyl phosphate using a convergent strategy involving a silver assisted nucleophilic substitution of tetra-*O*-acetyl-α-D-mannopyranosyl bromide by a conveniently protected *O*-serinyl phosphate is described. The straightforward synthesis of phosphoserine under Mitsunobu conditions is also reported.

1. Introduction

The congenital disorders of glycosylation[1] (CDG) belong to a group of inherited multisystem metabolic disorders characterised by the abnormal glycosylation of a number of serum glycoproteins.[2] Amongst the CDG, CDG-Ia is most frequently encountered and is related to a deficiency in the production of guanosine-diphosphate-mannose (GDP-Man). Its biosynthesis notably requires conversion of mannose-6-phosphate (M6P) into mannose-1-phosphate (M1P) by phosphomannose mutase (PMM2). M1P is then at the origin of GDP-Man under the action of guanosyl-monophosphate transferase. The enzymatic deficiency in PMM2 is responsible for CDG-Ia[3] and there is actually no treatment for CDG-Ia affected patients. In this context, we carried out a programme aiming at generating membrane permeant M1P prodrugs. Targeted prodrugs (Fig. 1) display either mono, di or tri-mannosyl phosphate structure and we have recently reported an efficient and general synthetic route towards these derivatives.[4] Preliminary biological evaluation of these prodrugs showed notably that dimannosyl phosphates appear as promising candidates. Indeed, these molecules have the advantage that after action by cellular esterases or phosphoesterases, the probability that they will generate M1P is higher than with monomannosyl phosphate.[5]

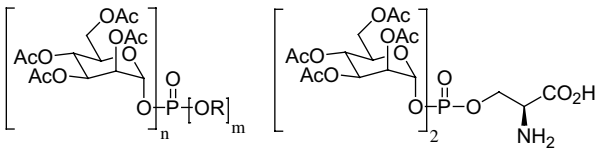

Figure 1. Prodrugs of M1P and targeted compound **1**.

In order to reduce the toxicity of such prodrugs displaying a dimannosyl phosphate structure, an emphasis is now placed upon the introduction of R groups to phosphate, which after liberation by cellular esterases, would lead to metabolites exhibiting weak toxicity. For that purpose, a serinyl moiety, expected to have a reduced toxicity has been chosen (Fig. 1). Furthermore, such a serinyl moiety should improve the hydrosolubility and bioavailability of the resulting prodrugs. Indeed, aminophosphoglycans have already been used as drug carriers.[6] It should be noted that the synthesis of α-D-monomannosyl-serine phosphate has already been described[7] through the formation of an α-D-mannosylphosphoramidite intermediate, followed by reaction with a protected serine derivative and subsequent oxidation of the resulting phosphite. However, to the best of our knowledge this strategy has only been applied to the synthesis of monomannosyl serinyl phosphate,[7] while no access to the targeted dimannosyl serinyl phosphate has

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been described. In order to reach such a compound, a complementary strategy is reported.

2. Results and discussion

Retrosynthetic analysis of the target compound **1** (Fig. 2) involves a coupling between bis-(peracetyl mannosyl)-phosphate **2** as a nucleophile and a conveniently O-activated L-serine derivative **3**. The protective groups of the serine derivative have been chosen to be cleavable in a single non-acidic step to preserve the phosphate at the anomeric position of mannose.

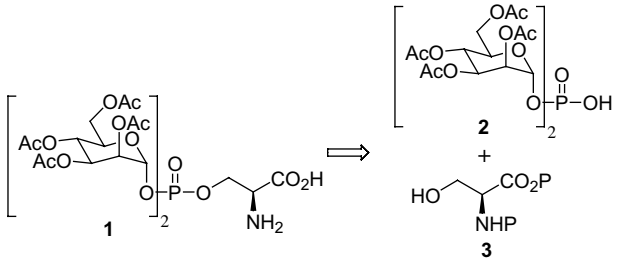


Figure 2. Retrosynthetic pathway of the targeted compound **1**.

Activation of the primary alcohol of serine **3** was intended to be performed under Mitsunobu conditions. To test this new reaction, a model study was carried out from benzyl *N*-benzyloxycarbonyl serine **3** and the commercially available dibenzyl phosphate (Scheme 1).

First, the esterification of the *N*-benzyloxycarbonyl L-serine by successive treatment with cesium carbonate in methanol followed by concentration and addition of benzyl bromide in DMF afforded **3**[8] (94% yield). Then, Mitsunobu coupling of **3** and dibenzyl phosphate in the presence of triphenyl phosphine and diethyl azodicarboxylate gave the expected dibenzylphosphoserine **4** in 50% yield. This new strategy to obtain serinyl phosphate in a single one-pot reaction is an interesting alternative compared to the phosphoramidite-oxidation methodology. Furthermore, it should be noted that on the one hand the hydroxyl activation of serine could be possible on a *N*-carbamate serine derivative in spite of the low nucleophilicity of its hydroxyl usually claimed for such N-protected serine derivatives, while on the other hand the β-elimination commonly observed with O-activated serine derivatives[9] was circumvented. Having succeeded in the synthesis of the phosphoserine according to Mitsunobu conditions, we next turned to the same reaction involving bis-(peracetylmannosyl)-phosphate **7**. This latter one was readily obtained by peracetylation of D-mannose followed by bromination and subsequent silver assisted nucleophilic substitution with benzyl phosphate as its disilver salt[10] followed by hydrogenolysis of the *O*-benzyl bond. Unfortunately the previous Mitsunobu conditions involving **3** and **7** proved to be unsuccessful. The lack of reactivity of phosphate **7** compared to that of the dibenzyl phosphate can tentatively be explained by the weak nucleophilicity of mannosyl phosphate as already reported in other alkylation reactions where the described yields are generally low.[5a]

To overcome this difficulty, we next explored a new strategy involving the condensation of *O*-serinyl phosphate **9** as a nucleophile with the peracylated α-D-mannopyranosyl bromide **5** as an electrophile (Fig. 3).

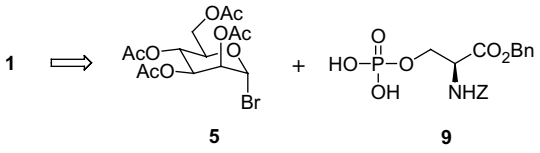
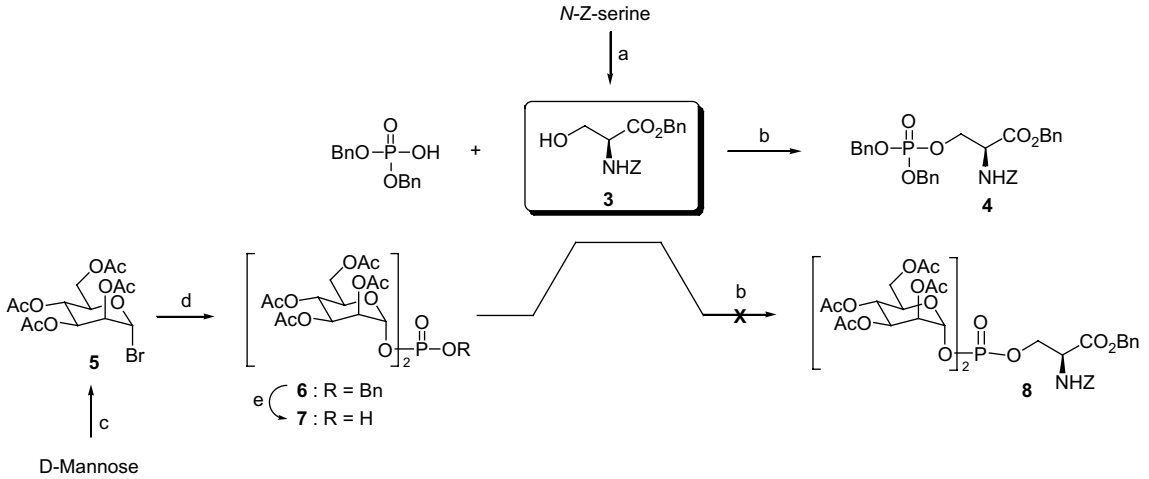
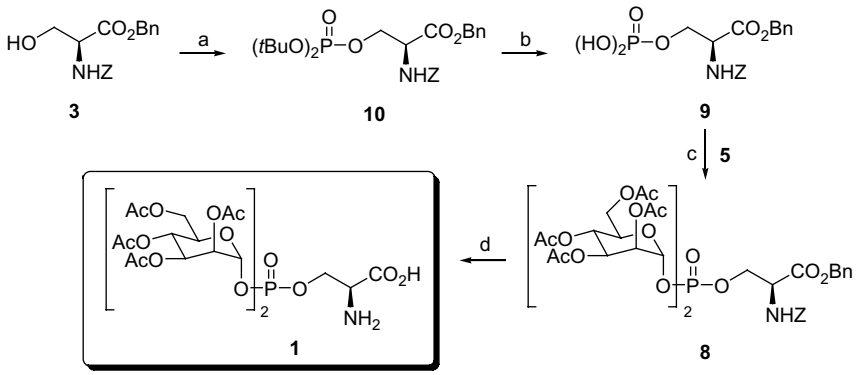


Figure 3. Retrosynthetic pathway of the targeted compound **1**.

The *O*-serinyl phosphate **9** was prepared in two steps (Scheme 2) involving first, an *N*,*N*′-di-isopropyl di-*tert*-butyl



Scheme 1. Reagents and conditions: (a) (i) Cs_2CO_3, MeOH, (ii) BnBr, DMF (94%); (b) Ph_3P, DEAD, $PhCH_3$, THF (50% for **4**); (c) (i) Ac_2O, DMAP, Et_3N, CH_2Cl_2, rt, (ii) HBr, AcOH (86% overall); (d) $BnOP(O)O_2Ag_2$, $PhCH_3$, molecular sieves 4 Å, rt (70%); (e) H_2, Pd/C, THF, MeOH (60%).



Scheme 2. Reagents and conditions: (a) (i) $(t\mathrm{BuO})_2\mathrm{PN}i\mathrm{Pr}_2$, 1*H*-tetrazole, CH_2Cl_2, THF; (ii) H_2O_2 (60%); (b) TFA, CH_2Cl_2 (100%); (c) **5** (2.2 equiv), Ag_2CO_3 (2.2 equiv), $PhCH_3$, molecular sieves 4 Å (45%); (d) H_2, Pd/C, THF, MeOH (100%).

phosphoramidite reaction with benzyl *N*-benzyloxycarbonyl-L-serine **3** followed by in situ H_2O_2 oxidation of the resulting phosphite to phosphate **10** (60%).[11] Next, acidolysis by trifluoroacetic acid in dichloromethane afforded the corresponding phosphate **9** in quantitative yield.[12] The silver assisted nucleophilic substitution of tetra-*O*-acetyl-α-D-mannopyranosyl bromide **5** by phosphate **9** was then carried out. The expected protected di-mannosyl serinyl phosphate **8** was obtained as an enantiomerically pure α-anomer after flash chromatographic purification[13] (45% yield, $J_{H1,H2} = 1.4$ Hz, $J_{H1,P} = 6.6$ Hz, $J_{H1',H2'} = 1.2$ Hz, $J_{H1',P} = 5.9$ Hz). Finally, simultaneous removal of both benzyl and benzyloxycarbonyl protective groups was performed by hydrogenolysis in the presence of palladium on charcoal in THF–methanol to afford the targeted di-mannosyl serinylphosphate **1** in quantitative yield.

3. Conclusion

In conclusion, we have described an efficient synthesis of di-mannosyl serinyl phosphate in a convergent strategy involving five steps from the commercially available *N*-benzyloxycarbonyl-L-serine and peracetyl α-D-mannopyranosyl bromide in 25% overall yield. To the best of the knowledge, this is the first synthesis of a phosphotriester involving two sugars linked at their anomeric position and an aminoacid. Based on this strategy, further work will focus upon the introduction of various aminoacid or peptide derivatives on the di-mannosyl phosphate to investigate their biological properties related to a potential CDG-Ia treatment. Furthermore, we also reported the successful synthesis of the phosphoserine according to Mitsunobu conditions. The versatility and the scope of that direct O-phosphorylation towards both phosphate reagents and aminoacids are currently being studied.

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11. Selected physical data for **10**: $[\alpha]_D^{20} = -6$ (*c* 1.0, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 250 MHz): δ 7.33–7.26 (m, 10H, 2Ph), 6.03 (d, 1H, NH), 5.19 (s, 2H, CO_2CH_2Ph), 5.12 (s, 2H, $NHCO_2CH_2Ph$), 4.59–4.56 (m, 1H, H_α), 4.45–4.30 (m, 1H, CH_a–OP), 4.27–4.19 (m, 1H, CH_b–OP), 1.48 (18H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 63 MHz): δ 169.1 (CO_2), 155.9 (CONH),

136.2, 135.0 (2 C_{ar}), 128.5, 128.4, 128.1, 128.0 ($CH_{ar.}$), 83.1, 83.0 (2d, $J_{tBu,P} = 3.7$ Hz, 2 $C(CH_3)_3$), 67.4 (CO_2CH_2Ph), 66.9 ($NHCOCH_2Ph$), 66.4 (d, $J_{C,P} = 4.6$ Hz, CH_2OP), 54.6 (d, $J_{C,P} = 6.9$ Hz, C_α), 29.7 (d, $J_{C,P} = 3.6$ Hz, $C(CH_3)_3$); ^{31}P NMR ($CDCl_3$, 250 MHz): δ −9.19 ppm; MS (ESI^+) *m/z* 522 for $C_{26}H_{37}NO_8P$ $[M+H]^+$; 410 for $C_{18}H_{19}NO_8P$ $[M-2tBu+H]^+$; HRMS (ESI^+) *m/z* calcd for $C_{26}H_{36}NNaO_8P$ $[M+Na]^+$: 544.2076. Found: 544.2050.

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13. Selected physical data for **8**: $[\alpha]_D^{20} = +45$ (*c* 1.0, CH_2Cl_2); ^1H NMR ($CDCl_3$, 250 MHz): δ 7.30–7.35 (m, 10H, 2Ph), 6.11 (d, 1H, NH), 5.71 (dd, 1H, $J_{1A,2A} = 1.4$ Hz, $J_{1A,P} = 6.6$ Hz, H_{1A}), 5.66 (dd, 1H, $J_{1B,2B} = 1.2$ Hz, $J_{1b,P} = 5.9$ Hz, H_{1B}), 5.37–5.29 (m, 6H, H_2, H_3, H_4), 5.23, 5.13 (2d, 4H, $J = 10.7$ Hz, $J = 7.8$ Hz, CO_2CH_2Ph, $NHCO_2CH_2Ph$), 4.66–4.58 (m, 2H, H_α,C*Ha*–OP), 4.50–4.47 (m 1H, C*Hb*–OP), 4.36–4.10 (6H, H-5, 2 H-6), 2.15–1.98 (24H, $COCH_3$); ^{13}C NMR ($CDCl_3$, 63 MHz): δ 170.4, 169.4, 168.4, 168.0 (CO), 155.8 (CONH), 134.9, 136.1 ($C_{ar.}$), 128.6, 128.4, 128.3, 128.0, 127.8 ($CH_{ar.}$), 95.6, 96.0 (2d, $J_{C1,P} = 3.6$ Hz, C_{1A}, C_{1B}), 70.8, 70.7 (C_{5A}, C_{5B}), 68.5, 68.6 (C_{2A}, C_{2B}), 68.4 (CO_2CH_2Ph), 68.0, 67.9 (C_{3A}, C_{3B}), 67.8 ($NHCOCH_2Ph$), 67.0 (br s, CH_2–OP), 65.0, 64.9 (C_{4A}, C_{4B}), 61.5, 61.7 (C_{6A}, C_{6B}), 54.3 (d, $J_{C,P} = 8.9$ Hz, C_α), 20.5 (CH_3); ^{31}P NMR ($CDCl_3$, 250 MHz): δ 3.67 ppm; MS (ESI^+) *m/z* 1092 for $C_{46}H_{56}NNaO_{26}P$ $[M+Na]^+$; 762 for $C_{32}H_{38}NNaO_{17}P$ $[M-mannosyl+Na]^+$; HRMS (ESI^+) calcd for $C_{46}H_{56}NO_{26}NaP$ $[M+Na]^+$: 1092.2726. Found: 1092.2712.