Chemical Synthesis of α-D-Mannosylphosphate Serine Derivatives: A New Class of Synthetic Glycopeptides

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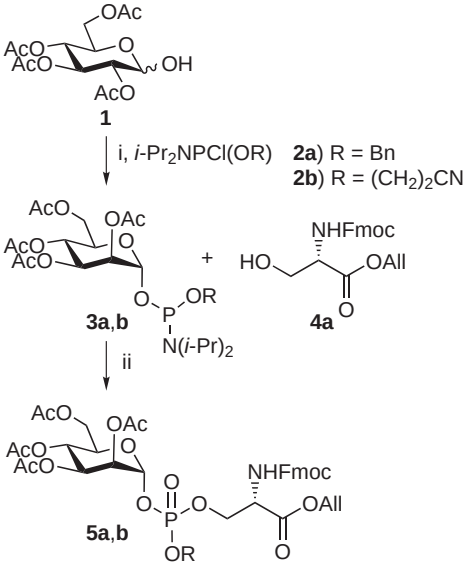
Dedicated to the memory of Professor Raymond U. Lemieux.

Abstract: α-D-Mannosylphosphate serine derivatives were conveniently synthesized by reaction of benzyl or cyanoethyl phosphochloroamidites with 2,3,4,6-tetra-*O*-acetyl-D-mannose to give intermediate α-mannosyl phosphoramidites, which were successfully reacted with properly protected serine derivatives in the presence of 1*H*-tetrazole to give phosphite triesters which could be oxidized to phosphotriesters using *t*-BuOOH.

Key words: carbohydrates, glycopeptides, glycosyl phosphates, phosphoglycosylation, phosphochloroamidites

Most eukaryotic cellular proteins with the exception of some hormones and enzymes are reliant on sugar units covalently attached to them to confer a broad range of important biological functions such as immunogenicity, solubility, cell-cell communication, protection from proteolytic attack, and induction and maintenance of the protein conformation in a biologically active forms.[1–6] The vast majority of glycoproteins can be divided into two principal groups: the N-linked glycoproteins having an N-glycosidic linkage to the side chain of L-asparagine and the more diverse O-linked group, bearing an O-glycosidic linkage to L-serine, L-threonine, 4-hydroxy-L-proline, or -L-tyrosine.

Recently, several new classes of glycoproteins have been identified and an intriguing group contains oligosaccharides linked to serine or threonine moieties via a phosphodiester linkage and this type of protein modification has been referred to as protein phosphoglycosylation.[7] The first reported example of a protein modified by a phosphoglycoside was an endopeptidase known as proteinase I, isolated from the slime mold *Dicyostelium dicoideum*.[8] It was shown that this glycoprotein contains a serine moiety modified by a phosphodiester linked to *N*-acetyl glucosamine (α-D-GlcNAc-1-PO_4-Ser). Two other cysteine proteinases isolated from *D. discoideum* have also been shown to carry GlcNAc-1-PO_4 modifications.[9] Phosphoglycosylation of serine was also observed in a secreted acid phosphatase of *Leishmania mexicana*.[10–12] In this case, serine is modified by α-mannosidic phosphodiester linkages which can either be monomeric or consist of a series of neutral α(1-2)-linked oligomannanes or phosphorylated oligosaccharides composed of PO_4-6Galβ(1-4)Man and PO_4-6[Glcβ(1-3)]Galβ(1-4)Man repeating units capped with a neutral mannosyl oligosaccharide.



Scheme 1 *Reagents and conditions*: i) DIPEA, CH_2Cl_2; ii) 1*H*-tetrazole, CH_3CN followed by addition of *t*-BuOOH at –40 °C.

In order to study in detail the biological significance of protein phosphoglycosylation, reasonable quantities of well-defined compounds are required. We report here, for the first time, a convenient synthetic approach for the preparation of properly protected α-D-mannosylphosphate serine derivatives that can be used for the synthesis of glycosylphosphopeptides.

It was envisaged that the preparation of α-D-mannosylphosphate serine derivatives would be complicated by the inherent acid lability of anomeric phosphates and the possibility of base-mediated β-elimination of the serine phosphotri(di)ester linkage. Furthermore, due to unfavorable hydrogen bonding, the side-chain hydroxyl of carbamate-protected serine and threonine derivatives are generally of low nucleophilicity[13–15] complicating the phosphorylation. In addition, anomeric phosphorylations are challenging due to the fact that these reactions need to be performed with control of anomeric configuration.

A number of strategies have been reported for the preparation of inter-glycosidic phosphodiesters and the most successful approaches involve the use of H-

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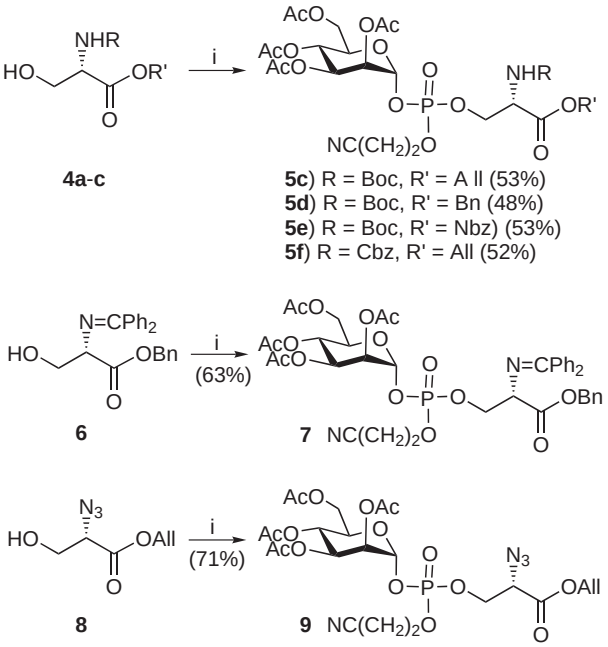
phosphonates[16,17] and phosphoamidites.[13,18,19] In first instance, 2,3,4,6-tetra-*O*-acetyl-D-mannose was reacted with salicylchlorophosphite and although after hydrolysis the expected H-phosphonate was obtained, further activation with pivaloyl chloride (PivCl) and reaction with serine derivative **4a** followed by oxidation with *t*-BuOOH did not provide the expected phosphodiester. Also an inverse procedure, in which serine was first phosphonylated followed by reaction with 2,3,4,6-tetra-*O*-acetyl-D-mannose, did also not lead to the expected compound. Fortunately, reaction of **1** with benzyl or cyanoethyl phosphochloroamidites **2** and **3** in the presence of diisopropylethylamine (DIPEA) gave the expected phosphoamidites **3a**,**b**, respectively. These derivatives could be further activated by 1*H*-tetrazole and reaction with serine derivative **4a** gave the expected intermediate phosphites, which could be oxidized to phosphotriesters **5a** and **5b**, respectively using *t*-BuOOH. After purification, by silica gel column chromatography, **5a** and **5b** were obtained in yields of 50% and 53%, respectively. ^{1}H NMR of **5a**,**b** showed H-1 at ca 5.8 ppm ($J_{H1,P}$ = 6.7 Hz) and deshielding of H-3 and H-5 by ca 0.2 ppm compared to the same protons in hemiacetal **1** confirming the α-configuration of the phosphites. Encouraged by these results, a range of Boc and Cbz protected serine derivatives were prepared that have allyl-, benzyl- or Nbz protected carboxylic acids. As can be seen in Scheme 2, the coupling of these derivatives with phosphoramidite **3b** followed by oxidation with *t*-BuOOH, gave the expected phosphotriesters **5c**–**5f** in acceptable yields.

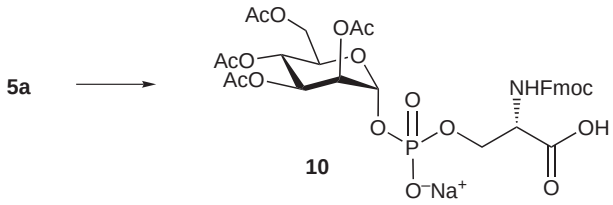
In order to increase the yields of the phosphorylations, serine derivatives **6** was employed which is protected by a benzophenone Schiff base. This compound could easily be prepared in good yield (75%) by reaction of the benzyl ester of serine with benzophenone imine.[20] It was expected that the hydroxyl of **6** would be more nucleophilic than a hydroxyl of similar serine derivatives that are protected by carbamates. In the latter compounds, unfavorable hydrogen bonding between the lone pair of the serine hydroxyl and the amide moiety of the protecting group reduces the electron density and hence nucleophilicity of the hydroxyl. On the other hand, it has been shown that replacement of carbamates (H-bond donor) with an imine type protecting group (H-bond acceptor) provides favorable hydrogen bonding that increases the electron density of the serine hydroxyl.[14] This finding has been elegantly exploited in high yielding glycosylations of benzophenone Schiff base protected serine. As expected, coupling of **1** with **6** followed by oxidation with *t*-BuOOH gave **7** in an improved yield of 63%. Even more gratifying was the finding that the use of azido-modified serine **8**, which was obtained by an azido transfer reaction using TfN_3,[21] gave even a higher yield (71%) of the coupling product **9**.

Having established a convenient approach for the phosphoglycosylation of serine derivatives, attention was focused on partial deprotection to obtain a building block that can be used in stepwise glycosylphosphopeptide assembly. It is well know that serine and threonine protected



Scheme 2 *Reagents and conditions*: i) compound **1**, 1*H*-tetrazole, CH_3CN followed by the addition of *t*-BuOOH at –40 °C.

phosphotriesters undergo piperidine mediated β-elimination to dehydroalanine and α-amino-butenoate. However, it has been found that phosphodiesters are perfectly stable under these conditions and furthermore, it has been shown that phosphodiesters are compatible with stepwise phosphopeptide synthesis.[22,23] Thus, the allyl ester of **5a** was cleaved by treatment with $(Ph_3P)_4Pd$ and Bu_3SnH and the resulting compound was converted into phosphodiester **10** (Scheme 3)by removal of the benzyl protecting group of phosphate using NaI in refluxing acetonitrile. After purification by size exclusion column chromatography using Sephadex LH-20, compound **10** was isolated in an overall yield of 53%. It is to be expected that **10** will be a suitable building block for the stepwise assembly of glycosylphosphopeptides.



Scheme 3 *Reagents and conditions*: i) $Pd(Ph_3P)_4$, BuSnH, AcOH, THF; ii) NaI, CH_3CN.

In conclusion, it has been demonstrated that benzyl and cyanoethyl phosphochloroamidites are convenient reagents for the preparation of a wide range of α-D-mannosylphosphate serine derivatives. The best results were obtained with serine derivatives that were modified by amino protecting or masking groups that provide favorable hydrogen bonding. It is to be expected[24] that the methodology presented here can be employed for the glo-

bal glycosylphosphorylation of pre-assembled peptides. Alternatively, building blocks such as **10** should be useful for the stepwise synthesis of glycosylphosphopeptides.

Preparation compound 3a. To a stirred solution of **1** (0.55 g, 1.43 mmol) and DIPEA (0.52 mL, 2.86 mmol) in CH_2Cl_2 (5 mL) was added benzyl *N*,*N*-diisopropylchlorophosphoramidite **2a** (0.3 g, 1.4 mmol). When TLC analysis (ethyl acetate/hexane 2/1) showed completion of the reaction, the reaction mixture was diluted with CH_2Cl_2 (20 mL) and successively washed with ice-cold 10% aqueous $NaHCO_3$ (10 mL), brine (10 mL) and water (15 mL), followed by drying over $MgSO_4$. After evaporation of the solvent, compound **3a** was obtained as a colorless oil (0.5g, 59%). ^{31}P NMR δ: 14.9, 15.2 (2 × s, 2 diastereoisomers). ^{1}H NMR (300 MHz, $CDCl_3$): δ: 7.22–7.16 (m, 5 H, Ar-*H*), 5.84 (dd, 1 H, H-1, $J_{1,2}$ = 1.6 Hz, $J_{1,P}$ = 8.0 Hz), 5.61 (s, 2 H, OCH_2Ph), 5.38 (dd, 1 H, H-3, $J_{2,3}$ = 3.6 Hz, $J_{3,4}$ = 9.5 Hz). 5.11 (t, 1 H, H-4, $J_{4,5}$ = 10 Hz), 5.09 (dd, 1 H, H-2), 4.93 (dd, 1 H, H6a, $J_{5,6a}$ = 5.0 Hz, $J_{6a,6b}$ = 12.4 Hz), 4.27 (ddd, 1 H, H-5, $J_{5,6b}$ = 2.1 Hz), 3.96 (dd, 1 H, H-6b), 2.97–2.53 [m, 2 H, 2 × $CH(CH_3)_2$], 2.11–1.65 (4 × s, 12 H, 4 × $COCH_3$), 1.63–1.59 [d, 12 H, 2 × $CH(CH_3)_2$]. ^{13}C NMR (125 MHz, $CDCl_3$) δ: 172.13, 171.54, 170.19, 169.23, 139.14, 127.23, 126.78, 125.11, 123.45, 101.23, 98.34, 87.23, 77.12, 76.82, 72.14, 69.14, 69.11, 67.56, 58.33, 53.61, 44.16, 42.65, 41.86, 29.87, 24.56, 23.11, 22.13, 20.93, 20.84, 20.67, 20.55, 20.33, 19.89. FAB-MS: *m/z* 608.26 $[M + Na]^+$.

Preparation compound 5a. To a stirred mixture of phosphoramidite **3a** (100 mg, 0.17 mmol) and amino acid **4a** (62.4 mg, 0.17 mmol) in acetonitrile (1.5 mL) was added 1*H*-tetrazole (0.67 mL of 3 wt.% solution in acetonitrile). When TLC analysis (ethyl acetate/hexane, 2 /3) showed the consumption of **3a**, the reaction mixture was cooled (–40 °C) and a solution of *t*-BuOOH in decane (77 μL, 5.0–6.0 M) added. The reaction mixture was left stirring at –40 °C until TLC analysis (ethyl acetate/hexane, 2 /1) showed complete formation of triester **5a**. The reaction mixture was concentrated to a small volume in vacuo and applied to a column of Sephadex LH-20 (eluent: CH_2Cl_2/MeOH, 2/1, v/v) to afford **5a** as a colorless syrup (70 mg, 50%). ^{31}P NMR δ: –3.19, –3.29 (2 × s, 2 diastereoisomers). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.10 (dd, 1 H, N*H*, *J* = 9.7 Hz, *J* = 8.7 Hz), 7.87 (d, 2 H, Ar-*H*, *J* = 7.3 Hz), 7.71 (d, 2 H, Ar-*H*, *J* = 6.8 Hz), 7.40–7.30 (m, 9 H, Ar-*H*), 5.90–5.85 (m, 1 H, $OCH_2CH{=}CH_2$), 5.68 (dd, 1 H, H-1, $J_{1,2}$ = 1.5 Hz, $J_{1,P}$ = 7.3 Hz), 5.32–5.04 (m, 7 H, H-2, H-3, OCH_2Ph, H-9, Fmoc, $OCH_2CH{=}CH_2$), 4.61–4.55 (m, 3 H, $OCH_2CH{=}CH_2$, α-CH), 4.35–3.94 (m, 8 H, H-4, H-5, H-6a,b, β-CH_2, CH_2O, Fmoc), 2.10–1.94 (4 × s, 12 H, 4 × CH_3CO). ^{13}C NMR (125 MHz, DMSO) δ: 170.63, 170.15, 170.02, 169.56, 132.81, 118.69, 118.50, 95.55, 79.47, 70.60, 68.84, 68.69, 68.39, 67.45, 65.98, 65.26, 63.59, 62.12, 28.74, 21.19, 21.12, 21.05, 19.69, 19.59. FAB-MS: *m/z* 730.99 $[M + Na]^+$.

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