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Note

Comparison of chemical and enzymatic synthesis of 2-acetamido-2-deoxy-D-mannose 6-phosphate: a new approach

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Abstract

Chemical and enzymatic methods to synthesis of 2-acetamido-2-deoxy-D-mannose-6-phosphate (ManNAc-6-*P*) have been investigated. A new preparative method has been developed although some established procedures were tried. In this new method, a 6-*O*-acetyl or 4,6-di-*O*-acetyl group of the per-*O*-acetylated 2-acetamido-2-deoxy-D-mannose (ManNAc) were regioselectively removed with an esterase from the yellow yeast, *Rhodosporidium toruloides*, followed by phosphorylation and O-deacetylation under mild conditions. ^{1}H and ^{13}C NMR data spectra of ManNAc-6-*P* were recorded. © 2001 Elsevier Science Ltd. All rights reserved.

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N-Acetylneuraminic acid (Neu5Ac) is an important component of glycoconjugates in viruses, bacteria, and many higher animals, including humans.[1] In both prokaryotic and eukaryotic organisms, Neu5Ac plays a key role in many biological recognition processes such as the neural cell adhesion.[2a] Neu5Ac is particularly prevalent on the surface of tumor cells and is known to have antiallergic activity.[2b,c] 2-Acetamido-2-deoxy-D-mannose 6-phosphate (ManNAc-6-*P*, **1**) as the phosphate intermediate was crucially involved in multitudes of Neu5Ac synthetic biopathways in bacteria and mammals. It was recently reported that a high concentration of ManNAc-6-*P* (**1**) inhibits the lyase, which directly synthesizes Neu5Ac from 2-acetamido-2-deoxy-D-mannose (ManNAc) and phosphoenolpyruvate (PEP) for biosynthesis of capsular polysialic acid (PA), indicating that ManNAc-6-*P* (**1**) is able to regulate Neu5Ac lyase activity and modulate the PA synthesis in bacteria (Scheme 1, path **A**).[3] On the other hand, the amount of Neu5Ac in vitro apparently increases when lysates of insect *Spodoptera frugiperda* (SF-9) cells infected with the recombinant baculovirus AcSAS (*Autographa california* with human sialic acid synthase gene) were incubated with ManNAc-6-*P* (**1**), but not with ManNAc, representing that ManNAc-6-*P* (**1**) play an important role in biosynthesis of Neu5Ac (Scheme 1, path **B**).[2d,e]

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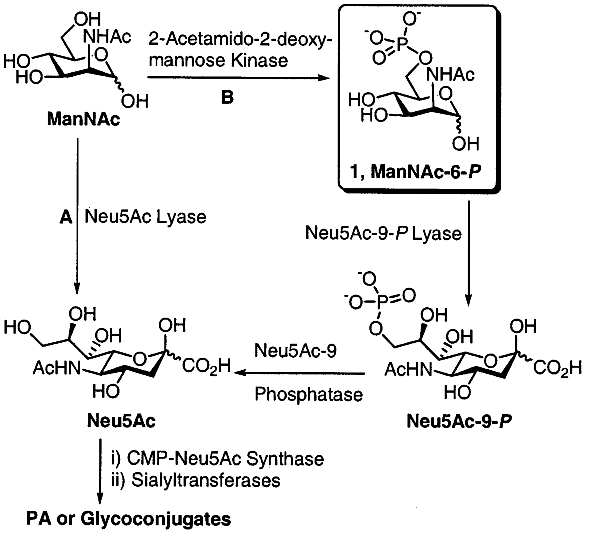
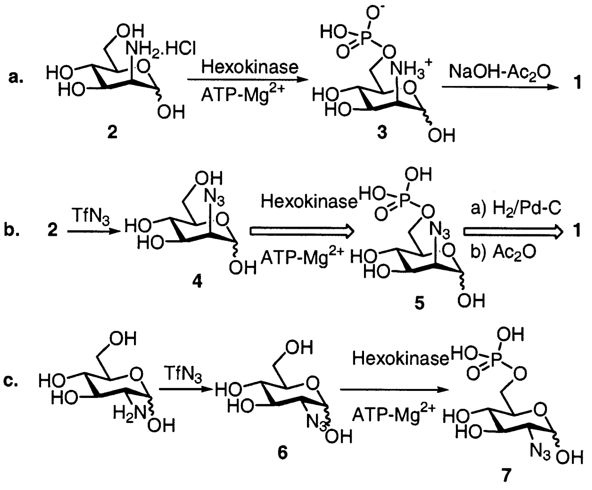


Scheme 1.



Scheme 2.

In order to investigate the biosynthetic schemes of Neu5Ac in various biological systems and enhance biosynthesis of Neu5Ac for glycoconjugates in the artificial systems such as insect cells, a substantial amount of authentic ManNAc-6-*P* (**1**) must be available. Although many chemical and enzymatic methods have been developed to prepare monosaccharide 6-phosphates efficiently,[4–6] ManNAc-6-*P* (**1**) is not commercially available, due perhaps to lack of the suitable and inexpensive enzymes or practical procedures for large-scale preparation. Among the known methods for preparation of aldohexose 6-phosphates, the most common is direct phosphorylation of the unprotected parent monosaccharide with commercially available hexokinase.[4,5] Hexokinase performs regiospecific phosphorylation of the primary hydroxyl group in many monosaccharides. It had been reported that hexokinase can phosphorylate mannose as effectively as glucose and is moderately tolerant of modification at C-2 and C-3 of the substrate structures.[5b] However, it is impractical to use such an enzymatic method for preparation of the 6-phosphates of ManNAc, GlcNAc, Gal, and GalNAc because hexokinase has very low activity towards these sugars. In an alternative small-scale procedure, 2-amino-2-deoxy-D-mannose (**2**) was directly phosphorylated by hexokinase to yield 2-amino-2-deoxy-D-mannose-6-phosphate (**3**)[4a] in a reasonable yield, which was then N-acetylated to afford the title compound (Scheme 2).[3,4b,5b] Our attempts to scale-up this procedure were not successful, as the products were contaminated by impurities such as an epimer generated during N-acetylation under strong alkaline conditions in the final product, according to NMR analysis.[2a] To alleviate these problems, we developed a new chemoenzymatic method for preparation of highly pure ManNAc-6-*P* (**1**), as shown by its ^{1}H and ^{13}C NMR data. The detailed procedures are reported herein.

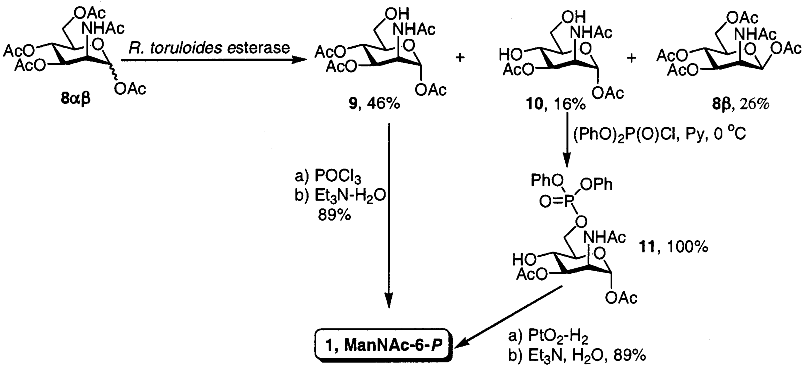
Encouraged by the acceptable yield of 6-O-phosphorylation of **2**, we tested 2-azido-2-deoxy-D-mannose (**4**), based on the reasoning that if **4** is a good substrate for hexokinase to yield 2-azido-2-deoxy-D-mannose 6-phosphate (**5**), its subsequent reduction and acetylation should readily lead to ManNAc-6-*P* (**1**). Thus, **4** and its epimer, 2-azido-2-deoxy-D-glucose (**6**), were prepared from **2** and 2-amino-2-deoxy-D-glucose, respectively, by treatment with triflyl azide (TfN_3).[7] By spectrophotometric and TLC assays,[5b] we found that **4** was neither a substrate nor an inhibitor of hexokinase, while **6** was a good substrate, giving 2-azido-2-deoxy-D-glucose 6-phosphate (**7**) in 54% yield (Scheme 2).

Searching for a different approach, we turned to selective enzymatic hydrolysis of the 6-*O*-acyl groups of per-*O*-acylated sugars. As is well known, selective O-deacylation by commercially available esterases and lipases usually follows the order of anomeric esters, the primary esters, and secondary esters.[8] However, Crout and co-workers recently reported that a crude esterase from yellow yeast, *Rhodosporidium toruloides*, regioselectively cleaved esters at C-6 position of the α anomer of per-O-acetylated D-hexopyranosides to give the 6-OH derivatives without affecting the 1-acetate.[9] Using the similar strategy, 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-D-mannose (**8αβ**), readily prepared from 2-acetamido-2-deoxy-D-mannose or **2** by treatment with acetic anhydride and pyridine,[8b,10] was regioselectively O-deacetylated at C-6 or C-4/C-6 to afford the derivatives **9** (46% yield) and **10** (16% yield), respectively. Due to the esterase specificities, there still was a considerable amount of unreacted β anomer of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-D-mannose (**8β**, 26%) in the reaction mixture. However, with the increase in the reaction time and the ratio of enzyme to substrate, **9** was further O-deacetylated at C-4 and increased the yield of **10** at the expense of **9**. Subsequently, **9** was treated with phosphoryl chloride, hydrolyzed, and O-deacetylated with a mixture of triethylamine and water in one pot to give ManNAc-6-*P* (**1**) in 89% yield after purification by anion-exchange chromatography or Sephadex G-10 column chromatography. Also, **10** was regioselectively phosphorylated with diphenyl chlorophosphate at 0 °C to afford the intermediate **11** in high yield, catalytically hydrogenolyzed in the presence of platinum oxide, and O-deacetylated with a mixture of triethylamine and water to furnish ManNAc-6-*P* (**1**) in 89% yield (Scheme 3).

In summary, we describe here a new method to synthesis of 2-acetamido-2-deoxy-D-mannose 6-phosphate (ManNAc-6-*P*, **1**) via the regioselective O-deacetylation of the per-*O*-acetylated ManNAc (**8αβ**), followed by phosphorylation ManNAc-6-*P* (**1**) in a total yield of 41%. Its purity was proven by ^{1}H and ^{13}C NMR data spectra.

1. Experimental

General methods.—Adenosine 5′-triphosphate (ATP, disodium salt) and hexokinase were purchased from Sigma-Aldrich. The crude esterase of *R. toruloides* was kindly provided by Mr R. Jinkens and Professor D. Crout (Department of Chemistry, University of Warwick, Coventry, CV4 7AL, UK). Melting points were determined with a Fisher–Johns apparatus and are not corrected. ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded with a Varian-400 NMR spectrometer at nominal resonance frequencies of 400, 100, and 162 MHz, respectively. The chemical shifts (δ) are expressed in parts per million (ppm) relative to internal standards: Me_4Si (0 ppm, ^{1}H), H_3PO_4 (0 ppm, ^{31}P). FABHRMS data were run at the mass spectrometry facility in the University of California, Riverside. Thin-layer chromatography (TLC) was performed on aluminum sheets coated with Silica Gel 60 F_{254} (thickness, 0.25 mm, E. Merck). Column chromatography was carried out on Silica Gel 60 (E. Merck). The ratios of solvents used for TLC or column chromatography are expressed in vol/vol.



Scheme 3.

*2-Amino-2-deoxy-*D*-mannose 6-phosphate* (**3**).—The procedure was essentially based on that of Jourdian and Roseman:[4a] 2-Amino-2-deoxy-D-mannose hydrochloride (**2**) (4.3 g, 20.00 mmol), $MgCl_2 \cdot 6H_2O$ (2.9 g, 14.0 mmol), and ATP (12.1 g, 22.0 mmol) were dissolved in 540 mL of distilled water. The solution was adjusted to pH 7.5 with 0.5 M KOH, and hexokinase (60 mg, 1320 U) dissolved in 10 mL water was added. The reaction mixture was continuously stirred for 2–3 h at rt. During the reaction, the pH of the solution was maintained at 7.5 by addition of 0.5 M KOH each half-hour until the pH became stable. TLC (2:1:1 *n*-BuOH–HOAc–H_2O) showed that the starting material completely disappeared, and a new compound (R_f 0.25) appeared as a ninhydrin-positive and UV-negative spot. The mixture (total 600 mL) was then adjusted to pH 2.0 by addition of concd HCl and one third portion (200 mL) was loaded onto a column of Dowex 50W × 8 (H^+ form, 200–400 mesh) cation-exchange resin (2.5 × 70 cm). The column was eluted with water, and the major anions were immediately eluted in the fifth through 45th 6-mL fractions, which were either nihydrin-negative or UV-positive. 2-Amino-2-deoxy-D-mannose 6-phosphate (**3**) came out in the 121st through 194th fractions, which were both ninhydrin-positive and UV-negative. The fractions containing the desired product were pooled and concentrated with a rotary evaporator(at 35 °C to 50–60 mL, which was lyophilized to give a white powder, **3** (4.3 g, 82%): mp 141–143 °C. ^1H NMR (400 MHz, D_2O) δ 5.20 (d, 0.6 H, *J* 1.0 Hz, H-1β), 5.02 (d, 1 H, *J* 1.6 Hz, H-1α), 4.01–3.87 (m, 3.8 H, H-6α, H-6′α, H-6β, H-6′β, H-3β), 3.82 (dd, 1 H, *J* 4.8, 9.6 Hz, H-3α), 3.53 (dd, 1 H, *J* 1.6, 4.4 Hz, H-2α), 3.52 (t, 0.6 H, *J* 9.6, 10.0 Hz, H-4β), 3.46 (dd, 0.6 H, *J* 1.0, 4.8 Hz, H-2β), 3.45 (t, 1 H, *J* 9.6, 9.6 Hz, H-4α), 3.40 (m, 1 H, H-5α), 3.37 (m, 0.6 H, H-5β). ^{13}C NMR (100 MHz, D_2O) δ 92.12, 91.44, 75.76 (d, *J* 8.4 Hz), 71.50 (d, *J* 8.4 Hz), 70.19, 67.63, 66.60, 66.45, 64.78, 64.50, 56.44, 55.30. ^{31}P (162 MHz, D_2O) δ 4.28, lit. 4.01.[5b] FABHRMS: *m*/*z* Anal. Calcd $C_6H_{15}NO_8P$ $[M+H]^+$ 260.0535. Found: 260.0532.

*2-Acetamido-2-deoxy-*D*-mannose 6-phosphate* (**1**).—To a solution of **3** (4.3 g, 16.4 mmol) in 250 mL of distilled water maintained at 0 °C was added, in portions, $NaHCO_3$ (5.5 g, 65.6 mmol), followed by dropwise addition of Ac_2O (1.86 mL, 19.7 mmol). The pH was maintained at 7.6 with the addition of 2.5 M NaOH, and the reaction mixture was stirred at 0 °C for 5–6 h and passed through a column of Dowex 50W × 8 resin (H^+ form, 100–200 mesh, 2.5 × 20 cm). The sugar-containing fractions were collected and concentrated to 50–60 mL and lyophilized to give a solid. The solid was redissolved in 150 mL of distilled water and adjusted to pH 4–5 with 2 M NaOH. The solution was passed through a short charcoal–silica gel column, concentrated, and lyophilized to yield a white solid, **1** (4.8 g, 90% based on monosodium salt). For further purification, 500 mg of the solid was dissolved in 5 mL of water and loaded onto a Sephadex G-10 column (1.5 × 95 cm) equilibrated with 50 mM HOAc. The column was eluted with 50 mM HOAc. The fractions with the same R_f were collected, concentrated, and lyophilized to give a white powder, **1** (400 mg, 80%): mp 53–55 °C (free acid), 133–135 °C (monosodium salt). ^1H NMR (400 MHz, D_2O) δ 4.70 (d, 1.8 H, *J* 1.6 Hz, H-1α), 4.62 (d, 1 H, *J* 1.2 Hz, H-1β), 4.04 (m, 1 H, H-2β), 3.90 (m, 1.8 H, H-2α), 3.89–3.74 (m, 6.6 H, H-6β, H-6′β, H-6α, H-6′α, H-5β), 3.65 (m, 1 H, H-5α), 3.63 (m, 1.8 H), 3.42 (m, 1 H), 3.25 (t, 1.8 H, *J* 10.0 Hz, H-4α), 3.15 (m, 1 H), 1.68 (d, 3 H, *J* 3.2 Hz, Acβ), 1.64 (s, 5.4 H, *J* 3.2 Hz, Acα). ^{13}C NMR (100 MHz, D_2O) δ 176.57, 175.61, 93.87 (C-1α), 93.82 (C-1β), 75.62 (d, *J* 7.6 Hz), 72.55, 71.44 (d, *J* 7.6 Hz), 69.41, 67.14, 66.91, 65.71, 65.58, 54.77, 53.94, 22.78, 22.66. ^{31}P (162 MHz, D_2O) δ 4.09 (sodium salt), lit. 3.94, diammonium.[5b] FABHRMS: *m*/*z* Anal. Calcd $C_8H_{17}NO_9P$ $[M+H]^+$ 302.0641. Found: 302.0646; $C_8H_{16}NNaO_9P$ $[M+Na]^+$ 324.0460. Found: 324.0466.

*2-Azido-2-deoxy-*D*-glucose 6-phosphate* (**7**).—2-Azido-2-deoxy-D-glucose (**6**) (133 mg, 0.65 mmol), $MgCl_2 \cdot 6H_2O$ (92 mg, 0.46 mmol), and ATP (464 mg, 0.84 mmol) were dissolved in 20 mL of distilled water. The solution was adjusted to pH 7.5 with 0.5 M KOH, and hexokinase (2 mg, 44 U) dissolved in 2 mL water was added. The reaction mixture was stirred for 2–3 h at rt. During the reaction, the pH of the solution was maintained at 7.5 by addition of 0.5 M KOH each half-hour until the pH became stable. TLC showed that the starting material remained, but a new compound appeared at R_f 0.30. The mixture (total 25 mL) was adjusted to pH 2.0 by addition of concd HCl and was loaded onto a column of Dowex 50W × 8 resin(H^+ form, 200–400 mesh, 1.5 × 30 cm), and eluted with water. The sugar-containing fractions were pooled and concentrated with a rotary evaporator at 35 °C to 5–10 mL, and then loaded onto a Sephadex G-10 column (1.5 × 95 cm) equilibrated with 50 mM HOAc. The column was eluted with 50 mM HOAc. The fractions with the same R_f were collected, concentrated, and lyophilized to give a white powder, **7** (100 mg, 54%): mp 59–61 °C. ^{1}H NMR (400 MHz, D_2O) δ 5.15 (d, 1 H, *J* 3.6 Hz, H-1α), 4.52 (d, 1.6 H, *J* 8.0 Hz, H-1β), 3.94 (dd, 1.6 H, *J* 5.6, 12.0 Hz, H-6β), 3.93–3.82 (m, 3.6 H, H-6′β, H-6α, H-6′α), 3.76 (m, 1 H, H-5α), 3.66 (t, 1 H, *J* 10.0 Hz, H-3α), 3.38 (t, 1.6 H, *J* 10.0 Hz, H-3β), 3.70–3.27 (m, 5.2 H, H-5β, H-4α, H-4β, H-2α), 3.11 (dd, 1.6 H, *J* 8.0, 9.6 Hz, H-2β). ^{13}C NMR (100 MHz, D_2O) δ 96.11, 92.18, 75.75 (d, *J* 7.6 Hz), 75.06, 72.27, 72.54 (d, *J* 8.3 Hz), 70.27, 69.95, 67.75, 64.92, 64.87, 64.45. ^{31}P (162 MHz, D_2O) δ 0.57 (free acid). FABHRMS: *m*/*z* Anal. Calcd $C_6H_{12}KN_3O_8P$ $[M+K]^+$ 323.9999. Found: 324.0009.

*2-Acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-α-*D*-mannose* (**9**).—2-Acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-D-mannose (**8αβ**) (3.89 g, 10.00 mmol) was dissolved in 40 mL of 0.2 M citrate–phosphate buffer (pH 5.0). After addition of the crude *R. toruloides* esterase (118 mg), the mixture was stirred at rt for 30 h and concentrated to dryness. The dried residue was suspended in 1:1 MeOH–CH_2Cl_2 (70 mL × 3). The solution was stirred at rt for 30 min and filtered. The combined filtrates were concentrated to give a residue, which was flash chromatographed (20: 1 $CHCl_3$–CH_3OH) to yield a waxy solid of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-mannose (**8β**) (1.00 g, 26%), a white solid of 2-acetamido-1,3-di-*O*-acetyl-2-deoxy-α-D-mannose (**10**) (475 mg, 16%), and a white solid of the title compound (1.61 g, 46%,). Compound **9**: mp 80–81 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.27 (d, 1 H, *J* 9.2 Hz, NH), 5.82 (d, 1 H, *J* 1.6 Hz, H-1), 5.09 (t, 1 H, *J* 9.6, 10.4 Hz, H-4), 5.07 (dd, 1 H, *J* 4.4, 9.6 Hz, H-3), 4.74 (dt, 1 H, *J* 1.6, 3.2, 9.2 Hz, H-2), 3.76 (dd, 1 H, *J* 2.0, 12.8 Hz, H-6), 3.55 (dd, 1 H, *J* 3.2, 12.8 Hz, H-6), 3.52 (m, 1 H, H-5), 2.07, 2.06, 2.04, 1.98 (s each, 3 H each, Ac). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.32, 172.22, 171.32, 169.73, 91.61, 76.36, 72.36, 66.56, 61.49, 50.69, 24.10, 21.83, 21.81, 21.78. FABHRMS: *m*/*z* Anal. Calcd $C_{14}H_{21}NNaO_9$ $[M+Na]^+$ 370.1114. Found: 370.1113.

*2-Acetamido-1,3-di-*O*-acetyl-2-deoxy-α-*D*-mannose* (**10**).—2-Acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-D-mannose (**8αβ**) (10.00 g, 25.71 mmol) was dissolved in 150 mL of 0.2 M citrate–phosphate buffer (pH 5.0). After addition of the crude *R. toruloides* esterase (1.01 g), the reaction mixture was stirred at rt for 50 h and concentrated to give a residue. The residue was suspended in 1:1 MeOH–CH_2Cl_2 (150 mL × 3). The solution was stirred at rt for 30 min and filtered. The combined filtrates were concentrated to a syrup, which was flash chromatographed (20: 1 $CHCl_3$–CH_3OH) to afford **8β** (1.50 g, 15%), **9** (3.36 g, 38%), and **10** (3.18 g, 41%). Compound **10**: mp 91–92 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.37 (d, 1 H, *J* 9.6 Hz, NH), 5.82 (d, 1 H, *J* 1.6 Hz, H-1), 4.87 (dd, 1 H, *J* 4.0, 10.0 Hz, H-3), 4.69 (dt, 1 H, *J* 1.2, 4.0, 9.2 Hz, H-2), 3.82 (t, 1 H, *J* 9.6, 10.0 Hz, H-4), 3.56 (m, 1 H, H-5), 3.52–3.44 (m, 2 H, H-6), 2.06, 2.04, 2.02 (s each, 3 H each, Ac). ^{1}H NMR (100 MHz, $CDCl_3$) δ 172.32, 172.16, 169.92, 92.01, 78.29, 75.28, 65.86, 62.28, 50.79, 24.15, 21.99, 21.89. FABHRMS: *m*/*z* Anal. Calcd $C_{14}H_{21}NNaO_9$ $[M+Na]^+$ 328.1008. Found: 328.0993.

*2-Acetamido-1,3,-di-*O*-acetyl-6-*O*-diphenoxyphosphoryl-2-deoxy-α-*D*-mannose* (**11**).—To a solution of **10** (1.28 g, 4.20 mmol) in 10 mL of dry pyridine was added dropwise a solution of diphenyl chlorophosphate (1.30 mL, 6.30 mmol) in 5 mL of dry pyridine at 0 °C. The mixture was stirred at rt overnight and concentrated to give a syrup, which was chromatographed to afford a white solid, **11**

(2.00 g, 89%): mp 61–62 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29–7.11 (m, 10 H, Ph), 5.75 (s, 1 H, H-1), 4.85 (dd, 1 H, J 4.0, 10.0 Hz, H-3), 4.63 (m, 1 H, H-2), 4.53 (m, 1 H, H-6), 4.48 (m, 1 H, H-6), 3.96 (t, 1 H, J 10.0, 9.6 Hz, H-4), 3.59 (m, 1 H, H-5), 3.33 (s, 1 H, OH), 1.96, 1.89, 1.88 (s each, 3 H each, Ac). ^{1}H NMR (100 MHz, D_2O) δ 172.69, 171.95, 169.68, 151.50 (d, J 2.3 Hz), 151.45 (d, J 2.5 Hz), 130.92, 130.80, 126.67, 126.55, 121.20, 121.15, 121.10, 121.05, 91.82, 76.83 (d, J 3.6 Hz), 75.12, 69.48 (d, J 7.6 Hz), 64.58, 50.59, 23.85, 21.93, 21.77. FABHRMS: m/z Anal. Calcd $C_{24}H_{28}NNaO_{11}P$ $[M+Na]^+$ 560.1298. Found: 560.1314.

*2-Acetamido-2-deoxy-*D*-mannose 6-phosphate* (**1**) *from* **9**.—To a solution of **9** (1.04 g, 2.99 mmol) in dry CH_2Cl_2 (30 mL) maintained under N_2 at 0 °C was added Et_3N (1.38 mL, 9.87 mmol) and $POCl_3$ (0.61 mL. 6.57 mmol). The mixture was stirred at rt overnight. The solution was mixed with ice water (5 mL), stirred for another 30 min, and extracted with CH_2Cl_2 (3 × 30 mL). The combined extracts were dried over anhyd Na_2SO_4, filtered, and evaporated to give a residue, which showed only one spot on TLC. The residue was dissolved in a mixture of Et_3N (10 mL) and water (60 mL), stirred for 24 h, and concentrated to a syrup. The solution of the syrup in 10 mL of water was passed through a column of Dowex 50W × 8 resin (H^+ form, 100–200 mesh, 2.5 × 20 cm). The sugar-positive fractions were collected and concentrated to 5 mL, which was loaded onto a column of Dowex 1 × 4 resin (HCO_3^- form, 200–400 mesh, 2.5 × 40 cm), and eluted with 200 mM ammonium bicarbonate. The fractions with the same R_f were pooled and concentrated to 5 mL, and then lyophilized to give the title compound (891 mg, 89% based on diammonium salt).

*2-Acetamido-2-deoxy-*D*-mannose 6-phosphate* (**1**) *from* **11**.—Compound **11** (1.99 g, 3.70 mmol) was dissolved in 60% HOAc (20 mL) and stirred with Adams' catalyst (PtO_2, 650 mg) for 4 days under H_2 at the end of which time TLC showed the presence of a single charring but non-UV absorbing compound with R_f 0.4 (1:1:0.1 $CHCl_3$–MeOH–H_2O), the catalyst was removed by filtration and washed with water and MeOH. The filtrates and washings were combined and concentrated to give a syrup. The syrup was dissolved in a mixture of triethylamine (10 mL) and water (60 mL), stirred for 24 h, and concentrated to a syrup. A solution of the syrup in 15 mL water was passed through a column of Dowex 50W × 8 resin (H^+ form, 100–200 mesh, 2.5 × 20 cm). The sugar-positive fractions were collected, concentrated to 5 mL, loaded onto a column of Dowex 1 × 4 resin (2.5 × 40 cm, HCO_3^- form), and eluted with 200 mM ammonium bicarbonate. The fractions with the same R_f were pooled and concentrated to 5 mL, which was then lyophilized to give the title compound (1.10 g, 89%).

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