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SYNTHESIS OF A POTENTIALLY INSULIN-MIMETIC PHOSPHODISACCHARIDE

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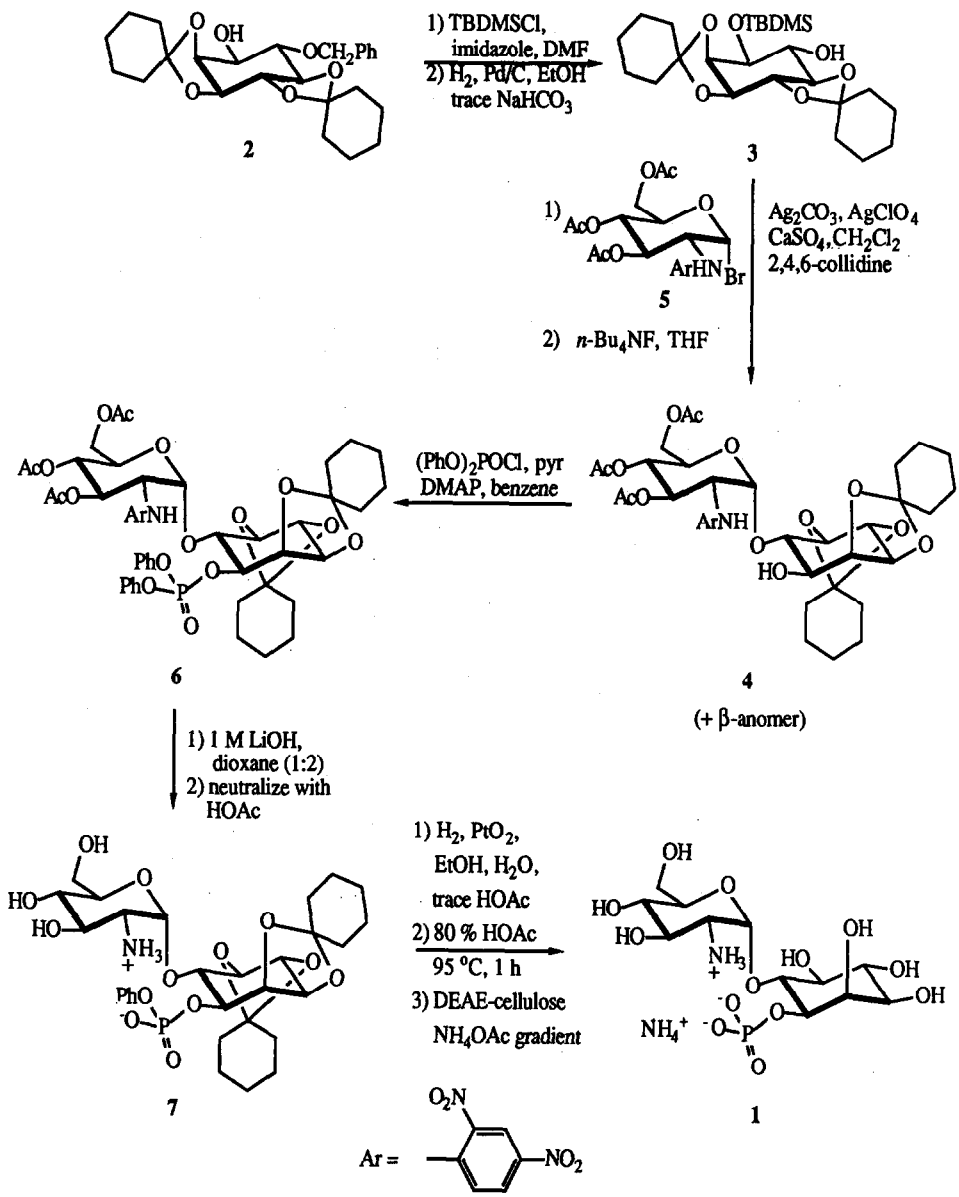
Summary: The synthesis of phosphodisaccharide **1** from *myo*-inositol and *D*-glucosamine has been accomplished. This compound is the terminal (and presumably essential) portion of both the putative insulin second messenger and the phosphatidylinositol glycan protein membrane anchor.

In 1986 two related insulin-mimetic phosphooligosaccharides (POS) were detected in insulin-stimulated rat hepatocytes.[1] While the complete structures of these compounds have not been elucidated, a large body of work over the past three years has suggested that: 1) POS may be the intracellular second messenger(s) for insulin action;[2] 2) POS contains a non-*N*-acetylated glucosamine glycosidically linked to an inositol phosphate;[1] 3) the structure of POS bears striking resemblance to the phosphatidylinositol-glycan protein membrane anchors (some of whose structures have been determined[3,4]) and POS may actually be derived from these anchors;[5] 4) compounds very closely related (or identical with) POS isolated from various cell types contain galactose and are insulin-mimetic;[6] and 5) the activity of POS is blocked by inositol-2-phosphate.[7]

These observations, taken together, suggested to us that the disaccharide consisting of *D*-glucosamine glycosidically linked (α1-6) to *D*-inositol-1-phosphate may be a sufficient substructure of POS to possess insulin-mimetic activity. To test this hypothesis we have prepared enantiomerically pure phosphodisaccharide **1** and report on the synthesis here.

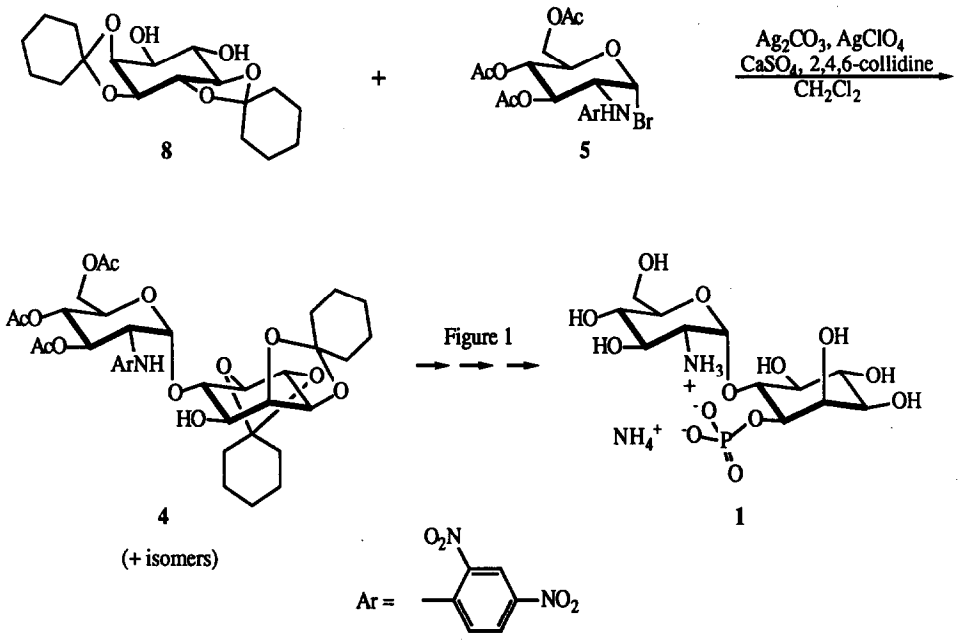
Protected *D*-inositol **2** (Figure I) was obtained in optically pure form from *myo*-inositol by the literature procedure.[8] Protection of the free 1-hydroxyl as its *t*-butyldimethylsilyl ether (TBDMSCl, imidazole, DMF, 48 °C) followed by hydrogenolysis (48 PSIG H_2, 10% Pd/C, EtOH, trace $NaHCO_3$) afforded the 1,2,3,4,5-protected inositol **3**[9] in 25% yield. Koenigs-Knorr coupling of **3** with protected *D*-glucosamine bromide **5**[10] provided a mixture of α- and β-linked disaccharides in quantitative yield. Deprotection of the mixture with tetrabutylammonium fluoride (THF) followed by chromatographic separation resulted in a 33% isolated yield of the desired α-anomer **4** as well as 14% of the β-anomer and 29% of recovered silylated disaccharide. Phosphorylation of **4** with diphenylphosphoryl chloride (pyridine, DMAP, benzene) afforded fully protected phosphodisaccharide **6** in 71% yield. Treatment with 1 M LiOH in dioxane for 18 h at 25 °C and for 1 h at 95 °C resulted in removal of the acetyl groups, the dinitrophenyl protecting group, and one of the phenyl groups of the phosphotriester to generate zwitterion **7**[11] (76%), which was

FIGURE I



purified by preparative thin layer chromatography (BuOH:H_2O:EtOH, 5:4:1, upper phase). Removal of the remaining protecting groups was accomplished by hydrogenolysis over Adams' catalyst in EtOH-H_2C (8:2) containing a trace of AcOH followed by warming at 95 °C in 80% HOAc for 1 h. The resulting

FIGURE II



phosphodisaccharide **1**[12] was purified by anion exchange chromatography (DEAE-cellulose, 0.025 - 0.300 M ammonium acetate buffer, pH = 6.0) in 45% yield from **7**. The correct chromatographic fractions were identifed by assaying for organic phosphate using the colorimetric phosphomolybdate method.[13] Those fractions containing organic phosphate were pooled and repeatedly evaporated from water to remove excess ammonium acetate.

While it was necessary to begin the synthesis with a glucosamine and an inositol of known absolute configuration (i.e. **2**) so that the desired *D,D*-isomer of the disaccharide could be unequivocally assigned, once the correct diastereomer of **4** was identified the synthesis could be shortened considerably. Koenigs-Knorr reaction of racemic inositol diol **8**[14] (Figure II) with protected glucosamine bromide **5** afforded a mixture of eight isomeric disaccharides which were separated by careful flash chromatography (40 μ SiO_2, acetone - methylene chloride, 1:99). The desired *D*-(α1-6)-*D* isomer **4** (Rf = 0.33, ether : petroleum ether, 1 : 1) was obtained from this mixture in 30% yield (based on 50% of **8**). This sequence obviates the inositol benzylation, the resolution of inositol enantiomers, the silylation, the hydrogenolysis, and the desilylation, a savings of six synthetic steps in all. The synthesis of **1** was then completed exactly as in Figure I by phosphorylation of **4** followed by deprotections. With this modification, the optically pure disaccharide **1** was prepared from racemic **8** in overall 7% of the theoretical yield.

Compound **1** is being tested for insulin mimetic activity *in vitro* and in whole cells. The results of these studies will be reported elsewhere.

Acknowledgment

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9. Satisfactory ^{1}H and ^{31}P (where applicable) NMR spectra were obtained for all new compounds. In addition, satisfactory high resolution mass spectra were obtained for compounds **3**, **4**, and **1**.

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11. The loss of one phenyl group was established both by integration of the ^{1}H NMR spectrum and by electophoresis on plastic-backed cellulose TLC plates (NH_4OAc buffer, pH 6.0, 200 V - 4 mA, staining with ninhydrin) on which the compound did not migrate due to its zwitterionic character at this pH.

12. 300 MHz ^{1}H NMR (D_2O) δ 5.53 (d, J = 3.6 Hz, 1 H, anomeric H), 4.00 (br s, 1 H), 3.82 - 3.95 (m, 2 H), 3.61 - 3.74 (m, 4 H), 3.50 (ψt, J = 9.6 Hz, 1 H), 3.30 -3.41 (m, 2 H), 3.21 (ψt, J = 9.4 Hz, 1 H), 3.11 (dd, J = 10.5, 3.9 Hz, 1 H). ^{31}P NMR (D_2O): 7.0 ppm (relative to 85% H_3PO_4). HR FAB MS (negative ion mode): Found, 420.09080; Calcd for $C_{12}H_{24}NO_{13}P$ - H, 420.09068. Compound **1** contained only ashable phosphate and no inorganic phosphate by the phosphomolybdate method.[13]

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