SYNTHESIS OF PHOSPHONO AND PHOSPHATE DERIVATIVES OF HYDROXYIMINO-D-ALDITOLS AS NEW POTENTIAL ANTIFUNGAL AGENTS

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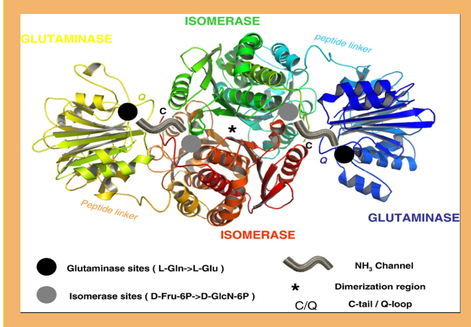
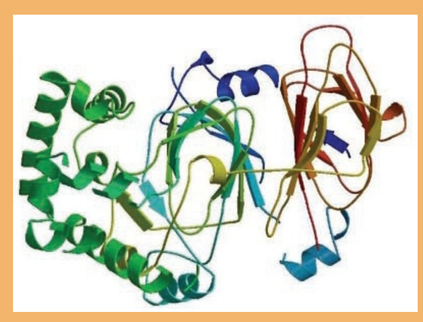


GlcN-6P synthase (GlmS)
[J. Mol. Biol. (2009) 385, 653–664]

In search for the new effective antifungals we focus on two enzymes involved in biosynthesis of the fungal cell wall. The first one is glucosamine--6-phosphate synthase (GlmS), enzyme catalyzing transformation of D-fructose-6-phosphate (Fru-6P) to D-glucosamine-6-phosphate (GlcN-6P) (Fig. 1) in chitin biosynthesis pathway [1]. The second one is phosphomanose isomerase (PMI), reported to play a crucial role in biosynthesis of many mannosylated structures, including the cell wall components of fungi. PMI is aldose-ketose isomerase and catalyzes reversible isomerization of D-manno-6-phosphate (Man-6P) to D-fructose-6-phosphate (Fru-6P) (Fig. 2) [2]. Both enzymes are proposed as the targets for antifungal chemotherapy and a search for their selective inhibitors has been continued. Mechanism of the reactions catalyzed by both enzymes are known and similar. The reaction performed by GlmS is believed to proceed through the formation of imine intermediate **I** [3] whereas the reaction catalyzed by PMI proceeds *via* a *cis*-endiol intermediate **II** [4].



Phosphomannose isomerase (PMI)
[J. Biol. Chem. (2004) Vol. 279, No. 2, p. 1491–1498]

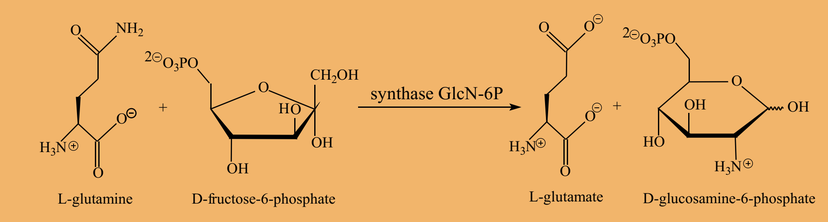
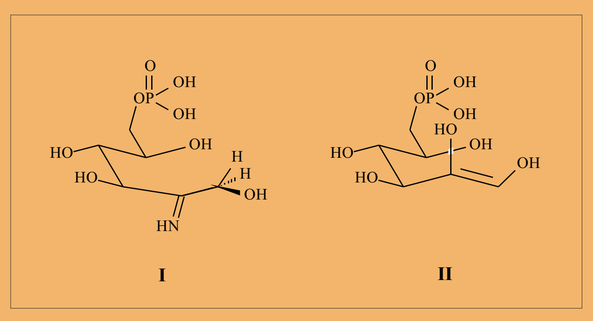


Fig. 1 The reaction catalyzed by synthase GlcN-6P



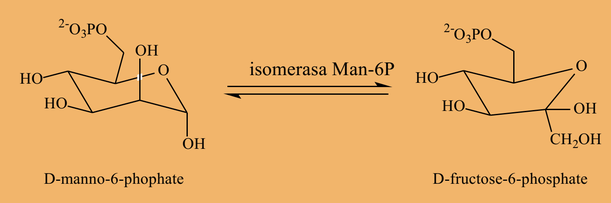
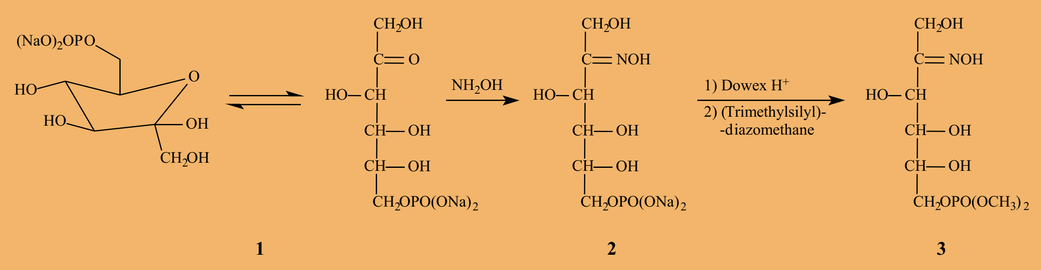


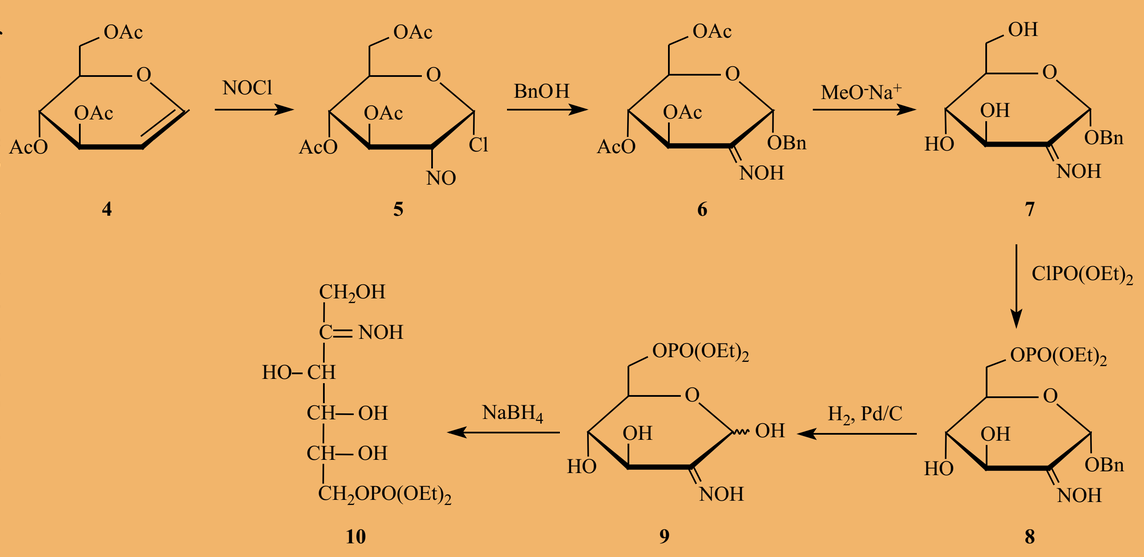
Fig. 2 The reaction catalyzed by isomerase Man-6P



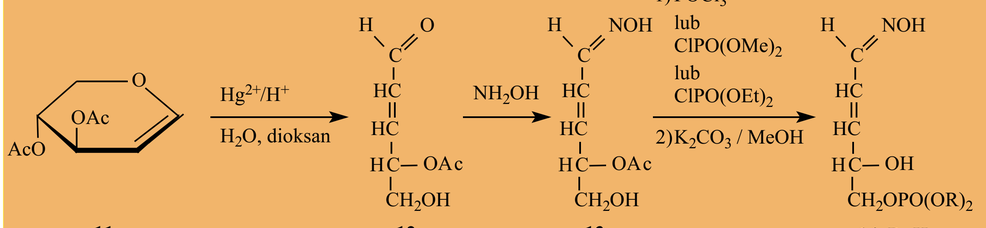
1 **2** **3**

In search of mimetics of intermediates **I** and **II** we synthesize phosphono and phosphate derivatives of the hydroxyimino-D-glucitols. Similarity in the structures of the planed compounds to intermediates **I** and **II** allow us to assume that they can be the potential inhibitors of the both enzymes. Dimethyl and diethyl ester analogs will have more lipophilic character, which make them easier to penetrate through the cytoplasmic cell membrane. It was proved that similar esters are hydrolyzed inside a cell. Our syntheses involve the transformationof D-fructose-6-phosphate disodium salt (**1**) into 2-deoxy-2-hydroxyimino-6-*O*-phosphono--D-glucitol disodium salt (**2**). Subsequent esterification of **2** by diazomethane provides ester **3**.

Synthesis of 6-*O*-diethylphosphonate-D-fructose (**10**) demands the other procedure. Thus, starting of commercially available 3,4,6-tri-*O*-acetyl-D-glucal (**4**) I obtained 3,4,6-tri-*O*-acetyl-2-deoxy-2-nitroso-*alpha*--D-glucopyranosyl chloride (**5**). Reaction of chloride **5** with benzyl alcohol provided benzyl 3,4,6-tri-*O*-acetyl--2-deoxy-2-hydroxyimino-*alpha*-D-hexopyranosides (**6**). My intention was to gain such the glycosides, which would be easy deglycosydated. Synthesized glycosides had proven to be the *alpha* or *beta* anomers with the *Z* or *E* configuration of the 2-hydroxyimino group. At this stage, stereoisomers of glycosides **6** were separated and identified. The main product was *alpha* anomer with the *Z* configuration of the 2-hydroxyimino group. Next, this stereoisomer was *O*-deacetylated, providing glycoside **7**. Benzyl 2-deoxy-2-hydroxyimino-*alpha*--D-*arabino*-hexopyranoside (**7**) was phosphorylated at the terminal hydroxyl group. I used diethyl chlorophosphate for the phosphorylation. It provided benzyl 2-deoxy-6-*O*-diethylphosphonate-2-hydroxyimino-*alpha*--D-glucopyranoside (**8**). In the next step, the aglycone part of 6-*O*-diethylphosphonate **8** was removed by hydrogenolysis and in this way 2-deoxy-6-*O*-diethylphosphonate-2-hydroxyimino-D-hexopyranose (**9**) was obtained. The last step of my synthesis was a reduction of 2-deoxy-6-*O*-diethylphosphonate-2--hydroxyimino-D-hexopyranoses (**9**) with $NaBH_4$, which was provide desired 2-deoxy-6-*O*-diethylphosphonate-2--hydroxyiminohexitol (**10**), with D-*arabino* configuration.



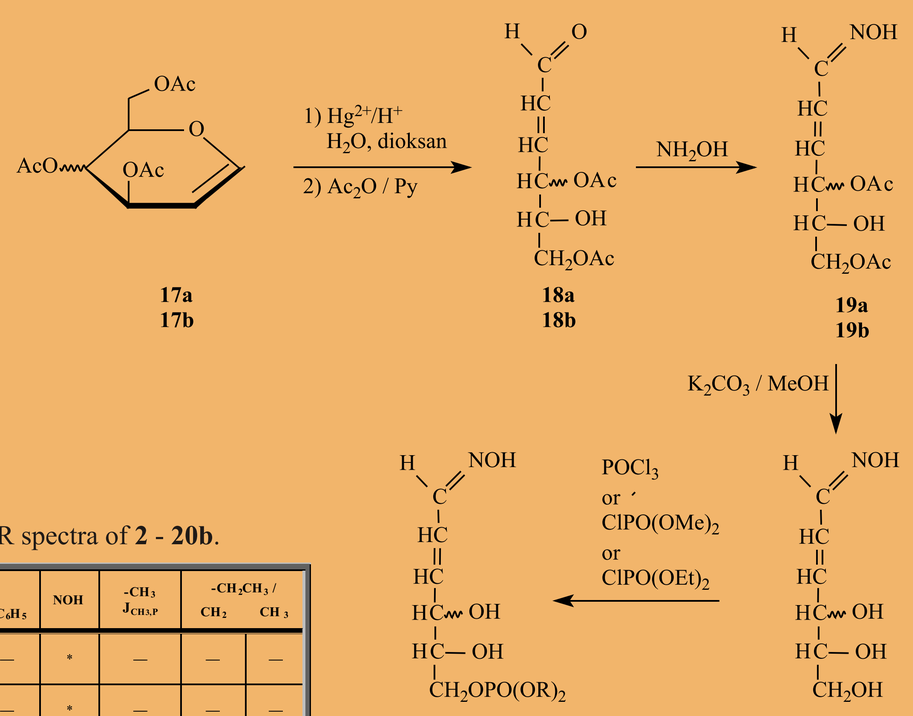
4 **5** **6** **7** **8** **9** **10**



11 **12** **13** **14** R=H **15** R=Me **16** R=Et

To get oxime of 2,3-dideoxy-6-*O*-phosphono-D-*glicero*-pent-2-enose (**14**) and its ester analogs (**15**, **16**) I was started from obtaining of 3,4-di-*O*-acetyl-D-arabinal (**11**), the known sugar substrate. This compound was gotten from D-arabinose. Hydrolysis of D-arabinal **11** followed by the elimination of the acetic acid was provided 4-*O*-acetyl-2,3-dideoxy-D-*glicero*-pent--2-enose (**12**). Reaction of D-pent-2-enose **12** with hydroxyloamine was provided oxime of 4-*O*-acetyl-2,3-dideoxy-D-*glicero*-pent--2-enose (**13**). Next, oxime **13** will be phosphorylated at the terminal hydroxyl group and then 4-*O*-deacetylated. I will use three different reagents for the phosphorylation. First one is phosphoryl chloride, which will provide oxime of 2,3-dideoxy--6-*O*-phosphono-D-*glicero*-pent-2-enose (**14**). Second one is dimethylchlorophosphate, which will provide oxime of 2,3-dideoxy-6-*O*-dimethylphosphate-D-*glicero*-pent-2-enose (**15**). The third phosphorylation will be carried out with diethyl chlorophosphate. In this way I will synthesized oxime of 2,3-dideoxy-6-*O*-diethylphosphate-D-*glicero*-pent-2-enose (**16**). Those oximes will be shorter by one carbon atom in comparison with the others synthesized by me compounds. I want to check if the length of alditol may influence its activity.

I plan to synthesized two stereoisomers of oximes of 2,3-dideoxy-6-*O*-phosphono-D-hex-2-enoses. First one (**a**) will have the same configuration as the configuration of the putative intermediate of the reaction catalyzed by Glcn-6P synthase and isomerase Man-6P. The second one (**b**) will have a configuratuion changed at the C4 carbon atom. To get oximes of 2,3-dideoxy-6-*O*-phosphono-D-hex-2-enoses (**21a,b**) and their ester analogs (**22a,b**, **23a,b**) I started from obtaining of 3,4,6-tri-*O*-acetyl-D-glucal (**17a**) and -D-galactal (**17b**), the known sugar substrates. These compounds was gotten from D-glucose (**a**) and D-galactose (**b**), respectively. Hydrolysis of D-glucal **17a** and D-galactal **17b** followed by the elimination of the acetic acid was provided respective 4,6-di-*O*-acetyl-2,3-dideoxy-D-hex-2-enoses (**18a** and **18b**). Reaction of D-hex-2-enoses **18a** and **18b** with hydroxyloamine was provided respective oximes of 4,6-di-*O*-acetyl-2,3-dideoxy-D-hex-2-enoses (**19a** and **19b**). Then oxime **19b** was *O*-deacetylated provide oxime**20b**. Oxime **19a** will be *O*-deacetylated provide oxime **20a**. Next, oximes **20a** and **20b** will be phosphorylated at the terminal hydroxyl group. I will use three different reagents for the phosphorylation. First one is phosphoryl chloride, which will provide oximes of 2,3-dideoxy-6-*O*-phosphono-D-*erytro*-hex-2-enose (**21a**) and -D-*treo*-hex-2-enose (**21b**). Second one is dimethylchlorophosphate, which will provide oximes of 2,3-dideoxy-6-*O*-dimethylphosphate--D-*erytro*-hex-2-enose (**22a**) and -D-*treo*-hex-2-enose (**22b**). The third phosphorylation will be carried out with diethylchlorophosphate. In this way I will synthesized oximes of 2,3-dideoxy-6-*O*-diethylphosphate-D-*erytro*-hex-2-enose (**23a**) and -D-*treo*-hex-2-enose (**23b**).



17a **17b** **18a** **18b** **19a** **19b** **20a** **20b**

21a **21b** R=H
22a **22b** R=Me
23a **23b** R=Et

Tabele 1
Characteristic and Malditof MS data for **2** - **10**.

No.	Yield %	Form (mp [°C])	M (g/mol)	MALDITOF MS
2 (*Z*. *E*)	98	solid	319	432 (M^+ + 23)
3	17	syrup	303	325 (M^+ + 23)
5	85	syrup	337	360 (M^+ + 23)
6	26	syrup	409	432 (M^+ + 23)
7	43	solid	282	305 (M^+ + 23)
8	42	syrup	419	442 (M^+ + 23)
9	17	syrup	329	352 (M^+ + 23)
10	20	syrup	331	354 (M^+ + 23)

Tabele 2
Chemical shifts (ppm) and ^{1}H - ^{1}H, and ^{1}H - ^{31}P coupling constants (Hz) in the ^{1}H NMR spectra of **2** - **20b**.

No. (Solv.)	H-1 $J_{1,2}$	H-1' $J_{1,1'}$	H-2 $J_{2,3}$	H-3 $J_{3,4}$	H-4 $J_{4,5}$	H-5 $J_{5,6}$ / $J_{5,6'}$	H-6' $J_{6,6'}$ / $J_{6',P}$	H-6 $J_{6,P}$	OAc	O-CH_2-Ph / $J_{A,B}$ H-A	H-B	-C_6H_5	NOH	-CH_3 $J_{CH3,P}$	-CH_2CH_3 / CH_2	CH_3
2 (*Z*) (D_2O)	4.28 (s) 1H	—	—	5.24 (d) 1H 2.4	3.98 (dd) 1H 6.8	3.80 (ddd) 1H *	3.90 (m) 2H *		—	—	—	—	*	—	—	—
2 (*E*) (D_2O)	4.46 (d) 1H	4.33 (d) 1H 14.8	—	4.54 (d) 1H 2.8	3.85 (dd) 1H 8.8	3.75 (ddd) 1H *	3.94 (m) 2H *		—	—	—	—	*	—	—	—
3 (CD_3OD)	3.71 (d) 1H	3.63 (d) 1H 12.0	—	4.04 (d) 1H 4.0	3.85 (dd) 1H 6.8	4.16 (ddd) 1H *	4.22 (m) 1H *	3.95 (m) 1H *	—	—	—	—	*	3.81 (d) 3H 3.78 (d) 3H 2.4	—	—
5 ($CDCl_3$)	6.65 (d) 1H 3.6	—	5.43 (dd) 1H 10.2	6.03 (t) 1H 9.6	6.17 (t) 1H 10.4	4.34 (m) 1H 4.0/2.0	4.14 (dd) 1H 12.8/–	4.38 (dd) 1H	2.08 (s) 3H 2.07 (s) 3H 1.99 (s) 3H	—	—	—	—	—	—	—
6 ($CDCl_3$)	6.09 (s) 1H	—	—	5.82 (d) 1H 10.0	5.21 (t) 1H 9.6	4.19 (ddd) 1H 4.4/2.4	4.02 (dd) 1H 12.0/–	4.29 (dd) 1H	2.09 (s) 3H 2.06 (s) 3H 2.04 (s) 3H	4.68 (d) 1H 12.0	4.75 (d) 1H	7.36 (m) 5H	8.22 (s) 1H	—	—	—
7 (CD_3OD)	6.08 (s) 1H	—	—	4.34 (d) 1H 9.2	3.41 (t) 1H 9.6	3.78 (ddd) 1H 5.2/2.0	3.84 (dd) 1H 11.6/–	3.70 (dd) 1H	—	4.61 (d) 1H 12.0	4.78 (d) 1H	7.34 (m) 5H	*	—	—	—
8 (CD_3OD)	6.07 (s) 1H	—	—	4.51 (d) 1H 9.6	3.66 (t) 1H 9.6	3.91 (m) 1H 8.4/*	4.16 (ddd) 1H 11.6/*	4.37 (ddd) 1H 3.6	—	4.61 (d) 1H 11.6	4.72 (d) 1H	7.30 (m) 5H	*	—	4.11 (m) 4H	1.31 (k) 6H
9 (CD_3OD)	6.25 (s) 1H	—	—	4.38 (d) 1H 9.2	3.41 (t) 1H 9.6	4.05 (m) 1H 4.8/2.0	4.29 (ddd) 1H 11.2/6.4	4.23 (ddd) 1H 7.2	—	—	—	—	*	—	4.13 (m) 4H	1.33 (k) 6H
12 ($CDCl_3$)	9.55 (d) 1H 7.6	—	6.26 (dd) 1H 16,0	6.78 (dd) 1H 4,0	5.57 (ddd) 1H 4.0; 6.8	4.26; 4.14 (2xdd) 2H $J_{5,5}$=11,6	—	—	2.09 (s) 3H	—	—	—	—	—	—	—
13 (*Z*/*E*) (CD_3OD)	7.72 (d) 1H 10.0	—	6.33 (dd) 1H 16.0	6.04 (dd) 1H 5.6	5.37 (m) 1H *	3.64 (m) 1H *	—	—	2.05 (s) 3H	—	—	—	*	—	—	—
19a (*Z*/*E*) (CD_3OD)	7.75 (d) 1H 10.0	—	6.38; 6.32 (2xdd) 1H 16.0	6.03; 6.07 (2xdd) 1H 6.8	5.38 (m) 1H *	3.94 (m) 1H 6.8/4.8	4.12 (dd) 1H 12.0	4.16 (dd) 1H	—	—	—	—	*	—	—	—
19b (*Z*/*E*) (CD_3OD)	7.76; 7.73 (2xd) 1H 10.0	—	6.41; 6.34 (2xdd) 1H 15.6	6.05; 6.03 (2xdd) 1H 6.4	5.41 (m) 1H 4.8	3.93 (ddd) 1H 6.4/4.4	4.16 (dd) 1H 11.6	4.11 (dd) 1H	2.05 (s) 6H	—	—	—	*	—	—	—
20b (*Z*/*E*) (CD_3OD)	7.76 (d) 1H 10.0	—	6.39 (dd) 1H 16.0	6.13 (dd) 1H 6.0	4.23 (m) 1H 4.8	3.58 (m) 1H 6.4/4.0	3.65 (dd) 1H 10.4	3.52 (dd) 1H	—	—	—	—	*	—	—	—

* not determined

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