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Hydrophobic Derivatives of 2-Amino-2-deoxy-D-glucitol-6-phosphate: A New Type of D-Glucosamine-6-phosphate Synthase Inhibitors with Antifungal Action

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Abstract—Several *N*-acyl and ester derivatives of 2-amino-2-deoxy-D-glucitol-6-phosphate (ADGP) have been synthesised and tested as inhibitors of fungal enzymes involved in early steps of chitin biosynthesis and for antifungal activity. All the tested derivatives were found to be much poorer inhibitors of the enzyme, D-glucosamine-6-phosphate (GlcN-6-P) synthase, than the parent compound but some of them exhibited much better antifungal activity. MIC values for the investigated compounds ranged between 10 mg mL^{-1}, found for ADGP and 0.3 mg mL^{-1} for the most active derivative, namely ADGP dimethyl ester. Increased affinity of ADGP derivatives to the artificial immobilised cell membrane was correlated with their enhanced ability to be taken up by fungal cells by free diffusion. It was found that some of the examined derivatives behaved as 'pro-drugs' and after internalisation were converted into ADGP in the cell-free extract. This conversion was relatively rapid for ADGP esters but very slow for *N*-acyl derivatives. Results of our studies demonstrate a possibility of design and preparation of GlcN-6-P synthase inhibitors exhibiting antifungal activity.

Introduction

Disseminated infections caused by human pathogenic fungi remain one of the most difficult problems in modern chemotherapy. Our ability to treat such infections with established agents do not satisfy medical needs.[1] The situation becomes more complicated because of the rapidly increasing number of immunocompromised patients and the emerging challenge of multidrug resistance. Only a very limited number of antifungal chemotherapeutics are in clinical use. The high toxicity of Amphotericin B for mammalian cells and fungistatic but not fungicidal action of azole antifungals limit their clinical applications. Therapeutic potential of the latter is also severely limited due to the substantial development of resistance, especially of the multidrug character.[2] One of the possible solutions to this problem is to consider an exploitation of new antifungal targets,[3] including enzymes involved in fungal cell wall biosynthesis. One of them is GlcN-6-P synthase, enzyme catalysing the first committed step in chitin biosynthesis pathway, that is transformation of D-fructose-6-phosphate (Fru-6-P) to D-glucosamine-6-phosphate. Although the enzyme is also present in mammalian systems, substantial difference in physiological consequences of GlcN-6-P synthase inhibition in fungi and in mammals, constitute a firm molecular basis for the selective toxicity of specific enzyme inhibitors.[4]

Known specific inhibitors of GlcN-6-P synthase belong to two different structural groups: L-glutamine mimics and analogues of the putative transition state intermediates. Among a number of known glutamine analogues, some are selective inhibitors of GlcN-6-P synthase, not interacting with other enzymes utilising L-glutamine as a substrate. One of them, N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid (FMDP), gave rise to oligopeptidic compounds demonstrating remarkable antifungal activity.[5] Incorporation of FMDP into peptide structure allowed effective internalisation of the enzyme inhibitor by the way of peptide permeases, but on the other hand was a reason for

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substantial specific resistance,[6] since peptides permeases are not essential for fungal cells.[7]

The second group of compounds comprises derivatives of phosphorylated aminosugars, including: 2-amino-2-deoxy-D-glucitol-6-phosphate (ADGP),[8] arabinose-5-phosphate oxime[9] and 5-methylenephosphono-D-arabino hydroximolactone,[10] as the most powerful GlcN-6-P synthase inhibitors. These compounds exhibit very poor, if any, antifungal activity. We have recently demonstrated that the antifungal activity of ADGP is limited due to the highly inefficient uptake of this compound by an unidentified active transport system and apparent inability to cross the membrane by free diffusion.[11] In the present paper, we describe a method of improving an antifungal activity of ADGP by modifications of its structure, giving rise to derivatives of enhanced lipophilicity, able to diffuse through the fungal cytoplasmic membrane.

Results and Discussion

Rationale for modification

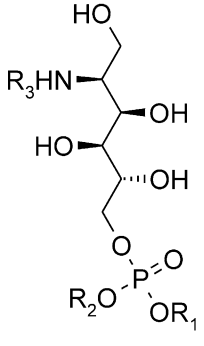
In the 2-amino-2-deoxy-D-glucitol-6-phosphate (**1**) molecule there are two sites charged at physiological conditions: the amino group and the phosphate group. These are also possible sites of modifications, resulting in increase of a general lipophilicity of the molecule, supposed to be advantageous for better penetration of the derivatives through the cytoplasmic cell membrane. We have therefore decided to prepare and test several *N*-acyl and ester derivatives of ADGP of general formula showed in Figure 1.

Synthesis

Compound **1** was prepared from D-glucosamine-6-phosphate upon reduction with sodium borohydride.[12] *N*-Acyl derivatives of ADGP **2**–**6** were obtained by acylation of the 2-amino group with respective acyl anhydrides under alkaline conditions. Compound 2 was also alternatively synthesised by acetylation of D-glucosamine-6-phosphate, followed by the selective reduction of the aldehyde group. All the compounds were obtained as white solids. Dimethyl ester derivatives of ADGP (**7**, **8**) were obtained upon the action of diazomethane on *N*-benzyloxycarbonyl-ADGP or *N*-acetyl-ADGP (2). In the former case, hydrogenolytic de-protection of the amino function afforded the required compound **7**. Synthesis of ethyl ester derivatives of ADGP (**9**, **10**, and **11**) was accomplished by multi-step procedures. D-Glucosamine was first *N*-protected upon acylation with benzylchloroformate or acetic anhydride and the obtained compounds were converted into methyl or benzyl glycosides **9a** or **10a**, respectively. The protected glucosamine derivatives were selectively phosphorylated at 6-OH with diethylchlorophosphate in the presence of AgCN, thus giving rise to derivatives **9b** and **10b**. Hydrogenolysis of **10b** afforded diethyl ester of *N*-acetyl-D-glucosamine-6-phosphate **10c**, while treatment of **9b** with traces of hydrochloric acid gave the diethyl ester of *N*-benzyloxycarbonyl-D-glucosamine-6-phosphate **9c**. Both compounds (**9c** and **10c**) were reduced with sodium borohydride to give products **9d**, **10** or **11**. Product **9d** was isolated and treated with H_2/Pd/C to give finally the required diethyl ester of ADGP **9**. Reduction of **10c** for a short time (2 h) afforded the diethyl ester of AcADGP **10**, but prolonged treatment of **10c** with $NaBH_4$ (overnight) led to the partial hydrolysis of the diethyl ester and eventual formation of the monoethyl ester of AcADGP **11**. Graphical representation of synthetic strategies is shown in Scheme 1. All the final ester products: **7**, **8**, **9**, **10** and **11** were obtained as the oily substances. All final compounds **1**–**11** were at least 99% pure, as judged by HPLC testing (see below).

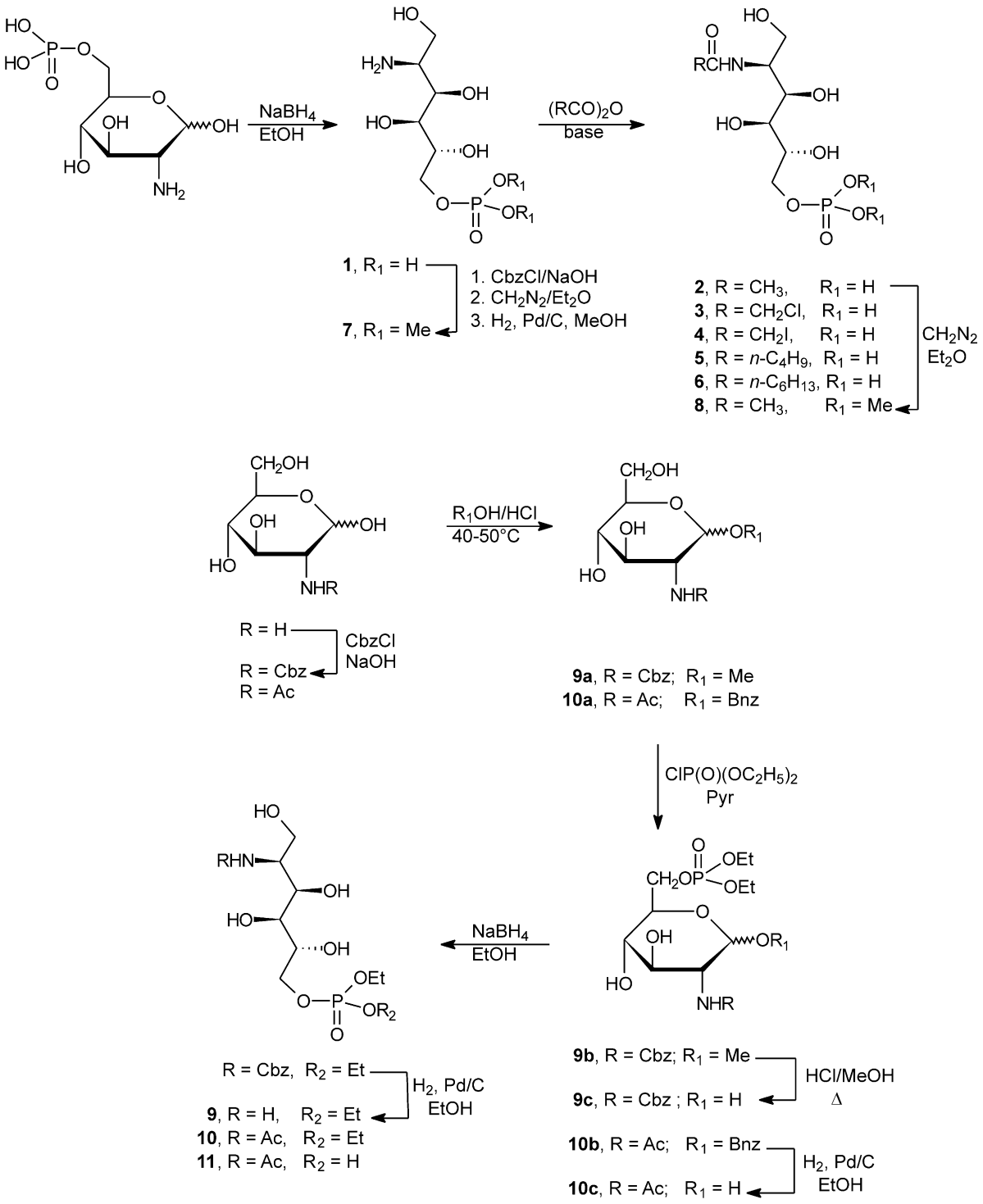
Comparison of apparent lipophilicity of the obtained compounds

The general idea of our approach was to convert the good GlcN-6-P synthase inhibitor ADGP into its derivatives demonstrating higher apparent lipophilicity than the parent compound and, in consequence, an increased potential to cross the cytoplasmic membrane by free diffusion. In order to compare the relative lipophilicity of ADGP and its derivatives, we determined their affinity to the artificial biological membrane immobilised on the bed of the IAM PC DD2 column. The column bed is especially designed for investigation of low and moderately lipophilic compounds.[13] It is composed of phosphatidylcholine residues attached to the silica



(**1**) $R_1=R_2=R_3=H$
(**2**) $R_1=R_2=H$ $R_3=C(O)CH_3$
(**3**) $R_1=R_2=H$ $R_3=C(O)CH_2Cl$
(**4**) $R_1=R_2=H$ $R_3=C(O)CH_2I$
(**5**) $R_1=R_2=H$ $R_3=C(O)C_4H_9$
(**6**) $R_1=R_2=H$ $R_3=C(O)C_6H_{13}$
(**7**) $R_1=R_2=CH_3$ $R_3=H$
(**8**) $R_1=R_2=CH_3$ $R_3=C(O)CH_3$
(**9**) $R_1=R_2=C_2H_5$ $R_3=H$
(**10**) $R_1=R_2=C_2H_5$ $R_3=C(O)CH_3$
(**11**) $R_1=C_2H_5$; $R_2=H$ $R_3=C(O)CH_3$

Figure 1. Structures of the investigated compounds.



Scheme 1. Synthetic strategies applied in this work. Cbz, benyloxycarbonyl; Bnz, benzyl.

support, thus forming a lipidic surface mimicking the monolayer of the fluidic cell membrane. The retention time measured in this system for a given compound, is not only a measure of lipophilicity but reflects also possible interactions with the artificial membrane, like hydrogen or electrostatic bond formation. We have determined the retention parameters, k'_{IAM}, for ADGP and its derivatives. It should be noted that all examined compounds gave single peaks, with noise level not exceeding 1%, thus confirming their at least 99% purity. The obtained data are presented in Table 1. The extreme values of log k'_{AM} were found for reference compounds: −0.7583 for GlcN-6-P and +0.8893 for benzene. Apparent lipophilicity of ADGP was intermediate, surprisingly enough much higher than that of the closely related reference compound, that is, GlcN-6-P. Acylation of the amino group or conversion of the phosphate group into dimethyl or diethyl esters caused further increase of apparent lipophilicity, as well as the elongation of the carbon chain in the series of *N*-acyl

Table 1. Data characterising affinity of the investigated compounds to the IAM.PC.DD2 column and their TLC mobility. ND = not determined

Compound	$\log k'_{IAM}$	R_f
GlcN-6-P	−0.7583	0.34 (B)
Benzene	0.8893	ND
1	−0.0437	0.28 (B)
2	0.2408	0.67 (B)
3	0.3170	0.65 (B)
5	0.3942	0.75 (B)
6	0.4407	0.80 (B)
7	0.2493	0.81 (A)
8	0.4074	0.95 (A)
9	0.2802	0.89 (A)
10	0.4238	0.85 (A)
11	0.3602	0.67 (A)

derivatives **2**–**6**. The log k'_{IAM} value found for the most lipophilic compound in the series, that is *N*-hexanoil-ADGP (**6**) was slightly higher than those of the *N*-acetyl esters **8** and **10**. The compounds **2**–**11** could be therefore considered as moderately lipophilic and thus potentially able to cross the cell membrane by free diffusion.

Enzyme inhibitory activity

Inhibitory activity of all the synthesised compounds towards *Candida albicans* GlcN-6-P synthase was determined. Some of these compounds were also tested as potential inhibitors of two other enzymes, catalysing respective: preceding and following steps in the chitin biosynthesis pathway, namely phosphoglucose isomerase and GlcN-6-P *N*-acetylase. The obtained data have been summarised in Table 2. All the derivatives appeared to be poorer inhibitors of GlcN-6-P synthase than ADGP. The enzyme inhibitory activity was especially reduced upon *N*-acylation of ADGP and the resulting loss of the positive charge (compare compounds **1** and **2**), whereas the elongation of the acyl chain was of the secondary importance (compounds **2**, **5** and **6**). The former observation is consistent with the earlier suggestion of Bearne and Blouin,[14] based on the results of analysis of the other GlcN-6-P synthase inhibitors. Surprisingly enough, enzyme inhibitory activity of *N*-haloacetyl ADGP derivatives **3** and **4** was very low. This is in contrast with the observations made by Badet-Denisot et al., who investigated the inhibitory potential of compounds **1**, **2**, **5** and **6** towards *Escherichia coli* GlcN-6-P synthase.[15] This discrepancy may suggest differences between the active centres of eukaryotic and prokaryotic GlcN-6-P synthases. Esterification of the phosphate group also reduced the inhibitory potential of ADGP, but the effect was less pronounced than in the case of *N*-acylation. Since Teplyakov et al.[16] previously demonstrated that interactions of the ADGP phosphate oxygen atoms with amino acid side chains at the GlcN-6-P synthase active centre involved exclusively hydrogen bonds, one may assume that such interactions are still possible for ADGP esters, provided no steric hindrance is implemented. Moreover, the IC_{50} values of ADGP esters were generally lower when determined for the enzyme present in the crude extract, in comparison to those found for the homogenous enzyme. One of the likely explanations for this finding is the possibility of relatively rapid conversion of ADGP esters into ADGP, catalysed by esterases present on the cell-free extract.

Table 2. Inhibition of *C. albicans* GlcN-6-P synthase by ADGP and its derivatives

Compd	IC_{50} (mM)	
	Pure enzyme	In cell-free extract
1	0.23	0.24
2	6.0	5.5
3	5.2	5.2
4	>20	>20
5	11	10.1
6	19	17.5
7	4.75	1.7
8	12	5.2
9	4.7	3.8
10	19	12
11	13	6.5

Both acylation of the amino group and esterification of the phosphate group in ADGP molecule had no influence on the inhibition manner. In all cases inhibition was competitive with regard to D-fructose-6-phosphate and non-competitive with regard to L-Gln (data not shown). It is therefore clear that the investigated compounds interact with GlcN-6-P synthase at the D-fructose-6-phosphate binding site.

Phosphoglucose isomerase catalyses a reaction preceding the GlcN-6-P synthase-catalysed step in the chitin biosynthesis pathway. On the other hand, the isolated Fru-6-P-binding domain of GlcN-6-P synthase exhibits the phosphoglucose isomerase-like activity.[17] It seemed thus reasonable to check whether the tested compounds demonstrated phosphoglucose isomerase-inhibiting activity. We found that ADGP (**1**), at 5 mM, reduced the yeast enzyme activity by only 10%. Surprisingly, *N*-acetyl-ADGP (**2**) appeared a relatively good inhibitor of phosphoglucose isomerase, inhibiting 50% of the enzyme activity at 76 ± 2 μM. Actually, the phosphoglucose isomerase inhibitory activity of **2** was much higher than that against GlcN-6-P synthase. Other derivatives did not affect the phosphoglucose isomerase activity.

GlcN-6-P acetylase follows GlcN-6-P synthase in the chitin biosynthesis pathway. Amino sugar phosphates and their derivatives can be potential inhibitors of GlcN-6-P acetylase as potential analogues of its substrate. However, none of the tested compounds inhibited enzyme activity up to 20 mM. Thus, with the exception of compound **2**, investigated compounds were shown to be specific inhibitors of GlcN-6-P synthase and did not significantly inhibit activity of enzymes catalysing step adjacent to formation of GlcN-6-P by GlcN-6-P synthase.

Antifungal activity of ADGP and its derivatives

Antifungal activity of compounds **1**–**3** and **5**–**11** was determined by a serial dilution microplate method. *N*-iodoacetyl-ADGP (**4**) was not tested, as this compound

Table 3. Antifungal in vitro activity of ADGP and its derivatives

Compd	MIC (mg mL^{-1})					
	C. albicans ATCC 10261	*C. albicans* ATCC 26278	*C. famata*	*C. humicola*	*C. glabrata*	*S. cerevisiae* ATCC 9763
1	5	5	5	5	>10	2.5
2	5	5	>10	5	2.5	0.75
3	>10	>10	>10	>10	>10	>10
5	2.5	2.5	2.5	5	5	2.5
6	10	10	5	10	10	5
7	0.3	0.3	0.6	ND	2.5	0.6
8	5	10	>10	ND	5	1.25
9	0.6	0.6	1.25	1.25	2.5	0.6
10	10	5	10	ND	5	1.25
11	2.5	2.5	10	5	1.25	10

ND, not determined.

did not show any GlcN-6-P synthase inhibitory activity. Minimal inhibitory concentrations (MICs) were determined in YNBG minimal medium with glucose as carbon source. Obtained results are summarised in Table 3. Practically identical results were obtained when the antifungal activity was determined in buffered RPMI 1640 medium (exact data not shown).

Most of the investigated compounds exhibited rather weak antifungal activity (MICs ranging from 2.5 to 10 mg mL^{-1}). Notable exceptions are the dimethyl and diethyl esters of ADGP (**7** and **9**), demonstrating growth inhibitory effect at much lower concentrations. On the other hand, it is worth noticing that *N*-acetyl-ADGP (**2**) inhibited fungal growth at concentrations similar to that of ADGP or even lower, despite its significantly poorer GlcN-6-P synthase inhibitory activity (see Table 2). Actually, the MIC values for this compound, as well as those for other *N*-acyl derivatives of ADGP (**5** and **6**) were quite comparable to their IC_{50} values determined in respect to *C. albicans* GlcN-6-P synthase. On the other hand, the MICs for ADGP esters 7 and 9 were even lower than their IC_{50}s against the enzyme. Antifungal activity of the monoethyl ester of AcADGP (**11**) was very similar or slightly better than that of *N*-acetyl-ADGP (**2**), while that of the dimethyl and diethyl esters of *N*-acetyl-ADGP (**8** and **10**) was poorer.

The microscopic examination of cells treated with ADGP, *N*-acetyl-ADGP or ADGP dimethyl ester revealed morphological changes characteristic for yeast cells affected by compounds inhibiting chitin and/or mannoprotein biosynthesis. These alterations included cell agglutination, visible destruction of cell integrity, cell swelling and inhibition of septum formation (data not shown). Similar morphological changes were previously described as a result of the action of oligopeptides containing an inhibitor of GlcN-6-P synthase.[18] On the other hand, known inhibitors of chitin synthase, nikkomycins and polyoxins, inhibit septum formation and cause cell swelling and lysis.[19, 20] while tunicamycin, a selective inhibitor of mannoprotein biosynthesis, causes mainly cell agglutination.[21] It should be noted that the presence of 10 mM D-glucosamine in the medium completely abolished the antifungal activity of investigated compounds, thus indicating that GlcN-6-P synthase is their primary target in fungal cells.

Uptake of ADGP, AcADGP and dimethyl ester of ADGP

The initial uptake rates of compounds **1**, **2** and **7** to *C. albicans* cells were determined in the presence of D-glucose, D-glucose and NaN_3, and in the absence of the carbon source. Results are summarised in Table 4.

Presence of D-glucose reduced *N*-acetyl-ADGP (**2**) uptake rates, but had no influence on ADGP (**1**) and ADGP dimethyl ester (**7**) uptake. In the presence of sodium azide, uptake of ADGP was completely inhibited but uptake of compounds **2** and **7** was only slowed down. The initial uptake rate of **2**, determined in the presence of sodium azide, was linearly dependent on the initial concentration of the former, while in the absence of NaN_3 was hyperbolic (Fig. 2). Similar phenomenon was observed for the compound **7** (details not shown). Maximal uptake rates were: 6.6 nmol (min mg dry weight)$^{-1}$, 20 nmol (min mg dry weight)$^{-1}$ and 15.5 nmol (min mg dry weight)$^{-1}$, for compounds **1**, **2** and **7**, respectively.

One may therefore conclude that *N*-acetyl-ADGP and ADGP ester are transported into *C. albicans* cells both by an active transport system and by free diffusion. We have not studied the uptake of other ADGP derivatives but it is very likely that their accumulation in fungal cells is also in part possible by free diffusion. On the other hand, ADGP is transported exclusively by an unidentified active transport system.

Table 4. Initial rates of uptake of ADGP, AcADGP and ADGP diethyl ester by *C. albicans* cells

Buffer/additives	Initial uptake rate[a] (nmol/min/mg dry weight)		
	(1)	**(2)**	**(7)**
Tris/glucose	1.1±0.2	3.1±0.2	2.8±0.3
Tris	1.0±0.2	5.0±0.3	2.7±0.2
Tris/glucose, NaN_3	<0.01	0.5±0.06	1.6±0.1

[a]Initial compound concentration 5 mg mL^{-1}.

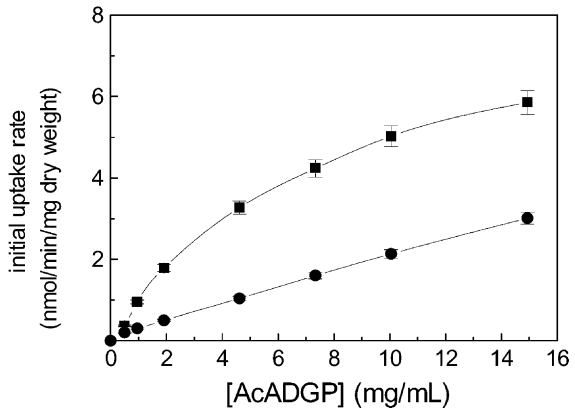


Figure 2. Kinetics of *N*-acetyl-ADGP uptake by *C. albicans* cells. Fungal cells harvested from the exponentially growing culture were washed, suspended in an appropriate buffer system and the cell suspension was incubated at 30 °C. *N*-Acetyl-ADGP was added and samples of the cell culture were collected at time intervals. Cells were harvested by filtration and concentration of the remaining ADGP derivative was determined in filtrates. The initial uptake velocities, determined from the plots of remaining AcADGP concentration versus time were re-plotted against the initial compound concentration. Values are the means of three independent determinations ±S.D. Buffer composition: (■) 50 mM Tris/HCl, pH 6.5, containing 1% glucose; (●) 50 mM Tris/HCl, pH 6.5, containing 1% glucose and 100 μM NaN_3.

Metabolism of ADGP, *N*-acetyl-ADGP and ADGP dimethyl ester

Composition of the cell-free extracts prepared from the *C. albicans* cells grown in the presence of ADGP, *N*-acetyl-ADGP or ADGP dimethyl ester was investigated by thin layer chromatography (TLC) analysis. These studies showed that ADGP (**1**) was not metabolised to any detectable derivative. On the other hand, *N*-acetyl-ADGP (**2**) and ADGP ester (**7**) were metabolised to compound chromatographically identical with ADGP. The conversion rate of **2** was very low, since the presence of ADGP was detected only in the cell-free extract prepared from the cells harvested after 8-h incubation with *N*-acetyl-ADGP. The ADGP ester (**7**) was metabolised much faster, since a spot corresponding to ADGP was detected as early as after 1 h incubation.

Similar results were obtained when compositions of the *C. albicans* cell-free extracts, incubated with **1**, **2** or **7**, were analysed by TLC. However, in that case it was found that the rate of conversion of **2** into **1** substantially increased when the cell free extract was additionally supplemented with ATP, 10 mM.

Low rate of intracellular deacetylation of *N*-acetyl-ADGP suggests that this compound, as a good inhibitor of phosphoglucose isomerase, may target this enzyme upon its action on fungal cells. Such inhibition should, in principle, result in growth inhibitory effect. However, we believe that this possibility seems rather unlikely, since the lowest MIC value of this compound (0.6 mg mL^{-1} against *Saccharomyces cerevisiae*) is almost three orders of magnitude higher than its IC_{50} against pure yeast phosphoglucose isomerase.

Conclusion

We have designed and synthesised a number of 2-amino-2-deoxy-D-glucitol-6-phosphate (ADGP) derivatives, modified at 2-amino and/or 6-phosphate groups. All the obtained derivatives demonstrated higher apparent affinity to the artificial immobilised cell membrane IAM PC DD2 than the parent compound and therefore a higher potential to cross the real cell membrane by free diffusion. The log k'_{IAM} values determined for these compounds were in the 0.2–0.5 range, thus indicating their moderately lipophilic character. In general opinion, log k'_{IAM} values ranging between 0 and 2 are considered advantageous since compounds demonstrating negative log k'_{IAM} are unlikely to diffuse through the biological membranes, while those showing log $k'_{IAM} > 2$ may be easily retained in the membrane environment.[13]

ADGP, a relatively strong inhibitor of GlcN-6-P synthase, was previously found to be a very poor antifungal agent, transported into *C. albicans* cells by unidentified, energy-dependent transport system.[11] We have been able to improve the antifungal activity of ADGP by its conversion into more lipophilic *N*-acyl and ester derivatives, able to cross the cell membrane by free diffusion. Although the obtained derivatives demonstrated lower inhibitory potential against the target enzyme, GlcN-6-P synthase, their intracellular metabolism, demonstrated for *N*-Acetyl-ADGP and ADGP dimethyl ester and suspected for other derivatives, gives rise to formation of the more active enzyme inhibitor. This mode of action is therefore consistent with the 'pro-drug' concept.[22,23] However, intracellular deacetylation of N-acetyl-ADGP is very slow and that of other *N*-acyl derivatives is possibly even slower. On the other hand, the ADGP dimethyl ester is much faster metabolised to ADGP, thus giving rise to intracellular accumulation of the more efficient enzyme inhibitor. We can therefore conclude that esterification is a more promising way of preparation of potential antifungal agents based on amino sugar phosphate analogues. Further studies, aimed at design and synthesis of novel GlcN-6-P synthase inhibitors, more effective than ADGP, should result in development of their ester derivatives as potential antifungals. These studies are in progress in our laboratory.

Experimental

Chemistry

Butanoic acid anhydride, hexanoic acid anhydride, chloroacetyl chloride, 2-amino-2-deoxy-D-glucose-6-phosphate and sodium borohydride were purchased from Sigma-Aldrich Chemical Co. All other chemicals were of the highest purity commercially available.

^1H and ^{31}P NMR spectra were obtained with Varian Unity Plus 500 at 200 and 500 MHz, respectively. The deuterated solvents were used as an internal lock for ^1H NMR and external aq 85% H_3PO_4 for ^{31}P NMR.

Specific rotations were measured on a Rudolph Autopol II digital polarimeter. TLC was carried out on Kieselgel 60 F 254 plates (Merck) in solvent systems: A (methanol–25% aq ammonia–water, 6:2:1 v/v/v), B (*n*-butanol–acetic acid–water 4:1:1 v/v/v) and C (chloroform–methanol 5:1, v/v). The spots were visualised with ninhydrin, cerium sulphate or Hanes' reagent.[24]

2-Amino-2-deoxy-D-glucitol-6-phosphate (1). This compound was synthesised according to the method described by Bearne[12] with 80% yield (literature yield[12] 60%).

***N*-Acetyl-2-amino-2-deoxy-D-glucitol-6-phosphate (2), *N*-chloroacetyl-2-amino-2-deoxy-D-glucitol-6-phosphate (3), *N*-iodoacetyl-2-amino-2-deoxy-D-glucitol-6-phosphate (4), *N*-*n*-butanoil-2-amino-2-deoxy-D-glucitol-6-phosphate (5) and *N*-*n*-hexanoil-2-amino-2-deoxy-D-glucitol-6-phosphate (6).** To the solution of **1** (100 mg, 0.38 mmol) and a base (1.78 mmol) in an appropriate solvent system (5 mL), an acylating agent was added for 20–60 min and then the reaction mixture was stirred for another 20–60 min. A course of the reaction was followed with TLC (solvent system A or B). Further details are shown in Table 5. Products **2**, **3** and **4** were purified by ion-exchange chromatography on Dowex 50 WX4 (H^+) column and obtained finally from the lyophilised filtrate. Compounds **5** and **6** were purified by consecutive adsorption (Silicagel) and ion-exchange (Dowex 50 WX4/H^+/) column chromatography Fractions containing product were pooled and lyophilised.

***N*-Acetyl-2-amino-2-deoxy-D-glucitol-6-phosphate (2) Method 2.** To the solution of 2-amino-2-deoxy-D-glucose-6-phosphate (100 mg, 0.36 mmol) and $NaHCO_3$ (150 mg, 1.78 mmol) in water (5 mL), acetic acid anhydride (10% solution in acetone, 0.6 mL) was added during 10 min. After another 10 min of stirring at room temperature, the reaction mixture was diluted with 2-propanol (5 mL) and then the solvent was evaporated. The oily residue was dissolved in water (3 mL) and cooled to 0 °C. Then $NaBH_4$ (150 mg, 4 mmol) was added portionwise for 2 h. The reaction mixture was stirred at room temperature for another 1 h and then the solvent was evaporated. The residue was dissolved in a solvent system A and chromatographed over Silicagel column in the same solvent system. Fractions containing the product were evaporated, re-dissolved in water and chromatographed over Dowex 50 WX4 (H^+) column. Fractions containing the product were pooled and lyophilised. Compound **2** was obtained with 86% yield (94 mg, 0.31 mmol).

Compound 2. ^{1}H NMR (D_2O) δ: 1.84 (s, 3H, CH_3CO); 3.48–3.55 (m, 4H); 3.6 (m, 1H); 3.74–3.82 (m, 2H), 3.9 (m, 1H); R_f (A) 0.56; R_f (B) 0.67. Compound **3**: ^{1}H NMR (D_2O) δ: 3.58–3.64 (m, 1H); 3.65–3.78 (m, 2H), 3.84–4.02 (m, 3H), 4.09 (s, 2H, CH_2ClCO); 4.18–4.28 (m, 2H); R_f (B) 0.65. Compound **4**: ^{1}H NMR (D_2O) δ: 3.48–3.64 (m, 4H); 3.63 (s, 2H, CH_2ICO); 3.72–3.85 (m, 3H); 3.87–3.93 (m, 1H); R_f (B) 0.62. Compound **5**: ^{1}H NMR (D_2O) δ: 0.96 (t, 3H, J = 7.3 Hz, CH_3CH_2); 1.66 (m, 2H, J = 7.3 Hz, $CH_3CH_2CH_2CO$); 2.23 (t, 2H, J = 7.9 Hz, $-CH_2CO$); 3.35 (m, 2H, J = 1.7 Hz); 3.68 (*m*, 2H); 3.76 (m, 1H); 4.07 (m, 3H); R_f (B) 0.75. Compound **6**: ^{1}H NMR (D_2O) δ: 0.9 (t, 2H, J = 6.67 Hz, CH_3CH_2); 1.3 (m, 4H, $CH_3(CH_2)_2$), 1.6 (m, 2H, J = 7.5 Hz, $CH_3(CH_2)_2CH_2CH_2$); 2.4 (t, 2H, J = 7.3 Hz, CH_2CH_2CO); 3.31 (m, 2H, J = 1.74 Hz); 3.64 (m, 2H); 3.74 (m, 1H); 4.07 (m, 3H); R_f (B) 0.80.

2-Amino-2-deoxy-D-glucitol-6-phosphate dimethyl ester (7). 2-Amino-2-deoxy-D-glucitol-6-phosphate (281 mg, 1.1 mmol) was dissolved in 1 M NaOH solution (1 mL) and benzylchloroformate (0.21 mL) was added. The reaction mixture was stirred at room temperature for 24 h, maintaining pH at 9–10 with 1 M NaOH water solution. The resulting solution was extracted 3× with ethyl ether. The remaining water layer was chromatographed over Dowex 50 WX8 (H) column. Fractions containing 2-(*N*-benzyloxycarbonylamino)-2-deoxy-D-glucose-6-phosphate were evaporated in vacuo. Product (200 mg) was dissolved in water (5 mL) and the diazomethane ethyl ether solution was added to permanent yellow tint. Solvent was distilled off and the product was purified by silicagel column chromatography in solvent system chloroform/methanol 5:1, v/v, affording after evaporation 90 mg of 2-(*N*-benzyloxycarbonylamino)-2-deoxy-D-glucose-6-phosphate dimethyl ester (yield 41%). This product was dissolved in methanol (6 mL), $NaBH_4$ (30 mg, 0.78 mmol) was added and the mixture was kept for 4 h at room temperature. The resulting solution was purified on a Dowex 50 WX4 (H^+) ion-exchange resin developed with methanol and then the methanolic solution of the product was subjected to catalytic hydrogenation for 4 h. Final overall yield: 25%.^{1}H NMR (D_2O) δ: 3.32 (m, 1H); 3.41 (m, 1H); 3.70 (2d, 6H, J = 10.7 Hz, $2\times CH_3OP$); 3.78 (m, 2H); 3.86 (m, 1H); 3.91 (m, 1H); 4.06 (m, 1H); 4.11 (m, 1H); R_f (A) 0.9.

2-*N*-Acetylamino-2-deoxy-D-glucitol-6-phosphate dimetyl ester (8). To the solution of D-glucosamine-6-phosphate (140 mg, 0.5 mmol) in 0.5 M NaOH (1 mL), acetic anhydride (95 μL, 1 mmol) was added. The reaction mixture was stirred for 2 h at room temperature and then chromatographed over Dovex 50 WX8 (H^+) column. Fractions containing reaction product were pooled and evaporated in vacuo, affording 150 mg of crude 2-*N*-acetylamino-2-deoxy-D-glucose-6-phosphate.

Table 5. Acylation of ADGP — details

Compd	Solvent	Base	Acylating agent	Reaction conditions	Yield (%)
2	Water	$NaHCO_3$	10% Acetic anhydride in acetone, 0.6 mL	40 min, room temp	96
3	Water	$NaHCO_3$	10% Chloroacetic anhydride in acetone, 0.8–1 mL	1 h, room temp	66
4	Water	$NaHCO_3$	Iodoacetic anhydride, 0.2 mL	2 h, 0 °C	49
5	Water/methanol	Triethylamine	Butanoic anhydride, 0.2 mL	2 h, room temp	60
6	Water/methanol	Triethylamine	Hexanoic anhydride, 0.5 mL	2 h, 60 °C	70

This product was dissolved in methanol (5 mL) and an ethyl ether solution of diazomethane was added dropwise to permanent yellow tint. Solvent was distilled off in vacuo affording 165 mg of the crude 2-*N*-acetylamino-2-deoxy-D-glucose-6-phosphate dimethyl ester. This product was purified by silicagel column chromatography in solvent system chloroform–methanol 5:1 (v/v). After evaporation of the solvent, the purified product was dissolved in methanol (2 mL) and $NaBH_4$ (20 mg, 0.52 mmol) was added. The reaction mixture was stirred for 4 h at room temperature and then chromatographed over Dowex 50 WX4 (H^+) ion-exchange resin. Product was eluted with methanol. Solvent was distilled off in vacuo, affording compound **8** (cummulative yield 63%). ^{1}H NMR (MeOD) δ: 1.99 (s, 3H, C**H**$_3$CO); 3.52 (d, 1H, $J=7.3$ Hz); 3.65 (d, 2H, $J=4.88$ Hz); 3.80 (d, 6H, $J=11.2$ Hz, 2×C**H**$_3$OP); 3.84 (m, 1H); 4.02 (m, 1H); 4.04 (t, 1H, $J=4.88$ Hz); 4.14 (m, 1H); 4.27 (m, 1H); R_f (C) 0.2.

2-Deoxy-2-(*N*-benzyloxycarbonylamino)-D-glucopyranose. This compound was prepared as white solid, mp 205–207 °C; $[\alpha]_D^{20}+73$ (*c* 1, Pyr) [lit. 214 °C; $[\alpha]_D^{20}+75.4$ (*c* 3.42, Pyr)], using the described procedure.[25]

Methyl 2-deoxy-2-(*N*-benzyloxycarbonylamino)-D-glucopyranoside (9a). 2-Deoxy-2-(*N*-benzyloxycarbonylamino)-D-glucopyranose (626 mg, 2 mmol) was suspended in methanol (12 mL) saturated with dry HCl. The reaction mixture was stirred for 3 h at 40 °C. Suspension containing 50 mg $PbCO_3$ in 2 mL of water was added. The resulting precipitate was filtered off. The filtrate was concentrated in vacuo, and the residue was crystallised from methanol/diethyl ether mixture. The product **9a** was obtained with 75% yield (490 mg), mp. 138–40 °C, $[\alpha]_D^{22}-4$ (*c* 1, MeOH). ^{1}H NMR (DMSO-d_6) δ: 3.08 (m, 2H, 2×OH); 3.16 (t, 1H, $J=9.1$ Hz, H4); 3.22 (m, 2H, H$_3$ + OH); 3.36 (s, 3H, CH$_3$O); 3.44 (m, 1H, $J=6.1$ Hz, H6); 3.66 (dd, 1H, 6.1 and $J=11.3$ Hz, H6′); 4.12 (d, 1H, $J=8.2$ Hz, H2); 4.52 (*t*, 1H, $J=6.1$ Hz, H5); 4.97 (AB + m, 3H, H1 + C**H**$_2$C$_6$H$_5$); 7.12 (d, 1H, $J=9.1$ Hz, N**H**); 7.34 (m, 5H, C$_6$**H**$_5$).

Benzyl 2-deoxy-2-(*N*-acetylamino)-D-glucopyranoside (10a). To the solution containing 884 mg (4 mmol) of *N*-acetyl-D-glucosamine in dry benzyl alcohol (10 mL), diethyl ether saturated with dry HCl was added (2 mL). The reaction mixture was vigorously stirred for 3 h at 50–60 °C. The crude product was precipitated by addition of benzene (10 mL) and diethyl ether (40 mL). The product was filtered off and crystallised twice from anhydrous ethanol. Product **10a** was obtained as a white solid (930 mg, yield 75%), mp. 183 °C, $[\alpha]_D^{20}+104$ (*c* 1, 94% EtOH); $[\alpha]_D^{20}+112$ (*c* 1, Pyr). ^{1}H NMR (MeOD) δ: 1.95 (s, 3H, C**H**$_3$CO); 3.36 (t, 1H, $J=9.1$ Hz, H4); 3.65–3.72 (m, 3H, H5, H3, H6); 3.81 (dd, 1H, $J=10.6$ Hz, H6′); 3.88 (dt, 1H, $J=10.6$ and 3.3 Hz, H2); 4.50 and 4.75 (AB 2d, 2H, C**H**$_2$C$_6$H$_5$); 4.86 (d, 1H, $J=3.6$ Hz, H1); 7.27–7.39 (m, 5H, C$_6$**H**$_5$); 8.03 (d, $J=8.2$ Hz, N**H**)

6-[Methyl-2-deoxy-2-(*N*-benzyloxycarbonylamino)-D-glucopyranosidyl]diethyl phosphate (9b) and 6-[benzyl-2-deoxy-2-(*N*-acetylamino)-D-glucopyranosidyl]diethyl phosphate (10b). Suspension containing diethyl chlorophosphate (418 mg, 2.4 mmol) and AgCN (5 mg, 0.04 mmol) in anhydrous pyridine (6 mL) was stirred at room temperature. After 30 min benzyl 2-deoxy-2-(*N*-acetylamino)-D-glucopyranoside **10a** (750 mg, 2.4 mmol) or methyl 2-deoxy-2-(*N*-benzyloxycarbonylamino)-D-glucopyranoside **9a** (370 mg, 1.13 mmol) was added and stirring was continued. After 72 h, an excess of pyridine was distilled off in vacuo and the residue was dissolved in water (10 mL) and extracted with chloroform (3×5 mL). The organic layer was washed with water (7 mL), hydrochloric acid (2% in water), and water (7 mL) again, and dried over anhydrous $MgSO_4$. After solvent evaporation the residue was purified on the Silicagel column (Merck 70–270 mesh) with chloroform as mobile phase.

Compound 9b. Yield 40% (200 mg), colourless oil, $[\alpha]_D^{20}-20$ (*c* 0.8, $CHCl_3$), $[\alpha]_D^{20}-135$ (*c* 1, MeOH). ^{1}H NMR ($CDCl_3$) δ: 1.31 (dt, 6H, $J=7.3$ and 10.3 Hz, 2×C**H**$_3$CH$_2$OP); 3.45 (s, 3H, C**H**$_3$O); 3.47 (d, 1H, $J=8.4$ Hz, H4); 3.67 (t, 1H, $J=9.3$ Hz, H3); 3.88 (m, 3H, H2 + 2×OH); 4.10 (qv, 4H, $J=7.3$ Hz, 2×CH$_3$C**H**$_2$OP); 4.24 (ddd, 1H, $J=4.4$, 9.3 and 11.2 Hz, H6); 4.3 (m, 2H, H5 + H6′); 5.08 and 5.10 (AB + m, 3H, H1 + C**H**$_2$C$_6$H$_5$); 5.7 (d, 1H, N**H**); 7.29 (m, 5H, C$_6$**H**$_5$).

Compound 10b. Yield 35% (375 mg), colourless oil, $[\alpha]_D^{20}+63$ (*c* 2.25, EtOH), ^{1}H NMR (MeOD) δ: 1.35 (dt, 6H, 3.0 and $J=7.1$ Hz, 2×CH$_3$C**H**$_2$OP); 1.95 (s, 3H, C**H**$_3$CO); 3.39 (t, 1H, $J=9.2$ Hz, H4); 3.71 (*t*, 1H, $J=9.8$ Hz, H3); 3.80 (*dd*, 1H, $J=4.8$ and 9.8 Hz, H2); 3.89 (m, 1H, H5); 4.14 (qv, 4H, $J=7.1$ Hz, 2×CH$_3$C**H**$_2$OP); 4.21 (m, 1H, H6); 4.28 (m, 1H, H6′); 4.51 and 4.73 (AB, 2H, C**H**$_2$C$_6$H$_5$); 7.09–7.38 (m, 5H, C$_6$H$_5$); 8.06 (d, $J=8.3$ Hz, N**H**).

6-[2-Deoxy-2-(*N*-benzyloxycarbonylamino)-D-glucopyranosidyl]diethyl phosphate (9c). Compound **9a** (87 mg, 0.2 mmol) was suspended in methanol (5 mL) and one drop of hydrochloric acid was added. The reaction mixture was stirred for 4–5 h at 70 °C, then the suspension of $PbCO_3$ (50 mg) in water (2 mL) was added. The resulting precipitate was filtered off. The filtrate was concentrated in vacuo and the residue was purified by ion-exchange chromatography on Dowex 50X W[H^+] column. Compound **9c** was obtained with 60% yield (50 mg), $[\alpha]_D^{22}+20$ (*c* 1.5, H_2O). ^{1}H NMR (MeOD) δ: 1.18 (dt, 6H, $J=7.3$ and 10.3 Hz, 2×C**H**$_3$CH$_2$OP); 3.21–3.95 (m, 7H); 4.05 (qv, 4H, $J=7.3$ Hz, 2×C**H**$_3$CH$_2$OP); 4.18 (m, 2H); 5.08 and 5.10 (AB + m, 3H, H1 + C**H**$_2$C$_6$H$_5$); 7.28 (m, 5H, C$_6$**H**$_5$).

6-[2-Deoxy-2-(*N*-acetylamino)-D-glucopyranosyl]diethyl phosphate (10c). Suspension of compound **10b** (112 mg, 0.254 mmol) and 10% Pd/C (60 mg) in methanol (5 mL) was saturated with H_2 for 6 h at room temperature. The catalyst was then filtered off and the solvent was distilled off in vacuo. Compound **10c** was obtained as a colourless oil with 90% yield (80 mg), $[\alpha]_D^{20}+22.9$ (*c* 1, EtOH). ^{1}H NMR (MeOD) δ: 1.35 (t, 6H, $J=7$ Hz, 2×C**H**$_3$CH$_2$OP); 2.03 (s, 3H, C**H**$_3$CO); 3.38 (t, 1H,

$J=9.2$ Hz, H4); 3.73 (t, 1H, $J=9.6$ Hz, H3); 3.85 (dd, 1H, 2.9 and $J=10.8$ Hz, H2); 3.97 (m, 1H, H5); 4.14 (qv, 4H, $J=7.2$ Hz, $2\times CH_3C\mathbf{H}_2OP$); 4.21 (m, 1H, H6); 4.27 (m, 1H, H6′); 5.10 (d, 1H, $J=2.9$ Hz, H1).

2-Amino-2-deoxy-D-glucitol-6-phosphate diethyl ester (9), 2-(*N*-acetylamino)-2-deoxy-D-glucitol-6-phosphate diethyl ester (10) and 2-(*N*-acetylamino)-2-deoxy-D-glucitol-6-phosphate ethyl ester (11). Solution of compound **9c** (50 mg, 0.12 mmol) or **10c** (53 mg, 0.15 mmol) in ethanol (4 mL) was cooled to 0 °C and $NaBH_4$ (35 mg) was added. The reaction mixtures were stirred for 2 h at room temperature (for preparation of compounds **9d** or **10**, respectively) or overnight for preparation of compound **11** from **10c**. The resulting solutions were chromatographed over the ion-exchange resin Amberlit IR-120 [H^+]. Fractions containing either of the products were pooled, combined and evaporated in vacuo with methanol (2×10 mL).

Compound 9d. Yield 65% (34 mg), colourless oil, ^{1}H NMR (D_2O) δ: 1.12 (qv, 3H, $J=7.3$ Hz, $C\mathbf{H}_3CH_2OP$); 1.20 (qv, 3H, $J=7.3$ Hz, $C\mathbf{H}_3CH_2OP$); 3.48 (m, 1H); 3.52 (m, 1H); 3.59 (m, 2H); 3.7 (m, 2H); 3.8 (m, 3H, $C\mathbf{H}_3CH_2OP+H_{glucitol}$); 3.92 (m, 1H); 4.2 (m, 2H, $CH_3C\mathbf{H}_2OP$); 5.0 (AB, 2H, $C\mathbf{H}_2C_6H_5$); 7.25 (s, 5H, $C_6\mathbf{H}_5$).

Compound 10. Yield 60% (32 mg), colourless oil, ^{1}H NMR (D_2O) δ: 1.12 (qv, 3H, $C\mathbf{H}_3CH_2OP$); δ: 1.18 (qv, 3H, $C\mathbf{H}_3CH_2OP$); 1.87 (s, 3H, $C\mathbf{H}_3CO$); 3.48 (m, 2H); 3.57 (m, 1H); 3.79 (m, 1H); 3.81 (m, 4H, $CH_3C\mathbf{H}_2OP+2H_{glucitol}$); 3.91 (m, 1H); 4.04 (m, 3H, $CH_3C\mathbf{H}_2OP+H_{glucitol}$).

Compound 11. Yield 40% (13 mg), colourless oil, $[\alpha]_D^{20}-5$ (*c* 0.4, EtOH), ^{1}H NMR (MeOD) δ: 1.1 (dt, 3H, 2.9 and $J=6.8$ Hz, $C\mathbf{H}_3CH_2OP$); 1.87 (s, 3H, $C\mathbf{H}_3CO$); 3.48 (m, 2H); 3.57 (dd, 1H, $J=11.3$ and 4.8 Hz); 3.79 (m, 1H); 3.81 (m, 4H, $CH_3C\mathbf{H}_2OP+2H_{glucitol}$); 3.91 (dd, 1H, $J=4.8$ and 6.3 Hz); 4.04 (t, 1H, $J=7.3$ Hz).

Compound **9d** was dissolved in ethanol (5 mL) and 10% Pd/C (30 mg) was added. The suspension was saturated with H_2 for 2 h at room temperature. Then the catalyst was filtered off, and the solvent was evaporated in vacuo to give **9** as a colourless oil with 55% yield (19 mg). ^{1}H NMR (D_2O) δ: 1.12 (qv, 3H, $J=7.3$ Hz, $C\mathbf{H}_3CH_2OP$); 1.20 (qv, 3H, $J=7.3$ Hz, $CH_3C\mathbf{H}_2OP$); 3.48 (m, 1H); 3.52 (m, 1H); 3.59 (m, 2H); 3.7 (m, 2H); 3.8 (m, 3H, $CH_3C\mathbf{H}_2OP+H_{glucitol}$); 3.92 (m, 1H); 4.2 (m, 2H, $CH_3C\mathbf{H}_2OP$).

Determination of the affinity to the artificial biological membrane

Interactions between examined compounds and immobilised artificial biological membrane were investigated with the use of a HPLC column IAM PC DD2 (Regis Techologies, Inc., Morton Grove, IL, USA), containing as a stationary phase 1-myristoyl-2-[13-carbonylimidazolide-tridecanoyl]-*sn*-3-glycerophosphocholine (lecithinimidazolide) bonded to silica-propylamine, with the unreacted propylamine moieties end-capped with C_{10} and C_3 alkyl chains. The column dimensions were 3 cm×4.6 mm, particle diameter 12 μm and pore diameter 300 Å. For all studies the injection volume was ca. 10 μL of a solute aqueous solution. The standard solvent system was acetonitrile/50 mM potassium-phosphate buffer pH 7.2 (50:50, 30:70, 20:80, 10:90, 5:95 v/v) containing 0.1% acetone. The flow rate was 1 mL min^{-1} and solute detection was at 495 nm. The chromatographic system consisted of a Model L-6200 A pump, a Model L-4250 UV/VIS detector and a Model D-2500 chromatointegrator (Merck-Hitachi, Vienna, Austria). The dead volume of the column was determined by the t_0 value, i.e., retention time of citric acid (applied as an aqueous solution, 50 mg mL^{-1}). Retention times of the studied compounds (t_R) were determined for different compositions of the mobile phase and used to calculate the capacity parameters, $k'_{IAM}=(t_r-t_0)/t_0$. Values of log k'_{IAM} were plotted against the acetonitryle content in the mobile phase and the resulting linear plots were extrapolated to give the log k^0_{IAM} value for the hypothetical purely aqueous mobile phase.

Determination of enzyme activity

GlcN-6-P synthase activity was determined according to the modified Elson-Morgan method described previously.[26] GlcN-6-P *N*-acetylase activity was assayed in solution containing 5 mM phosphate buffer pH 6.9, 8 mM glucosamine-6-phosphate, 2 mM acetyl-coenzyme A, appropriately diluted *C. albicans* cell-free extract and enzyme inhibitor in appropriate concentration. The reaction was carried out at 30 °C for 30 min and stopped by heating. Concentration of the *N*-acetyl-D-glucosamine-6-phosphate formed was determined as described previously.[26] Phosphoglucose isomerase activity was measured according to the procedure described by Noltmann et al.[27,28] using the yeast enzyme preparation (Sigma-Aldrich Chemical Co).

Determination of antifungal in vitro activity

Clinical strains of different *Candida* species were from the collection of the Department of Clinical Microbiology, Medical Academy of Gdańsk. *Candida* clinical and reference strains and *S. cerevisiae* ATCC 9763 (American Type Culture Collection, Manassas, Va, USA) were stored on Sabouraud Agar slants. Fungal *inocula* were grown in Sabouraud broth at 30C with shaking. MICs were determined by a serial dilution microtitrer plate method in Yeast Nitrogen Base medium containing 1% glucose as a carbon source. Wells containing serially diluted compound and control wells were inoculated with 10^5 cells mL^{-1} of an overnight culture of fungal cells and incubated for 24 h at 30 °C. MIC was defined as the lowest antifungal agent concentration preventing visible growth. Alternatively, MICs were determined in RPMI 1640 medium buffered with 3-[*N*-morpholino]propanesulfonic acid (MOPS) to pH 7.0, under conditions recommended by NCCLS.[29] In all cases, reproducible sharp end points were obtained and trailing effects were not observed.

Uptake studies

C. albicans ATCC 10261 cells, grown exponentially in YNB medium at 30C, were harvested, washed with saline and re-suspended in 50 mM Tris/HCl buffer, pH 6.5 containing 1% glucose to the final cell density of 10^8 cells mL^{-1} (about 1.0 mg dry weight cells mL^{-1}). The suspension was pre-incubated at 30 °C for 10 min, investigated compounds (**1**, **2** or **7**) dissolved in a minimal buffer aliquot were added to the final concentration of 5 mg mL^{-1} and the incubation was continued. At that moment and after 5, 10, 15, 30, 60 and 90 min, 100-μL samples were withdrawn and diluted 1:100. The diluted suspensions were immediately filtered through GF/C glass fibre filters (Whatman International Ltd., Maidstone, UK) under suction and filtrates were collected. Concentration of all investigated compounds was measured by determination of phosphate (V) ester concentration with Fiske Subba Row reagent.[30] Data were plotted as nanomoles of ADGP or its derivative taken up by 1 mg (dry weight) of cells versus time. The initial uptake velocities were determined from the slopes of the linear part of the curves originating at 0.0 point and then re-plotted against the initial compound concentration.

Investigation of metabolism of ADGP derivatives in a cell-free extract or by *C. albicans* cells

A concentrated solution of compound **1**, **2** or **7** was added to the cell-free extract, prepared from *C. albicans* ATCC 10261 cells as described previously, to give a final concentration of 5 mg mL^{-1}. The mixture was incubated at 30 °C. Samples of 1 mL were collected at hourly intervals and de-proteinised by addition of 1 mL of ethanol. The protein precipitate was removed by filtration and the composition of the filtrate was determined by TLC analysis of the filtrates (solvent system A). Alternatively, *C. albicans* ATCC 10261 cells were grown in YNB liquid medium at 30 °C in the presence or absence of an investigated compound, 1 mg mL^{-1}. The compound was added when the density of the cell suspension measured at $\lambda = 660$ nm reached 0.6. At hourly time intervals samples of the cell suspension were collected, cells were harvested by centrifugation and broken by the small-scale glass beads procedure.[31] Crude extracts were analysed TLC (solvent system A, spots visualised with ninhydrin or Hanes reagent).

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