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Note

N-Alkyl derivatives of 2-amino-2-deoxy-D-glucose

Beata Liberek,[a,*] Anna Melcer,[a] Anna Osuch,[a] Roland Wakieć,[b] Sławomir Milewski[b] and Andrzej Wiśniewski[a]

[a]*Faculty of Chemistry, University of Gdańsk, Sobieskiego 18, PL-80-952 Gdańsk, Poland*
[b]*Department of Pharmaceutical Technology and Biochemistry, Gdańsk University of Technology, Narutowicza 11/12, 80-952 Gdańsk, Poland*

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Abstract—Mono- and di-*N*-alkylated derivatives of 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucose (alkyl = methyl, ethyl, propyl, butyl, pentyl, hexyl, benzyl) were synthesised by the reductive alkylation of per-*O*-acetyl-D-glucosamine. (*N*-ethyl, *N*-propyl, *N*-butyl, *N*-pentyl and *N*-hexyl)-1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucoses were deacetylated in order to attempt an enzymatic phosphorylation. All products were characterised by means of IR, NMR and MS spectra. *N*-Ethyl- and *N*-pentyl-D-glucosamines were found to exhibit weak antifungal activity.

Keywords: D-Glucosamine; Reductive alkylation; Deacetylation; NMR analysis; Enzymatic phosphorylation; Antifungal activity

D-Glucosamine-6-phosphate (GlcN-6-P) synthase, an enzyme catalysing the first committed step in the chitin biosynthesis pathway, is a potential target in antifungal chemotherapy.[1] A structural analogue of the transition state *cis*-enolamine intermediate, 2-amino-2-deoxy-D-glucitol-6-phosphate (ADGP) specifically inhibits GlcN-6-P synthase.[2] Unfortunately, despite its strong enzyme inhibitory potency, this compound exhibits weak antifungal activity owing to its very slow uptake.[3] On the other hand, *N*-acyl and ester derivatives of ADGP were found to be much weaker inhibitors of GlcN-6-P synthase than the parent compound. Some of them, however, did exhibit a more pronounced antifungal activity.[4] Our molecular modelling studies on enzyme–inhibitor interactions have indicated that introduction of alkyl functional groups at the nitrogen atom of ADGP should not affect the bonding of the thus obtained *N*-alkyl derivatives of ADGP at the enzyme active centre, in comparison to the parent molecule (Wojciechowski M., et al., unpublished data). One may therefore expect that the strong enzyme inhibitory properties of *N*-alkyl derivatives of ADGP could be combined with good antifungal activity, because the charge distribution is similar to and the lipophilicity better than that of the parent ADGP molecule.

One of the pathways that we examined in order to obtain *N*-alkyl derivatives of ADGP involved *N*-alkylation of 2-amino-2-deoxy-D-glucose followed by an enzymatic phosphorylation and reduction (Scheme 1). Previously reported preparations of alkylated D-glucosamine derivatives include the reduction of the amido function,[5,6] nucleophilic cleavage of the oxazolidine ring[7] or reductive alkylation.[8] None of these *N*-alkylations satisfies our needs. On the other hand, reductive alkylation of amines is one of the convenient methods for secondary and tertiary amine formation.[9–12] In this method, a primary amine is treated with an aldehyde or a ketone in the presence of a suitable reducing agent (most often $NaCNBH_3$). Unfortunately, application of this strategy to the direct alkylation of 2-amino-2-deoxy-D-glucose turned out to be impossible, since reduction of the aldehyde group in the open-chain form of D-glucosamine occurred and 2-amino-2-deoxy-D-glucitol was formed under the reaction conditions ($NaCNBH_3$,

* Corresponding author. Tel.: +48 58 3450344; fax: +48 58 3410357; e-mail: beatal@chemik.chem.univ.gda.pl

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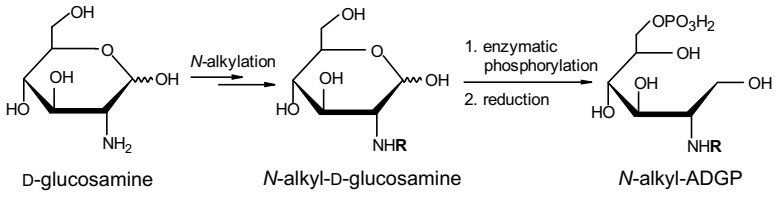
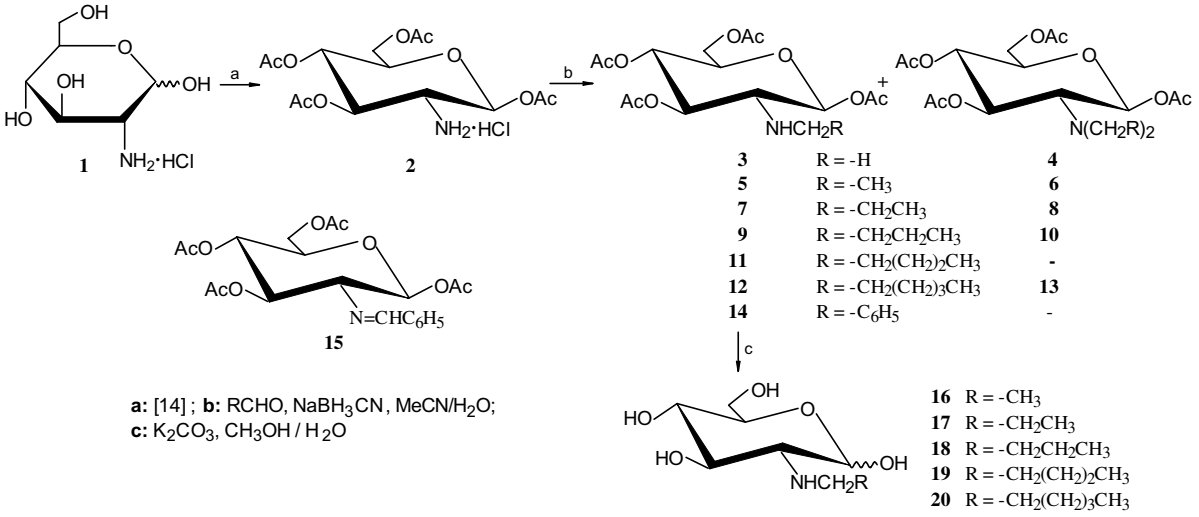


Scheme 1.

$CH_3CN–H_2O \sim 3:1$.[13]) To overcome this difficulty a procedure was applied involving the reductive alkylation of 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucose followed by deacetylation. $NaCNBH_3$ was chosen as reducing agent since the reductive alkylation of 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucose with other agents [H_2/Pd, $NaBH_4$ or $NaBH(OAc)_3$] was unsuccessful.

1,3,4,6-Tetra-*O*-acetyl-2-amino-2-deoxy-D-glucose hydrochloride (**2**, Scheme 2) was prepared from 2-amino-2-deoxy-D-glucose hydrochloride (**1**) according to the described procedure.[14] Reductive alkylation of **2** with the appropriate aldehydes was performed using a twofold molar excess of aldehyde and a threefold molar excess of $NaCNBH_3$ with respect to **2**, and was completed within 30 min. The reaction was not selective under these conditions and in most cases a mixture of mono- (**3**, **5**, **7**, **9**, **11**, **12** and **14**) and dialkylated products (**4**, **6**, **8**, **10** and **13**) was obtained. However, reaction of **2** with valeric aldehyde and benzaldehyde yielded only monoalkylated glucosamines (**11** and **14**). As expected, the longer the alkyl residue of an aldehyde, the smaller the quantity of dialkylated product (37% for **4** and 7% for **13**). Reaction of **2** with benzaldehyde afforded a mixture of the expected *N*-benzylamino- (**14**) but also the unexpected *N*-benzylideneamino derivatives (**15**). Isolation of **15** confirms the formation of an intermediate carbinol amine, which undergoes dehydration to yield the imine **15** following reductive alkylation.[10] This also provides evidence for the much greater stability of the aromatic imines in comparison with the aliphatic ones.

The structures of the synthesised *N*-alkyl and *N*,*N*-dialkyl derivatives of 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucose were confirmed by IR, ^1H and ^{13}C NMR spectroscopy (Tables 1–5), as well as mass spectrometry. Analysis of the NMR spectra of compounds **3**–**14** permitted the conclusion that the length of the alkyl chain does not affect the chemical shifts of the sugar ring protons or carbons. These are almost identical in the group of monoalkyl (Tables 1 and 3) or dialkyl (Tables 2 and 4) derivatives of D-glucosamine. The only exception can be seen in the NMR spectra of compounds **3** (the smallest alkyl chain) and **14** (a sterically and electronically different alkyl group). Interestingly, the number of alkyl groups has a significant influence on the resonance frequency of the sugar ring protons and carbons. The ^1H and ^{13}C NMR spectra of mono- and dialkyl compounds, which carry the same alkyl group, are clearly different. The presence of two alkyl groups causes deshielding of the H-1 ($\Delta\delta \sim 0.15$ ppm), H-2 ($\Delta\delta \sim 0.1$ ppm) and H-3 ($\Delta\delta \sim 0.2$ ppm) protons in comparison with the corresponding protons of the monoalkyl products. Conversely, the second alkyl group screens the C-1 ($\Delta\delta \sim 2.5$ ppm) and C-3 ($\Delta\delta \sim 2.8$ ppm)



Scheme 2.

Table 1. Chemical shifts (ppm) of the protons in the ^{1}H NMR spectra ($CDCl_3$) of **3**, **5**, **7**, **9**, **11**, **12**, **14** and **15**

	H-1	H-2	H-3	H-4	H-5	H-6	H-6′	OAc	NCH_2	CH_2	CH_3	Ph
3	5.88 (d) 1H	3.26 (t) 1H	5.44 (t) 1H	5.06 (t) 1H	3.89 (br d) 1H	4.31 (dd) 1H	4.10 (dd) 1H	2.22 2.12 2.08 2.04 (4s) 12H	—	—	2.36 (s) 3H	—
5	5.55 (d) 1H	2.85 (dd) 1H	(m) 2H	5.06	3.77 (ddd) 1H	4.31 (dd) 1H	4.07 (dd) 1H	2.16 2.08 2.03 (3s) 12H	2.74 (dq) 2.64 (dq) 2H	—	1.00 (t) 3H	—
7	5.55 (d) 1H	2.84 (dd) 1H	(m) 2H	5.06	3.77 (ddd) 1H	4.31 (dd) 1H	4.07 (dd) 1H	2.16 2.08 2.03 (4s) 12H	2.65 (dt) 2.57 (dt) 2H	1.38 (sex) 2H	0.86 (t) 3H	—
9	5.54 (d) 1H	2.84 (dd) 1H	(m) 2H	5.05	3.77 (ddd) 1H	4.30 (dd) 1H	4.07 (dd) 1H	2.16 2.08 2.03 (4s) 12H	2.64 (dt) 2.59 (dt) 2H	1.31 (m) 4H	0.88 (t) 3H	—
11	5.55 (d) 1H	2.84 (dd) 1H	(m) 2H	5.06	3.77 (ddd) 1H	4.31 (dd) 1H	4.07 (dd) 1H	2.16 2.08 2.03 (3s) 12H	2.67 (dt) 2.58 (dt) 2H	1.31 (m) 6H	0.88 (t) 3H	—
12	5.54 (d) 1H	2.84 (dd) 1H	(m) 2H	5.06	3.77 (ddd) 1H	4.31 (dd) 1H	4.07 (dd) 1H	2.16 2.08 2.03 (4s) 12H	2.67 (dt) 2.59 (dt) 2H	1.30 (m) 8H	0.88 (t) 3H	—
14	5.60 (d) 1H	2.94 (dd) 1H	5.09 (t) 1H	5.04 (t) 1H	3.78 (ddd) 1H	4.31 (dd) 1H	4.07 (dd) 1H	2.14 2.08 2.03 2.01 (4s) 12H	3.89 (d) 3.79 (d) 2H	—	—	7.20 (m) 5H
15	5.97 (d) 1H	3.50 (dd) 1H	5.46 (t) 1H	5.16 (t) 1H	3.99 (ddd) 1H	4.40 (dd) 1H	4.14 (dd) 1H	2.11 2.05 2.03 1.89 (4s) 12H	8.23 (s) 1H	—	—	7.72 (d) 2H 7.44 (m) 3H

carbon atoms and deshields carbon atom C-2 ($\Delta\delta \sim 2$ ppm) in comparison with the corresponding carbons of the monoalkyl derivatives.

Neither the length nor the number of alkyl groups has any influence on the conformation of the alkylated products. All of them (**3**–**14**), as well as by-product **15**, adopt the $^4C_1$ form, as demonstrated by the respective coupling constants (Table 5).

It is worth noting that the protons of the methylene group bound to the nitrogen atom in *N*-alkyl derivatives of 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucose are diastereotopic. Thus, they absorb at different fields and are coupled with geminal coupling constant. Therefore, the signal of each proton is a doublet of quartets (**5** and **6**), a doublet of triplets (**7**–**13**) or a doublet (**14**).

In order to obtain monoalkyl derivatives of D-glucosamine, 1,3,4,6-tetra-*O*-acetyl-2-(*N*-alkylamino)-2-deoxy-β-D-glucoses (**5**, **7**, **9**, **11** and **12**) were subjected to de-*O*-acetylation. Such a reaction is difficult in the case of D-glucosamine derivatives, since acetyl groups readily migrate to the nitrogen atom and tend to stay there. Additionally, methyl acetate, one of the products of deprotection carried out in methanol solution, may react with the amino group of D-glucosamine. Thus, after deacetylation, a mixture of products was always obtained, irrespective of whether the reaction was

Table 2. Chemical shifts (ppm) of the protons in the ^{1}H NMR spectra ($CDCl_3$) of **4**, **6**, **8**, **10** and **13**

	H-1	H-2	H-3	H-4	H-5	H-6	H-6′	OAc	NCH_2	CH_2	CH_3
4	5.75 (d) 1H	2.82 (dd) 1H	5.25 (dd) 1H	5.04 (t) 1H	3.72 (ddd) 1H	4.29 (dd) 1H	4.06 (dd) 1H	2.16 2.08 2.05 2.03 (4s) 12H	—	—	2.38 (s) 6H
6	5.74 (d) 1H	2.95 (dd) 1H	5.23 (dd) 1H	5.05 (t) 1H	3.72 (ddd) 1H	4.30 (dd) 1H	4.06 (dd) 1H	2.14 2.08 2.04 2.03 (4s) 12H	2.64 (dq) 2.55 (dq) 4H	—	0.96 (t) 6H
8	5.72 (d) 1H	2.97 (dd) 1H	5.25 (dd) 1H	5.03 (t) 1H	3.73 (ddd) 1H	4.31 (dd) 1H	4.05 (dd) 1H	2.15 2.08 2.04 2.02 (4s) 12H	2.52 (dt) 2.46 (dt) 4H	1.36 (sex) 4H	0.82 (t) 6H
10	5.70 (d) 1H	2.97 (dd) 1H	5.24 (dd) 1H	5.03 (t) 1H	3.73 (ddd) 1H	4.31 (dd) 1H	4.06 (dd) 1H	2.14 2.08 2.03 2.02 (4s) 12H	2.55 (dt) 2.48 (dt) 4H	1.28 (m) 8H	0.89 (t) 6H
13	5.70 (d) 1H	2.98 (dd) 1H	5.24 (t) 1H	5.03 (t) 1H	3.73 (ddd) 1H	4.31 (dd) 1H	4.05 (dd) 1H	2.14 2.08 2.03 2.02 (3s) 12H	2.54 (dt) 2.48 (dt) 4H	1.28 (m) 16H	0.88 (t) 6H

carried out in NaOMe in methanol, $(CH_3)_2NH$ in methanol or K_2CO_3 in methanol–water solution.[15] Applying potassium carbonate in the de-*O*-acetylation reaction enabled the desired *N*-alkyl-D-glucosamines with the best yields to be obtained within 30 min. Prolonging the deacetylation time led to the formation of more byproducts.

2-(*N*-Alkylamino)-2-deoxy-D-glucoses were obtained as mixtures of anomers, in which the thermodynamically more stable α stereoisomer predominated. Their structures were established on the basis of IR, ^{1}H and ^{13}C NMR spectroscopy (Tables 6–8) and mass spectrometry. The α:β ratio was determined by comparison of the areas of the H-1 peaks of the respective anomers in the ^{1}H NMR spectra. All the *N*-alkyl-D-glucosamines (**16**–**20**) adopt the $^4C_1$ conformation, as indicated by the relevant coupling constants (Table 8).

Analysis of the NMR spectra of **16**–**20** drew our attention to the empirical rules in the NMR spectroscopy of carbohydrates. Firstly, the H-1 signal of α anomers appears at higher δ values than the H-1 signal of β anomers, owing to their different equatorial and axial orientations ($\Delta\delta \sim 0.7$ ppm). Next, the axial orientation of the anomeric OH group in the α anomers causes absorption at lower fields of axially oriented H-2 and H-3 protons in comparison with the corresponding β anomers ($\Delta\delta \sim 0.4$ ppm). The C-1 carbon atom of the β anomers resonates at lower fields than the C-1 carbon atom of the α anomers ($\Delta\delta \sim 7$ ppm). Moreover, the C-2 carbon atom of the β anomers is deshielded in comparison with the corresponding C-2 carbon atom of the α anomers ($\Delta\delta \sim 3$ ppm).

The protons of the methylene group bound to the nitrogen atom in *N*-alkyl-D-glucosamines (**16**–**20**) are diastereotopic, as in the *O*-acetyl-*N*-alkyl-D-glucosamines (**4**–**14**). However, in the case of **18**–**20**, they behave as if they were chemically and magnetically equivalent and appear in one position as triplets.

Systematic attempts were made to carry out the enzymatic phosphorylation of 2-(*N*-alkylamino)-2-deoxy-D-glucoses (**16**–**20**) in order to obtain *N*-alkyl-D-glucosamine-6-phosphates. Unfortunately, none of the expected products were formed during incubation of compounds **16**–**20** with ATP in the presence of yeast hexokinase. Under the same conditions, D-glucosamine and D-mannosamine were efficiently phosphorylated by the enzyme but *N*-acetyl-D-glucosamine was not. It is known that yeast hexokinase has a relatively broad

Table 3. Chemical shifts (ppm) of the carbon atoms in the ^{13}C NMR spectra ($CDCl_3$) of **3**, **5**, **7**, **9**, **11**, **12**, **14** and **15**

	C-1	C-2	C-3	C-4	C-5	C-6	OAc		NCH_2/N=C	CH_2	CH_3	Ph
							C=O	CH_3				
3	90.49	57.66	70.95	68.20	72.71	61.46	171.25 170.78 169.80 169.20	21.17 21.10 20.90 20.77	—	—	41.79	—
5	95.00	60.91	74.14	68.52	72.66	61.99	171.07 170.92 169.90 169.40	21.29 21.01 20.97 20.88	42.63	—	16.10	—
7	94.95	61.10	74.08	68.55	72.67	61.99	171.04 170.92 169.90 169.39	21.28 21.01 20.97 20.87	50.11	23.77	11.63	—
9	95.00	61.16	74.12	68.52	72.66	61.99	171.02 170.91 169.90 169.39	21.27 20.98 20.96 20.86	47.92	32.83 20.19	14.06	—
11	95.00	61.14	74.10	68.54	72.67	61.99	171.02 170.92 169.91 169.39	21.29 21.00 20.98 20.88	48.23	30.45 29.31 22.70	14.25	—
12	95.00	61.14	74.10	68.53	72.67	61.99	171.02 170.92 169.91 169.39	21.29 21.01 20.98 20.88	48.25	31.86 30.73 26.82 22.84	14.26	—
14	95.34	60.33	74.15	68.42	72.68	61.93	171.15 170.92 169.86 169.37	21.29 20.98 20.86	52.42	31.86 30.73 26.82 22.84	—	128.70 128.25 127.41
15	93.26	73.30 73.18		68.18	72.99	61.99	170.93 170.13 169.76 168.97	21.03 21.00 20.91 20.73	165.36	—	—	135.51 131.76 128.91 128.79

spectrum of substrate specificity,[16] although D-glucose is a primary substrate of this enzyme. Our studies show that hexokinase-catalysed phosphorylation of *N*-substituted D-glucosamines is not possible. On looking for the most likely explanation for this, it should be noted that the charge status of the amino groups in D-glucosamine and *N*-alkyl-D-glucosamines is the same. One may therefore assume that an additional alkyl group in **16**–**20** introduces a steric hindrance and in this way precludes the desired substrate–enzyme interactions. Additionally, substitution of a proton by an alkyl group in the amino group of **16**–**20** may result in the lack of proper H-bonding between substrate and enzyme.

All *N*-alkyl-D-glucosamines were tested for in vitro antifungal activity using the microtitre serial dilution method. *N*-Ethyl- (**16**) and *N*-pentyl-D-glucosamines (**19**) were found to inhibit the growth of *S. cerevisiae* ATCC 9763 and *C. albicans* ATCC 10261 cells at 10 mg/mL. No growth inhibition was found with the other compounds or with the commercially available D-glucosamine. The mechanism of the observed antifungal effect is to be the subject of our further studies.

1. Experimental

1.1. General methods

Melting points were uncorrected. The IR spectra were recorded as Nujol mulls with a Bruker IFS 66 spectrophotometer. The ^1H and ^{13}C NMR spectra ($CDCl_3$, DMSO or CD_3OD, internal Me_4Si) were measured with a Varian Mercury 400 (400.49/100.70 MHz) instrument. Positive-ion mode MALDITOF mass spectra were obtained using a Bruker Biflex III spectrometer with 4-cyano-4-hydroxycinnamic matrix. Thin-layer chromatography (TLC) was performed on the E. Merck Kieselgel 60 F-254 plates using the following eluent systems (v/v): A, 1:2 toluene–AcOEt; B, 3:1 $CHCl_3$–MeOH; C, 1:1

Table 4. Chemical shifts (ppm) of the carbon atoms in the ^{13}C NMR spectra ($CDCl_3$) of **4**, **6**, **8**, **10** and **13**

	C-1	C-2	C-3	C-4	C-5	C-6	OAc		NCH_2	CH_2	CH_3
							C=O	CH_3			
4	92.03	66.16	70.82	69.10	72.60	62.06	170.94	21.49	—	—	41.77
							170.37	21.20			
							169.96	20.96			
							169.17	20.87			
6	92.60	62.91	71.18	69.12	72.63	62.06	170.94	21.35	42.59	—	14.99
							170.24	21.10			
							169.95	20.98			
							169.14	20.90			
8	92.52	63.30	71.34	69.28	72.43	62.00	170.97	21.37	53.21	22.61	11.64
							170.29	21.15			
							170.02	20.98			
							169.25	20.88			
10	92.57	63.24	71.37	69.36	72.47	62.06	170.97	21.35	51.07	31.76	14.27
							170.25	21.10		20.34	
							170.02	20.97			
							169.21	20.88			
13	92.54	63.18	71.34	69.33	72.43	62.03	170.96	21.35	51.34	32.03	14.27
							170.22	21.12		29.54	
							170.02	20.95		26.95	
							169.19	20.86		22.93	

Table 5. The ^1H–^1H coupling constants (Hz) for compounds **3**–**15**

	Sugar							Alkyl	
	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,6'}$	$J_{6,6'}$	J_{gem}	J_{vic}
3	8.8	9.6	9.6	9.6	4.0	[a]	12.4	—	—
4	8.8	10.8	9.2	10.0	4.8	2.0	12.4	—	—
5	8.4	10.4	[a]	9.6	4.8	2.0	12.4	11.6	7.0
6	8.8	10.8	9.2	10.0	4.8	2.4	12.4	13.6	6.8
7	8.4	10.0	[a]	[a]	4.8	2.0	12.4	11.6	6.8
8	8.8	10.8	9.2	10.4	4.4	2.0	12.4	12.8	7.2
9	8.8	10.4	[a]	10.0	4.8	2.0	12.4	11.6	6.8
10	8.8	10.4	9.2	10.4	4.8	2.0	12.4	13.3	6.6
11	8.8	10.4	[a]	[a]	4.4	2.0	12.4	11.6	6.8
12	8.8	10.4	[a]	10.0	4.8	2.4	12.4	11.6	6.8
13	8.8	10.8	9.2	10.4	4.4	2.0	12.4	13.6	6.8
14	8.8	10.0	9.2	9.6	4.4	2.0	12.4	13.6	—
15	8.4	9.6	9.6	10.0	4.4	2.0	12.4	—	—

[a] Not determined.

$CHCl_3$–MeOH, D, 5:3:1:1 *i*-ProOH-25% NH_3aq–H_2O–AcOH. Column chromatography was performed on MN Kieselgel 60 (<0.08 mm).

1.2. 1,3,4,6-Tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucose HCl (2)

Prepared according to the literature procedure.[14] Spectral data for **2**: IR: *v* 3435 (N–H), 2924, 2853 (C–H), 1760, 1747 (ester C=O), 1368 (ester CH_3), 1248, 1208 (ester C–O) cm^{-1}; ^1H NMR (DMSO): δ 8.87 (br s, 3H, NH_3^+), 5.93 (d, 1H, $J_{1,2}$ 8.8 Hz, H-1), 5.37 (dd, 1H, $J_{3,4}$ 9.6 Hz, H-3), 4.93 (t, 1H, $J_{4,5}$ 9.6 Hz, H-4), 4.19 (dd, 1H, $J_{6,6'}$ 12.8 Hz, H-6), 4.03 (ddd, 1H, $J_{5,6}$ 4.8, $J_{5,6'}$ 2.4 Hz, H-5), 4.00 (dd, 1H, H-6′), 3.54 (dd, 1H, $J_{2,3}$ 10.0 Hz, H-2), 2.17, 2.03, 1.99, 1.97 (4s, 12H, OAc); ^{13}C NMR (DMSO): δ 169.88, 169.69, 169.24, 168.56 (4C=O), 90.05 (C-1), 71.56 (C-5), 70.29 (C-3), 67.79 (C-4), 61.22 (C-6), 52.11 (C-2), 20.88, 20.79, 20.41, 20.27 ($4CO\mathit{CH}_3$).

1.3. General procedure for *N*-alkylation

1,3,4,6-Tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucose HCl (**2**) (500 mg, 1.3 mmol) and appropriate aldehyde (2.6 mmol) were stirred in the mixture of acetonitrile and water (3:1, 56 mL) at rt for 30 min. Then $NaCNBH_3$ (240 mg, 3.8 mmol) was added and stirring was continued for 30 min at rt. The end of the reaction was detected by TLC (solvent A). The mixture was diluted with water (30 mL) and extracted with $CHCl_3$ (3×50 mL). The $CHCl_3$ extract was dried over Na_2SO_4,

Table 6. Chemical shifts (ppm) of the protons in the ^{1}H NMR spectra (CD_3OD) of **16**–**20**

	$H\text{-}1_\alpha$	$H\text{-}1_\beta$	$H\text{-}2_\alpha$	$H\text{-}2_\beta$	$H\text{-}3_\alpha$	$H\text{-}3_\beta$	$H\text{-}4_\alpha$	$NCH_{2\alpha}$	$NCH_{2\beta}$	$CH_{2\alpha}$	$CH_{3\alpha}$	$CH_{3\beta}$
16	5.32 (d) 1H	4.57 (d) 1H	2.79 (dd) 1H	2.39 (dd) 1H	3.72 (m) 1H	3.30 (m) 1H	[a]	2.97 (dq) 2.94 (dq) 2H	2.90 (m) 2H	—	1.22 (t) 3H	1.15 (t) 3H
17	5.40 (d) 1H	4.70 (d) 1H	2.99 (dd) 1H	2.56 (dd) 1H	3.84 (dd) 1H	3.45 (dd) 1H	3.37 (t) 1H	3.01 (m) 2H	2.98 (m) 2H	1.69 (m) 2H	1.00 (t) 3H	0.98 (t) 3H
18	5.35 (d) 1H	4.60 (d) 1H	2.85 (dd) 1H	2.42 (dd) 1H	3.77 (m) 1H	3.37 (t) 1H	3.38 (t) 1H	2.96 (t) 2H	[a]	1.62 (m) 2H 1.41 (sex) 2H	0.97 (t) 3H	0.92 (t) 3H
19	5.34 (d) 1H	4.60 (d) 1H	2.86 (dd) 1H	2.42 (dd) 1H	3.76 (m) 1H	[a]	3.35 (t) 1H	2.94 (t) 2H	[a]	1.59 (m) 2H 1.37 (m) 4H	0.94 (t) 3H	[a]
20	5.37 (d) 1H	4.65 (d) 1H	2.93 (dd) 1H	2.48 (dd) 1H	3.81 (t) 1H	3.36 (t) 1H	[a]	2.99 (t) 2H	[a]	1.63 (m) 2H 1.35 (m) 6H	0.92 (t) 3H	[a]

[a] Not determined.

Table 7. Chemical shifts (ppm) of the carbon atoms in the ^{13}C NMR spectra (CD_3OD) of **16**–**20**

	C-1	C-2	C-3, C-4, C-5	C-6	NCH	CH_2	CH_3
16	91.08 (α) 98.48 (β)	62.77 (α)	73.20–72.26	62.70 (α)	42.64 (α) 44.52 (β)	—	13.64 (α) 14.99 (β)
17	90.21 (α) 96.88 (β)	62.35 (α)	73.27–71.97	62.49 (α)	48.96 (α) 49.09 (β)	21.32	11.54 (α) 11.72 (β)
18	90.71 (α) 97.98 (β)	62.78 (α) 65.75 (β)	73.24–72.20	62.61 (α) 62.96 (β)	47.22 (α)	30.77 (α) 32.31 (β) 21.25 (α) 21.36 (β)	14.15 (α) 14.32 (β)
19	90.73 (α)	62.78 (α)	73.25–72.22	62.60 (α) 62.98 (β)	47.85 (α)	30.25 (α) 28.44 (α) 23.50 (α)	14.34 (α)
20	90.46 (α) 97.46 (β)	62.58 (α) 65.56 (β)	73.26–72.14	62.54 (α) 62.90 (β)	47.76 (α)	32.67 (α) 32.85 (β) 28.26 (α) 29.72 (β) 27.69 (α) 27.85 (β) 23.66 (α) 23.97 (β)	14.42 (α)

filtered off and evaporated. The residue was chromatographed on silica gel (solvent A).

1.3.1. 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-methylamino- (3) and -2-dimethylamino-β-D-glucoses (4). Reductive alkylation of **2** with methanal (37% water solution) gave two products. First was **3** (6% syrup); R_f 0.16 (solvent A); IR: *v* 3352 (N–H), 2927 (C–H), 1753 (ester C=O), 1370 (ester CH_3), 1222 (ester C–O) cm^{-1}. MALDI-TOF-MS: *m*/*z* 362.3 $(M+H)^+$.

Second was **4** (37% white solid); R_f 0.47 (solvent A); IR: *v* 2940, 2876, 2792 (C–H), 1750 (ester C=O), 1368 (ester

Table 8. The ^1H–^1H coupling constants (Hz) for compounds **16**–**20**

	α Anomer				β Anomer			Alkyl	
	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	J_{gem}	J_{vic}
16	3.6	10.0	[a]	[a]	8.0	9.2	[a]	11.4	7.2
17	3.6	9.6	10.4	[a]	8.4	10.0	[a]	[a]	7.2
18	3.6	10.0	[a]	9.6	8.4	9.6	9.6	[a]	7.2
19	3.2	10.4	[a]	9.6	8.0	10.0	[a]	[a]	7.2
20	3.2	10.4	9.6	[a]	8.0	[a]	9.6	[a]	7.2

[a] Not determined.

CH_3), 1224 (ester C–O) cm^{-1}. MALDITOF-MS: *m/z* 376.0 $(M+H)^+$.

1.3.2. 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-ethylamino- (5) and -2-diethylamino-β-D-glucoses (6). Alkylation of **2** with ethanal yielded two products. First was **5** (68% syrup); R_f 0.31 (solvent A); IR: *v* 3354 (N–H), 2969 (C–H), 1752 (ester C=O), 1368 (ester CH_3), 1224 (ester C–O) cm^{-1}. MALDITOF-MS: *m/z* 376.3 $(M+H)^+$, 398.3 $(M+Na)^+$, 414.3 $(M+K)^+$.

Second was **6** (14% syrup); R_f 0.80 (solvent A); IR: *v* 2970, 2936, 2873 (C–H), 1752 (ester C=O), 1368 (ester CH_3), 1224 (ester C–O) cm^{-1}. MALDITOF-MS: *m/z* 404.0 $(M+H)^+$, 425.9 $(M+Na)^+$, 441.9 $(M+K)^+$.

1.3.3. 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-propylamino- (7) and -2-dipropylamino-β-D-glucoses (8). Alkylation of **2** with propanal led to two products. First was **7** (74% syrup); R_f 0.47 (solvent A); IR: *v* 3354 (N–H), 2961, 2936, 2875 (C–H), 1752 (ester C=O), 1368 (ester CH_3), 1224 (ester C–O) cm^{-1}. MALDITOF-MS: *m/z* 390.3 $(M+H)^+$, 412.3 $(M+Na)^+$, 428.2 $(M+K)^+$.

Second was **8** (9% syrup); R_f 0.81 (solvent A); IR: *v* 2961, 2935, 2874 (C–H), 1753 (ester C=O), 1368 (ester CH_3), 1224 (ester C–O) cm^{-1}. MALDITOF-MS: *m/z* 432.4 $(M+H)^+$, 455.4 $(M+Na)^+$, 471.4 $(M+K)^+$.

1.3.4. 1,3,4,6-Tetra-*O*-acetyl-2-butylamino- (9) and -2-dibutylamino-2-deoxy-β-D-glucoses (10). Alkylation of **2** with butanal yielded two products. First was **9** (59% syrup); R_f 0.46 (solvent A); IR: *v* 3353 (N–H), 2958, 2933, 2873 (C–H), 1752 (ester C=O), 1368 (ester CH_3), 1223 (ester C–O) cm^{-1}. MALDITOF-MS: *m/z* 404.2 $(M+H)^+$, 426.2 $(M+Na)^+$, 442.2 $(M+K)^+$.

Second was **10** (8% syrup); R_f 0.78 (solvent A); IR: *v* 2958, 2933, 2872 (C–H), 1753 (ester C=O), 1367 (ester CH_3), 1223 (ester C–O) cm^{-1}. MALDITOF-MS: *m/z* 460.4 $(M+H)^+$, 482.4 $(M+Na)^+$, 498.4 $(M+K)^+$.

1.3.5. 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-pentylamino-β-D-glucose (11). Alkylation of **2** with pentanal yielded **11** (57% syrup); R_f 0.55 (solvent A); IR: *v* 3352 (N–H), 2957, 2932, 2860 (C–H), 1753 (ester C=O), 1367 (ester CH_3), 1224 (ester C–O) cm^{-1}. MALDITOF-MS: *m/z* 418.1 $(M+H)^+$.

1.3.6. 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-hexylamino- (12) and -2-dihexylamino-β-D-glucoses (13). Alkylation of **2** with hexanal gave two products. First was **12** (61% syrup); R_f 0.64 (solvent A); IR: *v* 3353 (N–H), 2957, 2930, 2858 (C–H), 1753 (ester C=O), 1368 (ester CH_3), 1224 (ester C–O) cm^{-1}. MALDITOF-MS: *m/z* 432.0 $(M+H)^+$, 454.0 $(M+Na)^+$, 470.0 $(M+K)^+$.

Second was **13** (7% syrup); R_f 0.86 (solvent A); IR: *v* 2956, 2930, 2859 (C–H), 1755 (ester C=O), 1367 (ester CH_3), 1224 (ester C–O) cm^{-1}. MALDITOF-MS: *m/z* 516.4 $(M+H)^+$, 538.4 $(M+Na)^+$, 554.4 $(M+K)^+$.

1.3.7. 1,3,4,6-Tetra-*O*-acetyl-2-benzylamino- (14) and -2-benzylideneamino-2-deoxy-β-D-glucoses (15). Alkylation of **2** with benzaldehyde yielded two products. First was **14** (16% syrup); R_f 0.59 (solvent A); IR: *v* 1751 (ester C=O), 1367 (ester CH_3), 1222 (ester C–O), 746, 701 (C_6H_5) cm^{-1}. MALDITOF-MS: *m/z* 438.0 $(M+H)^+$, 460.0 $(M+Na)^+$, 475.9 $(M+K)^+$.

Second was **15** (21% white solid); R_f 0.69 (solvent A); IR: *v* 1755 (ester C=O), 1646 (C=N), 1367 (ester CH_3), 1221 (ester C–O), 759, 696 (C_6H_5) cm^{-1}.

1.4. General procedure for deacetylation

A mixture of appropriate 1,3,4,6-tetra-*O*-acetyl-2-(*N*-alkylamino)-2-deoxy-β-D-glucose (1 mmol) and K_2CO_3 (552 mg, 4 mmol) in MeOH–H_2O (5:2) solution (35 mL) was stirred at rt for 30 min. The end of deacetylation was detected by TLC (solvent B). Then, the reaction mixture was neutralized with MeOH saturated with HCl and concentrated under vacuum. The crude products were chromatographed on silica gel (solvent first B, next C).

1.4.1. 2-Deoxy-2-ethylamino-D-glucose (16). Deacetylation of **5** gave **16** (58% syrup, α:β ~ 2:1); R_f 0.05 (solvent B); IR: *v* 3429 (O–H), 2926 (C–H), 1028 (alcohol C–O) cm^{-1}. MALDITOF-MS: *m/z* 208.2 $(M+H)^+$.

1.4.2. 2-Deoxy-2-propylamino-D-glucose (17). Deacetylation of **7** yielded **17** (68% syrup, α:β ~ 1.3:1); R_f 0.09 (solvent B); IR: *v* 3439 (O–H), 2922, 2853 (C–H), 1066, 1030 (alcohol C–O) cm^{-1}. MALDITOF-MS: *m/z* 222.2 $(M+H)^+$, 244.1 $(M+Na)^+$.

1.4.3. 2-Deoxy-2-butylamino-D-glucose (18). Deacetylation of **9** led to **18** (58% white solid, $\alpha{:}\beta \sim 2.7{:}1$); R_f 0.11 (solvent B); IR: *v* 3267 (O–H), 2924, 2856 (C–H), 1040 (alcohol C–O) cm^{-1}. MALDITOF-MS: *m*/*z* 236.1 $(M+H)^+$.

1.4.4. 2-Deoxy-2-pentylamino-D-glucose (19). Deacetylation of **11** gave **19** (40% white solid, $\alpha{:}\beta \sim 2.4{:}1$); R_f 0.10 (solvent B); IR: *v* 3277 (O–H), 2957, 2925, 2855 (C–H), 1072 (alcohol C–O) cm^{-1}. MALDITOF-MS: *m*/*z* 250.0 $(M+H)^+$.

1.4.5. 2-Deoxy-2-hexylamino-D-glucose (20). Deacetylation of **12** yielded **20** (59% white solid, $\alpha{:}\beta \sim 2.3{:}1$); R_f 0.14 (solvent B); IR: *v* 3302 (O–H), 2955, 2924, 2854 (C–H), 1073 (alcohol C–O) cm^{-1}. MALDITOF-MS: *m*/*z* 264.0 $(M+H)^+$.

1.5. General procedure for an enzymatic phosphorylation

N-Alkyl D-glucosamine derivatives (25 μmol of each) were incubated in 0.75 mL aliquots containing 50 mM ATP, 50 mM $MgCl_2$ and yeast hexokinase (Sigma, product No H 4502) 20 U in 0.1 M Tris–HCl buffer, pH 8.0. The course of reaction was followed by TLC analysis (solvent D). Components of the reaction mixture were detected with ninhydrin, Hanes reagent and Elson–Morgan reagent.

1.6. Antifungal susceptibility testing

Antifungal in vitro activity was determined by the serial twofold dilution method in 96-well microtitre plates in the minimal Yeast Nitrogen Base medium (Difco) containing 2% glucose. The inoculum size was 10^4 cells/mL. Plates were incubated for 24 h at 30 °C and results were read visually.

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