STUDIA UNIVERSITATIS BABEŞ-BOLYAI, CHEMIA, LI, 1, 2006

# PSEUDOCERAMIDES AND THEIR DERIVATIVES. 3. BENZYLIDENE ACETALS OF N-ACETYL-N-METHYL GLUCAMINE – PRELIMINARY STUDY.

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**ABSTRACT.** Acetalization of N-acetyl-N-methyl-1-amino-1-deoxy-D-glucitol with 2.5 equivalents of 1,1-dimethoxybenzaldehide in N,N-dimethylformamide at reflux temperature with camphorsulphonic acid as catalyst yielded 5,6-O-benzylidene-N-acetyl-N-methyl-glucamine as the major product. 2,3:4,6-di-O-benzylidene-N-acetyl-N-methyl-glucamine, 4,6-O-benzylidene-N-acetyl-N-methyl-glucamine and a mixture of 2,3:5,6- and 3,4:5,6-di-O-benzylidene-N-acetyl-N-methyl-glucamine byproducts have been also isolated. Separation and purification conditions of these new products are described. The structures have been assigned by $^{13}$C-NMR and MS-EI.

**Keywords:** aminoalditol acetals, pseudoceramide, flash chromatography, 5,6-O-benzylidene-N-acetyl-N-methyl-glucamine, 2,3:4,6-di-O-benzylidene-N-acetyl-N-methyl-glucamine, 4,6-O-benzylidene-N-acetyl-N-methyl-glucamine, 2,3:5,6-di-O-benzylidene-N-acetyl-N-methyl-glucamine, 3,4:5,6-di-O-benzylidene-N-acetyl-N-methyl-glucamine.

## Introduction

The development of new biocompatible and biodegradable surfactants based on natural feedstocks is subject of an increasing scientific and industrial interest [1]. Aminoalditol derivatives represent a class of compounds that can be used both as surfactants as well as pseudoceramides [2, 3]. The use of N-methyl-glucamine (an accessible and cheap aminoalditol) allows two strategies in order to obtain a compound with amphiphatic character that could exhibit ceramide properties:

a) attachment of a nonpolar tail to the amine function followed by the attachment of a second nonpolar tail to the alcohol function

b) creation of an amide bond at the amine function (by acylation) followed by the attachment of two nonpolar tails at the alcohol functions.

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These two options can be realized with biocatalysts, using the catalytic capacity and the specificity of lipases [4, 5]. A new approach in the synthesis of these compounds starts from N-acetyl-N-methyl glucamine and consists in the enzymatic attachment of fatty acids by esterification or transesterification reactions catalyzed by lipases [6]. The reaction monitorization can easily performed by HPLC or GC, whether mono- and di-esters of N-acetyl-N-methyl-glucamine are available as standard compounds. The selective esterification of each alcohol function in the aminoalditol requires acetal derivatives as intermediates. This paper presents the results of acetalization of N-acetyl-N-methyl-1-amino-1-deoxy-D-glucitol with dimethoxy benzaldehyde in acid catalysis.

**Results and Discussion**

The condensation of aldehydes and ketones with alcohols is one of the first reactions studied in organic chemistry. E. Fischer obtained in 1895 for the first time, fructose acetals and then glucose acetals, in acid catalysis [7]. Acetals can be obtained in acidic, basic or neutral conditions or using other methods [8]. A series of well documented reviews present results of the acetalization reaction for alditols [9], aldoses, aldosides [10,11] and ketoses [12] but not of aminoalditols.

The acetalization of N-acetyl-N-methyl-glucamine was performed by transacetalization reaction in acidic media using dimethoxy benzaldehyde (see the experimental part). The reaction was monitorized by TLC.and the inspection of the plate showed 5 spots at the following Rf values: spot 1 (0.73), spot 2 (0.67), spot 3 (0.55), spot 4 (0.24) and spot 5 (0.15) (AcOEt :MeOH = 90:10).

Considering the TLC plate polarity and the eluent polarity we used, the spots on the TLC plate can be assigned as follows: spots 1, 2 and 3 for di-benzylidene derivatives and spots 4 and 5 for mono-benzylidene derivatives. After separation by flash chromatography, the compounds have been analyzed by MS-EI. Spots 1, 2 and 3 are di-benzylidene derivatives and have the molecular peak at m/z= 413($M^{+\cdot}$) for a 22 eV ionization energy, thus confirming the proposed assignments. Spot 1 was characterized as acetylated derivative, having the molecular peak at m/z= 455 ($M^{+\cdot}$), 24 eV.

The analysis of $^{1}$H-NMR spectra is difficult because the 8 protons in the linear side chain are localized in the 3.5-4.3 ppm interval and the coexistence of cis-trans conformers of the amide bond (Figure 1) determines the doubling of the total number of signals.

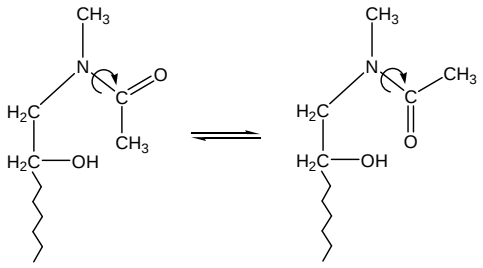


**Figure 1.**
Cis-trans conformers of N-acetyl-N-metyl-glucamine and derivatives

The $^{13}$C-NMR spectroscopy offers valuable data concerning the nature of the acetal rings as well as their positions [13, 14]. Buchanan and coworkers [15, 16] observed that the chemical shift of the acetal carbon atom is strongly influenced by the size of the ring and is situated in the following intervals:

108.1-115.7 ppm for 1,3-dioxolane rings
97.1-101.1 ppm for 1,3–dioxane rings
100.8-102.3 ppm for 1,3-dioxepane rings

The $^{13}$C-NMR spectra analysis of spot 1 show 8 signals in the169.4 - 171.2 ppm interval, which indicate the existence of 2 isomers, each one with 2 conformers. The 4 signals situated in the 103.7 – 104.2 ppm interval correspond to 4 acetalic C atoms from 1,3-dioxolanic rings and belong to 2 dibenzylidene isomers, both with 2 conformers. These chemical shifts are deshielded (they are present at lower ppm values: 103.7 - 104.2 ppm) comparing with literature data (108.1 - 115.7 ppm for 3-dioxolanic rings [15, 16]) because of the influence of Ph. Hereby the 2 isomers from spot 1 can be only the compounds **2** and **3** from Figure 2: 2,3:5,6-di-O-benzylidene-N-acetyl-N-methyl-glucosamine and 3,4:5,6-di-O-benzylidene-N-acetyl-N-methyl-glucosamine. Assigning position 5,6 to one of the benzylidene groups will be explained below.

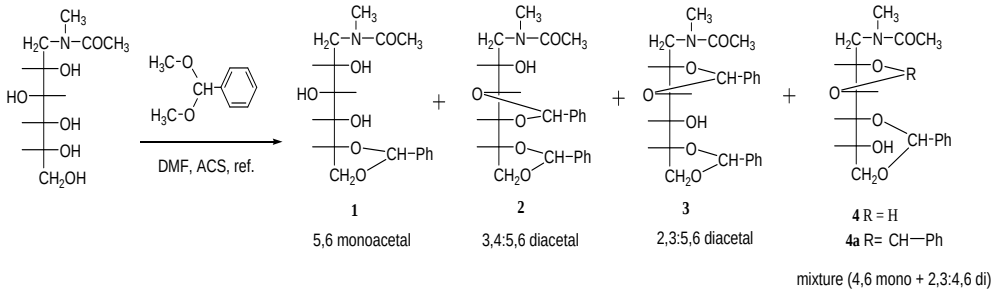


**Figure 2.**
Mono- and diacetal reaction products

$^{13}$C-NMR spectra analysis of spot 2 relieves 4 signals:

- at 103.7 and 104.5 ppm the signals correspond to an acetalic C from 1,3-dioxolane ring (the signals are shifted to a higher value of the magnetic field because of the influence of the phenyl group comparing with the literature data [15, 16])

- at 100.7 and 101.0 ppm the signals correspond to an acetalic C from 1,3-dioxane ring.

Admitting that the initial and kinetically more stable 5, 6-benzylidene derivative is stabilized during time to the thermodynamically more stable 4,6-benzylidene isomer, results that spot 2 can be assigned solely to compound **4a** (Figure 2) namely 2,3:4,6-di-O-benzylidene-N-acetyl-N-methyl-glucamine. The 3,5:4,6 and 2,5:4,6 isomers are excluded because the chemical shifts of acetalic C at 105.0 and 104.1 ppm are clearly assigned to a dioxolanic ring.

Although isopropyliden and cyclohexyliden aminoalditol acetals have been already described [20, 21] there are no data about benzylidene acetals, consequently the position of benzylidene group had to be established. The analysis of $^{1}$H-NMR spectra is difficult and doesn't give any valuable information. To spread the hydrogen atom attached on carbon atom with free hydroxyl groups from compound of spot 4 the free hydroxyl groups were derivatized by acetilation and than analysed by $^{1}$H-NMR.

The type of ring was deduced from chemical shifts of the acetalic C atom from the $^{13}$C-NMR spectra. There are 2 signals at 103.5 ppm and 103.9 ppm which indicate a 1,3-dioxolane ring and thereby the spot 4 correspond to compound **1** (Figure 2)**:** 5,6-O-benzyliden-N-acetyl-N-methyl-glucamine. The position of the 1,3-dioxolane ring on the linear side chain was determined by NMR experiments: monodimesional APT and bidimensional COSY and HETCOR. The assignment of C atoms from the linear side chain was established by HETCOR and APT experiments only for C1 and C6.

The presence of cis-trans conformers was proved previously by our group [20, 21]. Obtaining mainly 5,6-O-acetals of N-acetyl-N-methyl-glucamine

is in agreement with the literature concerning kinetic acetalization of diethyl-dithioacetals of glucose, galactose and arabinose [17-19] and for isopropyliden and cyclohexyliden acetals of N-acetyl-N-methyl-glucosamine [20, 21].

The $^{13}$C-NMR spectra analysis of spot 5 relieve the existence of 1,3-dioxane ring (100.4 and 100.5 ppm) and it was assigned to compound **4** from Figure 2 (4, 6-benzilidene isomer of N-acetyl-N-methyl-glucamine). The benzylation of compound **1** (spot 4) (NaH, BnBr) followed by hydrolysis of benzyliden acetal ($I_2$/MeOH) leads finally to 2,3,4-tri-O-benzyl-N-acetyl-N-methyl-glucozamine. This compound was also obtained from 5,6-isopropilydene-N-acetyl-N-methyl-glucozamine [21] and from 5,6-cyclohexylidene-N-acetyl-N-methyl-glucozamine[22], their structure were previously established [21]. These compounds were obtained by the same reaction path, the obtained compounds presenting identical IR spectra. Therefore the assigning structure of compound **4** (spot 5) were confirmed also by chemical way.

The procedure and the complete description of the $^{13}$C-NMR spectra are presented in the experimental part. The description of structure was done for both conformers together and the notation used is: (cis, trans), meaning that both signals are present and also that the number of signals is double.

**Equipment and Methods**

NMR spectra were recorded on a Varian Gemini 300 MHz apparatus against the line of the solvent ($CDCl_3$). Chemical shifts are expressed in ppm. Mass spectra were recorded on a Varian Finnigan Mat 212 mass spectrometer. TLC analysis was performed on Kieselgel Merck plates with fluorescence indicator. Separation by flash chromatography was performed on 220-400 mesh silica (Aldrich). Visualization of the chromatograms was accomplished with UV lamp and by developing with a 20% $H_2SO_4$ in ethanol solution, then heating the plate at 120ºC. Anhydrous solvents have been used.

**Experimental**

2 g (8.4 mmol) N-acetyl-N-methyl-glucamine is solved in 5 ml DMF (kept on 4Å molecular sieves) and a catalytic amount of camphorsulphonic acid is added under stirring. The reaction mixture is cooled to 0ºC and 3.6 ml (24 mmol) dimethoxy benzaldehyde are drop by drop added under argon atmosphere and stirring. The reaction mixture is stirring at reflux temperature (48 h) until TLC control (AcOEt:MeOH = 90:10) indicates the disappearance of the starting material. The reaction is stopped by adding 0.5 ml $Et_3N$ and the solvents are vacuum distilled with toluene. The mixture is then separated and purified by flash chromatography (Table 1).

The 5,6-O-benzyliden-N-acethyl-N-methyl glucosamine was obtained in 52% yield. All products are as consistent clays at room temperature.

**Table 1.**
Flash chromatography and TLC separation and analysis conditions of the acetalization products

| Spots separation | FC elution system | TLC control system |
|---|---|---|
| 1+2+3 spots by 4+5 spots | Tol:AcOEt=22:78 | AcOEt :MeOH=95:10 |
| 1+2 spots by spot 3 | Tol:AcOEt=22:78 | AcOEt :MeOH=95:10 |
| spot 1 by spot 2 | Tol:AcOEt=22:78 | AcOEt :MeOH=95:10 |
| spot 3 by spot 2 | Tol:AcOEt=25:75 | AcOEt :MeOH=95:5 |
| spot 4 by spot 5 | Tol:AcOEt=25:75 | AcOEt :MeOH=95:5 |
| spot 5 by 4+5 spots | Tol:AcOEt=25:75 | AcOEt :MeOH=95:5 |

**Spot 1:** *2,3:5,6- di-O-benzylidene-(4-O-acetyl)-N-acetyl-N-methyl-glucosamine* + *3,4:5,6-di-O-benzylidene-(2-O-acetyl)-N-acetyl-N-methyl-glucosamine* (compounds **2** and **3**, Figure 2)
**MS(EI),** 22eV, (characterized as monoacetylated derivative $C_{25}H_{29}NO_7$) m/z= 455($M^{+\cdot}$)
**$^{13}$CRMN (75MHz, $CDCl_3$)**
171.25, 171.19, 170.82, 170.40, 169.96, 169.64, 169.52 (4C, 2X $\underline{C}H_3$-CO, (cis, trans), (the isomers 2 and 3))
137.34, 136.80 (4C, 2x $\underline{C}$ from Ph, (cis, trans), the isomers 2 and 3))
129.71, 129.66, 129.38, 129.28, 129.07, 128.34, 128.29, 128.20, 126.30, 126.21, 126.16, 126.09, (20C, 10x $\underline{C}$H from Ph, (cis, trans), the isomers 2 and 3))
104.20, 104.01, 103.90, 103.74, (4C, 2x $\underline{C}$H-Ph, (cis, trans), the isomers 2 and 3)),
77.42, 76.99, 76.57, 74.98, 74.37, 74.24, 70.33, 70.28, 70.20, 70.06, 69.88, 68.09, 68.03, (8C, $\underline{C}$2, $\underline{C}$3, $\underline{C}$4, $\underline{C}$5, (cis, trans), the isomers 2 and 3))
67.37, 67.25, 67.10, 66.59, (2C, $\underline{C}$6, (cis, trans), the isomers 2 and 3))
50.77, 50.45, (2C, $\underline{C1}$, (cis, trans), the isomers 2 and 3))
37.19, 37.07, 33.52, 33.41, (2C, $\underline{C}H_3$-N, (cis, trans), the isomers 2 and 3))
21.54, 21.50, 21.10, 20.75, 20.71, 20.67, 20.58, 20.53, 20.45, 20.38, (4C, $\underline{C}H_3$-CO, (cis, trans) the isomers 2 and 3))

**Spot 2:** *2,3 :4 6-di-O-benzylidene-N-acetyl-N-methyl-glucosamine* (compound **4a**, Figure 2)
**MS(EI),** 22eV, ($C_{23}H_{27}NO_6$), m/z= 413($M^{+\cdot}$)
**$^{13}$CRMN (75MHz, $CDCl_3$)**
171.97, 171.46, (1C, N-$\underline{C}$=O, (cis, trans))
137.97, 137.90, (2C, $\underline{C}$ from Ph, (cis, trans))
129.61, 129.57, 129.41, 129.35, 129.09, 128.99, 128.38, 128.28, 128.27, 128.17, 126.71, 126.61, 126.55, 126.43, 126.34, 125.92, (10C, $\underline{C}$H from Ph, (cis, trans))
104.52, 104.33, 103.70, (1C, $\underline{C}$H-Ph, (cis, trans)), from dioxolane ring
101.02, 100.90, 100.76, (1C, $\underline{C}$H-Ph, (cis, trans)), from dioxane ring
78.69-68.56 (multiple signals), (4C, $\underline{C}$2, $\underline{C}$3, $\underline{C}$4, $\underline{C}$5, (cis, trans))
67.91, 67.55, (1C, $\underline{C}$6, (cis, trans))
49.02, 48.91, (1C, $\underline{C1}$, (cis, trans))
38.42, 38.16, (1C, $\underline{C}H_3$-N, (cis, trans))
21.75, 21.63, (1C, $\underline{C}H_3$-CO, (cis, trans))

**Spot 4**: *5, 6-O-benzylidene-N-acetyl-N-methyl-glucosamine* (compound **1**, Figure 2)
**MS(EI),** (24eV) ($C_{16}H_{23}NO_6$), m/z=325, ($M^{+\cdot}$)
**$^{13}$CRMN (75MHz, $CDCl_3$)**
173.06, (1C, N-C=O, (cis, trans))
137.87, 137.29, (1C, C from Ph, (cis, trans))
129.28, 129.13, 128.26, 128.19, 126.54, 126.44, 126.33, 126.31, 126.03, (5C, CH from Ph, (cis, trans))
103.98, 103.57, (1C, CH-Ph, (cis, trans))
74.02, 73.60, 72.47, 72.43, 70.09, 69.74, 69.67, 68.17, (4C, C2, C3, C4, C5, (cis, trans))
68.71, (1C, C6, (cis, trans))
53.77, 51.63, (1C, C1, (cis, trans))
38.11, 37.95, (1C, $CH_3$-N, (cis, trans))
21.63, 21.58, (1C, $CH_3$-CO, (cis, trans))

**Spot 5:** *4, 6-O-benzylidene-N-acetyl-N-methyl-glucosamine* (compound **4,** Figure 2)
**MS(EI),** (24 eV) ($C_{16}H_{23}NO_6$), m/z=325, ($M^{+\cdot}$)
**$^{13}$CRMN (75MHz, $CDCl_3$)**
172.18, (1C, N-C=O, (cis, trans))
137.73, 137.33, (1C, C from Ph, (cis, trans))
128.38, 128.27, 128.20, 126.75, 126.67, 126.56, 126.42, 126.23, 125.97, (5C, CH from Ph, (cis, trans))
100.59, 100.47, (1C, CH-Ph, (cis, trans))
79.53, 79.09, 78.40, 78.34, 78.04, 72.38, 72.24, 70.13, 69.57, 69.30, (4C, C2, C3, C4, C5, (cis, trans))
63.44, 62.71, (1C, C6, (cis, trans))
49.50, 49.11, (1C, C1, (cis, trans))
38.47, 38.41, (1C, $CH_3$-N, (cis, trans))
21.72, 21.59, (1C, $CH_3$-CO, (cis, trans))

## Conclusions

The reaction between N-acetyl-N-methyl-glucamine and dimethoxy benzaldehyde in acid catalysis (camphorsulphonic acid) gives mainly the 5,6-monobenzylidene acetal (compound **1**, Figure 2) with 52% yield. The new compounds: 5,6-O-benzylidene-N-acetyl-N-methyl-glucosamin (as main product), 2,3:4,6-di-O-benzylidene-N-acetyl-N-methyl-glucosamine, 4,6-O-benzylidene-N-acetyl-N-methyl-glucosamine and a mixture of 2,3:5,6- with 3,4:5,6-di-O-benzylidene-N-acetyl-N-methyl-glucosamine (not previously described in the literature), were characterized by MS-EI, $^1$H-NMR, and $^{13}$C-NMR. The identity of products was confirmed by MS and the ring type was established by $^{13}$C-NMR, COSY and HETCOR experiments. The careful study of the spectra indicates the existence of cis-trans conformers. The existence of conformers makes difficult their characterization by $^1$H-NMR, therefore they were characterized mainly by $^{13}$C-RMN. The optimal conditions for reaction monitorization and for flash chromatography separation and purification have been also established.

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