September 2006 *Volume 2 Issues 1-3*

Trade Science Inc.

Organic CHEMISTRY

An Indian Journal

Full Paper

OCAIJ, 2(1-3), 2006 [21-27]

Synthesis And Biological Evaluation Of Diamines Containing Monosaccharides

Corresponding Author

Afaf R.Genady
Department of Chemistry, Faculty of Science,
University of Tanta, 31527-Tanta (EGYPT)
Ph.: +2-040-3297974; Fax: +2-040-3350804,
E-mail: afafgenady@hotmail.com

Received: 10th April, 2006
Accepted: 28th April, 2006

Web Publication Date : 23rd September, 2006

ABSTRACT

A convenient synthetic route to diglycosylamines derived from aliphatic diamines [$NH_2(CH_2)_nNH_2$, where n=2,3, and 4] and α-D-glucose or D-ribose is presented . In a one step process, treatment of diamines with α-D-glucose and D-ribose in methanol in the presence of a catalytic amount of glacial acetic acid gave N,N′-diglycosylamines in high yield. The structures of the new compounds were confirmed by elemental analysis, IR, NMR, and mass spectrometry. The *in vitro* toxicity studies using B16 melanoma cells showed that N,′-Di-α-D-ribofuransoyl 1,2-ethanediamine, N,N′-Di-α-D-ribofuransoyl 1,3-propanediamine as well as N,N′-Di-α-D-ribofuransoyl 1,4-butanediamine are not toxic at low concentration (LD_{50} > 6 mM). The toxicity values for N,N′-Di-α-D-glucopyansoyl 1,2-ethanediamine, N,N′-Di-α-D-glucopyansoyl 1,3-propanediamine, and N,N′-Di-α-D-glucopyansoyl 1,4-butanediamine were lower than that of compounds rest compounds. It was observed that the toxicity increases with increasing number of carbon atoms in the chain of diamines.

KEYWORDS

N-glycosylamine;
Diamines;
B16 melanoma cells;
NMR-spectroscopy.

INTRODUCTION

Naturally occurring polyamines and their derivatives are biologically important. They are known to have high affinity for nucleic acids, and to exhibit a variety of effects on nucleic acid biosynthesis and metabolism[1-4]. On the other hand, glycoproteins and glycolipids are widely expressed on cell surfaces and participates in many molecular recognition and binding processes in both healthy and diseased states[5].

Full Paper

In particular, some bacterial surface proteins show specific binding for carbohydrates expressed human cells, and such interactions form an essential part of the infection pathway. It has been demonstrated that administration of synthetic or naturally carbohydrate derivatives can disrupt this infective, pathway, so long as the administered derivatives have high affinities for bacterial lectins[6]. The site of action of many cytotoxic agent is intracellular and a such these agents must first cross the cell membrane and having done so they must recognize and interact selectively with their cellular targets[7]. The development of novel strategies for drug delivery and targeting therefore presents a major challenge. Polyamines represent attractive candidates for the targeting of drugs whose site of action is nuclear and DNA because of two unique features: (i) the nature of their interaction with DNA, and (ii) the existence of an active uptake system for polyamines in a variety of cell types[8-10].

Carbohydrates are also important molecules for cell-cell interactions and cellular recognition. Due to the increased metabolism of tumors, sugars might be taken up to a larger extent there than in surrounding tissue[11]. Sugars are potentially attractive compounds because they either **(1)** may be taken up selectively into tumors due to the high metabolic rate of tumor cells vs normal cells, e.g. through the Na-glucose cotransporter[12], or **(2)** their nucleosides may be intracellularly converted to the corresponding nucleotides through phosphorylation by appropriate enzymes, or **(3)** they may potentially be incorporated into tumor DNA. Preparation of N-glycosylamines by condensation of amines with reducing sugars is well described in the literature and condensation of a small range of reducing sugars with polyamines has also been previously reported to allow efficient access to divalent carbohydrate derivatives in excellent yields[13-15]. A range of multivalent mannose mono-and disaccharides have been prepared in an efficient manner using a range of different methodology[16]. The use of boronated di-and polyamines containing sugars for boron neutron capture therapy (BNCT) has been recently described[17]. Here we report the synthesis of new N-glycosides of the sugars (D-glucose, and D-ribose) to diamines [$NH_2(CH_2)_nNH_2$, where n = 2, 3, and 4] by one post reaction with aim of obtaining compounds with enhanced biological significance (Figure 1). The study of their structure-activity relationship with respect to their in vitro toxicities by using B16 melanoma cells was investigated.

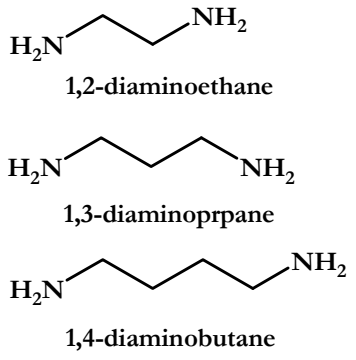


Figure 1: Schematic structure of 1,2diamino-ethane, 1,3-diaminopropane, and 1,4-diamino-butane

EXPERIMENTAL

Material and methods

The reagents, dry solvents methanol and THF were used as presented directly without further purification. 1,2-diaminoethane, 1,3-diaminopropane, 1,4-diaminobutane, α-D-glucose, and D-ribose were commercially available. Plate chromatography was conducted on silica gel 60 F_{254} (Merck). Elemental analyses were performed by a Perkin-Elmer 2400 automatic elemental analyzer. The measurements for NMR (^1H and ^{13}C) were carried out on a Bruker DPX 200 spectrometer. The chemical shifts δ are given in ppm relative to Ξ = 200 MHz for δ (^1H) (nominally $SiMe_4$), and Ξ = 50 MHz for δ (^{13}C) (nominally $SiMe_4$) in D_2O. IR (cm^{-1}) spectra were determined as KBr disc on a Biorad FTS-7 spectrometer. Electron spray ionization (ESI) mass spectra were recorded with a Bruker Esquire in CH_3OH.

Synthesis of compounds (1-6)

General procedure

A solution of diamines ($NH_2(CH_2)_nNH_2$, where n=2, 3, and 4 (1 mmol) was added to a solution of sugar (2 mmol) in 20 ml anhydrous CH_3OH with one drop of a catalytic glacial acetic acid under ni-

trogen atmosphere. The mixture was stirred for 8 h at room temperature. All volatile components of the solution were removed under vacuum to give colorless oil. The residue was chromatographed on silica gel using THF as eluent to yield the desired product.

N,N′-Di-α-D-glucopyansoyl 1,2-ethanediamine (1)

Yield: (0.52 g, 89 %) as a colorless solid; ν_{max}(KBr disc) cm^{-1} 3512s, 3313s (N-H str), 3929s, 2885s, 2842s (O-H str), 1621m (N-H bend), 694s (N-H wagging); δ_H(200 MHz; D_2O; Me_4Si) +6.23 (2H, bs, HN-), 4.88 (2H, bs, H-1, H-1'), 3.98 (2H, dd, H-6, H-6'), 3.82 (2H, d, H-2, H-2'), 3.69 (2H, dd, H-6, H-6'), 3.58 (2H, dd, H-3, H-3'), 3.54 (2H, t, H-4, H-4'), 3.7-3.45 (2H, m, H-5, H-5'), +2.78 (4H, m, -H_2CNH); δ_C(50 MHz; D_2O; Me_4Si), 89.59 (2C, C-1), 82.56 (2C, C-2), 76.34 (2C, C-3), 73.67 (2C, C-4), 69.01 (2C, C-5), 63.96 (2C, C-6), 48.16, 47.45 (2C, CH_2NH); *m/z* (ESI) 384 (M^+, 65%); (Found: C, 43.52; H, 6.92; N 7.16. $C_{14}H_{28}N_2O_{10}$ requires C, 43.75; H, 7.29; N, 7.29 %).

N,N′-Di-α-D-glucopyansoyl 1,3-propanediamine (2)

Yield: (0.48 g, 87 %) as a colorless solid; ν_{max}(KBr disc) cm^{-1} 3379s (N-H str), 2928s (O-H str), 1656w NH-bend), 695m (N-H wagging); δ_H(200 MHz; D_2O; Me_4Si) +6.19 (2H, bs, HN-), 4.84 (2H, bs, H-1, H-1'), 3.95 (2H, dd, H-6, H-6'), 3.84 (2H, d, H-2, H-2'), 3.72 (2H, dd, H-6, H-6'), 3.59 (2H, dd, H-3, H-3'), 3.56 (2H, t, H-4, H-4'), 3.72-3.44 (2H, m, H-5, H-5'), +2.73 (4H, m, -H_2CNH), +1.55 (2H, t, -CH_2-); δ_C(50 MHz; D_2O; Me_4Si), 89.57 (2C, C-1), 83.26 (2C, C-2), 77.05 (2C, C-3), 73.65 (2C, C-4), 68.94 (2C, C-5), 63.93 (2C, C-6), 48.18, 47.25 (2C, CH_2NH), 30.14 (C, -CH_2-); *m/z* (ESI) 398 (M^+, 57%); (Found: C, 44.96; H, 7.42; N 6.97. $C_{15}H_{30}N_2O_{10}$ requires C, 45.22; H, 7.53; N, 7.03 %).

N,N′-Di-α-D-glucopyansoyl 1,4-butanediamine (3)

Yield: (0.56 g, 93 %) as a colorless solid; ν_{max}(KBr disc) cm^{-1} 3305s (N-H str), 2919s, 2856s (O-H str), 1653w (N-H bend), 691m (N-H wagging); δ_H(200 MHz; D_2O; Me_4Si) +6.14 (2H, bs, HN-), 4.89 (2H, bs, H-1, H-1'), 3.98 (2H, dd, H-6, H-6'), 3.84 (2H, d, H-2, H-2'), 3.68 (2H, dd, H-6, H-6'), 3.56 (2H, dd, H-3, H-3'), 3.51 (2H, t, H-4, H-4'), 3.68-3.43 (2H, m, H-5, H-5'), +2.75 (4H, m, -H_2CNH), 1.58 (4H, m, -CH_2CH_2-); δ_C(50 MHz; D_2O; Me_4Si), 89.67 (2C, C-1), 82.31 (2C, C-2), 76.28 (2C, C-3), 73.28 (2C, C-4), 68.87 (2C, C-5), 63.96 (2C, C-6), 47.98, 47.11 (2C, CH_2NH), 30.2, 29.32 (2C, -CH_2CH_2-); *m/z* (ESI) 412 (M^+, 62%); (Found: C, 46.27; H, 7.53; N 6.48. $C_{16}H_{32}N_2O_{10}$ requires C, 46.60; H, 7.76; N, 6.79%).

N,N′-Di-α-D-ribofuransoyl 1,2-ethanediamine(4)

Yield: (0.51 g, 86 %) as a colorless solid; ν_{max}(KBr disc) cm^{-1} 3510s, 3311s (N-H str), 3927s, 2887s, 2841s (O-H str), 1622m (N-H bend), 689s (N-H wagging); δ_H(200 MHz; D_2O; Me_4Si), +6.18 (2H, bs, HN-), 5.12 (2H, bs, H-1), 3.99 (2H, dd, H-6, H-6'), 3.86 (2H, d, H-2, H-2'), 3.74 (2H, dd, H-6, H-6'), 3.65 (2H, dd, H-3, H-3'), 3.57 (2H, t, H-4, H-4'), 3.71-3.36 (2H, m, H-5, H-5'), +2.76 (4H, m, -H_2CNH); δ_C(50 MHz; D_2O; Me_4Si), 87.95 (2C, C-1), 82.43 (2C, C-2), 75.12 (2C, C-3), 69.5 (2C, C-4), 64.14 (2C, C-5), 48.29, 49.14 (2C, CH_2NH); *m/z* (ESI) 324 (M^+, 63%); (Found: C, 44.31; H, 7.28; N, 8.33. $C_{12}H_{24}N_2O_8$ requires C, 44.44; H, 7.40; N, 8.64 %).

N,N′-Di-α-D-ribofuransoyl 1,3-propanediamine (5)

Yield: (0.53 g, 88 %) as a colorless solid; ν_{max}(KBr disc) cm^{-1} 3373s (N-H str), 2925s (O-H str), 1666w NH-bend), 697m (N-H wagging); δ_H(200 MHz; D_2O; Me_4Si), +6.21 (2H, bs, HN-), 5.14 (2H, bs, H-1), 3.95 (2H, dd, H-6, H-6'), 3.85 (2H, d, H-2, H-2'), 3.75 (2H, dd, H-6, H-6'), 3.61 (2H, dd, H-3, H-3'), 3.58 (2H, t, H-4, H-4'), 3.65-3.39 (2H, m, H-5, H-5'), +2.72 (4H, m, -H_2CNH), 1.62 (2H, t, -CH_2-); δ_C(50 MHz; D_2O; Me_4Si), 87.93 (2C, C-1), 82.53 (2C, C-2), 75.22 (2C, C-3), 69.56 (2C, C-4), 64.18 (2C, C-5), 48.39, 49.19 (2C, CH_2NH), 30.71 (C, -CH_2-); *m/z* (ESI) 338 (M^+, 75%); (Found: C, 46.08; H, 7.58; N, 8.07. $C_{13}H_{26}N_2O_8$ requires C, 46.15; H, 7.69; N, 8.28 %)

N,N′-Di-α-D-ribofuransoyl 1,4-butanediamine (6)

Yield: (0.55 g, 91 %) as a colorless solid; ν_{max}(KBr

Full Paper

disc) cm^{-1} 3512s, 3314s (N-H str), 3929s, 2889s, 2845s (O-H str), 1625m (N-H bend), 693s (N-H wagging); δ_H(200 MHz; D_2O; Me_4Si), +6.20 (2H, bs, HN-), 5.10 (2H, bs, H-1), 3.95 (2H, dd, H-6, H-6'), 3.83 (2H, d, H-2, H-2'), 3.77 (2H, dd, H-6, H-6'), 3.66 (2H, dd, H-3, H-3'), 3.59 (2H, t, H-4, H-4'), 3.72-3.37 (2H, m, H-5, H-5'), +2.70 (4H, m, -H_2CNH), +1.47-1.62 (4H, m, -CH_2CH_2-); δ_C(50 MHz; D_2O; Me_4Si), 87.97 (2C, C-1), 82.45 (2C, C-2), 75.16 (2C, C-3), 69.56 (2C, C-4), 64.17 (2C, C-5), 48.17, 49.15 (2C, CH_2NH), 30.54, 29.97 (2C, -CH_2CH_2-); *m/z* (ESI) 352 (M^+, 83%); (Found: C, 47.39; H, 7.71; N, 7.82. $C_{14}H_{28}N_2O_8$ requires C, 47.72; H, 7.95; N, 7.95 %).

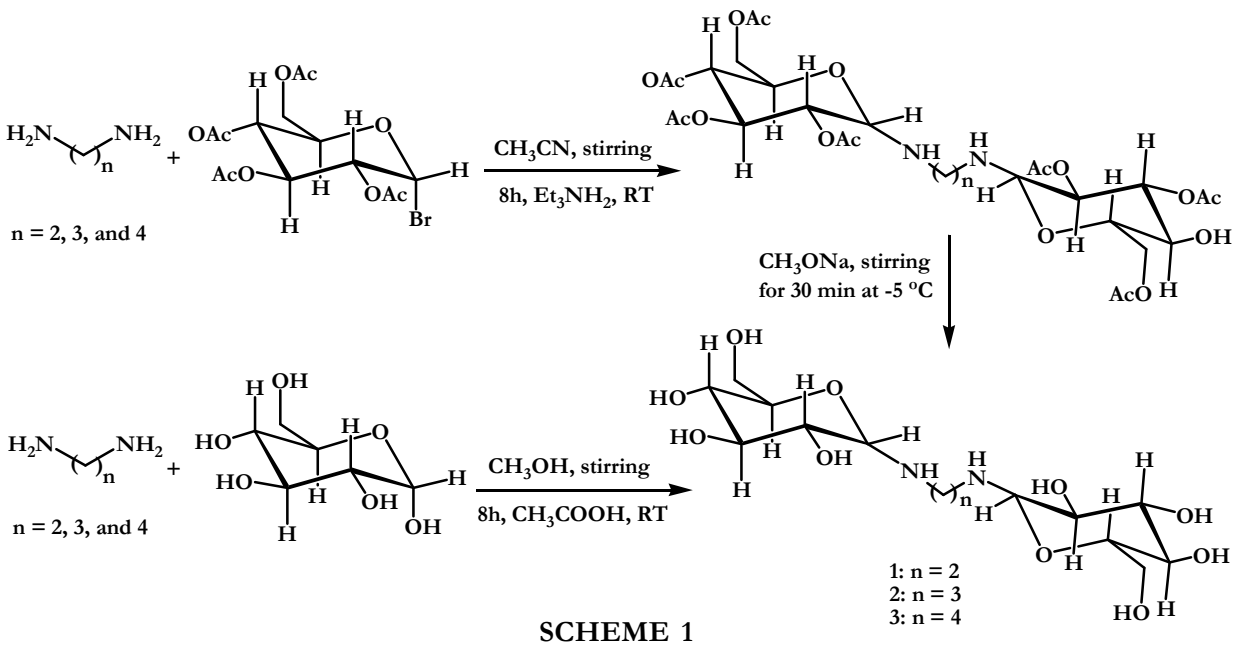
BIOLOGICAL STUDIES

All tests were repeated 2-3 times. For each compound Petri dishes were seeded with B16 melanoma cells grown in 9.69 g l^{-1} Eagle minimum essential medium (Biochrom KG) supplemented (EMEM) 10 ml l^{-1} Penicillin-Streptomycin (10 000 U-10 000 μg ml^{-1}, Biochrom KG), 2.2 g l^{-1} $NaHCO_3$ and 10 % fetal calf serum (FCS). Dishes were incubated overnight at 37 °C in a humidified atmosphere containing 5 % CO_2. The medium was replaced with medium containing varying concentrations of the N-glycosylamine compounds and incubated for an additional 24 h at 37 °C. The medium was removed from the dishes. The cells were suspended by trypsinization, counted and seeded out into new dishes at different dilutions. The numbers of colonies formed after one week were compared to the numbers of colonies formed in the control without boron. The medium was removed, washed with phosphate-buffered saline (PBS), dyed with GI-EMSA stain for 10-15 minutes and washed again with ethanol.

RESULTS AND DISCUSSION

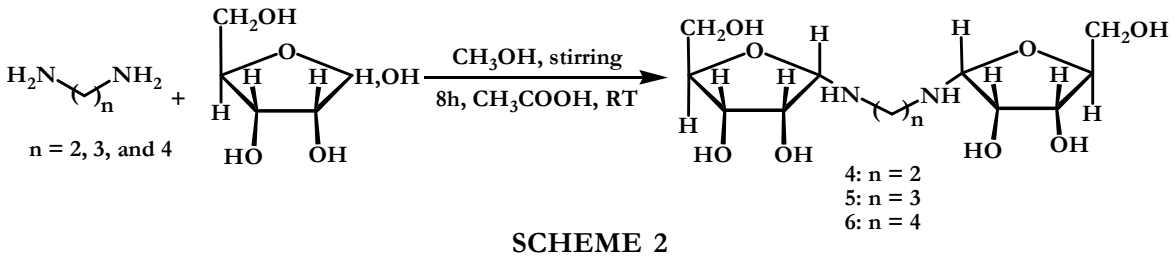
Chemistry

Of particular interest is the development of a one pot methodology that allow entry to multivalent derivatives without any need for protecting strategies. Diamines such as $NH_2(CH_2)_nNH_2$ where n = 2, 3, and 4, were treated with α-D-glucose, and D-ribose in methanol in the presence of glacial acetic acid as a catalyst to afford the corresponding *N*-D-glycosylamines in high yields (SCHEME 1 and 2). The *N*-glycosides could also be obtained by the reaction of the diamines with acetobromo-α-D-glucose in CH_3CN in the presence of triethylamine at room temperature affording N,N′-Di-α-(2,3,4,6-



SCHEME 1

Full Paper



SCHEME 2

tetra-*O*-acetyl)-D-glucopyranosyl diamine a good yield (SCHEME 1). Deacetylation of these compounds with freshly prepared CH_3ONa at -5 °C gave *N,N'*-Di- α -D-glucopyranosyl diamine (SCHEME 1).

The constitution and purity of each of the compounds **(1-6)** were established by IR, NMR, and mass spectroscopy. The IR spectra of compounds **(1-6)** showed absorption bands within the 2925-3932 cm^{-1} region characteristics for OH of carbohydrate moieties overlapped with NH of glucosidic or ribosidic band. NMR spectra of all compounds showed that the anomeric protons appeared as broad signals at δ = 4.83-4.89 ppm, while the anomeric carbons resonate at 89.8-89.52 ppm. ^1H NMR spectrum of compounds **(4-6)** showed that the anomeric proton resonates as sharp signal at δ = 4.85 ppm. While its ^{13}C NMR spectrum showed C-1' resonate at δ=87.95 ppm. These values are in consistent with α-configuration with $^4C_1$(D) configuration[15,18]. The anomeric configurations of the glycosylamines were determined by measuring $^1J[^{13}CH(1)]$ coupling constants for representative targets. Comparison of these with values reported in the literature[19] indicated that the glycosylamines were with α-configuration. The NMR spectroscopic data of the series of compounds among all the family number **(1-6)** are also very similar (see EXPERIMENTAL), although there are some minor variations in the proton shielding as the organodiamine group or sugar moiety changes. Moreover the ESI-MS spectra showed the most intense molecular ion peak (M^+) corresponding to isotopic pattern (see EXPERIMENTAL for details). As for the reaction of diamines and the carbohydrate in ratio 1:1, the mass spectra and elemental analyses do not support at all that only adducts are formed.

Biology

Among various solubilizing substituents, sugars have attracted most attention. Indeed, in addition to providing the molecule with polar hydroxyl groups, its targeting to cellular receptors for sugars might be expected[11]. The high hydrophilic properties of the compounds **(1-6)** allow their direct administration in aqueous solution at suitable concentration levels. No cosolvents such as DMSO, various alcohols or HCl are necessary. The most interesting, and potentially useful compounds for BNCT, would be those that attain a high concentration in tumor cells and are minimally toxic to the host and normal cells.

TABLE 1: *In vitro* toxicity of *N*-glycosylamines (1-6) in B16 melanoma cells[a]

C_{media}	Percentage of survival (%)					
*m*M	1	2	3	4	5	6
0.1	100 ± 2.12	100 ± 1.37	100 ± 1.34	100 ± 1.56	100 ± 2.03	100 ± 0.73
0.5	100 ± 1.89	100 ± 0.98	100 ± 1.32	100 ± 1.02	100 ± 2.19	100 ± 0.75
1.0	100 ± 2.14	96 ± 0.96	91 ± 1.98	100 ± 1.31	96 ± 1.69	92 ± 0.73
2.5	89 ± 2.05	73 ± 1.13	69 ± 1.99	94 ± 1.45	88 ± 1.53	79 ± 0.81
5.0	70 ± 1.25	61 ± 1.78	54 ± 1.15	79 ± 2.14	68 ± 2.18	62 ± 1.25
10.0	65 ± 1.66	51 ± 1.45	33 ± 2.61	68 ± 1.64	57 ± 2.26	38 ± 1.11
25.0	11 ± 2.57	2 ± 1.14	3 ± 2.17	21 ± 0.86	5 ± 1.66	3 ± 1.20

[a] B16 cells were incubated with *N*-glycosylamines for 24 h at different concentrations indicated. Cells were washed (PBS), trypsinized and seeded out for colony formation. After one week, colonies were washed, stained, washed again (ethanol) and counted

Full Paper

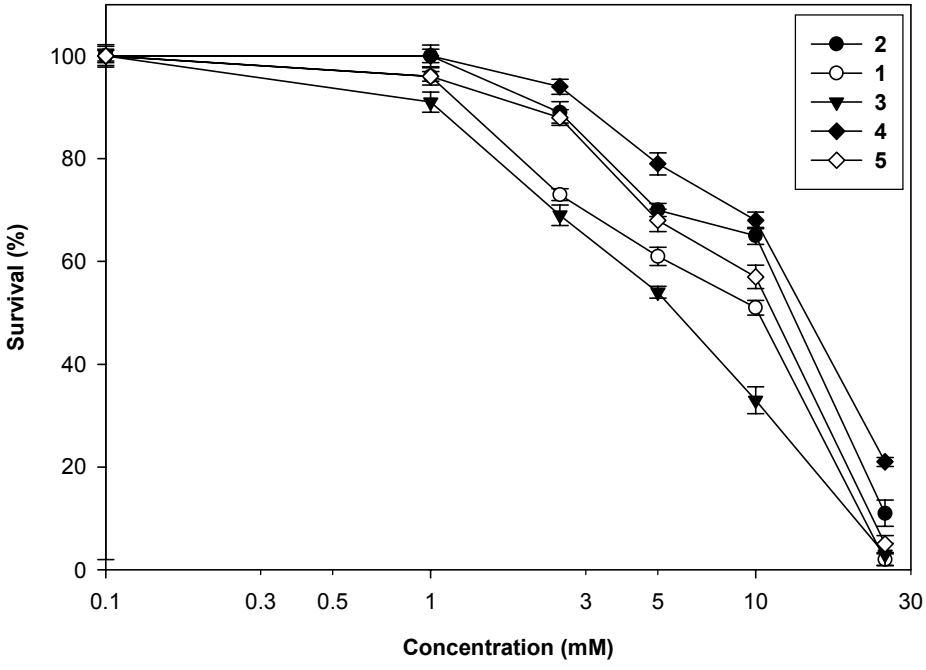


Figure 2: Percentage (± SD) of *in vitro* survival cell with respect to the concentration of the corresponding compounds (1-5)

The *in vitro* toxicity test was carried out to determine whether these compounds were sufficiently nontoxic. Previous experience with the assay has shown it to be a useful *in vitro* test for identifying nontoxic compounds that subsequently could be evaluated *in vivo*[20]. Evaluation of *in vitro* toxicity was made by employing quantified number of B16 melanoma cells, and, following a 24 h exposure to the test compounds, by measuring the number of surviving cells and comparing that to the number of surviving cells not exposed to the test compounds. The results of the *in vitro* toxicities of N-glcosylamines are shown in figure 2 and summarized in TABLE 1. All compounds were tested up to a maximum concentration of the *N*-glycosylamines (10 mM). It was observed that the survival ratio of the compounds **(1-6)** decreased when its concentrations in the medium increased from 0.01 to 10.0 mM with $LD_{50} > 6$ mM (TABLE 1 and Figure 2). Even at the higher level concentration (5 mM) of *N*-glycopyransoylamines, no toxicity from the drugs **(4-6)** was observed. Toxicity was increased also with increasing the number of carbon atoms in the diamine chains. The *in vitro* toxicities of the compounds **(4-6)** are lower than those of compounds **(1-3)**.

CONCLUSION

A simple and convenient procedure for the synthesis of some novel N-glycopyranosylamines and N-glycofuransoylamines was investigated. The structure of the newly prepared compounds were confirmed by both analytical and spectral data. The compounds **(4-6)** appear not to be toxic at wide range of *N*-glycosylamine concentrations even at (5 mM). These compounds might be useful as delivery agents for cancer treatment.

ACKNOWLEDGMENTS

Professor doctor Detlef Gabel and the financial support from the University of Bremen, Germany, are gratefully acknowledged.

REFERENCES

[1] C.W.Tabor, H.Tabor; Annu.Rev.Biochem., **53**, 749-790 **(1984)**.

[2] J.L.Holley, A.Mather, R.T.Wheelhouse, P.M.Cullis, J.A. Hartley, J.P.Bingham, G.M.Cohen; Cancer Res., **52**, 4190-4195 **(1992)**.

Full Paper

[3] Y.Li, J.L.Eisenman, D.L.Sentz, F.A.Rogers, S.S.Pan, L.T.Hu, M.L.Egorin, P.S.Callery; J.Med.Chem., **39**, 339-341 **(1996)**.

[4] P.A.Stark, B.D.Thrall, G.G.Meadows, M.M.Abdel-Monem; J.Med.Chem., **35**, 4264-4269 **(1992)**.

[5] B.G.Davis; Chem.Rev., **102**, 579-601 **(2002)**.

[6] D.Zopf, S.Roth; Lancet, **347**, 1017-1021 **(1996)**.

[7] G.M.Cohen, P.M.Cullis, J.A.Hartley, A.Mather, M.C. R.Symons, R.T.Wheelhouse; J.Chem.Soc., Chem. Commun., 298-300 **(1992)**.

[8] N.Seiler, J.G.Delcros, J.P.Moulinoux; Int.J.Biochem. Cell Biol., **28**, 843-861 **(1996)**.

[9] P.M.Cullis, M.C.R.Symons, G.M.Cohen, P.Wardmar; Med.Sci.Res., **8**, 87 **(1990)**.

[10] C.W.Porter, R.J.Bergeron, N.J.Stolowich; Cancer Res. **42**, 4072 **(1982)**.

[11] M.Monsigny, A.C.Roche, P.Midoux, R.Mayer; Adv. Drug Delivery Rev., **14**, 1-24 **(1994)**.

[12] M.Veyhl, K.Wagner, C.Volk, V.Gorboulev, K. Baumgarten, W.M.Weber, M.Schaper, B.Bertram, M. Wiessler, H.Koepsell; Proc.Natl.Acad.Sci.U S A., **95**, 2914-9 **(1998)**.

[13] J.M.MacLeod; Carbohydr.Res., **75**, 71-81 **(1979)**.

[14] S.P.Gaucher, S.F.Perdersen, J.A.Leary; J.Org.Chem., **64**, 4012-4015 **(1999)**.

[15] N.R.El-brollosy, M.F.Abdel-Megeed, G.A.El-Hiti, A.R.Genady; Afinidad, **60(504)**, 199-205 **(2003)**.

[16] W.Hayes, H.M.I.Osborn, S.D.Osborne, R.A.Rastall, B.Romagnoli; Tetraheron, **59**, 7983-7996 **(2003)**.

[17] M.E.El-Zaria, A.R.Genady, D.Gabel; New J.Chem., **1**, 597–602 **(2006)**.

[18] M.F.Abdel-Megeed, M.A.Saleh, Y.L.Aly, I.M.Abdo; Nucleos.Nucleot., **14**, 1985 **(1995)**.

[19] K.Block, C.Pedersen; J.Chem.Soc.Perkin Trans., **2**, 293-297 **(1974)**.

[20] R.J.Bergeron, A.H.Neims, J.S.McManis, T.R. Hawthorne, J.R.T.Vinson, R.Bortell, M.J.Ingeno; J. Med.Chem., **31**, 183-1190 **(1988)**.