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Preparation of Amino Alcohols Condensed with Carbohydrates: Evaluation of Cytotoxicity and Inhibitory Effect on NO Production

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This work reports the preparation of several amino alcohols condensed with D-arabinose, D-glucose, and D-galactose derivatives. These compounds were evaluated *in vitro* for their cytotoxicity and ability to decrease nitric oxide production in J774A.1 cells. Arabinofuranoside derivatives 5a, 5b and 5c showed a significant inhibition of nitric oxide production (>80% at 5 μg/mL), while the galactopyranoside derivative 8d showed a notable nitric oxide inhibitory activity (126% at 0.5 μg/mL).

Key words: amino alcohols, amphiphilicity, carbohydrate, cytotoxicity, nitric oxide production

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The immune system consists of the innate and the acquired immune responses (1). The knowledge of the main immune defense mechanisms allows an understanding of the body's immune response against various diseases (1). Nitric oxide (NO) is an important molecule in innate immunity, mediating diverse functions and also regulating the adaptive response. NO acts as an essential multifunctional mediator in various biologic systems (2). Immunosuppressive drugs are the principal compounds that can affect the immune response, reducing the activation of the innate and the acquired immunity (3). These drugs play an important role in clinical transplant patients and in the treatment of autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus (2,4). The sugar component participates in the molecular recognition of the cellular target, in many cases being essential for the biologic activity of the drugs (5). Zhang *et al.* (6,7) have investigated the effects of *N*-alkylated iminosugars on immune system, showing that these compounds hold potential as immunosuppressive agents. A large number of lipidic amino alcohols and derivatives have been described in the literature and their apoptotic and immunosuppressive activities tested (4,5,8–11). In this context, this work describes the synthesis of several amino alcohols condensed with three different carbohydrates and the biologic evaluation of their cytotoxic properties and their ability to decrease NO production. The aim of this study was to evaluate the influence of amino alcohol and carbohydrate moieties in this activity.

Experimental

General methods

Melting points were determined on a Microquímica MQAPF apparatus (Microquímica Ltda, Cotia, SP-Brazil) and are uncorrected. IR spectra were recorded using a BOMEM-FTIR MB102 spectrometer (ABB Bomem Inc., Quebec, Canada). Optical rotations were measured with a Perkin-Elmer 341 polarimeter (Perkin Elmer, Waltham, MA, USA), using a sodium lamp at 20 °C. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Advance DRX300 spectrometer (Bruker Corporation, Billerica, MA, USA). Thin-layer chromatography (TLC) was performed on glass plates and silica gel sheets (Silica Gel F254; Merck, Whitehouse Station, NJ, USA), visualized with iodine vapor, and/or revealed with ethanolic H_2SO_4 solution. Column chromatography was carried out on silica gel 60 (E. Merck 70–230 mesh). Solvents were purchased from Vetec Química (Vetec, Xerem, RJ, Brazil) and were distilled prior to use. Reagents were purchased from Aldrich (Sigma Aldrich, Saint Louis, MI, USA) and used without further purification.

Preparation of methyl 5-O-p-toluenesulfonyl-α-D-arabinofuranoside 3

p-Toluenesulfonyl chloride (22 mmol) was added to a mixture of alcohol **2** (20 mmol) in pyridine (15 mL) at 0 °C. The mixture was stirred at room temperature for 24 h and then concentrated under reduced pressure. The residue was dissolved in methylene chloride and washed three times with water. After evaporation of the organic phase, the residue was purified by column chromatography to furnish the desired compound **3** in 56% yield (11.2 mmol).

$[\alpha]_D$: +3.0 (*c* 0.69, CH_2Cl_2); IR (ν, cm^{-1}, KRS-5): 3435, 1396, 863, 665; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.80 (d, 2H, $J_{2',3'}$ 6.0 Hz, H2′ and

H6′); 7.35 (d, 2H, $J_{3',2'}$ 6.0 Hz, H3′ and H5′); 4.81 (s, 1H, H1); 4.22–4.05 (m, 4H, H2, H3, H4, H5a); 3.95–3.92 (m, 1H, H5b); 3.34 (s, 3H, OCH_3); 2.44 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 145.3 (C4′); 132.5 (C1′); 130.0 (C3′ and C5′); 128.1 (C2′and C6′); 108.8 (C1); 82.3, 80.7, 77.6 (C2, C3, C4); 69.4 (C5); 55.2 (OCH_3); 21.7 (CH_3).

General procedure for the preparation of amino alcohols 4, 5a-c, and 6

Tosylate **3** (1 mmol) was dissolved in ethanol (3 mL) and slowly added to a solution of the amino alcohols: ethanolamine, *N*-alkyl-aminoethanols (*N*-decyl-aminoethanol, *N*-dodecyl-aminoethanol), or 1-amino-dodecan-2-ol (1.2–2 mmol). The mixture was stirred at reflux for 24–48 h, and the solution was concentrated under reduced pressure. The crude product was dissolved in methylene chloride and washed three times with water. After drying with sodium sulfate, the organic phase was concentrated under reduced pressure. The residue was chromatographed on silica gel (methylene chloride/methanol) to furnish the desired compounds **5a–c** and **6**. To obtain amino alcohol **4**, the crude product was chromatographed directly on silica gel (CH_2Cl_2/MeOH).

Spectral data

Methyl 5-(2-hydroxyethylamino)-5-deoxy-α-D-arabinofuranoside 4. yield, 89%; $[\alpha]_D$: +37.3 (*c* 0.5, CH_3OH); IR (*v*, cm^{-1}, KRS-5): 3390, 1624, 1067; ^1H NMR (CD_3OD, 300 MHz): δ 4.69 (s, 1H, H1); 3.93 (s, 1H, H4); 3.85 (s, 1H, H2); 3.65–3.52 (m, 3H, H3 and C*H*$_2$OH); 3.29 (s, 3H, OCH_3); 2.86–2.82 (m, 1H, H5); 2.75–2.69 (m, 3H, H5′ and CH_2N); ^{13}C NMR (CD_3OD, 75 MHz): δ 110.9 (C1); 83.4, 83.1, 81.1 (C4, C2, C3); 60.6 (CH_2OH); 55.5 (OCH_3); 52.2; 52.1 (C5 and CH_2N).

Methyl 5-[(*N*-octyl)-2-hydroxyethylamino]-5-deoxy-α-D-arabinofuranoside 5a. yield, 32%; $[\alpha]_D$: +39.7 (*c* 0.3, CH_3OH); IR (*v*, cm^{-1}, KRS-5): 3379, 2924, 1466, 1020; ^1H NMR ($CDCl_3$, 300 MHz): δ 4.90 (s, 1H, H1); 4.22 (s, 1H, H4); 3.98 (s, 1H, H3); 3.92 (s, 1H, H2); 3.68 (t, 2H, $J_{7,6}$ 3.5 Hz, C*H*$_2$OH); 3.39 (s, 3H, OCH_3); 2.92–2.78 (m, 3H, H5 and CH_2N); 2.72–2.65 (m, 2H, CH_2N); 2.63–2.47 (m, 1H, H5′); 1.50 (s, 2H, $NCH_2CH_2CH_2$); 1.28 (s, 10H, CH_2aliph); 0.88 (t, 3H, *J* 6.8 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 109.9 (C1); 87.5, 79.3, 78.0 (C4, C2, C3); 60.4–56.7 (C5, CH_2N and CH_2OH); 55.2 (OCH_3); 32.0–22.8 (CH_2aliph); 14.3 (CH_3).

Methyl 5-[(*N*-decyl)-2-hydroxyethylamino]-5-deoxy-α-D-arabinofuranoside 5b. yield, 39%; $[\alpha]_D$: +17.7 (*c* 0.23, CH_3OH); IR (*v*, cm^{-1}, KRS-5): 3375, 2922, 1464, 1024; ^1H NMR ($CDCl_3$, 300 MHz): δ 4.91 (s, 1H, H1); 4.24 (s, 1H, H4); 3.97 (s, 1H, H3); 3.92 (s, 1H, H2); 3.69 (s, 3H, C*H*$_2$OH and OH); 3.40 (s, 3H, OCH_3); 2.92–2.76 (m, 3H, H5 and CH_2N); 2.67–2.62 (m, 2H, CH_2N); 2.55–2.50 (m, 1H, H5′); 1.49 (s, 2H, $NCH_2CH_2CH_2$); 1.26 (s, 14H, CH_2aliph); 0.88 (t, 3H, *J* 6.4 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 109.9 (C1); 88.0, 79.2, 77.6 (C4, C2, C3); 60.6–56.7 (C5, CH_2N and CH_2OH); 55.2 (OCH_3); 32.1–22.9 (CH_2aliph); 14.3 (CH_3).

Methyl 5-[(*N*-dodecyl)-2-hydroxyethylamino]-5-deoxy-α-D-arabinofuranoside 5c. yield, 30%; $[\alpha]_D$: −25.9 (*c* 0.15, CH_3OH); IR (*v*, cm^{-1}, KRS-5): 3385, 2923, 1466, 1028; ^1H NMR ($CDCl_3$, 300 MHz): δ 4.73 (s, 1H, H1); 4.16 (s, 1H, H4); 3.90 (s, 1H, H3 or H2); 3.85 (s, 1H, H3 or H2); 3.60 (s, 2H, C*H*$_2$OH); 3.33 (s, 3H, OCH_3); 2.80–2.38 (m, 6H, H5, H5′ and CH_2N); 1.42 (s, 2H, $NCH_2CH_2CH_2$aliph); 1.19 (s, 18H, CH_2aliph); 0.81 (t, 3H, *J* 6.6 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 109.9 (C1); 87.6, 79.3, 77.8 (C4, C2, C3); 60.4–56.7 (C5, CH_2N and CH_2OH); 55.2 (OCH_3); 32.1–22.9 (CH_2aliph); 14.3 (CH_3).

Methyl 5-[*N*-2-hydroxy-dodecylamino]-5-deoxy-α-D-arabinofuranoside 6. yield, 30%; $[\alpha]_D$: +35.5 (*c* 0.39, CH_3OH); IR (*v*, cm^{-1}, KRS-5): 3366, 2922, 1099; ^1H NMR ($CDCl_3$, 300 MHz): δ 4.89 (d, 1H, $J_{1,2}$ 3.1 Hz, H1); 4.23 (s, 1H, H4); 3.98–3.71 (m, 5H, H2, H3, C*H*OH, OH and NH); 3.38 (s, 3H, OCH_3); 3.13–3.03 (m, 1H, H5); 2.94–2.80 (m, 2H, H5′ and H6); 2.70–2.59 (m, 1H, H6′) 1.40 (s, 2H, $NCH_2CH_2CH_2$aliph); 1.25 (s, 16H, CH_2aliph); 0.88 (t, 3H, *J* 6.1 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 110.1 (C1); 86.2, 79.9, 78.7 (C4, C2, C3); 69.9 (*C*H(OH)CH_2aliph); 56.0 and 56.2 (OCH_3); 55.2, 49.7, 49.8 (C5, CH_2N); 35.5–22.9 (CH_2aliph); 14.3 (CH_3).

General procedure for the preparation of compounds 8a-e, 9, 11, and 12

A solution of the amino alcohol (3.0 mmol) in ethanol (5 mL) was added to a solution of **7** or **10** (2 mmol) in ethanol (3 mL). The mixture was stirred at reflux for 48–120 h, and the solution was concentrated under reduced pressure. The crude product was dissolved in methylene chloride and washed three times with water. After drying with sodium sulfate, the organic phase was concentrated under reduced pressure. The residue was chromatographed on silica gel (methylene chloride/methanol) to furnish the desired compounds **8a–e** and **9**. To obtain amino alcohols **11** and **12**, the crude products were directly chromatographed on silica gel (CH_2Cl_2/MeOH).

Spectral data

6-(2-Hydroxypropylamino)-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose 8a. yield, 68%; $[\alpha]_D$: −33.6 (*c* 0.5, CH_2Cl_2); IR (*v*, cm^{-1}, KRS-5): 3305, 2985, 1064; ^1H NMR ($CDCl_3$, 300 MHz): δ 5.50 (d, 1H, $J_{1,2}$ 4.8 Hz, H1); 4.57 (d, 1H, $J_{3,4}$ 7.9 Hz, H3); 4.28 (d, 1H, $J_{2,1}$ 4.8 Hz, H2); 4.15 (d, 1H, $J_{4,3}$ 7.9 Hz, H4); 3.87 (s, 1H, H5); 3.77 (m, 2H, C*H*$_2$OH); 3.06 (m, 2H, CH_2N); 2.88–2.76 (m, 4H, H6 and OH/NH); 1.68 (s, 2H, CH_2); 1.31; 1.43; 1.50 (3s, 12H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 109.5 and 108.8 (CiPr); 96.5 (C1); 72.1, 71.0, 70.7 (C4, C3, C2); 66.8 (C5); 64,2 (CH_2OH); 49.5 and 49.4 (CH_2N); 30.7 (CH_2); 26.2; 26.1; 25.1; 24.5 (CH_3iPr).

6-[(2′-Hydroxy-1′,1′-dimethyl)-ethylamino]-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose 8b. White crystals; mp, 78.5–79.6 °C; yield, 74%; $[\alpha]_D$: −60.5 (*c* 0.6, CH_2Cl_2); IR (*v*, cm^{-1}, KRS-5): 3294, 3211, 2871, 1067; ^1H NMR ($CDCl_3$, 300 MHz): δ 5.52 (d, 1H, $J_{1,2}$ 3.9 Hz, H1); 4.59 (d, 1H, $J_{3,4}$ 7.7 Hz, H3); 4.31–4.23 (m, 2H, H2 and H4); 3.80 (s, 1H, H5); 3.34 (d, 1H, *J* 10.3 Hz, C*H*$_2$OH); 3.23 (d, 1H, *J* 10.3 Hz, C*H*$_2$OH); 2.82 (dd, 1H, $J_{6,5}$ 8.8 Hz, $J_{6,6'}$ 10.5 Hz, H6); 2.63–2.61 (m, 1H, H6′); 2.28 (s, 2H, OH and NH); 1.53; 1.44; 1.31 (3s, 12H, CH_3); 1.06 (s, 6H, CH_3) ^{13}C NMR ($CDCl_3$, 75 MHz): δ 109.5 and 108.8 (CiPr); 96.5 (C1); 72.0, 70.9, 70.7 (C4, C3, C2); 68.0 (C5); 67.9 (CH_2OH); 54.0

($NC(CH_3)_2$); 42.0 (CH_2N); 26.3, 25.4, 25.1, 24.9 (CH_3); 24.6 and 23.6 (CH_3).

***N,N*-bis-6-(deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose)-3-hydroxypropylamino 9.** yield, 12%; $[\alpha]_D$: −69.5 (*c* 0.5, CH_2Cl_2); IR (*v*, cm^{-1}, KRS-5): 3365, 2983, 1068; ^1H NMR ($CDCl_3$, 300 MHz): δ 5.49 (d, 2H, $J_{1,2}$ 4.8 Hz, H1); 4.54 (d, 2H, $J_{3,4}$ 4.5 Hz, H3); 4.23–4.20 (m, 4H, H2, and H4); 3.92 (s, 2H, H5); 3.71 (s, 2H, CH_2OH); 3.17 (s, 1H, OH); 2.83–2.63 (m, 6H, CH_2N); 1.73–1.64 (s, 2H, CH_2); 1.55; 1.44; 1.33 (3s, 24H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 109.1 and 108.5 (CiPr); 96.6 (C1); 72.0, 70.9, 70.6 (C4, C3, C2); 65.9 (C5); 62.4 (CH_2OH); 54.7 and 53.9 (CH_2N); 29.1 (CH_2); 26.2, 25.2, 25.0, 24.4 (CH_3).

Methyl 6-(2-hydroxypropylamino)-6-deoxy-α-D-glucopyranoside 11. yield, 62%; $[\alpha]_D$: +128.1 (*c* 0.5, CH_3OH); IR (*v*, cm^{-1}, KRS-5): 3361, 2920, 1047; ^1H NMR (CD_3OD, 300 MHz): δ 4.53 (d, 1H, $J_{1,2}$ 6.0 Hz, H1); 3.52–3.44 (m, 3H, H2, H3, H5) 3.28–3.21 (m, 5H, C*H*$_2$OH and OCH_3); 3.0 (t, 1H, $J_{4,5}$ 9.0 Hz, H4); 2.87–2.82 (dd, 1H, $J_{6,5}$ 3.0 Hz, $J_{6,6'}$ 12.0 Hz, H6); 2.61–2.52 (m, 3H, H6′ and C*H*$_2$N); 2.01–1.99 (m, 1H, NH/OH); 1.63–1.58 (m, 2H, CH_2); ^{13}C NMR (CD_3OD, 75 MHz): δ 101.4 (C1); 75.1, 74.4, 73.7 (C4, C3, C2); 71.1 (C5); 61.7 (CH_2OH); 56.0 (OCH_3); 52.2, 48.2 (C6 and CH_2N); 33.1 (CH_2).

Methyl 6-[(2′-hydroxy-1′,1′-dimethyl)-ethylamino]-6-deoxy-α-D-glucopyranoside 12. White crystals; mp, 98–99.6 °C; yield, 50%; $[\alpha]_D$: +92.3 (*c* 0.33, CH_3OH); IR (*v*, cm^{-1}, KBr): 3541, 3263, 2970, 1043; ^1H NMR (CD_3OD, 300 MHz): δ 4.64 (d, 1H, $J_{1,2}$ 3.6 Hz, H1); 3.62–3.56 (m, 2H, H3 and H5) 3.40–3.31 (m, 5H, H2, NH or OH and OCH_3); 3.28 (d, 2H, *J* 9.9 Hz, C*H*$_2$OH); 2.93 (t, 1H, $J_{4,5}$ 9.0 Hz, H4); 2.94–2.89 (dd, 1H, $J_{6,5}$ 2.4 Hz, $J_{6,6'}$ 11.6 Hz, H6); 2.62 (dd, 1H, $J_{6',5}$ 2.4 Hz, H6′); 1.04 (s, 6H, CH_3); ^{13}C NMR (CD_3OD, 75 MHz): δ 101.4 (C1); 75.0, 74.8, 73.7 (C4, C3, C2); 72.0 (C5); 69.4 (CH_2OH); 56.0 (OCH_3); 54.8 ($NC(CH_3)_2CH_2OH$); 45.0 (C6); 24.2; 23.1 (CH_3).

Cytotoxicity and inhibition of NO production

MTT assay

Cell proliferation was measured using the MTT [3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide] test. Culture plates without supernatants were incubated with 100 μL of supplemented RPMI medium and 10 μL of MTT (5 mg/mL) for 4 h at 37 °C in 5% CO_2. After this time, the reaction was stopped by adding to each well 100 μL of an acidic isopropanol solution (100 mL of isopropyl alcohol/0.4 mL of HCl 10 N). Following 10-min incubation at room temperature, the optical density (OD) values at 570 nm were determined (Spectramax 190; Molecular Devices, Sunnyvale, CA, USA).

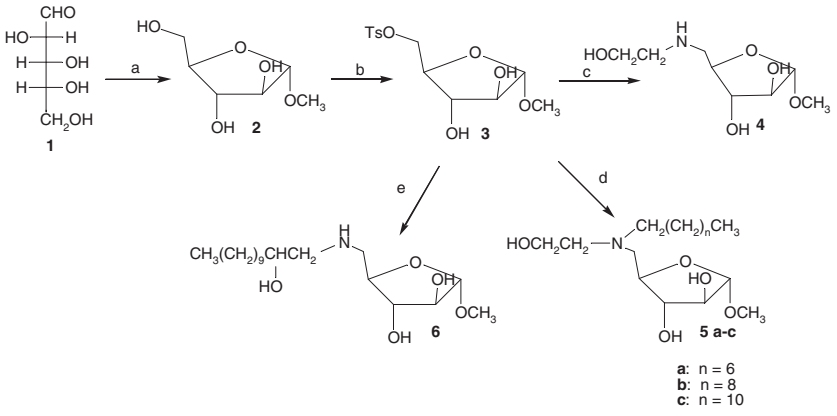
Griess method

Supernatants of J774A.1 cells were analyzed for the quantification of nitrites (NO_2^-) through the Griess method. Aliquots of supernatants were plated with 1% of sulfanilamide and 0.1% of *N*-(1-naphthyl) ethylenediamine. NO_2^- production was quantified by comparing with a standard curve, made with different concentrations of $NaNO_2$. The reading was made in the microplate reader (Spectramax 190; Molecular Devices) at 540 -nm wavelength.

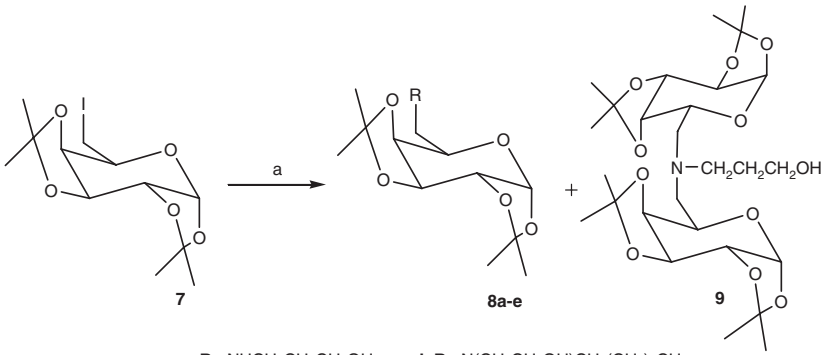
Results and Discussion

Initially, arabinofuranoside amino alcohol derivatives were synthesized (Scheme 1). D-arabinose **1** was first reacted with methanol under acidic conditions (Amberlite IR-120, Sigma-Aldrich) leading to methyl α-D-arabinofuranoside **2** (73% yield), which was subsequently treated with *p*-toluenesulfonyl chloride in pyridine to give compound **3** at 56% yield. This tosylated derivative was then treated with different amino alcohols in ethanol under reflux, furnishing the desired compounds **4**, **5a–c**, and **6** at 30–89% yields. The low yield in the preparation of the amino alcohols **5a–c** and **6** was mainly because of the formation of the methyl 2,5-anydro-α-D-arabinofuranoside.

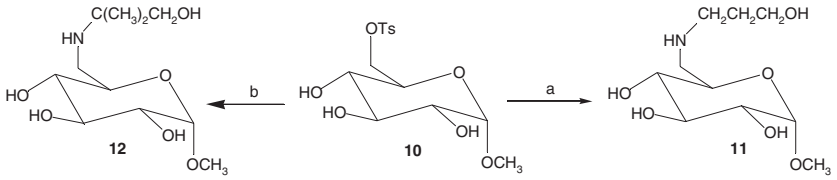
D-Galactose derivatives were prepared using a method previously described (10). D-galactose was first converted into 6-deoxy-6-iodo-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose **7** according to the literature procedure (11–13) and then treated with five different amino alcohols in ethanol under reflux (Scheme 2). Purification of the crude products was carried out by column chromatography (hexane:ethyl acetate) resulting in the isolation of amino alcohols **8a–e** at 40–80% yields. In this series, reaction of iodided derivative **7** with 1,3-propanolamine resulted in the formation of amino alcohol **9** at 12% yield.



Scheme 1: Preparation of D-arabinose derivatives. Reagents and conditions: (a) Amberlite-IR120, MeOH, 60 °C (73%); (b) TsCl, pyridine, 0 °C-r.t. (56%); (c) ethanolamine, MeOH, reflux (89%); (d) $CH_3(CH_2)_nCH_2NHCH_2CH_2OH$, EtOH, reflux (30–39%); (e) $NH_2CH_2CH(OH)(CH_2)_9CH_3$, EtOH, reflux (30%).



Scheme 2: Preparation of D-galactose derivatives. Reagents and conditions: (a) **8a**: NH_2CH_2-CH_2CH_2OH, EtOH, reflux (68%); **8b**: $NH_2C(CH_3)_2CH_2OH$, EtOH, reflux (74%); **8c:** $NH_2CH_2CH_2OH$, EtOH, reflux (80%); **8d–e**: $NH(CH_2CH_2OH)CH_2(CH_2)_nCH_3$, DMSO, 90 °C (40–56%).



Scheme 3: Preparation of D-glucose derivatives. Reagents and conditions: (a) $NH_2CH_2CH_2CH_2OH$, EtOH, reflux (62%); (b) $NH_2C(CH_3)_2CH_2OH$, EtOH, reflux (50%).

Amino alcohol derivatives **11** and **12** were obtained from methyl α-D-glucopyranoside, which was first converted into its tosylate **10**. The later compound was treated with 1,3-propanolamine and 2-amino-2-methylpropanol giving amino alcohols **11** and **12**, respectively (Scheme 3). All the compounds were purified by column chromatography. The structure was assigned by Infrared, ^{1}H NMR, ^{13}C NMR, and COSY experiments.

Cytotoxicity and inhibition of NO production

The cytotoxicity of the amino alcohols on J774A.1 cell culture was determined by a colorimetric MTT assay. The determination was based on the cell viability in the presence of serial concentrations of the tested compounds. The percentage of cytotoxicity was calculated from the mean of optical density (OD) measures according to the formula CTX (%) = $[1-(OD_{(J774A.1\ +\ compound)}/OD_{(J774A.1)})] \times 100$. None of the tested compounds demonstrated cytotoxic activity at low concentrations (0.5–0.00005 μg/mL) (Table 1).

The ability of the compounds to decrease NO production was evaluated by the Griess method using J744A.1 cell culture stimulated with interferon-gamma (IFNγ) plus lipopolysaccharide (LPS). N^G-monomethyl-L-arginine (LNMA) was used as a positive standard

Table 1: Inhibition of nitric oxide (NO) production and cytotoxicity (CTX) evaluation of glycosylated amino alcohols. J774A.1 cells were stimulated by IFN-γ/LPS and treated by serial concentrations of glycosylated amino alcohols per 48 h

	Inhibition of NO, and cytotoxicity (%) by different compound concentrations											
	5 μg/mL		0.5 μg/mL		0.05 μg/mL		0.005 μg/mL		0.0005 μg/mL		0.00005 μg/mL	
Compound	NO[a]	CTX[b]	NO	CTX	NO	CTX	NO	CTX	NO	CTX	NO	CTX
4	53.9	27.9	50.6	ns	56.2	ns	51.4	ns	53.4	ns	38.7	ns
5[a]	81.2	ns[c]	48.8	ns	60.2	ns	59.2	ns	47.3	ns	ns	ns
5[b]	106.6	ns	71.8	ns	61.7	ns	54.4	ns	41.5	ns	ns	ns
5[c]	121.0	ns	30.6	ns	ns	ns	ns	ns	ns	ns	ns	ns
6	132.2	36.3	71.3	ns	60.1	ns	62.3	ns	63.7	ns	42.0	ns
8[a]	67.5	24.9	62.9	ns	62.0	ns	55.8	ns	53.0	ns	25.0	ns
8[d]	–[d]	–	126	ns	17	ns	13	ns	–	–	–	–
9	51.5	ns	52.4	ns	60.1	ns	56.4	ns	47.6	ns	30.3	ns
11	46.3	ns	42.6	ns	34.3	ns	34.8	ns	21.8	ns	ns	ns
12	45.3	ns	44.9	ns	41.7	ns	42.3	ns	46.3	ns	ns	ns

[a]Inhibition of NO production in percentage NO (%) = $[1-(NO_{(J774A.1\ +\ stimulus\ +\ compound)}-NO_{(J774A.1\ +\ LNMA)}/NO_{(J774A.1\ +\ stimulus)}-NO_{(J774A.1\ +\ LNMA)})] \times 100$.
[b]Cytotoxicity percentage CTX (%) = $[1-(OD_{(J774A.1\ +\ compound)}/OD_{(J774A.1)})] \times 100$.
[c]Non-significant values.
[d]Non-tested dosages.

for NO inhibition (100%). The percentage of NO inhibition was calculated using the following formula, using the mean of NO results: NO (%) = [1− ($NO_{(J774A.1 + stimulus + compound)}$−$NO_{(J774A.1 + LNMA)}$/$NO_{(J774A.1 + stimulus)}$−$NO_{(J774A.1 + LNMA)}$)] × 100. The highest inhibition of NO production was obtained by arabinofuranoside derivative **6** at 5 μg/mL. However, it was cytotoxic at this concentration. In lower concentrations (0.5–0.0005 μg/mL), this compound was not cytotoxic and inhibited macrophage NO production by more than 60%. Other arabinofuranoside derivatives **5a**, **5b,** and **5c** also showed a significant inhibitory effect on NO production, above 80%, at 5 μg/mL concentration. The derivative **8d** showed a notable NO inhibition (126% at 0.5 μg/mL). Although not cytotoxic, the glucopyranoside derivatives **11** and **12** did not display significant NO inhibitory activity in relation to other substances tested. Secondary amines **4** (arabinofuranoside) and **8a** (galactopyranoside) were less active than tertiary amines **5a**, **5b**, **5c,** and **8d**, at the concentration of 5 μg/mL. Compounds **4** and **8a** showed elevated cytotoxicity at this same concentration.

All alkylated compounds containing an unprotected carbohydrate (compounds **5a–c** and **6**) displayed a good inhibition of NO production at 5 μg/mL, while non-alkylated compounds **11** and **12** or alkylated amino alcohols with a protected carbohydrate were less active. These results suggest that the carbohydrate moieties of the molecule play a part in the biologic activity and that amphiphilicity could be essential.

A correlation between biologic activity and hydrophobic character is frequently observed (14). The partition coefficient *P* of a compound in an *n*-octanol/water system represents the hydrophobic properties of this compound and can be determined experimentally or calculated. Considering the inhibition of NO production for the non-toxic compounds at a concentration of 5 μg/mL, we could establish a linear correlation between NO inhibition (%) and log (*P*) (Table 2, Figure 1).

Linear regression led to the following function:

$$\text{NO inhibition} = 14.94 \log(P) + 75.45 \text{ with a correlation coefficient of } 95\%$$

Table 2: Nitric oxide (NO) inhibition (%) and log (*P*) at a concentration of 5 μg/mL

Compound	Inhibition of NO	Log (*P*)
11	46.3	−2.39
4	53.9	−1.86
12	45.3	−1.75
9	51.5	−1.05
5a	81.2	1.02
5b	106.6	1.85
5c	121.0	2.68

Log (*P*) values were calculated using Chemdraw Ultra 12 software (trial version).

These results confirm the existence of a relationship between lipophilicity and inhibition of NO production using a 5 μg/mL concentration of the tested compounds.

The studies on detailed immunosuppressive mechanism of amino alcohols condensed with carbohydrate are still under investigation.

Conclusion

In summary, a series of amino alcohols condensed with D-arabinose, D-glucose, and D-galactose derivatives were prepared and evaluated *in vitro* for their cytotoxicity and ability to influence NO production in J774A.1 cells. Arabinofuranoside derivatives **5a**, **5b**, and **5c** showed a significant inhibition of NO production (>80% at 5 μg/mL concentration), while galactopyranoside derivative **8d** showed a notable inhibitory activity on NO production (126% at 0.5 μg/mL). It seems that amphiphilicity is important for the biologic activity.

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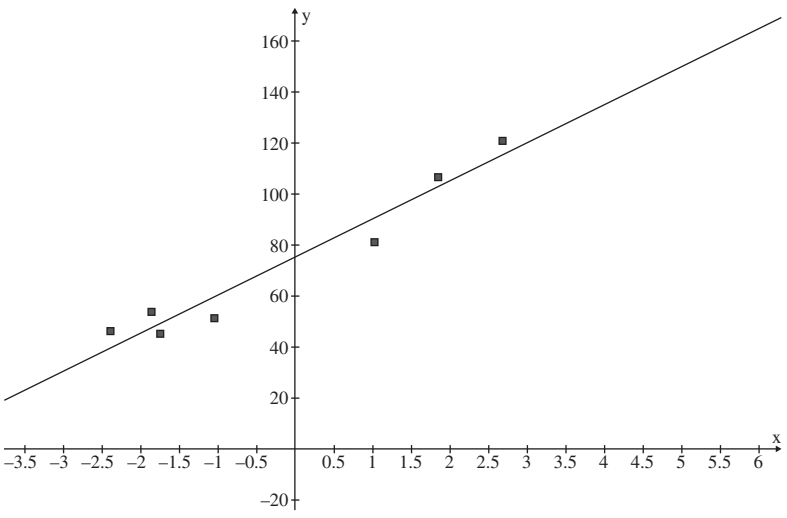

Figure 1: Linear correlation between nitric oxide (NO) inhibition (%) and log (*P*) at a concentration of 5 μg/mL.

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