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Note

An easy stereospecific synthesis of 1-amino-2,5-anhydro-1-deoxy-D-mannitol and arylamino derivatives

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Abstract

1-Amino-2,5-anhydro-1-deoxy-D-mannitol and a series of arylamino derivatives were prepared by nitrous acid deamination of 2-amino-2-deoxy-D-glucose and subsequent reductive amination of the resulting 2,5-anhydro-D-mannose. Some of these compounds showed an enhanced affinity for the hexose transporter of *Trypanosoma brucei* as compared to D-fructose. © 1999 Elsevier Science Ltd. All rights reserved.

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1. Introduction

In the course of a programme devoted to glycolytic enzymes inhibitors and D-glucose intake in the trypanosome [1], we became interested in the synthesis of D-fructose analogues [2] for the following reasons: (i) the hexose transporter of *Trypanosoma brucei* (THT1) recognizes not only D-glucose but also D-fructose, whereas that of human erythrocyte has only affinity for D-glucose [3,4]. Because of this peculiarity, one can consider the possibility of the specific intake of active compounds into the parasite via the transporter by binding such active compounds to a D-fructose unit, or to the analogue of β-D-fructose, 2,5-anhydro-D-mannitol, which also has a significant affinity for the hexose transporter THT1 [3,4]. Bringaud and Baltz [5,6] have shown that genes encoding for this transporter were highly conserved among kinetoplastidae, a feature which underlines the importance of this protein as a target for chemotherapy of trypanomiasis (sleeping sickness). These authors have also shown that these transporters are different in structure from that of the erythrocyte GLUT1 as revealed by a distinct sensitivity towards classical inhibitors; (ii) three of the enzymes of the glycolytic cascade, phosphoglucoisomerase, phosphofructokinase and aldolase, involve a phosphorylated fructose as substrate or product. In addition, the

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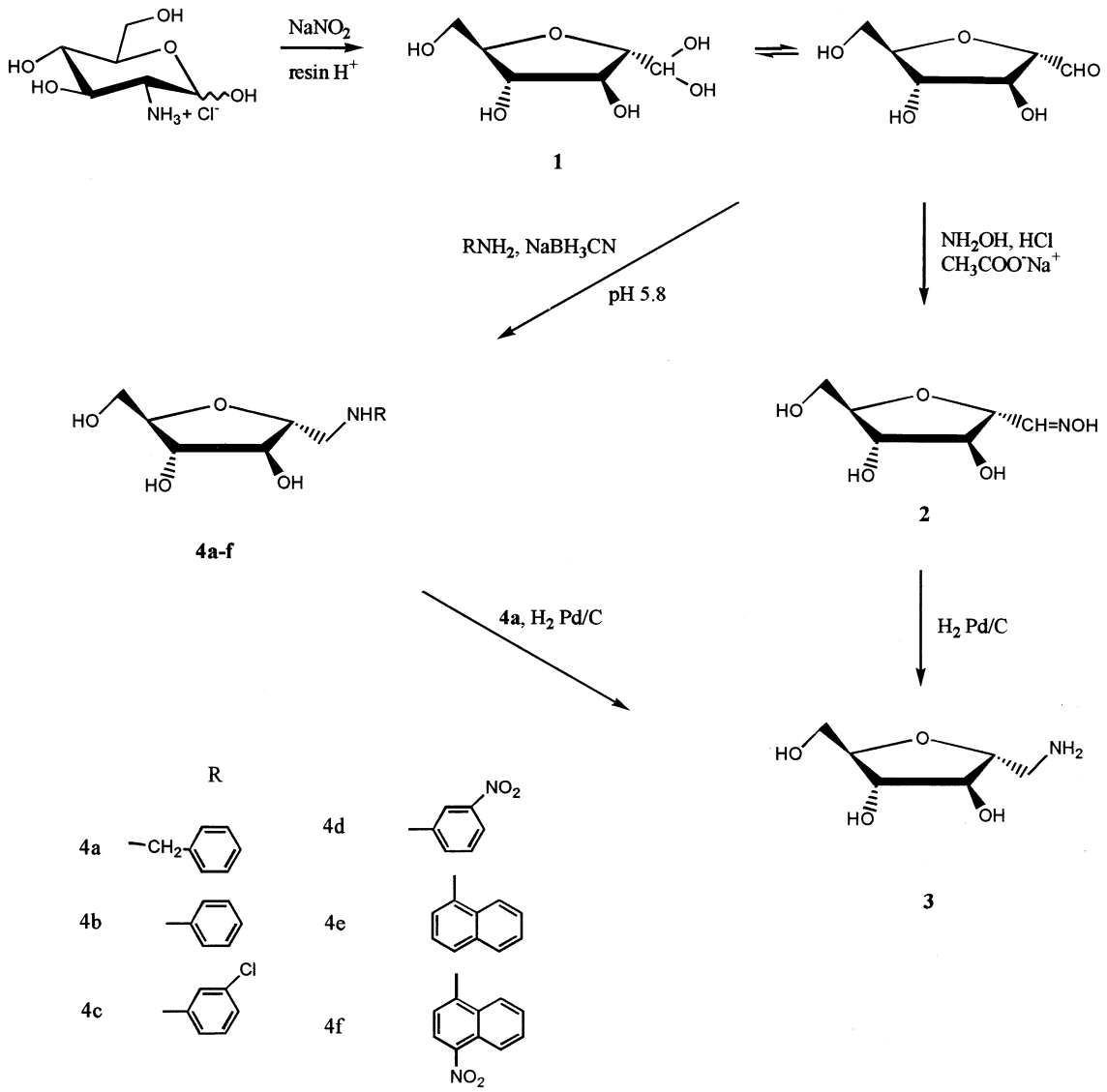
phosphofructokinase responsible for the synthesis of 2,6-diphospho-D-fructose, which is a powerful effector in the glycolysis pathway, involves a phosphorylated fructose as substrate. Potential inhibitors for these enzymes are likely to be D-fructofuranose analogues. In view of this, we devised a stereospecific synthesis of 1-amino-2,5-anhydro-1-deoxy-D-mannitol and derivatives (Scheme 1). A recent method described in the literature for the preparation of 1-amino-2,5-anhydro-1-deoxy-hexitols requires drastic conditions and leads to a mixture of compounds [7]. We therefore considered it preferable to develop an improved methodology involving 2,5-anhydro-D-mannose (**1**) [8] as starting material and reductive amination with the cyanohydridoborate anion [9].

2,5-Anhydro-D-mannose (**1**) was prepared by nitrous acid deamination of 2-amino-2-deoxy-D-glucose [10] following a modification of the Horton and Philips procedure [8]. We found it convenient to use sodium nitrite in the presence of a strong cation-exchange resin as nitrosating agent, which improved the workup and the yield.

Reductive amination of **1** with primary arylamine was carried out at optimum pH 5–6, resulting in the target arylamino derivatives **4a–f**. The stereospecificity of the reaction was confirmed by ^{13}C NMR spectroscopy of the crude extract (see Section 2). This method is



Scheme 1.

Table 1
Inhibition constants (K_i mM) on THT1 with respect to 2-deoxy-D-glucose (2-dGlc)

Compound	D-Glucose	2-Deoxy-D-*arabino*-hexose ('2-deoxy-D-glucose', 2-dGlc)	D-Fructose	2,5-Anhydro-D-mannitol					
K_m or K_i (mM)	0.76	0.54	4	8.6					
Compound	**1**	**2**	**3**	**4a**	**4b**	**4c**	**4d**	**4e**	**4f**
K_i[a] (mM)	>100	>100	>100	15.6 ± 1.5	4.5 ± 0.8	0.48 ± 0.1	1.4 ± 0.2	1 ± 0.2	0.2 ± 0.05

[a] The K_i values (mM), expressed as means S.D. ($n = 3$ or 4), were determined by inhibition of 2-dGlc uptake using a 30 s time point assay at 25 °C.

general and can be applied to different amino compounds (Scheme 1).

Attempts to synthesize 1-amino-2,5-anhydro-1-deoxy-D-mannitol (**3**) from **1** via the Leuckart–Wallach reductive amination with formamide or ammonium acetate were unsuccessful, probably due to the drastic conditions required, which involve a treatment at 180–230 °C in a sealed tube [11]. An alternative synthesis was therefore developed involving either hydrogenolysis of the benzylamino–mannitol derivative **4a** or hydrogenolysis of the intermediate oxime **2** formed from **1** and hydroxylamine. The latter method turned out to be simpler since the intermediate did not need purification.

Affinity measurements on the hexose transporter (Table 1) indicate that: (i) compounds bearing aliphatic polar substituents at C-1 have no affinity; this result appears particularly surprising for compound **1**, largely in the hydrate form and therefore close structurally to the reference 2,5-anhydro-D-mannitol; (ii) in contrast, those bearing a hydrophobic phenyl or naphthyl group on nitrogen bind at the transporter; this affinity is still increased further—up to 20 times that of the reference fructose—by substituents on the ring inducing a dipole, the negative part of which is external.

Other work is in progress for the design of inhibitors that will allow us to explore the binding site of this hexose carrier.

2. Experimental

General methods.—Optical rotations were determined with a Perkin–Elmer 241 polarimeter for solutions in water or MeOH. TLC was conducted on Silica Gel 60 F_{254} plates (E. Merck) with appropriate eluents. The products were detected by UV irradiation ($\lambda = 254$ nm) or by spraying the plates with an aqueous solution of 10% HCl containing 5% ammonium cerium (IV) nitrate or, for amino sugars, an ethanolic solution of ninhydrin (0.1 g, 100 mL) and heating. Final products were purified by preparative HPLC on a silica column (Macherey–Nagel Polygoprep C18 12–25 μm 60 Å). ^1H and ^{13}C NMR spectra in D_2O or CD_3OD were recorded at 200 MHz on a Bruker AC 200 spectrometer. Chemical shifts (δ) were measured in ppm from tetramethylsilane. High-resolution mass spectra were recorded on an AUTOSPEC 6F [Micromass, Altricham, UK] instrument using LSIMS (resolution 5000–10,000) as ionisation mode and glycerol, trichloroacetic acid 1% in H_2O as matrix and polyethyleneglycol (PEG) or PEG-Na as reference. Elementary analyses were performed on an Eager 200 instrument by the INP-ENSCT of Toulouse, France.

2,5-Anhydro-D-mannose (**1**).—A solution of 2-amino-2-deoxy-D-glucose hydrochloride (4.22 g, 19.6 mmol) in water (100 mL) at mutarotational equilibrium ($\cong 5$ h at 25 °C) was cooled to 0 °C. Sodium nitrite (3.45 g,

50 mmol) was added in one batch, followed by cation-exchange resin (Amberlite IR-120, H^+, 100 mL) in portions, the temperature being maintained at 0–5 °C during the addition. The mixture was stirred for 4 h at 0 °C, at which point the amino sugar was no longer detectable. The resin was filtered and the filtrate was neutralised by addition of an anion-exchange resin (Dowex $1 \times 8-50$, CO_3^{2-}). The solution was filtered and the filtrate was lyophilized to give a hygroscopic, yellow–white solid (3.06 g, 17 mmol, 87%) containing **1** and its hydrated form: $[\alpha]_D^{25} +$ 31.8° (c 1,43; MeOH), lit. + 33° [10]; R_f 0.43 (4:1 CH_2Cl_2–MeOH); ^1H NMR (D_2O): δ 8.40 (s, 0.6 H, H-1 aldehyde), 5.05 (d, 1 H, $J_{1,2}$ 7 Hz, H-1 *gem*-diol), 4.11 (t, 1 H, $J_{2,3} = J_{3,4}$ 5 Hz, H-3), 4.01 (t, 1 H, $J_{4,5}$ 5 Hz, H-4), 3.84 (m, 2 H, H-2 and H-5), 3.54–3.74 (2 dd, 2 H, $J_{5,6}$ 6 Hz and $J_{6a,6b}$ 12 Hz, H-6a and H-6b); ^{13}C NMR (D_2O): δ 188.2 (C-1 aldehyde), 92.31 (C-1 *gem*-diol), 86.66 (C-2), 85.92 (C-5), 80.05 (C-3), 79.17 (C-4), 63.70 (C-6); HRMS: Calcd for $C_6H_{13}O_6$ $[M + H]^+$ 181.0712; Found 181.1394. Anal. Calcd for $C_6H_{11}O_5$ $[M + H]^+$ 163.0606; Found 162.9981. Anal. Calcd for $C_6H_{12}O_6 \cdot 1.5H_2O$: C, 34.78; H, 7.29. Found: C, 34.56; H, 7.47.

2,5-Anhydro-D-*mannose oxime* (**2**).—To a solution of **1** (3.06 g, 17 mmol) in MeOH were successively added hydroxylamine hydrochloride (1.76 g, 25.5 mmol) and sodium acetate (2.79 g, 33.6 mmol). The solution was stirred for 6 h at room temperature. The methanol was removed under reduced pressure and the resulting syrup was dissolved in abs EtOH to precipate the salts. After filtration, complete removal of solvent gave **2** (2.63 g, 14.9 mmol, 87%): $[\alpha]_D^{25} + 9.9°$ (c 1, MeOH); R_f 0.56 (4:1 CH_2Cl_2–MeOH); ^1H NMR (CD_3OD): δ 8.49 (s, 1 H, N–OH), 7.41 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1), 4.38 (dd, 1 H, $J_{2,3}$ 4.8 Hz, H-2), 4.11 (dd, 1 H, $J_{3,4}$ 7 Hz, H-3), 4.03 (t, 1 H, H-4), 3.90 (m, 1 H, H-5), 3.69 (2 dd, 2 H, $J_{5,6}$ 6 Hz and $J_{6a,6b}$ 12 Hz, H-6a and H-6b); ^{13}C NMR (CD_3OD): δ 150.51 (C-1), 85.65 (C-5), 82.04 (C-2), 81.14 (C-3), 78.67 (C-4), 63.26 (C-6); HRMS: Calcd for $C_6H_{12}NO_5$ $[M + H]^+$ 178.0715; Found 178.0712.

1-Amino-2,5-anhydro-1-deoxy-D-*mannitol* (**3**)

Procedure A. Oxime **2** (2.63 g, 14.9 mmol) was dissolved in a solution of formic acid (4.4% in MeOH); Pd–C 10% (0.75 g) was added and the reduction was achieved by hydrogenation (at atmospheric pressure, room temperature, 10 h). Filtration and evaporation yielded **3** as a colourless oil (2.21 g, 13.6 mmol, 91%), purified on cation-exchange resin (Dowex 1×8, H^+) with 0.5 M acetic acid as eluent: $[\alpha]_D^{25} + 28.7°$ (*c* 1, MeOH); R_f 0.15 (EtOH + 10% AcOH); ^1H NMR (CD_3OD): δ 4.06 (t, 1 H, $J_{2,3} = J_{3,4}$ 5.5 Hz, H-3), 3.96–3.92 (m, 2 H, H-2 and H-5), 3.90 (dd, 1 H, $J_{4,5}$ 5 Hz, H-4), 3.74 (2 dd, 2 H, $J_{5,6}$ 6 Hz and $J_{6a,6b}$ 12 Hz, H-6a and H-6b), 3.69 (2 dd, 2 H, $J_{1a,1b}$ 14.5 Hz, H-1a and H-1b); ^{13}C NMR (CD_3OD): δ 86.57 (C-5), 81.70 (C-3), 80.49 (C-2), 78.91 (C-4), 63.24 (C-6), 42.76 (C-1); HRMS: Calcd for $C_6H_{14}NO_4$ $[M + H]^+$ 164.0923; Found 164.0934. Anal. Calcd for $C_6H_{13}NO_4 \cdot 1H_2O$: C, 39.79; H, 8.35; N, 7.73. Found: C, 39.93; H, 8.16; N, 7.86.

Procedure B. Pd–C 10% (0.69 g) was added to a solution of **4a** (3.43 g, 13.8 mmol) in MeOH and the suspension was stirred under H_2 at atmospheric pressure, room temperature for 16 h to yield compound **3** (1.59 g, 9.8 mmol, 71%), identical to the product obtained in procedure A.

N-*Aryl-1-amino-2,5-anhydro-1-deoxy-1*-D-*mannitols* (**4a–f**)

General procedure. $NaBH_3CN$ (0.7 equiv) was added to a solution of **1** (1 equiv) and the primary arylamine (1 equiv) in absolute MeOH at pH 5.8. The solution was stirred at room temperature while the pH was maintained at 5.8 by dropwise addition of 0.5 M HCl in MeOH until the starting material was no longer detectable. The mixture was concentrated to a small volume by rotatory evaporation under reduced pressure and resulting **4** was purified by preparative HPLC using various proportion of water–MeCN as eluent.

2,5-Anhydro-1-benzylamino-1-deoxy-D-*mannitol* (**4a**).—From benzylamine (2 g; 18.7 mmol); HPLC purification (95:5 water–MeCN) gave a colourless oil (3.49 g; 13.8

mmol; 74%): $[\alpha]_D^{25} + 75.3°$ (*c* 1.1, MeOH); ^{1}H NMR (CD_3OD): δ 7.45 (m, 5 H, H-2′ to H-6′), 4.02 (t, 1 H, $J_{2,3} = J_{3,4}$ 4.5 Hz, H-3), 3.92 (m, 2 H, H-2 and H-5), 3.85 (dd, 1 H, $J_{4,5}$ 5 Hz, H-4), 3.59–3.70 (4 dd, 4 H, $J_{6a,6b}$ 12 Hz and $J_{1a,1b}$ 14.5 Hz, H-6a, H-6b, H-1a and H-1b), 3.20 (d, 2 H, $J_{7a,7b}$ 12 Hz, H-7′a and H-7′b); ^{13}C NMR (CD_3OD): δ 133.38 (C-1′), 131 and 131.09 (C-3′ and C-5′), 130.24 (C-2′ and C-6′), 130.03 (C-4′), 86.39 (C-5), 81.07 (C-2), 80.86 (C-3), 78.74 (C-4), 63.22 (C-6), 50.43 (C-7), 44.40 (C-1); HRMS: Calcd for $C_{13}H_{19}NO_4Na$ $[M + Na]^+$ 276.1212; Found 276.1220. Anal. Calcd for $C_{13}H_{19}NO_4 \cdot 0.5H_2O$: C, 59.56; H, 7.70; N, 5.34. Found: C, 59.71; H, 7.42; N, 5.22.

2,5-Anhydro-1-deoxy-1-phenylamino-D-*mannitol* (**4b**).—From aniline (0.51 g; 5.5 mmol); HPLC purification (9:1 water–MeCN) gave a hygroscopic, pale-yellow solid (1.02 g; 4.3 mmol; 78%): $[\alpha]_D^{25} + 35.6°$ (*c* 1.15, H_2O); ^{1}H NMR (CD_3OD): δ 7.10 and 6.66 (2 m, 2 H and 3 H, H-2′ to H-6′), 4.01 (m, 3 H, H-3, H-4 and H-2), 3.86 (m, 1 H, H-5), 3.75–3.59 (2 dd, 2 H, $J_{5,6}$ 6 Hz, and $J_{6a,6b}$ 12 Hz, H-6a and H-6b), 3.40–3.19 (2 dd, 2 H, $J_{1a,1b}$ 14,5 Hz and $J_{1,2}$ 6 Hz, H-1a and H-1b); ^{13}C NMR (CD_3OD): δ 150.08 (C-1′), 130.19 (C-2′ and C-6′), 118.55 (C-3′ and C-5′), 114.40 (C-4′), 85.11 (C-5), 83.18 (C-2), 80.61 (C-3), 78.99 (C-4), 63.39 (C-6), 47.41 (C-1); HRMS: Calcd for $C_{12}H_{18}NO_4$ $[M + H]^+$ 240.1236; Found 240.1234. Anal. Calcd for $C_{12}H_{17}NO_4 \cdot 1H_2O$: C, 56.05; H, 7.45; N, 5.45. Found: C, 55.93; H, 7.6; N, 5.34.

*2,5-Anhydro-1-(*m-*chlorophenylamino)-1-deoxy*-D-*mannitol* (**4c**).—From 3-chloroaniline (0.64 g; 5 mmol); HPLC purification (7:3 water–MeCN) gave a pale-brown oil (0.98 g; 3.6 mmol; 72%): $[\alpha]_D^{25} + 48.6°$ (*c* 1.24, MeOH); ^{1}H NMR (CD_3OD): δ 7.03 (t, 1 H, $J_{4',5'} = J_{5',6'}$ 8 Hz, H-5′), 6.64 (t, 1 H, $J_{2',4'} = J_{2',6'}$ 2.5 Hz, H-2′), 6.57 (2dd, 2 H, H-4′ and H-6′), 4.01–3.97 (m, 3 H, H-3, H-4 and H-2), 3.79 (m, 1 H, H-5), 3.67 (2 dd, 2 H, $J_{5,6}$ 6 Hz, and $J_{6a,6b}$ 12 Hz, H-6a and H-6b), 3.42 (2 dd, 2 H, $J_{1a,1b}$ 14.5 Hz and $J_{1,2}$ 6 Hz, H-1a and H-1b); ^{13}C NMR (CD_3OD): δ 151.63 (C-1′), 135.92 (C-3′), 131.24 (C-5′), 117.54 (C-4′), 113.37 (C-2′), 112.3 (C-6′), 85.19 (C-5), 83.15 (C-2), 80.48 (C-3), 78.96 (C-4), 63.33 (C-6), 46.83 (C-1); HRMS: Calcd for $C_{12}H_{17}ClNO_4$ $[M + H]^+$ 274.0846; Found 274.0848. Anal. Calcd for $C_{12}H_{16}ClNO_4 \cdot 1.5H_2O$: C, 48.02; H, 6.38; N, 4.67. Found: C, 47.94; H, 6.51; N, 4.58.

*2,5-Anhydro-1-deoxy-1-(*m-*nitrophenylamino)*-D-*mannitol* (**4d**).—From 3-nitroaniline (0.48 g; 3.5 mmol); HPLC purification (4:1 water–MeCN) gave an orange oil (0.87 g; 3.08 mmol; 88%): $[\alpha]_D^{25} + 76°$ (*c* 1.1, MeOH); ^{1}H NMR (CD_3OD): δ 7.42 (m, 2 H, H-2′ and H-6′), 7.27 (t, 1 H, $J_{4',5'} = J_{5',6'}$ 8.3 Hz, H-5′), 7.03 (dd, 1 H, $J_{2',4'} = J_{4',6'}$ 2 Hz, H-4′), 3.98 (m, 3 H, H-2, H-3 and H-4), 3.86 (m, 1 H, H-5), 3.61–3.70 (2 dd, 2 H, $J_{5,6}$ 6 Hz and $J_{6a,6b}$ 12 Hz, H-6a and H-6b), 3.30–3.35 (2 dd, 2 H, $J_{1a,1b}$ 14.5 Hz and $J_{1,2}$ 6 Hz, H-1a and H-1b); ^{13}C NMR (CD_3OD): δ 151.19 (C-1′), 151 (C-3′), 130.84 (C-5′), 119.90 (C-6′), 112.10 (C-4′), 107.27 (C-2′), 85.25 (C-5), 83.12 (C-2), 80.35 (C-3), 78.86 (C-4), 63.29 (C-6), 46.75 (C-1); HRMS: Calcd for $C_{12}H_{17}N_2O_6$ $[M + H]^+$ 285.1087; Found 285.1087. Anal. Calcd for $C_{12}H_{16}N_2O_6 \cdot 1H_2O$: C, 47.71; H, 6.18; N, 9.27. Found: C, 47.63; H, 6.18; N, 9.32.

2,5-Anhydro-1-deoxy-1-(1-naphthylamino)-D-*mannitol* (**4e**).—From naphthylamine (0.43 g; 3 mmol); HPLC purification (MeCN) gave a colourless oil (0.35 g; 1.2 mmol; 40%): $[\alpha]_D^{25} + 15.3°$ (*c* 1.2, MeOH); ^{1}H NMR (CD_3OD): δ 7.77 and 7.63 (2 m, 2 H, H-4′ and H-5′), 7.30–7.09 (m, 4 H, H-2′, H-3′, H-6′ and H-7′), 8.47 (dd, 1 H, $J_{7',8'}$ 7.7 Hz and $J_{6',8'}$ 2 Hz, H-8′), 4.20 (t, 1 H, H-3), 4.11 (t, 1 H, $J_{4,5}$ 6.5 Hz, H-4), 3.95 (m, 1 H, H-5), 3.73–3.68 (m, 3 H, $J_{5,6}$ 6.5 Hz, $J_{6a,6b}$ 12 Hz, H-2, H-6a and H-6b), 3.35–3.30 (2 dd, 2 H, $J_{1a,1b}$ 14 Hz and $J_{1,2}$ 6 Hz, H-1a and H-1b); ^{13}C NMR (CD_3OD): δ 143.54 (C-1′), 134.27 (C-9′), 128.46 (C-3′), 126.66 (C-5′), 125.79 (C-6′), 124.73 (C-8′), 123.69 (C-10′), 120.50 (C-7′), 117.59 (C-4′), 104.67 (C-2′), 83.72 (C-5), 82.16 (C-2), 79.83 (C-3), 77.86 (C-4), 62.23 (C-6), 46.37 (C-1); HRMS: Calcd for $C_{16}H_{20}NO_4$ $[M + H]^+$ 290.1392; Found 290.1369. Anal. Calcd for $C_{16}H_{19}NO_4$: C, 66.47; H, 6.62; N, 4.84. Found: C, 66.15; H, 6.77; N, 4.79.

2,5-Anhydro-1-deoxy-1-(4-nitro-1-naphthylamino)-D-*mannitol* (**4f**).—From 4-nitro-1-naphthylamine (0.6 g; 3.2 mmol); HPLC purification (9:1 water–MeCN) gave an orange solid (0.5 g; 1.5 mmol; 47%): $[\alpha]_D^{25} + 2°$ (*c* 1.1, MeOH); ^{1}H NMR (CD_3OD): δ 8.88 (dd, 1 H, $J_{7',8'}$ 8 Hz and $J_{6',8'}$ 2 Hz, H-2′), 8.39 (d, 1

H, $J_{2',3'}$ 8.3 Hz, H-8′), 8.12 (d, 1 H, $J_{5',6'}$ 7 Hz, H-5′), 7.63 and 7.46 (2 t, 2 H, H-6′ and H-7′), 6.59 (d, 1 H, H-3′), 4.62 (1 H, NH), 4.21 (dd, 1 H, H-3), 4.09–3.96 (m, 3 H, H-2, H-5 and H-4), 3.77–3.58 (4 dd, 4 H, $J_{5,6}$ 6.5 Hz, $J_{6a,6b}$ 13 Hz, $J_{1a,1b}$ 14.5 Hz and $J_{1,2}$ 6 Hz, H-1a, H-1b, H-6a and H-6b); ^{13}C NMR (CD_3OD): δ 152.66 (C-1′), 134.72 (C-4′), 131.27 (C-6′), 130.80 (C-7′), 129.93 (C-10′), 126.60 (C-3′), 125.31 (C-8′), 123.84 (C-9′), 122.86 (C-5′), 102.24 (C-2′), 86.13 (C-5), 83.32 (C-2), 80.79 (C-3), 79.03 (C-4), 63.23 (C-6), 44.12 (C-1); HRMS: Calcd for $C_{16}H_{19}N_2O_6$ $[M+H]^+$ 335.1243; Found 335.1231. Anal. Calcd for $C_{16}H_{18}N_2O_6$: C, 57.52; H, 5.43; N, 8.38. Found: C, 57.71; H, 5.31; N, 8.50.

Determination of inhibition constants (K_i) of the analogues with the Trypanosoma brucei *glucose transporter.*—Long slender bloodstream forms of *Trypanosoma brucei* strain 427 were grown in rats and isolated by DEAE ion-exchange chromatography, as previously described by Lanham et al. [12]. The cells were washed three times in a phosphate-buffered saline solution (PBS: 0.15 M NaCl, 5 mM potassium phosphate, pH 7.4) at 4 °C and resuspended in PBS at a concentration of 10^9 cells ml^{-1}. After incubation for 2 min at 25 °C, 100 μL of cell suspension were incubated during 30 s with 2-deoxy-D-[1-^{3}H]-glucose as substrate (100 μM, 0.1 μCi per point) and various 2,5-anhydro-D-mannitol analogues as inhibitors (0.01–10 mM). The uptake was stopped by centrifugation, spinning the cells through an oil cushion of 1-bromododecane, as described by Wille et al. [13]. The amount of radioactivity contained in the cell pellet was determined by liquid scintillation counting. Inhibition constant (K_i) values of inhibiting analogues were determined from the relationship $V_0/V = 1 + ([I]/K_i)$ given by Eisenthal et al. [14], where V_0 and V are the uninhibited and inhibited initial rates respectively and $[I]$ is the concentration of inhibitor.

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