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Synthesis and antitubercular activity of novel Schiff bases derived from D-mannitol

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ABSTRACT

Six Schiff base derivatives of D-mannitol, 1,6-dideoxy-1,6-bis-{[(*E*)-arylmethylidene]amino}-D-mannitol (6: aryl = XC_6H_4: X = *o*-, *m*- and *p*- Cl or NO_2), have been synthesized and evaluated for their in vitro antibacterial activity against *Mycobacterium tuberculosis* $H_{37}Rv$ using the Alamar Blue susceptibility test and the activity expressed as the minimum inhibitory concentration (MIC) in μg/mL. All three nitro derivatives exhibit significant activities: activities of (**6d**: X = *o*-NO_2), (**6e**: X = *m*-NO_2) and (**6f**: X = *p*-NO_2) are 12.5, 25.0 and 25.0 μg/mL, respectively. When compared with first line drugs, such as ethambutol, they can be considered as a good starting point to develop new lead compounds for the treatment of multidrug-resistant tuberculosis. Characterization of the new compounds **6** is generally achieved spectroscopically. The structure of compound **3** has been confirmed by X-ray crystallography.

1. Introduction

Amino alcohols are very important and versatile compounds with significant applications in many fields, such as in synthetic and medicinal chemistry.[1–6] For example, ethambutol is an important amino alcohol (Fig. 1) used in the treatment of tuberculosis (TB). This disease is again a serious problem world-wide, and is responsible for almost 2 million deaths each year. Factors involved in the resurgence of TB[7] include the AIDS epidemic, which emerged in the mid-1980s, and the rapid spread of the multidrug-resistant (MDR) TB strain against all major anti-tuberculosis drugs in the market.[8] Particularly worrisome is the super bacterium XDR-TB (extensively drug-resistant tuberculosis), which is resistant to all first and second line anti-TB drugs.[9] Thus there is an urgency to develop new drugs and strategies to fight against tuberculosis or a tragedy will happen.

Continuing our search for new antitubercular compounds, we have investigated Schiff base derivatives derived from D-mannitol (Fig. 1), namely 1,6-dideoxy-1,6-bis-{[(*E*)-arylmethylidene]amino}-D-mannitol (**6**: aryl = XC_6H_4: X = *o*-, *m*- and *p*- Cl or NO_2), as possible active agents.

There are significant reasons for investigating Schiff base derivatives based on carbohydrates, for example, their functional similarities with amino alcohols and hydrazones, which can exhibit remarkable anti-TB activity.[10–16] Other reasons that can be mentioned are their significant lipophilicities, which could facilitate their entry into the intracellular environment and the carbohydratic nature of the cell wall of *Mycobacterium tuberculosis,* which has led to the investigation of carbohydrate derivatives as potential anti-TB agents.[17–19] Based upon our previous work with other classes of antitubercular agents, we restricted the substituents used in **6**, 1,6-dideoxy-1,6-bis-{[(*E*)-arylmethylidene]amino}-D-mannitol, to chloro and nitro groups.[20–26]

2. Results and discussion

2.1. The synthesis of the key intermediate 1,6-diazido-1,6-dideoxy-D-mannitol (3)

The key intermediate **3** was synthesized from D-mannitol. The use of this carbohydrate in organic synthesis has been highlighted in several reviews.[27–31] It has also other important applications in areas such as polymer science, cosmetics, medicine and pharmaceuticals.[32–34] Significant features of this carbohydrate are its functionality, versatility, low price and abundance.

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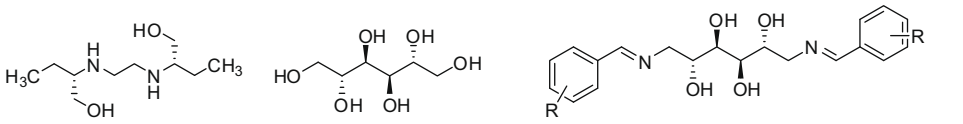
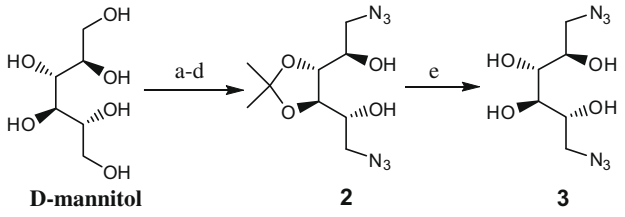


Figure 1. Structures of ethambutol, D-mannitol and the proposed Schiff bases derivatives (**6a–f**).



Scheme 1. Reagents and conditions: (a) acetone, H_2SO_4, 2 h, 77%; (b) AcOH 40%, 45 °C, 2 h, 98%; (c) TsCl, Py, 0 °C, 4 h; (d) NaN_3, DMF, 70 °C, 3,5 h, 70% (two steps); (e) HCl 2.5 N, 20 h; 95%.

1,6-Diazido-1,6-dideoxy-3,4-*O*-isopropylidene-D-mannitol **2** was obtained in four steps in 36.9% overall yield, using the methodology developed by Le Merrer and co-workers.[35,36] The key intermediate **3** was prepared by removal of the isopropylidene group from **2** under acidic conditions. Thus overall, 1,6-diazido-1,6-dideoxy-D-mannitol **3** was prepared in five steps in 50.2% overall yield, as shown in Scheme 1.

Characterization of **3** was achieved spectroscopically and by X-ray crystallography. The IR spectrum showed O–H and N_3 stretching vibrations at 3334 and 2105 cm^{-1}, respectively. The ^1H NMR spectrum exhibited peaks for the OH protons (doublets at 4.33 ppm, J = 8.4 Hz and 5.07 ppm, J = 6.4 Hz) and four sets of peaks for the C–H protons (between 3.26 and 3.65 ppm). The ^{13}C NMR spectrum showed three signals at 69.5 (C_3 and C_4), 69.9 (C_2 and C_5) and 54.4 ppm (C_1 and C_6).

The asymmetric unit of **3**, found in the X-ray crystallography study[37–43] comprised two molecules (Mol.A and Mol.B) having slightly different conformations. The atom arrangements and numbering scheme of Mol.A are shown in Figure 2, those for Mol.B have suffix B replacing A. Each molecule exhibits some disorder about one of its azido groups: N14–N15–N16 in Mol.A and N24–N25–N26 in Mol.B. A complex network of O–H···O hydrogen bonds involving all four hydroxyl groups of each independent molecule is present. Table 1 lists out the geometric parameters for the main hydrogen bonds and Figure 3 shows the supramolecular arrangement arising from the O–H···O intermolecular hydrogen bonds.

2.2. Synthesis of 1,6-dideoxy-1,6-bis-{[(*E*)-phenylmethylidene]-amino}-D-mannitol derivatives (6a–f)

Compound **3** was reduced using H_2–Pd (10% on C) in methanol solution to amino alcohol **4** in 98% yield. As **4** had only limited thermal stability, it was converted to the dihydrochloride salt, **5** (Scheme 2). The IR spectrum of **5** indicates the loss of the azido group, by the disappearance of the azido vibration at 2105 cm^{-1}, and the formation of the NH_3^+ derivative, ν (3202 cm^{-1}).

The 1,6-dideoxy-1,6-bis-{[(*E*)-arylmethylidene]amino}-D-mannitol derivatives, **6,** were obtained from reactions of **4** and substituted benzaldehydes at room temperature (0.8–4 h), in 78–92% yields (Scheme 2 and Table 2).

All the compounds were identified by the spectral data. In general, IR spectra show the N=C stretching vibration at 1633–1650 cm^{-1}. Specifically, in the ^1H NMR spectra, the imine proton (N=C–*H*) appears as a singlet in the range 8.83–8.33 ppm, while the C=N signals in the ^{13}C NMR spectra occur between 156.6 and 160.1 ppm.

A mixture of two geometric isomers at the imine site was found for the (**6**: X = Cl) compounds: the *E* and *Z* ratios were in the approximate ratio of 80:20. However for the (**6**: X = NO_2) compounds, only *E* isomers were isolated, as confirmed by NOE NMR analysis of the N=C*H* and NCH_2 protons.

2.3. Antimycobacterial activity

The antimycobacterial tests against *M. tuberculosis* ATTC 27294 of the derivatives **3**, **5** and **6a–f** were performed[42] using the micro plate Alamar Blue assay (MABA).[43] This methodology is nontoxic, uses thermally stable reagent and shows good correlation with proportional and BACTEC radiometric methods.[44,45] The results are shown in Table 3. Compounds **6d**, **6e** and **6f** exhibited a prom-

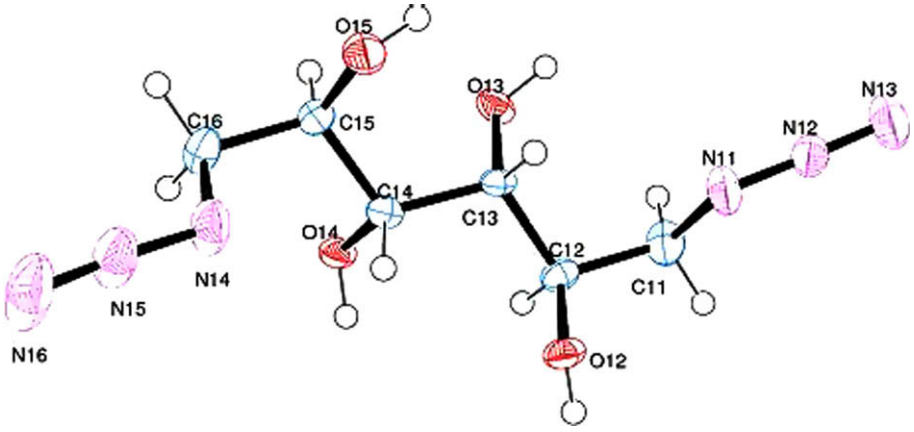


Figure 2. Atoms numbering scheme and atom arrangements for **3**. Probability ellipsoids are drawn at the 50% level. Hydrogen atoms are drawn as spheres of arbitrary radius.

Table 1
Selected hydrogen-bond parameters (Å, °) for **3**

D–H···A	D–H	H···A	D···A	D–H···A
O(13)–H(13)···O(24)^{i}	0.84	2.09	2.717(5)	131
O(15)–H(15)···O(23)^{i}	0.84	1.99	2.795(6)	161
O(23)–H(23)···O(12)^{ii}	0.84	1.90	2.699(6)	158
O(24)–H(24)···O(14) ^{iii}	0.84	1.99	2.794(5)	161
O(12)–H(12)···O(25)	0.84	1.92	2.686(7)	151
O(14)–H(14)···O(22)	0.84	1.86	2.675(5)	165
O(25)–H(25)···O(12)	0.84	2.02	2.675(5)	134
O(22)–H(22)···O(14)	0.84	1.96	2.686(7)	144

Symmetry codes: (i) $x, y+1, z$; (ii) $1-x, -1/2+y, 2-z$; (iii) $2-x, -1/2+y, 2-z$.

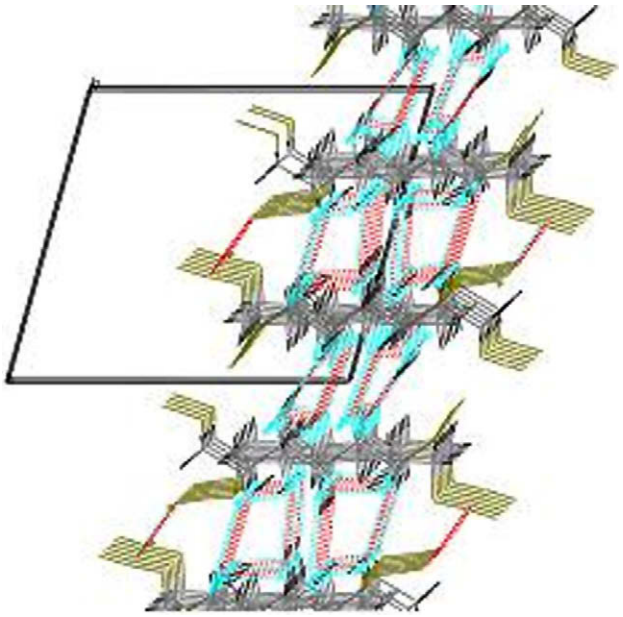


Figure 3. Supramolecular arrangement of the H-bonds network along *b*-axis for compound **3**.

ising inhibitory activity, when compared with ethambutol, the drug used as the positive control.

Table 2
Yields and reaction times of the Schiff bases derivatives **6**

Number	Substituents					Yield (%)	Time (h)
	R_1	R_2	R_3	R_4	R_5		
6a	Cl	H	H	H	H	80	1
6b	H	Cl	H	H	H	78	4
6c	H	H	Cl	H	H	82	0.8
6d	NO_2	H	H	H	H	85	1
6e	H	NO_2	H	H	H	80	3
6f	H	H	NO_2	H	H	92	0.6

2.4. Cell viability assay

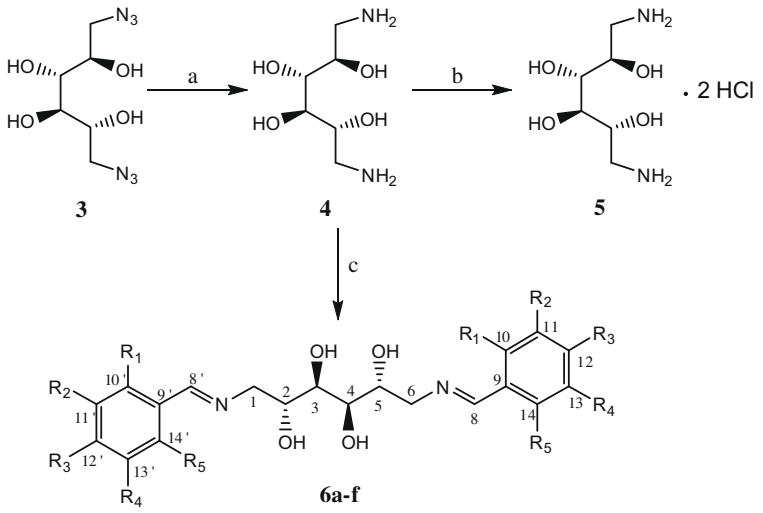
The cytotoxicity of the active compounds **6d**, **6e** and **6f** was examined by Mosmans's MTT(3-(4,5-dimethylthylthiazol-2yl)-2,5-dimethyl tetrazolium bromide; Merck) microcultured tetrazolium assay,[46] and the results were represented as percentage cell viability (Table 4). All the compounds were non-cytotoxic at all concentrations tested.

Compounds **6d**, **6e** and **6f** exhibited activity of 12.5, 25.0 and 25.0 μg/mL, respectively. The antimycobacterial activities of D-mannitol derivatives suggest that they may be selectively targeted to *M. tuberculosis* growth. These compounds are not cytotoxic to host cells at the concentrations effective in inhibiting *M. tuberculosis* infection. When compared with first line drugs such as ethambutol (MIC = 3.12 μg/mL), these derivatives could be considered as a good starting point for the development of new lead compounds in the fight against multidrug-resistant tuberculosis.

3. Experimental

3.1. General methods

Melting points were determined on a Buchi apparatus and are uncorrected. Infrared spectra were recorded on a Thermo Nicolet Nexus 670 spectrometer in KBr disks and frequencies are expressed in cm^{-1}. Mass spectra (MS) were carried out using a Waters model ZQ-LC/MS 2000. NMR spectra were recorded at ambient temperature on a Bruker Avance 500 spectrometer operating at 400.00 MHz (^{1}H) and 100.0 MHz (^{13}C), in deuterated dimethyl sulfoxide. Chemical shifts are reported in ppm (δ) relative to tetramethylsilane. Elemental analyses were performed at ICSN, CNRS, Gif-sur-Yvette, France.



Scheme 2. Reagents and conditions: (a) MeOH, Pd–C, H_2, 4 h, 98%; (b) HCl $_{(g)}$, 10 min, 100%; (c) EtOH–H_2O, different benzaldehydes, 78–92%.

Table 3
The in vitro activity of compounds **3**, **5** and **6a–f** against *M. tuberculosis* $H_{37}Rv$ strain (ATCC 27294, susceptible to both rifampin and isoniazid)

Number	Compound	MIC (μg/mL)
3	—	Resistant
5	—	Resistant
6a	R_1 = Cl and R_2 = R_3 = R_4 = R_5 = H	Resistant
6b	R_2 = Cl and R_1 = R_3 = R_4 = R_5 = H	Resistant
6c	R_3 = Cl and R_1 = R_2 = R_4 = R_5 = H	Resistant
6d	R_1 = NO_2 and R_2 = R_3 = R_4 = R_5 = H	12.5
6e	R_2 = NO_2 and R_1 = R_3 = R_4 = R_5 = H	25.0
6f	R_3 = NO_2 and R_1 = R_2 = R_4 = R_5 = H	25.0
Ethambutol	—	3.12

Table 4
Data of cytotoxicity on murine macrophages cells 18 h after the treatment

Compound	% Cell viability/doses (μg/mL)				
	0.01	0.1	1.0	10	100
6d	100	98	98	98	98
6e	100	98	98	97	96
6f	100	98	98	98	98

3.2. Synthesis of 1,6-diazido-1,6-dideoxy-D-mannitol (3)

A solution of 1,6-diazido-1,6-dideoxy- 3,4-*O*-isopropylidene-D-mannitol **2**[35,36] (15 g, 55.1 mmol) in HCl 2.5 M (50 mL) was stirred at room temperature for 20 h, concentrated in vacuo, the crude product was chromatographed ($CHCl_3$/MeOH, 95:5) to yield **3** (12.1 g, 95%) as a white crystalline solid. Mp: 93 °C (91–93 °C).[47] $[\alpha]_D$ +17.8 (*c* 1.0, MeOH). IR (KBr pellets; cm^{-1}): 3334 ν_{OH}; 2105 ν_{N3}. ^1H NMR (400 MHz; DMSO/Me_4Si; δ (ppm)): 3.26 (2H, dd, J = 12.6 and 6.4 Hz; $H_{1'}$ and $H_{6'}$); 3.44 (2H, dd, J = 12.6 and 2.0 Hz; H_1 and H_6); 3.55 (2H, dd, J = 8.4 and 8.4 Hz; H_3 and H_4); 3.65 (2H, dddd, J = 8.4; 6.4; 6.4 and 2.0 Hz; H_2 and H_5); 4.33 (2H, d, J = 8.4 Hz; OH_3 and OH_4); 5.07 (2H, d, J = 6.4 Hz; OH_2 and OH_5).; ^{13}C NMR (100 MHz, DMSO/Me_4Si; δ (ppm)): 54.36 (C_1 and C_6); 69.53 (C_3 and C_4); 69.87 (C_2 and C_5). ESIMS: [M−H]: 231.2.

3.3. Synthesis of 1,6-diamino-1,6-dideoxy-D-mannitol (4)

To a solution of **3** (200 mg, 0.86 mmol) in MeOH (10 mL) was added 10% Pd–C (25 mg), and the mixture was treated with H_2 for 4 h. The catalyst was filtered off and washed with MeOH (5 mL), and the combined organic extracts were concentrated to yield **4** as an oil (185 mg, 98%). The crude product was used in the next step without further purification.

3.4. Synthesis of 1,6-diamino-1,6-dideoxy-D-mannitol dihydrochloride (5)

Hydrogen chloride was bubbled into the solution of **4** (1.0 g, 5.5 mmol) in MeOH (50 mL) for 5 min. The formed white precipitate was collected, filtered and finally washed with cold water (1.3 g, 100%). Mp: 218–220 °C (217–220 °C).[48]

3.5. General procedure for the preparation of 1,6-dideoxy-1,6-bis-{[(*E*)-phenylmethylidene]amino}-D-mannitol derivatives (6a–f)

Solutions of the appropriate benzaldehyde (2.2 equiv) in EtOH and **4** (0.15 g, 0.83 mmol, 1.0 equiv) in water were mixed. The resulting mixture was stirred for 0.6–4 h, at room temperature and was concentrated under reduced pressure. The residues were purified by washing with distilled water (2 mL) and cold diethyl ether (10 mL) to give **6**.

3.6. 1,6-Dideoxy-1,6-bis-{[(E)-2-chlorophenylmethylidene]-amino}-D-mannitol (6a)

Yield: 80%; mp: 163–165 °C. IR (KBr; cm^{-1}): 3333 $\nu_{OH;}$ 1633 $\nu_{N=C-H}$. ^1H NMR (400 MHz; DMSO); δ (ppm): 3.55 (2H, dd, J = 11.6 and 7.2 Hz; $H_{1'}$ and $H_{6'}$); 3.66 (2H, dd, J = 9.2 and 9.2 Hz; H_3 and H_4); 3.79 (2H, dd, J = 7.2 and 6.0 Hz; H_2 and H_5); 4.09 (2H, d, J = 11.6 Hz; H_1 and H_6); 4.28 (2H, d, J = 9.2 Hz; OH_3 and OH_4); 4.56 (2H, d, J = 6.0 Hz; OH_2 and OH_5); 7.40 (8H, m; H_{11}, H_{12}, H_{13}, H_{14} and $H_{11'}$, $H_{12'}$, $H_{13'}$, $H_{14'}$); 8.46 (2H, s; H_8 and $H_{8'}$). ^{13}C NMR (100 MHz, DMSO); δ (ppm): 64.9 (C_1 and C_6); 70.1 (C_2 and C_5); 70.5 (C_3 and C_4); 127.2 (C_{12} and $C_{12'}$ or C_{13} and $C_{13'}$); 128.2 (C_{12} and $C_{12'}$ or C_{13} and $C_{13'}$); 129.7 (C_{11} and $C_{11'}$ or C_{14} and $C_{14'}$); 131.8 (C_{11} and $C_{11'}$ or C_{14} and $C_{14'}$); 132.9 (C_9 and $C_{9'}$); 133.8 (C_{10} and $C_{10'}$); 157.5 (C_8 and $C_{8'}$). ESIMS: $[M+H]^+$: 425.3. Anal. Calcd for $C_{20}H_{22}Cl_2N_2O_4{\cdot}1.0H_2O$: C, 54.19; H, 5.46; N, 6.32. Found: C, 54.28; H, 5.04; N, 6.41.

3.7. 1,6-Dideoxy-1,6-bis-{[(E)-3-chloro-phenylmethylidene]-amino}-D-mannitol (6b)

Yield: 78%; mp: 155–157 °C. IR (KBr; cm^{-1}): 3320 $\nu_{OH;}$ 1650 $\nu_{N=C-H}$. ^1H NMR (400 MHz; DMSO; δ (ppm): 3.49 (2H, dd, J = 11.0 and 6.7 Hz; $H_{1'}$ and $H_{6'}$); 3.66 (2H, dd, J = 8.2 and 7.5 Hz; H_3 and H_4); 3.80 (2H, dd, J = 6.7 and 6.0 Hz; H_2 and H_5); 4.05 (2H, d, J = 11.0 Hz; H_1 and H_6); 4.33 (2H, d, J = 7.5 Hz; OH_3 and OH_4); 4.55 (2H, d, J = 6,0 Hz; OH_2 and OH_5); 7.47 (4H, m; H_{12}, $H_{12'}$, H_{13} and $H_{13'}$); 7.69 (2H, d, J = 7.0 Hz; H_{11} and $H_{11'}$); 7.81 (2H, s; H_{10} and H_{10}); 8.31 (2H, s; H_8 and $H_{8'}$). ^{13}C NMR (100 MHz, DMSO/Me_4Si; δ (ppm): 64.5 (C_1 and C_6); 70.2 (C_2 and C_5); 70.5 (C_3 and C_4); 126.6 (C_{14} and $C_{14'}$); 127.0 (C_{10} and $C_{10'}$); 130.0 (C_{12} and $C_{12'}$ or C_{13} and $C_{13'}$); 130.4 (C_{12} and $C_{12'}$ or C_{13} and $C_{13'}$); 133.4 (C_9 and $C_{9'}$); 138.4 (C_{11} and $C_{11'}$); 160.1 (C_8 and $C_{8'}$). ESIMS: $[M+H]^+$: 425.4. Anal. Calcd for $C_{20}H_{22}Cl_2N_2O_4$: C, 56.48; H, 5.21; N, 6.59. Found: C, 56.46; H, 5.18; N, 6.52.

3.8. 1,6-Dideoxy-1,6-bis-{[(*E*)-4-chloro-phenylmethylidene]-amino}-D-mannitol (6c)

Yield: 82%; mp: 186 °C. IR (KBr; cm^{-1}): 3417 $\nu_{OH;}$ 1650 $\nu_{N=C-H}$. ^1H NMR (400 MHz; DMSO); δ (ppm): 3.48 (2H, dd, J = 11.6 and 8.0 Hz; $H_{1'}$ and $H_{6'}$); 3.67 (2H, dd, J = 8.0 and 8.0 Hz; H_3 and H_4); 3.79 (2H, ddd, J = 8.0; 8.0 and 6.4 Hz; H_2 and H_5); 4.03 (2H, d, J = 11.6 Hz; H_1 and H_6); 4.31 (2H, d, J = 8.0 Hz; OH_3 and OH_4); 4.52 (2H, d, J = 6.4 Hz; OH_2 and OH_5); 7.76 (4H, d, J = 8.4 Hz; H_{11} and $H_{11'}$ or H_{13} and $H_{13'}$); 7.50 (4H, d, J = 8.4 Hz; H_{10} and $H_{10'}$ or H_{14} and $H_{14'}$); 8.31 (2H, s; H_8 and $H_{8'}$). ^{13}C NMR (100 MHz, DMSO); δ (ppm): 64.5 (C_1 and C_6); 70.2 (C_2 and C_5); 70.6 (C_3 and C_4); 128.6 (C_{10} and $C_{10'}$; C_{14} and $C_{14'}$); 129.4 (C_{11} and $C_{11'}$; C_{13} and $C_{13'}$); 134.9 (C_9 and $C_{9'}$ or C_{12} and $C_{12'}$); 135.1 (C_9 and $C_{9'}$ or C_{12} and $C_{12'}$); 160.3 (C_8 and $C_{8'}$). ESIMS: $[M+H]^+$: 425.5. Anal. Calcd for $C_{20}H_{22}Cl_2N_2O_4{\cdot}2.0H_2O$: C, 52.07; H, 5.68; N, 6.07. Found: C, 52.04; H, 5.81; N, 5.98.

3.9. 1,6-Dideoxy-1,6-bis-{[(*E*)-2-nitro-phenylmethylidene]-amino}-D-mannitol (6d)

Yield: 85%; mp: 167–168 °C (decomposition). IR (KBr; cm^{-1}): 1521 and 1348 ν_{NO2}; 3415 $\nu_{OH;}$ 1634 $\nu_{N=C-H}$. ^1H NMR (400 MHz; DMSO); δ (ppm): 3.52 (2H, dd, J = 11.6 and 8.0 Hz; $H_{1'}$ and $H_{6'}$); 3.66 (2H, dd, J = 8.0 and 8.0 Hz; H_3 and H_4); 3.81 (2H, dd, J = 8.0 and 6.4 Hz; H_2 and H_5); 4.01 (2H, d, J = 11.6 Hz; H_1 and H_6); 4.35 (2H, d, J = 8.0 Hz; OH_3 and OH_4); 4.55 (2H, d, J = 6.4 Hz; OH_2 and OH_5); 7.70 (2H, dd, J = 7.6 and 7.6 Hz; H_{12} and $H_{12'}$); 7.79 (2H, dd, J = 7.6 and 7.6 Hz; H_{13} and $H_{13'}$); 8.02 (2H, d, J = 7.6 Hz; H_{14} and $H_{14'}$); 8.06 (2H, d, J = 7.6 Hz; H_{11} and $H_{11'}$); 8.56 (2H, s; H_8 and

$H_{8'}$). ^{13}C NMR (100 MHz, DMSO); δ (ppm): 64.3 (C_1 and C_6); 69.6 (C_2 and C_5); 70.0 (C_3 and C_4); 123.5 (C_{11} and $C_{11'}$); 128.9 (C_9 and $C_{9'}$); 129.5 (C_{14} and $C_{14'}$); 130.5 (C_{12} and $C_{12'}$); 132.8 (C_{13} and $C_{13'}$); 148.3 (C_{10} and $C_{10'}$); 156.6 (C_8 and $C_{8'}$). ESIMS: [M+H]: 447.0. Anal. Calcd for $C_{20}H_{22}N_4O_8$: C, 53.81; H, 4.97; N, 12.55. Found: C, 53.64; H, 5.12; N, 12.23.

3.10. 1,6-Dideoxy-1,6-bis-{[(*E*)-3-nitro-phenylmethylidene]-amino}-D-mannitol (6e)

Yield: 80%; mp: 152–153 °C (with decomposition). IR (KBr; cm^{-1}): 1532 and 1342 ν_{NO2}; 3488 $\nu_{OH;}$ 1650 $\nu_{N=C-H}$. ^1H NMR (400 MHz; DMSO); δ (ppm): 3.55 (2H, dd, J = 11.6 and 8.0 Hz; $H_{1'}$ and $H_{6'}$); 3.69 (2H, dd, J = 8.0 and 8.0 Hz; H_3 and H_4); 3.84 (2H, dd, J = 8.0 and 4.4 Hz; H_2 and H_5); 4.09 (2H, d, J = 11.6 Hz; H_1 and H_6); 4.37 (2H, d, J = 8,0 Hz; OH_3 and OH_4); 4.62 (2H, d, J = 4.4 Hz; OH_2 and OH_5); 7.75 (2H, dd, J = 7.6 and 7.6 Hz; H_{13} and $H_{13'}$); 8.17 (2H, d, J = 7.6 Hz; H_{14} and $H_{14'}$); 8.29 (2H, d, J = 7.6 Hz; H_{12} and $H_{12'}$); 8.46 (2H, s; H_8 and $H_{8'}$); 8.58 (2H, s; H_{10} and $H_{10'}$). ^{13}C NMR (100 MHz, DMSO); δ (ppm): 64,5 (C_1 and C_6); 70,1 (C_2 and C_5); 70,5 (C_3 and C_4); 121,7 (C_{10} and $C_{10'}$); 124,7 (C_{14} and $C_{14'}$);130,2 (C_{12} and $C_{12'}$);134,2 (C_{13} and $C_{13'}$); 137,9 (C_9 and $C_{9'}$);148,1 (C_{11} and $C_{11'}$); 159,7 (C_8 and $C_{8'}$). MS/ESI: $[M+H]^+$: 447.0. Anal. Calcd for $C_{20}H_{22}N_4O_8{\cdot}1.0.H_2O$: C, 51.72; H, 5.21; N, 12.06. Found: C, 51.53; H, 5.11; N, 11.98.

3.11. 1,6-Dideoxy-1,6-bis-{[(*E*)-4-nitro-phenylmethylidene]-amino}-D-mannitol (6f)

Yield: 92%; mp: 213 °C (decomposition). IR (KBr; cm^{-1}): 1527 and 1344 ν_{NO2}; 3445 ν_{OH}; 1645 $\nu_{N=C-H}$. ^1H NMR (400 MHz; DMSO); δ (ppm): 3.54 (2H, dd, J = 11.6 and 8.0 Hz; $H_{1'}$ and $H_{6'}$); 3.66 (2H, dd, J = 8.0 and 8.0 Hz; H_3 and H_4); 3,85 (2H, dd, J = 8.0 and 6.0 Hz; H_2 and H_5); 4.11 (2H, d, J = 11.6 Hz; H_1 and H_6); 4.35 (2H, d, J = 8.0 Hz; OH_3 and OH_4); 4.60 (2H, d, J = 6.0 Hz; OH_2 and OH_5); 8.01 (4H, d, J = 8.8 Hz; H_{11} and $H_{11';}$ H_{13} and $H_{13'}$); 8.30 (4H, d, J = 8.8 Hz; H_{10} and $H_{10';}$ H_{14} and $H_{14'}$); 8.45 (2H, s; H_8 and $H_{8'}$). ^{13}C NMR (100 MHz, DMSO); δ (ppm): 64.8 (C_1 and C_6); 70.1 (C_2 and C_5); 70.5 (C_3 and C_4); 123.8 (C_{10} and $C_{10'}$; C_{14} and $C_{14'}$); 128.8 (C_{11} and $C_{11'}$; C_{13} and $C_{13'}$); 141.9 (C_9 and $C_{9'}$); 148.3 (C_{12} and $C_{12'}$); 160.0 (C_8 and $C_{8'}$). ESIMS: $[M+H]^+$: 447.2. Anal. Calcd for $C_{20}H_{22}N_4O_8{\cdot}1.0.H_2O$: C, 51.72; H, 5.21; N, 12.06. Found: C, 51.48; H, 5.18; N, 12.21.

3.12. X-ray crystallographic study

Intensity data for 1,6-diazido-1,6-dideoxy-D-mannitol **3** were collected at 120 K with Mo Kα radiation using the κ-goniostat Bruker–Nonius CCD camera of the EPSRC crystallographic service, based at the University of Southampton, UK. Data collection was carried using the program COLLECT[37] and data reduction and unit cell refinement were achieved with the COLLECT[37] and DENZO programs.[38] No correction for absorption was applied. The program ORTEP-3 for Windows[39] was used in the preparation of Figure 3 and SHELXL-97[40] and PLATON[41] in the calculation of molecular geometry. The structure was solved by direct methods using SHELXS-97 and was fully refined by means of the program SHELXL-97.[40] Stereospecific synthesis using a chiral reagent unambiguously produced a single stereoisomer. All hydrogen atoms were placed in the calculated positions and were refined with a riding model. Crystal data and structure refinement details are listed below.

Empirical formula: $C_6H_{12}N_6O_4$, Formula weight: 232.22, Temperature: 120(2) K, Wavelength: 0.71073 Å, Crystal system: Monoclinic, Space group: $P2_1$, Unit cell dimensions: a = 10.0131(3) Å, b = 9.6906(3) Å, c = 11.2650(4) Å, α = 90°, β = 105.745(2)°, γ = 90°, Volume: 1052.06(6) Å^3, Z: 4, Density (calculated): 1.466 Mg/m^3, Absorption coefficient: 0.123 mm^{-1}, F(0 0 0): 488, Crystal size: 0.20 × 0.20 × 0.20 mm, θ range for data collection, 2.11–25.00°, Index ranges: $-11 \leqslant h \leqslant 11$; $-11 \leqslant k \leqslant 11$; $-13 \leqslant l \leqslant 13$, Reflections collected: 11084, Independent reflections: 3696, [R(int) = 0.0322], Reflections observed (>2σ): 3313, Data Completeness: 0.997, Refinement method: Full-matrix least-squares on F^2, Data/restraints/parameters: 3696/1/293, Goodness-of-fit on F^2: 1.035, Final R indices [$I > 2\sigma(I)$]: R_1 = 0.0518 wR_2 = 0.1218, R indices (all data): R_1 = 0.0604 wR_2 = 1268, Largest diff. peak and hole, 0.785 and −0.492 e Å^{-3}. CCDC deposition no.: 727270.

3.13. Biological activity assays

3.13.1. Antimycobacterial activity

Briefly, 200 μL of sterile deionized water was added to all outer-perimeter wells of sterile 96 well plates (Falcon, 3072: Becton Dickinson, Lincoln Park NJ) to minimize evaporation of the medium in the test wells during incubation. The 96 plates received 100 μL of the Middlebrook 7H9 broth (Difco laboratories, Detroit, MI, USA) and a serial dilution of the compounds **3**, **5** and **6a–f** was made directly on the plate. The final drug concentrations tested were 0.01–100.0 μL/mL. Plates were covered and sealed with parafilm and were incubated at 37 °C for 5 days. After this time, 25 μL of a freshly prepared 1:1 mixture of Alamar Blue (Accumed International, Westlake Ohio) reagent and 10% Tween 80 was added to the plate and was incubated for 24 h. A blue colour in the well was interpreted as no bacterial growth, and a pink colour was scored as growth. The MIC (Minimal Inhibition Concentration) was defined as the lowest drug concentration, which prevented a colour change from blue to pink.

3.13.2. Cell viability

The cells were plated in flat bottom 96 well plates (2.5×10^6 cells/mL) cultured for 1 h in a controlled atmosphere (CO_2 5% at 37 °C), and non-adherent cells were washed by gentle flushing with RPMI 1640. Adherent cells were cultured in the presence of medium alone, Tween 20 (3%) (live and dead controls, respectively) or different concentrations of compounds (0.1, 1.0, 10.0 and 100 mg/mL) in a triplicate assay. After 18 h, stock MTT solution (5 mg/mL of saline; 20 mL/well) was added to the culture and 4 h later, the supernatant was discharged and DMSO (100 mL/well) was added for formazan crystals solubilization and the absorbance was read at 540 nm in a plate reader (Biorad—450) relationship and their in vivo antibacterial activity test is in progress. The results were represented as percentage cell viability (Table 4).

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Appendix

CCDC 727270 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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