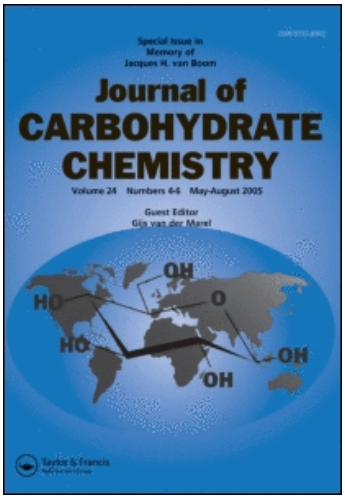

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Synthesis and Antitubercular Evaluation of New Bis-1,2,3-Triazoles Derived from D-Mannitol

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Five new bis-1,2,3-triazole derivatives from D-mannitol, namely 2,3,4,5-tetra-*O*-acetyl-1,6-dideoxy-1,6-bis-(4-substituted-1*H*-1,2,3-triazol-1-yl)-D-mannitol (**4**), have been synthesized from 2,3,4,5-tetra-*O*-acetyl-1,6-diazido-1,6-dideoxy-D-mannitol (**3**) and alkynes, employing copper (I)-catalyzed azide-alkyne cycloaddition (CuAAC) methodology. Evaluation of their in vitro antitubercular activity against *Mycobacterium tuberculosis* H_{37}Rv using the Alamar Blue susceptibility test indicated poor activities. However, this study has provided information about the SAR of D-mannitol derivatives in the search for new anti-TB drugs based on this carbohydrate.

Keywords D-mannitol; 1,2,3-Triazole; Antitubercular activity; Drugs.

INTRODUCTION

Tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium tuberculosis* that infects more than 2 billion people, equal to one-third of the

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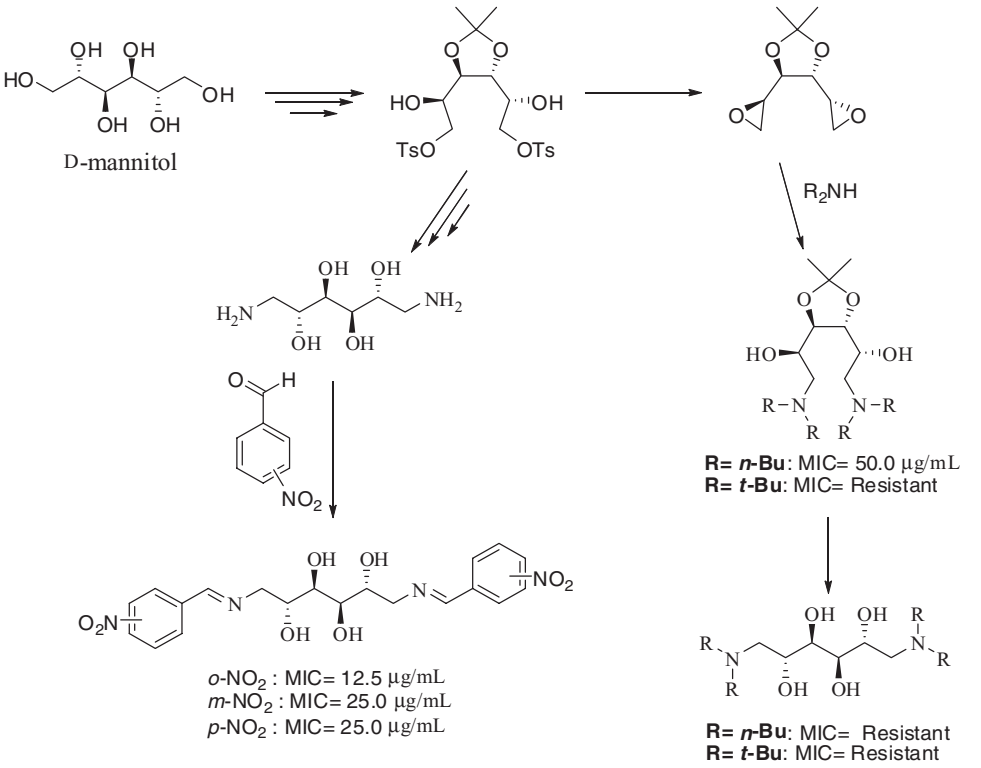
world's population. According to the World Health Organization (WHO), 1.77 million people died from TB in 2007. Because of the spread of HIV infection and the advent of resistant strains of TB bacilli, the global scene is even more alarming. For instance, there were an estimated 511,000 new multidrug-resistant (MDR) TB cases (strains that are resistant to first-line drugs) in 2007. The spread of MDR-TB can cost between 100 and 1,400 times the cost of standard treatment, and its mismanagement led to the appearance of another resistant strain called extensively drug-resistant TB (XDR-TB), which occurs when there is resistance to first- and second-line drugs. The increase of XDR-TB cases can restrict the treatment options and consequently further threatens to make TB incurable.[1] Due to the seriousness of this situation, we urgently need to develop new drugs to treat MDR-TB and XDR-TB. In this context, 1,2,3-triazoles are a significant class of heterocyclic compounds, which show a wide range of pharmacological activities, such as trypanocidal,[2,3] anti-HIV,[4] antiplatelet,[5] anticonvulsant,[6] and antimicrobial.[7,8]

After investigating amino-alcohols and Schiff bases derived from D-mannitol as possible antitubercular agents (Sch. 1),[9,10] we investigated symmetric bis-1,2,3-triazoles from D-mannitol because of the promising anti-TB activity exhibited by this heterocyclic nucleus.[11,12] Based on our previous work and the vital importance of lipophilicity for the biological activity of anti-TB compounds, we proposed another important modification in the D-mannitol core, that is, the acetylation of the hydroxyl groups. This modification will increase the lipophilicity of the derivatives, which could facilitate their entry into the intracellular environment. We now report the synthesis and evaluation of the antitubercular activity of 2,3,4,5-tetra-*O*-acetyl-1,6-dideoxy-1,6-bis-(4-substituted-1*H*-1,2,3-triazol-1-yl)-D-mannitol derivatives (**4a–e**), formed using copper (I)-catalyzed azide-alkyne cycloaddition methodology (CuAAC), known as "click chemistry."

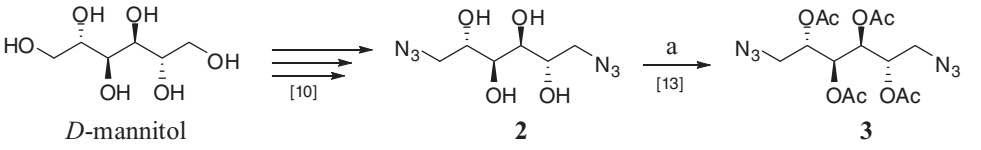
RESULTS AND DISCUSSION

Synthesis of the Key Intermediate 2,3,4,5-Tetra-O-acetyl-1,6-diazido-1,6-dideoxy-D-mannitol (3)

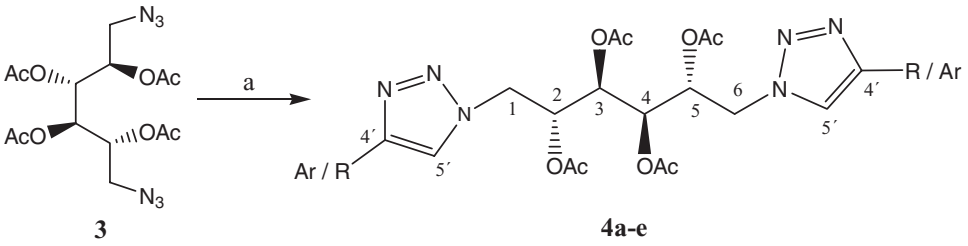
The synthesis of 1,6-diazido-1,6-dideoxy-D-mannitol **2** was obtained in five steps in 50% overall yield, as we previously described.[10] Next, the key intermediate **3** was prepared by acetylation of **2**, using acetic anhydride and pyridine (95%)[13] in 48% overall yield, as shown in Scheme 2. Characterization of **3** was achieved from spectroscopic data. ^{1}H and ^{13}C NMR data were identical to those described.[13]



Scheme 1: Amino-alcohols and Schiff bases derived from D-mannitol previously synthesized by our research group.



Scheme 2: Reagents and conditions: (a) Ac_2O, Py, rt, 20 h, 95%.



Scheme 3: Reagents and conditions: (a) CH_2Cl_2/H_2O, $CuSO_4.5H_2O$, sodium ascorbate, different alkynes, 72%–91%.

Synthesis of 2,3,4,5-Tetra-*O*-acetyl-1,6-dideoxy-1,6-bis-(4-substituted-1*H*-1,2,3-triazol-1-yl)-D-mannitol Derivatives (4a–e)

The 2,3,4,5-tetra-*O*-acetyl-1,6-dideoxy-1,6-bis-(4-substituted-1H-1,2,3-triazol-1-yl)-D-mannitol derivatives (**4a–e**) were obtained by coupling **3** with different alkynes, employing the CuAAC methodology (Sch. 3 and Table 1). This methodology has several advantages over older reactions, in that it is more rapid (mainly if we compare alkynes that are poor 1,3-dipolar acceptors), is highly efficient, can be performed in various solvents (including water), and is a regioselective process, since copper(I) binds to terminal alkynes to produce the 1,4-adduct exclusively.[14,15]

The selection of the substituents in the 1,2,3-triazole nucleus was chosen with the purpose of analyzing the influence of these substituents on the biological activity. Because of this, we synthesized 1,2,3-triazoles from alkynes having (1) an aromatic apolar group, for example, **4a**; (2) polar groups, for example, **4b** and **4d**; and (3) apolar groups, for example, **4c** and **4e**.

All these compounds were identified by spectroscopic means. In general, the ^{1}H NMR spectra occur with the 1,2,3-triazole protons (−N−C<u>H</u>=C−) as singlets in the range of 7.70 to 8.55 ppm, while the C=C signals in the ^{13}C

Table 1: Identification and yields of the 1,2,3-triazole derivatives (**4a–e**)

Entry	Substituents	Yield (%)
4a	Ar = phenyl	85
4b	R = 1-hydroxy-cyclohexyl	82
4c	R = 1-cyclohexen-1-yl	91
4d	R = 2-hydroxy-4-methylpentan-2-yl	72
4e	R = hexyl	78

Table 2: In vitro activity of compounds **2**, **3**, and **4a–e** against *M. tuberculosis* $H_{37}Rv$ strain (ATCC 27294, susceptible to both rifampin and isoniazid)

Entry	Substituents	MIC (μg/mL)[a]	clogP[b]
2	----------	>100.0[c]	–0.43
3	----------	>100.0	2.08
4a	Ar = phenyl	>100.0	3.10
4b	R = 1-hydroxy-cyclohexyl	>100.0	2.86
4c	R = 1-cyclohexen-1-yl	>100.0	3.54
4d	R = 2-hydroxy-4-methylpentan-2-yl	>100.0	2.61
4e	R = hexyl	>100.0	5.49
Ethambutol	----------	3.12	–0.71

[a]Minimum inhibitory concentration.
[b]Calculated using online www.molinspiration.com site.
[c]The strain is considered resistant to the tested substance.

NMR spectra occur between 155.6 and 146.9 ppm ($C_{4'}$) and 120.5 and 122.8 ppm ($C_{5'}$).

Formation of the 1,4-regioisomer in each case was confirmed by NOE NMR analysis of the 1,2,3-triazole (−N−C<u>H</u>=C−) and NC<u>H</u>$_2$ protons, which clearly indicated their close proximity.

Antimycobacterial Activity

The antimycobacterial test of derivatives **2**, **3**, and **4a–e** against *M. tuberculosis* ATTC 27294 was performed[16] using the micro plate Alamar Blue assay (MABA).[17] This methodology is nontoxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric methods.[18] The results are shown in Table 2.

In conclusion, we synthesized five new bis-1,2,3-triazole derivatives from D-mannitol **(4a–e)** prepared by CuAAC methodology ("click chemistry"). The bis-1,2,3-triazole derivatives **2**, **3**, and **4a–e** did not exhibit any antitubercular activity in spite of their increased lipophilicity (see clogP values in Table 2). Thus, lipophilicity was not so important for the modulation of the biological activity of the D-mannitol derivatives, as we had hoped. Other molecular characteristics such as the larger size and volume of these substances could be affecting their interactions with the active site of their cellular target. However, despite these results, we have obtained important information about the SAR of D-mannitol derivatives, which will be useful in the search for new anti-TB drugs based on this carbohydrate.

EXPERIMENTAL

General Procedures

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 MHz (^1H) and 100 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.

1,6-Diazido-1,6-dideoxy-D-mannitol (2)

Yield: 95% (white crystalline solid); mp: 93°C (91–93°C, ethanol)[10]

*2,3,4,5-Tetra-*O*-acetyl-1,6-diazido-1,6-dideoxy-D-mannitol (3)*

Yield: 95% (brown oil); $[\alpha]_D = +32.0°$ (c = 1.00, CH_2Cl_2), $[\alpha]_D = +33.0°$ (c = 1.00, CH_2Cl_2)[13]

General Procedure for the Preparation of 2,3,4,5-tetra-O-acetyl -1,6-dideoxy-1,6-bis-(4-substituted-1*H*- 1,2,3-triazol-1-yl)-D-mannitol Derivatives (4a–e)

Compound **3** (180.0 mg, 0.45 mmol) in 10 mL CH_2Cl_2/H_2O 1:1 is reacted with $CuSO_4.5H_2O$ (5.4 mg, 0.02 mmol) and sodium ascorbate (12 mg, 0.06 mmol) and the desired alkyne (0.90 mmol). The mixture was maintained under agitation at rt until the total formation of the product, as shown by thin layer chromatography. The organic phase was extracted with dichloromethane, dried with Na_2SO_4, and concentrated under reduced pressure. The residue obtained was purified by column chromatography on silica gel using as eluent a gradient mixture of hexane/ethyl acetate with increasing polarity up to 100% ethyl acetate.

*2,3,4,5-Tetra-O-acetyl-1,6-dideoxy-1,6-bis-(4-phenyl-1H-1,2,3-triazol-1-yl)-*D*-mannitol (4a)*

Yield: 85% (white solid); mp: 107°C (ethanol). ^1H NMR (400 MHz; DMSO-d_6); δ (ppm): 1.93 (6H, s; $COC\underline{H}_3$); 2.13 (6H, s; $COC\underline{H}_3$); 4.59 (2H, dd, J = 14.6 and 8.6 Hz; $H_{1'}$ and $H_{6'}$); 4.77 (2H, dd, J = 14.6 and 2.8 Hz; H_1 and H_6); 5.29 (2H, dd, J = 8.6 and 2.8 Hz; H_2 and H_5); 5.37 (2H, d, J = 7.2 Hz; H_3 and H_4); 7.33 (2H, dd, 7.4 and 7.4 Hz; $H_{9'}$); 7.45 (4H, dd, 7.4 and 7.4 Hz; $H_{8'}$ and $H_{11'}$); 7.83 (4H, d, 7.4 Hz; $H_{7'}$ and $H_{10'}$); 8.55 (2H,s; $H_{5'}$). ^{13}C NMR (100 MHz, DMSO-d_6); δ (ppm): 20.3 ($CO\underline{C}H_3$); 20.6 ($CO\underline{C}H_3$); 49.7 (C_1 and C_6); 68.2 (C_3 and C_4);

68.7 (C_2 and C_5); 122.1 ($C_{5'}$); 125.1 ($C_{7'}$ and $C_{10'}$); 127.9 ($C_{9'}$); 128.9 ($C_{8'}$ and $C_{11'}$); 130.5 ($C_{6'}$); 146.4 ($C_{4'}$); 169.0 ($\underline{C}OCH_3$); 169.7 ($\underline{C}OCH_3$). MS/ESI: [M+23]: 627.4; [M + 1]: 605.5. Anal. Calcd for $C_{30}H_{32}N_6O_8.H_2O.CH_2Cl_2$: C, 52.62; H, 5.13. Found: C, 52.20; H, 5.10.

2,3,4,5-Tetra-O-acetyl-1,6-dideoxy-1,6-bis[4-(cyclohexen-1-yl)-1H-1,2,3-triazol-1-yl]-D-*mannitol (4b)*

Yield: 91% (white solid); mp: 126–127°C (ethanol). ^{1}H NMR (400 MHz; DMSO-d_6); δ (ppm): 1.60 (2H, m; $H_{9'}$); 1.68 (2H, m; $H_{10'}$); 1.91 (6H, s; $COC\underline{H}_3$); 2.10 (6H, s; $COC\underline{H}_3$); 2.14 (2H, m; $H_{11'}$); 2.23 (2H, m; $H_{8'}$); 4.48 (2H, dd, J = 14.6 and 8.4 Hz; $H_{1'}$ and $H_{6'}$); 4.65 (2H, dd, J = 14.6 and 2.9 Hz; H_1 and H_6); 5.20 (2H, dd, J = 8.4 and 2.9 Hz; H_2 and H_5); 5.28 (2H, d, J = 7.2 Hz; H_3 and H_4); 6.39 (2H, sl; $H_{7'}$); 8.00 (2H, s; $H_{5'}$). ^{13}C NMR (100 MHz, DMSO-d_6); δ (ppm): 20.3 ($CO\underline{C}H_3$); 20.6 ($CO\underline{C}H_3$); 21.8 ($C_{9'}$); 21.9 ($C_{10'}$); 24.6 ($C_{11'}$); 25.7 ($C_{8'}$); 49.3 (C_1 and C_6); 68.1 (C_3 and C_4); 68.7 (C_2 and C_5); 120.5 ($C_{5'}$); 123.5 ($C_{7'}$); 127.2 ($C_{6'}$); 148.0 ($C_{4'}$); 168.9 ($\underline{C}OCH_3$); 169.7 ($\underline{C}OCH_3$). MS/ ESI: [M+H]: [M+23]: 635.5; [M+1]: 613.5. Anal. Calcd for $C_{30}H_{40}N_6O_8$: C, 58.81; H, 6.58. Found: C, 59.14; H, 6.12.

2,3,4,5-Tetra-O-acetyl-1,6-dideoxy -1,6-bis[4-(1-hydroxy-cyclohexyl)-1H-1,2,3-triazol-1-yl]-D-*mannitol (4c)*

Yield: 82% (white solid); mp: 84–86°C (ethanol). ^{1}H NMR (400 MHz; DMSO-d_6); δ (ppm): 1.37 (8H, m; $H_{8'}$ and $H_{10'}$); 1.63 (8H, m; $H_{7'}$ and $H_{11'}$); 1.85 (4H, m; $H_{9'}$); 1.88 (6H, s; $COC\underline{H}_3$); 2.09 (6H, s; $COC\underline{H}_3$); 4.47 (2H, dd, J = 14.5 and 8.6 Hz; $H_{1'}$ and $H_{6'}$); 4.65 (2H, dd, J = 14.5 and 3.0 Hz; H_1 and H_6); 4.85 (2H, s; $O\underline{H}$); 5.19 (2H, dd, J = 8.6 and 3.0 Hz; H_2 and H_5); 5.28 (2H, d, J = 7.2 Hz; H_3 and H_4); 7.81 (2H,s; $H_{5'}$). ^{13}C NMR (100 MHz, DMSO-d_6); δ (ppm): 20.3 ($CO\underline{C}H_3$); 20.6 ($CO\underline{C}H_3$); 21.6 ($C_{8'}$ and $C_{10'}$); 25.2 ($C_{9'}$); 37.9 ($C_{7'}$ and $C_{11'}$); 49.1 (C_1 and C_6); 67.9 ($C_{6'}$); 68.2 (C_3 and C_4); 68.7 (C_2 and C_5); 121.8 ($C_{5'}$); 155.6 ($C_{4'}$); 168.7 ($\underline{C}OCH_3$); 169.7 ($\underline{C}OCH_3$). MS/ESI: [M+H]: [M+23]: 671.4; [M+1]: 649.5. Anal. Calcd for $C_{30}H_{40}N_6O_8.H_2O$: C, 54.04; H, 6.95; N, 12.61. Found: C, 53.88; H, 6.76; N, 12.33.

2,3,4,5-Tetra-O-acetyl-1,6-dideoxy-1,6-bis-[4-(2-hydroxy-4-methylpentan-2-yl)-1H-1,2,3-triazol-1-yl]-D-*mannitol (4d)*

Yield: 85% (white solid); mp: 98–99°C (ethanol). ^{1}H NMR (400 MHz; DMSO-d_6); δ (ppm): 0.66 (6H, d, J = 6.4 Hz; $H_{9'}$); 0.80 (6H, d; J = 6.4 Hz; $H_{11'}$); 1.40 (6H, s; $H_{10'}$); 1.55 (4H, m; $H_{7'}$); 1.87 (6H, s; $COC\underline{H}_3$); 2.10 (6H, s; $COC\underline{H}_3$); 4.48 (2H, dd, J = 14.3 and 8.5 Hz; $H_{1'}$ and $H_{6'}$); 4.65 (2H, dd, J = 14.3 and 2.8 Hz; H_1 and H_6); 4.91 (2H, m, $H_{6'}$); 5.19 (2H, dd, J = 8.5 and 2.8 Hz; H_2 and H_5); 5.27 (2H, d, J = 7.3 Hz; H_3 and H_4); 5.75 (2H, s; $O\underline{H}$) 7.77 (2H,s; $H_{4'}$).^{13}C NMR (100 MHz, DMSO-d_6); δ (ppm): 20.3 ($CO\underline{C}H_3$); 20.6 ($CO\underline{C}H_3$); 23.7 ($C_{9'}$ or $C_{9'}$ or $C_{11'}$); 24.2 ($C_{9'}$ or $C_{9'}$ or $C_{11'}$); 24.4 ($C_{9'}$ or $C_{9'}$ or $C_{11'}$); 30.0 ($C_{10'}$); 49.0 (C_1

and C_6); 51.4 ($C_{7'}$); 68.1 (C_3 and C_4); 68.8 (C_2 and $C_{5'}$); 69.9 ($C_{6'}$); 121.8 ($C_{5'}$); 155.3 ($C_{4'}$); 168.7 ($\underline{C}OCH_3$); 169.7 ($\underline{C}OCH_3$). MS/ESI: [M+23]: 549.2; [M+1]: 526.1. Anal. Calcd for $C_{32}H_{52}N_6O_{10}.1.5H_2O$: C, 53.68; H, 7.21; N, 12.12. Found: C, 54.03; H, 7.25; N, 12.58.

2,3,4,5-Tetra-O-acetyl-1,6-dideoxy-1,6-bis[4-(1-hexyl)-1H-1,2,3-triazol-1-yl]-D-*mannitol (4e)*

Yield: 72% (white solid); mp: 92–94°C (ethanol). ^{1}H NMR (400 MHz; DMSO-d_6); δ (ppm): 0.84 (6H, t, $J = 6.5$ Hz); 1.25 (12H, m; $H_{10}/H_{10'}$; $H_9/H_{9'}$; and $H_8/H_{8'}$); 1.54 (4H, m; $H_7/H_{7'}$); 1.89 (6H, s; $COC\underline{H}_3$); 2.09 (6H, s; $COC\underline{H}_3$); 2.57 (4H, t, $J = 7.3$ Hz; H_6 and $H_{6'}$); 4.45 (2H, dd, $J = 14.5$ and 8.3 Hz; $H_{1'}$ and $H_{6'}$); 4.64 (2H, dd, $J = 14.5$ and 2.6 Hz; H_1 and H_6); 5.18 (2H, dd, $J = 8.3$ and 2.6 Hz; H_2 and H_5); 5.28 (2H, d, $J = 7.2$ Hz; H_3 and H_4); 7.80 (2H,s; $H_{4'}$).^{13}C NMR (100 MHz, DMSO-d_6); δ (ppm): 13.9 ($C_{11'}$); 20.3 ($CO\underline{C}H_3$); 20.6 ($CO\underline{C}H_3$); 22.0 ($C_{10'}$); 24.8 ($C_{9'}$); 28.0 ($C_{8'}$); 38.9 ($C_{7'}$); 31.0 ($C_{6'}$); 49.1 (C_1 and C_6); 68.1 (C_3 and C_4); 68.7 (C_2 and C_5); 121.5 ($C_{5'}$); 146.9 ($C_{4'}$); 168.7 ($\underline{C}OCH_3$); 169.7 ($\underline{C}OCH_3$). MS/ESI: [M+23]: 533.4; [M+1]: 510.2. Anal. Calcd for $C_{30}H_{48}N_6O_8$: C, 58.05; H, 7.79; N, 13.54. Found: C, 57.68; H, 8.02; N, 13.97.

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) and a serial dilution of the compounds **2**, **3**, and **4a–e** was made directly on the plate. The final drug concentrations tested were 0.01 to 100.0 μL/mL. Plates were covered and sealed with parafilm and 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, OH) reagent and 10% Tween 80 was added to the plate and incubated for 24 h. A blue color in the well was interpreted as no bacterial growth, and a pink color was scored as growth. The minimal inhibition concentration was defined as the lowest drug concentration, which prevented a color change from blue to pink.

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