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Synthesis of new sugar derivatives and evaluation of their antibacterial activities against *Mycobacterium tuberculosis*

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ABSTRACT

A series of sugar derivatives **(1–13)** were synthesized and evaluated for antibacterial activity against *Mycobacterium tuberculosis* (MTB), especially multi-drug resistant (MDR) MTB, and the structure–activity relationships of these compounds were studied. The results showed that the compound OCT313 (2-acetamido-2deoxy-β-D-glucopyranosyl *N,N*-dimethyldithiocarbamate) **(4)** exhibited significant in vitro bactericidal activity, and that the dithiocarbamate group at C-1 position of the glucopyranoside ring was requisite for the antibacterial activity.

Tuberculosis (TB) has become an important worldwide problem: about two million people die each year, particularly in developing countries. It is estimated that about one-third of the world population is currently infected with the bacillus in its latent form and that nearly nine million new cases develop each year.[1] According to WHO, multi-drug resistant tuberculosis is responsible for approximately 460 thousand new cases per year and for about 740 thousand new patients infected by both *Mycobacterium tuberculosis* and HIV/AIDS. Recent estimates show that 10% of all new TB infections are resistant to at least one anti-TB drug.[2] To treat an infection, a cocktail of drugs including, for example, isoniazid, rifampin, ethambutol and pyrazinamide are prescribed for two months followed by a continuation phase in which isoniazid and rifampin are taken. Long-term therapies lasting for between six and nine months have frequently led to patients' non-compliance and, in turn, contributed to the emergence of multi-drug resistant TB (MDR-TB).[3] The ever-increasing drug resistance, toxicity, and side effects of currently used anti-tuberculosis drugs, and the disappearance of their bactericidal activity necessitate new, safer, and more effective antimycobacterial compounds. In the last 10 years the research on *M. tuberculosis* and possible drug candidates have made much progress with the genome unrevealed and the discovery of different biological targets.[4,5]

Over 200 sugar derivatives were investigated for antibacterial activity by the broth dilution method. Two candidates were obtained after this random screening. One of them, OCT359 (allyl *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-α-D-galactopyranosyl)-(1→6)-*O*-2,3,4-tri-*O*-acetyl-β-D-glucopyranoside), has been reported previously.[6] In this study another compound, OCT313, 2-acetamido-2deoxy-β-D-glucopyranosyl *N,N*-dimethyldithiocarbamate **(4)**, was investigated for structure–activity relationships and antibacterial activities against MTB, including multi-drug resistant (MDR) MTB. OCT313 **(4)** consists *N*-acetyl-D-glucosamine and dimethyldithiocarbamate. This compound was prepared from acetylated glucosamine **(1)**, followed by chlorination,[7] substitution of the dimethyldithiocarbamate group and de-O-acetylation (Scheme 1).[8–10] OCT313 **(4)** was white crystals, mp 184–185 °C, and obtained in a 22.5% yield from **(1)**. In its NMR spectrum one proton doublet of H-1 appeared at δ 5.67 ($J_{1,2}$ = 11.0 Hz), indicative of β-configuration. The *N*-acetyl group appeared at δ 1.95 as a three proton singlet. Dimethyl signals of the dimethyldithiocarbonate group appeared at δ 3.37, 3.51 as each 3H singlet.

The isomers of OCT313 **(4)** were synthesized (Scheme 2). The general methods of synthesis of 2-acetamido-2-deoxy-α-D-mannopyranosyl *N,N*-dimethyldithiocarbamate **(5)** and 2-acetamido-2-deoxy-β-D-galactopyranosyl *N,N*-dimethyldithiocarbamate **(6)** are as follows. Instead of *N*-acetyl-D-glucosamine, *N*-acetyl-D-mannosamine or *N*-acetyl-D-galactosamine was used as a starting material. The reaction steps were the same as the synthesis of OCT313

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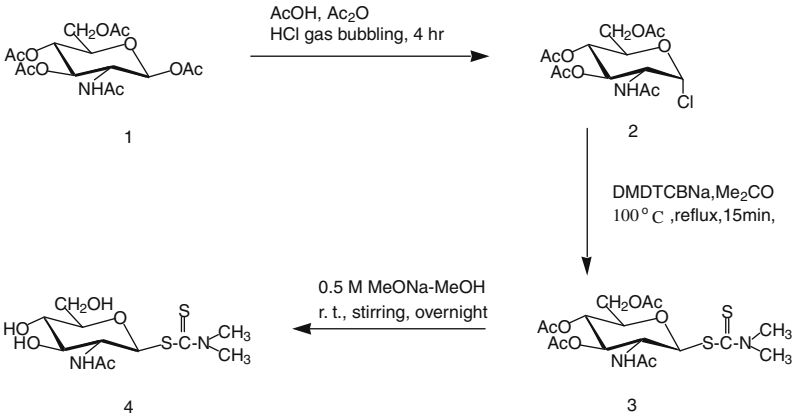
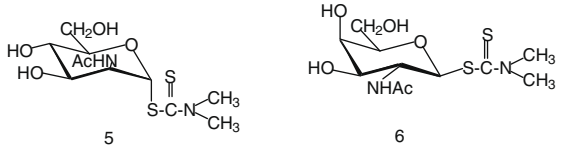


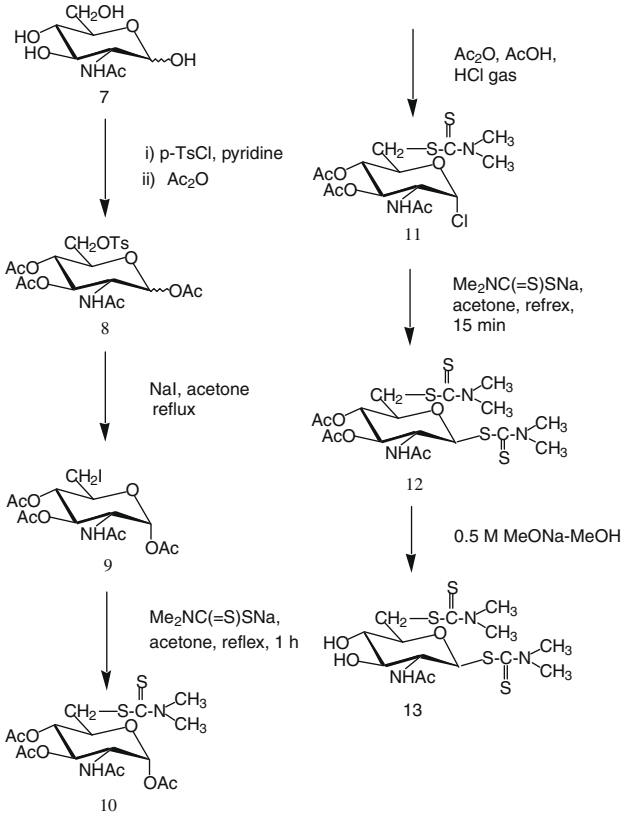
Scheme 1. Synthesis of 2-acetamido-2-deoxy-β-D-glucopyranosyl *N,N*-dimethyldithiocarbamate (OCT313) (**4**).



Scheme 2. Isomer of OCT313 2-acetamido-2-deoxy-α-D-mannopyranosyl *N,N*-dimethyldithiocarbamate (**5**) and 2-acetamido-2-deoxy-β-D-galactopyranosyl *N,N*-dimethyldithiocarbamate (**6**).

(4). Their yields were 53.6% and 29.7% from their peracetates, respectively.

Two dimethyldithiocarbamate groups were substituted at C-1 and C-6 position of *N*-acetyl-D-glucosamine **(13)** (Scheme 3). The general method of synthesis of 2-acetamido-6-*N,N*-dimethyldithiocarbamyl-2,6-dideoxy-β-D-glucopyranosyl *N,N*-dimethyldithiocarbamate **(13)** is as follows. *N*-Acetyl-D-glucosamine **(7)** was selectively tosylated, and then acetylated to give the tosylated peracetate **(8)**. Treatment of the tosylated peracetate with sodium iodite in boiling acetone caused replacement of the sulfonyloxy group by iodine and crystalline 2-acetamido-1,3,4-tri-*O*-acetyl-2,6-dideoxy-6-iodo-α-D-glucopyranose **(9)** was obtained in a yield of 87.8%. This indicated that the sulfonyl group in the tosylated peracetate was located on the primary alcohol group. A mixture of **(9)** and sodium dimethyldithiocabamate in acetone was refluxed for 15 min. After work-up, 6-dimethyldithiocabamate **(10)** was obtained as an amorphous powder in a 92.3% yield. Compound **10** was similarly treated as described for the preparation of **4** to afford 13 in a 30.9% yield from **9**.



Scheme 3. Synthesis of 2-acetamido-6-*N,N*-dimethyldithiocarbamyl-2,6-dideoxy-β-D-glucopyranosyl *N,N*-dimethyldithiocarbamate (**13**).

The antibacterial activity of compounds **1–13** was investigated by using *M. tuberculosis* $H_{37}Rv$, *Mycobacterium bovis* BCG (Tokyo-172), *Mycobacterium avium* 724S, *M. avium* SmO, *Mycobacterium smegmatis*, *Staphylococcus aureus*, and *Escherichia coli* as target bacteria (Table 1). OCT313 **(4)** was specifically effective to slow the grows of *Mycobacterium* species, such as *M. tuberculosis* and *M. bovis* BCG (Table 1). The character of a narrow spectrum of anti-bacterial activity is appropriate to anti-tuberculosis drugs, because the therapeutic term should be at least six months. The MIC of OCT313 **(4)** to *M. tuberculosis* and *M. bovis* BCG was between 25 and 32 μg/ml (Table 1). The MIC of C-4 isomer of OCT313 **(4)**, 2-acetamido-2-deoxy-β-D-galactopyranosyl *N,N*-dimethyldithiocarbamate **(6)** was 2 times lower than that of OCT313 **(4)**. The MIC of 2-acetamido-6-*N,N*-dimethyldithiocarbamyl-2,6-dideoxy-β-D-glucopyranosyl *N,N*-dimethyldithiocarbamate **(13)**, a derivative of OCT313 with two dimethyldithiocarbamate groups at C-1 and C-6 position was the same as that of OCT 313 **(4)**. The acetylated compounds, **3**, lost the antibacterial activity. A finding worthy of note was that sodium dimethyldithiocarbamate (DMSTCA.SS) exhibited strong anti-bacterial activity (Table 1).

The dimethyldithiocarbamate group at C-1 position of OCT313 **(4)** was responsible for the bactericidal effect. Sodium dimethyldithiocarbamate exhibited toxicity to human cell lines; however, the sugar bound to the dimethyldithiocarbamate compound, OCT313, reduced the cytotoxicity (Supplementary Fig. 1 and Table 1). The ratio of the toxic to effective dose of OCT 313 was from 28 to 242 in OCT313, however that of the sodium dimethyldithiocarbamate was from 14 to 318 (Supplementary Table 1). Degradation of OCT 313 (**4**) in the assay medium of anti-bacterial activity was not observed during assay period (data not shown).

In conclusion, the dimethyldithiocarbamate group at C-1 of *N*-acetyl-D-glucosamine is critical for antibacterial activity (Table 1).

Table 1
Antibacterial activities of glucosamine derivatives (MIC, μg/ml)[a]

	Organisms						
	M. tuberculosis $H_{37}Rv$	*M. bovis* BCG (Tokyo)	*M. avium* 724S	*M. avium* SmO	*M. smegmatis*	*S. aureus*	*E. coli*
Compounds							
1 Glc-N-Ac free	>100	>100	ne	ne	>100	ne	ne
3 OCT313-peracetate	>100	>100	>100	>100	>100	>100	>100
4 OCT313 (Glc-N-Ac DMDTCB)	25	31.3	>100	>100	>100	>100	>100
5 Man-*N*-Ac DMDTCB	>100	>100	>100	>100	>100	>100	>100
6 Gal-*N*-Ac DMDTCB	12.5	25	>100	>100	>100	>100	>100
13 Glc-*N*-Ac 1,6 DMDTCB	25	>100	>100	>100	>100	>100	>100
Raw materials							
DMDTCA.SS	0.78	0.78	ne	ne	>100	>100	>100
Dimethylamine	>100	>100	ne	ne	>100	ne	ne
Dimethylamine HCl	>100	>100	ne	ne	>100	ne	ne
anti-MTB Antibiotics							
INH	0.04	0.04	50	3.13	6.25	>100	>100
RFP	0.004	0.004	<0.05	0.004	1.56	0.002	50
SM	0.39	0.20	0.39	0.39	0.39	50	50
EB	2.5	1.5	3.13	1.56	12.5	>100	>100
PAS	0.16	50	100	100	1000	>100	>100
AMK	1.75	0.78	>100	1.56	1.25	100	50
KM	1.56	0.3	>100	1.56	3.13	12.5	12.5
GM	3.13	1.56	25	1.56	1.56	25	6.25

[a] Broth dilution methods using MiddleBrook 7H9 broth containing albumin, dextrose, and catalase for derivatives (ne, not examined). For *Staphylococcus aureus*, we used the heat-infusion broth. INH, isoniazid; RFP, rifampicin; SM, streptomycin; EB, ethambutol; AMK, amikacin; KM, kanamycin; GM, gentamycin.

Table 2
Antimycobacterial activity of OCT313 on drug-sensitive and resistant clinical isolates of *M. tuberculosis*

Clinical isolates	Resistance to	MIC for OCT313 (μg/ml)
Drug-susceptive strains		
A-1-1		6.25
A-1-2		6.25
A-3-5		6.25
A-3-11		6.25
A-3-12		6.25
A-3-13		6.25
A-3-15		6.25
A-3-16		6.25
A-3-20		6.25
A-3-21		6.25
A-3-22		6.25
A-3-9		6.25
A-3-22		6.25
A-1-3		6.25
A-2-5		6.25
A-3-1		6.25
A-3-2		6.25
A-3-6		6.25
A-3-17		12.5
A-3-19		6.25
Drug-resistant strains		
A-3-47	SM	6.25
A-4-8	SM	6.25
A-2-1	INH	6.25
A-2-3	INH, RFP	3.12
J-1-19	INH, RFP	6.25
K-3-6	INH, SM, EB	6.25
M-1-32	INH, RFP, SM, EB	6.25
N-4-11	INH, RFP, EB	6.25
N-5-2	INH, RFP, SM, EB	1.56
P-1-50	INH, RFP, SM, EB	6.25
P-4-11	INH, RFP, SM, EB	6.25
Q-4-1	INH, RFP, SM, EB	6.25
R-1-38	INH, RFP, SM, EB	6.25
U-2-15	INH, RFP, SM, EB	3.12
U-4-6	INH, RFP, SM, EB	6.25
Z-1-4	INH, RFP, SM, EB	6.25
A-2-4	RFP	6.25
A-2-6	INH, RFP, SM, EB	6.25
A-4-25	INH, SM	6.25

Proportion methods using Middlebrook 7H11 agar plates for INH, RFP, SM, EB and 7H9 broth for OCT313. Cut off concentrations of each antibiotic were 10, 10, 100 and 100 μg/ml, respectively.

Compounds having dimethyldithiocarbamate groups have been used for pesticides and their toxicity for humans was due to inhibition of choline esterase. However, the inhibitory effect of OCT313 on choline esterase was undetectable (Supplementary Fig. 2). The precise mechanism of the anti-tuberculosis effect of dimethyldithiocarbamate is unknown; however, OCT313 **(4)** exhibits bactericidal and lytic activities against *M. tuberculosis* and *M. bovis* BCG (Supplementary Fig. 3), strongly suggesting that OCT313 **(4)** exerts antibacterial activity by the mechanism distinct from that of dimethyldithiocarbamate. Furthermore, 25 clinical isolates of drug-resistant MTB and 19 drug-sensitive MTB were sensitive to OCT313 **(4)** (Table 2). The MICs of OCT313 **(4)** to these clinical isolates were from 1.56 to 12.5 μg/ml. Cross-resistance of OCT313 **(4)** to currently used anti-TB drugs was not observed (Table 2). These results strongly indicate that OCT359 possesses novel drug targets and may be a useful lead compound for MDB, especially MDR-MTB.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.09.095.

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8. *General procedures*—Melting points were determined with a Yamagimoto MP-S2 micro melting point apparatus and uncorrected. Solutions were concentrated in a rotary evaporator below 50 °C under vacuum. Optical rotations were measured with a JASCO P-1020 automatic digital polarimeter in a 0.1 dm tube. IR spectra were recorded with a JASCO FT/IR-4100 Spectrometer. ^{1}H NMR spectra were recorded at 500 MHz with a JNM-α500 spectrometer and JNM-ECA500/KJ, at 600 MHz with a BRUKER-AV600. ^{13}C NMR spectra were recorded at 125 MHz with a JNM-α500 spectrometer. Tetramethylsilane was used as an internal standard. Chemical shift are given on the δ scale. TLC was performed on precoated silica gel plates 0.25 mm thick (Kieselgel 60F_{254}, Merck). Detection was effected with H_2SO_4 or by UV irradiation at 254 nm. Column chromatography was performed on Silica Gel BW-820MH (Fuji-Silysia Chemical Ltd, Nagoya, Japan).
9. *2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl N,N-dimethyldithiocarbamate* (**3**)—To a solution of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride[7] (26.77 g, 73.2 mmol) in dry acetone (200 ml) was added sodium *N,N*-dimethyldithiocarbamate dihydrate (21 g, 146.6 mmol) and the mixture was refluxed for 15 min. After checked the disappearance of the starting materials by TLC ($CHCl_3$/acetone 6:1, v/v), the reaction mixture was evaporated to afford a syrup which was dissolved in water and $CHCl_3$. The organic layer was separated, washed with H_2O, dried ($MgSO_4$), and evaporated to afford a syrup. The syrup was dissolved in a small amount of $CHCl_3$ and chromatographed on a column with $CHCl_3$/acetone (10:1–3:1, v/v). Evaporation of the solvent gave (**1**) (12.4 g, 37.6 %) as an amorphous powder, $[\alpha]_D^{20}$ +49.6 (*c* 1.36, $CHCl_3$), IR (KBr) cm^{-1}: 3282 (NH), 1749 (C=O), 1666 (amide I), 1547 (amide II). ^{1}H NMR ($CDCl_3$) δ : 1.92,2.05 (×2), 2.08 (s, 12H, Ac × 4), 3.37, 3.54 (each s, 6H, NCH_3 × 2), 3.85 (m, 1H, H-5), 4.13 (dd, 1H, $J_{5,6a}$ = 2.1 Hz, $J_{6a,6b}$ = 12.5 Hz, H-6a), 4.25 (dd, 1H, $J_{5,6b}$ = 4.6 Hz,,H-6b), 4.57 (ddd, 1H, $J_{1,2}$ = 11.0 Hz, $J_{2,NH}$ = 9.8 Hz, $J_{2,3}$ = 9.8 Hz, H-2), 5.16 (dd, 1H, $J_{3,4}$ = 9.2 Hz, $J_{4,5}$ = 9.8 Hz, H-4),5.20 (dd, 1H, H-3), 5.79 (d, 1H, H-1), and 6.13 (d, 1H, NH). ^{13}C NMR ($CDCl_3$) δ: 20.8, 20.9, 21.0, 23.3 ($COCH_3$ × 4), 42.1, 45.8 (NCH_3 × 2), 52.2 (C-2), 62.1 (C-6), 68.1 (C-4), 74.8 (C-3), 76.7 (C-5), 89.3 (C-1), 169.5, 170.3, 170.9, 171.4 ($COCH_3$ × 4), and 193.7 (C=S).
10. *2-Acetamido-2-deoxy-β-D-glucopyranosyl N,N-dimethyldithiocarbamate* [(**4**), OCT313]—A 0.5 M methanolic MeONa (1 ml) was added to a suspension of (**3**) (9.3 g, 20.6 mmol) in dry MeOH (93 ml), and the mixture was stirred at room temperature for 2 h under exclusion of moisture. After neutralization with Amberlite IR-120B(H^+) resin and removal of the resin by filtration, the filtrate was evaporated to syrup which was crystallized from EtOH. Recrystallization from EtOH gave pure (**4**) (4.0 g, 59.7 %) as white crystals, mp 184–185°, $[\alpha]_D^{24}$ +50.7 (*c* 1.16, H_2O), IR(KBr) cm^{-1}: 3600–3100 (br OH), 1656 (amide I), 1562 (amide II). ^{1}H NMR (CD_3OD) δ: 1.95 (s, 3H, $NCOCH_3$), 3.37, 3.51 (each s, 6H, NCH_3 × 2), 3.39 (m, 1H, H-5), 3.44 (dd, 1H, $J_{3,4}$ = 8.6 Hz, $J_{4,5}$ = 9.5 Hz, H-4), 3.56 (dd, 1H, $J_{2,3}$ = 9.8 Hz, H-3), 3.69 (dd, 1H, $J_{5,6a}$ = 2.1 Hz, $J_{6a,6b}$ = 12.2 Hz, H-6a), 3.83 (dd, 1H, $J_{5,6b}$ = 4.9 Hz, H-6b), 4.07 (dd, 1H, $J_{1,2}$ = 11.0 Hz,H-2), and 5.67 (d, 1H, H-1). ^{13}C NMR (CD_3OD) δ: 22.9 ($COCH_3$), 42.0, 45.7 (NCH_3 × 2), 54.6 (C-2), 62.6 (C-6), 71.6 (C-4), 77.6 (C-3), 82.3 (C-5), 90.4 (C-1), 173.7 (C=O), and 195.4 (C=S).