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Synthesis and evaluation of anti-tubercular activity of new dithiocarbamate sugar derivatives

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ABSTRACT

The present study was undertaken to optimize the anti-tubercular activity of 2-acetamido-2-deoxy-β-D-glucopyranosyl *N,N*-dimethyldithiocarbamate (OCT313, Glc-NAc-DMDC), a lead compound previously reported by us. Structural modifications of OCT313 included the replacements of the DMDC group at C-1 by pyrrolidine dithiocarbamate (PDTC) and the acetyl group at C-2 by either propyl, butyl, benzyl or oleic acid groups. The antimycobacterial activities of these derivatives were evaluated against *Mycobacterium tuberculosis* (MTB). Glc-NAc-pyrrolidine dithiocarbamate (OCT313HK, Glc-NAc-PDTC) exhibited the most potent anti-tubercular activity with the minimal inhibitory concentration (MIC) of 6.25–12.5 μg/ml. The antibacterial activity of OCT313HK was highly specific to MTB and *Mycobacterium bovis* BCG, but not against *Mycobacterium avium*, *Mycobacterium smegmatis*, *Staphylococcus aureus* or *Escherichia coli*. Importantly, OCT313HK was also effective against MTB clinical isolates, including multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains. Interestingly, OCT313HK was exerted the primary bactericidal activity, and it was also exhibited the bacteriolytic activity at high concentrations. We next investigated whether the mycobacterial monooxygenase EthA, a common activator of thiocarbamide-containing anti-tubercular drugs, also activated OCT313HK. Contrary to our expectations, the anti-tubercular activity of dithiocarbamate sugar derivatives and dithiocarbamates were not dependent on *ethA* expression, in contrast to thiocarbamide-containing drugs. Overall, this study presents OCT313HK as a novel and potent compound against MTB, particularly promising to overcome drug resistance.

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More than 9.4 million people develop tuberculosis (TB) annually, and 1.7 million die each year.[1] New case of TB is still increasing all over the world, especially in low-income countries, and TB infection including both multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) is a leading cause of death worldwide. The spread of both MDR-TB and XDR-TB, due to poor compliance of anti-TB drugs, becomes a global health problem. Forty years have passed since the last development of anti-TB drug and the development of novel and innovative compounds is urgently needed.[2] We have recently reported that 2-acetamido-2-deoxy-β-D-glucopyranosyl *N,N*-dimethyldithiocarbamate (Glc-NAc-DMDC), named as OCT313, exhibited the potent antimycobacterial activity.[3]

Studies on the structure–activity relationships (SAR) at C-1, C-4 and C-6 positions of OCT313 established that the DMDC group at C-1 position was critical to the bactericidal activity. In this study, in order to improve the antimycobacterial activity of OCT313, we synthesized the derivative of dithiocarbamate group at the C-1 position and its antimycobacterial activity was evaluated. We first examined whether the elongation of alkyl side chain of dimethyldithiocarbamate, for example, diethyl and dibutyl, improved the antimycobacterial activity against *Mycobacterium tuberculosis* (MTB) H_{37}Rv. The elongation of carbon chain resulted in decreasing of anti-tubercular activity (Table 1). Previously, the antimycobacterial activity of pyrrolidine dithiocarbamate (PDTC) and dialkyldithiocarbamate derivatives have been demonstrated.[4–6] Next, we investigated whether dithiocarbamates containing heterocyclic ring, for example, 4-imidazodithiocarboxylic acid (IMTC) and PDTC were effective against MTB. As a result, PDTC was the most potent compound in our experiments, which was similar to first-line drugs in vitro.

Based on these findings, we synthesized C-1 derivative of OCT313, 2-acetamido-2-deoxy-β-D-glucopyranosyl pyrrolidine-1-

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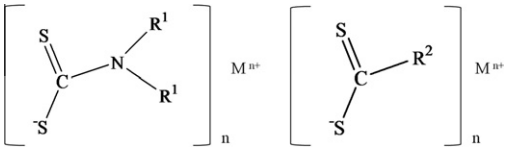
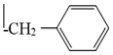
Table 1
Antimycobacterial activity of dithiocarbamate derivatives



Agent	*n*	M	R^1	R^2	MIC for (MIC, μg/ml)		
					M. tuberculosis $H_{37}Rv$	*M. bovis* BCG str. Tokyo 172	*M. smegmatis* JATA 64-01
Carbon chain							
DMDC	1	Na	$-CH_3$	—	1.56	1.56	>100
DDC	1	Na	$-CH_3CH_3$	—	3.13	3.13	>100
DDC	2	Zn	$-CH_2CH_3$	—	1.56	1.56	>100
DBuDC	2	Zn	$-CH_2CH_2CH_2CH_3$	—	12.5	12 5	>I00
Aromatic ring							
DBzDC	2	Zn			25	25	>100
Heterocyclic ring							
IMTC	1	Na			6.25	12 5	>I00
PDTC	1	NH_3			0.2	0.4	>I00

DMDC. Na, sodium dimethyldithiocarbamate; DDC. Na, sodium diethyldithiocarbamate; DDC. Zn, zinc bis (diethyldithiocarbamate); DBuDC. Zn, zinc bis(dibutyldithiocarbamate); DBzDC. Zn, zinc bis(dibenzyldithiocarbamate); IMTC. Na, sodium 4-imidazodithiocarboxylic acid; PDTC. NH_3, ammonium 1-pyrrolidine dithiocarbamate.

carbodithioate (OCT313HK, Glc-NAc-PDTC),[14,15] which is the substitution of the DMDC group at C-1 position of OCT313 to the PDTC group and was determined the antibacterial activity (Table 2). OCT313HK exhibited the potent antimycobacterial activity against both MTB and *Mycobacterium bovis* BCG Tokyo with MICs of 6.25 μg/ml and 12.5 μg/ml, respectively (Table 2). However, OCT313HK failed to inhibit the growth of *Mycobacterium smegmatis*, *Staphylococcus aureus*, *Escherichia coli, Enterococcus faecalis, Enterococcus faecium* and *Pseudomonas aeruginosa*. (Table 2 and Supplementary Table 1). Meanwhile, PDTC exhibited the anti-tubercular activity (MIC = 0.2 μg/ml) and antibacterial activities against *S. aureus* (MIC = 8–12.5 μg/ml), *E. faecalis* (MIC = 32 μg/ml), *E. faecium* (MIC = 32 μg/ml), but not *P. aeruginosa* (Table 2 and Supplementary Table 1). These data indicate that the antibacterial spectrum of OCT313HK is narrow compared to PDTC and exhibit 2 to 4-fold higher anti-tubercular activity than OCT313 (MIC = 25 μg/ml, Table 2).

We next evaluated the antimycobacterial activities of OCT313HK and PDTC against 40 MTB clinical isolates, including drug-sensitive and drug-resistant strains. As shown in Table 3, OCT313HK exhibited the anti-tubercular activity with the MIC of 6.25–12.5 μg/ml, whereas the MIC value of PDTC was 0.2–0.4 μg/ml. Importantly, no cross resistance to almost currently used anti-TB drugs was demonstrated, that OCT313HK and PDTC exhibited comparable activities against all these strains, including five

Table 2
Antibacterial activity of OCT313HK in vitro (MIC, μg/ml)[a]

Compound	Organisms							
	M tuberculosis $H_{37}RV$	*M. bovis* BCG str. Tokyo 172	*M. avium* subsp. hominissuis 104	*M. avium* subsp. avium ATCC2529I	*M. smegmatis* JATA 64-01	*S. aureus*	MRSA 873	*E. coli* DH5α
Synthetic derivative								
OCT313HK	6.25	12.5	100	50	>100	>100	>100	>100
OCT313	25	31.3	>100	>100	>100	>100	>100	>100
Raw material								
Glc-NAc free	>100	>100	>100	>100	>100	>100	>100	>100
DMDC. Na	0.78	1.56	>100	>100	>100	>100	>100	>100
PDTC. NHi	0.2	0.4	3.13	3 13	100	12.5	12.5	12.5
anti-IB drug								
INH	0.04	0.04	1.56	3 13	6.25	>100	>100	>100
RFP	0.004	0.004	0.25	<0.05	1.56	0.002	0.004	50
SM	0.39	0.2	1.56	3,13	0.39	50	>100	50
EB	2.5	1.5	1.6	1.6	12.5	>100	>100	>100
KM	1.56	0.3	3.13	3.13	3.13	12.5	>100	12.5
CPFX	0.39	0.1	0.39	1.56	0.39	0.2	ne	0.2
β-Lactam antibiotics								
PCG	500	500	ne	ne	ne	31.3	>500	25
ABPC	12.5	12.5	ne	ne	ne	50	>100	>100
IPM	3.13	3.13	ne	ne	ne	0.1	0.1	0.1

[a] Broth dilution methods using MiddleBrook 7H9 broth containing albumin, dextrose, and catalase for derivatives (ne, not examined). For *Staphylococcus aureus*, we used the LB broth. OCT313HK, Glc-NAc-PDTC; OCT313, Glc-NAc-DMDC, Glc-NAc free, *N*-acetyl glucosamine; DMDC. Na, sodium dimethyldithiocarbamate; PDTC. NH_3, ammonium 1-pyrrolidine dithiocarbamate; INH, isoniazid; RFP, rifampicin, SM, streptomycin; EB, ethambutol; KM, kanamycin, PAS, para-aminosalicylic acid; CPFX, ciprofloxacin; PCG, penicillin G; ABPC, aminobenzyl penicillin; IPM, imipenem.

Table 3
Antimycobacterial activities of OCT313HK and PDTC against clinical isolates of *M. tuberculosis*

Clinical isolates	Resistance to	MIC for (μg/ml)	
		OCT313HK	PDTC
Drug sensitive strain			
1		6.25	0.2
2		6.25	0.2
3		6.25	0.2
4		6.25	0.2
5		6.25	0.2
6		6.25	0.2
7		12.5	0.4
8		12.5	0.4
9		6.25	0.2
10		6.25	0.2
11		6.25	0.2
12		6.25	0.2
13		12.5	0.4
14		6.25	0.2
15		6.25	0.2
16		6.25	0.2
17		6.25	0.2
I8		6.25	0.2
19		6.25	0.4
20		12.5	0.4
Drug-resistant strain			
1	INH, RFP, EB, LVFX, SPFX, CPFX	6.25	0.2
2	INH, RFP, EB, LVFX, SPFX, CPFX	6.25	0.2
3	RFP, EB	6.25	0.2
4	INH, RFP, EB, LVFX, SI'I'X, CPFX	12.5	0.4
5	INH, RFP, EB, KM, LVFX, SPFX, CPFX	6.25	0.2
6	INH, RFP, SM, EB, KM, LVFX, SPFX, CPFX	6.25	0.2
7	RFP	6.25	0.2
8	RFP	6.25	0.2
9	INH, RFP, KM, LVFX, SPFX, CPFX	6.25	0.2
10	RFP	12.5	0.4
11	INH, RFP, EB, KM, LVFX, SPFX, CPFX	12.5	0.2
12	INH, RFP, EB	6.25	0.2
13	INH, RFP, SM, EB	6.25	0.2
14	INH, RFP, EB	6.25	0.2
15	INH, RFP, EB, LVFX, SPFX, CPFX	6.25	0.2
16	INH, RFP, SM, EB, KM, PAS, EVM, LVFX	6.25	0.2
17	INH, RFP	6.25	0.2
18	INH, RFP, SM, EB, PAS	6.25	0.2
19	INH, RFP, SM, EB, PAS, LVFX	12.5	0.4
20	INH, RFP, ETH	6.25	0.2
H_3-Rv		6.25–12.5	0.2–0.4

OCT313HK, Glc-NAc-PDTC; PDTC, ammonium 1-pyrrolidine dithiocarbamate; INH, isoniazid; RFP, rifampicin; SM, streptomycin; EB, ethambutol; KM, kanamycin; PAS, *para*-aminosalicylic acid; ETH, ethionamide; EVM, emviomycin; LVFX, levofloxacin; SPFX, sparfloxacin; CPFX, ciprofloxacin.

XDR-TB strains. XDR-TB is defined as TB that is resistant to at least rifampicin and isoniazid plus fluoroquinolones, and at least one of three injectable second line anti-TB drugs, that is, amikacin, kanamycin or capreomycin.[1] These results suggest that OCT313HK and PDTC are effective against strains, resistant to fluoroquinolones, e.g. levofloxacin (LVFX), sparfloxacin (SPFX), ciprofloxacin (CPFX), which have been considered as candidate compounds for new anti-TB therapy. Therefore, OCT313HK and PDTC may represent attractive drug candidates to be included in future pharmacological developments against XDR-TB.

Next, we investigated the primary mode of action of OCT313HK and OCT313. Each compound reduced not only the colony forming units, but also the optical density (OD) at both log-phase and stationary phase in time-rather than dose-dependent manner (Fig. 1 and Table 4). Both OCT313HK and OCT313 remarkably decreased the turbidity compared to other bactericidal drugs, that is, isoniazid (INH), ethionamide (ETH), streptomycin (SM), and kanamycin (KM) (data not shown). Consequently, we determined whether the lytic activity was suppressed by the presence of either dextran or sucrose, which was used to increase the extracellular osmotic pressure.[7] The lytic activity of OCT313HK and OCT313 against *M. bovis* BCG were inhibited in the presence of these reagents from day 2 (Fig. 1A and B). Taken together, these results suggest that OCT313HK and OCT313 exert bactericidal and bacteriolytic activities. Of note, this feature has not been observed with the currently used anti-TB drugs.

PDTC and DMDC belong to dithiocarbamates. Thiocarbamide-containing drugs, for example, ETH, thiacetazone (TAC) or isoxyl (ISO), which have been used as second line drugs are activated by the monooxygenase EthA.[8] Approximately, 50% of ETH-resistant clinical isolates possessed mutations in the *ethA* gene.[9] Thereby, we further studied whether *ethA* expression was required for antimycobacterial activity of dithiocarbamates and dithiocarbamate sugar derivatives. This was achieved by using *M. bovis* BCG strains carrying either the pMV261-*ethA* or the pMV261-*ethR*, designed to overexpress either EthA or EthR under the control of the constitutive *hsp60* promoter, respectively.[8] The MICs against these strains were compared to those of a BCG strain harboring the empty construct (Table 5). The EthR-overexpressing strain expressed high levels of resistance to ETH, whereas the EthA-overexpressing strain was hypersusceptible to ETH, consistently with previous reports.[8,10] In contrast, the MICs of dithiocarbamate-containing agents OCT313HK, OCT313 and PDTC against the EthA- or EthR-overexpressing strains were similar to those of the control strain, indicating that the anti-tubercular activity of these two compounds does not rely on EthA expression. In addition, OCT313HK and PDTC demonstrated no cross-resistance to ETH, because they were effective against ETH-resistant clinical isolate No. 20 (Table 3). These results suggest that the mode of action of both dithiocarbamate sugar derivatives and dithiocarbamates are different from the currently used anti-tubercular drugs, including ETH.

Finally, in order to study the SAR for sugar moieties of OCT313, we further synthesized the chemically modified derivatives at C-2 position of OCT313 (Table 6).[14,16] Previously, it was demonstrated that C-4 isomers of OCT313 was more potent anti-tubercular activity compared to OCT313. Nevertheless, the anti-tubercular activity of C-2 derivatives, which were modified to some other types of functional groups namely propionamido (R^2), butyramido (R^3), benzamido (R^4) and oleamido (R^5) were lower than original compound OCT313 (MIC = 50–100 μg/ml). Contrary to the strategy, 2-amino derivative of OCT313 (Glc-NH_2-DMDC) (R^6) was synthesized by de-*O*-acetylation of 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranosyl *N,N*-dimethyldithiocarbamate hydrochloride with an anion exchange resin.[17] The anti-tubercular activity of Glc-NH_2-DMDC was similar to C-2 derivatives (MIC = 50 μg/ml). These results suggest that the acetyl group at C-2 position of OCT313 was optimal for anti-tubercular activity.

In conclusion, this study has unraveled the potential of OCT313HK and OCT313 as valuable compounds for future pharmacological developments against MDR-TB and XDR-TB. Interestingly, OCT313HK exhibited unstained bacteriolytic activity compared to OCT313. The lytic activity of dithiocarbamate sugar derivatives is probably due to dithiocarbamate structure. Surprisingly, dithiocarbamate sugar-resistant colonies were unable to grow on 7H11 agar plate whereas, both anti-TB drug- and dithiocarbamate-resistant colonies were observed spontaneously (data not shown). The resistant strains to anti-TB drug, for example, INH, RFP, SM, KM, PAS and CPFX, can be subcultured in liquid medium, commonly. Nevertheless, we were not able to subculture the resistant strains to dithiocarbamate, for example, PDTC and DDC, in any broth containing each agent. This phenomenon was caused by robust clumping of dithiocarbamate-resistant bacilli compared to other strains. Actually, the colonization and morphology of dithiocarbamate-resistant strains

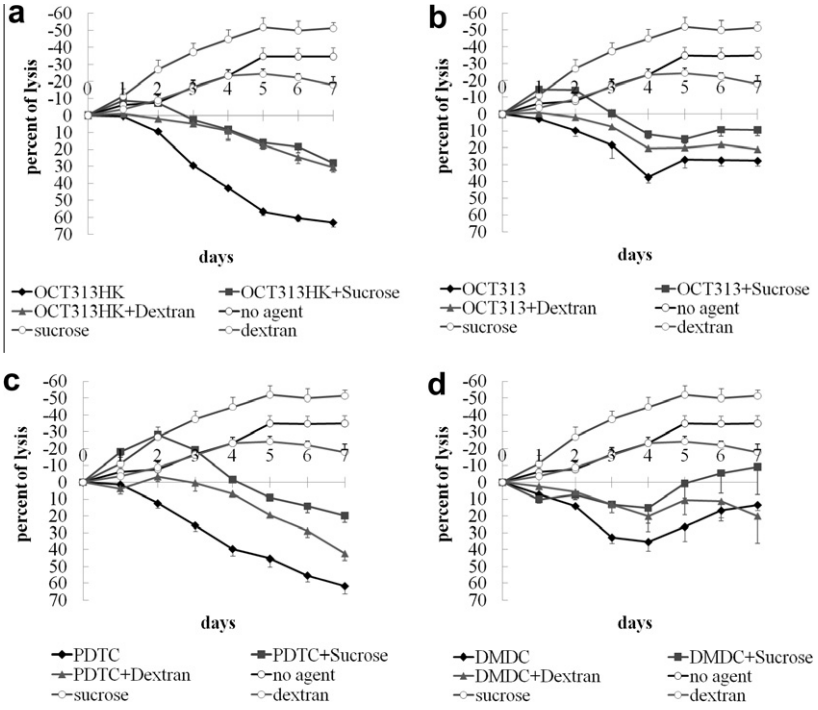


Figure 1. Bacteriolytic activity of each agent under the presence of either dextran or sucrose at log phase. Lysis of *M. bovis* BCG was determined by incubation with 125 mg/ml OCT313HK (A), 313 mg/ml OCT313 (B), 4 mg/ml PDTC (C) and 15.6 mg/ml DMDC (D) with or without 150 mM sucrose or 2.5% dextran (molecular weight = >500,000) for 7 days. Lysis of dilute suspensions of cells was determined by following spectrophotometrically the loss of reading at O.D. 530 nm. Experiments were carried out more than three times and representative data were shown. Error bars represent means ± SD (n = 3).

Table 4
Bactericidal activity of each agent under in the presence of either dextran or sucrose at both log phase and stationary phase

	Agent and condition	Ave. log CFU/ml ± SD[a]	
		day 7	day 21
A			
	OCT313HK	4.55 ± 0.14	4.43 ± 0.08
	OCT313HK + sucrose	6.25 ± 0.03	5.76 ± 0.10
	OCT313HK + dextran	6.07 ± 0.13	5.79 ± 0.05
B			
	OCT313	5.91 ± 0.07	5.20 ± 0.14
	OCT313 + sucrose	6.41 ± 0.08	5.78 ± 0.04
	OCT313 + dextran	6.44 ± 0.08	5.91 ± 0.03
C			
	PDTC	4.65 ± 0.08	4.41 ± 0.08
	PDTC + sucrose	6.05 ± 0.03	5.56 ± 0.04
	PDTC + dextran	6.93 ± 0.06	5.56 ± 0.09
D			
	DMDC	6.94 ± 0.05	8.21 ± 0.23
	DMDC + sucrose	6.18 ± 0.07	8.22 ± 0.03
	DMDC + dextran	6.09 ± 0.04	8.12 ± 0.09
E			
	No agent	8.44 ± 0.35	7.92 ± 0.06
	Sucrose	8.50 ± 0.23	8.13 ± 0.06
	Dextran	8 34 ± 004	8.26 ± 004

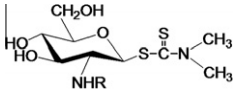
[a] Bactericidal activity against *M. bovis* BCG was determined by incubation with 125 μg/ml OCT313HK (A), 313 μg/ml OCT313 (B), 4 μg/ml PDTC (C) and 15.6 μg/ml DMDC (D) with or without 150 nM sucrose or 2.5% dextran for 7 days or 21 days, respectively. Values represent means ± SD. OCT313HK, Glc-NAc-PDTC; OCT313. Glc-NAc-DMDC; PDTC, ammonium 1-pyrrolidine dithiocarbamate; DMDC, sodium dimethyldithiocarbamate.

Table 5
MICs of ethionamide and dithiocarbamate-containing drugs against *M. bovis* BCG overexpressing either EthA or EthR in 7H11 agar supplemented with OADC

Strain	MIC for (MIC, μg/ml)			
	OCT313HK	OCT313	PDTC	ETH
BCG pMV261	1–2.5	10–20	0.5	1–5
BCG pMV261 ::*ethA*	1–2.5	10–20	0.5	0.5
BCG pMV261::*ethR*	1–2.5	10–20	0.25–0.5	10–25

OCT313HK, Glc-NAc-PDTC; OCT313. Glc-NAc-DMDC; PDTC, ammonium 1-pyrrolidine dithiocarbamate; ETH. ethionamide.

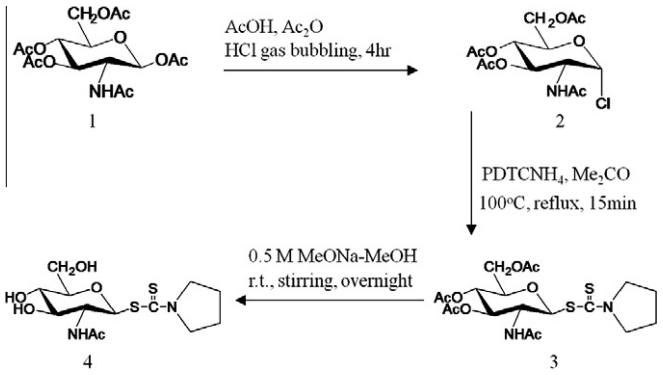
Table 6
Anti-tubercular activity of C2-derivatives of OCT313 in vitro[a]



R	Compound	MIC for (MIC, μg/ml) *M. tuberculosis* H_{37}-Rv
$R^1 = COCH_3$	OCT313 (Glc-NAc-DMDC)	25
$R^2 = COC_2H_5$	Glc-NPro-DMDC	50
$R^3 = COC_3H_5$	Glc-NBt-DMDC	50
$R^4 = COC_6H_6$	Glc-NBz-DMDC	100
$R^5 = COC_{17}H_{33}$	Gk-NOle-DMDC	100
R^6=H	Glc-NH_2-DMDC	50

[a] Broth dilution methods using MiddleBrook 7H9 broth containing albumin, dextrose, and catalase for derivatives.

were remarkably different from anti-TB drug-resistant strains. These findings suggested that the anti-tubercular activity of dithiocarbamate and dithiocarbamate sugar might be not caused due to some common known mechanism. Therefore, further work is required to clarify the specific targets of dithiocarbamate and dithiocarbamate sugar.



Scheme 1. Synthesis of 2-acetamido-2-deoxy-2-deoxy-β-D-glucopyranosyl pyrrolidine-1-carbodithioate (OCT313HK, Glc-NAc-PDTC) (**4**).

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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.2010.12.084.

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14. *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).
15. 2-Acetamido-2-deoxy-β-D-glucopyranosyl pyrrolidine-1-carbodithioate (**4**) (Scheme 1) was synthesized as follows. Reaction of literature known 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride[11] and ammonium 1-pyrrolidinecarbodithioate in acetone gave thioglycoside peracetate in 51.4% yields. In its ^{1}H NMR spectrum one proton doublet of H-1 appeared at δ 5.72 ($J_{1,2}$ = 11.0 Hz), indicative of β-configuration. De-*O*-acetylation of thioglycoside peracetate with 0.5 M sodium methoxide-methanol gave compound (2-acetamido-2-deoxy-β-D-glucopyranosyl pyrrolidine-1-carbodithioate) as colorless needles, mp 198–199° (decomp.) in 78% yields. $[\alpha]_D^{23}$ +46.4° (*c* 1.04, H_2O), IR(KBr) cm^{-1}: 3500–3300 (br OH, NH), 1639 (amide I), 1532 (amide II). ^{1}H NMR (CD_3OD) δ: 1.94 (s, 3H, $NCOCH_3$), 1.97, 2.07, 3.59, 3.70, 3.86 (m, 8H, methylene protons of pyrrolidine group), 3.38 (ddd, 1H, $J_{4,5}$ = 9.7 Hz, $J_{5,6a}$ = 5.0 Hz, $J_{5,6b}$ = 2.3 Hz, H-5), 3.42 (dd, 1H, $J_{3,4}$ = 8.6 Hz, H-4), 3.54 (dd, 1H, $J_{2,3}$ = 9.8 Hz, H-3), 3.68 (dd, 1H, $J_{6a,6b}$ = 12.0 Hz, H-6a), 3.82 (dd, 1H, H-6b), 4.05 (dd, 1H, $J_{1,2}$ = 11.0 Hz, H-2), and 5.72 (d, 1H, H-1). ^{13}C NMR(CD_3OD) δ 22.9 ($COCH_3$), 25.1, 26.9, 52.0, 56.3 (methylene carbons of pyrrolidine group), 54.5 (C-2), 62.6 (C-6), 71.6 (C-4), 77.6 (C-3), 82.3 (C-5), 89.4 (C-1), 173.6 (C=O), and 195.4 (C=S). HR-FAB-MS: *m/z* 351.1046 $[M+H]^+$ (calcd for $C_{13}H_{23}O_5N_2S_2$: 351.1049). 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl pyrrolidine-1-carbodithio ate (**3**): $[\alpha]_D^{25}$ +50.8° (*c* 1.06, $CHCl_3$), IR (KBr) cm^{-1}: 3283 (NH), 1747 (C=O), 1667 (amide I), 1544 (amide II). ^{1}H NMR($CDCl_3$) δ: 1.92 (s, 3H, $NCOCH_3$), 1.99, 2.05, 3.60, 3.73, 3.90 (m, 8H, methylene protons of pyrrolidine group), 2.05 (×2), 2.08 (s, 9H, $COCH_3$×3), 3.86 (ddd, 1H, $J_{4,5}$ = 9.8 Hz, $J_{5,6a}$ = 2.1 Hz, $J_{5,6b}$ = 4.9 Hz, H-5), 4.13 (dd, 1H, $J_{6a,6b}$ = 12.5 Hz, H-6a), 4.25 (dd, 1H, H-6b), 4.55 (ddd, 1H, $J_{1,2}$ = 11.0 Hz, $J_{2,NH}$ = $J_{2,3}$ = 9.8 Hz, H-2), 5.16 (dd, 1H, $J_{3,4}$ = 9.5 Hz, H-4), 5.21 (dd, 1H, H-3), 5.86 (d, 1H, H-1), and 6.25 (d, 1H, NH). ^{13}CNMR($CDCl_3$) δ 20.7 (×2), 20.8 ($OCOCH_3$×3), 23.2 ($NCOCH_3$), 24.2, 26.0, 51.1, 55.4 (methylene carbons of pyrrolidine group), 52.0 (C-2), 62.0 (C-6), 68.0 (C-4), 74.6 (C-3), 76.5 (C-5), 88.0 (C-1), 169.3, 170.2, 170.8, 171.2 ($NCOCH_3$, $OCOCH_3$×3), and 189.2 (C=S).
16. Acetamido group of OCT313 was chemically modified to some other types of functional groups namely propionamido (R^2), butyramido (R^3), and benzamido (R^4), oleamido (R^5) (Table 6). Synthetic method was as follows. After acyl chlorides or anhydride were reacted to literature known 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranose hydrochloride[12] resulting *N*-acyl-β-acetates were chlorinated with HCl gas in acetic acid and anhydride, and reacted with sodium *N,N*-dimethyldithiocarbamate in acetone. After de-*O*-acetylation some *N*-acyl derivatives of OCT313 were obtained. 2-Deoxy-2-propionamido-β-D-glucopyranosyl *N,N*-dimethyldithiocarbamate (R^2): $[\alpha]_D^{22}$ +53.9° (*c* 1.11, MeOH), IR (KBr) cm^{-1}: 3510–3100 (br OH, NH), 1641 (amide I), 1571 (amide II). ^{1}H NMR (CD_3OD + D_2O, 3:4, v/v) δ: 1.11 (t, 3H, *J* = 7.6 Hz, CH_2CH_3), 2.24 (m, 2H, CH_2CH_3), 3.38, 3.53 (s, 6H, NCH_3×2), 3.49 (m, 1H, H-4), 3.50 (m, 1H, H-5), 3.64 (dd, 1H, $J_{2,3}$ = 9.9 Hz, $J_{3,4}$ = 8.7 Hz, H-3), 3.73 (dd, 1H, $J_{5,6a}$ = 0.6 Hz, $J_{6a,6b}$ = 12.4 Hz, H-6a), 3.86 (dd, 1H, $J_{5,6b}$ = 4.5 Hz, H-6b), 4.08 (dd, 1H, $J_{1,2}$ = 11.0 Hz, H-2), and 5.70 (d, 1H, H-1). ^{13}C NMR (CD_3OD + D_2O, 3:4, v/v) δ 10.4 (CH_2CH_3), 30.3 (CH_2CH_3), 42.5, 46.1 (NCH_3×2), 54.0 (C-2), 61.9 (C-6), 70.8 (C-4), 76.5 (C-3), 81.7 (C-5), 90.0 (C-1), 178.5 (C=O), and 194.7 (C=S). HR-FAB-MS: *m/z* 339.1053 $[M+H]^+$ (calcd for $C_{12}H_{23}O_5N_2S_2$:339.1049). 2-Butyramido-2-deoxy-β-D-glucopyranosyl *N,N*-dimethyldithiocarbamate (R^3): mp 171–172 °C (decomp.), $[\alpha]_D^{22}$ +49.6° (*c* 1.40, MeOH), IR(KBr) cm^{-1}: 3500–3150 (br OH, NH), 1643 (amide I), 1535 (amide II). ^{1}H NMR (CD_3OD + D_2O, 2.5:1, v/v) δ 0.93 (t, 3H, *J* = 7.4 Hz, CH_2CH_3), 1.62 (m, 2H, CH_2CH_3), 2.21 (m, 2H, $NCOCH_2$), 3.38, 3.53 (s, 6H, NCH_3×2), 3.46 (m, 1H, H-5), 3.48 (m, 1H, H-4), 3.62 (dd, 1H, $J_{2,3}$ = 9.2 Hz, $J_{3,4}$ = 8.5 Hz, H-3), 3.73 (dd, 1H, $J_{5,6a}$ = 4.6 Hz, $J_{6a,6b}$ = 12.4 Hz, H-6a), 3.86 (dd, 1H, $J_{5,6b}$ = 1.8 Hz, H-6b), 4.10 (dd, 1H, $J_{1,2}$ = 11.0 Hz, H-2), and 5.70 (d, 1H, H-1). ^{13}C NMR (CD_3OD + D_2O, 2.5:1, v/v) δ 13.9 (CH_2CH_3), 20.1 (CH_2CH_3), 39.0 ($NCOCH_2$), 42.3, 45.9 (NCH_3×2), 54.2 (C-2), 62.2 (C-6), 71.1 (C-4), 76.9 (C-3), 82.0 (C-5), 90.2 (C-1), 172.1 (C=O), and 195.1 (C=S). HR-FAB-MS: *m/z* 353.1209 $[M+H]^+$ (calcd for $C_{13}H_{25}O_5N_2S_2$: 353.1205). 2-Benzamido-2-deoxy-β-D-glucopyranosyl *N,N*-dimethyldithiocarbamate (R^4): $[\alpha]_D^{22}$ +85.1° (*c* 1.31, MeOH), IR(KBr) cm^{-1}: 3500–3200 (br OH, NH), 1641 (amide I), 1534 (amide II). ^{1}H NMR (D_2O) δ 3.25, 3.40 (s, 6H, NCH_3×2), 3.63 (dd, 1H, $J_{3,4}$ = 8.8 Hz, $J_{4,5}$ = 9.9 Hz, H-4), 3.69 (ddd, 1H, $J_{5,6a}$ = 5.1 Hz, $J_{5,6b}$ = 2.1 Hz, H-5), 3.82 (dd, 1H, $J_{6a,6b}$ = 12.5 Hz, H-6a), 3.93 (dd, 1H, $J_{2,3}$ = 9.9 Hz, H-3), 3.95 (dd, 1H, H-6b), 4.39 (dd, 1H, $J_{1,2}$ = 10.9 Hz, H-2), 5.96 (d, 1H, H-1), and 7.40–7.90 (m, 5H, aromatic protons). ^{13}C NMR (D_2O) δ 44.8, 48.3 (NCH_3×2), 56.8 (C-2), 63.6 (C-6), 72.5 (C-4), 78.0 (C-3), 83.4 (C-5), 91.8 (C-1), 130.0, 131.7, 135.3, 136.1 (aromatic carbons), 174.0 (C=O), and 195.7 (C=S). HR-FAB-MS: m/z 387.1041 $[M+H]^+$ (calcd for $C_{16}H_{23}O_5N_2S_2$:387.1049). 2-Deoxy-2-oleamido-β-D-glucopyranosyl *N,N*-dimethyldithiocarbamate (R^5): $[\alpha]_D^{22}$ +40.9° (*c* 1.33, MeOH), IR (KBr) cm^{-1}: 3500–3200 (br OH, NH), 2924, 2853 (CH_2), 1643 (amide I), 1536 (amide II). ^{1}H NMR (CD_3OD) δ 0.90 (t, 3H, *J* = 6.8 Hz, CH_2CH_3), 1.23–2.25, 3.90 (m, 30H, methine and methylene protons of oleoyl group), 3.35, 3.50 (s, 6H, NCH_3×2), 3.38 (ddd, 1H, $J_{4,5}$ = 9.8 Hz, $J_{5,6a}$ = 5.1 Hz, $J_{5,6b}$ = 2.1 Hz, H-5), 3.43 (dd, 1H, $J_{3,4}$ = 8.6 Hz, H-4), 3.55 (dd, 1H, $J_{2,3}$ = 9.8 Hz, H-3), 3.68 (dd, 1H, $J_{6a,6b}$ = 12.0 Hz, H-6a), 3.82 (dd, 1H, H-6b), 4.09 (dd, 1H, $J_{1,2}$ = 11.0 Hz, H-2), and 5.67 (d, 1H, H-1). ^{13}C NMR (CD_3OD) δ 14.5 (CH_2CH_3), 23.7, 26.9, 27.5, 27.6, 30.1, 30.2, 30.3, 30.4, 30.6 (×2), 30.7, 33.0 (×2), 37.2, 39.6, 65.1 (methine and methylene carbons of oleoyl group), 41.9, 45.9 (NCH_3×2), 54.4 (C-2), 62.6 (C-6), 71.6 (C-4), 77.5 (C-3), 82.3 (C-5), 90.6 (C-1), 176.5 (C=O), and 195.4 (C=S). MS: *m/z* 569 $[M+Na]^+$.
17. 2-Amino derivative of OCT313 was synthesized by de-*O*-acetylation of 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranosyl *N,N*-dimethyldithiocarbamate hydrochloride with an anion exchange resin DOWEX 1-X4 (OH^-) in methanol in 60.2% yields, which was obtained by the reaction of 2-*N*-anisilidene-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl bromide[13] and sodium *N,N*-dimethyldithiocarbamate in acetone, followed by deanisilidenation with 5 M hydrochloric acid. 2-Amido-2-deoxy-β-D-glucopyranosyl *N,N*-dimethyldithiocarbamate (R^6): mp 161–162 (decomp.), $[\alpha]_D^{23}$ −77.4° (*c* 1.04, H_2O), IR(KBr) cm^{-1}: 3500–3100 (br OH, NH). ^{1}H NMR (D_2O) δ 3.02 (dd, 1H, $J_{1,2}$ = 10.7 Hz, $J_{2,3}$ = 9.0 Hz, H-2), 3.44, 3.55 (s, 6H, NCH_3×2), 3.47 (dd, 1H, $J_{3,4}$ = 9.2 Hz, $J_{4,5}$ = 9.5 Hz, H-4), 3.50 (dd, 1H, H-3), 3.59 (ddd, 1H, $J_{5,6a}$ = 5.3 Hz, $J_{5,6b}$ = 2.2 Hz, H-5), 3.74 (dd, 1H, $J_{6a,6b}$ = 12.5 Hz, H-6a), 3.89 (dd, 1H, H-6b), and 5.62 (d, 1H, H-1). ^{13}C NMR (D_2O) δ 44.9, 48.4 (NCH_3×2), 57.3 (C-2), 63.5 (C-6), 72.2 (C-4), 80.4 (C-3), 83.3 (C-5), 93.2 (C-1), and 195.7 (C=S). HR-FAB-MS: *m/z* 283.0776 $[M+H]^+$ (calcd for $C_9H_{19}O_4N_2S_2$: 283.0786).