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Synthesis of Mycothiol, 1D-1-*O*-(2-[*N*-Acetyl-L-cysteinyl]amino-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol, Principal Low Molecular Mass Thiol in the Actinomycetes

M. Anwar Jardine,[a] Hendrik S.C. Spies,[b,✠] Comfort M. Nkambule,[c]
David W. Gammon[c] and Daniel J. Steenkamp[a,*]

[a]*Department of Chemical Pathology, University of Cape Town Medical School, Observatory 7925, South Africa*
[b]*NMR Laboratory, University of Stellenbosch, Matieland 7602, South Africa*
[c]*Department of Chemistry, University of Cape Town, Rondebosch, South Africa*

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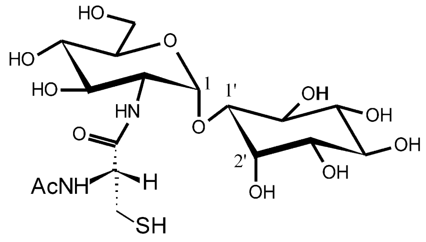
Abstract—Members of the actinomycetes produce 1D-1-*O*-(2-[*N*-acetyl-L-cysteinyl]amino-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol or mycothiol **1** as principal low molecular mass thiol. Chemical synthesis of a biosynthetic precursor of mycothiol, the pseudo-disaccharide 1D-1-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol **13** was achieved by the following steps: (1) Enantioselective synthesis gave the glycosyl acceptors (−)-2,3,4,5,6-penta-*O*-acetyl-D-*myo*-inositol **D-7** and the corresponding L-isomer **L-7**. (2) Condensation of **D-7** and **L-7** with the glycosyl donor 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitrophenylamino)-α-D-glucopyranosylbromide afforded the corresponding α and β anomeric products, which could be resolved by silica gel chromatography. (3) Deprotection of these by hydrolysis using an anion exchange resin gave 1D- and 1L-1-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol **13** and **15** and the corresponding β-coupled anomers **14** and **16**. Only **13**, and to a much lesser extent **15**, were used by enzymes present in an ammonium sulphate fraction of a cellfree extract of *Mycobacterium smegmatis* for the enzymatic synthesis of mycothiol. In the absence of acetyl-SCoA, the immediate biosynthetic precursor of **1**, desacetylmycothiol, was the major product. © 2002 Elsevier Science Ltd. All rights reserved.



Mycothiol

Introduction

All organisms capable of growth in an aerobic environment produce one or more low molecular mass thiols as a major metabolite. Studies on the distribution of these compounds in procaryotes[1,2] have brought to light significant differences between gram negative and gram positive bacteria. As in the vast majority of eukaryotes, glutathione is the principal antioxidant thiol in gram negative bacteria. Fahey et al.[2,3] proposed that the need to incorporate cysteine into the form of this proteolytically stable tripeptide stemmed from the rapid rate of autooxidation of free cysteine, which generates peroxide as a harmful product. Many gram positive bacteria, however, lack glutathione and, instead, produce a

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*Corresponding author. Tel.: +27-21-406-6105; fax: +27-21-406-6153; e-mail: daan@chempath.uct.ac.za
✠Deceased.

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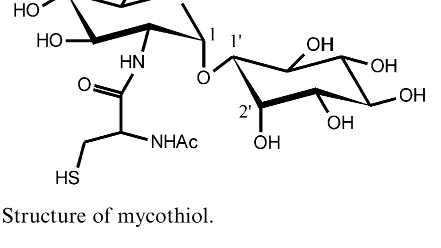
number of alternate thiols. Most Actinomycetes produce mycothiol (1D-1-*O*-(2-[*N*-acetyl-L-cysteinyl]amino-2-deoxy-α-D-glucopyranosyl-*myo*-inositol) (Scheme 1) as their principal low molecular mass thiol.[4–6]

Interest in mycothiol derives from several considerations. In its functions, mycothiol probably substitutes for glutathione as in the gram-positive bacteria, *Amycolatopsis methanolica and Rhodococcus erythropolis*, which use mycothiol, instead of glutathione, as the factor in the NAD/factor-dependent formaldehyde dehydrogenase.[7] The role of glutathione in bacteria is mainly a protective one and mutants defective in its synthesis show significantly enhanced sensitivity to oxidative stress and alkylating reagents. Glutathione also plays an important role in the metabolism of drugs. In the enteric bacteria the expression of several antioxidant enzymes, including glutathione reductase, γ-glutamylcysteine synthetase, catalase/peroxidase and alkylhydroperoxidase, is governed by the oxidation state of the *oxyR* transcription factor.[8] Inactivating mutations with respect to *oxyR* in the enterobacteria results in sensitivity to isoniazid,[9] a frontline drug for the treatment of tuberculosis. Since *Mycobacterium tuberculosis* WT carries an inactive *oxyR* gene[10] this phenomenon is probably a contributing factor in the exceptional sensitivity of *M. tuberculosis* to isoniazid. The relationship between the levels of expression of the antioxidant enzymes and isoniazid sensitivity, however, seems to be a complex one which is not entirely understood.[10] The recent observation that a mycothiol deficient mutant of *M. smegmatis* is considerably more resistant to isoniazid than the wildtype, can, for instance, not readily be rationalized in terms of current knowledge concerning mechanisms of resistance against the drug[11] and points to the need for more detailed studies on the metabolism and functions of mycothiol.

The biosynthesis of mycothiol proceeds with the early formation of the pseudodisaccharide, 1D-1-*O*-{2-*N*-acetylamino-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol (*N*-acetyl-α-D-GI), which is deacetylated and further metabolized by the sequential addition of first cysteine and then acetate, from acetyl-SCoA, to form mycothiol (Scheme 2).[12,13] The steps involved in the formation of *N*-acetyl-α-D-GI have not yet been characterized. Mycothiol is maintained in the reduced form by a NAD(P)H dependent disulfide reductase[4] encoded by *gorA* in the mycobacterial genome.[14,15] Mycothiol and its biosynthetic intermediates are required for the further characterization of the enzymes involved in its synthesis and of the newly discovered disulfide reductase.

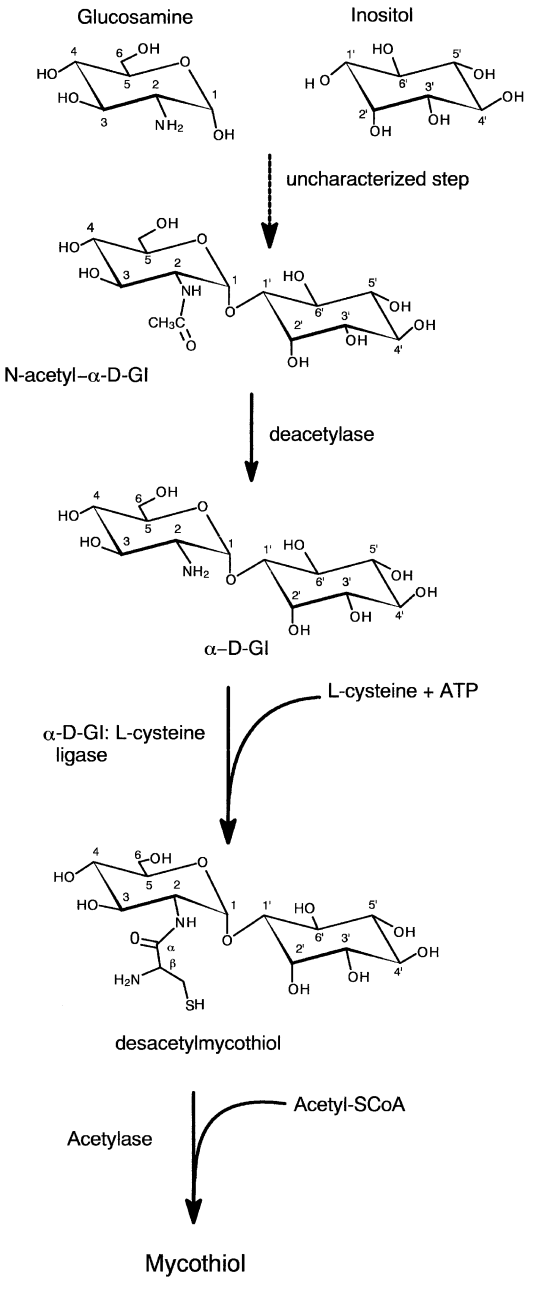


Scheme 1. Structure of mycothiol.

In this paper, we describe the synthesis of α-D-GI and its enzymatic conversion to mycothiol, which allowed an elucidation of the mycothiol biosynthetic pathway, as reported in a previous paper.[12] Our synthetic plan gave us access to enantiomeric and stereoisomeric analogues of α-D-GI, which were used to investigate the substrate specificity of the α-D-GI-L-cysteine ligase.[12]

Results and Discussion

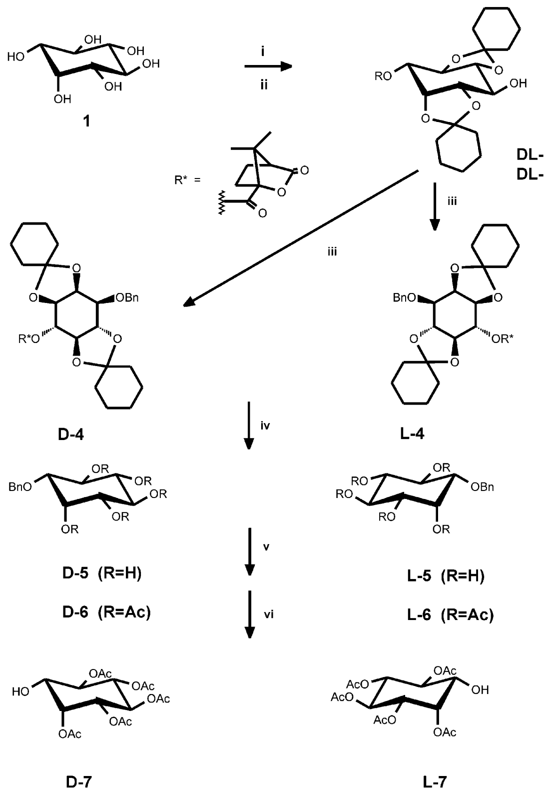
The pseudo-disaccharide moiety of mycothiol consists of D-glucosamine glycosidically coupled (α1-1′) to D-*myo*-inositol.[4] Such a coupling can be achieved synthetically, using an appropriately protected glycosyl donor and a *myo*-inositol acceptor. This synthetic route



Scheme 2. Biosynthesis of mycothiol.

necessitated the selective protection of *myo*-inositol in such a manner that it renders the 1′-OH available for glycosidic coupling. Commercially available *myo*-inositol (**1**; Scheme 3) was converted into racemic 1,2:4,5-dicyclohexylidene-*myo*-inositol (DL-**2**) (26%).[16] The protected *myo*-inositol (DL-**2**) was subsequently selectively benzylated to give the 3-benzylated product (DL-**3**) (60%).[17] The 3-benzylated products (DL-**3**) were resolved as the camphanate esters, using known literature procedures to give (D-**4**) and (L-**4**), respectively.[17] Acid hydrolysis secured the removal of the cyclohexylidene groups to give the monobenzylated *myo*-inositol (D-**5** and L-**5**), which was subsequently acetylated to give D/L-1-*O*-benzyl-2,3,4,5,6-penta-*O*-acetyl-*myo*-inositol (D-**6** and L-**6**). Reduction of the benzyl group gave the D/L-2,3,4,5,6-penta-*O*-acetyl-*myo*-inositol (D-**7** and L-**7**). Koenigs–Knorr coupling of products D-**7** and L-**7** with 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitrophenylamino)-α-D-glucopyranosyl bromide (**8**),[18] respectively, gave a mixture of α- and β-linked pseudo-disaccharides (α/β 1:1, w/w) (60%) (**9** and **11**) and (**10** and **12**), respectively (Scheme 4). Treatment of the protected pseudo-disaccharides (**9**–**12**), with an anion exchange resin (IR400-OH), gave pseudo-disaccharides (**13**–**16**).

The synthesis of α-D-GI (**13**), without the resolution of the protected *myo*-inositol (D,L-**3**) was also attempted. The racemic D,L-2,3,4,5,6-penta-*O*-acetyl-*myo*-inositol (D,L-**7**) were synthesised, using the same reaction sequence as before. Glycosidic coupling of 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitrophenylamino)-α-D-glucopyranosyl bromide (**8**) with (D,L-**7**), gave a racemic mixture of α- and β-linked pseudo-disaccharides (**9**–**12**).

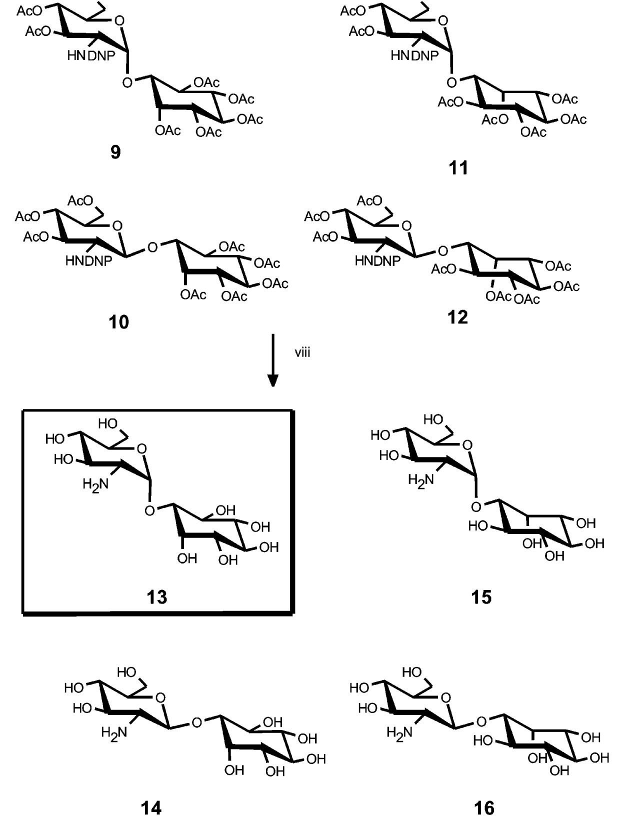


Scheme 3.

The α-coupled D,D (**9**) and D,L (**11**) diastereomers were separated, with great effort, using thin-layer chromatography, whereas the β-coupled products D,D (**10**) and D,L (**12**), were inseparable. Due to these purification difficulties, the enantioselective synthesis of α-D-GI (**13**) seemed a more practical route to follow.

Our synthetic plan gave us access to stereoisomeric analogues of the mycothiol precursor, α-D-GI (**13**), namely compounds **14**–**16**. These analogues where used to investigate the substrate specificity of the α-D-GI-L-cysteine ligase.[12]

The final step in mycothiol synthesis, namely the coupling of cysteine, was achieved enzymatically (Scheme 2). A crude, undialysed, cell-free extract of *M. smegmatis* catalysed the conversion of α-D-GI (**13**) into a mixture of desacetyl mycothiol and mycothiol (1:4, 40% conversion).[12] The products were quantified by [^{35}S]-cysteine incorporation, and characterised as the bimane derivative.[4] The α-D-GI-L-cysteine ligase produced mycothiol from α-D-GI (**13**) [$V_{\max}$ (app) = 0.92 ± 0.02 nmol/min/



Scheme 4. Reagents: (i) 1-ethoxycyclohexane, *p*-toluenesulphonic acid and dimethylformamide; (ii) NaH, BnBr and toluene; (iii) camphanic acid chloride and Et_3N in CH_2Cl_2; (iv) (a) KOH in ethanol; (b) 80% acetic acid (v/v); (v) acetic anhydride and pyridine; (vi) H_2 and Pd/C; (vii) AgOTf, 2,6-*tert*-dibutylpyridine and CH_2Cl_2; (viii) Amberlite IR400 (OH).

mg, K_m (app) = 140 ± 9 μM], whereas α-L-GI (**15**) displayed poor binding [V_{max} (app) = 0.51 ± 0.22 nmol/min/mg, K_m (app) = 2.69 ± 1.34 μM]. The β-coupled products (**14** and **16**), were inactive. The synthetic coupling of *N*-acetyl-cysteine to α-D-GI (**13**), using a variety of water soluble coupling and activating reagents was hitherto unsuccessful. This could be due to a combination of insolubility and steric demand of the various reactants. An alternative synthetic approach to mycothiol is currently in progress. This involves the synthesis of an appropriately protected α-D-GI (**13**), with the 2-amino group available for coupling with cysteine. Coupling of a S-protected *N*-acetyl-cysteine to the aforementioned protected α-D-GI (**13**), may be successful in an organic solvent. All products were characterised by NMR and electron spray mass spectrometry.

Experimental

General methods

All reactions were carried out under a nitrogen atmosphere, unless otherwise specified. Reactions were monitored by TLC using Merck F_{254} precoated silica gel plates. Detection was by means of a UV lamp (wavelength 254 nm) and by heating the plate at 200 °C after spraying with a solution of 5% vanillin/90% ethanol/5%H_2SO_4. All combined organic reactions were dried over anhydrous magnesium sulphate before evaporation on a Buchi rotary evaporator with bath temperature of 50 °C or below. Column chromatography was carried out on silica gel and the eluent mixture used is specified in each experiment. Commonly used solvents were purified and dried as follows: Methanol was distilled from magnesium powder under a nitrogen atmosphere and stored over molecular sieves type 3 Å. Triethylamine and pyridine was distilled from potassium hydroxide under a nitrogen atmosphere and stored over potassium hydroxide pellets. Dichloromethane was distilled from phosphorus pentoxide and stored over molecular sieves type 4 Å. Diethyl ether was distilled from sodium wire under a nitrogen atmosphere immediately before use. Ethyl acetate was washed with saturated aqueous sodium carbonate and brine, dried over sodium sulfate and distilled. *N,N*-Dimethylformamide was distilled from calcium hydride under reduced pressure and stored over molecular sieves type 4 Å. All melting points were determined in open capillary tubes and are uncorrected. Specific rotations ($[\alpha]_D$) were determined at 20 °C, unless otherwise specified, using a Perkin-Elmer 141 polarimeter and are recorded in units of $10^{-1}\,^\circ\,cm^2\,g^{-1}$. Mass spectra were recorded on a VG Autospec 4600.

NMR spectroscopy

NMR spectra were obtained on a Varian VXR 300 spectrometer operating at 299.9 and 75.42 MHz for the observation of ^1H and ^{13}C, respectively. All spectra were recorded at 25 °C in either D_2O or $CDCl_3$ as lock solvent. ^1H chemical shifts in D_2O were measured relative to sodium 4,4-dimethyl-4-silapentane-1-sulfonate (δ 0.0 ppm) as external reference. ^1H and ^{13}C chemical shifts in $CDCl_3$ were obtained relative to tetramethylsilane (δ 0.0 ppm) as internal reference. ^{13}C chemical shifts in D_2O were measured using 2.5% dioxane (δ 67.3 ppm) as external reference. ^{13}C multiplicities were determined using the APT[19] technique. Two-dimensional ^1H–^1H correlation spectra (COSY) were obtained using the pulse sequence: D1–90°–(t_1 + D3)–60°–AQ,[20] where D3 = 0.25 s was used to enhance long-range coupling effects. Two-dimensional ^1H–^{13}C correlation spectra (HETCOR) were obtained by means of the pulse sequence described previously.[21]

NMR spectral assignments for compounds **L-6** to **16** are presented in tabular form (Tables 1–6).

Synthetic procedures

(+)-1-*O*-Benzyl-2,3,4,5,6-penta-*O*-acetyl-D-*myo*-inositol (D-6). The (−)-3-benzyl-1,2:4,5-dicyclohexylidene-D-*myo*-inositol (**D-4**)[17] (1.68 g, 3.91 mmol) was treated with a solution of KOH (2.24 g, 40 mmol) in ethanol for 16 h at 20 °C. The solvent was evaporated, followed by the addition of 80% aqueous acetic acid (89 mL). The reaction mixture was allowed to stir at 95 °C for 1 h. Evaporation of the solvents, gave the crude 1-*O*-benzyl-D-*myo*-inositol (**D-5**). The latter compound was dissolved in pyridine (20 mL) and acetic anhydride (20 mL) and allowed to stir at 25 °C for 16 h. The reaction mixture was subsequently poured on ice, resulting in the precipitation of the crude white crystalline product (**D-6**), which was filtered and dried (1.7 g, 91%); mp 185–187 °C (ethyl acetate–hexane); $[\alpha]_D$ + 30.7° (±0.6°) (*c* 0.44, $CHCl_3$).

(−)-1-*O*-Benzyl-2,3,4,5,6-penta-*O*-acetyl-L-*myo*-inositol (L-6). Repetition of the above procedure, using (+)-3-benzyl-1,2:4,5-dicyclohexylidene-L-*myo*-inositol (**L-4**)[17]

Table 1. ^{13}C NMR data for compounds **6** and **7** (δ, ppm) at 25 °C in $CDCl_3$

	D-6 or **L-6**	**D-7** or **L-7**
Inositol[a]		
	71.84	72.50
	71.21	70.77
	70.93	70.49
	69.53	69.47
	69.17	69.20
	66.69	68.85
Acetyl		
CH_3	20.87	20.83
	20.69	20.76
	20.57×2	20.56
	20.53	20.52×2
CO	170.10	171.02
	169.94	170.27
	169.90	169.91
	169.70	169.81
	169.63	169.70
OBn		
OCH_2	74.45	—
1	136.90	
2, 6	127.82	—
3, 5	128.52	
4	128.09	—

[a] No assignments were made for inositol ring.

Table 2. ^{13}C NMR data of compounds **9**–**12** (δ, ppm) at 25 °C in CDCl$_3$

	9	**10**	**11**	**12**
Dinitrohenyl				
1	147.45	146.88	148.18	[a]
2	131.32	131.74	131.46	[a]
3	124.16	124.51	123.79	123.42
4	136.96	136.82	137.36	[a]
5	130.01	130.01	129.58	130.04
6	114.17	112.95	115.23	115.96
Glucosamine				
1	92.77	96.92	103.36	99.26
2	54.52	54.89	57.44	57.63
3	71.96	71.17	72.37	72.24*
4	71.02[†]	67.36	67.96	67.86*
5	68.82	69.45	72.30	72.07*
6	61.63	61.71	61.61	61.53*
Inositol				
1′	69.02[†]	68.54[‡]	68.87[‡]	68.77*
2′	65.30[‡]	69.17	69.93	69.25*
3′	70.02[‡]	71.99[‡]	74.96[‡]	73.24*
4′	69.64*	72.35*	70.87[*†]	70.34*
5′	67.96[†]	70.90	70.83[†]	69.79*
6′	69.49*	68.98*	68.96*	66.35*
Acetyl				
CH$_3$	20.72, 20.57 20.51×4 20.44, 20.3	20.65×2, 20.62 20.50×2, 20.41 20.39, 20.09	20.96, 20.66 20.54, 20.50×2 20.42, 20.34 20.10	20.76, 20.66 20.56, 20.49×2 20.42, 20.38 19.67
CO	170.58, 170.51 169.97, 169.74 169.55, 169.45 169.45, 169.06	170.63, 170.56 169.91, 169.76 169.69, 169.69 169.37, 168.66	170.71, 170.34 169.77, 169.71 169.44, 169.32 169.28, 168.91	

[a] Not observed due to low concentration.

[*,†,‡] Assignments are interchangeable in the same column.

Table 3. ^{13}C NMR data for compounds **13**–**16** (δ, ppm) at 25 °C in D$_2$O

	Mycothiol[4]	**13**	**14**	**15**	**16**
Glucosamine					
1	99.85	98.57	107.31	103.11	98.52
2	54.73	57.49	59.53	57.83	58.22
3	71.46	73.70*	72.26*	74.46*	74.67
4	70.85	72.36*	73.48*	73.77*	78.96*
5	73.29	70.87*	74.33*	72.50*	72.34*
6	61.33	63.12*	63.28	63.28	63.03
Inositol					
1′	79.86	78.13*	83.72*	82.03*	79.55
2′	72.46	76.88*	78.55*	76.83*	71.79
3′	71.81	76.40*	78.42*	75.59 *	73.52
4′	72.83	74.89*	76.75*	75.40*	74.77
5′	74.99	74.81*	74.83*	74.77*	76.78
6′	72.86	73.70*	74.36*	74.63*	73.22

*Assignments are interchangeable in the same column.

afforded product (**L-5**) and subsequently, the title compound (**L-6**) (95%); mp 185–186 °C (ethyl acetate–hexane); [α]$_D$ −30.1° (±0.4°) (*c* 0.39, CHCl$_3$).

(−)-2,3,4,5,6-Penta-*O*-acetyl-D-*myo*-inositol (D-7). To a solution of (**D-6**) (1.7 g, 3.54 mmol) in ethyl acetate (78 mL) were added 10% Pd on charcoal (1.2 g). The suspension was shaken under an atmosphere of hydrogen at 20 psi for 48 h. The catalyst was then removed by filtration and the filtrate evaporated under reduced pressure, to give (**D-7**) (1.2 g, 87%); mp 185–186 °C (chloroform–diethyl ether); [lit.[22] mp 185–187 °C); [α]$_D$ −5.8° (*c* 1.8, CHCl$_3$) (lit.[22] [α]$_D$ −11.2° (*c* 0.05, CHCl$_3$)].

(+)-2,3,4,5,6-Penta-*O*-acetyl-L-*myo*-inositol (L-7). Repetition of the above procedure, using (**L-6**) afforded the title product (**L-7**) (87%); mp 185–187 °C (chloroform–diethyl ether); (lit.[22] mp 185–187 °C); [α]$_D$ +8.5° (*c* 1.6, CHCl$_3$) [lit.[22] [α]$_D$ +11.2° (*c* 0.1, CHCl$_3$)].

2,3,4,5,6-Penta-*O*-acetyl-1-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitrophenylamino)-α-D-glucopyranosyl)-D-*myo*-inositol (9) and the-β-D-glucopyranosyl derivative (10). A mixture of (**D-7**) (1.0 g, 2.56 mmol), 2,6-di-*tert*-butylpyridine (0.65 mL) and dichloromethane (15 mL) was allowed to stir at 25 °C for 1 h. Silver triflate (1.0 g, 3.89 mmol) and a solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitrophenylamino)-α-D-glucopyranosyl bromide (**8**)[18] (1.95 g, 3.64 mmol) in dichloromethane (23 mL) were then added. The mixture was allowed to stir at 20 °C for 30 h, followed by neutralisation with triethylamine and then filtration. The filtrate was concentrated under reduced pressure to give a crude product mixture (3.65 g). Elution of the mixture of products from a silica gel (500 g) column (5 cm diameter) (diethyl ether–chloroform 2:98) gave (**9**) (1.06 g, 49%) as a yellow amorphous solid; (R_f 0.25 diethyl ether–chloroform 1:9); [α]$_D$ +11° (*c* 1.3, CHCl$_3$). Found: MNH$_4^+$, 861.2524, $C_{34}H_{41}O_{33}N_3$ requires MNH$_4$, 861.2525, followed by (**10**) (626 mg, 29%) as a yellow amorphous solid; (R_f 0.10, diethyl ether–chloroform 1:9); [α]$_D$ −34°

Table 4. ^{1}H Chemical shifts (δ, ppm), multiplicities and ^{1}H–^{1}H coupling constants (*J*, Hz) for compounds **6** and **7** at 25 °C in $CDCl_3$

	D-6 or **L-6**	**D-7** or **L-7**
Inositol		
1′	3.608 (dd, 10.1/2.8)	3.901 (ddd, 10.0/7.0/3.0)
2′	5.757 (t, 2.8)	5.594 (t, 2.9)
3′	4.938 (dd, 10.6/2.8)	4.978 (dd, 10.6/2.8)
4′	5.480 (dd, 10.6/9.7)	5.457 (dd, 10.6/9.6)
5′	5.079 (t, 9.8)	5.148 (t, 9.8)
6′	5.424 (t, 10.0)	5.310 (t, 10.0)
OBn		
OCHA	4.664 (d, 12.1)	—
OCHB	4.399 (d, 12.1)	—
Phenyl	7.2–7.4 (m, 5H)	—
OH	—	2.569 (d, 7.0)
CH_3	2.199, 2.015	2.213, 2.088
	2.001, 1.998	2.023, 2.012
	1.989	1.997

(*c* 1.1, $CHCl_3$). Found: MNH_4^+, 861.2550, $C_{34}H_{41}O_{33}N_3$ requires MNH_4, 861.2525.

2,3,4,5,6-Penta-*O*-acetyl-1-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitrophenylamino)-α-D-glucopyranosyl)-L-*myo*-inositol (11) and the-β-D-glucopyranosyl derivative (12). Repetition of the above procedure, using (**L7**) afforded the title products (**11**) (45%) as a yellow amorphous solid; (R_f 0.21, diethyl ether–chloroform 1:9); $[\alpha]_D$ +11° (*c* 1.0, $CHCl_3$). Found: MNH_4^+, 861.2537, $C_{34}H_{41}O_{33}N_3$ requires MNH_4, 861.2525, followed by (**12**) (25%) as a yellow amorphous solid; (R_f 0.25, diethyl ether–chloroform 1:9); $[\alpha]_D$ −60° (*c* 2.1, $CHCl_3$). Found: MNH_4^+, 861.2547, $C_{34}H_{41}O_{33}N_3$ requires MNH_4, 861.2525.

1D-1-*O*-(2-Amino-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol (13). A mixture of (**9**) (580 mg, 0.69 mmol), metha-

Table 5. ^{1}H Chemical shifts (δ, ppm), multiplicities and ^{1}H–^{1}H coupling constants (*J*, Hz) for compounds **9**–**12** at 25 °C in $CDCl_3$

	9	**11**	**10**	**12**
Inositol				
1′	4.015 (dd, 10.5/2.5)	4.157 (dd, 9.9/2.9)	3.941 (dd, 10.1/3.0)	4.042 (d, 10.1/2.8)
2′	5.611 (t, 2.6)	5.660 (t, 2.9)	5.706 (t, 2.8)	5.514 (t, 2.8)
3′	4.974 (dd, 10.4/2.7)	5.031 (dd, 10.6/2.9)	4.963 (dd, 10.6/2.7)	4.894 (dd, 10.6/2.8)
4′	5.532 (dd, 10.4/9.8)	5.523 (dd, 10.6/9.8)	5.362 (dd, 10.6/9.7)	5.353 (dd, 10.5/9.5)
5′	5.167 (t, 9.8)	5.077 (t, 9.9)	5.037 (t, 9.8)	5.157 (t, 9.7)
6′	5.652 (dd, 10.5/9.8)	5.536 (t, 9.9)	5.186 (t, 10.0)	5.274 (t, 10.0)
Glucosamine				
1	5.224 (d, 3.5)	5.169 (d, 3.9)	4.665 (d, 8.1)	4.675 (d, 8.0)
2	4.142 (ddd, 9.7/9.6/3.5)	4.086 (ddd, 10.0/10.1/3.9)	3.884 (ddd, 10.2/9.0/8.1)	3.761 (ddd, 10.1/9.1/8.0)
3	5.243 (t, 9.6)	5.293 (t, 9.9)	5.278 (dd, 10.2/9.2)	5.133 (dd, 10.1,9.2)
4	5.151 (t, 9.7)	5.146 (t, 9.8)	5.147 (t, 9.6)	5.327 (dd, 10.3,9.3)
5	4.099 (ddd, 9.9/4.5/2.5)	4.209 (ddd, 9.8/4.7/1.9)	3.793 (dt, 9.9/3.7,3.7)	3.813 (ddd, 10.0/4.2/2.3)
6A	4.297 (dd, 12.5/4.5)	4.348 (dd, 12.1/4.7)	4.241 (d, 3.7)	4.412 (dd, 12.5/4.2)
6B	4.237 (dd, 12.5/2.5)	4.143 (dd, 12.1/1.9)	4.241 (d, 3.7)	4.142 (dd, 12.5/2.3)
2,4-Dinitrophenyl				
3	9.106 (d, 2.7)	9.157 (d, 2.6)	9.113 (d, 2.6)	9.076 (d, 2.7)
5	8.265 (dd, 9.6/2.6)	8.251 (dd, 9.5/2.6)	8.194 (dd, 9.6/2.6)	8.263 (dd, 9.6/2.7)
6	7.097 (d, 9.6)	7.010 (d, 9.5)	7.092 (d, 9.6)	7.155 (d, 9.6)
NH	8.571 (d, 9.7)	8.635 (d, 10.0)	8.496 (d, 9.0)	8.341 (d, 9.1)
CH_3	2.181, 2.139	2.396, 2.154	2.207, 2.152	2.110, 2.105
	2.120, 2.052	2.069, 2.038	2.051, 1.992	2.049, 2.014
	2.025, 2.015	2.007, 1.953	1.975, 1.919	1.973, 1.935
	1.973, 1.798	1.786, 1.549	1.919, 1.756	1.908, 1.445

Table 6. ^{1}H Chemical shifts (δ, ppm), multiplicities and ^{1}H–^{1}H coupling constants (*J*, Hz) for compounds **13**–**16** at 25 °C in D_2O

	13	**15**	**14**	**16**
Inositol				
1′	3.584 (dd, 10.1/2.8)	3.586 (dd, 9.9/2.8)	3.636 (dd, 10.0/2.8)	3.862 (dd, 10.1/2.6)
2′	4.236 (t,2.8)	4.188 (t, 2.8)	4.281 (t, 2.8)	4.236 (t, 2.6)
3′	3.491 (dd, 10.0/2.8)	3.515 (dd, 10.0/2.8)	3.515 (dd, 10.0/2.8)	3.495 (dd, 10.0,2.6)
4′	3.643 (dd, 9.9/9.2)	3.614 (dd, 9.9/9.2)	3.608 (dd, 10.0/9.2)	3.640 (dd, 10.0/9.3)
5′	3.296 (t, 9.3)	3.286 (t, 9.3)	3.278 (t, 9.2)	3.291 (t, 9.3)
6′	3.707 (dd, 10.0/9.2)	3.755 (dd, 10.0/9.4)	3.743 (dd, 10.0/9.2)	3.734 (dd, 10.1/9.3)
Glucosamine				
1	5.031 (d, 3.60)	5.168 (d, 3.8)	4.551 (d, 8.3)	4.872 (d, 8.3)
2	2.755 (dd, 10.2/3.6)	2.854 (dd, 10.3/3.8)	2.678 (t, 8.2)	3.132 (dd, 10.6/8.3)
3	3.637 (dd, 10.0/9.3)	3.674 (dd, 10.2/9.0)	3.31–3.42	3.68[a]
4	3.414 (dd, 10.0/9.1)	3.389 (t, 9.4)	3.31–3.42	3.47[a,b]
5	3.963 (ddd, 10.0/4.7/2.6)	3.832 (ddd, 9.4/6.1/2.1)	3.31–3.42	3.48[a,b]
6A	3.813 (dd, 12.3/2.6)	3.850 (dd, 12.7/2.1)	3.880 (dd, 12.3/2.0)	3.901 (dd, 12.4/1.9)
6B	3.752 (dd, 12.3/4.7)	3.738 (dd, 12.7/6.1)	3.723 (dd, 12.3/5.2)	3.74[a]

[a] Value obtained from the COSY spectrum.

[b] Value obtained from the HETCOR spectrum.

nol (73 mL), acetone (45 mL), water (27 mL), and Amberlite IRA400 (OH) resin (90 mL) was allowed to stir at 25 °C for 3 days. After filtration of the mixture, the filtrate was concentrated under reduced pressure, followed by lyophilisation to give the product (**13**) (200 mg, 85%) as a white solid; $[\alpha]_D$ +109° (c 1.0, H_2O). Found: MH^+, 342.1409, $C_{12}H_{23}O_{10}N$ requires MH, 342.1400.

1D-1-*O*-(2-Amino-2-deoxy-β-D-glucopyranosyl)-*myo*-inositol (14). Repetition of the above procedure, using (**10**) afforded product (**14**) (62%); as a white solid; $[\alpha]_D$ +0.17° (c 1.2, H_2O). Found: MH^+, 342.1410, $C_{12}H_{23}O_{10}N$ requires MH, 342.1400.

1L-1-*O*-(2-Amino-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol (15). Repetition of the above procedure, using (**11**) afforded the title product (**15**) (88%); as a white solid; $[\alpha]_D$ +73° (c 1.1, H_2O). Found: MH^+, 342.1385, $C_{12}H_{23}O_{10}N$ requires MH, 342.1400.

1D-1-*O*-(2-Amino-2-deoxy-β-D-glucopyranosyl)-*myo*-inositol (16). Repetition of the above procedure, using (**12**) afforded the title product (**16**) (90%); as a white solid; $[\alpha]_D$ −27° (c 0.45, H_2O). Found: $MH^{+,}$ 342.1411, $C_{12}H_{23}O_{10}N$ requires MH, 342.1400. Enzymatic formation of mycothiol, 1D-1-*O*-(2-[*N*-acetyl-L-cysteinyl]-amido-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol, has been fully described in a previous publication.[12]

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