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Note

Synthesis of fused bicyclic thioglycosides of N-acylated glucosamine as analogues of mycothiol

Rikard Slättegård,[a] David W. Gammon[b] and Stefan Oscarson[a,*]

[a]*Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden*
[b]*Department of Chemistry, University of Cape Town, Rondebosch 7701, South Africa*

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Abstract—The synthesis of a fused bicyclic thioglycoside analogue of mycothiol, (3*R*)-3-acetylamino-4-one-6,7-dihydro-(1′,2′-dideoxy-β-D-glucopyranoso)[2′,1′-*f*′]-1,5-thiazepane (**5**), is reported. Treatment of phthalimido-protected peracetylated glucosamine with *N*-acetyl-cysteine and boron trifluoride-etherate gave the β-linked thioglycoside, which was deprotected and cyclized, using HOBt and EDCl to form the lactam and giving the target structure. This mycothiol mimic and its tri-*O*-acetate will be investigated as potential inhibitors of enzymes involved in the biosynthesis of mycothiol. The protected derivative also has the potential to be an α-selective *N*-cysteinyl glucosamine donor; however, initial glycosylation attempts failed due to the apparent stability of the fused bicyclic system.

Keywords: Glycomimetics; 1,2-*cis*-Glycosides; *Mycobacterium tuberculosis*; Mycothiol

Tuberculosis (TB) is a contagious disease caused by *Mycobacterium tuberculosis* and affects significant numbers of people worldwide. The global incidence of TB in 2002 was estimated at 8.8 million cases, with 1.8 million TB-related deaths, while in Africa, the estimates for 2002 were of 2.3 million cases with 550,000 deaths. The high mortality rates in Africa have been attributed to the association of TB with HIV and AIDS.

This project is based on the finding by one of our collaborators (Professor Daan Steenkamp, University of Cape Town) that *M. tuberculosis* in common with other actinomycetes produces the pseudodisaccharide mycothiol, 1D-1-*O*-(2-[*N*-acetyl-L-cysteinyl]amino-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol, apparently in defense against xenobiotics and oxidative stress.[1] The enzymes involved in the biosynthesis of this substance[2,3] are therefore potential drug targets, and analogues of the biosynthetic intermediates have been suggested as potential drug leads.

The synthesis of mycothiol is not trivial and contains several synthetic challenges including regio-selective protection and resolution of *myo*-inositol, stereospecific formation of the α-D-glucosamine glycosidic linkage, and introduction of the cysteine residue. So far only three total syntheses of mycothiol have been reported in overall yields of about 6% calculated from *myo*-inositol.[4–6] Hence, new methodology to increase the yield and to simplify the synthesis both to mycothiol and mimics thereof would be of value. Because the introduction of the cysteine moiety is often quite low-yielding,[5,6] we envisaged a route where this event would take place early in the synthetic pathway. This would allow synthesis of mimics containing variations of the inositol component, which have been shown to be interesting potential inhibitors for the mycothiol biosynthesis.[7] To control the stereoselectivity in the introduction of various aglycones, a cyclic thioglycoside donor, compound **4** (Scheme 1), was considered, with the thiol group of the cysteine residue β-linked to the anomeric position. Activation of this donor with thiophilic reagents in the presence of hydroxyl acceptors would hopefully then yield 1,2-*cis*-linked α-glycosides through a direct displacement reaction in a similar way to that described by Boons and co-workers with 2-*O*-(1*S*)-phenyl-2-(phenylsulfanyl)ethyl substituted glycosyl donors.[8]

* Corresponding author. E-mail: s.oscarson@organ.su.se

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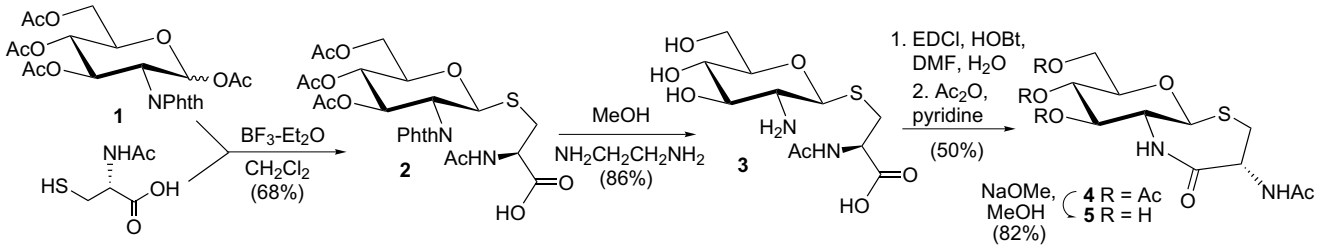

Scheme 1.

The synthesis of donor **4** was attempted in two ways, either through first formation of the amide bond followed by the construction of the thioglycosidic linkage or vice versa. There is precedence in the literature, with the corresponding *O*-glycoside, that at least the latter pathway should work.[9–12] Problems were encountered at several stages in the first approach where coupling of the cysteine moiety to various 2-amino-2-deoxy-D-glucose derivatives was ineffective. Addition of *S*-trityl protected N-acetylated L-cysteine to free 2-amino-2-deoxy-D-glucose using DCC as coupling reagent was the most promizing with a 33% yield in the amide bond formation. However, after acetylation and detritylation of this intermediate no conditions could be found to form the cyclic thioglycoside **4**.

This approach was therefore abandoned and attention turned instead to the initial introduction of the cysteine residue at the anomeric center (Scheme 1). To ensure a β-linkage the well-known phthalimido-protected derivative **1** was used as donor, and N-acetylated L-cysteine with a free carboxyl group as acceptor.[9] The desired β-thioglycoside was obtained in a 68% yield using boron trifluoride-etherate as the promoter. Removal of the phthalimido protecting group and de-O-acetylation was accomplished by treatment with ethylenediamine in methanol to yield the intermediate **3** (86%). As mentioned above, there are reports in the literature of formation of the cyclic product from the corresponding serine *O*-glycoside; during N-acetylation, as a by-product when attempting intermolecular peptide bond formation, and during removal of the 2-amino protecting group when the carboxyl group is in the form of an activated ester.[9–12] Furthermore, it was found that the intramolecular cyclic amide was only formed when the β-glycoside was used as precursor.[12] As expected, this also worked for our thioanalogue. Treatment of compound **3** with HOBt and EDCl afforded the desired bicyclic product in 50% yield, isolated as its tri-*O*-acetate **4** after acetylation with Ac_2O in pyridine, both to simplify purification and also to obtain a suitable donor candidate directly. Deacetylation through methoxide treatment then yielded the crystalline target compound **5** in 82% yield.

Initial attempts to use compound **4** as a donor were performed using either NIS/AgOTf or DMTST as the promoter, with the idea that this would afford a thiol or a methyl disulfide group in the product, which might then be further functionalized. However, donor **4** was found to be quite stable, with no reaction observed using an inositol acceptor (2,3,4,5,6-penta-*O*-benzyl-D-inositol). Under forcing conditions (high temperature, large excesses of promoter, and methanol as acceptor) donor **4** was activated and formation of small amounts of the methyl glycoside could be identified by MALDI-TOF MS. However, although donor **4** was consumed, no major product could be detected (by TLC or MALDI-TOF) or purified. The results with methyl triflate as promoter were also disappointing, with no major product detectable either in the presence (to form a glycoside) or absence (to form a sulfonium salt) of methanol.

In conclusion, a straightforward synthesis of fused bicyclic thioglycoside mimics of mycothiol has been worked out. The mimics will be evaluated as inhibitors of various enzymes involved in the biosynthesis of mycothiol (MshB, MshC, MshD) and others such as mycothione reductase, involved in maintaining mycothiol in its reduced form.[2,3] The cyclic thioglycoside is also an interesting α-directing glycosyl donor candidate, but so far effective activation conditions have not been found due to the obvious stability of the bicyclic system. To try to explain the stability of the bicyclic system and also to be of assistance in docking experiments into crystal structures of the various enzymes involved in mycothiol biosynthesis attempts to solve the X-ray crystal structure of compound **5** are in progress.

1. Experimental

1.1. General methods

TLC was carried out on Merck precoated 60 F_{254} plates using AMC (ammonium molybdate–cerium(IV) sulfate–10% sulfuric acid, 100 g:2 g:2 L) or 8% H_2SO_4 for visualization. Column chromatography was performed on silica gel (40–63 μm, Amicon) or reversed phase gel (C_{18} 60A 40–63 μm). NMR spectra were recorded in $CDCl_3$ (Me_4Si, $\delta = 0.00$) or D_2O (acetone ^{13}C $\delta = 30.89$, $^1H = 2.22$) at 25 °C on a Varian 300 MHz

or 400 MHz instrument. Organic solutions were concentrated at 30 °C under reduced pressure.

1.2. (*N*′-Acetyl-L-cysteinyl) 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (2)

To a solution of compound **1** (1.595 g, 3.34 mmol) in dry CH_2Cl_2 (15 mL) were added *N*-acetyl-L-cysteine (1.09 g, 6.68 mmol) and $BF_3{\cdot}Et_2O$ (1.27 mL, 10.1 mmol). The reaction mixture was heated at reflux under argon overnight. The mixture was diluted with CH_2Cl_2 and washed with satd aq $NaHCO_3$. The water phase was then acidified with HCl (1 M) and washed with CH_2Cl_2. The combined organic phases were dried ($MgSO_4$), filtered, and concentrated. Purification by silica gel chromatography (1:0→5:1, $CHCl_3$–MeOH) gave compound **2** (1.311 g, 2.26 mmol, 68%); $[\alpha]_D$ +11 (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$): δ 7.84–7.86 (m, 2H), 7.74–7.76 (m, 2H), 6.71 (d, $J = 6.8$ Hz, 1H), 5.78 (dd, $J = 0.8$, 9.2 Hz, 1H), 5.53 (d, $J = 10.5$ Hz, 1H), 5.15 (t, $J = 9.3$ Hz, 1H), 4.73–4.77 (m, 1H), 4.25–4.33 (m, 3H), 3.82–3.87 (m, 1H), 3.22 (dd, $J = 4.8$, 10 Hz, 1H), 3.08 (dd, $J = 4.8$, 10 Hz, 1H) 2.12 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 1.85 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 171.9, 171.4, 171.3, 170.2, 169.7, 134.8, 124.0, 82.1, 76.2, 71.4, 69.0, 62.4, 53.7, 52.4, 32.3, 22.8, 21.0, 20.7, 20.5; HRMS Calcd for $C_{25}H_{29}N_2O_{12}S$ $[M+H]^+$: 581.1436. Found: 581.1415.

1.3. (3*R*)-3-Acetylamino-4-one-6,7-dihydro-(3′,4′,6′-tri-*O*-acetyl-1′,2′-dideoxy-β-D-glucopyranoso)[2′,1′-*f*]-1,5-thiazepane (4)

Compound **2** (1.082 g, 1.87 mmol) was dissolved in MeOH (20 mL). 20 equiv of ethylenediamine (2.5 mL, 37.4 mmol) was added and the reaction mixture was stirred overnight at 60 °C. The solvents were removed under reduced pressure and the remaining ethylenediamine together with salts was removed from the residue by reversed phase chromatography using H_2O as the eluent. The fractions containing the product were concentrated and freeze-dried to give (*N*′-acetyl-L-cysteinyl) 2-amino-2-deoxy-1-thio-β-D-glucopyranoside (**3**, 0.52 g, 1.60 mmol, 87%); ^{13}C NMR (D_2O): δ 177.3, 174.3, 86.1, 80.8, 77.7, 70.2, 61.5, 56.3, 55.5, 32.4, 22.6. Compound **3** (481 mg, 1.48 mmol) was dissolved in DMF–H_2O (9:3, 12 mL). EDCl (576 mg, 3.0 mmol) and HOBt (400 mg, 2.96 mmol) were added and the mixture was stirred for 1 h at rt. The mixture was concentrated and the residue was then purified by silica gel chromatography (3:1, $CHCl_3$–MeOH) to give compound **5** (see below for data), which was dissolved in pyridine and Ac_2O added to the solution. The acetylation was quenched by the addition of MeOH after completion (TLC: 9:1 $CHCl_3$–MeOH) and concentrated. Silica gel chromatography (1:0→5:1, $CHCl_3$–MeOH) gave compound **4** (320 mg, 0.74 mmol, 50%); $[\alpha]_D$ +31 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.14 (d, $J = 5.5$ Hz, 1H, *H*NCOCH$_3$), 6.37 (d, $J = 9.5$ Hz, 1H, *H*NCOCH$_2$), 5.13–5.16 (m, 2H, H-3, H-4), 5.02–5.06 (m, 1H, H$_\alpha$-Cys), 4.53 (d, $J = 9.5$ Hz, 1H, H-1), 4.22 (dd, $J = 5.0$, 7.5, 1H, H-6$_a$), 4.13 (dd, $J = 2.0$, 10.5 Hz, 1H, H-6$_b$), 3.68–3.74 (m, 3H, H-5, H-2, H$_\beta$-Cys), 2.54 (dd, $J = 3.5$, 12.5 Hz, 1H, H$_\beta$-Cys), 2.10 (s, 3H, CH_3CO), 2.07 (s, 3H, CH_3CO), 2.05 (s, 6H, CH_3CO); ^{13}C NMR ($CDCl_3$): δ 171.3, 171.1, 170.8, 169.9, 169.5, 82.5 (C-1), 76.7 (C-5), 72.5, 68.3, (C-3, C-4), 62.2 (C-6), 57.3 (C-2), 53.1 (C$_\alpha$-Cys), 29.6 (C$_\beta$-Cys), 23.0, 20.9, 20.9, 20.7; HRMS Calcd for $C_{17}H_{24}N_2NaO_9S$ $[M+Na]^+$: 455.1095. Found: 455.1080.

1.4. (3*R*)-3-Acetylamino-4-one-6,7-dihydro-(1′,2′-dideoxy-β-D-glucopyranoso)[2′,1′-*f*]-1,5-thiazepane (5)

To a solution of compound **4** (74 mg, 0.171 mmol) in MeOH (1.5 mL) was added a catalytic amount of NaOMe (1 M). After the completion of the reaction, the mixture was neutralized with acetic acid and concentrated. Silica gel chromatography (5:1→3:1, $CHCl_3$–MeOH) gave compound **5** (43 mg, 0.140 mmol, 82%), which was crystallized from EtOH; mp 178 °C; $[\alpha]_D$ +69 (*c* 1.0, MeOH); ^{1}H NMR (D_2O): δ 5.19 (d, $J = 6.4$ Hz, 1H), 4.67 (d, $J = 9.6$ Hz, 1H), 3.88–3.91 (m, 1H), 3.65–3.76 (m, 3H), 3.52–3.59 (m, 3H), 2.55 (d, $J = 16$ Hz, 1H), 2.05 (s, 3H); ^{13}C NMR (D_2O): δ 174.0, 173.7, 82.3, 81.3, 74.1, 70.8, 61.4, 58.0, 53.6, 27.2, 22.4; HRMS Calcd for $C_{11}H_{19}N_2O_6S$ $[M+H]^+$: 307.0958. Found: 307.0950.

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