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Conjugates of plumbagin and phenyl-2-amino-1-thioglucoside inhibit MshB, a deacetylase involved in the biosynthesis of mycothiol

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ABSTRACT

N-Acetylglucosaminylinositol (GlcNAc-Ins)-deacetylase (MshB) and mycothiol-*S*-conjugate amidase (Mca), structurally related amidases present in mycobacteria and other Actinomycetes, are involved in the biosynthesis of mycothiol and in the detoxification of xenobiotics as their mycothiol-*S*-conjugates, respectively. With substrate analogs of GlcNAc-Ins, MshB showed a marked preference for inositol as the aglycon present in GlcNAc-Ins. The inhibition of MshB and Mca by 10 thioglycosides, 7 cyclohexyl-2-deoxy-2-*C*-alkylglucosides, and 4 redox cyclers was evaluated. The latter contained plumbagin tethered via 2 to 5 methylene carbons and an amide linkage to phenyl-2-deoxy-2-amino-1-thio-α-D-glucopyranoside. These proved to be the most potent amongst the 21 compounds tested as inhibitors of MshB. Their inhibitory potency varied with the length of the spacer, with the compound with longest spacer being the most effective.

1. Introduction

Tuberculosis is presently recognized as a worldwide cause of mortality in underprivileged communities. Owing to the development of strains of *Mycobacterium tuberculosis* that are resistant to various combinations of the drugs currently used in the treatment of tuberculosis, studies on the biochemistry of the mycobacteria have assumed great urgency, since metabolic processes that are present in pathogens, but not in their mammalian hosts, can be explored as potential targets for the development of new drugs.

While all eukaryotes and many bacteria produce glutathione as a major metabolite, members of the Actinomycetes, the phylum to which mycobacteria belong, uniquely produce mycothiol.[1] The discovery of mycothiol in the mycobacteria[2] has prompted detailed investigations of its biosynthetic enzymes and of enzymatic reactions that involve mycothiol (reviewed in Refs. 3–6). While the full spectrum of activities which utilize mycothiol has undoubtedly not yet been explored, the available information suggest that its role in mycobacterial metabolism mirrors that of glutathione in other organisms.

Early attempts to exploit mycothiol as a potential drug target focused on the enzyme mycothiol-*S*-conjugate amidase (Mca), a Zn-dependent amidase thought to play a role in the detoxification of alkylating agents and antibiotics such as rifampicin and cerulenin.[7] A number of bromotyrosine alkaloids of marine origin are as yet the most potent inhibitors of Mca that have been described.[8] Research subsequently moved on to the design of substrate-based inhibitors of Mca and of the related amidase, MshB, which catalyzes the third step in the biosynthesis of mycothiol.[9] To facilitate the synthesis of such inhibitors a viable alternative was sought which would obviate the necessity for costly preparation and resolution of the partially-protected *myo*-inositol unit, and its troublesome stereospecific glycosylation reaction, required during synthesis of the pseudodisaccharide moiety present in mycothiol and its precursors.[10] It had indeed earlier been found that the presence of inositol is not an absolute requirement for substrate recognition by the mycothioldisulfide reductase (mtr).[11] Knapp et al. introduced a S-linked cyclohexane as 'spacefilling' substitute for the inositol moiety present in mycothiol and its specursors.[12] As an alternative, Metaferia et al. replaced inositol with a six-membered ring derived from quinic acid.[13] This allowed retention of at least some of the hydrophilic character of inositol, while affording the advantage that only a single dicyclohexylidene derivative is obtained upon reaction with ethoxycyclohexene as a means of selective protection of its hydroxyl groups. Either approach

Abbreviations: Mycothiol, 1-D-*myo*-inosityl-2-(L-cysteinyl)amido-2-deoxy-α-D-glucopyranoside; MshB, 1-D-*myo*-inosityl-2-acetamido-2-deoxy-α-D-glucopyranoside *N*-deacetylase; GlcNAc-Ins, 1-D-*myo*-inosityl-2-acetamido-2-deoxy-α-D-glucopyranoside; MSSNaph, 2-*S*-mycothiolyl-6-hydroxynaphthyl-disulfide; Mca, mycothiol-*S*-conjugate amidase; mtr, mycothioldisulfide reductase.

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afforded compounds with activity as substrates or inhibitors of Mca, but there seemed to be little advantage in using the quinic acid derived ring rather than cyclohexane as an inositol surrogate. Following identification of the Mca homolog, 1-D-*myo*-inosityl-2-acetamido-2-deoxy-D-glucopyranoside (GlcNAc-Ins) deacetylase (MshB), as a mycothiol biosynthetic enzyme,[9,14] the latter enjoyed temporary prominence as a potential drug target, and its three-dimensional structure was reported independently by two groups during 2003 and 2004.[15,16] It was, however, contemporaneously found that MshB deficient mutants still produced significant amounts of mycothiol.[17] The continued production of low levels of mycothiol in such mutants was ascribed to deacetylation of *N*-acetyl-glucosaminylinositol (GlcNAc-Ins) by Mca or an alternate, as yet unidentified amidase.[16,18] This meant that complete blockage of the N-deacetylation of GlcNAc-Ins, and therefore of mycothiol synthesis, can only be achieved by inhibition of both MshB and the alternate amidase activity responsible for GlcN-Ins formation in MshB-mutants.

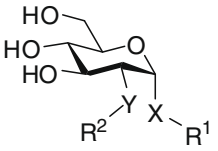
The most important determinant of the substrate specificities of Mca and MshB appears to be the presence or absence of a substituent on the amino group of the cysteine moiety, which is present in mycothiol. Thus the bimane derivative of desacetylmycothiol proved to be a better substrate of MshB than the natural substrate, GlcNAc-Ins, which implies that MshB, like Mca, has a binding site for aromatic groups distal from the binding pocket that accommodates the pseudodisaccharide moiety.[14] It therefore, seemed a reasonable assumption that inhibitors which simultaneously inhibit both enzymes can be developed. Such compounds were, indeed, recently reported by Metaferia et al.[19] in an exhaustive study of a series of cyclohexylthioglycosides bearing various substituents on the amino group of the glucosamine moiety. The most potent inhibitor of both MshB and Mca was a compound in which the amino group substituent was 2-carbonyl-5-(*p*-chlorophenyl)-furan. Variations in the structure of this aromatic substituent impacted significantly on the inhibitory potency of this series of inhibitors. The study by Metaferia et al.[19] thus provided further confirmation of the presence of an apolar binding pocket in proximity to the substrate binding site of MshB.

In this study we evaluate the substrate specificity of MshB for a limited series of substrates in which different six-membered rings substitute for the inositol moiety present in GlcNAc-Ins. We report the synthesis and evaluation of a number of substrate-mimic inhibitors, including potential transition state analogs, as inhibitors of Mca and MshB. It was also of interest to establish whether the range of aromatic substituents in MshB inhibitors can be extended to include functional groups with known cytotoxicity, such as the quinone redox cyclers. A limited range of 2,4-naphthoquinones, tethered via spacers of varying length to the amino group of 1-*S*-thiophenyl-2-deoxy-2-aminoglucopyranose, were synthesized as potential inhibitors of MshB using a strategy similar to that reported by Salmon-Chemin et al.[20] Some of these compounds were amongst the most potent inhibitors of MshB yet found. The substrate specificity of Mca was found to overlap not only with that of MshB, as previously reported, but also with that of the disulfide reductase, mtr, since both mtr and Mca utilized the heterodisulfide 2-*S*-mycothiolyl-6-hydroxynaphthyldisulfide[21] as a substrate.

2. Rationale and chemical synthesis

2.1. Alternate substrates of MshB

All inhibitors in this study, designed as substrate analogs (**I**) of either Mca or MshB, incorporated glucosamine or glucose as a substructure.



I X = O or S
Y = NH or CH_2

In previous studies, substituent X in compound **I** has mostly been sulfur[12,19] and R^1 cyclohexane, but no comparative analysis of the suitability of different aglycons as surrogates of the inositol moiety has as yet been undertaken. We were able to obtain a number of substrate analogs of GlcNAc-Ins in which a limited range of sulfur- or oxygen-linked six-membered rings had been introduced in lieu of inositol (see below in Table 1.)

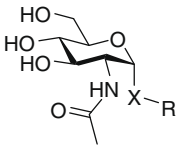
2.2. Synthesis of mycothiol analogs in which naphthoquinolyl carboxylic acids replaces *N*-acetylcysteine

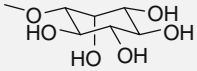
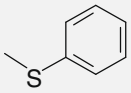
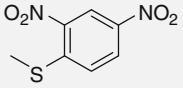
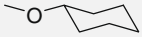
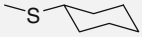
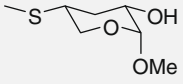
Previous efforts to obtain mycothiol deficient mutants of *M. tuberculosis*, by targeted disruption of the mshC[22] and mshA[23] genes, have been unsuccessful and, seen together with the results of high density mutagenesis,[24] indicated that mycothiol biosynthesis is essential for the growth of *M. tuberculosis* in culture. The recent isolation of mycothiol deficient, clinical isolates of *M. tuberculosis* that were resistant to ethionamide and isoniazid, however, caste doubt on the validity of the earlier studies.[25] In view of these contradictory findings it seemed prudent to attempt the synthesis of compounds that would not only inhibit MshB, thus lowering the levels of mycothiol, but which would also serve as subversive substrates of mtr. Subversive substrates are substrate analogs bearing groups that facilitate the transfer of single electrons between the flavin present in flavoenzymes and oxygen, with the resultant formation of superoxide. That such an approach could be successful was suggested by the finding that the mtr reduces various naphthoquinones at measurable rates comparable to those reported for other disulfide reductases.[26]

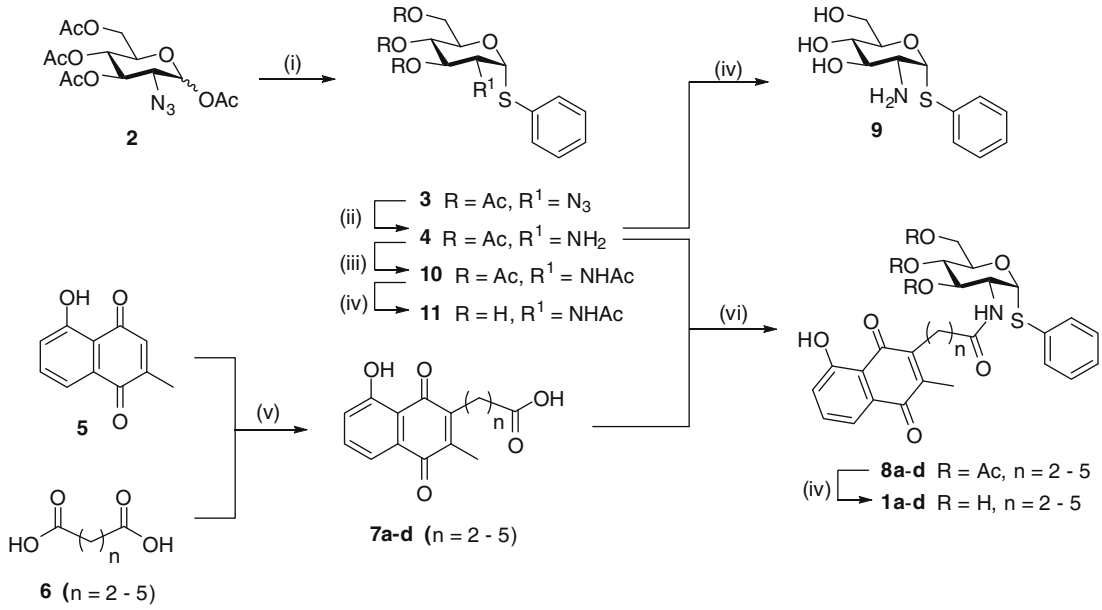
In a previous study of the substrate specificity of mtr the suitability of a few different aglycons in lieu of inositol in mycothiol analogs was explored.[27] The benzyl group was found to be the most suitable. This finding was rationalized on the basis that inositol has a hydrophobic 'patch' on the surface of the six-membered ring which displays three axial hydrogens. In the present study we found that thiophenylglycosides were also the most active substrate analogs in the reaction catalyzed by MshB. Therefore, a set of compounds **1a–d** (Scheme 1) was designed and prepared, in which the phenylthioglycoside is tethered to the naphthoquinone, plumbagin (**5**, Scheme 1). The choice of plumbagin as the redox cycling naphthoquinone was suggested by its high activity when used in subversive substrates of trypanothione reductase, and from the point of view of its synthesis, the possibility of selective monoalkylation of the naphthoquinone due to the presence of the methyl group at the 2-position.

The strategy chosen for preparation of **1a–d** envisaged initial alkylation at the 3-position of plumbagin to form a series of naphthyl-substituted alkanoic acids, followed by amide-coupling to a free amine on the sugar unit. Thus, as illustrated in Scheme 1, thiophenylglycoside **4** was first prepared by treatment of the readily available acetylated 2-azido glucose **2**[28] with thiophenol under BF_3 catalysis, followed by hydrogenation of the azide group. The naphthoquinonyl carboxylic acids **7** were then prepared by treatment of plumbagin (**5**) with a series of di-carboxylic acids **6** in a silver-mediated oxidative decarboxylation.[20] Amine **4** and plumbagin derivatives **7** were then combined under standard peptide-coupling

Table 1
Evaluation of a range of GlcNAc glycosides as substrates of MshB



Compound	RX	Substrate?	Activity at 250 μM (nmol/min/mg)
12		Yes	106
11		Yes	27
13		No	
14		Yes	6.07
15		Yes	6.11
16		No	



Scheme 1. Reaction conditions: (i) PhSH, $BF_3{\cdot}Et_2O$, 55 °C, 12 h (61%); (ii) H_2, Pd/C, rt, 5 h (93%); (iii) Ac_2O, pyridine (88%); (iv) cat. NaOMe, MeOH, rt, 1 h (yields of **11**, **1a**, **1b**, **1c**, **1d** were 98%; 34%, 46%, 60%, 52%, respectively); (v) $HO_2C(CH_2)_nCO_2H$, 30% aq CH_3CN, $AgNO_3$, $NH_4(S_2O_8)_2$, 70 °C, 8 h (yields of **7a**, **7b**, **7c**, **7d** were 98%; 32%, 40%, 40%, 50%, respectively); (vi) EDC, HOBt, CH_2Cl_2/THF, rt, 18 h (yields of **8a**, **8b**, **8c**, **8d** were 57%; 84%, 64%, 78%, respectively).

conditions to give the desired amides **1a–d** after de acetylation of the intermediate acetates **8a–d**.

2.3. Synthesis of substrates analogs and substrate-based inhibitors of Mca and MshB

In addition to the potential redox cyclers described above a total of 21 compounds were evaluated as substrates or inhibitors of Mca and MshB. These included (a) the natural substrate, GlcNAc-Ins (**12**[9]) of MshB, (b) a set of substrate analogs retaining the *N*-acetylglucosamine unit, but with the inositol unit replaced by a range of O- and S-linked alternatives (**11**, **13–16** (Table 1), **20**, **21**), (c) a series of cyclohexyl-1-thioglycosides (**22–26**) with variable substituents at C-2, and (d) a series of C-2-alkylated cyclohexyl glycosides (**29**, **30**, **31**, **33**, **35**, **37**, **38**), potential substrate or transition state isosteres of the natural substrate of MshB.

Substrate analog phenyl-2-acetamido-1-thio-α-glucoside **11** was prepared by N-acetylation and then de-O-acetylation of **4**, which could also be converted directly to the unprotected 2-aminoglucoside **9** (Scheme 1). The analogous β-thioglucosides **20** and **21** were prepared from acetylated glucosamine **17** via documented procedures[29,30] (Scheme 2).

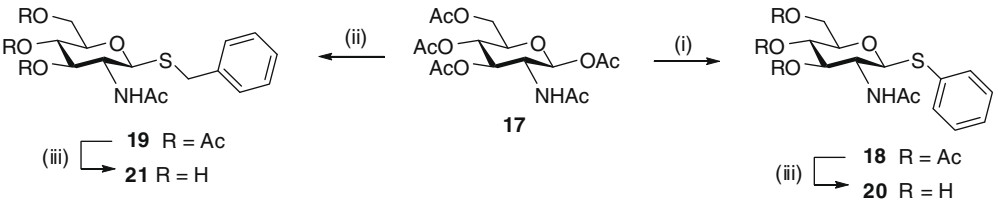
The set of cyclohexyl-2-*C*-alkylglucosides was prepared as shown in Scheme 3. Acetylated 2-*C*-allylglucosyl fluoride **27**[31]

was treated with cyclohexanol in the presence of $BF_3{\cdot}Et_2O$ to give the anomeric mixture of cyclohexyl glycosides **28a** and **28b**, which could be readily separated and deacetylated to provide cyclohexyl-2-*C*-allylglucosides **29** and **30**, respectively. Hydrogenation of **29** gave 2-*C*-propylglucoside **31**, while Wacker oxidation of the allyl group in **28a** yielded ketone **32** which was deacetylated to give the 2-*C*-oxopropylglucoside **33** or reduced with $NaBH_4$ to give, after deprotection, the inseparable mixture of diastereomeric alcohols **35**. The latter was also obtained by $LiAlH_4$ reduction of the mixture of epoxides **36**, available by treatment of 2-*C*-allylglucoside **28a** with *m*-chloroperbenzoic acid. Deacetylation of **36** using catalytic sodium methoxide in methanol gave a moderate yield of epoxide **37** accompanied by a bicyclic product **38**, presumed to arise from attack by the intermediate C-3 alkoxide formed upon deacetylation of the glucose unit, on the terminal carbon of the epoxypropyl unit.

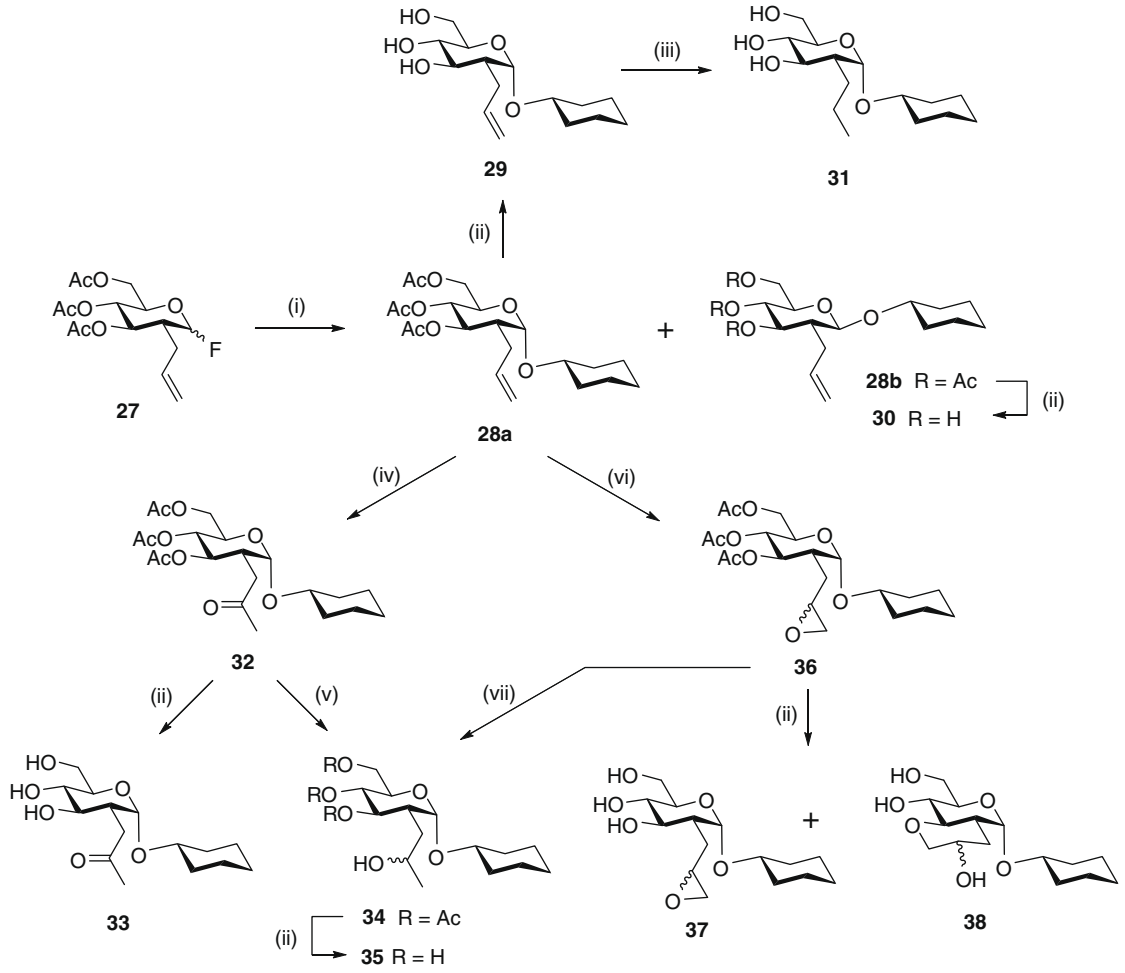
3. Results and discussion

3.1. Alternate substrates of MshB

Compounds evaluated as substrates of MshB are presented in Table 1. The K_m value for all compounds other than GlcNAc-Ins **12** and phenyl-2-acetamido-2-deoxy-1-thio-α-D-glucopyranoside (**11**) could not be established, because of limited availability and poor solubility (Fig. 1), but a comparison of their rate of cleavage, when present at a similar concentration of 250 μM, is presented in Table 1.



Scheme 2. Reaction conditions: (i) PhSH, $SnCl_4$, CH_2Cl_2, reflux, 24 h (75%); (ii) $(NH_2)_2CS$, CH_3CN, 80 °C, 15 min, then $PhCH_2Br$, Et_3N, rt, 3 h (75%); (iii) cat. NaOMe, MeOH, rt (yields of **20**, **21** were 91%, 89%, respectively).



Scheme 3. Reagents and conditions: (i) cyclohexanol, $BF_3{\cdot}Et_2O$, CH_2Cl_2, rt, 1 h (yields of **28a**, **28b** were 42%, 36%, respectively); (ii) NaOMe, MeOH. rt (yields of **29**, **30**, **33**, **35**, **37**, **38** were 99%, 98%, 84%,89%, 50%, 42%, respectively); (iii) H_2, Pd/C, EtOAc–MeOH (97%); (iv) O_2, $PdCl_2$, $CuCl_2$, DMF–H_2O (50%); (v) $NaBH_4$, THF–MeOH, −20 °C to 0 °C, 2 h (88%); (vi) *m*-CPBA, CH_2Cl_2 (91%).

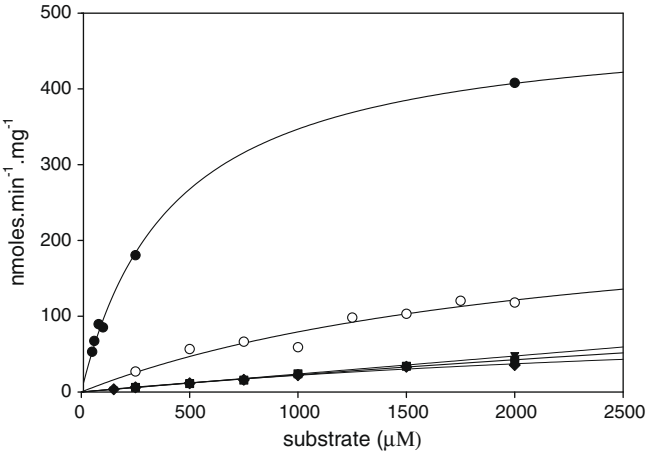


Figure 1. Dependence of the rate of amidase cleavage of substrate analogs on the nature of the aglycon in *N*-acetylglucosamine glycosides. The curves represent the dependence of GlcN-Ins formation with the natural substrate GlcNAc-Ins **12** (●), phenyl-2-acetamido-2-deoxy-1-thio-α-D-glucopyranoside **11** (○), cyclohexyl-2-acetamido-2-deoxy-1-thio-α-D-glucopyranoside **15** (■), cyclohexyl-2-acetamido-2-deoxy-α-D-glucopyranoside **14** (▼) and cyclohexyl-2-chloroacetamido-2-deoxy-1-thio-α-D-glucopyranoside **22** (▽).

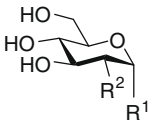
For cleavage of GlcNAc-Ins **12** by MshB the kinetic constants were estimated to be, $K_m = 347.7 \pm 27.2$ μM, $V_{max} = 436.6 \pm 148$ nmol/min/mg, $k_{cat} = 0.24 \pm 0.08$ s^{-1} and $k_{cat}/K_m = 690.3$ M^{-1} s^{-1}. For the deacetylation of phenylthioglycoside **11** the corresponding values were $K_m = 1695 \pm 151$ μM, $V_{max} = 214 \pm 57$ nmol/min/mg, $k_{cat} = 0.120 \pm 0.03$ s^{-1} and $k_{cat}/K_m = 69.4$ M^{-1} s^{-1}.

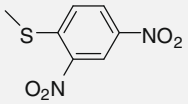
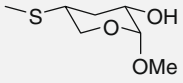
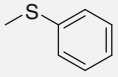
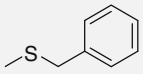
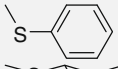
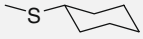
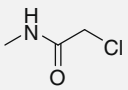
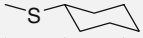
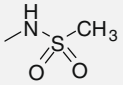
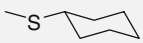
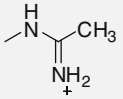
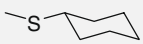
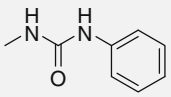
From the results presented in Table 1 and Figure 1 it is evident that the choice of aglycon is an important determinant of the recognition of substrate analogs of GlcNAc-Ins by MshB, the planar phenyl ring in a thiophenyl aglycon, as in phenylthioglycoside **11**, being better tolerated than the thiocyclohexyl group. By contrast the corresponding β-phenylthioglycoside was not a substrate (Table 2) The introduction of an electron withdrawing substituent, even though located in proximity to the scissile bond in cyclohexyl-2-chloroacetamido-2-deoxy-1-thio-α-D-glucopyranoside **22** (Table 2), did not seem to alter the rate of amidase cleavage by MshB significantly.

3.2. Evaluation of a series of thioglycosides as inhibitors of MshB and Mca

Thioglycosides tested as inhibitors of MshB and Mca (Table 2) included compounds that were not substrates, despite the

Table 2
Inhibition of Mca and MshB by thioglycosides: percentage inhibition was determined at substrate and inhibitor concentrations of 250 μM each for Mca and 500 μM each for MshB



Compound	R_1	R_2	Mca (%)	MshB (%)
13			0	29.4
16			27	0
20 (β-linked)			0	15.1
21 (β-linked)			0	13.3
9		–NH_2	17	6.1
22			0	14.9
23		–NH_2	0	16.4
24			0	0
25			6.8	0
26			15	17

presence of a cleavable *N*-acetyl group as in **13**, **16**, **20** and **21**. These compounds were also poor inhibitors, which confirms again the importance of the linkage to the glucosamine and of the structure of the aglycon for binding to the active sites of these enzymes. These observations are in agreement with those of Metaferia et al.[19] in that it was found that inhibitors lacking an aglycon were much poorer inhibitors than the corresponding structures in which a spacefilling, thiocyclohexyl moiety served as inositol surrogate. Thioglycosides **9** and **23**, analogs of the product Glc-Ins, were also poor inhibitors.

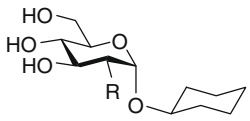
3.3. Evaluation of cyclohexyl-2-deoxy-2-*C*-alkylglucosides as inhibitors of MshB and Mca

Amongst the cyclohexyl-2-deoxy-2-*C*-alkylglucosides listed in Table 3 compound **29** stood out as the only inhibitor with reasonable potency, and then only against Mca. This group of inhibitors included the transition state analog **35** and **33** in which an isosteric 2-oxo-propyl group substituted for the acetamide group present in the substrate. While it may be inferred from the results presented in Figure 1 and Table 1 that the cyclohexyl moiety present in the compounds listed in Table 3 is not the preferred aglycon for interaction with MshB, the results presented in Table 3 do suggest that transition state analogs bearing the hydroxyethylene isostere, as in **35**, or a noncleavable mimic of the amide bond, as in **33**, hold little promise for further development into effective inhibitors of Mca or MshB.

3.4. Thiophenylglycosides linked to plumbagin are potent inhibitors of MshB

The naphthoquinone glycosides were amongst the most potent inhibitors of MshB yet described and also inhibited Mca, but to a lesser extent (Table 4 and Fig. 2). The less potent inhibition of Mca by **1a–d** is consistent with earlier reports[14,25] that the binding of substrates to Mca is influenced to a major extent by the presence of the acetyl group in the *N*-acetylcysteine substructure of mycothiol. When tested as subversive substrates compounds **1a–d** were also much poorer substrates of mtr than plumbagin itself. By contrast plumbagin tethered to spermidine was a much better

Table 3
Inhibition of Mca and MshB by cyclohexyl-2-deoxy-2-C-alkylglucosides: percentage inhibition was determined at substrate and inhibitor concentrations of 250 μM each for Mca and 500 μM each for MshB



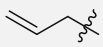
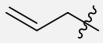
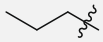
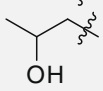
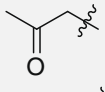
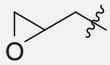
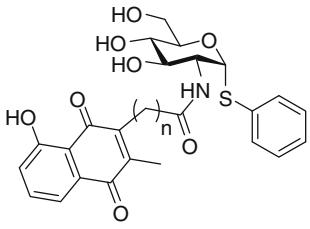
Compound	R	Mca	MshB
29		73.8	0
30		9.3	0
31		22.0	0
35		20.5	11.4
33		17.2	6.7
37		19.4	19.7
38	See structure (Scheme 3)	35.0	6.6

Table 4
Inhibition of Mca and MshB by substituted naphthoquinones: percentage inhibition was determined at substrate and inhibitor concentrations of 250 μM each for Mca and 500 μM each for MshB



1a n = 2
1b n = 3
1c n = 4
1d n = 5

Substrate	Mca	MshB
1a	28.8	57.4
1b	37.8	81.6
1c	23.2	81.4
1d	44.5	94.8

substrate of trypanothione reductase than plumbagin alone.[20] It should be noted that the presence and nature of a substituent on the cysteine amino group present in mycothiol was found to be an important determinant of the utilization of substrates not only by Mca, but also by mtr. Thus desacetylmycothiol or N-succinylated mycothiol (succ-Cys-GlcN-Ins) were surprisingly poor substrates of the enzyme and the current findings are, therefore, consistent with previous studies on the substrate specificity of mtr.[32] It should, therefore, be possible to overcome this difficulty by appropriate modification of the spacers used in **1a–d**.

M. tuberculosis mtr reduced the heterodisulfide, 2-*S*-mycothiol-yl-6-hydroxynaphthyldisulfide (MSSNaph), with a V_{max} that, under the conditions of the assay, was similar to that observed for the natural substrate, mycothioldisulfide, but with a $K_{m,app}$ value of 23 μM, which is 3–5-fold lower than values previously reported for the reduction of mycothioldisulfide,[33] thus indicating the presence of an apolar binding site distal from the site that accommodates the more hydrophilic mycothiol. As a result MSSNaph is a particularly useful substrate for the assay of mtr, because it allows a more economical use mycothiol, which is available only in limited amounts when isolated from natural sources. 6,6′-dihydroxynaphthyldisulfide was, however, not reduced by mtr. MSSNaph was also an efficient substrate of Mca with a K_m value of 328 ± 22 μM and a V_{max} that exceeded the rate of cleavage of MSmB by a factor of about 3.[34] Seen together it may be concluded that the substrate binding sites of MshB, Mca and mtr all interact favorably with molecules bearing aromatic groups distal from the more polar GlcN-Ins pseudodisaccharide.

More detailed studies of the inhibition of MshB by the series of naphthoquinone derivatives were undertaken to determine inhibition constants, and the results obtained in the case of **1d** are shown in Figure 3. Inhibition by the series of naphthoquinones seems to have been competitive. Inhibition constants for **1a**, **1c** and **1d** were estimated to be 167 ± 15 μM, 94 ± 11 μM and 16.8 ± 1.9 μM, respectively. These values may be compared to the K_m values of 348 ± 27 μM for the natural substrate, GlcNAc-Ins (**12**), and, more appropriately, with an estimated value of 1.695 ± 0.151 mM for the deacetylation of phenyl-2-acetamido-2-deoxy-1-thio-α-D-glucopyranoside **9**, which has the same aglycon that is present in the naphthoquinone derivatives.

The presence of the aromatic naphthoquinone, therefore, has a pronounced effect on the affinity with which these compounds are bound to the active site of MshB. The K_i values of the naphthoqui-

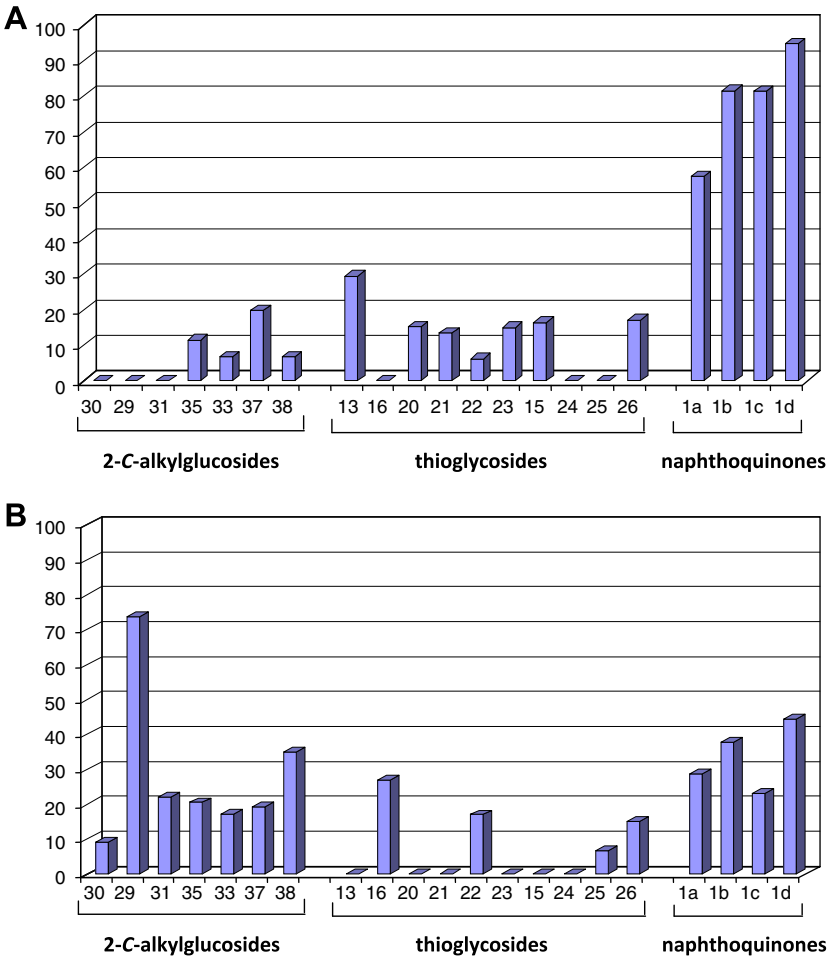


Figure 2. Comparison of inhibition of MshB (Panel A) and Mca (Panel B) by cyclohexyl-2-deoxy-2-C-alkylglycosides, various thioglycosides and compounds having plumbagin tethered via 2 to 5 methylene carbons by amide linkage to phenyl-2-deoxy-2-amino-1-thio-α-D-glucopyranoside. In the case of MshB the substrate was 500 μM GlcNAc-Ins and inhibitors were present at 500 μM, whereas 250 μM MSmB was the substrate for Mca and inhibitors were included at 250 μM. The product GlcN-Ins was detected by hplc of the AccQ-fluor derivative.

nones indicated that their binding affinity increases with the length of the spacer. To understand this phenomenon, the naphthoquinones with 2 carbons and 5 carbon spacers, **1a** and **1d**, were docked to the crystal structure of MshB previously published by Maynes et al.[15] Preliminary results showed that the longer carbon chain of **1d** extends the molecule such as to allow interaction of the naphthoquinone group with a hydrophobic dipeptide of Val 184 and Leu 185, when the carbonyl group of the potentially cleavable amide link is positioned such as to interact with the active site Zn atom. These studies are still continuing. A similar pattern of inhibition was observed for Mca in that naphthoquinones with longer spacers were also better inhibitors of Mca.

4. Experimental

4.1. Isolation and assays of enzymes

4.1.1. Expression and isolation of MshB

The gene encoding MshB, Rv1170, was amplified from *M. tuberculosis* DNA using pfu DNA polymerase and ligated into pET28 using the Nde1 and BamH1 restriction sites. The forward primer was 5′-gcccatatggtgtctgagacgccgcg-3′ and the reverse primer 5′-gttgggatcctacgtgccggacgcg-3′. The resultant construct was transformed into *Escherichia coli* BL21(DE3) pLysS and MshB expression was induced by addition of 0.4 mM IPTG at 18 °C. The induced cells (3.5 g) were suspended in 20 ml of lysis buffer, which contained 50 mM NaCl and 1.0 mM each of the protease inhibitors, TPCK, TLCK and PMSF in 50 mM Hepes, pH 7.5 and disrupted by sonication. The mixture was clarified by centrifugation at 15,000*g* for 30 min and the supernatant was dialyzed overnight against 25 mM Tris–Cl, pH 8.0. The preparation was applied to a DEAE–Sepharose column (45 × 4.5 cm) which was then eluted with eluted with 350 ml of 25 mM NaCl in 25 mM Tris–Cl, pH 8.0 and then with a 1 l gradient from 25 mM to 600 mM NaCl in 25 mM Tris–Cl, pH 8.0. Active fractions were pooled, concentrated by ultrafiltration and dialyzed overnight against 1 l of 50 mM phosphate buffer, pH 7.0 containing 0.5 M NaCl. The preparation was applied to a Zn-IMAC column (1.6 × 12.5 cm) which was then eluted with 75 ml of 1 mM imidazole in 50 mM K-phosphate buffer, pH 7.0 containing 0.5 M NaCl and then with a gradient to 20 mM imidazole in the same buffer. Active fractions were concentrated by ultrafiltration and chromatographed on Sephacryl-S300 (95 × 0.9 cm) using 50 mM K-phosphate pH 7.0 as eluent. The resultant preparation was electrophoretically homogeneous and was stored in 20% glycerol at −20 °C.

4.1.2. Isolation of Mca

Recombinant mca had previously been purified from the *E. coli* host cells by a three step procedure and the last chromatographic step on Phenyl-Sepharose, in agreement with our own results, entailed considerable loss of activity.[34] A theoretical basis for partial denaturation of proteins during hydrophobic interaction

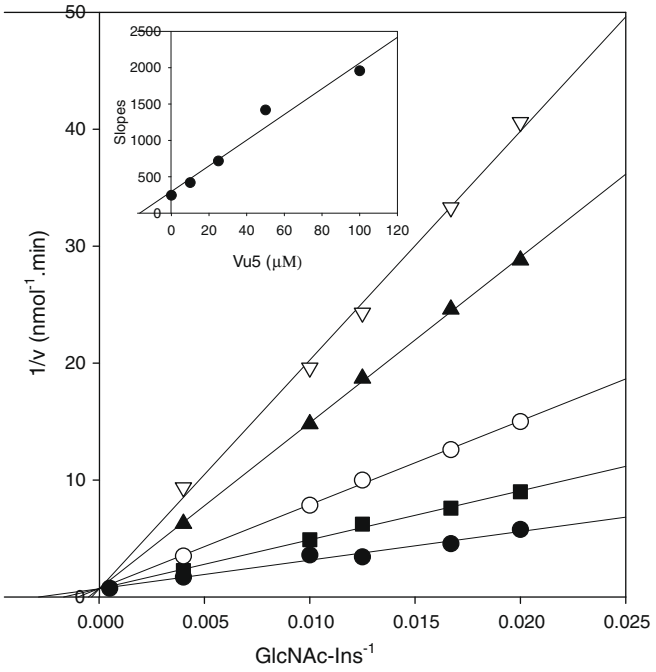


Figure 3. Inhibition of MshB by **1d**. The substrate GlcNAc-Ins was varied in the range 50–2000 μM and **1d** concentrations as follows: no inhibitor (●), 10 μM (■), 25 μM (○), 50 μM (▲) and 100 μM (▼). Assays were as described in Section 4 and the product GlcN-Ins was determined by hplc as the AccQ-fluor derivative.

chromatography had earlier been provided by Jungbauer et al.[35] Mca was therefore isolated from *Mycobacterium smegmatis* using $(NH_4)_2SO_4$ precipitation and chromatography on DEAE cellulose, hydroxyapatite and a Biosep SEC2000 hplc column to obtain an electrophoretically homogeneous preparation. 50 grams *M. smegmatis* was suspended in 160 ml of 50 mM Hepes buffer, pH 7.5, which contained 3 mM 2-mercaptoethanol and 0.1 mM each of the protease inhibitors, TPCK, TLCK and PMSF. The cells were disrupted by sonication for 10 min on ice and the mixture was clarified by centrifugation. The supernatant was fractionated with $(NH_4)_2SO_4$ and the fraction precipitated between 15% and 50% was collected and dialyzed against 25 mM Tris–Cl buffer, pH 8.0. The dialyzed preparation was applied to a DEAE–Sepharose column that had been equilibrated against the same buffer and was eluted using a linear gradient to 600 mM NaCl. Active fractions from DEAE–cellulose chromatography were pooled and concentrated by ultrafiltration using a membrane with 30 kDa exclusion limit. The concentrated enzyme preparation was dialyzed overnight against 10 mM potassium phosphate (KPi), pH 7.2 and was then loaded onto a hydroxyapatite column (2.2 × 30 cm) that had been equilibrated with 6 volumes of the starting buffer. The column was eluted with a 1000 ml gradient from 10 mM to 400 mM KPi, pH 7.2. Further purification of aliquots of this preparation by chromatography on Biosep Sec-S2000 was used to obtain the electrophoretically homogeneous enzyme.

4.1.3. Mycothiol disulfide reductase

The gene encoding *M. tuberculosis* mtr was expressed in *M. smegmatis* as described,[33] with minor modifications: The forward primer was 5′-CCC CTA CAA GTT TAA ACG TAC GAC-3′. To lower the level of constitutive expression of Rv2855 the transformed cells were cultured at 30 °C and expression was induced by raising the temperature to 42 °C. Under these conditions a ~160-fold increase in the level of mtr specific activity was achieved as compared to *M. smegmatis* lacking the pMV261 plasmid with Rv2855 insert.

4.1.4. Enzyme assays

4.1.4.1. Mycothiol-*S*-conjugate amidase (Mca). Mca activity was determined by hplc analysis of the bimane derivative of *N*-acetylcysteine derived from the cleavage of mycothiol–bimane, MSmB. Assay mixtures contained 8.0 nmol MSmB, where required, 8.0 nmol of an inhibitor and 1.0 μmol each of Na–Hepes buffer, pH 7.5, and NaCl and 7.7 μg Mca in a final volume of 40 μl. The reaction mixtures were incubated for 10 min at 37 °C. The reaction was stopped by adding 20 μl acetonitrile and 20 μl 5.0% TFA followed by heating at 60 °C for 10 min. Samples were stored at −80 °C until analyzed. Aliquots of the assay mixtures were injected onto a Phenomenex C-18 hplc column (250 × 4.6 mm). The column was eluted (Method 1) at a rate of 0.8 ml/min for 5 min with 5% B, then with a linear gradient to 90%B with a 5 min plateau at 90%B, followed by a return to initial conditions (A: 0.1% trifluoro acetic acid, B: 100% acetonitrile). The excitation and emission wavelengths on the fluorescence detector were 396 nm and 482 nm, respectively and the results were analyzed using Chromperfect software.

4.1.4.2. GlcNAc-Ins deacetylase, MshB. Assay mixtures contained, in a final volume of 40 μl, 1.0 μmol of Na–Hepes buffer pH 7.5, 1.0 μmol of NaCl, 3.26 μg of MshB and GlcNAc-Ins within a concentration range of 50–2000 μM The mixtures were incubated for 10 min at 37 °C. The reaction was terminated by the addition of 20 μl acetonitrile and 20 μl 0.1% TFA followed by heating for 10 min at 60 °C. Seventy microliters of the clarified supernatant were derivatized with AccQ-Fluor. The samples were then analyzed by hplc for GlcN-Ins, as the AccQ-fluor derivative, using a Phenomenex C-18 column (250 × 4.6 mm).

The column was eluted at a rate of 0.8 ml/min for 5 min with 5% B, then with a 30 min linear gradient to 90%B with a 5 min plateau at 90%B followed by a return to initial conditions (A: 0.1% TFA, B: acetonitrile).

In order to construct calibration curves that would allow quantitation of the amount of product formed, amidase cleavage of known amounts of alternate substrates by MshB were allowed to go completion. Compounds having a phenyl group as aglycon could be detected in the ultraviolet region and thus quantified by hplc without further derivatization, while compounds having a cyclohexyl aglycon were dansylated prior to analysis by hplc. The assay mixtures contained 1 μmol NaCl, 1 μmol Hepes buffer, pH 7.5, the substrate and 8.15 μg of MshB in a total volume of 40 μl. The mixtures were incubated for 10 min at 37 °C. The reactions were then terminated by addition of 40 μl of a 1:1 mixture of a TEA–acetic acid buffer (0.088%:0.57%) and acetonitrile and the samples were injected onto a Phenomenex luna C-18 hplc column (250 × 4.6 mm). The column was eluted using Method 1 described above, but substituting TEA–acetic acid (0.088%:0.57%) for 0.1% TFA.

4.1.4.3. Mycothioldisulfide reductase (Mtr). Assay mixtures contained 0.150 μmol of NADPH, 44 nmol of 2-*S*-mycothiolyl-6-hydroxynaphthyl disulfide (MSSNaph), 50 μmol Tris–Cl, pH 7.6 (25 °C) and 2 μmol EDTA in 1 ml. Reaction rates were determined at 30 °C. Assays were started by addition of the enzyme and the decrease in absorbance at 340 nm was followed by means of an Ocean Optics Mini-diode array spectrometer. Absorbance traces were imported into Sigma plot and the rates determined by linear regression.

5. Synthesis of substrate analogs and inhibitors

5.1. General

Cyclohexyl-1-thio-glycosides **22**, **23**, **24**, **25**, **26** and **15** were kindly provided[12] by Professor Spencer Knapp from the Department

of Chemistry & Chemical Biology, Rutgers University, Piscataway, New Jersey, USA. Thioglycosides **13** and **16** were provided Dr M. Anwar Jardine from the Department of Chemistry, University of Cape Town, Rondebosch, South Africa. All other compounds were synthesized as described below.

All reactions solvents were dried and distilled before use. Commercially available reagents were used without purification unless otherwise stated. All reactions were performed under an inert atmosphere of nitrogen in flame dried glassware. Reactions were monitored by thin-layer chromatography (TLC) carried out on Merck F254 silica plates and products visualized under UV light and by wetting the plate with a solution of anisaldehyde and sulfuric acid in ethanol, followed by heating. Column chromatography was performed on glass columns loaded with Merck Silica Gel 60 (70–230 mesh) eluting with mixtures of light petroleum and ethyl acetate. Nuclear magnetic resonance spectra (^{1}H and ^{13}C) were recorded on either a Varian Mercury (300 MHz) or Varian Unity (400 MHz) with $CDCl_3$ as the solvent and TMS ($\delta = 0$ ppm) as the internal standard. All chemical shifts are reported in ppm. Melting points were determined using a Reichert-Jung Thermovar hot plate microscope. The authenticity and purity of compounds that had not previously been reported in the literature were verified by elemental analysis.

5.2. Phenyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-1-thio-α-D-glucopyranoside (3)[‡]

$BF_3{\cdot}OEt_2$ (2.6 ml, 18.1 mmol,) was added to a solution of **2**[28] (1.5 g, 4.0 mmol) and thiophenol (0.862 ml, 8.0 mmol) in CH_2Cl_2 (30 ml) at 0 °C. The reaction mixture was stirred at 55 °C for 12 h. On completion, the reaction mixture was diluted with CH_2Cl_2, washed with brine and the organic layer separated. The aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. Silica gel chromatography (EtOAc–petroleum ether 10:90) of the crude mixture afforded **3** as an α/β mixture (1.03 g, 61%). The α-anomer was obtained as white crystals by crystallization from absolute ethanol: mp 93–97 °C; ^{1}H NMR (400 MHz) δ 7.51–7.47 (m, 2H, Ar-H), 7.34–7.29 (m, 3H, Ar-H), 5.61 (d, 1H, H-1, $J = 5.6$ Hz), 5.31 (dd, 1H, H-3, $J = 9.2$ Hz, $J = 10.5$ Hz), 5.01 (dd, 1H, H-4, $J = 9.2$ Hz, $J = 10.3$ Hz), 4.56 (ddd, 1H, H-5, $J = 2.3$ Hz, $J = 5.1$ Hz, $J = 10.3$ Hz), 4.26 (dd, 1H, H-6a, $J = 5.1$ Hz, $J = 12.4$ Hz), 4.04 (dd, 1H, H-6b, $J = 6.1$ Hz, $J = 11.1$ Hz), 2.07 (s, 3H, C*H*$_3$), 2.02 (s, 3H, C*H*$_3$), 1.99 (s, 3H, C*H*$_3$). ^{13}C NMR ($CDCl_3$) δ 170.4, 169.7, 132.4, 132.2, 129.2, 128.0, 86.5 (C-1), 76.6, 72.0, 68.7, 68.5, 61. 9, 61.5, 20.6, 20.5. Anal. Calcd for $C_{18}H_{21}N_3O_7S$: C, 51.06; H, 5.19; N, 9.92; S, 7.57. Found: C, 51.64; H, 5.11; N, 9.94; S, 7.23.

5.3. Phenyl-3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-1-thio-α-D-glucopyranoside (4)

A suspension of **3** (2.0 mmol, 0.844 g) and palladium on carbon (0.424 g) in ethanol (48 ml) was stirred under hydrogen (1 atm) for 5 h at room temperature. The reaction mixture was then filtered through Celite and further purified by silica column chromatography (EtOAc–petroleum ether 60:40) to yield **4** as a white solid (0.739 g, 93%), mp 83–85 °C. ^{1}H NMR ($CDCl_3$): δ 7.42–7.23 (m, 5H, phenyl), 5.56 (d, 1H, $J = 5.2$ Hz, H-1), 5.05 (dd, 2H, $J = 2.2$, 10.3 Hz, H-4, H-6a), 4.6 (m, 1H, H-5), 4.32 (dd, 1H, $J = 5.1$, 12.3 Hz, H-2), 4.07 (dd, 1H, $J = 2.3$, 12.3 Hz, H-3), 3.32 (dd, 1H, $J = 5.2$, 10.2 Hz, H-6b), 2.1 (s, 3H), 2.04 (s, 6H). ^{13}C NMR ($CDCl_3$): δ 170.70, 170.57, 169.78, 133.50, 131.88, 129.13, 127.74, 91.23, 74.72, 69.00, 62.30, 55.15, 20.68. [M+H] calcd for $C_{18}H_{23}NO_7S$: 398.1268; found 398.1283.

5.4. Phenyl-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-α-D-glucopyranoside (10)

A solution of **4** (37 mg, 0.093 mmol) in pyridine (2 ml) was treated at room temperature with acetic anhydride (0.9 ml, 9.52 mmol) for 2 h. The reaction mixture was then diluted with CH_2Cl_2 (10 ml), then the organic phase washed with 1 M HCl (2 × 10 ml), aqueous $NaHCO_3$ (2 × 10 ml) and brine (10 ml), before drying over $MgSO_4$. Compound **10** was obtained as a white solid (36 mg, 88%), mp 124–126 °C (lit.[37] mp 204–205 °C); $[\alpha]_D = +160.1$ (c 1.25, $CHCl_3$); lit.[37] $[\alpha]_D = -22.1$ (c 1.26, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.44–7.42 (m, 5H, phenyl), 5.88 (d, 1H, $J = 8.79$, N–H), 5.69 (d, 1H, $J = 5.13$, H-1), 5.15 (m, 2H, H-3, H-4), 4.59 (m, 1H, H-2), 4.50 (ddd, 1H, $J = 5.1$, 7.3, 12.5, H-5), 4.27 (dd, 1H, $J = 2.56$, 12.45, H-6a), 4.08 (dd, 1H, $J = 2.56$, 12.45, H-6b), 2.05, 2.04, 1.98 (s, 12H, 4×–$COCH_3$). [M+H] calcd for $C_{20}H_{25}NO_8S$: 440.1374; found: 440.1393.

5.5. Phenyl-2-acetamido-2-deoxy-1-thio-α-D-glucopyranoside (11)

A suspension of **10** (30 mg, 0.07 mmol 0.65 g, 1.48 mmol) in methanol (2 ml) was treatment with a catalytic amount of sodium methoxide in methanol at room temperature. The reaction was complete after 2 h, whereupon the solution was treated with methanol-washed Amberlite H^+ resin, then filtered and the filtrate concentrated under vacuum to give **11** as a white solid (22 mg, 98%); mp 220–222 °C (lit.[37] mp 230 °C); $[\alpha]_D = +108.6$ (c 0.5, MeOH) (lit.[37] $[\alpha]_D = +5.7$ (c 0.51, MeOH)); ^{1}H NMR (400 MHz, CD_3OD) δ 7.52–7.22 (*m*, 5H, phenyl), 5.68 (d, 1H, $J = 5.25$ Hz, H-1), 4.15–3.99 (m, 2H), 3.77 (dq, 2H, $J = 11.94$, 11.89, 11.89, 3.66 Hz,), 3.64 (dd, 1H, $J = 11.02$, 8.71 Hz), 3.43 (dd, 1H, $J = 9.84$, 8.70 Hz), 3.36–3.18 (m, 1H), 3.36–3.07 (m, 1H), 2.00 (*s*, 3H, 1×–COC*H*$_3$); ^{13}C NMR (CD_3OD): δ 172.53, 134.84, 131.94, 131.00, 128.64, 127.24, 88.26, 73.85, 71.52, 71.26, 61.25, 55.06, 21.34. Anal. Calcd for $C_{14}H_{19}NO_5S$: C, 53.66; H, 6.11; N, 4.47; S, 10.23. Found: C, 52.72; H, 5.98; N, 4.28; S, 9.90. [M+H] calcd for $C_{14}H_{19}NO_5S$: 314.1062; found: 314.1085.

5.6. Preparation of acid derivatives 7a–d

The naphthalenyl carboxylic acids **7a**–**d** were prepared by combining plumbagin **5** and dicarboxylic acids **6** following the procedure of Salmon-Chemin et al., with analytical data corresponding to that reported.[20]

5.6.1. 2-Carboxymethyl-8-hydroxy-3-methyl-1,4-naphthoquinone (7a)

Obtained as an orange solid (32%); ^{1}H NMR (acetone-d_6) δ (300 MHz) 12.09 (OH, 1H), 7.70 (dd, 1H, H-6, $J = 7.5$ Hz, $J = 8.4$ Hz), 7.57 (dd, 1H, H-7, $J = 1.1$ Hz, $J = 7.5$ Hz), 7.26 (dd, 1H, H-5, $J = 1.2$ Hz, $J = 8.4$ Hz), 2.96 (t, $J = 8.2$, 2H, C*H*$_2$–COOH), 2.58 (t, 2H, C*H*$_2$–CH$_2$–COOH, $J = 7.9$ Hz), 2.22 (s, 3H, C*H*$_3$). ^{13}C NMR (acetone-d_6) δ 190.4, 184, 173, 162, 145.7, 145, 136.4, 133, 123.5, 118.6, 114.9, 31.9, 22.1, 12.

5.6.2. 2-Carboxyethyl-8-hydroxy-3-methyl-1,4-naphthoquinone (7b)

Obtained as an orange solid (40%). ^{1}H NMR (acetone-d_6) δ (400 MHz) ppm 12.09 (OH, 1H), 7.69 (dd, 1H, H-6, $J = 7.5$ Hz, $J = 8.4$ Hz), 7.57 (dd, 1H, H-7, $J = 1.1$ Hz, $J = 7.5$ Hz),7.25 (dd, 1H, H-5, $J = 1.1$ Hz, $J = 8.4$ Hz), 2.74 (t, $J = 7.9$, 2H, C*H*$_2$–COOH), 2.44 (t, $J = 7.2$, 2H, C*H*$_2$–(CH$_2$)$_2$–COOH), 2.21 (s, 3H, C*H*$_3$), 1.84 (m, 2H, C*H*$_2$–CH$_2$–COOH). ^{13}C NMR 190.4, 184, 173.4, 161, 146, 145, 136, 132, 123, 118, 115, 32.9, 25.5, 23.3, 12.

[‡] We note that this compound has been reported as a 2.1:1 α/β mixture[36a] and as a 3:1 α/β mixture,[36b] but to our knowledge this is the first reported isolation and characterization of the pure α-anomer.

5.6.3. 2-Carboxypropyl-8-hydroxy-3-methyl-1,4-naphthoquinone (7c)

Obtained as an orange solid (40%). ^{1}H NMR (acetone-d_6) δ (400 MHz) ppm 12.1 (OH, 1H), 7.69 (dd, 1H, H-6, J = 7.5 Hz, J = 8.4 Hz), 7.56 (dd, 1H, H-7, J = 1.1 Hz, J = 7.5 Hz), 7.25 (dd, 1H, H-5, J = 1.1 Hz, J = 8.4 Hz), 2.69 (t, J = 7.8, 2H, CH_2–(CH$_2$)$_3$–COOH), 2.36 (t, J = 7.3, 2H, CH_2–COOH), 2.2 (s, 3H, CH_3), 1.73 (td, 2H, CH_2–CH$_2$–COOH, J = 7.1 Hz, J = 14.5 Hz), 1.60 (m, 2H, CH_2–(CH$_2$)$_2$–COOH). ^{13}C NMR 190.4, 184, 174, 162, 146.5, 144.7, 136, 133, 124, 119, 115, 33, 25.9, 25, 11.9.

5.6.4. 2-Carboxybutyl-8-hydroxy-3-methyl-1,4-naphthoquinone (7d)

Obtained as a red solid (50%). ^{1}H NMR (acetone-d_6) δ (400 MHz) ppm 12.1 (OH, 1H), 7.69 (dd, 1H, H-6, J = 7.5 Hz, J = 8.4 Hz), 7.56 (dd, 1H, H-7, J = 1.1 Hz, J = 7.5 Hz), 7.25 (dd, 1H, H-5, J = 1.1 Hz, J = 8.4 Hz), 2.67 (t, J = 7.9, 2H, CH_2–COOH), 2.31 (t, J = 7.3, 2H, CH_2–(CH$_2$)$_4$–COOH), 2.2 (s, 3H, CH_3), 1.67 (td, 2H, CH_2–CH$_2$–COOH, J = 7.2 Hz, J = 14.6 Hz), 1.54 (m, 4H, (CH_2)$_2$–(CH$_2$)$_2$–COOH). ^{13}C NMR 190.2, 184.4, 173.8, 161, 146.6, 145, 136.3, 132.3, 12.43, 118.4, 114.9, 33.3, 25.9, 24.3, 11.9.

5.7. General procedure for coupling naphthoquinonyl carboxylic acids 7 with phenylthioglycoside 4

Portions of acid **7** (1 equiv), phenylthioglycoside **4** (1.1 equiv), EDC (1.2 equiv), and HOBt (1.2 equiv) were combined in a mixture of CH_2Cl_2 and THF (1:2, 75 ml/g of **7**) under nitrogen. After stirring for 18 h at room temperature, the solvents were evaporated under reduced pressure and the residue re-dissolved in CH_2Cl_2, washed with 5% NaOH), 0.5 M HCl (2.5 ml), and then dried under reduced pressure. The acetylated products **8a–d** were each purified by preparative TLC (MeOH–CH_2Cl_2 2.5:97.5).

5.7.1. Phenyl-3,4,6-tri-*O*-acetyl-2-deoxy-2-[3′-(8″-hydroxy-3″-methyl-1″,4″-dioxo-1″,4″-dihydronaphthalen-2″-yl) propanamido]-1-thio-α-D-glucopyranoside (8a)

Obtained as an orange solid (57%, mp 163–165 °C). ^{1}H NMR ($CDCl_3$) δ (300 MHz) 12.0 (s, 1H, OH), 7.59–7.52 (m, 2H, Ar-H), 7.42–7.39 (m, 2H, Ar-H), 7.31–7.26 (m, 5H, Ar-H), 6.00 (d, 1H, J = 8.5 Hz, NH), 5.72 (d, 1H, J = 5.2 Hz, H-1,), 5.18–5.14 (m, 2H, H-3 & H-4), 4.61–4.47 (m, 2H, H-2 & H-5), 4.27 (dd, 1H, J = 5.0 Hz, J = 12.4 Hz H-6a), 4.07 (dd, 1H, H-6b, J = 2.3 Hz, J = 12.3 Hz), 2.93 (dd, J = 6.6 Hz, J = 8.5 Hz, 2H, CH_2–CONH), 2.41 (dd, 2H, CH_2–CH$_2$–CONH, J = 6.6 Hz, J = 8.5 Hz), 2.2 (3H, CH_3), 2.05, 2.04, 2.03 (s, 9H, 3×–COCH_3). ^{13}C NMR 190, 184, 171.8, 171.4, 170.7, 169.4, 161.5, 145.9, 145.1, 136.2, 132.9, 132.3, 131.7, 129.4, 128.09, 124.1, 119.2, 115.0, 87.6 (C-1), 71.5, 69.2, 68.3, 62.2, 53.0, 34.9, 22.7, 20.8, 20.8, 13.0. Anal. Calcd for $C_{32}H_{33}NO_{11}S$: C, 60.08; H 5.2; N, 2.19; S, 5.01. Found: C, 59.21; H, 5.21; N, 1.65; S, 4.27. [M+H]$^+$ calcd for $C_{32}H_{33}NO_{11}S$: [M+H]$^+$ cacld for $C_{32}H_{33}NO_{11}S$: 640.1847; found: 640.1843.

5.7.2. Phenyl-3,4,6-tri-*O*-acetyl-2-deoxy-2-[3′-(8″-hydroxy-3″-methyl-1″,4″-dioxo-1″,4″-dihydronaphthalen-2″-yl)butanamido]-1-thio-α-D-glucopyranoside (8b)

Obtained as a yellow solid (84%, mp 157–158 °C). ^{1}H NMR ($CDCl_3$) δ (300 MHz) 12.0 (s, 1H, OH), 7.6–7.56 (m, 2H, Ar-H), 7.44–7.41 (m, 2H, Ar-H), 7.30–7.20 (m, 5H, Ar-H), 6.08 (d, 1H, NH, J = 8.5 Hz), 5.76 (d, 1H, H-1, J = 5.4 Hz), 5.21–5.16 (m, 2H, H-3&4), 4.65–4.48 (m, 2H, H-2&5), 4.28 (dd, 1H, H-6a, J = 4.9 Hz, J = 12.4 Hz), 4.09 (dd, 1H, H-6b, J = 2.3 Hz, J = 12.3 Hz), 2.73–2.56 (m, 2H, CH_2–CONH), 2.29 (t, 1H, J = 7.1 Hz, CH_2–(CH$_2$)$_2$–CONH), 2.15 (3H, CH_3), 2.06, 2.049, 2.048 (s, 9H, 3×–COCH_3), 1.80 (m, 2H, CH_2–CH$_2$–CONH). ^{13}C NMR δ 189.0, 184.0, 172, 171.5, 170.5, 169.2, 161.2, 145.9, 145.4, 136, 132.8, 132.1, 131.4, 129.2, 127.8, 123.9, 118.9, 114.9, 87.5 (C-1), 71.2, 68.9, 68.2, 62, 52.7, 35.9, 25.6, 24.1, 20.7, 20.6, 20.5, 12.7. Anal. Calcd for $C_{33}H_{35}NO_{11}S$: C, 60.63; H, 5.4; N, 2.14; S, 4.91. Found: C, 59.78; H, 5.75; N, 1.71; S, 4.0. [M−H]$^+$ calcd for $C_{33}H_{35}NO_{11}S$: 652.1852; found 652.1981.

5.7.3. Phenyl-3,4,6-tri-*O*-acetyl-2-deoxy-2-[3′-(8″-hydroxy-3″-methyl-1″,4″-dioxo-1″,4″-dihydronaphthalen-2″-yl)pentanamido]-1-thio-α-D-glucopyranoside (8c)

Obtained as a red solid (64%, mp 127–130 °C). ^{1}H NMR ($CDCl_3$) δ (300 MHz) 12.0 (s, 1H, OH), 7.6–7.56 (m, 2H, Ar-H), 7.44–7.41 (m, 2H, Ar-H), 7.30–7.20 (m, 5H, Ar-H), 5.9 (d, 1H, NH, J = 8.5 Hz), 5.76 (d, 1H, H-1, J = 5.4 Hz), 5.17–5.13 (m, 2H, H-3&4), 4.65–4.48 (m, 2H, H-2&5), 4.28 (dd, 1H, H-6a, J = 4.9 Hz, J = 12.4 Hz), 4.09 (dd, 1H, H-6b, J = 2.3 Hz, J = 12.3 Hz), 2.63 (dt, 1H, J = 1.5 Hz, J = 7.1 Hz, CH_2–CONH), 2.25 (dt, 1H, J = 2.3 Hz, J = 7.2 Hz, –CH_2–(CH$_2$)$_3$–CONH), 2.15 (3H, CH_3), 2.05, 2.04, 2.03 (s, 9H, 3×–COCH_3), 1.7–1.6 (m, 2H, CH_2–CH$_2$–CONH), 1.5–1.4 (m, 2H, CH_2–(CH$_2$)$_2$–CONH). ^{13}C NMR 190, 184, 172, 171.6, 170.5, 169.2, 161.2, 146.4, 144.8, 136.8, 135.9, 132.8, 132.1, 131.4, 129.2, 127.8, 123.8, 118.8, 114.9, 87.6 (C-1), 71.3, 68.9, 68.1, 62, 52.7, 36.1, 27.9, 25.9, 25.5, 20.7, 20.66, 20.6, 12.7. Anal. Calcd for $C_{34}H_{37}NO_{11}S$: C, 61.16; H, 5.59; N, 2.1; S, 4.8. Found: C, 60.27; H, 5.67; N, 1.63; S, 3.91. [M−H]$^+$ calcd for $C_{34}H_{37}NO_{11}S$: 666.2008; found 665.8366.

5.7.4. Phenyl-3,4,6-tri-*O*-acetyl-2-deoxy-2-[3′-(8″-hydroxy-3″-methyl-1″,4″-dioxo-1″,4″-dihydronaphthalen-2″-yl)hexanamido]-1-thio-α-D-glucopyranoside (8d)

Obtained as a red solid (78%, mp 55–58 °C). ^{1}H NMR ($CDCl_3$) δ (300 MHz) 12.0 (s, 1H, OH), 7.6–7.56 (m, 2H, Ar-H), 7.44–7.41 (m, 2H, Ar-H), 7.30–7.20 (m, 5H, Ar-H), 5.9 (d, 1H, NH, J = 8.5 Hz), 5.76 (d, 1H, H-1, J = 5.4 Hz), 5.17–5.13 (m, 2H, H-3&4), 4.65–4.48 (m, 2H, H-2&5), 4.28 (dd, 1H, H-6a, J = 4.9 Hz, J = 12.4 Hz), 4.09 (dd, 1H, H-6b, J = 2.3 Hz, J = 12.3 Hz), 2.6–2.5 (m, 2H, CH_2–CONH), 2.2–2.18 (m, 2H, CH_2–(CH$_2$)$_4$–CONH), 2.2 (3H, CH_3), 2.058, 2.056, 2.04 (s, 9H, 3×–COCH_3), 1.6–1.5 (m, 2H, CH_2–CH$_2$–CONH), 1.5–1.4 (m, 4H, (CH_2)$_2$–CH$_2$–CONH). ^{13}C NMR 190, 184, 172, 171.6, 170.5, 169.2, 161.2, 146.4, 144.8, 136.8, 135.9, 132.8, 132.1, 131.4, 129.2, 127.8, 123.8, 118.8, 114.9, 87.6 (C-1), 71.3, 68.9, 68.1, 62, 52.7, 36.1, 27.9, 25.9, 25.5, 20.7, 20.66, 20.6, 12.7. Anal. Calcd for $C_{35}H_{39}NO_{11}S$: C, 61.66; H, 5.77; N, 2.05; S, 4.7. Found: C, 63.9; H, 6.14; N, 1.6; S, 3.47. [M−H]$^+$ calcd for $C_{35}H_{39}NO_{11}S$: 680.2165; found 679.5982.

5.8. General procedure for deacetylations

A portion of 0.2 M NaOMe in MeOH was added to a stirred suspension of acetylated glycoside **8** in MeOH at room temperature. When TLC showed complete conversion to a single product with lower polarity (typically after 1 h) Dowex-50WX-200(H$^+$) ion exchange resin was added to the reaction mixture until a neutral pH was reached. The dowex resin was then removed by filtration and the clear filtrate concentrated in vacuo to give deacetylated product.

5.8.1. Phenyl-2-deoxy-2-[3′-(8″-hydroxy-3″-methyl-1″,4″-dioxo-1″,4″-dihydronaphthalen-2″-yl)propanamido]-1-thio-α-D-glucopyranoside (1a)

Obtained as a red/brown solid (34%, 238–241 °C). ^{1}H NMR (DMSO-d_6) δ (400 MHz) 12.0 (s, 1H, OH),8.09 (d, 1H, NH, J = 6.5 Hz), 7.69 (t, 1H, H-7, J = 8.0 Hz), 7.5-7.4 (m, 1H, H-5,1H), 7.38–7.35 (2H, m, Ar-H), 7.31–7.24 (4H, m, Ar-H), 5.60 (d, 1H, H-1, J = 5.1 Hz), 5.12 (d, 1H, OH, J = 5.6 Hz), 4.85 (d, 1H, OH, J = 5.6 Hz), 4.51 (t, 1H, OH, J = 6.0 Hz), 3.87–3.80 (2H, m, H-2&3), 3.58–3.54 (2H, m, H-5&6$_a$), 3.46–3.40 (1H, m,H-6$_b$), 3.3–3.2 (1H, m, H-4), 2.8–2.7 (m, 2H, CH_2–CONH), 2.38–2.34 (m, 2H, CH_2–CH$_2$–CONH), 2.1 (3H, CH_3). ^{13}C NMR 183.8, 172, 171.9, 159.7, 145.1, 136.2, 134.2, 131.6, 130.9, 129.08, 129.03, 128.9, 127, 123.3, 118.4, 114.5,

87 (C-1), 73.8, 70.37, 70.30, 70, 60, 54.5, 54.4, 33.52, 33.48, 22.2, 12.4. [M+H] calcd for $C_{26}H_{27}NO_8S$: 514.1536; found 514.1544.

5.8.2. Phenyl-2-deoxy-2-[3′-(8″-hydroxy-3″-methyl-1″,4″-dioxo-1″,4″-dihydronaphthalen-2″-yl)butanamido]-1-thio-α-D-glucopyranoside (1b)

Obtained as a yellow/brown solid (46% mp 196–199 °C). ^{1}H NMR (DMSO-d_6) δ (300 MHz) 12.0 (s, 1H, OH), 7.86 (d, 1H, N*H*,*J* = 6.6 Hz), 7.69 (dd, 1H,*J* = 7.6 Hz,*J* = 8.3 Hz), 7.51 (dd, 1H,*J* = 1.1 Hz,*J* = 7.5 Hz), 7.40–7.37 (m, 2H, Ar-H), 7.29–7.18 (m, 4H, Ar-H), 5.64 (d, 1H, H-1, *J* = 5.1 Hz), 5.01 (d, 1H, O*H*, *J* = 5.6 Hz), 4.76 (d, 1H, O*H*, *J* = 5.6 Hz), 4.39 (t, 1H, O*H*, *J* = 5.6 Hz), 3.9–3.8 (m, 2H, H-2&3), 3.6–3.5 (m, 2H, H-5&6$_a$), 3.49–3.41 (m, 1H, H-6$_b$), 3.29–3.23 (m, 1H, H-4), 2.6–2.5 (2H, m, C*H*$_2$–CONH), 2.3–2.2 (2H, m, C*H*$_2$–(CH$_2$)$_2$–CONH), 2.0 (3H, s, CH$_3$), 1.7–1.6 (2H, m, C*H*$_2$–CH$_2$–CONH). ^{13}C NMR 189, 184, 172, 160, 146, 145, 136, 135, 132, 131, 129, 127, 123, 118, 88 (C-1), 74, 71, 70, 61, 55, 34, 28, 25, 24, 12. [M−H]$^+$ calcd for $C_{27}H_{29}NO_8S$ 526.1536; found 526.1526.

5.8.3. Phenyl-2-deoxy-2-[3′-(8″-hydroxy-3″-methyl-1″,4″-dioxo-1″,4″-dihydronaphthalen-2″-yl)pentanamido]-1-thio-α-D-glucopyranoside (1c)

Obtained as a brown solid (60%, mp 170–173 °C). ^{1}H NMR (DMSO-d_6) δ (300 MHz) 12.0 (s, 1H, OH), 7.86 (d, 1H, N*H*, *J* = 6.5 Hz), 7.69 (dd, 1H, *J* = 7.6 Hz, *J* = 8.3 Hz), 7.51 (d, 1H, *J* = 7.4 Hz), 7.40–7.3 (m, 2H, Ar-H), 7.26–7.16 (m, 4H, Ar-H), 5.6 (d, 1H, H-1, *J* = 5.1 Hz), 5.1 (d, 1H, O*H*, *J* = 5.5 Hz), 4.76 (d, 1H, O*H*, *J* = 5.5 Hz), 4.39 (t, 1H, O*H*, *J* = 5.8 Hz), 3.9–3.8 (m, 2H, H-2&3), 3.6–3.5 (m, 2H, H-5&6$_a$), 3.5–3.4 (m, 1H, H-6$_b$), 3.3–3.2 (m, 1H, H-4), 2.6–2.5 (2H, m, C*H*$_2$–CONH), 2.2–2.1 (2H, m, –C*H*$_2$–(CH$_2$)$_3$–CONH), 2.0 (3H,s, CH$_3$), 1.63–1.57 (2H, m, C*H*$_2$–CH$_2$–CONH), 1.5–1.4 (2H, m, C*H*$_2$–(CH$_2$)$_2$–CONH). ^{13}C NMR 189, 184, 173, 160, 146, 144, 136, 135, 132, 131, 129, 128, 127, 123, 118, 114, 87 (C-1), 74, 70.4, 70.1, 61, 55, 35, 27, 26, 25,12. [M−H]$^+$ calcd for $C_{28}H_{31}NO_8S$: 540.1692; found 540.1685.

5.8.4. Phenyl-2-deoxy-2-[3′-(8″-hydroxy-3″-methyl-1″,4″-dioxo-1″,4″-dihydronaphthalen-2″-yl)hexanamido]-1-thio-α-D-glucopyranoside (1d)

Obtained as a brown/orange solid (52%, mp 157–159 °C). ^{1}H NMR (DMSO-d_6) δ (300 MHz) 12.0 (s, 1H, OH), 7.86 (d, 1H, N*H*, *J* = 6.5 Hz), 7.7 (t, 1H, *J* = 8.3 Hz), 7.51 (d, 1H, *J* = 7.5 Hz), 7.4 (td, 2H, *J* = 1.2 & 8.2, Ar-H), 7.3–7.2 (m, 4H, Ar-H), 5.6 (d, 1H, H-1, *J* = 4.8 Hz), 5.1 (d, 1H, O*H*, *J* = 5.7 Hz), 4.8 (d, 1H, O*H*, *J* = 5.8 Hz), 4.5 (t, 1H, O*H*, *J* = 6.1 Hz), 3.9–3.8 (m, 2H, H-2&3), 3.6–3.5 (m, 2H, H-5&6$_a$), 3.5–3.4 (m, 1H, H-6$_b$), 3.3–3.2 (m, 1H, H-4), 2.6–2.5 (2H, m, C*H*$_2$–CONH), 2.2–2.1 (2H, m, C*H*$_2$–(CH$_2$)$_4$–CONH), 2.1 (3H, s, CH$_3$), 1.63–1.57 (2H, m, C*H*$_2$–CH$_2$–CONH), 1.5–1.4 (4H, m, (C*H*$_2$)$_2$–CH$_2$–CONH). ^{13}C NMR 189.7, 184, 173.5, 160, 146.8, 144.5, 136.6, 135, 132, 131.5, 129, 127, 123.6, 118, 114, 87.9 (C-1), 74, 70.8, 70.6, 60.7, 55, 35, 29, 27.9, 26, 25,12.6. [M−H]$^+$ calcd for $C_{29}H_{33}NO_8S$: 554.1849; found 554.1843.

5.9. Phenyl-3,4,6-tri-*O*-acetyl-2-deoxy-2-acetamido-1-thio-β-D-glucopyranoside (18)

Thiophenol (0.208 ml, 2.14 mmol) and $SnCl_4$ (1.25 ml, 1,25 mmol) were added to a stirred solution of **17** (1.0 g, 1.79 mmol) in CH_2Cl_2 (20 ml). The reaction mixture was heated at reflux for 24 h, cooled to room temperature and quenched by addition of saturated aqueous $NaHCO_3$. The aqueous layer was separated and extracted with DCM. The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure, and the solid residue was recrystallized from Et_2O–hexane (10:1) to give **18** (0.59 g, 75%); mp 186–187 °C (lit.[29b] 199 °C); $[\alpha]_D = -24.9$ (*c* 1.0, $CHCl_3$), lit.[29b] $[\alpha]_D = -20.4$ (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.52–7.50 (2H, m, Ph-*H*), 7.32–7.30 (3H, m, Ph-*H*), 5.60 (1H, d, *J* 9.3 Hz, N*H*), 5.23 (1H, t, *J* = 9.8 Hz, *H*-3), 5.06 (1H, t, *J* = 9.7 Hz, *H*-4), 4.87 (1H, d, *J* = 10.3 Hz, *H*-1), 4.20 (1H, dq, *J* = 4.2 &12.2 Hz, *H*-6a &b), 4.04 (1H, m, *H*-2), 3.73 (1H, ddd, *J* = 2.5, 5.2 & 10.0 Hz, *H*-5), 2.08 (3H, s, C*H*$_3$), 2.03 (3H, s, C*H*$_3$), 2.02 (3H, s, C*H*$_3$), 1.99 (3H, s, C*H*$_3$); (100 MHz, $CDCl_3$): δ_C 170.9, 170.5,169.9, 169.2, 132.4 (2×C), 128.8 (2×C),128 (2×C), 86.6 (C-1), 75.7, 73.7, 68.4, 62.3, 53.3, 23.2, 20.68, 20.62, 20.5. Anal. Cacld for $C_{20}H_{25}NO_8S$: C, 54.66; H, 5.73; N, 3.19; S, 7.30. Found: C, 54.30; H, 5.74; N, 2.71; S, 6.89. [M+H] calcd for $C_{20}H_{25}NO_8S$: 440.1379; found: 440.1396.

5.10. Phenyl-2-deoxy-2-acetamido-1-thio-β-D-glucopyranoside (20)

NaOMe (0.2 M) in MeOH (10 ml) was added to a suspension of **18** (0.4 g, 0.911 mmol) in methanol (20 ml) with stirring at room temperature. TLC showed complete conversion to a single more polar product within 10 min. The reaction mixture was then stirred with resin (Dowex-50WX-200(H^+), then the resin removed by filtration, and the filtrate concentrated under reduced pressure. The product **20** was obtained as white powder (260 mg, 91%); mp 231–234 °C (lit.[29b] 222 °C; lit.[29a] 196–199 °C); ^{1}H NMR (400 MHz, D_2O): δ_H 8.36 (1H, s, N*H*), 7.44 (2H, dd, *J* = 1.7 Hz and 7.8 Hz, Ph-*H*), 7.34–7.29 (3H, m, Ph-*H*), 4.80 (1H, d, *J* = 10.4 Hz, *H*-1), 3.82 (1H, d, *J* = 12.0 Hz, *H-6a*), 3.71–3.66 (1H, m, *H*-5 & 6b), 3.51 (1H, t, *J* 9.3 Hz, *H*-4), 3.41 (2H, m, *H*-2 & 3), 1.95 (3H, s, C*H*$_3$); (100 MHz, $CDCl_3$): δ_C 171 (*C*═O), 132, 130 (2×C), 128 (2×C), 127, 87 (C-1), 81, 76, 71, 61.5, 53.7, 21.5 (–*C*H$_3$). [M+H] calcd for $C_{14}H_{19}NO_5S$: 314.1062; found: 314.1079.

5.11. Benzyl-3,4,6-tri-*O*-acetyl-2-deoxy-2-acetamido-1-thio-β-D-glucopyranoside (19)

$BF_3{\cdot}Et_2O$ (0.16 ml, 1.34 mmol) was added in one portion to a cold suspension of **17** (0.5 g, 0.895 mmol). An exothermic reaction was immediately observed and the reaction mixture was allowed to stir for 50 min when TLC showed complete consumption of **17**. Anhydrous CH_3CN (20 ml) was then added followed by thiourea (0.136 g, 1.79 mmol) and the reaction mixture placed on a pre-heated oil bath at 80 °C for 15 min. with constant stirring. After full consumption of the starting material as judged by TLC, the reaction mixture was cooled to room temperature and BnBr (0.458 g, 0.318 ml, 1.34 mmol) and Et_3N (0.89 ml, 6.44 mmol) were added in succession and the reaction allowed to stir for 3 h at ambient temperature. After standard workup, the crude product was purified by flash column chromatography (EtOAc as eluent) to give **19** as a white powder (0.31 g, 75%), mp: 178–179 °C (lit.[30b] 199–202 °C). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.33–7.28 (5H, m, Ph-*H*); 5.32 (1H, d, *J* = 9.3 Hz, N*H*), 5.10 (1H, t, *J* = 9.6 Hz, C*H*–), 5.02 (1H, t, *J* = 9.5 Hz, C*H*–), 4.28–4.15 (4H, m, *H*-1,2,3 & 4), 3.96 (1H, d, *J* = 13.1 Hz, *H*-6a), 3.96 (1H, d, *J*13.1 Hz, *H*-6b), 3.57 (1H, ddd, *J* = 2.3, 4.9 & 9.6 Hz, *H*-5); (100 MHz, $CDCl_3$): δ_C 171 (*C*═O), 170.9 (*C*═O), 170 (*C*═O), 169.5 (*C*═O), 137, 129 (2×C), 128.6 (2×C),127, 83 (C-1), 76, 74.1, 68.2, 62.1, 53.2, 34.2 (Ph–*C*H$_2$–S), 23.2, 20.68, 20.62, 20.5. Anal. Calcd for $C_{21}H_{27}NO_8S$: C, 55.62; H, 6.00; N, 3.09; S, 7.07. Found: C, 55.74; H, 5.90; N, 2.36; S, 7.01. [M+H] calcd for $C_{21}H_{27}NO_8S$: 454.1536; found: 454.1556.

5.12. Benzyl 2-deoxy-2-acetamido-1-thio-β-D-glucopyranoside (21)

NaOMe (0.2 M) in MeOH (10 ml) was added to a suspension of **19** (0.4 g, 0.911 mmol) in methanol (20 ml) with stirring at room temperature. TLC showed complete conversion to a single more polar product within 10 min. The reaction mixture was then stirred

with resin (Dowex-50WX-200(H^+), then the resin removed by filtration, and the filtrate concentrated under reduced pressure to give **21** as white powder (0.27 g, 89%), mp: 125–127 °C (lit.[30b] 225–227 °C) ^{1}H NMR (400 MHz, D_2O): δ_H 8.36 (1H, s, N*H*), 7.7–7.6 (5H, m, Ph-*H*), 4.25 (1H, d, *J* = 10.2 Hz, *H*-1), 3.9 (1H, d, *J* = 12.2 Hz, *H*-6*a*), 3.8 (2H, m, –CH_2–), 3.65 (2H, m, *H*-5 & 6b), 3.5 (2H, m, *H*-3 & 4), 3.41 (2H, m, *H*-2 & 3), 3.3 (1H, m, *H*-2), 2.22 (3H, s, CH_3); (100 MHz, $CDCl_3$): δ_C 172.5 (C=O), 137, 128 (2×C), 127.7 (2×C), 126, 82 (C-1), 79, 74, 68, 59, 53.5, 23 (CH_2–), 21.5 (–CH_3). Anal. Calcd for $C_{15}H_{21}NO_5S$: C, 55.03; H, 6.47; N, 4.28; S, 9.79. Found: C, 53.66; H, 6.11; N, 4.47; S, 10.23. [M+H] calcd for $C_{15}H_{21}NO_5S$: 328.1219; found: 328.1214.

5.13. Cyclohexyl 3,4,6-tri-*O*-acetyl-2-*C*-allyl-2-deoxy-α-D-glucopyranoside (28a) and cyclohexyl 3,4,6-tri-*O*-acetyl-2-*C*-allyl-2-deoxy-β-D-glucopyranoside (28b)

A mixture of glucosyl fluoride **27**[31] (550 mg, 1.65 mmol), cyclohexanol (2.62 ml, 24.84 mmol) and molecular sieves 4 Å (1 g) in CH_2Cl_2 (13 ml) was stirred at room temperature for 1 h before $BF_3{\cdot}Et_2O$ (1.22 ml, 9.94 mmol) was added dropwise. The stirring was continued for 18 h, then Et_3N (1.1 ml) added and the solids removed by filtration. The filtrate was diluted with dichloromethane, and the CH_2Cl_2 phase washed sequentially with water, saturated $NaHCO_3$ and water, then dried over $MgSO_4$ and concentrated. Purification by column chromatography using ethyl acetate–petroleum ether (3:17) gave an inseparable mixture of anomers **28a** and **28b** (α:β ≈ 1:1 by ^{13}C NMR) as well as excess acceptor (cyclohexanol). The β-anomer **28b** crystallized from pentane at low temperature (244 mg, 36%). Separation of the mother liquor by column chromatography using ethyl acetate–petroleum ether (7:93) as eluent afforded α-anomer **28a** (287 mg, 42%) as an oil together with a mixture of anomers (105 mg, 15%) (overall yield 636 mg, 93%).

α-Anomer **28a**: $[\alpha]_D$ = +115.6 (*c* 2.6, $CHCl_3$); δ_H (400 MHz; $CDCl_3$) 5.73–5.63 (1H, m, H-2″), 5.22 (1H, dd, *J* = 11.2, 9.2 Hz, H-3), 5.06–4.91 (3H, m, H-4, H-3″$_a$, H-3″$_b$), 4.92 (1H, d, *J* = 3.6 Hz, H-1), 4.24 (1H, dd, *J* = 12.2, 4.6 Hz, H-6$_a$), 4.09–4.02 (2H, m, H-5, H-6$_b$), 3.54–3.47 (1H, m, H-1′), 2.14–2.10 (2H, m, H-1″$_a$, H-1″$_b$), 2.06, 2.00, 1.99 (9H-3 × $COOCH_3$), 1.99 (1H, m, H-2), 1.87–1.19 (10H, m, 5 × –CH_2–, cyclohexyl); δ_C (100 MHz; $CDCl_3$) 170.59, 170.42, 169.89 (3 × $COOCH_3$), 135.25 (C-2″), 116.64 (C-3″), 96.84 (C-1), 75.95 (C-1′), 72.45 (C-3), 70.11 (C-4), 67.66 (C-5), 62.52 (C-6), 44.31 (C-2), 33.31 (C–CH_2–, cyclohexyl), 31.73 (C-1″), 31.47, 25.48, 24.02, 23.79 (4 × –CH_2–, cyclohexyl), 20.70, 20.60, 20.59 (3 × $COOCH_3$); HRFABMS: *m/z* 435.199633 (M+Na)$^+$. Calcd for $C_{21}H_{32}O_8Na$ 435.199477 (M+Na)$^+$.

β-Anomer **28b**: mp 100 °C (from pentane); $[\alpha]_D$ = +16.7 (*c* 1.7, $CHCl_3$); δ_H (300 MHz; $CDCl_3$) 5.84–5.70 (1H, m, H-2″), 5.06 (1H, dd, *J* = 10.8, 9.0 Hz, H-3), 5.02–4.89 (3H, m, H-4, H-3″$_a$, H-3″$_b$), 4.39 (1H, d, *J* = 8.7 Hz, H-1), 4.25 (1H, dd, *J* = 12.1, 5.3 Hz, H-6$_a$), 4.07 (1H, dd, *J* = 12.1, 2.6 Hz, H-6$_b$), 3.65–3.55 (2H, m, H-5, H-1′), 2.25–2.21 (2H, m, H-1″$_a$, H-1″$_b$), 2.05, 1.99, 1.99 (9H, 3 s, 3 × $COOCH_3$), 2.03–1.21 (11H, m, H-2, 5 × –CH_2–, cyclohexyl); δ_C (75 MHz; $CDCl_3$) 170.70, 170.37, 169.84 (3 × *C*$OOCH_3$), 134.21 (C-2″), 117.20 (C-3″), 100.58 (C-1), 77.55 (C-1′), 72.90 (C-3), 71.34 (C-5), 70.34 (C-4), 62.68 (C-6), 45.24 (C-2), 33.57, 31.80 (2 × –CH_2–, cyclohexyl), 31.12 (C-1″), 25.55, 24.08, 24.01 (3 × –CH_2–, cyclohexyl), 20.75, 20.72, 20.66 (3 × $COOCH_3$); HRFABMS: *m/z* 435.199633 (M+Na)$^+$. Calcd for $C_{21}H_{32}O_8Na$ 435.199477 (M+Na)$^+$.

5.14. Cyclohexyl 2-*C*-allyl-2-deoxy-α-D-glucopyranoside (29)

Methanolic NaOMe (0.2 M, 0.7 ml, 0.14 mmol) was added to a solution of **28a** (287 mg, 0.69 mmol) in CH_2Cl_2–MeOH (4:6 ml) at room temperature. After 4 h, the solution was neutralized with Amberlite IR-120H$^+$ resin. The resin was removed by filtration, the solvent evaporated and the crystalline residue chromatographed on silica using methanol–ethyl acetate (5:95) as eluent to afford **29** as colorless crystals (196 mg, 99%), mp 165–168 °C (from ethyl acetate and petroleum ether); $[\alpha]_D$ = +120.8 (*c* 2.3, MeOH); δ_H (400 MHz; CD_3OD) 5.86–5.76 (1H, m, H-2″), 5.07–4.98 (2H, m, H-3″$_a$, H-3″$_b$), 4.87 (1H, d, *J* = 3.2 Hz, H-1), 3.77 (1H, m, H-6$_a$), 3.69–3.63 (2H, m, H-5, H-6$_b$), 3.58 (1H, m, H-1′), 3.49 (1H, dd, *J* = 10.6, 9.1 Hz, H-3), 3.24 (1H, t, *J* = 9.1 Hz, H-4), 2.51 (1H, m, H-1″$_a$), 2.14–2.05 (1H, m, H-1″$_b$), 1.90–1.21 (11H, m, H-2, 5 × –CH_2–, cyclohexyl); δ_C (100 MHz; CD_3OD) 136.84 (C-2″), 115.38 (C-3″), 96.65 (C-1), 74.62 (C-1′), 72.82 (C-3), 72.73 (C-5), 72.15 (C-4), 61.87 (C-6), 46.59 (C-2), 33.39 (C–CH_2–, cyclohexyl), 31.56 (C-1″), 31.45, 25.69, 23.88, 23.71 (4 × –CH_2–, cyclohexyl); HRFABMS: *m/z* 309.168001 (M+Na)$^+$. Calcd for $C_{15}H_{26}O_5Na$ 309.167784 (M+Na)$^+$.

5.15. Cyclohexyl 2-*C*-allyl-2-deoxy-β-D-glucopyranoside (30)

Methanolic NaOMe (0.2 M, 0.5 ml, 0.1 mmol) was added to a solution of **28b** (200 mg, 0.48 mmol) in CH_2Cl_2–MeOH (3:5 ml) at room temperature. After 2 h, the solution was neutralized with Amberlite IR-120H$^+$ resin. The resin was removed by filtration, the solvent evaporated and the crystalline residue was chromatographed on silica using methanol–ethyl acetate (5:95) as eluent to afford 2-*C*-allylglucoside **30** as white crystals (135 mg, 98%); mp 136–138 °C (from ethyl acetate and petroleum ether); $[\alpha]_D$ = −35.4 (*c* 2.2, MeOH); δ_H (400 MHz; CD_3OD) 5.97–5.87 (1H, m, H-2″), 5.10–4.99 (2H, m, H-3″$_a$, H-3″$_b$), 4.41 (1H, d, *J* = 8.8 Hz, H-1), 3.83 (1H, dd, *J* = 11.7, 2.4 Hz, H-6$_a$), 3.71 (1H, m, H-1′), 3.65 (1H, dd, *J* = 11.7, 5.4 Hz, H-6$_b$), 3.33 (1H, dd, *J* = 10.8, 8.4 Hz, H-3), 3.22 (1H, dd, *J* = 9.6, 8.4 Hz, H-4), 3.18–3.13 (1H, m, H-5), 2.38 (2H, m, H-1″$_a$, H-1″$_b$), 1.93–1.21 (11H, m, H-2, 5 × –CH_2–, cyclohexyl); δ_C (100 MHz; CD_3OD) 135.34 (C-2″), 116.06 (C-3″), 100.48 (C-1), 76.43 (C-5), 76.32 (C-1′), 73.80 (C-3), 71.97 (C-4), 61.89 (C-6), 47.32 (C-2), 33.59, 31.70 (2 × –CH_2–, cyclohexyl), 30.39 (C-1″), 25.65, 23.94, 23.84 (3 × –CH_2–, cyclohexyl); HRFABMS: *m/z* 309.168001 (M+Na)$^+$. Calcd for $C_{15}H_{26}O_5Na$ 309.167784 (M+Na)$^+$.

5.16. Cyclohexyl 2-deoxy-2-*C*-propyl-α-D-glucopyranoside (31)

A solution of **29** (279 mg, 0.50 mmol) in ethyl acetate–methanol (5:6 ml) was treated with H_2 (1 atm) at room temperature in the presence of 10% palladium on charcoal (400 mg) for 18 h. TLC analysis (ethyl acetate–petroleum ether, 4:1) showed the complete conversion of the starting material into the deprotected derivative. The catalyst was removed by filtration through Celite, the Celite washed with methanol and the combined filtrates evaporated to dryness. The remaining residue was passed through a reverse phase isolute® C18 column using methanol as an eluent to afford **31** as colorless crystals (140 mg, 97%), mp 184–186 °C (from ethyl acetate and petroleum ether); $[\alpha]_D$ = +82.8 (*c* 2.3, MeOH); δ_H (400 MHz; CD_3OD) 4.92 (1H, d, *J* = 3.2 Hz, H-1), 3.77 (1H, d (br), *J* = 9.6 Hz, H-6$_a$), 3.69–3.60 (3H, m, H-1′, H-5, H-6$_b$), 3.46 (1H, dd, *J* = 10.4, 9.0 Hz, H-3), 3.22 (1H, t, *J* = 9.0 Hz, H-4), 1.87–1.25 (15H, m, H-2, 5 × –CH_2–, cyclohexyl, -(CH_2)$_2$CH_3), 0.92 (3H, t, *J* = 7.2 Hz, H-3″); δ_C (100 MHz; CD_3OD) 96.75 (C-1), 74.20 (C-1′), 73.32 (C-3), 72.54 (C-5), 72.14 (C-4), 61.91 (C-6), 46.23 (C-2), 33.34, 31.13 (2 × –CH_2–, cyclohexyl), 29.44 (C-1″), 25.70, 23.75, 23.55 (3 × –CH_2–, cyclohexyl), 19.89 (C-2″), 13.59 (C-3″); HRFABMS: *m/z* 311.182957 (M+Na)$^+$. Calcd for $C_{15}H_{28}O_5Na$ 311.183433 (M+Na)$^+$.

5.17. Cyclohexyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-*C*-(2″-oxopropyl)-α-D-glucopyranoside (32)

A mixture of $PdCl_2$ (1.79 g, 10.10 mmol) and $CuCl_2$ (3.5 g, 26.03 mmol) in DMF–H_2O (1:7, 15 ml) was stirred at room temper-

ature under an atmosphere of oxygen (balloon) for 2 h. A solution of **28a** (1.05 g, 2.54 mmol) in CH_2Cl_2–DMF (1:1, 6 ml) was added dropwise and stirring was continued for 4 days under an atmosphere of oxygen. The reaction was stopped by the addition of a solution of 3 M HCl, extracted with CH_2Cl_2, dried over $MgSO_4$ and concentrated. Purification by column chromatography using ethyl acetate–petroleum ether afforded compound **32** as colorless crystals (550 mg, 50%); mp 72–73 °C (from petroleum ether); $[\alpha]_D$ = +131.9 (*c* 2.9, $CHCl_3$); ν_{max}/cm^{-1} 1744 (C=O); δ_H (300 MHz; $CDCl_3$) 5.14 (1H, dd, *J* = 11.0, 9.6 Hz, H-3), 5.08 (1H, d, *J* = 3.3 Hz, H-1), 4.95 (1H, t, *J* = 9.6 Hz, H-4), 4.22 (1H, dd, *J* = 12.0, 5.0 Hz, H-6_a), 4.05 (1H, dd, *J* = 12.0, 2.6 Hz, H-6_b), 4.02 (1H, m, H-5), 3.47 (1H, m, H-1′), 2.60 (1H, dd, *J* = 17.0, 9.0 Hz, H-$1''_a$), 2.54–2.45 (1H, m, H-2), 2.37 (1H, dd, *J* = 17.0, 3.4 Hz, H-$1''_b$), 2.09 (3H, s, H-3″), 2.05, 1.98, 1.97 (9H, 3 s, 3 × $COOCH_3$), 1.84–1.15 (10H, m, 5 × –CH_2–, cyclohexyl); δ_C (75 MHz; $CDCl_3$) 206.43 (C-2″), 170.68, 170.69, 169.78 (3 × *C*$OOCH_3$), 96.16 (C-1), 75.65 (C-1′), 71.99 (C-3), 69.85 (C-4), 67.48 (C-5), 62.43 (C-6), 41.36 (C-1″), 40.03 (C-2), 33.28, 31.32 (2 × –CH_2–, cyclohexyl), 30.13 (C-3″), 25.48, 24.01, 23.76 (3 × –CH_2–, cyclohexyl), 20.73, 20.66, 20.63 (3 × $COOCH_3$); HRFABMS: *m/z* 451.194600 $(M+Na)^+$. Calcd for $C_{21}H_{32}O_9Na$ 451.194392 $(M+Na)^+$.

5.18. Cyclohexyl 2-deoxy-2-*C*-(2″-oxopropyl)-α-D-glucopyranoside (33)

A methanolic sodium methoxide (0.2 M, 0.2 ml, 0.04 mmol) was added to a solution of **32** (200 mg, 0.47 mmol) in CH_2Cl_2–MeOH (4:5 ml) at room temperature. After 18 h, water (1.7 ml) was added and the solvent evaporated. Co-evaporation with methanol (×2) gave a solid residue that was recrystallized from ethyl acetate–petroleum ether (4:1) to afford **33** as white crystals (120 mg, 84%); mp 151–154 °C (from ethyl acetate and petroleum ether); $[\alpha]_D$ = +128.8 (*c* 2.1, MeOH); δ_C (400 MHz; CD_3OD) 4.97 (1H, d, *J* = 3.6 Hz, H-1), 3.77 (1H, dd, *J* = 11.6, 2.4 Hz, H-6_a), 3.67 (1H, dd, *J* = 11.6, 5.6 Hz, H-6_b), 3.65–3.60 (1H, m, H-5), 3.55 (1H, m, H-1′), 3.44 (1H, dd, *J* = 10.8, 8.8 Hz, H-3), 3.26 (1H, dd, *J* = 10.0, 8.8 Hz, H-4), 2.84 (1H, dd, *J* = 17.9, 4.2 Hz, H-$1''_a$), 2.58 (1H, dd, *J* = 17.9, 9.2 Hz, H-$1''_b$), 2.14 (3H, s, H-3″), 2.12 (1H, m, H-2), 1.87–1.19 (10H, m, 5 × –CH_2–, cyclohexyl); δ_C (100 MHz; CD_3OD) 209.81 (C-2″), 96.44 (C-1), 74.38 (C-1′), 72.68 (C-5), 72.37 (C-3), 71.98 (C-4), 61.81 (C-6), 42.39 (C-2), 41.52 (C-1″), 33.26, 31.09 (2 × –CH_2–, cyclohexyl), 29.12 (C-3″), 25.63, 23.79, 23.59 (3 × –CH_2–, cyclohexyl); HRFABMS: *m/z* 325.162838 $(M+Na)^+$. Calcd for $C_{15}H_{26}O_6Na$ 325.162699 $(M+Na)^+$.

5.19. Cyclohexyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-*C*-(2″-hydroxypropyl)-α-D-glucopyranoside (34)

$NaBH_4$ (0.19 g, 5.02 mmol) was added portion-wise to a solution of compound **32** (200 mg, 0.47 mmol) in THF–MeOH (2:5 ml) at −20 °C and the reaction allowed to warm to 0 °C over 2 h. The reaction was quenched with saturated $NaHCO_3$, extracted with CH_2Cl_2, and the CH_2Cl_2 solution dried over $MgSO_4$ and concentrated. Purification by column chromatography using ethyl acetate–petroleum ether (3:7) afforded an inseparable mixture of diastereomers **34** as an oil (178 mg, 88%, 1:1 by ^1H NMR); ν_{max}/cm^{-1} 3467 (br) (OH). Signals for the two diastereomers could be distinguished in the ^1H NMR spectrum of the mixture, and assigned with the aid of COSY and HSQC experiments: *Diastereomer* **34a**: δ_C (400 MHz; $CDCl_3$) 5.19 (1H, dd, *J* = 11.2, 8.9 Hz, H-3), 5.05 (1H, d, *J* = 3.6 Hz, H-1), 4.93 (1H, dd, *J* = 10.2, 8.9 Hz, H-4), 4.22 (1H, t, *J* = 4.4 Hz, H-6_a), 4.08–4.03 (2H, m, H-5, H-6_b), 3.85–3.77 (1H, m, H-2″), 3.57–3.50 (1H, m, H-1′), 2.07 (1H, m, H-2), 2.06, 2.01, 2.00 (9H, 3 s, 3 × $COOCH_3$), 1.88–1.19 (12H, m, 5 × –CH_2–, cyclohexyl, H-$1''_a$, H-$1''_b$), 1.17 (3H, d, *J* = 6.0 Hz, H-3″); δ_C (100 MHz; $CDCl_3$) 170.61, 170.46, 169.91 (3 × *C*$OOCH_3$), 97.51 (C-1), 75.90 (C-1′), 72.97 (C-3), 70.14 (C-4), 67.47 (C-5), 66.74 (C-2″), 62.49 (C-6), 42.44 (C-2), 36.94 (C-1″), 33.30, 31.34, 25.48 (3 × –CH_2–, cyclohexyl), 24.03 (C-3″), 24.03, 23.79 (2 × –CH_2–, cyclohexyl), 20.76, 20.61, 20.60 (3 × $COOCH_3$); *Diastereomer* **34b**: δ_H (400 MHz; $CDCl_3$) 5.22 (1H, dd, *J* = 11.2, 9.3 Hz, H-3), 5.08 (1H, d, *J* = 3.6 Hz, H-1), 4.95 (1H, dd, *J* = 10.4, 9.3 Hz, H-4), 4.25 (1H, t, *J* = 4.4 Hz, H-6_a), 4.08–4.03 (2H, m, H-5, H-6_b), 3.85–3.77 (1H, m, H-2″), 3.57–3.50 (1H, m, H-1′), 2.27–2.19 (1H, m, H-2), 2.06, 2.01, 2.00 (9H, 3 s, 3 × $COOCH_3$), 1.88–1.19 (12H, m, 5 × –CH_2–, cyclohexyl, H-$1''_a$, H-$1''_b$), 1.16 (3H, d, *J* = 6.0 Hz, H-3″); δ_C (100 MHz; $CDCl_3$) 170.61, 170.46, 169.85 (3 × *C*$OOCH_3$), 96.61 (C-1), 75.67 (C-1′), 72.43 (C-3), 70.14 (C-4), 67.57 (C-5), 64.49 (C-2″), 62.49 (C-6), 40.89 (C-2), 36.28 (C-1″), 33.30, 31.34, 25.48 (3 × –CH_2–, cyclohexyl), 24.49 (C-3″), 24.03, 23.79 (2 × –CH_2–, cyclohexyl), 20.76, 20.61, 20.60 (3 × $COOCH_3$); HRFABMS: *m/z* 453.210259 $(M+Na)^+$. Calcd for $C_{21}H_{34}O_9Na$ 453.210041 $(M+Na)^+$.

5.20. Cyclohexyl 2-deoxy-2-*C*-(2″-hydroxypropyl)-α-D-glucopyranoside (35)

Methanolic sodium methoxide (0.2 M, 0.3 ml, 0.60 mmol) was added to a solution of **34** (155 mg, 0.36 mmol) in CH_2Cl_2–MeOH (3:5 ml) at room temperature. After 18 h, the solution was neutralized with Amberilite IR-120H^+ resin, the solids were removed by filtration, the filtrate concentrated and the residue recrystallized from ethyl acetate–petroleum ether (4:1) to afford an inseparable mixture of diastereomers **35** as a solid (98 mg, 89%, 1:1 by ^1H NMR). Signals for the two diastereomers could be distinguished in the ^1H NMR spectrum of the mixture, and assigned with the aid of COSY and HSQC experiments: *Diastereomer* **35a**: δ_H (300 MHz; CD_3OD) 4.90 (1H, d, *J* = 3.3 Hz, H-1), 3.92 (1H, m, H-2″), 3.81–3.57 (4H, m, H-1′, H-5, H-6_a, H-6_b), 3.52 (1H, dd, *J* = 10.1, 9.0 Hz, H-3), 3.26 (1H, t, *J* = 9.0 Hz, H-4), 1.89–1.24 (13H, m, H-2, 5 × –CH_2–, cyclohexyl, H-$1''_a$, H-$1''_b$), 1.16 (3H, d, *J* = 6.0 Hz, H-3″); δ_C (75 MHz; CD_3OD) 99.07 (C-1), 75.78 (C-1′), 74.67 (C-3), 73.77 (C-5), 73.25 (C-4), 67.32 (C-2″), 63.05 (C-6), 44.95 (C-2), 38.46 (C-1″), 34.56, 32.33, 26.89, 25.06, 24.86 (5 × –CH_2–, cyclohexyl), 23.54 (C-3″); *Diastereomer* **35b**: δ_H (300 MHz; CD_3OD) 4.96 (1H, d, *J* = 3.6 Hz, H-1), 3.83 (1H, m, H-2″), 3.82–3.57 (4H, m, H-1′, H-5, H-6_a, H-6_b), 3.50 (1H, dd, *J* = 10.8, 9.1 Hz, H-3), 3.27 (1H, t, *J* = 9.1 Hz, H-4), 1.89–1.24 (13H, m, H-2, 5 × –CH_2–, cyclohexyl, H-$1''_a$, H-$1''_b$), 1.17 (3H, d, *J* = 6.3 Hz, H-3″); δ_C (75 MHz; CD_3OD) 98.49 (C-1), 75.46 (C-1′), 74.50 (C-3), 73.77 (C-5), 73.25 (C-4), 66.30 (C-2″), 63.05 (C-6), 44.87 (C-2), 38.87 (C-1″), 34.52, 32.33, 26.87, 24.98, 24.79 (5 × –CH_2–, cyclohexyl), 24.61 (C-3″); HRFABMS: *m/z* 327.177488 $(M+Na)^+$. Calcd for $C_{15}H_{28}O_6Na$ 327.178348 $(M+Na)^+$.

5.21. Cyclohexyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-*C*-(2″,3″-epoxypropyl)-α-D-glucopyranoside (36)

To a solution of **28a** (308 mg, 0.75 mmol) in CH_2Cl_2 (10 ml) at 0 °C was slowly added a solution of *m*-CPBA (70%, 0.32 g, 1.29 mmol) in CH_2Cl_2 (3.0 ml) (*m-CPBA was dissolved in CH_2Cl_2, dried with $MgSO_4$, filtered and concentrated before used*). The temperature was raised to room temperature and after stirring for 18 h, a further portion of *m*-CPBA (70%, 0.1 g, 0.40 mmol) in CH_2Cl_2 (2 ml) was added slowly to the mixture at 0 °C. The reaction was stirred for 6 h at room temperature, then saturated $Na_2S_2O_3$ added, the aqueous mixture extracted with CH_2Cl_2, and the CH_2Cl_2 washed with saturated $NaHCO_3$, dried over $MgSO_4$ and concentrated. The crude residue was purified by column chromatography using ethyl acetate–petroleum ether (1:4) as eluent to afford an inseparable mixture of diastereomers **36** (292 mg, 91%, 1:1 by ^1H NMR) as an oil. Signals for the two diastereomers could be

distinguished in the ^{1}H NMR spectrum of the mixture, and assigned with the aid of COSY and HSQC experiments: *Diastereomer* **36a**: δ_H (400 MHz; $CDCl_3$) 5.15 (1H, dd, J = 11.0, 9.4 Hz, H-3), 5.00 (1H, d, J = 3.2 Hz, H-1), 4.86 (1H, t, J = 9.4 Hz, H-4), 4.18–4.14 (1H, m, H-6$_a$), 4.03–3.96 (2H, m, H-5, H-6$_b$), 3.48 (1H, m, H-1′), 2.82 (1H, m, H-2″), 2.66 (1H, t, J = 5.0 Hz, H-3″$_a$), 2.36 (1H, dd, J = 5.0, 2.6 Hz, H-3″$_b$), 2.07 (1H, m, H-2), 1.98, 1.93, 1.92 (9H, 3 s, 3 × $COOCH_3$), 1.82–1.10 (12H, m, 5 × –CH_2–, cyclohexyl, H-1″$_a$, H-1″$_b$); δ_C (100 MHz; $CDCl_3$) 170.85, 170.83, 170.06 (3 × $COOCH_3$), 96.94 (C-1), 75.99 (C-1′), 72.41 (C-3), 70.23 (C-4), 67.85 (C-5), 62.66 (C-6), 49.97 (C-2″), 47.53 (C-3″), 42.39 (C-2), 33.51 (C-1″), 31.56, 30.69, 25.66, 24.27, 24.04 (5 × –CH_2–, cyclohexyl), 20.94, 20.78, 20.77 (3 × $COOCH_3$); *Diastereomer* **36b**: δ_H (400 MHz; $CDCl_3$) 5.15 (1H, dd, J = 11.0, 9.5 Hz, H-3), 5.04 (1H, d, J = 3.2 Hz, H-1), 4.87 (1H, t, J = 9.5 Hz, H-4), 4.18–4.14 (1H, m, H-6$_a$), 4.03–3.96 (2H, m, H-5, H-6$_b$), 3.48 (1H, m, H-1′), 2.82 (1H, m, H-2″), 2.62 (1H, t, J = 4.8 Hz, H-3″$_a$), 2.31 (1H, dd, J = 4.8, 2.6 Hz, H-3″$_b$), 2.07 (1H, m, H-2), 1.98, 1.93, 1.92 (9H, 3 s, 3 × $COOCH_3$), 1.82–1.10 (12H, m, 5 × –CH_2–, cyclohexyl, H-1″$_a$, H-1″$_b$); δ_C (100 MHz; $CDCl_3$) 170.75, 170.63, 170.11 (3 × $COOCH_3$), 97.69 (C-1), 76.68 (C-1′), 72.48 (C-3), 70.29 (C-4), 67.79 (C-5), 62.68 (C-6), 51.06 (C-2″), 46.82 (C-3″), 43.89 (C-2), 33.51 (C-1″), 31.52, 30.88, 25.71, 24.22, 24.00 (5 × –CH_2–, cyclohexyl), 20.94, 20.78, 20.77 (3 × $COOCH_3$); HRFABMS: *m/z* 451.195037 $(M+Na)^+$. Calcd for $C_{21}H_{32}O_9Na$ 451.194392 $(M+Na)^+$.

5.22. Cyclohexyl 2-deoxy-2-*C*-(2″,3″-epoxypropyl)-α-D-glucopyranoside (37) and cyclohexyl (2″*R*),(2″*S*)-3,3″-anhydro-2-deoxy-2-*C*-(2″,3″-dihydroxypropyl)-α-D-glucopyranoside (38)

Methanolic sodium methoxide (0.2 M, 0.3 ml, 0.06 mmol) was added to a solution of **36** (200 mg, 0.47 mmol) in CH_2Cl_2–MeOH (3:5 ml) at room temperature. After 6 h, water was added (1 ml) and the solvent evaporated to dryness. TLC indicated the formation of two products and separation was achieved by column chromatography using methanol–ethyl acetate (3:97) to obtain a diastereomeric mixture of epoxides **37** as an oil (71 mg, 50%, 4:1 by ^{1}H NMR) and a diastereomeric mixtures of alcohols **38** as crystals (60 mg, 42%, 1:1 by ^{1}H NMR). For compound **37** signals for each diastereomer could be distinguished in the ^{1}H NMR spectrum, and assigned with the aid of COSY and HSQC experiments. *Diastereomer* **37a**: δ_H (300 MHz; CD_3OD) 5.15 (1H, d, J = 3.0 Hz, H-1), 4.11 (1H, m, H-2″), 3.84–3.42 (8H, m, H-1′, H-3, H-3″$_a$, H-3″$_b$, H-4, H-5, H-6$_a$, H-6$_b$), 2.05–1.30 (13H, m, 5 × –CH_2–, cyclohexyl, H-2, H-1″$_a$, H-1″$_b$); δ_C (75 MHz; CD_3OD) 97.75 (C-1), 81.05 (C-1′), 80.40 (C-2″), 76.33 (C-3), 74.93 (C-4), 72.63 (C-5), 65.66 (C-3″), 62.32 (C-6), 47.92 (C-2), 34.50, 32.58 (2 × –CH_2–, cyclohexyl), 29.71 (C-1″), 26.82, 24.94, 24.76 (3 × –CH_2–, cyclohexyl); *Diastereomer* **37b**: δ_H (300 MHz; CD_3OD) 5.14 (1H, d, J = 3.0 Hz, H-1), 4.11 (1H, m, H-2″), 3.84–3.42 (8H, m, H-1′, H-3, H-3″$_a$, H-3″$_b$, H-4, H-5, H-6$_a$, H-6$_b$), 2.05–1.30 (13H, m, 5 × –CH_2–, cyclohexyl, H-2, H-1″$_a$, H-1″$_b$); δ_C (75 MHz; CD_3OD) 97.87 (C-1), 81.96 (C-1′), 80.15 (C-2″), 76.33 (C-3), 74.83 (C-4), 72.52 (C-5), 65.84 (C-3″), 62.35 (C-6), 46.65 (C-2), 34.50, 32.58 (2 × –CH_2–, cyclohexyl), 29.14 (C-1″), 26.82, 24.94, 24.76 (3 × –CH_2–, cyclohexyl); HRFABMS: *m/z* 325.162227 $(M+Na)^+$. Calcd for $C_{15}H_{26}O_6Na$ 325.162699 $(M+Na)^+$. Compound **38**: δ_H (400 MHz; CD_3OD) 5.04 (1H, d, J = 3.2 Hz, H-1), 3.80–3.60 (4H, m, H-1′, H-5, H-6$_a$, H-6$_b$), 3.50 (1H, dd, J = 10.8, 8.8 Hz, H-3), 3.26 (1H, dd, J = 9.2, 8.8 Hz, H-4), 3.07–3.00 (1H, m, H-2″), 2.77 (1H, dd, J = 5.0, 4.0 Hz, H-3″$_a$), 2.51 (1H, d, J = 5.0, 2.8 Hz, H-3″$_b$), 1.95–1.24 (13H, m, 5 × –CH_2–, cyclohexyl, H-2, H-1″$_a$, H-1″$_b$); δ_C (100 MHz; CD_3OD) 96.88 (C-1), 74.51 (C-1′), 72.77 (C-3), 72.71 (C-5), 72.01 (C-4), 61.84 (C-6), 50.42 (C-2″), 47.51 (C-3″), 44.07 (C-2), 33.40, 31.27 (2 × –CH_2–, cyclohexyl), 30.56 (C-1″), 25.68, 23.95, 23.77 (3 × –CH_2–, cyclohexyl); HRFABMS: *m/z* 325.162227 $(M+Na)^+$. Calcd for $C_{15}H_{26}O_6Na$ 325.162699 $(M+Na)^+$.

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