

# Synthesis of sulfoquinovose and sulfoquinovosyl diacylglycerides, and a fluorogenic substrate for sulfoquinovosidases

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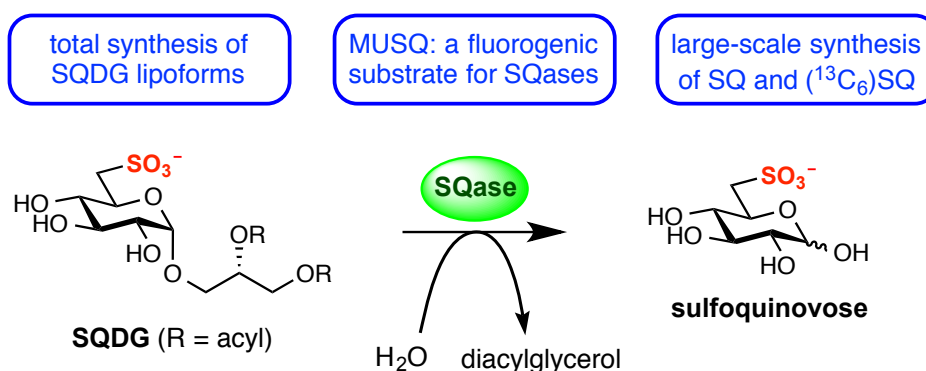
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Graphical abstract:

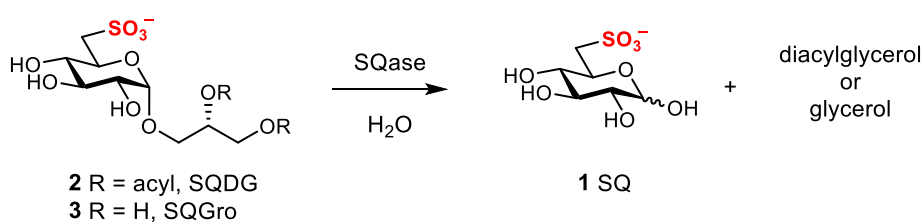


**Abstract:**

The sulfolipid sulfoquinovosyl diacylglycerol (SQDG) and its headgroup, the sulfosugar sulfoquinovose (SQ), are estimated to harbour up to half of all organosulfur in the biosphere. SQ is liberated from SQDG and related glycosides by the action of sulfoquinovosidases (SQases). We report a 10-step synthesis of SQDG that we apply to the preparation of saturated and unsaturated lipofoms. We also report an expeditious synthesis of SQ and (<sup>13</sup>C<sub>6</sub>)SQ, and X-ray crystal structures of sodium and potassium salts of SQ. Finally, we report the synthesis of a fluorogenic SQase substrate, methylumbelliferyl α-D-sulfoquinovoside, and examination of its cleavage kinetics by two recombinant SQases.

## Introduction:

The sulfosugar sulfoquinovose (SQ, **1**) is produced by photosynthetic organisms at a rate of 10 billion tonnes annually (Figure 1).<sup>1</sup> Approximately half of all organosulfur in terrestrial biomass is estimated to reside within SQ and consequently SQ metabolism comprises a significant arm of the global biogeochemical sulfur cycle. SQ is rarely found as the free sugar and is mainly present as the head group of the plant and cyanobacterial sulfolipid sulfoquinovosyl diacylglycerol (SQDG, **2**) (Figure 1).<sup>2,3</sup> SQ is liberated from SQDG (and its delipidated form sulfoquinovosyl glycerol, SQGro, **3**) by the action of specialized glycoside hydrolases known as sulfoquinovosidases (Figure 1).<sup>4,5</sup>



**Fig. 1** Sulfoquinovosyl diacylglycerol (SQDG) and sulfoquinovosyl glycerol (SQGro) undergo sulfoquinovosidase (SQase) catalysed hydrolysis to sulfoquinovose (SQ) and the corresponding aglycon.

The biosynthesis of SQDG has been well-characterized in plants, photosynthetic protists and cyanobacteria, and involves synthesis of the sugar nucleotide UDP-SQ from UDP-Glc and its transfer to diacylglycerol.<sup>2</sup> In the last five years two pathways for catabolism of SQ, a process known as sulfoglycolysis,<sup>6</sup> have been identified in bacteria. Both pathways involve the initial cleavage of SQ glycosides by SQases, which are typically encoded within sulfoglycolytic operons. The two pathways can be considered variants of classical glycolytic pathways and are termed the sulfoglycolytic Entner-Doudoroff<sup>7</sup> and the sulfoglycolytic Embden-Meyerhof-Parnas<sup>8</sup> pathways. Bioinformatics analysis suggests that sulfoglycolysis plays important roles in sulfur-cycling in complex microbial ecosystems, including those in soils, the gut and the ocean.

Our knowledge of the biochemical properties of enzymes involved in SQDG degradation and sulfoglycolysis remains in its infancy. Little is known about the broader metabolic consequences of sulfoglycolysis on cellular metabolism.<sup>9</sup> Such studies could benefit new methods for the preparation of SQDG and from large-scale methods for the synthesis of SQ and <sup>13</sup>C-labelled isotopologues. As the operons encoding the sulfo-ED and sulfo-EMP

pathways typically encode an SQase, detection of the activity of this highly specific class of enzyme<sup>4,5</sup> can therefore be considered a marker for the possible presence of a sulfoglycolytic pathway. Consequently, sensitive substrates for SQases that can detect enzymatic activity in cell lysates could allow study of expression of sulfoglycolytic pathways and support the untargeted discovery of new sulfoglycolytic organisms.

Here we disclose a new synthesis of SQDG, the shortest yet reported, enabling the synthesis of saturated and unsaturated lipofoms. We disclose an improved method for the synthesis of SQ and (<sup>13</sup>C<sub>6</sub>)SQ, facilitated by the identification of a crystalline sodium salt. We also report the first single-crystal X-ray structures of SQ. Finally, as part of an effort to develop sensitive fluorogenic substrates for SQase, we report the synthesis and kinetic characterization of the methylumbelliferyl glycoside of SQ and its kinetic parameters for two recombinant SQases.

## Results and Discussion:

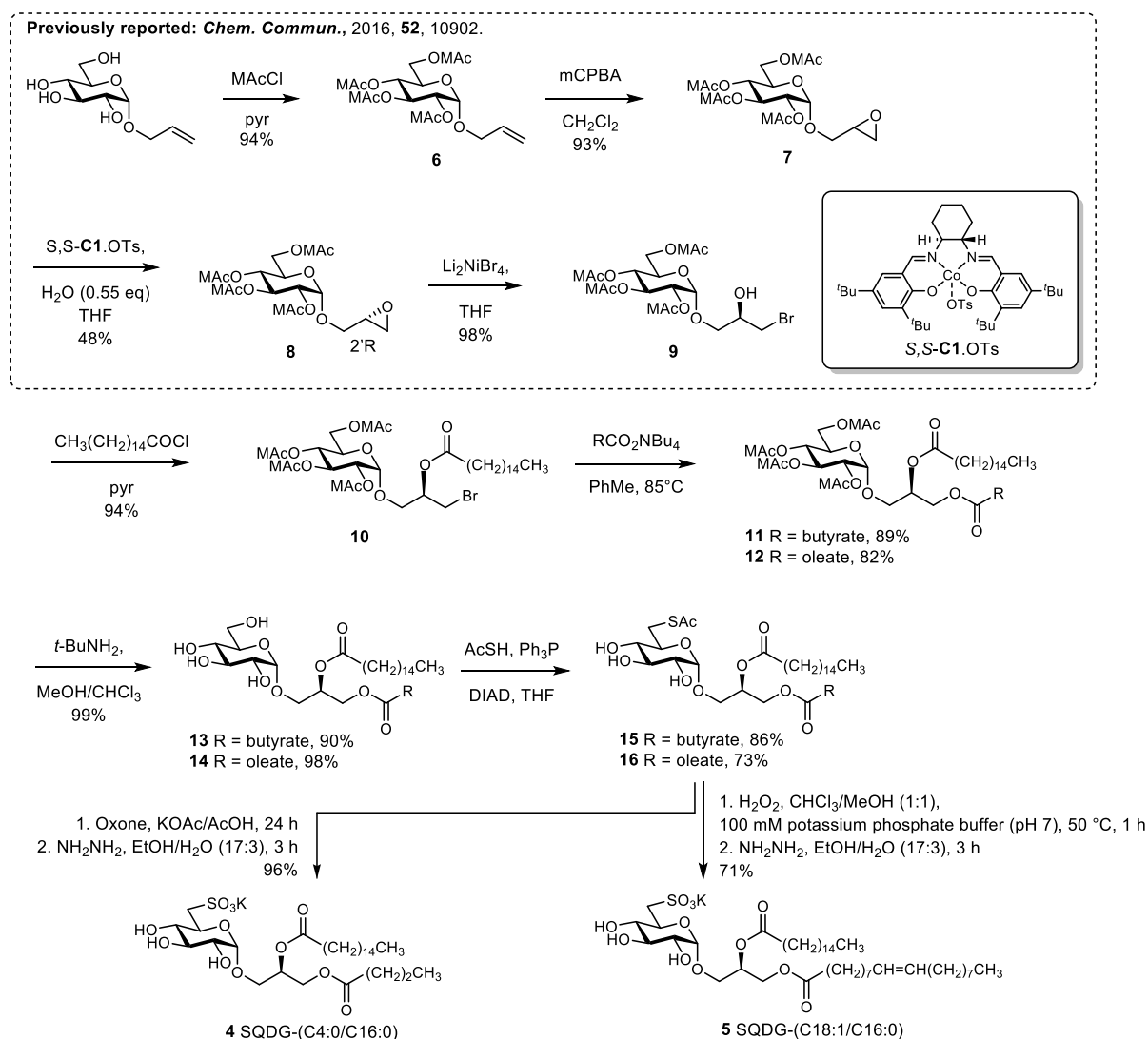
### 1. Synthesis of SQDG

Three approaches have been reported for the synthesis of SQDG as single diastereoisomers but all are lengthy and involve multiple protecting group interchanges. Gigg and co-workers developed a seventeen-step route,<sup>10</sup> and two independent fourteen-step routes have been disclosed by Danishefsky<sup>11</sup> and by Hanashima and co-workers.<sup>12</sup> All three approaches involve the glycosylation of a glycerol moiety but owing to the nature of the protecting groups, only the approach of Danishefsky provides access to unsaturated lipofoms. Hanashima and co-workers subsequently developed an alternative approach involving dihydroxylation of an allyl  $\alpha$ -D-glucoside; this method, while more direct, affords SQDG as a mixture of diastereomers epimeric at C2 of the glycerol moiety.<sup>13</sup> We have previously developed an efficient approach to synthesis of unsaturated  $\alpha$ -glucosyl diglycerides through a non-stereoselective epoxidation of allyl  $\alpha$ -D-glucoside followed by a Jacobsen hydrolytic kinetic resolution that delivers a 2'*R*-glyceryl  $\alpha$ -D-glucoside.<sup>14</sup> We proposed to apply this approach to synthesize a naturally occurring lipofom of SQDG-(sn1/sn2), SQDG-(C18:1/C16:0) **5**<sup>4</sup> as well as the simplified analogue SQDG-(C4:0/C16:0) **4**.

The first four steps proceeded in an identical fashion to that originally reported<sup>14</sup> and are reproduced here for completeness (Figure 2): (1) methoxyacetylation of allyl  $\alpha$ -glucopyranoside ( $\rightarrow$  **6**); (2) oxidation with mCPBA to afford epoxide **7** as a mixture of 2'-epimers; (3) Jacobsen hydrolytic kinetic resolution of **7** using *S,S*-C1.OTs to afford 2'*R*-epoxide **8** (and the 2'*S*-diol, not shown); and (4) ring-opening of the epoxide **8** to the bromohydrin **9** using Li<sub>2</sub>NiBr<sub>4</sub>.

Towards the SQDG target, we next treated bromohydrin **9** with palmityl chloride afforded the bromo-ester **10**. Nucleophilic substitution of the bromide of **10** using the tetrabutylammonium salts of butyric and oleic acids in toluene<sup>14</sup> gave the diglycerides **11** and **12**, respectively. Deprotection using tBuNH<sub>2</sub>/MeOH<sup>15</sup> afforded the glucosyl diglycerides **13** and **14**. A thioacetate was introduced at C6 using a Mitsunobu reaction with AcSH and DIAD/Ph<sub>3</sub>P to give the thioacetates **15** and **16**. Oxidation of the thioacetate group of **15** was achieved using Oxone buffered with KOAc/AcOH.<sup>16</sup> This provided the target SQDG analogue, contaminated with a less polar material tentatively assigned as the 4-*O*-acetyl-SQDG derivative. Careful deacetylation was achieved using 0.04 mM hydrazine in EtOH/H<sub>2</sub>O to give SQDG-(C4:0/C16:0) **4**. Oxidation of the thioacetate **16** proved more challenging. Oxidation with Oxone under the same conditions used for **15** resulted in epoxidation of the alkene in

addition to oxidation to the sulfonate. After considerable experimentation we established an alternative protocol for oxidation of **16** using H<sub>2</sub>O<sub>2</sub> in potassium phosphate buffer (pH 7). Again, contamination with the 4-*O*-acetyl SQDG was observed but was readily overcome by deacetylation with 0.04 mM hydrazine in EtOH/H<sub>2</sub>O to afford SQDG-(C18:1/C16:0) **5**.



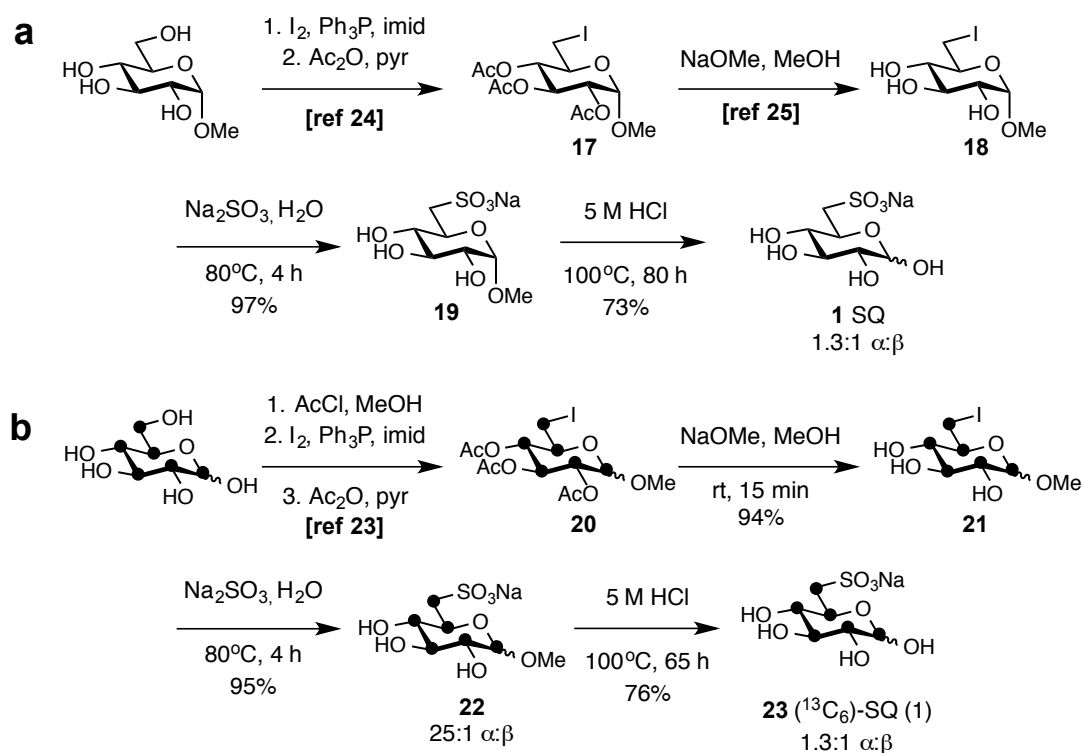
**Fig. 2** Synthesis of sulfoquinovosyl diacylglycerol (SQDG).

## 2. Synthesis of SQ (I)

Multiple publications have reported the synthesis of SQ. However, most reported methods can trace their roots to early work by either Ohle and Mertens<sup>17</sup> or Helferich and Ost.<sup>18</sup> Ohle and Mertens utilized 1,2-*O*-isopropylidene- $\alpha$ -D-glucopyranose as starting material and introduced divalent sulfur at C6, which was then oxidized to the sulfonate, followed by deprotection. Subsequent workers have modified this procedure to directly introduce the sulfonate moiety

using a sulfite substitution reaction.<sup>17, 19, 20</sup> Helferich and Ost used methyl  $\alpha$ -D-glucopyranoside as a substrate and introduced a sulfonate directly by substitution with sulfite.<sup>18, 21</sup> In early work crystalline potassium<sup>17, 22</sup> and sodium salts,<sup>18</sup> were identified. However, subsequent studies did not take advantage of these isolation methods; in some cases there was either no purification of the final SQ isolated,<sup>19-21</sup> or it was purified through tedious methods involving interconversion of toxic salts.<sup>22</sup> We have explored an alternative approach to SQ that involved introduction of sulfur into methyl  $\alpha$ -D-glucopyranoside as a thioacetate and then oxidation to the sulfonate.<sup>23</sup> This approach involved use of acetate protecting groups throughout most of the sequence to allow normal-phase chromatography of advanced intermediates, and of SQ sodium salt, an approach that we successfully applied to the synthesis of (<sup>13</sup>C<sub>6</sub>)SQ.<sup>23</sup> However, this route is long, and purification of the final SQ product via chromatography proved challenging to scale up. Here we have revisited the method of Helferich and Ost, and report a streamlined synthesis of SQ that involves just two chromatographic steps and direct crystallization of sodium salt of SQ, which we also use for the synthesis of (<sup>13</sup>C<sub>6</sub>)SQ.

Our approach commenced with methyl  $\alpha$ -D-glucopyranoside, which was converted to the acetylated iodide **17** using the method of Garegg and Samuelsson (Figure 3a).<sup>24</sup> Zemplen deacetylation using NaOMe/MeOH afforded the known iodide **18**.<sup>25</sup> The sulfonate group was introduced by nucleophilic substitution using sodium sulfite to give the methyl glycoside **19**. Hydrolysis using aqueous HCl afforded crude salt of SQ **1**, which crystallized upon prolonged storage. With access to seed crystals we were able to develop a reliable crystallization of the sodium salt of SQ from MeOH/H<sub>2</sub>O on a multigram scale in 73% yield.



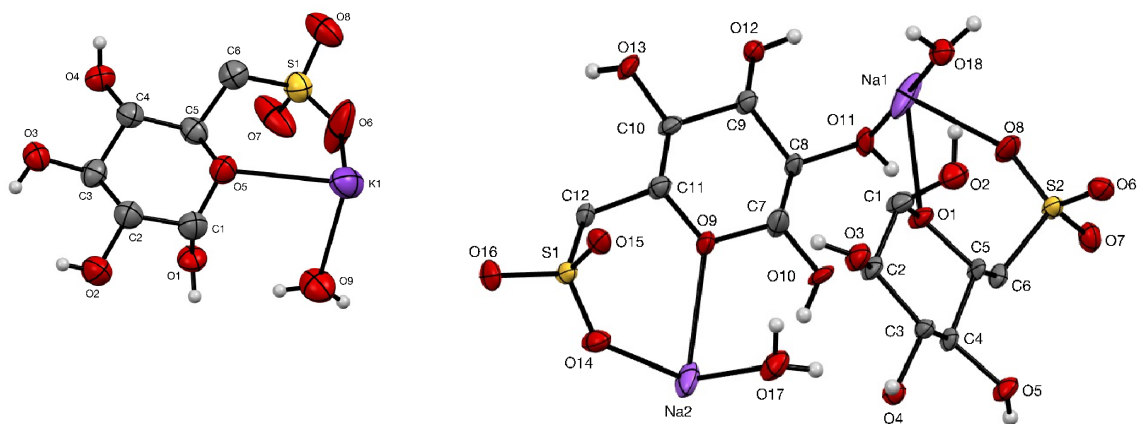
**Fig. 3** (a) Synthesis of sulfoquinovose (SQ) and (b) synthesis of ( $^{13}\text{C}_6$ )-sulfoquinovose ( $(^{13}\text{C}_6)\text{SQ}$ ). ● =  $^{13}\text{C}$ .

This approach was applied to the synthesis of ( $^{13}\text{C}_6$ )SQ. D-( $^{13}\text{C}_6$ )glucose was converted to the acetylated iodide **20** as previously reported (Figure 3b).<sup>23</sup> Deacetylation using NaOMe/MeOH gave the iodide **21** and the sulfonate group was introduced by sulfite substitution to produce **22**. Hydrolysis of **22** under acidic conditions, followed by crystallization from MeOH/H<sub>2</sub>O afforded ( $^{13}\text{C}_6$ )SQ **23** as the sodium salt. This route consists of five chemical steps from D-( $^{13}\text{C}_6$ )glucose and involves three chromatographic purifications, an improvement over our previous method.<sup>23</sup>

The acquisition of several crystalline forms of SQ prompted us to determine their single crystal X-ray structure of this compound. SQ potassium salt (prepared by ion-exchange) crystallizes as a monohydrate that forms a co-crystal consisting of the  $\alpha$ - and  $\beta$ -anomers in the ratio 0.70(2): 0.30(2) respectively, which occupy the same crystallographic site in the crystal, with one molecule in the asymmetric unit. The structure is shown in Figure 4 (left) and shows the major  $\alpha$ -anomer form. Molecules in the crystal array are extensively crosslinked by coordination of the sulfonate, hemiacetal and hydroxyl oxygens to the potassium counterion. SQ sodium salt also crystallises as a monohydrate with two independent molecules in the asymmetric unit linked by coordination to one of the two independent sodium counterions. One



of the molecules (defined by carbon atoms C1-C6) exists entirely as the  $\alpha$ -anomer, while the second molecule (defined by carbon atoms C7-C12) exists as a mixture of  $\alpha$ - and  $\beta$ -anomers in the ratio 0.34(1):0.66(1), respectively, at the same crystallographic site in the crystal. The structure is presented in Figure 4 (right) and shows the major  $\beta$ -anomer at the variable site. As for the potassium salt, the sodium salt structure is extensively crosslinked by coordination of sulfonate, hemiacetal and hydroxyl oxygens to the sodium counterion.



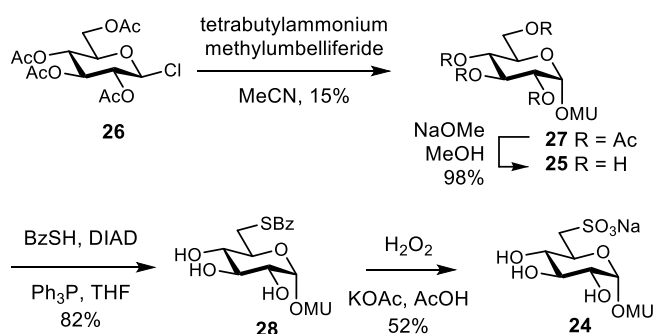
**Fig. 4** (Left) Thermal ellipsoid plot of sulfoquinovose potassium salt. The minor  $\beta$ -anomer which exists at the same crystallographic site is not shown for clarity. (Right) Thermal ellipsoid plot of sulfoquinovose sodium salt. The minor  $\alpha$ -anomer for the LHS sugar, which exists at the same crystallographic site is not shown for clarity purposes.

### 3. A fluorogenic substrate for SQases

Aryl glycosides are widely used as substrates for glycosidases due to their potential to release coloured, chromophoric or fluorescent phenol/phenolate dyes. We have previously reported that PNPSQ is an effective substrate for SQases, allowing real-time measurement of enzyme rates in a UV/Vis spectrophotometer.<sup>4, 5</sup> However, chromogenic substrates have limited utility in crude cell lysates where background absorption and low rates provide poor sensitivity. 4-Methylumbelliferyl glycosides are widely used as sensitive substrate for a range of glycosidases. For this reason we set out to synthesize methylumbelliferyl  $\alpha$ -sulfoquinovoside (MUSQ) **24**. Our approach sought to first prepare methylumbelliferyl  $\alpha$ -glucoside (MUGlc) **25**, then to introduce the sulfonate group at the 6-position.

The synthesis of MUGlc has been reported in the peer-reviewed<sup>26</sup> and patent literature<sup>27, 28</sup> but all methods suffer from low yields. We report a new method for its

preparation via the  $\beta$ -chloride **26**.<sup>29</sup> Nucleophilic substitution of **26** using the tetrabutylammonium salt of methylumbelliferone in MeCN afforded the tetraacetate **27** in modest but reproducible yield of 15%. While low, this represents an improvement on the literature approach, which reports just 10% yield.<sup>26</sup> Deacetylation under Zemplen conditions gave **25**.



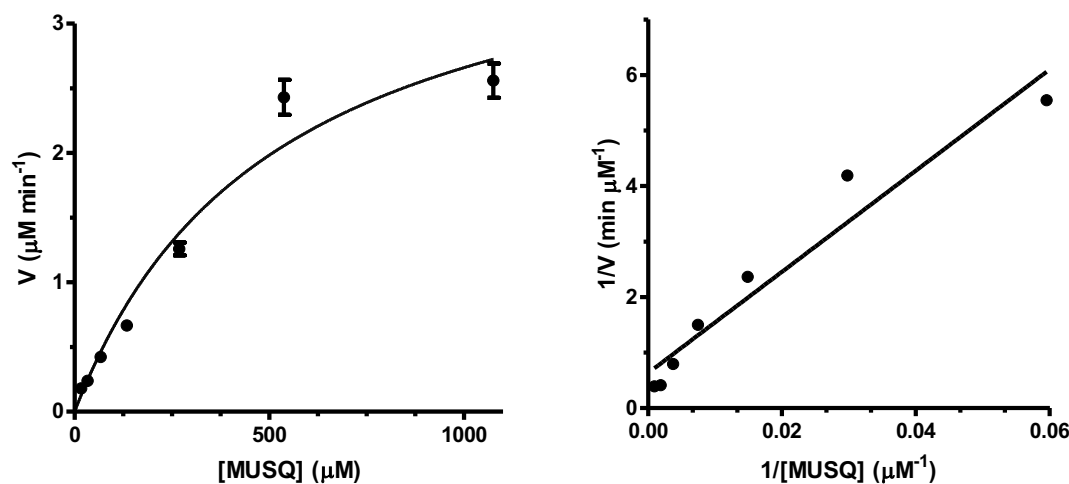
**Fig. 5** Synthesis of methylumbelliferyl  $\alpha$ -sulfoquinovoside (MUSQ).

Introduction of the sulfonate into **25** required some exploration. Initially, treatment of MUGlc with CBr<sub>4</sub>/Ph<sub>3</sub>P under Appel conditions afforded the 6-bromide in good yield. However, attempted substitution with Na<sub>2</sub>SO<sub>3</sub> in H<sub>2</sub>O led to cleavage of the glycoside. Instead, Mitsunobu reaction of **25** using BzSH and DIAD/Ph<sub>3</sub>P afforded the thiobenzoate, which was cleanly oxidized using H<sub>2</sub>O<sub>2</sub> buffered with KOAc/AcOH. Chromatography, followed by recrystallization afforded the potassium salt of MUSQ **24**.

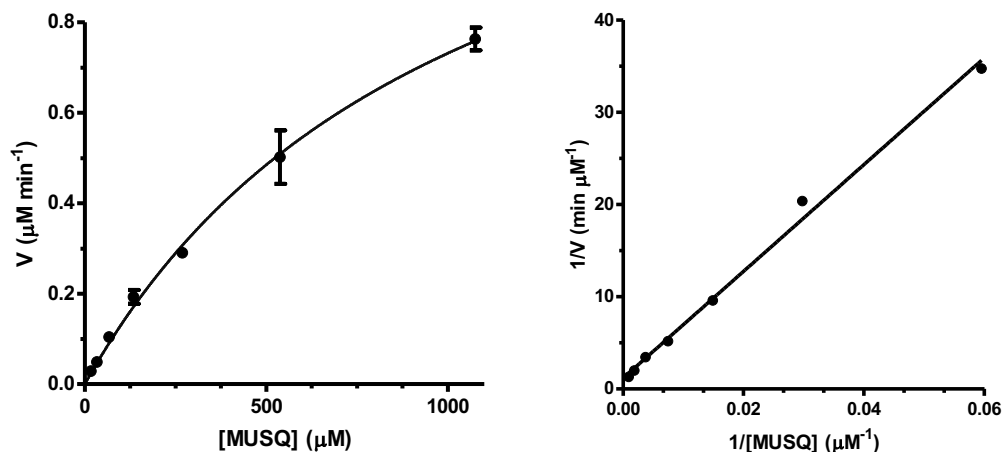
In order to explore the potential for use of MUSQ as a fluorogenic substrate for SQase, we measured the kinetic parameters for cleavage of MUSQ by two recombinant SQase, *Ec*YihQ from *Escherichia coli* and the sulfoquinovosidase *At*SQase (NCBI accession WP\_035199431) from *Agrobacterium tumefaciens*.<sup>5</sup> We used a stopped assay involving incubation of varying concentrations of MUSQ with each SQase over a period in which rates were linear, and then quenching of the reaction with a high pH stop buffer, followed by measurement of fluorescence, which was quantified by reference to a calibration curve. Michaelis-Menten and Lineweaver-Burk plots are shown in Figure 6. In both cases limited saturation was observed up to 1 mM MUSQ preventing accurate determination of  $K_M$  and  $V_{max}$  values. However,  $k_{cat}/K_M$  values could be accurately determined and are shown in Table 1 versus the equivalent values for PNPSQ with the same enzymes. This data reveals that MUSQ is approximately 10<sup>4</sup>–10<sup>5</sup>-fold poorer as a substrate for these two SQases. This data is unexpected given that the p*K*<sub>a</sub> values of 4-nitrophenol (7.15) and 4-methylumbelliferone (7.79) are similar, and so should have similar nucleofugacity. Possibly, the large differences in  $k_{cat}/K_M$

values between PNPSQ and MUSQ arise from the geometry of the active site. The active site of SQases have evolved to accommodate the aglycon of the natural substrate SQDG, a slender glycerol/diacylglyceride, meaning that the active site may have difficulty accommodating the more sterically demanding bicyclic aromatic system of methylumbelliferone. This interesting observation is, however, disappointing as the low  $k_{cat}/K_M$  values means that MUSQ is unlikely to be suitable as a sensitive substrate for SQases.

### A YihQ from *E. coli*



### B AtSQase from *A. tumefaciens*



**Fig. 6** Michaelis-Menten (left) and Lineweaver-Burk plots for MUSQ hydrolysis by (A) YihQ from *E. coli* and (B) SQase from *A. tumefaciens*.

**Table 1.** Kinetic parameters for MUSQ and PNPSQ cleavage by sulfoquinovosidases.

Enzyme	$k_{cat}/K_M$ for MUSQ ( $\text{M}^{-1} \text{s}^{-1}$ )	$k_{cat}/K_M$ for PNPSQ ( $\text{M}^{-1} \text{s}^{-1}$ ) (Ref. 6)
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<i>EcYihQ</i>	$(1.07 \pm 0.21) \times 10^1$	$(1.05 \pm 0.10) \times 10^5$
<i>AtSQase</i>	$1.97 \pm 0.01$	$(2.18 \pm 0.18) \times 10^5$

## Conclusion

As major species within the biogeochemical sulfur cycle, the biosynthesis and catabolism of SQDG and its headgroup SQ, are of considerable interest. SQDG can only be obtained from natural sources as mixtures of lipofoms, and consequently there is a need for streamlined methods for its synthesis. The route described herein is compatible for the preparation of both saturated and unsaturated lipofoms and at 10-steps is considerably shorter than previously reported methods. Practical access to large quantities of SQ and the ( $^{13}\text{C}_6$ ) isotopologue, facilitated by the identification of a readily crystallized sodium salt, will support future metabolic studies where this compound is used as a carbon source for microbial culture, as well as metabolomic studies of the effects of sulfoglycolysis on cellular metabolism. Finally, we report the first efforts to develop fluorogenic substrates of SQases. Disappointingly, MUSQ lacks the reactivity necessary to support its use in crude cell extracts, possibly as a result of the preference of SQases for elongated glycolipid substrates. Future design of fluorogenic SQase substrates will need to overcome the tendency of these enzymes to discriminate against bulky aglycons.

## Experimental

### General

Pyridine was distilled over KOH before use. Dichloromethane and THF were dried over alumina according to the method of Pangborn *et al.*<sup>30</sup> Reactions were monitored using thin layer chromatography (TLC), performed with silica gel 60 F<sub>254</sub>. Detection was effected by charring in a mixture of 5% sulfuric acid in methanol, ceric ammonium molybdate, and/or with UV light. Flash chromatography was performed according to the method of Still *et al.*<sup>31</sup> using silica gel 60. Melting points were determined by a capillary apparatus.  $[\alpha]_D$  values are given in deg 10<sup>-1</sup> cm<sup>2</sup> g<sup>-1</sup>. NMR experiments were conducted on 400 and 500 MHz instruments, with chemical shifts referenced relative to residual protiated solvent and in parts per million (ppm). <sup>1</sup>H–<sup>1</sup>H COSY and HMSC spectra were used to confirm proton and carbon assignments, respectively. Mass spectra were acquired in the ESI-QTOF mode.

### (2'*R*)-3'-Bromo-2'-palmitoyloxypropyl 2,3,4,6-tetra-*O*-methoxyacetyl- $\alpha$ -D-glucopyranoside (**10**)

Palmitoyl chloride (0.133 g, 0.484 mmol) was added to a stirred solution of (2'*R*)-3'-bromo-2'-hydroxypropyl 2,3,4,6-tetra-*O*-methoxyacetyl- $\alpha$ -D-glucopyranoside **9**<sup>14</sup> (0.150 g, 0.248 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (5 mL) and pyridine (0.20 mL, 0.248 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirring was continued overnight. The reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub>, washed sequentially with water, sat. aq. CuSO<sub>4</sub> and sat. aq. NaHCO<sub>3</sub>, then dried (MgSO<sub>4</sub>). The solvent was evaporated under reduced pressure and flash chromatography of the residue (pet. ether to 1:1 EtOAc/pet. ether) afforded **10** as colourless oil (0.201 g, 94%).  $[\alpha]_D^{20}$  +64.9° (*c* 0.217, EtOAc); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  0.81 (3 H, t, *J* 6.7 Hz, CH<sub>2</sub>CH<sub>3</sub>), 1.14–1.29 (26 H, m, alkyl), 1.52–1.62 (2 H, m,  $\beta$ -CH<sub>2</sub>), 2.26–2.32 (2 H, m,  $\alpha$ -CH<sub>2</sub>), 3.33, 3.34, 3.36, 3.38 (4  $\times$  3 H, 4 s, CH<sub>3</sub>O), 3.46 (1 H, dd, *J*<sub>1',2'</sub> 4.7, *J*<sub>1',1''</sub> 10.6 Hz, H1'), 3.54 (1 H, dd, *J*<sub>1',2'</sub> 6.2, *J*<sub>1',1''</sub> 10.7 Hz, H1'), 3.67 (1 H, dd, *J*<sub>2',3'</sub> 4.9, *J*<sub>3',3''</sub> 10.8 Hz, H3'), 3.84 (1 H, dd, *J*<sub>2',3'</sub> 4.7, *J*<sub>3',3''</sub> 10.9 Hz, H3'), 3.90–4.02 (4  $\times$  2 H, 4 m, 4  $\times$  CH<sub>3</sub>OCH<sub>2</sub>), 4.02–4.06 (1 H, m, H5), 4.13 (1 H, dd, *J*<sub>5,6a</sub> 1.8, *J*<sub>6a,6b</sub> 12.3 Hz, H6a), 4.33 (1 H, dd, *J*<sub>5,6b</sub> 4.3, *J*<sub>6a,6b</sub> 12.4 Hz, H6b), 4.94 (1 H, dd, *J*<sub>1,2</sub> 3.8, *J*<sub>2,3</sub> 10.1 Hz, H2), 5.03–5.13 (3 H, m, H1,2',4), 5.47 (1 H, t, *J*<sub>2,3</sub> = *J*<sub>3,4</sub> 9.7 Hz, H3); <sup>13</sup>C{<sup>1</sup>H} NMR (CDCl<sub>3</sub>, 100 MHz)  $\delta$  14.1 (CH<sub>2</sub>CH<sub>3</sub>), 22.7, 24.0, 29.1, 29.2, 29.3, 29.4, 29.58, 29.62, 29.7, 29.8, 31.9, 34.1 (alkyl-CH<sub>2</sub>), 59.3, 59.37, 59.39, 59.40 (4 C, OCH<sub>3</sub>), 61.6, 67.27, 68.30, 69.25, 69.30, 69.4, 70.35, 70.39, 70.6, 96.1 (C1), 169.2, 169.4,

169.5, 169.9 (4 C, MeOCH<sub>2</sub>C=O), 172.7 (sn2-CO<sub>2</sub>); HRMS (ESI<sup>+</sup>) calcd for C<sub>37</sub>H<sub>63</sub>BrO<sub>16</sub>Na<sup>+</sup> (M+Na) 865.3196. Found 865.3402.

**(2'R)-1'-O-Butyl-2'-O-palmitoyl-glyceryl 2,3,4,6-tetra-O-methoxyacetyl- $\alpha$ -D-glucopyranoside (11)**

Tetrabutylammonium hydroxide solution in H<sub>2</sub>O (1.5 M, 2 mL, 3.05 mmol) was added to a suspension of butyric acid (0.291 g, 3.3 mmol) in H<sub>2</sub>O. The resulting mixture was vigorously stirred at rt overnight. The solvent was evaporated and the crude residue was co-evaporated with toluene several times to give the tetrabutylammonium salt of oleic acid. A mixture of tetrabutylammonium butyrate (0.055 g, 0.180 mmol) and **10** (0.134 g, 0.159 mmol) in toluene (1 mL) was heated to 85 °C and stirred vigorously for 4 h. The solvents were evaporated and flash chromatography of the residue (CH<sub>2</sub>Cl<sub>2</sub> to 5:95 MeOH/CH<sub>2</sub>Cl<sub>2</sub>) afforded **11** as a colourless oil (0.120 g, 89%). [ $\alpha$ ]<sub>D</sub><sup>20</sup> +61.6° (*c* 0.27, EtOAc); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  0.86 (3 H, t, *J* 6.6 Hz, sn2-CH<sub>2</sub>CH<sub>3</sub>), 0.94 (3 H, t, *J* 7.4 Hz, sn1-CH<sub>2</sub>CH<sub>3</sub>), 1.13–1.33 (24 H, m, alkyl), 1.56–1.68 (4 H, m,  $\beta$ -CH<sub>2</sub>), 2.25–2.34 (4 H, m,  $\alpha$ -CH<sub>2</sub>), 3.38, 3.39, 3.41, 3.44 (4  $\times$  3 H, 4 s, CH<sub>3</sub>O), 3.62 (1 H, dd, *J*<sub>2',3'</sub> 5.4, *J*<sub>3',3''</sub> 11.1 Hz, H3'), 3.79 (1 H, dd, *J*<sub>2',3'</sub> 4.5, *J*<sub>3',3''</sub> 11.1 Hz, H3'), 3.92–4.09 (8 H, m, CH<sub>3</sub>OCH<sub>2</sub>), 4.04–4.07 (1 H, m, H5), 4.10–4.20 (2 H, m, H1a',6a), 4.29–4.40 (2 H, m, H1b',6b), 4.96 (1 H, dd, *J*<sub>1,2</sub> 3.7, *J*<sub>2,3</sub> 10.1 Hz, H2), 5.09–5.23 (3 H, m, H1,2',4), 5.51 (1 H, t, *J*<sub>2,3</sub> = *J*<sub>3,4</sub> 9.7 Hz, H3); <sup>13</sup>C {<sup>1</sup>H} NMR (CDCl<sub>3</sub>, 100 MHz)  $\delta$  13.8, 14.2 (2  $\times$  CH<sub>2</sub>CH<sub>3</sub>), 18.5, 22.8, 25.0, 29.3, 29.4, 29.5, 29.55, 29.62, 29.77, 29.81, 32.0, 34.3, 36.0, 59.5, 59.6 (4 C, OCH<sub>3</sub>), 61.7, 62.0, 66.9, 67.0, 67.4, 68.5, 69.3, 69.4, 69.5, 69.6, 69.8, 70.5, 70.7, 96.2 (C1), 169.2, 169.6, 169.7, 170.1 (4 C, MeOCH<sub>2</sub>C=O), 172.9, 173.0 (sn1-CO<sub>2</sub>, sn2-CO<sub>2</sub>); HRMS (ESI<sup>+</sup>) calcd for C<sub>41</sub>H<sub>70</sub>O<sub>18</sub>Na<sup>+</sup> (M+Na) 873.4459. Found 873.4451.

**(2'R)-1'-O-Butyl-2'-O-palmitoyl-glyceryl  $\alpha$ -D-glucopyranoside (13)**

A solution of t-butylamine (0.154 mL, 1.48 mmol) and **11** (0.120 g, 0.141 mmol) in CHCl<sub>3</sub> (0.32 mL) and MeOH (0.8 mL) was stirred at 0 °C for 10 min and then at 10 °C for 3 h at which time tlc indicated that the starting material was completely consumed. The solvents were evaporated under high vacuum without heating. Flash chromatography of the residue (pet. ether to EtOAc/pet. ether 50:50) afforded **13** as a white semisolid (0.071 g, 90%). [ $\alpha$ ]<sub>D</sub><sup>20</sup> +67.6° (*c* 0.857, MeOH); <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz)  $\delta$  0.92 (3 H, t, *J* 6.3 Hz, sn2-CH<sub>2</sub>CH<sub>3</sub>), 0.97 (3 H, t, *J* 7.4 Hz, sn1-CH<sub>2</sub>CH<sub>3</sub>), 1.26–1.42 (24 H, m, alkyl), 1.59–1.69 (4 H, m,  $\beta$ -CH<sub>2</sub>), 2.30–2.38 (4 H, m,  $\alpha$ -CH<sub>2</sub>), 3.36–3.37 (1 H, m, H3), 3.41 (1 H, dd, *J*<sub>2,3</sub> 2.9, *J*<sub>3,4</sub> 9.7 Hz, H4), 3.54–

3.72 (4 H, m, H<sub>2,5,6a,1a'</sub>), 3.78–3.82 (1 H, m, H<sub>6b</sub>), 3.89 (1 H, dd,  $J_{1b',2'}$  5.3,  $J_{1b',1a'}$  10.2 Hz, H<sub>1b'</sub>), 4.20 (1 H, dd,  $J_{2',3a'}$  6.1,  $J_{3a',3b'}$  11.5 Hz, H<sub>3a'</sub>), 4.48 (1 H, dd,  $J_{2',3'}$  2.3,  $J_{3',3'}$  12.0 Hz, H<sub>3b'</sub>), 4.81 (1 H, d, 3.3 Hz, H<sub>1</sub>), 5.23–5.30 (1 H, m, H<sub>2'</sub>); <sup>13</sup>C{<sup>1</sup>H} NMR (CDCl<sub>3</sub>, 100 MHz) δ 14.0, 23.7 (2 × CH<sub>2</sub>CH<sub>3</sub>), 19.4, 23.7, 26.0, 30.1, 30.2, 30.4, 30.5, 30.6, 30.8, 33.1, 35.1, 36.8 (alkyl-CH<sub>2</sub>, SCOCH<sub>3</sub>), 62.6, 63.8, 67.1, 71.58, 71.62, 73.4, 73.9, 74.9, 100.7 (C<sub>1</sub>), 174.7, 174.8 (sn1–CO<sub>2</sub>, sn2–CO<sub>2</sub>); HRMS (ESI<sup>+</sup>) calcd for C<sub>29</sub>H<sub>54</sub>O<sub>10</sub>Na<sup>+</sup> (M+Na) 585.3614. Found 585.3629.

**(2'R)-1'-O-Butyl-2'-O-palmitoyl-glyceryl 6-S-acetyl-6-deoxy-6-thio-α-D-glucopyranoside (15)**

A solution of triphenylphosphine (0.072 g, 0.279 mmol) and diethyl azodicarboxylate (0.044 mL, 0.279 mmol) in THF (10 mL) was prepared and held at 0 °C under nitrogen for 30 min. This solution was then transferred by cannula to a solution of thioacetic acid (0.0063 mL, 0.09 mmol) and **13** (0.050 g, 0.089 mmol) in THF (10 mL) at 0 °C under nitrogen. The resultant reaction mixture was stirred at 4 °C under nitrogen overnight. The solvents were evaporated and flash chromatography of the residue (CH<sub>2</sub>Cl<sub>2</sub> to 7:93 MeOH/CH<sub>2</sub>Cl<sub>2</sub>) afforded **15** as a pale-yellow oil (0.047 g, 86%). [ $\alpha$ ]<sup>20</sup><sub>D</sub> +60.4° (*c* 0.815, MeOH); <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz) δ 0.93 (3 H, t,  $J$  6.7 Hz, sn2-CH<sub>2</sub>CH<sub>3</sub>), 0.98 (3 H, t,  $J$  7.4 Hz, sn1-CH<sub>2</sub>CH<sub>3</sub>), 1.29–1.40 (20 H, m, alkyl), 1.60–1.72 (4 H, m, β-CH<sub>2</sub>), 2.31–2.39 (4 H, m, α-CH<sub>2</sub>), 2.36 (3 H, s, SAc), 2.96 (1 H, dd,  $J_{6b,5}$  8.6,  $J_{6b,6a}$  13.7 Hz, H<sub>6b</sub>), 3.17 (1 H, t,  $J_{2,3} = J_{3,4}$  9.2 Hz, H<sub>3</sub>), 3.42 (1 H, dd,  $J_{2,3}$  3.7,  $J_{3,4}$  9.7 Hz, H<sub>4</sub>), 3.54–3.67 (4 H, m, H<sub>2,5,6a,3a'</sub>), 3.88 (1 H, dd,  $J_{2',3b'}$  5.2,  $J_{3a',3b'}$  10.7 Hz, H<sub>3b'</sub>), 4.26 (1 H, dd,  $J_{1a',2'}$  6.5,  $J_{1a',1b'}$  12.0 Hz, H<sub>1a'</sub>), 4.46 (1 H, dd,  $J_{1b',2'}$  3.3,  $J_{1a',1b'}$  12.0 Hz, H<sub>1b'</sub>), 4.76 (1 H, d,  $J_{1,2}$  3.7 Hz, H<sub>1</sub>), 5.22–5.29 (1 H, m, H<sub>2'</sub>); <sup>13</sup>C{<sup>1</sup>H} NMR (CD<sub>3</sub>OD, 100 MHz) δ 14.0, 14.5 (2 × CH<sub>2</sub>CH<sub>3</sub>), 19.4, 23.7, 26.0, 30.2, 30.4, 30.48, 30.49, 30.6, 30.7, 30.76, 30.79, 30.80, 32.0, 33.1, 35.1, 36.8 (alkyl-CH<sub>2</sub>), 63.8, 67.0, 71.5, 72.2, 73.4, 74.6, 74.9, 100.4 (C<sub>1</sub>), 174.6, 174.8 (sn1–CO<sub>2</sub>, sn2–CO<sub>2</sub>), 196.8 (O=C–S); HRMS (ESI<sup>+</sup>) calcd for C<sub>31</sub>H<sub>56</sub>O<sub>10</sub>SNa<sup>+</sup> (M+Na) 643.3491. Found 643.3518.

**(2'R)-1'-O-Butyl-2'-O-palmitoyl-glyceryl 6-deoxy-6-sulfonato-α-D-glucopyranoside, potassium salt (4)**

Oxone (0.17 g, 0.028 mol) was added to a solution of **15** (0.04 g, 0.064 mmol) and KOAc (0.11 g, 0.0011 mol) in glacial AcOH (1 mL) and the resultant mixture was stirred at rt for 24 h. The mixture was concentrated and the residue purified by flash chromatography (EtOAc to 14:2:1 EtOAc/MeOH/H<sub>2</sub>O). The product was treated with hydrazine (0.04 mL, 0.04 mM) in

EtOH/H<sub>2</sub>O (17:3, 4.7 mL) at 40 °C for 3 h until tlc indicated there is only one compound. The solvent was evaporated and flash chromatography of the residue (EtOAc to EtOAc/MeOH/H<sub>2</sub>O 14:2:1) afforded **4** as a colourless semisolid (0.041 g, 96%). [ $\alpha$ ]<sup>20</sup><sub>D</sub> + 42.9° (*c* 0.263, MeOH); <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz) 0.92 (3 H, t, *J* 6.6 Hz, sn2-CH<sub>2</sub>CH<sub>3</sub>), 0.97 (3 H, t, *J* 7.4 Hz, sn1-CH<sub>2</sub>CH<sub>3</sub>), 1.28–1.38 (20 H, m, alkyl), 1.59–1.70 (4 H, m, β-CH<sub>2</sub>), 2.30–2.40 (4 H, m, α-CH<sub>2</sub>), 2.95 (1 H, dd, *J*<sub>5,6a</sub> 9.3, *J*<sub>6a,6b</sub> 13.3 Hz, H<sub>6a</sub>), 3.13 (1 H, t, *J*<sub>2,3</sub> = *J*<sub>3,4</sub> 9.2 Hz, H<sub>3</sub>), 3.35–3.46 (2 H, m, H<sub>4,6b</sub>), 3.57–3.72 (2 H, m, H<sub>2,3a'</sub>), 4.01–4.15 (2 H, m, H<sub>5,3b'</sub>), 4.22 (1 H, dd, *J*<sub>1b',2'</sub> 6.7, *J*<sub>1a',1b'</sub> 12.0 Hz, H<sub>1a'</sub>), 4.51 (1 H, dd, *J*<sub>1b',2'</sub> 2.8, *J*<sub>1a',1b'</sub> 12.0 Hz, H<sub>1b'</sub>), 4.79 (1 H, d, 3.4 Hz, H<sub>1</sub>), 5.30–5.36 (1 H, m, H<sub>2'</sub>); <sup>13</sup>C{<sup>1</sup>H} NMR (CD<sub>3</sub>OD, 100 MHz) δ 14.0, 14.4 (2 × CH<sub>2</sub>CH<sub>3</sub>), 19.4, 23.7, 26.0, 30.2, 30.5, 30.6, 30.7, 30.8, 33.1, 35.2, 36.8 (alkyl-CH<sub>2</sub>), 64.2, 67.1, 69.8, 71.7, 73.4, 74.86, 74.93, 100.0 (C<sub>1</sub>), 175.0 (2 C, sn1-CO<sub>2</sub>, sn2-CO<sub>2</sub>); HRMS (ESI<sup>-</sup>) calcd for C<sub>29</sub>H<sub>53</sub>O<sub>12</sub>S (M-H) 625.3263. Found 625.3176.

**(2'R)-1'-O-Oleoyl-2'-O-palmitoyl-glyceryl 2,3,4,6-tetra-O-methoxyacetyl-α-D-glucopyranoside (12)**

Tetrabutylammonium hydroxide solution in H<sub>2</sub>O (1.5 M, 1 mL, 1.525 mmol) was added to a suspension of oleic acid (0.45 g, 1.60 mmol) in H<sub>2</sub>O. The resulting mixture was vigorously stirred at r.t. overnight. The solvent was evaporated and the crude residue was co-evaporated with toluene several times to give the tetrabutylammonium salt of oleic acid. A mixture of tetrabutylammonium oleate (0.082 g, 0.160 mmol) and **10** (0.081 g, 0.078 mmol) in toluene (1 mL) was heated to 85 °C and stirred vigorously for 3 h. The solvents were evaporated and flash chromatography of the residue (CH<sub>2</sub>Cl<sub>2</sub> to 2:98 MeOH/CH<sub>2</sub>Cl<sub>2</sub>) afforded **12** as a colourless oil (0.048 g, 82%). [ $\alpha$ ]<sup>20</sup><sub>D</sub> + 61.6° (*c* 0.27, EtOAc); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 0.83–0.89 (2 × 3 H, m, CH<sub>2</sub>CH<sub>3</sub>), 1.20–1.38 (46 H, m, alkyl-CH<sub>2</sub>), 1.56–1.63 (4 H, m, β-CH<sub>2</sub>), 1.96–2.03 (4 H, m, H<sub>2</sub>CHC=CHCH<sub>2</sub>), 2.26–2.32 (2 × 2 H, m, α-CH<sub>2</sub>), 3.37, 3.39, 3.41, 3.43 (4 × 3 H, 4 s, 4 × CH<sub>3</sub>O), 3.62 (1 H, dd, *J*<sub>2',3'</sub> 5.4, *J*<sub>3',3'</sub> 11.2 Hz, H<sub>3'</sub>), 3.79 (1 H, dd, *J*<sub>2',3'</sub> 4.3, *J*<sub>3',3'</sub> 11.1 Hz, H<sub>3'</sub>), 3.92–4.07 (4 × 2 H, 4 m, CH<sub>3</sub>OCH<sub>2</sub>), 4.06–4.08 (1 H, m, H<sub>5</sub>), 4.09–4.19 (2 H, m, H<sub>1',6</sub>), 4.29–4.40 (2 H, m, H<sub>1',6</sub>), 4.95 (1 H, dd, *J*<sub>1,2</sub> 3.6, *J*<sub>2,3</sub> 10.1 Hz, H<sub>2</sub>), 5.09–5.21 (3 H, m, H<sub>1,2',4</sub>), 5.27–5.37 (2 H, m, HC=CH), 5.50 (1 H, t, *J*<sub>2,3</sub> = *J*<sub>3,4</sub> 9.7 Hz, H<sub>3</sub>); <sup>13</sup>C{<sup>1</sup>H} NMR (CDCl<sub>3</sub>, 100 MHz) 14.2 (2 × CH<sub>2</sub>CH<sub>3</sub>), 22.8, 24.96, 25.01, 27.27, 27.32, 29.18, 29.21, 29.23, 29.3, 29.4, 29.5, 29.6, 29.76, 29.78, 29.80, 29.9, 32.0, 32.02, 34.1, 34.3 (alkyl-CH<sub>2</sub>), 59.4, 59.50, 59.51 (4 C, OCH<sub>3</sub>), 61.7, 62.1, 67.0, 67.4, 68.4, 69.3, 69.38, 69.43, 69.5, 69.8, 70.5, 70.7, 96.2 (C<sub>1</sub>),



129.8, 130.1 (2 C, C=C), 169.3, 169.6, 170.0 (4 C, MeOCH<sub>2</sub>C=O), 173.0, 173.3 (sn1-CO<sub>2</sub>, sn2-CO<sub>2</sub>); HRMS (ESI<sup>+</sup>) calcd for C<sub>55</sub>H<sub>96</sub>O<sub>18</sub>Na<sup>+</sup> (M+Na) 1067.6494. Found 1067.6518.

**(2'R)-1'-O-Oleoyl-2'-O-palmitoyl-glycerol  $\alpha$ -D-glucopyranoside (14)**

A solution of t-butylamine (0.154 mL, 1.48 mmol) and **12** (0.20 g, 0.082 mmol) in CHCl<sub>3</sub> (0.32 mL) and MeOH (0.80 mL) was stirred at 0 °C for 10 min and then at 10 °C for 3 h at which time tlc indicated that the starting material was completely consumed. The solvent was evaporated under high vacuum without heating. Flash chromatography of the residue (CH<sub>2</sub>Cl<sub>2</sub> to 5:95 MeOH/CH<sub>2</sub>Cl<sub>2</sub>) afforded **14** as a white syrup (0.060 g, 98%). [ $\alpha$ ]<sup>20</sup><sub>D</sub> +59.4° (*c* 0.865, EtOAc); <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz)  $\delta$  0.88–0.93 (2  $\times$  3 H, m, CH<sub>2</sub>CH<sub>3</sub>), 1.26–1.40 (46 H, m, alkyl-CH<sub>2</sub>), 1.56–1.66 (4 H, m,  $\beta$ -CH<sub>2</sub>), 2.00–2.07 (4 H, m, H<sub>2</sub>CHC=CHCH<sub>2</sub>), 2.29–2.36 (2  $\times$  2 H, m,  $\alpha$ -CH<sub>2</sub>), 3.27–3.35 (1 H, m, H4), 3.39 (1 H, dd, *J*<sub>1,2</sub> 3.5, *J*<sub>2,3</sub> 9.7 Hz, H2), 3.55–3.71 (4 H, m, H3,5,6a,3'), 3.76–3.82 (1 H, m, H6b), 3.87 (1 H, dd, *J*<sub>3',3''</sub> 10.7, *J*<sub>2',3'</sub> 5.4 Hz, H3'), 4.20 (1 H, dd, *J*<sub>1',2'</sub> 6.5, *J*<sub>1',1''</sub> 12.0 Hz, H1'), 4.48 (1 H, dd, *J*<sub>1',2'</sub> 2.6, *J*<sub>1',1''</sub> 12.0 Hz, H1'), 4.80 (1 H, d, *J*<sub>1,2</sub> 3.5 Hz, H1), 5.23–5.28 (1 H, m, H2'), 5.33–5.37 (2 H, m, HC=CH); <sup>13</sup>C {<sup>1</sup>H} NMR (CD<sub>3</sub>OD, 150 MHz)  $\delta$  14.4, 14.5 (2  $\times$  CH<sub>3</sub>), 23.8, 26.0, 28.2, 30.2, 30.3, 30.37, 30.38, 30.48, 30.50, 30.6, 30.7, 30.80, 30.84, 30.9, 33.09, 33.10, 35.0, 35.2 (alkyl-CH<sub>2</sub>), 62.6, 63.8, 67.1, 71.58, 71.62, 73.5, 73.9, 74.9, 73.5 (C2,3,4,5,6,1',2',3'), 100.7 (C1), 130.8, 130.9 (2 C, C=C), 174.6, 174.9 (C=O); HRMS (ESI<sup>+</sup>) calcd for C<sub>43</sub>H<sub>82</sub>O<sub>10</sub>Na<sup>+</sup> (M+Na) 781.5805. Found 781.5819.

**(2'R)-1'-O-oleoyl-2'-O-palmitoyl-glycerol 6-S-acetyl-6-deoxy-6-thio- $\alpha$ -D-glucopyranoside (16)**

A solution of triphenylphosphine (0.048 g, 0.186 mmol) and diethyl azodicarboxylate (0.029 mL, 0.186 mmol) in THF (10 mL) was prepared and held at 0 °C under nitrogen for 30 min. This solution was then transferred by cannula into a solution of thioacetic acid (0.0039 mL, 0.056 mmol) and **14** (0.040 g, 0.053 mmol) in THF (5 mL) at 0 °C under nitrogen. The resultant reaction mixture was stirred at 4 °C under nitrogen overnight. The solvents were evaporated and flash chromatography of the residue (CH<sub>2</sub>Cl<sub>2</sub> to 3:97 MeOH/CH<sub>2</sub>Cl<sub>2</sub>) afforded **16** as a white oil (0.027 g, 73%). [ $\alpha$ ]<sup>20</sup><sub>D</sub> +62.0° (*c* 0.47, MeOH); <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz)  $\delta$  0.87–0.94 (2  $\times$  3 H, m, CH<sub>2</sub>CH<sub>3</sub>), 1.25–1.42 (46 H, m, alkyl), 1.57–1.66 (4 H, m,  $\beta$ -CH<sub>2</sub>), 1.99–2.08 (4 H, m, H<sub>2</sub>CHC=CHCH<sub>2</sub>), 2.29–2.37 (2  $\times$  2 H, m,  $\alpha$ -CH<sub>2</sub>), 2.33 (3H, s, SAc), 2.93 (1 H, dd, *J*<sub>5,6a</sub> 8.6, *J*<sub>6a,6b</sub> 13.6 Hz, H6a), 3.14 (1 H, t, *J*<sub>3,4</sub> = *J*<sub>4,5</sub> 9.2 Hz, H4), 3.39 (1 H, dd, *J*<sub>1,2</sub> 3.2, *J*<sub>2,3</sub> 9.5

Hz, H2), 3.50–3.65 (4 H, M, H3,5,6b,3'), 3.86 (1 H, dd,  $J_{3,3'}$  10.5,  $J_{2,3'}$  5.3 Hz, H3'), 4.22 (1 H, dd,  $J_{1a',2'}$  6.6,  $J_{1a',1b'}$  12.0 Hz, H1a'), 4.45 (1 H, dd,  $J_{1b',2'}$  2.3,  $J_{1b',1a'}$  11.9 Hz, H1b'), 4.73 (1 H, d,  $J_{1,2}$  3.2 Hz, H1), 5.20–5.27 (1 H, m, H2'), 5.30–5.41 (2 H, m, HC=CH);  $^{13}\text{C}\{^1\text{H}\}$  NMR ( $\text{CD}_3\text{OD}$ , 150 MHz)  $\delta$  14.52, 14.54 ( $2 \times \text{CH}_3$ ), 23.8, 26.05, 26.07, 28.2, 30.2, 30.3, 30.38, 30.41, 30.51, 30.53, 30.67, 30.71, 30.8, 30.9, 32.1, 33.1, 35.0, 35.2 (C6, alkyl- $\text{CH}_2$ ), 63.8, 67.0, 71.5, 72.2, 73.4, 74.6, 74.9 (C2,3,4,5,1',2',3'), 100.4 (C1), 130.8, 130.9 (2 C, C=C), 174.5, 174.8 (C=O), 196.8 (O=C-S); HRMS (ESI<sup>+</sup>) calcd for  $\text{C}_{45}\text{H}_{82}\text{O}_{10}\text{SNa}^+$  (M+Na) 837.5526. Found 837.5513.

**(2'R)-1'-O-Oleoyl-2'-O-palmitoyl-glyceryl 6-deoxy-6-sulfonato- $\alpha$ -D-glucopyranoside, potassium salt (5)**

$\text{H}_2\text{O}_2$  (9.8 M, 0.05 mL, 0.2 mmol) was added to a suspension of **16** (0.02 g, 0.025 mmol) in potassium phosphate buffer (what pH 7, 100 mM, 0.1 mL) and  $\text{CHCl}_3/\text{MeOH}$  (1:1 1 mL) and the resulting mixture was stirred at 50 °C for 1 h. Aqueous 1 M  $\text{Na}_2\text{S}_2\text{O}_5$  (1 mL) was added, the mixture was stirred briefly, and then extracted with  $\text{CHCl}_3$  ( $5 \times 5$  mL) and the organic layer was dried ( $\text{MgSO}_4$ ), filtered and evaporated. The crude residue, comprised of a mixture of the target SQDG and 4-O-acetyl-SQDG, was treated with hydrazine (0.04 mL, 0.04 mM) in  $\text{EtOH}/\text{H}_2\text{O}$  (17:3, 4.7 mL) at 40 °C for 3 h until tlc indicated there is only one compound. The solvent was evaporated and flash chromatography of the residue ( $\text{EtOAc}$  to 37:6:3  $\text{EtOAc}/\text{MeOH}/\text{H}_2\text{O}$ ) afforded **5** as a white gum (0.015 g, 71%).  $[\alpha]_D^{20} + 36.2^\circ$  ( $c$  0.29,  $\text{MeOH}$ );  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 400 MHz)  $\delta$  0.88–0.96 ( $2 \times 3$  H, m,  $\text{CH}_2\text{CH}_3$ ), 1.22–1.43 (46 H, m, alkyl), 1.56–1.67 (4 H, m,  $\beta\text{-CH}_2$ ), 2.02–2.08 (4 H, m,  $\text{H}_2\text{CHC}=\text{CHCH}_2$ ), 2.31–2.40 ( $2 \times 2$  H, m,  $\alpha\text{-CH}_2$ ), 2.90–2.98 (1 H, m, H6a), 3.11 (1 H, t,  $J_{3,4} = J_{4,5}$  8.9 Hz, H4), 3.34–3.39 (1 H, m, H6b), 3.42 (1 H, dd,  $J_{1,2}$  3.3,  $J_{2,3}$  9.8 Hz, H2), 3.56–3.73 (3 H, m, H3,5,3'), 4.01–4.16 (2 H, m, H4,3'), 4.19 (1 H, dd,  $J_{1a',2'}$  6.5,  $J_{1a',1b'}$  12.1 Hz, H1a'), 4.52 (1 H, dd,  $J_{1b',2'}$  2.2,  $J_{1b',1a'}$  11.9 Hz, H1b'), 4.78 (1 H, d,  $J_{1,2}$  3.5 Hz, H1), 5.30–5.34 (1 H, m, H2'), 5.34–5.39 (2 H, m, HC=CH);  $^{13}\text{C}\{^1\text{H}\}$  NMR ( $\text{CD}_3\text{OD}$ , 150 MHz)  $\delta$  14.5 ( $2 \times \text{CH}_3$ ), 23.8, 26.0, 28.2, 30.1, 30.16, 30.19, 30.3, 30.36, 30.38, 30.47, 30.50, 30.6, 30.7, 30.8, 33.1, 35.0, 35.2 (alkyl- $\text{CH}_2$ ), 54.3 (C6), 64.3, 67.1, 69.9 (3 C, C1',2',3'), 71.7, 73.5, 74.9, 75.0 (C2,3,4,5), 100.0 (C1), 130.8, 130.9 (2 C, C=C), 175.0, 175.1 (C=O); HRMS (ESI<sup>-</sup>) calcd for  $\text{C}_{43}\text{H}_{79}\text{O}_{12}\text{S}^-$  (M-H) 819.5298. Found 819.5152.

**Sodium methyl 6-deoxy-6-sulfonato- $\alpha$ -D-glucopyranoside (19)**

A solution of **18**<sup>25</sup> (3.04 g, 10.0 mmol) and Na<sub>2</sub>SO<sub>3</sub> (5.06 g, 40.2 mmol) in H<sub>2</sub>O (90 mL) was stirred at 80 °C for 4 h, then evaporated to dryness under reduced pressure. MeOH (75 mL) was added to the residue and the mixture was stirred at rt for 16 h. The filtrate was evaporated to dryness under reduced pressure and the residue purified by flash chromatography (EtOAc/MeOH/H<sub>2</sub>O, 9:2:1 → 3:2:1) to afford **19** as a colourless solid (2.72 g, 97%). [ $\alpha$ ]<sub>D</sub> +91.3° (*c* 0.855, MeOH; lit.<sup>32</sup> +82.1); <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O)  $\delta$  3.08 (1 H, dd,  $J_{6a,6b}$  14.7,  $J_{5,6a}$  9.8 Hz, H6a), 3.26 (1 H, t,  $J_{3,4} = J_{4,5}$  9.4 Hz, H4), 3.40 (1 H, d,  $J_{6a,6b}$  14.7 Hz, H6b), 3.47 (3 H, s, Me), 3.59 (1 H, dd,  $J_{2,3}$  9.8,  $J_{1,2}$  3.8 Hz, H2), 3.68 (1 H, t,  $J_{2,3} = J_{3,4}$  9.4 Hz, H3), 4.04 (1 H, t,  $J_{4,5} = J_{5,6a}$  9.5 Hz, H5); <sup>13</sup>C{<sup>1</sup>H} NMR (101 MHz, D<sub>2</sub>O)  $\delta$  52.0 (1 C, C6), 55.1 (1 C, Me), 67.7 (1 C, C5), 71.1 (1 C, C4), 72.3 (1 C, C2), 72.9 (1 C, C3), 98.9 (1 C, C1); HRMS (ESI)<sup>-</sup> *m/z* 257.0290 [C<sub>7</sub>H<sub>13</sub>O<sub>8</sub>S (M–Na)]<sup>-</sup> requires 257.0331].

### Sodium 6-deoxy-6-sulfonato-D-glucopyranose (**1**)

A solution of **19** (3.26 g, 11.6 mmol) in H<sub>2</sub>O (20.0 mL) was treated with aq. HCl (5 M, 3.3 mL, 16.3 mmol) and stirred at 100 °C for 80 h, then evaporated to dryness under reduced pressure. The residue was dissolved in H<sub>2</sub>O (128 mL) and stirred with IR-120 cation-exchange resin (68.5 g, Na<sup>+</sup> form) for 1 h. The filtrate was evaporated to dryness. Crystallization from hot 3:1 MeOH:H<sub>2</sub>O (44 mL) afforded **1** as a mixture of anomers (1.3  $\alpha$ : 1  $\beta$ ) as brown crystals (2.25 g, 73% over two crops). m.p 160–165°C; [ $\alpha$ ]<sub>D</sub> +40.7° (*c* 0.885, H<sub>2</sub>O); <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O)  $\delta$  3.13–3.04 (3 H, m, H6b- $\alpha$ ,6- $\alpha$ ,6b- $\beta$ ), 3.32–3.24 (3 H, m, H4- $\alpha$ ,2- $\beta$ ,4- $\beta$ ), 3.41 (1 H, d,  $J_{6a,6b}$  14.7 Hz, H6- $\beta$ ), 3.50 (1 H, t,  $J_{2,3} = J_{3,4}$  9.3 Hz, H3- $\beta$ ), 3.56 (1 H, dd,  $J_{2,3}$  9.8,  $J_{1,2}$  3.8 Hz, H2- $\alpha$ ), 3.73 (1 H, t,  $J_{2,3} = J_{3,4}$  9.4 Hz, H3- $\alpha$ ), 3.80 (1 H, t,  $J_{4,5} = J_{5,6a}$  9.5 Hz, H5- $\beta$ ), 4.24 (1 H, t,  $J_{4,5} = J_{5,6a}$  9.7 Hz, H5- $\alpha$ ), 4.68 (1 H, d,  $J_{1,2}$  8.0 Hz, H1- $\beta$ ), 5.22 (1 H, d,  $J_{1,2}$  3.7 Hz, H1- $\alpha$ ); <sup>13</sup>C{<sup>1</sup>H} NMR (101 MHz, D<sub>2</sub>O)  $\delta$  52.2, 52.1 (2C, C6- $\alpha$ , C6- $\beta$ ), 67.6 (1 C, C2- $\alpha$ ), 71.3 (1 C, C2- $\alpha$ ), 72.1 (1 C, C5- $\beta$ ), 72.5, 72.3 (2C, C4- $\beta$ , C4- $\alpha$ ), 72.6 (1 C, C3- $\alpha$ ), 74.0 (1 C, C2- $\beta$ ), 75.4 (1 C, C3- $\beta$ ), 91.9 (1 C, C1- $\alpha$ ), 95.8 (1 C, C1- $\beta$ ); HRMS (ESI)<sup>-</sup> *m/z* 243.0150 [C<sub>6</sub>H<sub>11</sub>O<sub>8</sub>S (M–Na)]<sup>-</sup> requires 243.0180].

### Methyl 6-deoxy-6-iodo-D-(<sup>13</sup>C<sub>6</sub>)glucopyranoside (**21**)

A solution of methyl 2,3,4-tri-*O*-acetyl-6-deoxy-6-iodo-D-(<sup>13</sup>C<sub>6</sub>)glucopyranoside **20**<sup>23</sup> (2.90 g, 6.6 mmol) in MeOH (23 mL) was treated with a solution of NaOMe in MeOH (0.66 mL, 1 M, 0.66 mmol) and stirred at rt for 15 min. The mixture was neutralized with AcOH (0.1 mL, 1.7 mmol) and evaporated to dryness under reduced pressure. The residue was purified by flash chromatography (EtOAc/pet. spirits, 80:20 → EtOAc → EtOAc/MeOH, 90:10) to afford **21** as

a mixture of anomers (20  $\alpha$ : 1  $\beta$ ) as a white solid (1.99 g, 94%).  $[\alpha]_D +104.8^\circ$  ( $c$  0.835, CH<sub>3</sub>OH); <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD)  $\delta$  4.90–4.42 (1 H, m, H1- $\alpha$ ), 4.20 (1 H, dd, <sup>1</sup> $J_{C1,H1}$  158.8, <sup>3</sup> $J_{H1,H2}$  7.6 Hz, H1- $\beta$ ), 3.88–3.51 (3 H, m, H3- $\alpha$ , H6a- $\alpha$ , H6a- $\beta$ ), 3.54 (3 H, d, <sup>3</sup> $J_{C1,H-Me}$  4.7 Hz, OMe- $\beta$ ), 3.45 (3 H, d, <sup>3</sup> $J_{C1,H-Me}$  4.4 Hz, OMe- $\alpha$ ), 3.50–3.26 (3 H, m, H5- $\alpha$ , 2- $\alpha$ , 5- $\beta$ ), 3.38–3.16 (6 H, m, H6b- $\alpha$ , 3- $\beta$ , 4- $\alpha$ , 2- $\beta$ , 6b- $\beta$ , 4- $\beta$ ); <sup>13</sup>C {<sup>1</sup>H} NMR (101 MHz, CD<sub>3</sub>OD)  $\delta$  105.2 (1 C, dt, <sup>1</sup> $J_{1,2}$  46.8, <sup>2</sup> $J_{1,3}$  4.8 Hz, C1- $\beta$ ), 102.3–100.7 (1 C, m, C1- $\alpha$ ), 78.0–77.0 (1 C, m, C5- $\beta$ ), 76.7 (1 C, dd, <sup>1</sup> $J_{1,2}$  40.5, <sup>2</sup> $J_{3,4} = J_{4,5}$  4.0 Hz, C4- $\beta$ ), 76.2–75.1 (1 C, m, C4- $\alpha$ ), 75.6–74.7 (2 C, m, C3- $\beta$ , C2- $\beta$ ), 75.1–74.1 (1 C, m, C3- $\alpha$ ), 73.5 (1 C, ddd, <sup>1</sup> $J_{1,2}$  41.9, <sup>1</sup> $J_{2,3}$  37.2, <sup>2</sup> $J_{2,4}$  5.1 Hz, C2- $\alpha$ ), 73.0–71.7 (1 C, m, C5- $\alpha$ ), 55.8 (1 C, s, OMe- $\alpha$ ), 7.2 (1 C, dt, <sup>1</sup> $J_{5,6}$  42.7, <sup>2</sup> $J_{4,6}$  4.0 Hz, C6- $\alpha$ ), 6.5 (1 C, dt, <sup>1</sup> $J_{5,6}$  42.0, <sup>2</sup> $J_{4,6}$  4.3 Hz, C6- $\beta$ ); HRMS (ESI)<sup>+</sup>  $m/z$  332.9926 [<sup>13</sup>C<sub>6</sub><sup>12</sup>CH<sub>13</sub>O<sub>5</sub>INa (M + Na)<sup>+</sup> requires 332.9907].

### Sodium methyl 6-deoxy-6-sulfonato-D-(<sup>13</sup>C<sub>6</sub>)glucopyranoside (**22**)

A solution of **21** (1.99 g, 6.4 mmol) and Na<sub>2</sub>SO<sub>3</sub> (3.23 g, 25.6 mmol) in H<sub>2</sub>O (60 mL) was stirred at 80°C for 4 h, then evaporated to dryness under reduced pressure. MeOH (47 mL) was added to the residue and the mixture was stirred at rt for 16 h. The filtrate was evaporated to dryness under reduced pressure and the residue purified by flash chromatography (EtOAc/MeOH/H<sub>2</sub>O, 9:2:1 → 4:2:1) to afford **22** as a mixture of anomers (25  $\alpha$ : 1  $\beta$ ) as a colourless solid (1.75 g, 95%).  $[\alpha]_D +92.1^\circ$  ( $c$  0.935, MeOH); <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O)  $\delta$  5.04–4.54 (1 H, m, H1- $\alpha$ ), 4.26–3.81 (1 H, m, H5- $\alpha$ ), 3.91–3.49 (1 H, m, H3- $\alpha$ ), 3.80–3.43 (1 H, m, H2- $\alpha$ ), 3.57 (3 H, d, <sup>3</sup> $J_{C1,H-Me}$  4.8 Hz, OMe- $\beta$ ), 3.47 (3 H, d, <sup>3</sup> $J_{C1,H-Me}$  4.2 Hz, OMe- $\alpha$ ), 3.60–3.20 (1 H, m, H6a- $\alpha$ ), 3.44–3.01 (1 H, m, H4- $\alpha$ ), 3.31–2.86 (1 H, m, H6b- $\alpha$ ); <sup>13</sup>C {<sup>1</sup>H} NMR (101 MHz, D<sub>2</sub>O)  $\delta$  102.9 (1 C, dt, <sup>1</sup> $J_{1,2}$  46.7, <sup>2</sup> $J_{1,3}$  4.5 Hz, C1- $\beta$ ), 98.9 (ddd, <sup>1</sup> $J_{1,2}$  45.9, <sup>2</sup> $J_{1,3}$  3.0, <sup>3</sup> $J_{1,4}$  1.7 Hz, C1- $\alpha$ ), 75.4 (1 C, d, <sup>1</sup> $J_{2,3} = J_{3,4}$  42.2 Hz, C3- $\beta$ ), 73.5–71.7 (5 C, m, C3- $\alpha$ , C2- $\alpha$ , C2- $\beta$ , C4- $\beta$ , C5- $\beta$ ), 71.6–70.4 (1 C, m, C4- $\alpha$ ), 68.4–67.1 (1 C, m, C5- $\alpha$ ), 55.1 (1 C, s, OMe- $\alpha$ ), 51.9 (2 C, dt, <sup>1</sup> $J_{5,6}$  41.8, <sup>2</sup> $J_{4,6}$  3.1 Hz, C6- $\alpha$ , C6- $\beta$ ); HRMS (ESI)<sup>+</sup>  $m/z$  263.0479 [<sup>13</sup>C<sub>6</sub><sup>12</sup>CH<sub>13</sub>O<sub>8</sub>S (M-H)<sup>-</sup> requires 263.0532].

### Sodium 6-deoxy-6-sulfonato-D-(<sup>13</sup>C<sub>6</sub>)glucopyranose (**23**)

A solution of sodium **22** (1.75 g, 6.13 mmol) in H<sub>2</sub>O (10.7 mL) was treated with aq. HCl (1.78 mL, 5 M, 8.9 mmol) and stirred at 100°C for 64 h, then evaporated to dryness under reduced pressure. The residue was dissolved in H<sub>2</sub>O (67 mL) and stirred with Na<sup>+</sup> IR-200 resin (36.0 g) for 1 h. The filtrate was evaporated to dryness. Crystallization from hot 3:1 MeOH:H<sub>2</sub>O (31 mL) afforded **23** as a mixture of anomers (1.3  $\alpha$ : 1  $\beta$ ) as light brown crystals (1.14 g, 69% over

two crops). m.p 163–166°C;  $[\alpha]_D^{25} +37.4^\circ$  ( $c$  0.785, H<sub>2</sub>O); <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O)  $\delta$  3.29–2.82 (2 H, m, H4- $\alpha$ , H2- $\beta$ ), 3.49–3.02 (3 H, m, H6b- $\alpha$ ,4- $\beta$ ,6b- $\beta$ ), 3.40 (2 H, dd, <sup>1</sup> $J_{C6,H6}$  134.8, <sup>2</sup> $J_{H6a,H6b}$  14.4 Hz, H6a- $\alpha$ ,6a- $\beta$ ), 4.00–3.27 (4 H, m, H2- $\alpha$ ,3- $\alpha$ ,3- $\beta$ ,5- $\beta$ ), 4.23 (1 H, dt, <sup>1</sup> $J_{C5,H5}$  146.8, <sup>3</sup> $J_{H4,H5} = J_{H5,H6b}$  9.3 Hz, H5- $\alpha$ ), 4.67 (1 H, dd, <sup>1</sup> $J_{C1,H1}$  161.4, <sup>3</sup> $J_{H1,H2}$  7.8 Hz, H1- $\beta$ ), 5.21 (1 H, d, <sup>1</sup> $J_{C1,H1}$  171.5 Hz, H1- $\alpha$ ); <sup>13</sup>C {<sup>1</sup>H} NMR (101 MHz, D<sub>2</sub>O)  $\delta$  52.7–51.4 (2 C, m, C6- $\alpha$ , C6- $\beta$ ), 68.5–66.7 (1 C, m, C5- $\alpha$ ), 71.8–70.8 (1 C, m, C2- $\alpha$ ), 73.1–71.8 (4 C, m, C3- $\alpha$ , C4- $\alpha$ , C4- $\beta$ , C5- $\beta$ ), 73.9 (1 C, ddd, <sup>1</sup> $J_{1,2}$  45.2, <sup>1</sup> $J_{2,3}$  38.7, <sup>2</sup> $J_{2,4}$  2.4 Hz, C2- $\beta$ ), 76.0–74.9 (1 C, m, C3- $\beta$ ), 92.3–91.4 (1 C, m, C1- $\alpha$ ), 95.8 (1 C, dt, <sup>1</sup> $J_{1,2}$  45.7, <sup>2</sup> $J_{1,3}$  4.8 Hz, C1- $\beta$ ); HRMS (ESI)<sup>+</sup>  $m/z$  249.0323 [<sup>13</sup>C<sub>6</sub>H<sub>11</sub>O<sub>8</sub>S (M–H)<sup>+</sup> requires 249.0376].

### Tetrabutylammonium methylumbelliferide

Tetrabutylammonium hydroxide solution in water (1.50 M, 2.89 g, 2.91 mL, 0.98 mmol) was added to a solution of methylumbelliferone (2.00 g, 1.00 mmol) in methanol. The mixture was stirred for 1 h at rt under nitrogen. The solvent was evaporated under reduced pressure, and the residue was co-evaporated with toluene several times to remove water to give the tetrabutylammonium salt of methylumbelliferone as a pale yellow solid (4.64 g, 98%). <sup>1</sup>H NMR (600 MHz, d<sub>4</sub>-methanol)  $\delta$  1.04 (12 H, t,  $J$  7.3 Hz, CH<sub>3</sub>), 1.43 (8 H, sextet,  $J$  7.5 Hz, CH<sub>2</sub>CH<sub>3</sub>), 1.64–1.71 (8 H, m, CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 2.41 (3 H, s, CH<sub>3</sub>-Ar), 3.22–3.27 (8 H, m, CH<sub>2</sub>N), 5.98 (1 H, s, H3-Ar), 6.59 (1 H, d,  $J$  2.4 Hz, H8-Ar), 6.74 (1 H, dd,  $J$  8.7,  $J$  2.4 Hz, H6-Ar), 7.52 (1 H, d,  $J$  8.7 Hz, H5-Ar).

### 4-Methylumbelliferyl 2,3,4,6-tetra-*O*-acetyl- $\alpha$ -D-glucopyranoside (27)

A mixture of 2,3,4,6-tetra-*O*-acetyl- $\beta$ -glucopyranosyl chloride **26**<sup>29</sup> (1.50 g, 4.09 mmol) and tetrabutylammonium methylumbelliferide (3.42 g, 8.18 mmol) in dry acetonitrile (400 mL) was stirred at room temperature for 3 d under a nitrogen atmosphere. The solvent was evaporated under reduced pressure and the residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (100 mL), washed with 5% NaOH (3  $\times$  100 mL) and brine, and dried (Na<sub>2</sub>SO<sub>4</sub>). The solvent was evaporated under reduced pressure and the residue was purified with flash chromatography (CH<sub>2</sub>Cl<sub>2</sub>: toluene:PS, 1:1:1  $\rightarrow$  7:1:1) to afford **27** as an off-white solid (400 mg, 15%). m.p. 129–131 °C;  $[\alpha]_D^{25} +181.4$ , ( $c$  1.00, chloroform); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  2.03, 2.04, 2.05, 2.07 (12 H, s, Ac), 2.40 (3 H, s, Me-Ar), 4.00–4.09 (2 H, m, H5,6a), 4.25 (1 H, dd,  $J_{6a,6b}$  12.1,  $J_{5,6b}$  4.4 Hz, H6b), 5.06 (1 H, dd,  $J_{2,3}$  10.3,  $J_{1,2}$  3.6 Hz, H2), 5.16 (1 H, t,  $J_{3,4} = J_{4,5}$  9.7 Hz, H4), 5.68 (1 H, t,  $J_{2,3} = J_{3,4}$  9.8 Hz, H3), 5.79 (1 H, d,  $J_{1,2}$  3.6 Hz, H1), 6.19 (1 H, s, H3-Ar), 7.04 (1 H, dd,  $J$

8.7,  $J$  2.3 Hz, H6-Ar), 7.08 (1 H, d,  $J$  2.2 Hz, H8-Ar), 7.54 (1 H, d,  $J$  8.7 Hz, H5-Ar);  $^{13}\text{C}\{^1\text{H}\}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  18.8 (Me-Ar), 20.70, 20.74, 20.76, 20.82 (4C,  $\text{COCH}_3$ ), 61.6 (C6), 68.2 (C4), 68.6 (C5), 69.9 (C3), 70.3 (C2), 94.5 (C1), 104.8 (C8-Ar), 113.4 (C3,6-Ar), 115.7 (C9-Ar), 126.0 (C5-Ar), 152.2 (C4-Ar), 155.0 (C10-Ar), 158.8 (C7-Ar), , 160.9 (C=O, C2-Ar), 169.7, 170.2, 170.3, 170.6 (4C, C=O, Ac).

#### 4-Methylumbelliferyl $\alpha$ -D-glucopyranoside (**MUGlc**, **25**)

Sodium methoxide (1 M in methanol) was added to a solution of **27** (400 mg, 1.18 mmol) in dry methanol (7 mL) to adjust to pH 10 and was stirred for 3 h at rt under a nitrogen atmosphere. The mixture was neutralized with 10% AcOH and was then concentrated in *vacuo* to give **25** as a white solid (392 mg, 98%). m.p. 208-211 °C;  $[\alpha]_{\text{D}}^{23} +216.4$  ( $c$  0.51, MeOH);  $^1\text{H}$  NMR (400 MHz,  $\text{d}_4$ -methanol)  $\delta$  2.48 (3H, s, Me-Ar), 3.45 (1 H, t,  $J_{3,4} = J_{4,5}$  9.4 Hz, H4), 3.58–3.61 (1 H, m, H5), 3.63 (1 H, dd,  $J_{2,3}$  9.7,  $J_{1,2}$  3.6 Hz, H2), 3.67–3.79 (2 H, m, H6a,6b), 3.87 (1 H, t,  $J_{2,3} = J_{3,4}$  9.3 Hz, H3), 5.65 (1 H, d,  $J_{1,2}$  3.5 Hz, H1), 6.22 (1 H, s, H3-Ar), 7.18–7.23 (2 H, m, H6,8-Ar), 7.71–7.76 (1 H, d,  $J$  9.6 Hz, H5-Ar);  $^{13}\text{C}\{^1\text{H}\}$  NMR 150 MHz,  $\text{d}_4$ -methanol)  $\delta$  18.6 (Me-Ar), 62.3 (C6), 71.3 (C4), 73.1 (C2), 74.8 (C3), 75.0 (C5), 99.3 (C1), 105.15 (C8-Ar), 112.9 (C3-Ar), 115.3 (C6-Ar), 116.0 (C9-Ar), 127.3 (C5-Ar), 155.5 (C4-Ar), 156.1 (C10-Ar), 161.6 (C7-Ar), 163.4 (C=O, C2-Ar).

#### 4-Methylumbelliferyl 6-*S*-benzoyl-6-deoxy-6-thio- $\alpha$ -D-glucopyranoside (**28**)

A solution of **25** (300 mg, 0.887 mmol) in DMF was added to a solution of  $\text{PPh}_3$  (582 mg, 2.22 mmol), DIAD (0.435 mL, 2.22 mmol) and thiobenzoic acid (0.262 mL, 2.22 mmol) in THF at 0° C under a nitrogen atmosphere. The mixture was stirred for 4 h at rt, and the solvent was evaporated under reduced pressure. The resulting reddish-brown gum was purified by flash chromatography (EtOAc:pet. spirit:MeOH, 1:1:0  $\rightarrow$  9.5:0: 0.5), affording **28** as a white solid (332 mg, 82%). m.p. 180-184 °C;  $[\alpha]_{\text{D}}^{24} +158.5$  ( $c$  0.625, acetone);  $^1\text{H}$  NMR (400 MHz,  $\text{d}_6$ -DMSO)  $\delta$  2.29 (3 H, d,  $J$  1.1, Me-Ar), 2.94 (1 H, dd,  $J_{6a,6b}$  13.8,  $J_{5,6b}$  9.7 Hz, H6a), 3.13–3.18 (1 H, m, H5), 3.45–3.49 (1 H, m, H4), 3.53 (1 H, td,  $J$  9.7,  $J$  2.5 Hz, H3), 3.59–3.64 (2 H, m, H2,6b), 5.13 (1 H, d,  $J$  5.1 Hz, OH-3), 5.26 (1 H, d,  $J$  6.3 Hz, OH-2), 5.29 (1 H, d,  $J$  5.8 Hz, OH-4), 5.63 (1 H, d,  $J_{1,2}$  3.6 Hz, H1), 6.16 (1 H, d,  $J$  1.2 Hz, H3-Ar), 7.00 (1 H, dd,  $J$  8.8,  $J$  2.4 Hz, H6-Ar), 7.07 (1 H, d,  $J$  2.4 Hz, H8-Ar), 7.39–7.43 (2 H, m, H3,5-Ph), 7.56 (1 H, d,  $J$  8.8 Hz, H5-Ar), 7.60 (1 H, tt,  $J$  7.7,  $J$  1.2 Hz, H4-Ph), 7.65 (2 H, dd,  $J$  8.3,  $J$  1.2 Hz, H2,6);  $^{13}\text{C}\{^1\text{H}\}$  NMR 150 MHz,  $\text{d}_6$ -DMSO)  $\delta$  18.0 (Me-Ar), 30.6 (C6), 71.3, 71.4 (C3, 4), 73.0 (C2), 73.4 (C5), 96.9 (C1), 104.0 (C8-Ar), 111.7 (C9-Ar), 111.8 (C3-Ar) 114.0 (C6-Ar), 126.1 (C5-Ar), 126.4

(C2,6-Ph), 128.8 (C3,5-Ph), 133.7 (C4-Ph), 136.2 (C1-Ph), 153.1 (C4-Ar), 154.1 (C10-Ar), 158.9 (C7-Ar), 160.0 (C=O-Ar), 190.4 (SC=O).

#### **Potassium 4-methylumbelliferyl 6-deoxy-6-sulfonato- $\alpha$ -D-glucopyranoside (MUSQ, 24)**

30% H<sub>2</sub>O<sub>2</sub> (3.5 mL) was added to a mixture of **28** (332 mg, 0.70 mmol) and KOAc (82 mg, 0.84 mmol) in glacial acetic acid. The mixture was stirred at 50 °C for 5 h under a nitrogen atmosphere. The mixture was diluted with water and quenched by the addition of PPh<sub>3</sub> in ether (3 × 2M, 20 mL). The aqueous layer was separated, and organic layer was extracted once with water. The solvent was evaporated under reduced pressure from combined aqueous solution, and purified by flash chromatography (0.2% Et<sub>3</sub>N in chloroform: MeOH:water, 1:0:0 → 4:12:1). The product was crystallised from hot ethanol to afford **24** as a white powder. (151 mg, 52%). m.p. 201-205 °C; [ $\alpha$ ]<sub>D</sub><sup>24</sup> +93.9 (*c* 0.725, Acetone); <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O)  $\delta$  2.44 (3 H, s, Me-Ar), 3.09 (1 H, dd,  $J_{6a,6b}$  14.8,  $J_{5,6b}$  9.5 Hz, H6a), 3.30–3.44 (2 H, m, H5,6b), 3.79 (1 H, dd,  $J_{2,3}$  9.8,  $J_{1,2}$  3.6 Hz, H2), 3.94 (1 H, t,  $J_{3,4} = J_{4,5}$  9.5 Hz, H4), 4.11 (1 H, t,  $J_{2,3} = J_{3,4}$  9.7 Hz, H3), 5.67 (1 H, d,  $J_{1,2}$  3.4 Hz, H1), 6.24 (1 H, s, H3-Ar), 7.16–7.24 (2 H, m, H6,8-Ar), 7.71 (1 H, d,  $J$  8.7 Hz, H5-Ar); <sup>13</sup>C{<sup>1</sup>H} NMR (100 MHz, D<sub>2</sub>O)  $\delta$  17.9 (Me-Ar), 52.0 (C6), 69.1 (C3), 70.8 (C2), 72.1 (C5), 72.6 (C4), 97.0 (C1), 104.5 (C8-Ar), 111.0 (C3-Ar), 114.5 (C6-Ar), 115.1 (C9-Ar), 126.3 (C5-Ar), 153.7 (C4-Ar), 156.3 (C10-Ar), 159.3 (C7-Ar), 165.7 (C=O, C2-Ar).

#### **Kinetic analysis of sulfoquinovosidases using MUSQ**

AtSQase and YihQ enzymes were obtained as previously described.<sup>4,5</sup> Reaction buffer contains 50 mM NaH<sub>2</sub>PO<sub>4</sub>/Na<sub>2</sub>HPO<sub>4</sub> and 150 mM of NaCl at pH 7.4. Stop buffer contains 1 M NaOH and 1 M glycine at pH 10.

#### **Calibration curve for 4-methylumbelliferone**

A calibration curve for the 4-methylumbelliferone fluorophore was constructed as follows. Solutions of 4-methylumbelliferone were prepared at 2.35, 4.70, 7.05, 9.40  $\mu$ M in Reaction buffer. 500  $\mu$ l of each standard solution was diluted with 500  $\mu$ l of Stop buffer to a total volume of 1000  $\mu$ l. The fluorescence was measured in a quartz cuvette using a Cary Eclipse fluorescence spectrophotometer, with an excitation wavelength of 365 nm and an emission wavelength of 445 nm. Data was plotted and subjected to least squares linear fitting to obtain a line of best fit.

### Enzyme kinetic assays with MUSQ

Enzyme kinetic assays were conducted in triplicate in a total of 150  $\mu\text{l}$  at 25  $^{\circ}\text{C}$  in a 0.5 mL Eppendorf tube. Each reaction contained 0.05% bovine serum albumin, and MUSQ concentrations of 16.8, 33.6, 67.2, 134.4, 268.8, 537.6, 1075.2  $\mu\text{M}$ . Reactions were initiated by addition of an aliquot of *At*SQase or *Yih*Q to a final enzyme concentration of 1.22  $\mu\text{M}$ . After 15-60 min, the reaction mixture was quenched by addition of 150  $\mu\text{l}$  of stop buffer. 250  $\mu\text{l}$  of the quenched mixture was diluted with 250  $\mu\text{l}$  Reaction buffer and 500  $\mu\text{l}$  Stop buffer to a final volume of 1000  $\mu\text{l}$ . The fluorescence was measured in a quartz cuvette using a Cary Eclipse fluorescence spectrophotometer as for the calibration curve. Control experiments were performed to demonstrate that reaction rates were linear over the chosen time period to ensure measurement of initial rates.

### X-ray crystallography

Crystals of SQ sodium and potassium salts were mounted in low temperature oil then flash cooled using an Oxford low temperature device. Intensity data were collected at 100 K on the MX2 beamline at the Australian Synchrotron.<sup>33</sup> The structures were solved by direct methods and difference Fourier synthesis using the SHELX suite of programs<sup>34</sup> as implemented within the WINGX<sup>35</sup> software. Thermal ellipsoid plots were generated using the program ORTEP-3.

Crystal data for SQ potassium salt:  $\text{C}_6\text{H}_{11}\text{O}_8\text{SK}\cdot(\text{H}_2\text{O})$   $M = 300.32$   $T = 100.0(1)$  K,  $\lambda = 0.710757$ , Orthorhombic, space group  $\text{P}2_12_12_1$   $a = 7.1850(14)$   $b = 10.000(2)$ ,  $c = 15.059(3)$   $\text{\AA}$   $V = 1082.0(4)$   $\text{\AA}^3$ ,  $Z = 4$ ,  $D_c = 1.844$   $\text{Mg M}^{-3}$   $\mu = 0.721$   $\text{mm}^{-1}$ ,  $F(000) = 624$ , crystal size  $0.03 \times 0.02 \times 0.02$  mm. 10195 reflections measured, 3142 independent reflections ( $R_{\text{int}} = 0.1100$ ), the final  $R$  was 0.0746 [ $I > 2\sigma(I)$ , 1838 data] and  $wR(F^2)$  (all data) was 0.2177, Absolute structure parameter -0.02(7), GOF 1.038. CCDC: 1967143.

Crystal data for SQ sodium salt:  $\text{C}_6\text{H}_{11}\text{O}_8\text{SNa}\cdot(\text{H}_2\text{O})$   $M = 284.21$   $T = 100.0(1)$  K,  $\lambda = 0.799895$ , monoclinic, space group  $\text{P}2_1$   $a = 6.9360(14)$   $b = 15.065(3)$ ,  $c = 9.817(2)$   $\text{\AA}$ ,  $\beta = 90.38(3)$ ,  $V = 1025.8(4)$   $\text{\AA}^3$ ,  $Z = 4$ ,  $Z' = 2$ ,  $D_c = 1.840$   $\text{Mg M}^{-3}$   $\mu = 0.542$   $\text{mm}^{-1}$ ,  $F(000) = 592$ , crystal size  $0.06 \times 0.04 \times 0.02$  mm. 12133 reflections measured, 4037 independent reflections ( $R_{\text{int}} = 0.1237$ ), the final  $R$  was 0.0713 [ $I > 2\sigma(I)$ , 3847 data] and  $wR(F^2)$  (all data) was 0.1718, Absolute structure parameter 0.06(5), GOF 1.067. CCDC: 1967144.

### Conflicts of interest

There are no conflicts to declare.



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