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Comprehensive synthesis of substrates, intermediates and products of the sulfoglycolytic Embden-Meyerhoff-Parnas pathway

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Abstract

Sulfoglycolysis is a metabolic pathway dedicated to the catabolism of the sulfosugar sulfoquinovose (SQ) into smaller organosulfur fragments. An estimated 10 billion tonnes of SQ fluxes through sulfoglycolysis pathways each year, making it a significant aspect of the biogeochemical sulfur cycle. Delineating the molecular details of sulfoglycolysis requires authentic samples of the various metabolites in these pathways. To this end, we have established chemical and chemoenzymatic methods for the synthesis of the key organosulfur metabolites sulfoquinovosylglycerol (SQGro), SQ (also in $^{13}C_6$-labelled form), sulfofructose (SF), sulfofructose-1-phosphate (SFP), sulfolactaldehyde (SLA) and 2,3-dihydroxypropanesulfonate (DHPS), as well as an improved route to the chromogenic sulfoquinovosidase substrate 4-nitrophenyl α-sulfoquinovoside.

INTRODUCTION

The sulfoglycolipid sulfoquinovosyl diacylglyceride (SQDG) is a significant chemical species in the global biogeochemical sulfur cycle,[1-2] along with the sulfur-containing amino acids cysteine and methionine, and the osmolytes dimethylsulfoniopropionate[3] and dimethylsulfoxonium propionate.[4] The abundance and ubiquity of SQDG has fostered the evolution of specialised microbial pathways for the catabolism of its unusual sulfoquinovose (SQ, **1**) head-group. Benson first proposed the existence of sulfoglycolytic pathways in 1961; named by analogy with the glycolytic processes for glucose metabolism.[5] Two sulfoglycolytic processes capable of catabolizing SQ have since been described.[6-7] In both cases, sulfoglycolysis is preceded by liberation of SQ from its glycosides SQDG and its delipidated forms *lyso*-SQDG and α-sulfoquinovosyl glycerol (SQGro, **2**),[8-9] and rapid equilibration of SQ anomers by an SQ mutarotase to overcome the intrinsically slow uncatalyzed mutarotation of this sugar.[10] The first sulfoglycolysis pathway was identified in *Escherichia coli*.[6, 11] It shares broad similarity with the classical Embden-Meyerhoff-Parnas (EMP) glycolysis pathway (which converts glucose to ATP and pyruvate) and thus is referred to as the sulfoglycolytic EMP (sulfo-EMP) pathway. The sulfo-EMP pathway consists of a series of enzymes that catalyse isomerization of SQ to sulfofructose (SF, **3**; YihS, SQ-SF-isomerase), phosphorylation of SF to provide sulfofructose-1-phosphate (SFP, **4**; YihV, SF-kinase), retro-aldol cleavage of SFP to dihydroxyacetonephosphate (DHAP; which enters central carbon metabolism) and sulfolactaldehyde (SLA, **5**; YihT, SFP-aldolase), and finally reduction of SLA to *S*-2,3-dihydroxypropanesulfonate (*S*-DHPS, **6**; YihU, SLA-reductase) (Figure 1).[6] This system is under control of a transcriptional regulator (CsqR), which represses expression of sulfoglycolytic proteins in the absence of SQ.[12] An alternative sulfoglycolytic pathway mimicking the Entner-Douderoff pathway has also been identified, as first described in *Pseudomonas putida* SQ1.[7]

Biochemical and structural studies of the sulfo-EMP pathway would benefit from the development of methods to synthesize and purify the various substrates, intermediates and products of the pathway. Current methods for the synthesis of SQGro are iterations of an arduous procedure first developed by Miyano and Benson[13] and later modified by Roy and Hewlins.[14] Several syntheses of SQ have been reported,[13-16] and while these methods are sufficient for the preparation of small amounts of material, larger quantities are needed to support metabolic studies. A two-step method reported for the synthesis of the chromogenic SQase substrate, 4-nitrophenyl α-sulfoquinovoside (PNPSQ, **7**), while direct, is challenging in

practice due to the poor stability of a thioacetate intermediate.[8] To our knowledge no methods for the synthesis of pure samples of SF, SFP or SLA have been reported (for previous efforts see: SFP,[5] SLA[5, 7]). Finally, while there are several reports for the synthesis of racemic DHPS,[17-18] these are arduous and time-consuming, and do not provide effective ways to purify the final product. As part of ongoing efforts to understand the molecular details of the sulfo-EMP pathway, we herein disclose improved methods for the synthesis of the known compounds SQGro, PNPSQ, SQ (also in ^{13}C-labelled form) and DHPS, and the first syntheses of the sulfoglycolytic intermediates SF, SFP and SLA.

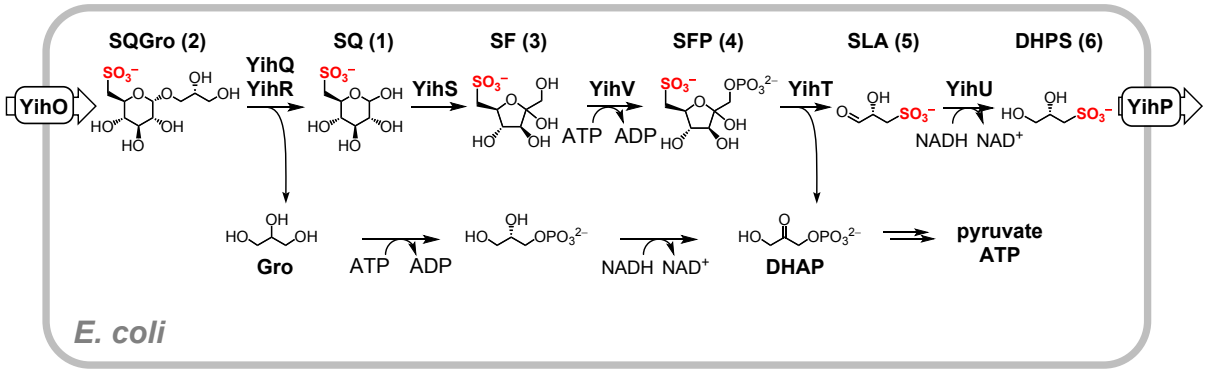
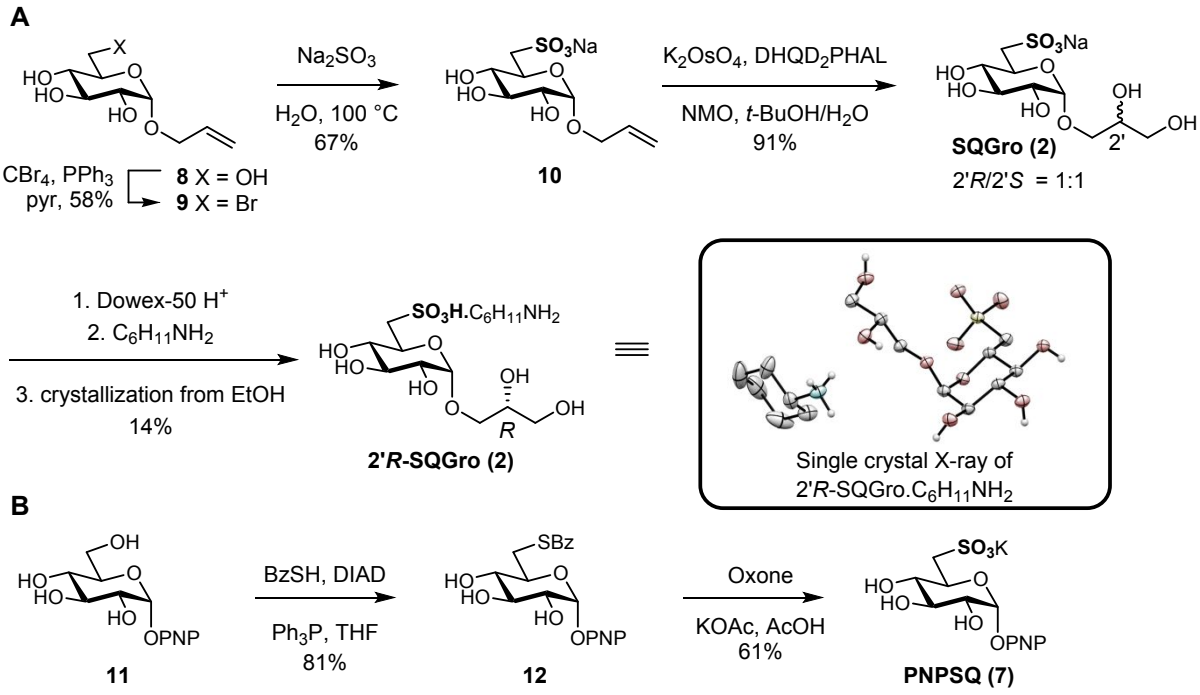


Figure 1. The sulfoglycolytic Embden-Meyerhof-Parnas pathway for metabolism of sulfoquinovosylglycerol (SQGro) and sulfoquinovose (SQ) in *E. coli*. YihO and YihP are putative influx/efflux proteins.

Results and discussion

Synthesis of the SQ glycosides SQGro and PNPSQ. Miyano and Benson reported the synthesis of SQGro in a multi-step process involving the preparation of SQ from 1,2-*O*-isopropylidene-α-D-glucofuranose in three steps, conversion to the allyl α-sulfoquinovoside, permanganate oxidation and multiple ion-exchange steps that ultimately provided the cyclohexylammonium salt.[13] Roy and Hewlins applied the same general approach, but made some improvements to the final purification through the isolation of the brucine salt prior to conversion to the cyclohexylammonium salt.[14] Inspired by approaches to related molecules,[19-20] we instead sought to utilize commercially available allyl α-D-glucopyranoside **8** as a precursor, as it possesses the underlying carbon framework of the target. Accordingly, Appel bromination of the 6-position of **8** afforded the bromide **9** in good yield (Scheme 1A). Direct substitution of the bromide with sodium sulfite in water afforded the known allyl α-D-sulfoquinovoside **10**.[13-14] While prior syntheses employ $KMnO_4$ to dihydroxylate this alkene,[13-14] we were inspired by related work using catalytic OsO_4 and Me_3NO[19] to adopt a ligand-catalyzed Sharpless dihydroxylation, which provides a rate enhancement for the oxidation of terminal alkenes versus osmium tetroxide alone.[21] We were, however, aware of the limited ability of chiral catalysts used in related transformations of unprotected allyl α-glycosides to impart any meaningful diastereoselectivity on the dihydroxylation reaction.[22] While preliminary experiments with commercially available AD-mix reagents provided a successful transformation, we had difficulty purifying the highly polar SQGro products from the large amounts of salts derived from the potassium ferricyanide co-oxidant. In the spirit of the original Sharpless asymmetric dihydroxylation process,[21] we therefore applied an Upjohn-type process using *N*-methylmorpholine *N*-oxide (NMO) as terminal oxidant but with K_2OsO_4.[23] Treatment of **10** with catalytic K_2OsO_4, $(DHQD)_2PHAL$ ligand, and NMO smoothly provided SQGro, and facilitated simple purification to provide the sodium salt in a 1:1 ratio of the 2'*R* and 2'*S*-isomers. Benson has reported that the cyclohexylammonium salt can be recrystallized to provide the pure 2'*R*-stereoisomer.[13] Therefore we converted the sodium salt to the sulfonic acid by passage through a column of Dowex-50 H^+ resin, added cyclohexylamine to neutralize the eluent, and concentrated the crude salt. Repeated fractional crystallization afforded a pure diastereoisomer, with melting-point and NMR data matching that reported previously.[13] Benson identified this material as the 2'*R*-stereoisomer by mixed melting point analysis with the cyclohexylammonium salt of naturally derived material, for which the stereochemistry was defined by use of the radiotracer method with co-precipitation with authentic L-, but not D-

glycerate,[24] a conclusion subsequently confirmed by X-ray analysis of the rubidium salt of naturally-derived SQGro.[25] We confirmed the assignment of our synthetic material as 2'*R*-SQGro through direct single crystal X-ray crystallographic analysis of the recrystallized material (Scheme 1A, Inset; Figure S1).

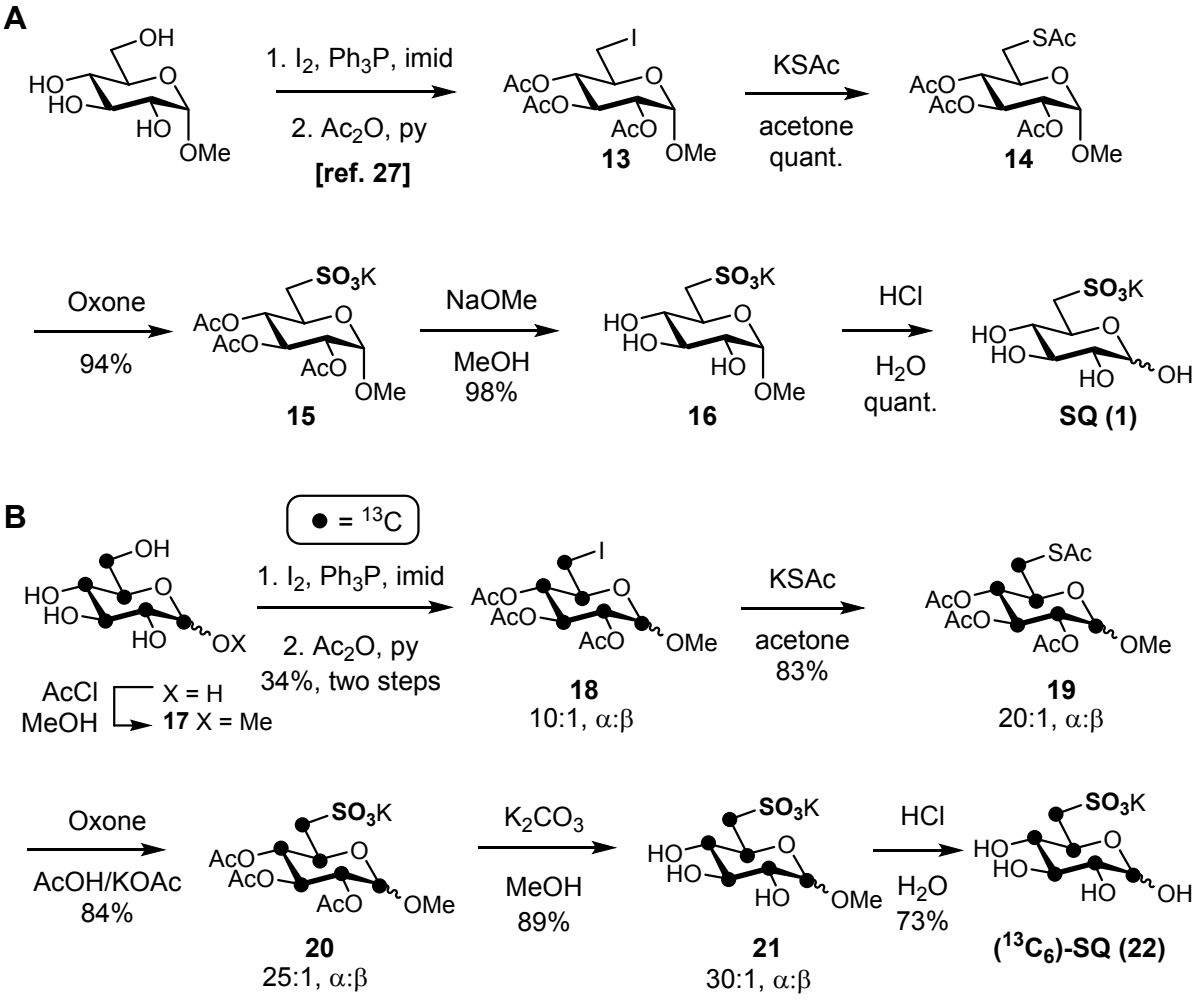


Scheme 1. Synthesis of sulfoquinovosylglycerol (SQGro) and 4-nitrophenyl α-D-sulfoquinovoside (PNPSQ). Inset: thermal ellipsoid plot for 2'*R*-SQGro, ellipsoids are at the 50% probability level.

We have previously reported the synthesis of PNPSQ (**7**) from PNPGlc (**11**), through a two-step procedure involving Mitsunobu reaction with thioacetic acid, followed by oxidation with KOAc/AcOH buffered mCPBA.[8] However, this procedure suffered from poor stability of the intermediate thioacetate, leading to variable yields for the subsequent oxidation. Careful analysis of reaction mixtures suggested that part of the reason for the instability of the thioacetate was its facile hydrolysis to the thiol and oxidation to the disulfide. Additionally, during the oxidation reaction, the acetyl group was found to migrate to the 4-position, to yield small amounts of 4-*O*-acetyl-PNPSQ. We sought to improve this reaction by use of thiobenzoic acid, which we anticipated may provide a more stable thioester that should be less prone to acyl migration in the oxidation step. Accordingly, Mitsunobu reaction of **11** with thiobenzoic acid afforded the thiobenzoate **12** in 81% yield (Scheme 1B). While this could be oxidized to

PNPSQ with KOAc/AcOH buffered mCPBA, better yields were obtained with Oxone in AcOH,[26] affording PNPSQ in 61% yield.

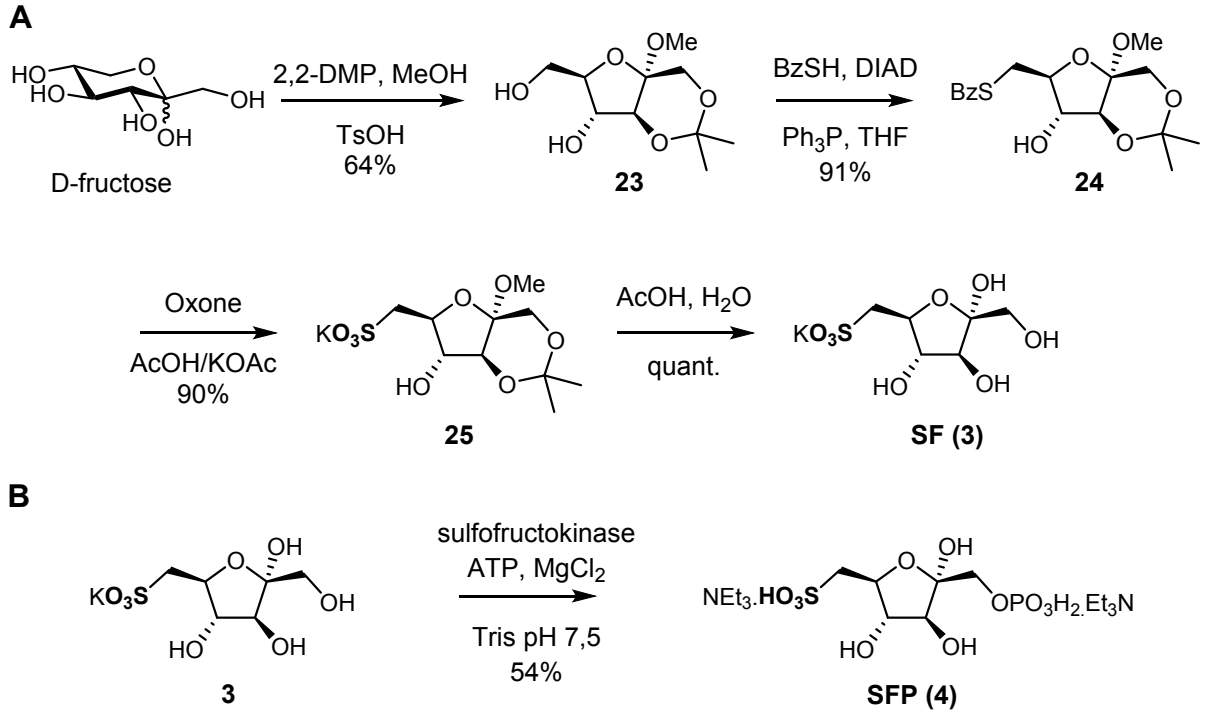
Synthesis of SQ. Several syntheses of SQ have been reported, which use either 1,2-*O*-isopropylidene-α-D-glucofuranose[13-15] or methyl glucopyranoside[16] as starting materials, with the sulfonate group introduced directly by nucleophilic substitution of a leaving group with sulfite. While direct, these methods provide products that can be difficult to purify. We herein report a longer route, but one that in our hands has proven to be easily scaled to multigram quantities of SQ, and through which the use of protecting groups enables purification of intermediates by crystallization or silica gel chromatography. Using the method of Garegg and Sameulsson (I_2, Ph_3P, imidazole; then Ac_2O, pyr), methyl α-D-glucopyranoside was converted to the iodide **13**,[27] then substituted with potassium thioacetate to afford the thioester **14** (Scheme 2A). Oxidation with Oxone afforded the protected sulfonate **15**, and treatment with NaOMe/MeOH allowed deprotection to give the methyl glycoside **16**. Finally, SQ (**1**) was obtained by treatment with aqueous HCl.



Scheme 2. Synthesis of sulfoquinovose (SQ) and ($^{13}C_6$)-SQ.

A modified version of this approach was used for the synthesis of ($^{13}C_6$)-SQ (**22**). ($^{13}C_6$)-Glucose was converted to a mixture of methyl glucopyranosides **17** by treatment with HCl in MeOH at reflux. Next, iodination using I_2, Ph_3P and imidazole in toluene,[27] followed by work-up and then acetylation and recrystallization, afforded the iodide **18**. Substitution with KSAc (→**19**), oxidation with Oxone (→**20**), deacetylation with K_2CO_3 (→**21**) and finally acid-catalyzed glycoside hydrolysis afforded ($^{13}C_6$)-SQ (**22**).

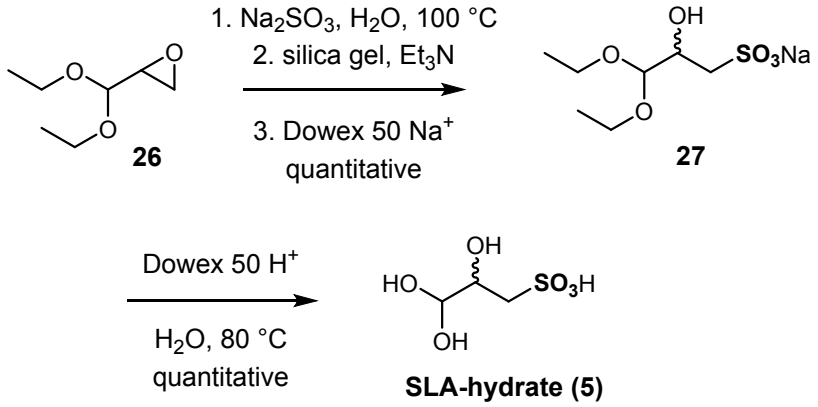
Synthesis of SF and SFP. For the synthesis of SF, we were attracted to a key intermediate, the bisacetal **23**, which can be prepared in one step from D-fructose, as reported by Hong and co-workers.[28] Treatment of D-fructose with MeOH/TsOH, and then 2,2-dimethoxypropane (2,2-DMP), afforded **23** in 64% yield (Scheme 3A). Compound **23** was treated with thiobenzoic acid under Mitsunobu conditions to afford the thiobenzoate **24**, and this was oxidized with AcOH/KOAc buffered Oxone, to afford the sulfonate **25**. Finally, careful hydrolysis of the protected sulfonate in AcOH/water afforded SF (**3**) in quantitative yield. SF exists as a 3.7:1 α:β ratio of anomers, as determined by ^{1}H NMR spectroscopy.



Scheme 3. Synthesis of sulfofructose (SF) and sulfofructose-1-phosphate (SFP).

For SFP (**4**) we utilized an enzymatic approach catalysed by *E. coli* SF kinase YihV. YihV was expressed from a pET plasmid as a His_6-fusion and purified by immobilized metal ion affinity chromatography. Incubation of a solution of SF (**3**) with 2 equivalents of ATP and $MgCl_2$ in the presence of YihV afforded a solution containing SFP (Scheme 3B). This was purified using ion-paired HLPC with triethylammonium bicarbonate buffer, affording **4** as the bis(triethylammonium) salt, as determined by ^1H NMR spectroscopy. SF exists as a 4:1 α:β ratio of anomers.

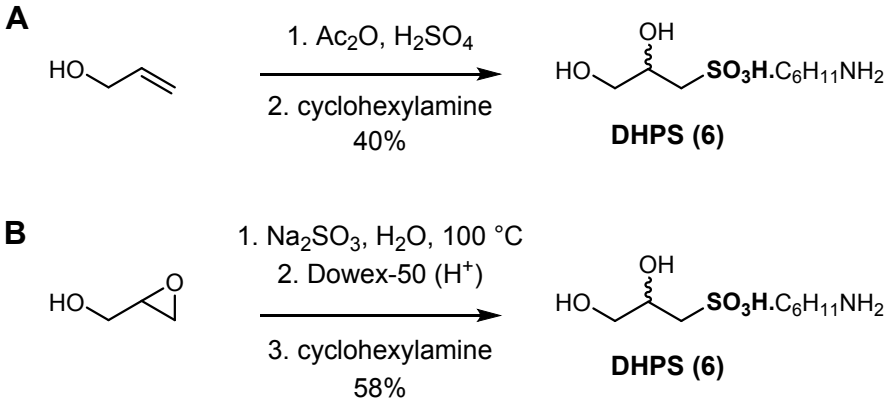
Synthesis of SLA. For the synthesis of SLA (**5**) we were inspired by processes used for the preparation of glyceraldehyde-3-phosphate. These prepare the diethyl acetal for long term storage, and then liberate the glyceraldehyde on demand through a brief treatment with acid resin.[29] Accordingly, we treated glycidol diethyl acetal (**26**) with sodium sulfite in water at reflux, which provided the diethyl acetal **27** after silica gel chromatography and ion-exchange (Scheme 4). To convert **27** to SLA, a solution of the diethyl acetal in H_2O or D_2O was treated at 80 °C with Dowex 50 H^+ resin. The resulting SLA was determined to be a hydrate, as indicated by a characteristic singlet at δ 4.44 ppm. No H/D exchange was noted at C2 over the period of several days.



Scheme 4. Synthesis of sulfolactaldehyde (SLA).

Synthesis of DHPS. Friese prepared DHPS in a direct, one-step fashion by treatment of allyl alcohol with Ac_2O and H_2SO_4,[17] and this procedure has been modernized by Mayer *et al.*[18] However, these procedures have a complex work-up involving multiple steps with evaporation of Ac_2O, water, conversion to the barium salt and back to the free acid before careful titration to the sodium salt, with no purification steps. While we were able to successfully reproduce this procedure, the lack of purification at the end left doubt as to the purity of the isolated

material. Consequently, we sought to simplify this approach and to develop a crystallization process. We were attracted to a short remark in an early paper by Benson who noted the potential for formation of a cyclohexylammonium salt.[30] After some experimentation we were able to reproduce this observation and confirm that this salt is crystalline, and here provide complete details. Our preferred process involves heating a solution of allyl alcohol, Ac_2O and H_2SO_4 as described by Mayer *et al*.,[18] then evaporation of the solvent and co-evaporation with H_2O until the pH of the distillate reached pH 6 (Scheme 5A). The residue is dissolved in EtOH and neutralized with cyclohexylamine and can be directly crystallized from EtOH/toluene to afford the pure cyclohexylammonium 2,3-dihydroxypropanesulfonate. While the yield is modest at 40%, the low cost of the reagents and the ease of the process readily allows preparation of decagram quantities.



Scheme 5. Synthesis of 2,3-dihydroxypropanesulfonate (DHPS).

We also devised an alternative approach for the synthesis of DHPS, in which glycidol is treated with sodium sulfite in water at reflux (Scheme 5B).[12] Passage of the reaction mixture through a Dowex-50 H^+ column is used to remove excess sulfite as sulfurous acid, and then conversion to the cyclohexylammonium salt allows isolation of the product in 58% yield.

Conclusions

The sulfoglycolytic Embden-Meyerhof-Parnas pathway is a major conduit through which organosulfur cycles in the terrestrial biosphere. However, detailed molecular studies have not been possible owing to the lack of methods to synthesize the substrates, intermediates and products of this pathway. The present disclosure of improved and in some cases new methods

for the preparation of this complete series of compounds will assist future studies to understand how SQ is processed by enzymes in this pathway.

Experimental

General

Pyridine was distilled over KOH before use. Dichloromethane and THF were dried over alumina according to the method of Pangborn *et al*.[31] Reactions were monitored using thin-layer chromatography (TLC), performed with silica gel 60 F254. Detection was effected by charring in a mixture of 5% sulfuric acid in methanol, 10% phosphomolybdic acid in EtOH, and/or visualizing with UV light. Flash chromatography was performed according to the method of Still *et al*.[32] using silica gel 60. Melting points were determined by a capillary apparatus. $[\alpha]_D$ values are given in deg 10^{-1} cm^2 g^{-1}. NMR experiments were conducted on 400 and 500 MHz instruments, with chemical shifts referenced relative to residual protiated solvent and are in parts per million (ppm). ^{1}H−^{1}H COSY and HMQC spectra were used to confirm proton and carbon assignments, respectively. Mass spectra were acquired in the ESI-QTOF mode.

1. Synthesis of SQGro

Allyl 6-bromo-6-deoxy-α-D-glucopyranoside (9). Carbon tetrabromide (2.10 g, 6.40 mmol) and triphenylphosphine (1.90 g, 7.20 mmol) were added to a solution of allyl α-D-glucopyranoside **8** (1.00 g, 4.50 mmol) in dry pyridine (12 mL) at 0 °C. The solution was warmed to room temperature and was stirred overnight under a nitrogen atmosphere. After the completion of the reaction, methanol (2 ml) was added and the solvent was removed under reduced pressure to give a gum. The gum was partitioned between toluene (25 mL) and water (25 mL). The aqueous phase was collected, and the toluene phase was extracted with water (10 mL). The combined aqueous layers were washed with additional toluene (10 mL). The combined aqueous layers were then evaporated to give a gum that was purified by flash chromatography (75-100% EtOAc/hexanes). Evaporation of the eluent yielded **9** as an off-white solid (0.710 g, 58%). m.p. 80-82 °C; $[\alpha]_D^{25}$ +114.9 ° (*c* 0.47, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD) δ 6.04–5.90 (1 H, m, H2'), 5.34 (1 H, d, $J_{2',3'trans}$ = 18.2 Hz, H3'a), 5.18 (1 H, d, $J_{2',3'cis}$ = 10.5 Hz, H3'b), 4.83 (1 H, d, $J_{1,2}$ = 3.8 Hz, H1), 4.26 (1 H, dd, $J_{1'a,1'b}$ = 13.0, $J_{1'a,2'}$ = 5.1 Hz, H1'a), 4.05 (1 H, dd, $J_{1'b,2}$ = 6.1 Hz, H1'b), 3.78–3.68 (2 H, m, H5,6a), 3.65 (1 H, t, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, H3), 3.52 (1 H, dd, $J_{6a,6b}$ = 10.4, $J_{5,6a}$ = 6.4 Hz, H6b), 3.40 (1 H, dd, H2), 3.24 (1 H, t, $J_{4,5}$ = 9.1 Hz, H4); ^{13}C{^{1}H} NMR (101 MHz, CD_3OD) δ 134.0 (C2'), 116.4 (C3'), 97.7 (C1), 73.4 (C3), 72.5 (C4), 72.0 (C2), 71.3 (C5), 67.9 (C1'), 32.9 (C6); HR-ESI-MS calcd for $C_{10}H_{16}BrO_5$ $[M+HCO_2]^-$ 327.0085, found 327.0057.

Sodium allyl 6-deoxy-6-sulfonato-α-D-glucopyranoside (10). A solution of anhydrous sodium sulfite (0.937 g, 7.40 mmol) and compound **9** (0.500 g, 1.86 mmol) in water (15 mL) was heated under nitrogen at reflux for 5 h. The solution was cooled to room temperature and the solvent was evaporated under reduced pressure. A slurry of the resulting white solid in methanol (10 mL) was stirred overnight, filtered and the filtrate evaporated to give a crude residue (0.700 g). The residue was purified by flash

chromatography (27:2:1 to 6:2:1 EtOAc/MeOH/H_2O), to give **10** as a clear viscous oil (0.380 g, 67% yield). $[\alpha]_D^{25}$ +94.9° (*c* 0.32, CH_3OH); ^1H NMR (400 MHz, CD_3OD) δ 6.04–5.92 (1 H, m, H2'), 5.38 (1 H, d, $J_{2',3'trans}$ = 18.7 Hz, H3'a), 5.15 (1 H, d, $J_{2',3'cis}$ = 11.5 Hz, H3'b), 4.81 (1 H, d, $J_{1,2}$ = 3.8 Hz, H1), 4.40 (1 H, dd, $J_{1'a,1'b}$ = 12.9, $J_{1'a,2}$ = 5.2 Hz, H1'a), 4.14–4.01 (2 H, m, H5,1'b), 3.67 (1 H, t, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, H3), 3.42 (1 H, dd, $J_{1,2}$ =3.8 Hz, H2), 3.35 (1 H, dd, $J_{6a,6b}$ = 14.4, $J_{5,6a}$ = 2.1 Hz, H6a), 3.10 (1 H, t, $J_{4,5}$ = 9.4 Hz, H4), 2.94 (1 H, dd, $J_{5,6b}$ = 8.9 Hz, H6a); $^{13}C\{^1H\}$ NMR (101 MHz, CD_3OD) δ 134.2 (C2'), 116.4 (C3'), 97.1 (C1), 73.7 (C4), 73.5 (C2), 72.0 (C3), 68.2 (C5), 67.7 (C1'), 53.0 (C6); HR-ESI-MS calcd for $C_9H_{15}O_8S$ [M^-] 283.0488, found 283.0481.

Sodium 2'*R*/*S*-glyceryl 6-deoxy-6-sulfonato-α-D-glucopyranoside (2). A solution of **10** (0.300 g, 0.980 mmol) in water (5 mL) was added to a stirred mixture of $K_2OsO_4.2H_2O$ (0.007 g, 0.0196 mmol), $(DHQD)_2PHAL$ (0.031 g, 0.0392 mmol), *N*-methylmorpholine *N*-oxide (0.230 g, 1.96 mmol) in *t*-butanol/water (4:5) (9 mL). The resulting mixture was stirred under nitrogen for 24 h. The reaction was quenched by the addition of anhydrous sodium sulfite (0.246 g, 1.96 mmol), and the mixture was stirred for 30 min. The solvent was evaporated under reduced pressure and the crude residue was subjected to flash chromatography (EtOAc/MeOH/H_2O, 8:2:1 to 5:2:1), to give **2** as a gum, 1:1 mixture of diastereoisomers (0.300 g, 91%). ^1H NMR (600 MHz, CD_3OD) δ 4.77 (1 H, d, *J* = 4.1 Hz, (H1-2'*R*), 4.76 (1 H, d, *J* = 4.1 Hz, (H1-2'*S*), 4.11–3.99 (4 H, m, H5,1'a-2'*R*,5,1'a-2'*S*), 3.92–3.85 (1 H, m, H2'-2'*R*), 3.83–3.78 (1 H, m, H2'-2'*S*), 3.71 (1 H, dd, *J* = 11.4, 5.4 Hz, H3'b-2'*S*), 3.66–3.48 (6 H, m, H3,3'a,3'b-2'*R*,3,3'a,1'b-2'*S*), 3.41 (2 H, ddd, *J* = 9.7, 3.8, 1.7 Hz, H2-2'*R*,2-2'*S*), 3.36–3.32 (3 H, m, H6a-2'*R*,6a-2'*S*,1'b-2'*R*), 3.10–3.03 (2 H, m, H4-2'*R*,4-2'*S*), 2.93–2.87 (2 H, m, H6b-2'*R*,6b-2'*S*); $^{13}C\{^1H\}$ NMR (101 MHz, CD_3OD) δ 98.7, 98.2 (2 C, C1-2'*R*/*S*), 73.7, 73.6 (2 C, C3-2'*R*/*S*), 73.4, 73.3 (2 C, C4-2'*R*/*S*), 72.1 (2 C, C2-2'*R*/*S*), 71.0, 70.8 (2 C, C2'-2'*R*/*S*), 69.3 (C1'-2'*R*), 68.5, 68.4, 68.3 (3 C, C1'-2'*S*,5-2'*R*/*S*), 62.8, 62.7 (2 C, C3'-2'*R*/*S*), 52.7, 52.6 (2 C, C6-2'*R*/*S*); HR-ESI-MS calcd for $C_9H_{17}O_{10}S$ [M^-] 317.0542, found 317.0536.

2'*R*-Glyceryl 6-deoxy-6-sulfonato-α-D-glucopyranoside, cyclohexylammonium salt (2'*R*-2). Sodium 2'*R*/*S*-glyceryl 6-deoxy-6-sulfonato-α-D-glucopyranoside **2** (0.300 g, 0.880 mmol) in water was passed through an Dowex 50 (H^+ form) to obtain an aqueous solution of the sulfonic acid, which was immediately treated with cyclohexylamine (0.104 g, 1.05 mmol) and evaporated to dryness to yield the cyclohexylamine salt of the mixture of diastereomers, as a semicrystalline solid. This was fractionally crystallized from ethanol (3 times) to yield the pure 2'*R*-stereoisomer (0.050 g, 14% for the combined fractional recrystallizations). mp 190.5-192 °C (lit.[30] 191-193 °C); $[\alpha]_D^{25}$ +75.3° (*c* 0.32, CH_3OH) (lit.[30] $[\alpha]_D^{25}$ +74.5° (*c* 18, H_2O, pH 4)); ^1H NMR (400 MHz, CD_3OD) δ 4.77 (1 H, d, $J_{1,2}$ = 3.7 Hz, H1), 4.14–4.01 (2 H, m, H5,1'a), 3.93–3.85 (1 H, m, H2'), 3.69–3.51 (3 H, m, H3,3'a,3'b), 3.44–3.32 (3 H, m, H2,1'b,6a), 3.11–3.00 (2 H, m, H4, cyclohexyl-C**H**), 2.91 (1 H, dd, $J_{6b,6a}$ = 14.2, $J_{5,6b}$ = 9.3 Hz, H6b), 2.06–1.94 (2 H, m, cyclohexyl-C$\mathbf{H}_2$), 1.92–1.79 (2 H, m, cyclohexyl-C$\mathbf{H}_2$), 1.74–1.64 (1 H, m, cyclohexyl-C$\mathbf{H}_2$), 1.46–1.12 (5 H, m, cyclohexyl-C$\mathbf{H}_2$); $^{13}C\{^1H\}$ NMR (101 MHz, CD_3OD) δ 98.8 (C1),

73.7 (C3), 73.6 (C4), 72.3 (C2), 71.1 (C2'), 69.4 (C1'), 68.4 (C5), 62.8 (C3'), 52.8 (C6), 50.1 (1 C, cyclohexyl-**C**H), 30.5, 24.5, 23.9 (5 C, cyclohexyl-**C**H_2); HR-ESI-MS calcd for $C_9H_{17}O_{10}S$ [M^-] 317.0542, found 317.0533.

2. PNPSQ synthesis

4-Nitrophenyl 6-*S*-benzoyl-6-thio-α-D-glucopyranoside (12). A solution of 4-nitrophenyl α-D-glucopyranoside **11** (0.100 g, 0.332 mmol) and thiobenzoic acid (59 µl, 0.50 mmol) in dry THF (2.0 ml) at 0 °C was added to a mixture of PPh_3 (0.13 g, 0.50 mmol) and DIAD (98 µl, 0.50 mmol) in THF (3.0 ml) at 0 °C. The reaction mixture was allowed to warm to r.t. and was stirred overnight. The mixture was concentrated, and the residue was purified by flash chromatography (EtOAc/pet. spirit, 30–100%), to afford **12** as a white solid (0.114 g, 81%). m.p. 103–105 °C); $[\alpha]_D^{23}$ +118.4° (*c* 0.50, CH_3OH); ^1H NMR (500 MHz, CD_3OD) δ 8.07–8.04 (2 H, m, Ar), 7.78–7.76 (2 H, m, Ph), 7.61–7.57 (1 H, m, Ph), 7.45–7.41 (2 H, m, Ph), 7.27–7.23 (2 H, m, Ar), 5.69 (1 H, d, $J_{1,2}$ = 3.7 Hz, H1), 3.85 (1 H, dd, $J_{2,3}$ = $J_{3,4}$ 9.2 Hz, H3), 3.74–3.68 (3 H, m, H2,5,6b), 3.36–3.34 (1 H, m, H4), 3.07–3.02 (1 H, m, H6a); $^{13}C\{^1H\}$ NMR (101 MHz, CD_3OD) δ 192.4 (C=O), 162.6, 143.6, 138.0, 134.7, 129.7, 127.9, 126.4, 118.0 (8 C, Ar,Ph), 98.2 (C1), 75.0 (C4), 74.8 (C3), 73.1, 73.0 (2 C, C2,5), 31.6 (C6); HR-ESI-MS calcd for $C_{19}H_{19}NO_8S$ [M + H]$^+$ 422.0904, found 422.0904.

Potassium 4-nitrophenyl 6-deoxy-6-sulfonato-α-D-glucopyranoside (7). Oxone (0.14 g, 0.22 mmol) was added to a solution of **12** (37.0 mg, 0.088 mmol) and KOAc (72 mg, 0.73 mmol) in glacial AcOH (3.0 ml). The mixture was stirred at rt for 24 h. The mixture was concentrated and the residue was purified by flash chromatography (EtOAc/MeOH/H_2O, 19:2:1→7:2:1) and C_{18} reversed phase chromatography (H_2O/CH_3CN, 95:5), affording **7** as a white solid (21.2 mg, 61%). m.p. 163 °C; $[\alpha]_D^{23}$ +166.2° (*c* 0.42, CH_3OH); ^1H NMR (400 MHz, CD_3OD) δ 8.22 (2 H, d, *J* 9.1 Hz, Ar), 7.44 (2 H, d, *J* 9.1 Hz, Ar), 5.52 (1 H, d, $J_{1,2}$ 3.6 Hz, H1), 4.16 (1 H, ddd, $J_{4,5}$ = 9.3, $J_{5,6a}$ = 8.1, $J_{5,6b}$ = 2.2 Hz, H5), 3.88 (1 H, dd, $J_{3,4}$ = 9.4, $J_{2,3}$ = 9.3 Hz, H3), 3.65 (1 H, dd, H2), 3.37 (1 H, dd, $J_{6a,6b}$ = 14.4 Hz, H6b), 3.28 (1 H, dd, H4), 3.02 (1 H, dd, H6a); $^{13}C\{^1H\}$ NMR (101 MHz, CD_3OD) δ 164.1, 144.0, 126.5, 118.9 (4 C, Ar), 100.0 (C1), 74.9 (C4), 74.5 (C3), 73.0 (C2), 71.2 (C5), 54.5 (C6); HR-ESI-MS calcd for $C_{12}H_{14}NO_{10}S$ [M^-] 364.0344 found 364.0331.

3. Synthesis of SQ and ($^{13}C_6$)-SQ

Methyl 2,3,4-tri-*O*-acetyl-6-*S*-acetyl-6-deoxy-6-thio-α-D-glucopyranoside (14). A mixture of methy1 2,3,4-tri-*O*-acetyl-6-deoxy-6-iodo-α-D-glucopyranoside[27] **13** (6.70 g, 0.016 mol) and KSAc (4.50 g, 0.039 mol) in acetone (200 ml) was refluxed for 24 h. The reaction mixture was concentrated using the rotary evaporator, dissolved in ethyl acetate, washed with water and brine and dried ($MgSO_4$). The mixture was evaporated and purified with flash chromatography (gradient elution, EtOAc/pet. spirit., 30:70 to 70:30), affording **14** as a yellow oil (5.93 g, 100%). $[\alpha]_D^{23}$ +94.1° (*c* 0.75, $CHCl_3$; lit.[33]

$[\alpha]_D$ +80.8°); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.43 (1 H, dd, $J_{3,4}$ = 9.3, $J_{2,3}$ = 10.1 Hz, H3), 4.93 (1 H, dd, $J_{4,5}$ = 9.9 Hz, H4), 4.89–4.83 (2 H, m, H1,2), 3.91 (1 H, ddd, $J_{5,6a}$ = 7.0, $J_{5,6b}$ = 3.0, $J_{4,5}$ = 10.0 Hz, H5), 3.39 (3 H, s, OCH_3), 3.20 (1 H, dd, $J_{6a,6b}$ = 14.2 Hz, H6b), 3.06 (1 H, dd, H6a), 2.34 (3 H, s, SAc), 2.07, 2.06, 1.99 (9 H, 3s, 3 x OAc); ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 194.7 (SC=O), 170.3, 170.2, 170.0 (3 C, OC=O), 96.7, 71.1, 71.0 (3 C, C1,2,4), 70.1 (C3), 68.4 (C5), 55.5 (OCH_3), 30.6 (SAc), 30.2 (C6), 20.9–20.8 (3 C, CH_3); HR-ESI-MS calcd for $C_{15}H_{22}O_9S$ $[M+H]^+$ 379.1057, found 379.1056.

Potassium methyl 2,3,4-tri-*O*-acetyl-6-deoxy-6-sulfonato-α-D-glucopyranoside (15). Oxone (21.0 g, 0.0340 mol) was added to a solution of **14** (5.10 g, 0.0130 mol) and NaOAc (18.0 g, 0.130 mol) in glacial AcOH (40 ml). The mixture was stirred at rt for 24 h. The mixture was concentrated and the residue was purified by flash chromatography (EtOAc/MeOH/H_2O, 15:2:1→5:2:1), affording **15** as a white solid (5.36 g, 94%). m.p. 152 °C (lit.[34] 113 °C); $[\alpha]_D^{23}$ +100.4° (*c* 1.31, $CHCl_3$; lit.[34] +129.3°); ^{1}H NMR (499 MHz, $CDCl_3$) δ 5.36 (1 H, t, $J_{2,3}$ = $J_{3,4}$ 9.4 Hz, H3), 4.97 (2 H, br. s, H1,4), 4.87 (1 H, d, H2), 4.23 (1 H, br. s, H5), 3.40 (3 H, s, OCH_3), 3.21 (1 H, br. s, H6b), 3.11 (1 H, d, $J_{5,6a}$ = 8.1 Hz, H6a), 2.05–1.95 (6 H, br. s, 2 x Ac), 1.91 (3 H, s, Ac); ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 171.0, 170.6, 170.1 (3 C, C=O), 96.6, 71.1 (2 C, C1,4), 70.8 (C2), 70.5 (C3), 65.9 (C5), 55.9 (OCH_3), 51.0 (C6), 21.0, 20.9, 20.8 (3 C, CH_3); HR-ESI-MS calcd for $C_{13}H_{19}O_{11}S$ $[M^-]$ 383.0654 found 383.0664.

Potassium methyl 6-deoxy-6-sulfonato-α-D-glucopyranoside (16). Potassium methyl 2,3,4-tri-*O*-acetyl-6-deoxy-6-sulfonato-α-D-glucopyranoside **15** (5.30 g, 0.0130 mol) was dissolved in MeOH and treated with Na (50 mg) and stirred at rt for 2 h. The mixture was neutralized with resin and purified by flash chromatography (EtOAc/MeOH/H_2O/, 10:2:1→5:2:1), affording **16** as a low-melting, hygroscopic white solid (3.63 g, 98%). $[\alpha]_D^{23}$ +87.1° (*c* 0.90, H_2O; lit.[35] $[\alpha]_D^{22}$ +82.1° in MeOH); ^{1}H NMR (500 MHz, D_2O) δ 4.80–4.78 (1 H, br. s, H1), 4.04 (1 H, dt, $J_{4,5}$ = $J_{5,6b}$ = 1.3, $J_{5,6a}$ = 9.9 Hz, H5), 3.68 (1 H, dd, $J_{2,3}$ = 8.0, $J_{3,4}$ = 10.8 Hz, H3), 3.60 (1 H, dd, $J_{1,2}$ = 3.8 Hz, H2), 3.48 (3 H, s, OCH_3), 3.41 (1 H, dd, $J_{6b,6a}$ = 14.7 Hz, H6b), 3.27 (1 H, dd, H4), 3.09 (1 H, dd, H6a); ^{13}C{^{1}H} NMR (101 MHz, D_2O) δ 98.9 (C1), 72.9 (C3), 72.3 (C4), 71.1 (C2), 67.7 (C5), 55.1 (OCH_3), 52.0 (C6); HR-ESI-MS calcd for $C_7H_{13}O_8S[M^-]$ 257.0337, found 257.0319.

Potassium 6-deoxy-6-sulfonato-D-glucopyranose (1). Compound **16** (2.40 g, 8.60 mmol) was dissolved in 2 M HCl and stirred at 100 °C for 24 h. The mixture was concentrated under reduced pressure and the residue was purified by flash chromatography (EtOAc/MeOH/H_2O, 10:2:1→2:2:1), affording **1** as a low-melting, hygroscopic light yellow solid (2.29 g, 100%). $[\alpha]_D^{23}$ +42.1° (*c* 0.64, H_2O; lit.[36] $[\alpha]_{546}^{20}$ +71.7°); ^{1}H NMR (400 MHz, D_2O) δ 5.22 (1 H, d, $J_{1,2}$ 3.7 Hz, H1α), 4.68 (1 H, d, $J_{1,2}$ 8.0 Hz, H1β), 4.24 (1 H, t, $J_{4,5}$ = $J_{5,6}$ = 9.4 Hz, H5α), 3.80 (1 H, t, $J_{4,5}$ = $J_{5,6}$ = 9.2 Hz, H5β), 3.73 (1 H, t, $J_{2,3}$ = $J_{3,4}$ = 9.4 Hz, H3α), 3.56 (1 H, dd, H2α), 3.50 (1 H, dd, $J_{2,3}$ = 5.9, $J_{3,4}$ = 15.3 Hz, H3β), 3.41 (1 H, d, $J_{6b,6a}$ = 14.7 Hz, H6bβ), 3.35–3.25 (3 H, m, H4α,2β,4β), 3.13–3.04 (3 H, m, H6bα,6aα,6aβ); ^{13}C{^{1}H} NMR (101 MHz, D_2O) δ 95.9 (C1β), 91.9 (C1α), 75.5 (C3β), 74.0 (C2β), 72.6 (C4α), 72.5 (C3α), 72.3

(C4β), 72.1 (C5β), 71.3 (C2α), 67.7 (C5α), 52.22, 52.16 (2 C, C6α,6β); HR-ESI-MS calcd for $C_6H_{11}O_8S$ [M^-] 243.0180, found 243.0202.

Methyl D-($^{13}C_6$)glucopyranoside (17). Acetyl chloride (0.80 mL, 11.2 mmol) was added to a solution of D-($^{13}C_6$)glucose (2.93 g, 15.8 mmol) in MeOH (60 mL). The solution was refluxed for 16 h and evaporated to dryness under reduced pressure to afford the title compound as a mixture of anomers (2.5:1, α:β) as a colourless glass. This was not purified, but was used directly in the next step. ^{1}H NMR (400 MHz, D_2O) δ 4.80 (1 H, dd, $^1J_{C1,H1}$ = 173.2 Hz, H1-α), 4.39 (1 H, dd, $^1J_{C1,H1}$ = 166.0, $J_{H1,H2}$ = 7.7 Hz, H1-β), 4.14–3.80 (2 H, m, H6a-α,6a-β), 3.97–3.69 (2 H, m, H6b-α,6b-β), 3.86–3.55 (1 H, m, H2-α), 3.78–3.45 (2 H, m, H3-α,5-α), 3.58 (3 H, d, $^3J_{C1,H-Me}$ = 4.3 Hz, OMe-β), 3.69–3.38 (2 H, m, H3-β,5-β), 3.42 (3 H, d, $^3J_{C1,H-Me}$ = 3.9 Hz, OMe-α), 3.41–3.17 (2 H, m, H4-α,4-β), 3.33–3.01 (1 H, m, H2-β); $^{13}C\{^1H\}$ NMR (101 MHz, D_2O) δ 103.1 (1 C, d, $^1J_{1,2}$ = 46.5 Hz, C1-β), 99.2 (1 C, dd, $^1J_{1,2}$ = 46.0, $^2J_{1,3}$ = 9.2 Hz, C1-α), 76.9–74.9 (2 C, m, C3-β,5-β), 73.7–72.2 (2 C, m, C2-β,3-α), 72.1–70.4 (2 C, m, C5-α,2-α), 70.3–68.8 (2 C, m, C4-α,4-β), 61.3–59.4 (2 C, m, C6-α,6-β), 55.0–54.8 (2 C, m, OMe-α,OMe-β); HR-ESI-MS calcd for $^{13}C_6{}^{12}CH_{14}O_6Na$ [M + Na]$^+$ 223.0889, found 223.0883.

Methyl 2,3,4-tri-*O*-acetyl-6-deoxy-6-iodo-D-($^{13}C_6$)glucopyranoside (18). A solution of **1** (3.15 g, 15.8 mmol), PPh_3 (6.26 g, 23.9 mmol), I_2 (5.65 g, 22.3 mmol) and imidazole (3.30 g, 48.5 mmol) in toluene (75 mL) was heated at 75°C for 2.5 h with vigorous stirring. The mixture was cooled, H_2O (90 mL) was added and the mixture was stirred vigorously for 15 min. The toluene phase was extracted with H_2O (3 × 30 mL), and the combined aqueous phase was washed with toluene (30 mL) and evaporated to dryness under reduced pressure. Ac_2O (33 mL) and pyridine (67 mL) were added to the residue and the mixture was stirred at rt for 16 h and then evaporated under reduced pressure. The residue was partitioned between toluene (40 mL) and H_2O (30 mL). The aqueous phase was washed with toluene (3 × 20 mL). The combined toluene phase was dried ($MgSO_4$) and evaporated to dryness under reduced pressure. The residue was crystallized from hot EtOH (26 mL) to afford **18** as a mixture of anomers (10:1, α:β) as colourless crystals (2.30 g, 34%). m.p. 137-139°C; $[\alpha]_D$ +103.3 (*c* 0.575, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.72–5.24 (1 H, m, H3-α), 5.20–4.61 (3 H, m, H1-α,2-α,4-α), 4.02–3.57 (1 H, m, H5-α), 3.48 (3 H, d, $^3J_{C1,H-Me}$ = 4.5 Hz, OMe-α), 3.41 (3 H, d, $^3J_{C1,H-Me}$ = 4.4 Hz, OMe-β), 3.52–3.07 (1 H, m, H6a-α), 3.36–2.90 (1 H, m, H6b-α), 2.10–2.06 (6 H, m, 2 × Ac-β), 2.09–2.04 (6 H, m, 2 × Ac-α), 2.02 (3 H, s, Ac-β), 2.00 (3 H, s, Ac-α); $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 170.5–169.4 (3 C, m, C=O-α), 101.6 (1 C, dd, $^1J_{1,2}$ = 46.9, $^2J_{1,3}$ = 25.8 Hz, C1-β), 96.9 (1 C, d, $^1J_{1,2}$ = 45.4 Hz, C1-α), 74.4–73.2 (1 C, m, C4-β), 72.6 (1 C, t, $^1J_{3,4}$ = $^1J_{4,5}$ = 39.7 Hz, C4-α), 72.1–71.7 (1 C, m, C2-β), 71.1 (1 C, dd, $^1J_{1,2}$ = 45.0, $^1J_{2,3}$ = 41.5 Hz, C2-α), 69.7 (1 C, t, $^1J_{2,3}$ = $^1J_{3,4}$ = 40.0 Hz, C3-α), 68.7 (1 C, t, $^1J_{4,5}$ = $^1J_{5,6}$ = 41.5 Hz, C5-α), 67.2 (1 C, t, $^1J_{3,4}$ = $^1J_{4,5}$ = 42.7 Hz, C3-β), 62.0 (1 C, d, $^1J_{4,5}$ = $^1J_{5,6}$ = 44.2 Hz, C5-β), 55.9 (1 C, s, OMe-α), 20.9 (3 C, s, COMe-α), 3.8 (1 C, d, $^1J_{5,6}$ = 42.7 Hz, C6-α), 3.1 (1 C, d, $^1J_{5,6}$ = 40.8 Hz, C6-β); HR-ESI-MS calcd for $^{13}C_6{}^{12}C_7H_{19}O_8INa$ [M + Na]$^+$ 459.0224, found 459.0216.

Methyl 2,3,4-tri-*O*-acetyl-6-*S*-acetyl-6-deoxy-6-thio-D-($^{13}C_6$)glucopyranoside (19). A solution of **2** (2.30 g, 5.3 mmol) and KSAc (1.53 g, 13.4 mmol) in acetone (80 mL) was refluxed for 16 h. The mixture was evaporated to dryness under reduced pressure and dissolved in EtOAc (42 mL). The organic phase was washed with H_2O (3 × 11 mL) and saturated $NaHCO_3$ solution (3 × 11 mL), dried ($MgSO_4$) and evaporated to dryness under reduced pressure. The residue was purified by flash chromatography (EtOAc/pet. spirit, 30:70 → 70:30) to afford **19** as a mixture of anomers (20:1, α:β) as a reddish-yellow oil (1.68 g, 83%). $[\alpha]_D$ +90.2 (*c* 1.04, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.63–5.25 (1 H, m, H3-α), 5.12–4.66 (3 H, m, H4-α,1-α,2-α), 4.06–3.75 (1 H, m, H5-α), 3.48 (3 H, d, $^3J_{C1,H\text{-}Me}$ = 4.7 Hz, OMe-β), 3.38 (3 H, d, $^3J_{C1,H\text{-}Me}$ = 4.6 Hz, OMe-α), 3.33–3.04 (1 H, m, H6a-α), 3.21–2.91 (1 H, m, H6b-α), 2.33 (3 H, s, SAc-α), 2.10–2.05 (6 H, m, 2 × OAc-α), 1.98 (3 H, s, OAc-α); ^{13}C{^{1}H} NMR (151 MHz, $CDCl_3$) δ 194.8 (1 C, s, SC=O-α), 172.2–169.6 (3 C, m, OC=O-α), 101.5 (1 C, d, $^1J_{1,2}$ = 48.6 Hz, C1-β), 96.7 (1 C, d, $^1J_{1,2}$ = 46.6 Hz, C1-α), 73.6–72.5 (2 C, m, C2-β,4-β), 71.6–69.5 (3 C, m, C2-α,3-α,4-α), 68.3 (1 C, t, $^1J_{4,5}$ = $^1J_{5,6}$ = 41.2 Hz, C5-α), 67.2 (1 C, t, $^1J_{2,3}$ = $^1J_{3,4}$ = 42.8 Hz, C3-β), 62.1 (1 C, d, $^1J_{4,5}$ = $^1J_{5,6}$ = 44.5 Hz, C5-β), 55.5 (1 C, s, OMe-α), 30.6 (1 C, s, SAc-α), 30.5–30.4 (1 C, m, C6-β), 30.2 (1 C, d, $^1J_{5,6}$ = 42.0 Hz, C6-α); HR-ESI-MS calcd for $^{13}C_6{}^{12}C_9H_{22}O_9SNa$ $[M + Na]^+$ 407.1084, found 407.1077.

Potassium methyl 2,3,4-tri-*O*-acetyl-6-deoxy-6-sulfonato-D-($^{13}C_6$)glucopyranoside (20). Oxone (5.53 g, 9.0 mmol) was added to a mixture of **3** (1.68 g, 4.4 mmol) and KOAc (4.32 g, 44.0 mmol) in AcOH (17 mL) and H_2O (2.4 mL). The mixture was stirred at rt for 20 h, then evaporated to dryness under reduced pressure. The residue was purified by flash chromatography (EtOAc/MeOH/H_2O, 12:2:1 → 5:2:1) to afford **20** as a mixture of anomers (25:1, α:β) as a colourless solid (1.57 g, 84%). $[\alpha]_D$ +108.1° (*c* 1.06, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.44 (1 H, d, $^1J_{C3,H3}$ = 150.2 Hz, H3-α), 5.39–4.70 (3 H, m, H1-α,4-α,2-α), 4.30 (1 H, d, $^1J_{C5,H5}$ = 148.7 Hz, H5-α), 3.59 (3 H, s, OMe-β), 3.47 (3 H, s, OMe-α), 3.40–2.95 (2 H, m, H6a-α, H6b-α), 2.09–2.03 (6 H, m, 2 × OAc-α), 1.99 (3 H, s, OAc-α); ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 170.9, 170.5, 170.1 (3 C, 3 x s, C=O-α), 102.1 (1 C, d, $^1J_{1,2}$ = 42.5 Hz, C1-β), 96.5 (1 C, d, $^1J_{1,2}$ = 41.6 Hz, C1-α), 72.1–69.6 (3 C, m, C4-α,3-α,2-α), 65.9 (1 C, t, $^1J_{4,5}$ = $^1J_{5,6}$ = 40.1 Hz, C5-α), 55.7 (1 C, s, OMe-α), 51.2 (1 C, d, $^1J_{5,6}$ = 40.1 Hz, C6-α), 21.0–20.7 (3 C, m, O=CMe-α); HR-ESI-MS calcd for $^{13}C_6{}^{12}C_7H_{19}O_{11}S$ $[M^-]$ 389.0849, found 389.0863.

Potassium methyl 6-deoxy-6-sulfonato-D-($^{13}C_6$)-glucopyranoside (21). A mixture of **4** (1.57 g, 3.7 mmol) and K_2CO_3 (0.37 g, 2.7 mmol) in MeOH (12 mL) was stirred at rt for 4 h. The mixture was neutralized with AcOH (0.20 mL, 3.5 mmol) and evaporated to dryness under reduced pressure. The residue was purified by flash chromatography (EtOAc/MeOH/H_2O, 6:2:1 → 3:2:1) to afford **21** as a mixture of anomers (30:1, α:β) as a colourless solid (0.99 g, 89%). $[\alpha]_D$ +85.7° (*c* 0.82, MeOH); ^{1}H NMR (400 MHz, D_2O) δ 4.99–4.46 (1 H, m, H1-α), 4.22–3.75 (1 H, m, H5-α), 3.85–3.46 (1 H, m, H3-α), 3.75–3.39 (1 H, m, H2-α), 3.42 (3 H, d, $^3J_{C1,H\text{-}Me}$ = 4.2 Hz, OMe-α), 3.55–3.14 (1 H, m, H6a-α), 3.44–2.94 (1 H, m, H4-α), 3.26–2.80 (1 H, m, H6b-α); ^{13}C{^{1}H} NMR (101 MHz, D_2O) δ 102.9 (1 C, d,

$^{1}J_{1,2}$ = 41.9 Hz, C1-β), 98.8 (1 C, dd, $^{1}J_{1,2}$ = 45.8, $^{2}J_{1,3}$ = 6.9 Hz, C1-α), 73.6–71.6 (2 C, m, C3-α,2-α), 71.0 (1 C, dd, $^{1}J_{3,4}$ = 44.3, $^{1}J_{4,5}$ = 38.3 Hz, C4-α), 67.6 (1 C, dd, $^{1}J_{5,6}$ = 41.3, $^{1}J_{4,5}$ = 35.7 Hz, C5-α), 55.0 (1 C, s, OMe-α), 51.9 (1 C, d, $^{1}J_{5,6}$ = 41.5 Hz, C6-α); HR-ESI-MS calcd for $^{13}C_6{}^{12}CH_{13}O_8S$ [M^-] 263.0532, found 263.0521.

Potassium 6-deoxy-6-sulfonato-D-($^{13}C_6$)glucose (22). Aq. HCl (1.6 mL, 2 M, 3.1 mmol) was added to a solution of **5** (0.97 g, 3.2 mmol) in H_2O (8 mL). The mixture was heated at 100°C for 24 h and evaporated to dryness under reduced pressure. The residue was purified by reverse phase chromatography (100% H_2O) and treated with Dowex 50 ion exchange resin (K^+ form) to afford **22** as a mixture of anomers (1:1, α:β) as a pale yellow solid (0.68 g, 73%). $[\alpha]_D$ +26.8° (*c* 1.30, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 5.21 (1 H, d, $^{1}J_{C1,H1}$ = 171.5 Hz, H1-α), 4.67 (1 H, dd, $^{1}J_{C1,H1}$ = 161.5, $J_{H1,H2}$ = 7.8 Hz, H1-β), 4.44–4.02 (1 H, m, H5-α), 4.02–3.28 (4 H, m, H5-β,3-α,2-α,3-β), 3.41 (2 H, dd, $^{1}J_{C6,H6}$ = 134.7, $^{2}J_{H6,H6'}$ = 14.5 Hz, H6a-β,6a-α), 3.49–3.06 (3 H, m, H6b-β,6b-α,4-β), 3.30–2.86 (2 H, m, H4-α,2-β); ^{13}C{^{1}H} NMR (101 MHz, D_2O) δ 95.8 (1 C, dd, $^{1}J_{1,2}$ = 45.1, $^{2}J_{1,3}$ = 4.8 Hz, C1-β), 91.9 (1 C, dd, $^{1}J_{1,2}$ = 44.5, $^{2}J_{1,3}$ = 13.5 Hz, C1-α), 76.1–74.9 (1 C, m, C3-β), 74.6–73.3 (1 C, m, C2-β), 73.1–71.9 (4 C, m, C3-α,4-β,4-α,5-β), 71.8–70.6 (1 C, m, C2-α), 68.4–66.9 (1 C, m, C5-α), 53.2–51.5 (2 C, m, C6-α,6-β); HR-ESI-MS calcd for $^{13}C_6H_{11}O_8S$ [M^-] 249.0376, found 249.0363.

4. Synthesis of SF

Methyl 1,3-*O*-isopropylidene-α-D-fructofuranoside (23). A mixture of fructose (1.0 g, 5.6 mmol) and TsOH (48 mg, 0.28 mmol) in anhydrous MeOH (5.6 ml) was stirred overnight. The reaction mixture was treated with 2,2-dimethoxypropane (5.6 ml) and stirred at rt for 45 min. Anhydrous $NaHCO_3$ to neutralize the solution, and the mixture was filtered, evaporated and the residue was purified by flash chromatography ($CHCl_3$/MeOH/Et_3N, 100:0:5→80:20:5) to afford **23** as a yellow oil (0.832 g, 64%). $[\alpha]_D^{23}$ +34.5° (*c* 0.50, $CHCl_3$; lit.[28] $[\alpha]_D$ +38.6° in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.11 (1 H, dt, $J_{5,6}$ = 3.3, $J_{4,5}$ = 4.9 Hz, H5), 4.04–3.89 (4 H, m, H1b,1a,3,4), 3.84–3.75 (2 H, m, H6b,6a), 3.30 (3 H, s, OCH_3), 1.45, 1.36 (6 H, 2s, 2 x CH_3); ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 101.4, 98.9 (2 C, C2,7), 87.7 (C5), 79.8, 77.8, 61.9 (3 C, C1,3,4), 62.9 (C6), 48.8 (OCH_3), 27.9, 19.4 (2 C, CH_3); HR-ESI-MS calcd for $C_{10}H_{18}O_6$ [M+H]$^+$ 235.1176, found 235.1176.

Methyl 6-*S*-benzoyl-1,3-*O*-isopropylidene-6-thio-α-D-fructofuranoside (24). A solution of **23** (0.40 g, 1.7 mmol) and thiobenzoic acid (0.30 ml, 2.6 mmol) in dry THF (10 ml) at 0 °C was added to a mixture of PPh_3 (0.67 g, 2.6 mmol) and DIAD (0.50 ml, 2.6 mmol) in THF (10 ml) at 0 °C. The reaction mixture was allowed to warm to r.t. and stirred for 1.5 h. The mixture was concentrated and the residue was purified by flash chromatography (EtOAc/pet. spirit/Et_3N, 1:9:0.1→3:7:0.1), to afford **24** as a yellow oil (0.550 g, 91%). $[\alpha]_D^{23}$ +43.4° (*c* 0.62, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.93-7.89 (2 H, m, Ph), 7.51–7.46 (1 H, m, Ph), 7.39–7.33 (2 H, m, Ph), 4.11 (1 H, dt, $J_{5,6}$ = 3.2, $J_{4,5}$ = 6.5 Hz, H5), 3.99–3.82 (4 H, m, H1a,1b,3,4), 3.41–3.34 (2 H, m, H6b,6a), 3.22 (3 H, s, OCH_3), 1.37, 1.33 (6 H, 2s,

2 x CH_3); $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$) δ 191.2 (C=O), 136.6, 133.4, 128.5, 127.2 (4 C, Ph), 102.3, 98.7 (2 C, C2,7), 85.1 (C5), 80.3, 79.8, 61.6 (3 C, C1,3,4), 48.5 (OCH_3), 31.6 (C6), 27.4, 19.9 (2 C, CH_3); HR-ESI-MS calcd for $C_{17}H_{22}O_6S$ $[M+H]^+$ 355.1201, found 355.1209.

Potassium methyl 6-deoxy-1,3-*O*-isopropylidene-6-sulfonato-α-D-fructofuranoside (25). Oxone (2.4 g, 3.9 mmol) was added to a solution of **24** (0.55 g, 1.6 mmol) and KOAc (1.5 g, 15 mmol) in glacial AcOH (8.0 ml) and the mixture was stirred at rt for 24 h. The mixture was concentrated and the residue was purified by flash chromatography (EtOAc/MeOH/H_2O, 10:2:1→7:2:1), affording **25** as a white crystalline solid (0.470 g, 90%). m.p. 177 °C; $[\alpha]_D^{23}$ +21.2° (*c* 0.55, MeOH); ^1H NMR (500 MHz, CD_3OD) δ 4.36–4.30 (1 H, m, H5), 4.09 (1 H, dd, $J_{3,4}$=1.2, $J_{4,5}$= 4.6 Hz, H4), 4.04 (1 H, d, $J_{3,4}$ = 1.3 Hz, H3), 3.98 (1 H, d, $J_{1a,1b}$ = 12.2 Hz, H1b), 3.82 (1 H, d, H1a), 3.35 (3 H, s, OCH_3), 3.28–3.18 (2 H, m, H6b,6a), 1.47 (3 H, s, CH_3), 1.38 (3 H, s, CH_3); $^{13}C\{^1H\}$ NMR (126 MHz, CD_3OD) δ 103.9, 100.4 (2 C, C2,7), 82.8 (C3), 82.0, 81.9 (2 C, C4,5), 62.8 (C1), 55.8 (C6), 48.8 (OCH_3), 27.1, 21.2 (2 C, CH_3); HR-ESI-MS calcd for $C_{10}H_{17}O_8S$ $[M^-]$ 297.0650, found 297.0656.

Potassium 6-deoxy-6-sulfonato-D-fructofuranose (3). Methyl 6-deoxy-1,3-*O*-isopropylidene-6-sulfonato-α-D-fructofuranoside potassium salt (0.13 g, 0.39 mmol) was dissolved in 1:1 acetic acid/H_2O (3 mL) and stirred at 25–30 °C for 72 h. The mixture was concentrated to afford **3** (3.7:1 α:β ratio of anomers) as a colourless oil (0.107 g, 100%). $[\alpha]_D^{23}$ +13.9° (*c* 0.86, H_2O); ^1H NMR (500 MHz, D_2O) δ 4.35 (1 H, ddd, $J_{4,5}$ = 4.8, $J_{5,6a}$ = 7.1, $J_{5,6b}$ = 8.1 Hz, H5-β), 4.21–4.09 (4 H, m, H3-β,3-α,4-α,5-α), 4.00 (1 H, dd, $J_{4,5}$ = 5.4, $J_{3,4}$ = 6.9 Hz, H4-β), 3.70 (1H, d, $J_{1b,1a}$ = 12.1 Hz, H1bβ) 3.63 (1 H, d, $J_{1b,1a}$ = 12.1 Hz, H1aβ), 3.60 (1 H, d, $J_{1b,1a}$ = 12.2 Hz, H1bα), 3.57 (1 H, d, $J_{1b,1a}$ = 12.2 Hz, H1aα), 3.37–3.20 (4 H, m, H6); $^{13}C\{^1H\}$ NMR (101 MHz, D_2O) δ 104.5 (C2-β), 101.7 (C2-α), 81.5 (C3-β), 79.5 (C4-β), 77.8, 76.1, 74.6 (3 C, C3-α,4-α,5-α), 76.7 (C5-β), 62.8 (C1-β), 62.7 (C1-α), 55.2 (C6-α), 53.7 (C6-β); HR-ESI-MS calcd for $C_6H_{11}O_8S$ $[M^-]$ 243.0180, found 243.0171.

5. Synthesis of SFP

Sulfofructose-1-phosphate, bis(triethylammonium) salt (4). A solution was prepared containing **3** (1000 µL of 172 mM stock; 0.048 g, 0.172 mmol), Na_3ATP (800 µL of 442 mM stock, 0.252 g, 0.353 mmol), Tris-buffer pH 7.5 (800 µL of 1.00 M stock), $MgCl_2$ (600 µL of 2000 mM stock), deionized water (Milli-Q; 775 µL). The final volume was 4.0 mL; the final concentrations were: SF (**3**) 43 mM, ATP 88 mM, Tris-buffer pH 7.5 100 mM and $MgCl_2$ 150 mM. YihV (12 mg/mL 25 µL) was added to the solution to start the reaction, which was kept at rt for 72 h. When the reaction was completed the solution was heated at 80 °C for 3 min. The supernatant was filtered through a 0.22 micron filter (Merck Millipore Ltd.). The reaction mixture was purified by HPLC-MS on a Shimadzu LCMS-2020 equipped with an LC-30AD pump, SIL-30AC autosampler and FRC-10A fraction collector, using a 150 x 21.2 mm ACE 10 SuperC18 column (part no. ACE-1311-1520). Solvent A: 50 mM aqueous triethylammonium bicarbonate pH 8.2; Solvent B: 50 mM triethylammonium bicarbonate in methanol.

The solvent program was 0-40% solvent B over 26 minutes, 40-100% solvent B over 0.5 minutes, 100% solvent B for 5.5 minutes and finally 0% solvent B for 8 minutes, flow rate of 8 mL/min. Fractions containing the product were combined and lyophilized to afford **4** (4:1 α:β ratio of anomers) as a colourless glass (49 mg, 54%). $[\alpha]_D^{23}$ +0.23° (*c* 3.65, H_2O); ^{1}H NMR (600 MHz, D_2O) δ 4.36 (0.22 H, ddd, *J* = 7.4, 6.3, 4.7 Hz, H5-β), 4.18–4.09 (2.74 H, m, H3,4-α,5-α), 4.05 (0.22 H, dd, $J_{4,5}$ = 6.2, $J_{3,4}$ = 5.1 Hz, H4-β), 3.95–3.80 (2 H, m, H1a-α,1a-β,1b-α,1b-β), 3.33–3.31 (2 H, m, H6a-α,6a-β,6b-α,6b-β), 3.26 (1 H, td, *J* = 7.9, 5.9 Hz, H6b), 3.21 (12 H, q, *J* = 7.3 Hz, $CH_3C\mathbf{H}_2$), 1.29 (18 H, t, *J* = 7.3 Hz, $C\mathbf{H}_3$); ^{13}C{^{1}H} NMR (151 MHz, D_2O) δ 104.1 ($^3J_{C,P}$ = 9.2 Hz, C2-β), 100.8 (d, $^3J_{C,P}$ = 9.8 Hz), 81.1, 79.2 (C3-β,4-β), 77.9 (C3-α,4-α,5-α), 77.7 (C5-β), 76.3, 75.1 (C3- α,4-α,5-α), 65.8 (C1-α), 65.3 (C1-β), 55.3 (C6-α), 54.0 (C6-β), 46.6 ($CH_3\mathbf{C}H_2$), 8.1 ($\mathbf{C}H_3$); ^{31}P{^{1}H} NMR (202 MHz, D_2O) δ 0.70; HR-ESI-MS calcd for $C_6H_{13}O_{11}PSNa$ [M+Na^+] 346.9808 found 346.9808.

6. Synthesis of SLA

Sodium DL-sulfolactaldehyde diethyl acetal (27). A solution of DL-glycidaldehyde diethylacetal **26** (0.100 g, 0.680 mmol) and anhydrous sodium sulfite (0.430 g, 3.40 mmol) in water (5 mL) was heated under reflux under nitrogen for 18 h. The solvent was evaporated under reduced pressure to afford a white solid that was purified by flash chromatography (27:2:1 EtOAc/MeOH/H_2O + 2% Et_3N to 15:8:4 EtOAc/MeOH/H_2O + 2% Et_3N) to give the product as the triethylammonium salt (0.150 g). This material was passed through a column of Dowex 50 (Na^+ form; 9 x 1.5 cm), and the eluent was evaporated to dryness to yield the sodium salt **27** as a glassy film (0.120 g, 71%). ^{1}H NMR (600 MHz, CD_3OD) δ 4.44 (1 H, d, $J_{1,2}$ = 4.7 Hz, H1), 4.10–4.06 (1 H, m, H2), 3.76–3.69 (2 H, m, H1'b,1"b), 3.64–3.56 (2 H, m, H1'a,1"a), 3.10 (1 H, dd, *J* = 14.2, 2.3 Hz, H3a), 2.89 (1 H, dd, *J* = 14.2, 9.5 Hz, H3b), 1.20 (3 H, m, *J* = 6.9 Hz, CH_3), 1.19 (3 H, t, *J* = 6.9 Hz, CH_3); ^{13}C{^{1}H} NMR (151 MHz, CD_3OD) δ 103.6 (C1), 68.6 (C2), 63.6 (C1"), 63.0 (C1'), 52.3 (C3), 14.2 (CH_3); HR-ESI-MS calcd for $C_7H_{15}O_6S$ [M^-] 227.0589, found 227.0572.

DL-Sulfolactaldehyde hydrate, free acid (5). Dowex 50 resin (H^+ form, 150 mg) was added to a solution of **27** (0.013 g, 0.050 mmol) in D_2O (0.50 ml). The mixture was stirred at 80 °C for 10 min and was filtered to remove the resin. NMR analysis indicated complete removal of acetal groups affording **5** as the hydrate in D_2O, along with 2 equivalents of EtOH. The product was stable as a solution at room temperature for several days, but decomposed upon removal of solvent. ^{1}H NMR (400 MHz, D_2O) δ 4.95 (1 H, d, $J_{1,2}$ 4.3 Hz, H1), 4.01–3.91 (1 H, m, H2), 3.63 (4 H, q, *J* 7.1 Hz, CH_2 of 2 × EtOH), 3.20 (1 H, dd, $J_{2,3b}$ = 2.7, $J_{3a,3b}$ = 14.5 Hz, H3b), 2.98 (1 H, dd, $J_{2,3a}$ = 8.9 Hz, H3a), 1.15 (6 H, t, *J* 7.1 Hz, CH_3 of 2 × EtOH); ^{13}C{^{1}H} NMR (101 MHz, D_2O) δ 90.6 (C1), 70.2 (C2), 52.6 (C3); HR-ESI-MS calcd for $C_3H_7O_6S$ [M^-] 170.9969, found 170.9974.

7. Synthesis of DHPS

Cyclohexylammonium 2,3-dihydroxypropanesulfonate (6).

(a) From allyl alcohol: A mixture of allyl alcohol (3.5 mL, 52 mmol), Ac_2O (15 mL, 159 mmol) and AcOH (5 mL, 88 mmol) was treated dropwise with H_2SO_4 (96%, 3 mL, 56 mmol). The mixture was heated for 3 h at 80°C. The solvent (approx 20 mL) was evaporated under reduced pressure and the residue was azeotroped with H_2O (12 × 15 mL) until the distillate's pH was 6. The residue was dissolved in EtOH (16 mL) and neutralized by addition of cyclohexylamine (7 mL, 61 mmol), at which point the solution was basic. The solution was evaporated to dryness under reduced pressure. The crude cyclohexylammonium DHPS was crystallized from EtOH/toluene (14 mL/34 mL) to afford **6** as white, powdery crystals (5.3 g, 40% over 2 crops). m.p. 52-53 °C; 1H NMR (400 MHz, D_2O) δ 4.09–3.99 (1 H, m, H2), 3.58 (1 H, dd, $J_{3a,3b}$ = 11.8, $J_{2,3b}$ = 3.6 Hz, H3b), 3.47 (1 H, dd, $J_{3a,3b}$ = 11.8, $J_{2,3a}$ = 6.4 Hz, H3a), 3.07–3.00 (1 H, m, cyclohexyl-CH), 3.00 (1 H, dd, $J_{1a,1b}$ = 14.6, $J_{1b,2}$ = 4.3 Hz, H1b), 2.91 (1 H, dd, $J_{1a,1b}$ = 14.4, $J_{1a,2}$ = 7.5 Hz, H1a), 1.90–0.97 (10 H, m, cyclohexyl-CH_2); $^{13}C\{^1H\}$ NMR (101 MHz, D_2O) δ 68.0 (C2), 64.5 (C3), 53.7 (C1), 50.3 (cyclohexyl-CH_2), 30.3, 24.2, 23.7 (cyclohexyl-CH_2); HR-ESI-MS calcd for $C_3H_7O_5S$ [M^-] 155.0014, found 155.0008.

(b) From glycidol: Anhydrous sodium sulfite (8.50 g, 67 mmol) was added to a stirred solution of glycidol (1.00 g, 13 mmol) in water (50 mL) and the solution was heated under nitrogen at reflux for 24 hours. After cooling to room temperature the reaction mixture was evaporated under reduced pressure. The resulting white solid was slurried in methanol (50 mL) for 4 hours, filtered and evaporated to give crude sodium 2,3-dihydroxypropanesulfonate (2.2 g). This was passed through Dowex 50 (H^+ form) and evaporated to obtain crude 2,3-dihydroxypropanesulfonic acid. This was dissolved in water (50 mL) again and treated with cyclohexylamine (1.41 g, 14.3 mmol) and followed by evaporating to dryness. The resulting slightly coloured viscous oil was purified by flash chromatography (30:2:1 to 5:2:1 EtOAc/MeOH/H_2O) to obtain **6** as a viscous oil (1.90 g, 58%). This can be recrystallized from 2-propanol/diethyl ether. m.p. 51-52 °C.

Cloning, expression and purification of SF kinase YihV. *Escherichia coli yihV* (NC_000913.3) was amplified through colony PCR using 'TOP10' *E. coli* cells (ThermoFisher) as template with the primers 5'-ATATACATATGATTCGTGTTGCTTGTGTAGG-3' and 5'-TGGTGCTCGAGTACAAAAAGTGACAAAAAAGATCG-3'. The product was cloned into pET21b(+) (Novagen) using the *NdeI* and *XhoI* restriction sites to provide pET21b-YihV. This plasmid was transformed into chemically competent 'T7 Express' *E. coli* cells (NEB), plated onto LB-agar (100 μg/ml ampicillin) and incubated at 37 °C for 16 h. A single colony was used to inoculate 10 ml of LB media (100 μg/ml ampicillin), which was incubated at 37 °C for 16 h with shaking (180 rpm). This culture was used to inoculate 1000 ml of S-broth media (100 μg/ml ampicillin), which was incubated at 37 °C with shaking (250 rpm). When the culture reached an OD_{600} of 0.7, it was cooled to room temperature and isopropyl β-D-thiogalactopyranoside added to a final concentration of 100 μM. This

culture was incubated at 18 °C for 19 h with shaking (220 rpm). The cells were harvested by centrifugation at 8000 g for 20 min at 4 °C then resuspended in 40 ml of binding buffer (50 mM NaPi, 500 mM NaCl, 5 mM imidazole, pH 7.4) containing protease inhibitor (Roche complete EDTA-free protease inhibitor cocktail) and lysozyme (0.1 mg/ml) by nutating at 4 °C for 30 min. Benzonase (1 µl) was added to the mixture and cell lysis achieved using sonication. The lysate was clarified by centrifugation (18,000 g for 20 min at 4 °C) and the supernatant filtered (0.45 µm) before loading onto a 1 ml HiTrap TALON column (GE Healthcare). The column was washed with 15 ml binding buffer then YihV was eluted using elution buffer (50 mM NaPi, 500 mM NaCl, 500 mM imidazole, pH 7.4). Fractions containing YihV (as determined by SDS-PAGE) were further purified by size exclusion chromatography on a HiLoad 16/600 Superdex 200 column using 50 mM NaPi, 150 mM NaCl, pH 7.4. Protein purity was estimated at >95% by Image Lab (Bio-Rad) analysis of Coomassie-stained SDS-PAGE and concentration determined by absorbance at 280 nm. The yield of YihV was 10 mg per litre of culture.

Single crystal X-ray structure determination of cyclohexylammonium 2'*R*-glyceryl a-D-sulfoquinovoside. Intensity data were collected at the Australian Synchrotron MX1 beamline[37] the temperature during data collection was maintained at 100.0(1) using an Oxford Cryosystems cooling device. The structure was solved by direct methods and difference Fourier synthesis.[38] Thermal ellipsoid plots were generated using the program ORTEP-3[39] integrated within the WINGX[40] suite of programs. Crystal data: $C_{24}H_{25}N_3O_4$, M = 419.47, T = 130.0 K, l = 0.71073 Å, Orthorhombic, space group $P2_12_12_1$, a = 6.9640(14) b = 9.1560(18), c =31.185(6) *Å*, V = 1988.4(7) *Å*^{3}, Z = 4, D_c = 1.395 mg M^{-3} m = 0.215 mm^{-1}, q_{max} = 32° F(000) = 896, crystal size 0.10 x 0.10 x 0.01 mm^3, 36298 reflections measured, 6184 independent reflections [R(int) = 0.098], the final R was 0.0465, [I > 2s(I), 5317 data] and wR(F^2) was 0.1276 (all data), GOOF = 1.045, Absolute structure parameter -0.08(4). The structure has been deposited at the CCDC (deposition number 1884470).

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Supporting Information

Supporting information: Figure S1, X-ray crystallography data and CIF file, ^{1}H, $^{13}C\{^{1}H\}$ and $^{31}P\{^{1}H\}$ (where relevant) NMR spectra for all new compounds and compounds prepared by new procedures.

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