A short gram-scale synthesis of Sulfavant A

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ABSTRACT

Recently we reported the promising activity as molecular vaccine adjuvants of a novel class of sulfoquinovosyl-diacylglycerols named Sulfavants. Herein we describe a modified and improved chemical synthesis of the lead product Sulfavant A (**1**), with the aim to produce from milligrams to ten grams of the pure compound that are necessary for the preclinical development. Starting from the versatile synthesis based on the trichloroacetimidate methodology, up-scaled preparation of Sulfavant A (**1**) was achieved in 11 steps by elimination and modification of those reactions that negatively affected the overall yield by the previous procedure. The novel strategy gave 17% overall yield of the target compound **1** and also paved the way for the gram-scale preparation of a wide range of other charged and neutral glycoglycerolipids.

Keywords: Sulfavants, adjuvant, sulfoquinovosyl-diacylglycerols, scale-up, glycolipids

INTRODUCTION

The importance of vaccines in elimination of human and veterinary infectious diseases has been dramatically brought to the forefront by the pandemic of COVID-19. On the other hand, vaccine research has greatly changed over the last years from an empirical approach to a rational design based on both molecular techniques and increasing knowledge of immune response. Currently, the identification and development of vaccine adjuvants have become a research priority in the field.[1–4] Adjuvants are substances that enhance the immune response to antigens by improving the capacity of the immune system to build a long standing and efficient response.[1–6] These products are mandatory to trigger the immune response and the resulting protection induced by synthetic

antigens, as well as they are considered crucial in the research of therapeutic vaccines to reduce the burden of chronic diseases such as cancer, obesity and neurodegenerative diseases. Sulfavant A (1) and the analogues Sulfavant S (2) and R (3), collectively named Sulfavants, are synthetic sulfolipids representing a new family of molecular vaccine adjuvants characterized by a sulfoquinovoside residue linked to a distearoylglycerol moiety through a beta anomeric linkage.[6-8]

Sulfavant A (1)

Sulfavant S (2)

Sulfavant R (3)
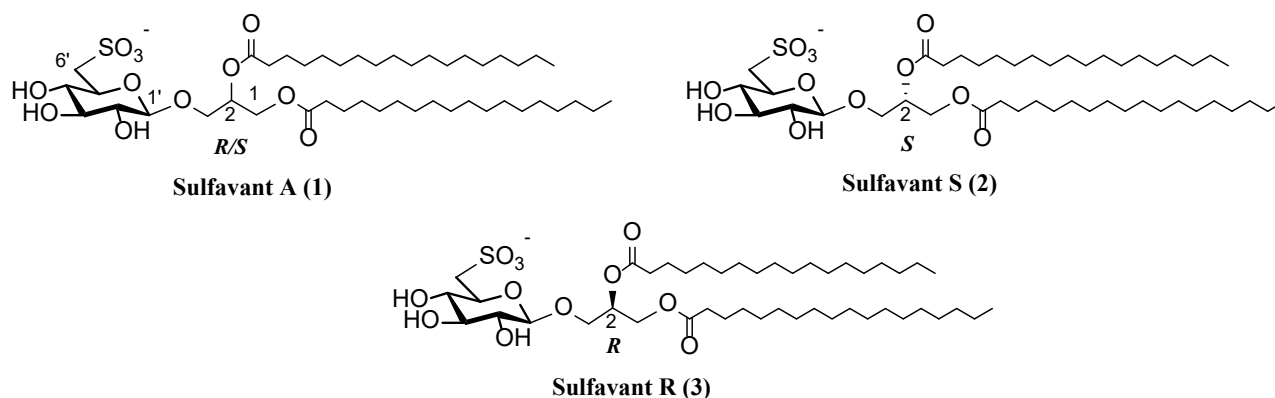
These molecules are able to effectively activate human Dendritic Cells (hDCs) determining an unusual maturation subset with up-regulation of MHC Class II and co-stimulatory molecules (HLA-DR, CD83, CD86, CD54), release of chemokines (CCL2, CCR7) and absence of cytokine synthesis except for interleukin-6 (IL-6) (manuscript in preparation). First preclinical tests of the lead compound Sulfavant A (**1**) highlighted induction of antigen-specific immunization with antibody titers comparable to commercial adjuvants in mice (e.g TiterMax).[6] In addition, vaccination with melanoma $hgp100_{25-33}$ peptide antigen and Sulfavant A (**1**) activated a protective response with reduction of tumour growth and increase of animal survival in a murine model of melanoma.[6]

Further biological studies on this new class of adjuvants are needed to define the mechanism of action and to carry out extensive preclinical trials for the preliminary evaluation of efficacy,

toxicity, pharmacokinetic and safety. The aim of the current work was the design and development of an improved synthesis to provide grams of Sulfavant A (**1**).

RESULT AND DISCUSSION

We have previously reported three slightly different synthesis of Sulfavants.[6-8] Unfortunately, none of these procedures was satisfactory for the gram-scale production of Sulfavant A (**1**) since we only achieved a total yield of 5.6%. In particular, the efficiency of the process was mostly lowered by the two hydrazinolysis reactions that, along with the following purification steps, caused the partial hydrolysis of the stearoyl chains and loss of product.

In order to bypass this issue, we designed a novel synthetic sequence that avoided one of the two limiting hydrazinolysis reactions and ameliorated several other steps by employing modified conditions. In particular, after the β-orienting coupling by trichloroacetimidate technology, the synthesis proceeded with deprotection of the sugar portion to give 1,2-*O*-isopropylidene-3-*O*-β-D-glucosyl-glycerol (**4**). This derivative is a versatile building block, useful for the preparation of a wide range of neutral and charged glycoglycerolipids (**Figure 1**). Specifically, compound **4** permits simple access to the functionalization of the entire sugar portion and the concomitant orthogonal protection of the glycerol moiety for the reaction with several acyl and alkyl chains.
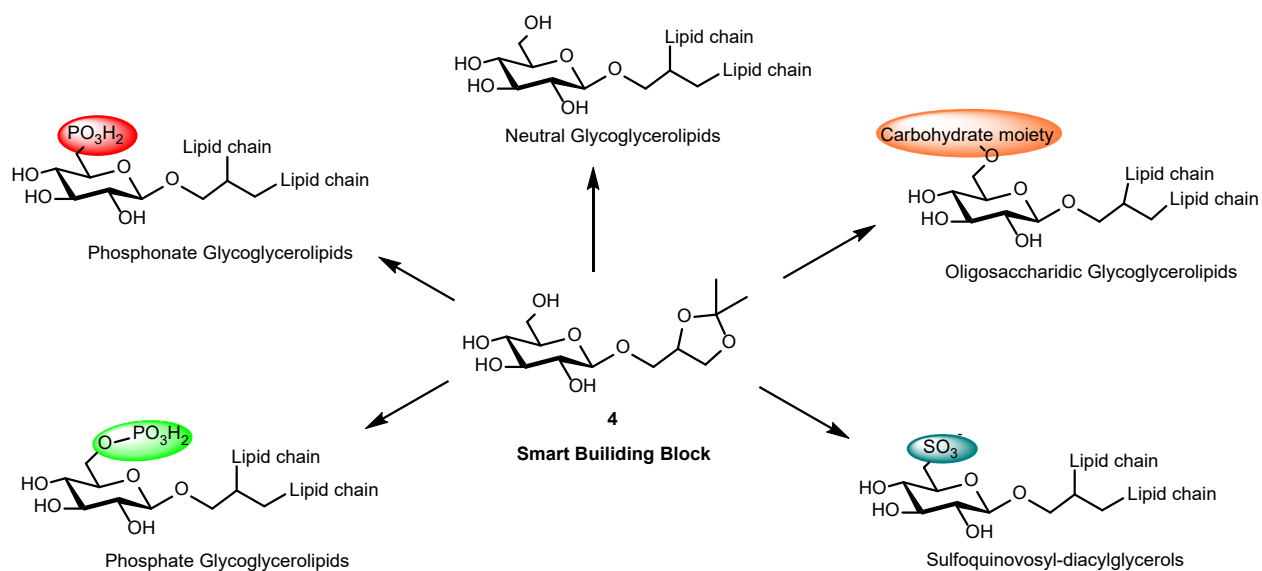
Figure 1. Putative use of compound **4** for the access to biologically active glycoglycerolipids**.**

As reported in **Scheme 1**, the first four steps of the new synthetic strategy for Sulfavant A (**1**) were common to the previous procedure[7] but with essential improvement in the yield of the intermediate **6** (from 79% to 97%) due to change of the conditions for the anomeric deacetylation by using additional equivalents of benzylamine and longer reaction time (40 hours). Preparation of the glucosyl-trichloroacetimidate donor under strictly anhydrous conditions allowed to reduce the amount of trichloroacetonitrile reagent (from 10 to 3 equivalents) with consequent lower costs and almost quantitative conversion into 1,2-*O*-isopropylidene-3-*O*-β-(2',3',4',6'-tetra-*O*-acetyl)-D-glucosyl-glycerol (**8**). The subsequent Zemplén deacetylation (MeO$^-$Na$^+$/MeOH)[9] led to the key intermediate **4** that was directly functionalized on carbon 6' by consecutive iodination and thioacetylation.[7,10] It is worth noting, that the scaling up of the methodology allowed the use of a much lower excess of lutidine in the iodination step as an additional improvement with respect to the previous synthesis. The opening of glycerol acetonide by Lewis acid, followed by DCC-based condensation with a slight excess of stearic acid gave the thioacetate **12**. The new sequence of

reactions for the conversion of **8** to **12** allowed to avoid the limiting hydrazinolysis step with 55% overall yield. This was exactly two-fold the yield we reported for the same route by the previous method at milligram scale.[7]
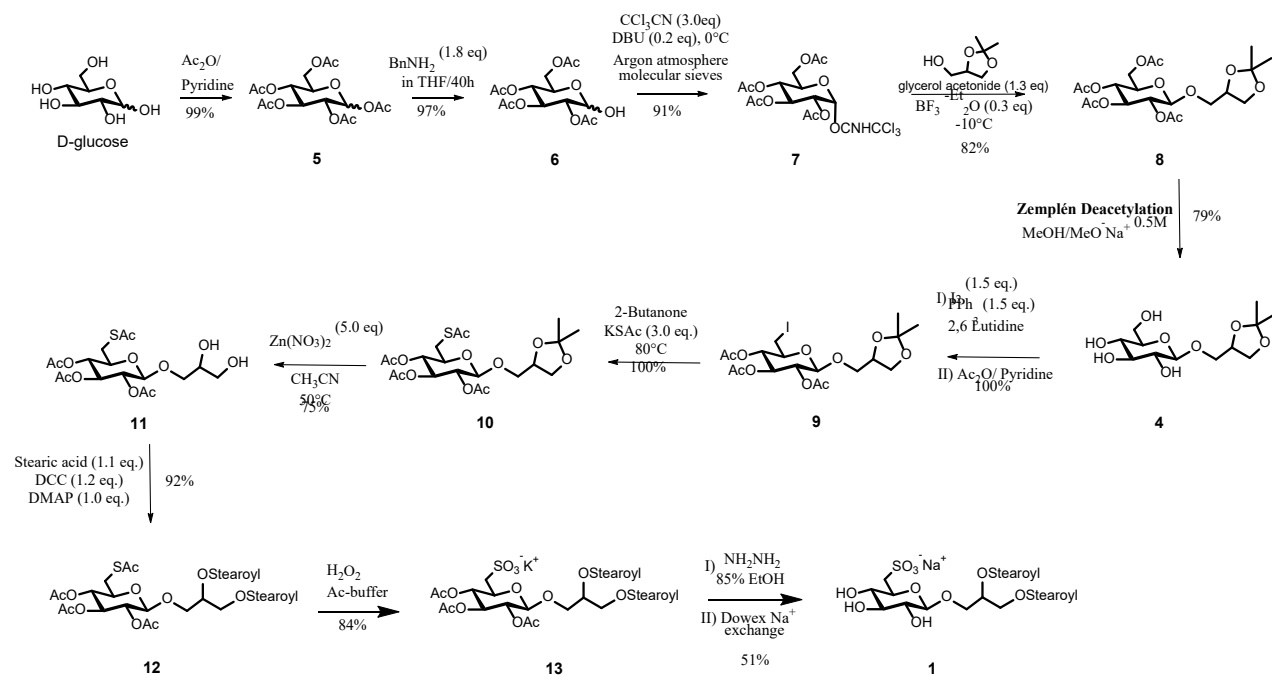
Scheme 1. Up-scaled synthesis of 15g Sulfavant A (**1**). Conversion of D-glucose to Na$^+$ form of the molecular adjuvant Sulfavant A (**1**) with 17% overall yield.

Oxidation of **12** with hydrogen peroxide gave the 1,2-*O*-distearoyl-3-*O*-β-(2',3',4'-tria-*O*-acetyl)-D-sulfoquinovosyl-glycerol (**13**) that was deacetylated to Sulfavant A (**1**) by hydrazinolysis. The conclusive deacetylation was also the hardest step to scale-up due to the presence of major quantity of hydrazine compared to the analytical procedure.[7] Use of the previously reported approach[7] led to a significant decrease of the reaction yield due to the difficulties to purify the product during the work-up and to partial deacylation. For this reason, the hydrazinolysis work up was improved quenching immediately the reaction with an excess of benzaldehyde. The process gave the less

toxic azines, benzaldehyde-diimine, that was also easily removed by chromatography on silica gel. Treatment with Na$^+$ Dowex gave 15 g of sodium salt of (1) as white powder, that is the pharmaceutical form used for the formulation of this adjuvant.

CONCLUSIONS

Sulfavants are a novel class of molecular adjuvants based on the sulfoquinovoside-glycerol skeleton. Herein, we reported an improved and scalable process that allows the synthesis of Sulfavant A (**1**) by a simple procedure ensuring high yield and purity on gram scale. The novel route relied on optimization of the synthetic conditions, as well as on a sequence of reactions that was designed to reduce the impact of limiting steps due to hydrazinolysis in the previous procedures.[6-8] The novel synthesis gave 15 grams of Sulfavant A (**1**) as sodium salt with an overall yield of 17%. The procedure does not have any limitation and it can be scaled to achieve hundreds of grams or kilograms of product, thus paving the way to the preclinical and clinical studies of this adjuvant.

The improved methodology also opens a straightforward approach to the synthesis of a wide class of glycoglicerolipids derivatives and other sulfolipids through an easy access to 1,2-*O*-isopropylidene-3-*O*-β-D-glucosyl-glycerol (**4**). Indeed, this precursor was properly designed as a smart building block, allowing an easy orthogonal functionalization of the sugar portion and the glycerol moiety. We anticipate that this strategy will be applied for the preparation of other members of the sulfavant family.

EXPERIMENTAL SECTION

General Experimental Procedures. NMR spectra were recorded on a Bruker Avance-400 (400.13 MHz) and on a Bruker DRX-600 equipped with a TXI CryoProbe in CDCl$_3$ and in CDCl$_3$:CD$_3$OD 1:1 (δ values are referred to CHCl$_3$ and CH$_3$OH at 7.26 and 3.34 ppm respectively). HR-MS spectra were acquired by a Q-Exactive Hybrid Quadrupole-Orbitrap mass spectrometer (Thermo Scientific). TLC plates (Kieselgel 60 F$_{254}$) and silica gel powder (Kieselgel 60, 0.063–0.200 mm) were from Merck.

All the reagents were purchased from Sigma-Aldrich and used without any further purification.

Glycerol acetonide: glycerol (40.0 g, 0.44 mol) was dissolved in *N,N*-Dimethylformamide (20.0 mL); 2,2-dimethoxypropane (80.0 mL) and *p*-toluenesulfonic acid (5.3 g) were added. After stirring overnight at 50 °C, the mixture was portioned between cold distilled water and dichloromethane (300 mL; 1:1 v/v). The organic phase was purified by silica gel chromatography using a gradient of petroleum ether/diethyl ether (9:1 to 8:2 v/v) to give glycerol acetonide (45.9 g, 0.348 mol, 80%) as colorless oil. ^{1}H NMR (CDCl$_3$): δ 4.24 (1H, m, H-2), 4.09 (1H, dd, *J*= 6.7, 8.5 Hz, H-3a), 3.82 (1H, dd, *J*= 6.4, 8.5 Hz, H-3b), 3.65 (2H, m, H$_2$-1), 1.46 (3H, s, CH$_3$), 1.40 (3H, s, CH$_3$); HRESIMS *m/z* 155.0699 [M+Na]$^+$ (calcd for C$_6$H$_{12}$O$_3$Na, 155.0684).

Peracetylated glucose (5): D-glucose (20.9 g, 0.116 mol) was dissolved in pyridine (52.0 mL, 0.638 mol) and acetic anhydride (61.0 mL, 0.638 mol) was added. The reaction mixture was stirred at room temperature overnight. After portioning in water and chloroform, the organic phase was evaporated under reduced pressure and purified by silica gel chromatography using a gradient of petroleum ether/diethyl ether (9:1 to 3:2 v/v) to give peracetylated glucose (**5**) (44.8 g, 0.115 mol,

99%) as white powder. ^{1}H NMR (CDCl$_3$): δ 6.36 (1H, d, J=3.5 Hz, H-1'), 5.54 (1H, t, J= 9.6 Hz, H-4'), 5.39 (1H, t, J= 9.3 Hz, H-3'), 5.28 (1H, dd, J= 3.5, 9.3 Hz, H-2'), 4.28 (1H, dd, J= 6.37, 11.1 Hz, H-6'a), 4.17 (1H, dd, J= 6.7, 11.1 Hz, H-6'b), 3.85 (1H, m, H-5'), 2.20-2.00 (15H, -COCH$_3$); HRESIMS m/z 413.1074 [M+Na]$^+$ (calcd for C$_{16}$H$_{22}$O$_{11}$Na, 413.1060).

2',3',4',6'-*O*-tetra-acetyl-glucose (6): Peracetylated glucose (42.3 g, 0.108 mol) was dissolved in tetrahydrofuran (50 mL) and 1.5 equivalents of Benzylamine (17.7 mL, 0.162 mol) were added dropwise; the reaction mixture was stirred overnight at room temperature. After 24 h further 0.3 equivalents of Benzylamine (3.54 mL, 0.032 mol) were added dropwise and the reaction mixture was stirred overnight at room temperature. After 40 h the mixture was portioned between water and chloroform, the organic phase was evaporated under reduced pressure and purified by silica gel chromatography using a gradient of petroleum ether/diethyl ether (9:1 to 3:7 v/v) to give 2',3',4',6'-tetra-acetyl-glucose (36.5 g, 0.105 mol, 97%) as yellowish foam. ^{1}H NMR (CDCl$_3$) main signals α anomer: δ 5.52 (1H, t, J= 9.6 Hz, H-3'), 5.45 (1H, d, J=3.5 Hz, H-1'),5.09 (1H, t, J= 9.3 Hz, H-4'), 4.90 (1H, dd, J=3.5, 9.6 Hz, H-2'), 4.35-4.20 (2H, m, H-6'a, H-6'b), 4.15-4.05 (1H, m, H-5'), 2.20-2.00 (12H, -COCH$_3$); HRESIMS m/z 371.0954 [M+Na]$^+$ (calcd for C$_{14}$H$_{20}$O$_{10}$Na, 371.0949).

1'-*O*-trichloroacetimidate-2',3',4',6'-*O*-tetra-acetyl-glucose (7): 2',3',4',6'-*O*-tetra-acetyl-glucose (**6**) (36.5 g, 0.105 mol) was dissolved in anhydrous dichloromethane (100 mL), followed by addition of 3.0 equivalents of trichloroacetonitrile (31.6 mL, 0.315 mol) and 0.2 equivalents of 1,8-Diazabiciclo[5.4.0]undec-7-ene (DBU) (3.1 mL, 0.021 mol). The reaction mixture was stirred under Argon atmosphere for 2 h at 0 °C on activated 4 Å molecular sieves. After evaporation under

reduced pressure, the mixture was purified by silica gel chromatography using a gradient of petroleum ether/diethyl ether (9:1 to 7:3 v/v) to give 1'-*O*-trichloroacetimidate-2',3',4',6'-*O*-tetra-acetyl-glucose (46.4 g, 0.095 mol, 90%) as yellowish foam. ^{1}H NMR (CDCl₃): δ 6.60 (1H, d, *J*=3.2 Hz, H-1'), 5.54 (1H, t, *J*= 9.3 Hz, H-4'), 5.30-5.10 (2H, overlapped, H-2', H-3'), 4.30-4.00 (3H, m, H-5', H₂-6'), 2.14-1.95 (12H, COCH₃); HRESIMS *m/z* 514.0037 [M+Na]⁺ (calcd for C₁₆H₂₀Cl₃NO₁₀Na, 514.0050).

1,2-*O*-isopropyliden-3-*O*-β-[(2',3',4',6'-*O*-tetra-acetyl)-D-glucosyl]-(*R*/*S*)-glycerol (8): 1'-*O*-trichloroacetimidate-2',3',4',6'-tetra-acetyl-glucose (**7**) (46.4 g, 0.095 mol) was dissolved in anhydrous dichloromethane (100 mL) and 1.3 equivalents of glycerol acetonide (16.5 g, 0.125 mol) was added; the reaction mixture was kept under Argon atmosphere on activated 4 Å molecular sieves at -20 °C; subsequently, 0.2 equivalents of boron trifluoride etherate were divided in two different portions. The first one (1.89 mL, 0.015 mol) was added dropwise and stirring was maintained for 3h. After a second addiction of boron trifluoride etherate (0.98 mL, 0.008 mol), the temperature was raised to -10 °C and the reaction mixture was stirred overnight. After neutralization with triethylamine (2.0 mL), the mixture was evaporated under reduced pressure and purified by silica gel chromatography using a gradient of petroleum ether/diethyl ether (8:2 to 1:1 v/v) to give 1,2-*O*-isopropylidene-3-*O*-β-[(2',3',4',6'-*O*-tetra-acetyl)-D-glucosyl]-(*R*/*S*)-glycerol (35.5 g, 0.077 mol, 81%) as white foam. ^{1}H NMR (400 MHz, CDCl₃): δ 5.13 (1H, bt, J = 9.4 Hz, H-3), 4.99 (1H, bt, J = 9.8 Hz, H-4), 4.89 (1H, bt, J = 8.4 Hz, H-2), 4.53 (1H, d, J = 7.8 Hz, H-1), 4.18−3.95 (3H, overlapped, H₂-1, H-2) 3.72−3.51 (5H, overlapped, H₂-6, H-5, H₂-3), 2.00 (3H, s, OAc), 1.97 (3H, s, OAc), 1.94 (3H, s, OAc), 1.92 (3H, s, OAc), 1.35

(3H, s, CH_3), 1.30 (3H, s, CH_3); HRESIMS m/z: 485.1635 $[M+Na]^+$ (calcd for $C_{20}H_{30}O_{12}Na$, 485.1629).

1,2-*O*-isopropylidene-3-*O*-β-D-glucosyl-(*R/S*)-glycerol (4): 1,2-*O*-isopropyliden-3-*O*-β-[(2',3',4',6'-*O*-tetra-acetyl)-D-glucosyl]-(*R/S*)-glycerol (**8**) (35.5 g, 0.077 mol) was dissolved in 0.5M methanolic solution of CH_3ONa (100 mL); After 2 h stirring at room temperature, the solution was diluted with H_2O (100 mL), neutralized with Dowex 50WX2 (H^+ form), and centrifuged at 15 °C (4000 rpm, 5 min). The supernatant was evaporated and purified by silica gel chromatography (10:0 to 9:1 v/v $CHCl_3$–MeOH) to give 1,2-*O*-isopropyliden-3-*O*-β-D-glucosyl-(*R/S*)-glycerol (17.7 g, 0.06 mol, 78%) as colorless oil.^{1}H NMR (400 MHz, CD_3OD): δ 4.36 (1H, m, H-2), 4.32 (1H, d, J = 7.6 Hz, H-1'), 4.10 (1H, dd, J = 6.6, 12.6 Hz, H-3b), 3.93 (1H, dd, J = 5.4, 10.2 Hz, H-1a), 3.89 (1H, bd, J = 11.0 Hz, Ha-6') 3.81 (1H, m, H-3a), 3.70-3.65 (2H, overlapped, Hb-6', H-1b), 3.39-3.30 (3H, overlapped, H-5', H-4', H-3'), 3.22 (1H, bt, J = 8.0 Hz, H-2'), 1.42 (3H, s, CH_3), 1.36 (3H, s, CH_3). ^{13}C NMR (100 MHz, CD_3OD): 110.3 (C, quaternary), 104.3 (CH, C1'), 77.8 (CH, C5'), 77.9 (CH, C3'), 75.7 (CH, C2), 74.7 (CH, C2'), 71.3 (CH, C4'), 71.1 (CH_2, C1), 67.3 (CH_2, C3), 62.5 (CH_2, C6'), 26.4 (CCH_3), 25.2 (CCH_3). HRESIMS *m/z* 317.1241, $[M+Na]^+$ (calcd for $C_{12}H_{22}O_8Na$), 317.1207).

1,2-*O*-isopropylidene-3-*O*-β-[(2',3',4'-tri-*O*-acetyl-6'-iodo)-D-glucosyl]-(*R/S*)-glycerol (9): Iodine (22.9 g, 0.09 mol) was added to a mixture of 1,2-*O*-isopropylidene-3-*O*-β-D-glucosyl-(*R/S*)-glycerol (**4**) (17.7 g 0.06 mol), triphenylphosphine (23.6 g, 0.09 mol) in 2,6-dimethylpyridine (34 g, 0.31 mol) at temperature of 80 °C. The mixture was stirred for 6 h at 80 °C and subsequently acetylated by addition of pyridine (19.0 mL) and acetic anhydride (19.0 mL). The reaction mixture

was stirred at room temperature overnight. After evaporation of the solvent under a stream of nitrogen, the mixture was purified by silica gel chromatography using a gradient of petroleum ether/diethyl ether (9:1 to 1:1 v/v) to give 1,2-*O*-isopropyliden-3-*O*-β-[(2',3',4'-tri-*O*-acetyl-6'-iodo)-D-glucosyl]-(*R/S*)-glycerol (31.8 g, 0.06 mol, 100%) as yellowish oil. ^{1}H NMR (400 MHz, CDCl$_3$): δ 5.20 (1H, m, H-3'), 5.00 (1H, bt, J = 9.0 Hz, H-2'), 4.90 (1H, bt, J = 9.35 Hz, H-4'), 4.64 (1H, d, J = 7.80 Hz, H-1'), 4.30 (1H, m, H-2), 4.05 (2H, overlapped, Ha-3, Hb-3), 3.85 (1H,dd, J = 5.9, 11.0 Hz, Ha-1), 3.70 (1H, m, Hb-1, H-1b), 3.53 (1H, m, H-5'), 3.30 (1H, bd, J = 11.0 Hz, Ha-6'), 3.18 (1H, bd, J = 10.9 Hz, Hb-6'), 2.05 (3H, s, OAc), 2.02 (3H, s, OAc), 2.00 (3H, s, OAc), 1.40 (3H, s, CH3), 1.34 (3H, s, CH3). ^{13}C NMR (100 MHz, CDCl$_3$): δ 170.9, 170.6, 170.3 (*COCH3*, acyl esters), 110.0 (C, quaternary), 100.2 (CH, C1'), 73.9 (CH, C2), 72.9 (CH, C5'), 71.8 (CH, C3'), 71.6 (CH, C4'), 70.9 (CH, C2'), 70.1 (CH$_2$, C1), 66.0 (CH$_2$, C3), 26.1 (C*CH3*), 24.8 (C*CH3*) 20.0 (CO*CH3*), 2.1 (CH$_2$, C6'). HRESIMS *m/z* 553.0583, [M+Na]$^+$ (calcd for C$_{18}$H$_{27}$INaO$_{10}$, 553.0541).

1,2-*O*-isopropylidene-3-*O*-β-[(2′,3′,4′-tri-*O*-acetyl-6′-thioacetyl)-D-glucosyl]-(*R/S*)-glycerol (10): 1,2-*O*-isopropylidene-3-*O*-β-[(2',3',4'-tri-*O*-acetyl-6'-iodo)-D-glucosyl]-(*R/S*)-glycerol (**9**) (31.8 g, 0.06 mol) was dissolved in 2-butanone (100 mL) and potassium thioacetate (34 g, 0.30 mol) was added. The reaction mixture was stirred at 80 °C for 2 h, and then, the solvent was evaporated under reduced pressure. The residue was purified by silica gel chromatography using a gradient of petroleum ether/diethyl ether (9:1 to 1:1 v/v) to give 1,2-*O*-isopropylidene-3-*O*-β-[(2′,3′,4′-tri-acetyl-6′-thioacetyl)-D-glucosyl]-(*R/S*)-glycerol (28.7 g, 0.06 mol, 100%) as yellowish oil. ^{1}H NMR (400 MHz, CDCl$_3$): δ 5.18 (1H, m, H-3'), 5.00 (2H, overlapped, H-2', H-4'), 4.55 (1H, d, J = 7.98 Hz, H-1'), 4.24 (1H, m, H-2), 4.03 (2H, overlapped, Ha-3, Hb-3), 3.77-3.68 (2H, overlapped, Ha-1, Hb-1), 3.63 (1H, m, H-5'), 3.25 (1H, bd, J = 12.0 Hz, Ha-6'), 3.05

(1H, bd, J = 10.9 Hz, Hb-6'), 2.34 (3H, s, SAc), 2.09 (3H, s, OAc), 2.04 (3H, s, OAc), 2.00 (3H, s, OAc), 1.41 (3H, s, CCH_3), 1.34 (3H, s, CCH_3). ^{13}C NMR (100 MHz, CDCl$_3$): δ 195.0 (SCOCH$_3$), 170.9, 170.6, 170.2 (COCH$_3$, acyl esters), 110.0 (C, quaternary), 100.0 (CH, C1'), 73.5 (CH, C2), 71.6 (CH, C3'), 70.5 (CH, C2'), 69.4 (CH$_2$, C1), 68.9 (CH, C5'), 67.9 (CH, C4'), 65.9 (CH$_2$, C3), 29.6 (SCOCH$_3$), 29.2 (CH$_2$S, C6') 25.7 (CCH$_3$), 25.5 (CCH$_3$) 20.0 (COCH$_3$). HRESIMS m/z 501.1463, [M+Na]$^+$ (calcd for C$_{20}$H$_{30}$NaO$_{11}$S, 501.1401).

3-O-β-[(2′,3′,4′-tri-O-acetyl-6′-thioacetyl)-D-glucosyl]-(R/S)-glycerol **(11):** 1,2-O-isopropylidene-3-O-β-[(2',3',4'-tri-O-acetyl-6'-thioacetyl)-D-glucosyl]-(R/S)-glycerol (**10**) (28.7 g, 0.06 mol) was dissolved in acetonitrile (200 mL) and 5.0 equivalents of zinc nitrate hexa-hydrate (89.2 g, 0.3 mol) were added. The reaction mixture was stirred and heated at 50 °C for 6 h. After evaporation of the organic solvent under reduced pressure, the mixture was diluted with CH$_2$Cl$_2$ (200 mL) and treated with 1M NaHCO$_3$ (200 mL 1:1 v/v). The organic layer was dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated to give 3-O-β-[(2',3',4'-tri-O-acetyl-6'-thioacetyl)-D-glucosyl]-(R/S)-glycerol (19.7 g, 0.045 mol, 75%) as yellowish foam. ^{1}H NMR (400 MHz, CDCl$_3$): δ 5.18 (1H, bt, J = 9.51 Hz, H-3'), 4.99 (2H, overlapped, H-2', H-4'), 4.52 (1H, d, J = 8.01 Hz, H-1'), 3.84 (3H, overlapped, H-5', Ha-3, Hb-3), 3.67-3.60 (3H, overlapped, Ha-1, Hb-1, H-2), 3.26 (1H, bd, Ha-6'), 3.03 (1H, bd, Hb-6'), 2.35 (3H, s, SAc), 2.10 (3H, s, OAc), 2.05 (3H, s, OAc), 2.00 (3H, s, OAc). ^{13}C NMR (100 MHz, CDCl$_3$): δ 195.0 (SCOCH$_3$), 170.9, 170.6, 170.2 (COCH$_3$, acyl esters), 100.3 (CH, C1'), 72.2 (CH, C2), 71.6 (CH, C3'), 71.5 (CH$_2$, C3), 70.1 (CH, C2', C4'), 69.0 (CH, C5'), 62.6 (CH$_2$, C1), 29.3 (CH$_2$S, C6'), 29.1 (SCOCH$_3$) 19.9 (COCH$_3$). HRESIMS m/z 461.1097, [M+Na]$^+$ (calcd for C$_{17}$H$_{26}$NaO$_{11}$S, 461.1088).

1,2-*O*-stearoyl-3-*O*-β-[(2',3',4'-tri-*O*-acetyl-6'-thioacetyl)-D-glucosyl]-(*R/S*)-glycerol (12): 3-*O*-β-[(2',3',4'-tri-*O*-acetyl-6'-thioacetyl)-D-glucosyl]-(*R/S*)-glycerol (**11**) (19.7 g, 0.045 mol) was dissolved, under Argon atmosphere, in anhydrous dichloromethane (200 mL), followed by 1.2 equivalents of dicyclohexylcarbodiimide (22.3 g, 0.11 mol), 1.0 equivalent of DMAP (11.0 g, 0.09 mol) and 1.1 equivalents of stearic acid (28.4 g, 0.1 mol). The reaction mixture was stirred overnight at room temperature; after evaporation under reduced pressure, the mixture was purified by silica gel chromatography using a gradient of petroleum ether/diethyl ether to give 1,2-*O*-stearoyl-3-*O*-β-[(2',3',4'-tri-*O*-acetyl-6'-thioacetyl)-D-glucosyl]-(R/S)-glycerol (40.2 g, 0.041 mol, 92%) as a colorless oil. ^{1}H NMR (400 MHz, CDCl$_3$): δ 5.20−5.14 (2H, m, H-2, H-3′), 4.96−4.89 (2H, m, H-2′, H-4′), 4.50 (1H, d, *J* = 8.0 Hz, H-1′), 4.28 (1H, dd, *J* = 4.1, 11.8 Hz, H-1a), 4.09 (1H, dd, *J* = 5.7, 11.8 Hz, H-1b), 3.91 (1H, dd, *J* = 4.5, 11.1 Hz, H-3a), 3.65 (1H, dd, *J* = 5.4, 11.1 Hz, H-3b), 3.62 (1H, m, H-5′), 3.25 (1H, bd, *J* = 11.4 Hz, H-6′a), 3.06 (1H, dd, *J* = 2.4 Hz, 11.4 Hz), 2.35 (3H, s, SAc), 2.33−2.29 (4H, m, α-methylene of stearoyl portion), 2.13−1.99 (9H, s, 3OAc), 1.64−1.57 (4H, m, β-methylene of stearoyl portion), 1.32−1.23 (60H, aliphatic methylenes), 0.93−0.87 (6H, overlapped, 2CH$_3$); HRESIMS m/z: 993.6329 [M + Na]$^+$ (calcd for C$_{53}$H$_{94}$O$_{13}$NaS, 993.6307).

1,2-*O*-stearoyl-3-*O*-β-[(2',3',4'-tri-*O*-acetyl)-D-sulfoquinovosyl]-(*R/S*)-glycerol (13): 1,2-*O*-stearoyl-3-*O*-β-[(2',3',4'-tri-*O*-acetyl-6'-thioacetyl)-D-glucosyl]-(*R/S*)-glycerol (**12**) (40.2 g, 0.041 mol) was dissolved in acetic acid (260 mL), potassium acetate (4.8 g, 0.049 mol) and 30% (w/v) H$_2$O$_2$ (20.0 mL) were added. The reaction mixture was stirred overnight at 40 °C. After evaporation, the oily residue was purified by silica gel chromatography using a gradient of chloroform/methanol (99:1 to 8:2 v/v) to give 1,2-*O*-stearoyl-3-*O*-β-[(2',3',4'-tri-*O*-acetyl-)-D-

sulfoquinovosyl]-(R/S)-glycerol (34.9 g, 0.034 mol, 84%, potassium salt) as a colourless oil; ^{1}H NMR (400 MHz, CDCl$_3$): δ 5.30 (2H, m, H-2, H-3'), 5.00-4.80 (2H, m, H-2', H-4′), 4.68 (1H, d, 7.3 Hz, H-1'), 4.31 (1H, bd, *J*=11.1 Hz, H-1a), 4.15-4.00 (3H, overlapped, H-1b, H-3a, H-3b), 3.75 (1H, m, H-5'), 3.20 (2H, overlapped, H$_2$-6'), 2.33-2.28 (4H, m, α-methylene), 2.06-1.98 (9H, s, 3 OAc), 1.64-1.55 (4H, m, β-methylene), 1.34-1.22 (acyl chain), 0.91-0.88 (6H, overlapped, 2CH$_3$); HRESIMS *m/z* 975.6097, [M]$^-$ (calcd for C$_{51}$H$_{91}$O$_{15}$S$^-$, 975.6084).

1,2-*O*-stearoyl-3-*O*-β-D-sulfoquinovosyl]-(*R/S*)-glycerol (Sulfavant A) (1): 1,2-*O*-stearoyl-3-*O*-β-[(2',3',4'-tri-*O*-acetyl)-D-sulfoquinovosyl]-(*R/S*)-glycerol (**13**) (34.9 g, 0.034 mol) was dissolved in aq. ethanol (85%) (1.5 L), 1.5 equivalents of hydrazine monohydrate (7.4 g, 0.153 mol) were added dropwise, and the reaction mixture was stirred for 3 h at 40 °C and there after stirred overnight at room temperature. The hydrazine excess was quenched with benzaldehyde (38.9 g, 0.383 mol) to give the less toxic benzaldehyde-diimine (azine), the mixture was stirred for 1 h at room temperature. After evaporation, the crude mixture was then purified by silica gel chromatography using a gradient of chloroform/methanol (98:2 to 8:2 v/v). The compound was then subjected to a standard ion exchange, diluted in methanol and treated with Dowex 50WX2 Na$^+$-form, filtered and evaporated to give 1,2-*O*-stearoyl-3-*O*-β-D-sulfoquinovosyl]-(*R*/S)-glycerol (Sulfavant A) (15.2 g, 0.017 mol, 51%) as a white solid; R$_f$ (chloroform/methanol 7:3) = 0.15; IR (liquid film) v$_{max}$ 3400, 2940, 2862, 1750, 1351, 1343 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$/CD$_3$OD 1/1): δ 5.29 (1H, m, H-2), 4.47 (1H, m, H-1a), 4.34 and 4.32 (each for 1H, d, 7.8 Hz, H-1'), 4.19 (1H, m, H-1b), 4.13-4.03 (1H, m, H-1a), 3.79-3.75 (2H, m, H-3b, H-5'), 3.42 (1H, m, H-3'), 3.32 (1H, m, H-6'a), 3.26 (1H, m, H-2'), 3.14 (1H, m, H-4'), 2.98 (1H, m, H-6'b), 2.43-

2.35 (4H, m, α-methylene), 1.69-1.58 (4H, m, β-methylene), 1.43-1.29 (acyl chain), 0.94 (6H, overlapped, 2CH$_3$); HRESIMS *m/z* 849.5777, [M]$^-$ (calcd for C$_{45}$H$_{85}$O$_{12}$S$^-$, 849.5767).

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Notes

The authors declare no competing financial interests.

Ethical Issue

The authors declare no ethical issue.

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