Supporting Information

for

Synthesis, docking study and biological evaluation of
D-fructofuranosyl and D-tagatofuranosyl sulfones as potential inhibitors of the mycobacterial galactan synthesis targeting the galactofuranosyltransferase GlfT2

Marek Baráth, Jana Jakubčinová, Zuzana Konyariková, Stanislav Kozmon, Katarína Mikušová and Maroš Bella

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Experimental and analytical data
Figure S1: The biochemical pathway for biosynthesis of mycobacterial galactan. The synthesis is initiated by a transfer of GlcNac-1-phosphate onto a decaprenyl-phosphate by the enzyme WecA and continues with the sequential addition of glycosyl residues to this lipid carrier by the enzymes WbbL, GltT1 and GltT2. GL-1 – GL-5 – glycolipid 1-5
Figure S2: Superimposition of the studied D-fructofuranosyl and D-tagatofuranosyl scaffolds (green carbons) with the probable transition state structure (gray carbons) of the GlfT2 catalytic reaction.

Figure S3: Superimposition of the docked UDP-Galf (green carbons) with UDP in the crystallographic GlfT2 structure (light gray carbons).
Table S1. Evaluation of the effects of the target compounds on synthesis of the lipid-linked galactan polymer.*

|       | Experiment 1 |       | Experiment 2 |       |
|-------|--------------|-------|--------------|-------|
|       | dpm          | % incorporation | dpm          | % incorporation |
| Control | 28,650       | 100          | 21,058       | 100          |
| 1aα    | 28,285       | 99           | 23,612       | 112          |
| 1bα    | 34,840       | 122          | 26,723       | 127          |
| 1cα    | 27,370       | 96           | 18,752       | 89           |
| 1cβ    | 30,505       | 106          | 24,498       | 116          |
| 2a     | 35,125       | 123          | 16,950       | 80           |
| 2b     | 29,720       | 104          | 15,667       | 74           |
| 2c     | 30,045       | 105          | 23,475       | 111          |
| 3a     | 33,440       | 117          | 20,962       | 100          |
| 3b     | 28,685       | 100          | 19,267       | 91           |
| 3c     | 27,670       | 97           | 19,693       | 94           |

*The values in the table represent the total incorporation of radioactive galactose from UDP-[\(^{14}\)C]-Gal into lipid-linked galactan polymer extracted by the solvents TT3 and E-soak (see Experimental part) in two independent experiments. The experiments were performed with 500 µg of the cell envelope protein in the reaction mixtures. The compounds were added at a 500 µM concentration. The lines in bold correspond to the compounds with high binding affinities.
Experimental

Docking study

The structures of all docked compounds were optimized at the DFT B3LYP/6-31+G* level allowing full relaxation of the structures. After the geometric optimization the ESP charges were associated with all atoms at the same level. The ESP charges were calculated on the atom centers and values were fitted to reproduce the molecular quadruple moment. All calculations were done employing the Jaguar program as a part of the Schrodinger Suite 2018.04 [1] and were done in gas phase without the solvent effects. Such prepared molecules were docked into the receptor structure. Two structures of the GlfT2 were used as a receptor for the docking. The first one was a crystal structure of the GlfT2 in complex with UDP (PDB ID: 4FIY). The second structure used as a receptor was the structure representing the transition state of the catalytic reaction. This structure was obtained in the previous study by QM/MM molecular dynamics simulations [2]. Both structures were prepared using the Protein Preparation Wizard procedure implemented in the Schrodinger Suite 2018.04. The protein structures were protonated to pH 7 and the proton positions were optimized to create the optimal hydrogen bond network. The OPLS03 force field atom types and charges were associated to the protein structures. The center of the docking grid was placed close to the β-phosphate position or molecular center of the transferring galactofuranose molecule in case of the 4FIY or modeled structure, respectively. The docking box dimensions were set to 15 × 15 × 15 Å. The docking was done in the Glide program using the default setup for the standard precision flexible ligand docking. The best ten poses for each docked compound were kept for further analysis by the Glide Pose Viewer. The presented predicted $K_i$ values of the
docked molecules were calculated based on the equation $\Delta G = RT \ln(K_i)$, where $\Delta G$ is the calculated predicted binding energy represented by the docking score, $R$ is the gas constant and $T$ is the temperature.

**Chemistry**

All reagents and anhydrous solvents were commercially sourced, and were used as received. The starting alcohols 4α, 5α, 5β, 6α and 10 were prepared according to a published method [3]. All solvents were of technical grade, and were distilled before use. Specific optical rotations were determined with a Jasco P-2000 polarimeter. $^1$H and $^{13}$C NMR spectra were recorded with a Bruker AVANCE III HD 400 spectrometer operating at 400 and 100 MHz working frequencies, respectively. Chemical shifts are given in ($\delta$), and tetramethylsilane (TMS) was used as an internal standard for deuterated solvents used (CDCl$_3$/CD$_3$OD/D$_2$O). Coupling constants are given in Hz. All given $^{13}$C spectra are proton decoupled. 2D NMR experiments (HSQC and COSY) were used for signal assignments. High-resolution mass spectra were recorded with an Orbitrap Elite (Thermo Scientific) mass spectrometer with ESI ionization in positive mode. Air humidity sensitive reactions were performed under inert atmosphere (nitrogen). Thin-layer chromatography (TLC) was carried out on glass plates pre-coated with TLC Silica gel 60 F$_{254}$ (E. Merck). The plates were visualized by immersing into phosphomolybdic acid (PMA; 10% solution in ethanol) or H$_2$SO$_4$ (5% H$_2$SO$_4$ in ethanol) and heating at ca 200 °C with a heat gun. Column chromatography was carried out as flash chromatography on Silica gel 60 (E. Merck, 0.040–0.063 mm). Solvents used for flash chromatography were of technical grade,
and were distilled before use. If both anomers are mentioned the alpha anomer is always indicated by “α” and beta anomer by “β” in compound numbering.

*General procedure for preparation of 1-O-dibenzyloxyphosphoryl d-fructo- and d-tagatofuranosyl sulfones 7α, 8α, 9α, 9β, 14 and 21*

To a solution of alcohol (1 equiv) in anhydrous acetonitrile (ca. 200 mg of saccharide per 5 mL) cooled in an ice-water bath to 0 °C, a solution of 1H-tetrazole in acetonitrile (0.45 M, 4 equiv) was added dropwise followed by the addition of dibenzyl (N,N-dimethyl)phosphoramidite (3 equiv). After 5 min of stirring, the ice-water bath was removed and the mixture was slowly warmed up to room temperature (1 h). Next, the reaction was cooled in the ice-water bath to 0 °C and m-CPBA (12 equiv, 70%) was added in one portion. After 5 min of stirring, the ice-water bath was removed and the mixture was slowly warmed up to room temperature (1.5–2 h). The reaction mixture was diluted with CH₂Cl₂ (20 mL), washed with 5% aqueous solution of Na₂S₂O₃ (2 × 15 mL), saturated aqueous solution of NaHCO₃ (2 × 15 mL) and with brine (2 × 15 mL). Organic layer was dried over Na₂SO₄, filtered and the solvent was evaporated under reduced pressure. The crude products were isolated and purified by column chromatography on silica gel using AcOEt/hexanes (1:3, v/v) for fructofuranosyl sulfones 7α, 8α, 9α, 9β and (1:4, v/v) for tagatofuranosyl sulfones 16 and 23.

*Ethyl 3,4,6-tri-O-benzyl-1-O-dibenzyloxyphosphoryl-α-D-fructofuranosyl sulfone (7α)*

Yield 120 mg, 81 %, colorless oil, [α]D = +58.6 (c = 1.0, CHCl₃), ¹H NMR (CDCl₃): δ 1H NMR (400 MHz, CDCl₃) δ 7.35 – 7.13 (m, 25H, Hα), 5.06 – 4.87 (m, 5H, 2×CH₂Ph, H-3), 4.68 (d, J = 11.5 Hz, 1H, CH₂Ph), 4.53 – 4.24 (m, 7H, 3×CH₂Ph, H-1,
H1a), 4.20 (ddd, J = 9.2, 5.0, 2.3 Hz, 1H, H-5), 4.07 − 3.97 (m, 1H, H-4), 3.54 (dd, J = 11.4, 2.4 Hz, 1H, H-6), 3.45 (dd, J = 11.4, 5.1 Hz, 1H, H-6a), 3.21 (dq, J = 13.7, 7.6 Hz, 1H, CH2CH3), 2.87 (dq, J = 13.7, 7.4 Hz, 1H, CH2CH3), 1.28 (t, J = 7.5 Hz, 3H, CH2CH3). 13C NMR (CDCl3): δ 137.8, 137.5, 136.8, 135.7, 135.6, 128.6, 128.5, 128.4, 128.2, 128.1, 128.0, 127.9, 127.8, 128.7 and 128.5 (CAr), 97.6 (C-2), 83.4 (C-3), 82.0 (C-4), 80.4 (C-5), 73.8, 73.3, 72.7, 69.5 and 69.4 (5×CH2Ph), 68.7 (C-6), 66.5 (C-1), 44.7 (CH2CH3), 5.3 (CH2CH3). HRMS: m/z calcd for C43H47O10PS: 809.2520 [M+Na]+; found: 809.2518.

Phenyl 3,4,6-tri-O-benzyl-1-O-dibenzyloxyphosphoryl-α-D-fructofuranosyl sulfone (8α)

Yield 158 mg, 82 %, colorless oil, [α]D = +33.9 (c = 1.0, CHCl3), 1H NMR (CDCl3): δ 7.98-7.95 (m, 2H, HA), 7.62 (t, 1H, HA), 7.31 (m, 27H, HA), 5.16 (d, 1H, J = 7.2 Hz, H-3), 5.00 (d, 1H, J = 11.4 Hz, CH2Ph), 4.90−4.67 (m, 6H, CH2Ph), 4.65−4.53 (m, 2H, CH2Ph, H-1), 4.51−4.34 (m, 4H, CH2Ph, H-5), 4.19−4.07 (m, 2H, H-4, H-1a), 3.66 (dd, J = 11.4, 2.4 Hz, 1H, H-6), 3.55 (dd, J = 11.4, 5.7 Hz, 1H, H-6a). 13C NMR (CDCl3) δ 137.9, 137.6, 137.1, 135.5, 134.2, 130.4, 128.8, 128.5, 128.4, 128.39, 128.34, 128.1, 127.8, 127.77, 127.71, 127.6, 127.5 and 127.4 (CAr), 98.5 (C-2), 83.8 (C-3), 82.3 (C-4), 80.0 (C-5), 74.1, 73.0, 72.8, 69.2 and 69.1, (5×CH2Ph), 69.0 (C-6), 65.2 (C-1). HRMS: m/z calcd for C47H47O10PS: 857.2520 [M+Na]+; found: 857.2531.
Isopropyl 3,4,6-tri-O-benzyl-1-O-dibenzyloxyphosphoryl-α-D-fructofuranosyl sulfone (9a)

Yield 110 mg, 85 %, colorless oil, [α]D = +47.2 (c = 1.0, CHCl3), 1H NMR (CDCl3): δ 7.39-7.24 (m, 23H, HAr), 7.19-7.17 (m, 2H, HAr), 5.07-5.01 (m, 5H, 2×CH2Ph, H-3), 4.82 (ABq, 1H, CH2Ph), 4.61 (ABq, 1H, CH2Ph), 4.52-4.44 (m, 6H, H-1, H-1a, 2×CH2Ph), 4.37-4.27 (m, 1H, H-5), 4.09 (dd, J = 9.4, 7.0 Hz, 1H, H-4), 3.65 (dd, J = 11.5, 2.4, 1H, H-6), 3.66-3.52 [m, 2H, CH(CH3)2], 1.45 and 1.39 [2d, each 3H, CH(C(CH3)2)]. 13C NMR (CDCl3): δ 137.8, 137.5, 136.9, 135.6, 135.5 and 135.5 (Car), 99.6 (C-2), 84.2 (C-3), 81.8 (C-4), 80.0 (C-5), 73.7, 73.1, 72.6, 69.5 and 69.4 (5×CH2Ph), 68.9 (C-6), 66.4 (C-1), 53.9 [CH(CH3)2], 17.3 and 15.3 [CH(CH3)2]. HRMS: m/z calcd for C44H49O10PS: 823.2676 [M+Na]+; found: 823.2681.

Isopropyl 3,4,6-tri-O-benzyl-1-O-dibenzyloxyphosphoryl-β-D-fructofuranosyl sulfone (9β)

Yield 165 mg, 79 %, colorless oil, [α]D = -65.5 (c = 1, CHCl3), 1H NMR (CDCl3) δ 7.39–7.26 (m, 23H, HAr), 7.21 (dd, J = 7.3, 2.3 Hz, 2H, HAr), 5.08 (dd, J = 8.3, 2.4 Hz, 4H, 2×CH2Ph), 4.83 (d, J = 11.4 Hz, 1H, CH2Ph), 4.68–4.43 (m, 9H, H-3, H-4, H-1, H-1a, 2×CH2Ph), 4.21 (td, J = 7.0, 3.7 Hz, 1H, H-5), 3.70 (dd, J = 10.5, 6.8 Hz, 1H, H-6), 3.62–3.57 (m, 1H, H-6a), 3.51 [p, J = 6.9 Hz, 1H, CH(CH3)2], 1.24 [s, 3H, CH(CH3)2], 1.18 [d, J = 9.6 Hz, 3H, CH(CH3)2]; 13C NMR (CDCl3) δ 137.7, 137.6, 137.1, 135.5, 135.5, 128.7, 128.67, 128.64, 128.57, 128.53, 128.06, 128.00, 127.87 and 127.84 (14CAr), 99.9 (C-2), 85.0 (C-3), 82.48 (C-5), 82.41 (C-4), 74.1, 73.3, 72.9, 69.8 and 69.6 (5×CH2Ph), 70.3 (C-6), 66.4 (C-1), 53.4 [CH(CH3)2], 16.6 and 16.5 [CH(CH3)2]. HRMS: m/z calcd for C44H49O10PS: 823.2676 [M+Na]+; found: 823.2678.
Ethyl 1-O-dibenzyloxyphosphoryl-3,4-O-isopropylidene-6-O-pivaloyl-α-D-tagatofuranosyl sulfone (14)

Yield 300 mg, 83 %, colorless oil, [α]D = +72.1 (c = 1.0, CHCl₃), ¹H NMR (CDCl₃): δ 7.43–7.31 (m, 10H, HAr), 5.29 (d, J = 6.1 Hz, 1H, H-3), 5.17–5.07 (m, 4H, 2×CH₂Ph), 4.94 (dd, J = 6.1, 4.1 Hz, 1H, H-4), 4.69 (dt, J = 7.4, 4.1 Hz, 1H, H-5), 4.56 (dd, J = 12.0, 4.4 Hz, 1H, H-1), 4.43 (dd, J = 12.0, 4.2 Hz, 1H, H-6), 4.35 (dd, J = 12.1, 5.5 Hz, 1H, H-1a), 4.18 (dd, J = 12.0, 7.5 Hz, 1H, H-6a), 3.35 (dd, J = 12.1, 5.5 Hz, 1H, H-1b), 1.43 and 1.32 [2s, each 3H, C(CH₃)₂], 1.37 (t, J = 7.5 Hz, 3H, CH₂CH₃), 3.06 (dq, J = 14.0, 7.5 Hz, 1H, CH₂CH₃); ¹³C NMR (CDCl₃): δ 177.9 (C=O), 135.5, 135.4, 128.7, 128.5, 127.98 and 127.94 (CAr), 114.0 [C(CH₃)₂], 101.5 (C-2), 82.1 (C-5), 81.5 (C-3), 80.9 (C-4), 69.57 and 69.52 (2×CH₂Ph), 67.0 (C-1), 62.3 (C-6), 46.1 (CH₂CH₃), 38.7 [C(CH₃)₃], 27.1 [C(CH₃)₃], 25.6 and 24.0 [C(CH₃)₂], 5.4 (CH₂CH₃). HRMS: m/z calcd for C₃₀H₄₁O₁₁PS: 679.1739 [M+K]⁺; found: 679.1725.

Phenyl 6-O-benzyl-1-O-dibenzyloxyphosphoryl-3,4-O-isopropylidene-α-D-tagatofuranosyl sulfone (21a)

Yield 335 mg, 86 %, colorless oil, [α]D = +24.7 (c = 1.0, CHCl₃), ¹H NMR (CDCl₃): δ 8.00–7.83 (m, 2H, HAr), 7.57–7.45 (m, 1H, HAr), 7.43–7.21 (m, 15H, HAr), 7.23–7.09 (m, 2H, HAr), 5.53 (d, J = 6.0 Hz, 1H, H-3), 5.08 (dd, J = 6.0, 4.2 Hz, 1H, H-4), 5.03 (dt, J = 7.2, 4.0 Hz, 1H, H-5), 4.90 (t, J = 7.8 Hz, 2H, CH₂Ph), 4.86–4.78 (m, 1H, CH₂Ph), 4.62 (dd, J = 11.9, 7.1 Hz, 1H, CH₂Ph), 4.56–4.45 (m, 3H, CH₂Ph, H-1), 4.33 (dd, J = 12.0, 6.7 Hz, 1H, H-1a), 3.85 (dd, J = 10.9, 4.0 Hz, 1H, H-6), 3.64 (dd, J = 10.9, 7.1 Hz, 1H, H-6a), 1.47 and 1.36 [2s, each 3H, C(CH₃)₂]; ¹³C NMR (CDCl₃): δ 138.0, 136.0, 135.8, 135.78, 135.73, 134.0, 130.3, 128.58, 128.55, 128.50, 128.4,
128.36, 128.30, 127.7, 127.64, 127.61 and 127.4 (C_\text{Ar}), 113.9 [C(CH_3)_2], 102.1 (C-2), 83.6 (C-5), 81.7 (C-3), 81.4 (C-4), 73.2, 69.1 and 69.0 (2\times CH_2 Ph), 68.4 (C-6), 66.0 (C-1), 25.6 and 24.2 [2s, each 3H, C(CH_3)_2]. HRMS: m/z calcd for C_{36}H_{39}O_8PS: 685.1995 [M+Na]^+; found: 685.1997.

Isopropyl 6-O-benzyl-1-O-dibenzylxyphosphoryl-3,4-O-isopropylidene-\alpha-D-tagatofuranosyl sulfone (21b)

Yield 330 mg, 82 %, colorless oil, [\alpha]_D = +12.4 (c = 1.0, CHCl_3), ^1H NMR (CDCl_3): 1H NMR (400 MHz, CDCl_3) δ 7.38 – 7.26 (m, 15H, Har), 5.33 (d, J = 6.1 Hz, 1H, H-3), 5.17 – 5.07 (m, 4H, 2\times CH_2 Ph), 4.94 (dd, J = 6.1, 4.1 Hz, 1H, H-4), 4.79 (dt, J = 7.2, 4.1 Hz, 1H, H-5), 4.64 – 4.39 (m, 4H, CH_2 Ph, H-1, H-1a), 3.79 (dd, J = 10.8, 4.2 Hz, 1H, H-6), 3.73 – 3.56 [m, 2H, H-6a, CH(CH_3)_2], 1.53 – 1.41 (m, 6H, 2\times CH_3), 1.38 – 1.29 (m, 6H, 2\times CH_3). 13C NMR (101 MHz, CDCl_3) δ 138.01, 135.56, 128.62, 128.57, 128.37, 127.96, 127.94, 127.85, 127.64, and 127.42 (Car) and 113.72 [C(CH_3)_2], 103.2 (C-2), 83.1 (C-5), 82.2 (C-3), 81.0 (C-4), 73.2 (CH_2 Ph), 69.5, 69.4 (2\times CH_2 Ph), 68.3 (C-6), 67.3 (C-1), 55.2 [CH(CH_3)_2], 25.7 (CH_3), 24.1 (CH_3), 17.2 (CH_3), 15.0 (CH_3). HRMS: m/z calcd for C_{33}H_{41}O_{10}PS: 699.1790 [M+K]^+; found: 699.1795.

General procedure for preparation of target compounds 1aa, 1ba, 1ca and 1cβ

A mixture of perbenzyl monosaccharide (1 equiv) and Pd/C (10%, 50 mg) in MeOH (5-8 mL) was stirred at room temperature under a hydrogen atmosphere for 3–4 h [TLC, CHCl_3/MeOH, (3:1, v/v)]. When the reaction was complete, the mixture was filtered through a pad of Celite® and a filter cake was washed with a mixture of MeOH/distilled water [2:1 (v/v), 7 mL]. The filtrate was evaporated under reduced pressure and the residue was dissolved in distilled water (5 mL). The resulting
mixture was filtered through a PTFE syringe filter (0.45 μm) and the filtrate was lyophilized to afford compounds 1aa, 1ba, 1ca and 1cβ.

Ethyl 1-O-phosphono-α-D-fructofuranosyl sulfone (1aa)
Yield 41 mg, 99 %, white powder, [α]D = +6.0 (c = 1.0, H2O), 1H NMR (D2O): δ 4.79 (s, 1H, H-3), 4.18 (m, 2H, H-1, H-1a), 4.10 (s, 1H, H-4), 3.99-3.95 (m, 1H, H-5), 3.88-3.82 (m, 1H, H-6), 3.68-3.60 (m, 1H, H-6a), 3.48-3.41 (m, 1H, CH2CH3), 3.29-3.20 (m, 1H, CH2CH3), 1.32 (s, 3H, CH2C2H5); 13C NMR (D2O) δ 97.8 (C-2), 82.0 (C-5), 75.9 (C-3), 74.1 (C-4), 63.7 (C-1), 60.0 (C-6), 44.9 (CH2CH3), 4.3 (CH2CH3). HRMS: m/z calcd for C8H17O10PS: 359.0172 [M+Na]+; found: 359.0177.

Phenyl 1-O-phosphono-α-D-fructofuranosyl sulfone (1ba)
Yield 11 mg, 98 %, white powder, [α]D = +3.5 (c = 0.5, H2O), 1H NMR (D2O): δ 7.81 (m, 5H, HAr), 4.49 (d, J = 7.9 Hz, 1H, H-3), 4.19-4.09 (m, 1H, H-1), 4.04 (t, J = 8.8 Hz, 1H, H-5), 3.78 (dd, J = 11.8, 5.4 Hz, 1H, H-1a), 3.66 (d, J = 11.5 Hz, 1H, H-6), 3.51 (dd, J = 13.6, 5.7 Hz, 2H, H-4, H-6a); 13C NMR (D2O) δ 135.6, 130.1 and 129.7 (CAr), 100.8 (C-2), 81.5 (C-4), 77.2 (C-3), 73.7 (C-5), 62.5 (C-1), 60.1 (C-6). HRMS: m/z calcd for C12H17O10PS: 407.0172 [M+Na]+; found: 407.0180.

Isopropyl 1-O-phosphono-α-D-fructofuranosyl sulfone (1ca)
Yield 15 mg, 99 %, white powder, [α]D = +8.0 (c = 1.0, CHCl3), 1H NMR (D2O): δ 4.71 (d, J = 7.4 Hz, 1H, H-3), 4.18 (dd, J = 12.2, 5.6 Hz, 1H, H-1), 4.09 (dd, J = 12.0, 4.9 Hz, 1H, H-1a), 4.06 (d, J = 8.2 Hz, 1H, H-5), 3.91 (d, J = 4.5 Hz, 1H, H-4), 3.74 (d, J = 12.8 Hz, 1H, H-6), 3.64 [p, J = 9.6, 6.6 Hz, 1H, CH(CH3)2], 3.54 (dd, J = 13.0, 4.6 Hz, 1H, H-6a), 1.29 and 1.23 [2s, each 3H, CH(CH3)2]; 13C NMR (D2O): δ 99.9 (C-2),
Isopropyl 1-O-phosphono-β-D-fructofuranosyl sulfone (1cβ)

Yield 35 mg, 99 %, white powder, [α]D = -12.1 (c = 1.0, CHCl₃), ¹H NMR (D₂O): δ 3.90 (m, 2H, H-5, H-4), 3.84 (m, 3H, H-3, H-6, H-6a), 3.73 (m, 2H, H-1, H-1a), 2.88 [p, J = 9.6, 6.6 Hz, 1H, CH(CH₃)₂], 1.18 and 1.12 [2s, each 3H, CH(CH₃)₂]; ¹³C NMR (D₂O): δ 97.5 (C-2), 83.7 (C-4), 80.6 (C-3), 71.7 (C-5), 62.1 (C-1), 60.3 (C-6), 51.1 [CH(CH₃)₂], 16.7 [CH(CH₃)₂]. HRMS: m/z calcd for C₉H₁₉O₁₀PS: 389.0068 [M+K]+; found: 389.0074.

1,2:3,4-Di-O-isopropylidene-6-O-pivaloyl-α-D-tagatofuranose (11)
Pivaloyl chloride (3.8 mL, 3.74 g, 31.0 mmol) was added dropwise to a stirred solution of diisopropylidene-α-D-tagatofuranose 10 [4] (6.70 g, 25.7 mmol) and pyridine (2.7 mL, 2.64 g, 33.4 mmol) in CH₂Cl₂ (100 mL) while being cooled in an ice-water bath. Next, the ice-water was removed and the reaction mixture was stirred at room temperature overnight. Then, the reaction mixture was washed with water (2×100 mL). The organic layer was dried with Na₂SO₄, and filtered, and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using EtOAc/hexanes (1:4, v/v) as the eluent to afford 6-O-pivaloyl-α-D-tagatofuranose 11 (7.53 g, 85%) as a thick colorless oil which crystallized in a refrigerator. M.p. 34-35 °C, [α]D = +66.9 (c 1.16, CHCl₃), ¹H NMR (CDCl₃): δ 4.79 (dd, J = 5.8, 3.6 Hz, 1H, H-4), 4.61 (d, J = 5.8 Hz, 1H, H-3), 4.45 (dd, J = 11.6, 4.1 Hz, 1H, H-6), 4.26 (d, J = 9.7 Hz, 1H, H-1), 4.20 (dd, J = 11.6, 7.6 Hz,
6-O-Benzyl-1,2:3,4-di-O-isopropylidene-d-tagatofuranose (12)

To a solution of diisopropylidene-D-tagatofuranose 10 [4] (1.8 g, 6.92 mmol) and BnBr (1.25 mL, 10.27 mmol) in THF (5 mL), 50% aqueous solution of NaOH (1.6 mL) was added followed by TBABr (250 mg, 0.692 mmol) and the resulting mixture was heated under reflux for 3 h. Then, the mixture was diluted with EtOAc (20 mL) and washed with 5% aqueous solution of citric acid (15 mL) and brine (15 mL). Organic layer was dried over Na$_2$SO$_4$, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using AcOEt/hexanes (1:10, v/v) as the eluent to afford 6-O-benzyl-D-tagatofuranose 12 (2.4 g, 91%) as a colorless oil. [α]$_D$ = +22.1 (c=1.0, CHCl$_3$). $^1$H NMR (CDCl$_3$): δ 7.48–7.02 (m, 5H, H$_{Ar}$), 4.84 (dd, $J$ = 5.9, 3.8 Hz, 1H, H-4), 4.69 (d, $J$ = 12.1 Hz, 1H, CH$_2$Ph), 4.64 (d, $J$ = 5.8 Hz, 1H, H-3), 4.59 (d, $J$ = 12.0 Hz, 1H, CH$_2$Ph), 4.31 (d, $J$ = 9.6 Hz, 1H, H-1a), 4.22 (ddd, $J$ = 6.6, 5.0, 3.7 Hz, 1H, H-5), 4.10 (d, $J$ = 9.7 Hz, 1H, H-1a), 3.86 (dd, $J$ = 10.5, 5.0 Hz, 1H, H-6), 3.72 (dd, $J$ = 10.6, 6.6 Hz, 1H, H-6a), 1.52, 1.44, 1.42 and 1.34 [4s, each 3H, C(CH$_3$)$_2$]; $^{13}$C NMR (CDCl$_3$): δ 138.2, 128.6, 128.3, 127.7 and 127.5 (C$_{Ar}$), 112.7 [C(CH$_3$)$_2$], 111.8 [C(CH$_3$)$_2$], 111.6 (C-2), 85.1 (C-3), 80.2 (C-4), 78.4 (C-5), 73.3 (CH$_2$Ph), 69.2 (C-1), 67.9 (C-6), 26.54, 26.50, 26.0 and 25.0 [C(CH$_3$)$_2$]; HRMS: m/z calcd for C$_{19}$H$_{27}$O$_6$: 351.1802 [M+H]$^+$; found: 351.1810.
1,2-Di-O-acetyl-6-O-benzyl-3,4-O-isopropylidene-β-D-tagatofuranose (18) and 1,2,6-tri-O-acetyl-3,4-O-isopropylidene-β-D-tagatofuranose (19)

To a solution of diacetonide 12 (3.0 g, 8.56 mmol) in acetic anhydride (36 mL), BF₃-OEt₂ (110 µL, 0.85 mmol) was added as a solution in anhydrous CH₂Cl₂ (1 mL) at 0 °C over 5 min and the reaction mixture was stirred at 0 °C for 1.5 h [TLC, EtOAc/hexanes, (1:3, v/v)]. Then the mixture was poured into ice cold saturated solution of NaHCO₃ (50 mL) and was vigorously stirred at 0 °C for 2 h. The mixture was extracted with CH₂Cl₂ (3 × 20 mL). The combined extracts were washed with brine (2 × 10 mL), dried with Na₂SO₄, and filtered, and the solvent was evaporated under reduced pressure. Particular products were separated and purified by column chromatography on silica gel using EtOAc/hexanes (1:5→1:1, v/v) as the eluent to afford title compounds 18 and 19.

Compound 18: Yield 1.99 g, 59%, colorless oil, Rᵣ = 0.36 (EtOAc/hexanes, 1:3), [α]₀ = +55.4 (c =1, CHCl₃). ¹H NMR (CDCl₃): δ 7.40–7.30 (m, 5H, H₅Ar), 4.97 (d, J = 6.0 Hz, 1H, H-3), 4.91 (dd, J = 6.0, 3.9 Hz, 1H, H-4), 4.70–4.52 (m, 4H, CH₂Ph, H-1, H-1a), 4.48 (ddd, J = 6.7, 5.0, 3.9 Hz, 1H, H-5), 3.81 (dd, J = 10.4, 5.1 Hz, 1H, H-6), 3.69 (dd, J = 10.4, 6.7 Hz, 1H, H-6a), 3.69 (dd, J = 10.4, 6.7 Hz, 1H, H-6a), 2.11 and 2.07 (2×COCH₃), 1.46 and 1.32 [2s, each 3H, C(CH₃)₂]; ¹³C NMR (CDCl₃): δ 170.2 and 169.7 (2×C=O), 138.0, 128.3, 127.8 and 127.6 (C₆Ar), 113.3 [C(CH₃)₂], 109.6 (C-2), 84.8 (C-3), 81.8 (C-5), 80.2 (C-4), 73.4 (CH₂Ph), 67.8 (C-6), 62.7 (C-1), 25.8 and 24.5 [C(CH₃)₂], 21.7 and 20.8 (2×COCH₃). HRMS: m/z calcd for C₂₀H₂₇O₈: 395.1700 [M+H]⁺; found: 395.1698.

Compound 19: Yield 0.81 g, 28%, colorless oil, Rᵣ = 0.22 (EtOAc/hexanes, 1:3), [α]₀ = +23.0 (c =1, CHCl₃) ¹H NMR (CDCl₃): δ 4.99 (d, J = 6.0 Hz, 1H, H-3), 4.94 (dd, J = 6.0, 4.0 Hz, 1H, H-4), 4.65 (d, J = 11.9 Hz, 1H, H-1), 4.57–4.42 (m, 3H, H-1a, H-5, H-
Ethyl 3,4-O-isopropylidene-6-O-pivaloyl-2-thio-α-D-tagatofuranoside (13)

BF₃·Et₂O (179 μL, 1.45 mmol) was added to a stirred solution of methyl pivalate 11 (500 mg, 1.45 mmol) and ethanethiol (157 μL, 2.17 mmol) in anhydrous CH₂Cl₂ (10 mL) at 0 °C and the reaction mixture was stirred at this temperature until all starting material was consumed (2 h). Next, the reaction mixture was diluted with CHCl₃ (20 mL) and was washed with a saturated aqueous solution of NaHCO₃ (30 mL). The organic layer was dried with Na₂SO₄, and filtered, and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography with EtOAc/hexanes (1:4, v/v) as the eluent to afford 2-thio-α-D-tagatofuranoside 13 (298 mg, 59%) as a colorless oil. [α]D = +124.6 (c = 1.75, CHCl₃), ¹H NMR (CDCl₃): δ 4.79 (dd, J = 6.0, 3.8 Hz, 1H, H-4), 4.49 (dd, J = 11.7, 3.6 Hz, 1H, H-6), 4.43 (d, J = 6.0 Hz, 1H, H-3), 4.33 (dt, J = 7.5, 3.7 Hz, 1H, H-5), 4.22 (dd, J = 11.7, 7.7 Hz, 1H, H-6a), 3.91 (d, J = 11.7 Hz, 1H, H-1), 3.75 (d, J = 11.7 Hz, 1H, H-1a), 2.51 (m, 2H, SCH₂CH₃), 1.48 and 1.29 [2s, each 3H, C(CH₃)₂], 1.23 (t, J = 7.5 Hz, 3H, SCH₂CH₃), 1.19 [s, 9H, C(CH₃)₃]; ¹³C NMR (100 MHz, CDCl₃): δ 178.1 (C=O), 113.1 [C(CH₃)₂], 96.7 (C-2), 85.7 (C-3), 80.4 (C-4), 77.9 (C-5), 61.98 (C-1), 61.92 (C-6), 38.6 [C(CH₃)₃], 27.0 [C(CH₃)₃], 25.7 and 24.4 [C(CH₃)₂], 21.2 (SCH₂CH₃), 15.1 (SCH₂CH₃); HRMS: m/z calcd for C₁₆H₂₈O₆S: 349.1679 [M+H]⁺; found: 349.1682.
**Ethyl 1-O-dibenzylxoyphosphoryl-3,4-O-isopropylidene-α-D-tagatofuranosyl sulfone (15)**

To a solution of pivalate 14 (170 mg, 0.31 mmol) in MeOH (3 mL), a freshly prepared 1 M solution of NaOMe (160 µL) was added. The reaction mixture was stirred at room temperature for 90 min [TLC, EtOAc/hexanes, (1:1, v/v)]. Then, the mixture was carefully neutralized with Amberlite® IR-120 H⁺. Next, the Amberlite was filtered off, washed with methanol (3 mL) and the filtrate was evaporated under reduced pressure. The crude product was purified by column chromatography with EtOAc/hexanes (1:1, v/v) as the eluent to afford alcohol 15 (137 mg, 93%) as a colorless oil. [α]₀ = +24.9 (c = 1.0, CHCl₃), ¹H NMR (CDCl₃): δ 7.41–7.33 (m, 10H, H₆Ar), 5.29 (d, J = 6.2 Hz, 1H, H-3), 5.18–5.02 (m, 4H, 2×C₆H₂Ph), 4.97 (dd, J = 6.2, 4.4 Hz, 1H, H-4), 4.69–4.50 (m, 2H, H-5, H-1), 4.42 (dd, J = 12.1, 6.4 Hz, 1H, H-1a), 3.85 (d, J = 5.6 Hz, 2H, H-6, H-6a), 3.30 (dq, J = 13.9, 7.6 Hz, 1H, CH₂CH₃), 3.00 (dq, J = 13.9, 7.6 Hz, 1H, CH₂CH₃), 1.46 and 1.32 [2s, each 3H, C(CH₃)₂]; ¹³C NMR (CDCl₃): δ 135.57, 135.51, 128.73, 128.71, 128.6, 127.99 and 127.97 (C₆Ar), 114.1 [C(CH₃)₂], 101.0 (C-2), 84.1 (C-5), 81.7 (C-3), 81.3 (C-4), 69.65 and 69.63, (2×CH₂Ph), 66.9 (C-1), 61.0 (C-6), 45.4 (CH₂CH₃), 25.6 and 23.9 [C(CH₃)₂], 5.03 (CH₃CH₂). HRMS: m/z calcd for C₂₅H₃₃O₁₀PS: 557.1605 [M+H]+; found: 557.1611.

**General procedure for preparation of compounds 16 and 22**

To a solution of acetonids 15 (120 mg, 0.22 mmol), 21a (112 mg, 0.17 mmol) and 21b (115 mg, 0.17 mmol) in THF (4 mL), 3 M HCl (1.4 mL for 15 and 1.1 mL for 21a and 21b) was added dropwise and the reaction mixture was stirred at 40 °C for 8 h [TLC, EtOAc/hexanes, (6:1, v/v)]. Then, the solid NaHCO₃ was carefully added to neutralize the reaction and the solvents were evaporated under reduced pressure.
The crude products were purified by column chromatography with EtOAc/hexanes (6:1→EtOAc, v/v) as the eluent to afford triol 16 and diols 22.

*Ethyl 1-O-dibenzyloxyphosphoryl-α-D-tagatofuranosyl sulfone (16)*

Yield 91 mg, 85 %, colorless oil, [α]_D = +2.1 (c = 1.0, MeOH), ^1^H NMR (CD_3OD): δ 7.42–7.36 (m 10H, H_{Ar}), 5.13 (m, 4H, 2×CH_2Ph), 5.05 (d, J = 4.9 Hz, 1H, H-3), 4.68 (dd, J = 11.9, 5.7 Hz, 1H, H-1), 4.55 (dd, J = 11.9, 5.0 Hz, 1H, H-1a), 4.31 (dd, J = 4.9, 3.1 Hz, 1H, H-4), 4.24 (ddd, J = 6.5, 4.6, 3.1 Hz, 1H, H-5), 3.88 (dd, J = 11.9, 4.5 Hz, 1H, H-6), 3.80 (dd, J = 11.8, 6.5 Hz, 1H, H-6a), 3.38 (m, 1H, C_2H_5CH_3), 2.98 (m, 1H, C_2H_5CH_3), 1.30 (t, J = 7.5 Hz, 3H, CH_2C_6H_5). ^13^C NMR (CD_3OD): δ 135.5, 128.38, 128.32 and 127.8 (C_{Ar}), 98.5 (C-2), 83.8 (C-5), 72.8 (C-3), 71.8 (C-4), 69.7 and 69.6 (2×CH_2Ph), 67.5 (C-1), 60.0 (C-6), 45.5 (CH_2CH_3), 4.12 (CH_2CH_3). HRMS: m/z calcd for C_{22}H_{29}O_{10}PS: 539.1115 [M+Na]^+; found: 539.1120.

*Phenyl 6-O-benzyl-1-O-dibenzyloxyphosphoryl-α-D-tagatofuranosyl sulfone (22a)*

Yield 80 mg, 78 %, colorless oil, [α]_D = +39.8 (c = 1.75, MeOH), ^1^H NMR (CD_3OD): δ 7.99–7.89 (m, 2H, H_{Ar}), 7.61–7.54 (m, 1H, H_{Ar}), 7.46–7.40 (m, 2H, H_{Ar}), 7.36 (dt, J = 4.6, 2.1 Hz, 3H, H_{Ar}), 7.33–7.25 (m, 10H, H_{Ar}), 7.22–7.15 (m, 2H, H_{Ar}), 5.23 (d, J = 4.9 Hz, 1H, H-3), 4.84–4.71 (m, 3H, 2×CH_2Ph), 4.71–4.56 (m, 2H, H-1, CH_2PH), 4.55–4.49 (m, 2H, H-1a, H-5), 4.47 (bs, 2H, CH_2Ph), 4.35 (dd, J = 4.9, 3.0 Hz, 1H, H-4), 3.84 (dd, J = 10.9, 3.7 Hz, 1H, H-6), 3.70 (dd, J = 10.9, 7.3 Hz, 1H, H-6a); ^13^C NMR (CD_3OD): δ 138.1, 137.6, 135.6, 135.5, 133.6, 130.1, 128.3, 128.27, 128.24, 128.1, 127.9, 127.6, 127.5, 127.2 (C_{Ar}), 99.6 (C-2), 82.5 (C-5), 73.4 (C-3), 72.8, (CH_2Ph), 69.3 (C-4), 69.24 and 69.22 (2×CH_2Ph), 68.5 (C-6), 66.5 (C-1). HRMS: m/z calcd for C_{33}H_{36}O_8PS: 623.1863 [M+H]^+; found: 623.1825.
Isopropyl 6-O-benzyl-1-O-dibenzyloxyphosphoryl-α-d-tagatofuranosyl sulfone (22b)

Yield 77 mg, 74 %, colorless oil, \([\alpha]_D^0 = +43.2 \ (c = 0.1, \ \text{MeOH})\), 1H NMR (400 MHz, CD\(_3\)OD): \(\delta 7.44–7.20 \ (m, 15\ H, \text{H}_\text{Ar})\), \(5.19–5.05 \ (m, \ 5\ H, 2\times \text{CH}_2\text{Ph}, \text{H}-3)\), \(4.70 \ (dd, \ J = 12.0, 6.1 \ Hz, 1\ H, \text{H}-1)\), \(4.56–4.47 \ (m, 3\ H, \text{CH}_2\text{Ph}, \text{H-1a})\), \(4.39 \ (dd, \ J = 7.0, 4.0, 2.9 \ Hz, 1\ H, \text{H}-5)\), \(4.25 \ (dd, \ J = 4.9, 2.9 \ Hz, 1\ H, \text{H}-4)\), \(3.82 \ (dd, \ J = 10.8, 4.0 \ Hz, 1\ H, \text{H}-6)\), \(3.70 \ (dd, \ J = 10.8, 7.2 \ Hz, 1\ H, \text{H}-6a)\), \(3.53 \ [p, \ J = 6.8 \ Hz, 1\ H, \text{CH}(\text{CH}_3)_2]\), \(1.40 \ [d, \ J = 6.7 \ Hz, 3\ H, \text{CH}(\text{CH}_3)_2]\), \(1.28 \ [d, \ J = 7.0 \ Hz, 3\ H, \text{CH}(\text{CH}_3)_2]\); \(^{13}\text{C}\) NMR (101 MHz, CD\(_3\)OD): \(\delta 138.1, 128.3, 128.3, 127.9, 127.79, 127.70 \) and \(127.2 \ (\text{C}_\text{Ar})\), \(100.4 \ (\text{C}-2)\), \(82.3 \ (\text{C}-5)\), \(73.6 \ (\text{C}-3)\), \(72.7 \ (\text{CH}_2\text{Ph})\), \(71.6 \ (\text{C}-4)\), \(69.7, 69.49 \ (2\times \text{CH}_2\text{Ph})\), \(68.4 \ (\text{C}-6)\), \(67.5 \ (\text{C}-1)\), \(54.7 \ [\text{CH}(\text{CH}_3)_2]\), \(16.62 \) and \(13.85 \ [\text{CH}(\text{CH}_3)_2]\). HRMS: m/z calcd for C\(_{30}\)H\(_{37}\)O\(_{10}\)PS: 659.1477 [M]+K\(^+\); found: 659.1472.

General procedure for the preparation of target compounds 2 and 3

A mixture of alcohol 15, triol 16 or compounds 21 and 22 (1 equiv) and Pd/C (10%, 50 mg) in MeOH (8 mL per 100 mg of substrate) was stirred at room temperature under a hydrogen atmosphere for 3–4 h [TLC, CHCl\(_3\)/MeOH, (3:1, v/v)]. When the reaction was complete, the mixture was filtered through a pad of Celite\(^\circledR\) and a filter cake was washed with a mixture of MeOH/distilled water [2:1 (v/v), 7 mL]. The filtrate was evaporated under reduced pressure and the residue was dissolved in distilled water (5 mL). The resulting mixture was filtered through a PTFE syringe filter (0.45 μm) and the filtrate was lyophilized to afford compounds 2 and 3.

Ethyl 1-O-phosphono-α-d-tagatofuranosyl sulfone (2a)

Yield 15 mg, 98 %, white powder, \([\alpha]_D = +10.7 \ (c = 1.0, \ H_2O)\), 1H NMR (CD\(_3\)OD): \(\delta 5.03 \ (m, 1\ H, \text{H}-3)\), \(4.52-4.40 \ (m, 2\ H, \text{H}-1, \text{H}-1a)\), \(4.34 \ (s, 1\ H, \text{H}-5)\), \(4.24 \ (s, 1\ H, \text{H}-4)\), 518
3.91-3.80 (m, 2H, H-6, H-6a), 3.51 (s, 1H, CH₂CH₃), 3.25 (s, 1H, CH₂CH₃), 1.38 (s, 3H, CH₂CH₃); ¹³C NMR (CD₃OD): δ 99.5 (C-2), 83.4 (C-4), 72.6 (C-3), 71.7 (C-5), 65.9 (C-1), 60.1 (C-6), 45.6 (CH₂CH₃), 4.1 (CH₂CH₃). HRMS: m/z calcd for C₉H₁₇O₁₀PS: 359.0172 [M+Na]⁺; found: 359.0175.

Phenyl 1-O-phosphono-α-D-tagatofuranosyl sulfone (2b)

Yield 17 mg, 96 %, white powder, [α]D = +6.9 (c = 1.0, H₂O), ¹H NMR (CD₃OD): δ 7.99 (d, J = 7.7 Hz, 1H, HA), 7.63 (t, J = 7.6 Hz, 2H, H₂A), 7.49–7.37 (m, 2H, H₂A), 5.10 (d, J = 5.0 Hz, 1H, H-3), 4.31 (d, J = 5.1 Hz, 1H, H-5), 4.25 (p, J = 5.1, 4.6 Hz, 2H, H-4, H-1), 4.09–4.01 (m, 1H, H-1a), 3.89–3.80 (m, 2H, H-6, H-6a). ¹³C NMR (CD₃OD): δ 136.7, 132.7, 130.1, 128.7 (CA), 101.9 (C-2), 84.8 (C-4), 74.8 (C-3), 72.4 (C-5), 65.2 (C-1), 61.1 (C-6). HRMS: m/z calcd for C₁₂H₁₇O₆PS: 375.0274 [M+H]⁺; found: 375.0275.

Isopropyl 1-O-phosphono-α-D-tagatofuranosyl sulfone (2c)

Yield 14 mg, 95 %, white powder, [α]D = +9.8 (c = 1.0, H₂O), ¹H NMR (CD₃OD): δ 4.98 (m, 1H, H-3), 4.58-4.42 (m, 2H, H-1, H-1a), 4.37–4.30 (m, 1H, H-4), 4.30–4.22 (m, 1H, H-5), 3.92 (p, J = 12.1, 4.4 Hz, 1H, CH(CH₃)₂), 3.83 (m, 2H, H-6, H-6a), 1.48 (d, J = 6.7 Hz, 3H, CH₃), 1.40 (d, J = 6.8 Hz, 3H, CH₃). ¹³C NMR (CD₃OD): δ 101.9 (C-2), 83.1 (C-5), 73.4 (C-3), 71.5 (C-3), 64.6 (C-1), 59.8 (C-6), 54.4 [CH(CH₃)₂], 16.5 and 13.9 [CH(CH₃)₂]. HRMS: m/z calcd for C₉H₁₉O₁₀PS: 373.0329 [M+Na]⁺; found: 373.0325.
**Ethyl 3,4-O-isopropylidene-1-O-phosphono-α-D-tagatofuranosyl sulfone (3a)**

Yield 16 mg, 98 %, white powder, \([\alpha]_D^o = +3.1\ (c = 0.5, \text{H}_2\text{O})\), \(^1\text{H}\) NMR (CD\(_3\)OD): \(\delta\) 5.34–5.17 (m, 1H, H-3), 4.96 (m, 1H, H-4), 4.56 (s, 1H, H-5), 4.44 (bs, 1H, H-1), 4.27 (d, \(J = 14.9\) Hz, 1H, H-1a), 3.85 (d, \(J = 11.0\) Hz, 1H, H-6), 3.79–3.68 (m, 1H, H-6a), 3.53 (s, 1H, CH\(_2\)CH\(_3\)), 3.28–3.19 (m, 1H, CH\(_2\)CH\(_3\)), 1.49 (s, 3H CH\(_2\)CH\(_3\)), 1.36 (bs, \(J = 10.6\) Hz, 6H, \([\text{C(CH}_3]\)_2]); \(^{13}\text{C}\) NMR (CD\(_3\)OD): \(\delta\) 113.6 \([\text{C(CH}_3]\)_2], 101.5 (C-2), 84.7 (C-5), 81.5 (C-3), 80.9 (C-4), 65.8 (C-1), 60.1 (C-6), 45.4 (CH\(_2\)CH\(_3\)), 24.6 and 22.9 [C(CH\(_3\))\(_2\)]. HRMS: m/z calcd for C\(_{11}\)H\(_{21}\)O\(_{10}\)PS: 399.0485 [M+Na]\(^+\); found: 399.0490.

**Phenyl 3,4-O-isopropylidene-1-O-phosphono-α-D-tagatofuranosyl sulfone (3b)**

Yield 17 mg, 98 %, white powder, \([\alpha]_D^o = +2.8\ (c = 1.0, \text{H}_2\text{O})\), \(^1\text{H}\) NMR (CD\(_3\)OD): \(\delta\) 7.98 (d, \(J = 7.3\) Hz, 2H, H\(_{\text{Ar}}\)), 7.71 (t, \(J = 6.9\) Hz, 1H, H\(_{\text{Ar}}\)), 7.60 (d, \(J = 7.4\) Hz, 2H, H\(_{\text{Ar}}\)), 5.49 (d, \(J = 5.9\) Hz, 1H, H-3), 5.10 (s, 1H, H-4), 4.80 (s, 1H, H-5), 4.43–4.36 (m, 1H, H-1), 4.16–4.06 (m, 1H, H-1a), 3.93–3.81 (m, 1H, H-6), 3.70 (t, \(J = 9.4\) Hz, 1H, H-6a), 1.49 and 1.38 [C(CH\(_3\))\(_2\)]; \(^{13}\text{C}\) NMR (CD\(_3\)OD): \(\delta\) 136.2, 133.8, 130.3 and 128.4 (C\(_{\text{Ar}}\)), 113.6 [C(CH\(_3\))\(_2\)], 102.2 (C-2), 84.9 (C-5), 81.9 (C-3), 81.1 (C-4), 64.5 (C-1), 60.1 (C-6), 24.5 and 22.9 [C(CH\(_3\))\(_2\)]. HRMS: m/z calcd for C\(_{15}\)H\(_{21}\)O\(_8\)PS: 415.0587 [M+Na]\(^+\); found: 415.0588.

**Isopropyl 3,4-O-isopropylidene-1-O-phosphono-α-D-tagatofuranosyl sulfone (3c)**

Yield 12 mg, 95 %, white powder, \([\alpha]_D^o = +14.1\ (c = 0.5, \text{H}_2\text{O})\), \(^1\text{H}\) NMR (CD\(_3\)OD): \(\delta\) 5.30 (d, \(J = 5.9\) Hz, 1H, H-3), 4.96 (m, 1H, H-4), 4.61 (dt, \(J = 7.8, 4.0\) Hz, 1H, H-5), 4.44 (d, \(J = 10.4\) Hz, 1H, H-1), 4.30 (d, \(J = 9.9\) Hz, 1H, H-1a), 3.98–3.88 [m, 1H, CH(CH\(_3\))\(_2\)], 3.84 (dd, \(J = 12.0, 4.1\) Hz, 1H, H-6), 3.74 (dd, \(J = 11.9, 7.4\) Hz, 1H, H-6a),
1.50 (bs, 6H, 2× CH₃), 1.36 (bs 6H, 2×CH₃). ¹³C NMR (CD₃OD): δ 113.5 [C(CH₃)₂], 103.7 (C-2), 84.6 (C-5), 82.4 (C-3), 80.7 (C-4), 65.8 (C-1), 60.1 (C-6), 55.2 [CH(CH₃)₂], 24.5 and 22.9 [C(CH₃)₂], 16.3 and 13.8 [CH(C(CH₃)₂)]. HRMS: m/z calcd for C₁₂H₂₃O₁₀PS: 413.0642 [M+Na]+; found: 413.0649.

**General procedure for the thioglycosylation of diacetonide 12**

BF₃·Et₂O (1.8 equiv.) was added to a stirred solution of diacetonide 12 (1 eq.) and corresponding thiol [3 equiv, (PhSH, iPrSH)] in anhydrous CH₂Cl₂ (5 mL per 100 mg) at −5 °C and the reaction mixture was stirred at this temperature until all starting material was consumed (1 h). Next, the reaction mixture was washed with saturated aqueous solution of NaHCO₃ (20 mL per 100 mg) and brine (20 mL per 100 mg). The organic layer was dried with Na₂SO₄, and filtered, and the solvent was evaporated under reduced pressure. The crude products were purified by column chromatography with EtOAc/hexanes (1:5, v/v) as the eluent to afford 2-thio-α-D-tagatofuranosides 17.

**Phenyl 6-O-benzyl-3,4-O-isopropylidene-2-thio-α-D-tagatofuranoside (17a)**

Yield 380 mg, 44 %, colorless oil, [α]D = +107.0 (c = 1.0, CHCl₃), ¹H NMR (CDCl₃): δ 7.59–7.44 (m, 2H, HAr), 7.44–7.24 (m, 8H, HAr), 4.91 (dd, J = 6.0, 3.8 Hz, 1H, H-4), 4.77 (dt, J = 7.0, 4.1 Hz, 1H, H-5), 4.67 (d, J = 12.0 Hz, 1H, CH₂Ph), 4.63 (d, J = 6.0 Hz, 1H, H-5), 4.57 (d, J = 12.0 Hz, 1H, CH₂Ph), 3.96 (dd, J = 10.7, 4.4 Hz, 1H, H-6), 3.85–3.74 (m, 2H, H-1, H-6a), 3.57 (d, J = 11.8 Hz, 1H, H-1a), 1.50 and 1.36 [C(CH₃)₂]; ¹³C NMR (CDCl₃): δ 138.2, 135.9, 129.2, 129.0, 128.8, 128.3, 127.6 and 127.5 (CAr), 113.1 [C(CH₃)₂], 99.4 (C-2), 85.6 (C-3), 80.6 (C-4), 79.5 (C-5), 73.3
(CH\textsubscript{2}Ph), 67.6 (C-6), 61.5 (C-1), 25.8 and 24.5 [C(CH\textsubscript{3})\textsubscript{2}]. HRMS: m/z calcd for C\textsubscript{22}H\textsubscript{26}O\textsubscript{5}S: 425.1393 [M+Na]; found: 425.1395.

Isopropyl 6-O-benzyl-3,4-O-isopropylidene-2-thio-\(\alpha\)-D-tagatofuranoside (17b)
Yield 255 mg, 54 %, colorless oil, [\(\alpha\)]\textsubscript{D} = +95.2 (c = 1.0, CHCl\textsubscript{3}), \(^1\)H NMR (CDCl\textsubscript{3}): \(\delta\) 7.38–7.25 (m, 5H, H\textsubscript{Ar}), 4.80 (dd, \(J = 6.0, 3.9\) Hz, 1H, H-4), 4.65 (d, \(J = 12.1\) Hz, 1H, CH\textsubscript{2}Ph), 4.55 (d, \(J = 12.1\) Hz, 1H, CH\textsubscript{2}Ph), 4.43 (dt, \(J = 6.0, 3.8\) Hz, 1H, H-5), 4.40 (d, \(J = 6.0\) Hz, 1H, H-3), 3.96 (dd, \(J = 11.6, 10.5\) Hz, 1H, H-1), 3.88 (dd, \(J = 10.7, 4.7\) Hz, 1H, H-6), 3.79 (dd, \(J = 11.6, 3.2\) Hz, 1H, H-1a), 3.75 (dd, \(J = 10.7, 7.0\) Hz, 1H, H-6a), 3.10 [p, \(J = 6.9\) Hz, 1H, CH(CH\textsubscript{3})\textsubscript{2}], 2.51 (dd, \(J = 10.5, 3.2\) Hz, 1H, OH), 1.48 and 1.31 [C(CH\textsubscript{3})\textsubscript{2}], 1.33 and 1.32 [2d, \(J = 6.9\) Hz, each 3H, CH(CH\textsubscript{3})\textsubscript{2}]; \(^{13}\)C NMR (CDCl\textsubscript{3}): \(\delta\) 138.1, 128.2 and 127.5 (C\textsubscript{Ar}), 112.8 [C(CH\textsubscript{3})\textsubscript{2}], 97.6 (C-2), 85.7 (C-3), 80.5 (C-4), 79.2 (C-5), 73.3 (CH\textsubscript{2}Ph), 67.5 (C-6), 61.8 (C-1), 32.8 [CH(CH\textsubscript{3})\textsubscript{2}], 25.7 [CH(CH\textsubscript{3})\textsubscript{2}], 25.1 and 24.4 [C(CH\textsubscript{3})\textsubscript{2}]. HRMS: m/z calcd for C\textsubscript{19}H\textsubscript{28}O\textsubscript{5}S: 391.1550 [M+Na]; found: 391.1555.

Phenyl 1-O-acetyl-6-O-benzyl-3,4-O-isopropylidene-2-thio-\(\alpha\)-D-tagatofuranoside (20)
To a solution of diacetate 18 (1.5 g, 3.81 mmol) and PhSH (550 \(\mu\)L, 5.33 mmol) in anhydrous CHCl\textsubscript{3} (12 mL), BF\textsubscript{3}.OEt\textsubscript{2} (1.5 mL, 11.4 mmol) was added dropwise at –20 °C and the reaction mixture was stirred for 90 min at this temperature [TLC, EtOAc/hexanes, (2:1, v/v)]. Then the mixture was diluted with CHCl\textsubscript{3} (50 mL) and washed with saturated solution of NaHCO\textsubscript{3} (2 \(\times\) 25 mL) and brine (2\(\times\) 15 mL). The organic layer was dried with Na\textsubscript{2}SO\textsubscript{4}, and filtered, and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography with EtOAc/hexanes (1:4, v/v) as the eluent to afford 2-thio-\(\alpha\)-D-tagatofuranoside 20.
(1.2 g, 72%) as a colorless oil. \([\alpha]_D^{20} = +77.1\ (c = 1.0, \text{CHCl}_3)\), \(^1\text{H} \text{NMR (CDCl}_3\): δ 7.61–7.43 (m, 2H, H\text{Ar}), 7.44–7.14 (m, 8H, H\text{HAr}), 4.91 (dd, \(J = 6.0, 3.8\) Hz, 1H, H-4), 4.76 (dt, \(J = 7.0, 4.2\) Hz, 1H, H-5), 4.65 (d, \(J = 12.1\) Hz, 1H, CH\text{Ph}), 4.60 (d, \(J = 6.0\) Hz, 1H, H-3), 4.56 (d, \(J = 12.0\) Hz, 1H, CH\text{Ph}), 4.24 (m, 2H, H1, H-1a), 3.95 (dd, \(J = 10.8, 7.1\) Hz, 1H, H-6a), 2.10 (s, 3H, COCH\text{3}), 1.46 and 1.35 [2s, each 3H, C(CH\text{3})\text{2}]; \(^{13}\text{C} \text{NMR (CDCl}_3\): δ 170.2 (COCH\text{3}), 138.3, 135.9, 129.7, 128.9, 128.7, 128.3, 127.59 and 127.54 (C\text{Ar}), 113.10 [C(CH\text{3})\text{2}], 96.7 (C-2), 85.5 (C-3), 80.8 (C-4), 79.5 (C-5), 73.3 (CH\text{2Ph}), 67.6 (C-6), 62.5 (C-1), 25.8 and 24.8 [C(CH\text{3})\text{2}], 21.00 (COCH\text{3}). HRMS: \(m/z\) calcld for C\text{24H28O6S}: 467.1499 [M+Na]+; found: 467.1501.

**Biological assays**

*Preparation of enzymatically active cell envelope fraction of Mycobacterium smegmatis mc\text{2}155*

*M. smegmatis mc\text{2}155* were grown in nutrient broth at 37 °C, 120 min\(^{-1}\). The cells were suspended in 50 mM MOPS buffer (pH 7.9) containing 5 mM β-mercaptoethanol and 10 mM MgCl\text{2} (Buffer A) in the ratio 3 g/8 mL of the final suspension, and the mixture was subjected to probe sonication (Soniprep 150; Sanyo, MSE Ltd., Sussex, United Kingdom) 10 times 60 s pulses with 90 s cooling intervals. The lysate was centrifuged at 100 000 × g for 60 min at 4 °C. The pellet was resuspended in buffer A (250 μL for 1 mL of the centrifuged suspension) to obtain the enzymatically active fraction containing mycobacterial membrane and cell wall proteins (L100) with a concentration of ≈ 20 mg/mL.
Reaction mixtures and analysis of reaction products

Enzyme reactions, extractions and analyses of the reaction products were carried out as described by Mikusova et al. [5] with minor modifications. 250–500 μg of L100 proteins were pre-incubated 10 min on ice with the inhibitors at the final concentration in the reaction mixture of 0.5–10 mM. The reaction mixture was supplemented with UDP-GlcNAc (200 μM), TDP-Rha (200 μM), NADH (2.5 mM) and UDP-[14C]Galp (0.125 μCi, specific activity 55 mCi/mmol, ARC) for monitoring of the incorporation of the radioactive substrate into the lipid-linked intermediates of galactan synthesis. The volume of the reaction was adjusted to 40 μL with Buffer A. The mixtures were incubated for 1 h at 37 °C and stopped by addition of 1.5 mL CHCl₃-CH₃OH (2:1; v/v). The suspensions were rocked at room temperature for 20 min and centrifuged. The supernatant, CHCl₃-CH₃OH phase containing lower lipid-linked galactan precursors (GL3–5) was removed from the pellet and subjected to Folch wash, as described previously [5]. The final CHCl₃-CH₃OH phase was dried and dissolved in 40–50 μL of CHCl₃/CH₃OH/H₂O/NH₄OH (65:25:3.6:0.5); 10 μL were subjected to scintillation counting and 10 μl were analysed by thin layer chromatography on silica gel plates [Silica gel 60 F₂₅₄ (Merck)] in CHCl₃-CH₃OH-NH₄OH-1 M ammonium acetate-H₂O (180:140:9:9:23). The radiolabeled lipid bands were visualized by autoradiography.

The pellets were extracted with 50% CH₃OH in H₂O with 0.9% NaCl, 50% CH₃OH in H₂O and 100% CH₃OH (1 mL each), as described previously [5], to remove the residual radiolabel. The lipid-linked galactan polymer was obtained by the extraction of the resulting pellets with 1 ml of “TT3” CHCl₃-CH₃OH-H₂O (10:10:3; v/v) and then with 1 ml of “E-soak” (water-ethanol-diethyl ether-pyridine-concentrated ammonium
hydroxide [15:15:5:1:0.017]) [5]. 10-20% of these fractions were then subjected to quantification by scintillation counting.

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