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Title: Synthesis and biological evaluation of arabinose 5-phosphate mimics modified at position five.

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Abstract. A set of new metabolically stable arabinose 5-phosphate analogues possessing phosphate mimetic groups at position 5 was synthesised. Their ability to interact with arabinose 5-phosphate isomerase from *Pseudomonas aeruginosa* was evaluated by STD-NMR studies. The synthesised compounds were also characterised for their activity *in vivo* on *P. aeruginosa* and *E. coli* strains. Unfortunately, none of the synthesised compounds was able neither to bind API nor to inhibit bacterial growth.

Keywords. Arabinose 5-phosphate / sugar isomerase / NMR interaction studies / phosphate analogues / Pseudomonas aeruginosa

Lipopolysaccharide (LPS) is a complex glycolipid essential for bacterial survival and responsible of eliciting immune responses in the host. Among the LPS's glycidic residues, 3-deoxy-D-manno-octulosonic acid (Kdo) is an essential component of LPSs in all Gram-negative bacteria known to date,[1] therefore its biosynthesis is a significant target for new therapeutics design;[2] it has been shown that the breakdown of Kdo biosynthesis results in an accumulation of Lipid A precursors

inside the cell, affecting bacterial viability.[3] Arabinose 5-phosphate isomerase (API) is the first committed enzyme of the Kdo biosynthetic pathway that catalyses the reversible isomerization of ribulose 5-phosphate (Ru5P) into arabinose 5-phosphate (A5P). Few API inhibitors have been designed to date;[4,5,6] among them the most effective compounds *in vitro* were described by Woodard and co-workers in 2011.[5,7] However, none of the reported inhibitors showed promising activity *in vivo,* thus the search for potent inhibitors against arabinose 5-phosphate isomerase is still open. We present herein the design, synthesis and biological evaluation of a set of new A5P analogues, modified at position 5 by the introduction of sulphur- or carboxylate-based phosphate mimetic groups (Fig. 1). Previous NMR studies revealed that an acidic group is fundamental for the binding with the enzyme,[8] due to its involvement in salt-bridging with basic residue in the catalytic pocket.[4] Since the phosphate group is known to have poor *in vivo* stability toward enzymatic hydrolysis,[9] the finding of a good and metabolically resistant phosphate mimetic is a key point for the design of stable potential inhibitors of API.

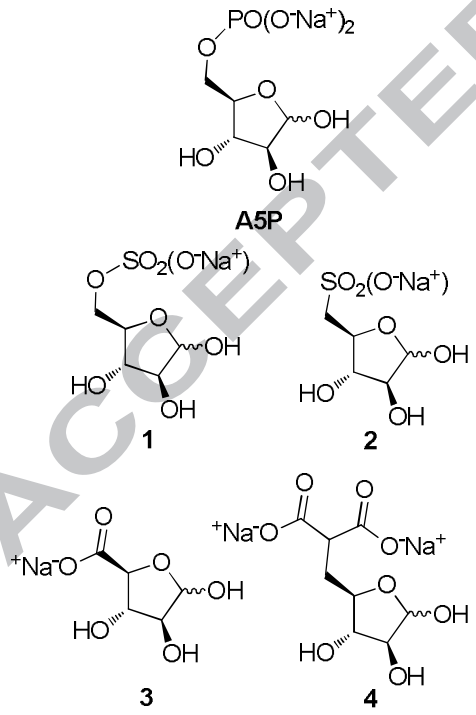
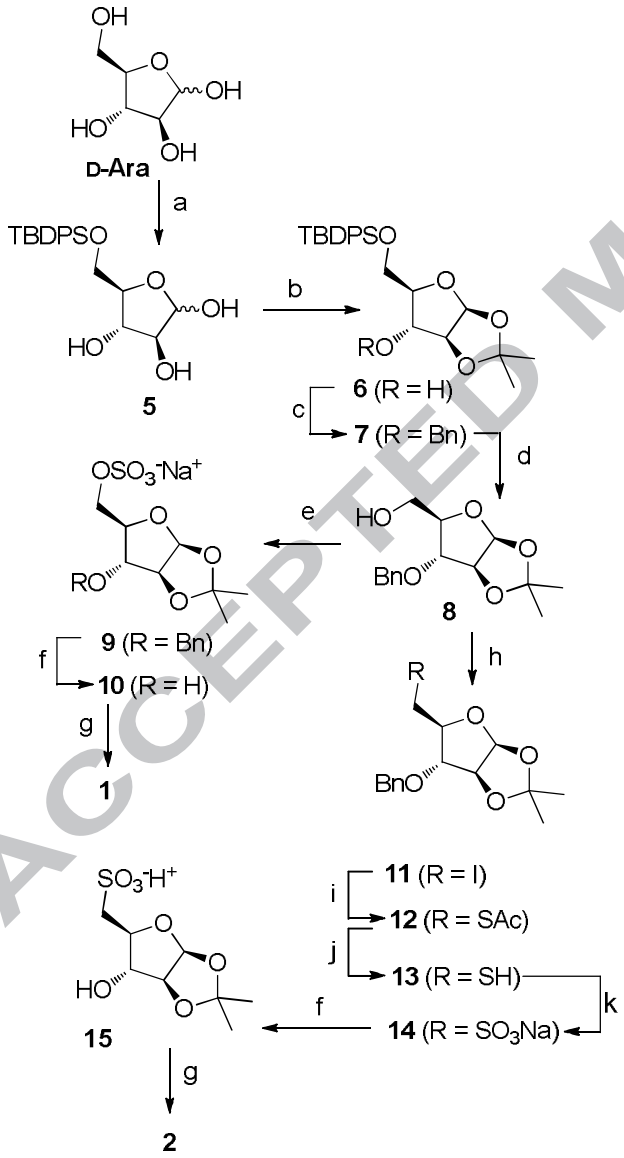


Fig. 1. Set of A5P analogues with different acidic group at position 5.

The sulphate derivative **1**, the non-isosteric sulphonate **2**, the non-isosteric carboxylate **3** and the malonate analogue **4** (malonate group has been already used as a good phosphate mimetic)[10] were synthesised.

The key intermediate **8** was synthesised from D-arabinose (Scheme 1), by protection at the primary hydroxyl group with *t*-butyldiphenylsilyl chloride (TBDPSCl) affording compound **5**, and then with acetone dimethyl acetal, in the presence of catalytic camphorsulphonic acid (CSA) (compound **6**). Benzylation of the hydroxyl group at position 3 (compound **7**) and subsequent selective cleavage of the silyl ether with TBAF, gave compound **8**.

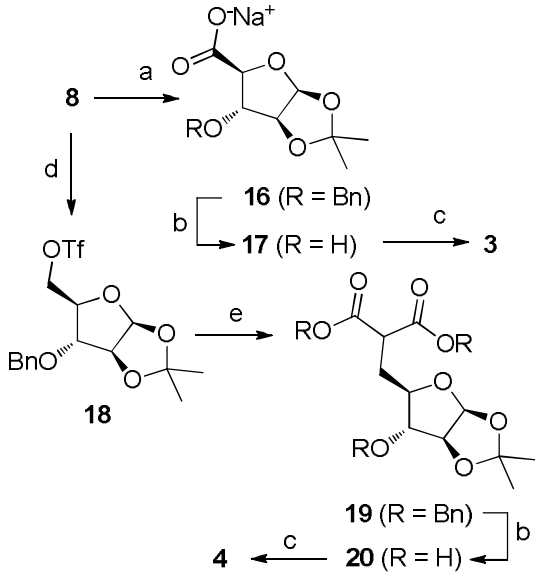


Scheme 1. *Reagents and conditions*. a) TBDPSCl, dry pyridine (4 °C, 75 %); b) DMP, CSA, dry dichloromethane (4 °C, 76 %); c) BnBr, NaH, dry DMF (93 %); d) TBAF, dry THF (80 %); e) SO_3·Py, DMF (90 %); f) H_2, $Pd(OH)_2$, EtOH, (quant. yield); g) i: D_2O (pH 4, 90 °C), ii: aq. 5 % NaOH (quant. yield); h) I_2, imidazole, PPh_3, dry toluene (100 °C, 94 %); i) KSAc, DMF (98 %); j) MeONa, dry MeOH (86 %); k) TEMPO, NaOCl, KBr, sat aq. $NaHCO_3$ (85 %).

Reaction of intermediate **8** with SO_3-Pyridine complex (Scheme 1) as sulphating agent[11] in DMF, followed by hydrogenolysis of the benzyl group to compound **10**, and final acetal hydrolysis gave compound **1**. The final deprotection step was performed in a D_2O solution in order to follow the reaction by ^{1}H-NMR.

Mitzunobu reaction of intermediate **8** with iodine, imidazole and triphenylphospine afforded iodide **11** (Scheme 1). Reaction of **11** with a DMF solution of potassium thioacetate to **12**, followed by Zemplèn methanolysis to thiol **13**, and oxidation with TEMPO gave sulphonate **14**. Final deprotection steps in usual conditions afforded deprotected sulphonate **2**.

On the other hand, direct oxidation of the intermediate **8** with TEMPO[12] gave the corresponding carboxylate derivative **16** (Scheme 2). Hydrogenolysis of the benzyl group to compound **17**, followed by deprotection of the dimethyl acetal as previously described afforded the non-isosteric carboxylate analogue **3**.



Scheme 2. *Reagents and conditions*. a) TEMPO, NaOCl, KBr, satd aq. $NaHCO_3$ (95 %); b) H_2, $Pd(OH)_2$, EtOH, (quant. yield); c) i: D_2O (pH 2-4, rt-90 °C), ii: aq. 5 % NaOH (quant. yield); d) Tf_2O, DTBMP, dichloromethane, -78 °C; e) dibenzyl malonate, LiHMDS, dry THF (50 % over two steps).

Finally, intermediate **8** was treated with triflic anhydride (Tf_2O) and di-tert-butyl methyl pyridine (DTBMP), obtaining the triflate **18** (Scheme 2).[13] The triflate was immediately reacted with dibenzylmalonate[14] and LiHMDS as the base, in dry THF, giving malonate **19**. Hydrogenolysis of benzyl groups to compound **20**, followed by acetal hydrolysis gave malonate **4**.

Compounds **1**-**4** were evaluated for their ability to bind to API from *Pseudomonas aeruginosa* by STD-NMR spectroscopy as previously described[15,7] and to inhibit the growth of *P. aeruginosa*, wild type strain *E. coli* (BW25113) and *E. coli* mutant strain (AS19).[6,16] The absence of small molecule-derived ^1H NMR signals in STD spectra indicates the absence of enzyme–small molecule associations. Unfortunately, none of the synthesised compounds was able to bind the target enzyme nor to inhibit bacterial growth.

1. Experimental

1.1. General methods

Solvents were dried over molecular sieves, for at least 24 h prior to use, when required. When dry conditions were required, the reaction was performed under Ar or N_2 atmosphere. Thin-layer chromatography (TLC) was performed on silica gel $60F_{254}$ coated glass plates (Merck) with UV detection when possible, charring with a conc. H_2SO_4/EtOH/H_2O solution (10:45:45 v/v/v), or with a solution of $(NH_4)_6Mo_7O_{24}$ (21 g), $Ce(SO_4)_2$ (1 g), conc. H_2SO_4 (31 mL) in water (500 mL) and then heating to 110°C for 5 min. Flash column chromatography was performed on silica gel 230-400 mesh (Merck). Platinum oxide was filtered by Acrodis® Premium 25 mm Syringe Filter, with 0.45 um Nylon membrane. Routine ^1H and ^{13}C NMR spectra were recorded at 400 MHz (^1H) and at 100.57 MHz (^{13}C) or on a Varian Mercury instrument. Chemical shifts are reported in parts per million downfield from TMS as an internal standard; *J* values are given in Hz. Mass spectra were recorded on a System Applied Biosystems MDS SCIEX instrument (Q TRAP, LC/MS/MS, turbon ion spray) or on a System Applied Biosystem MDS SCIEX instrument (Q STAR elite nanospray). ESI full MS were recorded on a Thermo LTQ instrument by direct inlet; relative percentages are shown in brackets. Elementar analysis (C, H, N) were performed with Perkin-Elmer series II 2400 analyse.

1.2. (3S,4S,5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)tetrahydrofuran-2,3,4-triol (5). To a stirred suspension of D-arabinose (1.83 g, 12.24 mmol, 1 equiv) in dry Py (30 mL), TBDPSCl (3.83 mL, 14.9 mmol, 1.2 equiv) was added dropwise at 0 °C under argon atmosphere. Afer 10 min the reaction was stirred at 4 °C overnight. EtOH was added to quench the reaction and the mixture was stirred for 15 min. The mixture was concentrated under reduced pressure. The product was purified by flash column chromatography (5:5, PE:EtOAc) affording **5** (3.547 g, 75 % yield) as a yellow oil (mixture of α- and β- anomers). NMR and mass data are in agreement with those reported.[17]

1.3. (3aS,5R,6R,6aS)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-6-ol (6). To a stirred solution of **5** (3.216 g, 8.278 mmol, 1 equiv) in dry CH_2Cl_2 (190 mL), 2,2- dimethoxypropane (2.18 mL, 17.79 mmol, 2 equiv) and camphorsulphonic acid (0.3 g,

0.828 mmol, 0.1 equiv) were added at 4 °C under argon atmosphere. The reaction mixture was stirred overnight at 4 °C. Then Et_3N was added to neutralize the reaction and the mixture was stirred for 15 min. The mixture was concentrated under reduced pressure. The product was purified by flash column chromatography (8:2, PE:EtOAc) giving **6** (2.69 g, 76 % yield) as a yellow oil. NMR and mass data are in agreement with those reported.[17]

1.4. (((3aS,5R,6R,6aS)-6-(benzyloxy)-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)methoxy)(tert-butyl)diphenylsilane (7). To a stirred solution of **6** (1.250 g, 2.919 mmol, 1 equiv) in dry THF (20 mL), benzyl bromide (1.84 mL, 11.676 mmol, 4 equiv) and sodium hydride 60 % (584 mg, 14.595 mmol, 5 equiv) were slowly added portion-wise. The reaction was stirred for three hours at room temperature and then the mixture was concentrated under reduced pressure. The product was purified by flash column chromatography (100 % PE →9:1, PE:EtOAc) giving **7** (1.40 g, 93 % yield) as a yellowish oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 – 7.54 (m, 5H, H Ph), 7.38 – 7.17 (m, 10H, H Ph), 5.82 (d, $J_{1,2}$ = 4.1 Hz, 1H, H1), 4.59 (d, $J_{2,1}$ = 4.1 Hz, 1H, H2), 4.54 (d, J = 1.5 Hz, 2H, CH_2 Bn), 4.21 – 4.11 (m, 2H, H3, H4), 3.80 – 3.67 (m, 2H, H5), 1.27 (s, 3H, Me), 1.22 (s, 3H, Me), 0.97 (s, 9H, tBu). ^{13}C NMR (101 MHz, $CDCl_3$) δ 137.77 (1C, C_{quat} C Ph), 135.93 (1C, C Ph), 133.50 (1C, C_{quat} C Ph), 133.38 (1C, C_{quat} C Ph), 130.09 (1C, C Ph), 130.03 (1C, C Ph), 128.83 (1C, C Ph), 128.18 (1C, C Ph), 128.07 (1C, C Ph), 128.04 (1C, C Ph), 128.02 (1C, C Ph), 112.77 (1C, C_{quat}), 106.07 (1C, C1), 85.52 (1C, C2), 85,43(1C, C4), 189 83.15 (1C, C3), 71.99 (1C, CH_2 Bn), 63.69 (1C, C5), 27.28 (1C, Me), 27.14 (3C, Me tBu), 26.45 (1C, Me), 19.52 (1C, C_{quat} tBu). MS: m/z = 541.4 $[M+Na]^+$, 557.4 $[M+K]^+$. $C_{31}H_{38}O_5Si$ (518,72): calcd C, 71.78; H, 7.38, found C, 71.69; H, 7.39.

1.5. ((3aS,5R,6R,6aS)-6-(benzyloxy)-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)methanol (8). To a stirred solution of **7** (1.29 g, 2.49 mmol, 1 equiv) in dry THF (20 mL), tetrabutyl ammonium fluoride (1 M THF solution, 7.47 ml, 7.47 mmol, 3 equiv) was added. The reaction mixture was stirred for 1 hour and then concentrated under reduced pressure. The product was purified by flash column chromatography (PE:EtOAc 7:3→6:4) giving **8** (560 mg, 80 % yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.03 (m, 5H, H Ph), 5.83 (d, $J_{1,2}$ = 3.9 Hz, 1H, H1), 4.60 (d, $J_{2,1}$ = 3.9 Hz, 1H, H2), 4.58 – 4.45 (m, 2H, CH_2 Bn), 4.15 – 4.08 (m, 1H, H4), 3.89 (d, J = 2.5 Hz, 1H, H3), 3.72 – 3.59 (m, 2H, H5), 2.71 (bs, 1H, OH), 1.44 (s, 3H, Me), 1.25 (s, 3H, Me). ^{13}C NMR (101 MHz, $CDCl_3$) δ 137.39 (1C, C_{quat} C Ph), 128.73 (1C, C Ph), 128.20 (1C, C Ph), 127.98 (1C, C Ph), 113.06 (1C, C_{quat}), 105.78 (1C, C1), 85.79 (1C, C4), 85.38 (1C, C2), 82.92 (1C, C3), 72.01 (1C, CH_2 Bn), 62.79 (1C, C5), 27.29 (1C, Me), 26.52 (1C, Me). MS: m/z = 303.4 $[M+Na]^+$, 319.3 $[M+K]^+$, 583.3 $[2M+Na]^+$. $C_{15}H_{20}O_5$ (280,32): calcd C, 64.27; H, 7.19; found C, 64.19; H, 7.18.

1.6. Sodium ((3aS,5R,6R,6aS)-6-(benzyloxy)-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)methyl sulfate (9). To a stirred solution of **8** (80 mg, 0.285 mmol, 1 equiv) in dry DMF (3 mL), SO_3-Py (454 mg, 2.854 mmol, 10 equiv) was added. The reaction was stirred for 1 hour and then it was neutralized adding NaOH 5 % solution. The mixture was extracted 6 times with brine and EtOAc; the organic phase was dried with Na_2SO_4, filtered and concentrated under reduced pressure. The product was purified by flash column chromatography (95:0.5, EtOAc:MeOH) giving **9** (85 mg, 90 % yield). ^{1}H NMR (400 MHz, CD_3OD) δ 7.46 – 7.17 (m, 5H, H Ph), 5.89 (d, $J_{1,2}$ = 3.9 Hz, 1H, H1), 4.69 (d, $J_{2,1}$ = 3.9 Hz, 1H, H2), 4.60 (s, 2H, CH_2 Bn), 4.35 (td, $J_{4,5}$ = 6.7 Hz, $J_{4,3}$ = 1.8 Hz, 1H, H4), 4.15 (d, $J_{5,4}$ = 6.7 Hz, 2H, H5), 4.09 (d, $J_{3,4}$ = 1.8 Hz, 1H, H3), 1.51 (s, 1H, Me), 1.30 (s, 1H, Me). ^{13}C NMR (101 MHz, CD_3OD) δ 138.87 (1C, C_{quat} Ph), 129.37 (1C, C Ph), 129.00 (1C, C Ph), 128.79 (1C, C Ph), 113.70 (1C, C_{quat}), 107.32 (1C, C1), 85.77 (1C, C2), 84.67 (1C, C4), 84.13 (1C, C3), 72.58 (1C, CH_2 Bn), 68.46 (1C, C5), 27.18 (1C, Me), 26.23 (1C, Me). MS: m/z = 359.2 $[M-H]^-$. $C_{15}H_{19}NaO_8S$ (382,36): calcd C, 47.12; H, 5.01, found C, 47.16; H, 5.02.

1.7. Sodium ((3aS,5R,6R,6aS)-6-(benzyloxy)-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)methyl sulfate (10). Compound **9** (70 mg, 0.212 mmol) was dissolved in MeOH (4 mL). Palladium hydroxide (70 mg) was added, the mixture was stirred under vacuum in order to remove the residual gas, and then H_2 was added. The reaction was stirred at room temperature for 24 hours and then the catalyst was filtered on a syringe filter and washed with MeOH. The solvent was evaporated giving **10** (53 mg, 98 % yield). ^{1}H NMR (400 MHz, CD_3OD) δ 5.90 (d, $J_{1,2}$ = 3.8 Hz,

1H, H1), 4.53 (d, $J_{2,1}$ = 3.8 Hz, 1H, H2), 4.22 (s, 1H, H3), 4.20 – 4.09 (m, 3H, H4, H5), 1.50 (s, 3H, Me), 1.29 (s, 3H, Me). ^{13}C NMR (101 MHz, CD_3OD) δ 113.51 (1C, C_{quat}), 107.34 (1C, C1), 87.86 (1C, C2), 87.30 (1C, C4), 76.52 (1C, C3), 68.60 (1C, C5), 27.03 (1C, Me), 26.10 (1C, Me). MS: m/z = 269.1 $[M-H]^-$. $C_8H_{13}NaO_8S$ (292,24): calcd. C, 32.88; H, 4.48, found C, 31.91; H, 4.49.

1.8. Sodium ((2R,3S,4S)-3,4,5-trihydroxytetrahydrofuran-2-yl)methyl sulfate (1). Compound **10** (40 mg, 0.137 mmol) was dissolved in D_2O (0.7 mL) and the solution at pH 4 was transferred into an NMR tube. The reaction was followed by ^{1}H-NMR until completion. Then the pH was neutralized adding NaOH 5 % and the solvent was lyophilised to give **1** (34 mg, quantitative yield). ^{1}H NMR (400 MHz, D_2O) δ 5.13 (bs, 1H, H1β), 5.08 (d, J = 2.4 Hz, 1H, H1α), 4.12 – 4.02 (m, 3H, H5'α, H5'β, H3a), 3.99 – 3.91 (m, 3H, H5"α, H5"β, H2β), 3.90 – 3.81 (m, 3H, H2α, H3β, H4α). ^{13}C NMR (101 MHz, D_2O) δ 100.93 (1C, C1α), 95.19 (1C, C1β), 80.97 (1C, C2α), 80.44 (1C, 3α), 78.62 (1C, C3β), 75.76 (1C, C2β), 75.27 (1C, C4α), 73.92 (1C, C4β), 68.77 (1C, C5β), 67.38 (1C, C5α). MS: m/z = 229.1 $[M-H]^-$. $C_5H_9NaO_8S$ (252,17): calcd. C, 23.81; H, 3.60, found: C, 23.88; H, 3.59.

1.9. (3aS,5S,6S,6aS)-6-(benzyloxy)-5-(iodomethyl)-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxole (11). To a stirred solution of **8** (600 mg, 2.140 mmol, 1 equiv) in dry toluen (20 mL), tryphenylphosphine (2.95 g, 10.7 mmol, 5 equiv), imidazole (0.459 mg, 6.42 mmol, 3 equiv) and then iodine (2.28 g, 8.56 mmol, 4 equiv) were added. The reaction mixture was stirred for 1 hour at 100 °C, then 20 mL of $NaHCO_3$ saturated solution were added. Under vigorous stirring, iodine was added until the mixture become dark coloured, in order to oxidize the tryphenylphosphine in excess. Then, iodine in excess was reduced adding a saturated solution of $Na_2S_2O_4$ (about 50 mL). The mixture was extracted 3 times with toluen; the organic phase was dried with Na_2SO_4, filtered and concentrated under reduced pressure. The product was purified by flash column chromatography (from 9.5:0.5 to 8:2, PE:EtOAc) giving **11** (784 mg, 94 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 – 7.27 (m, 5H, H Ph), 5.98 (d, $J_{1,2}$ = 3.7 Hz, 1H, H1), 4.69 – 4.60 (m, 3H, H2, CH_2 Bn), 4.38 (t, J = 7.5 Hz, 1H, H4), 4.15 (s, 1H, H3), 3.40 (d, J = 2.7 Hz, 1H, H5'), 3.38 (s, 1H, H5"), 1.54 (s,

3H, Me), 1.33 (s, 3H, Me). ^{13}C NMR (101 MHz, $CDCl_3$) δ 137.08 (1C, C_{quat} Ph), 128.55 (1C, C Ph), 128.47 (1C, C Ph), 128.05 (1C, C Ph), 127.90 (1C, C Ph), 112.70 (1C, C_{quat}), 106.52 (1C, C1), 85.68 (1C, C4), 84.66 (1C, C2), 83.82 (1C, C3), 71.59 (1C, CH_2 Bn), 27.06 (1C, Me), 25.95 (1C, Me), 6.32 (1C, C5). MS: m/z = 413.2 $[M+Na]^+$, 803.2 $[2M+Na]^+$. $C_{15}H_{19}IO_4$ (390,21): calcd C, 46.17; H, 4.91; found C, 46.09; H, 4.92;

1.10. S-(((3aS,5S,6S,6aS)-6-(benzyloxy)-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)methyl) ethanethioate (12). Compound **11** (530 mg, 1.359 mmol, 1 equiv) was dissolved in dry DMF (4 mL). Potassium thioacetate (388 mg, 3.398 mmol, 2.5 equiv) was added and the reaction was stirred at 80 °C for 1 hour. Then the solvent was evaporated under reduced pressure and the product was purified by flash column chromatography (9:1, PE:EtOAc) giving **12** (450 mg, 98 % yield) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 – 7.09 (m, 5H, H Ph), 5.89 (d, $J_{1,2}$ = 3.9 Hz, 1H, H1), 4.63 (d, $J_{2,1}$ = 3.9 Hz, 1H, H2), 4.60 – 4.52 (m, 2H, CH_2 Bn), 4.17 (td, J = 7.0, 2.1 Hz, 1H, H4), 3.89 (d, J = 2.1 Hz, 1H, H3), 3.23 (ddd, J = 33.3, 13.7, 7.1 Hz, 2H, H5), 2.33 (s, 3H, OAc), 1.55 (s, 3H, Me), 1.32 (s, 3H, Me). ^{13}C NMR (101 MHz, $CDCl_3$) δ 192.29 (1C, C_{quat} CO), 134.51 (1C, C_{quat} Ph), 125.94 (1C, C Ph), 125.42 (1C, C Ph), 125.28 (1C, C Ph), 110.20 (1C, C_{quat}), 103.38 (1C, C1), 82.30 (1C, C2), 81.58 (1C, C3), 81.02 (1C, C4), 69.07 (1C, CH_2 Bn), 29.38 (1C, C5), 27.96 (1C, CH_3 OAc), 24.41 (1C, Me), 23.51 (1C, Me). MS: m/z = 361.2 $[M+Na]^+$. $C_{17}H_{22}O_5S$ (338,42): calcd: C, 60.33; H, 6.55; found C, 60.25; H, 6.54;

1.11. ((3aS,5S,6S,6aS)-6-(benzyloxy)-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)methanethiol (13). Compound **12** (357 mg, 1.056 mmol, 1 equiv) was dissolved in dry MeOH (10 mL). Na (2,5 mg, 0.106 mmol, 0.1 equiv) was added and the reaction was stirred for 1 hour. Then silica gel was added, the solvent was evaporated under reduced pressure and the product was purified by flash column chromatography (8:2, PE:EtOAc) giving **13** (270 mg, 86 % yield) as a yellow oil. The corresponding disulfide compound was formed as secondary product (78 mg, 13 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 – 6.94 (m, 5H, H Ph), 5.92 (d, $J_{1,2}$ = 3.9 Hz, 1H, H1),

4.63 (d, $J_{2,1}$ = 3.9 Hz, 1H, H2), 4.58 (d, J = 3.9 Hz, 2H, CH_2 Bn), 4.46 – 4.38 (m, 1H, H4), 4.05 (d, J = 1.1 Hz, 1H, H3), 3.00 (ddd, J = 22.4, 13.6, 7.3 Hz, 2H, H5), 1.50 (s, 3H, Me), 1.30 (s, 3H, Me). ^{13}C NMR (101 MHz, $CDCl_3$) δ 137.17 (1C, C_{quat} Ph), 128.54 (1C, C Ph), 128.02 (1C, C Ph), 127.85 (1C, C Ph), 112.72 (1C, C_{quat}), 105.87 (1C, C1), 86.46 (1C, C4), 84.87 (1C, C2), 83.55 (1C, C3), 71.61 (1C, CH_2 Bn), 27.28 (1C, C5), 27.07 (1C, Me), 26.14 (1C, Me). MS: m/z = 319.2 $[M+Na]^+$. $C_{15}H_{20}O_4S$ (296,38): calcd. C, 60.79; H, 6.80, found C, 60.68; H, 6.79.

1.12. Sodium ((3aS,5S,6S,6aS)-6-(benzyloxy)-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)methanesulfonate (14). Compound **13** (128 mg, 0.432 mmol 1 equiv) was suspended in $NaHCO_3$ saturated solution (1 mL). A 0.1 M TEMPO solution in ACN (864 µL, 0.086 mmol, 0.2 equiv) and a 0.5 M KBr solution (173 µL, 0.086 mmol, 0.2 equiv) were added; then NaOCl 0.35 M solution (2.5 mL, 0.864 mmol, 2 equiv) was added and the reaction was stirred for 10 minutes. Then EtOH was added and the solvent was evaporated under reduced pressure; the product was purified by flash column chromatography (from 100 % EtOAc to 8:2, EtOAc:EtOH) giving **14** (135 mg, 85 % yield). ^{1}H NMR (400 MHz, CD_3OD) δ 7.81 – 6.63 (m, 5H, H Ph), 5.89 (d, $J_{1,2}$ = 3.8 Hz, 1H, H1), 4.69 – 4.57 (m, 4H, H2, H4, CH_2 Bn), 4.34 (s, 1H, H3), 3.35 (dd, $J_{5',5''}$ = 13.8, $J_{5',4}$ = 8.9 Hz, 1H, H5'), 3.17 (dd, $J_{5'',5'}$ = 13.8, $J_{5'',4}$ = 4.6 Hz, 1H, H5''), 1.49 (s, 3H, Me), 1.29 (s, 3H, Me). ^{13}C NMR (101 MHz, CD_3OD) δ 137.82 (1C, C_{quat} Ph), 127.96 (1C, C Ph), 127.59 (1C, C Ph), 127.37 (1C, C Ph), 111.92 (1C, C_{quat}), 106.11 (1C, C1), 84.68 (1C, C3), 84.30 (1C, C2), 81.92 (1C, C4), 71.25 (1C, CH_2 Bn), 54.65 (1C, C5), 25.49 (1C, Me), 24.50 (1C, Me). MS: m/z = 343.3 $[M-H]^-$. $C_{15}H_{19}NaO_7S$ (366,36): calcd C, 49.18; H, 5.23; found C, 49.10; H, 5.22.

1.13. Sodium ((3aS,5S,6S,6aS)-6-hydroxy-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)methanesulfonate (15). Compound **14** (88 mg, 0.240 mmol) was dissolved in EtOH (3 mL). Palladium hydroxide (80 mg) was added, the mixture was stirred under vacuum in order to remove the residual gas and then H_2 was added. The reaction was stirred at room temperature for 24 hours and then the catalyst was filtered on a syringe filter and washed with EtOH. The solvent was evaporated giving **15** (64 mg, 97 % yield). ^{1}H NMR (400 MHz, CD_3OD) δ 5.89 (d, $J_{1,2}$ = 3.8 Hz,

1H, H1), 4.53 (d, $J_{2,1}$ = 3.8 Hz, 1H, H2), 4.46 – 4.39 (m, 2H, H3, H4), 3.34 (dd, $J_{5',5''}$ = 14.0, $J_{5',4}$ = 5.7 Hz, 1H, H5'), 3.14 (dd, $J_{5'',5'}$ = 14.0, $J_{5'',4}$ = 4.9 Hz, 1H, H5''), 1.50 (s, 3H, Me), 1.30 (s, 3H, Me). ^{13}C NMR (101 MHz, CD_3OD) δ 111.89 (1C, C_{quat}), 105.95 (1C, C1), 86.36 (1C, C2), 84.37 (1C, C4), 77.23 (1C, C3), 54.64 (1C, C5), 25.48 (1C, Me), 24.51 (1C, Me). MS: m/z = 253.1 [M-H]$^-$. $C_8H_{13}NaO_7S$ (276,24): calcd C, 34.78; H, 4.74; found. C, 34.83; H, 4.75.

1.14. Sodium ((2S,3S,4S)-3,4,5-trihydroxytetrahydrofuran-2-yl)methanesulfonate (2). Compound **15** (40 mg, 0.145 mmol) was dissolved in D_2O (0.7 mL) and the solution at pH 4 was transferred into an NMR tube and heated at 90 °C for 1 day. The reaction was followed by ^{1}H-NMR until completion, then the pH was neutralized adding NaOH 5 % and the solvent was lyophilised to give **2** (33 mg, quantitative yields). ^{1}H NMR (400 MHz, D_2O) δ 5.10 (d, J = 3.2 Hz, 1H, H1β), 5.08 (d, $J_{1,2}$ = 2.2 Hz, 1H, H1α), 4.22 (ddd, J = 7.9, 5.7, 4.6 Hz, 1H, H4α), 3.96 – 3.88 (m, 3H, H4β, H3β, H2β), 3.85 (dd, J = 4.0, $J_{2,1}$ = 2.2 Hz, 1H, H2α), 3.83 – 3.77 (m, 1H, H3α), 3.15 – 2.97 (m, 2H, H5). ^{13}C NMR (101 MHz, D_2O) δ 101.09 (1C, C1α), 95.33 (1C, C1β), 80.78 (1C, C2α), 79.08 (1C, C3α), 78.80 (1C, C4α), 77.44, 76.79, 75.34 (3C, C2β, C3β, C4β), 55.27 (1C, C5β), 53.79 (1C, C5α). MS: m/z = 213.1 [M-H]$^-$. $C_5H_9NaO_7S$ (236,18): calcd. C 25.43, H 3.84, found C 25.39, H 3.85.

1.15. Sodium (3aS,5S,6S,6aS)-6-(benzyloxy)-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxole-5-carboxylate (16). Compound **8** (133 mg, 0.475 mmol, 1 equiv) was suspended in $NaHCO_3$ saturated solution (1 mL). A 0.1 M TEMPO solution in ACN (950 µL, 0.095 mmol, 0.2 equiv) and a 0.5 M KBr solution (190 µL, 0.095 mmol, 0.2 equiv) were added; then NaOCl 0.35 M solution (2.7 mL, 0.949 mmol, 2 equiv) was added and the reaction was stirred for 40 minutes. EtOH was added, the solvent was evaporated under reduced pressure, and the product was purified by flash column chromatography (100 % EtOAc) giving **16** (143 mg, 95 % yield). ^{1}H NMR (400 MHz, CD_3OD) δ 7.39 – 7.24 (m, 5H, H Ph), 5.97 (d, $J_{1,2}$ = 3.6 Hz, 1H, H1), 4.68 (d, $J_{2,1}$ = 3.6 Hz, 1H, H2), 4.65 – 4.61 (m, 3H, H4, CH_2 Bn), 4.48 (s, 1H, H3), 1.45 (s, 3H, Me), 1.27 (s, 3H, Me). ^{13}C NMR (101 MHz, CD_3OD) δ 177.49 (1 C, C5), 141.38 (1 C, C_{quat} Ph), 132.03 (1 C, C Ph), 131.59 (1

C, C Ph), 131.51 (1 C, C Ph), 116.63 (1 C, C_{quat}), 110.54 (1 C, C1), 88.48 (1 C, C3), 87.44 (1 C, C4), 86.62 (1 C, C2), 75.20 (1C, CH_2 Bn), 28.60 (1C, Me), 28.45 (1C, Me). MS: m/z = 293.2 [M-H]$^-$. $C_{15}H_{17}NaO_6$ (316,28): calcd. C, 56.96; H, 5.42; found. C, 56.88; H, 5.43.

1.16. Sodium (3aS,5S,6S,6aS)-6-hydroxy-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxole-5-carboxylate (17). Compound **16** (125 mg, 0.395 mmol) was dissolved in MeOH (5 mL). Palladium hydroxide (100 mg) was added, the mixture was stirred under vacuum in order to remove the residual gas and then H_2 was added. The reaction was stirred at room temperature for 24 hours and then the catalyst was filtered on a syringe filter and washed with MeOH. The solvent was evaporated giving **17** (88 mg, quantitative yields). ^{1}H NMR (400 MHz, D_2O) δ 5.89 (d, $J_{1,2}$ = 3.6 Hz, 1H, H1), 4.53 (d, J = 0.5 Hz, 1H, H4), 4.51 (d, $J_{2,1}$ = 3.6 Hz, 1H, H2), 4.32 (s, 1H, H3), 1.30 (s, 1H, Me), 1.16 (s, 1H, Me). ^{13}C NMR (101 MHz, D_2O) δ 174.79 (1 C, C5), 113.15 (1 C, C_{quat}), 105.74 (1C, C1), 85.87 (1C, C4), 83.97(1C, C2), 76.42 (1C, C3), 24.71 (1C, Me), 24.39 (1C, Me). MS: m/z = 203.2 [M-H]$^-$. $C_8H_{11}NaO_6$ (226,16): calcd. C, 42.49; H, 4.90; found C, 42.52; H, 4.89.

1.17. Sodium (2S,3S,4S)-3,4,5-trihydroxytetrahydrofuran-2-carboxylate (3). Compound **17** (40 mg, 0.177 mmol) was dissolved in D_2O (0.7 mL) and the pH 4 solution was heated at 90 °C overnight into an NMR tube. After reaction's completion the pH was neutralized adding NaOH 5 % and the solvent was lyophilised to give **3** (32 mg, quantitative yields). ^{1}H NMR (400 MHz, D_2O) δ 5.38 (d, J = 4.3 Hz, 1H, H1β), 5.34 (d, J = 1.3 Hz, 1H, H1α), 4.48 (d, J = 4.2 Hz, 1H, H4), 4.28 (t, J = 5.8 Hz, 1H, H3β), 4.23 – 4.17 (m, 2H, H3α, H4β), 4.03 (dd, J = 5.8, 4.3 Hz, 1H, H2β), 4.00 (dd, J = 2.7, 1.8 Hz, 1H, H2α). ^{13}C NMR (101 MHz, D_2O) δ 176.25 (1C, C5), 102.08 (1C, C1α), 96.55 (1C, C1β), 82.91 (1C, C4α), 80.12 (1C, C4β), 80.04 (1C, C2α), 78.82 (1C, C3α), 77.30 (1C, C3β), 75.49 (1C, C2β). MS: m/z = 163.1 [M-H]$^-$. $C_5H_7NaO_6$ (186,10): calcd. C, 32.27; H, 3.79; found. C, 32.22; H, 3.80.

1.18. ((3aS,5R,6R,6aS)-6-(benzyloxy)-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)methyl trifluoromethanesulfonate (18). To a stirred solution of **8** (110 mg, 0.389 mmol, 1 equiv) in dry DCM (4 mL), DTBMP (200 mg, 0.975 mmol, 2.5 equiv) was added. The solution was

cooled at -75 °C and triflic anhydride (132 µL, 0.785 mmol, 2 equiv) was added by syringe; the temperature was increased to 0 °C and after 30 minutes the reaction mixture was directly loaded on a silica gel column and quickly filtrated with (6:4, PE:EtOAc, 0.1 % TEA) giving **18**. The compound was used immediately for the next step because of its instability.

1.19. Dibenzyl 2-(((3aS,5R,6R,6aS)-6-(benzyloxy)-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)methyl)malonate (19). To an -80 °C solution of LiHMDS (2.05 mL, 2.037 mmol, 3 equiv) in dry THF (3.6 mL), dibenzylmalonate (509 µl, 2.037 mmol, 3 equiv) was added and then the mixture was stirred at -80 °C for 30 minutes. The freshly prepared triflate **18** (239 mg, 0.853 mmol, 1 equiv) was dissolved in dry THF (3.6 mL) and slowly added by syringe to the cold dibenzylmalonate solution. The temperature was slowly increased to 0 °C for 1 hour, then the reaction was stirred at 0°C for other 20 minutes, and finally at room temperature for 1.5 hours. The reaction was quenched adding NH_4Cl saturated solution and extracted with EtOAc; the organic phase was dried with Na_2SO_4, filtered and concentrated under reduced pressure. The product was purified by flash column chromatography (8.5:1.5, PE:EtOAc) giving **19** (183 mg, 50 % yield over two steps) as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.24 (m, 15H, H Ph), 5.87 (d, $J_{1,2}$ = 3.9 Hz, 1H, H1), 5.21 (d, J = 12.3 Hz, 1H, H Bn), 5.16 – 5.04 (m, 3H, H Bn), 4.61 (d, $J_{2,1}$ = 3.9 Hz, 1H, H2), 4.57 – 4.47 (m, 2H, H Bn), 4.21 – 4.13 (m, 1H, H4), 3.88 – 3.76 (m, 2H, H3, H6), 2.46 – 2.30 (m, 1H, H5'), 2.28 – 2.19 (m, 1H, H5"),, 1.43 (s, 3H, Me), 1.30 (s, 3H, Me). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.22 (1C, C_{quat} CO), 168.85 (1C, C_{quat} CO), 137.15 (1C, C_{quat} Ph), 135.42 (1C, C_{quat} Ph), 135.34 (1C, C_{quat} Ph), 128.65 (1C, C Ph), 128.62 (1C, C Ph), 128.40 (1C, C Ph), 128.39 (1C, C Ph), 128.29 (1C, C Ph), 128.25 (1C, C Ph), 128.06 (1C, C Ph), 127.86 (1C, C Ph), 127.73 (1C, C Ph), 127.08 (1C, C Ph), 112.70 (1C, C_{quat}), 105.95 (1C, C1), 85.53 (1C, C3), 84.85 (1C, C2), 82.10 (1C, C4), 71.64 (1C, CH_2 Bn), 67.33 (2C, CH_2 Bn), 48.74 (1C, C6), 32.98 (1C, C5), 26.74 (1C, Me), 26.18 (1C, Me). MS: m/z = 569.4 $[M+Na]^+$. $C_{32}H_{34}O_8$ (546,61): calcd. C, 70.31; H, 6.27; found. C, 70.25; H, 6.26.

1.20. 2-(((3aS,5R,6R,6aS)-6-hydroxy-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)methyl)malonic acid (20). Compound **19** (119 mg, 0.218 mmol) was dissolved in EtOH (4 mL). Palladium hydroxide (100 mg) was added, the mixture was stirred under vacuum in order to remove the residual gas and then H_2 was added. The reaction was stirred at room temperature for 24 hours and then the catalyst was filtered on a syringe filter and washed with MeOH. The solvent was evaporated giving **20** (59 mg, quantitative yields). ^{1}H NMR (400 MHz, CD_3OD) δ 5.88 (d, $J_{1,2}$ = 3.7 Hz, 1H, H1), 4.50 (d, $J_{2,1}$ = 3.7 Hz, 1H, H2), 4.07 – 3.96 (m, 2H, H3, H4), 3.59 (dd, $J_{6,5}$ = 10.4, 4.3 Hz, 1H, H6), 2.38 – 2.26 (m, 1H, H5'), 2.20 – 2.07 (m, 1H, H5''), 1.49 (s, 3H, Me), 1.29 (s, 3H, Me). ^{13}C NMR (101 MHz, CD_3OD) δ 173.18 (1C, C_{quat} CO), 172.76 (1C, C_{quat} CO), 113.26 (1C, C_{quat}), 107.41 (1C, C1), 88.10 (1C, C2), 86.98 (1C, C4), 79.31 (1C, C3), 49.66 (1C, C6) 33.95 (1C, C5), 26.74 (1C, Me), 26.00 (1C, Me). MS: m/z = 275.2 $[M-H]^-$. $C_{11}H_{16}O_8$ (276,24): calcd. C, 47.83; H, 5.84; found C, 47.79; H, 5.85.

1.21. Sodium 2-(((2R,3S,4S)-3,4,5-trihydroxytetrahydrofuran-2-yl)methyl)malonate (4). Compound **20** (40 mg, 0.157 mmol) was dissolved in D_2O (0.7 mL) and the solution was transferred into an NMR tube. The reaction was checked by ^{1}H-NMR and the pH 2 solution was reacted for 48 hours at room temperature. Then the pH was neutralized adding NaOH 5 % and the solvent was lyophilised to give **4** (33 mg, 99 %). ^{1}H NMR (400 MHz, D_2O) δ 5.04 (d, J = 4.6 Hz, 1Hβ), 5.01 (d, J = 2.5 Hz, 1Hα), 3.86 – 3.74 (m, 3H, H2α, H2β, H4), 3.64 (dd, J = 6.2, 4.6 Hz, 1H, H3), 3.52 – 3.47 (m, 1H, H6), 2.00 – 1.91 (m, 1H, H5'), 1.85 – 1.75 (m, 1H, H5''). ^{13}C NMR (101 MHz, D_2O) δ 178.80 (1C, C_{quat}), 178.66 (1C, C_{quat}), 100.61 (1C, C1α), 94.84 (1C, C1β), 81.63 (1C, C2α), 79.58 (1C, C3), 79.54 (1C, C6), 78.00 (1C, C2β), 76.11 (1C, C4), 35.62 (1C, C5β), 33.87 (1C, C5β). MS: m/z = 235.1 $[M-H]^-$, 257.1 $[M-2H+Na]^-$. $C_8H_{12}O_8$ (236,18): calcd. C, 40.68; H, 5.12; found. C, 40.60; H, 5.11.

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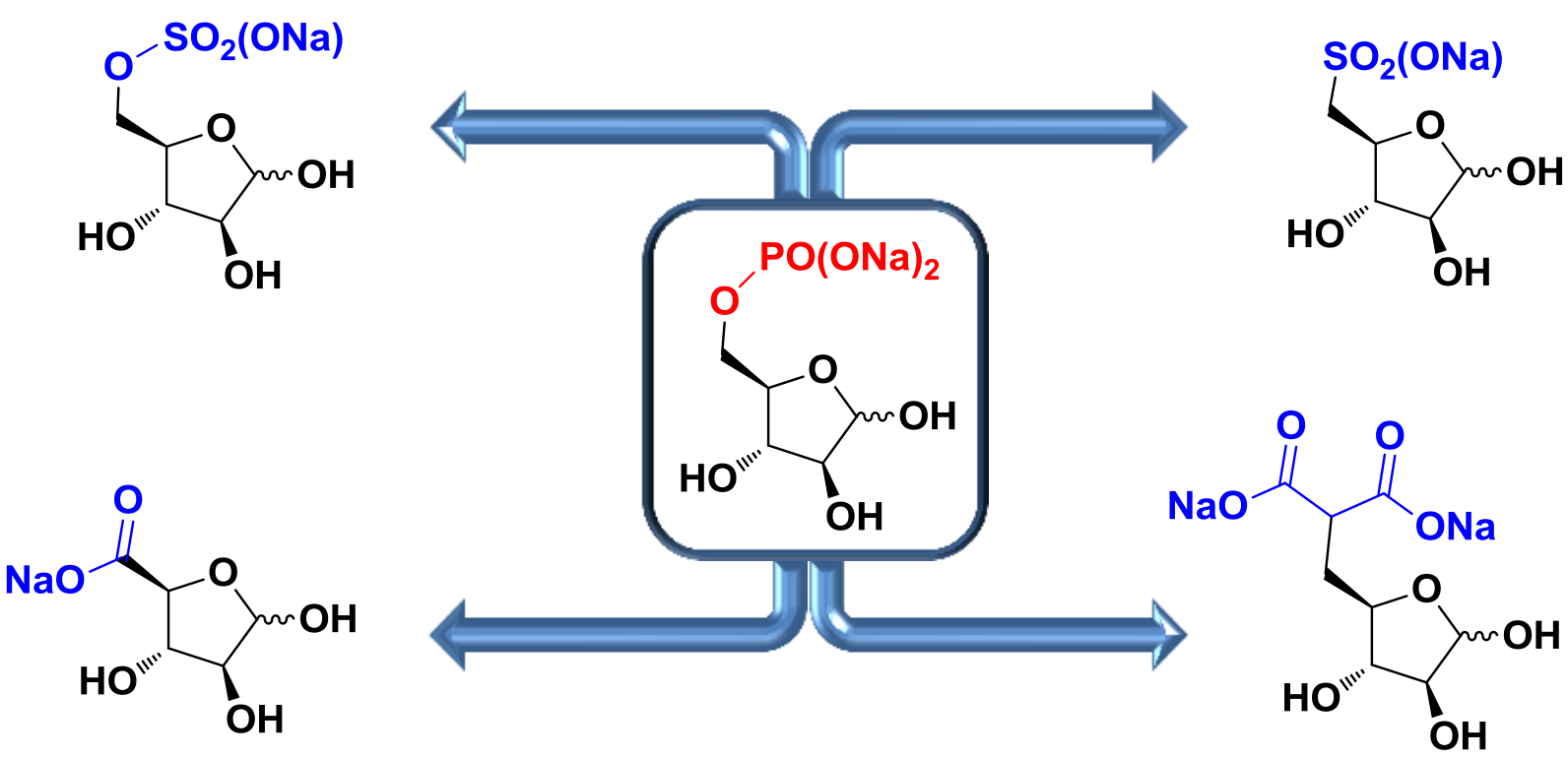
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HIGHLIGHTS

- We designed and synthesized a set of new Ara-5P analogues, modified at position 5.
- We tested the affinity of the synthesised compounds by STD-NMR spectroscopy studies.
- The synthesised compounds were studied *in vivo* on *P. aeruginosa* and *E. coli* strains.
- None of the compounds was active, either *in vivo* or on isolated API.

Best regards,

Luca Gabrielli