A Natural Carbohydrate Substrate for *Mycobacterium tuberculosis* Methionine Sulfoxide Reductase A

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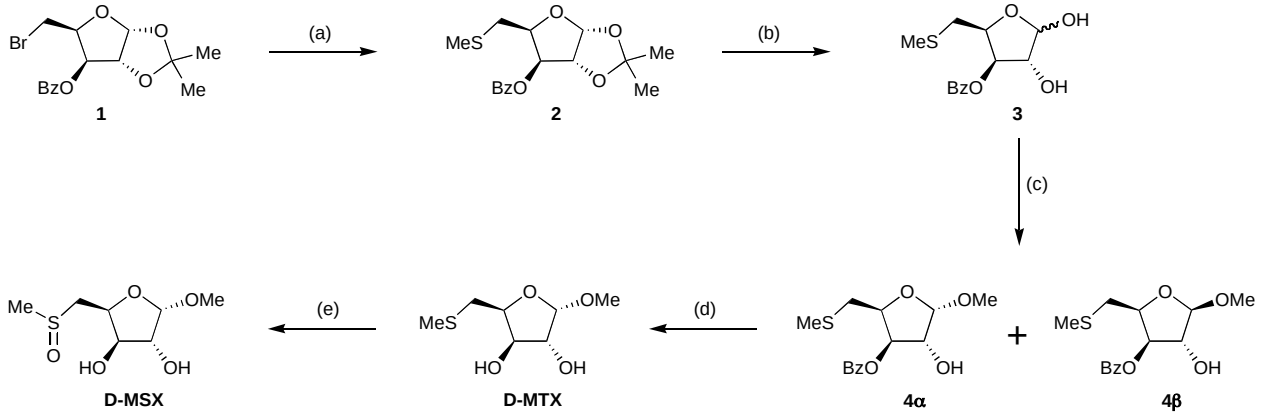
Supplementary Information

Synthesis of methyl 5-deoxy-5-methylsulfinyl-α-D/L-xylofuranoside

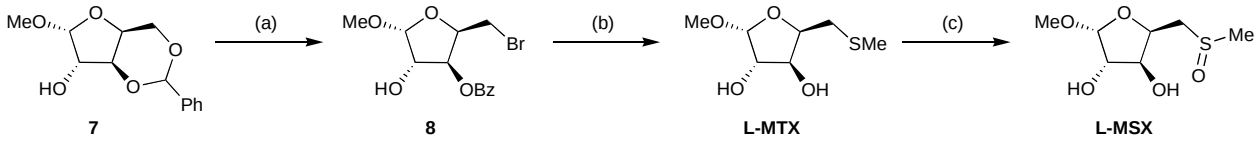
Two different routes were used to access the D- and L-isomers of MSX. The D-isomer was prepared from the known acetonide-protected xylose derivative **1** (Scheme S1).[1] Partial de-esterification occurred upon substitution of the bromide leaving group by thiomethoxide. Therefore, the crude product mixture was treated with benzoyl bromide and pyridine prior to isolation of sulfide **2**. The acetonide protecting group was then removed by acidic hydrolysis to give hemiacetal **3** which was converted to a mixture of methyl glycosides **4α** and **4β** which could be separated by silica gel chromatography. The benzoate ester provides a useful UV-active hydrophobic tag that is useful for the isolation and purification of compounds **3** and **4**. The ester was removed to give the **D-MTX** methyl glycoside, which was oxidized with hydrogen peroxide to yield a 1:1 mixture of **D-MSX** sulfoxides.



Scheme S1 Reagents: (a) (i) NaSMe / DMF; (ii) BzCl / pyridine, 90% (over two steps); (b) 60% aq. AcOH / H_2SO_4, 70 °C, 52%; (c) TMSCl / MeOH, 91% **4α**:**4β** 1:2; (d) NaOMe / MeOH, 82%; (e) H_2O_2 / H_2O, 92%.

The L-isomer was synthesised from the known benzylidene acetal-protected methyl glycoside **7** (Scheme S2).[2] Oxidative ring opening of the benzylidene acetal provided the 5-bromo derivative **8** which was treated with sodium thiomethoxide. In this case a longer reaction time (4 days) allowed complete removal of the benzoate protecting group in addition to substitution of the bromide

leaving group. The **L-MTX** glycoside was isolated in a modest yield before quantitative oxidation to give a 1:1 mixture of **L-MSX** sulfoxides.



Scheme S2 Reagents: (a) NBS / Ba_2CO_3 / CCl_4, 60%; (b) NaSMe / DMF, 38%; (c) H_2O_2 / H_2O, 100%.

General experimental methods

All solvents were dried prior to use, according to standard methods.[3] Otherwise, commercial reagents were used without further purification. All reactions were performed at room temperature under a nitrogen atmosphere unless stated otherwise. All concentrations were performed in vacuo. Analytical TLC was performed on silica gel 60-F_{254} (Merck) with detection by fluorescence and/or by charring following immersion in 5% H_2SO_4/MeOH. Flash chromatography was performed with silica gel 60 (Merck). Melting points were determined on a Reichert hot stage apparatus. Optical rotations were measured at the sodium D-line with an Optical Activity AA-1000 polarimeter. $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}. ^1H and ^{13}C NMR spectra were recorded at 25 °C on either a Bruker Avance 500 spectrometer or Varian Unity Inova 500 Spectrometer (at 500 MHz and 125 MHz, respectively), or a Bruker DPX 300 spectrometer (at 300 MHz and 75 MHz, respectively). ^1H NMR and ^{13}C NMR spectra were referenced using either residual solvent signals,[4] or tetramethylsilane in organic solvents or trimethylsilylpropane sulfonic acid in D_2O.[5] Signals were assigned using a combination of COSY and HMQC experiments, and where appropriate NOESY experiments. Coupling constants are given in Hz. The following abbreviations were used to explain the signal multiplicities or characteristics: s, singlet; bs, broad singlet; d, doublet; dd, double doublet; ddd, double double doublet; dt, doublet of triplets; m, multiplet; t, triplet. Mass spectra were acquired on a Micromass LCT-KA111 electrospray mass spectrometer or a Bruker MicrOTOF electrospray mass spectrometer. Infra-red spectra were recorded on a Perkin Elmer Spectrum One FT-IR Spectrometer. Elemental Analyses were performed by the microanalysis service in the School of Chemistry, University of Leeds using a Carlo Erba 1108 Elemental Analyzer. UV-vis absorption and chemiluminescence measurements were made using a PerkinElmer EnVision plate reader.

3-*O*-benzoyl-5-deoxy-1,2-isopropylidene-5-methylthio-α-D-xylofuranose 2

A mixture of sodium thiomethoxide (6.39 g, 91.1 mmol), 3-*O*-benzoyl-5-bromo-5-deoxy-1,2-*O*-isopropylidene-α-D-xylofuranose **1**[1] (15.5 g, 43.4 mmol) and DMF (80 mL) was stirred for 2 days. The reaction mixture was diluted with EtOAc (250 mL) and washed with saturated aq. $NaHCO_3$ solution (250 mL) and saturated aq. NaCl solution (2 × 250 mL). The organic extracts were dried ($MgSO_4$), filtered and evaporated to give an orange oil. A solution of the crude product and benzoyl chloride (5 mL) in pyridine (75 mL) was stirred for 16 h. The mixture was diluted with EtOAc (250 mL) and washed with 1 M HCl solution (4 × 250 mL), saturated aq. $NaHCO_3$ solution (250 mL) and saturated aq. NaCl solution (2 × 250 mL). The organic extracts were dried ($MgSO_4$), filtered and evaporated to give a brown oil. Flash chromatography (silica gel; Hex-EtOAc, 2:1) gave the *sulfide* **2** (12.62 g, 90%) as a syrup, $[\alpha]_D^{23}$ -58.4 (*c* 1.0 in $CHCl_3$); Found: C, 59.2; H, 6.3. $C_{16}H_{20}SO_5$ requires C, 59.2; H, 6.2%; ν_{max}/cm^{-1} 1725 (C=O); δ_H(500 MHz, $CDCl_3$); 8.04-7.46 (5 H, m, *Ph*CO), 5.99 (1 H, d, $J_{1,2}$ 3.8, H-1), 5.49 (1 H, d, $J_{3,4}$ 2.9, H-3), 4.65 (1 H, d, $J_{1,2}$ 3.8, H-2), 4.57 (1 H, ddd, $J_{4,5'}$ 7.1, $J_{4,5}$ 6.8, $J_{3,4}$ 2.9, H-4), 2.87 (1 H, dd, $J_{5,5'}$ 13.7, $J_{4,5}$ 6.8, H-5), 2.81 (1 H, dd, $J_{5,5'}$ 13.7, $J_{4,5'}$ 7.1, H-5'), 2.15 (3 H, s, *Me*SCH_2), 1.57 and 1.33 (6 H, s, 2 × (*Me*)$_2$C); δ_C(75 MHz, $CDCl_3$); 165.4 (Ph*C*O), 133.8-128.7 (*Ph*CO), 112.3 ((Me)$_2$*C*), 104.9 (C-1), 83.5 (C-2), 79.2 (C-4), 76.8 (C-3), 31.9 (C-5), 26.8 and 26.3 ((*Me*)$_2$C), 16.5 (*Me*SCH_2); *m/z* (ES) 347.1 ($[M + Na]^+$), 671.2 ($[2M + Na]^+$); Found: $[M + Na]^+$ 347.0933, $C_{16}H_{20}NaSO_5$ requires 347.0924.

3-*O*-Benzoyl-5-deoxy-5-methylthio-D-xylofuranose 3

Sulfuric acid (3 drops) was added to a solution of benzoylated sulfide **2** (1.0 g, 3.08 mmol) in 60% v/v aqueous acetic acid (10 mL). The mixture was heated for 2 h at 70 ºC. After cooling to room temperature, the mixture was neutralised with saturated aq. $NaHCO_3$ solution and extracted with EtOAc (125 mL). The organic extracts were washed with saturated aq. NaCl solution (2 × 125 mL), dried ($MgSO_4$), and evaporated to give an orange oil, which was purified by flash chromatography (silica gel; Hex-EtOAc, 3:2→2:3) to give the *diol* **3** (456 mg, 52%) as a colourless syrup, α:β 87:13; Found: C, 40.0; H, 6.8. $C_{13}H_{16}SO_5$ requires C, 40.0; H, 6.7%; ν_{max}/cm^{-1} 3412 (OH), 1720 (C=O); δ_H(500 MHz, $CDCl_3$); α-anomer 8.05-7.46 (5 H, m, *Ph*CO), 5.58 (1 H, dd, $J_{1,OH}$ 5.8, $J_{1,2}$ 4.4, H-1), 5.40 (1 H, dd, $J_{3,4}$ 4.6, $J_{2,3}$ 2.5, H-3), 4.71 (1 H, dt, $J_{4,5}$ 6.8, $J_{3,4}$ 4.6, H-4), 4.35 (1 H, ddd, $J_{2,OH}$ 4.4, $J_{1,2}$ 4.4, $J_{2,3}$ 2.5, H-2), 3.97 (1 H, d, $J_{1,OH}$ 5.8, OH-1), 3.52 (1 H, d, $J_{2,OH}$ 4.4, OH-2), 2.82 (2 H, d, $J_{4,5}$ 6.8, H-5 and H-5'), 2.16 (3 H, s, *Me*S); β-anomer 8.05-7.46 (5 H, m, *Ph*CO), 5.37 (1 H, d, $J_{1,OH}$ 5.3, H-1), 5.34 (1 H, dd, $J_{3,4}$ 4.6, $J_{2,3}$ 1.8, H-3), 4.68 (1 H, m, H-4), 4.32 (1 H, bs, H-2), 3.23 (1 H, d, $J_{1,OH}$ 5.3, OH-1), 2.95 (1 H, dd, $J_{5,5'}$ 13.7, $J_{4,5}$ 7.1, H-5), 2.91 (1 H, dd, $J_{5,5'}$ 13.7,

$J_{4,5'}$ 6.6, H-5'), 2.18 (3 H, s, *Me*S); δ_C(75 MHz, $CDCl_3$); α-anomer 134.0-128.9 (*Ph*CH_2), 96.2 (C-1), 80.2 (C-3), 77.1 (C-4), 76.4 (C-2), 33.3 (C-5), 16.6 (*Me*S); β-anomer 134.0-128.9 (*Ph*CH_2), 96.2 (C-1), 80.2 (C-3), 77.1 (C-4), 76.4 (C-2), 33.3 (C-5), 16.6 (*Me*S); *m/z* (ES); 307.1 ($[M + Na]^+$), 591.1 ($[2M + Na]^+$), 267.1 ($[M - OH]^+$); Found: $[M + Na]^+$ 307.0608, $C_{13}H_{16}NaSO_5$ requires 307.0611.

Methyl 3-*O*-benzoyl-5-deoxy-5-methylthio-D-xylofuranoside 4α, 4β

Chlorotrimethylsilane (13.4 μL, 0.150 mmol) was added to a stirred solution of diol **3** (0.85 g, 2.99 mmol) in anhydrous MeOH (15 mL). After 24 h the mixture was neutralised with triethylamine and concentrated to give an orange oil, which was subjected to flash chromatography (silica gel; CH_2Cl_2-EtOAc, 10:1→7:1) to give *α-xylofuranoside* **4α** (272 mg, 30%) as a colourless syrup $[\alpha]_D^{28}$ 109.0 (*c* 0.4 in $CHCl_3$); Found: C, 56.2; H, 6.2. $C_{14}H_{18}SO_5$ requires C, 56.4; H 6.1%; ν_{max}/cm^{-1} 3486 (OH), 1722 (C=O); δ_H(500 MHz, $CDCl_3$) 8.06-7.45 (5 H, m, *Ph*CO), 5.42 (1 H, dd, $J_{3,4}$ 5.0, $J_{2,3}$ 3.6, H-3), 5.08 (1 H, d, $J_{1,2}$ 4.5, H-1), 4.58 (1 H, dt, $J_{3,4}$ 5.0, $J_{4,5/5'}$ 6.4, H-4), 4.38 (1 H, ddd, $J_{2,OH}$ 6.1, $J_{1,2}$ 4.5, $J_{2,3}$ 3.6, H-2), 3.55 (3 H, s, CH_3O), 3.06 (1 H, d, $J_{2,OH}$ 6.1, OH-2), 2.76 (2 H, d, $J_{4,5/5'}$ 6.4, H-5 and H-5'), 2.16 (3 H, s, CH_3S); δ_C(125 MHz, $CDCl_3$) 166.3 (Ph*CO*), 133.6-128.7 (*Ph*CO), 101.5 (C-1), 80.0 (C-3), 77.2 (C-4), 77.0 (C-2), 55.9 (CH_3O), 33.6 (C-5), 16.6 (CH_3S); *m/z* (ES) 321.1 ($[M + Na]^+$), 619.2 ($[2M + Na]^+$), 267.1 ($[M - OMe]^+$); Found: $[M + Na]^+$ 321.0779, $C_{14}H_{18}NaSO_5$ requires 321.0767, and the *methyl β-xylofuranoside* **4β** (546 mg, 61%) as a colourless syrup $[\alpha]_D^{28}$ -107.3 (*c* 2.4 in $CHCl_3$); Found: C, 56.1; H, 6.1. $C_{14}H_{18}SO_5$ requires C, 56.4; H, 6.1%; ν_{max}/cm^{-1} 3436 (OH), 1720 (C=O); δ_H(500 MHz, $CDCl_3$) 8.04-7.45 (5 H, m, *Ph*CO), 5.28 (1 H, dd, $J_{3,4}$ 5.5, $J_{2,3}$ 1.9, H-3), 4.93 (1 H, d, $J_{1,2}$ 1.0, H-1), 4.66 (1 H, dt, $J_{4,5/5'}$ 6.8, $J_{3,4}$ 5.5, H-4), 4.29 (1 H, bs, H-2), 3.46 (3 H, s, CH_3O), 3.02 (1 H, bs, OH-2), 2.90 (2 H, m, H-5 and H-5'), 2.18 (3 H, s, CH_3S); δ_C(125 MHz, $CDCl_3$) 166.7 (Ph*CO*), 133.7-128.7 (*Ph*CO), 109.3 (C-1), 80.7 (C-2), 79.8 (C-4), 79.7 (C-3), 55.9 (CH_3O), 34.6 (C-5), 16.4 (CH_3S); *m/z* (ES) 321.1 ($[M + Na]^+$), 619.2 ($[2M + Na]^+$), 267.1 ($[M - OMe]^+$); Found: $[M + Na]^+$ 321.0774, $C_{14}H_{18}NaSO_5$ requires 321.0767.

Methyl 5-deoxy-5-methylthio-α-D-xylofuranoside D-MTX

Sodium methoxide in MeOH (368 μL, 0.18 mmol) was added to a solution of benzoylated xylofuranoside **4α** (183 mg, 0.6 mmol) in anhydrous MeOH (2 mL). After 3 h the reaction mixture was neutralized with Amberlite IRC-50 H^+ ion exchange resin and diluted with MeOH (25 mL), before filtration and evaporation to give an orange glass, which was purified by anion exchange chromatography (Dowex 1×2 OH^- resin, 1.5 × 50 cm; H_2O) to give α-*methyl xylofuranoside* **D-**

MTX (98 mg, 82%) as a colourless syrup; $[\alpha]_D^{27}$ +102.4 (*c* 1.0 in D_2O), lit.[6] $[\alpha]_D^{22}$ +119.6 (c 1.0 in H_2O); ν_{max}/cm^{-1} 3401 (OH); δ_H(500 MHz, D_2O); 5.03 (1 H, d, $J_{1,2}$ 4.4, H-1), 4.37 (1 H, ddd, $J_{4,5'}$ 8.1, $J_{4,5}$ 5.3, $J_{3,4}$ 5.2, H-4), 4.24 (1 H, dd, $J_{3,4}$ 5.2, $J_{2,3}$ 4.4, H-3), 4.17 (1 H, dd, $J_{2,3}$ 4.4, $J_{1,2}$ 4.4, H-2), 3.46 (3 H, s, O*CH*$_3$), 2.79 (1 H, dd, $J_{5,5'}$ 13.8, $J_{4,5}$ 5.3, H-5), 2.68 (1 H, dd, $J_{5,5'}$ 13.8, $J_{4,5'}$ 8.1, H-5'), 2.16 (3 H, s, *CH*$_3$S); δ_C(125 MHz, D_2O); 104.9 (C-1), 79.9 (C-4), 79.1 (C-2), 78.0 (C-3), 58.4 (*CH*$_3$O), 35.4 (C-5), 17.4 (*CH*$_3$S); *m/z* (ES); 217.1 ($[M + Na]^+$), 411.1 ($[2M + Na]^+$); Found: $[M + Na]^+$ 217.0505, $C_7H_{14}NaSO_4$ requires 217.0505.

Methyl 5-deoxy-5-*R,S*-methylsulfinyl-α-D-xylofuranoside D-MSX

Hydrogen peroxide (30% w/w, 39.4 μL, 0.39 mmol) was added to a solution of methyl xylofuranoside **D-MTX** (50 mg, 0.26 mmol) in water (0.5 mL) and stirred for 4 hours. The mixture was frozen and freeze-dried to give the *methylsulfinyl xylofuranoside* **D-MSX** as a colourless syrup comprising a 1:1 mixture of diastereomers (49 mg, 91%); ν_{max}/cm^{-1} 3302 (OH); δ_H(500 MHz, D_2O); *S*-diastereomer: 5.06 (1 H, d, $J_{1,2}$ 5.0, H-1), 4.59 (1 H, m, H-4), 4.32 (1 H, dd, $J_{3,4}$ 5.1, $J_{2,3}$ 4.7, H-3), 4.19 (1 H, dd, $J_{1,2}$ 5.0, $J_{2,3}$ 4.7, H-2), 3.46 (3 H, s, O*CH*$_3$), 3.12 (2 H, m, H-5 and H-5'), 2.79 (3 H, s, *CH*$_3$SO); *R*-stereoisomer: 5.05 (1 H, d, $J_{1,2}$ 4.7, H-1), 4.62 (1 H, m, H-4), 4.31 (1 H, dd, $J_{3,4}$ 5.0, $J_{2,3}$ 4.4, H-3), 4.17 (1 H, dd, $J_{1,2}$ 4.7, $J_{2,3}$ 4.4, H-2), 3.45 (3 H, s, O*CH*$_3$), 3.28 (1 H, dd, $J_{5,5'}$ 14.0, $J_{4,5}$ 4.6, H-5) 3.10 (1 H, dd, $J_{5,5'}$ 14.0, $J_{4,5'}$ 8.1, H-5'), 2.79 (3 H, s, *CH*$_3$S); δ_C(75 MHz, D_2O); 105.1, 104.9 (C-1), 79.3, 79.1 (C-2), 78.4, 78.2 (C-3), 75.7, 75.1 (C-4), 58.3, 58.3 (OMe), 56.9, 55.3 (C-5), 40.2, 39.8 (SOMe); *m/z* (ES); 233.0($[M + Na]^+$), 443.1 ($[2M + Na]^+$), 211.1 ($[M + H]^+$); Found: $[M + Na]^+$ 233.0455, $C_7H_{14}NaSO_5$ requires 233.0454.

Methyl 3-*O*-benzoyl-5-bromo-5-deoxy-α-L-xylofuranoside 8

Barium carbonate (1.95 g, 9.9 mmol) and *N*-bromosuccinimide (0.78 g, 4.4 mmol) were added to a solution of *methyl 3,5-O-benzylidene-α-L-xylofuranoside* **7**[2] (1 g, 3.96 mmol) in carbon tetrachloride (20 mL). The reaction mixture was heated for 90 min under reflux. After cooling the mixture to room temperature, the solution was diluted with CH_2Cl_2 (50 mL), and filtered through celite. The filtrate was then washed with aq. sodium thiosulfate (50 mL), and aq. NaCl (2 x 50mL), dried ($MgSO_4$) and concentrated to a yellow syrup. The syrup was purified by flash chromatography (silica gel; EtOAc-hexane 1:1) to afford *bromide* **8** (780 mg, 60 %) as a colourless syrup. $[\alpha]_D^{27}$ -102.7 (*c* 1, $CHCl_3$); Found: C, 47.15; H, 4.6. $C_{13}H_{15}BrO_5$ requires C, 46.9; H, 4.6%; ν_{max}/cm^{-1} 3435 (OH), 1644 (C=C); δ_H(500 MHz, $CDCl_3$); 8.06 (2 H, d, *J* 7.4, *Ph*CO), 7.60 (1 H, t, *J* 7.4, *Ph*CO), 7.47 (2 H, t, *J* 7.4, *Ph*CO), 5.45 (1 H, dd, $J_{2,3}$ 4.1, $J_{3,4}$ 6, H-3), 5.10 (1 H, d, $J_{1,2}$ 4.5, H-1), 4.67 (1 H,

q, $J_{3,4} = J_{4,5} = J_{4,5'}$ 6.0, H-4), 4.41 (1 H, ddd, $J_{2,3}$ 4.1, $J_{2,1}$ 4.5, $J_{2,2\text{-OH}}$ 6.5, H-2), 3.55 (1 H, s, OCH_3), 3.54 (1 H, dd, $J_{5,4}$ 6, $J_{5,5'}$ 10.0, H-5), 3.47 (1 H, dd, $J_{4,5'}$ 6.0, $J_{5',5}$ 10.0, H-5'), 3.00 (1 H, d, $J_{2\text{-OH},2}$ 6.5, OH-2); δ_C(75 MHz, $CDCl_3$); 133.7, 129.8, 129.1, 128.6 (*Ph*CO), 101.6 (C-1), 79.3 (C-3), 76.9 (C-2), 76.9 (C-4), 56.0 (OCH_3), 28.9 (CH_2Br); *m/z* (ES); 331 ($[M + H]^+$).

Methyl 5-deoxy-5-methylthio-α-L-xylofuranoside L-MTX

Sodium thiomethoxide (169 mg, 2.4 mmol) was added to a solution of bromide **8** (320 mg, 0.96 mmol) in DMF (2.5 mL). The reaction mixture was stirred for 96 h before diluting with EtOAc (10 mL) and concentration to leave a crude yellow syrup which was purified by anion exchange chromatography (Dowex 1×2 OH^- resin, 1.5 × 50 cm; H_2O) to give α-*methyl xylofuranoside* **L-MTX** (70 mg, 38%) as a colourless syrup. $[\alpha]_D^{25}$ –118.0 (*c* 0.2, H_2O); NMR spectral data were identical to those for D-MTX; *m/z* (ES); 194.7 ($[M + H]^+$); Found: $[M + Na]^+$ 217.0508, $C_7H_4O_4S$ requires 217.0505.

Methyl 5-deoxy-5-methylsulfinyl-α-L-xylofuranoside L-MSX

Hydrogen peroxide (30% w/w, 42 μL, 0.38 mmol) was added to a solution of *methyl xylofuranoside* **L-MTX** (10mg, 0.38 mmol) in water (2 mL) and stirred for 15 min. The mixture was frozen and freeze-dried to give the *methylsulfinyl xylofuranoside* **L-MSX** as a colourless syrup comprising a 1:1 mixture of diastereomers (10 mg, 93%) ν_{max}/cm^{-1} 3429 (OH); NMR spectral data were identical to those for D-MSX; *m/z* (ES); Found $[M + Na]^+$ 233.0464, $C_8H_{14}O_5S$ requires 233.0454.

Oxidation of CSU20 LAM with hydrogen peroxide

Hydrogen peroxide (5 μL, 3% w/w solution in D_2O, 4.6 μmol) was added to CSU20-LAM (2.5 mg, 0.23 μmol), in NMR buffer (700 μL, 50 mM sodium phosphate, D_2O, pD 7.4). ^{1}H-NMR spectroscopy showed essentially complete conversion of MTX to MSX after 16 hours at 25 °C.

Attempted oxidation of methyl α-D-mannopyranoside with hydrogen peroxide

Hydrogen peroxide (41 μL, 30% w/w solution in H_2O diluted to 3% with D_2O, 38.5 μmol) was added to methyl α-D-mannopyranoside (7.0 mg, 35.9 μmol) in D_2O (700 μL) in an NMR tube. After 2 hours ^{1}H-NMR showed no change to the mannose sugar. After 24 hours the same sample showed that no reaction had occurred.

Attempted oxidation of methyl α/β-D-arabinofuranoside (5:1 mixture of anomers) with hydrogen peroxide

Hydrogen peroxide (38 μL, 30% w/w solution in H_2O diluted to 3% with D_2O, 35.6 μmol) was added to a 5:1 mixture of α/β methyl D-arabinofuranoside (5.5 mg, 33.8 μmol) in D_2O (700 μL). After 2 hours ^{1}H-NMR showed no change to the arabinose sugars. After 24 hours the same sample showed that no reaction had occurred.

Bacterial Expression and Purification of MsrA

MsrA was produced using *E. coli* BL21 clones described previously.[7] Briefly, the cells were grown in LB media containing carbenicillin (100 mg/L). The flasks were shaken at 37 °C until the optical density of the growth culture was approx. 0.2, at which time expression was induced by adding IPTG to a final concentration of 1 mM. The cultures were incubated for a further three hours, before pelleting the cells by centrifugation (16780 × g at 4 °C for 30 minutes). The pellets were stored at -20 °C before purification. An MsrA cell pellet from 1 L of cell culture was resuspended in lysis buffer (15 mL, 50 mM sodium phosphate, 150 mM NaCl, 2 mM $MgSO_4$, pH 7.4) containing lysozyme (20 mg). The mixture was cooled on ice for ten minutes before deoxycholic acid (20 mg) and Benzonase Nuclease (Novagen, 250 U/μL, 2 μL) were added, and then shaken at 37 °C for one hour. The mixture was centrifuged at 75600 × g for 1 hour at 4 °C. The supernatant was then circulated through a NiNTA resin column (1 mL) for one hour at 4 °C. The column was then eluted successively with wash buffer (10 mL, 50 mM sodium phosphate, 500 mM NaCl, 20 mM imidazole, pH 7.4), elution buffer (2 × 10 mL, 50 mM sodium phosphate, 500 mM NaCl, 250 mM imidazole, pH 7.4), and wash buffer (2 × 10 mL). Fractions containing the protein were dialysed against either phosphate or tris dialysis buffer (3 × 1 L), and freeze-dried in 1 mL aliquots for storage at -80 °C.

Immunoblot anlaysis of MsrA and MsrB in *M. tuberculosis*

M. tuberculosis culture was grown in 7H10-OADC-TW broth (100 ml) up to OD_{600nm} = 0.6. After centrifugation and washing the pellet with lysis buffer, the pellet was resuspended in lysis buffer (5 ml), bead beaten and an aliquot (3 ml) was used to prepare the cytosolic and membrane fractions according to the method of Mueller-Oritz et al.[8] Samples (15 μL) of whole, soluble and membrane fractions were subjected to SDS-PAGE, and after transferring the protein bands to a nitrocellulose membrane, they were finally probed with anti-MsrA and anti-MsrB antibodies and the blot was developed using chemiluminescence.

MsrA Reduction Experiments

The buffer for all NMR experiments comprised 50 mM sodium phosphate in D_2O (pD 7.4). In each case the enzyme reactions were incubated overnight at 37 °C before analysis by ^{1}H-NMR spectroscopy. Compositions of each experiment were as follows:

D-MSX (0.6 mg, 2.7 μmol), DTT (1.5 mg, 7 μmol), MsrA (2.8 nmol) and NMR Buffer (700 μL).

L-MSX (1.4 mg, 6.7 μmol), DTT (1.5 mg, 7 μmol), MsrA (2.8 μmol) and NMR Buffer (700 μL).

MSX-LAM (2.5 mg, 0.23 μmol), DTT (1.1 mg, 5.0 μmol), MsrA (2.0 nmol) and NMR Buffer (700 μL).

***in silico* docking experiments**

Three dimensional models for 20 different ring conformations of methyl α-D-MTX were generated manually using Maestro (Schrödinger software). Each molecule was docked into the active site of the crystal structure of Mtb MsrA[7] using eHiTS (SymBioSys software), which generated and scored the twenty best poses. Each of these poses were analysed and scored using SPROUT (SymBioSys software), and the best poses for each ring conformation that satisfied the structural requirements of the enzyme-substrate complex were selected and are displayed in Fig. S1.

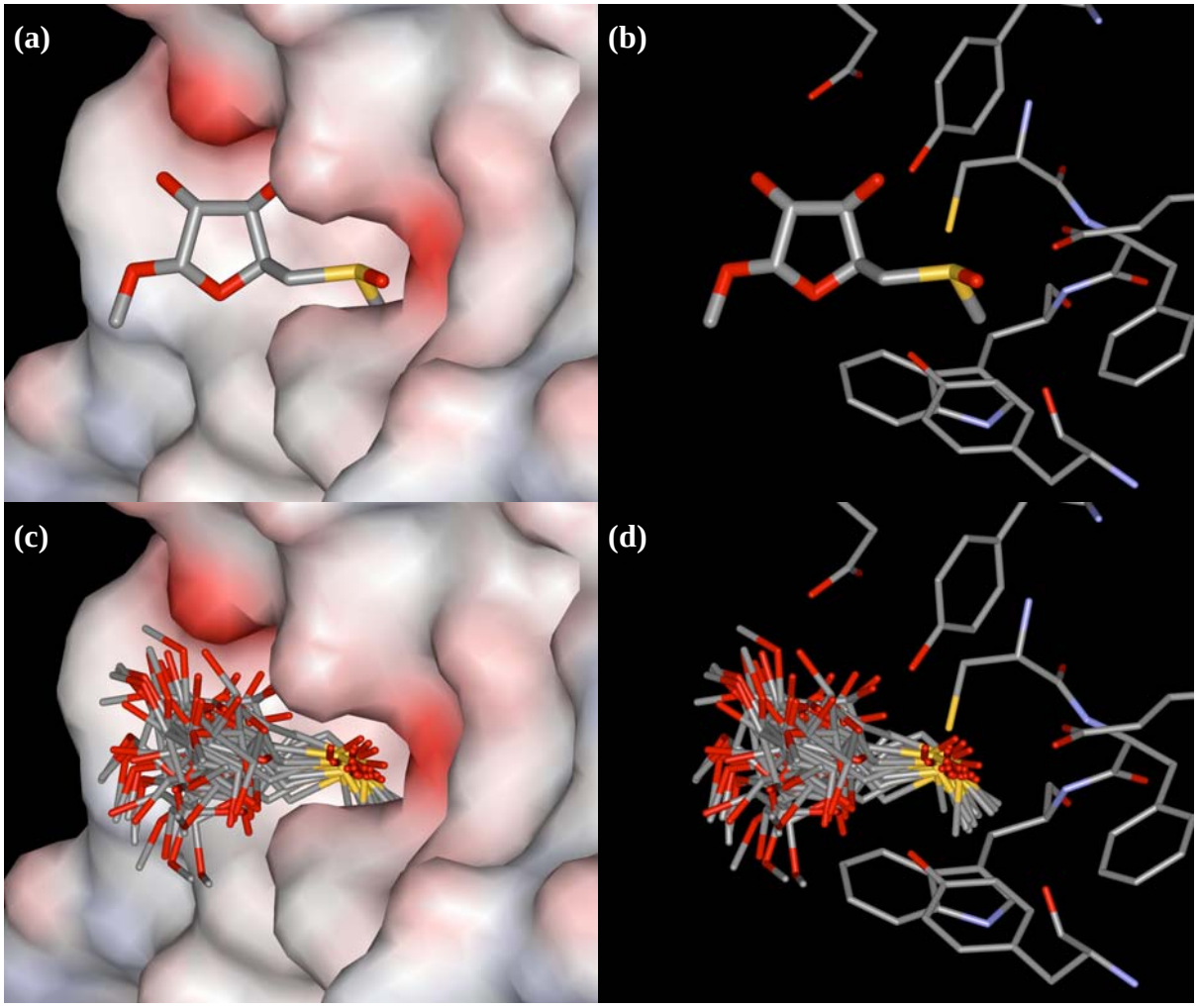


Fig. S1 Results of docking different MTX conformations into the Mtb MsrA crystal structure. The lowest energy pose is shown in (a) and (b), while the best poses for all twenty ring conformations are overlaid in (c) and (d).

References

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	Parameter	Value
1	NAME	Sam49427
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3	PROCNO	1
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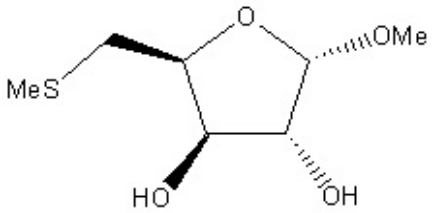
5	SF	500.2300000 MHz
6	Solvent	D2O
7	Temp	300.0 K
8	PULPROG	zg0f2pr
9	Nucleus	1H
10	SFO1	500.2327513 MHz
11	SWH	7002.801 Hz
12	NS	64



D-MTX

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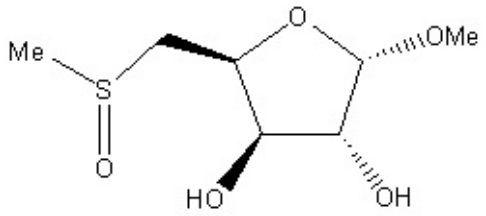
11	SWH	7002.801 Hz
12	NS	64



D-MSX

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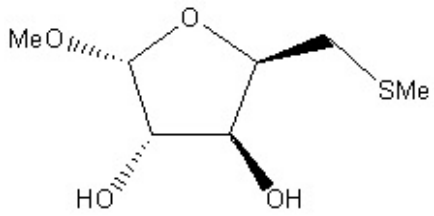
11	SWH	7002.801 Hz
12	NS	64



L-MTX

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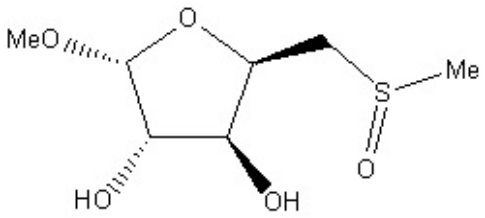

L-MSX