Supplementary Material for

Structural elucidation of 2-(6-(diethylamino)benzofuran-2-yl)-3-hydroxy-4*H*-chromen-4-one and labelling of *Mycobacterium aurum* cells

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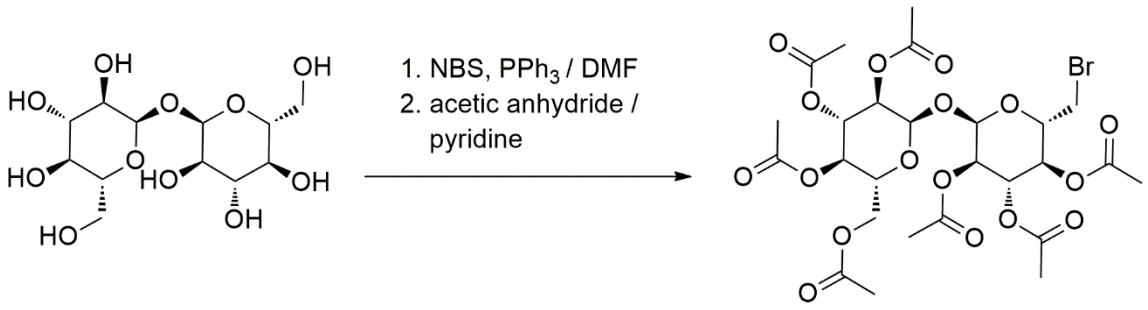
General

Solvents were distilled (with the exception of DMF) and stored over 4 Å molecular sieves. *N*-Bromosuccinimide and triphenylphosphine were recrystallized before use. Glassware was oven-dried at 110 °C prior to use. Flash chromatography was performed with a puriFlash® 430 instrument (Interchim, Montluçon, France). Columns were packed in 90 g (v = 40 mL min^{-1}) cartridges with 40 - 63 µm normal phase silica gel (Carl Roth GmbH, Karlsruhe, Germany). Column loading was performed with the dry load method. NMR spectra were recorded on an Agilent Technologies VNMRS 400 MHz spectrometer. Chemical shifts are reported relative to the residual solvent signal (chloroform-*d*: δ_H = 7.26 ppm; δ_C = 77.4 ppm; methanol-*d*4 δ_H = 3.31 ppm). APCI-MS was performed using an expression® CMS mass spectrometer (Advion Inc., Ithaca, NY, USA) with atmospheric solids analysis probe (ASAP) sampling. The HPLC apparatus consisted of two LC-40D pumps (analytical), two LC-20AP pumps (preparative), a SPD-M40 PDA detector, and a SIL-40C autosampler, all from the manufacturer Shimadzu (Kyoto, Japan). Purity was measured by UV absorbance at 254 nm.

Synthesis of 6-Bromo-6-deoxy-D-trehalose heptaacetate (6-Br-Ac$_7$-Tre)

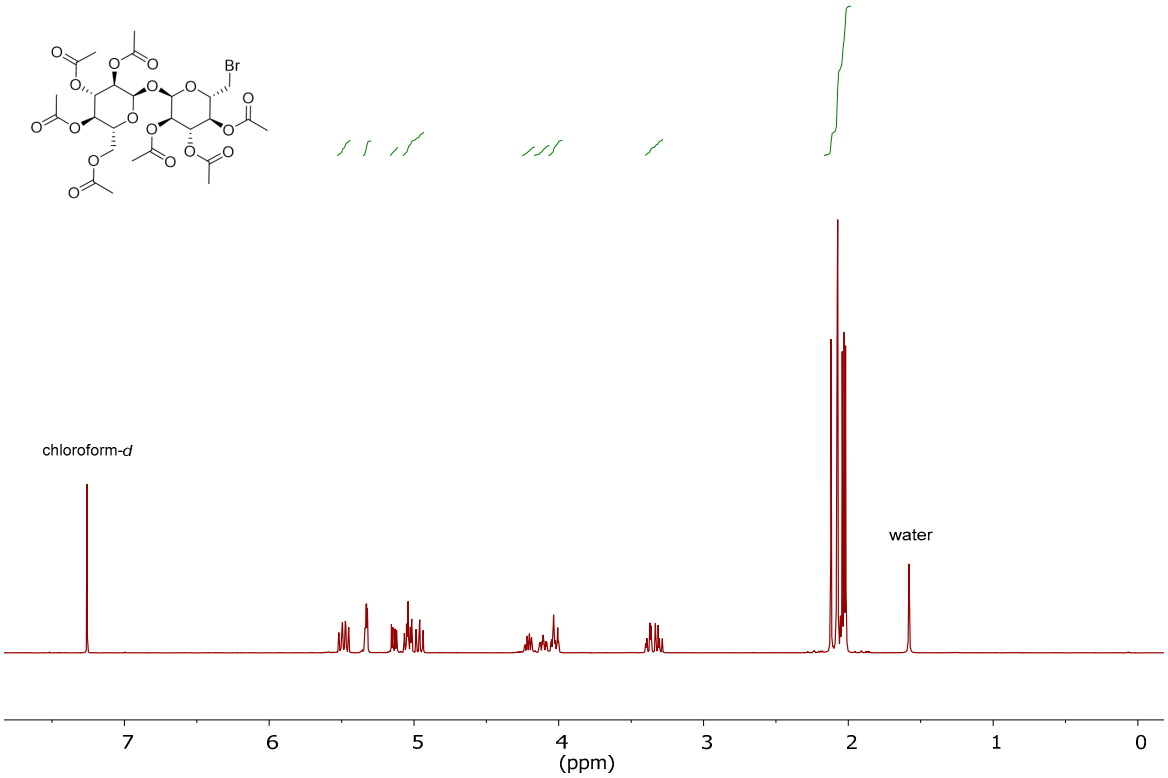


Tre **6-Br-Ac$_7$-Tre**

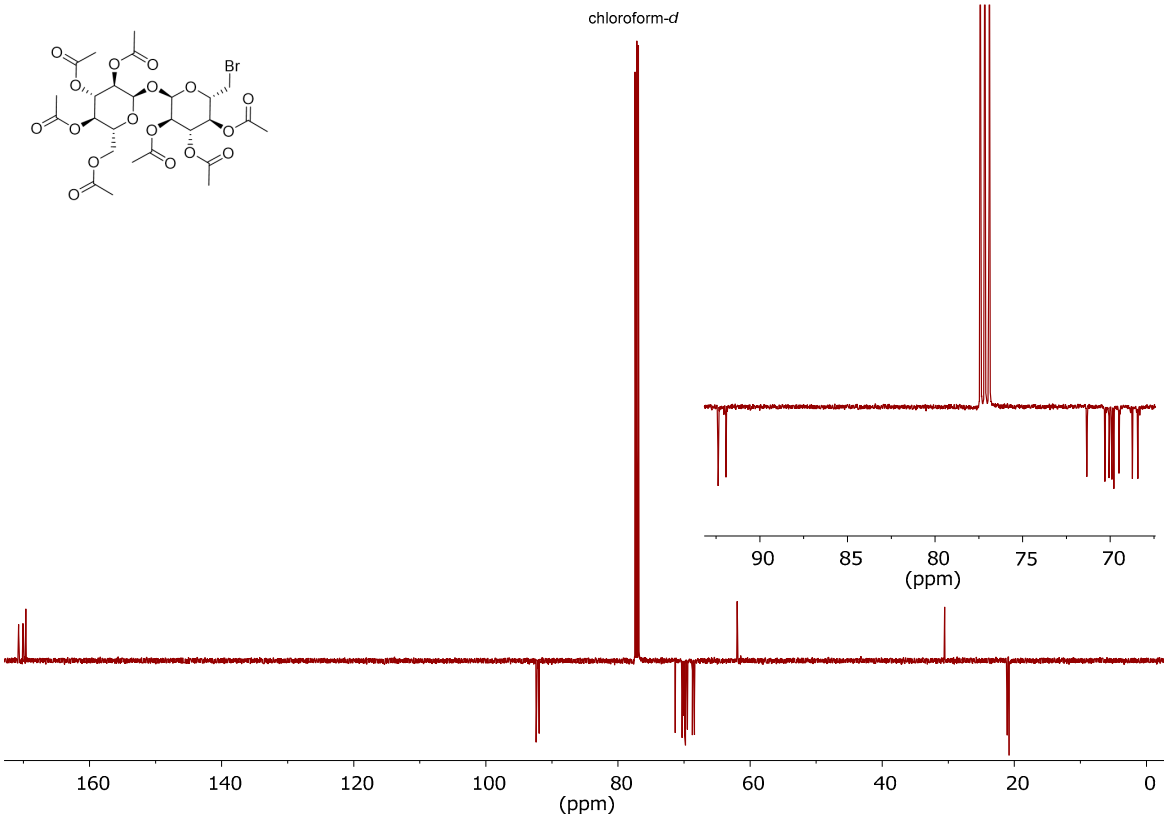
In a 250 mL flask, anhydrous DMF (100 mL) was heated to 85 °C, then anhydrous trehalose (4000 mg, 11.7 mmol) and *N*-bromosuccinimide (2290 mg, 12.9 mmol, 1.1 eq.) were added. After 5 min, triphenylphosphine (6130 mg, 23.4 mmol, 2.0 eq.) was added. The mixture was allowed to stir for 48 h at room temperature. Subsequently, the solvent was removed in vacuum to yield a colourless oil, which was taken up with anhydrous pyridine (100 mL). The resulting solution was cooled to 0 °C and acetic anhydride (15.5 mL, 164 mmol, 2.0 eq. relative to seven hydroxy groups of **6-Br-Tre**) was added dropwise with stirring. The solution was allowed to stir for 48 h at room temperature, and subsequently the solvent was removed under reduced pressure. The resulting orange oil was dissolved in ethyl acetate, washed twice with a saturated aqueous $NaHCO_3$ solution and twice with brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuum. The crude product was purified by flash chromatography (ethyl acetate/dichloromethane gradient from 5 % ethyl acetate to 30 % over ten column volumes). Yield: 1283 mg (1.77 mmol, 16 %, colourless solid).

^{1}H NMR (400 MHz, chloroform-*d*) δ 5.53 – 5.44 (m, 2H), 5.33 (dd, *J* = 4.0, 2.3 Hz, 2H), 5.14 (dd, *J* = 10.3, 3.8 Hz, 1H), 5.07 – 4.93 (m, 3H), 4.25 – 4.17 (m, 1H), 4.11 (ddd, *J* = 10.2, 7.6, 2.6 Hz, 1H), 4.07 – 3.98 (m, 2H), 3.40 – 3.28 (m, 2H), 2.16 – 1.98 (m, 21H) ppm. ^{13}C APT NMR (126 MHz, chloroform-*d*) δ 170.7, 170.1, 170.0, 169.7, 169.7, 169.7, 92.4, 91.9, 71.3, 70.3, 70.1, 69.9, 69.8, 69.5, 68.7, 68.4, 62.0, 30.6, 21.1, 20.8, 20.8, 20.7, 20.7 ppm.

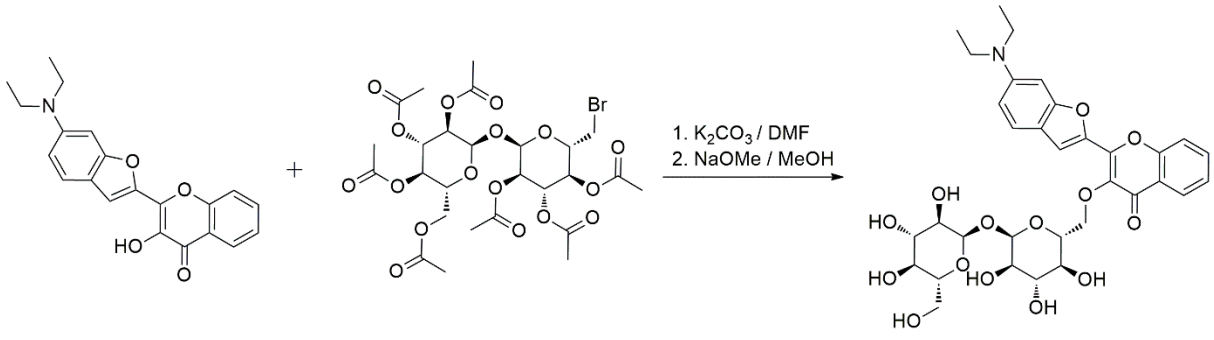
^{1}H NMR spectrum of 6-Br-Ac$_7$-Tre in chloroform-*d*



^{13}C APT NMR spectrum of 6-Br-Ac$_7$-Tre in chloroform-*d*



Synthesis of 3HC-2-Tre



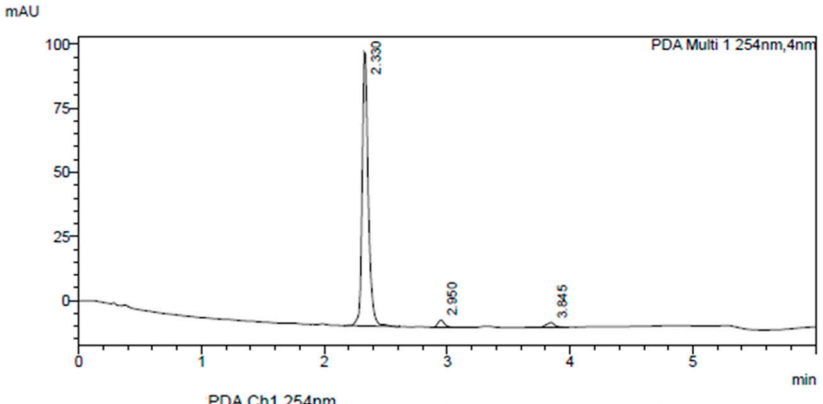
3HC-2 **6-Br-Ac$_7$-Tre** **3HC-2-Tre**

In a 10 mL flask, **3HC-2** (25 mg, 0.07 mmol), anhydrous K_2CO_3 (69 mg, 0.13 mmol, 1.8 eq.) and anhydrous DMF (2 mL) were mixed and stirred for 1 h at room temperature. Subsequently, **6-Br-Ac$_7$-Tre** (65 mg, 0.10 mmol, 1.4 eq.) was added, and the reaction was stirred for 24 h at 80 °C under light protection. The solvent was then removed under reduced pressure, and anhydrous 0.5 M NaOMe in methanol (2 mL) was added to the residue. After stirring for 1.5 h at room temperature, Amberlite IR120 H^+ resin was added with stirring until neutral pH was reached. The reaction mixture was filtered, and the solvent was removed under reduced pressure. The crude product was purified by reversed-phase C18 chromatography (250 mm Polaris C18-A, 21.2 mm, acetonitrile/water 15%-85% acetonitrile, v = 15.0 mL min^{-1}, t = 20 min). Yield: 8 mg (0.01 mmol, 17 %, orange solid).

HPLC purity: 95.1 % t_R = 2.33 min; 50 mm Eclipse Plus C18 1.8 µm, 4.6 mm, acetonitrile/water 15% - 85% acetonitrile, v = 1.0 mL min^{-1}, t = 6 min, λ = 220 nm).

^{1}H NMR (400 MHz, methanol-*d*4) δ 8.16 (dd, J = 8.0, 1.6 Hz, 1H), 7.95 (s, 1H), 7.78 (ddd, J = 8.7, 7.0, 1.7 Hz, 1H), 7.69 (d, J = 8.6 Hz, 1H), 7.51 (d, J = 9.5 Hz, 1H), 7.46 (ddd, J = 8.1, 7.0, 1.1 Hz, 1H), 6.84 – 6.79 (m, 2H), 5.26 (d, J = 3.8 Hz, 1H), 5.17 (d, J = 3.8 Hz, 1H), 4.51 – 4.40 (m, 2H), 4.22 – 4.17 (m, 1H), 3.95 – 3.78 (m, 5H), 3.75 – 3.68 (m, 2H), 3.60 (dd, J = 9.7, 3.8 Hz, 1H), 3.53 – 3.43 (m, 5H), 1.23 (t, J = 7.0 Hz, 6H). MS (APCI$^+$): m/z [M+H]$^+$ calcd. for $C_{33}H_{40}NO_{14}^+$, 674.2; found: 673.9.

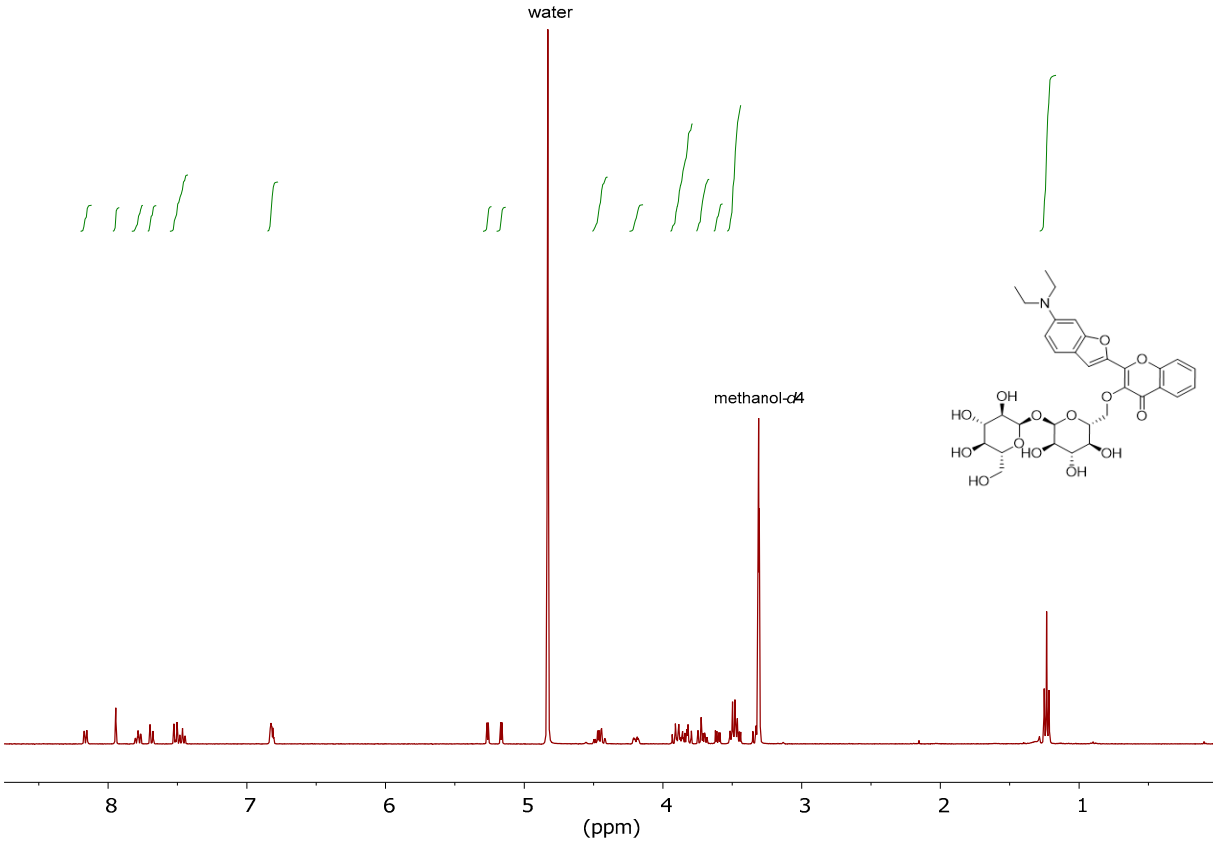
HPLC analysis of 3HC-2-Tre



PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	2.330	370563	106803	95.140
2	2.950	10414	2776	2.674
3	3.845	8515	1832	2.186
Tota		389492	111411	100.000

^{1}H NMR spectrum of 3HC-2-Tre in methanol-*d*4



APCI mass spectrum of 3HC-2-Tre