



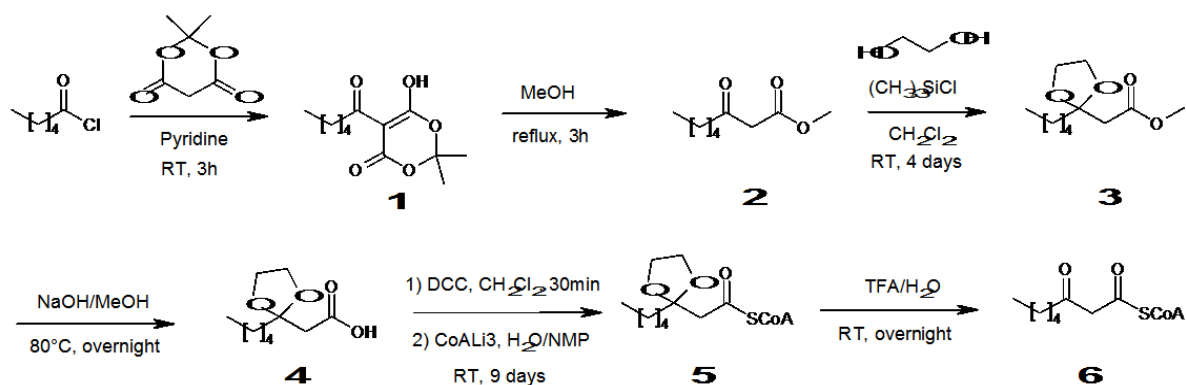
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**Supporting information for article:**

**Structural rearrangements occurring upon cofactor binding in the  
*Mycobacterium smegmatis*  $\beta$ -ketoacyl-acyl carrier protein  
reductase MabA**

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The procedure for the synthesis of  $\beta$ -keto-octanoyl-CoA (6) has been adapted from (Kanchanabanca *et al.*, 2013)

### 5-hexanoyl-6-hydroxy-2,2-dimethyl-1,3-dioxin-4-one (1)

Meldrum's acid (10 mmol, 1 eq.) was added to 10 mL of anhydrous pyridine and cooled to 0°C. Hexanoyl chloride (15 mmol, 1.5 eq.) was added dropwise at 0°C. The reaction mixture was stirred 3h at room temperature and then evaporated under reduced pressure. The residue was dissolved in diethyl ether and the organic layer was washed once with water and once with brine. The organic layer was dried over anhydrous magnesium sulfate (MgSO<sub>4</sub>) and the solvent was removed under reduced pressure resulting in yellow solution (100% yield). The product was used in the next step without further purification. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  0.91 (t, *J*= 6.8 Hz, 3H), 1.31-1.44 (m, 4H), 1.63 -1.73 (m, 2H), 1.74 (s, 6H), 3.07 (t, *J*= 7.8 Hz, 2H).

### Methyl 3-oxooctanoate (2)

Compound 1 (10 mmol, 1 eq.) was mixed with methanol (MeOH) (15 mL) and heated under reflux for 3h. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (cyclohexane/ethyl acetate 100/0 to 80/20). The solvents were removed under reduced pressure resulting in pale yellow oil (855 mg, 50% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  0.89 (t, *J*= 6.9 Hz, 3H), 1.25-1.36 (m, 4H), 1.56-1.65 (m, 2H), 2.53 (t, *J*= 7.3 Hz, 2H), 3.45 (s, 2H), 3.74 (s, 3H).

### Methyl 2-(2-pentyl-1,3-dioxolan-2-yl)acetate (3)

Compound 2 (850 mg, 4.93 mmol, 1 eq.) was mixed with 8.26 mL of ethylene glycol (148 mmol, 30 eq.) in 40 mL of CH<sub>2</sub>Cl<sub>2</sub>, and then 3.8 mL of chlorotrimethylsilane (29.6 mmol, 6 eq.) was added dropwise. The mixture was stirred at room temperature for 4 days, and then

washed twice with water. The organic layer was dried over anhydrous magnesium sulfate (MgSO<sub>4</sub>), and the solvent was removed under reduced pressure resulting in yellow oil (100% yield). The product was used in the next step without further purification. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 0.89 (t, J= 6.7 Hz, 3H), 1.29-1.34 (m, 4H), 1.35-1.45 (m, 2H), 1.77-1.82 (m, 2H), 2.67 (s, 2H), 3.70 (s, 3H), 3.96-4.01 (m, 4H).

#### **2-(2-pentyl-1,3-dioxolan-2-yl)acetic acid (4)**

Compound **3** (2.47 mmol, 1 eq.) was dissolved in 45 mL of methanol. 15 mL of sodium hydroxide (1 M) were added and the reaction mixture was heated at 80°C overnight. The solution was cooled to 0°C and then the pH was adjusted to 5 by the addition of ammonium chloride with 20 mL of water, then acetic acid. The methanol was removed under reduced pressure. The aqueous phase was extracted twice with ethyl acetate (70 mL). The organic layer was washed once with brine and then evaporated under reduced pressure. The remaining acetic acid was azeotropically removed by co-evaporating it twice with 50 mL of toluene resulting in pale yellow solid (400 mg, 80% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 0.89 (t, J= 6.8 Hz, 3H), 1.26-1.45 (m, 6H), 1.77-1.82 (m, 2H), 2.72 (s, 2H), 3.98-4.08 (m, 4H).

#### **2-(2-pentyl-1,3-dioxolan-2-yl)-acetyl-S-CoA (5)**

Compound **4** (0.51 mmol, 3.3 eq), *N,N'*-dicyclohexylcarbodiimide (DCC) (0.262 mmol, 1.68 eq) were dissolved in 1 mL of anhydrous dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>). The reaction mixture was stirred at 0°C for 30 minutes and then the precipitated *N,N'*-dicyclohexylurea (DCU) was removed by filtration and washed with CH<sub>2</sub>Cl<sub>2</sub>. The filtrate was evaporated and the residue was dissolved in 400 µL of *N*-methyl-2-pyrrolidone (NMP). A solution of coenzyme A trilithium salt (0.156 mmol, 1 eq) in 200 µL of water was added under argon. The solution was stirred at room temperature under argon for 9 days. Water was added and the aqueous phase was washed twice with diethyl ether. The aqueous phase was frozen and then lyophilized. The crude product was purified by preparative HPLC (H<sub>2</sub>O pH = 9.2/CH<sub>3</sub>CN 100/0 to 20/80) resulting in white solid (37 mg, 25% yield). [M+H]<sup>+</sup> = 952.

#### **β-keto-octanoyl-CoA (6)**

Compound **5** (36 µmol) was dissolved in 480 µL of water and 20 µL of trifluoroacetic acid (TFA) was added dropwise. The reaction mixture was stirred overnight at room temperature. Water (3 mL) was added, the sample was frozen and then lyophilized to afford a white powder (32 mg, 99% yield). [M+H]<sup>+</sup> = 908.