Supplementary Information

Figure S1

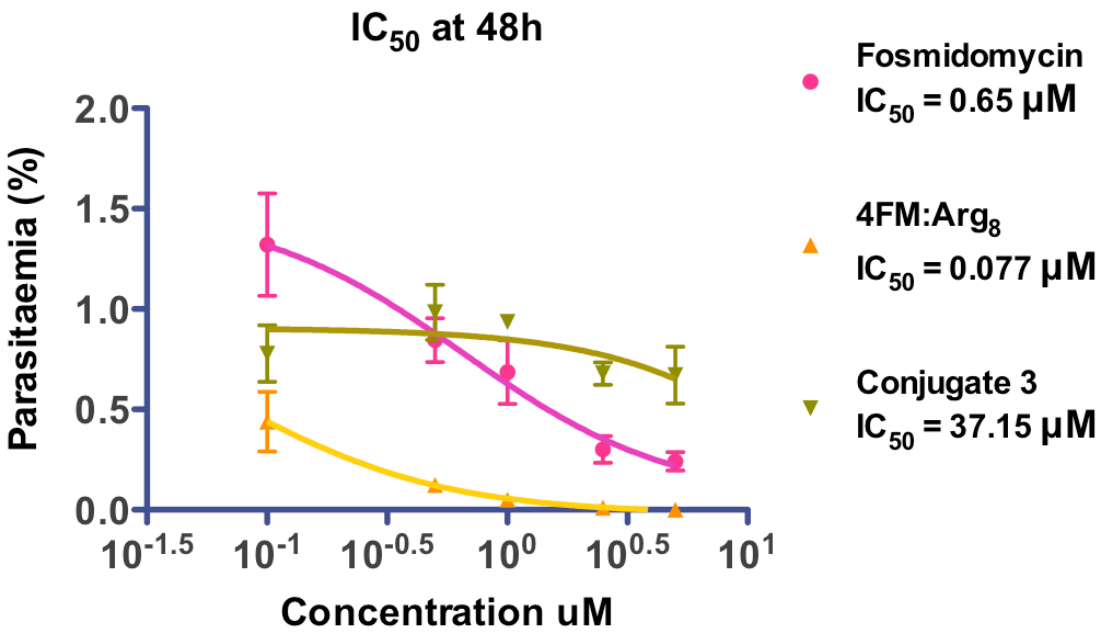


Fig. S1: IC_{50} chart of the *P. falciparum* growth curve at 48h in presence of either fosmidomycin, Salt **2** or conjugate **3**. Each data point represents the mean and standard deviation of three replicates. Graph was generated by non-linear one site logIC_{50} fit using the software Graphpad Prism.

Figure S2

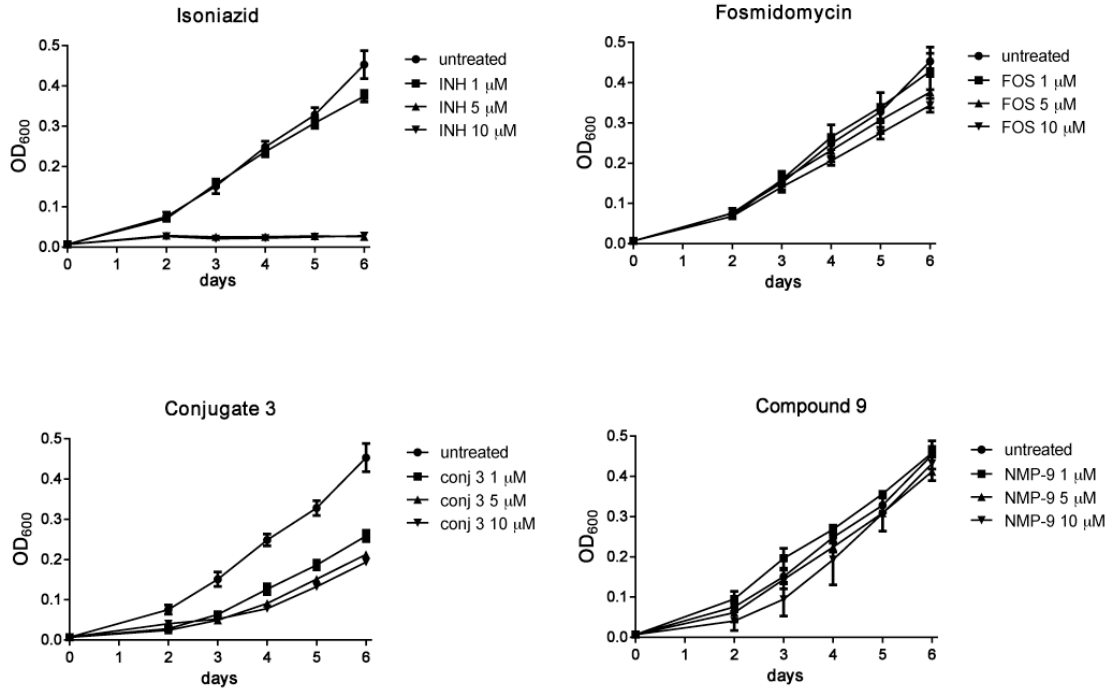


Fig. S2: Diethyl ester of fosmidomycin does not inhibit growth of *Mycobacterium bovis* BCG.

(A) *M. bovis* BCG was grown in presence of isoniazid, fosmidomycin, conjugate 3, or the diethyl ester derivative of fosmidomycin (compound 9 in the synthesis scheme). Similar to fosmidomycin compound 9 did not show any significant inhibitory effect on mycobacterial growth. Each data point represent the mean (± S.D.) of three biological replicates.

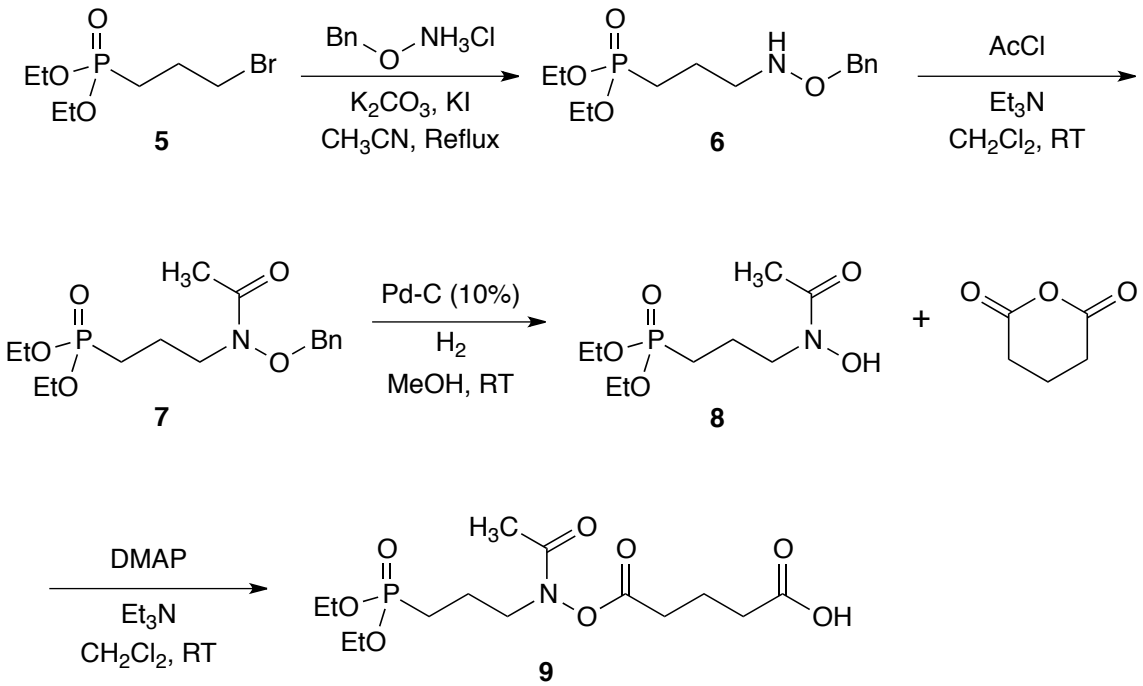
General methods

All reactions were monitored by TLC using silica gel 60 F_{254} plates. Visualization of the reaction components was achieved using UV fluorescence (254 nm) and Hanessian's stain. Silica gel chromatography was carried out over Kieselgel 60. The yields reported are after purification. ^{1}H, ^{13}C NMR spectra were recorded in deuterated solvents and chemical shifts (δ) are quoted in parts per million (ppm) calibrated to solvent residual peak (^{1}H and ^{13}C). Coupling constants (*J*) are measured in Hertz (Hz). The following abbreviations are used to describe multiplicities: *s* = singlet, *d* = doublet, *t* = triplet, *q* = quartet, *b* = broad, *m* = multiplet. Purification of final compounds was done by RP-HPLC (*Supelco Discovery BIO Wide Pore C18 column* (25 cm x 21.2 mm, 10 *μ*m *Sigma-Aldrich*) flow rate: 10 ml/min, detection wavelength: 220 nm).

Chemistry:

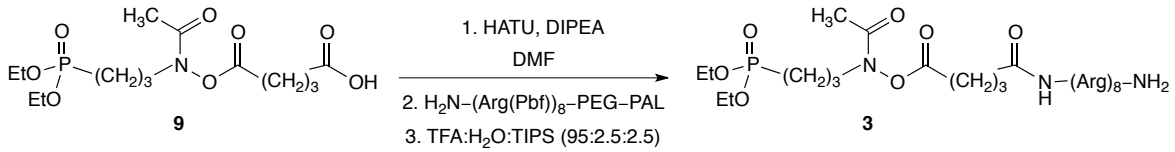
Synthesis of the targeted derivative of the fosmidomycin compound **9** (Scheme 1) was achieved starting from the commercially available 3-Bromopropylphosphonic acid diethylester **5**. Compound **5** was first transformed to the corresponding *O*-benzylhydroxylamino compound **6** by the nucleophilic substitution (S_N2) reaction of the bromide group by *O*-benzylhydroxylamine at refluxing acetonitrile. *N*-acetylated compound **7** was achieved by the treatment of acetyl chloride (AcCl) in the presence of triethylamine (Et_3N). Subsequent hydrogenolysis with H_2 gas in

presence of catalytic Pd-C (10%), removed the benzyl protection to give the free *N*-hydroxylamine compound **8**.



Scheme 1

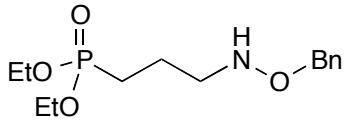
Treatment of compound **4** with glutaric acid anhydride in presence of Et_3N and 4-*N,N*-dimethylaminopyridine (DMAP) yielded the targeted derivative of fosmidomycin.



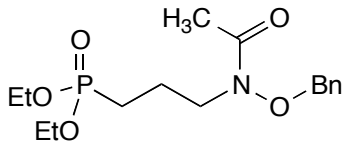
Scheme 2

Coupling of the fosmidomycin derivative **9** with the resin-bound carrier (octaarginine) was performed on a PAL-PEG solid support using *O*-(7-Aza-1H-

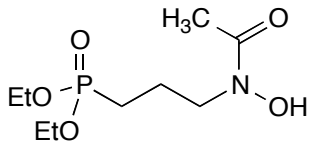
benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) as the coupling agent and diisopropyl ethyl amine (DIPEA) as the base in anhydrous DMF. Subsequent Pbf deprotection and cleavage from the resin with a cocktail mixture of TFA:H_2O:TIPS provided the fosmidomycin-Arg_8-conjugate **3** (Scheme 2).



(3-Benzyloxyamino-propyl)-phosphonic acid diethylester (**6**)**:** To a mixture of O-benzyl-hydroxylamine hydrochloride (3.07 g, 19.25 mmol), K_2CO_3 (19.25 g, 19.25 mmol) and KI (0.63 g, 3.85 mmol) in CH_3CN (50 mL) was added the 3-Bromopropylphosphonic acid diethylester **5** (1 g, 3.85 mmol) at room temperature and the reaction mixture was allowed to reflux for overnight. The reaction was quenched by the addition of H_2O and the aqueous phase was extracted with $CHCl_3$ (3 x 30 mL). The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure to give the crude compound. The residue was purified by column chromatography on silica gel using pure EtOAc as an eluent to give (3-benzyloxyamino-propyl)-phosphonic acid diethyl ester **6** as colourless oil (790 mg, 68%).**^{1}H-NMR** (400 MHz, $CDCl_3$): δ 1.30 (*t*, *J* = 7.2, 6H), 1.74-1.85 (*m*, 4H), 2.96 (*t*, *J* = 6.4, 2H), 4.03-4.12 (*m*, 4H), 4.68 (*s*, 2H), 5.58 (*bs*, 1H), 7.28-7.34 (*m*, 5H). **^{13}C-NMR** (100 MHz, $CDCl_3$): δ 16.4 (*d*, $^3J_{C\text{-}P}$ = 5.9), 20.6 (*d*, $^2J_{C\text{-}P}$ = 4.9), 23.2 (*d*, $^1J_{C\text{-}P}$ = 141.2), 52.1 (*d*, $^3J_{C\text{-}P}$ = 16.6), 61.4 (*d*, $^2J_{C\text{-}P}$ = 6.5), 76.4, 127.8, 128.4, 137.8. **HR-MS-ESI:** *m/z* Calcd. for $C_{14}H_{24}NO_4P$: 302.1521 $[M+H]^+$: *m/z* Found: 302.1518 $[M+H]^+$.

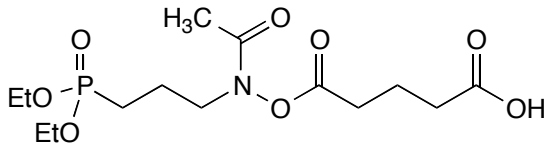


[3-(acetylbenzyloxy-amino)-propyl]-phosphonic acid diethyl ester (**7**): Acetylchloride (0.12 mL, 1.55 mmol) was added dropwise to a solution of **6** (390 mg, 1.29 mmol) and triethylamine (0.22 mL, 1.55 mmol) in dichloromethane. The reaction mixture was stirred at room temperature for 4 h. The reaction mixture was quenched with saturated $NaHCO_3$ and then washed with H_2O. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure to give the crude compound. The residue was purified by column chromatography on silica gel using CH_2Cl_2-MeOH (90:10) as an eluent to give **7** as a yellow oil (390 mg, 88%).**^{1}H-NMR** (400 MHz, $CDCl_3$): δ 1.31 (*t*, *J* = 7.2, 6H), 1.69-1.78 (*m*, 2H), 1.90-2.01 (*m*, 2H), 2.10 (*s*, 3H), 3.72 (*t*, *J* = 6.8, 2H), 4.05-4.11 (*m*, 4H), 4.83 (*s*, 2H), 7.37-7.40 (*m*, 5H). **^{13}C-NMR** (100 MHz, $CDCl_3$): δ 16.4 (*d*, $^3J_{C\text{-}P}$ = 5.9), 20.2 (*d*, $^2J_{C\text{-}P}$ = 4.7), 20.5, 22.3, 23.7, 61.6 (*d*, $^2J_{C\text{-}P}$ = 6.5), 76.4, 128.7, 129.0, 129.2, 134.3. **HR-MS-ESI:** *m/z* Calcd. for $C_{16}H_{27}NO_5P$: 344.1627 $[M+H]^+$: *m/z* Found: 344.1621 $[M+H]^+$.



[3-(acetyl-hydroxyamino)-propyl]-phosphonic acid diethyl ester (**8**): To a solution of the benzyl protected compound **7** (3.1 g, 9.02 mmol) in MeOH (100 mL) was added 10% Pd/C (184 mg) and hydrogenated at atmospheric pressure for 3h. The mixture was filtered over celite, and the solvent was removed by rotary evaporation to yield compound **8** as colorless oil (1.9 g, 84%).**^{1}H-NMR** (400 MHz,

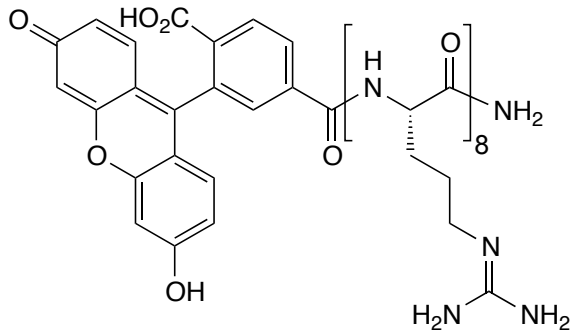
$CDCl_3$): δ 1.29 (*t*, *J* = 6.8, 6H), 1.75-1.83 (*m*, 2H), 1.87-1.95 (*m*, 2H), 2.12 (*s*, 3H), 3.69 (*t*, *J* = 6.0, 2H), 4.01-4.06 (*m*, 4H), 9.69 (*bs*, 1H). **^{13}C-NMR** (100 MHz, $CDCl_3$): δ 16.3 (*d*, $^{3}J_{C\text{-}P}$ = 6.0), 19.1 (*d*, $^{2}J_{C\text{-}P}$ = 5.2), 20.5, 22.2 (*d*, $^{1}J_{C\text{-}P}$ = 139.9), 47.4 (*d*, $^{3}J_{C\text{-}P}$ = 9.2), 62.1 (*d*, $^{2}J_{C\text{-}P}$ = 6.7), 172.6. **HR-MS-ESI:** *m/z* Calcd. for $C_9H_{21}NO_4P$: 254.1157 $[M+H]^+$: *m/z* Found: 254.1154 $[M+H]^+$.



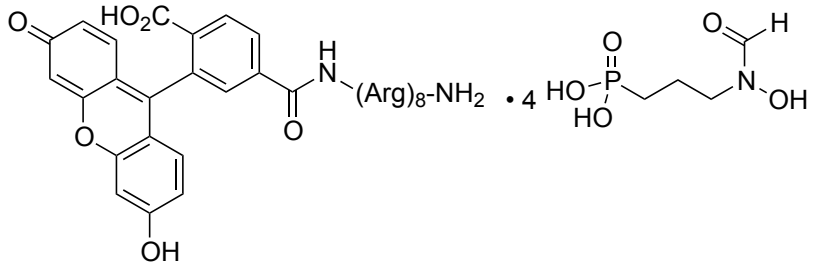
5-((N-(3-(diethoxyphosphoryl)propyl)acetamido)oxy)-5-oxopentanoic acid (**9**): A mixture of compound **8** (253 mg, 1.0 mmol), Glutaric acid anhydride (111 mg, 0.98 mmol), DMAP (122 mg, 1.0 mmol) and Et_3N (0.08 mL, 1.2 mmol) in CH_2Cl_2 (10 mL) were stirred at room temperature for overnight. The reaction was quenched by the addition of saturated $NaHCO_3$ (~ pH-9). The organic layer was separated and the aqueous layer was washed with CH_2Cl_2. The aqueous layer was acidified by the addition of 6N HCl (~ pH-2) and extracted with CH_2Cl_2 (2 x 30 mL). The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure to give the crude compound as faint pink liquid (140 mg, 38%) which was pure enough for the next step. **^{1}H-NMR** (400 MHz, $CDCl_3$): δ 1.28 (*t*, *J* = 6.8, 6H), 1.74-2.02 (*m*, 9H), 2.40 (*t*, *J* = 7.2, 2H), 2.53 (*t*, *J* = 7.2, 2H), 3.72 (*t*, *J* = 6.4, 2H), 4.03-4.09 (*m*, 4H), 9.69 (*bs*, 1H). **^{13}C-NMR** (100 MHz, $CDCl_3$): δ 16.3 (*d*, $^{3}J_{C\text{-}P}$ = 6.0), 19.1, 19.6, 20.3, 20.1 (*d*, $^{2}J_{C\text{-}P}$ = 17.1), 22.4 (*d*, $^{1}J_{C\text{-}P}$ = 141.8), 32.8, 30.7,

62.0 (*d*, $^{2}J_{C\text{-}P}$ = 6.6), 170.9, 172.7, 174.8. **HR-MS-ESI:** *m/z* Calcd. for $C_{14}H_{27}NO_8P$: 368.1474 $[M+H]^+$: *m/z* Found: 368.1468 $[M+H]^+$.

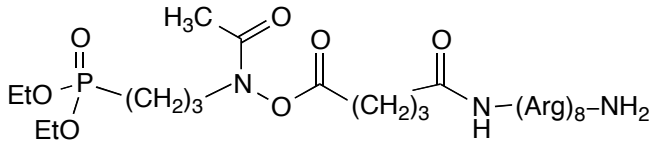
H_2N-(Arg(Pbf))$_8$-PAL-PEG-resin: Fmoc-(Arg(Pbf)$_8$-PAL-PEG-resin was prepared on an AB synthesiser using *FastMoc®* chemistry and Fmoc-Arg(Pbf)-OH (4.0 eq.), HBTU (3.9 eq.) on a 0.25 mmol scale of PAL-PEG-resin. Fmoc deprotection was performed by shaking the resin (220 mg, 0.21 mmol/g loading, 0.05 mmol) with three 10 mL-portions of 20% piperidine in DMF (10 min per treatment, draining between treatments), washed with DMF (3 x 10 mL), CH_2Cl_2 (3 x 10 mL).



6-Fam-octaarginine amide (**1**): A small amount of the above Fmoc-deprotected resin was coupled to the isomerically pure 6-carboxyfluorescein (6-FAM) on solid support. The peptide was cleaved from the resin by treatment with TFA:H_2O:TIPS (95:2.5:2.5) and the crude product was purified by HPLC on *Supelco Discovery BIO Wide Pore C18 column* (25 cm x 21.2 mm, 10 μm *Sigma-Aldrich*) with MeCN (0.1 % TFA) : H_2O (0.1 % TFA) ratio of 20 : 80 for 10 min and subsequently with a gradient from 20 : 80 to 25 : 75 in 5 min: t_R 12 min; **HR-MS-ESI:** *m/z* Calcd. for $C_{69}H_{119}N_{33}O_{14}$: 1624.8905 $[M+H]^+$: *m/z* Found: $[M+H]^+$.



6-Fam-octaarginine-Fosmidomycin-Salt (**2**): A solution of 6-Fam-Arg_8-NH_2 (**1**) (1.25 μmol) in H_2O (5 mL) was treated with a solution of fosmidomycin (5.00 μmol) in H_2O (5 mL) and the mixture was lyophilized to yield the 6-Fam-octaarginine-fosmidomycin salt (**2**).



Fosmidomycin-Arg_8-conjugate (**3**): The above H_2N-(Arg(Pbf))$_8$-PAL-PEG-resin was shaken with a mixture of **9** (55 mg, 0.15 mmol), HATU (57 mg, 0.15 mmol) and DIPEA (0.05 mL, 0.3 mmol) in DMF (5 mL) for 5 h. The solution was then drained, washed with DMF (3 x 10 mL), MeOH (3 x 10 mL) and CH_2Cl_2 (3 x 10 mL). The Pbf deprotection and cleavage from the resin was achieved by treatment with a cocktail mixture of TFA:H_2O:TIPS for 5 h. The crude product was purified by RP-HPLC on *Supelco Discovery BIO Wide Pore C18 column* (25 cm x 21.2 mm, 10 μm *Sigma-Aldrich*, eluent system MeCN (0.1 % TFA): H_2O (0.1 % TFA)) by applying a linear gradient of 0%-18% (MeCN) in 15 min followed by an isocratic gradient of 18 : 80 up to 30 min: t_R 25.3 min. **HR-MS-ESI:** *m/z* Calcd. for $C_{62}H_{123}N_{34}O_{15}P$: 1615.9723 $[M+H]^+$: *m/z* Found: 1615.9696 $[M+H]^+$.