MEPicides: α, β-Unsaturated Fosmidomycin Analogs as DXR inhibitors against Malaria

Xu Wang[†], Rachel L. Edwards[§], Haley Ball[#], Claire Johnson[#], Amanda Haymond[#], Misgina Girma[#], Michelle Manikkam[‡], Robert C. Brothers[†], Kyle T. McKay[†], Stacy D. Arnett[Δ], Damon M. Osbourn[Π], Sophie Alvarez[∞], Helena I. Boshoff[‡], Marvin J. Meyers[Δ,Σ], Robin D. Couch[#], Audrey R. Odom John[§], Cynthia S. Dowd[*,†]

[†]Department of Chemistry, George Washington University, Washington DC 20052
[§]Department of Pediatrics, Washington University School of Medicine, Washington University, St. Louis, MO 63110
[#]Department of Chemistry and Biochemistry, George Mason University, Manassas, VA 20110
[‡]Tuberculosis Research Section, LCIM, NIAID/NIH, Bethesda, MD 20892
[Δ]Department of Pharmacology and Physiology, Saint Louis University, St. Louis, MO 63104
[Π]Department of Molecular Microbiology and Immunology, Saint Louis University, St. Louis, MO 63104
[∞]Proteomics & Metabolomics Facility, Center for Biotechnology, Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE 68588
[Σ]Department of Chemistry, Saint Louis University, St. Louis, MO 63103

TABLE OF CONTENTS

General. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$, CD_3OD or $_{D2O}$ on Agilent spectrometer at 400 and 101 MHz, respectively, with TMS, H_2O or solvent signal as internal standard. Chemical shifts are given in parts per million (ppm). Spin multiplicities are given with the following abbreviations: s (singlet), br s (broad singlet), d (doublet), dd (doublet of doublets), ddd (doublet of doublets of doublets), t (triplet), dt (doublet of triplets), ddt (doublet of doublet of triplets), q (quadruplet), qt (quintuplet), m (multiplet). Mass spectra were measured in the ESI mode on an HPLC-MS (Agilent 1100) or in the EI mode on an GC-MS (Shimadzu GCMS-QP2010S). Thin layer chromatography (TLC) was performed on Baker-flex Silica Gel IB2-F silica plates and flash column chromatography was carried out using SiliCycle SiliaFlash P60 silica gel (40-63 μm). All reagents were purchase from commercial suppliers and used without further purification. Anhydrous solvents were purified by MBRAUN MB-SPS solvent purification system before use. All air sensitive reactions were carried out under nitrogen atmosphere. The purity of synthesized compounds (>95%) was determined by $^{1}H/^{13}C$ NMR in combination with HPLC-MS (Agilent 1100). Column: Thermo Fisher Scientific Hypersil GOLD aQ C-18 3 μm particle (250 mm × 4.6 mm). Mobile phase (containing 0.1% formic acid as the additive): linear gradient of acetonitrile (50%-100%) in water at a flow rate of 0.8 mL/min over 12.5 min, followed by 100% acetonitrile that was maintained for another 12.5 min. The UV detection wavelength was 210 nm and 254 nm. High-resolution mass spectroscopy spectra (HRMS) were recorded in positive or negative ESI mode on a Waters Q-TOF Ultima mass spectrometer (UIUC Mass Spectrometry Laboratory) or in positive FAB mode on a VG Analytical VG70SE magnetic sector mass spectrometer (JHU Mass Spectrometry Facility).

Synthesis of α, β-unsaturated fosmidomycin analogs.

Diethyl (prop-2-en-1-yl)phosphonate (6).[1] Triethyl phosphite (10 mL, 58 mmol, 1 eq) and allyl bromide (6.5 mL, 75 mmol, 1.3 eq) were added to an oven-dried round bottom flask covered with foil. The reaction mixture was stirred at 60 ^{o}C for 2 days, purified by fractional distillation under reduced pressure using a Kugelrohr to afford the title compound as a colorless oil (8.8 g, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.95 – 5.69 (m, 1H), 5.31 – 5.13 (m, 2H), 4.25 – 3.99 (m, 4H), 2.62 (ddt, *J* = 21.9, 7.4, 1.3 Hz, 2H), 1.32 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 127.7 (d, *J* = 11.3 Hz), 120.0 (d, *J* = 14.4 Hz), 62.1 (d, *J* = 6.5 Hz), 32.0 (d, *J* = 139.4 Hz), 16.6 (d, *J* = 6.0 Hz). GC-MS (EI): 178 *m*/*z* [M].

Diethyl (2,3-dibromopropyl)phosphonate (7).[2] To a solution of **6** (2.9 g, 16 mmol, 1 eq) in dry CH_2Cl_2 (30 mL) under N_2 was added Bromine (1 mL, 19.6 mmol, 1.2 eq) at 0 ^{o}C dropwise. The reaction mixture was stirred at room temperature for 2 h, quenched with saturated Na_2SO_3 (aq, 30 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were dried with

[1] Fourgeaud, P.; Midrier, C.; Vors, J.; Volle, J.; Pirat, J.; Virieux, D. Oxaphospholene and oxaphosphinene heterocycles via RCM using unsymmetrical phosphonates or functional phosphonates. *Tetrahedron* 2010, **66**, 758.

[2] Laureyn, I.; Stevens, C. V.; Soroka, M.; Malysa, P. Synthesis of γ-amino-α,β-unsaturated phosphonates via a substitution-elimination sequence of dibromophosphonates. *ARKIVOC* 2003, ***iv***, 102.

anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to afford the title compound as a light yellow oil (4.9 g, 88%) without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.49 – 4.37 (m, 1H), 4.23 – 4.07 (m, 4H), 3.93 (ddd, *J* = 10.8, 4.4, 2.1 Hz, 1H), 3.78 (dd, *J* = 10.8, 7.3 Hz, 1H), 2.78 (ddd, *J* = 18.8, 15.8, 6.1 Hz, 1H), 2.39 (ddd, *J* = 18.4, 15.8, 7.3 Hz, 1H), 1.37 – 1.32 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 69.2 (d, *J* = 7.5 Hz), 55.2 (d, *J* = 7.5 Hz), 43.4 (d, *J* = 2.8 Hz), 38.0 (d, *J* = 11.1 Hz), 33.9 (d, *J* = 141.3 Hz), 17.0 (d, *J* = 4.8 Hz), 15.8 (d, *J* = 4.8 Hz). LC-MS (ESI^+): 337.0, 339.0, 341.0 *m*/*z* $[M+H]^+$.

***tert*-Butyl *N*-(benzyloxy)carbamate (8).**[3] To a stirred solution of *O*-benzylhydroxylamine hydrochloride (9.6 g, 60 mmol, 1 eq) and triethylamine (9.0 mL, 66 mmol, 1.1 eq) in a 1:1 mixture of THF/H_2O (100 mL), was added di-*tert*-butyl dicarbonate (30% in dioxane, 43.7 mL, 60 mmol, 1 eq). The reaction mixture was stirred at room temperature for 2.5 h, and then concentrated under reduced pressure to eliminate THF. The residue was extracted with EtOAc (3 × 50 mL), the combined organic layers were washed with 0.5 M citric acid (aq, 2 × 50 mL) and H_2O (50 mL). The organic layer was dried with anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give the crude solid. The crude solid was then recrystallized in hexanes to afford the title compound as a white solid (12 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97-7.29 (m, 5H), 5.28 (s, 2H), 1.90 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.7, 135.7, 129.1, 128.5, 81.7, 78.4, 28.2. LC-MS (ESI^+): 245.9 *m*/*z* $[M+Na]^+$, 460.0 *m*/*z* $[2M+Na]^+$.

***tert*-butyl *N*-(benzyloxy)-*N*-[(2*E*)-3-(diethoxyphosphoryl)prop-2-en-1-yl]carbamate (9).**[4] To a solution of **8** (1.2 g, 5.4 mmol, 1 eq) in dry THF (15 mL) under N_2 at 0 °C was added dropwise a suspension of NaH (60% in oil, 430 mg, 10.8 mmol, 2 eq) in dry THF (10 mL). This mixture was stirred at 0 °C for 30 min at which point **7** (2 g, 5.9 mmol, 1.1 eq) in dry THF (3 mL) was added as well as NaI (16 mg, 0.11 mmol, 0.02 eq) at 0 °C. The reaction mixture was stirred at room temperature for 20 h, quenched with saturated $NaHCO_3$ (aq, 30 mL), concentrated under reduced pressure to eliminate THF and extracted with EtOAc (3 × 30 mL). The combined organic layers were dried with anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. Chromatographic separation on silica gel (EtOAc/CH_2Cl_2 = 2/1) gave the title compound as a light yellow oil (1.6 g, 77%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.31 (m, 5H), 6.69 (ddt, *J* = 22.4, 17.2, 5.3 Hz, 1H), 5.82 (ddt, *J* = 18.9, 17.2, 1.7 Hz, 1H), 4.83 (s, 2H), 4.12 – 4.02 (m, 6H), 1.49 (s, 9H), 1.31 – 1.27 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.3, 146.4 (d, *J* = 5.3 Hz), 135.4, 129.3, 128.6, 128.4, 119.5 (d, *J* = 187.6 Hz), 82.0, 61.8 (d, *J* = 5.6 Hz), 52.5 (d, *J* = 24.6 Hz), 28.2, 16.3 (d, *J* = 6.5 Hz). LC-MS (ESI^+): 799.2 *m*/*z* $[2M+H]^+$.

General procedure A for synthesis of amide 10a-c and 13. To a solution of MeOH (10.1 eq) in dry CH_2Cl_2 (1 M) under N_2 was added acetyl chloride (10 eq) dropwise at room temperature and

[3] Kadi, N.; Oves-Costales, D.; Barona-Gomez, F.; Challis, G. L. A new family of ATP-dependent oligoerization-macrocyclization biocatalysts. *Nat. Chem. Biol.* 2007, **3**, 652.

[4] Jackson, E.R.; San Jose, G.; Brothers, R.C.; Edelstein, E.K.; Sheldon, Z.; Haymond, A.; Johny, C.; Boshoff, H.I.; Couch, R.D.; Dowd, C.S. The effect of chain length and unsaturation on Mtb DXR inhibition and antitubercular killing activity of FR900098 analogs. *Bioorg. Med. Chem. Lett.* 2014, **24**, 649.

the mixture was stirred for 10 min. The reaction mixture was then added a solution of **9** (1 eq) in dry CH_2Cl_2 (1 M) and stirred at room temperature for 30 min. After the completion of deprotection, dry Na_2CO_3 (12 eq) was added at 0 ^oC and the mixture was stirred at the same temperature for 10 min. The reaction mixture at 0 ^oC was added dropwise RCOCl, $(CF_3CO)_2O$ or *N*-formylimidazole* (2 eq). The mixture was then warmed up to room temperature and stirred for 30 min to 24 h, quenched with saturated $NaHCO_3$ (aq) and extracted with CH_2Cl_2 (3×). The combined organic layers were dried with anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude concentrate was then purified by column chromatography on silica gel using EtOAc and CH_2Cl_2 (with a ratio from 1/10 to 2/1) to give the pure title compound.

***N*-Formylimidazole** To a suspension of 1,1'-carbonyldiimidazole (2.1 eq) in dry CH_2Cl_2 (2 M) under N_2 was added formic acid (2 eq) slowly at room temperature. The mixture was then stirred for 30 min to give a solution of *N*-formylimidazole in CH_2Cl_2 *in situ*, which was used immediately.

Diethyl [(1*E*)-3-[*N*-(benzyloxy)formamido]prop-1-en-1-yl]phosphonate (10a) Light yellow oil (594 mg, 73%). ^1H NMR (400 MHz, $CDCl_3$) δ 8.24 (s, 1H), 7.41 – 7.29 (m, 5H), 6.72 – 6.57 (m, 1H), 5.82 (ddd, *J* = 12.4, 10.0, 6.3 Hz, 1H), 4.84 (s, 2H), 4.30 – 4.20 (m, 2H), 4.13 – 3.98 (m, 4H), 1.32 – 1.27 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.3, 144.40 (d, *J* = 5.7 Hz), 134.1, 129.4, 129.2, 128.8, 120.8 (d, *J* = 188.7 Hz), 78.3, 61.9 (d, *J* = 5.6 Hz), 47.2 (d, *J* = 26.7 Hz), 16.3 (d, *J* = 6.3 Hz). LC-MS (ESI^+): 328.2 *m*/*z* $[M+H]^+$, 655.2 *m*/*z* $[2M+H]^+$.

Diethyl [(1*E*)-3-[*N*-(benzyloxy)-2,2,2-trifluoroacetamido]prop-1-en-1-yl]phosphonate (10b) Light yellow solids (720 mg, 73%) ^1H NMR (400 MHz, $CDCl_3$) δ 7.53 – 7.30 (m, 5H), 6.66 (ddt, *J* = 21.6, 17.2, 5.2 Hz, 1H), 5.91 – 5.80 (m, 1H), 4.94 (s, 2H), 4.43 – 4.36 (m, 2H), 4.12 – 4.01 (m, 4H), 1.32 – 1.28 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.7 (q, *J* = 33.6 Hz), 142.7 (d, *J* = 5.9 Hz), 129.4, 129.2, 128.8, 121.6 (d, *J* = 187.5 Hz), 115.9 (q, *J* = 286.9 Hz), 78.4, 62.0 (d, *J* = 5.7 Hz), 49.5 (d, *J* = 24.9 Hz), 16.3 (d, *J* = 6.2 Hz). LC-MS (ESI^+): 396.2 *m*/*z* $[M+H]^+$, 791.2 *m*/*z* $[2M+H]^+$.

Methyl *N*-(benzyloxy)-*N*-[(2*E*)-3-(diethoxyphosphoryl)prop-2-en-1-yl]carbamate (10c) Light yellow oil (444 mg, 50%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.29 (m, 5H), 6.73 – 6.59 (m, 1H), 5.86 – 5.74 (m, 1H), 4.84 (s, 2H), 4.13 – 4.09 (m, 2H), 4.07 – 3.98 (m, 4H), 3.77 (s, 3H), 1.30 – 1.24 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.7, 145.8 (d, *J* = 5.3 Hz), 135.1, 129.2, 128.7, 128.4, 120.0 (d, *J* = 187.5 Hz), 77.5, 61.8 (d, *J* = 5.6 Hz), 53.4, 52.5 (d, *J* = 24.7 Hz), 16.3 (d, *J* = 6.3 Hz). LC-MS (ESI^+): 358.2 *m*/*z* $[M+H]^+$, 715.2 *m*/*z* $[2M+H]^+$.

Diethyl [(1*E*)-3-[*N*-(benzyloxy)acetamido]prop-1-en-1-yl]phosphonate (13) Light yellow oil (722 mg, 85%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.31 (m, 5H), 6.77 – 6.63 (m, 1H), 5.81 (ddt, *J* = 20.6, 17.2, 1.7 Hz, 1H), 4.83 (s, 2H), 4.38 – 4.31 (m, 2H), 4.12 – 4.01 (m, 4H), 2.14 (s, 3H), 1.30 (ddd, *J* = 5.9, 5.0, 0.4 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.5, 145.7 (d, *J* = 5.5 Hz), 134.2, 129.2, 129.1, 128.7, 119.9 (d, *J* = 187.8 Hz), 77.0, 61.9 (d, *J* = 5.6 Hz), 48.7 (d, *J* = 25.3 Hz), 20.4, 16.3 (d, *J* = 6.3 Hz). LC-MS (ESI^+): 342.2 *m*/*z* $[M+H]^+$, 683.2 *m*/*z* $[2M+H]^+$.

General procedure B for synthesis of 11a-c, 14 and 18a-c.[4] To a solution of **10**, **13** or **17** (1 eq) in dry CH_2Cl_2 (0.1 M) under N_2 was added boron trichloride (1 M in CH_2Cl_2, 4 eq) at -78 °C dropwise. The reaction mixture was stirred at -78 °C for 30 min to 3 h, quenched with saturated $NaHCO_3$ (aq) and extracted with EtOAc (5×). The combined organic layers were dried with anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was then purified by column chromatography on silica gel using EtOAc and MeOH (EtOAc and CH_2Cl_2 for **18a-c**) to give the pure title compound.

Diethyl [(1*E*)-3-(*N*-hydroxyformamido)prop-1-en-1-yl]phosphonate (11a) Light yellow oil (106 mg, 55%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.06 (s, 1H), 8.32 (s, 1H), 6.75 – 6.55 (m, 1H), 5.99 – 5.74 (m, 1H), 4.32 – 4.25 (m, 2H), 4.09 – 3.92 (m, 4H), 1.25 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.5, 162.78, 146.3 (d, *J* = 5.5 Hz), 118.7 (d, *J* = 189.1 Hz), 62.3 (d, *J* = 5.7 Hz), 48.9 (d, *J* = 25.3 Hz,), 16.17 (d, *J* = 6.4 Hz). LC-MS (ESI$^+$): 238.0 *m*/*z* $[M+H]^+$, 475.2 *m*/*z* $[2M+H]^+$.

Diethyl [(1*E*)-3-(2,2,2-trifluoro-*N*-hydroxyacetamido)prop-1-en-1-yl]phosphonate (11b) Light yellow oil (44 mg, 19%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.78 (s, 1H), 6.89 (ddt, *J* = 22.8, 17.2, 5.7 Hz, 1H), 5.94 (ddt, *J* = 18.8, 17.2, 1.5 Hz, 1H), 4.50 – 4.46 (m, 2H), 4.08 – 3.99 (m, 4H), 1.35 – 1.28 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.8 (q, *J* = 36.3 Hz), 145.8 (d, *J* = 6.0 Hz), 119.6 (d, *J* = 191.2 Hz), 116.3 (q, *J* = 286.9 Hz), 62.5 (d, *J* = 5.9 Hz), 51.8 (d, *J* = 26.5 Hz), 16.1 (d, *J* = 6.5 Hz). LC-MS (ESI$^+$): 306.0 *m*/*z* $[M+H]^+$, 611.0 *m*/*z* $[2M+H]^+$.

Methyl *N*-[(2*E*)-3-(diethoxyphosphoryl)prop-2-en-1-yl]-*N*-hydroxycarbamate (11c) Colorless oil (135 mg, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.27 (s, 1H), 6.78 (ddt, *J* = 22.2, 17.2, 4.9 Hz, 1H), 5.94 (ddt, *J* = 19.8, 17.2, 1.7 Hz, 1H), 4.35 – 4.28 (m, 2H), 4.11 – 4.03 (m, 4H), 3.76 (s, 3H), 1.32 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.9, 147.4 (d, *J* = 5.5 Hz), 118.0 (d, *J* = 189.0 Hz), 62.0 (d, *J* = 5.6 Hz), 53.2, 53.1 (d, *J* = 23.8 Hz), 16.2 (d, *J* = 6.4 Hz). LC-MS (ESI$^+$) 268 *m*/*z* $[M+H^+]$, 535 *m*/*z* $[2M+H^+]$.

Diethyl [(1*E*)-3-(*N*-hydroxyacetamido)prop-1-en-1-yl]phosphonate (14) Colorless oil (512 mg, 77%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.91 (s, 1H), 6.72 (ddt, *J* = 22.3, 17.2, 5.1 Hz, 1H), 5.96 – 5.82 (m, 1H), 4.44 – 4.39 (m, 2H), 4.09 – 4.01 (m, 4H), 2.19 (s, 3H), 1.32 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.5, 147.6 (d, *J* = 5.3 Hz), 117.9 (d, *J* = 189.0 Hz), 62.2 (d, *J* = 5.7 Hz), 50.4 (d, *J* = 25.1 Hz), 20.3, 16.2 (d, *J* = 6.4 Hz). LC-MS (ESI$^+$): 252.2 *m*/*z* $[M+H]^+$, 503.2 *m*/*z* $[2M+H]^+$.

[({[(2,2-dimethylpropanoyl)oxy]methoxy}[(1*E*)-3-(*N*-hydroxyformamido)prop-1-en-1-yl]phosphoryl)oxy]methyl 2,2-dimethylpropanoate (18a) Light yellow oil (103 mg, 55%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.41 (s, 1H), 8.42 and 7.94 (s, 1H), 6.87 – 6.69 (m, 1H), 6.07 – 5.88 (m, 1H), 5.71 – 5.59 (m, 4H), 4.37 – 4.32 (m, 2H), 1.21 (s, 18H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.1, 163.0, 157.3, 147.3 (d, *J* = 6.1 Hz), 118.6 (d, *J* = 191.8 Hz), 81.6 (d, *J* = 4.9 Hz), 48.8 (d, *J* = 26.1

Hz), 38.7, 26.8. LC-MS (ESI^+): 410.2 *m/z* $[M+H]^+$, 819.2 *m/z* $[2M+H]^+$. HRMS (ESI^+) calculated for $C_{16}H_{28}NO_9P$, 409.1502; found, 432.1388 $[M+Na]^+$.

[({[(2,2-dimethylpropanoyl)oxy]methoxy}[(1*E*)-3-(2,2,2-trifluoro-*N*-hydroxyacetamido)prop-1-en-1-yl]phosphoryl)oxy]methyl 2,2-dimethylpropanoate (18b) Light yellow oil (15 mg, 34%). ^1H NMR (400 MHz, $CDCl_3$) δ 9.59 (bs, 1H), 6.86 (ddt, *J* = 22.9, 17.2, 5.4 Hz, 1H), 6.04 – 5.89 (m, 1H), 5.69 – 5.54 (m, 4H), 4.50 – 4.45 (m, 2H), 1.21 (s, 18H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.1, 157.0 (q, *J* = 36.4 Hz), 146.2 (d, *J* = 5.8 Hz), 119.0 (d, *J* = 192.8 Hz), 116.1 (q, *J* = 286.9 Hz), 81.6 (d, *J* = 5.1 Hz), 51.4 (d, *J* = 26.7 Hz), 38.7, 26.7. LC-MS (ESI^+): 478.2 *m/z* $[M+H]^+$, 955.2 *m/z* $[2M+H]^+$. HRMS (ESI^+) calculated for $C_{16}H_{28}NO_9P$, 409.1502; found, 432.1388 $[M+Na]^+$.

[({[(2,2-dimethylpropanoyl)oxy]methoxy}[(1*E*)-3-[hydroxy(methoxycarbonyl)amino]prop-1-en-1-yl]phosphoryl)oxy]methyl 2,2-dimethylpropanoate (18c) Light yellow oil (27 mg, 34%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.98 (s, 1H), 6.88 – 6.71 (m, 1H), 6.03 – 5.88 (m, 1H), 5.68 – 5.58 (m, 4H), 4.32 – 4.27 (m, 2H), 3.75 (s, 3H), 1.20 (s, 18H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.9, 157.8, 148.1 (d, *J* = 6.0 Hz), 117.8 (d, *J* = 192.2 Hz), 81.5 (d, *J* = 5.1 Hz), 53.5, 52.8 (d, *J* = 25.6 Hz), 38.7, 26.8. LC-MS (ESI^+): 440.2 *m/z* $[M+H]^+$, 879.2 *m/z* $[2M+H]^+$. HRMS (FAB^+) calculated for $C_{17}H_{30}NO_{10}P$, 439.1607; found, 440.1671 $[M+H]^+$.

General procedure C for synthesis of 12a-b and 16d-g. To a solution of **11a-b** or **15d-g** (1 eq) in dry CH_2Cl_2 (0.1 M) under N_2 was added TMSBr (10 eq) dropwise at 0 °C. The reaction mixture was warmed to room temperature, stirred overnight, and then concentrated under reduced pressure. The mixture was dissolved in CH_2Cl_2, evaporated under reduced pressure and dried under vacuum. The crude residue was then stirred in 0.5 M NaOH (1 eq) in H_2O at room temperature for 1 h, washed with Et_2O three times and lyophilized to give the title compounds.

Sodium hydrogen [(1*E*)-3-(*N*-hydroxyformamido)prop-1-en-1-yl]phosphonate (12a) Light yellow solids (29 mg, quantitative yield). ^1H NMR (400 MHz, CD_3OD) δ 8.32 and 7.99 (s, 1H), 6.52 – 6.30 (m, 1H), 6.11 – 5.93 (m, 1H), 4.32 – 4.17 (m, 2H). ^{13}C NMR (101 MHz, CD_3OD) δ 162.6, 137.5 (d, *J* = 5.0 Hz), 127.3 (d, *J* = 178.0 Hz), 48.7 (d, *J* = 23.6 Hz). LC-MS (ESI^-): 361.0 *m/z* $[2M-2Na+H]^-$, 542.2 *m/z* $[3M-3Na+2H]^-$. HRMS (ESI^-) calculated for $C_4H_7NNaO_5P$, 202.9960; found, 180.0065 $[M-Na]^-$.

Sodium hydrogen [(1*E*)-3-(2,2,2-trifluoro-*N*-hydroxyacetamido)prop-1-en-1-yl]phosphonate (12b) Light yellow solids (22 mg, quantitative yield). Rotamers with a ratio of 3:1. ^1H NMR (400 MHz, CD_3OD) δ 6.71 – 6.52 (m, 1H), 6.31 (t, *J* = 17.4 Hz, 0.75H), 6.01 (t, *J* = 17.4 Hz, 0.25H), 4.48-4.36 (m, 0.5H), 4.08 – 3.97 (m, 1.5H). ^{13}C NMR (101 MHz, CD_3OD) δ 156.5 (q, *J* = 35.9 Hz), 155.0 (q, *J* = 34.6 Hz), 140.0, 134.6, 129.2 (d, *J* = 183.2 Hz), 123.1 (d, *J* = 184.2 Hz), 116.4 (q, *J* = 286.2 Hz), 111.3 (q, *J* = 285.5 Hz), 52.3 (d, *J* = 25.2 Hz), 51.3 (d, *J* = 25.2 Hz). LC-MS (ESI^-): 248.0 *m/z* $[M-Na]^-$, 497.0 *m/z* $[2M-2Na+H]^-$. HRMS (ESI^-) calculated for $C_5H_6F_3NNaO_5P$,

270.9833; found, 247.9937 $[M-Na]^-$.

Sodium hydrogen [(1*E*)-3-[*N*-(1-phenylethoxy)acetamido]prop-1-en-1-yl]phosphonate (16d) White solids (28 mg, 77%). ^{1}H NMR (400 MHz, D_2O) δ 7.55 – 7.42 (m, 5H), 6.32 (ddt, *J* = 22.2, 17.3, 5.0 Hz, 1H), 5.91 – 5.76 (m, 1H), 5.13 (q, *J* = 6.6 Hz, 1H), 4.41 – 4.30 (m, 1H), 4.10 – 3.98 (m, 1H), 2.04 (s, 3H), 1.61 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (101 MHz, D_2O) δ 173.1, 140.0, 139.4, 129.3, 128.9, 127.9, 123.9 (d, *J* = 179.3 Hz), 83.3, 49.5, 19.8, 19.0. LC-MS (ESI^+): 599.2 *m*/*z* $[2M-2Na+3H]^+$. HRMS (FAB^+) calculated for $C_{13}H_{17}NNaO_5P$, 321.0742; found, 322.0824 $[M+H]^+$.

Sodium hydrogen [(1*E*)-3-(*N*-{[4-(propan-2-yl)phenyl]methoxy}acetamido)prop-1-en-1-yl]phosphonate (16e) White solids (22 mg, 51%). ^{1}H NMR (400 MHz, DMSO) δ 7.38 – 7.13 (m, 4H), 6.21 – 6.03 (m, 1H), 5.87 – 5.68 (m, 1H), 4.82 (s, 2H), 4.32 – 4.19 (m, 2H), 2.93 – 2.80 (m, 1H), 2.01 (s, 3H), 1.18 (d, *J* = 6.8 Hz, 6H). ^{13}C NMR (101 MHz, DMSO) δ 171.6, 149.4, 136.9, 130.2, 128.6, 127.7 (d, *J* = 189.8 Hz), 126.4, 75.9, 47.7, 33.7, 24.3. LC-MS (ESI^+): 328.2 *m*/*z* $[M-Na+2H]^+$, 350.2 *m*/*z* $[M+H]^+$. HRMS (FAB^+) calculated for $C_{15}H_{21}NNaO_5P$, 349.1055; found, 350.1130 $[M+H]^+$.

Sodium hydrogen [(1*E*)-3-{*N*-[(naphthalen-2-yl)methoxy]acetamido}prop-1-en-1-yl]phosphonate (16f) White solids (20 mg, 95%). ^{1}H NMR (400 MHz, D_2O) δ 7.94 – 7.74 (m, 4H), 7.61 – 7.39 (m, 3H), 6.28 – 6.07 (m, 1H), 5.90 – 5.71 (m, 1H), 4.97 (s, 2H), 4.36 – 4.16 (m, 2H), 1.97 (s, 3H). ^{13}C NMR (101 MHz, D_2O) δ 174.5, 135.9, 133.0, 132.6, 131.1, 129.2, 128.3, 127.9, 127.5, 127.3 (d, *J* = 162.2 Hz), 127.0, 126.8, 126.4, 76.5, 48.3 (d, *J* = 22.0 Hz), 19.2. LC-MS (ESI^+): 336.0 *m*/*z* $[M-Na+2H]^+$, 671.2 *m*/*z* $[2M-2Na+3H]^+$. HRMS (FAB^+) calculated for $C_{16}H_{17}NNaO_5P$, 357.0742; found, 358.0820 $[M+H]^+$.

Sodium hydrogen [(1*E*)-3-{*N*-[(4-phenylphenyl)methoxy]acetamido}prop-1-en-1-yl]phosphonate (16g) White solids (24 mg, 66%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.67 – 7.58 (m, 4H), 7.54 – 7.30 (m, 5H), 6.64 – 6.45 (m, 1H), 6.08 – 5.89 (m, 1H), 4.95 (s, 2H), 4.46 – 4.38 (m, 2H), 2.12 (s, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 173.2, 141.7, 140.8, 140.3, 133.5, 129.9, 128.5, 127.2, 126.8, 126.6, 123.8 (d, *J* = 182.8 Hz), 76.0, 47.7, 19.1. LC-MS (ESI^-): 721.0 *m*/*z* $[2M-2Na+H]^-$. HRMS (FAB^+) calculated for $C_{18}H_{19}NNaO_5P$, 383.0899; found, 384.0974 $[M+H]^+$.

Diammonium [(1*E*)-3-[hydroxy(methoxycarbonyl)amino]prop-1-en-1-yl]phosphonate (12c) To a solution of **11c** (1 eq) in dry CH_2Cl_2 (0.1 M) under N_2 was added TMSBr (10 eq) dropwise at 0 ^oC. The reaction mixture was warmed to room temperature, stirred overnight, and then concentrated under reduced pressure. The mixture was dissolved in CH_2Cl_2, evaporated under reduced pressure and dried *in vacuo*. The crude residue was then stirred in 5% NH_4OH in H_2O at room temperature for 1 h, washed with Et_2O (3×) and lyophilized to give the title compounds.

Light yellow solids (30 mg, quantitative yield). ^{1}H NMR (400 MHz, D_2O) δ 6.40 – 6.06 (m, 1H), 6.01 – 5.67 (m, 1H), 4.20 – 4.00 (m, 2H), 3.62 (s, 3H). ^{13}C NMR (101 MHz, D_2O) δ 158.6, 138.0 (d, *J* = 4.7 Hz), 126.0 (d, *J* = 175.2 Hz), 53.6, 52.9 (d, *J* = 22.9 Hz). LC-MS (ESI$^-$): 209.8 *m/z* [M-2NH_4+H]$^-$, 421.0 *m/z* [2M-4NH_4+3H]$^-$. HRMS (ESI$^-$) calculated for $C_5H_{16}N_3O_6P$, 245.0777; found, 210.0169 [M-2NH_4+H]$^-$.

General procedure D for synthesis of 15d-g. To a solution of **14** (1 eq) in dry CH_2Cl_2 (0.1 M) in a pressure tube under N_2 was added dry Na_2CO_3 (2 eq), RCH_2Br or $RCH(CH_3)Br$ (1.2 eq) and NaI (0.1 eq). The reaction mixture was then sealed and stirred at 60 ^{o}C for 48 h, filtered and concentrated under reduced pressure. The crude residue was then purified by column chromatography on silica gel using EtOAc and CH_2Cl_2 to give the pure title compound.

Diethyl [(1*E*)-3-[*N*-(1-phenylethoxy)acetamido]prop-1-en-1-yl]phosphonate (15d) Light yellow oil (63 mg, 25%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.25 (m, 5H), 6.50 (ddt, *J* = 22.1, 17.2, 5.2 Hz, 1H), 5.68 – 5.52 (m, 1H), 4.84 – 4.72 (m, 1H), 4.29 (m, 1H), 4.08 – 3.94 (m, 4H), 3.74 – 3.63 (m, 1H), 2.02 (s, 3H), 1.52 (d, *J* = 6.6 Hz, 3H), 1.29 – 1.23 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.4, 145.8 (d, *J* = 5.4 Hz), 140.1, 128.9, 128.6, 127.2, 119.4 (d, *J* = 187.8 Hz), 83.3, 61.8 (d, *J* = 5.6 Hz), 49.5 (d, *J* = 25.0 Hz), 20.6, 20.5, 16.3 (d, *J* = 6.3 Hz). LC-MS (ESI$^+$): 356.2 *m/z* [M+H] $^+$, 711.2 *m/z* [2M+H]$^+$.

Diethyl [(1*E*)-3-(*N*-{[4-(propan-2-yl)phenyl]methoxy}acetamido)prop-1-en-1-yl]phosphonate (15e) Light yellow oil (56 mg, 26%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 – 7.16 (m, 4H), 6.66 (ddt, *J* = 22.2, 17.2, 5.3 Hz, 1H), 5.77 (ddt, *J* = 18.8, 17.2, 1.5 Hz, 1H), 4.75 (s, 2H), 4.34 – 4.25 (m, 2H), 4.08 – 3.95 (m, 4H), 2.93 – 2.81 (m, 1H), 2.08 (s, 3H), 1.26 (t, *J* = 7.1 Hz, 6H), 1.21 (d, *J* = 6.9 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.8, 150.0, 145.7 (d, *J* = 5.4 Hz), 131.5, 129.3, 126.7, 119.9 (d, *J* = 187.8 Hz), 76.8, 61.8 (d, *J* = 5.6 Hz), 48.5, 33.9, 23.8, 20.3, 16.3 (d, *J* = 6.4 Hz). LC-MS (ESI$^+$): 384.2 *m/z* [M+H]$^+$.

Diethyl [(1*E*)-3-{*N*-[(naphthalen-2-yl)methoxy]acetamido}prop-1-en-1-yl]phosphonate (15f) Light yellow oil (76 mg, 42%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 – 7.78 (m, 4H), 7.56 – 7.41 (m, 3H), 6.72 (ddt, *J* = 22.4, 17.2, 5.2 Hz, 1H), 5.83 (ddt, *J* = 17.2, 12.2, 1.7 Hz, 1H), 5.00 (s, 2H), 4.39 – 4.34 (m, 2H), 4.11 – 4.02 (m, 4H), 2.17 (s, 3H), 1.32 – 1.27 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.8, 145.7 (d, *J* = 5.5 Hz), 133.4, 133.1, 131.6, 128.6, 128.6, 128.0, 127.7, 126.7, 126.5, 126.3, 120.0 (d, *J* = 187.8 Hz), 77.2, 61.9 (d, *J* = 5.5 Hz), 48.8 (d, *J* = 23.2 Hz), 20.5, 16.3 (d, *J* = 6.3 Hz). LC-MS (ESI$^+$): 392.2 *m/z* [M+H$^+$], 783.2 *m/z* [2M+H$^+$].

Diethyl [(1*E*)-3-{*N*-[(4-phenylphenyl)methoxy]acetamido}prop-1-en-1-yl]phosphonate (15g) Light yellow oil (158 mg, 38%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 – 7.55 (m, 4H), 7.47 – 7.32 (m, 5H), 6.70 (ddt, *J* = 22.4, 17.2, 5.3 Hz, 1H), 5.87 – 5.76 (m, 1H), 4.86 (s, 2H), 4.39 – 4.32 (m, 2H), 4.10 – 4.00 (m, 4H), 2.15 (s, 3H), 1.32 – 1.26 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.0, 145.7

(d, J = 5.4 Hz), 142.0, 140.3, 133.1, 129.6, 128.8, 127.6, 127.4, 127.1, 120.0 (d, J = 187.9 Hz), 76.8, 61.9 (d, J = 5.5 Hz), 48.8, 20.4, 16.3 (d, J = 6.3 Hz). LC-MS (ESI^+): 418.2 m/z $[M+H]^+$, 835.2 m/z $[2M+H]^+$.

General procedure E for synthesis of 17a-c. To a solution of **10a-c** (1 eq) in dry CH_2Cl_2 (0.1 M) under N_2 was added TMSBr (10 eq) dropwise at 0 ^oC. The reaction mixture was warmed to room temperature, stirred overnight, and then concentrated under reduced pressure. The mixture was dissolved in CH_2Cl_2, evaporated under reduced pressure and dried under vacuum. The crude residue was then stirred in 0.5 M NaOH (2 eq) in H_2O at room temperature for 1 h, washed with Et_2O (3×) and lyophilized to give disodium salts as white solids. The crude solid was then dissolved in dry DMF (0.1 M), and TEA (6 eq), chloromethylpivalate (6 eq) and NaI (0.1 eq) were added. The reaction mixture was stirred at 60 ^oC for 24 h, quenched with H_2O, and extracted with Et_2O (3×). The combined organic layers were dried with anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was then purified by column chromatography on silica gel using hexanes and EtOAc or CH_2Cl_2 and EtOAc to give the pure title compound.

({[(1*E*)-3-[*N*-(benzyloxy)formamido]prop-1-en-1-yl]({[(2,2-dimethylpropanoyl)oxy]methoxy})phosphoryl}oxy)methyl 2,2-dimethylpropanoate (17a) Light yellow oil (38 mg, 9%). ^1H NMR (400 MHz, $CDCl_3$) δ 8.23 (s, 1H), 7.43 – 7.30 (m, 5H), 6.72 (ddt, J = 22.4, 17.2, 5.1 Hz, 1H), 5.96 – 5.82 (m, 1H), 5.66 (dd, J = 13.1, 0.8 Hz, 4H), 4.84 (s, 2H), 4.30 – 4.20 (m, 2H), 1.21 (s, 18H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.7, 163.2, 146.0 (d, J = 6.0 Hz), 129.9, 129.5, 129.3, 128.8, 119.4 (d, J = 193.0 Hz), 81.5 (d, J = 5.4 Hz), 78.3, 47.0 (d, J = 25.8 Hz), 38.7, 26.8. LC-MS (ESI^+): 500.2 m/z $[M+H]^+$, 999.2 m/z $[2M+H]^+$.

({[(1*E*)-3-[*N*-(benzyloxy)-2,2,2-trifluoroacetamido]prop-1-en-1-yl]({[(2,2-dimethylpropanoyl)oxy]methoxy})phosphoryl}oxy)methyl 2,2-dimethylpropanoate (17b) Light yellow oil (6 mg, 1%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.28 (m, 5H), 6.71 (ddt, J = 22.4, 17.2, 5.1 Hz, 1H), 5.95 – 5.80 (m, 1H), 5.64 (d, J = 13.0 Hz, 4H), 4.92 (s, 2H), 4.44 – 4.31 (m, 2H), 1.18 (s, 18H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.7, 144.4 (d, J = 6.2 Hz), 129.4, 129.2, 128.8, 120.0 (d, J = 192.2 Hz), 115.9 (q, J = 286.7 Hz), 81.5 (d, J = 5.3 Hz), 78.5, 49.5 (d, J = 26.8 Hz), 38.7, 26.7. LC-MS (ESI^+): 568.2 m/z $[M+H]^+$.

({[(1*E*)-3-[(benzyloxy)(methoxycarbonyl)amino]prop-1-en-1-yl]({[(2,2-dimethylpropanoyl)oxy]methoxy})phosphoryl}oxy)methyl 2,2-dimethylpropanoate (17c) Light yellow oil (95 mg, 30%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.28 (m, 5H), 6.79 – 6.64 (m, 1H), 5.89 – 5.77 (m, 1H), 5.67 – 5.58 (m, 4H), 4.83 (s, 2H), 4.10 – 4.06 (m, 2H), 3.76 (s, 3H), 1.17 (s, 18H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.7, 157.6, 147.4 (d, J = 5.6 Hz), 135.0, 129.4, 128.7, 128.5, 118.5 (d, J = 192.6 Hz), 81.5 (d, J = 5.5 Hz), 77.6, 53.4, 52.4 (d, J = 26.1 Hz), 38.7, 26.8. LC-MS (ESI^+): 530.2 m/z $[M+H]^+$.

General procedure F for synthesis of 19e-g. To a solution of **16e-g** (1 eq) in dry DMF (0.1 M) was added TEA (6 eq), chloromethylpivalate (6 eq) and NaI (0.1 eq). The reaction mixture was stirred at 60 ^{o}C for 24 h, quenched with H_2O, and extracted with Et_2O (3×). The combined organic layers were dried with anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was then purified by column chromatography on silica gel using hexanes and EtOAc or CH_2Cl_2 and EtOAc to give the pure title compound.

[({[(2,2-dimethylpropanoyl)oxy]methoxy}[(1*E*)-3-(*N*-{[4-(propan-2-yl)phenyl]methoxy}acetamido)prop-1-en-1-yl]phosphoryl)oxy]methyl 2,2-dimethylpropanoate (19e) Light yellow oil (10.6 mg, 8%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.16 (m, 4H), 6.83 – 6.67 (m, 1H), 5.89 – 5.77 (m, 1H), 5.68 – 5.59 (m, 4H), 4.76 (s, 2H), 4.36 – 4.29 (m, 2H), 2.97 – 2.83 (m, 1H), 2.10 (s, 3H), 1.23 (d, *J* = 6.9 Hz, 6H), 1.18 (s, 18H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.8, 150.1, 147.3 (d, *J* = 5.7 Hz), 131.3, 129.4, 126.8, 118.5 (d, *J* = 192.7 Hz), 81.4 (d, *J* = 5.4 Hz), 76.9, 48.3 (d, *J* = 26.3 Hz), 38.7, 33.9, 26.8, 23.9, 20.3. LC-MS (ESI$^+$): 556.2 *m/z* [M+H]$^+$. HRMS (FAB$^+$) calculated for $C_{27}H_{42}NO_9P$, 555.2597; found, 556.2663 [M+H]$^+$.

[({[(2,2-dimethylpropanoyl)oxy]methoxy}[(1*E*)-3-{*N*-[(naphthalen-2-yl)methoxy]acetamido}prop-1-en-1-yl]phosphoryl)oxy]methyl 2,2-dimethylpropanoate (19f) Light yellow oil (16 mg, 12%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 – 7.76 (m, 4H), 7.54 – 7.40 (m, 3H), 6.76 (ddt, *J* = 22.5, 17.3, 5.1 Hz, 1H), 5.91 – 5.79 (m, 1H), 5.67 – 5.59 (m, 4H), 4.97 (s, 2H), 4.34 – 4.29 (m, 2H), 2.14 (s, 3H), 1.17 (s, 18H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.7, 147.3 (d, *J* = 5.7 Hz), 133.4, 133.1, 131.6, 128.7, 128.6, 128.0, 127.7, 126.7, 126.5, 126.3, 118.7 (d, *J* = 192.6 Hz), 81.5 (d, *J* = 5.4 Hz), 77.4, 48.8 (d, *J* = 25.0 Hz), 38.7, 26.8, 20.4. LC-MS (ESI$^+$): 564.2 *m/z* [M+H]$^+$. HRMS (FAB$^+$) calculated for $C_{28}H_{38}NO_9P$, 563.2284; found, 564.2363 [M+H]$^+$.

[({[(2,2-dimethylpropanoyl)oxy]methoxy}[(1*E*)-3-{*N*-[(4-phenylphenyl)methoxy]acetamido}prop-1-en-1-yl]phosphoryl)oxy]methyl 2,2-dimethylpropanoate (19g) Light yellow oil (30 mg, 15%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65-7.55 (m, 4H), 7.48-7.32 (m, 5H), 6.78 (ddt, *J* = 22.3, 17.2, 5.1 Hz, 1H), 5.87 (ddt, *J* = 17.2, 12.7, 1.6 Hz, 1H), 5.68 – 5.61 (m, 4H), 4.85 (s, 2H), 4.38 – 4.32 (m, 2H), 2.15 (s, 3H), 1.19 (s, 18H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.7, 147.3 (d, *J* = 5.7 Hz), 142.1, 140.3, 133.0, 129.7, 128.8, 127.6, 127.5, 127.1, 118.7 (d, *J* = 192.7 Hz), 81.5 (d, *J* = 5.4 Hz), 77.3, 48.6, 38.7, 26.8, 20.4. LC-MS (ESI$^+$): 590.2 *m/z* [M+H]$^+$. HRMS (FAB$^+$) calculated for $C_{30}H_{40}NO_9P$, 589.2441; found, 590.2506 [M+H]$^+$.

[({[(2,2-dimethylpropanoyl)oxy]methoxy}(prop-2-en-1-yl)phosphoryl)oxy]methyl 2,2-dimethylpropanoate (20) To a solution of **6** (5 g, 28 mmol, 1 eq) in dry CH_2Cl_2 (30 mL) under N_2 at 0 ^{o}C was added TMSBr (14.8 mL, 112 mmol, 4 eq) dropwise. The reaction mixture was warmed to room temperature and stirred overnight, and concentrated under reduced pressure. The mixture was dissolved in CH_2Cl_2, evaporated under reduced pressure and dried under vacuum. The crude

material was then stirred in CH_3OH (93 mL) at room temperature for 1 h, and concentrated under reduced pressure. The crude product was then dissolved in dry DMF (93 mL), to which was added TEA (6 eq), chloromethylpivalate (6 eq) and NaI (0.1 eq). The reaction mixture was stirred at 60 ^oC for 72 h, quenched with H_2O (100 mL), and extracted with Et_2O (3 × 100 mL). The combined organic layers were dried with anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was then purified by column chromatography on silica gel (hexanes/EtOAc = 3/1 to 1/1) to give the pure title compound as a colorless oil (5.1 g, 52%) with identical NMR spectroscopic data to that reported previously.[5] ^1H NMR (400 MHz, $CDCl_3$) δ 5.78 – 5.62 (m, 5H), 5.28 – 5.20 (m, 2H), 2.70 (dd, *J* = 22.6, 7.3 Hz, 2H), 1.23 (s, 18H). LC-MS (ESI^+): 351.2 *m*/*z* $[M+H]^+$, 701.2 *m*/*z* $[2M+H]^+$.

{[(2,3-dibromopropyl)({[(2,2-dimethylpropanoyl)oxy]methoxy})phosphoryl]oxy}{[(2,3-dibromopropyl)({[(2,2-dimethylpropanoyl)oxy]methoxy})phosphoryl]oxy}methyl 2,2-dimethylpropanoate (21) To a solution of **20** (5.1 g, 14.6 mmol, 1 eq) in dry CH_2Cl_2 (100 mL) under N_2 at 0 ^oC was added Bromine (0.9 mL, 17.5 mmol, 1.2 eq) dropwise. The reaction mixture was stirred at room temperature for 1 h, quenched with saturated Na_2SO_3 (aq, 100 mL) and extracted with CH_2Cl_2 (3 × 150 mL). The combined organic layers were dried with anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Chromatographic separation on silica gel (hexanes/EtOAc = 3/1) gave the title compound as a colorless oil (5.8 g, 78%). ^1H NMR (400 MHz, $CDCl_3$) δ 5.81 – 5.51 (m, 4H), 4.45 – 4.32 (m, 1H), 3.94 – 3.86 (m, 1H), 3.78 – 3.68 (m, 1H), 2.89 (ddd, *J* = 19.6, 15.9, 5.7 Hz, 1H), 2.54 – 2.41 (m, 1H), 1.23 (s, 18H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.8 (d, *J* = 1.2 Hz), 81.6 (dd, *J* = 6.2, 1.2 Hz), 42.2 (d, *J* = 3.2 Hz), 38.7, 37.4 (d, *J* = 12.8 Hz), 34.6 (d, *J* = 142.9 Hz), 26.8. LC-MS (ESI^+): 511.0 *m*/*z* $[M+H]^+$.

({[(1*E*)-3-bromoprop-1-en-1-yl]({[(2,2-dimethylpropanoyl)oxy]methoxy})phosphoryl}oxy)methyl 2,2-dimethylpropanoate (22) To a suspension of NaH (121 mg, 2.8 mmol, 1.2 eq) in dry THF (10 mL) at 0 ^oC was added **21** (1.2 g, 2.4 mmol, 1 eq) in dry THF (5 mL) dropwise. The reaction mixture was warmed to room temperature and stirred overnight, quenched with saturated aqueous $NaHCO_3$ (30 mL), and extracted with EtOAc (3 × 30 mL). The combined organic layers were dried with anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Chromatographic separation on silica gel (hexanes/EtOAc = 5/1 to 2/1) gave the title compound as a colorless oil (704 mg, 70%). ^1H NMR (400 MHz, $CDCl_3$) δ 6.93 – 6.76 (m, 1H), 6.04 – 5.91 (m, 1H), 5.71 – 5.62 (m, 4H), 4.00 – 3.96 (m, 2H), 1.21 (s, 18H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.7, 146.8 (d, *J* = 7.0 Hz), 120.4 (d, *J* = 191.5 Hz), 81.5 (d, *J* = 5.4 Hz), 38.7, 26.8. LC-MS (ESI^+): 429.0, 431.0 *m*/*z* $[M+H]^+$.

***O*-Benzylhydroxylamine (23)** To a suspension of *O*-benzylhydroxylamine hydrochloride (5 g, 31

[5] Pradère, U.; Clavier, H.; Roy, V.; Nolan, S. P.; Agrofoglio, L. A. The Shortest Strategy for Generating Phosphonate Prodrugs by Olefin Cross-Metathesis – Application to Acyclonucleoside Phosphonates. *European Journal of Organic Chemistry* **2011,** 2011, 7324-7330.

mmol, 1 eq) in Et_2O (125 mL) was added 5% aqueous NaOH (45 mL). The reaction mixture was stirred at room temperature for 30 min. The organic layer was separated and the aqueous layer was extracted with Et_2O (2 × 50 mL). The combined organic layers were dried with anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to afford the title compound as a colorless liquid (3.8 g, 97%) with identical NMR spectroscopic data to that reported previously.[6] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40-7.27 (m, 5H), 5.39 (bs, 2H), 4.69 (s, 2H). GC-MS (EI) 123 *m/z* [M].

({[(1*E*)-3-[(benzyloxy)amino]prop-1-en-1-yl]({[(2,2-dimethylpropanoyl)oxy]methoxy})phosphoryl}oxy)methyl 2,2-dimethylpropanoate (24) To a solution of **22** (2.8 g, 6.5 mmol, 1 eq) in dry THF (50 mL) was added **23** (980 mg, 8 mmol, 1.2 eq) and TEA (1.73 mL, 13 mmol, 2 eq). The reaction mixture was stirred at reflux for 3 h, and then concentrated under reduced pressure. Chromatographic separation on silica gel (hexanes/EtOAc = 2/1 to 1/2) gave the title compound as a colorless oil (1.0 g, 34%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.26 (m, 5H), 6.87 (ddt, 1H), 6.02 – 5.85 (m, 1H), 5.70 – 5.62 (m, 4H), 4.68 (s, 2H), 3.67 – 3.63 (m, 2H), 1.21 (s, 18H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.8, 150.1 (d, *J* = 5.3 Hz), 137.5, 128.4, 128.4, 128.0, 117.8 (d, *J* = 192.1 Hz), 81.5 (d, *J* = 5.5 Hz), 76.4, 54.0 (d, *J* = 24.2 Hz), 38.7, 26.8. LC-MS (ESI^+): 472.2 *m/z* $[M+H]^+$, 943.2 *m/z* $[2M+H]^+$.

Optimized synthesis for ({[(1*E*)-3-[*N*-(benzyloxy)formamido]prop-1-en-1-yl]({[(2,2-dimethylpropanoyl)oxy]methoxy})phosphoryl}oxy)methyl 2,2-dimethylpropanoate (17a) To a suspension of 1,1'-carbonyldiimidazole (688 mg, 4.3 mmol, 2 eq) in CH_2Cl_2 (3 mL) was added formic acid (0.16 mL, 4.3 mmol, 2 eq) dropwise at room temperature. The mixture was stirred at room temperature for 5 min, and then transferred dropwise to a solution of **24** (1.0 g, 2.1 mmol, 1 eq) and TEA (0.85 mL, 6.4 mmol, 3 eq) in CH_2Cl_2 (15 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min, quenched with saturated aqueous $NaHCO_3$ (30 mL) and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried with anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Chromatographic separation on silica gel (hexanes/EtOAc = 2/1 to 1/3) gave the title compound as a light yellow oil (862 mg, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (s, 1H), 7.43 – 7.30 (m, 5H), 6.72 (ddt, *J* = 22.4, 17.2, 5.1 Hz, 1H), 5.96 – 5.82 (m, 1H), 5.66 (dd, *J* = 13.1, 0.8 Hz, 4H), 4.84 (s, 2H), 4.30 – 4.20 (m, 2H), 1.21 (s, 18H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.7, 163.2, 146.0 (d, *J* = 6.0 Hz), 129.9, 129.5, 129.3, 128.8, 119.4 (d, *J* = 193.0 Hz), 81.5 (d, *J* = 5.4 Hz), 78.3, 47.0 (d, *J* = 25.8 Hz), 38.7, 26.8. LC-MS (ESI^+): 500.2 *m/z* $[M+H]^+$, 999.2 *m/z* $[2M+H]^+$.

Optimized synthesis for ({[(1*E*)-3-[*N*-(benzyloxy)-2,2,2-trifluoroacetamido]prop-1-en-1-yl]({[(2,2-dimethylpropanoyl)oxy]methoxy})phosphoryl}oxy)methyl 2,2-dimethylpropanoate (17b) To a solution of **24** (60

[6] Janza, B.; Studer, A. Stereoselective Cyclization Reactions of IBX-Generated Alkoxyamidyl Radicals. *The Journal of Organic Chemistry* **2005,** 70, 6991-6994.

mg, 0.13 mmol, 1 eq) in CH_2Cl_2 (2 mL) at 0 °C was added TEA (0.025 mL, 0.19 mmol, 1.5 eq) and trifluoroacetic anhydride (0.02 mL, 0.15 mmol, 1.2 eq) dropwise. The reaction mixture was stirred at 0 °C for 15 min, quenched with saturated aqueous $NaHCO_3$ (3 mL), and extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were dried with anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Chromatographic separation on silica gel (hexanes/EtOAc = 5/1 to 1/1) gave the title compound as a white solid (53 mg, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.28 (m, 5H), 6.71 (ddt, *J* = 22.4, 17.2, 5.1 Hz, 1H), 5.95 – 5.80 (m, 1H), 5.64 (d, *J* = 13.0 Hz, 4H), 4.92 (s, 2H), 4.44 – 4.31 (m, 2H), 1.18 (s, 18H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.7, 144.4 (d, *J* = 6.2 Hz), 129.4, 129.2, 128.8, 120.0 (d, *J* = 192.2 Hz), 115.9 (q, *J* = 286.7 Hz), 81.5 (d, *J* = 5.3 Hz), 78.5, 49.5 (d, *J* = 26.8 Hz), 38.7, 26.7. LC-MS (ESI$^+$): 568.2 *m*/*z* $[M+H]^+$.

Table S1. Inhibition of diethyl phosphonate analogs against *P. falciparum*

Compound	*P.f.* IC_{50} [μM]
1a	0.634 ± 0.103
11a	>250
11b	>250
11c	>250
15d	66.3 ± 12.5
15e	55.1 ± 2.7
15f	75.7 ± 6.3
15g	32.8 ± 1.3

Pf = *P. falciparum*; IC_{50} = concentration giving 50% inhibition of *Pf* growth

Bacterial strains and growth conditions

Recombinant protein was expressed in *Escherichia coli* Rosetta2(DE3) cells obtained from Novagen (San Diego, CA). *E. coli* was cultured at 37°C in Luria-Bertani (LB) media supplemented with 100 µg/mL ampicillin and 34 µg/ml chloramphenicol with constant shaking at 250 rpm. Agar (1.5% wt/vol) was added to prepare solid media.

***Cloning, expression, and purification of P. falciparum* DXR**

The *P. falciparum dxr* gene was truncated to begin at Lys 75 to remove the apicoplast signaling sequence. A *P. falciparum* 3D7 trophozoite cDNA library (MRA-297) was acquired from BEI resources and used as the template for amplification of the *Pf dxr* gene. The gene was PCR amplified using primers 5' CACC AAG AAA CCA ATT AAT GTA GCA 3' forward and 5' CTA TAG AGA ATT ATG TTT GTT GTA TAT ATC GGT AG 3' reverse and cloned into a pET100/D-TOPO vector to yield pPfDXR, facilitating the expression of an N-terminal His_6-tagged protein.

The expression plasmid (pPfDXR) was transformed into chemically competent *E. coli* Rosetta2(DE3) cells for protein expression. To express the His-tagged protein, a 10 mL overnight seed culture was added to 1 L of LB media and then incubated with shaking at 37 °C and 250 rpm. At an OD_{600} of 1.8, protein expression was induced with addition of isopropyl b-D-thiogalactopyranoside (IPTG) to 0.5 mM and the culture was further incubated with shaking at 37 °C and 250 rpm for an additional 18 h. Cells were harvested via centrifugation (4648 x g, 20 min, 4 °C) and stored at -80 °C. Protein was subsequently isolated and purified from the cells via chemical lysis and affinity chromatography.

Cells were lysed with lysis buffer A (100 mM Tris pH 8.0, 0.032% lysozyme, 3 mL per gram cell pellet), followed by lysis buffer B (0.1 M $CaCl_2$, 0.1 M $MgCl_2$, 0.1 M NaCl, 0.020% DNase, 0.3 mL per gram cell pellet). Clarified cell lysate was collected after centrifugation (48,000 x g, 20 min, 4 °C) and passed through a TALON immobilized metal affinity column (Clontech Laboratories, Mountain View, CA).

The column was washed with 20 column volumes of 1x equilibrium buffer (50 mM HEPES pH 7.5, 300 mM NaCl), 10 column volumes of 1x wash buffer (50 mM HEPES pH 7.5, 300 mM NaCl, 10 mM imidazole), and 15 column volumes of 2x wash buffer (100 mM HEPES pH 7.5, 600 mM NaCl, 20 mM imidazole). The protein was eluted with 5 column volumes of 1x elution buffer (150 mM imidazole pH 7.0, 300 mM NaCl). Buffer was exchanged with 0.1 M Tris pH 7.5, 1 mM NaCl, 5 mM DTT during concentration by ultrafiltration. Protein concentration was determined using Advanced Protein Assay Reagent (Cytoskeleton, Denver CO) with γ-globulins (Sigma-Aldrich) as the standard. Purified protein was visualized via Coomassie stained SDS-PAGE. The yield of PfDXR averages 1 mg per 1 L shake flask.

P. falciparum* DXR *Kinetic Characterization

To determine the apparent K_M for DOXP, 120 µL assay solutions contained 100 mM Tris pH 7.8, 25 mM $MgCl_2$, 150 mM NADPH, 0.86 µM Pf DXR, and variable concentrations of DOXP. The assay solution was incubated at 37 °C for 10 min prior to addition of DOXP, to facilitate the

association of NADPH with the enzyme. To determine the apparent K_M for NADPH, assays were performed with a fixed DOXP concentration (0.4 mM) and a variable concentration of NADPH. Nonlinear regression fitting of enzyme velocity versus substrate concentration was used to determine the apparent kinetic constants (Figure S1).

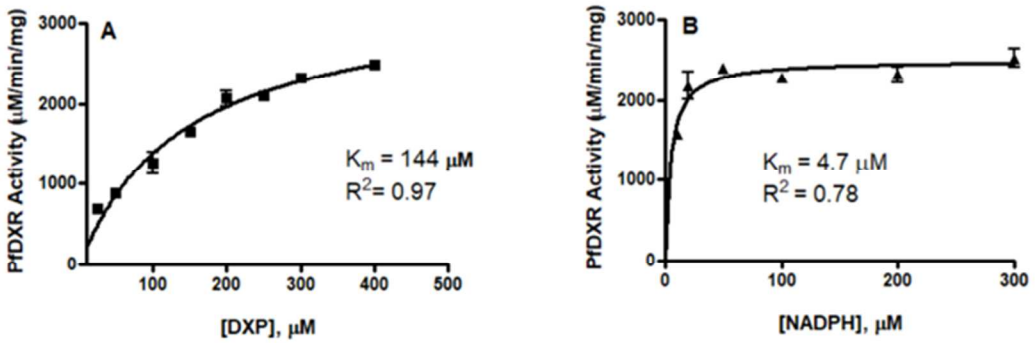

Figure S1. The substrate dependent catalytic activity of Pf DXR. Shown are Michaelis-Menten plots of reaction velocity as a function of A) DOXP concentration and B) NADPH concentration. Least-squares best fit of the data to the Michaelis-Menten equation produces the kinetic parameters listed in Table 1. The R^2 value for each plot is indicated. All assays were performed in duplicate.

P. falciparum culture

P. falciparum strain 3D7 (wild-type, WT) was obtained through MR4 as part of the BEI Resources Repository, NIAID, NIH (www.mr4.org). A *P. falciparum* strain containing increased levels of MEP pathway metabolites, *had1* (MRA-1257), and its isogenic compliment, *had1* + PfHad1-GFP (MRA-1258), were generated in strain 3D7, as reported.[7] Parasites were cultured in a 2% suspension of human erythrocytes and RPMI 1640 (Sigma) medium supplemented with 27 mM sodium bicarbonate, 11 mM glucose, 5 mM HEPES, 1 mM sodium pyruvate, 0.37 mM hypoxanthine, 0.01 mM thymidine, 10 μg/mL gentamicin, and 0.5% Albumax (Gibco) at 37 °C, 5% O_2/5% CO_2/90% N_2 atmosphere as previously described.[8]

[7] Trager, W. & Jensen, J. Human malaria parasites in continuous culture. *Science.* **193,** 673–675 (1976); Zhang, B. *et al.* A second target of the antimalarial and antibacterial agent fosmidomycin revealed by cellular metabolic profiling. *Biochemistry* **50,** 3570–3577 (2011).

[8] Trager, W. & Jensen, J. Human malaria parasites in continuous culture. *Science.* **193,** 673–675 (1976); Zhang, B. *et al.* A second target of the antimalarial and antibacterial agent fosmidomycin revealed by cellular metabolic profiling. *Biochemistry* **50,** 3570–3577 (2011).

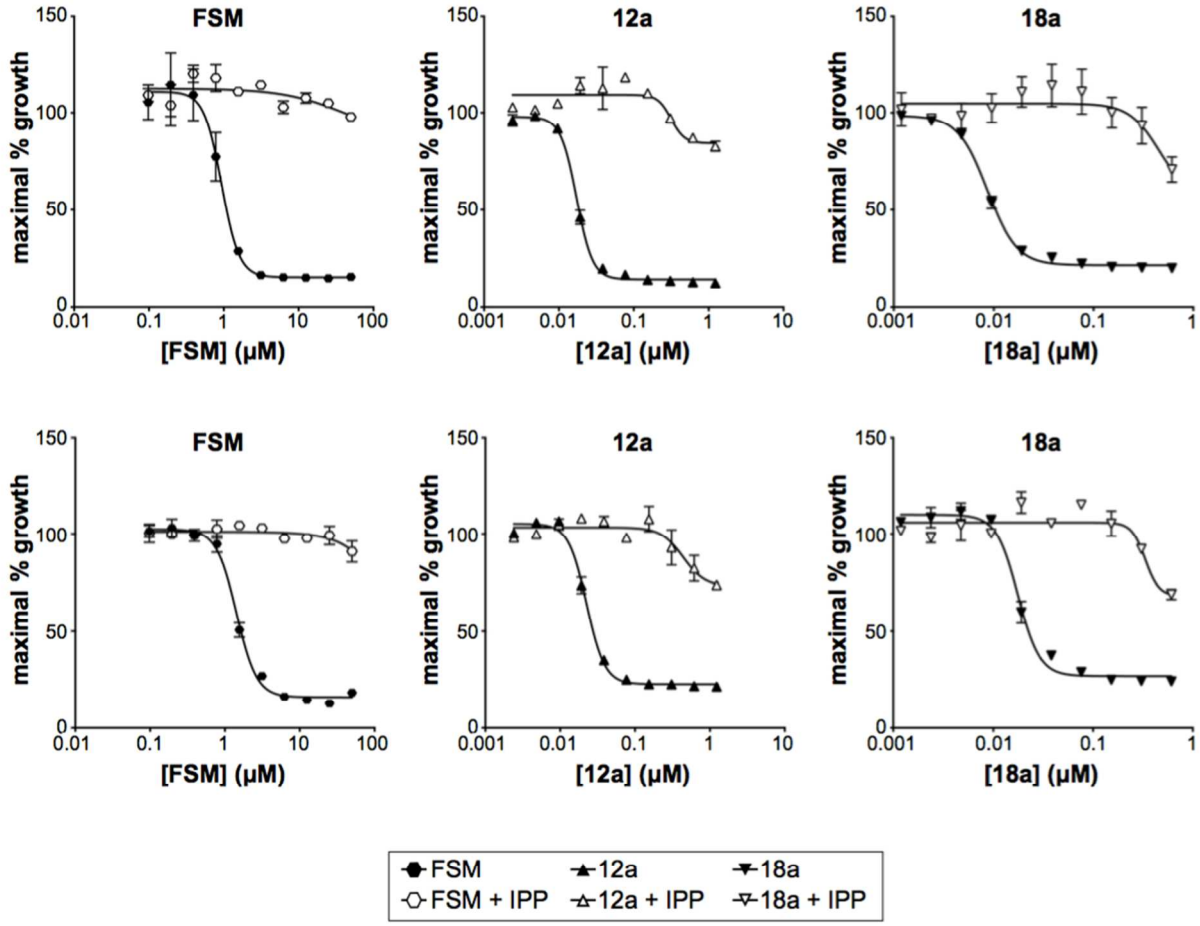

Figure S2. *P. falciparum* growth inhibited by analogs **12a** and **18a** is restored by IPP supplementation. Shown are graphs from two additional independent experiments. Data from third independent experiment is shown in Figure 4.

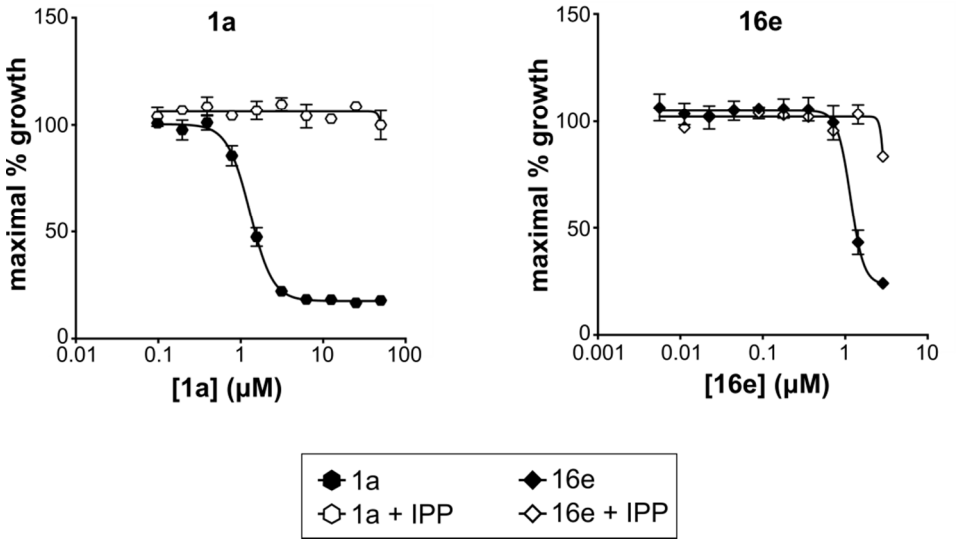

Figure S3. *P. falciparum* growth inhibited by the *N*-alkoxy analog **16e** is also restored by IPP supplementation. Shown are representative graphs from at least 3 independent experiments.

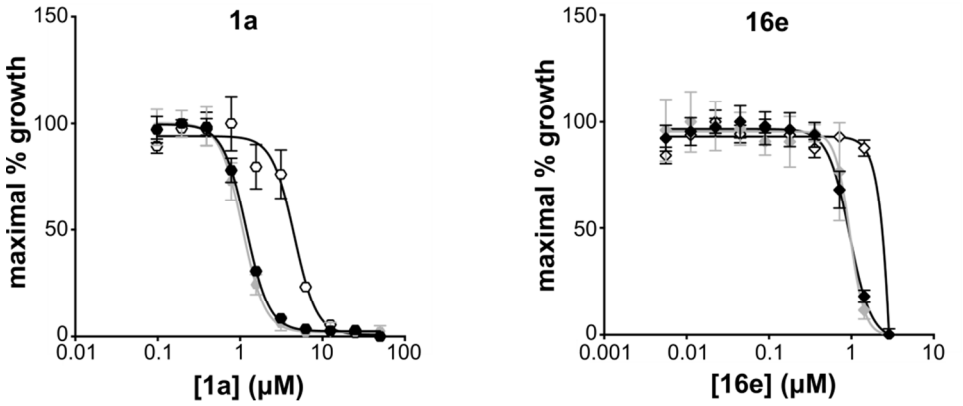

Figure S4. *P. falciparum* strains with high levels of DOXP confer resistance to DXR inhibitors. Dose-dependent growth inhibition was determined for *P. falciparum* strains treated with inhibitors as previously described.[9] Strains with higher levels of the DXR substrate DOXP are more resistant to DXR inhibitors as indicated by a shift in the IC_{50} curve (*had1*; open shapes, black line) when compared to WT *P. falciparum* (3D7; closed shapes, grey line). Sensitivity was restored if a WT copy of HAD1 was supplied in the mutant strain (*had1* + HAD1-GFP; closed shapes, black line). Data are representative of at least 3 independent biological replicates performed in duplicate.

[9] Guggisberg, A. M. *et al.* A sugar phosphatase regulates the methylerythritol phosphate (MEP) pathway in malaria parasites. *Nat. Commun.* **5,** 4467 (2014).

HepG2 cell inhibition assays

For cytotoxicity assays, HepG2 cells (ATCC HB-8065) were grown in DMEM supplemented with 4 mM L-glutamine (Gibco #11966-025) with either 4.5 g/L D-glucose or 1.8 g/L galactose as carbon source. Cells were trypsinized, resuspended in the respective medium (DMEM/glutamine/glucose or DMEM/glutamine/galactose) to 4 x 10^5 cells/mL and 50 µL/well transferred to flat-bottom white opaque tissue culture plates (Falcon #353296) containing 50 µL/well of the respective medium with test compound. Compound concentrations were two-fold dilutions ranging from 50 µM to 0.049 µM as well as the drug-free DMSO-only control. All concentrations were tested in duplicate for each carbon source. After 24 h incubation at 5% CO_2, 37 ^oC, 10 µL/well of Celltiter-Glo reagent (Promega #G9241) was added and luminescence recorded after 20 min incubation in the dark.

MEP pathway metabolite assay

Sample preparation. *P. falciparum* strain 3D7 was cultured at 37 °C in 30 mL volumes in 100 mm tissue culture dishes (Techno Plastic Products) at 4% hematocrit until >8% parasitemia. Cultures were synchronized until >75% of parasites were in ring stage growth, and then treated for 10 h with or without 18a at 65 nM (5x the 3D7 IC50) in triplicate. Cultures were lysed with 5% saponin, the parasite pellets washed with 1x phosphate-buffered saline (PBS), and the pellets stored at -80 °C. MEP pathway intermediates were extracted via the addition of glass beads (212-300 u) and 600 µL chilled H_2O: chloroform: methanol (3:5:12 v/v) spiked with PIPES (piperazine-N,N′-bis(2-ethanesulfonic acid) as internal standard. The cells were disrupted with the TissueLyser II instrument (Qiagen) using a microcentrifuge tubes adaptor set pre-chilled for 2 min at 20 Hz. The samples were then centrifuged at 16,000 g at 4 ºC, the supernatants collected, and pellet extraction repeated once more. The supernatants were pooled and 300 µL chloroform and 450 µL of chilled water were added to the supernatants. The tubes were vortexed and centrifuged. The upper layer was transferred to a new tube and dried using a speed-vac. The pellets were re-dissolved in 100 µL of 50% acetonitrile.

LC-MS/MS analysis. For LC separation, a Luna-NH_2 column (3 um, 150 x 2 mm, Phenomenex) was used flowing at 0.4 mL/min. The gradient of the mobile phases A (20 mM ammonium acetate, pH 9.8, 5% ACN) and B (100% acetonitrile) was as follows: 60% B for 1 min, to 6% B in 3 min, hold at 6% B for 5 min, then back to 60% B in 0.5 min. The LC system was interfaced with a Sciex QTRAP 6500+ mass spectrometer equipped with a TurboIonSpray (TIS) electrospray ion source. Analyst software (version 1.6.3) was used to control sample acquisition and data analysis. The QTRAP 6500+ mass spectrometer was tuned and calibrated according to the manufacturer's recommendations. Metabolites were detected using MRM transitions that were previously optimized using standards. The instrument was set-up to acquire in negative mode. For quantification, an external standard curve was prepared using a series of standard samples containing different concentrations of metabolites and fixed concentration of the internal standard. The limit of detection for deoxyxylulose 5-phosphate (DOXP), methylerythritol phosphate (MEP), cytidine diphosphate methylerythritol (CDP-ME), and methylerythritol cyclodiphosphate (MEcPP) was 0.0064 µM for a 10 µL injection volume.

Mouse liver microsomes and plasma stability

In this protocol, the metabolic stability of compounds at 1 μM was determined in mouse liver microsomes (MLM) and mouse plasma. For microsomal stability each test compound was incubated in an aqueous reaction mixture consisting of 0.25 μM microsomal protein CYP450 activity, 1.2 mM NADPH, 3.3 mM $MgCl_2$, and 100 mM potassium phosphate buffer (pH 7.4). For plasma stability each test compound was incubated in mouse plasma (VWR). After incubation at 37 ºC a 50 μL aliquot of the reaction was transferred to 200 μL ice cold acetonitrile containing internal standard (Enalapril, 100 ng/mL). The quenched reaction mixtures were centrifuged at 3200 rpm for 5 min, and 100 μL of the supernatant were transferred to 96-well plate and analyzed by LC-MS/MS using an Applied Biosystems-Sciex API 4000. Analyte/internal standard peak area ratios were used to evaluate stability. The MRM transitions for enalapril, **12a**, and **18a** were m/z: 376.9 > 91.2, 511.197 > 102.1 and 283.259 > 102.1, respectively. An Armor C18 column (2.1 × 30 mm, 5 μm; Analytical Sales and Services, Pompton Plains, NJ) was used for chromatographic separation. Mobile phases were 0.1% formic acid, 1 mM triethylamine in water and acetonitrile with a flow rate of 0.35 mL/min. The starting phase was 0% acetonitrile increased to 100% acetonitrile over 3 minutes. Peak areas were integrated using Analyst Software (AB Sciex, Foster City, CA). Determination of *in vitro* half-life assumed first-order kinetics, where half-life is equivalent to -0.693/k, where -k is the slope of the linear regression of log percentage remaining versus incubation time.[10]

***In vivo* exposure study**

Animal care and all procedures were conducted at Charles River Laboratories (Wilmington, MA) and performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the institutional animal care and use committee (Protocol No. I019). Compound **18a** was added to 2% methylcellulose 0.5% Tween80 and sonicated to make a 10 mg/mL suspension. The suspension was administered to unfasted female Swiss Weber mice (n=3) at 20 mg/kg i.p. Plasma samples (10 μL) were removed at 0.25, 0.5, 1, 2, 4, 6 and 8 h and stored at -80 °C. Plasma samples were added to ice cold acetonitrile containing the internal standard, glafenine, as appropriate to bring samples into the standard curve range (50–10,000 ng/mL), then centrifuged for 5 minutes at 3200 rpm and the supernatant transferred to a 96-well sample plate for analysis by liquid chromatography-tandem mass spectrometry. The MRM transitions for glafenine and **12a** were m/z: 370.9 > 296.9 and 180.075 > 119.9, respectively. A Synergi 4 μm Hydro-RP column (250 x 4.6 mm, 80 Å) was used for chromatographic separation. Mobile phases were 0.1% formic acid in water and acetonitrile with a flow rate of 1.2 mL/min. The starting phase was 0% acetonitrile for 3 min, increased to 60% acetonitrile over 6 min. Peak areas were integrated using Analyst Software (AB Sciex, Foster City, CA).

[10] Obach, R. S.; Baxter, J. G.; Liston, T. E.; Silber, B. M.; Jones, B. C.; Macintyre, F.; Rance, D. J.; Wastall, P. The Prediction of Human Pharmacokinetic Parameters from Preclinical and In Vitro Metabolism Data. *Journal of Pharmacology and Experimental Therapeutics* **1997,** 283, 46-58.