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Supporting Information

Non-phosphate inhibitors of IspE protein, a kinase in the non-mevalonate pathway for isoprenoid biosynthesis and a potential target for antimalarial therapy

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Supporting Information

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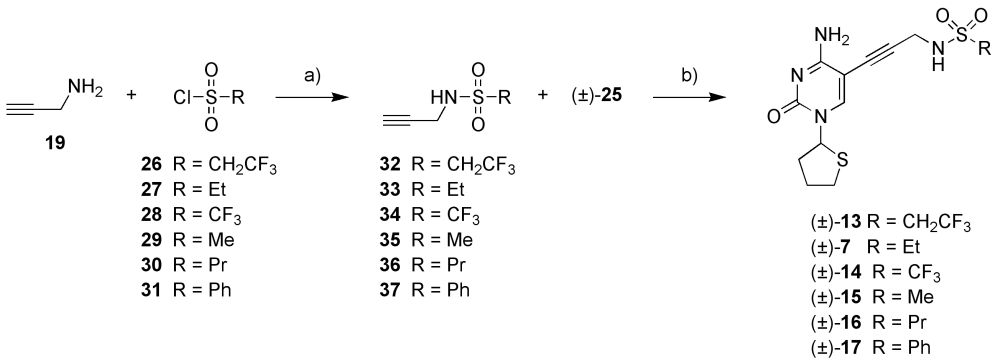
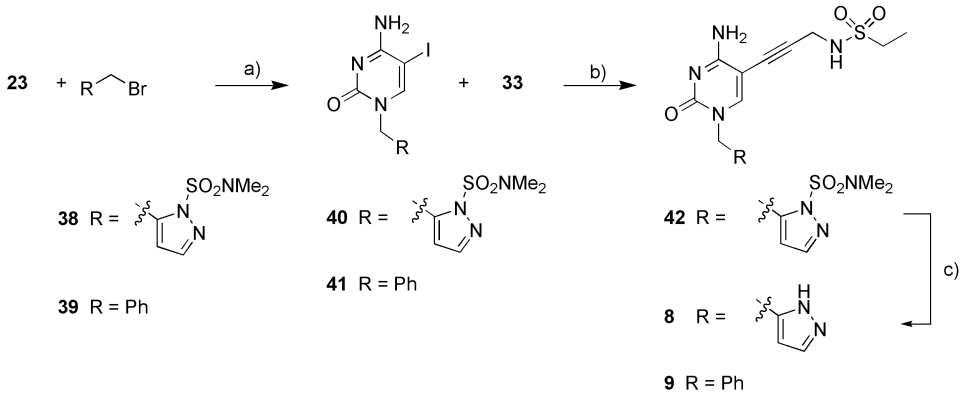


26 R = CH_2CF_3
27 R = Et
28 R = CF_3
29 R = Me
30 R = Pr
31 R = Ph

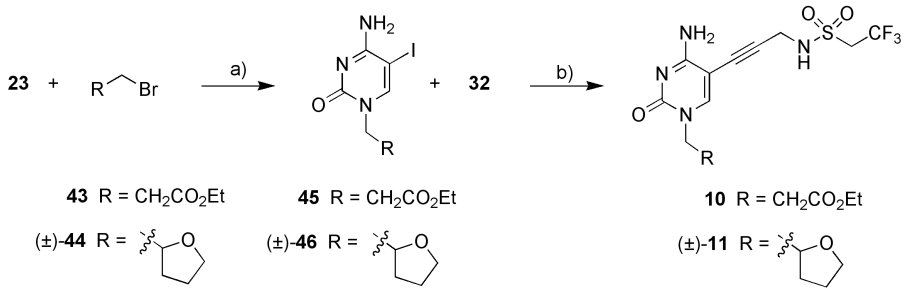
32 R = CH_2CF_3
33 R = Et
34 R = CF_3
35 R = Me
36 R = Pr
37 R = Ph

(±)-**13** R = CH_2CF_3
(±)-**7** R = Et
(±)-**14** R = CF_3
(±)-**15** R = Me
(±)-**16** R = Pr
(±)-**17** R = Ph

Scheme 1SI. Synthesis of inhibitors (±)-**7** and (±)-**13**-(±)-**17**. a) Et_3N, CH_2Cl_2, 25 °C, 5 min, **32** (79%), **33** (75%), **34** (50%), **35** (88%), **36** (82%), **37** (88%); b) Et_3N, $[PdCl_2(PPh_3)_2]$, CuI, DMF, 25 °C, 5-26 h, (±)-**13** (50%), (±)-**7** (70%), (±)-**14** (68%), (±)-**15** (90%), (±)-**16** (76%), (±)-**17** (56%). DMF = *N*,*N*-Dimethylformamide.



Scheme 2SI. Synthesis of inhibitors **8** and **9**. a) NaH, DMF, 25 °C, 16-24 h, **40** (95%), **41** (57%); b) Et_3N, $[PdCl_2(PPh_3)_2]$, CuI, DMF, 25 °C, 19-26 h, **42** (84%), **9** (75%); c) HCl in MeOH (1.4M), 0 °C → 25 °C, 2 h, 97%.



Scheme 3SI. Synthesis of inhibitors **10** and (±)-**11**. a) NaH, DMF, 25-70 °C, 22-24 h, **45** (89%), (±)-**46** (43%); b) Et_3N, $[PdCl_2(PPh_3)_2]$, CuI, DMF, 25 °C, 25-24 h, **10** (4%), (±)-**11** (52%).

Scheme 4SI. Synthesis of inhibitor **18**. a) Et_3N, $[PdCl_2(PPh_3)_2]$, CuI, DMF, 25 °C, 24 h, 51%.

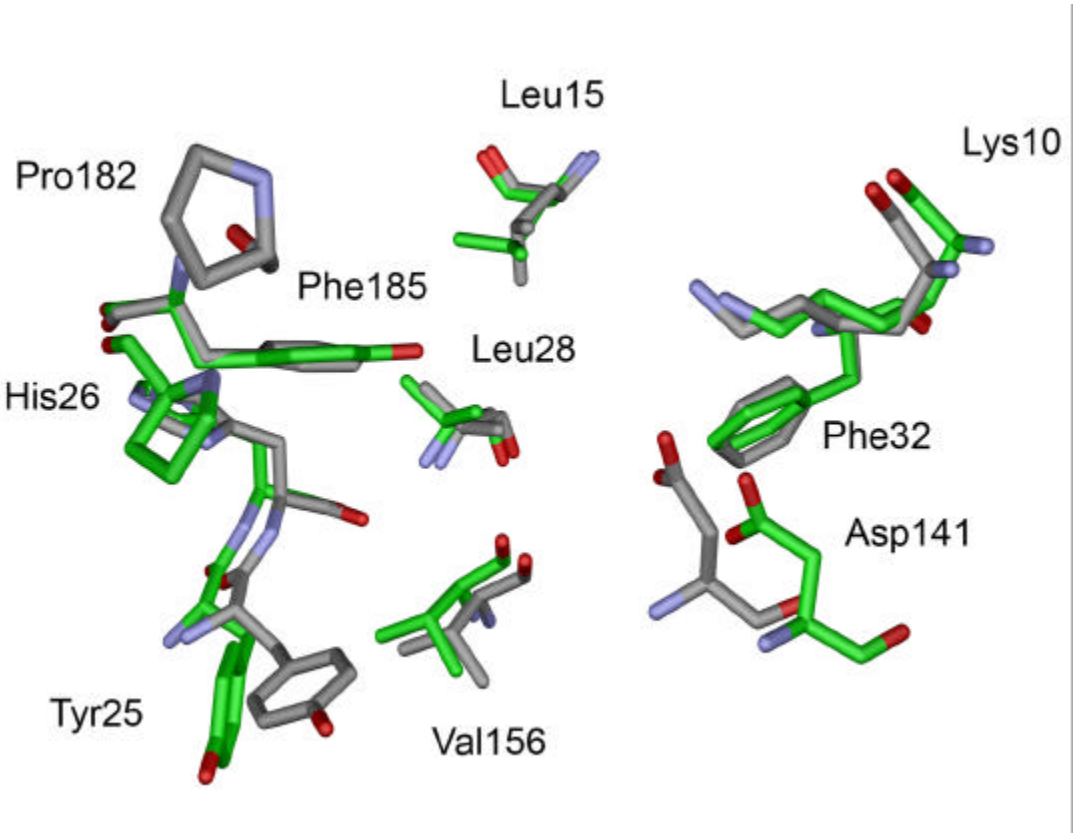


Figure 1SI. Superposition of the two published X-ray crystal structures of IspE.[1]

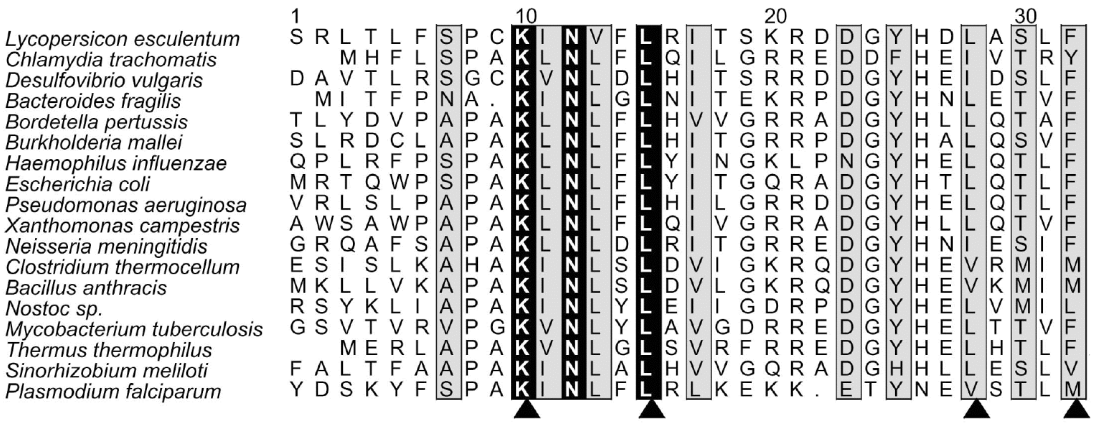


Figure 2SI. Amino acid sequence alignment of IspE proteins from various organisms. The alignment was constructed from IspE amino acid sequences of 16 representative members from bacterial groups, from one plant (*Lycopersicon esculentum*), and from one apicomplexan protist (*Plasmodium falciparum*) using the program PileUp (GCG, Madison, Wisconsin). The numbering is according to the amino acid sequence of the *Escherichia coli* protein. Arrows indicate the residues lining the hydrophobic pocket as illustrated in Figure 3.

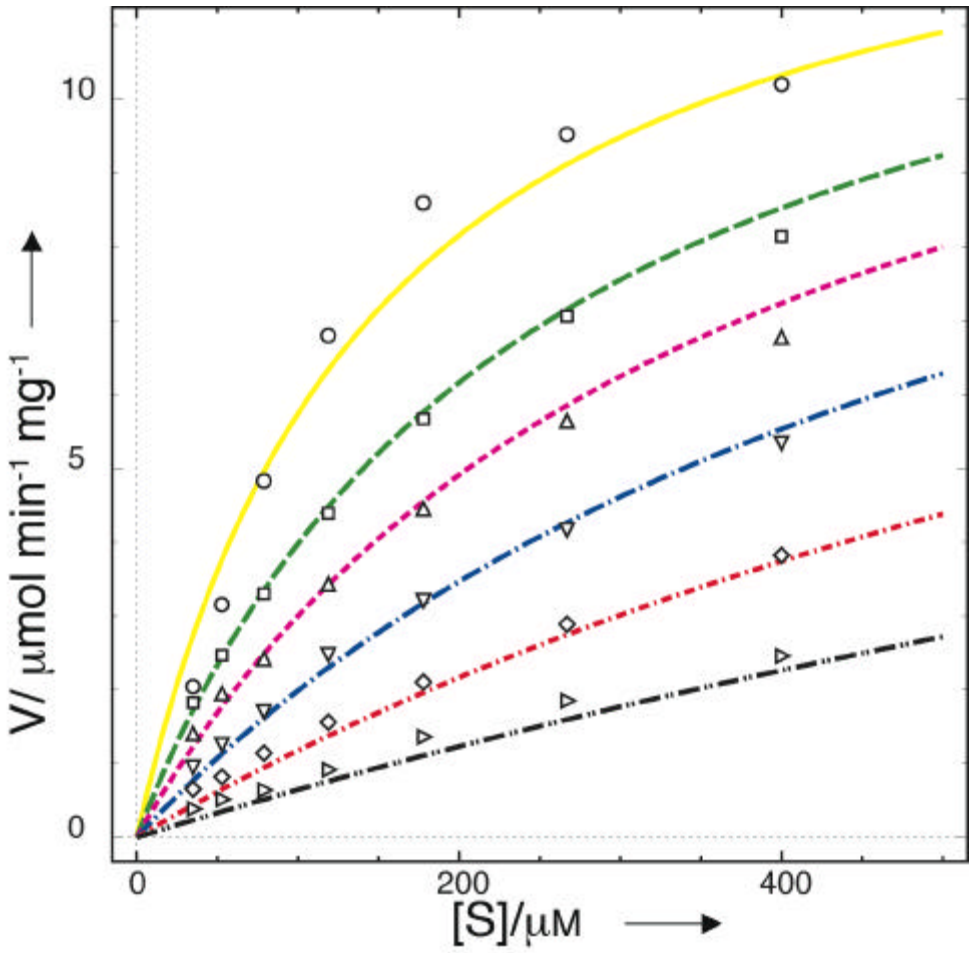


Figure 3SI. Exemplary kinetics for the inhibition of IspE by (±)-**12** used to calculate the K_{ic}-value.

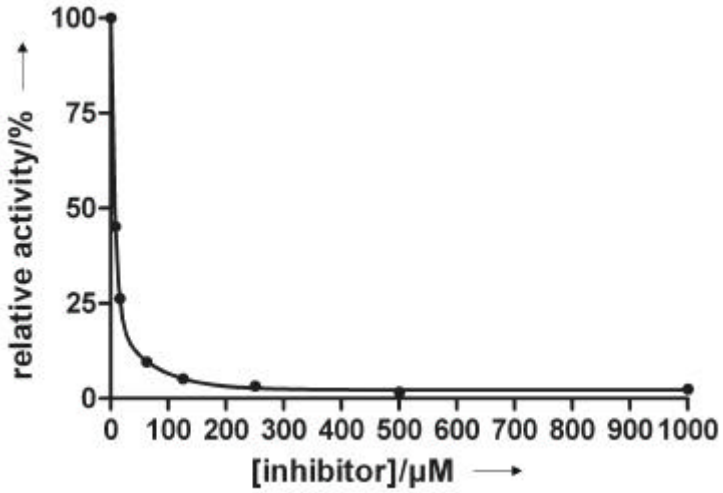


Figure 4SI. Exemplary IC_{50} curve for inhibition of IspE by ligand (±)-**12**.

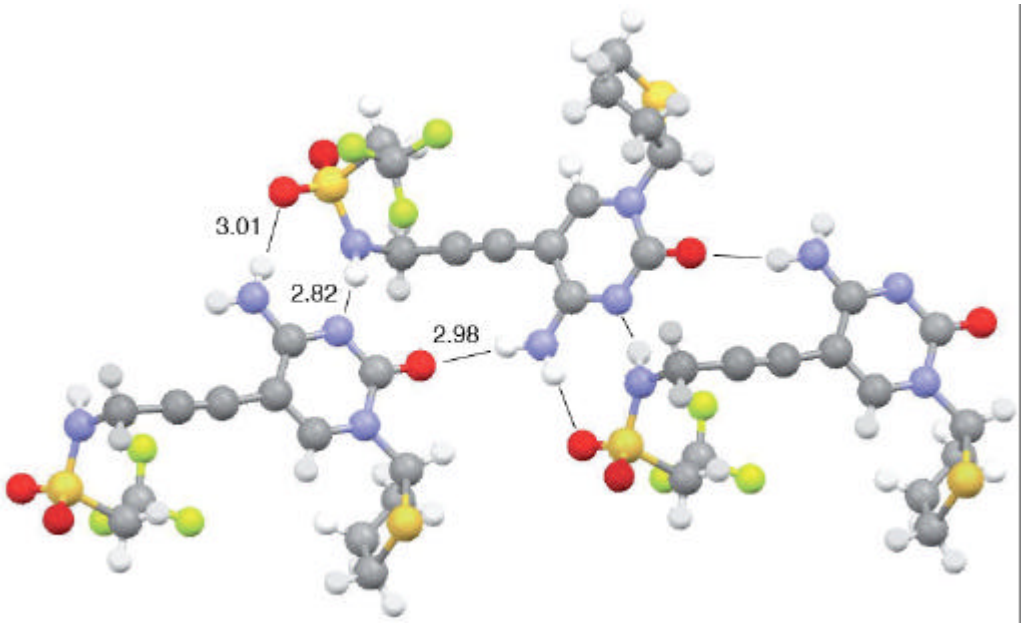


Figure 5SI. Crystal packing of inhibitor (±)-**13**. Arrangement of three neighboring molecules in the crystal of (±)-**13**, showing an extended H-bonding network involving both cytosine and sulfonamide moieties. Distances are given in Å between heavy atoms. The two enantiomers each build H-bonded chains of molecules along a two-fold screw axes in the direction of the crystallographic b-axis. Color code: C: grey; O: red; N: blue; S: yellow; F: green.

Experimental section of the supporting information

General: Solvents and reagents were purchased reagent-grade and used without further purification. Compound **23** was synthesized according to a literature procedure.[2] All reactions were carried out under an Ar atmosphere unless otherwise stated. CH_2Cl_2 and toluene were freshly distilled over CaH_2 and sodium, respectively. All products were dried under high vacuum (10^{-2} Torr) before analytical characterization. TLC: Aluminium sheets coated with SiO_2-60 UV_{254} from *Macherey-Nagel*, visualization by UV light at 245 nm and staining with a solution of $KMnO_4$ (1.5 g), K_2CO_3 (10 g), 5% NaOH (2.5 mL) in H_2O (150 mL); or a solution of ninhydrin (0.3 g) in butanol (100 mL) and glacial acetic acid (3 mL). Column chromatography (CC): SiO_2-60 (230-400 mesh, 0.040-0.063 mm) from *Fluka*. Analytical HPLC was performed on a *Knauer Prontosil 120 C18* column (259 x 4 mm, 5 µm, 100 Å); products were eluted with a linear gradient (5-55%) of CH_3CN in H_2O containing 0.1% TFA over 20 min with a flow rate of 1 mL/min with UV detection at l = 254 nm. Preparative HPLC was performed on a *Knauer Prontosil 120-5 C18* column (250 x 25 mm, 7 µm, 100 Å); products were eluted with a linear gradient (5-100%) of CH_3CN in H_2O containing 0.1% TFA with a flow rate of 10 mL/min with UV

detection at *l* = 254 nm. Melting points (mp): *Büchi-510* apparatus; uncorrected. IR Spectra: *Perkin Elmer Spectrum BX FTIR System* spectrometer (*ATR-unit, Attenuated Total Reflection, Golden Gate*). NMR spectra (^{1}H, ^{13}C): *Varian Gemini-300*, Bruker AMX-400, and *Bruker AMX-500*; spectra were recorded at 25 °C using the solvent peak as an internal reference. Coupling constants (*J*) are given in Hz. The resonance multiplicity is described as s (singlet), br. s (broad singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). In the ^{13}C NMR spectrum of compound **10**, the signal from the CF_3 group is not visible. High-resolution mass spectra (HRMS): *IonSpec Ultima FT-ICR* with 3-hyrdroxypicolinic acid (3-HPA) as matrix (MALDI); *Micromass AutoSpec-Ultima* (EI); *Finnigan TSQ 7000* (ESI). Elemental analyses were performed by the Mikrolabor at the Laboratorium für Organische Chemie, ETH Zürich. The nomenclature was generated with the computer program *ACD/Name* (*ACD/Labs*).

General procedure A for the *Sonogashira* cross coupling of 5-iodocytosine derivatives: To an Ar-degassed *Schlenk* flask charged with iodocytosine (1.0 eq.), the acetylene (1.1-3 eq.), and Et_3N (2.0 Äquiv.) in anhydrous DMF, $[PdCl_2(PPh_3)_2]$ (0.1 eq.) and CuI (0.2 eq.) were added at 25 °C. The mixture was left to stir at 25 °C in the

dark, the solvent evaporated in vacuo, and the residue purified by CC.

If purification by CC yielded the triethylammonium salt, the solid was dissolved in CH_2Cl_2/*i*-PrOH 3:1 washed with saturated aqueous NaCl solution (3x), dried over Na_2SO_4, filtered, and concentrated in vacuo.

General Procedure B for the alkylation of 5-iodocytosine derivatives: To a suspension of the cytosine derivative (1.1 eq.) and NaH (1.1 eq., as a 60% dispersion in mineral oil) in anhydrous DMF stirred for 1.5 h at 25 °C, the alkyl bromide (1.0 eq.) in anhydrous DMF was added slowly. The mixture was stirred at 25 °C and concentrated in vacuo.

General Procedure C for the formation of a sulfonamide: To a solution of propargyl amine (1 eq.) and Et_3N (1.1 eq.) in dry CH_2Cl_2 (0.13 M), the sulfonyl chloride (1 eq.) was added dropwise at 0 °C. The mixture was left to stir at 25 °C for 5 min and concentrated in vacuo.

(±)-*N*-{3-[4-Amino-2-oxo-1-(tetrahydro-2-thienyl)-1,2-dihydropyrimidin-5-yl]prop-2-yn-1-yl}ethanesulfonamide ((±)-7): *General procedure A*, starting from (±)-**25** (50 mg, 0.155 mmol), **33** (25 mg, 0.171 mmol), Et_3N (31 mg, 0.310 mmol), $[PdCl_2(PPh_3)_2]$ (11 mg, 0.016 mmol), and CuI (6 mg, 0.031 mmol) in anhydrous DMF (3.5 mL). The mixture was left to stir for 26 h. Purification by CC (CH_2Cl_2/MeOH 95:5) to yield (±)-**7** (37 mg, 70%) as an

orange solid. Mp: 192-197 °C; ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$ 7:1): δ = 1.31 (t, *J* = 7.5 Hz, 3 H), 1.78-1.97 (m, 1 H), 1.99-2.11 (m, 2 H), 2.20-2.28 (m, 1 H), 2.85-2.93 (m, 1 H), 3.04 (q, *J* = 7.5 Hz, 2 H), 3.09-3.14 (m, 1 H), 4.02 (s, 2 H), 6.17-6.20 (m, 1 H), 8.10 ppm (s, 1 H); ^{13}C NMR (125 MHz, $(CD_3)_2SO$): δ = 7.9, 28.9, 32.7, 32.8, 36.8, 45.8, 64.3, 75.3, 89.3, 91.3, 145.2, 153.7, 164.1 ppm; IR (neat): $\tilde{n}$ = 3383, 3296, 2968, 1639, 1599, 1494, 1403, 1343, 1310, 1278, 1228, 1184, 1136, 1081, 972, 878, 847, 778, 715, 639 cm^{-1}; HRMS (MALDI): calculated for $C_{13}H_{19}N_4O_3S_2^+$ (*M*H$^+$): 343.0893, found: 343.0899.

***N*-{3-[4-Amino-2-oxo-1-(1*H*-pyrazol-3-ylmethyl)-1,2-dihydropyrimidin-5-yl]prop-2-yn-1-yl}ethanesulfonamide (8)**: To **42** (45 mg, 0.101 mmol), HCl in MeOH (1.4 M, 2.41 mL, 3.38 mmol) was added and the mixture stirred at 0 °C for 1 h and at 25 °C for 1 h. The mixture was concentrated in vacuo. Purification by CC (CH_2Cl_2/MeOH 90:10, 2% NH_3 (25% in H_2O)) to yield **8** (33 mg, 97%) as an off-white solid. Mp: 219-224 °C; ^{1}H NMR (300 MHz, $(CD_3)_2SO$, 1 drop D_2O, 1 drop TFA): δ = 1.19 (t, *J* = 7.5 Hz, 3 H), 3.08 (q, *J* = 7.5 Hz, 2 H), 4.03 (s, 2 H), 5.00 (s, 2 H), 6.28 (d, *J* = 2.3 Hz, 1 H), 7.68 (d, *J* = 2.3 Hz, 1 H), 8.23 (s, 1 H), 8.41 ppm (s, 1 H); ^{13}C NMR (125 MHz, $(CD_3)_2SO$): δ = 7.9, 32.6, 45.9, 75.2, 79.1, 88.4, 91.2,

103.6, 129.3, 147.3, 149.1, 153.9, 164.6 ppm; IR (neat): $\tilde{n}$ = 3329, 3073, 2231, 1626, 1494, 1450, 1428, 1393, 1309, 1237, 1210, 1180, 1130, 1081, 1054, 994, 920, 842, 772, 719, 662 cm^{-1}; HRMS (MALDI): calculated for $C_{13}H_{17}N_6O_3S^+$ (MH^+): 337.1077, found: 337.1084; Anal. calculated for $C_{13}H_{16}N_6O_3S$ (336.37): C 46.42, H 4.79, N 24.98; found: C 46.37, H 4.87, N 24.65.

***N*-[3-(4-Amino-1-benzyl-2-oxo-1,2-dihydropyrimidin-5-yl)prop-2-yn-1-yl]ethanesulfonamide (9):** *General procedure A*, starting from **41** (100 mg, 0.306 mmol), **33** (49 mg, 0.336 mmol), Et_3N (62 mg, 0.611 mmol), $[PdCl_2(PPh_3)_2]$ (22 mg, 0.031 mmol), and CuI (12 mg, 0.061 mmol) in anhydrous DMF (7 mL). The mixture was left to stir for 26 h. Purification by CC (CH_2Cl_2/MeOH 95:5) to yield **9** (79 mg, 75%) as a white solid. Mp: 238-240 °C; ^1H NMR (300 MHz, $CDCl_3/CD_3OD$ 7:1): δ = 1.27 (t, *J* = 7.4 Hz, 3 H), 3.00 (q, *J* = 7.4 Hz, 2 H), 3.96 (s, 2 H), 4.88 (s, 2 H), 7.20-7.31 (m, 5 H), 7.39 ppm (s, 1 H); ^{13}C NMR (75 MHz, $(CD_3)_2SO$): δ = 8.0, 32.6, 45.8, 51.6, 75.1, 88.7, 91.4, 127.5, 127.6 (2C), 128.5 (2C), 137.4, 149.2, 154.1, 164.7 ppm; IR (neat): $\tilde{n}$ = 3444, 3084, 1665, 1621, 1504, 1450, 1393, 1359, 1319, 1278, 1235, 1210, 1196, 1144, 1075, 1048, 915, 825, 777, 751, 695, 674 cm^{-1}; HRMS (MALDI): calculated for $C_{16}H_{19}N_4O_3S^+$ (MH^+): 347.1172, found: 347.1164.

Ethyl [4-amino-2-oxo-5-(3-{[(2,2,2-trifluoroethyl)sulfonyl]amino}prop-1-yn-1-yl)pyrimidin-1(2*H*)-yl]acetate (10): *General procedure A*, starting from 45 (112 mg, 0.347 mmol), 32 (80 mg, 0.398 mmol), Et_3N (80 mg, 0.796 mmol), $[PdCl_2(PPh_3)_2]$ (25 mg, 0.036 mmol), and CuI (14 mg, 0.072 mmol) in anhydrous DMF (8 mL). The mixture was left to stir for 25 h. Purification by CC (CH_2Cl_2/MeOH 95:5), followed by reversed-phase HPLC (0.1% TFA, H_2O/CH_3CN 95:5 → 60:40 in 12 min, 60:40 → 50:50 in 24 min, 50:50 → 0:100 in 4 min, 14 min at 0:100) to yield 10 (7 mg, 4%) as a white solid. Mp: 254-258 °C; ^{1}H NMR (300 MHz, $(CD_3)_2SO$): δ = 1.19 (t, *J* = 7.2 Hz, 3 H), 4.11 (q, *J* = 7.2 Hz, 2 H), 4.12 (s, 2 H), 4.46 (s, 2 H), 4.49 (q, *J* = 10.0 Hz, 2 H), 6.91 (br. s, 1 H), 7.81 (br. s, 1 H), 7.97 (s, 1 H), 8.33 ppm (br. s, 1 H); ^{13}C NMR (125 MHz, $(CD_3)_2SO$): δ = 14.0, 32.8, 50.0, 52.8, 61.0, 75.2, 88.6, 90.7, 149.8, 154.0, 165.0, 168.3 ppm (the signals from the CF_3 group were not visible); IR (thin film): $\tilde{n}$ = 3440, 2358, 1738, 1634, 1494, 1425, 1378, 1343, 1325, 1276, 1251, 1215, 1157, 1131, 1090, 1053, 1024, 910, 819, 782, 668 cm^{-1}; HRMS (MALDI): calculated for $C_{13}H_{16}F_3N_4O_5S^+$ (*M*H$^+$): 397.0788, found: 397.0784.

(±)-*N*-{3-[4-Amino-2-oxo-1-(tetrahydrofuran-2-ylmethyl)-1,2-dihydropyrimidin-5-yl]prop-2-yn-1-yl}-2,2,2-trifluoroethanesulfonamide ((±)-11): *General procedure A*,

starting from (±)-**46** (140 mg, 0.44 mmol), **32** (176 mg, 0.87 mmol), Et_3N (0.18 mL, 1.31 mmol), $[PdCl_2(PPh_3)_2]$ (30.6 mg, 0.044 mmol), and CuI (16.6 mg, 0.087 mmol) in anhydrous DMF (10.1 mL). The mixture was left to stir for 24 h. Purification by CC (CH_2Cl_2/MeOH 95:5) to yield (±)-**11** (89 mg, 52%) as a brown solid. Mp: > 134 °C (dec.); ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$ 3:1): δ = 1.30-1.45 (m, 2H), 1.67-1.76 (m, 1H), 1.80-1.92 (m, 1H), 3.30-3.45 (m, 1H), 3.55-3.68 (m, 2H), 3.81 (q, *J* = 9.1, 2H), 3.95 (s, 2H), 4.09-4.22 (m, 2H), 7.50 ppm (s, 1H); ^{13}C NMR (125 MHz, $(CD_3)_2SO$): δ = 25.0, 28.1, 32.7, 52.1, 53.0 (q, *J* = 29.5 Hz), 67.0, 69.7, 75.7, 75.8, 90.4, 122.4 (q, *J* = 275.5 Hz), 150.1, 154.1, 164.6 ppm; IR (thin film): $\tilde{n}$ = 3734, 3628, 2994, 2359, 2342, 1718, 1653, 1506, 1394, 1351, 1322, 1251, 1159, 1131, 1082, 891, 844, 809, 781, 736, 702, 668 cm^{-1}; HRMS (MALDI): calculated for $C_{14}H_{18}F_3N_4O_4S^+$ (*M*H$^+$): 395.0995, found: 395.0993.

(±)-*N*-{3-[4-Amino-2-oxo-1-(tetrahydro-2-thienyl)-1,2-dihydropyrimidin-5-yl]prop-2-yn-1-yl}cyclopropanesulfonamide ((±)-12): *General procedure A*, starting from (±)-**25** (112 mg, 0.35 mmol), **21** (110 mg, 0.69 mmol), Et_3N (0.15 mL, 1.04 mmol), $[PdCl_2(PPh_3)_2]$ (24.4 mg, 0.035 mmol), and CuI (13.1 mg, 0.07 mmol) in anhydrous DMF (8 mL). The mixture was left to stir for 22 h. Purification by CC (CH_2Cl_2/MeOH 96:4) to yield (±)-

12 (116 mg, 94%) as a brown solid. Mp: 160-161 °C. ^{1}H NMR (300 MHz, $(CD_3)_2SO$): δ = 0.94 (d, *J* = 6.1, 4H), 1.93-2.05 (m, 3H), 2.15-2.20 (m, 1H), 2.65 (q, *J* = 6.1, 1H), 2.82-2.88 (m, 1H), 3.15-3.30 (m, 1H), 4.05 (d, *J* = 5.7 Hz, 2H), 6.12-6.15 (m, 1H), 6.84 (br. s, 1H), 7.56 (t, *J* = 5.7 Hz, 1H), 7.82 (br. s, 1H), 8.11 ppm (s, 1H); ^{13}C NMR (75 MHz, $(CD_3)_2SO$): δ = 4.8 (2C), 28.8, 29.3, 32.6, 32.8, 36.7, 64.1, 75.2, 89.2, 91.4, 145.0, 153.6, 163.9 ppm; IR (neat): $\tilde{n}$ = 3383, 3098, 2946, 2227, 1667, 1637, 1610, 1505, 1429, 1404, 1230, 1188, 1137, 1080, 1038, 974, 884, 853, 778, 698, 641 cm^{-1}; HRMS (ESI): calculated for $C_{14}H_{18}N_4NaO_3S_2^+$ ([*M* + Na]$^+$): 377.0713, found: 377.0714. Anal. calculated for $C_{14}H_{18}N_4O_3S_2$ (354.08): C 47.44, H 5.12, N 15.81, found: C 47.31, H 5.14, N 15.54.

(±)-*N*-{3-[4-Amino-2-oxo-1-(tetrahydro-2-thienyl)-1,2-dihydropyrimidin-5-yl]prop-2-yn-1-yl}-2,2,2-trifluoroethanesulfonamide ((±)-13): *General procedure A*, starting from (±)-**25** (80 mg, 0.25 mmol), **32** (54 mg, 0.27 mmol), Et_3N (70 µL, 0.5 mmol), $[PdCl_2(PPh_3)_2]$ (17.5 mg, 0.025 mmol), and CuI (9.5 mg, 0.05 mmol) in anhydrous DMF (5.7 mL). The mixture was left to stir for 5 h. Purification by CC (CH_2Cl_2/MeOH 96:4) to yield (±)-**13** (50 mg, 50%) as an off-white solid. Mp: 194-195 °C; ^{1}H NMR (300 MHz, $(CD_3)_2SO$): δ = 21.94-2.06 (m, 3H), 2.17-2.23 (m, 1H), 2.82-2.89 (m, 1H), 3.16-3.26 (m, 1H), 4.12 (d, *J* =

4.8 Hz, 2H), 4.52 (q, *J* = 9.9 Hz, 2H), 6.13-6.17 (m, 1H), 6.89 (br. s, 1H), 7.83 (br. s, 1H), 8.14 (s, 1H), 8.33 ppm (t, *J* = 4.8 Hz, 1H); ^{13}C NMR (125 MHz, $(CD_3)_2SO$): δ = 28.9, 32.8 (2C), 36.9, 53.0 (q, *J* = 29.4 Hz), 64.3, 75.7, 89.1, 90.6, 122.4 (*q*, *J* = 275.6 Hz), 145.4, 153.7, 164.0 ppm; IR (thin film): $\tilde{n}$ = 3294, 2972, 2926, 2360, 2342, 1652, 1432, 1376, 1230, 1195, 1147, 10734, 668, 612 cm^{-1}; HRMS (MALDI): calculated for $C_{13}H_{16}F_3N_4O_3S_2^+$ (*M*H$^+$): 397.0616, found: 397.0605.

(±)-*N*-{3-[4-Amino-2-oxo-1-(tetrahydro-2-thienyl)-1,2-dihydropyrimidin-5-yl]prop-2-yn-1-yl}-1,1,1-trifluoromethanesulfonamide ((±)-14): *General procedure A*, starting from (±)-**25** (112 mg, 0.35 mmol), **34** (130 mg, 0.69 mmol), Et_3N (0.15 mL, 1.04 mmol), $[PdCl_2(PPh_3)_2]$ (24.4 mg, 0.035 mmol), and CuI (13.1 mg, 0.07 mmol) in anhydrous DMF (8 mL). The mixture was left to stir for 22 h. Purification by CC (CH_2Cl_2/MeOH 96:4) to yield (±)-**14** (90 mg, 68%) as a yellow solid. Mp: > 155 °C (dec.); ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$ 7:1, 45 °C): δ = 1.65-1.85 (m, 1H), 1.87-2.10 (m, 2H), 2.12-2.32 (m, 1H), 2.78-2.92 (m, 1H), 3.03-3.14 (m, 1H), 4.06-4.19 (m, 2H), 6.06-6.20 (m, 1H), 8.07 ppm (s, 1H); ^{13}C NMR (75 MHz, $(CD_3)_2SO$): δ = 29.6, 33.5, 34.6, 37.6, 62.7, 65.2, 77.0, 90.5, 120.3 (*q*, *J* = 322.9 Hz), 146.3, 154.3, 164.8 ppm; IR (neat): $\tilde{n}$ = 3425, 3336, 2967, 2360, 1644, 1603, 1505, 1404, 1368,

1299, 1224, 1181, 1146, 1074, 972, 859, 780, 680 cm^{-1}; HRMS (MALDI): calculated for $C_{12}H_{14}F_3N_4O_3S_2^+$ (MH^+): 383.0454, found: 383.0449.

(±)-*N*-{3-[4-Amino-2-oxo-1-(tetrahydro-2-thienyl)-1,2-dihydropyrimidin-5-yl]prop-2-yn-1-yl}methanesulfonamide ((±)-15): *General procedure A*, starting from (±)-**25** (120 mg, 0.37 mmol), **35** (98.8 mg, 0.74 mmol), Et_3N (0.16 mL, 1.04 mmol), $[PdCl_2(PPh_3)_2]$ (26.1 mg, 0.037 mmol), and CuI (14.1 mg, 0.074 mmol) in anhydrous DMF (8.6 mL). The mixture was left to stir for 21 h. Purification by CC (CH_2Cl_2/MeOH 97:3) to yield (±)-**15** (110 mg, 90%) as a brown solid. Mp: 165-166 °C; ^1H NMR (300 MHz, $(CD_3)_2SO$): δ = 1.94-2.06 (m, 3H), 2.15-2.22 (m, 1H), 2.80-2.88 (m, 1H), 2.99 (s, 3H), 3.15-3.26 (m, 1H), 4.04 (d, *J* = 5.6 Hz, 2H), 6.12-6.16 (m, 1H), 6.88 (br. s, 1H), 7.50 (t, *J* = 5.6 Hz, 1H), 7.82 (br. s, 1H), 8.13 ppm (s, 1H); ^{13}C NMR (75 MHz, $(CD_3)_2SO$): δ = 29.0, 32.8, 32.9, 36.8, 64.3 (2C), 75.5, 89.3, 91.2, 145.3, 153.7, 164.1 ppm; IR (neat): $\tilde{n}$ = 3416, 3310, 3065, 2923, 2862, 2362, 2227, 1636, 1592, 1504, 1482, 1431, 1403, 1298, 1251, 1229, 1140, 1057, 1011, 971, 851, 780, 667 cm^{-1}; HRMS (MALDI): calculated for $C_{12}H_{16}N_4NaO_3S_2^+$ ($[M + Na]^+$): 351.0556, found: 351.0561; Anal. calculated for $C_{12}H_{16}N_4O_3S_2$ (328.07): C 43.89, H 4.91; found: C 43.89, H 5.15.

(±)-*N*-{3-[4-Amino-2-oxo-1-(tetrahydro-2-thienyl)-1,2-dihydropyrimidin-5-yl]prop-2-yn-1-yl}propane-1-sulfonamide ((±)-16): *General procedure A*, starting from (±)-**25** (112 mg, 0.35 mmol), **36** (111.8 mg, 0.69 mmol), Et_3N (0.15 mL, 1.04 mmol), $[PdCl_2(PPh_3)_2]$ (24.4 mg, 0.035 mmol), and CuI (13.1 mg, 0.069 mmol) in anhydrous DMF (8 mL). The mixture was left to stir for 21 h. Purification by CC (CH_2Cl_2/MeOH 97:3) to yield (±)-**16** (94 mg, 76%) as an off-white solid. Mp: >201 °C (dec.); ^{1}H NMR (300 MHz, $(CD_3)_2SO$): δ = 0.93 (t, *J* = 7.4 Hz, 3H), 1.62-1.73 (m, 2H), 1.90-2.05 (m, 3H), 2.15-2.22 (m, 1H), 2.82-2.88 (m, 1H), 3.06-3.11 (m, 2H), 3.18-3.25 (m, 1H), 4.01 (d, *J* = 5.8 Hz, 2H), 6.12-6.16 (m, 1H), 6.89 (br. s, 1H), 7.55 (t, *J* = 5.8 Hz, 1H), 7.82 (br. s, 1H), 8.12 ppm (s, 1H); ^{13}C NMR (75 MHz, $(CD_3)_2SO$): δ = 12.6, 16.9, 28.9, 32.7, 32.8, 36.9, 53.1, 64.3, 75.3, 89.3, 91.4, 145.3, 153.7, 164.1 ppm; IR (neat): $\tilde{n}$ = 3393, 3317, 3210, 3051, 2962, 2872, 2707, 2359, 2235, 1666, 1642, 1604, 1534, 1503, 1462, 1400, 1342, 1312, 1294, 1248, 1225, 1178, 1080, 1055, 970, 925, 892, 876, 844, 779, 737, 696, 668, 643 cm^{-1}; HRMS: calculated for $C_{14}H_{20}N_4NaO_3S_2^+$ ($[M + Na]^+$): 379.0869, found: 379.0873; Anal. calculated for $C_{14}H_{20}N_4O_3S_2$ (356.10): C 47.17, H 5.56; found: C 47.06, H 5.73.

(±)-*N*-{3-[4-Amino-2-oxo-1-(tetrahydro-2-thienyl)-1,2-dihydropyrimidin-5-yl]prop-2-yn-1-yl}benzenesulfonamide ((±)-17): *General procedure A*, starting from (±)-**25** (80 mg, 0.25 mmol), **37** (53 mg, 0.27 mmol), Et_3N (70 μL, 0.5 mmol), $[PdCl_2(PPh_3)_2]$ (17.5 mg, 0.025 mmol), and CuI (9.5 mg, 0.05 mmol) in anhydrous DMF (5.7 mL). The mixture was left to stir for 5 h. Purification by CC (CH_2Cl_2/MeOH 96:4) to yield (±)-**17** (55 mg, 56%) as a yellow solid. Mp: 173-174 °C; ^{1}H NMR (300 MHz, $(CD_3)_2SO$): δ = 1.89-2.02 (m, 3H), 2.16-2.20 (m, 1H), 2.83-2.91 (m, 1H), 3.19-3.31 (m, 1H), 3.95 (d, *J* = 5.7 Hz, 2H), 6.11-6.14 (m, 1H), 6.64 (br. s, 1H), 7.54-7.65 (m, 3H), 7.76 (br. s, 1H), 7.83 (s, 1H), 7.76-7.87 (m, 2H), 8.11 ppm (t, *J* = 5.7 Hz, 1H); ^{13}C NMR (125 MHz, $(CD_3)_2SO$): δ = 28.9, 32.8, 33.0, 37.0, 64.2, 75.5, 89.2, 90.3, 126.7 (2C), 129.1 (2C), 132.5, 140.2, 144.8, 153.6, 163.8 ppm; IR (thin film): $\tilde{n}$ = 3307, 2973, 2879, 2360, 2341, 1376, 1225, 1195, 1150, 1074, 668 cm^{-1}; HRMS (ESI): calculated for $C_{17}H_{18}N_4NaO_3S_2^+$ ($[M + Na]^+$): 413.0713, found: 413.0735.

4-Amino-1-benzyl-5-(3-hydroxyprop-1-yn-1-yl)pyrimidin-2(1*H*)-one (18): *General procedure A*, starting from **41** (100 mg, 0.306 mmol), propargyl alcohol (**47**) (51 mg, 0.917 mmol), Et_3N (62 mg, 0.611 mmol), $[PdCl_2(PPh_3)_2]$ (22 mg, 0.031 mmol), and CuI (12 mg, 0.061 mmol) in anhydrous DMF (7 mL). The mixture was left to stir for 24 h.

Purification by CC (CH_2Cl_2/MeOH 95:5) to yield **18** (40 mg, 51%) as an off-white solid. Mp: 184-187 °C; ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD 7:1): δ = 4.31 (s, 2 H), 4.90 (s, 2 H), 7.21-7.34 (m, 5 H), 7.40 ppm (s, 1 H); ^{13}C NMR (75 MHz, $(CD_3)_2SO$): δ = 49.7, 51.6, 75.5, 89.1, 94.9, 127.3, 127.5 (2C), 128.3 (2C), 137.2, 148.8, 153.9, 164.4 ppm; IR (thin film): $\tilde{n}$ = 2360, 1643, 1496, 113, 1024, 649 cm^{-1}; HRMS (MALDI): calculated for $C_{14}H_{14}N_3O_2^+$ (MH$^+$): 256.1081, found: 256.1084.

***N*-Prop-2-yn-1-ylcyclopropanesulfonamide (21):** *General procedure C*, starting from propargyl amine (**19**) (0.46 mL, 7.1 mmol), cyclopropanesulfonyl chloride (**20**) (1g, 7.1 mmol), and Et_3N (1.1 mL, 7.8 mmol) in dry CH_2Cl_2 (53 mL). Purification by CC (EtOAc/cyclohexane 2:3) to yield **21** (814 mg, 72%) as a colorless oil. ^{1}H NMR (300 MHz, ($CDCl_3$): δ = 0.95-1.05 (m, 2H), 1.08-1.19 (m, 2H), 2.34 (t, J = 2.5 Hz, 1H), 2.48-2.57 (m, 1H), 3.89 (m, 2H), 5.14 ppm (br. s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 5.7 (2C), 30.4, 32.5, 72.7, 79.4 ppm; IR (neat): $\tilde{n}$ = 3276, 2924, 2360, 2336, 1429, 1327, 1300, 1146, 1074, 891, 835, 668 cm^{-1}; HRMS (ESI): calculated for $C_6H_9NNaO_2S^+$ ([M + Na]$^+$: 182.0246, found: 182.0242. Anal. calculated for $C_6H_9NO_2S$ (159.04): C 45.27, H 5.70, N 8.80; found: C 44.99, H 5.73, N 8.82.

(±)-4-Amino-5-iodo-1-(tetrahydro-2-thienyl)pyrimidin-2-(1*H*)-one ((±)-25): To a suspension of **23** (474 mg, 2 mmol) in dry toluene (20 mL) were added Et_3N (1.12 mL, 8 mmol) and trimethylsilyl trifluormethanesulfonate (1.54 mL, 8 mmol) at 25 °C. Tetramethylene sulfoxide (**24**) (0.18 mL, 2 mmol) and ZnI_2 (384 mg, 1.2 mmol) were added at 0 °C. The mixture was stirred at 25 °C for 17 h. The resulting mixture was washed with saturated aqueous $NaHCO_3$ solution (90 mL). The aqueous layer was extracted with CH_2Cl_2/*i*-PrOH 3:1 (3 x 80 mL). The combined organic phases were washed with saturated aqueous NaCl solution (3 x 80 mL), dried over Na_2SO_4, and concentrated in vacuo. Purification by CC (CH_2Cl_2/MeOH 99:1 → 96:4) to yield (±)-**25** (388 mg, 60%) as a white solid. Mp: 183-187 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.78-1.93 (m, 1 H), 2.02-2.18 (m, 2 H), 2.28-2.40 (m, 1 H), 2.91-2.99 (m, 1 H), 3.16-3.23 (m, 1 H), 5.53 (br. s, 1 H), 6.26-6.30 (m, 1 H), 8.21 (s, 1 H), 8.24 ppm (br. s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$/CD_3OD 7:1): δ = 28.1, 33.2, 38.4, 56.2, 65.6, 147.8, 155.5, 163.4 ppm; IR (neat): $\tilde{n}$ = 2948, 1630, 1470, 1386, 1273, 1224, 1169, 1130, 1076, 876, 771, 638 cm^{-1}; HRMS (MALDI): calculated for $C_8H_{11}IN_3OS^+$ (*M*H$^+$): 323.9662, found: 323.9660.

2,2,2-Trifluoro-*N*-prop-2-yn-1-ylethanesulfonamide (32): *General procedure C*, starting from propargyl amine (**19**)

(0.26 mL, 4 mmol), 2,2,2-trifluoroethanesulfonyl chloride (**26**) (0.44 mL, 4 mmol), and Et_3N (0.61 mL, 4.4 mmol) in dry CH_2Cl_2 (30 mL) to yield **32** (633 mg, 79%) as an orange solid. Purification by CC (EtOAc/cyclohexane 1:1). Mp: 56-59 °C; ^1H NMR (300 MHz, $CDCl_3$): δ = 2.45 (t, *J* = 2.5 Hz, 1 H), 3.99 (q, *J* = 8.8 Hz, 2 H), 4.04 (dd, *J* = 6.0, 2.5 Hz, 2 H), 4.87 ppm (br. s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 32.9, 55.1 (q, *J* = 31.1 Hz), 74.2, 77.7, 117.7 ppm (q, *J* = 277.1 Hz); IR (neat): $\tilde{n}$ = 3321, 3306, 3019, 2961, 2853, 2127, 1430, 1400, 1367, 1325, 1260, 1241, 1154, 1139, 1084, 1070, 992, 926, 888, 857, 817, 674, 655, 630 cm^{-1}; HRMS (ESI): calculated for $C_5H_5F_3NO_2S^+$ ([*M*-H]$^+$): 199.9999, found: 200.0034; Anal. calculated for $C_5H_6F_3NO_2S$ (201.01): C 29.85, H 3.01, N 6.96; found: C 29.78, H 3.06, N 6.99.

***N*-Prop-2-yn-1-ylethanesulfonamide (33):**[3] *General procedure C*, starting from propargyl amine (**19**) (110 mg, 2.0 mmol), ethanesulfonyl chloride (**27**) (257 mg, 2.0 mmol), and Et_3N (223 mg, 2.2 mmol) in dry CH_2Cl_2 (15 mL) to yield **33** (222 mg, 75%) as an orange oil. Purification by CC (EtOAc/cyclohexane 1:1). ^1H NMR (300 MHz, $CDCl_3$): δ = 1.42 (t, *J* = 7.5 Hz, 3 H), 2.35 (t, *J* = 2.5 Hz, 1 H), 3.18 (q, *J* = 7.5 Hz, 2 H), 3.97 (dd, *J* = 3.7, 2.5 Hz, 2 H), 4.48 ppm (br. s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 8.3, 32.6, 47.8, 72.9, 78.9 ppm; IR (neat): $\tilde{n}$ =

3275, 3136, 3062, 2944, 2360, 1770, 1694, 1645, 1609, 1565, 1496, 1434, 1380, 1350, 1315, 1284, 1233, 1182, 1138, 1063, 930, 909, 833, 777, 715, 690, 648. Spectral data consistent with known data.

1,1,1-Trifluoro-*N*-prop-2-yn-1-ylmethanesulfonamide (34): *General procedure C*, starting from propargyl amine (**19**) (0.51 mL, 8 mmol), trifluoromethanesulfonyl chloride (**28**) (0.85 mL, 8 mmol), and Et_3N (1.22 mL, 8.8 mmol) in dry CH_2Cl_2 (60 mL). Purification by CC (EtOAc/cyclohexane 1:1) to yield **34** (748 mg, 50%) as an orange oil. ^1H NMR (300 MHz, ($CDCl_3$): δ = 2.42. (t, *J* = 2.5 Hz, 1H), 4.07-4.13 (m, 2H), 5.55 ppm (br. s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 33.9, 74.1, 76.7, 119.29 (*q*, *J* = 320.4 Hz), 145.4, 153.7, 164.0 ppm; IR (neat): $\tilde{n}$ = 3300, 2923, 2360, 2342, 1653, 1436, 1375, 1232, 1197, 1145, 1068, 635, 616 cm^{-1}; HRMS (ESI): calculated for $C_4H_3F_3NO_2S^-$ ([*M*-H]$^-$): 185.9842, found: 185.9840.

***N*-Prop-2-yn-1-ylmethanesulfonamide (35)**:[4] *General procedure C*, starting from propargyl amine (**19**) (0.51 mL, 8 mmol), methanesulfonyl chloride (**29**) (0.62 mL, 8 mmol), and Et_3N (1.22 mL, 8.8 mmol) in dry CH_2Cl_2 (60 mL). Purification by CC (EtOAc/cyclohexane 1:1) to yield **35** (940 mg, 88%) as a colorless crystalline solid (EtoAc/cyclohexane). ^1H NMR (300 MHz, $CDCl_3$): δ = 2.38 (t, *J* = 2.6 Hz, 1H), 3.09 (t, *J* = 1.2 Hz, 3H), 3.91-3.98

(m, 2H), 4.87 ppm (br. s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 32.6, 41.4, 73.3, 78.9 ppm; IR (neat): $\tilde{n}$ = 3298, 3269, 3020, 2937, 2122, 1758, 1438, 1412, 1327, 1302, 1245, 1149, 1061, 1004, 972, 923, 828, 764, 710, 680, 639, 616; MS (ESI): calculated for $C_4H_6NO_2S$ ([*M*-H]$^+$): 132.0, found: 132.0. Spectral data consistent with known data.

***N*-Prop-2-yn-1-ylpropane-1-sulfonamide (36):**[3] *General procedure C*, starting from propargyl amine (**19**) (0.51 mL, 8 mmol), 1-propanesulfonyl chloride (**30**) (0.90 mL, 8 mmol), and Et_3N (1.22 mL, 8.8 mmol), in dry CH_2Cl_2 (60 mL). Purification by CC (EtOAc/cyclohexane 1:2) to yield **36** (1.05 g, 82%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.00 (dt, *J* = 2.8, 7.5 Hz, 3H), 1.74-1.86 (m, 2H), 2.33 (t, *J* = 2.6 Hz, 1H), 3.04-3.10 (m, 2H), 3.86 (dd, *J* = 2.6, 5.9 Hz, 2H), 5.26 ppm (br. s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.8, 17.1, 32.2, 54.8, 72.6, 79.0 ppm; IR (neat): $\tilde{n}$ = 3274, 2937, 2361, 1431, 1322, 1258, 1045, 924, 867, 668, 634 cm^{-1}; HRMS (ESI): calculated for $C_6H_{11}NNaO_2S^+$ ([*M* + Na]$^+$): 184.0403, found: 184.0398. Spectral data consistent with known data.

***N*-Prop-2-yn-1-ylbenzenesulfonamide (37):**[3] *General procedure C*, starting from propargyl amine (**19**) (0.26 mL, 4 mmol), phenylsulfonyl chloride (**31**) (0.51 mL, 4 mmol), and Et_3N (0.61 mL, 4.4 mmol) in dry CH_2Cl_2 (30 mL).

Purification by CC (EtOAc/cyclohexane 1:3) to yield **37** (689 mg, 88%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.08 (t, *J* = 2.5 Hz, 1H), 3.83 (dd, *J* = 2.5, 5.9 Hz, 2H), 5.23 (br. s, 1H), 7.47-7.60 (m, 3H), 7.88-7.92 ppm (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 32.8, 72.9, 77.8, 127.1 (2C), 128.9 (2C), 132.8, 139.2 ppm; IR (neat): $\tilde{n}$ = 3283, 3012, 2672, 1448, 1326, 1159, 1094, 1070, 926, 835, 756, 722, 689, 633 cm^{-1}; HRMS (ESI): calculated for $C_9H_9NNaO_2S^+$ ([*M* + Na]$^+$): 218.0246, found: 218.0247. Spectral data consistent with known data.

3-[(4-Amino-5-iodo-2-oxopyrimidin-1(2*H*)-yl)methyl]-*N*,*N*-dimethyl-1*H*-pyrazole-1-sulfonamide (40): *General procedure B*, starting from **23** (97 mg, 0.410 mmol), NaH (16 mg, 0.410 mmol), and 3-(bromomethyl)-*N*,*N*-dimethyl-1*H*-pyrazol-1-sulfonamide (**38**)[5] (100 mg, 0.373 mmol) in anhydrous DMF (2 x 4 mL). The mixture was left to stir for 16 h. Purification by CC (CH_2Cl_2/MeOH 99:1 → 96:4) to yield **40** (151 mg, 95%) as a white solid. Mp: 196-198 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.05 (s, 6 H), 5.28 (s, 2 H), 6.43 (d, *J* = 1.9 Hz, 1 H), 7.60 (d, *J* = 1.9 Hz, 1 H), 7.86 ppm (s, 1 H); ^{13}C NMR (75 MHz, $(CD_3)_2SO$): δ = 38.6 (2C), 45.2, 56.5, 106.9, 142.7, 144.0, 151.8, 154.2, 164.4 ppm; IR (neat): $\tilde{n}$ = 3438, 2948, 1633, 1455, 1418, 1393, 1382, 1355, 1325, 1275, 1206, 1169, 1128, 1094, 1055, 1027, 971, 944, 920, 886, 810, 789, 779, 728,

714, 640, 625 cm^{-1}; HRMS (MALDI): calculated for $C_{10}H_{14}IN_6O_3S^+$ (*M*H^+): 424.9887, found: 424.9875; Anal. calculated for $C_{10}H_{13}IN_6O_3S$ (424.22): C 28.31, H 3.09, N 19.81; found: C 28.36, H 3.05, N 19.55.

4-Amino-1-benzyl-5-iodopyrimidin-2(1*H*)-one (41): General *procedure B*, starting from **23** (474 mg, 2.00 mmol), NaH (48 mg, 2.00 mmol), and benzylbromide (**39**) (311 mg, 1.82 mmol) in anhydrous DMF (2 x 20 mL). The mixture was left to stir for 24 h. Purification by CC (CH_2Cl_2/MeOH 98:2 → 90:10) to yield **41** (337 mg, 57%) as a white solid. Mp: 225-228 °C; ^{1}H NMR (300 MHz, $(CD_3)_2SO$): δ = 4.85 (s, 2 H), 6.54 (br. s, 1 H), 7.26-7.33 (m, 5 H), 7.72 (br. s, 1 H), 8.22 ppm (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3/CD_3OD$ 7:1): δ = 52.5, 56.6, 127.8 (2C), 128.2, 128.8 (2C), 135.1, 150.5, 156.4, 163.5 ppm; IR (thin film): $\tilde{n}$ = 2359, 2341, 1626, 1543, 1365, 1280, 1218, 1070, 1023, 827, 733, 681, 645 cm^{-1}; HRMS (MALDI): calculated for $C_{11}H_{11}IN_3O^+$(*M*H^+): 327.9941, found: 327.9944.

3-{[4-Amino-5-{3-[(ethylsulfonyl)amino]prop-1-yn-1-yl}-2-oxopyrimidin-1(2*H*)-yl]methyl}-*N*,*N*-dimethyl-1*H*-pyrazole-1-sulfonamide (42): *General procedure A*, starting from **40** (110 mg, 0.259 mmol), **33** (42 mg, 0.285 mmol), Et_3N (52 mg, 0.518 mmol), $[PdCl_2(PPh_3)_2]$ (18 mg, 0.052 mmol), and CuI (18 mg, 0.026 mmol) in anhydrous DMF (6 mL). The mixture was left to stir for 19 h. Purification by CC

(CH_2Cl_2/MeOH 99:1 → 96:4) to yield **42** (97 mg, 84%) as a yellow solid. Mp: 210-212 °C. ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD 7:1): δ = 1.32 (t, *J* = 7.5 Hz, 3 H), 2.98 (s, 6 H), 3.05 (q, *J* = 7.5 Hz, 2 H), 4.02 (s, 2 H), 5.18 (s, 2 H), 6.32 (d, *J* = 1.3 Hz, 1 H), 7.56 (d, *J* = 1.3 Hz, 1 H), 7.65 ppm (s, 1 H); ^{13}C NMR (75 MHz, CH_2Cl_2/MeOH 9:1): δ = 10.0, 33.0, 39.0 (2C), 44.7, 47.5, 74.5, 90.8, 91.6, 109.7, 140.7, 142.1, 147.9, 154.7, 164.7 ppm; IR (neat): $\tilde{n}$ = 3379, 3101, 1665, 1621, 1505, 1455, 1373, 1318, 1281, 1235, 1215, 1173, 1140, 1068, 976, 919, 786, 720, 695, 676, 655, 608 cm^{-1}; HRMS (MALDI): calculated for $C_{15}H_{22}N_7O_5S_2^+$ (*M*H$^+$): 444.1118, found: 444.1111.

Ethyl(4-amino-5-iodo-2-oxopyrimidin-1(2*H*)-yl)acetate (45): *General procedure B*, starting from **23** (948 mg, 4 mmol), NaH (176.4 mg, 4.4 mmol), and ethyl bromoacetate (**43**) (735 mg, 4.4 mmol) in anhydrous DMF (60 + 20 mL). The mixture was left to stir for 22 h. Purification by CC (CH_2Cl_2/MeOH 97.5:2.5) to yield **45** (1.15 g, 89%) as a white solid. Mp: 207-209 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.30 (t, *J* = 7.2 Hz, 3 H), 4.25 (q, *J* = 7.2 Hz, 2 H), 4.51 (s, 2 H), 7.57 ppm (s, 1 H); ^{13}C NMR (125 MHz, $(CD_3)_2SO$): δ = 14.0, 49.6, 55.6, 60.9, 152.2, 154.5, 164.3, 168.4 ppm; IR (neat): $\tilde{n}$ = 3460, 2977, 2250, 1723, 1633, 1478, 1412, 1397, 1368, 1356, 1330, 1282, 1230, 1207, 1093, 1012, 958, 920, 876, 819, 776, 731, 710, 645,

625; HRMS (MALDI): calculated for $C_8H_{11}IN_3O_3^+$ (*M*H$^+$): 323.9840, found: 323.9846; Anal. calculated for $C_8H_{10}IN_3O_3$ (323.09): C 29.74, H 3.15, N 13.01; found: C 29.78, H 3.06, N 13.13.

(±)-4-Amino-5-iodo-1-(tetrahydrofuran-2-ylmethyl)pyrimidin-2(1*H*)-one ((±)-46): *General procedure B*, starting from **23** (176 mg, 0.74 mmol), NaH (31 mg, 0.81 mmol), and tetrahydrofurfuryl bromide ((±)-**44**) (134 mg, 0.81 mmol) in anhydrous DMF (11 + 4 mL). The mixture was left to stir at 70 °C for 24 h. Purification by CC (CH_2Cl_2/MeOH 96:4) to yield (±)-**46** (101 mg, 43%) as a white solid. Mp: > 162 °C (dec.); ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$ 7:1): δ = 1.37-1.49 (m, 1H), 1.72-1.81 (m, 2H), 1.88-1.97 (m, 1H), 3.38-3.46 (m, 1H), 3.60-3.75 (m, 2H), 3.96-4.03 (m, 2H), 7.66 ppm (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3/CD_3OD$ 7:1): δ = 25.5, 28.4, 52.7, 55.4, 68.0, 76.7, 152.0, 156.0, 163.7 ppm; IR (neat): $\tilde{n}$ = 3385, 3070, 2975, 2885, 2503, 2359, 2323, 1803, 1651, 1615, 1557, 1538, 1527, 1471, 1417, 1386, 1373, 1363, 1316, 1270, 1214, 1189, 1162, 1095, 1068, 1020, 987, 949, 916, 904, 822, 778, 766, 668, 636, 615 cm^{-1}; HRMS (MALDI): calculated for $C_9H_{13}IN_3O_2^+$ (*M*H$^+$): 322.0047, found: 322.0041; Anal. calculated for $C_9H_{12}IN_3O_2$ (321.00): C 33.66, H 3.77; found: C 33.45, H 4.05.

Biological assays.

Materials. [1,3,4-$^{13}C_3$]4-Diphosphocytidyl-2*C*-methyl-D-erythritol (CDP-ME) was prepared as described earlier.[6] IspE *E. coli* was prepared according to the published procedure.[7] NADH and phosphoenolpyruvate potassium salt were purchased from *Biomol*, ATP and pyruvate kinase/lactate dehydrogenase from *Sigma-Aldrich*.

Determination of the IC_{50} value by initial rate measurements. Assay mixtures were prepared as described earlier[8] with some modifications: 60 µL of a solution containing 100 mM Tris hydrochloride, pH 8.0, 10 mM $MgCl_2$, 2 mM dithiothreitol, 2.5 mM phosphoenolpyruvate potassium salt, 2 mM ATP, 0.46 mM NADH, 1 U of lactate dehydrogenase, 1 U of pyruvate kinase, and IspE protein were added to 60 µl of the inhibitor solutions (final concentration varied from 8 – 1000 µM). The reaction was started by addition of 60 µL of CDP-ME (final concentration 1 mM) and monitored at 340 nm.

Determination of the K_i value by initial rate measurements. Assay mixtures were prepared as follows: 50 µL of a solution containing 100 mM Tris hydrochloride, pH 8.0, 10 mM $MgCl_2$, 2 mM dithiothreitol, 2.5 mM phosphoenolpyruvate potassium salt, 2 mM ATP, 0.46 mM NADH, 1 U of lactate dehydrogenase, and 1 U of pyruvate kinase were added to 50 µl each of the inhibitor solution of IspE protein. The reaction was started by addition of

50 µL CDP-ME. For the determination of each single K_M value in the presence of the inhibitor, the concentration of CDP-ME was varied from 35 to 400 µM, while keeping the concentration of inhibitor fixed. The mixtures were incubated at 27 °C, and the reaction was monitored photometrically at 340 nm. The K_{ic} and K_{iu} values of the inhibitors were obtained by observing the behavior of K_M values at different inhibitor concentrations (0 – 32 µM) and calculated using the program Dynafit.[9]

Monitoring the inhibition of the reaction catalyzed by IspE *via* ^{13}C NMR spectroscopy. Assay mixtures contained 100 mM Tris hydrochloride, pH 8.0, 10 mM $MgCl_2$, 5 mM ATP, 10 % (v/v) D_2O, 2 mM dithiothreitol, 1.5 mM [1,3,4-$^{13}C_3$]-CDP-ME, and 13 µg IspE protein in a volume of 500 µL. Inhibitory substances were added at final concentrations ranging from 0 to 1 mM. The mixtures were incubated at 37 °C and terminated by the addition of EDTA to a final concentration of 20 mM. The solution was analyzed by ^{13}C NMR spectroscopy on a *Bruker DRX 500* (125 MHz) and referenced to an internal standard of [1-$^{13}C_1$]-glucose (0.9 mM).

X-ray crystal structure of (±)-13.

Crystal data at 223 K for ($C_{13}H_{15}F_3N_4O_3S_2$) [M_r = 396.41]: monoclinic, space group C2/c, Z = 8, r_{calcd} = 1. 565 g cm$^-$

^{3}, a = 23.4052 (7), b = 13.5616 (5), c = 10.7568 (4) Å, ß = 99.700 (2)°, V = 3365.5 (2) Å^3. Bruker-Nonius Kappa-CCD diffractometer, Mo-Ka radiation, ? = 0.7107 Å. Number of reflections measured = 6470, independent = 3844, R= 0.022. The structure was solved by direct methods (SIR97)[10] and refined by full-matrix least-squares analysis (SHELXL- 97),[11] calculated H-atoms were included in the final structure factor calculation. Both the trifluoromethyl group and the tetrahydrothiophenylring are heavily disordered leading to a relatively bad agreement factor. Final R(*F*) = 0.103, wR(F^2) = 0.318 for 226 parameters and 3844 reflections with I > 2s (I) and T < 27.5°. CCDC- 632407 contains the supplementary crystallographic data for this paper and is available free of charge from the Cambridge Crystallographic Data Centre (CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; Tel.: (+44) 1223-336-408; Fax: (+44) 1223-336-033; E-mail: deposit@ccdc.cam.ac.uk).

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