Supporting information. Discovery of a Series of Indane-Containing NBTIs with Activity Against Multidrug-Resistant Gram-Negative Pathogens

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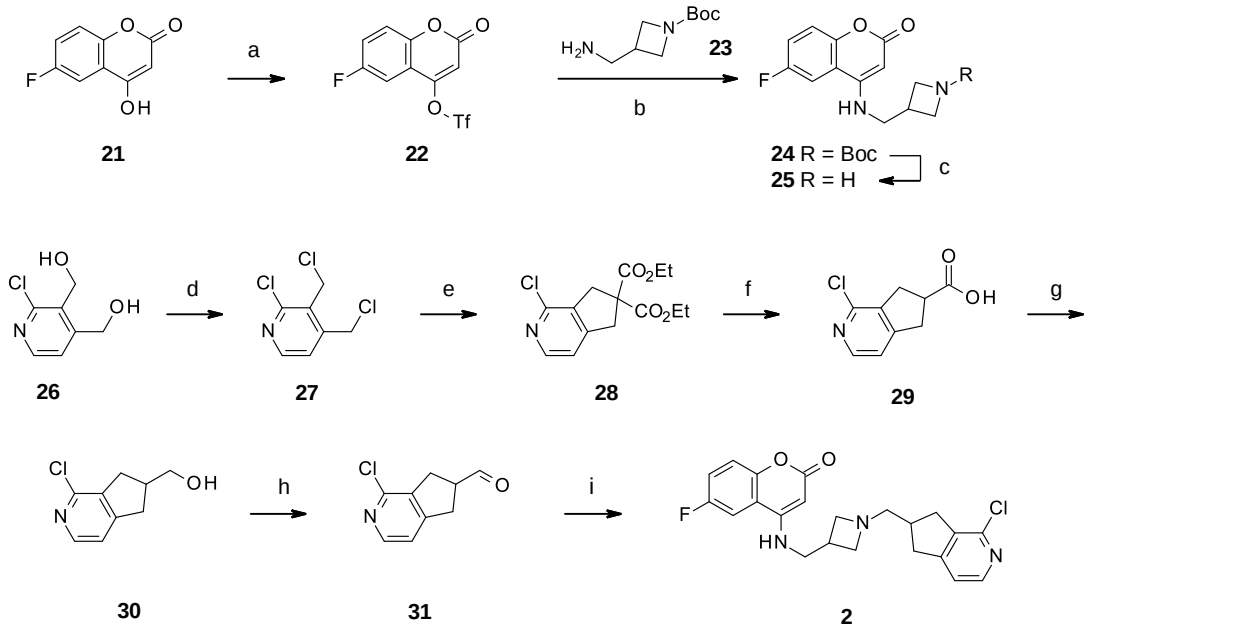
Experimental Procedures

General Methods for Chemistry. All solvents and reagents were obtained from commercial sources and used as received. All reactions were followed by TLC (TLC plates F254, Merck) or LCMS (liquid chromatography–mass spectrometry) analysis. Silica gel chromatography was either performed using cartridges packed with silica gel (ISOLUTE columns, TELOS flash columns) or silica-NH_2 gel (TELOS flash NH_2 columns) on ISCO Combi flash companion or on glass columns using silica gel 60 (32–60 mesh, 60 Å). ^{1}H and ^{13}C NMR spectra were obtained on a Bruker Avance 300, 400, or 600 MHz spectrometer. The samples were prepared by dissolving the material into 0.85 mL of deuterated solvent. The sample temperature was maintained at 25 °C for all data collections, and spectra were acquired and processed using Bruker TopSpin software, version 3. Chemical shifts (δ in ppm) are reported relative to tetramethylsilane with the residual solvent peak as the internal reference (i.e., chloroform-*d* = 7.26 ppm, DMSO-d_6 = 2.50 ppm). ^{1}H resonances are reported to the nearest 0.01 ppm. NMR abbreviations are as follows: s, singlet; d, doublet; t, triplet; q, quadruplet; quint, quintuplet; sext, sextuplet; m, multiplet; and br, broadened. Coupling constants (*J*) are reported to the nearest 0.1 Hz. All assay compounds had a purity of >95%. LC-MS (ESI, positive or negative ion) data were recorded on Waters UPLC-MS systems equipped with Waters Acquity, a CTC PAL autosampler and a Waters single quadrupole mass (SQD) spectrometer using ESI modes (positive and/or negative). The separation was achieved on a Zorbax Eclipse Plus C18 1,7 μm 2.1 × 30 mm column at 50 °C; A = 0.01% formic acid in water, B = acetonitrile at flow 1; gradient: 0 min 3% B, 0.2 min 3% B, 2 min 97% B, 1.7 min 97% B, and 2.0 min 97% B. The injection volume was 2 μL. MS (ESI, positive or negative ion): FIA (flow injection analysis)–MS was recorded on an Applied Biosystem API150 mass spectrometer. Sample introduction was made with a CTC PAL autosampler and a Shimadzu LC-10ADVP pump. LC-MS high-resolution spectra were recorded with an Agilent LC system consisting of an Agilent 1290 high-pressure gradient system and an Agilent 6545B QTOF. The separation was achieved on a Zorbax Eclipse Plus C18 1,8 μm 2.1 × 50 mm column at 55 °C; A = 0.02% formic acid in water and B = acetonitrile with 0.01% formic acid at flow 0.8 mL/min. gradient: 0 min 5% B, 0.3 min 5% B, 4.5 min 99% B 5 min 99% B. The injection volume was 2 μL. Ionization was performed in an Agilents Multimode source. The mass spectrometer was run in the "2 GHz extended dynamic range" mode, resulting in a resolution of about 20 000 at m/z = 922. Mass accuracy was ensured by internal drift correction. All of the reported yields are for

isolated products and not optimized. No unexpected or unusually high safety hazards were encountered.

Synthetic procedure for the synthesis of compound 2

Scheme S1. Synthesis of compound **2**.



Reagents and conditions: (a) Tf_2O, NEt_3, DCM, 0 ºC; (b) **23**, $iPrNEt_2$, MeCN, 90 ºC; (c) HCl, dioxane, 25 ºC; (d) $SOCl_2$, DCM, 25 ºC; (e) $(EtO_2C)_2CH_2$, NaH, THF, 0 ºC to 25 ºC; (f) HCl, 100 ºC; (g) $LiAlH_4$, THF, 0 ºC; (h) Dess-Martin periodinane, DCM, 25 ºC; (i) **25**, $NaBH(OAc)_3$, 3Å mol. sieves, AcOH, THF, MeOH, 25 ºC.

Step a: To a suspension of 6-fluoro-4-hydroxy-2*H*-chromen-2-one **21** (6.45 g, 35.4 mmol, 1 eq) in DCM (100 mL) was added triethylamine (4.3 g, 5.93 mL, 42.5 mmol, 1.2 eq). The resulting solution was cooled to 0 °C and trifluoromethanesulfonic anhydride (12.2 g, 7.33 ml, 42.5 mmol, 1.2 eq) was added dropwise, then the mixture was stirred at 0 °C for 3 h. Water was added to the reaction mixture and the resulting mixture extracted twice with DCM. The combined organic layers were washed with water, dried over Na_2SO_4 and concentrated *in vacuo* to give 6-fluoro-2-oxo-2*H*-chromen-4-yl trifluoromethanesulfonate **22** (11.3 g, 36.2 mmol, quant. yield) as a light brown solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.45–7.35 (m, 3H), 6.57 (d, J = 0.6 Hz, 1H).

Step b: To a mixture of 6-fluoro-2-oxo-2*H*-chromen-4-yl trifluoromethanesulfonate **22** (1.3 g, 4.16 mmol, 1 eq) and *tert*-butyl 3-(aminomethyl)azetidine-1-carboxylate **23** (776 mg, 758 µL, 4.16 mmol, 1 eq) in MeCN (70 mL) was added *N*,*N*-diisopropylethylamine (1.08 g, 1.45 ml, 8.33

mmol, 2 eq) and the mixture heated to 90 °C with stirring for 1 h 30 min. The crude reaction mixture was evaporated. DCM was added and the product was filtered off and dried *in vacuo* to give *tert*-butyl 3-(((6-fluoro-2-oxo-2*H*-chromen-4-yl)amino)methyl)azetidine-1-carboxylate **24** (1.24 g, 3.56 mmol, 85.4 % yield) as a white solid. LC-MS (ESN) m/z: $[M–H]^-$ calculated for $[C_{18}H_{21}FN_2O_4–H]^-$: 347.1, found: 347.3. ^{1}H NMR (300 MHz, DMSO-d6) δ ppm 7.96 (dd, J = 2.8, 9.9 Hz, 1H), 7.60 (t, J = 5.1 Hz, 1H), 7.47 (dd, J = 2.8, 8.1 Hz, 1H), 7.41–7.35 (m, 1H), 5.31 (s, 1H), 3.92 (m, 2H), 3.60 (m, 2H), 3.52–3.40 (m, 2H), 2.87 (t, J = 7.8 Hz, 1H), 1.37 (s, 9H).

Step c: To *tert*-butyl 3-(((6-fluoro-2-oxo-2*H*-chromen-4-yl)amino)methyl)azetidine-1-carboxylate **24** (1.24 g, 3.56 mmol, 1 eq) eq was added HCl 4M in dioxane (17.8 mL, 71.1 mmol, 20 eq) and the mixture stirred at 25 ºC for 2 h. The mixture was evaporated totally and the residue taken up in DCM. $NaHCO_3$ (sat. aq.) was added and the aqueous layer was extracted 5 times with DCM. The combined organic extracts were dried over Na_2SO_4 and evaporated to give the crude product which was purified by silica cartridge chromatography (20 g SiO_2, DCM to 10% MeOH/DCM to 1% NH_4OH in 10% MeOH/DCM) to give 4-((azetidin-3-ylmethyl)amino)-6-fluoro-2*H*-chromen-2-one **25** (200 mg, 0.806 mmol, 22.7 % yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d6) δ ppm 7.99 (dd, J = 2.8, 10.1 Hz, 1H), 7.61 (br s, 1H), 7.46 (dd, J = 2.8, 8.1 Hz, 1H), 7.40–7.34 (m, 1H), 5.23 (s, 1H), 3.57 (t, J = 7.8 Hz, 2H), 3.48–3.41 (m, 2H), 3.27–3.21 (m, 2H), 3.06–2.83 (m, 1H).

Step d: To a solution of [2-chloro-3-(hydroxymethyl)-4-pyridyl]methanol **26** (for preparation, see WO 2013127268 A1) (15.0 g, 86.4 mmol, 1 eq) in DCM was added thionyl chloride (20.6 g, 12.6 mL, 173 mmol, 2 eq) at 25 °C, and then the solution was stirred at 25 °C for 2 h. The mixture was evaporated totally; to the residue was added DCM (200 mL) and water (100 mL), then Na_2CO_3 (sat. aq.) was added to reach pH 8. The mixture was extracted twice with DCM, the combined organic extracts were washed with brine, and dried over Na_2SO_4. The solvent was removed to give crude 2-chloro-3,4-bis(chloromethyl)pyridine **27** (7.7 g, 36.6 mmol, 42.3 % yield) as a dark red oil which was used directly without purification. LC-MS (ESP) m/z: $[M+H]^+$ calculated for $[C_7H_6Cl_3N+H]^+$: 210.0, found: 210.0.

Step e: To a solution of diethyl malonate (5.86 g, 5.58 ml, 36.6 mmol, 1 eq) in THF (200 mL) was added sodium hydride (2.93 g, 73.2 mmol, 2 eq) at 0 °C, and the solution was stirred at 0 °C for 1 h. After that the solution was added to a stirred cooled to 0 °C solution of 2-chloro-3,4-bis(chloromethyl)pyridine **27** (7.7 g, 36.6 mmol, 1 eq) in THF (145 mL), and then the solution

was stirred at 25 °C for 16 h. The mixture was poured into ice-water (50 mL) and the solution was extracted with EtOAc (30 mL x 2) and the combined organic layers were dried with Na_2SO_4. The solvent was removed to give crude product which was purified by silica cartridge chromatography (70 g SiO_2, heptane to EtOAc) to give diethyl 1-chloro-5,7-dihydrocyclopenta[c]pyridine-6,6-dicarboxylate **28** (4.5 g, 15.1 mmol, 41.3 % yield) as a light yellow liquid. LC-MS (ESP) m/z: $[M+H]^+$ calculated for $[C_{14}H_{16}ClNO_4+H]^+$: 298.1, found: 298.2. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 8.21 (d, J = 5.0 Hz, 1H), 7.10 (d, J = 5.0 Hz, 1H), 4.24 (q, J = 7.5 Hz, 4H), 3.68–3.63 (m, 3H), 3.45–3.22 (m, 1H), 1.28 (t, J = 7.5 Hz, 6H).

Step f: To diethyl 1-chloro-5,7-dihydrocyclopenta[c]pyridine-6,6-dicarboxylate **28** (4.5 g, 15.1 mmol, 1 eq) was added hydrochloric acid (55.1 g, 49.2 mL, 378 mmol, 25 eq), and then the solution was stirred at 100 °C for 16 h. The cooled mixture was poured onto water and washed twice with DCM. The aqueous layer was evaporated and the residue triturated with toluene twice followed by evaporation to give crude 1-chloro-6,7-dihydro-5*H*-cyclopenta[c]pyridine-6-carboxylic acid **29** (2.86 g, 14.5 mmol, 95.8 % yield) as a light brown solid which was used in the next step without any further purification. LC-MS (ESP) m/z: $[M+H]^+$ calculated for $[C_9H_8ClNO_2+H]^+$: 198.0, found: 198.1. ^{1}H NMR (300 MHz, DMSO-d6) δ ppm 11.12 (br s, 1H), 8.19 (d, J = 4.9 Hz, 1H), 7.33 (d, J = 4.9 Hz, 1H), 3.45–2.92 (m, 5H).

Step g: To an ice-cooled solution of 1-chloro-6,7-dihydro-5*H*-cyclopenta[c]pyridine-6-carboxylic acid **29** (1.2 g, 6.07 mmol, 1 eq) in THF (50 mL) was added 2M lithium aluminum hydride in THF (4.6 mL, 9.2 mmol, 1.5 eq) and then the solution was stirred at 0 °C for 2 h. The reaction was quenched by the dropwise addition of acetone (2.5 mL). To the mixture was added water (100 mL) and the mixture was stirred vigorously at 25 ºC for 2 h. The aqueous phase was extracted with Et_2O twice and the combined organic layers were dried over Na_2SO_4 and evaporated to give a crude product which was purified by silica cartridge chromatography (20 g SiO_2, heptane to EtOAc) to give (1-chloro-6,7-dihydro-5*H*-cyclopenta[c]pyridin-6-yl)methanol **30** (700 mg, 3.81 mmol, 62.8 % yield) as a white solid. LC-MS (ESP) m/z: $[M+H]^+$ calculated for $[C_9H_{10}ClNO+H]^+$: 184.0, found: 184.0. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 8.16 (d, J = 5.0 Hz, 1H), 7.09 (d, J = 5.0 Hz, 1H), 3.68 (br s, 2H), 3.24–3.02 (m, 2H), 2.93–2.70 (m, 3H), 1.87–1.72 (m, 1H).

Step h: (1-Chloro-6,7-dihydro-5*H*-cyclopenta[c]pyridin-6-yl)methanol **30** (700 mg, 3.81 mmol, 1 eq) was dissolved in DCM (38 mL) and the solution was cooled to 0 °C. Dess-Martin reagent

(1.7 g, 4 mmol, 1.05 eq) was added and the ice bath was removed. After 1 h at 25 ºC the reaction was quenched by the addition of sat. $Na_2S_2O_3$ (aq) and sat. $NaHCO_3$ (aq). The mixture was extracted 3 x with DCM and the combined organic layers were dried with $MgSO_4$ and evaporated. The residue was purified by silica cartridge chromatography eluting with 0-50% EtOAc in heptane to give 1-chloro-6,7-dihydro-5*H*-cyclopenta[c]pyridine-6-carbaldehyde **31** (530 mg, 2.92 mmol, 76.6 % yield) as light yellow oil. LC-MS (ESP) m/z: $[M+H]^+$calculated for $[C_9H_8ClNO+H]^+$: 182.0, found: 182.1. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 9.79 (d, J = 0.8 Hz, 1H), 8.21 (d, J = 4.9 Hz, 1H), 7.13 (d, J = 4.9 Hz, 1H), 3.52–3.14 (m, 5H).

Step i: To a solution of 4-((azetidin-3-ylmethyl)amino)-6-fluoro-2*H*-chromen-2-one **25** (200 mg, 0.806 mmol, 1 eq) in THF (4 mL) and methanol (4 mL) was added powdered molecular sieves 3Å (200 mg), 1-chloro-6,7-dihydro-5*H*-cyclopenta[c]pyridine-6-carbaldehyde **31** (176 mg, 0.967 mmol, 1.2 eq) and acetic acid (82.2 mg, 78.4 µL, 1.37 mmol, 1.7 eq) and the mixture was stirred for 30 minutes at 25 ºC. Sodium triacetoxyborohydride (290 mg, 1.37 mmol, 1.7 eq) was added and the mixture stirred for 30 minutes at 25 ºC. Na_2CO_3 (sat. aq.) was added to the reaction mixture, which was extracted 3 x with DCM. The combined organic layers were washed with NaCl (sat. aq.), dried over Na_2SO_4 and concentrated *in vacuo* to give the crude product which was purified by flash chromatography (20 g SiO_2, 0 % to 10 % MeOH/DCM) to give 4-(((1-((1-chloro-6,7-dihydro-5*H*-cyclopenta[c]pyridin-6-yl)methyl)azetidin-3-yl)methyl)amino)-6-fluoro-2*H*-chromen-2-one **2** (260 mg, 0.628 mmol, 78% yield, 100% purity) as a white solid. LC-HRMS (m/z): $[M+H]^+$ calculated for $[C_{22}H_{21}ClFN_3O_2+H]^+$: 414.1379, found: 414.1383. ^{1}H NMR (600 MHz, DMSO-d6) δ ppm 8.16 (dt, J = 4.9, 1.0 Hz, 1H), 7.98 (dd, J = 10.0, 2.9 Hz, 1H), 7.63 (t, J = 5.4 Hz, 1 H), 7.48 (ddd, J = 9.1, 8.0, 2.9 Hz, 1H), 7.38 (dd, J = 9.1, 4.8 Hz, 1H), 7.26–7.30 (m, 1H), 5.24 (s, 1H), 3.46 (dd, J = 6.7, 5.6 Hz, 2H), 3.22–3.30 (m, 2H), 3.04–3.12 (m, 1H), 2.94–3.02 (m, 1H), 2.94–3.03 (m, 2H), 2.71–2.81 (m, 2H), 2.63–2.70 (m, 1H), 2.48 (br dd, J = 3.8, 1.8 Hz, 2H), 2.47 (br s, 1H). ^{13}C NMR (151 MHz, DMSO-d6, 25 ºC) δ ppm 161.7, 158.1 (d, J = 238 Hz), 156.5, 153.0, 149.8, 148.1, 147.0, 137.9, 120.3, 119.6 (d, J = 27 Hz), 119.3, 115.8, 108.9 (d, J = 27 Hz), 82.4, 63.7, 58.3, 46.1, 37.8, 36.5, 35.7, 29.3. ^{19}F NMR (565 MHz, DMSO-d6) δ ppm –118.79 (dd, J = 4.9, 9.4 Hz, 1F).

Figure S1. ^{1}H NMR spectrum of compound **2**.

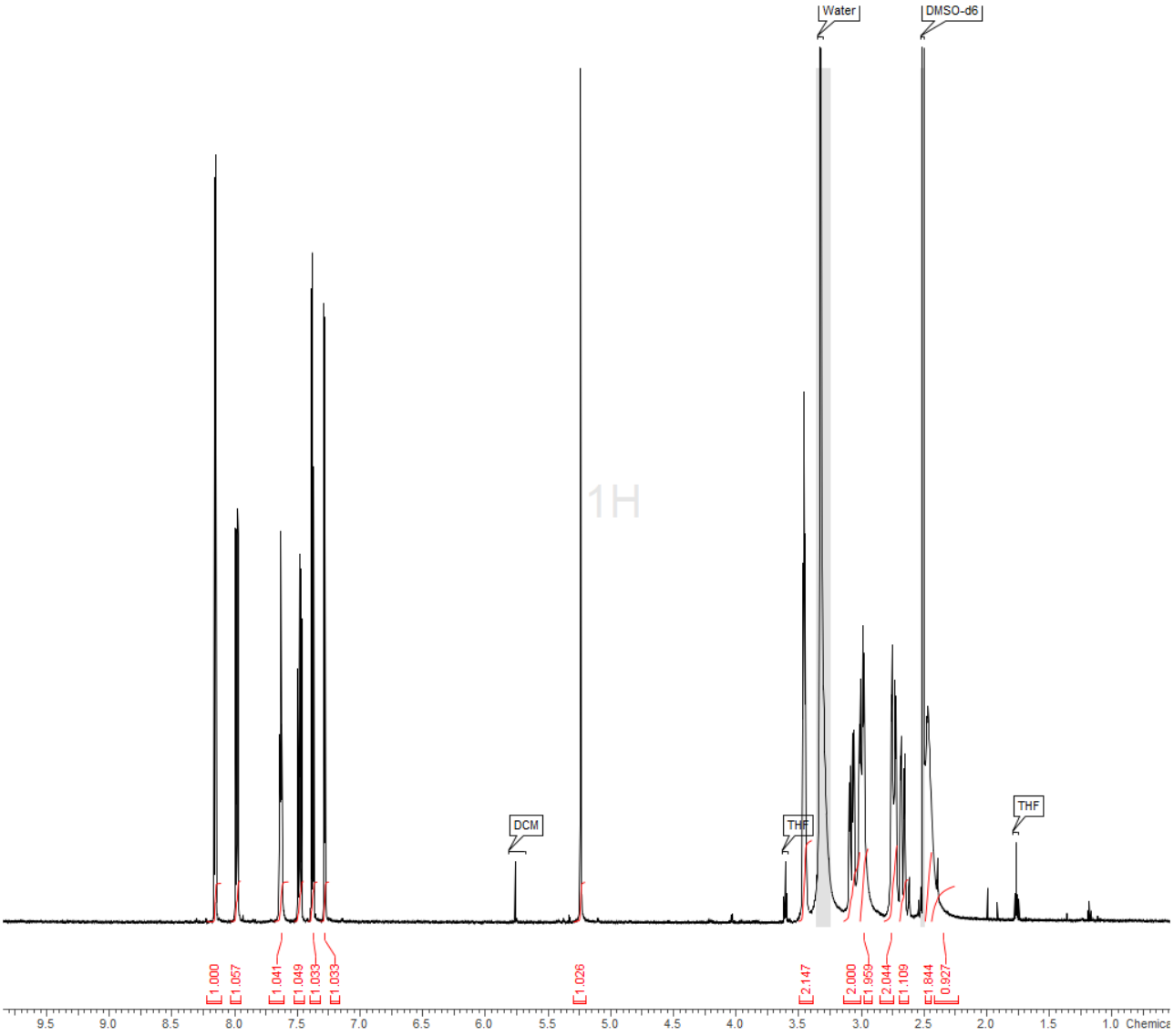
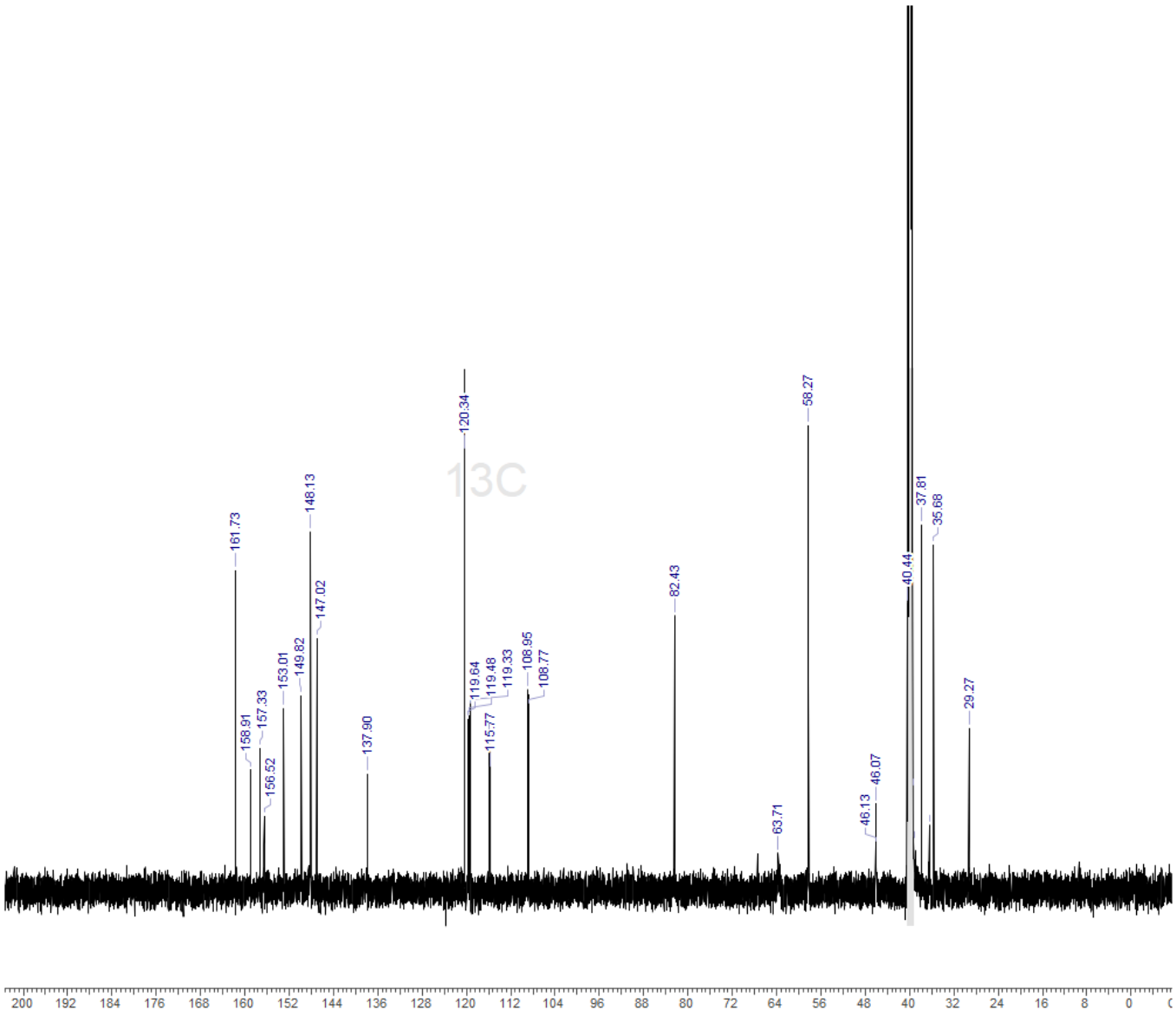
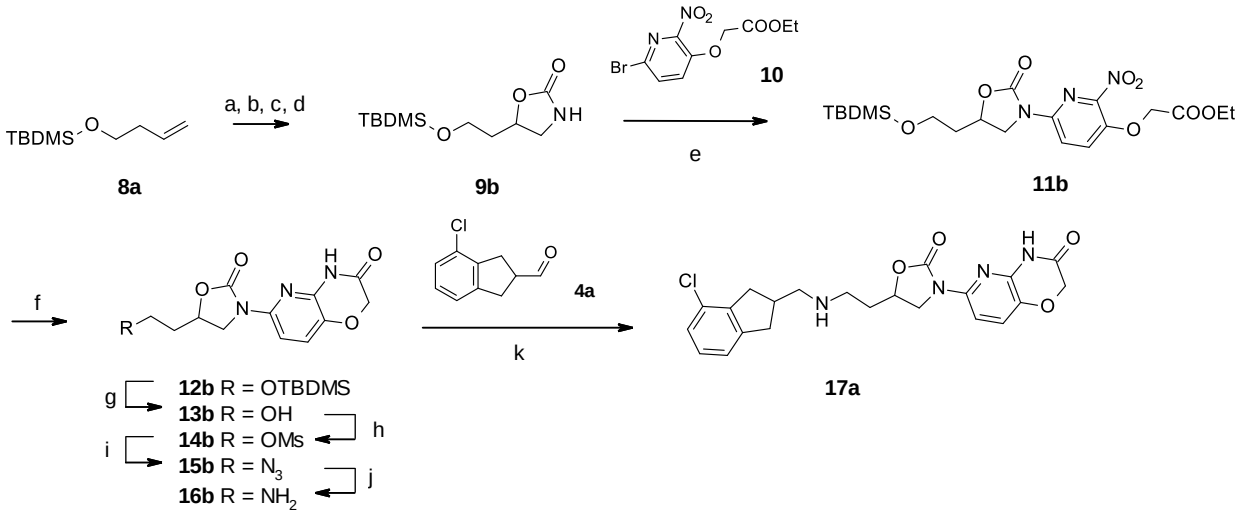


Figure S2. ^{13}C NMR spectrum of compound **2**.



Synthetic procedure for the synthesis of compound 17a and its four individual stereoisomers

Scheme S2. Synthesis of compound **17a**.



Reagents and conditions: (a) mCPBA, CH_2Cl_2, 0 ºC; (b) NaN_3, NH_4Cl, MeOH, 80 ºC; (c) H_2, Pd/C, THF, 25 ºC; (d) CDI, THF, 50 ºC; (e) **10**, CuI (0.2 eqiv), $Me_2NCH_2CH_2NMe_2$ (0.4 equiv), K_2CO_3, 1,4-dioxane, 100 ºC; (f) Fe, AcOH, 70 ºC; (g) HCl, MeOH, 25 ºC; (h) MsCl, NEt_3, CH_2Cl_2, 0 ºC to 25 ºC; (i) NaN_3, DMF, 60 ºC; (j) H_2, Pd/C, MeOH, 25 ºC; (k) **4a**, $Na(CN)BH_3$, MeOH, 25 ºC.

Step a: To a stirred solution of but-3-enoxy-*tert*-butyl-dimethyl-silane **8a** (17.8 g, 95.5 mmol, 1 eq) in DCM (250 mL) cooled to 0 °C, was added portion-wise 3-chloroperbenzoic acid (23.3 g, 114 mmol, 1.2 eq) and the mixture stirred at 25 °C for 16 h. The reaction mixture was filtered to remove inorganics. Then, the filtrate was quenched with 10% sodium thiosulphate solution and extracted with DCM (100 mL x 3). The organic layers were combined and washed with 10% sodium bicarbonate solution (100 mL), dried over $MgSO_4$, filtered and concentrated to provide *tert*-butyl-dimethyl-[2-(oxiran-2-yl)ethoxy]silane (19.3 g, 95.4 mmol, 99.9% yield) as a light brown oil which was used in the next step directly. ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 3.71 (dd, J = 5.7, 6.7 Hz, 2H), 2.99–2.91 (m, 1H), 2.73–2.66 (m, 1H), 2.48–2.44 (m, 1H), 1.72–1.56 (m, 2H), 0.88 (s, 9H), 0.00 (s, 6H).

Step b: To a solution of *tert*-butyl-dimethyl-[2-(oxiran-2-yl)ethoxy]silane (19.3 g, 95.4 mmol, 1.0 eq) in methanol (600 mL) was added sodium azide (10.0 mL, 286 mmol, 3.0 eq) and ammonium chloride (10.2 g, 191 mmol, 2.0 eq), and then the solution was stirred at 80 °C for 16 h. The solvent was evaporated under reduced pressure and the residue was partitioned between EtOAc (200 mL) and water (100 mL). The organic layer was washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure, affording crude 1-azido-4-[*tert*-butyl(dimethyl)silyl]oxy-butan-2-ol (23.0

g, 93.7 mmol, 98.3% yield) as a yellow oil which was used in the next step directly. ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 4.96 (br d, J = 5.3 Hz, 1H), 3.72–3.61 (m, 3H), 3.23–3.09 (m, 2H), 1.68–1.39 (m, 2H), 0.83 (s, 9H), 0.01 (s, 6H).

Step c: To a solution of 1-azido-4-[*tert*-butyl(dimethyl)silyl]oxy-butan-2-ol (23.0 g, 93.7 mmol, 1.0 eq) in THF (500 mL) was added 10% Pd/C catalyst (2 g), and then the mixture was stirred under a hydrogen balloon at 25 °C for 4 h. The catalyst was filtered off and the filtrate was concentrated to give crude 1-amino-4-[*tert*-butyl(dimethyl)silyl]oxy-butan-2-ol (18.0 g, 82.0 mmol, 87.5% yield) as a brown oil which was used in the next step directly. ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 4.37 (br s, 1H), 3.71–3.56 (m, 2H), 3.53–3.40 (m, 1H), 2.58–2.52 (m, 1H), 2.46–2.34 (m, 1H), 1.86–1.64 (m, 1H), 1.64–1.31 (m, 3H), 0.87 (s, 9H), 0.04 (s, 6H).

Step d: To a solution of 1-amino-4-[*tert*-butyl(dimethyl)silyl]oxy-butan-2-ol (6.0 g, 27.4 mmol, 1.0 eq) in THF (60 mL) was added *N*,*N'*-carbonyldiimidazole (6.7 g, 41.0 mmol, 1.5 eq), and the solution was stirred at 50 °C for 16 h. The solvent was removed by evaporation and the residue was taken up in EtOAc (100 mL) and the solution was washed with brine (100 mL) and dried over Na_2SO_4. The solvent was removed by evaporation and the crude product was purified by chromatography on silica gel (petroleum ether: EtOAc = 2:1) to give 5-[2-[*tert*-butyl(dimethyl)silyl]oxyethyl]oxazolidin-2-one **9b** (3.8 g, 15.5 mmol, 57% yield) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 5.76 (br s, 1H), 4.82 (dq, J = 5.7, 7.6 Hz, 1H), 3.83–3.69 (m, 3H), 3.35 (t, J = 7.9 Hz, 1H), 2.06–1.81 (m, 2H), 0.90 (s, 9H), 0.08 (s, 6H).

Step e: To a solution of 5-[2-[*tert*-butyl(dimethyl)silyl]oxyethyl]-1,3-oxazolidin-2-one **9b** (2614 mg, 10.7 mmol, 1.3 eq) and ethyl 2-[(6-bromo-2-nitro-3-pyridyl)oxy]acetate **10** (2.5 g, 8.19 mmol, 1.0 eq) in 1,4-dioxane (50 mL) was added potassium carbonate (2380 mg, 17.3 mmol, 2.1 eq), copper (I) iodide (312 mg, 1.64 mmol, 0.2 eq) and *N*,*N'*-dimethyl-ethylenediamine (289 mg, 3.28 mmol, 0.4 eq). The mixture was degassed with nitrogen three times and stirred at 100 °C for 2 h. The mixture was filtered and the filtrate was concentrated under vacuum to give the crude product (3.0 g). The crude product was purified by flash chromatography on silica gel (petroleum ether: EtOAc =10:1 to 3:1) to give ethyl 2-[[6-[5-[2-[*tert*-butyl(dimethyl)silyl]oxyethyl]-2-oxo-oxazolidin-3-yl]-2-nitro-3-pyridyl]oxy]acetate **11b** (2.60 g, 5.54 mmol, 67.6% yield) as a yellow solid. LC-MS (ESP) m/z: $[M+H]^+$ calculated for $[C_{20}H_{31}N_3O_8Si+H]^+$: 470.2, found: 470.1.

Step f: A mixture of ethyl 2-[6-[5-[2-[*tert*-butyl(dimethyl)silyl]oxyethyl]-2-oxo-1,3-oxazolidin-3-yl]-2-nitropyridin-3-yl]oxyacetate **11b** (2.60 g, 5.54 mmol, 1 eq) and iron (1.55 g, 27.7 mmol, 5

eq) in acetic acid (50 mL) was stirred at 70 °C for 2 h. The mixture was cooled to 25 ºC. The solid was filtered off and washed with EtOAc (100 mL) then the filtrate was concentrated and the residue was taken up in water (50 mL) and the pH of the solution was adjusted to around 10 by progressively adding $NaHCO_3$ (aq). The solution was extracted with EtOAc (3 x 50 mL) and the combined organics dried over Na_2SO_4. The solvent was removed to give the crude product which was purified by flash chromatography on silica gel (petroleum ether: EtOAc =2:1) to give 6-[5-[2-[*tert*-butyl(dimethyl)silyl]oxyethyl]-2-oxo-oxazolidin-3-yl]-4*H*-pyrido[3,2-b][1,4]oxazin-3-one **12b** (1500 mg, 3.81 mmol, 68.8% yield) as a white solid. LC-MS (ESP) m/z: $[M+H]^+$ calculated for $[C_{18}H_{27}N_3O_5Si+H]^+$: 394.2, found: 394.1.

Step g: To a solution of 6-[5-[2-[*tert*-butyl(dimethyl)silyl]oxyethyl]-2-oxo-oxazolidin-3-yl]-4*H*-pyrido[3,2-b][1,4]oxazin-3-one **12b** (1500 mg, 3.81 mmol, 1.0 eq) in methanol (10 mL) was added 4M HCl in MeOH (9.7 mL, 38.8 mmol, 10.2 eq), and then the solution was stirred at 25 °C for 0.5 h. The solid was collected by filtration and then was dried under reduced pressure to give 6-[5-(2-hydroxyethyl)-2-oxo-oxazolidin-3-yl]-4*H*-pyrido[3,2-b][1,4]oxazin-3-one hydrochloride **13b** (1200 mg, 3.8 mmol, 99.7% yield) as a white solid. LC-MS (ESP) m/z: $[M+H]^+$ calculated for $[C_{12}H_{13}N_3O_5+H]^+$: 280.1, found: 280.1.

Step h: To a solution of 6-[5-(2-hydroxyethyl)-2-oxo-oxazolidin-3-yl]-4*H*-pyrido[3,2-b][1,4]oxazin-3-one hydrochloride **13b** (500 mg, 1.58 mmol, 1.0 eq) and triethylamine (0.88 mL, 6.33 mmol, 4.0 eq) in DCM (50 mL) and THF (20 mL) was added methanesulfonyl chloride (0.37 mL, 4.75 mmol, 3.0 eq) at 0 °C, and then the solution was stirred at 25 °C for 2 h. The solution was washed with water (10 mL) and dried over Na_2SO_4. The solvent was removed to give crude 2-[2-oxo-3-(3-oxo-4*H*-pyrido[3,2-b][1,4]oxazin-6-yl)oxazolidin-5-yl]ethyl methanesulfonate **14b** (565 mg, 1.58 mmol, quant. yield) as a colorless oil which was used directly in the next step.

Step i: To a solution of 2-[2-oxo-3-(3-oxo-4*H*-pyrido[3,2-b][1,4]oxazin-6-yl)oxazolidin-5-yl]ethyl methanesulfonate **14b** (565 mg, 1.58 mmol, 1.0 eq) in DMF (20 mL) was added sodium azide (0.28 mL, 7.9 mmol, 5.0 eq), and then the mixture was stirred at 50 °C for 16 h. The mixture was poured into water (50 mL) and extracted with EtOAc (2 x 50 mL). The combined organics were dried over Na_2SO_4. The solvent was removed to give the crude product which was washed with MTBE (30 mL) and dried under reduced pressure to give 6-[5-(2-azidoethyl)-2-oxo-oxazolidin-3-yl]-4*H*-pyrido[3,2-b][1,4]oxazin-3-one **15b** (430 mg, 1.41 mmol, 89.5% yield) as a yellow solid. LC-MS (ESP) m/z: $[M+H]^+$ calculated for $[C_{12}H_{12}N_6O_4+H]^+$: 305.1, found: 305.1.

Step j: To a solution of 6-[5-(2-azidoethyl)-2-oxo-oxazolidin-3-yl]-4*H*-pyrido[3,2-b][1,4]oxazin-3-one **15b** (430 mg, 1.41 mmol, 1.0 eq) in THF (40 mL) was added 10% Pd/C catalyst (100 mg), then the mixture was stirred at 25 °C under a hydrogen balloon for 2 h. The catalyst was filtered off and the filtrate was concentrated to give the crude product, which washed with MTBE (30 mL) and dried under reduced pressure to give 6-[5-(2-aminoethyl)-2-oxo-oxazolidin-3-yl]-4*H*-pyrido[3,2-b][1,4]oxazin-3-one **16b** (350 mg, 1.26 mmol, 80.1% yield) as an off-white solid. LC-MS (ESP) m/z: $[M+H]^+$ calculated for $[C_{12}H_{14}N_4O_4+H]^+$: 279.1, found: 279.1.

Step k: To a solution of 6-[5-(2-aminoethyl)-2-oxo-oxazolidin-3-yl]-4*H*-pyrido[3,2-b][1,4]oxazin-3-one **16b** (30.0 mg, 0.11 mmol, 1.0 eq) in methanol (3 mL) was added 4-chloroindane-2-carbaldehyde **4a** (26.0 mg, 0.13 mmol, 1.2 eq) and sodium cyanoborohydride (13.6 mg, 0.22 mmol, 2.0 eq). The mixture was stirred at 25 °C for 16 h. The solvent was removed by evaporation to give a residue, which was dissolved in DMF (1 mL) and purified by preparative-HPLC (formic acid conditions) to give 6-[5-[2-[(4-chloroindan-2-yl)methylamino]ethyl]-2-oxo-oxazolidin-3-yl]-4*H*-pyrido[3,2-b][1,4]oxazin-3-one; formic acid **17a** (12.2 mg, 0.03 mmol, 23.2 % yield, 99% purity) as a white solid. LC-HRMS (m/z): $[M+H]^+$ calculated for $[C_{22}H_{23}ClN_4O_4+H]^+$: 443.1481, found: 443.1488. ^{1}H NMR (600 MHz, DMSO-d6) δ ppm 11.21 (br s, 1H), 8.17 (s, 1H), 7.60 (d, J = 8.7 Hz, 1H), 7.44 (d, J = 8.7 Hz, 1H), 7.13–7.21 (m, 3H), 4.71–4.86 (m, 1H), 4.62 (d, J = 1.1 Hz, 2H), 4.24 (dd, J = 10.0, 8.5 Hz, 1H), 3.78 ddd, J = 10.0, 7.0, 1.4 Hz, 1H), 3.02–3.10 (m, 1H), 3.00–3.12 (m, 1H), 2.73–2.80 (m, 1H), 2.69–2.73 (m, 1H), 2.68–2.80 (m, 2H), 2.67–2.85 (m, 2H), 2.63–2.68 (m, 1H), 1.92–2.02 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d6, 27 ºC): δ ppm 166.5, 163.7, 154.4, 145.8, 144.2, 141.1, 140.0, 135.3, 130.1, 128.8, 126.5, 125.9, 123.7, 106.9, 72.2, 67.3, 53.8, 49.4, 45.0, 38.0, 37.8, 36.6, 34.0.

The four individual stereoisomers of **17a** were prepared by separating the two enantiomers of compound **13b** by chiral SFC (instrument: ACS-WH-SFC-A; column: Daicel Chiralpak AD (25 cm x 3.0 cm, 10 µm; mobile phase: (0.1% $NH_3.H_2O$)/MeOH 50/50 % v/v), and separating each resulting 1:1 mixture of diastereoisomers of compound **17a** by semi-preparative chiral HPLC (column: Chiralpak AD-H (25 x 2.0 cm), 5 µm; mobile phase: n-hexane/(ethanol/methanol 1/1 + 0.1% isopropylamine) 50/50 % v/v; flow rate: 17 mL/min; DAD detection: 220 nm). The individual isomers were characterized by ^{1}H NMR, LC-MS and chiral HPLC and were all pure (≥98%) single stereoisomers (ee ≥ 94%).

Figure S3. ^{1}H NMR spectrum of compound **17a**.

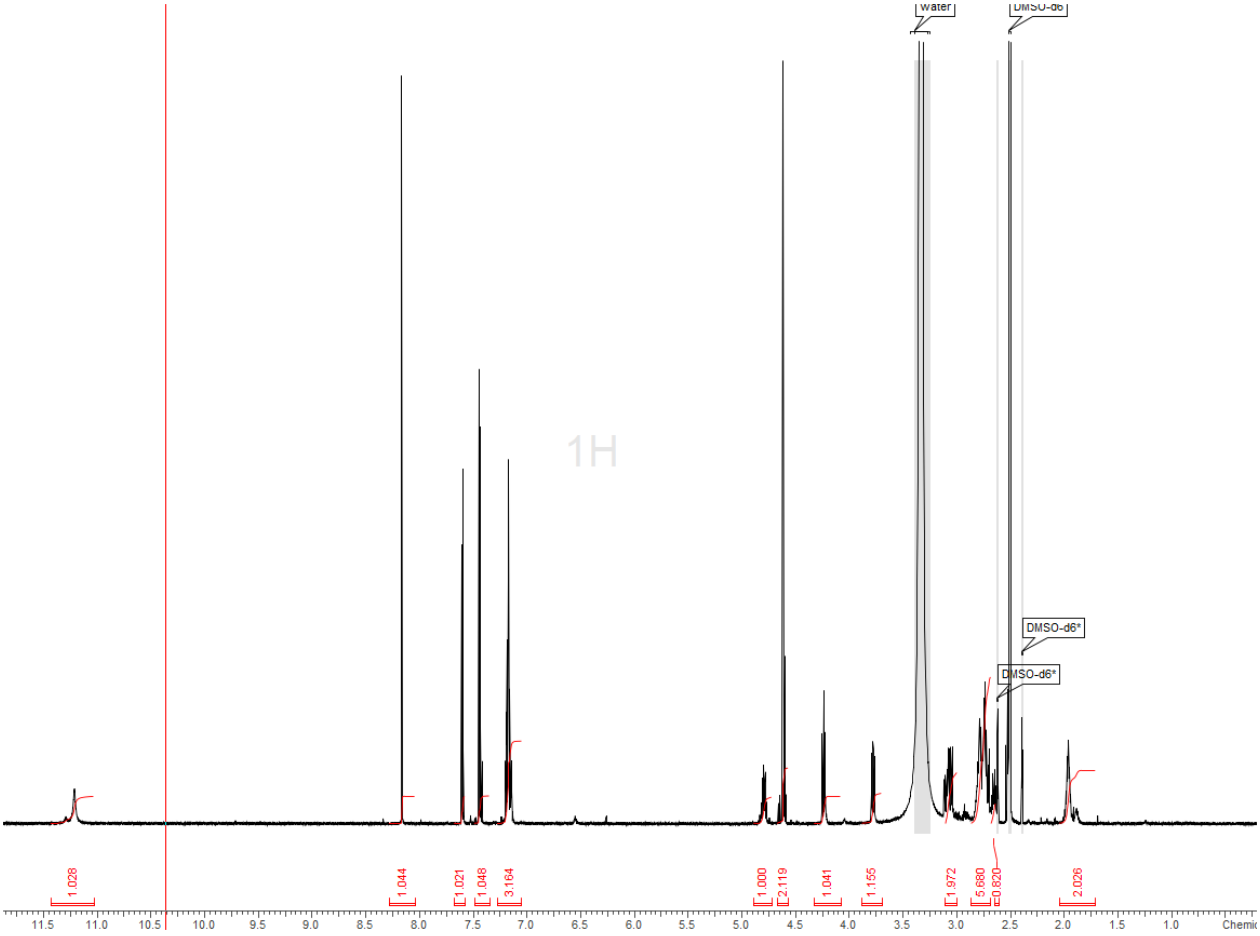
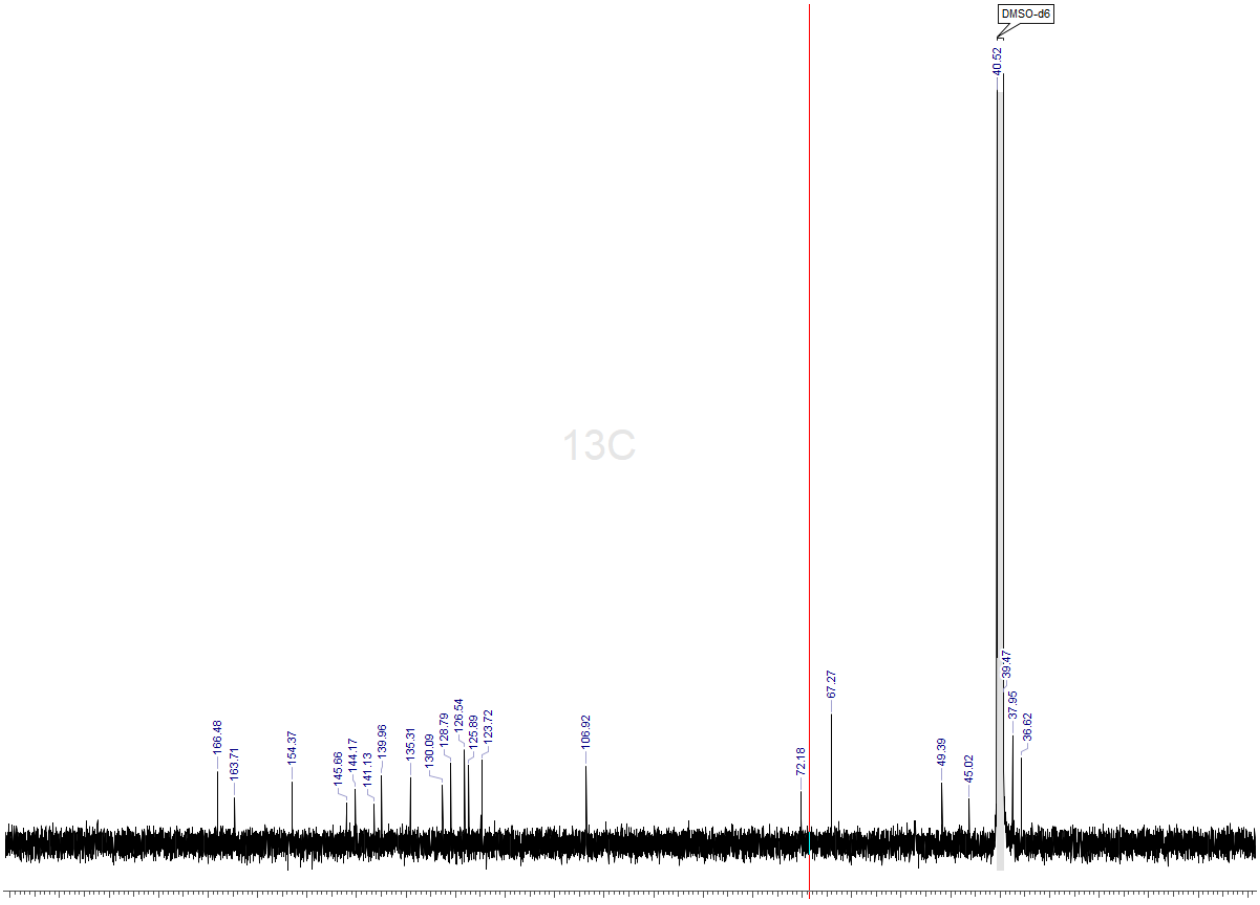
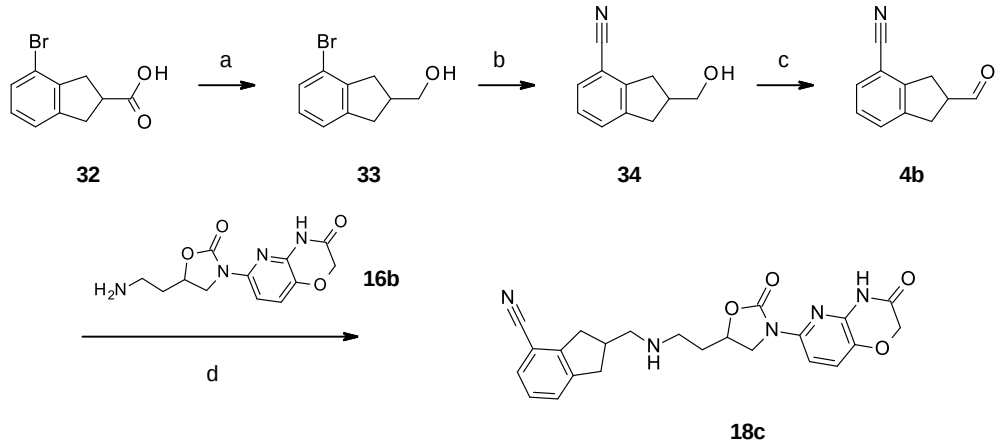


Figure S4. ^{13}C NMR spectrum of compound **17a**.



Synthetic procedure for the synthesis of compound 18c

Scheme S3. Synthesis of compound **18c**.



Reagents and conditions: (a) $BH_3.Me_2S$, THF, -78 to 20 ºC; (b) $Zn(CN)_2$, Zn (0.1 eq), dppf (0.1 eq), $Pd(PPh_3)_4$ (0.1 eq), DMA, 100 ºC; (c) Dess-Martin periodinane, DCM, 0 ºC to 25 ºC; (d) **16b**, $Na(CN)BH_3$, MeOH, 25 ºC.

Step a: To a solution of 4-bromoindane-2-carboxylic acid **32** (14.1 g, 58.6 mmol, 1 eq) in THF (400 mL) was added dropwise borane-methyl sulfide complex (17.6 mL, 176 mmol, 3.0 eq) at −78 °C, and then the mixture was warmed slowly to 20 °C during 2 h. The reaction was quenched slowly by addition of MeOH (100 mL) then refluxed for 0.5 h. The mixture was purified directly by column chromatography (petroleum ether/ethyl acetate=5:1) to give (4-bromoindan-2-yl)methanol **33** (10.2 g, 44.9 mmol, 76.7 % yield) as a light yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm 7.32 (d, J = 7.91 Hz, 1H), 7.15 (d, J = 7.40 Hz, 1H), 6.99–7.06 (m, 1H), 3.58–3.78 (m, 2H), 3.04–3.30 (m, 2H), 2.61–2.95 (m, 3H).

Step b: A suspension of (4-bromoindan-2-yl)methanol **33** (10.0 g, 44.1 mmol, 1 eq), zinc cyanide (1.68 mL, 26.5 mmol, 0.60 eq), zinc (289 mg, 4.41 mmol, 0.10 eq), 1,1'-bis(diphenylphosphino) ferrocene (2.45 g, 4.41 mmol, 0.10 eq) and tetrakis(triphenylphosphine)palladium(0) (5.1 g, 4.41 mmol, 0.10 eq) in *N*,*N*-dimethylacetamide (400 mL) was stirred at 100 °C under a nitrogen atmosphere for 16 h. The reaction was quenched with water (400 mL) and the mixture was extracted with EtOAc (2 x 500 mL). The combined organic phase was washed with H_2O (3 x 300 mL) and brine (500 mL). The combined organic layer was dried over sodium sulfate, then concentrated to give a residue, which was purified by column chromatography (petroleum ether/EtOAc=5:1 to 3:1) to give 2-(hydroxymethyl)indane-4-carbonitrile **34** (7.5 g, 43.3 mmol, 98.1 % yield) as a light brown oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm 7.45-7.43 (m, 2H), 7.25 (dd,

J = 8.0, 8.0 Hz, 1H), 3.63–3.76 (m, 2H), 3.09–3.33 (m, 2H), 3.03 (s, 1H), 2.99 (br d, *J* = 6.15 Hz, 1H), 2.76–2.92 (m, 1H).

Step c: To a solution of 2-(hydroxymethyl)indane-4-carbonitrile **23** (400 mg, 2.31 mmol, 1 eq) in DCM (40 mL) was added Dess-Martin periodinane (1270 mg, 3.0 mmol, 1.3 eq) at 0 °C. Then the solution was stirred at 25 °C for 2 h. The mixture was filtered and the filtrate was diluted with DCM (30 mL). The mixture was washed with saturated Na_2CO_3 (aq), dried over Na_2SO_4 and concentrated under vacuum to give a crude product which was purified by chromatography column on silica gel (petroleum ether/EtOAc=10/1 to 3/1) to give 2-formylindane-4-carbonitrile **4b** (250 mg, 1.46 mmol, 61.2% yield) as a white solid. LC-MS (ESP) m/z: $[M+H]^+$ calculated for $[C_{11}H_9NO+H]^+$: 172.1, found: 172.2. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 9.82 (s, 1H), 7.51–7.44 (m, 2H), 7.33–7.25 (m, 1H), 3.58–3.47 (m, 1H), 3.46–3.35 (m, 3H), 3.31–3.20 (m, 1H).

Step d: To a solution of 2-formylindane-4-carbonitrile **4b** (246 mg, 1.44 mmol, 1.0 eq), 6-[5-(2-aminoethyl)-2-oxo-oxazolidin-3-yl]-4*H*-pyrido[3,2-b][1,4]oxazin-3-one **16b** (400 mg, 1.44 mmol, 1.0 eq) in methanol (20 mL) was added sodium cyanoborohydride (271 mg , 4.31 mmol, 3 eq) .The mixture was stirred at 25 °C for 16 h. The reaction mixture was concentrated under vacuum to give the crude product, which was purified by preparative-HPLC (formic acid system) with freeze-drying to give 2-[[2-[2-oxo-3-(3-oxo-4*H*-pyrido[3,2-b][1,4]oxazin-6-yl)oxazolidin-5-yl]ethylamino]methyl]indane-4-carbonitrile; formic acid **18c** (80.0 mg, 0.17 mmol, 11.5% yield, 99% purity) as a white solid. LC-HRMS (m/z): $[M+H]^+$ calculated for $[C_{23}H_{23}N_5O_4+H]^+$: 434.1823, found: 434.1827. ^{1}H NMR (600 MHz, DMSO-d6) δ ppm 10.66–11.80 (m, 1H), 8.22 (s, 1H), 7.59–7.61 (m, 1H), 7.58 (dd, *J* = 7.7, 0.9 Hz, 1H), 7.54 (d, *J* = 7.5 Hz, 1H), 7.44 (d, *J* = 8.6 Hz, 1H), 7.33 (t, *J* = 7.6 Hz, 1H), 4.73–4.89 (m, 1H), 4.62 (d, *J* = 0.6 Hz, 2H), 4.23 (dd, *J* = 10.0, 8.5 Hz, 1H), 3.74–3.81 (m, 2H), 3.17 (br dd, *J* = 16.1, 7.1 Hz, 1H), 3.05–3.12 (m, 1H), 2.86 (br dd, *J* = 16.4, 4.5 Hz, 1H), 2.72–2.80 (m, 3H), 2.65–2.71 (m, 3H), 1.88–2.02 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d6, 27 ºC): δ ppm 166.5, 164.2, 154.4, 147.5, 145.0, 144.2, 140.0, 135.3, 130.2, 129.9, 127.8, 125.9, 118.4, 108.2, 106.9, 72.3, 67.3, 53.7, 49.4, 45.1, 38.5, 37.3, 36.7, 34.2.

Figure S5. ^{1}H NMR spectrum of compound **18c**.

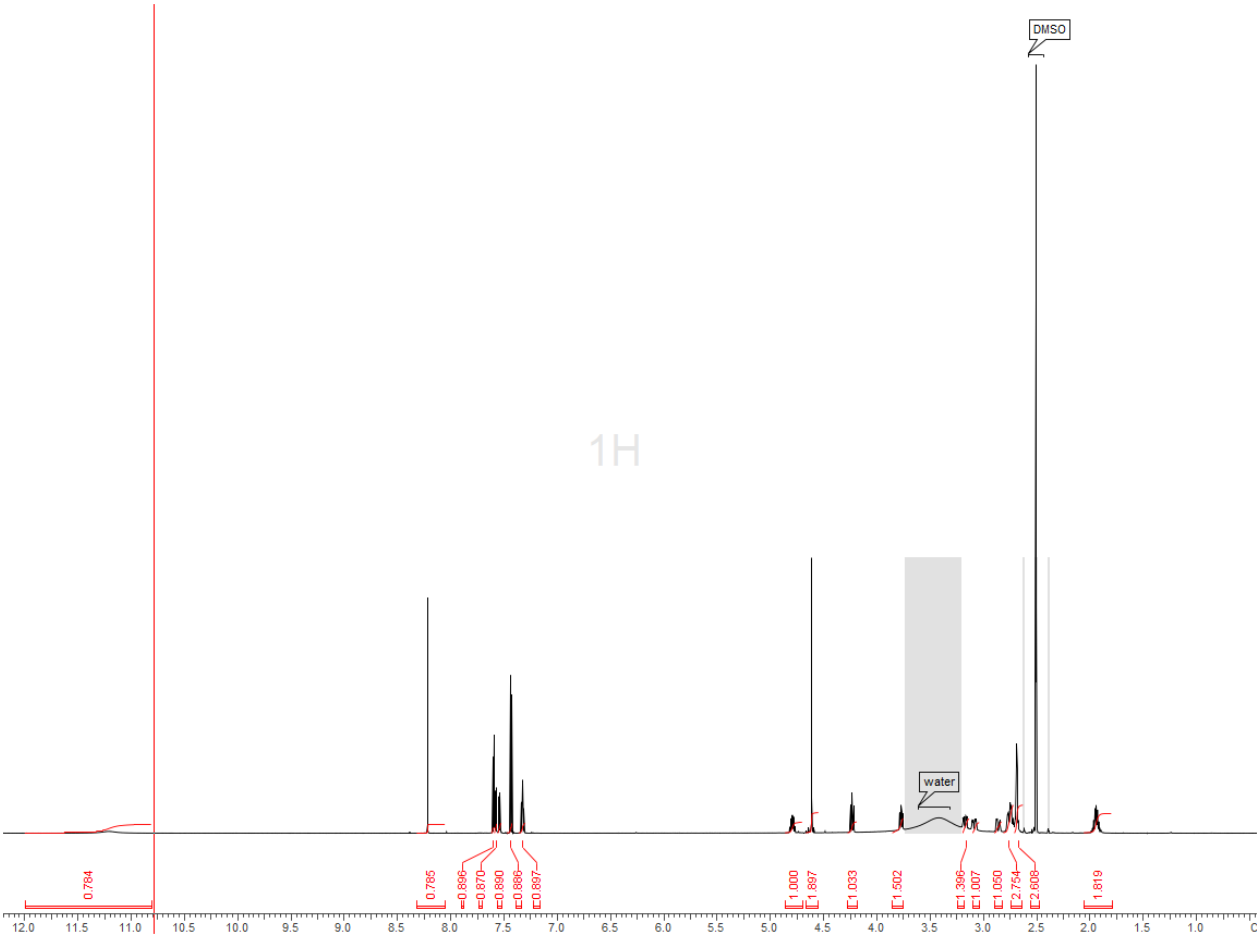


Figure S6. ^{13}C NMR spectrum of compound **18c**.

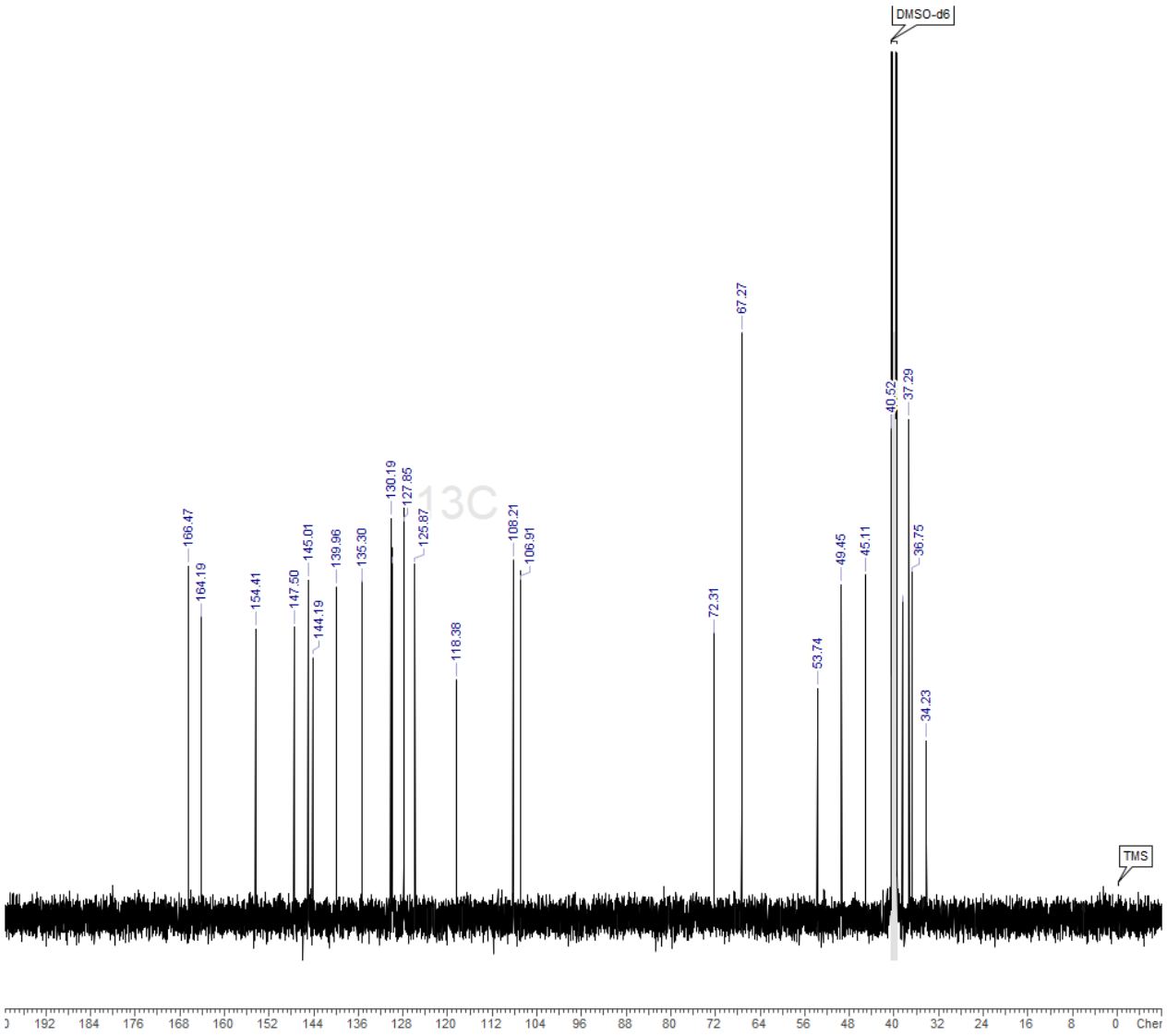


Table S1. LC-MS and data purity for compounds **17b**-**17g**, **18a**-**b**, **19a**-**19e** and **20**.

Compound	LC-MS	Purity
17b	$[M+H]^+$ calculated for $[C_{21}H_{21}ClN_4O_4+H]^+$: 429.1, found: 429.0	98%
17c	$[M+H]^+$ calculated for $[C_{22}H_{23}ClN_4O_4+H]^+$: 443.1, found: 443.1	98%
17d	$[M+H]^+$ calculated for $[C_{20}H_{19}ClN_4O_4+H]^+$: 415.1, found: 415.1	99%
17e	$[M+H]^+$ calculated for $[C_{22}H_{23}ClN_4O_4+H]^+$: 443.1, found: 443.2	98%
17f	$[M+H]^+$ calculated for $[C_{23}H_{25}ClN_4O_4+H]^+$: 457.2, found: 457.1	99%
17g	$[M+H]^+$ calculated for $[C_{21}H_{21}ClN_4O_4+H]^+$: 429.1, found: 429.2	100%
18a	$[M+H]^+$ calculated for $[C_{22}H_{24}N_4O_4+H]^+$: 409.2, found: 409.2	100%
18b	$[M+H]^+$ calculated for $[C_{22}H_{23}FN_4O_4+H]^+$: 427.2, found: 427.3	97%
19a	$[M+H]^+$ calculated for $[C_{22}H_{24}N_4O_4+H]^+$: 409.2, found: 409.2	100%
19b	$[M+H]^+$ calculated for $[C_{22}H_{23}FN_4O_4+H]^+$: 427.2, found: 427.2	100%
19c	$[M+H]^+$ calculated for $[C_{22}H_{23}ClN_4O_4+H]^+$: 443.1, found: 443.2	98%
19d	$[M+H]^+$ calculated for $[C_{22}H_{23}FN_4O_4+H]^+$: 427.2, found: 427.2	95%
19e	$[M+H]^+$ calculated for $[C_{23}H_{23}N_5O_4+H]^+$: 434.2, found: 434.2	98%
20	$[M+H]^+$ calculated for $[C_{22}H_{23}ClN_4O_4+H]^+$: 443.1, found: 443.2	99%

Antimicrobial susceptibility testing

MICs were determined using the Clinical Laboratory and Standard Institute (CLSI) reference broth microdilution method (Clinical and Laboratory Standards Institute, 2022M100 – Ed32). Test articles solutions were prepared at 50x the final desired concentration in 100% DMSO. Standards (ciprofloxacin and moxifloxacin) were obtained from Sigma-Aldrich and were prepared in ultrapure water. The compounds, along with standard antibiotics, were serially diluted 2-fold across the 96-well plates (Greiner Bio-One): compounds ranged from 64 μg/mL to 0.06 μg/mL or from 32 μg/mL to 0.03 μg/mL, standards ranged from 32 μg/mL to 0.03 μg/mL. Gram-positive and Gram-negative bacteria were cultured overnight in Tryptone Soya agar (TSA, Thermo Fisher Diagnostics Spa). The inoculum was prepared by making a direct saline suspension of isolated colonies selected from agar plates and it was adjusted to achieve a turbidity equivalent to the 0.5 McFarland standard. The suspension was then diluted 1:200 in cation-adjusted Mueller Hinton Broth (CAMHB, Thermo Fisher Diagnostics Spa) within 15 minutes. A positive control of just the

bacteria and a negative control of only the medium were included for every plate tested. All the plates were covered and incubated at 35±2 °C in ambient air for 20 to 24 hours. MICs were determined visually with the MIC defined as the lowest concentration at which no growth was visible after incubation. The appropriate ATCC strains were used in each assay as quality controls (QC) to confirm the validity of the results based on the current CLSI breakpoints.

ATPase enzyme assay

The quantification of ATP consumption (or ADP generation) can be used to monitor enzyme activity and determine compound inhibition. Transcreener ADP2 FI is a commercially available kit (Bellbrook Labs) that allows to detect the ADP formed during the reaction.

Test articles and standards solutions were prepared at 100x the final top concentration in 100% DMSO. To create a 10-point dose response curves the compounds, along with standard antibiotics, were serially diluted (half log dilution) in DMSO across 384 Microplate (Beckman Coulter); compounds and standards ranged from 10 µM to 0.00032 µM in 1% final DMSO concentration. Using the Echo 550 acoustic dispenser (Labcyte, Beckman) 50 nL of each compound was transferred into the 384 microplate (Greiner Bio-One). Assay buffer, at 2x, were prepared: *E. coli* gyrase buffer contained 35 mM Tris-HCl (pH 7.5), 24 mM KCl, 4 mM $MgCl_2$, 2 mM DTT, 1.8 mM spermidine, 6.5% (w/v) glycerol and 0.1 mg/mL albumin. *E. coli* Topoisomerase IV buffer included 40 mM HEPES-KOH (pH 7.6), 100 mM potassium glutamate, 10 mM magnesium acetate, 1 mM DTT and 50 µg/mL albumin. Enzyme solution and substrate solution (Inspiralis) were prepared in ice using the appropriate assay buffer. Gyrase and Topoisomerase reactions were performed in 5 µL final assay volume and incubated for 80 and 120 minutes respectively at 25 ºC. The reaction was stopped by adding the ADP detection mixture (Bellbrook Labs) and after 60 minutes incubation, the FI signal was read with plate reader (ex 577.2-15 nm / em 641-44 / dichroic 604.5 CLARIOstar, BMG Labtech). Each assay was validated by the response of the reference controls and the robust Z' and the concentration of the sample that produces 50% inhibition (IC_{50}) was reported.

DNA supercoiling assay for *E. coli* DNA gyrase

DNA Gyrase was reconstituted by mixing purified *E. coli* GyrA and GyrB (1:1) subunits in dilution buffer on ice for 15 minutes. A substrate mixture of 6 µL of assay buffer (comprising 175 mM Tris.HCl (pH 7.4), 120 mM KCl, 20 mM MgCl2, 10 mM DTT, 9 mM Spermidine, 5 mM ATP, 32.5% (w/v) Glyrcerol, 500 µg/ml BSA) and 1.5 µL Relaxed pBR322 DNA (Inspiralis, 200 ng/µL) made up to 28 µL with water was added into each well of a 96-well V bottom plate. 1 µL of test compound in DMSO was added to the wells and incubated at 25 ºC for 5 minutes. 1 µL of DNA Gyrase was added to each well and the enzyme reaction mixture was incubated at 37ºC for 1 hour. Final compound concentrations ranged from 100 µM down to 0.03 µM in half-log steps resulting in an eight point dose response curve. The reaction was stopped by adding 3.5 µL of dye mix (10X BlueJuice Gel Loading Buffer). The DNA products were separated by electrophoresis on 1% agarose gel in TAE buffer run overnight at 2 V/cm. The gels were stained with 1x SYBR® Gold Nucleic Acid Gel Stain (10,000x) in TAE buffer at 25 ºC for 30 minutes. The gels were photographed under UV irradiation using a Bio-Rad Gel DocTM EZ Imager gel documentation system. The supercoiled pBR322 DNA bands were quantitated by Image Lab 5.2.1 and the bands intensity of the converted supercoiled DNA bands for each sample were collected and analyzed by GraphPad Prism. The enzyme activities inhibition% were calculated by the following formula:

$$\text{Enzyme inhibition (\%)} = 100\% - \frac{\text{Intensity of compound treated well}}{\text{Intensity of DMSO treated well}} \times 100\%$$

The calculated values were applied for the non-linear regression curve analysis. IC_{50} values of each test were fitted automatically in IDBS E-WorkBook Suite.

Decatenation Assay for *E. coli* DNA Topoisomerase IV

The assay was performed as for the DNA supercoiling assay for *E. coli* DNA gyrase using purified *E. coli* ParC and ParE (1:1) subunits instead of purified *E. coli* GyrA and GyrB (1:1) subunits.

Decatenation assay for human Topoisomerase II alpha

A substrate mixture of 6 µL of assay buffer (comprising 250 mM Tris.HCl (pH 8.0), 600 mM KCl, 50 mM $MgCl_2$, 20 mM DTT, 10 mM Spermidine, 5 mM ATP and 150 µg/mL BSA) and 1µl kDNA (200ng/µL, Inspiralis) made up to 28 µL with water was added into each well of a 96-well V bottom plate. 1 µL of test compound in DMSO was added to the wells and incubated at 25 ºC for 5 minutes. 1 µL of human Topoisomerase II alpha (Thermo Scientific™) was added to each well and the enzyme reaction mixture was incubated at 37ºC for 1 hour. Final compound concentrations ranged from 100 µM down to 0.03 µM in half-log steps resulting in an eight point dose response curve. The reaction was stopped by adding 3.5 µL of dye mix (10X BlueJuice Gel Loading Buffer). The DNA products were separated by electrophoresis on 1% agarose gel in TAE buffer run overnight at 2 V/cm. The gels were stained with 1x SYBR® Gold Nucleic Acid Gel Stain (10,000x) in TAE buffer at 25 ºC for 30 minutes. The gels were photographed under UV irradiation using a Bio-Rad Gel DocTM EZ Imager gel documentation system. The decatenated DNA bands were quantitated by Image Lab 5.2.1 and the bands intensity of the converted decatenated DNA bands for each sample were collected and analyzed. The enzyme activities inhibition% were calculated by the following formula:

$$\text{Enzyme inhibition (\%)} = 100\% - \frac{\text{Intensity of compound treated well}}{\text{Intensity of DMSO treated well}} \times 100\%$$

The calculated values were applied for the non-linear regression curve analysis. IC50 values of each test were fitted automatically in IDBS E-WorkBook Suite.

hERG electrophysiology assay

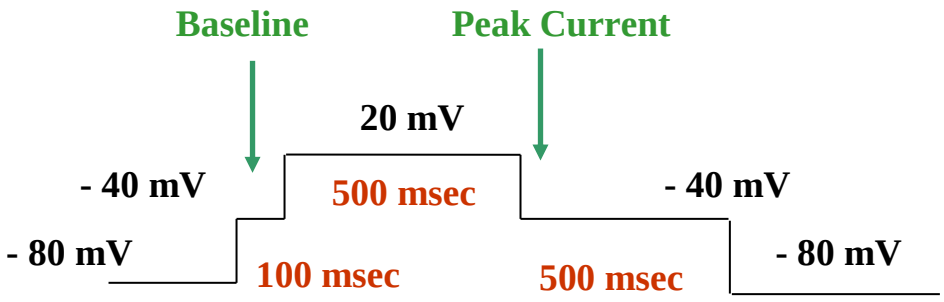
HERG K^+ channels have been cloned from human heart and recombinant channels are stably expressed in CHO cells (CHO_{hERG}). CHO_{hERG} are grown in sterile tissue flasks in DMEM/F-12 (1:1) medium (Invitrogen, USA) supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS) (Hyclone, USA) and 500 µg/ml Gentamycin solution (Gibco, UK)) at 35-39°C in 5% CO2 (SOP SP.M.007). The confluent cells cultures are sub-cultured every 3-4 days using Accumax (Innovative Cell Technologies, USA).

Ready-to-use frozen instant CHO_{hERG} cells are cryopreserved at Evotec AG (Germany). For the experimental use, the vials with cryopreserved cells are thawed at 37°C, incubated in the pre-warmed IMDM cell culture medium (Gibco Life Technologies, USA) for 30 min at 37°C and re-suspended in the 2/3 HBSS (Gibco, UK) and 1/3 external solution to get 150-200 000 cells/mL.

The effects of a compound on hERG K^+-currents parameters were evaluated at 2 concentrations in at least 4 cells (typically 5 to 7 cells).

The hERG test is performed using automated patch clamp system SynchroPatch® 384 (Nanion Technologies GmbH, Germany). On the day of the experiment, an aliquot of the cell suspension in a 2:1 mixture of the HBSS and external solution is placed in the Cellhotel. K^+ currents are measured with the patch-voltage-clamp technique in the whole-cell configuration at RT or at 35-37°C using the built-in 384 channel amplifier and associated software (PatchControl 384). Currents are low-pass filtered using the analog 3 kHz Bessel filter and the digital 3kHz Lanczos filter and are digitized at 5 kHz. Series resistance is typically 2-9 MΩ and is compensated by 80%. The reported current amplitudes represent the maximal amplitude of a peak current.

Figure S7. Pulse pattern used to elicit outward K^+ current at 35-37 °C.



Cells are held at a resting voltage of -80 mV and they are stimulated by a voltage pattern to activate hERG channels and conduct outward IK_{hERG} current, at a stimulation frequency of 0.1 Hz (6 bpm). After the cells have stabilized for a few minutes and the currents are steady, the amplitude and kinetics of IK_{hERG} are recorded under control conditions (vehicle control) for 3-5 min. Thereafter, the test item is tested at ascending concentrations (1 and 10 µM). Cells are exposed to each test item concentration for 3 min. In parallel to drug experiments, a 0.03 and 0.3 µM solution of the standard IK_{hERG} blocker E-4031 is applied for 3 min in order to entirely suppress IK_{hERG}. E-4031 is tested as a positive control for the IK_{hERG} block within each experiment.

The amplitudes of IK_{hERG} are recorded in each concentration of drug and they are compared to the vehicle control values (taken as 100%) to define fractional blocks. The fractional block values from single cells are saved in the appropriate .xls file generated by DataControl® 384 Software (Nanion Technologies GmbH). Data are expressed as mean±SEM for each drug concentration. The effect of the solvent (DMSO) on the hERG K^+ current is studied in a vehicle control group. The relative current values are corrected to the mean vehicle effect at each test item concentration as follows:

$$\mathrm{I}_{corrected}(\%) = \frac{I_{test}}{meanI_{vehicle}} \times 100$$

where I is a relative current amplitude.

If the effect of a drug on the currents was equal to or above 20% at the highest concentration tested, then concentration-response data were fitted with the following relationship:

$$\mathrm{I}(C) = \frac{100}{1 + (C / IC50)^h}$$

where C is the concentration, IC_{50} is the concentration producing 50% block and h is the Hill coefficient.

Concentration-response curves are fitted by non-linear regression analysis using EworkBook suite (ID Business Solutions Ltd, UK). Data fit is done with the 4 Parameter Logistic Model (fit = (A+(B/(1+((x/C)^D)))), where A=0 and B=100).

Protein purification of *S. aureus* GyrB27–A56(GKdel/Tyr123Phe)

The construct design and purification procedures are as described by Bax *et al.* (2010). Briefly the *S. aureus* GyrB (residues 409-644) was fused to *S. aureus* GyrA (residues 2-491) and the flexible Greek key domain (residues 544–579) was replaced with two amino acids threonine-glycine, the catalytic Tyrosine 123 was mutated to a phenylalanine. It was constructed in the vector pET-30a without any affinity tag, and was expressed well in *E.coli* as soluble protein. After cell lysis and clarification, the purification procedures were followed with columns of Q-HP, Heparin-HP, Resource Q, Superdex200 and Mono-Q. The final protein was concentrated to 14.5 mg/mL in buffer 20 mM HEPES, pH 7.0, 100 mM Na_2SO_4, 3 mM $MnCl_2$, 0.5 mM TCEP.

Crystallization of GyrB27–A56 ternary complex with DNA and small molecular inhibitors

The GyrB27–A56 ternary complex with DNA and small molecular inhibitor was formed *in vitro* by mixing 14.5 mg/ml protein with two times molar ratio of DNA duplex (5-AGCCGTAGGGCCCTACGGCT-3, 3-TCGGCATCCCGGGATGCCGA-5) and six times molar ratio of the small molecular inhibitor. Crystallization was performed using microbatch method in 96-well plate by mixing 0.5 µL ternary complex with 0.5 µL crystallization buffer (14% PEG5000 MME, 100 mM Bistris pH 5.5), and covered with 20 µL paraffin oil. Crystals appeared within 2 days and were collected 10 days after setting up.

X-ray crystallography of GyrB27–A56 ternary complex with DNA and small molecular inhibitors

Data was collected at Swiss Light Source beamline PX-II using an ADSC Quantum CCD or a Pilatus II detector. X-ray wavelength was 1A and oscillation width 0.25-0.3°. Intensities were integrated with XDS (Kabsch, 2010; PMID 20124692), scaled with AIMLESS (PMID 23793146) and treated for anisotropy using STARANISO (GlobalPhasing). High resolution limits for the data were selected based on $I/s(I) \geq 1$ and $CC1/2 \geq 0.3$ in the outer shell. The space group is P61 with a=93 Å and c=408 Å. Some datasets show signs of twinning with twin law k,h, -l, which was taken into account during refinement. Phases were generated by molecular replacement with PHASER (McCoy et al., 2007; PMID 19461840) using an in-house structure as the search model. Models were rebuilt in Coot (Casanal et al., 2020; PMID 31730249) and refined with Phenix (Liebschner et al., 2019; PMID 31588918) using individual and TLS protocols for B-values. The final structures contain a symmetric dimer per asymmetric unit with a C_2-symmetrically disordered ligand.

Additional Data

Table S2. MIC values (µg/mL) for all panel strains

Compound	*E. coli* K-12 BW25113	*E. coli* K-12 BW25113 ΔTolC	*E. coli* ATCC 35218	*E. coli* ATCC BAA-2340	*E. coli* ATCC 25922	*A. baumannii* ATCC 19606	*A. baumannii* ATCC 51432	*A. baumannii* ATCC BAA-747	*K. pneumonia* ATCC 10031	*K. pneumonia* ATCC 700603	*K. pneumonia* ATCC BAA-1705	*K. pneumonia* ATCC BAA-2146	*P. aeruginosa* NCTC 13437	*P. aeruginosa* NCTC 11451	*P. aeruginosa* ATCC 27853	*S. aureus* ATCC BAA-1556	*S. aureus* ATCC 29213
17a	≤0.06	≤0.06	0.25	0.5	0.125	0.125	0.5	≤0.06	≤0.06	4	2	4	1	2	2	≤0.06	≤0.06
17b	4	0.25	4	8	2	4	8	1	0.125	16	16	16	16	32	16	4	2
17c	0.25	≤0.03	0.5	1	0.25	0.5	0.5	0.06	≤0.03	2	2	4	2	4	2	0.125	0.06
17d	8	≤0.06	4	32	8	4	8	2	≤0.06	32	>64	>64	>64	>64	>64	4	1
17e	≤0.06	≤0.06	0.25	0.5	0.25	0.25	0.5	≤0.06	≤0.06	2	4	8	2	4	1	≤0.06	≤0.06
17f	0.25	≤0.03	0.5	2	0.5	1	1	0.25	≤0.03	8	8	16	4	16	4	1	0.5
17g	1	≤0.06	1	2	0.5	1	2	1	≤0.06	4	32	32	8	16	8	0.5	0.25
18a	1	≤0.06	1	4	1	2	2	0.25	0.125	8	8	32	8	16	8	2	1
18b	0.25	≤0.06	0.25	1	0.5	0.5	0.5	≤0.06	≤0.06	2	4	8	2	8	4	0.25	0.125
18c	≤0.06	≤0.06	0.125	≤0.06	≤0.06	0.125	≤0.06	≤0.06	≤0.06	1	1	4	0.5	1	0.5	≤0.06	≤0.06
19a	2	0.25	4	8	2	8	8	1	0.125	16	16	16	16	32	8	4	2
19b	0.250	≤0.06	0.5	1	0.5	1	1	0.125	≤0.06	4	4	16	4	8	4	0.5	0.25
19c	1	≤0.06	1	4	2	4	4	0.5	0.125	8	8	32	8	16	16	4	2
19d	1	≤0.06	1	4	1	4	4	0.5	0.125	8	8	32	8	16	16	2	1
19e	0.125	≤0.06	0.25	0.5	0.25	0.5	0.5	≤0.06	≤0.06	2	2	8	1	4	2	0.25	≤0.06
20	2	0.06	2	4	2	8	4	0.5	0.06	8	16	16	8	16	8	2	1
ciprofloxacin	≤0.03	≤0.03	≤0.03	>32	≤0.03	1	8	0.5	≤0.03	0.5	>32	>32	32	0.25	0.25	16	0.5
meropenem	≤0.03	0.06	≤0.03	2	≤0.03	2	4	0.5	≤0.03	≤0.03	16	>32	>32	0.125	0.5	2	0.125

Table S3. ATPase enzyme activities

Compound	*E. coli* DNA Gyrase IC_{50} (μM)	*E. coli* TopoIV IC_{50} (μM)
2	0.16	0.060
17a	0.090	0.013
17b	0.29	0.098
17c	0.072	0.022
17d	0.58	0.20
17e	0.090	0.034
17f	0.12	0.035
17g	0.23	0.063
18a	0.18	0.047
18b	0.10	0.023
18c	0.021	0.0041
19a	0.27	0.14
19b	0.12	0.034
19c	0.25	0.050
19d	0.16	0.060
19e	0.057	0.0079
20	0.19	0.079
novobiocin	0.010	0.28

Figure S8. Scatter plot of *E. coli* DNA Gyrase IC_{50} (μM) vs *E. coli* TopoIV IC_{50} (μM)

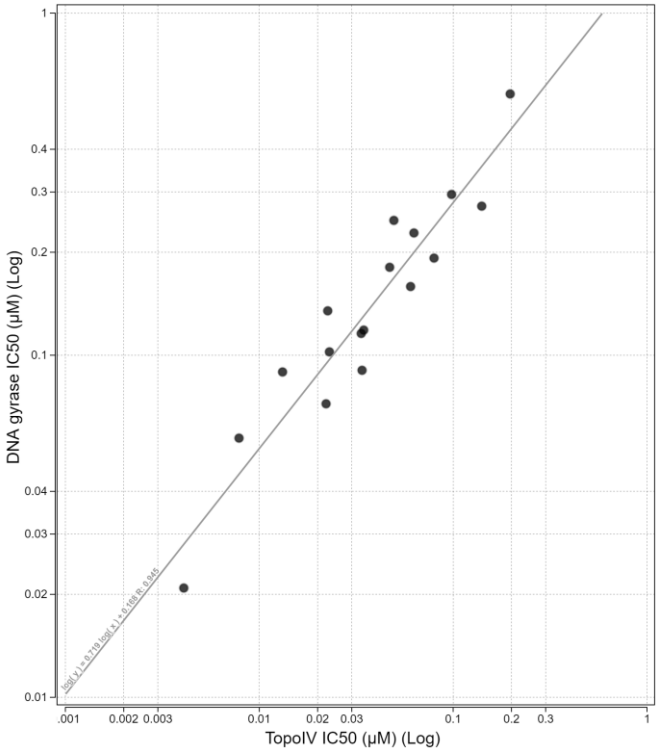


Table S4. Topological enzyme activities

Compound	*E. coli* DNA Gyrase supercoiling IC_{50} (μM)	*E. coli* TopoIV decatenation IC_{50} (μM)	Human TopoIIa decatenation IC_{50} (μM)
18c	0.10	0.16	>100
ciprofloxacin	0.69	23	>100
doxorubicin	0.34	0.36	0.75

Table S5. X-ray crystallographic data collection and refinement statistics

	7FVS (**2**)	7FVT **(17a)**
Wavelength	0.9791	1
Resolution range	46.46 - 2.16 (2.237 - 2.16)	63.16 - 2.081 (2.155 - 2.081)
Space group	P 61	P 61
Unit cell	92.915 92.915 407.576 90 90 120	92.938 92.938 407.651 90 90 120
Total reflections	1321189 (121918)	1238667 (127502)
Unique reflections	105704 (6727)	118528 (1672)
Multiplicity	12.5 (11.8)	10.5 (10.8)
Completeness (%)	95.56 (63.50)	86.82 (14.10)
Mean I/sigma(I)	11.36 (0.95)	9.43 (0.80)
Wilson B-factor	37.04	33.93
R-merge	0.1632 (2.122)	0.2054 (2.783)
R-meas	0.1703 (2.216)	0.2162 (2.922)
R-pim	0.04832 (0.6319)	0.06705 (0.8897)
CC1/2	0.997 (0.771)	0.997 (0.216)
CC*	0.999 (0.933)	0.999 (0.596)
Reflections used in refinement	101241 (6692)	102929 (1672)
Reflections used for R-free	5096 (350)	5184 (84)
R-work	0.1851 (0.3378)	0.1996 (0.3085)
R-free	0.2205 (0.3892)	0.2484 (0.3327)
CC(work)	0.872 (0.430)	0.937 (0.622)
CC(free)	0.851 (0.419)	0.906 (0.631)
Number of non-hydrogen atoms	11927	12033
macromolecules	11428	11451
ligands	37	37
solvent	462	545
Protein residues	1341	1341
RMS(bonds)	0.082	0.082
RMS(angles)	1.12	1.48
Ramachandran favored (%)	95.27	97.3
Ramachandran allowed (%)	4.5	2.7
Ramachandran outliers (%)	0.23	0
Rotamer outliers (%)	1.83	1.13
Clashscore	12.94	4.14
Average B-factor	32.93	42.16
macromolecules	32.98	42.06
ligands	36	68.04
solvent	34.81	43.01
Number of TLS groups	0	11

Figure S9. Supplementary image for **2** bound to DNA gyrase-DNA, with 2Fo-Fc map contoured at 1 rmsd. DNA on the left, protein to the right.

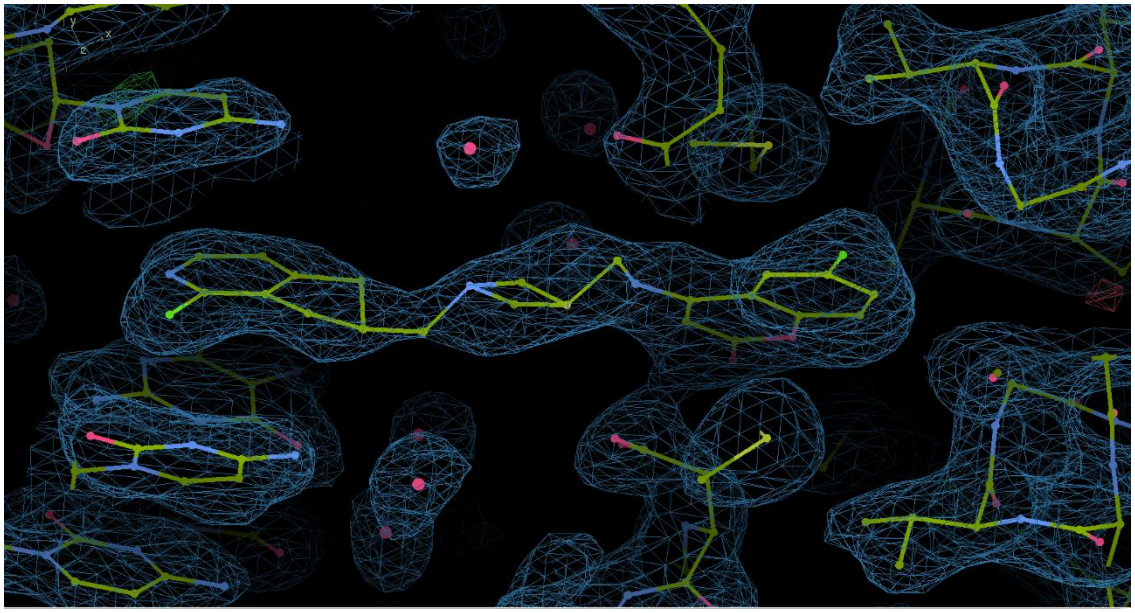


Figure S10. Supplementary image for **17a** bound to DNA gyrase-DNA with 2Fo-Fc map contoured at 1 rmsd. DNA on the left, protein to the right.