Supporting Information

Oxabicyclooctane Linked Novel Bacterial Topoisomerase Inhibitors as Broad Spectrum Antibacterial Agents

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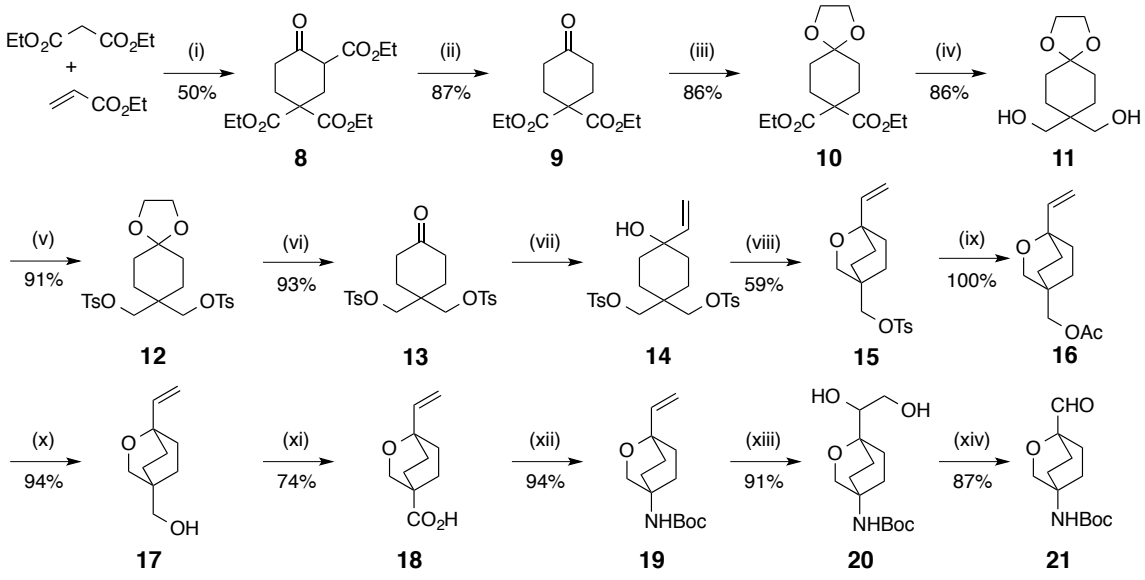
Scheme 1



Triethyl 4-oxocyclohexane-1,1,3-tricarboxylate (8)

A suspension of sodium hydride (112.3 g, 2.34 mol) in anhydrous tetrahydrofuran (1 L) was added a solution of diethyl malonate (150 g, 0.937 mol) in anhydrous tetrahydrofuran (300 mL) at 40–45 °C,

the suspension was stirred at the same temperature for 15 minutes. A solution of ethyl acrylate (215 mL, 1.97 mol) in anhydrous tetrahydrofuran (300 mL) was added to the suspension, the resulting mixture was stirred for 15 minutes. The mixture was poured onto ice water, adjusted to pH 3 by addition of concentrated hydrochloric acid and extracted with ethyl acetate. The organic extracts were dried over anhydrous sodium sulfate, filtered, and then concentrated in vacuo. Flash chromatography (hexane/ethyl acetate = 4:1) of the residue gave **8** (147.8 g, 50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23–1.33 (m, 9H), 2.34–2.46 (m, 6H), 4.19–4.28 (m, 6H).

Diethyl 4-oxocyclohexane-1,1-dicarboxylate (9)

A mixture of **8** (158.4 g, 0.504 mol) and sodium chloride (86.3 g, 1.48 mol) in dimethyl sulfoxide (720 mL) and water (21.6 mL) was heated at 160 °C for 1.7 hours. The mixture was poured onto ice water and extracted with ethyl acetate. The organic extracts were dried over anhydrous sodium sulfate, filtered, and then concentrated in vacuo. Flash chromatography (hexane/ethyl acetate = 3:1) of the residue gave **9** (111.7 g, 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.24–1.30 (m, 6H), 2.34–2.48 (m, 8H), 4.25 (q, J = 7.4 Hz, 4H).

Diethyl 1,4-dioxaspiro[4.5]decane-8,8-dicarboxylate (10)

A mixture of **9** (105.5 g, 435 mmol), ethylene glycol (29.1 mL, 523 mmol) and *p*-toluenesulfonic acid hydrate (827 mg, 4.35 mmol) in toluene (870 mL) was heated under reflux for 4 hours with using Dean-Stark apparatus. The mixture was poured onto saturated sodium hydrogen carbonate solution and extracted with ethyl acetate. The organic extracts were dried over anhydrous sodium sulfate, filtered, and then concentrated in vacuo. Flash chromatography (hexane/ethyl acetate = 5:1) of the residue gave **10** (106.6 g, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25 (t, J = 7.3 Hz, 6H), 1.69 (t, J = 6.1 Hz, 4H), 2.18 (t, J = 6.1 Hz, 4H), 3.94 (s, 4H), 4.18 (q, J = 7.3 Hz, 4H).

1,4-Dioxaspiro[4.5]decane-8,8-diyldimethanol (11)

To a solution of lithium aluminum hydride (738 mL, 1 M in diethyl ether) was added a solution of **10** (105.7 g, 369 mmol) in anhydrous diethyl ether (738 mL) at –20 °C, the resulting suspension was stirred at 0 °C for 3 hours. After quenching the reaction by adding water/tetrahydrofuran (1:1, 132.8 mL) and 5N sodium hydroxide solution (33.2 mL) under cooling with ice, the mixture was stirred at room temperature for overnight. After dilution of the mixture with dichloromethane/methanol (5:1, 1

L), the insoluble materials were filtered off and washed with dichloromethane/methanol (5:1, 500mLx2). The combined mixture of the filtrate and washing was added silica-gel (220 g). The suspension was stirred for 15 minutes. The insoluble materials were filtered off and washed with (dichloromethane/methanol = 5:1). The combined filtrate and the washing were concentrated in vacuo to give **11** (64.0 g, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.53–1.58 (m, 4H), 1.60–1.65 (m, 4H), 2.37 (t, J = 5.5 Hz, 2H), 3.65 (d, J = 5.5 Hz, 4H), 3.95 (s, 4H).

1,4-Dioxaspiro[4.5]decane-8,8-diylbis(methylene) bis(4-methylbenzenesulfonate) (12)

To a solution of **11** (112.0 g, 554 mmol) in anhydrous pyridine (700 mL) was added *p*-toluenesulfonyl chloride (232.3 g, 1.22 mol) under cooling with ice, the resulting suspension was stirred at room temperature for overnight. After dilution of the mixture with ethyl acetate, the mixture was washed with 10% aqueous citric acid solution (1 Lx4) and brine. The organic extracts were concentrated in vacuo. Treatment of the residue with ethanol (1.5 L) gave **12** (343.5 g, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 146–1.52 (m, 8H), 2.46 (s, 6H), 3.84 (s, 4H), 3.88 (s, 4H), 7.35 (d, J = 8.0 Hz, 4H), 7.71–7.76 (m, 4H).

(4-Oxocyclohexane-1,1-diyl)bis(methylene) bis(4-methylbenzenesulfonate) (13)

A mixture of **12** (240.1 g, 470 mmol), 1N hydrochloric acid (1.8 L) and tetrahydrofuran (3.6 L) was heated under reflux for 5 hours. The mixture was extracted with ethyl acetate. The organic extracts were washed with water, dried over anhydrous sodium sulfate, filtered, and then concentrated in vacuo to give the crude product. A suspension of the crude product in hexane (1 L) was stirred at room temperature for 30 minutes. The precipitates were collected by filtration to give **13** (219.0 g, 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.72 (t, J = 7.3 Hz, 4H), 2.22 (t, J = 7.3 Hz, 4H), 2.47 (s, 6H), 3.94 (s, 4H), 7.37 (d, J = 7.9 Hz, 4H), 7.72–7.76 (m, 4H).

(4-Hydroxy-4-vinylcyclohexane-1,1-diyl)bis(methylene) bis(4-methylbenzenesulfonate) (14)

To a solution of vinylmagnesium bromide (203 mL, 1 M in tetrahydrofuran) was added drop wise a solution of **13** (73.0 g, 156 mmol) in anhydrous tetrahydrofuran (312 mL) at –78 °C for 5 hours, the mixture was stirred at the same temperature for 15minutes. After quenching the reaction by adding saturated ammonium chloride solution, the mixture was evaporated in vacuo to remove tetrahydrofuran. The mixture was extracted with diethyl ether. The organic extracts were washed

with brine, dried over anhydrous sodium sulfate, filtered, and then concentrated in vacuo to give the crude alcohol **14**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.36–1.46 (m, 8H), 2.46 (s, 3H), 2.47 (s, 3H), 3.76 (s, 2H), 3.92 (s, 2H), 5.05 (d, J = 11 Hz, 1H), 5.18 (d, J = 18 Hz, 1H), 5.85 (dd, J = 18, 11 Hz, 1H), 7.32–7.38 (m, 4H), 7.70–7.77 (m, 4H).

(1-Vinyl-2-oxabicyclo[2.2.2]octan-4-yl)methyl 4-methylbenzenesulfonate (15)

To a solution of **14** in anhydrous dimethoxyethane (3.2 L) was added sodium hydride (22.5 g, 468 mmol, 50% oil dispersion) under cooling with ice, the mixture was stirred at the same temperature for 30 minutes. The mixture was heated under reflux for 2.5 hours. After quenching the reaction by adding saturated ammonium chloride solution, the mixture was extracted with ethyl acetate. The organic extracts were dried over anhydrous sodium sulfate, filtered, and then concentrated in vacuo. Flash chromatography (hexane/ethyl acetate = 3:1) of the residue gave **15** (26.7 g, 52%, 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47–1.53 (m, 2H), 1.60–1.72 (m, 4H), 1.82–1.92 (m, 2H), 2.45 (s, 3H), 3.66–3.68 (m, 2H), 3.69 (s, 2H), 5.01 (dd, J = 11, 1.2 Hz, 1H), 5.12 (dd, J = 18, 1.2 Hz, 1H), 5.78 (dd, J = 18, 11 Hz, 1H), 7.35 (d, J = 8.0 Hz, 2H), 7.76 (d, J = 8.0 Hz, 1H).

(1-Vinyl-2-oxabicyclo[2.2.2]octan-4-yl)methyl acetate (16)

A mixture of **15** (27.0 g, 81.3 mmol) and cesium carbonate (52.7 g, 275 mmol) in anhydrous *N*,*N*-dimethylformamide (500 mL) was heated at 100 °C for overnight. After dilution of the mixture with water, the mixture was extracted with ethyl acetate. The organic extracts were dried over anhydrous sodium sulfate, filtered, and then concentrated in vacuo to give **16** (17.7 g, quant). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.52–1.62 (m, 2H), 1.66–1.77 (m, 4H), 1.85–1.95 (m, 2H), 2.05 (s, 3H), 3.79–3.81 (m, 4H), 5.03 (dd, J = 11, 1.8 Hz, 1H), 5.15 (dd, J = 18, 1.2 Hz, 1H), 5.82 (dd, J = 11, 1.8 Hz,1H).

(1-Vinyl-2-oxabicyclo[2.2.2]octan-4-yl)methanol (17)

To a solution of **16** (17.0 g, 80.8 mmol) in methanol (265 mL) was added a solution of potassium carbonate (55.8 g, 404 mmol) in water (340 mL) under cooling, the mixture was stirred at room temperature for 2 hours and was evaporated in vacuo to remove methanol. The aqueous mixture was extracted with ethyl acetate. The organic extracts were dried over anhydrous sodium sulfate, filtered, and then concentrated in vacuo. Flash chromatography (hexane/ethyl acetate = 1:2) of the residue

gave **17** (13.9 g, 94%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.49–1.59 (m, 2H), 1.64–1.76 (m, 4H), 1.85–1.95 (m, 2H), 3.35 (d, J = 5.5 Hz, 2H), 3.81–3.82 (m, 2H), 3.79–3.81 (m, 4H), 5.02 (dd, J = 11, 1.2 Hz, 1H), 5.16 (dd, J = 18, 1.2 Hz, 1H), 5.82 (dd, J = 18, 11 Hz, 1H).

1-Vinyl-2-oxabicyclo[2.2.2]octane-4-carboxylic acid (18)

To a solution of **17** (22.7 g, 135 mmol) in *N*,*N*-dimethylformamide (360 mL) was added pyridinium dichromate (177.8 g, 473 mmol) under cooling with ice, the mixture was stirred at 25–40 °C for 3.5 hours. After dilution of the mixture with water, the mixture was extracted with ethyl acetate. The organic extracts were extracted with 1N potassium hydroxide solution. The aqueous solution was adjusted to pH 1 by adding concentrated hydrochloric acid and extracted with ethyl acetate. The organic extracts were dried over anhydrous sodium sulfate, filtered, and then concentrated in vacuo. Flash chromatography (hexane/ethyl acetate/acetic acid = 1:1:0.02) of the residue gave **18** (18.1 g, 74%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.67–1.87 (m, 8H), 3.83 (s, 2H), 4.96 (dd, J = 11, 1.8 Hz, 1H), 5.08 (dd, J = 18, 1.8 Hz, 1H), 5.77 (dd, J = 18, 11 Hz, 1H).

***t*-Butyl 1-vinyl-2-oxabicyclo[2.2.2]octan-4-ylcarbamate (19)**

To a suspension of **18** (10.0 g, 54.9 mmol) and dried molecular sieves (4A, 11.0 g, powder) in anhydrous toluene (280 mL) was added triethylamine (8.42 mL, 60.4 mmol) and diphenylphosphoryl azide (DPPA, 13.0 mL, 60.4 mmol), the mixture was stirred at room temperature for 2 hours and heated at reflux for 2 hours. After insoluble materials were filtered off, the filtrate was concentrated in vacuo. To a solution of the residue in anhydrous tetrahydrofuran (230 mL) was added potassium *t*-butoxide (13.6 g, 121 mmol) under cooling with ice, the mixture was stirred at room temperature for overnight. After quenching the reaction by addition of 10% aqueous citric acid solution, the mixture was concentrated in vacuo. After dilution of the residue with ethyl acetate, the mixture was washed with saturated sodium hydrogen carbonate solution, water and brine, dried over anhydrous sodium sulfate, filtered, and then concentrated in vacuo. Flash chromatography (toluene/tetrahydrofuran = 10:1) of the residue gave **19** (13.12 g, 94%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (s, 9H), 1.61–2.01 (m, 6H), 2.06–2.12 (m, 2H), 3.99 (s, 2H), 4.28 (s, 1H), 5.02 (dd, J = 11, 1.2 Hz, 1H), 5.15 (dd, J = 18, 1.8 Hz, 1H), 5.81 (dd, J = 18, 11 Hz, 1H). IR (ATR) cm^{-1}: 3314, 1713. MS (CI^+) *m/z*: 254.2 (MH^+). HRMS (CI^+) for $C_{14}H_{24}NO_3$ (MH^+): calcd, 254.1756; found, 254.1726.

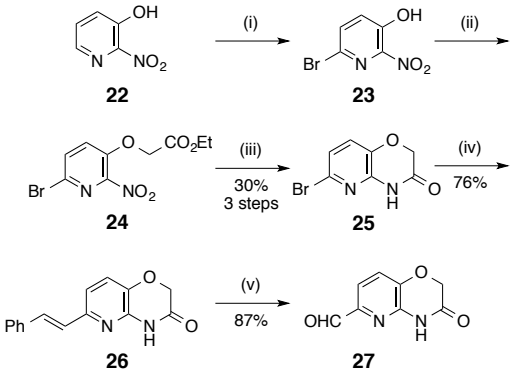
***t*-Butyl 1-(1,2-dihydroxyethyl)-2-oxabicyclo[2.2.2]octan-4-ylcarbamate (20)**

To a solution of **19** (5.00 g) in acetone (84.3 mL) and water (16.9 mL) were added a solution of 4-methylmorphline oxide (20.6 mL, 4.8 M in water) and a solution of osmium tetroxide (10.0 mL, 2.5 wt% in *t*-butanol), the mixture was stirred at room temperature for 5 hours. After quenching the reaction by adding a solution of sodium sulfite (73 mL, 17 wt% in water), the mixture was concentrated in vacuo. After dilution of the residue with ethyl acetate, the mixture was washed with water and brine, dried over anhydrous sodium sulfate, filtered, and then concentrated in vacuo. Treatment of the residue with ether gave **20** (5.18 g, 91%). ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9H), 1.60–1.69 (m, 2H), 1.75–1.85 (m, 2H), 1.96–2.17 (m, 4H), 2.38 (dd, J = 8.6, 3.7 Hz, 1H), 2.55 (d, J = 6.1 Hz, 1H), 3.39–3.45 (m, 1H), 3.60–3.72 (m, 2H), 3.93 (dd, J = 7.9, 3.1 Hz, 1H), 3.98 (dd, J = 7.9, 2.4 Hz, 1H), 4.28 (br, 1H). IR (ATR) cm^{-1}: 3358, 1688. MS (CI^+) *m/z*: 288 (MH^+). HRMS (CI^+) for $C_{14}H_{26}NO_5$ (MH^+): calcd, 288.1811; found, 288.1818.

***t*-Butyl 1-formyl-2-oxabicyclo[2.2.2]octan-4-ylcarbamate (21)**

To a solution of **20** (3.00 g) in tetrahydrofuran (131 mL) was added sodium periodate, the resulting mixture was stirred at room temperature for 30 minutes. After dilution of the mixture with water, the mixture was extracted with ethyl acetate. The organic extracts were washed with brine, dried over anhydrous sodium sulfate, filtered, and then concentrated in vacuo. Treatment of the residue with ether gave **21** (2.33 g, 87%). ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 9H), 1.81–1.91 (m, 4H), 1.94–2.06 (m, 2H), 2.07–2.17 (m, 2H), 4.06 (s, 2H), 4.31 (br, 1H), 9.56 (s, 1H). IR (ATR) cm^{-1}: 3362, 1742, 1689. MS (CI^+) *m/z*: 256 (MH^+). HRMS (CI^+) for $C_{13}H_{22}NO_4$ (MH^+): calcd, 256.1549; found, 256.1537.

Scheme 2



6-Bromo-2-nitropyridin-3-ol (23)

To a solution of **22** (140 g, 1.00 mol) in methanol (2.5 L) was added a solution of sodium methoxide [prepared from Na (24.2 g) and methanol (215 mL)] at room temperature. The mixture was stirred at the same temperature for 30 min. Bromine (51.4 mL, 1.00 mol) was added dropwise to the mixture at 0 °C, the mixture was stirred at the same temperature for 2 hours. After quenching the reaction by adding acetic acid (18 mL), the mixture was concentrated in vacuo to give **23**, which was used for the next step without further purification.

Ethyl 2-(6-bromo-2-nitropyridin-3-yloxy)acetate (24)

To a suspension of the crude **23** and potassium carbonate (277 g, 2.00 mol) in acetone (1.4 L) was added ethyl bromoacetate (111 mL, 1.00 mol), the mixture was heated at reflux for 8 hours. After dilution of the mixture with t-butyl methyl ether (1.4 L), the resulting precipitates were filtered off. The filtrate was concentrated in vacuo to give **24**, which was used for the next step without further purification.

6-Bromo-2H-pyrido[3,2-b][1,4]oxazin-3(4H)-one (25)

A suspension of the crude **24** and iron powder (162 g, 2.90 mol) in acetic acid (1.2 L) was heated at 90 °C for 1.5 hours. After dilution of the mixture with ethyl acetate (2.4 L), the resulting precipitates were filtered off. The filtrate was concentrated in vacuo. Flash column chromatography (hexane/ethyl acetate = 2:1) of the residue gave **25** (69.0 g, 30%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.67 (s, 2H), 1.89 (d, J = 18.3 Hz, 1H), 7.11 (d, J = 18.3 Hz, 1H), 7.13 (d, J = 18.3 Hz, 1H), 8.12 (brs, 1H).

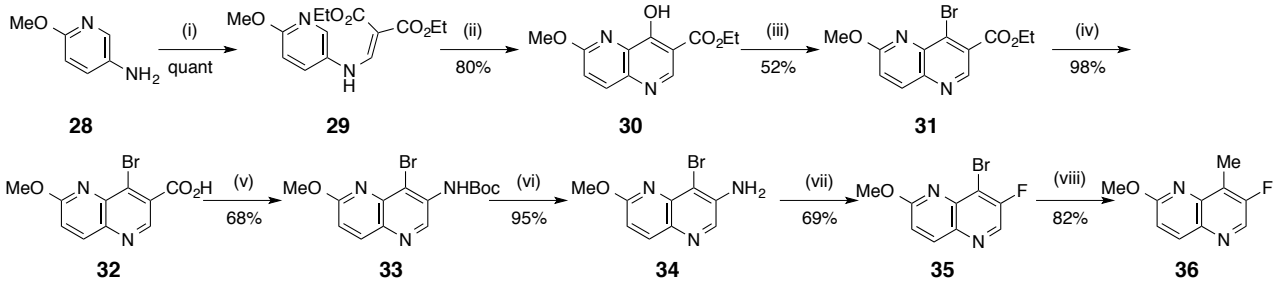
(*E*)-6-Styryl-2H-pyrido[3,2-b][1,4]oxazin-3(4H)-one (26)

To a degassed solution of **25** (28.9 g 126 mmol) in 1,4-dioxane (630 mL) and water (100 mL) was added phenylvinylboronic acid (19.2 g, 126 mmol), potassium carbonate (35.6 g, 252 mmol) and tetrakis(triphenylphosphine)palladium (4.42 g, 3.79 mmol), the mixture was heated at reflux for 24 hours. After dilution of the mixture with water (720 mL), the resulting precipitates were collected by filtration and washed with water (180 mL). Flash column chromatography (NH silica-gel, hexane/1,4-dioxane = 2:1) of the crude product gave **26** (24.3 g, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.68 (s, 2H), 7.01 (d, J = 7.9 Hz, 1H), 7.03 (d, J = 15.9 Hz, 1H), 7.23 (d, J = 7.9 Hz, 1H), 7.36 (t, J = 7.3 Hz, 2H), 7.46 (d, J = 15.9 Hz, 1H), 7.53 (d, J = 7.3 Hz, 1H), 8.09 (brs, 1H).

3-Oxo-3,4-dihydro-2H-pyrido[3,2-b][1,4]oxazine-6-carbaldehyde (27)

A suspension of **26** (24.0 g, 95.2 mmol) in dichloromethane (1.2 L) and methanol (420 mL) was bubbled with ozone at –71 °C until a pale blue colour appeared. The excess ozone was removed by bubbling air through the suspension for 30 min. Dimethyl sulfide (36 mL, 490 mmol) was added to the suspension. The mixture was stirred at room temperature for overnight and concentrated in vacuo. After dilution of the mixture with diethyl ether (130 mL) and 0.5 M hydrochloric acid (65 mL), the resulting precipitates were collected by filtration and washed with water (40 mL x 3) and diethyl ether (40 mL). Treatment of the crude product with acetone (80 mL) gave **27** (14.7 g, 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.80 (s, 2H), 7.39 (d, J = 7.9 Hz, 1H), 7.69 (d, J = 7.9 Hz, 1H), 8.35 (brs, 1H), 9.89 (s, 1H).

Scheme 3



Diethyl 2-((6-methoxypyridin-3-ylamino)methylene)malonate (29)

A mixture of **28** (100g, 0.806 mol) and diethyl ethoxymethylenemalonate (178 g, 0.806 mol) in ethanol (1 L) was heated under reflux for 2 hours. The mixture was concentrated in vacuo to give **29** (244 g, quant). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.32 (t, J = 7.4 Hz, 3H), 1.38 (t, J = 7.4 Hz, 3H), 3.94

(s, 3H), 4.24 (q, *J* = 7.4 Hz, 2H), 4.31 (q, *J* = 7.4 Hz, 2H), 6.78 (d, *J* = 8.6 Hz, 1H), 7.43 (dd, *J* = 9.2, 3.1 Hz, 1H), 8.03 (d, *J* = 3.1 Hz, 1H), 8.37 (d, *J* = 3.1 Hz, 1H), 10.90–11.10 (m, 1H).

Ethyl 4-hydroxy-6-methoxy-1,5-naphthyridine-3-carboxylate (30)

The diester **29** (60.0g, 0.20 mol) was added portion wise to diphenyl ether (300 mL) at 260 °C for 5 minutes. After cooling, the mixture was diluted with pentane. The resulting precipitates were collected by filtration and washed with hexane to give crude **3**. Another two experiments at the same reaction scale gave the crude product **3**. The combined crude **3** was stirred in hexane (1.2 L), the precipitates were collected by filtration and washed with hexane to give **30** (157.2 g, 80%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.27 (t, *J* = 6.7 Hz, 3H), 3.94 (s, 3H), 4.21 (t, *J* = 6.7 Hz, 2H), 7.20 (d, *J* = 8.6 Hz, 1H), 7.99 (d, *J* = 9.2 Hz, 1H), 8.49 (brs, 1H).

Ethyl 4-bromo-6-methoxy-1,5-naphthyridine-3-carboxylate (31)

To a suspension of **30** (312 g, 1.26 mol) in anhydrous *N*,*N*-dimethylformamide (1.1 L) was added phosphorous tribromide (175 mL, 1.51 mol) under cooling with water, the mixture was stirred at room temperature for 2.5 hours. The mixture was poured into ice water (4 L), the mixture was adjusted to pH 8 with saturated sodium hydrogen carbonate solution. The resulting precipitates were collected by filtration, washed with water, and dried. Flash chromatography (toluene/ethyl acetate = 5:1) of the crude product gave **31** (203 g, 52%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.37 (t, *J* = 7.3 Hz, 3H), 4.09 (s, 3H), 4.43 (q, *J* = 7.3 Hz, 2H), 7.43 (d, *J* = 9.1 Hz, 1H), 8.36 (d, *J* = 9.1 Hz, 1H), 8.91 (s, 1H).

4-Bromo-6-methoxy-1,5-naphthyridine-3-carboxylic acid (32)

A suspension of **31** (192 g, 0.617 mol) in tetrahydrofuran (1.9 L) was added 2N sodium hydroxide solution (694 mL, 1.39 mol) under cooling with ice, the mixture was stirred at room temperature for 3 hours. After quenching the reaction by adding 2N hydrochloric acid (375 mL, pH 6), the mixture was evaporated in vacuo to remove tetrahydrofuran. The aqueous mixture was adjusted to pH 2 with 2N hydrochloric acid (400 mL) and diluted with water (1.3 L). The resulting precipitates were collected by filtration and washed with water to give **32** (171 g, 98%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.09 (s, 3H), 7.41 (d, *J* = 9.1 Hz, 1H), 8.35 (d, *J* = 8.5 Hz, 1H), 14.03 (s, 1H).

_t_-Butyl 4-bromo-6-methoxy-1,5-naphthyridin-3-ylcarbamate (33)

A mixture of **32** (169 g, 0.593 mol), diphenyl phosphoryl azide (DPPA, 141 mL, 0.653 mol), triethylamine (744 mL, 5.34 mol) and anhydrous *t*-butanol (886 mL, 9.26 mol) in anhydrous *N*,*N*-dimethylformamide (2 L) was heated at 100 °C for 1 hour and concentrated in vacuo. After dilution of the residue with ethyl acetate, the mixture was washed with saturated sodium hydrogen carbonate solution and brine, dried over anhydrous sodium sulfate, filtered, and then concentrated in vacuo. Flash chromatography (hexane/ethyl acetate = 3:1) of the residue gave **33** (144 g, 68%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.49 (s, 9H), 4.06 (s, 3H), 7.26 (d, J = 9.2 Hz, 1H), 8.29 (d, J = 9.2 Hz, 1H), 8.83 (s, 1H), 9.15 (s, 1H).

4-Bromo-6-methoxy-1,5-naphthyridin-3-amine (34)

To a solution of **33** (98.0 g, 0.277 mol) in dichloromethane (280 mL) was added trifluoroacetic acid (166 mL) at –10 °C, the mixture was stirred at room temperature for overnight and concentrated in vacuo. After dilution of the residue with chloroform, the mixture was poured onto saturated sodium hydrogen carbonate solution (2.3 L, pH 8). The resulting precipitates were collected by filtration and washed with water to give **7** (54.0 g). The combined mixture of the filtrate and washing was extracted with chloroform (1 L). The organic extracts were dried over anhydrous sodium sulfate, filtered, and then concentrated in vacuo to give **34** (13.1 g, total 67.1 g, 95%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.00 (s, 3H), 6.21 (brs, 2H), 6.88 (d, J = 8.6 Hz, 1H), 8.05 (d, J = 8.6 Hz, 1H), 8.34 (s, 1H).

8-Bromo-7-fluoro-2-methoxy-1,5-naphthyridine (35)

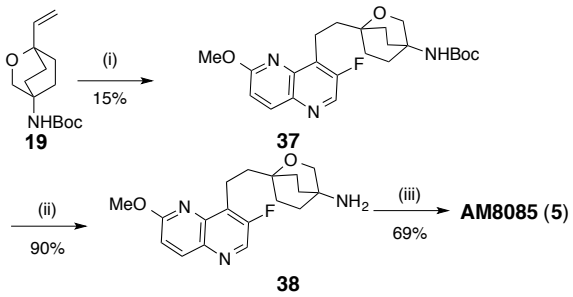
To a solution of **34** (37.1 g, 0.146 mol) in anhydrous tetrahydrofuran (580 mL) was added nitrosyl tetrafluoroborate (20.8 g, 0.169 mol) at –10 °C, the mixture was stirred at the same temperature for 50 minutes. Another nitrosyl tetrafluoroborate (5.39 g, 43.8 mmol) was added to the mixture at the same temperature. After stirring for 35 minutes, another nitrosyl tetrafluoroborate (1.80 g, 14.6 mmol) was added to the mixture. After stirring for 5 minutes, the resulting precipitates were collected by filtration and washed with cold tetrahydrofuran to give diazonium salt as yellow solid (49.1 g). A suspension of the salt (49.1 g) in decalin (730 mL) was heated at 100 °C for 1 hour. After cooling with NaCl-ice bath, the precipitates were collected by filtration and dissolved with ethyl acetate. The mixture was washed with saturated sodium hydrogen carbonate solution, dried over

anhydrous sodium sulfate, filtered, and then concentrated in vacuo. Flash chromatography (toluene/ethyl acetate = 30:1) of the residue gave **35** (22.0 g, 59%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.09 (s, 3H), 7.32 (d, J = 9.2 Hz), 8.36 (d, J = 9.2 Hz), 8.87 (s, 1H).

7-Fluoro-2-methoxy-8-methyl-1,5-naphthyridine (36)

A degassed mixture of **35** (15.0 g), methylboronic acid (6.99 g), tetrakis(triphenylphosphine)palladium (6.74 g), saturated potassium carbonate solution (45.6 mL) and 1,4-dioxane (70.7 mL) was stirred at 100 ºC for 100 hours, and then concentrated in vacuo. After dilution of the residue with ethyl acetate, the mixture was washed with water and brine, dried over anhydrous sodium sulfate, filtered, and then concentrated in vacuo. Flash chromatography (hexane/ethyl acetate = 4:1) of the residue gave **36** (9.25 g, 83%). Mp: 79 ºC (from hexane). ^{1}H NMR (DMSO-d_6) δ 2.64 (d, J = 1.8 Hz, 3H), 4.10 (s, 3H), 7.07 (d, J = 9.2 Hz, 1H), 8.17 (d, J = 9.2 Hz, 1H), 8.61 (s, 1H). MS (EI$^+$) *m/z*: 192 (M$^+$). HRMS (EI$^+$) for $C_{10}H_9FN_2O$ (M$^+$): calcd, 192.0699; found, 192.0715. Anal. Calcd for $C_{10}H_9FN_2O$: C, 62.49; H, 4.72; N, 14.58. Found: C, 61.25; H, 4.73; N, 14.33.

Scheme 4



***t*-Butyl-1-(2-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)ethyl)-2-oxabicyclo[2.2.2]octan-4-ylcarbamate (37)**

To a solution of **19** (250 mg, 0.987 mmol) in anhydrous tetrahydrofuran (4.3 mL) was added a solution of 9-borabicyclo[3.3.1]nonane dimer (3.9 mL, 1.97 mmol, 0.5 M in tetrahydrofuran) under cooling with ice, the mixture was stirred at room temperature for 1 hour. After quenching the reaction by adding water (1 drop) under cooling, the mixture was added **35** (253 mg, 0.987 mmol), tetrakis(triphenylphosphine)palladium (228 mg, 0.197 mmol), tripotassium phosphate (1.49 g, 7.00

mmol) and ethanol/water (2.3 mL, 4:1), and degassed. The mixture was heated at 70 °C for 18 hours and concentrated in vacuo. After dilution of the residue with ethyl acetate, the mixture was washed with water and brine, dried over anhydrous sodium sulfate, filtered, and then concentrated in vacuo. Flash chromatography (hexane/ethyl acetate = 3:1) of the residue gave **37** (127 mg, 30%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (s, 9H), 1.74–1.87 (m, 6H), 1.91–2.17 (m, 4H), 3.15–3.22 (m, 2H), 3.96 (s, 2H), 4.08 (s, 3H), 4.29 (brs, 1H), 7.05 (d, J = 9.2 Hz, 1H), 8.16 (d, J = 9.2 Hz, 1H), 8.59 (s, 1H).

1-(2-(3-Fluoro-6-methoxy-1,5-naphthyridin-4-yl)ethyl)-2-oxabicyclo[2.2.2]octan-4-amine (38)

To a solution of **37** (80.0 mg, 0.185 mmol) in dichloromethane (0.84 mL) was added trifluoroacetic acid (0.84 mL) under cooling with ice, the mixture was stirred at the same temperature for 30 minutes and concentrated in vacuo. After dilution of the residue with water, the mixture was adjusted to pH 13 with 1 M aqueous sodium hydroxide solution. The resulting precipitates were collected by filtration and washed with water to give **38** (55.5 mg, 90%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.37 (brs, 2H), 1.45–1.54 (m, 6H), 1.89–1.98 (m, 6H), 4.05 (s, 3H), 7.29 (d, J = 9.2 Hz, 1H), 8.27 (d, J = 9.2 Hz, 1H), 8.81 (s, 1H).

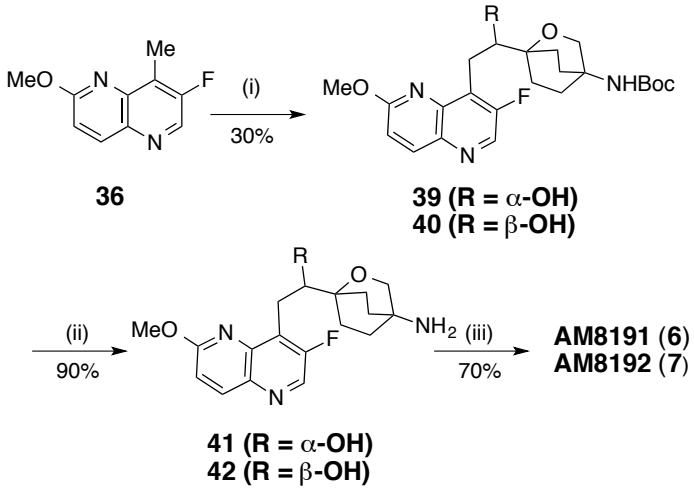
6-((1-(2-(3-Fluoro-6-methoxy-1,5-naphthyridin-4-yl)ethyl)-2-oxabicyclo[2.2.2]octan-4-ylamino) methyl)-2H-pyrido[3,2-b][1,4]oxazin-3(4H)-one (5fb)

A mixture of **38** (350 mg, 1.06 mmol) and **27** (198 mg, 1.11 mmol) in anhydrous *N*,*N*-dimethylformamide (8.4 mL) was added acetic acid (1.2 mL), the mixture was stirred at room temperature for 10 minutes. The resulting solution was added three times of sodium triacetoxyborohydride (112 mg x3, 0.528 mmol x3) hourly under cooling with ice, the mixture was stirred at room temperature for overnight. The mixture was concentrated in vacuo. After dilution of the residue with dichloromethane, the mixture was washed with saturated sodium carbonate solution, water and brine. The organic extracts were dried over anhydrous sodium sulfate, filtered, and then concentrated in vacuo. Flash chromatography (dichloromethane/methanol = 30:1) of the residue gave **5fb** (357 mg, 69%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.58–1.77 (m, 8H), 1.83–1.92 (m, 3H), 3.08–3.15 (m, 2H), 3.58 (s, 2H), 3.62 (d, J = 6.1 Hz, 2H), 4.02 (s, 3H), 4.59 (s, 2H), 7.01 (d, 7.9 Hz, 1H), 7.22 (d, J = 9.2 Hz, 1H), 7.27 (d, J = 7.9 Hz, 1H), 8.26 (d, J = 9.2 Hz, 1H), 8.74 (s, 1H), 11.41 (brs, 1H).

6-((1-(2-(3-Fluoro-6-methoxy-1,5-naphthyridin-4-yl)ethyl)-2-oxabicyclo[2.2.2]octan-4-ylamino) methyl)-2H-pyrido[3,2-b][1,4]oxazin-3(4H)-one hydrochloride (5)

To a solution of **5fb** (40.0 mg, 0.081 mmol) in dichloromethane (2 mL) and ethanol (0.5 mL) was added a solution of hydrogen chloride (22 uL, 0.081 mmol, 4 M in 1,4-dioxane) under cooling with ice, the mixture was stirred at room temperature for 2 hours and concentrated in vacuo. Treatment of the residue with ethanol gave **5** (40.0 mg, 93%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.66–1.73 (m, 2H), 1.79–1.91 (m, 2H), 1.93–2.10 (m, 6H), 3.09–3.18 (m, 2H), 3.91 (s, 2H), 4.04 (s, 3H), 4.07–4.15 (m, 2H), 4.69 (s, 2H), 7.22 (d, J = 8.0 Hz, 1H), 7.24 (d, J = 8.6 Hz, 1H), 7.45 (d, J = 8.0 Hz, 1H), 8.27 (d, J = 8.6 Hz, 1H), 8.76 (s, 1H), 9.25–9.36 (m, 2H), 11.32 (s, 1H).

Scheme 5



***t*-Butyl-1-(2-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)-1-hydroxyethyl)-2-oxabicyclo[2.2.2]octan-4-ylcarbamate (39 and 40)**

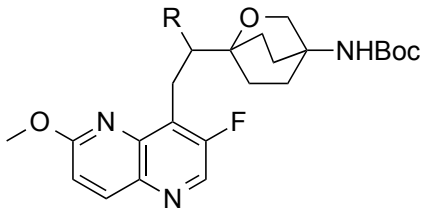
To a suspension of **36** (1.13 g) in tetrahydrofuran (58.8 mL) was added a solution of LDA (5.88 mL, 1.0 M in tetrahydrofuran) at –78 ºC, the mixture was stirred at the same temperature for 50 minutes. *t*-Butyl 1-formyl-2-oxabicyclo[2.2.2]octan-4-ylcarbamate **21** (500 mg) was added to the mixture at –78 ºC, the resulting mixture was stirred at the same temperature for 1.5 hours. After quenching the reaction by adding 10% citric acid solution, the mixture was extracted with dichloromethane. The organic extracts were washed with water and brine, dried over anhydrous sodium sulfate, filtered, and then concentrated in vacuo. Flash chromatography (hexane/ethyl acetate = 1:1) of the residue gave the racemate (**39** and **40**, 446 mg). Optical resolution (CHIRALPAK IA,

hexane/isopropanol/methyl *t*-butyl ether = 20:50:30) of the racemate (400 mg) gave **39** (206 mg) and **40** (197 mg).

39: $[\alpha]^{26}_{D}$ –61.1 º (*c* 0.201, MeOH). ^{1}H NMR (DMSO-d_6) δ 1.38 (s, 9H), 1.71–2.08 (m, 8H), 2.96–3.03 (m, 1H), 3.70–3.77 (m, 3H), 4.02 (s, 3H), 4.48 (d, J = 6.1 Hz, 2H), 6.59 (br, 1H), 7.20 (d, J = 9.2 Hz, 1H), 8.25 (d, J = 9.2 Hz, 1H), 8.72 (s, 1H). IR (ATR) cm^{-1}: 3339, 1685. MS (ESI^{+}) *m/z*: 448 (MH^{+}). HRMS (ESI^{+}) for $C_{23}H_{31}FN_3O_5$ (MH^{+}): calcd, 448.22477; found, 448.22493.

40: $[\alpha]^{26}_{D}$ +72.3 º (*c* 0.200, MeOH). ^{1}H NMR (DMSO-d_6) δ 1.36 (s, 9H), 1.72–2.01 (m, 8H), 2.99 (dd, J = 12.1, 10.3 Hz, 1H), 3.28–3.36 (m, 1H), 3.70–3.80 (m, 3H), 4.02 (s, 3H), 4.48 (d, J = 5.5 Hz, 2H), 6.59 (br, 1H), 7.20 (d, J = 9.1 Hz, 1H), 8.25 (d, J = 9.1 Hz, 1H), 8.72 (s, 1H). IR (ATR) cm^{-1}: 3339, 1685. MS (ESI^{+}) *m/z*: 448 (MH^{+}). HRMS (ESI^{+}) for $C_{23}H_{31}FN_3O_5$ (MH^{+}): calcd, 448.22477; found, 448.22475.

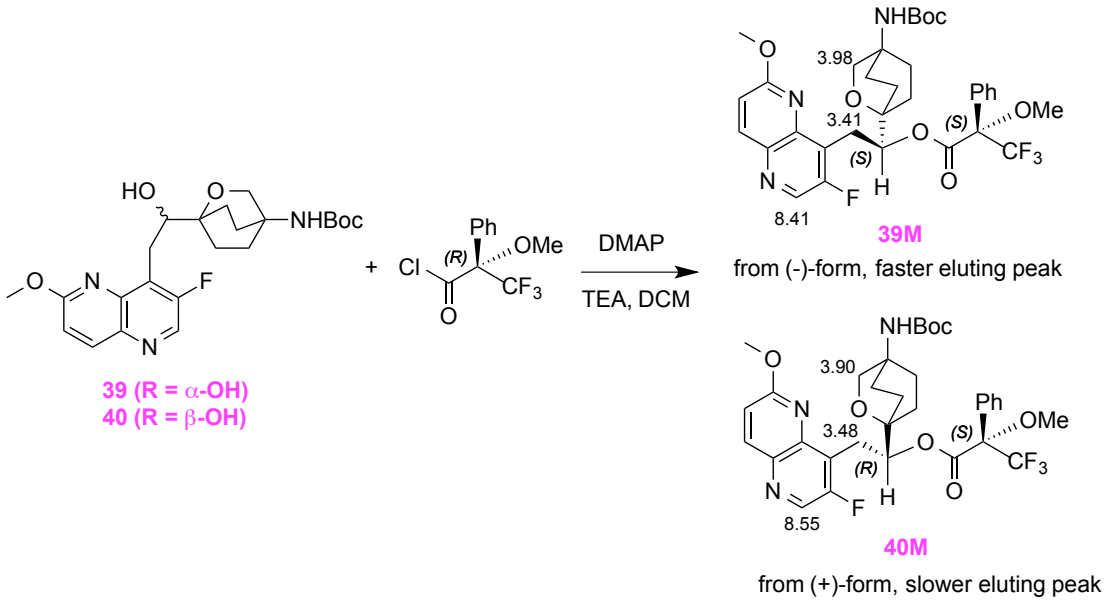
Alternative Optical Resolution Condition



37 (R = α-OH) **39 (R = α-OH)**
38 (R = β-OH) **40 (R = β-OH)**

The mixture of **39 & 40** (please note **37** and **38** was duplicated in the draft) was resolved by SFC (Chiralpak AD 250*50 mm I.D., 5 um; Mobile phase: Supercritical CO_2/IPA = 60/40; Flow rate: 160 mL/min; Wavelength: 220nm) to give **39** ((-)-form**)** as faster peak and **40** ((+)-form) as slow peak.

2. Mosher Ester Preparation and NMR analysis



To a solution of **39** or **40** (17.2 mg, 0.04 mmol), TEA (20 mg, 0.2 mmol) in DCM (4 mL) was added (R)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl chloride (25 mg, 0.1 mmol) followed by adding DMAP (5 mg, 0.04 mmol). The resulting mixture was stirred at r.t. for 1 h before diluted with DCM (10 mL). The mixture was washed with aq. Na_2CO_3, citric acid and brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified via prep-TLC (DCM:MeOH = 10:1) to give the desired Mosher ester.

39M **^{1}H-NMR** (400 MHz, $CDCl_3$) δ ppm 8.41 (s, 1 H), 8.13 (d, J = 9.2 Hz, 1 H), 7.32-7.34 (m, 1H), 7.19-7.24 (m, 4 H), 7.01-7.04 (m, 1 H), 5.91-5.94 (m, 1H), 4.32 (brs, 1H), 4.08 (s, 3 H), 3.95-4.01 (m, 2 H), 3.40-3.42 (m, 2 H), 3.26 (s, 3H), 2.08-2.14 (m, 4H), 1.86-1.92 (m, 4H), 1.43 (s, 9H). **MS** m/z 664.1 $(M+1)^+$.

40M **^{1}H-NMR** (400 MHz, $CDCl_3$) δ ppm 8.55 (s, 1 H), 8.16 (d, J = 9.2 Hz, 1 H), 7.30-7.28 (m, 1H), 7.19-7.22 (m, 2 H), 7.06-7.08 (m, 3 H), 5.90-5.93 (m, 1H), 4.29 (brs, 1H), 4.08 (s, 3 H), 3.86-3.93 (m, 2 H), 3.42-3.54 (m, 2 H), 3.32 (s, 3H), 2.01-2.11 (m, 4H), 1.60-1.86 (m, 4H), 1.26 (s, 9H). **MS** m/z 664.1 $(M+1)^+$.

1-(4-Amino-2-oxabicyclo[2.2.2]octan-1-yl)-2-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)ethanol (41)

The title compound **41** (122 mg) was prepared from *t*-butyl

1-(2-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)-1-hydroxyethyl)-2-oxabicyclo[2.2.2]octan-4-ylcarbamate **39** (170 mg) in the same manner as described for **38**. $[\alpha]^{27}_{D}$ –85.4 º (*c* 0.199, MeOH). ^{1}H NMR ($CDCl_3$) δ 1.30 (s, 2H), 1.46–1.62 (m, 4H), 1.68–1.81 (m, 3H), 1.86–1.98 (m, 1H), 3.01 (dd, *J* = 12.2, 10.4 Hz, 1H), 3.29–3.32 (m, 1H), 3.43 (s, 2H), 3.73 (ddd, *J* = 9.8, 6.1, 3.1 Hz, 1H), 4.41 (d, *J* = 6.1 Hz, 1H), 7.20 (d, *J* = 9.2 Hz, 1H), 8.25 (d, *J* = 9.2 Hz, 1H), 8.72 (s, 1H). IR (ATR) cm^{-1}: 3350, 3279. MS (CI^+) *m/z*: 348 (MH^+). HRMS (CI^+) for $C_{18}H_{23}FN_3O_3$ (MH^+): calcd, 348.1723; found, 348.1721.

The antipode **42** (100 mg) was prepared in the same manner from **40** (145 mg). $[\alpha]^{27}_{D}$ +94.5 º (*c* 0.200, MeOH). ^{1}H NMR ($CDCl_3$) δ 1.31 (s, 2H), 1.46–1.62 (m, 4H), 1.69–1.81 (m, 3H), 1.89–1.98 (m, 1H), 3.01 (dd, *J* = 12.2, 10.4 Hz, 1H), 3.29–3.37 (m, 1H), 3.43 (s, 2H), 3.73 (ddd, *J* = 9.8, 6.1, 3.1 Hz, 1H), 4.40 (d, *J* = 6.1 Hz, 1H), 7.20 (d, *J* = 9.2 Hz, 1H), 8.25 (d, *J* = 8.6 Hz, 1H), 8.72 (s, 1H). IR (ATR) cm^{-1}: 3350, 3273. MS (CI^+) *m/z*: 348 (MH^+). HRMS (CI^+) for $C_{18}H_{23}FN_3O_3$ (MH^+): calcd, 348.1723; found, 348.1701.

6-((1-(2-(3-Fluoro-6-methoxy-1,5-naphthyridin-4-yl)-1-hydroxyethyl)-2-oxabicyclo[2.2.2]octan-4-ylamino)methyl)-2H-pyrido[3,2-b][1,4]oxazin-3(4H)-one (6fb)

The title compound **6fb** (120 mg) was prepared from **41** (100 mg) and **27** (53.8 mg) in the same manner as described for **5fb**. $[\alpha]^{28}_{D}$ –59.9 º (*c* DMF, 0.302). ^{1}H NMR (DMSO-d_6) δ 1.56–2.03 (m, 9H), 3.03 (t, *J* = 10.4 Hz, 1H), 3.29–3.37 (m, 1H), 3.57 (s, 2H), 3.63 (d, *J* = 4.3 Hz, 1H), 3.71–3.79 (m, 1H), 4.02 (s, 3H), 4.44 (d, *J* = 6.1 Hz, 1H), 4.59 (s, 2H), 7.01 (d, *J* = 7.9 Hz, 1H), 7.21 (d, *J* = 9.2 Hz, 1H), 7.28 (d, *J* = 7.9 Hz, 1H), 8.25 (d, *J* = 9.2 Hz, 1H), 8.72 (s, 1H), 11.15 (s, 1H). IR (ATR) cm^{-1}: 1698. MS (ESI^+) *m/z*: 510 (MH^+). HRMS (ESI^+) for $C_{26}H_{29}FN_5O_5$ (MH^+): calcd, 510.21527; found, 510.21492.

The antipode **7fb** (114 mg) was prepared in the same manner from **42** (90.0 mg). $[\alpha]^{28}_{D}$ +71.0 º (*c* DMF, 0.302). ^{1}H NMR (DMSO-d_6) δ 1.54–2.03 (m, 9H), 3.02 (dd, *J* = 12.2, 11.0 Hz, 1H), 3.28–3.38 (m, 1H), 3.57 (s, 2H), 3.63 (d, *J* = 4.3 Hz, 1H), 3.73–3.79 (m, 1H), 4.02 (s, 3H), 4.44 (d, *J* = 6.1 Hz, 1H), 4.59 (s, 2H), 7.01 (d, *J* = 7.9 Hz, 1H), 7.21 (d, *J* = 9.2 Hz, 1H), 7.28 (d, *J* = 7.9 Hz, 1H), 8.26 (d, *J* = 9.2 Hz, 1H), 8.72 (s, 1H), 11.15 (s, 1H). IR (ATR) cm^{-1}: 1698. MS (ESI^+) *m/z*: 510 (MH^+). HRMS (ESI^+) for $C_{26}H_{29}FN_5O_5$ (MH^+): calcd, 510.21527; found, 510.21587.

6-((1-(2-(3-Fluoro-6-methoxy-1,5-naphthyridin-4-yl)-1-hydroxyethyl)-2-oxabicyclo[2.2.2]octan-4-ylamino)methyl)-2H-pyrido[3,2-b][1,4]oxazin-3(4H)-one hydrochloride (6).

The title compound **6** (200 mg) was prepared from **6fb** (210 mg) in the same manner as described for **5**. Mp: 239–240 ºC (from EtOH/DCM). $[\alpha]^{25}_{D}$ –54.6 º (*c* 0.200, DMSO). ^{1}H NMR (DMSO-d_6) δ 1.82–2.16 (m, 8H), 3.04 (t, *J* = 12.2 Hz, 1H), 3.80 (br, 1H), 3.88 (s, 2H), 4.03 (s, 3H), 4.10 (br, 2H), 4.69 (s, 2H), 4.70 (br, 3H), 7.20 (br, 1H), 7.22 (d, *J* = 9.2 Hz, 1H), 7.45 (d, *J* = 7.9 Hz, 1H), 8.27 (d, *J* = 9.2 Hz, 1H), 8.74 (s, 1H), 9.26 (br, 2H), 11.32 (s, 1H). IR (ATR) cm^{-1}: 1703. MS (ESI^{+}) *m/z*: 510 (MH^{+}) (as free base). HRMS (ESI^{+}) for $C_{26}H_{29}FN_5O_5$ (MH^{+}) (as free base): calcd, 510.21527; found, 510.21491.

The antipode **7** (219 mg) was prepared in the same manner from **7fb** (210 mg). Mp: 239–240 ºC (from EtOH/DCM). $[\alpha]^{26}_{D}$ +53.8 º (*c* 0.202, DMSO). ^{1}H NMR (DMSO-d_6) δ 1.81–2.17 (m, 8H), 3.04 (t, *J* = 11.6 Hz, 1H), 3.80 (br, 1H), 3.88 (s, 2H), 4.03 (s, 3H), 4.10 (br, 2H), 4.69 (s, 2H), 4.69 (s, 3H), 7.16–7.20 (m, 1H), 7.22 (d, *J* = 8.6 Hz, 1H), 7.45 (d, *J* = 7.9 Hz, 1H), 8.27 (d, *J* = 9.2 Hz, 1H), 8.74 (s, 1H), 9.27 (br, 2H), 11.32 (s, 1H). IR (ATR) cm^{-1}: 1703. MS (ESI^{+}) *m/z*: 510 (MH^{+}) (as free base). HRMS (ESI^{+}) for $C_{26}H_{29}FN_5O_5$ (MH^{+}) (as free base): calcd, 510.21527; found, 510.21453.

Systemic infection model. ICR mice (male, 4 weeks old, n=5) were inoculated intraperitoneally with 2 x10^{7} CFU/mouse of *S. aureus* Smith or 7 x10^{6} CFU/mouse of *E. coli* ML4707. Mice were treated orally or intravenously with the compounds at 1hour post infection. ED_{50} (effective dose at which 50% animals survived) was determined from the survival rate on 7-day after the infection.

Measurement of hERG channel activity: PatchXpress automated patch clamp

Experimental procedures for measurement of hERG currents with PatchXpress were previously described in detail elsewhere[1,2]. Briefly, whole-cell hERG currents were measured from CHO cells stably expressing hERG channels using the automated patch clamp system, PatchXpress® 7000A (Molecular Devices), at ambient temperature. Resistance of the planar patch plate (SealChip16™[AVIVA]) chambers (holes) were between 1 and 3 MΩ. Currents were elicited with a voltage-step protocol at 20-s inter-pulse intervals. hERG current was measured from a holding potential (Vh) of −80 mV. A 20-ms depolarizing pre-pulse to −50 mV was applied for measurement of baseline current, followed by a return to Vh for 80 ms, an activating 4-s depolarizing step to a test

potential (Vt) of +20mV, and a 4-s repolarizing step (Vtail) to −50 mV. hERG currents were quantified as peak deactivating tail current amplitude during Vtail. Currents were monitored for stability for 5 min before addition of the vehicle alone (DMSO control) or drug diluted from stocks in DMSO. Drug was applied (60 µL) at sequentially increasing concentrations at a rate of 25 µL/s. For each condition or drug concentration, duplicate or triplicate 60 µL additions were made to each test well at 11-s intervals in order to achieve equilibrium and current was monitored for 5 min at each drug concentration. The effects of drug on currents were normalized to the vehicle control current level for each cell, and were expressed as a percent inhibition using DataXpress (Molecular Devices Corp., Union City, California,U.S.A.). The averaged concentration-response data (means ± SEM, n ≥ 3 for each data point) for current inhibition were fitted with a Hill equation to determine the half-inhibitory concentration (IC50).

Inhibition of macromolecular synthesis. The assay was performed as previously described [3-5]. Briefly, mid-log (A_{600} = 0.5–0.6) *S. aureus* growth was incubated with increasing concentration of inhibitor at 37 °C for 20 min with 1 µCi/mL 2-[^{3}H]-glycerol, 1 µCi/mL [^{14}C]-thymidine, 1 µCi/mL [^{3}H]-uridine, 5 µCi/mL 4,5-[^{3}H]-leucine, or [^{14}C]-glycine to measure phospholipids, DNA, RNA, protein, and cell wall synthesis respectively. Cell wall labeling with [^{14}C]-glycine (*S. aureus*) was performed in the presence of 100 µg/mL chloramphenicol, which prevents protein synthesis and disengages the stringent response. The reaction was stopped by addition of 10% trichloroacetic acid and the cells were harvested using a glass fiber filter (PerkinElmer Life Sciences, 1205-401). The filter was dried and counted with scintillation fluid.

Determination of time kill kinetics. Time kill kinetics of kibdelomycin was conducted using *S. aureus* ATCC29213 at 0.5x, 1x, 2x 4x, and 8x of MIC (determined by broth dilution method) corresponding to concentrations of 0.03, 0.06, 0.12, 0.25, 0.5 and 1 µg/mL, respectively by CLSI guidelines[6]. Briefly, a stock solution of 5.12 mg/mL of kibdelomycin was prepared in DMSO. The inoculum suspension was prepared from growth on a TSA/5% sheep blood plate to equal the turbidity of a 0.5 McFarland Standard in Mueller Hinton II Broth (MHBII, Becton Dikinson, Sparks, MD), diluted 1:3, and grown in fresh MHBII at 35°C and incubated while shaker at 150 rpm. After approximately 2 hr, the suspension was again adjusted to equal a 0.5 McFarland Standard, was diluted 1:2 in fresh MHBII, and 1 mL was used to inoculate each 125 mL Erlenmeyer flasks

containing 8.75 mL of sterile MHBII. The final target cell density was approximately 10^6 – 10^7 CFU/mL. Just prior to T0, 0.25 mL of the appropriate concentration of kibdelomycin solution was added to each flask, gently mixed, followed by 1.0 mL of diluted inoculum. Thus, test drug vessels contained 8.75 mL MHBII, 1.0 mL inoculum, and 0.25 mL drug solution (40X). A total of 22 vessels were prepared in this fashion, immediately swirled to mix, and 0.03 mL was removed from the initial 0.5 McFarland Standard tube for determination of the viable count at baseline (T0) by serial ten-fold dilution in MHB II. All vessels were incubated at 35°C in a New Brunswick Scientific Series 25 Incubated Shaker rotating at 150 rpm to provide gentle mixing. The vessels were sampled at 2 hr (T2), 4 hr (T4), 6 hr (T6), 8 hr (T8), and 24 hr (T24) for determination of viable count. Viable counts were determined by removing 0.3 mL from each vessel and serially diluting 10-fold in 0.27 mL of MHBII with the Biomek 2000, then plating duplicate 10 µL samples onto TSA with 5% sheep blood plates using the track dilution method. All plates were incubated for 20-24 hr at 35°C. Colonies were manually counted, and the CFU/ml was determined from the average count from the duplicate plates, followed by calculation of the log10 CFU/ml. A bactericidal effect was defined as a 3 log10 CFU/mL decrease in viable count at 24 hr relative to the starting inoculum.

(1) Trepakova, E. S.; Malik, M. G.; Imredy, J. P.; Penniman, J. R.; Dech, S. J.; Salata, J. J. Application of PatchXpress planar patch clamp technology to the screening of new drug candidates for cardiac KCNQ1/KCNE1 (I Ks) activity. *Assay Drug Develop. Technol.* **2007**, *5*, 617–27.

(2) Zeng, H.; Penniman, J. R.; Kinose, F.; Kim, D.; Trepakova, E. S.; Malik, M. G.; Dech, S. J.; Balasubramanian, B.; Salata, J. J. Improved throughput of PatchXpress hERG assay using intracellular potassium fluoride. *Assay Drug Develop. Technol.* **2008**, *6*, 235-41.

(3) Wang, J.; Galgoci, A.; Kodali, S.; Herath, K. B.; Jayasuriya, H.; Dorso, K.; Vicente, F.; Gonzalez, A.; Cully, D.; Bramhill, D.; Singh, S. Discovery of a Small Molecule That Inhibits Cell Division by Blocking FtsZ, a Novel Therapeutic Target of Antibiotics. *J. Biol. Chem.* **2003**, *278*, 44424-28.

(4) Wang, J.; Soisson, S. M.; Young, K.; Shoop, W.; Kodali, S.; Galgoci, A.; Painter, R.; Parthasarathy, G.; Tang, Y.; Cummings, R.; Ha, S.; Dorso, K.; Motyl, M.; Jayasuriya, H.; Ondeyka, J.; Herath, K.; Zhang, C.; Hernandez, L.; Alloco, J.; Basilio, Á.; Tormo, J. R.; Genilloud, O.; Vicente, F.; Pelaez, F.; Colwell, L.; Lee, S. H.; Michael, B.; Felcetto, T.; Gill, C.; Silver, L. L.; Hermes, J.; Bartizal, K.; Barrett, J.; Schmatz, D.; Becker, J. W.; Cully, D.; Singh, S. B. Platensimycin is a selective FabF inhibitor with potent antibiotic properties. *Nature* **2006**, *441*, 358-61.

(5) Phillips, J. W.; Goetz, M. A.; Smith, S. K.; Zink, D. L.; Polishook, J.; Onishi, R.; Salowe, S.; Wiltsie, J.; Allocco, J.; Sigmund, J.; Dorso, K.; Lee, S.; Skwish, S.; de la Cruz, M.; Martin, J.; Vicente, F.; Genilloud, O.; Lu, J.; Painter, R. E.; Young, K.; Overbye, K.; Donald, R. G.; Singh, S. B. Discovery of kibdelomycin, a potent new class of bacterial type II topoisomerase inhibitor by chemical-genetic profiling in Staphylococcus aureus. *Chem. Biol.* **2011**, *18*, 955-65.

(6) Clinical and Laboratory Standards Institute. Methods for determining bactericidal activity of antimicrobial agents; approved guideline. CLSI document M26-A, CLSI, Wayne, Pa, USA. **1999**.