Supplementary Information

Features of HIV-1 Integrase Strand Transfer Inhibitors with Reduced Susceptibility to Resistant Mutant Forms of Integrase

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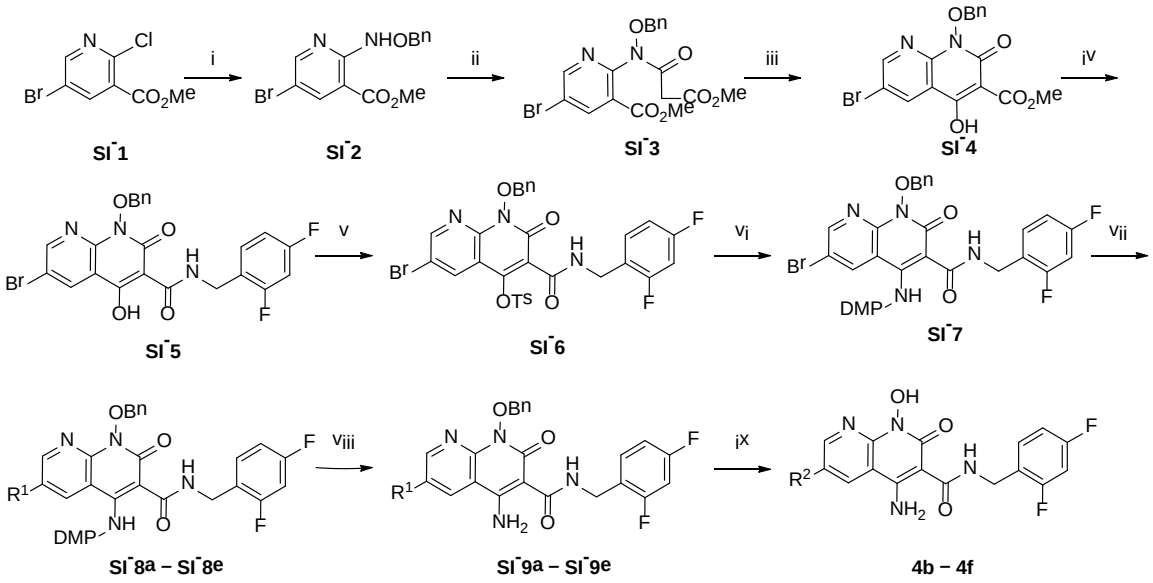
Synthetic

General. ^{1}H and ^{13}C NMR data were obtained on a Varian 400 MHz spectrometer or a Varian 500 MHz spectrometer and are reported in ppm relative to TMS and referenced to the solvent in which the spectra were collected. Solvent was removed by rotary evaporation under reduced pressure and anhydrous solvents were obtained commercially and used without further drying. Purification by silica gel chromatography was performed using a Teledyne Isco Combiflash with EtOAc–hexane solvent systems. Preparative high performance liquid chromatography (HPLC) was conducted using a Waters Prep LC4000 system having photodiode array detection and Phenomenex C_{18} columns (Cat. No. 00G-4436-P0-AX: 250 mm x 21.2 mm; 10 μm particle size; 110 Å pore) at a flow rate of 10 mL/min. Binary solvent systems consisting of A = 0.1% aqueous TFA and B = 0.1% TFA in CH_3CN were employed with gradients as indicated. Products were obtained as amorphous solids following lyophilization. Electrospray ionization-mass spectra (ESI-MS) were acquired with an Agilent LC/MSD system equipped with a multimode ion source. High-resolution LC/MS and LC/MS/MS analyses were conducted on a Thermo-Fisher LTQ-XL Orbitrap hybrid mass spectrometer system operated under Xcalibur (version 2.1.0 SP1) control for data acquisition and qualitative analysis. A similar narrow-bore, small particle (3.5 μm) Zorbax Rapid-Resolution reversed-phase C_{18} column (100 x 2.1 mm) - guard column (12.5 x 2.1 mm) combination was eluted with CH_3CN:H_2O containing 0.1% AcOH at 250 μL/min.

Scheme S1



Scheme S1. Preparation of compounds **4b – 4f.** *Reagents and conditions:* i) $BnONH_2$; ii) $ClCOCH_2CO_2CH_3$, NEt_3, CH_2Cl_2; iii) NaOMe, MeOH; iv) 2,4-diF-$BnNH_2$; v) TsCl, NEt_3, CH_3CN; vi) 2,4-dimethoxylbenzyl amine, DIEA, DMF; vii) alkyne, $Pd(PPh_3)Cl_2$, DIEA, CuI (Sonogashira reaction[1]) or (vinylsulfonyl)benzene, $Pd_2(dba)_3$, $^tBu_3P$-BF_4, $^cHex_2NMe$ (Heck reaction[2]); viii) TFA, CH_2Cl_2; ix) H_2, 10% Pd•C, MeOH.

Methyl 2-((benzyloxy)amino)-5-bromonicotinate (**SI-2**). A solution of commercially available methyl 5-bromo-2-chloronicotinate (**SI-1**) (2.01 g, 8.02 mmol) in *O*-benzylhydroxylamine (4.67 mL, 40.1 mmol) was heated at 110 °C (overnight). The reaction mixture was cooled to room temperature and purified by silica gel chromatography to yield **SI-2** as a colorless oil (1.53 g, 57% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.00 (s, 1H), 8.51 (d, *J* = 2.4 Hz, 1H), 8.24 (d, *J* = 2.4 Hz, 1H), 7.47 (dd, *J* = 8.0, 1.2 Hz, 2H), 7.40 – 7.32 (m, 3H), 5.06 (s, 2H), 3.84 (s, 3H). ^{13}C NMR (101

MHz, $CDCl_3$) δ 165.60, 158.06, 154.29, 141.72, 136.01, 128.91 (2C), 128.48 (2C), 128.41, 108.50, 108.11, 78.14, 52.46. ESI-MS m/z: 337.0, 339.0 ($M+H^+$).

Methyl 2-(*N*-(Benzyloxy)-3-methoxy-3-oxopropanamido)-5-bromonicotinate (**SI-3**). Methyl 3-chloro-3-oxopropanoate (**SI-2**) (1.00 ml, 9.04 mmol) was added dropwise to a solution of methyl 2-((benzyloxy)amino)-5-bromonicotinate (1.52 g, 4.52 mmol) and NEt_3 (1.27 ml, 9.04 mmol) in CH_2Cl_2 (10 mL). The reaction mixture was stirred at room temperature (1 h). The mixture was filtered and the filtrate was concentrated and purified by silica gel chromatography to provide **SI-3** as a colorless oil (1.2 g, 61% yield). ^1H NMR (400 MHz, $CDCl_3$) δ 8.63 (s, 1H), 8.24 (d, *J* = 2.1 Hz, 1H), 7.32 – 7.27 (m, 5H), 4.95 (s, 2H), 3.82 (s, 3H), 3.67 (s, 3H), 3.55 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.00, 167.27, 167.01, 164.06, 152.00, 141.66, 133.59, 129.66 (2C), 129.09, 128.50 (2C), 124.78, 118.85, 78.46, 52.63, 52.44, 40.64. ESI-MS m/z: 437.0, 439.0 ($M+H^+$).

Methyl 1-(Benzyloxy)-6-bromo-4-hydroxy-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxylate (**SI-4**). Sodium methoxide (1.44 mL, 25% in MeOH, 6.32 mmol) was added drop-wise to a solution of **SI-3** (1.14 g, 2.53 mmol) in MeOH (5.0 mL) at room temperature. The resulting yellow suspension turned white after being stirred at room temperature (1 h), then stirring was continued at room temperature (overnight). The reaction mixture was brought to pH 4 by the addition of aqueous 2 N HCl and stirring was continued (15 minutes), then the formed solids were collected by filtration and the filtrate was washed (H_2O) and dried to yield. **SI-4** as a white solid (1.0 g, 98% yield). ^1H NMR (400 MHz, $CDCl_3$) δ 8.76 (d, *J* = 2.4 Hz,

1H), 8.51 (d, *J* = 2.4 Hz, 1H), 7.66 – 7.63 (m, 2H), 7.37 – 7.30 (m, 3H), 5.20 (s, 2H), 4.04 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.06, 169.01, 156.03, 155.37, 148.03, 136.42, 133.78, 130.02 (2C), 129.11, 128.41(2C), 113.94, 110.71, 99.52, 78.30, 53.46. ESI-MS m/z: 405.0, 407.0 ($M+H^+$).

1-(Benzyloxy)-6-bromo-*N*-(2,4-difluorobenzyl)-4-hydroxy-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**SI-5**). A mixture of **SI-4** (2.22 g, 5.48 mmol) and (2,4-difluorophenyl)methanamine (6.51 mL, 54.8 mmol) in DMF (3 mL) was subjected to microwave irradiation (140 °C, 2 h). The resulting mixture was filtered and the filtrate was washed (MeOH) and purified by silica gel chromatography. The purified material was crystallized (MeOH) to yield **SI-5** as a white solid **(**2.18 g, 77% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.21 (s, 1H), 8.73 (dd, J = 2.4, 1.2 Hz, 1H), 8.51 (dd, J = 2.3, 1.2 Hz, 1H), 7.62 – 7.50 (m, 2H), 7.37 – 7.25 (m, 4H), 6.86 – 6.71 (m, 2H), 5.18 (s, 2H), 4.59 (d, J = 5.9 Hz, 2H). ESI-MS m/z: 516.0, 518.0 ($M+H^+$).

1-(Benzyloxy)-6-bromo-3-((2,4-difluorobenzyl)carbamoyl)-2-oxo-1,2-dihydro-1,8-naphthyridin-4-yl 4-Methylbenzenesulfonate (**SI-6**). A solution of NEt_3 (4.38 mL, 31.3 mmol) and 4-methylbenzene-1-sulfonyl chloride (2.98 g, 15.6 mmol) was added to a solution of **SI-5** (.69 g, 5.21 mmol) in CH_3CN (6 mL) and CH_2Cl_2 (3 mL) at room temperature. The reaction mixture was stirred at room temperature (overnight). Purification of the reaction product by silica gel chromatography provided **SI-6** as a yellow solid (2.76 g, 79% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.75 (d, *J* = 2.2 Hz, 1H), 8.28 (t, *J* = 5.8 Hz, 1H), 8.03 (d, *J* = 2.2 Hz, 1H),

7.89 (d, *J* = 8.3 Hz, 2H), 7.64 (dd, *J* = 6.6, 2.7 Hz, 2H), 7.49 (dd, *J* = 15.1, 8.5 Hz, 1H), 7.40 (dd, *J* = 5.0, 3.1 Hz, 5H), 6.85 (ddd, *J* = 19.1, 10.0, 4.3 Hz, 2H), 5.29 (s, 2H), 4.59 (d, *J* = 5.9 Hz, 2H), 2.51 (s, 3H). ESI-MS m/z: 670.0, 672.0 ($M+H^+$).

1-(Benzyloxy)-6-bromo-*N*-(2,4-difluorobenzyl)-4-((2,4-dimethoxybenzyl)amino)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**SI-7**). Commercially available (2,4-dimethoxyphenyl)methanamine (0.63 mL, 4.2 mmol) was added to a solution of **SI-6** (565 mg, 0.84 mmol) in DMF (2 mL) with *N*-ethyl-*N*-isopropylpropan-2-amine (1.5 mL, 8.4 mmol). The reaction mixture was heated (50 °C, 1 h) then cooled to room temperature. Purification of the reaction product by silica gel chromatography provided **SI-7** as a white solid (498 mg, 0.75 mmol, 89% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.05 (t, *J* = 6.2 Hz, 1H), 10.61 (t, *J* = 5.7 Hz, 1H), 8.58 (d, *J* = 2.1 Hz, 1H), 8.34 (d, *J* = 2.2 Hz, 1H), 7.57 (dd, *J* = 7.3, 1.9 Hz, 2H), 7.39 – 7.22 (m, 4H), 7.17 (dd, *J* = 8.9, 4.3 Hz, 1H), 6.85 – 6.63 (m, 2H), 6.47 – 6.34 (m, 2H), 5.15 (s, 2H), 4.64 (d, *J* = 6.3 Hz, 2H), 4.52 (d, *J* = 5.7 Hz, 2H), 3.74 (s, 3H), 3.73 (s, 3H).

General Procedure A. Sonogashira Synthesis of 1-(Benzyloxy)-*N*-(2,4-difluorobenzyl)-4-((2,4-dimethoxybenzyl)amino)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamides (SI-8a – SI-8d).[1,3] To a mixture of 6-bromo-containing heterocycle (**SI-7**) (0.30 mmol), *bis*(triphenylphosphine)palladium (II) dichloride (6 mg, 9 μmol), *N*-ethyl-*N*-isopropylpropan-2-amine (0.05 mL, 0.30 mmol) and copper(I) iodide (6 mg, 0.03 mmol) in DMF (1.5 mL) was added an appropriate alkyne (0.45 mmol) and the mixture was flushed with argon, sealed with cap and

heated at 70 °C (4 h). The resultant dark mixture was cooled to room temperature and purified by silica gel chromatography to yield 6-substituted products **(SI-8a – SI-8d)**.

1-(Benzyloxy)-*N*-(2,4-difluorobenzyl)-4-((2,4-dimethoxybenzyl)amino)-6-(3-hydroxyprop-1-yn-1-yl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (SI-8a). Treatment of **SI-7** with commercially available prop-2-yn-1-ol as outlined in General Procedure A provided **SI-8a** a white solid (85% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.02 (t, *J* = 5.8 Hz, 1H), 10.65 (t, *J* = 5.8 Hz, 1H), 8.64 (d, *J* = 1.9 Hz, 1H), 8.33 (d, *J* = 1.9 Hz, 1H), 7.66 (d, *J* = 1.9 Hz, 1H), 7.65 (d, *J* = 1.5 Hz, 1H), 7.40 – 7.33 (m, 4H), 7.20 (d, *J* = 8.3 Hz, 1H), 6.85 – 6.78 (m, 2H), 6.46 (q, *J* = 2.1 Hz, 2H), 5.24 (s, 2H), 4.71 (d, *J* = 5.9 Hz, 2H), 4.59 (d, *J* = 5.7 Hz, 2H), 4.46 (d, *J* = 6.1 Hz, 2H), 3.80 (s, 3H), 3.78 (s, 3H).

1-(Benzyloxy)-*N*-(2,4-difluorobenzyl)-4-((2,4-dimethoxybenzyl)amino)-6-(5-hydroxypent-1-yn-1-yl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (SI-8b). Treatment of **SI-7** with commercially available pent-4-yn-1-ol as outlined in General Procedure A provided **SI-8b** as a white solid (52% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.02 (t, *J* = 5.9 Hz, 1H), 10.69 (t, *J* = 5.7 Hz, 1H), 8.65 (d, *J* = 1.4 Hz, 1H), 8.30 (d, *J* = 1.5 Hz, 1H), 7.67 (d, *J* = 6.5 Hz, 2H), 7.40 – 7.36 (m, 4H), 7.26 – 7.25 (m, 1H), 6.82 (dt, *J* = 11.9, 9.2 Hz, 2H), 6.49 (d, *J* = 8.1 Hz, 2H), 5.25 (s, 2H), 4.74 (d, *J* = 6.0 Hz, 2H), 4.60 (d, *J* = 5.6 Hz, 2H), 3.83 (s, 3H), 3.80 (s, 3H), 3.78 – 3.73 (m, 2H), 2.56 (t, *J* = 7.0 Hz, 2H), 1.86 (p, *J* = 6.6 Hz, 2H). ESI-MS m/z: 669.2 ($M+H^+$).

1-(Benzyloxy)-*N*-(2,4-difluorobenzyl)-4-((2,4-dimethoxybenzyl)amino)-6-(6-hydroxyhex-1-yn-1-yl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (SI-8c). Treatment of **SI-7** with commercially available hex-5-yn-1-ol as outlined in General Procedure A provided **SI-8c** as a white solid (54% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.01 (t, *J* = 5.9 Hz, 1H), 10.69 (t, *J* = 5.7 Hz, 1H), 8.65 (dd, *J* = 1.8, 0.9 Hz, 1H), 8.31 – 8.30 (m, 1H), 7.67 (d, *J* = 6.8 Hz, 2H), 7.40 – 7.35 (m, 4H), 7.23 (s, 1H), 6.85 – 6.78 (m, 2H), 6.48 (d, *J* = 7.8 Hz, 2H), 5.24 (s, 2H), 4.74 (d, *J* = 6.0 Hz, 2H), 4.59 (d, *J* = 5.7 Hz, 2H), 3.82 (d, *J* = 0.9 Hz, 3H), 3.79 (d, *J* = 0.8 Hz, 3H), 3.71 (dd, *J* = 5.8, 5.3 Hz, 2H), 2.47 (t, *J* = 6.2 Hz, 2H), 1.72 – 1.70 (m, 4H). ESI-MS m/z: 683.3 ($M+H^+$).

1-(Benzyloxy)-*N*-(2,4-difluorobenzyl)-4-((2,4-dimethoxybenzyl)amino)-6-(8-hydroxyoct-1-yn-1-yl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (SI-8d). Treatment of **SI-7** with commercially available oct-7-yn-1-ol as outlined in General Procedure A provided **SI-8d** as a white solid (83% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.00 (t, *J* = 5.8 Hz, 1H), 10.69 (t, *J* = 5.7 Hz, 1H), 8.65 (d, *J* = 1.7 Hz, 1H), 8.33 (d, *J* = 1.8 Hz, 1H), 7.67 (dd, *J* = 7.4, 1.3 Hz, 2H), 7.40 – 7.33 (m, 4H), 7.23 (d, *J* = 7.9 Hz, 3H), 6.85 – 6.781 (m, 2H), 6.48 – 6.46 (m, 2H), 5.24 (s, 2H), 4.75 (d, *J* = 5.9 Hz, 2H), 4.59 (d, *J* = 5.6 Hz, 2H), 3.81 (s, 3H), 3.79 (s, 3H), 3.66 (t, *J* = 6.5 Hz, 2H), 2.43 (t, *J* = 7.1 Hz, 2H), 1.66 – 1.57 (m, 4H), 1.50 – 1.41 (m, 4H). ESI-MS m/z: 711.3 (MH^+).

Heck Synthesis[2] of (*E*)-1-(Benzyloxy)-*N*-(2,4-difluorobenzyl)-4-((2,4-dimethoxybenzyl)amino)-2-oxo-6-(2-(phenylsulfonyl)vinyl)-1,2-dihydro-1,8-

naphthyridine-3-carboxamide (SI-8e). A mixture of 6-bromo-containing heterocycle (**SI-7**) (110 mg, 0.17 mmol), *tris*(dibenzylideneacetone)dipalladium(0) (17 mg , 0.02 mmol), commericially available (vinylsulfonyl)benzene (28 mg, 0.17 mmol) and tri-*tert*-butylphosphonium tetrafluoroborate (3 mg, 0.01 mmol), *N*-cyclohexyl-*N*-methylcyclohexanamine (58 μL, 0.33 mmol) in dioxane (1.0 mL) in a sealed vessel charged with Argon was subjected to microwave irradiation (120 °C, 12 h) was microwave-heated at 120 °C for 12 hr. The resultant mixture was cooled to room temperature and purified by silica gel chromatography to yield **SI-8e** as a yellow solid (51 mg, 0.07 mmol, 41% yield)**.** ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.33 (t, *J* = 6.7 Hz, 1H), 10.70 (s, 1H), 8.67 (d, *J* = 1.5 Hz, 1H), 8.34 (d, *J* = 1.5 Hz, 1H), 7.90 (d, *J* = 8.1 Hz, 3H), 7.74 - 7.55 (m, 8H), 7.41-7.35 (m, 5H), 6.85 (dd, *J* = 18.9, 9.2 Hz, 2H), 6.57 (d, *J* = 1.9 Hz, 1H), 6.52 - 6.43 (m, 1H), 6.05 (d, *J* = 15.4 Hz, 1H), 5.25 (s, 2H), 4.71 (d, *J* = 6.8 Hz, 2H), 4.64 (d, *J* = 5.3 Hz, 2H), 3.87 (s, 3H), 3.79 (s, 3H), 3.47 (s, 2H).

General Procedure C. Synthesis of 4-Amino-1-(benzyloxy)-*N*-(2,4-difluorobenzyl)- 2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamides (SI-9a – SI-9e). To a solution of 2,4-dimethoxybenzylamino-protected carboxamide (**SI-8a – SI-8e**) (0.25 mmol) in CH_2Cl_2 (2 mL) was added TFA (2 mL) and the solution was stirred at room temperature (5 minutes). Volatiles were removed by rotary evaporation and the resulting residue was purified by silica gel chromatography to yield 6-substituted products **(SI-9a – SI-9e)**.

4-Amino-1-(benzyloxy)-N-(2,4-difluorobenzyl)-6-(3-hydroxyprop-1-yn-1-yl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (SI-9a). Treatment

of **SI-8a** as outlined in General Procedure C provided **SI-9a** as a white solid (56% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.55 (s, 1H), 8.75 (s, 1H), 8.06 (s, 1H), 7.67 – 7.65 (m, 2H), 7.41 – 7.37 (m, 4H), 6.84 (dd, *J* = 19.5, 9.2 Hz, 2H), 5.26 (s, 2H), 4.64 (d, *J* = 5.6 Hz, 2H), 4.53 (s, 2H). ESI-MS m/z: 491.1 (MH^+).

4-Amino-1-(benzyloxy)-*N*-(2,4-difluorobenzyl)-6-(5-hydroxypent-1-yn-1-yl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (SI-9b). Treatment of **SI-8b** as outlined in General Procedure C provided **SI-9b** as a white solid (65% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.53 (t, *J* = 5.8 Hz, 1H), 8.68 (d, *J* = 1.7 Hz, 1H), 7.98 (d, *J* = 1.8 Hz, 1H), 7.62 (dd, *J* = 7.3, 1.9 Hz, 2H), 7.38 – 7.328 (m, 4H), 6.78 (dt, *J* = 9.0, 5.3 Hz, 2H), 5.21 (s, 2H), 4.59 (d, *J* = 5.7 Hz, 2H), 3.79 (t, *J* = 6.1 Hz, 2H), 2.55 (t, *J* = 7.0 Hz, 2H), 1.85 (p, *J* = 6.6 Hz, 2H). ESI-MS m/z: 519.2 ($M+H^+$).

4-Amino-1-(benzyloxy)-N-(2,4-difluorobenzyl)-6-(6-hydroxyhex-1-yn-1-yl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (SI-9c). Treatment of **SI-8c** as outlined in General Procedure C provided **SI-9c** as a white solid (94% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.59 (s, 1H), 8.75 (bs, 1H), 8.01 (s, 1H), 7.67 (d, *J* = 6.7 Hz, 2H), 7.42 – 7.35 (m, 4H), 6.87 - 6.81 (m, 2H), 5.26 (s, 2H), 4.64 (d, *J* = 5.6 Hz, 2H), 3.78 (d, *J* = 13.5 Hz, 2H), 2.52 (t, *J* = 6.5 Hz, 2H), 1.76 – 1.74 (m, 2H), 1.66 - 1.60 (m, 2H). ESI-MS m/z: 533.2 ($M+H^+$).

4-Amino-1-(benzyloxy)-*N*-(2,4-difluorobenzyl)-6-(8-hydroxyoct-1-yn-1-yl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (SI-9d). Treatment of **SI-8d** as outlined in General Procedure C provided **SI-9d** as a white solid (61% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.55 (t, *J* = 5.7 Hz, 1H), 8.65 (s, 1H), 8.11 (s, 1H),

7.61 – 7.59 (m, 2H), 7.37 – 7.29 (m, 4H), 6.82 – 6.74 (m, 2H), 5.19 (s, 2H), 4.58 (d, *J* = 5.6 Hz, 2H), 3.63 (bs, 2H), 2.38 (t, *J* = 6.9 Hz, 4H), 1.59 - 1.54 (m, 4H), 1.47 – 1.40 (m, 4H). ESI-MS m/z: 561.2 (MH^+).

(*E*)-4-Amino-1-(benzyloxy)-*N*-(2,4-difluorobenzyl)-2-oxo-6-(2-(phenylsulfonyl)vinyl)-1,2-dihydro-1,8-naphthyridine-3-carboxamide (SI-9e). Treatment of **SI-8e** as outlined in General Procedure C provided **SI-9e** as a white solid (73% yield)**.** ^1H NMR (400 MHz, $CDCl_3$) δ 10.99 (bs, 1H), 10.40 (t, J = 5.5 Hz, 1H), 8.71 (s, 1H), 8.36 (s, 1H), 7.85 (d, J = 7.5 Hz, 2H), 7.67 (d, J = 15.4 Hz, 1H), 7.59 - 7.46 (m, 5H), 7.31 - 7.25 (m, 4H), 7.09 (d, J = 15.4 Hz, 1H), 6.76 - 6.69 (m, 2H), 5.15 (s, 2H), 4.52 (d, J = 5.4 Hz, 2H). ESI-MS m/z: 603.1 (MH^+), 626.1 (MNa^+).

General Procedure D. Synthesis of *N*-(2,4-Difluorobenzyl)-1-hydroxy-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamides (4b – 4f). To a suspension of benzyl-protected hydroxylamine (**SI-9a – SI-9e**) (0.1 mmol) in MeOH (10 mL) and EtOAc (3 mL) was added Pd•C (30 mg, 10%) and the mixture was stirred at room temperature under hydrogen. When the reaction was complete (as indicated by the disappearance of starting material to TLC), the mixture was filtered and the filtered solid washed (MeOH) and the combined filtrate was concentrated. The resulting yellow residue was taken up in DMF and purified by HPLC to provide product (**4b** – **4f**) as a floccular white solid.

4-Amino-N-(2,4-difluorobenzyl)-1-hydroxy-6-(3-hydroxypropyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (4b). Treatment of **SI-9a** as described under General Procedure D and purification by preparative HPLC (linear

gradient of 30% B to 70% B over 30 minutes; retention time = 15.2 minutes) provided **4b** as floccular white solid (76% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.70 (t, *J* = 5.7 Hz, 1H), 10.52 (bs, 1H), 8.58 (s, 1H), 8.55 (s, 1H), 7.42 (dd, *J* = 15.5, 8.1 Hz, 1H), 7.24 (t, *J* = 9.9 Hz, 1H), 7.07 (t, *J* = 8.5 Hz, 1H), 4.52 (d, *J* = 5.7 Hz, 2H), 3.46 – 3.43 (m, 2H), 2.73 (t, *J* = 7.7 Hz, 2H), 1.83 – 1.77 (m, 2H). ESI-MS m/z: 405.1 (MH^+). HRMS calcd. for $C_{19}H_{19}F_2N_4O_4$ [MH^+], 405.1369; found, 405.1362.

4-Amino-*N*-(2,4-difluorobenzyl)-1-hydroxy-6-(5-hydroxypentyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (4c). Treatment of **SI-9b** as described under General Procedure D and purification by preparative HPLC (linear gradient of 30% B to 60% B over 30 minutes; retention time = 19.8 minutes) provided **4c** as floccular white solid (75% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.70 (t, *J* = 5.8 Hz, 1H), 8.58 (d, *J* = 1.8 Hz, 1H), 8.54 (d, *J* = 1.8 Hz, 1H), 7.42 (dd, *J* = 15.4, 8.7 Hz, 1H), 7.24 (ddd, *J* = 10.6, 9.4, 2.6 Hz, 1H), 7.09 – 7.05 (m, 1H), 4.52 (d, *J* = 5.8 Hz, 2H), 3.39 (t, *J* = 6.5 Hz, 2H), 2.68 (t, *J* = 7.6 Hz, 2H), 1.68 – 1.62 (m, 2H), 1.49 – 1.43 (m, 2H), 1.36 – 1.30 (m, 2H). ESI-MS m/z: 433.2 (MH^+). HRMS calcd. for $C_{21}H_{23}F_2N_4O_4$ [MH^+], 433.1682; found, 433.1667.

4-Amino-*N*-(2,4-difluorobenzyl)-1-hydroxy-6-(6-hydroxyhexyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (4d). Treatment of **SI-9c** as described under General Procedure D and purification by preparative HPLC (linear gradient of 30% B to 60% B over 30 minutes; retention time = 23.2 minutes) provided **4d** as floccular white solid (65% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.70 (t, *J* = 5.5 Hz, 1H), 10.53 (bs, 1H), 8.58 (s, 1H), 8.55 (s, 1H), 7.43 (dd, *J* = 15.3,

8.0 Hz, 1H), 7.25 (t, *J* = 9.9 Hz, 1H), 7.07 (t, *J* = 8.3 Hz, 1H), 4.52 (d, *J* = 5.1 Hz, 2H), 3.38 (t, *J* = 6.3 Hz, 2H), 2.68 (t, *J* = 7.5 Hz, 2H), 1.65 (s, 2H), 1.41 (d, *J* = 6.4 Hz, 2H), 1.33 (s, 4H). ESI-MS m/z: 447.2 ($M+H^+$). HRMS calcd. $C_{22}H_{25}F_2N_4O_4$ [MH^+], 447.1838; found, 447.1838.

4-Amino-*N*-(2,4-difluorobenzyl)-1-hydroxy-6-(8-hydroxyoctyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (4e). Treatment of **SI-9d** as described under General Procedure D and purification by preparative HPLC (linear gradient of 35% B to 60% B over 30 minutes; retention time = 25.8 minutes) provided **4e** as floccular white solid (88% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.63 (t, *J* = 5.7 Hz, 1H), 8.51 (s, 1H), 8.47 (s, 1H), 7.36 (dd, *J* = 15.5, 8.4 Hz, 1H), 7.17 (dd, *J* = 14.2, 5.7 Hz, 1H), 7.00 (t, *J* = 7.5 Hz, 1H), 4.45 (d, *J* = 5.7 Hz, 2H), 3.29 (d, *J* = 6.6 Hz, 2H), 2.61 (t, *J* = 7.6 Hz, 2H), 1.58 (bs, 2H), 1.32 (d, *J* = 6.5 Hz, 2H), 1.24 (bs, 4H), 1.19 (bs, 4H). ESI-MS m/z: 475.2 (MH^+). HRMS calcd. for $C_{24}H_{29}F_2N_4O_4$ [MH^+], 475.2151; found, 475.2151.

4-Amino-*N*-(2,4-difluorobenzyl)-1-hydroxy-2-oxo-6-(2-(phenylsulfonyl)ethyl)-1,2-dihydro-1,8-naphthyridine-3-carboxamide (4f). Treatment of **SI-9e** as outlined in General Procedure D provided **4f** as a white solid after purification by preparative HPLC (linear gradient of 30% B to 60% B over 30 minutes; retention time = 25.5 minutes). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.58 (t, J = 5.7 Hz, 1H), 10.47 (bs, 2H), 8.52 (s, 1H), 8.43 (s, 1H), 7.83 (d, J = 7.4 Hz, 2H), 7.65 (t, J = 7.4 Hz, 1H), 7.57 (t, J = 7.6 Hz, 2H), 7.35 (dd, J = 15.4, 8.4 Hz, 1H), 7.20 - 7.14 (m, 1H), 7.00 (t, J = 8.3 Hz, 1H), 4.44 (d, J = 5.7 Hz, 2H), 3.74 - 3.70 (m, 2H), 2.98 - 2.95 (m,

2H). ESI-MS m/z: 515.1 (MH^+). HRMS cacld. for $C_{24}H_{21}F_2N_4O_5S$: 515.1195; Found 515.1193.

Biological

HIV-1 IN Biochemical Assays. As previously described,[5] inhibitors or an equivalent volume of DMSO were added to a reaction mixture containing 20 nM ^{32}P-labeled DNA substrate and 400 nM IN in 50 mM MOPS pH 7.2, 7.5 mM $MgCl_2$, and 14 mM 2-mercaptoethanol. Reactions were performed at 37 °C (2 h) and stopped by the addition of an equal volume of loading buffer [formamide containing 1% SDS (sodium dodecyl sulfate), 0.25% bromophenol blue, and xylene cyanol]. Products were separated in 16% polyacrylamide denaturing sequencing gels. Dried gels were visualized using a Typhoon 8600 (GE Healthcare). Densitometric analyses were performed using the ImageQuant 5.1 software from GE Healthcare. Data analyses (linear regression, IC_{50} determination, and standard deviation) were performed using Prism 5.0 software from GraphPad.

HIV-1 Vector Constructs. pNLNgoMIVR$^-$ΔEnv.LUC has been described previously.[6] The IN reading frame was removed from pNLNgoMIVR$^-$ΔEnv.LUC by digestion with KpnI and SalI and inserted between the KpnI and SalI sites of pBluescript II KS+. Using this construct as the wild-type template, the following HIV-1 IN mutants were prepared using the QuikChange II XL (Stratagene, La Jolla, CA) site-directed mutagenesis protocol: H51Y, T66I, E92Q, G118R, Y143R, Q148H, N155H, R263K, G140S + Q148H, and H51Y + R263K. The following sense and cognate antisense (not shown) oligonucleotides (Integrated DNA Technologies, Coralville, IA) were used in

the mutagenesis: H51Y, 5'-CTAAAAGGGGAAGCCATGTATGGACAAGTAGACTGTA-3'; T66I, 5'-CCAGGAATATGGCAGCTAGATTGTATACATTTAGAAGGAAAAGTT-3'; E92Q, 5'-GCAGAAGTAATTCCAGCACAGACAGGGCAAGAAA -3'; G118R, 5'-GTACATACAGACAATCGCAGCAATTTCACCAGTAC-3'; G140S, 5'-GGGGATCAAGCAGGAATTTAGCATTCCCTACAATC-3'; Y143C, 5'-GCAGGAATTTGGCATTCCCCGCAATCCCCAAAGTCAAGGA-3'; Q148H, 5'-CATTCCCTACAATCCCCAAAGTCATGGAGTAATAGAATCTA -3'; N155H, 5'-CCAAAGTCAAGGAGTAATAGAATCTATGCATAAAGAATTAAAGAAAATTATAGGACA-3'; R263K 5'- AAAGTAGTGCCAAGAAAAAAAGCAAAGATCATC -3'. The double mutation G140S + Q148H was constructed using the previously generated Q148H mutant and the appropriate oligonucleotides to introduce the second mutation, G140S. The double mutation H51Y + R263K was constructed by using the previously generated H51Y mutant and the appropriate oligonucleotides for the second mutation, R263K. The DNA sequence of each construct was verified by DNA sequencing. The mutant IN coding sequences from pBluescript II KS+ were subcloned into pNLNgoMIVR$^-$ ΔEnv.LUC (between the KpnI and SalI sites) to produce the full-length mutant HIV-1 IN constructs. These DNA sequences were also checked by DNA sequence determination.

Single-round HIV-1 Infectivity Assay. The human embryonyl kidney cell culture cell line 293 was acquired from the American type Culture Collection (ATCC). The human osteosarcoma cell line, HOS, was obtained from Dr. Richard Schwartz (Michigan State University, East Lansing, MI) and grown in Dulbecco's modified

Eagle's medium (Invitrogen, Carlsbad, CA) supplemented with 5% (v/v) fetal bovine serum, 5% newborn calf serum, and penicillin (50 units/mL) plus streptomycin (50 µg/mL; Quality Biological, Gaithersburg, MD). The transfection vector, pNLNgoMIVR^{-}ΔLUC was made from pNLNgoMIVR^{-}ΔEnv.HSA by removing the HSA reporter gene and replacing it with a luciferase reporter gene, which was inserted between the NotI and XhoI restriction sites.[6]

VSV-g-pseudotyped HIV was produced by transfection of 293 cells as indicated above.[7] On the day prior to transfection, 293 cells were plated on 100-mm-diameter dishes at a density of 1.5 X 10^6 cells per plate and transfected with 16 µg of pNLNgoMIVR^{-}ΔLUC and 4 µg of pHCMV-g (obtained from Dr. Jane Burns, University of California, San Diego) using the calcium phosphate method. At approximately 6 h after the calcium phosphate precipitate was added, 293 cells were washed twice with phosphate-buffered saline (PBS) and incubated with fresh media for 48 h. The virus-containing supernatants were harvested, clarified by low-speed centrifugation, filtered, and diluted for preparation in antiviral infection assays. On the day prior to the screen, HOS cells were seeded in 96-well luminescence cell culture plates at a density of 4000 cells in 100 µL per well. On the day of the assay for cellular cytotoxicity, cells were treated with compounds from a concentration range of 250 µM to 0.05 µM and then incubated at 37 ˚C (48 h). On the day of the assay for antiviral activity, cells were treated with compounds from a concentration range of 5 µM to 0.0001 µM using 11 serial dilutions and then incubated at 37 ˚C (3 h). After 3h, 100 µL of virus-stock [diluted to achieve a

luciferase signal between 0.2 and 1.5 Relative Luciferase Units (RLUs)] was added to each well and incubation was continued (37 ˚C, 48 h). Cellular cytotoxicity was measured by using the ATP Lite Luminescence detection system and monitored by adding 50 μL of cell lysis buffer from the Luminescence ATP detection assay to each well followed by mixing at 700 rpm at room temperature using a compact thermomixer (5 minutes). After addition of 50 μL of reconstituted Luminescence ATP detection assay reagent to all wells except for the negative control/background wells, the plates were mixed at 700 rpm at room temperature using a compact thermomixer (5 minutes), incubated at room temperature to allow time for signal development (20 minutes), and cytotoxicity was determined using the microplate reader. Infectivity was measured using the Steady-lite plus luminescence reporter gene assay system (PerkinElmer, Waltham, MA). Luciferase activity was measured by adding 100 μL of Steady-lite plus buffer (PerkinElmer) to the cells, incubating at room temperature (20 minutes), and measuring luminescence using a microplate reader. Both cytotoxicity and antiviral activity were normalized to the cellular cytotoxicity and infectivity using cells incubated in absence of the respective target compounds. KaleidaGraph (Synergy Software, Reading, PA) was used to perform non-linear regression analysis on the data. Final IC_{50} values were determined from the fit model.

X-ray Crystallography

Table S1. X-ray data collection and refinement statistics.

	4a	4c	4f
Data Collection			
Space group	$P4_12_12$	$P4_12_12$	$P4_12_12$
Cell dimensions a, b, c (Å)	159.7, 159.7, 123.5	159.6, 159.9, 123.8	159.9, 159.9, 123.8
Resolution range (Å)	48.90 - 2.58	57.73 - 2.85	56.53 - 2.67
R_{merge}	0.081 (1.7)	0.056 (0.89)	0.078 (0.91)
$I/\sigma(I)$	19.4 (1.9)	21.6 (2.1)	14.4 (2.0)
Completeness (%)	99.9 (99.9)	99.7 (99.2)	96.58 (98.0)
Redundancy	11.5 (11.9)	11.5 (11.9)	3.6 (3.7)
Refinement			
Reflections (total/free)	96,038/4876	37,914/1,899	84,540/4,274
R/R_{free}	0.185/0.203	0.178/0.200	0.181/0.200
No. Atoms			
Protein, DNA	5,090	5,086	5,099
Ligand	113	95	95
Water	135	91	195
Average B-factors (Å^2)			
Protein, DNA	79.2	75.6	67.8
Ligands	93.3	91.5	79.2
Water	73.4	64.2	62.2

R.m.s. deviations			
Bond lengths (Å)	0.005	0.012	0.006
Bond angles (°)	0.64	0.75	0.61
Ramachandran plot (%)			
Favored	98	98	98
Outliers	0	0.18	0

Each structure was determined from a single crystal. Data for the highest resolution shells are given in parenthesis.

Table S2. Contact areas of PFV-intasome-bound ligands.

No.	Residue[c]	Contact Area (Å^2)[a,b]				
		RAL[d]	**DTG[e]**	**4a**	**4c**	**4f**
1	Ligand	266 (52)[f]	246 (54)[f]	241 (63)[f]	272 (56)[f]	270 (53)[f]
2	A17	49.2	57	64.3	63.2	58.6
3	Tyr 212 (Tyr 143)	41.8	12.4	--	20.5	25.4
4	Pro 214 (Pro 145)	38.4	34.7	34.2	33.7	40.6
5	C16	31.8	30.9	36.7	30	33.1
6	Glu 221 (Glu 152)	19.7	18.9	18.7	18	18.1
7	Mg(A)[g]	16.6	16.9	17.6	17.3	18
8	Mg(B)[h]	13.8	12.4	12.1	12.4	12.8
9	Gln 186 (Asn 117)	10.4	3.7	--	16.8	7.2

10	Gln 215 (Gln 146)	10.3	13.8	12	11.3	7.2
11	G4	9.2	12.4	9	9.5	7
12	Pro 211 (Pro 141)	8.7	--	--	4.7	--
13	Asp 185 (Asp 116)	5.4	6.1	6.5	7.5	18
14	Asp 128 (Asp 65)	0.5	0.3	0.9	1.3	1.8
15	Gly 187 (Gly 118)	--	9.5	0.3	4.7	6.1
16	Arg329	--	7.3	--	--	--
17	His213	--	--	--	--	3.8

[a]Contact areas were calculated using the standard protocols in MolSoft ICM Software (version 3.8-4a for Mac OSX); [b]Contact areas showing notable differences are highlighted in yellow; [c]Correpsonding IN residues are shown in parenthesis; [d]PDB accession code 3OYA; [e]PDB accession code 3S3M; [f]Percent of total ligand exposed area; [g]Magnesium ion chelated by residues D128 and D185; [h]Magnesium ion chelated by residues E221 and D128.

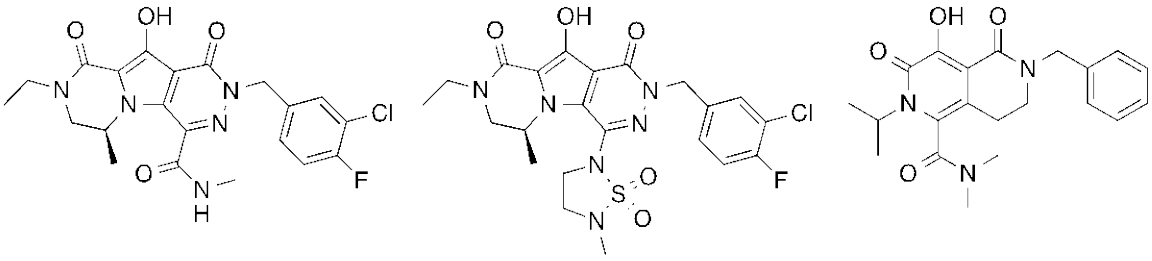


Figure S2. Structures of 2nd generation INSTIs mentioned in the text.

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