Probing Chelation Motifs in HIV Integrase Inhibitors

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SUPPORTING INFORMATION

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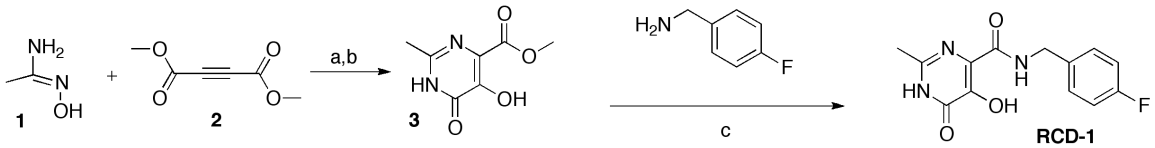
Materials and Methods

General. Unless otherwise noted, starting materials were purchased from commercial suppliers (Sigma-Aldrich, ChemBridge, Acros Organics, TCI America) and were used without further purification. Chromatography was preformed using a CombiFlash Rf 200 from TeledyneISCO. ^{1}H NMR spectra were recorded on one of several Varian FT-NMR spectrometers, property of the Department of Chemistry and Biochemistry, University of California San Diego. Mass spectrometry was performed at the Small Molecule Spectrometry Facility in the Department of Chemistry and Biochemistry, University of California San Diego. Compounds **RCD-2**, **RCD-3**, **RCD-4**, **RCD-4S**, **RCD-4S^2**, **RCD-5**, **RCD-7**, **4**, and **12** were all synthesized as previously described (Agrawal, A.; De Oliveira, C. A. F; et al. *J. Med. Chem.* **2009**, *52*, 1063; Agrawal, A.; Romero-Pereze, D.; et al. *ChemMedChem* **2008**, *3*, 812; Yan, Y. et al. *Org. Lett*. **2007**, *9*, 2517; Yan, Y.; Miller, M.; et al. *Bioorg. Med. Chem. Lett*. **2009**, *19*, 1970; Karpishin, T. B. et al. *J. Am. Chem. Soc*. **1993**, *115*, 182; K. Raymond, J. Xu, in United States Patent and Trademark Office (Ed.: U. S. P. T. Office), The Regents of the University of California (Oakland, CA) Pat. No. 5,892,029, US, 1999.).

Synthetic Chemistry

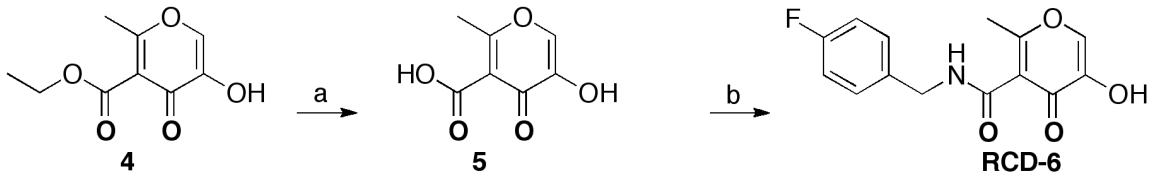


a) MeOH, 2h rt; b) Xylenes, 2h 90°C, 6h 135°C, c) FMBA, DMF 90°C, O/N

Methyl 5-hydroxy-2-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxylate (3): The synthesis of this compound was adapted from a literature procedure (Belyk, K. M.; Morrison, H. G.; Jones, P.; Summa, V. Preparation of N-(4-fluorobenzyl)-5-hydroxy-1-methyl-2-(1-methyl-1-{[(5-methyl-1,3,4-oxadiazol-2-yl)carbonyl]amino}ethyl)-6-oxo-1,6-dihydropyrimidine-4-carboxamide potassium salts as HIV integrase inhibitors. PCT Int. Appl. WO/2006/060712, 2006). To a solution of (*E*)-N'-hydroxyacetimidamide **(1)**

(500 mg, 6.75 mmol) in 8 mL of MeOH, was added 900 μL dimethyl but-2-ynedioate (**2**). After 1 h at room temperature, 6 mL of xylenes was added and the MeOH was removed. The solution was then refluxed at 135 °C for 16 h. The solution was cooled to 60 °C, and 3 mL of MeOH was added with stirring. After 30 minutes, 8 mL of methyl *t*-butyl ether (MTBE) was added dropwise and the solution was kept at 0 °C for 16 h. The black precipitate was filtered off and rinsed with cold 10% MeOH/MTBE. Yield = 44%. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 2.51 (s, 3H), 4.04 (s, 3H), 10.73 (br, 1H; N*H*). ESI-MS(+) *m/z* 184.9 $[M+H]^+$.

***N*-(4-Fluorobenzyl)-5-hydroxy-2-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide (RCD-1).** The synthesis of this compound was adapted from literature procedure (Summa, V.; Petrocchi, A.; Matassa, V. G.; et al. *J. Med. Chem.* 2006, *49*, 6646). 5,6-Dihydroxy-2-methyl-pyrimidine-4-carboxylic acid methyl ester (**1c)** (100 mg, 0.54 mmol) and (4-fluorophenyl)methanamine (FPMA, 124 μL, 1.1 mmol) were combined in 3 mL DMF and refluxed at 90 °C for 16 h. The reaction was then cooled to room temperature, and 1M HCl was added until precipitate formed. The solution was cooled further to 0 °C for 30 minutes. The precipitate was filtered and rinsed with ether. A dark brown solid obtained. Yield= 38%. ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): δ = 2.23 (s, 3H), 4.42 (d, *J*= 4.0 Hz, 2H), 7.13 (t, *J*=8.0 Hz, 2H; ArH), 7.35 (t, *J*=6.0 Hz, 2H; ArH), 9.33 (brt, *J*=8.0 Hz, 1H; N*H*). ESI-MS(+) *m/z* 278.0 $[M+H]^+$. Anal. Calcd for $C_{13}H_{12}FN_3O_3$: C, 56.32; H, 4.36; N, 15.16. Found: C, 56.31; H, 4.38; N, 15.11.

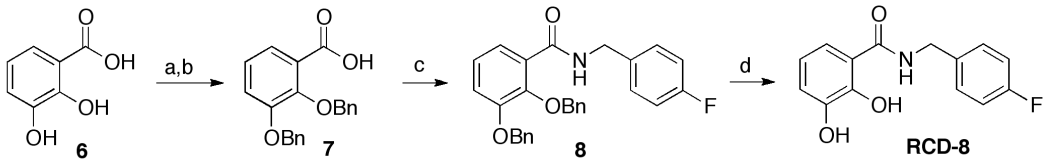


a) 6M NaOH, H2O RT b) FMBA, EDCI, HOBt, CH_2Cl_2, N_2, c)

5-Hydroxy-2-methyl-4-oxo-4H-pyran-3-carboxylic acid (5): To a solution of **4** (250 mg, 1.26 mmol) in 5 mL of H_2O was added, 3 mL of a 6M NaOH solution. The mixture was stirred for 3 h at room temperature under nitrogen. The reaction was evaporated under vacuum and the product (**5**) was extracted with CH_2Cl_2 and washed with 6M HCl.

The organic phase was dried over anhydrous $MgSO_4$ and concentrated to a yellow solid (150 mg, 0.88 mmol). Yield = 70%. ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): δ = 2.29 (s, 2H; CH_3), 8.05 (s, 1H; ArH), 9.36 (s, 1H; ArOH). ESI-MS(-) *m/z* 169.22 $[M-H]^-$.

4-Fluorobenzyl 5-hydroxy-2-methyl-4-oxo-4H-pyran-3-carboxylate (RCD-6): To a solution of **5** (60 mg, 0.35 mmol) in 10 mL of dry CH_2Cl_2 was added 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI, 81 mg, 0.42 mmol), hydroxybenzotriazole (HOBt, 57 mg, 0.42 mmol), and FPMA (48 µL, 0.42 mmol). The mixture was stirred overnight at room temperature under nitrogen and extracted with 1M HCl and CH_2Cl_2. The organic phase was dried over anhydrous $MgSO_4$, filtered, and concentrated to a yellow solid. The crude solid was purified via silica column chromatography (0-5% MeOH/CH_2Cl_2) to obtain the product as a yellow solid (28 mg, 0.10 mmol). Yield = 29%. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C): δ = 2.31 (s, 3H; CH_3), 5.64 (d, *J* = 2.8 Hz, 2H; CH_2), 7.08 (dd, *J* = 9.2, 2.8 Hz, 2H; ArH), 7.35 – 7.37 (m, 2H; ArH), 7.99 (s, 1H; ArH), 7.20 (brt, 1H; CON*H*CH_2). ESI-MS(-) *m/z* 276.25 $[M-H]^-$. Anal. Calcd for $C_{14}H_{12}FNO_4$: C, 60.65; H, 4.36; N, 5.05. Found: C, 61.04; H, 4.76; N, 5.13.



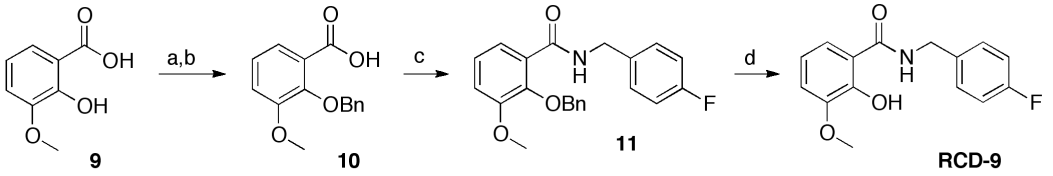
a) benzyl chloride, K_2CO_3, DMF, 95°C,N_2, b) 6M NaOH, MeOH, rt c) FMBA, EDCI, HOBt, CH_2Cl_2, N_2. d) HCl/HOAc (1:1), rt

2,3-Bis(benzyloxy)benzoic acid (7): To a solution of dihydroxybenzoic acid (**6)** (500 mg, 3.24 mmol) in 30 mL of DMF, benzyl chloride (1.33 mL, 11.6 mmol) and K_2CO_3 (1.71 g, 12.4 mmol) was added. The resulting mixture was then heated to reflux at 120 °C under nitrogen and stirred overnight. The reaction mixture was filtered and the filtrate was evaporated under vacuum to obtain a brown oil. The crude oil was purified via a silica plug using CH_2Cl_2 as eluant. Evaporation of the solvent gave a clear oil (1.36 g, 3.12 mmol). Yield = 96%. To a solution of the oil (1.32 g, 3.11 mmol) in 10 mL of MeOH, was added 6 mL of 6M NaOH. The mixture was stirred overnight at room temperature under nitrogen. The solvent was evaporated under vacuum and the product (**7**) was extracted into CH_2Cl_2 and washed with 6M HCl. The organic phase was

collected, dried over anhydrous $MgSO_4$, and evaporated under vacuum to give a white solid. Yield = 99%. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 5.20 (s, 2H; C*H*$_2$), 5.27 (s, 2H; C*H*$_2$), 7.17 (t, *J* = 8.0 Hz, 1H; ArH), 7.27 - 7.50 (m, 10H; ArH), 7.73 (dd, *J* = 7.6, 1.6 Hz, 1H; ArH). ESI-MS(-) *m/z* 332.92 $[M-H]^-$.

2,3-Bis(benzyloxy)-N-(4-fluorobenzyl)benzamide (8): To a solution of **7** (500 mg, 1.49 mmol) in 15 mL of dry CH_2Cl_2, was added EDCI (343 mg, 1.79 mmol), HOBt (242 mg, 1.79 mmol), and FPMA (204 μL, 1.79 mmol). The mixture was stirred overnight at room temperature under nitrogen. The reaction was extracted with CH_2Cl_2 and washed with 1M HCl. The organic phase was collected, dried over anhydrous $MgSO_4$, and concentrated under vacuum to obtain a brown oil. The oil was purified via silica column chromatography with 0-1% MeOH/CH_2Cl_2 as eluant to yield a white solid. Yield = 58%. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 4.42 (d, *J* = 5.6 Hz, 2H; NHC*H*$_2$), 4.99 (s, 2H; C*H*$_2$), 5.09 (s, 2H; C*H*$_2$), 6.93 (t, *J* = 8.6 Hz, 2H; ArH), 7.14 - 7.18 (m, 5H; ArH), 7.23 (d, *J* = 7.0 Hz, 2H; ArH), 7.27 (d, *J* =7.6 Hz, 1H; ArH), 7.31 (t, *J* = 7.4 Hz, 2H; ArH), 7.37 – 7.43 (m, 2H; ArH), 7.47 (d, *J* = 7.6 Hz, 2H; ArH), 7.81 (dd, *J* = 6.0, 3.2 Hz, 1H; ArH), 8.42 (t, *J* = 5.4 Hz, 1H; CON*H*CH_2). ESI-MS(+) *m/z* 441.91 $[M+H]^+$, 464.01 $[M+Na]^+$.

N-(4-Fluorobenzyl)-2,3-dihydroxybenzamide (RCD-8): Compound **8** (372 mg, 0.84 mmmol) was stirred in 25 mL of a 1:1 solution of HCl:HOAc at room temperature for 5 d to obtain a turbid mixture. The solution was evaporated to dryness and the resulting residue was co-evaporated with 3×5 mL of MeOH and the resulting solid was dried overnight in a vacuum oven to yield the product as a white solid (186 mg, 0.71 mmol). Yield = 85%. ^{1}H NMR (400 MHz, DMSO-*d*$_6$, 25 °C): δ = 4.45 (d, *J* = 6.0 Hz, 2H; NHC*H*$_2$), 6.65 (t, *J* = 8.0 Hz, 1H; ArH), 6.89 (d, *J* = 7.6 Hz, 1H; ArH), 7.12 (t, *J* = 8.8, Hz, 2H; ArH), 7.29 (d, *J* = 8.4 Hz, 1H; ArH), 7.33 (dd, *J* = 8.4, 2.8 Hz, 2H; ArH), 9.31 (t, *J* = 6.0 Hz, 1H; CON*H*CH_2). APCI-MS(+) *m/z* 262.11 $[M+H]^+$. Anal. Calcd for $C_{14}H_{12}FNO_3$•0.5 H_2O: C, 62.22; H, 4.85; N, 5.18. Found: C, 62.36; H, 5.09; N, 5.23.



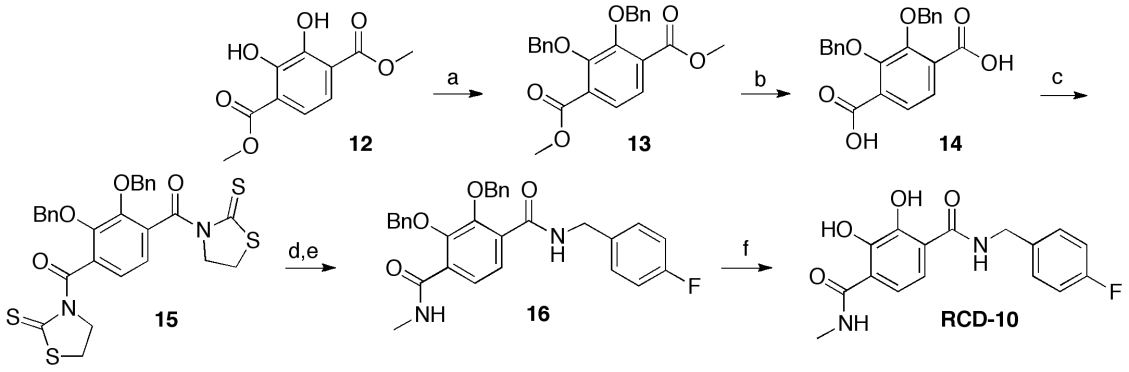
a) benzyl chloride, K_2CO_3, DMF, 95°C,N_2, b) 6M NaOH, MeOH, rt, c) FMBA, EDCI, HOBt, CH_2Cl_2, N_2, d) HCl/HOAc (1:1), rt

2-(Benzyloxy)-3-methoxybenzoic acid (10): To a solution of 3-methoxysalicylic acid (**9**, 500 mg, 2.97 mmol) in 10 mL of DMF was added benzyl chloride (880 μL, 7.63 mmol) and K_2CO_3 (1.16 g, 8.41 mmol). The resulting mixture was heated to reflux at 120 ˚C under nitrogen and stirred overnight. The reaction was vacuum filtered and the filtrate was concentrated to a dark brown oil. The oil was purified via a silica plug using CH_2Cl_2 as an eluant, after which removal of solvent under vacuum gave an off-white oil (763 mg, 2.19 mmol). Yield = 74%. To a solution of the oil (763 mg, 2.19 mmol) in 5 mL of MeOH was added 3 mL of 6M NaOH . The mixture was stirred overnight at room temperature under nitrogen. The reaction was evaporated under vacuum and the product was extracted with CH_2Cl_2 and washed with 6M HCl. The organic phase was collected, dried over anhydrous $MgSO_4$, and concentrated under vacuum to an off-white solid (566 mg, 2.19 mmol). Yield = 99%. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 3.97 (s, 3H; OC*H*$_3$), 5.27 (s, 2H; C*H*$_2$), 7.19 (d, *J* = 3.6 Hz, 1H; ArH), 7.36 - 7.41 (m, 5H; ArH), 7.43 (d, *J* = 2.1 Hz, 1H; ArH), 7.68 (dd, *J* = 6.3, 3.0 Hz, 1H; ArH). ESI-MS(+) *m/z* 259.11 $[M+H]^+$, 276.10 $[M+NH_4]^+$.

2,3-Bis(benzyloxy)-N-(4-fluorobenzyl)benzamide (11): To a solution of **10** (566 mg, 2.19 mmol) in 15 mL of dry CH_2Cl_2 was added EDCI (504 mg, 2.63 mmol), HOBt (335 mg, 2.63 mmol), and FPMA (301 μL, 2.63 mmol). The mixture was stirred overnight at room temperature under nitrogen, after which the solution was extracted with CH_2Cl_2 and washed with 1M HCl. The organic phase was collected, dried over anhydrous $MgSO_4$, and concentrated under vacuum to give a yellow oil. The oil was purified via silica column chromatography using 0-2% MeOH/CH_2Cl_2 as eluant, after which removal of solvent under vacuum gave an off-white solid (383 mg, 1.05 mmol). Yield = 48%. ^{1}H NMR (400 MHz, $CDCl_3$-*d*$_1$, 25 °C): δ = 3.92 (s, 3H; OC*H*$_3$), 4.41 (d, *J* = 5.6 Hz, 2H; NHC*H*$_2$), 4.99 (s, 2H; C*H*$_2$), 6.91 (t, *J* = 8.8 Hz, 2H; ArH), 7.07 (dd, *J* = 8.0, 1.6 Hz, 1H; ArH), 7.11 (dd, *J* = 8.4, 5.2 Hz, 2H; ArH), 7.16 (t, *J* = 8.2 Hz, 1H; ArH), 7.22 (dd, *J* =

7.2, 1.6 Hz, 2H; ArH), 7.29 - 7.37 (m, 3H; ArH), 7.74 (dd, *J* = 7.6, 1.6, Hz, 1H; ArH), 8.31 (brs, 1H; CON*H*CH_2). ESI-MS(+) *m/z* 366.27 $[M+H]^+$, 388.25 $[M+Na]^+$.

N-(4-Fluorobenzyl)-2-hydroxy-3-methoxybenzamide (RCD-9): Compound **11** (300 mg, 0.82 mmol), was stirred in 10 mL of a 1:1 solution of HCl:HOAc at room temperature for 5 d to obtain a turbid mixture. The solution was evaporated to dryness and the resulting residue was co-evaporated with 3×5 mL of MeOH and the resulting solid was dried overnight in a vacuum oven to yield the product as a white solid (163 mg, 0.59 mmol). Yield = 72%. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C): δ = 3.74 (s, 3H; OCH_3), 4.43 (d, *J* = 6.3 Hz, 2H; NHCH_2), 6.78 (t, *J* = 8.0 Hz, 1H; ArH), 7.07 (d, *J* = 7.4 Hz, 1H; ArH), 7.11 (t, *J* = 8.9, Hz, 2H; ArH), 7.31 (dd, *J* = 8.6, 3.4 Hz, 2H; ArH), 7.41 (dd, *J* = 8.0, 1.1 Hz, 2H; ArH), 9.32 (t, *J* = 6.0 Hz, 1H; CON*H*CH_2). ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C): 42.1 (CH_2), 56.2 (OCH_3), 115.5 (ArC), 115.6 (ArC), 115.9 (ArC), 118.4 (ArC), 119.1 (ArC), 129.7 (ArC), 129.8 (ArC), 135.5 (ArC), 148.9 (ArC), 151.2 (ArC), 169.8 (C=O). ESI-MS(+) *m/z* 276.20 $[M+H]^+$. Anal. Calcd for $C_{15}H_{14}FNO_3$: C, 65.45; H, 5.13; N, 5.09. Found: C, 65.76; H, 5.51; N, 5.12.



a) Benzyl bromide, K_2CO_3, DMF, 85°C,N_2, b) 4% KOH aq, 3h, rt, c) TAT, DMAP, DCC, DCM, O/N, rt, d) FPMA, CH_2Cl_2, 5h, rt e) CH_3NH_2, DCM, 30min, rt f) HCl/HOAc (1:1), rt

Dimethyl 2,3-bis(benzyloxy)terephthalate (13): To a solution of **12** (1 g, 4.4 mmol) in 20 mL DMF was added K_2CO_3 (2.43 mg, 17.6 mmol) and benzyl bromide (120 μL, 10 mmol). The mixture was refluxed for 10 h at 85 °C, at which time the insoluble salts were filtered off. Approximately 10 mL of H_2O was added to the filtrate and the resulting off-white precipitate was collected. Yield = 90%. ^{1}H NMR (400 MHz, $CDCl_3$,

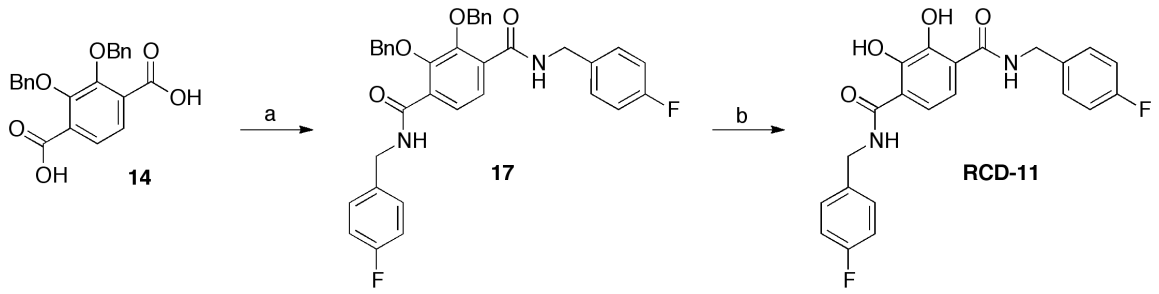
25 °C): δ = 4.49 (d, *J*=8.0 Hz, 4H), 6.86 (t, *J*= 8.0 Hz, 4H; ArH), 7.16 (t, *J*=6.0 Hz, 4H; ArH), 7.99 (t, *J*=8.0 Hz, 1H; ArH), 8.32 (d, *J*=8.0 Hz, 2H; ArH), 8.37 (brt, *J*=8.0 Hz, 2H; N*H*). ESI-MS(+) *m/z* 381.99 $[M+H]^+$.

2,3-Bis(benzyloxy)terephthalic acid (14): To a solution of **13** (1.1 g, 2.7 mmol) in 60 mL THF was added 20 mL of 4% KOH/H_2O. The solution was stirred for 4 h at room temperature, after which 40 mL of water was added. The solution was then washed with EtOAc and acidified with 6M HCl until a precipitate formed. The product was isolated by filtration as a white solid**.** Yield = 91%. ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): δ = 5.02 (s, 4H), 7.33 (m, 6H; ArH), 7.39 (m, 4H; ArH), 7.48 (s, 2H; ArH). ESI-MS(-) *m/z* 376.83 $[M-H]^-$.

(2,3-Bis(benzyloxy)-1,4-phenylene)bis((2-thioxothiazolidin-3-yl)methanone) (15): The synthesis of this compound was adapted from a literature procedure (Cohen, S. M.; Petoud, S.; et al. *Inorg. Chem.* **1999**, *38*, 4522) starting from **14** (800 mg, 2.11 mmol) and producing a yellow solid as the product. Yield = 89%. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 2.95 (t, *J*=8.0 Hz, 4H), 4.31 (t, *J*= 8.0 Hz, 4H), 5.07 (s, 4H), 7.20 (s, 2H; ArH), 7.35 (m, 10H; ArH). ESI-MS(+) *m/z* 580.74 $[M+H]^+$.

2,3-Bis(benzyloxy)-N1-(4-fluorobenzyl)-N4-methylterephthalamide (16). Compound **15** (1.1 g, 1.9 mmol) was combined with FPMA (80 μL, 0.7 mmol) in 120 mL of CH_2Cl_2. After 3 h, the reaction mixture was evaporated to dryness and partially purified by passage through a silica plug using 5% MeOH/CH_2Cl_2 as eluant. The semi-purified material was dissolved in 12 mL of CH_2Cl_2 to which 800 mL of CH_3NH_2 (40% aqueous solution) was added. After 30 min, the reaction mixture was evaporated to dryness and purified by silica column chromatography using 0-5% MeOH/CH_2Cl_2 as eluant. After removal of solvent the desired product was isolated as a white solid (343 mg, 0.69 mmol). Yield = 98%. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 2.82 (d, *J*=4.0 Hz, 3H), 4.44 (d, *J*= 8.0 Hz, 2H), 5.08 (d, *J*=4.0 Hz, 4H), 6.93 (t, *J*=8.0 Hz, 2H; ArH), 7.20 (m, 12H; ArH), 7.66 (brt, *J*=8.0 Hz, 1H; N*H*), 7.93 (q, *J*=8.0 Hz, 2H; ArH), 8.10 (brt, *J*=8.0 Hz, 1H; N*H*). ESI-MS(+) *m/z* 498.90 $[M+H]^+$.

N1-(4-Fluorobenzyl)-2,3-dihydroxy-N4-methylterephthalamide (RCD-10): Compound **16** (340 mg, 0.68 mmol), was stirred in 18 mL of a 1:1 solution of HCl:HOAc at room temperature for 3 d to obtain a turbid mixture. Addition of water resulted in precipitation of a white solid that was isolated by filtration and washed with water (159 mg, 0.5 mmol). Yield = 73%. ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): δ = 2.80 (d, *J*=4.0 Hz, 3H), 4.47 (d, *J*=8.0 Hz, 2H), 7.15 (t, *J*= 8.0 Hz, 2H; ArH), 7.33 (d, *J*=8.0 Hz, 2H; ArH), 7.36 (t, *J*=8.0 Hz, 2H; ArH), 8.87 (brt, *J*=4.0 Hz, 1H; N*H*), 9.36 (brt, *J*=4.0 Hz, 1H; N*H*). ESI-MS(+) *m/z* 318.96 $[M+H]^+$. Anal. Calcd for $C_{16}H_{15}FN_2O_4$: C, 60.37; H, 4.75; N, 8.80. Found: C, 60.54; H, 4.79; N, 8.89.

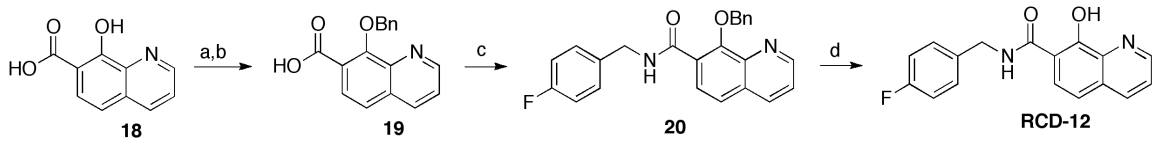


a) FPMA, EDCI, HOBt, CH_2Cl_2, N_2, b) (1:1) HCl/HoAC, 3 d

2,3-Bis(benzyloxy)-N1,N4-bis(4-fluorobenzyl)terephthalamide (17): This compound was prepared from **14** (300 mg, 0.79 mmol) according to the procedure outlined for **32** (see below). The product was isolated as a white solid. Yield = 54%. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 4.42 (d, *J*=10.0 Hz, 4H), 5.05 (s, 4H), 6.95 (t, *J*= 8.0 Hz, 4H; ArH), 7.14 (m, 10H; ArH), 7.29 (t, *J*=8.0 Hz, 4H; ArH), 7.35 (s, 2H; ArH), 8.06 (brt, *J*=8.0 Hz, 2H; N*H*). ESI-MS(+) *m/z* 592.95 $[M+H]^+$.

N1,N4-Bis(4-fluorobenzyl)-2,3-dihydroxyterephthalamide (RCD-11): Compound **17** (250 mg, 0.42 mmol), was stirred in 16 mL of a 1:1 solution of HCl:HOAc at room temperature for 3 d to obtain a turbid mixture. Addition of water resulted in precipitation of a white solid that was isolated by filtration and washed with water (143 mg, 0.35 mmol). Yield = 83%. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 4.62 (d, *J*=4.0 Hz, 4H), 7.05 (t, *J*= 8.0 Hz, 4H; ArH), 7.14 (s, 2H; ArH), 7.20 (brt, *J*=6.0 Hz, 2H; N*H*), 7.33 (t,

J=6.0 Hz, 4H; ArH), 10.74 (brs, 2H, O*H*). ESI-MS(+) *m/z* 412.96 $[M-H]^+$. Anal. Calcd for $C_{22}H_{18}F_2N_2O_4$: C, 64.07; H, 4.40; N, 6.79. Found: C, 63.87; H, 4.45; N, 6.89.



18 19 20 RCD-12

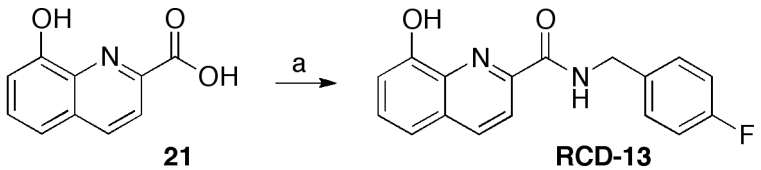
a) Benzyl chloride, K_2CO_3, DMF, 95°C,N_2, b) 6M NaOH, MeOH, rt, c) FPMA, EDCI, HOBt, CH_2Cl_2, N_2, d) HCl/HOAc (1:1), rt

8-(Benzyloxy)quinoline-7-carboxylic acid (19): To a solution of 8-hydroxyquinoline-7-carboxylic acid (**18**) (500 mg, 2.64 mmol) in 10 mL of DMF was added benzyl chloride (782 µL, 6.78 mmol) and K_2CO_3 (1.03 g, 7.47 mmol). The resulting mixture was heated to reflux at 120 ˚C under nitrogen and stirred overnight. The mixture was then vacuum filtered and the filtrate was concentrated under vacuum to a reddish-brown oil. The oil was purified via a silica plug using CH_2Cl_2 as eluant, after which removal of solvent gave an orange oil (585 mg, 1.58 mmol). Yield = 60%. To a solution of the oil (585 mg, 1.58 mmol) in 5 mL of MeOH was added, 3 mL of 6M NaOH. The solution was stirred overnight at room temperature under nitrogen. The solution was then evaporated under vacuum and the residue was dissolved in CH_2Cl_2 and washed with 6M HCl. The organic phase was collected, dried over anhydrous $MgSO_4$, and concentrated under vacuum to give a yellow solid (444 mg, 1.58 mmol). Yield = 99%. ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): δ = 5.43 (s, 2H; CH_2), 7.33 (d, *J* = 7.2 Hz, 2H; ArH), 7.37 (t, *J* = 7.2 Hz, 2H; ArH), 7.58 (d, *J* = 6.8 Hz, 2H; ArH), 7.63 (dd, *J* = 8.4, 4.4 Hz, 1H; ArH), 7.77 (d, *J* = 2.4 Hz, 1H; ArH), 8.42 (dd, *J* = 8.4, 1.4 Hz, 1H; ArH), 9.01 (dd, *J* = 4.4, 2.0 Hz, 1H; ArH). ESI-MS(-) *m/z* 278.32 $[M-H]^-$.

8-(Benzyloxy)-N-(4-fluorobenzyl)quinoline-7-carboxamide (20): To a solution of **19** (400 mg, 1.43 mmol) in 15 mL of dry CH_2Cl_2 was added EDCI (329 mg, 1.72 mmol), HOBt (232 mg, 1.72 mmol), and FPMA (197 µL, 1.72 mmol). The mixture was stirred overnight at room temperature under nitrogen, after which the solution was extracted with CH_2Cl_2 and washed with 1M HCl. The organic phase was collected, dried over anhydrous $MgSO_4$, and concentrated under vacuum to give a yellow oil. The oil was purified via silica column chromatography using 0-2% MeOH/CH_2Cl_2 as eluant. After

removal of solvent the product was obtained as a yellow solid (171 mg, 0.44 mmol). Yield = 31%. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 4.43 (d, *J* = 5.6 Hz, 2H; NHC*H*$_2$), 5.51 (s, 2H; C*H*$_2$), 6.92 (t, *J* = 8.8 Hz, 2H; ArH), 7.13 (dd, *J* = 6.4, 3.0 Hz, 2H; ArH), 7.32 (d, *J* = 5.2 Hz, 5H; ArH), 7.48 (dd, *J* = 8.4, 4.0 Hz, 1H; ArH), 7.64 (d, *J* = 8.4 Hz, 1H; ArH), 8.18 (dd, *J* = 8.4, 2.0, Hz, 1H; ArH), 8.28 (d, *J* = 8.8 Hz, 1H; ArH), 8.60 (brt, 1H; CON*H*CH_2), 8.99 (dd, *J* = 4.0, 1.6 Hz, 1H; ArH). ESI-MS(+) *m/z* 387.11 $[M+H]^+$.

***N*-(4-Fluorobenzyl)-2,3-dihydroxybenzamide (RCD-12):** Compound **20** (154 mg, 0.40 mmmol) was stirred in 10 mL of a 1:1 was stirred in 25 mL of a 1:1 solution of HCl:HOAc at room temperature for 5 d to obtain a turbid mixture. The solution was evaporated to dryness and the resulting residue was co-evaporated with 3×5 mL of MeOH and the resulting solid was dried overnight in a vacuum oven to yield the product as a yellow solid (101 mg, 0.34 mmol). Yield = 85%. ^{1}H NMR (500 MHz, DMSO-*d*$_6$, 25 °C): δ = 4.54 (d, *J* = 4.6 Hz, 2H; NHC*H*$_2$), 7.13 (t, *J* = 8.6 Hz, 2H; ArH), 7.38 (t, *J* = 6.0 Hz, 2H; ArH), 7.57 (d, *J* = 9.1, Hz, 1H; ArH), 7.85 (brt, 1H; ArH), 8.17 (d, *J* = 8.6 Hz, 1H; ArH), 8.67 (d, *J* = 8.0 Hz, 1H; ArH), 9.00 (brs, 1H; ArH), 9.75 (brt, 1H; CON*H*CH_2. ^{13}C NMR (125 MHz, DMSO-*d*$_6$, 25 °C): 42.4 (CH_2), 113.6 (ArC), 115.5 (ArC), 115.7 (ArC), 117.7 (ArC), 124.4 (ArC), 126.1 (ArC), 130.0 (ArC), 131.4 (ArC), 135.4 (ArC), 148.2 (ArC), 155.9 (ArC), 160.7 (ArC), 162.7 (ArC), 168.8 (C=O). ESI-MS(+) *m/z* 297.12 $[M+H]^+$. Anal. Calcd for $C_{17}H_{13}FN_2O_2$•2.25 H_2O: C, 60.62; H, 5.24; N, 8.32. Found: C, 60.53; H, 4.83; N, 8.33.

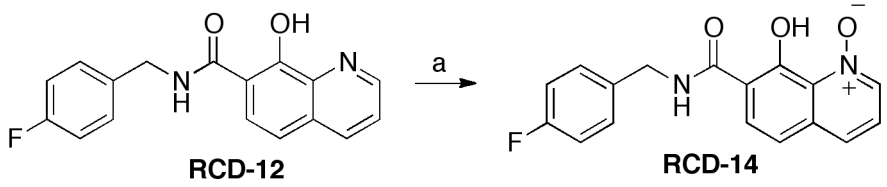


21 **RCD-13**

a) FPMA, EDCI, HOBt, CH_2Cl_2, N_2

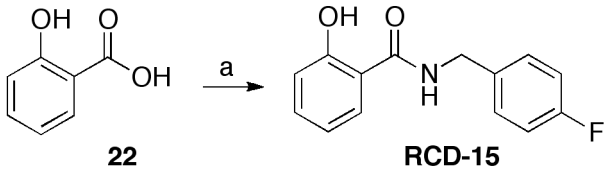
***N*-(4-Fluorobenzyl)-8-hydroxyquinoline-2-carboxamide (RCD-13):** To a solution of 8-hydroxyquinoline-2-carboxylic acid, (**21**, 400 mg, 2.1 mmol) in 20 mL of CH_2Cl_2 was added EDCI (487 mg, 2.5 mmol), HOBt (343 mg, 2.5 mmol), and FPMA (290 µL, 2.5

mmol). The resulting mixture was stirred at room temperature for 16 h under nitrogen. The mixture was washed with 1M HCl and brine. The organic phase was collected and dried over anhydrous $MgSO_4$. The crude product was evaporated under vacuum and purified via flash silica column chromatography using 0-5% MeOH/CH_2Cl_2 as eluant to give the product as a pale yellow solid (383 mg, 1.3 mmol). Yield = 61%. ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): δ = 4.59 (d, *J*=8.0 Hz, 2H), 7.17 (t, J= 8.0 Hz, 2H; ArH), 7.19 (d, *J*=8.0 Hz, 1H; ArH), 7.40 (t, *J*=4.0 Hz, 2H; ArH), 7.46 (d, *J*=8.0 Hz, 1H; ArH), 7.55 (t, *J*=8.0 Hz, 1H; ArH), 8.15 (d, *J*=8.0 Hz, 1H; ArH), 8.49 (d, *J*=8.0 Hz, 1H; ArH), 10.14 (brt, *J*=8.0 Hz, 1H; N*H*). ESI-MS(+) *m/z* 297.09 $[M+H]^+$. Anal. Calcd for $C_{17}H_{13}FN_2O_2$: C, 68.91; H, 4.42; N, 9.45. Found: C, 68.99; H, 4.81, N, 9.56.



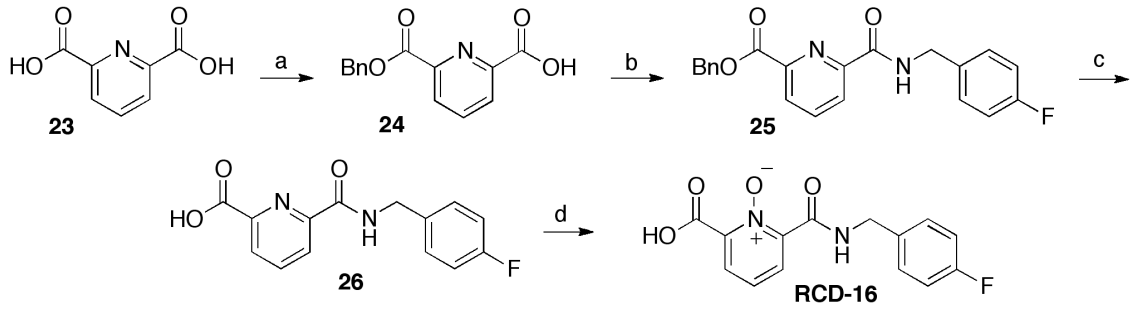
a) 30% H_2O_2, TFA, 80°C, O/N

7-((4-Fluorobenzyl)carbamoyl)-8-hydroxyquinoline 1-oxide (RCD-14): This compound was prepared from **RCD-12** as adapted from a literature procedure (Agrawal, A. et al. *J. Med. Chem*. **2009**, *52*, 1063); a detailed procedure is provided for **RCD-16** (see below)**.** **RCD-12** (183 mg, 0.5 mmol) was combined with TFA and H_2O_2 to produce a dark brown solid. Yield = 35%. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 4.72 (d, *J*=8.0 Hz, 2H), 7.05 (t, *J*= 8.0 Hz, 2H; ArH), 7.39 (m, 3H; ArH), 7.61 (dd, *J*=8.0 Hz, *J*=4.0 Hz, 1H; ArH), 8.17 (d, *J*=8.0 Hz, 1H; ArH), 8.24 (d, *J*=8.0 Hz, 1H), 8.27 (br, 1H; N*H*), 8.89 (d, *J*=4.0 Hz, 1H). ESI-MS(+) *m/z* 296.97 $[M-O-]^+$. Anal. Calcd for $C_{17}H_{13}FN_2O_3$: C, 63.19; H, 4.43; N:8.67. Found: C, 63.42; H, 4.85; N, 8.17.



a) FMBA, EDCI, HOBt, CH_2Cl_2, N_2

N-(4-Fluorobenzyl)-2-hydroxybenzamide (RCD-15): To a solution of 2-hydroxybenzoic acid (**22**, 500 mg, 3.6 mmol) in 20 mL of CH_2Cl_2 was added EDCI (833 mg, 4.3 mmol), HOBt (585 mg, 4.3 mmol), and FPMA (495 μL, 4.3 mmol). The mixture was stirred at room temperature for 16 h under nitrogen. The reaction was then rinsed with 1M HCl and brine. The organic phase was collected and dried over anhydrous $MgSO_4$. The crude product was evaporated under vacuum and purified via flash silica column chromatography using CH_2Cl_2 as eluant, which after removal of solvent gave the product as a white solid (302 mg, 1.2 mmol). Yield = 34%. ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): δ = 4.48 (d, *J*=4.0 Hz, 2H), 6.88 (t, *J*= 8.0 Hz, 2H; ArH), 7.13 (t, *J*=8.0 Hz, 2H; ArH), 7.38 (m, 3H; ArH), 7.86 (d, *J*=8.0 Hz, 1H; ArH), 9.34 (brt, *J*=8.0 Hz, 1H; N*H*). ESI-MS(+) *m/z* 245.99 $[M+H]^+$. Anal. Calcd for $C_{14}H_{12}FNO_2$: C, 68.56; H, 4.93; N, 5.71. Found: C, 68.18; H, 5.35; N, 5.87.



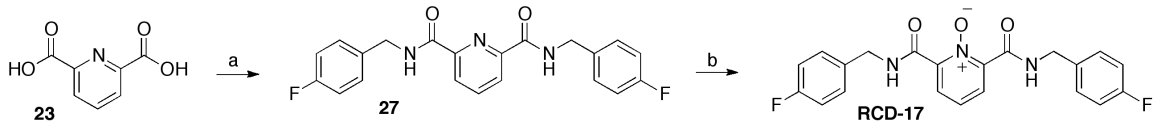
a) BnBr, $NaHCO_3$, DMF, b) FPMA, EDCI, HOBt, CH_2Cl_2, N_2, c) KOH, 85°C, 3h, d) 30% H_2O_2, TFA, 80°C, O/N

6-((Benzyloxy)carbonyl)picolinic acid (24): The synthesis of this compound was adapted from a literature procedure (Gardiner, J. et al. *Chem. Biodiversity*, **2006**, *3*, 1181). To pyridine-2,6-dicarboxylic acid (**23**, 2 g, 12 mmol) in 40 mL DMF was added $NaHCO_3$ (1.18 g, 14.4 mmol) and benzyl bromide (1.7 mL, 14.4 mmol). The reaction mixture was heated to 60 °C for 16 h, after which the solution was cooled to room temperature. To the reaction, 40 mL of H_2O was added and the aqueous layer was rinsed with EtOAc before being acidified to pH 3 with 1M HCl. The solution was extracted with EtOAc, the organic phase was collected, dried over anhydrous $MgSO_4$, and the crude mixture was evaporated under vacuum to give a white solid. Yield = 16%. ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): δ = 5.41 (s, 2H), 7.45 (m, 5H; ArH), 8.22 (m, 3H; ArH). ESI-MS(-) *m/z* 255.92 $[M-H]^-$.

***N*-(4-Fluorobenzyl)-6-(2-phenylacetyl)picolinamide (25):** This compound was prepared according to the coupling procedure outlined for compound **8**. Compound **24** (400 mg, 1.56 mmol) was combined with 1.2 eq of FPMA to give the desired product (58 mg, 0.52 mmol). Yield = 10%. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 4.65 (d, *J*=8.0 Hz, 2H), 5.43 (s, 2H), 7.02 (t, *J*= 8.0 Hz, 2H; ArH), 7.36 (m, 7H; ArH), 8.01 (t, *J*=8.0 Hz, 1H; ArH), 8.22 (d, *J*=8.0 Hz, 1H; ArH), 8.40 (d, *J*=8.0 Hz, 1H; ArH), 8.54 (brt, *J*=8.0 Hz, 1H; N*H*). ESI-MS(+) *m/z* 364.90 $[M+H]^+$.

6-((4-fluorobenzyl)carbamoyl)picolinic acid (26): To a solution of **25** (300 mg, 0.82 mmol) in 20 mL MeOH was added KOH (157 mg, 2.8 mmol). The reaction mixture was heated to 85 °C for 4 h, then neutralized with HCl. The solvent was removed under vacuum and the resulting solid was dissolved in 5% MeOH/CH_2Cl_2. Insoluble particles were hot filtered and the solution was dried under vacuum to produce a white solid. Yield = 90%. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ= 4.32 (d, *J*=4.0 Hz, 2H), 6.72 (t, J= 8.0 Hz, 2H; ArH), 7.01 (t, *J*=6.0 Hz, 2H; ArH), 7.54 (brt, *J*=6.0 Hz, 1H; N*H*), 7.87 (d, *J*=8.0 Hz, 1H; ArH), 7.95 (d, *J*=4.0 Hz, 1H), 8.82 (brt, *J*=6.0 Hz, 1H; N*H*). ESI-MS(-) *m/z* 272.90 $[M-H]^-$.

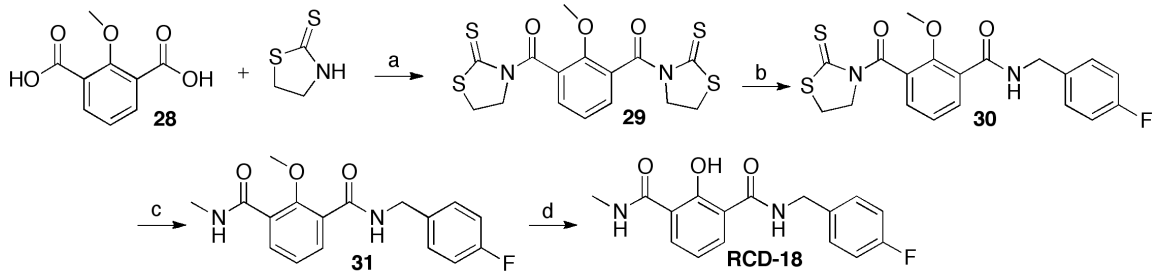
2-Carboxy-6-((4-fluorobenzyl)carbamoyl)pyridine 1-oxide (RCD-16): The synthesis of this compound was adapted from a literature procedure (Agrawal, A. et al. *J. Med. Chem*. **2009**, *52*, 1063). A mixture of 1.5 mL TFA and 220 μL of 30% H_2O_2 was added to **26** (100 mg, 0.55 mmol). The solution was refluxed at 80 °C for 16 h, and then cooled to room temperature. Approximately 7 mL of water was added and the brown precipitate that formed was filtered off and collected. Yield = 19%. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 4.67 (d, *J*=4.0 Hz, 2H), 7.06 (t, *J*= 8.0 Hz, 2H; ArH), 7.35 (t, *J*=6.0 Hz, 2H; ArH), 7.82 (t, *J*=8.0 Hz, 1H; ArH), 8.60 (dd, *J*=8.0 Hz, *J*=4.0 Hz, 1H; ArH), 8.74 (dd, *J*=8.0 Hz, *J*=4.0 Hz, 1H; ArH), 10.29 (brt, *J*=8.0 Hz, 1H; N*H*). ESI-MS(-) *m/z* 288.65 $[M-H]^-$. Anal. Calcd for $C_{14}H_{11}FN_2O_4$: C, 57.93; H, 3.82; N, 9.65. Found: C, 58.19; H, 4.10; N, 9.37.



a) FPMA, EDCI, HOBt, CH_2Cl_2, N_2, b) 30% H_2O_2, TFA, 80°C, O/N

N2,N6-Bis(4-fluorobenzyl)pyridine-2,6-dicarboxamide (27): To a solution **23** (400 mg, 2.4 mmol) in 15 mL of CH_2Cl_2 was added EDCI (1 g, 5.3 mmol), HOBt (712 mg, 5.3 mmol), and FPMA (620 µL, 5.3 mmol). The mixture was stirred at room temperature for 16 h under nitrogen. The reaction was then washed with 1M HCl and brine. The organic phase was collected and dried over anhydrous $MgSO_4$. The crude product was evaporated under vacuum and purified via flash silica column chromatography using 0-5% MeOH/CH_2Cl_2 as eluant. Yield = 65%. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 4.49 (d, *J*=8.0 Hz, 4H), 6.86 (t, *J*= 8.0 Hz, 4H; ArH), 7.16 (t, *J*=6.0 Hz, 4H; ArH), 7.99 (t, *J*=8.0 Hz, 1H; ArH), 8.32 (d, *J*=8.0 Hz, 2H; ArH), 8.37 (brt, *J*=8.0 Hz, 2H; N*H*). ESI-MS(+) *m/z* 381.99 $[M+H]^+$.

2,6-Bis((4-fluorobenzyl)carbamoyl)pyridine 1-oxide (RCD-17): **RCD-17** was prepared according to the procedure outlined for **RCD-16** using **27** (580 mg, 1.5mmol) as the starting material. The desired compound was purified via flash silica column chromatography using 0-5% MeOH/CH_2Cl_2 as the eluant. Yield = 10%. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 4.63 (d, *J*=6.0 Hz, 4H), 7.03 (t, *J*= 9.0 Hz, 4H; ArH), 7.33 (t, *J*=7.5 Hz, 4H; ArH), 7.62 (t, *J*=6.0 Hz, 1H; ArH), 8.60 (d, *J*=6.0 Hz, 2H; ArH), 10.93 (br, 2H; N*H*). ESI-MS(+) *m/z* 397.98 $[M+H]^+$. Anal. Calcd for $C_{21}H_{17}F_2N_3O_3$: C, 63.47; H, 4.31; N, 10.57. Found: C, 63.10; H, 4.40; N, 10.72.



a) DMAP, DCC, TAT, DCM, 5h, rt b) FPMA, DCM. 1h, rt, c) 40% CH_3NH_2, DCM, 30min, rt, d) BBr_3, DCM, 0°C - rt, 3d

(2-Methoxy-1,3-phenylene)bis((2-thioxothiazolidin-3-yl)methanone) (29): The synthesis of this compound was adapted from a literature procedure (Cohen, S. M. et al.

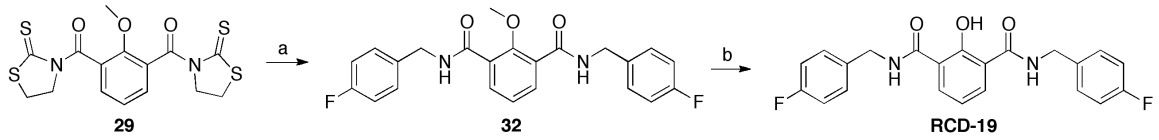
Inorg. Chem. **1999**, *38*, 4522). To a solution of 2-methoxyisophthalic acid (2.7 g, 13.8 mmol) (**28**) in 120 mL of CH_2Cl_2 was added thiazolidine-2-thione (3.3 g, 28 mmol), a catalytic amount of N,N-dimethylaminopyridine (DMAP), and N,N′-dicyclohexylcarbodiimide (DCC, 5.7 g, 28 mmol) at room temperature. The mixture was stirred for 5 h under nitrogen. The solution was then filtered and the solvent was removed from the filtrate under vacuum. The compound was purified via flash silica column chromatography using CH_2Cl_2 as eluant to give the product as a bright yellow solid (1.6 g, 3.9 mmol). Yield = 29%. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 3.42 (t, *J*=8.0 Hz, 4H), 3.90 (s, 3H), 4.60 (t, *J*=8.0 Hz, 4H), 7.14 (t, *J*=8.0 Hz, 1H; ArH), 7.43 (d, *J*=4.0 Hz, 2H; ArH). ESI-MS(+) *m/z* 398.67 $[M+H]^+$.

***N*-(4-Fluorobenzyl)-2-methoxy-3-(2-thioxothiazolidine-3-carbonyl)benzamide (30):** The synthesis of this compound was adapted from literature procedure (Cohen, S. M. et al. *Inorg. Chem.* **1999**, *38*, 4522). To a solution of **29** (300 mg, 0.73 mmol) in 100 mL of CH_2Cl_2 was added FPMA (29 μL, 0.24 mmol). The reaction mixture was stirred overnight at room temperature under nitrogen. The solvent was then removed under vacuum and the resulting mixture was purified via flash silica column chromatography using CH_2Cl_2 as eluant to give the product as a bright yellow solid. Yield = 88%. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 3.43 (t, *J*=6.0 Hz, 2H), 3.75 (s, 3H), 4.60 (d, *J*=4.0 Hz, 2H), 4.65 (t, *J*= 8.0 Hz, 2H), 7.02 (t, *J*=8.0 Hz, 2H; ArH), 7.21 (t, *J*=8.0 Hz, 1H; ArH), 7.25 (t, *J*=4.0 Hz, 2H; ArH), 7.33 (d, *J*=8.0 Hz, 1H; ArH), 7.80 (brt, *J*=8.0 Hz, 1H; N*H*), 8.15 (d, *J*=8.0 Hz, 1H; ArH). ESI-MS(+) *m/z* 404.81 $[M+H]^+$.

N1-(4-Fluorobenzyl)-2-methoxy-N3-methylisophthalamide (31): To a solution of **30** (150 mg, 0.37 mmol) in 4 mL CH_2Cl_2 was added 230 μL of CH_3NH_2 (40% aqueous solution) at room temperature. The mixture was stirred vigorously for 30 min under nitrogen. The solution was washed with water and the crude material was purified via flash silica column chromatography using 0-10% MeOH/CH_2Cl_2 as eluant. Yield = 76%. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 2.93 (d, *J*=4.0 Hz, 3H), 3.69 (s, 3H), 4.55 (d, *J*= 4.0 Hz, 2H), 6.99 (t, *J*=8.0 Hz, 2H; ArH), 7.22 (t, *J*=8.0 Hz, 1H; ArH), 7.29 (t, *J*=8.0 Hz,

2H; ArH), 7.75 (brt, *J*=8.0 Hz, 1H; N*H*), 7.94 (d, *J*=8.0 Hz, 1H; ArH), 7.98 (d, *J*=9.0 Hz, 1H; ArH). ESI-MS(+) *m/z* 317.0 $[M+H]^+$.

N1-(4-Fluorobenzyl)-2-hydroxy-N3-methylisophthalamide (RCD-18): RCD-18 was prepared according to the detailed procedure outlined for **RCD-19** (see below) and was isolated as a white solid (30.6 mg, 0.10 mmol). Yield = 36%. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 3.01 (d, *J*=4.0 Hz, 3H), 4.61 (d, *J*= 4.0 Hz, 2H), 6.87 (t, *J*=8.0 Hz, 1H; ArH), 7.01 (t, *J*=8.0 Hz, 2H; ArH), 7.30 (t, *J*=6.0 Hz, 2H; ArH), 7.61 (br, 1H; N*H*), 7.90 (d, *j*=8.0 Hz, 1H), 8.05 (d, *J*=8.0 Hz, 1H; ArH), 8.29 (br, 1H; N*H*). ESI-MS(+) *m/z* 302.95 $[M+H]^+$. Anal. Calcd for $C_{16}H_{14}FNO_4$: C, 63.57; H, 5.00; N, 9.27. Found: C, 63.32; H, 5.10; N, 9.28.



a) FPMA, DCM. 1h, rt, b) BBr_3, DCM, 0°C - rt, 3d

N1,N3-Bis(4-fluorobenzyl)-2-methoxyisophthalamide (32): To a solution of **29** (300 mg, 0.75 mmol) in 100 mL CH_2Cl_2 was added FPMA (215 µL, 1.87 mmol). The reaction was stirred at room temperature overnight. The solvent was then removed under vacuum and the crude product was purified via flash silica column chromatography with CH_2Cl_2 as eluant. Yield = 23%. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 3.43 (t, *J*=6.0 Hz, 2H), 3.75 (s, 3H), 4.60 (d, *J*= 8.0 Hz, 2H), 4.64 (t, *J*=6.0 Hz, 2H), 7.03 (t, *J*=8.0 Hz, 2H; ArH), 7.24 (t, *J*=8.0 Hz, 1H; ArH), 7.31 (t, *J*=8.0 Hz, 2 H; ArH), 7.41 (d, *J*=8.0 Hz, 1H; ArH), 7.76 (brt, *J*=8.0 Hz, 1H; N*H*), 8.16 (d, *J*=8.0 Hz, 1H; ArH). ESI-MS(+) *m/z* 404.79 $[M+H]^+$.

N1,N3-Bis(4-fluorobenzyl)-2-hydroxyisophthalamide (RCD-19): To a solution of **32** (70 mg, 0.17 mmol) in 15 mL CH_2Cl_2 was added BBr_3 (58 mg, 0.23 mmol) under nitrogen at 0 °C. The mixture was stirred for 3 d, the reaction was then quenched with MeOH, and the mixture was diluted with water. The solution was boiled until the yellow color dissipated and the volume of the solution was reduced by half. MeOH was added to induce precipitation and the resulting white solid was isolated by filtration. Yield =

21%. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 4.64 (d, *J*=4.0 Hz, 4H), 6.97 (t, *J*= 8.0 Hz, 1H; ArH), 7.04 (t, *J*=8.0 Hz, 4H; ArH), 7.33 (t, *J*=6.0 Hz, 4H; ArH), 7.70 (br, 2H; N*H*), 7.97 (d, *J*=8.0 Hz, 2H). ESI-MS(+) *m/z* 396.93 $[M+H]^+$. Anal. Calcd for $C_{22}H_{18}F_2N_2O_3$: C, 66.66; H, 4.58; N, 7.07. Found: C, 66.54; H, 4.98; N, 6.86.

In Vitro Integrase Catalytic Assays

Recombinant HIV-1 IN and oligonucleotide substrates were obtained as previously reported (Marinello et al. *Biochemistry* **2008**, *47*, 9345-9354; Metifiot et al. *Antimicrob. Agents Chemother.* **2011**, *55*, 5127-5133; Hare et al. *Mol. Pharmacol.* **2011**, *80*, 565-572). Integrase reactions were performed in 10 μL total volume including 400 nM HIV-1 IN, 20 nM 5'-end [^{32}P]-labeled oligonucleotide substrate, and 1 μL inhibitor solution in 50 mM MOPS, pH 7.2, 7.5 mM $MgCl_2$, and 14.3 mM 2-mercaptoethanol. Inhibitor dilutions were in DMSO, and DMSO without drug was used as a control. Reactions were incubated at 37 °C for 60 min, terminated by adding 10 μL loading dye (10 mM EDTA, 98% deionized formamide, 0.025% xylene cyanol, and 0.025% bromophenol blue), and were subjected to electrophoresis in 20% polyacrylamide–7 M urea gels. Gels were dried and reaction products were visualized and quantified with a Typhoon 8600 (GE Healthcare, Little Chalfont, Buckinghamshire, UK). Densitometric analyses were performed using ImageQuant from Molecular Dynamics Inc. The concentrations at which enzyme activity was reduced by 50% (IC_{50}) were determined using "Prism" software (GraphPad Software, San Diego, CA) for nonlinear regression to fit dose-response data to logistic curve models.

Computational Docking Studies

The coordinates for the X-ray crystal structure of PFV-IN were taken from the RCSB Protien Data Bank (entry: 3OYA) and prepared using the Protein Preparation Wizard, which is a part of the Maestro software package (Maestro v9.1; Schrodinger, Inc.). The Protein Prepartion Wizard was used to add bond order assignments and formal charges for heterogroups (amino acid residues, metal-ligand bonds) and hydrogen atoms to the system. To optimize the hydrogen bonding network histidine tautomers and ionization states were predicted, and manual corrections were made when necessary to ensure

correct coordination with the two Mg (II) ions. Proper assignment of Asn and Gln sidechains was assessed by rotating 180º around the terminal χ angle of these residues while adding hydrogen atoms to sample the hydrogen-bonding network around the residues to determine if the oxygen and nitrogen atoms were properly assigned. All water molecules in the structure were removed.

Three-dimensional structures of the RCD fragments and Raltegravir were prepared using LigPrep (LigPrep v2.4 Schrodinger, Inc.) with Epik (Epik v2.1 Schrodinger, Inc.) to generate multiple protonation and tautomeric states for the ligands at pH values of 7.0±2.0.

The metal binding state (i.e. deprotonated hydroxyl groups) of the RCD compounds were docked flexibly into the active site of the prepared PFV-IN structure. Docking was preformed with Glide 5.5 (Glide v5.5; Schrodinger, Inc.) with the standard precision scoring function to estimate protein-ligand binding affinities. A maximum of ten scoring poses were saved for each fragment. The top scoring poses for each fragment were found to possess the expected binding modes with reasonable metal-ligand bond distances based on the 3OYA crystal complex.

To calculate the RMSD of the various compounds, the superposition tool within Maestro was used. The two compounds of interest were selected and the atoms to be compared were manually selected to generate the RMSD value. The calculations were conducted using the 'in place' option, which omits a post-docking minimization of the compounds that is designed to move the structures in order get the lowest possible RMS difference between the two superimposed fragments.

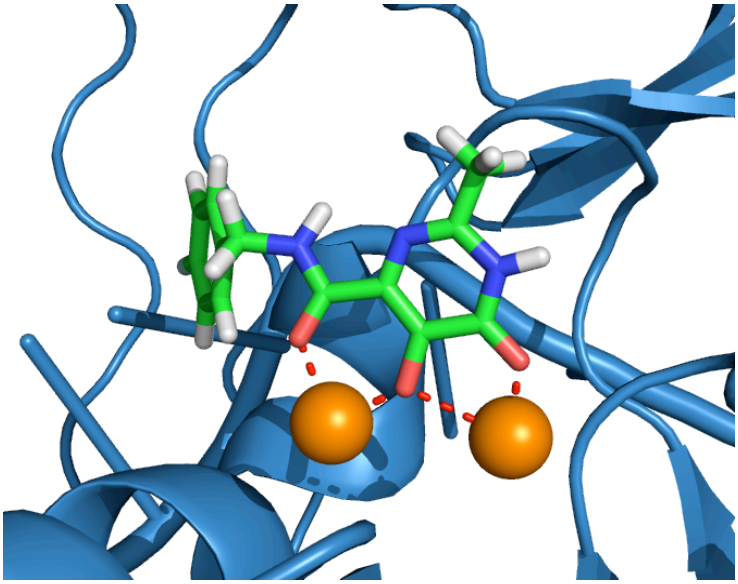


Figure S1. Docked structure of RCD-1 in the active site of PFV-IN (PDB: 3OYA). The inhibitor is shown in sticks (colored by atom), the enzyme as a blue ribbon, and the Mg^{2+} ions as orange spheres.

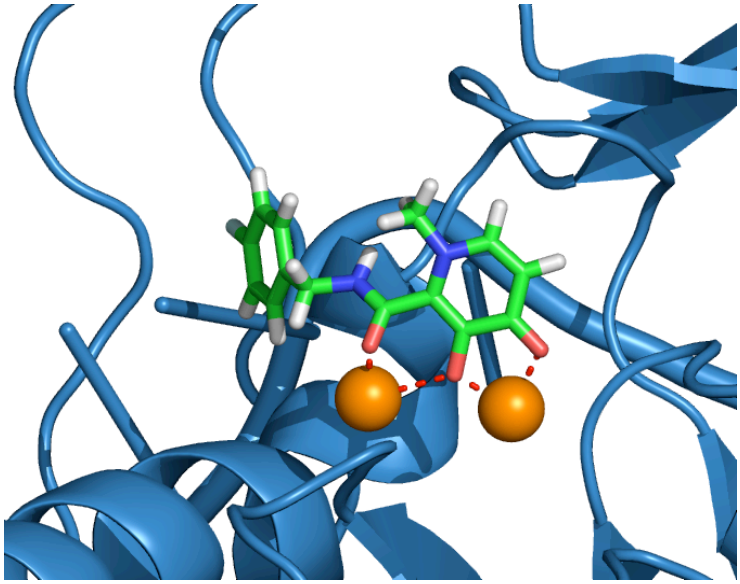


Figure S2. Docked structure of RCD-2 in the active site of PFV-IN (PDB: 3OYA). The inhibitor is shown in sticks (colored by atom), the enzyme as a blue ribbon, and the Mg^{2+} ions as orange spheres.

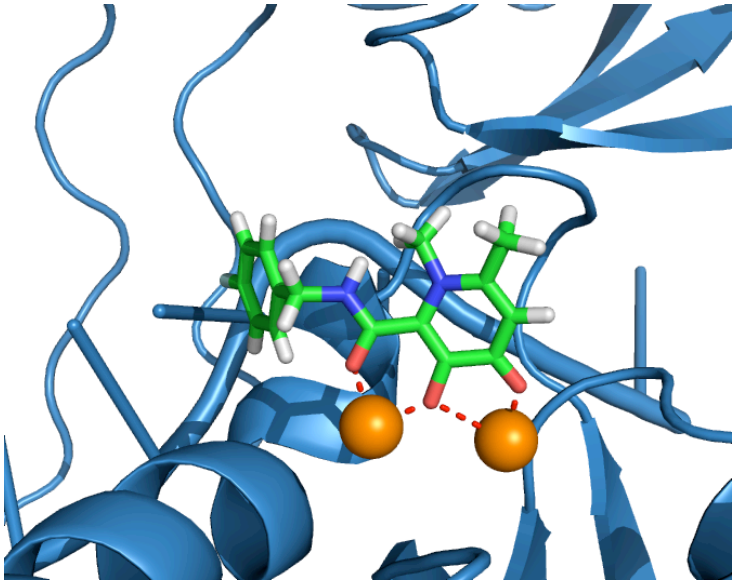


Figure S3. Docked structure of RCD-3 in the active site of PFV-IN (PDB: 3OYA). The inhibitor is shown in sticks (colored by atom), the enzyme as a blue ribbon, and the Mg^{2+} ions as orange spheres.

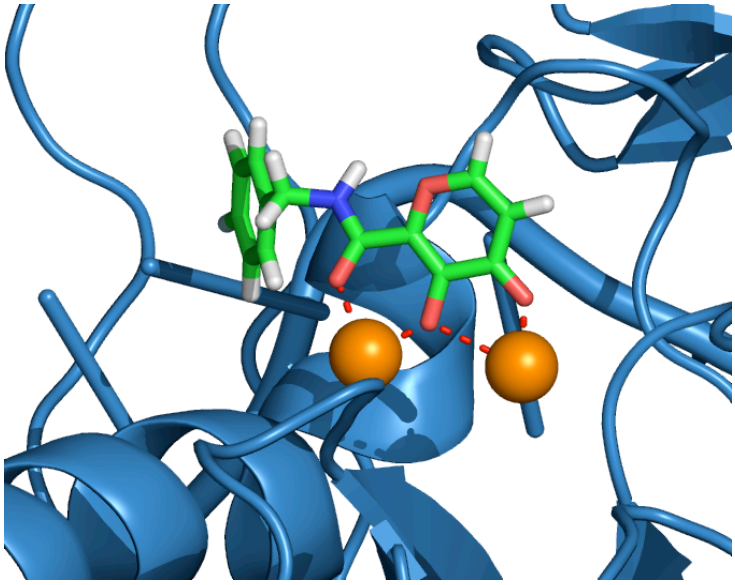


Figure S4. Docked structure of RCD-4 in the active site of PFV-IN (PDB: 3OYA). The inhibitor is shown in sticks (colored by atom), the enzyme as a blue ribbon, and the Mg^{2+} ions as orange spheres.

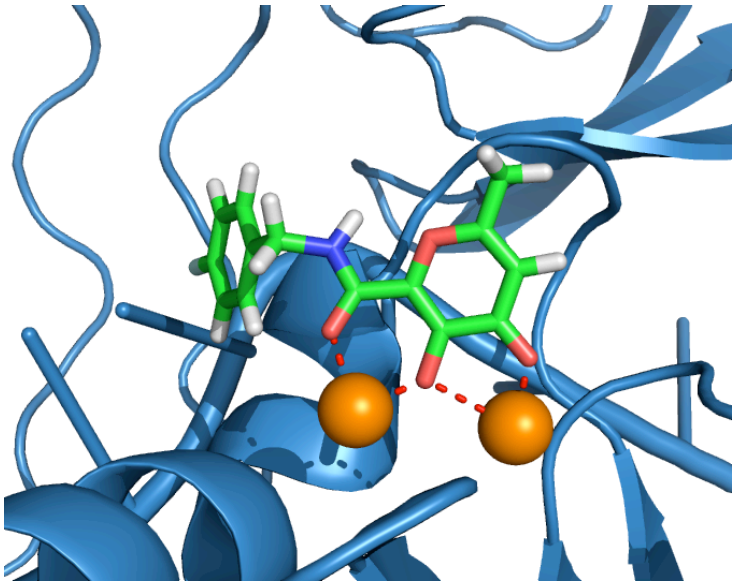


Figure S5. Docked structure of RCD-5 in the active site of PFV-IN (PDB: 3OYA). The inhibitor is shown in sticks (colored by atom), the enzyme as a blue ribbon, and the Mg^{2+} ions as orange spheres.

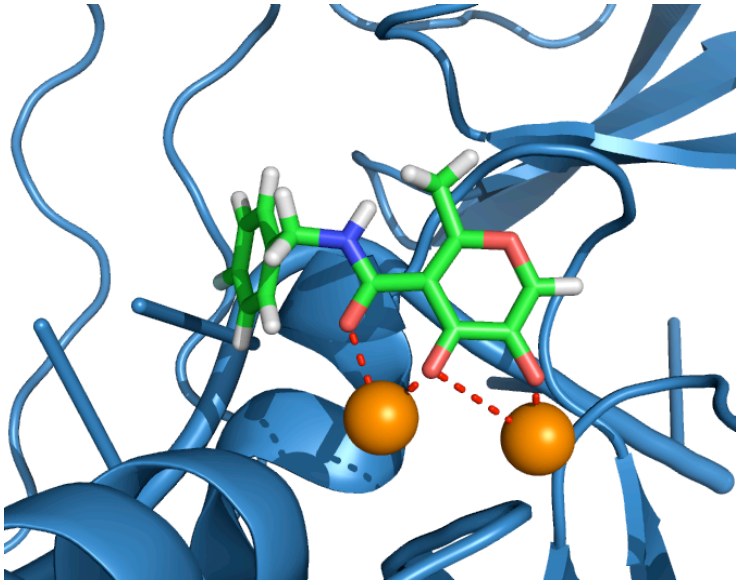


Figure S6. Docked structure of RCD-6 in the active site of PFV-IN (PDB: 3OYA). The inhibitor is shown in sticks (colored by atom), the enzyme as a blue ribbon, and the Mg^{2+} ions as orange spheres.

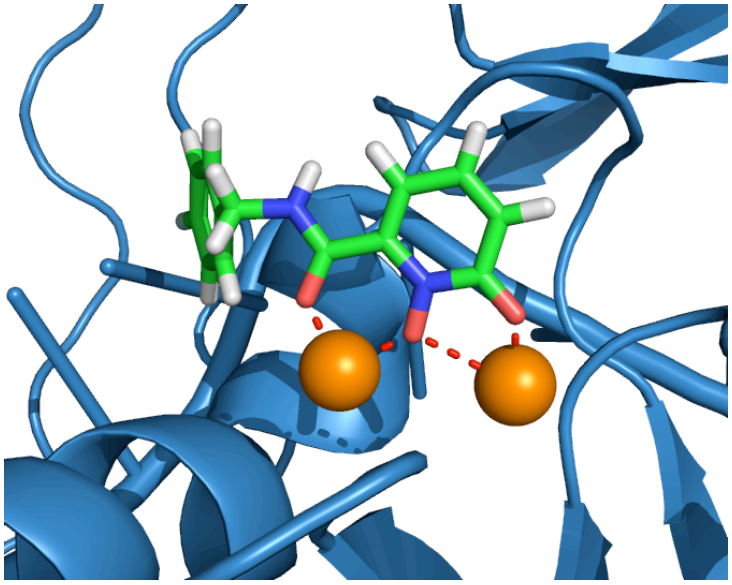


Figure S7. Docked structure of RCD-7 in the active site of PFV-IN (PDB: 3OYA). The inhibitor is shown in sticks (colored by atom), the enzyme as a blue ribbon, and the Mg^{2+} ions as orange spheres.

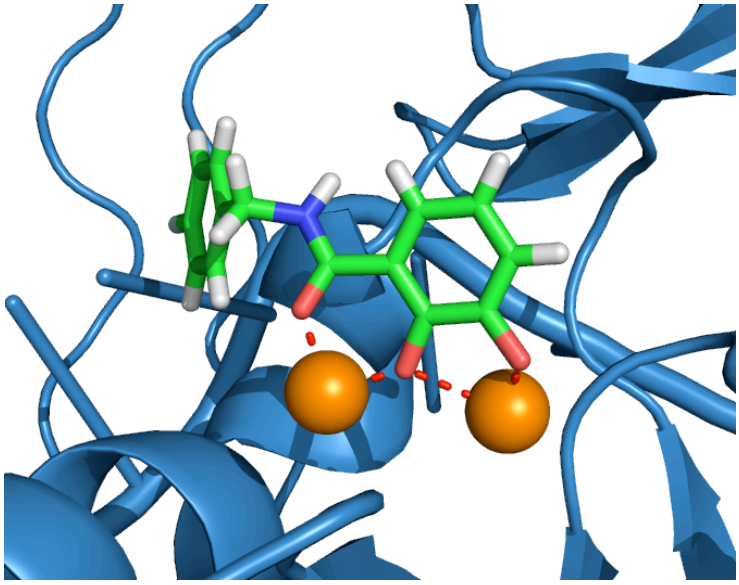


Figure S8. Docked structure of RCD-8 in the active site of PFV-IN (PDB: 3OYA). The inhibitor is shown in sticks (colored by atom), the enzyme as a blue ribbon, and the Mg^{2+} ions as orange spheres.

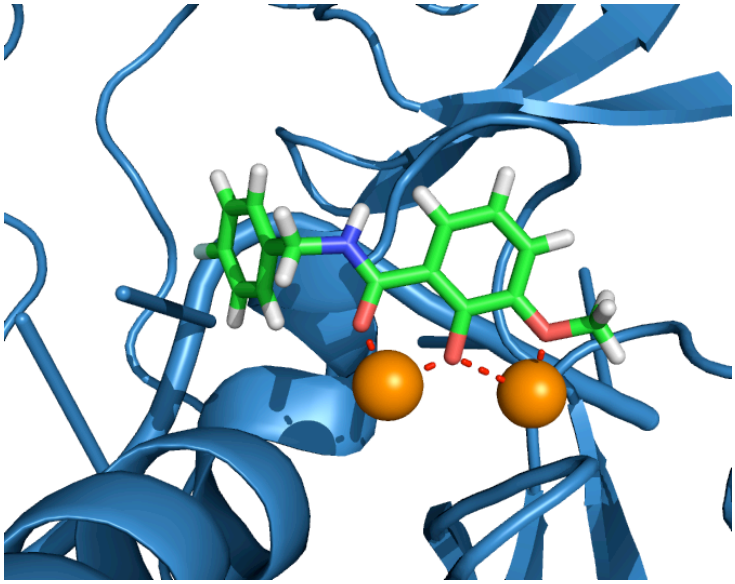


Figure S9. Docked structure of RCD-9 in the active site of PFV-IN (PDB: 3OYA). The inhibitor is shown in sticks (colored by atom), the enzyme as a blue ribbon, and the Mg^{2+} ions as orange spheres.

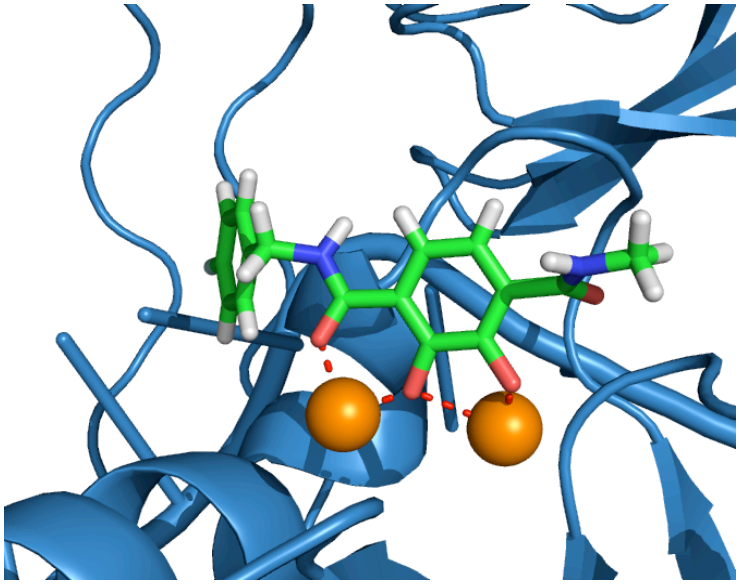


Figure S10. Docked structure of RCD-10 in the active site of PFV-IN (PDB: 3OYA). The inhibitor is shown in sticks (colored by atom), the enzyme as a blue ribbon, and the Mg^{2+} ions as orange spheres.

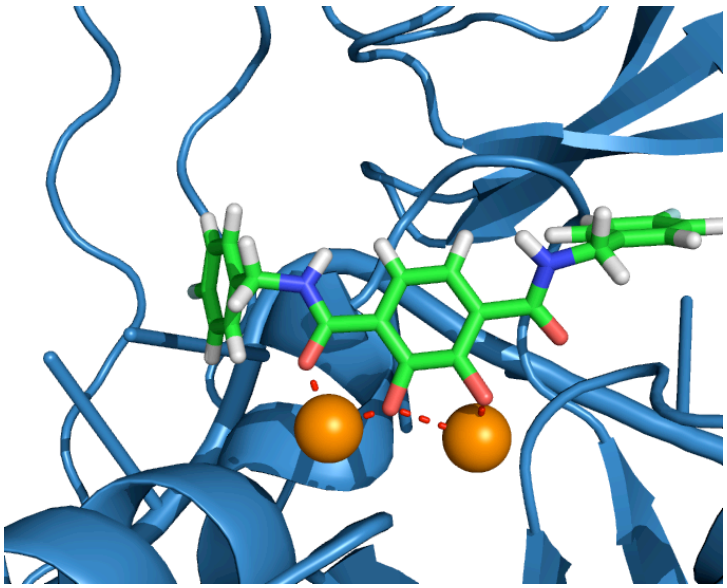


Figure S11. Docked structure of RCD-11 in the active site of PFV-IN (PDB: 3OYA). The inhibitor is shown in sticks (colored by atom), the enzyme as a blue ribbon, and the Mg^{2+} ions as orange spheres.

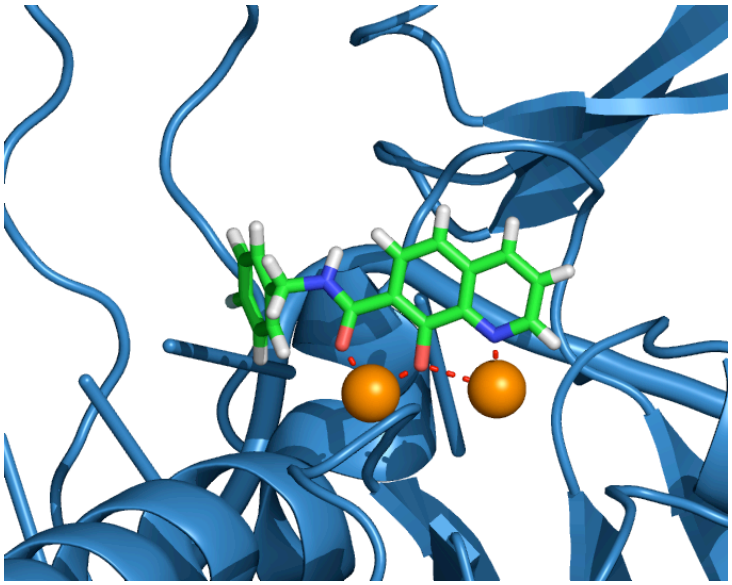


Figure S12. Docked structure of RCD-12 in the active site of PFV-IN (PDB: 3OYA). The inhibitor is shown in sticks (colored by atom), the enzyme as a blue ribbon, and the Mg^{2+} ions as orange spheres.

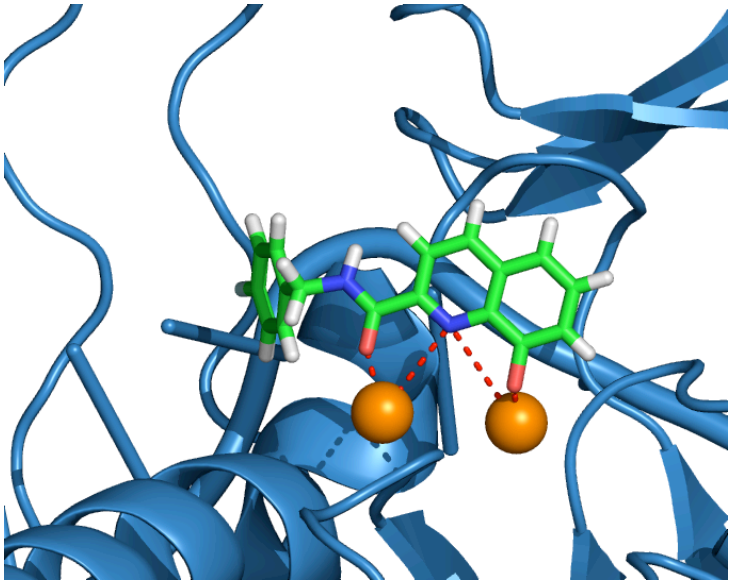


Figure S13. Docked structure of RCD-13 in the active site of PFV-IN (PDB: 3OYA). The inhibitor is shown in sticks (colored by atom), the enzyme as a blue ribbon, and the Mg^{2+} ions as orange spheres.

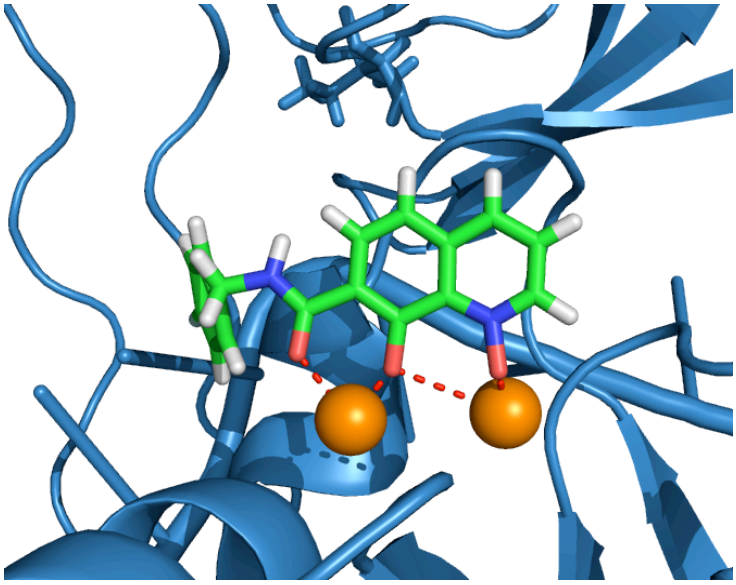


Figure S14. Docked structure of RCD-14 in the active site of PFV-IN (PDB: 3OYA). The inhibitor is shown in sticks (colored by atom), the enzyme as a blue ribbon, and the Mg^{2+} ions as orange spheres.

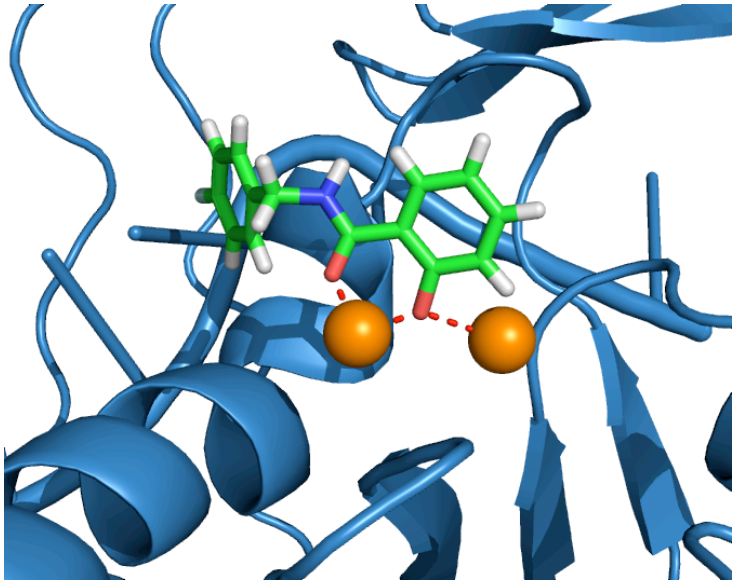


Figure S15. Docked structure of RCD-15 in the active site of PFV-IN (PDB: 3OYA). The inhibitor is shown in sticks (colored by atom), the enzyme as a blue ribbon, and the Mg^{2+} ions as orange spheres.

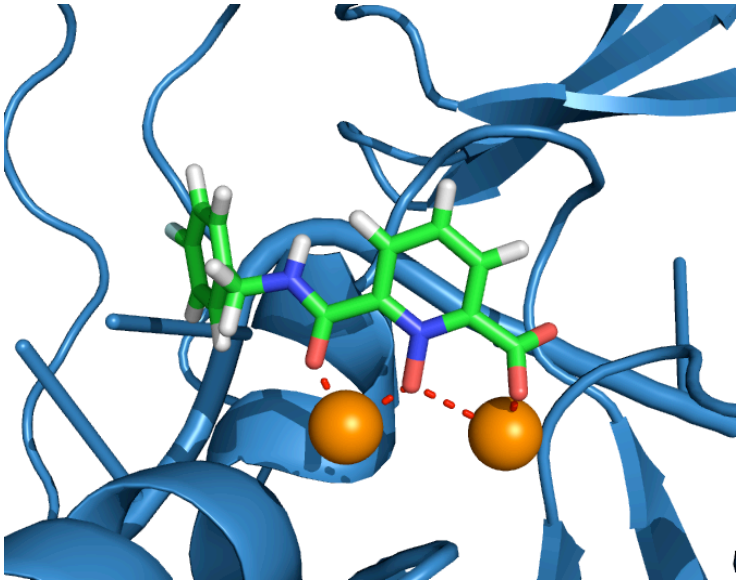


Figure S16. Docked structure of RCD-16 in the active site of PFV-IN (PDB: 3OYA). The inhibitor is shown in sticks (colored by atom), the enzyme as a blue ribbon, and the Mg^{2+} ions as orange spheres.

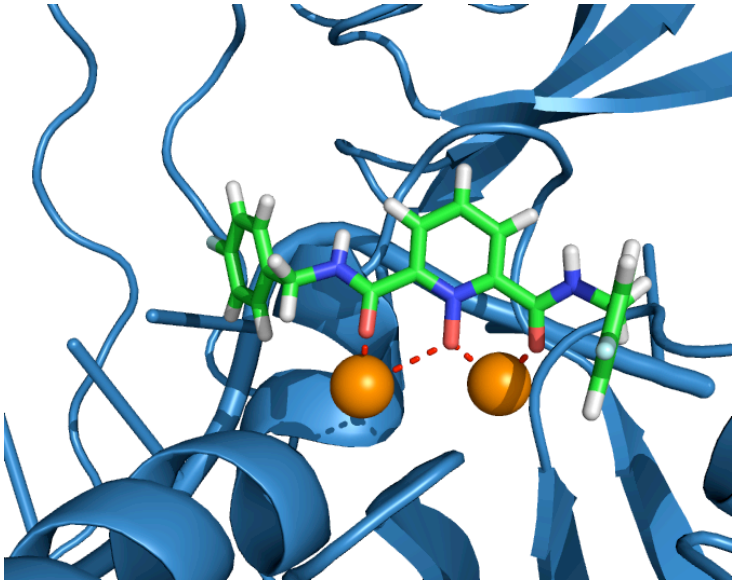


Figure S17. Docked structure of RCD-17 in the active site of PFV-IN (PDB: 3OYA). The inhibitor is shown in sticks (colored by atom), the enzyme as a blue ribbon, and the Mg^{2+} ions as orange spheres.

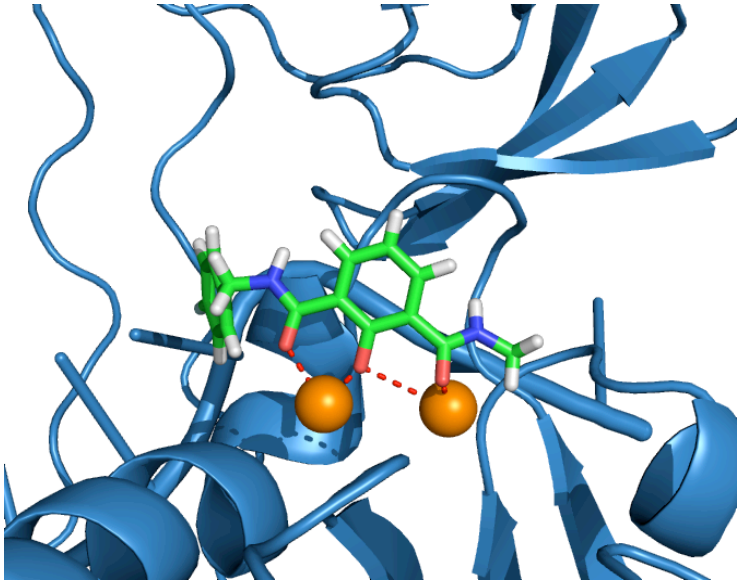


Figure S18. Docked structure of RCD-18 in the active site of PFV-IN (PDB: 3OYA). The inhibitor is shown in sticks (colored by atom), the enzyme as a blue ribbon, and the Mg^{2+} ions as orange spheres.

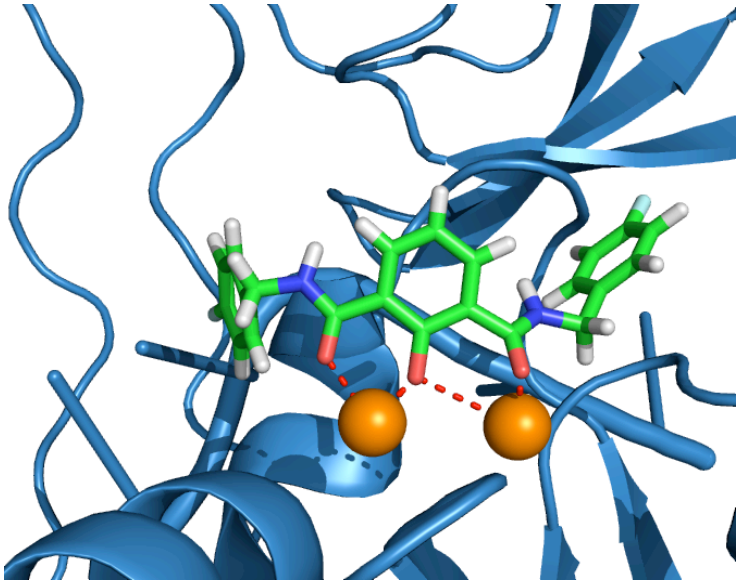


Figure S19. Docked structure of RCD-19 in the active site of PFV-IN (PDB: 3OYA). The inhibitor is shown in sticks (colored by atom), the enzyme as a blue ribbon, and the Mg^{2+} ions as orange spheres.

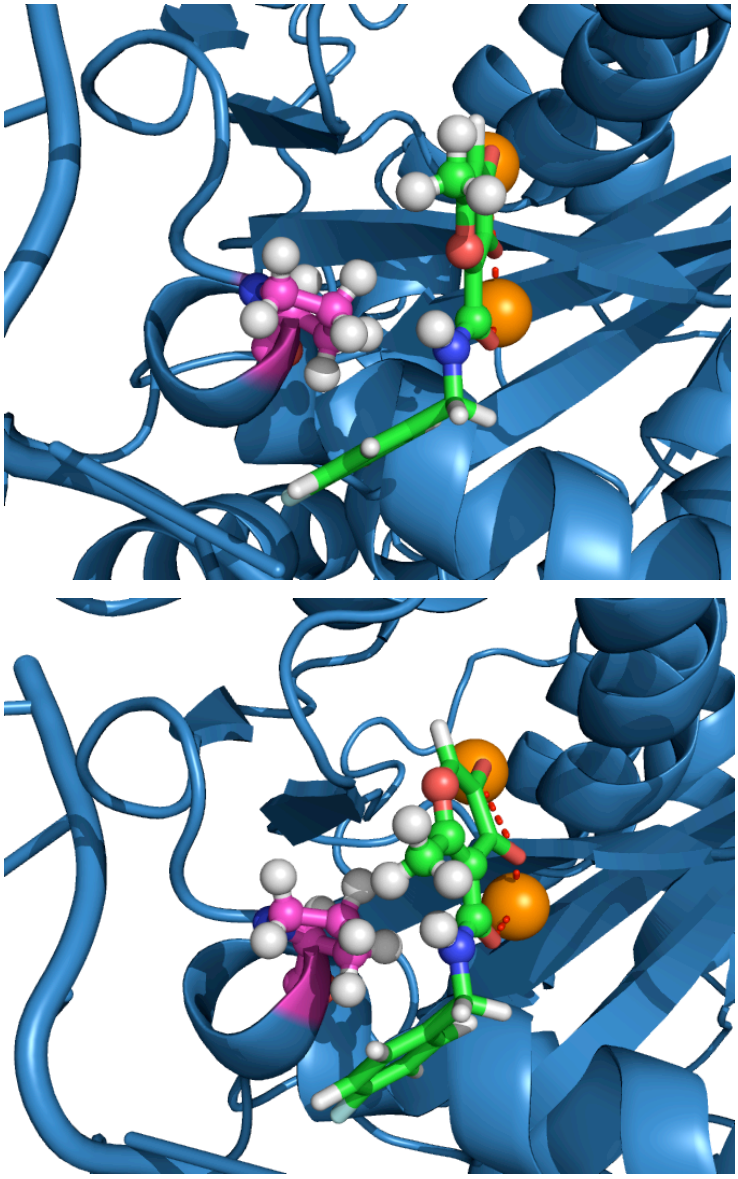


Figure S20. Docked structure of RCD-5 (top) and RCD-6 (bottom) in the active site of PFV-IN (PDB: 3OYA). From this perspective, the steric clash between the inhibitor methyl group in RCD-6 and Pro124 is apparent; no such clash exists for RCD-5. The inhibitor is shown in stick (colored by atom, some atoms shown as balls), the enzyme as a blue ribbon, and the Mg^{2+} ions as orange spheres.

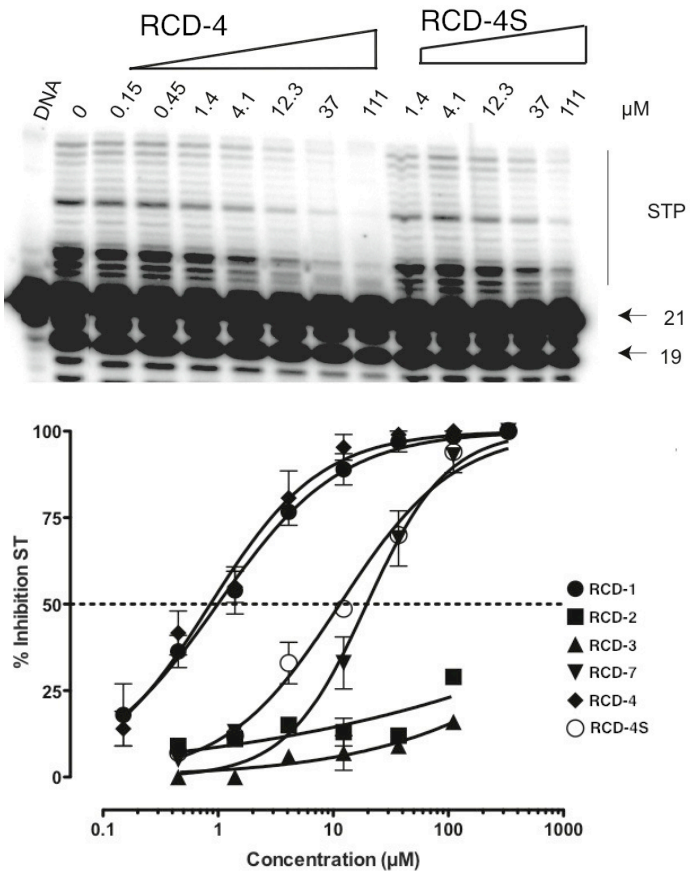


Figure S21. Representative denaturing sequencing gel (top) and titration curves (bottom) for RCD compounds. Strand transfer products (labeled 'STP'), full-length DNA substrate (labeled '21'), and 3'-processed products (labeled '19') are noted on the gel. Strand transfer inhibition shows a clear dependence on the MBG.