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Approaches to the Synthesis of a Novel, anti-HIV Active Integrase Inhibitor

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Abstract

The novel HIV-1 integrase inhibitor **1**, discovered in our laboratory, exhibits potent anti-HIV activity against a diverse set of HIV-1 isolates and also against HIV-2 and SIV. In addition, this compound displays low cellular cytotoxicity and possesses a favorable *in vitro* drug interaction profile with respect to isozymes of cytochrome P450 (CYP) and uridine 5'-diphospho-glucuronosyltransferase (UGT). However, the total synthesis of this significant HIV integrase inhibitor has not been reported. This contribution describes an optimized, reproducible, multi-step, synthetic route to inhibitor **1**. The yield for the separate steps averaged about 80%. The methodologies utilized in the synthesis were, among others, a palladium-catalyzed cross-coupling reaction, a crossed-Claisen condensation, and a hydrazino amide synthesis step. Successful alternative synthetic methodologies for some of the steps are also described.

Introduction

A major issue that complicates the global therapeutic response to HIV/AIDS involves HIV co-infections with both viral and microbial agents.¹⁻³ Some of these co-infections (e.g., with *Mycobacterium tuberculosis*) represent particularly deadly liaisons. HIV co-infections have introduced additional requirements in HIV combination therapeutics and one significant requirement is that of a favorable drug-drug interaction profile,^{4,5} especially those associated with cytochrome P450 (CYP) and uridine 5'-diphospho-glucuronosyltransferase (UGT).⁶⁻⁹ Isozymes and isoforms of CYP and UGT are among the more significant determining factors in the incidence of adverse drug-drug interactions.

We have discovered a novel HIV-1 integrase inhibitor (compound **1**), that exhibits remarkable anti-HIV activity against a diverse set of HIV-1 isolates of major group M, and also against HIV-2 and SIV (mean EC₅₀ 35.0 nM).^{10,11} For key Group M subtypes A, B, C and F, found in a substantial part of the world, the mean EC₅₀ was 18.9 nM.¹¹ The mean selectivity index (SI) was 4,618 and the highest SI was for HIV type B (US), which was almost 14,000. Cytotoxicity data (CC₅₀ 96,200 nM) gave strong evidence that compound **1**

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possessed low toxicity in human cell cultures. The interaction of integrase inhibitor **1** with the HIV-1 intasome is illustrated in Figure 1.

In addition, the compound **1** displays a favorable drug interaction profile with respect to isozymes of CYP and UGT,¹¹ which is of significant potential relevance in HIV combination therapeutics. However, the total synthesis of this compound has not been reported. This contribution describes a concise, reproducible and high-yielding approach to the synthesis of this potent HIV integrase inhibitor.

Results and Discussion

The total synthesis utilized 5-bromo-2-methoxypyridine (**2**) as the starting material. This compound was first treated with *n*-BuLi in anhydrous diethyl ether at -50°C , followed by the addition of 2,6-difluorobenzaldehyde,¹² which afforded the alcohol **3** in 90% yield (Scheme 1). In the next step, deoxygenation of the benzylic hydroxyl group and deprotection of the methoxy group was achieved with trimethylsilyl iodide (generated *in situ* from TMSI and NaI), trifluoroacetic acid (TFA) and triethylsilane (TES)^{12,13} to give the pyridinone **4** in 88% yield. The reagents used in this step were in excess to ensure completion of both alterations in the molecule.

In subsequent steps, precursors **5a** and **5b** were prepared by two routes and the methodology for each route was designed to provide efficient and reproducible access to key intermediate **7** (Scheme 2). The bromo compound, **5a**, was prepared by treatment of **4** with *N*-bromosuccinimide (NBS) in CHCl_3 (77% yield), while the iodo compound, **5b**, was produced in 90% yield when compound **4** was treated with *N*-iodosuccinimide (NIS) under acidic conditions. Introduction of the *o*-fluorobenzyl group at the *N*-H position of **5a** using sodium hydride and 2-fluorobenzyl bromide afforded compound **6** in an overall yield of 83 %.

A number of potential methodologies are available for the introduction of an acetyl group at the 3-position of intermediate **6**. After initial exploratory studies, we settled on two procedures for achieving this transformation (Scheme 2). The first involved the palladium-catalyzed cross-coupling reaction of **6** with 1-ethoxyvinyl(tributyl)stannane,^{13,14} which proceeded smoothly to give, after acidic work-up, the acetylpyridinone **7**. The crude product from this reaction was easily purified by silica gel chromatography to afford pure compound **7** in 83% yield. It should be noted that the optimal equivalent ratio of compound **6** to the organostannane is 1:1.2. Larger excesses of the organostannane resulted in lower efficiency in chromatographic purifications. The iodinated intermediate related to **6** can also be used in this cross-coupling step, but the bromo derivative was preferred because of the ease of purification, in this and the two preceding steps.

An alternative approach to introduction of the acetyl group is also shown in Scheme 2. This approach avoids the use of heavy and toxic metals (palladium and tin), in the synthesis, although there is not a problem for contamination of the final target molecule because of

product purifications, starting with **7** and all subsequent products including the target molecule. In this alternative approach to the palladium-catalyzed cross-coupling, compound **5b** was treated with isopropyl magnesium chloride followed by reaction with N-methoxy-N-methylacetamide¹⁵ in toluene at -10 °C, which converted **5b** to **8** in 68% yield. Benzylation of the latter gave the acetyl compound **7** (82 %). Interestingly, the benzylated derivative of **5b** gave lower and less reproducible yields in the Grignard reaction compared to compound **5b**. Other Grignard approaches that we investigated, though successful, increased the number of steps in the synthesis. Overall, in the three-step conversion of compound **4** to the key intermediate **7**, the palladium-catalyzed cross-coupling approach gave slightly higher yields (53%) compared to the Grignard approach (50%).

In the following step of the synthesis, which was designed to lead to ester **9** (Scheme 3), previously reported approaches, including those from our earlier work,^{16,17} were adapted and further modified for our particular substrate **7**. This crossed-Claisen condensation of **7** was carried out with dimethyl oxalate in the presence of sodium *tert*-butoxide. The latter must be free of sodium hydroxide for higher efficiency in this reaction. The resulting diketo acid methyl ester **9**, after appropriate work-up and purification, was produced in 70% yield. It is very important for the ester to be carefully purified at this stage through triturations with methanol, because impurities in the ester are difficult to remove in the step that follows. This next step was the aqueous acid hydrolysis of the ester **9** in 1,4-dioxane at 95-100 °C for a minimum reaction time (2 h) to produce the diketo acid **10**. The reaction can be followed easily by TLC. This immediate precursor for the target compound was isolated through work-up and the resulting residue was washed with water and purified by trituration (3 times) with a minimum amount of chloroform. Crystalline diketo acid was isolated in a final purified yield of 78%. Highly purified **10** is essential for the final amidation step as even small amounts of impurities are difficult to remove at the target compound stage.

In parallel to the acid-catalyzed hydrolysis step, we also investigated the base-catalyzed hydrolysis of ester **9**. However, the base-catalyzed hydrolysis gave significantly lower yields (<50%), which resulted from decomposition of the starting ester through a retro-Claisen cleavage reaction to regenerate the acetyl compound **7**. Thus, the base-catalyzed hydrolysis reaction is clearly not as efficient as the acid-catalyzed approach.

The final step of the synthesis leading to the hydrazino amide (target compound **1**), required preparation of the reagent, 1-(amino)-2-pyrrolidinone *p*-toluenesulfonate (**11**), which was achieved following a modification of the literature method.^{18,19} For introduction of the amide functionality, compound **10** in DMF was treated with hydroxybenzotriazole (HOBt) followed by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI-HCl).²⁰ The resulting activated intermediate was then treated with **11** followed by sodium bicarbonate and the reaction mixture was stirred between 0-5 °C for 2.5 h. After completion of amide formation, as indicated by TLC, the reaction mixture was quenched with water and worked up as described in the experimental section. Purification involved triturations, first with methanol and then with 1:1 chloroform: pentane, which produced the pure hydrazino amide **1** as yellow crystals in 68% yield. Purity of the final product was established by HPLC, ¹H and ¹³C NMR and quantitative UV data (see experimental and supporting information for HPLC, HRMS and spectral data). Finally, in contrast to the preferred enolic form of its precursor, target molecule **1** exists in the enolic form shown in Scheme 3. This is supported by NMR correlation spectroscopy and a single-crystal X-ray structure determination.²¹

Conclusion

We have developed efficient and reproducible methodologies for the synthesis of our potent anti-HIV active integrase inhibitor **1**. The average yield for the steps to the target compound was about 80%. The order of addition of reagents was also investigated for some of the steps and the optimal order is given in the experimental. Crystallization provided the most efficient approach for purification in almost all of the steps. Key highlights of the total synthesis were the palladium-catalyzed cross-coupling step and the alternative Grignard approach to produce the key intermediate **7**, a crossed-Claisen condensation to elaborate the side chain of **7**, the optimization of the hydrolysis step involving ester **9**, and the final hydrazino amidation step. Target molecule **1** was obtained in exceptionally high purity (>99.6%).

Experimental

All chemicals and solvents used in the synthetic steps and in crystallizations and triturations were purchased from Aldrich and used as received. Commercial solvents for extraction and column chromatography were purchased from EMD chemicals or Fisher Scientific and used as received. The reactions were monitored by thin layer chromatography (TLC) using appropriate developing solvents and pre-coated silica gel plates (UV 254 nm) purchased from Merck and Co. Each synthetic reaction was performed under an inert atmosphere of dry nitrogen unless stated otherwise. ¹H NMR and ¹³C NMR spectra were recorded on a Varian Unity Inova 500 MHz NMR spectrometer. Chemical shifts are reported in δ (ppm) relative to tetramethylsilane (TMS) as internal standard and multiplicities are given as singlet (s), doublet (d), quartet (q), multiplet (m) and broad singlet (br s). High resolution mass spectral data were obtained on a Q-TOF Ion Mobility mass spectrometer. Ultraviolet spectra were recorded on Varian Cary Model 3 spectrophotometer. Melting points were determined on an Electrothermal 9100 melting point apparatus and are uncorrected. Chromatographic separations were carried out using a Biotage® SP1 flash chromatography system with mounted 230-400 mesh silica gel columns. HPLC analyses for establishing purity were performed on an analytical Delta Pak C18 column (15 μ m, 300 mm $\times$ 3.9 mm, 100 Å) using a Beckman Coulter Gold 127 HPLC system with a Gold 166 UV analytical detector. The data were collected and processed using a Gold software package.

(2,6-Difluorophenyl)(6-methoxypyridin-3-yl)methanol (**3**)

To a solution of **2** (15 g, 79.78 mmol) in anhydrous diethyl ether (300 mL) at -50°C under nitrogen, was added dropwise and over 15 min, *n*-BuLi in hexane [2.5 M solution, 38.9 mL (6.1 g), 95.73 mmol]. The resulting yellow slurry was stirred at -50°C for 20 minutes and then followed with dropwise addition of 2,6-difluorobenzaldehyde (12.45 g, 87.60 mmol) while maintaining the temperature at -50°C . The reaction mixture was then stirred for 2 h at -50°C . After the completion of reaction, as indicated by TLC (developed with 30% ethyl acetate: hexane, v/v), the reaction was allowed to warm up to -10°C and then quenched with saturated NH_4Cl (200 mL). The phases were allowed to separate and the aqueous layer was extracted with diethyl ether (2 $\times$ 200 mL). The combined organic extracts were washed with H_2O (200 mL), brine (100 mL) and then dried over anhydrous Na_2SO_4 . The solvent was evaporated *in vacuo* and the crude compound was purified by trituration with 10% ethyl acetate in hexane to afford intermediate **3** as a white solid (18.0 g, 90 % yield); mp $72-74^{\circ}\text{C}$; ¹H NMR (CDCl_3 , 500 MHz): δ 8.14 (s, 1H), 7.70 - 7.68 (dd, $J = 2.5$ Hz, 1.5 Hz, 1H), 7.33-7.27 (m, 1H), 6.96-6.92 (m, 2H), 6.76 (d, $J = 8.5$ Hz, 1H), 6.23 (d, $J = 6.0$ Hz, 1H), 3.94 (s, 3H), 3.00 (d, $J = 5.5$ Hz, 1H); ¹³C NMR (CDCl_3 , 125 MHz): δ 163.7, 161.7, 161.6, 159.7, 159.6, 144.4, 136.8, 130.5, 129.9, 129.8, 129.7, 118.8, 112.1, 112.1, 111.9, 111.9, 110.7, 65.5, 53.5; HRMS: calcd for $\text{C}_{13}\text{H}_{12}\text{F}_2\text{NO}_2$ $[\text{M}+\text{H}]^+$ 252.0836, found 252.0834.

5-(2,6-Difluorobenzyl)pyridin-2(1H)-one (4)

A mixture of **3** (18 g, 71.64 mmol), NaI (53.7 g, 358.2 mmol) and triethylsilane (25 g, 215 mmol) in anhydrous acetonitrile (500 mL) was stirred at room temperature for 10 min. The solution was then cooled to 0 °C and TFA (24.5 g, 15.96 mL, 214.8 mmol) was added dropwise and slowly followed by slow addition of trimethylsilyl chloride (46.7 g, 429.8 mmol). The reaction mixture was allowed to come to room temperature and then heated at 70 °C for 8 h. After the completion of the reaction, as indicated by TLC (CHCl₃: MeOH, 95:5 v/v, developing solvent), the reaction mixture was cooled to room temperature and saturated Na₂SO₃ solution (400 mL) was added until the reaction mixture became colorless. The phases were separated and the aqueous layer was extracted with ethyl acetate (2 × 300 mL). The combined organic phase was washed with water (300 mL), followed by brine (200 mL) and then the organic phase was dried over anhydrous Na₂SO₄. The solvent was evaporated *in vacuo* and the residue was triturated with methanol to afford **4** as a white solid (14.0 g, 88% yield): mp 193-194 °C; ¹H NMR (CDCl₃, 500 MHz): δ 13.17 (br s, 1H), 7.44 - 7.42 (dd, *J* = 2.5 Hz, 3.0 Hz, 1H), 7.22-7.16 (m, 2H), 6.91-6.84 (m, 2H), 6.51 (d, *J* = 9.0 Hz, 1H), 3.75 (s, 2H); ¹³C NMR (CDCl₃, 125 MHz): δ 164.8, 162.2, 162.1, 160.2, 160.1, 143.2, 132.9, 128.6, 128.4, 120.1, 118.0, 115.4, 111.5, 111.4, 111.3, 111.3, 24.0; HRMS: calcd for C₁₂H₁₀F₂NO [M+H]⁺ 222.0730, found 222.0730.

3-Bromo-5-(2,6-difluorobenzyl)pyridin-2(1H)-one (5a)

To a solution of **4** (14 g, 63.29 mmol) in CHCl₃ (300 mL) was added *N*-bromosuccinimide (NBS) (11.5 g, 64.6 mmol) and the reaction mixture was heated under reflux for 2 h. The reaction was cooled to room temperature, washed with water and the organic phase was dried over anhydrous Na₂SO₄ and concentrated *in vacuo*. The resulting brown solid was purified by triturating with methanol to afford product **5a** as a pale yellow solid (14.6 g, 77 % yield): mp 209-211 °C; ¹H NMR (CDCl₃, 500 MHz): δ 13.42 (br s, 1H), 7.83 (d, *J* = 2.5 Hz, 1H), 7.30 (d, *J* = 2.0 Hz, 1H), 7.25-7.19 (m, 1H), 6.93-6.88 (m, 2H), 3.76 (s, 2H); ¹³C NMR (CDCl₃, 125 MHz): δ 162.1, 162.0, 160.6, 160.1, 160.1, 145.0, 132.3, 128.9, 128.8, 128.7, 118.8, 115.5, 114.8, 111.6, 111.6, 111.5, 111.4, 23.8; HRMS: calcd for C₁₂H₉BrF₂NO [M+H]⁺ 299.9836, found 299.9822.

3-Bromo-5-(2,6-difluorobenzyl)-1-(2-fluorobenzyl)pyridin-2(1H)-one (6)

To a solution of **5** (14 g, 46.64 mmol) in anhydrous DMF (300 mL) was added, in several portions at room temperature and under nitrogen, NaH (2.05 g, 51.30 mmol of NaH which was a 60 % suspension in mineral oil). After stirring the reaction mixture for 30 min at room temperature, 2-fluorobenzyl bromide (9.69 g, 51.30 mmol) was added, and stirring was continued for an additional 2 h. After the completion of the reaction, as indicated by TLC (hexane: ethyl acetate, 70:30, v/v, developing solvent), the solvent was removed *in vacuo* and the resulting residue was dissolved in ethyl acetate (400 mL), washed with water (300 mL), brine (200 mL) and then dried over anhydrous Na₂SO₄. The solvent was then removed to afford the crude product which was purified by trituration with 30% ethyl acetate:hexane (v/v) to afford compound **6** as a pale yellow solid (15.8 g, 83% yield): mp 107-109 °C; ¹H NMR (CDCl₃, 500 MHz): δ 7.65 (d, *J* = 2.5 Hz, 1H), 7.52 (t, *J* = 7.5 Hz, 1H), 7.34 (br s, 1H), 7.32-7.27 (m, 1H), 7.24-7.18 (m, 1H), 7.13-7.04 (m, 2H), 6.92-6.87 (m, 2H), 5.15 (s, 2H), 3.71 (s, 2H); ¹³C NMR (CDCl₃, 125 MHz): δ 162.1, 160.1, 158.3, 142.8, 135.4, 132.1, 132.1, 130.3, 130.2, 128.8, 124.6, 124.5, 122.6, 122.5, 117.0, 116.9, 115.5, 115.3, 115.0, 111.6, 111.5, 111.4, 111.4, 48.0, 24.0; HRMS: calcd for C₁₉H₁₄BrF₃NO [M+H]⁺ 408.0211, found 408.0228.

3-Acetyl-5-(2,6-difluorobenzyl)-1-(2-fluorobenzyl)pyridin-2(1H)-one (7)

To a mixture of compound **6** (15.8 g, 38.7 mmol) and bis(triphenylphosphine)palladium(II) chloride (2.71 g, 3.87 mmol) in anhydrous DMF (450 mL) was added 1-ethoxyvinyl(tributyl)stannane (16.79 g, 46.4 mmol). The resulting reaction mixture was stirred at 70 °C under nitrogen for 1h. Once the reaction was complete, as indicated by TLC (CHCl₃: MeOH, 95:5 v/v, developing solvent), the solvent was removed *in vacuo*. The resulting residue was dissolved in ethyl acetate (400 mL), filtered through a pad of celite and the filtrate was stirred with 1N HCl (200 mL) for 15 min. The organic phase was separated and washed with water (2 × 200 mL), brine (200 mL) and then dried over anhydrous Na₂SO₄. The crude product was purified by silica gel column chromatography using 30% ethylacetate: hexane (v/v) as eluent to afford **7** as a white solid (12.0 g, 83% yield): mp 121-123 °C; ¹H NMR (CDCl₃, 500 MHz): δ 8.04 (d, *J* = 3.0 Hz, 1H), 7.56 (s, 1H), 7.43 (t, *J* = 7.5 Hz, 1H), 7.32-7.30 (m, 1H), 7.22-7.19 (m, 1H), 7.15-7.06 (m, 2H), 6.90-6.87 (m, 2H), 5.16 (s, 2H), 3.76 (s, 2H), 2.65 (s, 3H); ¹³C NMR (CDCl₃, 125 MHz): δ 197.7, 162.2, 162.1, 162.1, 160.4, 160.2, 160.2, 160.1, 144.4, 141.2, 131.4, 131.4, 130.3, 130.2, 128.7, 128.6, 127.8, 124.6, 124.6, 122.6, 122.5, 116.5, 115.6, 115.5, 115.0, 111.6, 111.5, 111.4, 111.4, 47.2, 30.9, 24.2; HRMS: calcd for C₂₁H₁₇F₃NO₂ [M+H]⁺ 372.1211, found 372.1211.

5-(2,6-Difluorobenzyl)-3-iodopyridin-2(1H)-one (5b)

To a solution of 5-(2,6-difluorobenzyl)pyridin-2(1H)-one, **4** (1.71 g, 7.73 mmol), in glacial AcOH (32 mL), TFA (2 mL) was added *N*-iodosuccinimide (1.74 g, 7.73 mmol) and the resulting red homogenous solution was stirred at room temperature for 16 h. The reaction mixture was poured onto crushed ice, neutralized by dropwise addition of conc. NH₄OH and the resulting solution extracted with CHCl₃ (3 × 50 mL). The combined organic extract was washed with brine (100 mL) and then dried over Na₂SO₄ and concentrated. The product was triturated with Et₂O to obtain **5b** as a pale yellow solid (2.40 g, 90% yield): mp 207-208 °C; ¹H NMR (CDCl₃, 500 MHz): δ 13.20 (br s, 1H), 8.05 (d, *J* = 3.0 Hz, 1H), 7.32 (unresolved dd, 1H), 7.24-7.21 (m, 1H), 6.90 (unresolved dd, 2H), 3.78 (s, 2H); ¹³C NMR (CDCl₃, 125 MHz): δ 162.3, 162.3, 162.2, 160.3, 160.3, 152.1, 133.9, 128.9, 119.7, 115.2, 115.0, 114.9, 111.8, 111.8, 111.7, 111.6, 91.4, 23.8, 23.8, 23.8; HRMS: calcd for C₁₂H₉F₂INO [M+H]⁺ 347.9697, found 347.9695.

3-Acetyl-5-(2,6-difluorobenzyl)pyridin-2(1H)-one (8)

Compound **5b** (2.80 g, 8.06 mmol) was placed in dry 500 mL round-bottom flask with a magnetic stirring bar and septum and dried under vacuum prior to the beginning of the reaction, which was conducted under dry nitrogen. Anhydrous toluene (250 mL) was added, the suspension cooled to -10 °C and treated dropwise with ⁱPrMgCl·LiCl (1.3 M in THF, 24.8 mL, 32.24 mmol) over a period of 20 min. The resulting clear solution was stirred at room temperature for 1 h during which turbidity gradually appeared. The reaction mixture was cooled to -10 °C and a solution of *N*-methyl-*N*-methoxyacetamide (1.65 g, 16.11 mmol) in anhydrous toluene (10 mL) was added dropwise over 30 min. The reaction mixture was stirred at room temperature for 4 h, quenched by addition of saturated NH₄Cl (100 mL) and then transferred to a separating funnel containing EtOAc (200 mL). After extraction, the organic layer was separated, washed with 1N HCl (100 mL), water (2 × 100 mL), brine (100 mL) and then dried over Na₂SO₄ and concentrated. The crude product was purified by column chromatography using 20% acetone in chloroform (v/v) as the eluent to get **8** as a white solid (1.44 g, 68% yield): mp 169-170 °C; ¹H NMR (CDCl₃, 500 MHz): δ 12.93 (br s, 1H), 8.16 (d, *J* = 2.5 Hz, 1H), 7.53 (unresolved dd, 1H), 7.25-7.19 (m, 1H), 6.90 (unresolved dd, 2H), 3.84 (s, 2H), 2.68 (s, 3H); ¹³C NMR (DMSO-*d*₆, 125 MHz): δ 197.1, 162.1, 162.0, 160.9, 160.1, 160.0, 144.6, 140.0, 140.0, 129.8, 129.8, 129.7, 129.6, 127.1, 116.0, 115.8,

115.7, 112.4, 112.3, 112.3, 112.2, 112.1, 112.0, 31.0, 30.9, 23.5, 23.5, 23.4. HRMS: calcd for $C_{14}H_{12}F_2NO_2$ $[M+H]^+$ 264.0836, found 264.0823.

3-Acetyl-5-(2,6-difluorobenzyl)-1-(2-fluorobenzyl)pyridin-2(1H)-one (7)

To a stirred cold solution of **8** (1.90 g, 7.22 mmol), anhydrous K_2CO_3 (0.997 g, 7.22 mmol) in anhydrous DMF (50 mL) was added dropwise, 2-fluorobenzylbromide (1.36 g, 7.22 mmol). The reaction mixture was stirred at room temperature under argon for 3 h and then quenched by addition of water (100 mL) and extracted with EtOAc (3×100 mL). The combined organic extract was washed with water (100 mL), brine (100 mL) and dried (Na_2SO_4) and concentrated. The crude product was triturated with hexane (2×500 mL) followed by 10% ethyl acetate in hexane (50 mL) and the product filtered and dried under vacuum to give **7** as an off-white solid (2.20 g, 82% yield). The data for this compound matched with that obtained for compound **7** prepared through the palladium-catalyzed cross-coupling route shown above.

Methyl 4-(5-(2,6-difluorobenzyl)-1-(2-fluorobenzyl)-2-oxo-1,2-dihydropyridin-3-yl)-2-hydroxy-4-oxobut-2-enoate (9)

To a stirred solution of sodium *tert*-butoxide (8.69 g, 87.77 mmol) in anhydrous THF (400 mL) was added a solution of dimethyl oxalate (10.47 g, 87.77 mmol) in anhydrous THF (80 mL) under a nitrogen atmosphere and the yellow turbid reaction mixture was stirred for 30 min. A solution of 3-acetyl-5-(2,6-difluorobenzyl)-1-(2-fluorobenzyl) pyridin-2(1H)-one **7** (8.15 g, 21.94 mmol) in anhydrous THF (80 mL) was then added rapidly and the resulting red reaction mixture was stirred for 3 h at room temperature. After completion of reaction as indicated by TLC ($CHCl_3$: MeOH, 95:5, v/v, developing solvent), the reaction mixture was cooled to 0-5 °C in an ice bath and 1N HCl (100 mL) was added in one portion. The resulting product was extracted with ethyl acetate (2×300 mL) and the combined organic phase was washed with water (2×200 mL) and brine (300 mL) and then dried over anhydrous Na_2SO_4 . The solvent was removed and the resulting solid was triturated with methanol to afford **9** as a yellow solid (7.02 g, 70 % yield): mp 142-144 °C; 1H NMR ($CDCl_3$, 500 MHz): δ 15.21 (br s, 1H), 8.25 (d, $J = 1.5$ Hz, 1H), 7.87 (s, 1H), 7.61 (s, 1H), 7.46 (t, $J = 7.5$ Hz, 1H), 7.34-7.29 (m, 1H), 7.23-7.19 (m, 1H), 7.15-7.06 (m, 2H), 6.92-6.89 (m, 2H), 5.19 (s, 2H), 3.89 (s, 3H), 3.80 (s, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 185.4, 172.3, 162.7, 162.1, 160.1, 159.4, 144.7, 141.9, 131.6, 131.6, 130.5, 130.4, 128.9, 128.9, 128.8, 124.7, 124.7, 123.5, 122.4, 122.3, 116.9, 115.6, 115.5, 115.1, 115.0, 111.6, 111.7, 111.5, 111.5, 101.8, 53.0, 53.0, 47.4, 47.3, 24.3, 24.3, 24.3; HRMS: calcd for $C_{24}H_{19}F_3NO_5$ $[M+H]^+$ 458.1215, found 458.1235.

4-(5-(2,6-Difluorobenzyl)-1-(2-fluorobenzyl)-2-oxo-1,2-dihydropyridin-3-yl)-2-hydroxy-4-oxobut-2-enoic acid (10)

To a stirred solution of **9** (7.4 g, 16.2 mmol) in 1,4-dioxane (110 mL) was added 1N HCl (100 mL) and the reaction mixture was heated between 95- 100 °C for 2 h. After completion of the reaction as monitored by TLC ($CHCl_3$: MeOH, 95:5, v/v, developing solvent), the hot reaction mixture was filtered quickly through filter paper. The solvent was evaporated and distilled H_2O (200 mL) was added to the residue. The resulting precipitated yellow product was filtered, washed with copious amounts of water and dried under vacuum. This solid was purified by trituration ($3 \times$) with a minimum amount of chloroform to afford **10** as a yellow solid (5.6 g, 78 % yield): mp 189-190 °C; UV (methanol) λ 391 nm (ϵ 13,491), 320 nm (ϵ 5,671); 1H NMR ($DMSO-d_6$, 500 MHz): δ 15.09 (br s, 1H), 14.04 (br s, 1H), 8.20 (d, $J = 8.0$ Hz, 2H), 7.78 (s, 1H), 7.42-7.37 (m, 2H), 7.26-7.14 (m, 5H), 5.26 (s, 2H), 3.90 (s, 2H); ^{13}C NMR ($DMSO-d_6$, 125 MHz): δ 184.9, 173.8, 163.7, 162.1, 162.0, 161.7, 160.1, 160.1, 159.7, 159.0, 144.7, 144.5, 130.5, 130.5, 130.4, 130.3, 129.9, 125.0, 125.0, 123.6, 123.5,

122.1, 116.6, 115.9, 115.8, 115.4, 112.3, 112.3, 112.2, 112.18, 101.4, 47.9, 23.8; HRMS: calcd for $C_{23}H_{17}F_3NO_5$ $[M+H]^+$ 444.1059, found 444.1107.

4-(5-(2,6-Difluorobenzyl)-1-(2-fluorobenzyl)-2-oxo-1,2-dihydropyridin-3-yl)-4-hydroxy-2-oxo-N-(2-oxopyrrolidin-1-yl)but-3-enamide (1)

To a solution of **10** (13.0 g, 29.32 mmol) in DMF (120 mL) was added 1-hydroxybenzotriazole hydrate (HOBt, 4.35 g, 32.25 mmol), followed by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI.HCl, 6.16 g, 32.25 mmol) at 0 °C. The resulting mixture was stirred at 0 °C for 30 min. and 1-(amino)-2-pyrrolidinone *p*-toluenesulfonate, **11** (9.58 g, 35.18 mmol) was added followed by the addition of $NaHCO_3$ (2.95 g, 35.18 mmol). The reaction mixture was stirred for 2.5 h between 0-5 °C. After completion of the reaction, as indicated by TLC (5% methanol in chloroform, v/v, developing solvent), the reaction was quenched with water (200 mL). The product was extracted with ethylacetate (2 × 100 mL). Combined organic layer was washed successively with 10% aq. $NaHCO_3$ (75mL), 10% aq. HCl (75 mL) followed by brine solution (75 mL). Organic layer was dried over sodium sulfate and concentrated under reduced pressure to give crude compound which was purified by trituration with methanol followed by further trituration with chloroform:pentane (1:1 v/v) to afford compound **1** as yellow crystals (10.5 g, 68 % yield): mp 175-176 °C; UV (methanol) λ 401 nm (ϵ 9,139), 318 nm (ϵ 6,225); 1H NMR ($CDCl_3$, 500 MHz): δ 15.24 (br s, 1H), 8.78 (s, 1H), 8.21 (d, J = 2.0 Hz 1H), 7.98 (s, 1H), 7.61 (br s, 1H), 7.51 (t, J = 7.0 Hz, 1H), 7.32-7.28 (m, 1H), 7.25-7.19 (m, 1H), 7.14-7.05 (m, 2H), 6.93-6.88 (m, 2H), 5.17 (s, 2H), 3.79 (s, 2H), 3.69-3.64 (m, 2H), 2.46 (t, J = 8.0 Hz, 2H), 2.18-2.12 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 179.3, 173.4, 162.2, 162.1, 162.1, 160.2, 160.2, 160.1, 159.5, 159.0, 144.0, 141.7, 141.6, 132.1, 132.0, 130.4, 130.4, 128.8, 124.8, 124.7, 122.5, 122.4, 122.3, 116.6, 115.6, 115.4, 115.0, 111.7, 111.6, 111.5, 111.4, 98.5, 47.8, 47.4, 47.4, 28.4, 24.3, 16.8; HRMS: calcd for $C_{27}H_{23}F_3N_3O_5$ $[M+H]^+$ 526.1590, found 526.1589. Purity: 99.61% by HPLC (see "Supplementary Information").

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

A molecular modeling picture of the HIV intasome (pdb code 3OYA) depicting the interaction of compound **1** (green) with both the viral enzyme [α -helix (red), β -sheets (blue) and connecting strands (yellow)] and viral DNA (orange-red, light blue and gray atom colors). Compound **1** interacts through chelation with two magnesium ions (purple spheres) in the integrase active site. Other interactions involve integrase amino acid residues Tyr 143, Pro 145 and Asp 116. There is also a pi stacking interaction of one phenyl ring of compound **1** with the cytosine ring of deoxycytidine 16 of viral DNA. All of these interactions within the active-site pocket combine to block the ability of the HIV intasome to incorporate viral DNA into human DNA.

Scheme 1.
Synthesis of intermediate **5a** and **5b**

Scheme 2. Two possible synthetic pathways to key intermediate **7**

Scheme 3.
Conversion of intermediate **7** to target compound **1**