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Structure-Guided Optimization of HIV Integrase Strand Transfer Inhibitors

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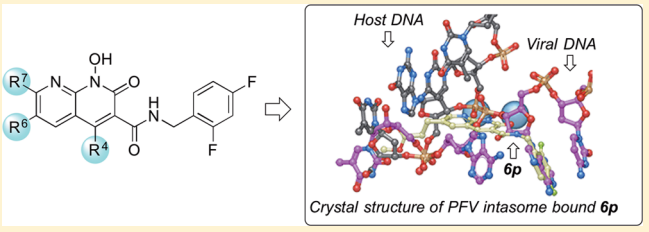
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Supporting Information

ABSTRACT: Integrase mutations can reduce the effectiveness of the first-generation FDA-approved integrase strand transfer inhibitors (INSTIs), raltegravir (RAL) and elvitegravir (EVG). The second-generation agent, dolutegravir (DTG), has enjoyed considerable clinical success; however, resistance-causing mutations that diminish the efficacy of DTG have appeared. Our current findings support and extend the substrate envelope concept that broadly effective INSTIs can be designed by filling the envelope defined by the DNA substrates. Previously, we explored 1-hydroxy-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamides as an INSTI scaffold, making a limited set of derivatives, and concluded that broadly effective INSTIs can be developed using this scaffold. Herein, we report an extended investigation of 6-substituents as well the first examples of 7-substituted analogues of this scaffold. While 7-substituents are not well-tolerated, we have identified novel substituents at the 6-position that are highly effective, with the best compound (**6p**) retaining better efficacy against a broad panel of known INSTI resistant mutants than any analogues we have previously described.



INTRODUCTION

HIV-1 integrase (IN) plays a key role in the viral life cycle, inserting the double-stranded DNA that is generated by reverse transcription of the viral RNA genome into the genome of the host cell.[1] Integration is essential for viral replication, and for this reason, IN is a therapeutic target for the treatment of HIV infections. To date, three HIV IN antagonists have been approved for clinical use: raltegravir (RAL, **1**), elvitegravir (EVG, **2**), and dolutegravir (DTG, **3**) (Figure 1).[2−4] These drugs belong to a class of compounds called integrase strand transfer inhibitors (INSTIs) because they inhibit DNA strand transfer (ST), the second step of integration catalyzed by IN, rather than the first step, the 3′-processing reaction (**3′-P**).[5−8] Development of drug resistance mutations is a common problem in antiviral therapy and, not surprisingly, mutations affecting the susceptibility of the virus to RAL and EVG have rapidly emerged.[9−11] However, the second-generation inhibitor, DTG, retains potency against some but not all RAL/EVG resistant HIV variants.[12−16] Therefore, the development of new small molecules that have minimal toxicity and improved efficacy against the existing resistant mutants remains an important research objective.[17]

Retroviral integration is mediated by IN multimers that are assembled on the viral DNA ends, forming a stable synaptic complex, also referred to as the intasome.[18−21] The INSTIs only bind to the active site of IN when the processed viral DNA ends are appropriately bound to the intasome.[8,22] The way in which INSTIs bind to the intasome was elucidated by solving crystal structures of the orthologous retroviral IN from the prototype foamy virus (PFV).[19,23,24] The INSTIs are "interfacial" inhibitors; they bind to the active site of IN and interact with the bound viral DNA following the 3′-processing step.[8,19,25] Essential structural features that contribute to the binding of INSTIs include an array of three heteroatoms (highlighted in red, Figure 1) that chelate the two catalytic Mg^{2+} ions in the IN active site and a halobenzyl side chain (halophenyl portion highlighted in blue, Figure 1) that stacks with the penultimate nucleotide (a deoxycytidine) at the 3′ end of the viral DNA.[8,19] We have recently shown that the 1-hydroxy-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide motif (**4**) can serve a useful platform for developing HIV-1 IN

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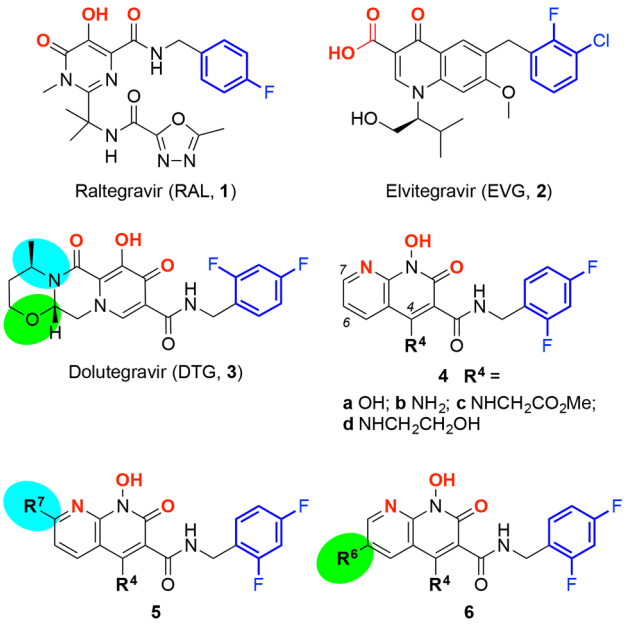
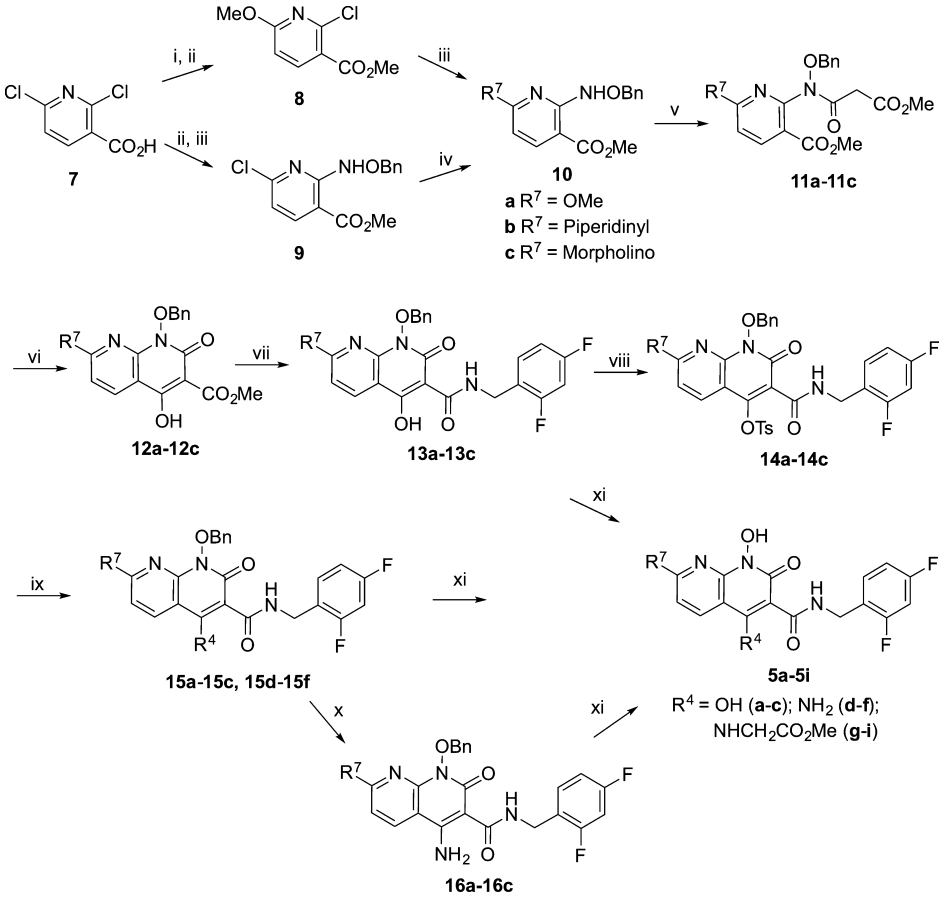


Figure 1. HIV-1 integrase inhibitors. Colored areas indicate regions of intended correspondence.

inhibitors that retain high efficacy against the RAL/EVG-resistant mutants.[26,27] We initially examined the properties of a series of analogues related to structure **4** by varying the substituents at the 4-position. Our objective was to identify compounds that retain efficacy against the mutations Y143R, N155H, and Q148H/G140S, which have been associated with clinical resistance to RAL,[27] and some of these mutations also play a role in the development of resistance against DTG.[28] This approach yielded compounds including **4a−d**, which are approximately equivalent to RAL in their potency against recombinant wild-type (WT) HIV-1 IN in biochemical assays. However, the small molecules also showed improved antiviral efficacies against the Y143R and N155H mutants in cell-based assays.[26,27] Although antiviral efficacies against the Q148H/G140S double mutant were also improved relative to RAL, the new compounds were inferior to DTG, prompting us to continue our developmental efforts.

Structural studies using the PFV intasome have revealed that the tricyclic system of DTG is sufficiently extended to make contacts with G187 in the $\beta4-\alpha2$ loop of PFV IN (G118 in IN).[23] It has been argued that the interactions with this region may contribute to the improved properties of DTG and other second-generation INSTIs.[4,23,29,30] Therefore, we considered that adding functionality to either the 6- or 7-positions of **4** could interact with the same region of the catalytic site (highlighted in green and cyan, respectively, in the structures of

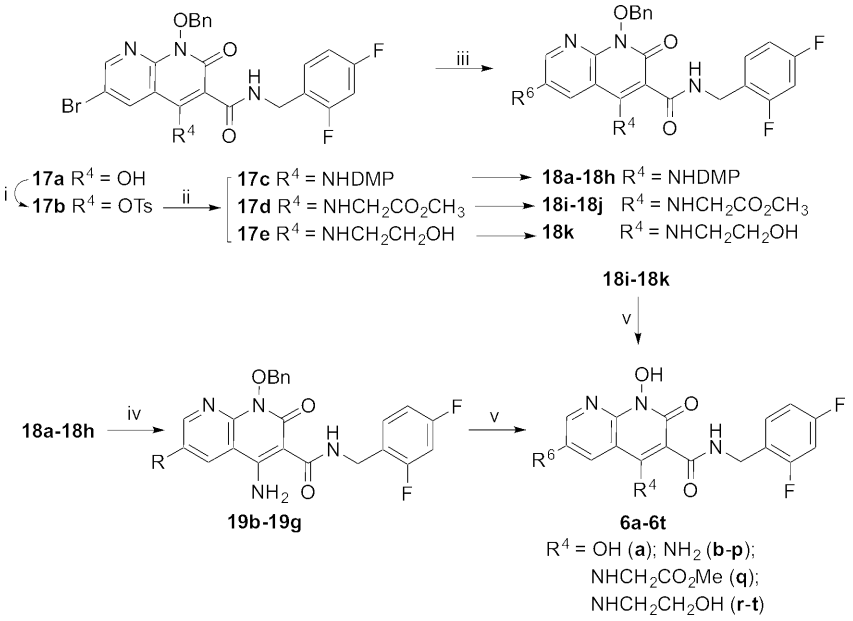
Scheme 1. Synthesis of 4,7-Bis-Substituted Analogues 5a−5i[a]



[a]Reagents and conditions: (i) KO^{t}Bu, MeOH, 65 °C; (ii) H_2SO_4, MeOH; (iii) $BnONH_2$, DIEA, dioxane, 110 °C; (iv) (b) piperidine or (c) morpholine, DMF, 80 °C; (v) $ClCOCH_2CO_2CH_3$, TEA, DCM; (vi) NaOMe, MeOH; (vii) 2,4-diF-$BnNH_2$, DMF, 140 °C; (viii) TsCl, DIEA, MeCN; (ix) RNH_2, DIEA, DMF, 50 °C, R = 2,4-diMeOBn (a) or CH_2CO_2Me (b); (x) TFA, DCM; (xi) H_2, 10% Pd/C, MeOH.

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Scheme 2. Synthesis of 4,6-Bis-Substituted Analogues 6a−6t[a]



[a]Reagents and conditions: (i) TsCl, Et_3N, CH_3CN; (ii) RNH_2, DIEA, DMF, 50 °C, R = 2,4-diMeOBn (DMP) (a), CH_2CH_2OH (b), or CH_2CO_2Me (c); (iii) alkyne, $Pd(PPh_3)_2Cl_2$, DIEA, CuI, DMF, 70 °C or alkene, $Pd(OAc)_2$, TEA, PPh_3, DMF, 100 °C; (iv) TFA, DCM; (v) H_2, 10% Pd/C, MeOH.

DTG and **4**, Figure 1). In a preliminary work, we modified the 6-position of **4** and showed that adding linear side chains bearing terminal hydroxyl groups can improve antiviral efficacies against the Q148H/G140S double mutant to levels approaching that of DTG.[31] Furthermore, depending on the 6-substituent, compounds could retain essentially all of their antiviral potency against a more extensive panel of HIV-1-based vectors that carry the major DTG-resistant IN mutants, including the G118R, T66I, E92Q, R263K, and H51Y single mutants and the H51Y/R263K double mutant.[17,32−34] These data have two important implications: First, 6-substituents can have an important role in maintaining antiviral efficacy against resistant mutant forms of IN. Second, compounds that are broadly effective against mutant forms of IN bind in ways that involve substrate mimicry and, when bound, fit within the "substrate envelope".[31,35] However, the data were confined to a small number of 6-modificaitons that had limited chemical diversity. Given the potential promise of 6-substituents, we felt that it was important to more thoroughly examine this position and, accordingly, we describe here an extensive set of 6-modifications (designated as the **6** series analogues, Figure 1). We were also interested in examining substituents at the 7-position of **4** because these could potentially afford an alternate way to engage G118 in the $\beta4-\alpha2$ loop of IN. We present the first examination of compounds that have modifications at the 7-position (designated as the **5** series analogues). To provide a more complete SAR study, we have also introduced various functionalities at the 4-position in combination with various 6- and 7-substitutents. These efforts have resulted in one of the most potent and effective compounds that we have developed to date. This work sheds additional light on enzyme−inhibitor interactions that can contribute to retention of efficacy against strains of virus that contain mutant forms of IN resistant to the approved INSTIs.

RESULTS AND DISCUSSION

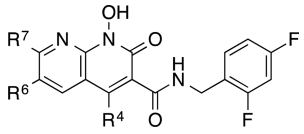
Chemistry. To prepare our 7-substituted analogues **5a−i**, we employed 2,6-dichloronicotinic acid (**7**) as the starting material. The 6-chloro group of **7** was converted into the final 7-substituent in two ways. In one route, the 6-chloro moiety was displaced with methoxyl (KO^tBu in MeOH),[36] and this was followed by esterification of the 3-carboxy group to yield **8** (Scheme 1). In the second route, the 3-carboxyl group of **7** was first converted directly to the 3-methyl ester. In both cases, the 2-chloro group was then displaced with $BnONH_2$ to give the corresponding 2-benzyloxyamine-containing derivatives (**10a** and **9**, respectively). The 6-chloro-substituents of the second series **9** were transformed into the corresponding 6-piperidinyl and 6-morpholino derivatives (**10b** and **10c**, respectively) by heating with DMF solutions of piperidine or morpholine. Using a procedure similar to those we previously reported (Scheme 1),[26,27,31] intermediates **10a−c** were transformed to the 4-hydroxy (**5a−c**), 4-amino (**5d−f**), and 4-[2-(methyl glycinate)] (**5g−i**) final products.

We transformed the 6-bromo analogues (**17a−c**, Scheme 2) into a variety of 6-substituted INSTIs by coupling alkynes and alkenes using Sonogashira and Heck coupling chemistries.[31] In addition to the 6-substituted products with a 4-hydroxyl (**6a**), we prepared analogues having a free amine at the 4-position (**6b−p**) as well as analogues with 4-[2-(methyl glycinate)]-amine (**6q**) and 4-(2-hydroxyethyl)amine (**6r−t**) (Scheme 2).

Evaluation of New Derivatives Using in Vitro IN Catalytic Assays and Single-Round Virus Replication Assays. We designed compounds **5a−i** to examine the effects of substituents at the 4-position (hydroxyl, amine, or methyl glycinate) in combination with modifications of the 7-position (methoxyl, piperidinyl, or morpholino). We evaluated the compounds by in vitro IN activity assays that utilize ^{32}P-radiolabeled oligonucleotides (Table 1).[37] We also tested the potency of the compounds in single-round replication assays that employ HIV-1 vectors carrying WT IN. We evaluated the

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Table 1. Inhibitory Potencies of Compounds Using an in Vitro IN Assay[a]



no.	R^4	R^6	R^7	in vitro (IC_{50} μM) 3′-P	in vitro (IC_{50} μM) ST
5′a[b]	OH	H	H	1.2 ± 0.2	0.055 ± 0.008
5a	OH	H	OCH_3	32 ± 4	0.033 ± 0.005
5b	OH	H	piperidinyl	>333	70 ± 16
5c	OH	H	morpholino	>333	1.9 ± 0.2
5′d[c]	NH_2	H	H	2.5 ± 0.3	0.019 ± 0.002
5d	NH_2	H	OCH_3	18 ± 2	0.011 ± 0.002
5e	NH_2	H	piperidinyl	>333	54 ± 18
5f	NH_2	H	morpholino	>333	54 ± 10
5′g[c]	$NHCH_2CO_2CH_3$	H	H	0.71 ± 0.10	0.021 ± 0.011
5g	$NHCH_2CO_2CH_3$	H	OCH_3	20 ± 2	0.042 ± 0.005
5h	$NHCH_2CO_2CH_3$	H	piperidinyl	>333	96 ± 40
5i	$NHCH_2CO_2CH_3$	H	morpholino	>333	6.2 ± 0.8
6a	OH	$(CH_2)_5OH$	H	14.3 ± 1.1	0.012 ± 0.003
6′b[d]	NH_2	$(CH_2)_5OH$	H	8.2 ± 1.2	0.0027 ± 0.0004
6b	NH_2	$(CH_2)_3OAc$	H	14.3 ± 2.1	0.010 ± 0.002
6c	NH_2	$(CH_2)_6OBz$	H	143 ± 14	3.3 ± 0.6
6d	NH_2	$(CH_2)_3{}^cHex$	H	289 ± 49	3.3 ± 0.5
6e	NH_2	$(CH_2)_4Ph$	H	42 ± 4	2.0 ± 0.4
6f	NH_2	$(CH_2)_2Ph$	H	48 ± 4	0.014 ± 0.003
6g	NH_2	$(CH_2)_3N(CH_3)_2$	H	3.0 ± 0.4	0.014 ± 0.002
6h	NH_2	$(CH_2)_3O(CH_2)_2OH$	H	1.5 ± 0.4	0.0020 ± 0.0004
6i	NH_2	$(CH_2)_2CO_2H$	H	ND[e]	0.004 ± 0.001
6j	NH_2	$(CH_2)_2CON(CH_3)_2$	H	ND	0.008 ± 0.001
6k	NH_2	$(CH)_2CON(CH_3)_2$	H	ND	0.005 ± 0.001
6l	NH_2	$(CH_2)_2CONH^iPr$	H	ND	0.002 ± 0.007
6m	NH_2	$(CH)_2CONH^iPr$	H	ND	0.006 ± 0.005
6n	NH_2	$(CH_2)_2CONH(CH_2)_2OH$	H	ND	0.002 ± 0.001
6o	NH_2	$(CH)_2CONH(CH_2)_2OH$	H	ND	0.007 ± 0.001
6p	NH_2	$(CH_2)_2CO_2CH_3$	H	2.9 ± 0.6	0.0031 ± 0.0005
6q	$NHCH_2CO_2CH_3$	$(CH_2)_5OH$	H	2.1 ± 0.5	0.004 ± 0.001
6r	$NH(CH_2)_2OH$	$(CH_2)_5OH$	H	4.0 ± 0.5	0.0025 ± 0.0002
6s	$NH(CH_2)_2OH$	$(CH_2)_5OAc$	H	ND	ND
6t	$NH(CH_2)_2OH$	$(CH_2)_2CO_2CH_3$	H	3.2 ± 0.3	0.0031 ± 0.0003

[a]Assays were performed using a gel-based protocol with a Mg^{2+} cofactor as described.[51] [b]Data have been reported previously.[26] [c]Data have been reported previously.[27] [d]Data have been reported previously.[31] [e]Not determined.

antiviral potencies of a subset of the compounds against HIV-1 vectors carrying the canonical Y143R, N155H, and Q148H/G140S mutations that are associated with high-level HIV-1 resistance to RAL and virological failure in patients (Table 2).[17,32−34,38,39] As we recently reported, compounds that lack substituents at the 6- and 7-positions and have a hydroxyl group at the 4-position (**5′a**) showed lower potencies in the ST assay and poorer antiviral potencies than the corresponding 4-amino-containing compound (**5′d**) (Tables 1 and 2).[26,27] In our current work, we found that a compound with a 4-[2-(methyl glycinate)] modification of the 4-amino group (**5′g**) retained its enzymatic potency in these assays relative to **5′d**. Bulky substituents at the 7-position (**5b**, **5c**, **5e**, **5f**, **5h**. and **5i**, Table 1) diminished potency by three orders of magnitude relative to the corresponding unsubsituted analogues (**5′a**, **5′d**, and **5′g**, respectively). Although a 7-methoxyl group was better tolerated (compare with **5a**, **5d**, and **5g**), in all cases there was a reduction in the antiviral potency (Table 2).

We recently described a series of structurally related 4-amino-containing INSTIs, which possess 6-substituents consisting of primary hydroxyl or sulfone groups tethered by alkyl chains of various lengths.[31] The chemical diversity of these substituents was limited. To extend the diversity of the modifications at the 6-position, our current study examines analogues containing tethered acetoxy (**6b**), benzyloxy (**6c**), cyclohexyl (**6d**), phenyl (**6e** and **6f**), dimethylamino (**6g**), carboxyl (**6i**), methyl ester (**6p**), and carboxamido having a variety of alkylamide groups (**6j−6o**) groups (Tables 1 and 2). Several of the resulting compounds retained low-nanomolar IC_{50} values in ST reactions. *N*,*N*-Dimethylpropylamine (**6g**, ST IC_{50} = 14 nM), propanoic acid (**6i**), propanoic amides (**6j−o**), and propanoic acid methyl ester (**6p**) as well as the reverse ester **6b** displayed IC_{50} values of 10 nM or less in the ST assay. Tethering a phenyl ring with an ethylene chain maintained good inhibitory potency (**6f**, ST IC_{50} = 14 nM). However, appending phenyl or cyclohexyl groups by increasing spacer

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Table 2. Antiviral Potencies in Cells Infected with HIV-1 Vectors That Carry WT or Resistant IN Mutants[a]

	EC_{50} (nM/FC[e])				
no.	WT	Y143R	N155H	G140S/Q148H	CC_{50} (μM)
RAL (1)[b]	4 ± 2	162 ± 16 (41×)	154 ± 33 (39×)	1900 ± 300 (475×)	>250
EVG (2)[b]	6.4 ± 0.8	7.9 ± 2.3 (1×)	90 ± 18 (14×)	5700 ± 1100 (891×)	>250
DTG (3)[b]	1.6 ± 0.9	4.3 ± 1.2 (3×)	3.6 ± 1.3 (2×)	5.8 ± 0.5 (4×)	>250
5′a[c]	6.2 ± 2.9	11 ± 2 (2×)	31 ± 8 (5×)	308 ± 125 (50×)	137 ± 20
5a	215 ± 31	ND[f]	ND	ND	12 ± 5
5′d[c]	1.1 ± 0.7	2.5 ± 0.6 (2×)	5.3 ± 2.3 (5×)	35 ± 9 (32×)	>250
5d	30 ± 9	62 ± 26 (2×)	400 ± 101 (13×)	3600 ± 1600 (120×)	2.2 ± 0.4
5′g	3.8 ± 1.2	4.6 ± 2.2 (1×)	19 ± 7 (5×)	36 ± 16 (9×)	>250
5g	32 ± 13	52 ± 13 (2×)	1260 ± 320 (39×)	ND	12 ± 3
6a	24 ± 4	8.3 ± 1.7 (0.3×)	32 ± 3 (1×)	29 ± 10 (1×)	>250
6′b[d]	1.3 ± 0.2	3.0 ± 0.5 (2×)	2.4 ± 0.8 (2×)	9.4 ± 3.6 (7×)	>250
6b	1.4 ± 0.4	2.9 ± 0.9 (2×)	5.4 ± 2.5 (4×)	13 ± 8 (9×)	>250
6c	4.8 ± 1.6	3.5 ± 1.2 (0.7×)	5.4 ± 1.9 (1×)	21 ± 7.8 (4×)	14.4 ± 4.8
6d	53 ± 14	112 ± 14 (2×)	146 ± 37 (3×)	ND	27.4 ± 3.1
6e	11 ± 2	4.7 ± 0 (0.4×)	39 ± 10 (4×)	128 ± 44 (12×)	13 ± 2
6f	5.6 ± 1.9	5.5 ± 2.4 (1×)	21 ± 5 (4×)	140 ± 34 (25×)	18 ± 2
6g	6.1 ± 2.1	7.0 ± 2.3 (1×)	29 ± 9 (5×)	32 ± 5 (5×)	5.0 ± 1.8
6h	1.6 ± 0.2	1.2 ± 0.6 (0.8×)	4.2 ± 1.5 (3×)	35 ± 3.7 (22×)	>250
6i	1.9 ± 0.5	4.9 ± 1.5 (3×)	10 ± 3 (5×)	195 ± 25 (103×)	>250
6j	1.3 ± 0.5	6 ± 1 (5×)	6.8 ± 1.6 (5×)	16 ± 5 (12×)	>250
6k	1.6 ± 0.9	7.2 ± 0.3 (5×)	15 ± 2 (9×)	58 ± 19 (36×)	>250
6l	5 ± 1.3	8.7 ± 2.2 (2×)	11 ± 4 (2×)	13 ± 1 (3×)	>250
6m	2.1 ± 0.7	11 ± 1 (5×)	12 ± 3 (6×)	286 ± 8 (136×)	>250
6n	263 ± 52	ND	ND	ND	>250
6o	196 ± 32	ND	ND	ND	>250
6p	0.67 ± 0.15	0.67 ± 0.23 (1×)	2.3 ± 0.2 (3×)	5.3 ± 1.8 (8×)	>250
6q	3.3 ± 1.7	3.5 ± 1.6 (1×)	11 ± 2 (3×)	42 ± 2 (13×)	>250
6r	13 ± 4.2	12 ± 2 (0.9×)	15 ± 4 (1×)	16 ± 7 (1×)	>250
6s	5.3 ± 1.2	4.1 ± 1 (0.8×)	10 ± 2 (2×)	5.3 ± 2.1 (1×)	>100
6t	4.1 ± 1	3.8 ± 1.9 (0.9×)	3.9 ± 2.1 (1×)	16 ± 4 (4×)	>250

[a]Cytotoxic concentration resulting in 50% reduction in the level of ATP in human osteosarcoma (HOS) cells; EC_{50} values obtained from cells infected with lentiviral vector harboring WT or indicated IN mutants as previously reported. [b]Data has been reported previously.[27] [c]Data have been reported previously.[26] [d]Data have been reported previously.[31] [e]FC, fold change relative to WT = EC_{50} of mutants/EC_{50} of WT. [f]Not determined.

lengths reduced potency by as much as three orders of magnitude: three methylenes (cyclohexyl-containing **6d**, ST IC_{50} = 3.3 μM), four methylenes (phenyl-containing **6e**, ST IC_{50} = 2.0 μM), or longer (**6c**, ST IC_{50} = 3.3 μM) (Table 1). This result contrasts with our previous finding that appending a phenyl ring using a three-unit chain that contains a sulfone moiety can result in retention of high potency. In the latter case, a cocrystal structure with the PFV intasome revealed that the sulfone group causes the phenyl ring to adopt an unusual $\pi-\pi$ stacking orientation, in which it is folded up under the naphthyridine ring system and fills the catalytic space more completely.[31]

Most 6-substituted analogues in our current study showed low nanomolar potencies against the WT enzyme in the in vitro ST assay and against a WT HIV-1 vector in the single-round infectivity assay (Tables 1 and 2, respectively). However, for the 6-tethered 2-hydroxyethylamides (**6n** and **6o**), in vitro ST inhibitory potencies and antiviral potencies against a vector that replicates using WT IN differed considerably (IC_{50} < 10 nM as compared to $EC_{50} \geq$ 200 nM). Additionally, while analogues **6c**, **6d**, and **6e** showed micromolar ST-inhibitory potencies in the in vitro assay, they were significantly more potent in the antiviral assay. The reasons for these discrepancies are not clear. Among the remaining compounds, **6p**, with a 6-$(CH_2)_2CO_2CH_3$ substituent, exhibited the best antiviral profile against the panel of resistant mutants (Table 2). This derivative displayed a modest improvement in antiviral potency against a vector carrying a WT IN (EC_{50} = 0.67 nM) as compared to **6′b**, which had been the most promising compound in work we recently reported.[31] Inhibitor **6′b** contains a 6-$(CH_2)_5OH$ group and a primary amine at the 4-position. Adding 4-[2-(methyl glycinate)] or 2-hydroxyethyl substituents to the 4-amino group of **6′b** did not improve the inhibitory profiles (compounds **6q** and **6r**, respectively), nor were the antiviral potencies improved by the addition of a 2-hydroxyethyl substituent to the 4-amino group of **6p** to yield **6t** (Table 2). Most 6-subsituted analogues were not cytotoxic within the range tested (Table 2). Exceptions were found with **6c**, **6d**, and **6e**, which also showed anomalous discrepancies between their in vitro and antiviral potencies, and with **6f** and **6g**. The reasons for the greater cytotoxicities of these analogues are not clear.

An important component of our current study is to test the effects of substituents at the 7-position. Given proximity to the metal-chelating 8-aryl nitrogen, we were uncertain of what the effects such modifications would have. Bulky groups at the 7-position (**5b**, **5c**, **5e**, **5f**, **5h**, and **5i**) resulted in from two to three orders of magnitude loss of inhibitory potency against the WT enzyme in vitro. These latter data were consistent with the bulky substituents causing a disruption of essential interactions with the catalytic metal ions. Of greater interest was our finding

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Table 3. Antiviral Potencies in Cells Infected with HIV-1 Vectors That Carry DTG-Resistant IN Mutants[a]

	EC_{50} (nM/FC[c])						
no.	WT	G118R	T66I	E92Q	R263K	H51Y	H51Y/R263K
RAL (1)[b]	4 ± 2	36 ± 5 (9×)	2.8 ± 0.4 (0.7×)	30 ± 10 (8×)	5.7 ± 2.3 (1×)	3.4 ± 0.2 (0.9×)	6 ± 2.3 (2×)
EVG (2)[b]	6.4 ± 0.8	21 ± 10 (3×)	66 ± 1 (10×)	154 ± 34 (24×)	10 ± 6 (2×)	4.5 ± 2.1 (0.7×)	53 ± 18 (8×)
DTG (3)[b]	1.6 ± 0.9	13 ± 5 (8×)	0.9 ± 0.8 (0.6×)	2.3 ± 0.4 (1×)	11 ± 3 (7×)	3.2 ± 0.2 (2×)	16 ± 2 (10×)
6′b[b]	1.3 ± 0.2	5.3 ± 1.6 (4×)	0.93 ± 0.24 (0.7×)	3.8 ± 2.3 (3×)	2.6 ± 0.1 (2×)	3.8 ± 0.6 (3×)	2.6 ± 1.4 (2×)
6b	1.4 ± 0.4	5.9 ± 1.4 (4×)	0.75 ± 0.07 (0.5×)	1.2 ± 0.1 (1×)	1.8 ± 0.3 (1×)	0.8 ± 0.2 (0.6×)	3.9 ± 1.9 (3×)
6p	0.67 ± 0.15	4.8 ± 1.5 (7×)	0.53 ± 0.06 (0.8×)	2.0 ± 1.1 (3×)	0.5 ± 0.0 (0.7×)	0.63 ± 0.30 (0.9×)	2.4 ± 0.8 (3×)
6r	13 ± 4.2	24 ± 8 (2×)	1.9 ± 0.07 (0.1×)	6.5 ± 0.8 (0.5×)	18 ± 2 (1×)	11 ± 1.4 (0.8×)	31 ± 10 (2×)
6s	5.3 ± 1.2	15 ± 2 (3×)	1.2 ± 0.5 (0.2×)	3.8 ± 1.3 (0.7×)	3.6 ± 0.9 (0.7×)	7.4 ± 0.9 (1×)	15 ± 1.8 (3×)
6t	4.1 ± 1	4.8 ± 0.6 (1×)	0.62 ± 0.15 (0.2×)	2.7 ± 1.2 (0.7×)	6.5 ± 0.8 (2×)	6.9 ± 2.9 (2×)	2.5 ± 0.1 (0.9×)

[a]EC_{50} values obtained from cells infected with a lentiviral vector harboring WT or the indicated IN mutants as previously reported. [b]Data for these compounds have been reported previously.[27,31] [c]FC, fold change relative to WT = EC_{50} of mutants/EC_{50} of WT.

that 7-OCH_3 substituents were well tolerated against WT enzyme in in vitro assays (see **5a**, **5d**, and **5g**, Table 1).

Potencies of the New Compounds Against an Extended Set of Drug Resistant IN Mutants. We selected a subset of the analogues (**6b**, **6p**, **6r**, **6s**, and **6t**) for additional evaluation in single-round viral replication assays using HIV-1 vectors harboring single G118R, T66I, E92Q, R263K, and H51Y, or the double H51Y/R263K substitutions associated with DTG resistance (Table 3).[17,32−34,38,39] For comparison, we also included **6′b**, which was one of the most broadly effective of our previously reported inhibitors.[31] The new compounds showed good antiviral potencies against the entire panel of mutants. Although several compounds appear to be promising, the most potent analogue (by a small margin) was the 6-methyl 3-propanoate-containing **6p**, which showed a slightly better inhibitory profile against the first panel of mutants (Table 2) while also retaining greater inhibitory potency than DTG against the second, more extended panel of mutants (Table 4).

Table 4. Comparison of Antiviral Potencies and Fold Improvement of Compounds 6′b and 6p Relative to DTG

	EC_{50} (nM)[a] (fold improvement[b])		
integrase mutants	DTG (3)[c]	6′b[c]	6p
WT	1.6 ± 0.9	1.3 ± 0.2 (0.81×)	0.67 ± 0.15 (0.42×)
Y143R	4.3 ± 1.2	3.0 ± 0.5 (0.71×)	0.67 ± 0.23 (0.16×)
N155H	3.6 ± 1.3	2.4 ± 0.8 (0.67×)	2.3 ± 0.2 (0.64×)
G140S/Q148H	5.8 ± 0.5	9.4 ± 3.6 (1.62×)	5.3 ± 1.8 (0.91×)
G118R	13 ± 5	5.3 ± 1.6 (0.41×)	4.8 ± 1.5 (0.37×)
T66I	0.9 ± 0.8	0.93 ± 0.24 (1.03×)	0.53 ± 0.06 (0.59×)
E92Q	2.3 ± 0.4	3.8 ± 2.3 (1.65×)	2.0 ± 1.1 (0.87×)
R263K	11 ± 3	2.6 ± 0.1 (0.24×)	0.5 ± 0.0 (0.05×)
H51Y	3.2 ± 0.2	3.8 ± 0.6 (1.19×)	0.63 ± 0.30 (0.20×)
H51Y/R263K	16 ± 2	2.6 ± 1.4 (0.16×)	2.4 ± 0.8 (0.15×)

[a]EC_{50} values obtained from cells infected with a lentiviral vector harboring WT or the indicated IN mutants as previously reported. [b]Fold improvement relative to DTG = EC_{50} of compounds/EC_{50} of DTG. [c]Data for these compounds have been reported previously.[27,31]

Crystal Structures of the PFV Intasome in Complex with 5′g, 5g, and 6p. To understand how some of the current analogues interact with the active site of IN, we soaked PFV intasome crystals in the presence of **5′g**, **5g**, or **6p** and refined the resulting structures (Table 5). We recently described a crystal structure of **5′d** bound to the active site of PFV intasome.[31] The inhibitor **5′g** bound to PFV IN differs from the previously reported **5′d** structure only in having a *N*-(methyl 2-glycinate)amine moiety at its 4-position and, predictably, the two compounds bind to the active site in a very similar fashion. Superposition with the DTG-bound structure confirmed that 8-naphthyridine nitrogen and 1-*N*-hydroxyl of **5′g** take the place of the 6-oxo and 7-hydroxyl heteroatoms of DTG. The naphthyridine 2-oxo carbonyl of **5′g** corresponds to the ring 8-oxo carbonyl of DTG (Figure 2A).[23] However, the 4-*N*-(methyl 2-glycinate)amine group of **5′g** extends into the region which is occupied by the first base of the scissile dinucleotide in viral DNA prior to 3′-P (designated A18 in the crystal structure, Figure 2A), which may be related to the ability of the compound to inhibit the 3′-P reaction.[8] Therefore, an extended form of substrate mimicry may arise from the modification at the 4-position of **5′g**, which was not seen in the cocrystal structure of **5′d**.[31] The idea that inhibitors that remain within the "substrate envelope" are particularly effective against resistant forms of the target enzyme was proposed by Schiffer and colleagues and is based on the idea that to be able to support viral replication, any mutant form of a viral enzyme must be capable of binding its normal substrate(s).[35] Thus, if the bound inhibitor remains within an envelope defined by the substrate(s), the inhibitor is expected to retain some efficacy against mutant forms of the enzyme.[40] Even though the original hypothesis was based on work with protease inhibitors, it seems likely that this principle can be applied broadly to antiviral compounds that bind at the active sites of essential viral enzymes. The 4-*N*-(methyl 2-glycinate) amine group of **5′g** is situated near (within 5 Å) to the side chains of Y212 and P214 (residues corresponding to Y143 and P145 in HIV-1 IN) and to the adenosine base of the 3′ terminal viral DNA residue (A17), which adopts two alternative conformations in the crystal structure (Figure 2B). The interactions with the side chains are much less extensive in the cases of DTG (**3**) and **5′d**. Extension into this region of the catalytic site is also seen with several highly potent INSTIs having diverse structures (see Figure S1 in Supporting Information).

Prior structural studies using the PFV intasome showed that the tricyclic system of DTG makes contacts with G187 in the $\beta4-\alpha2$ loop of PFV IN (G118 in IN), and it has been argued that the interactions with this region may contribute to the improved properties of DTG and other second-generation INSTIs.[4,23,29,30] As has already been discussed, we thought that it might be possible to make useful contacts with the same region of the catalytic site by adding functionality to the 6- and 7-positions of **4**. To this end, we introduced substituents at the

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Table 5. X-Ray Data Collection and Refinement Statistics[a]

	5′g	5g	6p
Data Collection			
space group	$P4_12_12$	$P4_12_12$	$P4_12_12$
cell dimensions *a*, *b*, *c* (Å)	159.6, 159.6, 124.1	158.6, 158.6, 123.1	160.5, 160.5, 123.8
resolution range (Å)	71.36−2.55 (2.62−2.55)	79.29−2.60(2.67−2.60)	71.77−2.77 (2.84−2.77)
R_{merge}	0.062 (1.49)	0.025 (0.55)	0.072 (1.075)
$I/\sigma(I)$	26.6 (2.0)	19.5 (1.4)	18.9 (2.0)
completeness (%)	99.8 (98.8)	99.9 (99.7)	98.8 (97.4)
redundancy	11.5 (11.0)	14.4 (14.2)	7.2 (7.3)
Refinement			
reflections (total/free)	52565/2601	48670/2438	40988/2057
R/R_{free}	0.179/0.207	0.176/0.204	0.177/0.205
no. atoms			
protein, DNA	5150	5129	5127
ligand	159	132	146
water	229	109	87
average *B*-factors (Å^2)	71.7	84.3	75.7
protein, DNA	71.3	83.9	74.9
ligands	91.8	117.23	107.3
water	67.2	78.9	68.3
rmsd			
bond lengths (Å)	0.038	0.039	0.012
bond angles (deg)	0.56	0.76	1.38
Ramachandran plot (%)			
favored	99	97	98
outliers	0	0	0.2

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

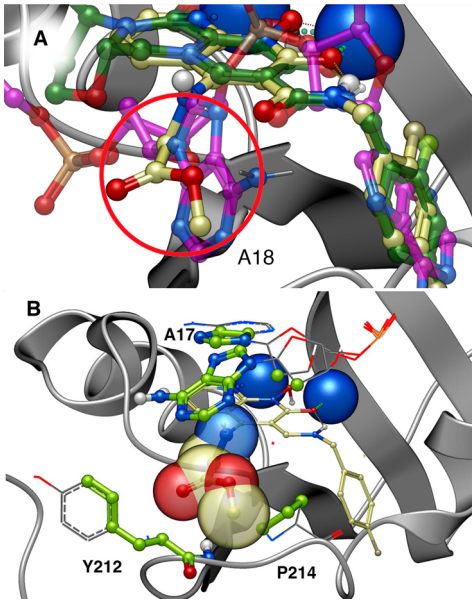


Figure 2. Crystal structures of PFV intasome-bound inhibitors. (A) Bound **5′g** (cream) with overlays showing the relative positions of pre-3′-P viral DNA (violet; PDB 4E7I) and DTG (**3**) (green; PDB 3S3M). The 4-*N*-(methyl 2-glycinate) moiety of **5′g** is circled in red, highlighting its correspondence with the T_{+2} adenosine (A18) base of pre-3′-P viral DNA. (B) Bound **5′g** with its 4-*N*-(methyl 2-glycinate) moiety shown as transparent spheres. Atoms within 5 Å (Y212 and P214 and A17 of 3′-P DNA) are shown in green as a stick diagram. For both A and B, metal ions are shown as solid blue spheres.

6- and 7-positions of **4** that could potentially recapitulate some of the desirable features of DTG (highlighted in green and cyan, respectively, in the structures of DTG and **4**, Figure 1).

We obtained the crystal structure of **6p** bound to the PFV intasome because it represents the most potent 6-substituted analogue in our current study. The structure revealed that the 6-methyl 3-propanoate side chain of the compound is situated within the region defined by an envelope defined by both the pre-3′-P viral DNA and the host target DNA substrate (Figure 3). This is consistent with our recent report that 6-substituents of the compounds exhibiting the best antiviral profiles against the panel of resistant mutants bind within an envelope defined

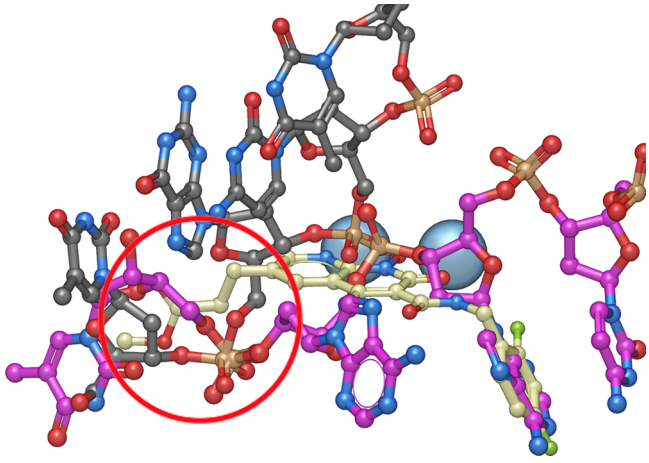


Figure 3. Crystal structures of **6p** bound to the PFV intasome. Structures of the pre-3′-P viral DNA (violet; PDB 4E7I) and target complex host DNA substrate (gray; PDB 4E7K) are overlaid. The 6-(methyl 3-propanoate) group of **6p** is circled in red, showing its correspondence with aspects of both pre-3′-P viral DNA and the host target DNA. Cofactor Mg^{2+} ions are shown as blue spheres; the structures of bound viral DNAs have been omitted for clarity.

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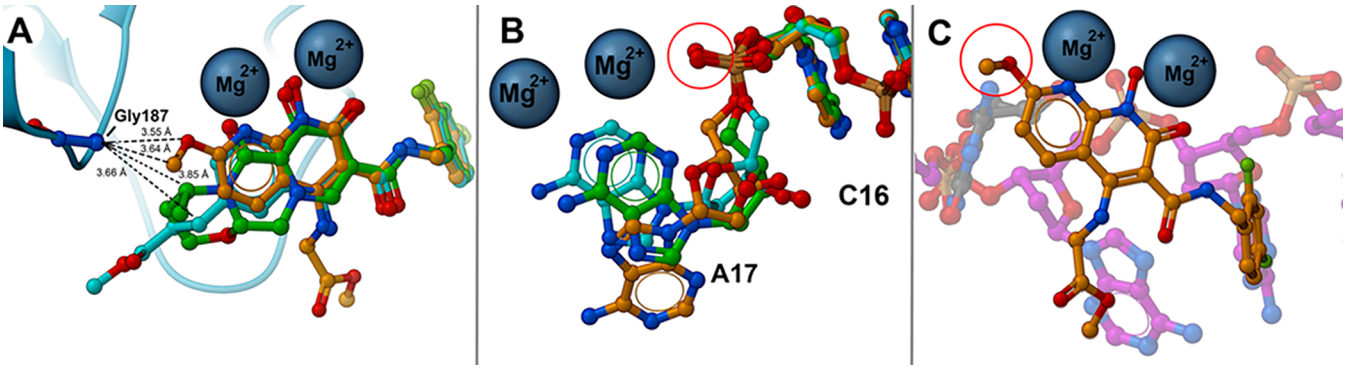


Figure 4. Crystal structures of PFV intasome-bound inhibitors. (A) Bound **5g** (orange) with superimposed **6p** (cyan) and DTG (**3**) (green; PDB 3S3M) showing closest distance to the Gly187 α-methylene (side chains of Asp186 and Gln187 shown in semitransparent), bound 3′-P DNAs have been omitted for clarity. (B) Superimposed bound 3′-P DNAs associated with A, circled in cyan are the oxygens in the C16−A17 phosphoryl linkages, whose engagement in electrostatic interactions with the side chain imidazole nitrogen of the N224H mutant (corresponding to IN mutant N155H) would be broken on binding of the INSTIs. (C) Bound **5g** with superimposed pre-3′-P viral DNA (violet; PDB 4E7I) and target complex host DNA substrate (gray; PDB 4E7K), circled in red is the 7-OCH_3, which protrudes outside the substrate envelope. Metal ions are shown as solid blue spheres.

as the confluence of viral DNA substrates.[31] The structure also shows that the methyl carboxylate group of **6p** makes van der Waals interactions with the β4−α2 loop of IN, becoming sandwiched between main chain atoms of Q186 and G187 (residues corresponding to HIV-1 N117 and G118, respectively) and the side chain of Y212 (Figure 4).

To understand the effects of introducing a 7-OCH_3 substituent, we solved the structure of **5g** bound to the PFV intasome and overlaid the corresponding structure of DTG bound to the PFV intasome (Figure 4). We observed that the 7-OCH_3 group of **5g** is approximately 3.6 Å from the G187 residue in the β4−α2 loop, which compares favorably with DTG and **6p** (approximately 3.9 and 3.7 Å, respectively, Figure 4A). The 7-OCH_3 group of **5g** did not appear to significantly alter the position of the metal chelating heteroatoms of **5g** relative to **6p**. However, it is not immediately obvious why **5g** is more susceptible to the N155H mutation compared to **6p** or to the more closely related **5′g**. Because the N155 residue of HIV-1 IN corresponds to N224 in PFV IN, the X-ray crystal structure with the mutation N224H in PFV IN did show that the side chain of His224 interacts with a phosphoryl oxygen atom in viral DNA and that INSTI binding is accompanied by a loss of this contact.[24] The associated energetic penalty may explain the loss potency of some compounds against N155H HIV-1 IN. In the cocrystal structure of PFV intasome with **5g**, the location of this phosphoryl oxygen is nearly identical to that seen for **6p**, **5′g**, and DTG (Figure 4C). The abilities of some INSTIs to readjust in the active site of IN may help explain variations among in their susceptibilities of the different INSTIs to the N155H mutation.[23,24,41]

CONCLUSIONS

Earlier, we reported that the 1-hydroxy-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide scaffold represents a potentially attractive platform for the development of INSTIs that retain good antiviral efficacy against a panel of viral vectors carrying resistant mutant forms of IN.[26,27] More recently, we began an exploration of the effects of modifying the 6-position of our earlier compounds.[31] We found that 6-substituents may play an important role in enhancing the maintenance of antiviral efficacy against drug-resistant mutant forms of IN. Furthermore, the most broadly effective compounds are substrate mimics that fit within the "substrate envelope." Our original purpose in synthesizing 6-substituents was to create additional interactions within areas of the catalytic region accessed by the third ring of DTG. However, the range of functionality reported in the prior paper was quite limited, consisting of primary hydroxyl groups tethered by linear polymethylene chains of varying lengths and a single tethered phenyl sulfone. We now report a much more extensive exploration of functionalities at the 6-position as well as the first examination of derivatives with modifications at the 7-position. We also tested whether the compounds could be improved by introduced by varying functionalities at the 4-position.

We observed that −OCH_3 groups are somewhat better tolerated than bulky subsituents at the 7-position when examined in vitro against WT enzyme. However, compounds with 7-OMe groups showed significantly reduced antiviral potency when tested against viral vectors that carry the N155H mutation. We found that a variety of substituents can be added at the 6-position, which enhance the antiviral potencies of the compounds against the major RAL-, EVG-, and DTG-resistant IN mutants. Many of these compounds are not cytotoxic at the tested concentrations, and the best analogue, **6p**, is the most effective in terms of its ability to inhibit the entire spectrum of IN mutants of any of the compounds we have described. The crystal structure of the PFV intasome with **6p** bound supports the idea that interactions with the β4−α2 loop are important and that compounds that bind within the envelope defined by both the viral and host DNA substrates helps the compounds retain broad efficacy. We found that the 4-amine substituent in **5′g** accesses a region of the active site of IN that would normally be occupied by the base of the T_{+2} adenosine of the viral DNA prior to the 3′-P cleavage, providing further support that binding within an envelope defined by the DNA substrates helps the compounds retain broad efficacy. Thus, our results will inform the design and development of next-generation INSTIs with broader efficacy against the known drug resistant mutants.

EXPERIMENTAL SECTION

General Procedures. Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded 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

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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 Combiflash with EtOAc–hexanes solvent systems. Preparative high pressure liquid chromatography (HPLC) was conducted using a Waters Prep LC4000 system having photodiode array detection and Phenomenex C_{18} columns (catalogue no. 00G-4436-P0-AX, 250 mm × 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 acetonitrile were employed with gradients as indicated. Products were obtained as amorphous solids following lyophilization. Electrospray ionization-mass spectrometric (ESI-MS) were acquired with an Agilent LC/MSD system equipped with a multimode ion source. Purities of samples subjected to biological testing were assessed using this system and shown to be ≥95%. High resolution mass spectrometric (HRMS) were acquired by LC/MS-ESI using LTQ-Orbitrap-XL at 30K resolution.

General Procedure I for the Synthesis of Carboxamides (5a–i and 6a–t). Benzyl protected compounds (**13**, **15**, **16**, and **19**) (0.1 mmol) were suspended in methanol (10 mL) and ethyl acetate (3 mL). One equivalent of Pd/C (10%) was added. The mixture was stirred at room temperature under hydrogen. When the starting material was disappeared (TLC), the crude mixture was filtered and washed by methanol. The filtrate was concentrated and purified by HPLC to provide final carboxamides (**5a–i** and **6a–t**).

*N-(2,4-Difluorobenzyl)-1,4-dihydroxy-7-methoxy-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**5a**).* Treatment of **13a** as outlined in general procedure I and purification by preparative HPLC (with a linear gradient of 30% B to 90% B over 30 min; retention time = 25.5 min) provided **5a** as a white fluffy solid (22% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.00 (s, 1H), 10.42 (d, J = 5.8 Hz, 1H), 8.29 (dd, J = 8.7, 0.9 Hz, 1H), 7.48 (dd, J = 15.3, 8.6 Hz, 1H), 7.30–7.25 (m, 1H), 7.10 (dd, J = 9.8, 7.3 Hz, 1H), 6.83–6.81 (m, 1H), 4.62 (d, J = 6.0 Hz, 2H), 4.02 (s, 3H). ESI-MS m/z: 378.1 (MH^+).

*N-(2,4-Difluorobenzyl)-1,4-dihydroxy-2-oxo-7-(piperidin-1-yl)-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**5b**).* Treatment of **13b** as outlined in general procedure I, and purification by preparative HPLC (with a linear gradient of 50% B to 90% B over 30 min; retention time = 24.0 min) provided **5b** as a white fluffy solid (13% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.52 (bs, 1H), 10.36 (t, J = 5.9 Hz, 1H), 7.93 (d, J = 9.1 Hz, 1H), 7.41 (dd, J = 15.3, 8.7 Hz, 1H), 7.25–7.19 (m, 1H), 7.04 (dd, J = 9.3, 6.7 Hz, 1H), 6.82 (d, J = 9.2 Hz, 1H), 4.54 (d, J = 5.8 Hz, 2H), 3.74 (s, 4H), 1.61 (bs, 2H), 1.52 (bs, 4H). ESI-MS m/z: 431.2 (MH^+).

*N-(2,4-Difluorobenzyl)-1,4-dihydroxy-7-morpholino-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**5c**).* Treatment of **13c** as outlined in general procedure I, and purification by preparative HPLC (with a linear gradient of 30% B to 90% B over 30 min; retention time = 24.3 min) provided **5c** as a white fluffy solid (30% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.60 (s, 1H), 10.36 (t, J = 6.0 Hz, 1H), 8.01 (d, J = 9.1 Hz, 1H), 7.41 (dd, J = 15.3, 8.6 Hz, 1H), 7.25–7.19 (m, 1H), 7.06–7.02 (m, 1H), 6.83 (d, J = 9.1 Hz, 1H), 4.54 (d, J = 6.0 Hz, 2H), 3.73 (s, 4H), 3.65 (t, J = 4.5 Hz, 4H). ESI-MS m/z: 433.1 (MH^+).

*4-Amino-N-(2,4-difluorobenzyl)-1-hydroxy-7-methoxy-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**5d**).* Treatment of **16a** as outlined in general procedure I, and purification by preparative HPLC (with a linear gradient of 30% B to 75% B over 30 min; retention time = 22.4 min) provided **5d** as a white fluffy solid (37% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.60 (t, J = 5.6 Hz, 1H), 10.51 (bs, 1H), 8.48 (d, J = 8.8 Hz, 1H), 7.38 (dd, J = 15.4, 8.7 Hz, 1H), 7.22–7.17 (m, 1H), 7.02 (td, J = 8.5, 2.5 Hz, 1H), 6.72 (d, J = 8.8 Hz, 1H), 4.46 (d, J = 5.7 Hz, 2H), 3.94 (s, 3H). ESI-MS m/z: 377.1 (MH^+).

*4-Amino-N-(2,4-difluorobenzyl)-1-hydroxy-2-oxo-7-(piperidin-1-yl)-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**5e**).* Treatment of **16b** as outlined in general procedure I, and purification by preparative HPLC (with a linear gradient of 50% B to 70% B over 30 min; retention time = 20.6 min) provided **5e** as a white fluffy solid (49% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.63 (t, J = 5.8 Hz, 2H), 8.19 (d, J = 9.2 Hz, 1H), 7.36 (dd, J = 15.4, 8.7 Hz, 1H), 7.21–7.16 (m, 1H), 7.02 (t, J = 9.0 Hz, 1H), 6.75 (d, J = 9.2 Hz, 1H), 4.44 (d, J = 5.7 Hz, 2H), 3.69 (bs, 4H), 1.59 (bs, 2H), 1.50 (bs, 4H). ESI-MS m/z: 430.2 (MH^+).

*4-Amino-N-(2,4-difluorobenzyl)-1-hydroxy-7-morpholino-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**5f**).* Treatment of **16c** as outlined in general procedure I, and purification by preparative HPLC (with a linear gradient of 30% B to 65% B over 30 min; retention time = 24.0 min) provided **5f** as a white fluffy solid (28% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.62 (t, J = 5.7 Hz, 1H), 10.27 (bs, 1H), 10.19 (bs, 1H), 8.26 (d, J = 9.1 Hz, 1H), 7.36 (dd, J = 15.4, 8.7 Hz, 1H), 7.21–7.16 (m, 1H), 7.02 (td, J = 8.2, 2.0 Hz, 1H), 6.76 (d, J = 9.1 Hz, 1H), 4.44 (d, J = 5.7 Hz, 2H), 3.66 (d, J = 3.3 Hz, 8H). ESI-MS m/z: 432.1 (MH^+).

*Methyl 2-((3-((2,4-Difluorobenzyl)carbamoyl)-1-hydroxy-7-methoxy-2-oxo-1,2-dihydro-1,8-naphthyridin-4-yl)amino)acetate (**5g**).* Treatment of **15a** as outlined in general procedure I, and purification by preparative HPLC (with a linear gradient of 30% B to 80% B over 30 min; retention time = 23.7 min) provided **5g** as a white fluffy solid (20% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.17 (bs, 1H), 10.61 (bs, 1H), 10.45 (t, J = 5.7 Hz, 1H), 8.31 (d, J = 9.0 Hz, 1H), 7.45 (dd, J = 15.3, 8.7 Hz, 1H), 7.22–7.17 (m, 1H), 7.02 (td, J = 8.5, 1.6 Hz, 1H), 6.65 (d, J = 8.9 Hz, 1H), 4.48 (d, J = 5.4 Hz, 2H), 4.45 (d, J = 5.9 Hz, 2H), 3.94 (s, 3H), 3.63 (s, 3H). ESI-MS m/z: 449.1 (MH^+).

*Methyl 2-((3-((2,4-Difluorobenzyl)carbamoyl)-1-hydroxy-2-oxo-7-(piperidin-1-yl)-1,2-dihydro-1,8-naphthyridin-4-yl)amino)acetate (**5h**).* Treatment of **15b** as outlined in general procedure I, and purification by preparative HPLC (with a linear gradient of 50% B to 70% B over 30 min; retention time = 22.9 min) provided **5h** as a white fluffy solid (29% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.45 (bs, 1H), 10.70 (t, J = 5.7 Hz, 1H), 7.98 (d, J = 9.4 Hz, 1H), 7.39 (dd, J = 15.3, 8.7 Hz, 1H), 7.22–7.16 (m, 1H), 7.02 (td, J = 8.6, 2.1 Hz, 1H), 6.65 (d, J = 9.4 Hz, 1H), 4.45 (bs, 2H), 4.44 (s, 2H), 3.70 (bs, 4H), 3.64 (s, 3H), 1.60 (bs, 3H), 1.51 (bs, 3H). ESI-MS m/z: 502.2 (MH^+).

*Methyl 2-((3-((2,4-Difluorobenzyl)carbamoyl)-1-hydroxy-7-morpholino-2-oxo-1,2-dihydro-1,8-naphthyridin-4-yl)amino)acetate (**5i**).* Treatment of **15c** as outlined in general procedure I, and purification by preparative HPLC (with a linear gradient of 30% B to 70% B over 30 min; retention time = 26.0 min) provided **5i** as a white fluffy solid (18% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.43 (bs, 1H), 10.66 (t, J = 5.8 Hz, 1H), 10.31 (bs, 1H), 8.06 (d, J = 9.3 Hz, 1H), 7.40 (dd, J = 15.4, 8.7 Hz, 1H), 7.21–7.16 (m, 1H), 7.02 (dd, J = 9.8, 7.4 Hz, 1H), 6.66 (d, J = 9.3 Hz, 1H), 4.47 (d, J = 5.4 Hz, 2H), 4.45 (d, J = 5.7 Hz, 2H), 3.66 (d, J = 5.2 Hz, 8H), 3.63 (s, 3H). ESI-MS m/z: 504.2 (MH^+).

*N-(2,4-Difluorobenzyl)-1,4-dihydroxy-6-(5-hydroxypentyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**6a**).* Treatment of 1-(benzyloxy)-6-bromo-*N*-(2,4-difluorobenzyl)-4-hydroxy-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide **17a**[31] with commercially available pent-4-yn-1-ol as outlined in general procedures J and I and purification by preparative HPLC (linear gradient of 30% B to 70% B over 30 min; retention time = 23.8 min) provided **6a** as a white fluffy solid (46% yield, two steps). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.96 (bs, 1H), 10.52 (t, J = 6.1 Hz, 1H), 8.70 (d, J = 2.0 Hz, 1H), 8.26 (d, J = 2.0 Hz, 1H), 7.48 (dd, J = 15.3, 8.6 Hz, 1H), 7.30–7.25 (m, 1H), 7.09 (td, J = 8.5, 2.5 Hz, 1H), 4.63 (d, J = 5.9 Hz, 2H), 3.37 (t, J = 6.5 Hz, 2H), 2.73 (t, J = 7.6 Hz, 2H), 1.65–1.59 (m, 2H), 1.46–1.42 (m, 2H), 1.35–1.28 (m, 2H). ESI-MS m/z: 434.1 (MH^+). HRMS calcd $C_{21}H_{22}F_2N_3O_5$ [MH_+], 434.1522; found, 434.1507.

*3-(5-Amino-6-((2,4-difluorobenzyl)carbamoyl)-8-hydroxy-7-oxo-7,8-dihydro-1,8-naphthyridin-3-yl)propyl Acetate (**6b**).* Treatment of **19a** as outlined in general procedure I and purification by preparative HPLC (linear gradient of 30% B to 70% B over 30 min; retention time = 20.6 min) provided **6b** as a white fluffy solid (71% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.69 (t, J = 5.7 Hz, 1H), 8.60 (s, 1H), 8.57 (s, 1H), 7.42 (dd, J = 15.6, 7.5 Hz, 1H), 7.24 (t, J = 9.9 Hz, 1H), 7.07 (t, J = 8.5 Hz, 1H), 4.52 (d, J = 5.5 Hz, 2H), 4.05 (t, J = 6.3 Hz, 2H), 2.75 (t, J = 7.9 Hz, 2H), 2.00 (s, 3H), 1.97 (d, J = 7.7 Hz, 2H). ESI-MS m/z: 447.1 (MH^+). HRMS calcd $C_{21}H_{21}F_2N_4O_5$ [MH^+], 447.1475; found, 447.1477.

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*6-(5-Amino-6-((2,4-difluorobenzyl)carbamoyl)-8-hydroxy-7-oxo-7,8-dihydro-1,8-naphthyridin-3-yl)hexyl Benzoate (**6c**).* Protection of 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[31] (50 mg, 0.073 mmol) using benzoic anhydride (25 mg, 0.11 mmol) in pyridine (0.5 mL) (room temperature, 2 h) provided 6-(8-(benzyloxy)-6-((2,4-difluorobenzyl)carbamoyl)-5-((2,4-dimethoxybenzyl)amino)-7-oxo-7,8-dihydro-1,8-naphthyridin-3-yl)-hex-5-yn-1-yl benzoate as a white solid (56 mg, 96% yield). [^{1}H NMR (500 MHz, $CDCl_3$) δ 11.99 (t, J = 5.8 Hz, 1H), 10.69 (t, J = 5.8 Hz, 1H), 8.67 (d, J = 1.4 Hz, 1H), 8.34 (d, J = 1.7 Hz, 1H), 8.07−8.05 (m, 2H), 7.39−7.68 (m, 2H), 7.57 (t, J = 7.4 Hz, 1H), 7.45 (t, J = 7.8 Hz, 2H), 7.40−7.36 (m, 4H), 7.23 (d, J = 8.9 Hz, 1H), 6.86−6.79 (m, 2H), 6.48−6.46 (m, 2H), 5.26 (s, 2H), 4.75 (d, J = 5.9 Hz, 2H), 4.60 (d, J = 5.6 Hz, 2H), 4.41 (t, J = 6.4 Hz, 2H), 3.81 (s, 3H), 3.78 (s, 3H), 2.54 (t, J = 7.0 Hz, 2H), 1.99−1.93 (m, 2H), 1.84−1.78 (m, 2H). ESI-MS m/z: 787.3 (MH^+).] After treatment as outlined in general procedure H and I and purification by preparative HPLC (linear gradient of 55% B to 75% B over 30 min; retention time = 25.4 min), **6c** was provided as a white fluffy solid (45% yield, two steps). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.69 (t, J = 5.7 Hz, 1H), 8.58 (d, J = 1.9 Hz, 1H), 8.54 (s, 1H), 7.94 (dd, J = 8.4, 1.3 Hz, 2H), 7.64 (dt, J = 8.7, 1.3 Hz, 1H), 7.53−7.50 (m, 2H), 7.42 (dd, J = 15.3, 8.5 Hz, 1H), 7.26−7.21 (m, 1H), 7.06 (dd, J = 9.8, 7.3 Hz, 1H), 4.51 (d, J = 5.6 Hz, 2H), 4.26 (t, J = 6.5 Hz, 2H), 2.70−2.67 (m, 2H), 1.73−1.66 (m, 4H), 1.45−1.42 (m, 2H), 1.39−1.38 (m, 2H). ESI-MS m/z: 551.2 (MH^+). HRMS calcd $C_{29}H_{29}F_2N_4O_5$ [MH^+], 551.2089; found, 551.2089.

*4-Amino-6-(3-cyclohexylpropyl)-N-(2,4-difluorobenzyl)-1-hydroxy-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**6d**).* Treatment of **19b** as outlined in general procedure I and purification by preparative HPLC (linear gradient of 50% B to 80% B over 30 min; retention time = 27.5 min) provided **6d** as a white fluffy solid (40% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.63 (t, J = 5.8 Hz, 1H), 8.51 (s, 1H), 8.47 (s, 1H), 7.36 (dd, J = 15.5, 8.6 Hz, 1H), 7.20−7.15 (m, 1H), 7.00 (t, J = 8.5 Hz, 1H), 4.45 (d, J = 5.7 Hz, 2H), 2.59 (t, J = 7.7 Hz, 2H), 1.63−1.52 (m, 7H), 1.16−1.00 (m, 6H), 0.82−0.75 (m, 2H). ESI-MS m/z: 471.2 (MH^+). HRMS calcd $C_{25}H_{29}F_2N_4O_3$ [MH^+], 471.2202; found, 471.2204.

*4-Amino-N-(2,4-difluorobenzyl)-1-hydroxy-2-oxo-6-(4-phenylbutyl)-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**6e**).* Treatment of **19c** as outlined in general procedure I and purification by preparative HPLC (linear gradient of 40% B to 80% B over 30 min; retention time = 26.0 min) provided **6e** as a white fluffy solid (27% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.69 (t, J = 5.8 Hz, 1H), 8.58 (s, 1H), 8.54 (s, 1H), 7.42 (dd, J = 15.5, 8.5 Hz, 1H), 7.26 (t, J = 7.6 Hz, 2H), 7.24−7.21 (m, 1H), 7.19−7.14 (m, 3H), 7.07 (t, J = 8.5 Hz, 1H), 4.51 (d, J = 5.7 Hz, 2H), 2.72 (t, J = 7.3 Hz, 2H), 2.62 (t, J = 7.5 Hz, 2H), 1.69 (dd, J = 14.8, 7.1 Hz, 2H), 1.65−1.53 (m, 2H). ESI-MS m/z: 479.2 (MH^+). HRMS calcd $C_{26}H_{25}F_2N_4O_3$ [MH^+], 479.1889; found, 479.1880.

*4-Amino-N-(2,4-difluorobenzyl)-1-hydroxy-2-oxo-6-phenethyl-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**6f**).* Treatment of **19d** as outlined in general procedure I and purification by preparative HPLC (linear gradient of 40% B to 80% B over 30 min; retention time = 22.5 min) provided **6f** as a white fluffy solid (65% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.69 (bs, 1H), 10.51 (bs, 1H), 8.61 (s, 1H), 8.53 (s, 1H), 7.45−7.42 (m, 1H), 7.35−7.32 (m, 1H), 7.27 (d, J = 7.0 Hz, 1H), 7.23 (d, J = 6.7 Hz, 2H), 7.20−7.17 (m, 1H), 7.08−7.05 (m, 1H), 4.52 (d, J = 5.5 Hz, 2H), 2.99 (bs, 4H). ESI-MS m/z: 451.1 (MH^+). HRMS calcd $C_{24}H_{21}F_2N_4O_3$ [MH^+], 451.1576; found, 451.1576.

*4-Amino-N-(2,4-difluorobenzyl)-6-(3-(dimethylamino)propyl)-1-hydroxy-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**6g**).* Treatment of **19e** as outlined in general procedure I and purification by preparative HPLC (linear gradient of 30% B to 55% B over 30 min; retention time = 17.2 min) provided **6g** as a white fluffy solid (52% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.68 (t, J = 5.8 Hz, 1H), 10.57 (s, 1H), 8.62 (s, 1H), 8.57 (s, 1H), 7.42 (dd, J = 15.5, 8.5 Hz, 1H), 7.27−7.22 (m, 1H), 7.07 (dd, J = 9.8, 7.3 Hz, 1H), 4.52 (d, J = 5.8 Hz, 2H), 3.06 (d, J = 7.7 Hz, 2H), 2.78 (s, 6H), 2.74 (t, J = 7.5 Hz, 2H), 2.01 (dt, J = 15.7, 7.8 Hz, 2H). ESI-MS m/z: 432.2 (MH^+). HRMS calcd $C_{21}H_{24}F_2N_5O_3$ [MH^+], 432.1842; found, 432.1824.

*4-Amino-N-(2,4-difluorobenzyl)-1-hydroxy-6-(3-(2-hydroxyethoxy)propyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**6h**).* Treatment of **19f** as outlined in general procedure I and purification by preparative HPLC (linear gradient of 30% B to 60% B over 30 min; retention time = 20.5 min) provided **6h** as a white fluffy solid (86% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.69 (t, J = 5.8 Hz, 1H), 8.59 (d, J = 1.9 Hz, 1H), 8.56 (d, J = 1.9 Hz, 1H), 7.52−7.35 (m, 1H), 7.24 (ddd, J = 10.5, 9.4, 2.6 Hz, 1H), 7.07 (ddd, J = 10.4, 8.1, 2.2 Hz, 1H), 4.52 (d, J = 5.7 Hz, 2H), 3.51 (t, J = 5.3 Hz, 2H), 3.41 (dt, J = 7.5, 5.7 Hz, 4H), 2.76−2.73 (m, 2H), 1.92−1.86 (m, 2H). ESI-MS m/z: 449.1 (MH^+). HRMS calcd $C_{21}H_{23}F_2N_4O_5$ [MH^+], 449.1631; found, 449.1631.

*3-(5-Amino-6-((2,4-difluorobenzyl)carbamoyl)-8-hydroxy-7-oxo-7,8-dihydro-1,8-naphthyridin-3-yl)propanoic Acid (**6i**).* Treatment of **19g** with aqueous NaOH and then as outlined in general procedure I and purification by preparative HPLC (with a linear gradient of 30% B to 50% B over 30 min; retention time = 19.5 min) provided **6i** as a white fluffy solid (80% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.63 (t, J = 5.4 Hz, 1H), 8.68−8.66 (m, 1H), 8.63−8.60 (m, 1H), 8.57 (d, J = 1.9 Hz, 1H), 8.53 (d, J = 1.9 Hz, 1H), 7.40−7.34 (m, 1H), 7.21−7.16 (m, 1H), 7.04−6.99 (m, 1H), 4.47 (d, J = 5.5 Hz, 2H), 2.87 (t, J = 7.5 Hz, 2H), 2.62 (t, J = 7.6 Hz, 2H). ESI-MS m/z: 419.1 (MH^+). HRMS calcd $C_{19}H_{17}F_2N_4O_5$ [MH^+], 419.1162; found, 419.1147.

*4-Amino-N-(2,4-difluorobenzyl)-6-(3-(dimethylamino)-3-oxopropyl)-1-hydroxy-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**6j**) and (E)-4-Amino-N-(2,4-difluorobenzyl)-6-(3-(dimethylamino)-3-oxoprop-1-en-1-yl)-1-hydroxy-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**6k**).* Treatment of **18e** as outlined in general procedures I and H and purification by preparative HPLC (with a linear gradient of 30% B to 45% B over 30 min) provided **6j** and **6k** as white fluffy solids. For **6j**: retention time = 20.7 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.62 (t, J = 5.8 Hz, 1H), 10.46 (brs, 1H), 8.56 (d, J = 1.8 Hz, 1H), 8.50 (d, J = 1.8 Hz, 1H), 7.36 (dd, J = 15.4, 8.6 Hz, 1H), 7.20−7.14 (m, 1H), 7.00 (td, J = 8.6, 1.9 Hz, 1H), 4.45 (d, J = 5.7 Hz, 2H), 2.90 (s, 3H), 2.84 (t, J = 7.4 Hz, 2H), 2.74 (s, 3H), 2.65 (t, J = 7.5 Hz, 2H). ESI-MS m/z: 446.2 (MH^+). HRMS calcd $C_{21}H_{22}F_2N_5O_4$ [MH^+], 446.1634; found, 446.1617. For **6k**: retention time = 24.1 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.63 (s, 1H), 10.53 (t, J = 5.8 Hz, 1H), 8.94 (s, 2H), 7.49 (d, J = 15.4 Hz, 1H), 7.37 (dd, J = 12.2, 5.3 Hz, 1H), 7.31 (d, J = 15.5 Hz, 1H), 7.21−7.15 (m, 1H), 7.01 (td, J = 8.9, 2.3 Hz, 1H), 4.46 (d, J = 5.7 Hz, 2H), 3.14 (s, 3H), 2.89 (s, 3H). ESI-MS m/z: 444.1 (MH^+). HRMS calcd $C_{21}H_{20}F_2N_5O_4$ [MH^+), 444.1478; found, 444.1475.

*4-Amino-N-(2,4-difluorobenzyl)-1-hydroxy-6-(3-(isopropylamino)-3-oxopropyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**6l**) and (E)-4-Amino-N-(2,4-difluorobenzyl)-1-hydroxy-6-(3-(isopropylamino)-3-oxoprop-1-en-1-yl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**6m**).* Treatment of **18f** as outlined in general procedures I and H and purification by preparative HPLC (with a linear gradient of 30% B to 45% B over 30 min) provided **6l** and **6m** as white fluffy solids. For **6l**: retention time = 23.0 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.62 (t, J = 5.7 Hz, 1H), 10.46 (brs, 1H), 8.50 (d, J = 1.8 Hz, 1H), 8.47 (s, 1H), 7.61 (d, J = 7.5 Hz, 1H), 7.36 (dd, J = 15.4, 8.7 Hz, 1H), 7.20−7.14 (m, 1H), 7.02−6.98 (m, 1H), 4.45 (d, J = 5.7 Hz, 2H), 3.76−3.68 (m, 1H), 2.84 (t, J = 7.5 Hz, 2H), 2.36 (t, J = 7.6 Hz, 2H), 0.90 (d, J = 6.6 Hz, 6H). ESI-MS m/z: 460.2 (MH^+). HRMS calcd $C_{22}H_{24}F_2N_5O_4$ [MH^+], 460.1791; found, 460.1772. For **6m**: retention time = 27.9 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.63 (s, 1H), 10.53 (t, J = 5.8 Hz, 1H), 8.84 (s, 1H), 8.82 (s, 1H), 8.01 (d, J = 7.7 Hz, 1H), 7.41 (d, J = 15.8 Hz, 1H), 7.37−7.33 (m, 1H), 7.20−7.15 (m, 1H), 7.00 (td, J = 8.6, 2.3 Hz, 1H), 6.66 (d, J = 15.9 Hz, 1H), 4.46 (d, J = 5.7 Hz, 2H), 3.91 (td, J = 13.3, 6.6 Hz, 1H), 1.05 (d, J = 6.6 Hz, 6H). ESI-MS m/z: 458.2 (MH^+). HRMS calcd $C_{22}H_{22}F_2N_5O_4$ [MH^+], 458.1634; found, 458.1628.

*4-Amino-N-(2,4-difluorobenzyl)-1-hydroxy-6-(3-((2-hydroxyethyl)amino)-3-oxopropyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**6n**) and (E)-4-Amino-N-(2,4-difluorobenzyl)-1-hydroxy-6-(3-((2-hydroxyethyl)amino)-3-oxoprop-1-en-1-yl)-*

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*2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**6o**).* Treatment of **18g** as outlined in general procedures I and H and purification by preparative HPLC (with a linear gradient of 20% B to 50% B over 30 min) provided **6n** and **6o** as white fluffy solids. For **6n**: retention time = 22.1 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.56 (d, J = 1.9 Hz, 1H), 8.49 (d, J = 1.8 Hz, 1H), 7.42 (dd, J = 15.4, 8.6 Hz, 1H), 7.25−7.19 (m, 1H), 7.06 (dd, J = 9.4, 7.7 Hz, 1H), 4.50 (s, 2H), 3.32 (t, J = 6.1 Hz, 2H), 3.08 (t, J = 6.1 Hz, 2H), 2.92 (t, J = 7.5 Hz, 2H), 2.48 (t, J = 7.5 Hz, 2H). ESI-MS m/z: 462.1 (MH^+), 484.1 (MNa^+). HRMS calcd $C_{21}H_{22}F_2N_5O_5$ [MH^+], 462.1584; found, 462.1562. For **6o**: retention time = 23.9 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.63 (s, 1H), 10.53 (t, J = 5.8 Hz, 1H), 8.85 (s, 1H), 8.83 (s, 1H), 8.09 (t, J = 5.8 Hz, 1H), 7.43 (d, J = 15.8 Hz, 1H), 7.36 (dd, J = 15.4, 8.6 Hz, 1H), 7.20−7.15 (m, 1H), 7.03−6.98 (m, 1H), 6.73 (d, J = 15.9 Hz, 1H), 4.65 (brs, 1H), 4.46 (d, J = 5.6 Hz, 2H), 3.40 (brs, 2H), 3.20 (dd, J = 11.9, 6.0 Hz, 2H). ESI-MS m/z: 460.1 (MH^+), 482.1 (MNa^+). HRMS calcd $C_{21}H_{20}F_2N_5O_5$ [MH^+], 460.1427; found, 460.1421.

*Methyl 3-(5-Amino-6-((2,4-difluorobenzyl)carbamoyl)-8-hydroxy-7-oxo-7,8-dihydro-1,8-naphthyridin-3-yl)propanoate (**6p**).* Treatment of **19g** as outlined in general procedure I and purification by preparative HPLC (linear gradient of 30% B to 65% B over 30 min; retention time = 23.4 min) provided **6p** as a white fluffy solid (72% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.68 (t, J = 5.8 Hz, 1H), 8.62 (d, J = 1.4 Hz, 1H), 8.58 (s, 1H), 7.42 (dd, J = 15.3, 8.6 Hz, 1H), 7.26−7.22 (m, 1H), 7.07 (t, J = 8.5 Hz, 1H), 4.51 (d, J = 5.8 Hz, 2H), 3.58 (s, 3H), 2.95 (t, J = 7.5 Hz, 2H), 2.77 (t, J = 7.6 Hz, 2H). ESI-MS m/z: 433.1 (MH^+). HRMS calcd $C_{20}H_{19}F_2N_4O_5$ [MH^+], 433.1318; found, 433.1311.

*Methyl 2-((3-((2,4-Difluorobenzyl)carbamoyl)-1-hydroxy-6-(5-hydroxypentyl)-2-oxo-1,2-dihydro-1,8-naphthyridin-4-yl)amino)-acetate (**6q**).* Treatment of **18i** as outlined in general procedure I and purification by preparative HPLC (linear gradient of 30% B to 60% B over 30 min; retention time = 20.5 min) provided **6q** as a white fluffy solid (82% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.22 (bs, 1H), 10.05 (bs, 1H), 8.48 (s, 1H), 8.22 (s, 1H), 7.52 (dd, J = 15.6, 8.6 Hz, 1H), 7.24−7.11 (m, 1H), 7.00 (t, J = 8.5 Hz, 1H), 4.41 (t, J = 5.5 Hz, 4H), 3.59 (s, 3H), 3.32 (t, J = 6.5 Hz, 4H), 2.62 (t, J = 7.6 Hz, 2H), 1.61−1.47 (m, 2H), 1.45−1.33 (m, 2H), 1.32−1.18 (m, 2H). ESI-MS m/z: 505.2 (MH^+). HRMS calcd $C_{24}H_{27}F_2N_4O_6$ [MH^+], 505.1893; found, 505.1889.

*N-(2,4-Difluorobenzyl)-1-hydroxy-4-((2-hydroxyethyl)amino)-6-(5-hydroxypentyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**6r**).* Treatment of **18j** as outlined in general procedure I and purification by preparative HPLC (linear gradient of 25% B to 40% B over 30 min; retention time = 24.5 min) provided **6r** as a white fluffy solid (22% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.98 (s, 1H), 10.52 (s, 1H), 8.54 (s, 1H), 8.37 (s, 1H), 7.50−7.47 (m, 1H), 7.24 (t, J = 10.0 Hz, 1H), 7.07 (t, J = 8.4 Hz, 1H), 4.50 (d, J = 5.6 Hz, 2H), 3.70 (bs, 2H), 3.61 (bs, 2H), 3.38 (t, J = 6.5 Hz, 2H), 2.69 (t, J = 7.4 Hz, 2H), 1.63−1.60 (m, 2H), 1.46−1.44 (m, 2H), 1.33−1.24 (m, 2H). ESI-MS m/z: 477.2 (MH^+). HRMS calcd $C_{23}H_{26}F_2N_4O_5$ [MH^+], 477.1944; found, 477.1944.

*5-(6-((2,4-Difluorobenzyl)carbamoyl)-8-hydroxy-5-((2-hydroxyethyl)amino)-7-oxo-7,8-dihydro-1,8-naphthyridin-3-yl)-pentyl Acetate (**6s**).* The mixture of **18j** (74 mg, 0.13 mmol), triethylamine (109 μL, 0.79 mmol), and acetic anhydride (37 μL, 0.40 mmol) in CH_2Cl_2 (3 mL) was stirred at room temperature (3 h). The crude mixture was purified by silica gel chromatography to provide 5-(8-(benzyloxy)-6-((2,4-difluorobenzyl)carbamoyl)-5-((2-hydroxyethyl)amino)-7-oxo-7,8-dihydro-1,8-naphthyridin-3-yl)pent-4-yn-1-yl acetate as a colorless oil (31 mg, 39% yield). [^{1}H NMR (500 MHz, $CDCl_3$) δ 12.00 (brs, 1H), 10.69 (t, J = 5.6 Hz, 1H), 8.31 (s, 1H), 7.67 (d, J = 6.9 Hz, 2H), 7.41−7.36 (m, 4H), 6.86−6.80 (m, 2H), 5.24 (s, 2H), 4.62 (d, J = 5.6 Hz, 2H), 4.35 (t, J = 5.4 Hz, 2H), 3.94 (dd, J = 10.6, 5.3 Hz, 2H), 3.83 (t, J = 5.9 Hz, 2H), 2.60 (t, J = 7.0 Hz, 2H), 2.11 (s, 3H), 1.90 (p, J = 6.5 Hz, 2H). ESI-MS m/z: 605.2 (MH^+).] This compound was treated as outlined in general procedure I and purified by preparative HPLC (linear gradient of 30% B to 55% B over 30 min; retention time = 24.1 min) to provide **6s** as a white fluffy solid (12% yield, two steps from **18j**). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.56 (brs, 1H), 10.29 (brs, 1H), 8.53 (s, 1H), 8.30 (s, 1H), 7.53−7.48 (m, 1H), 7.22 (t, J = 9.9 Hz, 1H), 7.07−7.03 (m, 1H), 4.48 (d, J = 5.8 Hz, 2H), 4.17−4.15 (m, 2H), 3.81 (d, J = 4.5 Hz, 2H), 3.38−3.36 (m, 2H), 2.68 (t, J = 7.4 Hz, 2H), 1.97 (s, 3H), 1.64−1.57 (m, 2H), 1.47−1.41 (m, 2H), 1.33−1.29 (m, 2H). ESI-MS m/z: 519.2 (MH^+), 541.1 (MNa^+).

*Methyl 3-(6-((2,4-Difluorobenzyl)carbamoyl)-8-hydroxy-5-((2-hydroxyethyl)amino)-7-oxo-7,8-dihydro-1,8-naphthyridin-3-yl)-propanoate (**6t**).* Treatment of **18k** as outlined in general procedure I and purification by preparative HPLC (linear gradient of 30% B to 50% B over 30 min; retention time = 21.0 min) provided **6t** as a white fluffy solid (20% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.09 (bs, 1H), 10.54 (t, J = 5.7 Hz, 1H), 8.57 (d, J = 1.8 Hz, 1H), 8.43 (d, J = 1.7 Hz, 1H), 7.46 (dd, J = 15.4, 8.6 Hz, 1H), 7.26−7.21 (m, 1H), 7.09−7.05 (m, 1H), 4.50 (d, J = 5.7 Hz, 2H), 3.71 (s, 2H), 3.61 (t, J = 5.1 Hz, 2H), 3.58 (s, 3H), 2.96 (t, J = 7.4 Hz, 2H), 2.73 (t, J = 7.4 Hz, 2H). ESI-MS m/z: 477.1 (MH^+). HRMS calcd $C_{23}H_{26}F_2N_4O_5$ [MH^+], 477.1580; found, 477.1581.

Methyl 2-Chloro-6-methoxynicotinate (8). Commercially available 2,6-dichloronicotinic acid **7** (3.1 g, 16 mmol) was added to the mixture of potassium *t*-butoxide (5.7 g, 48 mmol) in methanol (75 mL). The reaction mixture was stirred (65 °C, 24 h). The reaction mixture was concentrated and acidified using concentrated aqueous HCl. The crude mixture was filtered. The formed solid was collected to provide 2-chloro-6-methoxynicotinic acid as a white solid (2.8 g, 94% yield).[36] [^{1}H NMR (400 MHz, DMSO-d_6) δ 13.33 (bs, 1H), 8.19 (d, J = 8.5 Hz, 1H), 6.92 (d, J = 8.5 Hz, 1H), 3.92 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.52, 164.54, 147.69, 143.80, 120.02, 109.82, 54.86. ESI-MS m/z: 188.0 (MH^+).] 2-Chloro-6-methoxynicotinic acid (2.8 g, 15 mmol) was suspended in thionyl chloride (20 mL). The suspension was stirred and refluxed (3 h). The reaction mixture was concentrated. The residue was mixed with methanol (20 mL). The mixture was refluxed (3 h) and concentrated. The crude residue was purified by silica gel chromatography to provide **8** as a colorless oil (2.5 g, 81% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (d, J = 8.5 Hz, 1H), 6.48 (d, J = 8.5 Hz, 1H), 3.77 (s, 3H), 3.70 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.56, 164.25, 148.96, 142.60, 118.11, 108.96, 54.30, 52.09.

General Procedure A for the Synthesis of Methyl 2-((Benzyloxy)amino)-nicotinates (9 and 10a). 2-Chloronicotinates (**7** and **8**) (12 mmol) were mixed with *O*-benzylhydroxylamine (48 mmol) and DIEA (36 mmol). The reaction mixture was stirred (110 °C, 18 h). The crude mixture was purified by silica gel chromatography to provide methyl 2-((benzyloxy)amino)-nicotinates (**9** and **10a**).

*Methyl 2-((Benzyloxy)amino)-6-chloronicotinate (**9**).* Treatment of methyl 2,6-dichloronicotinate, which was prepared from commercially available 2,6-dichloronicotinic acid **7** in 91% yield based on known method.[42] ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, J = 8.1 Hz, 1H), 7.31 (d, J = 8.1 Hz, 1H), 3.90 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.95, 152.88, 149.74, 142.46, 125.12, 122.79, 52.94.], as outlined in general procedure A provided **9** as a colorless oil (55% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.08 (s, 1H), 8.07−7.97 (m, 1H), 7.52−7.46 (m, 2H), 7.41−7.25 (m, 3H), 6.77−6.68 (m, 1H), 5.03 (s, 2H), 3.80 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.13, 159.29, 155.57, 142.02, 135.92, 129.17(2C), 128.45(3C), 114.10, 104.88, 78.29, 52.26. ESI-MS m/z: 293.1 (MH^+).

*Methyl 2-((Benzyloxy)amino)-6-methoxynicotinate (**10a**).* Treatment of **8** as outlined in general procedure A provided **10a** as a colorless oil (51% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.22 (s, 1H), 7.99 (d, J = 8.6 Hz, 1H), 7.47−7.45 (m, 2H), 7.38−7.29 (m, 3H), 6.16 (d, J = 8.6 Hz, 1H), 5.09 (s, 2H), 3.99 (s, 3H), 3.78 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.82, 166.70, 160.47, 142.09, 136.36, 128.81 (2C), 128.42 (2C), 128.26, 101.26, 98.90, 78.39, 53.74, 51.67.

General Procedure B for the Synthesis of 6-Substituted Methyl 2-((Benzyloxy)amino)-nicotinates (10b and 10c). Methyl 2-((benzyloxy)amino)-6-chloronicotinate (**9**) (1 mmol) was dissolved in DMF (1 mL). Piperidine or morphiline (4 mmol) was added. The mixture was stirred and heated (80 °C, 1 h). The crude mixture was

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purified by silica gel chromatography to provide 6-substituted methyl 2-((benzyloxy)amino)-nicotinates (**10b** and **10c**).

*Methyl 2-((Benzyloxy)amino)-6-(piperidin-1-yl)nicotinate (**10b**).* Treatment of **9** with piperidine as outlined in general procedure B provided **10b** as a colorless oil (88% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.12 (s, 1H), 7.85 (dd, J = 9.0, 0.7 Hz, 1H), 7.46−7.44 (m, 2H), 7.36−7.30 (m, 4H), 6.05 (d, J = 9.0 Hz, 1H), 5.07 (s, 2H), 3.74 (d, J = 0.7 Hz, 3H), 3.39−3.66 (m, 4H), 1.67−1.65 (m, 2H), 1.63−1.61 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.99, 160.55, 159.92, 140.74, 136.79, 128.74 (2C), 128.60, 128.42, 128.33 (2C), 128.05, 97.10, 94.46, 78.11, 51.22, 45.70, 25.66, 24.74. ESI-MS m/z: 342.2 (MH^+).

*Methyl 2-((Benzyloxy)amino)-6-morpholinonicotinate (**10c**).* Treatment of **9** with morpholine as outlined in general procedure B provided **10c** as a colorless oil (99% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.13 (s, 1H), 7.88 (d, J = 8.8 Hz, 1H), 7.41 (dd, J = 8.0, 1.5 Hz, 2H), 7.34−7.23 (m, 3H), 5.99 (d, J = 8.9 Hz, 1H), 5.03 (s, 2H), 3.73 (s, 3H), 3.73−3.71 (m, 4H), 3.63−3.60 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.86, 160.19 (2C), 141.05, 136.70, 128.63 (2C), 128.36 (2C), 128.11, 96.84, 95.76, 78.11, 66.57 (2C), 51.36, 44.73 (2C). ESI-MS m/z: 344.2 (MH^+).

General Procedure C for the Synthesis of Methyl Nicotinates (11a−c). Methyl 3-chloro-3-oxopropanoate (12 mmol) was added dropwise to a solution of methyl 2-((benzyloxy)amino)-nicotinates (**10a−c**) (6 mmol) and triethylamine (12 mmol) in CH_2Cl_2 (40 mL). The mixture was stirred at room temperature (1 h). The crude mixture was filtered and the filtrate was concentrated. The crude residue was purified by silica gel chromatography to provide methyl nicotinates (**11a−c**).

*Methyl 2-(N-(Benzyloxy)-3-methoxy-3-oxopropanamido)-6-(piperidin-1-yl)nicotinate (**11b**).* Treatment of **10b** as outlined in general procedure C provided **11b** as a yellow oil (76% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (d, J = 8.9 Hz, 1H), 7.30 (d, J = 3.8 Hz, 2H), 7.24−7.23 (m, 3H), 6.49 (d, J = 9.0 Hz, 1H), 4.99 (s, 2H), 3.90 (s, 1H), 3.80 (s, 1H), 3.72 (s, 3H), 3.62 (s, 3H), 3.55−3.56 (m, 4H), 1.61−1.58 (m, 2H), 1.55−1.54 (m, 4H). ESI-MS m/z: 442.2 (MH^+).

*Methyl 2-(N-(Benzyloxy)-3-methoxy-3-oxopropanamido)-6-morpholinonicotinate (**11c**).* Treatment of **10c** as outlined in general procedure C provided **11c** as a yellow oil (79% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, J = 8.8 Hz, 1H), 7.32−7.28 (m, 5H), 6.52 (dd, J = 8.9, 0.9 Hz, 1H), 5.01 (s, 2H), 3.85−3.83 (m, 1H), 3.78 (s, 3H), 3.77−3.75 (m, 4H), 3.72−3.71 (m, 1H), 3.68 (s, 3H), 3.60−3.57 (m, 4H). ESI-MS m/z: 444.2 (MH^+).

General Procedure D for the Synthesis of Methyl Carboxylates (12a−c). A solution of sodium methanolate (16 mmol, 25% in methanol) was added to a solution of methyl nicotinates (**11a−c**) (6 mmol) in methanol (4 mL). The mixture was stirred at room temperature (18 h). The reaction mixture was brought to pH 4 by the addition of aqueous HCl (2N) and stirred (0 °C, 15 min). The crude suspension was filtered, and the solid was collected to provide methyl carboxylates (**12a−c**).

*Methyl 1-(Benzyloxy)-4-hydroxy-7-methoxy-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxylate (**12a**).* Treatment of **10a** as outlined in general procedure C and D provided **12a** as a white solid (54% yield, two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (d, J = 8.7 Hz, 1H), 7.62−7.59 (m, 2H), 7.35−7.32 (m, 3H), 6.61 (d, J = 8.7 Hz, 1H), 5.21 (s, 2H), 4.05 (s, 3H), 4.00 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.60, 170.32, 167.69, 156.93, 149.75, 136.69, 134.30, 129.68 (2C), 128.94, 128.42 (2C), 107.37, 102.64, 96.48, 77.90, 54.52, 53.01.

*Methyl 1-(Benzyloxy)-4-hydroxy-2-oxo-7-(piperidin-1-yl)-1,2-dihydro-1,8-naphthyridine-3-carboxylate (**12b**).* Treatment of **11b** as outlined in general procedure D provided **12b** as a white solid in 62% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.91 (s, 1H), 8.00 (d, J = 9.1 Hz, 1H), 7.61 (dd, J = 7.4, 1.7 Hz, 2H), 7.34 (d, J = 7.0 Hz, 3H), 6.49 (d, J = 9.1 Hz, 1H), 5.18 (s, 2H), 3.98 (s, 3H), 3.75−3.73 (m, 4H), 1.71−1.70 (m, 2H), 1.65−1.63 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.95, 170.00, 160.00, 157.59, 150.44, 135.06, 134.75, 129.66(2C), 128.71, 128.33(2C), 102.62, 98.44, 94.11, 77.47 (2C), 52.65, 46.08, 25.76 (2C), 24.56. ESI-MS m/z: 410.2 (MH^+).

*Methyl 1-(Benzyloxy)-4-hydroxy-7-morpholino-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxylate (**12c**).* Treatment of **11c** as outlined in general procedure D provided **12c** as a white solid (84% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.97 (d, J = 9.3 Hz, 1H), 7.53−7.51 (m, 2H), 7.39−7.33 (m, 4H), 6.60 (d, J = 9.3 Hz, 1H), 5.00 (s, 2H), 3.72−3.54 (m, 11H). ESI-MS m/z: 412.2 (MH^+).

General Procedure E for the Synthesis of Carboxyamides (13a−c). A solution of methyl carboxylates (**12a−c**) (0.3 mmol) and (2,4-difluorophenyl)methanamine (1.5 mmol) in DMF (1 mL) was heated (140 °C, 2 h) in microwave reactor. The crude mixture was purified by silica gel chromatography and recrystallized from methanol to provide carboxamides (**13a−c**).

*1-(Benzyloxy)-N-(2,4-difluorobenzyl)-4-hydroxy-7-methoxy-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**13a**).* Treatment of **12a** as outlined in general procedure E provided **13a** as a white solid (45% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.24 (t, J = 5.6 Hz, 1H), 8.22 (dd, J = 8.7, 0.4 Hz, 1H), 7.56−7.53 (m, 2H), 7.35−7.29 (m, 4H), 6.81−6.73 (m, 2H), 6.62 (dd, J = 8.7, 0.4 Hz, 1H), 5.20 (d, J = 0.6 Hz, 2H), 4.58 (d, J = 6.0 Hz, 2H), 4.02 (d, J = 0.5 Hz, 3H). ESI-MS m/z: 468.2 (MH^+).

*1-(Benzyloxy)-N-(2,4-difluorobenzyl)-4-hydroxy-2-oxo-7-(piperidin-1-yl)-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**13b**).* Treatment of **12b** as outlined in general procedure E provided **13b** as a white solid (90% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.30 (t, J = 6.0 Hz, 1H), 10.03 (s, 1H), 8.05 (dd, J = 9.1, 0.7 Hz, 1H), 7.58−7.56 (m, 2H), 7.38−7.33 (m, 4H), 6.85−6.76 (m, 2H), 6.54 (dd, J = 9.1, 0.7 Hz, 1H), 5.20 (s, 2H), 4.60 (d, J = 6.0 Hz, 2H), 3.76−3.74 (m, 4H), 1.71−1.69 (m, 2H), 1.67−1.61 (m, 4H). ESI-MS m/z: 521.2 (MH^+).

*1-(Benzyloxy)-N-(2,4-difluorobenzyl)-4-hydroxy-7-morpholino-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**13c**).* Treatment of **12c** as outlined in general procedure E provided **13c** as a white solid (89% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.22 (bs, 1H), 8.08 (d, J = 9.1 Hz, 1H), 7.50−7.48 (m, 2H), 7.33−7.24 (m, 4H), 6.79−6.69 (m, 2H), 6.48 (d, J = 9.1 Hz, 1H), 5.15 (s, 2H), 4.56 (d, J = 6.2 Hz, 2H), 3.73−3.66 (m, 8H). ESI-MS m/z: 523.2 (MH^+).

General Procedure F for the Synthesis of *p*-Methylbenzenesulfonates (14a−c). Compound 4-hydroxyl analogues (**13a−c**) (0.5 mmol) were dissolved in CH_3CN (3 mL). DIEA (2.8 mmol), CH_2Cl_2 (2 mL), and *p*-methylbenzene-1-sulfonyl chloride (1.4 mmol) were added. The reaction mixture was stirred at room temperature (18 h). The mixture crude was purified by silica gel chromatography to provide *p*-methylbenzenesulfonates (**14a−c**).

*1-(Benzyloxy)-3-((2,4-difluorobenzyl)carbamoyl)-7-methoxy-2-oxo-1,2-dihydro-1,8-naphthyridin-4-yl 4-Methylbenzenesulfonate (**14a**).* Treatment of **13a** as outlined in general procedure F provided **14a** as a colorless oil (68% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (t, J = 5.9 Hz, 1H), 8.01 (d, J = 8.8 Hz, 1H), 7.87 (d, J = 8.3 Hz, 2H), 7.58−7.56 (m, 2H), 7.46−7.44 (m, 1H), 7.37−7.35 (m, 3H), 7.32 (dd, J = 8.6, 0.7 Hz, 2H), 6.83−6.74 (m, 2H), 6.64 (d, J = 8.8 Hz, 1H), 5.25 (s, 2H), 4.48−4.46 (m, 2H), 4.06 (s, 3H), 2.43 (s, 3H). ESI-MS m/z: 622.1(MH^+).

*1-(Benzyloxy)-3-((2,4-difluorobenzyl)carbamoyl)-2-oxo-7-(piperidin-1-yl)-1,2-dihydro-1,8-naphthyridin-4-yl 4-Methylbenzenesulfonate (**14b**).* Treatment of **13b** as outlined in general procedure F provided **14b** as a colorless oil (53% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.83 (t, J = 5.9 Hz, 1H), 7.88 (dd, J = 8.8, 3.2 Hz, 3H), 7.58−7.55 (m, 2H), 7.44−7.35 (m, 4H), 7.31−7.29 (m, 2H), 6.82−6.73 (m, 2H), 6.57 (d, J = 9.3 Hz, 1H), 5.22 (s, 2H), 4.44 (s, 2H), 3.76 (bs, 4H), 2.42 (s, 3H), 1.72−1.69 (m, 2H), 1.66−1.64 (m, 4H). ESI-MS m/z: 675.2 (MH^+).

*1-(Benzyloxy)-3-((2,4-difluorobenzyl)carbamoyl)-7-morpholino-2-oxo-1,2-dihydro-1,8-naphthyridin-4-yl 4-Methylbenzenesulfonate (**14c**).* Treatment of **13c** as outlined in general procedure F provided **14c** as a colorless oil (27% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (t, J = 5.9 Hz, 1H), 8.19−8.18 (m, 2H), 7.94 (d, J = 9.2 Hz, 1H), 7.86 (d, J = 8.4 Hz, 2H), 7.36−7.50 (m, 2H), 7.36−7.28 (m, 3H), 6.82−6.74 (m, 3H), 6.55 (dd, J = 9.2, 0.7 Hz, 1H), 5.21 (s, 2H), 4.43−4.41

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(m, 2H), 3.78−3.73 (m, 8H), 2.42 (s, 3H). ESI-MS *m/z*: 677.2 (MH^+).

General Procedure G for the Synthesis of 4-Substituted Amines (15a−f, 17d, and 17e). A solution of *p*-methylbenzenesulfonates (**14a−c** and **17b**) (0.2 mmol), DIPEA (2 mmol), and methyl 2-aminoacetate hydrochloride or 2,4-dimethoxyphenyl)methanamine (1 mmol) in DMF (2 mL) was stirred and heated (50 °C, 1 h). The crude mixture was purified by silica gel chromatography to provide 4-substituted amines (**15a−f**, **17d**, and **17e**).

*Methyl 2-((1-(Benzyloxy)-3-((2,4-difluorobenzyl)carbamoyl)-7-methoxy-2-oxo-1,2-dihydro-1,8-naphthyridin-4-yl)amino)acetate (**15a**).* Treatment of **14a** with methyl 2-aminoacetate hydrochloride as outlined in general procedure G provided **15a** as a white solid (91% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.20 (t, J = 5.5 Hz, 1H), 10.70 (t, J = 5.7 Hz, 1H), 8.02 (d, J = 8.9 Hz, 1H), 7.59−7.56 (m, 2H), 7.41−7.32 (m, 4H), 6.82−6.74 (m, 2H), 6.55 (d, J = 8.9 Hz, 1H), 5.20 (s, 2H), 4.61 (d, J = 5.8 Hz, 2H), 4.35 (d, J = 5.5 Hz, 2H), 4.02 (s, 3H), 3.76 (s, 3H). ESI-MS *m/z*: 539.2 (MH^+).

*Methyl 2-((1-(Benzyloxy)-3-((2,4-difluorobenzyl)carbamoyl)-2-oxo-7-(piperidin-1-yl)-1,2-dihydro-1,8-naphthyridin-4-yl)amino)-acetate (**15b**).* Treatment of **14b** with methyl 2-aminoacetate hydrochloride as outlined in general procedure G provided **15b** as a white solid (80% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.99 (t, J = 5.7 Hz, 1H), 10.78 (t, J = 5.8 Hz, 1H), 7.81 (d, J = 9.3 Hz, 1H), 7.58−7.55 (m, 2H), 7.41−7.31 (m, 4H), 6.81−6.72 (m, 2H), 6.40 (d, J = 9.3 Hz, 1H), 5.18 (s, 2H), 4.60 (d, J = 5.7 Hz, 2H), 4.32 (d, J = 5.6 Hz, 2H), 3.76 (s, 3H), 3.71−3.69 (m, 4H), 1.71−1.67 (m, 2H), 1.64−1.59 (m, 4H). ESI-MS *m/z*: 592.2 (MH^+).

*Methyl 2-((1-(Benzyloxy)-3-((2,4-difluorobenzyl)carbamoyl)-7-morpholino-2-oxo-1,2-dihydro-1,8-naphthyridin-4-yl)amino)-acetate (**15c**).* Treatment of **14c** with methyl 2-aminoacetate hydrochloride as outlined in general procedure G provided **15c** as a white solid (63% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.06 (t, J = 5.6 Hz, 1H), 10.73 (t, J = 5.8 Hz, 1H), 7.88 (dd, J = 9.2, 0.8 Hz, 1H), 7.53−7.50 (m, 2H), 7.41−7.27 (m, 3H), 6.81−6.73 (m, 3H), 6.38 (dd, J = 9.3, 0.8 Hz, 1H), 5.16 (s, 2H), 4.60 (d, J = 5.8 Hz, 2H), 4.32 (d, J = 5.5 Hz, 2H), 3.76 (s, 3H), 3.75−3.73 (m, 4H), 3.68−3.65 (m, 4H). ESI-MS *m/z*: 594.2 (MH^+).

*1-(Benzyloxy)-N-(2,4-difluorobenzyl)-4-((2,4-dimethoxybenzyl)-amino)-7-methoxy-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**15d**).* Treatment of **14a** with (2,4-dimethoxyphenyl)-methanamine as outlined in general procedure G provided **15d** as a white solid which was directly used in next step. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.88 (t, J = 6.2 Hz, 1H), 10.77 (t, J = 5.8 Hz, 1H), 8.10 (dd, J = 8.9, 0.7 Hz, 1H), 7.61−7.59 (m, 2H), 7.38−7.31 (m, 4H), 7.24−7.21 (m, 1H), 6.81−6.73 (m, 2H), 6.47−6.42 (m, 3H), 5.24 (s, 2H), 4.67 (d, J = 6.1 Hz, 2H), 4.56 (d, J = 5.7 Hz, 2H), 4.02 (s, 3H), 3.77 (s, 3H), 3.75 (s, 3H). ESI-MS *m/z*: 617.2 (MH^+).

*1-(Benzyloxy)-N-(2,4-difluorobenzyl)-4-((2,4-dimethoxybenzyl)-amino)-2-oxo-7-(piperidin-1-yl)-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**15e**).* Treatment of **14b** with (2,4-dimethoxyphenyl)-methanamine as outlined in general procedure G provided **15e** as a white solid (65% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.71 (t, J = 6.2 Hz, 1H), 10.85 (t, J = 5.8 Hz, 1H), 7.88 (d, J = 9.3 Hz, 1H), 7.60−7.57 (m, 2H), 7.37−7.32 (m, 4H), 7.27−7.24 (m, 1H), 6.81−6.72 (m, 2H), 6.44−6.42 (m, 2H), 6.30 (d, J = 9.3 Hz, 1H), 5.20 (s, 2H), 4.64 (d, J = 6.2 Hz, 2H), 4.55 (d, J = 6.1 Hz, 2H), 3.77 (s, 3H), 3.76 (s, 3H), 3.70−3.67 (m, 4H), 1.70−1.66 (m, 2H), 1.63−1.58 (m, 4H). ESI-MS *m/z*: 670.3 (MH^+).

*1-(Benzyloxy)-N-(2,4-difluorobenzyl)-4-((2,4-dimethoxybenzyl)-amino)-7-morpholino-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**15f**).* Treatment of **14c** with (2,4-dimethoxyphenyl)-methanamine as outlined in general procedure G provided **15f** as a white solid which was directly used in next step. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.73 (t, J = 6.1 Hz, 1H), 10.75 (t, J = 5.8 Hz, 1H), 7.95 (d, J = 9.2 Hz, 1H), 7.52−7.50 (m, 2H), 7.33−7.21 (m, 5H), 6.80−6.71 (m, 4H), 6.30 (d, J = 9.2 Hz, 1H), 5.16 (s, 2H), 4.63 (d, J = 6.1 Hz, 2H), 4.51 (d, J = 5.8 Hz, 2H), 3.76 (s, 3H), 3.74 (s, 3H), 3.73−3.71 (m, 4H), 3.65−3.63 (m, 4H). ESI-MS *m/z*: 672.3 (MH^+).

General Procedure H for the Synthesis of Amine Analogues (16a−c, 19b−g). The 2,4-dimethoxybenzyl protected compounds (**15d−f** and **18a−h**) (0.25 mmol) were dissolved in CH_2Cl_2 (2 mL). TFA (2 mL) was added at room temperature. The reaction mixture was concentrated. The crude residue was purified by silica gel chromatography to provide amine analogues (**16a−c** and **19b−g**).

*4-Amino-1-(benzyloxy)-N-(2,4-difluorobenzyl)-7-methoxy-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**16a**).* Treatment of **15d** as outlined in general procedure H provided **16a** as a colorless oil (72% yield, two steps (general procedures G and H). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.60 (t, J = 5.9 Hz, 1H), 7.85 (dd, J = 8.7, 0.7 Hz, 1H), 7.58 (dd, J = 7.3, 2.2 Hz, 2H), 7.36−7.32 (m, 4H), 6.81−6.74 (m, 2H), 6.58 (dd, J = 8.8, 0.7 Hz, 1H), 5.21 (s, 2H), 4.58 (d, J = 5.8 Hz, 2H), 4.03 (s, 3H). ESI-MS *m/z*: 467.2 (MH^+).

*4-Amino-1-(benzyloxy)-N-(2,4-difluorobenzyl)-2-oxo-7-(piperidin-1-yl)-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**16b**).* Treatment of **15e** as outlined in general procedure H provided **16b** as a colorless oil (46% yield, two steps (general procedures G and H). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.62 (t, J = 5.9 Hz, 1H), 7.58 (d, J = 9.1 Hz, 1H), 7.54−7.52 (m, 2H), 7.35−7.26 (m, 4H), 6.78−6.69 (m, 2H), 6.44 (d, J = 9.2 Hz, 1H), 5.15 (s, 2H), 4.54 (d, J = 5.8 Hz, 2H), 3.70−3.65 (m, 4H), 1.66−1.63 (m, 2H), 1.61−1.57 (m, 4H). ESI-MS *m/z*: 520.2 (MH^+).

*4-Amino-1-(benzyloxy)-N-(2,4-difluorobenzyl)-7-morpholino-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**16c**).* Treatment of **15f** as outlined in general procedure H provided **16c** as a colorless oil (32% yield, two steps (general procedures G and H). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.61 (t, J = 5.7 Hz, 1H), 7.70 (d, J = 9.0 Hz, 1H), 7.57−7.49 (m, 2H), 7.41−7.28 (m, 3H), 6.83−6.71 (m, 3H), 6.48 (dd, J = 9.1, 0.7 Hz, 1H),5.18 (s, 2H), 4.58 (d, J = 5.8 Hz, 2H), 3.81−3.75 (m, 4H), 3.69−3.67 (m, 4H). ESI-MS *m/z*: 522.2 (MH^+).

*Methyl 2-((1-(Benzyloxy)-6-bromo-3-((2,4-difluorobenzyl)-carbamoyl)-2-oxo-1,2-dihydro-1,8-naphthyridin-4-yl)amino)-acetate (**17d**).* Treatment of 1-(benzyloxy)-6-bromo-3-((2,4-difluorobenzyl)carbamoyl)-2-oxo-1,2-dihydro-1,8-naphthyridin-4-yl 4-methylbenzenesulfonate **17b**[31] with methyl glycinate as outlined in general procedure G provided **17d** as a white solid (74% 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). ESI-MS *m/z*: 587.1, 589.1 (MH^+).

*1-(Benzyloxy)-6-bromo-N-(2,4-difluorobenzyl)-4-((2-hydroxyethyl)amino)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**17e**).* Treatment of 1-(benzyloxy)-6-bromo-3-((2,4-difluorobenzyl)carbamoyl)-2-oxo-1,2-dihydro-1,8-naphthyridin-4-yl 4-methylbenzenesulfonate **17b**[31] with 2-aminoethan-1-ol as outlined in general procedure G provided **17e** as a white solid (93% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.65 (bs, 1H), 10.65 (bs, 1H), 8.64 (d, J = 1.9 Hz, 1H), 8.47 (d, J = 1.9 Hz, 1H), 7.58 (d, J = 6.1 Hz, 2H), 7.31 (t, J = 7.2 Hz, 4H), 6.79−6.73 (m, 2H), 5.15 (s, 2H), 4.55 (d, J = 5.6 Hz, 2H), 3.88−3.86 (m, 2H), 3.78−3.75 (m, 2H). ESI-MS *m/z*: 559.1, 561.1 (MH^+).

General Procedure J for the Synthesis of 6-Alkylated Analogues (18a, 18b, 18d, 18i, and 18j) Using Sonogashira Reaction. Bromides (**17a** and **17c−e**) (0.3 mmol) were mixed with bis(triphenylphosphine)palladium(II) dichloride (9 μmol), DIEA (0.3 mmol), and copper(I) iodide (0.03 mmol) in DMF (1.5 mL) in a reaction tube. The related terminal alkynes (0.45 mmol) were added. The reaction mixture was flushed with argon and sealed with a cap and heated (70 °C, 4 h). The crude mixture was purified by silica gel chromatography to provide 6-alkylated analogue (**18a**, **18b**, **18d**, **18i**, and **18j**).

General Procedure K for the Synthesis of 6-Alkylated Analogues (18c, 18e−h, and 18k) Using Heck Reaction. The mixture of bromides (**17c** and **17e**) (0.2 mmol), alkenes (0.4 mmol), triethylamine (0.6 mmol), diacetoxypalladium (0.04 mmol), and triphenylphosphine (0.08 mmol) in DMF (0.5 mL) was flushed with argon and sealed in a reaction tube. The reaction mixture was microwave-heated (100 °C, 5 h). The crude mixture was purified by silica gel chromatography to provide 6-alkylated analogues (**18c**, **18e−h**, and **18k**).

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*1-(Benzyloxy)-6-(3-cyclohexylprop-1-yn-1-yl)-N-(2,4-difluorobenzyl)-4-((2,4-dimethoxybenzyl)amino)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**18a**).* Treatment of 1-(benzyloxy)-6-bromo-*N*-(2,4-difluorobenzyl)-4-((2,4-dimethoxybenzyl)amino)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide **17c**[31] with commercially available prop-2-yn-1-ylcyclohexane as outlined in general procedure J provided **18a** as a colorless oil (79% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.02 (t, J = 5.8 Hz, 1H), 10.70 (t, J = 5.7 Hz, 1H), 8.66 (t, J = 1.6 Hz, 1H), 8.32 (s, 1H), 7.68 (d, J = 7.7 Hz, 2H), 7.40−7.36 (m, 4H), 7.25−7.24 (m, 1H), 6.85−6.78 (m, 2H), 6.48−6.45 (m, 2H), 5.25 (s, 2H), 4.75 (d, J = 6.0 Hz, 2H), 4.60 (d, J = 5.6 Hz, 2H), 3.82 (s, 3H), 3.80 (s, 3H), 2.32 (d, J = 6.6 Hz, 2H), 1.85 (d, J = 12.3 Hz, 2H), 1.78−1.68 (m, 3H), 1.57 (ddd, J = 10.9, 7.0, 3.6 Hz, 1H), 1.31−1.16 (m, 3H), 1.05 (qd, J = 12.4, 3.3 Hz, 2H).

*1-(Benzyloxy)-N-(2,4-difluorobenzyl)-4-((2,4-dimethoxybenzyl)amino)-2-oxo-6-(4-phenylbut-1-yn-1-yl)-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**18b**).* Treatment of 1-(benzyloxy)-6-bromo-*N*-(2,4-difluorobenzyl)-4-((2,4-dimethoxybenzyl)amino)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide **17c**[31] with commercially available but-3-yn-1-ylbenzene as outlined in general procedure J provided **18b** as a colorless oil (98% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.99 (t, J = 5.6 Hz, 1H), 10.68 (t, J = 5.7 Hz, 1H), 8.62 (s, 1H), 8.31 (s, 1H), 7.68−7.66 (m, 2H), 7.39−7.31 (m, 6H), 7.27−7.21 (m, 4H), 6.85−6.77 (m, 2H), 6.47−6.46 (m, 2H), 5.24 (s, 2H), 4.73 (d, J = 5.8 Hz, 2H), 4.59 (d, J = 5.7 Hz, 2H), 3.81 (s, 3H), 3.76 (s, 3H), 2.92 (d, J = 7.4 Hz, 2H), 2.73 (t, J = 7.4 Hz, 2H).

*(E)-1-(Benzyloxy)-N-(2,4-difluorobenzyl)-4-((2,4-dimethoxybenzyl)amino)-2-oxo-6-styryl-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**18c**).* Treatment of 1-(benzyloxy)-6-bromo-*N*-(2,4-difluorobenzyl)-4-((2,4-dimethoxybenzyl)amino)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide **17c**[31] with commercially available styrene as outlined in general procedure K provided **18c** as a colorless oil (27% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.14 (t, J = 6.7 Hz, 1H), 10.81 (t, J = 5.9 Hz, 1H), 8.71 (s, 1H), 8.44 (s, 1H), 7.71 (d, J = 6.7 Hz, 2H), 7.44−7.38 (m, 10H), 6.99 (d, J = 16.4 Hz, 1H), 6.88−6.81 (m, 2H), 6.58−6.52 (m, 3H), 5.29 (s, 2H), 4.80 (d, J = 6.7 Hz, 2H), 4.65 (d, J = 5.6 Hz, 2H), 3.86 (s, 3H), 3.77 (s, 3H).

*1-(Benzyloxy)-N-(2,4-difluorobenzyl)-4-((2,4-dimethoxybenzyl)amino)-6-(3-(2-hydroxyethoxy)prop-1-yn-1-yl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**18d**).* Treatment of 1-(Benzyloxy)-6-bromo-*N*-(2,4-difluorobenzyl)-4-((2,4-dimethoxybenzyl)amino)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide **17c**[31] with 2-(prop-2-yn-1-yloxy)ethanol as outlined in general procedure J provided **18d** as a colorless oil (64% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.06 (t, J = 5.9 Hz, 1H), 10.66 (t, J = 5.7 Hz, 1H), 8.70 (d, J = 1.2 Hz, 1H), 8.38 (d, J = 1.4 Hz, 1H), 7.67 (d, J = 6.6 Hz, 2H), 7.41−7.34 (m, 4H), 7.24 (d, J = 7.9 Hz, 1H), 6.85−6.79 (m, 2H), 6.49−6.43 (m, 2H), 5.25 (s, 2H), 4.75 (d, J = 6.0 Hz, 2H), 4.60 (d, J = 5.7 Hz, 2H), 4.45 (s, 2H), 3.82 (s, 3H), 3.84−3.81 (m, 2H), 3.80 (s, 3H), 3.71−3.69 (m, 2H).

*(E)-1-(Benzyloxy)-N-(2,4-difluorobenzyl)-4-((2,4-dimethoxybenzyl)amino)-6-(3-(dimethylamino)-3-oxoprop-1-en-1-yl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**18e**).* Treatment of 1-(benzyloxy)-6-bromo-*N*-(2,4-difluorobenzyl)-4-((2,4-dimethoxybenzyl)amino)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide **17c**[31] with commercially available *N*,*N*-dimethylacrylamide as outlined in general procedure K provided **18e** as a colorless oil (41% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.17 (t, J = 6.5 Hz, 1H), 10.74 (t, J = 5.7 Hz, 1H), 8.79 (d, J = 1.8 Hz, 1H), 8.39 (d, J = 1.8 Hz, 1H), 7.72−7.67 (m, 3H), 7.60−7.55 (m, 1H), 7.51−7.46 (m, 1H), 7.44−7.38 (m, 4H), 6.88−6.81 (m, 2H), 6.51−6.48 (m, 2H), 5.28 (s, 2H), 4.77 (d, J = 6.6 Hz, 2H), 4.64 (d, J = 5.7 Hz, 2H), 3.83 (s, 3H), 3.83 (s, 3H), 3.07 (s, 3H), 3.00 (s, 3H).

*(E)-1-(Benzyloxy)-N-(2,4-difluorobenzyl)-4-((2,4-dimethoxybenzyl)amino)-6-(3-(isopropylamino)-3-oxoprop-1-en-1-yl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**18f**).* Treatment of 1-(benzyloxy)-6-bromo-*N*-(2,4-difluorobenzyl)-4-((2,4-dimethoxybenzyl)amino)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide **17c**[31] with commercially available *N*-isopropylacrylamide as outlined in general procedure K provided **18f** as a colorless oil (93% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.14 (t, J = 6.8 Hz, 1H), 10.75 (t, J = 5.7 Hz, 1H), 8.65 (d, J = 1.8 Hz, 1H), 8.35 (d, J = 1.8 Hz, 1H), 7.69−7.64 (m, 2H), 7.51−7.51 (m, 1H), 7.48−7.42 (m, 3H), 7.40−7.33 (m, 3H), 6.87−6.79 (m, 2H), 6.58 (dd, J = 8.3, 2.2 Hz, 1H), 6.54 (d, J = 2.2 Hz, 1H), 5.44 (d, J = 15.6 Hz, 1H), 5.25 (s, 2H), 4.71 (d, J = 6.8 Hz, 2H), 4.61 (t, J = 9.6 Hz, 2H), 4.24−4.16 (m, 1H), 3.86 (s, 3H), 3.79 (s, 3H), 1.24 (s, 3H), 1.23 (s, 3H). ESI-MS m/z: 698.2 (MH^+), 720.2 (MNa^+).

*(E)-1-(Benzyloxy)-N-(2,4-difluorobenzyl)-4-((2,4-dimethoxybenzyl)amino)-6-(3-((2-hydroxyethyl)amino)-3-oxoprop-1-en-1-yl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**18g**).* Treatment of 1-(benzyloxy)-6-bromo-*N*-(2,4-difluorobenzyl)-4-((2,4-dimethoxybenzyl)amino)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide **17c**[31] with commercially available *N*-(2-hydroxyethyl)-acrylamide as outlined in general procedure K provided **18g** (84% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.54 (s, 1H), 6.31 (d, J = 17.0 Hz, 1H), 6.16 (dd, J = 17.0, 10.2 Hz, 1H), 5.69 (d, J = 10.2 Hz, 1H), 3.77 (t, J = 5.0 Hz, 2H), 3.51 (dd, J = 9.8, 4.9 Hz, 2H), 3.31 (d, J = 25.1 Hz, 1H).

*(E)-Methyl 3-(8-(Benzyloxy)-6-((2,4-difluorobenzyl)carbamoyl)-5-((2,4-dimethoxybenzyl)amino)-7-oxo-7,8-dihydro-1,8-naphthyridin-3-yl)acrylate (**18h**).* Treatment of 1-(benzyloxy)-6-bromo-*N*-(2,4-difluorobenzyl)-4-((2,4-dimethoxybenzyl)amino)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide **17c**[31] with commercially available methyl acrylate as outlined in general procedure K provided **18h** as a colorless oil (38% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.35 (t, J = 6.6 Hz, 1H), 10.73 (t, J = 5.6 Hz, 1H), 8.72 (s, 1H), 8.40 (s, 1H), 7.69−7.66 (m, 2H), 7.59 (d, J = 16.1 Hz, 1H), 7.42−7.38 (m, 4H), 7.23 (d, J = 8.0 Hz, 1H), 6.88−6.82 (m, 2H), 6.57 (d, J = 1.9 Hz, 1H), 6.54−6.50 (m, 1H), 6.34−6.32 (d, J = 8.3 Hz, 1H), 6.02 (d, J = 16.0 Hz, 1H), 5.27 (s, 2H), 4.78 (d, J = 6.7 Hz, 2H), 4.64 (d, J = 5.7 Hz, 2H), 3.85 (s, 3H), 3.85 (s, 3H), 3.83 (s, 3H).

*Methyl 2-((1-(Benzyloxy)-3-((2,4-difluorobenzyl)carbamoyl)-6-(5-hydroxypent-1-yn-1-yl)-2-oxo-1,2-dihydro-1,8-naphthyridin-4-yl)-amino)acetate (**18i**).* Treatment of **17d** with commercially available pent-4-yn-1-ol as outlined in general procedure J provided **18i** as a colorless oil (45% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.33 (s, 1H), 10.65 (t, J = 5.7 Hz, 1H), 8.69 (s, 1H), 8.19 (s, 1H), 7.67−7.65 (m, 2H), 7.43−7.37 (m, 4H), 6.87−6.80 (m, 2H), 5.24 (s, 2H), 4.67 (d, J = 5.6 Hz, 2H), 4.46 (d, J = 4.9 Hz, 2H), 3.84 (s, 3H), 3.85−3.83 (m, 2H), 2.61 (t, J = 7.0 Hz, 2H), 1.91 (t, J = 6.5 Hz, 2H). ESI-MS m/z: 591.2 (MH^+).

*1-(Benzyloxy)-N-(2,4-difluorobenzyl)-4-((2-hydroxyethyl)amino)-6-(5-hydroxypent-1-yn-1-yl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**18j**).* Treatment of **17e** with commercially available pent-4-yn-1-ol as outlined in general procedure J provided **18j** as a colorless oil (57% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.68 (s, 1H), 10.69 (t, J = 5.5 Hz, 1H), 8.61 (d, J = 1.8 Hz, 1H), 8.32 (d, J = 1.8 Hz, 1H), 7.61−7.57 (m, 2H), 7.32−7.29 (m, 4H), 6.79−6.72 (m, 2H), 5.16 (s, 2H), 4.55 (d, J = 5.5 Hz, 2H), 3.86−3.84 (m, 2H), 3.80−3.75 (m, 2H), 2.52 (t, J = 7.0 Hz, 2H), 1.84−1.82 (m, 2H), 1.53−1.49 (m, 2H). ESI-MS m/z: 563.2 (MH^+).

*(E)-Methyl 3-(8-(Benzyloxy)-6-((2,4-difluorobenzyl)carbamoyl)-5-((2-hydroxyethyl)amino)-7-oxo-7,8-dihydro-1,8-naphthyridin-3-yl)-acrylate (**18k**).* Treatment of **17e** with commercially available methyl acrylate as outlined in general procedure K provided **18k** as a colorless oil (52% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.82 (s, 1H), 10.71 (s, 1H), 8.84 (s, 1H), 8.67 (s, 1H), 7.74 (d, J = 16.2 Hz, 1H), 7.58−7.55 (m, 2H), 7.41−7.38 (m, 4H), 6.87−6.81 (m, 2H), 6.55 (d, J = 16.1 Hz, 1H), 5.26 (s, 2H), 4.64 (d, J = 5.6 Hz, 2H), 3.85 (s, 3H), 3.60 (t, J = 4.9 Hz, 2H), 2.62−2.60 (m, 2H). ESI-MS m/z: 565.2 (MH^+).

*3-(5-Amino-8-(benzyloxy)-6-((2,4-difluorobenzyl)carbamoyl)-7-oxo-7,8-dihydro-1,8-naphthyridin-3-yl)prop-2-yn-1-yl Acetate (**19a**).* Compound 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[31] (29 mg, 0.06 mmol) was mixed with acetic anhydride (8.4 μL, 0.09 mmol) in pyridine (0.5 mL). The solution was stirred at room temperature (2 h). The crude mixture was purified by silica gel chromatography to provide **19a** as a colorless oil (24 mg, 76% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.54 (t, J = 5.7 Hz, 1H), 8.77 (d, J = 1.7 Hz, 1H), 8.16 (d, J = 1.7 Hz, 1H), 7.65 (dd, J = 7.5, 1.6 Hz, 2H),

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7.42−7.33 (m, 4H), 6.87−6.80 (m, 2H), 5.25 (s, 2H), 4.93 (s, 2H), 4.63 (d, J = 5.7 Hz, 2H), 2.16 (s, 3H). ESI-MS m/z: 533.2 (MH^+).

*4-Amino-1-(benzyloxy)-6-(3-cyclohexylprop-1-yn-1-yl)-N-(2,4-difluorobenzyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**19b**).* Treatment of **18a** as outlined in general procedure H provided **19b** as a colorless oil (91% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.60 (t, J = 5.8 Hz, 1H), 8.74 (d, J = 1.8 Hz, 1H), 8.03 (d, J = 1.9 Hz, 1H), 7.66 (dd, J = 7.5, 1.8 Hz, 2H), 7.42−7.35 (m, 4H), 6.87−6.80 (m, 2H), 5.25 (s, 2H), 4.63 (d, J = 5.8 Hz, 2H), 2.34 (d, J = 6.7 Hz, 2H), 1.88 (dd, J = 13.5, 1.9 Hz, 2H), 1.78−1.74 (m, 2H), 1.70 (ddd, J = 12.6, 5.0, 2.6 Hz, 1H), 1.62−1.55 (m, 1H), 1.34−1.25 (m, 2H), 1.22−1.16 (m, 1H), 1.07 (ddd, J = 24.4, 12.4, 3.3 Hz, 2H). ESI-MS m/z: 557.2 (MH^+).

*4-Amino-1-(benzyloxy)-N-(2,4-difluorobenzyl)-2-oxo-6-(4-phenylbut-1-yn-1-yl)-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**19c**).* Treatment of **18b** as outlined in general procedure H provided **19c** as a colorless oil (94% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.61 (t, J = 5.8 Hz, 1H), 8.69 (s, 1H), 8.00 (s, 1H), 7.65 (dd, J = 7.1, 1.7 Hz, 2H), 7.39−7.32 (m, 6H), 7.28−7.26 (m, 3H), 6.87−6.80 (m, 2H), 5.24 (s, 2H), 4.63 (d, J = 5.8 Hz, 2H), 2.94 (t, J = 7.4 Hz, 2H), 2.74 (t, J = 7.5 Hz, 2H). ESI-MS m/z: 565.2 (MH^+).

*(E)-4-Amino-1-(benzyloxy)-N-(2,4-difluorobenzyl)-2-oxo-6-styryl-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**19d**).* Treatment of **18c** as outlined in general procedure H provided **19d** as a colorless oil (68% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.65 (t, J = 5.4 Hz, 1H), 8.89 (s, 1H), 8.12 (s, 1H), 7.81 (d, J = 8.3 Hz, 1H), 7.68 (d, J = 7.1 Hz, 2H), 7.63 (d, J = 7.0 Hz, 1H), 7.53 (d, J = 8.1 Hz, 2H), 7.40−7.38 (m,4H), 7.33−7.30 (m, 1H), 7.17 (dd, J = 47.6, 17.5 Hz, 2H), 6.89−6.79 (m, 2H), 5.28 (s, 2H), 4.64 (d, J = 5.2 Hz, 2H). ESI-MS m/z: 539.2 (MH^+).

*4-Amino-1-(benzyloxy)-N-(2,4-difluorobenzyl)-6-(3-(dimethylamino)prop-1-yn-1-yl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**19e**).* Treatment of 1-(benzyloxy)-6-bromo-*N*-(2,4-difluorobenzyl)-4-((2,4-dimethoxybenzyl)amino)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide **17c**[31] with commercially available *N*,*N*-dimethylprop-2-yn-1-amine as outlined in general procedures J and H provided **19e** as a colorless oil (33% yield, two steps). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.57 (t, J = 5.7 Hz, 1H), 8.77 (d, J = 1.6 Hz, 1H), 8.11 (d, J = 1.6 Hz, 1H), 7.67−7.65 (m, 2H), 7.42−7.36 (m, 4H), 6.87−6.80 (m, 2H), 5.25 (s, 2H), 4.63 (d, J = 5.7 Hz, 2H), 3.51 (s, 2H), 2.42 (s, 6H). ESI-MS m/z: 518.2 (MH^+).

*4-Amino-1-(benzyloxy)-N-(2,4-difluorobenzyl)-6-(3-(2-hydroxyethoxy)prop-1-yn-1-yl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (**19f**).* Treatment of **18d** as outlined in general procedure H provided **19f** as a colorless oil (87% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.55 (t, J = 5.7 Hz, 1H), 8.79 (d, J = 1.7 Hz, 1H), 8.08 (d, J = 1.8 Hz, 1H), 7.68−7.67 (m, 2H), 7.41−7.38 (m, 4H), 6.88−6.82 (m, 2H), 5.27 (s, 2H), 4.64 (d, J = 5.8 Hz, 2H), 4.49 (s, 2H), 3.86−3.84 (m, 2H), 3.77−3.75 (m, 2H). ESI-MS m/z: 535.2 (MH^+).

*(E)-Methyl 3-(5-Amino-8-(benzyloxy)-6-((2,4-difluorobenzyl)-carbamoyl)-7-oxo-7,8-dihydro-1,8-naphthyridin-3-yl)acrylate (**19g**).* Treatment of **18h** as outlined in general procedure H provided **19g** as a colorless oil (38% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.54 (d, J = 5.6 Hz, 1H), 8.89 (d, J = 1.5 Hz, 1H), 8.13 (s, 1H), 7.76 (d, J = 16.1 Hz, 1H), 7.67 (d, J = 6.1 Hz, 2H), 7.41−7.37 (m, 4H), 6.87−6.81 (m, 2H), 6.59 (d, J = 16.1 Hz, 1H), 5.27 (s, 2H), 4.64 (d, J = 5.7 Hz, 2H), 3.86 (s, 3H). ESI-MS m/z: 521.2 (MH^+).

HIV-1 IN Biochemical Assays. As previously described,[43] inhibitors or an equivalent volume of DMSO were added to a reaction mixture containing 20 nM DNA substrate 5′ ^{32}P-labeled on the transferred strand and 400 nM IN in 50 mM MOPS pH 7.2, 7.5 mM $MgCl_2$, and 14 mM 2-mercaptoethanol. Reactions were allowed to proceed for 2 h at 37 °C and were stopped by the addition of an equal volume of loading buffer [1% sodium dodecyl sulfate, 0.025% bromophenol blue, and 0.025% xylene cyanol made in formamide]. Reaction products, separated in 16% polyacrylamide denaturing sequencing gels, were visualized using by phosphorimaging using a Typhoon 8600 instrument (GE Healthcare) and quantified using the ImageQuant 5.1 software (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.[44] The IN reading frame was removed from pNLNgoMIVR$^-$ΔEnv.LUC by digestion with *Kpn*I and *Sal*I and inserted between the *Kpn*I and *Sal*I sites of pBluescript II KS+. With this construct as the WT 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′-CTAAAAGGGGAAGCCATG-TATGGACAAGTAGACTGTA-3′; T66I, 5′-CCAGGAATATGG-CAGCTAGATTGTATACATTTAGAAGGAAAAGTT-3′; E92Q, 5′-GCAGAAGTAATTCCAGCACAGACAGGGCAAGAAA-3′; G118R, 5′-GTACATACAGACAATCGCAGCAATTTCACCAGTAC-3′; G140S, 5′-GGGGATCAAGCAGGAATTTAGCATTCCCTA-CAATC-3′; Y143C, 5′-GCAGGAATTTGGCATTCCCCGCAA-TCCCCAAAGTCAAGGA-3′; Q148H, 5′-CATTCCCTACAA-TCCCCAAAGTCATGGAGTAATAGAATCTA-3′; N155H, 5′-CCAAAGTCAAGGAGTAATAGAATCTATGCATAAAGA-ATTAAAGAAAATTATAGGACA-3′; R263K 5′-AAAGTAGTGCCA-AGAAAAAAAGCAAAGATCATC-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 *Kpn*I and *Sal*I sites) to produce the full-length mutant HIV-1 IN constructs. The mutant forms of the vector were validated by DNA sequence determination.

Single-Round HIV-1 Infectivity Assay. Assays were performed using the human embryonic kidney cell culture cell line 293 acquired from the American Type Culture Collection (ATCC) and the human osteosarcoma cell line, HOS, obtained from Dr. Richard Schwartz (Michigan State University, East Lansing, MI) using methods that have been previously reported.[44,45] The 293 and HOS cells were 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.[44] VSV-*g*-pseudotyped HIV was produced by transfection of 293 cells.[45] On the day prior to transfection, 293 cells were plated on 100 mm diameter dishes at a density of 1.5×10^6 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 use 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 using a concentration range from 250 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 using a concentration range from 5 to 0.0001 μM using 11 serial dilutions and then incubated at 37 °C (3 h). After 3 h, 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

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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 min). 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 min) and incubated at room temperature to allow time for signal development (20 min), 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 min), 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 nonlinear regression analysis on the data. Final IC_{50} values were determined from the fit model.

X-ray Crystallography. PFV intasome crystals were soaked in the presence of 0.5−1 mM INSTIs in cryoprotection solution prior to snap freezing in liquid nitrogen as described.[23,24] X-ray diffraction data collected on beamlines I04 and I03 of the Diamond Light Source (Oxfordshire, UK) were processed using XDS[46] and Aimless.[47] Structures were determined via rigid-body refinement of model generated from PDB ID 4BDZ by removing small molecule atoms, and the compounds were fitted into resulting F_o-F_c difference maps. The models were built in Coot,[48] refined in Phenix,[49] and validated using MolProbity.[50] Relevant data collection and refinement statistics are given in Table 5.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.7b00596.

Overlays of the PFV crystal structures of several bound INSTIs highlighting the correspondence of features corresponding to the 4-position of the current compounds; predicted molPAINS values for final products (PDF)

Molecular formula strings (CSV)

Accession Codes

Atomic coordinates and structure factors for PFV intasome-bound **5′g**, **5g**, and **6p** have been deposited in the RCSB Protein Data Bank (PDB codes 5MMA, 5NO1, and 5MMB, respectively).

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Notes

The authors declare the following competing financial interest(s): Certain of the reported compounds are included in patent applications.

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■ ABBREVIATIONS USED

HIV-1, human immunodeficiency virus type 1; PFV, prototype foamy virus; AIDS, acquired immune deficiency syndrome; FDA, Food and Drug Administration; IN, integrase; RAL, raltegravir; EVG, elvitegravir; DTG, dolutegravir; 3-P, 3-processing; ST, strand transfer; INSTIs, integrase strand transfer inhibitors; DNA, DNA; IC_{50}, half-maximum inhibitory concentration; EC_{50}, half-maximal effective concentration; WT, wild-type; DMF, dimethylformamide; HPLC, high-pressure liquid chromatography; HRMS, high-resolution mass spectrometry; PBS, phosphate-buffered saline; RLUs, relative luciferase units

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