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A potent and orally active HIV-1 integrase inhibitor

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Abstract—A 1,6-naphthyridine inhibitor of HIV-1 integrase has been discovered with excellent inhibitory activity in cells, good pharmacokinetics, and an excellent ability to inhibit virus with mutant enzyme.

Replication of the human immunodeficiency virus (HIV) is dependent upon the enzyme HIV Integrase (IN), one of three essential enzymes encoded in the viral genome. HIV IN catalyzes the insertion of the viral genome into the host genome. HIV IN subunits assemble onto double stranded viral DNA and process the 3′ ends to prepare the strand for integration. The pre-integration complex then enters the nucleus where it catalyzes the DNA insertion (strand transfer) step. Inhibitors of viral IN strand transfer inhibit HIV-1 viral replication.[1] HIV IN thus presents an attractive chemotherapeutic target for the treatment of HIV infection and AIDS.

Compound **1**, L-870810, an 8-hydroxy-1,6-naphthyridine, is the first HIV IN inhibitor to significantly decrease viral load and increase CD4 cell count in treatment naive or experienced patients infected with HIV. It is a potent inhibitor of the strand transfer process catalyzed by HIV-1 IN (IC_{50} = 10 nM, Table 1) and has shown excellent antiviral activity in cell culture in the absence of human serum proteins (IC_{95} = 15 nM, n = 237).[2] Addition of 50% normal human serum (NHS) to the cell culture results in a 7-fold loss of efficacy (IC_{95} = 102 nM, n = 237), presumably as a result of the compound's high affinity for serum protein (99% protein bound).[3] Compounds with greater potency against HIV-1 in the presence of 50% NHS were sought with the goal of improving efficacy and of lowering dose.

In our effort to decrease the affinity of **1** for serum protein, we were aware of work by Whitesides and co-workers, who have shown that self-assembling monolayers containing alkylated amide side chains demonstrate reduced binding to proteins like fibrinogen and lysozyme compared to monolayers containing alkyl side chains.[7] We explored substitution of the benzyl moiety of compound **1** with alkylated amides to lower protein binding. The 2-position of the benzyl phenyl was targeted after substitution at the 3- and 4-positions with polar groups resulted in losses of potency relative to **1** (R.P. Gomez, N.J. Anthony, S.D. Young, unpublished data). To allow

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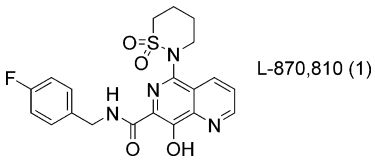
Table 1. Efficacy and physical properties of compound **1**



Inhibition of strand transfer IC_{50}[a] (nM)	Antiviral activity in cell culture IC_{95}[b] (nM)		% Protein binding[c]	Log *P*[d]	equiv sol pH 7.4[e] (mg/mL)
	FBS	NHS			
10	15	102	99	2.1	0.00021

[a] The inhibition of strand transfer assay was performed with recombinant HIV-IN (0.1 μM) preassembled on immobilized oligonucleotides using 0.5 nM DNA.[2b] Values are means of at least two experiments (for compound **1**, $n = 5$), lower limit of accuracy ~5 nM, standard deviation is ~2-fold.

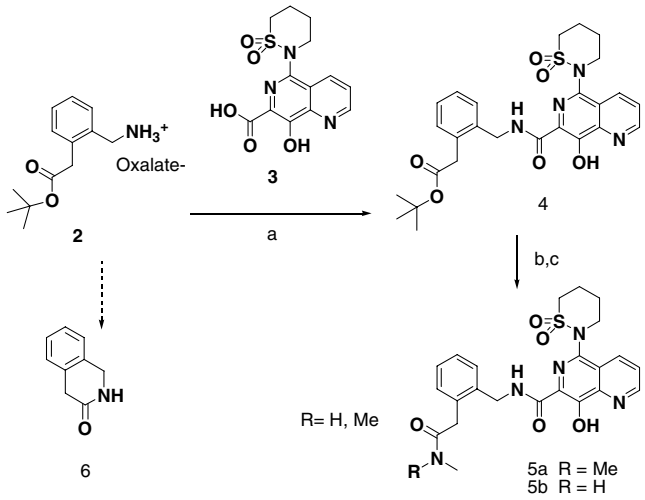
[b] Antiviral activity was assessed by measuring the decrease in HIV-1 p24 core antigen in MT-4 human T-lymphoid cells/HIV-1IIIb cultured in the presence of increasing concentrations of inhibitor. Antiviral activity in cell culture (IC_{95}) is the drug concentration which inhibits 95% viral growth relative to control in cell culture. Low protein conditions (FBS): run with 10% fetal bovine serum, high protein conditions (NHS): run with 50% normal human serum. For all compounds $n = 2$ or greater unless otherwise noted. For compound **1** $n = 237$. Lower limit ~7 nM. Standard deviation ~2-fold.[4]

[c] % Protein binding ultrafiltration method, see Ref. 3.

[d] Log *P* measurement, see Ref. 5.

[e] Equilibrium solubility assay, see Ref. 6.

maximum flexibility in the nature of the final amide, our synthesis attempted to incorporate the functional group as an ester-substituted benzylamine that, after coupling with the naphthyridine core, could be transformed into a variety of amides **5** (Scheme 1). Pre-activation of the acid **3**[8,9] with EDT/HOBt followed by treatment with the benzylamine **2** and base gave intermediate **4** in good yield and minimized loss of the benzylamine to the side product **6**. Compounds **5a** and **5b** were prepared in ~30% yield in three steps.



Scheme 1. Preparation of analogs in Table 2. Reagents and conditions: (a) EDT, HOBt, DMF, followed by **3** and NMM, rt (52%); (b) 1 N aq. NaOH, (8 equiv) /THF/MeOH, 1:1:1 vol, 50 °C (71%); (c) RNHMe, BOP reagent, TEA, DMF, rt (80%).

The 2-position acetamide analogs **5a** and **5b** retain intrinsic potency relative to the unsubstituted benzyl parent **7** (Table 2). For these compounds a reduction in protein binding and log *P* relative to parent **7** is also observed. For example **5b**, these changes are accompanied by improved antiviral activity with a smaller shift (4-fold vs 8-fold) observed between the low (FBS) and high (NHS) protein versions of the cell assay.

Attachment of a polar group directly to the 2-position of the benzyl ring was explored next. We decided to prepare analogs containing the 4-fluoro group, which is important for potency in HIV IN inhibitors.[10] The first polar groups to be explored were sulfonamide and sulfone. Amide bond formation with the amines 2-methanesulfonamide-4-fluoro benzylamine and 2-mono- and di-methylaminosulfonyl-4-fluorobenzylamine[11] using the conditions described for **5a** and **5b** gave compounds **8a**,**b** and **9** (Table 3).

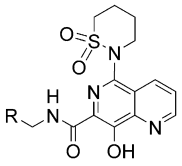
The results for the 2-sulfonamide and sulfone analogs are summarized in Table 3. The sulfonamides **8a** and **8b** were less active than **1** in strand transfer and showed only modest decreases in protein binding (0–3%). These small changes did not greatly affect the compound activity in cell culture in the presence of NHS relative to **1**. The methyl sulfone compound **9** was equipotent to **1** in strand transfer and showed an encouraging 2-fold improvement in cell culture in the presence of NHS (antiviral IC_{95} 49 vs 102 nM for **1**). The protein binding was decreased approximately 2% relative to **1**. All three compounds showed decreases in log *P*; the decrease was greatest for **9** (1.6 vs 2.1 for **1**).

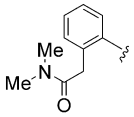
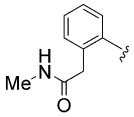
Incorporation of a 2-amide group required another synthetic approach, as we anticipated that 4-fluoro-2-carboxy benzylamine ester **10** would undergo facile intramolecular cyclization to the 5-membered lactam **11** under the conditions of the EDT-mediated peptide coupling (Scheme 2).

We therefore settled on masking the benzylamine, incorporating the desired amide at the 2-position, and then unveiling the benzylamine to do the coupling. Two routes were worked out in this fashion. In the first route, the benzylic nitrogen was introduced as bis-*tert*-butoxycarbonyl amine, then palladium-catalyzed carbonylative amide formation was used to give amide **15** as a mixture of mono- and bis-BOC products (Scheme 3). The course of the carbonylation reaction was sensitive to the amount of methylamine present, with greater than sixteen equivalents being necessary to inhibit the formation of the oxalate product **16**. Deprotection of the amine with HCl gave the stable hydrochloride salt **17a,** which can be stored as a solid at room temperature for months without significant degradation. The overall yield of **17a** after four steps was 40%.

The second route was developed to avoid complications associated with scaling up the palladium reaction. Bromination of the commercially available ester **18**[12] to give **19** was followed by displacement of the bromine with bis-*tert*-butoxycarbonyl amine to give **20** (Scheme 4).

Table 2. SAR of analogs including amide side chains[a,b,c,d]



Compound	R	Inhibition of strand transfer IC_{50}[a] (nM)	Antiviral activity in cell culture IC_{95}[b] (nM)		PB[d] (%)	Log *P*[c]
			10% FBS	50% NHS		
7		10	125	1000	99	1.9
5a		14	167	1000[e]	94	1.0
5b		7	109	438	92	1.0

[a] The inhibition of strand transfer assay was performed with recombinant HIV-IN (0.1 μM) preassembled on immobilized oligonucleotides using 0.5 nM DNA.[2b] Values are means of at least two experiments (for compound **1**, $n = 5$), lower limit of accuracy ~5 nM, standard deviation is ~2-fold.

[b] Antiviral activity was assessed by measuring the decrease in HIV-1 p24 core antigen in MT-4 human T-lymphoid cells/HIV-1IIIb cultured in the presence of increasing concentrations of inhibitor. Antiviral activity in cell culture (IC_{95}) is the drug concentration which inhibits 95% viral growth relative to control in cell culture. Low protein conditions (FBS): run with 10% fetal bovine serum, high protein conditions (NHS): run with 50% normal human serum. For all compounds $n = 2$ or greater unless otherwise noted. For compound **1** $n = 237$. Lower limit ~7 nM. Standard deviation ~2-fold.[4]

[c] % Protein binding ultrafiltration method, see Ref. 3.

[d] Log *P* measurement, see Ref. 5.

[e] $n = 1$.

The amide of interest could then be readily formed by heating the ester with the required amine in methanol under pressure. Deprotection with HCl gas gave the stable amine hydrochloride salts **17a** or **17b** in good yield (overall yield 56% for four steps).

The final compounds were prepared by pre-activating acid **3** with EDT/HOAT, then coupling with the amine hydrochlorides **17a** and **17b**, and base. The major products were the desired naphthyridine amides **21a** and **21b**, (Scheme 5) and the formation of **11** was minimized (yield 65%).

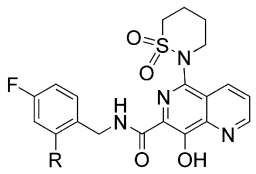
The results for the 2-amide analogs are shown in Table 3. Even though **21a** is less potent than **1** in the strand transfer assay (Table 3) and less potent in cell culture in FBS, the lower protein binding (90% vs 99% for **1**) contributes to improved antiviral properties in the presence of NHS (63 vs 102 nM for **1**).

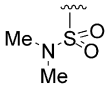
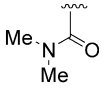
The mono-methylamide **21b** retains intrinsic activity against IN (strand transfer $IC_{50} = 10$ nM) and shows a decrease in protein binding (92% vs 99% for compound **1**). Antiviral activity in cell culture under low protein conditions improves 2-fold relative to **1** ($IC_{95} = 7$ vs 14 nM), and only a 2-fold loss of activity is observed in the presence of 50% NHS. The antiviral activity in cell culture in the presence of NHS is therefore improved 6-fold relative to **1** to give an IC_{95} of 16 nM.

An analysis of the amide's and sultam's effects on the potency and physical properties of the naphthyridine template is shown in Table 4.[14] The 2-methylamide has a minimal impact on intrinsic potency. The strand transfer potency of the unsubstituted naphthyridine **22** is similar to that of the 2-methylamido-substituted analog **23** and, likewise, unsubstituted **1** has the same potency as its 2-methylamido-substituted analog **21b**. The 2-methylamido group lowers the log *P* of **22** by 1 log unit and of **1** by 0.5 log units. The unsubstituted naphthyridine **22** is highly protein bound (99%). Addition to **22** of either the polar 2-methylamido group to give **23** or the sultam to give **1** results in a very small or undetectable change in the protein binding (0–2%). However, combination of both groups in compound **21b** has a synergistic effect and leads to a ~7% decrease in protein binding relative to **22**. This particular combination of protein binding and log *P* provides improved cell culture activity in this series.

The lower protein binding of **21b** was confirmed with a more accurate ultracentrifugation protocol using ^{14}C radiolabeled inhibitor. Under these conditions a 10-fold increase in free fraction is observed between radiolabeled

Table 3. SAR of analogs including polar side chains[a,b,c,d]



Compound	R	Inhibition of strand transfer IC_{50}[a] (nM)	Antiviral activity in cell culture[b]		PB[c] (%)	Log P[d]
			10% FBS	50% NHS		
1	H	10	15	102	99	2.1
8a		30	16	94	97	1.9
8b		45	<8[e]	125	99	1.9
9		10	12	49	98	1.6
21a		110	23	63	90	1.0
21b		10	7	16	92	1.6

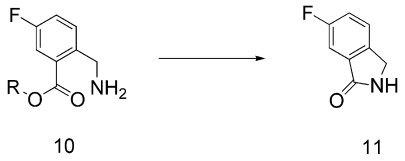
[a] The inhibition of strand transfer assay was performed with recombinant HIV-IN (0.1 μM) preassembled on immobilized oligonucleotides using 0.5 nM DNA.[2b] Values are means of at least two experiments (for compound **1**, $n = 5$), lower limit of accuracy ~5 nM, standard deviation is ~2-fold.

[b] Antiviral activity was assessed by measuring the decrease in HIV-1 p24 core antigen in MT-4 human T-lymphoid cells/HIV-1IIIb cultured in the presence of increasing concentrations of inhibitor. Antiviral activity in cell culture (IC_{95}) is the drug concentration which inhibits 95% viral growth relative to control in cell culture. Low protein conditions (FBS): run with 10% fetal bovine serum, high protein conditions (NHS): run with 50% normal human serum. For all compounds $n = 2$ or greater unless otherwise noted. For compound **1** $n = 237$. Lower limit ~7 nM. Standard deviation ~2-fold.[4]

[c] % Protein binding ultrafiltration method, see Ref. 3.

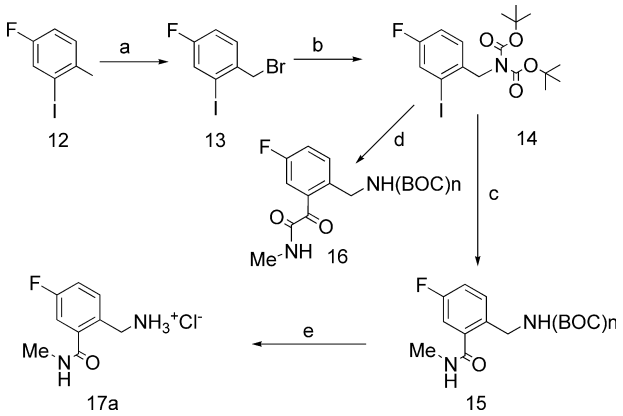
[d] Log P measurement, see Ref. 5.

[e] $n = 1$.



10 11

Scheme 2. Free base cyclization to lactam.



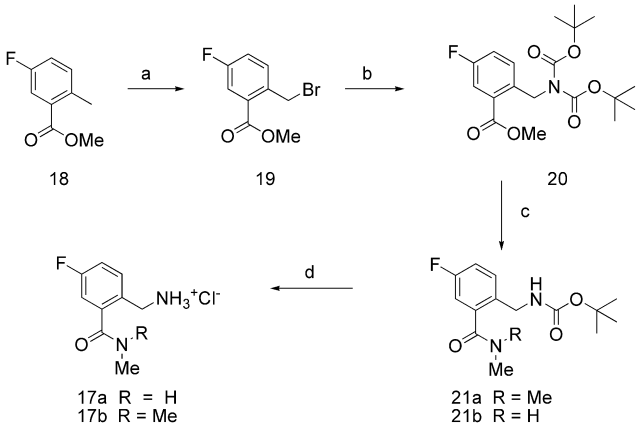
12 13 14 16 15 17a

Scheme 3. Synthesis of compound **17a**. Reagents and conditions: (a) NBS, CCl_4 benzoyl peroxide (71%); (b) NaH, $HN(BOC)_2$, DMF 50 °C (88%); (c) $Pd(OAc)_2$, dppf, DIEA, DMF, CO(g) 75 psi, $NH_2Me > 16$ equiv 70 °C (~65% mono plus bis-BOC products); (d) HCl (g) EtOAc, −78 to 0 °C (quant.).

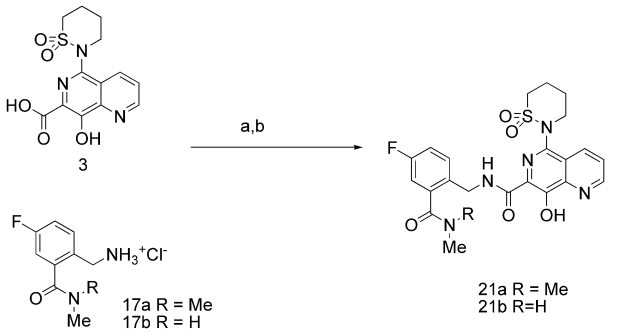
21b and **1** (15% vs 1.5%).[13,15] The solubility of **21b** at pH 7.4 is 2.3 μg/mL compared to 0.21 μg/mL for **1**.[6]

The pharmacokinetic profiles of **21b** are comparable to those of **1** in rat, dog, and rhesus (Table 5).[16] Figure 1 shows the pharmacokinetic profile of **21b** sodium salt in rats after oral dosing at 10 mg/kg in 1% aqueous methylcellulose. At 24 h, the concentration of drug remaining (78 ± 25 nM) is still ~5-fold greater than the IC_{95} for antiviral activity in cell culture (16 nM).

A desirable feature of a HIV IN drug candidate is the ability to display good antiviral activity against potential mutant viruses. Figure 2 shows the comparative resistance profile of compounds **1** and **21b** against virus containing site-directed mutations. These mutations

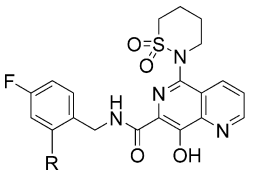


Scheme 4. Alternative synthesis of compounds **17**. Reagents and conditions: (a) NBS, CCl_4, benzoyl peroxide (71%); (b) NaH, $HN(BOC)_2$, DMF, 0 °C (88%); (c) $MeNH_2$, MeOH sealed tube, 70 °C, (95%); (d) HCl (g) EtOAc, −78 to 0 °C (95%).



Scheme 5. Preparation of compounds **21**. Reagents and conditions: (a) 1.3 equiv EDC, 1.3 equiv HOAT; (b) 1.1 equiv amine, 1.1 equiv Hunig's base (65%).

Table 5. Compounds **1** and **21b** pharmacokinetic profile[a]



R= H (**1**) CONHMe (**21b**)

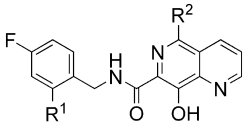
Pharmacokinetics, Na^+ salts[a]						
Species	*F* (%)[b]		$T_{1/2}$ iv (h)[c]		Cl[d] (mL/min/kg)	
	1	**21b**	**1**	**21b**	**1**	**21b**
Rat	41	45	1.2	1.2	2.8	8.6
Dog	24	65	2.9	1.8	2.0	2.8
Rhesus	51	23	0.7	1.4	6.6	18.3

[a] For dose and methods see Ref. 16.
[b] %*F*, percent bioavailability after oral administration.
[c] $T_{1/2}$, half-life of concentration in hours after intravenous (iv) administration.
[d] Cl, clearance in mL/min/kg after iv administration.

were observed to arise from serial passage in cell culture under exposure to diketoacid IN inhibitors and **1**.[2b] Compound **1** is somewhat susceptible to virus with mutations in the active-site residues F121 and N155 (4- and 12-fold loss in efficacy observed relative to wild type). Compound **21b** retains excellent activity against all these mutations, with only 2- to 4-fold shifts in the IC_{50} observed against the mutants compared to wild type virus.

The combination of favorable properties found in **21b** prompted us to learn more about its structure. Figure 3 shows the X-ray structure of **21b**.[17] The amide bond

Table 4. Group effects on antiviral potency and protein binding are additive[a,b,c,d]



Compound	R^1	R^2	Inhibition strand transfer IC_{50}[a] (nM)	Antiviral activity in cell culture (NHS) IC_{95}[b] (nM)	Protein binding[c]	Log *P*[d]
22	H	H	33	5000	99	2.4
23	CONHMe	H	51	1042	98	1.4
1	H		10	102	99	2.1
21b	CONHMe		10	16	92	1.6

[a] The inhibition of strand transfer assay was performed with recombinant HIV-IN (0.1 μM) preassembled on immobilized oligonucleotides using 0.5 nM DNA.[2b] Values are means of at least two experiments (for compound **1**, $n = 5$), lower limit of accuracy ~5 nM, standard deviation is ~2-fold.
[b] Antiviral activity was assessed by measuring the decrease in HIV-1 p24 core antigen in MT-4 human T-lymphoid cells/HIV-1IIIb cultured in the presence of increasing concentrations of inhibitor. Antiviral activity in cell culture (IC_{95}) is the drug concentration which inhibits 95% viral growth relative to control in cell culture. Low protein conditions (FBS): run with 10% fetal bovine serum, high protein conditions (NHS): run with 50% normal human serum. For all compounds $n = 2$ or greater unless otherwise noted. For compound **1** $n = 237$. Lower limit ~7 nM. Standard deviation ~2-fold.[4]
[c] % Protein binding ultrafiltration method, see Ref. 3.
[d] Log *P* measurement, see Ref. 5.

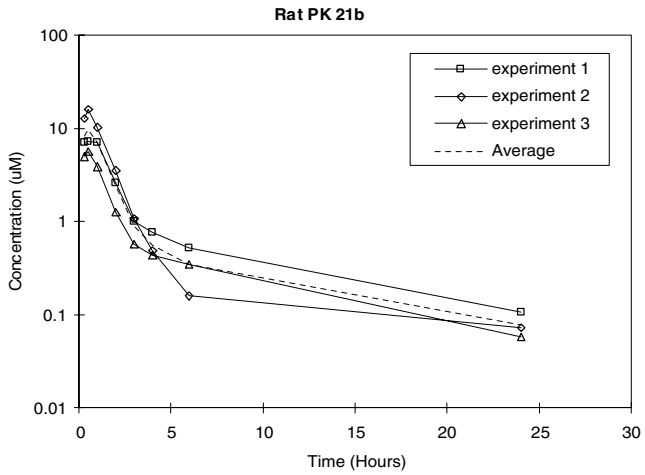


Figure 1. Pharmacokinetic profile for **21b** sodium salt dosed orally in rats (10 mg/kg, 1% aq methylcellulose suspension).[16]

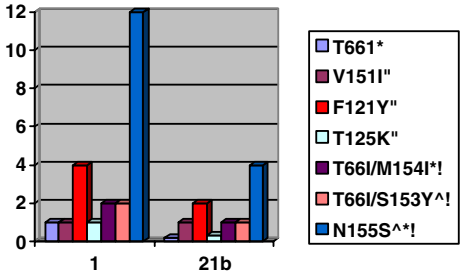


Figure 2. Comparative resistance profile of **1** and **21b** with HIV-1 containing site-directed IN mutations. Numbers denote shift in IC_{50} relative to wild type HIV-1 as derived from single cycle infectivity assays. ^Viruses selected by serial passage in the presence of diketo-acids. ''Viruses selected by serial passage in cell culture in the presence of **1**. *Viruses which exhibit >50% reduction in specific infectivity (impaired replication capacity).[2b]

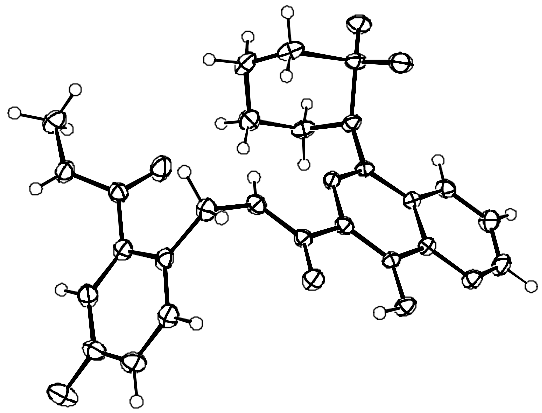


Figure 3. ORTEP representation of **21b**. Non-hydrogen atoms are represented by ellipsoids corresponding to 30% probability. Hydrogen atoms have been drawn at an arbitrary size.

linking the benzyl group to the naphthyridine is approximately coplanar with the naphthyridine and aligned to hydrogen bond with the phenolic oxygen. The monomethyl amide is twisted out of the plane of the benzyl group with a torsion angle of 49°. The sultam nitrogen is sp^3 hybridized, and the sultam ring is perpendicular to the plane of the naphthyridine.

In summary, optimization of **1** has led to **21b** (L-900564), an inhibitor of HIV IN, that has excellent cell potency in the presence of NHS (IC_{95} NHS = 16 nM, $n = 15$), excellent activity against mutants raised to HIV IN inhibitors, and a very good pharmacokinetic profile. Compound **21b** was selected for further pre-clinical evaluation.

Acknowledgments

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References and notes

1. For a recent review on the structure and function of HIV-1 IN, see: (a) Chiu, T. K.; Davies, D. R. *Curr. Top. Med. Chem.* **2004**, *4*, 965; (b) Pommier, Y.; Johnson, A. A.; Marchand, C. *Nat. Rev. Drug Disc.* **2005**, *4*, 236; For a recent review on HIV-1 IN inhibitors, see: (c) Anthony, N. J. *Curr. Top. Med. Chem.* **2004**, *4*, 979.
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3. *Human plasma binding*. Compound is dissolved in pH 7.4 buffer (NaH_2PO_4/Na_2HPO_4) to give a 5×10^{-5} M solution and filtered through a 0.45-mm filter. 4.5 mL is diluted with 0.5 mL, pH 7.4 buffer to give a control solution. 4.5 mL is diluted with 0.5 mL human plasma (final plasma conc. = 5.8×10^{-5} M) to give the test solution. The control and test solutions are pipetted into Centrifree® ultrafiltration devices and centrifuged. The control and test ultrafiltrates are assayed by HPLC compared to a 5×10^{-5} M reference solution of compound dissolved in

MeOH. $K_D = [C_f\ (HPd - C_i + C_f)]/(C_i - C_f)$ (calcd). % Binding = 100 * $[(C_i - C_f)/C_i]$ (obsd). % Binding = 100 * $[HP/(HP + K_D)]$ (calcd) where C_i = initial compound concentration (from control); C_f = final compound concentration (from plasma average); HP = assumed initial human plasma concentration (5.8×10^{-4} M); HPd = assumed diluted human plasma concentration (5.8×10^{-5} M); K_D, dissociation constant.

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5. UV detection method determining the relative concentration of a methanol solution of a compound partitioned between an octanol and pH 7.4 (KH_2PO_4/NaOH) water layer. HPLC methods are based on the maximum absorbance of each compound. Log *P* = log (Octanol HPLC area)(Octanol dilution)/(Buffer HPLC area)(buffer dilution).
6. *Solubility determination protocol*. A suspension of crystalline drug in 10 mM, pH 7.4, sodium phosphate buffer was allowed to equilibrate at room temperature for 5 days. Aliquots of the suspension were centrifuged at 15,000 rpm for 15 min. The solution drug concentration determined by HPCL at day 2 and 5 is taken as the solubility value.
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13. *Protein binding determination with radiolabeled material*. An ultracentrifugation method was used to determine the unbound fraction of radiolabeled compound in human plasma. Plasma (pH adjusted to 7.4) was treated with compound to yield final concentrations of 1, 5, and 20 μM. Following incubation at 37 °C for 30 min, aliquots of plasma were transferred immediately to 1.5 mL polyallomer centrifuge tubes. The tubes were centrifuged at 37 °C and 180,000*g* overnight (~18 h) using a high-speed centrifuge (Beckman Optima™, Model TLX). A six- or eight-place, fixed-angle rotor (TLA-100.3 or TLA-100.4) was used. Aliquots (0.2 mL each) of the supernatant were removed sequentially. Radioactivity in supernatants and original plasma samples was determined by liquid scintillation spectrometry with quench correction by external standardization. The percent unbound fraction (fu) was calculated as follows: fu (%) = dpm in protein-free section/dpm in original plasma × 100%.
14. Compounds **22** and **23** were prepared in a manner similar to that described for **21b**. See Ref. 9 for experimental details.
15. Compounds **1** and **21b** containing ^{14}C at the 8-carboxy carbon were prepared by methods outlined in reference 8, using methyl *N*-[(4-methylphenyl)sulfonyl]glycinate labeled with ^{14}C at glycine carbonyl carbon to give ^{14}C labeled **3**, which was coupled as described above to give the final compounds.
16. The sodium salts were ground in a mortar and pestle, and dosed orally as an aqueous solution/suspension in 1% aq methylcellulose in rats (10 mg/kg). In dogs and rhesus monkeys 0.5% aq methylcellulose suspensions were dosed at 1 mg/kg. Compound was dosed intravenously dissolved in dimethylsulfoxide (DMSO) at concentrations of 2 mg/kg in rats and 1 mg/kg in dogs and rhesus.
17. Crystallographic data (excluding structure factors) for **21b** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number 619397. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB@ 1EZ, UK [Fax: +44 1223 336033 or email: depost@ccdc.cam.ac.uk].