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Design and synthesis of substituted 4-oxo-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrazine-2-carboxamides, novel HIV-1 integrase inhibitors

H. Marie Langford,[a,*] Peter D. Williams,[a] Carl F. Homnick,[a] Joseph P. Vacca,[a] Peter J. Felock,[b] Kara A. Stillmock,[b] Marc V. Witmer,[b] Daria J. Hazuda,[b] Lori J. Gabryelski[c] and William A. Schleif[c]

[a]*Department of Medicinal Chemistry, Merck Research Laboratories, West Point, PA 19486, USA*
[b]*Department of Biological Chemistry, Merck Research Laboratories, West Point, PA 19486, USA*
[c]*Department of Vaccine and Biologics Research, Merck Research Laboratories, West Point, PA 19486, USA*

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Abstract—A series of 4-oxo-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrazine-2-carboxamides was synthesized and tested for their inhibition of HIV-1 integrase catalytic activity and HIV-1 replication in cells. Structure–activity studies around lead compound **5** indicated that a coplanar relationship of metal-binding heteroatoms provides optimal binding to the integrase active site. Identification of potency-enhancing substituents and adjustments in lipophilicity provided **17b** which inhibits integrase-catalyzed strand transfer with an IC_{50} value of 74 nM and inhibits HIV-1 replication in cell culture in the presence of 50% normal human serum with an IC_{95} value of 63 nM.

The causative agent of acquired immune deficiency syndrome (AIDS) is the human immunodeficiency virus type 1 (HIV-1). Currently marketed therapies for treating HIV-1 infection include drugs which target two of the three viral enzymes required for replication, reverse transcriptase and protease. Resistance to these drugs is increasing at an alarming rate, and thus there is a need to develop new agents which work by different mechanisms. The third viral enzyme, integrase, is used by the virus to insert double stranded proviral DNA into host chromosomal DNA. The three-step integration process includes assembly of integrase and proviral DNA, endonucleolytic processing of proviral DNA catalyzed by integrase, and strand transfer of proviral DNA into the host cell DNA catalyzed by integrase.[1] Several integrase inhibitors which work by inhibiting the strand transfer step have progressed to the stage of clinical testing in patients infected with HIV-1 and have shown very promising results.[2]

A recent report from our laboratories[3] described pyrrolopyrazinone **1** as a novel mimetic of the metal-binding diketoacid portion[4] of diketoacid integrase inhibitors (Fig. 1). Consistent with previous observations in another structural series,[5] modifications of **1** which reinforce a coplanar relationship between important metal-binding functionalities, for example, cyclization to lactam **2**, were found to improve potency in biochemical and cell-based assays. Another way of facilitating a coplanar arrangement between metal-binding functionalities in **1** was envisioned with pyrazolopyrazinone **3**.

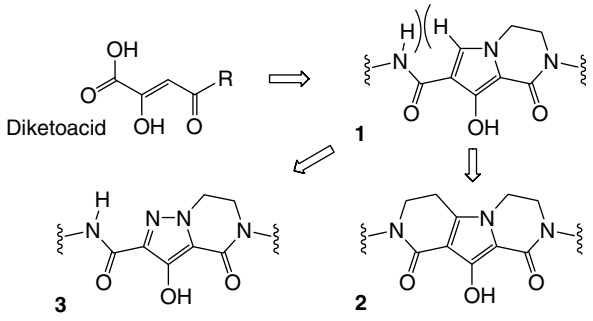

Figure 1.

Keywords: HIV-1; Integrase inhibitor; Antiviral.
* Corresponding author. Tel.: +1 215 652 8116; fax: +1 215 652 3971; e-mail: hmarie_langford@merck.com

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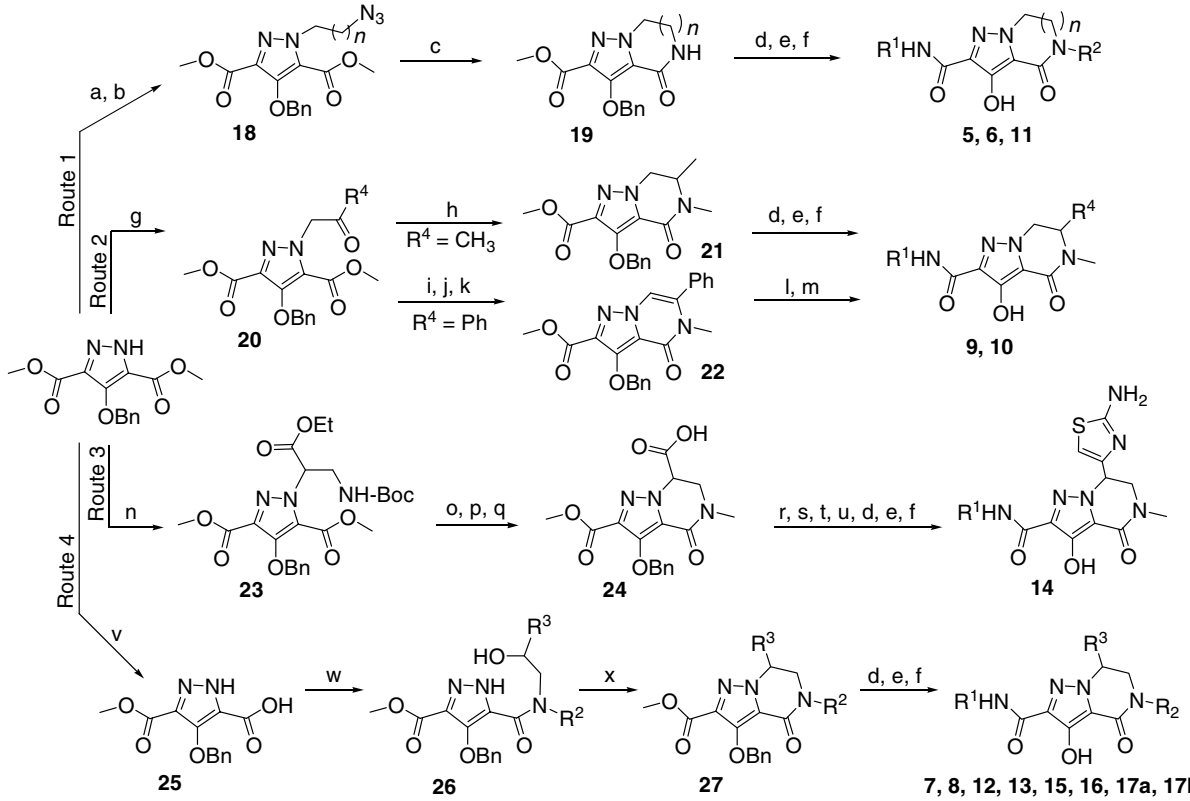
This modification replaces a non-bonded interaction between amide and pyrrole protons with a potentially attractive hydrogen bonding interaction between the amide proton and pyrazole nitrogen. Herein we describe the synthesis of novel pyrazolopyrazinone HIV-1 integrase inhibitors and structure–activity relationships which led to the discovery of analogs with nanomolar potency in biochemical and cell-based assays.

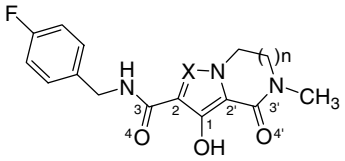
All of the inhibitors were synthesized starting with dimethyl 4-(benzyloxy)-1H-pyrazole-3,5-dicarboxylate[6] (see Scheme 1). In Route 1, the pyrazole nitrogen was alkylated using 1,2-dibromoethane or 1,3-dibromopropane followed by displacement of the bromine using sodium azide to give **18**. Staudinger reduction of the azide was accompanied by ring closure to **19**, and straightforward manipulations led to **5**, **6**, and **11**. In Route 2, the pyrazole nitrogen was alkylated using chloroacetone or 2-chloroacetophenone to give **20**. When R^4 was methyl, reductive amination of the ketone using sodium triacetoxyborohydride and methylamine proceeded smoothly and was accompanied by lactam ring closure to **21**. When R^4 was phenyl, the reductive amination/ring closure was problematic, and it was necessary to first form a mono-amide and then close the ring by heating in the presence of toluene sulfonic acid to form enamide **22**. The double bond was then reduced and straightforward manipulations provided **9** and **10**. In Route 3, the pyrazole nitrogen was alkylated with $BocNHCH_2CH(OMs)CO_2Et$[7] to give **23**. The Boc group was removed under acidic conditions and basic work-up induced closure of the lactam ring. The pyrazinone nitrogen was alkylated and the ethyl ester was selectively hydrolyzed using one equivalent of NaOH to give **24**. Formation of the acid chloride, followed by treatment with diazomethane and then HCl, gave an α-chloroketone which was cyclized to an aminothiazole. Straightforward manipulations gave **14**. Route 4 differed from the three previous routes in that the amide bond was formed first, followed by ring closure onto the pyrazole nitrogen. Mono acid **25** was produced in high yield using dimethylhydrazine.[8] Peptide coupling of **25** to an amino alcohol derivative, $R^2NHCH_2CHR^3OH$, provided **26** which then underwent intramolecular Mitsunobu reaction to give bicycle **27**. Straightforward manipulations provided **7**, **8**, **12**, **13**, **15**, and **16**. Asymmetric syntheses of **17a** and **17b** were achieved using the *S* and *R* isomers of *N*-methyl 2-hydroxy-2-phenylethylamine,[9] respectively. Enantiomerically pure **17a** and **17b** were obtained by final purification on a chiral column to remove traces of the other enantiomer (ChiralPak AS column, 80:10:10 hexane/MeOH/EtOH eluant with 0.1% TFA modifier, retention time **17a** = 13.96 min, **17b** = 11.09 min).

To address the question of the metal-binding pharmacophore geometry and binding to the integrase active site,



Scheme 1. Reagents and conditions: (a) $BrCH_2(CH_2)_nBr$ (n = 1 or 2), Cs_2CO_3, DMF; (b) NaN_3, DMF; (c) PPh_3, DMF, 0 °C, H_2O, 90 °C; (d) NaOH, MeOH; (e) R^1NH_2, EDC, HOBT, DMF; (f) H_2, 10% Pd/C, MeOH; (g) R^4COCH_2Cl, Cs_2CO_3, DMF; (h) $NaBH(OAc)_3$, $MeNH_2$, CH_2Cl_2; (i) NaOH, MeOH/THF, 50 °C; (j) $MeNH_2$, EDC, HOBT, DMF; (k) TsOH, toluene, 125 °C; (l) PtO_2, H_2, MeOH; (m) R^1NH_2, MeOH, reflux; (n) $BOCNHCH_2CH(OMs)CO_2Et$, Cs_2CO_3, CH_3CN; (o) TFA, CH_2Cl_2, basic work-up; (p) MeI, NaH, DMF; (q) NaOH, MeOH/THF; (r) oxalyl chloride, DMF, CH_2Cl_2, 0 °C; (s) CH_2N_2, CH_2Cl_2, 0 °C; (t) HCl in ether, CH_2Cl_2; (u) thiourea, MeOH, reflux; (v) Me_2NNH_2, reflux; (w) $R^2NHCH_2CHR^3OH$, EDC, HOBT, DMF; (x) DEAD, PPh_3, THF.

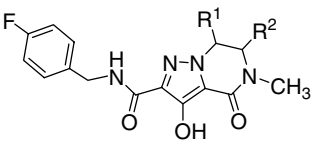
Table 1. Calculated conformations and inhibition of strand transfer activity for selected compounds



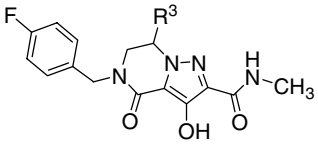
Compound	X	*n*	Dihedral angle[a]		Strand transfer IC_{50}[b] (nM)
			A	**B**	
4	CH	1	20°	5°	400
5	N	1	3°	4°	⩽10[c]
6	N	2	2°	23°	470

[a] Conformations from MMFF calculations in Spartan, dihedral A = C1–C2–C3–O4, dihedral B = C1–C2′–C3′–O4′.
[b] For assay details, see Ref. 11.
[c] Lower limit of assay is 10 nM.

Table 2. Inhibition of strand transfer activity for selected compounds



5, 7-10



11, 12

Compound	R^1	R^2	R^3	Strand transfer IC_{50}[a] (nM)
5	H	H	—	⩽10[b]
7	CH_3	H	—	13
8	Ph	H	—	⩽10[b]
9	H	CH_3	—	40
10	H	Ph	—	110
11	—	—	H	40
12	—	—	Ph	130

[a] For assay details, see Ref. 11.
[b] Lower limit of assay is 10 nM.

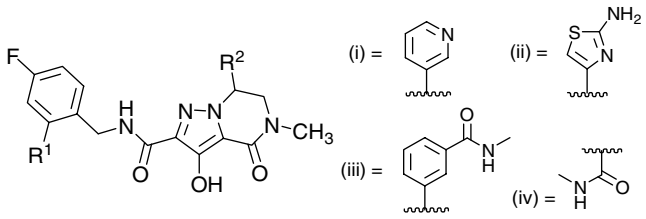
the conformational properties of pyrrolopiperazinone **4**, pyrazolopiperazinone **5**, and ring-expanded pyrazolodiazepinone **6** were investigated using molecular mechanics force field calculations contained in the Spartan molecular modeling program.[10] As shown in Table 1 with dihedral angle measurements A and B, pyrazolopyrazinone **5** adopts a low energy conformation in which both carbonyl groups are essentially coplanar with the central heterocycle (dihedral angles A and B are both near 0 degrees), whereas significant deviation from coplanarity is seen with the exocyclic carbonyl group in pyrrolopyrazinone **4** and the endocyclic carbonyl group in pyrazolodiazepinone **6** (dihedral angles A and B are approximately 20 degrees, respectively). Using an integrase-catalyzed strand transfer assay,[11] it is seen that **5** is greater than 40-fold more potent than **4** and **6**, thus providing additional evidence that optimal binding to integrase active site metals[4] is achieved with an inhibitor containing a coplanar arrangement of ligating heteroatoms.

Compound **5** was used as a starting point for further structure–activity studies (Table 2). In comparing the potencies of **5**, **7**, and **8** in the strand transfer assay, it was seen that methyl and phenyl substituents at the R^1 position on the pyrazinone ring are well tolerated. Similar substitutions at the adjacent R^2 position on the pyrazinone ring reduced potency (compare **5**, **9**, and **10**). Because the pyrazolopyrazinone scaffold is pseudo-symmetric about the axis containing the central C–OH bond, we also investigated 'reverse analogs' in which the fluorobenzyl group is attached to the pyrazinone lactam nitrogen. Potencies of the 'reverse analogs' **11** and **12** in the strand transfer assay were less than their isomeric counterparts **5** and **8**, respectively, and therefore additional structure–activity studies focused on the isomer series related to **5**.

In an assay which measures antiviral activity in MT4 cells conducted in the presence of 10% fetal bovine serum (FBS) or 50% normal human serum (NHS),[12] phenyl-substituted pyrazinone **8** proved to be 10-fold more potent than the unsubstituted analog **5**, thus demonstrating a beneficial role for substitution on the pyrazinone ring (see Table 3). The lesser potency of 'reversed analogs' **11** and **12** compared to **8** in the strand transfer assay was similarly manifested in the viral replication assay. Both **5** and **8** showed a 5-fold shift to lower potency in the antiviral assay conducted in the presence of higher concentrations of serum. We therefore sought analogs of **8** with modifications which might reduce binding to serum proteins. Modifying the potency-enhancing phenyl group in **8** by making it smaller and less lipophilic (**16**) did not significantly alter the antiviral potency or the serum shift. Modifications to the phenyl group in **8** which involved the inclusion of heteroatoms to make it more polar (**13**–**15**) did in fact reduce the magnitude of the serum shift, however, these modifications also reduced the antiviral potency relative to **8**, presumably by making the compounds less cell-penetrant. In a related series of naphthyridinone integrase inhibitors, inclusion of a hydrogen bond acceptor group at the *ortho* position of the fluorobenzyl amide was found to reduce plasma protein binding and improve antiviral potency in cell-based assays.[13] This tactic produced excellent results in the present series. Asymmetric syntheses of the pyrazolopyrazinone core of **8** were developed starting with the individual enantiomers of styrene epoxide, and final products were elaborated with the inclusion of an *ortho*-*N*-methylcarboxamido group to give *R* and *S* enantiomers, **17a** and **17b**, respectively. The two enantiomers did not differentiate from one another in the strand transfer assay, and both were greater than 5-fold less potent than the racemic des-carboxamido analog **8**. The antiviral potencies of **17a** and **17b** showed no serum shift, and the *S* isomer **17b** was 4-fold more potent than **17a**. The reduced serum shift for **17a** and **17b** relative to **8** in the antiviral assay may be related to their 10-fold greater free fraction as measured in a human plasma protein binding assay[13] (12%, 12%, 1.1% unbound, respectively). The greater free fraction of **17a** and **17b** relative to **8** likely contributes to their maintenance of antiviral potency despite being less potent than **8** in the strand transfer assay.

The antiviral IC_{95} value of 63 nM in the presence of 50% NHS for **17b** compares favorably with the potency of

Table 3. Inhibition of strand transfer activity and viral replication for selected compounds



Compound	R^1	R^2	Strand transfer[a] IC_{50} (nM)	Antiviral activity[b] IC_{95} (nM)	
				(10% FBS)	(50% NHS)
5	H	H	⩽10[d]	630	2500
8	H	Ph	⩽10[d]	50	310
11[c]			40	5000	>10,000
12[c]			130	10,000	>10,000
13	H	(i)	⩽10[d]	250	500
14	H	(ii)	⩽10[d]	500	1000
15	H	(iii)	⩽10[d]	1000	1000
16	H	*i*-Pr	20	63	250
17a	(iv)	Ph(*R*)	62	250	250
17b	(iv)	Ph(*S*)	74	63	63

[a] For assay details, see Ref. 11.
[b] Viral replication in MT4 cells, for assay details, see Ref. 12, FBS, fetal bovine serum; NHS, normal human serum.
[c] See Table 2 for structure.
[d] Lower limit of assay is 10 nM.

other integrase inhibitors measured in this assay that have progressed to clinical studies and demonstrated efficacy for reducing viral load in HIV-infected patients (e.g., MK-0518[2] IC_{95} = 33 nM, L-870810[14] IC_{95} = 100 nM).

Reported herein is the design and synthesis of a novel series of pyrazolopyrazinone HIV-1 integrase inhibitors. Structural features which facilitate a coplanar relationship between the central heterocycle and flanking carbonyl groups provided inhibitors with high potency in an in vitro biochemical assay (strand transfer IC_{50} is less than or equal to 10 nM). Inclusion of a lipophilic substituent on the pyrazinone ring improved antiviral activity in a cell-based assay, and addition of a polar group at the *ortho* position of the fluorobenzyl amide reduced plasma protein binding and improved antiviral potency in the presence of human serum in a cell-based assay. An optimal compound from this work, **17b**, exhibited antiviral activity in cell culture in the presence of human serum (IC_{95} = 63 nM) that is similar to integrase inhibitors which have demonstrated efficacy for reducing viral load in HIV-infected patients. Further manipulation around this series may aid in the development of next generation anti-HIV drugs.

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References and notes

1. Anthony, N. J. *Curr. Top. Med. Chem.* **2004**, *4*, 979.
2. (a) Grinsztejn, G.; Nguyen, B.-Y.; Katlama, C.; Gatell, J. M.; Lazzarin, A.; Vittecoq, D.; Gonzalez, C. J.; Chen, J.; Harvey, C. M.; Isaacs, R. D. *Lancet* **2007**, *369*, 1261; (b) Cahn, P.; Sued, O. *Lancet* **2007**, *369*, 1235; (c) Miller, M.; Witmer, M.; Stillmock, K.; Felock, P.; Ecto, L.; Flynn, J.; Schleif, W.; Donradula, G.; Danovich, R.; Hazuda, D. International AIDS Conference, 2006, Toronto, Canada, Abstract No. ThAa0302.
3. Fisher, T. E.; Kim, B.; Staas, D. D.; Lyle, T. A.; Young, S. D.; Vacca, J. P.; Zrada, M. M.; Hazuda, D. J.; Felock, P. A.; Schleif, W. A.; Gabryelski, L. J.; Anari, M. R.; Kochansky, C. J.; Wai, J. S. *Bioorg. Med. Chem. Lett*. **2007**, *17* 6511.
4. Grobler, J. A.; Stillmock, K. A.; Hu, B.; Witmer, M.; Felock, P.; Espeseth, A. S.; Wolfe, A.; Egbertson, M.; Bourgeois, M.; Melamed, J.; Wai, J. S.; Young, S. D.; Vacca, J. P.; Hazuda, D. J. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 6661.
5. Zhuang, L.; Wai, J. S.; Embrey, M. W.; Fisher, T. E.; Egbertson, M. S.; Payne, L. S.; Guare, J. P.; Vacca, J. P.; Hazuda, D. J.; Felock, P. J.; Wolfe, A. L.; Stillmock, K. A.; Witmer, M. V.; Moyer, G.; Schleif, W. A.; Gabryelski, L. J.; Leonard, Y. M.; Lynch, J. J., Jr.; Michelson, S. R.; Young, S. D. *J. Med. Chem.* **2003**, *464*, 453.
6. (a) Bertho, A.; Nüssel, H. *Liebigs Ann. Chem.* **1927**, *457*, 278; (b) Rodriguez-Franco, M. I.; San Lorenzo, P.; Martinez, A.; Navarro, P. *Tetrahedron* **1999**, *55*, 2763.
7. Prepared in two steps (hydrogenation in the presence of di-*tert*-butyldicarbonate, mesylation) from ethyl 3-azido-2-hydroxypropionate. See: McCort, G. A.; Pascal, J. C. *Tetrahedron Lett.* **1992**, *33*, 4443.
8. Nematollahi, J.; Kasina, S. *J. Chem. Soc., Chem. Commun.* **1974**, *19*, 775.
9. Prepared by addition of methylamine to 98% ee *R*- or *S*-styrene oxide (Aldrich). See: Gurjar, M. K.; Krishna, L. M.; Sarma, B. V. N. B. S.; Chorghade, M. S. *Org. Process Res. Dev.* **1998**, *2*, 422.
10. Spartan is a molecular modeling software package available from Wavefunction, Inc., 18401 Von Karman Avenue, Suite 370, Irvine, CA 92612, USA.
11. Hazuda, D. J.; Felock, P.; Hastings, J. C.; Pramanik, B.; Wolfe, A. *J. Virol.* **1997**, *71*, 7005.

12. Vacca, J. P.; Dorsey, B. D.; Schleif, W. A.; Levin, R. B.; McDaniel, S. L.; Darke, P. L.; Zugay, J.; Quintero, J. C.; Blahy, O. M.; Roth, E.; Sardana, V.; Schlabach, A. J.; Graham, P. I.; Condra, J. H.; Gotlib, L.; Holloway, M. K.; Lin, J.; Chen, I.; Vastag, K.; Ostovic, D.; Anderson, P. S.; Emini, E. A.; Huff, J. R. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 4096.
13. Egbertson, M. S.; Moritz, H. M.; Melamed, J. Y.; Han, W.; Perlow, D. S.; Kuo, M. S.; Embrey, M. W.; Vacca, J. P.; Zrada, M. M.; Cortes, A. R.; Wallace, A.; Leonard, Y.; Hazuda, D. J.; Miller, M. D.; Felock, P. J.; Stillmock, K. A.; Witmer, M. V.; Schleif, W.; Gabryelski, L. J.; Moyer, G.; Ellis, J. D.; Jin, L.; Xu, W.; Braun, M. P.; Kassahun, K.; Tsou, N. N.; Young, S. D. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1392.
14. (a) Hazuda, D. J.; Anthony, N. J.; Gomez, R. P.; Jolly, S. M.; Wai, J. S.; Zhuang, L.; Fisher, T. E.; Embrey, M. W.; Guare, J. P., Jr.; Egbertson, M. S.; Vacca, J. P.; Huff, J. R.; Felock, P. J.; Witmer, M. V.; Stillmock, K. A.; Danovich, R.; Grobler, J.; Miller, M. D.; Espeseth, A. S.; Jin, L.; Chen, I.-W.; Lin, J. H.; Kassahun, K.; Ellis, J. D.; Wong, B. K.; Xu, W.; Pearson, P. G.; Schleif, W. A.; Cortese, R.; Emini, E.; Summa, V.; Holloway, M. K.; Young, S. D. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 11233; (b) Little, S. J; Drusano, G.; Schooley, R.; Haas, D. W.; Kumar, P.; Hammer, S.; McMahon, D.; Squires, K.; Asfou, R.; Richman, D.; Chen, J.; Saah, A.; Leavitt, R.; Hazuda, D.; Nguyen, B.-Y.; Protocol 004 Study Team, 12th Conference on Retroviruses and Opportunistic Infections, Feb 22–25, 2005; Boston, MA, Abstract 161.