Structure-Based Design, Synthesis, and Biological Evaluation of Irreversible Human Rhinovirus 3C Protease Inhibitors. 8. Pharmacological Optimization of Orally Bioavailable 2-Pyridone-Containing Peptidomimetics

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The optimization of the pharmacokinetic performance of various 2-pyridone-containing human rhinovirus (HRV) 3C protease (3CP) inhibitors following oral administration to either beagle dogs or CM-monkeys is described. The molecules described in this work are composed of a 2-pyridone-containing peptidomimetic binding determinant and an α,β-unsaturated ester Michael acceptor moiety which forms an irreversible covalent adduct with the active site cysteine residue of the 3C enzyme. Modification of the ester contained within these compounds is detailed along with alteration of the P_2 substituent present in the peptidomimetic portion of the inhibitors. The pharmacokinetics of several inhibitors in both dogs and monkeys are described (7 h plasma concentrations after oral administration) along with their human plasma stabilities, stabilities in incubations with human, dog, and monkey microsomes and hepatocytes, Caco-2 permeabilities, and aqueous solubilities. Compounds containing an α,β-unsaturated ethyl ester fragment and either an ethyl or propargyl P_2 moiety displayed the most promising combination of 3C enzyme inhibition (k_{obs}/[I] 170 000−223 000 M^{-1} s^{-1}), antiviral activity (EC_{50} = 0.047−0.058 μM, mean vs seven HRV serotypes), and pharmacokinetics following oral administration (7 h dog plasma levels = 0.248−0.682 μM; 7 h CM-monkey plasma levels = 0.057−0.896 μM).

Introduction

The human rhinoviruses (HRVs) are members of the picornavirus family and are the single most significant cause of the common cold.[1−3] Presently, no marketed antiviral agents exist for treating rhinovirus-related illnesses, and the large number of HRV serotypes (>100) makes the development of a vaccine seem unlikely.[4] In contrast to earlier attempts at antirhinoviral identification,[5] our research efforts have focused on inhibiting a critical virus-encoded enzyme whose activity is essential for completion of the HRV replication cycle. This protein, the human rhinovirus 3C protease (3CP), is a cysteine protease which catalyzes the proteolytic processing of the large polypeptide produced by cellular translation of the rhinovirus RNA genome.[6] In addition, the 3CP enzyme exhibits structural similarity to the trypsin protein family, but displays minimal homology to prevalent mammalian enzymes.[7] Due to its importance in the viral replication cycle, 3CP is an ideal target for the development of novel antirhinoviral agents, and several examples of 3CP inhibitors have recently appeared in the literature.[8] Our previous 3CP inhibitor discovery efforts led to the identification of a novel class of substrate-inspired 2-pyridone-containing peptidomimetics which incorporate a Michael acceptor moiety capable of irreversibly forming a covalent adduct with the active site cysteine residue of the 3C enzyme (Figure 1).[9,10] These efforts culminated with the identification of several optimized pyridones which display potent in vitro antiviral activity against multiple rhinovirus serotypes (e.g., **1** and **2**, Figure 1 and Table 1,) and one such molecule (**2**) was shown to be orally bioavailable in the dog.[9]

As previously detailed, compound **2** exhibited good pharmacokinetics in beagle dogs following administration as an oral solution with 7 h plasma levels of the molecule in excess of its average in vitro antiviral potency (Table 1).[9,11,12] Similar compound exposures were also noted when **2** was administered to dogs using a suspension formulation (Table 1). In stark contrast, the inhibitor was poorly bioavailable when orally delivered in solution to CM-monkeys with 7 h postadministration plasma levels well below the compound's average in vitro antiviral activity (Table 1). This dramatic species difference may result from the greater in vitro metabolism of **2** observed upon exposure to simian hepatocytes relative to that noted during analogous canine experiments (Table 2). Since no animal model of HRV infection exists which strongly correlates human symptomatology with in vitro antirhinoviral activity, the significance of the observed differences in compound **2**

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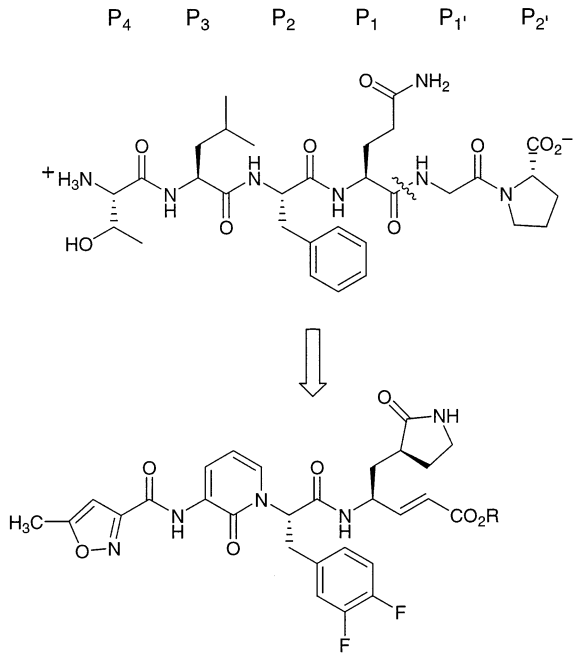


Figure 1. Design of 2-pyridone-containing HRV 3CP inhibitors.

exposure between dog and monkey is not known with certainty. However, data obtained from in vitro human hepatocyte metabolism studies conducted with **2** more closely paralleled results from the corresponding simian experiments (Table 2). We therefore sought to identify 3CP inhibitors which exhibited good oral bioavailability and pharmacokinetics in *both* dogs and monkeys in an effort to maximize their potential to display promising human exposures.[13]

Results and Discussion

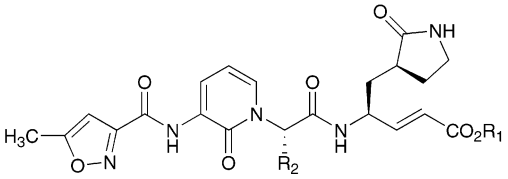
To accomplish our goal, we envisioned that further modification of the 3CP inhibitors identified by the project to date would be required. As mentioned above, rapid metabolism of compound **2** was suspected to be the cause of the poor oral bioavailability exhibited by the molecule in CM-monkeys. Accordingly, we sought to improve upon such parameters by reducing the lipophilicity of **2** in an effort to minimize its suspected association with metabolizing cytochrome P450 enzymes and/or esterases.[14,15] Our previous structure−activity studies also indicated that the Michael acceptor moiety, the P_1 γ-lactam, the P_3 pyridone, and the P_4 isoxazole fragment were all critical for imparting potent antiviral activity to this series of 3CP inhibitors.[9,16] We therefore focused our compound modification efforts on altering the lipophilic P_2 benzyl substituent present in both compounds **1** and **2**. Prior structure−activity studies conducted with related peptidyl molecules had also demonstrated that this substituent could be truncated to some degree without drastic loss of either 3CP inhibitory properties or antiviral activity.[17]

Replacement of the P_2 benzyl substituent present in **1** with a propargyl moiety afforded a molecule (**3**) which displayed somewhat reduced anti-3CP properties (Table 1).[18] This loss in 3CP inhibitor potency was consistent with similar reductions noted during earlier studies conducted with related peptidyl molecules.[17] Despite the anti-3CP potency alteration, compound **3** retained good absolute antiviral properties when tested against seven distinct rhinovirus serotypes in cell culture (Table 1). In addition, the molecule exhibited good stability toward human plasma (Table 1) and displayed improved stability in human and canine microsome and/or hepatocyte-based metabolism experiments relative to the benzyl-containing molecule **1** (Table 2). The former result suggested that large, lipophilic 3CP inhibitors such as **1** tended to exhibit greater affinity for human plasma esterases relative to the more hydrophilic compound **3**. The latter observations were consistent with improvements in oxidative metabolic lability effected through reduced cytochrome P450 association and/or removal of the metabolically labile P_2 benzyl substituent from the inhibitor series.[19] The permeability of compound **3** through Caco-2 cell monolayers was also examined, and its aqueous solubility was determined (Table 2). The permeability of **3** was observed to be somewhat reduced relative to that noted for compounds **1** and **2**, but its solubility was surprisingly enhanced. Importantly, the results of these physical property assessments collectively suggested that **3** might exhibit good systemic exposures in animals following oral administration.[20]

Encouragingly, compound **3** was orally bioavailable in the dog when administered in a solution formulation and afforded 7 h plasma concentrations in excess of its average in vitro antiviral activity (Table 1). Nearly identical 7 h plasma concentrations were observed when the molecule was administered orally to dogs using a suspension vehicle as well. As predicted by in vitro simian metabolic stability experiments (Table 2), solution-based oral administration of compound **3** to CM-monkeys afforded poorer 7 h plasma concentration levels relative to those observed in dog (Table 1). However, absolute plasma levels of the molecule 7 h post administration in the monkey substantially exceeded those previously noted for compound **2** and were nearly identical to the average antiviral activity of **3** as determined by cell culture testing. These promising results prompted a more detailed exploration of 3CP inhibitors which incorporated a propargyl substituent at the P_2 position in lieu of the benzyl fragment present in the parent molecules.

Accordingly, the ester moiety present in this class of 3CP inhibitors was extensively modified in the hopes of further improving the esterase-related metabolic stability of the molecules (Table 1, compounds **4**−**11**). Such improvements were noted during our previous studies involving 2-pyridone anti-3CP agents containing P_2 benzyl substituents although they were achieved with a simultaneous worsening of 3CP inhibition properties (cf., compare compounds **1** and **2**, Table 1).[9] As expected, incorporation of sterically demanding ester groups into the P_2-propargyl-pyridone inhibitor design typically afforded molecules which displayed reduced anti-3CP potencies relative to the ethyl ester-containing compound **3**. Unfortunately, and in contrast to results obtained with inhibitor **2**, the majority of the non-ethyl ester-containing analogues of **3** exhibited weak (mean $EC_{50} > 0.20$ μM) absolute antirhinoviral potency when tested against seven HRV serotypes in cell culture

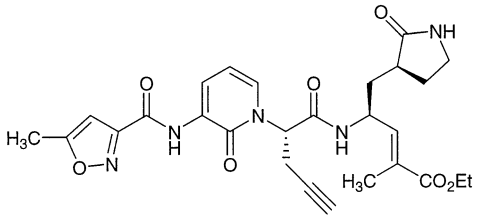
Table 1. 2-Pyridone-Containing HRV 3CP Inhibitors



compd no.	R_1	R_2	prep[a]	formula[b]	$k_{obs}/[I]$ (M^{-1} s^{-1})[c]	EC_{50} (μM)[d]	plasma $t_{1/2}$ (h)[e]	C_{7h} (μM) (dog)[f]	C_{7h} (μM) (monkey)[g]
1	CH_2CH_3	CH_2(3,4-F)Ph	ref 9	$C_{30}H_{31}F_2N_5O_7$	1 800 000	0.011	1.4	NA	NA
2	$CH(CH_3)_2$	CH_2(3,4-F)Ph	ref 9	$C_{31}H_{33}F_2N_5O_7 \cdot 0.75H_2O$	548 000	0.078	9.7	0.815 0.959[h]	0.012
3	CH_2CH_3	$CH_2C{\equiv}CH$	B	$C_{26}H_{29}N_5O_7 \cdot 0.75H_2O$	223 400	0.058	6.6	0.248 0.190[h]	0.057
4	$CH(CH_3)_2$	$CH_2C{\equiv}CH$	B	$C_{27}H_{31}N_5O_7 \cdot 0.50H_2O$	100 000	0.174[i]	ND	NA	NA
5	$C(CH_3)_3$	$CH_2C{\equiv}CH$	A	$C_{28}H_{33}N_5O_7$	60 500	0.361	ND	NA	NA
6	$CH_2C(CH_3)_3$	$CH_2C{\equiv}CH$	B	$C_{29}H_{35}N_5O_7 \cdot 0.25H_2O$	127 500	0.276	ND	NA	NA
7	cyclobutyl	$CH_2C{\equiv}CH$	B	$C_{28}H_{31}N_5O_7 \cdot 0.25H_2O$	207 100	0.090	3.3	0.073	ND
8	cyclopentyl	$CH_2C{\equiv}CH$	B	$C_{29}H_{33}N_5O_7 \cdot 0.75H_2O$	186 000	0.174	12.5	0.160	ND
9	cyclohexyl	$CH_2C{\equiv}CH$	B	$C_{30}H_{35}N_5O_7 \cdot 0.75H_2O$	34 000	0.406[j]	ND	NA	NA
10	cycloheptyl	$CH_2C{\equiv}CH$	B	$C_{31}H_{37}N_5O_7 \cdot 0.30H_2O$	47 000	0.812[j]	ND	NA	NA
11	CH_2Ph	$CH_2C{\equiv}CH$	B	$C_{31}H_{31}N_5O_7 \cdot 0.50H_2O$	300 000	0.036	4.3	0.563	0.003
13	CH_2CH_3	CH_2CH_3	B	$C_{25}H_{31}N_5O_7 \cdot 0.50H_2O$	170 000	0.047	5.6	0.682 <0.010[h]	0.896
14	$CH(CH_3)_2$	CH_2CH_3	B	$C_{26}H_{33}N_5O_7 \cdot 0.50H_2O$	36 000	0.322[j]	ND	NA	NA
15	$C(CH_3)_3$	CH_2CH_3	A	$C_{27}H_{35}N_5O_7 \cdot 0.50H_2O$	45 100	0.405	ND	NA	NA
16	$CH_2C(CH_3)_3$	CH_2CH_3	B	$C_{28}H_{37}N_5O_7 \cdot 0.50H_2O$	38 200	0.310	ND	NA	NA
17	cyclobutyl	CH_2CH_3	B	$C_{27}H_{33}N_5O_7 \cdot 0.75H_2O$	170 000	0.076	9.2	0.222	ND
18	cyclohexyl	CH_2CH_3	B	$C_{29}H_{37}N_5O_7 \cdot 0.50H_2O$	23 000	0.313[j]	ND	NA	NA
19	cycloheptyl	CH_2CH_3	B	$C_{30}H_{39}N_5O_7 \cdot 0.80H_2O$	15 000	ND	ND	NA	NA
20	CH_2Ph	CH_2CH_3	B	$C_{30}H_{33}N_5O_7 \cdot 0.50H_2O$	210 000	0.064	2.5	0.156	ND
Pirodavir				ref 31	NA	0.031[k]			
Pleconaril				ref 32	NA	0.136			

[a] Method of preparation: see Schemes 1 and 2. [b] Elemental analyses (C, H, N) of all compounds agreed to within ±0.4% of theoretical values. [c] Inhibition activity against HRV-14 3C protease; see ref 30 for assay method and error. [d] Antirhinoviral activity (mean of EC_{50} values determined against HRV serotypes 2, 3, 9, 14, 16, 25, and 39 unless otherwise noted); cytotoxicity values for all compounds were >10 μM; see ref 30 for assay method and error. [e] Half-life of compound upon exposure to human plasma; see Experimental Section for additional details. [f] Compound plasma concentration in dogs 7 h after oral administration (30 mg/kg, vehicle = 80:20 propylene glycol:H_2O); see Experimental Section for additional details. [g] Compound plasma concentration in CM-monkeys 7 h after oral administration (50 mg/kg, vehicle = 80:20 propylene glycol:H_2O); see Experimental Section for additional details. [h] Vehicle = 0.5% CMC suspension. [i] Compound was inactive when tested to 10 μM against HRV 39 (value is mean of activity against other six serotypes). [j] Mean of EC_{50} values determined against HRV serotypes 9, 14, and 25. [k] Compound was inactive when tested to 10 μM against HRV 25 (value is mean of activity against other six serotypes). ND = not determined. NA = not applicable.

(Table 1). A similar potency reduction was also exhibited by a molecule containing a methyl substituent adjacent to the Michael acceptor ester moiety (compound **12**),



12

$k_{obs}/[I]$ = 12,500 $M^{-1}s^{-1}$

EC_{50} = 1.46 μM

(HRV-14)

thus precluding this alternate approach to improving esterase-related metabolic stability. The three notable exceptions which emerged from the above activities were derivatives of **3** which incorporated cyclobutyl, cyclopentyl, and benzyl esters in lieu of the ethyl fragment (compounds **7**, **8**, and **11**, respectively). Although both inhibitors **7** and **8** displayed acceptable stability profiles toward human plasma (half-life >3 h, Table 1), they also exhibited relatively poor pharmacokinetics in the dog following oral administration. In each case, 7 h plasma concentrations were noted below the respective mean in vitro antiviral EC_{50}s of the molecules (Table 1). The poorer canine pharmacokinetic performance of **7** and **8** relative to **3** may be related to the increased in vitro canine metabolism and/or the lower water solubility exhibited by the former molecules (Table 2). In addition, the stability profiles of **7** and **8** toward canine and human liver microsomes were not significantly different from each other and suggested that dramatic pharmacokinetic improvement would not occur in the latter species (Table 2). These two molecules were therefore not examined further and were not studied in simian oral bioavailability experiments.

In contrast, the benzyl ester-containing inhibitor **11** displayed acceptable human plasma stability properties (half-life >3 h) and exhibited excellent exposure and pharmacokinetics after oral administration in the dog with 7 h plasma concentrations of the molecule far exceeding its in vitro antiviral potency (Table 1). These excellent pharmacokinetics were not obviously suggested by in vitro experiments which indicated a reduced stability of compound **11** toward canine microsomes and hepatocytes along with lesser aqueous

Table 2. In Vitro Properties of 2-Pyridone-Containing HRV 3CP Inhibitors

compd no.	dog (% metab)[a]		monkey (% metab)[a]		human (% metab)[a]		H_2O soln (μg/mL)[b]	Caco-2 P_{app} ($\times 10^{-6}$ cm/s)[c]	ClogP[d]	protein binding (% free)[e]	
	M	H	M	H	M	H				dog	human
1	57	ND	ND	ND	81	94	25	35	ND	ND	ND
2	57	16[f]	ND	92[f]	56	87[f]	21	36	2.07	8−19	4−9
3	45	12	55	79	40	85	377	6	0.18	54	34
7	53	ND	ND	ND	52	ND	25	14	0.57	ND	ND
8	76	ND	ND	ND	59	ND	15	36	1.12	20	7
11	67	61	ND	69	52	51	22	18	1.38	25	13
13	37	14	61	76[g]	61	82	38	5	0.66	ND	ND
17	46	ND	ND	ND	49	ND	192	10	1.04	42	33
20	69	55	ND	ND	56	27	41	11	1.86	20	8

[a] M = loss of compound (% metabolized) after 30 min exposure to liver microsomes from indicated species (25 μM compound concentration); see Experimental Section for additional details. H = loss of compound (% metabolized) after 2 h exposure to hepatocytes from indicated species (25 μM compound concentration); see Experimental Section for additional details. [b] Thermodynamic aqueous solubility; see Experimental Section for additional details. [c] Apical to basolateral permeability through Caco-2 cell monolayers; see Experimental Section for additional details. [d] LogP values calculated using Biobyte ClogP 4.0. [e] Plasma protein binding (% free); see Experimental Section for additional details. [f] 4 h exposure. [g] 5 μM compound concentration. ND = not determined.

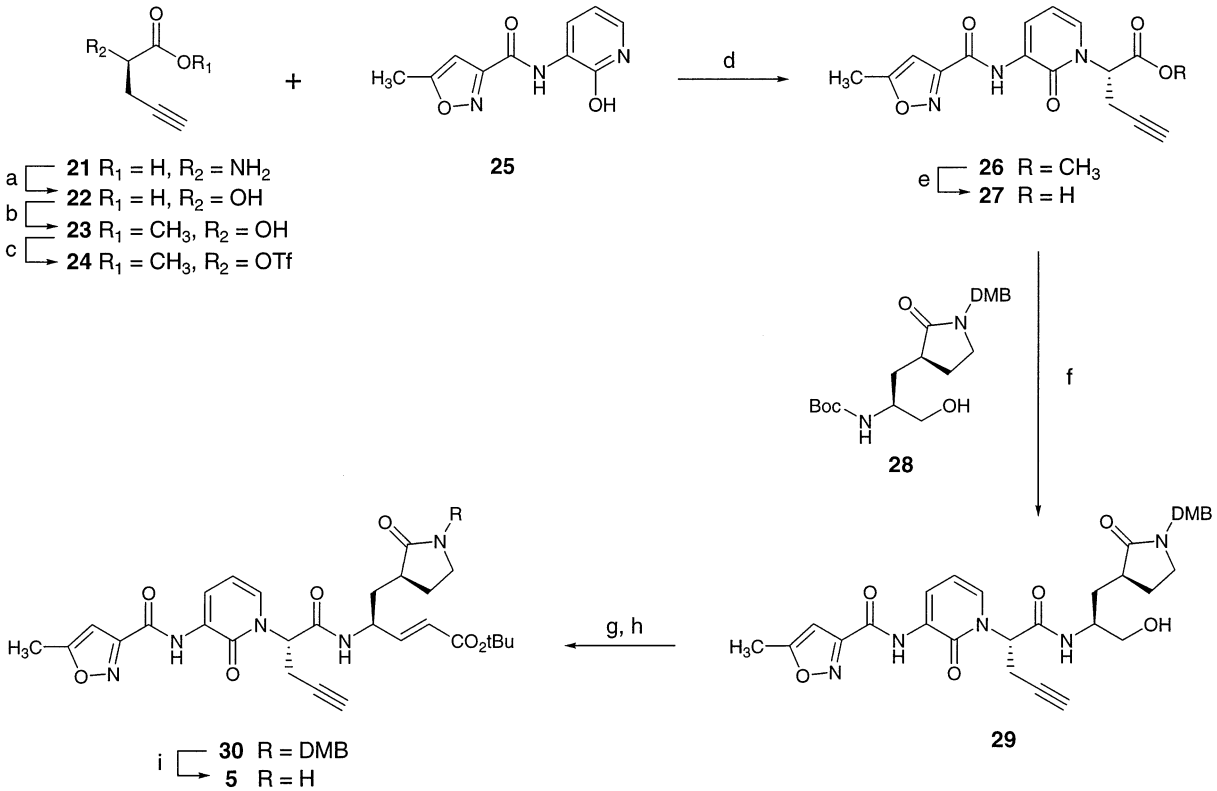
solubility relative to inhibitor **3** (Table 2). Although compound **11** performed well in the dog, the molecule was poorly bioavailable in CM-monkeys and provided 7 h simian plasma levels that were near the limits of detection. The strikingly poor performance of compound **11** in the monkey relative to **3** was also not easily predicted by in vitro experiments which suggested roughly equivalent hepatocyte stability and improved gut permeability of the former molecule (Table 2). Thus, a precise explanation of the oral pharmacokinetics observed for compound **11** in dogs and monkeys remains elusive but may involve additional metabolism of the molecule not easily detected using microsome and/or hepatocyte-based stability assessments. These results underscore the difficulty in accurately predicting the oral exposures of these peptidomimetic 3CP inhibitors using in vitro data and reaffirmed our desire to identify compounds which demonstrated good pharmacokinetic properties in both dogs and monkeys.

Accordingly, we also examined a series of 3CP inhibitors in which the P_2 benzylic substituent present in the lead compound **2** was replaced with an ethyl fragment (compounds **13**−**20**, Table 1). As was observed for the propargyl-containing molecules above, inhibitors which incorporated ethyl, cyclobutyl, or benzyl esters displayed the most potent 3CP inhibitory properties and in vitro antirhinoviral activities (compounds **13**, **17**, and **20**, respectively). Compound **13** also exhibited excellent exposure when orally administered to dogs using a solution formulation with 7 h plasma concentrations far exceeding its mean in vitro antirhinoviral activity (Table 1). Even greater exposures of the compound were noted in corresponding simian experiments which employed a solution-based delivery vehicle. Once again, the excellent pharmacokinetics of compound **13** in the monkey relative to the dog were not easily predicted from in vitro metabolic stability assessments which indicated that the molecule was more stable toward canine microsomes and hepatocytes (Table 2). Since additional assessments of **3** and **13** suggested comparable in vitro simian metabolism, equivalent Caco-2 permeabilities, and improved aqueous solubility of **3**, they did not readily clarify the >10-fold exposure difference between the two that was observed in the monkey (Tables 1 and 2). As mentioned above, we suspect that the in vivo metabolism of the compounds described in this work is complex and most likely involves processes that are not easily assessed with relatively simple in vitro metabolism assays. A potential concern with compound **13** was noted when the molecule was orally administered to dogs using a suspension formulation. In stark contrast to the solution-based experiment, suspension administration afforded very poor 7 h plasma levels of the compound (Table 1). This result suggested that the aqueous solubility of **13** may limit its oral exposure potential relative to **3** in the absence of a solution-based formulation and a significant solubility difference was indeed noted between the two compounds (Table 2). However, inhibitor **2** displayed aqueous solubility similar to that exhibited by **13** yet was readily bioavailable in the dog following oral suspension dosing (Table 1). Thus, the precise reasons for the poor exposures of **13** following suspension administration are not known with certainty.

As noted above, oral administration of compound **17** to the dog afforded 7 h plasma levels in excess of the molecule's in vitro antiviral potency (Table 1). The compound also demonstrated excellent in vitro stability toward human plasma (Table 1). However, the ratio of dog 7 h levels to antiviral potency was relatively low (<4-fold) compared to some of the other compounds described in this work (cf., compounds **2**, **3**, **11**, and **13**) and similar metabolic stability was observed for the compound in both human and canine in vitro microsome experiments (Table 2). Because of these limitations, inhibitor **17** was not selected for subsequent profiling in simian oral exposure assessments. Compound **20** also exhibited a relatively poor ratio between dog 7 h plasma concentration and antiviral potency and displayed unacceptable stability when exposed to human plasma as well (half-life <3 h; Table 1). The compound was therefore not selected for further experimentation. The latter result was particularly unfortunate given the molecule's good in vitro stability profile when exposed to human liver microsomes or hepatocytes (Table 2). The origins of the poor human plasma stability of compound **20** are presently not known.

Because of the difficulties encountered in predicting the in vivo pharmacokinetics of the 2-pyridone-containing 3CP inhibitors from the available in vitro data, we retrospectively examined whether extensive tissue compartmentalization and/or metabolism of the more lipophilic compounds might explain the molecules' observed plasma levels. No correlation was noted between the

Scheme 1[a]



[a] Synthetic Method A. Reagents and conditions (DMB = 2,4-dimethoxybenzyl, Tf = trifluoromethanesulfonyl: (a) $NaNO_2$, 1.0 M H_2SO_4, 0 → 23 °C, 16 h, 66%; (b) HCl, CH_3OH, 23 °C, 16 h, 79%; (c) 1.9 equiv of 2,6-lutidine, 1.7 equiv of Tf_2O, CH_2Cl_2, 0 °C, 25 min; (d) 1.1 equiv of **25**, 0.95 equiv of NaH, THF, 23 °C, 30 min, then 1.0 equiv of **24**, 23 °C, 30 min, 90%; (e) 2.5 equiv of LiI, pyridine, reflux, 30 min, 97%; (f) 1.1 equiv of **28**, HCl, 1,4-dioxane, 23 °C, 2 h, then 1.0 equiv of **27**, 1.4 equiv of HOBt, 7.0 equiv of $^iPr_2NEt$, 1.3 equiv of EDC, CH_2Cl_2, 0 → 23 °C, 16 h, 33%; (g) 1.15 equiv of Dess−Martin periodinane, CH_2Cl_2, 0 °C, 75 min; (h) 1.1 equiv of $Ph_3P{=}CHCO_2tBu$, THF, reflux, 45 min, 64%; (i) 4.2 equiv of DDQ, 10:1 $CHCl_3$:H_2O, 65 °C, 4 h, 71%.

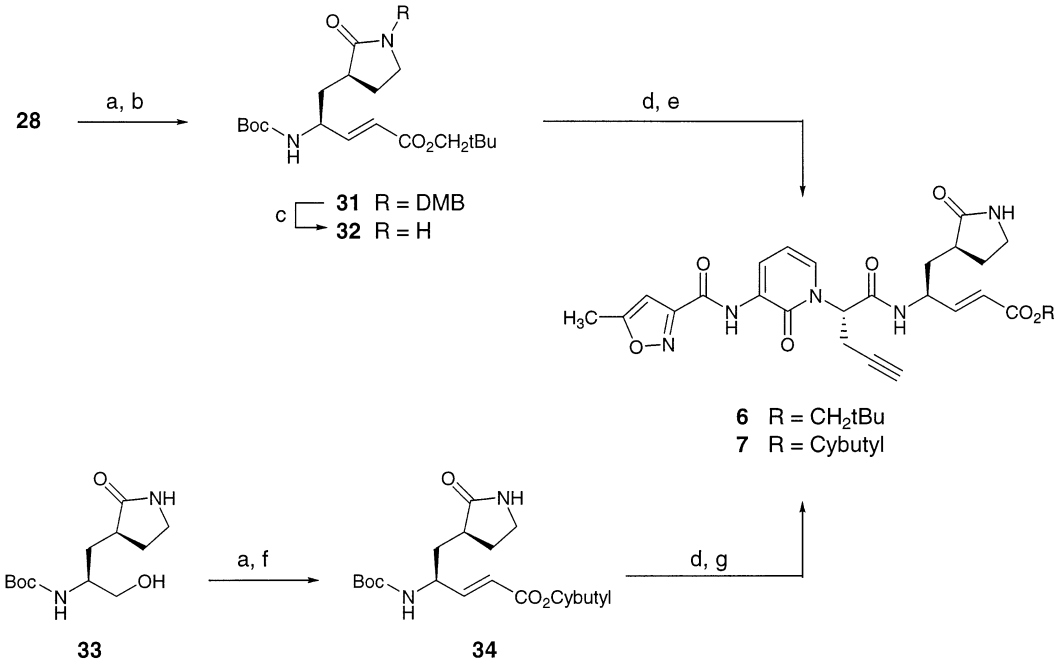
compounds' calculated logP values (Table 2) and their measured 7 h canine plasma concentrations ($R = -0.16$; not graphically depicted). Comparison of calculated polar surface area and canine plasma levels resulted in a similar lack of correlation ($R = 0.44$; data not shown). The calculated logP values did qualitatively parallel the propensity of the compounds to bind to plasma proteins in both dog and human (more lipophilic = smaller free fraction; Table 2), but the relevance of this observation to in vivo predictions is not known at this time. Thus, somewhat contrary to our original hypothesis and strategy, good in vivo pharmacokinetics were not always imparted to the 2-pyridone-containing 3CP inhibitors simply by reducing their lipophilicties.

Despite the above-mentioned uncertainties surrounding the use of in vitro data to predict in vivo pharmacokinetics, the optimization studies described above did succeed in achieving our primary objective of identifying potent 3CP inhibitors and antirhinoviral agents which display good exposures in both dogs and monkeys following oral administration. In particular, compounds **3** and **13** exhibited plasma concentrations in the dog 7 h after an oral administration more than 4-fold greater than their corresponding in vitro antirhinoviral potencies. Similarly, oral administration of these molecules to CM-monkeys afforded 7 h plasma concentrations that either far exceeded (compound **13**) or approximated (compound **3**) their respective in vitro antiviral EC_{50} values. The collective performance of both compounds in dogs and monkeys was superior to that exhibited by the P_2 benzyl-containing compound **2** (very low monkey exposures) and suggested that truncation of the P_2 benzyl substituent may enhance oral exposure of related molecules in these species. Thus, compounds **3** and **13** represent additional attractive options for the development of orally bioavailable human rhinovirus 3C protease inhibitors.

Synthesis

The 2-pyridone-containing 3CP inhibitors described in this study were prepared by two related synthetic methods (A and B). The particular method employed to synthesize a given compound is indicated in Table 1, and representative examples of each are given below. These syntheses differ primarily in the nature of the P_1 fragment utilized to complete each sequence and employ several synthetic transformations and intermediates from previously reported preparations of related pyridone-containing 3CP inhibitors.[9] The first method (Method A) is illustrated in Scheme 1 with the preparation of compound **5** and involves coupling of a P_1 amino-alcohol moiety to a $P_4-P_3-P_2$ fragment followed by oxidation and olefin formation.[16] Thus, commercially available D-propargylglycine (**21**) was converted to intermediate **22** using a known process for the transformation of α-amino acids to the corresponding α-hydroxy compounds.[21] This entity was subsequently esterified under acidic conditions to afford methyl ester **23** in good yield. The alcohol present in **23** was then

Scheme 2[a]



[a] Synthetic Method B. Reagents and conditions (DMB = 2,4-dimethoxybenzyl): (a) 1.1 equiv of Dess−Martin periodinane, CH_2Cl_2, 0 → 23 °C, 1.5 h; (b) 1.2 equiv of $Ph_3P{=}CHCO_2CH_2tBu$, THF, reflux, 1.5 h; (c) 4.2 equiv of DDQ, 10:1 $CHCl_3$:H_2O, 60 °C, 4 h, 67%; (d) TFA, CH_2Cl_2, 23 °C, 35 min; (e) 1.0 equiv of **27**, 1.4 equiv of HOBt, 1.25 equiv of EDC, 7.0 equiv of $^iPr_2NEt$, CH_2Cl_2, 0 → 23 °C, 24 h, 44%; (f) 0.97 equiv of $Ph_3P{=}CHCO_2$cyclobutyl, THF, reflux, 1.5 h, 44%; (g) 1.0 equiv of **27**, 1.4 equiv of HOBt, 1.25 equiv of EDC, 7.0 equiv of $^iPr_2NEt$, CH_2Cl_2, 0 → 23 °C, 16 h, 46%.

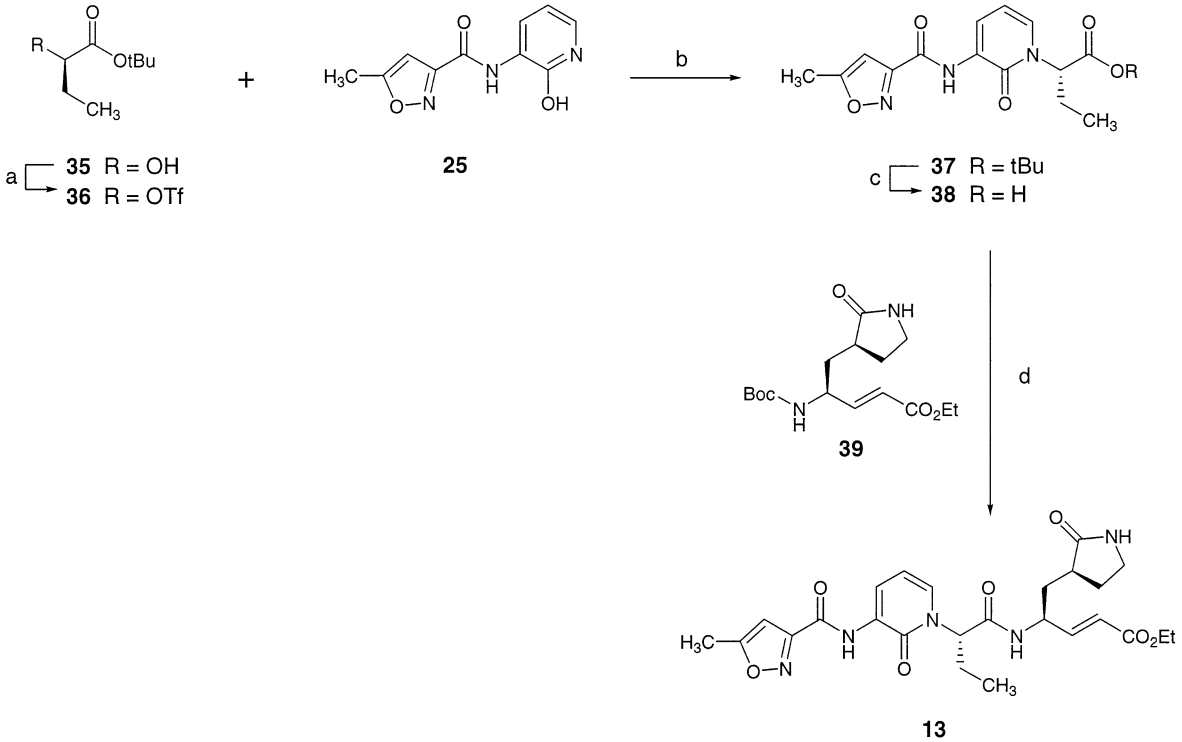
converted to the corresponding triflate (**24**) for use in the following coupling reaction. Although the described sequence to prepare **24** from D-propargylglycine was executed without purification, it routinely provided the desired triflate in quantities and purities sufficient for subsequent transformations.

Accordingly, condensation of **24** with the sodium salt of hydroxypyridine **25**[9] afforded coupling product **26** in good yield (95% ee). In direct analogy to our previous work, the described preparation of intermediate **26** predominantly provided the *N*-alkylated pyridone product as evidenced by TLC analysis of the reaction mixture and ^1H NMR analysis of the coupling product.[22,23] The methyl ester present in **26** was subsequently converted to the corresponding carboxylic acid (**27**) by treatment with LiI/pyridine. This reagent combination was chosen in order to minimize racemization of the chiral center present in **26** that was noted when employing more basic saponification conditions (e.g., NaOH). Coupling of **27** with the amine derived from deprotection of γ-lactam **28**[24] then afforded alcohol **29** as a single diastereomer in good yield following flash column purification.[25] This intermediate was then oxidized using the Dess−Martin reagent,[26] and the resulting crude aldehyde (not shown) was converted to olefin **30** in good overall yield. As was encountered during previous syntheses of peptidyl and peptidomimetic 3CP inhibitors,[17,24] the above olefination process afforded the desired *trans*-isomer with <5% of the corresponding *cis*-isomer as determined by ^1H NMR analysis of the crude reaction mixture. Oxidative removal[27] of the dimethoxybenzyl protecting group present in **30** afforded the desired 3CP inhibitor **5** in good yield after purification by silica gel chromatography. The described inhibitor preparation method was also used to synthesize compound **15** employing $P_4{-}P_3{-}P_2$ intermediate **38** (see Scheme 3 below) in lieu of carboxylic acid **27**.

The acid-labile esters present in inhibitors **5** and **15** necessitated the introduction of such functionalities after assembly of the $P_4{-}P_3{-}P_2{-}P_1$ inhibitor skeleton. In contrast, the remainder of the other 2-pyridone-containing 3CP inhibitors described in this work were prepared by coupling an olefin-containing P_1 fragment with an appropriate $P_4{-}P_3{-}P_2$ moiety (synthetic Method B).[16] This method was operationally simpler than Method A above since no compound modifications were required after effecting the described coupling. A representative example of Method B is illustrated in Scheme 2 with the preparation of compound **6**. Thus, γ-lactam **28**[24] was subjected to an oxidation/olefination sequence employing (triphenyl-λ^5-phosphanylidene)acetic acid 2,2-dimethylpropyl ester (see below and Experimental Section) to provide the *trans*-olefination product **31** in moderate yield. The dimethoxybenzyl moiety present in **31** was then removed under oxidative[27] conditions to afford γ-lactam **32**. This intermediate was subjected to an acidic Boc deprotection protocol, and the resulting amine salt (not shown) was coupled with carboxylic acid **27** to provide inhibitor **6** as a single diastereomer following silica gel purification.[25]

A variation of synthetic Method B is also illustrated in Scheme 2 with the preparation of inhibitor **7**. This variation utilizes γ-lactam **33**[28] in lieu of **28** and therefore did not require a debenzylation step analogous to the conversion of **31** to **32** described above. Thus, oxidation/olefination of **33** employing (triphenyl-λ^5-phosphanylidene)acetic acid cyclobutyl ester (see below and Experimental Section) afforded the *trans*-olefination product **34** in moderate yield. Acidic deprotection of this material followed by carbodiimide-mediated coupling of

Scheme 3[a]



[a] Synthetic Method B. Reagents and conditions: (a) 2.0 equiv of 2,6-lutidine, 1.9 equiv of Tf_2O, CH_2Cl_2, 0 °C, 25 min; (b) 1.1 equiv of **25**, 1.0 equiv of NaH, THF, 23 °C, 30 min, then 1.0 equiv of **36**, 23 °C, 30 min, 59%; (c) TFA, CH_2Cl_2, 23 °C, 30 min; (d) (from **39**) TFA, CH_2Cl_2, 23 °C, 2 h, then 1.0 equiv of **38**, 1.2 equiv of HOBt, 1.1 equiv of EDC, 7.0 equiv of $^iPr_2NEt$, CH_2Cl_2, 0 → 23 °C, 18 h, 58%.

the resulting amine salt (not shown) with carboxylic acid **27** provided inhibitor **7** in good yield after purification by flash column chromatography.[25]

Another application of Method B is provided in Scheme 3 with the preparation of inhibitor **13** and involves the synthesis of a $P_4-P_3-P_2$ intermediate containing a P_2-ethyl moiety. Thus, commercially available (*R*)-2-hydroxybutyric acid *tert*-butyl ester was converted to the corresponding triflate (**36**). In direct analogy with synthetic Method A above, condensation of crude **36** with the sodium salt of hydroxypyridine **25**[9] afforded coupling product **37** as a single enantiomer in good yield.[22,23] Subsequent acidic deprotection of **37** then provided carboxylic acid **38** which was used without purification in the next step. Coupling of **38** with the amine salt (not shown) derived from γ-lactam **39**[28] gave inhibitor **13** in good yield after purification by flash column chromatography.[25]

The remainder of the 3CP inhibitors described in this work were prepared by utilization of synthetic Method B to couple either carboxylic acid **27** (compounds **3** and **4** as well as **8**−**12**) or **38** (compounds **14**−**20**) to appropriately derivatized P_1 fragments. The phosphorus ylides required to effect these syntheses, along with those illustrated in Schemes 1, 2, and 3, were either commercially available (compounds **3**, **5**, **11**−**13**, **15**, and **20**), known in the literature (compounds **4**,[29] and **14**[29]) or were prepared by slight modifications of known syntheses[29] (compounds **6**−**10**, and **16**−**19**; see Experimental Section).

Conclusions

The studies presented above further demonstrate that peptidomimetic, 2-pyridone-containing irreversible inhibitors of the human rhinovirus 3C protease can function as potent, broad-spectrum, orally bioavailable antirhinoviral agents. Because of an apparent complex interplay between the metabolism profiles, gut permeabilities, and aqueous solubilities of these compounds, the physiochemical and biological properties which determine their pharmacokinetic performance following oral administration in both dogs and monkeys were not rigorously defined by our efforts. Two potent 3CP inhibitors were nevertheless identified which displayed promising pharmacokinetics in both dogs and monkeys following oral administration. Importantly, the plasma concentrations of these molecules in both species equaled or exceeded their average antirhinoviral activity as determined by cell culture assays (EC_{50}, 7 HRV serotypes) for at least 7 h postadministration. Collectively, these results suggest that additional examination of 2-pyridone-containing HRV 3CP inhibitors is warranted and that such study may lead to the identification of related molecules suitable for clinical development as orally delivered agents.

Experimental Section

General descriptions of experimental procedures, reagent purifications, and instrumentation along with conditions and uncertainties for enzyme and antiviral assays are provided elsewhere.[30] ^1H NMR chemical shifts are reported in ppm (δ) downfield relative to internal tetramethylsilane, and coupling constants are given in hertz. The following abbreviations also apply: HATU [*O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate], HOBt (1-hydroxybenzotriazole hydrate), EDC [1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride], CDI (1,1′-carbonyldiimidazole), MTBE (*tert*-butyl methyl ether), DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone), TFA (trifluoroacetic acid), DMAP [4-(dimeth-

ylamino)pyridine]. Pirodavir[31] was kindly provided by Janssen Pharmaceuticals. Pleconaril was prepared as described in the literature.[32]

Representative Example of Synthesis Method A. Synthesis of *trans*-(2′*S*,3‴′*S*,4*S*)-4-(2′-{3″-[(5‴-Methylisoxazole-3‴-carbonyl)amino]-2″-oxo-2″*H*-pyridin-1″-yl}pent-4′-ynoylamino)-5-(2‴′-oxopyrrolidin-3‴′-yl)pent-2-enoic Acid *tert*-Butyl Ester (5).

(*R*)-2-Hydroxypent-4-ynoic Acid (22). D-Propargylglycine (**21**) (1.50 g, 13.3 mmol, 1 equiv) was dissolved in 1 M H_2SO_4 (52 mL) and cooled to −2 °C. A solution of sodium nitrite (40% aq, 6.6 mL) was added dropwise, keeping the temperature below 10 °C. After addition was complete, the reaction mixture was held at 0 °C for 3 h and then allowed to warm to 23 °C and stirred 16 h more. The reaction mixture was extracted with MTBE (3 × 75 mL), and the organic phases were dried over Na_2SO_4 and evaporated to give **22** as a yellow oil (1.0 g, 66%) which was used without further purification.

(*R*)-2-Hydroxypent-4-ynoic Acid Methyl Ester (23). Compound **22** (0.714 g, 6.26 mmol, 1 equiv) was dissolved in CH_3OH (20 mL). A solution of HCl in 1,4-dioxane (4.0 M, 0.3 mL) was added. The reaction mixture was stirred 16 h, diluted with EtOAc (250 mL), washed with aq $NaHCO_3$ and brine (70 mL each), dried over Na_2SO_4, and evaporated to give **23** as a yellow oil (0.630 g, 79%) which was used without further purification.

(*R*)-2-Trifluoromethanesulfonyloxypent-4-ynoic Acid Methyl Ester (24). Compound **23** (0.460 g, 3.59 mmol, 1 equiv) was dissolved in CH_2Cl_2 (27 mL) and cooled to 0 °C. 2,6-Lutidine (0.795 mL, 6.83 mmol, 1.9 equiv) and trifluoromethanesulfonic anhydride (1.03 mL, 6.12 mmol, 1.7 equiv) were added successively. After being stirred 25 min, the reaction mixture was diluted with MTBE (250 mL), washed with a mixture of brine and 1 N HCl (3:1, 70 mL then 40 mL × 2), dried over $MgSO_4$, and evaporated to give **24** as a residue which was used without further purification.

(2*S*)-2-{3′-[(5″-Methylisoxazole-3″-carbonyl)amino]-2′-oxo-2′*H*-pyridin-1′-yl}pent-4-ynoic Acid Methyl Ester (26). 5-Methylisoxazole-3-carboxylic acid (2′-hydroxypyridin-3′-yl)amide (**25**[9]) (0.866 g, 3.95 mmol, 1.1 equiv) was dissolved in THF (15 mL). Sodium hydride (60% dispersion in mineral oil, 0.136 g, 3.4 mmol, 0.95 equiv) was added in portions, and the reaction mixture was stirred 30 min. To this mixture was added a solution of **24** prepared above (3.59 mmol theoretical) in THF (15 mL). After being stirred 30 min more, the reaction mixture was diluted with EtOAc (300 mL), washed with brine (2 × 75 mL), dried over Na_2SO_4, and evaporated. The residue was purified by flash column chromatography (40% EtOAc in hexanes) to give the product as an off-white solid (0.776 g, 90%): mp = 162−164 °C; R_f = 0.42 (50% EtOAc in hexanes); IR (cm^{-1}) 3342, 3284, 1748, 1697, 1650, 1597, 1533; ^1H NMR ($CDCl_3$) δ 2.01 (t, 1H, J = 2.7), 2.50 (d, 3H, J = 0.7), 3.06 (ddd, 1H, J = 17.6, 4.5, 2.7), 3.21 (ddd, 1H, J = 17.6, 9.6, 2.7), 3.78 (s, 3H), 5.11 (dd, 1H, J = 9.6, 4.5), 6.33 (t, 1H, J = 7.3), 6.48 (s, 1H), 7.16 (dd, 1H, J = 7.3, 1.7), 8.49 (dd, 1H, J = 7.3, 1.7), 9.56 (s, 1H); Anal. ($C_{16}H_{15}N_3O_5 \cdot 0.25H_2O$) C, H, N.

(2*S*)-2-{3′-[(5″-Methylisoxazole-3″-carbonyl)amino]-2′-oxo-2′*H*-pyridin-1′-yl}pent-4-ynoic Acid (27). Lithium iodide (3.13 g, 23.4 mmol, 2.5 equiv) and compound **26** (3.08 g, 9.35 mmol, 1 equiv) were combined in pyridine (12 mL) and refluxed 30 min. After being cooled, the reaction mixture was poured into 1 M HCl (300 mL) and extracted with CH_2Cl_2 (3 × 300 mL). The combined organic phases were washed with a mixture of brine and 1 M HCl (10:1, 3 × 50 mL), dried over $MgSO_4$, and evaporated to provide crude **27** (2.86 g, 97%) which was used without further purification.

(1″*S*,1‴*S*,3‴′*S*)-5-Methylisoxazole-3-carboxylic acid [1′-(1″-{1‴-[1‴′-(2‴′,4‴′-dimethoxybenzyl)-2‴′-oxopyrrolidin-3‴′-ylmethyl]-2‴-hydroxyethylcarbamoyl}but-3″-ynyl)-2′-oxo-1′,2′-dihydropyridin-3′-yl]amide (29). Compound **28**[24] (2.59 g, 6.34 mmol, 1.1 equiv) was dissolved in a solution of HCl in 1,4-dioxane (2.0 M, 54 mL) and stirred 2 h. The volatiles were evaporated to give the crude amine salt which was then dissolved in CH_2Cl_2 (140 mL) and cooled to 0 °C. Compound **27** (1.82 g, 5.77 mmol, 1 equiv), HOBt (1.09 g, 8.07 mmol, 1.4 equiv), $^iPr_2NEt$ (7.03 mL, 40.3 mmol, 7.0 equiv), and EDC (1.44 g, 7.51 mmol, 1.3 equiv) were added successively. The reaction mixture was allowed to warm to 23 °C and stirred 16 h then diluted with CH_2Cl_2 (600 mL) and washed with 2.5% $KHSO_4$, brine, aq $NaHCO_3$ and brine (100 mL each). After being dried over Na_2SO_4, the organic phase was concentrated, and the residue was purified by flash column chromatography (3% CH_3OH in CH_2Cl_2) to give **29** as a foam (1.16 g, 33%): R_f = 0.20 (5% CH_3OH in CH_2Cl_2); IR (cm^{-1}) 3389, 1649, 1596, 1531; ^1H NMR ($CDCl_3$) δ 1.48−1.69 (m, 2H), 1.90−2.02 (m, 2H), 2.11−2.22 (m, 1H), 2.39−2.53 (m, 1H), 2.50 (d, 3H, J = 0.7), 3.05−3.16 (m, 4H), 3.55−3.71 (m, 2H), 3.77 (s, 3H), 3.78 (s, 3H), 3.85−3.91 (m, 1H), 3.98−4.08 (m, 1H), 4.16 (d, 1H, J = 14.5), 4.40 (d, 1H, J = 14.5), 5.32−5.39 (m, 1H), 6.31 (t, 1H, J = 7.3), 6.40−6.44 (m, 2H), 6.47 (s, 1H), 6.99−7.03 (m, 1H), 7.28 (dd, 1H, J = 7.3, 1.7), 8.35 (s, 1H, J = 7.0), 8.44 (dd, 1H, J = 7.3, 1.7), 9.57 (s, 1H); Anal. ($C_{31}H_{35}N_5O_8 \cdot 0.5H_2O$) C, H, N.

***trans*-(2‴′*S*,3′*S*,4*S*)-5-[1′-(2″,4″-Dimethoxybenzyl)-2′-oxopyrrolidin-3′-yl]-4-(2‴-{3‴′-[(5‴′-methylisoxazole-3‴′-carbonyl)-amino]-2‴′-oxo-2‴′*H*-pyridin-1‴′-yl}pent-4‴′-ynoylamino)-pent-2-enoic Acid *tert*-Butyl Ester (30).** Dess−Martin periodinane (Lancaster, 0.917 g, 2.15 mmol, 1.15 equiv) was added to a 0 °C solution of **29** (1.13 g, 1.87 mmol, 1 equiv) in CH_2Cl_2 (20 mL). The reaction vessel was then warmed to 23 °C and stirred 75 min. The volatiles were evaporated, and the residue was evaporated from toluene (3 × 5 mL). The residue was then dissolved in THF (40 mL). (*tert*-Butoxycarbonylmethylene)triphenylphosphorane (0.773 g, 2.05 mmol, 1.1 equiv) was added, and the reaction mixture was refluxed 45 min and then cooled and concentrated. The residue was purified by flash column chromatography (2% CH_3OH in $CHCl_3$) to give **30** as a foam (0.844 g, 64%): R_f = 0.38 (5% CH_3OH in CH_2Cl_2); IR (cm^{-1}) 3331, 1678, 1649, 1596, 1531; ^1H NMR ($CDCl_3$) δ 1.46 (s, 9H), 1.50−1.70 (m, 1H), 1.83−1.96 (m, 1H), 2.00 (t, 1H, J = 2.6), 2.11−2.22 (m, 1H), 2.40−2.53 (m, 1H), 2.50 (s, 3H), 3.07−3.14 (m, 4H), 3.75 (s, 3H), 3.78 (s, 3H), 4.10 (d, 1H, J = 14.5), 4.38 (d, 1H, J = 14.5), 4.44−4.55 (m, 1H), 5.29−5.36 (m, 1H), 5.92 (dd, 1H, J = 15.6, 1.4), 6.32 (t, 1H, J = 7.2), 6.37−6.44 (m, 2H), 6.47 (s, 1H), 6.70 (dd, 1H, J = 15.6, 5.6), 7.00 (d, 1H, J = 8.6), 7.27 (dd, 1H, J = 7.2, 1.6), 8.45 (dd, 1H, J = 7.2, 1.6), 8.68 (d, 1H, J = 6.0), 9.59 (s, 1H); Anal. ($C_{37}H_{42}N_5O_9 \cdot 0.75H_2O$) C, H, N.

***trans*-(2′*S*,3‴′*S*,4*S*)-4-(2′-{3″-[(5‴-Methylisoxazole-3‴-carbonyl)amino]-2″-oxo-2″*H*-pyridin-1″-yl}pent-4′-ynoylamino)-5-(2‴′-oxopyrrolidin-3‴′-yl)pent-2-enoic Acid *tert*-Butyl Ester (5).** DDQ (0.372 g, 1.64 mmol, 1.4 equiv) was added to a solution of **30** (0.821 g, 1.17 mmol, 1 equiv) in a mixture of $CHCl_3$ and H_2O (10:1, 29 mL). The reaction vessel was placed in an oil bath maintained at 65 °C. After stirring 1.5 h, additional DDQ (0.372 g, 1.64 mmol, 1.4 equiv) was added. After an additional 1.5 h, still more DDQ (0.372 g, 1.64 mmol, 1.4 equiv) was added. After 4 h total, the reaction mixture was allowed to cool, diluted with EtOAc (500 mL), washed with a mixture of 10% $KHSO_4$ and brine (1:1, 100 mL) and a mixture of saturated $NaHCO_3$ and brine (1:1, 100 mL), and then dried over $MgSO_4$ and evaporated. The residue was purified by flash column chromatography (2% CH_3OH in CH_2Cl_2) to provide **5** (0.458 g, 71%) as a solid: mp = 195 °C, dec; R_f = 0.22 (5% CH_3OH in CH_2Cl_2); IR (cm^{-1}) 3295, 1690, 1649; ^1H NMR ($CDCl_3$) δ 1.47 (s, 9H), 1.50−1.79 (m, 2H), 2.04 (t, 1H, J = 2.6), 2.05−2.37 (m, 3H), 2.49 (s, 3H), 2.92 (ddd, 1H, J = 17.0, 8.6, 2.6), 3.02 (ddd, 1H, J = 17.0, 6.7, 2.6), 3.20−3.37 (m, 2H), 4.41−4.52 (m, 1H), 5.65−5.73 (m, 1H), 5.94 (dd, 1H, J = 15.6, 1.4), 6.33 (t, 1H, J = 7.3), 6.46 (s, 1 H), 6.70 (s, 1 H), 6.73 (dd, 1 H, J = 15.6, 5.3), 7.48 (dd, 1 H, J = 7.3, 1.7), 8.41 (dd, 1 H, J = 7.3, 1.7), 8.62 (d, 1 H, J = 6.6), 9.53 (s, 1H); Anal. ($C_{28}H_{33}N_5O_7$) C, H, N.

***trans*-(2′*S*,3‴′*S*,4*S*)-4-(2′-{3″-[(5‴-Methylisoxazole-3‴-carbonyl)amino]-2″-oxo-2″*H*-pyridin-1″-yl}butyrylamino)-5-(2‴′-oxopyrrolidin-3‴′-yl)pent-2-enoic Acid Ethyl Ester (15).** R_f = 0.27 (5% CH_3OH in CH_2Cl_2); IR (cm^{-1}) 3331, 3295, 1690, 1649, 1590, 1531, 1455, 1155; ^1H NMR ($CDCl_3$) δ 0.93

(t, 3H, $J = 7.3$), 1.40−1.60 (m, 1H), 1.49 (s, 9H), 1.62−1.78 (m, 1H), 1.87−2.02 (m, 1H), 2.09−2.36 (m, 4H), 2.50 (s, 3H), 3.26−3.43 (m, 2H), 4.39−4.50 (m, 1H), 5.78 (dd, 1H, $J = 8.9$, 6.7), 5.89 (dd, 1H, $J = 15.7$, 1.3), 6.35 (t, 1H, $J = 7.3$), 6.47 (s, 1H), 6.76 (dd, 1H, $J = 15.7$, 5.9), 7.33 (s, 1H), 7.64 (dd, 1H, $J = 7.3$, 1.8), 8.43 (dd, 1H, $J = 7.3$, 1.8), 8.59 (d, 1H, $J = 6.8$), 9.57 (s, 1H); Anal. ($C_{27}H_{35}N_5O_7 \cdot 0.5H_2O$) C, H, N.

Representative Example of Ylide Preparation. Synthesis of (Triphenyl-λ^5-phosphanylidene)acetic Acid Cyclobutyl Ester. EDC (14.4 g, 75.1 mmol, 1.1 equiv) and DMAP (0.833 g, 6.82 mmol, 0.1 equiv) were added sequentially to a solution of bromoacetic acid (9.48 g, 68.2 mmol, 1.0 equiv) and cyclobutanol (4.92 g, 68.2 mmol, 1 equiv) in CH_2Cl_2 at 0 °C. The reaction mixture was warmed to 23 °C, was stirred at that temperature for 16 h, and then was partitioned between water (150 mL) and CH_2Cl_2 (100 mL). The aqueous layers were subsequently extracted with a 1:1 mixture of EtOAc and hexanes (150 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure to a volume of approximately 35 mL (no heat was applied during this concentration in order to minimize loss of the desired intermediate).

The crude cyclobutyl ester thus obtained was dissolved in benzene (100 mL) at 23 °C, and triphenylphosphine (16.1 g, 61.4 mmol, 0.9 equiv) was added. The reaction mixture was then heated to 45 °C at which point a white precipitate began to form. After being stirred 22 h at 45 °C, the mixture was cooled to 23 °C. The precipitate was collected by filtration through medium weight paper, washed with benzene (2 × 50 mL), and air-dried.

The material obtained in the previous step was suspended in water (150 mL) at 23 °C. Sodium hydroxide (7.5 mL of a 2.0 M aqueous solution) was added via pipet over a 2 min period, resulting in additional precipitate formation. The reaction mixture was then transferred to a separatory funnel and extracted with EtOAc (2 × 150 mL). The combined organic layers were dried over Na_2SO_4 and concentrated to provide slightly impure (triphenyl-λ^5-phosphanylidene)acetic acid cyclobutyl ester (4.33 g, 19% from bromoacteic acid) as a colorless oil. This crude material was subsequently used in the preparation of inhibitors **7** and **17**. The ylides required for the synthesis of compounds **6** and **16**, **8**, **9** and **18**, and **10** and **19** were prepared in analogous manner utilizing neopentyl alcohol, cyclopentanol, cyclohexanol, and cycloheptanol, respectively, in lieu of the cyclobutanol employed above.

Representative Example of Synthesis Method B. Synthesis of *trans*-(2′*S*,3‴′*S*,4*S*)-4-(2′-{3″-[(5‴-Methylisoxazole-3‴-carbonyl)amino]-2″-oxo-2″*H*-pyridin-1″-yl}pent-4′-ynoylamino)-5-(2‴′-oxopyrrolidin-3‴′-yl)pent-2-enoic Acid 2,2-Dimethylpropyl Ester (6).

***trans*-(3′*S*,4*S*)-4-*tert*-Butoxycarbonylamino-5-[1′-(2″,4″-dimethoxybenzyl)-2′-oxopyrrolidin-3′-yl]pent-2-enoic Acid 2,2-Dimethylpropyl Ester (31).** Dess−Martin periodinane (2.30 g, 5.38 mmol, 1.1 equiv) was added to a 0 °C solution of **28**[24] (2.00 g, 4.90 mmol, 1 equiv) in CH_2Cl_2 (50 mL). The reaction vessel was then warmed to 23 °C and stirred 1.5 h. The volatiles were evaporated, and the residue was evaporated from toluene (2 × 20 mL). The residue was then dissolved in THF (100 mL). (Triphenyl-λ^5-phosphanylidene)acetic acid 2,2-dimethylpropyl ester (prepared using the general ylide synthesis described above, 2.29 g, 5.86 mmol, 1.2 equiv) was added, and the reaction mixture was refluxed 1.5 h, cooled, and evaporated. The residue was purified by flash column chromatography (3% CH_3OH in $CHCl_3$ then 40% EtOAc in hexanes) to give slightly impure **31** (1.50 g). This material was not characterized and was instead used directly in the next step.

***trans*-(3′*S*,4*S*)-4-*tert*-Butoxycarbonylamino-5-(2′-oxopyrrolidin-3′-yl)pent-2-enoic Acid 2,2-Dimethylpropyl Ester (32).** DDQ (0.925 g, 4.07 mmol, 1.4 equiv) was added to a solution of **31** (1.50 g, 2.89 mmol, 1 equiv) in a mixture of $CHCl_3$ and H_2O (10:1, 55 mL). The reaction vessel was placed in an oil bath maintained at 60 °C. After the mixture was stirred for 1.5 h, additional DDQ (0.925 g, 4.07 mmol, 1.4 equiv) was added. After an additional 1.5 h, still more DDQ (0.925 g, 4.07 mmol, 1.4 equiv) was added. After 4 h total, the reaction mixture was allowed to cool, diluted with EtOAc (500 mL), washed with a mixture of 10% $KHSO_4$ and brine (1:1, 100 mL) and a mixture of saturated $NaHCO_3$ and brine (1:1, 100 mL), then dried over $MgSO_4$ and evaporated. The residue was purified by flash column chromatography (2% CH_3OH in CH_2Cl_2) to provide **32** as a brown foam (0.717 g, 67%): $R_f = 0.42$ (5% CH_3OH in CH_2Cl_2); IR (cm^{-1}) 3295, 1713, 1690, 1666, 1272; ^1H NMR ($CDCl_3$) δ 0.95 (s, 9H), 1.44 (s, 9H), 1.55−1.66 (m, 1H), 1.78−1.86 (m, 1H), 1.95−2.06 (m, 1H), 2.39−2.56 (m, 2H), 3.30−3.40 (m, 2H), 3.83 (s, 2H), 4.31−4.42 (m, 1H), 5.32 (d, 1H, $J = 7.9$), 5.98 (dd, 1H, $J = 15.7$, 1.6), 6.38 (s, 1H), 6.86 (dd, 1H, $J = 15.7$, 5.2); Anal. ($C_{19}H_{32}N_2O_5 \cdot 0.25H_2O$) C, H, N.

***trans*-(2′*S*,3‴′*S*,4*S*)-4-(2′-{3″-[(5‴-Methylisoxazole-3‴-carbonyl)amino]-2″-oxo-2″*H*-pyridin-1″-yl}pent-4′-ynoylamino)-5-(2‴′-oxopyrrolidin-3‴′-yl)pent-2-enoic Acid 2,2-Dimethylpropyl Ester (6).** TFA (10 mL) was added to a solution of **32** (0.702 g, 1.91 mmol, 1 equiv) in CH_2Cl_2 (14 mL) and stirred 35 min at 23 °C. The volatiles were evaporated, and the residue was concentrated from CCl_4 (20 mL) to give the crude amine salt. This material was combined with intermediate **27** (0.601 g, 1.91 mmol, 1 equiv) and HOBt (0.360 g, 2.66 mmol, 1.4 equiv) in CH_2Cl_2 (34 mL) and cooled to 0 °C. EDC (0.457 g, 2.38 g, 1.25 equiv) and $^iPr_2NEt$ (2.32 mL, 13.3 mmol, 7.0 equiv) were then added sequentially. The reaction mixture was allowed to warm to 23 °C, stirred 24 h, diluted with EtOAc (550 mL), and washed with 2.5% $KHSO_4$, brine, aq $NaHCO_3$, and brine (75 mL each). The organic phase was dried over Na_2SO_4 and evaporated. The residue was purified by flash column chromatography (2% CH_3OH in $CHCl_3$) to give **6** as a white amorphous powder (0.470 g, 44%): $R_f = 0.48$ (5% CH_3OH in CH_2Cl_2); IR (cm^{-1}) 3295, 1690, 1649, 1596, 1531; ^1H NMR ($CDCl_3$) δ 0.95 (s, 9H), 1.54−1.80 (m, 2H), 2.05 (t, 1H, $J = 2.6$), 2.10−2.39 (m, 3H), 2.49 (s, 3H), 2.91 (ddd, 1H, $J = 17.0$, 8.2, 2.6), 3.01 (ddd, 1H, $J = 17.0$, 6.8, 2.6), 3.22−3.38 (m, 2H), 3.82 (s, 2H), 4.45−4.56 (m, 1H), 5.72−5.79 (m, 1H), 6.06 (dd, 1H, $J = 15.7$, 1.5), 6.33 (t, 1H, $J = 7.2$), 6.46 (s, 1H), 6.86 (dd, 1H, $J = 15.7$, 5.4), 6.93 (s, 1H), 7.51 (dd, 1H, $J = 7.2$, 1.7), 8.41 (dd, 1H, $J = 7.2$, 1.7), 8.69 (d, 1H, $J = 6.6$), 9.52 (s, 1H); Anal. ($C_{29}H_{35}N_5O_7 \cdot 0.25H_2O$) C, H, N.

Representative Example of Synthetic Method B. Synthesis of *trans*-(2′*S*,3‴′*S*,4*S*)-4-(2′-{3″-[(5‴-Methylisoxazole-3‴-carbonyl)amino]-2″-oxo-2″*H*-pyridin-1″-yl}pent-4′-ynoylamino)-5-(2‴′-oxopyrrolidin-3‴′-yl-pent-2-enoic Acid Cyclobutyl Ester (7).

***trans*-(3′*S*,4*S*)-4-*tert*-Butoxycarbonylamino-5-(2′-oxopyrrolidin-3′-yl)pent-2-enoic Acid Cyclobutyl Ester (34).** Dess−Martin periodinane (1.16 g, 2.72 mmol, 1 equiv) was added to a 0 °C solution of **33**[28] (0.700 g, 2.71 mmol, 1 equiv) in CH_2Cl_2 (25 mL). The reaction vessel was then warmed to 23 °C and stirred 1.5 h. The volatiles were evaporated, and the residue was evaporated from toluene (2 × 10 mL). The residue was then dissolved in THF (50 mL). (Triphenyl-λ^5-phosphanylidene)acetic acid cyclobutyl ester (0.982 g, 2.62 mmol, 0.97 equiv) was added, and the reaction mixture was refluxed 1.5 h and then cooled and concentrated. The residue was filtered through a plug of silica gel (5% CH_3OH in CH_2Cl_2 as eluent) to provide **34** (0.389 g, 41%) which was used without further purification.

***trans*-(2′*S*,3‴′*S*,4*S*)-4-(2′-{3″-[(5‴-Methylisoxazole-3‴-carbonyl)amino]-2″-oxo-2″*H*-pyridin-1″-yl}pent-4′-ynoylamino)-5-(2‴′-oxopyrrolidin-3‴′-yl-pent-2-enoic Acid Cyclobutyl Ester (7).** TFA (3 mL) was added to a solution of **34** (0.389 g, 1.10 mmol, 1 equiv) in CH_2Cl_2 (4 mL) and stirred 35 min. The volatiles were evaporated, and the residue was concentrated from CCl_4 (5 mL) to provide the crude amine salt. This material was combined with **27** (0.348 g, 1.10 mmol, 1 equiv) and HOBt (0.209 g, 1.55 mmol, 1.4 equiv) in CH_2Cl_2 (20 mL) and cooled to 0 °C. EDC (0.265 g, 1.38 g, 1.25 equiv) and $^iPr_2NEt$ (1.35 mL, 7.75 mmol, 7.0 equiv) were added. The reaction mixture was allowed to warm to 23 °C, was stirred 16 h, and then was partitioned between CH_2Cl_2 (3 × 125 mL) and a mixture of $NaHCO_3$ and brine (1:1, 100 mL). The

combined organic phases were washed with a mixture of $NaHCO_3$ and brine (1:1, 100 mL), dried over $MgSO_4$, and evaporated. The residue was purified by flash column chromatography (2% CH_3OH in $CHCl_3$) to give **7** as a glass (0.281 g, 46%): R_f = 0.33 (5% CH_3OH in CH_2Cl_2); IR (cm^{-1}) 3507, 3331, 3295, 1690, 1649, 1596, 1531; ^1H NMR ($CDCl_3$) δ 1.52−1.87 (m, 4H), 2.01−2.41 (m, 8H), 2.50 (s, 3H), 2.92 (ddd, 1H, J = 17.0, 8.5, 2.6), 3.02 (ddd, 1H, J = 17.0, 6.8, 2.6), 3.21−3.37 (m, 2H), 4.43−4.54 (m, 1H), 4.96−5.08 (m, 1H), 5.68−5.76 (m 1H), 6.00 (dd, 1H, J = 15.7, 1.5), 6.33 (t, 1H, J = 7.3), 6.45−6.48 (m, 1H), 6.83 (s, 1H), 6.84 (dd, 1H, J = 15.7, 5.4), 7.49 (dd, 1H, J = 7.3, 1.7), 8.41 (dd, 1H, J = 7.3, 1.7), 8.68 (d, 1H, J = 6.6), 9.52 (s, 1H); Anal. ($C_{28}H_{31}N_5O_7 \cdot 0.25H_2O$) C, H, N.

Representative Example of Synthetic Method B. Synthesis of *trans*-(2′*S*,3‴′*S*,4*S*)-4-(2′-{3″-[(5‴-Methylisoxazole-3‴-carbonyl)amino]-2″-oxo-2″*H*-pyridin-1″-yl}butyrylamino)-5-(2‴′-oxopyrrolidin-3‴′-yl)pent-2-enoic Acid Ethyl Ester (13).

(*R*)-2-Trifluoromethanesulfonyloxybutyric Acid *tert*-Butyl Ester (36). (*R*)-2-Hydroxybutyric acid *tert*-butyl ester (**35**) (0.133 g, 0.830 mmol, 1 equiv) was dissolved in CH_2Cl_2 (7 mL) and cooled to 0 °C. 2,6-Lutidine (0.193 mL, 1.66 mmol, 2.0 equiv) and trifluoromethanesulfonic anhydride (0.265 mL, 1.58 mmol, 1.9 equiv) were added successively. After being stirred 25 min, the reaction mixture was diluted with MTBE (100 mL) and washed with a mixture of brine and 1 N HCl (3:1, 3 × 30 mL) and then dried over $MgSO_4$ and concentrated to give **36** as a residue. This material was used in the next step without further purification.

(2*S*)-2-{3′-[(5″-Methylisoxazole-3″-carbonyl)amino]-2′-oxo-2′*H*-pyridin-1′-yl}butyric Acid *tert*-Butyl Ester (37). 5-Methylisoxazole-3-carboxylic acid (2′-hydroxypyridin-3′-yl)-amide (**25**[9]) (0.200 g, 0.912 mmol, 1.1 equiv) was dissolved in THF (6 mL). Sodium hydride (60% dispersion in mineral oil, 0.0332 g, 0.83 mmol, 1.0 equiv) was added, and the reaction mixture was stirred 30 min. To this mixture was added a solution of crude **36** prepared above (0.830 mmol theoretical) in THF (7 mL). After being stirred 30 min more, the reaction mixture was diluted with EtOAc (100 mL), washed with brine (2 × 50 mL), dried over Na_2SO_4, and evaporated. The residue was purified by flash column chromatography (25% EtOAc in hexanes) to give **37** as an oil (0.178 g, 59%): R_f = 0.30 (25% EtOAc in hexanes); IR (cm^{-1}) 3331, 3131, 1731, 1690, 1649, 1602, 1531, 1455; ^1H NMR ($CDCl_3$) δ 0.93 (t, 3H, J = 7.3), 1.45 (s, 9), 1.83−2.01 (m, 1H), 2.17−2.31 (m, 1H), 2.50 (s, 3H), 5.44−5.51 (m, 1H), 6.32 (t, 1H, J = 7.2), 6.48 (s, 1H), 7.10 (dd, 1H, J = 7.2, 1.8), 8.45 (dd, 1H, J = 7.2, 1.8), 9.64 (s, 1H); Anal. ($C_{18}H_{23}N_3O_5$) C, H, N.

***trans*-(2′*S*,3‴′*S*,4*S*)-4-(2′-{3″-[(5‴-Methylisoxazole-3‴-carbonyl)amino]-2″-oxo-2″*H*-pyridin-1″-yl}butyrylamino)-5-(2‴′-oxopyrrolidin-3‴′-yl)pent-2-enoic Acid Ethyl Ester (13).** TFA (2 mL) was added to a solution of **39**[28] (0.130 g, 0.398 mmol, 1 equiv) in CH_2Cl_2 (3 mL) and stirred 30 min. The volatiles were evaporated, and the residue was concentrated from CCl_4 (5 mL) to provide the crude amine salt which was set aside.

TFA (2 mL) was added to a solution of **37** (0.143 g, 0.397 mmol, 1.0 equiv) in CH_2Cl_2 (3 mL) and stirred 2 h. The volatiles were evaporated to provide the crude carboxylic acid (**38**) which was combined with the crude amine salt prepared above in CH_2Cl_2 (4 mL) and cooled to 0 °C. HOBt (0.064 g, 0.47 mmol, 1.2 equiv), $^iPr_2NEt$ (0.484 mL, 2.78 mmol, 7.0 equiv), and EDC (0.084 g, 0.44 mmol, 1.1 equiv) were added successively. The reaction mixture was allowed to warm to 23 °C, stirred 18 h, and then partitioned between CH_2Cl_2 (3 × 30 mL) and a mixture of $NaHCO_3$ and brine (1:1, 30 mL). The combined organic phases were washed with a mixture of $NaHCO_3$ and brine (1:1, 10 mL), dried over $MgSO_4$, and evaporated. The residue was purified by flash column chromatography (gradient elution, 2 to 3% CH_3OH in CH_2Cl_2) to give **13** as a white foam (0.119 g, 58%): R_f = 0.46 (10% CH_3OH in $CHCl_3$); IR (cm^{-1}) 3331, 1684, 1649, 1590, 1531; ^1H NMR ($CDCl_3$) δ 0.92 (t, 3H, J = 7.3), 1.29 (t, 3H, J = 7.1), 1.47−1.58 (m, 1H), 1.62−1.77 (m, 1H), 1.85−2.00 (m, 1H), 2.08−2.33 (m, 4H), 2.49 (s, 3H), 3.25−3.42 (m, 2H), 4.19 (q, 2H, J = 7.1), 4.39−4.50 (m, 1H), 5.73 (dd, 1H, J = 8.8, 6.8), 5.97 (dd, 1H, J = 15.7, 1.4), 6.31−6.37 (m, 1H), 6.46 (s, 1H), 6.86 (dd, 1H, J = 15.7, 5.9), 7.18 (s, 1H), 7.57−7.62 (m, 1H), 8.40−8.44 (m, 1H), 8.58−8.62 (m, 1H), 9.56 (s, 1); Anal. ($C_{25}H_{31}N_5O_7 \cdot 0.5H_2O$) C, H, N.

***trans*-(2′*S*,3‴′*S*,4*S*)-4-(2′-{3″-[(5‴-Methylisoxazole-3‴-carbonyl)amino]-2″-oxo-2″*H*-pyridin-1″-yl}pent-4′-ynoylamino)-5-(2‴′-oxopyrrolidin-3‴′-yl)pent-2-enoic Acid Ethyl Ester (3).** R_f = 0.31 (5% CH_3OH in CH_2Cl_2); IR (cm^{-1}) 3295, 1684, 1649, 1596, 1531; ^1H NMR ($CDCl_3$) δ 1.27 (t, 3H, J = 7.1), 1.52−1.62 (m, 1H), 1.64−1.80 (m, 1H), 2.04 (t, 1H, J = 2.6), 2.08−2.38 (m, 3H), 2.49 (s, 3H), 2.91 (ddd, 1H, J = 17.0, 8.4, 2.6), 3.01 (ddd, 1H, J = 17.0, 6.8, 2.6), 3.22−3.39 (m, 2H), 4.18 (q, 2H, J = 7.1), 4.44−4.55 (m, 1H), 5.71−5.78 (m, 1H), 6.03 (dd, 1H, J = 15.6, 1.5), 6.32 (t, 1H, J = 7.2), 6.46 (s, 1H), 6.85 (dd, 1H, J = 15.6, 5.4), 6.89 (s, 1H), 7.49 (dd, 1H, J = 7.2, 1.7), 8.41 (dd, 1H, J = 7.2, 1.7), 8.68 (d, 1H, J = 6.8), 9.52 (s, 1H); Anal. ($C_{26}H_{29}N_5O_7 \cdot 0.75H_2O$) C, H, N.

***trans*-(2′*S*,3‴′*S*,4*S*)-4-(2′-{3″-[(5‴-Methylisoxazole-3‴-carbonyl)amino]-2″-oxo-2″*H*-pyridin-1″-yl}pent-4′-ynoylamino)-5-(2‴′-oxopyrrolidin-3‴′-yl)pent-2-enoic Acid Isopropyl Ester (4).** R_f = 0.45 (10% CH_3OH in CH_2Cl_2); IR (cm^{-1}) 3304, 1692, 1650; ^1H NMR ($CDCl_3$) δ 1.25 (d, 6H, J = 6.2), 1.54−1.78 (m, 2H), 2.03 (t, 1H, J = 2.5), 2.06−2.33 (m, 3H), 2.49 (s, 3H), 2.88−3.07 (m, 2H), 4.47−4.49 (m, 1H), 5.00−5.08 (m, 1H), 5.66−5.71 (m, 1H), 6.00 (dd, 1H, J = 15.6, 1.4), 6.33 (t, 1H, J = 7.2), 6.46 (s, 1H), 7.73 (br, s, 1H), 6.83 (dd, 1H, J = 15.7, 5.4), 7.48 (dd, 1H, J = 7.2, 1.7), 8.41 (dd, 1H, J = 7.2, 1.7), 8.65 (d, 1H, J = 6.59), 9.53 (s, 1H); Anal. ($C_{27}H_{31}N_5O_7 \cdot 0.50H_2O$) C, H, N.

***trans*-(2′*S*,3‴′*S*,4*S*)-4-(2′-{3″-[(5‴-Methylisoxazole-3‴-carbonyl)amino]-2″-oxo-2″*H*-pyridin-1″-yl}pent-4′-ynoylamino)-5-(2‴′-oxopyrrolidin-3‴′-yl)pent-2-enoic Acid Cyclopentyl Ester (8).** R_f = 0.54 (10% CH_3OH in CH_2Cl_2); IR (cm^{-1}) 3302, 1690, 1649, 1632; ^1H NMR ($CDCl_3$) δ 1.62−1.80 (m, 7H), 1.87−1.92(m, 2H), 2.01−2.12 (m, 2H), 2.26−2.40 (m, 2H), 2.53 (s, 3H), 2.95−3.12 (m, 2H), 3.26−3.36 (m, 2H), 4.50−4.53 (m, 1H), 5.20−5.25 (m, 1H), 5.54−5.60 (m, 1H), 6.01 (dd, 1H, J = 15.6, 1.5), 6.31 (t, 2H, J = 7.2), 6.40 (s, br, 1H), 6.49 (s, 1H), 6.83 (dd, 1H, J = 15.6, 5.4), 7.43 (dd, 1H, J = 6.9, 1.8), 8.44 (dd, 1H, J = 6.9, 1.5), 8.58 (d, 1H, J = 6.6), 9.56 (s, 1H); Anal. ($C_{29}H_{33}N_5O_7 \cdot 0.75H_2O$) C, H, N.

***trans*-(2′*S*,3‴′*S*,4*S*)-4-(2′-{3″-[(5‴-Methylisoxazole-3‴-carbonyl)amino]-2″-oxo-2″*H*-pyridin-1″-yl}pent-4′-ynoylamino)-5-(2‴′-oxopyrrolidin-3‴′-yl)pent-2-enoic Acid Cyclohexyl Ester (9).** R_f = 0.48 (10% CH_3OH in CH_2Cl_2); IR (cm^{-1}) 3305, 1685, 1650, 1597, 1534; ^1H NMR ($CDCl_3$) δ 1.24−1.98 (m, 12H), 2.03 (t, 1H, J = 2.5), 2.05−2.39 (m, 3H), 2.50 (s, 3H), 2.90−3.08 (m, 2H), 3.20−3.34 (m, 2H), 4.45−4.52 (m, 1H), 4.75−4.83 (m, 1H), 5.59−5.64 (m, 1H), 6.01 (dd, 1H, J = 15.6, 1.2), 6.33 (t, 1H, J = 7.2), 6.46 (s, 1H), 6.54 (br, s, 1H), 6.82 (dd, 1H, J = 15.6, 5.3), 7.44 (dd, 1H, J = 7.2, 1.7), 8.41 (dd, 1H, J = 7.2, 1.7), 8.59 (d, 1H, J = 6.6), 9.53 (s, 1H); Anal. ($C_{30}H_{35}N_5O_7 \cdot 0.75H_2O$) C, H, N.

***trans*-(2′*S*,3‴′*S*,4*S*)-4-(2′-{3″-[(5‴-Methylisoxazole-3‴-carbonyl)amino]-2″-oxo-2″*H*-pyridin-1″-yl}pent-4′-ynoylamino)-5-(2‴′-oxopyrrolidin-3‴′-yl)pent-2-enoic Acid Cycloheptyl Ester (10).** ^1H NMR ($CDCl_3$) δ 1.30−1.90 (m, 15H), 2.01 (t, 1H, J = 2.4), 2.20−2.40 (m, 2H), 2.49 (s, 3H), 2.90−3.10 (m, 2H), 3.20−3.30 (m, 2H), 4.45−4.56 (m, 1H), 4.92−5.03 (m, 1H), 5.48 (t, 1H, J = 7.3), 5.98 (d, 1H, J = 15.6), 6.21 (s, 1H), 6.32 (t, 1H, J = 7.4), 6.46 (s, 1H), 6.80 (dd, 1H, J = 15.6, 5.3), 7.35−7.38 (dd, 1H, J = 7.0, 1.5), 8.39−8.43 (m, 2H), 9.52 (s, 1H); Anal. ($C_{31}H_{37}N_5O_7 \cdot 0.3H_2O$) C, H, N.

***trans*-(2′*S*,3‴′*S*,4*S*)-4-(2′-{3″-[(5‴-Methylisoxazole-3‴-carbonyl)amino]-2″-oxo-2″*H*-pyridin-1″-yl}pent-4′-ynoylamino)-5-(2‴′-oxopyrrolidin-3‴′-yl)pent-2-enoic Acid Benzyl Ester (11).** R_f = 0.36 (10% CH_3OH in CH_2Cl_2); IR (cm^{-1}) 3298, 1685, 1650, 1596, 1534; ^1H NMR ($CDCl_3$) δ 1.54−1.74 (m, 2H), 1.97 (t, 3H, J = 2.7), 2.00−2.19 (m, 1H), 2.22−2.39

(m, 2H), 2.49 (s, 3H), 2.88−3.07 (m, 2H), 3.19−3.32 (m, 2H), 4.44−4.52 (m, 1H), 5.16 (s, 2H), 5.55−5.60 (m, 1H), 6.07 (dd, 1H, J = 15.7, 1.5), 6.32 (t, 1H, J = 7.2), 6.45 (s, 1H), 6.48 (br, s, 1H), 6.89 (dd, 1H, J = 15.7, 5.3), 7.29−7.41 (m, 5H), 7.44 (dd, 1H, J = 6.2, 1.7), 8.40 (dd, 1H, J = 7.5, 1.7), 8.61 (d, 1H, J = 6.6), 9.52 (s, 1H); Anal. ($C_{31}H_{31}N_5O_7$·0.50H_2O) C, H, N.

***trans*-(2′*S*,3‴′*S*,4*S*)-2-Methyl-4-(2′-{3″-[(5‴-methylisoxazole-3‴-carbonyl)amino]-2″-oxo-2″*H*-pyridin-1″-yl}pent-4′-ynoylamino)-5-(2‴′-oxopyrrolidin-3‴′-yl)pent-2-enoic Acid Ethyl Ester (12).** R_f = 0.50 (10% CH_3OH in $CHCl_3$); IR (cm^{-1}) 3307, 1690, 1649; ^1H NMR ($CDCl_3$) δ 1.29 (t, 3H, J = 7.1), 1.36−1.47 (m, 1H), 1.65−1.80 (m, 1H), 1.95−2.00 (m, 4H), 2.14−2.40 (m, 3H), 2.50 (s, 3H), 2.87−3.03 (m, 2H), 3.23−3.38 (m, 2H), 4.18 (q, 2H, J = 7.1), 4.56−4.68 (m, 1H), 5.63−5.72 (m, 1H), 6.34 (t, 1H, J = 7.3), 6.47 (s, 1H), 6.52−6.58 (m, 1H), 6.81 (s, 1H), 7.46 (dd, 1H, J = 7.3, 1.6), 8.42 (dd, 1H, J = 7.3, 1.6), 8.65 (d, 1H, J = 6.4), 9.54 (s, 1H); Anal. ($C_{27}H_{31}N_5O_7$·0.5H_2O) C, H, N.

***trans*-(2′*S*,3‴′*S*,4*S*)-4-(2′-{3″-[(5‴-Methylisoxazole-3‴-carbonyl)amino]-2″-oxo-2″*H*-pyridin-1″-yl}butyrylamino)-5-(2‴′-oxopyrrolidin-3‴′-yl)pent-2-enoic Acid Isopropyl Ester (14).** IR (cm^{-1}) 3333, 1681, 1531, 1276; ^1H NMR ($CDCl_3$) δ 0.98 (t, 3H, J = 7.2), 1.29 (d, 6H, J = 6.6), 1.46−1.73 (m, 1H), 1.89−2.07 (m, 1H), 2.13−2.37 (m, 4H), 2.52 (s, 3H), 3.30−3.43 (m, 3H), 4.48 (m, 1H), 5.02−5.14 (m, 1H), 5.69 (t, 1H, J = 6.9), 5.96 (d, 1H, J = 15.6), 6.39 (t, 1H, J = 7.5), 6.49 (s, 1H), 6.86 (dd, 1H, J = 15.6, 6.0), 6.91 (s, br. 1H), 7.56 (d, 1H, J = 7.2), 8.44 (d, 1H, J = 7.5), 8.53 (d, 1H, J = 6.3), 9.59 (s, 1H); Anal. ($C_{26}H_{33}N_5O_7$·0.5H_2O) C, H, N.

***trans*-(2′*S*,3‴′*S*,4*S*)-4-(2′-{3″-[(5‴-Methylisoxazole-3‴-carbonyl)amino]-2″-oxo-2″*H*-pyridin-1″-yl}butyrylamino)-5-(2‴′-oxopyrrolidin-3‴′-yl)pent-2-enoic Acid 2,2-Dimethylpropyl Ester (16).** R_f = 0.27 (5% CH_3OH in CH_2Cl_2); IR (cm^{-1}) 3331, 3295, 1690, 1649; ^1H NMR ($CDCl_3$) δ 0.87−0.99 (m, 12H), 1.51−1.61 (m, 1H), 1.64−1.79 (m, 1H), 1.83−2.00 (m, 1H), 2.10−2.37 (m, 4H), 2.49 (s, 3H), 3.26−3.43 (m, 2H), 3.83 (s, 2H), 4.43−4.54 (m, 1H), 5.71 (dd, 1H, J = 8.7, 6.9), 6.00 (dd, 1H, J = 15.7, 1.3), 6.35 (t, 1H, J = 7.2), 6.46 (s, 1H), 6.86 (dd, 1H, J = 15.7, 5.9), 7.39 (s, 1H), 7.58 (dd, 1H, J = 7.2, 1.7), 8.42 (dd, 1H, J = 7.2, 1.7), 8.53 (d, 1H, J = 7.0), 9.55 (s, 1H); Anal. ($C_{28}H_{37}N_5O_7$·0.5H_2O) C, H, N.

***trans*-(2′*S*,3‴′*S*,4*S*)-4-(2′-{3″-[(5‴-Methylisoxazole-3‴-carbonyl)amino]-2″-oxo-2″*H*-pyridin-1″-yl}butyrylamino)-5-(2‴′-oxopyrrolidin-3‴′-yl)pent-2-enoic Acid Cyclobutyl Ester (17).** IR (cm^{-1}) 3334, 1690, 1632; ^1H NMR ($CDCl_3$) δ 0.95 (t, 3H, J = 7.5), 1.55−1.85 (m, 5H), 1.88−2.01 (m, 1H), 2.04−2.44 (m, 7H), 2.52 (s, 3H), 3.31−3.41 (m, 2H), 4.48 (m, 1H), 5.01−5.11 (m, 1H), 5.63−5.71 (m, 1H), 5.96 (dd, 1H, J = 15.0, 1.5), 6.36 (t, 1H, J = 7.5), 6.49 (s, 1H), 6.83−6.90 (m, 2H), 7.54 (d, 1H, J = 7.2), 8.45 (dd, 1H, J = 7.5, 1.8), 8.53 (dd, 1H, J = 6.6, 1.8), 9.59 (s, 1H); Anal. ($C_{27}H_{33}N_5O_7$·0.75H_2O) C, H, N.

***trans*-(2′*S*,3‴′*S*,4*S*)-4-(2′-{3″-[(5‴-Methylisoxazole-3‴-carbonyl)amino]-2″-oxo-2″*H*-pyridin-1″-yl}butyrylamino)-5-(2‴′-oxopyrrolidin-3‴′-yl)pent-2-enoic Acid Cyclohexyl Ester (18).** R_f = 0.37 (10% CH_3OH in CH_2Cl_2); IR (cm^{-1}) 3334, 1687, 1649, 1595, 1532; ^1H NMR ($CDCl_3$) δ 0.92 (t, 3H, J = 7.4), 1.24−1.99 (m, 14H), 2.06−2.30 (m, 3H), 2.49 (s, 3H), 3.28−3.39 (m, 2H), 4.45−4.47 (m, 1H), 4.77−4.84 (m, 1H), 5.65−5.70 (m, 1H), 5.95 (dd, 1H, J = 15.7, 1.1), 6.34 (t, 1H, J = 7.3), 6.46 (s, 1H), 6.83 (dd, 1H, J = 15.7, 5.8), 6.93 (br, s, 1H), 7.54 (dd, 1H, J = 7.3, 1.7), 8.42 (dd, 1H, J = 7.3, 1.7), 8.49 (d, 1H, J = 6.8), 9.57 (s, 1H); Anal. ($C_{29}H_{37}N_5O_7$·0.50H_2O) C, H, N.

***trans*-(2′*S*,3‴′*S*,4*S*)-4-(2′-{3″-[(5‴-Methylisoxazole-3‴-carbonyl)amino]-2″-oxo-2″*H*-pyridin-1″-yl}butyrylamino)-5-(2‴′-oxopyrrolidin-3‴′-yl)pent-2-enoic Acid Cycloheptyl Ester (19).** ^1H NMR ($CDCl_3$) δ 0.85−2.35 (m, 22H), 2.49 (s, 3H), 3.23−3.38 (m, 2H), 4.46−4.57 (m, 1H), 4.93−5.03 (m, 1H), 5.44−5.52 (m, 1H), 5.93 (dd, 1H, J = 15.6, 1.2), 6.12 (s, br, 1H), 6.32 (1H, t, J = 7.2), 6.46 (s, 1H), 6.80 (dd, 1H, J = 15.7, 5.7), 7.35 (dd, 1H, J = 7.2, 1.7), 8.15 (d, 1H, J = 6.8), 8.41 (dd, 1H, J = 7.4, 1.6), 9.58 (s, 1H); Anal. ($C_{30}H_{39}N_5O_7$·0.80H_2O) C, H, N.

***trans*-(2′*S*,3‴′*S*,4*S*)-4-(2′-{3″-[(5‴-Methylisoxazole-3‴-carbonyl)amino]-2″-oxo-2″*H*-pyridin-1″-yl}butyrylamino)-5-(2‴′-oxopyrrolidin-3‴′-yl)pent-2-enoic Acid Benzyl Ester (20).** R_f = 0.35 (10% CH_3OH in CH_2Cl_2); IR (cm^{-1}) 3335, 1685, 1649, 1595, 1533; ^1H NMR ($CDCl_3$) δ 0.91 (t, 3H, J = 7.4), 1.50−1.97 (m, 3H), 2.11−2.31 (m, 4H), 2.48 (s, 3H), 3.27−3.38 (m, 2H), 4.45−4.47 (m, 1H), 5.17 (s, 2H), 5.65−5.70 (m, 1H), 6.01 (dd, 1H, J = 15.6, 1.5), 6.33 (t, 1H, J = 7.1), 6.46 (s, 1H), 6.90 (dd, 1H, J = 15.6, 5.8), 7.00 (br, s, 1H), 7.30−7.39 (m, 5H), 7.54 (dd, 1H, J = 7.1, 1.8), 8.42 (dd, 1H, J = 7.4, 1.8), 8.56 (d, 1H, J = 6.8), 9.56 (s, 1H); Anal. ($C_{30}H_{33}N_5O_7$·0.50H_2O) C, H, N.

Pharmacokinetic Experiments. The in vivo pharmacokinetic results described in this work were determined after either intravenous (15 mg/kg, n = 3) and oral (30 mg/kg, n = 3) single agent dosing of the appropriate molecules to male beagle dogs (BW ∼9.3 kg) or intravenous (25 mg/kg, n = 3) and oral (50 mg/kg, n = 3) single agent dosing to male CM-monkeys (BW ∼3.9 kg). The compounds were typically administered in a vehicle consisting of 80% propylene glycol and 20% sterile water at a concentration of 15.0 mg/mL, although animals from several oral studies were also dosed using a 0.5% carboxymethyl cellulose suspension formulation. In each case, blood samples were collected periodically for up to 24 h postdosing, and the plasma concentrations of each test compound were determined by LCMS. Pharmacokinetic parameters were estimated by noncompartmental analysis of the individual plasma concentration−time data.

Plasma Stability Studies. Pooled heparinized human plasma from 10 individuals was obtained from Golden West Biologicals, Temecula, CA, and frozen at −70 °C until analysis. The plasma stability experiment was initiated by transferring 1990 μL of plasma (n = 3) or 100 mM potassium phosphate pH 7.4 buffer (n = 3) into separate test tubes. A 10 μL aliquot of a freshly made 5 mM acetonitrile solution of the individual test compounds was transferred into the plasma or buffer to achieve a final compound concentration of 25 μM. The test tubes were incubated at 37 °C for 1−3 h and 200 μL samples were collected at regular intervals. The samples were mixed with 2 mL of acetonitrile and vortexed to ensure protein precipitation and immediate termination of metabolic transformations. After centrifugation for 10 min at 4000 rpm at 10 °C in a Sorvall RT 7 centrifuge, the clear supernatant was decanted into a new set of tubes, and the volatiles were removed under a stream of nitrogen using a Dri-block sample concentrator (Techne, Princeton, NJ). The samples were reconstituted in 250 μL of mobile phase (60% 25 mM pH = 5.1 $NH_4H_2PO_4$ buffer and 40% CH_3CN). Chromatographic analysis was performed using a 1100 Hewlett-Packard HPLC with a Primesphere reversed phase column (5 μm, 4.6 × 15 mm, Phenomenex, Torrance, CA) at a flow rate of 1 mL/min using a gradient elution. A volume of 100 μL was injected onto the column, and the test molecules were detected by UV absorption at 212 nm. The standard curve of the compounds ranged from 0.05 μg/mL to 40 μg/mL. The half-life of a given compound's metabolic conversion rate was determined by linear regression (WinNonlin Professional, Pharsight, Mountainview, CA) of the mean plasma concentration−time data obtained from the incubation studies.

Microsome Stability Studies. Canine, simian, or human pooled liver microsomes (1 mg/mL, consisting of 6−8 livers) and 2 mM NADPH were incubated in 100 mM potassium phosphate buffer, pH 7.4, at 37 °C in a shaking water bath for 1 min. The reaction was initiated by the addition of 25 μM test compound and proceeded for 30 min followed by the addition of 500 μL acetonitrile to terminate the reaction. The incubation volume was 500 μL. Separate control samples were also prepared in a manner similar to the time zero samples with 500 μL of acetonitrile added to the test tube prior to the test compound. All samples were vortexed (2 min) on a SP Multi-tube Vortexer and then centrifuged at 2500 × g for 20 min. The supernatant was concentrated and then analyzed by HPLC. Chromatographic separation was achieved for the test compounds using either a Hewlett-Packard 1050 HPLC with

a Primesphere C_{18} HC reversed phase column (5μ, 4.6 × 150 mm) or a Zorbax Eclipse XDB C_{18} column (5 μm, 4.6 × 150 mm). Analyte elution was conducted with the former using 25 mM $NH_4H_2PO_4$ buffer, pH 4.5, with constant 5% methanol and a time gradient for acetonitrile of 0−2 min: 5%; 2−10 min: 5−50%; 10−20 min: 50%; 20−21 min: 50−5%; 21−24 min 5%, with total run time of 24 min. Alternatively, compounds examined using the Zorbax column were eluted using 25 mM $NH_4H_2PO_4$ buffer, pH 4.5 with constant 10% methanol and a time gradient for acetonitrile of time 0−10 min: 10−55%; 10−16 min: 55−75%; 16−18 min: 75−10%; 18−20 min: 10%, with a total run time of 25 min. All molecules were monitored at 215 nm.

Caco-2 Permeability Studies. Caco-2 cells were cultivated under aseptic conditions at 37 °C in an atmosphere of 90% relative humidity (95% air and 5% CO_2). The culture medium consisted of 10% fetal bovine serum, 100 IU/mL penicillin, 100 μg/mL streptomycin, and 1% MEM nonessential amino acid solution. The culture medium was replaced every 3−4 days, and cells were harvested with trypsin−EDTA (0.25%) upon reaching 70−80% confluency. The Caco-2 cells from passages 30−50 were seeded onto six-well transwell plates (63 000 cells/cm^2) and grown for 21−25 days until formation of a confluent monolayer was apparent (TEER values between 400 and 600 Ω). Sanpwell filters were soaked in Hanks balanced salt solution (HBSS) for 1 h and then were mounted onto the diffusion chamber.

The apical to basolateral permeability of test compounds was studied using the above apparatus as follows. A donor buffer consisting of HBSS with 25 mM mannitol (pH 6.5), 0.5% DMSO, and 2% ethanol was prepared as a vehicle for the molecules under study. Independently, 5 mL of a buffer solution containing the test compound (100 μM) or permeability reference markers (metoprolol and atenolol; 100 μM each) was added to the apical side of the cell monolayer (n = 3). Blank HBSS receiver buffer containing 25 mM glucose (5 mL, pH = 7.4) was quickly added to the basolateral side of the monolayer, and 1 mL aliquots were subsequently removed from this compartment at 5, 45, 90, and 120 min. After removal of each aliquot, 1 mL of blank HBSS was added to the basolateral side of the monolayer. Buffer solutions were maintained at 37 °C during the course of the experiment and were bubbled with a mixture of air−CO_2 (95/5) to facilitate oxygenation and stirring. The concentrations of the test compounds and permeability marker (atenolol) present in the samples were determined by HPLC and P_{app} values were calculated as described in the literature.[33] Using the above apparatus and procedure, the P_{app} of internal atenolol was determined to be $1.0 \pm 0.1 \times 10^{-6}$ cm/s while that of metoprolol (measured independently of test compounds) was found to be $11.1 \pm 0.5 \times 10^{-6}$ cm/s.

At the end of the transport experiment, ^{14}C-radiolabeled mannitol (1.7 μCi) was added to each donor chamber to evaluate the integrity of the cell monolayers. Samples were collected from the donor (100 μL) and receiver (1 mL) side at 0 and 60 min after adding ^{14}C-mannitol. These samples were diluted by adding 5 mL of liquid scintillation cocktail, and the amount of ^{14}C-mannitol present in each was measures using a liquid scintillation counter. The P_{app} of internal mannitol was determined to be 0.07×10^{-6} cm/s.

Plasma Protein Binding Determinations. For each tested compound, 1 mM and 0.1 mM stock solutions (containing 50% acetonitrile, 20% DMSO, and 30% 100 mM potassium phosphate buffer, pH 7.4) were prepared, and 10 μL of each was added to 1 mL of heparinized dog or human plasma or 1 mL buffer (100 mM potassium phosphate pH 7.4; final compound concentrations of 1 and 10 μM, respectively). The samples were incubated at 37 °C in a shaking water bath for 1 h. An aliquot (100 μL) of plasma or buffer was removed and placed in separate test tubes containing methanol (1 mL) for estimation of total compound concentration. The remainder (900 μL) of each plasma or buffer sample was then transferred to a Millipore Centrifree device. The Millipore tubes were centrifuged at 2200 rpm for 20 min using a Sorvall Centrifuge RC 5C with a swinging bucket rotor SH-3000. Following centrifugation, 100 μL was removed from the ultrafiltrate side and transferred to a test tube containing methanol (1 mL). An internal standard, pentoxifylline (50 μL of 20 μg/mL solution in methanol), was added to all tubes. All samples were vortexed (5 min) on a SP Multi-tube Vortexer (Baxter, McGaw Park, IL) and centrifuged at 2500 × g for 30 min. The organic layer was removed and evaporated under a gentle stream of nitrogen using a Dri-Block sample concentrator (Techne, Princeton, NJ) at 45 °C. Samples were reconstituted with 100 mL of a 1:1 mixture of methanol and 0.1% formic acid. All samples were vortexed a second time for 5 min and centrifuged at 2500 × g for 20 min. The supernatants were transferred into a 96-well plate for analysis by LCMS. The protein binding value (percent free) was calculated by dividing the measured amount of compound present in the filtrate by the amount detected in whole plasma and multiplying the product by 100. Three replicates were evaluated per assay for each species at each concentration.

Water Solubility Determinations. To a buffer solution (pH = 7.0, 50 mM phosphate, 1 mL) in a vial was added 1−2 mg of test compound. The mixture was stirred at room temperature for 4 h and then was filtered using a 0.45 μm syringe filter. The concentration of the drug in the filtrate was determined by HPLC analysis employing a standard solution of a known concentration.

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References

(1) For part 7 in this series, see: Dragovich, P. S.; Prins, T. J.; Zhou, R.; Johnson, T. O.; Brown, E. L.; Maldonado, F. C.; Fuhrman, S. A.; Zalman, L. S.; Patick, A. K.; Matthews, D. A.; Hou, X.; Meador, J. W., III; Ferre, R. A.; Worland, S. T. Structure-Based Design, Synthesis, and Biological Evaluation of Irreversible Human Rhinovirus 3C Protease Inhibitors. 7. Structure−Activity Studies of Bicyclic 2-Pyridone-Containing Peptidomimetics. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 733−738.

(2) (a) Couch, R. B. Rhinoviruses. In *Fields Virology*, 4th ed.; Knipe, D. M., Howley, P. M., et al., Eds.; Lippincott, Williams, & Wilkins Publishers: Philadelphia, 2001; Vol. 1, Chapter 25, pp 777−797. (b) McKinlay, M. A.; Pevear, D. C.; Rossmann, M. G. Treatment of the Picornavirus Common Cold by Inhibitors of Viral Uncoating and Attachment. *Annu. Rev. Microbiol.* **1992**, *46*, 635−654. (c) Phillpotts, R. J.; Tyrrell, D. A. J. Rhinovirus Colds. *Br. Med. Bull.* **1985**, *41*, 386−390. (d) Gwaltney, J. M. The Common Cold. In *Principles and Practices of Infectious Diseases*; Mandell, G. L., Douglas, R. G., Bennett, J. E., Eds.; John Wiley & Sons: New York, 1985, Chapter 38, pp 351−355. (e) Gwaltney, J. M. Rhinoviruses. In *Viral Infections of Humans*; Evans, A. S., Ed.; Plenum Publishing Corp.: New York, 1982; Chapter 20, pp 491−517.

(3) (a) Rueckert, R. R. *Picornaviridae*: The Viruses and Their Replication. In *Fields Virology*, 4th ed.; Knipe, D. M., Howley, P. M., et al., Eds.; Lippincott, Williams, & Wilkins Publishers: Philadelphia, 2001; Vol. 1, Chapter 23, pp 685−722. (b) Kräusslich, H.-G.; Wimmer, E. Viral Proteinases. *Annu. Rev. Biochem.* **1988**, *57*, 701−754.

(4) Hamparian, V. V.; Colonno, R. J.; Cooney, M. K.; Dick, E. C.; Gwaltney, J. M., Jr.; Hughes, J. H.; Jordan, W. S., Jr.; Kapikian, A. Z.; Mogabgab, W. J.; Monto, A.; Phillips, C. A.; Rueckert, R. R.; Schieble, J. H.; Stott, E. J.; Tyrrell, D. A. J. A Collaborative Report: Rhinoviruses-Extension of the Numbering System From 89 to 100. *Virology* **1987**, *159*, 191−192.

(5) (a) Turner, R. B.; Wecker, M. T.; Pohl, G.; Witek, T. J.; McNally, E.; St. George, R.; Winther, B.; Hayden, F. G. Efficacy of Tremacamra, a Soluble Intercellular Adhesion Molecule 1, for Experimental Rhinovirus Infection. *JAMA* **1999**, *281*, 1797−1804. (b) Rogers, J. M.; Diana, G. D.; McKinlay, M. A. Pleconaril, a Broad Spectrum Antipicornaviral Agent. *Antiviral Chemother.* **1999**, *5*, 69−76. (c) Hamdouchi, C.; Ezquerra, J.; Vega, J. A.; Vaquero, J. J.; Alvarez-Builla, J.; Heinz, B. A. Short Synthesis and Anti-Rhinoviral Activity of Imidazo[1,2-*a*]Pyridines: The Effect of Acyl Groups at 3-Position. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1391−1394. (d) Carrasco, L. Picornavirus Inhibitors. *Pharmacol. Ther.* **1994**, *64*, 215−290.

(6) (a) Bergmann, E. M.; James, M. N. G. Proteolytic Enzymes of the Viruses of the Family Picornaviridae. *Proteases Infect. Agents* **1999**, 139−163. (b) Cordingley, M. G.; Callahan, P. L.; Sardana, V. V.; Garsky, V. M.; Colonno, R. J. Substrate Requirements of Human Rhinovirus 3C Protease for Peptide Cleavage *in Vitro*. *J. Biol. Chem*. **1990**, *265*, 9062−9065. (c) Orr, D. C.; Long, A. C.; Kay, J.; Dunn, B. M.; Cameron, J. M. Hydrolysis of a Series of Synthetic Peptide Substrates by the Human Rhinovirus 14 3C Proteinase, Cloned and Expressed in *Escherichia coli*. *J. Gen. Virol*. **1989**, *70*, 2931−2942. (d) Cordingley, M. G.; Register, R. B.; Callahan, P. L.; Garsky, V. M.; Colonno, R. J. Cleavage of Small Peptides In Vitro by Human Rhinovirus 14 3C Protease Expressed in *Escherichia coli*. *J. Virol*. **1989**, *63*, 5037−5045.

(7) (a) Matthews, D. A.; Smith, W. W.; Ferre, R. A.; Condon, B.; Budahazi, G.; Sisson, W.; Villafranca, J. E.; Janson, C. A.; McElroy, H. E.; Gribskov, C. L.; Worland, S. Structure of Human Rhinovirus 3C Protease Reveals a Trypsin-like Polypeptide Fold, RNA-Binding Site, and Means for Cleaving Precursor Polyprotein. *Cell* **1994**, *77*, 761−771. (b) Allaire, M.; Chernaia, M. M.; Malcolm, B. A.; James, M. N. G. Picornaviral 3C Cysteine Proteinases Have a Fold Similar to Chymotrypsin-like Serine Proteinases. *Nature* **1994**, *369*, 72−76. (c) Bazan, J. F.; Fletterick, R. J. Viral Cysteine Proteases are Homologous to the Trypsin-like Family of Serine Proteases: Structural and Functional Implications. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 7872−7876. (d) Gorbalenya, A. E.; Blinov, V. M.; Donchenko, A. P. Poliovirus-encoded Proteinase 3C: A Possible Evolutionary Link Between Cellular Serine and Cysteine Proteinase Families. *FEBS Lett*. **1986**, *194*, 253−257.

(8) (a) Dragovich, P. S. Recent Advances in the Development of Human Rhinovirus 3C Protease Inhibitors. *Exp. Opin. Ther. Pat.* **2001**, *11*, 177−184. (b) Wang, Q. M. Protease Inhibitors as Potential Antiviral Agents for the Treatment of Picornaviral Infections. *Prog. Drug Res*. **1999**, *52*, 197−219. (c) Wang, Q. M. Human Rhinovirus 3C Protease Inhibitors: Recent Developments. *Exp. Opin. Ther. Pat.* **1998**, *8*, 1151−1156.

(9) Dragovich, P. S.; Prins, T. J.; Zhou, R.; Brown, E. L.; Maldonado, F. C.; Fuhrman, S. A.; Zalman, L. S.; Tuntland, T.; Lee, C. A.; Patick, A. K.; Matthews, D. A.; Hendrickson, T. F.; Kosa, M. B.; Liu, B.; Batugo, M. R.; Gleeson, J.-P. R.; Sakata, S. K.; Chen, L.; Guzman, M. C.; Meador, J. W., III; Ferre, R. A.; Worland, S. T. Structure-Based Design, Synthesis, and Biological Evaluation of Irreversible Human Rhinovirus 3C Protease Inhibitors. 6. Structure−Activity Studies of Orally Bioavailable, 2-Pyridone-Containing Peptidomimetics. *J. Med. Chem*. **2002**, *45*, 1607−1623.

(10) For other examples of Michael acceptor-containing cysteine protease inhibitors, see: (a) Roush, W. R.; Gwaltney, S. L., II; Cheng, J.; Scheidt, K. A.; McKerrow, J. H.; Hansell, E. Vinyl Sulfonate Esters and Vinyl Sulfonamides: Potent, Irreversible Inhibitors of Cysteine Proteases. *J. Am. Chem. Soc.* **1998**, *120*, 10994−10995. (b) McGrath, M. E.; Klaus, J. L.; Barnes, M. G.; Brömme, D. Crystal Structure of Human Cathepsin K Complexed with a Potent Inhibitor. *Nature Struct. Biol*. **1997**, *4*, 105−109. (c) Brömme, D.; Klaus, J. L.; Okamoto, K.; Rasnick, D.; Palmer, J. T. Peptidyl Vinyl Sulphones: A New Class of Potent and Selective Cysteine Protease Inhibitors. *Biochem. J*. **1996**, *315*, 85−89. (d) Palmer, J. T.; Rasnick, D.; Klaus, J. L.; Brömme, D. Vinyl Sulfones as Mechanism-Based Cysteine Protease Inhibitors. *J. Med. Chem*. **1995**, *38*, 3193−3196. (e) Liu, S.; Hanzlik, R. P. Structure−Activity Relationships for Inhibition of Papain by Peptide Michael Acceptors. *J. Med. Chem*. **1992**, *35*, 1067−1075. (f) Hanzlik, R. P.; Thompson, S. A. Vinylogous Amino Acid Esters: A New Class of Inactivators for Thiol Proteases. *J. Med. Chem*. **1984**, *27*, 711−712.

(11) The 7 h plasma concentration of a given compound observed in the dog and monkey following oral administration was chosen as a convenient measure of both oral bioavailability and pharmacokinetic performance in these species. In general, this parameter correlated well with C_{max} and AUC values across the series of molecules under study. Since no correlation between 3CP inhibitor in vitro antirhinoviral activity, human plasma level, and clinical efficacy has been established, the relevance of the 7 h concentration values toward assessing the therapeutic efficacy potential of the described 3CP inhibitors is not known.

(12) The absolute oral bioavailabilities (defined as the ratios of AUCs observed following oral and IV administration) of the compounds described in this work varied from 27 to 55% in the dog and from 1 to 25% in the monkey. Such absolute bioavailability values did not correlate well with observed pharmacokinetics (i.e., 7 h plasma levels) and were therefore determined not to be a reliable predictor of potential compound exposures in other species.

(13) Representative examples of the 2-pyridone-containing 3CP inhibitors described in this work were shown to be highly unstable toward rodent plasma due to facile ester hydrolysis.[9] Pharmacological evaluation of these molecules was therefore not conducted in rodents.

(14) (a) de Groot, M. J.; Ekins, S. Pharmacophore Modeling of Cytochromes P450. *Adv. Drug Deliv. Rev*. **2002**, *54*, 367−383. (b) Lewis, D. F. V.; Dickins, M. Factors Influencing Rates and Clearance in P450-Mediated Reactions: QSARs for Substrates of the Xenobiotic-metabolizing Hepatic Microsomal P450s. *Toxicology* **2002**, *170*, 45−53. (c) Lewis, D. F. V. Structural Characteristics of Human P450s Involved in Drug Metabolism: QSARs and Lipophilicity Profiles. *Toxicology* **2000**, *144*, 197−203. (d) Rendic, S.; Di Carlo, F. J. Human Cytochrome P450 Enzymes: A Status Report Summarizing Their Reactions, Substrates, Inducers, and Inhibitors. *Drug Met. Rev*. **1997**, *29*, 413−580.

(15) In vitro microsome experiments conducted in both the presence and absence of the NADPH cytochrome cofactor suggested that the metabolism of the majority of the compounds described in this study was effected by a combination of P450-mediated oxidative transformations and esterase-mediated ester hydrolysis (Lee, C. A., unpublished results).

(16) The nomenclature used for describing the individual amino acid residues of a peptide substrate (P_2, P_1, $P_{1'}$, $P_{2'}$, etc.) and the corresponding enzyme subsites (S_2, S_1, $S_{1'}$, $S_{2'}$, etc.) is described in: Schechter, I.; Berger, A. On the Size of the Active Site in Proteases. I. Papain. *Biochem. Biophys. Res. Commun*. **1967**, *27*, 157−162.

(17) Dragovich, P. S.; Webber, S. E.; Babine, R. E.; Fuhrman, S. A.; Patick, A. K.; Matthews, D. A.; Reich, S. H.; Marakovits, J. T.; Prins, T. J.; Zhou, R.; Tikhe, J.; Littlefield, E. S.; Bleckman, T. M.; Wallace, M. B.; Little, T. L.; Ford, C. E.; Meador, J. W., III; Ferre, R. A.; Brown, E. L.; Binford, S. L.; DeLisle, D. M.; Worland, S. T. Structure-Based Design, Synthesis, and Biological Evaluation of Irreversible Human Rhinovirus 3C Protease Inhibitors. 2. Peptide Structure−Activity Studies. *J. Med. Chem*. **1998**, *41*, 2819−2834.

(18) In the subsequent discussion of structure−activity relationships, the notation "3CP" indicates 3C protease derived from HRV-14 unless otherwise specified.

(19) Our previous research indicated that the P_2 benzyl substituent present in 2-pyridone-containing 3CP inhibitors such as **1** and **2** is subject to facile P450-mediated oxidative metabolism. Fluorination of this fragment was observed to improve the in vitro metabolic stability profiles of compounds which contain it.[9] Full details of these metabolism studies will be published elsewhere (Lee, C. A., manuscript in preparation).

(20) For some general discussions concerning the physiochemical attributes of organic molecules which lead to favorable oral bioavailability properties, see: (a) Veber, D. F.; Johnson, S. R.; Cheng, H.-Y.; Smith, B. R.; Ward, K. W.; Kopple, K. D. Molecular Properties That Influence the Oral Bioavailability of Drug Candidates. *J. Med. Chem*. **2002**, *45*, 2615−2623. (b) Egan, W. J.; Lauri, G. Prediction of Intestinal Permeability. *Adv. Drug. Deliv. Rev*. **2002**, *54*, 273−289. (c) Stenberg, P.; Norinder, U.; Luthman, K.; Artursson, P. Experimental and Computational Screening Models for the Prediction of Intestinal Drug Absorption. *J. Med. Chem*. **2001**, *44*, 1927−1937. (d) Lipinsky, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and Computational Approaches to Estimate Solubility and Permeability in Drug Discovery and Development Settings. *Adv. Drug. Deliv. Rev*. **1997**, *23*, 3−25. (e) Chan, O. H.; Stewart, B. H. Physiochemical and Drug-Delivery Considerations for Oral Drug Bioavailability. *Drug Discuss. Today* **1996**, *1*, 461−473.

(21) Cahiez, G.; Metais, E. Enantioselective Preparation of α-Acyloxy Ketones from α-Hydroxy and α-Amino Acids. *Tetrahedron Lett*. **1995**, *36*, 6449−6452 and references therein.

(22) No rigorous effort was made to detect and/or quantitate the possible *O*-alkylation reaction product using more stringent analytical methods.

(23) For general references on the alkylation of 2-hydroxypyridines, see: (a) Scriven, E. F. V. In *Comprehensive Heterocyclic Chemistry*; Katritzky, A. R., Rees, C. W., Eds.; Pergamon Press: Oxford, 1984; Vol. 2, pp 165−314. (b) Tieckelmann, H. In *Pyridine and its Derivatives*; Abramovitch, R. A., Ed.; Wiley-Interscience: New York, 1974; Vol. 14, Supplement 3, Chapter 12, pp 597−1180.

(24) Dragovich, P. S.; Prins, T. J.; Zhou, R.; Webber, S. E.; Marakovits, J. T.; Fuhrman, S. A.; Patick, A. K.; Matthews, D. A.; Lee, C. A.; Ford, C. E.; Burke, B. J.; Rejto, P. A.; Hendrickson, T. F.; Tuntland, T.; Brown, E. L.; Meador, J. W., III; Ferre, R. A.; Harr, J. E. V.; Kosa, M. B.; Worland, S. T. Structure-Based Design, Synthesis, and Biological Evaluation of Irreversible Human Rhinovirus 3C Protease Inhibitors. 4. Incorporation of P_1 Lactam Moieties as L-Glutamine Replacements. *J. Med. Chem*. **1999**, *42*, 1213−1224.

(25) The described coupling of the propargyl- and ethyl-containing P_4−P_3−P_2 moieties with the P_1 fragments typically resulted in greater racemization (20−30%) of the P_2 stereocenter than was noted in our previous work employing analogues which incorporated P_2 benzyl substituents.[9] Thus, careful flash column chromatography was often required to obtain the 3CP inhibitors described in the present work in diastereomerically pure form.

In all cases, the purified compounds were shown to be configurationally stable when subjected to routine handling and biological testing procedures. The cause of the differing racemization rates observed during the synthesis of the various 2-pyridone-containing 3CP inhibitors is not known.

(26) Dess, D. B.; Martin, J. C. A Useful 12-I-5 Triacetoxyperiodinane (the Dess−Martin Periodinane) for the Selective Oxidation of Primary or Secondary Alcohols and a Variety of Related 12-I-5 Species. *J. Am. Chem. Soc.* **1991**, *113*, 7277−7287.

(27) (a) Mori, S.; Iwakura, H.; Takechi, S. A New Amidoalkynylation Using Alkynlyzinc Reagent. *Tetrahedron Lett.* **1988**, *29*, 5391−5394 and references therein. (b) Overman, L. E.; Osawa, T. A Convenient Synthesis of 4-Unsubstituted β-Lactams. *J. Am. Chem. Soc.* **1985**, *107*, 1698−1701 and references therein.

(28) (a) Tian, Q.; Nayyar, N. K.; Babu, S.; Chen, L.; Tao, J.; Lee, S.; Tibbetts, A.; Moran, T.; Liou, J.; Guo, M.; Kennedy, T. An Efficient Synthesis of a Key Intermediate for the Preparation of the Rhinovirus Protease Inhibitor AG7088 via Asymmetric Dianionic Cyanomethylation of *N*-Boc-L-(+)-Glutamic Acid Dimethyl Ester. *Tetrahedron Lett.* **2001**, *42*, 6807−6809. (b) Tian, Q.; Nayyar, N. K.; Babu, S.; Tao, J.; Moran, T. J.; Dagnino, R., Jr.; Remarchuk, T. P.; Melnick, M. J.; Mitchell, L. J., Jr.; Bender, S. L. WO 01/14329, 2001.

(29) Okuma, K.; Tachibana, Y.; Sakata, J.-i.; Komiya, T.; Kaneko, I.; Komiya, Y.; Yamasaki, Y.; Yamamoto, S.-i.; Ohta, H. Reactions of Wittig Reagents With Episulfides or Elemental Sulfur. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 4323−4327 and references therein.

(30) Webber, S. E.; Okano, K.; Little, T. L.; Reich, S. H.; Xin, Y.; Fuhrman, S. A.; Matthews, D. A.; Love, R. A.; Hendrickson, T. F.; Patick, A. K.; Meador, J. W., III; Ferre, R. A.; Brown, E. L.; Ford, C. E.; Binford, S. L.; Worland, S. T. Tripeptide Aldehyde Inhibitors of Human Rhinovirus 3C Protease: Design, Synthesis, Biological Evaluation, and Cocrystal Structure Solution of P_1 Glutamine Isosteric Replacements. *J. Med. Chem.* **1998**, *41*, 2786−2805.

(31) Andries, K.; Dewindt, B.; Snoeks, J.; Willebrords, R.; Van Eemeren, K.; Stokbroekx, R.; Janssen, P. A. J. In Vitro Activity of Pirodavir (R 77975), a Substituted Phenoxy-Pyridazinamine with Broad-Spectrum Antipicornaviral Activity. *Antimicrob. Agents Chemother.* **1992**, *36*, 100−107.

(32) Diana, G. D.; Rudewicz, P.; Pevear, D. C.; Nitz, T. J.; Aldous, S. C.; Aldous, D. J.; Robinson, D. T.; Draper, T.; Dutko, F. J.; Aldi, C.; Gendron, G.; Oglesby, R. C.; Volkots, D. L.; Reuman, M.; Bailey, T. R.; Czerniak, R.; Block, T.; Roland, R.; Oppermann, J. Picornavirus Inhibitors: Trifluoromethyl Substitution Provides a Global Protective Effect Against Hepatic Metabolism. *J. Med. Chem.* **1995**, *38*, 1355−1371 and references therein.

(33) (a) Artursson, P.; Palm, K.; Luthman, K. Caco-2 Monolayers in Experimental and Theoretical Predictions of Drug Transport. *Adv. Drug Deliv. Rev.* **2001**, *46*, 27−43. (b) Yee, S. In Vitro Permeability Across Caco-2 Cells (Colonic) can Predict In Vivo (Small Intestinal) Absorption in Man-Fact or Myth. *Pharm. Res.* **1997**, *14*, 763−766. (c) Delie, F.; Rubas, W. A Human Colonic Cell Line Sharing Similarities With Enterocytes as a Model to Examine Oral Absorption: Advantages and Limitations of the Caco-2 Model. *Crit. Rev. Ther. Drug Car. Sys.* **1997**, *14*, 221−286. (d) Audus, K. L.; Bartel, R. L.; Hidalgo, I. J.; Borchardt, R. T. The Use of Cultured Epithelial and Endothelial Cells for Drug Transport and Metabolism Studies. *Pharm. Res.* **1990**, *7*, 435−451.

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