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Structure–Activity Relationships of Acyloxyamidine Cytomegalovirus DNA Polymerase Inhibitors

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Abstract—This paper describes the structure–activity relationships of a new class of cytomegalovirus DNA polymerase inhibitors having two aryl groups joined by an acyloxyamidine linker. Examination of a series of analogues in which the terminal groups are varied revealed a very narrow SAR around the 2,4-dichlorophenyl group of the lead compound, but a variety of replacements for the benzothiazole ring are compatible with activity. The most notable of these is the isoxazole ring of compound **78**, which provides a 30-fold enhancement in potency compared to the lead compound. We also describe the design, synthesis and evaluation of 10 analogues in which the acyloxyamidine linker is modified or replaced by an isosteric group. Structure–activity relationship studies identified the linker -NH_2 group as a critical pharmacophoric element. Ab initio molecular orbital calculations combined with qualitative estimates of steric interaction energies suggest that the lowest energy conformations of the acyloxyamidine linker are characterized by an extended planar C_{Ar}–C=N–O–C arrangement and either a *syn*-periplanar or *anti*-periplanar N–O–C–$C_{Ar'}$ arrangement. Only the *anti*-periplanar conformation was observed in the crystal structures of three acyloxyamidines. The most active of the linker-modified compounds designed on the basis of these studies is the amidine carbamate **20**, which is approximately one-third as potent in the cytomegalovirus DNA polymerase inhibition assay as the comparator acyloxyamidine **53.** The activity of **20** suggests that acyloxyamidines may bind to the cytomegalovirus DNA polymerase via an *anti*-periplanar conformation similar to that observed in the crystal structure of acyloxyamidine **36**.

Introduction

Cytomegalovirus (CMV) is a member of the herpesvirus family, a group of double-stranded DNA viruses which includes the herpes simplex viruses 1 and 2, Epstein–Barr virus, and varicella-zoster virus.[1] The members of this virus family share the ability to establish latent infections in otherwise healthy individuals, and to reactivate following certain environmental stimuli or host immunosuppression. Cytomegalovirus infects 40–100% of various human populations,[2] the frequency of infection varying with cultural and regional trends in breast feeding and other forms of physical intimacy.

In contrast to the relatively benign profile of CMV infection in immunocompetent individuals, CMV is among the most common causes of morbidity and mortality among the immunocompromised. The prevalence of CMV infection among AIDS patients is nearly 100%, and 25–40% will develop CMV-related morbidity.[3,4] Retinitis is among the most common manifestations of CMV infection in these patients. This syndrome, which is characterized by retinal lesions consisting of hemorrhagic exudates, will always lead to blindness in the absence of treatment.[5] Up to one third of bone marrow transplant patients and 13–38% of renal allograft patients will develop clinically apparent CMV disease.[6,7] The utility of currently available treatments for CMV disease is limited by their toxicity, their modest efficacy, and by the requirement of intraveneous administration for maximum efficacy.

Broad screening of the Pharmacia and Upjohn chemical library led to the identification of the acyloxyamidine **1** as a 20 μM competitive inhibitor of the CMV DNA polymerase. This compound does not detectably inhibit human DNA polymerase alpha at 100 μM concentration. In this report we describe a preliminary investigation into the effects on CMV polymerase inhibition potency of varying the terminal aryl groups of this lead

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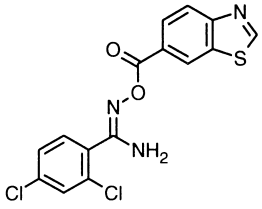
structure. We also report molecular orbital calculations and X-ray crystallographic investigations undertaken to elucidate the conformational behavior of the metabolically unstable acyloxyamidine linker, and the synthesis and evaluation of a series of analogues in which this linker is modified or replaced by an isosteric group.



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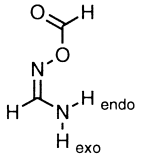
Results and Discussion

Conformational analysis

We elected to initiate our investigation into the conformational preferences of **1** with a series of ab initio molecular orbital calculations on the model compound *N*-(formyloxy)formamidine (**2**). This model system was chosen because it represents the smallest molecular entity which contains all the essential features of the *N*-(acyloxy)amidine linker of **1**. Ab initio geometry optimizations were performed with the 3-21G basis set and single point energy calculations were performed on the optimized structures using the 6-31G* basis set.[8,9]



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The four local conformational energy minima discovered in this effort are represented by structures A–D in Figure 1. In these four structures the C=N bond adopts an *E* or *Z* geometry and the geometry about the carboxyl C–O bond is *syn*-periplanar or *anti*-periplanar. The observed preference for the *Z* configuration of the C=N bond may result from attractive electrostatic interactions between the oxime oxygen and the endo hydrogen of the-NH_2 group. The preference for a *syn*-periplanar or *anti*-periplanar arrangement about the carboxyl C–O bond is in accord with expectations based on resonance interactions of the carbonyl group and a lone pair on the single-bonded carboxyl oxygen.

In all four local conformational energy minima the geometry about the N–O bond is *anti*-periplanar. Three structures having other torsion angles about the N–O bond were examined in this study and found not to be local conformational energy minima at the 3-21G level of theory. A cyclically hydrogen bonded conformation similar to E was found to be a local conformational energy minimum using AM1 calculations but optimization of this structure using the more rigorous ab initio calculations led to the planar structure B. In order to obtain an estimate of the relative energy of a cyclically hydrogen bonded structure at the 6-31G*//3-21G level of theory, the dihedral angle about the N–O bond was constrained to its value in the AM1 structure (56°) and the remaining geometrical parameters were optimized at the 3-21G level. Single point energy calculations using the 6-31G* basis set place this conformation (structure E) 9.0 kcal/mol above the lowest energy conformation.[10] A related structure in which the C=N–O–C dihedral angle is constrained to a value of 120° has a relative energy of 3.8 kcal/mol. This structure also minimized to structure B when the C=N–O–C dihedral angle constraint was removed from the calculation. A third structure similar to *E* but having an *E* configuration of the C=N bond minimized to conformation D upon geometry optimization. The energetic preference for an antiperiplanar geometry about the N–O bond may arise from electrostatic repulsion between the nitrogen sp^2 lone pair and the two sp^3 lone pairs on oxygen. These interactions are minimized in conformations in which the N–O bond adopts an *anti*-periplanar arrangement.

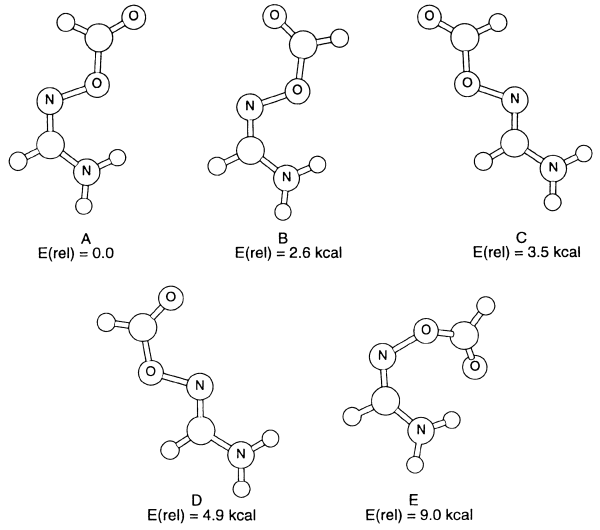


Figure 1. 6-31G*//3-21G conformational energy minima of **2**.

In order to use the data in Figure 1 to predict the conformational behavior of **1**, account must be taken of steric interactions that are present in **1** and absent in the model compound **2**. Conceptually this corresponds to replacing both C–H bonds in each of the structures of Figure 1 with C–C(aryl) bonds. Steric interactions involving the aryl rings which are present in some but not in all of the resulting *N*-(acyloxy)amidine conformers can then be estimated and appropriate correction factors applied to the relative conformational energies from Figure 1. As seen in Figure 2, all of the steric interactions satisfying this description are 1,4 eclipsing interactions between an aryl group and either an sp^3 oxygen or an sp^2 nitrogen. If these two types of interaction are assumed to be approximately equal and assigned a value x, the relative energies of conformers F, G, H, I and J are $(-2.6+x)$, 0.0, $(0.9+2x)$, $(2.3+x)$, and $(6.4+x)$ kcal/mol, respectively. In this approximation, values of x between 1 and 4 kcal/mol lead to a

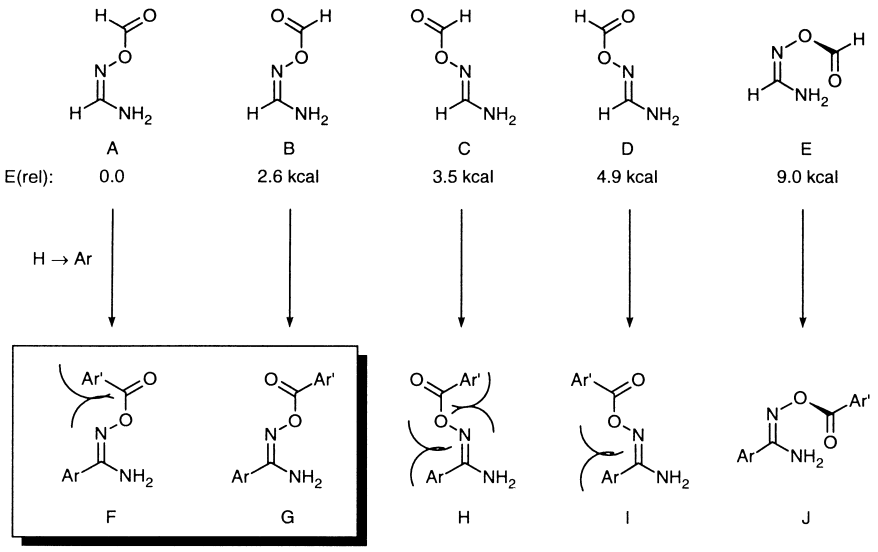


Figure 2.

prediction that the conformational equilibrium of **1** will be strongly dominated by conformers F and G. Values of *x* above and below this range lead to predictions that the conformational equilibrium will be dominated by F or G, respectively.

Crystallography

The X-ray crystal structure of a representative acyloxyamidine **36** is shown in Figure 3. Crystal structures were also determined for two other analogues of **1**, compounds **48** and **55**. All three of these compounds adopt a conformation in the solid state which is similar to conformer G of Figure 2, a result which is in accord with the results of our ab initio calculations. The atoms of the acyloxyamidine linker vary only slightly from coplanarity. In each of the crystal structures the carbonyl oxygen participates in an intermolecular hydrogen bond with the NH_2 group of an adjacent molecule. The ability of this hydrogen bonding network to stabilize conformation G in the solid state by reducing electrostatic repulsions between the imino nitrogen and the carbonyl oxygen may explain our failure to observe conformation F in any of these three crystal structures.

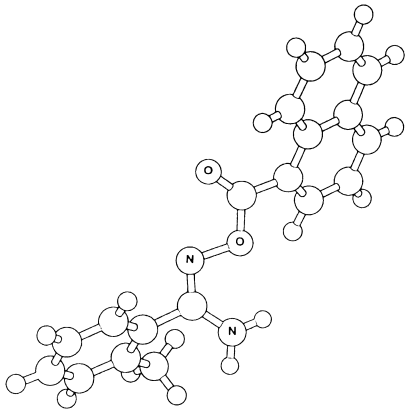


Figure 3. Crystal structure of compound **36**.

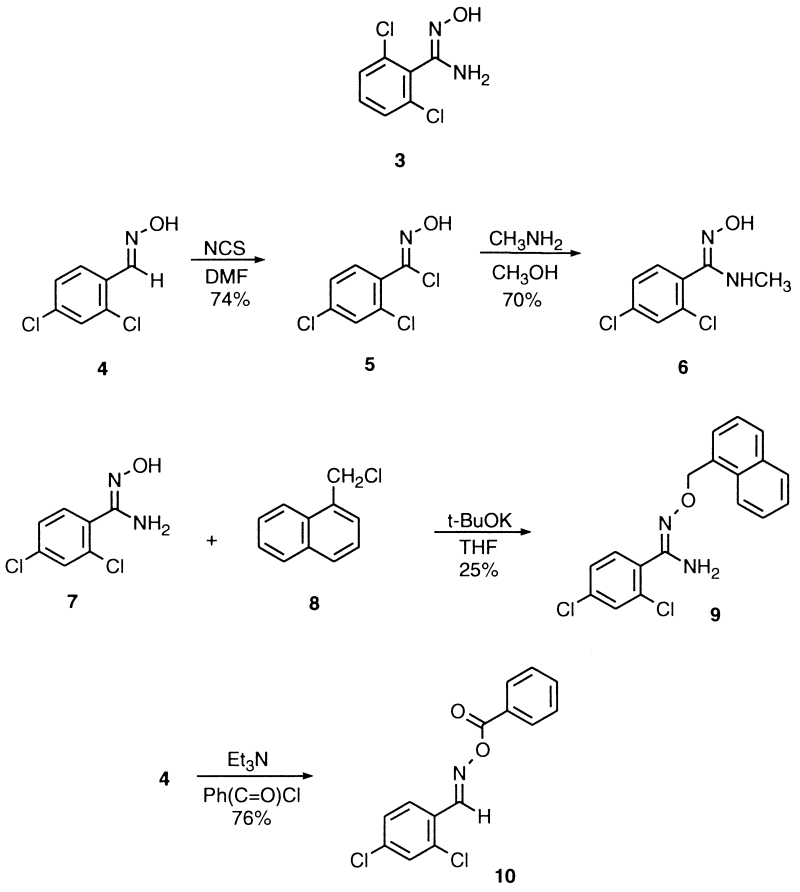
Synthesis

The acyloxyamidines described in this report were prepared by the acylation of hydroxyamidines with acid chlorides in the presence of triethylamine.[11] Most of the hydroxyamidines were commercially available or were synthesized by the reaction of a nitrile with anhydrous methanolic hydroxylamine. The 2,6-disubstituted hydroxybenzamidine **3** was prepared by a literature procedure involving nucleophilic attack of ammonia on 2,6-dichloro-*N*-hydroxybenzimidoyl chloride.[12,13] The *N*-substituted hydroxyamidine **6** was synthesized by treating 2,4-dichloro-*N*-hydroxybenzimidoyl chloride **5** with methylamine. The imidoyl chloride **5** was prepared by the action of NCS on the oxime **4**.[14] Analogue **9** was prepared by alkylation of the hydroxyamidine **7** with 1-chloromethylnaphthalene. Analogue **10** was prepared by acylating oxime **4** with benzoyl chloride (Scheme 1).

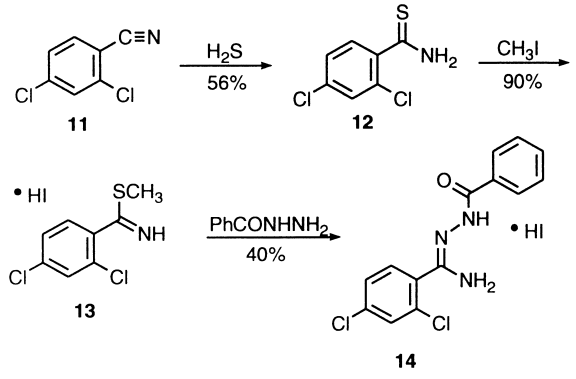
The aminoamidine **14** was prepared by the route shown in Scheme 2.

The amidine **17** was prepared by the action of methanolic ammonia on the imidate **16** (Scheme 3). Acylation of this amidine with phenyl chloroformate gave the amidine carbamate **20**. Treating amidine **17** with isocyanate **19** gave the amidine urea **21**.

The synthesis of the imidazole **23** (Scheme 4) was based on a method for the synthesis of methyl 2-phenylimidazole-4-carboxylate reported by Heindel and Chun.[15] In this approach an intermediate hydroxyamidine vinyl ether is obtained by conjugate addition of a hydroxyamidine to an electron-deficient alkyne. Thermolysis of this ether occurs with the loss of a molecule of water to produce the imidazole ring. In our hands the conjugate addition of the hydroxyamidine **7** to phenyl ethynyl ketone and the thermal rearrangement of the resulting vinylogous ester gave the imidazole **23** in 5% overall yield.



Scheme 1.



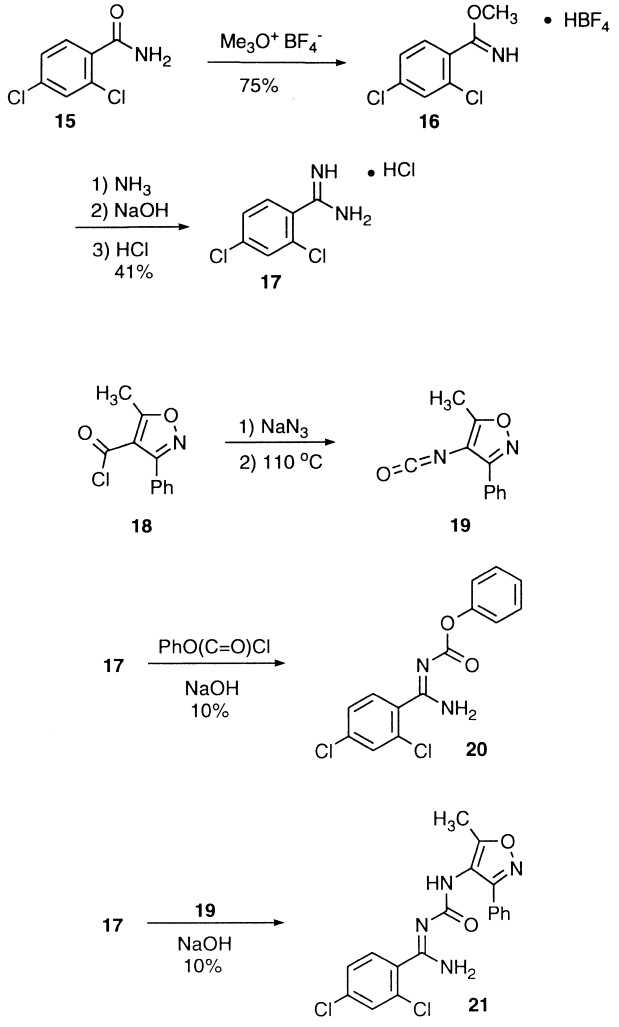
Scheme 2.

The tetrahydrotriazene **29** was prepared by a multistep synthetic route involving preparation of a suitably substituted aminoalkylhydrazine **28** and cyclization onto the imidate ester **16** (Scheme 5). The low yield in the final step of this synthetic sequence results in part from the formation of large amounts of a byproduct arising from the reaction of **28** with two equivalents of **16**. Presumably the formation of this byproduct could be reduced by performing the cyclization under high dilution conditions.

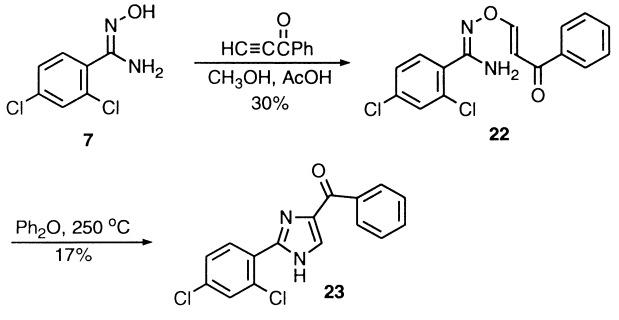
The hydroxyamidine **31** was prepared by treating the hydroxyimidoyl chloride **5** with 2-phenylmorpholine **30** (Scheme 6). The amidine **33** was prepared by treating the thioimidate **13** with the tetrahydrooxazine **32**.[16]

Structure–activity relationships

The compounds of this report were screened for their ability to inhibit CMV DNA polymerase using a scintillation proximity assay (Amersham) to measure incorporation of nucleotide into a template/primer. The effect of varying the substitution pattern of the left hand ring on activity in the CMV polymerase assay is described in Table 1. In this series the benzothiazole group of the lead compound **1** was replaced by a 1-naphthyl group because this substitution exerts a favorable effect on activity and because the requisite acid chloride is commercially available. Compound **42,** which retains the left hand 2,4-dichlorophenyl group of the lead compound **1**, serves as the benchmark for this series of compounds. Among the 19 left hand ring substitution patterns examined, none confers activity in the CMV polymerase assay greater than the 2,4-dichloro substitution pattern of **42**. The chlorine in the 2-position of the phenyl ring can be replaced by methyl (compound **44**) without significantly changing the activity, suggesting that the steric rather than the electronic properties of the 2-substituent are important for activity. Examination of molecular models suggests that the presence of a 2-substituent enforces a non-coplanar arrangement of the left hand ring and the attached $(C{=}N)NH_2$ fragment. In the absence of such a substituent, a coplanar



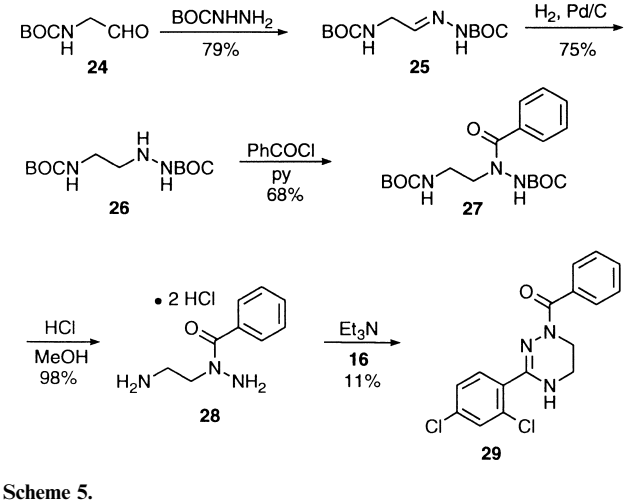
Scheme 3.



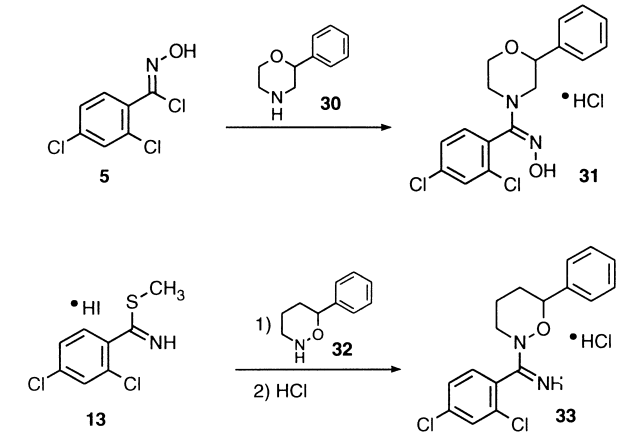
Scheme 4.

arrangement of these two groups is expected to be energetically favored due to resonance interactions.

Table 2 shows the structures and in vitro screening results for 19 analogues of **1** in which the benzothiazole ring is replaced by a substituted or unsubstituted phenyl ring. Replacement of the benzothiazole ring of **1** (CMV polymerase $IC_{50} = 20\,\mu M$) with phenyl gave a compound of equal potency (**53**). Attaching a methyl, phenyl, or chlorine substituent to the *ortho* position of this phenyl ring gave compounds of enhanced potency, the best of these substituents being phenyl (compound



Scheme 5.

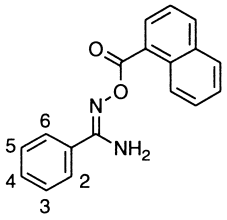


Scheme 6.

56). Examination of molecular models suggest that *ortho* substituents enforce an non-coplanar arrangement of the carboxylate group and the attached phenyl ring. A coplanar arrangement is expected to be favored by resonance interactions in the absence of an *ortho* substituent. Substitution in the *para* position with methyl, methoxy, or chloro gave compounds that were less active than **56.** These results, in combination with the activity of the lead compound **1**, suggest that a steric exclusion zone may exist in this part of the enzyme binding pocket that will tolerate no substituent larger than a divalent nitrogen.

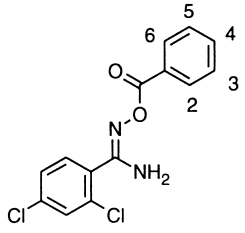
Table 3 shows the structures and in vitro screening results for eight analogues of **1** in which the benzothiazole moiety has been replaced by an araalkyl, cycloalkyl, or heteroaromatic group. The benzyl and cyclohexyl groups of compounds **72** and **73** gave compounds having very modest levels of activity, as did the relatively hydrophilic furyl and pyridyl groups of **74** and **75**. The modest activity of **76** and **77** may be related to the presence of a ring CH in the steric exclusion zone that was hypothesized to explain the inactivity of compounds **60–62**. The most interesting compound of this series is the isoxazole derivative **78**, which is approximately 30 times more potent than the lead compound **1**.

Table 1. Substituted phenyl rings as replacements for the 2,4-dichlorophenyl group of **14**



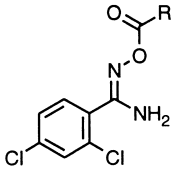
No.	Substituents					IC_{50} (μM)
	C-2	C-3	C-4	C-5	C-6	
34	Cl					>100
35	OCH_3					>100
36	CH_3					>100
37		Cl				>100
38			Cl			50
39			CH_3			>100
40	Cl	Cl				38.7
41	Cl		F			28.6
42	Cl		Cl			3.1
43	Cl		Br			2.6
44	CH_3		Cl			3.9
45	CH_3		Br			4.2
46	CH_3		CH_3			20.4
47	Cl			Cl		15.0
48	Cl				Cl	59.6
49		Cl	CH_3			>100
50		Cl		Cl		>100
51		OCH_3		OCH_3		32.7
52	Cl		Cl		Cl	13.7

Table 2. Substituted phenyl rings as replacements for the benzothiazole ring of **1**



No.	Substituents					IC_{50} (μM)
	C-2	C-3	C-4	C-5	C-6	
53						22
54	Cl					10
55	CH_3					6.1
56	Ph					5.4
57		Cl				11
58		OCH_3				7.7
59		CH_3				16
60			Cl			>50
61			OCH_3			>100
62			CH_3			>100
63	Cl	Cl				10
64	OCH_3	OCH_3				>100
65	CH_3	F				2.1
66	Cl			Cl		6.7
67	CH_3			F		4.4
68	Cl				Cl	37
69	CH_3				CH_3	32
70		Cl		Cl		25
71		OCH_3		OCH_3		5.3

Table 3. Araalkyl, cycloalkyl and aromatic groups as replacements for the benzothiazole ring of **1**



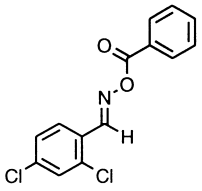
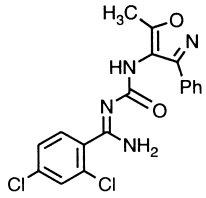
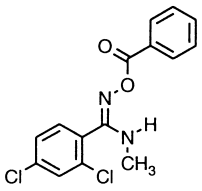
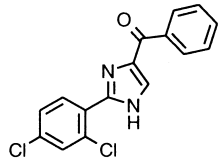
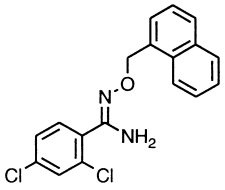
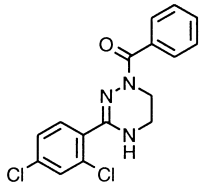
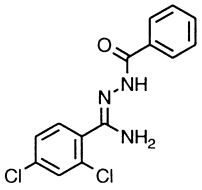
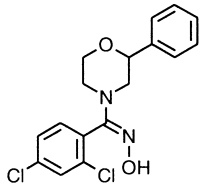
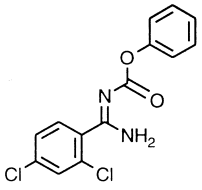
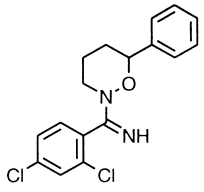
No.	R	IC_{50} (μM)
72	Benzyl	44
73	Cyclohexyl	>100
74	2-Furyl	>100
75	4-Pyridyl	52
76	2-Benzothiophenyl	72
77	2-Quinoxalinyl	>100
78	5-Methyl-3-phenylisoxazol-4-yl	0.7
79	3-(2,6-Dichlorophenyl)-5-methylisoxazol-4-yl	9.5

The synthesis of this analogue was undertaken because of its structural similarity to the active analogue **56**, and because it was hoped that the steric hinderance of the carbonyl group in this analogue would provide improved metabolic stability.

Compounds **42** and **78** were evaluated for their ability to inhibit CMV replication in human foreskin fibroblast (HFF) cells using standard antiviral assay conditions. Neither of these compounds exhibited reproducible antiviral activity at concentrations $\leq$100 μM. Because the antiviral assay is performed in a growth medium that includes 10% serum, we hypothesized that the lack of antiviral activity of these compounds might be due to rapid degradation by serum esterases. In order to test this hypothesis, the stability of compounds **42** and **78** in serum at 37 °C was evaluated by HPLC analysis. Each compound was rapidly degraded with the formation of two new compounds that coelute with the hydroxyamidine and carboxylic acid expected from hydrolysis of the linker C–O bond. Attempts to improve the serum stability of these compounds by increasing the steric hindrance around the carbonyl carbon as in compounds **68** and **69** led to reduced activity in the CMV polymerase assay. Based on these results our efforts to discover more metabolically stable compounds were focused on the discovery of replacements for the acyloxyamidine linker.

Table 4 describes the CMV polymerase inhibition activity of the 10 analogues of this report that incorporate structural modifications of the acyloxyamidine linker. These analogues can be divided into three structural classes. Class 1 consists of analogues in which only incremental changes have been made to the acyloxyamidine linker group (compounds **10**, **80**, **9**, **14**, **20** and **21**). Some members of this group incorporate structural changes which were intended to improve the metabolic stability of the resulting analogues relative to that of the corresponding acyloxyamidine. In others, the incremental change was intended to elucidate the pharmacophoric elements of the acyloxyamidine linker. Compounds of class 2 (**23** and **29**) incorporate the amidine

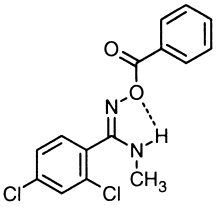
Table 4. Analogues incorporating modifications of the acyloxyamidine linker

No.	Structure	IC_{50} (μM)	No.	Structure	IC_{50} (μM)
10		>100[a]	**21**		>100[b]
80		>100[a]	**23**		174
9		19	**29**		>100[a]
14		>100	**31**		157
20		73	**33**		>100[a]

[a] < 10% inhibition at 100 μM concentration.
[b] < 5% inhibition at 50 μM concentration.

group into a ring structure which is intended to provide a metabolically stable framework for maintaining the left hand aryl group and the right hand aroyl group in the same relative orientation that is found in the two most energetically favored conformations of the acyloxyamidine parent. The design of these compounds incorporates resonance interactions between the carbonyl group and the atoms of the ring. This resonance restricts the carbonyl group to either of two coplanar orientations with respect to the amidine group, in close analogy with conformations F and G of Figure 2. In class 3 (compounds **31** and **33**), a six member ring constrains the conformation of the three atom linker between the amidine carbon and the right hand aryl ring. This conformational constraint, in combination with the amidine resonance, is expected to maintain a roughly coplanar arrangement of all the atoms of the linker group. The amidine carbon–nitrogen single bond in these compounds may adopt either an *s-cis* or *s-trans* arrangement with respect to the right hand phenyl ring and the imino nitrogen. Of these, only the *s-cis* conformer closely resembles one of the preferred conformations of the acyloxyamidine lead compounds.

Most of the linker-modified analogues described in this report incorporate phenyl as the right-hand aryl ring. As can be seen from data in Table 2, a variety of other aryl groups in this position lead to acyloxyamidines that are more potent inhibitors of CMV polymerase. Our decision to employ phenyl as the right hand aromatic ring in many of these analogues arose from the greater ease of synthesis of the phenyl compounds. It was our expectation that potency-enhancing right hand aryl groups could be incorporated after the best isosteric



80

Figure 4.

replacement for the acyloxyamidine group had been identified.

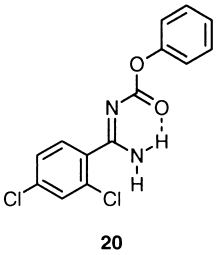
Compounds **10** and **80** of Table 4 were designed to evaluate the importance of the amidine -NH_2 group in binding to CMV polymerase. Replacement of the -NH_2 group of **53** with a hydrogen as in compound **10** or with an -$NHCH_3$ group as in compound **80** resulted in a greater than 5-fold decrease in affinity for the CMV polymerase binding site. These results can most easily be explained by the hypothesis that the *exo* N−H bond of the benchmark compound **53** is required for hydrogen bonding interactions with the enzyme. Compound **80** might reasonably be expected to adopt a conformation similar to that shown in Figure 4, with the CH_3 group occupying the *exo* position. This arrangement maintains a cyclic hydrogen bond between the oxime oxygen and the amino hydrogen while minimizing unfavorable steric interactions involving the oxime oxygen that are present in other arrangements.

Compounds **9**, **14**, **20** and **21** of Table 4 were designed to examine the effect on CMV polymerase inhibition activity of changes in the molecular fragment connecting the amidino group and right hand aryl ring of the acyloxyamidine parent compounds **42**, **53** and **78**. When the C=O fragment of naphthyl compound **42** was replaced by a CH_2 group the activity of the resulting analogue **9** was reduced about 5-fold. If one assumes that the most energetically favored conformation of the relatively rigid parent compound is similar to its ideal binding geometry, the decreased binding of the more conformationally mobile **9** can be explained by entropy considerations. This hypothesis implies that there are no energetically important hydrogen bonds between the carbonyl oxygen of **42** and the enzyme.

The amidine carbamate **20** was found to be about 3-fold less active than the benchmark compound **53**. Although we were initially encouraged that the amidine carbamate linker of this compound might serve as a metabolically stable replacement for the acyloxyamidine linker, HPLC studies demonstrated that **20** is not significantly more stable in serum than the acyloxyamidine **1**. Crystal structures of other compounds having an HN–C=N–(C=O)–O–C array reveal an extended, planar, cyclically hydrogen bonded geometry for this fragment similar to that drawn for **20** in Figure 5.[17–20] This proposed conformation is in accord with expectations based on resonance and steric effects, and places the two aryl rings and the -NH_2 group in relative orientations similar to that in conformer G of Figure 2. In the hopes of improving on the serum stability of **20** we prepared the amidine urea **21**, but this compound failed to exhibit any activity in the CMV polymerase inhibition assay.

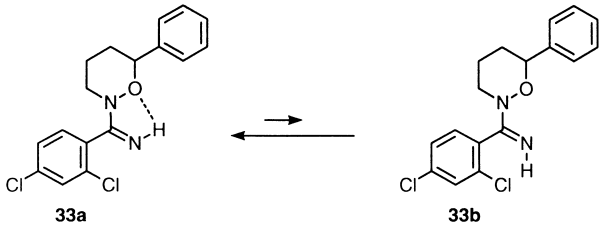
The two compounds of structural class 2 that were investigated in this study are the imidazole **23** and the tetrahydrotriazene **29**. Among these, **23** was observed to exhibit modest activity in the CMV polymerase assay. This compound incorporates the *exo* N–H, the carbonyl group, and the sp^2 hybridized nitrogen of the benchmark compound **53**, but molecular modeling results suggest that the 5-member imidazole ring introduces bond angle distortions that significantly alter the relative orientation of the two aromatic rings compared to that found in **53**. In order to determine the activity of a similar compound having bond angles more similar to those in **53**, the tetrahydrotriazene **29** was prepared. The inactivity of this compound may indicate the existence of a steric exclusion zone in the area transversed by the two CH_2 groups of the tetrahydrotriazene ring.

The two members of structural class 3 that were examined are the amidines **31** and **33**. The design of each of these two analogues includes an oxygen atom attached to one of the amidine nitrogens. The presence of this oxygen is intended to assure that these two compounds, like **53**, will not be ionized at physiological pH.[21,22] In compound **31**, the hydrogen bond donating site analogous to the *exo* hydrogen of **53** is shifted slightly relative to the position of the two aromatic rings because of the formal replacement of an N–H by N–OH. This compound exhibited about one-seventh the activity of **53** in the CMV polymerase inhibition assay. In hopes of preparing an analogue with greater structural similarity to **53** we prepared the tetrahydrooxazine derivative **33**. This compound proved completely inactive in the CMV polymerase assay. The inactivity of this analogue may



20

Figure 5.



33a 33b

Figure 6.

be due to formation of a cyclic hydrogen bond between the single N–H of this compound and the ring oxygen (Fig. 6). The resulting *Z* geometry about the C=N bond would place a lone pair in the position occupied by the *exo* N–H of **53**. This *exo* N–H was was previously hypothesized to be required for activity (vide supra). An alternative hypothesis, that the inactivity of **33** is due to its lack of a hydrogen bond acceptor analogous to the carbonyl oxygen of **53**, seems less attractive in light of the observed activity of **9**.

Conclusions

This report describes our preliminary SAR investigation of a new class of CMV polymerase inhibitors characterized by two aryl groups connected by an acyloxyamidine linker. Initial studies in which the two aryl groups were varied revealed little tolerance for modification of the left hand 2,4-dichlorophenyl group, but replacing the right hand benzothiazole ring with a disubstituted isoxazole ring as in **78** led to a 30-fold enhancement in activity. The favorable effect on activity of substituents adjacent to the linker on each aromatic ring may reflect a preferred binding conformation in which the aryl rings lie out of the plane of the adjacent linker atoms. The metabolic instability associated with the acyloxyamidine linker led us to undertake a research effort designed to discover more stable isosteric replacements for this functional group. Structure–activity relationship studies identified the linker-NH_2 group as a critical pharmacophoric element. Ab initio molecular orbital calculations combined with qualitative estimates of steric interaction energies suggest that the lowest energy conformations of the acyloxyamidine linker are characterized by an extended planar C_{Ar}–C=N–O–C arrangement and either a *syn*-periplanar or *anti*-periplanar N–O–C–$C_{Ar'}$ arrangement. Only the *anti*-periplanar conformation was observed in the crystal structures of three acyloxyamidines. Among the linker replacements designed on the basis of these studies, good activity in the cytomegalovirus DNA polymerase inhibition assay was most closely associated with the amidine carbamate linker of **20.** This compound is approximately one-third as potent in the cytomegalovirus DNA polymerase inhibition assay as the comparator acyloxyamidine **53.** The activity of **20** suggests that acyloxyamidines may bind to the cytomegalovirus DNA polymerase via the *anti*-periplanar conformation observed in the crystal structure of **36**.

Experimental

Synthesis

1-{[(Benzoyloxy)imino]methyl}-2,4-dichlorobenzene (10). A 0 °C solution of 0.75 g (3.95 mmol) 2,4-dichlorobenzaldehyde oxime[22] and 0.70 mL (5.00 mmol) diisopropylethylamine in 15 mL of tetrahydrofuran was treated with 0.46 mL (3.95 mmol) benzoyl chloride. The mixture was stirred at 25 °C for 18 h and then it was partitioned between 50 mL of dichloromethane and 50 mL of 3 N aqueous sodium hydroxide solution. The phases were separated and the organic phase was dried (Na_2SO_4). The solvent was evaporated at reduced pressure and the residue was recystallized from refluxing methanol. The precipitate was collected by filtration, washed with 5 mL of methanol, and dried at 20 torr/50 °C/48 h to give the title compound as 0.88 g (76%) of a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.91 (s, 1H), 8.11 (overlapping triplets, J = 7.5, 7.5 Hz, 3H), 7.60 (tt, J = 1.0, 7.5 Hz, 1H), 7.49–7.38 (m, 3H), 7.27 (dd, J = 2.0, 8.5 Hz, 1H). MS (EI) m/z (rel. intensity) 293 (M^+, 1), 173 (5), 171 (7), 123 (4), 109 (4), 106 (22), 105 (100), 77 (61), 75 (4), 51 (13), 50 (5). Anal. calcd for $C_{14}H_9Cl_2NO_2$: C, 57.17; H, 3.08; N, 4.76. Found: C, 57.06; H, 2.90; N, 4.54.

2,4-Dichloro-*N*′-hydroxy-*N*-methylbenzenecarboximidamide (6). A solution of 0.80 g (3.56 mmol) of **5**[23,24] in 10 mL of methanol was added all at once to 20 mL of a saturated solution of methylamine in methanol. After 30 min the reaction mixture was partitioned between 50 mL of dichloromethane and 50 mL of pH 12 phosphate buffer. The aqueous phase was washed with 10 mL of dichloromethane and then the combined organic extracts were washed with 50 mL of distilled water and dried (Na_2SO_4). The solvent was evaporated at reduced pressure and the residue was dissolved in 25 mL of refluxing methanol. Distilled water was added to the warm solution until it became cloudy and the solution was allowed to stand 12 h at 25 °C. The product was collected by filtration, yielding 0.55 g (70%) of the title compound as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.0 (broad s, 1H), 7.38 (d, J = 2.0 Hz, 1H), 7.30–7.19 (m, 2H), 5.30 (broad s, 1H), 2.54 (s, 3H). MS (EI) m/z (rel. intensity) 218 (M^+, 100), 220 (64), 189 (35), 188 (31), 187 (56), 186 (40), 174 (48), 172 (74), 136 (27), 124 (26). Anal. calcd for $C_8H_8Cl_2N_2O$: C, 43.86; H, 3.68; N, 12.79. Found: C, 43.87; H, 3.78; N, 12.78.

[(Benzoyloxy)imino](2,4-dichlorophenyl)-*N*-methylmethanamine (80). A solution of 0.25 g (1.14 mmol) of **6** in 5 mL of tetrahydrofuran was treated successively with 0.21 mL (1.50 mmol) of triethylamine and 0.132 mL (1.14 mmol) of benzoyl chloride. The mixture was stirred 20 h, then it was partitioned between 25 mL of dichloromethane and 25 mL of 1 N aqueous sodium hydroxide solution. The organic phase was dried (Na_2SO_4) and the solvent was evaporated at reduced pressure. The residue was recystallized from refluxing methanol and water. The product was collected by filtration and washed with 9:1 methanol:distilled water. This procedure yielded the title compound as 0.25 g (68%) of a white powder, mp 185–186 °C. An analytical sample was dried at 20 torr/55 (C/6 h. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (dt, J = 6.0, 1.0 Hz, 2H), 7.55 (tt, J = 1.5, 6.5 Hz, 1H), 7.50–7.37 (m, 4H), 7.32 (dd, J = 2.0, 8.0 Hz, 1H), 5.47 (broad q, J = indet., 1H), 2.71 (d, J = 5.0 Hz, 3H). MS (EI) m/z (rel. intensity) 322 (M^+, 13), 324 (8), 188 (5), 186 (8), 174 (3), 172 (4), 106 (8), 105 (100), 77 (14), 51 (3). Anal. calcd for $C_{15}H_{12}Cl_2N_2O_2$: C, 55.75; H, 3.74; N, 8.67. Found: C, 55.89; H, 3.77; N, 8.75.

2,4-Dichloro-*N*′-(1-naphthylmethoxy)benzenecarboximidamide (9). To a 0 °C solution of 0.400 g (1.95 mmol) 2,4-dichlorobenzamidoxime (**7**) in 3 mL of tetrahydrofuran was added over 1 min 2.0 mL (2.0 mmol) of a 1.0 M solution of potassium *tert*-butoxide in tetrahydrofuran. To the resulting thick suspension was added 0.91 mL (1.95 mmol) of neat 1-(chloromethyl)napthalene over 1 min, followed by 1.0 mL of DMPU. The mixture was stirred 19 h at 25 °C and then it was partitioned between 30 mL of distilled water and 30 mL of dichloromethane. The organic phase was dried ($MgSO_4$) and the solvent was evaporated at reduced pressure. The residue was purified by chromatography (55% dichloromethane in hexanes) followed by recrystallization from dichloromethane and hexane. This procedure yielded the product as 170 mg (25%) of a white solid, mp 78–80 °C. A sample was dried at 40 °C/20 torr/18 h for elemental analysis. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.18 (d, J = 7.0 Hz, 1H), 7.92-7.80 (m, 2H), 7.60–7.40 (m, 6H), 7.28 (dd, J = 2.0, 8.0 Hz, 1H), 5.57 (s, 2H), 4.86 (broad s, 2H). MS (FAB) m/z (rel. intensity) 345 (M + H, 71), 349 (7), 348 (11), 347 (46), 346 (28), 344 (21), 191 (7), 189 (12), 142 (13), 141 (100). Anal. calcd for $C_{18}H_{14}N_2OCl_2$: C, 62.62; H, 4.09; N, 8.11. Found: C, 62.43; H, 4.08; N, 8.01.

2,4-Dichlorobenzenecarbothioamide (12). A flask was loaded with a solution of 25g (145 mmol) 2,4-dichlorobenzonitrile (**11**) in a mixturre of 100 mL of pyridine and 28.0 mL (200 mmol) of triethhylammine. Hydrogen sulfide gas was bubbled through the reaction mixture at a moderately vigorous rate for 20 min, then the mixture was stirred 20 h at 25 °C. A vigorous flow of nitrogen was passed through the reaction mixture for 20 min, and then the reaction mixture was diluted with 500 mL of distilled water and 500 mL of 9:1 ethyl ether:ethyl acetate. The aqueous phase was extracted with 100 mL of ethyl ether and the combined organic extracts were washed successively with two 300 mL portions of 3.0 N hydrochloric acid and one 100 mL portion of brine. The organic phase was dried ($MgSO_4$), and the solvent was evaporated at reduced pressure. The residue was stirred in hexanes, and the resulting yellow solid was collected by filtration. It was then recrystallized from cyclohexane and collected by filtration to give 16.6 g (56%) of the title compound as bright yellow needles, mp 86–87 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.09 (broad s, 1H), 7.65 (d, J = 8.5 Hz, 1H), 7.39 (d, J = 2.0 Hz, 1H), 7.27 (dd, J = 8.5, 2.0 Hz, 1H), 7.22 (broad s, 1H). MS (EI) m/z (rel. intensity) 205 (M^+, 75), 207 (51), 174 (19), 172 (63), 170 (100), 136 (20), 75 (25), 74 (22), 60 (64), 50 (21). Anal. calcd for $C_7H_5Cl_2NS$: C, 40.80; H, 2.45; N, 6.80. Found: C, 40.70; H, 2.42; N, 6.68.

Methyl 2,4-dichlorobenzenecarbimidothioate hydroiodide (13). A mixture of 5.0 g (24.3 mmol) of **12**, 2.3 mL (36.0 mmol) of methyl iodide and 35 mL of acetone was stirred 3 days at 25 °C. The white solid precipitate which formed during this period was collected by filtration and washed with acetone. The product was obtained as 6.5 g (76%) of a white powder. ^{1}H NMR (300 MHz, CD_3OD) δ 7.80 (d, J = 2.0 Hz, 1H), 7.72 (d, J = 8.0 Hz, 1H), 7.63 (dd, J = 8.0, 2.0 Hz, 1H), 2.86 (s, 3 H). MS (FAB) m/z (rel. intensity) 220 (M + H, 100), 376 (3), 374 (4), 224 (13), 223 (7), 222 (69), 221 (11), 186 (2), 174 (3), 172 (4). Anal. calcd for $C_8H_8Cl_2INS$: C, 27.60; H, 2.32; N, 4.02. Found: C, 27.53; H, 2.22; N, 3.89.

***N*′-[(2,4-Dichlorophenyl)(imino)methyl]benzohydrazide hydroiodide (14).** A 25 °C suspension of 0.196 g (1.44 mmol) benzoic hydrazide in 1.0 mL of absolute ethanol was added all at once to a 0 °C solution of 0.50 g (1.44 mmol) **13** in 2.0 mL of ethanol. The mixture was stirred 26 h at 5 °C and then it was diluted with 30 mL of diethyl ether and 10 mL of cyclohexane. The mixture was allowed to stand 35 min and then the precipitate was collected by filtration. It was washed with 4:1 diethyl ether:absolute ethanol. The solid was dried in a stream of air and then further dried at 20 torr/80 °C for 4 h to give the title compound as 0.25 g (40%) of a white solid, mp 190–191.5 (C. ^{1}H NMR (CD_3OD) δ 8.01 (d, J = 7.5 Hz, 2H), 7.84 (d, J = 2.0 Hz, 1H), 7.80 (d, J = 8.5 Hz, 1H), 7.73–7.62 (m, 2H), 7.56 (t, J = 7.5 Hz, 2H). MS (FAB) m/z (rel. intensity) 308 (M + H, 100), 312 (11), 311 (6), 310 (63), 174 (3), 172 (5), 105 (13). Anal. calcd for $C_{14}H_{11}Cl_2N_3O \cdot HI$ 0.5 H_2O: C, 37.78; H, 2.95; N, 9.27. Found: C, 37.70; H, 2.94; N, 9.27.

Phenyl amino(2,4-dichlorophenyl)methylidenecarbamate (20). A solution of 3.7 g (25 mmol) trimethyloxonium tetrafluoroborate and 4.75 g (25 mmol) of 2,4-dichlorobenzamide in 20 mL of anhydrous dichloromethane was stirred three days at 25 °C. The reaction mixture was diluted with 30 mL of anhydrous diethyl ether and the mixture was filtered. The filtrant was washed anhydrous diethyl ether and dried in a stream of air for 10 min. This procedure gave the imidate hydrotetrafluoroborate (**16**) as 5.5 g of a white powder (75%). ^{1}H NMR (300 MHz, DMSO) δ 7.97 (d, J = 2.0 Hz, 1H), 7.82 (d, J = 8.5 Hz, 1H), 7.76 (dd, J = 2.0, 8.5 Hz, 1H), 4.23 (s, 3H).

A solution of 2.2 g (7.53 mmol) of this imidate in 25 mL of anhydrous methanol was treated with 25 mL of anhydrous ammonia-saturated methanol. The mixture was stirred 6 days at 25 °C and then it was partitioned between 100 mL of ethyl acetate and 50 mL of 10% aqueous sodium hydroxide solution. The aqueous phase was washed with 25 mL of ethyl acetate and the combined organic extracts were dried ($MgSO_4$). The solvent was evaporated at reduced pressure and the oily residue was dissolved in 10 mL of isopropanol. The solution was treated with 15 mL of 1.0 N hydrogen chloride in diethyl ether. After standing 15 min, the white solid precipitate was collected by filtration and washed with 1:1 diethyl ether:isopropanol. This procedure gave the amidine **17** as 0.69 g (41%) of a white solid, mp 252–254 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.61 (s, 2H), 9.57 (s, 2H), 7.95 (d, J = 2 Hz, 1H), 7.74 (d, J = 8.0 Hz, 1H), 7.68 (dd, J = 2.0, 8.0 Hz, 1H).

A solution of 300 mg (1.33 mmol) of **17** in 6 mL of 2:1 acetone:deionized water was cooled to 0 °C and treated with 0.175 mL of 15 M aqueous sodium hydroxide solution. After 3 min at 0 °C phenyl chloroformate (0.167 mL, 1.33 mmol) was added and the solution was stirred 1 h at 0 °C and 20 min at 25 °C. The mixture was

partitioned between 50 mL of diethyl ether and 50 mL of deionized water. The organic phase was washed with 10 mL of 2 M aqueous $NaHSO_4$ solution, then with 10 mL of 3.0 N aqueous sodium hydroxide solution, and finally with 10 mL of brine. The solution was dried ($MgSO_4$), and the solvent was evaporated at reduced pressure. The residue was purified by chromatography on silica gel (15–20% ethyl acetate in cyclohexane). This material was recrystallized from dichloromethane and cyclohexane. The white needles which formed were collected by filtration and dried at 45 °C/20 torr for 5 h. The yield is 40 mg (10%), mp 112–113 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72 (d, J = 8.5 Hz, 1H), 7.41 (d, J = 2.0 Hz, 1H), 7.37–7.27 (m, 4H), 7.20–7.09 (m, 4H). MS (FAB) m/z (rel. intensity) 309 (M + H, 100), 313 (11), 312 (11), 311 (69), 310 (17), 217 (24), 215 (35), 152 (4), 95 (6), 77 (5). Anal. calcd for $C_{14}H_{10}Cl_2N_2O_2 \cdot 0.25\ CH_2Cl_2$: C, 51.80; H, 3.20; N, 8.48. Found: C, 51.75; H, 2.94; N, 8.49. The presence of 0.25 mol of dichloromethane in the crystals was confirmed by ^{1}H NMR.

***N*-[Amino(2,4-dichlorophenyl)methylidene]-*N'*-(5-methyl-3-phenyl-4-isoxazolyl)urea (21).** Two millilitres of distilled water were added to a suspension of 1.7 g (7.7 mmol) of **18** and 0.52 g (8.0 mmol) of NaN_3 in 10 mL of acetone. After 16 h the mixture was partitioned between 1,2-dichloroethane and distilled water. The organic phase was washed with brine and then it was dried ($MgSO_4$). The volume of the solution was reduced 50% by evaporation at reduced pressure. The residue was refluxed for 45 min and then the solvent was evaporated at reduced pressure. Kugelrohr distillation (0.1 torr, 125 °C) of the residue gave 230 mg of colorless oil. This oil gave a strong isocyanate peak at 2264 cm^{-1} in its IR spectrum.

A suspension of 200 mg (0.89 mmol) of the amidine **17** in 2 mL of absolute ethanol was treated with 0.57 mL of 1.56 M sodium ethoxide in ethanol. After 5 min 180 mg (0.89 mmol) of the isocyanate was added and the mixture was stirred 18 h. The mixture was diluted with 15 mL of distilled water and the precipitate was collected by filtration. It was washed with three 3 mL portions of distilled water and dried in a stream of air. It was chromatographed on 50 mL of silica gel eluting with 10% EtOAc in CH_2Cl_2. Fractions containing the desired product were combined and the solvent was evaporated at reduced pressure. The solid was dissolved in 5 mL of methanol and precipitated with 10 mL of distilled water. The product was collected by filtration and dried in a stream of air. The product was obtained as 0.185 g of a white powder, mp 112–113 °C. ^{1}H NMR ($CDCl_3$) δ 7.8–7.62 (m, 3H), 7.52–7.30 (m, 7H), 2.47 (s, 3H). MS (ESI^+) for $C_{18}H_{14}Cl_2N_4O_2$ m/z 389 $(M+H)^+$. Anal. calcd for $C_{18}H_{14}Cl_2N_4O_2$: C, 55.54; H, 3.63; N, 14.39; Cl, 18.22. Found: C, 55.29; H, 3.62; N, 14.25.

[2-(2,4-Dichlorophenyl)-1*H*-imidazol-4-yl](phenyl)methanone (23). A solution of 1.57 g (7.69 mmol) of **7**, 1.0 g (7.69 mmol) phenylethynyl ketone, and 50 μL of acetic acid in 16 mL of methanol was stirred in the dark under a nitrogen atmosphere for 20 h. The reaction mixture was partitioned between 75 mL of distilled water and 50 mL of dichloromethane. The aqueous phase was washed with 25 mL of dichloromethane and the combined organic extracts were dried (Na_2SO_4). The solvent was evaporated at reduced pressure and the residue was chromatographed on 1500 mL of silica gel (15–20% ethyl acetate:cyclohexanes). Fractions containing the product were combined to give the addition product as 0.77 g (30%) of a slightly impure orange gum. ^{1}H NMR ($CDCl_3$) δ 8.05 (d, J = 12.0 Hz, 1H), 7.92 (d, J = 7.5 Hz, 2H), 7.60–7.42 (m, 5H), 7.35 (dd, J = 2.0, 8.0 Hz, 1H), 6.83 (d, J = 12.0 Hz, 1H), 5.23 (broad s, 2H).

A mixture of 0.63 g (1.88 mmol) of this gum and 4.0 mL of diphenyl ether were combined under a nitrogen atmosphere. The reaction mixture was immersed in a preheated 250 °C oil bath and stirred for 15 min. The mixture was cooled to 25 °C. The mixture was chromatographed on 100 mL of silica gel (0–20% ethyl acetate in cyclohexane). Fractions corresponding to the more polar of the two major products were combined and the solvent was evaporated at reduced pressure. The residue was dissolved in 20 mL of 3:1 methanol:distilled water and decanted from a small volume of insoluble dark oil. The supernatent was partitioned between distilled water and dichloromethane. The organic phase was dried ($MgSO_4$) and the solvent was evaporated at reduced pressure. The residue was recrystallized from cyclohexane, and the product was collected by filtration to give the title compound as 100 mg (17%) of a tan powder, mp 171.5–174 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.29 (d, J = 8.5 Hz, 1H), 7.95 (d, J = 7.5 Hz, 2H), 7.79 (s, 1H), 7.63 (d, J = 7.5 Hz, 1H), 7.58–7.42 (m, 4H), 7.39 (dd, J = 8.5, 2.0 Hz, 1H). MS (EI) m/z (rel. intensity) 316 (M^+, 100), 318 (65), 317 (23), 241 (28), 239 (42), 186 (19), 184 (29), 145 (56), 105 (31), 77 (43). Anal. calcd for $C_{16}H_{10}Cl_2N_2O$: C, 60.59; H, 3.18; N, 8.83. Found: C, 60.44; H, 3.25; N, 8.58.

***tert*-Butyl 2-{-2-[(*tert*-butoxycarbonyl)amino]ethylidene}-1-hydrazine-carboxylate (25).** To a freshly prepared solution of 2.6 g (16.4 mmol) Boc-glycinal[25,26] in 50 mL of cyclohexane as added 1.94 g (14.7 mmol) of solid *t*-butyl carbazate. The solution was rapidly heated to reflux. After 1 h it was allowed to slowly cool to 25 °C and stirred overnight. The resulting thick suspension was diluted with 75 mL of hexanes and filtered. The product was obtained as 3.5 g (79%) of a white solid, mp 107–108 °C. A sample was dried at 20 torr/60 °C for 16 h prior to elemental analysis. ^{1}H NMR (300 MHz, DMSO) δ 10.53 (s, 1H), 7.16 (broad s, 1H), 7.09 (broad t, J = indet., 1H), 3.62 (broad t, J = indet., 2H), 1.42 (s, 9H), 1.38 (s, 9H). MS (EI) m/z (rel. intensity) 200 (M^+–$(CH_3)_3CO$, 5%), 161 (36), 144 (14), 117 (12), 101 (6), 100 (5), 74 (4), 59 (4), 58 (6), 57 (100), 56 (4). Anal. calcd for $C_{12}H_{23}N_3O_4$ 0.15 C_6H_{12}: C, 54.18; H, 8.74; N, 14.69: Found: C, 54.13; H, 8.50; N, 14.55. The persistence of cyclohexane in the crystal after heating the compound at reduced pressure was confirmed by ^{1}H NMR.

***tert*-Butyl 2-{2-[(*tert*-butoxycarbonyl)amino]ethyl}-1-hydrazinecarboxylate (26).** A mixture of 2.0 g (7.33 mmol) of hydrazone **25**, 0.4 g 10% Pd/C and 75 mL of 95% ethanol was agitated under 50 psi hydrogen until **25**

could no longer be detected by TLC. At this time (ca. 5 h) the mixture was filtered through Celite and the solvent was evaporated from the filtrate at reduced pressure. The residue was chromatographed on silica gel (35–40% ethyl acetate in cyclohexane). The product was obtained as a colorless oil which solidified on standing to give 1.52 g (75%) of a white solid, mp 158–159 °C. A sample was dried at 20 torr/50 °C for 4 h prior to elemental analysis. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.37 (broad s, 1H), 5.23 (broad s, 1H), 3.86 (broad s, 1H), 3.15 (dt, J = 5.5 Hz, indeterminate, 2H), 2.83 (t, J = 5.5 Hz, 2H), 1.39 (s, 9H), 1.37 (s, 9H). MS (FAB) m/z (rel. intensity) 276 (M + H, 57), 275 (16), 220 (41), 176 (13), 164 (100), 163 (29), 120 (34), 57 (78), 41 (13). Anal. calcd for $C_{12}H_{25}N_3O_4$: C, 52.35; H, 9.15; N, 15.26; Found: C, 52.41; H, 9.02; N, 15.22.

***tert*-Butyl 2-benzoyl-2-{2-[(*tert*-butoxycarbonyl)amino]-ethyl}-1-hydrazinecarboxylate (27).** Benzoyl chloride (0.49 mL, 4.22 mmol) was added to a 0 °C solution of 1.16 g (4.22 mmol) **26** and 1.21 mL (15 mmol) pyridine in 50 mL of anhydrous THF. After 1.5 h the mixture was partitioned between 100 mL of dichloromethane and 100 mL of deionized water. The aqueous phase was washed with 10 mL of dichloromethane and the combined organic extracts were washed with 50 mL of 1 M aqueous KH_2PO_4 solution and then with 50 mL of saturated aqueous sodium bicarbonate solution. The solution was dried (Na_2SO_4). The solvent was evaporated reduced pressure. The residue was purified by silica gel chromatography (25–30% ethyl acetate in cyclohexane) followed by recrystallization from 1 mL of dichloromethane and the solution was diluted with 7 mL of hexanes. The product was collected by filtration to give 1.09 g (68%) of a white solid, mp 126–127.5 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48 (d, J = 7.7 Hz, 2H), 7.40–7.25 (m, 3H), 4.0–2.7 (broad, 4H), 1.43 (s, 9H), 1.25 (broad s, 9H). MS (EI) m/z (rel. intensity) 379 (M^+, 0.1), 279 (9), 250 (9), 223 (28), 149 (24), 145 (9), 106 (8), 105 (100), 101 (39), 77 (21), 57 (83). Anal. calcd for $C_{19}H_{29}N_3O_5$: C, 60.14; H, 7.70; N, 11.07. Found: C, 60.26; H, 7.76; N, 11.04.

***N*-(2-Aminoethyl)benzohydrazide dihydrochloride (28).** To a 50 mL round bottom flask loaded with 0.45 g (1.1 mmol) of solid **27** was added 15 mL of 25% methanolic HCl. After stirring for 5 min a heavy white precipitate formed. After 3 additional hours the mixture was diluted with 15 mL of diethyl ether and the solid was collected by filtration. After drying at 20 torr/45 °C the product was obtained as 0.27 g (98%) of a white solid, mp 175–178 °C. ^{1}H NMR (300 MHz, D_2O) δ 7.62–7.45 (m, 5H), 3.91 (broad s, 2H), 3.33 (broad s, 2H). MS (FAB) m/z (rel. intensity) 180 (M + H, 100), 360 (5), 359 (20), 334 (3), 181 (12), 179 (7), 165 (3), 163 (13), 148 (7), 105 (16). Anal. calcd for $C_9H_{13}N_3O \cdot 2\,HCl \cdot 0.7\,H_2O$: C, 40.83; H, 6.24; N, 15.87. Found: C, 40.74; H, 6.33; N, 16.07.

[3-(2,4-Dichlorophenyl)-5,6-dihydro-1,2,4-triazin-1(4*H*)-yl]-(phenyl)methanone (29). A suspension of 0.120 g (0.476 mmol)of **28** and 0.139 g (0.476 mmol) of **16** in 2 mL of anhydrous methanol was treated with 0.279 mL (2.0 mmol) triethylamine. The mixture was stirred 18 h at 25 °C and then 18 h at reflux. The mixture was cooled to 25 °C and partitioned between 15 mL of diethyl ether and 15 mL of water. The organic phase was washed with 15 mL of saturated sodium bicarbonate solution and then with 10 mL of brine. The solution was dried ($MgSO_4$), and the solvent was removed by evaporation at reduced pressure. The residue was chromatographed on silica gel (4–8% absolute ethanol in dichloromethane). Three fractions were obtained having R_f values (silica gel, 6% absolute ethanol in dichloromethane, I_2 visualization) of 0.57, 0.45 and 0.36, respectively. The least polar material was further purified by silica gel chromatography (15% ethyl acetate in dichloromethane). This material was recrystallized from dichloromethane and hexanes. The white solid was collected by filtration and dried in a stream of air to give the title compound as 18 mg (11%) of a white solid having mp 160–162 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, J = 8 Hz, 2H), 7.60–7.20 (m, 7H), 4.10 (broad s, 2H), 3.73 (broad s, 2H). IR 3276 (m), 1615 (s), 1575 (m), 1476 (m), 1450 (m), 1426 (s). MS (FAB) m/z (rel. intensity) 333 (M^+, 40), 338 (12), 337 (16), 336 (66), 335 (43), 334 (100), 332 (11), 330 (11), 105 (54), 77 (7). HRMS (EI) calcd for $C_{16}H_{13}Cl_2N_3O$: 333.0436, found: 333.0441. Anal. calcd for $C_{16}H_{13}Cl_2N_3O$: C, 57.50; H, 3.92; N, 12.57. Found: C, 57.69; H, 3.90; N, 12.43.

(2,4-Dichlorophenyl)(2-phenyl-4-morpholinyl)methanone oxime (31). This compound was prepared using 3-phenylmorpholine in place of methylamine in the procedure for the preparation of **6**. Mp 202–204 °C (dec.). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.79 (d, J = 8.0 Hz, 1H), 7.65–7.25 (m, 7H), 4.65–4.55 (m, 1H), 4.05–3.78 (m, 1H), 3.78–3.65 (m, 1H), 3.65–3.45 (m, 1H), 3.45–3.25 (m, 1H), 3.15–2.97 (m, 1H), 2.97–2.82 (m, 1H). MS (FAB) m/z (rel. intensity) 351 (M + H, 100), 355 (10), 354 (14), 353 (64), 352 (26), 350 (10), 337 (14), 336 (5), 335 (23), 162 (5). Anal. calcd for $C_{17}H_{16}Cl_2N_2O_2 \cdot HCl$: C, 52.67; H, 4.42; N, 7.22. Found: C, 52.67; H, 4.60; N, 7.18.

(2,4-Dichlorophenyl)(6-phenyl-1,2-oxazinan-2-yl)methanimine hydrochloride (33). Tetrahydrooxazine hydrochloride **32** (0.150 g, 0.752 mmol) was dissolved in 15 mL of water and treated with a large excess of 3.0 M sodium hydroxide solution. The free base was extracted into diethyl ether and the solvent was evaporated at reduced pressure. The residue was dissolved in 1.5 mL of methanol and treated with 0.257 g (0.740 mmol) of the isothiouronium hydroiodide salt **13**. After 15 min at 25 °C the reaction mixture was partitioned between pH 10 phosphate buffer and ethyl acetate. the organic phase was dried ($MgSO_4$) and the solvent was evaporated at reduced pressure. The residue was purified by silica gel chromatography (1:1 ethyl acetate:hexanes). The residue was dissolved in 10 mL of isopropanol and treated with 2 mL of 3 N hydrochloric acid. The solvent was evaporated in a stream of nitrogen and the residue was dissolved in 3 mL of ethyl acetate. Diluting this solution with diethyl ether gave a precipitate which was collected by filtration and washed with 2:1 ethyl ether:ethyl acetate. After drying at 70 °C/20 torr for 16 h the product

was obtained as 61 mg (23%) of a white solid, mp 219–220 °C. ^{1}H NMR (DMSO-d_6). The spectrum of this compound suggests that two slowly interconverting conformers are present in approximately equal amounts. The integrations in the peak list have been normalized to a single molecule. δ 9.99 (s, 0.5H), 9.96 (s, 0.5H), 9.55 (s, 0.5H), 9.54 (s, 0.5H), 8.01 (dd, J = 2.0, 6.5 Hz, 1H), 7.94 (d, J = 8.5 Hz, 0.5H), 7.82 (d, J = 6.5 Hz, 0.5H), 7.75 (dd, J = 2.0, 8.5 Hz, 0.5H), 7.73 (dd, J = 2.0, 8.5 Hz, 0.5H), 7.57–7.38 (m, 5H), 5.38 (d, J = 8.8 Hz, 0.5H), 5.16 (d, J = 10.6 Hz, 0.5H), 3.70–3.55 (m, 2H), 2.20–1.75 (m, 4H). MS (FAB) m/z (rel. intensity) 335 (M + H, 100), 671 (3), 669 (2), 340 (2), 339 (11), 338 (13), 337 (66), 336 (21), 301 (2), 131 (10). HRMS (FAB) calcd for $C_{17}H_{16}Cl_2N_2O + H_1$ 335.0718, found 335.0714. Anal. calcd for $C_{17}H_{16}Cl_2N_2O$·HCl: C, 54.93; H, 4.61; N, 7.54. Found: C, 54.58; H, 4.66; N, 7.48.

Acyloxyamidines **34**–**79** were prepared by the general procedures below. Mass spectral data, ^{1}H NMR data and combustion analysis results were compatible with the assigned structures.

Conversion of nitriles to hydroxyamidines (Procedure A). A solution of the nitrile in anhydrous 3.1 M methanolic NH_2OH (2.0 molar equivalents) was heated at reflux overnight. After cooling to room temperature, the solution was concentrated to dryness. The residual solid was dissolved in 3 M hydrochloric acid and the solution was filtered to remove insoluble material. The acid solution was washed with ether and then neutralized with solid K_2CO_3, which caused a solid to precipitate. The solution was allowed to stand for at least 20 min, after which the solid was collected via vacuum filtration and washed with water. The product was dried overnight in a 60 °C vacuum oven. The product obtained was used in the next step without further characterization.

Acylation of hydroxyamidines (Procedure B). To a mixture of a hydroxyamidine (2.44 mmol) and triethylamine (3.0 mmol) in anhydrous tetrahydrofuran (8 mL) was added the appropriate acid chloride (2.44 mmol) all at once. The mixture was stirred 24 h at 25 °C and then it was diluted with an organic solvent (50 mL of ethyl acetate, dichloromethane, or chloroform) and a saturated aqueous solution of sodium bicarbonate (50 mL). The organic phase was washed with brine and then it was dried with magnesium sulfate. The solution was filtered and the solvent was evaporated at reduced pressure. Except where otherwise noted, the following procedure was used to purify the product. The material was dissolved in a minimum volume of warm acetone (typically 15 mL) and cyclohexane was added until the solution became cloudy. The resulting mixture was stored at 5 °C for 12–48 h, and the crystals which formed were collected by filtration. The solid was washed with cyclohexane (5 mL), and dried in a stream of air.

Acylation of hydroxyamidines (Procedure C). This procedure was used in cases in which the required acid chloride is not commercially available. The required acid chloride was prepared from the corresponding carboxylic acid in the following manner. One drop of anhydrous dimethylformamide was added to a mixture of the appropriate carboxylic acid (2.44 mmol), anhydrous methylene chloride (5 mL), and oxalyl chloride (2.44 mmol). Within a few seconds of this addition, gas evolution was observed. The mixture was stirred 4 h, and the resulting clear solution was then added to a solution of a hydroxyamidine (2.44 mmol) and triethylamine (6.0 mmol) in anhydrous tetrahydrofuran (10 mL). The mixture was stirred 24 h at 25 °C, and then the product was isolated as described in Procedure B.

Crystallography

Crystal data for 36. $C_{19}H_{16}N_2O_2$; formula wt. = 304.4; monoclinic; space group $P\ 2_1$ $Z = 2$; $a = 5.194(5)$, $b = 11.115(13)$, $c = 13.209(17)$Å, $\beta = 91.67(6)$, $V = 762(1)$Å^3; calculated density = 1.33 g cm^{-3}; absorption coefficient $\mu = 0.62$ mm^{-1}; clear, thick plate 0.08×0.23×0.23–0.28 mm crystallized from acetone and H_2O and mounted on a glass fiber. The total number of reflections measured was 6649; there were 1624 unique reflections; 1307 had intensities $> 3\sigma$; Rint was 0.056.

Crystal data for 55. $C_{15}H_{12}N_2O_2Cl_2$; formula wt. = 323.2; monoclinic; space group $P\ 21$ /c, $Z = 4$; $a = 5.225(1)$, $b = 21.739(2)$, $c = 13.372(2)$Å, $\beta = 106.08(6)$, $V = 1459(1)$Å^3; calculated density = 1.47 g cm^{-3}; absorption coefficient $\mu = 0.45$ mm^{-1}; clear needle 0.08×0.13×0.62 mm crystallized from acetone and H_2O and mounted on a glass fiber. The total number of reflections measured was 7899; there were 2552 unique reflections; 1754 had intensities $> 3\sigma$; Rint was 0.061.

Crystal data for 48. $C_{18}H_{12}N_2O_2Cl_2$; formula wt. = 359.2; monoclinic; space group $P\ 2_1$, $Z = 2$; $a = 7.275(6)$, $b = 9.559(3)$, $c = 11.449(7)$Å, $\beta = 92.55(9)$, $V = 798(1)$Å^3; calculated density = 1.50 g cm^{-3}; absorption coefficient $\mu = 0.37$ mm^{-1}; clear prism 0.19×0.11×0.4 mm crystallized from chloroform and mounted on a glass fiber. The total number of reflections measured was 7724; there were 1837 unique reflections; 1600 had intensities $> 3\sigma$; Rint was 0.047.

General. Intensity data for all three structures were measured at low temperature, −120 °C, on a Mar imaging plate area detector from Area Detector Systems Corp., Poway, CA with a molybdenum long fine focus sealed tube X-ray source and graphite monochromator, ($\lambda o K$) = 0.7107Å). The resolution was 0.77Å; $2\theta_{max} = 55$. Data from 300 mm images using 5° oscillations in phi at a distance of 100.75 mm were reduced and corrected using the Denzo data processing package.[27] Exposure times were 3 min/image for **48**, and 4 min/image for **36** and **55**. Cell parameters are means and standard deviations of determinations from 36 independent images.

All three structures were solved by direct methods using SHELXS86.[28] Least squares refinements included coordinates for all atoms and anisotropic thermal parameters for nonhydrogen atoms. Temperature factors for

hydrogens were assigned as one-half unit higher than the equivalent isotropic temperature factors for the attached carbon. The function minimized in the refinement was $\Sigma w(Fo^2 - Fc^{*2})^2$, where weights w were $1/\sigma(^2(Fo^2)$ and Fc^{*2} was as defined by Larson,[29] except for **55** (no secondary extinction parameter was considered necessary for that refinement). In the final cycles all shifts were $<0.3\sigma$. Atomic form factors were from Doyle and Turner,[30] and, for hydrogen, from Stewart, Davidson and Simpson.[31] The CRYM[32] system of computer programs was used for the refinements. All three structures exhibit a similar hydrogen bonding pattern involving one hydrogen of the primary amine substituent and the carbonyl oxygen in a symmetry related molecule.

Final agreement parameters for **36** were: $R = 0.050$ for 1623 reflections, (one low angle reflection was given zero weight in the refinement), and 0.040 for the 1307 reflections having $Fo^2 \geq 3\sigma$; standard deviation of fit = 2.0. Translations in x relate hydrogen bonded molecules; N–O distance 2.983(3)Å.

Final agreement parameters for **55** were: $R = 0.073$ for 2550 reflections, (two low angle reflections were given zero weight in the refinement), and 0.047 for the 1754 reflections having $Fo^2 \geq 3\sigma$; standard deviation of fit = 2.0. Translations in x relate hydrogen bonded molecules; N–O distance 2.947(3)Å.

Final agreement parameters for **48** were: $R = 0.041$ for all 1837 reflections, and 0.035 for the 1600 reflections having $Fo^2 \geq 3\sigma$; standard deviation of fit = 1.82. In this case, the primary amine hydrogen forms a bifurcated hydrogen bond. It is approximately equidistant from the carbonyl oxygen and the imino nitrogen in the symmetry related molecule at 1-x, y-0.5, 2.0-z. (N–O distance: 3.018(3) Å; N–N distance: 2.968(3)Å); H–O distance: 2.16(3)Å; H–N distance: 2.28(3)Å.

Biology

The HCMV polymerase inhibition assay was performed using a scintillation proximity assay (SPA). Reactions were performed in 96-well plates. The assay was conducted in 100 μL volume with 5.4 mM HEPES (pH 7.5), 11.7 mM KCl, 4.5 mM $MgCl_2$, 0.36 mg/mL BSA, and 90 nM ^{3}H-dTTP and 2 mM (3-[(3-cholamidopropyl)-dimethylammonio]-1-propane-sulfonate. HCMV polymerase was diluted in enzyme dilution buffer containing 50% glycerol, 250 mM NaCl, 10 mM HEPES (pH 7.5), 100 μg/mL BSA, 0.01% sodium azide and added at 10% (or 10 μL) of the final reaction volume. Compounds were diluted in 50% DMSO and 10 μL were added to each well. Control wells contained an equivalent concentration of DMSO. Reactions were initiated via the addition of 6 nM biotinylated poly(dA)-oligo(dT) template/primer to reaction mixtures containing the enzyme, substrate, and compounds of interest. Plates were incubated in a 25 or 37 °C H_2O bath and terminated via the addition of 40 μL/reaction of 0.5 M EDTA (pH 8) per well. Reactions were terminated within the time-frame during which substrate incorporation was linear (30 min). Ten microlitres of streptavidin–SPA beads (20 mg/mL in PBS/10% glycerol) were added following termination of the reaction. Plates were incubated 10 min at 37 °C, then equilibrated to room temperature, and counted on a Packard Topcount. Linear regressions were performed and IC_{50}'s calculated using computer software.

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