Inhibition of Herpes Simplex Virus Type 1 Ribonucleotide Reductase by Substituted Tetrapeptide Derivatives

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It is known that peptides corresponding to the C-terminus of the small subunit of herpes simplex virus type 1 and 2 ribonucleotide reductase can inhibit enzymatic activity by preventing the association of the enzyme's two subunits. In a quest for smaller, more potent inhibitors, we have conducted a structure activity investigation based on the pentapeptide H-Val-Val-Asn-Asp-Leu-OH. Potency increases of up to 4000 times (IC_{50} 0.18 μM) have been achieved in an enzymatic assay by a combination of modifying the N-terminal valine to a diethylacetyl group, adding a methyl group to the β-carbon of the adjacent valine, dialkylating the asparagine side-chain nitrogen and dimethylating the β-carbon of the aspartic acid residue. In addition the relative contribution of various inhibitor functionalities to inhibitor potency has been investigated.

Introduction

Herpes simplex viruses (HSV-1 and HSV-2) are responsible for a variety of human maladies including genital and oral lesions, ocular diseases, and encephalitis. The majority of antiherpetic agents currently on the market or under development are nucleoside analogues that target the viral DNA polymerase.[1,2] In addition to this enzyme, there is a growing body of evidence supporting the essentiality of HSV ribonucleotide reductase (RR) in viral infectivity.[3-5] Consequently, a selective RR inhibitor is becoming an attractive target for drug development.[6,7]

The enzyme ribonucleotide reductase catalyzes the conversion of ribonucleoside diphosphates into their corresponding 2'-deoxy derivatives, the latter being key intermediates in DNA biosynthesis.[8] Many organisms, including herpes viruses, *E. coli*, and mammals, encode unique ribonucleotide reductases that nonetheless possess a number of common characteristics.[9,10] Two such characteristics include the presence of a stable tyrosyl free radical and the dependency on Fe(III) for catalytic activity. These reductases are also structurally characterized by the presence of two distinct subunits, the smaller (R2) containing the iron and tyrosyl radical and the larger (R1) containing redox active thiols that provide the hydrogen for nucleotide reduction. The association of these two subunits is required for catalytic activity. We are investigating the inhibition of this subunit association as a potential strategy for antiviral therapy.

It is known that the nonapeptide H-Tyr-Ala-Gly-Ala-Val-Val-Asn-Asp-Leu-OH, **1**, which corresponds to the nine C-terminal amino acids of the herpes simplex virus ribonucleotide reductase small subunit, can inhibit HSV RR (IC_{50} 38 μM) by preventing subunit association.[11,12] The significance of this inhibition is highlighted by the inactivity of **1** against a mammalian RR (IC_{50} > 2000 μM). Such selective inhibition is also reported for the nonapeptide H-Asn-Ser-Phe-Thr-Leu-Asp-Ala-Asp-Phe-OH, which corresponds to the nine C-terminal amino acids of the human RR small subunit.[13] This peptide selectively inhibits the human enzyme over the viral enzyme. These results and others[14,15] establish the involvement of the small subunit C-terminus in the association of ribonucleotide reductase subunits. Since there is low sequence homology between human and viral RR C-termini, the nature of subunit association is understandably species specific in this case and thus provides the basis for selective inhibition. One early step in further evaluation of the potential of inhibitors of HSV RR subunit association would be to use nonapeptide **1** as a lead to improve inhibitor potency and reduce inhibitor size.

Investigations by Gaudreau and co-workers demonstrated that truncation of the N-terminus of the lead nonapeptide (**1**) by up to four amino acids still provided inhibitors with measurable inhibitory activity (Table I, compounds **2–6**).[16,17] A similar study of the C-terminus, however, revealed that removal of just one amino acid was highly detrimental (compound **7**). This study suggested that pentapeptide **5** might contain the minimum structural requirements for effective binding to the large subunit, so consequently we undertook an SAR investigation based on an analogue of this peptide in the hope of improving inhibitor efficacy. This report describes our efforts to identify potent substituted tetrapeptide derivatives and to establish the relative importance of various inhibitor pharmacophores.

Results and Discussion

Our earlier investigations with longer peptide based inhibitors revealed that replacement of the asparagine side-chain NH_2 by pyrrolidine increased inhibitor potency approximately 50-fold.[18] This modification provided an important foundation upon which this current structure–activity investigation was based. A comparison between the pentapeptide H-Val-Ile-Asn-Asp-Leu-OH[19] (IC_{50} 610 ± 110 μM) and the pyrrolidine containing derivative **8** (Table II, IC_{50} 13 ± 4 μM) exemplifies the improvement in efficacy obtained upon modifying this region of the molecule. A more in-depth analysis of the structural requirements at this position will be presented later.

Compound **8** was used as a point of reference for an investigation and optimization of the structural requirements at the N-terminal position (valine). As shown in Table II, the terminal amino group is not optimal for activity since its removal (compound **9**) provides a more

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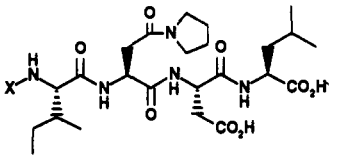
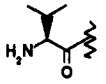
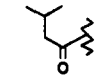
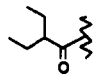
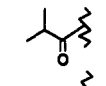
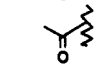
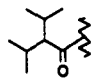
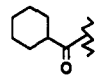
Table I[16]

compd		IC_{50} (μM)
1	H-Tyr-Ala-Gly-Ala-Val-Val-Asn-Asp-Leu-OH	38
2	H-Ala-Gly-Ala-Val-Val-Asn-Asp-Leu-OH	280
3	H-Gly-Ala-Aval-Val-Asn-Asp-Leu-OH	220
4	H-Ala-Val-Val-Asn-Asp-Leu-OH	190
5	H-Val-Val-Asn-Asp-Leu-OH	760
6	H-Val-Asn-Asp-Leu-OH	>2000
7	H-Tyr-Ala-Gly-Ala-Val-Val-Asn-Asp-OH	>2000

Table II

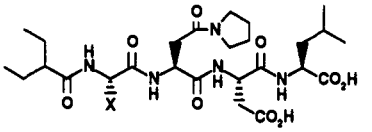


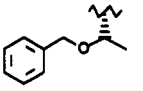
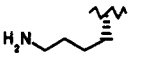
Compound	X	IC_{50} (μM)
8		13 ± 4
9		3.0 ± 0.5
10		1.5 ± 0.1
11		10 ± 1
12		>1000
13		2.0 ± 0.2
14		1.5 ± 0.1

potent inhibitor. A diethylacetyl group, however, is among the best N-terminal acyl substitutions found so far (compound 10). The substantial potency loss on sequential truncation of this diethylacetyl group to a dimethylacetyl group (compound 11) and an acetyl group (compound 12) demonstrates the importance of the size of the N-terminal lipophilic moiety. The exact shape of this lipophilic moiety, however, does not appear critical as indicated by the comparable activities of compounds **9**, **10**, **13**, and **14**. This study suggests that the inhibitor N-terminus may be involved in a strong lipophilic binding interaction with the large subunit.

Since the diethylacetyl group proved to be a better N-terminus than valine, compound **10** became a point of reference for a structure–activity investigation of the adjacent amino acid residue (isoleucine). As illustrated in Table III, there does not appear to be a strong preference for a particular side-chain functional group at this position, since replacement with most amino acid classes produces active inhibitors. However, derivatives with β-branched side chains such as compounds **10**, **17**, **18**, and **21** (IC_{50} 0.6–4.4 μM) appear slightly more potent than those bearing linear side chains such as **15**, **19**, and **20** (IC_{50} 6.1–14 μM). The most effective side chain at this position has the greatest degree of β-substitution, that is, a *tert*-butyl group (compound **21**), while the least effective side chain is the smallest, that is, a hydrogen (compound **16**). These results

Table III



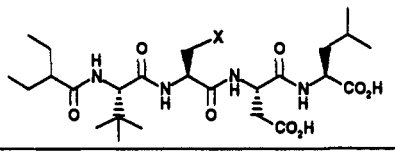
Compound	X	IC_{50} (μM)
10		1.5 ± 0.1
15		11 ± 3
16		77 ± 4
17		3.1 ± 0.4
18		4.4 ± 1.0
19		6.1 ± 1.2
20		14 ± 3
21		0.6 ± 0.1

suggest that the side chain at this position does not directly interact with the large subunit. Conceivably, the more highly β-substituted side chains decrease the conformational flexibility of the molecule and thus favor the inhibitor adopting its bioactive conformation.

As mentioned earlier, a significant enhancement of inhibitor potency can be achieved by replacing the asparagine side-chain NH_2 by pyrrolidine. A more detailed examination of the structural requirements at this position is depicted in Table IV, in which the newly optimized derivative **21** serves as a point of reference. The beneficial effect imparted by alkylation of the side-chain nitrogen is evident (cf. **22** with **21**, **26**, and **27**). Furthermore, the side-chain nitrogen is not essential for activity, since both carbon (cf. **23** and **21**) and oxygen analogues (cf. **28** and **27**) have potencies comparable to their nitrogen counterparts. It also appears from a comparison of **23** to **24** and **21** to **22** that both the carbonyl group and the five-membered ring provide similar contributions to inhibitor potency. However, if these two groups are positioned further from the peptide backbone by an additional methylene (cf. **21** and **25**), potency is dramatically reduced. Exactly how the side-chain carbonyl and the adjacent alkyl functionality contribute to inhibitor potency is not immediately obvious.

An investigation of the adjacent aspartic acid position revealed the considerable contribution of the side-chain carboxyl to inhibitor potency. As shown in Table V, removal of this functionality (cf. **21** and **29**) reduces inhibitor activity by more than a 1000-fold. Even replacement of the carboxylic acid OH by an NH_2 (cf. **21** and **30**) produces a 100-fold reduction in activity. In addition, the position of the carboxyl function appears critical, as indicated by the loss of activity resulting from the use of a longer side chain (cf. **21** and **31**). These

Table IV



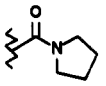
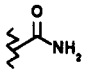
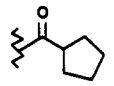
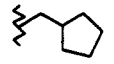
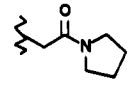
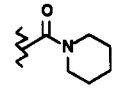
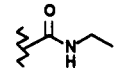
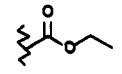
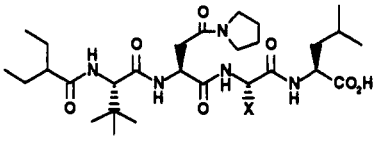
Compound	X	IC_{50} (μM)
21		0.6 ± 0.1
22		100 ± 12
23		1.3
24		64 ± 7
25		>1000
26		1.3 ± 0.3
27		6.9 ± 1.0
28		4.7 ± 1.0

Table V

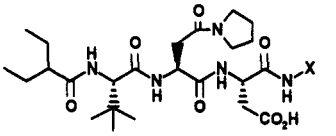


Compound	X	IC_{50} (μM)
21		0.6 ± 0.1
29		>1000
30		77 ± 16
31		>1000
32		0.18 ± 0.04

observations suggest the involvement of the aspartic acid side-chain carboxyl in a specific binding interaction with the RR large subunit. However, despite the apparent inflexibility of this carboxyl side chain to modification, an improvement in inhibitor efficacy can be achieved through geminal dimethylation of the aspartic acid β-carbon (cf. 21 and 32). Compound 32 constitutes the most potent inhibitor prepared in this investigation.

A structure–activity study of the C-terminal residue is shown in Table VI.[20] An appropriate lipophilic group at this end of the molecule is likely critical for activity, since

Table VI



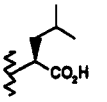
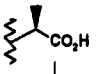
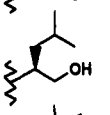
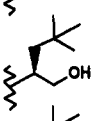
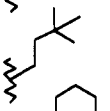
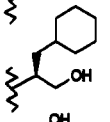
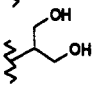
Compound	X	IC_{50} (μM)
21		0.6 ± 0.1
33		>1000
34		5.6 ± 1.6
35		1.9 ± 0.4
36		19 ± 5
37		8.1 ± 2.5
38		>1000

Table VII

	IC_{50} (μM)			IC_{50} (μM)	
compd	HSV-RR	Human-RR	compd	HSV-RR	Human-RR
10	1.5 ± 0.1	>1000	28	4.7 ± 1.0	>1000
18	4.4 ± 1.0	>1000	32	0.18 ± 0.04	>1000
21	0.6 ± 0.1	>1000	35	1.9 ± 0.4	>1000

removal of the leucine isopropyl group in compound 21 causes a severe loss of potency (compound 33). In contrast, the carboxylic acid function appears less critical, since reduction of the acid to an alcohol (cf. 21 and 34) or complete removal of the carboxyl group (cf. 21 and 36) still provides inhibitors with measurable activity (i.e., IC_{50} < 1000 μM). A 3-fold increase in inhibitor potency, however, can be obtained by the addition of an extra methyl to the terminal lipophilic group (cf. 34 and 35). These results suggest the involvement of the C-terminus in a lipophilic binding interaction and perhaps a less significant hydrogen bonding interaction with the RR large subunit. Moreover, the observation that the C-terminal carboxyl is not essential for inhibitor activity can be exploited to help reduce the overall inhibitor peptidic character.

To verify the selectivity of these smaller and more potent HSV ribonucleotide reductase inhibitors for HSV RR, a number of the most potent derivatives were tested against human RR.[6] As shown in Table VII, all the compounds tested clearly demonstrated specificity for HSV ribonucleotide reductase.

Conclusion

The structure–activity investigation described herein culminates in the identification of a substituted tetrapeptide derivative, compound 32, which is approximately 200 times more potent against HSV ribonucleotide reductase than the lead nonapeptide 1 and is more than 4000 times

more active than pentapeptide **5**. This accomplishment and the selectivity of these potent peptide derivatives for HSV RR over human RR, constitute an important first step in the design of inhibitors of HSV subunit association as potential antiherpes compounds.

Experimental Section

Ribonucleotide Reductase Inhibition Assays. The inhibitory effect of our peptide derivatives on HSV-1[16] and human[13] ribonucleotide reductases was measured according to published protocols. The reported IC_{50} values are the mean of at least three separate determinations, and the standard deviation from the mean is also reported. Compound **23** was only evaluated once.

Materials: *N*-Boc-L-amino Acids and Amino Alcohol Derivatives. Common *N*-Boc-L-amino acids were obtained from commercial sources with the following protected side chains: Asp-β-OBn, Glu-γ-OBn, Thr-OBn, and Lys-ϵ-NCbz. The C-terminal *N*-Boc-amino acids Leu and Ala were incorporated with their respective carboxylic acid groups protected as benzyl esters. *N*-Boc amino alcohols were prepared from the corresponding *N*-Boc-L-amino acid by reduction[21] and protection of the resultant alcohol as a benzyl ether.[22] *N*-Boc-L-cyclohexylalanine was obtained by catalytic hydrogenation of *N*-Boc-L-phenylalanine.[23] *N*-Boc-2(*S*)-amino-4-cyclopentyl-4-oxobutanoic acid[24] (amino acid residue in compound **23**) and *N*-Boc-β,β-dimethyl-D,L-aspartic acid[25] were prepared according to literature procedures. *N*-Boc-L-Asp-βOEt was prepared from commercial *N*-Boc-Asp-αOBn by esterification with ethyl iodide[26] and subsequent hydrogenolysis of the benzyl ester.

Preparation of Boc-2(*R*/*S*)-amino-4-cyclopentylbutanoic Acid. A mixture of 3-cyclopentylpropanal (0.88 g, 7.0 mmol), KCN (0.49 g, 7.6 mmol), and ammonium carbonate (1.83 g, 19.1 mmol) in 50/50 ethanol–water (30 mL) was stirred at 60 °C for 24 h. The ethanol was removed under vacuum, and the resultant aqueous solution was acidified to pH 2 with concentrated HCl. The resultant white solid was collected by filtration, washed with water, and dried under vacuum (0.85 g, 62%). The hydantoin product (0.50 g, 2.6 mmol) was refluxed in aqueous NaOH (15 mL, 0.7 M) for 23 h, after which the reaction mixture was concentrated to approximately 5 mL, and a solution of di-*tert*-butyldicarbonate (0.73 g, 3.3 mmol) in THF (20 mL) was added. After 2 h, the THF was removed under vacuum, the residue was diluted with water (30 mL), and the mixture was washed with ether (2×). The aqueous phase was acidified to pH 2 with 1 N aqueous HCl and then extracted 2× with ethyl acetate. Drying ($MgSO_4$), filtration, and concentration of the combined ethyl acetate layers afforded the *N*-Boc amino acid as a white solid (0.53 g, 75%): 400 MHz ^{1}H NMR ($CDCl_3$) δ 4.97 (d, J = 7.5 Hz, 1 H), 4.31–4.26 (m, 1 H), 1.91–1.35 (m, 11 H), 1.45 (s, 9 H), 1.12–1.03 (m, 2 H).

Preparation of Boc-2(*S*)-amino-4-pyrrolidino-4-oxobutanoic Acid. This procedure is applicable to the preparation of the related amino acid residues embodied in compounds **25**, **26**, and **27**. L-*N*-BOC-Asp-αOBn (47.6 g, 0.147 mol) was suspended in acetonitrile (500 mL) and 1,1′-carbonyldiimidazole (24.3 g, 0.150 mol) was added in small portions under a nitrogen atmosphere (caution: vigorous evolution of carbon monoxide). After 45 min, the reaction mixture was cooled to 0 °C, pyrrolidine (13.4 mL, 0.160 mol) was added dropwise, and the resulting mixture was stirred for 3 h. The solvent was evaporated under vacuum, and the residue was dissolved in ethyl acetate (500 mL). This solution was washed successively with 1 N aqueous HCl (3 × 100 mL), 1 N aqueous NaOH (2 × 100 mL) and brine (100 mL), dried ($MgSO_4$), filtered, and concentrated. The crude product was dissolved in methanol (400 mL) containing 20% $Pd(OH)_2$ (500 mg) and stirred under 1 atm of H_2 for 20 h. The reaction mixture was filtered through Celite, and the solvent was removed under vacuum to provide a white solid, which was crystallized from ethyl acetate–hexane (37 g, 88%): 400 MHz ^{1}H NMR ($CDCl_3$) δ 5.84 (br d, J = 6 Hz, 1 H), 4.50–4.45 (m, 1 H), 3.61–3.48 (m, 3 H), 3.46–3.38 (m, 1 H), 3.15 (dd, J = 17, 2 Hz, 1 H), 2.65 (dd, J = 9.5, 17 Hz, 1 H), 2.10–1.89 (m, 4 H), 1.44 (s, 9 H).

Inhibitor Synthesis. All inhibitors were prepared by solution phase peptide synthesis, in which *N*-Boc-amino acid derivatives were coupled sequentially from C- to N-terminus by using benzotriazol-1-yl-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) as the coupling agent and subsequent removal of the *N*-Boc protective group was effected by 6 N HCl in dioxane. The N-terminal acyl groups were also incorporated by using TBTU as the coupling agent. The following procedure is representative. To a solution of *N*-Boc-amino acid (1 mmol) in dry acetonitrile (2.5 mL) was added TBTU (1 mmol) and *N*-methylmorphine (1 mmol). After approximately 5 min, this solution was added to a solution of amino acid or peptide hydrochloride salt (1 mmol) in dry acetonitrile (2.5 mL) containing *N*-methylmorpholine (2 mmol). The reaction mixture was stirred at ambient temperature for 2–6 h (reaction monitored by TLC) and then poured into a mixture of ethyl acetate (50 mL) and saturated aqueous sodium bicarbonate (50 mL). The organic phase was washed with another portion of sodium bicarbonate, 1 N aqueous HCl (2 × 50 mL), and brine (50 mL). Drying ($MgSO_4$), filtration, and concentration provided the peptide, usually of sufficient purity, to continue to the next step without further purification. *N*-Boc peptide derivatives could be purified if necessary by conventional flash chromatography. The *N*-Boc-peptide product (1 mmol) was then treated with 6 N HCl in dioxane (5 mL) for 30 min. The solvent was removed under vacuum, and the resulting hydrochloride salt was subjected to high vacuum before its use in the next coupling reaction.

Subsequently, the fully protected peptide derivatives were purified by flash chromatography, and the various benzyl-related protective groups were removed by catalytic hydrogenolysis by using 10% Pd/C (10 mol %) in methanol and 1 atm of H_2 for 3 h. The resultant inhibitor was usually obtained in greater than 95% purity (HPLC and NMR), but if necessary it could be purified by preparative HPLC on a C18 reverse-phase column (Vydac, 15 μm particle size) eluting with 0.06% aqueous TFA–0.06% TFA in acetonitrile gradients.

The Boc-2-amino-4-cyclopentylbutanoic acid and *N*-Boc-β,β-dimethyl aspartic acid used in the preparation of inhibitors **24** and **32**, respectively, were incorporated as racemates. The diastereomers obtained on coupling these racemic amino acid derivatives to their appropriate chiral partner were separated by flash chromatography after removal of the *N*-Boc group. Both diastereomers were elaborated to the corresponding final peptide. For both **24** and **32**, only one of each pair of diastereomers proved to have measurable inhibitory activity. The active dias-

tereomer presumably had the configuration corresponding to the native amino acid stereochemistry.

Inhibitor Characterization and Purity. All peptide derivatives showed satisfactory 400 MHz ^{1}H NMR spectra, FAB mass spectra ($M^+ + H$) and/or ($M^+ + Na$), amino acid analysis including peptide recovery, and HPLC purity (>95%). A full tabulation of this data is available as supplementary material.

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Supplementary Material Available: Full tabulation of ^{1}H NMR, FAB mass spectra, amino acid analysis, and HPLC purity data for peptide derivatives (8 pages). Ordering information is given on any current masthead page.

References

(1) Herpes Simplex Virus, Infectious Disease Study #3. *Infectious Disease*, a Pharmacor Service, Decision Resources Inc.: USA, March 1992.
(2) Dunn, C. D. R. A review of recent developments in the chemotherapy of viral infections. *Scrip's Antiviral Rep.* **1991**, 9–61.
(3) Idowu, A. K.; Fraser Smith, E. B.; Poffenberger, K. L.; Herman, R. C. Deletion of the herpes simplex virus type 1 ribonucleotide reductase gene alters virulence and latency in vivo. *Antiviral Res.* **1992**, *17*, 145–146.
(4) Jacobson, J. G.; Leib, D. A.; Goldstein, D. J.; Bogard, C. L.; Schaffer, P. A.; Weller, S. K.; Coen, D. M. A herpes simplex virus ribonucleotide reductase deletion mutant is defective for productive acute and reactivatable latent infections of mice and for replication in mouse cells. *Virology* **1989**, *173*, 276–283.
(5) Cameron, J. M.; McDougall, I.; Marsden, H. S.; Preston, V. G.; Ryan, M. D.; Subak-Sharpe, J. H. Ribonucleotide reductase encoded by herpes simplex virus is a determinant of the pathogenicity of the virus in mice and a valid antiviral target. *J. Gen. Virol.* **1988**, *69*, 2607–2612.
(6) Spector, T.; Harrington, J. A.; Porter, D. J. T. Herpes and human ribonucleotide reductases Inhibition by 2-acetylpyridine 5-[(2-chloroanilino)thiocarbonyl]thiocarbonohydrazone (348U87). *Biochem. Pharmacol.* **1991**, *42*, 91–96.
(7) Spector, T. Ribonucleotide reductase encoded by herpesviruses: inhibitors and therapeutic considerations. In *Inhibitors of Ribonucleoside Reductase Activity*; Cory, J. G., Cory, A. H., Eds.; Pergamon Press Inc.: 1989; pp 235–243.
(8) Cory, J. G. Role of ribonucleotide reductase in cell division. In *Inhibitors of Ribonucleoside Reductase Activity*; Cory, J. G., Cory, A. H., Eds.; Pergamon Press Inc.: 1989; pp 1–17.
(9) Lammers, M.; Follman, H. The ribonucleotide reductases: a unique group of metalloenzymes essential for cell proliferation. *Struct. Bonding* **1983**, *54*, 27–91.
(10) Ingemarson, R.; Lankinen, H. The herpes simplex virus type 1 ribonucleotide reductase is a tight complex of the type composed of 40 K and 140 K proteins, of which the latter shows multiple forms due to proteolysis. *J. Virology* **1987**, *156*, 417–422.
(11) Dutia, B. M.; Frame, M. C.; Subak-Sharpe, J. H.; Clarke, W. N.; Marsden, H. S. Specific inhibition of herpes virus ribonucleotide reductase by synthetic peptides. *Nature* **1986**, *321*, 439–441.
(12) Cohen, E. A.; Gaudreau, P.; Brazeau, P.; Langelier, Y. Specific inhibition of herpes simplex virus ribonucleotide reductase activity by a nonapeptide derived from the carboxyl terminus of subunit 2. *Nature* **1986**, *321*, 441–443.
(13) Cosentino, G.; Lavallée, P.; Rakhit, S.; Plante, R.; Gaudette, Y.; Lawetz, C.; Whitehead, P. W.; Duceppe, J.-S.; Lépine-Frenette, C.; Dansereau, N.; Guilbault, C.; Langelier, Y.; Gaudreau, P.; Thelander, L.; Guindon, Y. Specific inhibition of ribonucleotide reductases by peptides corresponding to the C-terminal of their second subunit. *Biochem. Cell Biol.* **1991**, *69*, 79–83.
(14) Paradis, H.; Gaudreau, P.; Brazeau, P.; Langelier, Y. Mechanism of inhibition of herpes simplex virus (HSV) ribonucleotide reductase by a nonapeptide corresponding to the carboxyl terminus of its subunit 2. Specific binding of a photoaffinity analog, [4′-azido-Phe^6] HSV2-(6-15), to subunit 1. *J. Biol. Chem.* **1988**, *263*, 16045–16050.
(15) Liuzzi, M.; Scouten, E.; Ingermarson, R. Inhibition of herpes simplex virus ribonucleotide reductase by synthetic nonapeptides: a potential antiviral therapy. In *Proceedings of the American Society of Microbiology: Innovations in Antiviral Development and Detection of Viral Infections*; Walsh, L., Block, T. M., Crowell, R. L., Jungkind, D. L., Eds.; Plenum Publishing Corporation: New York, in press.
(16) Gaudreau, P.; Paradis, H.; Langelier, Y.; Brazeau, P. Synthesis and inhibitory potency of peptides corresponding to the subunit 2 C-terminal region of herpes virus ribonucleotide reductase. *J. Med. Chem.* **1990**, *33*, 723–730.
(17) Gaudreau, P.; Brazeau, P.; Richer, M.; Cormier, J.; Langlois, D.; Langelier, Y. Structure–function studies of peptides inhibiting the ribonucleotide reductase activity of herpes simplex virus type 1. *J. Med. Chem.* **1992**, *35*, 346–350.
(18) An analogous observation has been reported for hexa- and substituted pentapeptide inhibitors. Chang, L. L.; Hannah, J.; Ashton, W. T.; Rasmusson, G. H.; Ikeler, T. J.; Patel, G. F.; Garsky, V.; Uncapher, C.; Yamanaka, G.; McClements, W. L.; Tolman, R. L. Substituted penta- and hexapeptides as potent inhibitors of herpes simplex virus type 2 ribonucleotide reductase. *BioMed. Chem. Lett.* **1992**, *2*, 1207–1212.
(19) The pentapeptide containing Ile at the fourth amino acid from the C-terminus was used as a direct comparison point instead of pentapeptide **5**. Our earlier SAR with longer peptide inhibitors was based on the varicella zoster virus C-terminal sequence because the VZC C-terminal nonapeptide was found to be more potent against HSV RR than the corresponding HSV nonapeptide **1**.[16] However, the first five amino acids of both C-termini differ only by the presence of Ile for Val at the fourth amino acid from the C-terminus.
(20) For synthetic simplicity, compound **21** was used as a point of reference for structural modification of the C-terminal amino acid residue instead of the more potent derivative **32**. The use of **32** would have required the repeated use of racemic β,β-dimethylaspartic acid.
(21) Valerio, R. M.; Alewood, P. F.; Johns, R. B. Synthesis of optically active 2-(*tert*-butoxycarbonylamino)-4-dialkoxyphosphorylbutanoate protected isosteres of *O*-phosphonoserine for peptide synthesis. *Synthesis* **1988**, 786–789.
(22) Freedman, H. H.; Bubois, R. A. An improved Williamson ether synthesis using phase transfer catalysis. *Tetrahedron Lett.* **1975**, 3251–3254.
(23) Boger, J.; Payne, L. S.; Perlow, D. S.; Lohr, N. S.; Pol, M.; Blaine, E. H.; Ulm, E. H.; Schorn, T. W.; LaMont, B. I.; Lin, T.-Y.; Kawai, M.; Rich, D. H.; Verber, D. F. Renin inhibitors. Synthesis of subnonomolar, competitive, transition-state analogue inhibitors containing a novel analogue of statine. *J. Med. Chem.* **1985**, *28*, 1779–1790.
(24) Aubry, N.; Plante, R.; Deziel, R. A simple synthesis of γ and δ-keto α-amino acid derivatives. *Tetrahedron Lett.* **1990**, *31*, 6311–6312.
(25) Bochenska, M.; Biernat, J. F. Aminodicarboxylic acids. Part XI. A simple method for preparation of β-branched aspartic acids. *Roczniki Chem.* **1976**, *50*, 1195–1199.
(26) Moore, G. G.; Foglia, T. A.; McGahan, T. J. Preparation of hindered esters by the alkylation of carboxylate salts with simple alkyl halides. *J. Org. Chem.* **1979**, *44*, 2425–2429.