at 48 h, T_0 is the cell number for test wells at time zero (normally 10^5), C_{48} is the number for the control at 48 h, and C_0 is the number for the control at time zero (normally, $T_0 = C_0 = 10^5$ cells/mL). Compounds exhibiting an $ED_{50} > 5$ μg/mL are considered to be inactive in this screen.

In Vivo P388 Screen. Compounds were tested for their ability to prolong the survival of $B_6D_2F_1$ mice (six per group) receiving an implant of P388 mouse leukemia cells (10^6 cells in 0.2 mL saline) ip on Day 0. The test sample was constituted in saline/ethanol (6:1, v/v) and injected ip (20 mg/kg) on Days 1-5 (0.2 mL). The mice were observed until death, and the mean survival times were compared to those of the controls receiving the same number of cells at Day 0 and sterile saline (0.2 mL) for the five following days. Significant in vivo antineoplastic activity is ascribed to compounds exhibiting a %T/C > 125.

Transport Studies. The transport of 2'-deoxyuridine (DU), (*E*)-5-(2-iodovinyl)-2'-deoxyuridine (**4b**), **11a**, **12a**, **13**, **14**, and **16** were determined with fresh murine erythrocytes exposed to various concentrations of extracellular test nucleoside and [6-^{3}H]thymidine. The concentration of [6-^{3}H]thymidine in the extracellular fluid at various times after this exposure was determined by counting aliquots by liquid-scintillation counting on a Beckman LS9000 or Searle Mark III counter. These procedures and the interpretation of results have been described in detail elsewhere.[17,18] The results are summarized in Table I.

Acknowledgment. We are grateful to the Medical Research Council of Canada (Grant MT-5965) (L.I.W. and E.E.K.) and the National Cancer Institute of Canada (T.M.A.) for financial support of this work, and to the Alberta Heritage Foundation for Medical Research for a postdoctoral fellowship (R.K.) and graduate studentship (L.X.).

Registry No. **4b**, 69304-48-9; **7c**, 1024-99-3; **8c**, 123881-85-6; **9b**, 121563-64-2; **9c**, 99394-52-2; **10a**, 55520-67-7; **10b**, 123881-86-7; **10c**, 55520-64-4; (*R*)-**11a**, 123881-87-8; (*S*)-**11a**, 123881-88-9; (*R*)-**11b**, 123881-89-0; (*S*)-**11b**, 123881-90-3; (*R*)-**11c**, 123881-91-4; (*S*)-**11c**, 123881-92-5; (*R*)-**12a**, 123881-93-6; (*S*)-**12a**, 123881-94-7; (*R*)-**12b**, 123881-95-8; (*S*)-**12b**, 123881-96-9; (*R*)-**12c**, 123881-97-0; (*S*)-**12c**, 123881-98-1; (*R*)-**13**, 123881-99-2; (*R*)-**13** 3',5'-dibenzoate, 123882-03-1; (*S*)-**13**, 123882-00-8; (*S*)-**13** 3',5'-dibenzoate, 123882-04-2; (*R*)-**14**, 123882-01-9; (*S*)-**14**, 123882-02-0; (*R*)-**16**, 123882-05-3; (*S*)-**16**, 123882-06-4; ethyl acrylate, 140-88-5.

Synthesis and Inhibitory Potency of Peptides Corresponding to the Subunit 2 C-Terminal Region of Herpes Virus Ribonucleotide Reductases[1]

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H-Tyr^{329}-Ala^{330}-Gly^{331}-Ala^{332}-Val^{333}-Val^{334}-Asn^{335}-Asp^{336}-Leu^{337}-OH, the C-terminal end of herpes simplex virus ribonucleotide reductase subunit 2 (HSV R2), specifically inhibits viral enzyme activity by interacting with subunit 1 (HSV R1). In a previous structure–activity study, we identified four sites on the nonapeptide where the inhibitory potency could be modulated: a minimum active core 333–337, a spacer segment 330–332, and the N- and C-termini. To further explore the structural features of HSV R2-(329–337) that are required to obtain a potent inhibition, a series of analogues comprising modifications in these four regions were synthesized by solid-phase methodology. Changes in the segment 333–337 of the molecule decreased the inhibitory potency by more than 2-fold, except for the Ile^{334} substitution, which resulted in a 1.5-fold increase in potency. Replacement of Tyr^{329} by other aromatic or aliphatic amino acids diminished the nonapeptide activity from 1.4-fold to 5.9-fold. The spacer segment contributed to enhance potency. Modification with amino acids that could induce conformational changes, such as Pro or D-Ala, generated compounds with a similar or lower activity, respectively. Amidation or amino acyl addition at the carboxylic end was detrimental while acylation of the N-terminus was generally beneficial for the inhibitory potency. Disubstitution in position 332 and 334 by Thr and Ile, which are present in the C-terminal portion of varicella-zoster virus ribonucleotide reductase subunit 2, resulted in a peptide that is 4.0 times more potent than HSV R2-(329-337), while each monosubstitution alone generated peptides with 150% of the activity of HSV R2-(329-337) nonapeptide. These results indicate a synergistic effect of the disubstitution which confers to this analogue physicochemical properties enhancing its ability to interact with its R1 binding site.

Numerous herpesviruses, such as herpes simplex types I and II (HSV-1 and HSV-2), Epstein–Barr virus (EBV), varicella-zoster virus (VZV), pseudorabies virus (PRV), and equine herpesvirus type I (EHV-1), induce ribonucleotide reductase (RR) activities.[2-7] This enzyme, formed by the association of two nonidentical subunits (R1 and R2), catalyzes the reduction of ribonucleoside diphosphates to their deoxy forms and plays an important role in viral replication.[8-10] Cohen et al.[11,12] and Dutia et al.[13] demonstrated that a synthetic nonapeptide, H-Tyr^{329}-Ala^{330}-Gly^{331}-Ala^{332}-Val^{333}-Val^{334}-Asn^{335}-Asp^{336}-Leu^{337}-OH (HSV R2-(329–337)), corresponding to the RR enzyme's subunit 2 carboxyl terminus, specifically and reversibly inhibits HSV-1, HSV-2, and PRV RR activities. Studies on the mechanism of action of HSV R2-(329–337) have shown that it impedes binding of the RR subunits by interacting with R1,[14,15] thus impairing enzymatic activity. Previously, we determined the minimum active segment of this pep-

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(1) Symbols and abbreviations are in accord with the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (Eur. J. Biochem. 1984, 158, 9). All optically active amino acids are of the L configuration, unless otherwise specified. Additional abbreviations used are: Ac, acetyl; β-Ala, 3-aminopropionic acid; desamino-Tyr, 3-(4-hydroxyphenyl)-propionic acid; OMe, methoxy.

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0022-2623/90/1833-0723$02.50/0

tide and a number of structural requirements necessary to induce, in vitro, a potent inhibition of RR activity.[16] We are now presenting the solid-phase synthesis of these peptides and their purification and characterization along with the inhibitory potency of a new series of analogues. The synthesis includes monosubstitutions in the minimum active core region, in position 329, in the spacer region 330–332, modifications and elongation at the C- and N-termini, and monosubstitutions of the nonapeptide corresponding to the C-terminus of the VZV R2 subunit.

Results and Discussion

Peptides **1–54** were synthesized by solid-phase methodology originally described by Merrifield.[17] The scheme of synthesis, deprotection, and cleavage were based on *t*-Boc chemistry/acid-labile amino acid protecting groups. Final side chain deprotection and cleavage of peptides from resins were accomplished with HF. Yield of synthesis derived by weight-gain determination was superior to 90% for all the peptides and yield after cleavage was greater than 85%, except for peptides **37**, **47**, and **48–50** (≃60%). Amino acid analysis after acidic hydrolysis of the HF-treated resin of the latter peptides showed a peptide content lower than 10%, thus suggesting that the cleavage procedure was effective and was not accountable for the low recovery. The poor solubility of peptides **37**, **49**, and **50** in aqueous acidic solutions could, however, account for their low yield.

Purification, accomplished in one step by reverse-phase high-performance liquid chromatography (HPLC), yielded homogeneous products as determined in various TLC and HPLC solvent systems (Table I). Amino acid analysis of the pure peptides after acidic (data not shown) and enzymatic hydrolysis (Table II) confirmed their theoretical composition. Leucine aminopeptidase (LAP) digestion allowed the precise quantification of Asn, Val, and Ile content in several peptides bearing a free amino terminus and the detection of potential β-aspartyl residues, since LAP does not hydrolyse β-amino acids.

Inhibition of HSV-1 RR activity was evaluated for all peptides shown in Table III in the presence of 1 mM bacitracin, a concentration maintaining a high level of HSV R2-(329–337) throughout the assay period. Compounds **2–10** represent C- and N-terminal fragments of HSV R2-(329–337). As reported previously,[16] removal of Tyr^{329} drastically decreased the inhibitory potency (10% of that of compound 1). Deletions of Ala^{332}, Val^{333}, and Val^{334} gave a pentapeptide (**5**) with 5% of the activity of compound 1 and an inactive tetrapeptide (**6**) and tripeptide (**7**). N-terminal fragments 329–334 (**8**), 329–335 (**9**), and 329–336 (**10**) were inactive at a concentration of 2 mM, indicating that a single deletion at the nonapeptide's C-terminus is sufficient to reduce the activity beyond detection limits. Hence, the minimum active core of HSV R2-(329–337) corresponds to H-Val^{333}-Val^{334}-Asn^{335}-Asp^{336}-Leu^{337}-OH in our in vitro enzymatic assay.

The monosubstitution of these amino acids by Ala resulted in inactive compounds at up to 1 mM (**11**, **14–16**) or a weakly active (**12**) analogue. In addition, although a hydrophobic character seemed an important determinant of activity in the portion 333–334 of compound **1**, steric hindrance may also play a role. Indeed, compound **13**, where Val^{334} was substituted for Leu^{334}, exhibited 41% of the activity of compound **1**. Compounds **17–20**, which represent substitutions in position 337 by less or more hindered aliphatic and aromatic amino acids, such as Val, Ile, Phe, and Tyr, indicated that only isosteric moieties could be substituted for Leu^{337} to preserve inhibitory potency. These observations, together with those on fragments and Ala substitutions, pinpoint the importance of a hydrophobic core (Leu^{337} and Val^{333}-Val^{334}) for interaction of HSV R2-(329–337) with the subunit R1.

Another series of HSV R2-(329–337) analogues was designed to assess the importance of the phenolic hydroxyl function in position 329. Compouds **21** and **22**, in which the tyrosyl residue was substituted by an aliphatic amino acid (Ala, Leu), showed a reduced inhibitory potency (21–26% of **1**) which was almost as drastic as the deletion of tyrosine (**2**). The Leu^{329} analogue has a slightly greater activity than the Ala^{329} analogue, suggesting that an increase in hydrophobicity might be advantageous. This was further documented by [Phe^{329}]HSV R2-(329–337) (**23**), which retained 61% of the nonapeptide activity. Substitution of the phenyl ring by a heterocycle such as imidazole (**24**) resulted in a peptide with only 17% of the activity of compound **1**. This indicates that the aromatic character in position 329 is important for activity and that introduction of an electron-deficient heteroatom may create physicochemical changes that modify conformational requirements for maximal activity. A substitution in position 329 by the D isomer (**25**) reduced the potency of 30% of that of compound **1**, suggesting that a specific orientation of Tyr^{329} has to be maintained for high activity. Interestingly, a free hydroxyl on Tyr^{329} is not essential for inhibitory activity, since the methoxylated analogue (**26**) retained 68% of the activity of compound **1**. However, it contributed to an increase in potency.

The importance of the chain length between Tyr^{329} and the active core 333–337 was then verified with compounds **27–33**. Our results with **27** confirmed data reported by Dutia et al.[13] and suggest that, in addition to a specific hydrophobic character, a critical distance is required between Tyr^{329} and Ala^{332} to induce maximum activity. This was confirmed by compound **28**, which lacked Ala^{330} and Gly^{331} but gave an identical loss of potency with compound **27**. Interestingly, results with **28** also suggest that beyond a critical distance between these two amino acids, no drastic effect is observed. The introduction of a C8 alkyl spacer to replace Ala^{330}, Gly^{331}, and Ala^{332} generated a peptide (**29**) which had 70% of the activity of peptide **1**, indicating that a specific distance has to be maintained between Tyr^{329} and the minimum active core to obtain favorable orientation at the binding site on the R1 subunit. Replacement of Ala^{330} by its D isomer produced a weak analogue (**30**), retaining 30% of the HSV R2-(329–337) activity. Compound **31**, in which Ala^{330} was replaced by a β-alanyl residue, exhibited an activity reduced to 60% of that of compound **1**. Hence, structural changes induced at the portion 330–332 of peptide **1** do not seem to be favorable. Surprisingly, replacement of Gly^{331} or Ala^{332} by

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Table I. Physicochemical Data

no.	MW	% overall yield	TLC[a] I	TLC[a] II	TLC[a] III	TLC[a] IV	HPLC t_R, min	HPLC homogeneity (214 nm/280 nm)
1	921	65	0.55	0.83	0.65		16.6,[b] 26.7,[c] 24.5,[d] 16.9,[e] 18.4[f]	99.0/99.4,[b-f] 98.8/99.1[e]
2	841	55	0.42	0.69	0.55		22.7,[c] 11.0[e]	97.1,[c,g] 98.4[e,g]
3	771	71	0.46	0.77	0.55		22.2,[c] 10.9[e]	97.0,[c] 96.1[e]
4	715	82	0.51	0.80	0.58		21.6,[c] 12.0[e]	96.2,[c] 95.1[e]
5	559	88	0.52	0.73	0.61		17.3,[c] 5.1[e]	96.5,[c] 96.2[e]
6	459	100	0.47	0.66	0.48		13.2,[c] 1.9[e]	99.6,[c] 97.7[e]
7	360	36	0.36	0.63	0.33		6.9,[c] 1.1[e]	98.9,[c] 99.9[e]
8	579	64	0.45	0.84	0.73		19.0,[c] 15.0[e]	97.9/98.4,[c] 98.3/98.6[e]
9	693	28	0.48	0.77	0.41		16.4,[c] 14.5[e]	95.7/96.3,[c] 98.2/98.1[e]
10	808	42	0.32	0.63	0.46		17.3,[c] 11.1[e]	96.5/98.8,[c] 98.1/95.0[e]
11	893	21	0.54	0.71			13.5,[b] 15.8[e]	99.9/99.8,[b] 97.7/99.2[e]
12	935	31	0.61	0.81	0.64		19.5,[b] 19.5[e]	98.8/100,[b] 98.9/96.8[e]
13	878	63	0.53	0.77	0.68		19.4,[b] 20.0[e]	99.9/97.7,[b] 99.5/100[e]
14	877	50	0.56	0.78	0.70		17.0,[b] 23.4[e]	99.6/99.0,[b] 98.9/96.2[e]
15	879	44	0.28	0.60	0.51		7.9,[b] 25.0[e]	98.6/96.9,[b] 96.9/96.6[e]
16	907	43	0.45	0.73			11.4,[b] 14.8[e]	96.5/100,[b] 98.7/99.0[e]
17	921	47	0.58	0.71			14.7,[b] 16.3[e]	97.7/99.5,[b] 98.2/95.0[e]
18	955	57	0.60	0.79			17.3,[b] 17.8[e]	99.2/96.9,[b] 97.9/95.0[e]
19	971	61	0.52	0.84			11.8,[b] 15.7[e]	99.4/100,[b] 98.5/98.9[e]
20	829	36	0.36	0.60	0.53		12.9,[b] 14.3[e]	100,[b] 98.1[e]
21	871	28	0.59	0.80	0.67		15.9,[b] 19.9[e]	96.0,[b] 99.4[e]
22	905	23	0.56	0.80			18.0,[b] 20.0[e]	99.6,[b] 98.0[e]
23	940	13	0.59	0.58			19.3,[b] 19.5[e]	99.7,[b] 98.3[e]
24	921	37	0.51	0.75	0.67		9.1,[b] 12.4[e]	98.9/100,[b] 99.0/99.0[e]
25	935	35	0.60	0.83	0.67		20.6,[b] 21.7[e]	99.4/99.2,[b] 94.0/95.5[e]
26	793	31	0.62	0.84	0.60		17.3,[b] 18.1[e]	98.0/100,[b] 97.6/98.9[e]
27	850	15	0.59	0.84	0.60		16.5,[b] 18.0[e]	96.6/99.3,[b] 98.0/99.2[e]
28	922	29	0.65	0.85			21.4,[b] 22.6[e]	99.0/99.1,[b] 99.1/99.3[e]
29	921	27	0.53	0.76	0.68		18.3,[b] 18.6[e]	98.0/100,[b] 99.4/97.3[e]
30	921	37	0.45	0.71	0.64		16.4,[b] 19.5[e]	97.7/98.4,[b] 100/96.2[e]
31	961	69	0.59	0.81			16.0,[b] 17.4[e]	100/99.3,[b] 99.0/100[e]
32	947	44	0.59	0.80			14.8,[b] 17.2[e]	99.8/99.7,[b] 98.3/100[e]
33	893	26	0.44	0.66	0.62		14.0,[b] 16.0[e]	100/99.7,[b] 98.3/100[e]
34	920	80	0.57	0.82	0.70		14.7,[b] 22.7[e]	96.8/95.8,[b] 99.0/100[e]
35	1049	39	0.40	0.65			11.5,[b] 20.6[e]	99.2/100,[b] 100/98.1[e]
36	963	81	0.65	0.87		0.74	23.3,[b] 18.4[e]	99.6/99.2,[b] 99/100[e]
37	961	33	0.62	0.89	0.73		21.6,[b] 23.8[e]	97.4/97.9,[b] 100/100[e]
38	601	49	0.63	0.84		0.75	16.8,[b] 11.0[e]	97.0,[b] 98.9[e]
39	892	27	0.63	0.81			23.4,[b] 19.3[e]	98.9/98.9,[b] 99.4/100[e]
40	975	14	0.66	0.87			24.7,[b] 22.4[e]	98.0/100,[b] 97.8/98.5[e]
41	835	31	0.72	0.89			24.1,[b] 18.5[e]	96.9/97.5,[b] 99.0/98.0[e]
42	963	40	0.56	0.78	0.73		24.5,[b] 19.8[e]	98.2/97.7,[b] 99.4/97.4[e]
43	963	35	0.56	0.79	0.72		23.1,[b] 18.8[e]	98.9/97.7,[b] 99.7/97.9[e]
44	906	67	0.68	0.87	0.76		26.3,[b] 20.7[e]	96.7/95.0,[b] 95.3/94.4[e]
45	1061	22	0.62	0.83			20.1,[b] 23.2[e]	99.5/90.1,[b] 97.1/96.5[e]
46	1049	33	0.39	0.67			12.4,[b] 19.6[e]	99.2/99.0,[b] 99.1/99.0[e]
47	1196	22			0.60	0.68	30.0,[c] 18.3[e]	99.5/100,[c] 95.0/95.3[e]
48	1585	14			0.60	0.63	29.7,[c] 19.3[e]	95.5/95.5,[c] 95.2/95.9[e]
49	2094	8					30.4,[f] 27.2[e]	95.6/97.0,[f] 95.0/95.1[e]
50	1063	17	0.43	0.85	0.63	0.64	14.6,[d] 22.3[e]	98.2/97.9,[d] 99.0/99.0[e]
51	965	35	0.60	0.83	0.71		19.4,[b] 20.6[e]	99.8/99.4,[b] 98.6/98.5[e]
52	950	60	0.74	0.85			24.7,[b] 18.9[e]	95.8/96.1,[b] 99.2/98.5[e]
53	935	52	0.59	0.79	0.69		18.2,[b] 19.9[e]	99.6/96.4,[b] 99.0/98.1[e]
54	951	36	0.59	0.76	0.69		15.4,[b] 17.1[e]	99.7/100,[b] 100/97.6[e]

[a] Whatman silica gel 150A LK5DF glass plates, 0.22 mm thickness. Solvent systems: I, 1-butanol–water–acetic acid (4:1:1); II, 1-butanol–ethyl acetate–water–acetic acid (1:1:1:1); III, ethyl acetate–pyridine–water–acetic acid (5:5:3:1); IV, 1-butanol–pyridine–water–acetic acid (5:4:3:1). [b] Column: μ Bondapak C18 column (150 × 38 mm), 10 μ particles. Conditions: linear gradient, solvent A consisted of 0.01% aqueous TFA (pH 2.9) and solvent B consisted of CH_3CN–0.01% TFA, 0.75% B/min for 20 min, initial condition 10% B, flow rate 1.5 mL/min, 23 °C. [c] As in *b* except 0.83% B/min for 30 min, initial condition 0% B. [d] As in *b* except 0.67% B/min for 30 min, initial condition 20% B. [e] Column: as in *b*; conditions: linear gradient, solvent A consisted of 0.01 M aqueous NH_4OAc (pH 6.9) and solvent B consisted of CH_3CN, 0.83% B/min for 30 min, initial condition 0% B, flow rate 1.5 mL/min, 23 °C. [f] As in *b* except 0.75% B/min for 40 min, initial condition 10% B, 23 °C. [g] When only one value is given, it was determined at 214 nm.

a prolyl residue (compounds **32** or **33**), which could induce a conformational constraint, did not drastically affect the inhibitory potency of compound **1**. These results could indicate that the conformation of HSV R2-(329–337) at the R1 binding site is similar to that of analogue **32** or **33**.

Modifications or extension at the C- or N-terminus of HSV R2-(329–337) were also evaluated. Amidation of the carboxylic end of compound **1** (**34**) or its extension by a lysyl residue (**35**) was detrimental, reducing their activity to 32% and 12%, respectively. Lysine was chosen to study the effect of such an extension with the objective of subsequently enhancing HSV R2-(329–337) penetration in vitro or in vivo by coupling the nonapeptide to lysyl polymers.[18] The results indicate that the HSV R2-(329–337) binding site on R1 does not accommodate either an

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Table II. Quantitative Amino Acid Analysis after Leucine Aminopeptidase Digestion

no.	Asp[a,b]	Asn	Gln	Thr	Ser	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe	His	Lys	Arg	% rec
1	0.98 (1)	0.94 (1)				0.99 (1)	2.05 (2)	2.06 (2)			1.02 (1)	0.96 (1)					100
2	0.99 (1)	1.00 (1)				0.96 (1)	1.97 (2)	2.04 (2)			1.04 (1)						100
3	0.98 (1)	1.00 (1)				0.94 (1)	1.00 (1)	2.06 (2)			1.01 (1)						100
4	1.05 (1)	0.99 (1)					0.92 (1)	2.05 (2)			0.98 (1)						100
5	1.04 (1)	0.99 (1)						1.97 (2)			1.00 (1)						100
6	1.11 (1)	0.98 (1)						0.78 (1)			1.13 (1)						88
7	0.93 (1)	0.99 (1)									1.07 (1)						96
8						1.03 (1)	1.96 (2)	1.92 (2)				1.08 (1)					100
9		0.98 (1)				0.98 (1)	2.02 (2)	1.93 (2)				1.09 (1)					94
10	0.92 (1)	0.95 (1)				0.99 (1)	2.04 (2)	2.06 (2)				1.04 (1)					100
11	1.09 (1)	0.96 (1)				0.99 (1)	2.97 (3)	1.05 (1)			0.98 (1)	0.95 (1)					100
13	1.03 (1)	0.89 (1)				1.03 (1)	1.89 (2)	1.08 (1)			2.02 (2)	1.06 (1)					100
14	0.89 (1)					1.00 (1)	2.97 (3)	2.10 (2)			1.04 (1)	1.00 (1)					93
15		0.79 (1)				1.03 (1)	3.08 (3)	2.11 (2)			1.05 (1)	1.01 (1)					100
16	0.97 (1)	0.99 (1)				0.96 (1)	3.00 (3)	2.08 (2)				0.99 (1)					100
17	0.99 (1)	0.98 (1)				0.94 (1)	2.00 (2)	2.93 (3)				1.16 (1)					89
18	1.00 (1)	1.00 (1)				1.07 (1)	2.00 (2)	2.02 (2)		0.95 (1)		0.96 (1)					99
19	0.95 (1)	0.98 (1)				0.97 (1)	2.03 (2)	2.07 (2)				0.98 (1)	1.00 (1)				96
20	0.97 (1)	1.05 (1)				0.95 (1)	1.94 (2)	2.11 (1)				1.98 (2)					94
21	1.00 (1)	0.98 (1)				1.00 (1)	3.00 (3)	2.00 (2)			1.02 (1)						93
22	1.08 (1)	0.99 (1)				0.99 (1)	1.89 (2)	2.03 (2)			2.02 (2)						100
23	1.03 (1)	0.96 (1)				1.01 (1)	1.85 (2)	2.02 (2)			1.07 (1)		1.06 (1)				100
24	0.95 (1)	1.01 (1)				1.07 (1)	1.85 (2)	2.02 (2)			1.10 (1)			1.00 (1)			92
28	1.10 (1)	0.96 (1)					0.89 (1)	2.11 (2)			1.05 (1)	0.99 (1)					96
27	1.06 (1)	0.99 (1)				1.00 (1)	1.00 (1)	2.07 (2)			1.02 (1)	0.97 (1)					84
11	1.04 (1)	1.00 (1)				0.98 (1)	2.93 (3)	1.05 (1)			1.01 (1)	0.99 (1)					95
34	0.92 (1)	1.02 (1)				0.96 (1)	2.04 (2)	2.11 (2)			0.99 (1)	1.02 (1)					92
35	1.00 (1)	0.92 (1)				1.05 (1)	2.03 (2)	1.99 (2)			0.99 (1)	1.05 (1)			0.97 (1)		89
51	1.18 (1)	1.00 (1)		0.83 (1)		1.02 (1)	0.90 (1)	1.04 (1)		1.03 (1)	1.13 (1)	1.04 (1)					79
53	0.99 (1)	1.00 (1)				0.88 (1)	1.77 (2)	0.97 (1)		1.15 (1)	1.27 (1)	0.97 (1)					100
54	1.06 (1)	1.03 (1)		0.89 (1)		1.08 (1)	1.11 (1)	2.20 (2)			0.79 (1)	0.83 (1)					100

[a] Cation-exchange resin Waters column (0.40 × 25 cm); flow rate, 0.4 mL/min; temperature, 62 °C; detection, fluorescence (*o*-phthaldehyde). Elution program consisted of (1) linear gradient; solvent A contained 0.2 N sodium, pH 3.05, and solvent B contained 0.2 N sodium, pH 9.60; 1.67% /min for 60 min; initial condition 0% B and (2) isocratic elution; 100% B for 30 min. [b] Leucine aminopeptidase–peptide 1:10 (w/w), 37 °C, 48 h.

extra amino acid bearing a free amino group or a carboxamide function. However, substitutions with other amino acids in position 338 will be needed to demonstrate that Leu[329] must be the last amino acid at the C-terminus. Acetylation of the N-terminus of HSV R2-(329–337) and its analogues resulted in inhibitory activity changes of various magnitudes. It increased the potency of compounds **36–40** 2–3-fold but had little effect on compounds **41–43**, suggesting that, when properly oriented, the C-terminus benefits from an increase in hydrophobicity. This was further supported by the results obtained with compounds **44** and **45**, where Tyr[329] was replaced by a less (desaminotyrosyl) or more (8-aminooctanoyl) hydrophobic residue. These two peptides respectively were 1.8 and 3.6 times more potent than nonapeptide **1**. Addition of a lysyl residue at this end of the molecule (**46**) did not drastically affect the inhibitory potency (82% of compound **1**) as it did at the C-terminus. Extension of the nonapeptide HSV R2-(329–337) at the N-terminus to generate a dodecapeptide (**47**), pentadecapeptide (**48**), and nonadecapeptide (**49**) corresponding to the native C-terminus of the HSV R2 subunits increased its activity 1.4–3.6-fold. We have not investigated if this greater biological activity, especially of compound **49**, is related to an increased number of charged groups and/or hydrophobic residues or to a conformation favoring exposure of residues critical for the interaction on the R1 subunit. However, in a previous report,[19] it was shown that the elongation of peptide **1** by a sole seryl residue was sufficient to increase the inhibitory potency 2-fold, indicating that the nature of the specific amino acid added may influence the activity.

Finally, a series of peptides representing the C-terminus of EBV (**50**) and VZV R2 subunits (**51**), along with analogues of the VZV nonapeptide (**52–54**), were tested on HSV-1 RR activity. Compound **50** possessed 33% and compound **51** possessed 400% of HSV R2-(329–337) activity. In compound **51**, replacement of Tyr[298] by a 3-(4-hydroxyphenyl)propionyl residue (**52**) did not increase the inhibitory potency, as it did for compound **1**. Monosubstitution on compound **51** (Thr[332] for Ala and Ile[334] for Val) produced peptides (**53**, **54**) with 1.5 times the activity of compound **1**. Our data indicate that the disubstitution in the native VZV R2 nonapeptide has a synergistic effect. Replacement of an alanine by a threonyl residue concurrently with the substitution of a valine by an isoleucyl residue may have favorably increased the amphypathy.[20] These results also suggest that the R1 binding site for R2 C-terminal nonapeptides is homologues for the three herpes reductases.

In conclusion, this structure–activity study on peptides corresponding to the subunit 2 C-terminal region of viral RRs has elucidated the chemical requirements for a potent inhibition of HSV RR activity (peptides **2**, **15**, and **51**). Efforts should now be directed toward conformational studies and molecular modeling to help design peptides or peptidomimetics[21] that possess a higher affinity for their specific binding site on the HSV R1 subunit and a greater inhibitory potency.

Experimental Section

Materials. *p*-Methylbenzhydrylamine resin (0.55 mequiv of amine/g), *p*-chloromethyl-poly(styrene-*co*-divinylbenzene 1%) resin (0.65 mequiv of chlorine/g) and N^{α}-*tert*-butyloxycarbonyl (Boc) protected amino acid derivatives were purchased from Bio-Mega Inc. Amino acid reactive side chains were protected as follows: Asp, β-*O*-Bzl; Glu, λ-*O*-Bzl; Thr, *O*-Bzl; Ser, *O*-Bzl; Cys, *S*-4-MeBzl; Tyr, *O*-2-Br-Z; His, N^{im}-Tos; Lys, N^{ϵ}-2-Cl-Z; Arg, N^{g}-Tos. Prior to use, all amino acid derivatives were tested for purity by thin-layer chromatography (TLC) and melting point determination. N^{α}-Boc-8-aminooctanoic acid was prepared by acylation of 8-aminooctanoic acid (Aldrich Chemicals) by means of di-*tert*-butyl dicarbonate.[22,23] Reagent-grade 2-propanol and

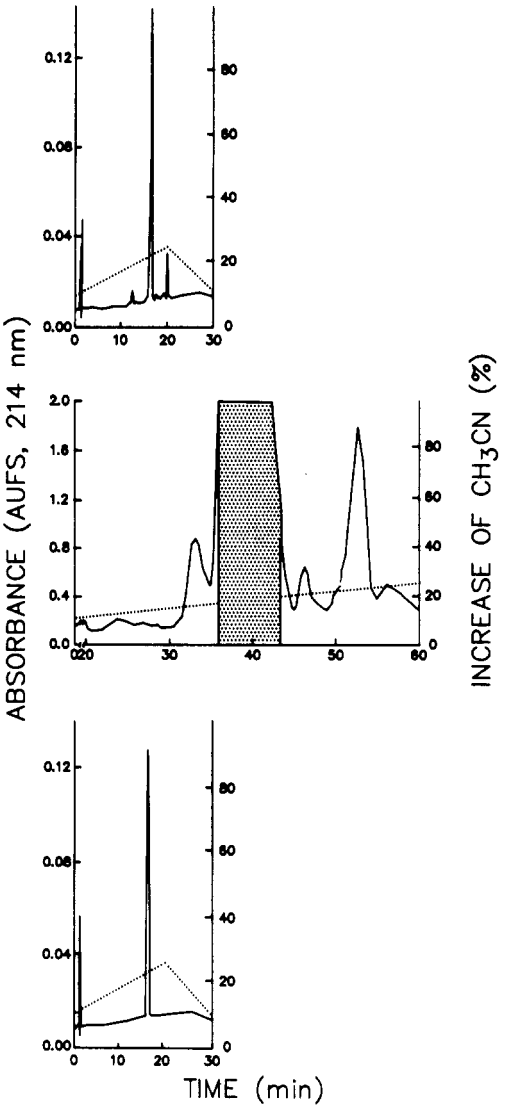

Figure 1. Analytical and preparative chromatographic profiles of HSV R2-(329–337) detected at 214 nm. Top panel, 30 μg of the post HF peptide was subjected to analysis on a C18 μ Bondapak column (150 mm × 38 mm) using a linear gradient. Solvent A consisted of 0.01% aqueous TFA (pH 2.9) and solvent B consisted of CH_3CN–0.01% TFA [0.75% B/min for 20 min, initial condition 10% B, flow rate 1.5 mL/min (1700 psi back pressure)]. Middle panel, 1.09 g of the post HF peptide was purified on a C18 Vydac column (30 cm × 5.7 cm) using a linear gradient. Solvent A consisted of 0.1% TFA (pH 1.9) and solvent B consisted of CH_3CN–0.1% TFA [0.25% B/min for 60 min, initial condition 10% B, flow rate 75 mL/min (684 psi back pressure)]. The dotted area represents homogeneous peptide fractions. Bottom panel, 25 μg of purified HSV R2-(329–337) were analyzed according to the conditions described in the top panel and showed a purity of 99.0%.

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Table III. Inhibitory Potency of Peptides Located at the C-terminus of HSV, EBV, and VZV Subunit 2 Ribonucleotide Reductases

no.	compound	primary structure	IC_{50},[a] μM	% RI[b]
1	HSV R2-(329–337)	Tyr-Ala-Gly-Ala-Val-Val-Asn-Asp-Leu	36–60	100[c]
2	HSV R2-(330–337)	Ala-Gly-Ala-Val-Val-Asn-Asp-Leu	283 (36)[d]	13[c]
3	HSV R2-(331–337)	Gly-Ala-Val-Val-Asn-Asp-Leu	225 (36)	16[c]
4	HSV R2-(332–337)	Ala-Val-Val-Asn-Asp-Leu	190 (36)	19[c]
5	HSV R2-(333–337)	Val-Val-Asn-Asp-Leu	760 (36)	5[c]
6	HSV R2-(334–337)	Val-Asn-Asp-Leu		0[c,e]
7	HSV R2-(335–337)	Asn-Asp-Leu		0[c,e]
8	HSV R2-(329–334)	Tyr-Ala-Gly-Ala-Val-Val		0[c,e]
9	HSV R2-(329–335)	Tyr-Ala-Gly-Ala-Val-Val-Asn		0[c,e]
10	HSV R2-(329–336)	Tyr-Ala-Gly-Ala-Val-Val-Asn-Asp		0[c,e]
11	[Ala^{333}]HSV R2-(329–337)	Tyr-Ala-Gly-Ala-Ala-Val-Asn-Asp-Leu		0[c,e]
12	[Ala^{334}]HSV R2-(329–337)	Tyr-Ala-Gly-Ala-Val-Ala-Asn-Asp-Leu	760 (60)	8[c]
13	[Leu^{334}]HSV R2-(329–337)	Tyr-Ala-Gly-Ala-Val-Leu-Asn-Asp-Leu		41
14	[Ala^{335}]HSV R2-(329–337)	Tyr-Ala-Gly-Ala-Val-Val-Ala-Asp-Leu		0[c,f]
15	[Ala^{336}]HSV R2-(329–337)	Tyr-Ala-Gly-Ala-Val-Val-Asn-Ala-Leu		0[c,f]
16	[Ala^{337}]HSV R2-(329–337)	Tyr-Ala-Gly-Ala-Val-Val-Asn-Asp-Ala		0[c,f]
17	[Val^{337}]HSV R2-(329–337)	Tyr-Ala-Gly-Ala-Val-Val-Asn-Asp-Val	846 (55)	6.5
18	[Ile^{337}]HSV R2-(329–337)	Tyr-Ala-Gly-Ala-Val-Val-Asn-Asp-Ile	153 (55)	36
19	[Phe^{337}]HSV R2-(329–337)	Tyr-Ala-Gly-Ala-Val-Val-Asn-Asp-Phe		0[f]
20	[Tyr^{337}]HSV R2-(329–337)	Tyr-Ala-Gly-Ala-Val-Val-Asn-Asp-Tyr		0[f]
21	[Ala^{329}]HSV R2-(329–337)	Ala-Ala-Gly-Ala-Val-Val-Asn-Asp-Leu	280 (60)	21[c]
22	[Leu^{329}]HSV R2-(329–337)	Leu-Ala-Gly-Ala-Val-Val-Asn-Asp-Leu	211 (55)	26
23	[Phe^{329}]HSV R2-(329–337)	Phe-Ala-Gly-Ala-Val-Val-Asn-Asp-Leu	90 (55)	61
24	[His^{329}]HSV R2-(329–337)	His-Ala-Gly-Ala-Val-Val-Asn-Asp-Leu	323 (55)	17
25	[D-Tyr^{329}]HSV R2-(329–337)	D-Tyr-Ala-Gly-Ala-Val-Val-Asn-Asp-Leu	200 (50)	30[c]
26	[Tyr^{329}(OMe)]HSV R2-(329–337)	Tyr(OMe)-Ala-Gly-Ala-Val-Val-Asn-Asp-Leu	88 (60)	68[c]
27	[Tyr^{330}]HSV R2-(330–337)	Tyr-Gly-Ala-Val-Val-Asn-Asp-Leu	330 (60)	18[c]
28	[Tyr^{331}]HSV R2-(331–337)	Tyr-Ala-Val-Val-Asn-Asp-Leu	340 (60)	18[c]
29	[octanoyl$^{330-332}$]HSV R2-(329–337)	Tyr-C8-Val-Val-Asn-Asp-Leu	63 (44)	70
30	[D-Ala^{330}]HSV R2-(329–337)	Tyr-D-Ala-Gly-Ala-Val-Val-Asn-Asp-Leu	200 (60)	30[c]
31	[β-Ala^{330}]HSV R2-(329–337)	Tyr-β-Ala-Gly-Ala-Val-Val-Asn-Asp-Leu	100 (60)	60[c]
32	[Pro^{331}]HSV R2-(329–337)	Tyr-Ala-Pro-Ala-Val-Val-Asn-Asp-Leu	37 (44)	119
33	[Pro^{332}]HSV R2-(329–337)	Tyr-Ala-Gly-Pro-Val-Val-Asn-Asp-Leu	48 (44)	92
34	HSV R2-(329–337) amide	Tyr-Ala-Gly-Ala-Val-Val-Asn-Asp-Leu-NH_2	190 (60)	32[c]
35	[Lys^{338}]HSV R2-(329–338)	Tyr-Ala-Gly-Ala-Val-Val-Asn-Asp-Leu-Lys	458 (55)	12
36	Ac-HSV R2-(329–337)	Ac-Tyr-Ala-Gly-Ala-Val-Val-Asn-Asp-Leu	20 (60)	300[c]
37	Ac-[Tyr^{329}(OMe)]HSV R2-(329–337)	Ac-Tyr(OMe)-Ala-Gly-Ala-Val-Val-Asn-Asp-Leu	40 (60)	150[c]
38	Ac-HSV R2-(329–337) amide	Ac-Tyr-Ala-Gly-Ala-Val-Val-Asn-Asp-Leu-NH_2	76 (60)	79[c]
39	Ac-[Tyr^{330}]HSV R2-(330–337)	Ac-Tyr-Gly-Ala-Val-Val-Asn-Asp-Leu	150 (60)	40[c]
40	Ac-HSV-(333–337)	Ac-Val-Val-Asn-Asp-Leu	400 (60)	15[c]
41	Ac-[Tyr^{331}]HSV R2-(331–337)	Ac-Tyr-Ala-Val-Val-Asn-Asp-Leu	330 (60)	18[c]
42	Ac-[D-Tyr^{329}]HSV R2-(329–337)	Ac-D-Tyr-Ala-Gly-Ala-Val-Val-Asn-Asp-Leu	165 (60)	36[c]
43	Ac-[D-Ala^{330}]HSV R2-(329–337)	Ac-Tyr-D-Ala-Gly-Ala-Val-Val-Asn-Asp-Leu	230 (60)	26[c]
44	[desamino-Tyr^{329}]HSV R2-(329–337)	desamino-Tyr-Ala-Gly-Ala-Val-Val-Asn-Asp-Leu	33 (60)	182[c]
45	[8-aminooctanoyl^{328}]HSV R2-(328–337)	H_2N-C8-Tyr-Ala-Gly-Ala-Val-Val-Asn-Asp-Leu	12 (44)	360
46	[Lys^{328}]HSV R2-(328–337)	Lys-Tyr-Ala-Gly-Ala-Val-Val-Asn-Asp-Leu	68 (55)	82
47	HSV R2-(326–337)	Ser-Thr-Ser-Tyr-Ala-Gly-Ala-Val-Val-Asn-Asp-Leu	29 (60)	207[c]
48	HSV R2-(323–337)	Glu-Cys-Arg-Ser-Thr-Ser-Tyr-Ala-Gly-Ala-Val-Val-Asn-Asp-Leu	42 (60)	183[c]
49	HSV R2-(319–337)	Thr-Asp-Phe-Phe-Glu-Cys-Arg-Ser-Thr-Ser-Tyr-Ala-Gly-Ala-Val-Val-Asn-Asp-Leu	15 (55)	360
50	EBV R2-(294–302)	Tyr-Thr-Met-Leu-Val-Val-Asp-Asp-Leu	110 (36)	33[c]
51	VZV R2-(298–306)	Tyr-Ala-Gly-Thr-Val-Ile-Asn-Asp-Leu	15 (60)	400[c]
52	[desamino-Tyr^{298}]VZV R2-(298–306)	desamino-Tyr-Ala-Gly-Thr-Ile-Asn-Asp-Leu	13 (42)	350
53	[Ala^{301}]VZV R2-(298–306)	Tyr-Ala-Gly-Ala-Val-Ile-Asn-Asp-Leu	33 (49)	148
54	[Val^{303}]VZV R2-(298–306)	Tyr-Ala-Gly-Thr-Val-Val-Asn-Asp-Leu	29 (44)	152

Footnotes to Table III

Results are expressed in percentage of the activity obtained in control experiments without peptide and represents the mean of two [(EBV-R2-(294-302), HSV R2-(323-337)] or four (others) determinations that varied less than 10% with each other. Specific activity of the RR preparations were 18.1, 21.6, and 30.0 units/mg. [a] IC_{50}, concentration of peptide producing 50% of the maximal inhibition. [b] RI, relative inhibition in percentage compared to HSV R2-(329-337). [c] Taken from Gaudreau, P.; Michaud, J.; Cohen, E.; Langelier, Y.; Brazeau, P. *J. Biol. Chem.* **1987**, *262*, 12413. [d] Respective IC_{50} of HSV R2-(329-337), in each experiment. [e] Inactive at 2 mM. [f] Inactive at 1 mM.

Table IV. Schedule of Operation for Solid-Phase Synthesis of HSV R2-(329-337) and Analogues with Preformed Symmetrical Anhydrides

step	operation	solvent or reagent	mixing time, min	repetition
1	wash	CH_2Cl_2	2	3
2	prewash	TFA-CH_2Cl_2 (40:60)	5	1
3	deprotection	TFA-CH_2Cl_2 (40:60)	25	1
4	wash	CH_2Cl_2	2	3
5	wash	CH_2Cl_2	2	3
6	wash	2-propanol-CH_2Cl_2 (50:50)	3	1
7	wash	2-propanol	3	2
8	wash	CH_2Cl_2	3	2
9	prewash	DIEA-CH_2Cl_2 (5:95)	1	1
10	neutralization	DIEA-CH_2Cl_2 (5:95)	5	1
11	wash	CH_2Cl_2	2	3
12	coupling	preformed symmetrical anhydrides 2-3 equiv,[a,b] except for Val^{334}, 5 equiv)	60-180	1
13	wash	CH_2Cl_2	2	2
14	wash	2-propanol	2	2
15	wash	CH_2Cl_2	2	2

[a] Asn^{335} was coupled with DCC/HOBT. [b] 4-Methylmorpholine (0.5 equiv) was added when a second coupling was needed, 30 min after beginning of the coupling.

methylene chloride (CH_2Cl_2) were bought from Anachemia Canada Inc. CH_2Cl_2 was distilled from anhydrous sodium carbonate. *N,N*-diisopropylethylamine (DIEA) and 4-methylmorpholine (Aldrich Chemicals) were distilled from ninhydrin and subsequently kept at 4 °C. *N,N*-dimethylformamide (DMF) (Anachemia Canada Inc.) was distilled from ninhydrin, in vacuo, after a 3-day storage over 4-A molecular sieves and kept under an argon atmosphere. Anisole was distilled and kept at 4 °C. Trifluoroacetic acid (TFA) (Halocarbon Products Co.) and acetic acid (HOAc) (Anachemia Canada Inc.) were distilled prior to use. *N,N'*-dicyclohexylcarbodiimide (DCC), acetic anhydride (Ac_2O) (Aldrich Chemicals), and accusolv-grade methanol (MeOH) and acetonitrile (CH_3CN) (Anachemia Canada Inc.) were used without further purification.

Peptide Synthesis. Merrifield resin was coupled to C-terminus-protected amino acid, by using the cesium salt method of Gisin.[24] The degree of substitution was determined by the picric acid colorimetric test[25] or by quantitative amino acid analysis after acidic hydrolysis (12 N HCl-propionic acid (1:1), containing 0.1% phenol at 150 °C, 90 min) and varied between 0.23 (Boc-Val) and 0.64 (Boc-Asp (*β-O*-Bzl) mmol of amino acyl/g. Compounds **1-54** were prepared by automated solid-phase synthesis (Vega 250) as described in Table IV. Couplings were achieved by preformed symmetrical anhydrides, except for Boc-Asn, which was coupled with DCC/1-hydroxybenzotriazole (HOBT) and desamino-Tyr, which was introduced as its pentafluorophenyl ester.[26,27] Their completion was ascertained by a ninhydrin colorimetric test.[28] Boc protecting groups were removed with TFA-CH_2Cl_2 (40:60) containing 1% D,L-methionine (w/v) when Boc-Met was incorporated in the growing peptide. This was followed by neutralization with DIEA-CH_2Cl_2 (5:95). After completion of the synthesis and removal of the last Boc group, peptides **36-43** were N-acetylated with Ac_2O. Deprotection of the amino acid side chains and cleavage of the peptides from the resin were performed with anhydrous hydrogen fluoride-anisole (9:1, v/v; 10 mL/g of peptide-resin intermediate) at −15 °C for 30 min and then at 0 °C for 30 additional min. When Met was present in the peptide chain, 0.5% D,L-methionine (w/v) was added to the reaction mixture. HF-anisole removal was done in vacuo followed by precipitation of the crude peptides with peroxide-free anhydrous ethyl ether and solubilization with 20% aqueous HOAc. Solutions were lyophilized to yield amorphous powders.

Peptide Purification and Physicochemical Characterization. The crude peptides were submitted to analytical HPLC to optimize the purification procedure. Then, a sample load ranging from 100 mg to 1.0 g was subjected to preparative HPLC on a Partisil 10 ODS-3 Whatman (10 μm particle size) column (2.2 cm × 50 cm) or on a Vydac C18 (15 μm particle size) column (5.7 cm × 30 cm), using a binary solvent system consisting of 0.01 M aqueous ammonium acetate (NH_4OAc), pH 6.9, and CH_3CN for peptides **36-44**, **50**, and **52** and of aqueous 0.01% TFA, pH 2.9, and 0.01% TFA-CH_3CN for the others. The concentration of TFA was increased to 0.1% in both mobile phases, when large samples were purified. A flow rate of 7.0-8.0 mL/min was used with the Whatman column, while 75 mL/min was used with the Vydac column. A typical chromatographic run for HSV R2-(329-337), using the Whatman column, was as follows: 0.16% B/min for 60 min, initial condition 10% B, flow rate 8.0 mL/min. A chromatographic profile of the same peptide, using the Vydac column, is shown in Figure 1 (middle panel). Elution of the peptides was monitored at 214 and/or 280 nm. Collected fractions were readily screened by analytical HPLC and pooled accordingly. The peptides thus obtained were subjected to rotary evaporation, in vacuo, to remove CH_3CN and then lyophilized twice. Purified peptides were analyzed for homogeneity by TLC and HPLC in appropriate solvent systems (Table I and Figure 1, bottom panel) and for amino acid composition by amino acid analysis after acidic hydrolysis (data not shown) and leucine amino peptidase digestion[26] (Table II).

HSV-1 Ribonucleotide Reductase Assay. The effect of synthetic peptides on HSV-1 RR activity was determined as previously described[11,29] using HSV-1 RR partially purified from quiescent BHK-21/C13 cells infected with strain F at 20 plaque-forming units (PFU) per cell. Samples of viral enzyme preparation (50 μg, specific activity 30 units/mg; 75 μg, specific activity 18.1 units/mg; or 65 μg, specific activity 21.6 units/mg) were mixed with increasing concentrations of peptides, and RR activity was assayed by monitoring the reduction of cytidine 5'-diphosphate (CDP). The standard reaction mixture, in a final volume of 60 μL, contained 50 mM HEPES, pH 7.8, 4 mM NaF,

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30 mM dithiothreitol, 1 mM bacitracin, 50 μM CDP, and 0.25 μCi of [^{3}H]CDP. After 30 min of incubation at 37 °C, the reaction was stopped by immersing the tube in boiling water for 4 min, and the precipitate was removed by centrifugation. Nucleotides in the supernatant were converted to nucleosides by enzymatic hydrolysis. The deoxyribonucleosides were subsequently separated from the ribonucleosides by ascending polyethyleneimine-cellulose chromatography.

Acknowledgment. We gratefully acknowledge Claire Guilbault for RR assays, Frances Lowry for editing assistance, and Marie-Françoise Marchal for secretarial work. This research was supported by the Medical Research Council of Canada and Bio-Mega Inc. Pierrette Gaudreau is the recipient of a scholarship from "Fonds de la Recherche en Santé du Québec".

Registry No. **1**, 103424-73-3; **2**, 112208-24-9; **3**, 112208-25-0; **4**, 112208-26-1; **5**, 112208-27-2; **6**, 112208-28-3; **7**, 112208-29-4; **8**, 112208-30-7; **9**, 112208-31-8; **10**, 112208-32-9; **11**, 112208-49-8; **12**, 115532-43-9; **13**, 124045-09-6; **14**, 112237-53-3; **15**, 112208-51-2; **16**, 112208-52-3; **17**, 124045-10-9; **18**, 124045-11-0; **19**, 124045-12-1; **20**, 124045-13-2; **21**, 112208-40-9; **22**, 124069-82-5; **23**, 120372-17-0; **24**, 124045-14-3; **25**, 112208-41-0; **26**, 112208-43-2; **27**, 103424-74-4; **28**, 112208-37-4; **29**, 124045-15-4; **30**, 112208-46-5; **31**, 112208-48-7; **32**, 120372-19-2; **33**, 124045-16-5; **34**, 112208-34-1; **35**, 124045-17-6; **36**, 112208-33-0; **37**, 112208-44-3; **38**, 112208-35-2; **39**, 112208-39-6; **40**, 112208-36-3; **41**, 112208-38-5; **42**, 112208-42-1; **43**, 112208-47-6; **44**, 112208-45-4; **45**, 124045-18-7; **46**, 124045-19-8; **47**, 112208-23-8; **48**, 112208-22-7; **49**, 124045-20-1; **50**, 112208-53-4; **51**, 112208-54-5; **52**, 124045-21-2; **53**, 124069-83-6; **54**, 124045-22-3.

Methylating Agents as Trypanocides

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Methylating agents, such as streptozotocin, procarbazine, *N*-methyl-*N*-nitrosourea, dimethyl sulfate, 1,2-dimethylhydrazine, and a series of 1,2-bis(sulfonyl)-1-methylhydrazines synthesized in this laboratory, were evaluated and shown to be therapeutically active against murine models of African trypanosomiasis. At high dose levels, methylating agents halted trypanosome proliferation and transformed cells into bizarre forms containing multiple nuclei and kinetoplasts. These cells disappeared from the bloodstream of mice bearing these organisms in 48–72 h. When administered at repetitive low doses, methylating agents induced the entire population of trypanosomes to differentiate into biochemically distinct short-stumpy forms in a synchronous manner. These results suggest that methylating agents may be used as biochemical tools in the study of trypanosome differentiation.

Trypanosomes of the brucei group are flagellated protozoa which produce lethal infections in humans and domestic mammals throughout much of sub-Saharan Africa. With the exception of α-(difluoromethyl)ornithine (DFMO), the trypanocidal drugs currently in use have been available for 25–80 years. Current treatment of early-stage infections consists of suramin for *Trypanosoma rhodesiense* and pentamidine for *Trypanosoma gambiense*.[1-3] These therapies require approximately 6 weeks of hospitalization due to drug toxicity.[1,2] The only drug available for late-stage sleeping sickness is melarsoprol.[1-4] This drug has serious side effects and up to 5% of patients die due to drug toxicity. Suramin, pentamidine, and melarsoprol are all administered by intravenous injection. Recently, DFMO has been shown to be effective against early-stage sleeping sickness in man and animals. However, there are doubts as to its efficacy in late-stage disease unless it is used in combination with other less desirable agents such as bleomycin.[5,6] Therefore, better drugs are needed to treat trypanosomiasis.

In the present study, we report the evaluation of a number of methylating agents, including several methylhydrazine derivatives synthesized in our laboratory[7], as antitrypanosomal agents against murine models of trypanosomiasis.

Biological Results and Discussion

The antitrypanosomal properties of compounds 1–16 were determined by measuring their effects on the survival time of mice bearing *T. rhodesiense*. The results are summarized in Table I. A representative compound, 1,2-bis(methylsulfonyl)-1-methylhydrazine (**5**), was also tested against a number of other trypanosome species, such as *T. gambiense*, which like *T. rhodesiense* causes a fatal disease in man,[1,2,4,8] and *Trypanosoma evansi* and *Trypanosoma equiperdum*, which are of veterinary importance[2,8] (Table II).

All of the methylating agents tested displayed significant trypanocidal activity. In general, compounds lacking a reactive methyl group, but structurally identical with the corresponding *N*-methyl analogues in all other respects (e.g., compounds **1** and **9**), or containing the methyl group, but lacking good leaving groups (compound **8**),[9] were inactive as trypanocides. Methanol was produced by these agents in aqueous solutions free from strong competing nucleophiles. Formation of this alcohol was used as a

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0022-2623/90/1833-0730$02.50/0