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SUBSTITUTED PENTA- AND HEXAPEPTIDES AS POTENT INHIBITORS OF HERPES SIMPLEX VIRUS TYPE 2 RIBONUCLEOTIDE REDUCTASE

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Abstract: Structural modifications of the Tyr, Asn, and Leu residues of YVVNDL, a peptide which is equipotent to YAGAVVNDL in the inhibition of herpes simplex virus type 2 ribonucleotide reductase (HSV-2 RR), have produced peptides which are as much as 90- to 120-times as potent as YAGAVVNDL *in vitro* against HSV-2 RR. The chemistry and the structure activity relationships of these inhibitors are described.

Ribonucleotide reductase (RR) is responsible for the reduction of a ribonucleoside diphosphate at the 2' position to the corresponding 2'-deoxyribonucleoside,[1] which provides the 2'-deoxynucleotides required for DNA synthesis.[2] Herpes simplex virus (HSV) RR consists of two non-identical virally encoded subunits.[1] Because the viral-specified enzyme differs markedly in sequence from its mammalian counterpart,[3] inhibition of HSV RR may be a valid target for antiherpetic therapy.[4,5] Moreover, it has been shown that the nonapeptide YAGAVVNDL,[6] corresponding to the C-terminal sequence of the small subunit of HSV RR, specifically inhibits HSV RR *in vitro*[7] by binding to the large subunit, thus antagonizing their association.[8] This peptide is essentially inactive against mammalian RR.[7] Recent structure activity relationship (SAR) studies have described *nona*peptides as much as 45-times as potent as YAGAVVNDL *in vitro* against HSV-1 RR.[9] In this communication, we report the development of highly potent RR inhibitors by modification of a hexapeptide, YVVNDL, which is equipotent to YAGAVVNDL *in vitro* against HSV-2 RR.[10]

Chemistry

The peptide portions of most of these molecules were prepared by Fmoc-based[§] SPPS[11] on a peptide synthesizer, using commercially available HO-PKA resin and the corresponding single amino acid units as their Fmoc-PFP ester derivatives. However, a number of acyl-VVNDL analogs were prepared by *N*-acylation of a resin-bound peptide unit prepared by t-Boc-based SPPS[12] using a Merrifield resin and the corresponding t-Boc-amino acids.

In the Fmoc-based method, the synthesis began with the coupling of the first amino acid onto HO-PKA (HOBT, DMAP, 4MM). Subsequently, a sequence of deprotection (piperidine /DMF) and coupling with the next Fmoc-PFP-amino acid (HOBT/DMF) on a semi-automated or automated peptide synthesizer, was continued until the

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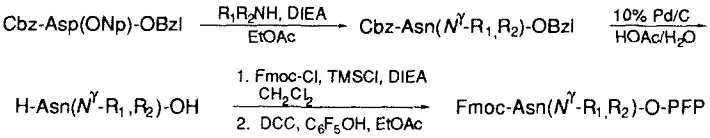
[§] Abbreviations used: Fmoc = N-9-fluorenylmethoxycarbonyl, SPPS = solid phase peptide synthesis, HO-PKA = polyamide-kieselguhr composite resin with type A linkage, PFP = pentafluorophenyl, t-Boc = t-butyloxycarbonyl, HOBT = 1-hydroxybenzotriazole, DMAP = dimethylaminopyridine, 4MM = 4-methylmorpholine, DMF = dimethylformamide, TFA = trifluoroacetic acid, DIEA = diisopropylethylamine, Cbz = benzyloxycarbonyl, ONp = p-nitrophenyl ester, Bzl = benzyl, BOP = benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate, OMerrifield = O-Polystyrene resin (copolymer of styrene with 1% divinylbenzene), Avl = 2-aminovaleric acid, TBDMSi = t-butyldimethylsilyl, LAH = lithium aluminum hydride, LDA = lithium diisopropylamide, HMPA = hexamethylphosphoramide.

desired sequence of amino acids was put in place. In cases where no acylation was required to reach the final product, the H-peptide-OPKA material from the synthesizer was treated with a 95:4:1 mixture of TFA : 1,2-ethanedithiol : thioanisole, to cleave the peptide from the resin, as well as remove any t-butyl groups present as secondary protection, to yield the peptide C-terminal acid. The crude peptide, obtained by extraction with 80% acetic acid, was purified by semi-preparative reversed phase HPLC on either a Vydac C-18 or a Zorbax C-8 column, eluting with acetonitrile/water gradient systems (in the presence of 0.1% TFA) containing 15-30% acetonitrile, to obtain the desired peptides in >97% purity by analytical HPLC. Each peptide thus purified was further characterized by high field (300 or 400 MHz) 1D and/or 2D ^{1}H-nmr, FAB-MS, and in certain cases, amino acid analysis.

When further manipulations of the resin-bound peptide were necessary, the protected resin-peptide was first deblocked and then coupled with an appropriate amino acid derivative or acylating agent. A number of acylating reagent combinations were used,[12,13] including: Fmoc-amino acid PFP ester and HOBT; acyl chloride and DIEA; Fmoc-amino acid, BOP, HOBT, and 4MM; symmetrical or mixed anhydride and DMAP. After removal of DMF, acid cleavage as described above, followed by reversed phase HPLC of the crude material via the previously mentioned systems gave the desired compounds which were characterized by ^{1}H-nmr, and FAB-MS.

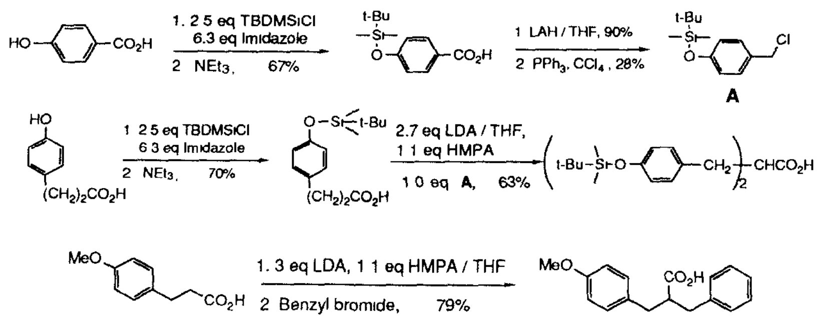
The requisite *N*γ-substituted asparagines were synthesized from Cbz-Asp(ONp)-OBzl according to the general scheme shown below:[13]



$$\text{Cbz-Asp(ONp)-OBzl} \xrightarrow[\text{EtOAc}]{\text{R}_1\text{R}_2\text{NH, DIEA}} \text{Cbz-Asn}(N^{\gamma}\text{-R}_1,\text{R}_2)\text{-OBzl} \xrightarrow[\text{HOAc/H}_2\text{O}]{\text{10\% Pd/C}}$$

$$\text{H-Asn}(N^{\gamma}\text{-R}_1,\text{R}_2)\text{-OH} \xrightarrow[\text{2. DCC, C}_6\text{F}_5\text{OH, EtOAc}]{\text{1. Fmoc-Cl, TMSCl, DIEA, CH}_2\text{Cl}_2} \text{Fmoc-Asn}(N^{\gamma}\text{-R}_1,\text{R}_2)\text{-O-PFP}$$

The (*N*-substituted)-YVVNDL analogs were prepared via alkylation of YVVNDL by reductive amination with the appropriate aldehyde and sodium borohydride.[13] For the substituted pentapeptides which are derived from H-VVN-D(OBzl)-L-OMerrifield, acylation with either the anhydride or the PFP ester of the appropriate acid (DIEA/dioxane)[13] was followed by HF treatment, which cleaved the resin-peptide linkage and removed the benzyl ester of Asp. Reversed phase HPLC of the crude product, obtained by extraction with acetic acid, as described above, gave the pure compound. Several of the carboxylic acids required for acylation were prepared as indicated below:



Enzyme Assay

For assay of ribonucleotide reductase activity, the reduction of $[^3\mathrm{H}]$-cytidine-5'-diphosphate $\{[^3\mathrm{H}]$-CDP$\}$ to $[^3\mathrm{H}]$-2'-deoxycytidine-5'-diphosphate $\{[^3\mathrm{H}]$-dCDP$\}$ was monitored. The HSV enzyme was prepared from HSV-2(strain 186)-infected baby hamster kidney cells essentially as described previously.[8a] Reactions containing the enzyme extract, 100 m*M* HEPES (pH 8), 20 μ*M* $\mathrm{FeCl_3}$, 10 m*M* dithiothreitol, 100 μ*M* $[^3\mathrm{H}]$-CDP (100 mCi/mmol), and the synthetic peptide at varying concentrations were incubated at 37°C for 30 min. After heat-quench, the nucleotides were converted to the corresponding nucleosides by incubation with *Crotalus adamanteus* venom (4 mg/mL final concentration). The tritiated deoxycytidine and the tritiated cytidine were separated by chromatography on Dowex-1-borate column and subsequently quantified by liquid scintillation counting.[8a] Each compound was assayed at least twice unless otherwise indicated.[14] The $\mathrm{IC_{50}}$ of the nonapeptide YAGAVVNDL was determined as a control for each experiment.[15] Potency relative to YAGAVVNDL was calculated as described in **Table 1**.

In Vitro Structure-Activity Relationships

The inhibition of HSV-2 RR activity by synthetic peptides **2**-**44** is reported in **Table 1**. Replacements or modifications of Y, N, and L in YVVNDL were studied.[16] While certain *N*-substituted-Tyr derivatives gave modest potency enhancement (entries **4** and **7**), alkyl substitutions at the α position decreased activity substantially (entries **9** and **10**). Apparently, the free $\mathrm{NH_2}$ group in Tyr is not necessary for good *in vitro* inhibition (entry **8**). Analogs **11**-**22** comprise [substituted acyl (or carbamyl)]-VVNDL derivatives. Bulky monosubstituted-acetyls gave limited improvement in activity (entries **12** and **13**). Di- or trisubstituted-acetyls (entries **14**-**19** and **22**) varied widely in activity, depending on such factors as size and hydrophobicity of the substituents. Small substituents gave poor inhibitors (entries **14** and **22**). The dibenzylacetyl derivative (entry

Table 1

		Average $\mathrm{IC_{50}}$(μM)		
Compound/ Entry	**Peptide**	**Test Compd.**	**YAGAVVNDL Control**[a]	**Relative Potency**[b]
	Variations on Tyrosine			
1	YAGA-VVNDL	25[c]	—	—
2	Y-VVNDL	15	15	1.0
3	(*N*-Me)Y-VVNDL	12	15	1.3
4	(*N*-Bzl)Y-VVNDL	6.0	30	5.0
5	[*N*-(Biphenyl-$\mathrm{CH_2}$)]Y-VVNDL	32	34	1.1
6	[*N*-(3-Phenoxybenzyl)]Y-VVNDL	12	40	3.3
7	[*N*-[(3-Thienyl)methyl]]Y-VVNDL	20	80	4.0
8	(des-$\mathrm{NH_2}$)Y-VVNDL	21	20	1.0
9	Y(α-Me)-VVNDL - diastereomer 1	120	30	0.25
10	Y(α-Me)-VVNDL - diastereomer 2	58	30	0.52
11	(Bzl)$\mathrm{CH_2}$CO-VVNDL	11[d]	17	1.5
12	(Ph)$_2$CH$\mathrm{CH_2}$CO-VVNDL	10	60	6.0
13	(β-Naph)$\mathrm{CH_2}$CO-VVNDL	5.0	30	6.0
14	$\mathrm{(CH_3)_2}$CHCO-VVNDL[e]	30	5	0.17
15	(Ph)$_2$CHCO-VVNDL	90[d]	24	0.27
16	(Bzl)$_2$CHCO-VVNDL[e]	0.5	19	38
17	(4-OH-Bzl)$_2$CHCO-VVNDL	6.5	35	5.4
18	(Bzl)(4-OMe-Bzl)CHCO-VVNDL-diastereomer 1	1.5[d]	9	6.0

Table 1 (continued)

		Average IC_{50}(μM)		
Compound/ Entry	**Peptide**	**Test Compd.**	**YAGAVVNDL Control**[a]	**Relative Potency**[b]
19	(Bzl)(4-OMe-Bzl)CHCO-VVNDL-diastereomer 2[f]	0.45	9.5	21
20	(Ph)$_2$NCO-VVNDL	170	24	0.14
21	(Bzl)$_2$NCO-VVNDL	11	24	2.2
22	(CH$_3$)$_3$CCO-VVNDL	21	5	0.24
	Variations on Leucine			
23	YVVND-A(Cyclohexyl)	55	16	0.29
24	YVVND-L(α-Me) (RS)	320	38	0.12
25	YVVND-G(Allyl)	>430	22	NA[g]
26	YVVND-(Avl)	160	25	0.16
27	YVVND-L(γ-Me)	13	43	3.3
	Variations on Asparagine			
28	YVV-N(N^{γ}-Me)-DL	2[d]	21	11
29	YVV-N(N^{γ}-Bzl)-DL	4	22	5.5
30	YVV-N(N^{γ}-Me$_2$)-DL[e]	0.3	27	90
31	YVV-N[N^{γ}-(CH$_2$)$_4$]-DL	1	32	32
32	YVV-N(N^{γ}-Me,*N*-Et)-DL	0.4	10	25
33	YVV-N(N^{γ}-Bzl$_2$)-DL	2.0	25	13
34	YVV-N[N^{γ}-[[(CH$_2$)$_2$]$_2$O]]-DL	0.6	22	36
35	FVV-D(OMe)-DL[e]	2.0	27	14
	Variations on More Than One Amino Acid Residue			
36	Y-VV-N(N^{γ}-Me$_2$)-D-L(γ-Me)[e,h]	0.2	22	110
37	(*N*-Bzl)Y-VV-N-D-L(γ-Me)[h]	1.5	35	23
38	(Bzl)$_2$CHCO-VV-N(N^{γ}-Bzl)-D-L	0.4	22	55
39	(Bzl)$_2$CHCO-VV-N-D-L(γ-Me)[h]	0.1	12	120
40	D-VV-N(N^{γ}-Me$_2$)-DL[e]	0.15	9.5	63
41	D-VV-N[N^{γ}-(CH$_2$)$_4$]-DL	1[d]	32	32
42	(*N*-Bzl)Y-VV-N(N^{γ}-Me$_2$)-D-L(γ-Me)	0.3	18	60
43	(Bzl)$_2$CHCO-VV-N(N^{γ}-Me$_2$)-D-L(γ-Me)[e]	0.3	21	70
44	(Bzl)$_2$CHCO-VV-N(N^{γ}-Bzl)-D-L(γ-Me)[e]	0.2	10	50

[a] IC_{50} of YAGAVVNDL determined as a control for each experiment.

[b] Relative potency compared to that of YAGAVVNDL for each entry.

[c] Average of 51 entries which ranged from 5 to 80. $\mu_{IC_{50}} = 25 \pm 3.6$ with 95% confidence level.

[d] This compound was assayed only once.

[e] Amino acid composition determined.

[f] 600 μ*M* against calf thymus RR.

[g] NA = not active

[h] No activity against calf thymus RR at 300μM, the highest concentration tested.

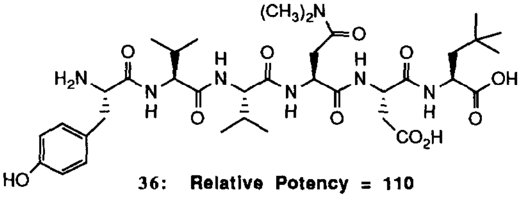
16) was a submicromolar inhibitor with a relative potency of 38-fold, but the diphenylacetyl derivative (entry **15**) was a poor inhibitor. Presumably, the benzyl groups in entry **16**, by virtue of length and/or conformational mobility, are able to reach a hydrophobic binding site not accessible to phenyl or small alkyl substituents. Entry **17** (vs. **16**) shows the deleterious effect of placing polar groups on the benzyl moieties. However, a 4-

methoxybenzyl group is apparently tolerated (entry **19**). Isosteric replacement of carbon by nitrogen in the acyl substituent gave lackluster inhibitors (compare entries **15** vs. **20**, and **16** vs. **21**).

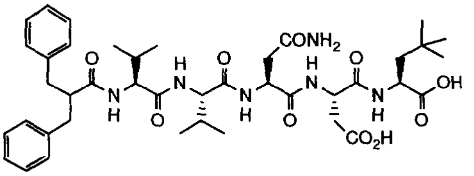
Of a number of replacements of the C-terminal Leu examined (entries **23-27**), only Leu(γ-Me) (entry **27**) gave an enhancement in potency over YVVNDL. For N^{γ}-monosubstitution on Asn (entries **28-29**), the less bulky substituent was preferred. N^{γ},N^{γ}-disubstituted Asn's (entries **30-34**) gave a number of submicromolar inhibitors. The best of these, Asn(N^{γ},N^{γ}-dimethyl) had a 90-fold relative potency (entry **30**). However, as steric bulk of the substituents increased, activity decreased (entries **30-33**). Analogs from Asn[N^{γ}-$[[(CH_2)_2]_2O]]$ and Asp(OMe) (where Phe was used in place of Tyr) also gave substantial increases in potency relative to YVVNDL (entries **34-35**).

Among the peptides with two modified residues (entries **36-41**), additive potency enhancement was observed in three cases where the modifications were at the terminal residues. Where the modified residues were closer together on the peptide chain, the observed increase in activity was less than additive. Similarly, in the cases where each of Y, N, and L was modified or replaced at the same time (entries **42-44**), the resulting increases in potency were disappointing considering those observed for each modification alone. Possibly, the interactions of the fairly bulky side chains may restrict these peptides to somewhat less favorable conformations with respect to binding.

In summary, by judicious replacement of Tyr or substitution on Asn or Leu in YVVNDL, HSV-2 RR inhibitors that are respectively 38-fold (**16**), 90-fold (**30**), and 3-fold (**27**) as potent as the nonapeptide YAGAVVNDL have been synthesized. When some or all of these favorable modifications were built into a single peptide, the best of the resulting analogs had a 110-fold (**36**) and 120-fold (**39**) potency enhancement over the nonapeptide.



36: Relative Potency = 110



39: Relative Potency = 120

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14. Each IC_{50} value was derived from a curve containing at least five points.

15. YAGAVVNDL and YVVNDL were also assayed against HSV-1 (KOS) RR. The IC_{50}'s obtained were within experimental error of those obtained with HSV-2 RR.

16. Replacement of Y, N, and L in YVVNDL by a number of other natural amino acids did not lead to more potent inhibitors than YVVNDL. In addition, limited SAR studies were also done on the V, V, and D positions of YVVNDL. These, at best, produced inhibitors equipotent with YVVNDL. Hannah, J.; Garsky, V.; Chang, L. L.; Ashton, W. T.; Sugg, E.; Yamanaka, G.; Uncapher, C.; McClements, W. L.; Tolman, R. L. unpublished results.