

DESIGN AND SYNTHESIS OF A TETRAHYDROPYRAN-BASED INHIBITOR OF MAMMALIAN RIBONUCLEOTIDE REDUCTASE

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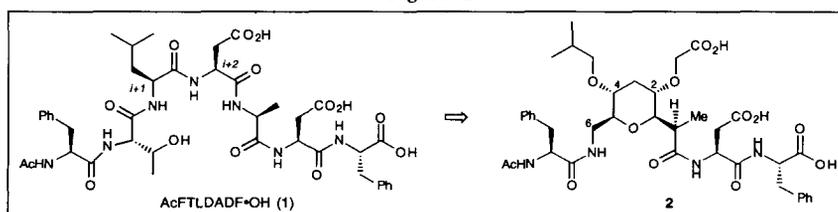
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Abstract: A tetrahydropyran-based inhibitor (**2**) of mammalian ribonucleotide reductase (mRR) has been designed and synthesized based on the heptapeptide, N-AcFTLDADF (**1**), corresponding to the C-terminus of the R2 subunit of mRR. Inhibition studies revealed that **2** is indeed a competent inhibitor, albeit less potent than **1**. © 1998 Elsevier Science Ltd. All rights reserved.

Ribonucleotide reductase (RR), an enzyme that plays a critical role in regulating DNA replication, as well as an indirect role in regulating other enzymes in the DNA synthetic pathway, represents an important target for the design and synthesis of antiviral and cancer chemotherapeutic agents.¹ Toward this end, several laboratories² have demonstrated that type 1 RRs, such as mammalian ribonuclease reductase (mRR), can be inhibited by peptides corresponding to the C-terminus of the R2 subunit. These peptides effectively compete with R2 for binding to the R1 subunit and thus prevent RR assembly.³ The NMR-derived structure⁴ of such a peptide, heptapeptide N-AcFTLDADF, bound to mouse R1 was found to share a common reverse turn conformation with the crystallographically determined structure⁵ of the C-terminus of *E. coli* R2 bound to *E. coli* R1. This result suggested that high affinity, peptidic inhibitors of mRR could be based on this common turn feature. We further reasoned that a properly constrained (i.e., preorganized) analog would likely have a greater affinity for R1 than the native peptide due to the conformational lability of small peptides in solution.⁶ Herein we describe the design of an inhibitor of mRR based on the heptapeptide N-AcFTLDADF exploiting the elements of a tetrahydropyran scaffold to constrain the β -turn.

Previously we demonstrated the effective use of monosaccharides as viable β -turn mimetics in the design of potent somatostatin, NK-1 and β -adrenergic agonists/antagonists.⁷ More recently, von Roedern and Kessler have exploited amino acid sugars as turn templates in cyclic peptidomimetics.⁸ From the perspective of design, monosaccharides also represent excellent chirons for the construction of diverse tetrahydropyran scaffolds. To exploit this tactic for ribonuclease reductase inhibitors, we envisioned attachment of the *i+1* and *i+2* functionality of the heptapeptide N-AcFTLDADF (i.e., the Leu³ and Asp⁴ side chains) to the 4- and 2-hydroxyls, respectively, of L-glucose. Conversion to the tetrahydropyran scaffold would then permit introduction of the methyl group

Figure 1



by activation of the primary hydroxyl and azide formation furnished (-)-**5**¹² in 72% yield for the two steps. Oxidative-removal of the C(4) PMB group with DDQ (74%), installation of the leucine-mimicking side chain (NaH, isobutyl bromide, tetra-*n*-butylammonium iodide, THF, 86% yield) and hydrolysis of the *tert*-butyl ether with aqueous acetic acid then provided **6**¹² in 72% yield as a mixture of α - and β -anomers (1.5:1). The alanine-mimicking methyl group was next introduced via a Wittig reaction (76%); ring closure^{13a} with concomitant base induced methyl equilibration^{13b} followed by ester hydrolysis furnished a mixture of (+)-**7a**¹² (66%, 2 steps) and the methyl diastereomer [(-)-**7b**,¹² 13%] separable by flash chromatography.¹⁴ Formation of the *tert*-butyl ester (N,N-dimethylformamide di-*tert*-butylacetal, toluene, 85 °C, 80%), removal of the C(2) benzyl group with simultaneous reduction of the azide (H₂, Pd/C) and *in situ* Boc protection of the resultant amine furnished (-)-**8**¹² in 73% yield. Installation of the aspartic acid-mimicking side chain at C(2) was next achieved via allylation of the secondary hydroxyl group (allyl bromide, NaH; 84%) followed by oxidative-cleavage of the olefin with *in situ* benzyl protection (RuO₂, NaIO₄; BnBr, K₂CO₃, 72%). Treatment of the derived ester with trifluoroacetic acid (TFA) removed both the C-terminal acid and N-terminal amine protecting groups; re-protection of the amine as the Boc derivative then gave acid (+)-**9**¹² in 95% yield. Coupling to the bis-protected dipeptide Asp(OBn)-Phe-OBn was next efficiently achieved (88%) via activation with diethylphosphoryl cyanide (DEPC);¹⁵ liberation of the primary amine with TFA, coupling to N-AcPhe (DEPC, Et₃N, THF) and removal of the benzyl protecting groups completed the synthesis of peptidomimetic (-)-**2**¹⁶ (62%, 3 steps).

The tetrahydropyran-based mimetic (**2**) was found to inhibit mRR, though considerably less well than N-AcFTLDADF (K_i of 400-500 μ M for **2** vs. K_i of 15-20 μ M for **1**, Table 1). We suspect the lower affinity results from steric conflicts with either or both of the 2-*O*-carboxymethyl and 4-*O*-isobutyl pendant groups. Structure-function^{3c} and modeling (unpublished) studies on the interaction of the parent N-AcFTLDADF peptide with mammalian R1 suggest that these groups are not essential for inhibitory activity, but that the N- and C-terminal Phe residues are important. These considerations are currently being incorporated in our efforts to design and synthesize second generation peptidomimetics with enhanced RR inhibitory activity.

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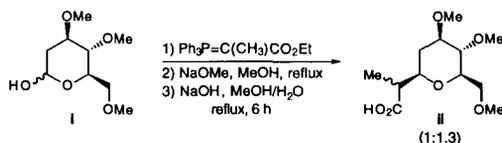
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Table 1. Ribonuclease reductase inhibitory activity of **2**.

[2], μ M	Residual RR Activity (%)
0	100
30	86 \pm 8
100	73 \pm 6
300	64 \pm 6
500	48 \pm 4
1000	32 \pm 6

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 - The structure assigned to each new compound is in accord with its infrared, 500 MHz ^1H NMR, and 125 MHz ^{13}C NMR spectra, as well as appropriate parent ion identification by high resolution mass spectrometry.
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- The absolute configuration at the methyl center was determined by X-ray analysis of the minor diastereomer (-)-**7b**.
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- Compound (-)-**2** was isolated as a white solid (mp 120–123 °C) possessing the following spectral data: $[\alpha]_{\text{D}}^{20}$ -1.90° (c 0.16, EtOH); ^1H NMR (500 MHz, CD_3OD) δ 7.14 (m, 4 H), 7.08 (m, 6 H), 4.62 (dd, $J = 5.3$, 8.0 Hz, 1 H), 4.56 (dd, $J = 5.2$, 7.6 Hz, 1 H), 4.51 (dd, $J = 6.2$, 8.8 Hz, 1 H), 4.07 (d, $J = 16.5$ Hz, 1 H), 4.02 (d, $J = 16.5$ Hz, 1 H), 3.47 (dd, $J = 2.4$, 13.8 Hz, 1 H), 3.23 (m, 3 H), 3.08 (m, 2 H), 3.00 (dd, $J = 6.6$, 8.5 Hz, 1 H), 2.96 (m, 2 H), 2.91 (dd, $J = 7.6$, 13.9 Hz, 1 H), 2.89 (m, 1 H), 2.76 (dd, $J = 7.8$, 13.9 Hz, 1 H), 2.75 (m, 1 H), 2.66 (dd, $J = 5.3$, 16.9 Hz, 1 H), 2.62 (m, 1 H), 2.60 (dd, $J = 8.1$, 16.9 Hz, 1 H), 1.78 (s, 3 H), 1.68 (m, 1 H), 1.10 (dd, $J = 11.3$, 22.1 Hz, 1 H), 1.00 (d, $J = 7.2$ Hz, 3 H), 0.81 (d, $J = 6.7$ Hz, 3 H), 0.80 (d, $J = 6.7$ Hz, 3 H); ^{13}C NMR (125 MHz, CD_3OD) δ 177.5, 174.0, 173.9, 173.9, 173.6, 173.0, 172.8, 138.5, 138.0, 130.5, 130.3, 129.5, 127.9, 127.7, 81.4, 80.3, 76.8, 75.4, 75.2, 66.2, 56.1, 55.0, 51.0, 42.5, 41.6, 39.1, 38.3, 35.4, 34.3, 30.0, 22.5, 19.7, 11.1; high-resolution mass spectrum (FAB, NBA) m/z 807.3442 $[(\text{M}+\text{Na})^+]$; calcd for $\text{C}_{39}\text{H}_{52}\text{N}_4\text{O}_{13}$: 807.3429].