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

Amos B. Smith, III,* Setsuya Sasho, Bari A. Barwis, Paul Sprengeler, Joseph Barbosa, Ralph Hirschmann,* and Barry S. Cooperman*

Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104, U.S.A.

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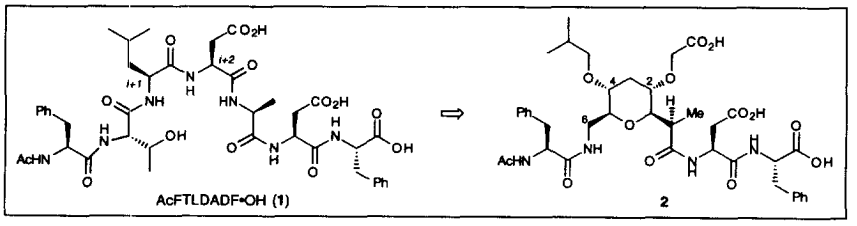
Abstract: A tetrahydropyran-based inhibitor (**2**) of mammalian ribonucleotide reductase (mRR) has been designed and synthesized based on the heptapeptide, N-AcFTLDADF (**1**), corresponding to the C-terminus of the R2 subunit of mRR. Inhibition studies revealed that **2** is indeed a competent inhibitor,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

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 laboratories[2] 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 structure[4] of such a peptide, heptapeptide N-AcFTLDADF, bound to mouse R1 was found to share a common reverse turn conformation with the crystallographically determined structure[5] of the C-terminus of *E. coli* R2 bound to *E. coli* R1. This result suggested that high affinity, peptidal 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 Leu[3] and Asp[4] 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



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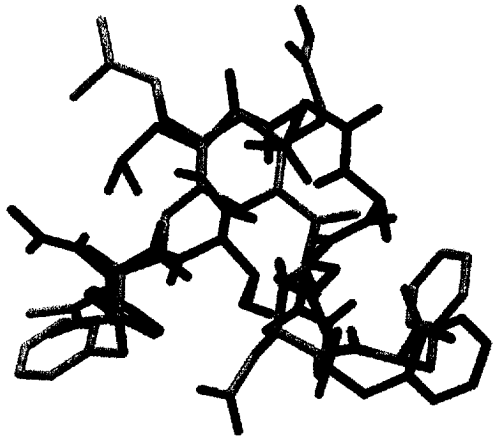
corresponding to the methyl of Ala[5] of **1**. The latter structural element was considered important due to the hydrophobic interaction observed between the analogous methyl and Phe[7] in the trNOE structure of **1**. The N-AcPhe was then attached at the 6-position and the C-terminal Asp and Phe residues connected by a two carbon linker at position 1.

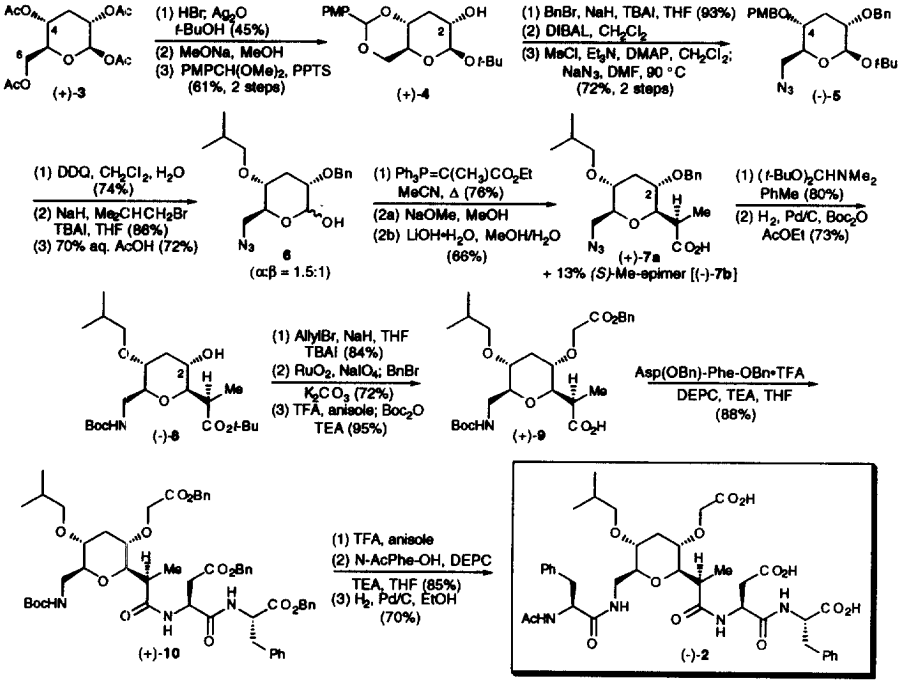
Figure 2. Overlay of the minimized structure of **2** (gray) with the NMR-derived conformation of peptide **1** (black).



Molecular modeling employing the MM2 force field[9] included with MacroModel (v3.1)[10] suggested that tetrahydropyran-based peptidomimetic **2** was capable of adopting a conformation similar to that required for the binding of peptide **1** as determined by NMR studies. Shown in Figure 2 is an overlay of the minimized structure of **2** (gray) with the NMR-derived conformation of peptide **1** (black).

The synthesis of **2** began with the known tetraacetate (+)-**3**.[7b] Silver(I) oxide mediated Koenigs-Knorr coupling[11] via the bromide with *tert*-butanol, followed by acetate methanolysis (NaOMe, MeOH) and formation of the C(4)-C(6) acetal afforded (+)-**4**[12] in 27% yield for the three step sequence. Protection of the C(2) hydroxyl as the benzyl ether (93%), reductive opening of the acetal followed

Scheme 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 (H_2, 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 (RuO_2, $NaIO_4$; BnBr, K_2CO_3, 72%). Treatment of the derived ester with trifluoroacetic acid (TFA) removed both the C-terminal acid and N-terminal amine protecting groups; reprotection 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, Et_3N, 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 μM for **2** vs. K_i of 15-20 μM 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.

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

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

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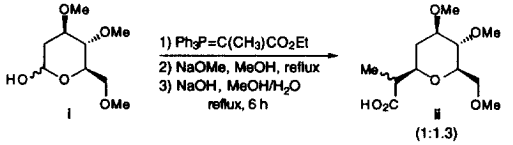
12. The structure assigned to each new compound is in accord with its infrared, 500 MHz ^{1}H NMR, and 125 MHz ^{13}C NMR spectra, as well as appropriate parent ion identification by high resolution mass spectrometry.
13. (a) Fraser-Reid, B.; Dawe, R. D.; Tulshian, D. B. *Can. J. Chem.* **1979**, *57*, 1746. (b) The observed diastereoselectivity (ca. 5:1) presumably arises via relief of $A^{1,3}$ strain between the methyl and OBn groups. A related Wittig reaction (**i** → **ii**) lacking a substituent in position 2 furnished a 1:1.3 mixture of methyl epimers.



14. The absolute configuration at the methyl center was determined by X-ray analysis of the minor diastereomer (-)-**7b**.
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16. Compound (-)-**2** was isolated as a white solid (mp 120–123 °C) possessing the following spectral data: $[\alpha]_D^{20}$ -1.90° (*c* 0.16, EtOH); ^{1}H 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 $[(M+Na)^+$; calcd for $C_{39}H_{52}N_4O_{13}$: 807.3429].