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Synthesis and Biological Activity of Cyclic Peptide Inhibitors of Ribonucleotide Reductase

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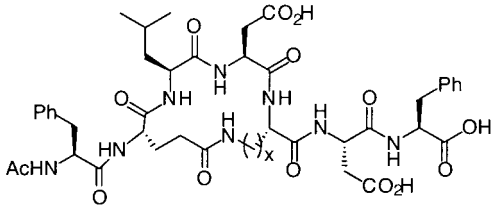
ABSTRACT



2: x = 1 **4**: x = 3
3: x = 2 **5**: x = 4

A series of lactam-bridged peptide inhibitors (2–6) of mammalian ribonucleotide reductase (mRR) has been designed and synthesized on the basis of the heptapeptide *N*-AcFTLDADF (1), corresponding to the C-terminus of the R2 subunit of mRR. Inhibition studies revealed a direct relation between ring size and activity, with peptide 5 being 2.5 times more potent than peptide 1.

Mammalian ribonucleotide reductase (mRR), a potential target for cancer intervention,[1] is inhibited by the binding of the heptapeptide *N*-AcFTLDADF (**1**), a mimic of the C-terminus of its R2 subunit, to its R1 subunit.[2] In 1995 we demonstrated by transfer-NOE NMR studies that peptide **1** bound to mRR exhibits a reverse turn structure consisting of the amino acids TLDA (positions 2−5).[3] We envisioned that constraining this turn through an *i*,*i*+3 interaction held the promise of significantly increasing the binding affinity of peptide **1** to R1 and would thereby yield a promising lead compound for the development of peptidomimetic inhibitors of mRR.[4]

Bioactive linear peptides have often been cyclized in order to reduce conformational freedom and enhance binding affinity by mimicking or inducing bound structural motifs.[5] Highly potent and selective opioid peptide analogues have

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been developed employing *i*,*i*+2 side chain to side chain lactamization.[6] Furthermore, *i*,*i*+3 and *i*,*i*+4 spaced side chain to side chain lactam bridges have been found to stabilize turn conformations[7] and helical segments,[8] respectively. Lactam bridges have been used most frequently to constrain peptide conformations, since they are readily synthesized and are not redox labile. The latter characteristic is particularly important for inhibitors of mammalian ribonucleotide reductase (mRR), given the reducing environment required for catalytic activity. In this Letter we describe the design and synthesis of inhibitors of mRR, based on the heptapeptide *N*-AcFTLDADF, exploiting a lactam bridge to constrain the β-turn (Figure 1).

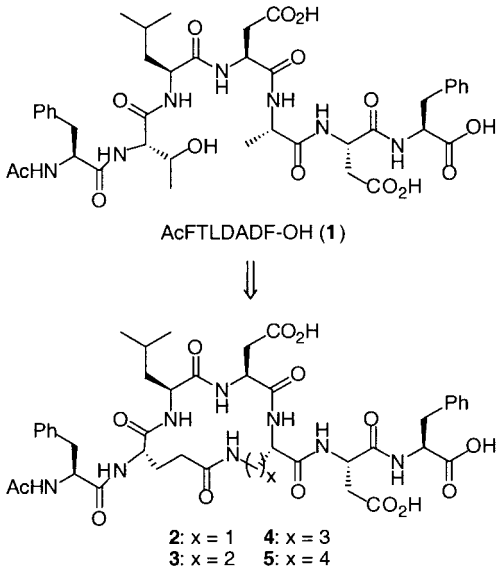
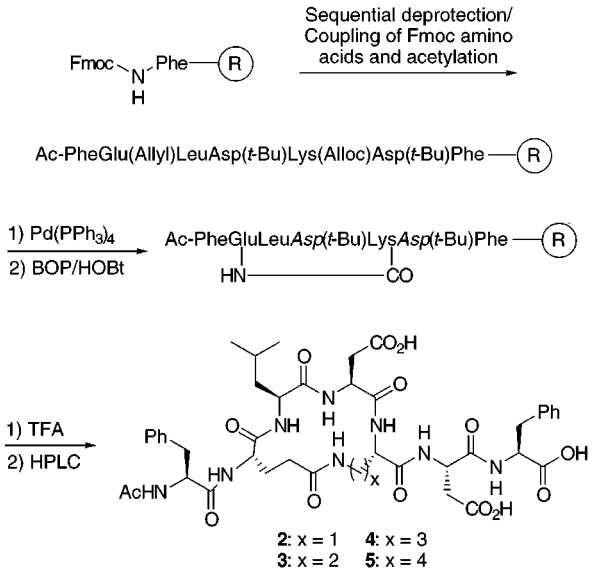


Figure 1.

To rigidify the natural β-turn in peptide **1**, we chose to incorporate an amide linkage between amino acid residues at the T (2) and A (5) positions, since these amino acids contribute only marginally to the inhibitory potency of peptide **1**.[2c] Glutamic acid was thus substituted in position 2, and the optimal length of the side chain link between positions 2 and 5 was tested by varying the amino-bearing side chain in position 5 from lysine (C_4) to ornithine (C_3), diamino butanoic acid (Daba, C_2), or diamino propanoic acid (Dap, C_1). The *N*-AcPhe residue at the N-terminus and the Asp-Phe moiety at the C-terminus were not varied since they are known to be essential for bioactivity of peptide **1**.[2c] For peptide **6**, a lysine was placed in position 2 and a glutamate in position 5 to test the importance of the placement of the lactam linkage.

To construct the peptides via C-to-N iteration, Fmoc L-Phe-OH linked to entagel resin (Novabiochem) was deprotected with piperidine and subjected to HBTU-mediated coupling cycles with Fmoc L-Asp(O*t*Bu)-OH, Fmoc L-Lys/Orn/Daba/Dap(Alloc)-OH (**5**, **4**, **3**, **2**, respectively) or Fmoc L-Glu-(Allyl)-OH (**6**), Fmoc L-Asp(O*t*Bu)-OH, Fmoc L-Leu-OH, Fmoc L-Glu(Allyl)-OH (**2**, **3**, **4**, **5**) or Lys(Alloc)-OH (**6**), and Fmoc L-Phe-OH with piperidine deprotection (Scheme 1). The resultant N-terminal free amine was then acetylated

Scheme 1



with acetic anhydride. Residues in positions 2 and 5, which were orthogonally protected with allyl ester and alloc carbamate groups, were selectively and simultaneously deprotected using tetrakis(triphenylphosphine), acetic acid, and *N*-methylmorpholine.[9] Initial attempts at simultaneous deprotection with tributyltin hydride/bis(triphenylphosphine)-palladium(II) chloride proved less effective;[10] while the allyl

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(13) Yields were not optimized. Each reaction step was checked for product formation by cleaving a small amount of resin with 95% aqueous TFA. The cleaved product was then analyzed using Maldi-TOF-MS, and the appropriate parent ion was identified. The final products were identified by 500 MHz ^{1}H NMR and/or Maldi-TOF−MS and MS/MS fragmentation studies. As a typical compound, **5** was isolated as a white powder and possessed the following spectral data: ^{1}H NMR (500 MHz, D_2O) 0.86 (2H, d, 6.0 Hz, Leu δ−CH_3), 0.90 (2H, d, 6.1 Hz, Leu δ-CH_3), 1.26−1.45 (4H, m, Lys γ-CH_2, δ-CH_2), 1.55−1.68 (5H, m, Leu β-CH_2, Leu γ-CH_2, Lys β-CH_2), 1.90 (3H, s, Ac), 1.91−1.97 (2H, m, Glu β-CH_2), 2.16−2.22 (2H, m, Glu γ-CH_2), 2.44−2.64 (4 H, m, Phe or Asp β-CH_2), 2.92−3.09 (4H, m, Phe or Asp β-CH_2), 3.11−3.17 (2H, m, Lys ϵ-CH_2), 4.19 (1H, t, 7.1 Hz, Glu α-CH), 4.29−4.38 (3H, α-CH), 4.45 (1H, t, 6.6 Hz, α-CH), 4.50−4.54 (2H, m, α-CH), 7.17-7.32 (10H, m, 2 Phe aromatics). ESI-HRMS: M + Na^+ expected 959.4127, found 959.4147.

Table 1

Cyclic Peptides	Binding affinity (μM)
Ac–FTLDADF–OH (**1**)	10±2
Ac–FELD(Dap)DF–OH (**2**)	60±10
Ac–FELD(Daba)DF–OH (**3**)	10±2
Ac–FELD(Orn)DF–OH (**4**)	10±2
Ac–FELDKDF–OH (**5**)	4±1
Ac–FKLDEDF–OH (**6**)	100±20

ester could be removed with ease, the alloc carbamate resisted complete cleavage. The peptides were then cyclized on the resin using BOP and HOBt,[11] conditions which proved better in this context than HBTU, DCC, or DPPA. Completion of the cyclization process was confirmed via the Kaiser test.[12] The cyclic peptides were then deprotected, removed from the resin with 95% aqueous trifluoroacetic acid, and purified to homogeneity by RP-HPLC (overall yields based on resin-bound Fmoc Phe were **2**, 10%; **3**, 3%; **4**, 26%; **5**, 33%; **6**, 8%).[13] An analogue of the protected form of peptide **2**, containing Asp in position 2 instead of Glu, did not cyclize, forming instead an aspartimidate in position 2 upon treatment with TFA.

To determine the binding affinities, we employed a novel assay,[14] based on the use of Sepharose-FTLDADF as an affinity column to purify R1.[15] Premixed solutions of R1 with potential ligands were loaded onto microcolumns containing the peptide-linked column material. The columns were spun in a microcentrifuge and washed, and the eluate was analyzed for R1 content. The amount of R1 eluted was correlated directly to ligand affinity, as the result of competition with column-bound peptide.

The binding studies revealed a direct relation between ring size and activity. Peptide **5** was found to be the most potent mRR inhibitor, with a K_i of 4−5 μM vs 10 μM for our previous lead peptide **1** (Table 1). The K_i value rises to 10 μM when the ring size is reduced by one methylene group to furnish cyclic peptide **4**. Interestingly, further reduction in ring size by a second methylene unit does not increase K_i (peptide **3**). This result suggests that a loss in interactions between the inhibitor and mRR is balanced by the more favorable entropy of binding a less flexible system (i.e., **3**). Further ring contraction as in peptide **2** results in a dramatic loss of activity. The large decrease in activity of peptide **6** vs peptide **5** demonstrates the importance of the position and orientation of the amide linkage within the lactam.

A Monte Carlo conformational search employing the AMBER[16] force field included with MacroModel (v.6.0)[17]

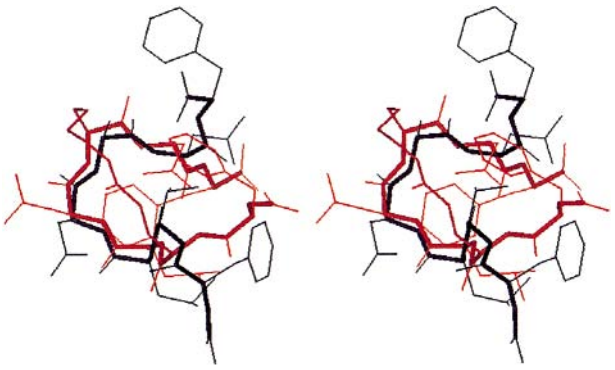

Figure 2. Stereoview of an overlay of the low-energy conformation of **5** (red) with the NMR-derived conformation of R1-bound peptide **1** (black). Backbones are highlighted.

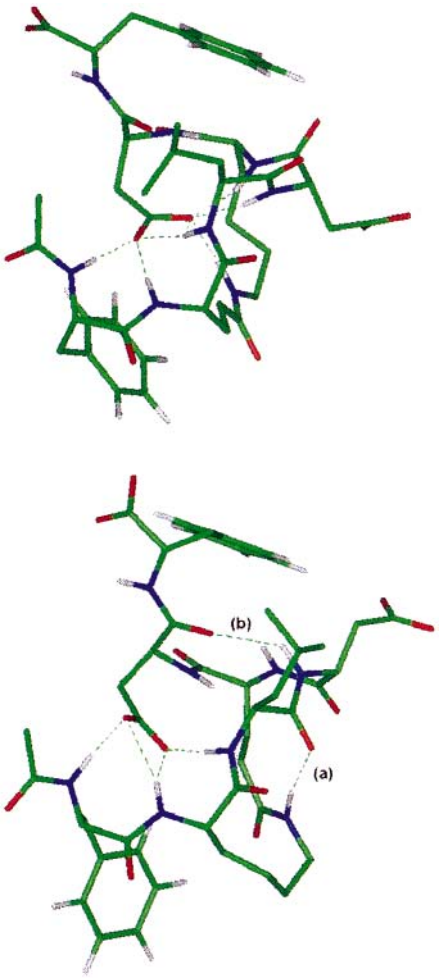

Figure 3. Hydrogen bonding pattern (N in blue, O in red) of the low-energy conformation of **5** (top) compared to the low-energy conformation of **6** (bottom).

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was performed for cyclic peptides **2**−**6**. The predicted low-energy conformations were then compared to the conformation of peptide **1** bound to mR1, which we had determined by transfer-NOE NMR studies.[3] Peptide **5** exhibited a predicted turn structure, similar to that found in peptide **1** (Figure 2). Peptides **3** and **4** also had predicted conformations nearly identical to that of peptide **5**, consistent with their binding affinities.

Previous studies found that both Phe 1 and Phe 7 are important for overall activity. We therefore expected the distance between the two phenyl rings to be similar for all active compounds. This distance (aromatic C_1 to aromatic C_1) for peptides **1**, **3**, **4**, and **5** was measured to be 9.85, 10.18, 10.14, and 9.94 Å, respectively. In peptide **2**, the predicted backbone structure was markedly altered and the overall distance between Phe 1 to Phe 7 collapsed to 6.81 Å, consistent with the lower binding affinity to mR1.

Several changes in hydrogen bonding were also seen on comparing the predicted structures of peptides **5** and **6** (Figure 3). The two most important were hydrogen bonds (a) between the carbonyl of Leu 3 and the ϵ-nitrogen of the lactam ring and (b) between the nitrogen of Asp 4 and the α-carbonyl of Asp 6, which were present in peptide **6** and which have no equivalent in peptide **5**. The absence of intra-ring hydrogen bonding thus appears critical for high-affinity binding. Other changes, involving the β-carbonyl of Asp 6, are less likely to be important, since this side chain appears to be nonessential for the peptide binding to R1.[2c]

While there is a strong overall correlation between the peptide backbones of peptides **1** and **3**−**5** (Figure 2), the rather poor side chain−side chain overlap between these cyclic compounds and peptide **1** suggests that compounds with improved affinity for R1, based on the lactam structure of peptide **5**, may be obtainable. Efforts in this direction are underway in our laboratory.

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