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Design, synthesis, and evaluation of octahydropyranopyrrole-based inhibitors of mammalian ribonucleotide reductase

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Abstract—Inhibitors of mammalian ribonucleotide reductase possessing a novel octahydropyranopyrrole scaffold based on a cyclic heptapeptide inhibitor have been designed, synthesized, and evaluated. Structure–function studies reveal that the bicyclic scaffold is indeed necessary to maintain inhibitory activity.

Ribonucleotide reductases (RR) constitute well-recognized targets for cancer and antiviral intervention, given the central role this enzyme plays in regulating DNA replication.[1] In 1990, Cooperman and co-workers demonstrated that the mammalian ribonucleotide reductase (mRR), a class Ia RR, can be inhibited via competitive binding at the R1 subunit by the heptapeptide N-AcFTLDADF (linear P7, IC_{50} = 20 μM), which corresponds to the C-terminus of the R2 subunit.[2] Transfer-NOE NMR studies demonstrated that P7 bound to mRR assumes a reverse β-turn structure consisting of the amino acids TLDA.[3] By exploiting a lactam bridge to constrain the turn, we subsequently reported the design, synthesis, and evaluation of several cyclic oligopeptides, of which cyclic peptide **1** (Fig. 1) revealed a higher binding affinity to mR1 by 2.5 times relative to the linear peptide P7.[4] More recently, we have focused on the design and synthesis of inhibitors of mRR based on non-peptidal scaffolds, which hold the promise of both higher affinity and selectivity, as well as bio-availability and stability, and hence would hold the promise of greater therapeutic potential. To this end, we reported the use of a tetrahydropyran scaffold to constrain the β-turn.[5] The resultant inhibitor **2**, however, proved to be significantly less potent (20×) than P7. In this letter, we describe the design, synthesis, and biological evaluation of an inhibitor of mRR based on cyclic oligopeptide **1**, employing an octahydropyranopyrrole scaffold to mimic the β-turn.

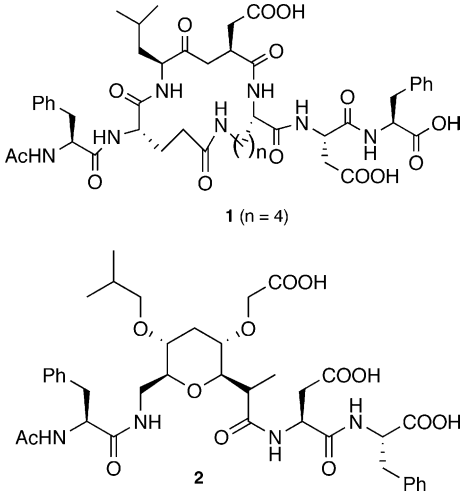

Figure 1. Top: cyclic-P7 (**1**); bottom: tetrahydropyran-based inhibitor **2**.

At the outset, we reasoned that the low binding affinity of **2** to mR1 resulted from steric conflicts with either or both of the 2-*O*-carboxymethyl and 4-*O*-isobutyl pendant groups. Structure–function studies on the interaction of the linear P7 peptide suggest that these groups may not be essential for inhibitory activity; we therefore redesigned and then constructed two pyran-based mimetics **3a** and **3b**, which (1) exclude the Asp^4 side-chain mimic and (2) accommodate a more concise synthetic approach (Scheme 1). The syntheses of **3a**

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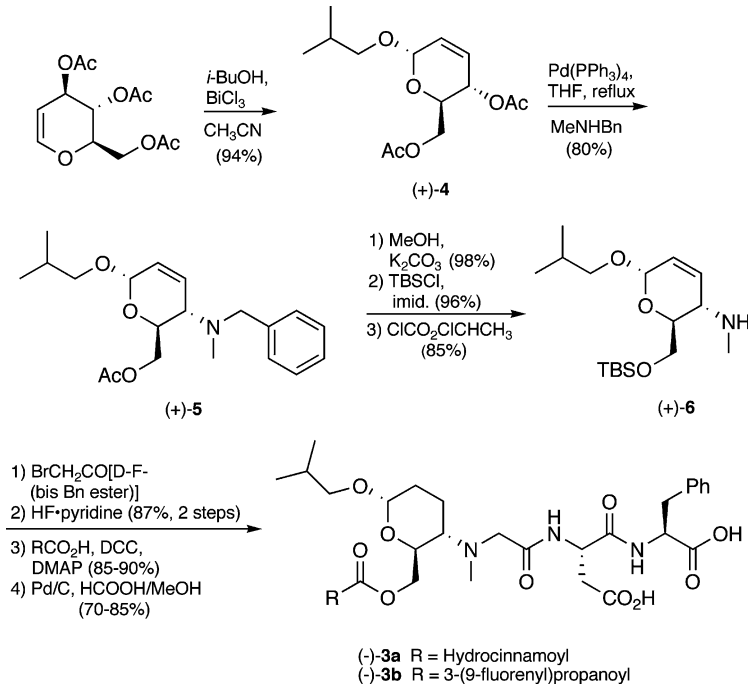


Scheme 1.

and **3b** began with a Ferrier glycosidation[6] involving tri-*O*-acetyl-D-glucal and isobutanol. Palladium-catalyzed allylic amination with *N*-benzyl methyl amine, employing the Baer and Hanna protocol,[7] provided amine (+)-**5** in 75% yield for the two steps. Acetate methanolysis[8] followed by sequential TBS protection of the resultant alcohol and N-debenzylation using the Olofson reagent[9] furnished amine (+)-**6** in 80% yield for the three-step sequence. Installation of the peptidyl side chain followed in turn by alkylation with BrAc-D-Asp-D-Phe bis-benzyl ester, TBS removal, DCC-mediated esterification, and hydrogenolysis led to prospective inhibitors (−)-**3a** and (−)-**3b**. The extent to which (−)-**3a**,**b** bind to mR1 and thereby inhibit enzyme activity was measured employing an assay previously developed in our laboratory based on an FTLDADF-Sepharose affinity column.[10] Assay results revealed that (−)-**3a** and (−)-**3b** exhibited little or no inhibitory activity. We therefore abandoned further efforts to exploit the tetrahydropyran as a scaffold for the development of mRR inhibitors.

Molecular design and modeling exploiting the MM2 force field included with MacroModel (v3.1) suggested that the octahydropyranopyrrole scaffold might be a more appropriate platform upon which to attach and thereby display the appropriate side chains corresponding to the key amino acid residues present in **1**. We initially targeted peptidomimetic **7** for synthesis; the appendages include a hydrocinnamate ester, an isobutyloxy group, and a free hydroxyl to mimic the N-AcPhe1, Leu[3], and Asp[4] residues, respectively, and the requisite Asp[6]-Phe[7] moiety for the C-terminus. Illustrated in Figure 2 is an overlay of the MM2 minimized structure of **7** (gray) on the NMR-derived conformation of cyclic peptide **1** (green).

The synthesis of **7** (Scheme 2) again began with the bismuth (III) trichloride mediated Ferrier glycosylation[6] of commercially available (−)-tri-*O*-acetyl-D-glucal with isobutanol. The corresponding glycoside (7.8:1 α/β ratio) was then subjected to acetate methanolysis[8] and monosilylation (TIPSOTf, 2,6-lutidine, −5 °C)[11] to furnish allylic alcohol (+)-**8** in 83% yield for the three steps. Swern oxidation,[12] followed by Johnson iodination,[13] next led to α-iodoenone (+)-**9** (92%, two steps), which upon Luche reduction ($NaBH_4$, $CeCl_3{\cdot}7H_2O$, MeOH)[14] produced a separable mixture (2.3:1) of alcohols, of which the major isomer was reacted under Mitsunobu conditions[15] with the

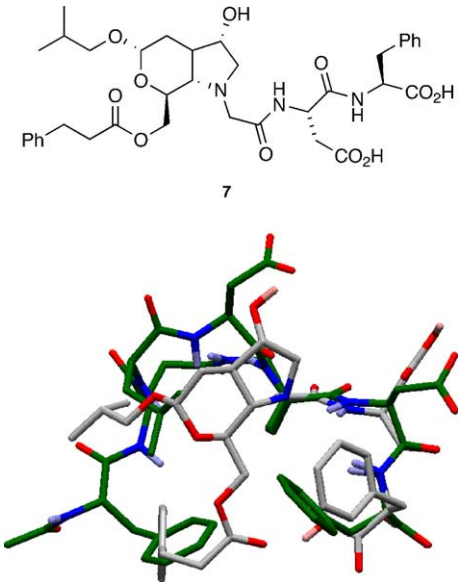
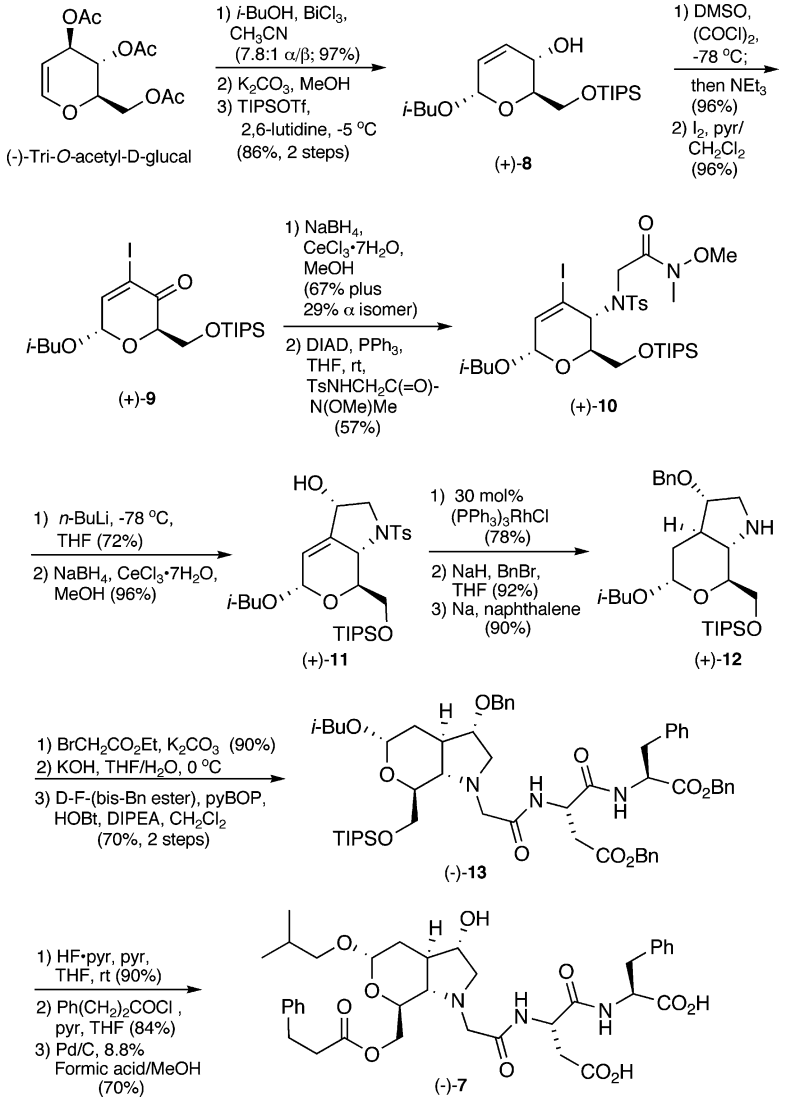


Figure 2. Top: peptidomimetic target **7**; bottom: overlay of the MM2 minimized structure of **7** (gray) on the NMR-derived conformation of cyclic peptide **1** (green).



Scheme 2.

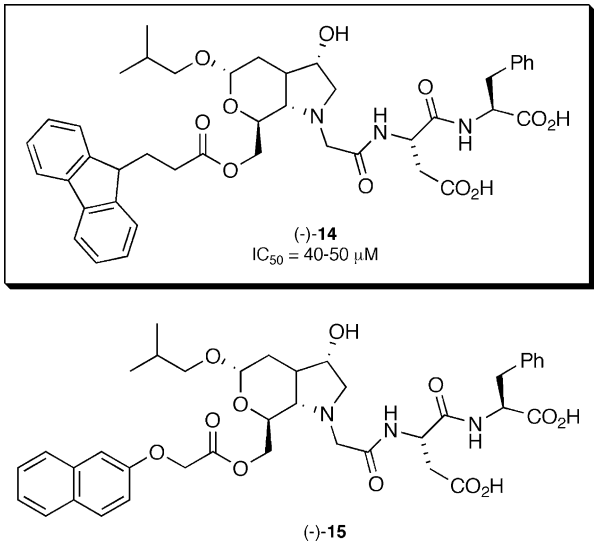


Figure 3. Active mRR inhibitor (−)-**14** and inactive compound (−)-**15**.

Weinreb amide derived from *N*-Ts glycine to furnish aminoglycolate (+)-**10** in 38% (two steps). Anionic cyclization (*n*-BuLi, THF, −78 °C, 72%),[16] followed by a second Luche reduction (96%) of the resultant enone, then gave allylic alcohol (+)-**11** as a single isomer. Hydroxyl-directed hydrogenation (30 mol % Wilkinson's catalyst),[17] protection of the alcohol as the benzyl ether (92%), and reductive removal of the tosyl group (Na, naphthalene, 90% yield)[18] cleanly furnished amine (+)-**12**. Installation of the C-terminal peptidal side chain was then achieved by sequential alkylation with ethyl bromoacetate (90% yield), basic hydrolysis (KOH, THF/H_2O), and coupling with D-Asp-D-Phe bis-benzyl ester (pyBOP, HOBt, DIPEA, CH_2Cl_2, 70% for two steps). Removal of the triisopropylsilyl-protecting group (HF·pyridine, 90%),[19] acylation of the resultant alcohol with hydrocinnamoyl chloride, and global deprotection employing transfer hydrogenation conditions (8.8% formic acid/MeOH, 10% Pd/C)[20] completed the synthesis of (−)-**7**.

Pleasingly, mimetic (−)-**7** did indeed inhibit mRR, although 5–6 times less potently than P7. Encouraged by this result, we prepared two related congeners, (−)-**14** and (−)-**15** (Fig. 3), wherein only the N-terminal carboxylate moiety was varied. Assay results revealed that (−)-**14** exhibited increased activity relative to (−)-**7**, whereas (−)-**15** had little or no inhibitory activity. Compound (−)-**14**, which contains the 3-(9-fluorenyl)propanoyl group, proved to be our most potent compound (IC_{50} = 40–50 μM), exhibiting an activity roughly half of P7.

In summary, we have identified octahydropyranopyrrole as a viable scaffold for the construction of β-turn mimetics. Moreover, we have exploited this structural motif to access our most potent small molecule nonpeptide peptidomimetic inhibitor of mRR to date. Further investigations to unveil additional structure–activity relationships of this scaffold, and thereby to maximize inhibition of mRR, continue in our laboratory and will be reported in due course.

Acknowledgments

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