UNIVERSITÀ DEGLI STUDI DI MILANO
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RSH Inhibitors to Hamper Persisters Formation

Marco Minneci,[a] Lorenzo Bisconti,[a] Luca Sorrentino[a] and Sara Sattin[a]*

Department of Chemistry, Università degli Studi di Milano,
Via Golgi 19, 20133 Milano (MI)
lorenzo.bisconti@unimi.it

Background: Persisters are phenotypic variants of regular cells in bacterial population that stochastically enter a dormant, drug-tolerant state [1]. Interestingly, when persisters awake, they give rise to an isogenic population still susceptible to antibiotics [1]. This phenomenon is linked to the difficult eradication of chronic infections in humans [2]. One of the working hypotheses for persisters formation is the survival signalling cascade called Stringent Response (SR).

Aim: The first step of SR is (p)ppGpp (guanosine tetra- and/or penta-phosphate accumulation, a transformation is catalysed by the RSH (RelA/ SpoT Homologue) enzyme superfamily (Figure 1) [3]. Our goal is to design and synthesize new RSH inhibitors, possibly hampering persisters formation; in order to test their inhibitory effect on RSH synthetase activity, we will have to detect the presence of (p)ppGpp in solution. We present the first results towards to set up of the appropriate enzymatic assay.

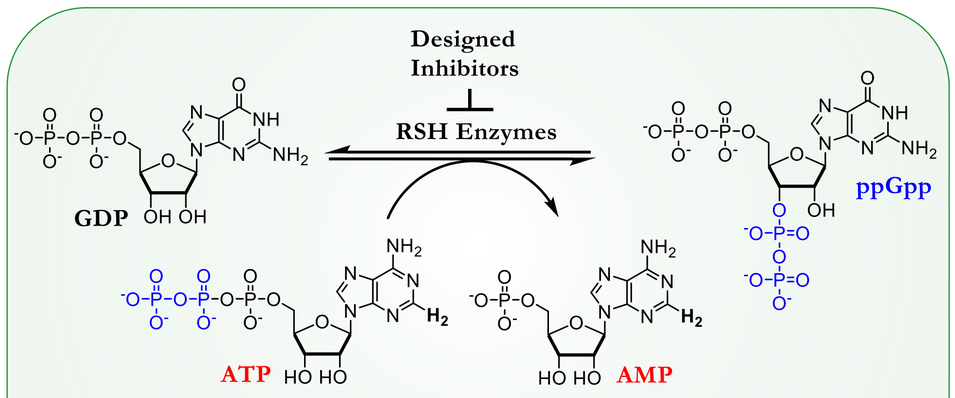


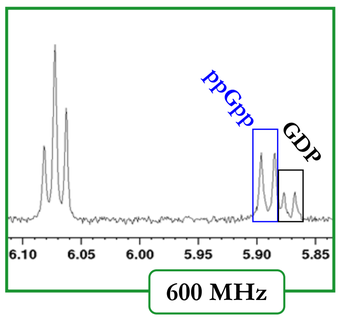
Fig. 1: ppGpp Synthesis catalysed by Rel_{Seq} Reaction conditions: Hepes 50mM, $MgCl_2$: 10 mM, DTT: 1 mM, **ATP:** 6 mM, **GDP:** 3 mM, **Rel_{Seq}:** 0.2 mg/L, **pH:** 7.5

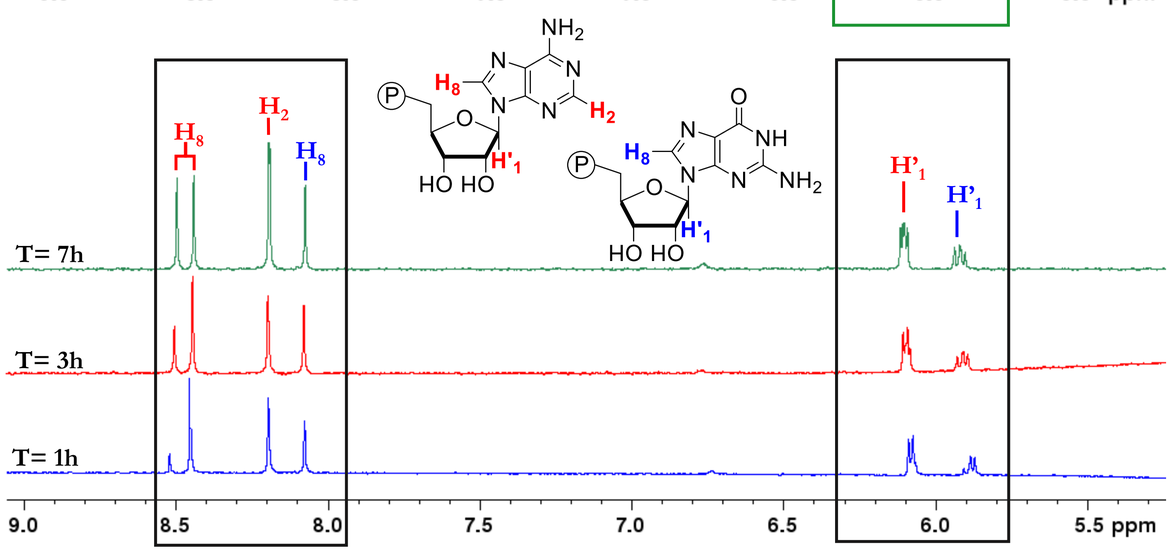
Work plan:

1. Production and purification of ppGpp as standard for analytical and biological assays.
2. Set up of an enzymatic assay for testing the inhibitory effect of the designed compounds on the synthetic activity of RSH proteins..

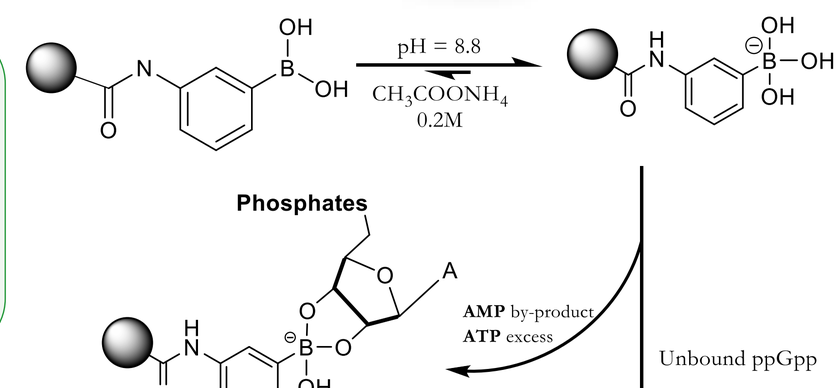
NMR Monitoring of ppGpp Synthesis

ppGpp Purification Protocol



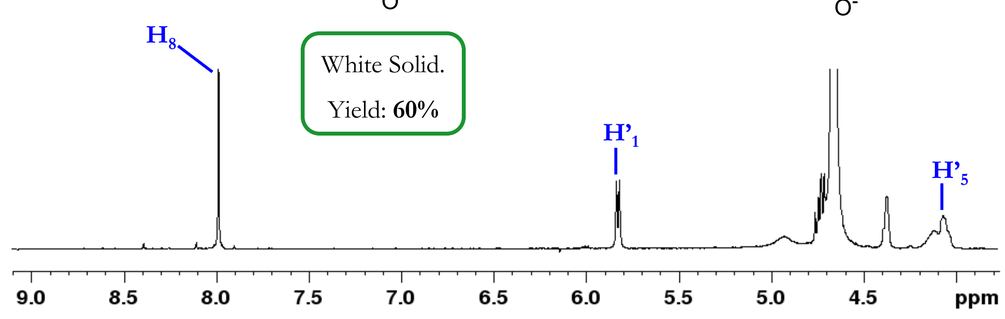


Affinity Chromatography with Immobilized Boronic Acid Gel: The boronate **$-B(OH)_3^-$** made covalent bond only with analytes possessing suitable *cis*-diols [4].

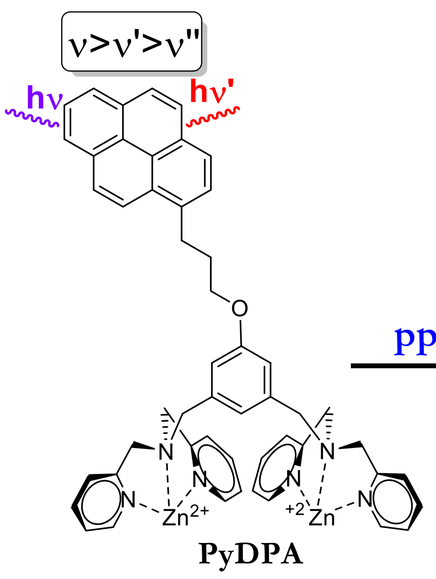


NMR Monitoring:
Instrument: 400 MHz
Solvent: H_2O-D_2O (10% -90%)
Mode: Water suppression was achieved using the excitation sculpting pulse sequence

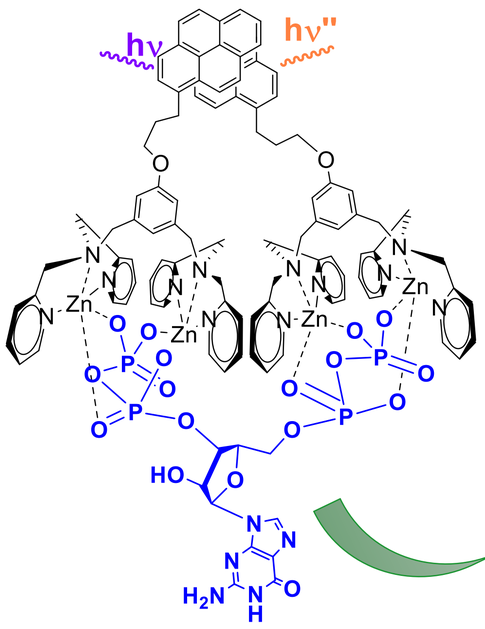
White Solid.
Yield: **60%**

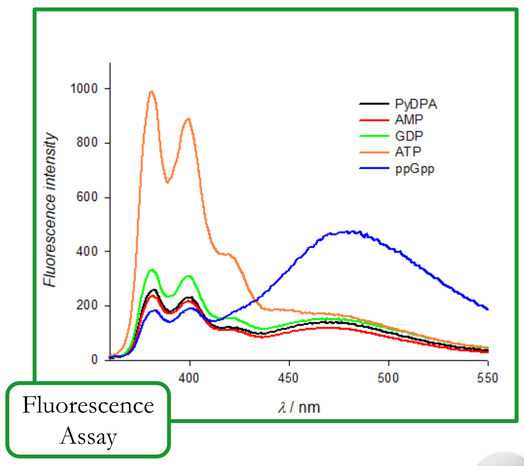


Selectively Detection of ppGpp: Usually this reaction is followed by **^{32}P-GDP** or qualitatively with PEI-Cellulose TLC. The NMR analysis needs long proton relaxation time (≥30 seconds) to gain good quality spectra. In a recent work we proposed the synthesis and the activity of PyDPA able to give selective detection of ppGpp in reaction conditions using fluorescence analysis [6].



ppGpp





Fluorescence Assay

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