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Supplementary Information

Hyperpolarization study on Remdesivir with its biological reaction monitoring via signal amplification by reversible exchange

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EXPERIMENTAL SECTION

Sample Preparation. Remdesivir was purchased from eNovation Chemicals LLC (New Jersey, United States) and used without further purification (Its purity was confirmed via ^{1}H-NMR). Methanol-d_4 (CD_3OD, 99.8 atom %D, Eurisotop) and dimethyl sulfoxide-d_6 (CD_3SOCD_3, 99.8 atom %D, Eurisotop) were also used in the form obtained. In the experiment using Pre-catalyst1 (IMes-Ir, [Ir(IMes)(COD)Cl], 2 mg, $3.1x10^{-3}$ mmol), remdesivir (18 mg, $3.1x10^{-2}$ mmol) was dissolved in CD_3OD (900 μL) and CD_3SOCD_3 (900 μL), individually[1]. Pre-catalyst2 (Crabtree's-Ir, $[Ir(COD)(PCy_3)(py)]PF_6$, 2 mg, $2.5x10^{-3}$ mmol) was resolved to solution of remdesivir(15 mg, $2.5x10^{-2}$ mmol) in CD_3OD (900 μL).

SABRE Catalyst Activation and Measurement of Hyperpolarized Signal. ^{1}H NMR spectra used for the characterization of remdesivir was acquired on a Bruker Avance □□□ NMR spectrometer operating at a ^{1}H resonance frequency of 300MHz and referenced to the residual CH_3 peak of methanol-d_4 (δ=3.31) or CH_3 peak of dimethyl sulfoxide-d_6 (δ =2.50). The *para*-hydrogen generator was composed as a home-built instrument, in which hydrogen gas (Hanmi gas, >99.9%, a mixture the spin isomers ortho-hydrogen and *para*-hydrogen) was allowed to pass through a heat exchanger filled with a FeO(OH) catalyst (Sigma Aldrich)[2-4]. This instrument was filled with liquid nitrogen in a Dewar flask generating ca. 50% *para*-hydrogen. In each experiment, *para*-hydrogen continuously flowed into the drug candidate sample at a rate of 6mL/min at 23 □C and 1 atm. The following system was established and developed to gain various magnetic field data: the power supply was GPS-1850D (Bench Power Supply, Linear DC). A shielded coil wound with copper-coated wire and a shielded coil on top was 200 mm in diameter and 190 mm in height. The magnetic field through the shielded coil was controlled by setting the current, which was in the range of 0-5 A. The magnetic field produced by the modulated current was measured using a Lakeshore Gauss meter.

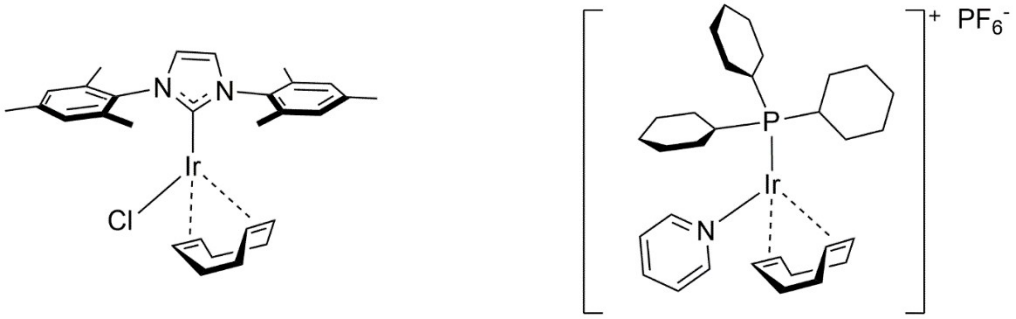
For the calculation of the ^{1}H signal enhancement factor (fold), the signals of samples amplified through hyperpolarization and those of non-amplified samples were compared by using the below equation[1]. The enhancement factor was estimated using the raw integral of the hyperpolarized and non-polarized spectra. Solvent peaks (CD_3OD, CD_3SOCD_3) were matched to determine the exact integral of the signal through the same chemical shift region.

$$signal\ enhancement\ factor\ (fold) = \frac{signal\ of amplified\ sample}{signal\ of\ non-amplified\ sample}$$

In order to activate the mixture of the substrate and the catalyst, the sample was bubbled by inserting *para*-hydrogen for 20 min in the NMR tube under the earth's magnetic field. Samples were injected rapidly in less than 5 sec into a 300 MHz NMR spectrometer to measure the hyperpolarized signals. Individually, measurements were made with differences in the magnetic field (the earth's magnetic field, 30G, 50G, 70G, 90G, 110G, and 130G) and signals of hyperpolarized hydrogen were regularly obtained. All NMR spectra were measured in each magnetic field by bubbling for another 1 min with 50% *para*-hydrogen at 23 □C and 1 atm with 1 scan. The various experiments of hyperpolarization were accompanied in the same manner as mentioned above.

Enzymatic Hydrolysis of Remdesivir and Reaction Monitoring using SABRE Hyperpolarization.

The esterase from the porcine liver (PLE, lyophilized powder, 15 units/mg) was purchased from Sigma-Aldrich. The PLE (20mg, 300units) was suspended in deuterium oxide (1mL). The sample for reaction monitoring was prepared with remdesivir (18mg, $3.1x10^{-2}$ mmol) and pre-catalyst1 (IMes-Ir, [Ir(IMes)(COD)Cl], 2mg, $3.1x10^{-3}$ mmol) in CD_3SOCD_3 (800μL) by adding enzymic stock solution (100μL). The sample solution was kept constant at pH 8 by the addition of 0.1N sodium hydroxide solution[5-7]. The reaction mixture was moved to a 5mm NMR tube and activated by *para*-hydrogen for 20 min at 110G. The bubbling process by *para*-hydrogen substituted the stirring of solution for homogeneity. After activation, individual hyperpolarized spectrum was obtained by using the same method of hyperpolarized signal measurement procedure described above, at 10min intervals over 2 hours.



IMes-Ir-catalyst (pre-catalyst1) **Crabtree's-Ir-catalyst (pre-catalyst2)**

Figure S1 Structures of Pre-catalysts

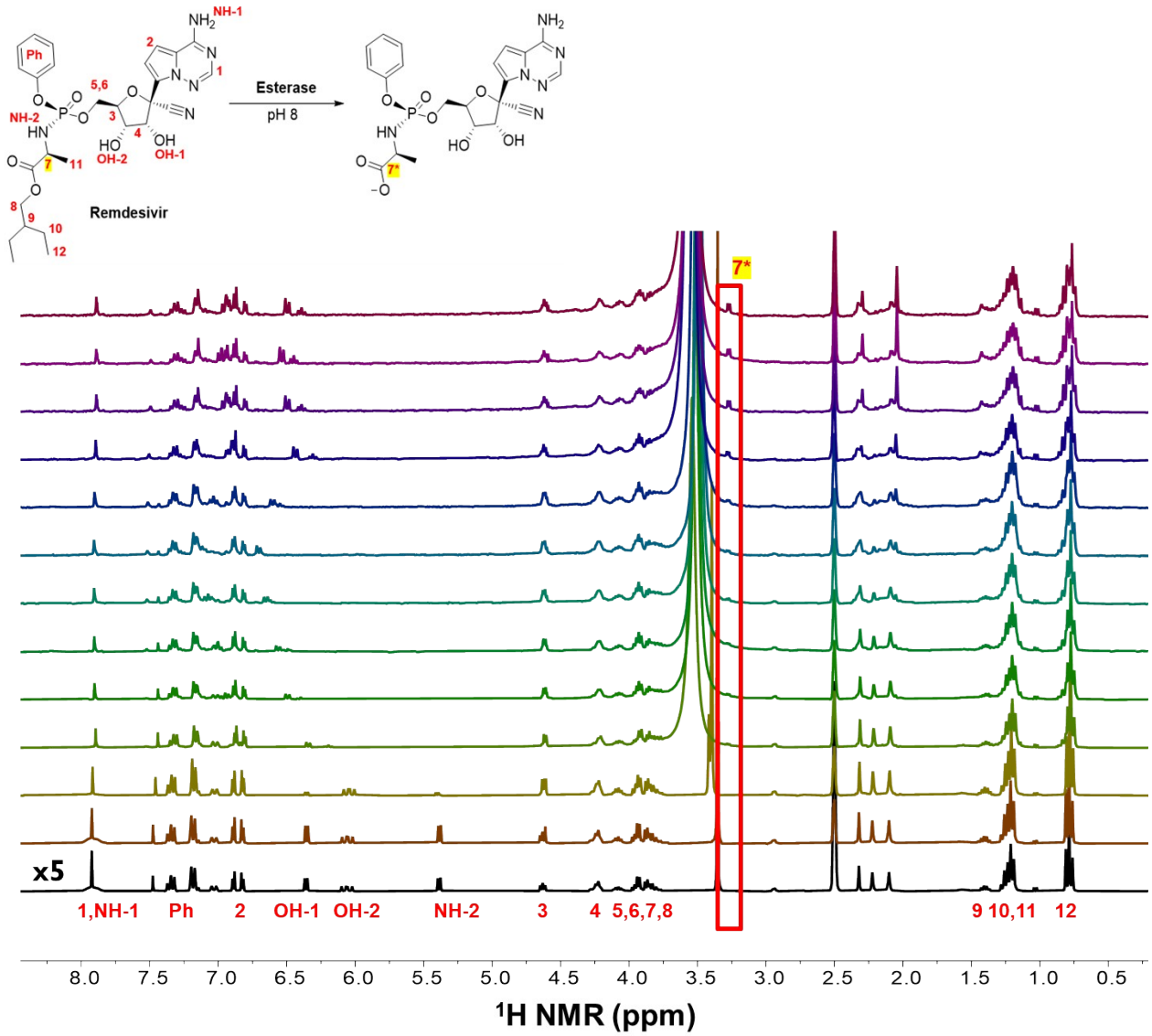


Figure S2 ^{1}H spectra of enzymatic hydrolysis monitoring of remdesivir for 120 min; elimination of 2-ethylbutyl ester group

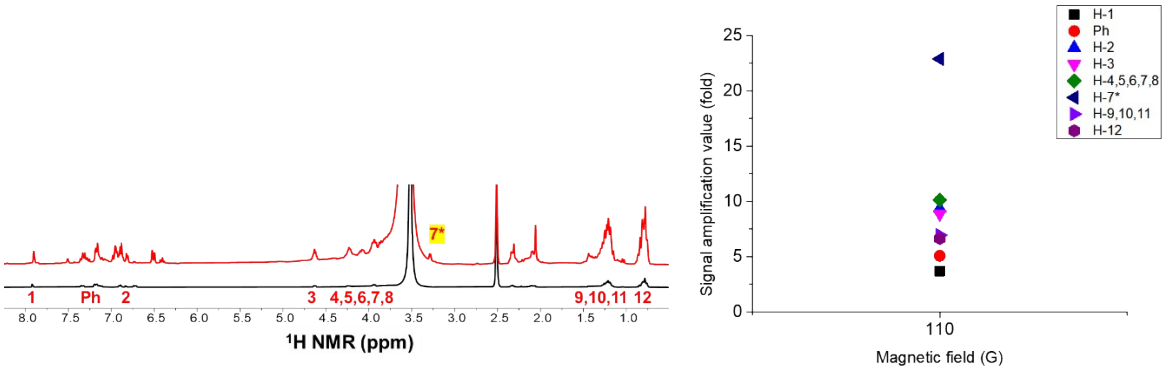


Figure S3 ^{1}H spectra of remdesivir: enzymatic hydrolysis after 120 min (black spectrum) and amplified through hyperpolarization (red spectrum) at the same time (left); Amplification number of protons from hyperpolarized enzymatic hydrolysis of remdesivir after 120min (right).

REFERENCES

1. L. SJ, J. K, S. JH, L. HJ, M. S, C. H, N. SK and K. K, *Scientific reports*, 2019, **9**, 12422-12429.
2. H. Chae, S. Min, H. J. Jeong, S. K. Namgoong, S. Oh, K. Kim and K. Jeong, *Analytical Chemistry*, 2020, **92**, 10902-10907.
3. H. J. Jeong, S. Min, H. Chae, S. Kim, G. Lee, S. K. Namgoong and K. Jeong, *Scientific Reports*, 2020, **10**, 1-13.
4. H. J. Jeong, S. Min and K. Jeong, *Analyst*, 2020, **145**, 6478-6484.
5. G. P and J. WP, *Biochemistry*, 1971, **10**, 1210-1222.
6. A. Pinto, I. Serra, D. Romano, M. L. Contente, F. Molinari, F. Rancati, R. Mazzucato and L. Carzaniga, *Catalysts*, 2019, **9**, 113-123.
7. P. Süss, S. Illner, J. v. Langermann, S. Borchert, U. T. Bornscheuer, R. Wardenga and U. Kragl, *Organic Process Research and Development*, 2014, **18**, 897-903.