Supporting Information For

Over 20% ^{15}N Hyperpolarization in Under One Minute for Metronidazole, an Antibiotic and Hypoxia Probe

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1. Experimental Details

1.a. Metronidazole stock solutions preparation

To an Eppendorf safe-lock tube (1.5 mL) metronidazole (0.026 g, 0.15 mmol, 0.10 M final concentration), non-activated iridium catalyst ([IrCl(COD)(IMes)], MW ~ 640, 3.2 mg, 0.0075 mmol, 0.0050 M final concentration, prepared in the previous studies[1]) was added. It was followed by the addition of methanol-d_4 (1.5 mL total volume). The tube with stock solution (**I**) was flushed with Argon and vortexed. In order to achieve 50% lower tracer and catalyst concentration, the stock solution (**II**), 0.50 mL the stock solution (**I**) was transferred via Ranin XLS pipet to another Argon-flushed Eppendorf safe-lock tube (1.5 mL) and 0.50 mL of methanol-d_4 was added.

1.b. Metronidazole activation

The stock solution (**I**) or (**II**) (0.50 mL) was transferred via Ranin XLS pipet into an Argon-filled medium-walled NMR sample tube (5 mm medium wall precision, 3.43 mm ID, 9 in. long, Wilmad glass P/N 503-PS-9) equipped with the Teflon tube extension (0.25 in. OD, 3/16 in. ID), which was approximately 7 cm long. The tube was attached to the previously described setup[2] through wye push-to-connect adapter.[2] The SABRE sample was activated by bubbling parahydrogen at 140 sccm for (~1 min) and at 20 sccm flow rate (~5 min) under ~6 atm parahydrogen (~50% *para*- fraction or ~80% *para*-fraction); flow rate was controlled by the mass flow controller (Sierra Instruments, Monterey, CA, model number C100L-DD-OV1-SV1-PV2-V1-S0-C0).

1.c. ^{1}H SABRE hyperpolarization

^{1}H SABRE hyperpolarization procedure (Figure S3) was performed similarly to that described earlier.[1] Briefly, the sample tube with activated catalyst and to-be-hyperpolarized substrate is placed in the fringe field of the magnet at 6±4 mT (measured with gauss meter), and parahydrogen is bubbled for ~30 seconds using the setup described above.

1.d. ^{15}N SABRE-SHEATH hyperpolarization

Activated at room temperature samples were cooled by placing them on ice for at least 3 minutes. This "temperature cycling" of samples yielded better signal enhancements in general. ^{15}N SABRE-SHEATH hyperpolarization procedure was conducted similarly to that described earlier.[1] The sample solution was bubbled with parahydrogen (~50% (SI) or 80% (main text) *para*- fraction 140 sccm (for ~1 min, at ~6 atm) inside the magnetic shield for a period of ~1 min. The Earth's magnetic field was attenuated using three-layered mu-metal shield (6 in. ID & 15 in. in length, part number ZG-206, Magnetic Shield Corp., Bensenville, IL), which was degaussed before use. The magnetic field was created using a custom-built solenoid coil and a power supply (GPRS series, GW INSTEK). After stopping parahydrogen bubbling the sample was quickly transferred from the shield to the Earth's magnetic field followed by sample insertion in the bore of 9.4 T magnet and acquisition of the ^{15}N NMR spectrum (Figures S4 and S5). ^{15}N chemical shifts were referenced to external urea-$^{15}N_2$ sample calibrated to 77.6 ppm. ^{15}N peaks integrals were integrated with respect to a sample of neat pyridine-^{15}N (integral value was set to 1.00).

1.e. Calculation of SABRE polarization enhancement factors

^{1}H SABRE enhancements (Table S1) were calculated by comparing integral signal intensities of corresponding NMR peaks of the spectra of hyperpolarized and thermally polarized conditions. ^{15}N

SABRE-SHEATH enhancements were calculated by comparing integral signal intensities of all hyperpolarized NMR peaks obtained from the hyperpolarized sample in a 5 mm medium-walled NMR tube at 100 mM or 50 mM concentration of metronidazole and referencing it to the ^{15}N NMR signal from a thermally polarized ~12.4 M solution of pyridine-^{15}N (neat, purchased from Isotec-Sigma-Aldrich) in a standard 5 mm NMR tube. The following formula was used for ^{15}N signal enchantments:

$$\varepsilon = (S_{HP}/S_{REF})*([REF]/[HP])* (A_{REF}/A_{HP}),$$

where S_{HP} is hyperpolarized signal, S_{REF} is a signal from the reference compound, [REF] and [HP] are concentrations of reference (~12.4 M) and hyperpolarized (0.150, 0.100 or 0.050 M times ^{15}N natural abundance factor) samples, respectively, A_{REF} and A_{HP}, are the effective cross sections (i.e. inner area) of the NMR tubes for reference and hyperpolarized samples. (A_{REF}/A_{HP}) was calculated as $4.14^2/(3.43^2 - 1.59^2) = 1.85$, where 4.14 mm is the ID of standard NMR tube, 3.43 mm is the ID of medium wall NMR tube, and 1.59 mm is the OD of the Teflon capillary (for parahydrogen bubbling) inserted in the HP sample.[3] For example, for the data presented in Figure 2b, ^{15}N signal enhancement was calculated as the following:

$$\varepsilon = (0.5804/1)*[(12.4*0.98)/(0.050*0.00364)]*1.85 \sim 7.2*10^4$$

Table S1. Calculations of ^{15}N signal enhancements and polarization levels (P).

	Figure 2b	Figure 2c		
		NO_2-group	N-group	N-CH_3-group
Thermal integral	1	1	1	1
HP integral	0.5804	0.0049	1.092	0.0196
Thermal concentration (M)	12.2	12.2	12.2	12.2
HP concentration (M)	0.000182	0.000546	0.000546	0.000546
Signal enhancement	~$7.2*10^4$	~$2*10^2$	~$4.5*10^4$	~$8*10^2$
P_{15N} (actual, 80% p-H_2)	~24	~0.07	~15	~0.26
P_{15N} (extrapolated to ~100% parahydrogen)	~32	~0.09	~20	~0.36

Experiments were performed with both ~80% parahydrogen (main text, maximum currently available in the laboratory) and ~50% parahydrogen (see Figures S3-S5).

2. Spectral Data

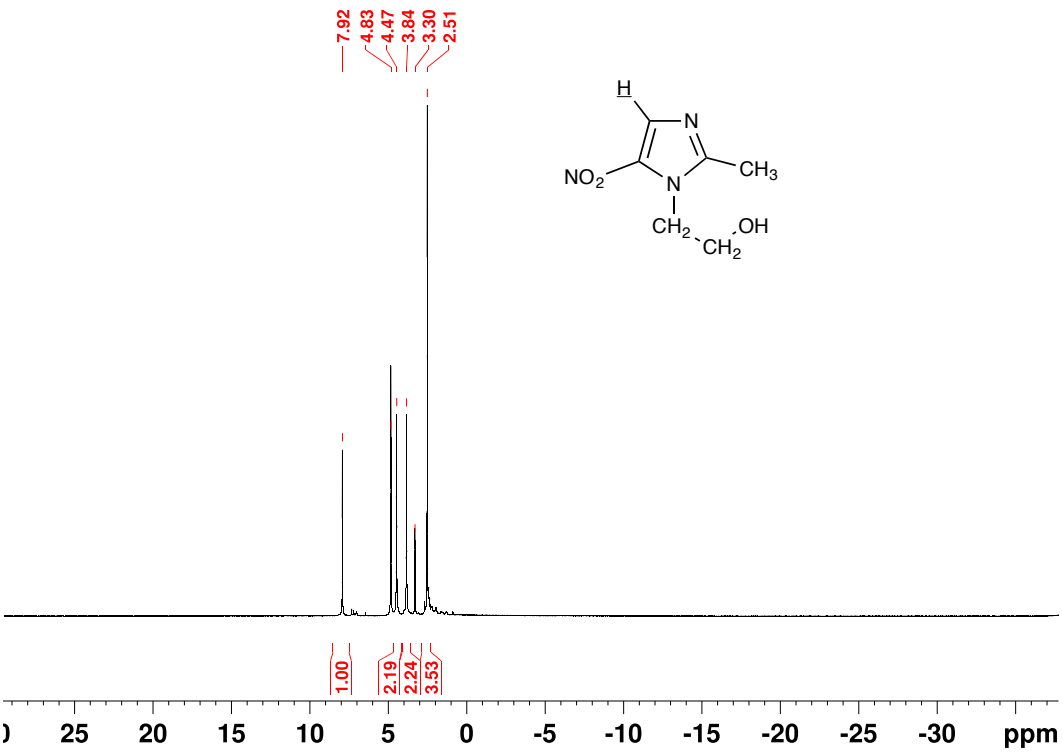

Figure S1. High-resolution thermal ^{1}H spectrum NMR spectrum of metronidazole (0.100 M) and Ir-IMes SABRE catalyst[4] (0.0050 M) in methanol-d_4.

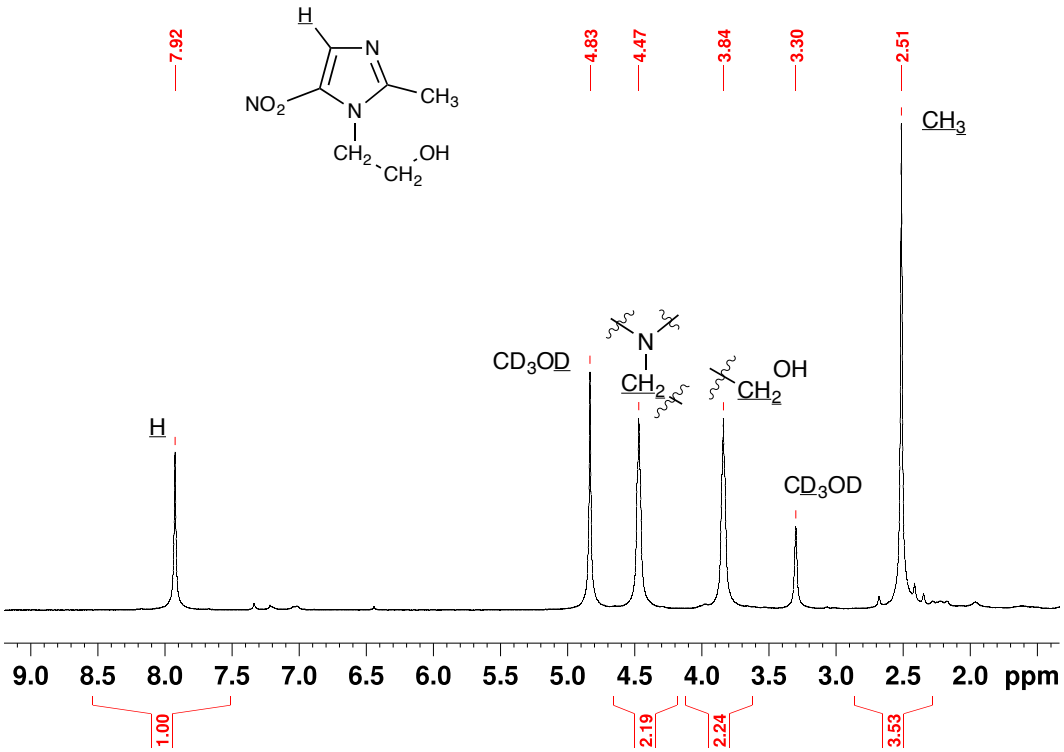

Figure S2. High-resolution ^{1}H NMR spectrum of metronidazole (0.100 M) and Ir-IMes SABRE catalyst[4] (0.0050 M) in methanol-d_4 (metronidazole peaks' region with the complete NMR peak assignments).

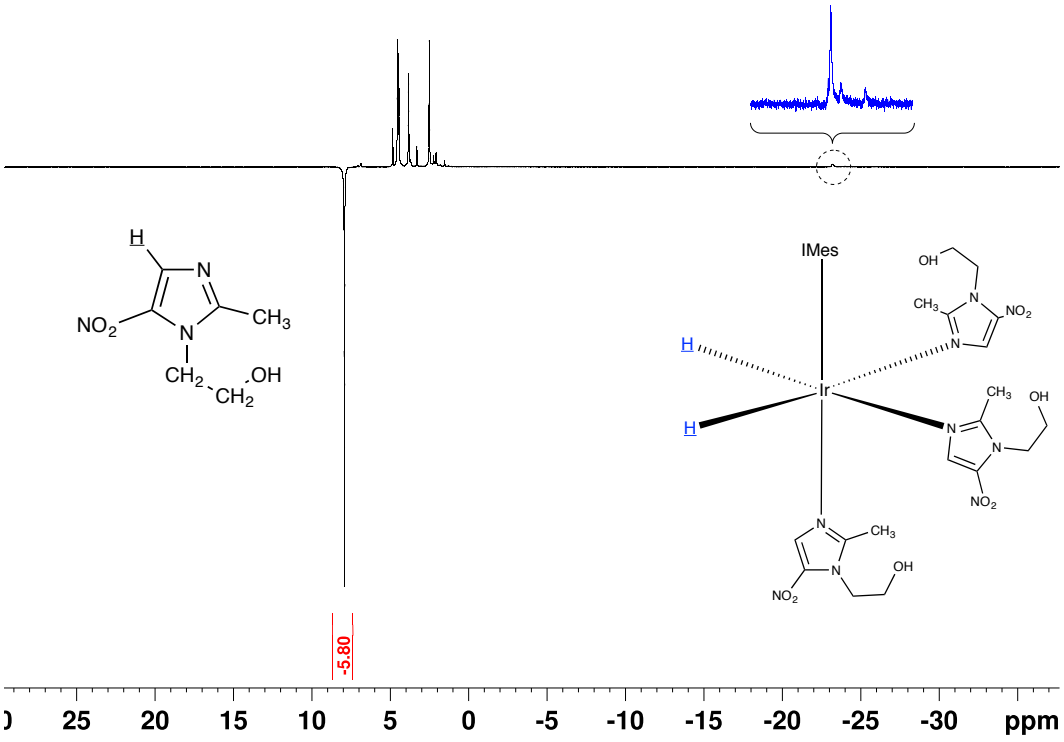

Figure S3. High-resolution Hyperpolarized (HP) ^{1}H NMR spectrum of metronidazole (0.10 M) and Ir-IMes SABRE catalyst (0.0050M) in methanol-d_4 (the spectral region of SABRE-active Ir-hydride is also shown). The data is obtained using 50% parahydrogen.

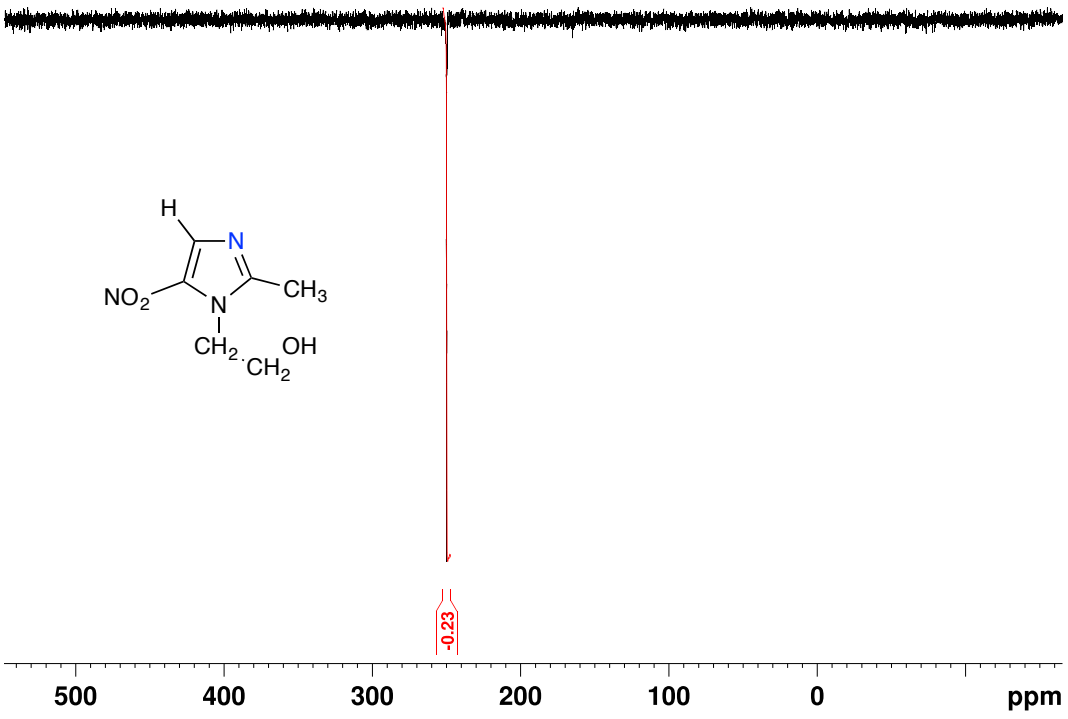

Figure S4. High-resolution hyperpolarized (HP) ^{15}N NMR spectrum of metronidazole (0.050 M) and Ir-IMes SABRE catalyst (0.0025M) in methanol-d_4 (with NMR peak assignment). The data is obtained using 50% parahydrogen.

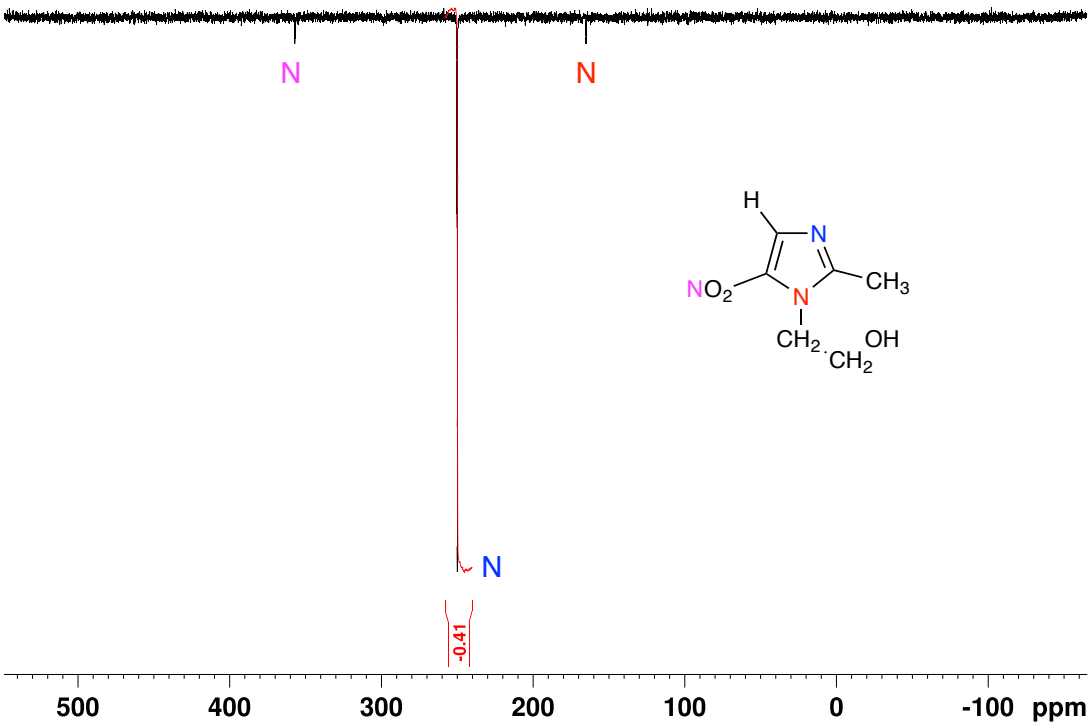

Figure S5. High-resolution hyperpolarized (HP) ^{15}N NMR spectrum of metronidazole (0.100 M) and Ir-IMes SABRE catalyst (0.0050 M) in methanol-d_4 (with peaks assignments). The data is obtained using 50% parahydrogen.

3. References Used in Supporting Information

(1) Theis, T.; Truong, M. L.; Coffey, A. M.; Shchepin, R. V.; Waddell, K. W.; Shi, F.; Goodson, B. M.; Warren, W. S.; Chekmenev, E. Y. *J. Am. Chem. Soc.* **2015**, *137*, 1404.

(2) Truong, M. L.; Shi, F.; He, P.; Yuan, B.; Plunkett, K. N.; Coffey, A. M.; Shchepin, R. V.; Barskiy, D. A.; Kovtunov, K. V.; Koptyug, I. V.; Waddell, K. W.; Goodson, B. M.; Chekmenev, E. Y. *J. Phys. Chem. B* **2014**, *18* 13882.

(3) Barskiy, D.; Kovtunov, K.; Koptyug, I.; He, P.; Groome, K.; Best, Q.; Shi, F.; Goodson, B.; Shchepin, R.; Truong, M.; Coffey, A.; Waddell, K.; Chekmenev, E. *ChemPhysChem* **2014**, *15*, 4100.

(4) Cowley, M. J.; Adams, R. W.; Atkinson, K. D.; Cockett, M. C. R.; Duckett, S. B.; Green, G. G. R.; Lohman, J. A. B.; Kerssebaum, R.; Kilgour, D.; Mewis, R. E. *J. Am. Chem. Soc.* **2011**, *133*, 6134.