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1 Article

## 2 Hyperpolarised $^1\text{H}$ - $^{13}\text{C}$ benchtop NMR spectroscopy

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8 **Abstract:** Benchtop NMR spectrometers with sub-ppm spectral resolution have opened up new  
9 opportunities for performing NMR outside of the standard laboratory environment. However, the  
10 relatively weak magnetic fields of these devices (1 - 2 T) results in low sensitivity and significant  
11 peak overlap in  $^1\text{H}$  NMR spectra. Here we use hyperpolarised  $^{13}\text{C}\{^1\text{H}\}$  NMR to overcome these  
12 challenges. Specifically, we demonstrate the use of the signal amplification by reversible exchange  
13 (SABRE) *parahydrogen*-based hyperpolarisation technique to enhance the sensitivity of natural  
14 abundance 1D and 2D  $^{13}\text{C}\{^1\text{H}\}$  benchtop NMR spectra. We compare two detection methods for  
15 SABRE-enhanced  $^{13}\text{C}$  NMR and observe an optimal  $^{13}\text{C}\{^1\text{H}\}$  signal-to-noise ratio (SNR) for a  
16 refocused INEPT approach, where hyperpolarisation is transferred from  $^1\text{H}$  to  $^{13}\text{C}$ . In addition, we  
17 exemplify SABRE-enhanced 2D  $^{13}\text{C}$  benchtop NMR through the acquisition of a 2D HETCOR  
18 spectrum of 260 mM of 4-methylpyridine at natural isotopic abundance in a total experiment time  
19 of 69 mins. In theory, signal averaging for over 300 days would be required to achieve a comparable  
20 SNR for a thermally polarised benchtop NMR spectrum acquired of a sample of the same  
21 concentration at natural abundance.

22 **Keywords:** NMR spectroscopy; Benchtop; low-field; *parahydrogen*; hyperpolarisation; SABRE  
23 (signal amplification by reversible exchange).  
24

### 25 1. Introduction

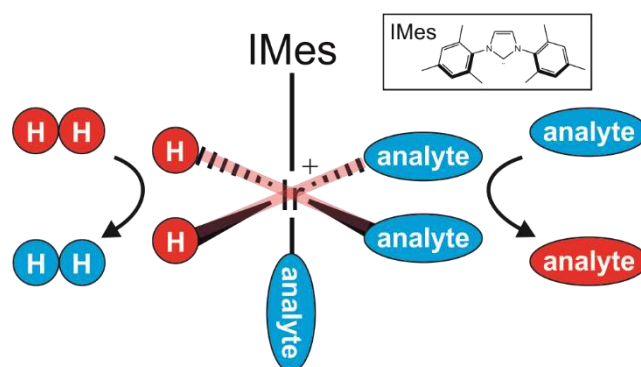
26 Benchtop NMR spectrometers have the potential to open up new applications for NMR  
27 spectroscopy outside of the traditional laboratory environment owing to the relative portability of  
28 these devices. Of particular interest are permanent magnet spectrometers with magnetic field  
29 strengths of 1 – 2 T that have the capability to record high-resolution NMR spectra [1]. Whilst several  
30 of the original NMR discoveries were made using permanent magnet NMR spectrometers [2–9], their  
31 usage became limited once strong and stable superconducting electromagnets became readily  
32 available [10–12]. In the early 2000s, the prospect of cheap, cryogen-free and compact NMR  
33 spectrometers prompted the resurgence of permanent magnet based systems [13]. A major obstacle  
34 to these spectrometers was the need for highly homogenous magnetic fields as high-resolution  
35 spectra require field spatial variations of less than tens of parts per billion [14]. It was through  
36 specifically designed Halbach arrays of magnets [15], advancements in shimming electronics and  
37 temperature stabilisation that these tens-of-ppb magnetic field homogeneities were achieved [16–18].  
38 From this point, a range of high-resolution benchtop NMR spectrometers with different field  
39 strengths and heteronuclear detection capabilities have been developed and implemented across a  
40 plethora of applications [19], such as industrial quality control [20–26],  $^1\text{H}$  and  $^{13}\text{C}$  NMR  
41 undergraduate teaching [27–32] and on-line reaction monitoring [33–39].

42 A significant limitation of these benchtop spectrometers is their low inherent sensitivity. All  
43 NMR experiments are considered to be insensitive due to the small Boltzmann population difference  
44 between the nuclear energy levels that form when NMR-active nuclei are placed within an external  
45 magnetic field. The energy level spacing is magnetic field strength dependent and so moving to lower

46 magnetic field strengths further reduces sensitivity [40]. Indeed overall NMR sensitivity scales with  
 47 field strength as approximately  $B_0^{3/2}$ . Additionally, lower magnetic field strengths reduce spectral  
 48 chemical shift dispersion, which scales linearly with field, and can cause strong coupling issues,  
 49 where coupling constants are similar in magnitude to chemical shift differences between coupled  
 50 spins. As a result, broad and overlapping peaks are common in low-field NMR spectra, even for  
 51 simple molecules. This is a particular challenge of  $^1\text{H}$  benchtop NMR spectroscopy because of the  
 52 relatively small chemical shift range of  $^1\text{H}$  nuclei.

53 To improve the general applicability of benchtop NMR spectrometers, novel methods are  
 54 required to overcome the challenges of sensitivity and resolution. In this work, we explore the  
 55 potential for natural abundance  $^{13}\text{C}$  NMR spectra to be used to surmount the issue of reduced  
 56 resolution. Due to isotopic dilution, the increased chemical shift range when compared to  $^1\text{H}$  NMR  
 57 and the ability to simplify spectra through broadband  $^1\text{H}$  decoupling, natural abundance  $^{13}\text{C}$  NMR  
 58 spectra can be as readily interpreted at 43 MHz (1 T) as at 300 MHz (7 T). However, benchtop  $^{13}\text{C}$   
 59 NMR spectroscopy poses a significant sensitivity challenge because the receptivity of natural  
 60 abundance  $^{13}\text{C}$  is  $1.7 \times 10^{-4}$  relative to  $^1\text{H}$ .

61 In principle, the low sensitivity of benchtop NMR can be overcome using hyperpolarisation.  
 62 Hyperpolarisation methods generate a population difference that is orders of magnitude larger than  
 63 at thermal equilibrium and so provide large NMR signal enhancements. Popular hyperpolarisation  
 64 techniques include Dynamic Nuclear Polarisation (DNP) [41–43] and Parahydrogen Induced  
 65 Polarisation (PHIP) [44–46]. One relatively inexpensive method, which has been successfully  
 66 implemented at low-field is a PHIP-based method called Signal Amplification by Reversible  
 67 Exchange (SABRE) [47–49]. SABRE catalytically transfers the latent polarisation in *para*-hydrogen (*p*-  
 68  $\text{H}_2$ , the NMR-silent singlet spin isomer of  $\text{H}_2$ ) to a target molecule without chemical alteration of the  
 69 target. The mechanism by which this occurs, a simplified scheme of which is shown in Figure 1, is  
 70 through reversible binding of *p*- $\text{H}_2$  and the target analyte to a metal complex (commonly iridium-  
 71 based) in the presence of a weak polarisation transfer field (PTF) in the range of 0 – 20 mT. Oxidative  
 72 addition to the metal complex breaks the symmetry of the *p*- $\text{H}_2$  molecule, allowing for its stored  
 73 polarisation to be transferred through the scalar coupling network of the complex to the target  
 74 molecule that is also bound to the metal. Both the *p*- $\text{H}_2$  and the target analyte bind reversibly,  
 75 leading to a build-up of hyperpolarised analyte in solution over a period of seconds [50].



76

77 **Figure 1.** A schematic representation of the SABRE polarisation transfer process. Hyperpolarisation  
 78 is catalytically transferred from the nuclear singlet isomer of hydrogen, *p*- $\text{H}_2$ , to the target analyte via  
 79 the  $J$  coupling network of the active SABRE catalyst. Rapid reversible exchange of the analyte and *p*-  
 80  $\text{H}_2$  on the catalyst leads to a build-up of hyperpolarised analyte in solution over a period of seconds.  
 81 In general, this process is optimised in a weak magnetic field of 0 – 20 mT. The active SABRE catalyst  
 82 is a positively charged Ir(III) di-hydride complex with two molecules of the analyte bound *trans* to  
 83 the hydrides and a N-heterocyclic carbene (IMes, shown in the inset) bound *trans* to a third molecule  
 84 of the analyte.

85 Many factors influence the effectiveness of the SABRE polarisation transfer but of significant  
 86 importance is the PTF [51]. The PTF determines the pathway of the magnetisation transfer through  
 87 the coupling network [52]. The resonance condition that leads to optimal transfer varies significantly

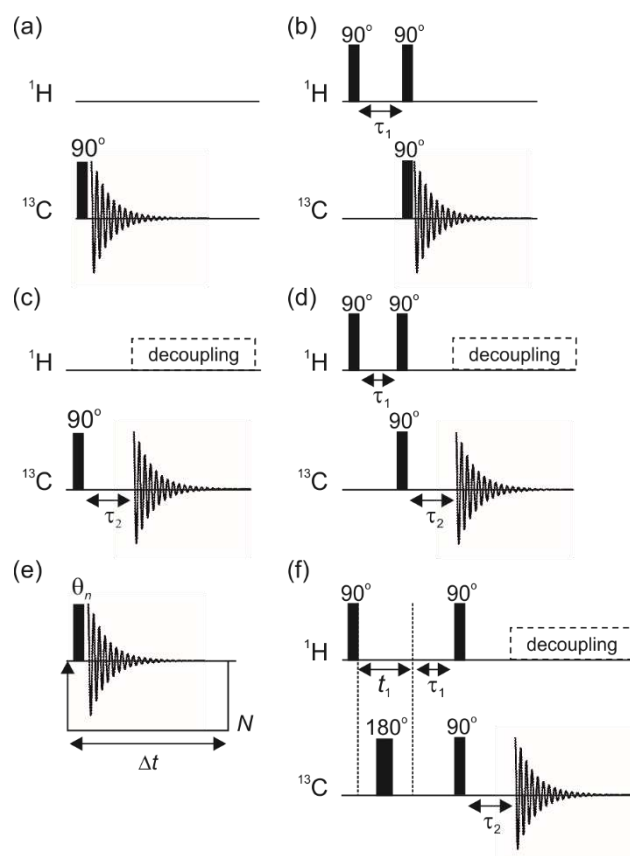
88 for  $^1\text{H}$  SABRE and for transfer to other nuclei [53]. In the SABRE-SHEATH (SABRE in Shield Enables  
89 Alignment Transfer to Heteronuclei) approach, the focus is polarisation transfer to heteronuclei,  
90 which requires a PTF below the Earth's magnetic field. In this case a  $\mu$ -metal shield coupled with an  
91 electromagnet is used to reach the microtesla PTF required for direct polarisation transfer to the  
92 heteronucleus (e.g.  $^{15}\text{N}$ ) [54–56]. In principle, SABRE-SHEATH can be used for direct polarisation  
93 transfer to  $^{13}\text{C}$ ; however, due to the coupling network within the active SABRE polarisation transfer  
94 complex, this direct transfer is often highly inefficient [57]. Optimal  $^{13}\text{C}$  hyperpolarisation is typically  
95 achieved through an indirect transfer mediated by  $^1\text{H}$  or  $^{15}\text{N}$  [58]. In this work, we investigate the  
96 feasibility of SABRE-hyperpolarised benchtop  $^{13}\text{C}\{^1\text{H}\}$  NMR experiments with a focus on the  
97 optimisation of the pulse sequences used for hyperpolarised  $^{13}\text{C}$  detection and an analysis of the  
98 challenges and opportunities provided by hyperpolarised 2D  $^{13}\text{C} - ^1\text{H}$  benchtop NMR spectroscopy.  
99

## 100 2. Materials and Methods

101 SABRE hyperpolarised NMR spectra are acquired by dissolving  $\text{H}_2$  gas, enriched in the *para*  
102 state, in a solution containing the active SABRE catalyst and the target analyte within a weak  
103 polarisation transfer field (PTF) typically in the range of 0 - 20 mT [59,60]. The introduction of  $\text{H}_2$  into  
104 the solution is achieved either manually, via sample shaking, or via bubbling. In the so-called shake-  
105 and-drop approach, the headspace of an NMR tube fitted with a Young's tap is filled with several  
106 bars of *p*- $\text{H}_2$ . The sample is shaken vigorously within the desired polarisation transfer field (PTF) for  
107 a period of a few seconds, allowing for the build-up of hyperpolarised analyte in solution. This  
108 polarisation transfer step is followed by rapid manual transfer of the sample into the NMR  
109 spectrometer for signal detection. In the automated flow-based approach, *p*- $\text{H}_2$  is bubbled through  
110 the solution for a period of seconds in a reaction chamber that sits within a small electromagnet that  
111 provides the desired PTF [61,62]. Following polarisation transfer, the sample is flowed pneumatically  
112 under a pressure of  $\text{N}_2$  gas into the NMR spectrometer for detection. In general, the automated  
113 approach provides a lower SABRE enhancement due to a combination of engineering limitations  
114 including less efficient mixing, longer sample transfer times and lower levels of *p*- $\text{H}_2$  enrichment in  
115 solution during bubbling. However, the flow system provides control over parameters such as the  
116 duration of polarisation, PTF and sample transfer time and therefore can generate the level of  
117 reproducibility required to achieve SABRE-enhanced 2D NMR spectroscopy. The level of  
118 reproducibility of this system has been assessed previously to be ~5 % [49]. In this work, the 1D NMR  
119 spectra acquired to investigate the efficacy of different  $^{13}\text{C}$  NMR detection strategies were carried out  
120 using the manual shaking approach and the SABRE-enhanced 2D spectroscopy was achieved using  
121 an automated flow system.

122 For manual SABRE experiments, the sample was made up in a NMR tube fitted with a Young's  
123 valve. The sample contained 260 mM of 4-methylpyridine (4-MP) and 5.2 mM of  $[\text{IrCl}(\text{COD})(\text{IMes})]$   
124 pre-catalyst (where COD is 1,5-cyclooctadiene and IMes is 1,3-bis(2,4,6-trimethyl-phenyl)-  
125 imidazolium) made up to 0.6 mL with methanol-*d*<sub>4</sub>. The sample was de-gassed under vacuum using  
126 a freeze-pump-thaw method (detailed by Shaver *et al.* [63] but with liquid  $\text{N}_2$  replaced with a dry-ice  
127 acetone bath) to allow the sample to be placed under an atmosphere of *p*- $\text{H}_2$  during SABRE  
128 experiments. Repeat shake-and-drop experiments were performed on a single sample by evacuating  
129 the head-space and refilling with *p*- $\text{H}_2$  between experiments. *Parahydrogen* was generated by cooling  
130  $\text{H}_2$  gas over a paramagnetic catalyst based on activated charcoal at 28 K (with a conversion efficiency  
131 of 99%). The design of this generator has been described previously in ref. [65]. A handheld magnetic  
132 array with a 6.1 mT field strength was used to supply the necessary PTF during SABRE transfer. [64]  
133 The sample shaking time was 10 s and the sample transfer time was  $2.0 \pm 0.5$  s in all experiments. On  
134 the addition of *p*- $\text{H}_2$  to the solution the pre-catalyst will convert to the active form,  $[\text{Ir}(\text{IMes})(\text{H})_2(4\text{-MP})_3]\text{Cl}$ . Full conversion to the active form is required prior to achieving quantitative SABRE results.  
135 The activation was monitored by acquiring 6 repeat  $^1\text{H}$  shake-and-drop experiments over a typical  
136 period of 10 minutes, with the addition of fresh *p*- $\text{H}_2$  to the headspace of the NMR tube between each  
137 experiment.  
138

139 Automated SABRE experiments were performed on a 3 mL solution containing 4-  
 140 methylpyridine (260 mM) and 5.2 mM of the pre-catalyst [IrCl(COD)(IMes)] in methanol-*d*<sub>4</sub>. Samples  
 141 were loaded into a flow system consisting of a mixing chamber held within an electromagnet capable  
 142 of generating magnetic field strengths between 0 and 14 mT and a custom designed flow cell that  
 143 holds the sample within the benchtop NMR spectrometer in the detection region (see Figure S1 in the  
 144 supporting information) [49]. These were connected by fluorinated ethylene propylene tubing with  
 145 sample transference being controlled by a pneumatic control unit (Bruker) with a supply of N<sub>2</sub> gas (6  
 146 bar absolute). The *para*hydrogen used in the automated SABRE experiments was generated using the  
 147 same home-built generator described above. More details on this automated SABRE system can be  
 148 found in ref. [49]. SABRE hyperpolarisation was achieved by bubbling *p*-H<sub>2</sub> at 4 bar (absolute)  
 149 through the sample within the mixing cell for a fixed period of time (15 s). The pressure was then  
 150 released and N<sub>2</sub> gas was used to transfer the sample into the flow cell within the benchtop NMR  
 151 spectrometer for detection. The sample transfer time, including a 3 s delay for the H<sub>2</sub> pressure release  
 152 step, was 4.1 s. For multiple-step experiments, an additional inter-scan delay of 16 s was included to  
 153 return the sample to the mixing chamber and to allow for relaxation and full recovery of the *p*-H<sub>2</sub>  
 154 pressure. In a similar fashion to the manual shaking method full conversion of the pre-catalyst to the  
 155 active form must be completed before performing quantitative experiments. 8 <sup>1</sup>H pulse-and-acquire  
 156 experiments on the flow system were conducted over 15 minutes to monitor the activation process.



157

158 **Figure 2.** NMR pulse sequences where  $\tau_1 = 1/2J_{CH}$  and  $\tau_2 = 1/3J_{CH}$ . (a) Pulse and acquire (PA) for  
 159 direct <sup>13</sup>C detection, (b) INEPT for transfer from <sup>1</sup>H to <sup>13</sup>C, (c) PA with refocusing delay and <sup>1</sup>H  
 160 decoupling, (d) INEPT with refocusing delay and <sup>1</sup>H decoupling, (e) single-shot variable flip angle  
 161 hyperpolarisation lifetime measurement (see Table S1 for values of  $\theta_n$ ), and (f) 2D HETCOR, where  $t_1$   
 162 is incremented to encode <sup>1</sup>H chemical shift into the indirect dimension. For hyperpolarisation  
 163 experiments, the pulse sequences are applied immediately following the SABRE hyperpolarisation  
 164 step and sample transfer into the benchtop NMR spectrometer.

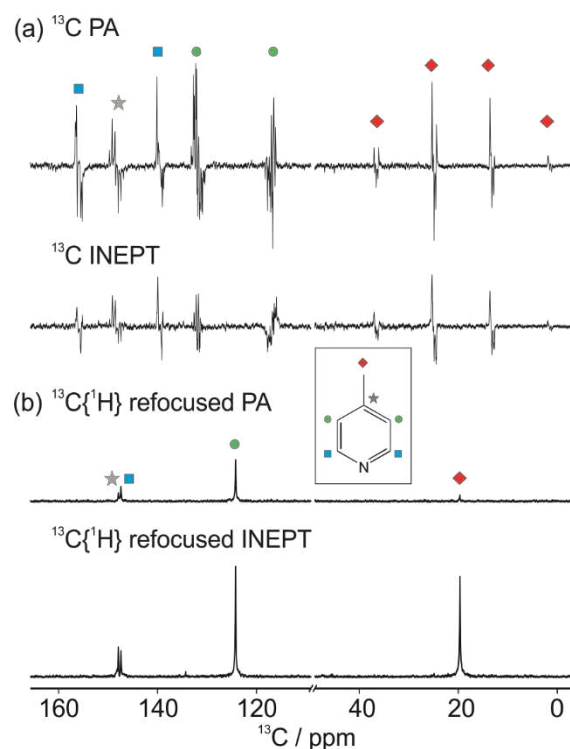
165 All NMR data were collected using a 43 MHz (1 T) NMR spectrometer (Spinolve Carbon,  
 166 Magritek) equipped with <sup>1</sup>H/<sup>19</sup>F and <sup>13</sup>C channels. At the start of each session, shimming and

167 frequency calibrations were performed on a reference sample containing a 10%:90% H<sub>2</sub>O:D<sub>2</sub>O  
168 mixture. All non-hyperpolarised benchtop NMR spectra were performed using a 0.6 mL sample of  
169 neat 4-methylpyridine (10.3 M) in a standard 5 mm NMR tube. The NMR detection sequences  
170 employed in this work are illustrated in Figure 2. In principle, 180° refocusing pulses should be used  
171 in the INEPT and refocused PA pulse sequences to improve performance by refocusing the chemical  
172 shift evolution. However, it was found for both hyperpolarised and thermally polarised experiments  
173 on the benchtop NMR spectrometer, the presence of additional 180° pulses led to lower SNR. We  
174 attribute this effect to poor RF pulse homogeneity. For all hyperpolarisation experiments, these  
175 detection sequences were applied immediately following SABRE hyperpolarisation using one of the  
176 two methods detailed above. A list of the variable flip angles used in the single-shot  
177 hyperpolarisation lifetime experiments (Figure 2e) are provided in Table S1 in the supporting  
178 information. These flip angles were chosen such that each pulse excited a fixed fraction of the  
179 available magnetisation, enabling a fit of the resultant signal integrals to a simple exponential decay  
180 function in order to determine the hyperpolarisation lifetime. The reported values of <sup>1</sup>H and <sup>13</sup>C  
181 hyperpolarisation lifetimes are the average of five repeated measurements and the standard error  
182 across the repetitions was used to define the error bars. The PTFs used for the <sup>1</sup>H and <sup>13</sup>C  
183 hyperpolarisation lifetime measurements were 6.1 mT (as described above) and ~50 μT (the Earth's  
184 magnetic field), respectively. All spectra were processed and <sup>13</sup>C SNR values were calculated using  
185 MestReNova (Mestrelab research).

### 186 3. Results

#### 187 3.1. Optimal detection of SABRE-enhanced <sup>13</sup>C benchtop NMR spectra

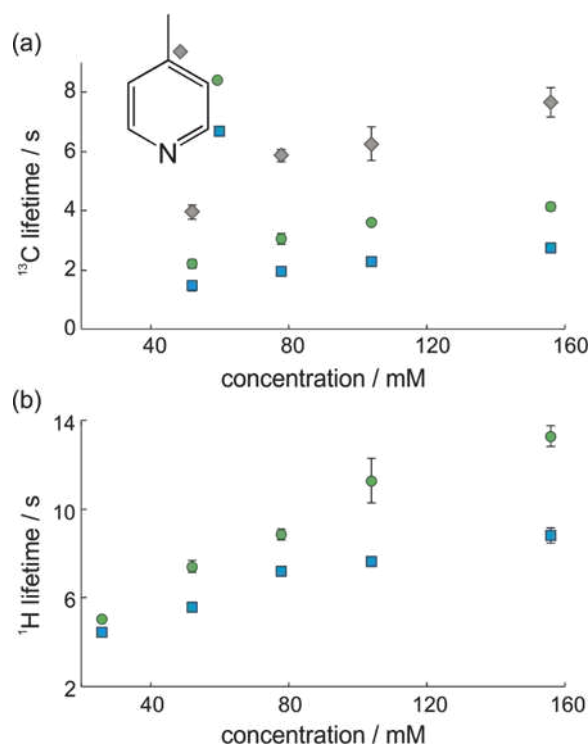
188 To explore the optimal detection approach for SABRE-hyperpolarised benchtop <sup>13</sup>C NMR  
189 spectroscopy, we compare two methods for hyperpolarised signal acquisition. In the first pulse-and-  
190 acquire (PA) approach, the <sup>13</sup>C NMR signal is detected directly following a single 90° excitation pulse  
191 (Figure 2a). SABRE-enhanced benchtop <sup>13</sup>C NMR spectra acquired using PA have been reported  
192 previously [49]. Here we compare this approach with a second method where the <sup>13</sup>C NMR signal is  
193 detected indirectly following a *J*-based INEPT transfer of hyperpolarisation from <sup>1</sup>H to <sup>13</sup>C (Figure  
194 2b). A comparison of the SABRE hyperpolarised <sup>13</sup>C benchtop NMR spectra of 4-methylpyridine (4-  
195 MP) using these two approaches is presented in Figure 3a. In both cases, SABRE hyperpolarisation  
196 was achieved using the manual sample shaking method.



197

198 **Figure 3.** 1D SABRE hyperpolarised  $^{13}\text{C}$  NMR spectra of 260 mM 4-methylpyridine at natural  
 199 abundance in MeOD with 5.2 mM active SABRE catalyst. Each spectrum was acquired in a single scan  
 200 on a benchtop (43 MHz) NMR spectrometer following SABRE hyperpolarisation in a PTF of 6.1 mT.  
 201 (a) Fully-coupled  $^{13}\text{C}$  NMR spectra acquired immediately following a  $90^\circ$   $^{13}\text{C}$  pulse (top) and following  
 202 INEPT transfer from  $^1\text{H}$  with  $J_{\text{CH}} = 10$  Hz. (b)  $^{13}\text{C}\{^1\text{H}\}$  NMR spectra acquired using PA (top) and INEPT  
 203 transfer from  $^1\text{H}$  (bottom). A refocusing delay of  $(3J_{\text{CH}})^{-1}$  with  $J_{\text{CH}} = 10$  Hz was included prior to signal  
 204 acquisition in both cases.

205 Inspection of the fully-coupled, SABRE-enhanced  $^{13}\text{C}$  NMR spectra in Figure 3a reveals that a  
 206 higher overall  $^{13}\text{C}$  NMR signal enhancement is observed for the PA detection scheme (Figure 3a, top)  
 207 when compared to the INEPT approach where polarisation is transferred from  $^1\text{H}$  to  $^{13}\text{C}$  (Figure 3b  
 208 bottom). This suggests that the efficiency of the spontaneous indirect transfer from  $^1\text{H}$  to  $^{13}\text{C}$  during  
 209 SABRE in the PTF is higher than the efficiency of the RF-driven transfer achieved by the INEPT  
 210 sequence following transfer of the sample to the NMR detector. One potential explanation for the  
 211 higher  $^{13}\text{C}$  signal observed in the PA experiment is relaxation. If the lifetime of the  $^1\text{H}$  polarisation is  
 212 shorter than for the  $^{13}\text{C}$  hyperpolarisation, a higher proportion of available signal will decay during  
 213 sample transport in the INEPT case when compared to the PA case. Figure 4 presents a comparison  
 214 of the  $^{13}\text{C}$  and  $^1\text{H}$  hyperpolarisation lifetimes as a function of concentration of 4-methylpyridine,  
 215 where the concentration of the catalyst is 5.2 mM in all cases. These single-shot lifetime measurements  
 216 were acquired on the benchtop NMR spectrometer using the variable flip angle sequence in Figure  
 217 2e immediately following hyperpolarisation using the manual shaking SABRE procedure. Contrary  
 218 to the hypothesis, we find that the lifetimes for the  $^1\text{H}$  hyperpolarisation are longer than for the  
 219 directly-detected  $^{13}\text{C}$  polarisation. Therefore these results do not support the proposition that  
 220 relaxation effects lead to higher observed  $^{13}\text{C}$  signals in the PA case. However, it should be noted that  
 221 these experiments do not distinguish between  $^1\text{H}$  hyperpolarisation in molecules with and without  
 222  $^{13}\text{C}$ .



**Figure 4.**  $^{13}\text{C}$  and  $^1\text{H}$  SABRE hyperpolarisation lifetimes measured using a single-shot pulse sequence (Figure 2e) at 1 T (43 MHz) for 4-methylpyridine in methanol- $d_4$  with 5.2 mM SABRE catalyst. Values are the average over 5 experiments and error bars are the standard error across the repeats measurements.

A second possible explanation for the lower signals observed following the INEPT transfer is that the efficiency of hyperpolarisation transfer to  $^1\text{H}$  is not optimised under these experimental conditions for molecules containing  $^{13}\text{C}$ . Both of the fully-coupled, SABRE-enhanced  $^{13}\text{C}$  NMR spectra in Figure 3a contain peaks that have anti-phase character with respect to a relatively long range  $^1\text{H}$ - $^{13}\text{C}$  coupling on the order of  $J_{\text{CH}} = 10$  Hz. This anti-phase character is consistent with previous observations in the literature involving indirect SABRE polarisation transfer to  $^{13}\text{C}$  via  $^1\text{H}$  [66]. In the case of the PA experiment, the anti-phase character indicates that the SABRE process has enhanced two-spin-order terms involving non-directly-bonded  $^1\text{H}$ - $^{13}\text{C}$  pairs within the analyte [49]. In the case of the INEPT transfer experiment, we find that the optimal  $^{13}\text{C}$  NMR signal is observed using a constant of  $J_{\text{CH}} = 10$  Hz for the transfer step. Interestingly, the  $^{13}\text{C}$  NMR signal observed for a larger one-bond coupling constant of  $J_{\text{CH}} = 140$  Hz is reduced. This is in contrast to INEPT experiments carried out on samples at thermal equilibrium, where the most efficient transfer of polarisation is achieved between directly bonded  $^1\text{H}$  and  $^{13}\text{C}$  nuclei. These results indicate that protons directly bonded to  $^{13}\text{C}$  are less efficiently hyperpolarised via the SABRE process under our experimental conditions.

The lower efficiency of  $^1\text{H}$  hyperpolarisation for protons directly bonded to  $^{13}\text{C}$  can be understood by considering the resonance condition that facilitates spontaneous polarisation transfer in SABRE. Efficient polarisation transfer requires that the difference in chemical shift between the source of the polarisation (the  $p$ - $\text{H}_2$ -derived hydrides in the polarisation transfer complex) and the target nuclei of the analyte bound to the complex be approximately equal to the dominant  $J$  coupling constant within the coupling network. Typically, this is  $J_{\text{HH}} \sim 8$  Hz between the pair of hydrides. This gives rise to an optimal PTF for transfer to aromatic  $^1\text{H}$  resonances in the analyte of  $\sim 6.5$  mT and of a few  $\mu\text{T}$  for direct transfer to other nuclei such as  $^{15}\text{N}$  and  $^{13}\text{C}$ . However, if the protons in the target analyte are directly bonded to  $^{13}\text{C}$ , the dominant coupling will be the one-bond  $^{13}\text{C}$ - $^1\text{H}$  coupling on the order of 140 Hz. This will significantly shift the resonance condition to a PTF on the order of  $\sim 0.1$  T. Therefore polarisation transfer to protons directly bonded to  $^{13}\text{C}$  is inefficient in the PTF of 6.1 mT used in these experiments. This effect has been observed previously in the case of SABRE

255 hyperpolarisation of  $^{13}\text{C}$  in acetonitrile, where no polarisation transfer to the methyl carbon is  
 256 observed [66]. In addition, Ivanov and co-workers have performed  $^{13}\text{C}$  SABRE experiments over a  
 257 wide range PTF values [52]. In their experiments, significant  $^{13}\text{C}$  SABRE hyperpolarisation was  
 258 observed in a relatively strong PTF field of 90 mT. Therefore, it is likely that the efficiency of the  
 259 INEPT approach could be greatly increased by carrying out the SABRE polarisation transfer in a  
 260 much stronger PTF and using a one-bond  $J$  coupling constant for the transfer.

261 In order to simplify the spectra in Figure 3a and to improve SNR we can apply broad-band  $^1\text{H}$   
 262 decoupling during  $^{13}\text{C}$  signal acquisition. Due to the anti-phase character of the SABRE-enhanced  $^{13}\text{C}$   
 263 NMR spectra, a refocusing delay is required prior to acquisition, as illustrated in the sequences in  
 264 Figure 2c and 2d. The resultant SABRE-enhanced  $^{13}\text{C}\{^1\text{H}\}$  benchtop NMR spectra acquired with the  
 265 refocused PA and INEPT pulse sequences are presented in Figure 3b. In both cases, the decoupling  
 266 has simplified the spectra and improved the signal-to-noise ratio (SNR), as expected. The narrow  
 267 single resonances for each of the four  $^{13}\text{C}$  environments are well resolved, including the very small  
 268 difference of 0.5 ppm between the *para* (gray star) and *ortho* (blue square) carbon positions. The  
 269 average SNR values for each  $^{13}\text{C}$  resonance of 4-MP, calculated for a set of repeat measurements  
 270 acquired with each of the two detection methods, are given in Table 1. In contrast to the fully-coupled  
 271 case, here the  $^{13}\text{C}\{^1\text{H}\}$  signal is much greater for the INEPT transfer when compared to the PA case.  
 272 This implies that the delay used to re-focus the anti-phase signals is insufficient to simultaneously re-  
 273 focus all of the signals in the PA case, leading to significant signal cancellation during  $^1\text{H}$  decoupling.  
 274 Therefore, despite the apparent SNR advantage of PA detection in the fully-coupled case, the INEPT  
 275 approach produces superior results for  $^{13}\text{C}\{^1\text{H}\}$  NMR spectra.

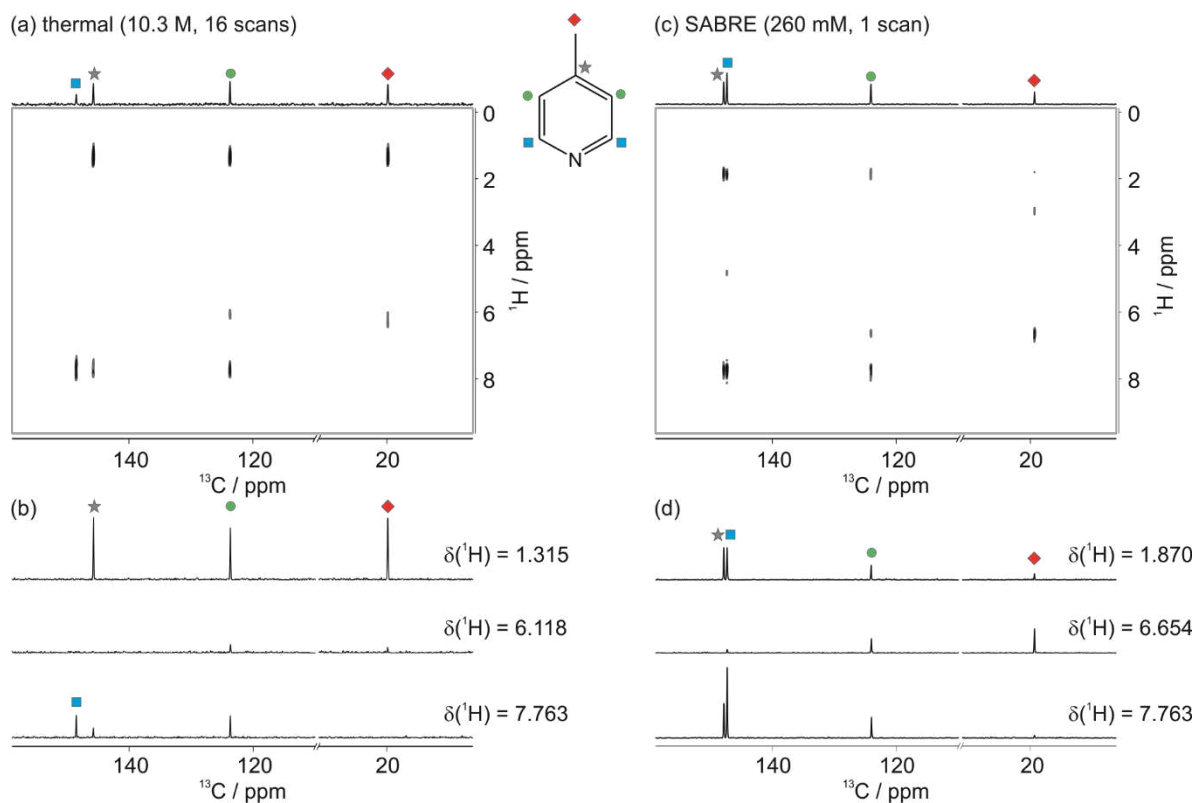
276 **Table 1.** Signal-to-noise ratios (SNR) for SABRE-enhanced  $^{13}\text{C}\{^1\text{H}\}$  NMR spectra of 4-MP acquired  
 277 with the refocused PA and refocused INEPT pulse sequences. Values are the average over 7 (PA) and  
 278 3 (INEPT) repeat experiments.

$^{13}\text{C}$ resonance	refocused PA	refocused INEPT
<i>para</i>	$9.9 \pm 0.5$	$31 \pm 2$
<i>ortho</i>	$18.9 \pm 0.9$	$23 \pm 2$
<i>meta</i>	$43 \pm 2$	$91 \pm 7$
<i>methyl</i>	$6.4 \pm 0.6$	$73 \pm 7$

279 We note that all of these experiments were carried out in a PTF of 6.1 mT. The distribution of  
 280 polarisation and the relative efficiency of the detection schemes will vary with the choice of PTF.  
 281 Indeed, in previous work, the maximum PA  $^{13}\text{C}$  signal intensity was observed for a PTF equal to the  
 282 Earth's magnetic field ( $\sim 50 \mu\text{T}$ ). In addition, as discussed above, it is probable that carrying out  
 283 SABRE in a much higher PTF could be beneficial for optimising the hyperpolarisation of  $^1\text{H}$  directly  
 284 bonded to  $^{13}\text{C}$ . This could significantly improve the over-all efficiency of the INEPT approach.  
 285

### 286 3.1. SABRE-enhanced 2D $^{13}\text{C}$ - $^1\text{H}$ benchtop NMR spectroscopy

287 In addition to the single-shot 1D  $^{13}\text{C}$  NMR experiments presented above, it is also of interest to  
 288 consider the feasibility of acquiring SABRE enhanced  $^{13}\text{C}$ - $^1\text{H}$  2D NMR spectra of samples at natural  
 289 isotopic abundance. SABRE-enhanced 2D  $^1\text{H}$ - $^1\text{H}$  benchtop NMR experiments have been reported  
 290 previously[49]. Here we extend this approach to heteronuclear experiments, exemplified by a  $^1\text{H}$ - $^{13}\text{C}$   
 291 HETCOR spectrum acquired using the pulse sequence in Figure 2f. Figure 5 presents a comparison  
 292 between a 2D HETCOR benchtop NMR spectrum acquired using polarisation at thermal equilibrium  
 293 for neat 4-methylpyridine (10.3 M, 16 scans, 307 min total experiment time, Figure 5a) and one  
 294 acquired using SABRE hyperpolarisation of 260 mM 4-methylpyridine (1 scan, 69 min total  
 295 experiment time, Figure 5c). The SABRE spectrum was achieved by re-hyperpolarising the solution  
 296 outside of the spectrometer between the acquisition of each transient, as described previously[49]  
 297



298

299 **Figure 5.** 2D  $^{13}\text{C}$ - $^1\text{H}$  HETCOR benchtop NMR spectra of 4-methylpyridine (4-MP). (a) Thermally  
 300 polarised spectrum of neat 4-MP (10.3 M) acquired with 64 steps each with 16 scans in a total  
 301 experiment time of 307 min (5.1 h). (b) 1D slices through the 2D spectrum in (a) at the chemical shift  
 302 of the *methyl* proton resonance (top), *meta* proton resonance (middle) and *ortho* proton resonance  
 303 (bottom). (c) SABRE hyperpolarised 2D spectrum of 260 mM 4-MP with 5.2 mM active SABRE catalyst  
 304 in methanol- $d_4$  acquired with 90 steps each with a single scan in a total experiment time of 69 mins.  
 305 (d) 1D slices through the 2D spectrum in (c) as in (b). Note, the differences in chemical shifts between  
 306 the two spectra are due to the presence of the solvent (methanol- $d_4$ ) in the SABRE case.

307 The 2D spectrum in Figure 5c demonstrates the ability of SABRE hyperpolarisation to enable  
 308 high-sensitivity 2D  $^{13}\text{C}$ - $^1\text{H}$  benchtop NMR on relatively low concentration samples at natural isotopic  
 309 abundance and in reasonable experiment times. In order to obtain a comparable SNR, a neat sample  
 310 (10.3 M) of 4-MP and 16 scans were used for the reference spectrum in Figure 5a. The use of  
 311 hyperpolarisation allows for a reduction in concentration by two orders of magnitude as well as a  
 312 reduction in experiment time by a factor of  $\sim 4.5$ . We note that in order to achieve a comparable result  
 313 to the SABRE spectrum at the lower concentration, signal averaging for over 300 days would have  
 314 been required. As in the 1D INEPT case, efficient hyperpolarisation transfer from  $^1\text{H}$  to  $^{13}\text{C}$  is observed  
 315 using a relatively long-range coupling constant of  $J_{\text{CH}} = 10$  Hz due to the inefficiency of SABRE  
 316 hyperpolarisation of protons directly bonded to  $^{13}\text{C}$ .

#### 317 4. Discussion and Conclusions

318 In this work we have demonstrated the acquisition of high-resolution 1D and 2D  $^{13}\text{C}\{^1\text{H}\}$   
 319 benchtop NMR spectra of relatively low concentrations of the target analyte at natural abundance in  
 320 a single scan. The SABRE-enhanced  $^{13}\text{C}\{^1\text{H}\}$  spectra are easily interpreted at 1 T, including the  
 321 separation of peaks with chemical shift differences of less than 0.5 ppm. Using SABRE  
 322 hyperpolarisation with a PTF of 6.1 mT, the optimal SNR was achieved by the PA approach in the  
 323 fully-coupled spectra but the refocused INEPT approach provided optimal SNR in the decoupled  
 324 spectra. The superior performance of the INEPT sequence is likely due to the wide range of anti-  
 325 phase  $^{13}\text{C}$ - $^1\text{H}$  terms that are excited in the PA approach and that are not easily refocused using a single  
 326 delay. While the INEPT approach provided the highest SNR for the  $^{13}\text{C}\{^1\text{H}\}$  spectra, our results

327 highlight that  $^1\text{H}$  nuclei directly bonded to  $^{13}\text{C}$  are not hyperpolarised efficiently under these  
328 experimental conditions. In future work, adopting the approach of Ivanov and coworkers [52] to  
329 perform SABRE in a much higher PTF within the bore of the benchtop NMR spectrometer could lead  
330 to much more efficient  $^1\text{H}$ - $^{13}\text{C}$  hyperpolarisation via a 1-bond INEPT transfer.

331 One of the key advantages of the SABRE approach over other hyperpolarisation methods for  
332 analytical applications is that it is reversible and so a single sample can be re-polarised multiple times.  
333 We have exploited this feature to acquire 2D  $^{13}\text{C}$ - $^1\text{H}$  benchtop NMR spectra, with re-polarisation  
334 achieved outside of the spectrometer between each step of the 2D experiment. However, in our flow-  
335 based approach to SABRE-enhanced 2D NMR, evaporation of the solvent during sample transfer and  
336  $p$ - $\text{H}_2$  bubbling ultimately limits the maximum number of transients that can be achieved for a single  
337 sample. In addition, the transfer of the sample between the spectrometer and the mixing chamber is  
338 time-consuming relative to the other steps in the experiment. These limitations could potentially be  
339 overcome by using more efficient sampling methods, such as the single-shot 2D methods [67], which  
340 have previously been demonstrated using SABRE and high-field detection [68]. Alternatively,  
341 hyperpolarisation within the bore of the benchtop spectrometer, as suggested above, would  
342 significantly limit the transfer time and distance between the SABRE polarisation transfer step and  
343 the signal detection step. This approach has the potential to make SABRE-enhanced 2D  $^{13}\text{C}$  benchtop  
344 NMR viable for more routine applications by significantly decreasing experiment times, increasing  
345 the maximum number of transients, and improving sensitivity by increasing the efficiency of the  
346 INEPT transfer.

347 SABRE hyperpolarisation was achieved here using a model analyte, 4-methylpyridine. In order  
348 for an analyte to be strongly enhanced by SABRE, it needs to reversibly bind to the catalyst on an  
349 appropriate timescale. The residence time on the catalyst must be long enough for significant  
350 polarisation transfer to occur but not too long such that NMR relaxation dominates. It is well  
351 established that N-heterocycles are good SABRE substrates. However, recent advances in SABRE  
352 catalysis have extended this approach to other functional groups, such as amines, using the standard  
353 SABRE mechanism [69]. In addition, a new mechanism for transfer, called SABRE-Relay, has been  
354 introduced, whereby a carrier with exchangeable protons, is hyperpolarised through direct  
355 association to the catalyst and then transfers polarisation to a target substrate via proton exchange.  
356 In principle, this provides a route to the hyperpolarisation method of any target substrate with  
357 exchangeable protons [69]. Thus the range of target analytes that are accessible to the SABRE  
358 approach are expected to increase going forward.

359 **Supplementary Materials:** The following are available online at [www.mdpi.com/xxx/s1](http://www.mdpi.com/xxx/s1), Figure S1: Schematic  
360 and photo of the automated flow system used for SABRE hyperpolarisation with benchtop NMR detection, Table  
361 S1: List of the variable RF pulse angles used in the single-shot hyperpolarisation lifetime measurements.

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363 and investigation, A.D.R., P.M.R. and M.E.H.; writing—original draft preparation, A.D.R. and M.E.H.; writing—  
364 review and editing, A.D.R., M.E.H. and P. M.R.; supervision and funding acquisition, M.E.H..

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