CHEMISTRY
A European Journal

A Journal of
ChemPubSoc Europe

Accepted Article

Title: Achieving High 1H Nuclear Hyperpolarization Levels with Long Lifetimes in a Range of Tuberculosis Drug Scaffolds

Authors: Simon Duckett, Philip Norcott, Peter J Rayner, and Gary G. R. Green

To be cited as: *Chem. Eur. J.* 10.1002/chem.201703278

Link to VoR: http://dx.doi.org/10.1002/chem.201703278

Supported by
ACES

WILEY-VCH

10.1002/chem.201703278

WILEY-VCH

Achieving High ^{1}H Nuclear Hyperpolarization Levels with Long Lifetimes in a Range of Tuberculosis Drug Scaffolds

Philip Norcott,[a] Peter J. Rayner, [a] Gary G. R. Green, [a] and Simon B. Duckett*[a]

Abstract: Despite the successful use of isoniazid, rifampicin, pyrazinamide and ethambutol in the treatment of tuberculosis (TB) it is a disease of growing global concern. We illustrate here a series of methods that will dramatically improve the magnetic resonance imaging (MRI) detectability of nineteen TB relevant agents and note that the future probing of their uptake and distribution *in vivo* would be expected to significantly enhance their efficacy in disease treatment. This improvement in detectability is achieved by use of the *para*hydrogen based SABRE protocol in conjunction with the ^{2}H-labelling of key sites within their molecular structures, and the ^{2}H-labelling of the magnetization transfer catalyst. The T_1 relaxation times and polarization levels of these agents are quantified under test conditions to produce a protocol to identify structurally optimized motifs for future detection. For example, deuteration of the 6-position of a pyrazinamide analogue leads to a structural form that exhibits T_1 values of 144.5 s for 5-H with up to 20% polarization. This represents a >7-fold extension in relaxation time and almost 10x improvement in polarization level when compared to its unoptimized structure.

Introduction

Tuberculosis (TB) is a bacterial infection that is recognized by the World Health Organization as one of the most significant infectious diseases, killing an estimated 1.8 million people in 2015 alone.[1] Pyrazinamide[2] (**1**) and isoniazid[3] (**2**), whose structures are detailed in Figure 1, are two first-line drugs that are used in conjunction with ethambutol or rifampicin in its treatment.[4] In the case of pyrazinamide, the proposed active form, pyrazinoic acid, is produced by the pyrazinamidase enzyme and accumulates within *Mycobacterium tuberculosis*.[5] While its precise mode of therapeutic action remains unclear, the prodrug isoniazid is activated by KatG catalase to form a complex which binds to the carrier protein InhA to reduce its activity.[6]

The ^{11}C radiolabeling of pyrazinamide, isoniazid and rifampicin in conjunction with subsequent imaging by positron emission tomography allows access to pharmacokinetic and drug distribution data that is used in TB diagnosis.[7] Imaging the uptake and distribution of these therapeutics by magnetic resonance imaging (MRI) might therefore aid in TB treatment. However, while nuclear magnetic resonance (NMR) spectroscopy and MRI are invaluable analytical techniques for research and medicine they suffer from an inherent insensitivity that means they cannot routinely provide access to this type of information.[8] This challenge stems from the fact that even in the 1.5 T magnetic field of a commonly used hospital scanner the associated nuclear spin interaction produces a signal that is derived positively from just 1 in 10^5 of the proton nuclei present. Hyperpolarization techniques change the associated Boltzmann spin state populations to improve detectability by several orders of magnitude.[9]

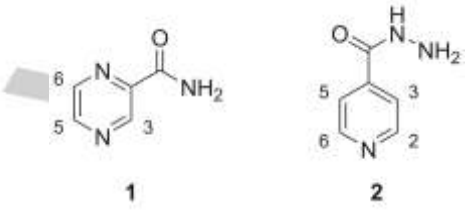
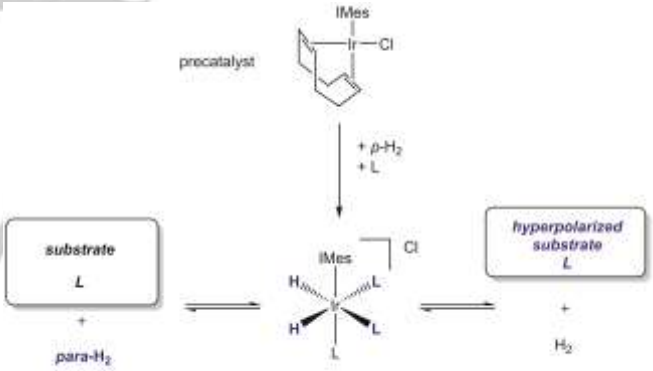


Figure 1. Structures of the tuberculosis drugs pyrazinamide (**1**) and isoniazid (**2**).



Scheme 1. Representation of the Signal Amplification by Reversible Exchange (SABRE) process for substrate L where IMes is 1,3-bis(2,4,6-trimethylphenyl)imidazol-2-ylidene.

A number of hyperpolarization methods have been developed which are beginning to find use in the study of living systems.[10] Those that utilize *para*hydrogen (p-H_2) as the polarization feedstock are known collective by the term PHIP (*para*hydrogen induced polarization) processes.[11] Although p-H_2 is itself undetectable by NMR spectroscopy as it possesses no angular spin momentum, when it is incorporated into a new molecule through a chemical reaction that breaks its nuclear symmetry the resulting environments can produce strongly hyperpolarized MR signals.[12] Signal Amplification By Reversible Exchange (SABRE) is a rapidly emerging and inexpensive method for hyperpolarizing materials that uses p-H_2.[13] It achieves this result through the catalytic transfer of polarization from p-H_2 to a target

[a] Dr P Norcott, Dr PJ Rayner, Prof. GGR Green, Prof SB Duckett
Centre for Hyperpolarisation in Magnetic Resonance
Department of Chemistry, University of York
YO10 5NY, UK
simon.duckett@york.ac.uk

Supporting information for this article is given via a link at the end of the document.

substrate (L) *via* an iridium complex without changing the material's chemical identity.

A common polarization catalyst that is used in the SABRE approach is formed by the reaction of [IrCl(COD)(IMes)] (where IMes = 1,3-bis(2,4,6-trimethylphenyl)imidazol-2-ylidene, and COD = *cis*,*cis*-1,5-cyclooctadiene), with the hyperpolarization target (L), and *p*-H_2 (Scheme 1).[14] Reversible polarization transfer then readily occurs through the *J*-coupling network[15] of the iridium catalyst from the hydride ligands into the target substrate at low field, although it can also be driven by radio frequency excitation at high field.[16] Developments in SABRE over the last few years have seen significant progress in the hyperpolarization of ^{1}H, ^{15}N, ^{31}P and ^{13}C nuclei amongst others.[17]

A key limitation that is associated with all hyperpolarization techniques is the rate at which the hyperpolarized nuclei relax back to their thermally distributed form.[18] This timescale is often elevated for heteronuclei but the ubiquity and high natural abundance of ^{1}H nuclei make its detection highly desirable in MRI. Hence the establishment of methods to hyperpolarize nuclei, in a long-lived form, reflect a scientifically challenging but very important research goal that has involved a range of approaches.[19] T_1 relaxation times of ^{1}H nuclei approaching 2 minutes have been reported in an isotopically labelled methyl nicotinate in conjunction with SABRE polarization of up to 50%.[20] Alternatively, ^{1}H singlet states have been used to store magnetization in a functionalized pyridazine with a T_{LLS} value of over 4 minutes.[21]

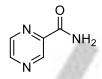
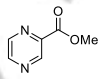
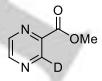
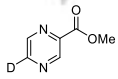
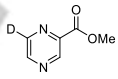
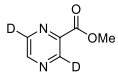
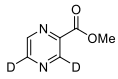
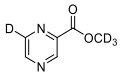
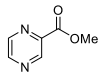
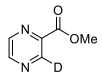
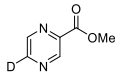
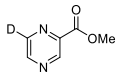
Both pyrazinamide and isoniazid have already been reported to participate in SABRE hyperpolarization,[22] but further optimization is needed prior to their potential *in vivo* detection. Herein we develop a strategy to increase the relaxation times of these key tuberculosis related pharmaceuticals, and some related structural analogues, through selective deuteration. By extending the relaxation times of these substrates, their suitability to SABRE could also improve.[20, 23] This hypothesis stems from the fact that each molecule of *p*-H_2 possesses a specific amount of underlying polarization and consequently transfer to fewer ^{1}H sites on a target could be beneficial. Consequently, a further benefit to this approach lies in the simplified and stronger MR response that might result.

Results and Discussion

SABRE investigation of pyrazinamide (1) and methyl pyrazine-2-carboxylate (3) We begin our study with the two pyrazinamide-class substrates pyrazinamide (**1**) and methyl pyrazine-2-carboxylate (**3**, Table 1). The latter, alongside numerous other ester derivatives, have been recognized as being active against several strains of *M. tuberculosis*.[24]

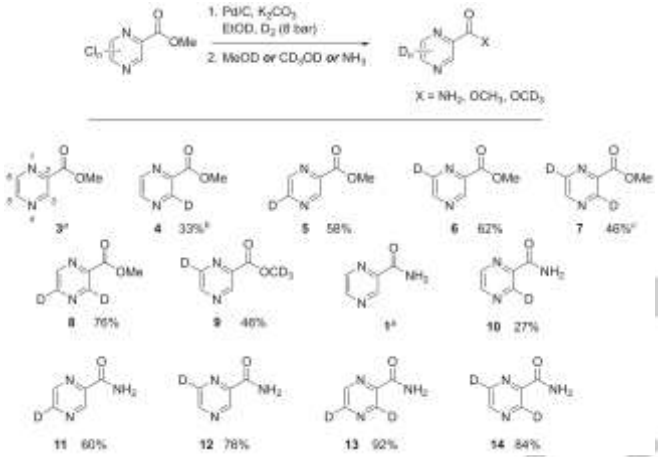
In methanol-d_4 solution, **1** was found to exhibit T_1 values of 80.6, 20.9 and 21.2 seconds for protons 3-H, 5-H and 6-H respectively. In contrast, the methyl ester **3** was found to exhibit T_1 values of 64, 23.4 and 23.5 seconds. NMR samples of these two agents were prepared at a concentration of 20 mM in a 4:1 ratio with the precatalyst [IrCl(COD)(IMes)] (5 mM). These samples were placed under 3 bar of *p*-H_2 and a series of SABRE polarization transfer experiments undertaken which involved shaking the sample for 10 seconds in the 65 gauss fringe field associated with a 9.4 T NMR spectrometer, followed by immediate transfer into the magnet for measurement. The average ^{1}H NMR signal intensities seen for **1** in the resulting measurements, under SABRE, corresponded to polarization levels of 1.7%, 2.6% and 2.7% respectively, while those for **3** were 2.4%, 4.3% and 3.9%. Hence, both of these materials are highly suitable for SABRE. As expected, the measured T_1 values

Table 1. T_1 values and hyperpolarization levels of **1**, **3** and a series of deuterated methyl pyrazinecarboxylate derivatives.

Compound		Site	$T_{1(\text{no cat.})}$ (s)	$T_{1(\text{with cat.})}$ (s)	Polarization Level (%)
			Methanol-d_4		
1		3-H: 5-H: 6-H:	80.6 20.9 21.2	32.7 12.1 12.3	2.7 ± 0.3 2.8 ± 0.3 1.6 ± 0.1
3		3-H: 5-H: 6-H:	64.1 23.4 23.5	20.6 9.3 12.0	4.3 ± 0.3 3.9 ± 0.2 2.4 ± 0.2
4		5-H: 6-H:	24.9 24.0	10.3 14.1	3.5 ± 0.3 2.8 ± 0.2
5		3-H: 6-H:	91.1 84.0	26.0 39.2	6.7 ± 0.3 6.2 ± 0.3
6		3-H: 5-H:	99.7 144.5	32.9 26.2	11.9 ± 0.6 9.2 ± 0.5
7		5-H:	39.5	22.6	7.8 ± 0.2
8		6-H:	98.1	43.1	2.5 ± 0.2
9		3-H: 5-H:	53.0 53.1	25.6 21.8	8.8 ± 0.3 7.5 ± 0.2
			Ethanol-d_6		
3		3-H: 5-H: 6-H:	55.4 17.2 17.2	8.3 4.6 7.4	3.16 ± 0.08 1.96 ± 0.05 0.95 ± 0.04
4		5-H: 6-H:	11.0 11.7	5.4 7.6	2.35 ± 0.05 0.99 ± 0.02
5		3-H: 6-H:	18.3 20.9	5.9 13.6	4.4 ± 0.2 3.9 ± 0.3
6		3-H: 5-H:	21.4 23.8	10.8 11.5	4.7 ± 0.3 2.9 ± 0.2

of **3** under SABRE conditions at 298 K were found to decrease in accordance with the fact that they reflect a weighted response from the free and bound forms.[23, 25] The corresponding average fall in T_1 for the sites in **1** under these conditions was 47%, while that for **3** was 60% which is consistent with the observation of better polarization transfer in **3** as these differences suggest a stronger interaction with the catalyst.

Synthesis of deuterated analogues of pyrazinamide (1) and methyl pyrazine-2-carboxylate (3) In order to improve on the hyperpolarization level, a series of deuterated derivatives of **1** and **3** were prepared by the palladium-catalysed reduction of the corresponding heteroaryl chlorides under D_2,[20, 26] followed by transesterification or amidation as shown in Scheme 2. Full synthetic procedures and characterization data are presented in the supplementary information.



Scheme 2. Synthetic route to the pyrazine carboxylate and amide isotopologues **3**-**12**. [a]Commercially available. [b]D_2O/THF instead of EtOD. [c]Methyl 3,6-dibromopyrazine-2-carboxylate used as the starting material and Na_2CO_3 used in place of K_2CO_3.

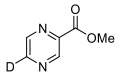
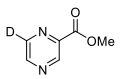
SABRE studies on deuterated analogues of methyl pyrazine-2-carboxylate (3) The relaxation and SABRE polarization data for the methyl ester derivatives **4**-**6** that contain a single ^{2}H-label are detailed in Table 1. Substrate **4**, with deuteration at the 3-position, gave a slight increase in the relaxation times of the remaining ^{1}H nuclei compared to **3**, whilst delivering comparable levels of SABRE polarization to it. In contrast, compound **5**, where the label is in the 5-position, exhibits significantly improved relaxation times and polarization levels (6.2–6.7%). In this case the effective T_1 values of the substrate in the presence of the hyperpolarization catalyst are 25 and 39 seconds, and they increase to 91 and 84 seconds in its absence. These numbers indicate that relaxation within the catalyst is therefore reduced by substrate deuteration and consequently higher hyperpolarization levels result.[20] The most striking result though was achieved with deuteration at the 6-position as compound **6** gave 11.9% polarization for 3-H (a ~3700-fold signal gain) and 9.2% polarization for 5-H. The T_1 values of the ^{1}H nuclei in **6** are now 100 and 145 seconds and hence we have achieved our initial goal of producing a strong and long lived response. This increase in T_1 relaxation time is consistent with the removal of the strong $^3J_{H\text{-}H}$ coupling that is present within **3**.

From an MRI perspective, substrates such as **7** and **8** which contain a single proton, and two ^{2}H labels, would be desirable probes due to the reduction in chemical shift artefacts that would be seen in an echo planar image. While these compounds exhibited higher proton T_1 values than **1** they were not a long as those of **6**. However, **7** produced an impressive SABRE response of 7.8% polarization which is higher than the 2.5% polarization achieved for **8** even though its T_1 values are shorter. These results confirm therefore that substrate binding (L, Scheme 1) occurs predominately at the nitrogen centre that is remote to ester, with the associated $^4J_{H\text{-}H}$ polarization transfer coupling in the SABRE catalyst being more efficient than the $^5J_{H\text{-}H}$ coupling that would be used for **8**.

We sought to further improve these results by replacing the CH_3 group in **6** with a CD_3 group to produce **9**. Despite our predictions, this d_3-methyl ester performed worse than **6**. Upon replacing the methanol-d_4 solvent with the more biologically amenable solvent ethanol-d_6, the T_1 values and signal enhancements achieved across this series of compounds fell, although the best results were still achieved with **6** (4.7%). This change is consistent with the associated increase in solvent viscosity and hence more facile relaxation that results.[27]

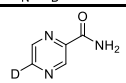
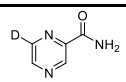
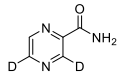
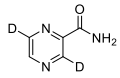
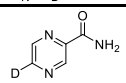
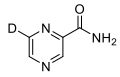
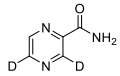
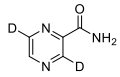
SABRE investigations harnessing deuterated catalysts with deuterated analogues of methyl pyrazine-2-carboxylate (3) In order to see if the effect of relaxation caused by the SABRE catalyst can be reduced, this process was re-examined with a series of deuterated analogues of [IrCl(COD)(IMes)] where the NHC isotopologues had the form shown in Figure 2. Results with **5** and **6**, when the catalyst bears a d_{22}-IMes ligand, proved optimal with both the T_1 values and hyperpolarization levels increasing as detailed in Table 2 (also see supplementary information). The polarization level of 13.4% seen for the 3-H response of **6** corresponds to a ~4200-fold signal enhancement at 9.4 T. The catalyst bearing the d_{24}-IMes ligand, however, was not as efficacious, slightly increasing T_1 values, but with a loss in observed polarization. In an effort to further improve the polarization transfer efficiency, we tested the effect of temperature. This revealed that when **6** was polarized with the d_{22}-IMes catalyst at 0°C a ~6300-fold signal gain for H-3 was realized (corresponding to 20.4% polarization, see supporting information). The temperature at which the SABRE catalysis is conducted has been shown to significantly affect the rate of

Table 2. Effect of a deuterated catalyst and substrate system in methanol-d_4 solution.

Compound	Ligand	Site	$T_{1(\text{with cat.})}$ (s)	Polarization Level (%)
5 	IMes	3-H: 6-H:	26.0 39.2	6.7 ± 0.3 6.2 ± 0.3
	d_{22}-IMes	**3-H:** **6-H:**	**40.8** **54.0**	**10.9 ± 0.5** **9.0 ± 0.5**
6 	IMes	3-H: 5-H:	32.9 26.2	11.9 ± 0.6 9.2 ± 0.5
	d_{22}-IMes	**3-H:** **5-H:**	**37.9** **31.3**	**13.4 ± 0.7** **9.5 ± 0.3**

dissociation of the substrate molecule.[14b] As such, the lifetime of the SABRE active complex will be longer at lower temperatures, which proves to be more efficient for polarization transfer to **6**.

Table 3. T_1 values and hyperpolarization levels of deuterated pyrazinamide derivatives

Compound		Site	$T_{1(\text{no cat.})}$ (s)	$T_{1(\text{with cat.})}$ (s)	Polarization Level (%)
		Methanol-d_4			
1		3-H: 5-H: 6-H:	80.6 20.9 21.2	32.7 12.1 12.3	2.7 ± 0.3 2.8 ± 0.3 1.6 ± 0.1
10		5-H: 6-H:	19.4 20.4	11.3 11.6	3.2 ± 0.2 2.1 ± 0.1
11		3-H: 6-H:	34.8 33.8	21.6 12.7	3.4 ± 0.1 2.8 ± 0.02
12		3-H: 5-H:	51.2 51.5	39.4 (49.6)* 39.3 (49.1)*	2.7 (3.92 ± 0.04)* 2.4 (3.92 ± 0.04)*
13		6-H:	102.8	39.8	0.9 ± 0.1
14		5-H:	55.9	24.5 (37.0)*	4.1 ± 0.3 (9.3 ± 0.6)*
		Ethanol-d_6			
1		3-H: 5-H: 6-H:	56.5 13.6 13.8	18.3 8.0 8.7	2.6 ± 0.2 2.0 ± 0.2 1.2 ± 0.1
10		5-H: 6-H:	13.4 13.1	6.9 6.8	1.7 ± 0.08 1.1 ± 0.1
11		3-H: 6-H:	25.6 30.3	8.3 4.1	3.0 ± 0.2 2.2 ± 0.1
12		3-H: 5-H:	32.8 34.9	18.4 (26.1)* 18.8 (26.6)*	4.5 ± 0.3 (7.7 ± 0.4)* 3.3 ± 0.2 (6.4 ± 0.4)*
13		6-H:	57.4	12.0	2.2 ± 0.1
14		5-H:	29.0	15.3 (16.9)*	6.1 ± 0.3 (7.6 ± 0.2)*

*[IrCl(COD)(d_{22}-Imes)] used as the catalyst

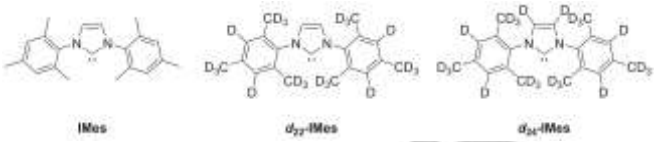


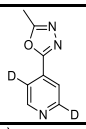
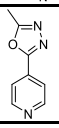
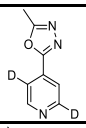
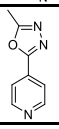
Figure 2. Deuterated analogues of the IMes ligand.

SABRE studies on deuterated analogues of pyrazinamide (1) Investigations were also conducted on a series of related ^{2}H-labelled derivatives of pyrazinamide (**1**), the results of which are detailed in Table 3. Polarization levels and relaxation times were again affected, with deuteration at the 6-position (compound **12**) yielding improved levels of polarization, especially in ethanol-d_6, with its two protons exhibiting 6.4 and 7.7% polarization with the d_{22}-IMes catalyst. Interestingly, the T_1 values for **12** in the presence of this catalyst proved to be remarkably close to that of the substrate alone.

Systematic study of SABRE utilising the isoniazid-type substrates 17-19 Our strategy for increasing the SABRE ^{1}H-polarization levels and T_1 relaxation times by isotopic labelling can also be applied to isoniazid-type substrates. Isoniazid (**2**) has a different structural motif to that of 1, with it containing a single nitrogen centre and substitution in the 4-position. Additionally, **2** exhibits more rapid relaxation than **1** and consequently significantly lower polarization levels are achieved. Optimal, isotopic substitution now results from ^{2}H labelling at the 2- and 5-positions, as shown in **17**, which acts to create two isolated ^{1}H spin systems that exhibit slow relaxation (ca. 34.3 s) whilst maintaining a $^4J_{\text{H-H}}$ transfer coupling that enables 2.0% polarization under similar SABRE conditions to those used previously. **17** was synthesised from dichloroisonicotinic acid **15** by esterification and subsequent palladium catalyzed reduction under D_2 to yield deuterated isonicotinate **16**. Reaction with hydrazine hydrate then gave 2,5-d_2-isoniazid **17** that could also be converted into oxadiazole **18** upon treatment with triethyl orthoacetate (Scheme 3).[28]

The ^{2}H-labelled derivative **18**, was compared to its unlabelled isotopologue **19** which has shown activity against a number of strains of *M. tuberculosis*.[29] As shown in Table 4, when compared to pyrazinamide (**1**), the unlabeled compounds **2** and **19** exhibited much shorter T_1 values; between 4 and 7 seconds in the presence of the SABRE catalyst. Furthermore, the signal enhancements produced by SABRE are low. However, upon deuteration of isoniazid to give **17**, these relaxation times are increased several-fold and polarization levels of 1.9–2.1% result in methanol-d_4. **18** also exhibited significantly longer relaxation times in both methanol-d_4 and ethanol-d_6 when compared to **19**, but the corresponding increase in polarization level is less than that seen with **17**. These values can be improved by the use of the d_{22}-IMes ligand, with the polarization level for **17** rising to 3.7%. Higher temperatures also result in improved responses, such that **14** yields a 9.7% return at 313 K with the d_{22}-catalyst (see the supporting information). Thus we suggest that, in contrast to **6**, the optimal exchange rate for **17** occurs at higher temperatures. Therefore we note that while varying the temperature clearly changes the levels of

Table 4. T_1 values and hyperpolarization levels of deuterated isoniazid derivatives

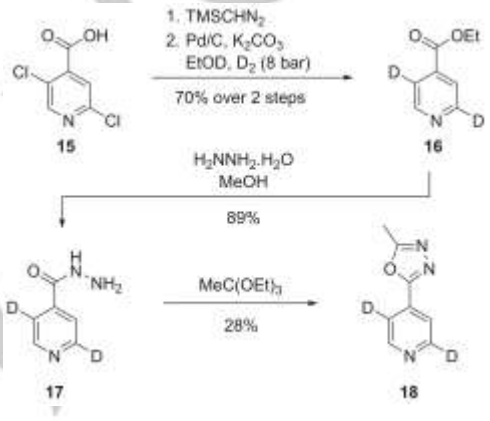
Compound	Site	$T_{1(\text{no cat.})}$ (s)	$T_{1(\text{with cat.})}$ (s)	Polarization Level (%)
Methanol-d_4				
2 	*o*-H: *m*-H:	7.4 7.5	4.9 5.8	1.2 ± 0.02 0.5 ± 0.09
17 	*o*-H: *m*-H:	34.4 34.3	16.5 (17.5)* 19.7 (21.0)*	2.1 ± 0.1 (3.7 ± 0.3)* 1.9 ± 0.1 (3.7 ± 0.4)*
18 	*o*-H: *m*-H:	9.5 40.7	19.3 24.3	0.35 ± 0.04 0.39 ± 0.04
19 	*o*-H: *m*-H:	7.9 9.2	6.4 6.7	0.37 ± 0.02 0.11 ± 0.01
Ethanol-d_6				
2 	*o*-H: *m*-H:	5.2 4.9	4.4 4.4	0.69 ± 0.07 0.06 ± 0.01
17 	*o*-H: *m*-H:	24.5 26.2	12.9 (15.5)* 14.0 (17.4)*	1.07 ± 0.09 (1.82 ± 0.08)* 1.38 ± 0.1 (1.41 ± 0.05)*
18 	*o*-H: *m*-H:	12.1 34.7	14.3 18.0	0.88 ± 0.07 0.93 ± 0.08
19 	*o*-H: *m*-H:	5.0 6.2	4.6 5.0	0.51 ± 0.01 0.06 ± 0.01

*[IrCl(COD)(d_{22}-Imes)] used as the catalyst

hyperpolarization achieved using SABRE in these systems a trend was not observed and each agent needs to be investigated individually to secure an optimal response.

Seeking improvement, SABRE studies on mixtures of these agents We hypothesized that if a mixture of two of these agents were to be employed, it might be possible to improve the detectability of one at the expense of the other. We therefore prepared three samples, containing equimolar amounts of **6** and **8**, **4** and **6** and **13** and **14**. While **6** and **8** produced individual returns of 9.2, 11.9 and 2.5% respectively this changed to 6.4, 6.6 and 1.8% in the mixture and hence both agents perform worse when mixed and agrees with the fall in relaxation times that is also seen.

For the mixture of **4** and **6**, the H-5 polarization of **4** remains constant but there is a significant fall in the other three response levels and the associated relaxation times. In contrast, the polarization level of **13** and **14** both improve, from 0.9% and 4.1% alone to 2.0% and 5.5% in the mixture. The T_1 of **13** falls as a consequence while that of **14** sees little change. This suggests that it may be possible to improve the levels of enhancement produced with the substrates that exhibit weaker polarization by this combination strategy. We therefore examined a sample of **8** and **13** as a further control and discovered that while the polarization of **8** fell to from 2.5% to 1.2% that of **13** increased to 1.7% from 0.9%. There were associated increases in the T_1 values of agent **8** and falls in the value for **13** in accordance with these changes.



Scheme 3. Synthesis of deuterated isoniazid analogues

Conclusions

We have undertaken a systematic SABRE study of the substrates pyrazinamide (**1**), methyl pyrazine-2-carboxylate (**3**) and isoniazid (**2**) and a series of their deuterated analogues. As a consequence, we have shown that the selective partial deuteration of these tuberculosis drug scaffolds results in both a dramatically improved SABRE response and significantly improved relaxation times. Further refinement through the use of a deuterated SABRE polarization transfer catalyst, results in ^{1}H signal gains of up to 6300 times those seen under Boltzmann conditions at 9.4 T result to yield 20.3% net polarization, an effect which is achieved in a matter of seconds. T_1 relaxation times as high as 145 s were seen in methanol-d_4 solution for agent **6**, thereby lengthening the observation window that is available to the detection of an MR response after the hyperpolarization transfer step. **6** represents the optimum isotopologue due to removal of the strong $^3J_{H\text{-}H}$ coupling that facilitates relaxation within **3**, whilst also maintaining an efficient $^4J_{H\text{-}H}$ coupling to the metal hydrides when bound to the catalyst enabling efficacious polarization transfer.

For the tuberculosis drug pyrazinamide (**1**), increases in T_1 relaxation of up to 340% and increases in average signal enhancements from SABRE of up to 360% were achieved in the biologically amenable solvent ethanol-d_6, which correspond to the detection of a ^{1}H signal that has greater than 2500 times the intensity received under Boltzmann detection conditions. This systematic study of a series of pyrazine-2-carboxylates, pyrazine-2-carboxamides, and isoniazid substrates reveals clear insight into the desired deuterium substitution patterns required to optimize the detection of these pharmaceutically important molecules for SABRE hyperpolarization. In the case of **1** and **3**, retaining a ^{1}H label at the 5-H or 1-H sites produce the optimum situation in accordance with the fact that SABRE transfer will proceed into this site via a large $J_{H\text{-}H}$ coupling in the associated catalyst.[15] In view of the fact that tandem treatments with these agents are also common, we also tested the effect of SABRE on a series of mixtures of these agents. It is clear from the presented data that it may be possible to further improve the response of weakly polarising systems under such conditions.

By extending the relaxation times and increasing polarization levels of the key TB drugs shown here we open up the potential for their *in vivo* detection. As SABRE can be a continuous process,[30] the polarization level can be maintained to the point of injection, and thus, despite the T_1 times being reduced in water (see supporting information), these signal gains and relaxation rates should give enough scope for clinically relevant information to be collected. We predict that it will be possible to improve on these responses still further by either working at higher pressures of *p*-H_2 or utilising alternative SABRE catalysts in conjunction with the ^{2}H-labelling strategy exemplified here.[14b, 14c, 23] We hope that ultimately this would contribute positively to the treatment of tuberculosis.

Experimental Section

Polarization transfer procedure: The polarization transfer experiments were conducted in a 5 mm NMR tube that was equipped with a J. Young's Tap. Samples were prepared as follows; a 5 mM solution of [IrCl(COD)(NHC)] and 20 mM of substrate (**1-19**) in methanol-d_4 or ethanol-d_6 (0.6 mL). The samples were degassed prior to the introduction of *para*hydrogen at a pressure of 3 bar. Samples were then shaken for 10 s in the specified fringe field of an NMR spectrometer before being rapidly transported into the magnet for subsequent interrogation by NMR spectroscopy.

Synthetic Procedures and characterization: Full experimental procedures and characterization data can be found in the supplementary information.

Acknowledgements

This work was supported by The Wellcome Trust (Grants 092506 and 098335), the MRC, Bruker Biospin UK and the University of York. We also thank Professor V. H. Perry for useful discussions.

Keywords: Hyperpolarization • SABRE • Tuberculosis • Catalysis • Isotopes

Supporting information via: DOI: 10.15124/5e44a9c2-c968-42c9-b8f0-885a552bafa0

[1] WHO, WHO Press: Geneva, **2016**.
[2] S. Kushner, H. Dalalian, J. L. Sanjurjo, F. L. Bach, S. R. Safir, V. K. Smith, J. H. Williams, *J. Am. Chem. Soc.* **1952**, *74*, 3617-3621.
[3] H. H. Fox, *Science* **1952**, *116*, 129-134.
[4] A. Zumla, P. Nahid, S. T. Cole, *Nat. Rev. Drug Discov.* **2013**, *12*, 388-404.
[5] Y. Zhang, M. M. Wade, A. Scorpio, H. Zhang, Z. Sun, *J. Antimicrob. Chemother.* **2003**, *52*, 790-795.
[6] A. Banerjee, E. Dubnau, A. Quemard, V. Balasubramanian, K. S. Um, T. Wilson, D. Collins, G. Delisle, W. R. Jacobs, *Science* **1994**, *263*, 227-230.
[7] L. Liu, Y. Xu, C. Shea, J. S. Fowler, J. M. Hooker, P. J. Tonge, *J. Med. Chem* **2010**, *53*, 2882-2891.
[8] (a) A. Viale, S. Aime, *Curr. Opin. Chem. Biol.* **2010**, *14*, 90-96; (b) S. B. Duckett, N. J. Wood, *Coord. Chem. Rev.* **2008**, *252*, 2278-2291.
[9] (a) J. H. Ardenkjær-Larsen, B. Fridlund, A. Gram, G. Hansson, L. Hansson, M. H. Lerche, R. Servin, M. Thaning, K. Golman, *Proc. Natal. Acad. Sci. (USA)* **2003**, *100*, 10158-10163; (b) R. R. Rizi, *Proc. Natal. Acad. Sci. (USA)* **2009**, *106*, 5453-5454.
[10] (a) L. Schröder, T. J. Lowery, C. Hilty, D. E. Wemmer, A. Pines, *Science* **2006**, *314*, 446-449; (b) A. Comment, B. van den Brandt, K. Uffmann, F. Kurdzesau, S. Jannin, J. A. Konter, P. Hautle, W. T. Wenckebach, R. Gruetter, J. J. van der Klink, *Concepts Magn. Reson.* **2007**, *31B*, 255-269; (c) M. E. Merritt, C. Harrison, Z. Kovacs, P. Kshirsagar, C. R. Malloy, A. D. Sherry, *J. Am. Chem. Soc.* **2007**, *129*, 12942-12943; (d) C. Gabellieri, S. Reynolds, A. Lavie, G. S. Payne, M. O. Leach, T. R. Eykyn, *J. Am. Chem. Soc.* **2008**, *130*, 4598-4599; (e) K. Golman, R. i. t. Zandt, M. Lerche, R. Pehrson, J. H. Ardenkjaer-Larsen, *Cancer Research* **2006**, *66*, 10855-10860; (f) S. J. Kohler, Y. Yen, J. Wolber, A. P. Chen, M. J. Albers, R. Bok, V. Zhang, J. Tropp, S. Nelson, D. B. Vigneron, J. Kurhanewicz, R. E. Hurd, *Magn. Reson. Med.* **2007**, *58*, 65-69; (g) J. S. Nelson, D. Vigneron, J. Kurhanewicz, A. Chen, R. Bok, R. Hurd, *Appl. Magn. Reson.* **2008**, *34*, 533-544; (h) E. Y. Chekmenev, J. Hövener, V. A. Norton, K. Harris, L. S. Batchelder, P. Bhattacharya, B. D. Ross, D. P. Weitekamp, *J. Am. Chem. Soc.* **2008**, *130*, 4212-4213.
[11] J. Natterer, J. Bargon, *Prog. Nucl. Magn. Reson. Spectrosc.* **1997**, *31*, 293-315.
[12] C. R. Bowers, D. P. Weitekamp, *J. Am. Chem. Soc.* **1987**, *109*, 5541-5542.
[13] (a) R. W. Adams, J. A. Aguilar, K. D. Atkinson, M. J. Cowley, P. I. P. Elliott, S. B. Duckett, G. G. R. Green, I. G. Khazal, J. López-Serrano, D. C. Williamson, *Science* **2009**, *323*, 1708-1711; (b) K. D. Atkinson, M. J. Cowley, P. I. P. Elliott, S. B. Duckett, G. G. R. Green, J. López-Serrano, A. C. Whitwood, *J. Am. Chem. Soc.* **2009**, *131*, 13362-13368; (c) R. E. Mewis, *Magn. Reson. Chem.* **2015**, *53*, 789-800.
[14] (a) M. J. Cowley, R. W. Adams, K. D. Atkinson, M. C. R. Cockett, S. B. Duckett, G. G. R. Green, J. A. B. Lohman, R. Kerssebaum, D. Kilgour, R. E. Mewis, *J. Am. Chem. Soc.* **2011**, *133*, 6134-6137; (b) L. S. Lloyd, A. Asghar, M. J. Burns, A. Charlton, S. Coombes, M. J. Cowley, G. J. Dear, S. B. Duckett, G. R. Genov, G. G. R. Green, L. A. R. Highton, A. J. J. Hooper, M. Khan, I. G. Khazal, R. J. Lewis, R. E. Mewis, A. D. Roberts, A. J. Ruddlesden, *Cat.*

Sci. Technol. **2014**, *4*, 3544-3554; (c) B. J. A. van Weerdenburg, S. Gloggler, N. Eshuis, A. H. J. Engwerda, J. M. M. Smits, R. de Gelder, S. Appelt, S. S. Wymenga, M. Tessari, M. C. Feiters, B. Blumich, F. Rutjes, *Chem. Commun.* **2013**, *49*, 7388-7390.

[15] N. Eshuis, R. Aspers, B. J. A. van Weerdenburg, M. C. Feiters, F. Rutjes, S. S. Wijmenga, M. Tessari, *J. Magn. Reson.* **2016**, *265*, 59-66.

[16] (a) A. N. Pravdivtsev, A. V. Yurkovskaya, H.-M. Vieth, K. L. Ivanov, *J. Phys. Chem. B* **2015**, *119*, 13619-13629; (b) R. W. Adams, S. B. Duckett, R. A. Green, D. C. Williamson, G. G. R. Green, *J. Chem. Phys.* **2009**, *131*; (c) A. N. Pravdivtsev, A. V. Yurkovskaya, H. M. Vieth, K. L. Ivanov, R. Kaptein, *ChemPhysChem* **2013**, *14*, 3327-3331; (d) T. Theis, M. Truong, A. M. Coffey, E. Y. Chekmenev, W. S. Warren, *J. Magn. Reson.* **2014**, *248*, 23-26.

[17] (a) R. V. Shchepin, M. L. Truong, T. Theis, A. M. Coffey, F. Shi, K. W. Waddell, W. S. Warren, B. M. Goodson, E. Y. Chekmenev, *J. Phys. Chem. Lett.* **2015**, *6*, 1961-1967; (b) T. Theis, M. L. Truong, A. M. Coffey, R. V. Shchepin, K. W. Waddell, F. Shi, B. M. Goodson, W. S. Warren, E. Y. Chekmenev, *J. Am. Chem. Soc.* **2015**, *137*, 1404-1407; (c) M. L. Truong, T. Theis, A. M. Coffey, R. V. Shchepin, K. W. Waddell, F. Shi, B. M. Goodson, W. S. Warren, E. Y. Chekmenev, *J. Phys. Chem. C* **2015**, *119*, 8786-8797; (d) D. A. Barskiy, R. V. Shchepin, A. M. Coffey, T. Theis, W. S. Warren, B. M. Goodson, E. Y. Chekmenev, *J. Am. Chem. Soc.* **2016**, *138*, 8080-8083; (e) A. W. J. Logan, T. Theis, J. F. P. Colell, W. S. Warren, S. J. Malcolmson, *Chem. Eur. J.* **2016**, *22*, 10777-10781; (f) R. E. Mewis, K. D. Atkinson, M. J. Cowley, S. B. Duckett, G. G. R. Green, R. A. Green, L. A. R. Highton, D. Kilgour, L. S. Lloyd, J. A. B. Lohman, D. C. Williamson, *Magn. Reson. Chem.* **2014**, *52*, 358-369; (g) R. E. Mewis, R. A. Green, M. C. R. Cockett, M. J. Cowley, S. B. Duckett, G. G. R. Green, R. O. John, P. J. Rayner, D. C. Williamson, *J. Phys. Chem. B* **2015**, *119*, 1416-1424; (h) M. J. Burns, P. J. Rayner, G. G. R. Green, L. A. R. Highton, R. E. Mewis, S. B. Duckett, *J. Phys. Chem. B* **2015**, *119*, 5020-5027; (i) V. V. Zhivonitko, I. V. Skovpin, I. V. Koptyug, *Chem. Commun.* **2015**, *51*, 2506-2509.

[18] P. Nikolaou, B. M. Goodson, E. Y. Chekmenev, *Chem. Eur. J.* **2015**, *21*, 3156-3166.

[19] (a) M. Carravetta, M. H. Levitt, *J. Am. Chem. Soc.* **2004**, *126*, 6228-6229; (b) G. Pileio, M. Carravetta, M. H. Levitt, *Proc. Natal. Acad. Sci. (USA)* **2010**, *107*, 17135-17139; (c) M. H. Levitt, in *Annual Review of Physical Chemistry, Vol 63, Vol. 63* (Eds.: M. A. Johnson, T. J. Martinez), Annual Reviews, Palo Alto, **2012**, pp. 89-105; (d) S. S. Roy, P. Norcott, P. J. Rayner, G. G. R. Green, S. B. Duckett, *Angew. Chem. Int. Ed.* **2016**, *55*, 15642-15645; (e) T. Theis, G. X. Ortiz, A. W. J. Logan, K. E. Claytor, Y. Feng, W. P. Huhn, V. Blum, S. J. Malcolmson, E. Y. Chekmenev, Q. Wang, W. S. Warren, *Sci. Adv.* **2016**, *2*.

[20] P. J. Rayner, M. J. Burns, A. M. Olaru, P. Norcott, M. Fekete, G. G. R. Green, L. A. R. Highton, R. E. Mewis, S. B. Duckett, *Proc. Natal. Acad. Sci. (USA)* **2017**, *114*, E3188-E3194.

[21] S. S. Roy, P. Norcott, P. J. Rayner, G. G. R. Green, S. B. Duckett, *Angew. Chem.* **2016**, *128*, 15871-15874.

[22] H. Zeng, J. Xu, J. Gillen, M. T. McMahon, D. Artemov, J.-M. Tyburn, J. A. B. Lohman, R. E. Mewis, K. D. Atkinson, G. G. R. Green, S. B. Duckett, P. C. M. van Zijl, *J. Magn. Reson.* **2013**, *237*, 73-78.

[23] D. A. Barskiy, A. N. Pravdivtsev, K. L. Ivanov, K. V. Kovtunov, I. V. Koptyug, *Phys. Chem. Chem. Phys.* **2016**, *18*, 89-93.

[24] (a) K. E. Bergmann, M. H. Cynamon, J. T. Welch, *J. Med. Chem* **1996**, *39*, 3394-3400; (b) M. H. Cynamon, R. Gimi, F. Gyenes, C. A. Sharpe, K. E. Bergmann, H. J. Han, L. B. Gregor, R. Rapolu, G. Luciano, J. T. Welch, *J. Med. Chem* **1995**, *38*, 3902-3907; (c) S. C. Ngo, O. Zimhony, W. J. Chung, H. Sayahi, W. R. Jacobs, J. T. Welch, *Antimicrob. Agents Chemother.* **2007**, *51*, 2430-2435.

[25] R. E. Mewis, M. Fekete, G. G. R. Green, A. C. Whitwood, S. B. Duckett, *Chem. Commun.* **2015**, *51*, 9857-9859.

[26] A. J. Holmes, P. J. Rayner, M. J. Cowley, G. G. R. Green, A. C. Whitwood, S. B. Duckett, *Dalton Transactions* **2015**, *44*, 1077-1083.

[27] (a) N. Bloembergen, E. M. Purcell, R. V. Pound, *Phys. Rev.* **1948**, *73*, 679-712; (b) G. Pileio, *Prog. Nucl. Magn. Reson. Spectrosc.* **2010**, *56*, 217-231.

[28] M. Gangapuram, K. K. Redda, *Journal of Heterocyclic Chemistry* **2009**, *46*, 309-316.

[29] G. Navarrete-Vázquez, G. M. a. Molina-Salinas, Z. V. Duarte-Fajardo, J. Vargas-Villarreal, S. Estrada-Soto, F. González-Salazar, E. Hernández-Núñez, S. Said-Fernández, *Biorg. Med. Chem.* **2007**, *15*, 5502-5508.

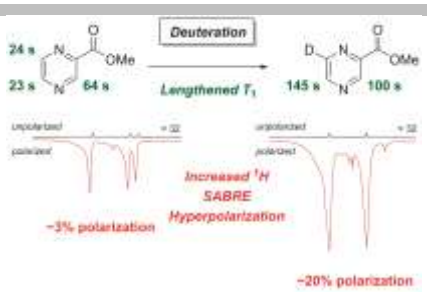
[30] (a) J.-B. Hovener, N. Schwaderlapp, T. Lickert, S. B. Duckett, R. E. Mewis, L. A. R. Highton, S. M. Kenny, G. G. R. Green, D. Leibfritz, J. G. Korvink, J. Hennig, D. von Elverfeldt, *Nat. Comm.* **2013**, *4*, 2946-2946; (b) P. Rovedo, S. Knecht, T. Bäumlisberger, A. L. Cremer, S. B. Duckett, R. E. Mewis, G. G. R. Green, M. Burns, P. J. Rayner, D. Leibfritz, J. G. Korvink, J. Hennig, G. Pütz, D. von Elverfeldt, J.-B. Hövener, *J. Phys. Chem. B* **2016**, *120*, 5670-5677.

10.1002/chem.201703278

WILEY-VCH

FULL PAPER

The tuberculosis drugs, pyrazinamide, isoniazid and their derivatives have been optimally hyperpolarized using SABRE. Through ^{2}H-labelling the drugs and catalysts T_1 lifetimes of over two minutes and ~20% polarization have been achieved which opens the door to *in vivo* assessment.



*Philip Norcott, Peter J. Rayner, Gary G. R. Green, Simon B. Duckett**

Page No. – Page No.

Achieving High ^{1}H Nuclear Hyperpolarization Levels with Long Lifetimes in a Range of Tuberculosis Drug Scaffolds