SHARPER-enhanced benchtop NMR: improving SNR by removing couplings and approaching natural linewidths

Claire L. Dickson[a], George Peat[a], Matheus Rossetto[b], Meghan E. Halse[b], Dušan Uhrín*[a]

[a] EaStCHEM School of Chemistry, University of Edinburgh, Edinburgh, UK.

[b] Department of Chemistry, University of York, York, UK.

Electronic Supplementary Information

Figures

Tables

1. Comparison of the SHARPER experiments with and without PFGs in the acquisition loop.

The increased sensitivity of SHARPER experiments without PFGs in the acquisition loops (**Fig. S1**) is caused by multiple factors including the reduced relaxation and diffusion effects (especially for the *sel*SHARPER experiments), eddy current effects and the fact that PFGs eliminate magnetisation that has not received perfect inversion, e.g. due to B_1 inhomogeneity of pulses, while the CPMG phase cycling without PFG preserves it.

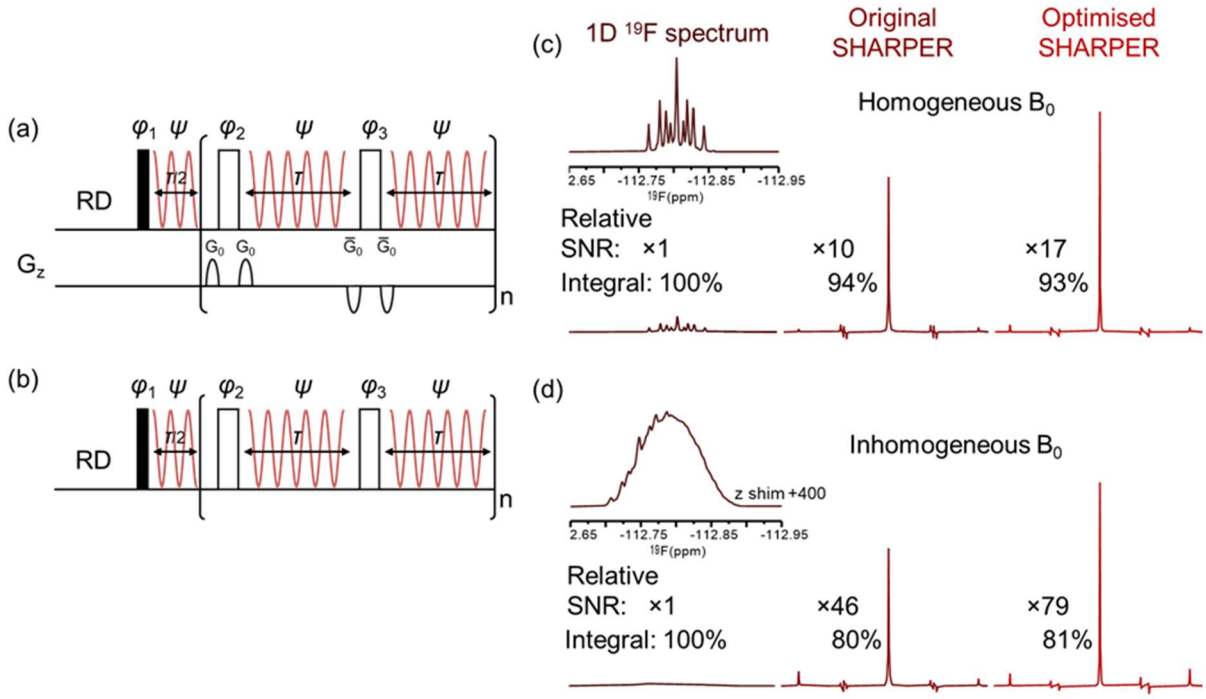

Fig. S1. A comparison of 376.5 MHz ^{19}F SHARPER spectra of fluorobenzene, **1**, obtained by (a) the original SHARPER pulse sequence with PFGs and (b) the optimised SHARPER pulse sequence (identical to **Fig. 1a** in the main paper) in (c) homogeneous and (d) and inhomogeneous magnetic fields. The insets in (c) and (d) show vertical expansion of ^{19}F multiplets from 1D spectra. The filled and empty rectangles represent 90° and 180° hard pulses. τ = AQ/(2n), where AQ is the total acquisition time and n is the total number of loops. The following phase cycle was used for (a): φ_1 = 2x, 2(-x), 2y, 2(-y); φ_2 = 2(y,-y), 2(x, -x); φ_3 = 2(-y, y), 2(-x, x); ψ = 2x, 2(-x), 2y, 2(-y), while for (b): φ_1 = x; φ_2 = y, -y; ψ = x.

Acquisition and processing parameters. The 376.5 MHz ^{19}F spectra of **1** in toluene-d_8 shown in **Fig. S1**, were acquired on a three-channel Bruker Avance III NMR spectrometer equipped with a 5 mm z-gradient BB TBO ^{1}H, ^{19}F probe using one scan, a total acquisition time (AQ) of 10.0 s, dwell time of 66.4 μs and a 90° rectangular pulse of 16.373 μs. The ^{19}F SHARPER loop used chunk lengths (τ) of 20.8 ms. When applicable, 300 μs sine-shaped PFGs set to ±1% of the nominal value followed by a 200 μs gradient recovery delay were used. The spectra were processed by retaining both real and imaginary data points and zero-filling to 524k points. Exponential line-broadening (1.15 Hz) was used prior to FT for the determination of integral values. The measurement of SNR was performed on spectra without any line-broadening.

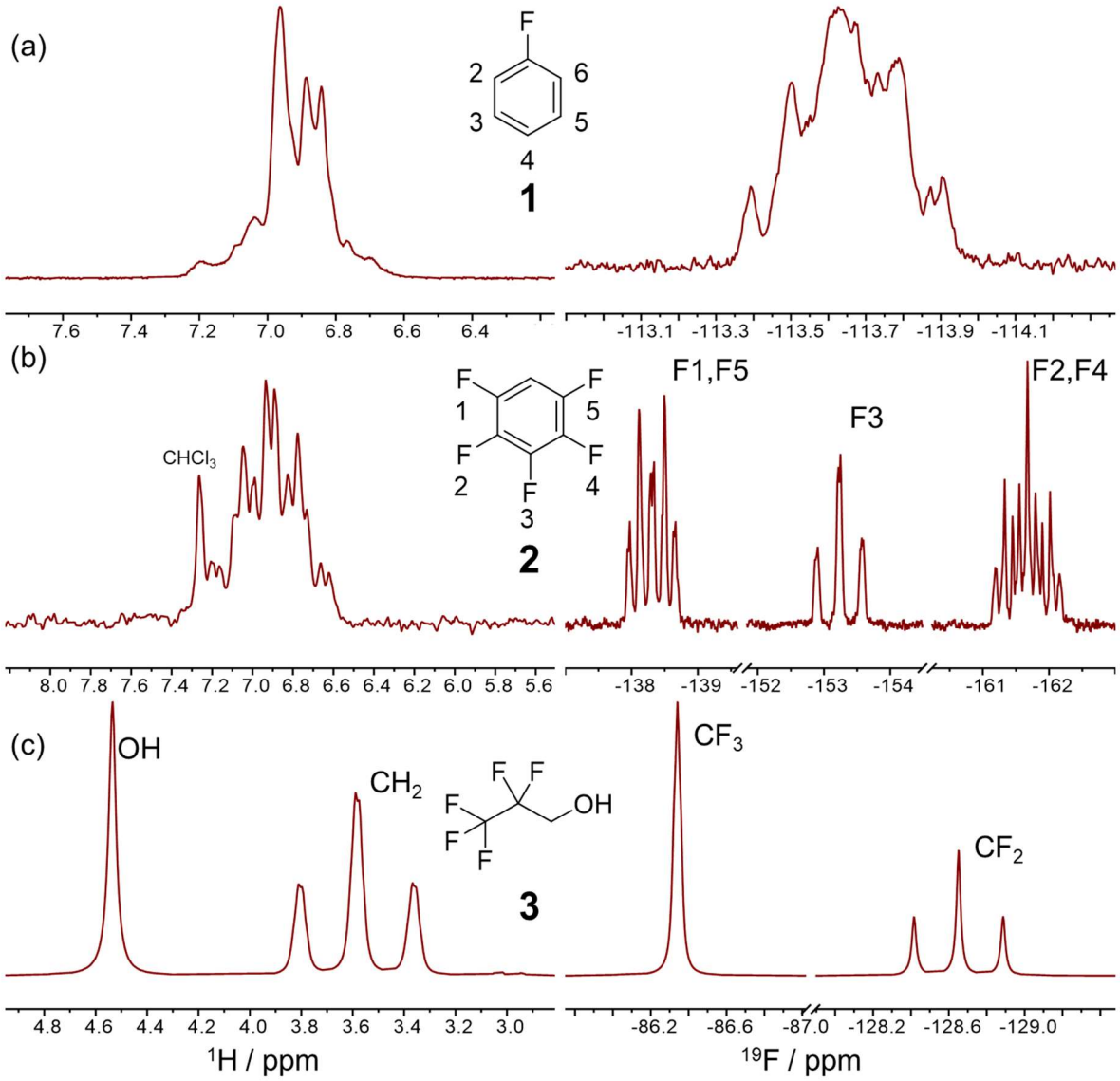

Fig. S2. 1D ^{1}H and ^{19}F spectra acquired on a 60 MHz NMR spectrometer. (a) fluorobenzene, **1**, in toluene-d_8; (b) pentafluorobenzene, **2**, in chloroform-d and (c) neat 2,2,3,3,3,-pentafluoropropanol, **3**.

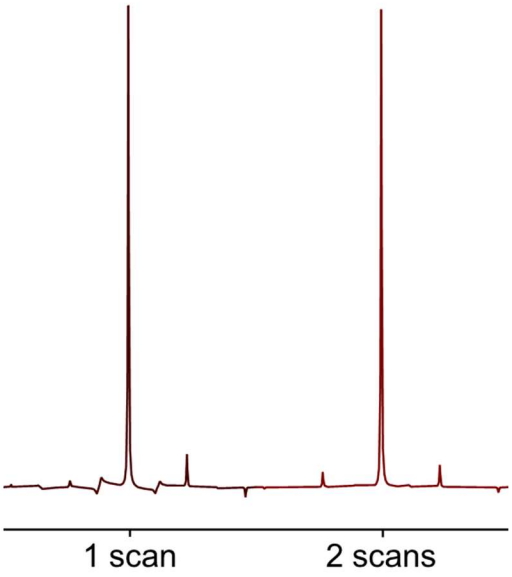

Fig. S3. A comparison of 1 and 2 scan 376.5 MHz spectra acquired using a rectangular ^{19}F *sel*SHARPER. The CF_2 signal of neat pentafluoropropanol, **2**, is shown. Both real and imaginary points were kept.

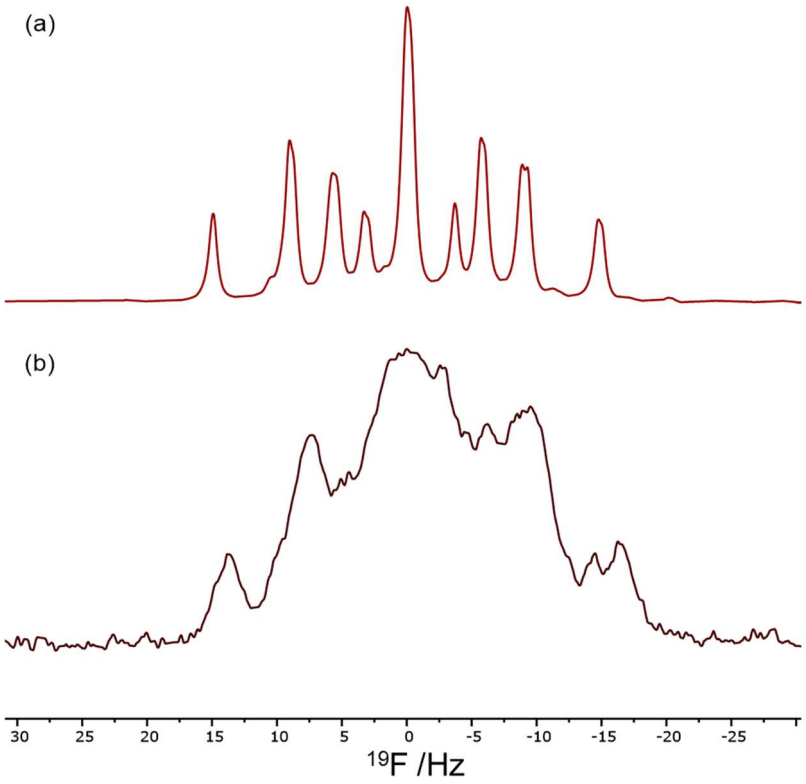

Fig. S4. A comparison ^{19}F NMR spectra of fluorobenzene, **1**, at (a) 376.5 and (b) 56.5 MHz.

2. Experimental parameters

Benchtop ^{19}F spectra of fluorobenzene, **1**, (50 μl and 550 μl of toluene-d_8) pentafluorobenzene, **2**, (30.8 mg in 0.6 ml $CDCl_3$) and 2,2,3,3,3,-pentafluoropropanol, **3**, neat or 43 μl in 550 μl of D_2O were acquired on a Spinsolve Ultra 60 MHz Carbon benchtop spectrometer. The full power 90° ^{19}F rectangular pulse was 132 *μs* for all samples. The ^{19}F SPFGSE/SE *sel*SHARPER experiment used 5 ms 180° Gaussian refocussing pulses; direct excitation in the 270G *sel*SHARPER was achieved by a 7.5 ms 270° Gaussian pulse. The rectangular *sel*SHARPER used a 90° and 180° rectangular pulse of 393 *μs* and 786 *μs* for both the neat and diluted samples of **3**. Rectangular PGFs in the SPFGSE-*sel*SHARPER experiment were applied for 300 *μs* using a 70% proportional change to the z shim coils. Parameters that varied across samples are tabulated below.

Table S1. Experimental parameters of SHARPER experiments.

Sample	Scans[a]	Repetition time /s	Acquisition time /s	Dwell time /μs	Chunk time, τ /ms
1	1, 2, 4	17	10.9445	167	20.04
2	16	23	8.192	125	20
3 (neat)	4	13	6.488	99	20
3 (D_2O)	16	29	19.6608	300	20

[a] two dummy scans were applied

The spectra were processed by zero-filling to 256k points, automatic baseline correction with a 3rd order polynomial and exponential line broadening to π / AQ, where AQ is the acquisition time of the spectra, to measure integrals and linewidth at half height ($\Delta^S_{1/2}$), the applied line broadening was subtracted to produce the reported values[1]. The measurements of SNR was performed on SHARPER spectra using matched filters line-broadening, LB = $\Delta^S_{1/2}$. The measurement of SNR on 1D ^{19}F and SPFGSE spectra for comparison to SHARPER spectra was done using line-broadening (LB = 0.64 Hz) determined to maximise SNR while retaining resolved multiplets and following the decay[2] of the FIDs that was visible up to 0.5 s (hence LB = 1/0.5π = 0.64 Hz). The removal of the data from the imaginary channel of the SHARPER FIDs was performed within the Spinsolve program by zeroing the imaginary channel prior to saving the FID. SNR calculations were performed in Mestrenova 14.2.1.27684 using its in-built manual SNR function. The tallest peak of the investigated multiplet in 1D spectra was considered. Identical chemical shift window was chosen to evaluate the white noise in the 1D and SHARPER spectra. This window was free from chunking sidebands, impurities or baseline issues.

Table S2. ^{19}F T_1 and T_2 relaxation times for compounds **1** - **3** measured using inversion recovery and CPMG methods, respectively.

Compound	Solvent	^{19}F δ/ppm	T_1 /s	T_2 /s
1	toluene-d_8	-112.96	3.2	3.0
2	$CDCl_3$, F1,5	-138.62	4.2	3.0
	F3	-153.54	4.3	3.4
	F2,4	-161.98	4.3	2.6
3	Neat, CF_3	-84.199	1.3	1.2
	CF_2	-126.51	1.3	1.3
3	D_2O, CF_3	-81.642	5.1	4.8
	CF_2	-123.75	5.7	5.8

3. Calibrating power levels of selective pules

If the response of amplifiers is not strictly linear, the longer lower power rectangular or shaped pulses in the *sel*SHARPER pulse sequences require calibration. The calibration procedure implemented on a 60 MHz Magritek spectrometer was as follows. Nutation curves were measured with a standard ProtonDurationSweep experiment (Spinsolve Expert version 1.40.8) modified for ^{19}F. For a given power level, this experiment records a series of pulse and acquire 1D spectra with incremented rectangular pulse length using 1 scan and a repetition time of 10 s. Spectra were integrated and integral values plotted against the pulse duration to determine the maximum response and therefore the 90° pulse up to the manufacture's limit of 1000 µs. As mostly the 180° pulses are used in SHARPER sequences these values were doubled and pw_{180} values plotted against the power level (dB scale) and extrapolated beyond the 2 ms value using a 6th order polynomial function. These power levels deviated slightly from the theoretical dependency also shown in **Figs. S5** and **S6**.

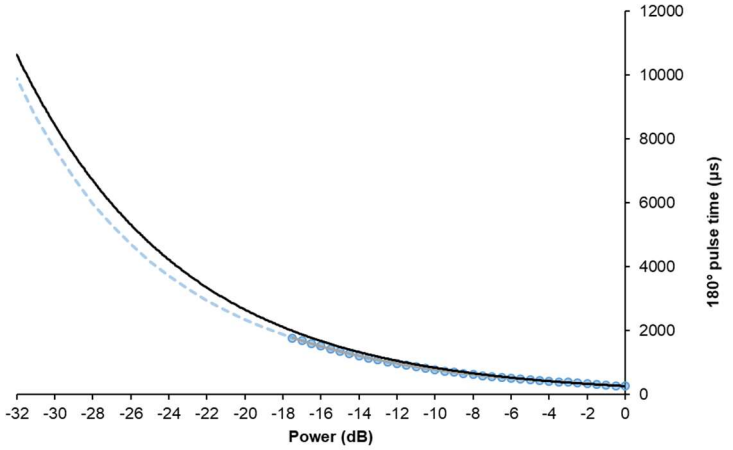

Fig. S5. Relationship between the attenuation and the pulse length. Experimental values are given between 0 and -17.6dB (blue circles). The dashed line represents extrapolation of the experimental values to -32 dB and the black line is the theoretical dependency, $pw_{180} = pw_{180}^{0dB} 10^{\frac{-dB}{20}}$, (here $pw_{180}^{0dB} = 264\mu s$).

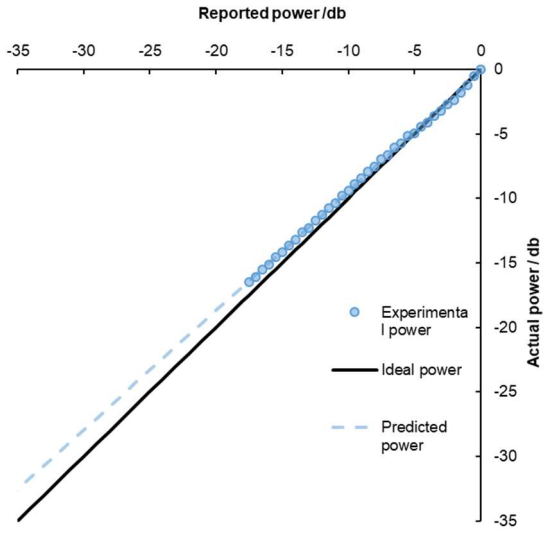

Fig. S6. The relationship between the produced and input power (in dB).

The power level for the 180° Gaussian pulses was adjusted to account for the differences in the power output of rectangular and Gaussian pulses of the same length as $dB_{Gauss} = dB_{rect} - 20 \log(0.41)$. It has been demonstrated previously,[3] that miss-setting of 180° pulses in SHARPER experiments leads to decreased intensity of the main signal and the deterioration of its lineshape. Capitalising on the sensitivity gains provided by SHARPER, it therefore is possible to fine tune the calibration using real samples; the neat liquid standards are not required. In case of fluorobenzene, **1**, the agreement between the general calibration and the SHARPER calibration was satisfactory (**Fig. S7**) showing small difference in signal intensity within ± 0.8 dB indicating a good tolerance of SHARPER experiments to slight miss calibration. This was also confirmed using other compounds as seen in **Table S3**.

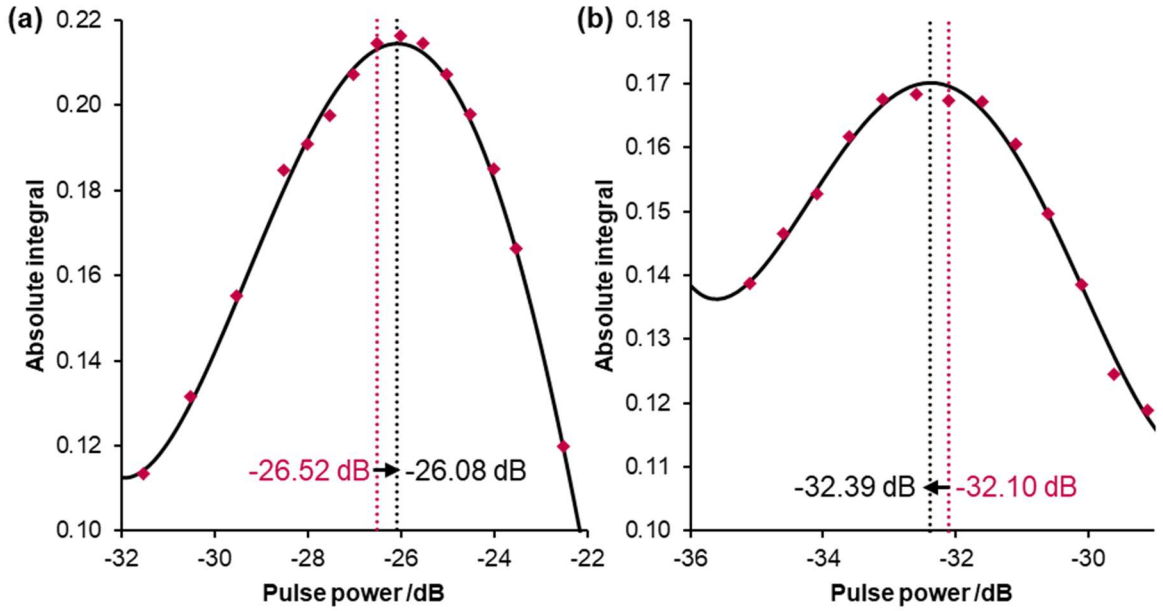

Fig. S7. Pulse calibration using SPFGSE *sel*SHARPER measured for **1** in toluene-d_8. (a) and (b) show 5 ms and 10 ms 180° Gaussian pulses, respectively. The red and black dotted lines indicate the power levels predicted by nutation experiments and the *sel*SHARPER calibrated values, respectively.

Table S3. SPFGSE *sel*SHARPER optimised powers levels for 180° Gaussian pulses.

Compound	Solvent	Pulse length /ms	Predicted power (PP) /dB	Optimised power (OP)/dB	PP-OP/dB
1	Toluene-d_8	5	-26.52	-26.08	-0.44
1	Toluene-d_8	10	-32.10	-32.39	0.29
2	$CDCl_3$	5	-26.52	-25.81	-0.71
3	Neat	5	-26.52	-25.92	-0.6
3	D_2O	5	-26.52	-26.00	-0.52

4. Relative increase of SNR in one scan SHARPER experiments.

The values of the SNR presented in **Fig. 2** for a 1D spectrum of fluorbenzene, **1**, were obtained using a 4 scan experiment, a 90° pulse ^{19}F and the repetition time dictated by the ^{19}F T_1 of **1**. As the same repetition time was also used for SHARPER experiment, spins could only relax for shorter time, mainly because the acquisition time does not count towards relaxation. Additional factors for *sel*SHARPER, were the selective pulses, which lengthen the acquisition. However, when considering a one scan experiment and allowing for different acquisition times to be used, enhancements achieved by SHARPER experiments per unit of time increase further. These enhancements are quantified in **Table S4** for different versions of SHARPER taking into account the T_1 relaxation time of **1**.

Table S4. A comparison of SNR enhancements for multiple- and single-scan experiments on **1** [a,b]

Experiment	Actual AQ	Available relaxation time /s	Relative SNR-multiple scans[c]	Relative SNR - single scan
1D ^{19}F	10.95	17.0	1.0 [d]	1.0 [d]
SPFGSE ^{19}F	10.95	17.0	0.9	0.9
Nonselective SHARPER	11.09	5.9	20.8	24.7
SPFGSE-*sel*SHARPER	13.68	3.3	12.8	19.9
SE-*sel*SHARPER	13.68	3.3	11.0	17.1
270G-*sel*SHARPER	13.68	3.3	10.4	16.1

[a] Achieved per unit of time; [b] Set acquisition time AQ = 10.95s; ^{19}F T_1 = 3.21 s; [c] Taken from Fig. 2; [d]Normalised to 1.

5. Python script for removing the imaginary component of SHARPER FIDs .

```
# -*- coding: utf-8 -*-
"""
Script to zero imaginary component of 1D Bruker FID
   @author: Claire Dickson
   @email: claire.l.dickson@ed.ac.uk
Requirements:
   Python 3.6 tested
   Bruker 1D FIDs tested
   numpy, nmrglue, matplotlib, shutil
Notes:
   - User must enter input path: "path".
   - A new folder path-proc/outstr will be made for the processed FID.
   - The output experiment number (expno) will match the input if inpath
     and outstr use the same number.
   - The script will fail if the output folder already exists.
   - The script only replaces the FID in the new output folder, any processed spectra
     in the output folder will be old until the new FID is processed in TopSpin.
   - Plots can be removed from the script without effecting the processing.
   - The script is set up for the processing of Bruker data. However NMR glue is
     capable of reading/writing Bruker, NMRPipe, Magritek, JCAMP-DX, etc.
     Therefore, it can be modified for use on different file types.

"""

# PREPARATION
import nmrglue as ng
import matplotlib.pyplot as plt
import numpy as np
import shutil


# USER INPUT REQUIRED
# Define the location of the experimental dataset up to but excluding expno
path = 'fill out with folder details'

# Location (expno) of the inital input data, eg 35
inpath = path+"/35"

# Output prefix, must be a number eg 351
outstr = "351"

# COPY ORIGINAL DATA

# Location (expno) for the final output data
outpath = path+"-proc/"+outstr   # for the 1i zeroed data

# Make Output folder, this will fail if the output folder already exists
shutil.copytree(inpath, outpath)

# PROCESSING OF FID
```

```
# Read in the raw data using nmrglue
dic,data = ng.bruker.read(inpath)

# separate the real data
real = data.real

# create a new imaginary component of zeroes, the same length as the original
imag = np.array([1j]*len(data))

# combine real and imaginary
new = real + imag

# write the new FID in a bruker format, overwriting what is already there.
ng.fileio.bruker.write(outpath,dic,new,overwrite=True)

# PLOT OF FIDS
fig, (ax1, ax2) = plt.subplots(2, 1, figsize=(30, 5))
fig.subplots_adjust(hspace=0.5)

# Plot the real/imaginary components of FID
ax1.plot(data.real)
ax1.plot(data.imag)

# plot the new real and imaginary components
ax2.plot(new.real)
ax2.plot(new.imag)

# label plots
ax2.set_xlabel('Data point')
ax2.set_ylabel('1i set to 0')
ax1.set_xlabel('Data point')
ax1.set_ylabel('Raw data')
```

6. Bruker AU program for removing the imaginary component of SHARPER FIDs.

```
/*
Claire Dickson
20 April 2021
claire.l.dickson@ed.ac.uk

 - Making a new folder (name, expno, procno) and coping the current data to it.
 - Opening the FID and making a "rawdata" array for further editing.
 - The imaginary data in "rawdata" is then set to zero.
- Notes:
        1. Both raw (FID) and processed (spectrum) data are copied.
           The FID must be reprocessed to see the result.
        2. For Bruker data (NEO console) use double (as below).
        3. For Bruker data (AVIII console) all instances of “double” should be replaced with “float”.
        4. Benchtop data has not been tested.
*/

// Initialisation.
#define MAXSIZE 800000

int expno1, procno1, expno2, procno2, td, tdtest, a, b, c;
double rawdata[MAXSIZE];
char oldname[500], newname[500], infile[PATH_MAX], outfile[PATH_MAX];
FILE *fpin, *fpout;


// Get the foreground data set.
GETCURDATA

// Set initial data variables from current dataset.
expno1 = expno;
procno1 = procno;
strcpy(oldname, name);
strcpy(infile, ACQUPATH("fid"));

// Set new data variables from current dataset.
expno2 = expno;
procno2 = procno;
strcpy(newname, name);

// Print some information.
printf("\tSTART \n\n");
printf("Current dataset: \t%s %d %d \n", name, expno, procno);
printf("Initial dataset: \t%s %d %d \n", oldname, expno1, procno1);
printf("Initial FID file path: \t%s\n", infile);

// Offer user the chance to change one or all of the variables for the new data.
```

```
GETSTRING("Enter a file name:", newname)
GETINT("Enter a experiment file number:", expno2)
GETINT("Enter a processed file number:", procno2)

// Write a copy of the inital dataset to the new location.
WRPA(newname, expno2, procno2, disk, user)
printf("\n\tCopy raw and processed data to new file location. \n");
printf("Current dataset: \t%s %d %d \n", name, expno, procno);
printf("Initial dataset: \t%s %d %d \n", oldname, expno1, procno1);
printf("New dataset: \t\t\t%s %d %d \n", newname, expno2, procno2);

// Load the new location.
DATASET(newname, expno2, procno2, disk, usr)
strcpy(outfile, ACQUPATH("fid"));
printf("\n\tGo to new file location. \n");
printf("Current dataset: \t%s %d %d \n", name, expno, procno);
printf("Initial dataset: \t%s %d %d \n", oldname, expno1, procno1);
printf("New dataset: \t\t\t%s %d %d \n", newname, expno2, procno2);
printf("New FID file path: \t%s\n\n", outfile);

// Load in TD (real+imaginary points) from the experiment parameters
FETCHPAR("TD", &td)

// Open the FID for reading using the outfile path, return an error message if it fails.
if((fpin = fopen(outfile,"r")) == NULL){STOPMSG("Open of new FID failed for reading.\n")};

// Read the data in the FID into rawdata
// Data in the FID is 8 bytes, with alternating real and imaginary rows up to TD.
fread(rawdata,sizeof(double),td,fpin);

//Close the FID which is in the outfile path
fclose(fpin);

// Print some of the initial points of the FID
printf("\n\t Check the first five rows of the initial FID. \nRow\t\tReal\t\tImaginary\n");
for(a=0;a<10;a++)
	{
		if (a%2 == 0)
			printf("%d\t\t%f\t\t", a/2, rawdata[a]);
		else
			printf("%f\n", rawdata[a]);
	}


// Set the imaginary points to 0
for(b=0;b<td;b++)
	{
		if (b%2 == 1)
```

```
                        rawdata[b] = 0;
        }


// Print some of the initial points of the processed FID
printf("\n\t Check the first five rows of the new FID. \nRow\t\tReal\t\tImaginary\n");
for(a=0;a<10;a++)
        {
                if (a%2 == 0)
                        printf("%d\t\t%f\t\t", a/2, rawdata[a]);
                else
                        printf("%f\n", rawdata[a]);
        }

// Open the FID for writing using the outfile path, return an error message if it fails.
if((fpout = fopen(outfile,"w")) == NULL){STOPMSG("Open of new FID failed for writing.\n")};

// Read the data in the FID into rawdata
// Data in the FID is 8 bytes, with alternating real and imaginary rows up to TD.
fwrite(rawdata,sizeof(double),td,fpout);

//Close the FID which is in the outfile path
fclose(fpout);

QUIT
```

7. Faster acquisition of SHARPER spectra with optimum SNRs

The long FIDs of **3** in D_2O obtained in rectangular *sel*SHARPER experiments (AQ = 20 s) were truncated to keep data points up to $AQ_T = nT_2^S$, where n is the truncation factor and T^S_2 is the effective T_2 relaxation of the SHARPER singlet calculated as $T_2^S = 1/\pi\Delta^S_{1/2}$ ($\Delta^S_{1/2}$ is linewidth at half height of the SHARPER singlet). The truncated FIDs were multiplied by an exponential function producing e^{-a} attenuation of their last point. The LB to achieve such attenuation can be calculated using Eqn. 1.

$$LB = \frac{a-n}{n}\Delta^S_{1/2} \qquad (1)$$

Table S5 contains two examples of the rectangular *sel*SHARPER spectra of **3** containing singlets of the CF_3 and CF_2 groups, respectively. It can be seen that (i) the maximum SNR is obtained for n=3 and LB = $\Delta^S_{1/2}$ (ii) The second maximum for n=1.5 is just beyond the theoretical maximum (i.e. n = 1.25, not considering apodisation); (iii) SNR plateaus between n = 1 and 2 staying above 90 % of the maximum achievable SNR; (iii) even for n = 0.25, SNR stays above 50% of the maximum. The much shortened acquisition times corresponding to these truncations are suitable for faster reactions.

Table S5. SNR of SHARPER spectra as a function of the acquisition time. Attenuation factor a = 3 was used, reducing the last point of the truncated FIDs to 5% of their starting values.[a]

Group	Truncation factor $n = AQ_T/T_2^S$	Truncated acquisition time AQ_T /s	$\frac{3-n}{n}$	Applied LB /Hz	Relative SNR[b]
CF_3	0.25	1.21	11.0	0.725	52%
	0.5	2.41	5.0	0.329	72%
	1	4.82	2.0	0.132	92%
	1.25	6.03	1.4	0.092	96%
	1.5	**7.23**	**1.0**	**0.066**	**98%**
	2	9.64	0.5	0.033	95%
	3	14.47	0.0	0.000	71%
	3	14.47	1.0	0.066	100%
CF_2	0.25	1.02	11.0	0.857	53%
	0.5	2.04	5.0	0.389	76%
	1	4.08	2.0	0.156	93%
	1.25	5.10	1.4	0.109	96%
	1.5	**6.12**	**1.0**	**0.078**	**97%**
	2	8.16	0.5	0.039	95%
	3	12.24	0.0	0.000	74%
	3	12.24	1.0	0.078	100%

[a] $\Delta^S_{1/2}$ (CF_3) = 0.066 Hz, $\Delta^S_{1/2}$ (CF_2) = 0.078 Hz, T_2^S (CF_3) = 4.82 s, T_2^S (CF_2) = 4.08 s.

[b] Relative to maximum achievable SNR for $AQ_T = 3T_2^S$ and the FID multiplied by a matched filter, LB=$\Delta^S_{1/2}$.

8. Eliminating the effects of *J* couplings on the integral intensities of SHARPER spectra

The spectrum of pentafluoropropanol, **3**, contains signals due to the CF_3 and CF_2 groups split by CH_2 protons (**Fig. S2c**). The CF_3 group is an unresolved triplet (J ~1 Hz), while the CF_2 is a large triplet (J = 13.8 Hz). Using the chunk length of 20 ms and integrating only the main CF_2 and CF_3 SHARPER signals produced differences of up to 10% relative to the corresponding CF_2 and CF_3 integrals obtained from a 1D ^{19}F spectrum of **3 (Fig. S8a)**. As anticipated, integration of the CF_2 SHARPER signal returned smaller values due to its large $^3J_{HF}$ coupling constant. Widening the integral region to include the first sidebands reduced these differences to ~1 % in all cases as illustrated in **Fig. S8b** for different variants of SHARPER pulse sequences.

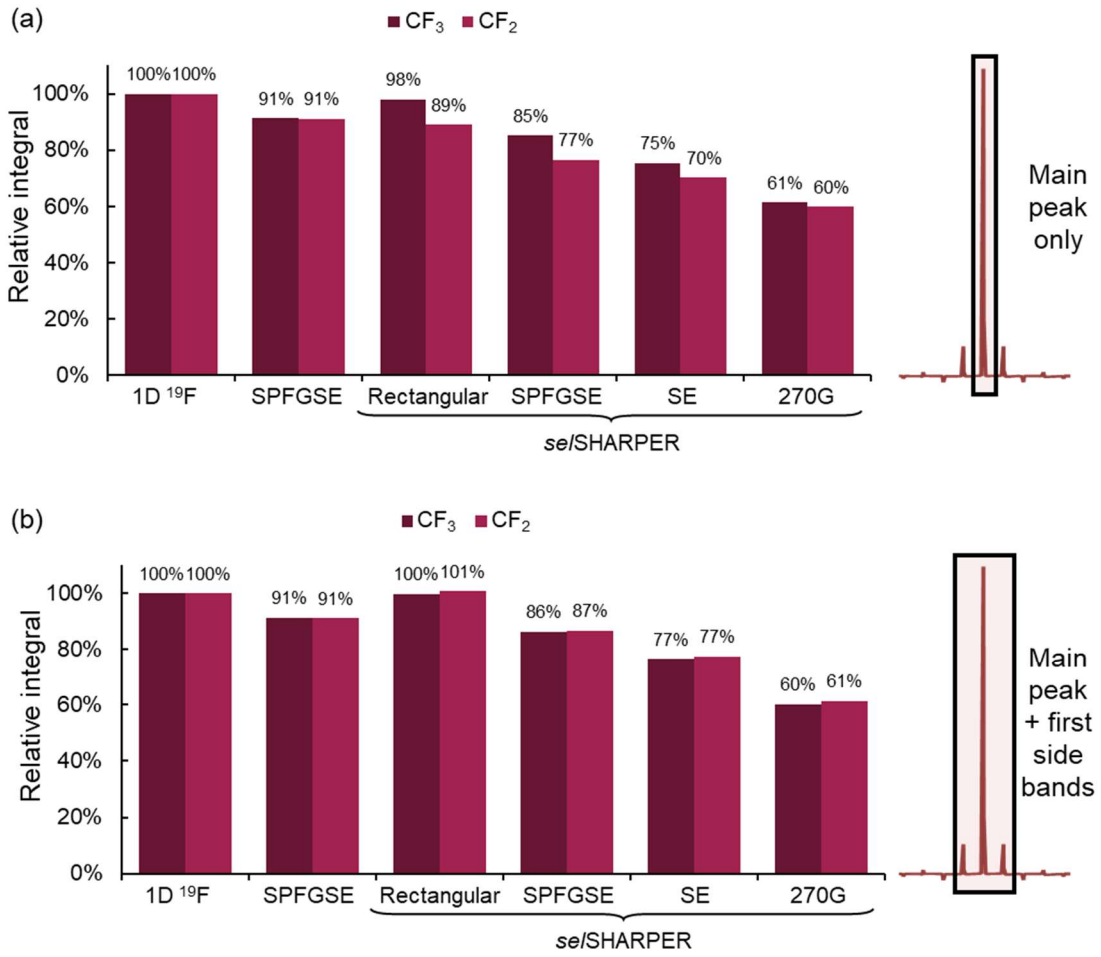

Fig. S8. The effect of *J* couplings on the integral intensities in *sel*SHARPER spectra of neat sample of **3**. Relative integrals for the CF_3 and CF_2 groups compared to those (set to 100%) in a 1D ^{19}F spectrum of **3**. (a) Integrals of the main *sel*SHARPER peak only; (b) integrals using wider integral region to include the first side bands.

References

1. R. G. Spencer, *Concepts in Magnetic Resonance Part A*, 2010, **36A**, 255-265.
2. J. C. Lindon and A. G. Ferrige, *Progress in Nuclear Magnetic Resonance Spectroscopy*, 1980, **14**, 27-66.
3. A. B. Jones, G. C. Lloyd-Jones and D. Uhrin, *Analytical Chemistry*, 2017, **89**, 10013-10021.