Electronic Supplementary Material (ESI) for ChemComm.

Supplementary Information

Measuring Couplings in Crowded NMR Spectra: Pure Shift NMR with Multiplet Analysis

M. Foroozandeh, R. W. Adams, P. Kiraly, M. Nilsson, and G. A. Morris

Contents

Experimental details

All experimental spectra were recorded at a nominal temperature of 298 K on a Bruker Avance II+ 500 MHz spectrometer with a 5 mm BBO probe equipped with a *z*-gradient coil with a maximum nominal gradient strength of 53 G cm^{-1}. For the PSYCHE-2D J experiment, G_1, G_3 and G_5 are half-sine gradient pulses, used for selection of the desired coherence transfer pathways and have amplitudes of 15 G cm^{-1}, 24 G cm^{-1} and 38 G cm^{-1} respectively and a duration of 1 ms each. G_4 is a rectangular gradient pulse, aligned with the midpoint of the pair of chirp pulses, and has an amplitude of 0.75 G cm^{-1}. G_2 and G_6 are rectangular gradient pulses, applied to dephase coherences that arise from strong couplings, and have an amplitude of 1.0 G cm^{-1}. The two 180° chirp pulses have a duration of 40 ms, a bandwidth of 10 kHz, and an RF amplitude of 447 Hz each. The PSYCHE pulse sequence element consists of two low flip angle saltire chirp pulses with flip angle of 15°, an RF amplitude of 50 Hz, a duration of 15 ms, and a bandwidth of 10 kHz each. The TSE-PSYCHE experiment was run with the same parameters as for PSYCHE-2D J, except that data were acquired using the pulse sequence shown in Figure S2b and were processed using a reconstruction macro.

For the *nemo*ZS experiment, an rsnob pulse with 2×40 non-equidistant modulations was used with a duration of 120 ms and an RF amplitude of 1.56 kHz. The spatial encoding gradient during this pulse was set to 0.65 G cm^{-1}.

Relationship of RF amplitude and flip angle for saltire chirp pulse

A normal chirp pulse with linear low-to-high frequency sweep over ΔF (Hz), duration of τ_p (sec), and RF amplitude of A (Hz) can be written as:

$$LH\_Chirp(t) = Ae^{i\pi\left(\Delta F/\tau_p\right)\times(t-t_0)^2} = A\left\{\cos\left(\pi\left(\Delta F/\tau_p\right)(t-t_0)^2\right) + i\sin\left(\pi\left(\Delta F/\tau_p\right)(t-t_0)^2\right)\right\} \qquad (1)$$

For a high-to-low frequency sweep this gives:

$$HL\_Chirp(t) = Ae^{-i\pi\left(\Delta F/\tau_p\right)\times(t-t_0)^2} = A\left\{\cos\left(\pi\left(\Delta F/\tau_p\right)(t-t_0)^2\right) - i\sin\left(\pi\left(\Delta F/\tau_p\right)(t-t_0)^2\right)\right\} \qquad (2)$$

where $t_0 = 0$ and t varies from - $\tau_p/2$ to $\tau_p/2$.

For a saltire chirp pulse, which is the average of *LH_Chirp*(t) and *HL_Chirp*(t), only the real part remains (Figure S1):

$$Saltire\_Chirp(t) = A\cos\left(\pi\left(\Delta F/\tau_p\right)(t-t_0)^2\right) \qquad (3)$$

transforming the pulse from phase modulated to amplitude modulated.

The flip angle β in degrees of a saltire chirp pulse can be calculated as:

$$\begin{aligned}\beta &= 360\times A\int_{-\infty}^{+\infty}\cos\left(\pi\left(\Delta F/\tau_p\right)(t-t_0)^2\right)dt \\ &= 360\times A\sqrt{\frac{\tau_p}{2\Delta F}}\end{aligned} \qquad (4)$$

and consequently, the RF amplitude of PSYCHE chirp element (in Hz) required for a flip angle $\beta°$ is:

$$A = \left(\frac{\beta}{360}\right)\times\sqrt{\frac{2\Delta F}{\tau_p}} \qquad (5)$$

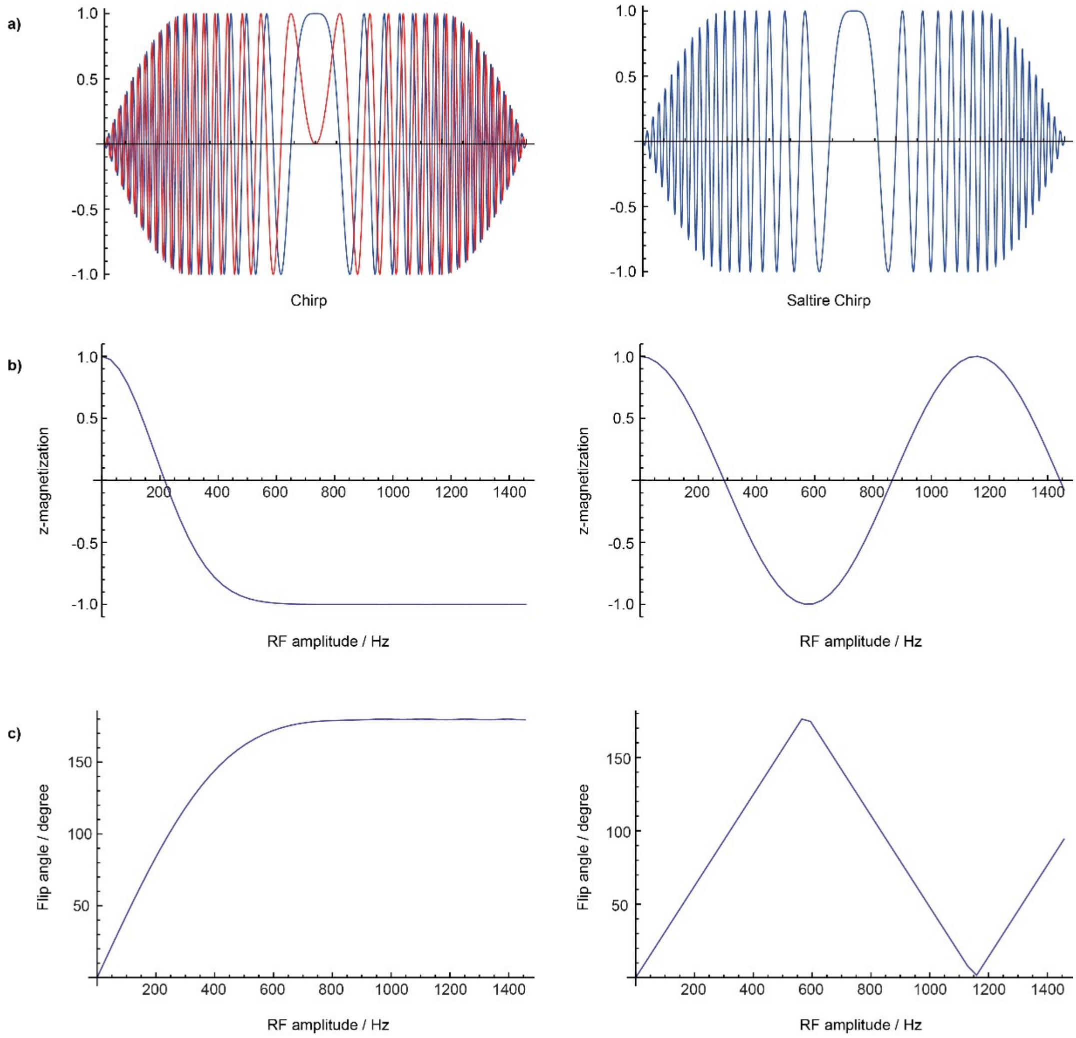

Figure S1. Graphs showing the **(a)** phase and amplitude modulation profiles (real part in blue, imaginary in red) as a function of time, **(b)** the degree of inversion of an initial equilibrium z magnetization as a function of RF amplitude *A*, and **(c)** net flip angle on resonance, modulo 180°, as a function of *A*, for a normal (left) and saltire chirp (right). All plots were calculated for a 15 ms pulse of 10 kHz sweep width.

Pulse sequence of 1D PSYCHE and 1D TSE-PSYCHE

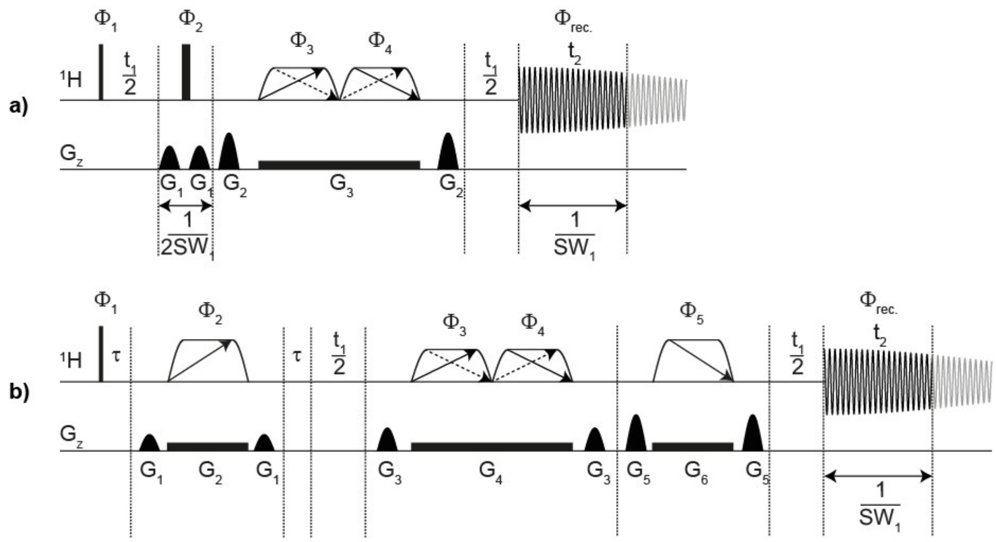

Figure S2. Pulse sequence for (a) 1D PSYCHE, and (b) 1D TSE-PSYCHE. Thin and thick filled rectangular shapes are 90° and 180° pulses respectively. Double chirp pulse elements with double arrows are PSYCHE pulse elements consisting of two low flip angle saltire chirp pulses. Trapezoidal shapes with single arrows are 180° swept-frequency chirp pulses, and τ is equal to $1/4*SW_1$. Both experiments are run in an interferogram-based fashion. All half-sine shape gradients are CTP gradients and rectangular shapes are weak gradients during chirp elements.

Experimental results for 1D TSE-PSYCHE

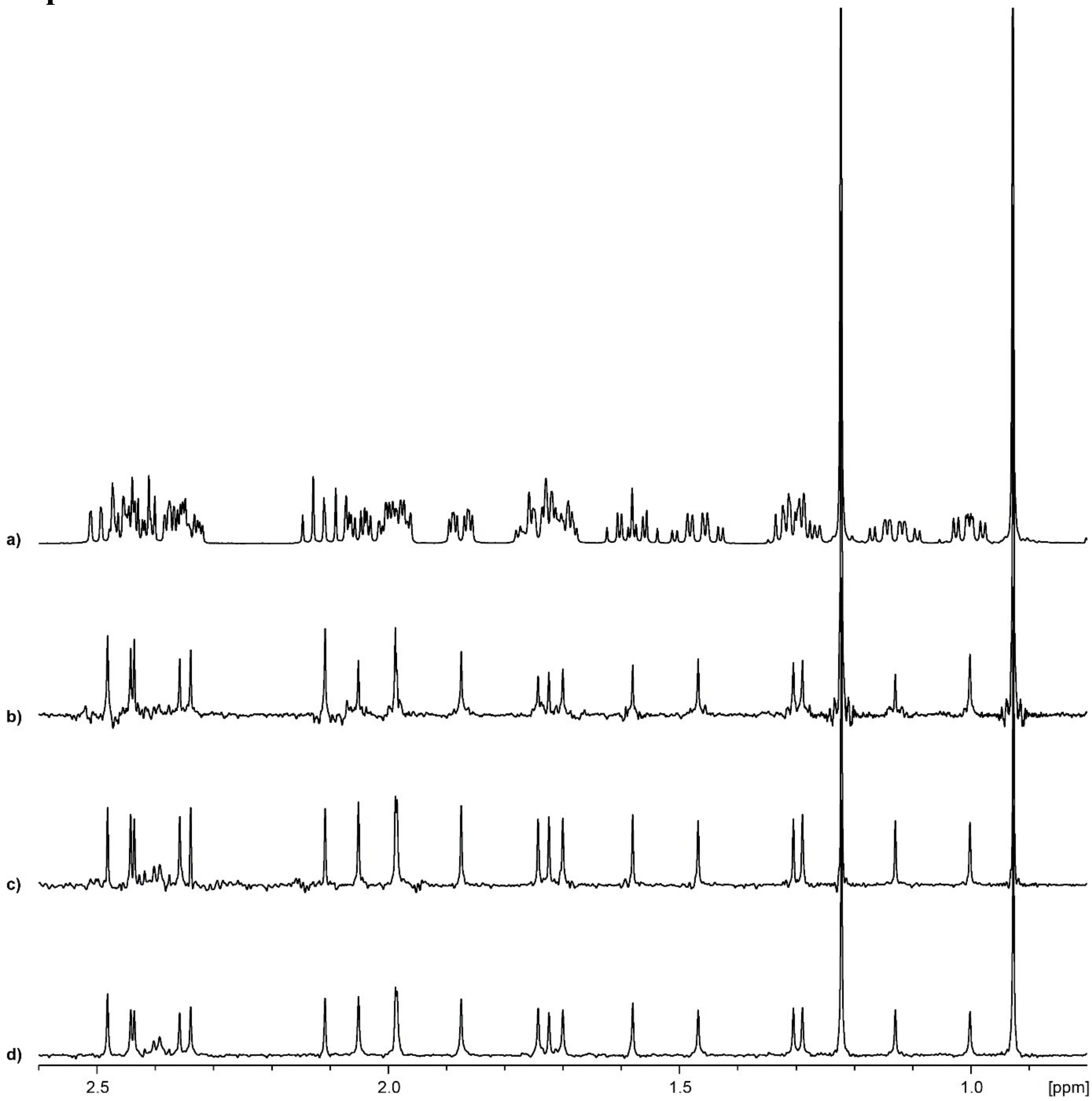

Figure S3. Spectra of a sample of androst-4-ene-3,17-dione in chloroform-*d*, acquired by (a) conventional 1D ^{1}H, (b) the nemoZS method using a 120 ms rsnob pulse with 2×40 non-equidistant modulations, (c) 1D PSYCHE, and (d) 1D TSE-PSYCHE. In (c) and (d) the flip angle β of the PSYCHE pulse element was set to 14° (corresponding to an RF amplitude of 45 Hz).

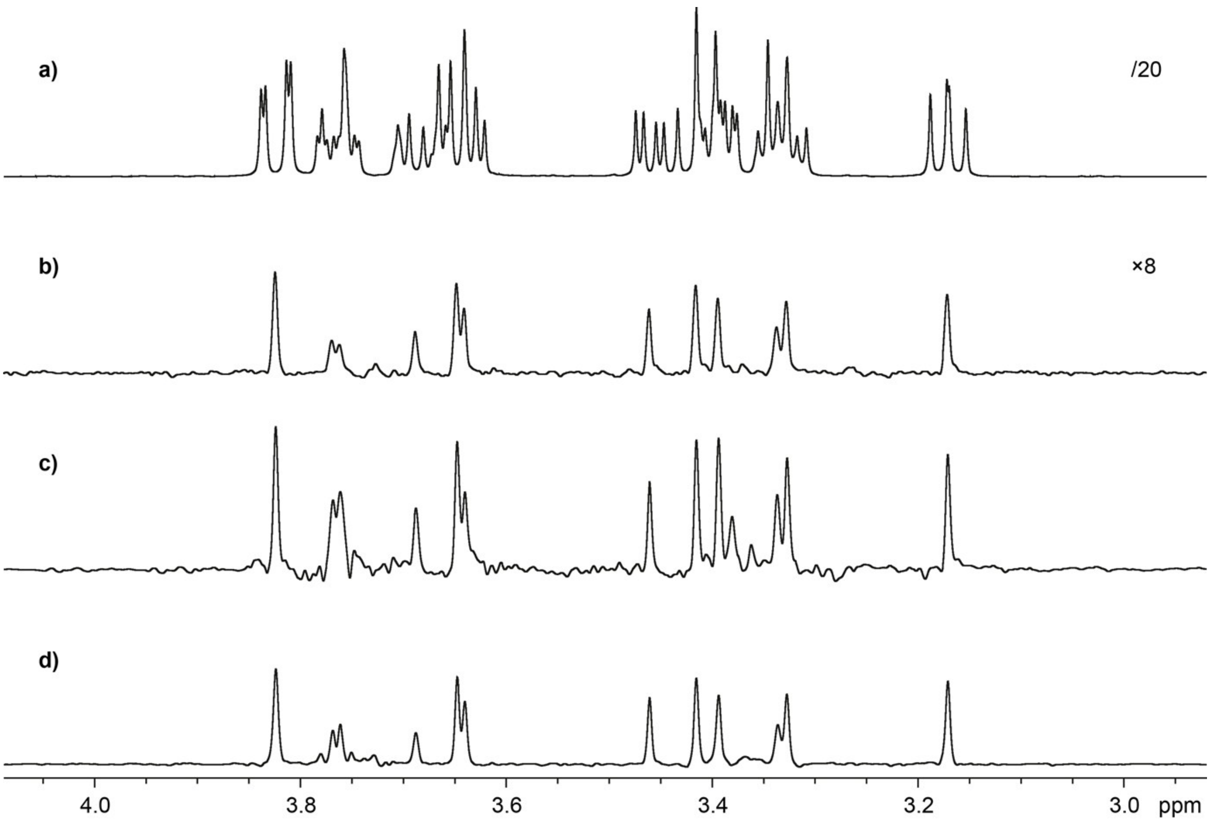

Figure S4. Spectra of a sample of glucose in D_2O, acquired by (a) conventional 1D ^1H, (b) the ZS method using a 120 ms rsnob pulse, (c) 1D PSYCHE, and (d) 1D TSE-PSYCHE. In (c) and (d) the flip angle β of the PSYCHE pulse element was set to 15° (corresponding to an RF amplitude of 48 Hz).

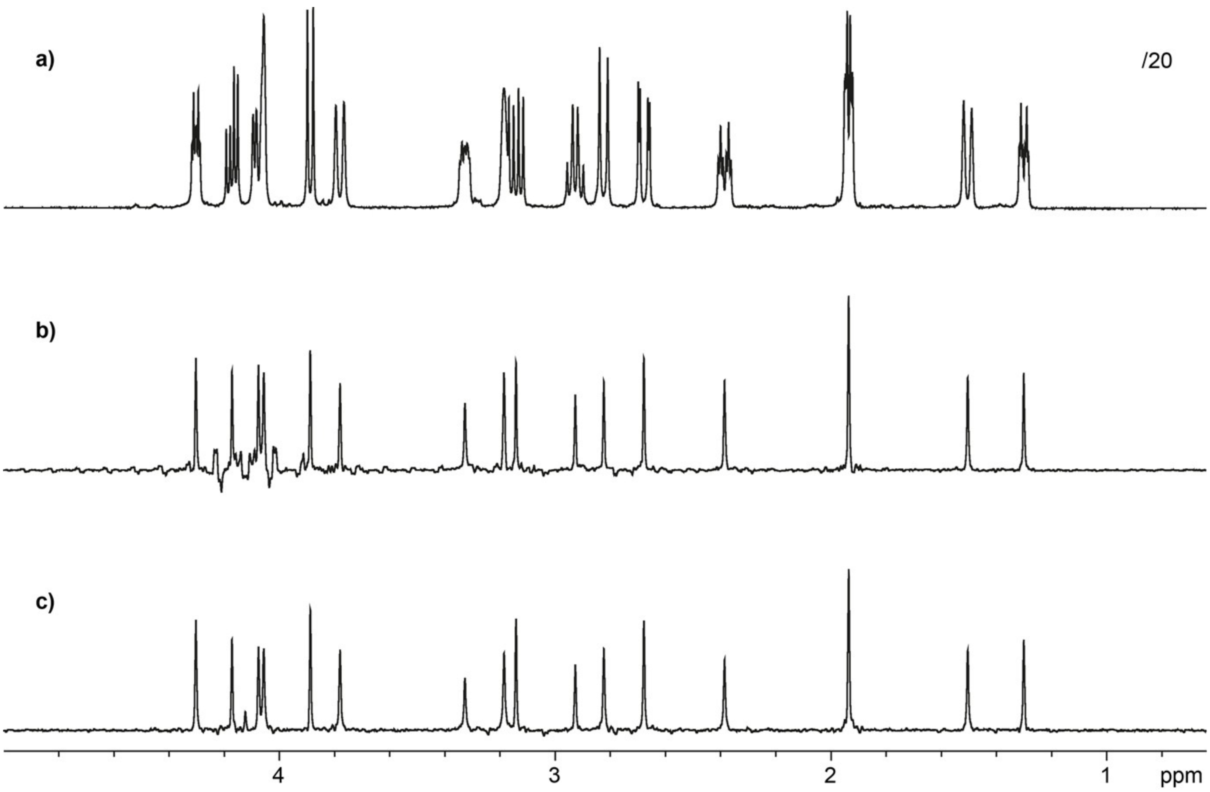

Figure S5. Spectra of a sample of strychnine in chloroform-*d*, acquired by (a) conventional 1D ^{1}H, (b) 1D PSYCHE, and (c) 1D TSE-PSYCHE. In (b) and (c) the flip angle β of the PSYCHE pulse element was set to 15° (corresponding to an RF amplitude of 48 Hz).

Tolerance of TSE-PSYCHE to B_1 inhomogeneity and pulse miscalibration

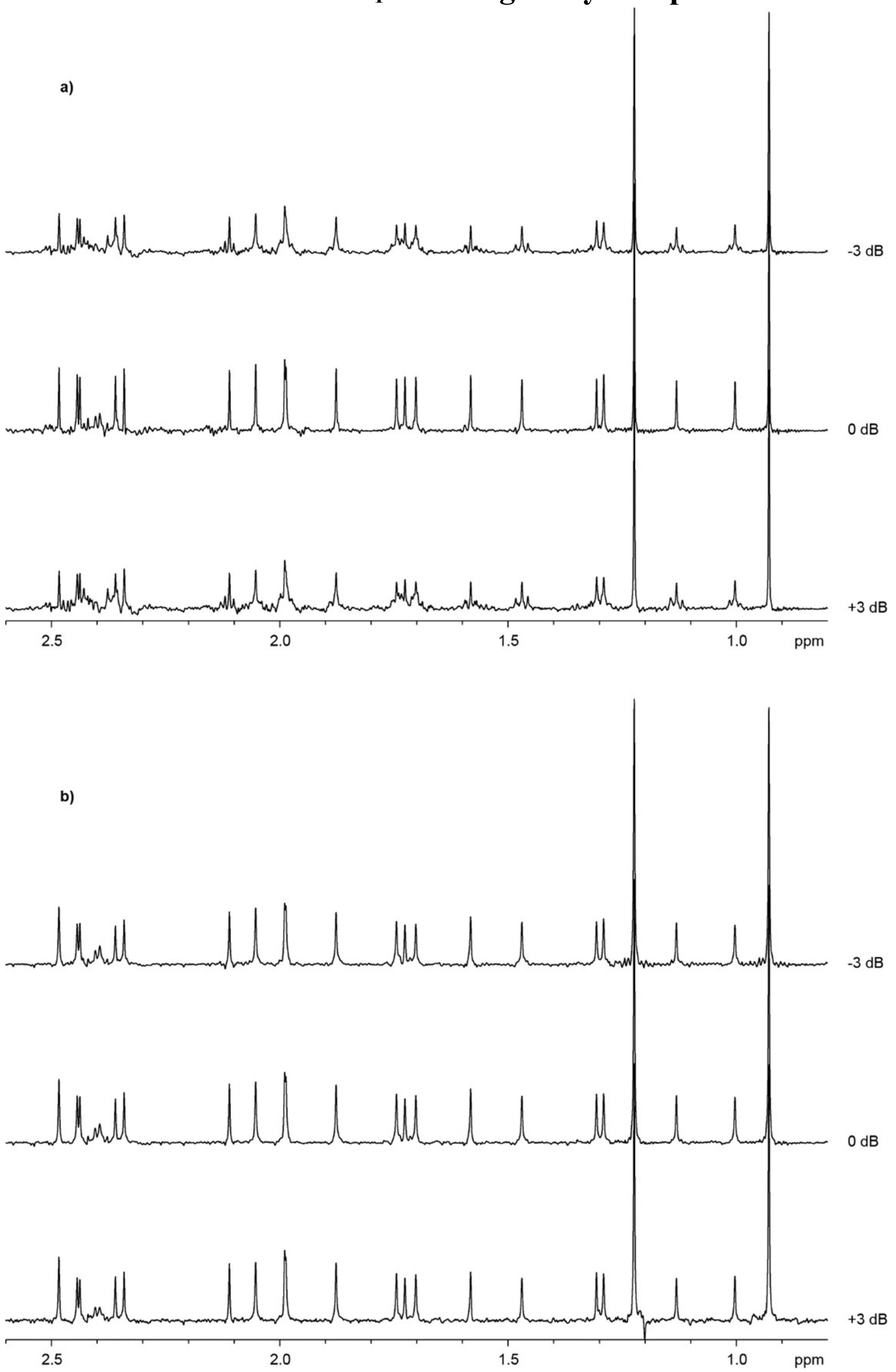

Figure S6. The effect of variation of B_1 field on **a)** 1D PSYCHE, and **b)** 1D TSE-PSYCHE for a sample of androst-4-ene-3,17-dione in chloroform-*d*. The values on the right side of each spectrum indicate the deviation of the RF power used from its optimum value. The TSE-PSYCHE experiment shows a very good tolerance of pulse miscalibration.

Comparison of PSYCHE 2D J to conventional 2D J and Pell-Keeler 2DJ using ZS

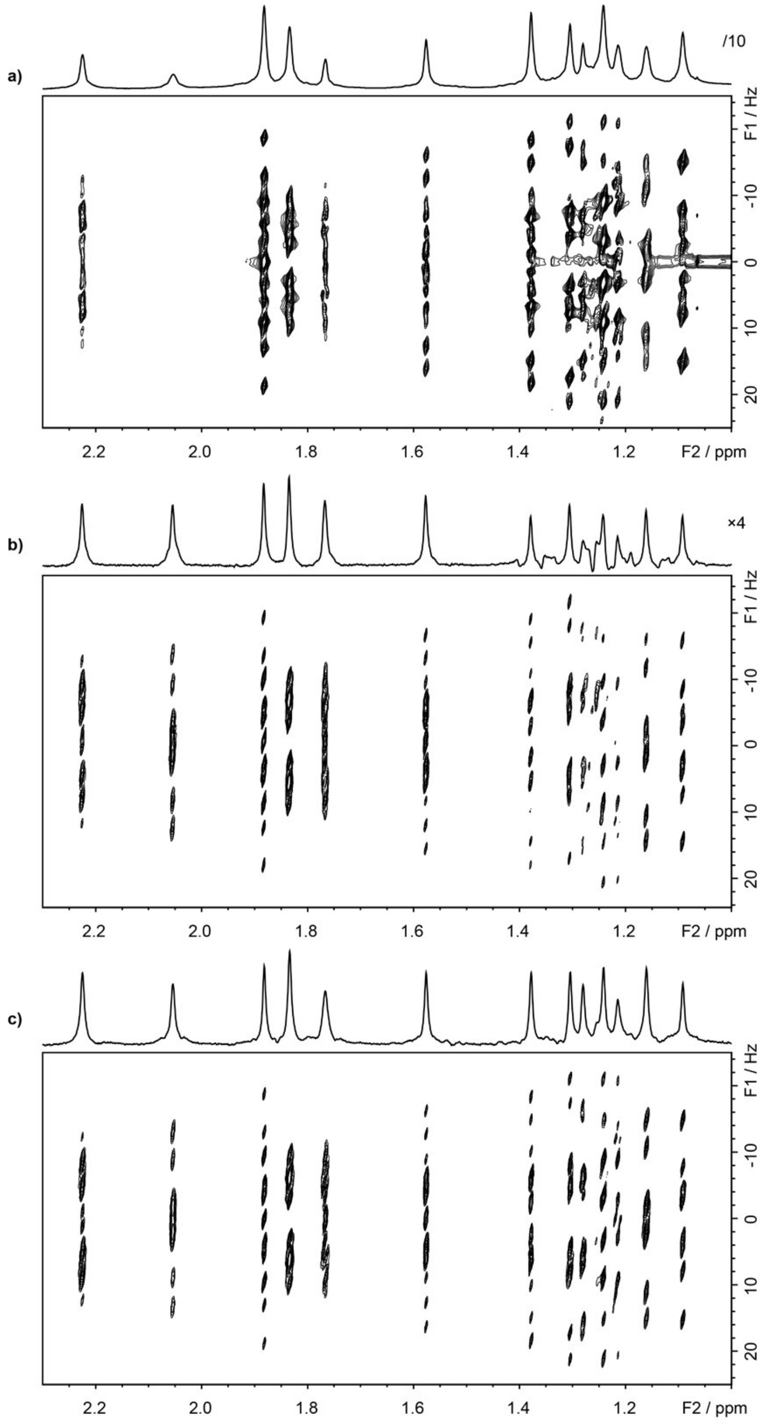

Figure S7. 2D J-resolved spectra of a sample of estradiol in DMSO-d_6, obtained using (**a**) the conventional 2D J-resolved pulse sequence, (**b**) the pulse sequence developed by Pell and Keeler, using the ZS method for J refocusing, and (**c**) the PSYCHE-2D J sequence. Spectrum (**a**) was processed in magnitude mode, using an unshifted sine bell window function in the indirect dimension (F_1) and a Gaussian window function in the direct dimension (F_2). Spectrum (**b**) was acquired using a 30 ms rsnob pulse, and processed using Gaussian window functions in both F_1 and F_2 dimensions. Spectrum (**c**) was acquired using a flip angle β of 15° for the PSYCHE pulse element and processed using Gaussian window functions in both F_1 and F_2 dimensions. All spectra were acquired with 128 and 16384 points in F_1 and F_2 respectively, with spectral windows of 50 Hz and 5000 Hz in F_1 and F_2 respectively, 8 transients, and an experimental time of 1.5 h each.

PSYCHE 2D J spectrum of cyclosporin A

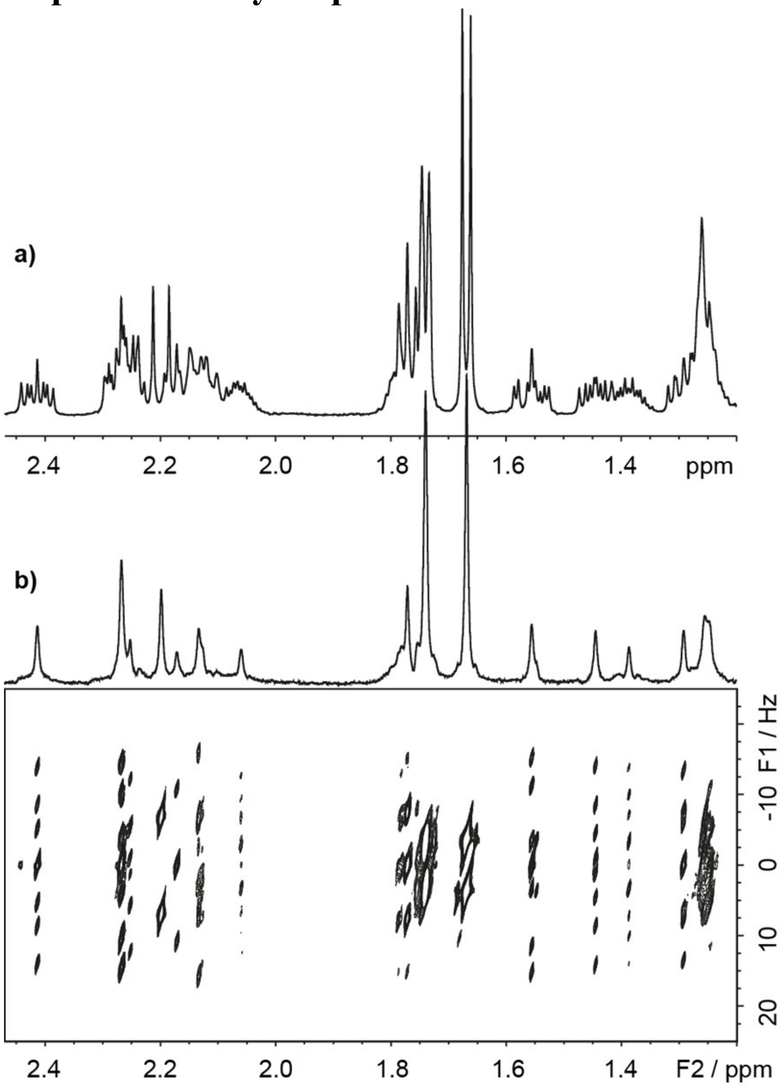

Figure S8. (**a**) 1D [1]H spectrum, and (**b**) absorption mode PSYCHE-2D J spectrum of a sample of cyclosporin A in benzene-d_6. The 2D J spectrum is tilted through 45°.

Multiplet structures for estradiol, extracted from PSYCHE 2D J spectrum

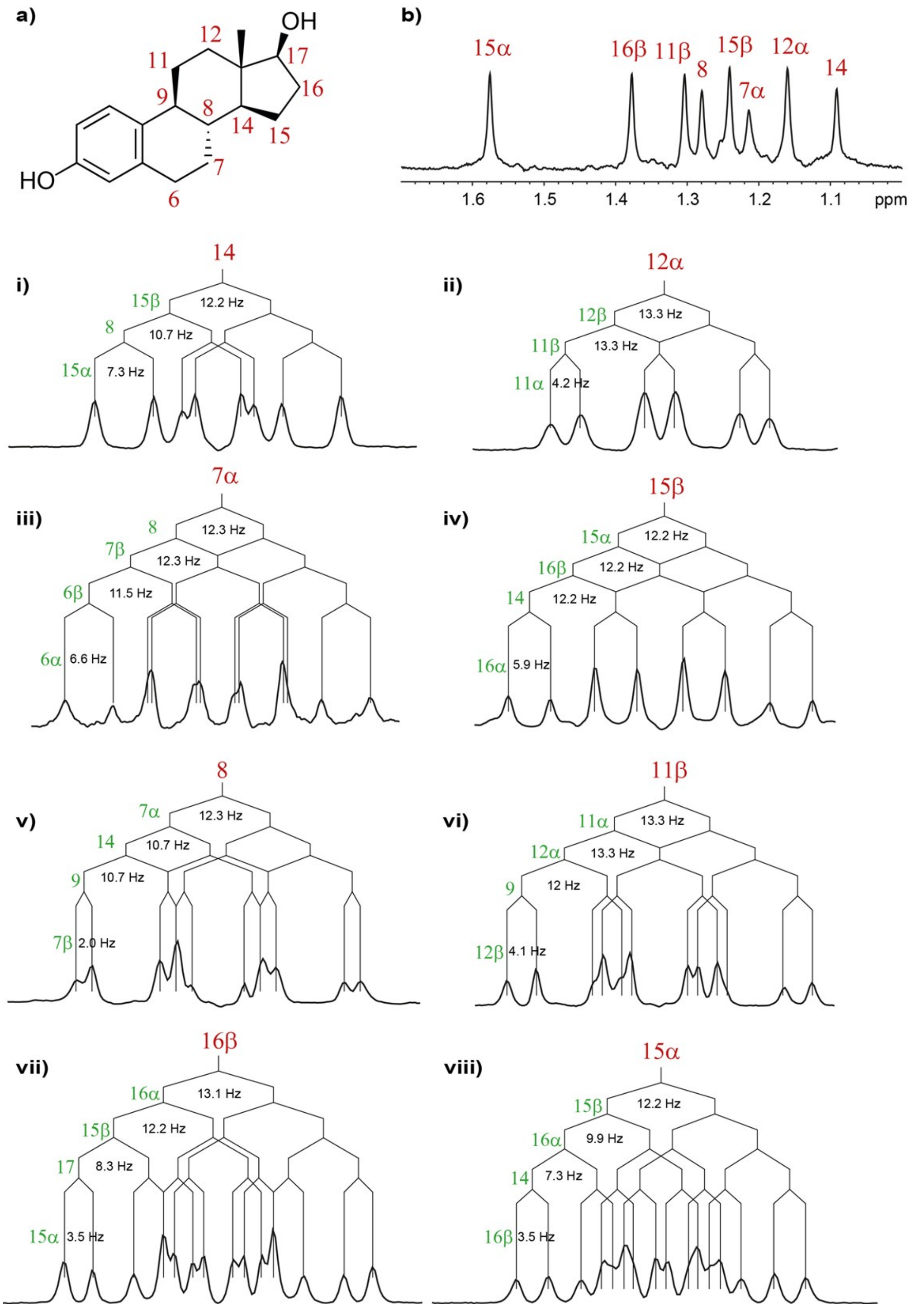

Figure S9. (**a**) Structure of 17β-estradiol (E2), (**b**) pure shift 1D ^{1}H spectrum, obtained by F_2 projection of an absorption mode PSYCHE 2D J spectrum, with assignments of signals, and (**i-viii**) the multiplets structures taken from the spectrum shown in **Figure 2b**. Each multiplet is marked with the proton number in red, coupling partner numbers in green, and the corresponding J values.

Chemical shift and J evolution during PSYCHE 2D J sequence

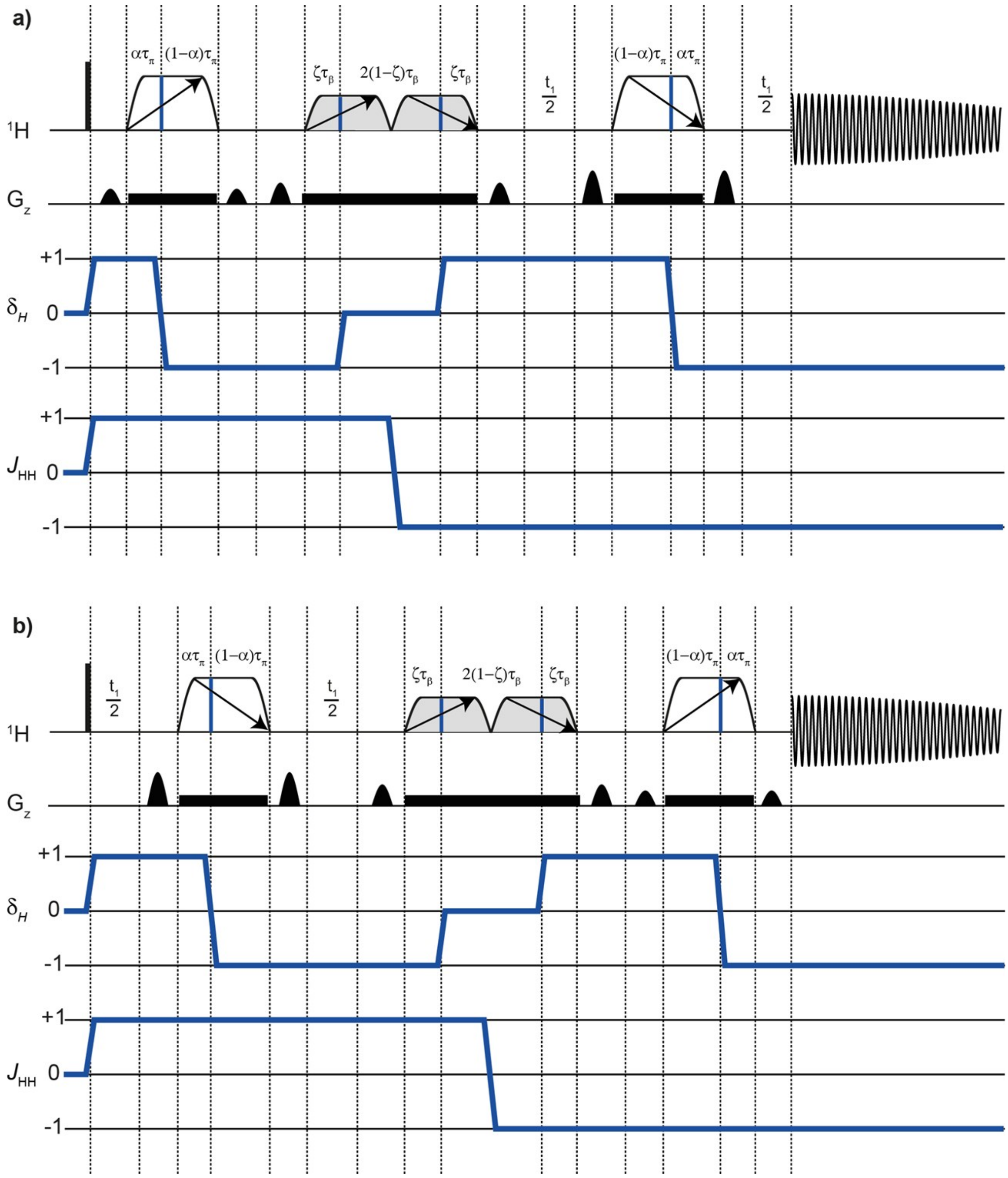

Figure S10. Pulse sequences for PSYCHE-2D J, to acquire (**a**) N-type, and (**b**) *J*-reversed N-type pathways with respect to the evolution of J-coupling, to obtain absorption mode lineshapes. Coherence transfer pathways are presented for each pulse for evolution of the chemical shift (δ_H), and J-couplings (J_{HH}). Time fractions α and ζ are used to specify the time at which a given offset is on resonance for the 180° and low flip angle swept-frequency chirp pulses in the pulse sequence.

PSYCHE 2D J spectrum and multiplets for 3-methoxyphenol

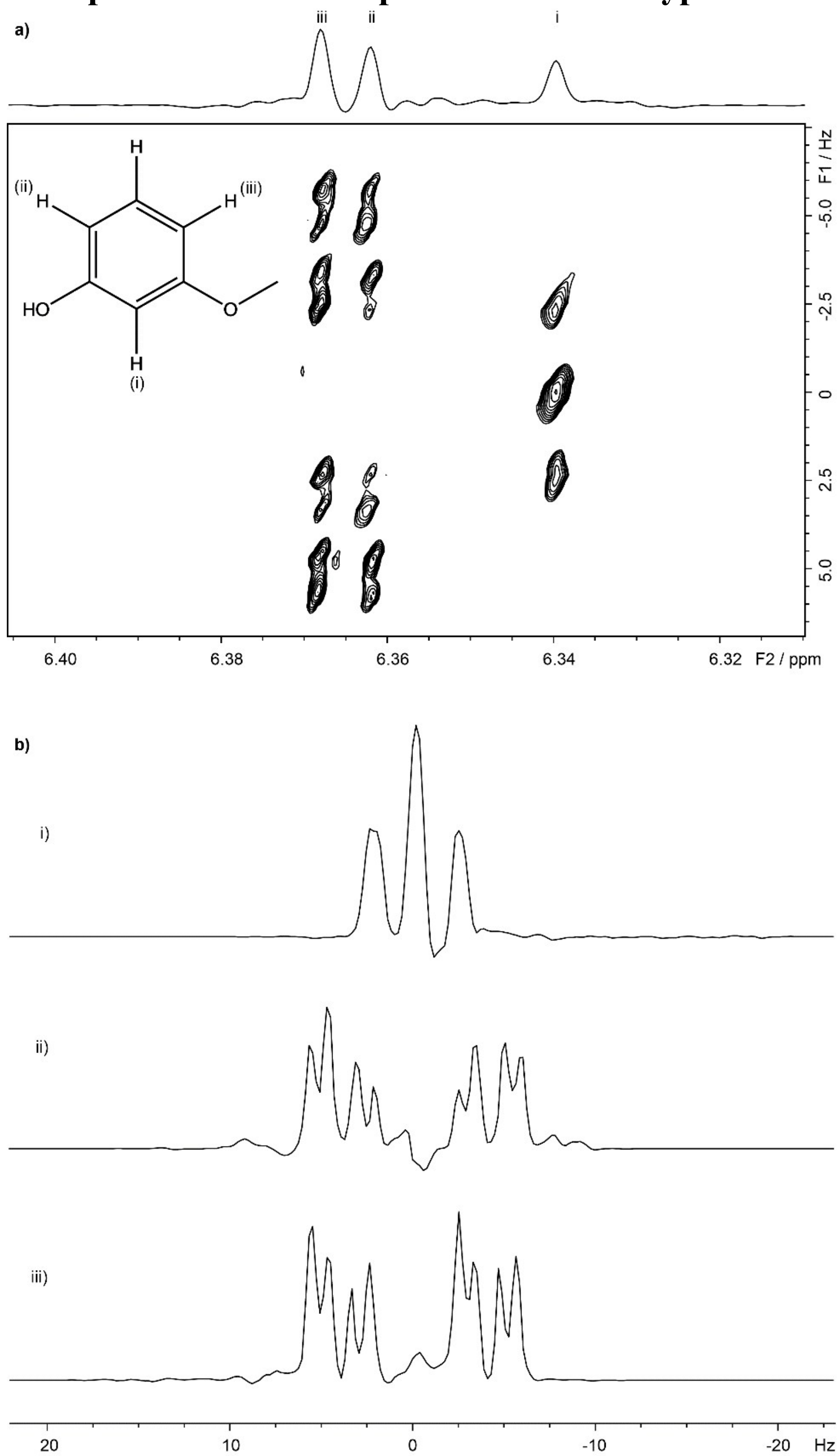

Figure S11. (a) Expansion of a crowded region of the PSYCHE-2D J spectrum of 3-methoxyphenol (inset) in DMSO-d_6, with the 45° projection on top with assignments. **(b)** Multiplet structures extracted from **(a)** for each of the signals indicated in the 1D projection.

Comparison of PSYCHE 2DJ, ZS, and *nemo*ZS for 3-methoxyphenol

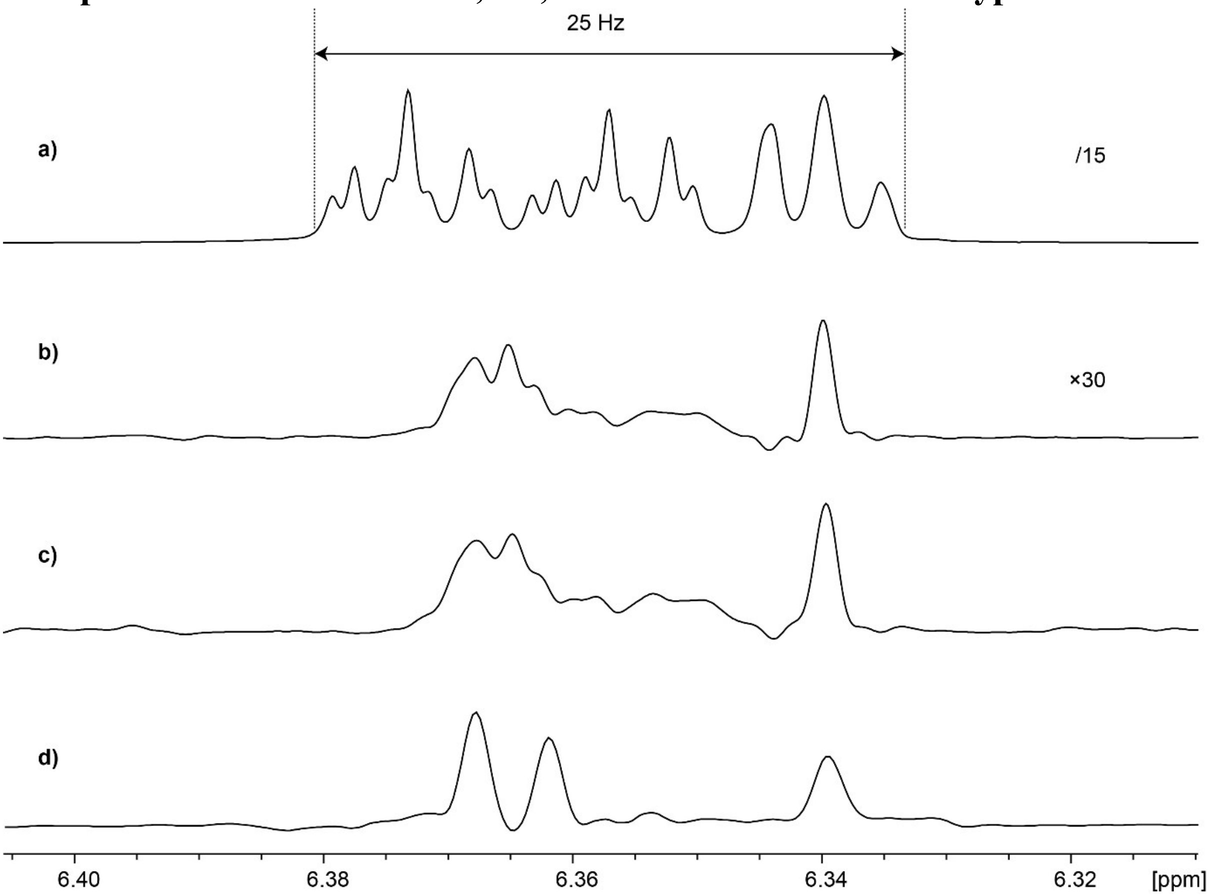

Figure S12. Comparison of the crowded region of the ^{1}H proton spectra of 3-methoxyphenol in DMSO-d_6, obtained using **(a)** conventional single pulse excitation, **(b)** a ZS pure shift experiment using a 120 ms rsnob pulse with an RF amplitude of 19.5 Hz, **(c)** a *nemo*ZS experiment using a 120 ms rsnob pulse with 2×40 non-equidistant modulations with an RF amplitude of 1560 Hz, and **(d)** the 45° projection of the PSYCHE 2D *J* spectrum shown in **Figure S9a**. The duration of the PSYCHE element was 80 ms, with a flip angle β of 20°, and an RF amplitude A of 39 Hz. The two 180° chirp pulses both had a duration of 40 ms and an RF amplitude A of 447 Hz.

Comparison of the accuracy of coupling measurement by conventional 2D J and PSYCHE 2D J

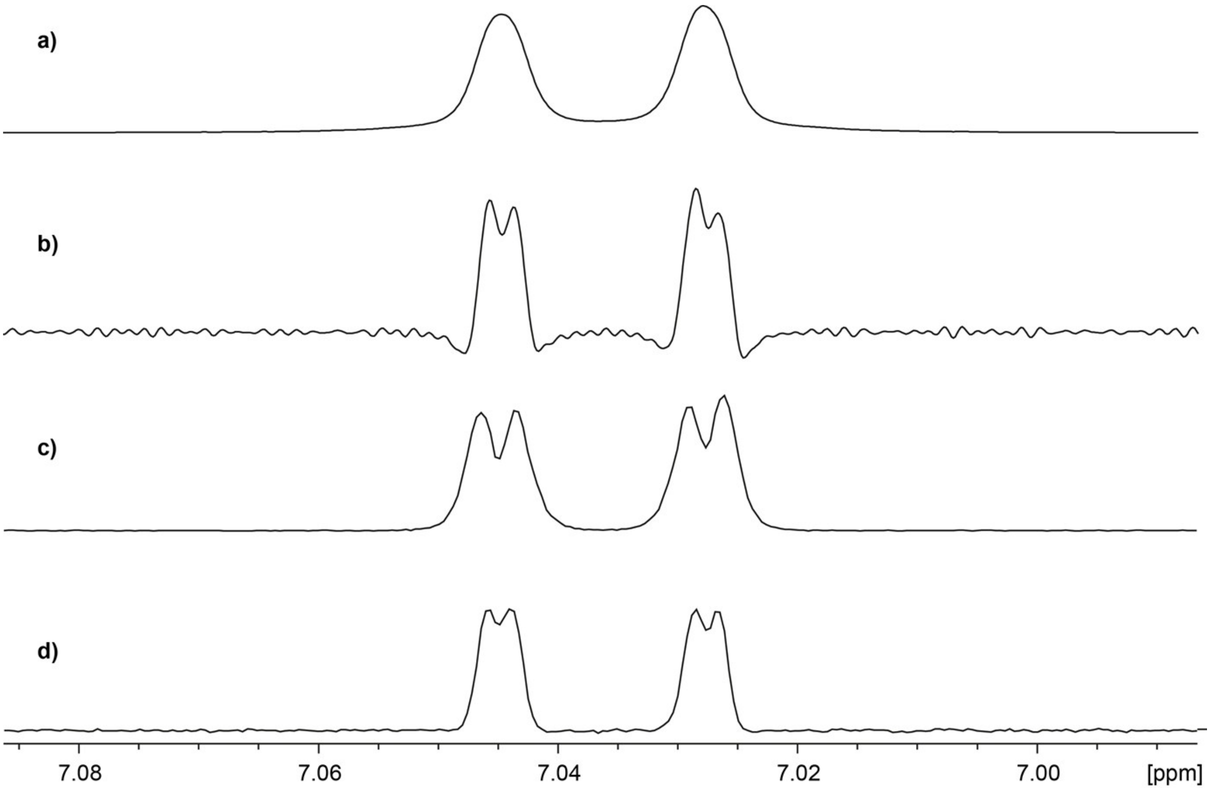

Figure S13. Comparison of traces showing one of the aromatic signals of estradiol in DMSO-d_6, obtained from **(a)** conventional single pulse excitation, **(b)** conventional single pulse excitation with strong Lorentz-Gauss resolution enhancement, in order to resolve the small J coupling, **(c)** slice from 45° projection of conventional 2D J spectrum, and **(d)** slice form 45° projection of the PSYCHE 2D *J* spectrum. A pronounced overestimation of the coupling constant is noticeable in the normal 2D J spectrum **(c)**, caused by the distorted lineshapes and absolute-value processing, whereas the trace through the PSYCHE 2D J spectrum (**d**), shown without resolution enhancement, is undistorted.

Pulse sequence codes

```
;psychejresph.mf

;PSYCHE 2D J-resolved
;phase sensitive 2D J-resolved with absorption mode lineshapes
;using PSYCHE as J-refocusing element
;to acqure N-type and J-reversed N-type ser files
;Using echo / anti-echo processing
;
;Mohammadali Foroozandeh
;University of Manchester
;Avance II+/III Version
;Topspin 3.x
;
;(1)  Foroozandeh, M.; Adams, R. W.; Meharry, N. J.; Jeannerat, D.; Nilsson, M.; Morris, G. A. Angew. Chem. Int. Ed. 2014, 53, 6990.
;(2)  Foroozandeh, M.; Adams, R. W.; Nilsson, M.; Morris, G. A. J. Am. Chem. Soc. 2014, 136, 11867.
;
;$CLASS=HighRes
;$DIM=2D
;$TYPE=
;$SUBTYPE=
;$COMMENT=

#include <Avance.incl>
#include <Delay.incl>
#include <Grad.incl>

"d0=0u"
"in0=inf1/2"
"d12=20u"
"l0=1"

"cnst50=(cnst20/360)*sqrt((2*cnst21)/(p40/2000000))"
"p30=1000000.0/(cnst50*4)"
"cnst31= (p30/p1) * (p30/p1)"
"spw40=plw1/cnst31"

"p31=1000000.0/(cnst51*4)"
"cnst32= (p31/p1) * (p31/p1)"
"spw41=plw1/cnst32"
"spw42=spw41"

"p20=p40"
"p21=p41"
"p22=p42"

1 ze
2 d1
3 d12 pl1:f1

  p1 ph1

  if "l0 %2 == 1"
    {
    50u UNBLKGRAD
    p16:gp1
    d16
    d16 pl0:f1
    ( center (p41:sp41 ph2):f1 (p21:gp11) )
    d16
    p16:gp1
    d16
    50u
    50u
    p16:gp2
    d16
    d16
    ( center (p40:sp40 ph3):f1 (p20:gp10) )
    d16
    p16:gp2
    d16
    50u
```

```
    d0
    50u
    p16:gp3
    d16
    d16
    ( center (p42:sp42 ph4):f1 (p22:gp12) )
    d16
    p16:gp3
    d16
    50u BLKGRAD
    d0
    }
  else
    {
    d0
    50u UNBLKGRAD
    p16:gp3
    d16
    d16
    ( center (p42:sp42 ph4):f1 (p22:gp12) )
    d16
    p16:gp3
    d16
    50u
    d0
    50u
    p16:gp2
    d16
    d16
    ( center (p40:sp40 ph3):f1 (p20:gp10) )
    d16
    p16:gp2
    d16
    50u
    50u
    p16:gp1
    d16
    d16
    ( center (p41:sp41 ph2):f1 (p21:gp11) )
    d16
    p16:gp1
    d16
    50u BLKGRAD
    }

  go=2 ph31
  d1 mc #0 to 2 F1EA(iu0, id0)
exit

ph1 = 0 2 0 2 0 2 0 2
ph2 = 0 0 0 0 0 0 0 0
ph3 = 0 0 1 1 0 0 1 1
ph4 = 0 0 0 0 1 1 1 1
ph31=0 2 2 0 2 0 0 2

;pl0 : zero power
;pl1 : high power
;p1  : 90 degree high power pulse
;p16 : duration of CTP gradients (1m)
;p20 : duration of weak gradient during PSYCHE pulse element
;p21 : duration of weak gradient during 1st 180-degree swept-frequency pulse
;p22 : duration of weak gradient during 2nd 180-degree swept-frequency pulse
;p40 : duration of double-chirp PSYCHE pulse element
;p41 : duration of 1st 180-degree swept-frequency pulse
;p42 : duration of 2nd 180-degree swept-frequency pulse
;d0  : incremented delay
;d1  : relaxation delay
;d16 : recovery delay for gradients
;spw40 : RF power of double-chirp PSYCHE pulse element
;spw41 : RF power of 1st 180-degree swept-frequency pulse
;spw42 : RF power of 2nd 180-degree swept-frequency pulse
;spnam40: file name for PSYCHE pulse element
;spnam41: file name for 1st 180-degree swept-frequency pulse
;spnam42: file name for 2nd 180-degree swept-frequency pulse
;gpz1: CTP gradient (35%)
```

```
;gpz2: CTP gradient (49%)
;gpz3: CTP gradient (77%)
;gpz10: weak gradient during PSYCHE element (1-3%)
;gpz11: weak gradient during 1st 180-degree chirp (1-3%)
;gpz12: weak gradient during 2nd 180-degree chirp (1-3%)
;gpnam1: SINE.100
;gpnam2: SINE.100
;gpnam3: SINE.100
;gpnam10: RECT.1
;gpnam11: RECT.1
;gpnam12: RECT.1
;cnst20: desired flip angle for PSYCHE pulse element (degree) (normally 10-25)
;cnst21: bandwidth of each chirp in PSYCHE pulse element (Hz) (normally 10000)
;cnst51: RF amplitude for 180-degree chirp pulses (Hz)
;l0  : loop for N/R cycle
;in0 : 1/(2 * SW) = DW
;nd0 : 2
;td1 : number of t1 increments
;MC2 : EA
```

```
;psychetse.mf

;TSE-PSYCHE
;Pure Shift Yielded by Chirp Excitation
;Using triple spin echo for suppression of strong coupling artefacts
;
;Mohammadali Foroozandeh
;University of Manchester
;Avance II+/III Version
;Topspin 3.x
;
;Data can be reconstructed using a macro available at http://nmr.chemistry.manchester.ac.uk
;
;(1)  Foroozandeh, M.; Adams, R. W.; Meharry, N. J.; Jeannerat, D.; Nilsson, M.; Morris, G. A. Angew. Chem. Int. Ed. 2014, 53, 6990.
;(2)  Foroozandeh, M.; Adams, R. W.; Nilsson, M.; Morris, G. A. J. Am. Chem. Soc. 2014, 136, 11867.
;
;$CLASS=HighRes
;$DIM=2D
;$TYPE=
;$SUBTYPE=
;$COMMENT=

#include <Avance.incl>
#include <Delay.incl>
#include <Grad.incl>

define delay tauA
define delay tauB

"in0=inf1/2"
"p2=p1*2"
"tauA=in0/2"
"tauB=dw*2*cnst4"

"cnst50=(cnst20/360)*sqrt((2*cnst21)/(p40/2000000))"
"p30=1000000.0/(cnst50*4)"
"cnst31= (p30/p1) * (p30/p1)"
"spw40=plw1/cnst31"

"p31=1000000.0/(cnst51*4)"
"cnst32= (p31/p1) * (p31/p1)"
"spw41=plw1/cnst32"
"spw42=spw41"

"p20=p40"
"p21=p41"
"p22=p42"

1 ze
2 d1 pl1:f1
3 p1 ph1
  tauA
  50u UNBLKGRAD
  p16:gp1
  d16
  10u pl0:f1
  d16
  ( center (p41:sp41 ph2):f1 (p21:gp11) )
  d16
  60u
  p16:gp1
  d16
  tauA
  d0
  p16:gp2
  d16
  10u
  d16
  ( center (p40:sp40 ph3):f1 (p20:gp10) )
  d16
  10u
  p16:gp2
  d16
  tauB
```

```
  p16:gp3
  d16
  60u
  d16
  ( center (p42:sp42 ph4):f1 (p22:gp12) )
  d16
  10u pl1:f1
  p16:gp3
  d16
  d0
  50u BLKGRAD
  go=2 ph31
  d1 mc #0 to 2 F1QF(id0)

exit

ph1= 0 2 0 2 0 2 0 2                        ; Hard_90
ph2= 0 0 0 0 1 1 1 1                        ; sweep-180
ph3= 0 0 1 1 0 0 1 1                        ; beta
ph4= 0 0 0 0 0 0 0 0                        ; sweep-180
ph31=0 2 2 0 2 0 0 2                        ; Receiver

;pl0 : zero power
;pl1 : high power
;p1  : 90 degree high power pulse
;p16 : duration of CTP gradients (1m)
;p20 : duration of weak gradient during PSYCHE pulse element
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;gpz2: CTP gradient (49%)
;gpz3: CTP gradient (77%)
;gpz10: weak gradient during PSYCHE element (1-3%)
;gpz11: weak gradient during 1st 180-degree chirp (1-3%)
;gpz12: weak gradient during 2nd 180-degree chirp (1-3%)
;gpnam1: SINE.100
;gpnam2: SINE.100
;gpnam3: SINE.100
;gpnam10: RECT.1
;gpnam11: RECT.1
;gpnam12: RECT.1
;cnst4: number of drop points
;cnst20: desired flip angle for PSYCHE pulse element (degree) (normally 10-25)
;cnst21: bandwidth of each chirp in PSYCHE pulse element (Hz) (normally 10000)
;cnst51: RF amplitude for 180-degree chirp pulses (Hz)
;in0 : 1/(2 * SW) = DW
;nd0 : 2
;td1 : number of t1 increments
;MC2 : QF
```

```
;psyche.mf
;
;1D PSYCHE
;Pure Shift Yielded by Chirp Excitation
;modified for internal calculation of RF amplitude of PSYCHE pulse element
;based on desired flip angle and bandwidth of chirp pulses
;
;Mohammadali Foroozandeh
;University of Manchester
;Avance II+/III Version
;Topspin 3.x
;
;Data can be reconstructed using a macro available at http://nmr.chemistry.manchester.ac.uk
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;
;$CLASS=HighRes
;$DIM=2D
;$TYPE=
;$SUBTYPE=
;$COMMENT=

#include <Avance.incl>
#include <Delay.incl>
#include <Grad.incl>

define delay tauA
define delay tauB

"in0=inf1/2"
"p2=p1*2"
"tauA=in0/2-p16-d16-50u"
"tauB=(dw*2*cnst4)+d16+50u"

"cnst50=(cnst20/360)*sqrt((2*cnst21)/(p40/2000000))"
"p30=1000000.0/(cnst50*4)"
"cnst31= (p30/p1) * (p30/p1)"
"spw40=plw1/cnst31"

"p10=p40"

1 ze
2 d1 pl1:f1
3 p1 ph1
  d0
  tauA
  50u UNBLKGRAD
  p16:gp1
  d16
  p2 ph2
  50u
  p16:gp1
  d16
  tauA
  p16:gp2
  d16
  10u pl0:f1
  tauB
  ( center (p40:sp40 ph3):f1 (p10:gp10) )
  d16
  10u pl1:f1
  p16:gp2
  d16
  50u BLKGRAD
  d0
  go=2 ph31
  d1 mc #0 to 2 F1QF(id0)
exit

ph1 = 0 2 0 2 0 2 0 2                    ; Hard_90
ph2 = 0 0 0 0 1 1 1 1                    ; Hard_180
ph3 = 0 0 1 1 0 0 1 1                    ; beta
ph31=0 2 2 0 2 0 0 2                     ; Receiver
```

```
;pl0 : zero power
;pl1 : high power
;p1  : 90 degree high power pulse
;p16 : duration of CTP gradients (1m)
;p10 : duration of weak gradient during PSYCHE pulse element
;p40 : duration of double-chirp PSYCHE pulse element
;d0  : incremented delay
;d1  : relaxation delay
;d16 : recovery delay for gradients
;spw40 : RF power of double-chirp PSYCHE pulse element
;spnam40: file name for PSYCHE pulse element
;gpz1: CTP gradient (77%)
;gpz2: CTP gradient (49%)
;gpz10: weak gradient during PSYCHE element (1-3%)
;gpnam1: SINE.100
;gpnam2: SINE.100
;gpnam10: RECT.1
;cnst4: number of drop points
;cnst20: desired flip angle for PSYCHE pulse element (degree) (normally 10-25)
;cnst21: bandwidth of each chirp in PSYCHE pulse element (Hz) (normally 10000)
;in0 : 1/(2 * SW) = DW
;nd0 : 2
;td1 : number of t1 increments
;MC2 : QF
```