Ultrahigh-resolution Total Correlation NMR Spectroscopy

Supporting Information

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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}. G_1, G_2, G_3, G_4 and G_5 are field gradient pulses with half sine shapes. Gradient pulses G_1 and G_2 are used for selection of desired coherence transfer pathways (CTP) and had amplitudes of 24 G cm^{-1} and 38 G cm^{-1} respectively and duration of 2.5 ms each. Gradient pulse G_3 is aligned with the center of the two chirp pulses and had an amplitude of 0.75 G cm^{-1}. Gradient pulses G_4 are applied to dephase zero quantum coherences and had an amplitude of 1.5 G cm^{-1}. Gradient pulse G_5 is a spoil pulse and had an amplitude of 12 G cm^{-1}. $\Phi_1=x,-x$, $\Phi_2=x$, $\Phi_3=\Phi_4=x,x,-x,-x,y,y,-y,-y$, $\Phi_5=\Phi_6=\Phi_7=\Phi_8=x$ and $\Phi_{rec.}=x,-x,-x,x,x,-x,-x,x$. Number of transients was set to 8, TD and TD1 were set to 2048, and SW and SW1 were set to 5000 Hz, in a total experiment time of 9 h.

Generation of Chirp pulses

Chirp pulse elements used in the PSYCHE pulse sequence were generated and integrated in the Shape Tool of Topspin software version 2.1. The two chirp pulses in the double chirp element were generated with the following parameters:

Size of Shape: 10000

Total Sweep-Width: 10000 Hz

Length of Pulse: 15000

% to be smoothed: 20 %

Q (for the middle of shape): 5.0

For the low-to-high pulse, the first option box (Low to High Field) was checked, while for the high-to-low pulse it was unchecked. Pulse elements Low-to-High/ High-to-Low and High-to-Low/Low-to-High were generated by concatenating the text files of individual pulses. The mixed pulse, which sweeps frequencies from low-to-high and high-to-low field simultaneously, was generated by using the Add Shape option in the Manipulate menu of Shape Tool, or by the command "manipul addshape", aligning at the centre and rescaling the amplitude to 100 %.

Calibration of Chirp pulses with small flip angle

The chirp pulses used may be generated by using the spectrometer software to calculate an adiabatic inversion (180°) pulse and then reducing the radiofrequency (RF) power. To find the RF field strength ($\gamma B_1/2\pi$) needed to generate a PSYCHE chirp pulse element of flip angle *β*, the RF field ($\gamma B_{1(\max)}/2\pi$) for a 180° rotation is first calculated by integrating the pulse as an adiabatic shape via the Analysis menu of Bruker Shape Tool, or by using the command "analyze integradia". For the pulse used here, with a duration, τ_p, of 15 ms, a sweep-width, *ΔF*, of 10 kHz, and an adiabaticity factor, *Q*, of 5, an RF field of 728 Hz is obtained for $\gamma B_{1(\max)}/2\pi$. $\gamma B_{1(\max)}/2\pi$ can also be calculated using Equation S1:

$$\frac{\gamma B_{1(\max)}}{2\pi} = \frac{\sqrt{\left(\tau_p \times \Delta F \times Q/2\pi\right)}}{\tau_p}$$ **Equation S1**

The relationship between flip angle and RF field strength for a chirp pulse of this shape but different amplitudes is illustrated in Figure S9. As ishown in Figure S9b, there is an approximately linear relationship between the desired flip angle and the RF amplitude of the chirp pulse for flip angles in the range 0° – 45°. For a given flip angle, the RF field strength required with the pulse shape described is given by Equation S2. For example, the PSYCHE chirp elements used in this work, with a flip angle of 20°, were obtained by reducing the RF field used for a chirp 180° pulse by a factor of 16, corresponding to an addition of 24 dB to the power attenuation of the shaped pulse.

$$\frac{\gamma B_{1(\beta)}}{2\pi} = 2.27 \times \beta \qquad \textbf{Equation S2}$$

where β is in degrees and the LHS in Hz.

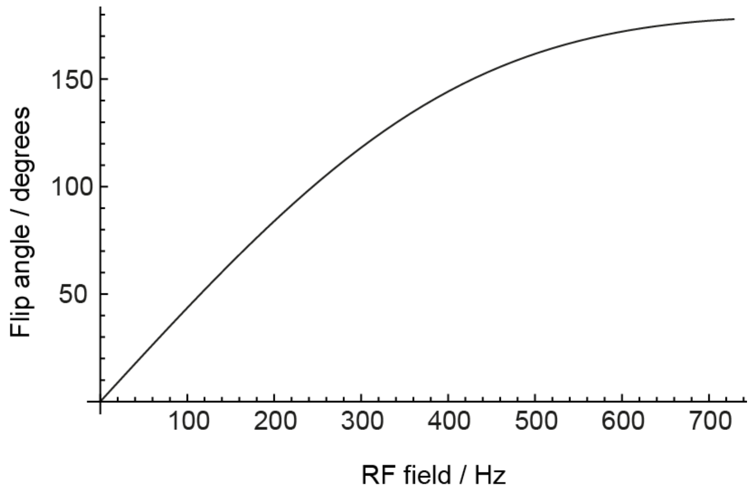

Figure S1. Graph illustrating the relationship between the flip angle (in degrees) and the RF field (in Hz) of the shaped pulse used in the PSYCHE chirp pulse element.

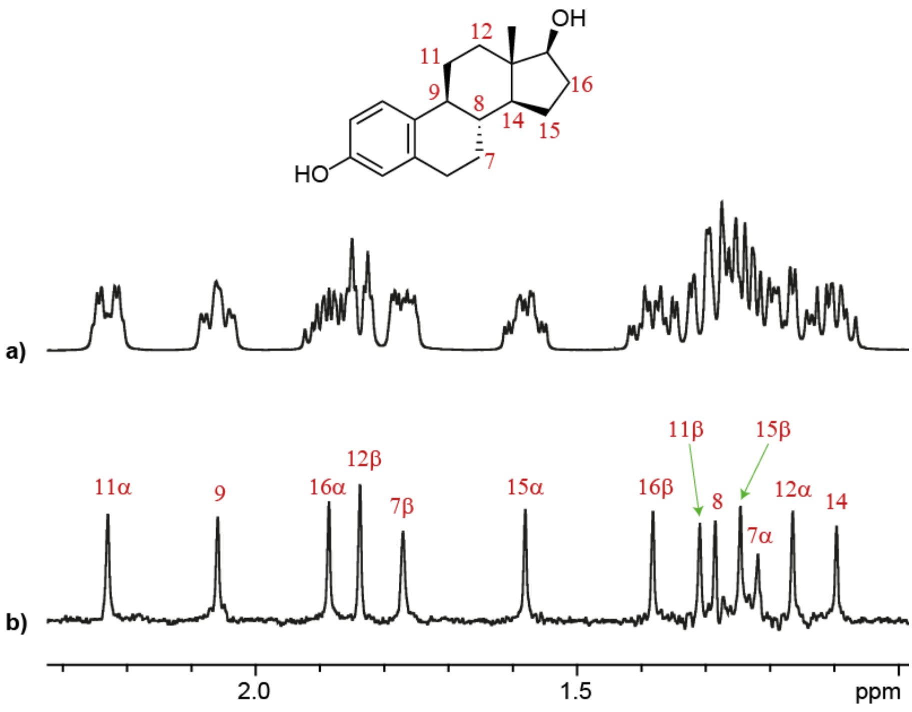

Figure S2. (**a**) Conventional 1D ^{1}H spectrum, and (**b**) 1D pure shift spectrum obtained by PSYCHE of a sample of estradiol (scheme) in DMSO-d_6. The assignments are given above the peaks in (**b**).

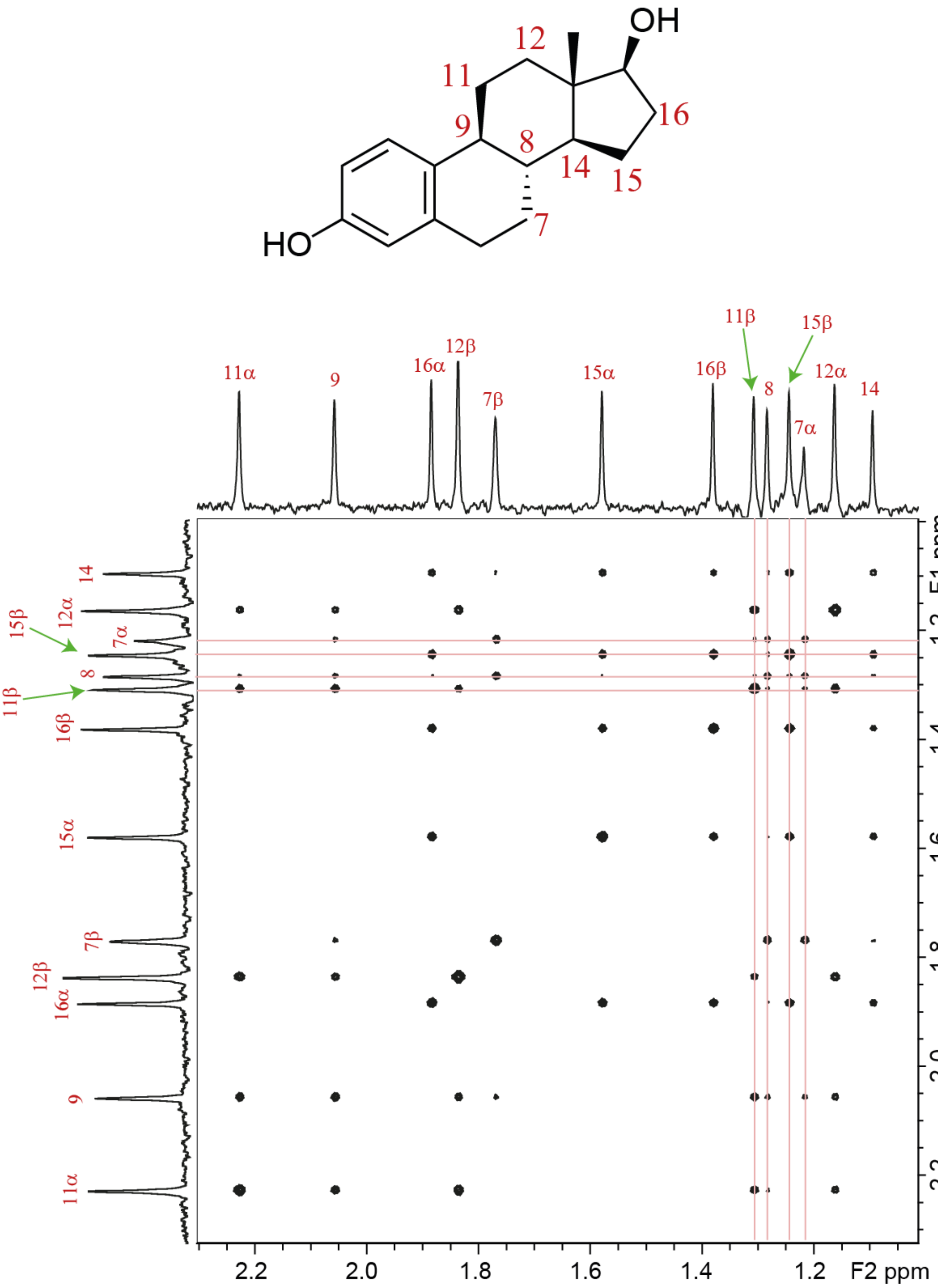

Figure S3. Assignments for signals in the region shown in Figure 2c in the manuscript of a sample of estradiol (scheme) in DMSO-d_6. Red lines are used to facilitate the tracking of connectivities.

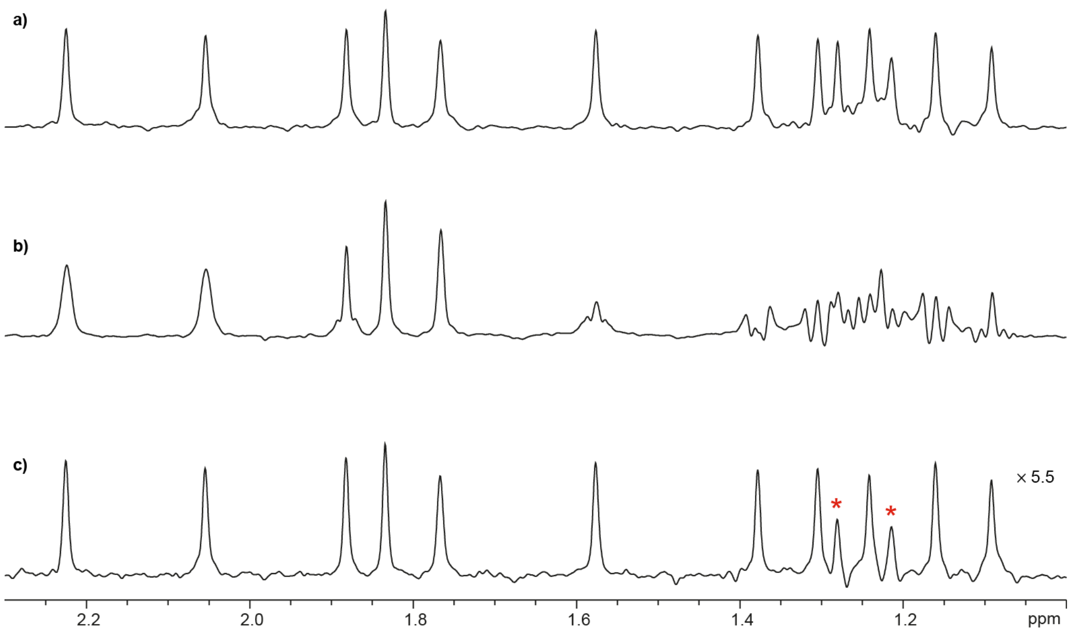

Figure S4. 1D ^{1}H spectra of a sample of estradiol in DMSO-d_6, obtained (**a**) with PSYCHE, (**b**) with, ZS using a 12 ms rsnob refocusing pulse, and (**c**) using ZS with a 50 ms rsnob refocusing pulse. In (**c**), ZS approaches PSYCHE in terms of cleanliness, albeit at a cost in sensitivity, but the selectivity of the refocusing pulse is still not enough to decouple the signals marked with red asterisks. 50 ms pulses were used in pseudo-3D F_2-PSYCHE-TOCSY and F_2-ZS-TOCSY for comparison with F_1-PSYCHE-TOCSY. (Figure S7)

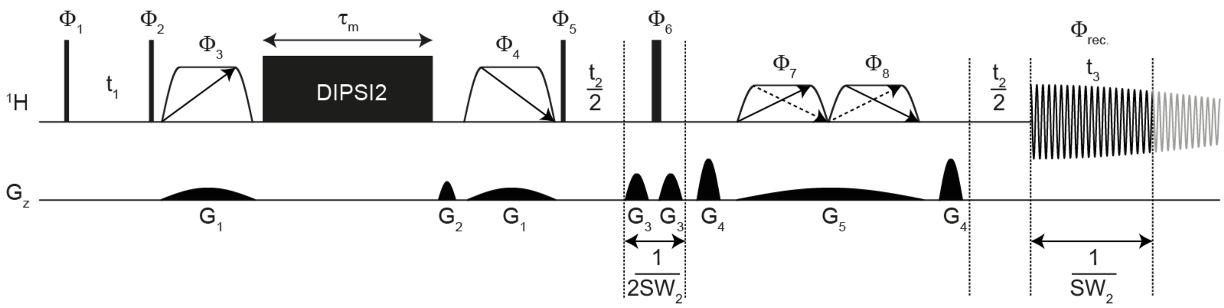

Figure S5. Pulse sequence for F_2-PSYCHE-TOCSY. 90° and 180° RF pulses are represented by narrow and wide rectangles respectively. Low-power chirp pulses with flip angle β are represented by trapezoids with cross-diagonal arrows. In this work each of the low-power chirp pulses has a frequency sweep in opposite directions simultaneously, a duration of 15 ms, an RF amplitude of 46 Hz, and a flip angle of 20°. Trapezoids either side of the mixing element (DIPSI2) are 180° chirp pulses of 30 ms duration and 894 Hz amplitude, to suppress zero quantum coherences. G_1, G_2, G_3, G_4 and G_5 are field gradient pulses with half-sine shapes. Gradient pulses G_1 are applied to dephase zero quantum coherences and had an amplitude of 1.5 G cm^{-1}. Gradient pulse G_2 is a spoil gradient and had an amplitude of 12 G cm^{-1}. Gradient pulses G_3 and G_4 are used for selection of desired coherence transfer pathways (CTP) and had amplitudes of 24 G cm^{-1} and 38 G cm^{-1} respectively and duration of 2.5 ms each. Gradient pulse G_5 was aligned with the center of the PYCHE low-power chirp pulse element and had an amplitude of 0.75 G cm^{-1}. Pulse phases: Φ_1=*x,-x*, Φ_2=Φ_3=Φ_4=Φ_5=Φ_6=*x*, Φ_7=Φ_8= *x,x,-x,-x,y,y,-y,-y,* and $\Phi_{rec.}$=*x,-x,-x,x,x,-x,-x,x* were used. For the F_2-ZS-TOCSY the double-chirp element was replaced by an rsnob refocusing pulse and gradient pulse G_5 had rectangular shape. Rsnob pulses had either 12 ms duration with RF amplitude of 195 Hz or 50 ms duration with RF amplitude of 47 Hz, as noted in text.

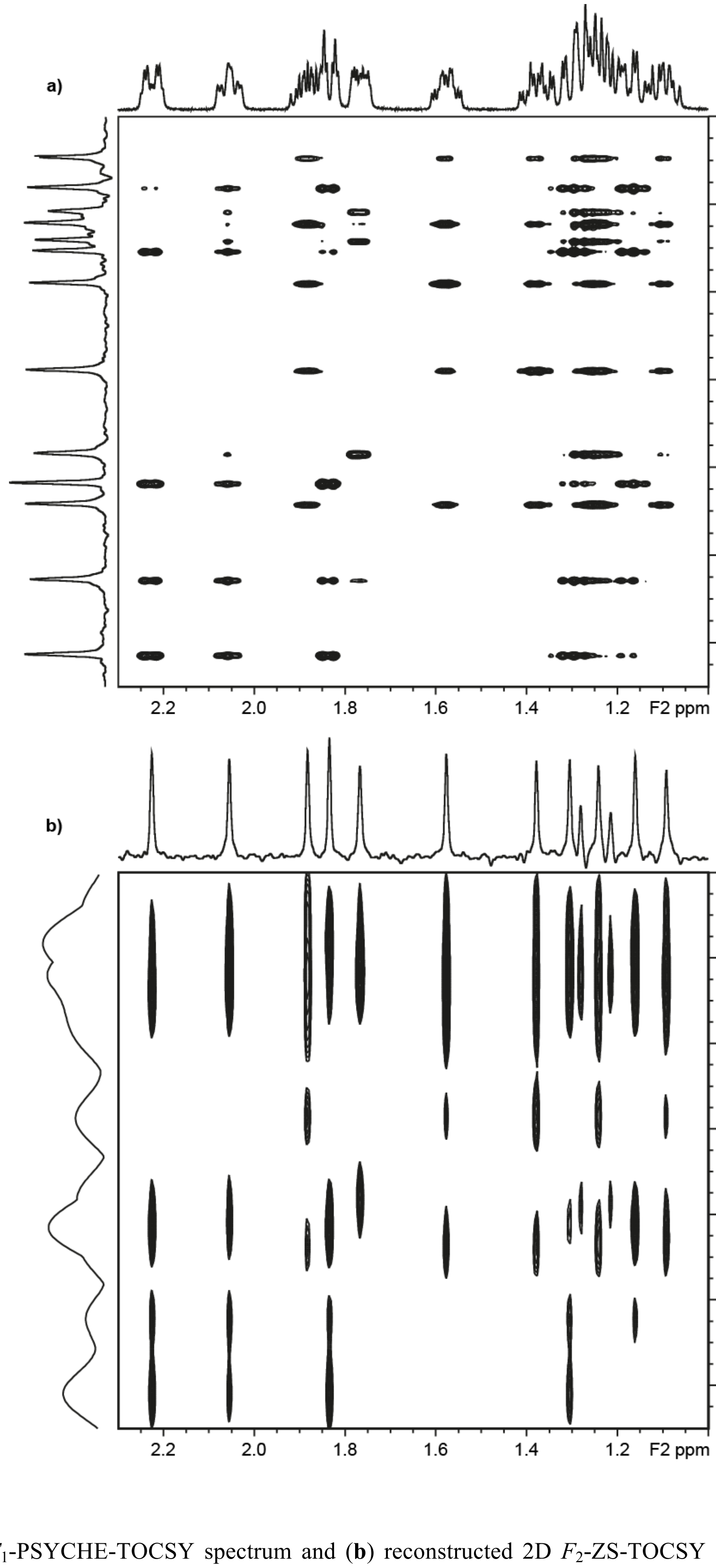

Figure S6. (**a**) 2D F_1-PSYCHE-TOCSY spectrum and (**b**) reconstructed 2D F_2-ZS-TOCSY spectrum of a sample of estradiol in DMSO-d_6. For (**a**) the PSYCHE element had the same settings as described in figure S5. For (**b**) the rsnob pulse had duration of 50 ms and RF amplitude of 47 Hz. For the same experiment time (9 h) the F_1-PSYCHE-TOCSY experiment had 2048 points in F_1 while F_2-ZS-TOCSY had 128 points.

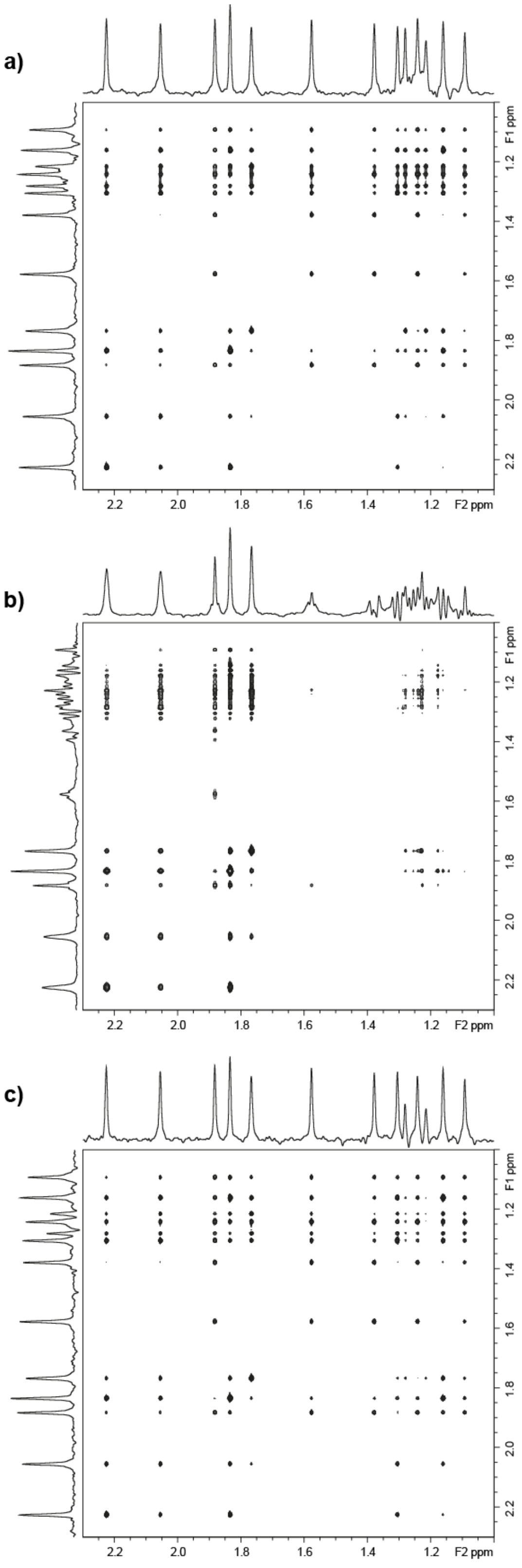

Figure S7. 2D spectra after covariance processing of (**a**) F_2-PSYCHE-TOCSY and (**b**) and (**c**) F_2-ZS-TOCSY of a sample of estradiol in DMSO-d_6. Spectra were acquired using the pulse sequences described in Figure S5. F_2-ZS-TOCSY spectra were acquired using an rsnob pulse of duration 12 ms and RF amplitude 195 Hz for (**b**) and duration 50 ms with RF amplitude 47 Hz for (**c**). (**c**) has a vertical scale magnification of 5.5x relative to (**a**) and (**b**). Note the spurious responses in (**b**) and (**c**).

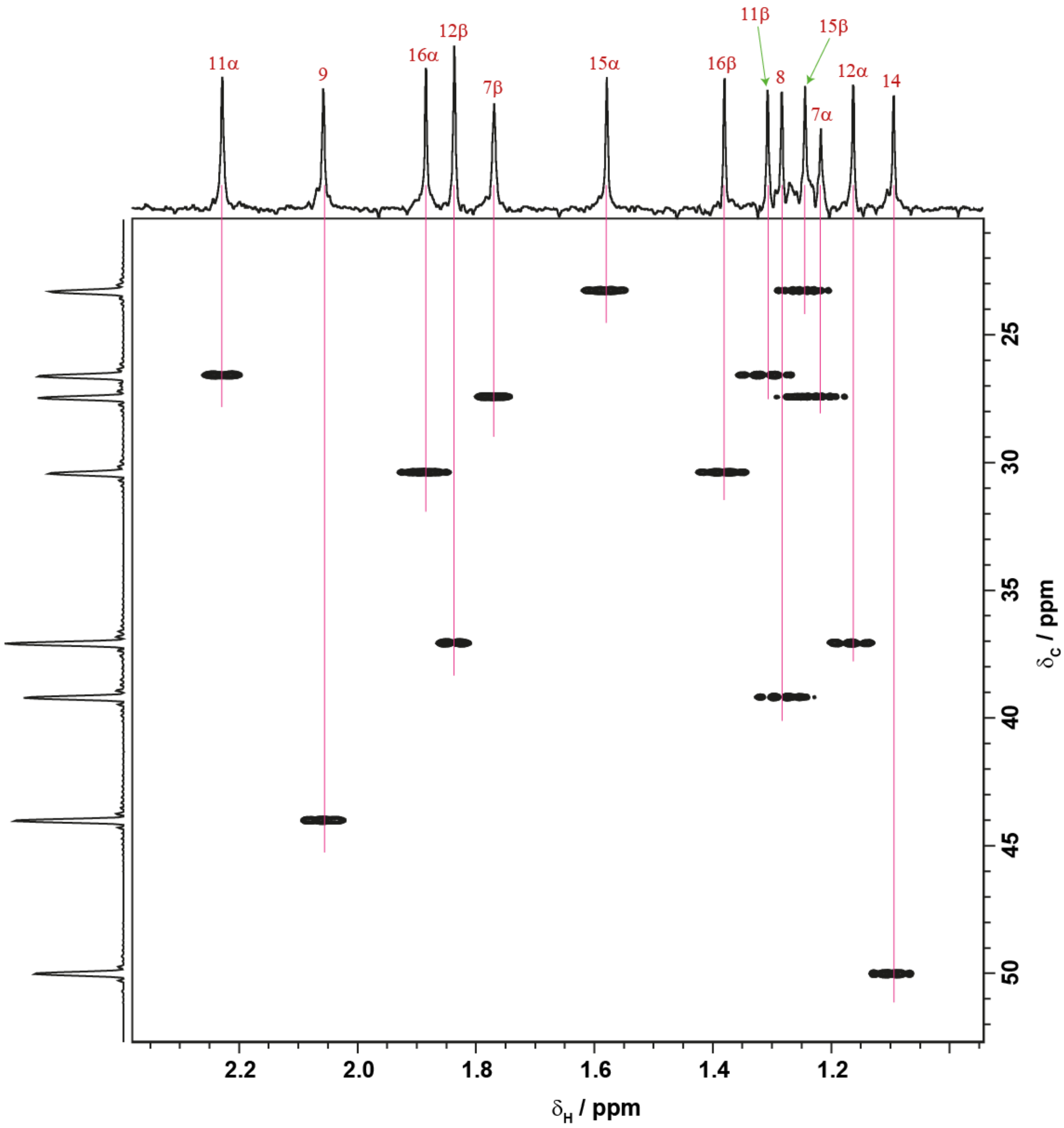

Figure S8. ^{1}H-^{13}C HSQC spectrum of a sample of estradiol in DMSO-d_6. To verify the accuracy of the chemical shifts observed in the PSYCHE experiment, a 1D PSYCHE pure shift spectrum is shown above the ^{1}H-^{13}C HSQC spectrum along F_2. Chemical shift changes due to second order isotope effects are too small to be seen in this comparison.

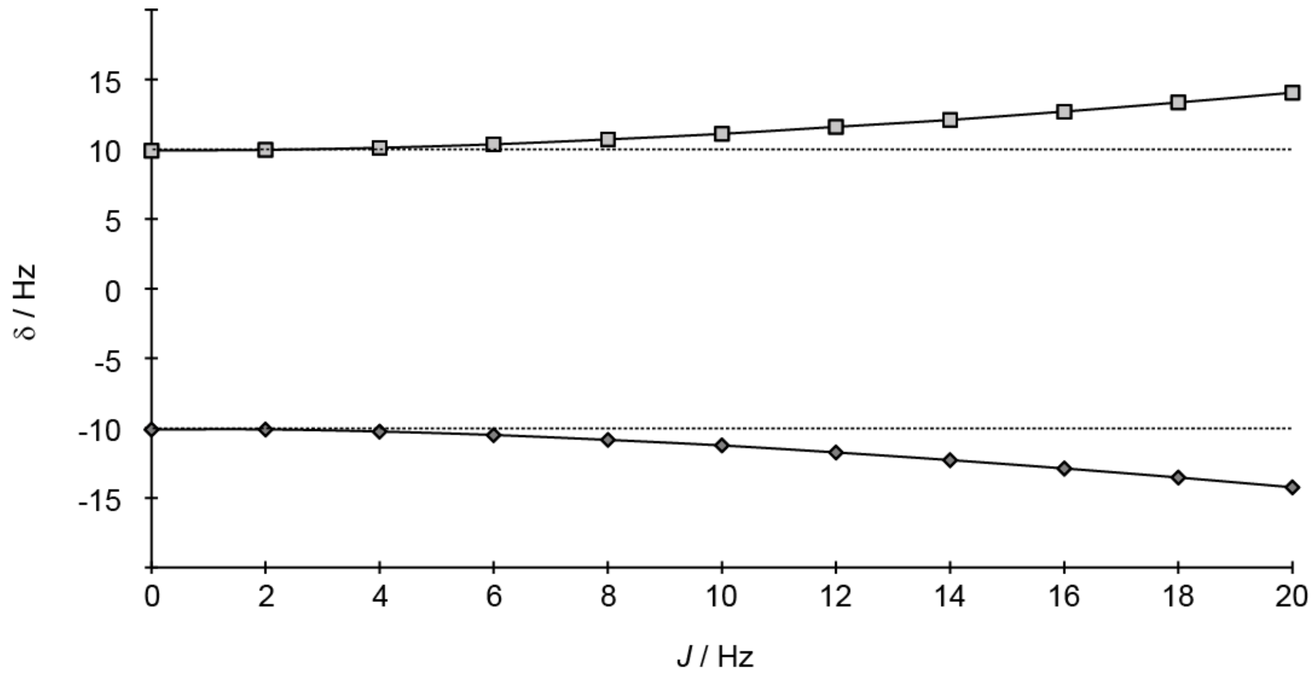

Figure S9. Apparent chemical shifts found by simulation of a strongly coupled AB spin system on application of the PSYCHE pulse sequence. The observed chemical shifts of the pure shift signals (squares and diamonds) for the two spins were calculated as a function of *J*. Their real chemical shifts (dashed lines) are shown for comparison. The calculation used a model AB spin system with $\Delta\delta$ = 20 Hz. Calculations were performed using the NMRSIM program in Bruker Topspin 3.2. Parameters for the PSYCHE pulse sequence were taken from Figure 1a in the manuscript, except that the program used does not include the effect of a field gradient pulse during the PSYCHE chirp pulse element.

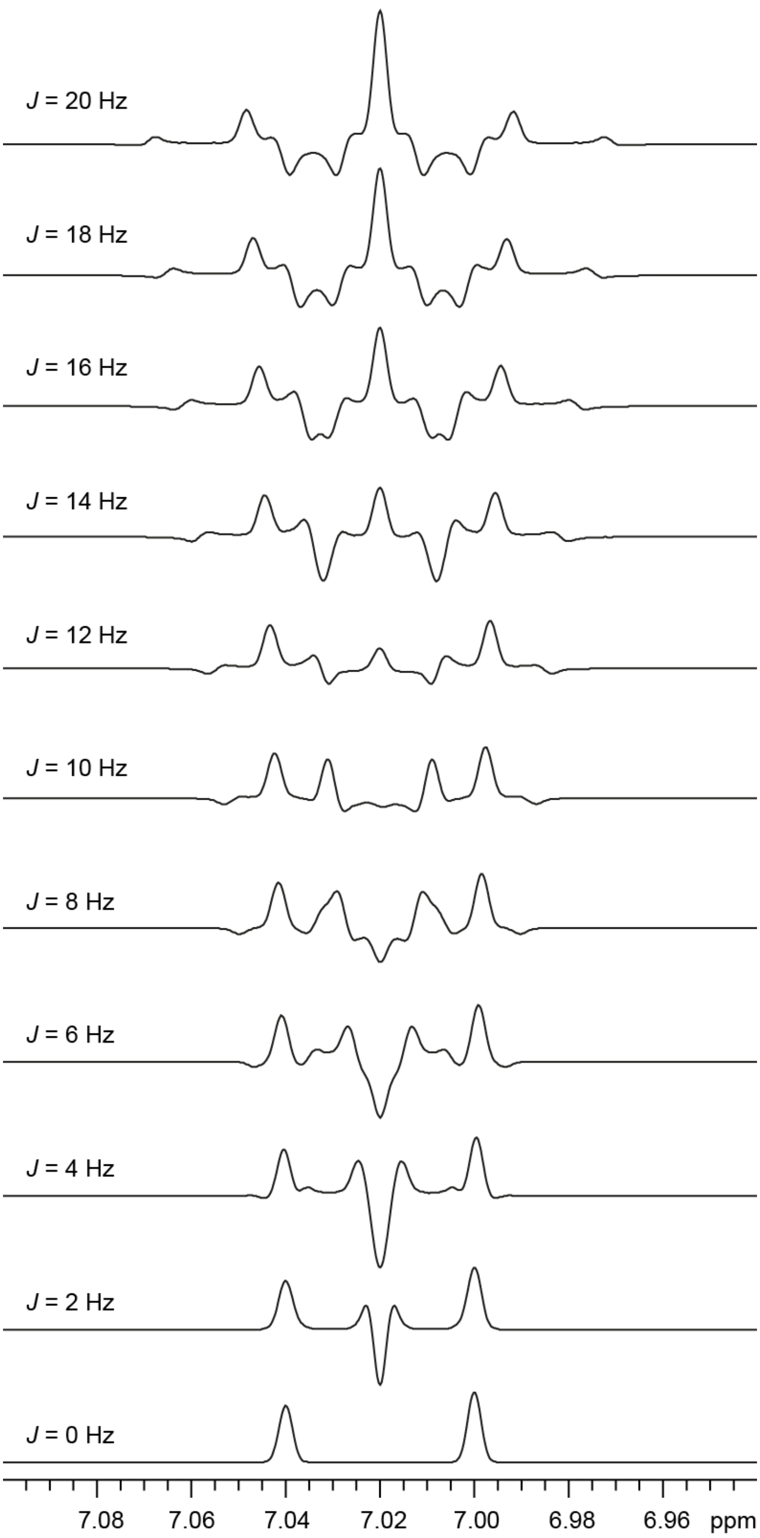

Figure S10. Simulated PSYCHE spectra of an AB system from which the values in Figure S9 were obtained. In a real experiment a pulsed field gradient during the PSYCHE element, omitted in these calculations, would result in attenuation of the strong coupling artifacts which appear at the average of the two chemical shifts.

Pulse sequence code

```
;psyche_tocsyzqs_f1.mf
;F1  decoupled TOCSY using PSYCHE
;With DIPSI and ZQF

; Mohammadali Foroozandeh
; University of Manchester
; (26/11/2013)
; Avance II+/III Version

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

"p2=2*p1"
"d0=0u"
"in0=inf1/2"
"FACTOR1=(d9/(p6*115.112))/2+0.5"
"l1=FACTOR1*2"
"d12=20u"
"p20=p10"
"p21=p11"
"p22=p12"


1 ze
2 d1
3 d12 pl1:f1
  p1 ph1
  d0
  50u UNBLKGRAD
  p16:gp1
  d16
  p2 ph5
  50u
  p16:gp1
  d16
  p17:gp2
  d17
  10u pl0:f1
  d16
  (center (p20:gp10) (p10:sp10 ph6):f1 )
  d16
  10u pl1:f1
  p17:gp2
  d17
  d0
  p1 ph2
  5u pl0:f1
  ( center (p21:gp11) (p11:sp1 ph4):f1 )
  d17
  5u pl10:f1
4 p6*3.556 ph23
  p6*4.556 ph25
  p6*3.222 ph23
  p6*3.167 ph25
  p6*0.333 ph23
  p6*2.722 ph25
  p6*4.167 ph23
  p6*2.944 ph25
  p6*4.111 ph23

  p6*3.556 ph25
  p6*4.556 ph23
  p6*3.222 ph25
  p6*3.167 ph23
  p6*0.333 ph25
  p6*2.722 ph23
  p6*4.167 ph25
  p6*2.944 ph23
  p6*4.111 ph25

  p6*3.556 ph25
  p6*4.556 ph23
```

```
  p6*3.222 ph25
  p6*3.167 ph23
  p6*0.333 ph25
  p6*2.722 ph23
  p6*4.167 ph25
  p6*2.944 ph23
  p6*4.111 ph25

  p6*3.556 ph23
  p6*4.556 ph25
  p6*3.222 ph23
  p6*3.167 ph25
  p6*0.333 ph23
  p6*2.722 ph25
  p6*4.167 ph23
  p6*2.944 ph25
  p6*4.111 ph23
  lo to 4 times l1

  5u pl0:f1
  p18:gp3
  d17
  ( center (p22:gp12) (p12:sp2 ph4):f1 )
  d17
  50u BLKGRAD
  5u pl1:f1
  p1 ph3
  go=2 ph31
  d1 mc #0 to 2 F1PH(ip1, id0)
exit


ph1= 0 2
ph2= 0
ph3= 0
ph4= 0
ph5= 0
ph6= 0 0 1 1 2 2 3 3
ph23=3
ph25=1
ph31=0 2 2 0 0 2 2 0

;p1: high power 90 pulse width
;p2: high power 180 pulse width
;p6  : 90 degree low power pulse
;p10: duration of PSYCHE chirp element
;p11: duration ZQ suppression chirp pulse
;p12: duration ZQ suppression chirp pulse
;p20: duration of PSYCHE gradient
;p21: duration ZQ suppression gradient
;p22: duration ZQ suppression gradient
;p16: duration of CTP gradient
;p17: duration of CTP gradient
;p18:duration of homospoil gradient
;pl1: f1 channel - power level for pulse (default)
;pl10: 120 dB
;pl10: DIPSI-2 power
;sp1: selective pulse power level
;spoffs1: selective pulse offset (0 Hz)
;spnam1:  file name for selective pulse
;sp2: selective pulse power level
;spoffs2: selective pulse offset (0 Hz)
;spnam2:  file name for selective pulse
;sp10: selective pulse power level
;spoffs10: selective pulse offset (0 Hz)
;spnam10:  file name for selective pulse
;gpz1: CTP gradient 49%
;gpz2: CTP gradient 77%
;gpz3: CTP gradient 25%
;gpz10: psyche gradient 1-3 %
;gpz11: ZQS gradient 1-3%
;gpz12: ZQS gradient 1-3 %
;gpnam1: SINE.100
;gpnam2: SINE.100
;gpnam3: SINE.100
```

```
;gpnam10: SINE.100
;gpnam11: SINE.100
;gpnam12: SINE.100
;d0  : incremented delay
;d1  : relaxation delay
;d9  : TOCSY mixing time
;d16: gradient stabilisation delay
;d17: gradient stabilisation delay
;l1  : loop for DIPSI cycle
;in0 : 1/(2 * SW) = DW
;NS  : number of scans
;DS  : number of dummy scans
;td1 : number of t1 increments
;MC2 : TPPI
```