Electronic Supplementary Material (ESI) for RSC Advances.

A new tool for NMR analysis of complex systems:

selective pure shift TOCSY

Electronic Supporting Information

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A. Experimental section

All spectra were recorded on a Bruker Avance II+ 500 MHz spectrometer with a 5mm BBO probe equipped with a z-gradient coil with a maximum nominal gradient strength of 53 G cm^{-1}.

1. Pulse sequence

The detailed pulse sequence for the 1D selective TOCSY-PSYCHE experiment is shown in Fig. S1. Narrow and wide filled rectangles represent hard 90° and 180° pulses, respectively. The duration of the 90° pulse (p1) was set to 10.7 μs and 10.5 μs for ^{1}H for the peppermint oil sample and for the mixture of provitamin D_3 and vitamin D_3, respectively. The first selective 180° pulse (p12) is applied to the isolated resonance of interest, and typically RSNOB (*1*) or REBURP (*2*) shapes are used. The TOCSY transfer is achieved by using the DIPSI-2 mixing scheme with a mixing time (d9) of 50-200 ms depending of the sample. Trapezoids on either side of the DIPSI-2 isotropic mixing element are low-power 180° chirp pulses of 20 kHz bandwidth, used to suppress zero quantum coherences (*3*) and their durations were set to 10 (p32) and 30 (p34) ms, respectively. Trapezoids with cross-diagonal arrows are low-power chirp pulses of net flip angle β<<90° which sweep frequency in opposite directions simultaneously (saltire elements) and their durations were set to 15 ms each, with a sweep width of 10 kHz in order to give uniform excitation over about a 6 kHz range, with an amplitude of 64 Hz, and a flip angle β of 10-20°. Gradient pulses G_1, G_3, and G_4 are used to select the desired coherence transfer pathways, with amplitudes of 7.0 G cm^{-1}, 41.2 G cm^{-1}, and 26.2 G cm^{-1}, and duration of 1 ms each. G_2 is a spoil gradient with amplitude of 16.6 G cm^{-1} and duration of 1 ms. G_0, G_{10}, and G_{11} are weak pulsed field gradients applied simultaneously with the chirp pulses and saltire elements, with amplitudes of 1.6 G cm^{-1}, 1.1 G cm^{-1}, and 1.6 G cm^{-1} respectively. All gradient pulses were followed by a recovery delay of 200 μs in the selective TOCSY element (d17) and 1 ms in the PSYCHE block (d16). The highlighted part of the FID, with duration of $1/SW_1$, shows the homodecoupled chunk of data acquired for each increment of t_1. Define the number of drop points (cnst4) as 4. The minimum phase cycle recommended is 8 steps, but 16 step phase cycling provides cleaner results. The full phase cycle is given in Table S1.

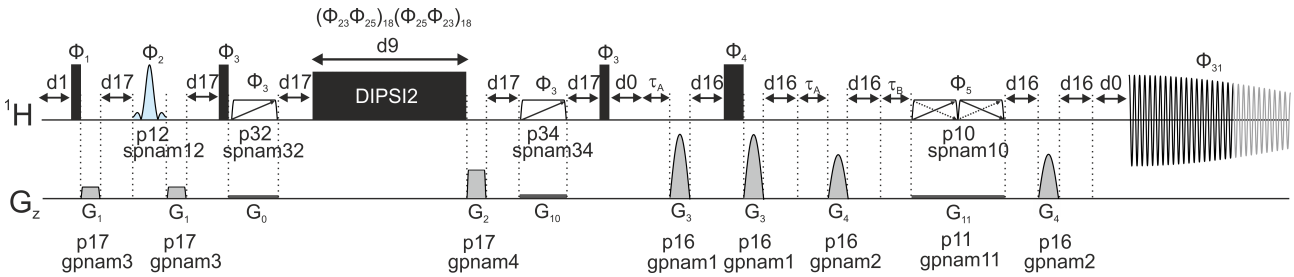

Fig. S1. Detailed pulse sequence for Bruker implementation of the 1D selective TOCSY-PSYCHE experiment. The definition of each parameter can be found at the end of the pulse sequence (Section C of the ESI), along with recommended standard values.

Table S1. 16 step phase cycle

Φ_1	x, -x
Φ_2	x_2, y_2, $-x_2$, $-y_2$
Φ_3	x
Φ_4	x
Φ_5	x_8, y_8
Φ_{23}	-y
Φ_{25}	y
Φ_{31}	$(x, -x, -x, x)_2$, $(-x, x, x, -x)_2$

2. Creating chirp and saltire pulses

These pulses are uncommon so a detailed description will be provided.

2.1 Chirp pulse (for ZQF)

In the *Shape Tool* of Topspin ('stdisp') open a shape of an adiabatic smoothed chirp. Use a low-to-high field, 20% smoothed, sweep width of 20 kHz (or at least twice the desired spectral window) and 2000 points. The duration of the pulse should be optimized for the sample. For the samples studied in this publication good results were obtained using 10 ms for the first and 30 ms for the second pulse (p32 and p34, respectively). Save both new pulses and select them in 'spnam32' and 'spnam34'. Their values of γB1 (gammaB1) must be used to define 'cnst53' and 'cnst54'. (*3*).

2.2 Saltire pulse (for PSYCHE)

In the *Shape Tool* of Topspin open a shape of an adiabatic smoothed chirp. Use a low-to-high field, 20% smoothed, sweep-width of 10 kHz (or at least twice the desired spectral window), 10000 points and 15 ms duration. Save and create a chirp pulse with the same properties, but now with high-to-low field. Open *Add Shape* ('manipul addshapes'), and open the two pulses created. Align the high-to-low pulse to the end of the low-to-high pulse, scale them to 100%, and save as AddShape1. Now align the low-to-high with the end of the high-to-low, scale as 100%, and save as AddShape2. Add the two new shapes created, aligning them to the middle of each other and scaling them to 100%. Save this pulse as PSYCHE_Saltire and select this pulse as 'spnam10'. The duration of the PSYCHE element (p10) must be defined as 30 ms, the bandwidth (cnst21) must be 10000 Hz this way, and the flip angle (cnst20) must be set as desired (normally between 10° and 30°). (*4*).

B. Experimental data

All experimental data for this paper are freely available for download from DOI: 10.15127/1.302716.

1. Peppermint oil, 25% in DMSO-d_6 (v/v)

The sample was prepared dissolving 150 μL of commercial peppermint oil (Obbekjaers) in 450 μL of DMSO-d_6. Experiments were run at 303 K. For all experiments the ^{1}H spectral window was set to 4409 Hz (8.81 ppm), the carrier frequency to 1750 Hz (3.5 ppm) and the duration of the hard 90° pulse was 10.7 μs. 1D Selective TOCSY spectra were recorded with 32k complex points using 32 transients in an experiment time around 4 min. Prior to Fourier transformation, zero-filling to 64k was applied. All PSYCHE and 1D selective TOCSY-PSYCHE ^{1}H NMR spectra were recorded with 16k complex points, 50 t_1 increments (with a chunk size of 11.3 ms) and a flip angle β of 13°. Menthol was recorded with 32 transients, in a total experiment time of 1 h 35 min, and both menthone and neomenthol were recorded with 64 transients, in a total experimental time of 3 h 10 min. Prior to Fourier transformation, zero-filling to 32k and Lorentz to Gauss transformation with LB of -0.01 Hz and GB of 0.002 were applied. In the 1D selective TOCSY experiments (conventional and pure shift) different signals were selected - H_2 of *trans*-menthone (2.10 ppm), H_1 of menthol (3.17 ppm), and H_1 of neomenthol (3.91 ppm) - by using RSNOB selective pulses (70 ms for menthol and neomenthol and 110ms for menthone); a 200 ms mixing period was used.

1.1. 2D experiments

From the TOCSY spectrum (Fig. S2) it is clear that a detailed analysis, even using homonuclear 2D methods (Fig. S3), is almost impossible. Note that due to the large dynamic range of the sample, only the menthol (majority compound) can be observed properly in the 2D display. t_1-noise from the menthol signals appears at similar intensity to some neomenthol and *trans*-menthone signals.

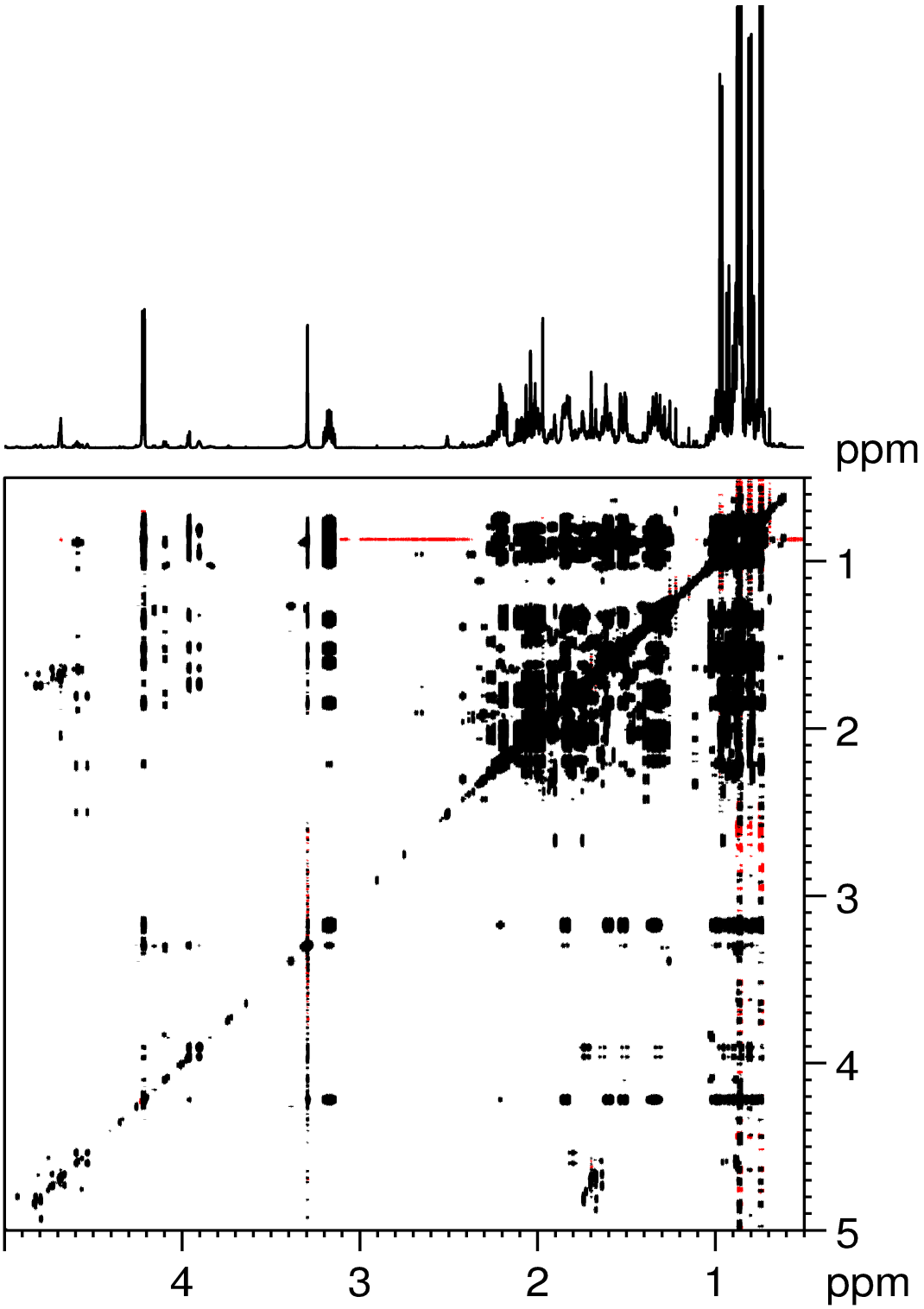

Fig. S2. 500 MHz 2D TOCSY spectrum of peppermint oil in DMSO-d_6. Data were recorded with 16 scans, 8k complex points in the direct dimension and 512 t_1 increments, with experiment time of 5 h 2 min. Both dimensions were zero-filled once prior to Fourier transformation. Lorentz to Gauss transformation was applied with LB of -0.01 Hz, GB of 0.003 for the direct dimension and LB of -0.01 Hz, GB of 0.005 for the indirect dimension.

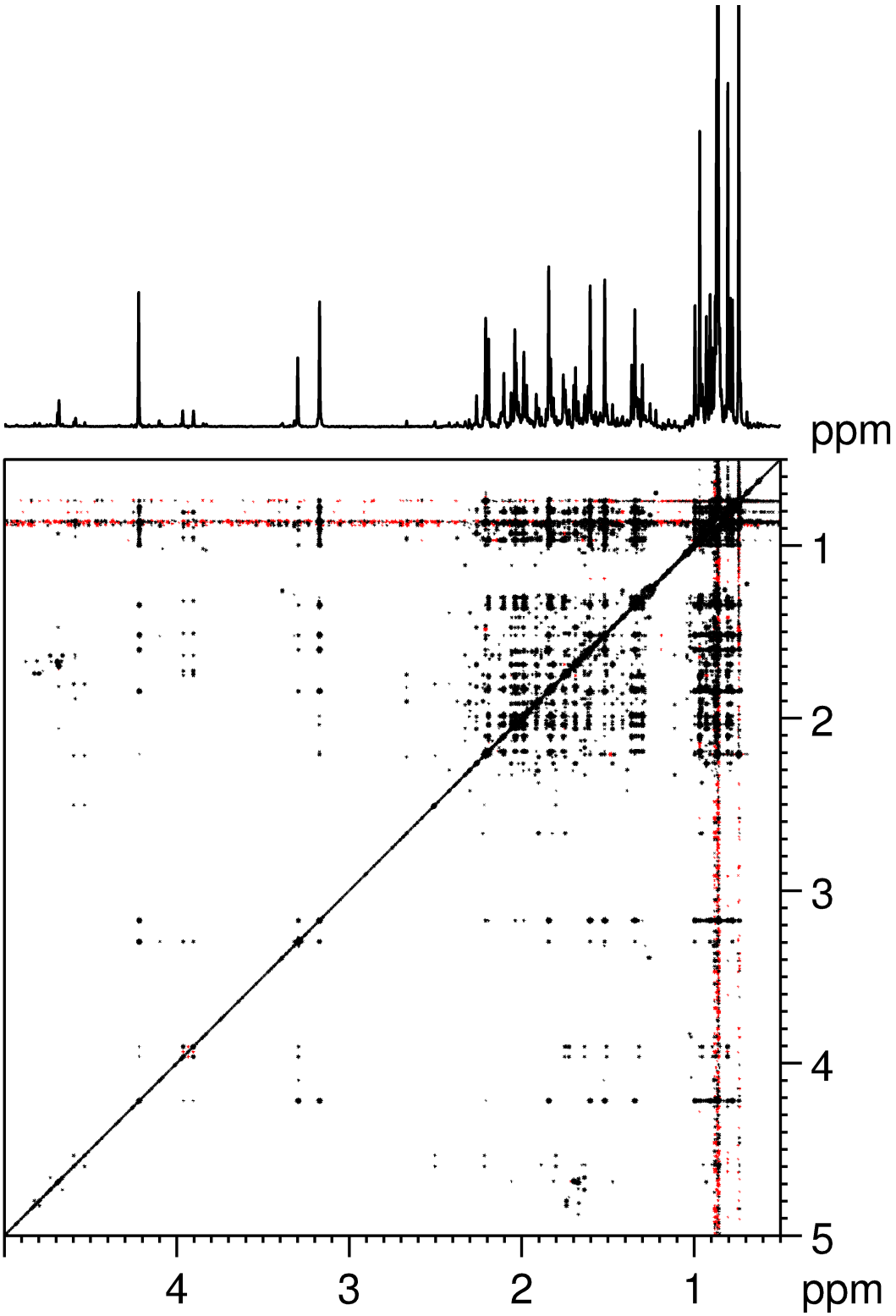

Fig. S3. 500 MHz 2D F_1-PSYCHE-TOCSY spectrum of peppermint oil in DMSO-d_6 after covariance processing. Data were recorded with 8 scans and 2k FID points in both dimensions, in an experiment time of 7 h 29 min. Both dimensions were zero-filled once prior to Fourier transformation, and Lorentz to Gauss transformation with LB of -0.01 Hz and GB of 0.002 was applied.

1.2. Comparison between 2D and 1D selective TOCSY-PSYCHE experiments

F_1 traces through 2D F_1-PSYCHE-TOCSY spectrum were compared with 1D selective TOCSY-PSYCHE spectra, to illustrate that long experiment times can be avoided if appropriate 1D experiments are chosen instead of the full 2D. Importantly, the results also benefit from significantly better spectral quality; to achieve the same resolution as the 1D spectra would require an impractically long 2D experiment.

1.2.1. Menthol

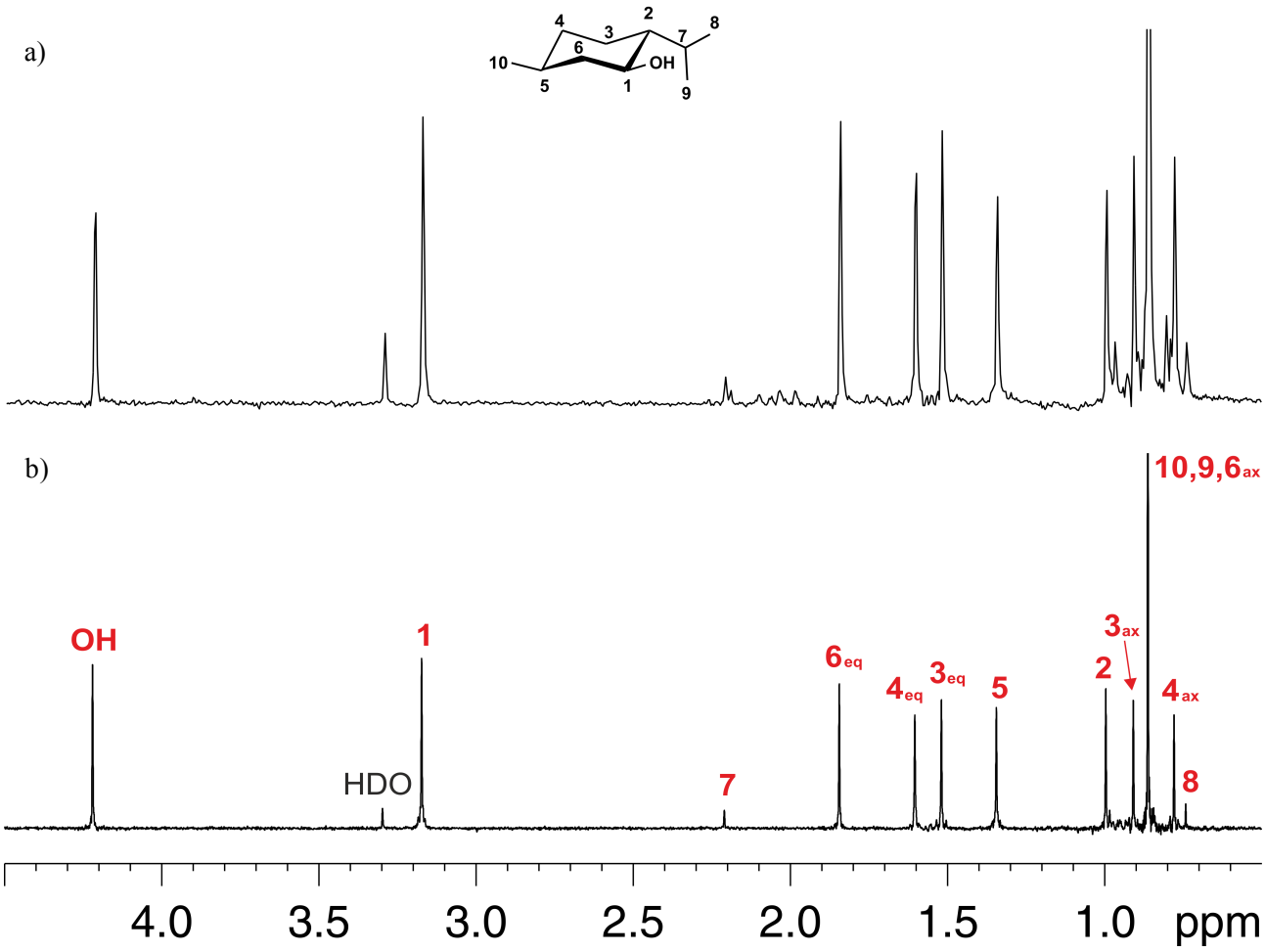

Fig. S4. ^{1}H spectra of menthol from peppermint oil: a) trace along F_2 from F_1-PSYCHE-TOCSY with covariance in F_2, and b) 1D selective TOCSY-PSYCHE. In the 1D experiments the refocusing selective pulse was applied at 3.17 ppm.

1.2.2. Neomenthol

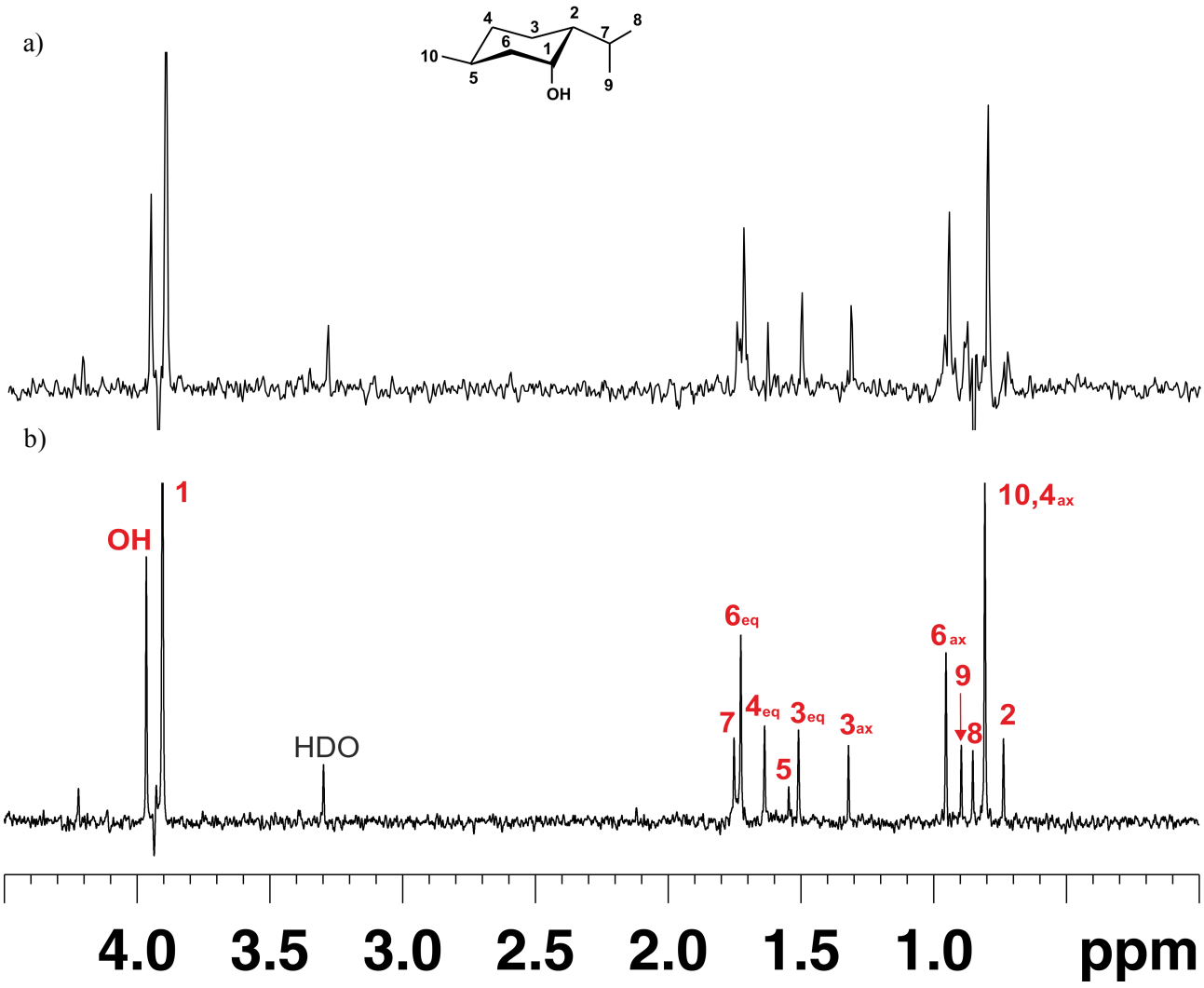

Fig. S5. ^{1}H spectra of neomenthol from peppermint oil: a) trace along F_2 from F_1-PSYCHE-TOCSY with covariance in F_2, and b) 1D selective TOCSY-PSYCHE. In the 1D experiments the refocusing selective pulse was applied at 3.95 ppm.

1.2.3. *trans*-Menthone

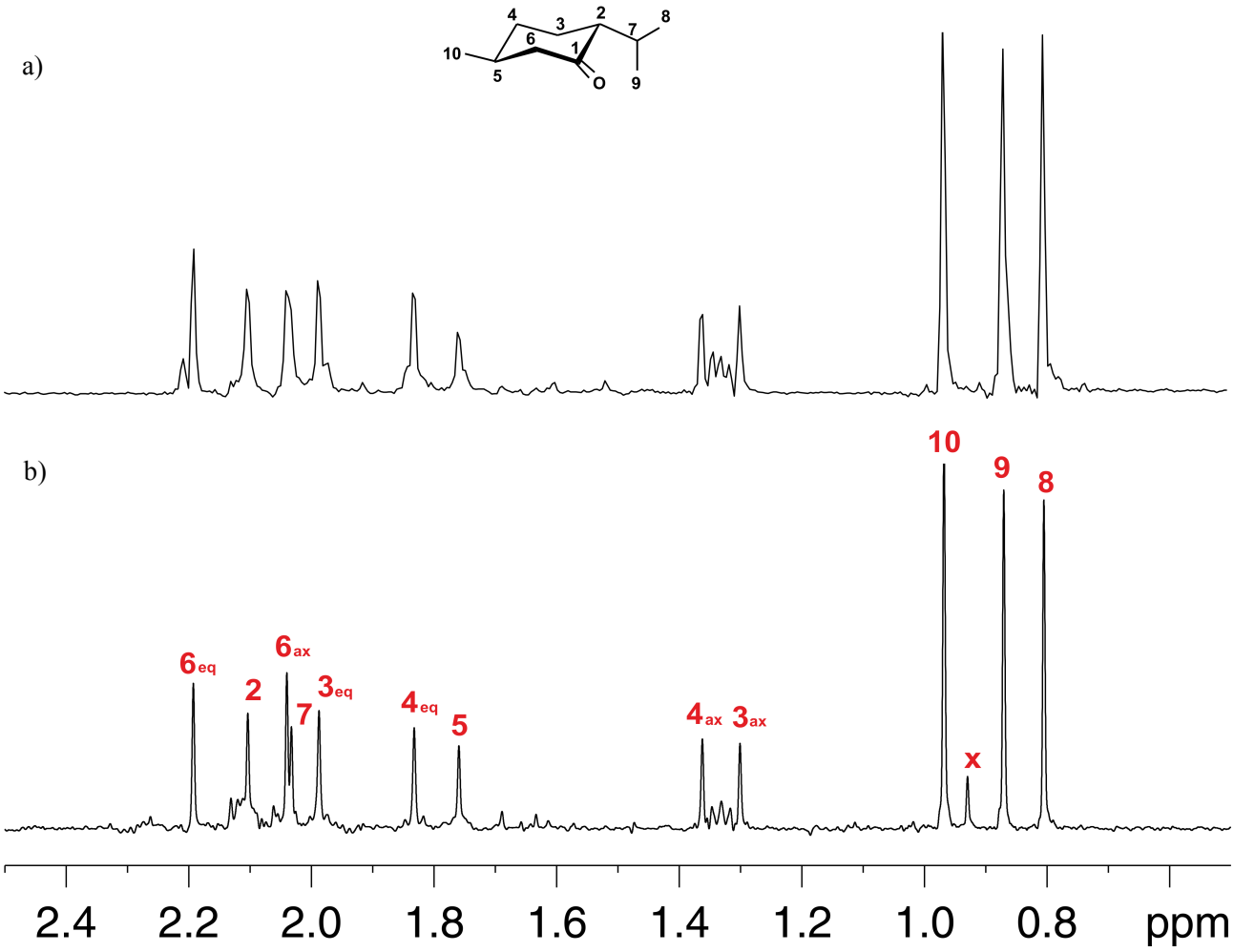

Fig. S6. ^{1}H spectra of *trans*-menthone from peppermint oil: a) trace along F_2 from F_1-PSYCHE-TOCSY with covariance in F_2, and b) 1D selective TOCSY-PSYCHE. In the 1D experiments the refocusing selective pulse was applied at 2.10 ppm.

1.3. Spectral assignment

Aided by HSQC and with the chemical shift values of the protons from the 1D selective TOCSY-PSYCHE spectra, each molecule was assigned and confirmed by literature values.(*5,6*) The HSQC experiment was recorded with 16 scans, 4k complex points and 512 t_1 increments. Direct and indirect dimensions were zero-filled to 32k and 1k points, respectively, and 90º phase-shifted squared sine-bell apodization was applied in both dimensions prior to Fourier transformation. The ^{13}C spectral window was fixed at 27669.48 Hz (219.2 ppm) centred on 13833 Hz (110 ppm).

Menthol		
Label	^{13}C δ (ppm)	^{1}H δ (ppm)
1	70.3	3.17
2	50.1	1.00
3	23.4	1.52 0.91
4	34.9	1.60 0.78
5	31.6	1.35
6	45.7	1.85 0.87
7	25.5	2.21
8	16.5	0.74
9	21.3	0.86
10	22.6	0.86

Neomenthol		
Label	^{13}C δ (ppm)	^{1}H δ (ppm)
1	65.8	3.90
2	48.4	0.74
3	24.3	1.51 1.29
4	35.5	1.64 0.81
5	29.0	1.54
6	43.4	1.71 0.96
7	25.6	1.75
8	20.0	0.85
9	21.1	0.89
10	22.8	0.81

Menthone		
Label	^{13}C δ (ppm)	^{1}H δ (ppm)
1	211.3	-
2	55.1	2.11
3	27.7	1.99 1.31
4	33.7	1.84 1.35
5	35.2	1.77
6	50.5	2.20 2.04
7	26.1	2.03
8	18.9	0.81
9	21.2	0.87
10	22.4	0.97

2. Mixture of provitamin D_3 and vitamin D_3 in $CDCl_3$

The mixture sample was prepared dissolving 10 mg each of commercial (Sigma Aldrich) 7-dehydrocholesterol (provitamin D_3) and cholecalciferol (vitamin D_3) in 600 µL of $CDCl_3$, producing a total concentration of 40 mM each. Experiments were run at 298 K. The ^1H spectral window was fixed at 8012 Hz (16 ppm) for all experiments, centred on 1498 Hz (3 ppm), and the duration of the 90º pulse of 10.5 µs. 1D selective TOCSY spectra were recorded with 32k complex points for 32 transients with an experiment time around 4 min. Prior to Fourier transformation zero-filling to 64k was applied. All PSYCHE and 1D selective TOCSY-PSYCHE ^1H NMR spectra were recorded with 16k complex points, 32 transients, and 100 t_1 increments (with a chunk size of 12.5 ms), with a total experiment time of 2 h 35 min (the time could have been reduced, acquiring only 40 chunks, without significant truncation). Prior to Fourier transformation, zero-filling to 32k and Lorentz to Gauss transformation with LB of -0.01 Hz and GB of 0.005 were applied. In the 1D selective TOCSY experiments (conventional and pure shift) different signals were selected - H_3 of provitamin D_3 (3.96 ppm) and H_3 of vitamin D_3 (3.65 ppm) - by using 46 ms RSNOB pulses; a 80 ms mixing period was used.

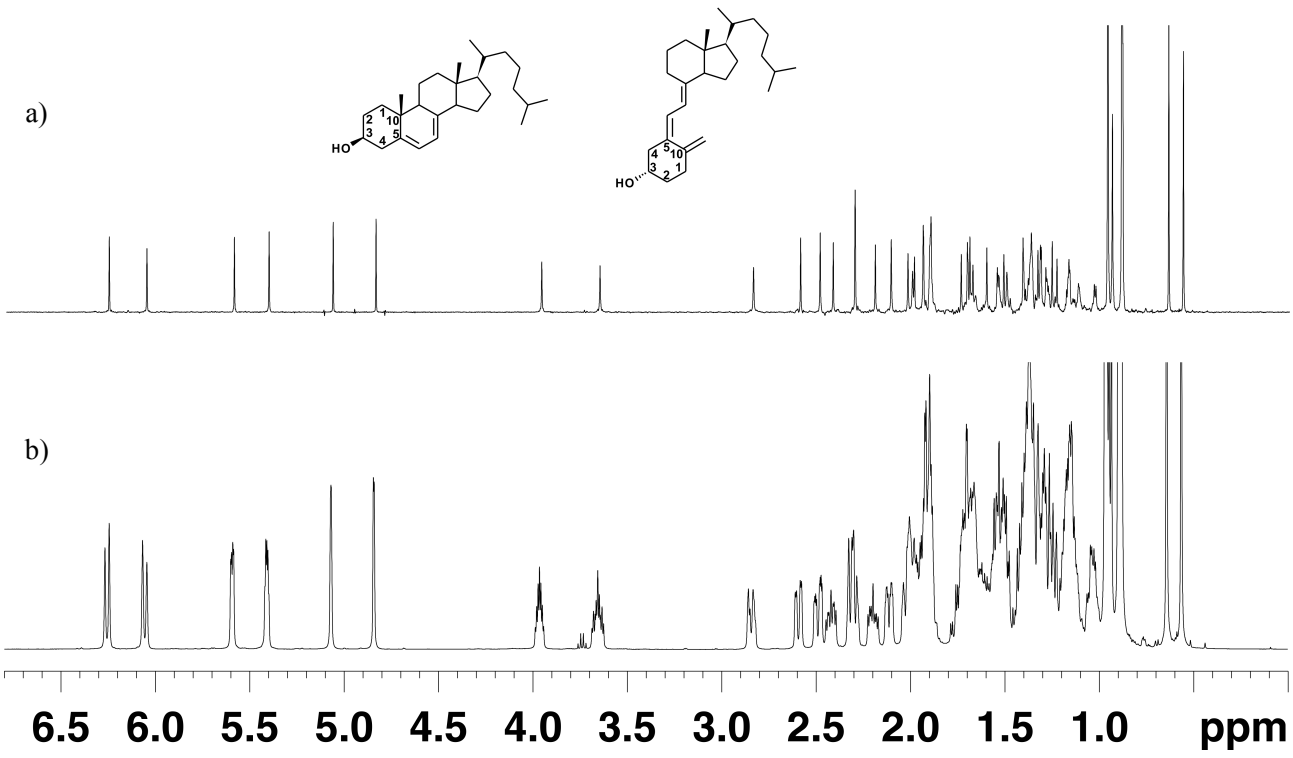

Fig. S7. Full spectra of the mixture containing provitamin D_3 (left) and vitamin D_3 (right). a) PSYCHE and b) conventional ^1H spectra.

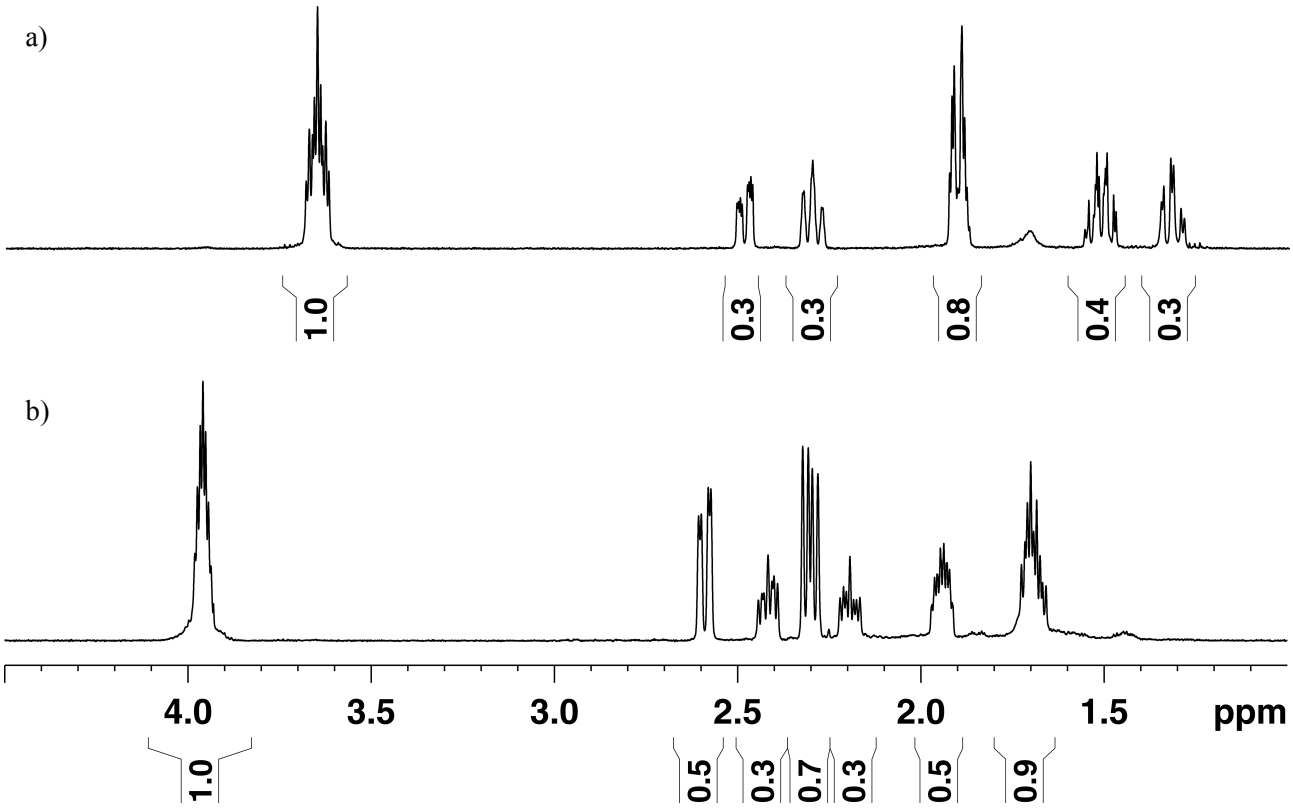

Fig. S8. 1D selective TOCSY spectra. Selecting carbinolic signal of provitamin D_3 (a) of vitamin D_3 (b).

C. Pulse sequence

1D selective PSYCHE TOCSY (for Bruker)

```
;seldipsy
; 13/04/2016
;
;   1D selective TOCSY PSYCHE
;   DIPSI-2 sequence for mixing
;   using selective refocussing with a shaped pulse
;   and with zero-quantum suppression
;
;  Guilherme Dal Poggetto and Laura Castañar
;  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
;
;References
;(1)  Foroozandeh, M.; Adams, R. W.; Meharry, N. J.; Jeannerat, D.; Nilsson, M.; Morris, G. A. Angew. Chem. Int. Ed. 2014, 53, 6990.
;(2)  Thrippleton, M.J.; Keeler,  J.; Angew. Chem. Int. Ed. 2003, 42, 3938.
;(3)  Kessler,H.; Oschkinat, H.; Griesinger, C.; Bermel, W.; J. Magn. Reson. 1986, 70, 106.
;
;$CLASS=HighRes
```

```
;$DIM=2D
;$TYPE=
;$SUBTYPE=
;$COMMENT= TOPSPIN3.x

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

define delay tauA
define delay tauB

 "in0=inf1/2"

"p2=p1*2"
"p11=p10"

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

"spoff10=0"
"spoff12=bf1*(cnst12/1000000)-o1"
"spoff32=0"
"spoff34=0"

"p33=1000000.0/(cnst53*4)"
"cnst33= (p33/p1) * (p33/p1)"
"spw32=plw1/cnst33"

"p35=1000000.0/(cnst54*4)"
"cnst35= (p35/p1) * (p35/p1)"
"spw34=plw1/cnst35"

"cnst50=(cnst20/360)*sqrt((2*cnst21)/(p10/2000000))"
"p30=1000000.0/(cnst50*4)"
"cnst31= (p30/p1) * (p30/p1)"
"spw10=plw1/cnst31"

"FACTOR1=(d9/(p6*115.112))/2"
"l1=FACTOR1*2"


1 ze
2 30m
  20u pl1:f1 BLKGRAD
  d1
  50u UNBLKGRAD
  (p1 ph1):f1
  3u
  p17:gp1
  d17 pl0:f1
  p12:sp12:f1 ph2:r                                          ;selective SE
  3u
  p17:gp1
  d17 pl1:f1
```

```
                                                        ;Begin TOCSY block
  p1 ph3
  10u gron0
  (p32:sp32 ph3):f1
  20u groff
  d17 pl10:f1
                                                        ;begin DIPSI2
3 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 3 times l1
                                                        ;end DIPSI2
  p17:gp2
  d17
  10u gron10
  (p34:sp34 ph3):f1
  20u groff
  d17 pl1:f1
  p1 ph3
```

```
                                                        ;end TOCSY
                                                        ;Begin PSYCHE
4 d0
  tauA
  50u
  p16:gp3
  d16
  p2 ph4
  50u
  p16:gp3
  d16
  tauA
  p16:gp4
  d16
  10u pl0:f1
  tauB
  ( center (p10:sp10 ph5):f1 (p11:gp11) ) ;PSYCHE element
  d16
  10u pl1:f1
  p16:gp4
  d16
  50u BLKGRAD
  d0
                                                        ;End PSYCHE
  go=2 ph31
  30m mc #0 to 2 F1QF(id0)
exit


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

;POWER LEVEL
;pl0  : zero power (0W)
;pl1  : power level for pulse (default)
;pl10 : power level for TOCSY-spinlock
;sp10 : power level of double-chirp PSYCHE pulse element
;sp12 : power level of refocusing shaped pulse
;sp32 : power level of adiabatic pulse of first ZQF element
;sp34 : power level of adiabatic pulse of last ZQF element

;PULSE DURATION
;p1  : 90 degree high power pulse
;p2  : 180 degree high power pulse
;p6  : 90 degree low power pulse
;p10 : duration of double-chirp PSYCHE pulse element                    [30 ms]
;p12 : 180 degree refocusing shaped pulse
;      choose p12 according to desired selectivity
;p32 : first ZQF 180 degree inversion shaped pulse (adiabatic)          [10 msec]
```

;p34 : second ZQF 180 degree inversion shaped pulse (adiabatic) [30 msec]

;GRADIENT DURATION
;p11 : duration of weak gradient during PSYCHE pulse element [p11=p10]
;p16 : duration of CTP gradients for PSYCHE [1 ms]
;p17 : duration of CTP gradients for Selective-TOCSY [0.9 ms]

;DELAY
;d0 : incremented delay
;d1 : relaxation delay [5*T1 s]
;d9 : TOCSY mixing time [50-200 ms]
;d16 : recovery delay for gradients in PSYCHE block [1 ms]
;d17 : selective spin-echo delay in selective-TOCSY block [200 us]

;PULSE SHAPE
;spnam10 : file name for PSYCHE pulse element [SALTIRE]
;spnam12 : file name for the selective 180 refocusing shaped pulse [RSNOB or REBURP]
;spnam32 : file name for the adiabatic shaped pulse using in first ZQF [CHIRP]
; smoothed chirp (low to high, 20% smoothing, 1000 points, 20KHz)
;spnam34 : file name for the adiabatic shaped pulse using in last ZQF [CHIRP]
; smoothed chirp (low to high, 20% smoothing, 1000 points, 20KHz)

;GRADIENT SHAPE
;gpnam1 : SMSQ10.100
;gpnam2 : SMSQ10.100
;gpnam3 : SINE.100
;gpnam4 : SINE.100
;gpnam11: RECT.1

;GRADIENT STRENGTH
;gpz0 : first ZQF gradient [3%]
;gpz1 : CTP gradient [13%]
;gpz2 : CTP gradient [31%]
;gpz3 : CTP gradient [77%]
;gpz4 : CTP gradient [49%]
;gpz10: last ZQF gradient [2%]
;gpz11: weak gradient during PSYCHE element [1-3%]

;CONSTANTS
;cnst4 : number of drop points from FID
;cnst12: chemical shift for selective pulse (offset, in ppm)
;cnst20: desired flip angle for PSYCHE pulse element (degree) [10-25]
;cnst21: bandwidth of each chirp in PSYCHE pulse element (Hz) [10000 Hz]
;cnst53: GammaB1 of first adiabatic ZQF shaped pulse
;cnst54: GammaB1 of last adiabatic ZQF shaped pulse

;OTHER
;ns : 8 * n, total number of scans: NS * TD0
;ds : 8
;td1 : number of t1 increments [16-64]
;sw1 : sw2/n (n has to be an integer number) [40-100 Hz]
;in0 : 1/(2 * SW) = DW
;nd0 : 2
;l1 : loop for DIPSI cycle: ((p6*115.112) * l1) = mixing time
;MC2 : QF

D. References

1. E. Kupce, J. Boyd, I. A. Campbell, "Short selective pulses for biological applications". *Journal of Magnetic Resonance* **106**, 300-303 (1995).
2. H. Geen, R. Freeman, "Band selective radiofrequency pulses". *Journal of Magnetic Resonance* **93**, 93-141 (1991).
3. M. J. Thrippleton, J. Keeler, "Elimination of zero-quantum interference in two-dimensional NMR spectra". *Angewandt Chemie International Edition* **42**, 3938-3941 (2003).
4. M. Foroozandeh *et al.*, "Ultrahigh-resolution NMR spectroscopy". *Angewandt Chemie International Edition* **53**, 6990-6992 (2014).
5. Y. Senda, S. Imaizumi, "^{13}C pulse Fourier transform NMR of menthol stereoisomers and related compounds". *Tetrahedron* **31**, 2905-2908 (1975).
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