Electronic Supplementary Material (ESI) for Faraday Discussions.

Electronic Supplementary Information

Reduced dimensionality hyphenated NMR experiments for the structure determination of compounds in mixtures

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Figure S2. 1D traces through C1-C5 carbons of α-D-glucopyranose taken from Ω_{13C} + $\kappa\Omega_{1H}$ the (3,2)D BIRD^{r,X}-HSQC-TOCSY/CLIP-COSY experiments.

Figure S3. Overlay of $\Omega_{13C} \pm \kappa\Omega_{1H}$ (3,2)D BIRD^{r,X}-HSQC-NOESY/ROESY spectra.

Figure S4. 1D traces through C1-C5 carbons of α-D-glucopyranose taken from the Ω_{13C} + $\kappa\Omega_{1H}$ (3,2)D BIRD^{r,X}-HSQC-HSQMBC spectra.

Figure S5. CH_2 region of the 2D (3,2)D BIRD^{r,X}-HSQC-HSQMBC spectra.

Scheme 1. Polarisation transfer analysis of the HSQC-HSQMBC experiment.

Pulse programs for the reduced dimensionality experiments

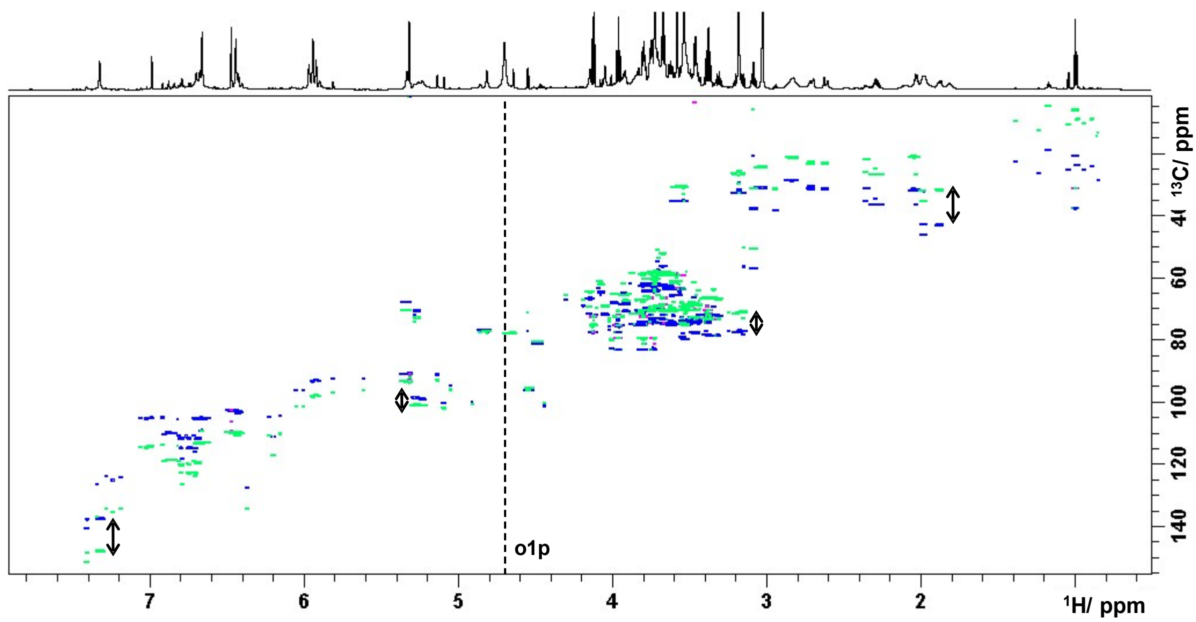


Fig. 1S. Overlay of Ω_{13C} - $\kappa\Omega_{1H}$ (blue) and Ω_{13C} + $\kappa\Omega_{1H}$ (green) (3,2)D BIRD^{r,X}-HSQC spectra of green tea. Arrows indicate the level of displacement of cross peaks as a function Ω_{1H} –

o1p, where o1p is the position of the [1]H r.f. carrier (4.7 ppm in this spectrum, indicated by the dashed line). Note the change of the direction of the displacement after o1p.

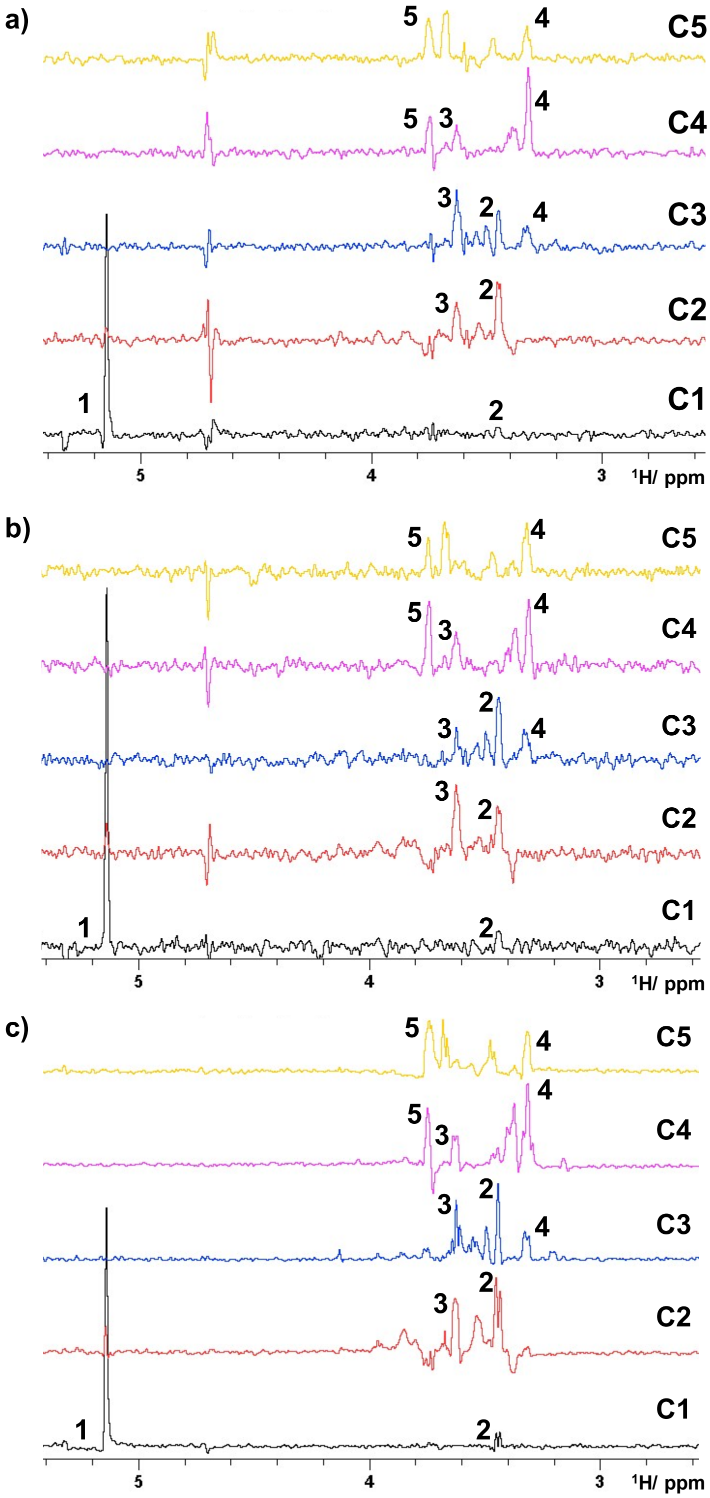


Fig. 2S. 1D traces through C1-C5 carbons of α-D-glucopyranose taken from $\Omega_{13C} + \kappa\Omega_{1H}$ the (3,2)D BIRD^{r,X}-HSQC-CLIP-COSY (a) Δ= 11.4 ms, (b) Δ= 16.7 ms and (c) 20ms mixing time (3,2)D BIRD^{r,X}-HSQC-TOCSY spectrum of green tea. The ^{1}H resonances are labelled.

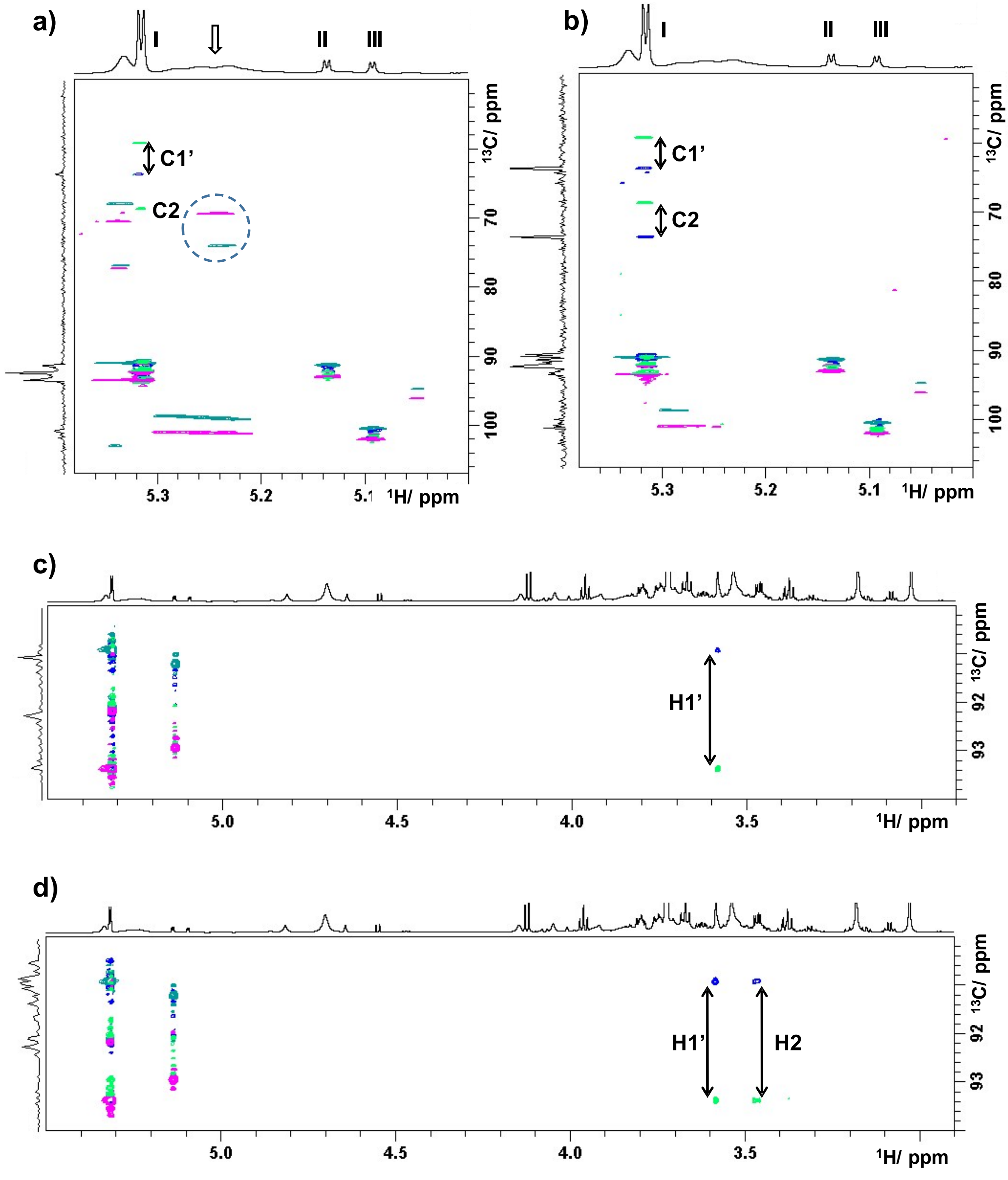


Fig. 3S. Overlay of the $\Omega_{13C} \pm \kappa\Omega_{1H}$ (3,2)D BIRD^{r,X}-HSQC-NOESY (a,c) and (3,2)D BIRD^{r,X}-HSQC-ROESY (b,d) spectra of green tea focusing on the anomeric regions. Spectra (a, b) show NOEs/ROEs from the other ring protons to the anomeric proton, while spectra (c, d) show the transfer the other way round (from H1 to H1’ or H2). The roman numerals refer to sucrose (I), α-glucose and 2-O-arabinopyranosyl-myo-inositol (III), while the arrow highlights the resonances belonging to a polysaccharide. The NOESY spectrum was phased to have the ^{1}H-^{13}C cross peaks negative and thus the NOESY cross peaks of small molecules as positive signals. Only sucrose NOESY cross peaks were above the noise level, while the

ROESY signals were much more intense. Broad polysaccharide signals show strong and negative NOEs (circled signals in (a)), while the corresponding ROEs were not detected.

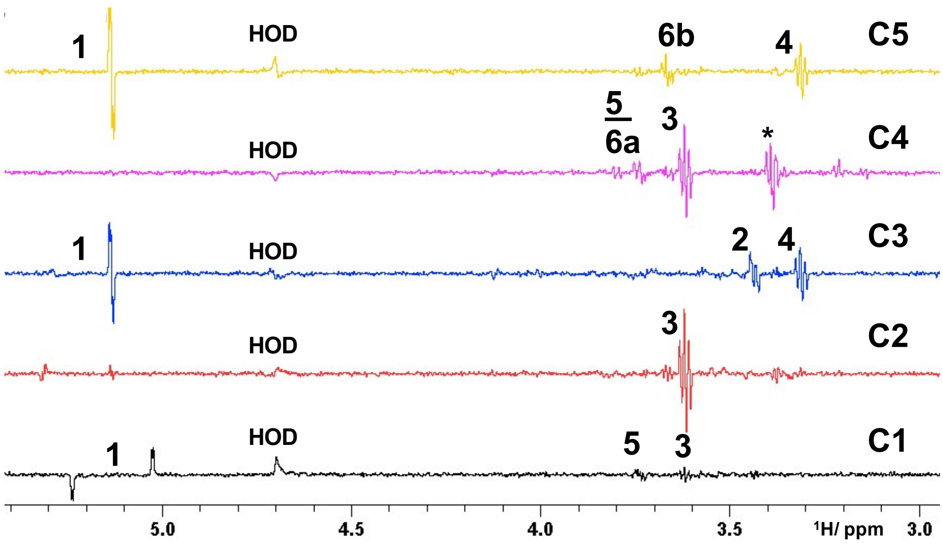


Fig. 4S. 1D traces through C1-C5 carbons of α-D-glucopyranose taken from the $\Omega_{13C} + \kappa\Omega_{1H}$ (3,2)D BIRD^{r,X}-HSQC-HSQMBC spectra of green tea. The signal labelled with an asterisk does not belong to glucose. The proton resonances and residual water signal are labelled.

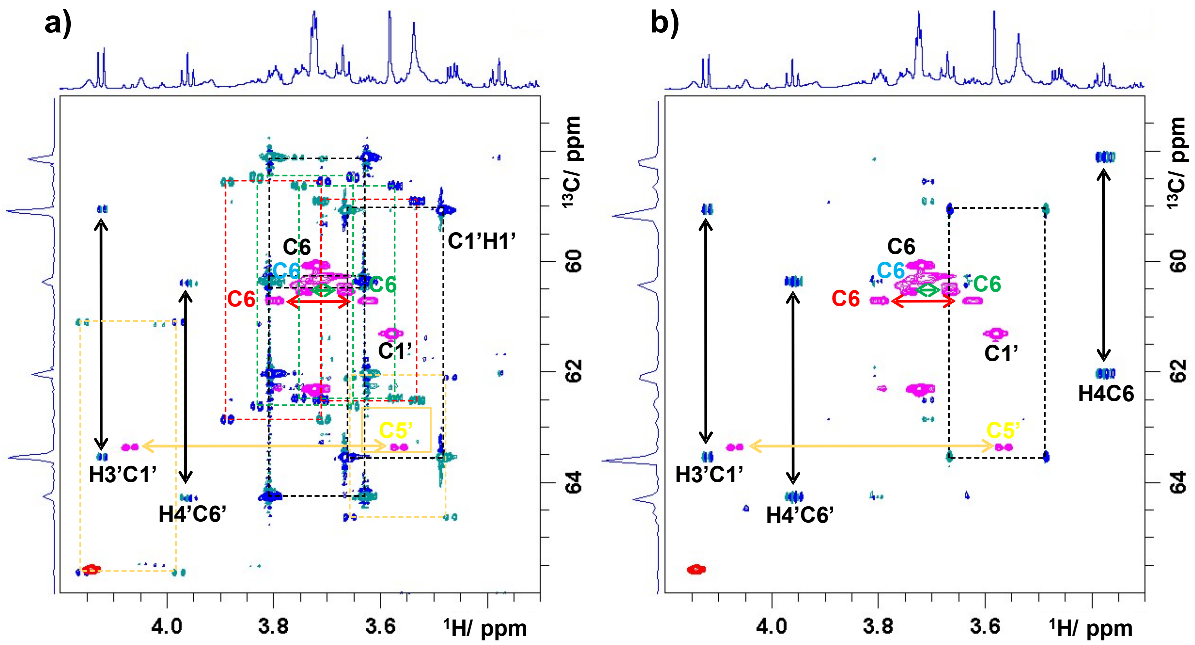
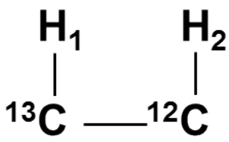
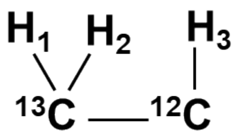
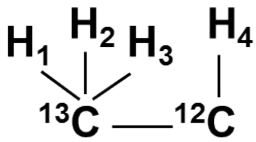


Fig. 5S. The CH_2 region of the 2D (3,2)D BIRD^{r,X}-HSQC-HSQMBC spectra of green tea acquired (a) with $\tau = 0.5/^1J_{CH}$ and (b) $\tau = 0.25/^1J_{CH}$. Cross peaks labels are colour coded as: black (sucrose), green (α-glucose), red (β-glucose), yellow (2-O-arabinopyranosyl-myo-inositol) and blue (polysaccharides). Arrows depict the separation between long-range cross peaks, while dashed boxes trace out the one-bond CH cross peaks. Note a significant suppression of one-bond cross peaks in (b) and enhanced intensity of long-range cross peaks.

Scheme 1. Intensity of one-bond (yellow) and long-range (orange) correlations in (3,2)D BIRD^{r,X}-HSQC-HSQMBC experiment.

CH	CH_2	CH_3
		
$-2S_yH_{1z}c_1c_2$	$-2S_yH_{1z}c_1c_2c_3$	$-2S_yH_{1z}c_1c_2c_3c_4$
$4S_xH_{1z}H_{2z}c_1s_2$	$4S_xH_{1z}H_{2z}c_1s_2c_3$	$4S_xH_{1z}H_{2z}c_1s_2c_3c_4$
$S_xs_1c_2$	$S_xs_1c_2c_3$	$S_xs_1c_2c_3c_4$
$2S_yH_{2z}s_1s_2$	$2S_yH_{2z}s_1s_2c_3$	$2S_yH_{2z}s_1s_2c_3c_4$
	$4S_xH_{1z}H_{3z}c_1c_2s_3$	$4S_xH_{1z}H_{3z}c_1c_2s_3c_4$
	$8S_yH_{1z}H_{2z}H_{3z}c_1s_2s_3$	$8S_yH_{1z}H_{2z}H_{3z}c_1s_2s_3c_4$
	$2S_yH_{3z}s_1c_2s_3$	$2S_yH_{3z}s_1c_2s_3c_4$
	$-4S_xH_{2z}H_{3z}s_1s_2s_3$	$-4S_xH_{2z}H_{3z}s_1s_2s_3c_4$
		$4S_xH_{1z}H_{4z}c_1c_2c_3s_4$
		$8S_yH_{1z}H_{2z}H_{4z}c_1s_2c_3s_4$
		$2S_yH_{4z}s_1c_2c_3s_4$
		$-4S_xH_{2z}H_{4z}s_1s_2c_3s_4$
		$8S_yH_{1z}H_{3z}H_{4z}c_1c_2s_3s_4$
		$-16S_xH_{1z}H_{2z}H_{3z}H_{4z}c_1s_2s_3s_4$
		$-4S_xH_{3z}H_{4z}s_1c_2s_3s_4$
		$-8S_yH_{2z}H_{3z}H_{4z}s_1s_2s_3s_4$

CH	CH_2	CH_3
c_1: $\cos\pi^1J_{CH}\tau$	c_1: $\cos\pi^1J_{CH}\tau$	c_1: $\cos\pi^1J_{CH}\tau$
c_2: $\cos\pi^nJ_{CH}(T-\tau)$	c_2: $\cos\pi^1J_{CH}\tau$	c_2: $\cos\pi^1J_{CH}\tau$
s_1: $\sin\pi^1J_{CH}\tau$	c_3: $\cos\pi^nJ_{CH}(T-\tau)$	c_3: $\cos\pi^1J_{CH}\tau$
s_2: $\sin\pi^nJ_{CH}(T-\tau)$	s_1: $\sin\pi^1J_{CH}\tau$	c_4: $\cos\pi^nJ_{CH}(T-\tau)$
	s_2: $\sin\pi^1J_{CH}\tau$	s_1: $\sin\pi^1J_{CH}\tau$
	s_3: $\sin\pi^nJ_{CH}(T-\tau)$	s_2: $\sin\pi^1J_{CH}\tau$
		s_3: $\sin\pi^1J_{CH}\tau$
		s_4: $\sin\pi^nJ_{CH}(T-\tau)$

For CH_2 and CH_3 only results for H_1 are presented. Only the active long-range proton–carbon couplings were considered. All observable terms should be multiplied by a $\Pi \cos\pi^nJ_{CH}(T-\tau)$ to reflect the effect of passive long-range proton-carbon couplings. T and τ are the one-bond and long-range evolution intervals, respectively. Non-highlighted terms do not yield observable signals. Table below gives the intensity of one-bond and long-range correlations for $\tau = 0.5/^1J_{CH}$ and $0.25/^1J_{CH}$. Term (2) given for CH_2 cross peaks applies for equivalent CH_2 protons. Identical $^nJ_{CH}$ were assumed for both CH_2 protons and an exact match between the actual $^1J_{CH}$ couplings and the one used for setting the τ interval is also assumed.

	CH	CH_2	CH_3	CH	CH_2	CH_3

τ	$0.5/^1J_{CH}$			$0.25/^1J_{CH}$		
One-bond correlation	0	$(2)*c_3$	0	$0.707*c_2$	0	$1.06*c_4$
Long-range correlation	s_2	0	0	$0.707*s_2$	$(2)*0.5s_3$	$1.06*s_4$

Pulse programs for the reduced dimensionality experiments

The Bruker pulse programs for the reduced dimensionality experiments are provided below except the (3,2) HSQC-TOCSY pulse program which can be found in: Brodaczewska, N. *et al.*, *Journal of Biomolecular NMR* (2018) 70:115 together with the processing script.

For all experiments: in Topspin3, TD1 must be set twice the increments as in a single 2D. In Topspin4 only normal TD1 should be used. Topspin4 recognises that (i) these are experiments with interleaved acquisition of 2D spectra and (ii) also allows one to inspect the in-phase and antiphase spectra by processing these separately. The PHC0 phase in F1 in the cosine and sine modulated experiments should be set to 0° and 90°, respectively.

<u>(3,2) HSQC-CLIP-COSY pulse program</u>

```
;HSQC_CLIPCOSY_Hmod
;Justinas Sakas  and  Nicholle G.A. Bell
;Faraday Discuss. 2019 https://doi.org/10.1039/C9FD00008A
;This modification is based on the HSQC-CLIP-COSY pulse sequence published in:
;Gyöngyösi T, Timári I, Haller J, Koos MR, Luy B, Kövér KE. ChemPlusChem. (2018)
;83:1:53-60.https://doi.org/10.1002/cplu.201700452
;By using this pulse sequence, or any modification of it in any published material
;you agree to acknowledge the above-mentioned publications.
;ek_ti_HSQC_CLIP_COSY_dc
;avance-version - Avance II, AQS system, SADC digitizer, RX22 receiver, TopSpin 2.1
;2D H-1/X correlation via double inept transfer
;phase sensitive using Echo/Antiecho-TPPI gradient selection
;with decoupling during acquisition
;using purging gradient in inept transfer
;using shaped pulses for inversion and refocussing on f2 – channel

;$CLASS=HighRes
;$DIM=2D
;$TYPE=
;$SUBTYPE=
;$COMMENT=
;July 27, 2016 KEK, IT
#include <Avance.incl>
#include <Grad.incl>
#include <Delay.incl>
"p2=p1*2"
"p4=p3*2"
"d4=1s/(cnst2*4)" ;CNST2 = 1J(XH)
"d11=30m"
"d14=1s/(cnst3*4)" ;CNST3 = ca. 20 Hz, duration of echo
"d24=d14-d4-p4"
"d26=p16+d16+4u"
# ifdef LABEL_CN
"p22=p21*2"
# else
# endif /*LABEL_CN*/
"d0=3u"
```

```
"d25=3u"
"in0=inf1/2"
"in25=cnst1*in0"
"l0=0"
"DELTA1=d4-p16-larger(p2,p14)/2-de-8u"
"DELTA2=d4-larger(p2,p14)/2"
"DELTA3=d4-larger(p2,p14)/2-p1*2/PI"
"DELTA5=d4*2-larger(p2,p14)/2-4u"
"TAU1=d4-p16-d16-larger(p2,p14)/2-4u"
"TAU2=d4+p16+d16-larger(p2,p14)/2-4u+2*d25"
# ifdef LABEL_CN
"DELTA=p16+d16+larger(p2,p22)+d0*2"
# else
"DELTA=p16+d16+p2+d0*2"
# endif /*LABEL_CN*/

1 ze
d11 pl12:f2
2 d11 do:f2
d1 BLKGRAD
if "l0 %2 == 0"
     {
   (p1 ph1)       ;cos
     }
  else
     {
   (p1 ph2)       ;sin
     }

;3 (p1 ph1)      ;cos
;3 (p1 ph2)      ;sin
d25
p16:gp7
d16 pl0:f2
(p1 ph1)      ;BIRDrX cos
DELTA5
4u
(center (p2 ph2) (p14:sp3 ph1):f2 )
4u
DELTA5
(p1 ph1)
p16:gp7*-1
d16
d25
TAU1 pl0:f2
4u
(center (p2 ph1) (p14:sp3 ph6):f2 )
4u
TAU2 pl2:f2
(p1 ph2)
;3u
```

```
;p16:gp3 ;gpz3 = 15 purging gradient
;d16
(p3 ph3):f2
d0
# ifdef LABEL_CN
(center (p2 ph5) (p22 ph1):f3 )
# else
(p2 ph5)
# endif /*LABEL_CN*/
d0
p16:gp1*EA
d16 pl0:f2
4u
(p24:sp7 ph4):f2
4u
DELTA pl2:f2
(p3 ph4):f2
;3u
;p16:gp6 ;gpz6 = 11 purging gradient
;d16
(p1 ph11)
;CLIP COSY starts here
;in-phase transfer block, planar mixing
d14 ;proton-proton coupling evolution allowed, antiphase proton magnetization
;with respect to X is refocused
p2 ph20
d24 ;d24 = d14 - d4 - p4
(p4 ph6):f2 ;d4 = 1/4J(XH)
d4
(p1 ph20)
d14
p2 ph13
d14
;second filter block
p1 ph21
4u gron4 pl9:f1
(p32:sp29 ph9):f1 ;p23 = 20 ms, SChirp20_20ms_30kHz_lh
40u groff
p16:gp5
d16 pl1:f1
(p1 ph14) (p3 ph7):f2
d26 pl12:f2
p2 ph2
4u
p16:gp2
;d16 BLKGRAD
d16
go=2 ph31 cpd2:f2
d11 do:f2 mc #0 to 2
F1I(iu0, 2)
```

```
F1EA(calgrad(EA), caldel(d0, +in0) & caldel(d25, +in25) & calph(ph3, +180) & calph(ph6,
+180) & calph(ph31, +180))
d16 BLKGRAD
exit

ph1=0
ph2=1
ph3=0 2
ph4=0 0 0 0 2 2 2 2
ph5=0 0 2 2
ph6=0
ph11=1
ph7=0 2
ph13=3
ph20=1 1 3 3
ph21=0
ph14=0
ph9=2
ph31=0 2 0 2 2 0 2 0
;pl0 : 0W
;pl1 : f1 channel - power level for pulse (default)
;pl2 : f2 channel - power level for pulse (default)
;pl3 : f3 channel - power level for pulse (default)
;pl12: f2 channel - power level for CPD/BB decoupling
;sp3: f2 channel - shaped pulse 180 degree for inversion
;sp7: f2 channel - shaped pulse 180 degree for refocussing
;p1 : f1 channel - 90 degree high power pulse
;p2 : f1 channel - 180 degree high power pulse
;p3 : f2 channel - 90 degree high power pulse
;p14: f2 channel - 180 degree shaped pulse for inversion
;p16: homospoil/gradient pulse
;p22: f3 channel - 180 degree high power pulse
;p24: f2 channel - 180 degree shaped pulse for refocussing
;p28: f1 channel - trim pulse [0 usec]
;d0 : incremented delay (2D) [3 usec]
;d1 : relaxation delay; 1-5 * T1
;d4 : 1/(4J)XH
;cnst1: scaling factor between incrementable periods
;cnst2 = 1JXH
;cnst3 = ca. 20 Hz, duration of echo
;d11: delay for disk I/O [30 msec]
;d16: delay for homospoil/gradient recovery
;cnst2: = J(XH)
;inf1: 1/SW(X) = 2 * DW(X)
;in0: 1/(2 * SW(X)) = DW(X)
;nd0: 2
;NS: 4 * n
;DS: >= 16
;td1: number of experiments
;FnMODE: echo-antiecho
;cpd2: decoupling according to sequence defined by cpdprg2
```

```
;pcpd2: f2 channel - 90 degree pulse for decoupling sequence
;use gradient ratio: gp 1 : gp 2
; 80 : 20.1 for C-13
; 80 : 8.1 for N-15
;for z-only gradients:
;gpz1: 80%
;gpz2: 20.1% for C-13, 8.1% for N-15
;gpz3: 15%
;gpz4: -5%
;gpz5: -17.9%
;gpz6: 11%
;gpz7: 7%
;use gradient files:
;gpnam1: SMSQ10.100
;gpnam2: SMSQ10.100
;gpnam5: SMSQ10.100
;gpnam7: SMSQ10.100
;preprocessor-flags-start
;LABEL_CN: for C-13 and N-15 labelled samples start experiment with
;option -DLABEL_CN (eda: ZGOPTNS)
;preprocessor-flags-end
;$Id: hsqcetgpsp.2,v 1.6.2.2 2011/02/24 17:27:49 ber Exp $
```

(3,2) HSQC-NOESY pulse program

```
;HSQC_NOESY_Hmod
;Justinas Sakas  and  Nicholle G.A. Bell
;Faraday Discuss. 2019 https://doi.org/10.1039/C9FD00008A
;By using this pulse sequence, or any modification of it in any published material
;you agree to acknowledge the above-mentioned publication.
;Based on BRULKER's:
;hsqcetgpnosp
;avance-version (12/01/11)
;2D H-1/X correlation via double inept transfer
;with correlation via dipolar coupling
;dipolar coupling may be due to noe or chemical exchange
;phase sensitive using Echo/Antiecho-TPPI gradient selection
;with decoupling during acquisition - using f2 (and f3)
;using trim pulses in inept transfer
;using shaped pulses for inversion on f2 - channel
;optional water suppresion during the mixing time: zgoptns -DPRESAT
;
;$CLASS=HighRes
;$DIM=2D
;$TYPE=
;$SUBTYPE=
;$COMMENT=

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

"p2=p1*2"
"p4=p3*2"
"d4=1s/(cnst2*4)"
"d11=30m"
"d12=20u"
"d0=3u"
"d25=3u"

"in0=inf1/2"
"in25=cnst1*inf1/2"

"DELTA1=p16+d16+8u"
"DELTA2=d4-larger(p2,p14)/2"
"DELTA3=d4-larger(p2,p14)/2-p16-d16-p3-14u"
"DELTA5=d4*2-larger(p2,p14)/2-4u"
"DELTA=p16+d16+p2+d0*2-4u"

"TAU=d8-p16-d16"
"TAU1=d4-p16-d16-larger(p2,p14)/2-4u"
"TAU2=d4+p16+d16-larger(p2,p14)/2-4u+2*d25"


1 ze
d11 pl12:f2
```

```
2 d11 do:f2
d1 BLKGRAD
10u pl0:f2

3 d12 pl1:f1 UNBLKGRAD
if "l0 %2 == 0"
     {
 (p1 ph10)     ;sin
     }
else
     {
 (p1 ph1)     ;cos
     }

d25
p16:gp5
d16 pl0:f2

(p1 ph1)      ;BIRD(r,X)
DELTA5
4u
(center (p2 ph2) (p14:sp3 ph1):f2 )
4u
DELTA5
(p1 ph1)

p16:gp5*-1
d16
d25

TAU1
4u
(center (p2 ph1) (p14:sp3 ph6):f2 )
4u
TAU2 pl2:f2

p28 ph1
4u
(p1 ph2) (p3 ph3):f2
d0

(p2 ph5)

d0
p16:gp1*EA
d16 pl0:f2
(p24:sp7 ph4:r):f2
4u
DELTA pl2:f2
(ralign (p1 ph1) (p3 ph4):f2 )
DELTA2 pl0:f2
(center (p2 ph1) (p14:sp3 ph1):f2 )
DELTA2
(p1 ph2)
```

```
# ifdef PRESAT
d12 pl9:f1
TAU cw:f1
4u do:f1
p16:gp1
d16 pl1:f1
# else
TAU
p16:gp3
d16
# endif

(p1 ph1)
DELTA1
(p2 ph2)
4u
p16:gp2
d16 pl12:f2
;4u BLKGRAD
4u
go=2 ph31 cpd2:f2
d11 do:f2 mc #0 to 2

F1I(iu0, 2)
F1EA(calgrad(EA), caldel(d0, +in0) & caldel(d25, +in25) & calph(ph3, +180) & calph(ph6,
+180) & calph(ph31, +180))
4u BLKGRAD
exit


ph1=0
ph2=1
ph3=0 2
ph4=0 0 0 0 2 2 2 2
ph5=0 0 2 2
ph6=0
ph10=1
ph14=0 0 0 0 0 0 0 0
    2 2 2 2 2 2 2 2
ph15=1
ph22=3
ph23=0
ph24=1
ph25=2
ph26=0
ph31=0 2 0 2 2 0 2 0


;pl0 : 0W
;pl1 : f1 channel - power level for pulse (default)
;pl2 : f2 channel - power level for pulse (default)
;pl3 : f3 channel - power level for pulse (default)
;pl12: f2 channel - power level for CPD/BB decoupling
;pl16: f3 channel - power level for CPD/BB decoupling
;sp3: f2 channel - shaped pulse 180 degree
```

```
;p1 : f1 channel -  90 degree high power pulse
;p2 : f1 channel - 180 degree high power pulse
;p3 : f2 channel -  90 degree high power pulse
;p4 : f2 channel - 180 degree high power pulse
;p14: f2 channel - 180 degree shaped pulse for inversion
;p16: homospoil/gradient pulse
;p22: f3 channel - 180 degree high power pulse
;p28: f1 channel - trim pulse in inept transfer      [1 msec]
;d0 : incremented delay (2D)                         [3 usec]
;d1 : relaxation delay; 1-5 * T1
;d4 : 1/(4J)XH
;d8 : mixing time
;d11: delay for disk I/O                             [30 msec]
;d12: delay for power switching                      [20 usec]
;d16: delay for homospoil/gradient recovery
;inf1: 1/SW(X) = 2 * DW(X)
;in0: 1/(2 * SW(X)) = DW(X)
;nd0: 2
;cnst1: scaling factor
;cnst2: 1JCH
;ns: 1 * n
;ds: >= 16
;td1: number of experiments
;FnMODE: echo-antiecho
;cpd2: decoupling according to sequence defined by cpdprg2
;pcpd2: f2 channel - 90 degree pulse for decoupling sequence
;cpd3: decoupling according to sequence defined by cpdprg3
;pcpd3: f3 channel - 90 degree pulse for decoupling sequence


;use gradient ratio:    gp 1 : gp 2 : gp 3
;                         40 : 10.05 :   50      for C-13

;for z-only gradients:
;gpz1: 40%
;gpz2: 10.05% for C-13, 8.1% for N-15
;gpz3: 11%
;gpz5:  7%

;use gradient files:
;gpnam1: SMSQ10.100
;gpnam2: SMSQ10.100
;gpnam3: SMSQ10.100
;gpnam5: SMSQ10.100

;preprocessor-flags-start
;LABEL_CN: for C-13 and N-15 labeled samples start experiment with
;option -DLABEL_CN (eda: ZGOPTNS)
;preprocessor-flags-end

;$Id: hsqcetgpnosp,v 1.7.6.1 2012/01/31 17:56:32 ber Exp $
```

(2,3) HSQC-ROESY pulse program

```
;hsqcetgprosp_Hmod
;Justinas Sakas  and  Nicholle G.A. Bell
;Faraday Discuss. 2019 https://doi.org/10.1039/C9FD00008A
;By using this pulse sequence, or any modification of it in any published material
;you agree to acknowledge the above-mentioned publication.
;Based on BRUKER's:
;hsqcetgprosp
;avance-version (12/01/11)
;2D H-1/X correlation via double inept transfer
;with cw spinlock for ROESY mixing
;phase sensitive using Echo/Antiecho-TPPI gradient selection
;with decoupling during acquisition
;using trim pulses in inept transfer
;using shaped pulses for inversion on f2 - channel
;A. Bax & D.G. Davis, J. Magn. Reson 63, 207-213 (1985)
;
;$CLASS=HighRes
;$DIM=2D
;$TYPE=
;$SUBTYPE=
;$COMMENT=
#include <Avance.incl>
#include <Grad.incl>
#include <Delay.incl>

"p2=p1*2"
"p4=p3*2"
"d4=1s/(cnst2*4)"
"d11=30m"
"d12=20u"

#   ifdef LABEL_CN
"p22=p21*2"
#   else
#   endif /*LABEL_CN*/

"d0=3u"
"d25=3u"

"in0=inf1/2"
"in25=cnst1*inf1/2"

"DELTA1=p16+d16+8u"
"DELTA2=d4-larger(p2,p14)/2"
"DELTA5=d4*2-larger(p2,p14)/2-4u"

#   ifdef LABEL_CN
"DELTA=p16+d16+larger(p2,p22)+d0*2-4u"
#   else
```

```
"DELTA=p16+d16+p2+d0*2-4u"
#   endif /*LABEL_CN*/
"TAU1=d4-p16-d16-larger(p2,p14)/2-4u"
"TAU2=d4+p16+d16-larger(p2,p14)/2-4u+2*d25"

1 ze

#   ifdef LABEL_CN
d11 pl12:f2 pl16:f3
2 d1 do:f2 do:f3 BLKGRAD
10u pl0:f2 pl3:f3
#   else
d11 pl12:f2
2 d1 do:f2 BLKGRAD
10u pl0:f2
#   endif /*LABEL_CN*/

3 d12 pl1:f1
if "l0 %2 == 0"
    {
(p1 ph2)     ;cos
    }
else
    {
(p1 ph1)     ;sin
    }
d25
p16:gp5
d16 pl0:f2

(p1 ph1)      ;BIRD(r,X)
DELTA5
4u
(center (p2 ph2) (p14:sp3 ph1):f2 )
4u
DELTA5
(p1 ph1)

p16:gp5*-1
d16
d25
TAU1
4u
(center (p2 ph1) (p14:sp3 ph6):f2 )
4u
TAU2 pl2:f2 UNBLKGRAD
p28 ph1
4u
(p1 ph2) (p3 ph3):f2
d0
```

```
#   ifdef LABEL_CN
  (center (p2 ph5) (p22 ph1):f3 )
#   else
  (p2 ph5)
#   endif /*LABEL_CN*/

d0
p16:gp1*EA
d16 pl0:f2
(p24:sp7 ph4:r):f2
4u
;(p4 ph4):f2
DELTA pl2:f2
(ralign (p1 ph1) (p3 ph4):f2 )
DELTA2 pl0:f2
(center (p2 ph1) (p14:sp3 ph1):f2 )
DELTA2 pl11:f1
(p15 ph1)
DELTA1 pl1:f1
(p2 ph2)
4u
p16:gp2

#   ifdef LABEL_CN
d16 pl12:f2 pl16:f3
4u BLKGRAD
go=2 ph31 cpd2:f2 cpd3:f3
d1 do:f2 do:f3 mc #0 to 2
#   else
d16 pl12:f2
; 4u BLKGRAD
go=2 ph31 cpd2:f2
d1 do:f2 mc #0 to 2
#   endif /*LABEL_CN*/

F1I(iu0, 2)
F1EA(calgrad(EA), caldel(d0, +in0) & caldel(d25, +in25) & calph(ph3, +180) & calph(ph6,
+180) & calph(ph31, +180))
4u BLKGRAD
exit


ph1=0
ph2=1
ph3=0 2
ph4=0 0 0 0 2 2 2 2
ph5=0 0 2 2
ph6=0
ph22=3
ph23=0
ph24=1
```

```
ph25=2
ph26=0
ph31=0 2 0 2 2 0 2 0


;pl0 : 0W
;pl1 : f1 channel - power level for pulse (default)
;pl2 : f2 channel - power level for pulse (default)
;pl3 : f3 channel - power level for pulse (default)
;pl11: f1 channel - power level for ROESY-spinlock
;pl12: f2 channel - power level for CPD/BB decoupling
;pl16: f3 channel - power level for CPD/BB decoupling
;sp3: f2 channel - shaped pulse 180 degree
;p1 : f1 channel -  90 degree high power pulse
;p2 : f1 channel - 180 degree high power pulse
;p3 : f2 channel -  90 degree high power pulse
;p4 : f2 channel - 180 degree high power pulse
;p14: f2 channel - 180 degree shaped pulse for inversion
;p15: f1 channel - pulse for ROESY spinlock
;p16: homospoil/gradient pulse
;p22: f3 channel - 180 degree high power pulse
;p28: f1 channel - trim pulse in inept transfer      [1 msec]
;d0 : incremented delay (2D)                         [3 usec]
;d1 : relaxation delay; 1-5 * T1
;d4 : 1/(4J)XH
;d11: delay for disk I/O                             [30 msec]
;d12: delay for power switching                      [20 usec]
;d16: delay for homospoil/gradient recovery
;cnst1: = scaling factor
;cnst2: = J(XH)
;inf1: 1/SW(X) = 2 * DW(X)
;in0: 1/(2 * SW(X)) = DW(X)
;nd0: 2
;ns: 1 * n
;ds: >= 16
;td1: number of experiments
;FnMODE: echo-antiecho
;cpd2: decoupling according to sequence defined by cpdprg2
;pcpd2: f2 channel - 90 degree pulse for decoupling sequence
;cpd3: decoupling according to sequence defined by cpdprg3
;pcpd3: f3 channel - 90 degree pulse for decoupling sequence

;use gradient ratio:     gp 1 : gp 2
;                          20 : 5.03      for C-13
;                          20 :  2.05     for N-15

;for z-only gradients:
;gpz1: 20%
;gpz2: 5.03% for C-13, 2.05% for N-15
;gpz5: 7.0%
```

```
;use gradient files:
;gpnam1: SMSQ10.100
;gpnam2: SMSQ10.100
;gpnam5: SMSQ10.100

;preprocessor-flags-start
;LABEL_CN: for C-13 and N-15 labelled samples start experiment with
;option -DLABEL_CN (eda: ZGOPTNS)
;preprocessor-flags-end

;$Id: hsqcetgprosp,v 1.7 2012/01/31 17:49:26 ber Exp $
```

(3,2) HSQC-HSQMBC pulse program

```
;HSQC_HSQMBC.2
;Justinas Sakas  and  Nicholle G.A. Bell
;Faraday Discuss. 2019 https://doi.org/10.1039/C9FD00008A

;This is a modification of a 3D HSQC-HSQMBC
;Uhrin, D. J. Magn. Reson. (2002):159:2:145-50

;$CLASS=HighRes
;$DIM=3D
;$TYPE=
;$SUBTYPE=
;$COMMENT=

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

"p2=p1*2"
"p4=p3*2"
"d4=1s/(cnst2*2)"
"d5=1s/(cnst2*4)"
"d11=30m"
"d12=20u"
"d15=1s/(cnst3*2)" ;T period
"d14=1/(2*cnst4*cnst2)"

"d20=d15/2-p16-d16"
"d21=d14-p19-d16"

"in0=inf1/2"
"in20=in0"
"in21=in0"

"d25=2u"          ; initial H evolution
"in25=cnst1*in0"

"acqt0=-p1*2/3.1416"

"DELTA=p14/2"
"DELTA3=d15/2-d14-p16-2*d16-p19-p2"

"TAU1=d4-p14/2"
"TAU2=d5-2*p19-2*d16-p14/2"
"TAU3=d5-p14/2-6u"

1 ze
2 d11
```

```
50u BLKGRAD
d1
10u pl2:f2
3
50u UNBLKGRAD
(p3 ph1):f2
p16:gp0
d16

; this is the real start
if "l0 %2 == 0"
    {
(p1 ph2):f1     ;cos
    }
else
    {
(p1 ph1):f1     ;sin
    }

d25
p19:gp1
d16 pl0:f2
(p1 ph1)                                  ;BIRD(r,X)
TAU1
(center (p2 ph2) (p14:sp3 ph1):f2 )
TAU1
(p1 ph1)
p19:gp1*-1
d16
d25

TAU2
p19:gp2
d16
4u
(center (p2 ph1) (p14:sp3 ph1):f2 )
4u
p19:gp2
d16 pl2:f2
TAU3
(p1 ph2)
p16:gp3
d16
(p3 ph3):f2  ; on 13C
d20
p16:gp4
d16

(p1 ph1) ;BIRD(r,X)
d4
(center (p2 ph2) (p4 ph3):f2)
```

```
d4
(p1 ph1)

p16:gp4
d16
DELTA3
p19:gp5
d16

(p2 ph1)
p19:gp5*-1
d16
d21

(p3 ph4):f2
p16:gp6
d16

(p1 ph1)

go=2 ph31
d11 mc #0 to 2

F1I(iu0, 2)
F1PH(calph(ph3, -90) & calph(ph31, +180), caldel(d21, +in21) & caldel(d20, -in20) &
caldel(d25, +in25))
50u BLKGRAD
exit

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

;pl0 : 0W
;pl1 : f1 channel - power level for pulse (default)
;pl2 : f2 channel - power level for pulse (default)
;pl3 : f3 channel - power level for pulse (default)
;sp3: f2 channel - shaped pulse 180 degree
;p1 : f1 channel -  90 degree high power pulse
;p2 : f1 channel - 180 degree high power pulse
;p3 : f2 channel -  90 degree high power pulse
;p4 : f2 channel - 180 degree high power pulse
;p14: f2 channel - 180 degree shaped pulse for inversion    [500us]
;p16: homospoil/gradient pulse
;d0 : incremented delay (2D)                  [3 usec]
;d25: incremented delay while on 1H                        [2 usec]
;d20: decremented delay while on 13C
;d21: incremented delay while on 13C
;d1 : relaxation delay; 1-5 * T1
```

```
;d4 : 0.5/(1J)XH
;d5 : 0.25/(1J)XH
;d11: delay for disk I/O                    [30 msec]
;d16: delay for homospoil/gradient recovery
;cnst1: scaling factor between incremental periods
;cnst2:1J(XH)
;cnst3:nJ(CH) for optimum transfer
;cnst4: 2 for CH, 4 for CH2/CH3
;inf1: 1/SW(X) = 2 * DW(X)
;in0: 1/(2 * SW(X)) = DW(X)
;nd0: 2
;ns: 2 * n
;ds: >= 16
;td1: number of experiments
;FnMODE: States-TPPI

;gpz0: 10.0
;gpz1: 30.0
;gpz2: 11.6
;gpz3: 26.0
;gpz4: 16.0
;gpz5: 50.0
;gpz6: 22.0

;use gradient files:
;gpnam0: SMSQ10.100
;gpnam1: SMSQ10.100
;gpnam2: SMSQ10.100
;gpnam3: SMSQ10.100
;gpnam4: SMSQ10.100
;gpnam5: SMSQ10.100
;gpnam6: SMSQ10.100

;$Id: hsqcetgpnosp,v 1.7.6.1 2012/01/31 17:56:32 ber Exp $
```