Electronic Supplementary Information (ESI)

Synergy and sensitivity-balance in concatenating experiments in NO Relaxation Delay NMR (NORD)

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NMR acronyms

BANGO: small angle or β-TANGO

BIRD: BIlinear Rotation Decoupling

TIG-BIRD: Triselective Independent Gyrations BIRD

BIG-BIRD: Biselective Independent Gyrations BIRD

HMBC: Heteronuclear Multiple Bond Correlation

HSQC: Heteronuclear Single Quantum Coherence

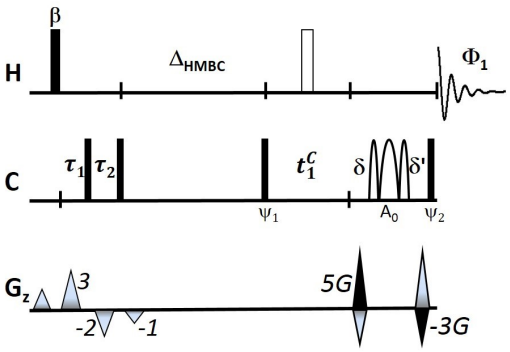
INEPT: Insensitive Nuclei Enhancement by Polarization Transfer

NORD: NO Relaxation Delay

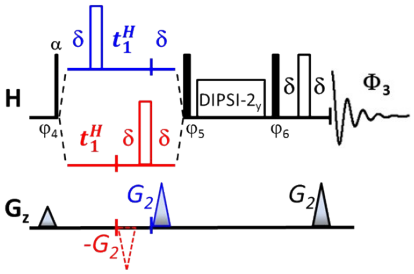
TOCSY: Total Correlation Spectroscopy

TANGO: Testing for Adjacent Nuclei with a Gyration Operator

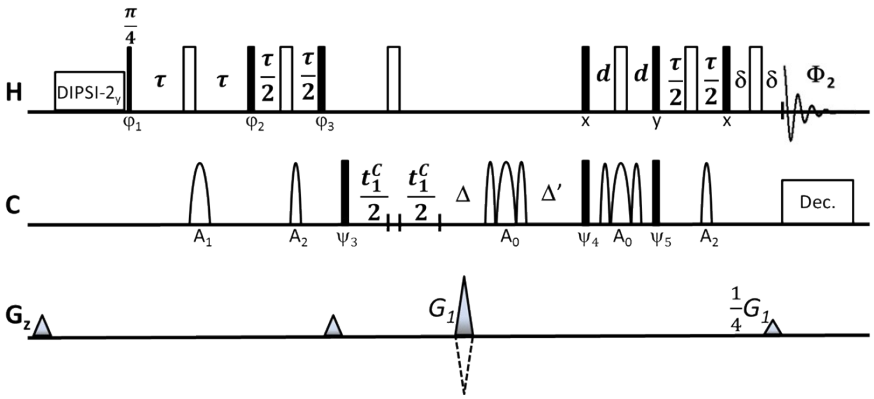
NORD {HMBC}



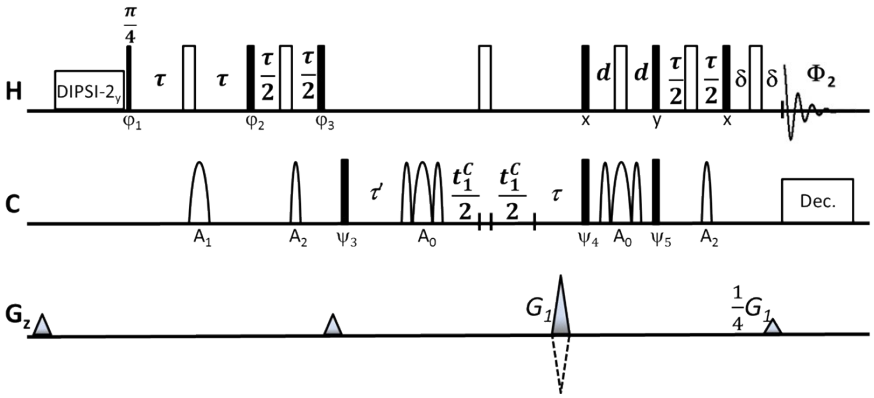
NORD {TOCSY}



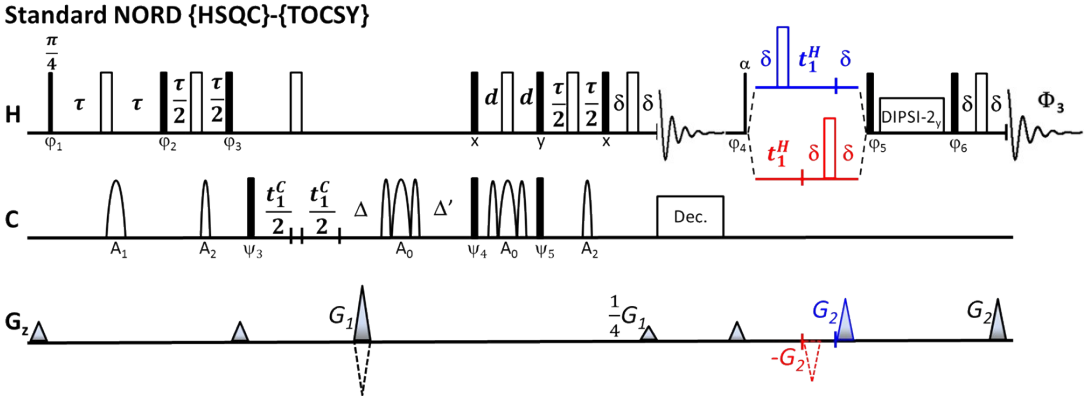
Standard NORD {HSQC}



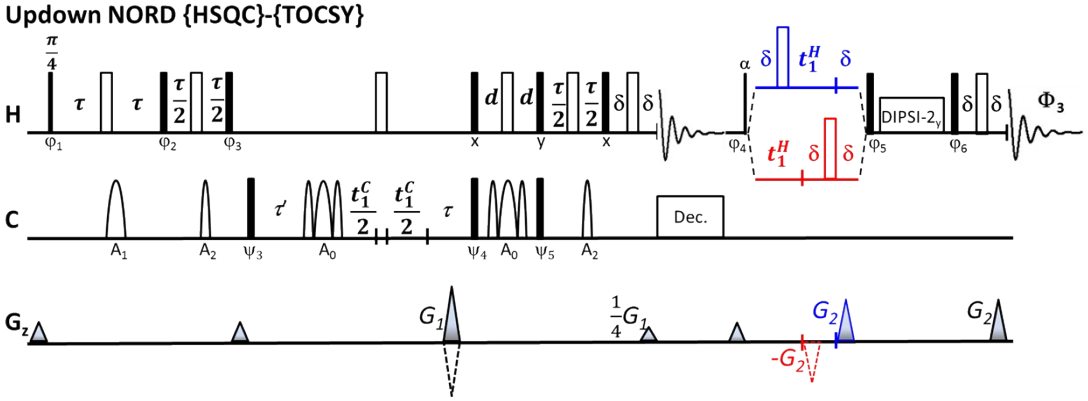
Updown NORD {HSQC}



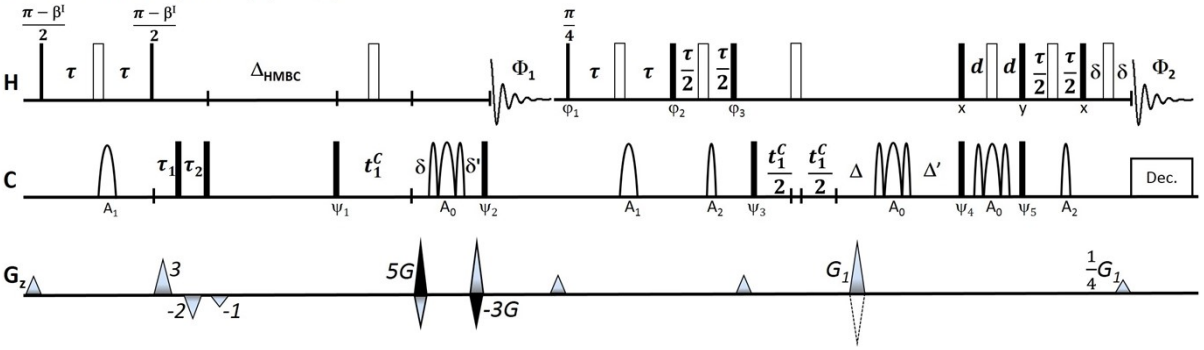
Standard NORD {HSQC}-{TOCSY}



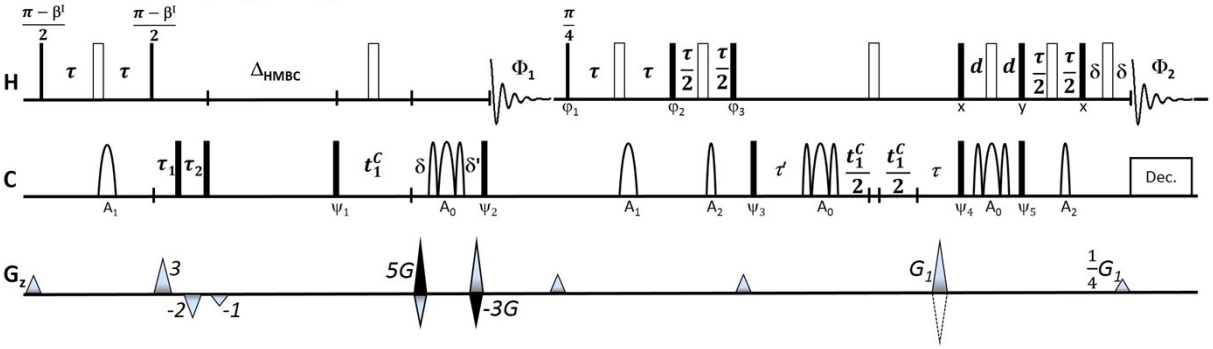
Updown NORD {HSQC}-{TOCSY}



Standard NORD {HMBC}-{HSQC}



Updown NORD {HMBC}-{HSQC}



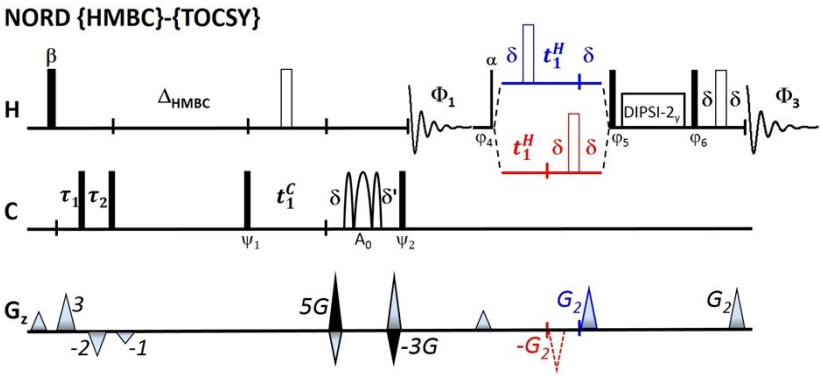


Figure S1 One- and two-module truncated versions of the NORD {HMBC}-{HSQC}-{TOCSY} experiment outlined in Fig. 1. See the caption to that figure for details.

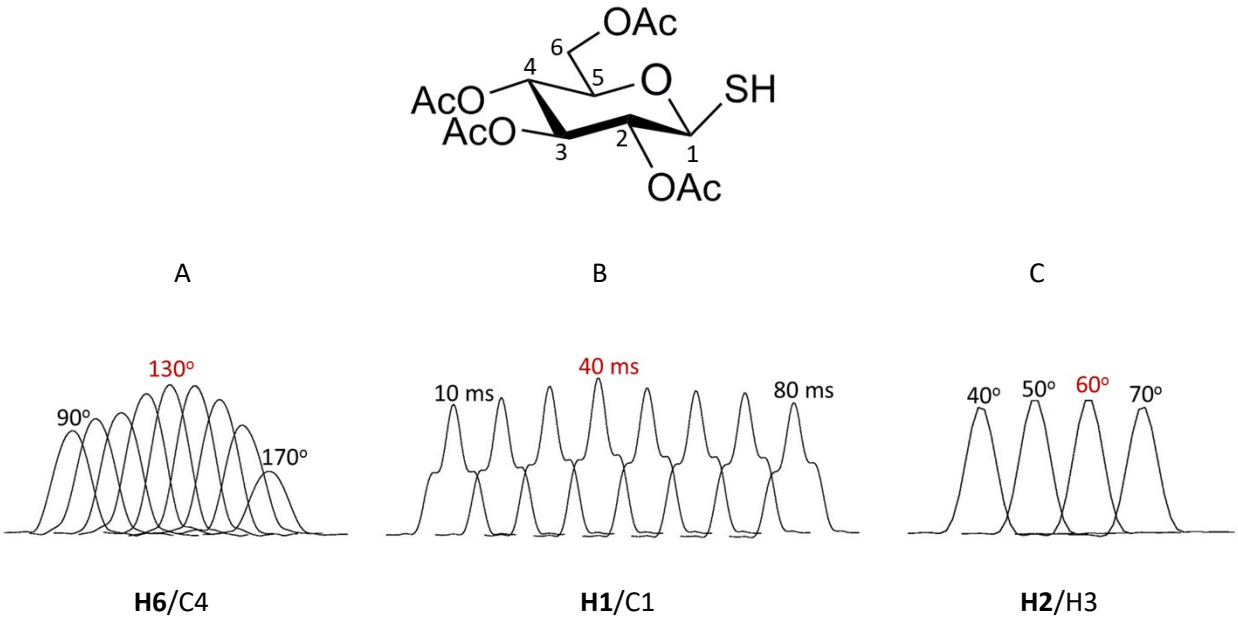


Figure S2. Optimization of single-module NORD experiments on a monosaccharide (34 mg/550µl $CDCl_3$). Firstly, ^1H excitation angle (β) dependence test measurements in the NORD {HMBC} (A) were carried out. The number of scans was 2 in the 9 minutes long experiments and the excitation flip angle was incremented in 10° steps from 90° to 170°. Prepolarization time (DIPSI-2) dependence in the NORD {HSQC} experiment (B) was also measured. The number of scans was 2 in the 7 minutes long experiments and the DIPSI-2 time was incremented in 10 ms steps between 10 ms and 80 ms. ^1H excitation angle (α) dependence in the NORD {TOCSY} (C) was also investigated. The number of scans was 2 in the 10.5 minutes long experiments and the excitation angle was incremented in 10° steps from 40° to 70°. The direct acqusition time ($t2_{max}$) was 143.4 ms.

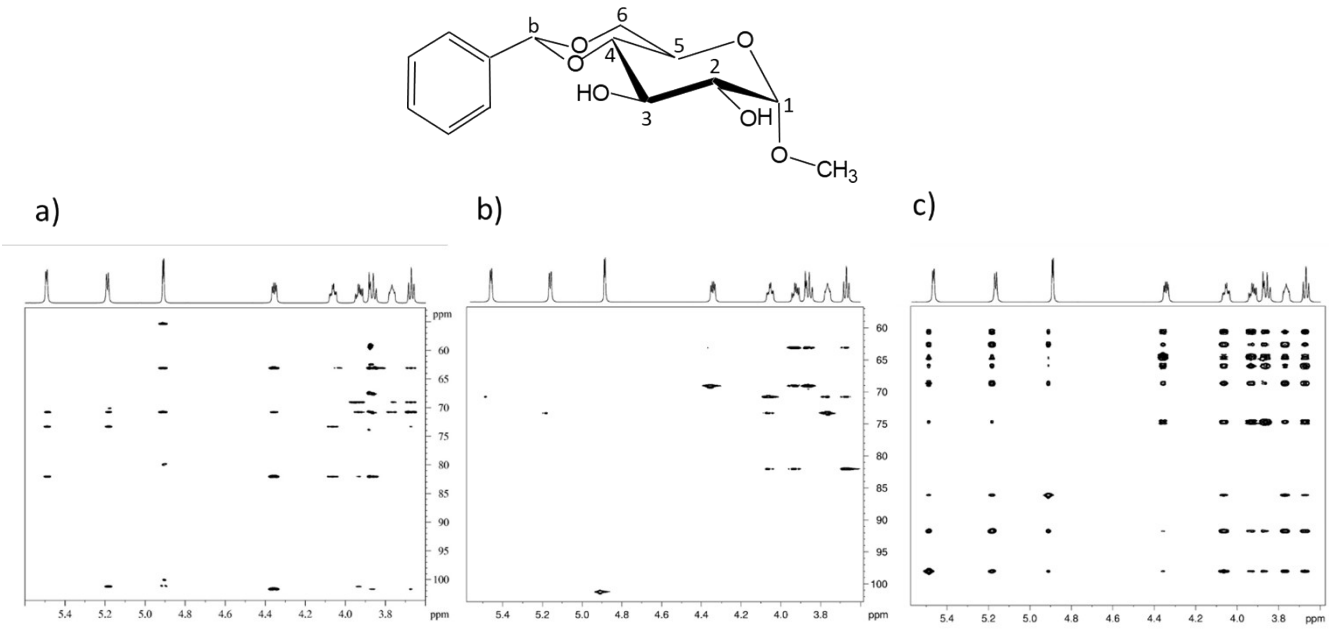


Figure S3. Excerpts from the NORD {HMBC}-{HSQC}-{TOCSY} experiment on a monosaccharide derivative (**1**, 60 mg dissolved in 550µl DMSO-d_6/C_6D_6). The measurement time was 26.5 minutes, two scans were recorded per increment, with 1024 points acquired in t_1 and t_2 in each module. The spectral width was 5.1 ppm in the ^{1}H and 100 ppm in the ^{13}C dimension. The direct acqusition time ($t2_{max}$) was 143.4 ms. The TOCSY mixing time was 80 ms and the excitation angle α was set to 30°. Adiabatic ^{13}C inversion pulses were applied for better sensitivity: CAWURST-20 (121 ppm, 1.94 ms; H2L) and CAWURST-20 (121 ppm, 0.97 ms; H2L). Spectral data were extracted and presented in three correlation maps (a-c): HMBC, HSQC and TOCSY, respectively.

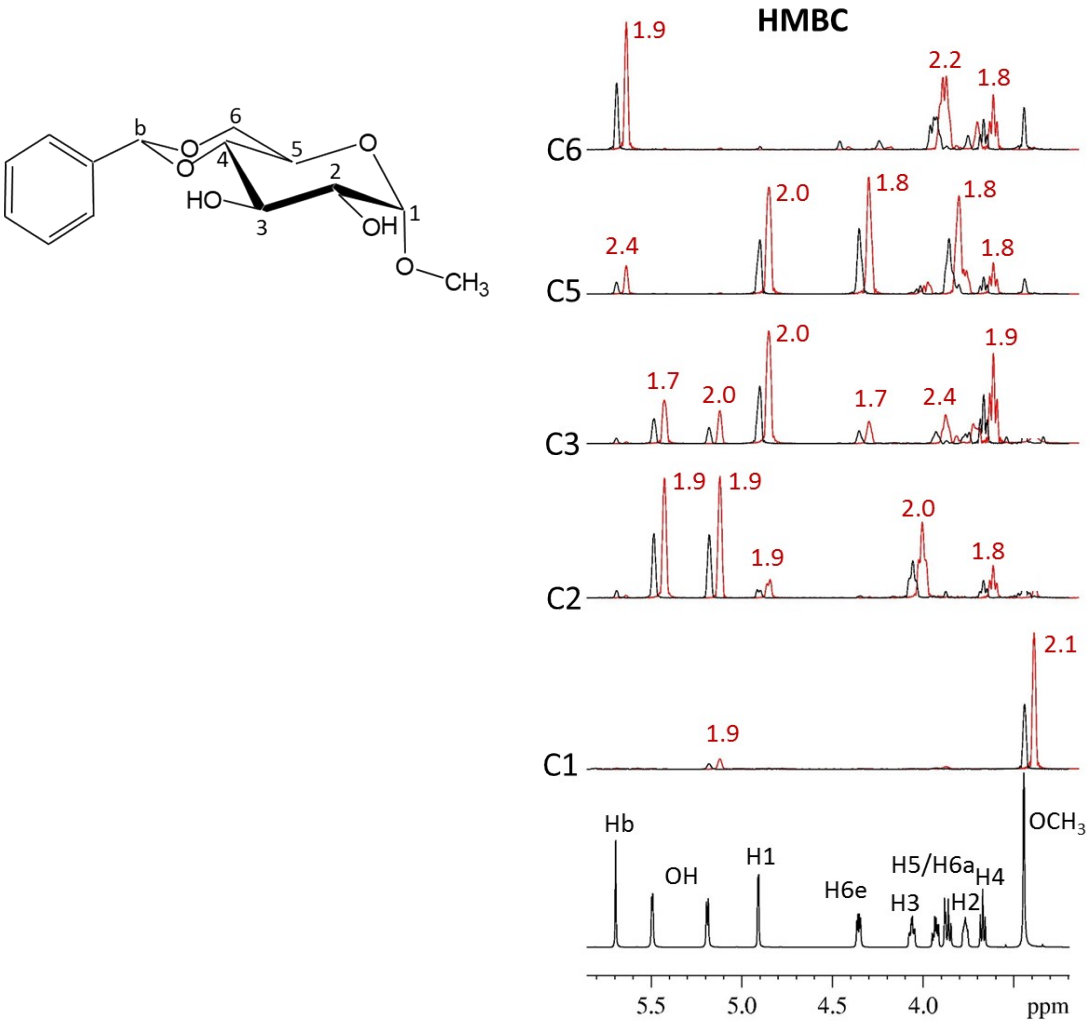


Figure S4. Sensitivity comparison of individual NORD {HMBC} (black) versus HMBC from NORD {HMBC}-{HSQC}-{TOCSY} three-module experiments (red). The measurements were carried out on a monosaccharide sample (**1**), 60 mg dissolved in 550 µL DMSO-d_6/C_6D_6. After 26.5 minutes runtime, 1024 increments were collected (t_1) with 1024 data points (t_2). The number of scans was 2 and the spectral width was set to 5.1 ppm (^{1}H) and 100 ppm (^{13}C). The direct acqusition time ($t2_{max}$) was 143.4 ms. To reach the optimal sensitivity balance, magnetization was excited with $\beta^I = \frac{\pi}{2}$ in BANGO of the HMBC module and α = 30° in the TOCSY module of NORD {HMBC}-{HSQC}-{TOCSY}. On the other hand, a 130° ^{1}H excitation pulse was used in the corresponding standalone NORD {HMBC}. A range of 1.7-2.4 S/N improvement was observed in the concatenated HMBC shown with five different sections relative to the corresponding individual optimized NORD {HMBC} experiment with the same number of scans. Red spectra are horizontally shifted for better visualization and the measured S/N values are given above the F_2 sections.

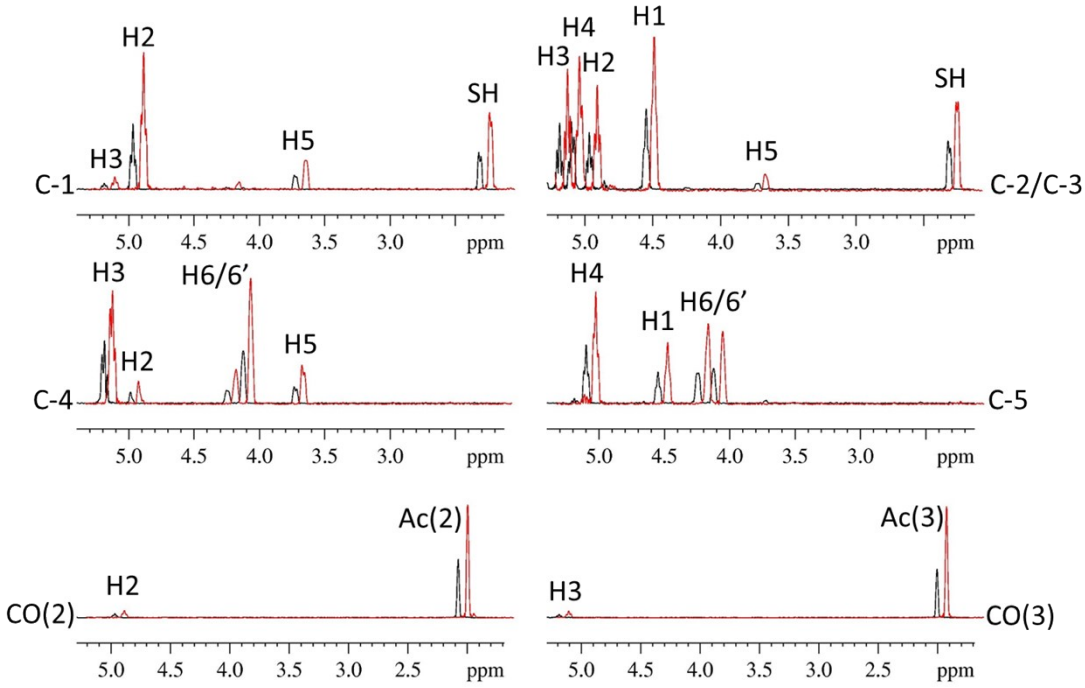


Figure S5. Sensitivity comparison of individual NORD {HMBC} (black) versus HMBC from NORD {HMBC}-{HSQC}-{TOCSY} experiment (red). Measurements were carried out on a monosaccharide sample (structure shown on the top), 30 mg dissolved in 550 µL DMSO-d_6 solution. In 26.5 minutes runtime, 1024 t_1 increments were collected with 1024 t_2 data points. The number of scans was 2 and the spectral widths were set to 5.1 ppm (^{1}H) and 100 ppm (^{13}C). The direct acqusition time ($t2_{max}$) was 143.4 ms. An average of 2.0 S/N improvement was observed in the HMBC spectrum from NORD {HMBC}-{HSQC}-{TOCSY} relative to the corresponding individual optimized NORD {HMBC} experiment with the same number of scans. Red spectra are horizontally shifted for better visualization.

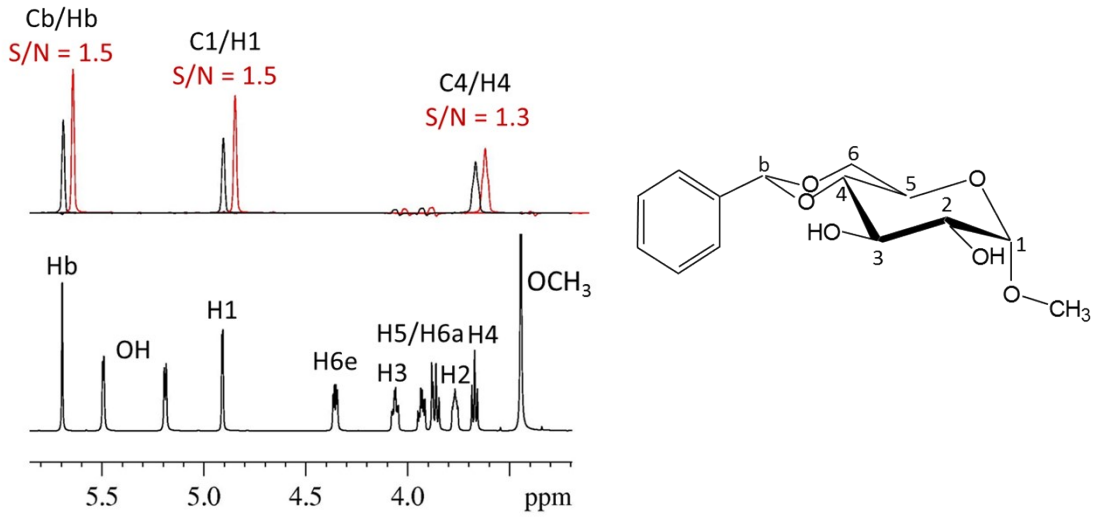


Figure S6. Sensitivity comparison of NORD {HSQC} (black) compared to the HSQC spectrum from NORD {HSQC}-{TOCSY} (red). S/N values measured in NORD {HSQC}-{TOCSY} are given above the F_2 sections relative to the corresponding individual optimized NORD {HSQC} experiment with the same number of scans. Red signals are shifted horizontally for better visualization. The demonstrated correlations were selected by their coupling patterns: the singlet Hb, H1 with a small homonuclear coupling (3.6 Hz) and H4 with a large coupling (9.4 Hz). The two-module experiment was recorded in 17 minutes on compound **1** (60 mg/ 550 µl DMSO-d_6/C_6D_6). 2 scans were recorded per increment, with 1024 points acquired in t_1 and t_2 in both HSQC and TOCSY modules. The spectral width was 5.1 ppm in the ^{1}H and 100 ppm in the ^{13}C dimension. The direct acqusition time ($t2_{max}$) was 143.4 ms. The TOCSY mixing time was 80 ms and the α angle was 30°. The stand-alone NORD {HSQC} was acquired in 8 minutes with identical experimental parameters.

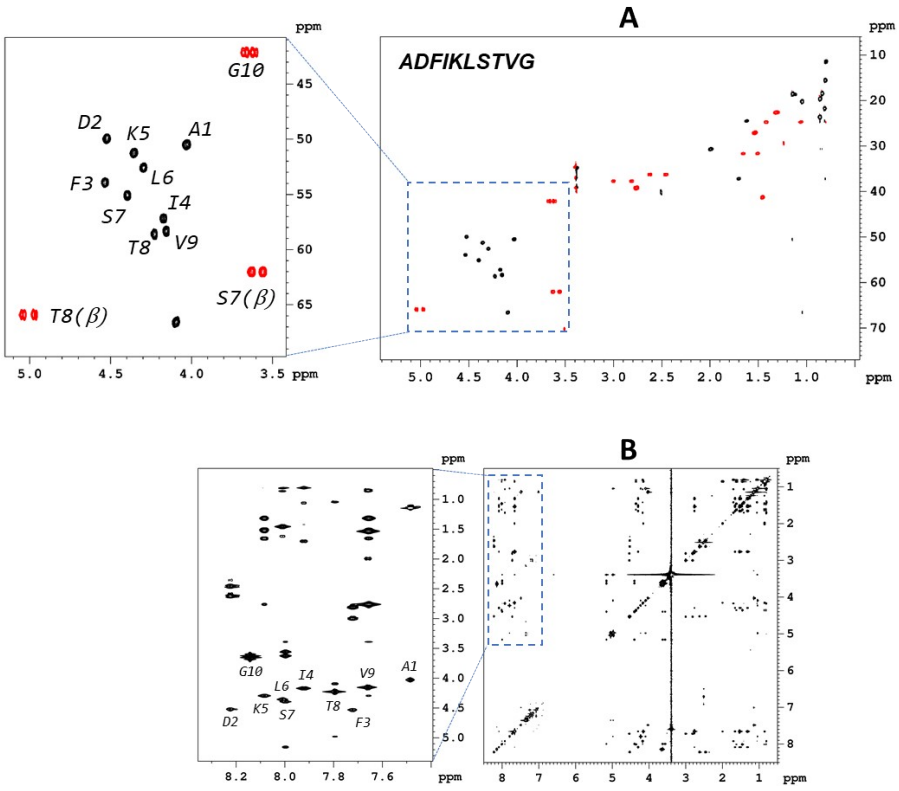


Figure S7. Spin-system assignment of a decapeptide with ten different residues (ADFIKLSTVG) using the edited NORD {HSQC}-{TOCSY} experiment. The sample contained 10.16 mg of peptide in 550 µl DMSO-d_6 solution. The plots shown are the overlay of CH/CH_3 (black) and CH_2 (red) ^{13}C-^{1}H HSQC subspectra in (A) with expansion of the Cα-Hα region and ^{1}H-^{1}H TOCSY (B) with expansion of the H-NH region to the left. The concatenated experiments were run in 24 minutes and 2 scans were recorded per increment (512 in t_1) in a time-shared two-step editing cycle. The number of t_2 data points was 2048 in both modules. The direct acqusition time ($t2_{max}$) was 174.1 ms. The TOCSY mixing time was 80 ms.

Pulse sequence code of NORD {HMBC}-{HSQC}-{TOCSY} experiment for Bruker spectrometers (*Avance III and NEO systems*)

```
;nord_hmbc_hsqc_tocsy
;avance-version – tested on NEO system, TopSpin 4.0.2

;This pulse sequence is part of István Timári, Tamás Milán Nagy, Katalin E. Kövér,
;Ole W. Sørensen, 'Synergy and sensitivity-balance in concatenating experiments
;in NO Relaxation Delay NMR (NORD)'

;June 17, 2021 tested by IT, TMN, KEK

;The pulse sequence has been coded for test purposes only and may contain errors.
;The functionality of the pulse sequence itself may differ depending on
;the hardware as well as the software used to execute it. Functionality
;on differing systems cannot be granted.
;Any use of this pulse sequence on a spectrometer is at your own risk.
;
;By using this pulse sequence, or any modification of it in any published material
;you agree to acknowledge the above-mentioned publication.

;NORD concatenation of THREE experiments: HMBC, HSQC and TOCSY

;BANGO, TIG-BIRD and INEPT elements include J-compensated adiabatic X-inversion pulse

;set FNmode – echo-antiecho and NBL=3

;PROCESSING:
;FIRST separate the three datasets using AU splitx
;the resulting datasets can be processed with xfb (set echo-antiecho, pk)
;then for hmbc use xf2m for magnitude calculation in F2
;the F1 chemical shift scale in TOCSY is corrected with au-program fixF1 prior to xfb.



;$CLASS=HighRes
;$DIM=2D
;$TYPE=
;$SUBTYPE=
;$COMMENT=

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


;use: wvm –a in TopSpin command line to setup adiabatic pulse parameters

"cnst2=113"         ;for J-compensated adiabatic pulse set CNST2 = 113

"cnst31=(1-sfo2/sfo1)/(1+sfo2/sfo1)"

define list<gradient> EA3 = { 1.000 -cnst31}    ;HMBC phase sensitive, echo-anti echo
define list<gradient> EA4 = { -cnst31 1.000}

"p2=p1*2"
"p4=p3*2"

"d4=1s/(cnst2*4)"    ;INEPT delay
"d6=1s/(cnst2*2)"    ;BANGO and TIG-BIRD delays

"d11=50u"

"d20=10u"      ;z-filter delays in TOCSY
"d21=10u"

"p11=p1/2"     ;1H-45 for TIG-BIRD with v = 0

"p13 = (p1/90)*cnst11"   ;cnst11 = 45 corresponds to beta = 90 in BANGO
```

```
"d0=3u"
"in0=inf1/2"

"d10=3u"

"cnst10=2*dw/inf1"    ;cnst10 is a scaling factor for SW(13C)/SW(1H) for TOCSY

"in10=inf1*cnst10"


"DELTA=p16+d16+p2+d0*2-4u"


"DELTA2=d4-4u"       ;INEPT
"d22 = DELTA2-p29"

"d25=p16+d16+4u-p1*0.78+de"

"d24 = 1.86m"

"DELTA3=d24-cnst17*p24/2-4u"   ;d24 = 1.86 ms, CNST17 = 1

"DELTA4=d6-4u"        ;BANGO, TIG-BIRD,
"d23 = DELTA4-p31"

"DELTA5=p16+d16+d10"   ;1H-1H TOCSY
"DELTA6=p16+d16+8u"


"d18=1s/(cnst14*2)"     ;cnst14 = 6 for HMBC long-range J(CH)

"DELTA21=1s/(2 * cnst6)-p16-d16"     ;HMBC
"DELTA22=1s/(2 * cnst7)-p16-d16"     ;CNST6 = 125 Hz, CNST7 = 165 Hz
"DELTA23=d18-p16-d16-4u"
"DELTA24=p2+d0*2"


"FACTOR3=(d7/(p6*115.112))/2"        ;d7 = 70-80 ms for 1H-1H TOCSY transfer
"l3=FACTOR3*2"

"l4 = td1/6"        ;NBL = 3, data from three experiments are stored in one file

"acqt0=0"

"p12 = (p1/90)*cnst12"   ;CNST12 = small flip angle excitation pulse of 1H-1H TOCSY

"l9=1"

baseopt_echo

1 ze
  d11 pl12:f2                   ;d11 = 50u
2 d11 do:f2 st0                 ;NBL = 3
  d11 pl2:f2 pl1:f1

 (p3 ph1):f2
  50u UNBLKGRAD
  p16:gp12*2.77         ;purging gradient, gpz12 = 17.13%
  d16

3 (p13 ph1):f1         ;HMBC starts with BANGO resulting in 90 degree excitation
                       ;of 1H-12C proton magn., CNST11 sets BANGO angle
  DELTA4 pl0:f2
  4u

  (p2 ph1)
  (p31:sp18 ph1):f2   ;J-compensated adiabatic pulse during BANGO, H2L sweep, 1.94 ms

  4u
  d23 pl2:f2
  (p13 ph1):f1
```

```
DELTA21              ;second order low-pass J-filter to suppress one-bond correlations
p16:gp23             ;gpz23 = 15
d16 pl2:f2
(p3 ph27):f2
DELTA22
p16:gp14             ;gpz14 = -10
d16
(p3 ph27):f2
4u
p16:gp15             ;gpz15 = -5
d16
DELTA23
(p3 ph28):f2
d0
(p2 ph26):f1
d0
p16:gp1*EA3           ;gpz1 = 80
d16
(p24:sp7 ph29):f2     ;p24 = 2msec for Crp80comp.4
DELTA24
p16:gp1*EA4
d16 pl2:f2
(p3 ph29):f2
4u BLKGRAD

goscnp ph15


12 d11 st
13 d11 do:f2

   50u UNBLKGRAD

   p16:gp13           ;purging gradient between two experiments
                      ;gpz13 = 17.13-71%
   d16 pl1:f1
                      ;HSQC starts with TIG-BIRD

14 (p11 ph11):f1      ;TIG-BIRD with v = 0, p11=45 degree 1H; ph11=phase 45
    DELTA4 pl0:f2
    4u

    (p2 ph1)
    (p31:sp18 ph6):f2    ;J-compensated adiabatic pulse, H2L sweep, 1.94 ms
    4u

    d23
    (p1 ph12):f1         ;ph12 = phase 225 degree

    DELTA2               ;INEPT delay
    4u

    (p2 ph1)
    (p29:sp20 ph6):f2    ;J-compensated adiabatic pulse, H2L sweep, 0.97ms
    4u

    d22 pl2:f2


    (p1 ph22)
    p16:gp11             ;purging gradient gpz11 = 31-51 during zz-magn.
    d16

    (p3 ph3):f2
    d0

    (p2 ph7)

    d0
    p16:gp1*EA
    d16 pl0:f2
```

```
  (p24:sp7 ph8:r):f2    ;composite CHIRP, 2 ms
  4u
  DELTA pl2:f2

  (center (p1 ph21) (p3 ph4):f2 )
  4u
  DELTA3 pl0:f2
  (center (p2 ph1) (p24:sp7 ph9:r):f2 )   ;composite CHIRP, 2 ms
  4u
  DELTA3 pl2:f2
  (center (p1 ph2) (p3 ph5):f2 )
  4u
  DELTA2 pl0:f2


  (p2 ph1)
  (p29:sp20 ph1):f2      ;J-compensated adiabatic pulse, H2L sweep, 0.97ms
  4u
  d22 pl1:f1

  (p1 ph1)

  d25

  (p2 ph1)

  4u pl12:f2
  p16:gp2              ;gpz2 = 20.1
  d16 BLKGRAD

  goscnp ph31 cpd2:f2

22 d11 st
23 d11 do:f2


  50u UNBLKGRAD
  p16:gp0           ;purging gradient between experiments
                    ;gpz0 = 17.13-71%

  d16 pl1:f1

  p12 ph16    ;1H-1H TOCSY with DIPSI-2 starts using small-flip angle excitation

  if "l9 %2 == 1"     ;TOCSY echo-antiecho selection

  {
  DELTA5     ;p12=(p1/90)*cnst12", CNST12=small flip angle excitation pulse
  p2 ph17
  d10              ;d10 incremented by in10 to get 1H-dimension for TOCSY in F1
  p16:gp3          ;gpz3 = 30
  d16

  }
  else
  {
  d10
  p16:gp3*EA
  d16
  p2 ph17
  DELTA5
  }

  p1 ph18
  d20 pl10:f1

                    ;begin DIPSI2
24 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 24 times l3
                                    ;end DIPSI2

d21 pl1:f1
p1 ph19
DELTA6

p2 ph17

4u
p16:gp4         ;;gpz4 = 30
d16
4u BLKGRAD
go=2 ph30

                                  ;PROCESSING
d11 do:f2 wr #0 if #0 zd          ;use AU: splitx to separate datasets

d11*0.15 igrad EA
d11*0.15 igrad EA3   ;HMBC
d11*0.15 igrad EA4   ;HMBC
d11*0.15 ip5*2

d11*0.15 iu9

lo to 2 times 2

d11*0.15 id0
d11*0.15 id10

d11*0.15 ip3*2
d11*0.15 ip6*2
d11*0.15 ip16*2
d11*0.15 ip28*2
d11*0.15 ip15*2
d11*0.15 ip30*2
d11*0.15 ip31*2
```

```
 d11*0.15 ru9

lo to 2 times l4    ; l4 = td1/6   NBL = 3 !!!


exit


ph1=0
ph2=1

ph21=0

ph3=0 2 2 0

ph4=2 2 0 0 0 0 2 2
ph5=1 1 3 3 3 3 1 1

ph6=0
ph7=0
ph8=0
ph9=0

ph22=1 1 1 1 3 3 3 3

ph23=3   ;DIPSI-2
ph25=1

ph11=(8) 1 1 1 1 7 7 7 7   ;TIG-BIRD phases - ph11 and ph12
ph12=(8) 5 5 5 5 3 3 3 3

ph16=0 2           ;1H-1H TOCSY with DIPSI-2
ph17=0
ph18=0 0 2 2
ph19=2 2 0 0

ph26=0   ;HMBC
ph27=0
ph28=0 2 2 0
ph29=0 0 2 2

ph15=0 2
ph30=0 2
ph31=0 2


;pl0 : 0W
;pl1 : f1 channel - power level for pulse (default)
;pl2 : f2 channel - power level for pulse (default)
;pl12: f2 channel - power level for CPD/BB decoupling
;sp7: 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
;p24: f2 channel - 180 degree shaped pulse for refocussing
; = 2msec for Crp80comp.4
;p16: homospoil/gradient pulse
;d0 : incremented delay (2D)                          [3 usec]
;d10 : incremented delay (2D)                         [3 usec]
;d16: delay for homospoil/gradient recovery

;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: echo-antiecho
;cpd2: decoupling according to sequence defined by cpdprg2
;pcpd2: f2 channel - 90 degree pulse for decoupling sequence
```

```
; ~~~~~~~~~~~~~~ WaveMaker Shapes ~~~~~~~~~~~~~~~~~~~~~~~~~~
;USE: wvm -a  command in TopSpin command line to generate CA-WURST adiabatic shape
pulses

;sp18:wvm:ad180Ccawurst: cawurst-20(121 ppm, 1.92 ms; H2L)

;sp20:wvm:ad180Ccawurstlh2: cawurst-20(121 ppm, 0.97 ms; L2H)



;for z-only gradients:

;use gradient files:
;gpnam: SMSQ10.100
```

Pulse sequence code of NORD {HSQC}-{TOCSY} experiment for Bruker spectrometers (*Avance III and NEO systems*)

```
;nord_hsqc_tocsy
;avance-version – tested on NEO system, TopSpin 4.0.2

;This pulse sequence is part of István Timári, Tamás Milán Nagy, Katalin E. Kövér,
;Ole W. Sørensen, 'Synergy and sensitivity-balance in concatenating experiments
;in NO Relaxation Delay NMR (NORD)'

;June 17, 2021 tested by IT, TMN, KEK

;The pulse sequence has been coded for test purposes only and may contain errors.
;The functionality of the pulse sequence itself may differ depending on
;the hardware as well as the software used to execute it. Functionality
;on differing systems cannot be granted.
;Any use of this pulse sequence on a spectrometer is at your own risk.
;
;By using this pulse sequence, or any modification of it in any published material
;you agree to acknowledge the above-mentioned publication.

;NORD concatenation of TWO experiments: HSQC and TOCSY

;TIG-BIRD and INEPT elements include J-compensated adiabatic X-inversion pulse

;set FNmode – echo-antiecho and NBL=2

;PROCESSING:
;FIRST separate the two datasets using AU splitx
;the resulting datasets can be processed with xfb (set echo-antiecho, pk)
;the F1 chemical shift scale in TOCSY is corrected with au-program fixF1 prior to xfb.


;$CLASS=HighRes
;$DIM=2D
;$TYPE=
;$SUBTYPE=
;$COMMENT=

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


;use: wvm –a in TopSpin command line to setup adiabatic pulse parameters

"cnst2=113"         ;for J-compensated adiabatic pulse set CNST2 = 113


"p2=p1*2"
"p4=p3*2"
```

```
"d4=1s/(cnst2*4)"    ;INEPT delay
"d6=1s/(cnst2*2)"    ;TIG-BIRD delay, CNST2 = 113 Hz with adiabatic 13C-pulse

"d11=50u"

"d20=10u"   ;z-filter delays in TOCSY
"d21=10u"

"p11=p1/2"  ;1H-45 for TIG-BIRD with v = 0

"d0=3u"

"in0=inf1/2"

"d10=3u"

"cnst10=2*dw/inf1"    ;cnst10 is a scaling factor for SW(13C)/SW(1H) for TOCSY

"in10=inf1*cnst10"


"DELTA=p16+d16+p2+d0*2-4u"


"DELTA2=d4-4u"       ;INEPT-delay
"d22 = DELTA2-p29"

"d25=p16+d16+4u-p1*0.78+de"

"d24 = 1.86m"

"DELTA3=d24-cnst17*p24/2-4u"   ;CNST17 = 1

"DELTA4=d6-4u"        ;TIG-BIRD delay
"d23 = DELTA4-p31"

"DELTA5=p16+d16+d10"   ;1H-1H TOCSY
"DELTA6=p16+d16+8u"


"FACTOR3=(d7/(p6*115.112))/2"        ;d7 = 70-80 ms for 1H-1H TOCSY transfer
"l3=FACTOR3*2"

"l4 = td1/4"    ;NBL = 2, data from two experiments are stored in one file

"acqt0=0"

"p12 = (p1/90)*cnst12"  ;CNST12 = small flip angle excitation pulse of 1H-1H TOCSY

"l9=1"

baseopt_echo


1 ze
  d11 pl12:f2
2 d11 do:f2 st0                ;NBL = 2
  d11 pl2:f2 pl1:f1

  (p3 ph1):f2
  50u UNBLKGRAD
  p16:gp12 *2.77              ;purging gradient
  d16                         ;gpz12 = 17.13%


4 (p11 ph11):f1            ;TIG-BIRD with v = 0, p11 = 45 degree 1H; ph11 = phase 45
  DELTA4 pl0:f2
  4u

  (p2 ph1)
  (p31:sp18 ph6):f2        ;J-compensated adiabatic pulse during TIG-BIRD,
  4u
```

```
  d23
  (p1 ph12):f1              ;ph12 = phase 225 degree

  DELTA2                    ;INEPT delay
  4u

  (p2 ph1)
  (p29:sp20 ph6):f2         ;J-compensated adiabatic pulse during INEPT
  4u

  d22 pl2:f2


  (p1 ph22)
  p16:gp11                  ;purging gradient gpz11 = 31-51
  d16

  (p3 ph3):f2
  d0

  (p2 ph7)

  d0
  p16:gp1*EA
  d16 pl0:f2
  (p24:sp7 ph8:r):f2                    ;composite CHIRP, 2 ms
  4u
  DELTA pl2:f2

  (center (p1 ph21) (p3 ph4):f2 )
  4u
  DELTA3 pl0:f2
  (center (p2 ph1) (p24:sp7 ph9:r):f2 )   ;composite CHIRP, 2 ms
  4u
  DELTA3 pl2:f2
  (center (p1 ph2) (p3 ph5):f2 )
  4u
  DELTA2 pl0:f2


  (p2 ph1)
  (p29:sp20 ph1):f2          ;J-compensated adiabatic pulse during back INEPT transfer
  4u
  d22 pl1:f1

  (p1 ph1)

  d25

  (p2 ph1)

  4u pl12:f2
  p16:gp2              ;gpz2 = 20 %
  d16 BLKGRAD

  goscnp ph31 cpd2:f2

22 d11
23 d11 do:f2

  50u UNBLKGRAD

  p16:gp13           ;purging gradient between two experiments
                     ;gpz13 = 17.13-71%
  d16 pl1:f1
  p12 ph16          ;1H-1H TOCSY with DIPSI-2 starts here
                    ;SECOND experiment with small-flip angle excitation, p12

   if "l9 %2 == 1"

{
```

```
  DELTA5            ;p12 should be optimized, ca. 10-30 degree 1H pulse
                    ;p12 = (p1/90)*cnst12", CNST12 = small flip angle excitation
  p2 ph17
  d10               ;d10 incremented by in10 to get 1H-dimension for TOCSY in F1
  p16:gp3           ;gpz3 = 30
  d16

}
else
{
  d10                  ;;d10 incremented by in10 to get 1H-dimension for TOCSY in F1
  p16:gp3*EA           ;;gpz3 = 30
  d16
  p2 ph17
  DELTA5
}

  p1 ph18
  d20 pl10:f1

                                          ;begin DIPSI2
24 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 24 times l3
                                          ;end DIPSI2

  d21 pl1:f1
  p1 ph19

  DELTA6
  p2 ph17
  4u
  p16:gp4        ;gpz4 = 30
  d16
  4u BLKGRAD
```

```
  go=2 ph30

                                    ;PROCESSING
  d11 do:f2 wr #0 if #0 zd          ;use AU: splitx to separate datasets

  d11*0.33 igrad EA
  d11*0.33 ip5*2

  d11*0.33 iu9

  lo to 2 times 2

  d11*0.15 id0
  d11*0.15 id10

  d11*0.15 ip3*2
  d11*0.15 ip6*2
  d11*0.15 ip16*2
  d11*0.15 ip30*2
  d11*0.15 ip31*2

  d11*0.15 ru9

  lo to 2 times l4    ; l4 = td1/4   NBL = 2

exit


ph1=0
ph2=1

ph21=0

ph3=0 2 2 0

ph4=2 2 0 0 0 0 2 2
ph5=1 1 3 3 3 3 1 1

ph6=0
ph7=0
ph8=0
ph9=0

ph22=1 1 1 1 3 3 3 3

ph23=3   ;DIPSI-2
ph25=1

ph11=(8) 1 1 1 1 7 7 7 7   ;TIG-BIRD phases - ph11 and ph12
ph12=(8) 5 5 5 5 3 3 3 3

ph16=0 2         ;1H-1H TOCSY with DIPSI-2
ph17=0
ph18=0 0 2 2
ph19=2 2 0 0

ph30=0 2
ph31=0 2


;pl0 : 0W
;pl1 : f1 channel - power level for pulse (default)
;pl2 : f2 channel - power level for pulse (default)
;pl12: f2 channel - power level for CPD/BB decoupling
;sp7: 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
;p24: f2 channel - 180 degree shaped pulse for refocussing
   ; = 2msec for Crp80comp.4
;p16: homospoil/gradient pulse
```

```
;d0 : incremented delay (2D)                          [3 usec]
;d10 : incremented delay (2D)                         [3 usec]
;d16: delay for homospoil/gradient recovery

;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: echo-antiecho
;cpd2: decoupling according to sequence defined by cpdprg2
;pcpd2: f2 channel - 90 degree pulse for decoupling sequence


; ~~~~~~~~~~~~~~ WaveMaker Shapes ~~~~~~~~~~~~~~~~~~~~~~~~~~
;USE: wvm -a  command in TopSpin command line to generate CA-WURST adiabatic shape
pulses

;sp18:wvm:ad180Ccawurst: cawurst-20(121 ppm, 1.92 ms; H2L)

;sp20:wvm:ad180Ccawurstlh2: cawurst-20(121 ppm, 0.97 ms; L2H)

;for z-only gradients:

;use gradient files:
;gpnam: SMSQ10.100
```