Supplement of

Exclusively heteronuclear NMR experiments for the investigation of intrinsically disordered proteins: focusing on proline residues

Isabella C. Felli et al.

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Supplementary information

The pulse sequences used to acquire the 3D experiments to focus on proline residues are reported hereafter:

1. Pulse sequence 1 – 3D (H)CBCACON_pro
2. Pulse sequence 2 – 3D (H)CCCCON_pro
3. Pulse sequence 3 – 3D (H)CBCANCO_pro
4. Pulse sequence 4 – 3D (H)CACOCON_pro
Pulse sequence 1 – 3D (H)CBCACON_pro

; c_hcbcacon_ia3d_pro
; avance-version (20/07/28)
; (H)CbCaCON
; 3D sequence with
; 13C detected correlation for triple resonance using
; multiple inept transfer steps
;
; F2(Ha/b) -> F1(Ca/b,t1) -> F1(Ca) -> F1(C=O)
; -> F3(N,t2) -> F1(C=O,t3)
;
; on/off resonance 13C pulses using shaped pulses
; phase sensitive (t1)
; phase sensitive (t2)
; using constant time in t1
; using IPAP scheme for virtual decoupling
; using selective N pulse for Pro
; (use parameterset )
;
; M.G. Murrali, A. Piai, W. Bermel, I.C. Felli & R. Pierattelli,
; ChemBioChem 19, 1625-1629 (2018)
; W. Bermel, I. Bertini, V. Csizmok, I. C. Felli, R. Pierattelli &
; P. Tompa, J. Magn. Reson. 198, 275-281 (2009)
; W. Bermel, I. Bertini, I.C. Felli, R. Kuemmerle
; & R. Pierattelli, J. Magn. Reson. 178, 56-64 (2006)
; (W. Bermel, I. Bertini, L. Duma, I.C. Felli, L. Emsley, R. Pierattelli,
; P.R. Vasos, Angew. Chem. Int. Ed. 44, 3089-3092 (2005)
; (L. Duma, S. Hediger, A. Lesage & L. Emsley,
; J. Magn. Reson. 164, 187-195 (2003)
;
; $CLASS=HighRes
; $DIM=3D
; $TYPE=
; $SUBTYPE=
; $COMMENT=

prosol relations=<triple_c>

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

"p4=p3*2"
"p22=p21*2"
"d11=30m"
"d12=20u"
"d3=1.1m"
"d4=1.8m"
"d22=4.5m"
"d23=16.6m"
"d25=4.0m"
"d0=3u"
"d10=3u"
"d20=d25-p12-4u"
in0=inf1/2
in10=inf2/2
in20=in0
td1=tdmax(td1,d20*2,in20)
DELTA=d10*2+larger(p4,p8)
DELTA1=d25-d0-larger(p12,p22)-d3-p4
DELTA2=d23-d22-p12
DELTA3=d23-p12-larger(p12,p59)/2-4u
DELTA4=d23/2-p12/2
DELTA5=d25-p12-4u
DELTA6=d23-d22-p12-larger(p12,p59)/2
l0=1
spoff13=bf1*((cnst22/2-cnst21/2)/1000000)
spoff23=0
spoff24=0
spoff25=0
spoff26=bf1*((cnst21-cnst23)/1000000)
spoff27=bf1*((cnst22-cnst21)/1000000)
o1_F1=bf1*cnst23/1000000
aqseq 321
1 ze
d11 pl12:f2 pl16:f3
d1 do:f2 do:f3
d1 fq=cnst23(bf ppm):f1
d12 pl2:f2 pl3:f3
50u UNBLKGRAD
d11 (p3 ph1):f2
d4
center (p12:sp24 ph1) (p4 ph1):f2
d4
(p3 ph2):f2
p16:gp1
d16
(p11:sp23 ph3)
d0
center (p12:sp26 ph6) (p22 ph1):f3
d3
(p4 ph6):f2
DELTA1
(p12:sp24 ph1)
d20
(p12:sp26 ph1)
4u
(p11:sp25 ph1)
4u
(p12:sp26 ph1)
DELTA5
(p12:sp24 ph1)
4u
(p12:sp26 ph1)
DELTA5
(p11:sp23 ph1)
p16:gp2
d16 fq=cnst21(bf ppm):f1
(p11:sp23 ph5)
d22
(p12:sp27 ph1)
DELTA6
center (p12:sp24 ph1) (p59:sp46 ph1):f3
DELTA3
(p12:sp27 ph1)
4u
(p11:sp25 ph1)
p16:gp3
d16 pl3:f3
d10
(center (p8:sp13 ph6) (p4 ph6):f2 )
d10
(p22 ph1):f3
DELTA
(p21 ph1):f3

if "l0 %2 == 1"
{
  (p11:sp23 ph1)
  DELTA4
  (p12:sp27 ph1)
  DELTA4
  (center (p12:sp24 ph1) (p22 ph1):f3 )
  DELTA4
  (p12:sp27 ph1)
  DELTA4 pl16:f3
}
else
{
  (p11:sp23 ph7)
  d22
  (p12:sp27 ph1)
  DELTA4
  (center (p12:sp24 ph1) (p22 ph1):f3 )
  DELTA4
  DELTA4 pl16:f3
  (p12:sp27 ph1)
}
4u BLKGRAD
go=2 ph31 cpd2:f2 cpd3:f3
d11 do:f2 do:f3 mc #0 to 2

#ifdef LABEL_F1
F1(iu0, 2)
#else
F2(iu0, 2)
#endif /*LABEL_F1*/
F1PH(calph(ph3, +90), caldel(d0, +in0) & caldel(d20, -in20))
F2PH(calph(ph4, +90), caldel(d10, +in10))
exit
ph1=0
ph2=1
ph3=0
ph4=0 0 2 2
ph5=0 0 0 2 2 2 2 2
ph6=0 0 0 0 0 0 2 2 2 2 2
ph7=3
ph31=0 2 2 0 2 0 2

;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
;sp13: f1 channel - shaped pulse 180 degree (adiabatic)
;sp23: f1 channel - shaped pulse 90 degree (on resonance)
;sp24: f1 channel - shaped pulse 180 degree (on resonance)
;sp25: f1 channel - shaped pulse 90 degree (on resonance) for time reversed pulse
;sp26: f1 channel - shaped pulse 180 degree (C=O off resonance)
;sp27: f1 channel - shaped pulse 180 degree (Ca off resonance)
;sp46: f3 channel - shaped pulse 180 degree (N, selective for Pro)
;p3 : f2 channel - 90 degree high power pulse
;p4 : f2 channel - 180 degree high power pulse
;p8 : f1 channel - 180 degree shaped pulse for inversion (adiabatic)
;p11: f1 channel - 90 degree shaped pulse
;p12: f1 channel - 180 degree shaped pulse
;p16: homospoil/gradient pulse [1 msec]
;p21: f3 channel - 90 degree high power pulse
;p22: f3 channel - 180 degree high power pulse
;p59: f3 channel - 180 degree shaped pulse (N, selective for Pro)
;d0 : incremented delay (F1 in 3D) [3 usec]
d1 : relaxation delay; 1-5 * T1
d3 : 1/(6J(HCa)) [1.1 msec]
d4 : 1/(4J(HCa)) [1.8 msec]
d10: incremented delay (F2 in 3D) [3 usec]
d11: delay for disk I/O [30 msec]
d12: delay for power switching [20 usec]
d16: delay for homospoil/gradient recovery
d20: decremented delay (F1 in 3D) = d25+d0-p12-4u
;d22: 1/(4J(COCa)) [4.5 msec]
d23: 1/(4J(NCO)) [16.6 msec]
;d25: 1/(8J(CaCb)) [4.0 msec]
;cnst21: CO chemical shift (offset, in ppm)
;cnst22: Calpha chemical shift (offset, in ppm)
;cnst23: Caliphatic chemical shift (offset, in ppm)
;o1p: CO chemical shift (cnst21)
;l0: flag to switch between inphase and antiphase
;in0: 1/(2 * SW(Ca/b)) = DW(Ca/b)
;nd0: 2
;in10: 1/(2 * SW(N)) = DW(N)
;nd10: 2
;in20: = in0
;ns: 16 * n
;ds: >= 32
;td1: number of experiments in F1
;td2: number of experiments in F2
;FnMODE: States-TPPI (or TPPI) in F1 td1 max = n * d20 / in20 (n = 4 or = 2 with LABEL_F2)
;FnMODE: States-TPPI (or TPPI) in F2
;cpd2: decoupling according to sequence defined by cpdprg2
;cpd3: decoupling according to sequence defined by cpdprg3
;pcpd2: f2 channel - 90 degree pulse for decoupling sequence
;pcpd3: f3 channel - 90 degree pulse for decoupling sequence

;for z-only gradients:
;gpz1: 50%
;gpz2: 30%
;gpz3: 19%
;gpz4: 13%

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

;preprocessor-flags-start
;LABEL_F2: to do ipap in F2 start experiment with
; option -DLABEL_F2 (eda: ZGOPTNS)
;preprocessor-flags-end

;use AU-program splitcomb [ipap 2] (F1 or F2 with LABEL_F2) to process data

;$Id:$
Pulse sequence 2 – 3D (H)CCON_pro

; c_hcccon_ia3d_pro
; advance-version (20/07/28)
;(H)CC(CO)N
; 3D sequence with
; 13C detected correlation for triple resonance using
; multiple inept transfer steps and
; C-C FLOPSY16 spinlock
; F1(Ca,t1) -> F1(Ca) -> F1(C=O)
; -> F3(N,t2) -> F1(C=O,t3)
; on/off resonance 13C pulses using shaped pulses
; phase sensitive (t1)
; phase sensitive (t2)
; using IPAP scheme for virtual decoupling
; using selective N pulse for Pro
; (use parameter set)
; M.G. Murrali, A. Piai, W. Bermel, I.C. Felli & R. Pierattelli,
; ChemBioChem 19, 1625-1629 (2018)
; W. Bermel, I. Bertini, I.C. Felli, R. Kuemmerle
; & R. Pierattelli, J. Magn. Reson. 178, 56-64 (2006)

$CLASS=HighRes
$DIM=3D
$TYPE=
$SUBTYPE=
$COMMENT=
prosol relations=<triple_c>

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

"p4=p3*2"
"p22=p21*2"
"d11=30m"
"d12=20u"
"d3=0.95m"
"d4=1.8m"
"d22=4.5m"
"d23=16.6m"

"d0=3u"
"d10=3u"

"in0=inf1/2"
"in10=inf2/2"

"DELTA=d10*2+p8"
"DELTA1=d3/2"
"DELTA2=d3/2+p22+p4+d0*2"
"DELTA3=d23-d22-p12"
"DELTA4=d23-p12-larger(p12,p59)/2-4u"
"DELTA5=d23/2-p12/2"

"FACTOR2=(d9/(p6*188.448))"
"l1=FACTOR2"

"l0=1"

"spoff13=bf1*((cnst22/2-cnst21/2)/1000000)"
"spoff22=bf1*((cnst21-cnst22)/1000000)"
"spoff23=0"
"spoff24=0"
"spoff25=0"
"spoff26=bf1*((cnst21-cnst23)/1000000)"
"spoff27=bf1*((cnst22-cnst21)/1000000)"
"spoff28=0"

"o1_F1=bf1*cnst23/1000000"

aqseq 321

1 ze
d11 pl12:f2 pl16:f3
d21 do:f2 do:f3
d1 fq=cnst23(bf ppm):f1
d12 pl2:f2 pl3:f3
50u UNBLKGRAD
d4
(p3 ph1):f2
d4
(center (p12:sp24 ph1) (p4 ph1):f2 )
d4
(p3 ph8):f2
d0
(p22 ph1):f3
DELTA1
(p12:sp26 ph1)
DELTA1
(p4 ph1):f2
d0
(p12:sp24 ph1)
DELTA1
(p12:sp26 ph1)
DELTA1
(p11:sp25 ph1)
p19:gp1
d16 pl10:f1
;begin FLOPSY16
4 p6*0.511 ph11
p6*1.067 ph12
p6*1.822 ph13
p6*1.767 ph14
p6*1.444 ph15
p6*1.767 ph14
p6*1.822 ph13
p6*1.067 ph12
p6*0.511 ph11
p6*0.511 ph21
p6*1.067 ph22
p6*1.822 ph23
p6*1.767 ph24
p6*1.444 ph25
p6*1.767 ph24
p6*1.822 ph23
p6*1.067 ph22
p6*0.511 ph21
p6*1.067 ph22
p6*0.511 ph21

p6*0.511 ph21
p6*1.067 ph22
p6*1.822 ph23
p6*1.767 ph24
p6*1.444 ph25
p6*1.767 ph24
p6*1.822 ph23
p6*1.067 ph22
p6*0.511 ph21

p6*0.511 ph21
p6*1.067 ph22
p6*1.822 ph23
p6*1.767 ph24
p6*1.444 ph25
p6*1.767 ph24
p6*1.822 ph23
p6*1.067 ph22
p6*0.511 ph21

p6*0.511 ph11
p6*1.067 ph12
p6*1.822 ph13
p6*1.767 ph14
p6*1.444 ph15
p6*1.767 ph14
p6*1.822 ph13
p6*1.067 ph12
p6*0.511 ph11

p6*0.511 ph11
p6*1.067 ph12
p6*1.822 ph13
p6*1.767 ph14
p6*1.444 ph15
p6*1.767 ph14
p6*1.822 ph13
p6*1.067 ph12
p6*0.511 ph11

lo to 4 times l1
;end FLOPSY16

4u
p19:gp2
d16 fq=cnst22(bf ppm):f1
20u pl12:f2
20u cpd2:f2
(p11:sp23 ph1)
d22
(p12:sp22 ph1)
4u
(p25:sp28 ph1)
d22
(p12:sp22 ph1)
4u
(p11:sp25 ph2)
4u do:f2
p16:gp3
d16 fq=cnst21(bf ppm):f1
20u cpd2:f2
(p11:sp23 ph1)
d22
(p12:sp27 ph1)
DELTA6
(center (p12:sp24 ph1) (p59:sp46 ph1):f3)
DELTA4
(p12:sp27 ph1)
4u
(p11:sp25 ph1)
4u do:f2
p16:gp4
d16 pl3:f3
20u cpd2:f2
(p21 ph4):f3
d10
(p8:sp13 ph6)
d10
(p22 ph1):f3
DELTA
(p21 ph5):f3
DELTA
if "!0 %2 == 1"
{
(p11:sp23 ph1)
DELTA5
(p12:sp27 ph1)
DELTA5
(center (p12:sp24 ph1) (p22 ph1):f3)
DELTA5
DELTA5 pl16:f3
}
else
{
  (p11:sp23 ph7)
d22
  (p12:sp27 ph1)
DELTA3
  (center (p12:sp24 ph1) (p22 ph1):f3 )
DELTA5
  DELTA5 pl16:f3
  (p12:sp27 ph1)
}

4u BLKGRAD
go=2 ph31 cpd3:f3
d11 do:f2 do:f3 mc #0 to 2

# ifdef LABEL_F1
  F1I(iu0, 2)
# else
  F2I(iu0, 2)
#else /*LABEL_F1*/
  F1PH(calph(ph3, +90), caldel(d0, +in0))
  F2PH(calph(ph4, +90), caldel(d10, +in10))
exit

ph1=0
ph2=1
ph3=1 3
ph4=0 0 0 0 2 2 2 2
ph5=0 0 0 0 0 0 0 0 2 2 2 2 2 2 2
ph6=0
ph7=3
ph8=1 1 3 3
ph11=(720) 0
ph12=(720) 90
ph13=(720) 135
ph14=(720) 630
ph15=(720) 45
ph21=(720) 360
ph22=(720) 450
ph23=(720) 495
ph24=(720) 270
ph25=(720) 405
ph31=0 2 2 0 0 2 2 0 2 0 2 0

;pl1 : f1 channel - power level for pulse (default)
;pl2 : f2 channel - power level for pulse (default)
;pl3 : f3 channel - power level for pulse (default)
;pl10: f1 channel - power level for TOCSY-spinlock
;pl12: f2 channel - power level for CPD/BB decoupling
;pl16: f3 channel - power level for CPD/BB decoupling
;sp13: f1 channel - shaped pulse 180 degree (adiabatic)
;sp22: f1 channel - shaped pulse 180 degree (C=O off resonance)
;sp23: f1 channel - shaped pulse 90 degree (on resonance)
;sp24: f1 channel - shaped pulse 180 degree (on resonance)
;sp25: f1 channel - shaped pulse 90 degree (on resonance)
;sp10: f1 channel - shaped pulse 180 degree (Ca, sp28)
;sp11: f1 channel - 90 degree shaped pulse
;sp12: f1 channel - 180 degree shaped pulse
;p3 : f2 channel - 90 degree high power pulse
;p4 : f2 channel - 180 degree high power pulse
;p6 : f1 channel - 90 degree low power pulse
;p8 : f1 channel - 180 degree shaped pulse for inversion (adiabatic)
;p11: f1 channel - 90 degree shaped pulse
;p12: f1 channel - 180 degree shaped pulse
;p16: homospoil/gradient pulse [1 msec]
p19: gradient pulse 2 [500 usec]
p21: f3 channel - 90 degree high power pulse
;p22: f3 channel - 180 degree high power pulse
;p25: f1 channel - 180 degree shaped pulse (Ca, sp28)
p59: f3 channel - 180 degree shaped pulse (N, selective for Pro)
;d0 : incremented delay (F1 in 3D) [3 usec]
d1 : relaxation delay; 1-5 * T1
;d3 : 1/(6J(HCa)) [950 usec]
d4 : 1/(4J(HCa)) [1.8 msec]
d9 : TOCSY mixing time [12 msec]
d10: incremented delay (F2 in 3D) [3 usec]
d11: delay for disk I/O [30 msec]
d12: delay for power switching [20 usec]
d16: delay for homospoil/gradient recovery [150 usec]
d22: 1/(4J(COCa)) [4.5 msec]
d23: 1/(4J(NCO)) [16.6 msec]
cnst21: CO chemical shift (offset, in ppm)
cnst22: Calpha chemical shift (offset, in ppm)
;cnst23: Caliphatic chemical shift (offset, in ppm)
;o1p: CO chemical shift (cnst21)
;i0: flag to switch between inphase and antiphase
;i1: loop for FLOPSY16 cycle: ((p6*188.448) * l1) = mixing time
;inf1: 1/SW(Cali) = 2 * DW(Cali)
;inf2: 1/SW(N) = 2 * DW(N)
;in0: 1/(2 * SW(Cali)) = DW(Cali)
;nd0: 2
;in10: 1/(2 * SW(N)) = DW(N)
;nd10: 2
;ns: 16 * n
;ds: >= 32
;td1: number of experiments in F1
;td2: number of experiments in F2
;FnMODE: States-TPPI (or TPPI) in F1
;FnMODE: States-TPPI (or TPPI) in F2
;cpd2: decoupling according to sequence defined by cpdprg2
;cpd3: decoupling according to sequence defined by cpdprg3
;pcpd2: f2 channel - 90 degree pulse for decoupling sequence
;pcpd3: f3 channel - 90 degree pulse for decoupling sequence

;for z-only gradients:
;gpz1: 60%
;gpz2: 50%
;gpz3: 19%
;gpz4: 11%

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

;preprocessor-flags-start
;LABEL_F2: to do ipap in F2 start experiment with
;option -DLABEL_F2 (eda: ZGOPTNS)
;preprocessor-flags-end

;use AU-program splitcomb [ipap 2] (F1 or F2 with LABEL_F2) to process data

;$Id: $
Pulse sequence 3 – 3D (H)CBCANCO__pro

:c_hcbcanco_ia3d_pro
;avance-version (20/07/28)
;(H)CbCaNCO
;3D sequence with
; 13C detected correlation for triple resonance using
; multiple inept transfer steps
;
;  F2(Ha/b) -> F1(Ca/b,t1) -> F1(Ca)
;   -> F3(N,t2) -> F1(C=O,t3)
;  
;on/off resonance 13C pulses using shaped pulses
;phase sensitive (t1)
;phase sensitive (t2)
;using constant time in t1
;using constant time in t2
;using IPAP scheme for virtual decoupling
;using selective N pulse for Pro
;(use parameterset )
;
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; P. Tompa, J. Magn. Reson. 198, 275-281 (2009)
;(W. Bermel, I. Bertini, I.C. Felli, R. Kuemmerle
; & R. Pierattelli, J. Magn. Reson. 178, 56-64 (2006) )
;
;CLASS=HighRes
;DIM=3D
;TYPE=
;SUBTYPE=
;COMMENT=

prosol relations=<triple_c>

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

"p4=p3*2"
"p22=p21*2"
"d11=30m"
"d12=20u"
"d3=1.1m"
"d4=1.8m"
"d22=4.5m"
"d23=11.0m"
"d25=4.0m"
"d27=16.0m"

"d0=3u"
"d10=d23" 
"d20=d25-p12-4u"
"d30=d27-larger(p12,p59)/2"

"in0=inf1/2"
"in10=inf2/2"

"in20=in0"
"in30=in10"

"td1=tdmax(td1,d20*2,in20)"
"td2=tdmax(td2,d30*2,in30)"

"DELTA1=d25-d0-larger(p12,p22)-d3-p4"
"DELTA2=d27-d10-p12-larger(p12,p59)/2"
"DELTA3=d27/2-p12/2"
"DELTA4=d27-d22-p12"

"l0=1"

"spoff23=0"
"spoff24=0"
"spoff25=0"

"spoff26=bf1*((cnst21-cnst23)/1000000)"
"spoff27=bf1*((cnst22-cnst21)/1000000)"

"o1_F1=bf1*cnst23/1000000"
d11 pl12:f2 pl16:f3
d11 do:f2 do:f3
d12 fq=cnst23(bf ppm):f1
d12 pl2:f2 pl3:f3
50u UNBLKGRAD

(p3 ph1):f2
d4
(center (p12:sp24 ph1) (p4 ph1):f2 )
d4
(p3 ph2):f2

p16:gp1
d16

(p11:sp23 ph3)
do
(center (p12:sp26 ph6) (p22 ph1):f3 )
d3
(p4 ph1):f2
DELTA1
(p12:sp24 ph1)
d20
(p12:sp26 ph1)
4u
(p11:sp25 ph1)

(d23
(center (p12:sp24 ph1) (p22 ph1):f3 )
d23 pl12:f2
(p11:sp23 ph1)

p16:gp2
d16 fq=cnst21(bf ppm):f1
20u cpd2:f2
(p21 ph4):f3
d10
(p12:sp27 ph6)
DELTA2
(center (p12:sp24 ph6) (p59:sp46 ph1):f3 )
d30 pl3:f3
(p21 ph1):f3
4u do:f2
p16:gp3
d16
20u cpd2:f2

if "l0 %2 == 1"
{
    (p11:sp23 ph5)
    DELTA3
    (p12:sp27 ph1)
    DELTA3
    (center (p12:sp24 ph1) (p22 ph1):f3 )
    DELTA3
    (p12:sp27 ph1)
    DELTA3 pl16:f3
}
else
{
    (p11:sp23 ph7)
    d22
    (p12:sp27 ph1)
    DELTA4
    (center (p12:sp24 ph1) (p22 ph1):f3 )
    DELTA3
    DELTA3 pl16:f3
    (p12:sp27 ph1)
}

4u BLKGRAD

go=2 ph31 cpd3:f3
d11 do:f2 do:f3 mc #0 to 2

# ifdef LABEL_F1
F1l(iu0, 2)
# else
F2l(iu0, 2)
# endif /*LABEL_F1*/

F1PH(calph(ph3, +90), caldel(d0, +in0) & caldel(d20, -in20))
F2PH(calph(ph4, +90), caldel(d10, +in10) & caldel(d30, -in30))
exit

ph1=0
ph2=1
ph3=0 2
ph4=0 0 2 2
ph5=0 0 0 2 2 2 2
ph6=0 0 0 0 0 0 0 2 2 2 2 2 2 2 2
ph7=3 3 3 1 1 1 1
ph31=0 2 2 0 0 0 2

;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
;sp23: f1 channel - shaped pulse 90 degree (on resonance)
;sp24: f1 channel - shaped pulse 180 degree (on resonance)
;sp25: f1 channel - shaped pulse 90 degree (on resonance) for time reversed pulse
;sp26: f1 channel - shaped pulse 180 degree (C=O off resonance)
;sp27: f1 channel - shaped pulse 180 degree (Ca off resonance)
;sp46: f3 channel - shaped pulse 180 degree (N, selective for Pro)
;p3 : f2 channel - 90 degree high power pulse
;p4 : f2 channel - 180 degree high power pulse
;p11: f1 channel - 90 degree shaped pulse
;p12: f1 channel - 180 degree shaped pulse
;p16: homospoil/gradient pulse [1 msec]
;p21: f3 channel - 90 degree high power pulse
;p22: f3 channel - 180 degree high power pulse
;p59: f3 channel - 180 degree shaped pulse (N, selective for Pro)
;d0 : incremented delay (F1 in 3D) [3 usec]
;d1 : relaxation delay; 1-5 * T1
;d3 : 1/(6J(HCa)) [1.1 msec]
;d4 : 1/(4J(HCa)) [1.8 msec]
;d10: incremented delay (F2 in 3D) = d23
;d11: delay for disk I/O [30 msec]
;d12: delay for power switching [20 usec]
;d16: delay for homospoil/gradient recovery
;d20: decremented delay (F1 in 3D) = d25-p12-4u
;d21: 1/(4J(COCa)) [4.5 msec]
;d23: 1/(4J(NCa)) [11.0 msec]
;d25: 1/(8J(CaCb)) [4.0 msec]
;d27: 1/(4J(NCO)) [16.0 msec]
;d30: decremented delay (F2 in 3D) = d27-larger(p12,p59)/2
;cnst21: CO chemical shift (offset, in ppm)
;cnst22: Calpha chemical shift (offset, in ppm)
;cnst23: Caliphatic chemical shift (offset, in ppm)
;o1p: CO chemical shift (cnst21)
;l0: flag to switch between inphase and antiphase
;inf1: 1/SW(Ca) = 2 * DW(Ca)
;inf2: 1/SW(N) = 2 * DW(N)
;in0: 1/(2 * SW(Ca)) = DW(Ca)
;nd0: 2
;in10: 1/(2 * SW(N)) = DW(N)
;nd10: 2
;in20: = in0
;in30: = in10
;ns: 16 * n
;ds: >= 32
;td1: number of experiments in F1
;td2: number of experiments in F2
;FnMODE: States-TPPI (or TPPI) in F1
;FnMODE: States-TPPI (or TPPI) in F2
;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

;for z-only gradients:
;gpz1: 50%
;gpz2: 30%
;gpz3: 19%

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

;preprocessor-flags-start
;LABEL_F2: to do ipap in F2 start experiment with
; option -DLABEL_F2 (eda: ZGOPTNS)
;preprocessor-flags-end

;use AU-program splitcomb [ipap 2] (F1 or F2 with LABEL_F2) to process data
;$Id: $
Pulse sequence 4 – 3D (H)CACOCON_pro

; c_hcacocon_ia3d_pro
; avance-version (20/07/28)
; (HCa)COCON-TOCSY
; 3D sequence with
; homonuclear Hartman-Hahn transfer using MOCCA-XY16
; sequence for mixing
; F2(H) -> F1(Ca) -> F1(C=O,t1, MOCCA) -> F3(N,t2) -> F1(C=O,t3)
; on/off resonance 13C pulses using hard and shaped pulses
; phase sensitive (t1)
; phase sensitive (t2)
; using semi-constant time in t1
; using IPAP scheme for virtual decoupling
; using selective N pulse for Pro
; (use parameter set)
;
; M.G. Murrali, A. Piai, W. Bermel, I.C. Felli & R. Pierattelli,
; ChemBioChem 19, 1625-1629 (2018)
; I.C. Felli, R. Pierattelli, S.J. Glaser & B. Luy,
; J. Biomol. NMR 43, 187-196 (2009)
; W. Bermel, I. Bertini, I.C. Felli, Y.-M. Lee, C. Luchinat & R.
; Pierattelli,
; J. Am. Chem. Soc. 128, 3918-3919
; S. Balayssac, B. Jimenez & M. Piccioli,
; J. Magn. Reson. 182, 325-329 (2006)
;
; $CLASS=HighRes
; $DIM=3D
; $TYPE=
; $SUBTYPE=
; $COMMENT=

prosol relations=<triple_c>

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

"p22=p21*2"
"d11=30m"
"d12=20u"
"d3=1.1m"
"d4=1.8m"
"d22=4.5m"
"d23=16.6m"
"d21=300u"
"d10=3u"
"d28=3u"
"d0=d22"
"d20=d22+d28-4u"
"in0=inf1/2"
"in10=inf2/2"
"FACTOR1=d20*10000000*2/td1"
"INCR1=FACTOR1/1000000"
"if ( INCR1 > in0 ) { in20 = in0; } else { in20 = INCR1; }
"if ( INCR1 > in0 ) { in28 = 0; } else { in28=in0-INCR1; }
"TAU=d21/2"
"FACTOR2=(d9/((d21+p14))*16)"
"l1=FACTOR2*16"
"d31=(d21+p14)*l1"
"DELTA=d10*2+p8"
"DELTA1=d4-larger(p4,p25)/2"
"DELTA2=d22-d3-p4"
"DELTA3=d23/2-p12/2"
"DELTA4=d23-d22-p12"
"DELTA5=d23-larger(p12,p59)/2"
"l0=0"
"spoff13=bf1*((cnst21/2+cnst22/2)/1000000)-o1"
"spoff23=0"
"spoff24=0"
"spoff25=0"
"spoff26=bf1*((cnst22-cnst21)/1000000)"
"spoff27=bf1*((cnst21-cnst22)/1000000)"
"o1_F1=bf1*cnst21/1000000"
aqseq 321
1 ze
d11 p112:f2 p116:f3
2 d11 do:f2 do:f3
4u BLKGRAD
1151 d1 pl1:f1 pl2:f2
1152 20u rpp11
1153 50u UNBLKGRAD
1154 d12 cpd3:f3
1155 20u fq=cnst22(bf ppm):f1
1156
1157 (p3 ph1):f2
1158 DELTA1
1159 (center (p25:sp28 ph1) (p4 ph1):f2)
1160 DELTA1
1161 (p3 ph2):f2
1162
1163 (p11:sp23 ph1)
1164 d3
1165 (p4 ph1):f2
1166 DELTA2
1167 (p12:sp27 ph1)
1168 4u
1169 (p25:sp28 ph1)
1170 d22
1171 (p12:sp27 ph1)
1172 4u
1173 (p11:sp25 ph5)
1174
1175 p16:gp4
1176 d16 fq=cnst21(bf ppm):f1
1177
1178 (p11:sp23 ph3)
1179 d0
1180 (p12:sp26 ph1)
1181 d28
1182 (p12:sp24 ph1)
1183 d20
1184 (p12:sp26 ph1)
1185 4u
1186 (p11:sp25 ph4)
1187
1188 4u do:f3
1189 p16:gp1
1190 d16 pl20:f1
1191
1192 ;begin MOCCA-XY16
1193 4 TAU
1194 (p14 ph11)
1195 TAU ipp11
1196 10 to 4 times 11
1197 ;end MOCCA-XY16
1198
1199 p16:gp2
1200 d16 pl12:f2 pl3:f3
1201 20u cpd2:f2
if "%2 == 0"
{
  (p11:sp23 ph1)
  DELTA3
  (p12:sp26 ph1)
  DELTA3
  (center (p12:sp24 ph1) (p22 ph1):f3 )
  DELTA3
  (p12:sp26 ph1)
  DELTA3 p116:f3
}
else
{
  (p11:sp23 ph7)
  d22
  (p12:sp26 ph1)
  DELTA4
  (center (p12:sp24 ph1) (p22 ph1):f3 )
  DELTA3
  DELTA3 p116:f3
  (p12:sp26 ph1)
}
4u BLKGRAD

#ifdef LABEL_F1
  F1I(iu0, 2)
#endif

F2I(iu0, 2)
#endif /*LABEL_F1*/
F1PH(calph(ph3, +90), caldel(d0, +in0) & caldel(d20, -in20) &
calph(d28, +in28))
F2PH(calph(ph6, +90), caldel(d10, +in10))
d31
exit

ph1=0
ph2=1
ph3=0 2
ph4=1 1 1 1 1 1 1 3 3 3 3 3 3
ph5=0 0 0 0 2 2 2 2
ph6=0 0 2 2
ph7=3
ph11=0 1 0 1 1 0 1 0 2 3 2 3 2 3 2
ph31=0 2 2 0 2 0 0 2 2 0 0 2 0 2 2 0

;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
;pl120: f1 channel - power level for TOCSY (high sel.)
;sp13: f1 channel - shaped pulse 180 degree (adiabatic)
;sp23: f1 channel - shaped pulse 90 degree (on resonance)
;sp24: f1 channel - shaped pulse 180 degree (on resonance)
;sp25: f1 channel - shaped pulse 90 degree (on resonance)
;sp28: f1 channel - shaped pulse 180 degree (Ca off resonance)
;sp29: f1 channel - shaped pulse 180 degree (C=O off resonance)
;sp28: f1 channel - shaped pulse 180 degree (Ca on resonance)
;sp46: f3 channel - shaped pulse 180 degree (N, selective for Pro)
;p3 : f2 channel - 90 degree high power pulse
;p4 : f2 channel - 180 degree high power pulse
;p8 : f2 channel - 180 degree shaped pulse for inversion (adiabatic)
;p11: f1 channel - 90 degree shaped pulse
;p12: f1 channel - 180 degree shaped pulse
;p14: f1 channel - 180 degree low power pulse at p120
;p16: homospoil/gradient pulse [1 msec]
p21: f3 channel - 90 degree high power pulse
;p22: f3 channel - 180 degree high power pulse
;p25: f1 channel - 180 degree shaped pulse (Ca, sp28)
p59: f3 channel - 180 degree shaped pulse (N, selective for Pro)
d0 : incremented delay (F1 in 3D): = d22
d1 : relaxation delay; 1-5 * T1
d3 : 1/(6J(HCa)) [1.1 msec]
;d4 : 1/(4J(HCa)) [1.8 msec]
;d9 : TOCSY mixing time
;d10: incremented delay (F2 in 3D) [3 usec]
;d11: delay for disk I/O [30 msec]
;d12: delay for power switching [20 usec]
;d16: delay for homospoil/gradient recovery
;d20: decremented delay (F1 in 3D) = d22+d28-4u
;d21: delay for MOCCA sequence [300 usec]
;d22: 1/(4J(COCa)) [4.5 msec]
;d23: 1/(4J(NCO)) [15.0 msec]
;d28: incremented delay (F1 in 3D) [3 usec]
;d31: total mixing time as executed
;cnst21: CO chemical shift (offset, in ppm)
;cnst22: Calpha chemical shift (offset, in ppm)
;olp: CO chemical shift (cnst21)
;l0: flag to switch between inphase and antiphase
;l1: loop for MOCCA-XY16 cycle: ((TAU*2+p14)*16 * l1) = mixing time
;inf1: 1/SW(CO) = 2 * DW(CO)
;inf2: 1/SW(N) = 2 * DW(N)
;in0: 1/(2 * SW(CO)) = DW(CO)
;nd0: 2
;in10: 1/(2 * SW(N)) = DW(N)
;nd10: 2
;in20: = k * in0
;in28: = (1 - k) * in0
;ns: 8 * n
;ds: >= 32
;td1: number of experiments in F1
;td2: number of experiments in F2
;FnMODE: States-TPPI (or TPPI) in F1
;FnMODE: States-TPPI (or TPPI) in F2
;cpd2: decoupling according to sequence defined by cpdprg2
;cpd3: decoupling according to sequence defined by cpdprg3
;pcpd2: f2 channel - 90 degree pulse for decoupling sequence
;pcpd3: f3 channel - 90 degree pulse for decoupling sequence
;for z-only gradients:
;gpz1: 80%
;gpz2: 70%
;gpz3: 30%
;gpz4: 50%
;use gradient files:
;gpnam1: SMSQ10.100
;gpnam2: SMSQ10.100
;gpnam3: SMSQ10.100
;gpnam4: SMSQ10.100
;use AU-program splitcomb [ipap 2] to process data
;$Id: $