Four-dimensional NOE-NOE spectroscopy of SARS-CoV-2 Main Protease to facilitate resonance assignment and structural analysis*

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Supporting Information

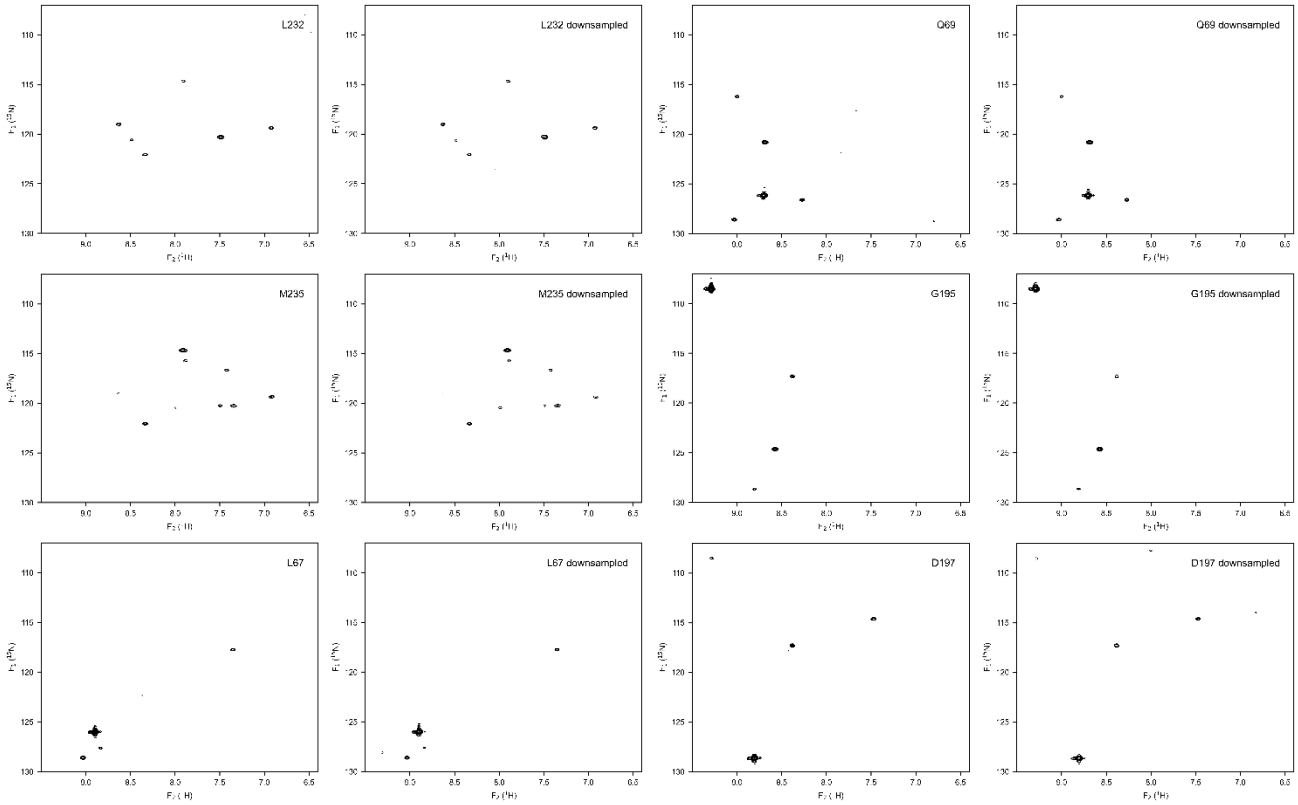
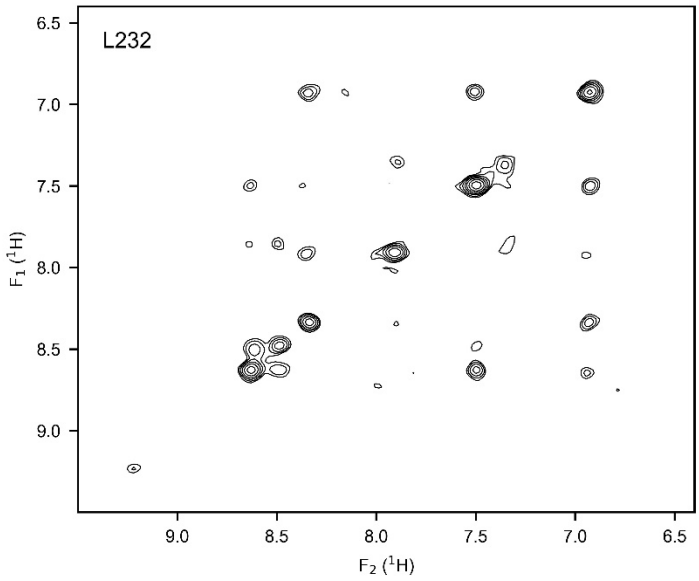
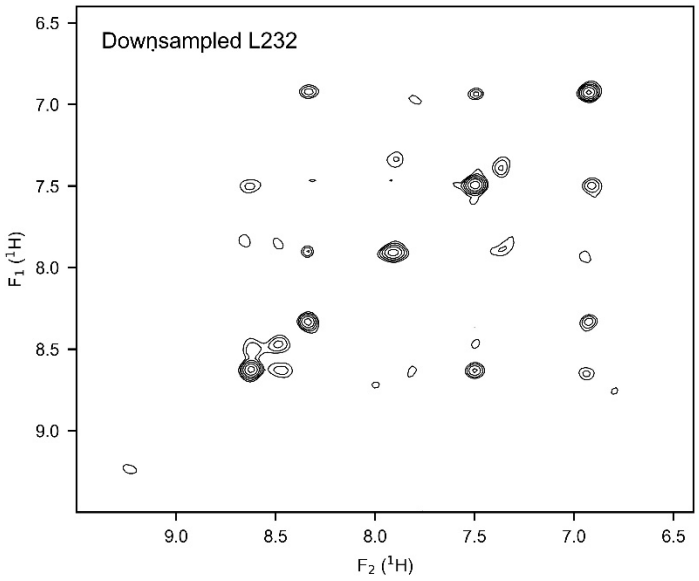
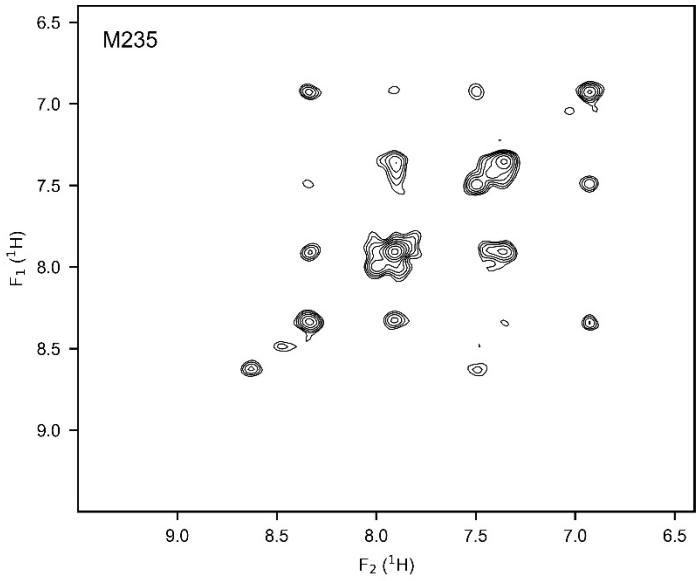
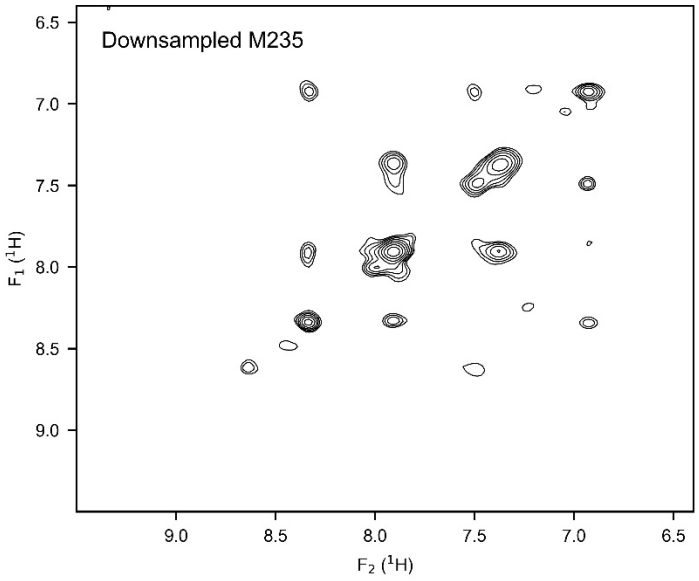


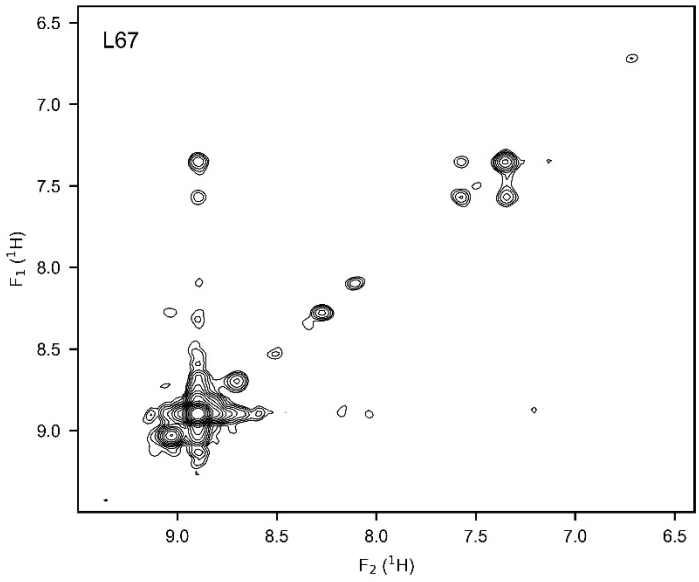
Figure S1. Comparison of the cross sections through the 4D TROSY-NOESY-TROSY spectrum shown in Figure 2 of the main text with the corresponding cross sections obtained from a 33% subset of the sampled time domain data, processed identically to the full dataset, and contoured at the same level relative to the noise. The time domain datapoints used for the "downsampled" spectrum were selected by limiting the length of the total time domain vector according to $[(t_1/t_{1,max})^2 + [(t_2/t_{2,max})^2 + [(t_3/t_{3,max})^2]^{0.5} \leq 0.84$, where $t_{1,max}$, $t_{2,max}$, and $t_{3,max}$ are the length of the time domain in the fully sampled data set.

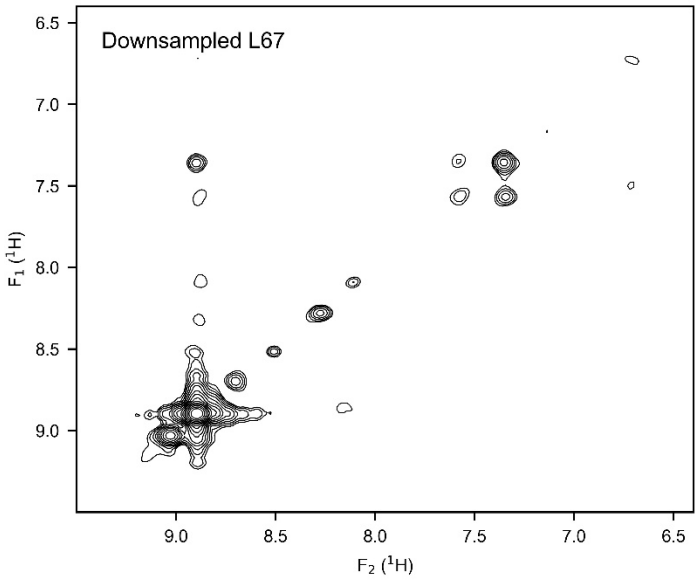

L232
F1 (1H)
F2 (1H)
6.5
7.0
7.5
8.0
8.5
9.0


Downsampled L232
F1 (1H)
F2 (1H)
6.5
7.0
7.5
8.0
8.5
9.0


M235
F1 (1H)
F2 (1H)
6.5
7.0
7.5
8.0
8.5
9.0


Downsampled M235
F1 (1H)
F2 (1H)
6.5
7.0
7.5
8.0
8.5
9.0


L67
F1 (1H)
F2 (1H)
6.5
7.0
7.5
8.0
8.5
9.0


Downsampled L67
F1 (1H)
F2 (1H)
6.5
7.0
7.5
8.0
8.5
9.0

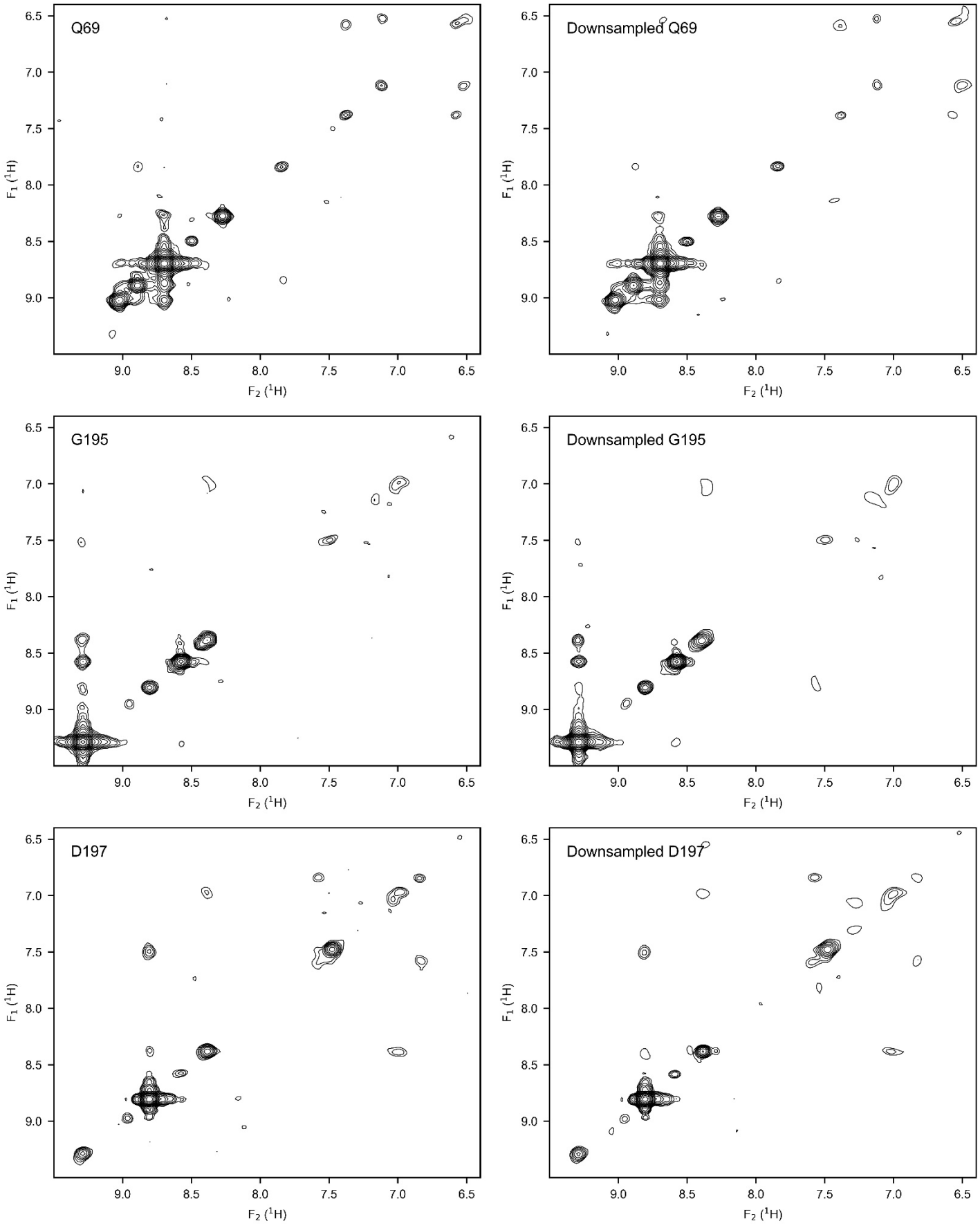

Q69
Downsampled Q69
G195
Downsampled G195
D197
Downsampled D197
F1 (1H)
F2 (1H)
6.5
7.0
7.5
8.0
8.5
9.0

Figure S2. Comparison of the same cross sections through the 4D NOESY-NOESY-TROSY spectrum shown in Figure 3 of the main text with the corresponding cross sections obtained from a 33% subset of the sampled time domain data, processed identically to the full dataset, and contoured at the same level relative to the noise. The time domain datapoints used for the "downsampled" spectrum were selected by limiting the length of the total time domain vector according to $[(t_1/t_{1,max})^2 + [(t_2/t_{2,max})^2 + [(t_3/t_{3,max})^2]^{0.5} \leq 0.84$, where $t_{1,max}$, $t_{2,max}$, and $t_{3,max}$ are the length of the time domain in the fully sampled data set.

Bruker pulse program and NMRPipe processing scripts for the 4D TROSY-NOESY-TROSY:

```
#include <bits.jfy>

define list<gradient> EA2 = { 1.0 -1.0 }

"in0=inf3*0.5"
"in10=inf2"
"in20=inf1*0.5"

"d25=p22"
"d26=p22+100u"

"d10=in10*0.5-p1"

"d0=105u+p7*0.635-p4*2-2u"
"d20=105u+p7*0.635-p4*2-2u"

"d11=50m"
"d21=2.1m-p21"
"d22=2.5m-p21-p2"
"d23=2.1m-p2-14u"

"d7=d8-p7-p27-1m"


1       ze
        1m
2       10m  do:C2 do:N LOCKH_OFF
        d11
        0.1m
3       3m
4       3m
5       3m
6       1m do:N
7       5u do:C1
        10u pl4:C1
        d1 BLKGRAD
        1m UNBLKGRAD
        10u pl1:H
        10u pl7:N

;------- start 90-degree on hn ----------------------
       (p1 ph0):H
        3u
        p21:gp1
        d21
        300u
       (center (p1*2 ph0):H (p7*2 ph0):N)
        d21
        p21:gp1
        303u
       (p1 ph1):H     ;INEPT to 15N
        3u
        3u pl12:H
       (p2 ph11:r):H
;goto 999
```

```
        6u
        4u pl1:H
        p20:gp0
        200u pl6:C2
        10u pl4:C1
;********15N evolution******************
       (p7 ph17):N
        10u
        p23:gp15*EA
        200u
       (p7 ph0 p7*2.4 ph1 p7 ph0):N
        p23:gp16*EA
        d20
       (p4*2 ph0):C1
        4u
       (p4*2:sp8 ph0):C2
        d20
       (p1 ph13):H
        3u
        3u pl2:H
       (p2:sp0 ph3:r):H
;goto 999
        6u gron2
        d23 pl1:H
        2u groff
       (center (p1*2 ph0):H (p7 ph0 p7*2.4 ph1 p7 ph0):N)
        3u gron2
        d23 pl2:H
        3u groff
       (p2:sp1 ph0:r):H
        6u
        2u pl1:H
       (p1 ph0):H (p7 ph5):N    ;DOUBLE 90
        5u
;goto 999
        p21:gp3
        d22 pl2:H
       (center (d25 p2 ph10:r 3u 2u pl1 p1*2 ph0 3u 2u pl2 p2 ph10:r):H (p7*2 ph0):N)
;goto 999
        5u pl1:H
        p21:gp3
        d22
       (p7 ph0):N
        p22:gp14
        d10       ;d10=in10*0.5-p1
       (p1 ph6):H
;--------NOE mixing starts--------------
        d7
       (p7 ph0):N
        100u
        p27:gp7
        900u
;------- start 90-degree on hn ---------------
       (p1 ph0):H
        3u
        p21:gp1
        d21
```

```
        300u
       (center (p1*2 ph0):H (p7*2 ph0):N)
        d21
        p21:gp1
        303u
       (p1 ph1):H    ;INEPT to 15N
        3u
        3u pl12:H
       (p2 ph11:r):H
;goto 999
        6u
        4u pl1:H
        p20:gp0
        200u pl6:C2
        10u pl4:C1
;******15N evolution******************
       (p7 ph7):N
        10u
        p23:gp5*EA2
        200u
       (p7 ph0 p7*2.4 ph1 p7 ph0):N
        p23:gp6*EA2
        d0
       (p4*2 ph0):C1
        4u
       (p4*2:sp8 ph0):C2
        d0
       (p1 ph12):H
        3u
        3u pl2:H
       (p2:sp0 ph2:r):H
;goto 999
        6u gron2
        d23 pl1:H
        2u groff
       (center (p1*2 ph0):H (p7 ph0 p7*2.4 ph1 p7 ph0):N)
        3u gron2
        d23 pl2:H
        3u groff
       (p2:sp1 ph0:r):H
        6u
        2u pl1:H
       (p1 ph0):H (p7 ph4):N    ;DOUBLE 90
        5u
;goto 999
        de
        p21:gp3
        d22 pl2:H
       (center (d26 p2 ph10:r 3u 2u pl1 p1*2 ph0 3u 2u pl2 p2 ph10:r):H (p7*2 ph0):N)
;goto 999
        5u pl1:H
        p21:gp3
        d22
       (p7 ph0):N
        p22:gp4
999     95u pl30:C2
        5u BLKGRAMP
```

```
       go=2 ph31 cpd2:C2
        10m do:C2 do:N LOCKH_OFF mc #0 to 2
      F3EA(calgrad(EA2) & calph(ph2, +180) & calph(ph12, +180) & calph(ph4, +180),
caldel(d0, +in0) & calph(ph7, +180) & calph(ph31, +180))
      F2PH(calph(ph6, +90), caldel(d10, +in10) & calph(ph6, +90)) ;second ph6+90 shifts
carrier by 3/4*sw
      F1EA(calgrad(EA) & calph(ph3, +180) & calph(ph13, +180) & calph(ph5, +180),
caldel(d20, +in20) & calph(ph17, +180) & calph(ph31, +180))

1m do:C1
1m do:N
exit

ph0=0
ph1=1
ph2=3
ph3=3
ph4=1
ph5=1
ph6=0 0 2 2
ph7=1 3
ph10=2
ph11=0
ph12=1
ph13=1
ph17=1
ph31=1 3 3 1


#!/bin/csh

#FLATT requires the latest SMILE Version 2.1 Rev 2019.337.11.19

nusExpand.tcl -mode bruker -sampleCount 3987 -off 0 \
 -in ./ser -out ./ser_full -sample ./nuslist

rm -fr tmp

bruk2pipe -verb -in ./ser_full \
  -bad 0.0 -ext -aswap -AMX -decim 1560 -dspfvs 20 -grpdly 67.9867858886719  \
  -xN         3072  -yN              180  -zN        182  -aN         180  \
  -xT         1536  -yT               90  -zT         91  -aT          90  \
  -xMODE       DQD  -yMODE Echo-AntiEcho  -zMODE Complex  -aMODE  Complex  \
  -xSW   12820.513  -ySW        2564.103  -zSW  4545.455  -aSW   2564.103  \
  -xOBS    800.134  -yOBS         81.086  -zOBS  800.134  -aOBS    81.086  \
  -xCAR      4.773  -yCAR        116.682  -zCAR    9.033  -aCAR   116.682  \
  -xLAB        Htr  -yLAB            Ntr  -zLAB     Hnoe  -aLAB      Nnoe  \
  -ndim          4  -aq2D        Complex                                    \
| pipe2xyz -x -out tmp/test%04d.fid -ov

rm -fr fid

xyz2pipe -in tmp/test%04d.fid -z                              \
| nmrPipe -fn MAC -macro $NMRTXT/bruk_ranceA.M -noRd -noWr \
| pipe2xyz -out fid/test%04d.fid -z -ov

rm -fr ft1
```

```
xyz2pipe -in fid/test%04d.fid -x                              \
| nmrPipe  -fn POLY -time                                     \
| nmrPipe  -fn EXT -xn 780 -sw                                \
| nmrPipe  -fn SP -off 0.4 -end 0.98 -pow 2 -elb 15 -c 0.5 \
| nmrPipe  -fn ZF -zf 1 -auto                                 \
| nmrPipe  -fn FT                                             \
| nmrPipe  -fn PS -p0 -40 -p1 0 -di                           \
| nmrPipe  -fn FLATT -x1 12.5ppm -xn 5.3ppm -noauto -m 1   \
             -fitNodes 12.5ppm to 10.5ppm, 6ppm to 5.3ppm \
| nmrPipe  -fn POLY -auto -ord 1 -x1 12.5ppm -xn 5.3ppm    \
| nmrPipe  -fn EXT -x1 10.8ppm -xn 6.0ppm -sw               \
| pipe2xyz -out ft1/test%04d.ft1 -a

rm -fr prof*.dat ser_full tmp fid

rm -fr ft1.smile

xyz2pipe -in ft1/test%04d.ft1 -x               \
| nmrPipe  -fn SMILE -thresh 0.95 -report 2    \
           -sample nuslist -sampleCount 3987   \
           -xP0 90 -xP1   0 -xzf 1             \
           -yP0 45 -yP1 180 -yzf 1 -yNeg -yAlt \
           -zP0 90 -zP1   0 -zzf 1 -zNeg -zAlt \
| pipe2xyz -out ft1.smile/test%04d.ft1 -x

rm -fr ft

xyz2pipe -in ft1.smile/test%04d.ft1 -x \
| nmrPipe  -fn ZF -zf 1 -auto          \
| nmrPipe  -fn FT                      \
| nmrPipe  -fn PS -p0 90 -p1 0 -di     \
| nmrPipe  -fn TP                      \
| nmrPipe  -fn ZF -zf 1 -auto          \
| nmrPipe  -fn FT -neg -alt            \
| nmrPipe  -fn PS -p0 45 -p1 180 -di   \
| pipe2xyz -out ft/test%04d.ft3 -y

xyz2pipe -in ft/test%04d.ft3 -z        \
| nmrPipe  -fn ZF -zf 1 -auto          \
| nmrPipe  -fn FT -neg -alt            \
| nmrPipe  -fn PS -p0 90 -p1 0 -di     \
| nmrPipe  -fn TP                      \
| pipe2xyz -out ft/test%04d.ft4 -z

proj4D.tcl -in ft/test%04d.ft4

rm -fr ft/test*.ft3
```

Bruker pulse program and NMRPipe processing scripts for the 4D NOESY-NOESY-TROSY:

```
#include <bits.jfy>

"in0=inf3*0.5"
"in10=inf2*0.5"
"in20=inf1*0.5"


"d25=p22"
"d26=p22+100u"

"d10=3u"
"d16=d10*2+6u+p7*4.4"

"d20=in20*0.5-10u"

"d0=105u+p7*0.635-p4*2-2u"

"d11=50m"
"d21=2.1m-p21"
"d22=2.5m-p21-p2"
"d23=2.1m-p2-14u"

"d7=d8-p7-p27-1m"
"d17=d18-p7-p27-0.9m"


1       ze
        1m
2       10m  do:C2 do:N LOCKH_OFF
        d11
        0.1m
3       3m
4       3m
5       3m
6       1m do:N
7       5u do:C1
        10u pl4:C1
        d1 BLKGRAD
        1m UNBLKGRAD
        10u pl0:H
        10u pl7:N
        10u fq=cnst8(bf ppm):H
;-------first NOESY dimension---------------
       (center (p15:sp5 ph0 5u d20 5u 5u d20 5u p15:sp6 ph5:r):H (p7 ph0 p7*2.4 ph1 p7
ph0):N)
;-------first NOE mixing--------------
        d17
       (p7 ph0):N
        100u
        p27:gp8
        800u
;-------second NOESY dimension ----------------
       (p15:sp5 ph6:r):H
        d10
       (p7 ph0 p7*2.4 ph1 p7 ph0):N
```

```
        d10 pl1:H
       (p1 ph0 p1*2.3 ph1 p1 ph0):H
        d16
       (p1 ph0 p1*2.3 ph1 p1 ph0):H
        3u
        3u pl0:H
       (p15:sp6 ph0):H
;-------second NOE mixing--------------
        d7
       (p7 ph0):N
        100u fq=0:f1
        p27:gp7  ;1.7m @44%
        900u pl1:H
;-------trosy starts with 90 on hn-----------------
       (p1 ph0):H
        3u
        p21:gp1
        d21
        300u
       (center (p1*2 ph0):H (p7*2 ph0):N)
        d21
        p21:gp1
        303u
       (p1 ph1):H                ;INEPT to 15N
        3u
        3u pl12:H
       (p2 ph11:r):H
;goto 999
        6u
        4u pl1:H
        p20:gp0
        200u pl6:C2
        10u pl4:C1
;******15N evolution******************
       (p7 ph7):N
        10u
        p23:gp5*EA
        200u
       (p7 ph0 p7*2.4 ph1 p7 ph0):N
        p23:gp6*EA
        d0
       (p4*2 ph0):C1
        4u
       (p4*2:sp8 ph0):C2
        d0
       (p1 ph12):H
        3u
        3u pl2:H
       (p2:sp0 ph2:r):H
;goto 999
        6u gron2
        d23 pl1:H
        2u groff
       (center (p1*2 ph0):H (p7 ph0 p7*2.4 ph1 p7 ph0):N)
        3u gron2
        d23 pl2:H
        3u groff
```

```
       (p2:sp1 ph0:r):H
        6u
        2u pl1:H
       (p1 ph0):H (p7 ph4):N    ;DOUBLE 90
        5u
;goto 999
        de
        p21:gp3
        d22 pl2:H
       (center (d26 p2 ph10:r 3u 2u pl1 p1*2 ph0 3u 2u pl2 p2 ph10:r):H (p7*2 ph0):N)
;goto 999
        5u pl1:H
        p21:gp3
        d22
       (p7 ph0):N
        p22:gp4
999     95u pl30:C2
        5u BLKGRAMP
       go=2 ph31 cpd2:C2
        10m do:C2 do:N LOCKH_OFF mc #0 to 2
      F3EA(calgrad(EA) & calph(ph2, +180) & calph(ph12, +180) & calph(ph4, +180),
caldel(d0, +in0) & calph(ph7, +180) & calph(ph31, +180))
      F2PH(calph(ph6, +90), caldel(d10, +in10))
      F1PH(calph(ph5, -90), caldel(d20, +in20))

1m do:C1
1m do:N
exit

ph0=0
ph1=1
ph2=3
ph4=1
ph5=0 0 2 2
ph6=0
ph7=1 3
ph10=2
ph11=0
ph12=1
ph31=1 3 3 1


#!/bin/csh

#FLATT requires the latest SMILE Version 2.1 Rev 2019.337.11.19

nusExpand.tcl -mode bruker -sampleCount 5482 -off 0 \
 -in ./ser -out ./ser_full -sample ./nuslist

rm -fr ft1

bruk2pipe -verb -in ./ser_full \
  -bad 0.0 -ext -aswap -AMX -decim 1248 -dspfvs 20 -grpdly 67.9840850830078  \
  -xN         3072  -yN              180  -zN         120  -aN         120  \
  -xT         1536  -yT               90  -zT          60  -aT          60  \
  -xMODE       DQD  -yMODE Echo-AntiEcho  -zMODE  Complex  -aMODE  Complex  \
  -xSW   16025.641  -ySW        2564.103  -zSW   5000.000  -aSW   5000.000  \
```

```
  -xOBS    800.134  -yOBS         81.086  -zOBS   800.134  -aOBS   800.134  \
  -xCAR      4.773  -yCAR        116.682  -zCAR     8.316  -aCAR     8.316  \
  -xLAB        Htr  -yLAB            Ntr  -zLAB        Hz  -aLAB        Ha  \
  -ndim          4  -aq2D        Complex                                     \
| nmrPipe  -fn POLY -time                                  \
| nmrPipe  -fn EXT -xn 960 -sw                             \
| nmrPipe  -fn SP -off 0.4 -end 0.98 -pow 2 -elb 15 -c 0.5 \
| nmrPipe  -fn ZF -zf 1 -auto                              \
| nmrPipe  -fn FT                                         \
| nmrPipe  -fn PS -p0 139 -p1 0 -di                       \
| nmrPipe  -fn FLATT -x1 14.5ppm -xn 5.2ppm -noauto -m 1   \
             -fitNodes 14.5ppm to 10.5ppm, 6ppm to 5.2ppm \
| nmrPipe  -fn POLY -auto -ord 1 -x1 14.5ppm -xn 5.2ppm    \
| nmrPipe  -fn EXT -x1 10.8ppm -xn 6.0ppm -sw              \
| pipe2xyz -out ft1/test%04d.ft1 -a

rm -fr prof*.dat ser_full

rm -fr ft1.smile

xyz2pipe -in ft1/test%04d.ft1 -x             \
| nmrPipe  -fn SMILE -thresh 0.95 -report 2  \
           -sample nuslist -sampleCount 5482 \
           -xP0  90 -xP1   0 -xzf 1          \
           -yP0 -45 -yP1   0 -yzf 1 -yAlt    \
           -zP0  90 -zP1 180 -zzf 1 -zAlt    \
| pipe2xyz -out ft1.smile/test%04d.ft1 -x

rm -fr ft.tmp

xyz2pipe -in ft1.smile/test%04d.ft1 -x \
| nmrPipe  -fn ZF -zf 2                \
| nmrPipe  -fn FT                      \
| nmrPipe  -fn PS -p0 90 -p1 0 -di     \
| nmrPipe  -fn TP                      \
| nmrPipe  -fn ZF -zf 2                \
| nmrPipe  -fn FT -alt                 \
| nmrPipe  -fn PS -p0 -45 -p1 0 -di    \
| pipe2xyz -out ft.tmp/test%04d.ft3 -y

xyz2pipe -in ft.tmp/test%04d.ft3 -z    \
| nmrPipe  -fn ZF -zf 2                \
| nmrPipe  -fn FT -alt                 \
| nmrPipe  -fn PS -p0 90 -p1 180 -di   \
| nmrPipe  -fn TP                      \
| pipe2xyz -out ft.tmp/test%04d.ft4 -z

rm -fr ft

xyz2pipe -in ft.tmp/test%04d.ft4 -a \
| nmrPipe -fn FLATT -first -last    \
| pipe2xyz -out ft/test%04d.ft4 -a

proj4D.tcl -in ft/test%04d.ft4

rm -fr ft.tmp/test*.ft3
```