Supporting Information

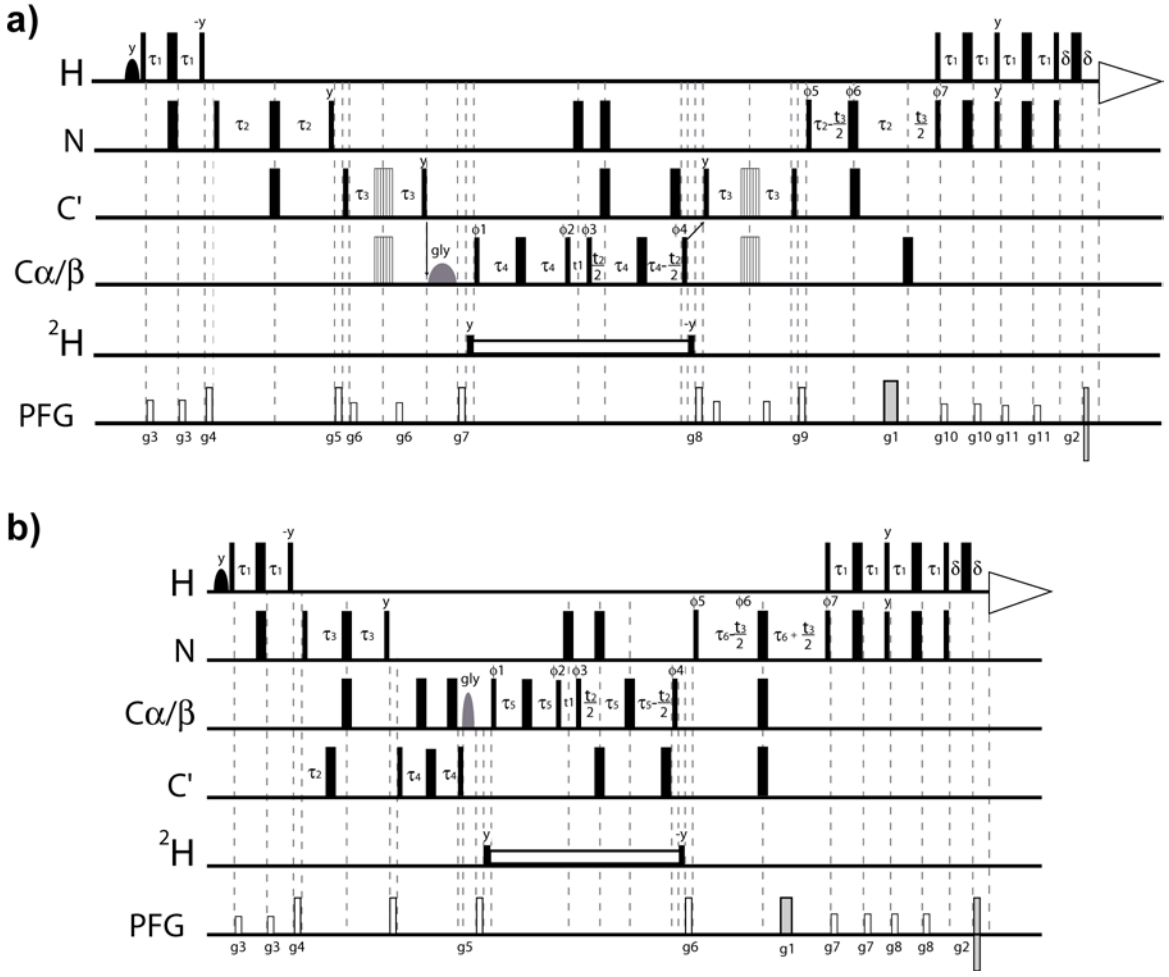

Figure S1: (4,2)D PR-NMR TROSY-based a) HN(CO)CACB and b) Intra-HNCACB pulse sequences for the assignment of larger perdeuterated proteins. Selective inversion of glycine C_α nuclei has been included in the sequences in order to ensure that the signs of these resonances are the same as the remainder of the residues. The selective glycine inversion is accomplished with an on resonance 4.63 ms IBURP2[37] pulse ($B1_{max}$ of 1077Hz) on our Inova 800 spectrometer. All pulses are applied along the x-axis unless otherwise indicated. The carrier frequencies are: 4.75 ppm in ^{1}H, 45.5 ppm in ^{13}C and 119.4 ppm in ^{15}N. The water selective pulse is accomplished with a 1.14 ms sinc pulse. ^{2}H decoupling is achieved with a 2000 Hz WALTZ16[38] field. **a) HN(CO)CACB**: The delays are: $\tau1 = 2.4$ ms, $\tau2 = 14.0$ ms, $\tau3 = 4.5$ ms, $\tau4 = 7.1$ ms, $\delta = 250$ μs. The phase cycle is: $\phi1 = x,-x$; $\phi2 = y$; $\phi3 = 4(y),4(-y)$; $\phi4$

= 2(x),2(-x); ϕ5 = y; ϕ6 = x; ϕ7 = x; rec = 2(x,-x,-x,x). Quadrature detection in C_β and C_α is achieved using States-TPPI phase cycling of ϕ1 and ϕ4 respectively. Quadrature detection in N is accomplished using the sensitivity enhanced pulse field gradient technique and ϕ7. The gradients are: g1 = 2.5 ms at 26.5 G/cm; g2 = 0.25 ms at 26.5 G/cm; g3 = 0.5 ms at 14.3 G/cm; g4 = 1.0 ms at 22.5 G/cm; g5 = 1.0 ms at 25.9 G/cm; g6 = 1.0 ms at 18.4 G/cm; g7 = 1.0 ms at 30.0 G/cm; g8 = 1.0 ms at 23.9 G/cm; g9 = 4.0 ms at 22.5 G/cm; g10 = 0.5 ms at 16.9 G/cm; g11 = 0.5 ms at 19.0 G/cm. **b) intra-HNCACB**: The delays are: τ1 = 2.4 ms, τ2 = 16.5 ms, τ3 = 26.0 ms, τ4 = 4.2 ms, τ5 = 7.1 ms, τ6 = 12.5 ms, δ = 250 μs. The phase cycle is: ϕ1 = x,-x; ϕ2 = y; ϕ3 = y; ϕ4 = 2(x),2(-x); ϕ5 = y; ϕ6 = 4(x),4(-x); ϕ7 = x; rec = x,-x,-x,x. Quadrature detection in C_β and C_α is achieved using States-TPPI phase cycling of ϕ1 and ϕ5 respectively. Quadrature detection in N is accomplished using the sensitivity enhanced pulse field gradient technique and ϕ7. The gradients are: g1 = 2.5 ms at 26.5 G/cm; g2 = 0.25 ms at 26.5 G/cm; g3 = 0.7 ms at 16.1 G/cm; g4 = 1.1 ms at 10.3 G/cm; g5 = 1.0 ms at 5.1 G/cm; g6 = 1.5 ms at -15.9 G/cm; g7 = 0.5 ms at 10.3 G/cm; g8 = 0.9 ms at 15.9 G/cm.

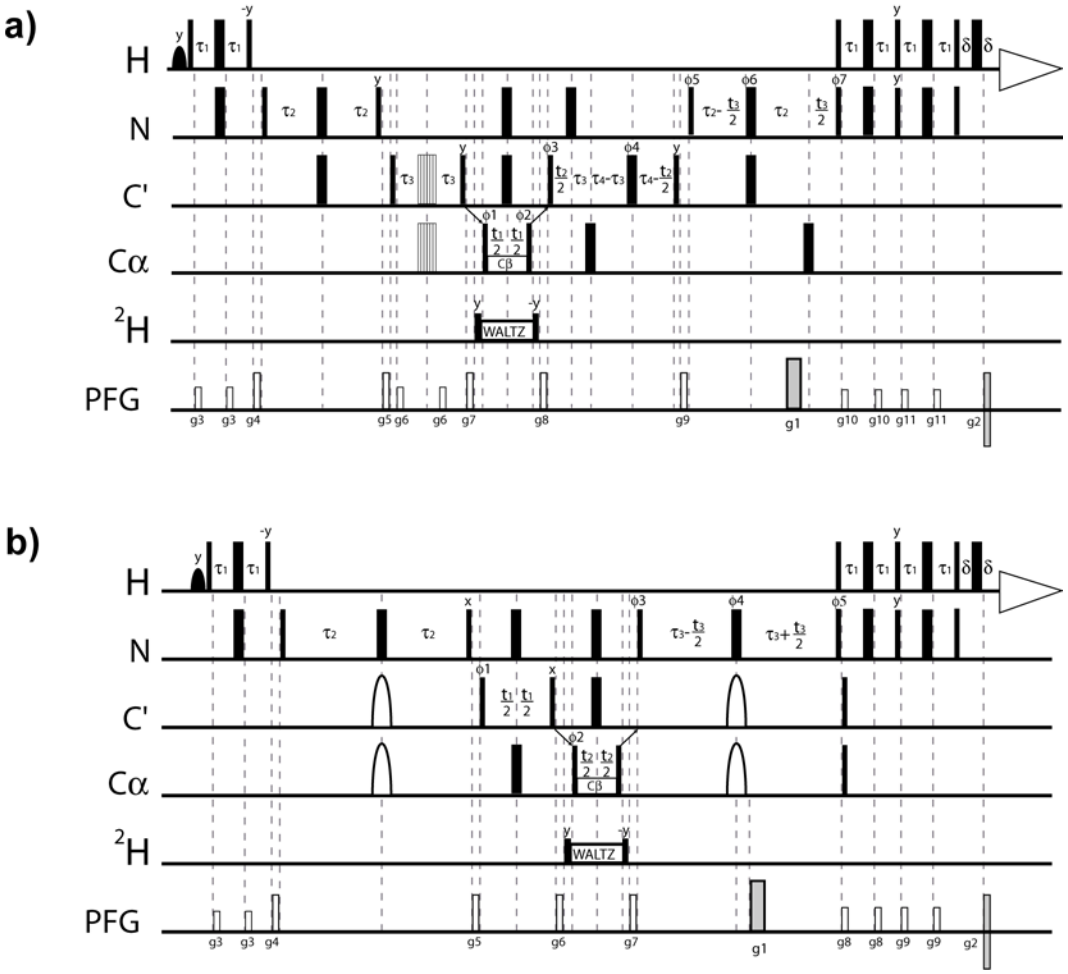

Figure S2: (4,2)D PR-NMR TROSY-based a) HNCOCA and b) $HNCO_{i-1}CA_i$ pulse sequences for the assignment of larger perdeuterated proteins. Selective C_β decoupling has been added to the sequences during those periods when C_α magnetization is in the transverse plane. This is accomplished with a three band WURST2 decoupling scheme.[43] The three bands cover the ranges: 69.5-65.5ppm for threonines, 17.5-13.5ppm for alanines and 40-20ppm for all other C_β nuclei. All pulses are applied along the x-axis unless otherwise indicated. The carrier frequencies are: 4.75 ppm in ^1H, 55.2 ppm in $^{13}C_\alpha$, 173.1 ppm in $^{13}C'$ and 119.4 ppm in ^{15}N. The water selective pulse is accomplished with a 7.1 ms EBURP-1[37] pulse. ^2H decoupling is achieved with a 573 Hz GARP1[44] field. **a) HNCOCA**: The delays are: $\tau1 = 2.2$ ms, $\tau2 = 12.0$ ms, $\tau3 = 4.0$ ms, $\tau4 = 4.3$ ms; $\delta = 250$ μs. The phase cycle is: $\phi1 = x$; $\phi2 =$

2(x)2(-x); ϕ3 = x; ϕ4 = x,y,-x,-y; ϕ5 = y; ϕ6 = 2(x)2(-x); ϕ7 = x; rec = x,-x,-x,x. Quadrature detection in C' and C_α is achieved using States-TPPI phase cycling of ϕ3 and ϕ1 respectively. Quadrature detection in N is accomplished using the sensitivity enhanced pulse field gradient technique and ϕ7. The gradients are: g1 = 1.25 ms at 26.5 G/cm; g2 = 0.125 ms at 25.3 G/cm; g3 = 0.4 ms at 10.2 G/cm; g4 = 1.0 ms at 15.3 G/cm; g5 = 0.7 ms at 20.4 G/cm; g6 = 0.5 ms at 12.3 G/cm; g7 = 0.6 ms at -15.3 G/cm; g9 = 0.5 ms at 15.3 G/cm; g10 = 0.4 ms at 6.3 G/cm; g11 = 0.4 ms at 10.9 G/cm. **b) $HNCO_{i-1}CA_i$**: The delays are: τ1 = 2.2 ms, τ2 = 13.0 ms, τ3 = 13.0 ms, δ = 250 μs. The phase cycle is: ϕ1 = x,-x; ϕ2 = 2(x),2(-x); ϕ3 = x,-x; ϕ4 = x; ϕ5 = 4(x),4(-x); rec = 2(x),4(-x),2(x). Quadrature detection in C' and C_α is achieved using States-TPPI phase cycling of ϕ1 and ϕ2 respectively. Quadrature detection in N is accomplished using the sensitivity enhanced pulse field gradient technique and ϕ5. The gradients are: g1 = 1.25 ms at 26.5 G/cm; g2 = 0.125 ms at 25.3 G/cm; g3 = 0.4 ms at 10.2 G/cm; g4 = 1.0 ms at 20.4 G/cm; g5 = 0.7 ms at 20.4 G/cm; g6 = 1.0 ms at -20.4 G/cm; g7 = 1.5 ms at 15.3 G/cm; g8 = 0.4 ms at 17.4 G/cm; g9 = 0.4 ms at 14.3 G/cm.

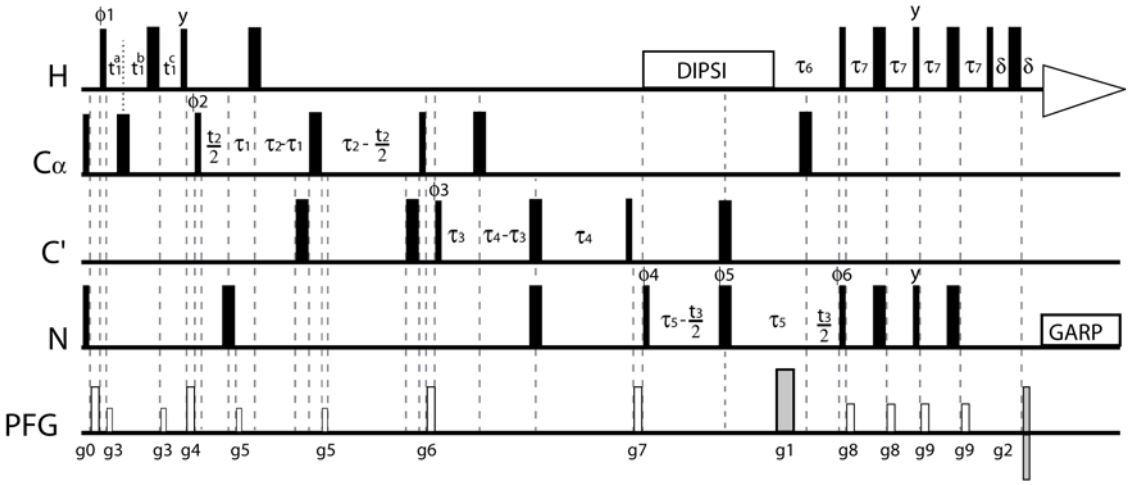

Figure S3: (4,2)D PR-NMR HACA(CO)NH pulse sequences for the assignment of $^{1}H^{13}C^{15}N$-labeled proteins. All pulses are applied along the x-axis unless otherwise indicated. The carrier frequencies are: 5.17 ppm in ^{1}H, 57.9 ppm in $^{13}C_{\alpha}$ and 118.6 ppm in ^{15}N. ^{1}H decoupling is achieved with a 7500 Hz DIPSI2[50] scheme. The delays are: $\tau1 = 1.7$ ms, $\tau2 = 4.7$ ms, $\tau3 = 14.0$ ms, $\tau4 = 14.0$ ms; $\tau5 = 5.4$ ms; $\delta = 250$ μs. The phase cycle is: $\phi1 = x$; $\phi2 = x$; $\phi3 = x,-x$; $\phi4 = x$; $\phi5 = 2(x),2(y)$; $\phi6 = x$; rec = x,-x,-x,x. Quadrature detection in H_{α} and C_{α} is achieved using States-TPPI phase cycling of $\phi1$ and $\phi2$ respectively. Quadrature detection in N is accomplished using the sensitivity enhanced pulse field gradient technique and $\phi6$. The gradients are: g0 = 0.5 ms at 16.3 G/cm; g1 = 2.5 ms at 26.5 G/cm; g2 = 0.25 ms at 26.5 G/cm; g3 = 0.5 ms at 19.0 G/cm; g4 = 0.5 ms at -26.5 G/cm; g5 = 0.5 ms at 21.8 G/cm; g6 = 0.5 ms at -30.6 G/cm; g7 = 0.5 ms at -28.0 G/cm; g8 = 0.5 ms at 4.1 G/cm; g9 = 0.5 ms at 6.1 G/cm.

Table S1 – TROSY-HN(CO)CACB Data Collection

Projection	ni	Phase_C	Phase_N	Angle_N	Angle_Ca	Angle_Cb	nt	SWtilt	Time(min.)
HN-N	64	1	1,2	0.0	90.0	90.0	8	2900	24
HN-CA	64	1,3	1	90.0	0.0	90.0	16	10200	48
HN-CB	64	1,2	1	90.0	90.0	0.0	16	12400	48
Tilt 1	64	1,2,3,4	1,2	86.0	15.5	75.0	32	14783	376
Tilt 2	64	1,2,3,4	1,2	73.9	33.7	61.3	32	16021	376
Tilt 3	64	1,2,3,4	1,2	54.7	54.7	54.7	32	14722	376
Tilt 4	64	1,2,3,4	1,2	33.7	73.9	61.3	32	10752	376
Tilt 5	64	1,2,3,4	1,2	15.5	86.0	75.0	32	6285	376

Total time = 33.3 hours

Table S2 – TROSY-Intra-HNCACB Data Collection

Projection	ni	Phase_C	Phase_N	Angle_N	Angle_Ca	Angle_Cb	nt	SWtilt	Time(min.)
HN-N	64	1	1,2	0.0	90.0	90.0	16	2900	48
HN-CA	64	1,3	1	90.0	0.0	90.0	32	11400	96
HN-CB	64	1,2	1	90.0	90.0	0.0	32	12400	96
Tilt 1	64	1,2,3,4	1,2	86.0	15.5	75.0	64	15092	773
Tilt 2	64	1,2,3,4	1,2	73.9	33.7	61.3	64	16598	773
Tilt 3	64	1,2,3,4	1,2	54.7	54.7	54.7	64	15415	773
Tilt 4	64	1,2,3,4	1,2	33.7	73.9	61.3	64	11328	773
Tilt 5	64	1,2,3,4	1,2	15.5	86.0	75.0	64	6595	773

Total time = 68.4 hours

Table S3 – TROSY-HNCACO Data Collection

Projection	ni	Phase_C	Phase_N	Angle_N	Angle_Ca	Angle_C'	nt	SWtilt	Time(min.)
HN-N	64	1	1,2	0.0	90.0	90.0	8	2900	48
HN-CA	45	1,3	1	90.0	0.0	90.0	16	4965	66
HN-C'	64	1,2	1	90.0	90.0	0.0	16	3256	95
Tilt 1	64	1,2,3,4	1,2	86.0	75.0	15.5	32	4620	751
Tilt 2	64	1,2,3,4	1,2	73.9	61.3	33.7	32	5899	751
Tilt 3	64	1,2,3,4	1,2	54.7	54.7	54.7	32	6420	751
Tilt 4	64	1,2,3,4	1,2	33.7	61.3	73.9	32	5701	751
Tilt 5	64	1,2,3,4	1,2	15.5	75.0	86.0	32	4301	751

Total time = 66 hours

Table S4 – TROSY-HNCOCA Data Collection

Projection	ni	Phase_C	Phase_N	Angle_N	Angle_Ca	Angle_C'	nt	SWtilt	Time(min.)
HN-N	64	1	1,2	0.0	90.0	90.0	8	2900	24
HN-CA	64	1,2	1	90.0	0.0	90.0	16	4965	48
HN-C'	27	1,3	1	90.0	90.0	0.0	16	3256	20
Tilt 1	64	1,2,3,4	1,2	86.0	15.5	75.0	32	5826	373
Tilt 2	64	1,2,3,4	1,2	73.9	33.7	61.3	32	6500	373
Tilt 3	64	1,2,3,4	1,2	54.7	54.7	54.7	32	6420	373
Tilt 4	64	1,2,3,4	1,2	33.7	73.9	61.3	32	5354	373
Tilt 5	64	1,2,3,4	1,2	15.5	86.0	75.0	32	3978	373

Total time=32.6

Table S5 – TROSY-HNCO$_{i-1}$CA$_i$ Data Collection

Projection	ni	Phase_C	Phase_N	Angle_N	Angle_Ca	Angle_C'	nt	SWtilt	Time(min.)
HN-N	64	1	1,2	0.0	90.0	90.0	8	2900	24
HN-CA	64	1,3	1	90.0	0.0	90.0	16	4965	48
HN-C'	64	1,2	1	90.0	90.0	0.0	16	3256	48
Tilt 1	64	1,2,3,4	1,2	86.0	75.0	15.5	32	4620	370
Tilt 2	64	1,2,3,4	1,2	73.9	61.3	33.7	32	5899	370
Tilt 3	64	1,2,3,4	1,2	54.7	54.7	54.7	32	6420	370
Tilt 4	64	1,2,3,4	1,2	33.7	61.3	73.9	32	5701	370
Tilt 5	64	1,2,3,4	1,2	15.5	75.0	86.0	32	4301	370

Total time = 32.8 hours

Table S6 – HACANH Data Collection

Projection	ni	Phase_C/H	Phase_N	Angle_N	Angle_Ca	Angle_Ha	nt	SWtilt	Time(min.)
HN-N	48	1	1,2	0.0	90.0	90.0	8	2100	17
HN-CA	64	1,3	1	90.0	0.0	90.0	16	4499	46
HN-HA	28	1,2	1	90.0	90.0	0.0	16	3000	21
Tilt 1	40	1,2,3,4	1,2	86.0	75.0	15.5	32	4198	228
Tilt 2	40	1,2,3,4	1,2	73.9	61.3	33.7	32	5240	228
Tilt 3	40	1,2,3,4	1,2	54.7	54.7	54.7	32	5542	228
Tilt 4	40	1,2,3,4	1,2	33.7	61.3	73.9	32	4741	228
Tilt 5	40	1,2,3,4	1,2	15.5	75.0	86.0	32	3393	228

Total time = 20.4 hours

Table S7 – HACA(CO)NH Data Collection

Projection	ni	Phase_C/H	Phase_N	Angle_N	Angle_Ca	Angle_Ha	nt	SWtilt	Time(min.)
HN-N	58	1	1,2	0.0	90.0	90.0	8	2100	21
HN-CA	36	1,3	1	90.0	0.0	90.0	16	4499	27
HN-HA	28	1,2	1	90.0	90.0	0.0	16	3000	21
Tilt 1	40	1,2,3,4	1,2	86.0	75.0	15.5	32	4198	230
Tilt 2	40	1,2,3,4	1,2	73.9	61.3	33.7	32	5240	230
Tilt 3	40	1,2,3,4	1,2	54.7	54.7	54.7	32	5542	230
Tilt 4	40	1,2,3,4	1,2	33.7	61.3	73.9	32	4741	230
Tilt 5	40	1,2,3,4	1,2	15.5	75.0	86.0	32	3393	230

Total time = 20.3 hours

Macros for Automatic PR-NMR Data Acquisition on a Varian Spectrometer

hncacb_start:

```
create('nN','real','global')
create('f_nam','string','global')
nN=1
hncacb_pulse
```

hncacb_pulse:

```
wexp='hncacb_series'
au
```

hncacb_series:

```
format(nN,1,0):f_nam
svf('/data/PR_NMR/hncacb_' + f_nam)
nN=nN+1
echo(nN)

if nN>8 then hncacb_end endif

if nN=1 then
   jexp80 angle_Ca=90.0 angle_Cb=90.0 nt=16 ni=64 phase=1 phase2=1,2
   hncacb_pulse
endif

if nN=2 then
   jexp80 angle_Ca=90.0 angle_Cb=0.0 nt=32 ni=64 phase=1,2 phase2=1
   hncacb_pulse
endif

if nN=3 then
   jexp80 angle_Ca=0.0 angle_Cb=90.0 nt=32 ni=64 phase=1,3 phase2=1
   hncacb_pulse
endif

if nN=4 then
   jexp80 angle_Cb=15.504 angle_Ca=75.037 nt=64 ni=64 phase=1,2,3,4 phase2=1,2
   hncacb_pulse
endif

if nN=5 then
   jexp80 angle_Cb=33.690 angle_Ca=61.289 nt=64 ni=64 phase=1,2,3,4 phase2=1,2
   hncacb_pulse
endif

if nN=6 then
   jexp80 angle_Cb=54.736 angle_Ca=54.736 nt=64 ni=64 phase=1,2,3,4 phase2=1,2
   hncacb_pulse
endif

if nN=7 then
   jexp80 angle_Cb=73.898 angle_Ca=61.289 nt=64 ni=64 phase=1,2,3,4 phase2=1,2
   hncacb_pulse
endif

if nN=8 then
   jexp80 angle_Cb=86.033 angle_Ca=75.037 nt=64 ni=64 phase=1,2,3,4 phase2=1,2
   hncacb_pulse
endif
```

hncacb_end:

```
destroy('f_nam','global')
destroy('nN','global')
```

NMRPipe Macros for Conversion and Processing of PR-NMR Data

Conversion macro for tilt angles:

```
#!/bin/csh
foreach x (4 5 6 7 8)
foreach i (--+ -++ +-+ +++)
var2pipe -in ../data/hacanh_$x.fid/fid_$i           \
  -xN           1024    -yN             80          \
  -xT            512    -yT             40          \
  -xMODE     Complex    -yMODE     Complex          \
  -xSW       8000.00    -ySW      11400.00          \
  -xOBS      599.727    -yOBS      201.172          \
  -xCAR        5.18     -yCAR        45.25          \
  -xLAB           NH    -yLAB         Tilt          \
  -ndim            2    -aq2D       States          \
        -out ../cfids/hacanh_$x$i.fid  -verb -ov
end
end
echo Done conversion.
```

NMRPipe processing macro for tilt angles:

```
#!/bin/csh
foreach x (4 5 6 7 8)
foreach i (--+ -++ +-+ +++)
nmrPipe    -in ../cfids/hacanh_$x$i.fid                                  \
| nmrPipe  -fn POLY -time                                                \
| nmrPipe  -fn SP -off 0.45 -end 0.95 -pow 2 -c 0.5                      \
| nmrPipe  -fn ZF -size 2048                                            \
| nmrPipe  -fn FT                                                       \
| nmrPipe  -fn PS -p0 -14.0 -p1 0.0 -di                                 \
| nmrPipe  -fn EXT -x1 6.00ppm -xn 11.8ppm -round 16 -sw                \
| nmrPipe  -fn TP                                                       \
| nmrPipe  -fn LP -ps0-0                                                \
| nmrPipe  -fn SP -off 0.45 -end 0.95 -pow 2 -c 0.5                      \
| nmrPipe  -fn ZF -auto                                                 \
| nmrPipe  -fn FT                                                       \
| nmrPipe  -fn PS -p0 0.0 -p1 0.0 -di                                   \
| nmrPipe  -fn TP                                                       \
| nmrPipe  -fn POLY -auto                                               \
   -out ../pdata/hacanh_$x$i.dat -verb 2 -ov
end
end
```