SUPPORTING INFORMATION

Facile measurement of ^{1}H-^{15}N residual dipolar couplings in larger perdeuterated proteins

Nicholas C. Fitzkee and Ad Bax

Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892-0520, U.S.A.

Origins of Antiphase Peaks in Protonated Samples

During the initial INEPT step with the ^{15}N 180º pulse in position *d* (in Fig.1, main text), remote protons with long range couplings, $^{lr}J_{NH}$, to ^{15}N evolve between time points *a* and *b* according to:

$$H_y \rightarrow H_y \cos(\pi^{lr}J_{NH}T) - 2H^J{}_xN_z \sin(\pi^{lr}J_{NH}T) \quad \text{(S1)}$$

The ^{1}H 90_y / ^{15}N $90_{\phi 2}$ pulse at time point *b* for e.g. ϕ_2 = y transforms the antiphase term according to:

$$2H^J{}_xN_z \rightarrow -2H^J{}_zN_x = -2H^J{}_zN_x\,(H^N{}_\alpha + H^N{}_\beta) \quad \text{(S2)}$$

where the $N_xH^N{}_\beta$ and $N_xH^N{}_\alpha$ product terms correspond to the regular ^{15}N TROSY and anti-TROSY ^{15}N components (both are antiphase with respect to remote proton H^J, as reflected in the $H^J{}_zN_xH^N{}_\alpha$ and $H^J{}_zN_xH^N{}_\beta$ products). Subsequent t_1 evolution of the TROSY component under $^{lr}J_{NH}$ coupling results in:

$$-2H^J{}_zN_xH^N{}_\beta \rightarrow \sin(\pi^{lr}J_{NH}t_1)\, N_yH^N{}_\beta - 2\cos(\pi^{lr}J_{NH}t_1)\, H^J{}_zN_xH^N{}_\beta \quad \text{(S3)}$$

Including both ^{15}N evolution at its regular ω_N TROSY frequency and the $^{lr}J_{NH}$ rephasing of the antiphase $H^J{}_zN_x$ component, the t_1 dependence of the first term on the right hand side of (S3), including ^{15}N evolution, can be rewritten as

$$\sin(\pi^{lr}J_{NH}t_1)\, N_yH^N{}_\beta \exp(i\omega_Nt_1) = \tfrac{1}{2}\{\exp[i\pi^{lr}J_{NH}t_1] - \exp[-i\pi^{lr}J_{NH}t_1]\}\exp(i\omega_Nt_1)\}\, N_yH^N{}_\beta =$$
$$\tfrac{1}{2}\{\exp[i(\omega_N+\pi^{lr}J_{NH}t_1)t_1] - \exp[i(\omega_N-\pi^{lr}J_{NH})t_1]\}\, N_yH^N{}_\beta \quad \text{(S4)}$$

The $N_yH^N{}_\beta$ terms at the right hand side of expression S4 are the regular TROSY terms, which are subsequently transferred back into two observable regular $H^N{}_{tr}N_\beta$ TROSY $^1H^N$ transverse magnetization components for detection, with oppositely signed amplitudes and modulated by $\omega_N \pm \pi^{lr}J_{NH}$ in the t_1 dimension. Note that similar antiphase contributions, approximately two-fold smaller in magnitude due to the shorter $^{lr}J_{NH}$ dephasing period, are present in the reference experiment. However, there they are dwarfed by the much stronger, regular one-bond $^1J_{NH}$ correlation.

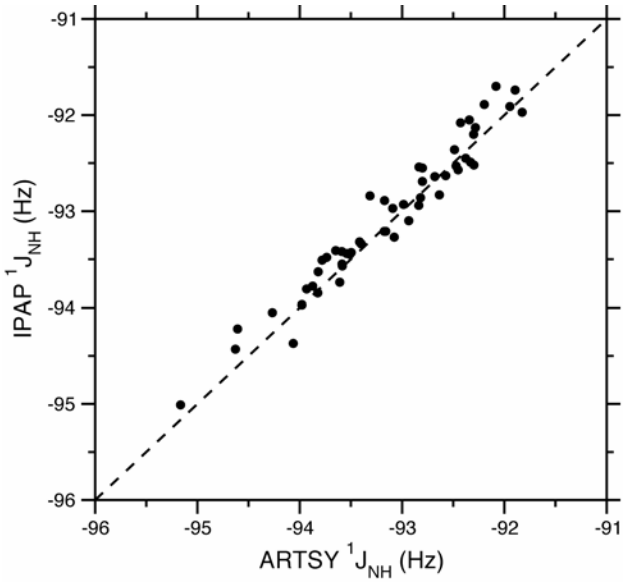

Figure S1. Comparison of isotropic $^1J_{NH}$ values measures in GB3. ^1H-^{15}N scalar couplings measured using the ARTSY pulse scheme are compared with those measured using a recently published IPAP-HSQC (Yao et al. 2009). ARTSY couplings were measured at 600 MHz for a ^2H, ^{15}N, ^{13}C sample of 1.8 mM wild-type GB3 at pH 6.6. IPAP couplings were recorded at 750 MHz for perdeuterated GB3 mutant K4A K19E V42E.

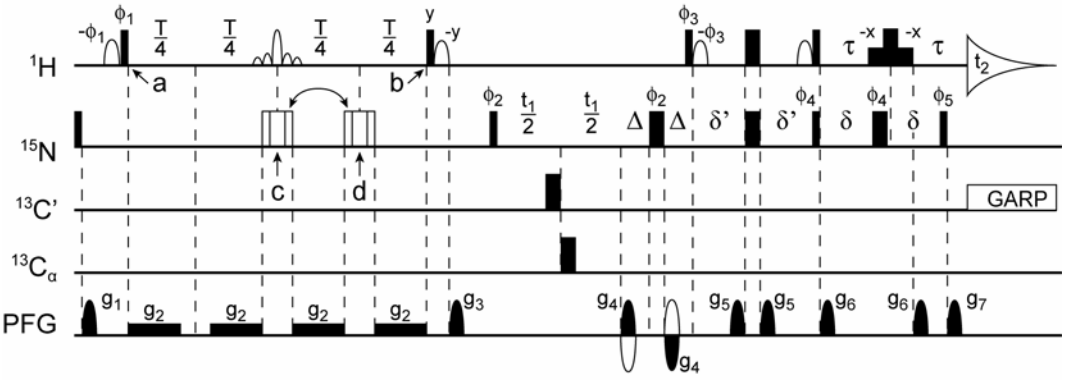

Figure S2. The ARTSY sequence, adapted for protonated proteins. Relative to the original sequence, the 180° ^{1}H refocusing pulse has been replaced with a 1.5 ms H^N-centered REBURP pulse (Geen and Freeman 1991) and an additional sinc-shaped water flip back pulse (following the 90°$_x$ pulse at time point *b*) has been employed to compensate. In the attenuated spectrum, pulses applied at time point *c* are centered, and J evolution during the pulses needs to be taken into account. The effective $^1J_{NH}$ evolution time during the 1.5-ms REBURP pulse and the centered 90_x-210_y-90_x composite ^{15}N inversion pulse was calculated to be 1.3 ms using the program SIGMA (Novak et al. 2010). All other details are identical to those described for the deuterated version of the pulse scheme (Fig. 1, main text).

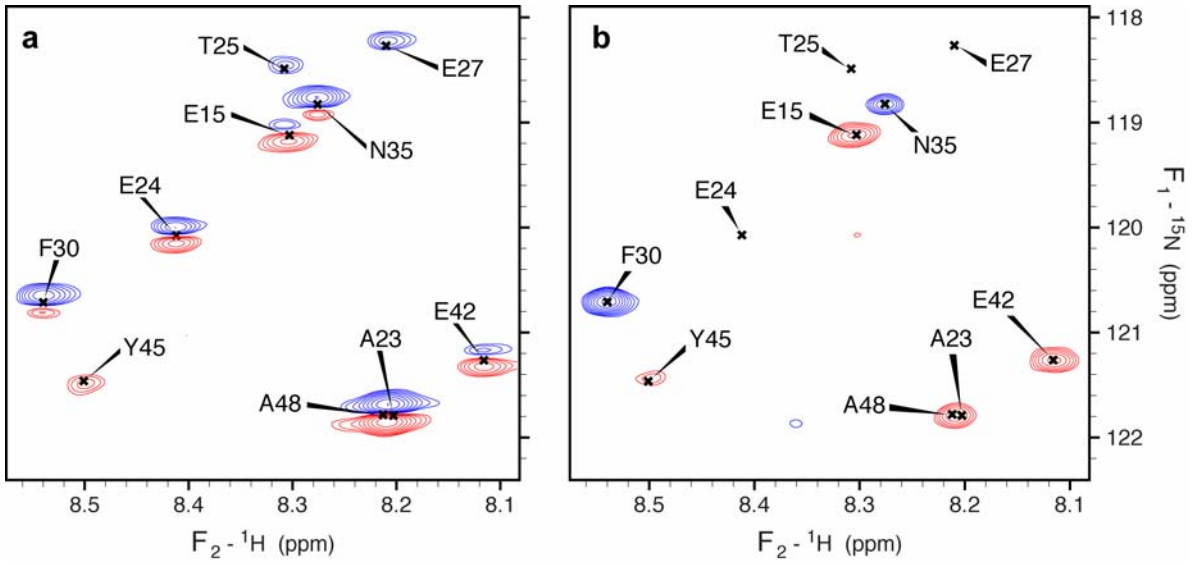

Figure S3. Spectral regions of the J-dephased (attenuated) ARTSY spectra, recorded on the ^{1}H, ^{13}C, ^{15}N-labeled, K19A V42E D47K mutant of GB3 under isotropic conditions at 600 MHz. Antiphase artifacts arising from long range ^{1}H-^{15}N couplings are present when the spectrum is (a) recorded with the scheme of Figure 1, main text, but absent (b) when recorded with the pulse scheme of Figure S2. In both panels, peak positions were identified using the non-attenuated reference spectra (not shown).

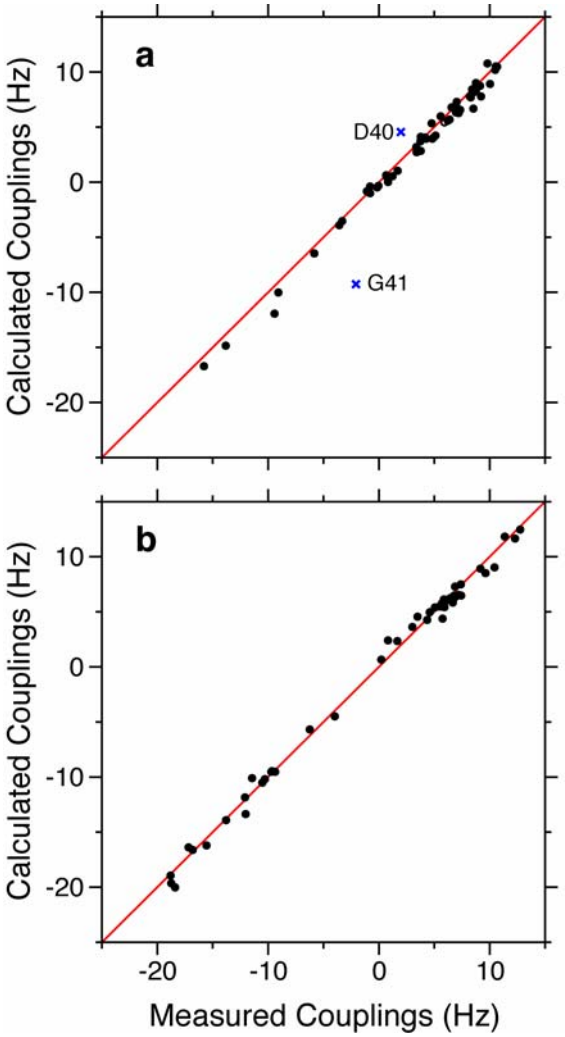

Figure S4. Comparison of measured $^{1}D_{NH}$ values in Pf1 for protonated and deuterated GB3. (a) Measured couplings in deuterated wild-type GB3 best-fitted to PDB entry 2OED (Ulmer et al. 2003). Residues 40 and 41 were excluded in the original 2OED refinement and were not used in fitting the alignment tensor. Couplings were measured at 600 MHz using the ^{15}N-^{1}H ARTSY sequence of Figure 1, main text. The resulting Q-factor is 0.092. (b) The same comparison for the protonated K19A V42E D47K mutant of GB3. RDCs were obtained using the ARTSY sequence modified for protonated proteins (Figure S2). Calculated couplings were determined using the N-H bond vector orientations, derived by Yao *et al*. (Yao 2010) for a set of six GB3 mutants. The Q-factor for this fit is 0.067, including all residues except for the overlapping amides of A23 and A48.

References

Geen H and Freeman R (1991) Band-selective radiofrequency pulses. J. Magn. Reson. 93: 93-141

Novak P, Zidek L, Motackova V, Padrta P, Svenkova A, Nuzillard JM, Krasny L and Sklenar V (2010) S3EPY: a Sparky extension for determination of small scalar couplings from spin-state-selective excitation NMR experiments. J. Biomol. NMR 46: 191-197

Ulmer TS, Ramirez BE, Delaglio F and Bax A (2003) Evaluation of backbone proton positions and dynamics in a small protein by liquid crystal NMR spectroscopy. J. Am. Chem. Soc. 125: 9179-9191

Yao LS, Ying JF and Bax A (2009) Improved accuracy of N-15-H-1 scalar and residual dipolar couplings from gradient-enhanced IPAP-HSQC experiments on protonated proteins. J. Biomol. NMR 43: 161-170

Table S1. $^{1}J_{NH}$ and $^{1}D_{NH}$ values measured at 900 MHz for IN^{50-212} in 4% PEG/hexanol.

Residue		$^{1}J_{NH}$	σ_{JNH}	$^{1}D_{NH}$	σ_{DNH}
52	GLY	-94.2	0.2	0.0	0.2
53	GLU	-92.6	0.0	-0.1	0.1
54	VAL	-92.6	0.1	-1.0	0.1
55	ASP	-93.1	0.1	-2.2	0.2
56	SER	-92.5	0.3	-3.0	0.4
57	SER				
58	PRO				
59	GLY	-94.6	0.6	0.9	0.8
60	ILE	-92.6	0.4	10.8	0.5
61	TRP	-93.5	0.6	6.7	0.8
62	GLN				
63	LEU				
64	ASP	-94.4	1.2		
65	CYS				
66	THR	-93.4	0.3	-17.7	0.4
67	HIS	-93.3	0.3	-7.7	0.4
68	LEU				
69	GLU	-93.9	0.2	-6.7	0.2
70	GLY	-94.2	0.2	-7.0	0.3
71	LYS	-92.1	0.2	6.8	0.2
72	VAL	-92.4	0.2	-5.7	0.2
73	ILE	-93.2	1.0	-14.8	2.1
74	LEU	-90.1	1.4		
75	VAL				
76	ALA	-93.0	1.2		
77	VAL	-93.3	1.8		
78	HIS	-94.0	0.5	0.8	0.8
79	VAL	-92.9	0.4	8.9	0.5
80	ALA	-93.2	0.6	6.7	0.8
81	SER	-94.4	1.6	11.4	2.3
82	GLY	-93.8	0.6	4.7	0.9
83	TYR	-92.4	0.7	7.3	1.2
84	ILE				
85	GLU	-90.1	1.0	-14.8	1.5
86	ALA				
87	GLU	-93.5	0.3	-14.0	0.6
88	VAL				
89	ILE	-94.8	0.2	-12.1	0.3
90	PRO				
91	ALA	-92.3	0.2	6.4	0.4
92	GLU	-92.9	0.3	3.4	0.4
93	THR	-92.6	0.3	-10.6	0.5
94	GLY				
95	GLN	-93.5	0.7	5.3	0.9
96	GLU	-94.1	0.8	-6.6	1.5
97	THR	-94.3	1.2	-0.4	1.7
98	ALA	-93.0	0.4	5.9	0.7
99	TYR	-93.8	0.4	4.3	0.6

Residue		$^1J_{NH}$	σ_{JNH}	$^1D_{NH}$	σ_{DNH}
100	PHE	-93.0	0.5	-2.0	0.8
101	LEU	-93.9	0.5	3.0	0.7
102	LEU				
103	LYS	-94.7	0.6	4.2	0.7
104	LEU	-93.6	0.6	0.7	0.9
105	ALA	-93.3	0.3	6.7	0.4
106	GLY	-95.2	0.5	9.5	0.8
107	ARG	-93.7	1.0	-3.3	1.3
108	TRP	-93.3	1.1		
109	PRO				
110	VAL	-93.7	0.3	-4.7	0.4
111	LYS	-92.5	0.7	5.6	1.3
112	THR	-91.0	0.5	3.4	0.9
113	VAL	-92.1	0.3	4.9	0.4
114	HIS	-92.2	0.4	-0.8	0.6
115	THR	-93.9	0.3	0.7	0.5
116	ASP				
117	ASN	-93.1	0.4		
118	GLY	-95.7	0.7	8.0	1.1
119	SER	-93.9	1.1	1.0	1.6
120	ASN	-94.5	0.3	4.5	0.4
121	PHE	-93.8	0.6	-6.6	0.8
122	THR				
123	SER	-92.6	0.3	6.6	0.4
124	THR				
125	THR				
126	VAL	-92.5	0.3	-6.2	0.4
127	LYS	-93.7	0.4	1.9	0.5
128	ALA	-93.7	0.2	6.3	0.2
129	ALA	-94.1	0.3	0.7	0.4
130	CYS	-94.9	0.7	-0.6	0.9
131	GLU	-95.5	0.6	7.7	0.8
132	TRP	-93.4	0.4	5.2	0.6
133	ALA	-92.8	0.6	-0.9	0.7
134	GLY	-94.3	0.5	6.2	0.6
135	ILE	-92.1	0.4	8.3	0.5
136	LYS	-92.3	0.3	7.1	0.4
137	GLN	-92.0	0.2	7.3	0.3
138	GLU	-91.9	0.3	-3.5	0.4
139	PHE	-91.8	0.2	2.9	0.2
140	GLY	-94.1	0.3	-3.6	0.4
141	ILE	-92.8	0.2	-0.4	0.3
142	PRO				
143	TYR	-92.6	0.6	1.9	0.8
144	ASN	-93.0	0.2	-0.4	0.3
145	PRO				
146	GLN	-92.6	0.2	0.0	0.2
147	SER	-92.7	0.4	-1.6	0.4
148	GLN	-92.7	0.3	-2.7	0.4

Residue		$^1J_{NH}$	σ_{JNH}	$^1D_{NH}$	σ_{DNH}
149	GLY	-94.6	0.3	1.2	0.4
150	VAL	-92.5	0.6	5.5	0.9
151	ILE	-92.6	0.4	-0.4	0.5
152	GLU	-93.0	0.7		
153	SER				
154	MET	-92.9	0.5	2.8	0.8
155	ASN	-94.2	0.7	-2.8	0.9
156	LYS				
157	GLU	-93.4	0.5	4.6	0.7
158	LEU	-93.2	0.6	-2.1	0.8
159	LYS	-92.4	0.6	-7.9	0.8
160	LYS	-93.5	1.2	5.7	2.0
161	ILE	-93.3	0.6	3.4	1.1
162	ILE	-93.1	0.9	-5.4	1.3
163	GLY	-94.6	0.6	-5.7	0.9
164	GLN	-92.8	0.4	4.9	0.6
165	VAL	-89.5	1.3	-4.3	2.1
166	ARG	-92.3	0.4	-11.9	0.7
167	ASP	-93.6	0.5	0.6	0.6
168	GLN	-91.2	0.3	-5.5	0.5
169	ALA				
170	GLU	-93.4	0.4		
171	HIS	-92.8	0.2	-2.7	0.3
172	LEU				
173	LYS				
174	THR	-92.3	0.7	11.3	1.4
175	ALA	-96.8	1.9		
176	VAL	-93.6	0.3	8.8	0.5
177	GLN	-92.7	0.4	7.1	0.7
178	MET	-93.4	0.4		
179	ALA	-94.3	0.4	8.4	0.5
180	VAL	-94.2	0.5	8.1	0.6
181	PHE	-94.1	0.5	8.8	0.7
182	ILE	-93.9	0.5	9.1	0.6
183	HIS	-93.6	0.8	9.2	1.1
184	ASN	-95.0	0.6	7.2	0.8
185	LYS	-93.4	0.4		
186	LYS	-93.8	0.4	5.1	0.6
187	ARG	-92.7	0.3	-0.7	0.5
188	LYS	-92.7	0.1	4.5	0.2
189	GLY	-94.2	0.3	1.9	0.4
190	GLY	-94.4	0.1	1.1	0.1
191	ILE	-92.7	0.1	0.5	0.1
192	GLY	-94.0	0.1	-4.5	0.1
193	GLY	-94.0	0.1	0.6	0.1
194	TYR	-92.6	0.1	-8.1	0.2
195	SER	-93.5	0.4	-9.5	0.6
196	ALA	-92.7	0.4	6.3	0.5
197	GLY	-94.1	0.5	8.4	0.6

Residue		$^1J_{NH}$	σ_{JNH}	$^1D_{NH}$	σ_{DNH}
198	GLU	-93.8	0.4	3.9	0.6
199	ARG	-93.5	0.4	6.5	0.5
200	ILE	-93.9	0.4	8.6	0.6
201	VAL	-92.6	0.5	8.4	0.8
202	ASP	-94.4	0.6	8.0	0.8
203	ILE	-93.6	0.5	7.4	0.7
204	ILE	-94.3	0.6	8.2	0.8
205	ALA	-93.4	0.6	8.4	0.8
206	THR				
206b	THR	-89.6	2.1	-8.4	2.6
207	ASP	-93.5	0.3	6.3	0.4
207b	ASP				
208	ILE	-92.8	0.2	4.0	0.3
208b	ILE	-93.5	0.5	-0.7	0.7
209	GLU	-93.2	0.2	2.3	0.3
209b	GLU	-92.7	0.6	-1.6	0.8
210	THR	-92.8	0.1	-0.3	0.1
210b	THR				
211	LYS	-92.6	0.1	-0.4	0.1
211b	LYS	-92.1	0.6	-2.4	0.7
212	GLU	-92.0	0.0	-1.3	0.0
212b	GLU	-92.0	0.2	-1.3	0.3

```
; PULSE SEQUENCE
; Parameters, processing, and analysis scripts are on
http://spin.niddk.nih.gov/bax/pp/ under ARTSY
; title:   artsy-qjnh.pp
; summary: ARTSY-2D TROSY for measuring JNH
; author:  Nick Fitzkee (nfitzkee@nih.gov)
; date:    June 29, 2010

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

;--- SWITCH -----
#define CARBON_LABEL
#define INTERLEAVE

#define H f1
#define N f3
#define C1 f2    ; 13C centered at 76 ppm (if available)
#define C2 f2    ; 13C centered at 156 ppm

define pulse PG1
define pulse PG3
define pulse PG4
define pulse PG5
define pulse PG6
define pulse PG7

;Gradient Pulses
"PG1=1m"       ;half-encoding
"PG3=500u"
"PG4=500u"     ;half-encoding
"PG5=250u"
"PG6=800u"
"PG7=101.3u"   ;decoding

"l5=1"
"l6=1"

"d0=3u"                          ;use if set up for (0,0) 15N phase

#ifdef CARBON_LABEL
"DELTA4=p14*2+4u+d0*2+p21*1.274-p1"   ;phc0=0 phc1=0 aliased peaks same sign
;"d0=in0*0.5-p21*0.637-2u-p14+p1*0.5" ;ph0=90deg ph1=-180deg and semi-colon
out DELTA4, below
#else
"DELTA4=d0*2+2u+p21*1.274-p1"    ;phc0=0 phc1=0  aliased peaks same sign
;"d0=in0*0.5-p21*0.637-1u"       ;ph0=90deg ph1=-180deg and semi-colon out
DELTA4, below
#endif

"p2=p1*2"
"p22=p21*2"

"d11=30m"
"d12=100u"
"d16=190u"                       ;Gradient recovery
"d28=p6+5u+p1-p21"

; Delays to compensate composite 15N 180
```

```
"d29=p21*4.34"

; Adjust for Clean TROSY (with phcor14) - total time for 1st S3E element
"d25=2.71m"

; Total time of 1st transfer step (1/JNH)
; Adjust this so 2nd FID is a null for isotropic sample
; In the attenuated spectrum, the coupling will evolve for d27, and in
; the reference spectrum, it will evolve for d27*0.5 + 0.637*p1.
"d27=10.75m"

"DELTA1=d27-d29*3-p1*1.237-80u"
"DELTA2=d25-PG5-p29-d16"
"DELTA3=2.71m-PG6-p6-205u"
"DELTA5=300u-PG7-20u-p1-3u"

; Comment out when multiple SGUs in use for C1/C2
; You will need to set the offset of sp3 manually (to 56 ppm)
"cnst21=176"
"cnst22=56"
"spoff3=bf2*((cnst22-cnst21)/1000000)"
"spoff5=0"


1       ze
        1m
2       10u do:C2
        1m BLKGRAD
        d11
        d12*6
3       d12*6
4       d12*6
5       d1
        1m UNBLKGRAD
        10u pl0:H
        10u pl3:N
        10u pl0:C1
        10u pl0:C2

;- start 90-degree on hn -------------------------------------

       (p21 ph0):N              ; along with cycling ph5,
        3u                      ; this thoroughly eliminates
        PG1:gp1                 ; 15N Boltzmann magnetization
        500u

       (p29:sp12 ph11:r):H
        3u
        3u pl1:H
10     (p1 ph5):H
        5u
        5u gron2
        DELTA1*0.25
        10u groff
        d29*2
        5u
        5u gron2
        DELTA1*0.25
        10u groff

        if "l6==1" goto 20
       (p2 ph0):H
       (p21 ph0 p21*2.34 ph1 p21 ph0):N
        5u
```

```
        5u gron2
        DELTA1*0.25
        10u groff
        d29
        goto 25

20      (p2 ph0):H
        d29*0.75
        5u
        5u gron2
        DELTA1*0.25
        10u groff
       (p21 ph0 p21*2.34 ph1 p21 ph0):N
        d29*0.25

25      5u
        5u gron2
        DELTA1*0.25
        10u groff
       (p1 ph1):H
30      3u

;goto 100               ;WARNING - make sure pl12 is set properly for C' dec
;                                  or comment out C' AQ cpd before using this
;                                  statement.  Ditto for all 'goto 100's'.
        PG3:gp3
        d16

;- quantitative-J finished, start 15N evolution ---------------

       if "l5==1" {
       (p21 ph17):N
        } else {
       (p21 ph7):N
        }
        d0

#ifdef CARBON_LABEL
       (p14:sp3 ph0):C1
        4u
       (p14:sp5 ph0):C2
#else
        2u
#endif
        d0

        PG4:gp4*EA        ;half-encoding pulse
        200u
       if "l5==1" {
       (p22 ph17):N
         } else {
       (p22 ph7):N
         }
        PG4:gp4*EA*-1     ;other half of encoding pulse
        200u
        DELTA4            ;comment out if 90,180 delay selected

;- start final TROSY back-transfer ----------------------------

       (p1 ph12):H
        3u
        2u pl0:H
       (p29:sp1 ph21:r):H
;goto 100
```

```
        5u
        PG5:gp5
        DELTA2
        d16 pl1:H
       (center (p2 ph0):H (p22 ph0):N)
        5u
        DELTA2
        PG5:gp5
        d16 pl0:H
       (p29:sp2 ph22:r):H
        3u
        2u pl1:H
       (p1 ph0):H (p21 ph15):N
        5u
;goto 100
        PG6:gp6
        195u pl7:H
        DELTA3
       (150u p6 ph10:r 3u 2u pl1 p2 ph0 3u 2u pl7 p6 ph10:r):H (d28 p22
ph15):N
        3u
        2u pl1:H
;goto 100
        PG6:gp6
        DELTA3
        45u
       (p21 ph14:r):N
        3u
        PG7:gp7               ;decoding gradient
        DELTA5
100     5u
        5u pl12:C2
        10u BLKGRAD
#ifdef CARBON_LABEL
        go=2 ph31 cpds2:C1
#else
        go=2 ph31
#endif
        10u do:C2
        1m BLKGRAD
        d11 wr #0 if #0 zd
#ifdef INTERLEAVE
        d12*6 iu6
       lo to 3 times 2
#else
        d12*6
#endif
        d12 igrad EA
        d12 ip12*2
        d12 ip21*2
        d12 ip14*2
        d12 iu5
        d12 ru6
       lo to 4 times 2
        d12 ip7*2
        d12 ip17*2
        d12 ip31*2
        d12 id0
        d12 ru5
        d12
       lo to 5 times l3

1m do:C1
1m do:N
```

```
1m BLKGRAD
exit

ph0=0
ph1=1
ph2=2
ph3=3

ph5=0 2
ph7=1 1 3 3
ph10=2
ph11=3
ph12=3
ph14=3 3 3 3 1 1 1 1
ph15=0 0 0 0 2 2 2 2
ph17=1 1 3 3
ph21=1
ph22=0
ph31=1 3 3 1 3 1 1 3


;---- NOTES -----

; tested on Bruker AVIII TS2-PL3 consoles
; Note: when used on older dmx/drx consoles, add 3u between pulses
; in the 15N composite pulse (and adjust d29, d30)

; All "goto 100" statements are at points where water flip-backs can
; be tuned.  See note above at first statement.

;1H pulses (H=f1, center on H2O):
;p1 = 90 deg (10us) hard 1H pulse @ pl1
;p6 = 1.2ms soft 90 deg pulse @ pl7
;p29 = 1.9m @ sp1, sp2, and sp12  (spnam1 = sinc1.0)

;15N pulses (N=f3, carrier=119ppm):
;p21 = 90 deg (~50us) 15N pulse @pl3

;13C pulses (C1=C2=f2, carrier=176ppm; must modify for two channels):
;p14 = selective 180 deg (23.7*2us @ 600MHz)
;spnam3 and spnam5 = RECT.1000  (~1000 points req'd for spoffs)
;cpdprg2 = garp  (aq C' dec program for 13C samples)
;pcpd2   = pw for C' aq dec (140u) @ pl12

;N  SW = 1/(2*in0)
;echo-antiecho in N15

;RECT pulse pairs do not work well on an AVIII
;gpz1 = +33%  sine.100   Boltzmann killer
;gpz2 = +0.5  (gron2)
;gpz3 = +17%  sine.100   zz crusher gradient
;gpz4 = +70%  sine.20    encoding gradient
;gpz5 = +15%  sine.50
;gpz6 = +23%  sine.50
;gpz7 = +70%  sine.20    decoding gradient
```