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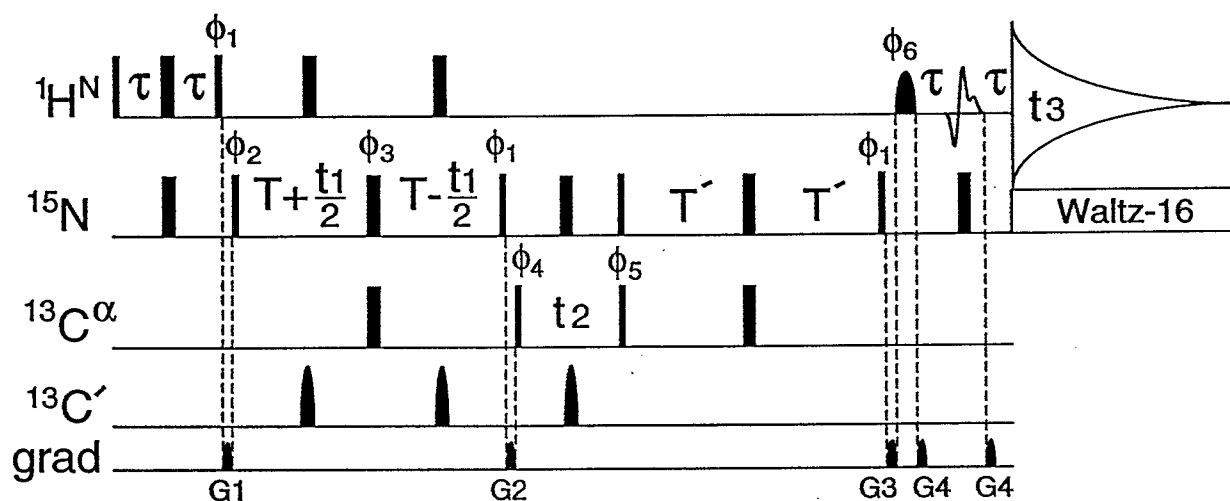


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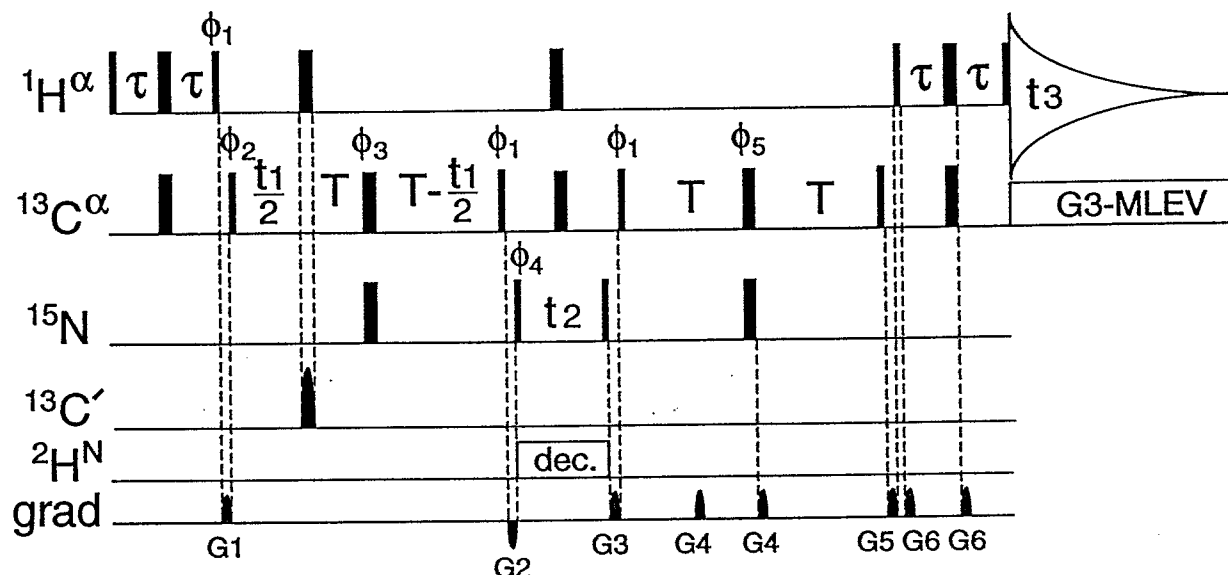
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HNCA[HA] - E.COSY

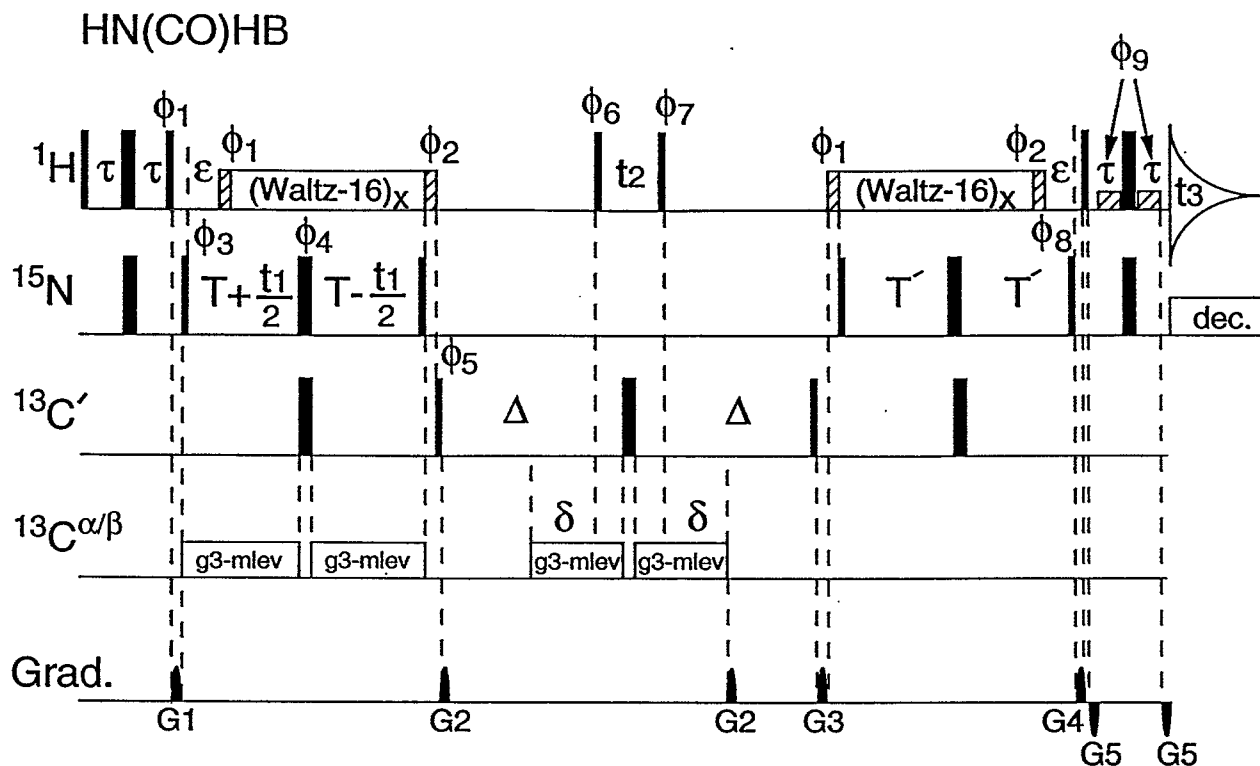


Supplementary Figure 1. Pulse sequence of the HNCA[HA]-E.COSY experiment. Pulses for which the phase is not indicated are applied along the x axis. The ^1H carrier is positioned at 8.36 ppm. The last two ^1H pulses are applied with power levels adjusted for minimal excitation of H_2O . The pulse with phase ϕ_6 has an amplitude profile shaped to correspond to the center lobe of a $\sin x/x$ function, a duration of 720 μs , and a flip angle of 90° . The final ^1H pulse is a Q3 180° shaped pulse (Emsley, L.; Bodenhausen, G. *J. Magn. Reson.* 1992, 97, 135-148) and has a duration of 1.78 ms. The carriers for the $^{13}\text{C}^{\alpha}$ and $^{13}\text{C}'$ pulses are positioned at 58 and 177 ppm, respectively. Carbonyl pulses have a shaped amplitude profile, corresponding to the center lobe of a $\sin x/x$ function and have 180° flip angles. Except for the $^{13}\text{C}'$ pulse during $^{13}\text{C}^{\alpha}$ evolution, which has a duration of 300 μs , all others have a duration of 195 μs . For the remainder, narrow and wide rectangular pulses correspond to 90° and 180° flip angles, respectively. The RF field strengths of the 90° and 180° $^{13}\text{C}^{\alpha}$ pulses are adjusted to 4.6 and 10.4 kHz such that they do not excite the $^{13}\text{C}'$ nuclei (for 150.9 MHz ^{13}C). The carrier for the ^{15}N pulses is positioned at 117.8 ppm. The high power ^{15}N pulses have a RF field strength of 5.3 kHz. Waltz-16 ^{15}N decoupling during acquisition was performed using a 1.4 kHz RF field. Pulsed field gradients are sine-bell shaped, with a peak amplitude of 25 G/cm. Phase cycling is as follows: $\phi_1 = y$; $\phi_2 = x$; $\phi_3 = x, y, -x, -y$; $\phi_4 = x$; $\phi_5 = x, x, -x, -x$; $\phi_6 = 4(x), 4(-x)$; Acq = $x, -x, -x, x, -x, x, x, -x$. Quadrature in the t_1 and t_2 domains is obtained by changing the phases ϕ_2 and ϕ_4 , respectively, in the usual States-TPPI manner. Delay durations are $\tau = 2.25$ ms, $T = 13.62$ ms, and $T' = 11$ ms. Pulsed field gradient durations are $G1 = 2$ ms, $G2 = 1.5$ ms, $G3 = 1$ ms, and $G4 = 0.2$ ms.

HCAN[C'] - E.COSY

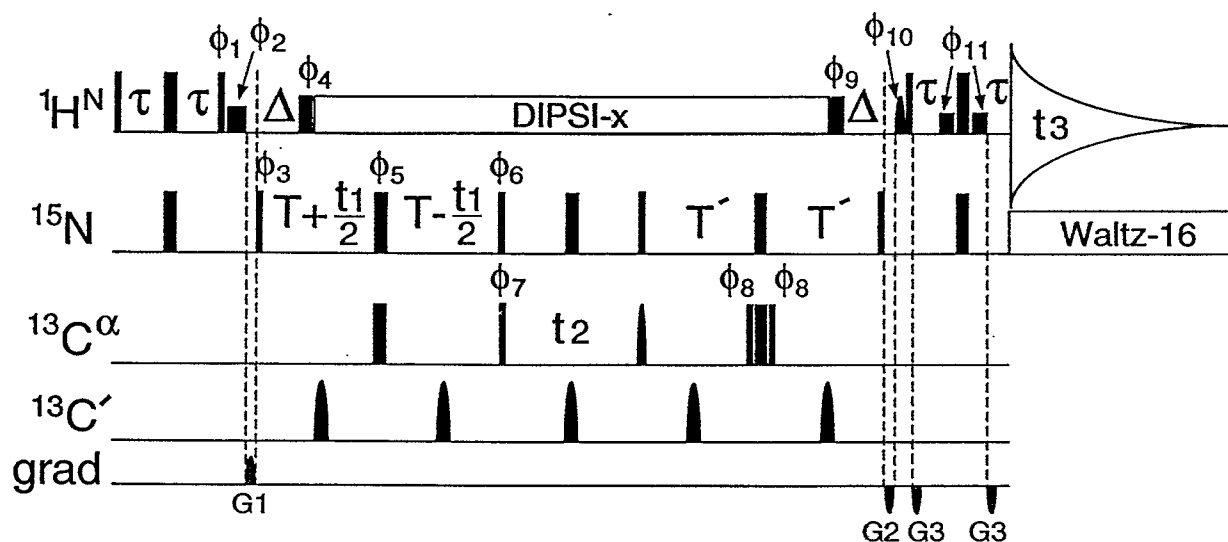


Supplementary Figure 2. Pulse sequence of the HCAN[C']-E.COSY experiment. Pulses for which the phase is not indicated are applied along the x axis. Narrow and wide rectangular pulses correspond to 90° and 180° flip angles, respectively. The ^1H carrier is positioned on the HDO resonance and the carriers for the $^{13}\text{C}^\alpha$ and $^{13}\text{C}'$ pulses are positioned at 58 and 177 ppm, respectively. The RF field strengths of the 90° and 180° $^{13}\text{C}^\alpha$ pulses are adjusted to 4.6 and 10.4 kHz such that they do not excite the $^{13}\text{C}'$ nuclei (for 150.9 MHz ^{13}C). The carbonyl pulse, with an amplitude profile corresponding to the center lobe of a $\sin x/x$ function, has a 180° flip angle, and a duration of 195 μs . The carrier for the ^{15}N pulses is positioned at 117.8 ppm and all ^{15}N pulses are applied using a RF field strength of 5.5 kHz. $^{13}\text{C}^\alpha$ decoupling was performed with a MLEV-sequence using G3 pulse cascades²⁵ adjusted such that there is no excitation of the $^{13}\text{C}'$ nuclei (each 180° G3 pulse has a duration of 512 μs). Pulsed field gradients are sine-bell shaped, with a peak amplitude of 25 G/cm. Phase cycling is as follows: $\phi_1 = y$; $\phi_2 = x$; $\phi_3 = x, x, y, y$; $\phi_4 = x, -x$; $\phi_5 = 3^\circ$; Acq = $x, -x, -x, x$. Phase ϕ_5 is programmed on the AMX-600 spectrometer as a non-integral multiple of 90° in order to compensate for the change in phase associated with the change in power level. Quadrature in the t_1 and t_2 domains is obtained by changing the phases ϕ_2 and ϕ_4 , respectively, in the usual States-TPPI manner. ^2H decoupling (2 W) is accomplished using a 1.7 kHz RF field, centered in the amide region of the deuterium spectrum (Wang, A. C.; Grzesiek, S.; Tschudin, R.; Lodi, P. J.; Bax, A. J. *Biomol. NMR*. 1995, 5, 376-382). Delay durations are $\tau = 1.5$ ms and $T = 14.28$ ms. Gradient strengths are $G1 = 1.5$ ms, $G2 = 0.5$ ms (negative polarity), $G3 = 1$ ms, $G4 = 2$ ms, $G5 = 0.5$ ms, and $G6 = 0.2$ ms.



Supplementary Figure 3. Pulse sequence of the improved HN(CO)HB experiment. Pulses for which the phase is not indicated are applied along the x axis. The low power ^1H pulses, partially shaded rectangles in the figure, all have 90° flip angles. For the remainder, narrow and wide rectangular pulses correspond to 90° and 180° flip angles, respectively. The ^1H carrier is positioned at the HDO frequency. The carriers for the $^{13}\text{C}^\alpha$ and $^{13}\text{C}'$ pulses are positioned at 46 and 177 ppm, respectively. The RF field strength of the $^{13}\text{C}'$ pulses is adjusted to 8.8 kHz such that the final 180° pulse has a null at 56 ppm (for a 500 MHz NMR spectrometer). $^{13}\text{C}^\alpha/\beta$ decoupling was accomplished with a MLEV composite G3 pulse cascade²⁵ using $614\ \mu\text{s}$ 180° G3 pulses (Emsley, L.; Bodenhausen, G. *Chem. Phys. Lett.* **1990**, *165*, 469-476). The carrier for the ^{15}N pulses is positioned at 116.5 ppm. ^{15}N pulses have an RF field strength of 5.3 kHz. Waltz-16 ^{15}N decoupling during acquisition was performed using a 1.4 kHz RF field. Pulsed field gradients are sine-bell shaped, with a peak amplitude of 25 G/cm. Phase cycling is as follows: $\phi_1 = y$; $\phi_2 = -y$; $\phi_3 = x$; $\phi_4 = x, y, -x, -y$; $\phi_5 = x, x, -x, -x$; $\phi_6 = x$; $\phi_7 = 8(x), 8(-x)$; $\phi_8 = 4(x), 4(-x)$; $\phi_9 = -x$; Acq = P, -P, -P, P (P = x, -x, -x, x). For acquisition of the 2D reference spectrum, Acq = P, -P. Quadrature in the t_1 and t_2 domains is obtained by changing the phases ϕ_3 and ϕ_6 , respectively, in the usual States-TPPI manner. Delay durations are $\tau = 2.25\ \text{ms}$, $\varepsilon = 5.4\ \text{ms}$, $T = 13.52\ \text{ms}$, $\Delta = 25\ \text{ms}$, $\delta = 7\ \text{ms}$, and $T' = 10\ \text{ms}$. Low power pulses with phases ϕ_1 and ϕ_2 and the ^1H Waltz-16 decoupling have a RF field strength of 4.8 kHz. Pulses with ϕ_9 have an RF field strength of 266 Hz. Gradient durations are $G1 = 5\ \text{ms}$, $G2 = 3.5\ \text{ms}$, $G3 = 2.5\ \text{ms}$, $G4 = 1.5\ \text{ms}$, and $G5 = 0.25\ \text{ms}$ (negative polarity). Note that, owing to software limitations, this pulse sequence did not function properly on our NIH Bruker AMX spectrometer (software UXNMR, version 940320) but has been executed successfully on a Bruker DMX-500 instrument.

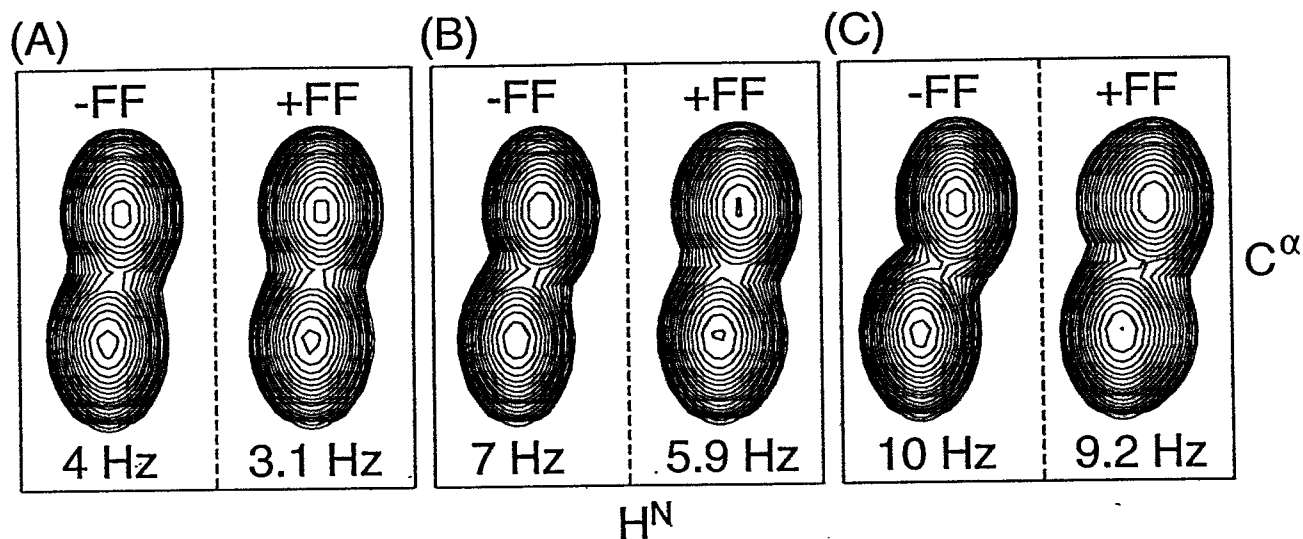
HNCA[CB] - E.COSY



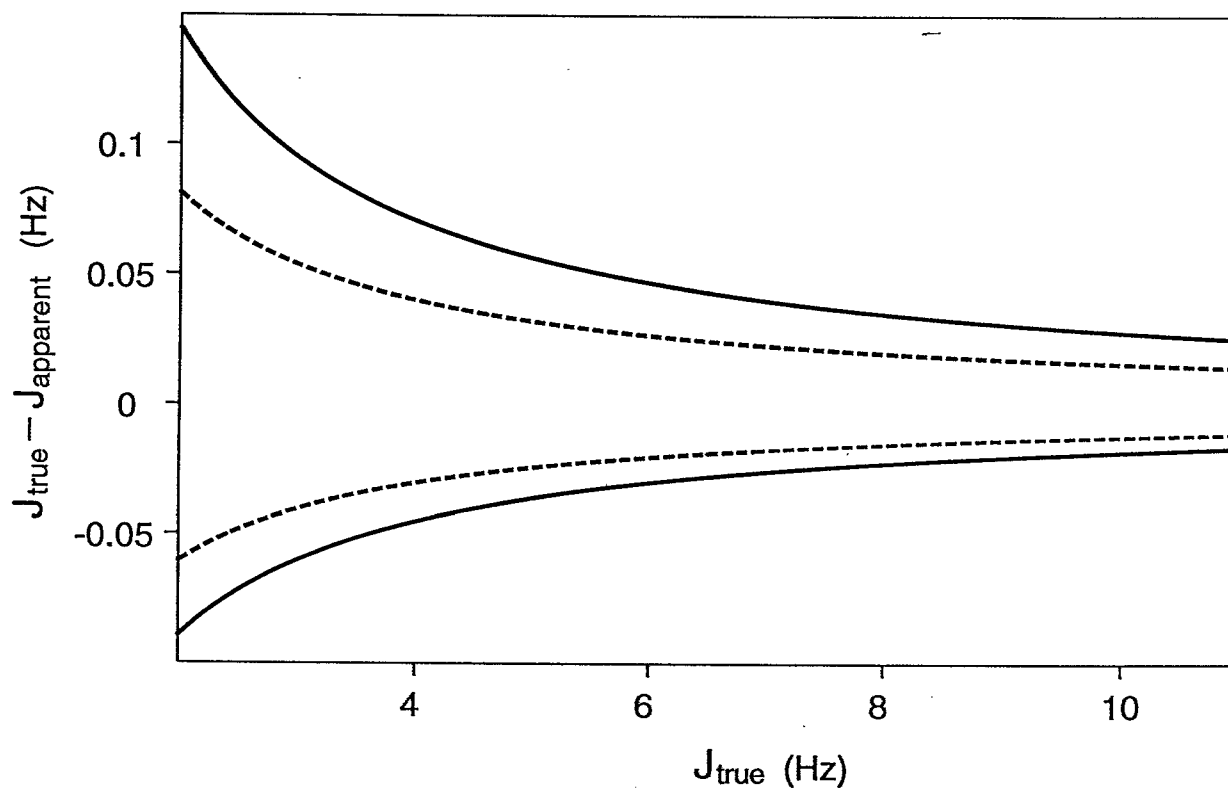
Supplementary Figure 4. Pulse sequence of the HNCA[CB]-E.COSY experiment. Pulses for which the phase is not indicated are applied along the x axis. The low power pulses with phases ϕ_2 , ϕ_4 , ϕ_9 , ϕ_{10} , and ϕ_{11} , all have 90° flip angles. The center pulse of the $^{13}\text{C}^\alpha$ composite pulse has a flip angle of 220° . For the remainder, narrow and wide rectangular pulses correspond to 90° and 180° flip angles, respectively. The ^1H carrier is positioned on the H_2O resonance. The carriers for the $^{13}\text{C}^\alpha$ and $^{13}\text{C}'$ pulses are positioned at 58 and 177 ppm, respectively. Except for the $^{13}\text{C}^\alpha$ shaped pulse (center lobe of $\sin x/x$ and duration of 753 μs , 90° flip angle, at 150.9 MHz ^{13}C) and the $^{13}\text{C}^\alpha$ composite 180° pulse (RF field = 17.9 kHz), the RF field strengths of the 90° and 180° $^{13}\text{C}^\alpha$ pulses are adjusted to 4.6 and 10.4 kHz such that they do not excite the $^{13}\text{C}'$ nuclei (for 150.9 MHz ^{13}C). Carbonyl pulses have 180° flip angles (195 μs) and have amplitude profiles shaped to correspond to the center lobe of a $\sin x/x$ function. The carrier for the ^{15}N pulses is positioned at 117.8 ppm. The ^{15}N high power pulses have an RF field strength of 5.3 kHz. Waltz-16 ^{15}N decoupling during acquisition was performed using a 1.3 kHz RF field. Phase cycling is as follows: $\phi_1 = y$; $\phi_2 = x$ (329°); $\phi_3 = x$; $\phi_4 = y$; $\phi_5 = x$, x , y , y , $-x$, $-x$, $-y$, $-y$; $\phi_6 = 4(x)$, $4(-x)$; $\phi_7 = x$, $-x$; $\phi_8 = y$; $\phi_9 = -y$; $\phi_{10} = 263^\circ$; $\phi_{11} = 149^\circ$; Acq = x , $-x$, $-x$, x , $-x$, x , x , $-x$. Phases ϕ_2 , ϕ_{10} , and ϕ_{11} are programmed on the AMX-600 spectrometer as non-integral multiples of 90° in order to compensate for changes in phase associated with changes in power level. Quadrature in the t_1 and t_2 domains is obtained by changing the phases ϕ_3 and ϕ_7 , respectively, in the usual States-TPPI manner. Delay durations are $\tau = 2.25$ ms, $\Delta = 5.4$ ms, $T = 13.62$ ms, and $T' = 11$ ms. Gradient durations are $G1 = 2.5$ ms, $G2 = 1$ ms (negative polarity), and $G3 = 0.25$ ms (negative polarity). Solvent suppression is accomplished by keeping the water magnetization along $+z$ between scans. This requires a combination of water flip-back pulses (90_{ϕ_2} , $90_{\phi_{10}}$, $90_{\phi_{11}}$) (Grzesiek, S.; Bax, A. *J. Am. Chem. Soc.* **1993**, *115*, 12593-12594), spin-locking the water magnetization along the x-axis (90_{ϕ_4} , DIPSI-2(x), 90_{ϕ_9}) (Kay, L. E.; Xu, G. Y.; Yamazaki, T. *J. Magn. Reson. Ser. A* **1994**, *109*, 129-133), and Watergate (Piotto, M.; Saudek, V.; Sklenar, V. *J. J. Biomol. NMR.* **1992**, *2*, 661-665). Pulses with phases ϕ_2 and ϕ_{11} have a RF field strength of 225 Hz and the pulse with phase ϕ_{10} has an amplitude profile corresponding to the center lobe of a $\sin x/x$ function and a duration of 2.9 ms. Pulses with phases ϕ_4 and ϕ_9 and the DIPSI-2 ^1H decoupling are applied with a RF field strength of 4.6 kHz.

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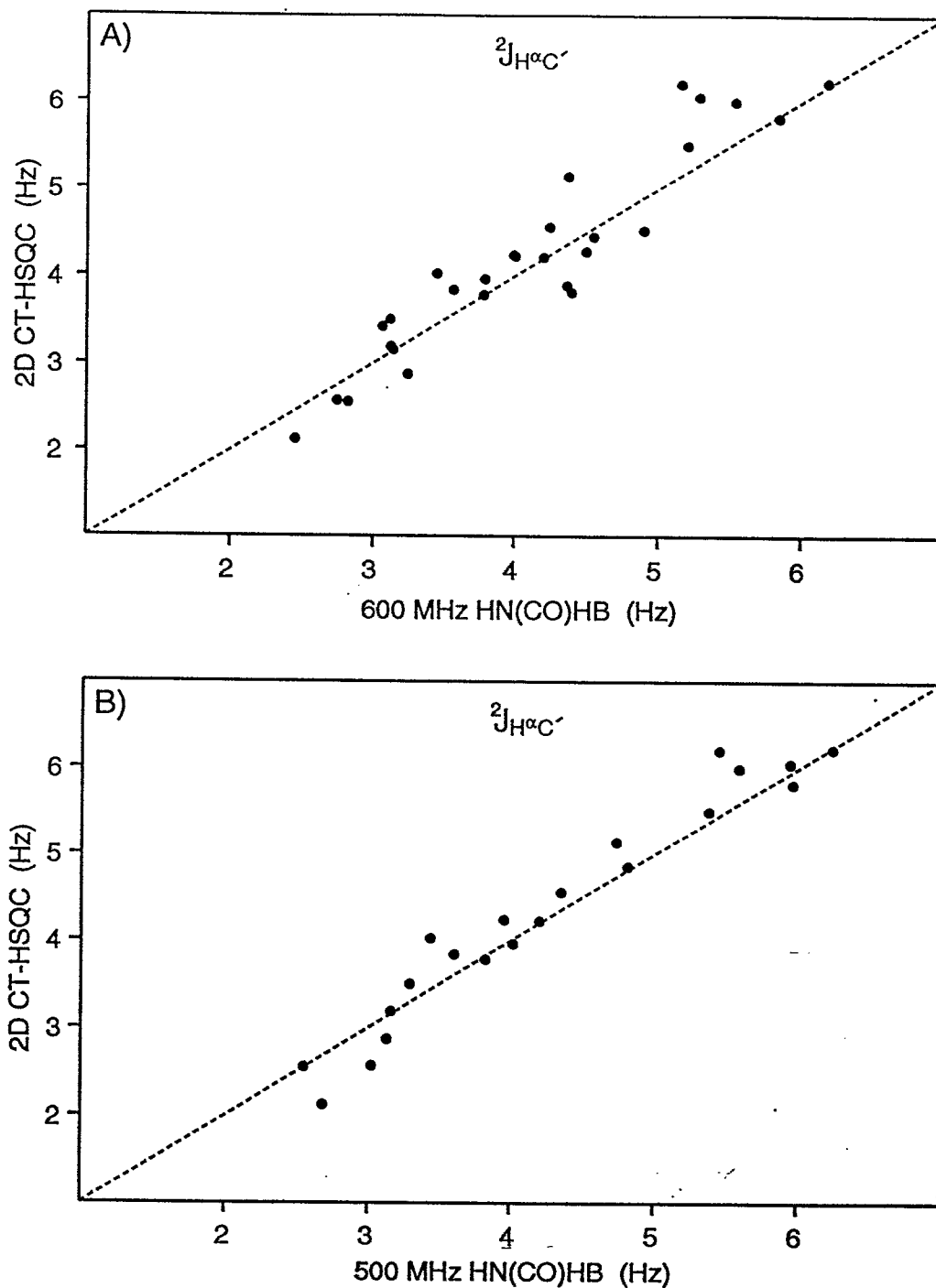


Supplementary Figure 5. Simulated HNCA[HA]-E.COSY intraresidue cross-peaks both with (+FF) and without (-FF) $^1H^{\alpha}$ spin flips for different values of $^3J_{HNH^{\alpha}}$. The average selective $R_{1\rho}$ of 4.6 s^{-1} for the $^1H^{\alpha}$ spins was used for the simulations. The true couplings are (A) 4 Hz, (B) 7 Hz, and (C) 10 Hz. The apparent couplings, reduced due to $^1H^{\alpha}$ spin flips, are (A) 3.1 Hz, (B) 5.9 Hz, and (C) 9.2 Hz, when using CAPP contour peak-picking as described in the experimental section.



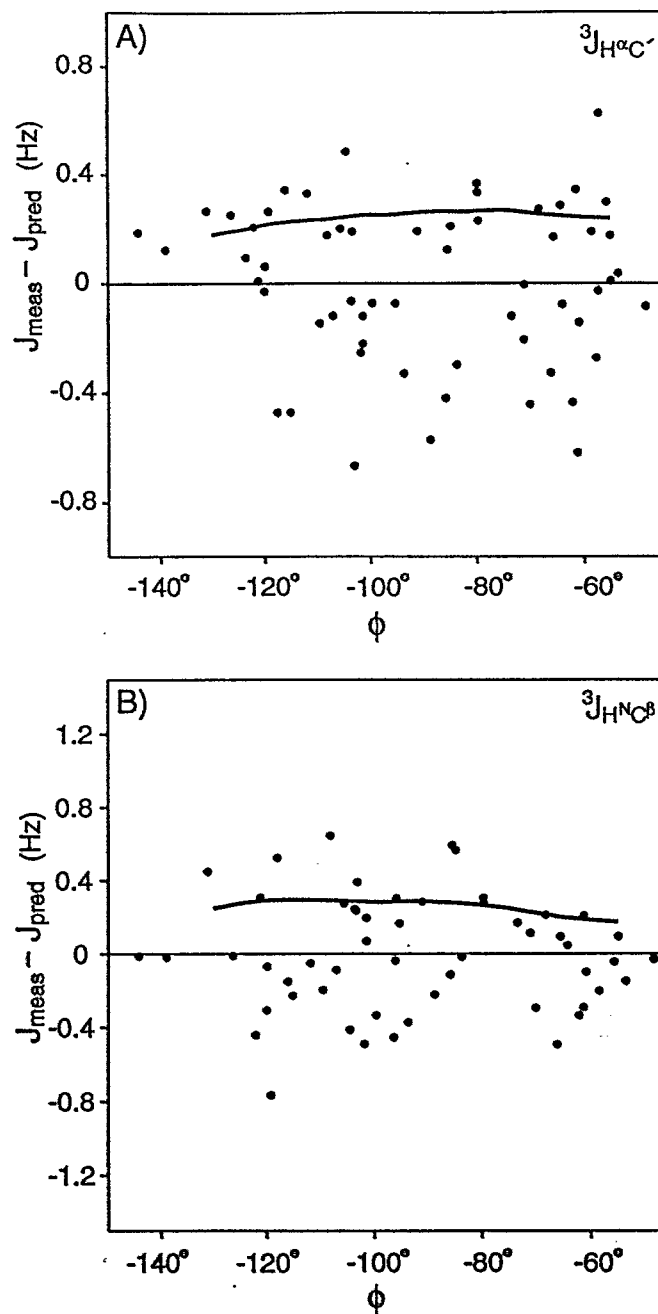
Supplementary Figure 6. $J_{\text{true}} - J_{\text{apparent}}$ versus J_{true} using eq 1 with a $T_{1\alpha}$ value of 217 ms for J_{true} and $T_{1\alpha}$ values of 152 and 382 ms (solid lines) and 172 and 294 ms (dashed lines) for J_{apparent} . The solid lines include 90% of the measured $T_{1\alpha}^{-1}$ values and the dashed lines include 70% (one standard deviation).

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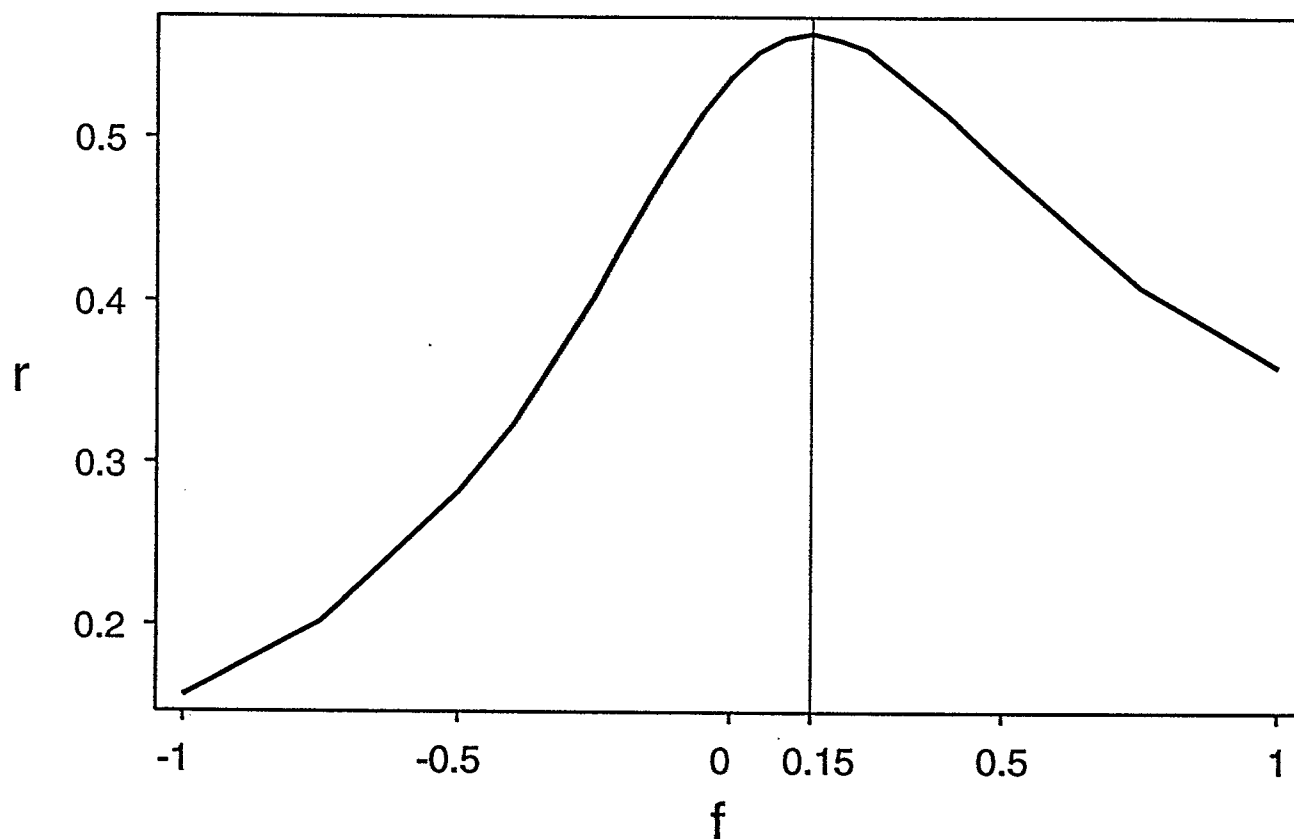
Supplementary Figure 7. Plots of $^2J_{H\alpha C'}$ from a 2D-HSQC E.COSY spectrum¹⁸ versus those from a 3D HN(CO)HB at (A) 600 MHz (pairwise rms difference = 0.29 Hz) and (B) 500 MHz (pairwise rms difference = 0.26 Hz).

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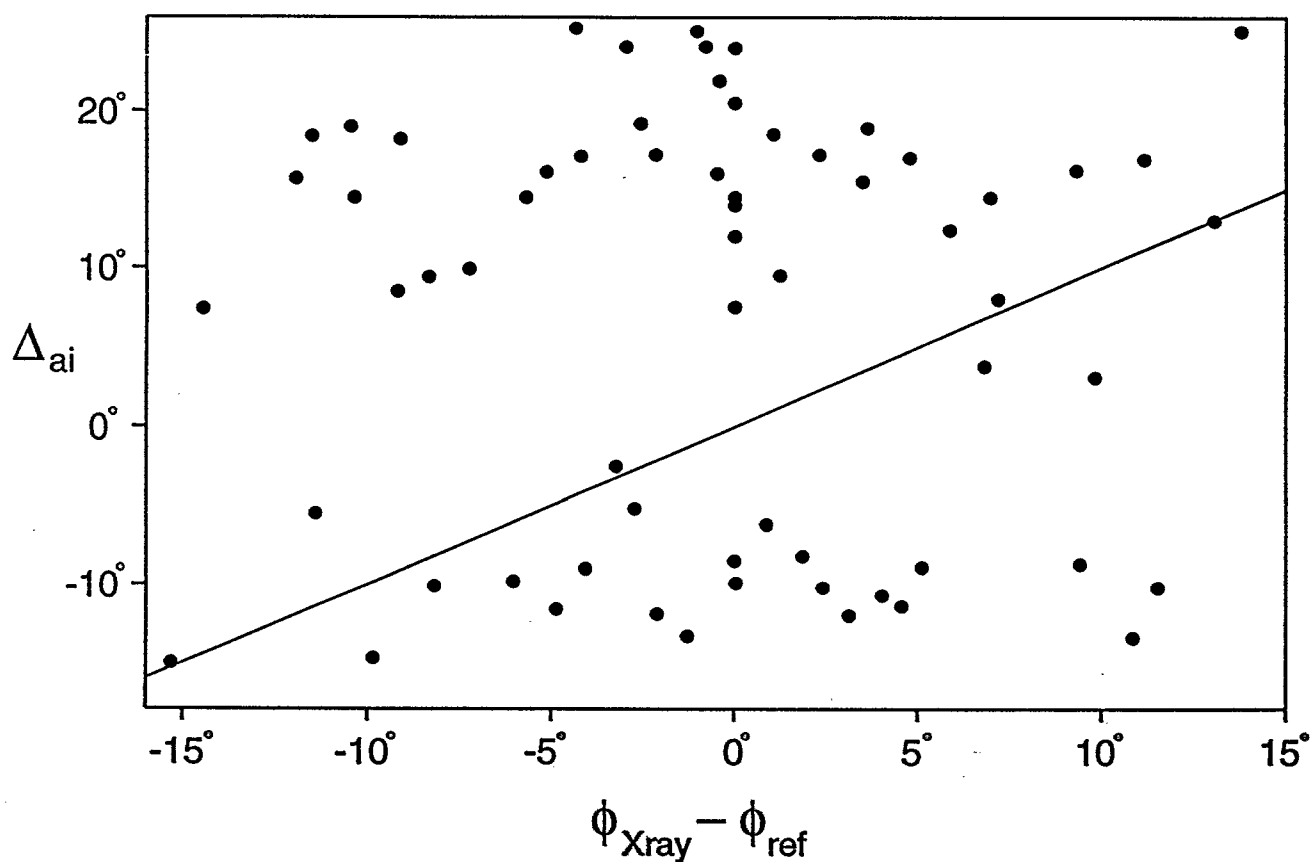


Supplementary Figure 8. Plots of the deviation between measured J values, J_{meas} , and those predicted by the Karplus curve, J_{pred} , for (A) ${}^3J_{\text{H}\alpha\text{C}'}$ and (B) ${}^3J_{\text{HNC}\beta}$, using X-ray ϕ angles. The solid line indicates the average deviation between measured and predicted J values has been generated as described in the legend to Figure 5 of the manuscript.

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Supplementary Figure 9. Correlation coefficient, r , between $J_{\text{meas}} - J_{\text{pred}}$ for $^3J_{\text{H}\alpha\text{C}}$ and $^3J_{\text{H}\text{N}\text{H}\alpha}$ (considering only residues in the range $-100^\circ \leq \phi \leq -60^\circ$, where both Karplus curves have a steep ϕ dependence), as a function of the fractional correction f used for adjusting the dihedral angles when calculating the best fit $^3J_{\text{H}\text{N}\text{H}\alpha}$ Karplus curve. Thus, only one Karplus curve was used for $^3J_{\text{H}\alpha\text{C}}$, but multiple f -dependent curves were calculated for $^3J_{\text{H}\text{N}\text{H}\alpha}$ by fitting all $^3J_{\text{H}\text{N}\text{H}\alpha}$ values measured for non-flexible residues to a Karplus curve, using dihedral angles $\phi - 60^\circ - f\Delta_{\text{ai}}$.



Supplementary Figure 10. Correlation between the difference of the X-ray and the NMR-refined ϕ angles ($\phi_{\text{XRAY}} - \phi_{\text{ref}}$) and the correction to the ϕ angle, Δ_{ai} , obtained from the plots presented in Figure 9 of Head-Gordon, T. et al. *J. Am. Chem. Soc.* **1991**, *113*, 5989-5997.

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Supplementary Table 1: Selective longitudinal relaxation rates, $R_{1\alpha}$ (s^{-1}), in human ubiquitin^a.

M1	2.2	I23	5.1	Q49	5.6	R74	4.5
F4	5.2	E24	4.1	L50	6.1		
V5	5.7	V26	4.3	R54	4.0		
K6	5.8	K27	5.6	T55	4.7		
T7	4.5	A28	3.7	L56	5.2		
L8	2.6	I30	4.2	S57	2.7		
T9	2.0	Q31	4.6	D58	1.8		
K11	3.1	K33	2.3	Y59	3.0		
T12	5.2	E34	2.0	N60	3.4		
I13	4.5	I36	5.8	I61	5.3		
T14	5.0	P37	5.8	Q62	3.2		
L15	4.3	Q41	7.3	K63	6.6		
E16	5.6	R42	5.2	S65	3.2		
V17	5.4	L43	7.1	T66	5.9		
E18	5.3	I44	6.5	L69	6.8		
D21	4.2	F45	6.5	V70	3.7		
T22	5.1	A46	3.3	R72	3.7		
I23	5.1	K48	3.8	L73	4.9		

^a Selective relaxation rates for ^{12}C -attached $^1\text{H}^\alpha$ nuclei in human ubiquitin at 30 °C. The rates were measured using scheme 2f of Peng and Wagner (1992) modified for ^{13}C -attached protons. Therefore, for the rates listed the relatively small, calculated contribution from one-bond ^{13}C - ^1H dipolar interaction ($\sim 0.49 \text{ s}^{-1}$) was subtracted from the experimentally determined rates. Also, as the measurement was conducted on a sample dissolved in D_2O , the $^1\text{H}^{\text{N}}$ - $^1\text{H}^\alpha$ dipolar contribution was calculated based on the X-ray structure (with protons built on with XPLOR (Brünger, 1992)) and the listed rates include this contribution.

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Supplementary Table 2: 3J coupling constants and backbone ϕ angles calculated from the X-ray structure and NMR data.

	$^3J_{\text{H}^{\text{N}}\text{H}^{\alpha}}$	$^3J_{\text{H}^{\alpha}\text{C}^{\prime}}$	$^3J_{\text{H}^{\text{N}}\text{C}^{\beta}}$	$^3J_{\text{H}^{\text{N}}\text{C}^{\prime}}$	X-ray ϕ^{a}	X-ray/NMR ϕ^{b}	NMR ϕ^{c}
	Hz				degree		
Q2 ^e	8.31	2.59	1.52	0.08	-91.0		
I3	9.27	2.98	-0.24	1.88	-131.1	-135.9	-135.9
F4	10.04	3.14	0.85	-0.13	-116.0	-110.3	-110.3
V5	10.00		0.10	0.71	-118.0	-124.8	-124.8
K6	9.30	2.29	1.43	-0.06	-95.2	-98.7	-99.0
T7	8.93	2.42	1.74	-0.04	-99.6	-95.3	-95.1
L8	4.15	1.43	2.15	1.59	-73.4	-62.0	-62.0
T9	8.63	2.32	1.26	0.11	-101.4	-97.3	-97.3
G10 ^d				0.14	77.4		
K11	7.26		2.00	0.31	-96.3	-84.8	-83.0
T12	9.67	2.87	0.62	0.35	-119.9	-111.5	-111.5
I13	9.91	2.57	1.17	0.31	-109.5	-107.3	-107.0
T14	9.62	2.42	1.14	-0.14	-101.4	-102.5	-103.4
L15	9.95	3.02	0.35	0.78	-126.4	-125.9	-122.1
E16	9.77	3.08	0.93	0.40	-111.8	-113.0	-113.0
V17	9.82	2.66	0.09	1.46	-139.0	-134.8	-132.5
E18	9.56	2.77	0.86	0.43	-120.0	-109.6	-109.6
P19 ^d		1.22			-54.9		
S20	7.62	2.16		0.18	-79.8	-83.8	-85.7
D21	5.52	1.46	2.25		-71.0	-71.0	-71.0
T22	7.57	1.65	2.05	0.01	-83.7	-82.9	-82.9

				14			
I23	3.94	1.52	2.75	1.54	-61.3	-61.3	-61.3
E24 ^d		0.82			-57.6		
N25	5.50	1.46	2.34	0.67	-65.5	-68.6	-70.3
V26	5.72	1.30	2.65	0.56	-58.4	-69.9	-69.9
K27	4.28	1.02	2.55	1.00	-60.8	-63.2	-63.2
A28	4.57	0.98	2.92		-66.1	-61.2	-61.2
K29	5.55	1.54	2.40	0.80	-64.2	-68.8	-70.5
I30	5.77	0.99	2.67	0.66	-70.0	-68.7	-68.7
Q31		0.76	2.79	1.19	-62.1	-60.0	-60.0
D32	3.82	1.06	2.57	1.72	-53.4	-55.3	-57.3
K33	7.13	1.98	2.04	0.26	-93.6	-83.7	-81.9
E34	9.54	2.89		-0.05	-123.6	-108.3	-106.8
G35 ^d				0.99	81.2		
I36	7.98	2.12	1.86		-79.7	-86.7	-88.6
P37 ^d		1.71			-57.0		
P38 ^d		1.06			-57.2		
D39	5.15	1.65	2.19	1.15	-68.2	-69.1	-69.1
Q40	9.45		1.27	0.59	-95.8	-106.7	-106.7
Q41	8.41	2.20	1.44	-0.19	-84.8	-94.1	-94.3
R42	9.81	2.81	0.20	1.52	-121.2	-131.0	-133.0
L43	9.66	2.54	0.99	0.25	-103.6	-103.6	-106.8
I44	10.27	3.01	0.92	0.09	-122.1	-112.9	-112.9
F45	8.73	2.57	0.04	1.67	-144.3	-141.1	-141.1
A46	6.47	7.12	0.27	3.58	48.2	41.0	41.0
G47 ^d				0.98	61.7		
K48	9.41	2.31	0.96	0.11	-115.1	-103.1	-103.1

				15			
Q49	6.97	1.61	2.08	0.05	-85.8	-80.7	-80.5
L50	7.11	2.02	1.89	-0.02	-79.6	-83.2	-83.4
E51	8.06	2.30	1.80	-0.07	-101.8	-91.3	-89.3
D52	2.48	0.90	2.38	2.92	-48.2	-45.5	-43.3
R54	9.59	2.13	1.39	0.19	-85.4	-99.2	-99.2
T55	9.20	3.11	1.60	-0.33	-104.5	-101.5	-101.5
L56	3.70	0.56	2.25	1.94	-61.2	-53.0	-53.0
S57	3.70	1.17		1.88	-63.9	-57.9	-56.0
D58	4.14	1.36	2.48	1.56	-55.6	-60.7	-61.3
Y59	9.45	2.41	1.49	0.07	-91.0	-100.4	-100.5
N60	7.26	6.49	0.80	2.52	57.9	44.9	44.9
I61	7.34	1.56	2.08	-0.11	-88.7	-81.4	-81.4
Q62	9.01	2.79	1.01	0.19	-103.4	-102.4	-102.4
K63	2.36	1.05	2.34	2.62	-54.8	-45.7	-44.9
E64	7.19	7.17	1.35	1.63	66.9	61.0	58.9
S65	5.63	1.26		0.74	-71.1	-71.1	-70.6
T66	9.82	3.07	1.34	-0.40	-119.2	-104.7	-104.7
L67	9.88	1.92	0.86	0.15	-103.1	-102.7	-102.7
H68	9.70	2.85	0.87	0.36	-105.6	-107.9	-110.2
L69	9.59	2.56	1.17		-107.0	-107.0	-104.7
V70	9.88	2.87	0.39	0.83	-108.1	-119.3	-123.0
L71	8.30		1.60	0.12	-96.0	-93.4	-91.9
R72 ^d		2.33			-117.6		
L73 ^e	7.52	2.11	1.39		-83.7		
R74 ^e	6.67	2.44	1.45		-97.7		

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- ^a X-ray structure-derived backbone ϕ angles (Vijay-Kumar et al., 1987).
- ^b Backbone ϕ angles were determined by choosing the value closest to the X-ray angle in the flat region of the square well potential (*c.f.* eq 4). The pairwise rms difference with the X-ray ϕ angles is 5.8°. These refined ϕ angles were used to reparametrize the Karplus curves shown in Figure 7.
- ^c Backbone ϕ angles were determined by choosing the value in the center of the square well. These ϕ angles were not used in any reparametrizations.
- ^d Residues for which less than three 3J values could be measured were not subjected to ϕ angle refinement, and the X-ray ϕ angle was used throughout.
- ^e Residues not included in the Karplus parametrizations because of flexibility in this region of the polypeptide backbone.

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Supplementary Table 3: $^3J_{\text{H}^{\text{N}}\text{H}^{\alpha}}$ coupling constants (Hz) as measured using the CT-HMQC-J, the HNHA, and the HNCA[HA]-E.COSY experiments.

	HMQC ^a	HNHA ^b	E.COSY ^c
Q2	8.31	7.71	
I3	9.27	8.40	7.61
F4	10.04	9.16	8.34
V5	10.00	9.40	8.58
K6	9.30	8.98	8.19
T7	8.93	8.29	7.79
L8	4.15	3.83	3.52
T9	8.63	8.34	8.23
K11	7.26	6.85	6.41
T12	9.67	9.29	8.74
I13	9.91	9.45	9.71
T14	9.62	8.93	8.67
L15	9.95	9.43	8.81
E16	9.77	9.22	8.77
V17	9.82	9.68	8.60
E18	9.55	9.26	8.52
S20	7.62	7.41	6.42
D21	5.52	5.05	
T22	7.57	7.07	6.04
I23	3.94	3.71	3.06
N25	5.50	5.15	4.69
V26	5.72	5.27	4.46
K27	4.28	3.97	3.31

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A28	4.57	4.16	
K29	5.55	5.36	4.47
I30	5.77	5.40	4.87
Q31			3.46
D32	3.82	3.42	3.07
K33	7.13	6.79	
E34	9.54	8.87	9.06
I36	7.98	7.57	6.58
D39	5.15	4.56	4.09
Q40	9.45	8.85	7.47
Q41	8.41	7.87	
R42	9.81	10.01	
L43	9.66	9.51	8.49
I44	10.27	9.91	8.96
F45	8.73	7.90	7.14
A46	6.47	6.22	5.91
K48	9.41	9.20	8.76
Q49	6.97	6.41	
L50	7.11	6.96	
E51	8.06	7.77	7.28
D52	2.48	2.31	
R54	9.59	9.06	
T55	9.20	9.05	7.53
L56	3.70	3.52	
S57	3.69	3.54	2.73
D58	4.13	4.03	3.47

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Y59	9.45	9.10	
N60	7.26	7.07	6.65
I61	7.34	7.51	6.54
Q62	9.01	8.71	8.60
K63	2.36	2.22	1.56
E64	7.19	6.43	
S65	5.63	5.27	4.65
T66	9.82	9.84	
L67	9.88	9.35	8.73
H68	9.70	9.45	
L69	9.59	8.96	
V70	9.88	9.33	8.84
L71	8.30		
L73	7.51	7.21	
R74	6.67	6.15	

^a $^3J_{\text{H}^{\text{N}}\text{H}^{\alpha}}$ coupling constants (Hz) measured from fitting the *J*-modulated peak intensities in a CT-HMQC-*J* data set consisting of eight 2D spectra with dephasing times of 45, 60, 80, 100, 120, 140, 170, and 200 ms. $T_{1\alpha}$ relaxation effects have been corrected for by using experimentally measured $T_{1\alpha}$ values.

^b $^3J_{\text{H}^{\text{N}}\text{H}^{\alpha}}$ coupling constants (Hz) measured from a 3D HNHA data set. $T_{1\alpha}$ relaxation effects have been corrected for by using experimentally measured $T_{1\alpha}$ values in the manner described in reference 31. These $T_{1\alpha}$ -corrected $^3J_{\text{H}^{\text{N}}\text{H}^{\alpha}}$ values are systematically lower than the $^3J_{\text{H}^{\text{N}}\text{H}^{\alpha}}$ CT-HMQC-*J* values by ~0.4 Hz.

^c $^3J_{\text{H}^{\text{N}}\text{H}^{\alpha}}$ splittings (Hz) measured from a 3D HNCA[HA]-E.COSY spectrum. No corrections of any kind have been applied to these splittings.

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Supplementary Table 4: X-ray crystal structure-derived ϕ , ψ backbone angles and the corrections to the ϕ angle as determined experimentally and theoretically.

	$\phi_{\text{xray}} (^{\circ})^{\text{a}}$	$\psi_{\text{xray}} (^{\circ})^{\text{a}}$	$\phi_{\text{X-ray}} - \phi_{\text{refined}} (^{\circ})^{\text{b}}$	$\Delta_{\text{ai}} (^{\circ})^{\text{c}}$
I3	-131.1	163.0	4.8	17
F4	-116.0	140.2	-5.7	14
V5	-118.0	114.2	6.8	4
K6	-95.2	127.5	3.5	15
T7	-99.6	170.8	-4.4	25
L8	-73.4	-6.9	-11.4	-6
T9	-101.4	14.9	-4.1	-9
G10	77.4	16.5		14
K11	-96.3	138.1	-11.5	18
T12	-119.9	131.8	-8.4	9
I13	-109.5	142.0	-2.2	17
T14	-101.4	139.7	1.0	18
L15	-126.4	154.0	-0.5	16
E16	-111.8	121.1	1.2	10
V17	-139.0	170.7	-4.2	17
E18	-120.0	144.5	-10.4	14
S20	-79.8	-8.1	4.0	-11
D21	-71.0	148.4	0.0	20
T22	-83.7	160.4	-0.8	24
I23	-61.3	-37.2	0.0	-10
N25	-65.5	-44.4	3.1	-12
V26	-58.4	-46.4	11.5	-10
K27	-60.8	-38.0	2.4	-10

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A28	-66.1	-38.1	-4.9	-12
K29	-64.2	-37.3	4.6	-11
I30	-70.0	-39.6	-1.3	-13
Q31	-62.1	-48.6	-2.1	-12
D32	-53.4	-41.8	1.9	-8
K33	-93.6	-24.4	-9.9	-15
E34	-123.6	-6.3	-15.3	-15
G35	81.2	5.3		12
I36	-79.7	124.9	7.0	14
D39	-68.2	-15.6	0.9	-6
Q40	-95.8	-10.5	10.8	-13
Q41	-84.8	129.7	9.3	16
R42	-121.2	116.0	9.8	3
L43	-103.6	130.2	0.0	14
I44	-122.1	131.8	-9.2	8
F45	-144.3	129.6	-3.2	-2
A46	48.2	46.0	7.2	8
G47	61.7	21.6		8
K48	-115.1	142.7	-12.0	16
Q49	-85.8	130.3	-5.2	16
L50	-79.6	138.3	3.6	19
E51	-101.8	140.0	-10.5	19
D52	-48.2	-42.2	-2.7	-5
R54	-85.4	165.5	13.8	25
T55	-104.5	164.6	-3.0	24
L56	-61.2	-36.2	-8.2	-10

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S57	-63.9	-29.6	-6.0	-10
D58	-55.6	-39.3	5.1	-9
Y59	-91.0	4.7	9.4	-9
N60	57.9	45.4	13.0	13
I61	-88.7	116.4	-7.3	10
Q62	-103.4	169.5	-1.0	25
K63	-54.8	143.1	-9.1	18
E64	66.9	19.1	5.9	12
S65	-71.1	159.5	0.0	24
T66	-119.2	126.7	-14.5	7
L67	-103.1	154.6	-0.4	22
H68	-105.6	135.7	2.3	17
L69	-107.0	115.8	0.0	-8
V70	-108.1	139.9	11.2	17
L71	-96.0	138.8	-2.6	19

^a X-ray crystal structure-derived backbone ϕ , ψ torsion angles³⁰ in degrees.

^b The $\phi_{\text{X-ray}} - \phi_{\text{refined}}$ difference as determined from ϕ angle refinement using experimentally determined 3J values as described in the main text.

^c Δ_{ai} , the difference between $\phi - 180^\circ$ and the calculated $\text{H}^{\text{N}}\text{-N-C}^{\alpha}\text{-C}'$ dihedral angle.^{55,56}

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;HNCA[HA]-E.COSY

;Andy C. Wang and Ad Bax, March 1995

;JACS submitted October 1995

;include "bits.sg"

#define CARBON

#define NITROGEN

#undef COADD_2D

#define CARBON_LOOP 24

#define NITROGEN_LOOP 32

#define ITERATIONS 9

;

;PROTON pulses:

;carrier at 8.36ppm

;p1 = 90 at hl1=3dB

;p2 = 720us proton 90 @ tp0=38.5dB and tpname0=sinc1.0

;p4 = 890us proton 90 @ tp1=29.5dB and tpname1=q3_128.acw

;p30 = 50us 90 at hl3 = 17dB for Dipsi-2 decoupling

;NITROGEN pulses:

;p3=nitrogen 90 at dbhi

;p31=nitrogen 90 at dbl0

;CARBON pulses:

;carrier at 58 ppm

;p5=53.9u Ca 90 @ dl0=29 dB for null at 177 ppm

;p6=24.1u Ca 90 @ dl1=21 dB for null at 177 ppm

;p7=97.4u C' 90-deg offset sinc used as 180-deg @ dp0=30 dB

;p9=150u C' 90-deg offset sinc used as 180-deg @ dp2=33 dB

;

;Nitrogen constant time:

d18=6.81m

d10=d18

d20=d18

;in10=in19=in20

;in10=in20=in19=1/(4*sw)

;CARBON:

;sw=1/(2*in0)

d0=in0-p9-4u-p5*0.636

;

;DELAYS:

d3=2.25m

;d7 ca 100u = dw

d8=aq

d11=50m

d12=8m

d23=p3-p6

;p3 > p6

d24=p7-p1

d26=p3-p1

d27=p9-p3

d28=p4-p3

;

;f1 = hydrogen frequency on water

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;f2 = Ca frequency 58 ppm

;f3 = nitrogen frequency

```

;
100u CLEAR_BITS ;switch 2H decoupler off, switch everything off
100u LOCK_ON ;switch lock on
ze
2 d11 dbo
d12
3 d12*3
4 d12*2
5 d12*4
6 d12*6
7 10u dbo
10u do
10u to
100u LOCK_ON ;switch lock on
d1 dlo
20m hl1
20u dbhi
;***** Start 90-degree *****
(p1 ph0):f1
d3
(d26 p1*2 ph10):f1 (p3*2 ph0):f3
d3
(p1 ph1):f1
50u LOCK_OFF
GRAD(10, POSITIV, 40)
3m
(p3 ph3):f3
;***** N15 evolution *****
d10 dl6
4u
(p7*2 ph10):dp0 (d24 p1*2 ph0):f1
d10 dl1
10u
(d23 p6*2 ph0):f2 (p3*2 ph5):f3
10u
d20 dl6
(p7*2 ph10):dp0 (d24 p1*2 ph0):f1
4u
d20 dl0
(p3 ph1):f3
GRAD(20, POSITIV, 30)
2.25m
(p5 ph4):f2
;***** C-alpha evolution *****
4u
d0 dl6 ;t1/2
(d27 p3*2 ph10):f3 (p9*2 ph20):dp2
d0
4u dl0
;***** back to N *****

```

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```

      (p5 ph6):f2
      4u
      (p3 ph10):f3
      11m
      4u dl1
      (d23 p6*2 ph0):f2 (p3*2 ph10):f3
      4u
      11m tlo
;*****      back to Hn      *****
      (p3 ph1):f3
      GRAD(30, POSITIV, 20)
      1.5m
      (p2 ph8):tp0
      GRAD(40, POSITIV, 4)
      d4
      (p4*2 ph7):tp1 (d28 p3*2 ph10):f3
      GRAD(50, POSITIV, 4)
      (2u ph0)
      d7 dblo
      2u dbl0
      p24:e
      3u adc
      d8 cpdb
10u do
10u to
10u dbo
rcyc=2 ph31
d11 wr #0 if #0 zd
;
#ifdef CARBON
d12 ip4
lo to 3 times 2
d12 id0
d12 ip31
d12 ip31
lo to 4 times CARBON_LOOP
#endif
;
#ifdef NITROGEN
d12 ip3
d12 rd0
lo to 5 times 2
d12 id10
d12 dd20
d12 ip31
d12 ip31
lo to 6 times NITROGEN_LOOP
#endif
;
Co-add 2D spectra
#ifdef COADD_2D
d12 rf #0
d12 rp4

```

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```
d12 rp31
d12 rd0
d12 CLEAR_BITS
d12 ze
lo to 7 times ITERATIONS
#endif
;
100u do
100u dbo
100u to
100u LOCK_ON ;switch lock on
100u CLEAR_BITS ;switch 2H decoupler off, switch everything off
exit
;
ph0=0
ph1=1
ph3=0
ph4=0
ph5=0 1 2 3
ph6=0 0 2 2
ph7=(360) 327
ph8=0 0 0 0 2 2 2 2
ph10=0
ph20=0
ph31=0 2 2 0 2 0 0 2
;
```

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;HCAN[C']-E.COSY

;3J(HaC') experiment requires D2O sample and deuterium decoupling.

;Uses shaped pulse decoupling of ¹³C during acquisition.

;Andy C. Wang and Ad Bax, March 1995

;JACS submitted October 1995

#include "bits.sg"

#define CARBON

#define NITROGEN

#define CARBON_LOOP 48

#define NITROGEN_LOOP 130

;

;Proton (f1 = hydrogen frequency on water)

;p1 = proton 90 10 us @ h11 = 3 dB

;Carbon (f2 = carbon frequency 58 ppm)

;p3 = low power carbon 90 11 us @ d10=10 dB

;p4 = low power carbon 90 53.92 us @ d11=29.5 dB

;p6 = low power carbon 90 24.11 us @ d12=21.3 dB

;p8 = low power g3mlev.acw cpd @ dp3=25 dB, dpoffs3=0 & p8=8192u

;calibrate 256 us 90-degree g3.acw pulse!!

;p11= low power shaped offset sinc 90 97.4 us @ dp0=29.6 dB

;in0=in20=1/(2sw)

;Nitrogen (f3 = nitrogen frequency)

;p7 = high power nitrogen 90 45 us

;in30=1/(2sw)

;

;Delays:

d0=4u

d5=8m

;universal dd, id, ip delay

d11=50m

d20=14.28m

d21=p3-p1

d22=p7-p6

d23=p6-p1

d24=p11-p1

p24=5u

d30=in30*0.5-p7*0.6366-p6

;-90/180 phase correction

;

100u CLEAR_BITS

ze

2 d11

d5

3 d5*3.0

4 d5*3.0

;

5 d5*4.0

6 100u CLEAR_BITS

10u h11

10u dbhi

10u d10

d1 d10

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```

;-----Start 90-degree on Ha
  (p1 ph0):f1
  1.5m
  2u
  (d21 p1*2 ph0):f1 (p3*2 ph10):f2
  2u
  1.5m dl1
;-----Inept to Ca
  (p1 ph1):f1
  10u HIGH( 11 )           ;lock off
  GRAD( 10, POSITIV, 30 )
  2.2m
  (p4 ph2):f2 ;.....End of first Inept
  2u
  d0 dl6
  (d24 p1*2 ph0):f1 (p11*2 ph10):dp0
  2u
  14.28m dl2
  (d22 p6*2 ph3):f2 (p7*2 ph0):f3
  8u
  (p11*2):c8
  d20 dl1
;-----Inept to 15N
  (p4 ph1):f2
  GRAD( 20, NEGATIV, 10 )
  5u HIGH( 12 )           ;2H decoupler ON
  (p7 ph4):f3 ;.....End of Inept to 15N
  d30 dl2
  (d23 p1*2 ph0):f1 (p6*2 ph10):f2
  d30
;-----Inept back to Ca
  (p7 ph0):f3
  5u LOW( 12 )           ;2H decoupler OFF
  GRAD( 30, POSITIV, 20 )
  3.37m dl1
  (p4 ph1):f2 ;.....End of back inept from 15N
  2u
  8.28m
  GRAD( 40, POSITIV, 40 )
  4m dl2
  (d22 p6*2 ph5):f2 (p7*2 ph0):f3
  2u
  GRAD( 50, POSITIV, 40 )
  12.28m dl1
;-----Inept back to Ha
  (p4 ph0):f2
  GRAD( 60, POSITIV, 10 )
  1.1m
  (p1 ph0):f1 ;.....End of reverse inept to Ha
  2u
  GRAD( 70, POSITIV, 4 )
  1.3m dl2

```

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```

      (d23 p1*2 ph0):f1 (p6*2 ph10):f2
2u
GRAD( 80, POSITIV, 4 )
  1.3m dl6
  (p1 ph0):f1
d7
  p24:e
  3u adc
88  (p8 ph0):dp3      ;81.92 ms of decoupling
  6u
  lo to 88 times 10
  30m
5u do
5u LOW( 11 )          ;lock on
rcyc=2 ph31
d11 wr #0 if #0 zd
;
#ifdef NITROGEN
d5 ip4
lo to 3 times 2
d5 id30
d5 ip31
d5 ip31
lo to 4 times NITROGEN_LOOP
#endif
;
#ifdef CARBON
d5 ip2
d5 rd30
d5 rp4
lo to 5 times 2
d5 id0
d5 dd20
d5 ip31
d5 ip31
lo to 6 times CARBON_LOOP
#endif
;
100u do
100u dbo
100u RESET
exit
;
ph0=0
ph1=1
ph2=0
ph3=(360) 10 10 100 100    ;power level switch + BS phase correction
ph4=0 2
ph5=(360) 3                ;power level switch correction
ph10=0
ph31=0 2 2 0
;

```

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```
;HN(CO)HB for the DMX-500
;Andy C. Wang and Ad Bax, March 1995
;JACS submitted October 1995
```

```
;
#include "bitsdmx.nt"
#define NITROGEN
#define PROTON
#define PROTON_LOOP 70
#define NITROGEN_LOOP 35
;
```

```
;Protons on f1 (at HDO frequency):
;f1 = hydrogen frequency on water
;p1 = proton 90 @ pl1
;p2 = proton 90 ca 940u Watergate @ pl2
;p30 = proton 90 ca 50u @ pl30 using cpd1:f1
;
```

```
;Nitrogen on f3 (at 116.5 ppm):
;p3 = 90 at pl3
;p31 = 90 at pl31 ca. 180us
;in20 = in21
;
```

```
;Carbons on f2 (at 177 ppm):
;p5 = 63.7u ;C' 90 @ pl5
;p6 = 28.5u ;90 used as 180 @ pl6 ;null at 56ppm
;p29=614u ;180 at 1 sp 1 @ ca. 12.4 dB w/ 2 spoofs 1 = -16464Hz
;pulse program cpd2:f2
;in0 = 1/2sw
;d0 = in0*0.5 - p6 - p1*0.636- 15u ; 90,-180 phase
;
```

```
;DELAYS:
d4=2.25m
d5=4.6m-p30
d3=d4-p2-250u
;d7 ca 100u = dw
d11=50m
d12=15m
d14=18m-50u*70 ;CO-Hb dephase
d20=13.5m
d21=d20-5.4m-p30
d24=p3-p6
d26=p3-p1
;
```

```
100u RESET
```

```
ze
2 d11 do:f3
d12
3 d12*3
4 d12*3
5 d12*4
6 10u do:f2
d1 do:f1
10u pl1:f1
```

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```

10u pl3:f3
10u blank ;lock off
;_____start 90-degree_____
    (p1 ph10):f1
    d4
    (d26 p1*2 ph0):f1 (p3*2 ph1):f3
    d4
    (p1 ph1):f1
    GRAD(10, POSITIV, 100)
    3.5m pl29:f2
    3.5m cpd2:f2 ;G3 mlev16 pulse decoupling of Cab (p29 @ 2 sp 1)
    (p3 ph3):f3
;_____15N evolution_____
    5.4m pl30:f1
    (p30 ph1):f1
    d21 cpd1:f1
    10u do:f2
    10u pl6:f2
    (d24 p6*2 ph10):f2 (p3*2 ph8):f3
    5u
    5u pl29:f2
    10u cpd2:f2
    d20
;_____INEPT to CO_____
    (p3 ph0):f3
    10u do:f2
    10u do:f1
    (p30 ph7):f1
    10u pl5:f2
    (p5 ph5):f2
;_____
    GRAD(20, POSITIV, 70)
    d14 pl29:f2
    10u cpd2:f2
    7m pl1:f1
;_____Hb evolution_____
    (p1 ph24):f1
    d0
    5u do:f2
    10u pl6:f2
    (p6*2 ph0):f2
    5u
    5u pl29:f2
    5u cpd2:f2
    d0
    (p1 ph25):f1
    7m
    10u do:f2
    GRAD(30, POSITIV, 70)
    d14 pl5:f2
;_____INEPT to 15N_____
    (p5 ph0):f2

```

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```

GRAD(40, POSITIV, 50)
3.4m pl30:f1
(p30 ph1):f1
5u cpd1:f1
(p3 ph10):f3
;
10m
10u pl6:f2
(d24 p6*2 ph0):f2 (p3*2 ph0):f3
d5
10u do:f1
(p30 ph7):f1
5.4m
;
INEPT to Hn
(p3 ph11):f3
GRAD(50, POSITIV, 30)
2m pl1:f1
(p1 ph0):f1
;
GRAD(60, NEGATIV, 5)
d3 pl2:f1
(p2 ph14):f1
2u
5u pl1:f1
(p1*2 ph0):f1
2u
5u pl2:f1
(p2 ph14):f1 (p3*2 ph10):f3
GRAD(70, NEGATIV, 5)
d3 pl31:f3
go=2 ph31 cpd3:f3
10u do:f2
10u do:f1
10u do:f3
10u unblank ;lock on
d11 wr #0 if #0 zd
;
#ifdef PROTON
d12 ip24
lo to 3 times 2
d12 id0
d12 ip31
d12 ip31
lo to 4 times PROTON_LOOP
#else
d12*4
#endif
;
#ifdef NITROGEN
d12 rd0
d12 rp24
d12 ip3

```

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```
lo to 5 times 2
d12 id21
d12 dd20
d12 ip31
d12 ip31
lo to 6 times NITROGEN_LOOP
#endif
;
exit
;
ph0=0
ph1=1
ph3=0
ph5=0 0 2 2
ph7=3
ph8=0 1 2 3
ph10=0
ph11=0 0 0 0 2 2 2 2
ph14=(3600) 1836 ;adjust for power level hl2
ph24=0
ph25=0 0 0 0 0 0 0 2 2 2 2 2 2 2 2
;
#ifdef PROTON
ph31=0 2 2 0 2 0 0 2
#else
ph31=0 2 2 0 2 0 0 2 2 0 0 2 0 2 2 0
#endif
;
```

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;HNCA[CB]-E.COSY

;Andy C. Wang and AdBax, March 1995

;JACS submitted October 1995

;Water is NOT saturated during experiment! Very low residual H2Oxy.

; 1. Flipped along z prior to gradients

; 2. Spin-locked along x during 1H decoupling

; 3. Uses sine-bell flip-back and Watergate prior to acquisition

;_____

#include "bits.sg"

#define CARBON

#define NITROGEN

#undef COADD_2D

#define CARBON_LOOP 56

#define NITROGEN_LOOP 32

#define ITERATIONS 9

;_____

;PROTON pulses:

;p1 = 90 at hl1=3dB

;p2 = 900us 90 at hl2 = 45dB for Watergate pulse

;p4 = 2.9m sinc 90 at tp0 = 45dB for water-flip-back pulse

;p30 = 50us 90 at hl3 = 17dB for Dipsi-2 decoupling

;NITROGEN pulses:

;p3=nitrogen 90 at dbhi

;p31=nitrogen 90 at dbl0

;CARBON pulses:

;carrier at 58 ppm

;p5=53.9u Ca 90 @ dl0=29 dB for null at 177 ppm

;p6=24.1u Ca 90 @ dl1=21 dB for null at 177 ppm

;p8=753u Ca 90-deg sinc @ dp1=46 dB

;p7=97.4u C' 90-deg offset sinc used as 180-deg @ dp0=30 dB

;p9=150u C' 90-deg offset sinc used as 180-deg @ dp2=33 dB

;p10=14.0u Ca pulse @ dl2=14 dB for composite 180

;p11=14.0u Ca pulse @ dl2=14 dB for composite 180

;p12=14.0u Ca pulse @ dl2=14 dB for composite 180

;_____

;Nitrogen constant time:

d18=6.81m

d10=d18-5.4m-p30

d19=d18

d20=d18

;in10=in19=in20

;in10=in20=in19=1/(4*sw)

;CARBON constant time:

;sw=1/(2*in0)

d0 = 4u

;_____

;DELAYS:

d2=5.6m-p9*2

d3=2.25m

d4=d3-250u-p2

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```

d5=5.5m-p9
d6=d5-p30-p9-4u
p24=d4
;d7 ca 100u = dw
d8=aq
d11=50m
d12=8m
d23=p3-p6
d25=p3-p11*2.22
d26=p3-p1
d27=p9-p3
;
;f1 = hydrogen frequency on water
;f2 = Ca frequency 58 ppm
;f3 = nitrogen frequency
;
100u CLEAR_BITS ;switch 2H decoupler off, switch everything off
100u LOCK_ON ;switch lock on
ze
2 d11 dbo
d12
3 d12*3
4 d12*2
5 d12*5
6 d12*6
7 10u dbo
10u do
10u to
100u LOCK_ON ;switch lock on
d1 dlo
20m hl1
20u dbhi
;***** Start 90-degree *****
(p1 ph0):f1
d3
(d26 p1*2 ph0):f1 (p3*2 ph10):f3
d3
(p1 ph2):f1
50u LOCK_OFF
10u hl2
(p2 ph9):f1 ;flip water to +z
GRAD( 10, POSITIV, 50.0 )
3.3m
(p3 ph3):f3
;***** N15 evolution *****
5.4m hl3
(p30 ph7):f1 ;put H2O on +x for spin-lock
d10 cpdts dl6 ;(T+t2)/4 & dec. must be along x for H2O suppr.
(p7*2 ph10):dp0
4u
2u
d19 dl1

```

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```

10u
(d23 p6*2 ph6):f2 (p3*2 ph5):f3
2u
10u
d20 dl6
(p7*2 ph10):dp0
4u
d20 dl0
(p3 ph11):f3
20u
(p5 ph4):f2
;***** C-alpha evolution *****
4u
d0 dl6 ;t1/2
(d27 p3*2 ph1):f3 (p9*2 ph20):dp2
d0
4u dl6
;***** back to N *****
(p8 ph17):dp1
(p3 ph10):f3
d5
(p9*2 ph20):dp2
d6
(p30):c8
4u dl2
(d25 p10 ph7 2u p11*2.44 ph0 2u p12 ph7):f2 (p3*2 ph10):f3
2u
d2 dl6
(p9*2 ph10):dp2
2u to
(p30 ph8):f1 ;put H2O back along +z
1m
4.4m hl1
;***** back to Hn *****
(p3 ph0):f3
2u
GRAD(70, NEGATIV, 20)
1.6m
5u tlo
(p4 ph13):tp0 ;half-gauss 90 to flip water to +y
2u
2u thi
50u hl1
(p1 ph0):f1
GRAD(80, NEGATIV, 5)
d4 hl2
(p2 ph14):f1 ;Watergate -x 90 pulse on water
2u
5u hl1
(p1*2 ph0):f1
2u
5u hl2

```

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```

      (p2 ph14):f1 (p3*2 ph10):f3
      GRAD(90, NEGATIV, 5)
      2u dblo
      (2u ph0)
      d7 dbl0
      p24:e
      3u adc
      d8 cpdb
10u do
10u to
10u dbo
rcyc=2 ph31
d11 wr #0 if #0 zd
;
#ifdef CARBON
d12 ip4
lo to 3 times 2
d12 id0
d12 ip31
d12 ip31
lo to 4 times CARBON_LOOP
#endif
;
#ifdef NITROGEN
d12 ip3
d12 rd0
lo to 5 times 2
d12 id10
d12 id19
d12 dd20
d12 ip31
d12 ip31
lo to 6 times NITROGEN_LOOP
#endif
;
Co-add 2D spectra
#ifdef COADD_2D
d12 rf #0
d12 rp4
d12 rp31
d12 rd0
d12 CLEAR_BITS
d12 ze
lo to 7 times ITERATIONS
#endif
;
100u do
100u dbo
100u to
100u LOCK_ON ;switch lock on
100u CLEAR_BITS ;switch 2H decoupler off, switch everything off
exit
;

```

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```
ph0=0
ph1=1 3
ph2=1
ph3=0
ph4=0 0 2 2
ph5=0 0 0 0 1 1 1 1
ph6=0
ph7=1
ph8=3
ph9=(360)329 ;adjusted +x pulse for hl2 water-flip-back
ph10=0
ph11=0 0 0 0 0 0 0 0 2 2 2 2 2 2 2 2
ph13=(360)263 ;adjusted -x half-gauss water-flip-back pulse at tp0
ph14=(360)149 ;adjusted -x Watergate pulse at hl2
ph15=0
ph17=2 ;2 2 2 2 2 2 2 0 0 0 0 0 0 0 0
ph20=0
ph31=0 0 2 2 2 2 0 0 2 2 0 0 0 0 2 2
;
```
