Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Enhancing the resolution of multi-dimensional heteronuclear NMR spectra of intrinsically disordered proteins by homonuclear broadband decoupling.

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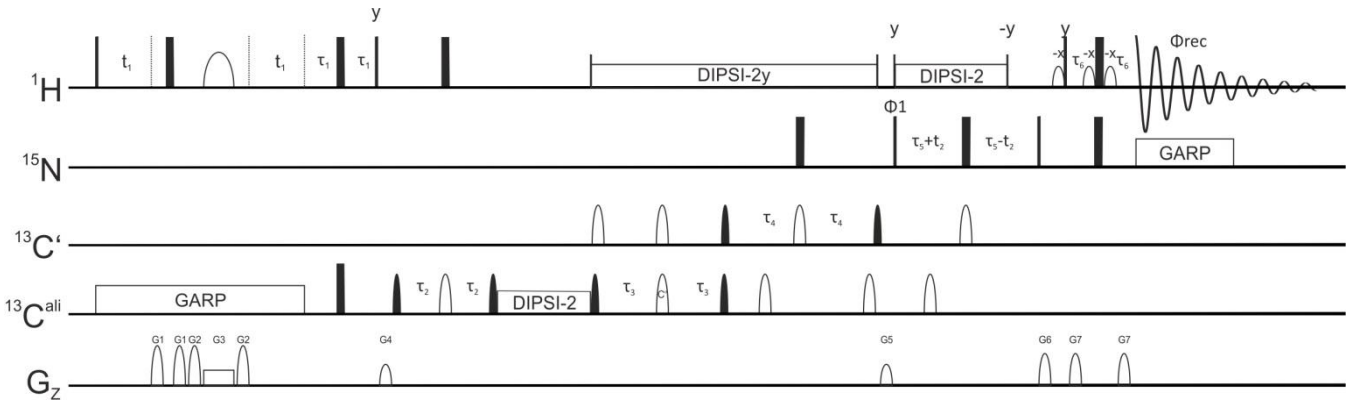
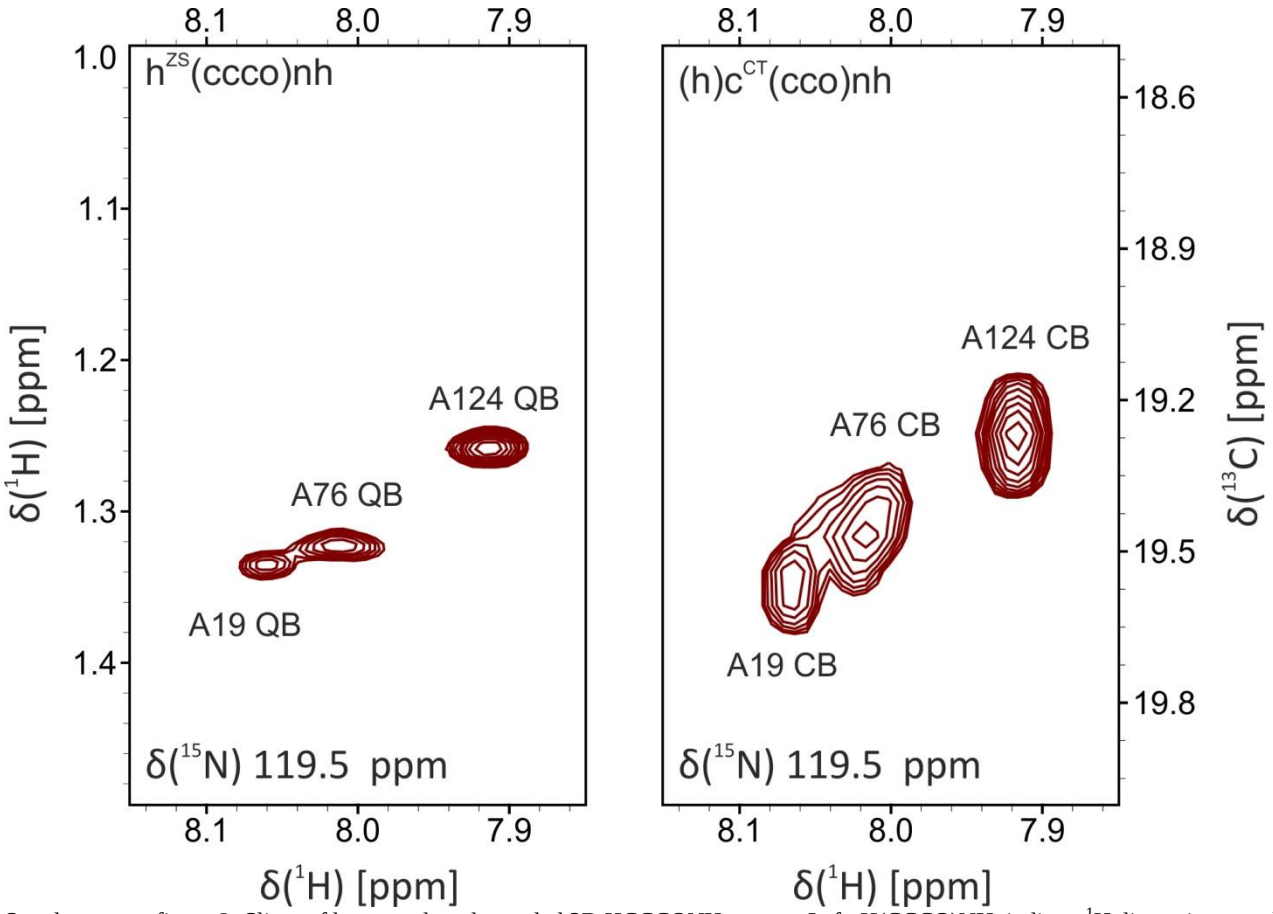
Experimental Section

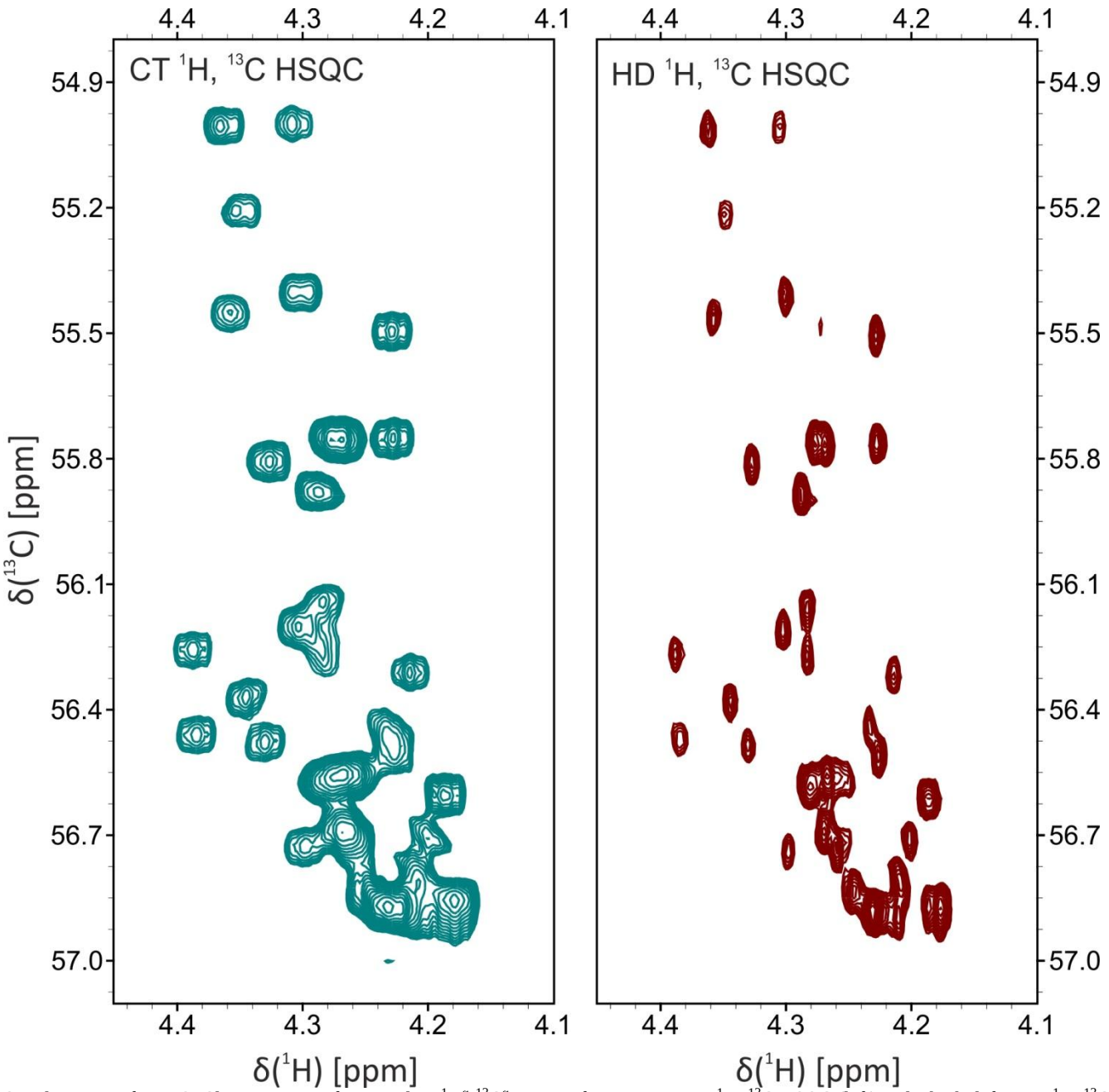
Alpha synuclein and SERF1a were expressed and purified as described previously (ref. 25 in the main text). All spectra were recorded on a Bruker Avance III 700 MHz spectrometer with a TCI cryogenic probe at 298 K. 3D (H)C(CCO)NH and H(CCCO)NH spectra were recorded on 1 mM uniformly ^{15}N and ^{13}C labeled α synuclein in 50 mM Bistris pH 6.5, 50 mM NaCl (10 % D_2O). Constant time ^{1}H, ^{13}C HSQCs and high definition ^{1}H, ^{13}C HSQCs were recorded on 500 μM uniformly ^{13}C and ^{15}N labeled α synuclein in 25 mM KPi, 25 mM NaCl (100 % D_2O). We used a 40 ms Eburp and a 8 ms Gauss pulse during a slice selective gradient to achieve slice selective excitation and refocusing, respectively. The gradient strength was 0.2 Gauss/cm to cover the H-methyl region and 0.4 Gauss/cm to cover the Hα region. Spectra were processed NMRpipe and analyzed using Sparky (T. D. Goddard and D. G. Kneller, SPARKY 3, University of California, San Francisco). NUS spectra were reconstructed using MDDNMR (refs 28 and 29 in the main text).



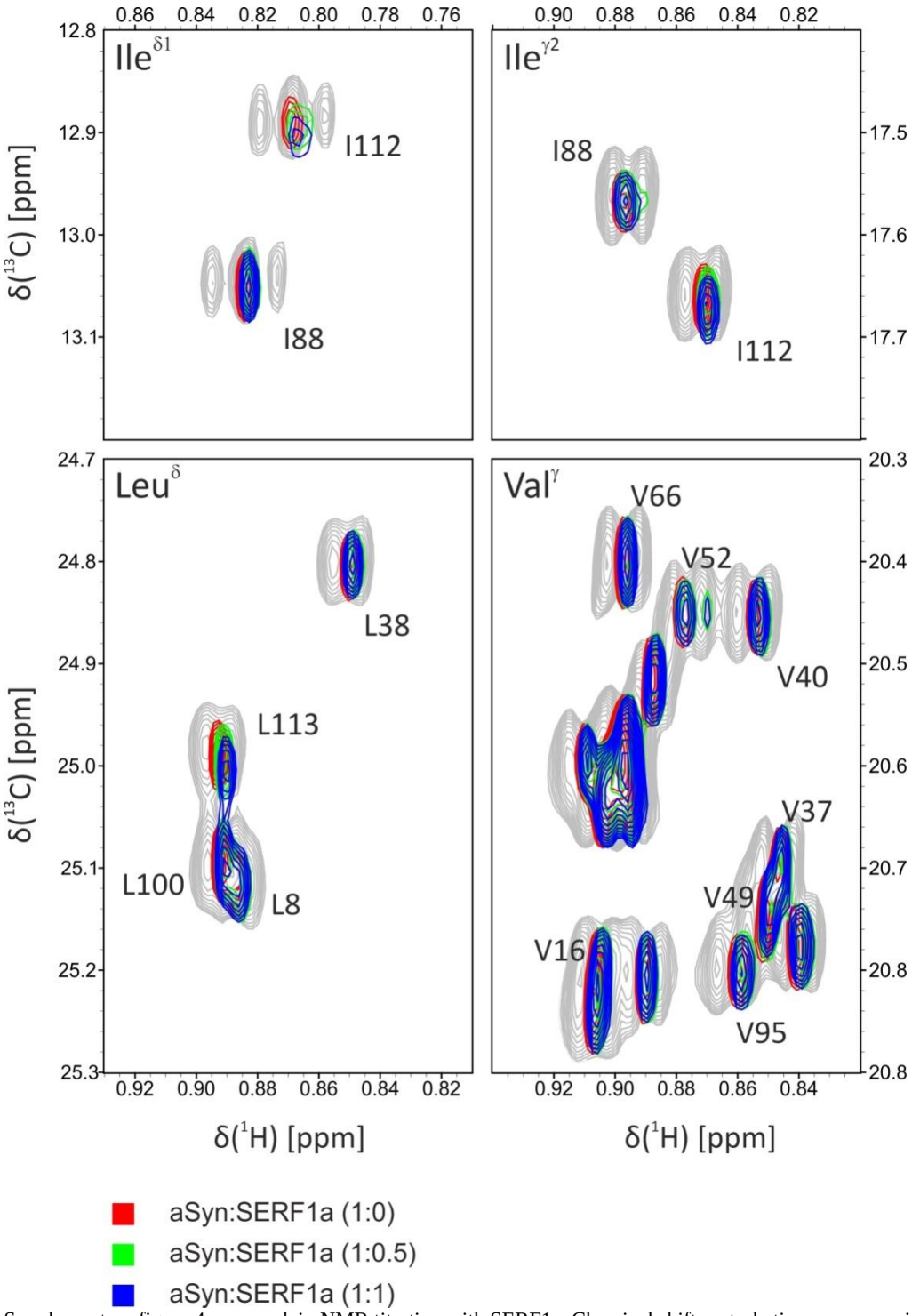
Supplementary figure 1: ZS-decoupled H(CCCO)NH experiment. The HCCCONH experiment has been described in detail elsewhere. In order to decouple the indirect proton dimension a ZS-decoupling scheme is placed in the middle of the proton chemical shift evolution delay (t_1) as described previously for homonuclear spectra (ref. 16 in the main text). ^{13}C is decoupled in the chemical shift evolution period using a GARP decoupling scheme. Subsequently magnetization is transferred to ^{13}C via INEPT. Narrow and wide rectangles represent 90° and 180° pulses. Filled and open half-ellipsoids denote shaped 90° and 180° pulses respectively.



Supplementary figure 2: Slices of homonuclear decoupled 3D HCCCONH spectra. Left: H(CCCO)NH, indirect ^{1}H dimension was decoupled using ZS-decoupling and a gradient strength of 0.2 Gauss/cm. The spectrum was recorded with 16 scans and 2048 increments in the indirect proton dimension. Total acquisition time was 36h using NUS (5% sampling) Right: (H)C(CCO)NH, ^{13}C dimension was decoupled using constant time evolution. The spectrum was recorded with 16 scans and 1024 increments in the carbon dimension. Total acquisition time was 28h using NUS (12% sampling).



Supplementary figure 3: Close-up view of α synuclein $^{1}H^{\alpha}$-$^{13}C^{\alpha}$ region of a constant time ^{1}H, ^{13}C HSQC (left) and a high definition ^{1}H, ^{13}C HSQC (right). HD ^{1}H, ^{13}C HSQC was recorded using a gradient strength of 0.2 Gauss/cm to decouple the acquisition dimension. Both experiments were recorded using 4 scans.



Supplementary figure 4. α synuclein NMR titration with SERF1a. Chemical shift perturbations were monitored using HD ^{1}H, ^{13}C HSQC spectra. Grey spectrum represents a conventional constant time ^{1}H ^{13}C HSQC, which shows much more overlap.

ESI Table 1: Alpha syncuclein chemical shifts of methyl groups

Alanin	CB	HB#
17	19,32	1,341
18	19,23	1,347
19	19,26	1,352
29	19,13	1,334
53	19,38	1,359
56	19,35	1,339
76	19,41	1,327
91	19,25	1,374
124	19,2	1,273

Isoleucin	CD1	CG2	HD1#	HG2#
88	13,05	17,57	0,824	0,878
112	12,89	17,66	0,81	0,852

Leucin	CD1	CD2	HD1#	HD2#
8	23,55	25,11	0,831	0,887
38	24,8	23,84	0,85	0,784
100	25,09	23,48	0,892	0,834
113	24,99	23,68	0,893	0,832

Threonine	CG2	HG2
33	21,54	1,169
60	21,75	1,183
64	21,57	1,149

Valine	CG1	CG2	HG1#	HG2#
3	21,19	20,29	0,798	0,791
15	21,22	20,79	0,839	0,841
16	-	20,84	-	0,907
26	-	20,64	-	0,896
37	21,19	20,7	0,816	0,847
40	21,21	20,45	0,865	0,854
48	21,14	20,78	0,782	0,841
49	-	20,74	-	0,851
52	21,23	20,45	0,9	0,878
63	21,35	20,6	0,899	0,899
66	21,19	20,4	0,897	0,897
70	21,27	-	0,872	-
82	21,22	20,57	0,891	0,898
95	21,21	20,8	0,804	0,86
118	20,62	21,24	0,9045	-

Pulse sequence for HD-HSQC experiment

```
; Pulse sequence starts here
; N. Helge Meyer, Klaus Zangger
; instant homonuclear decoupled, constant time HSQC
;based on Bruker release sequence hsqcctetgpsp
;avance-version (11/02/24)
;HSQC
;2D H-1/X correlation via double inept transfer
;phase sensitive using Echo/Antiecho-TPPI gradient selection
;with decoupling during acquisition
;constant time version
;using trim pulses in inept transfer
;using shaped pulses for inversion on f2 – channel
;(G.W. Vuister & A. Bax, J. Magn. Reson. 98, 428-435 (1992))
;with homonuclear broadband decoupling during acquisition
;$CLASS=HighRes
;$DIM=2D
;$TYPE=
;$SUBTYPE=
;$COMMENT=

prosol relations=<triple>

#include <Avance.incl>
#include <Grad.incl>
#include <Delay.incl>
#include <De.incl>
dwellmode explicit

"d12=6.5u"

"d2=aq/l0"
"d3=d2/2"
"l1=l0-1"
"p2=2*p1"
"p22=p21*2"
"d4=1s/(cnst2*4)"
"d11=30m"
"d12=20u"

"d0=3u"
"d20=d23-p16-d16-p14*1.5-4u-d12"

"in0=inf1/2"

"in20=in0"

"DELTA1=d4-larger(p2,p8)/2-p16-de-8u"
"DELTA2=d4-larger(p2,p8)/2-4u"
"DELTA3=d23-d0-p14/2-larger(p14,p22)-4u"
"DELTA4=d4-larger(p2,p8)/2-p1*2/PI-4u"
"DELTA5=d4-larger(p2,p8)/2-4u-d16-p16-d12*2"

"spoff3=0"
"spoff5=bf2*(cnst21/1000000)-o2"
"spoff13=0"

"acqt0=0"
baseopt_echo

1 ze
  d11 pl12:f2
2 d11
  4u BLKGRAD
  d1 do:f2

  d12 pl0:f1
  d12 UNBLKGRAD
3 d12 gron4
  d12 cpd2:f2
  (p11:sp11 ph1)
  d12 do:f2
  d12 groff
  ;p1 ph1
  p16:gp5
  d16 pl1:f1
  DELTA5 pl0:f2
```

```
  4u
  (center (p2 ph1) (p8:sp13 ph6):f2 )
  4u
  DELTA2 pl2:f2
  (p28 ph1)
  4u
  (p1 ph2) (p3 ph3):f2
  d0
  (center (p2 ph5) (p14:sp5 ph1):f2 (p22 ph1):f3 )
  4u
  DELTA3 pl0:f2
  (p14:sp3 ph4):f2
  d20
  p16:gp1*EA*-1
  d16 pl0:f2
  (p14:sp5 ph1):f2
  4u
  d12 pl2:f2
  (ralign (p1 ph1) (p3 ph4):f2 )
  4u
  DELTA4 pl0:f2
  (center (p2 ph1) (p8:sp13 ph1):f2 )
  4u
  p16:gp2
  DELTA1 pl12:f2
  4u cpd2:f2

  ACQ_START(ph30,ph31)

   0.05u DWL_CLK_ON
   0.1u REC_UNBLK
   d3:r
   0.1u REC_BLK
   0.05u DWL_CLK_OFF

   p16:gp6
   d16 pl1:f1
   p2 ph7
   p16:gp6
   d16
   p16:gp7
   d16 pl0:f1
   10u
   d12 gron4
   (p12:sp12 ph8)
   d12 groff
   10u
   p16:gp7
   d16

4  0.05u DWL_CLK_ON
   0.1u REC_UNBLK
   d2:r
   0.1u REC_BLK
   0.05u DWL_CLK_OFF

   p16:gp6
   d16 pl1:f1
   p2 ph7
   p16:gp6
   d16
   p16:gp7
   d16 pl0:f1
   10u
   d12 gron4
   (p12:sp12 ph8)
   d12 groff
   10u
   p16:gp7
   d16

lo to 4 times l1

   0.05u DWL_CLK_ON
   0.1u REC_UNBLK
   d3
   40m
   0.1u REC_BLK
```

```
  0.05u DWL_CLK_OFF

rcyc=2
  d11 do:f2 mc #0 to 2
    F1EA(calgrad(EA), caldel(d0, +in0) & caldel(d20, -in20) & calph(ph3, +180) & calph(ph6, +180) & calph(ph31, +180))
exit

ph0=0
ph1=0
ph2=1
ph3=0 2
ph4=0 0 0 0 2 2 2 2
ph5=0 0 2 2
ph6=0
ph7=0
ph8=0
ph30=0
ph31=0 2 0 2 2 0 2 0

;pl0 : 0W
;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
;sp3 : f2 channel - shaped pulse 180 degree (on resonance)
;sp5 : f2 channel - shaped pulse 180 degree (off resonance)
;sp11: f1 channel - shaped pulse 90 degree
;sp12: f2 channel - shaped pulse 180 degree
;sp13: f2 channel - shaped pulse 180 degree (adiabatic)
;p1 : f1 channel -  90 degree high power pulse
;p2 : f1 channel - 180 degree high power pulse
;p3 : f2 channel -  90 degree high power pulse
;p8 : f2 channel - 180 degree shaped pulse for inversion (adiabatic)
;p11: f1 channel -  90 degree shaped pulse for slice selective excitation
;p12: f1 channel - 180 degree shaped pulse for slice selective refocussing
;p14: f2 channel - 180 degree shaped pulse
;p16: homospoil/gradient pulse
;p22: f3 channel - 180 degree high power pulse
;p28: f1 channel - trim pulse                    [1 msec]
;d0 : incremented delay (2D)                     [3 usec]
;d1 : relaxation delay; 1-5 * T1
;d4 : 1/(4J)XH
;d11: delay for disk I/O                         [30 msec]
;d12: delay for power switching                  [20 usec]
;d16: delay for homospoil/gradient recovery
;d20 : = d23
;d23: d23 = T : 13.3 or 26.6 msec
;     2T (constant time period) = n/J(CC)
;cnst2: = J(XH)
;cnst21: CO chemical shift (offset, in ppm)
;inf1: 1/SW(X) = 2 * DW(X)
;in0: 1/(2 * SW(X)) = DW(X)
;in20: = in0
;nd0: 2
;NS: 4 * n
;DS: 32
;td1: number of experiments
;FnMODE: echo-antiecho
;cpd2: decoupling according to sequence defined by cpdprg2
;pcpd2: f2 channel - 90 degree pulse for decoupling sequence

;use gradient ratio:    gp 1 : gp 2 : gp5
;                       80 : 35.1 : 15    for C-13
;                       80 : 23.1 : 15    for N-15

;for z-only gradients:
;gpz1: 80%
;gpz2: 35.1% for C-13, 23.1% for N-15
;gpz4: 0.1-2% for slice selective excitation/refocussing
; gpz5: 15%
; gpz6: 7%
; gpz7: 9%
;use gradient files:
;gpnam1: SMSQ10.100
;gpnam2: SMSQ10.100
;gpnam5: SMSQ10.100
;gpnam6: SMSQ10.100
;gpnam7: SMSQ10.100
```