

## Supporting Information

# Backbone Assignment of Perdeuterated Proteins by Solid-State NMR Using Proton-Detection and Ultrafast Magic-Angle Spinning

Pascal Fricke<sup>1</sup>, Veniamin Chevelkov<sup>1\*</sup>, Maximilian Zinke<sup>1</sup>, Karin Giller<sup>2</sup>, Stefan Becker<sup>2</sup>, Adam Lange<sup>1,3\*</sup>

<sup>1</sup>Department of Molecular Biophysics, Leibniz-Institut für Molekulare Pharmakologie, 13125 Berlin, Germany

<sup>2</sup>Department of NMR-based Structural Biology, Max Planck Institute for Biophysical Chemistry, 37077 Göttingen, Germany

<sup>3</sup>Institut für Biologie, Humboldt-Universität zu Berlin, 10115 Berlin, Germany

\* [alange@fmp-berlin.de](mailto:alange@fmp-berlin.de) (A.L.) or [shevelkov@fmp-berlin.de](mailto:shevelkov@fmp-berlin.de) (V.S.)

---

## Supplementary Data 1

(H)NH pulse sequence example (on Bruker hardware running TopSpin version 2.1)

```
1 ; hNH2D_sp
2 ; water suppression by water saturation and spoil pulse
3 ; 2D version
4 ; o1 on water resonance
5 ; o2 in the middle of amide region
6 ; o3 in the middle of carbon region
7 ;*****
8 ;* The authors are not responsible for any damage caused *
9 ;* by the use of this pulse program or parts thereof. *
10 ;*****
11
12 #include <Avance.incl>
13 #include <Delay.incl>
14 #include <Grad.incl>
15
16
17 "p23=1.7*p22"
18 "d31=1s/cnst31-.5*p29-8u-de"
19 "d0=.5u"
20
21 "in0=inf1/4" ;d0=F1 -> ph17 (reverse)
22
23
24
25 1 ze
26 d18
27 30m LOCKDEC_ON
28 30m LOCKH_ON
29 30m H2_PULSE
30 2 40u do:f3
31 40u do:f2
32 40u do:f1
33 30m H2_LOCK
34 30m LOCKH_OFF
35 d1
36 30m LOCKH_ON
37 30m H2_PULSE
38 20u reset:f1 reset:f2 reset:f3
39
40
41
```

```

42 20u fq=cnst10:f1 ;1H offset to HN
43 5u pl6:f3 ;13C XY DC power
44 15u pl2:f2 ;15N hN CP power
45 5u pl3:f1 ;1H 90 power
46 p4:f1 ph1 ;1H 90 pulse
47 .6u pl5:f1 ;1H hN CP power
48
49 (p15 ph2):f2 (p15:spf3 ph10):f1 ;hN CP
50
51
52 .6u pl4:f2 ;15N 90 power
53 .6u pl12:f1 ;1H waltz16 DC power
54 .6u cpd1:f1 ;1H waltz16 DC scheme (pcpd1)
55 .6u cpd3:f3 ;13C xy DC scheme (p28, d28)
56 d0 ;15N incrementation
57 ;(p6 ph21):f2 ;15N 180 for 1H waltz16 DC optimization
58 d0 ;15N incrementation
59 .6u do:f3 ;13C DC power OFF
60 .6u do:f1 ;1H DC power OFF
61
62 p5:f2 ph17 ;15N 90 pulse
63 d20 ;water suppression delay d20
64 2u fq=cnst19:f1 ;1H offset to water for presat pulse
65
66 1u pl21:f1 ;1H spoil pulse power
67 (p21:spf12 ph13):f1 ;1H spoil pulse
68
69 d7 ;water suppression delay d7
70
71 2u pl16:f1 ;1H water saturation pulse power
72 16 p22:f1 ph22 ;water saturation
73 p23:f1 ph22 ;water saturation
74 p23:f1 ph23 ;water saturation
75 lo to 16 times 1
76 d7 ;water suppression delay d7
77
78 5u pl5:f1 ;1H nH CP power
79 d9 ;delay
80 5u fq=cnst10:f1 ;1H offset to HN
81 5u
82 p7:f2 ph18 ;15N 90 pulse
83 10u pl2:f2 ;15N nH CP power
84
85 (p16 ph12):f2 (p16:spf4 ph11):f1 ;nH CP
86
87 5u
88 8u pl3:f1 ;1H power
89 d31 ;1H refocusing delay
90 p29:f1 ph29 ;1H 180 refocusing pulse
91 d31 ;1H refocusing delay
92 6u pl29:f2 ;15N waltz16 DC power
93 1u cpd2:f2 ;15N waltz16 DC scheme (pcpd2)
94 1u
95 go=2 ph31 ; ;Acquisition
96
97 40u do:f1 ;decouplers off
98 40u do:f3 ;decouplers off
99 40u do:f2 ;decouplers off
100
101 10m wr #0 if #0 zd
102
103 1m ip17
104 10u id0
105 lo to 2 times td1
106
107 HaltAcqu, 1m
108
109 30m H2_LOCK
110 30m LOCKH_OFF
111 30m LOCKDEC_OFF
112
113 exit

```

```

114
115
116 ph1= 0 2
117 ph10= 3 3
118 ph2= 0 0 2 2
119 ph17= 1
120 ph18= 1 1 1 1 3 3 3 3
121 ph12= 0
122 ph13= 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3
123      1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
124      3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3
125      1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
126 ph11= 0 0 0 0 0 0 0 0 2 2 2 2 2 2 2 2
127 ph21= 2
128 ph29= 0 0 0 0 0 0 0 0 2 2 2 2 2 2 2 2
129 ph31= 0 2 2 0 2 0 0 2 2 0 0 2 0 2 2 0
130 ph22= 0 0 0 0 2 2 2 2
131 ph23= 1 1 1 1 1 1 1 1 3 3 3 3 3 3 3 3
132
133 ;cnst31: MAS speed in Hz
134 ;d1: recycle delay
135 ;p4: 1H 90° hard excitation pulse duration
136 ;pl3: 1H hard 90° excitation pulse r.f. power
137 ;p15: 1H-15N CP duration
138 ;p15: 1H CP r.f. power during 1H-15N CP and 15N-1H CP
139 ;p12: 15N CP r.f. power during 1H-15N CP
140 ;spnam3: ramp shape (on 1H) during 1H-15N CP
141 ;cnst10: distance between water and amide protons in Hz (positive)
142 ;pl12: 1H WALTZ-dec r.f. power during 15N evolution
143 ;pcpd1: 1H WALTZ-dec pulse duration during 15N evolution
144 ;cpdprg1: 1H WALTZ-16 decoupling scheme during 15N evolution
145 ;p16: 13C XY-dec r.f. power during 15N evolution
146 ;p28: 13C XY-dec pulse duration during 15N evolution
147 ;d28: 13C XY-dec delay during 15N evolution
148 ;cpdprg3: 13C XY decoupling scheme during 15N evolution
149 ;p5: 15N 90° flip pulse duration
150 ;p7: 15N 90° flip pulse duration (for optimization)
151 ;p14: 15N 90° flip pulse r.f. power
152 ;d20: water suppression T delay
153 ;cnst19: distance between 1H offset and water signal in Hz
154 ;p21: water suppression spoil pulse duration
155 ;p121: water suppression spoil pulse r.f. power
156 ;spnam12: water suppression spoil pulse ramp shape
157 ;p22: first pulse duration in water suppression train
158 ;p23: second pulse duration in water suppression train (automatically calculated)
159 ;pl16: water suppression train r.f. power
160 ;d7: small water suppression delay to optimize efficiency
161 ;d9: small water suppression delay to optimize efficiency
162 ;p16 15N-1H CP duration
163 ;spnam4: ramp shape (on 1H) during 15N-1H CP
164 ;d31: spin echo delay (automatically calculated)
165 ;p29: 1H 180° spin echo pulse duration
166 ;pl29: 15N WALTZ-dec r.f. power during acquisition
167 ;pcpd2: 15N WALTZ-dec pulse duration during acquisition
168 ;cpdprg2: 15N WALTZ-16 decoupling scheme during acquisition

```

## Supplementary Data 2

XY decoupling pulse sequence example (on Bruker hardware running TopSpin version 2.1)

```
1 ; XY_Decoupling
2 ;*****
3 ;* The authors are not responsible for any damage caused *
4 ;*   by the use of this pulse program or parts thereof.   *
5 ;*****
6
7 1  d28
8   p28:0
9   d28
10  p28:90
11
12  d28
13  p28:0
14  d28
15  p28:90
16
17  d28
18  p28:90
19  d28
20  p28:0
21
22  d28
23  p28:90
24  d28
25  p28:0
26
27  d28
28  p28:180
29  d28
30  p28:270
31
32  d28
33  p28:180
34  d28
35  p28:270
36
37  d28
38  p28:270
39  d28
40  p28:180
41
42  d28
43  p28:270
44  d28
45  p28:180
46
47 jump to 1
```

## Supplementary Data 3

(H)CANH pulse sequence example (on Bruker hardware running TopSpin version 2.1)

```
1 ; (H)CANH_3D_sp
2 ; water suppression by water saturation and spoil pulse
3 ; 3D version
4 ; o1 on water resonance
5 ; o2 in the middle of whole carbon region
6 ; o3 in the middle of amide region
7 ;*****
8 ;* The authors are not responsible for any damage caused *
9 ;* by the use of this pulse program or parts thereof. *
10 ;*****
11
12 #include <Avance.incl>
13 #include <Delay.incl>
14 #include <Grad.incl>
15
16
17 "d31=1s/cnst31-.5*p29-8u-de"
18 "p23=1.7*p22"
19 "d0=.5u"
20 "d10=.5u"
21
22 "in0=inf2/2" ;d0=F2 -> ph15 (reverse)
23 "in10=inf1/4" ;d10=F1-> ph16 (reverse)
24
25
26
27 1 ze
28 30m LOCKDEC_ON
29 30m LOCKH_ON
30 30m H2_PULSE
31 2 40u do:f3
32 40u do:f2
33 40u do:f1
34 30m H2_LOCK
35 30m LOCKH_OFF
36 d1
37 30m LOCKH_ON
38 30m H2_PULSE
39 20u reset:f1 reset:f2 reset:f3
40
41
42
43 5u pl29:f3 ;15N waltz16 DC power
44 15u pl2:f2 ;13C hCA CP power
45 5u pl3:f1 ;1H 90 power
46 2u fq=cnst10:f1 ;1H offset to HN
47 2u fq=cnst11:f2 ;13C offset to CA
48 p4:f1 ph1 ;1H 90 pulse
49 0.6u pl5:f1 ;1H hCA CP power
50
51 (p15 ph2):f2 (p15:spf1 ph10):f1 ;hCA CP
52
53 .6u cpd3:f3 ;15N waltz16 DC scheme
54 d0 ;13C incrementation
55 .6u do:f3 ;15N DC power OFF
56
57 .6u pl14:f3 ;15N caN CP power
58 .6u pl15:f2 ;13C caN CP power
59
60 (p25 ph15):f2 (p25:spf3 ph14):f3 ;caN CP
61
62 1u fq=0:f2 ;13C offset reset
63 .6u pl9:f2 ;13C xy DC power
64 .6u cpd5:f2 ;13C xy DC scheme
65 .6u pl12:f1 ;1H waltz16 DC power
66 .6u cpd1:f1 ;1H waltz16 DC scheme
67 d10 ;15N incrementation
```

```

68      d10                                ;15N incrementation
69      .6u pl6:f3                          ;15N 90 power
70      .6u do:f2                            ;13C DC power OFF
71      .6u do:f1                            ;1H DC power OFF
72
73      p5:f3 ph16                          ;15N 90 pulse
74      d20                                  ;water suppression delay d20
75      2u fq=cnst19:f1                     ;1H offset to water for presat pulse
76
77      1u pl21:f1                           ;1H spoil pulse power
78      (p21:spf12 ph18):f1                 ;1H spoil pulse
79
80      d7                                    ;water suppression delay d7
81
82      2u pl16:f1                            ;1H water saturation pulse power
83
84      16 p22:f1 ph22                        ;water saturation
85          p23:f1 ph23                      ;water saturation
86          p23:f1 ph23                      ;water saturation
87      lo to 16 times 1
88
89      d7                                    ;water suppression delay d7
90
91      5u pl7:f1                             ;1H nH CP power
92      d9                                    ;delay
93      5u fq=cnst10:f1                     ;1H offset back to HN
94      2u
95      p5:f3 ph17                           ;15N 90 pulse
96      .6u pl8:f3                          ;15N nH CP power
97
98      (p20 ph12):f3 (p20:spf4 ph11):f1    ;nH CP
99
100     5u
101     8u pl3:f1                             ;1H power
102     d31                                   ;1H refocusing delay
103     p29:f1 ph29                          ;1H 180 refocusing pulse
104     d31                                   ;1H refocusing delay
105
106     6u pl30:f3                            ;15N waltz16 DC power
107     1u cpd4:f3                            ;15N waltz16 DC scheme
108     1u
109     go=2 ph31 ;                            ;Acquisition
110
111     40u do:f1                             ;decouplers off
112     40u do:f3                             ;decouplers off
113     40u do:f2                             ;decouplers off
114
115     10m wr #0 if #0 zd
116
117     1m ip15
118     10u id0
119     lo to 2 times td2
120
121     800u rp15
122     800u rd0
123
124     1m ip16
125     800u id10
126     lo to 2 times td1
127
128
129     HaltAcqu, 1m
130
131     30m H2_LOCK
132     30m LOCKH_OFF
133     30m LOCKDEC_OFF
134
135     exit
136
137     ph1 = 0 2
138     ph10= 3 3 3 3 3 3 3 3 1 1 1 1 1 1 1 1
139     ph2 = 0 0 2 2

```

```

140 ph15= 0
141 ph14= 0
142 ph16= 1
143 ph17= 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
144      2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
145 ph18= 0 0 0 0 2 2 2 2
146 ph12= 1
147 ph11= 0 0 0 0 2 2 2 2
148 ph22= 0 0 0 0 2 2 2 2
149 ph23= 1 1 1 1 1 1 1 1 3 3 3 3 3 3 3 3
150 ph21= 0
151 ph29= 0 2 2 0 0 2 2 0
152 ph31= 0 2 2 0 2 0 0 2 2 0 0 2 0 2 2 0
153      2 0 0 2 0 2 2 0 0 2 2 0 2 0 0 2
154
155 ;cnst31: MAS speed in Hz
156 ;d1: recycle delay
157 ;p4: 1H 90° hard excitation pulse duration
158 ;p13: 1H hard 90° excitation pulse r.f. power
159 ;cnst10: distance between water and amide protons in Hz (positive)
160 ;cnst11: distance between 13C offset and 13CA in Hz (negative)
161 ;p15: 1H-13CA CP duration
162 ;p15: 1H CP r.f. power during 1H-13CA CP
163 ;p12: 13CA CP r.f. power during 1H-13CA CP
164 ;spnam1: ramp shape (on 1H) during 1H-13CA CP
165 ;p129: 15N WALTZ-dec r.f. power during 13CA evolution
166 ;pcpd3: 15N WALTZ-dec pulse duration during 13CA evolution and acquisition
167 ;cpdprg3: 15N WALTZ-16 decoupling scheme during 13CA evolution
168 ;p25: 13CA-15N CP duration
169 ;p115: 13CA CP r.f. power during 13CA-15N CP
170 ;p114: 15N CP r.f. power during 13CA-15N CP
171 ;spnam3: ramp shape (on 15N) during 13CA-15N CP
172 ;p19: 13C XY-dec r.f. power during 15N evolution
173 ;p28: 13C XY-dec pulse duration during 15N evolution
174 ;d28: 13C XY-dec delay during 15N evolution
175 ;cpdprg5: 13C XY decoupling scheme during 15N evolution
176 ;p112: 1H WALTZ-dec r.f. power during 15N evolution
177 ;pcpd1: 1H WALTZ-dec pulse duration during 15N evolution
178 ;cpdprg1: 1H WALTZ-16 decoupling scheme during 15N evolution
179 ;p5: 15N 90° flip pulse duration
180 ;p16: 15N 90° flip pulse r.f. power
181 ;d20: water suppression T delay
182 ;cnst19: distance between 1H offset and water signal in Hz
183 ;p21: water suppression spoil pulse duration
184 ;p121: water suppression spoil pulse r.f. power
185 ;spnam12: water suppression spoil pulse ramp shape
186 ;p22: first pulse duration in water suppression train
187 ;p23: second pulse duration in water suppression train (automatically calculated)
188 ;p116: water suppression train r.f. power
189 ;d7: small water suppression delay to optimize efficiency
190 ;d9: small water suppression delay to optimize efficiency
191 ;p20: 15N-1H CP duration
192 ;p18: 15N CP r.f. power during 15N-1H CP
193 ;p17: 1H CP r.f. power during 15N-1H CP
194 ;spnam4: ramp shape (on 1H) during 15N-1H CP
195 ;d31: spin echo delay (automatically calculated)
196 ;p29: 1H 180° spin echo pulse duration
197 ;p130: 15N WALTZ-dec r.f. power during acquisition
198 ;cpdprg4: 15N WALTZ-16 decoupling scheme during acquisition

```

## Supplementary Data 4

(H)CONH pulse sequence example (on Bruker hardware running TopSpin version 2.1)

```
1 ; (H)CONH_3D
2 ; water supression by water saturation and spoil pulse
3 ; 3D version
4 ; o1 on water resonance
5 ; o2 in the middle of CO
6 ; o3 in the middle of amide region
7 ;*****
8 ;* The authors are not responsible for any damage caused *
9 ;* by the use of this pulse program or parts thereof. *
10 ;*****
11
12 #include <Avance.incl>
13 #include <Delay.incl>
14 #include <Grad.incl>
15
16
17 "d31=1s/cnst31-.5*p29-8u-de"
18 "p23=p22*1.7"
19 "d0=0.5u"
20 "d10=.5u"
21
22 "in0=inf2/4" ;d0= F2 -> ph15
23 "in10=inf1/4" ;d10=F1 -> ph16 (reverse)
24
25
26 1 ze
27 30m LOCKDEC_ON
28 30m LOCKH_ON
29 30m H2_PULSE
30 2 40u do:f3
31 40u do:f2
32 40u do:f1
33
34 30m H2_LOCK
35 30m LOCKH_OFF
36 d1
37 30m LOCKH_ON
38 30m H2_PULSE
39 20u reset:f1 reset:f2 reset:f3
40
41
42
43 15u pl2:f2 ;13C hCO CP power
44 5u pl3:f1 ;1H 90 power
45 2u fq=cnst10:f1 ;1H offset to HN
46 1u fq=0:f2 ;13C offset reset
47 p4:f1 ph1 ;1H 90 pulse
48 0.4u pl5:f1 ;1H hCO CP power
49
50 (p15 ph2):f2 (p15:spf1 ph10):f1 ;hCO CP
51
52 10u
53 2u pl6:f3 ;15N power for refoc pulses
54 d0 ;13C incrementation
55 .6u pl26:f2 ;13C selective pulse power
56 0.1u
57 (center (p26:sp5 ph25):f2 (p11 ph25):f3) ;13CA selective pulse + 15N 180 refoc pulse
58 d0 ;13C incrementation
59 10u
60 (p26:sp6 ph25):f2 ;13CO selective pulse (spoffs6, sp6, spnam6)
61 10u
62 2u pl12:f1 ;1H waltz16 DC power
63 (center (p26:sp5 ph25):f2 (p11 ph25):f3) ;13CA selective pulse + 15N 180 refoc pulse
64
65 10u pl14:f3 ;15N coN CP power
66 2u pl15:f2 ;13C coN CP power
67
```

```

68      (p25 ph15):f2 (p25:spf3 ph14):f3      ;coN CP
69
70      .6u pl9:f2                          ;13C xy DC power
71      1u fq=cnst24:f2                      ;13C offset to center
72      .6u cpd5:f2                          ;13C xy DC scheme
73      .8u cpd1:f1                          ;1H waltz16 DC scheme
74
75      d10                                  ;15N incrementation
76      d10                                  ;15N incrementation
77      .6u pl6:f3                          ;15N 90 power
78      .6u do:f2                            ;13C DC power OFF
79      .6u do:f1                            ;1H DC power OFF
80
81      p5:f3 ph16                          ;15N 90 pulse
82      d20                                  ;water suppression delay d20
83      2u fq=cnst19:f1                     ;1H offset to water
84
85      1u pl21:f1                           ;1H spoil pulse power
86      (p21:spf12 ph7):f1                 ;1H spoil pulse
87
88      d7                                  ;water suppression delay d7
89
90      2u pl16:f1                           ;1H water saturation pulse power
91      16 p22:f1 ph22                      ;water saturation
92      p23:f1 ph23                         ;water saturation
93      p23:f1 ph23                         ;water saturation
94      lo to 16 times 1
95
96      d7                                  ;water suppression delay d7
97
98      5u pl7:f1                            ;1H nH CP power
99      d9                                  ;delay
100     5u fq=cnst10:f1                     ;1H offset back to HN
101     2u
102     p5:f3 ph17                          ;15N 90 pulse
103     .6u pl8:f3                          ;15N nH CP power
104
105     (p20 ph12):f3 (p20:spf4 ph11):f1    ;nH CP
106
107     5u
108     8u pl3:f1                            ;1H power
109     d31                                  ;1H refocusing delay
110     p29:f1 ph29                          ;1H 180 refocusing pulse
111     d31                                  ;1H refocusing delay
112
113     6u pl30:f3                           ;15N waltz16 DC power
114     1u cpd4:f3                           ;15N waltz16 DC scheme
115     1u
116     go=2 ph31                            ;Acquisition
117     40u do:f1                            ;decouplers off
118     40u do:f3                            ;decouplers off
119     40u do:f2                            ;decouplers off
120
121     10m wr #0 if #0 zd
122
123     1m ip15
124     10u id0
125     lo to 2 times td2
126
127     800u rp15
128     800u rd0
129
130     1m ip16
131     800u id10
132     lo to 2 times td1
133
134     HaltAcqu, 1m
135
136     30m H2_LOCK
137     30m LOCKH_OFF
138     30m LOCKDEC_OFF
139

```

```

140 exit
141
142 ph1= 0 2
143 ph10= 3 3 1 1
144 ph2= 0 0 0 0 2 2 2 2
145 ph7 = 1 1 1 1 3 3 3 3
146 ph15= 0
147 ph14= 0
148 ph16= 1
149 ph17= 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
150      2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
151 ph12= 1
152 ph11= 0 0 0 0 0 0 0 0 2 2 2 2 2 2 2 2
153 ph22= 2 0 0 2 0 2 2 0 1 3 3 1 3 1 1 3
154 ph23= 1 1 3 3 3 3 1 1 0 0 2 2 2 2 0 0
155 ph29= 0 2 2 0 0 2 2 0 2 0 0 2 2 0 0 2
156 ph25= 0
157 ph31= 0 2 2 0 2 0 0 2 2 0 0 2 0 2 2 0
158      2 0 0 2 0 2 2 0 0 2 2 0 2 0 0 2
159
160 ;cnst31: MAS speed in Hz
161 ;d1: recycle delay
162 ;p4: 1H 90° hard excitation pulse duration
163 ;pl3: 1H hard 90° excitation pulse r.f. power
164 ;cnst10: distance between water and amide protons in Hz (positive)
165 ;p15: 1H-13CO CP duration
166 ;pl5: 1H CP r.f. power during 1H-13CO CP
167 ;pl2: 13CO CP r.f. power during 1H-13CO CP
168 ;spnam1: ramp shape (on 1H) during 1H-13CO CP
169 ;pl6: 15N hard pulse r.f. power
170 ;p26: 13CA/CO selective pulse duration
171 ;pl26: 13CA/CO selective pulse r.f. power
172 ;sp5: 13CA selective pulse r.f. power
173 ;sp6: 13CO selective pulse r.f. power
174 ;spnam5: 13CA selective pulse shape
175 ;spnam6: 13CO selective pulse shape
176 ;spoffs5: distance between 13CO and 13CA (negative)
177 ;spoffs6: 0 Hz
178 ;pl1: 15N 180° inversion pulse duration
179 ;p25: 13CO-15N CP duration
180 ;pl15: 13CO CP r.f. power during 13CO-15N CP
181 ;pl14: 15N CP r.f. power during 13CO-15N CP
182 ;spnam3: ramp shape (on 15N) during 13CO-15N CP
183 ;cnst24: distance between CO and center (100ppm) in Hz (negative)
184 ;pl9: 13C XY-dec r.f. power during 15N evolution
185 ;p28: 13C XY-dec pulse duration during 15N evolution
186 ;d28: 13C XY-dec delay during 15N evolution
187 ;cpdprg5: 13C XY decoupling scheme during 15N evolution
188 ;pl12: 1H WALTZ-dec r.f. power during 15N evolution
189 ;pcpd1: 1H WALTZ-dec pulse duration during 15N evolution
190 ;cpdprg1: 1H WALTZ-16 decoupling scheme during 15N evolution
191 ;p5: 15N 90° flip pulse duration
192 ;d20: water suppression T delay
193 ;cnst19: distance between 1H offset and water signal in Hz
194 ;p21: water suppression spoil pulse duration
195 ;pl21: water suppression spoil pulse r.f. power
196 ;spnam12: water suppression spoil pulse ramp shape
197 ;p22: first pulse duration in water suppression train
198 ;p23: second pulse duration in water suppression train (automatically calculated)
199 ;pl16: water suppression train r.f. power
200 ;d7: small water suppression delay to optimize efficiency
201 ;d9: small water suppression delay to optimize efficiency
202 ;p20: 15N-1H CP duration
203 ;pl8: 15N CP r.f. power during 15N-1H CP
204 ;pl7: 1H CP r.f. power during 15N-1H CP
205 ;spnam4: ramp shape (on 1H) during 15N-1H CP
206 ;d31: spin echo delay (automatically calculated)
207 ;p29: 1H 180° spin echo pulse duration
208 ;pl30: 15N WALTZ-dec r.f. power during acquisition
209 ;cpdprg4: 15N WALTZ-16 decoupling scheme during acquisition

```

## Supplementary Data 5

Selective 180° <sup>13</sup>C pulse optimization sequence example (on Bruker hardware running TopSpin version 2.1)

```
1 ; Selective_180
2 ; selective soft pulse on either CA or CO and subsequent carbon 90 hard pulse
3 ; used for CA decoupling during CO evolution periods
4 ; ol in the middle of whole carbon region
5 ;*****
6 ;* The authors are not responsible for any damage caused *
7 ;* by the use of this pulse program or parts thereof. *
8 ;*****
9
10 #include <Avance.incl>
11 #include <Delay.incl>
12
13
14
15 1 ze
16 30m LOCKDEC_ON
17 30m LOCKH_ON
18 30m H2_PULSE
19 2 40u do:f2
20 30m H2_LOCK
21 30m LOCKH_OFF
22 d1
23 30m LOCKH_ON
24 30m H2_PULSE
25 1u fq=0:f2
26 1u pl11:f1 ;13C selective pulse power
27 5u
28 (p11:sp11 ph3):f1 ;13C selective pulse (spoffs11, sp11, spnam11)
29 5u
30 1u p11:f1 ;13C 90 power
31 p1:f1 ph1 ;13C 90 pulse
32 1u pl12:f2 ;1H spinal64 DC power (optional)
33 1u cpds2:f2 ;1H spinal64 DC scheme
34 go=2 ph31 ;Acquisition
35
36 1m do:f2 ;decouplers off
37
38 100m wr #0
39
40 HaltAcqu, 1m
41
42 30m H2_LOCK
43 30m LOCKH_OFF
44 30m LOCKDEC_OFF
45
46 exit
47
48 ph1 = 0 1 2 3
49 ph3 = 0 1 2 3
50 ph31= 2 3 0 1
51
52 ;d1: recycle delay
53 ;p11: 13CA/CO selective pulse duration
54 ;pl11: 13CA/CO selective pulse power
55 ;sp11: 13CA/CO selective pulse power
56 ;spnam11: 13CA/CO selective pulse shape
57 ;spoffs11: distance between carbon offset and CA/CO in Hz
58 ;p1: 13C hard excitation pulse duration
59 ;pl1: 13C hard excitation pulse r.f. power
60 ;pl12: 1H spinal 64 decoupling r.f. power (not needed, can be 0)
61 ;cpdprg2: 1H spinal 64 decoupling scheme
```

## Supplementary Data 6

(H)CACO(N)H pulse sequence example (on Bruker hardware running TopSpin version 2.1)

```
1 ; (H)CACO(N)H_3D_sp
2 ; water suppression by water saturation and spoil pulse
3 ; 3D version
4 ; o1 on water resonance
5 ; o2 in the middle of CA
6 ; o3 in the middle of amide region
7 ;*****
8 ;* The authors are not responsible for any damage caused *
9 ;* by the use of this pulse program or parts thereof. *
10 ;*****
11
12 #include <Avance.incl>
13 #include <Delay.incl>
14 #include <Grad.incl>
15
16
17 "d31=1s/cnst31-5u-.5*p29-de"
18 "p23=1.7*p22"
19 "d0=.5u"
20 "d10=.5u"
21
22 "in0=inf2/2" ;d0=F2 -> ph2
23 "in10=inf1/4" ;d10=F1-> ph15
24
25
26 1 ze
27 30m LOCKDEC_ON
28 30m LOCKH_ON
29 30m H2_PULSE
30 2 40u do:f3
31 40u do:f2
32 40u do:f1
33 30m H2_LOCK
34 30m LOCKH_OFF
35 d1
36 30m LOCKH_ON
37 30m H2_PULSE
38 1m reset:f1 reset:f2 reset:f3
39
40
41 1u fq=0:f2 ;13C offset reset
42 1u fq=cnst10:f1 ;1H offset to HN
43 10u pl2:f2 ;13C hCA CP power
44 10u pl1:f1 ;1H 90 power
45 10u pl29:f3 ;15N waltz16 DC power
46 p2:f1 ph1 ;1H 90 pulse
47 .6u pl19:f1 ;1H hCA CP power
48
49 (p15:spf1 ph0):f1 (p15 ph2):f2 ;hCA CP
50
51 .7u cpd4:f3 ;15N waltz16 DC scheme (pcpd3)
52 0.1u
53 d0 ;13C incrementation
54 .6u pl17:f2 ;13C caCO homo CP power
55 1u do:f3 ;15N DC power OFF
56
57 (p17:spf17 ph17):f2 ;13C caCO homo CP
58
59 .6u fq=cnst25:f2 ;13C offset to CO
60 .6u pl18:f2 ;13C trim pulse power
61 (p18 ph18):f2 ;13CO trim pulse
62 10u
63 2u pl6:f3 ;15N power
64 d10 ;13C incrementation
65 .6u pl26:f2 ;13C selective pulse power
66 0.1u
67 (center (p26:sp5 ph25):f2 (p11 ph25):f3) ;13CA selective pulse + 15N 180 refoc pulse
```

```

68      d10                                ;13C incrementation
69      10u
70      (p26:sp6 ph25):f2                  ;13CO selective pulse (spoffs6, sp6)
71      10u
72      (center (p26:sp5 ph25):f2 (p11 ph25):f3) ;13Calpha selective pulse + 15N 180 refoc pulse
73      10u pl14:f3                        ;15N coN CP power
74      2u pl15:f2                          ;13C coN CP power
75
76      (p25 ph15):f2 (p25:spf3 ph14):f3   ;coN CP
77
78      .6u pl6:f3                          ;15N 90 power
79      p5:f3 ph16                          ;15N 90 pulse
80      d20                                  ;water suppression delay d20
81      2u fq=cnst19:f1                    ;1H offset to water
82
83      1u pl7:f1                            ;1H spoil pulse power
84      (p14:spf12 ph24):f1                ;1H spoil pulse
85
86      d7                                  ;water suppression delay d7
87
88      2u pl16:f1                          ;1H water saturation power
89      16 p22:f1 ph22                      ;water saturation
90      p23:f1 ph23                        ;water saturation
91      p23:f1 ph23                        ;water saturation
92      lo to 16 times 1
93
94      d7                                  ;water suppression delay d7
95
96      5u pl7:f1                            ;1H nH CP power
97      5u fq=cnst10:f1                    ;1H offset to HN
98      d9                                  ;delay
99      p5:f3 ph20                          ;15N 90 pulse
100     .6u pl8:f3                          ;15N nH CP power
101
102     (p16 ph12):f3 (p16:spf4 ph11):f1    ;nH CP
103
104     5u
105     5u pl1:f1                            ;1H power
106     d31                                  ;1H refoc delay
107     p29:f1 ph29                          ;1H 180 refoc pulse
108     d31                                  ;1H refoc delay
109
110     6u pl30:f3                            ;15N waltz16 DC power
111     1u cpd6:f3                            ;15N waltz16 DC scheme (pcpd3)
112     1u
113     go=2 ph31                             ;Acquisition
114     40u do:f1                             ;decouplers off
115     40u do:f3                             ;decouplers off
116     40u do:f2                             ;decouplers off
117
118     10m wr #0 if #0 zd
119
120     1m ip2
121     10u id0
122     lo to 2 times td2
123
124     500u rp2
125     100u rd0
126
127     600u ip15
128     200u id10
129     lo to 2 times td1
130
131     HaltAcqu, 1m
132
133     30m H2_LOCK
134     30m LOCKH_OFF
135     30m LOCKDEC_OFF
136
137     exit
138
139     ph0= 3 3 3 3 3 3 3 3 1 1 1 1 1 1 1 1

```

```

140 ph1= 0 2
141 ph2= 0 0 2 2
142 ph17= 0 0 0 0 0 0 0 0 2 2 2 2 2 2 2 2
143 ph18= 3 3 3 3 3 3 3 3 1 1 1 1 1 1 1 1
144 ph14= 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
145      3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3
146 ph15= 0
147 ph16= 2
148 ph20= 1
149 ph12= 0
150 ph22= 0 0 0 0 2 2 2 2
151 ph23= 1 1 1 1 1 1 1 1 3 3 3 3 3 3 3 3
152 ph11= 0 0 0 0 2 2 2 2
153 ph24= 0 0 0 0 2 2 2 2
154 ph29= 0 0 2 2
155 ph25= 0
156 ph31= 0 2 2 0 2 0 0 2 2 0 0 2 0 2 2 0
157      2 0 0 2 0 2 2 0 0 2 2 0 2 0 0 2
158
159 ;cnst31: MAS speed in Hz
160 ;d1: recycle delay
161 ;p2: 1H 90° hard excitation pulse duration
162 ;p11: 1H hard pulse r.f. power
163 ;cnst10: distance between water and amide protons in Hz (positive)
164 ;p15: 1H-13CA CP duration
165 ;p19: 1H CP r.f. power during 1H-13CA CP
166 ;p12: 13CA CP r.f. power during 1H-13CA CP
167 ;spnam1: ramp shape (on 1H) during 1H-13CA CP
168 ;p129: 15N WALTZ-dec r.f. power during 13CA evolution
169 ;pcpd3: 15N WALTZ-dec pulse duration during 13CA evolution
170 ;cpdprg4: 15N WALTZ-16 decoupling scheme during 13CA evolution
171 ;p17: 13CA-13CO DREAM pulse duration
172 ;p117: 13CA-13CO DREAM pulse r.f. power
173 ;spnam17: 13CA-13CO DREAM pulse shape
174 ;cnst25: distance between 13CA and 13CO in Hz (positive)
175 ;p18: trim pulse duration
176 ;p118: trim pulse r.f. power
177 ;p26: 13CA/CO selective pulse duration
178 ;p126: 13CA/CO selective pulse r.f. power
179 ;sp5: 13CA selective pulse r.f. power
180 ;sp6: 13CO selective pulse r.f. power
181 ;spnam5: 13CA selective pulse shape
182 ;spnam6: 13CO selective pulse shape
183 ;spoffs5: distance between 13CO and 13CA (negative)
184 ;spoffs6: 0 Hz
185 ;p11: 15N 180° inversion pulse duration
186 ;p16: 15N hard pulse r.f. power
187 ;p25: 13CO-15N CP duration
188 ;p115: 13CO CP r.f. power during 13CO-15N CP
189 ;p114: 15N CP r.f. power during 13CO-15N CP
190 ;spnam3: ramp shape (on 15N) during 13CO-15N CP
191 ;p5: 15N 90° flip pulse duration
192 ;d20: water suppression T delay
193 ;cnst19: distance between 1H offset and water signal in Hz
194 ;p14: water suppression spoil pulse duration
195 ;p17: water suppression spoil pulse r.f. power and 1H CP r.f. power for 15N-1H CP
196 ;spnam12: water suppression spoil pulse ramp shape
197 ;p22: first pulse duration in water suppression train
198 ;p23: second pulse duration in water suppression train (automatically calculated)
199 ;p116: water suppression train r.f. power
200 ;d7: small water suppression delay to optimize efficiency
201 ;d9: small water suppression delay to optimize efficiency
202 ;p16: 15N-1H CP duration
203 ;p18: 15N CP r.f. power during 15N-1H CP
204 ;spnam4: ramp shape (on 1H) during 15N-1H CP
205 ;d31: spin echo delay (automatically calculated)
206 ;p29: 1H 180° spin echo pulse duration
207 ;p130: 15N WALTZ-dec r.f. power during acquisition
208 ;cpdprg6: 15N WALTZ-16 decoupling scheme during acquisition
209 ;pcpd3: 15N WALTZ decoupling pulse duration

```

## Supplementary Data 7

$^{13}\text{CA}$ - $^{13}\text{CO}$  or  $^{13}\text{CA}$ - $^{13}\text{CB}$  DREAM pulse optimization sequence example (on Bruker hardware running TopSpin version 2.1)

```
1 ; CACO/CACB_DREAM_calib
2 ; SPECIFIC CP on CA and subsequent DREAM transfer to CO or to CB
3 ; used for CACO or CACB transfer
4 ; trim pulse only needed for CACO DREAM
5 ; comment line 42 for transfer from CA to CB!
6 ; ol in the middle of whole carbon region
7 ;*****
8 ;* The authors are not responsible for any damage caused *
9 ;* by the use of this pulse program or parts thereof. *
10 ;*****
11
12 #include <Avance.incl>
13
14
15
16 1 ze
17 30m LOCKDEC_ON
18 30m LOCKH_ON
19 30m H2_PULSE
20 2 10u do:f2
21 30m H2_LOCK
22 30m LOCKH_OFF
23 d1
24 30m LOCKH_ON
25 30m H2_PULSE
26 10u fq=0:f2
27 10u
28 10u fq=cnst15:f1 ;13C offset to CA
29 10u pl2:f2 ;1H 90 power
30 5u pl5:f1 ;13C hCA CP power
31 p2:f2 ph1 ;1H 90 pulse
32 0.8u pl6:f2 ;1H hCA CP power
33
34 (p15 ph2):f1 (p15:spf0 pl6 ph0):f2 ;hCA CP
35
36 0.8u pl17:f1 ;13C caCO or caCB homo CP power
37
38 (p17:spf17 ph17):f1 ;13C caCO or caCB homo CP
39
40 1u pl18:f1 ;13CO trim pulse power
41 .8u fq=cnst25:f1 ;13C offset to CO
42 (p18 ph18):f1 ;13CO trim pulse (comment line for CACB DREAM!)
43 .8u fq=0:f1 ;13C offset reset
44
45 1u pl12:f2 ;1H spinal64 DC power (optional)
46 1u cpds2:f2 ;1H spinal64 DC scheme
47 go=2 ph31 ;Acquisition
48
49 1m do:f2 ;decouplers off
50
51 100m wr #0
52
53 HaltAcqu, 1m
54
55 30m H2_LOCK
56 30m LOCKH_OFF
57 30m LOCKDEC_OFF
58
59 exit
60
61
62 ph0= 0 2
63 ph1= 1 1 1 1 3 3 3 3
64 ph20=3 3 3 3 1 1 1 1
65 ph2= 0 0 2 2
66 ph3= 1 1 3 3
```

```
67 ph17=0
68 ph18=3
69 ph31=0 2 2 0 2 0 0 2
70
71 ;d1: recycle delay
72 ;cnst15: distance between carbon offset and 13CA in Hz (negative)
73 ;p2: 1H 90° excitation pulse duration
74 ;pl2: 1H 90° excitation pulse r.f. power
75 ;p15: 1H-13CA CP duration
76 ;pl5: 13CA CP r.f. power during 1H-13CA CP
77 ;pl6: 1H CP r.f. power during 1H-13CA CP
78 ;spnam0: ramp shape (on 1H) during 1H-13CA CP
79 ;p17: 13CA-13CO or 13CA-13CB DREAM pulse duration
80 ;pl17: 13CA-13CO or 13CA-13CB DREAM pulse r.f. power
81 ;spnam17: 13CA-13CO or 13CA-13CB DREAM pulse shape
82 ;p18: trim pulse duration
83 ;pl18: trim pulse r.f. power
84 ;cnst25: distance between carbon offset and 13CO in Hz (positive)
85 ;pl12: 1H spinal 64 decoupling r.f. power (not needed, can be 0)
86 ;cpdprg2: 1H spinal 64 decoupling scheme
```

## Supplementary Data 8

(H)COCA(N)H pulse sequence example (on Bruker hardware running TopSpin version 2.1)

```
1 ; (H)COCA(N)H_3D_sp
2 ; water suppression by water saturation and spoil pulse
3 ; 3D version
4 ; o1 on water resonance
5 ; o2 in the middle of CO
6 ; o3 in the middle of amide region
7 ;*****
8 ;* The authors are not responsible for any damage caused *
9 ;* by the use of this pulse program or parts thereof. *
10 ;*****
11
12 #include <Avance.incl>
13 #include <Delay.incl>
14 #include <Grad.incl>
15
16
17 "d31=1s/cnst31-5u-.5*p29-de"
18 "p23=1.7*p22"
19 "d0=.5u"
20 "d10=.5u"
21
22 "in0=inf2/4" ;d0=F2 -> ph15 (reverse)
23 "in10=inf1/4" ;d10=F1-> ph2
24
25
26 1 ze
27 30m LOCKDEC_ON
28 30m LOCKH_ON
29 30m H2_PULSE
30 2 40u do:f3
31 40u do:f2
32 40u do:f1
33 30m H2_LOCK
34 30m LOCKH_OFF
35 d1
36 30m LOCKH_ON
37 30m H2_PULSE
38 1m reset:f1 reset:f2 reset:f3
39
40
41 1u fq=0:f2 ;13C offset reset
42 1u fq=cnst10:f1 ;1H offset to HN
43 10u pl2:f2 ;13C hCO CP power
44 10u pl1:f1 ;1H 90 power
45 p2:f1 ph1 ;1H 90 pulse
46 .6u pl19:f1 ;1H hCO CP power
47
48 (p15:spf1 ph0):f1 (p15 ph2):f2 ;hCO CP
49
50 10u
51 2u pl6:f3 ;15N power for refoc pulses
52 d10 ;13C incrementation
53 .6u pl26:f2 ;13C selective pulse power
54 0.1u
55 (center (p26:sp5 ph25):f2 (p11 ph25):f3) ;13CA selective pulse + 15N 180 refoc pulse
56 d10 ;13C incrementation
57 10u
58 (p26:sp6 ph25):f2 ;13CO selective pulse (spoffs6, sp6, spnam6)
59 10u
60 (center (p26:sp5 ph25):f2 (p11 ph25):f3) ;13CA selective pulse + 15N 180 refoc pulse
61
62 2u pl29:f3 ;15N waltz16 DC power
63 .6u pl18:f2 ;13C trim pulse power
64 (p18 ph18):f2 ;13CO trim pulse
65 .6u fq=cnst0:f2 ;13C offset to CA
66 .6u pl17:f2 ;13C coCA homo CP power
67
```

```

68 (p17:spf17 ph17):f2 ;coCA homo CP
69
70 .6u cpd4:f3 ;15N waltz16 DC scheme (pcpd3)
71 d0 ;13C incrementation
72 d0 ;13C incrementation
73 1u do:f3 ;15N DC power OFF
74 2u pl15:f2 ;13C caN CP power
75 2u pl14:f3 ;15N caN CP power
76 2u
77
78 (p25 ph15):f2 (p25:spf3 ph14):f3 ;caN CP
79
80 2u pl6:f3 ;15N 90 power
81 p5:f3 ph16 ;15N 90 pulse
82 d20 ;water suppression delay d20
83 2u fq=cnst19:f1 ;1H offset to water
84
85 1u pl7:f1 ;1H spoil pulse power
86 (p14:spf12 ph24):f1 ;1H spoil pulse
87
88 d7 ;water suppression delay d7
89
90 2u pl16:f1 ;1H water saturation power
91 16 p22:f1 ph22 ;water saturation
92 p23:f1 ph23 ;water saturation
93 p23:f1 ph23 ;water saturation
94 lo to 16 times 1
95
96 d7 ;water suppression delay d7
97
98 5u pl7:f1 ;1H nH CP power
99 d9 ;delay
100 5u fq=cnst10:f1 ;1H offset to HN
101 2u
102 p5:f3 ph20 ;15N 90 pulse
103 .6u pl8:f3 ;15N nH CP power
104
105 (p16 ph12):f3 (p16:spf4 ph11):f1 ;nH CP
106
107 5u
108 5u pl1:f1 ;1H power
109 d31 ;1H refoc delay
110 p29:f1 ph29 ;1H 180 refoc pulse
111 d31 ;1H refoc delay
112
113 6u pl30:f3 ;15N waltz16 DC power
114 1u cpd6:f3 ;15N waltz16 DC scheme (pcpd3)
115 1u
116 go=2 ph31 ;Acquisition
117 40u do:f1 ;decouplers off
118 40u do:f3 ;decouplers off
119 40u do:f2 ;decouplers off
120
121 10m wr #0 if #0 zd
122
123 1m ip15
124 10u id0
125 lo to 2 times td2
126
127 100u rp15
128 10u rd0
129
130 100u ip2
131 100u id10
132 lo to 2 times td1
133
134 HaltAcqu, 1m
135
136 30m H2_LOCK
137 30m LOCKH_OFF
138 30m LOCKDEC_OFF
139

```

```

140 exit
141
142 ph0= 0 2
143 ph1= 1 1 1 1 3 3 3 3
144 ph2= 0 0 2 2
145 ph17= 0 0 0 0 0 0 0 0 2 2 2 2 2 2 2 2
146 ph18= 1 1 1 1 1 1 1 1 3 3 3 3 3 3 3 3
147 ph14= 1
148 ph15= 0
149 ph16= 2
150 ph20= 1 1 1 1 1 1 1 1 3 3 3 3 3 3 3 3
151 ph12= 0
152 ph22= 0 0 0 0 2 2 2 2
153 ph23= 1 1 1 1 1 1 1 1 3 3 3 3 3 3 3 3
154 ph24= 0 0 0 0 2 2 2 2
155 ph11= 0 0 0 0 0 0 0 0 1 1 1 1 1 1 1 1
156 2 2 2 2 2 2 2 2 3 3 3 3 3 3 3 3
157 1 1 1 1 1 1 1 1 0 0 0 0 0 0 0 0
158 3 3 3 3 3 3 3 3 2 2 2 2 2 2 2 2
159 ph29= 0 0 0 0 0 0 0 0 1 1 1 1 1 1 1 1
160 2 2 2 2 2 2 2 2 3 3 3 3 3 3 3 3
161 1 1 1 1 1 1 1 1 0 0 0 0 0 0 0 0
162 3 3 3 3 3 3 3 3 2 2 2 2 2 2 2 2
163 ph25= 0
164 ph31= 0 2 2 0 2 0 0 2 3 1 1 3 1 3 3 1
165 2 0 0 2 0 2 2 0 1 3 3 1 3 1 1 3
166 1 3 3 1 3 1 1 3 2 0 0 2 0 2 2 0
167 3 1 1 3 1 3 3 1 0 2 2 0 2 0 0 2
168
169 ;cnst31: MAS speed in Hz
170 ;d1: recycle delay
171 ;p2: 1H 90° hard excitation pulse duration
172 ;p11: 1H hard pulse r.f. power
173 ;cnst10: distance between water and amide protons in Hz (positive)
174 ;p15: 1H-13CO CP duration
175 ;p119: 1H CP r.f. power during 1H-13CO CP
176 ;p12: 13CO CP r.f. power during 1H-13CO CP
177 ;spnam1: ramp shape (on 1H) during 1H-13CO CP
178 ;p26: 13CA/CO selective pulse duration
179 ;p126: 13CA/CO selective pulse r.f. power
180 ;sp5: 13CA selective pulse r.f. power
181 ;sp6: 13CO selective pulse r.f. power
182 ;spnam5: 13CA selective pulse shape
183 ;spnam6: 13CO selective pulse shape
184 ;spoffs5: distance between 13CO and 13CA (negative)
185 ;spoffs6: 0 Hz
186 ;p11: 15N 180° inversion pulse duration
187 ;p16: 15N hard pulse r.f. power
188 ;p18: trim pulse duration
189 ;p118: trim pulse r.f. power
190 ;cnst0: distance between 13CO and 13CA in Hz (negative)
191 ;p17: 13CO-13CA DREAM pulse duration
192 ;p117: 13CO-13CA DREAM pulse r.f. power
193 ;spnam17: 13CO-13CA DREAM pulse shape
194 ;p129: 15N WALTZ-dec r.f. power during 13CA evolution
195 ;pcpd3: 15N WALTZ-dec pulse duration during 13CA evolution
196 ;cpdprg4: 15N WALTZ-16 decoupling scheme during 13CA evolution
197 ;p25: 13CA-15N CP duration
198 ;p115: 13CA CP r.f. power during 13CA-15N CP
199 ;p114: 15N CP r.f. power during 13CA-15N CP
200 ;spnam3: ramp shape (on 15N) during 13CA-15N CP
201 ;p5: 15N 90° flip pulse duration
202 ;d20: water suppression T delay
203 ;cnst19: distance between 1H offset and water signal in Hz
204 ;p14: water suppression spoil pulse duration
205 ;p17: water suppression spoil pulse r.f. power and 1H CP r.f. power for 15N-1H CP
206 ;spnam12: water suppression spoil pulse ramp shape
207 ;p22: first pulse duration in water suppression train
208 ;p23: second pulse duration in water suppression train (automatically calculated)
209 ;p116: water suppression train r.f. power
210 ;d7: small water suppression delay to optimize efficiency
211 ;d9: small water suppression delay to optimize efficiency

```

```
212 ;p16: 15N-1H CP duration
213 ;p18: 15N CP r.f. power during 15N-1H CP
214 ;spnam4: ramp shape (on 1H) during 15N-1H CP
215 ;d31: spin echo delay (automatically calculated)
216 ;p29: 1H 180°spin echo pulse duration
217 ;p130: 15N WALTZ-dec r.f. power during acquisition
218 ;cpdprg6: 15N WALTZ-16 decoupling scheme during acquisition
219 ;pcpd3: 15N WALTZ decoupling pulse duration
```

## Supplementary Data 9

(H)CBCA(N)H pulse sequence example (on Bruker hardware running TopSpin version 2.1)

```
1 ; (H)CBCA(N)H_3D_sp
2 ; water suppression by water saturation and spoil pulse
3 ; 3D version
4 ; o1 on water resonance
5 ; o2 in the middle of CB
6 ; o3 in the middle of amide region
7 ;*****
8 ;* The authors are not responsible for any damage caused *
9 ;* by the use of this pulse program or parts thereof. *
10 ;*****
11
12 #include <Avance.incl>
13 #include <Delay.incl>
14 #include <Grad.incl>
15
16
17 "d31=1s/cnst31-5u-.5*p29-de"
18 "p23=1.7*p22"
19 "d0=0.5u"
20 "d10=0.5u"
21
22 "in0=inf2/4" ;d0=F2 -> ph15 (reverse)
23 "in10=inf1/4" ;d10=F1-> ph2
24
25
26
27 1 ze
28 30m LOCKDEC_ON
29 30m LOCKH_ON
30 30m H2_PULSE
31 2 40u do:f3
32 40u do:f2
33 40u do:f1
34 30m H2_LOCK
35 30m LOCKH_OFF
36 d1
37 30m LOCKH_ON
38 30m H2_PULSE
39 1m reset:f1 reset:f2 reset:f3
40
41
42 1u fq=cnst25:f2 ;13C offset reset
43 1u fq=cnst10:f1 ;1H offset to HN
44 10u pl2:f2 ;13C hCB CP power
45 10u pl1:f1 ;1H 90 power
46 p2:f1 ph1 ;1H 90 pulse
47 .6u pl19:f1 ;1H hCB CP power
48
49 (p15:spf1 ph0):f1 (p15 ph2):f2 ;hCB CP
50
51 10u fq=cnst20:f2 ;13C offset to adjust indirect dimension
52 d10 ;13CB incrementation
53 d10 ;13CB incrementation
54 .6u pl29:f3 ;15N waltz16 DC power
55 .6u fq=cnst0:f2 ;13C offset to CA
56 .6u pl17:f2 ;13C cbCA homo CP power
57 (p17:spf17 ph17):f2 ;cbCA homo CP (spnam17)
58 .7u cpd4:f3 ;15N waltz16 DC (pcpd3)
59 d0 ;13CA incrementation
60 d0 ;13CA incrementation
61 1u do:f3 ;15N DC power OFF
62 .8u pl15:f2 ;13C caN CP power
63 2u
64 .8u pl14:f3 ;15N caN CP power
65
66 (p25 ph15):f2 (p25:spf3 ph14):f3 ;caN CP
67
```

```

68      2u do:f1                ;1H DC power OFF
69
70      .6u pl6:f3              ;15N 90 power
71      p5:f3 ph16              ;15N 90 pulse
72      d20                      ;water suppression delay d20
73      2u fq=cnst19:f1         ;1H offset to water
74
75      1u pl7:f1                ;1H spoil pulse power
76      (p14:spf12 ph24):f1     ;1H spoil pulse
77
78      d7                       ;water suppression delay d7
79
80      2u pl16:f1               ;1H water saturation power
81      16 p22:f1 ph22          ;water saturation
82      p23:f1 ph23            ;water saturation
83      p23:f1 ph23            ;water saturation
84      lo to 16 times 1
85      d7                       ;water suppression delay d7
86
87      5u pl7:f1                ;1H nH CP power
88      5u fq=cnst10:f1         ;1H offset to HN
89      d9                      ;delay
90      p5:f3 ph20              ;15N 90 pulse
91      .6u pl8:f3              ;15N nH CP power
92
93      (p16 ph12):f3 (p16:spf4 ph11):f1 ;nH CP
94
95      5u
96      5u pl1:f1                ;1H power
97      d31                      ;1H refoc delay
98      p29:f1 ph29             ;1H 180 refoc pulse
99      d31                      ;1H refoc delay
100     3u
101     1u pl30:f3                ;15N waltz16 DC power
102     1u cpd6:f3                ;15N waltz16 DC scheme (pcpd3)
103     1u
104     go=2 ph31                 ;Acquisition
105     40u do:f1                  ;decouplers off
106     40u do:f3                  ;decouplers off
107     40u do:f2                  ;decouplers off
108
109     10m wr #0 if #0 zd
110
111     1m ip15
112     100u id0
113     lo to 2 times td2
114
115     100u rp15
116     100u rd0
117
118     100u ip2
119     100u id10
120     lo to 2 times td1
121
122     HaltAcqu, 1m
123
124     30m H2_LOCK
125     30m LOCKH_OFF
126     30m LOCKDEC_OFF
127
128     exit
129
130     ph0= 0 2
131     ph1= 1 1 1 1 3 3 3 3
132     ph2= 0 0 2 2
133     ph17= 0 0 0 0 0 0 0 0 2 2 2 2 2 2 2 2
134     ph14= 1
135     ph15= 0
136     ph16= 2
137     ph20= 1 1 1 1 1 1 1 1 3 3 3 3 3 3 3 3
138     ph12= 0
139     ph22= 0 0 0 0 2 2 2 2

```

```

140 ph23= 1 1 1 1 1 1 1 1 3 3 3 3 3 3 3 3
141 ph24= 0 0 0 0 2 2 2 2
142 ph11= 0 0 0 0 0 0 0 0 1 1 1 1 1 1 1 1
143      2 2 2 2 2 2 2 2 3 3 3 3 3 3 3 3
144      1 1 1 1 1 1 1 1 0 0 0 0 0 0 0 0
145      3 3 3 3 3 3 3 3 2 2 2 2 2 2 2 2
146 ph29= 0 0 0 0 0 0 0 0 1 1 1 1 1 1 1 1
147      2 2 2 2 2 2 2 2 3 3 3 3 3 3 3 3
148      1 1 1 1 1 1 1 1 0 0 0 0 0 0 0 0
149      3 3 3 3 3 3 3 3 2 2 2 2 2 2 2 2
150 ph31= 0 2 2 0 2 0 0 2 3 1 1 3 1 3 3 1
151      2 0 0 2 0 2 2 0 1 3 3 1 3 1 1 3
152      1 3 3 1 3 1 1 3 2 0 0 2 0 2 2 0
153      3 1 1 3 1 3 3 1 0 2 2 0 2 0 0 2
154
155 ;cnst31: MAS speed in Hz
156 ;d1: recycle delay
157 ;cnst25: 0
158 ;p2: 1H 90° hard excitation pulse duration
159 ;pl1: 1H hard pulse r.f. power
160 ;cnst10: distance between water and amide protons in Hz (positive)
161 ;p15: 1H-13CB CP duration
162 ;pl19: 1H CP r.f. power during 1H-13CB CP
163 ;pl2: 13CB CP r.f. power during 1H-13CB CP
164 ;spnam1: ramp shape (on 1H) during 1H-13CB CP
165 ;cnst20: adjustment (in Hz) to correctly center 13CB indirect dimension
166 ;cnst0: distance between 13CB and 13CA in Hz (positive)
167 ;p17: 13CB-13CA DREAM pulse duration
168 ;pl17: 13CB-13CA DREAM pulse r.f. power
169 ;spnam17: 13CB-13CA DREAM pulse shape
170 ;pl29: 15N WALTZ-dec r.f. power during 13CA evolution
171 ;pcpd3: 15N WALTZ-dec pulse duration during 13CA evolution
172 ;cpdprg4: 15N WALTZ-16 decoupling scheme during 13CA evolution
173 ;p25: 13CA-15N CP duration
174 ;pl15: 13CA CP r.f. power during 13CA-15N CP
175 ;pl14: 15N CP r.f. power during 13CA-15N CP
176 ;spnam3: ramp shape (on 15N) during 13CA-15N CP
177 ;pl6: 15N 90° flip pulse r.f. power
178 ;p5: 15N 90° flip pulse duration
179 ;d20: water suppression T delay
180 ;cnst19: distance between 1H offset and water signal in Hz
181 ;p14: water suppression spoil pulse duration
182 ;pl7: water suppression spoil pulse r.f. power and 1H CP r.f. power for 15N-1H CP
183 ;spnam12: water suppression spoil pulse ramp shape
184 ;p22: first pulse duration in water suppression train
185 ;p23: second pulse duration in water suppression train (automatically calculated)
186 ;pl16: water suppression train r.f. power
187 ;d7: small water suppression delay to optimize efficiency
188 ;d9: small water suppression delay to optimize efficiency
189 ;p16: 15N-1H CP duration
190 ;pl8: 15N CP r.f. power during 15N-1H CP
191 ;spnam4: ramp shape (on 1H) during 15N-1H CP
192 ;d31: spin echo delay (automatically calculated)
193 ;p29: 1H 180° spin echo pulse duration
194 ;pl30: 15N WALTZ-dec r.f. power during acquisition
195 ;cpdprg6: 15N WALTZ-16 decoupling scheme during acquisition
196 ;pcpd3: 15N WALTZ decoupling pulse duration

```

## Supplementary Data 10

### Gradient water suppression pulse sequence snippet

```
1 ; Gradient for water suppression
2 ;*****
3 ;* The authors are not responsible for any damage caused *
4 ;*   by the use of this pulse program or parts thereof.   *
5 ;*****
6
7     50u UNBLKGRAD
8     p27:gp21           ;gradient application (gpnam21, gpz21, p17, d27)
9     d27               ;ringdown delay
10    5u BLKGRAD
```