Supplementary Information

Characterization of peptide O···HN hydrogen bonds via ^{1}H-detected ^{15}N/^{17}O solid-state NMR spectroscopy

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Experimental methods

The N-acetyl-[U-^{13}C,^{15}N,70%-^{17}O]-L-valyl-L-leucine (N-Ac-VL) sample used here was prepared by ^{17}O labeling U-^{13}C,^{15}N-FMOC-L-valine and U-^{13}C,^{15}N-FMOC-L-leucine with H$_2$^{17}O via a multiple turnover reaction.[1] The procedure involves reacting an FMOC, BOC, Trt, or OtBu amino acid with excess carbodiimide and in this case 70% H$_2$^{17}O. The reaction is a kinetically enhanced multiple turnover process that provides the ^{17}O-labeled FMOC amino acids in high yield and an isotopic enrichment equal to that of the starting H$_2$^{17}O. It appears to be a generally applicable approach for ^{17}O carboxyl groups. Further details of the procedure are available in Refs. 1,2.

NMR experiments were acquired at 18.8 T on a Bruker Avance III HD console using a triple-resonance probe designed and constructed at the NHMFL using a JEOL 0.75 mm MAS stator. The carrier frequencies for ^{1}H, ^{17}O, and ^{15}N were 800.12, 108.48, and 81.08 MHz, respectively. All experiments were performed at a spinning frequency $\omega_r/2\pi = 90$ kHz. In all instances, the initial ^{17}O polarization was enhanced by saturation/inversion of the ^{17}O satellite-transitions using a WURST pulse[3,4] with a sweep width equal to the MAS frequency of 90 kHz, 1 ms pulse duration, peak rf field $\omega_1/2\pi = 12.4$ kHz, and an offset of +450 kHz, leading to ^{17}O signal enhancement for the N-Ac-VL sample by a factor of approximately three. Other experimental parameters are given in Figs. S1-S3. The cosine low-power MQMAS sequence (cos-lpMQMAS)[5,6] with triple-/single-quantum split-t_1 evolution was used to obtain isotropic ^{17}O resolution using cosine pulses with a duration of two rotor periods, and a ratio $k = 19/12$ for $S = 5/2$ nuclei.[7,8] For ^{1}H-^{17}O heteronuclear dipolar recoupling during the RESPDOR[9] portion of the ohNH$^{\{O\}}$ experiment, the SR4$^2_1$ sequence[10] was used on the ^{1}H channel at an rf field amplitude $\omega_1/2\pi$ twice the spinning frequency, 180 kHz. The RESPDOR build-up curve was fit using the following equation[11]
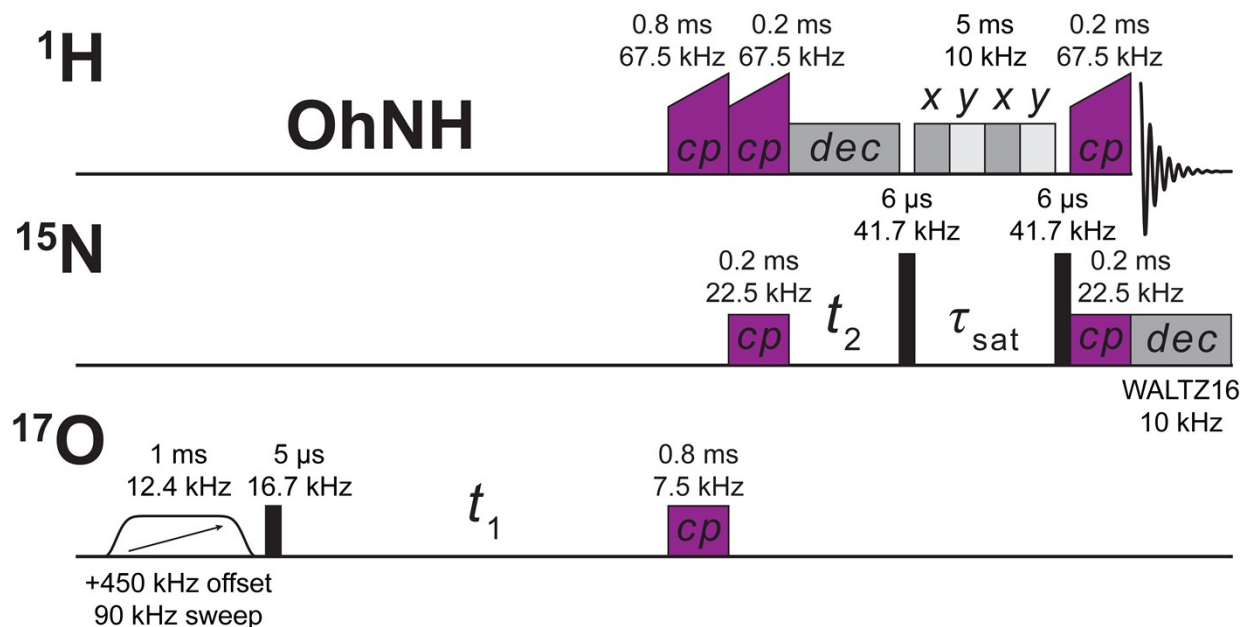
$$(1 - S_r/S_0) = \frac{5}{6} - \frac{\pi\sqrt{2}}{144} \sum_{k=1}^{5} [10 - 2(k-1)] J_{1/4}\left(\frac{k\pi d_{HO}t_{mix}}{4}\right) J_{-1/4}\left(\frac{k\pi d_{HO}t_{mix}}{4}\right)$$

where $J_{\pm 1/4}$ are Bessel functions of the first kind, d_{HO} is the ^{1}H-^{17}O dipolar coupling, and t_{mix} is the total duration of SR4$^2_1$ dipolar recoupling. The dipolar coupling in units of hertz is defined as

$$d_{HO} = -\frac{\gamma_H \gamma_O \hbar}{2\pi r_{HO}^3} \cdot 10^{-7}$$

where γ_H (26.75222×10^7 rad·T^{-1}·s^{-1}) and γ_O (-36.264×10^7 rad·T^{-1}·s^{-1}) are the gyromagnetic ratios of the ^{1}H and ^{17}O nuclei, $\hbar$ (1.05457×10^{-34} J·s) is Planck's constant divided by π, and r_{HO} is the distance in meters between the H and O sites. The dipolar coupling between ^{15}N and ^{17}O nuclei can be calculated using the above equation by replacing the ^{1}H related parameters with ^{15}N ones, e.g., γ_N = -2.7116×10^7 rad·T^{-1}·s^{-1}.

^{1}H chemical shifts were referenced indirectly to neat tetramethylsilane (δ_{iso} = 0 ppm) by setting the highest frequency peak of histidine·HCl·H$_2$O to 17.2 ppm.[12] ^{15}N and ^{17}O shifts were referenced to liquid NH3 and D$_2$O at 0 ppm, respectively, by using the reference frequency for ^{1}H at 0 ppm and the IUPAC recommended standard frequency ratios.[13] All spectra were processed using Bruker Topspin 4.1.1 and imported into MATLAB R2020b Update 6 (9.9.0.1718557) for analysis and plotting. The isotropic ^{17}O dimension of 3D spectra, the axis was scaled and referenced according to the 'unified' convention for MQMAS and STMAS.[14] Essentially, the ^{17}O spectral window was divided by a factor (3 - k) = 17/12, while keeping the Larmor frequency and shift reference the same as for conventional 1D ^{17}O spectra.

Fig. S1. Pulse sequence schematic and experimental parameters used for the three-dimensional OhNH experiment.

------------------------------- OhNH pulse sequence in Bruker format -------------------------------
```
; OhNH.ih - written by ivan
;  - 3D H/N/O correlation under fast MAS
;  - O(aniso) -(CP)-> H -(CP)-> N -(CP)-> H

;parameters:
;ns : 4*n
;p1 : 1H 90 pulse at PL1
;p2 : 15N 90 pulse at PL2
;p3 : 17O 90 pulse at PL3
;p8 : ST-saturation WURST pulse at SP8
;p14 : 17O->1H contact time at SP5(1H) + PL9(17O)
;p15 : 1H->15N contact time at SP0(1H) + PL5(15N)
;p16 : 15N->1H contact time at SP1(1H) + PL6(15N)
;p27 : duration of water suppression (MISSISSIPI)
;pcpd1 : =25us for 10kHz waltz16
;pcpd2 : =25us for 10kHz waltz16
;d0 : initial t2 delay
;d1 : recycle delay
;d20 : initial t1 delay
;in0 : = t2(15N) increment
;in20 : = t1(17O) increment
```

```
;pl1 : 1H pulse power level
;pl2 : 15N pulse power level
;pl3 : 17O pulse power level
;pl5 : 15N CP(1H->15N) power level
;pl6 : 15N CP(15N->1H) power level
;pl9 : CP(17O->1H) power level
;pl11 : 1H dec power level
;pl12 : 15N dec power level
;pl17 : 1H WS power level
;sp0 : 1H CP(1H->15N) power level
;sp1 : 1H CP(15N->1H) power level
;sp5 : 1H CP(1H->17O) power level
;sp8 : ST-saturation WURST power level
;spnam8 : WURST shape, linear frequency sweep
;spoffs8 : ST-saturation WURST offset
;cpdprg1 : waltz16 at PL11
;cpdprg2 : waltz16 at PL12
;l30 : # rotor periods for WURST pulse
;$TYPE=ivan

"p8=1s*l30/cnst31"
"d0=0"
"d20=0"

aqseq 312

1 ze
2 10m do:f2
  d1

  (p8:sp8 ph20):f3  ;WURST ST saturation pulse
  3u

  5u pl1:f1 pl5:f2 pl3:f3
  (p3 ph1):f3  ;17O 90 pulse

  d20  ; 17O t1 evolution

  (p14:sp5 ph22):f1 (p14 ph20 pl9):f3  ;17O->1H CP

  (p15:sp0 ph20):f1 (p15 ph2 pl5):f2  ;1H->15N CP
  1u pl11:f1
  (1u cpds1):f1
  (center (d0):f2 (p3*2 ph20 pl3):f3)  ;17O pi-pulse @ mid t2
  2u
  (ralign (1u do):f1 (p2 ph20 pl2):f2)  ;15N 90 for z-filter

  (p27*0.25 ph20 p217):f1  ;MISSISSIPI
  (p27*0.25 ph21):f1
  (p27*0.25 ph22):f1
  (p27*0.25 ph23):f1
  (p2 ph3):f2  ;15N 90

  (p16:sp1 ph4):f1 (p16 ph20 pl6):f2  ;15N->1H CP
  0.5u pl12:f2
  go=2 ph31 cpds2:f2 finally do:f2
  1u do:f2
```

```
   10m mc #0 to 2
     F1PH( calph(ph1,+90), caldel(d20,+in20) )
     F2PH( calph(ph2,+90), caldel(d0,+in0) )
exit

ph1= 1 3
ph2= 1
ph3= 1 1 3 3
ph4={1}*4 {3}*4 {0}*4 {2}*4
ph20=0
ph21=1
ph22=2
ph23=3
ph31={0 2 2 0}^2^3^1
```
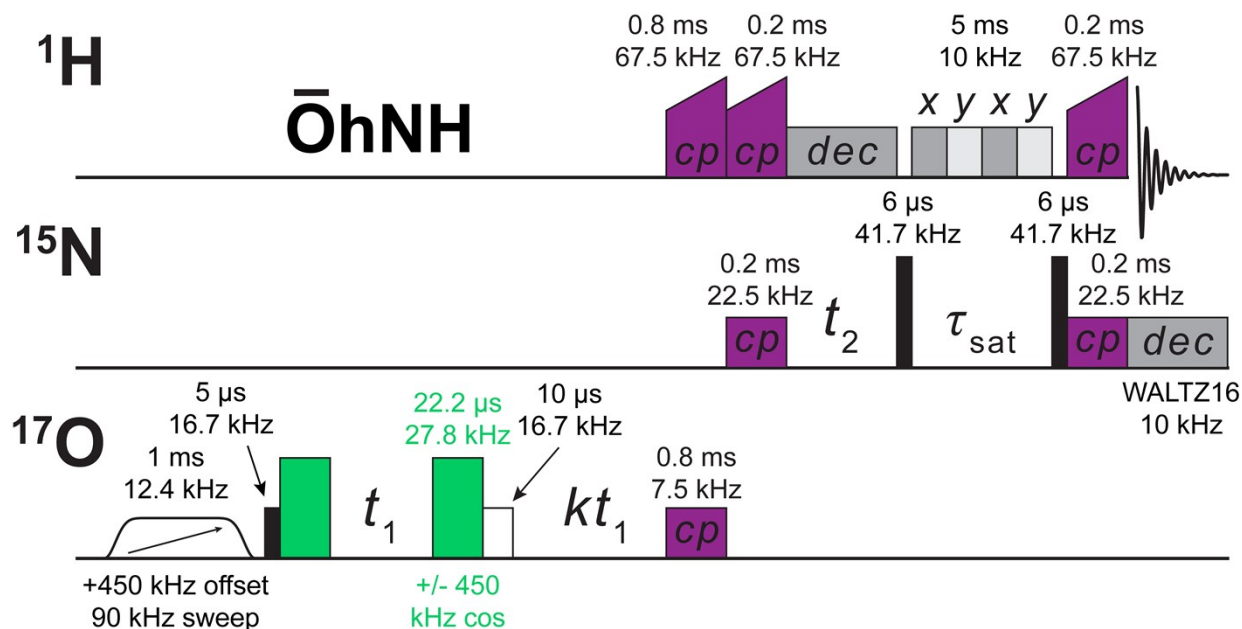
------------------------------- OhNH pulse sequence in Bruker format -------------------------------

Fig. S2. Pulse sequence schematic and experimental parameters used for the three-dimensional OͪhNH experiment.

------------------------------- OͪhNH pulse sequence in Bruker format -------------------------------
```
; OihNH.ih — written by ivan
;   - 3D H/N/O correlation under fast MAS
;   - O(iso) −(CP)-> H −(CP)-> N −(CP)-> H
```

```
;parameters:
;ns : 24*n
;p1 : 1H 90 pulse at PL1
;p2 : 15N 90 pulse at PL2
;p3 : 17O 90 pulse at PL3
;p7 : CT<->3Q conversion pulse at SP7
;p8 : ST-saturation WURST pulse at SP8
;p14 : 17O->1H contact time at SP5(1H) + PL9(17O)
;p15 : 1H->15N contact time at SP0(1H) + PL5(15N)
;p16 : 15N->1H contact time at SP1(1H) + PL6(15N)
;p27 : duration of water suppression (MISSISSIPI)
;pcpd1 : =25us for 10kHz waltz16
;pcpd2 : =25us for 10kHz waltz16
;d0 : initial t2 delay
;d1 : recycle delay
;d7 : =k*t1 delay for isotropic 17O evolution
;d20 : initial t1 delay
;in0 : =t2(15N) increment
;in7 : =k*t1 increment for isotropic 17O evolution
;in20 : =rotor period, t1(17O) increment
;pl1 : 1H pulse power level
;pl2 : 15N pulse power level
;pl3 : 17O pulse power level
;pl5 : 15N CP(1H->15N) power level
;pl6 : 15N CP(15N->1H) power level
;pl9 : CP(17O->1H) power level
;pl11 : 1H dec power level
;pl12 : 15N dec power level
;pl20 : 17O saturation pulse power level
;pl27 : 1H WS power level
;sp0 : 1H CP(1H->15N) power level
;sp1 : 1H CP(15N->1H) power level
;sp5 : 1H CP(1H->17O) power level
;sp7 : CT<->3Q conversion power level
;sp8 : ST-saturation WURST power level
;spnam7 : COS shape, cosine amplitude-modulated pulse
;spnam8 : WURST shape, linear frequency sweep
;spoffs8 : ST-saturation WURST offset
;cpdprg1 : waltz16 at PL11
;cpdprg2 : waltz16 at PL12
;cnst31 : MAS frequency [Hz]
;l30 : # rotor periods for WURST pulse
;$TYPE=ivan

"p7=2s/cnst31"
"p8=1s*l30/cnst31"
"d0=0"
"d20=0"
"d7=(2s/cnst31)*1.583333-p3"
"in7=in20*1.583333" ;k for S=5/2

aqseq 312

1 ze
2 10m do:f2
  d1
```

```
  (p8:sp8 ph20):f3  ;WURST ST saturation pulse
  3u


  5u pl1:f1 pl5:f2 pl3:f3
  (p3 ph1):f3  ;17O 90 pulse
  (p7:sp7 ph1):f3  ;17O 1Q->3Q
  d20  ; 17O t1 evolution
  (p7:sp7 ph20):f3  ;17O 3Q->1Q
  (p3*2 ph20 pl3):f3  ;17O 180 pulse
  d7

  (p14:sp5 ph22):f1 (p14 ph20 pl9):f3  ;17O->1H CP

  (p15:sp0 ph20):f1 (p15 ph2 pl5):f2  ;1H->15N CP
  1u pl11:f1
  (1u cpds1):f1
  (center (d0):f2 (p3*2 ph20 pl3):f3)  ;17O pi-pulse @ mid t2
  2u
  (ralign (1u do):f1 (p2 ph20 pl2):f2)  ;15N 90 for z-filter

  (p27*0.25 ph20 pl27):f1  ;MISSISSIPI
  (p27*0.25 ph21):f1
  (p27*0.25 ph22):f1
  (p27*0.25 ph23):f1
  (p2 ph3):f2  ;15N 90

  (p16:sp1 ph4):f1 (p16 ph20 pl6):f2  ;15N->1H CP
  0.5u pl12:f2
  go=2 ph31 cpds2:f2 finally do:f2
  1u do:f2
  10m mc #0 to 2
    F1PH( calph(ph1,+30), caldel(d20,+in20)&caldel(d7,+in7) )
    F2PH( calph(ph2,+90), caldel(d0,+in0) )
exit

ph1=(12) 0 2 4 6 8 10
ph2= 1
ph3={1}*6 {3}*6
ph4={1}*12 {3}*12 {0}*12 {2}*12
ph20=0
ph21=1
ph22=2
ph23=3
ph31={{0 2 0 2 0 2}^2}^2^3^1
```
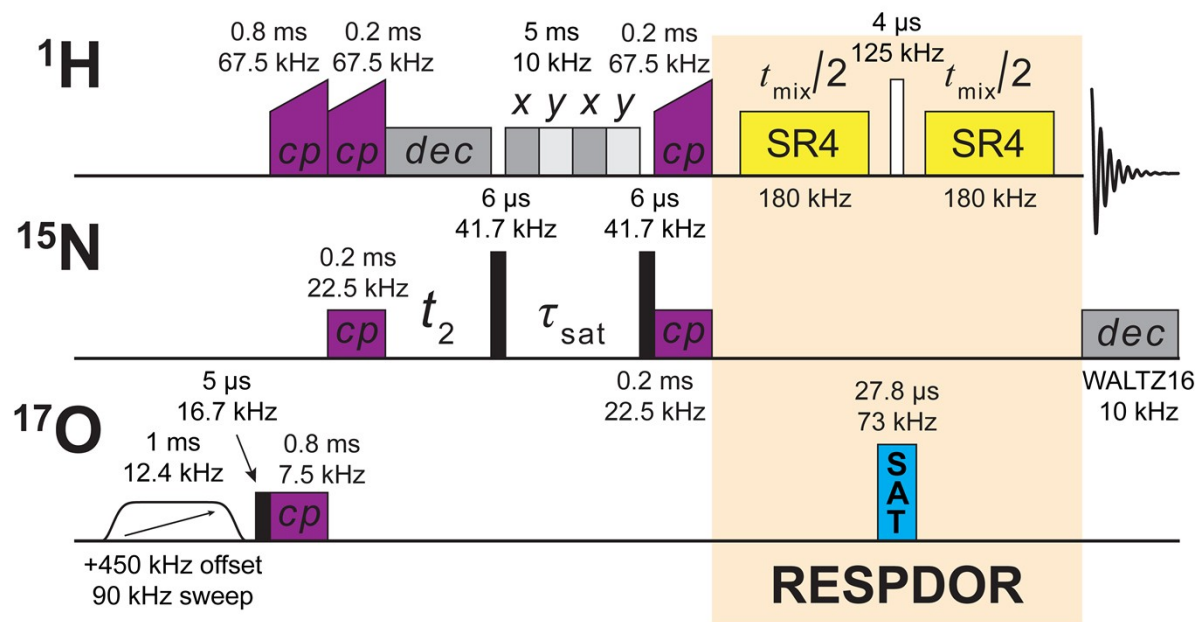------------------------------- OhNH pulse sequence in Bruker format -------------------------------

ohNH{O}

t_{mix}: 13 × 66.7 μs = 8.7 ms t_2: 32 × 800 μs = 25.6 ms

384 transients, 0.05 s recycle delay, experimental time = 7.7 hr

Fig. S3. Pulse sequence schematic and experimental parameters used for the three-dimensional ohNH{O} experiment.

-------------------------------- OhNH{O} pulse sequence in Bruker format --------------------------------

```
; OhNHo.ih - written by ivan
;  - 3D H/N/O correlation under fast MAS
;  - pseudo-3D hNH spectra with 17O/1H RESPDOR in the 3rd dimension
;  - SR4 1H/17O dipolar recoupling

;parameters:
;ns : 32*n
;p1 : 1H 90 pulse at PL1
;p2 : 15N 90 pulse at PL2
;p3 : 17O 90 pulse at PL3
;p8 : ST-saturation WURST pulse at SP8
;p14 : 17O->1H contact time at SP5(1H) + PL9(17O)
;p15 : 1H->15N contact time at SP0(1H) + PL5(15N)
;p16 : 15N->1H contact time at SP1(1H) + PL6(15N)
;p20 : 17O RESPDOR saturation pulse at PL20
;p21 : 1H SR4 pulse at PL10, =tr/4
;p27 : duration of water suppression (MISSISSIPI)
;pcpd1 : =25us for 10kHz waltz16
;pcpd2 : =25us for 10kHz waltz16
;d0 : initial t2 delay
```

9

```
;d1 : recycle delay
;in0 : =t2(15N) increment
;pl1 : 1H pulse power level
;pl2 : 15N pulse power level
;pl3 : 17O pulse power level
;pl5 : 15N CP(1H->15N) power level
;pl6 : 15N CP(15N->1H) power level
;pl9 : CP(17O->1H) power level
;pl10 : =2vr, 1H SR4 power level
;pl11 : 1H dec power level
;pl12 : 15N dec power level
;pl20 : 17O saturation pulse power level
;pl27 : 1H WS power level
;sp0 : 1H CP(1H->15N) power level
;sp1 : 1H CP(15N->1H) power level
;sp5 : 1H CP(1H->17O) power level
;sp8 : ST-saturation WURST power level
;spnam8 : WURST shape, linear frequency sweep
;spoffs8 : ST-saturation WURST offset
;cpdprg1 : waltz16 at PL11
;cpdprg2 : waltz16 at PL12
;cnst20 : # rotor periods for 17O RESPDOR saturation pulse
;cnst31 : MAS frequency [Hz]
;l8 : L8*2 = # SR4 rotor periods to increment mixing time per t1
;l10 : initial # SR4 rotor periods
;l30 : # rotor periods for WURST pulse
;$TYPE=ivan

"p8=1s*l30/cnst31"
"p20=1s*cnst20/cnst31"
"p21=0.25s/cnst31"

"d0=0"
"d25=(1s/cnst31)"
"d26=(0.25s/cnst31)"
"d28=(1s/cnst31)-0.5u"

"l0=0"
"l2=0"
"l8=l10"
"cnst6=0"
"cnst8=0"

aqseq 312

1 ze
2 10m do:f2

  5u rpp16
  5u rpp17
  5u rpp18
  5u rpp19

"cnst6=180*trunc(((l2)/16)%2)"
  5u ip16+cnst6
  5u ip17+cnst6
  5u ip18+cnst6
```

```
  5u ip19+cnst6

  d1

  (p8:sp8 ph20):f3  ;WURST ST saturation pulse
  3u

  5u pl1:f1 pl5:f2 pl3:f3
  (p3 ph1):f3  ;17O 90 pulse

  (p14:sp5 ph22):f1 (p14 ph20 pl9):f3  ;17O->1H CP

  (p15:sp0 ph20):f1 (p15 ph2 pl5):f2  ;1H->15N CP
  1u pl11:f1
  (1u cpds1):f1
  (center (d0):f2 (p3*2 ph20 pl3):f3)  ;17O pi-pulse @ mid t2
  2u
  (ralign (1u do):f1 (p2 ph20 pl2):f2)  ;15N 90 for z-filter

  (p27*0.25 ph20 pl27):f1  ;MISSISSIPI
  (p27*0.25 ph21):f1
  (p27*0.25 ph22):f1
  (p27*0.25 ph23):f1
  (p2 ph3):f2  ;15N 90

  (p16:sp1 ph4):f1 (p16 ph20 pl6):f2  ;15N->1H CP

if "l2%2==0"{
    d25 pl20:f3
}
if "l2%2==1"{
    d25 pl=0[Watt]:f3
}
15 (p21 ph16 pl10):f1
   (p21 ph17):f1
   (p21 ph16^):f1
   (p21 ph17^):f1
   lo to 15 times l10
  d26
  (center (p1*2 ph5 pl1):f1 (p20 ph20):f3)
  d26
25 (p21 ph18 pl10):f1
   (p21 ph19):f1
   (p21 ph18^):f1
   (p21 ph19^):f1
   lo to 25 times l10
  d28

  0.5u pl12:f2
  go=2 ph31 cpds2:f2 finally do:f2
  1u do:f2
  10m mc #0 to 2
    F1PH( calph(ph2,+90), caldel(d0,+in0) )
    F2PH( calclc(l2,+1), calclc(l10,+l8) )
exit

ph1= 1 3
```

```
ph2= 1
ph3= 1 1 3 3
ph4= 1
ph5={0}*4 {1}*4 {2}*4 {3}*4
ph16=(360) 90 270 210 30 330 150
ph17=(360) 270 90 30 210 150 330
ph18=(360) 90 270 210 30 330 150
ph19=(360) 270 90 30 210 150 330
ph20=0
ph21=1
ph22=2
ph23=3
ph31={0 2 2 0}^2
```

-------------------------------- OhNH$^{\{O\}}$ pulse sequence in Bruker format --------------------------------

Acknowledgements

Notes and references

1   M. S. Seyfried, B. S. Lauber and N. W. Luedtke, *Org. Lett.*, 2010, **12**, 104–106.
2   E. G. Keeler, V. K. Michaelis, M. T. Colvin, I. Hung, P. L. Gor'kov, T. A. Cross, Z. Gan and R. G. Griffin, *J. Am. Chem. Soc.*, 2017, **139**, 17953–17963.
3   E. Kupce and R. Freeman, *J. Magn. Reson. A*, 1995, **115**, 273–276.
4   N. M. Trease, K. K. Dey and P. J. Grandinetti, *J. Magn. Reson.*, 2009, **200**, 334–339.
5   I. Hung, *J. Magn. Reson.*, 2021, **324**, 106913.
6   I. Hung and Z. Gan, *J. Magn. Reson.*, 2021, **328**, 106994.
7   D. Massiot, B. Touzo, D. Trumeau, J. P. Coutures, J. Virlet, P. Florian and P. J. Grandinetti, *Solid State Nucl. Magn. Reson.*, 1996, **6**, 73–83.
8   S. P. Brown and S. Wimperis, *J. Magn. Reson.*, 1997, **128**, 42–61.
9   Z. Gan, *Chem. Commun.*, 2006, 4712–4714.
10 A. Brinkmann and A. P. M. Kentgens, *J. Am. Chem. Soc.*, 2006, **128**, 14758–14759.
11 X. Lu, O. Lafon, J. Trébosc and J.-P. Amoureux, *J. Magn. Reson.*, 2012, **215**, 34–49.
12 A. Venkatesh, M. P. Hanrahan and A. J. Rossini, *Solid State Nucl. Magn. Reson.*, 2017, **84**, 171–181.
13 R. K. Harris, E. D. Becker, S. M. C. De Menezes, R. Goodfellow and P. Granger, *Pure Appl. Chem.*, 2001, **73**, 1795–1818.
14 J. P. Amoureux, C. Huguenard, F. Engelke and F. Taulelle, *Chem. Phys. Lett.*, 2002, **356**, 497–504.