SUPPLEMENTARY MATERIAL

A NMR Experiment for Simultaneous Correlations of Valine and Leucine/Isoleucine Methyls with Carbonyl Chemical Shifts in Proteins.

Vitali Tugarinov*, Vincenzo Venditti, and G. Marius Clore*

Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892-0520, USA

Bruker pulse sequence for SIM-HMCM(CGCBCA)CO experiment

```
;;SIM-HMCM(CGCBCA)CO - Tugarinov, Venditti & Clore

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

#define CO
#define METHYL

define delay TAUB
define delay TAUBS
define delay TAU
define delay DEUT
define delay EPSI
define delay TAUD
define delay TAUD_1
define delay TAUC
define delay TAUC_1
define delay TAUCS
define delay pd15

define pulse C_REB
define pulse CA_REB
define pulse VME_REB
define pulse CO_REB
define pulse CG2_REB
define pulse Q5P

"C_REB=p5"      ; high power RE-BURP (~350u on-resonance at 600 MHz)
"CO_REB=p6"          ; high power RE-BURP (~350u at 600 MHz applied at 176 ppm)
"CG2_REB=p15"   ; 4.0 ms RE-BURP applied at 16 ppm for Ile Cg2 methyls (600 MHz)
"CA_REB=p19"    ; 1.8 ms RE-BURP applied at 60 ppm for ILV Ca (600 MHz)
"VME_REB=p9"    ; 3.0 ms RE-BURP applied at 21.5 ppm for Val Cg methyls (600
MHz)
"Q5P=p14"       ; 1.4 ms Q5 Gaussian Cascade applied on-resonance (33 ppm)

"pd15=p15"
"DEUT=1.4m"


"TAUB=3.5m-8u"
"TAUBS=TAUB-C_REB*0.5"
"TAU=3.5m-DEUT-p17-8u"

"TAUC=3.5m-8u-0.5*CG2_REB"
"TAUC_1=TAUC"
```

```
"TAUCS=3.5m-8u-0.5*CG2_REB"

"TAUD=2.50m-4u-CO_REB*0.5-VME_REB*0.5"
"TAUD_1=TAUD"

"d3=2.0m-p25-12u"
"d4=2.0m-p25-14u-p18-4u" ;  use for watergate (p18@pl9) in water
;"d4=2.0m-p25-14u-4u"

"d11=5m"
"d12=5m"

; carbonyl evolution delays
"d0=2u"

"EPSI=p21*0.5-p10"

; methyl evolution delays
"d22=3.5m - 4u"
"d23=3.5m - 4u - C_REB*0.5"
"d24=3.5m - 4u - C_REB*0.5"
"d25=3.5m - 4u"

;---------------------------------------------------------------------
"cnst21=2264"                          ; Ca-Cb offset (33 ppm)
"cnst22=0"                        ; offset of 13C carrier (18 ppm; Methyl)
;---------------------------------------------------------------------

1 ze
d11 LOCKDEC_ON
d11 H2_PULSE
2 10u
  d11 do:f2
  d12
3 2u
4 2u
5 2u
6 2u
7 2u
8 2u
  d11 H2_LOCK
  d11 LOCKH_OFF
  d1
  d12 UNBLKGRAD
  d12 H2_PULSE
  50u fq=cnst22:f2
```

```
 10u pl8:f2
 10u pl0:f1
 10u pl7:f3
 10u pl17:f4
 (p2:sp2 ph0:r):f1       ; 7-ms EBURP-1 on water
 2u pl0:f1
;;  (p3:sp3 ph0:r):f1
 2u
 (p8 ph0):f2
 2u
 p26:gp0
 1m pl1:f1


 (p1 ph0):f1
 2u
 p25:gp0
 d3
 10u
 (center (p8*2 ph0):f2 (p1*2 ph0):f1)
 2u
 p25:gp0
 d3
 10u fq=cnst21:f2

 (p8 ph2):f2
 4u
 TAUB
 4u
 (p1*2 ph0):f1
 4u
 TAUBS
 4u pl0:f2
 (C_REB:sp5 ph0):f2
 4u
 TAUBS
 4u
 (p1*2 ph0):f1
 2u
 2u pl8:f2
 TAUB
 2u pl18:f4
 2u cpds4:f4
 (lalign (p8 ph3):f2 (p1 ph11):f1)

 4u
```

```
  TAUC
  4u pl0:f2
  pd15                    ;(CG1_REB:sp15 ph0):f2
  4u
  TAUCS
  4u pl0:f2
  (C_REB:sp5 ph0):f2
  4u
  TAUCS
  4u pl0:f2
  pd15                    ;(CG1_REB:sp15 ph0):f2
  4u
  TAUC_1
  4u

  (Q5P:sp14 ph14):f2
  4u
  TAUC
  4u pl0:f2
  (CG2_REB:sp15 ph0):f2
  4u
  TAUCS
  4u pl0:f2
  (C_REB:sp5 ph0):f2              ; CHANGE THIS PHASE TO Y INVERT VAL VS.
ILE/LEU
  4u
  TAUCS
  4u pl0:f2
  (CG2_REB:sp15 ph0):f2
  4u
  TAUC
  4u pl8:f2
  (p8 ph3):f2

; ******* CARBON CA-CO TRANSFER ******
    TAUD pl0:f2
    (VME_REB:sp9 ph0):f2
    TAUD pl0:f2
    2u pl0:f2
    (CO_REB:sp6 ph0):f2
    2u pl0:f2
    (C_REB:sp5 ph0):f2
    2u pl0:f2
    TAUD_1 pl0:f2
    (VME_REB:sp9 ph0):f2
    TAUD_1 pl0:f2
```

```
   (CO_REB:sp6 ph0):f2
   2u pl0:f2
   (p10:sp10 ph1):f2
; ****** CARBON CA-CO TRANSFER END *****

   2u pl0:f2

; ******** CARBONYL EVOLUTION **********
  (p10:sp11 ph5):f2
  d0
  2u pl0:f2
  (center (CA_REB:sp19 ph15):f2 (p7*2 ph0):f3)
  2u pl0:f2
  d0
  2u
  (p10*2:sp13 ph0):f2
  2u
  4u pl0:f2
  (center (CA_REB:sp19 ph0):f2 (p7*2 ph0):f3)
  4u pl0:f2
  (p10:sp12 ph0):f2
; ********* CARBONYL END **************

   2u pl0:f2

; ******* CARBON CO-CA CT  ***************
   (p10:sp17 ph1):f2
   TAUD_1 pl0:f2
   (VME_REB:sp9 ph0):f2
   TAUD_1 pl0:f2
   2u pl0:f2
   (CO_REB:sp6 ph0):f2
   2u pl0:f2
   (C_REB:sp5 ph0):f2
   2u pl0:f2
   TAUD
   (VME_REB:sp9 ph0):f2
   TAUD pl0:f2
   (CO_REB:sp6 ph0):f2
   2u pl8:f2
; ******* CARBON CO-CA CT ENDS ************
   (p8 ph4):f2
   4u
   TAUC
   4u pl0:f2
   (CG2_REB:sp15 ph0):f2
```

```
  4u
  TAUCS
  4u pl0:f2
  (C_REB:sp5 ph0):f2
  4u
  TAUCS
  4u pl0:f2
  (CG2_REB:sp15 ph0):f2
  4u
  TAUC_1
  4u pl0:f2

 (Q5P:sp16 ph14):f2
  4u
  TAUC
  4u pl0:f2
  pd15
  4u
  TAUCS
  4u pl0:f2
  (C_REB:sp5 ph0):f2
  4u
  TAUCS
  4u pl0:f2
  pd15
  4u
  TAUC
  4u pl8:f2

; ******  METHYL EVOLUTION **********
 (ralign (p8 ph4):f2 (p1 ph11):f1)
 2u do:f4
 d22
 2u
 (p1*2 ph0):f1
 d23
 2u pl17:f4
 2u pl0:f2
 (C_REB:sp5 ph12):f2
 4u
 d24
 (p1*2 ph0):f1
 d25
 4u pl8:f2
; ********* back to protons **********
```

```
  (p8 ph6):f2
  2u
  p25:gp0
  d4
  10u fq=cnst22:f2
  2u pl9:f1
  (p18 ph9:r):f1
  2u pl1:f1
  (center (p8*2 ph0):f2 (p1*2 ph0):f1)
  2u pl9:f1
  (p18 ph9:r):f1
  2u pl1:f1
  p25:gp0
  10u pl31:f2
  d4

  go=2 ph31 cpd2:f2
  10u do:f2
  d11 wr #0 if #0 zd

#ifdef CO
  d12 ip5
  lo to 3 times 2
  d12 id0
  d12 ip31
  d12 ip31
  lo to 4 times l2
  d12 rd0
#endif

#ifdef METHYL
  d12 ip6
  lo to 5 times 2
  d12 id22
  d12 id23
  d12 dd24
  d12 dd25
  d12 ip31
  d12 ip31
  lo to 6 times l3
#endif

d11 H2_LOCK
d11 LOCKH_OFF
d11 LOCKDEC_OFF
exit
```

ph0=0
ph1=1
ph2=0 2
ph3=1 1 3 3
ph4=1 1 1 1 3 3 3 3
ph5=0 0 0 0 2 2 2 2
ph6=0
ph7=1
ph8=2
ph9=2
ph10=3
ph11=1
ph12=0
ph14=1
ph15=0 0 0 0 0 0 0 0 2 2 2 2 2 2 2 2
ph31=0 2 0 2 2 0 2 0 0 2 0 2 2 0 2 0