

Supplementary Information

Structure Activity Relationship of Imidazo-pyridinium Analogs as Antagonists of Neuropeptide S Receptor

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Table 1. Profile of **20e** against 55 other targets at @ 10 μ M at Cerep®.

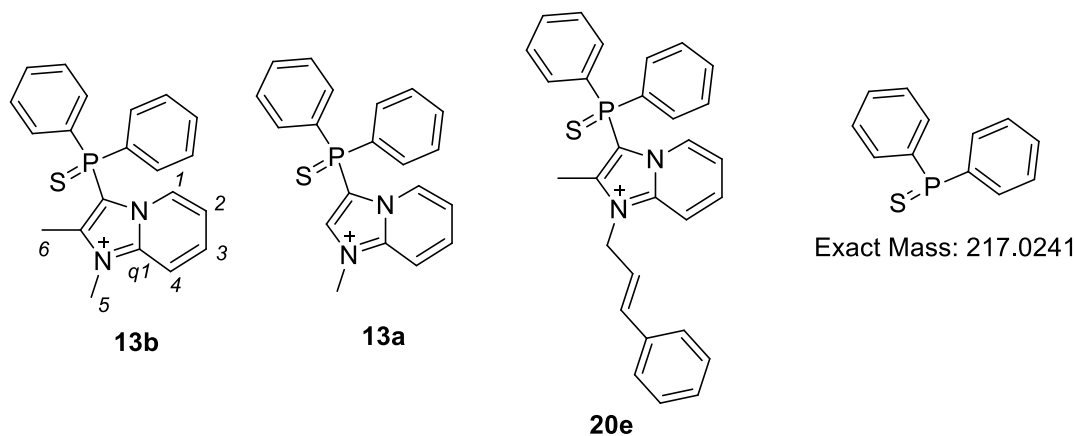
20e @ 10 μM	% Inhibition of Control Specific Binding
Assay	
A1 (h) (antagonist radioligand)	18
A2A (h) (agonist radioligand)	32
A3 (h) (agonist radioligand)	27
alpha 1 (non-selective) (antagonist radioligand)	46
alpha 2 (non-selective) (antagonist radioligand)	14
beta 1 (h) (agonist radioligand)	17
beta 2 (h) (agonist radioligand)	6
AT1 (h) (antagonist radioligand)	-25
B2 (h) (agonist radioligand)	2
CB1 (h) (agonist radioligand)	69
CCK1 (CCKA) (h) (agonist radioligand)	25
D1 (h) (antagonist radioligand)	72
D2S (h) (antagonist radioligand)	83
ETA (h) (agonist radioligand)	6
GABA (non-selective) (agonist radioligand)	25
GAL2 (h) (agonist radioligand)	-24
CXCR2 (IL-8B) (h) (agonist radioligand)	-9
CCR1 (h) (agonist radioligand)	-13
H1 (h) (antagonist radioligand)	37
H2 (h) (antagonist radioligand)	23
MC4 (h) (agonist radioligand)	48

MT1 (ML1A) (h) (agonist radioligand)	57
M1 (h) (antagonist radioligand)	87
M2 (h) (antagonist radioligand)	96, IC ₅₀ = 0.59 μM
M3 (h) (antagonist radioligand)	93, IC ₅₀ = 1.00 μM
NK2 (h) (agonist radioligand)	92, IC ₅₀ = 1.5 μM
NK3 (h) (antagonist radioligand)	15
Y1 (h) (agonist radioligand)	2
Y2 (h) (agonist radioligand)	41
NTS1 (NT1) (h) (agonist radioligand)	-1
delta 2 (DOP) (h) (agonist radioligand)	75
kappa (KOP) (agonist radioligand)	99, IC ₅₀ = 0.21 μM
mu (MOP) (h) (agonist radioligand)	99, IC ₅₀ = 0.06 μM
NOP (ORL1) (h) (agonist radioligand)	12
TP (h) (TXA2/PGH2) (antagonist radioligand)	-10
5-HT1A (h) (agonist radioligand)	77
5-HT1B (antagonist radioligand)	15
5-HT2A (h) (antagonist radioligand)	65
5-HT2B (h) (agonist radioligand)	6
5-HT3 (h) (antagonist radioligand)	21
5-HT5a (h) (agonist radioligand)	34
5-HT6 (h) (agonist radioligand)	20
5-HT7 (h) (agonist radioligand)	21
sst (non-selective) (agonist radioligand)	43
VPAC1 (VIP1) (h) (agonist radioligand)	-12
V1a (h) (agonist radioligand)	12
Ca ²⁺ channel (L, verapamil site) (phenylalkylamine) (antagonist radioligand)	83
KV channel (antagonist radioligand)	55
SKCa channel (antagonist radioligand)	-5
Na ⁺ channel (site 2) (antagonist radioligand)	106, IC ₅₀ = 0.2 μM
Cl ⁻ channel (GABA-gated) (antagonist radioligand)	32
norepinephrine transporter (h) (antagonist radioligand)	86
dopamine transporter (h) (antagonist radioligand)	91, IC ₅₀ = 2.9 μM
5-HT transporter (h) (antagonist radioligand)	75

2D NMR spectroscopy on 13b

We performed a detailed structural analysis of **13b** by 2D NMR spectroscopy to elucidate the regiochemistry of alkylation at the N1 imidazole nitrogen with compounds such as **12b** and **12d**. This was an expected outcome as the precedent in Tolmachev et al. (ref 26 in manuscript). The protons 1-4 (see figure **13b** below) were correlated to each other by the COSY experiment. The carbons associated with them and other protons were correlated with a HSQC experiment. A HMBC experiment showed a strong correlation of the quaternary carbon *q1* with the hydrogens at position 5 establishing the alkylation at the imidazole N1 nitrogen. The carbon *q1* also has correlations with the protons at positions 1, 4 and 3 in the HMBC experiment. Finally, a NOESY experiment shows a correlation between the hydrogens at positions 4 and 5 (methyl group).

Further support to the structural assignment was observed in the MS fragmentation of compound **20e**. Thus the LCMS analysis of compound **20e** used in our rat PK study shows $[M+H]^+$ m/z 465.2 and 217.1 MRM transition. Furthermore, exposing compounds **13a** and **13b** to a MS fragmentor with increasing voltage increments leads to formation of the same fragment with mass 217, a fragment common among the analogs.



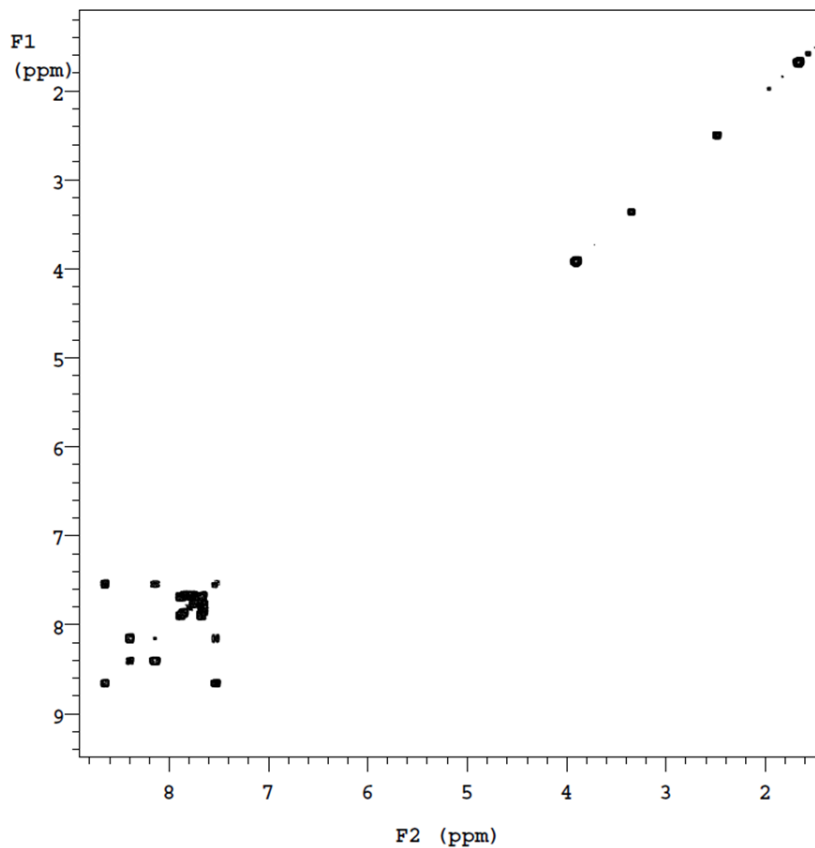
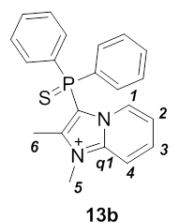
1. gCOSY of 13b

Sample Name:
SPA01-081
Data collected on:
nmr1.bogus-vmrs400
Archive directory:
/home/Walkup1/vnmrsys/data/patnaiks
Sample directory:
SPA01-081_20130906_01
Fidfile: SPA01-081_gCOSY_01

Pulse Sequence **gCOSY**
Solvent: dmsd
Data collected on: Sep 6 2013

Temp. 25.0 C / 298.1 K
Sample #26, operator: patnaiks

Relax. delay 1.000 sec
Acq. time 0.150 sec
Width 3858.0 Hz
2D Width 3858.0 Hz
Single scan
128 increments
OBSERVE H1, 399.9388525 MHz
DATA PROCESSING
Sq. sine bell 0.075 sec
F1 DATA PROCESSING
Sq. sine bell 0.033 sec
FT size 2048 x 2048
Total time 3 min 10 sec



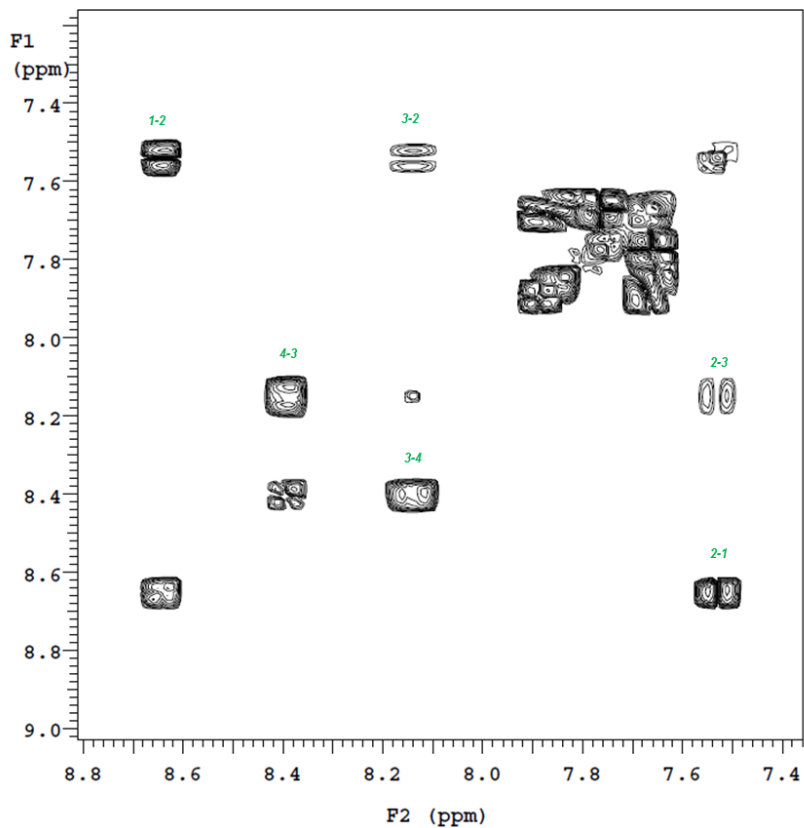
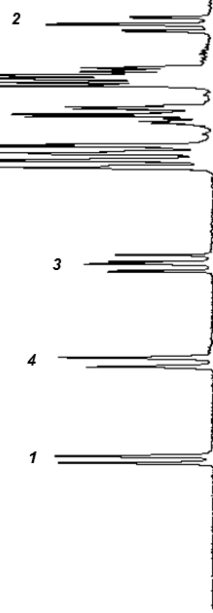
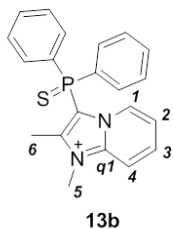
2. gCOSY of **13b** (expansion)

Sample Name:
SPA01-081
Data Collected on:
nmr1.bogus-vnmrs400
Archive directory:
/home/Walkup1/vnmrsys/data/patnaiks
Sample directory:
SPA01-081_20130906_01
FidFile: SPA01-081_gCOSY_01

Pulse Sequence: **gCOSY**
Solvent: dmsd
Data collected on: Sep 6 2013

Temp. 25.0 C / 298.1 K
Sample #26, Operator: patnaiks

Relax. delay 1.000 sec
Acq. time 0.150 sec
Width 3858.0 Hz
2D Width 3858.0 Hz
Single scan
128 increments
OBSERVE H1, 399.9388525 MHz
DATA PROCESSING
Sq. sine bell 0.075 sec
F1 DATA PROCESSING
Sq. sine bell 0.033 sec
FT size 2048 x 2048
Total time 3 min 10 sec



3. gHSQCAD of **13b** (expansion)

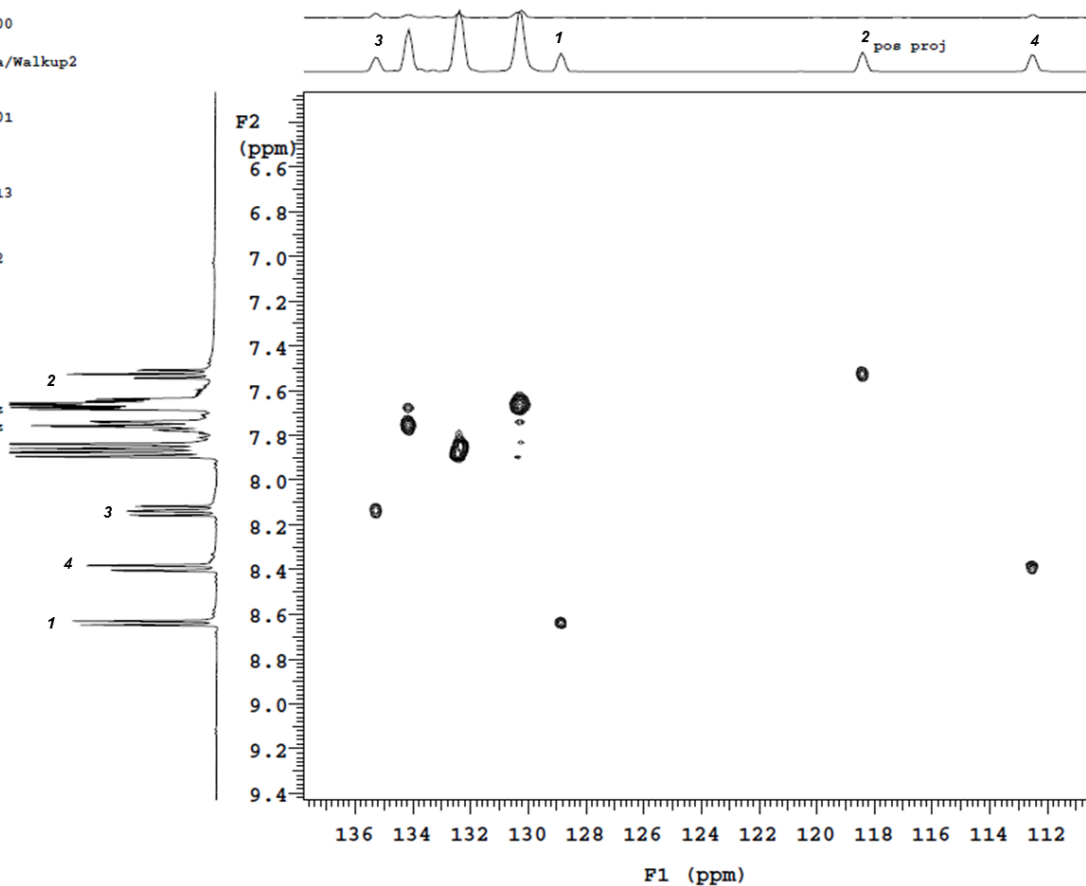
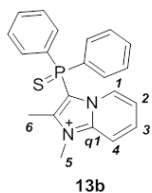
Sample Name:
 SPA01-081
 Data Collected on:
 nmr2.ncats.nih.gov-vmrns400
 Archive directory:
 /home/Walkup2/vmrnsys/data/Walkup2
 Sample directory:
 SPA01-081_20130913_01
 FidFile: SPA01-081_gHSQCAD_01

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Pulse Sequence: **gHSQCAD**
 Solvent: dmsd
 Data collected on: Sep 13 2013

Temp. 25.0 C / 298.1 K
 Sample #46, Operator: Walkup2

Relax. delay 1.000 sec
 Acq. time 0.150 sec
 Width 6410.3 Hz
 2D Width 20105.6 Hz
 32 repetitions
 2 x 512 increments
 OBSERVE H1, 399.8111706 MHz
 DECOUPLE C13, 100.5416467 MHz
 Power 34 dB
 on during acquisition
 off during delay
 W40_autocx modulated
 DATA PROCESSING
 Gauss apodization 0.069 sec
 F1 DATA PROCESSING
 Gauss apodization 0.024 sec
 FT size 2048 x 4096
 Total time 10 hr, 59 min



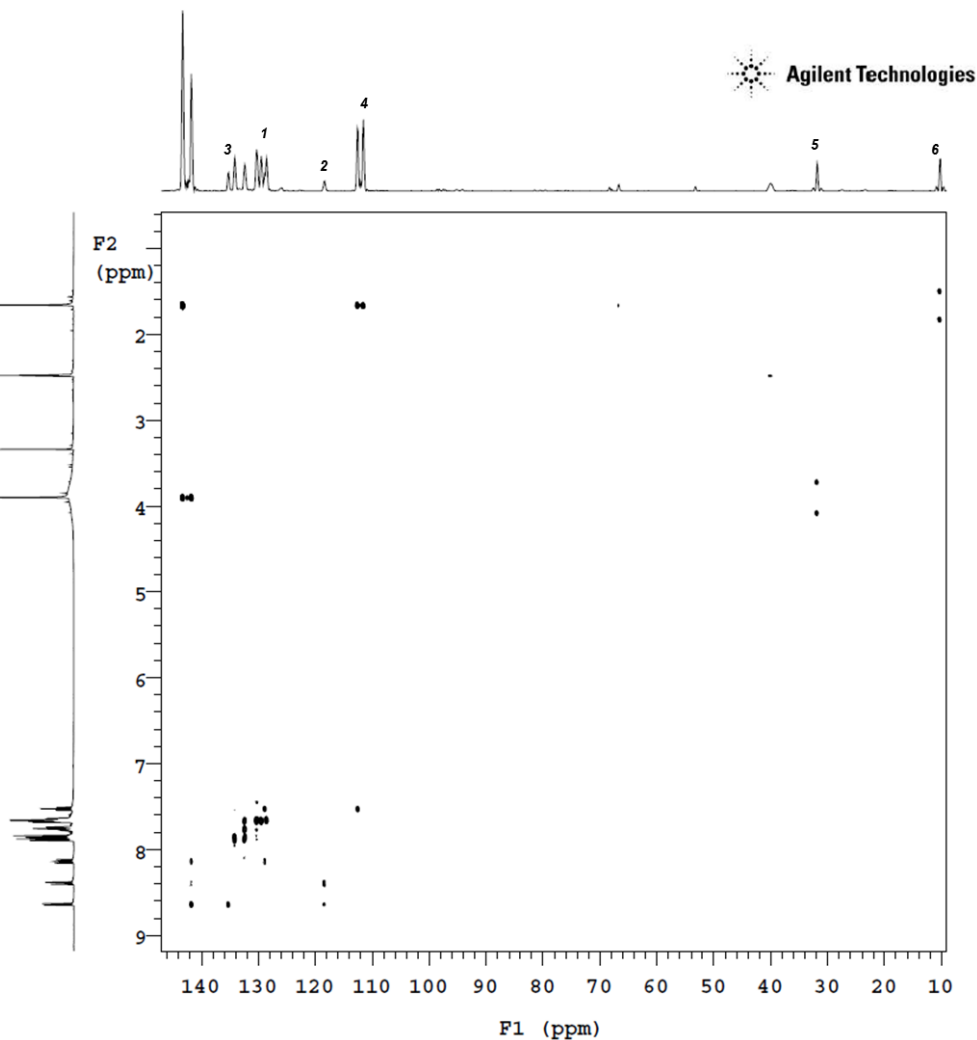
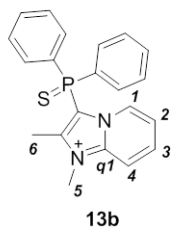
4. gHMBCAD of 13b

Sample Name:
SPA01-081
Data Collected on:
nmr2.ncats.nih.gov-vnmrs400
Archive directory:
/home/Walkup2/vnmrsys/data/Walkup2
Sample directory:
SPA01-081_20130913_01
FidFile: SPA01-081_gHMBCAD_01

Pulse Sequence: **gHMBCAD**
Solvent: dmsc
Data collected on: Sep 14 2013

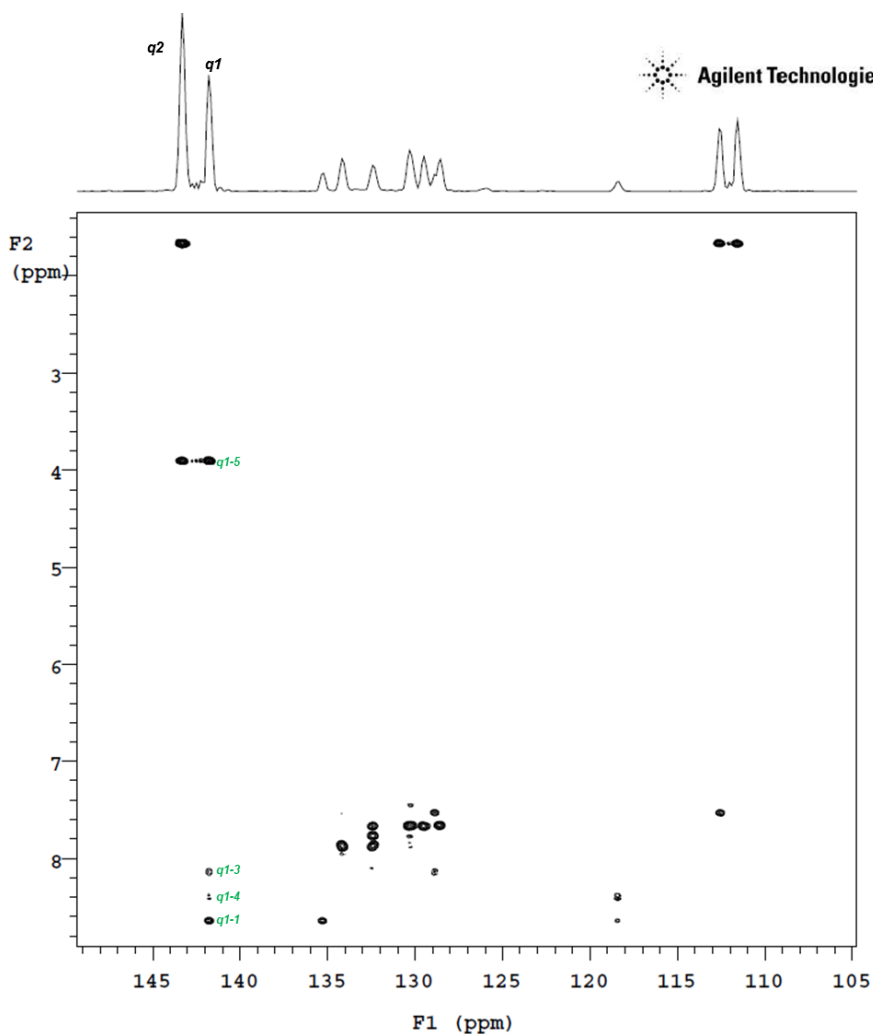
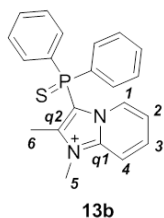
Temp. 25.0 C / 298.1 K
Sample #46, Operator: Walkup2

Relax. delay 1.000 sec
Acq. time 0.150 sec
Width 6410.3 Hz
2D Width 24125.5 Hz
64 repetitions
2 x 512 increments
OBSERVE H1, 399.8111706 MHz
DATA PROCESSING
Sq. sine bell 0.075 sec
F1 DATA PROCESSING
Gauss apodization 0.020 sec
FT size 2048 x 4096
Total time 22 hr, 37 min



5. gHMBCAD of **13b** (expansion)

Sample Name:
 SPA01-081
 Data Collected on:
 nmr2.ncats.nih.gov-vmrns400
 Archive directory:
 /home/Walkup2/vnmrsys/data/Walkup2
 Sample directory:
 SPA01-081_20130913_01
 FidFile: SPA01-081_gHMBCAD_01
 Pulse Sequence: **gHMBCAD**
 Solvent: dmsd
 Data collected on: Sep 14 2013
 Temp. 25.0 C / 298.1 K
 Sample #46, Operator: Walkup2
 Relax. delay 1.000 sec
 Acq. time 0.150 sec
 Width 6410.3 Hz
 2D Width 24125.5 Hz
 64 repetitions
 2 x 512 increments
 OBSERVE H1, 399.8111706 MHz
 DATA PROCESSING
 Sq. sine bell 0.075 sec
 F1 DATA PROCESSING
 Gauss apodization 0.020 sec
 FT size 2048 x 4096
 Total time 22 hr, 37 min



5. NOESY of 13b

Sample Name:
SPA01-081_NOESY
Data Collected on:
nmr1.bogus-vnmrs400
Archive directory:
/home/Walkup1/vnmrsys/data/patnaiks
Sample directory:
SPA01-081_NOESY_20130906_01
FidFile: SPA01-081_NOESY_NOESY_01

Pulse Sequence: **NOESY**
Solvent: dmsc
Data collected on: Sep 7 2013

Temp. 25.0 C / 298.1 K
Sample #26, Operator: patnaiks

Relax. delay 1.000 sec
Acq. time 0.150 sec
Width 3655.0 Hz
2D Width 3655.0 Hz
32 repetitions
2 x 400 increments
OBSERVE H1, 399.9388525 MHz
DATA PROCESSING
Gauss apodization 0.069 sec
F1 DATA PROCESSING
Gauss apodization 0.058 sec
FT size 4096 x 4096
Total time 10 hr, 1 min

