

1H-Imidazole-2,5-dicarboxamides as NS4A Peptidomimetics: Identification of a New Venue to Inhibit HCV-NS3 Protease

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S1. Superposition of NS4A in four different crystal structures

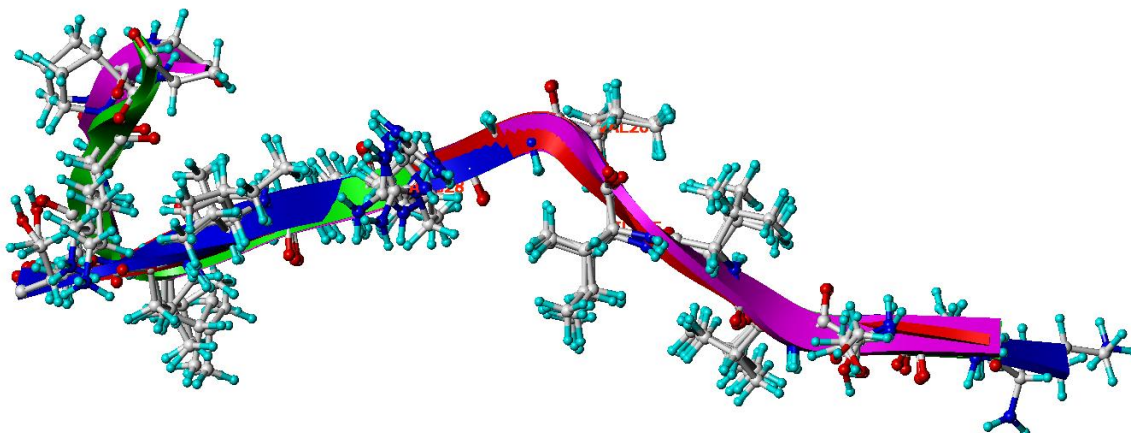


Figure S1-A: Conserved conformation of NS4A peptide in bound form with NS3 protease domain. Examples were downloaded from PDB website (rcsb.org) and illustrated as follows: 1A1R (Green), 2OC1 (magenta), 3OYP (Red) and 4U01 (Blue)

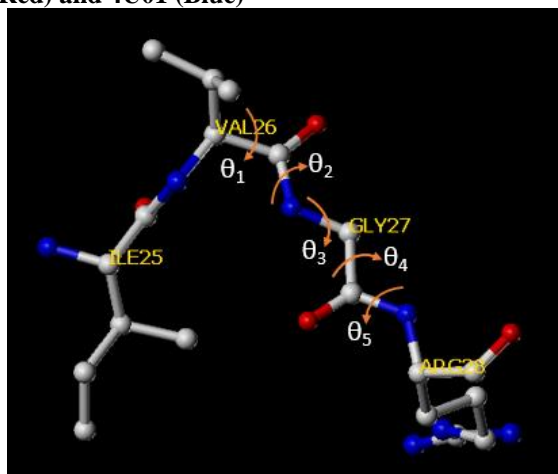


Figure S1-B. Dihedral angles θ_n of the bound NS4A's planar region (PDB Code: 1NS3). Numerical values of θ_1 to θ_5 are listed in Table S1-A.

Torsion	Actual	Deviation from Plane
θ_1	13.9	+13.9 (eclipsed cis)
θ_2	179.4	-0.6
θ_3	184.6	+4.6
θ_4	191.6	+11.6
θ_5	184.3	+4.3

Table S1. Dihedral angles of core part of bound NS4A (PDB Code: 1NS3).

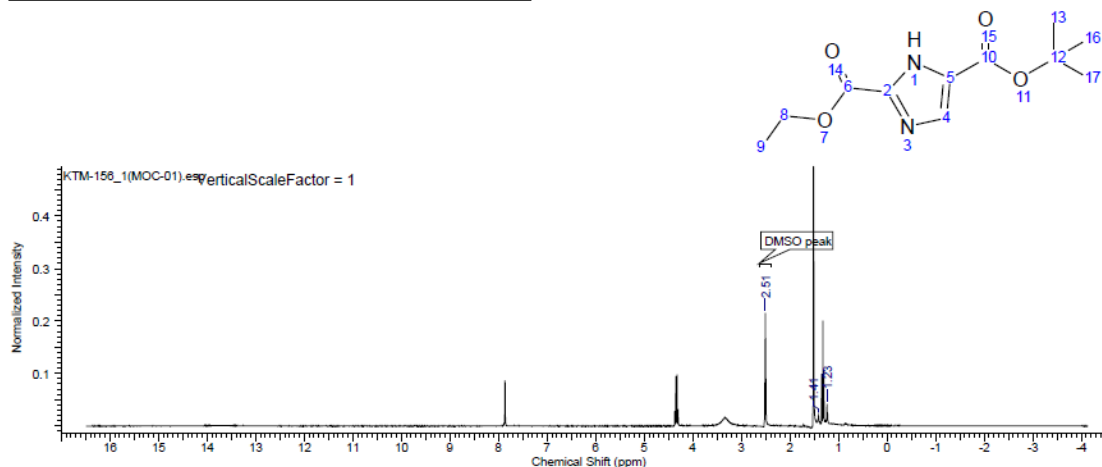
S2. Spectra of Intermediates and Final Compounds

5-(tert-butyl) 2-ethyl 1H-imidazole-2,5-dicarboxylate (2)

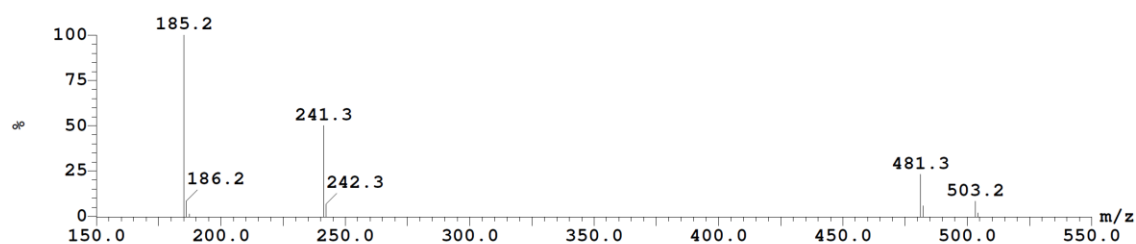
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3/2/2017 1:46:23 PM

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Owner	Administrator	Points Count	16384
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Sweep Width (Hz)	6187.74	Solvent	DMSO-d6
		Spectrum Offset (Hz)	1853.6917
		Receiver Gain	256.00
		Temperature (degree C)	26.160
		Spectrum Type	STANDARD



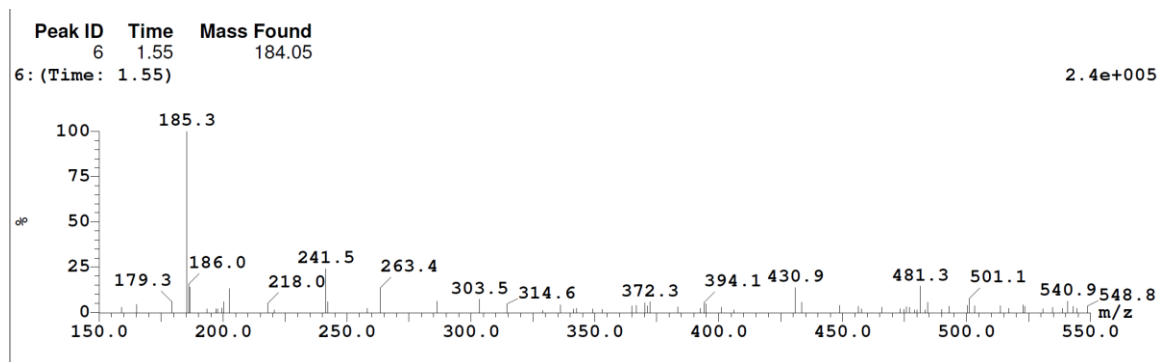
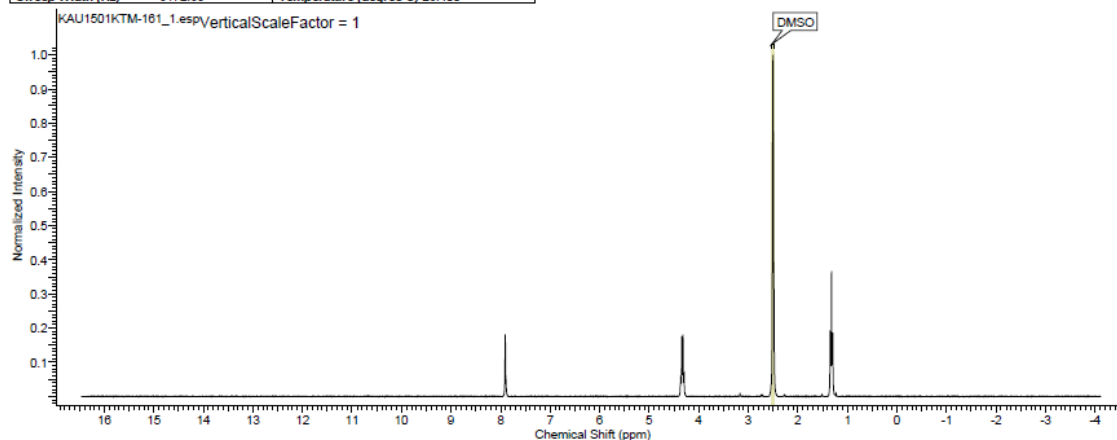
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3/2/2017 1:50:15 PM

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Nucleus	1H	Number of Transients	16	Origin	spect
Owner	quest	Points Count	32768	Pulse Sequence	zg30
SW (Hz)	6172.84	Solvent	DMSO-d6	Receiver Gain	645.10
Sweep Width (Hz)	6172.85	Temperature (degree C)	26.400	Spectrum Offset (Hz)	1853.4263
				Spectrum Type	STANDARD



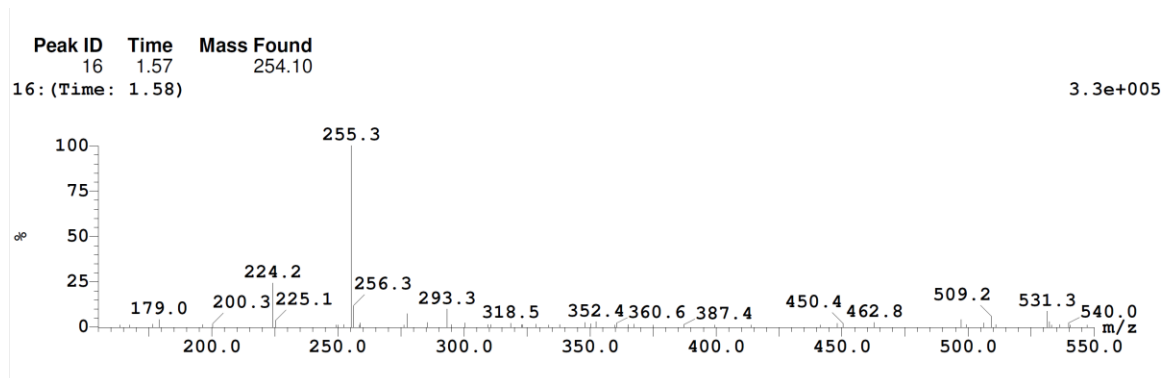
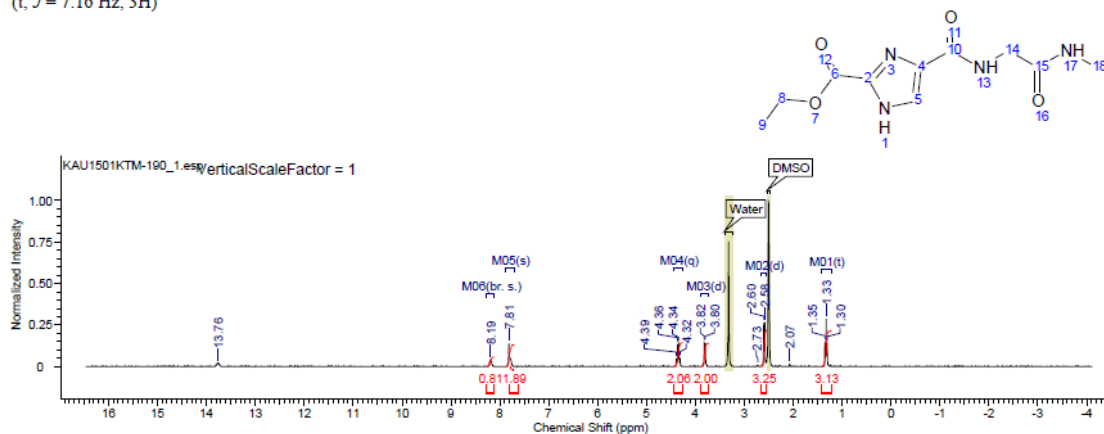
Ethyl 4-((2-(methylamino)-2-oxoethyl)carbamoyl)-1H-imidazole-2-carboxylate (4a)

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3/8/2017 3:58:45 PM

Formula	C ₁₄ H ₁₄ N ₄ O ₃	FW	254.2426
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Nucleus	1H	Number of Transients	16
Owner	quest	Points Count	32768
SW (cyclical) (Hz)	6172.84	Pulse Sequence	zg30
Sweep Width (Hz)	6172.65	Solvent	DMSO-d6
		Spectrum Offset (Hz)	1853.4263
		Temperature (degree C)	26.900
		Receiver Gain	512.00
		Spectrum Type	STANDARD

¹H NMR (300 MHz, DMSO-d₆) δ 8.19 (br. s., 1H), 7.81 (s, 2H), 4.35 (q, *J* = 7.16 Hz, 2H), 3.81 (d, *J* = 5.65 Hz, 2H), 2.59 (d, *J* = 4.52 Hz, 3H), 1.33 (t, *J* = 7.16 Hz, 3H)

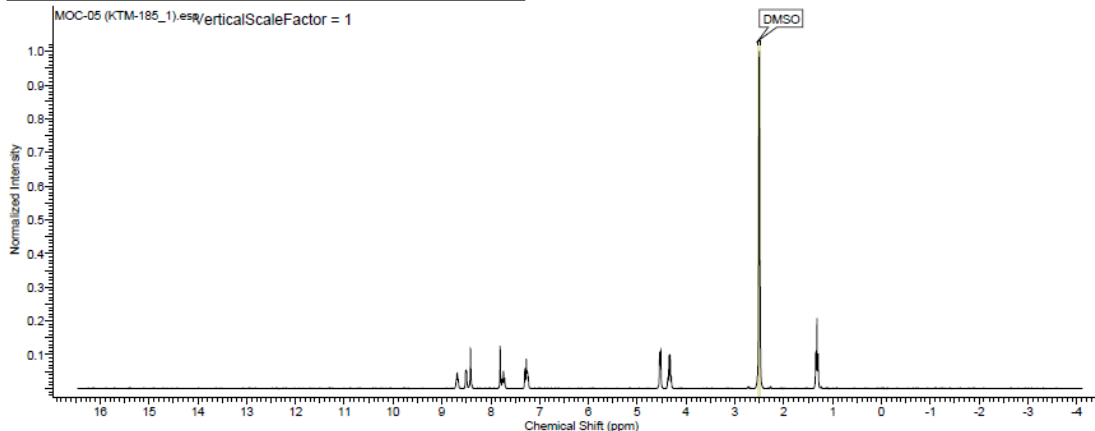


Ethyl 5-((pyridin-2-ylmethyl)carbamoyl)-1H-imidazole-2-carboxylate (4c)

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3/2/2017 2:00:53 PM

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Nucleus	1H	Number of Transients	16	Origin	spect	Original Points Count	32768
Owner	quest	Points Count	32768	Pulse Sequence	zg30	Receiver Gain	456.10
SW(cyclical) (Hz)	6172.84	Solvent	DMSO-d6	Spectrum Offset (Hz)	1853.4263	Spectrum Type	STANDARD
Sweep Width (Hz)	6172.65	Temperature (degree C)	25.500				

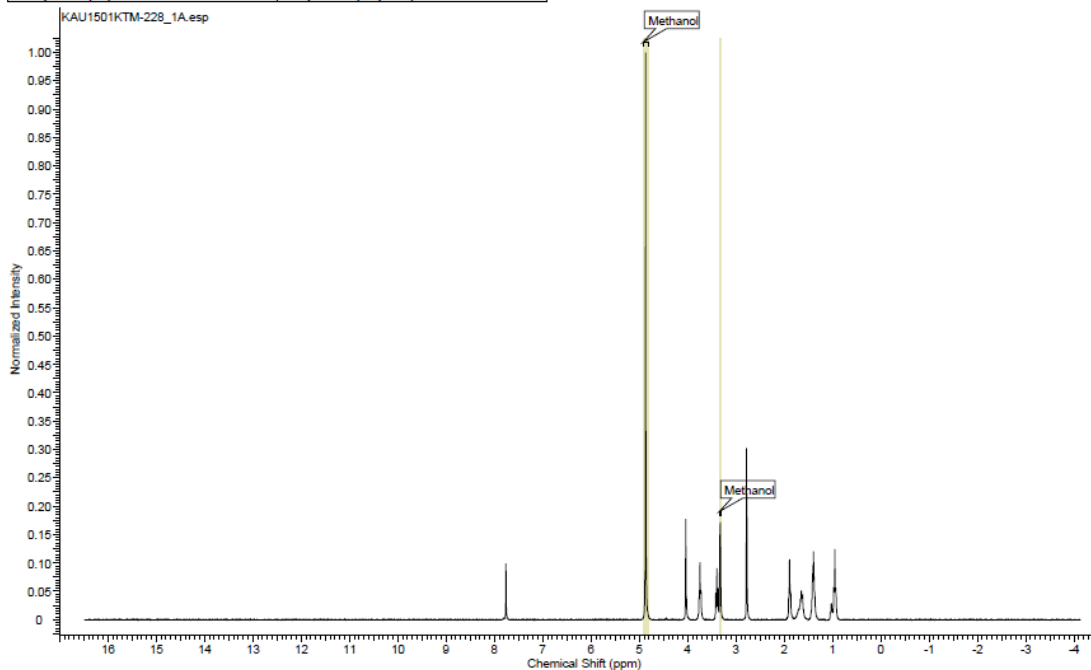


MOC-11

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5/6/2017 10:03:53 AM

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Date	16 Nov 2015 23:17:36		Date Stamp	16 Nov 2015 23:17:36	
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Nucleus	1H	Number of Transients	8	Origin	spect
Owner	Administrator	Points Count	16384	Pulse Sequence	zg
SW(cyclical) (Hz)	6188.12	Solvent	METHANOL-d4	Spectrum Offset (Hz)	1853.6917
Sweep Width (Hz)	6187.74	Temperature (degree C)	25.160	Receiver Gain	645.10
				Spectrum Type	STANDARD



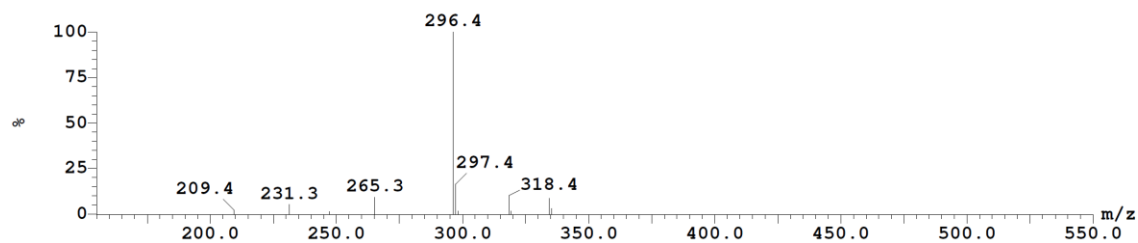
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24.946 —
28.852 —
28.967 —
38.918 —
41.727 —
46.781 —
47.064 —
47.349 —
47.512 —
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141.115 —
158.598 —
163.740 —
170.795 —

PPM

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Hz/cm: 627.979 ppm/cm: 8.32028

8.3e+005



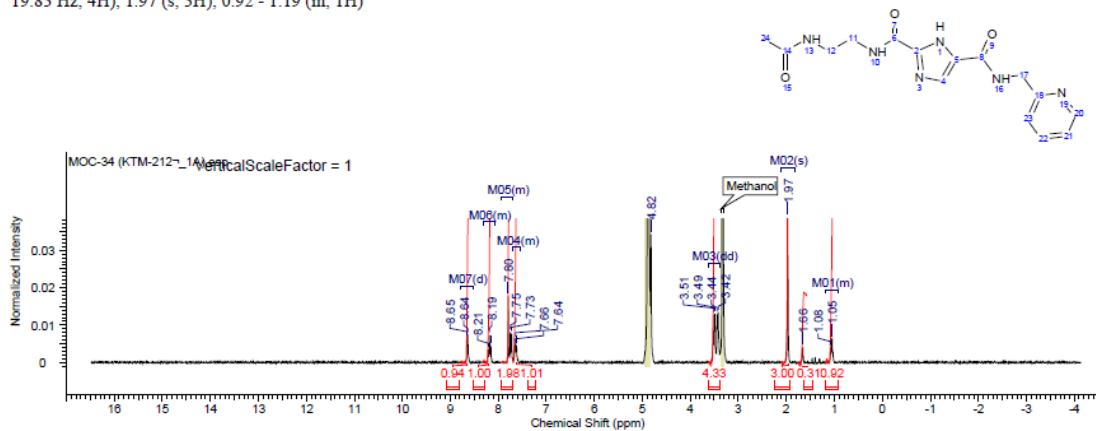
MOC-34

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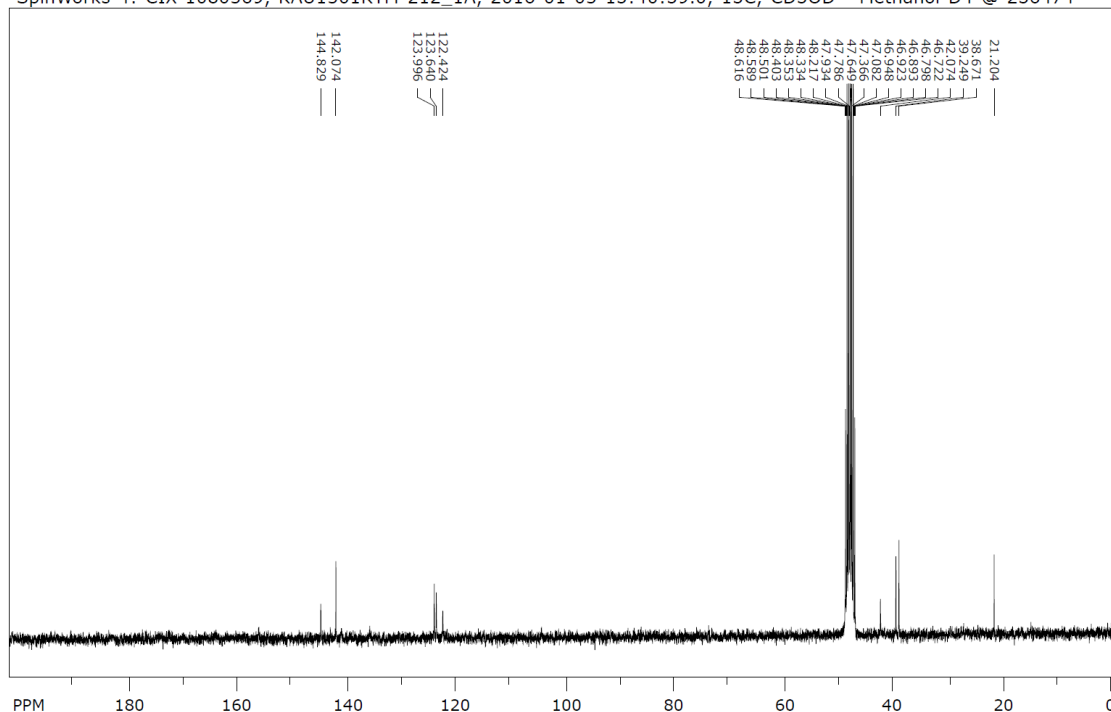
5/8/2017 8:03:26 AM

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Date	22 Oct 2015 11:42:24	Date Stamp	22 Oct 2015 11:42:24
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Nucleus	1H	Number of Transients	8
Owner	Administrator	Points Count	16384
SW (cyclical) (Hz)	6188.12	Pulse Sequence	zg
Sweep Width (Hz)	6187.74	Solvent	METHANOL-d4
		Spectrum Offset (Hz)	1853.8917
		Receiver Gain	812.70
		Spectrum Type	STANDARD
		Temperature (degree C)	25.160

¹H NMR (300 MHz, METHANOL-d₄) δ 8.65 (d, *J* = 4.91 Hz, 1H), 8.08 - 8.32 (m, 1H), 7.69 - 7.93 (m, 2H), 7.54 - 7.69 (m, 1H), 3.47 (dd, *J* = 5.85, 19.83 Hz, 4H), 1.97 (s, 3H), 0.92 - 1.19 (m, 1H)



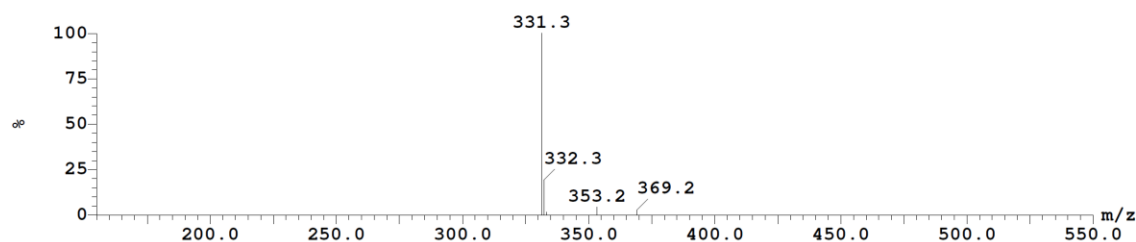
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number of scans: 640

freq. of 0 ppm: 75.467746 MHz
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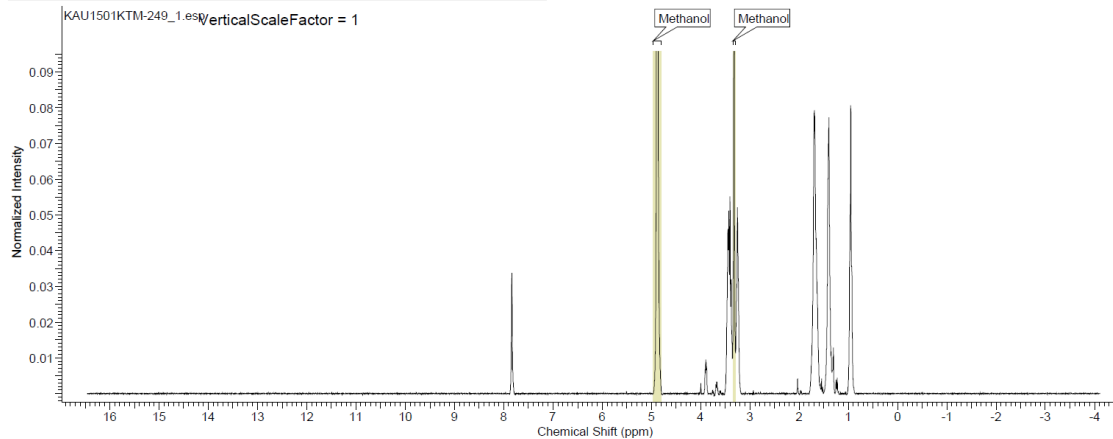


MOC-23

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5/9/2017 1:06:20 PM

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Nucleus	1H	Number of Transients	16
Owner	guest	Points Count	32768
SW(cyclical) (Hz)	6172.84	Solvent	METHANOL-d4
Sweep Width (Hz)	6172.65	Temperature (degree C)	26.100
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		Pulse Sequence	zg30
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		Spectrum Type	STANDARD

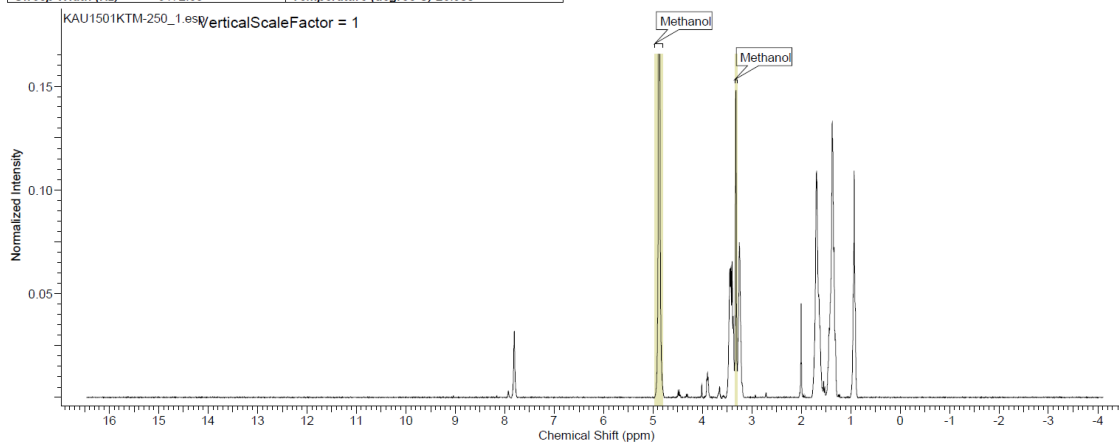


MOC-24

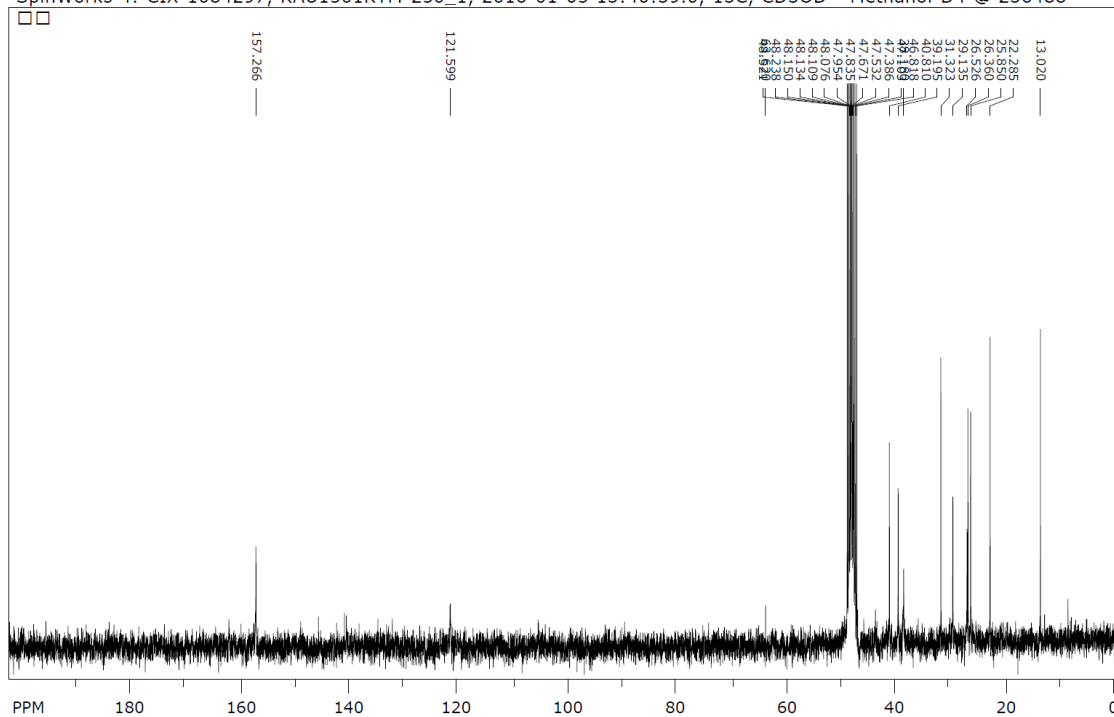
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5/9/2017 1:02:51 PM

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Nucleus	1H	Number of Transients	16	Origin	spect
Owner	guest	Points Count	32768	Pulse Sequence	zg30
SW(cyclical) (Hz)	6172.84	Solvent	METHANOL-d4	Spectrum Offset (Hz)	1853.4263
Sweep Width (Hz)	6172.65	Temperature (degree C)	26.900	Spectrum Type	STANDARD

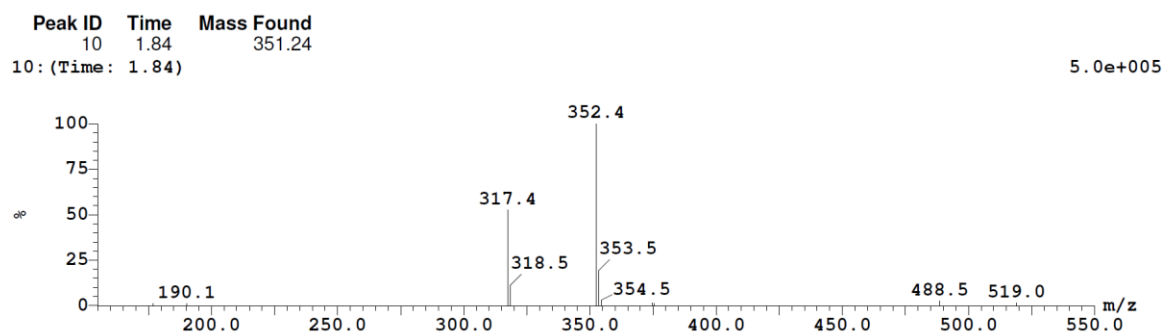


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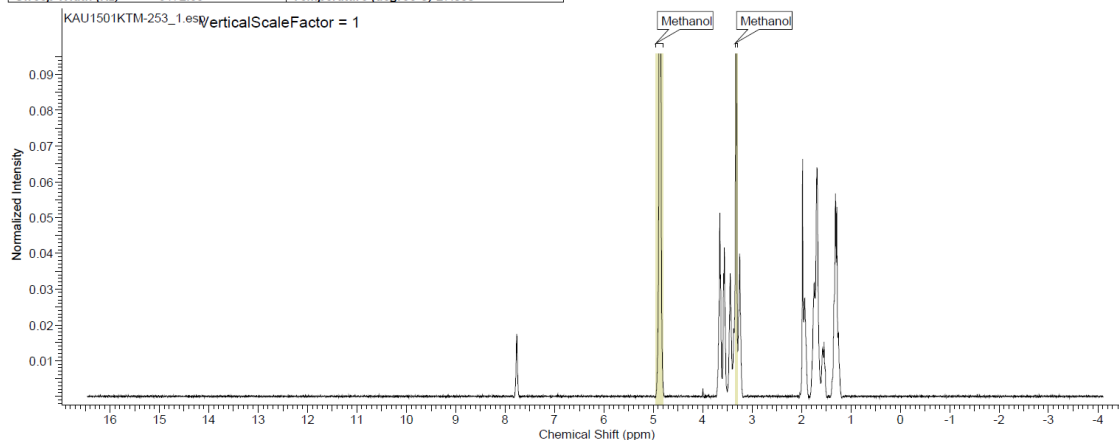


MOC-26

This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/

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Sweep Width (Hz)	6172.65	Temperature (degree C)	27.000	Frequency (MHz)	300.13
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				Receiver Gain	912.30
				Spectrum Type	STANDARD

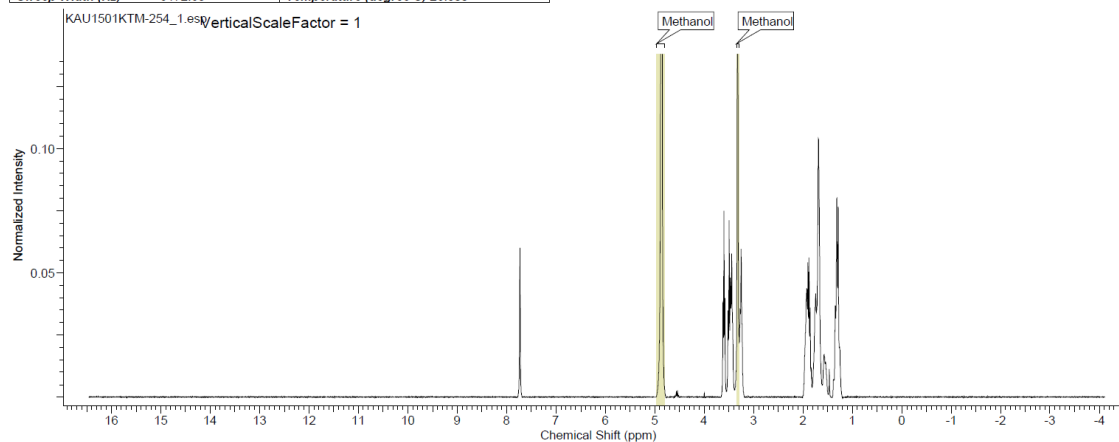


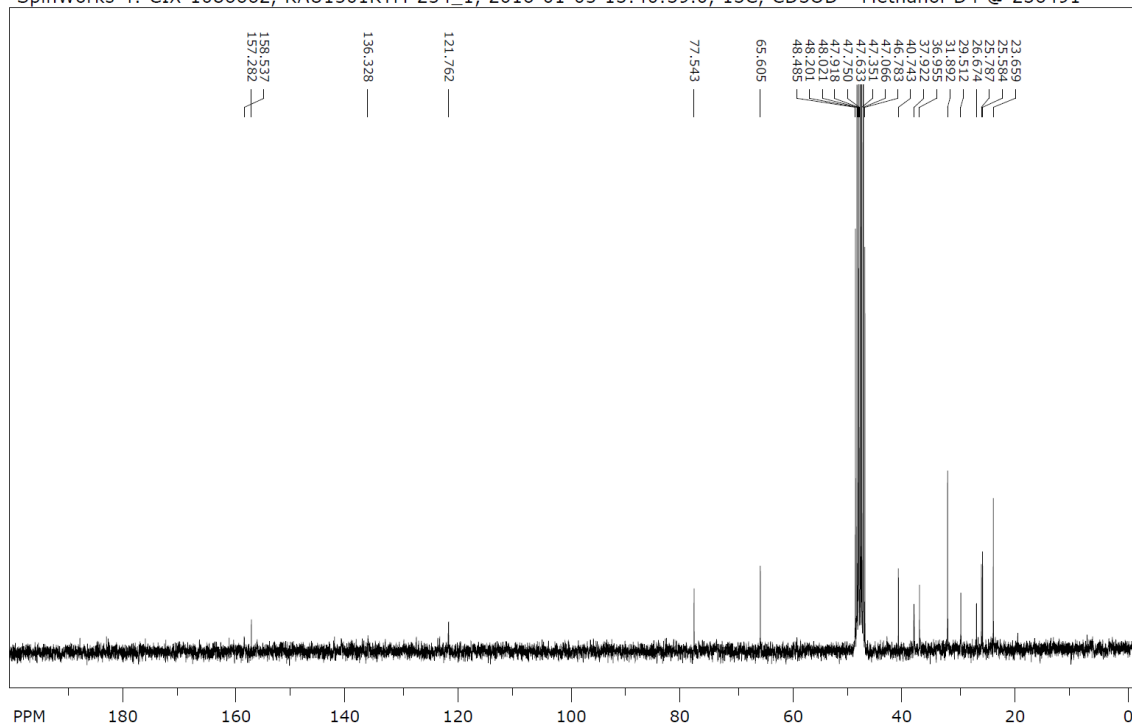
MOC-27

This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/

5/9/2017 12:48:13 PM

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Owner		guest		Points Count		32768		Pulse Sequence		zg30		Receiver Gain		724.10	
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Sweep Width (Hz)		6172.65		Temperature (degree C)		26.800									



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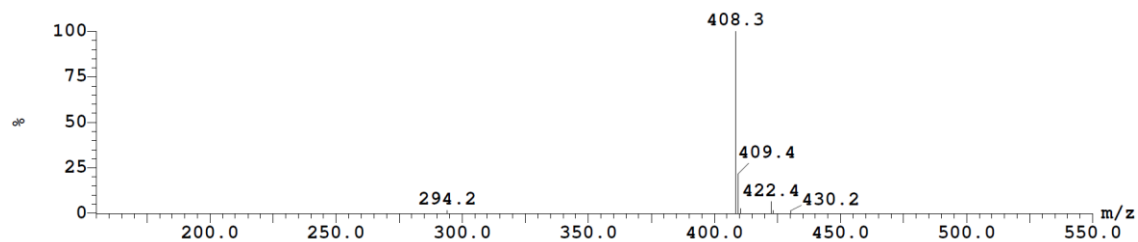
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number of scans: 640

freq. of 0 ppm: 75.467746 MHz
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7.8e+005

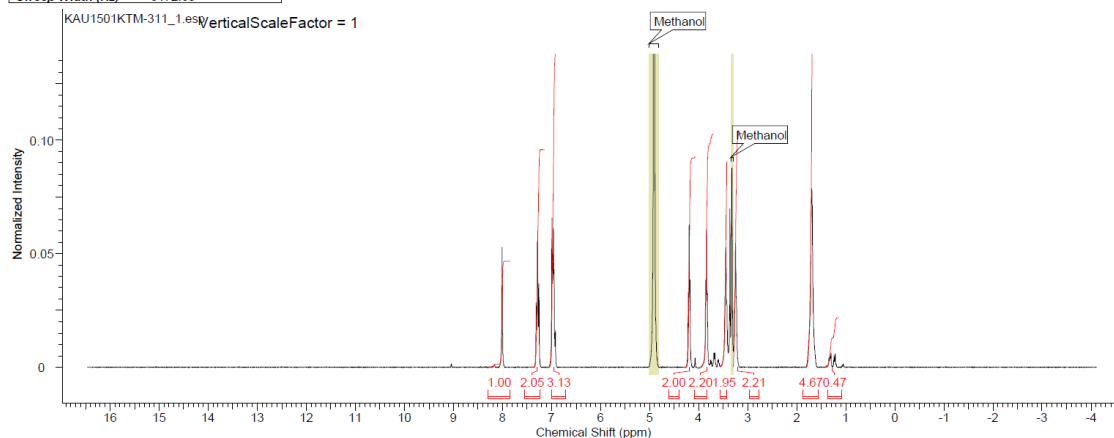


MOC-28

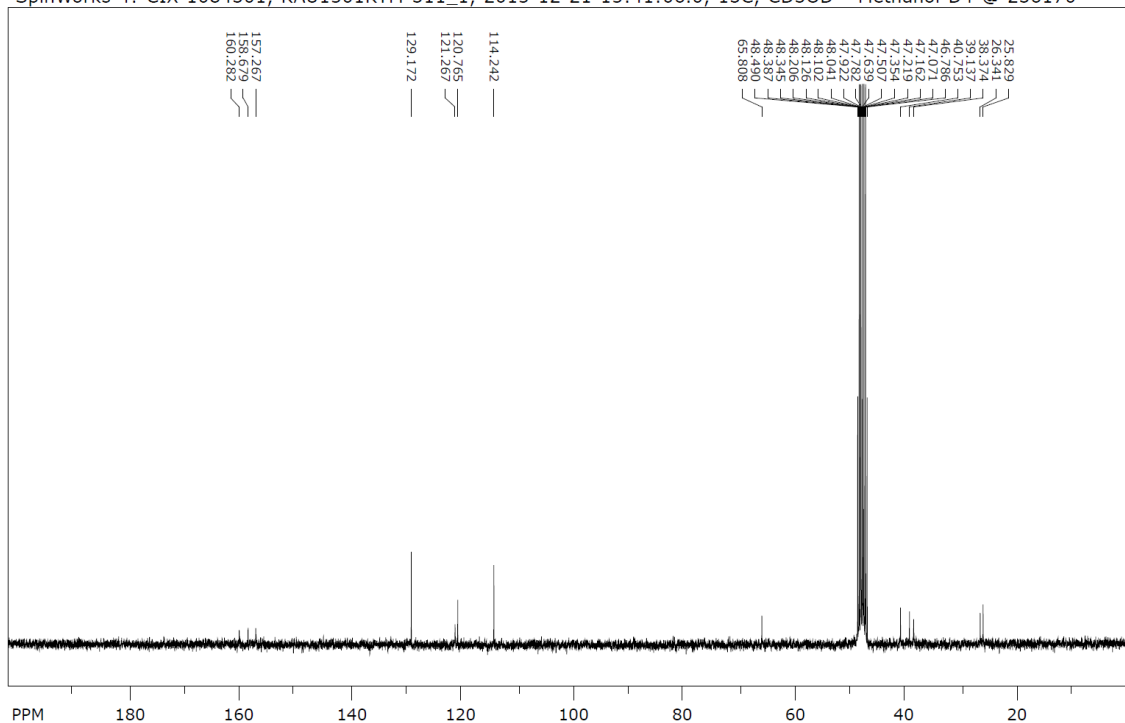
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/

5/9/2017 1:10:35 PM

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Nucleus	1H	Number of Transients	16	Origin
Owner	guest	Points Count	32768	Pulse Sequence
SW(cyclical) (Hz)	6172.84	Solvent	METHANOL-d4	Spectrum Offset (Hz)
Sweep Width (Hz)	6172.65			Spectrum Type



SpinWorks 4: CIX-1084301, KAU1501KTM-311_1, 2015-12-21 15:41:06.0, 13C, CD3OD - Methanol-D4 @ 236170

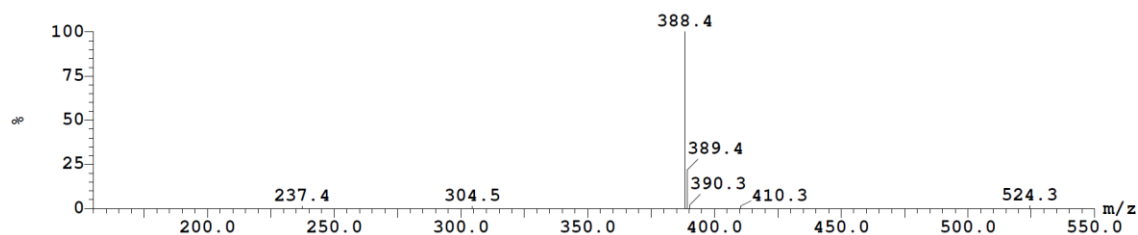


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number of scans: 600

freq. of 0 ppm: 75.467746 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000
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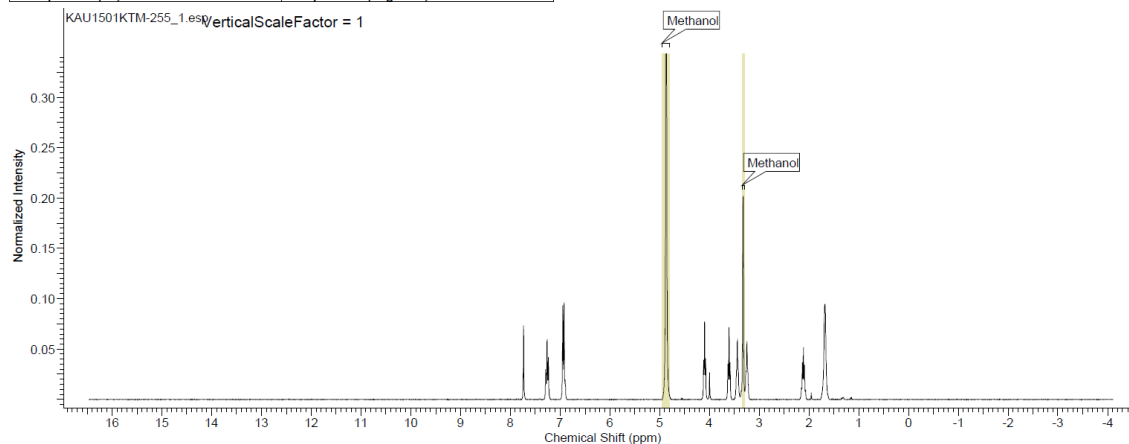


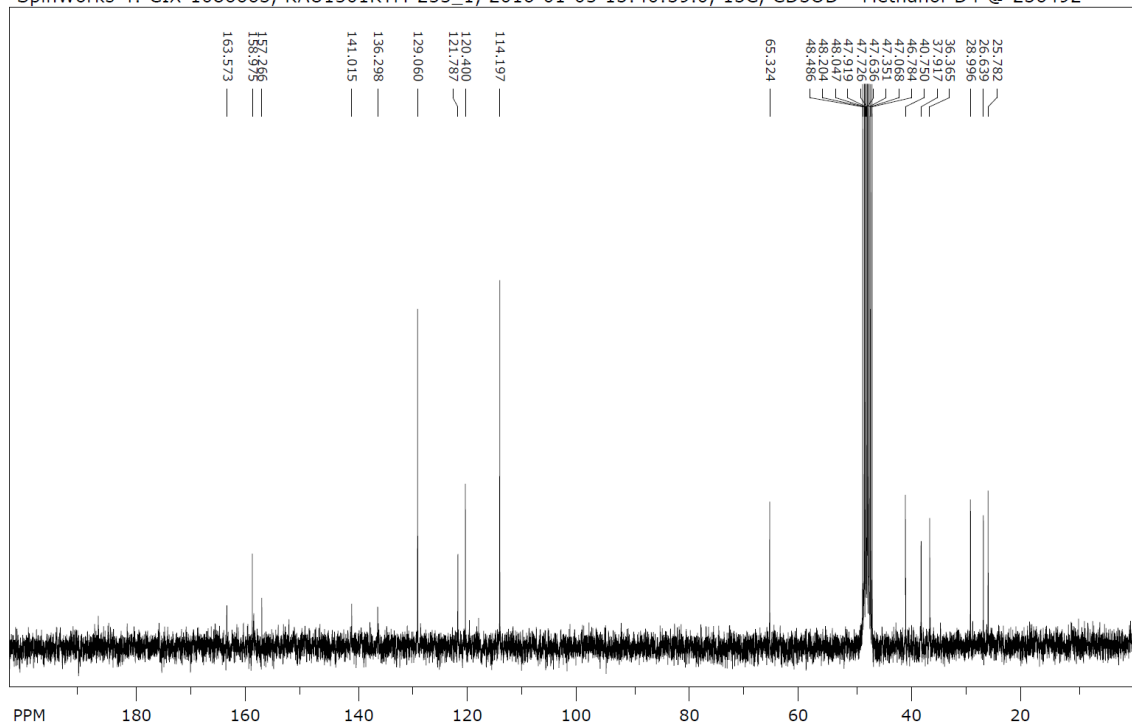
MOC-29

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5/9/2017 9:06:02 AM

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Nucleus	1H	Number of Transients	16	Origin	spect
Owner	quest	Points Count	32768	Pulse Sequence	zg30
SW(cyclical) (Hz)	6172.84	Solvent	METHANOL-d4	Receiver Gain	812.70
Sweep Width (Hz)	6172.65	Temperature (degree C)	26.600	Spectrum Offset (Hz)	1853.4263
				Spectrum Type	STANDARD



SpinWorks 4: CIX-1086663, KAU1501KTM-255_1, 2016-01-05 13:40:39.0, ¹³C, CD3OD - Methanol-D4 @ 236492

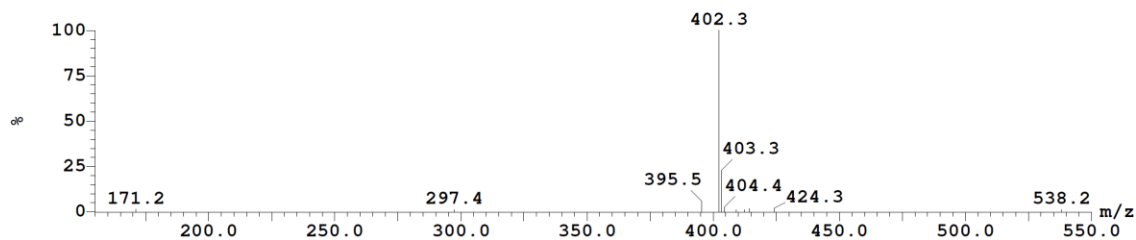
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transmitter freq.: 75.475670 MHz
time domain size: 65536 points
width: 20325.20 Hz = 269.2948 ppm = 0.310138 Hz/pt
number of scans: 640

freq. of 0 ppm: 75.467746 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000
Hz/cm: 616.813 ppm/cm: 8.17235

Peak ID	Time	Mass Found
12	1.89	401.22

12: (Time: 1.89)

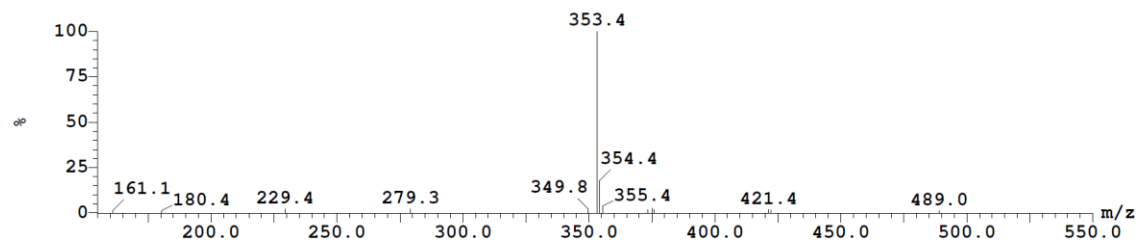
7.5e+005



Peak ID	Time	Mass Found
18	2.87	352.20

18: (Time: 2.87)

7.6e+005

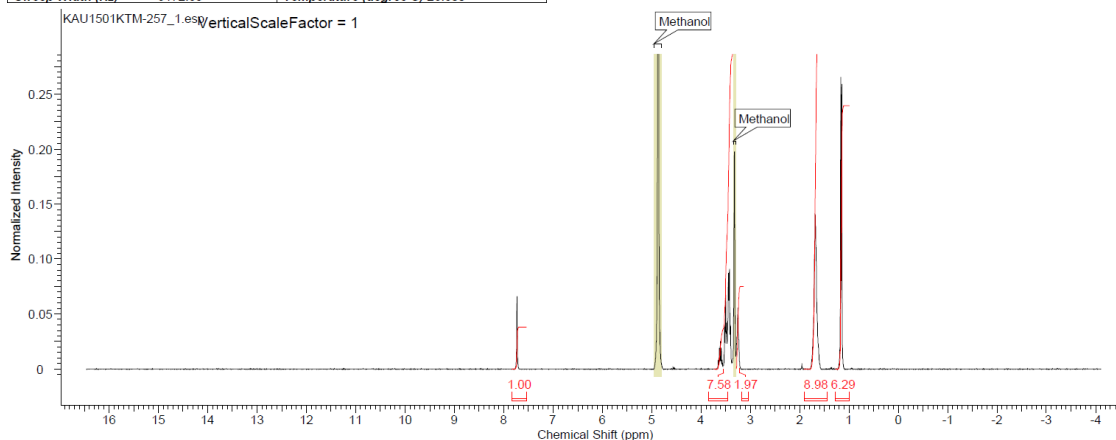


MOC-30

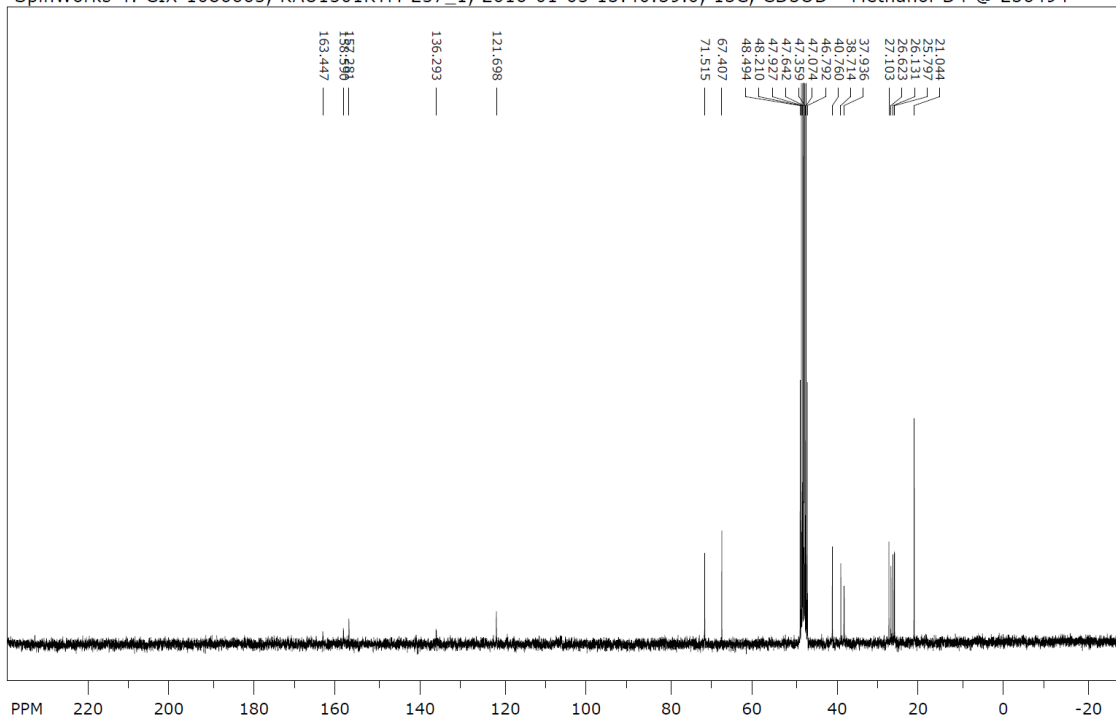
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/

5/9/2017 8:57:15 AM

Acquisition Time (sec)	5.3084	Comment	CIX-1086665, KAU1501KTM-257_1, 1H, CD3OD - Methanol-D4 @ 231798		
Date	03 Dec 2015 21:37:20	Date Stamp	03 Dec 2015 21:37:20		
File Name	C:\Users\mtk0005\Google Drive\KAU\MEI-Araby\12-BIO3193-03 Imidazole Cpds\1H-NMR\KAU1501KTM-257_1.fid	Frequency (MHz)	300.13		
Nucleus	1H	Number of Transients	16	Origin	spect
Owner	guest	Points Count	32768	Pulse Sequence	zg30
SW(cyclical) (Hz)	6172.84	Solvent	METHANOL-d4	Spectrum Offset (Hz)	1853.4263
Sweep Width (Hz)	6172.65	Temperature (degree C)	26.600	Spectrum Type	STANDARD



SpinWorks 4: CIX-1086665, KAU1501KTM-257_1, 2016-01-05 13:40:39.0, 13C, CD3OD - Methanol-D4 @ 236494

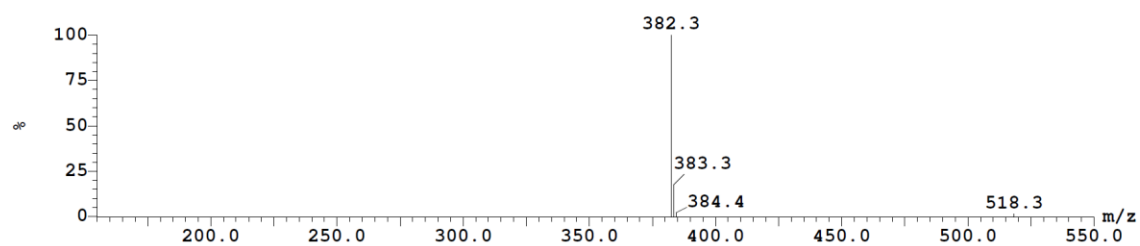


file: ...01KTM-257_1, 13C, 300 @ 236494\fid exp: <zpgp30>
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time domain size: 65536 points
width: 20325.20 Hz = 269.2948 ppm = 0.310138 Hz/pt
number of scans: 640

freq. of 0 ppm: 75.467746 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000
Hz/cm: 813.008 ppm/cm: 10.77179

Peak ID Time Mass Found
15 2.08 381.25
15: (Time: 2.06)

1.4e+006

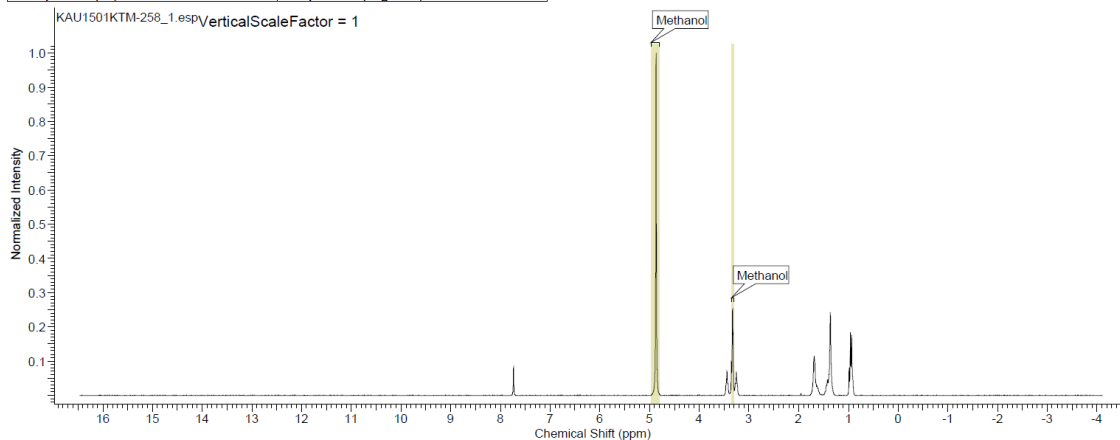


MOC-31

This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/

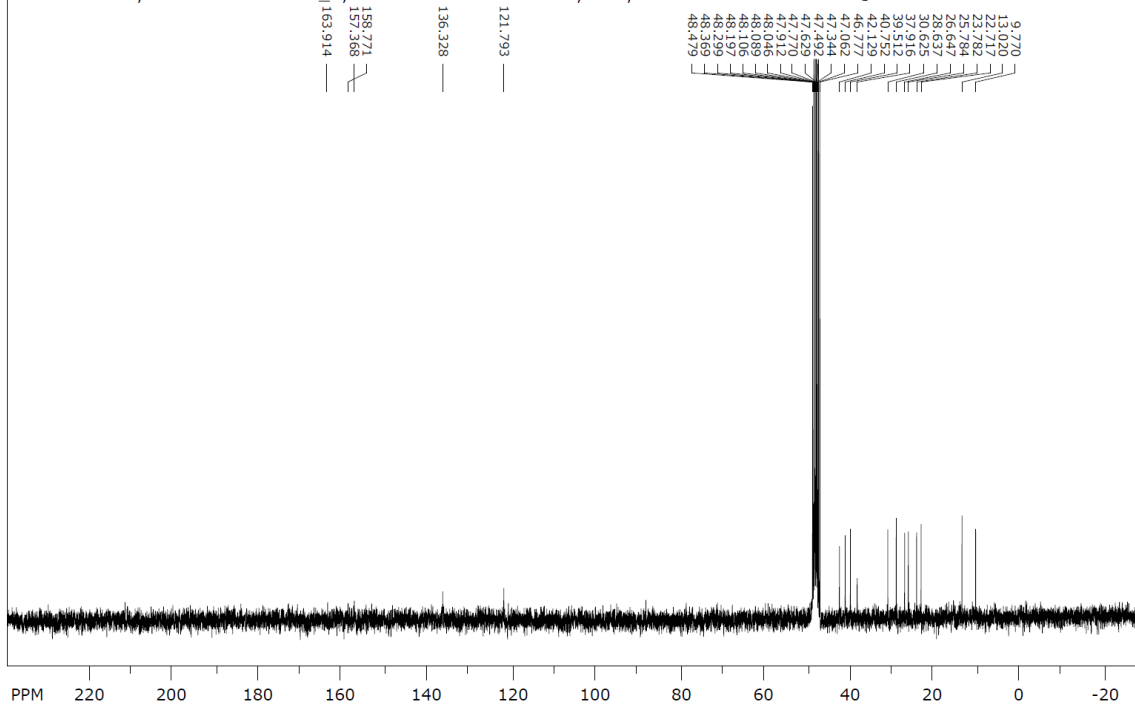
5/9/2017 8:44:35 AM

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Date	03 Dec 2015 21:43:44	Date Stamp	03 Dec 2015 21:43:44
File Name	C:\Users\mtk0005\Google Drive\KAU\MEI-Araby\12-BIO3193-03 Imidazole Cpd\1H-NMR\KAU1501KTM-258_1.fid	Frequency (MHz)	300.13
Nucleus	1H	Number of Transients	16
Owner	guest	Points Count	32768
SW(cyclical) (Hz)	6172.84	Pulse Sequence	zg30
Sweep Width (Hz)	6172.65	Solvent	METHANOL-d4
		Spectrum Offset (Hz)	1853.4263
		Receiver Gain	724.10
		Spectrum Type	STANDARD



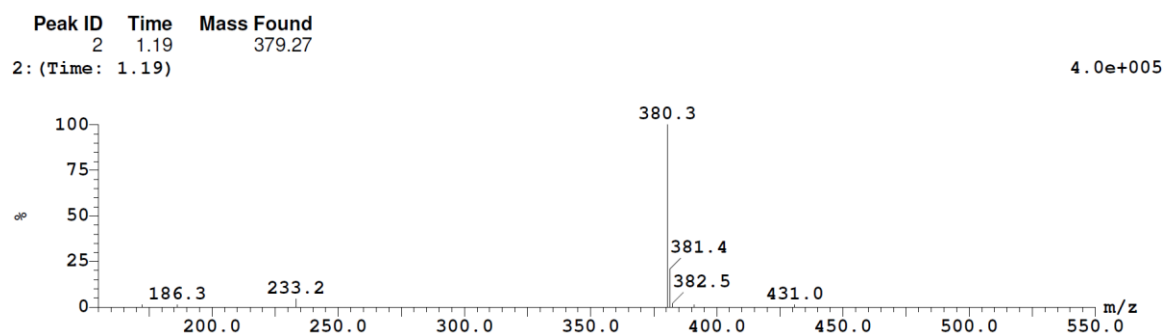
SpinWorks 4:

CIX-1086666, KAU1501KTM-258_1, 2016-01-05 13:40:39.0, 13C, CD3OD - Methanol-D4 @ 236495



file: ...01KTM-258_1, 13C, 310 @ 236495.fid exp: <zgpg30>
transmitter freq.: 75.475670 MHz
time domain size: 65536 points
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number of scans: 640

freq. of 0 ppm: 75.467746 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000
Hz/cm: 813.008 ppm/cm: 10.77179

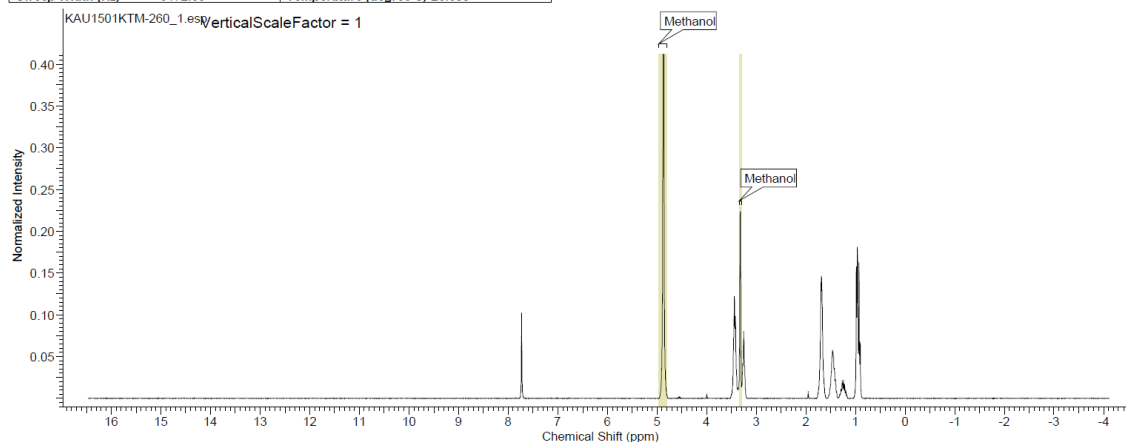


MOC-32

This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/

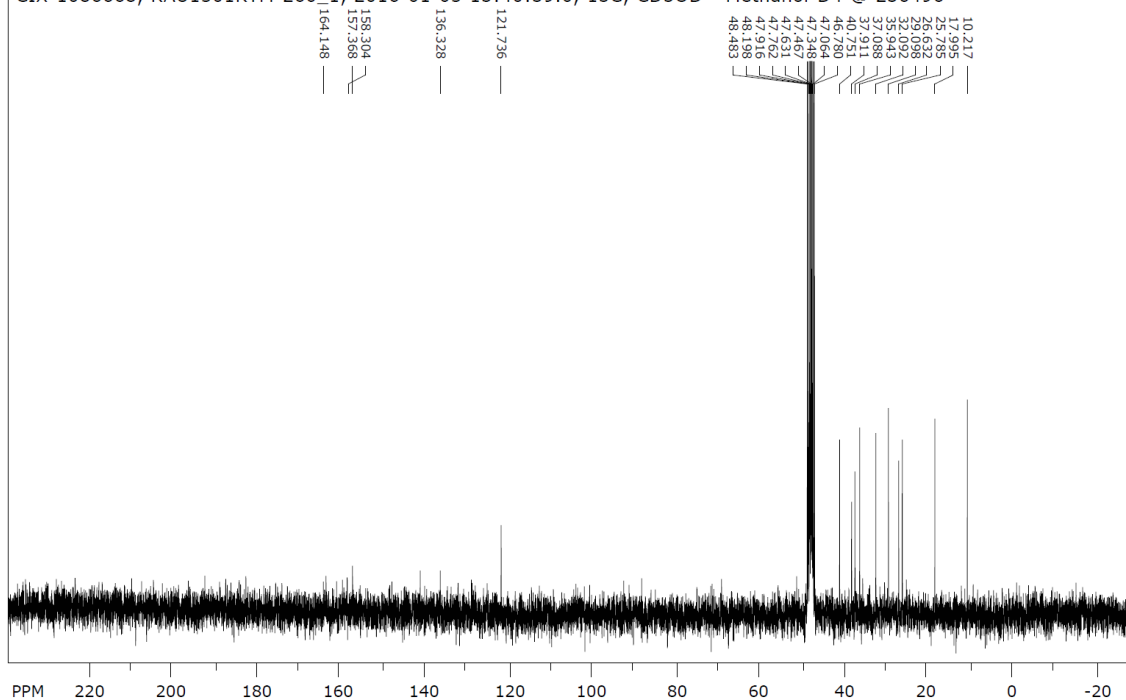
5/9/2017 8:40:15 AM

Acquisition Time (sec)	5.3084	Comment	CIX-1086668, KAU1501KTM-260_1, 1H, CD3OD - Methanol-D4 @ 231805		
Date	03 Dec 2015 21:54:24	Date Stamp	03 Dec 2015 21:54:24		
File Name	C:\Users\mtk0005\Google Drive\KAU\MEI-Araby\12-BIO3193-03 Imidazole Cpds\1H-NMR\KAU1501KTM-260_1.fid	Frequency (MHz)	300.13		
Nucleus	1H	Number of Transients	16	Origin	spect
Owner	guest	Points Count	32768	Pulse Sequence	zg30
SW(cyclical) (Hz)	6172.84	Solvent	METHANOL-d4	Spectrum Offset (Hz)	1853.4263
Sweep Width (Hz)	6172.65	Temperature (degree C)	26.600	Spectrum Type	STANDARD



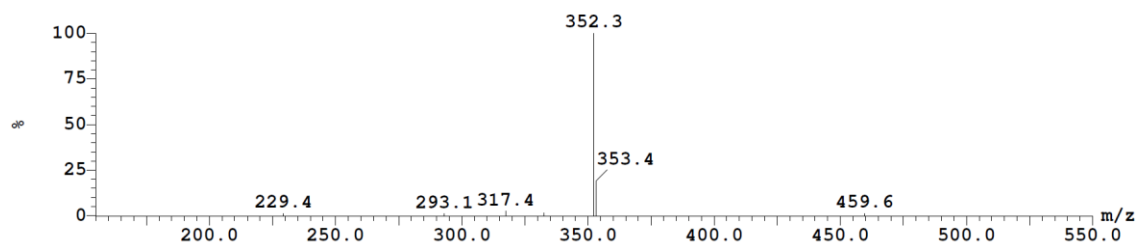
SpinWorks 4:

CIX-1086668, KAU1501KTM-260_1, 2016-01-05 13:40:39.0, 13C, CD3OD - Methanol-D4 @ 236496



Peak ID Time Mass Found
6 1.84 351.24
6: (Time: 1.86)

5.3e+005

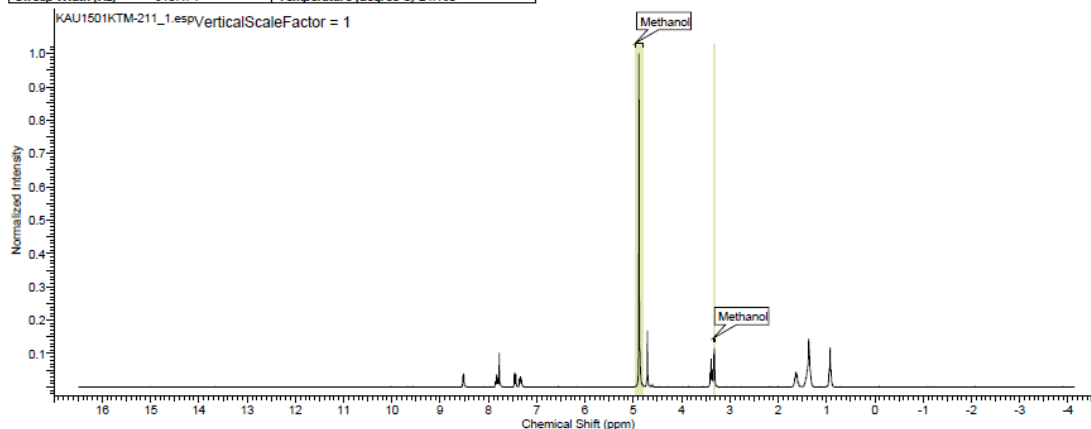


MOC-33

This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/

5/8/2017 8:06:31 AM

Acquisition Time (sec)	2.6477	Comment	CIX-1080568, KAU1501KTM-211 1, 1H, CD3OD - Methanol-D4 @ 219295		
Date	22 Oct 2015 11:50:56	Date Stamp	22 Oct 2015 11:50:56		
File Name	C:\Users\imtk0005\Google Drive\KAU\IMEI-Araby\12-BIO3193-03 Imidazole Cpd\1H-NMR\KAU1501KTM-211_1.fid		Frequency (MHz)	300.13	
Nucleus	1H	Number of Transients	8	Origin	spect
Owner	Administrator	Points Count	16384	Pulse Sequence	zg
SW (cycles) (Hz)	6188.12	Solvent	METHANOL-d4	Receiver Gain	512.00
Sweep Width (Hz)	6187.74	Temperature (degree C)	24.160	Spectrum Offset (Hz)	1853.6917
				Spectrum Type	STANDARD



SpinWorks 4: C1X 1000566, RAG1561RM 211_1, 2016-01-05 15:46:55.8, 150, CDCl₃, Methanol-d₄ 200472

12.990
22.278
25.544
29.230
31.324
38.962
39.676
43.676
46.793
47.077
47.661
47.868
47.489
47.645
47.928
48.066
48.107
48.212
48.289
48.376
48.495
48.686
48.702

121.560
121.863
121.936
122.452

136.291
136.511
137.498
141.144

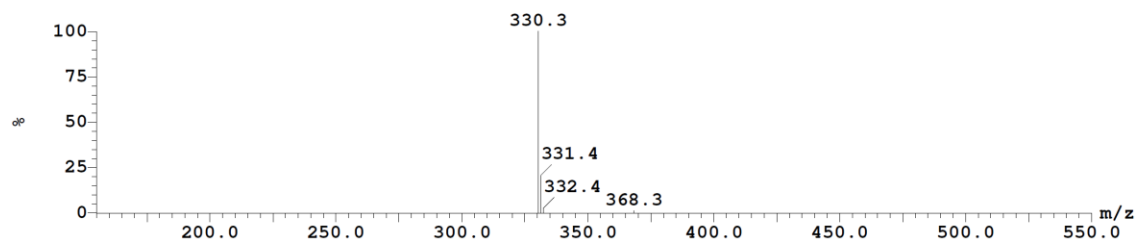
148.440

157.833
158.611
163.647
163.664

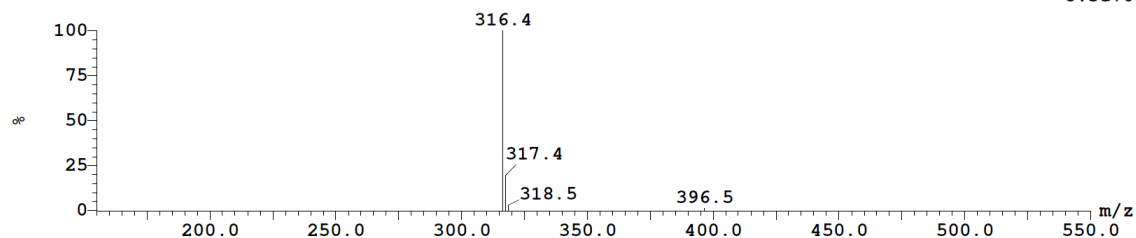
PPM

freq. of 0 ppm: 75.467746 MHz
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LB: 1.000 GF: 0.0000
Hz/cm: 613.961 ppm/cm: 8.13456

3.6e+006



```
1:MS AP+
8.5e+005
```



S3. Competition Assays of MOC Compounds with NS4A

Determination of NS4A₂₁₋₃₄ binding affinity

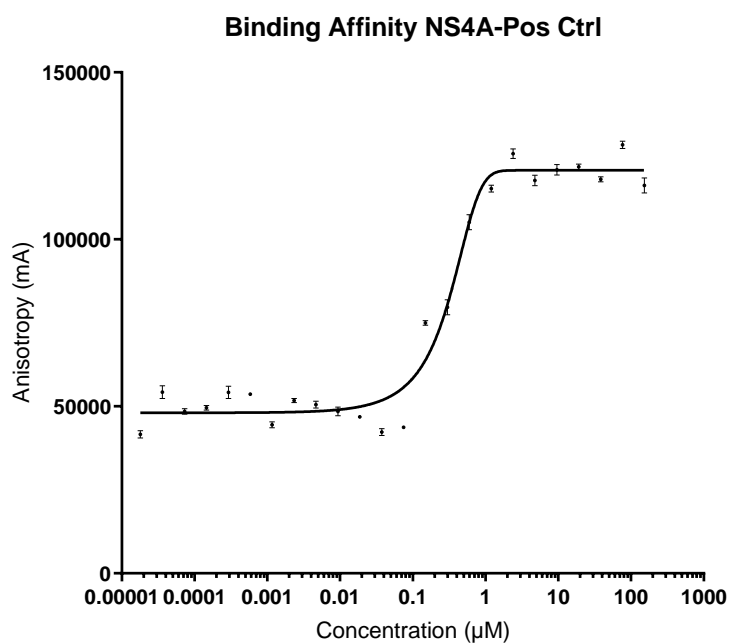


Figure S3. Fluorescence assay for binding of NS4A₂₁₋₃₄ to NS3 (Genotype 4).

S4. Molecular Dynamics

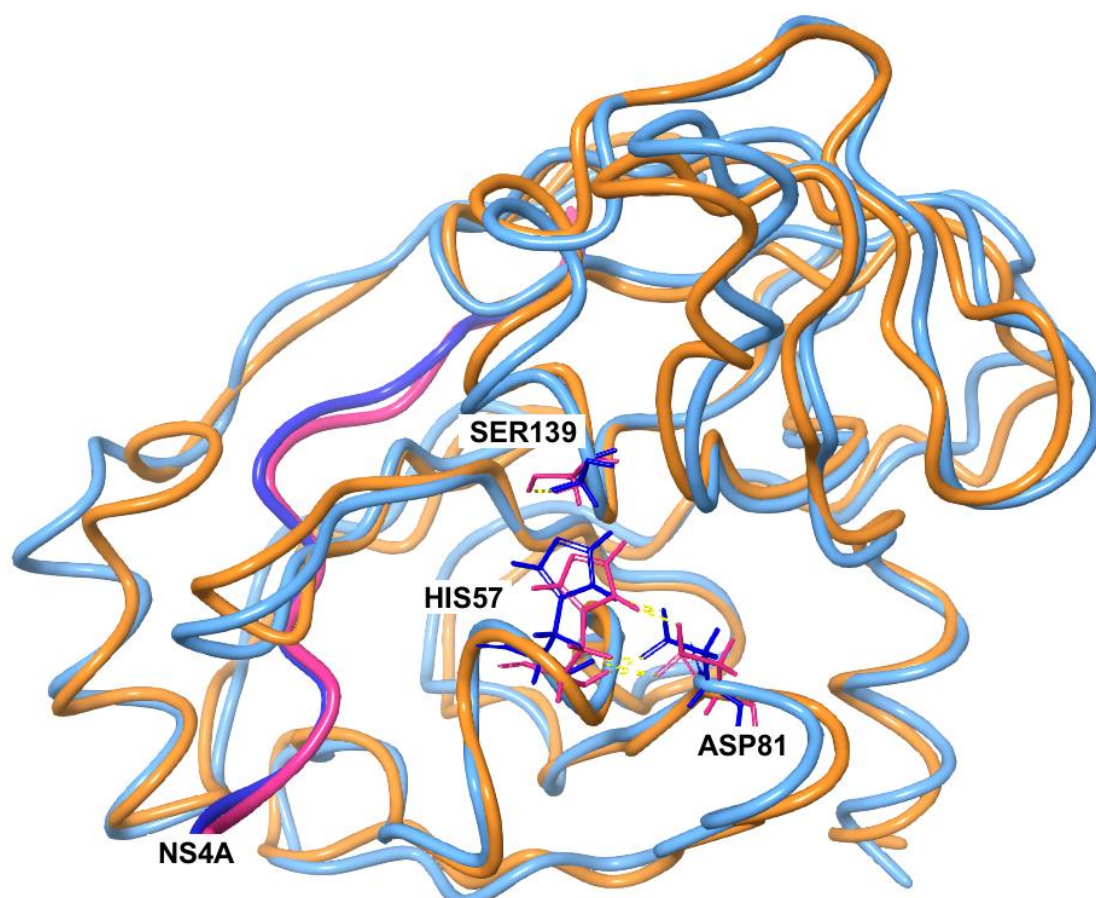


Figure S4-A: The crystal structure of Ns3 complexed with NS4A21'-32' was downloaded and prepared according to Protein Preparation protocol described in the Experimental Section 4.3.2. Note hydrogen bonding reserved in the catalytic triad His-57, Asp-81 and Ser-139.

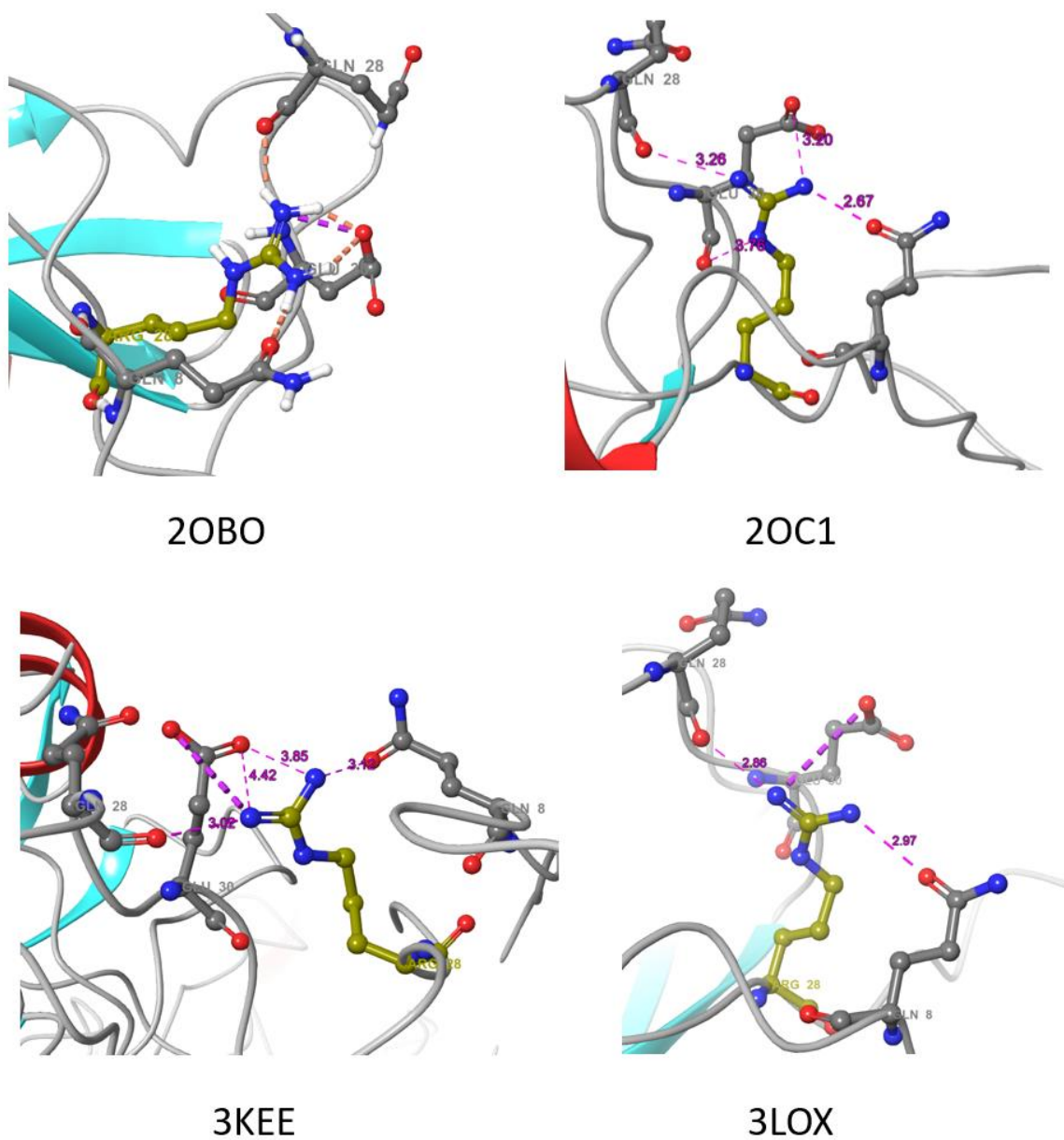
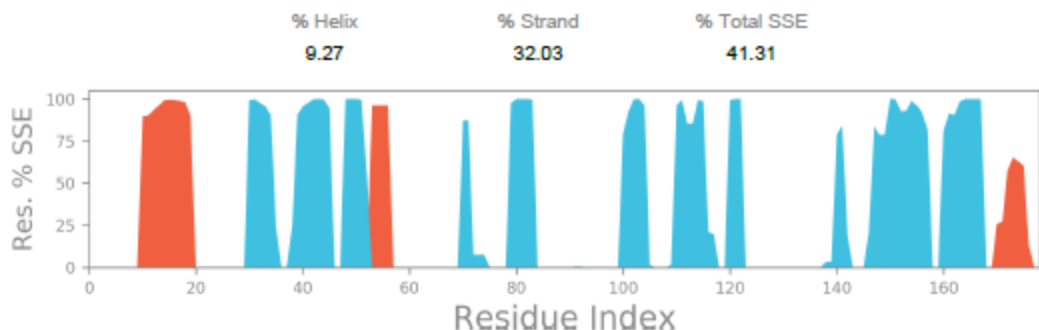


Figure S4-B. Interactions of Arg-28` terminal guanidine at four different reported NS3/4A crystal structures (PDB code are printed under each corresponding image). Note that it commonly makes ionic interactions with Glu-30 and hydrogen bonding with Gln-8 and Gln-28 but in different conformations.

SCHRODINGER

Protein Secondary Structure



Protein secondary structure elements (SSE) like **alpha-helices** and **beta-strands** are monitored throughout the simulation. The plot above reports SSE distribution by residue index throughout the protein structure. The plot below summarizes the SSE composition for each trajectory frame over the course of the simulation, and the plot at the bottom monitors each residue and its SSE assignment over time.

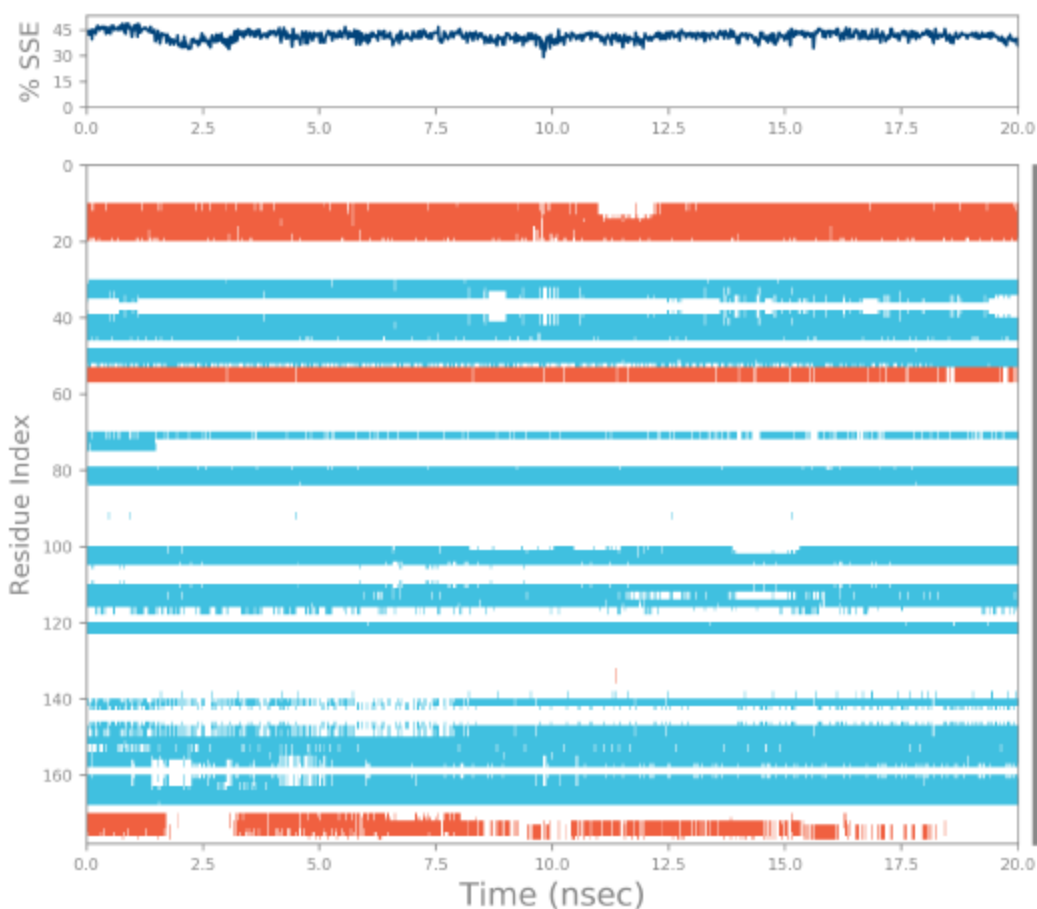


Figure S4-C. Report generated by Desmond on the secondary structure of the NS3 apoprotein during the MD simulation time. The preservation of secondary structure relates the quality of the model because a loss of secondary structure during conformational excitation would mean low quality of the binding model.