

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

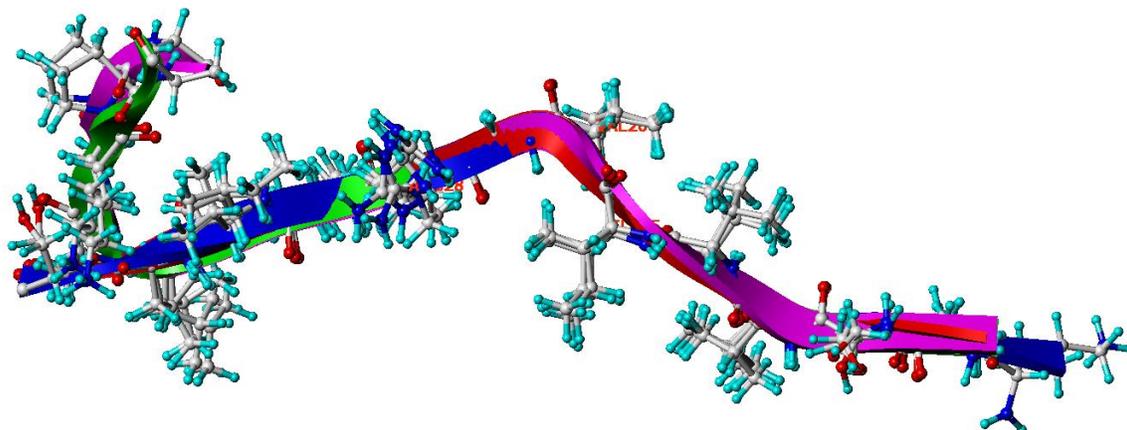


Figure S1-A: Conserved conformation of NS4A peptide in bound form with NS3 protease domain. Examples were downloaded from PDB website (rcsb.org) and illustrated as follows: 1A1R (Green), 2OC1 (magenta), 3OYP (Red) and 4U01 (Blue)

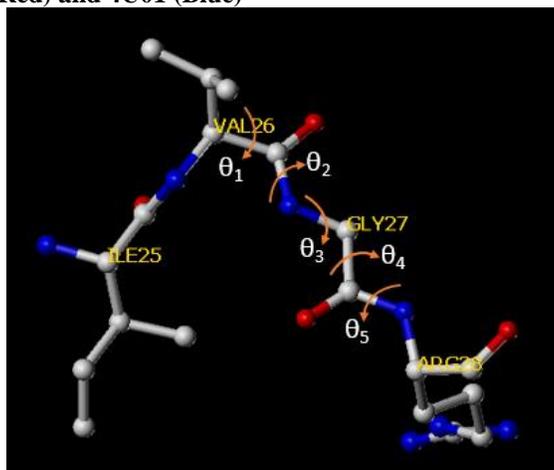


Figure S1-B. Dihedral angles θ_n of the bound NS4A's planar region (PDB Code: 1NS3). Numerical values of θ_1 to θ_5 are listed in Table S1-A.

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).

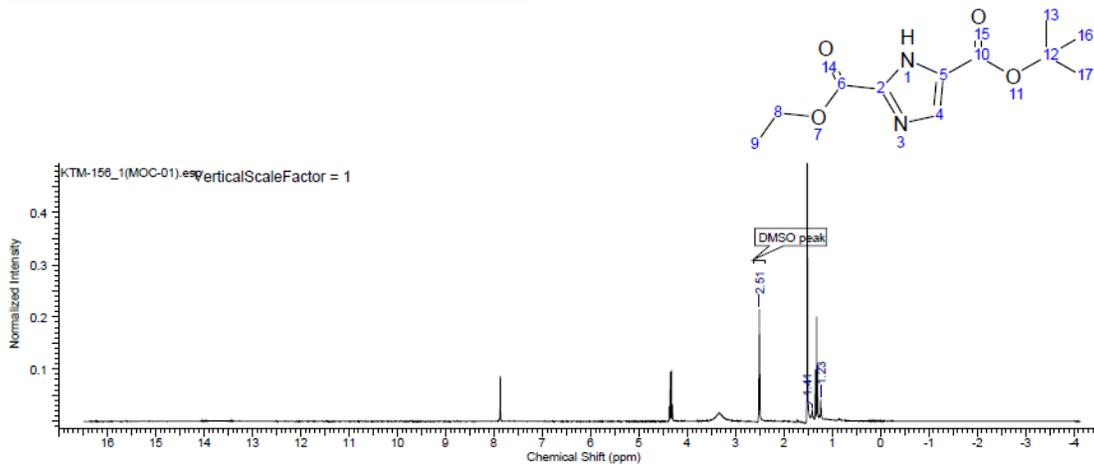
S2. Spectra of Intermediates and Final Compounds

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

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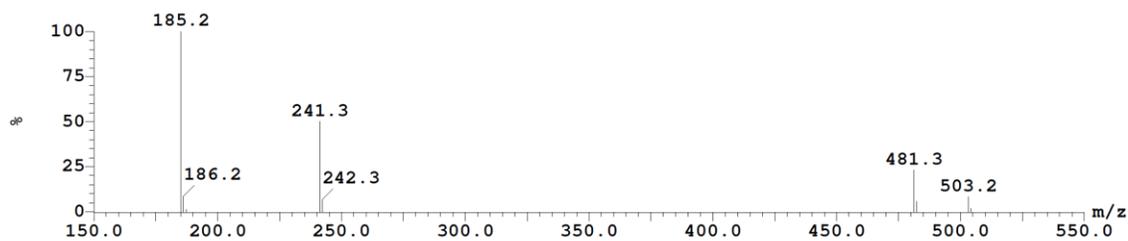
3/2/2017 1:46:23 PM

Formula C, H, N, O	FW	240.2557			
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Date Stamp	10 Sep 2015 20:25:04				
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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	DMSO-d6	Spectrum Offset (Hz)	1853.6917
Sweep Width (Hz)	6187.74	Temperature (degree C)	26.160	Spectrum Type	STANDARD



Peak ID Time Mass Found
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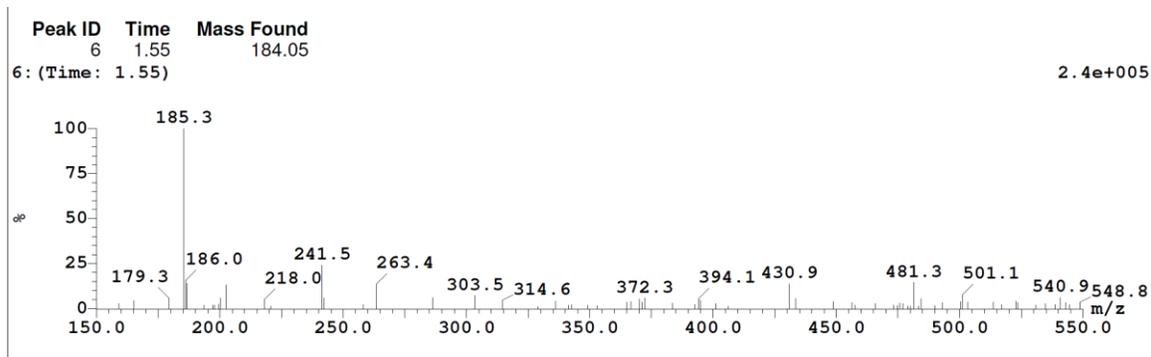
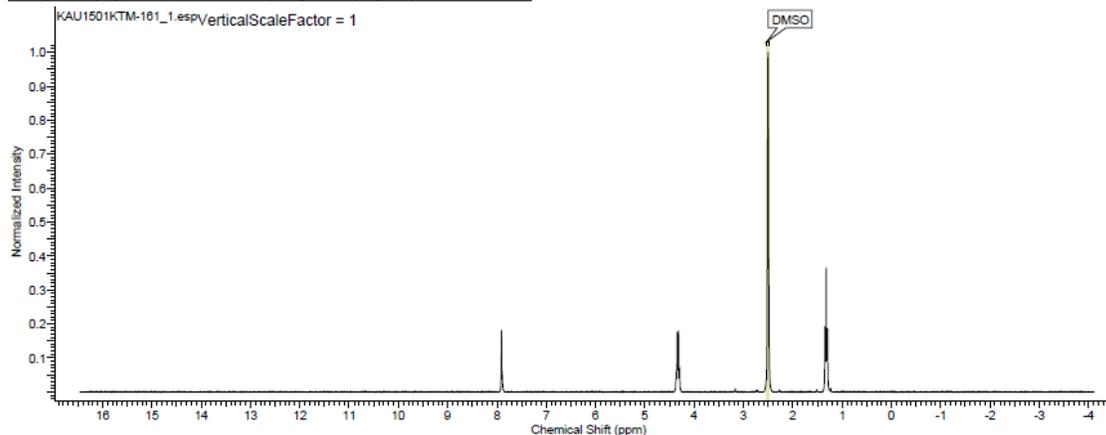
1.7e+007



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3/2/2017 1:50:15 PM

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Owner	quest	Points Count	32768	Pulse Sequence	zg30
SW(cyclical) (Hz)	8172.84	Solvent	DMSO-d6	Receiver Gain	645.10
Sweep Width (Hz)	8172.85	Temperature (degree C)	26.400	Spectrum Offset (Hz)	1853.4263
				Spectrum Type	STANDARD



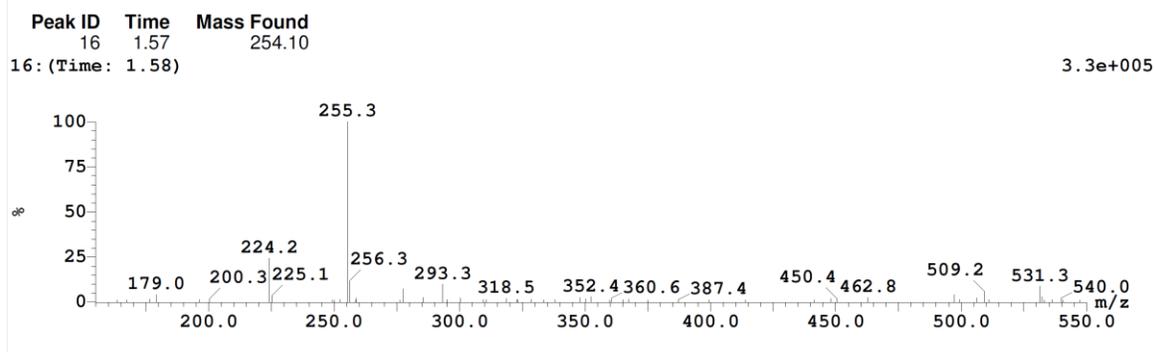
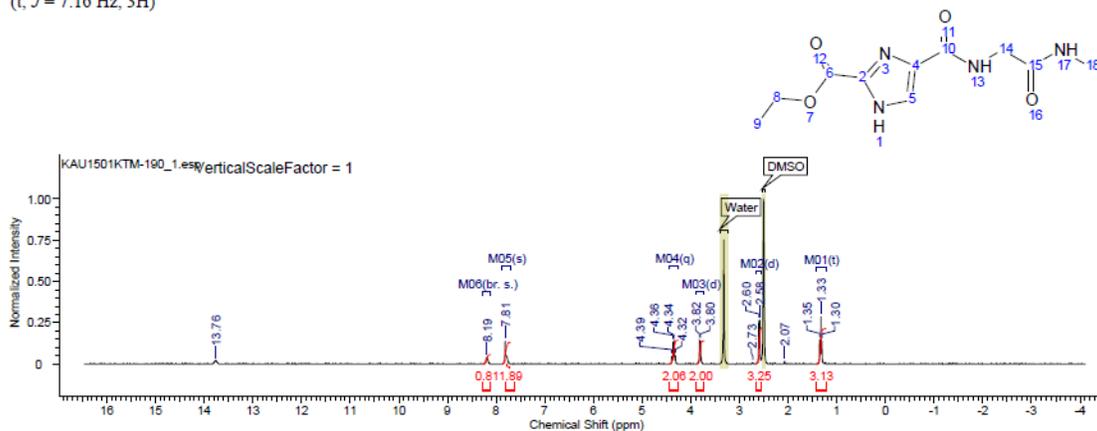
Ethyl 4-((2-(methylamino)-2-oxoethyl)carbamoyl)-1H-imidazole-2-carboxylate (4a)

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Date Stamp	13 Oct 2015 20:31:28										
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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	512.00
SW (cyclical) (Hz)	6172.84					Solvent	DMSO-d6	Spectrum Offset (Hz)	1853.4283	Spectrum Type	STANDARD
Sweep Width (Hz)	6172.66					Temperature (degree C)	26.900				

^1H NMR (300 MHz, DMSO- d_6) 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)

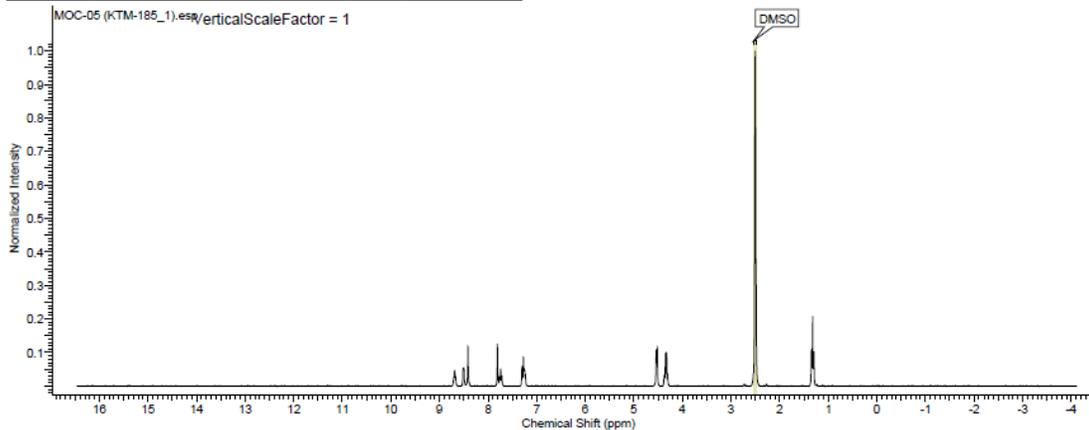


Ethyl 5-((pyridin-2-ylmethyl)carbamoyl)-1H-imidazole-2-carboxylate (4c)

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3/2/2017 2:00:53 PM

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Nucleus	1H	Number of Transients	16	Origin	spect
Owner	quest	Points Count	32788	Pulse Sequence	zg30
SW(cyclical) (Hz)	6172.84	Solvent	DMSO-d6	Spectrum Offset (Hz)	1853.4283
Sweep Width (Hz)	6172.85	Temperature (degree C)	25.500	Receiver Gain	456.10
				Spectrum Type	STANDARD

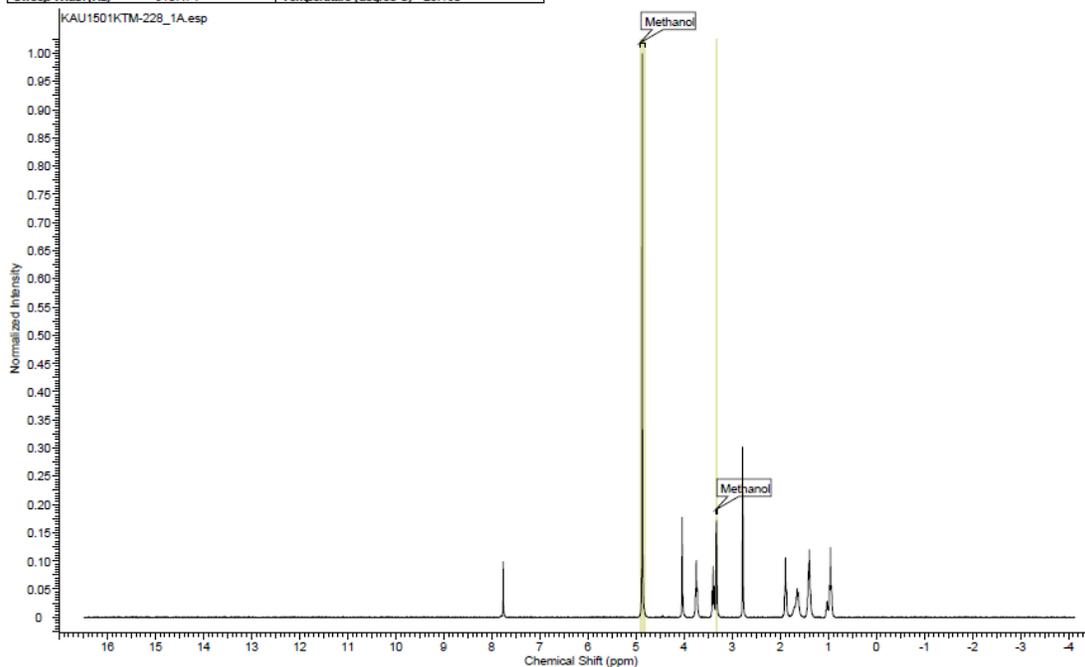


MOC-11

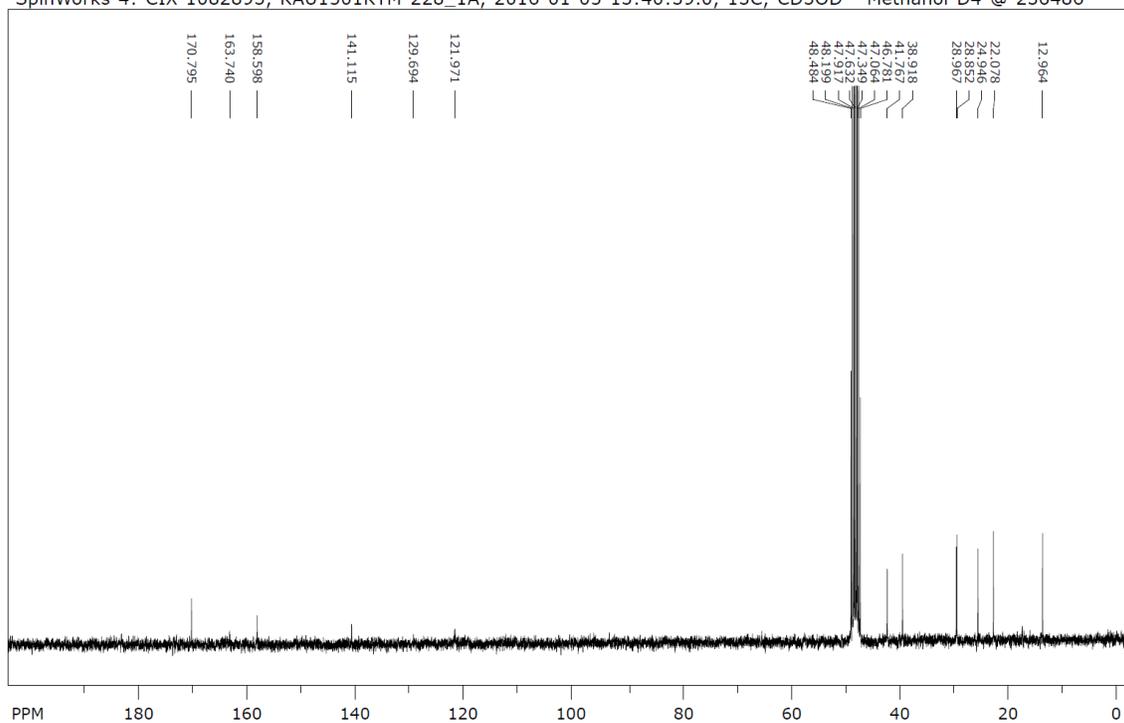
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Date	18 Nov 2015 23:17:36				
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Nucleus	1H	Number of Transients	8	Origin	spect
Owner	Administrator	Points Count	18384	Pulse Sequence	zg
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Sweep Width (Hz)	6187.74	Temperature (degree C)	25.160	Receiver Gain	645.10
				Spectrum Type	STANDARD



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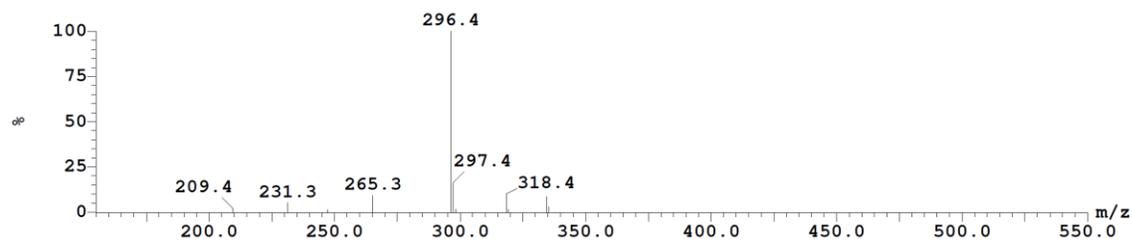
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number of scans: 640

freq. of 0 ppm: 75.467746 MHz
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8.3e+005



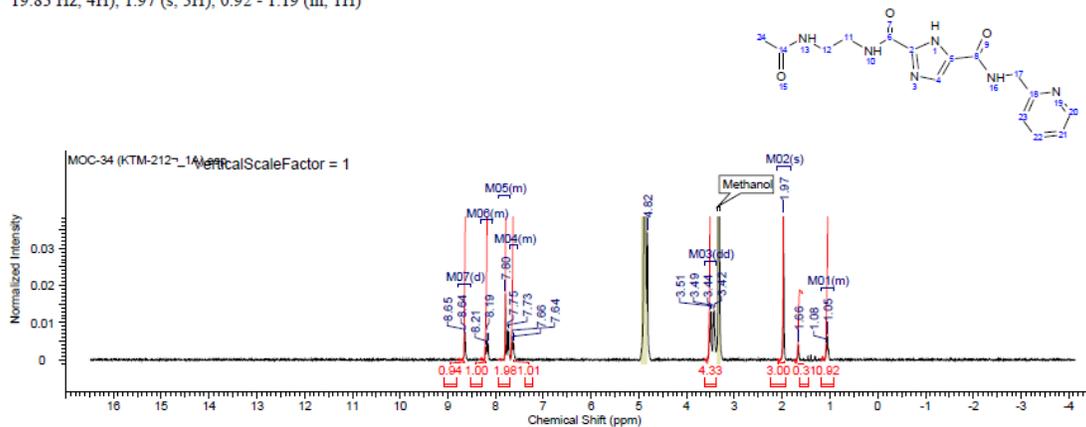
MOC-34

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

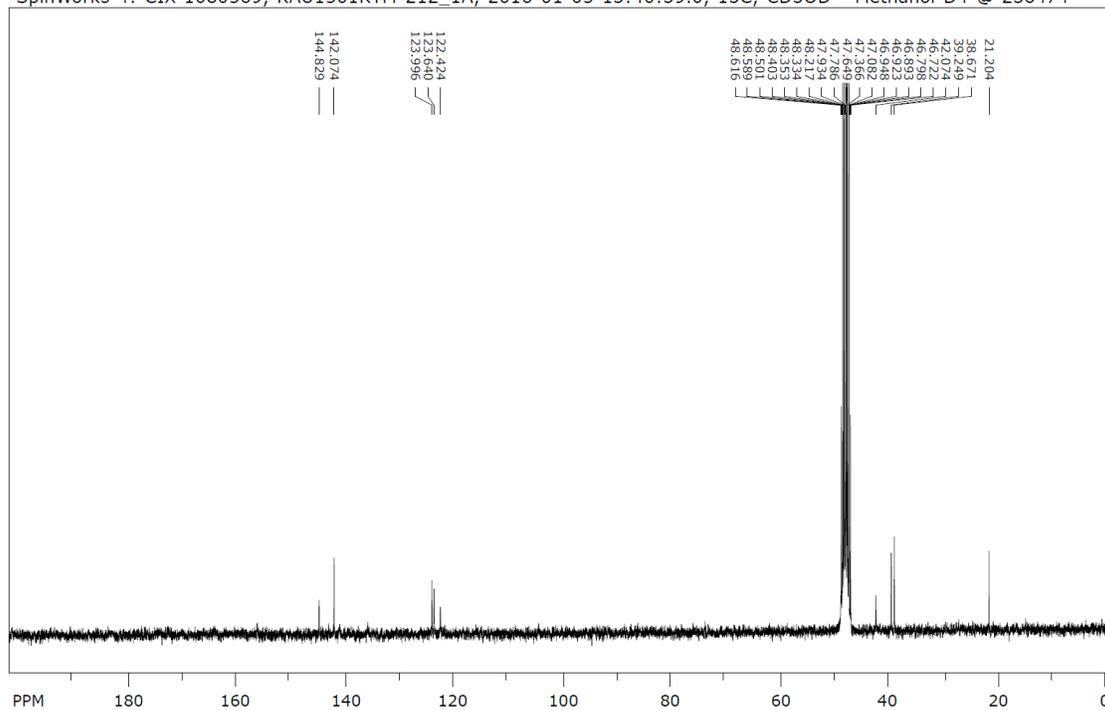
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Date Stamp	22 Oct 2015 11:42:24					
Origin	spect					
Pulse Sequence	zg					
Spectrum Offset (Hz)	1853.8917					
Frequency (MHz)	300.13					
Original Points Count	16384					
Receiver Gain	812.70					
Spectrum Type	STANDARD					

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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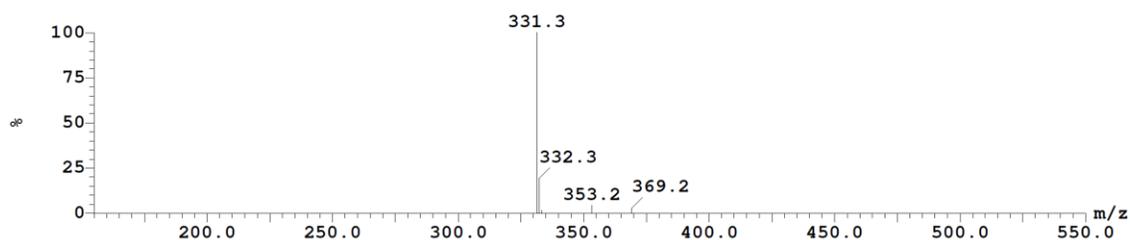


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6.3e+005

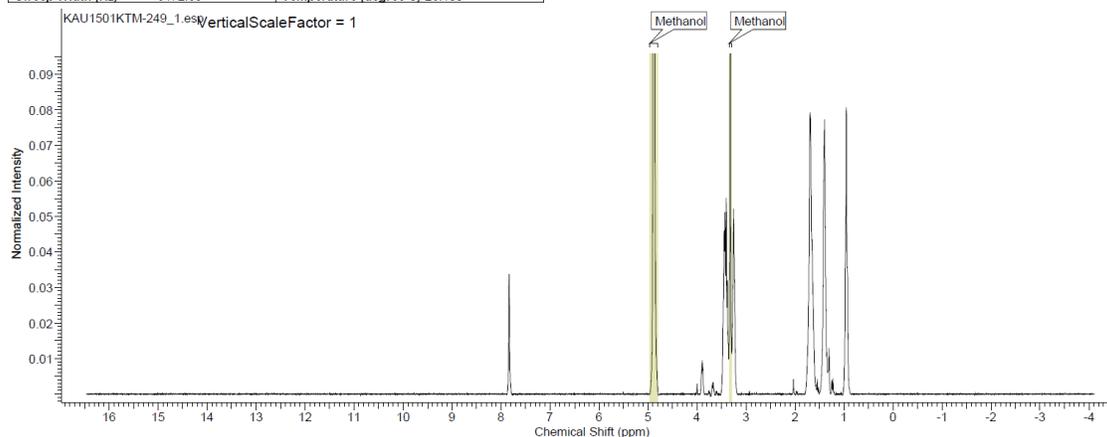


MOC-23

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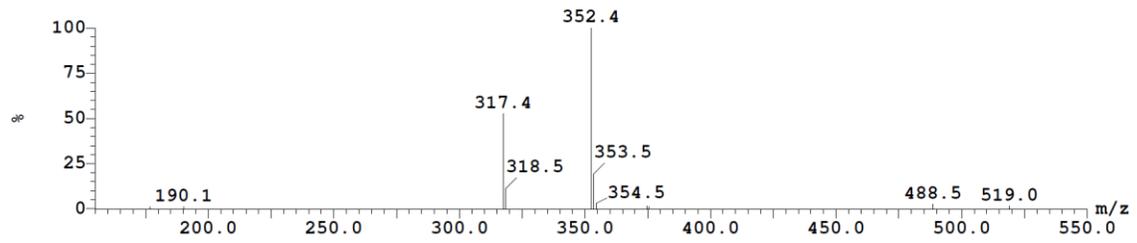
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Owner	guest	Points Count	32768	Pulse Sequence	zg30
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Sweep Width (Hz)	6172.65	Temperature (degree C)	26.100	Receiver Gain	912.30
				Spectrum Type	STANDARD



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5.0e+005

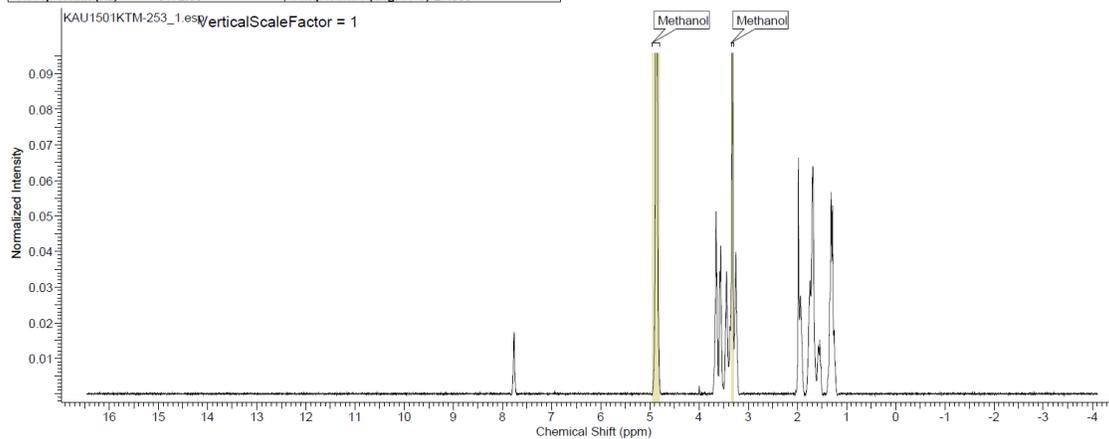


MOC-26

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

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				Spectrum Type	STANDARD

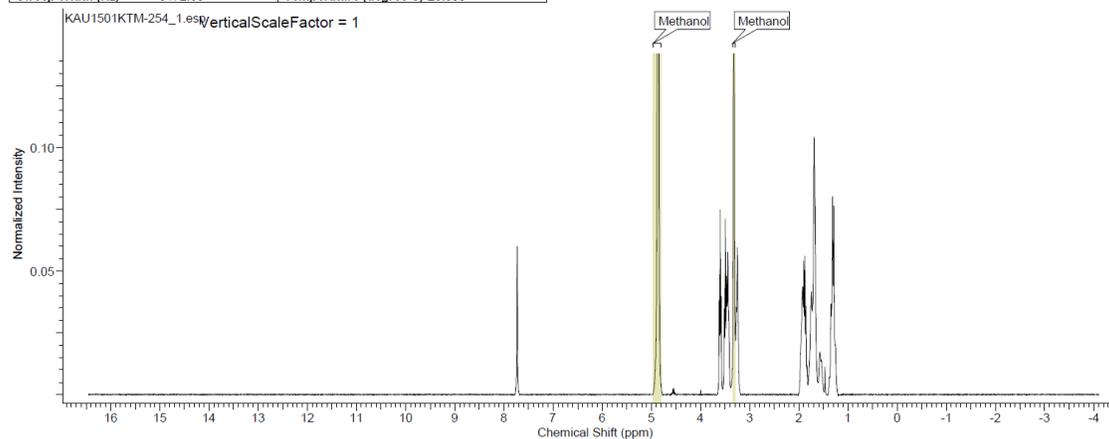


MOC-27

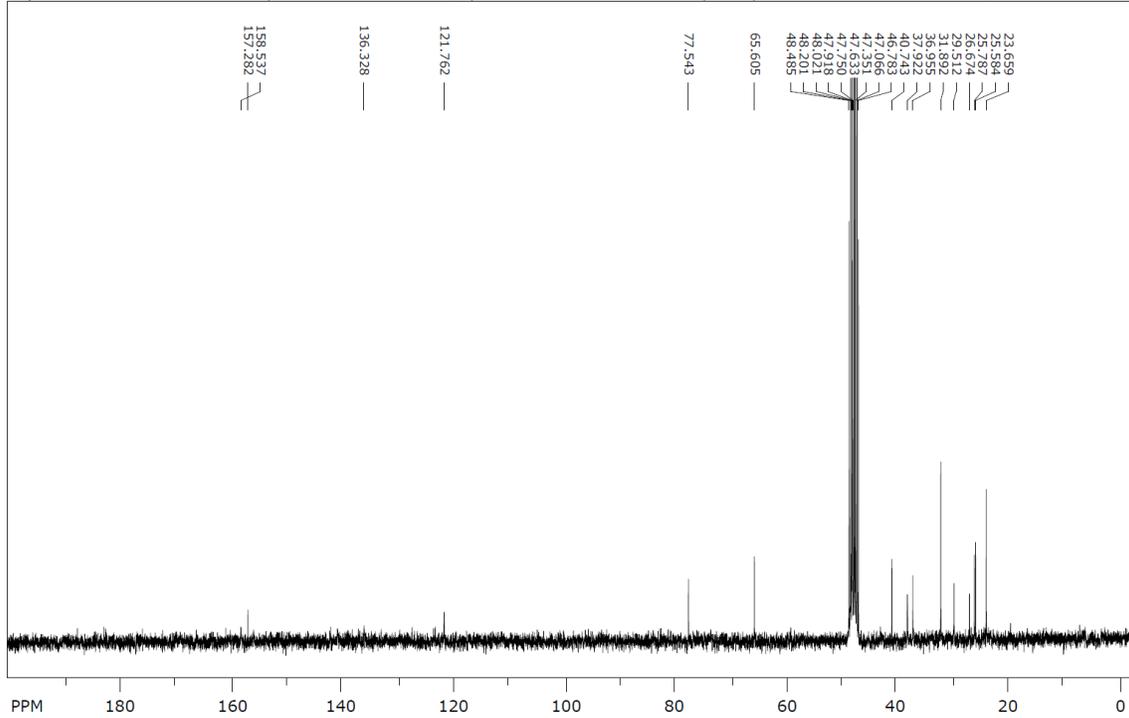
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5/9/2017 12:48:13 PM

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Owner	quest	Points Count	32768	Pulse Sequence zg30	
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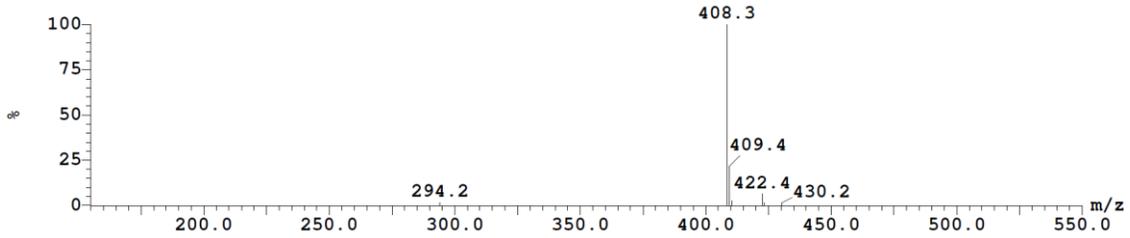


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7.8e+005

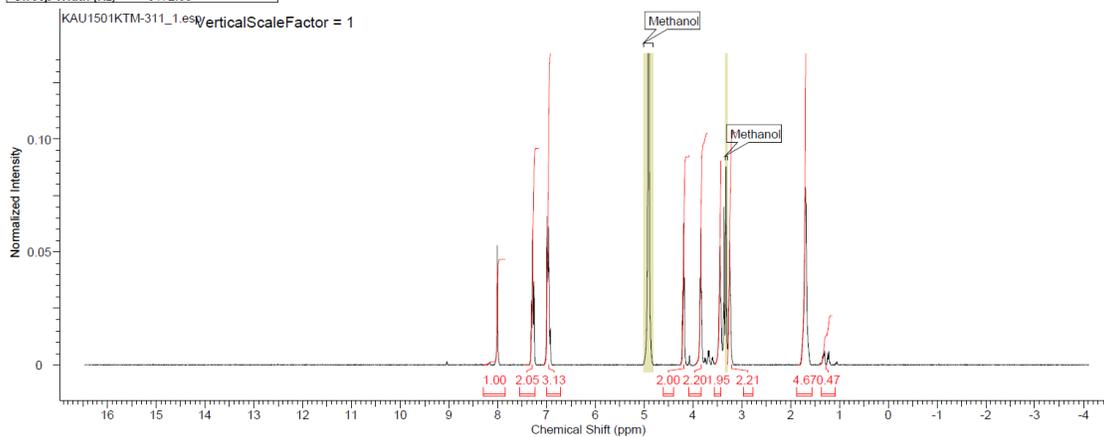


MOC-28

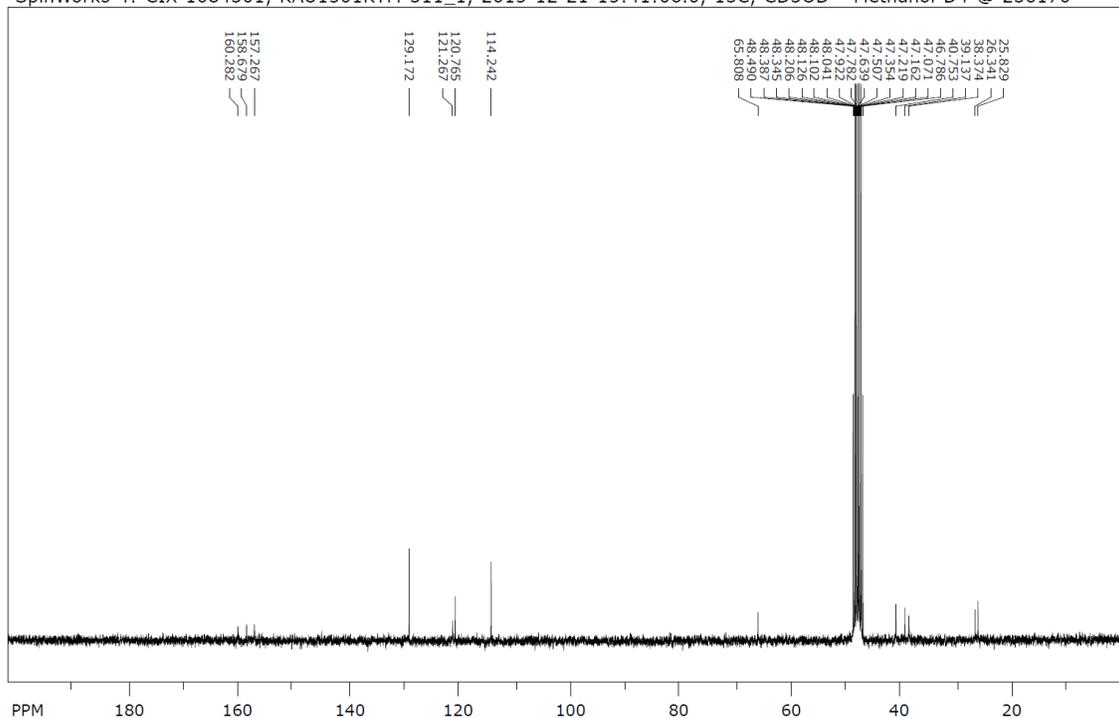
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Nucleus	¹ H	Number of Transients	16	Origin	spect	Original Points Count	32768
Owner	guest	Points Count	32768	Pulse Sequence	zg30	Receiver Gain	512.00
SW(cyclical) (Hz)	6172.84	Solvent	METHANOL-d4	Spectrum Offset (Hz)	1853.4263	Spectrum Type	STANDARD
Sweep Width (Hz)	6172.65						



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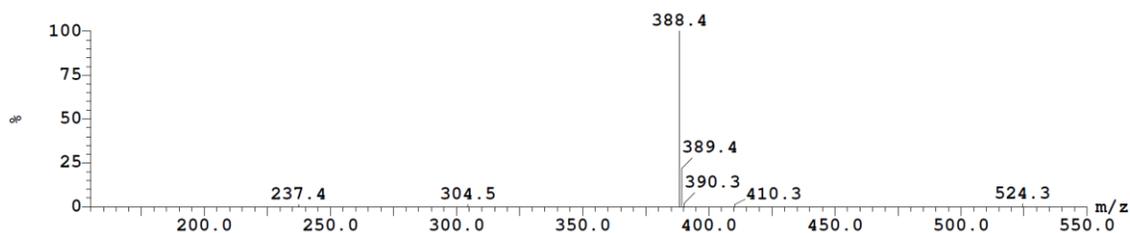


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8.6e+005

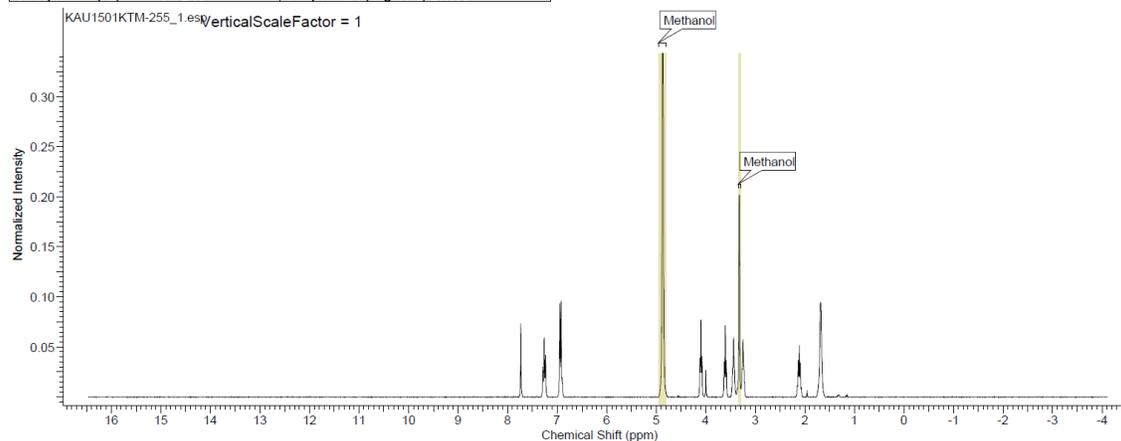


MOC-29

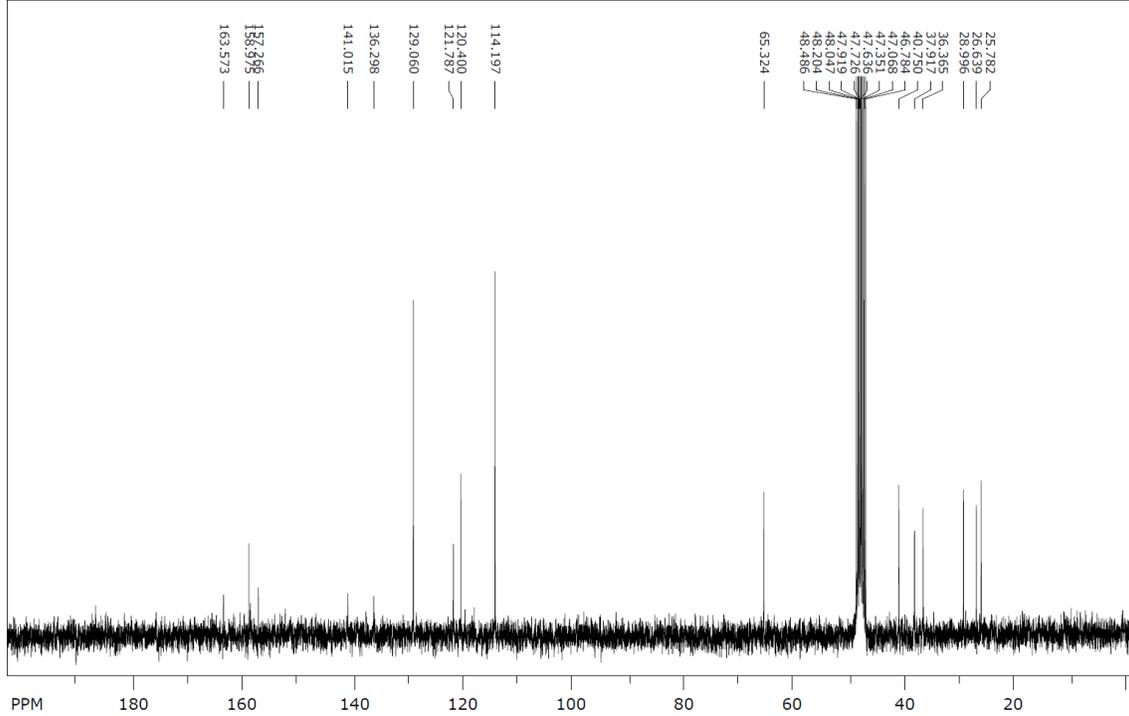
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Nucleus	1H	Number of Transients	16	Origin	spect	Original Points Count	32768
Owner	quest	Points Count	32768	Pulse Sequence	zg30	Receiver Gain	812.70
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Sweep Width (Hz)	6172.65	Temperature (degree C)	26.600				



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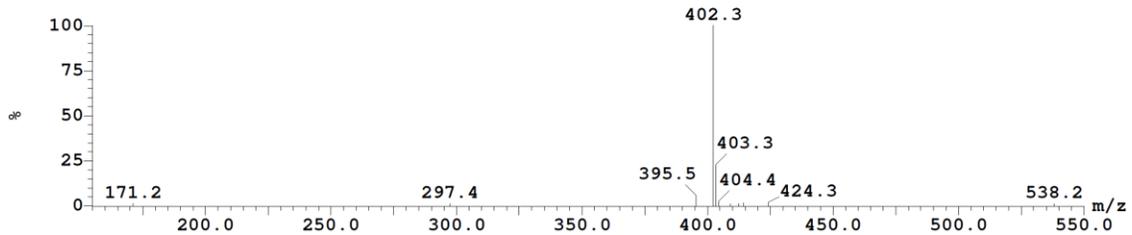


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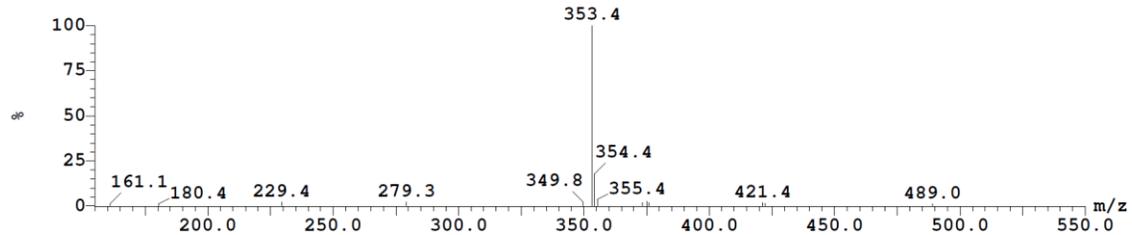
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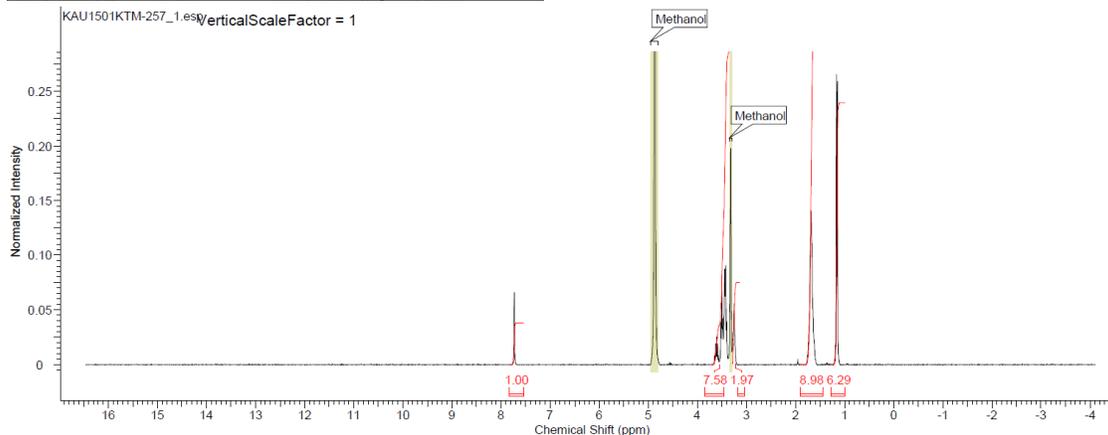


MOC-30

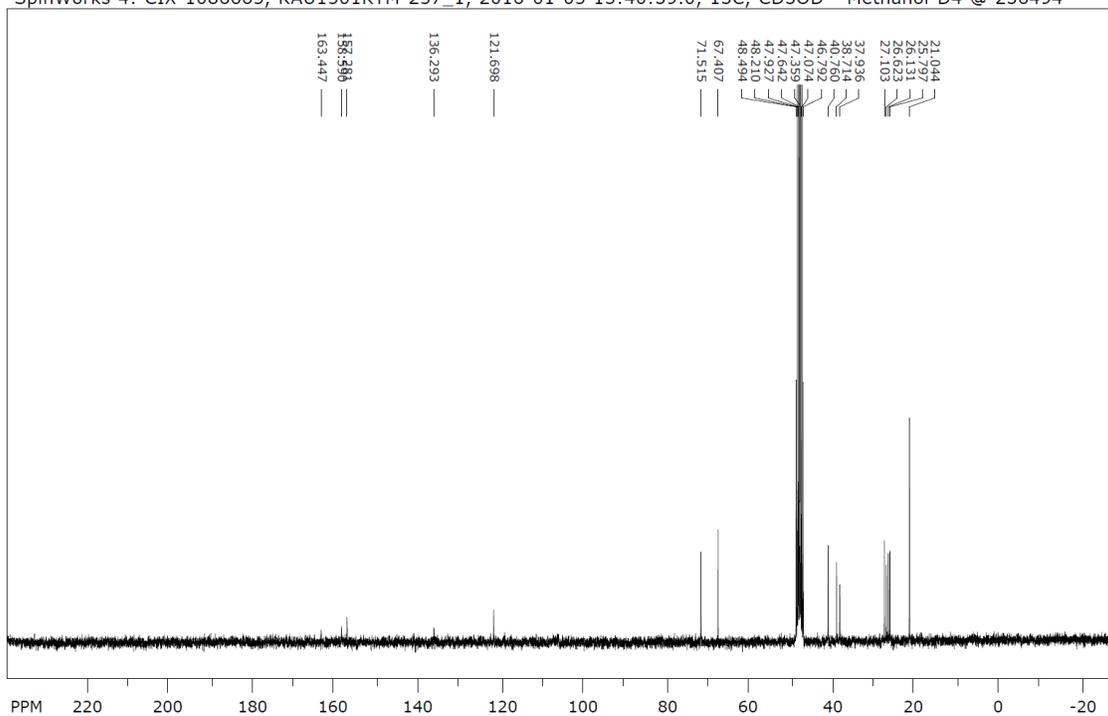
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/

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
Owner	quest	Points Count	32768	Original Points Count
SW(cyclical) (Hz)	6172.84	Solvent	METHANOL-d4	Receiver Gain
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



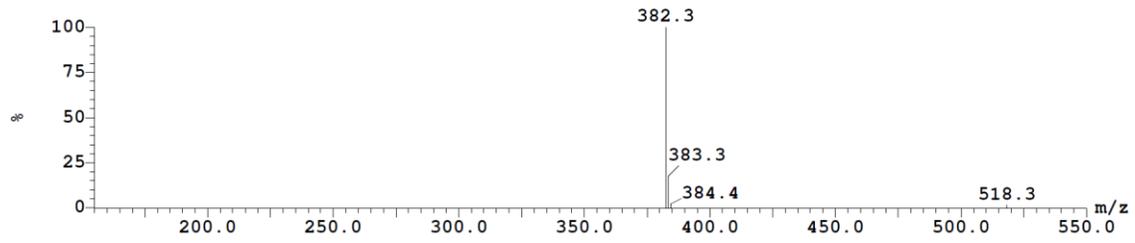
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 width: 20325.20 Hz = 269.2948 ppm = 0.310138 Hz/pt
 number of scans: 640

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
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15: (Time: 2.06)

1.4e+006

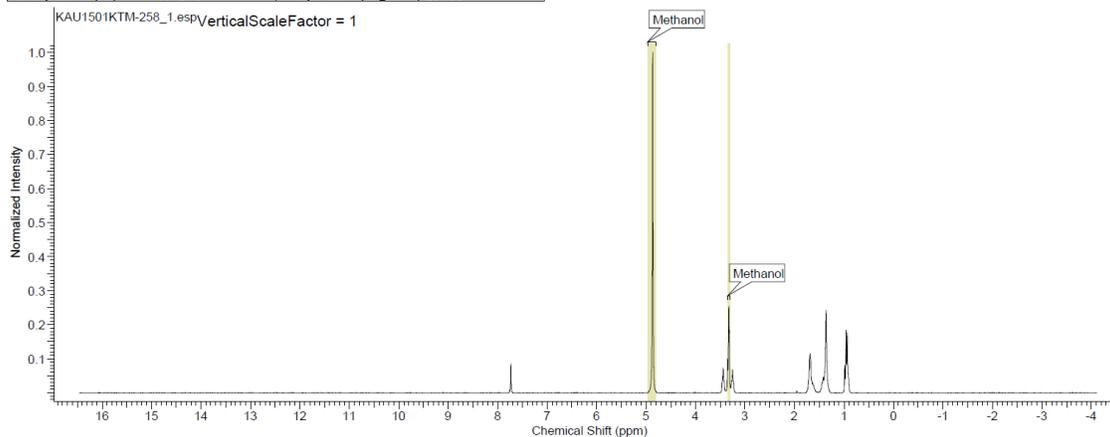


MOC-31

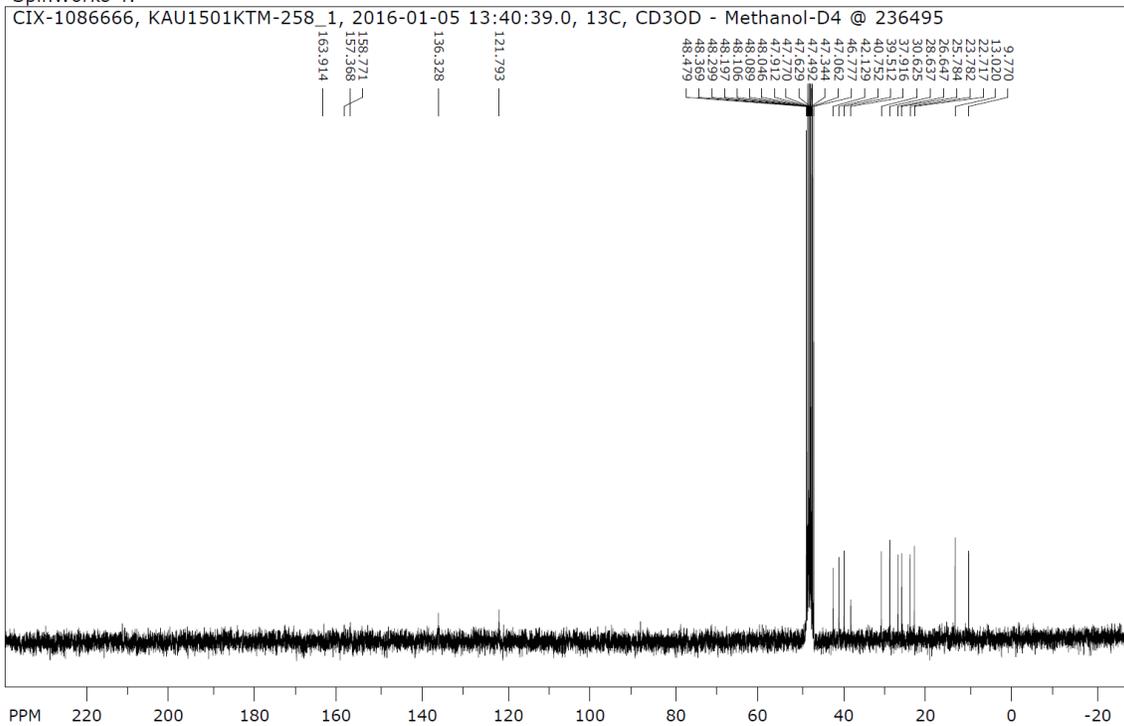
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/

5/9/2017 8:44:35 AM

Acquisition Time (sec)	5.3084	Comment	CIX-1086666, KAU1501KTM-258_1_1H, CD3OD - Methanol-D4 @ 231799	
Date	03 Dec 2015 21:43:44	Date Stamp	03 Dec 2015 21:43:44	
File Name	C:\Users\mitk0005\Google Drive\KAU\MEI-Araby\12-BIO3193-03 Imidazole Cpds\1H-NMR\KAU1501KTM-258_1.fid		Frequency (MHz)	300.13
Nucleus	1H	Number of Transients	16	Original Points Count
Owner	guest	Points Count	32768	Receiver Gain
SW(cyclical) (Hz)	6172.84	Solvent	METHANOL-d4	Spectrum Offset (Hz)
Sweep Width (Hz)	6172.65	Temperature (degree C)	26.700	Spectrum Type
				STANDARD



SpinWorks 4:



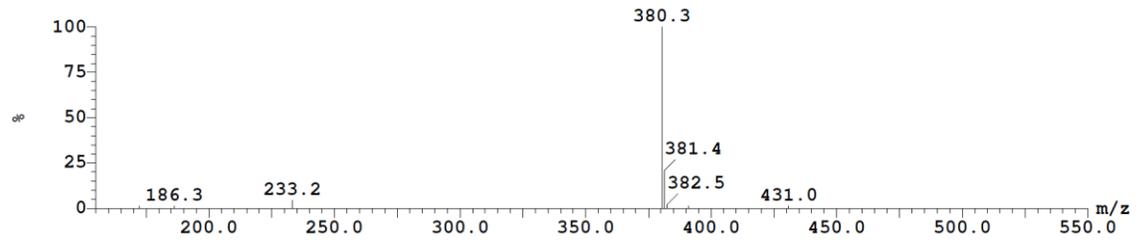
file: ...01KTM-258_1, 13C, 310 @ 236495.fid exp: <zpgg30>
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 number of scans: 640

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

Peak ID	Time	Mass Found
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2: (Time: 1.19)

4.0e+005

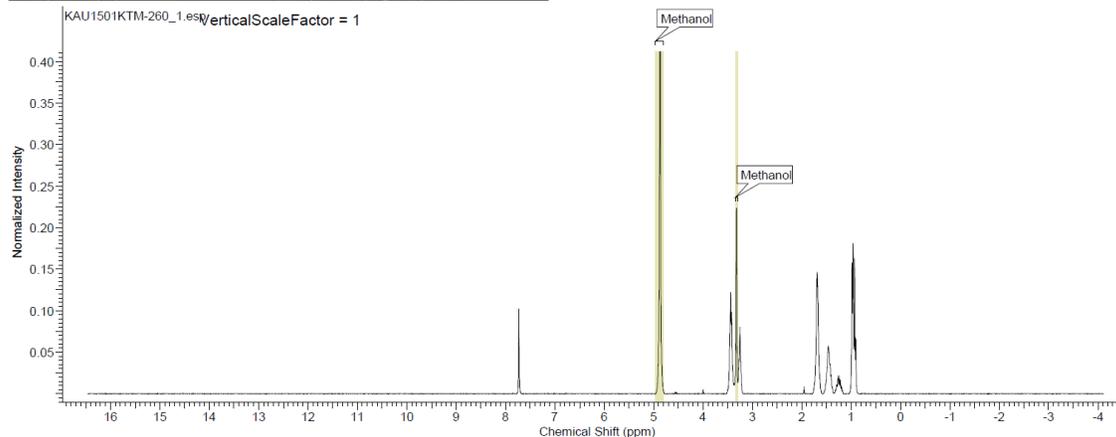


MOC-32

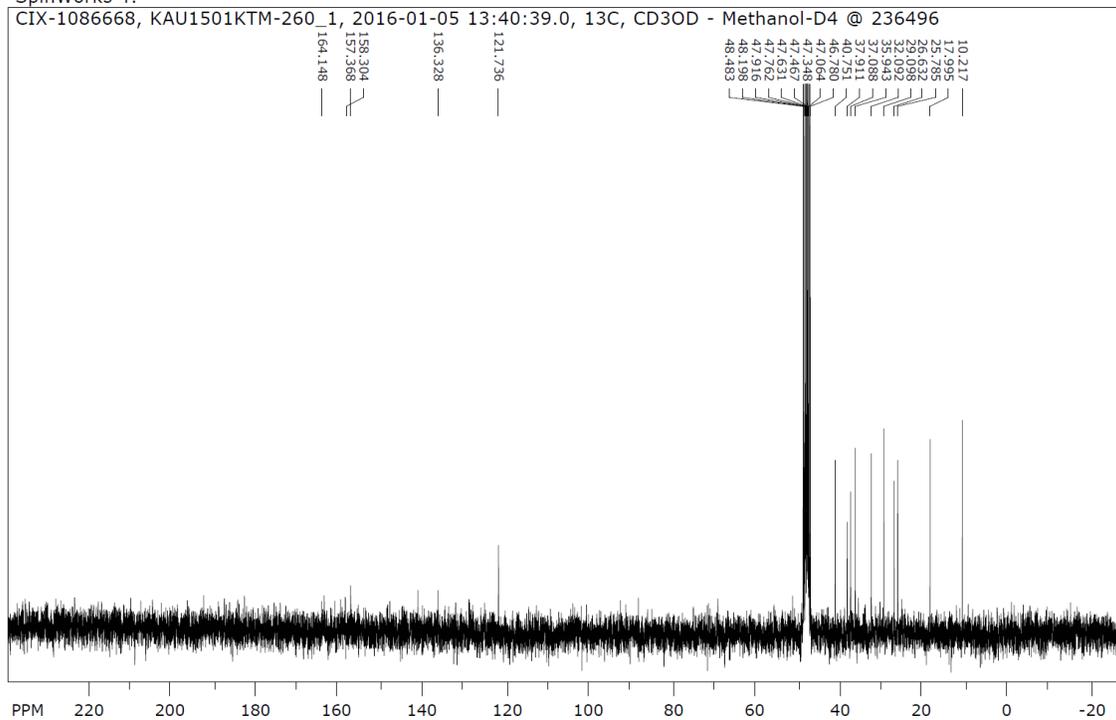
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/

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\imt0005\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	quest	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	Original Points Count	32768
				Receiver Gain	724.10
				Spectrum Type	STANDARD



SpinWorks 4:

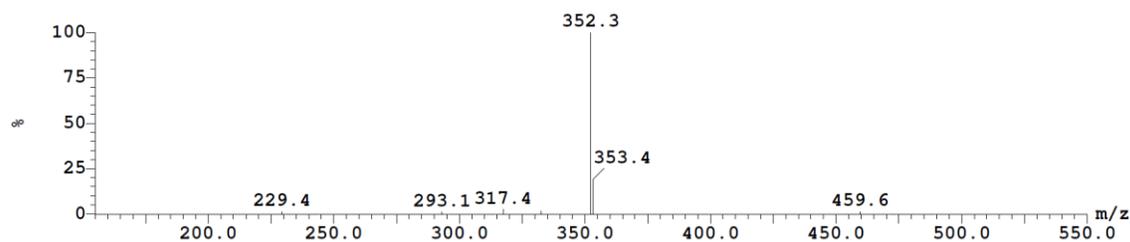


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 width: 20325.20 Hz = 269.2948 ppm = 0.310138 Hz/pt
 number of scans: 540

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
6 1.84 351.24
6: (Time: 1.86)

5.3e+005

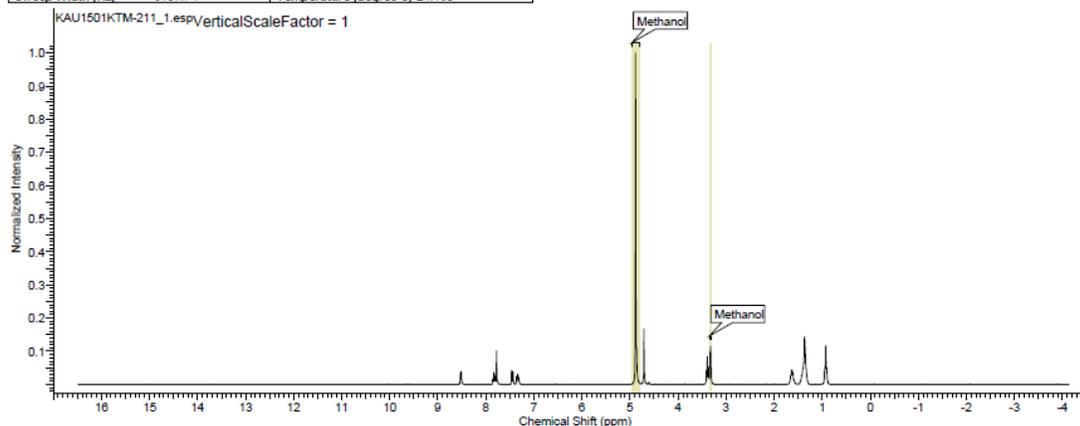


MOC-33

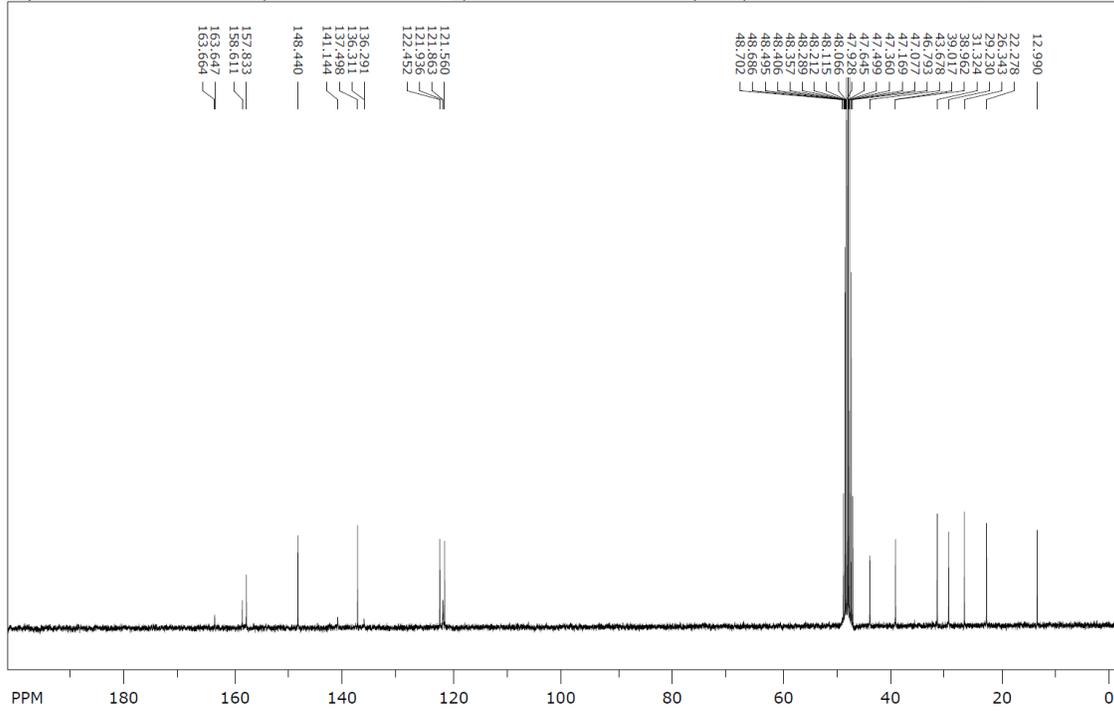
This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrproc/

5/8/2017 8:06:31 AM

Acquisition Time (sec)	2.6477	Comment	CIX-1080668, 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	Original Points Count	16384
Pulse Sequence	zgpg30	Receiver Gain	512.00	Spectrum Type	STANDARD
SW (cyclical) (Hz)	6188.12	Solvent	METHANOL-d4	Spectrum Offset (Hz)	1853.6917
Sweep Width (Hz)	6187.74	Temperature (degree C)	24.160		



SpinWorks 4: CIX-1080568, KAU1501KTM-211_1, 2016-01-05 13:40:39.0, 13C, CD3OD - Methanol-D4 @ 236472

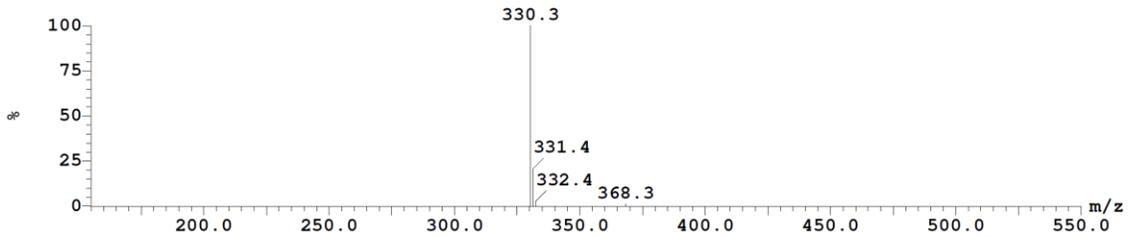


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 number of scans: 640

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 processed size: 32768 complex points
 LB: 1.000 GF: 0.0000
 Hz/cm: 613.961 ppm/cm: 8.13456

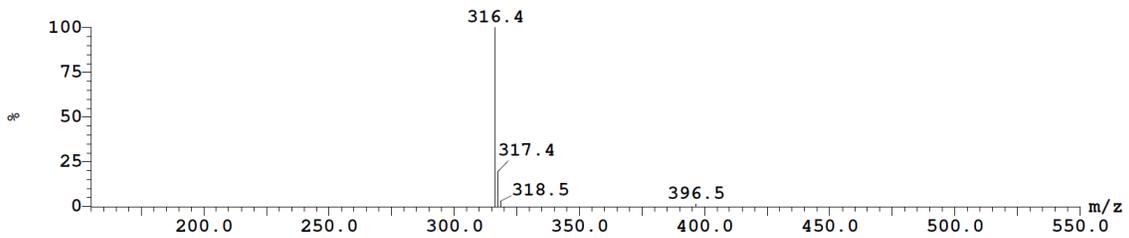
Peak ID Time Mass Found
 14 2.79 329.19
 14: (Time: 2.78)

3.6e+006



Peak ID Time Mass Found
 7 2.50 316.21
 7: (Time: 2.50)

1: MS AP+
 8.5e+005



S3. Competition Assays of MOC Compounds with NS4A

Determination of NS4A₂₁₋₃₄ binding affinity

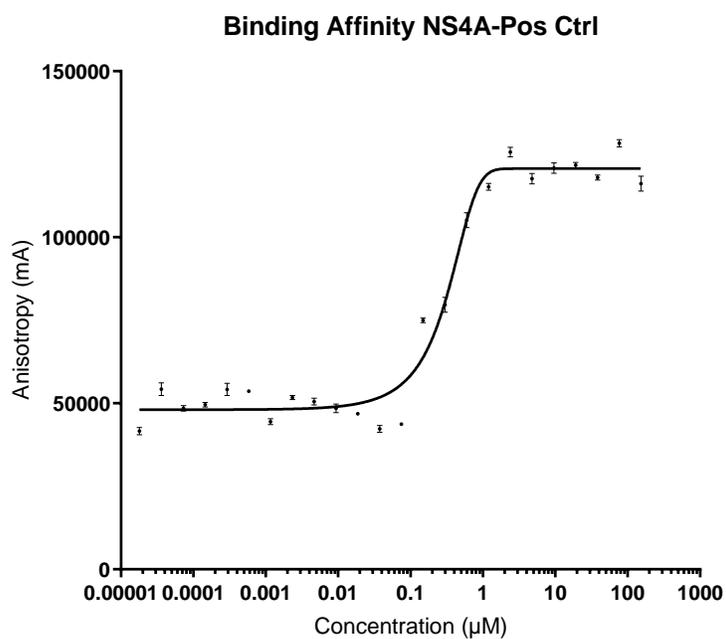


Figure S3. Fluorescence assay for binding of NS4A₂₁₋₃₄ to NS3 (Genotype 4).

S4. Molecular Dynamics

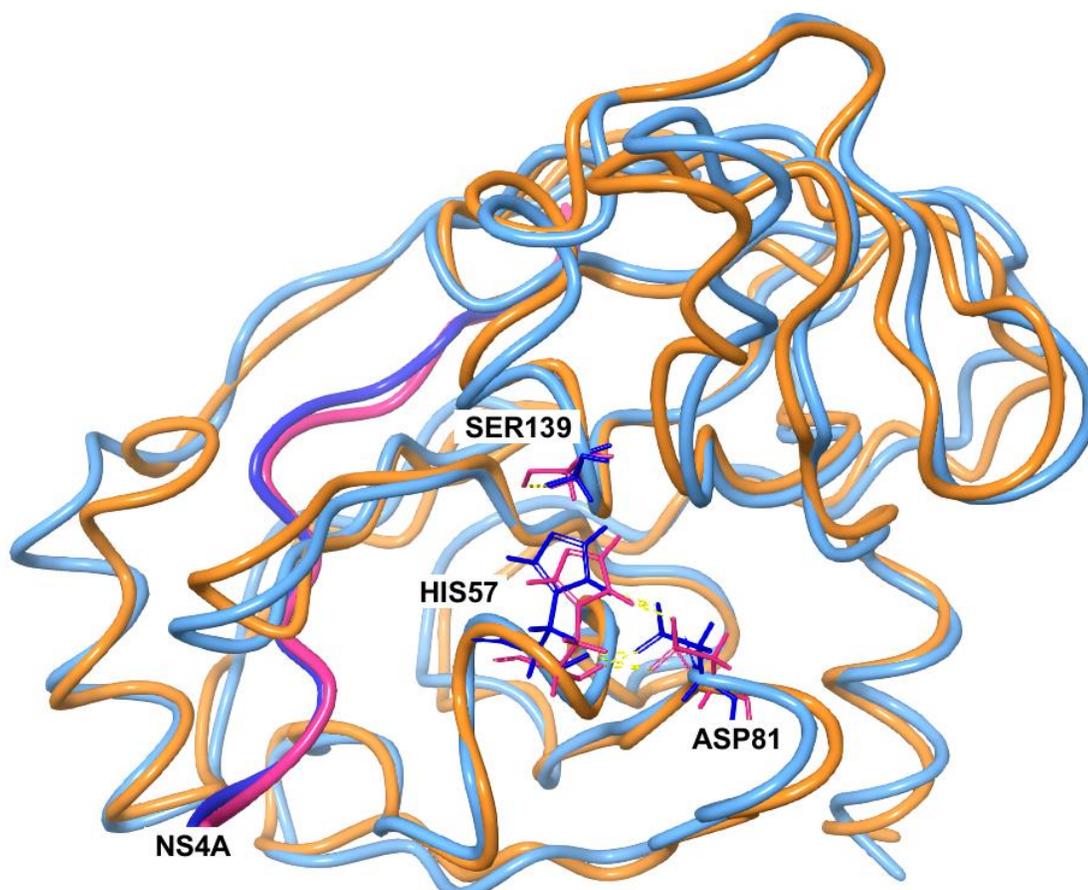


Figure S4-A: The crystal structure of Ns3 complexed with NS4A21'-32' was downloaded and prepared according to Protein Preparation protocol described in the Experimental Section 4.3.2. Note hydrogen bonding reserved in the catalytic triad His-57, Asp-81 and Ser-139.

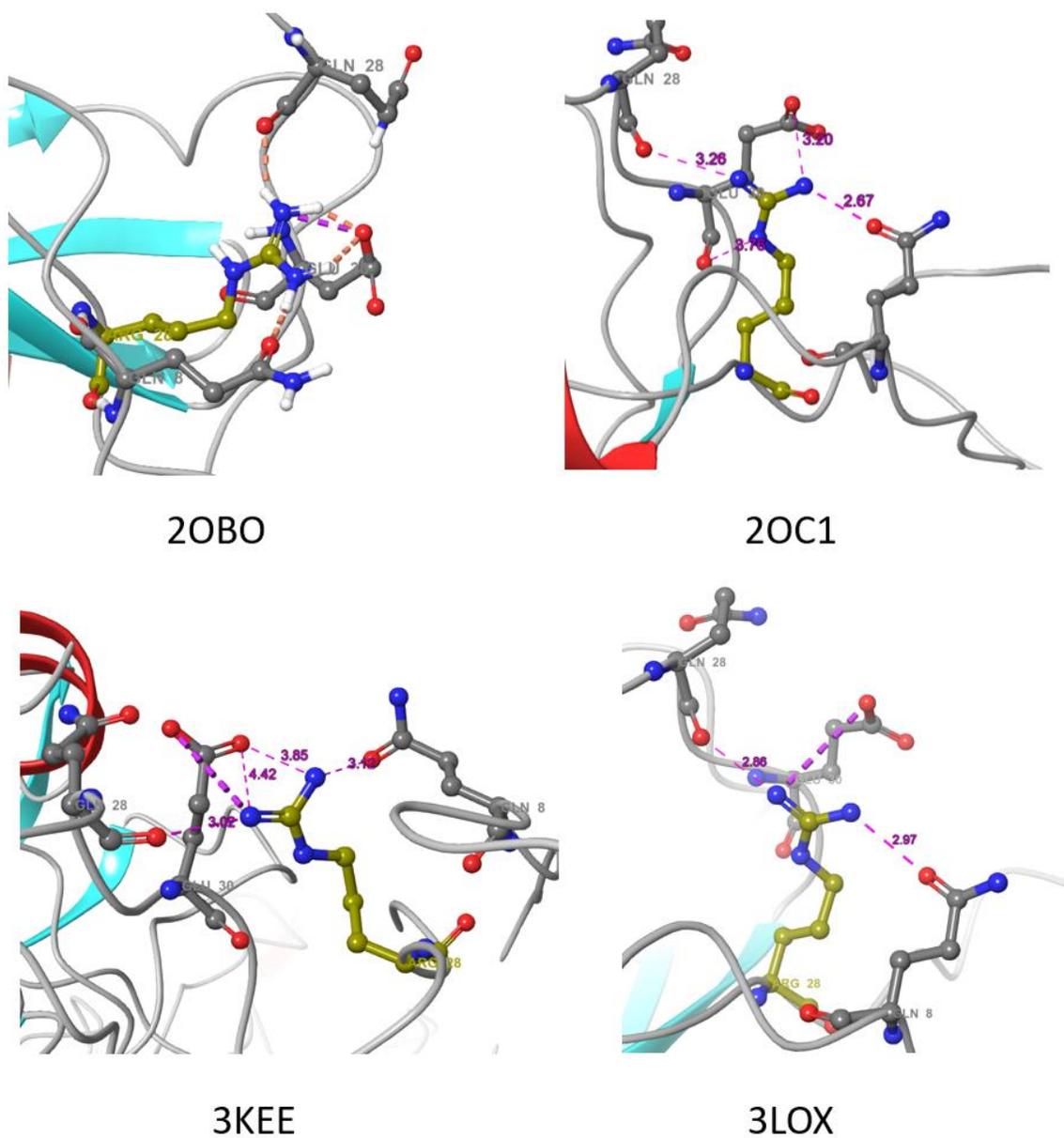
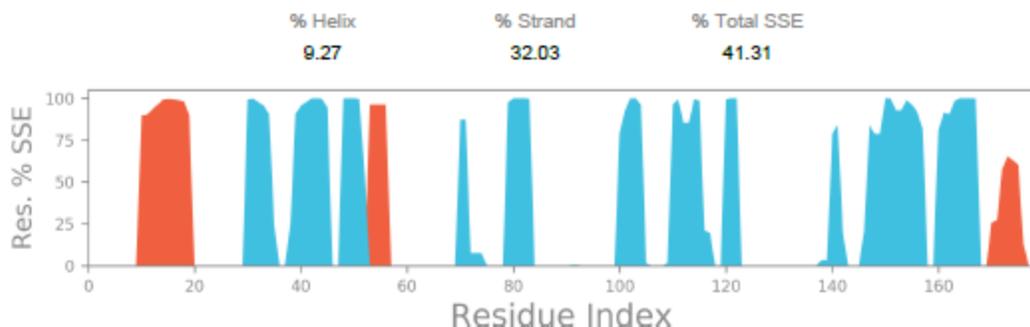


Figure S4-B. Interactions of Arg-28` terminal guanidine at four different reported NS3/4A crystal structures (PDB code are printed under each corresponding image). Note that it commonly makes ionic interactions with Glu-30 and hydrogen bonding with Gln-8 and Gln-28 but in different conformations.

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.

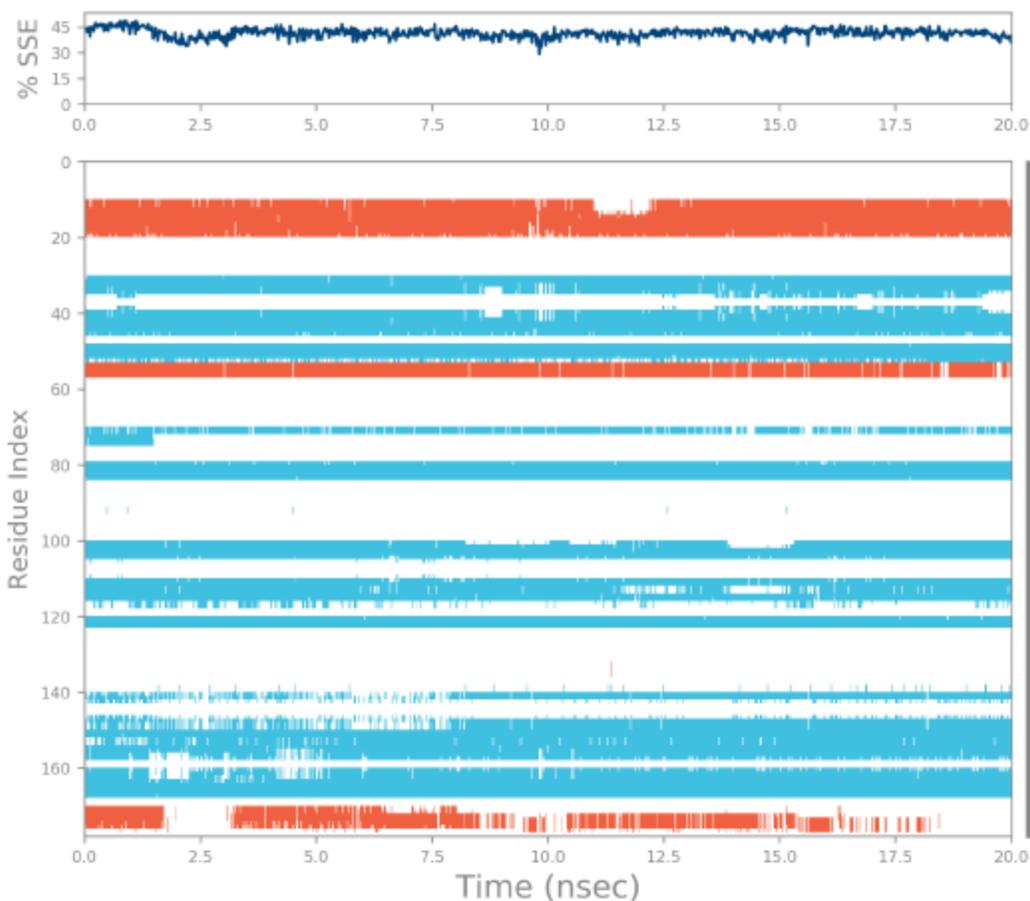


Figure S4-C. Report generated by Desmond on the secondary structure of the NS3 apoprotein during the MD simulation time. The preservation of secondary structure relates the quality of the model because a loss of secondary structure during conformational excitation would mean low quality of the binding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