Electronic Supplementary Material (ESI) for RSC Advances.

Investigation of a sensing approach based on a rapid reduction of azide to selectively measure bioavailability of H_2S

Gunwoo Kim,[a] Eunju Jang,[a] Alexis M Page,[b] Ting Ding,[a] Kimberly A. Carlson,[b] and Haishi Cao[a*]

Department of Chemistry, University of Nebraska at Kearney, Kearney NE 68849, USA
Department of Biology, University of Nebraska at Kearney, Kearney NE 68849, USA

Contents:

Experimental

Apparatus

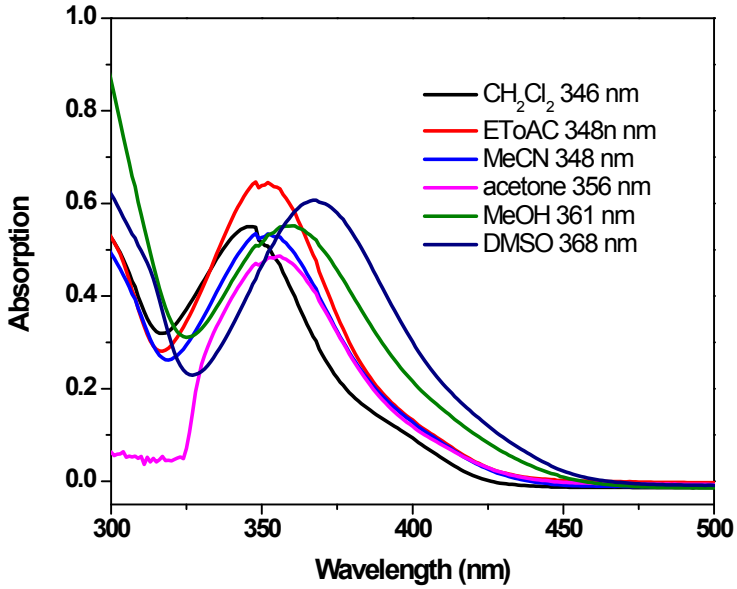
Absorbance spectra were collected by Cary Series Uv-vis Spectrophotometer (Agilent Technologies). Fluorescence measurements were all performed by using a FluoroMax-4 Spectrofluorometer (Horiba Jobin Yvon, USA). All of fluorescence spectra were recorded in a 1 cm quartz cuvette. The excitation and emission slits were set at 2 nm. ^{1}H and ^{13}C NMR spectra were recorded on (^{1}H 300MHz, ^{13}C 75MHz) Bruker 300 Ultra-Shield spectrometer at room temperature. A TE2000-S inverted fluorescent microscope (Nikon, Melville, NY) were used for cell imaging.

Reagents

All reagents used for synthesis and measurements were purchased from Sigma-Aldrich (MO, USA), Fisher Scientific (USA) and Acros Organics (USA) in analytical grade and used as received, unless otherwise stated.

Cell imaging

U937 culture. U937 cells (human monocyte cell line; American Type Culture Collection (ATCC), Manassas, VA) were maintained at 37°C with 5% CO_2 in T-25 flasks in RPMI 1640 (ATCC) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco Invitrogen Corporation, Grand Island, NY) and 1% penicillin-streptomycin (ATCC). For imaging, cells were placed in a 50-mL conical tube and centrifuged at 1,000xg for 5 min. The supernatant was removed and the pellet resuspended in 7 mL of supplemented RPMI 1640 medium. The cells were counted using trypan blue (Avocado Research Chemicals Ltd., Lancashire, England) and a hemocytometer (Bright-Line, Horsham, PA) and diluted to a density of 1 x 10^6 cells/mL. Into 8-well chamber slides (Falcon™, Bedford, MA), 1 x 10^5 cells were added to each well. The cells were incubated for 48h. After 48h, the **AHS** sensor was added to three wells at 5 mM, 4 wells at 10 mM, and 1 well did not have the sensor added. This was incubated for 10 min. Following incubation, H_2S was added to individual wells in concentrations of 0, 2.5, 5, or 10 mM (**AHS** at 5 mM with H_2S at either 2.5, 5, or 10 mM; **AHS** at10 mM with H_2S at either 0, 2.5, 5, or 10 mM; and **AHS** at 0 mM with H_2S at 10 mM). This was incubated for 10 min. After incubation, the supernatant was removed by aspiration and the slides were mounted (Immu-Mount; Thermo, Pittsburgh, PA). Following mounting of the slides, the cells were visualized using a TE2000-S inverted fluorescent microscope (Nikon, Melville, NY).



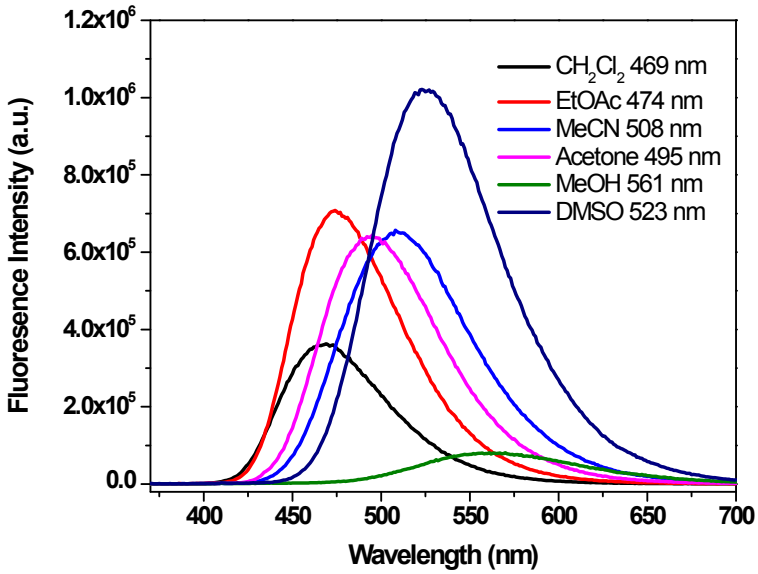
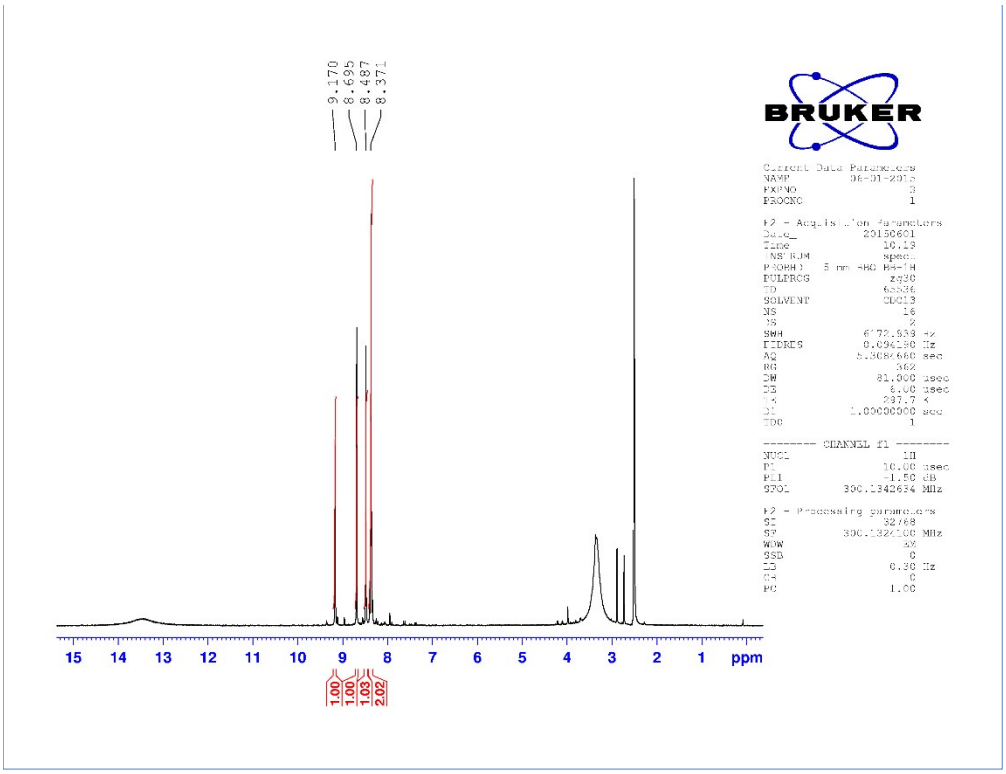
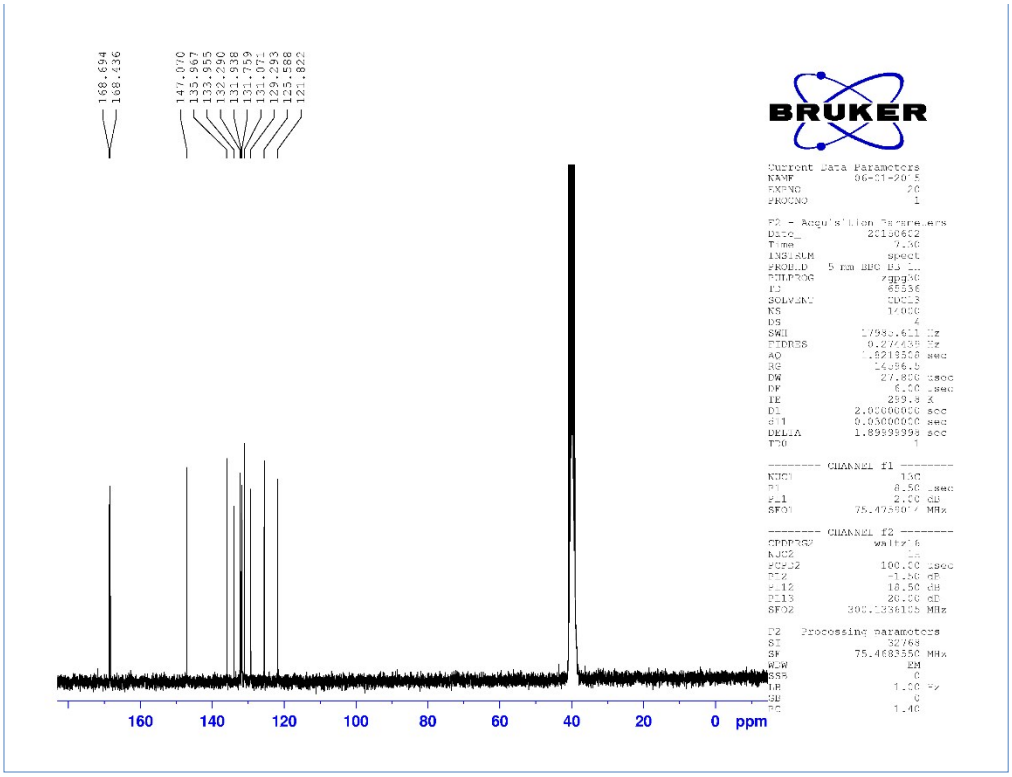


Fig. S1. The aborption and emission spectra of 6-amino-2,3-NI in different solvents at 25 °C.

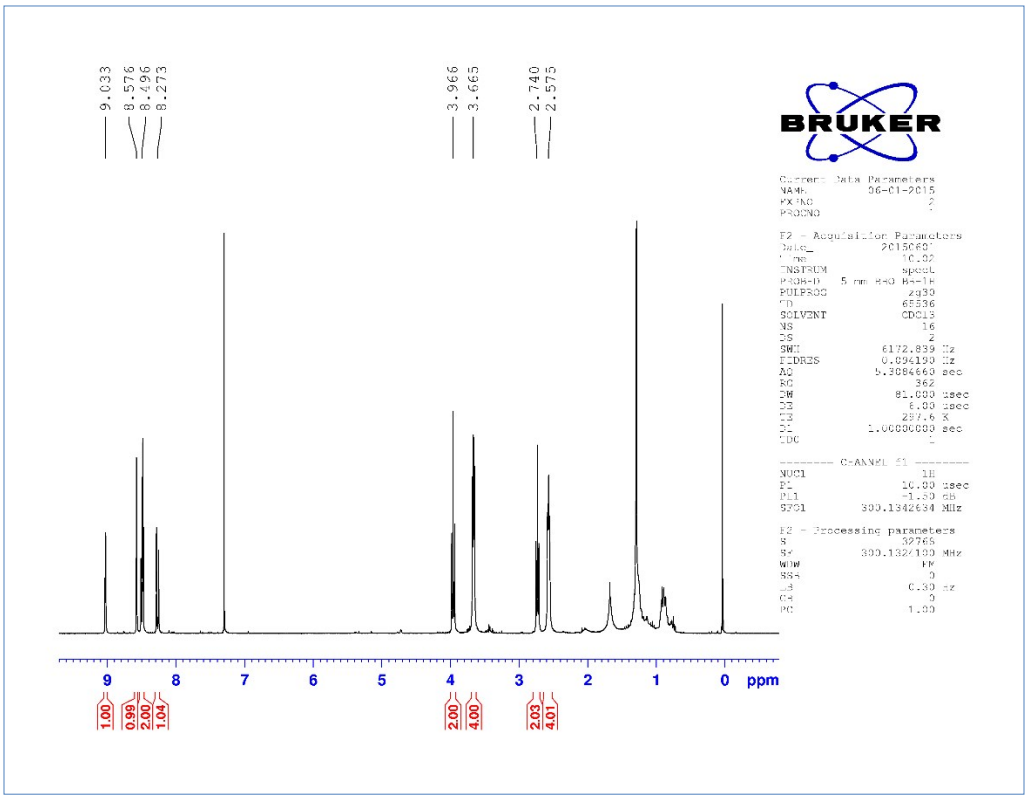
Compound 1

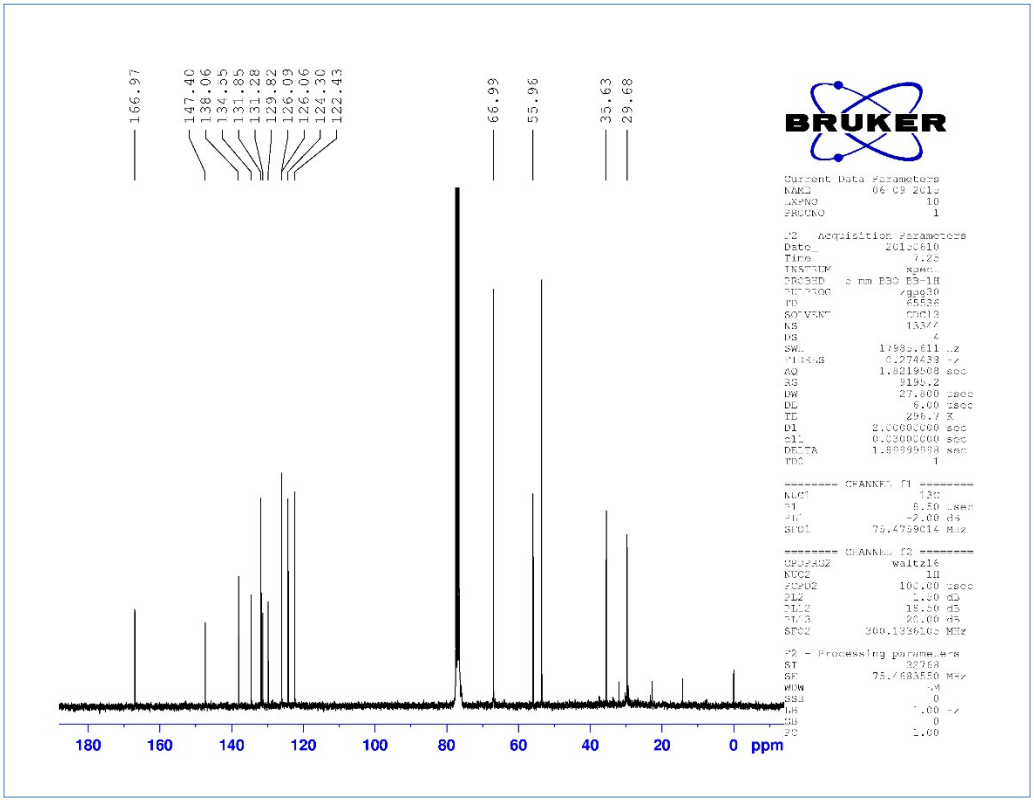

9.170
8.695
8.487
8.371
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
1.00 1.00 1.03 2.02
BRUKER

c

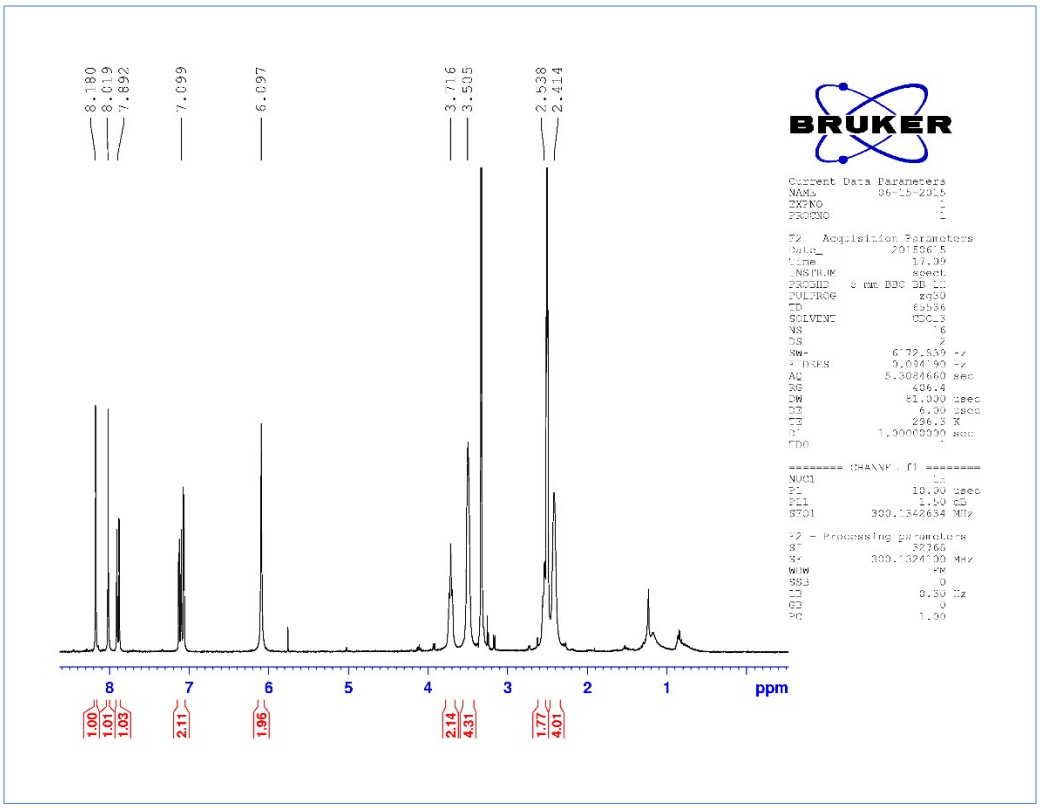

168.694
168.436
147.070
135.967
133.955
132.290
131.938
131.759
131.071
129.293
129.258
121.822
160 140 120 100 80 60 40 20 0 ppm
BRUKER

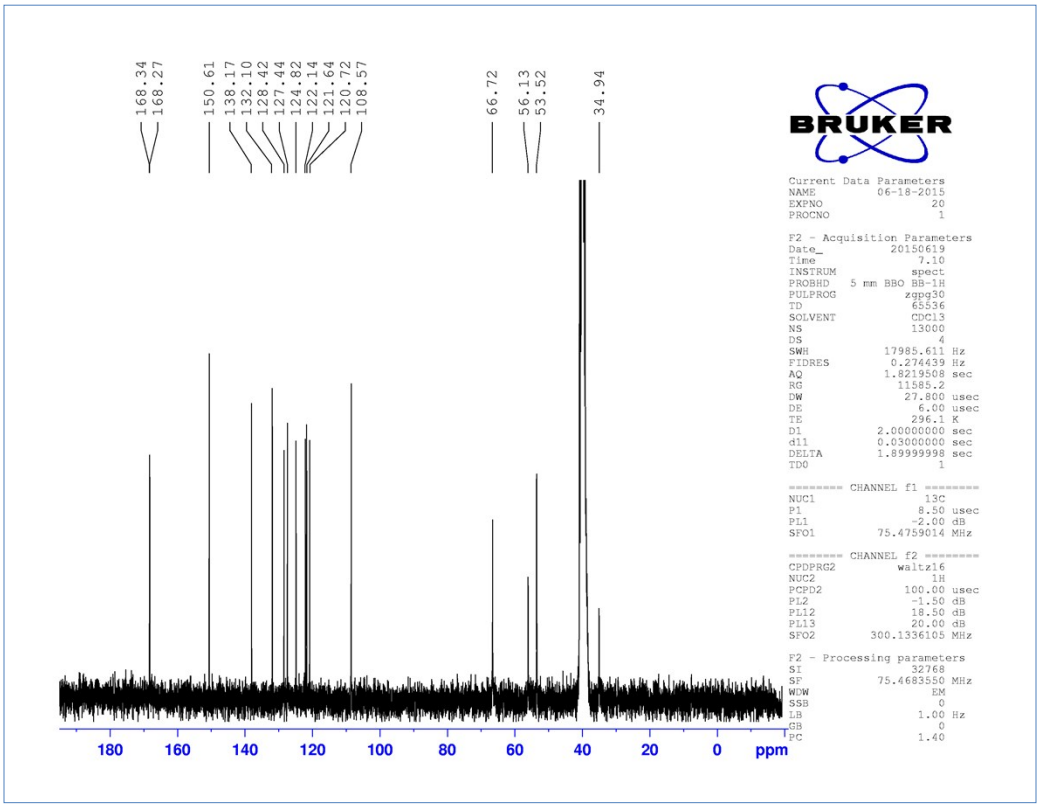
NHS


9.033
8.576
8.496
8.273
3.966
3.665
2.740
2.575
BRUKER
9
8
7
6
5
4
3
2
1
0
ppm
1.00
0.99
2.00
1.04
2.00
4.00
2.03
4.01

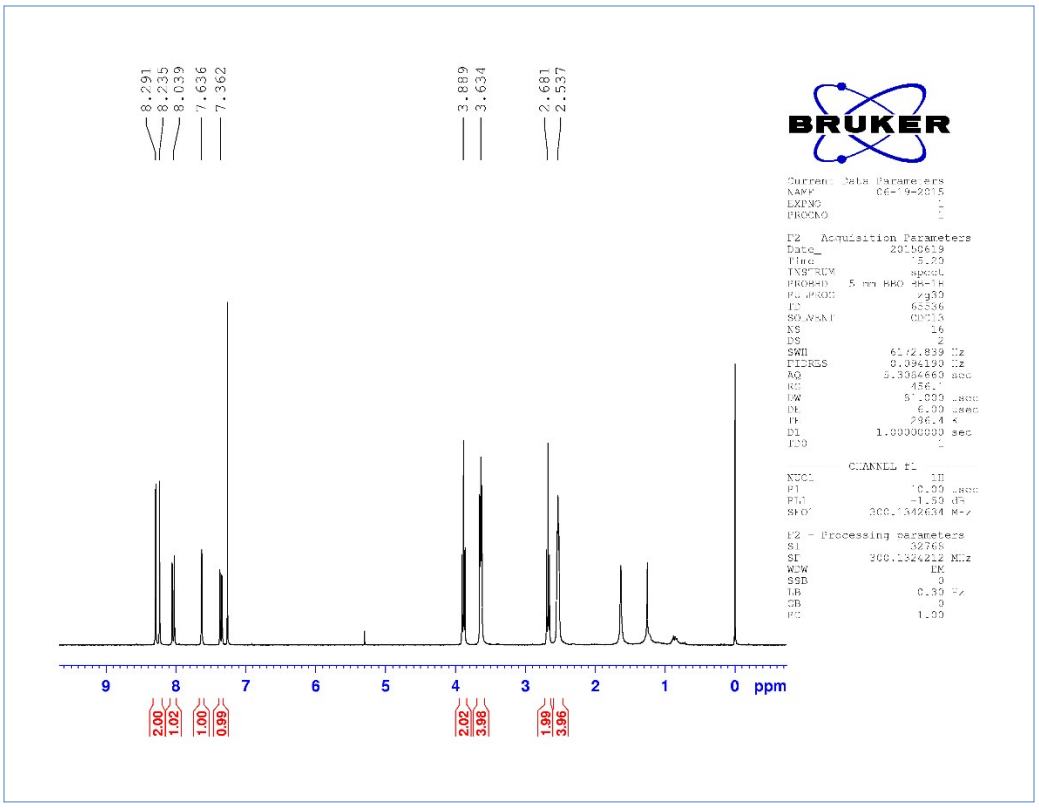

166.97
147.40
138.06
134.55
131.85
131.28
129.82
126.09
126.06
124.30
122.43
66.99
55.96
35.63
29.68
BRUKER
180
160
140
120
100
80
60
40
20
0
ppm

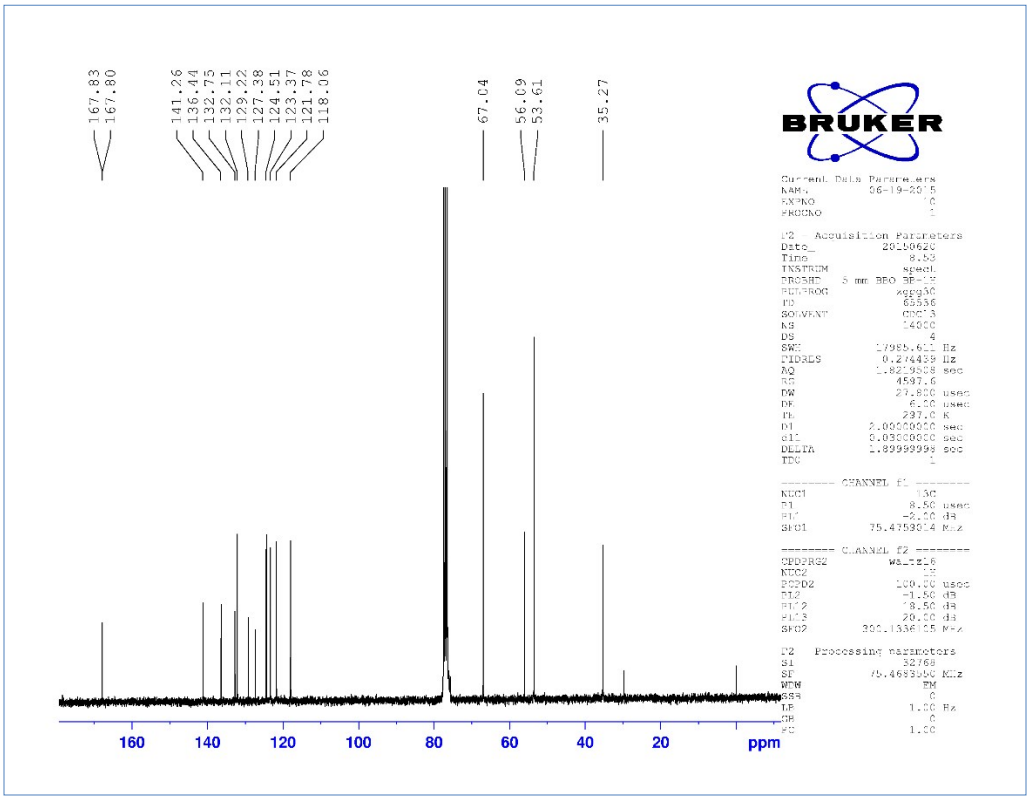
Compound 2


8.180
8.019
7.892
7.099
6.097
3.716
3.505
2.538
2.414
BRUKER
Current Data Parameters
NAME 06-25-2015
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20150625
Time 17.09
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 6172.839 Hz
FIDRES 0.094190 Hz
AQ 5.3084660 sec
RG 406.4
DW 81.000 usec
DE 6.00 usec
TE 296.3 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 10.30 usec
PL1 1.50 dB
SFO1 300.1342634 MHz
F2 - Processing parameters
SI 32768
SF 300.1324700 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
8
7
6
5
4
3
2
1
ppm
1.00
1.01
1.03
2.11
1.96
2.14
4.31
1.77
4.01


168.34
168.27
150.61
138.17
132.10
128.42
127.44
124.82
122.14
121.64
120.72
108.57
66.72
56.13
53.52
34.94
BRUKER
Current Data Parameters
NAME 06-18-2015
EXPNO 20
PROCNO 1
F2 - Acquisition Parameters
Date_ 20150619
Time 7.10
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 13000
DS 4
SWH 17985.611 Hz
FIDRES 0.274439 Hz
AQ 1.8219508 sec
RG 11585.2
DW 27.800 usec
DE 6.00 usec
TE 296.1 K
D1 2.00000000 sec
d11 0.03000000 sec
DELTA 1.89999998 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 8.50 usec
PL1 -2.00 dB
SFO1 75.4759014 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 100.00 usec
PL2 -1.50 dB
PL12 18.50 dB
PL13 20.00 dB
SFO2 300.1336105 MHz
F2 - Processing parameters
SI 32768
SF 75.4683550 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
180
160
140
120
100
80
60
40
20
0
ppm

AHS


8.291
8.235
8.039
7.636
7.362
3.889
3.634
2.681
2.537
BRUKER
9
8
7
6
5
4
3
2
1
0
ppm
2.00
1.02
1.00
0.99
2.02
3.98
1.99
3.96


167.83
167.80
141.26
136.44
132.75
132.11
129.22
127.38
124.51
123.37
121.78
118.06
67.04
56.09
53.61
35.27
BRUKER
160
140
120
100
80
60
40
20
ppm

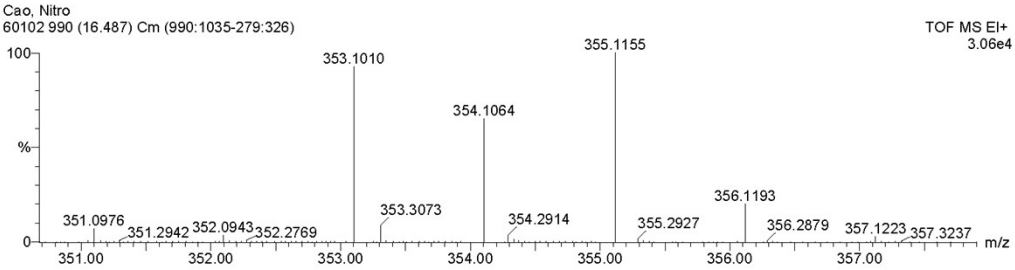
HRMS for NHS

Elemental Composition Report **Page 1**

Multiple Mass Analysis: 4 mass(es) processed
Tolerance = 15.0 PPM / DBE: min = -1.5, max = 50.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions
8444 formula(e) evaluated with 49 results within limits (all results (up to 1000) for each mass)

Cao, Nitro
60102 990 (16.487) Cm (990:1035-279:326)



Minimum:	20.00				-1.5		
Maximum:	100.00		200.0	15.0	50.0		
Mass	RA	Calc. Mass	mDa	PPM	DBE	Score	Formula
353.1010	92.72	353.1012	-0.2	-0.5	13.0	n/a	12C18 H15 N3 O5
		353.1007	0.3	0.8	17.5	n/a	12C22 13C H14 N O3
		353.0998	1.2	3.3	13.5	n/a	12C16 H13 N6 O4
		353.1025	-1.5	-4.3	12.5	n/a	12C20 H17 O6
		353.0994	1.6	4.6	18.0	n/a	12C20 13C H12 N4 O2
		353.1034	-2.4	-6.8	22.0	n/a	12C25 13C H12 N2
		353.1039	-2.9	-8.1	17.5	n/a	12C21 H13 N4 O2
		353.0980	3.0	8.4	13.0	n/a	12C19 13C H16 O6
		353.1052	-4.2	-11.9	17.0	n/a	12C23 H15 N O3
		353.0967	4.3	12.2	13.5	n/a	12C17 13C H14 N3 O5
		353.0966	4.4	12.3	21.5	n/a	12C27 H13 O
354.1064	65.13	354.1059	0.5	1.5	12.5	n/a	12C19 13C H17 O6
		354.1072	-0.8	-2.3	17.5	n/a	12C20 13C H13 N4 O2
		354.1077	-1.3	-3.5	13.0	n/a	12C16 H14 N6 O4
		354.1045	1.9	5.3	13.0	n/a	12C17 13C H15 N3 O5
		354.1045	1.9	5.5	21.0	n/a	12C27 H14 O
		354.1085	-2.1	-6.1	17.0	n/a	12C22 13C H15 N O3
		354.1090	-2.6	-7.3	12.5	n/a	12C18 H16 N3 O5
		354.1032	3.2	9.1	13.5	n/a	12C15 13C H13 N6 O4
		354.1031	3.3	9.3	21.5	n/a	12C25 H12 N3
		354.1103	-3.9	-11.1	12.0	n/a	12C20 H18 O6
		354.1112	-4.8	-13.6	21.5	n/a	12C25 13C H13 N2
		354.1117	-5.3	-14.9	17.0	n/a	12C21 H14 N4 O2
355.1155	100.00	355.1155	0.0	0.1	12.5	n/a	12C16 H15 N6 O4
		355.1150	0.5	1.3	17.0	n/a	12C20 13C H14 N4 O2
		355.1164	-0.9	-2.5	16.5	n/a	12C22 13C H16 N O3
		355.1168	-1.3	-3.7	12.0	n/a	12C18 H17 N3 O5
		355.1137	1.8	5.1	12.0	n/a	12C19 13C H18 O6
		355.1182	-2.7	-7.5	11.5	n/a	12C20 H19 O6
		355.1124	3.1	8.9	12.5	n/a	12C17 13C H16 N3 O5
		355.1123	3.2	9.0	20.5	n/a	12C27 H15 O
		355.1191	-3.6	-10.0	21.0	n/a	12C25 13C H14 N2
		355.1195	-4.0	-11.3	16.5	n/a	12C21 H15 N4 O2
		355.1110	4.5	12.6	13.0	n/a	12C15 13C H14 N6 O4
		355.1109	4.6	12.8	21.0	n/a	12C25 H13 N3
356.1193	20.03	356.1188	0.5	1.3	12.5	n/a	12C15 13C H15 N6 O4
		356.1188	0.5	1.5	20.5	n/a	12C25 H14 N3
		356.1201	-0.8	-2.3	20.0	n/a	12C27 H16 O
		356.1202	-0.9	-2.5	12.0	n/a	12C17 13C H17 N3 O5
		356.1215	-2.2	-6.2	11.5	n/a	12C19 13C H19 O6
		356.1161	3.2	9.0	16.0	n/a	12C22 H16 N2 O3
		356.1229	-3.6	-10.0	16.5	n/a	12C20 13C H15 N4 O2
		356.1156	3.7	10.3	20.5	n/a	12C26 13C H15 O
		356.1233	-4.0	-11.2	12.0	n/a	12C16 H16 N6 O4
		356.1147	4.6	12.8	16.5	n/a	12C20 H14 N5 O2
		356.1242	-4.9	-13.8	16.0	n/a	12C22 13C H17 N O3
		356.1143	5.0	14.0	21.0	n/a	12C24 13C H13 N3

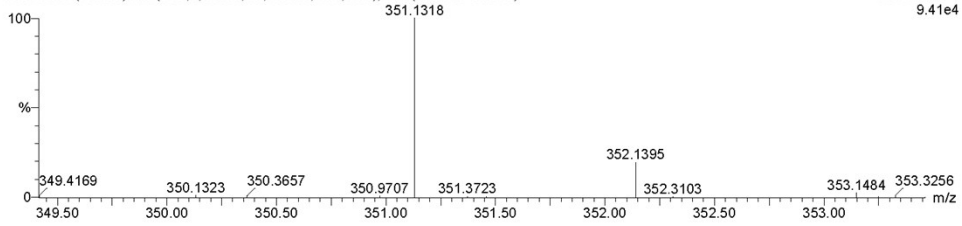
HRMS for AHS

Elemental Composition Report **Page 1**

Tolerance = 15.0 PPM / DBE: min = -1.5, max = 50.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions
1576 formula(e) evaluated with 8 results within limits (all results (up to 1000) for each mass)

Cao, Azide
60103 656 (10.936) AM (Cen,6, 90.00, Ht,7000.0,0.00,0.69); Cm (597:689-91:213) TOF MS EI+
9.41e4



Minimum:	20.00				-1.5		
Maximum:	100.00		200.0	15.0	50.0		
Mass	RA	Calc. Mass	mDa	PPM	DBE	Score	Formula
351.1318	100.00	351.1313	0.5	1.3	18.0	n/a	12C20 13C H14 N6
		351.1327	-0.9	-2.5	17.5	n/a	12C22 13C H16 N3 O
		351.1331	-1.3	-3.8	13.0	n/a	12C18 H17 N5 O3
		351.1300	1.8	5.1	13.0	n/a	12C19 13C H18 N2 O4
		351.1340	-2.2	-6.4	17.0	n/a	12C24 13C H18 O2
		351.1345	-2.7	-7.6	12.5	n/a	12C20 H19 N2 O4
		351.1287	3.1	8.9	13.5	n/a	12C17 13C H16 N5 O3
		351.1358	-4.0	-11.4	17.5	n/a	12C21 H15 N6