A Highly selective near-infrared fluorescencent probe for imaging H_2Se in living cells and in vivo

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Contents:

1. The pH dependence of the probe reaction

To apply NIR-H_2Se in more complicated systems, we also tested the effect of pH on the fluorescence response of the probe to H_2Se. It was found that NIR-H_2Se is stable and displays the obvious response for H_2Se in the region of 6.6–7.8. Thus, the probe could function properly at physiological pH.

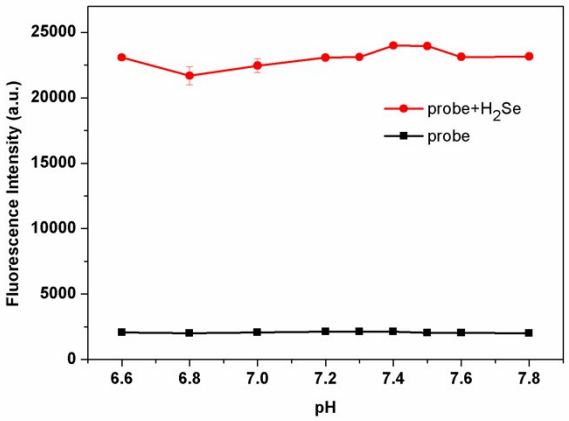


Figure S1. Fluorescence intensity changes of NIR-H_2Se (10 μM) at different pH values in the absence (black line) or presence (red line) of H_2Se (50 μM). The reactions were carried out for 5 min at room temperature in 10 mM PBS solution.

2. Effect of probe concentration

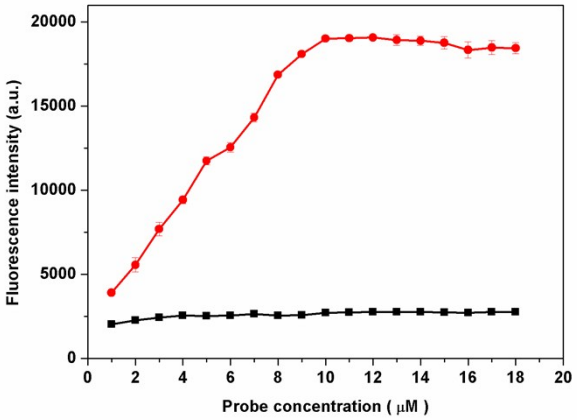


Figure S2. Effect of probe concentration (H_2Se concentration: 50 μM; PBS: pH 7.4,).

3. HPLC assay

The reaction of NIR-H_2Se with H_2Se was further analyzed by HPLC. The retention times for NIR-H_2Se and the product **2** are 6.01 and 10.55 min, respectively (Figure

S3a, b). After stirring the reaction containing NIR-H_2Se (10 μM) and H_2Se (50 μM) in PBS buffered (pH 7.4) solution at room temperature for 5 min, the HPLC profiles were illustrated in Figure S3c. The results indicated the probe indeed converted to the diamino product.

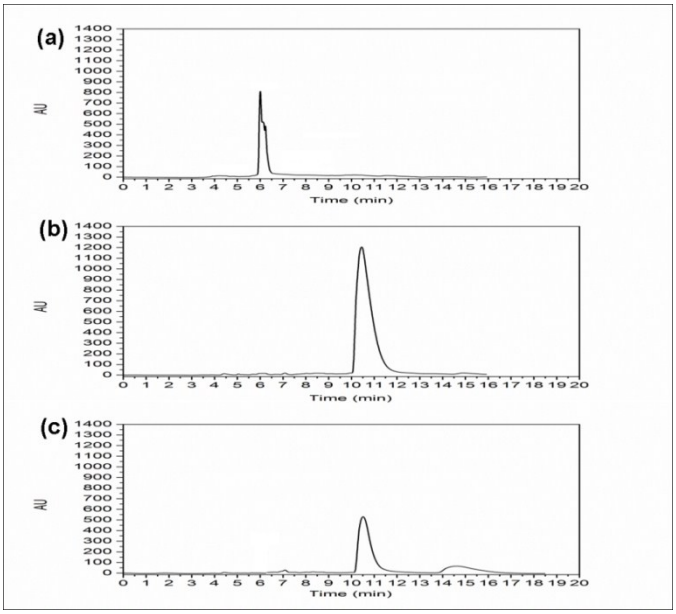


Figure S3. HPLC of (a) NIR-H_2Se (10 μM), (b) compound **2** (10 μM), and the reaction product of (c) NIR-H_2Se (10 μM) with H_2Se (50 μM) after incubation of them for 5 min in PBS buffered (pH 7.4) solution.

4. Mechanism study of the probe (NIR- H_2Se) with H_2Se by HR MS

To explore the sensing mechanism of NIR- H_2Se for H_2Se, the NIR- H_2Se and reaction mixture of NIR- H_2Se with H_2Se was characterized by HRMS spectrometry. The HR MS spectrum of NIR-H_2Se (10 μM) in Figure S 4a revealed a main peak at 488.1205 before the addition of H_2Se (50 μM), corresponding to the probe (m/z calcd = 488.1218). After the addition of H_2Se, a new peak at 412.2358 appeared, coinciding exactly with the diamino product (m/z calcd = 412.2383), which indicated that NIR- H_2Se was converted into compound **2** (Figure S 4b)

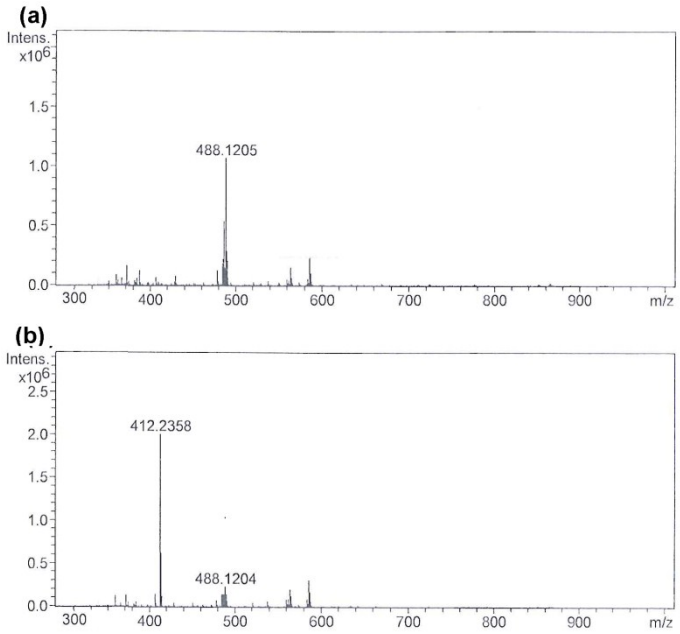


Figure S4 HRMS of NIR-H_2Se before (a) and after (b) addition of H_2Se.

5. Selectivity of NIR-H_2Se toward metal ions and amino acids

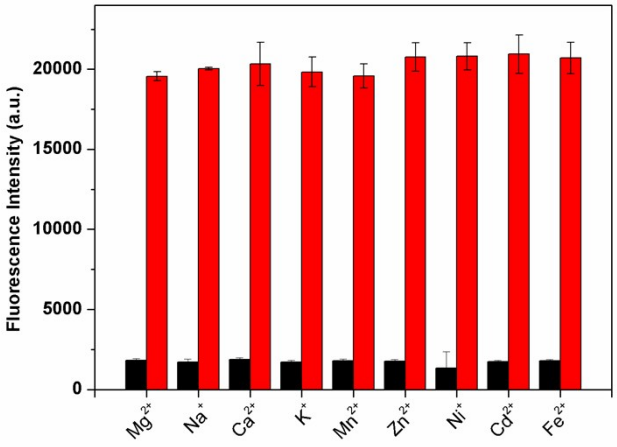


Figure S5. Fluorescence responses of NIR-H_2Se (10 μM) to diverse metal ions (5 mM for each) in PBS buffered (pH 7.4) solution.

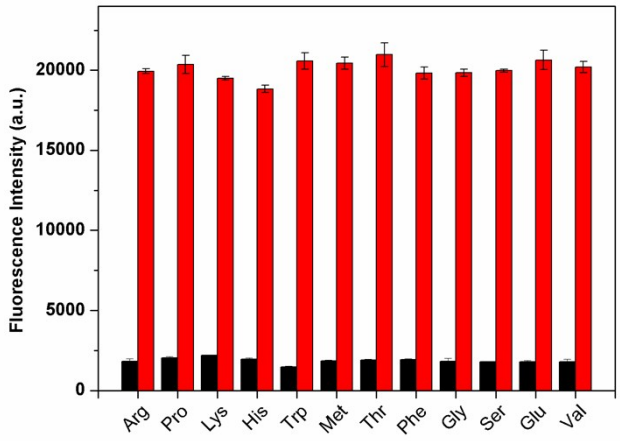


Figure S6. Fluorescence intensity changes of NIR-H_2Se (10 μM) upon addition of 100 equiv. amino acids in 10 mM phosphate buffer, pH 7.4 at room temperature. Black bars represent the addition of one of these interferents to a 10 μM solution of NIR-H_2Se. Red bars represent the addition of H_2Se plus one of these interferents to the probe solution.

6. MTT assay

To evaluate the cytotoxicity of NIR-H_2Se, we performed an MTT assay on HepG2 cells with probe concentrations from 10-500 μM. The results showed that NIR-H_2Se was of low toxicity towards cell cultures under experimental conditions.

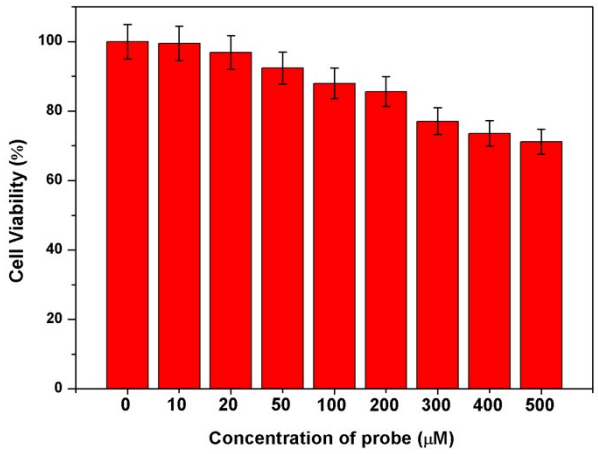


Figure S7. Cell viability of NIR-H_2Se at different concentrations.

7. Photo-bleaching test of the reaction product of NIR-H_2Se with H_2Se

The resistance to photobleaching experiments were also carried out to evaluate the stability of the probe. Exposure to the laser radiation for 500s, no significant fluorescence decrease was observed, which suggested that the probe was stable and

can be used for long-time cells imaging (Figure S7).

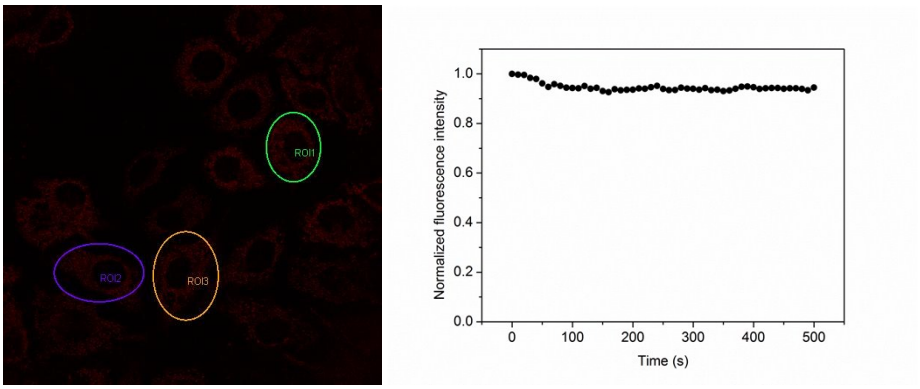
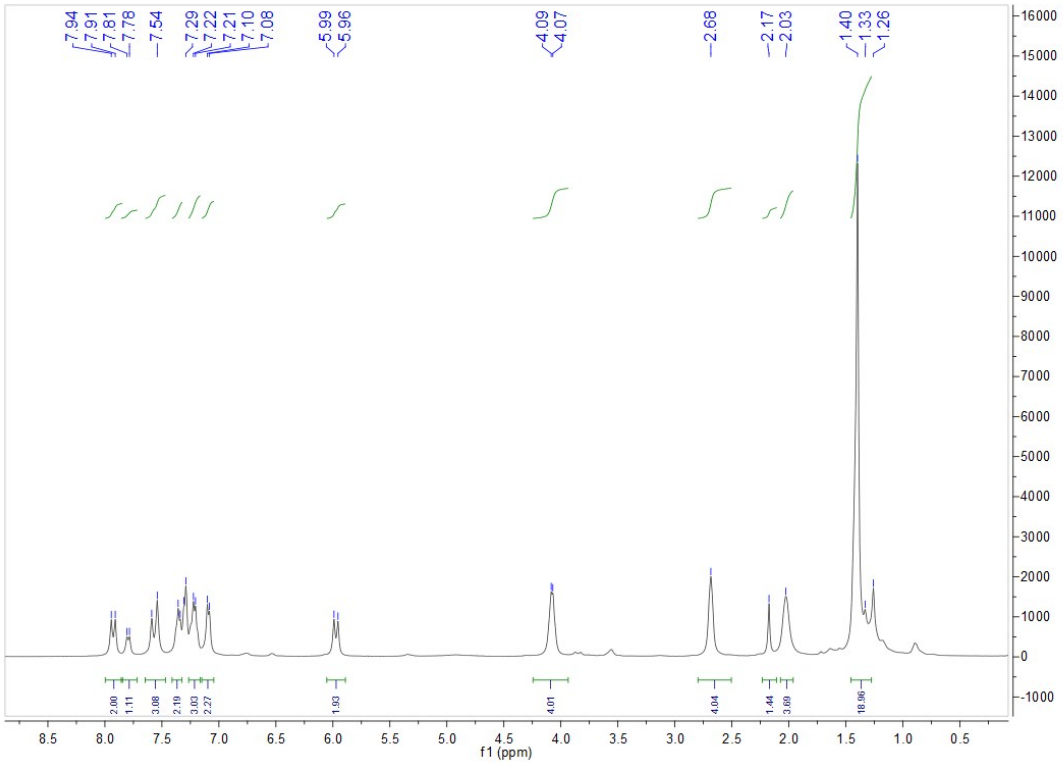
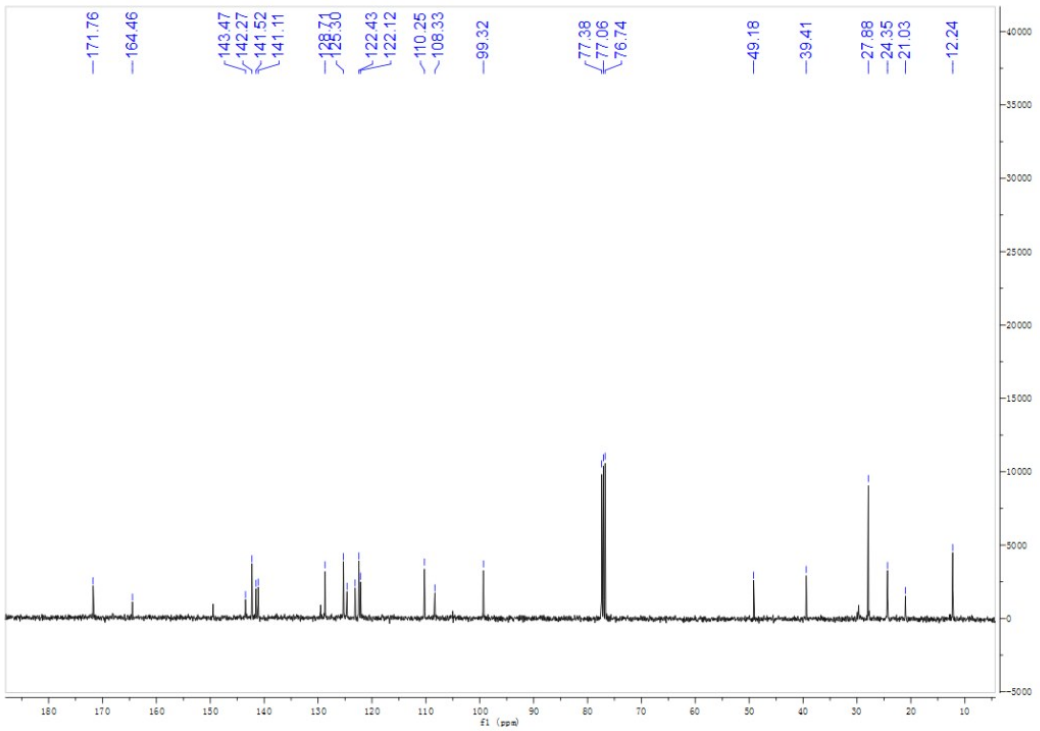


Figure S8. Test of photostability of NIR-H_2Se (10 μM) (a) Confocal fluorescence images (0-500 s) were achieved by means of time-sequential scanning of the probe-loaded HepG2 cells for 15 min (b) Normalized fluorescence intensity of the three selected regions of (a) from 0 to 500s.

8. ^{1}H-NMR, ^{13}C-NMRand HR-MS spectra of compound 1, 2 and NIR-H_2Se



^{1}H NMR of compound 1



^{13}C NMR of compound 1

Mass Spectrum List Report

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Comment			

Acquisition Parameter

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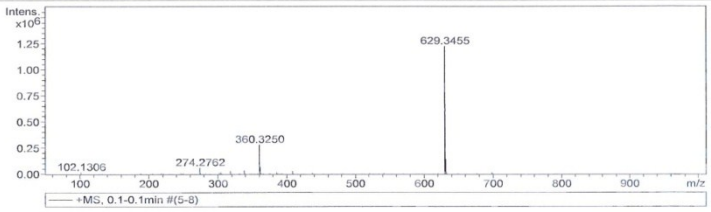
Scan End	1000 m/z	Set Collision Cell RF	300.0 Vpp	Set Divert Valve	Source



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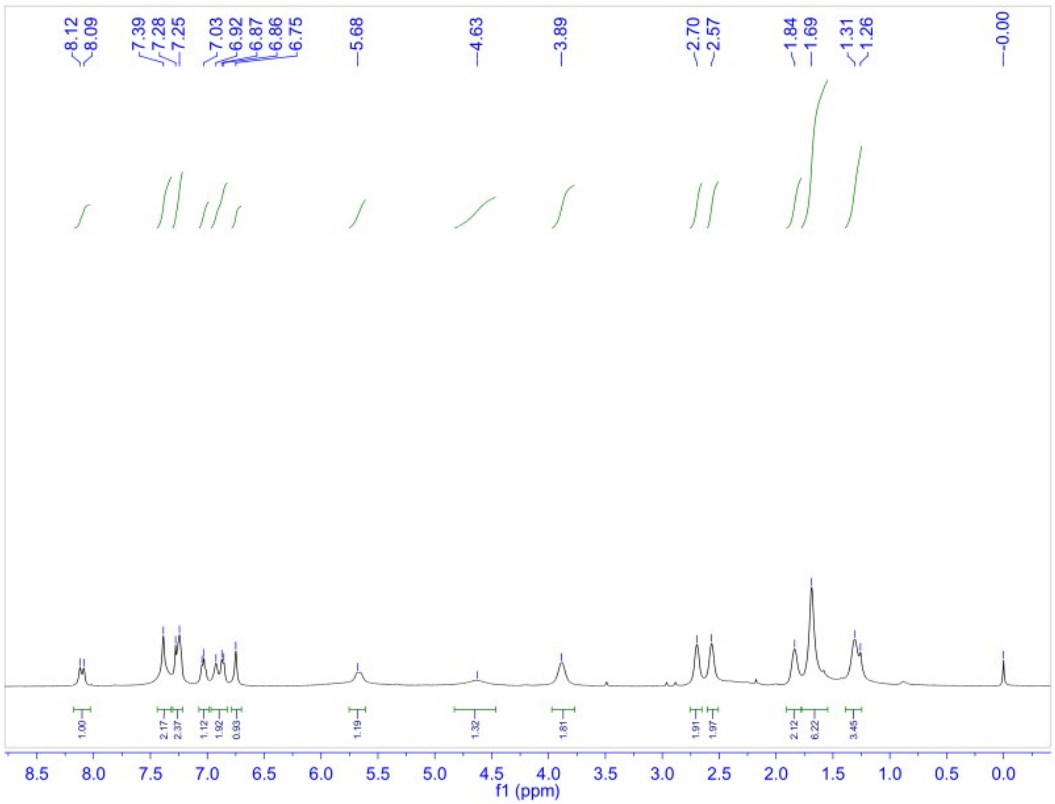
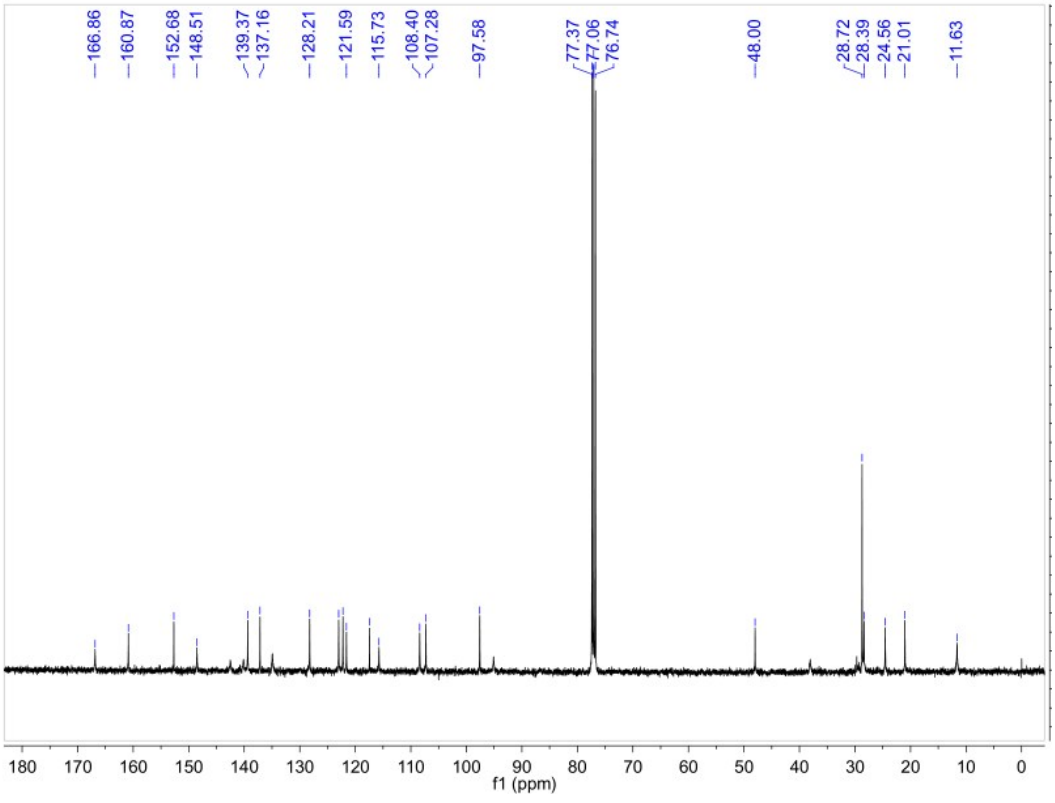
2	188.1460	16521	19133.0	9567	0.0114
3	218.2140	17618	15531.0	7766	0.0124
4	246.2452	17216	14653.0	7327	0.0143
5	250.1801	17793	14520.5	7260	0.0141
6	274.2762	18521	120366.5	60183	0.0148
7	275.2794	18096	21253.0	10627	0.0152
8	302.3074	19176	26329.0	13165	0.0158
9	304.2630	18851	33135.0	16568	0.0161
10	318.3020	18766	52523.5	26262	0.0170
11	330.3381	19785	14206.5	7103	0.0167
12	338.3433	19421	71453.5	35727	0.0174
13	339.3466	19549	17684.0	8842	0.0174
14	358.3099	18893	16159.0	8080	0.0190
15	360.3250	18835	563271.0	281636	0.0191
16	361.3284	19639	136699.0	68350	0.0184
17	362.3304	17167	26793.5	13397	0.0211
18	376.3050	9094	14349.5	7175	0.0414
19	385.2647	19742	33812.5	16906	0.0195
20	408.3091	18627	53025.5	26513	0.0219
21	409.3125	19875	14049.0	7025	0.0206
22	437.1937	17470	23606.5	11803	0.0250
23	520.3674	19384	22883.0	11442	0.0268
24	541.2669	19304	14798.5	7399	0.0280
25	619.5238	19436	13913.5	6957	0.0319
26	629.3455	17526	2443715.5	1221858	0.0359
27	630.3491	20766	1332275.0	666138	0.0304
28	631.3523	21182	280013.0	140457	0.0298
29	632.3554	21307	40588.0	20294	0.0297
30	697.6538	21523	29150.5	14575	0.0324

Bruker Compass DataAnalysis 4.0 printed: 5/7/2015 10:26:57 AM Page 1 of 1

HR MS of compound 1



^{1}H NMR of compound 2



^{13}C NMR of compound 2

Mass Spectrum List Report

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Comment			

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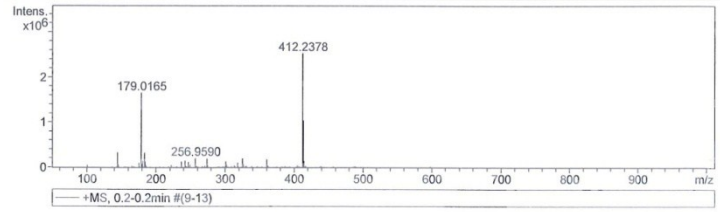

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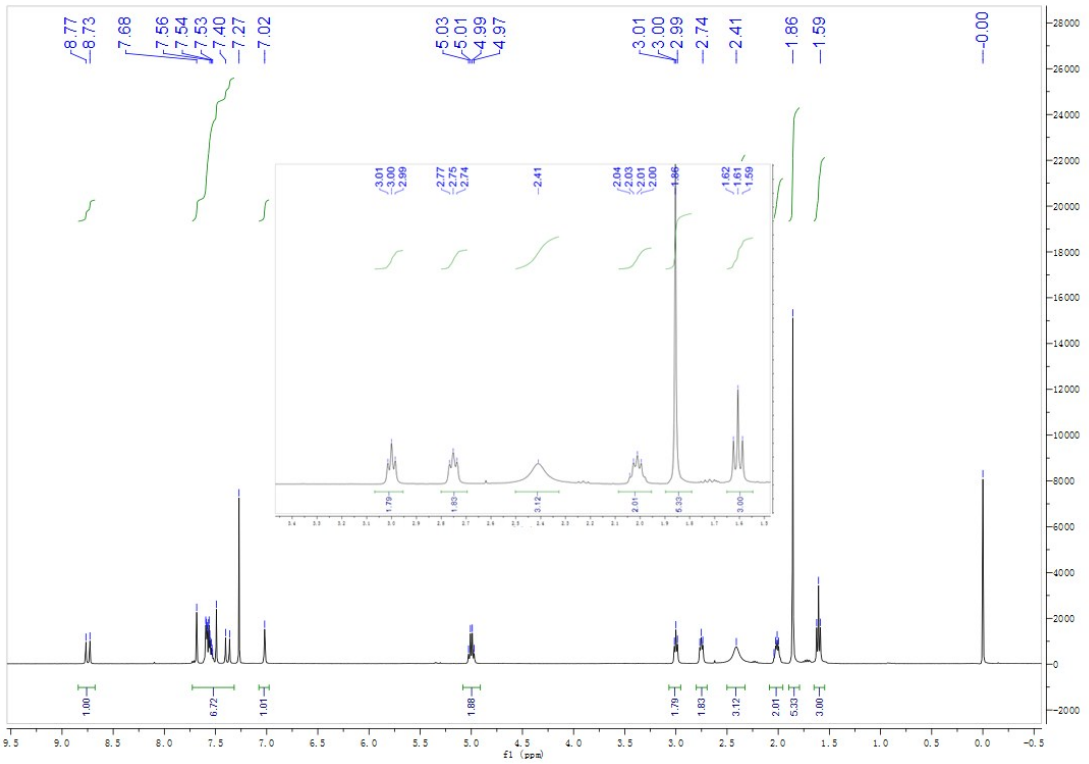
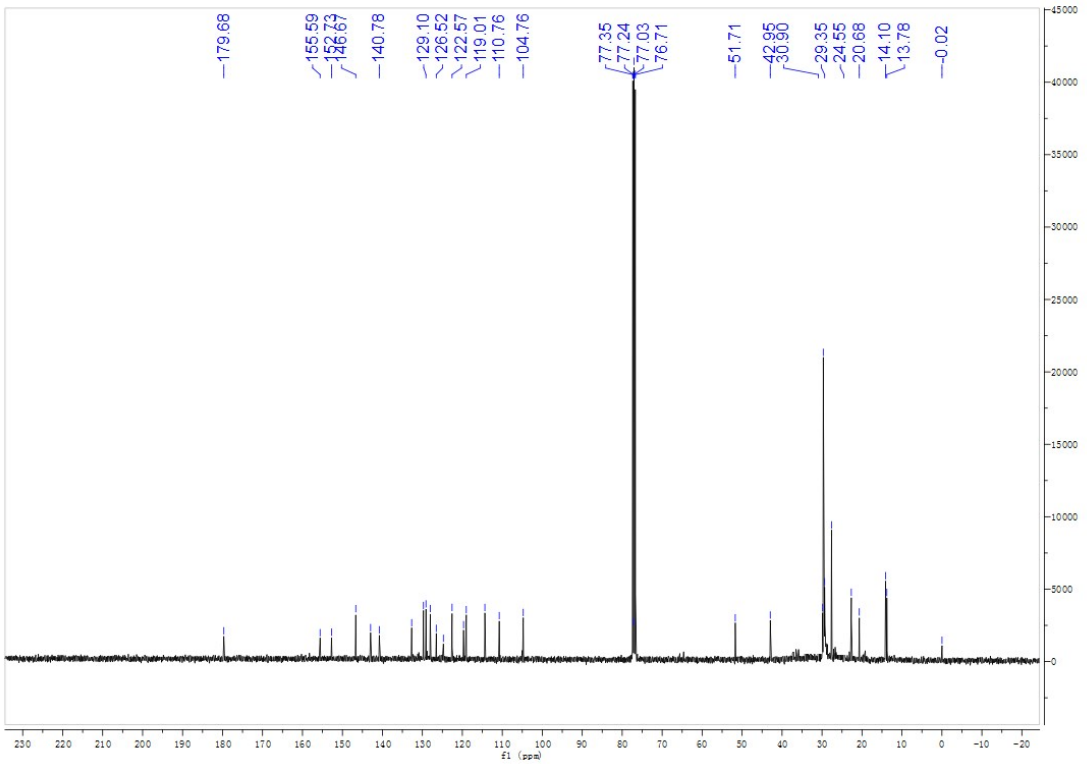
3	145.9857	19128	26703.2	58747	0.0076
4	176.0076	25801	39593.4	87105	0.0068
5	179.0165	38571	748082.9	1645782	0.0046
6	180.0187	21453	34631.3	76189	0.0084
7	181.0122	24408	64248.3	141346	0.0074
8	183.9938	25284	145139.8	319308	0.0073
9	184.4948	22413	20752.8	45656	0.0082
10	184.9932	22008	56316.5	123896	0.0084
11	185.9917	21125	19517.0	42937	0.0088
12	222.0550	24861	23877.2	52530	0.0089
13	236.9770	26481	53898.5	118577	0.0089
14	242.2832	30793	65181.8	143400	0.0079
15	246.9303	24522	50014.1	110031	0.0101
16	248.9276	25880	26914.6	59212	0.0096
17	256.9590	26477	91668.1	201670	0.0097
18	274.2731	27195	85056.6	187125	0.0101
19	274.6470	24022	19505.7	42913	0.0114
20	300.9852	26152	57238.0	125924	0.0115
21	302.3044	25292	24104.1	53029	0.0120
22	318.2992	26592	43992.4	96783	0.0120
23	324.9461	28586	86634.6	190596	0.0114
24	326.9457	24756	33313.4	73289	0.0132
25	360.3226	27977	79046.4	173902	0.0129
26	361.3259	25145	18950.3	41691	0.0144
27	404.9602	26360	19812.0	43586	0.0154
28	412.2378	40464	1145833.3	2520833	0.0102
29	413.2408	45532	468690.9	1031120	0.0091
30	414.2436	28297	60838.0	133844	0.0146

HR MS of compound 2



^{1}H NMR of NIR-H_2Se (the inset shows the detail of up-field spectrogram)



^{13}C NMR of NIR-H_2Se

Mass Spectrum List Report

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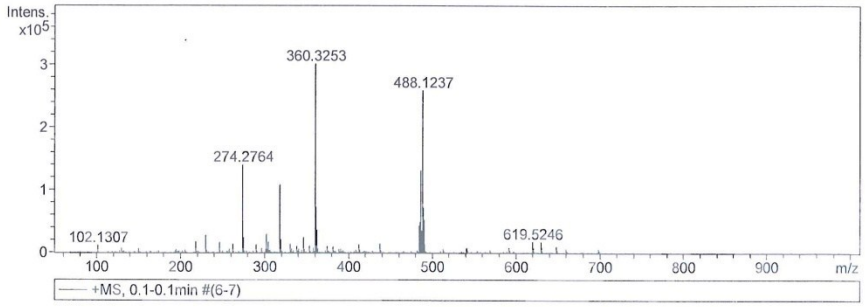

#	m/z	Res.	S/N	I	FWHM
1	102.1307	13939	7553.7	11331	0.0073
2	218.2142	17563	11559.7	17340	0.0124
3	230.2504	17773	18257.7	27387	0.0130
4	246.2453	17747	10789.0	16184	0.0139
5	262.2401	18477	8808.0	13212	0.0142
6	274.2764	18424	93244.7	139867	0.0149
7	275.2797	18176	16181.7	24273	0.0151
8	290.2712	18862	8572.0	12858	0.0154
9	302.3076	18863	20095.0	30143	0.0160
10	304.2632	18449	11561.3	17342	0.0165
11	318.3022	18948	72220.3	108331	0.0168
12	319.3055	18780	13633.3	20450	0.0170
13	330.3386	19151	9158.7	13738	0.0172
14	346.3332	19217	16066.7	24100	0.0180
15	353.2679	19123	7166.3	10750	0.0185
16	360.3253	18823	200332.0	300498	0.0191
17	361.3285	19594	48325.7	72489	0.0184
18	362.3292	18884	24049.0	36074	0.0192
19	412.2394	19212	8472.3	12709	0.0215
20	437.1943	19818	9316.0	13974	0.0221
21	484.1263	19477	28857.0	43286	0.0249
22	485.1278	20129	32841.3	49262	0.0241
23	486.1249	20084	86958.0	130437	0.0242
24	487.1276	19880	23871.7	35808	0.0245
25	488.1237	20182	172141.3	258212	0.0242
26	489.1267	19682	47263.3	70895	0.0249
27	490.1249	19677	34415.7	51624	0.0249
28	491.1272	19811	8733.0	13100	0.0248
29	619.5246	20073	11601.3	17402	0.0309
30	629.3461	21363	11468.3	17203	0.0295

HR MS of NIR-H_2Se