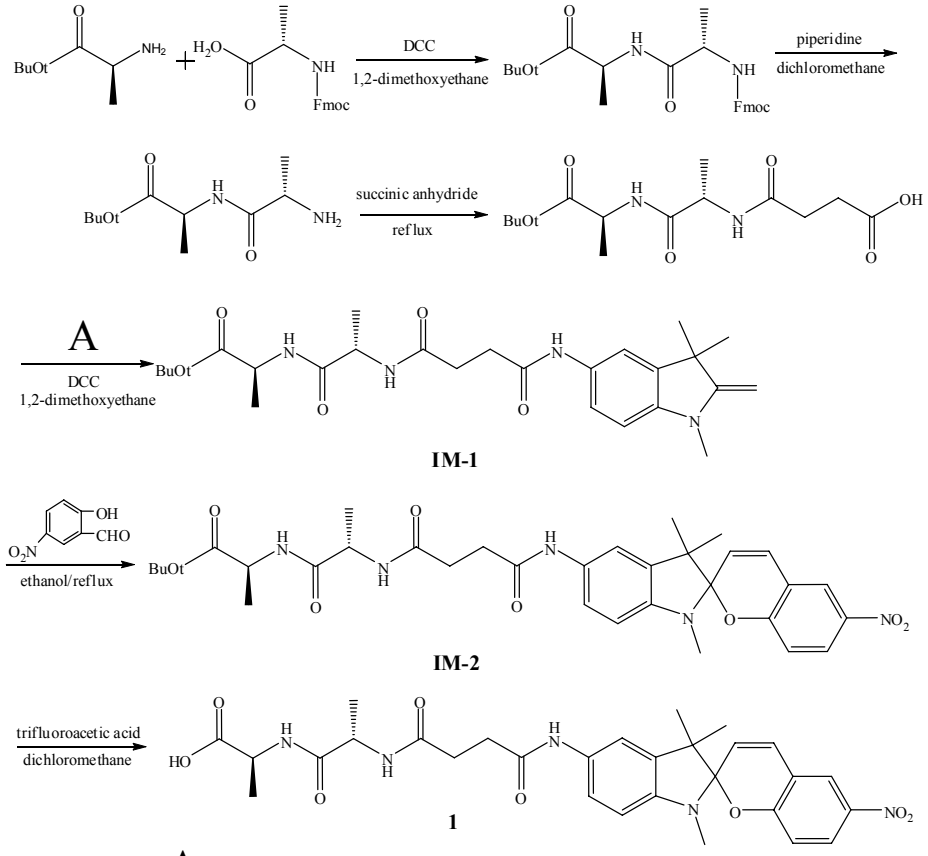
Spiropyran-linked dipeptide forms supramolecular hydrogel with dual responses to light and ligand-receptor interaction

Zhenjun Qiu, Haitao Yu, Jinbo Li, Yu Wang and Yan Zhang*

General: Chemical reagents and solvents were used as received from commercial sources. ^{1}HNMR spectra were obtained on a 300 MHz Bruker DPX 300, MS on a Finnigan Mat TSQ 7000, HRMS on Bruker Daltonics APEXIII 7.0 TESLA FTMS; SEM on Hitachi S-4800 scanning electron microscope, HPLC on Agilent 1200 with methanol and water as eluents, CD on a JASCO J-810 spectrometer, UV spectrum on a Shimadzu UV3600 UV-Vis-NIR Spectrophotometer.

*Synthesis of compound **1***:



Scheme 1 Synthesis of compound **1**

Detailed description on the synthetic procedure:
N-(fluorenyl-9-methoxycarbonyl)-D-Alanine, H-D-Ala-OtBu and N,N-Diisopropyl-ethylamine were dissolved in anhydrous dimethoxyethane. The solution was cooled to 4°C, and dicyclohexylcarbodiimide was added to the solution. After stirring for 24 hrs. at 4°C, the urea precipitate was filtered out and the solvent was subsequently removed under reduced pressure to give a colorless oil. The oil was used in the following step without further purification. Piperidine was added to the solution of the oil in chloroform the mixture was stirred at room temperature for 0.5h. The solvent was removed *in vacuo* and the residue was purified by flash chromatography on a silica gel column using 1/30 MeOH/CH_2Cl_2 to afford H-D-Ala-D-Ala-OtBu as colorless oil.

H-D-Ala-D-Ala-OtBu (432mg, 2mmol) and succinic anhydride (200mg, 2mmol) were dissolved in 30ml anhydrous dimethoxyethane. The solution was heated under reflux for 2hrs. Then the solution was cooled to 4°C, 1,3,3-trimethyl-2-methyleneindolin-5-amine (376mg, 2mmol) and dicyclohexylcarbodiimide (DCC, 453mg, 2.2mmol) was added to the solution. After 24 hours at 4°C, the precipitate was filtered out and the solvent was removed *in vacuo* to give a brown oil. And the brown oil was purified by flash chromatography on a silica gel column using 1/15 MeOH/CH_2Cl_2 to afford **IM-1** as yellow oil. (786mg, Yield: 81%)

^{1}HNMR of IM-1(300MHz, $CDCl_3$) δ (ppm): 8.82(s, 1H), 7.49(dd, 2H, J=8.4, 2.7Hz), 7.28(s,1H), 7.11(d, 1H, J=8.4Hz), 6.31(d, 1H, J=8.4Hz), 4.62(m, 1H), 4.34(m, 1H), 3.75(s, 2H), 2.93(s, 3H), 2.64(m, 4H,), 1.39(s, 9H), 1.26(m, 12H).
^{13}CNMR of IM-1 (300MHz, $CDCl_3$) δ (ppm): 172.3, 172.0, 170.4, 162.7, 143.0, 137.7, 129.8, 120.0, 115.4, 104.3, 81.6, 72.9, 48.6, 44.1, 32.1, 31.2, 29.7, 29.2, 28.7, 27.8, 18.3, 17.8.
ESI-MS of IM-1: 487 (M+1), **HRMS of IM-1:** 487.29067 (C 26 H 39 N 4 O 5)

IM-1 (486mg, 1mmol) and 2-hydroxy-5-nitrobenzaldehyde(184mg, 1.1mmol) were dissolved in 20ml of anhydrous ethanol. And the mixture was heated under reflux for 2

hrs. At the end of the reaction, solvent was removed and the resulted red oil was purified by flash chromatography on a silica gel column using ethyl acetate to afford **IM-2** as yellow wax. (451mg, Yield: 71%)

^{1}HNMR of IM-2 (300MHz, $CDCl_3$) δ (ppm): 8.62(s, 1H), 7.97(m, 2H), 7.34(m, 2H), 7.20(m, 3H), 6.90(d, 1H, J=10.5Hz), 6.69(d, 1H, J=9.6Hz), 6.41(d, 1H, J=8.4Hz), 5.81(d, 1H, J=10.5Hz), 4.60(m, 1H), 4.39(m, 1H), 2.67(m, 7H), 1.43(s, 9H), 1.33(m, 6H,), 1.22(s, 3H), 1.12(s, 3H).

^{13}CNMR of IM-2(300MHz, $CDCl_3$) δ (ppm): 172.2, 172.0, 170.3, 159.6, 144.4, 140.8, 136.5, 130.8, 128.2, 125.8, 122.6, 121.3, 120.0, 118.5, 115.3, 115.1, 106.8, 81.8, 52.2, 48.7, 48.6, 32.2, 31.3, 28.9, 27.8, 25.6, 19.7, 18.4, 18.0.

ESI-MS of IM-2: 658 (M+Na), **HRMS of IM-2:** 658.28416 (C 33 H 41 N 5 Na 1 O 8)

Trifluoroacetic acid (3ml) was added to the solution of **IM-2** (318mg, 0.5mmol) in 5ml chloroform and the mixture was stirred at room temperature for 2.5h. The solvent was removed *in vacuo* to give a yellow oil. 30ml Ethyl ether was added to the yellow oil to precipitate compound **1** as a yellow powder. (267mg, Yield: 92%)

^{1}HNMR of 1 (300MHz, CD_3OD) δ (ppm): 8.10(d, 1H, J=3.0Hz), 8.03(dd, 1H, J=9.0, 3.0Hz), 7.33(d, 1H, J=1.8Hz), 7.21(dd, 1H, J=8.4, 1.8Hz), 7.10(d, 1H, J=10.5Hz), 6.78(d, 1H, J=9.0Hz), 6.52(d, 1H, J=8.1Hz), 5.96(d, 1H, J=10.5Hz), 4.36(m, 2H), 2.63(m, 7H), 1.38(m, 6H,), 1.26(s, 3H), 1.18(s, 3H).

^{13}CNMR of 1 (300MHz, CD_3OD) δ (ppm): 174.3, 173.5, 173.2, 171.1, 159.6, 144.4, 140.8, 136.6, 131.2, 128.4, 125.3, 122.5, 121.0, 120.0, 119.0, 115.0, 114.9, 106.7, 52.0, 46.8, 32.3, 30.5, 28.1, 16.6, 16.2.

ESI-MS of 1: 602 (M+Na), **HRMS of 1:** 602.22102 (C 29 H 33 N 5 Na 1 O 8)

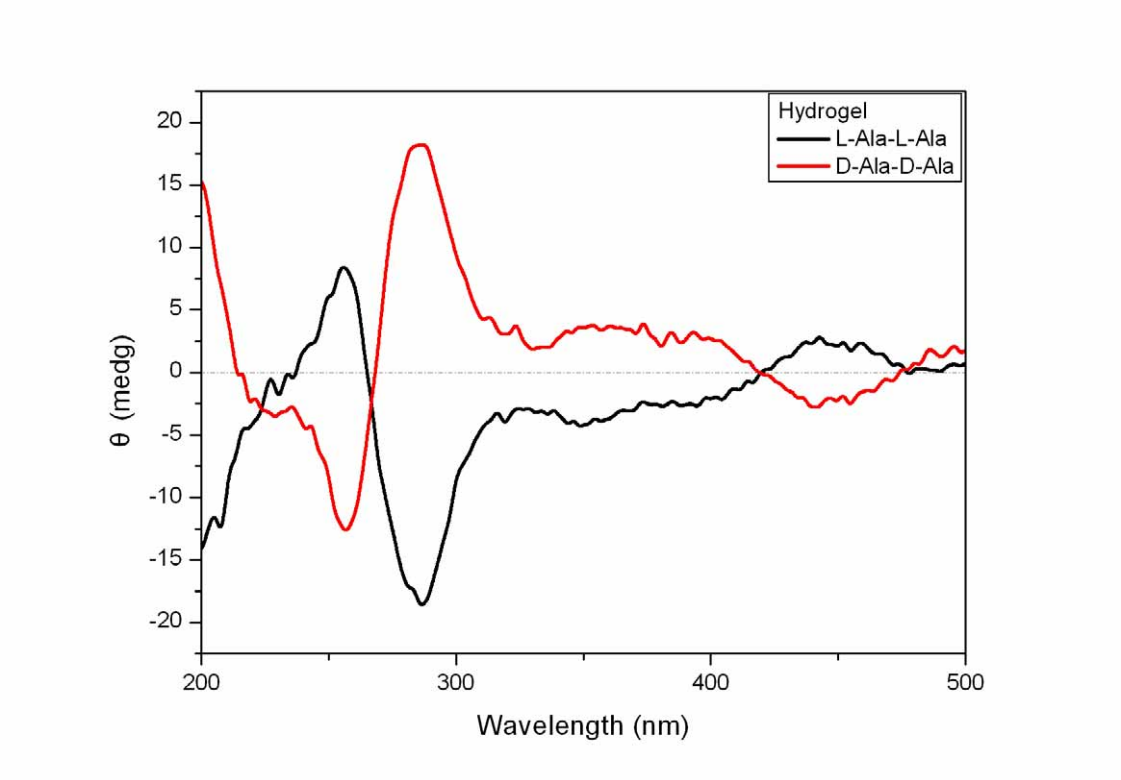
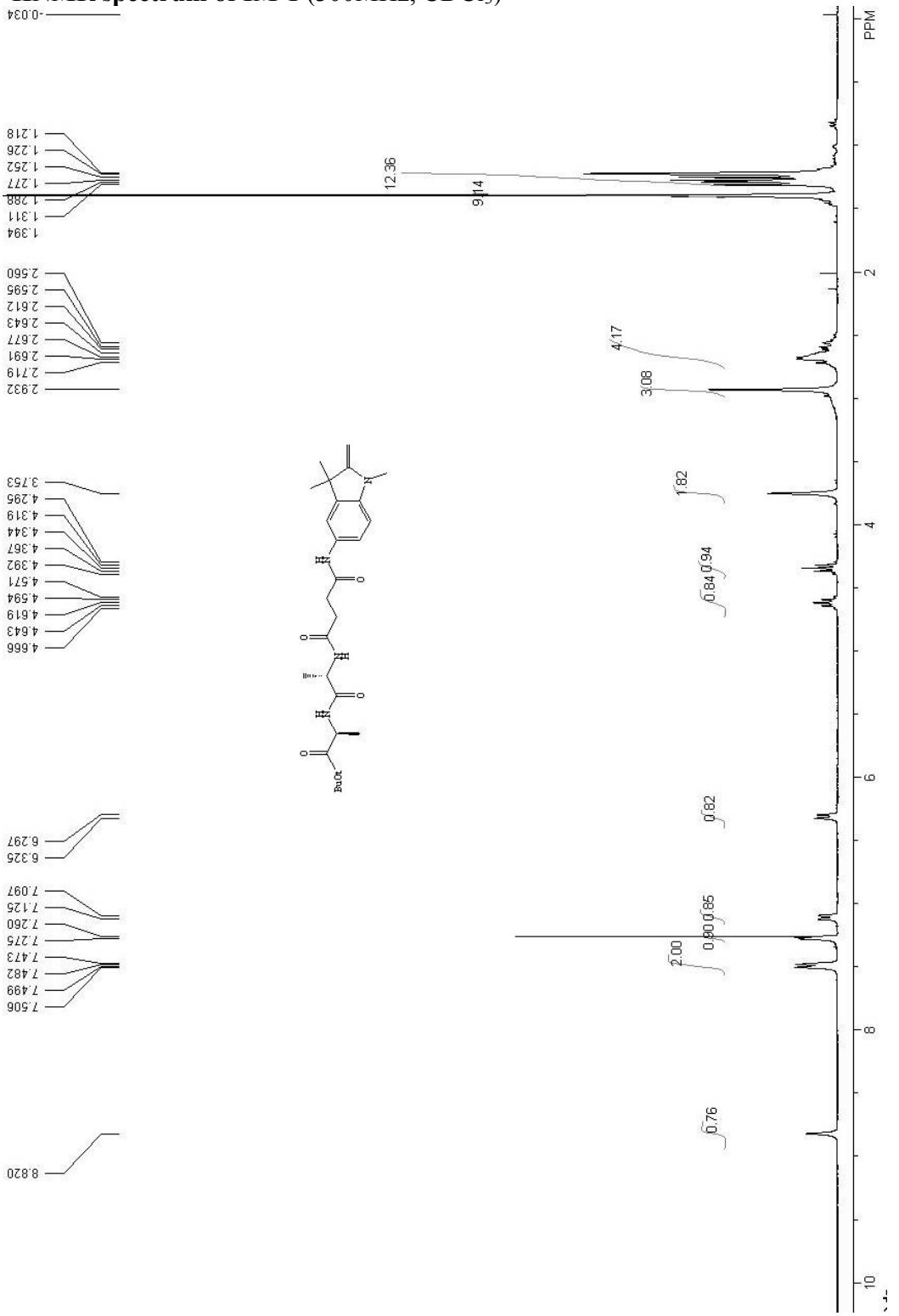
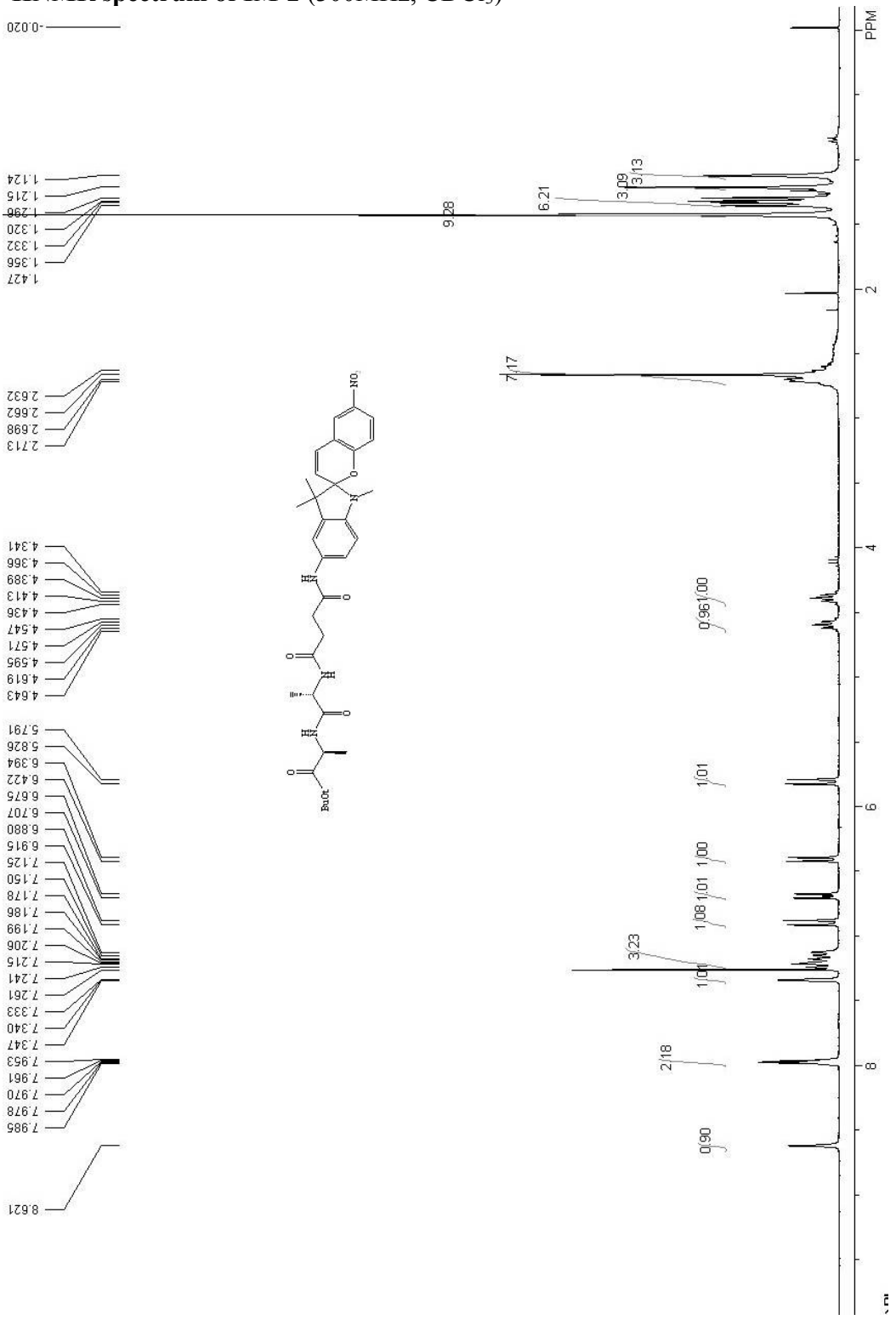


Fig. 1 Circular Dichronism spectra of the hydrogel formed by spiropyran-D-Ala-D-Ala and spiropyran-L-Ala-L-Ala

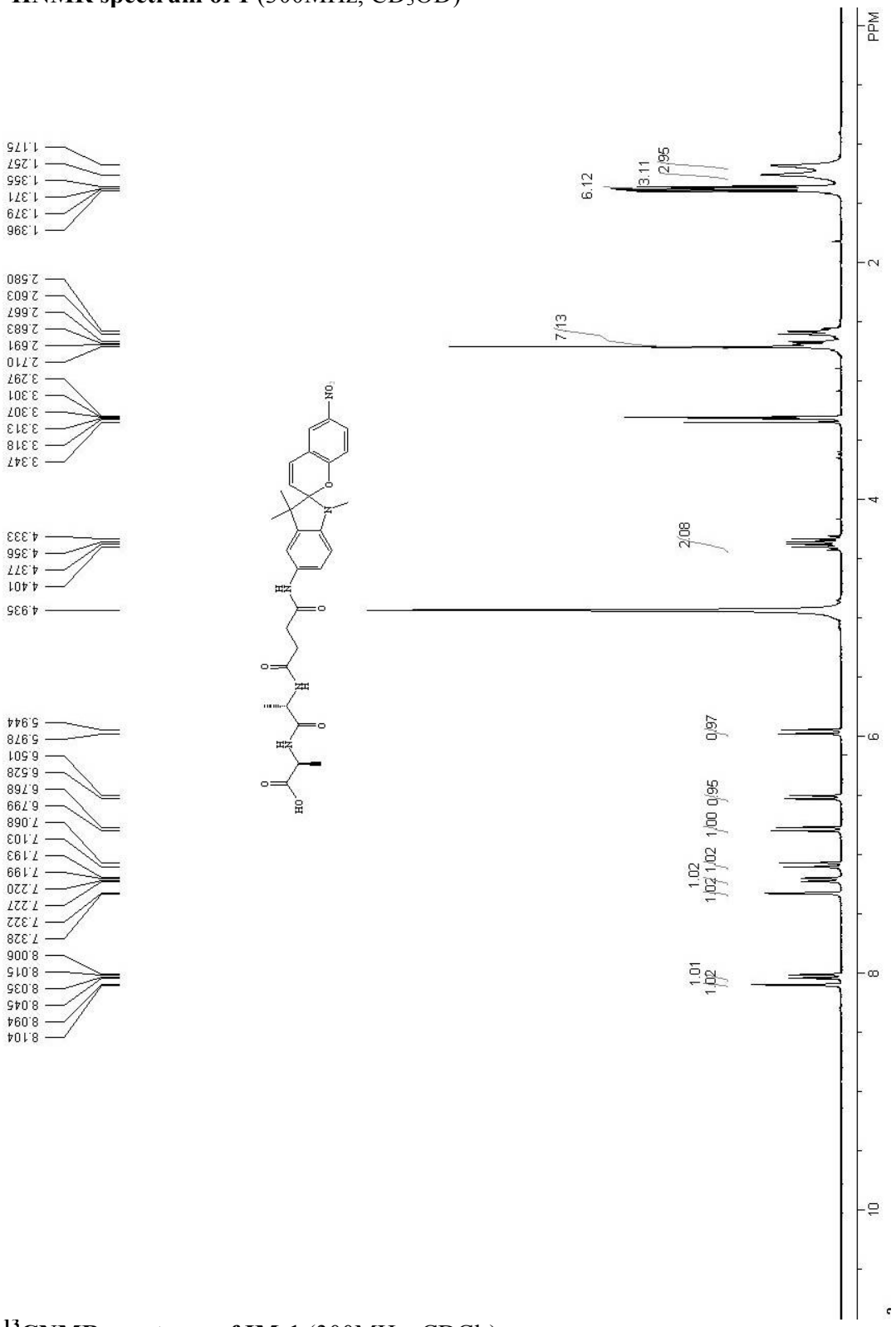
[1]HNMR spectrum of IM-1 (300MHz, $CDCl_3$)



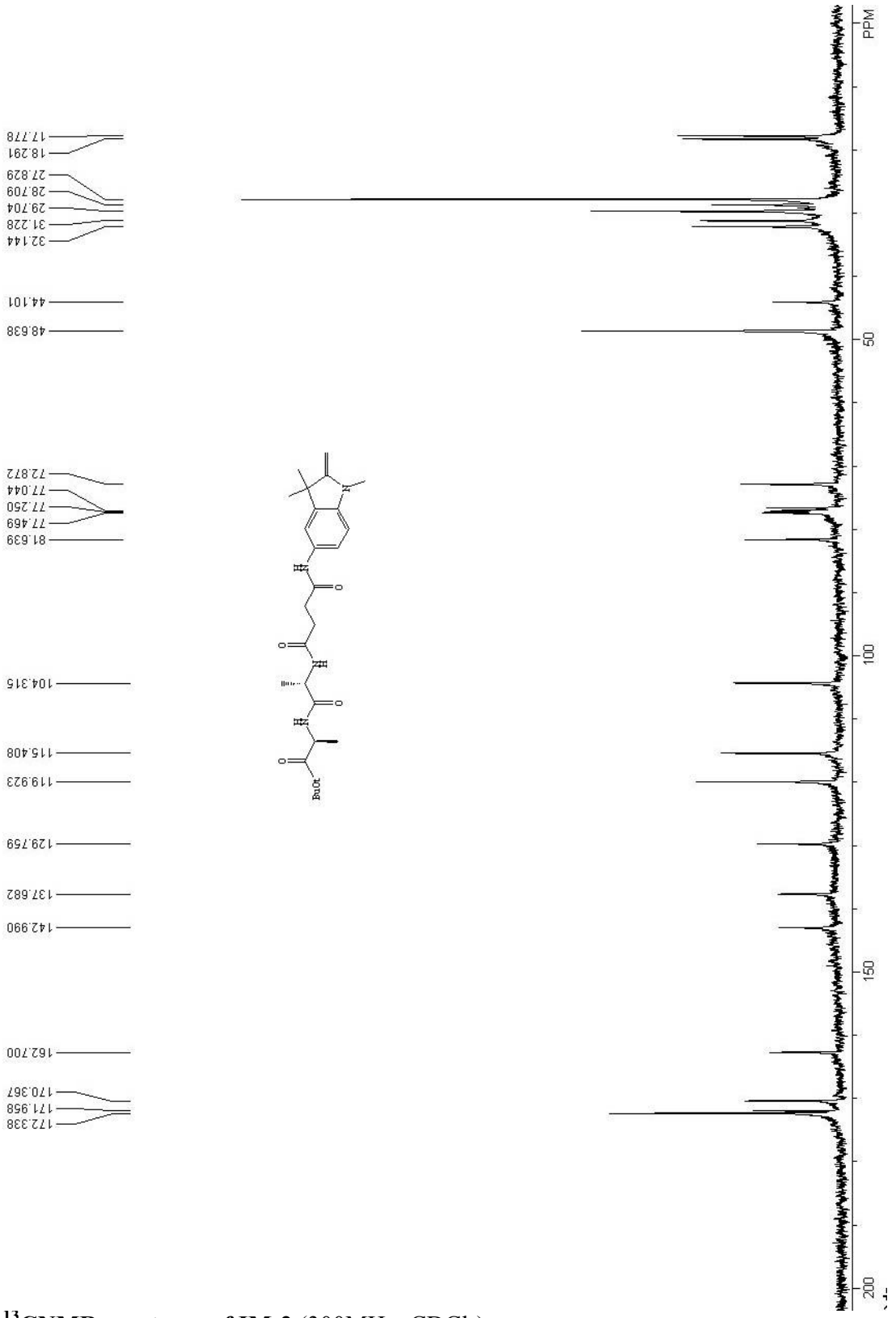
[1]HNMR spectrum of IM-2 (300MHz, $CDCl_3$)



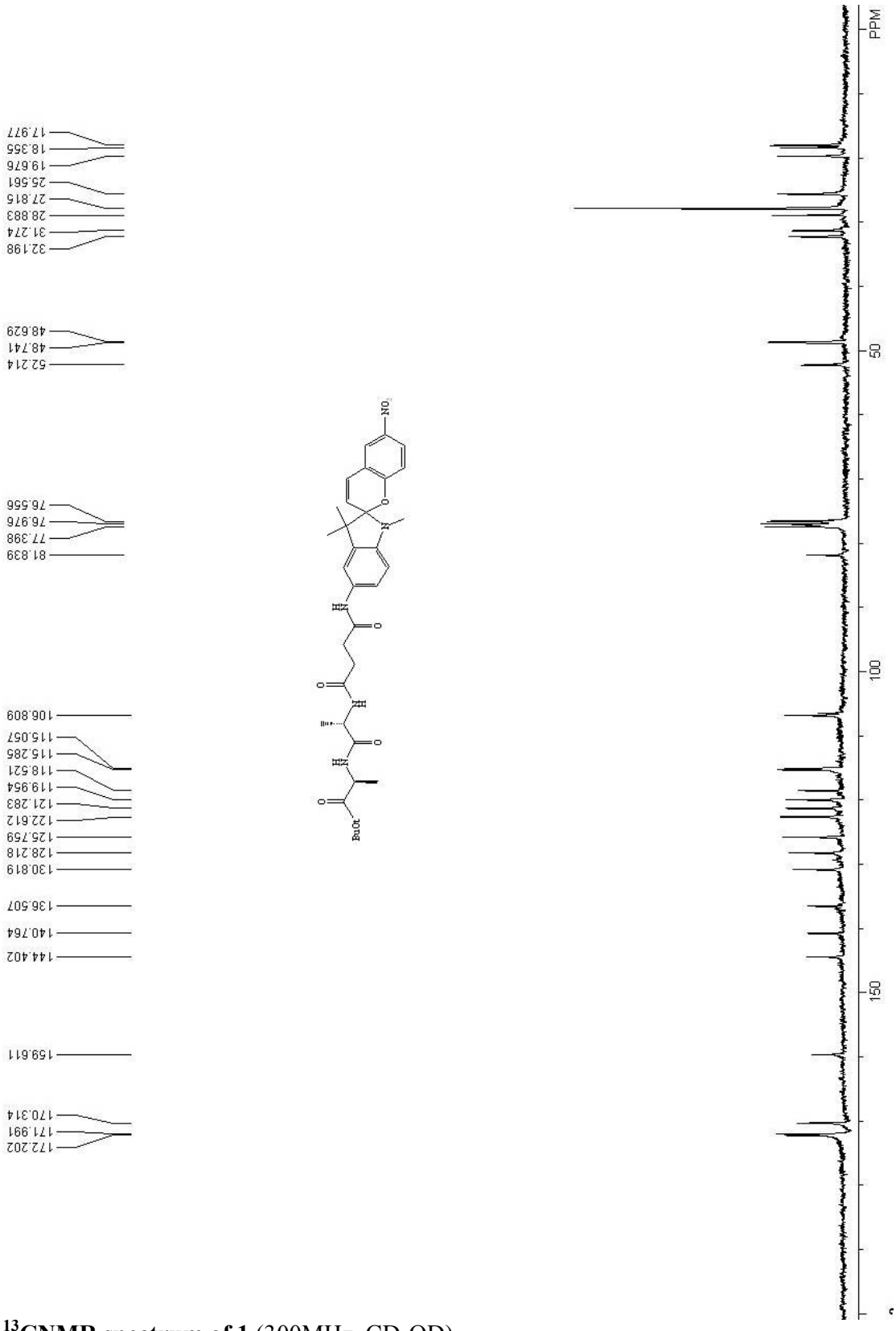
[1]HNMR spectrum of 1 (300MHz, CD_3OD)



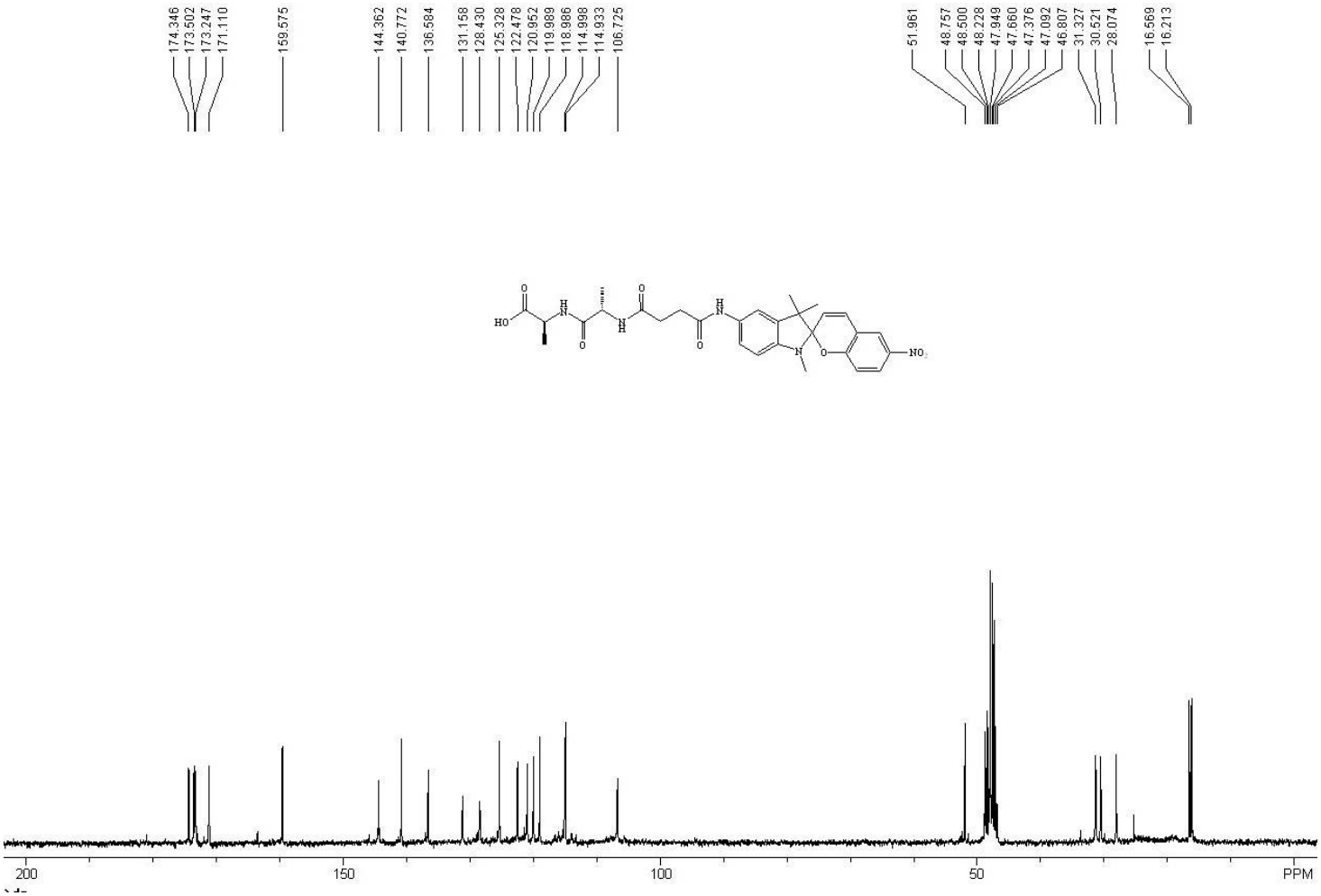
[13]CNMR spectrum of IM-1 (300MHz, $CDCl_3$)



^{13}CNMR spectrum of IM-2 (300MHz, $CDCl_3$)



^{13}CNMR spectrum of 1 (300MHz, CD_3OD)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173.247
171.110
159.575
144.362
140.772
136.584
131.158
128.430
125.328
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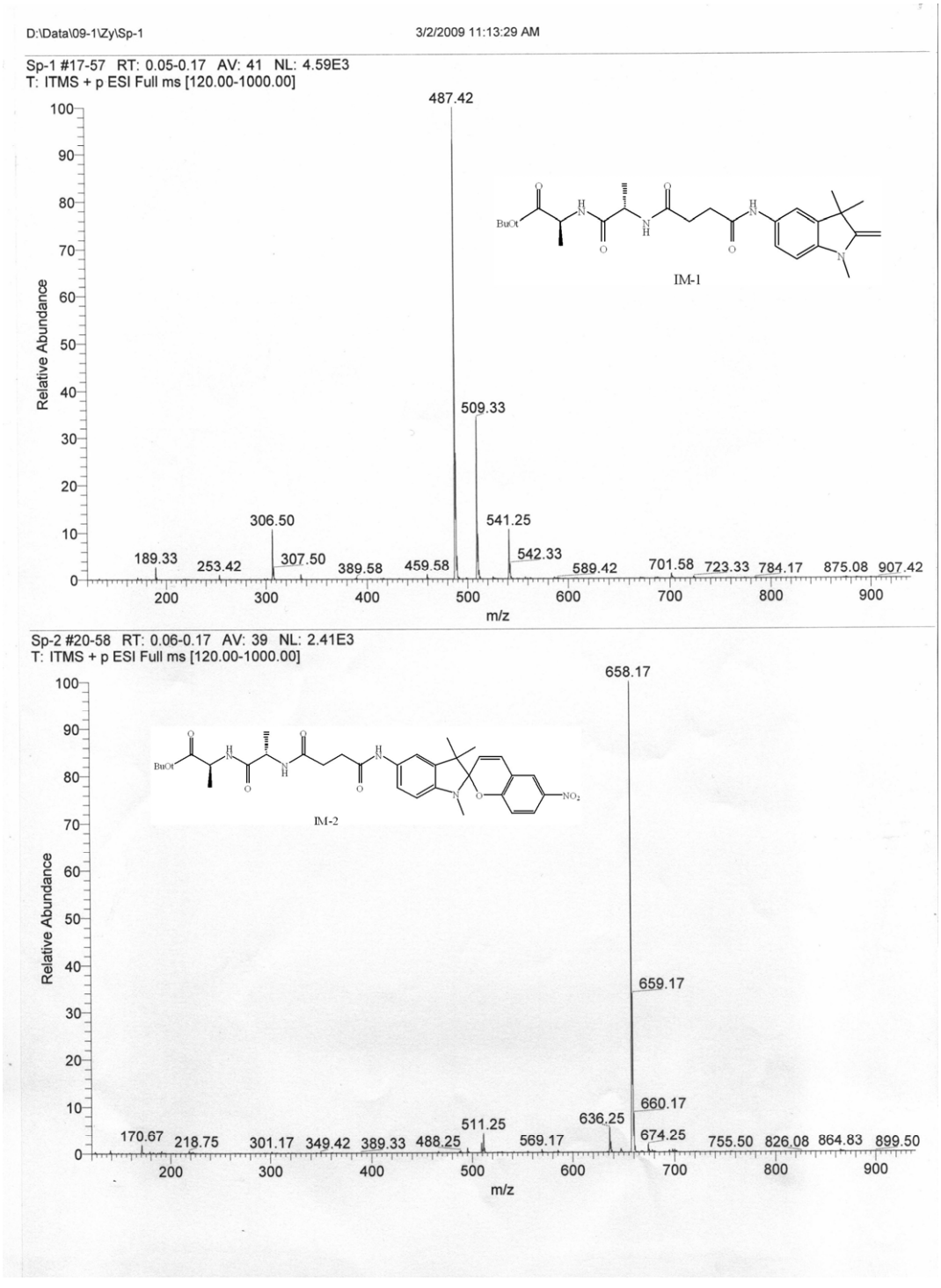
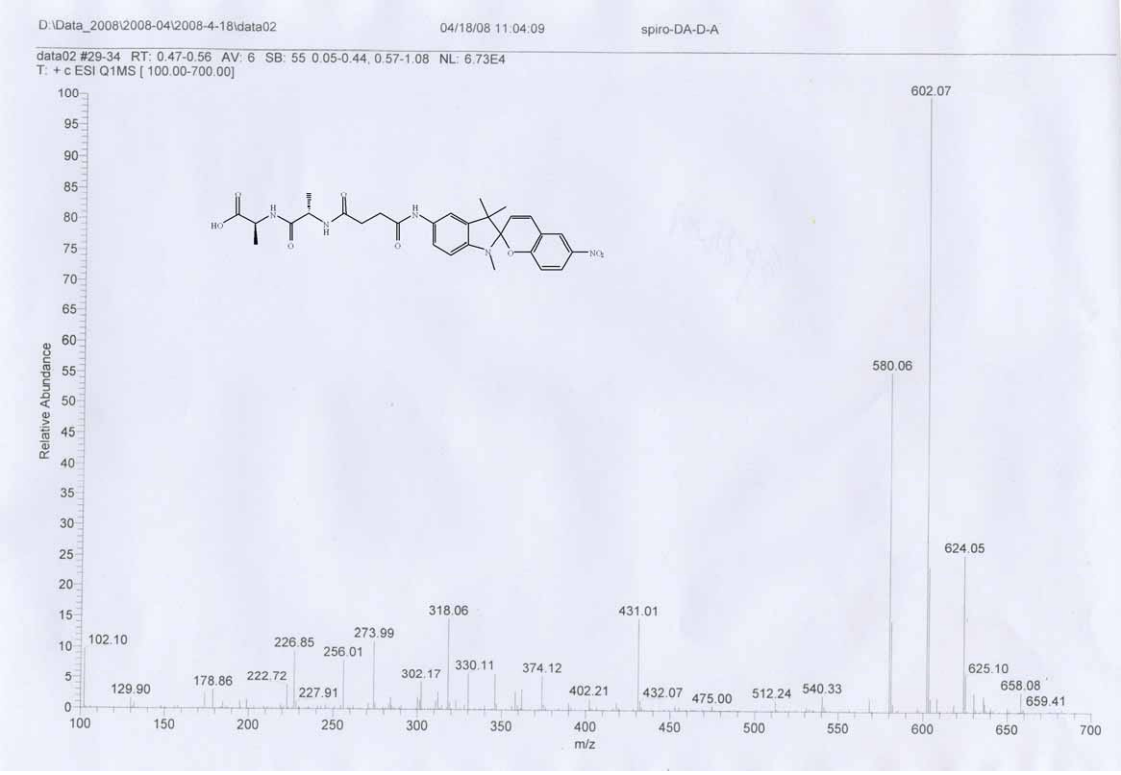
119.989
118.986
114.998
114.933
106.725
51.961
48.757
48.500
48.228
47.949
47.660
47.376
47.092
46.807
31.327
30.521
28.074
16.569
16.213
HO
O
H
N
O
H
N
O
O
H
N
N
O
NO2
200
150
100
50
PPM

ESI-MS of IM-1 and IM-2



ESI-MS of compound **1**



HRMS of IM-1

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High Resolution MS Data Report

Instrument

Bruker Daltonics, Inc. APEXIII 7.0 TESLA FTMS

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Sample Name	1
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Operator:	zfj
Ion Mass (Measured)	487.29067

Analysis Info

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C 25 H 43 O 9	0.012	487.29016	-1.04	-1.16	-0.51	4.50	ok	even
C 23 H 41 N 3 O 8	0.017	487.28882	-3.80	-4.05	-1.85	5.00	ok	odd

HRMS of IM-2

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High Resolution MS Data Report

Instrument

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Analysis Info

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C 34 H 163 N 5 O 1	0.216	658.28522	1.60	2.46	1.05	-44.00	ok	odd
C 35 H 43 N 2 Na 1 O 9	0.219	658.28608	2.91	3.86	1.91	15.00	ok	odd
C 34 H 166 N 2 Na 1 O 2	0.221	658.28415	-0.02	0.91	-0.01	-47.50	ok	even
C 34 H 44 N 1 O 12	0.225	658.28580	2.49	3.45	1.64	13.50	ok	even
C 33 H 41 N 5 Na 1 O 8	0.225	658.28473	0.87	1.54	0.57	15.50	ok	even
C 33 H 167 N 1 O 5	0.227	658.28388	-0.43	0.49	-0.28	-49.00	ok	odd
C 32 H 164 N 5 Na 1 O 1	0.227	658.28281	-2.05	-1.16	-1.35	-47.00	ok	odd
C 32 H 42 N 4 O 11	0.231	658.28446	0.45	1.13	0.30	14.00	ok	odd
C 32 H 45 N 1 Na 1 O 12	0.236	658.28340	-1.16	-0.42	-0.77	10.50	ok	even

HRMS of 1

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High Resolution MS Data Report

Instrument

Bruker Daltonics, Inc. APEXIII 7.0 TESLA FTMS

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Operator:	zfj
Ion Mass (Measured)	602.22102

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C 16 H 160 N 7 Na 1 O 8	0.129	602.22206	1.72	1.72	1.04	-60.00	ok	odd
C 29 H 159 N 1 O 5	0.172	602.22128	0.42	0.42	0.25	-49.00	ok	odd
C 29 H 33 N 5 Na 1 O 8	0.173	602.22213	1.84	1.84	1.11	15.50	ok	even
C 30 H 158 N 2 Na 1 O 2	0.178	602.22155	0.88	0.88	0.53	-47.50	ok	even
C 27 H 157 N 4 O 4	0.192	602.21994	-1.81	-1.81	-1.09	-48.50	ok	even
C 28 H 156 N 5 Na 1 O 1	0.199	602.22021	-1.35	-1.35	-0.81	-47.00	ok	odd
C 30 H 155 N 5 O 1	0.212	602.22262	2.64	2.64	1.59	-44.00	ok	odd