Electronic Supplementary Information (ESI)

Rational design of novel near-infrared fluorescent derivatives and its application for bioimaging

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1. General Information

Unless otherwise noted, materials were obtained from Aldrich and were used without further purification. All solvents were of analytical grade. The stock solution of **S-DCM-*N*** and **S-DCM-*P*** were prepared in DMSO and CH_2Cl_2. ^1H and ^{13}C NMR in $CDCl_3$ were obtained by a Bruker AV-400 spectrometer with tetramethylsilane (TMS) as internal standard. High Resolution Mass Spectra (HRMS) were obtained by a Waters LCT Premier XE spectrometer. Absorption spectra were measured on a Varian Cary 500 spectrophotometer at 25 °C. Fluorescence spectra were recorded on a Varian Cary Eclipse fluorescence spectrophotometer (1 cm quartz cell) at 25 °C. Deionized water was used to prepare all aqueous solutions. Cell imaging was performed with an inverted FL microscope (Nikon Eclipse Ti).

CCDC 1472796 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Table S1 Crystal data and structure refinement for **S-DCM-*N***

Crystal	**S-DCM-*N***
Molecular formula	$C_{37}H_{31}N_5O$
Formula weight	561.26
Temperature	293(2) K
Wavelength	0.71073 Å
Crystal system, space group	Monoclinic, P2(1)/n
Unit cell dimensions	a = 8.7921(9) Å alpha = 90 deg. b = 19.2348(19) Å beta = 98.119(2) deg. c = 20.091(2) Å gamma = 90 deg.
Volume	3363.7(6) $Å^3$
Z, Calculated density	4, 1.277 Mg/m^3
Absorption coefficient	0.231 mm^{-1}
F(000)	1352
Crystal size	0.176 × 0.121 × 0.059
Theta range for data collection	1.47 to 25.50 deg.
Limiting indices	$-10 \le h \le 10$, $-19 \le k \le 23$, $-24 \le l \le 24$
Reflections collected/unique	19540/6265 [R(int) = 0.0793]
Completeness to theta = 25.50	100.0%
Absorption correction	Empirical
Max. and min. transmission	1.00000 and 0.71820
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	6265/40/446
Goodness-of-fit on F^2	1.011
Final R indices [I>2sigma(I)]	R1 = 0.0663, wR2 = 0.1648
R indices (all data)	R1 = 0.1532, wR2 = 0.2018
Largest diff. peak and hole	0.204 and -0.377 $e.A^{-3}$

Table S2 Torsion angels [deg] for **S-DCM-*N***

atom	angel[deg]	atom	angel[deg]
C(5)-O(1)-C(1)-C(2)	-0.1(5)	C(7)-C(8)-C(17)-N(3)	-162(7)
C(5)-O(1)-C(1)-C(28)	-179.8(3)	C(16)-C(8)-C(17)-N(3)	20(7)
O(1)-C(1)-C(2)-C(3)	1.5(5)	C(4)-C(5)-C(18)-C(19)	-176.8(3)
C(28)-C(1)-C(2)-C(3)	-178.9(3)	O(1)-C(5)-C(18)-C(19)	3.2(5)
C(1)-C(2)-C(3)-C(6)	-178.9(3)	C(5)-C(18)-C(19)-C(20)	-178.3(3)
C(1)-C(2)-C(3)-C(4)	-1.6(5)	C(18)-C(19)-C(20)-C(25)	-0.6(5)
C(6)-C(3)-C(4)-C(5)	178.0(3)	C(18)-C(19)-C(20)-C(21)	174.9(3)
C(2)-C(3)-C(4)-C(5)	0.6(5)	C(25)-C(20)-C(21)-C(22)	2.3(5)
C(3)-C(4)-C(5)-O(1)	0.7(5)	C(19)-C(20)-C(21)-C(22)	-173.5(3)
C(3)-C(4)-C(5)-C(18)	-179.3(3)	C(20)-C(21)-C(22)-C(23)	0.9(6)
C(1)-O(1)-C(5)-C(4)	-0.9(5)	C(27)-N(4)-C(23)-C(22)	-178.1(4)
C(1)-O(1)-C(5)-C(18)	179.0(3)	C(26)-N(4)-C(23)-C(22)	-4.4(6)
C(4)-C(3)-C(6)-C(15)	0.4(5)	C(27)-N(4)-C(23)-C(24)	0.9(6)
C(2)-C(3)-C(6)-C(15)	177.5(3)	C(26)-N(4)-C(23)-C(24)	174.6(4)
C(4)-C(3)-C(6)-C(7)	178.3(3)	C(21)-C(22)-C(23)-N(4)	175.1(3)
C(2)-C(3)-C(6)-C(7)	-4.5(5)	C(21)-C(22)-C(23)-C(24)	-3.9(5)
C(3)-C(6)-C(7)-C(8)	150.3(3)	N(4)-C(23)-C(24)-C(25)	-175.3(3)
C(15)-C(6)-C(7)-C(8)	-31.7(5)	C(22)-C(23)-C(24)-C(25)	3.7(5)
C(3)-C(6)-C(7)-C(9)	-35.1(5)	C(23)-C(24)-C(25)-C(20)	-0.6(5)
C(15)-C(6)-C(7)-C(9)	142.8(3)	C(21)-C(20)-C(25)-C(24)	-2.4(5)
C(6)-C(7)-C(8)-C(16)	-17.5(5)	C(19)-C(20)-C(25)-C(24)	173.3(3)
C(9)-C(7)-C(8)-C(16)	167.8(3)	C(2)-C(1)-C(28)-C(29)	-176.3(4)
C(6)-C(7)-C(8)-C(17)	164.7(3)	O(1)-C(1)-C(28)-C(29)	3.4(5)
C(9)-C(7)-C(8)-C(17)	-10.1(5)	C(1)-C(28)-C(29)-C(30)	-177.2(3)
C(8)-C(7)-C(9)-C(10)	-47.5(5)	C(28)-C(29)-C(30)-C(35)	-173.3(3)
C(6)-C(7)-C(9)-C(10)	137.8(3)	C(28)-C(29)-C(30)-C(31)	8.8(6)
C(8)-C(7)-C(9)-C(14)	128.7(3)	C(35)-C(30)-C(31)-C(32)	1.7(5)
C(6)-C(7)-C(9)-C(14)	-46.0(5)	C(29)-C(30)-C(31)-C(32)	179.7(3)
C(14)-C(9)-C(10)-C(11)	-1.2(5)	C(30)-C(31)-C(32)-C(33)	0.4(6)
C(7)-C(9)-C(10)-C(11)	175.1(3)	C(37)-N(5)-C(33)-C(34)	-1.2(6)
C(9)-C(10)-C(11)-C(12)	-0.2(6)	C(36)-N(5)-C(33)-C(34)	176.5(4)
C(10)-C(11)-C(12)-C(13)	1.0(7)	C(37)-N(5)-C(33)-C(32)	178.7(4)
C(11)-C(12)-C(13)-C(14)	-0.4(7)	C(36)-N(5)-C(33)-C(32)	-3.6(6)

C(12)-C(13)-C(14)-C(9)	-1.1(6)	C(31)-C(32)-C(33)-N(5)	177.5(3)
C(10)-C(9)-C(14)-C(13)	1.9(5)	C(31)-C(32)-C(33)-C(34)	-2.5(5)
C(7)-C(9)-C(14)-C(13)	-174.5(4)	N(5)-C(33)-C(34)-C(35)	-177.5(4)
C(3)-C(6)-C(15)-N(1)	95(112)	C(32)-C(33)-C(34)-C(35)	2.6(5)
C(7)-C(6)-C(15)-N(1)	-83(12)	C(33)-C(34)-C(35)-C(30)	-0.5(6)
C(7)-C(8)-C(16)-N(2)	-118(7)	C(31)-C(30)-C(35)-C(34)	-1.7(5)
C(17)-C(8)-C(16)-N(2)	60(7)	C(29)-C(30)-C(35)-C(34)	-179.7(3)

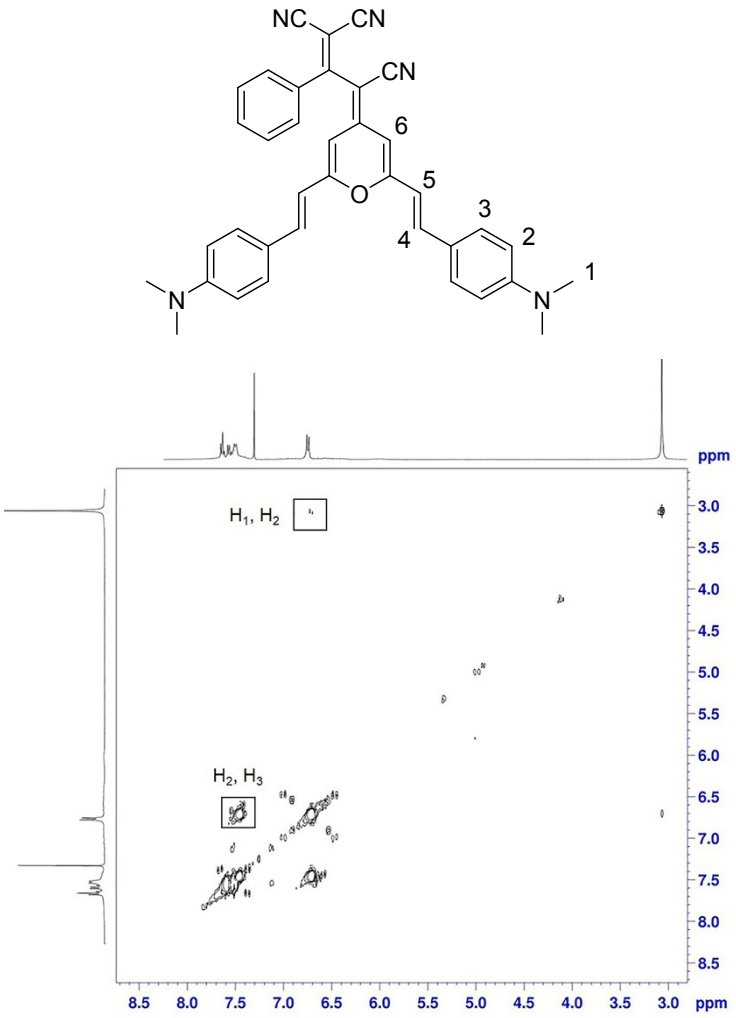


Fig. S1 Two-dimensional ^{1}H-^{1}H COSY NMR spectroscopy of **S-DCM-*N***

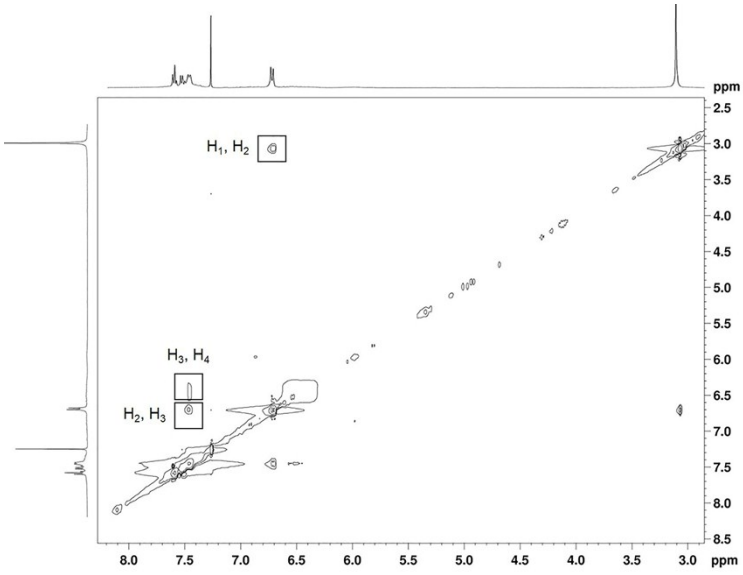


Fig. S2 Two-dimensional ^{1}H-^{1}H NOESY NMR spectroscopy of **S-DCM-*N***

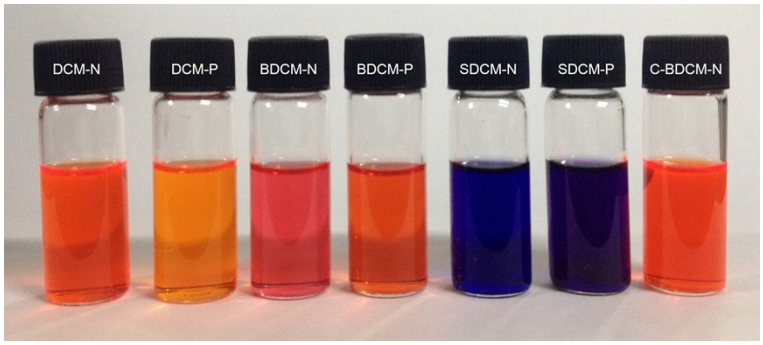


Fig. S3 Pictures of DCM analogues in CH_2Cl_2

S-DCM-*N*

Table S3 absorption and emission spectral properties of **S-DCM-*N*** in different solvents

solvent	λ_{max} (nm)	ε ($M^{-1}cm^{-1}$)	λ_{em} (nm)	Φ_f
CH_2Cl_2	580	38350	732	4.62
$CHCl_3$	581	53000	698	6.02
CH_3CN	571	43000	--	--
C_2H_5OH	575	40800	--	--
CH_3OH	575	51800	--	--
Toluene	566	44100	664	1.03
DMSO	590	47900	--	--

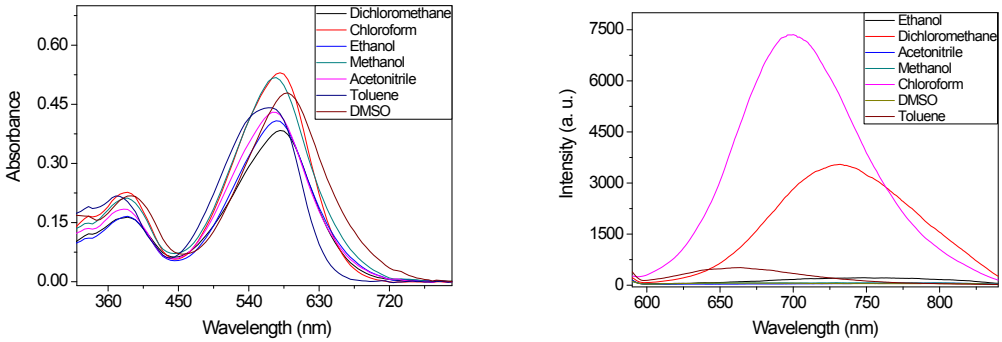


Fig. S4 Absorption and emission spectra of **S-DCM-*N*** (10 μM) in various solvents

S-DCM-*P*

Table S4 Absorption and emission spectral properties of **S-DCM-*P*** in various solvents

solvent	λ_{max} (nm)	ε ($M^{-1}cm^{-1}$)	λ_{em} (nm)	Φ_f
CH_2Cl_2	568	37300	728	3.52
$CHCl_3$	570	45100	704	4.36
CH_3CN	554	45900	--	--
C_2H_5OH	560	38400	--	--
CH_3OH	558	42500	--	--
Toluene	548	41200	656	0.92
DMSO	567	40700	--	--

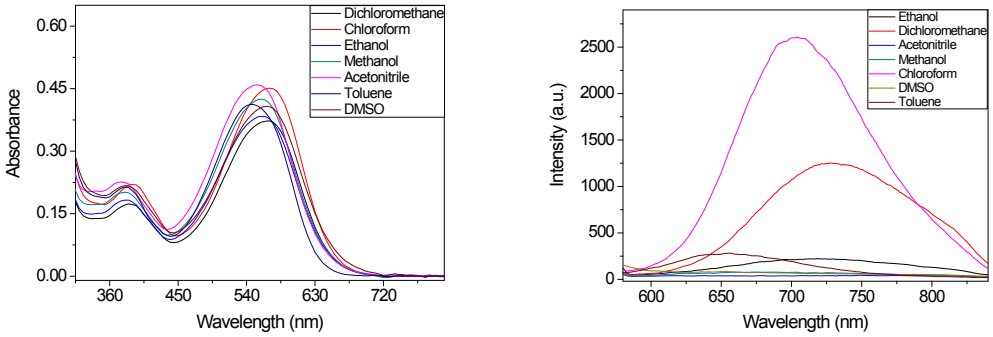


Fig. S5 Absorption and emission spectra of **S-DCM-*P*** (10 μM) in various solvents

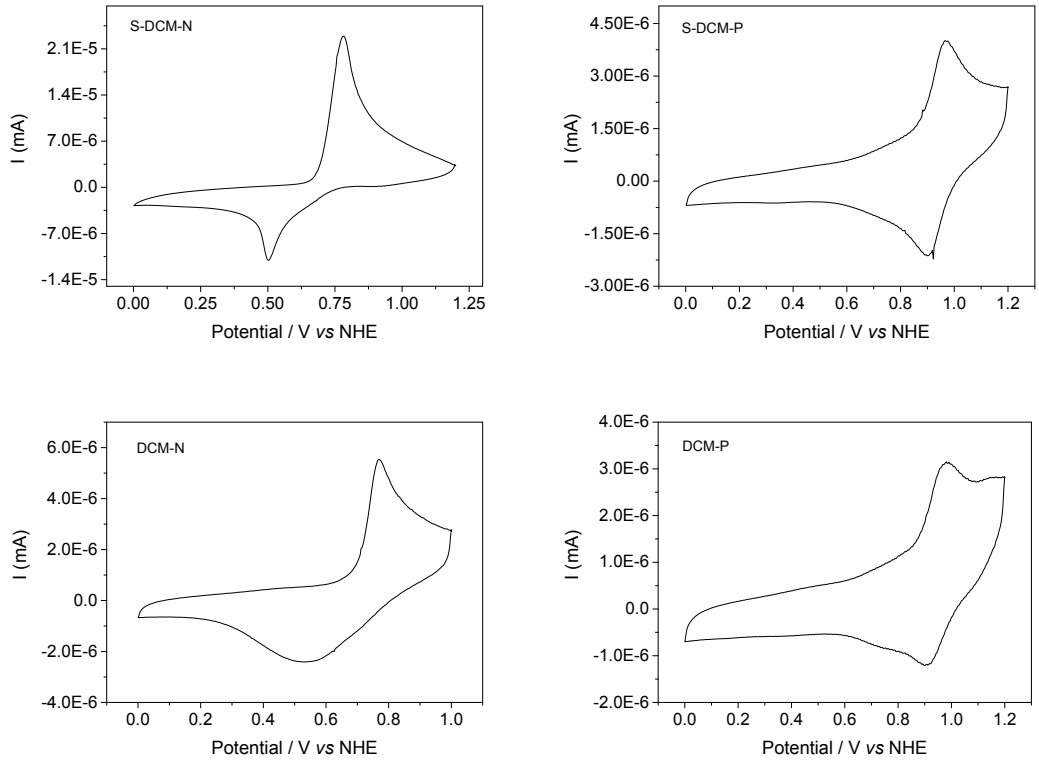
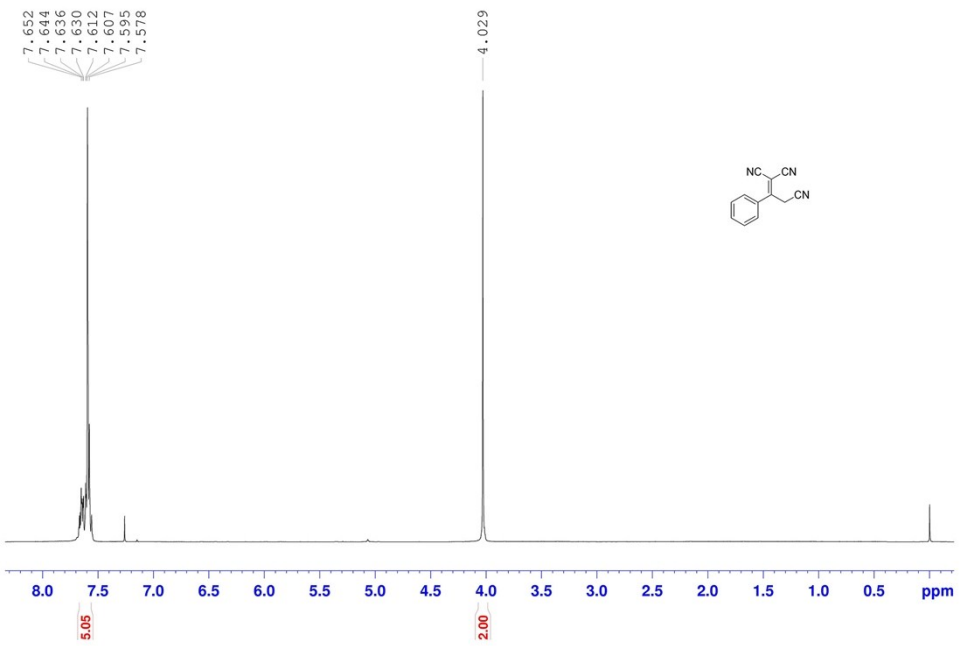


Fig. S6 Cyclic voltammograms of DCM analogues measured in CH_2Cl_2

(a)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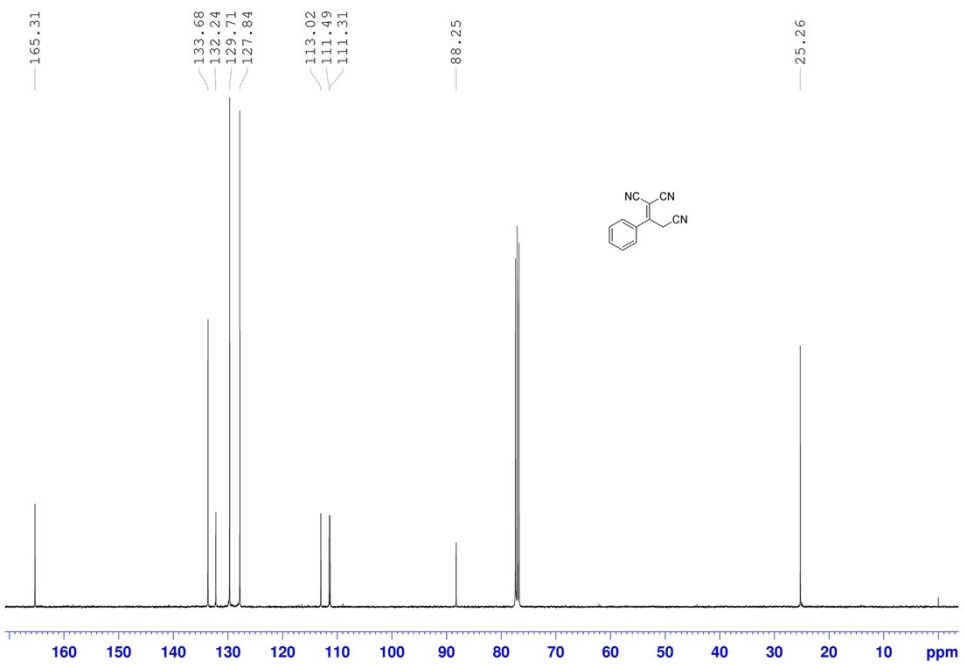
7.644
7.636
7.630
7.612
7.607
7.595
7.578
4.029
NC
CN
CN
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
5.05
2.00

(b)


165.31
133.68
132.24
129.71
127.84
113.02
111.49
111.31
88.25
25.26
NC
CN
CN
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

(c)

Single Mass Analysis
Tolerance = 100.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
6 formula(e) evaluated with 2 results within limits (up to 1 closest results for each mass)
Elements Used:
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WH-ZHU ECUST institute of Fine Chem 07-Jan-2013 20:31:14

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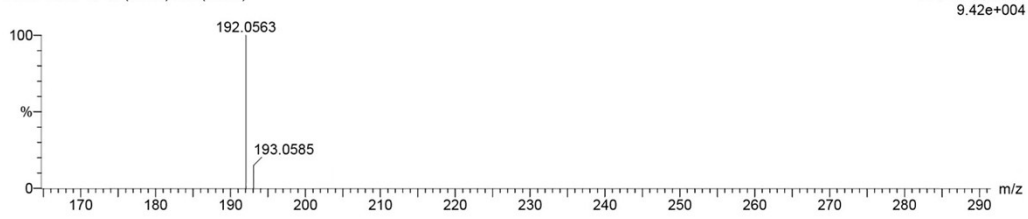
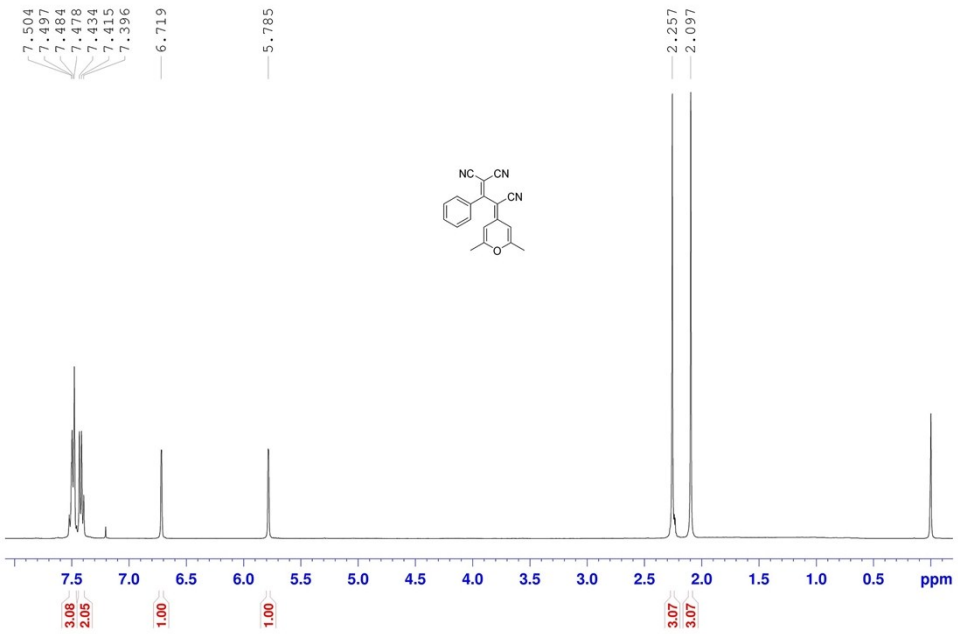
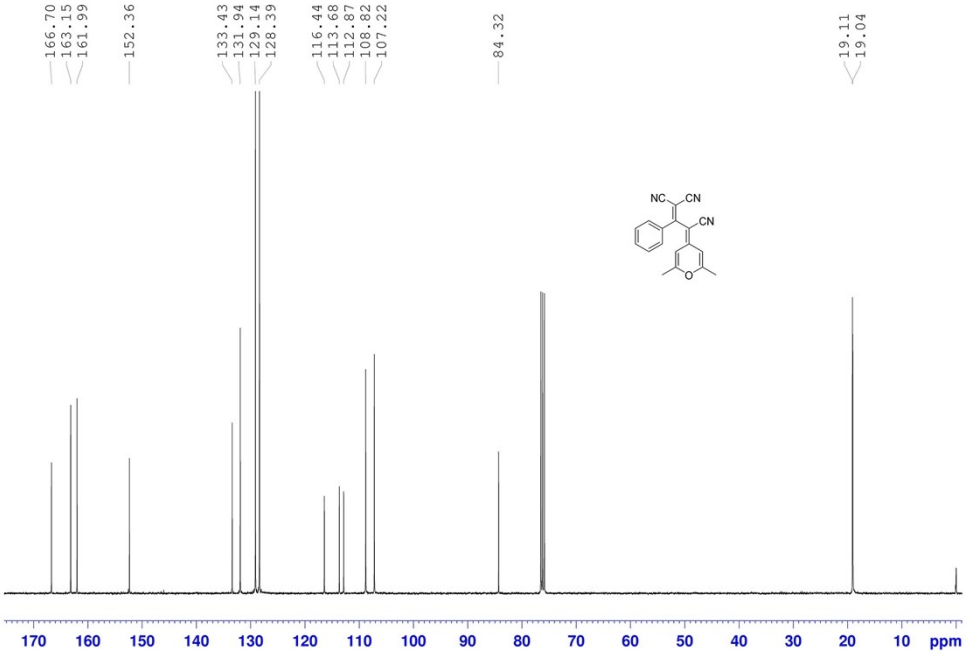


Minimum:				-1.5			
Maximum:		100.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
192.0563	192.0562	0.1	0.5	11.5	50.3	0.0	C12 H6 N3

Fig. S7 ^{1}H NMR (a), ^{13}C NMR (b) and HRMS (c) of **2**





Elemental Composition Report **Page 1**

Single Mass Analysis
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Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
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WH-ZHU ECUST institute of Fine Chem 06-Jan-2016 21:02:37
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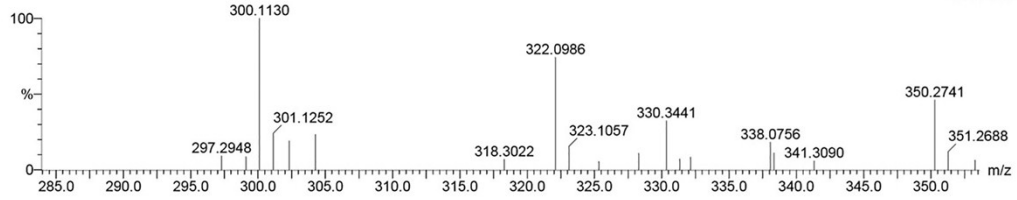

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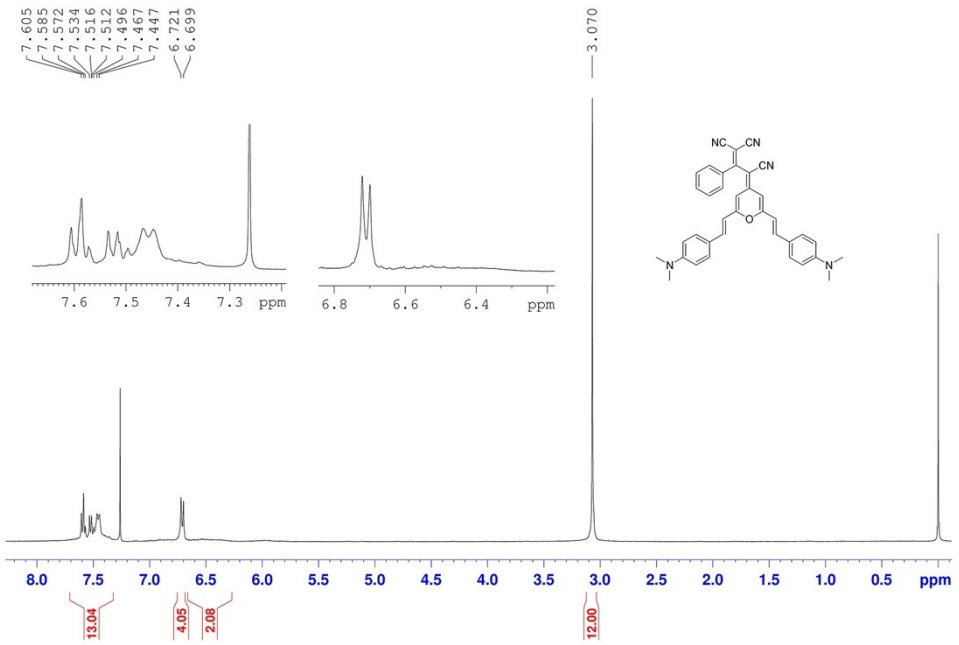
300.1130	300.1137	-0.7	-2.3	14.5	42.9	0.0	C19 H14 N3 O

Fig. S8 ^{1}H NMR, ^{13}C NMR and HRMS of **3**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7.572
7.534
7.516
7.512
7.496
7.467
7.447
6.721
6.699
3.070
NC
CN
CN
O
N
7.6 7.5 7.4 7.3 ppm
6.8 6.6 6.4 ppm
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
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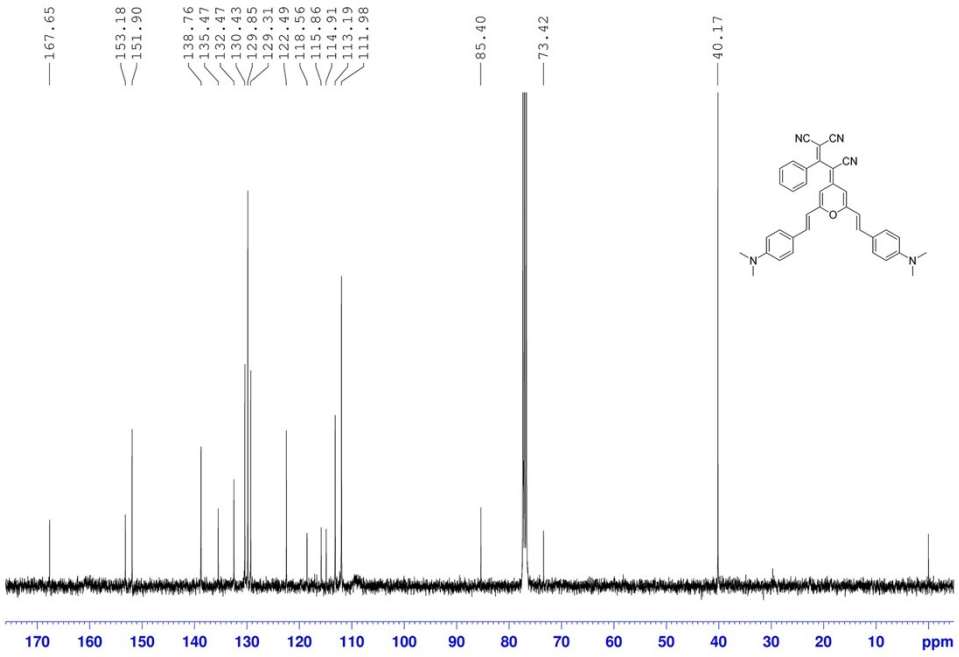
4.05
2.08
12.00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138.76
135.47
132.47
130.43
129.85
129.31
122.49
118.56
115.86
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113.19
111.98
85.40
73.42
40.17
NC
CN
CN
O
N
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

Single Mass Analysis
Tolerance = 50.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
68 formula(e) evaluated with 3 results within limits (up to 1 closest results for each mass)
Elements Used:
C: 0-37 H: 0-33 N: 0-5 O: 0-7

WH-ZHU ECUST institute of Fine Chem 10-Jun-2015 22:22:56

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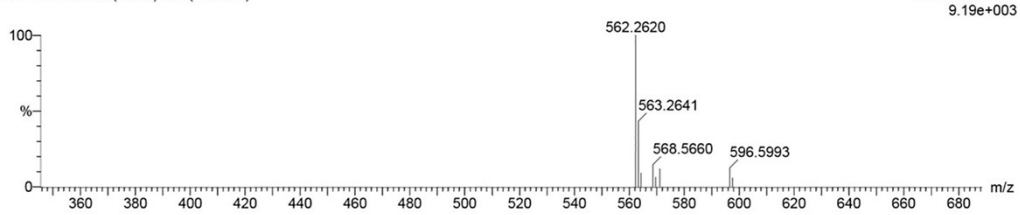

Minimum:				-1.5			
Maximum:		300.0	50.0	100.0			
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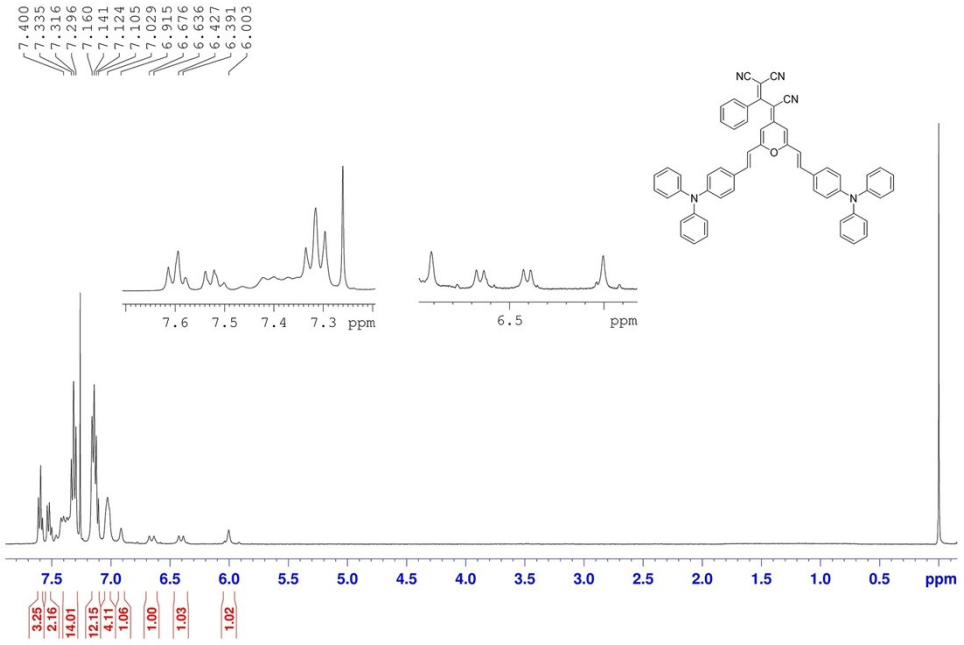
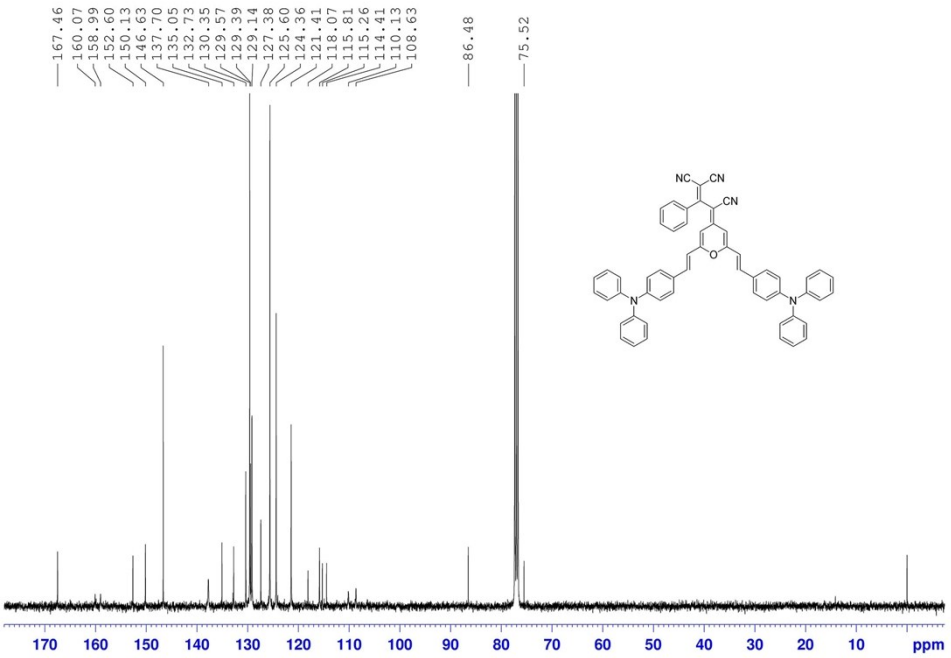
562.2620	562.2607	1.3	2.3	24.5	8.2	0.0	C37 H32 N5 O

Fig. S9 ^{1}H NMR, ^{13}C NMR and HRMS of **S-DCM-*N***





Single Mass Analysis
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Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
73 formula(e) evaluated with 6 results within limits (up to 1 closest results for each mass)
Elements Used:
C: 0-57 H: 0-40 N: 0-5 O: 0-7

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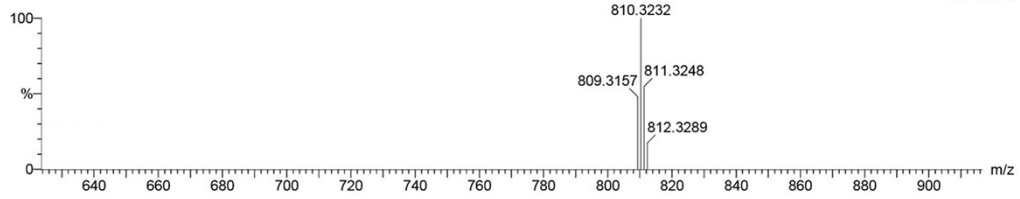

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Maximum:		300.0	50.0	100.0			
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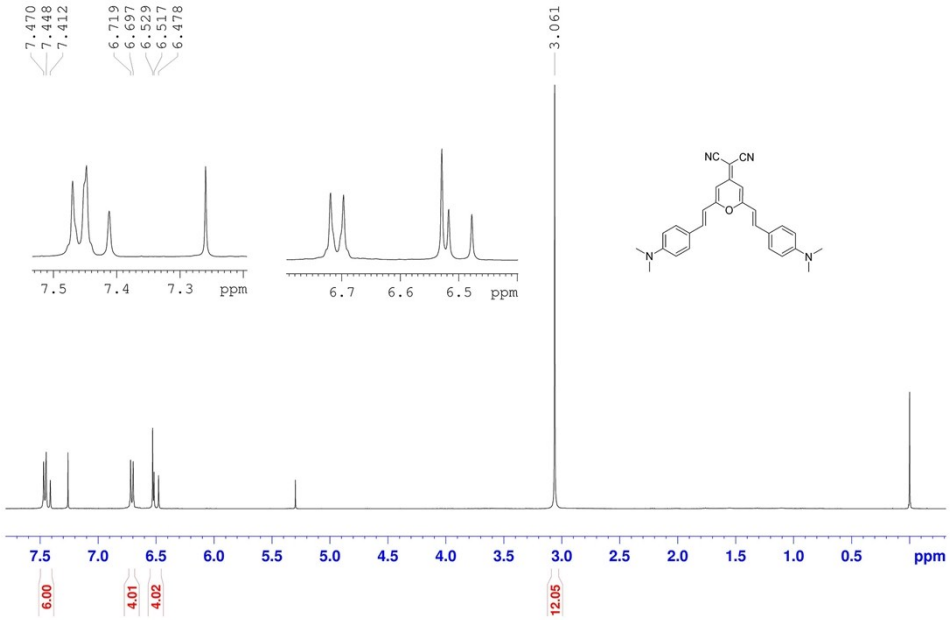
Fig. S10 ^{1}H NMR, ^{13}C NMR and HRMS of **S-DCM-*P***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6.517
6.478
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CN
O
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7.3
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6.6
6.5
ppm
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6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
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1.0
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ppm
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4.01
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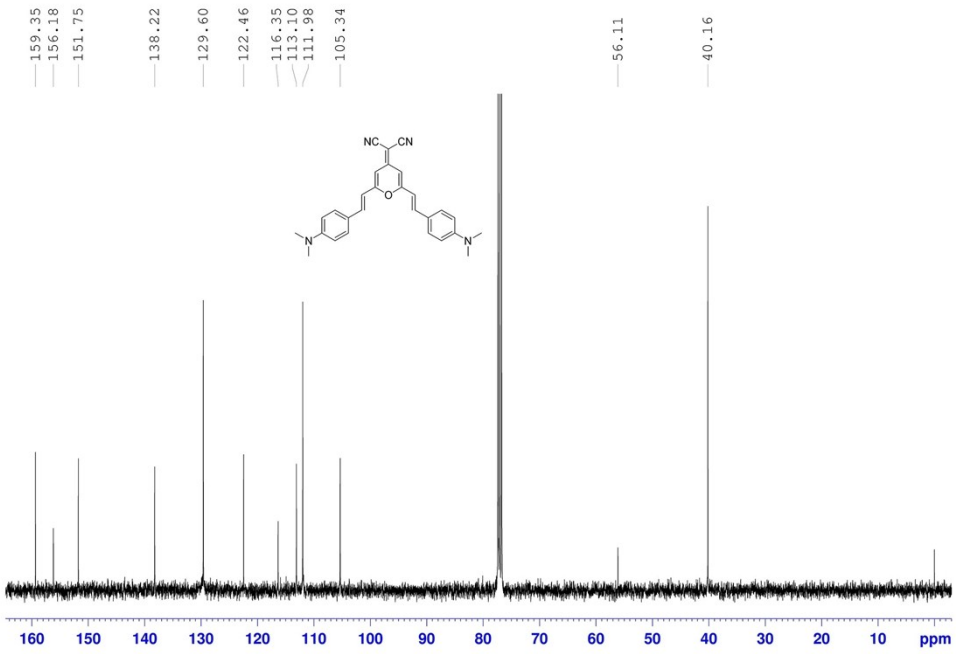
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113.10
111.98
105.34
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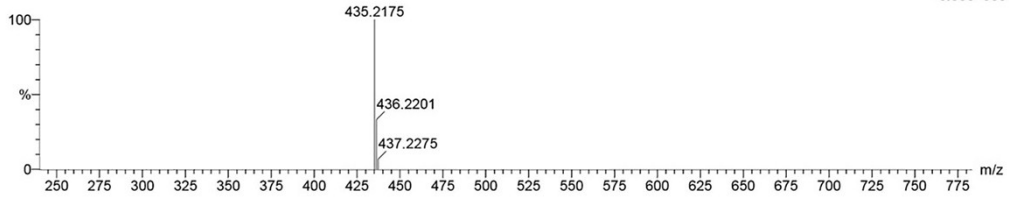
40.16
NC
CN
O
N
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

Single Mass Analysis
Tolerance = 50.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
6 formula(e) evaluated with 1 results within limits (up to 1 closest results for each mass)
Elements Used:
C: 0-28 H: 0-40 N: 0-4 O: 0-1

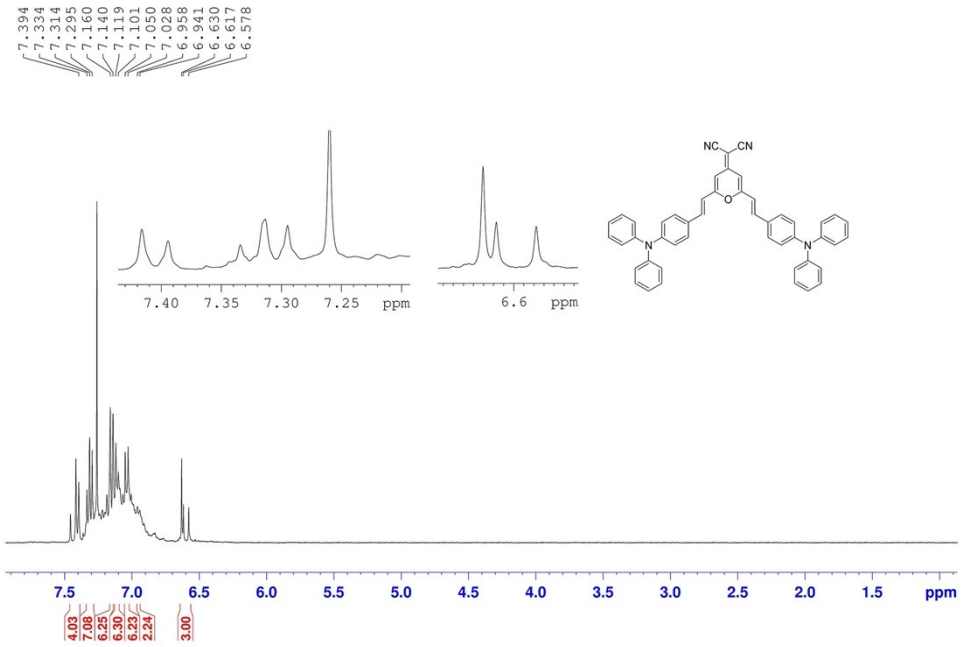
WH-ZHU ECUST institute of Fine Chem 10-Jun-2015 22:30:14

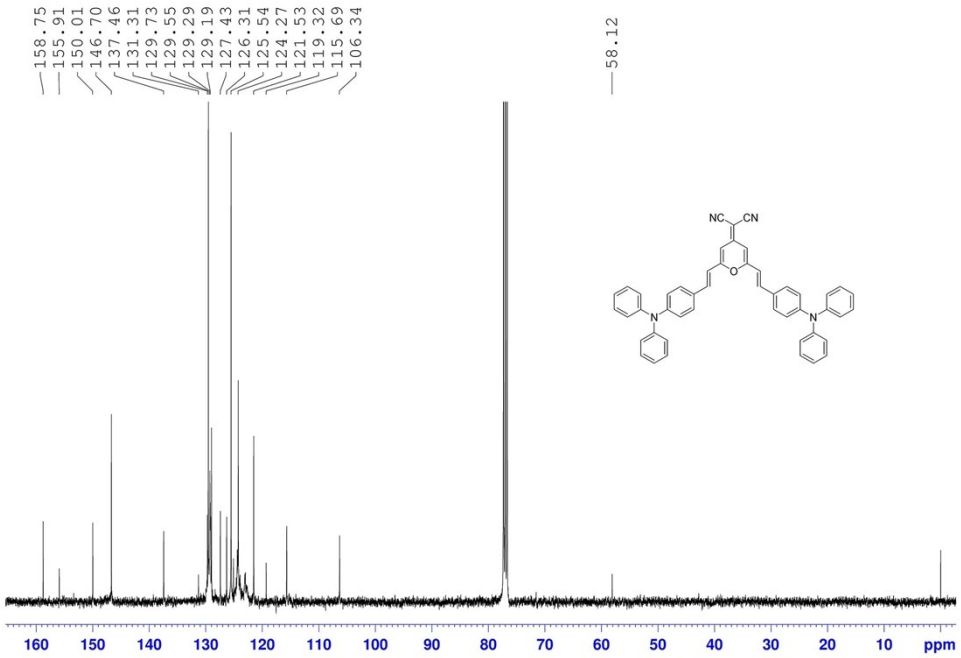
ZW-WXH-3 206 (1.365) Cm (204:206) 1: TOF MS ES+ 6.08e+003



Minimum:		300.0	50.0	-1.5			
Maximum:				100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
435.2175	435.2185	-1.0	-2.3	17.5	7.1	0.0	C28 H27 N4 O

Fig. S11 ^{1}H NMR, ^{13}C NMR and HRMS of **DCM-*N***

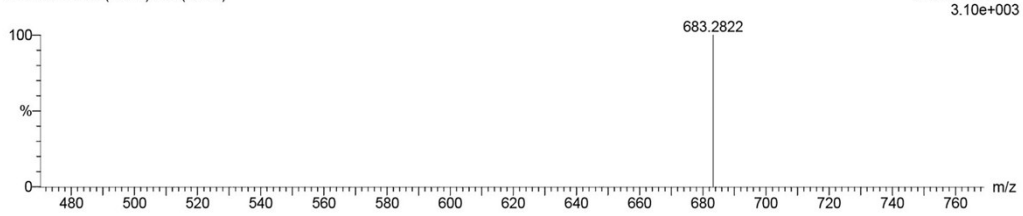




Single Mass Analysis
Tolerance = 50.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
5 formula(e) evaluated with 1 results within limits (up to 1 closest results for each mass)
Elements Used:
C: 0-48 H: 0-44 N: 0-4 O: 0-2

WH-ZHU ECUST institute of Fine Chem 03-Jun-2015 21:47:12
ZW-WXH-1 41 (0.344) Cm (39:41) 1: TOF MS ES+ 3.10e+003



Minimum:				-1.5			
Maximum:		300.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
683.2822	683.2811	1.1	1.6	33.5	8.5	0.0	C48 H35 N4 O

Fig. S12 ^{1}H NMR, ^{13}C NMR and HRMS of **DCM-*P***

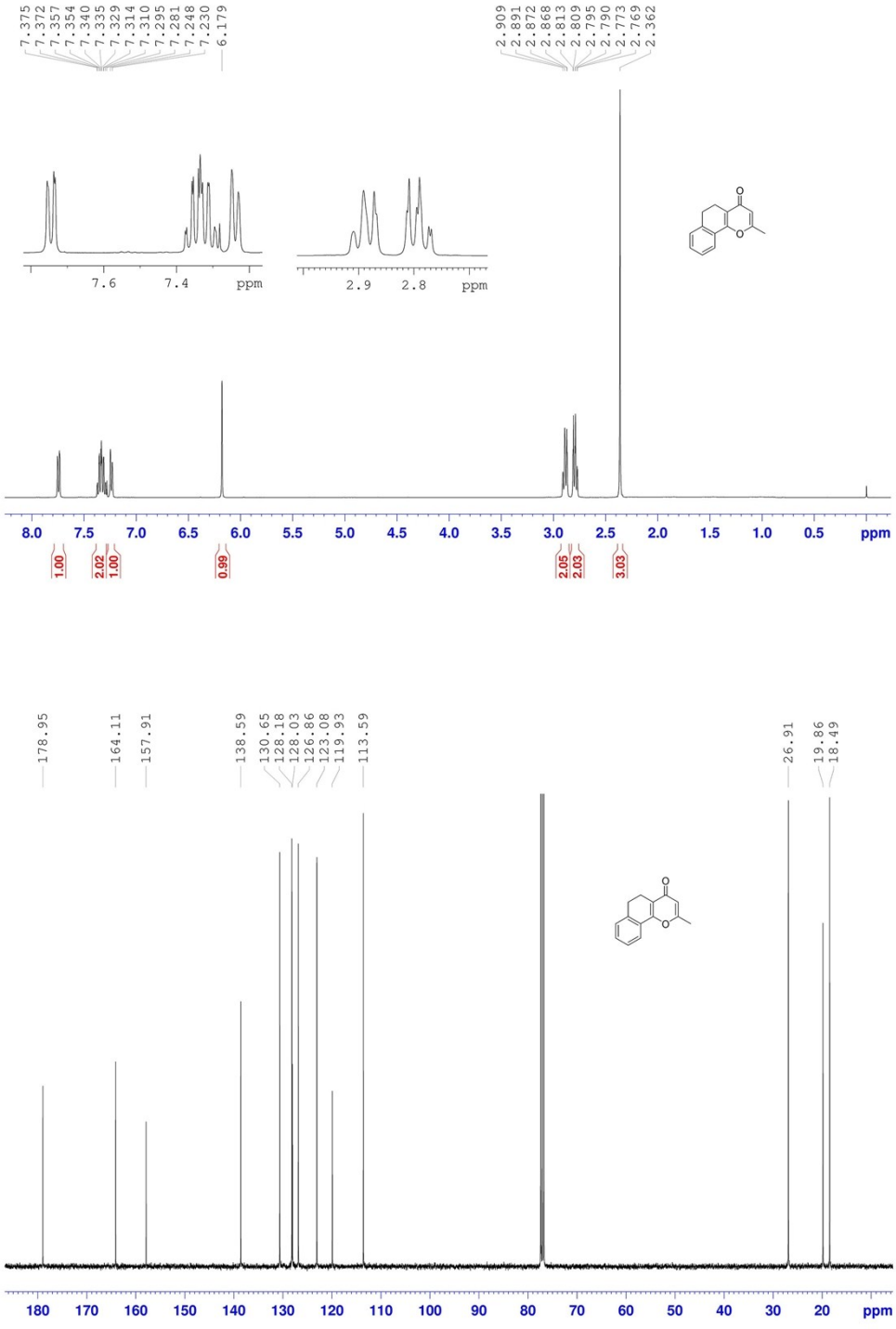


Fig. S13 ^{1}H NMR and ^{13}C NMR of **6**

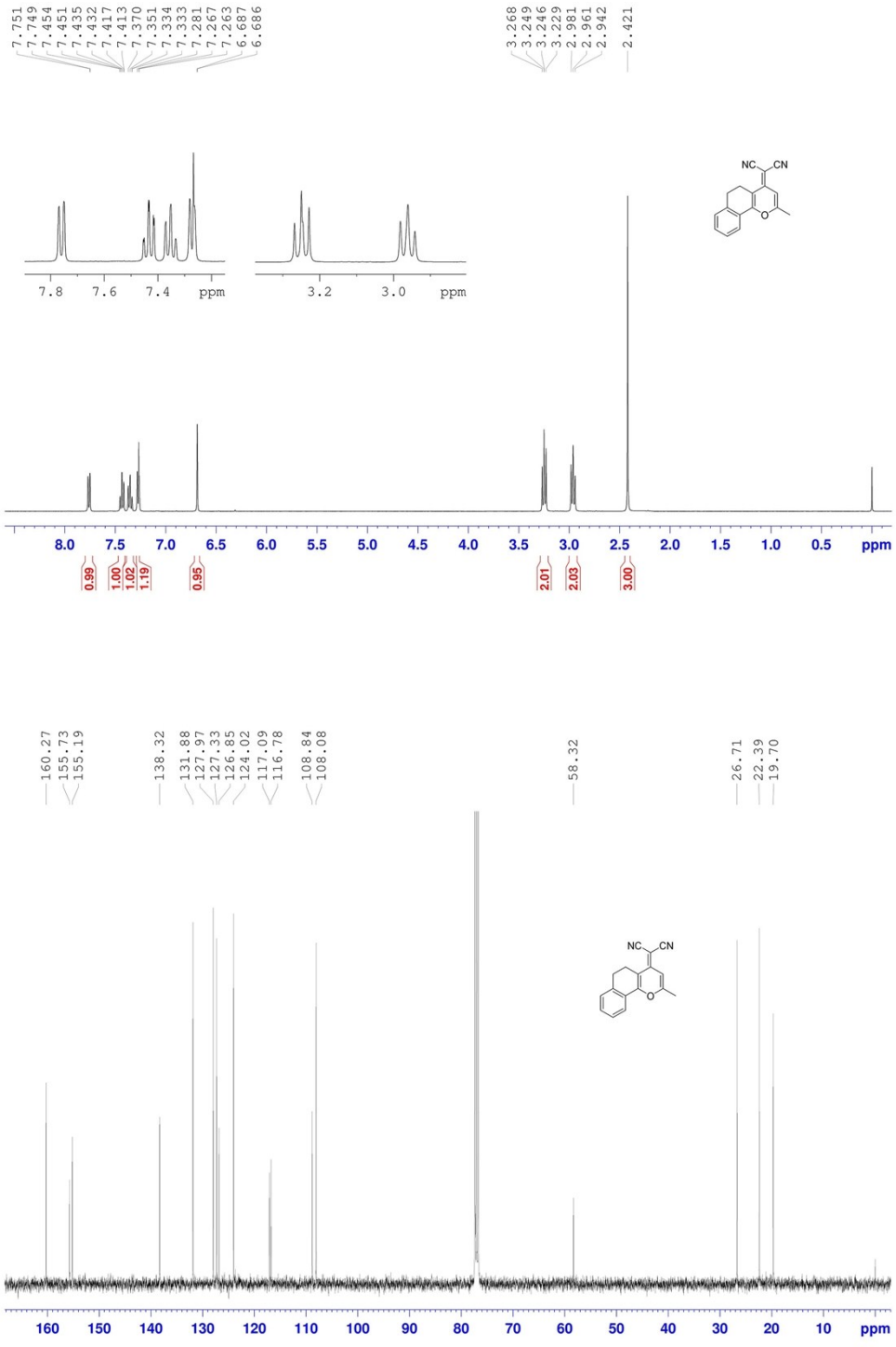
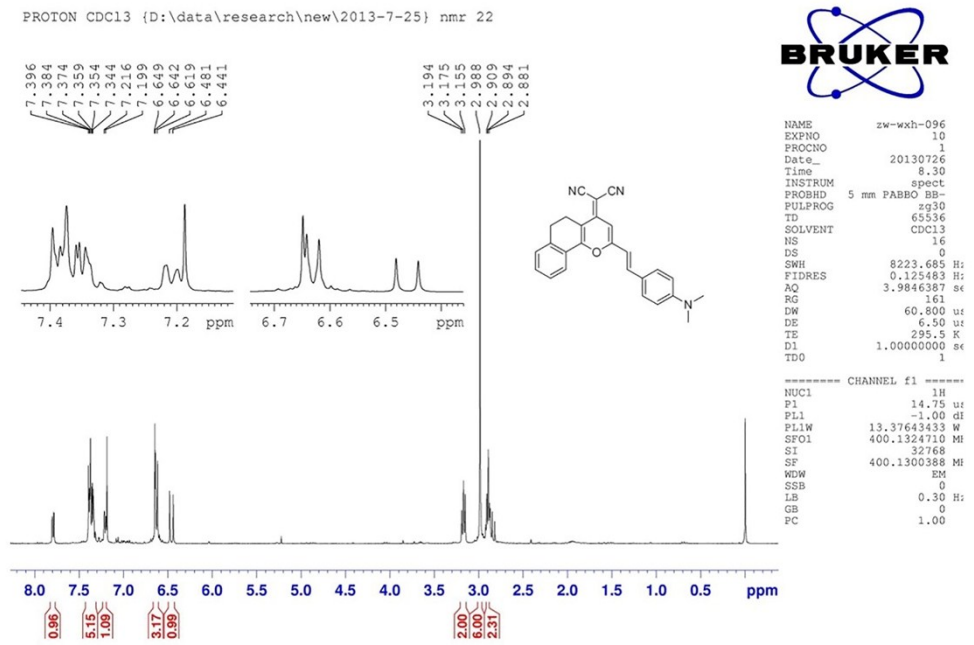


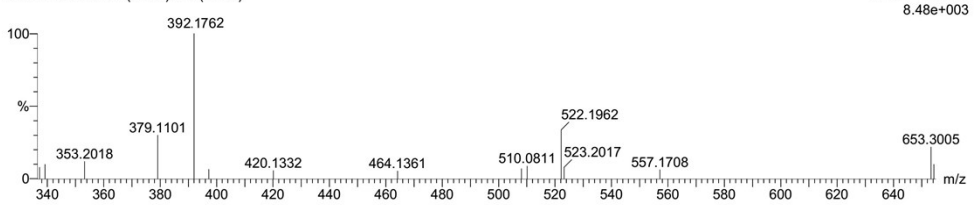
Fig. S14 ^{1}H NMR and ^{13}C NMR of *7*



Monoisotopic Mass, Even Electron Ions
26 formula(e) evaluated with 1 results within limits (up to 1 best isotopic matches for each mass)
Elements Used:
C: 0-26 H: 0-22 N: 0-3 O: 0-1

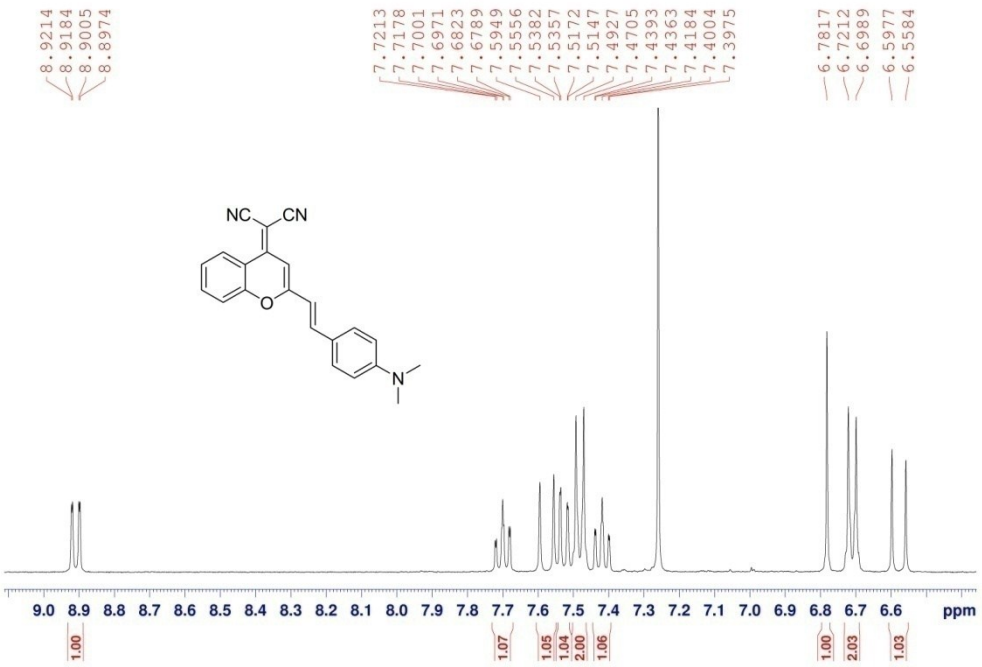
WH-ZHU ECUST institute of Fine Chem 16-Jan-2014 17:57:37

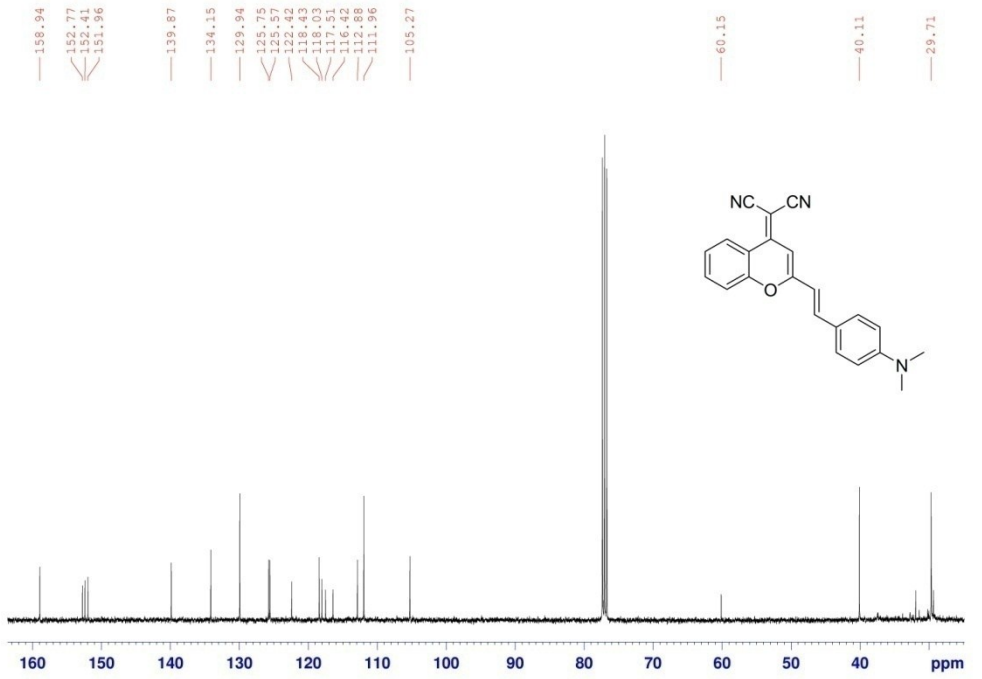
ZWH-YCX-0141 41 (0.963) Cm (40:63) 1: TOF MS ES+ 8.48e+003



Minimum:				-1.5			
Maximum:		30.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
392.1763	392.1763	0.0	0.0	20.5	7.9	0.0	C26 H22 N3 O1

Fig. S15 ^{1}H NMR and HRMSof **C-DCM-*N***

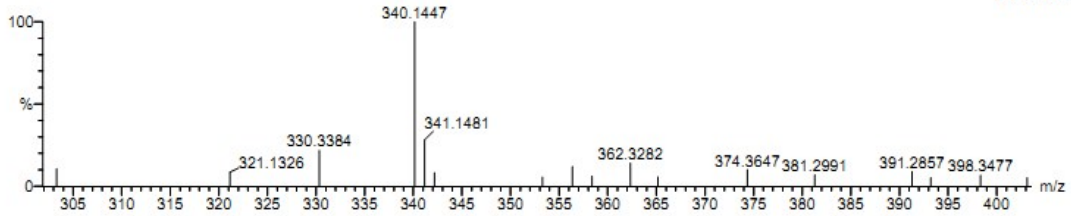

8.9214
8.9184
8.9005
8.8974
7.7213
7.7178
7.7001
7.6971
7.6823
7.6789
7.5949
7.5556
7.5382
7.5357
7.5172
7.5147
7.4927
7.4705
7.4393
7.4363
7.4184
7.4004
7.3975
6.7817
6.7212
6.6989
6.5977
6.5584
NC
CN
O
N
9.0 8.9 8.8 8.7 8.6 8.5 8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3 7.2 7.1 7.0 6.9 6.8 6.7 6.6 ppm
1.00
1.07
1.05
1.04
2.00
1.06
1.00
2.03
1.03


158.94
152.77
152.41
151.96
139.87
134.15
129.94
125.75
125.57
122.42
118.43
118.03
117.51
116.42
112.88
111.96
105.27
60.15
40.11
29.71
NC
CN
O
N
160 150 140 130 120 110 100 90 80 70 60 50 40 ppm

Elemental Composition Report **Page 1**

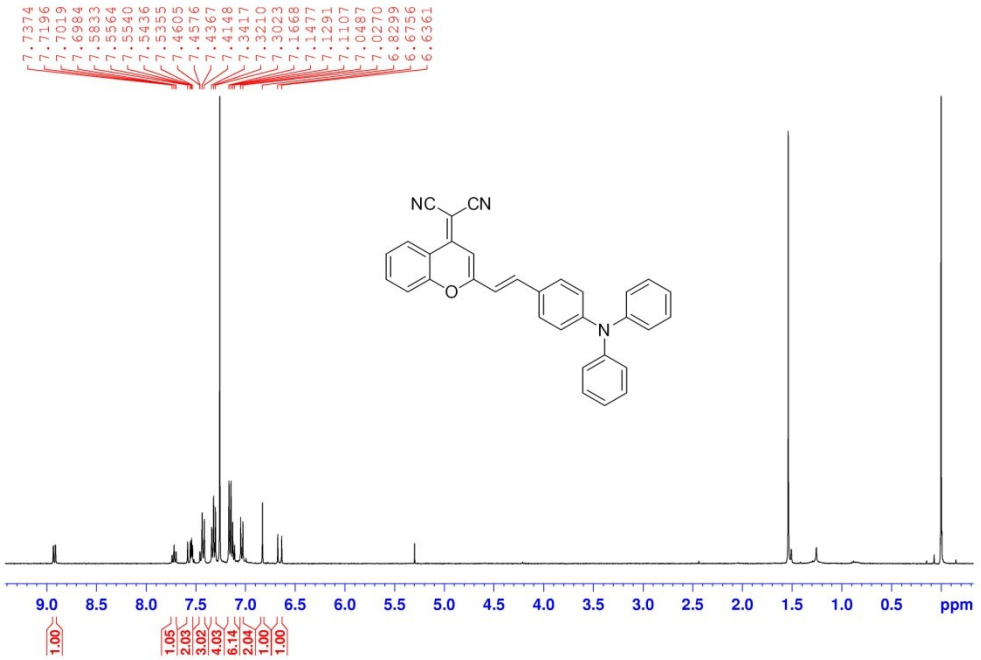
Single Mass Analysis
Tolerance = 3.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 4

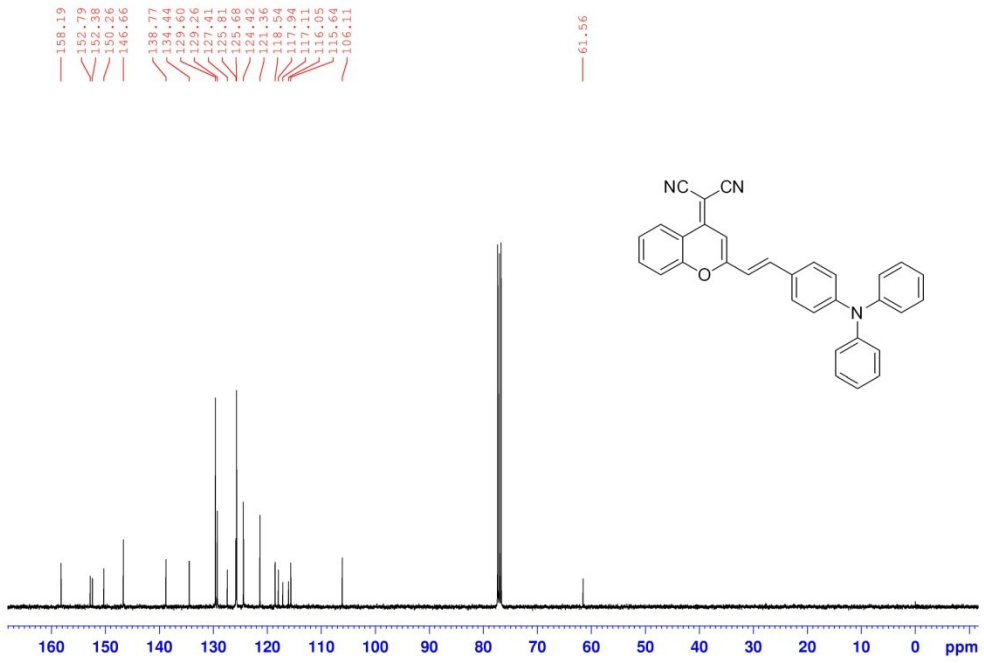
Monoisotopic Mass, Even Electron Ions
95 formula(e) evaluated with 1 results within limits (up to 1 best isotopic matches for each mass)
Elements Used:
C: 20-32 H: 1-50 N: 0-4 O: 0-8
ZHU-WH LCT Premier
Key Lab for Advanced Materials --- ECUST
ZWH-SCX-002 44 (1.658) Cm (40:48) 1: TOF MS ES+
4.56e+004



Minimum:				-1.5			
Maximum:		3.0	50.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
340.1447	340.1450	-0.3	-0.9	15.5	39.1	0.0	C22 H18 N3 O

Fig. S16 ^{1}H NMR, ^{13}C NMR and HRMS of **B-DCM-*N***

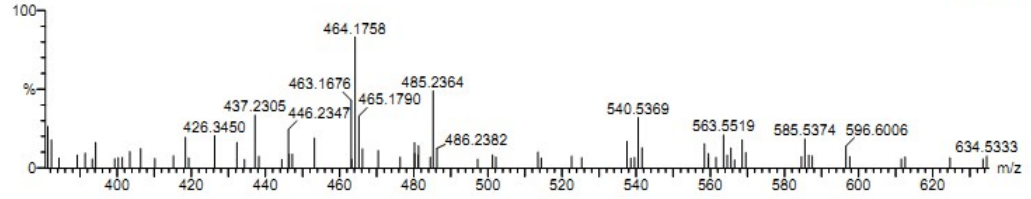




Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 3.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 4

Monoisotopic Mass, Even Electron Ions
150 formula(e) evaluated with 1 results within limits (up to 1 best isotopic matches for each mass)
Elements Used:
C: 20-32 H: 1-50 N: 0-4 O: 0-8
ZHU-WH LCT Premier
Key Lab for Advanced Materials --- ECUST
ZWH-SCX-001 48 (1.780) Cm (44:49) 1: TOF MS ES+
2.41e+003



Minimum:				-1.5			
Maximum:		3.0	50.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
464.1758	464.1763	-0.5	-1.1	23.5	19.6	0.0	C32 H22 N3 O

Fig. S17 ^{1}H NMR, ^{13}C NMR and HRMS of **B-DCM-*P***