Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

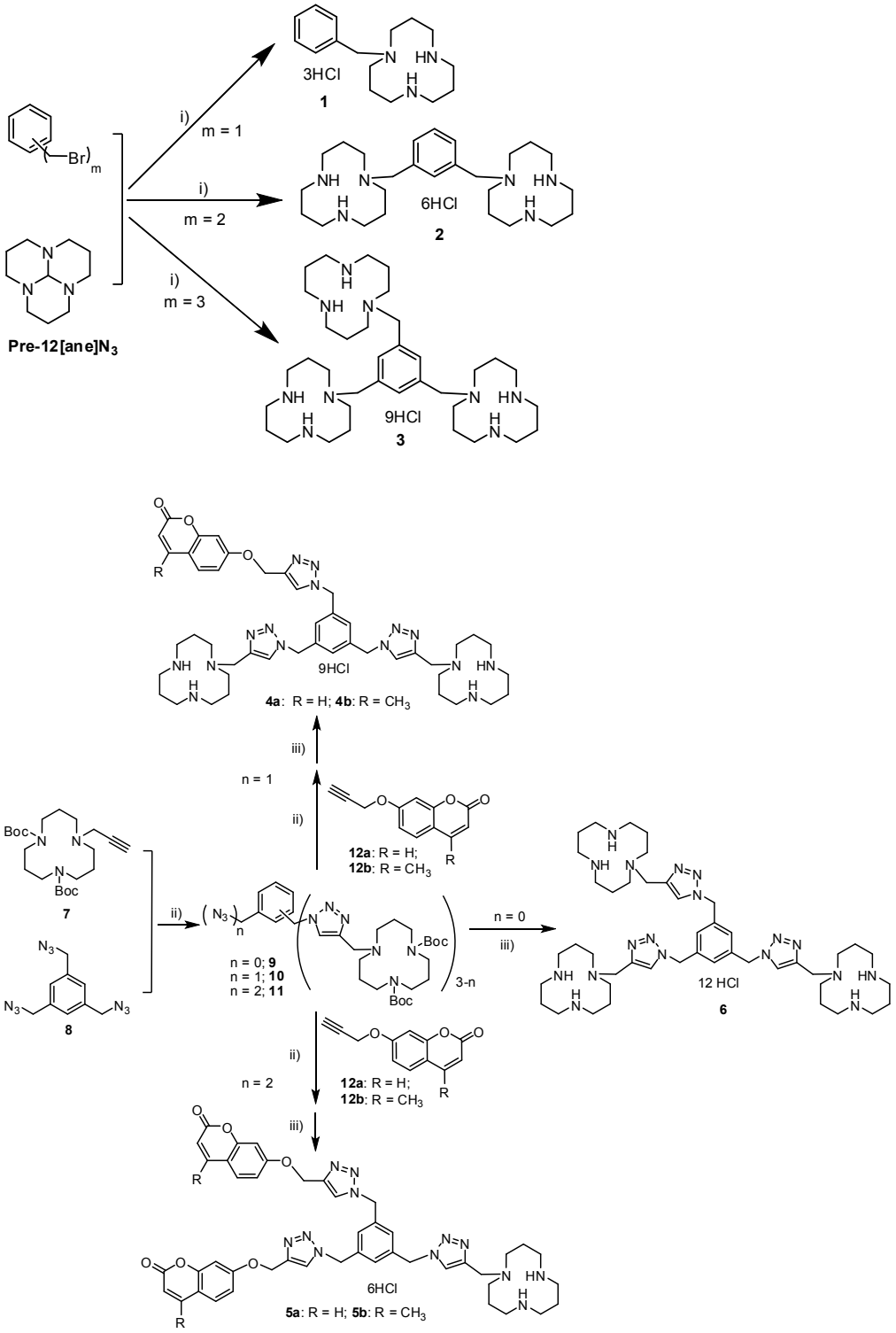
Supporting Information

Synthesis of bifunctional molecules containing [12]aneN_3 and coumarin moieties as effective DNA condensation agents and new non-viral gene vectors

Contents:

1. Synthesis and characterization

1.1 Synthesis route



Scheme S1 Syntheses of 1-6: i) a) $CHCl_3$, overnight; b) 3M HCl, reflux 12 h; ii) THF/H_2O, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, overnight; iii) CH_3COCl, CH_3OH.

1.2 Syntheses of the Compounds **1**, **2**, **7-12**

1.2.1 Syntheses of compound **1**, **2**, **7**

Compound **1**, **2**, **7** were syntheses according the literature [1, 2].

1): Yield: 61%. M.p.: 206 ℃-208 ℃. ^{1}H NMR (400 MHz, D_2O) δ 7.56 (s, 5H), 4.38 (s, 2H), 3.47 – 3.26 (m, 12H), 2.32 – 2.11 (m, 6H). ^{13}C NMR (101 MHz, D_2O) δ 131.12, 130.59, 129.61, 128.54, 58.97, 46.77, 42.29, 41.19, 20.59, 17.62. ESI-MS Calcd. for $C_{16}H_{28}N_3(M+H)^+$: 262.2, found: 262.4.

2): Yield: 82%. M.p.:199 ℃-200 ℃. ^{1}H NMR (400 MHz, D_2O) δ 7.76 – 7.61 (m, 4H), 4.49 (s, 4H), 3.53 – 3.43 (m, 8H), 3.43 – 3.32 (m, 16H), 2.35 – 2.18 (m, 12H). ^{13}C NMR (101 MHz, D_2O) δ 133.72, 133.14, 130.69, 129.75, 58.28, 46.85, 42.25, 41.11, 20.54, 17.57. ESI-MS Calcd. for $C_{26}H_{49}N_6(M+H)^+$: 445.4, found: 445.8.

7): Yield: 44%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.38 (d, *J* = 2.2 Hz, 2H), 3.26 – 3.33 (m, 8H), 2.51 – 2.54 (m, 4H), 2.15 (t, *J* = 2.2 Hz, 1H), 1.88 – 1.75 (m, 6H), 1.46 (s, 18H).

1.2.2 Synthesis of compound **8**

Compound **8** was synthesis according to literature [3].

8): Yield: 97%. ^{1}H NMR (400 MHz, $CDCl_3$): 7.25 (s, 3H), 4.40 (s, 6H).

1.2.3 Synthesis of compound **12**

Compound **12** was synthesis according to literature [4].

12a): Yield: 86%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (d, *J* = 9.5 Hz, 1H), 7.40 (d, *J* = 8.5 Hz, 1H), 6.96 – 6.85 (m, 2H), 6.28 (d, *J* = 9.5 Hz, 1H), 4.76 (d, *J* = 2.4 Hz, 2H), 2.58 (t, *J* = 2.4 Hz, 1H).

12b): Yield: 83%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (dd, *J* = 7.6, 1.8 Hz, 1H), 6.96 – 6.89 (m, 2H), 6.16 (d, *J* = 1.0 Hz, 1H), 4.76 (d, *J* = 2.4 Hz, 2H), 2.57 (t, *J* = 2.4 Hz, 1H), 2.40 (d, *J* = 1.0 Hz, 3H).

1.2.4 Syntheses of compound **9-11**

Azide compound **8** (0.44 g, 1.8 mmol) and compound **7** (1.12 g, 2.7 mmol) were added into THF/H_2O (v/v = 2:1), $CuSO_4 \cdot 5H_2O$ (0.045 g, 0.18 mmol) and sodium ascorbate (0.044 g, 0.37 mmol) were also added into the solution as catalysis. The mixture was stirred over night at room temperature, saturated with NaCl, and extracted with ethyl acetate. The organic layers were washed once with brine, dried over Na_2SO_4 and evaporated under reduced pressure. The crude products were purified by flash chromatography on silica gel with PE/Acetone (2:1) to yield the Boc-protected compound **9-11** as pale yellow solid.

9): 0.31 g, Yield: 12%. M.p.:35 ℃-36 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (s, 3H), 7.06 (s, 3H), 5.45 (s, 6H), 3.75 (s, 6H), 3.52 – 3.17 (m, 24H), 2.55 – 2.33 (m, 12H), 2.07 – 1.61 (m, 18H), 1.42 (s, 54H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.36, 144.42, 137.19, 127.12, 122.77, 79.36, 53.26, 49.81, 46.97, 45.48, 43.96, 28.56, 27.35, 26.17. IR (KBr, cm^{-1}): 2967, 2930, 1727, 1690, 1476, 1454, 1414, 1365, 1283, 1165, 1073. ESI-MS Calcd. for $C_{75}H_{128}N_{18}O_{12}(M+2H)^+$: 1473.0,

found: 1473.2.

10): 0.75 g, Yield: 40 %. M.p.:79 ℃-81 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (s, 2H), 7.12 (s, 3H), 5.51 (s, 4H), 4.31 (s, 2H), 3.77 (s, 4H), 3.38 – 3.30 (m, 16H), 2.42 – 2.34 (m, 8H), 1.84 – 1.81 (m, 12H), 1.43 (s, 36H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.43, 144.61, 137.86, 136.92, 127.50, 126.90, 122.58, 79.42, 54.08, 53.49, 49.93, 46.96, 45.55, 43.99, 28.62, 27.28, 26.29. IR (KBr, cm^{-1}): 3433, 2976, 2929, 2097, 1682, 1484, 1412, 1368, 1164, 1044. HR-MS Calcd. for $C_{53}H_{88}N_{15}O_8(M+H)^+$: 1062.6940, found: 1062.6906.

11): 0.30 g, Yield: 26 %. M.p.:52 ℃-64 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (s, 1H), 7.26 (s, 1H), 7.21 (s, 2H), 5.56 (s, 2H), 4.36 (s, 4H), 3.77 (s, 2H), 3.61 – 3.03 (m, 8H), 2.72 – 2.19 (m, 4H), 2.19 – 1.53 (m, 6H), 1.43 (s, 18H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.38, 144.53, 137.48, 136.50, 127.94, 127.19, 122.42, 79.36, 54.21, 53.62, 49.93, 46.97, 45.55, 43.99, 28.58, 27.29, 26.27. IR (KBr, cm^{-1}): 2974, 2926, 2098, 1686, 1478, 1454, 1414, 1365, 1248, 1165, 1048. HR-MS Calcd. for $C_{31}H_{49}N_{12}O_4(M+H)^+$: 653.4000, found: 653.4011.

2. Agarose gel electrophoresis (Time effects)

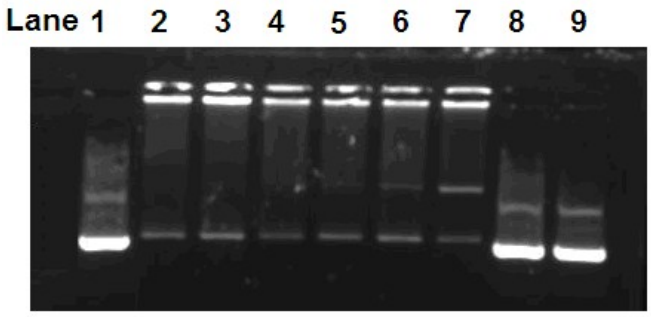


Figure S1. Agarose gel electrophoresis assay to investigate the pUC18 DNA condensation induced by different time of **3** in 50mM Tris-HCl buffer (pH = 7.4, 37 ºC). [DNA] = 9 μg/mL, Lane 1, 8, 9: DNA control, 0, 4, 12 h; lane 2-7: [**3**]= 60 μM, 0.5, 1, 2, 3, 4, 12 h.

3. Dynamic light scattering

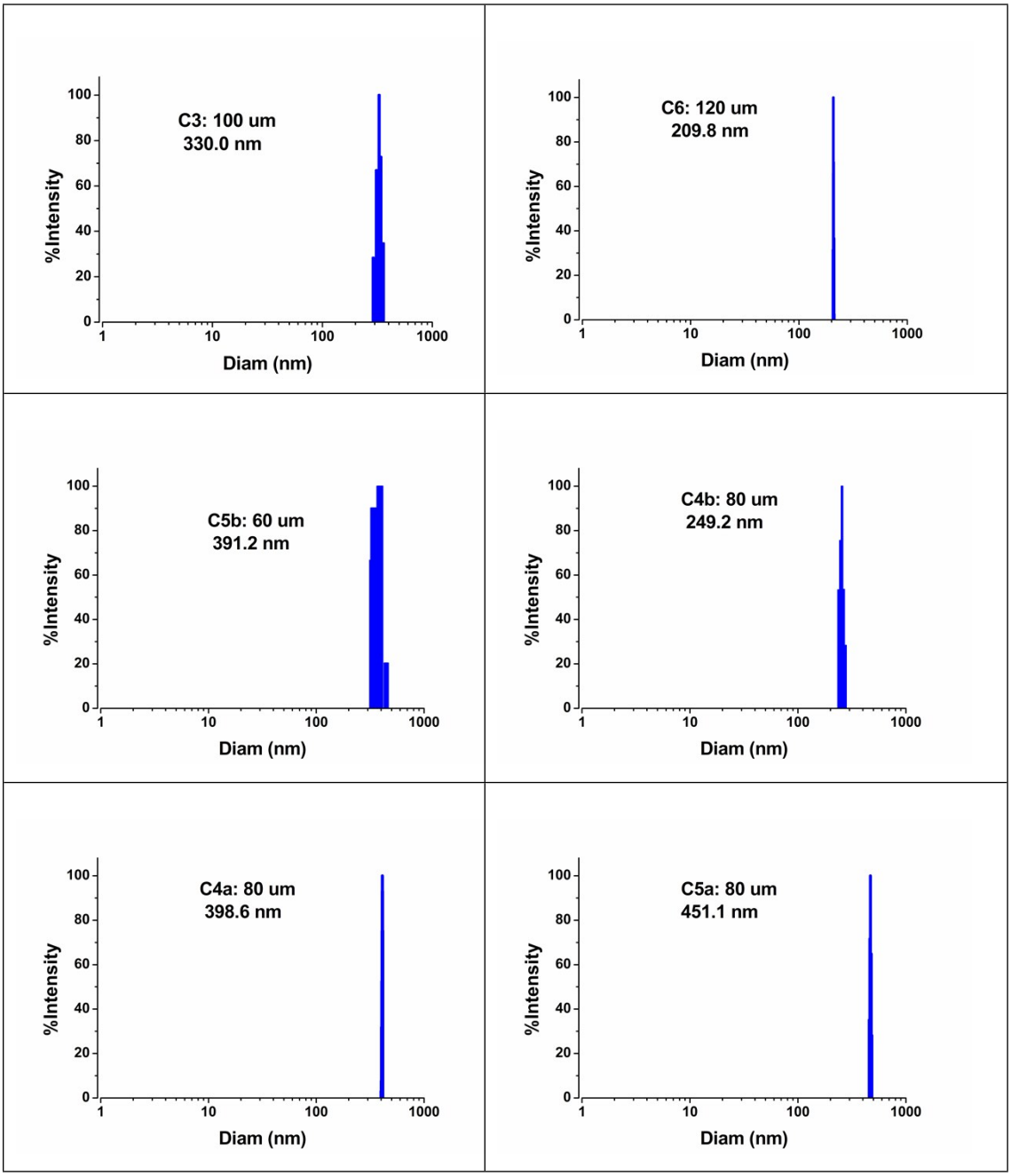


Figure S2. Hydrodynamic diameter distributions of pUC18 DNA particles condensed by **3**-6 at different concentrations. The DNA concentration is 1 μg/mL.

4. EB displacement assay

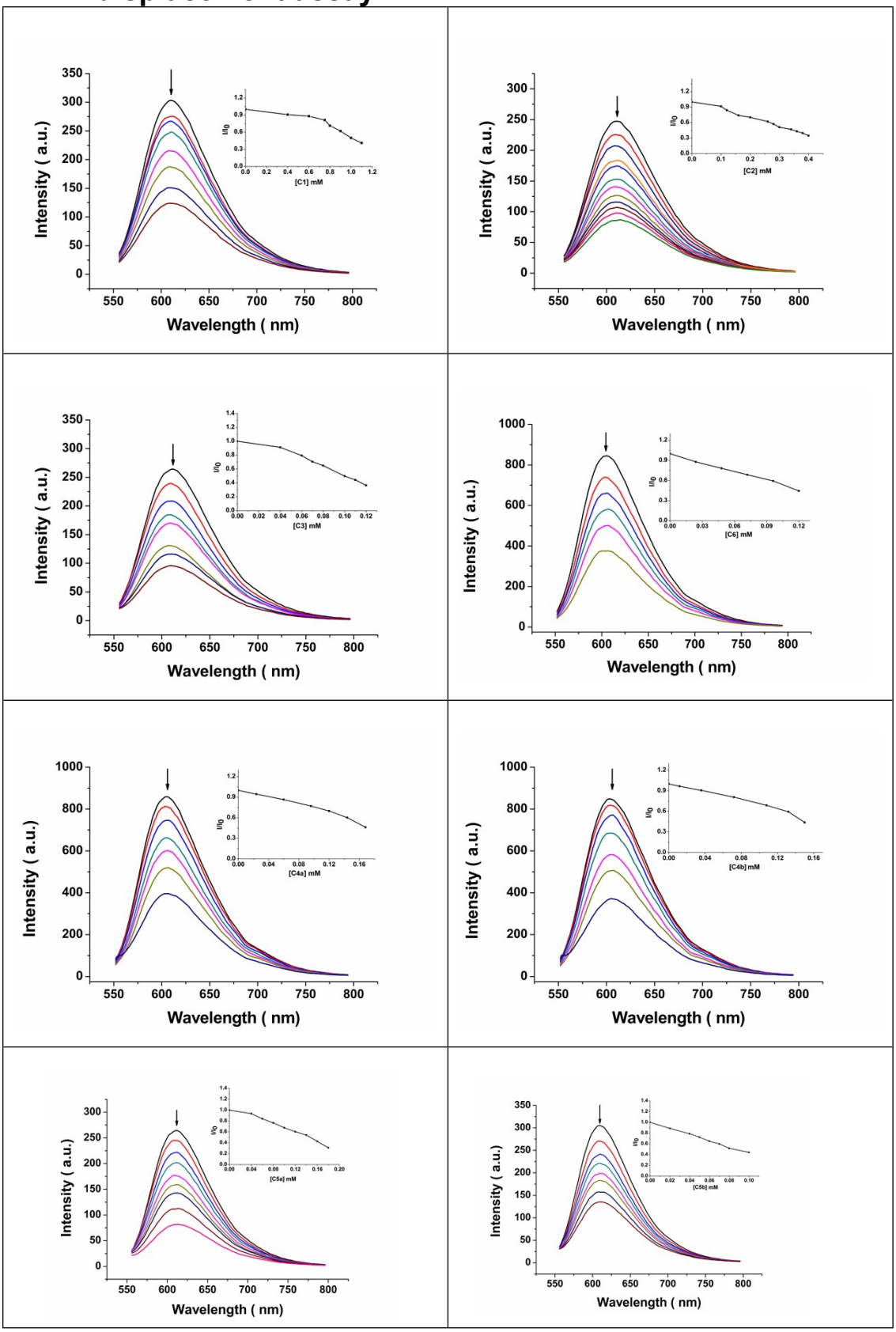


Figure S3. Fluorescence quenching curves of EB bound CT-DNA by **1-6** in 5 mM Tris-HCl/50 mM NaCl (pH 7.4, λ_{ex}=537 nm, [EB] = 20 μM, [DNA] = 100 μM, 25.0 °C). The arrows show the intensity changes on increasing the concentration of the condensing agents.

5. Cellular uptake study

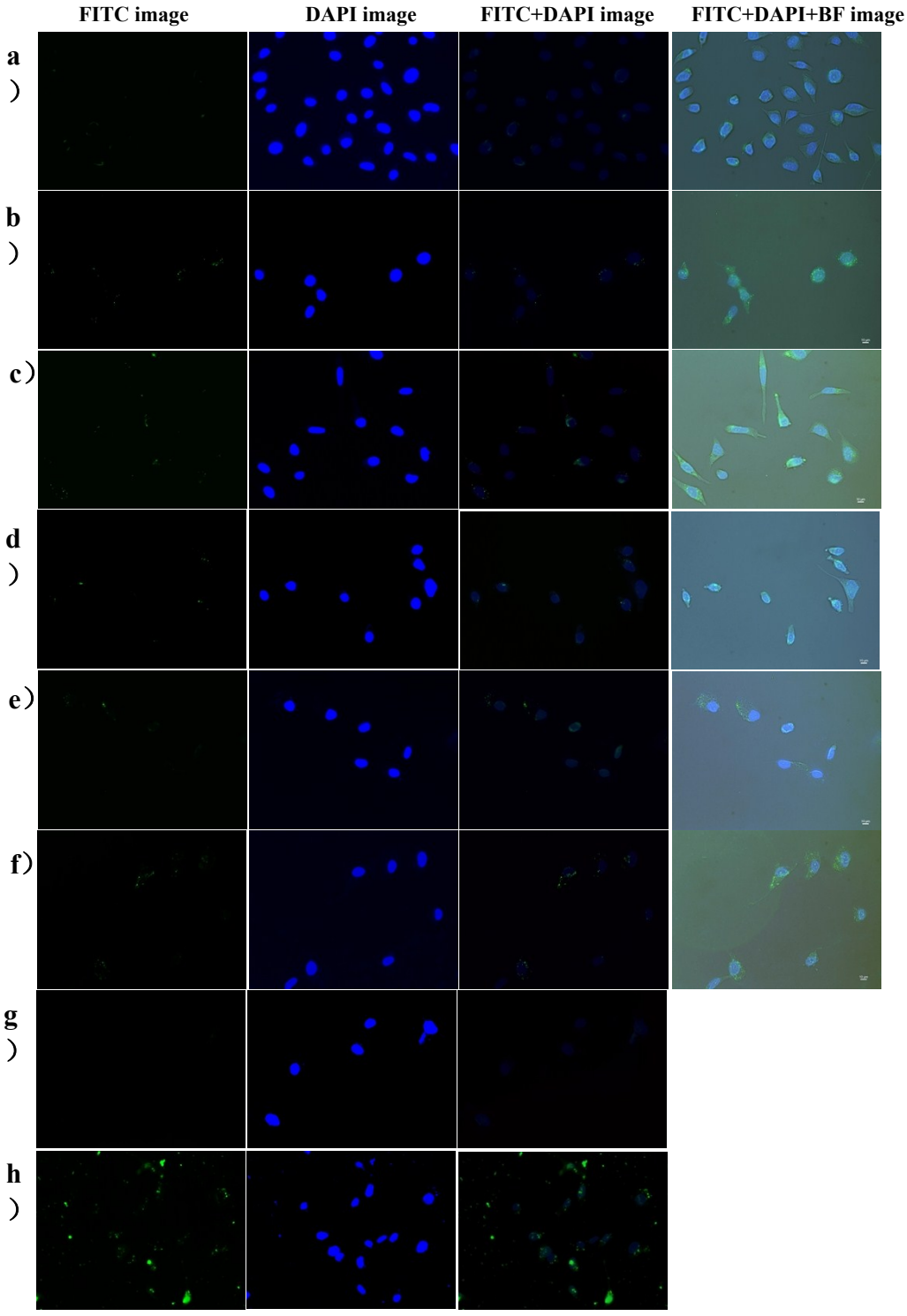


Figure S4. Fluorescence microscope image (40×) of Hela cells transfected with FITC-DNA by NLS-free **4b** at different DOPE ratios and concentrations. The concentration of FITC-DNA was 5 μg/dish. (a-d) **[4b]** = 60 μM, the **4b**/DOPE ratios are 1:0, 2:1, 1:1, 1:2, respectively; (e-f) **4b**/DOPE = 1:1, **[4b]** = 40, 60 μM,, respectively; (g) NLS-plasmid assemblies control; (h) Lipofectamine 2000™.

6. References

[1] Z. -F. Guo, H. Yan, Z. -F. Li, Z. -L. Lu, *Org. Biomol. Chem*., **2011**, *9*, 6788.

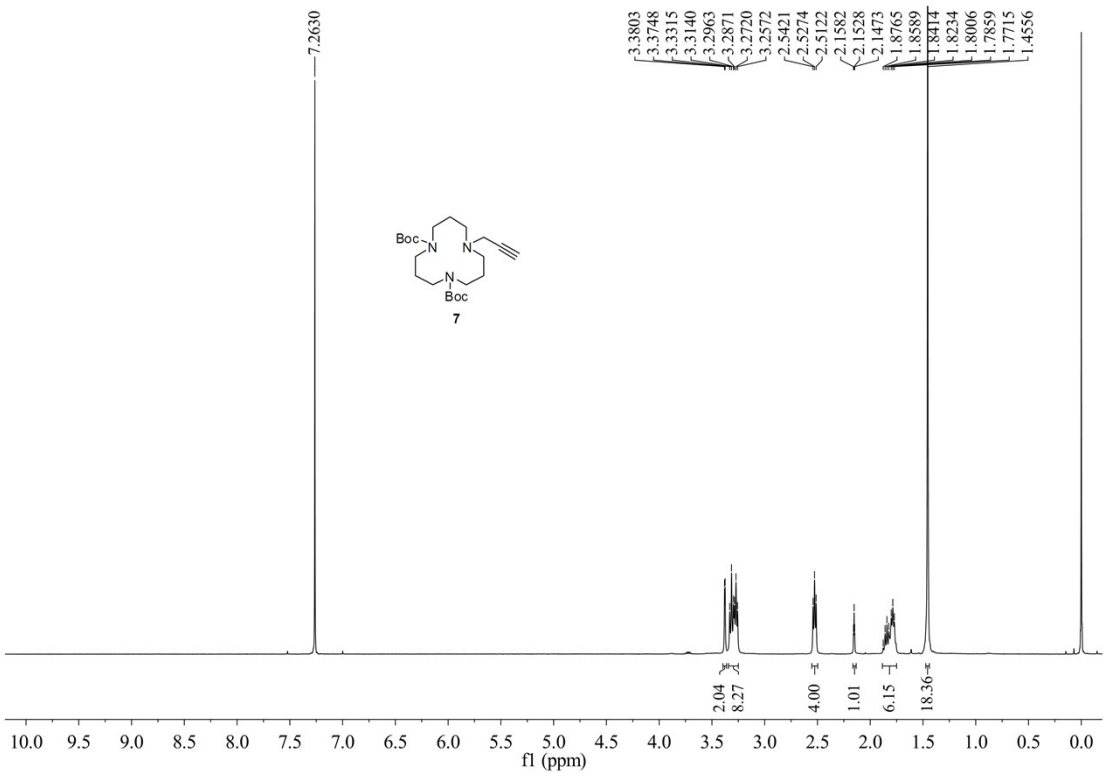
[2] P. Brunet, J. D. Wuest, *J. Org. Chem.*, **1996**, *61*, 1847.

[3] Y. Song, E. K. Kohlmeir, T. J. Meade, *J. Am. Chem. Soc.*, **2008**, *130*, 6662.

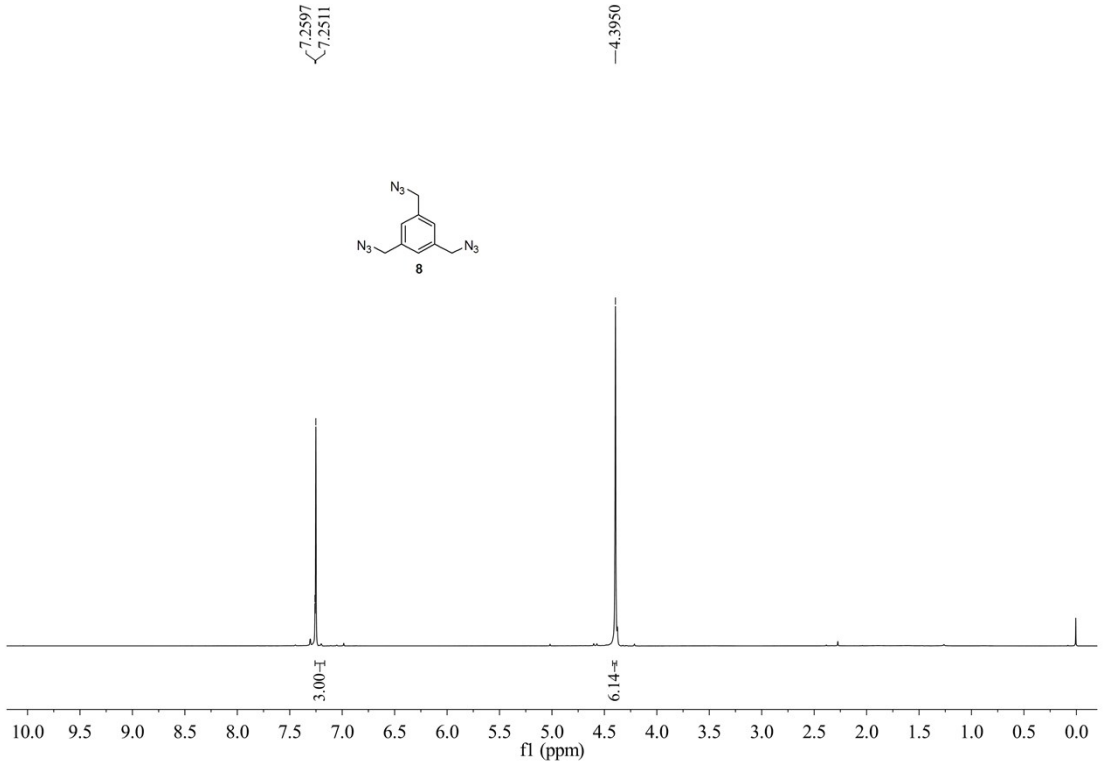
[4] K. Ivana, K. Sona, K. Pavol, *Tetrahedron*, **2007**, *63*, 312.

7. Spectra of the compounds synthesized

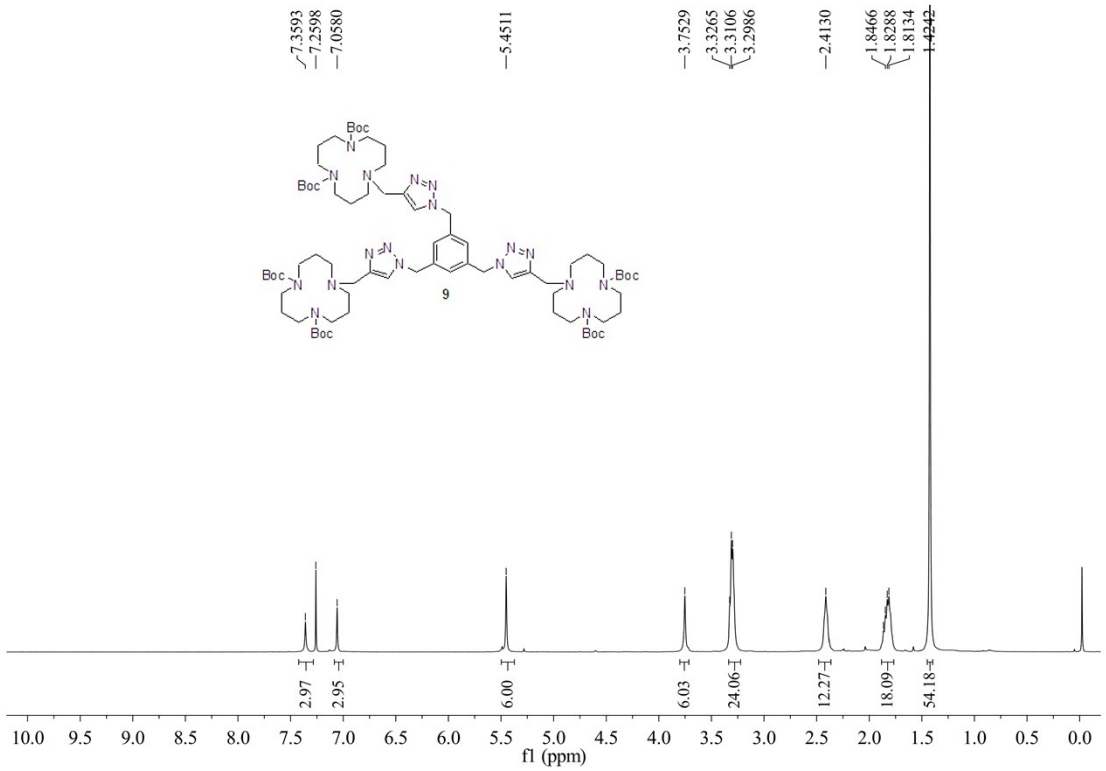
7.1 Spectra data for compound **7**

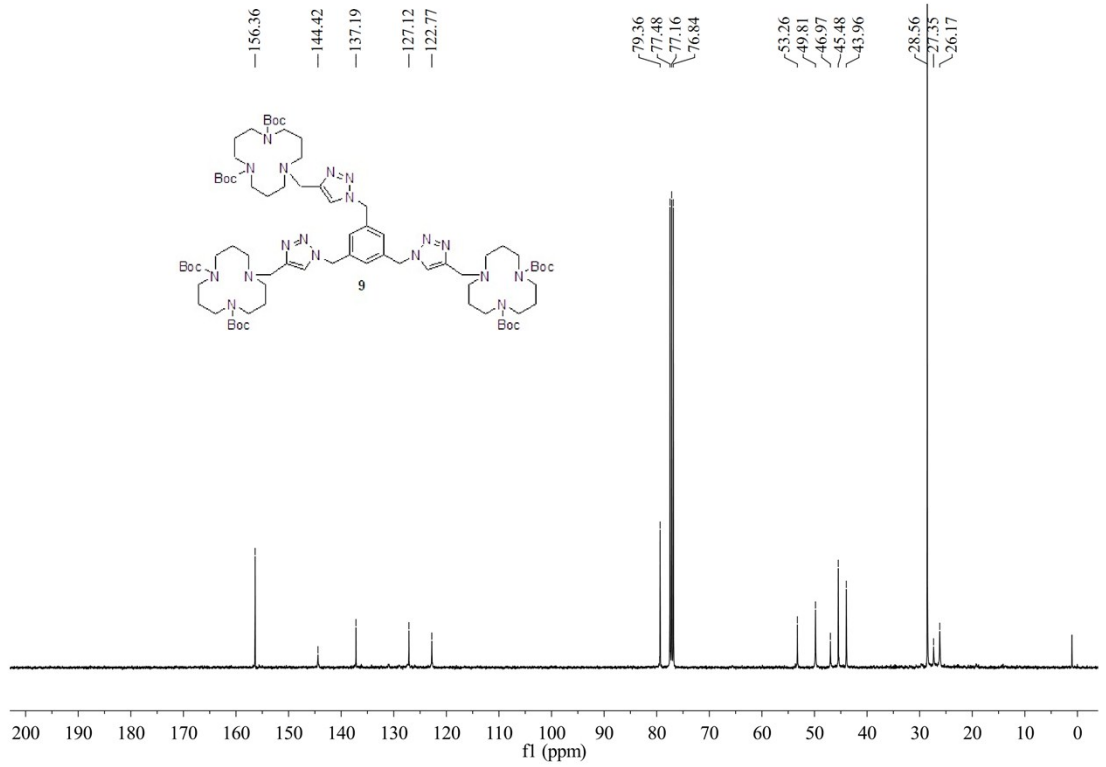


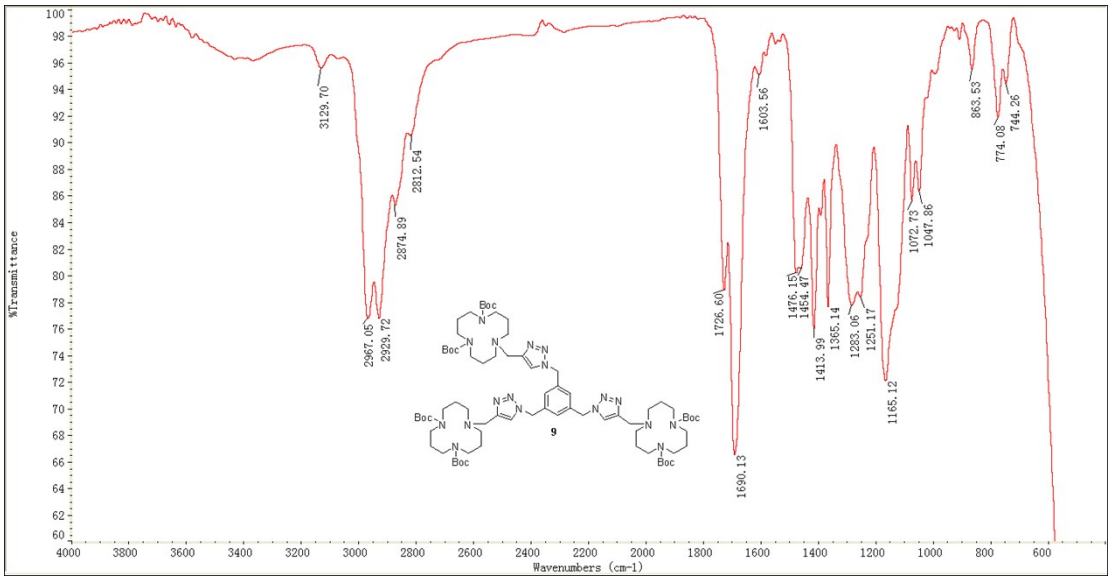
7.2 Spectra data for compound **8**

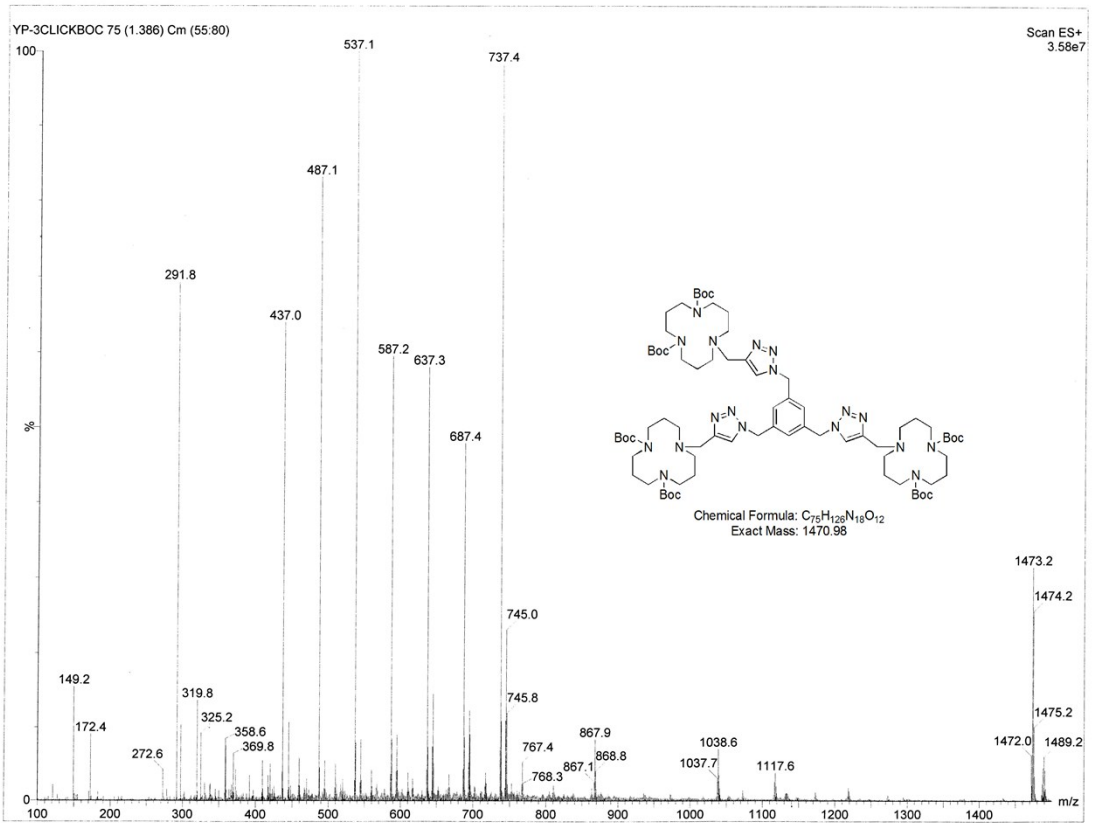


7.3 Spectra data for compound **9**

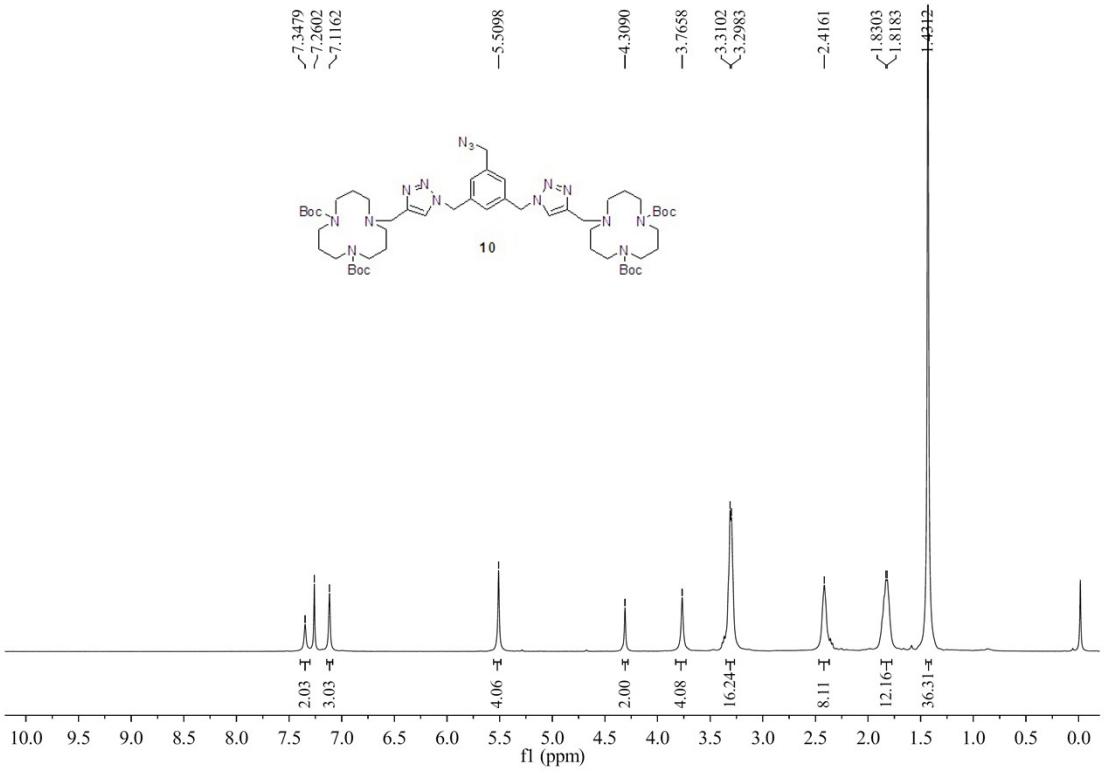


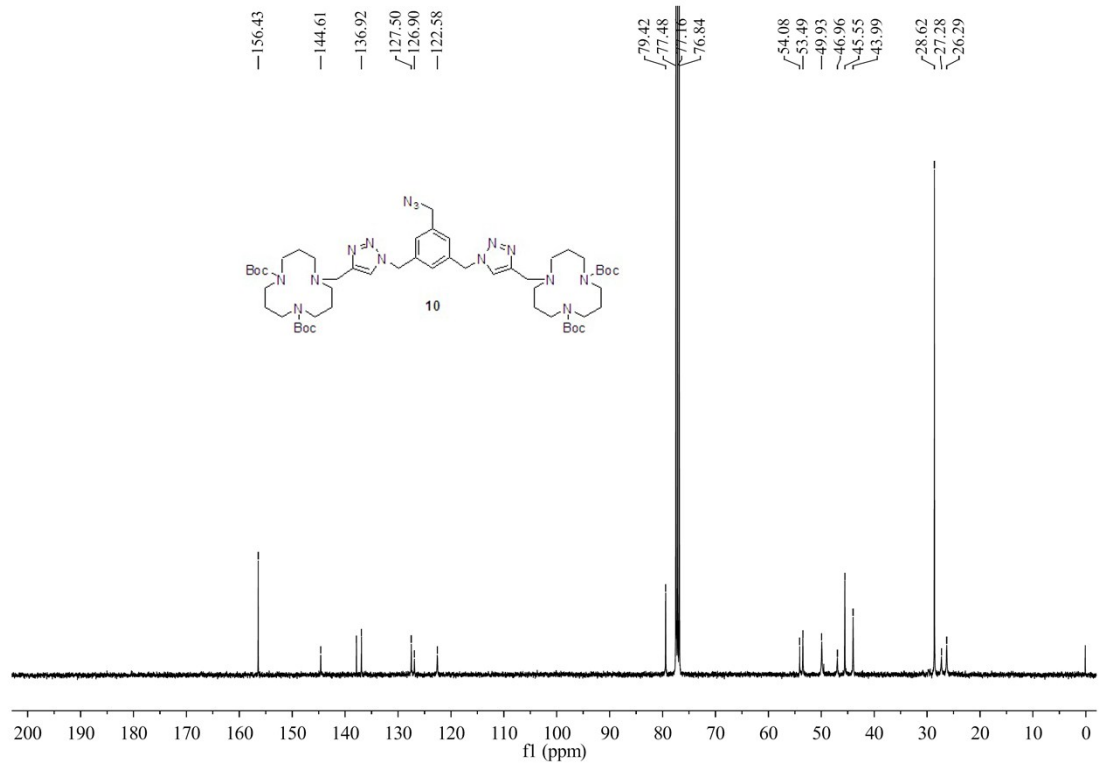


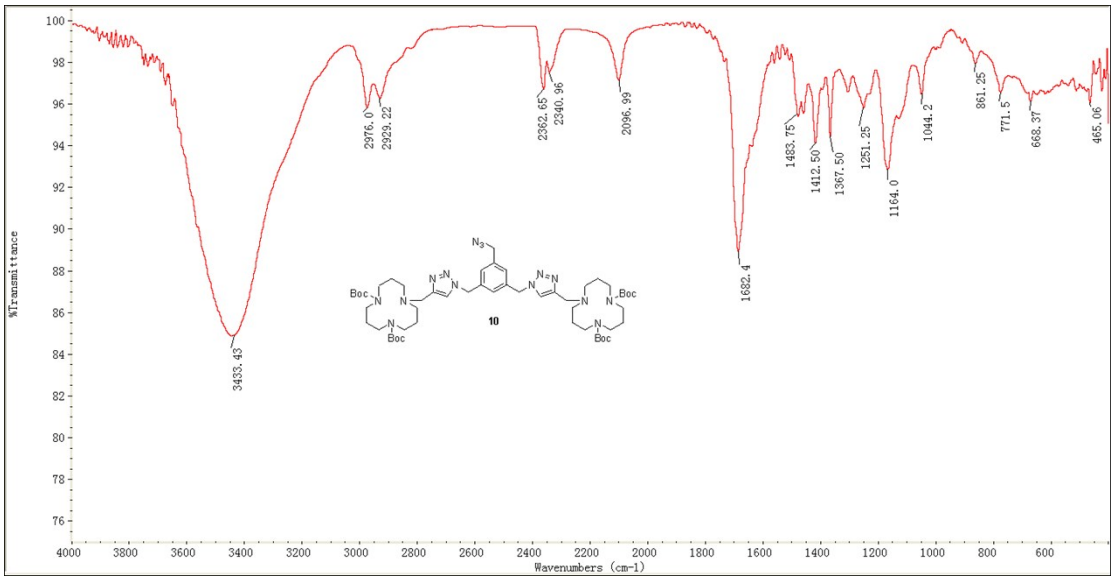

%Transmittance
Wavenumbers (cm-1)
3129.70
2967.05
2929.72
2874.89
2812.54
1726.60
1690.13
1603.56
1476.15
1454.47
1413.99
1365.14
1283.06
1251.17
1165.12
1072.73
1047.86
863.53
774.08
744.26
9


YP-3CLICKBOC 75 (1.386) Cm (55:80)
Scan ES+
3.58e7
%
m/z
537.1
737.4
487.1
291.8
437.0
587.2
637.3
687.4
745.0
745.8
149.2
319.8
325.2
358.6
369.8
172.4
272.6
767.4
867.9
868.8
867.1
768.3
1038.6
1037.7
1117.6
1473.2
1474.2
1475.2
1472.0
1489.2
Chemical Formula: C75H126N18O12
Exact Mass: 1470.98

7.4 Spectra data for compound **10**



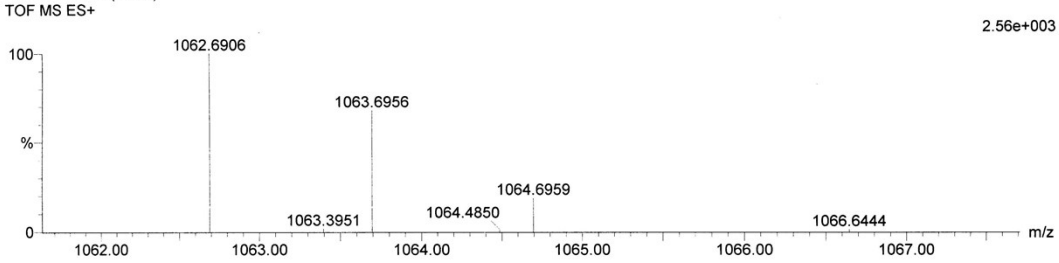




Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

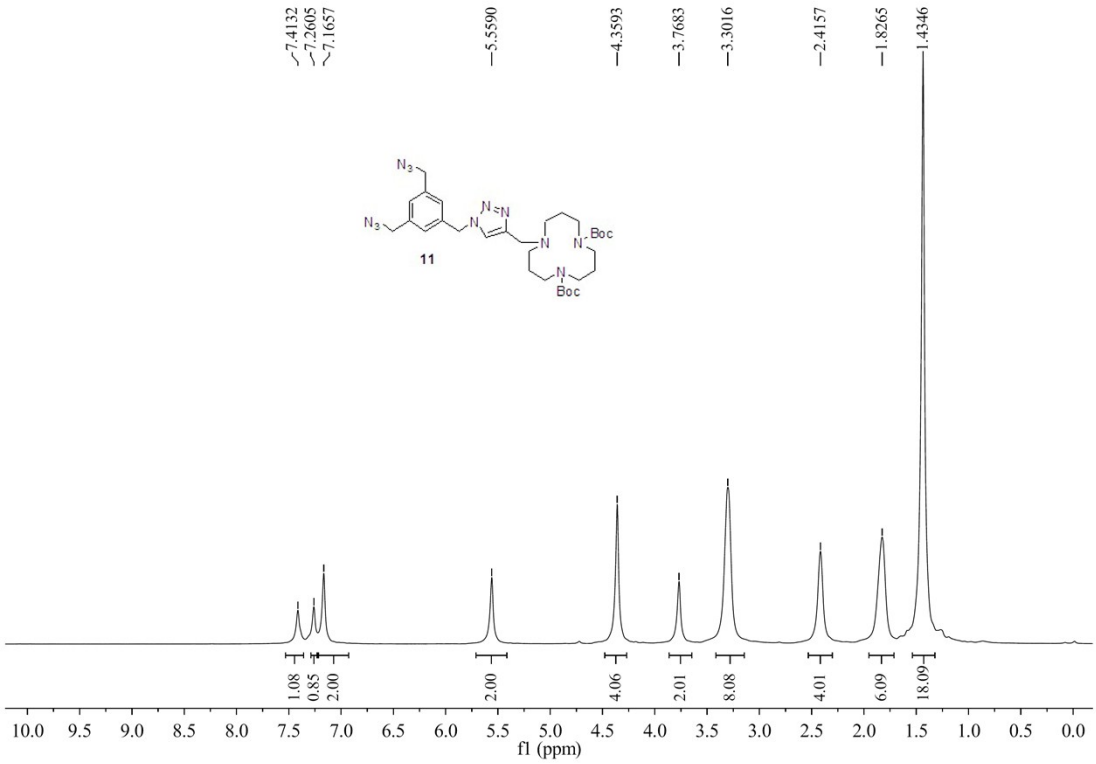
Monoisotopic Mass, Even Electron Ions
140 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-55 H: 0-100 N: 0-15 O: 0-8
YP-N3DBOC 9 (0.154)
TOF MS ES+

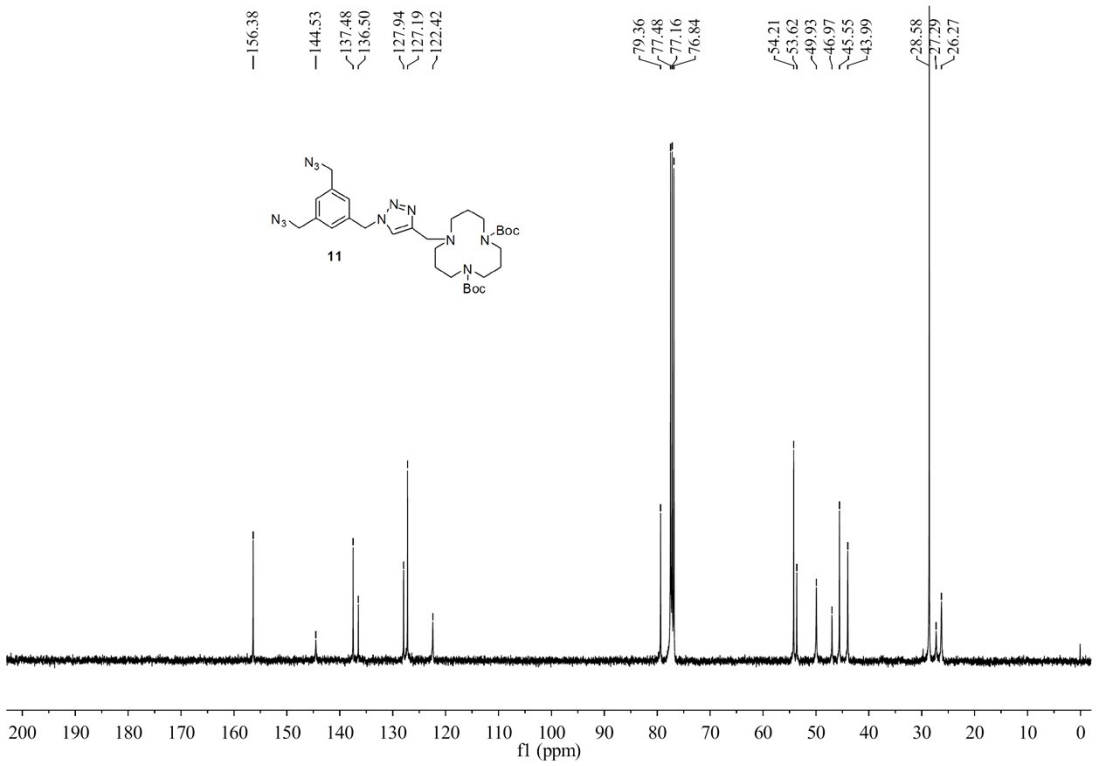


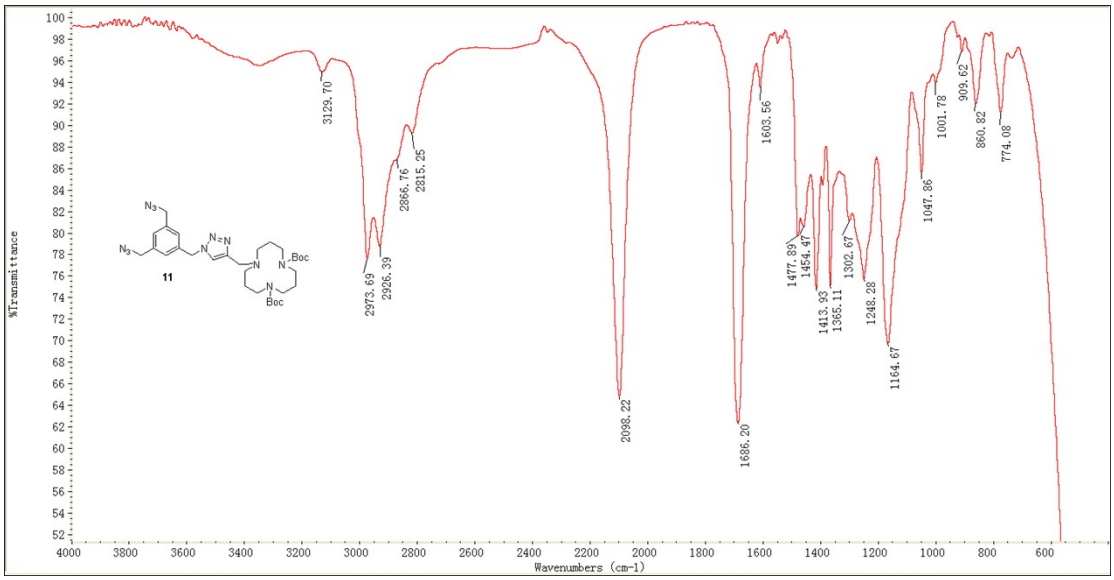
Minimum:				-1.5		
Maximum:		5.0	5.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
1062.6906	1062.6940	-3.4	-3.2	17.5	12.0	C53 H88 N15 O8

$[M+H]^+$

7.5 Spectra data for compound **11**





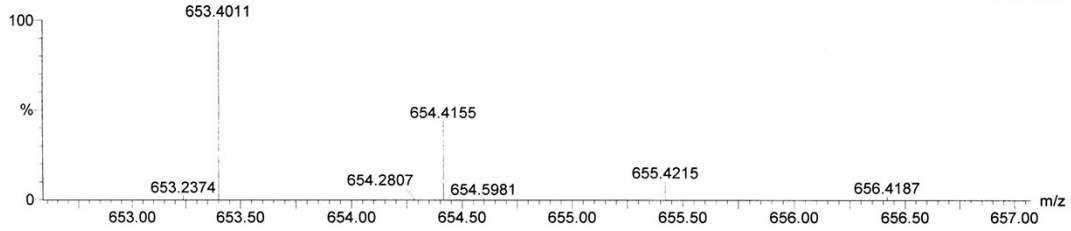


Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 2.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

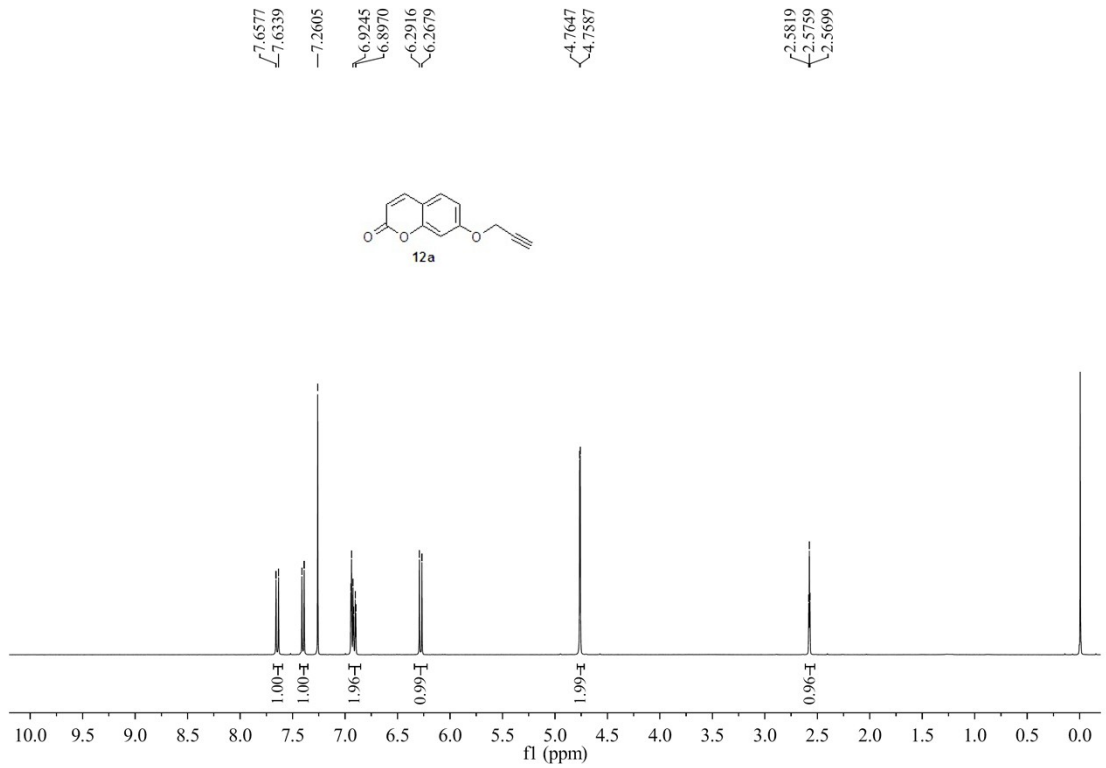
Monoisotopic Mass, Even Electron Ions
249 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-41 H: 0-50 N: 0-15 O: 0-5
YP-N3SBOC 6 (0.102)
TOF MS ES+

7.27e+003

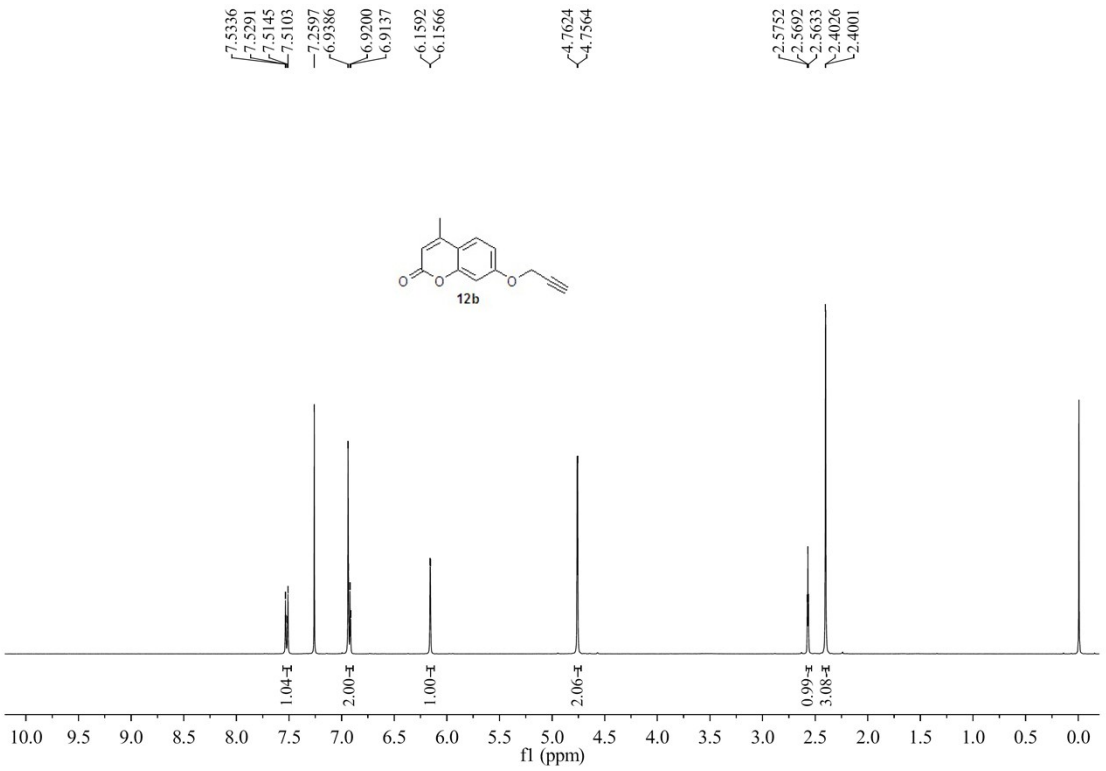


Minimum:				-1.5		
Maximum:		5.0	2.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
653.4011	653.4000	1.1	1.7	13.5	19.9	C31 H49 N12 O4

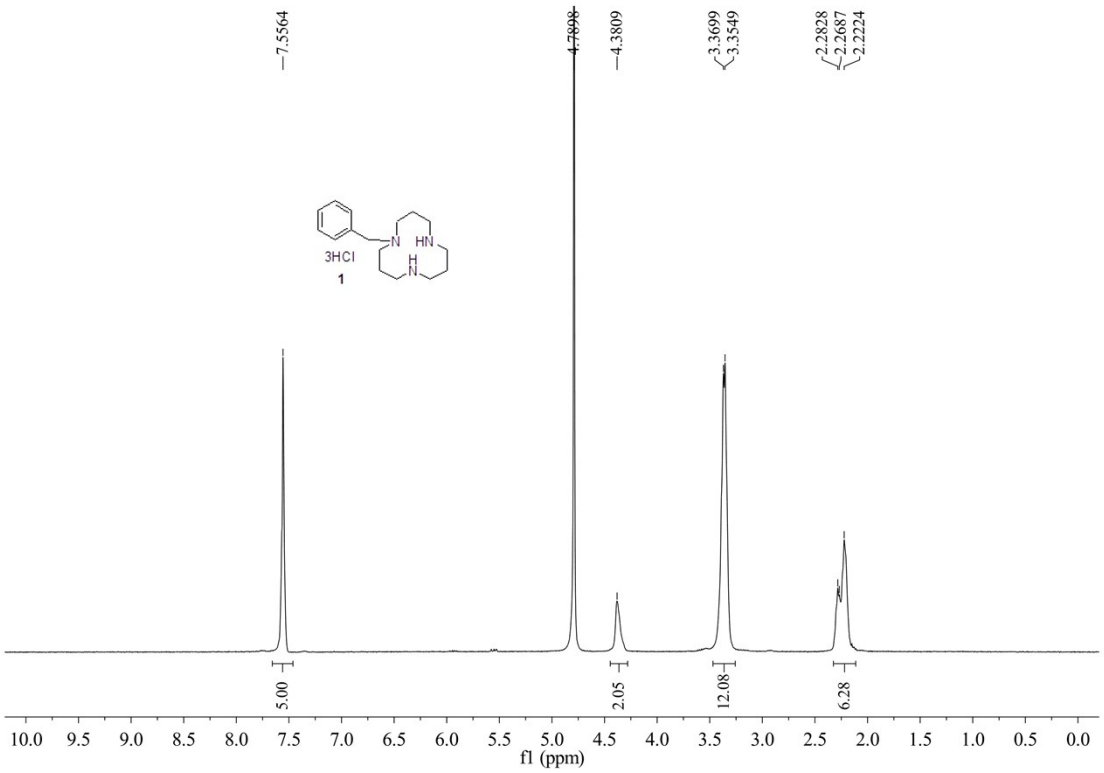
7.6 Spectra data for compound **12a**

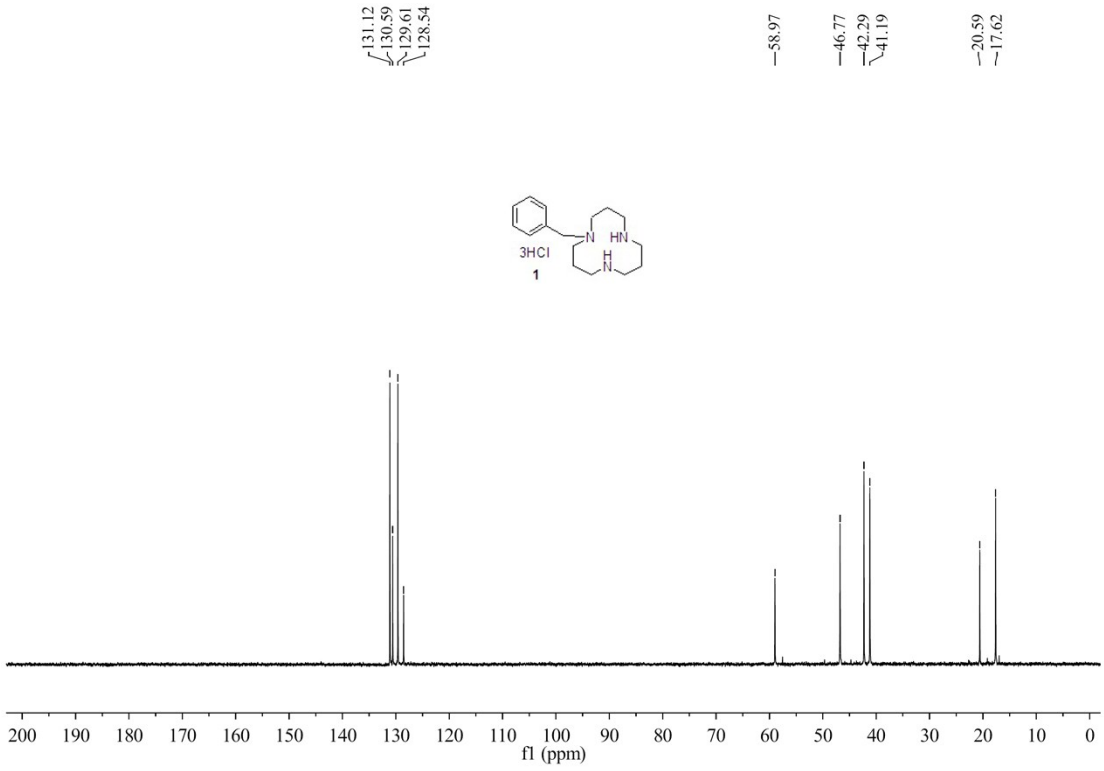


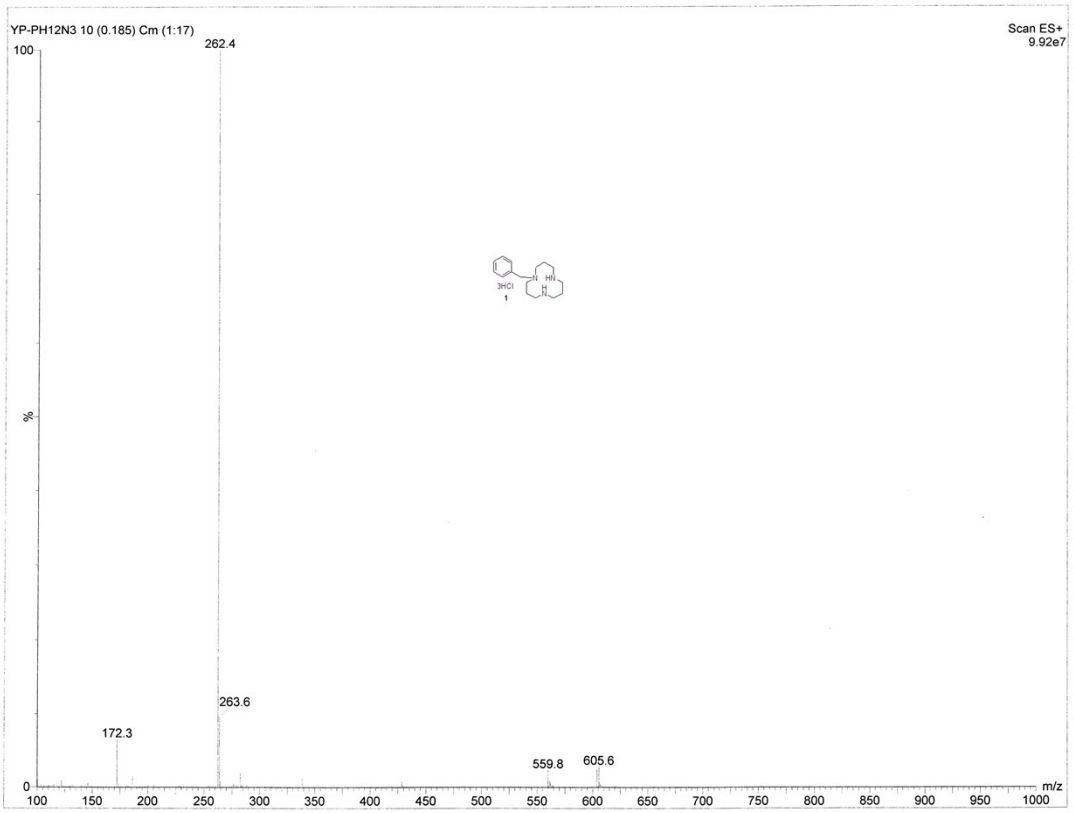
7.7 Spectra data for compound **12b**



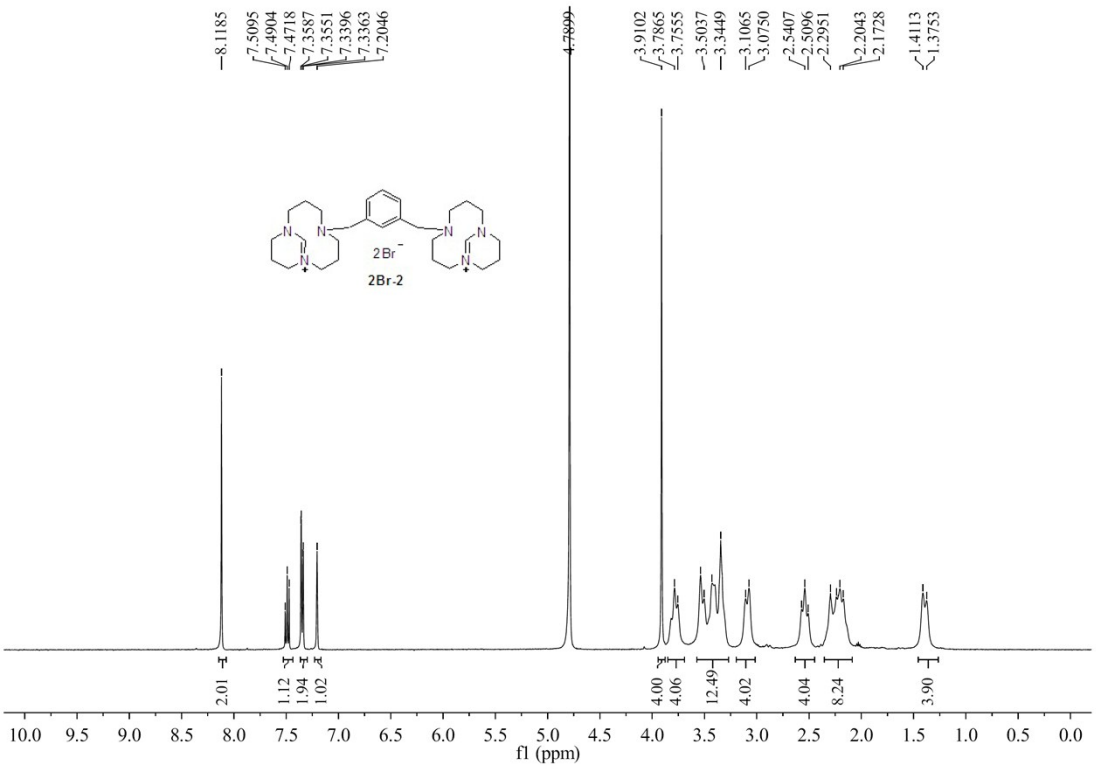
7.8 Spectra data for compound **1**



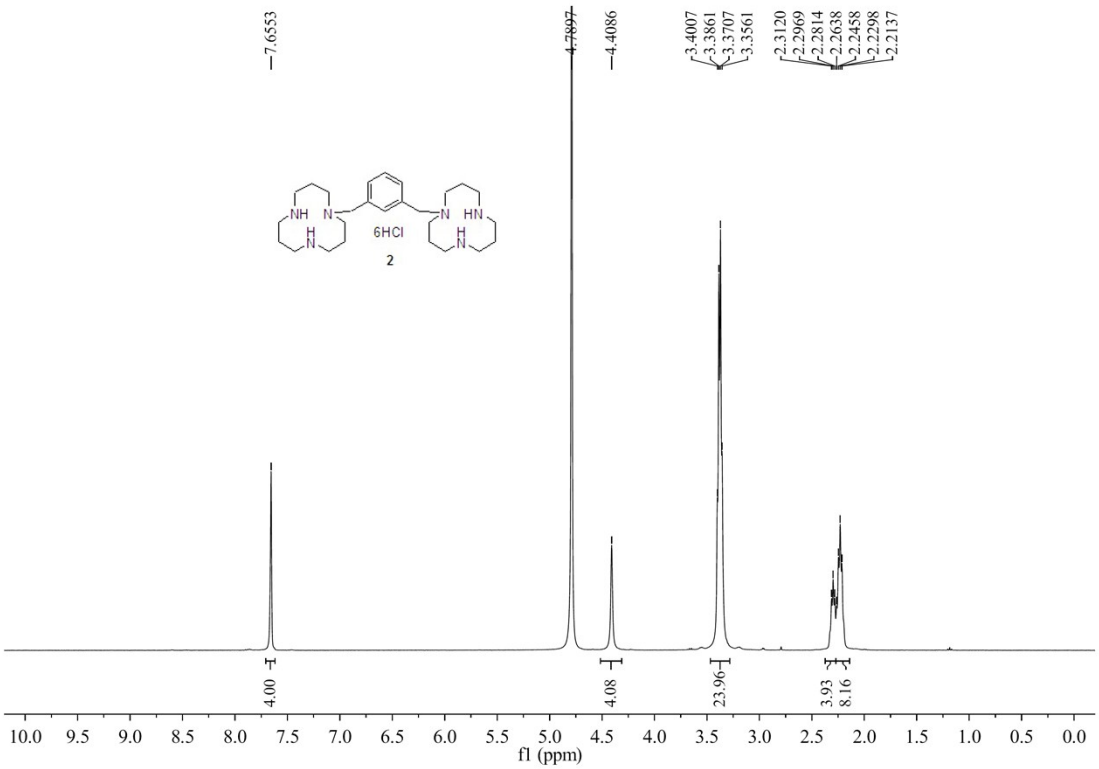


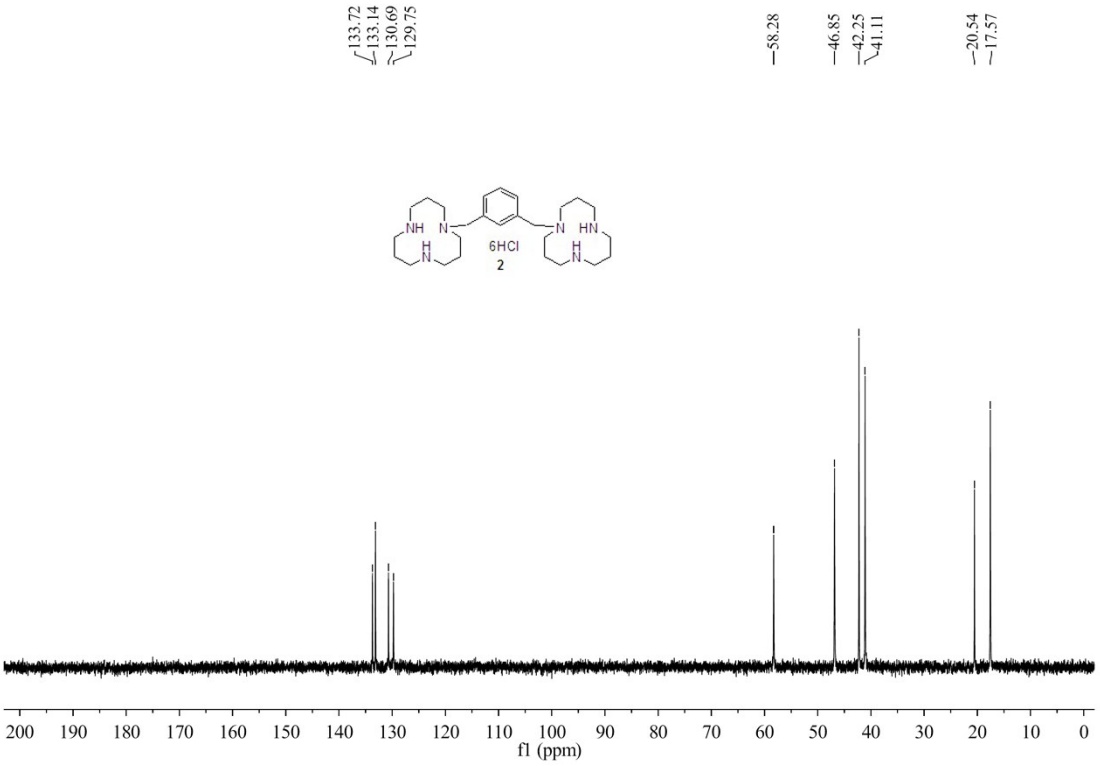


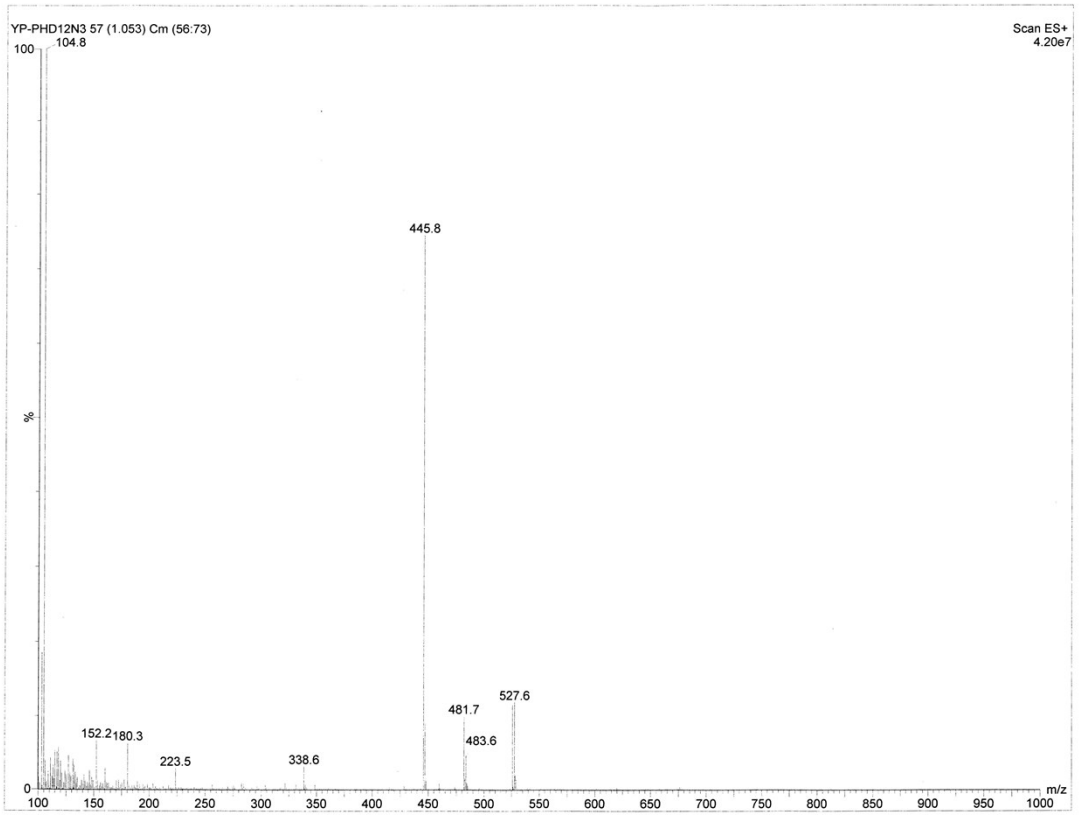
7.9 Spectra data for compound **2Br-2**



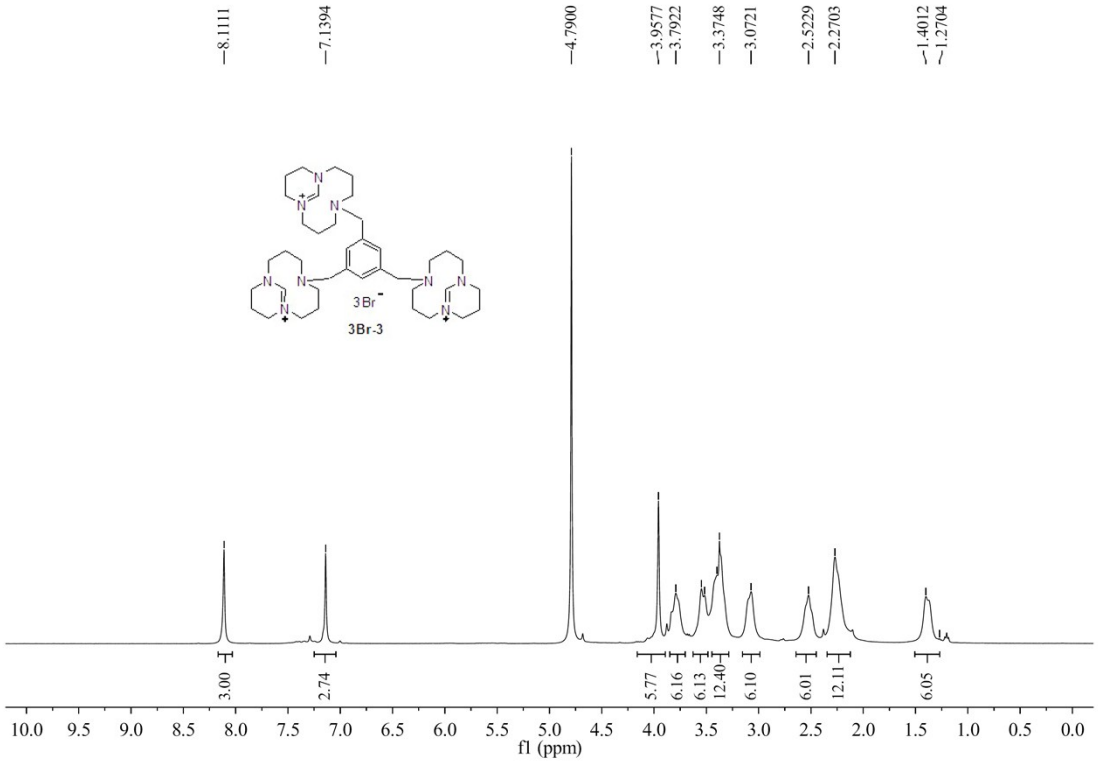
7.10 Spectra data for compound **2**

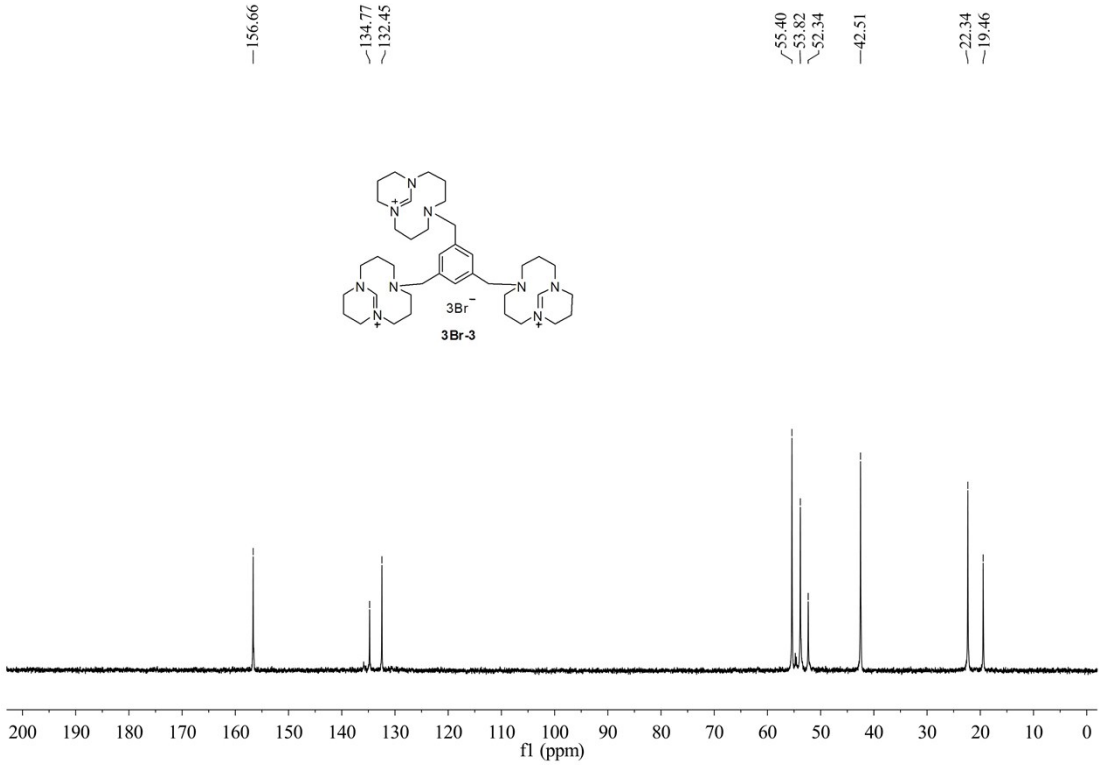


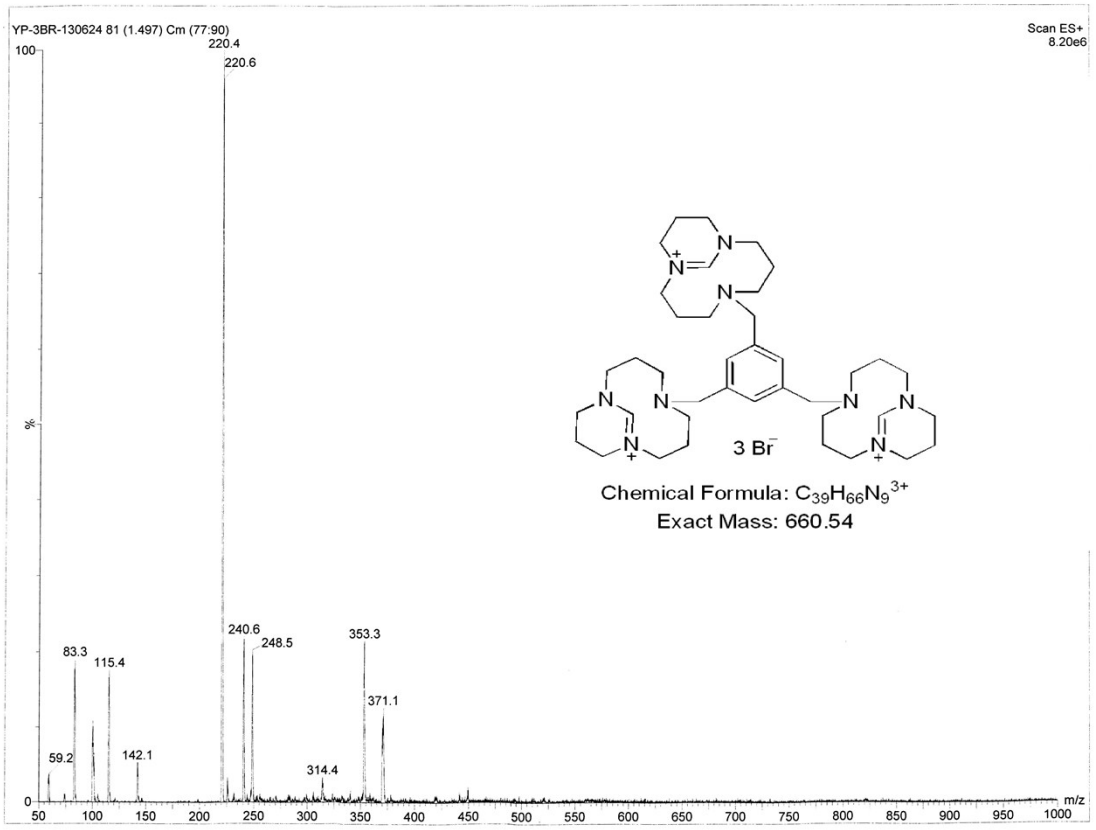




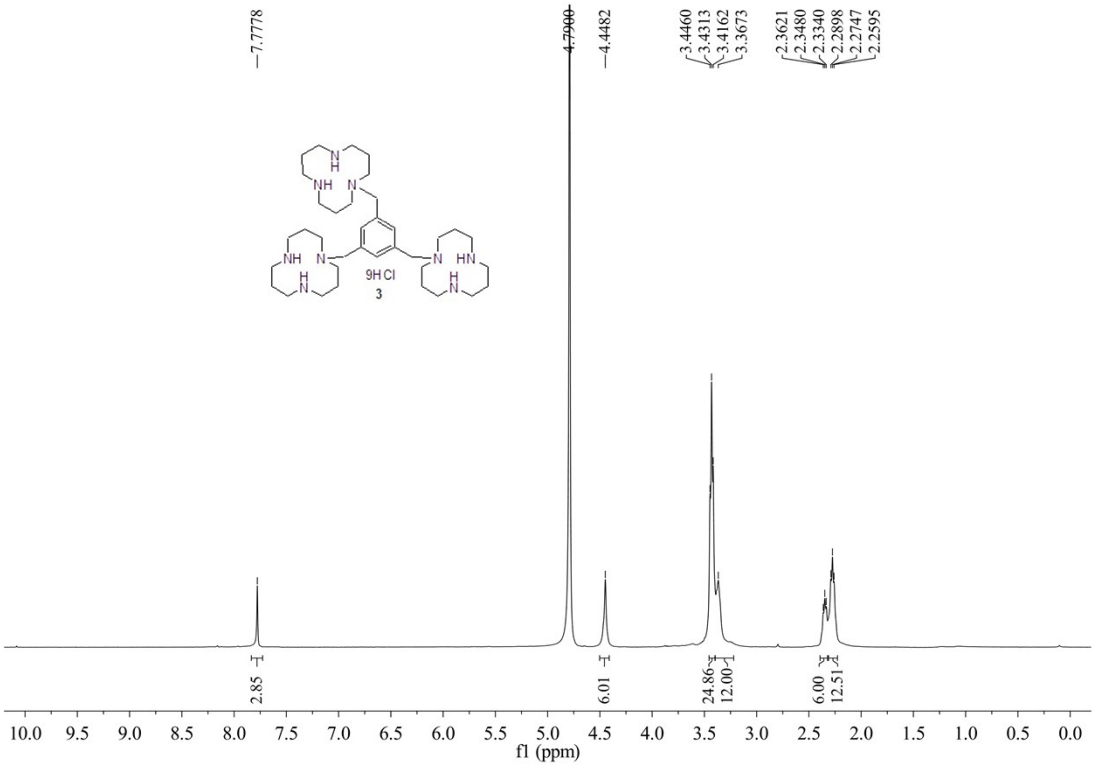
7.11 Spectra data for compound **3Br-3**

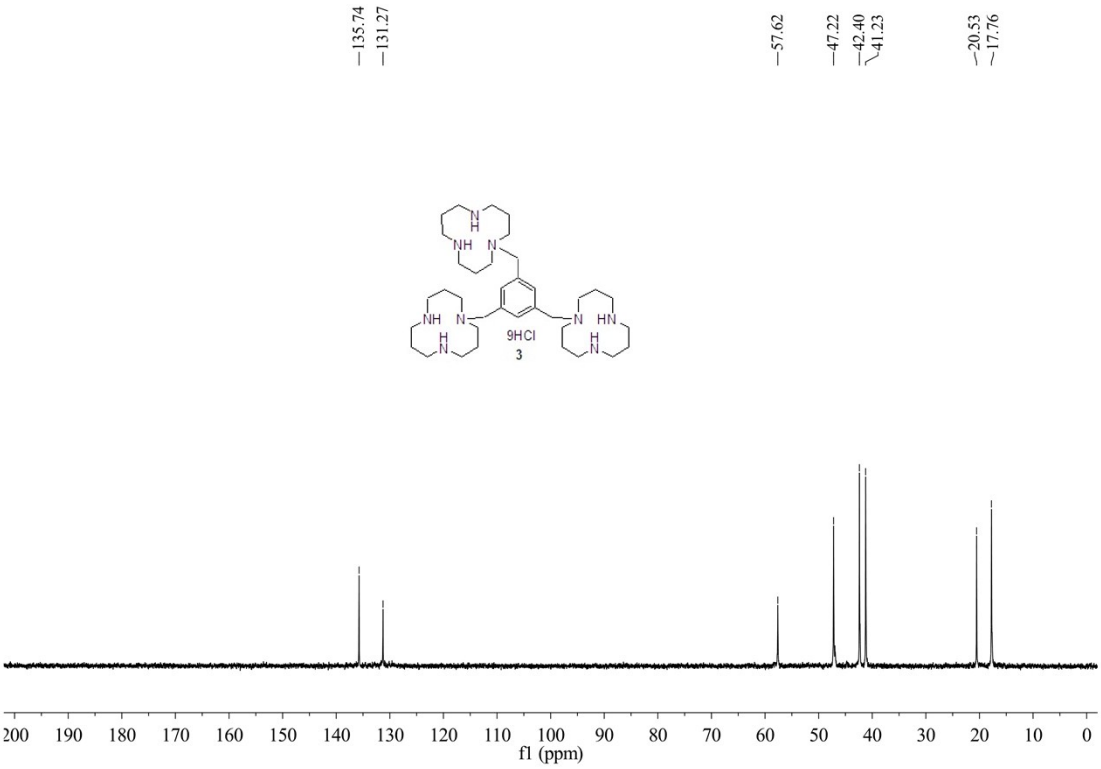


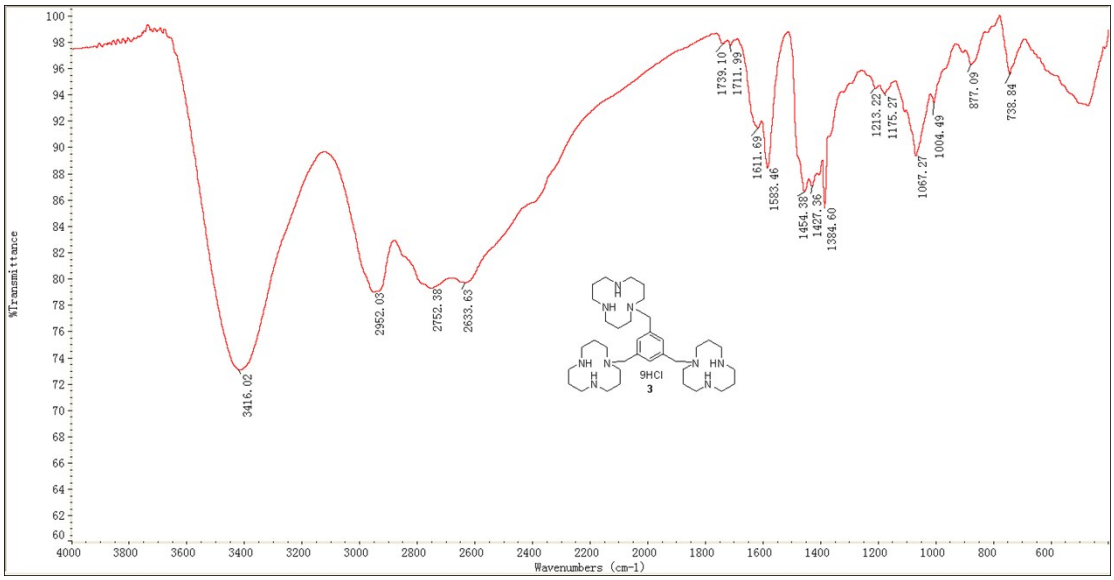

156.66
134.77
132.45
55.40
53.82
52.34
42.51
22.34
19.46
3Br⁻
3Br-3
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


YP-3BR-130624 81 (1.497) Cm (77:90)
Scan ES+
8.20e6
100
%
0
220.4
220.6
240.6
248.5
353.3
371.1
83.3
115.4
59.2
142.1
314.4
50 100 150 200 250 300 350 400 450 500 550 600 650 700 750 800 850 900 950 1000
m/z
3 Br⁻
Chemical Formula: C39H66N9 3+
Exact Mass: 660.54

7.12 Spectra data for compound **3**



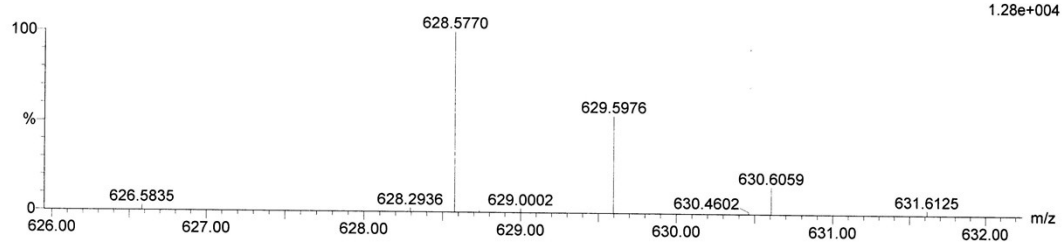




Elemental Composition Report **Page 1**

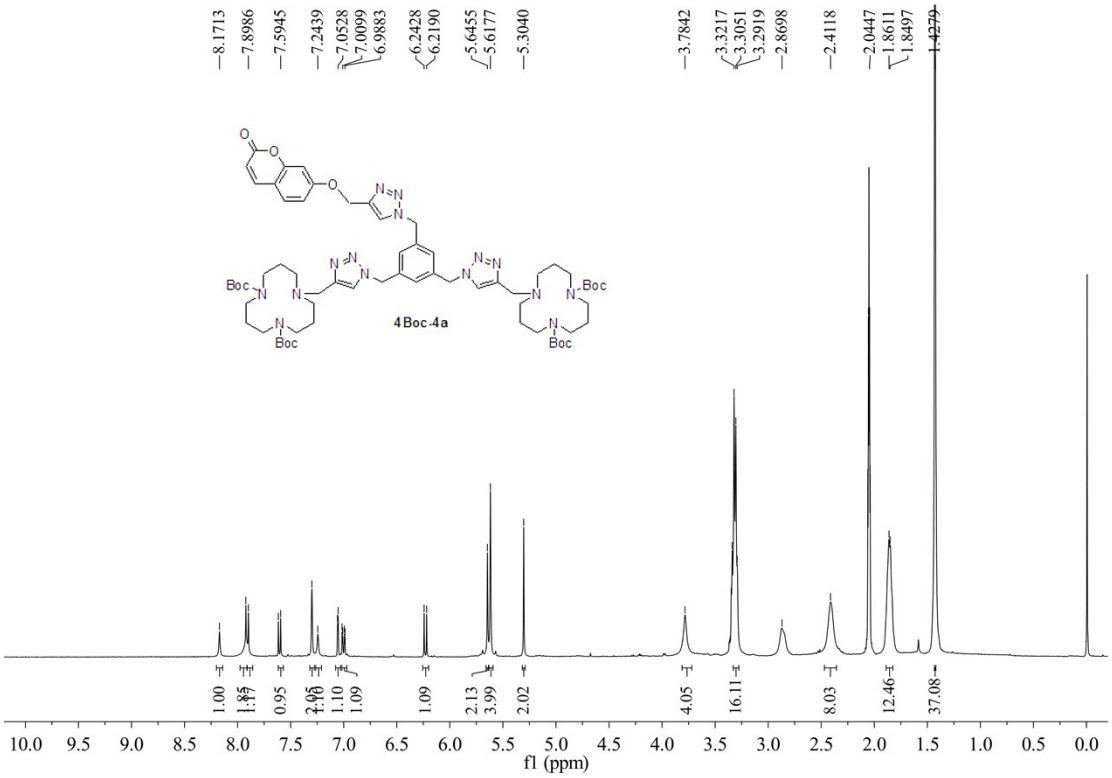
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

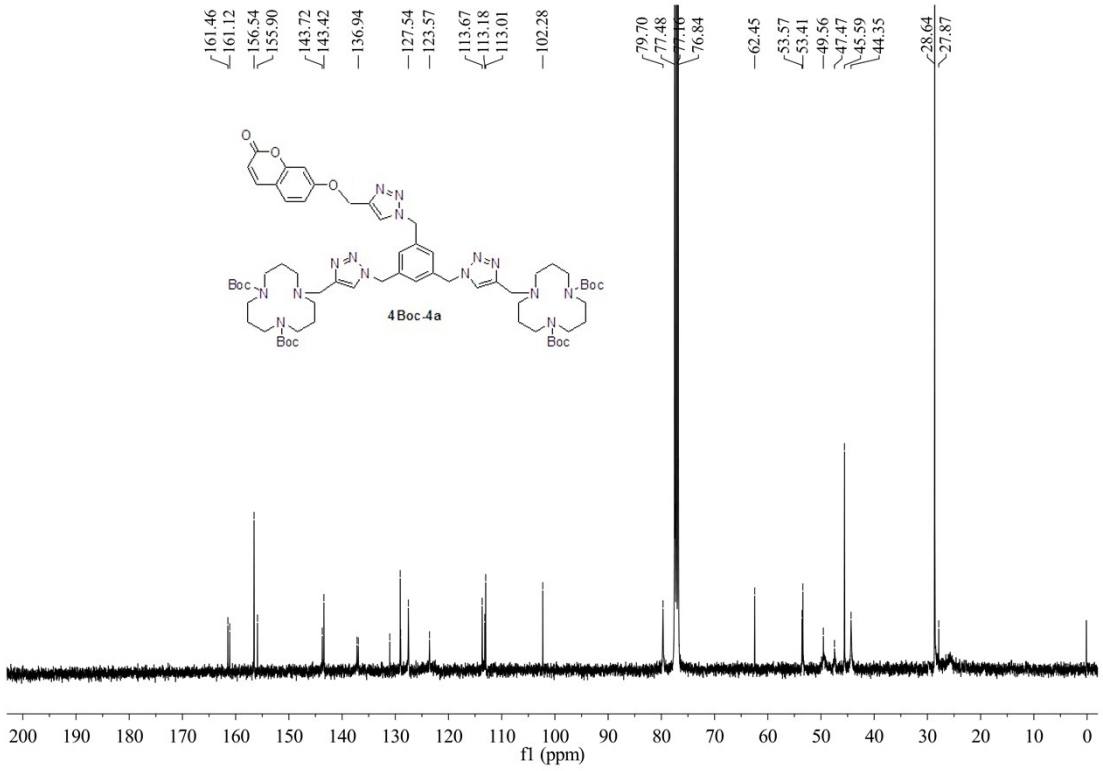
Monoisotopic Mass, Even Electron Ions
328 formula(e) evaluated with 3 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-40 H: 0-80 N: 0-10 O: 0-6
YP-PHT12N3HCL-130625 10 (0.170)
TOF MS ES+

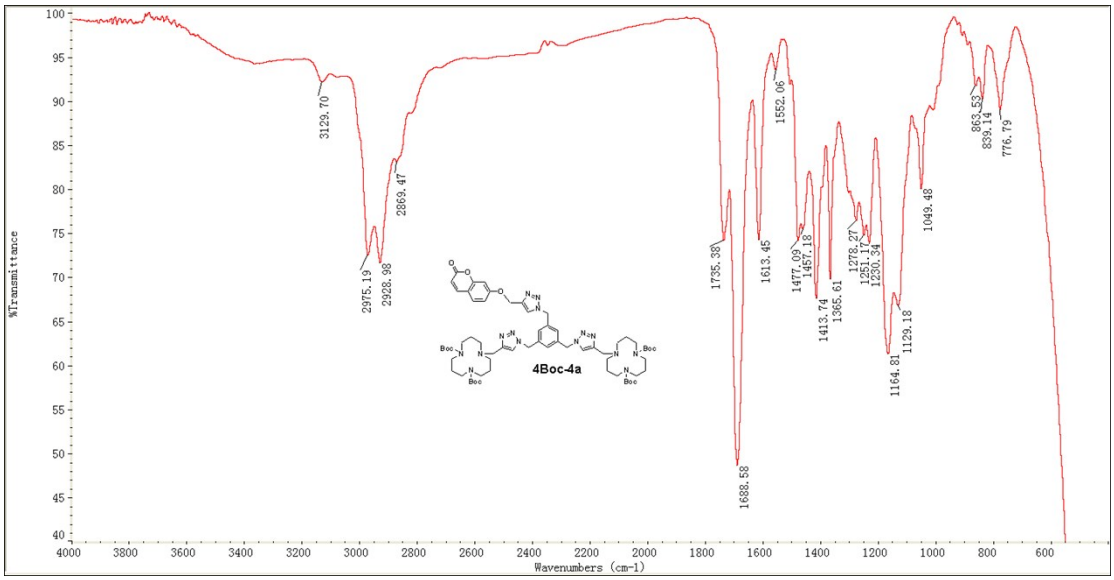


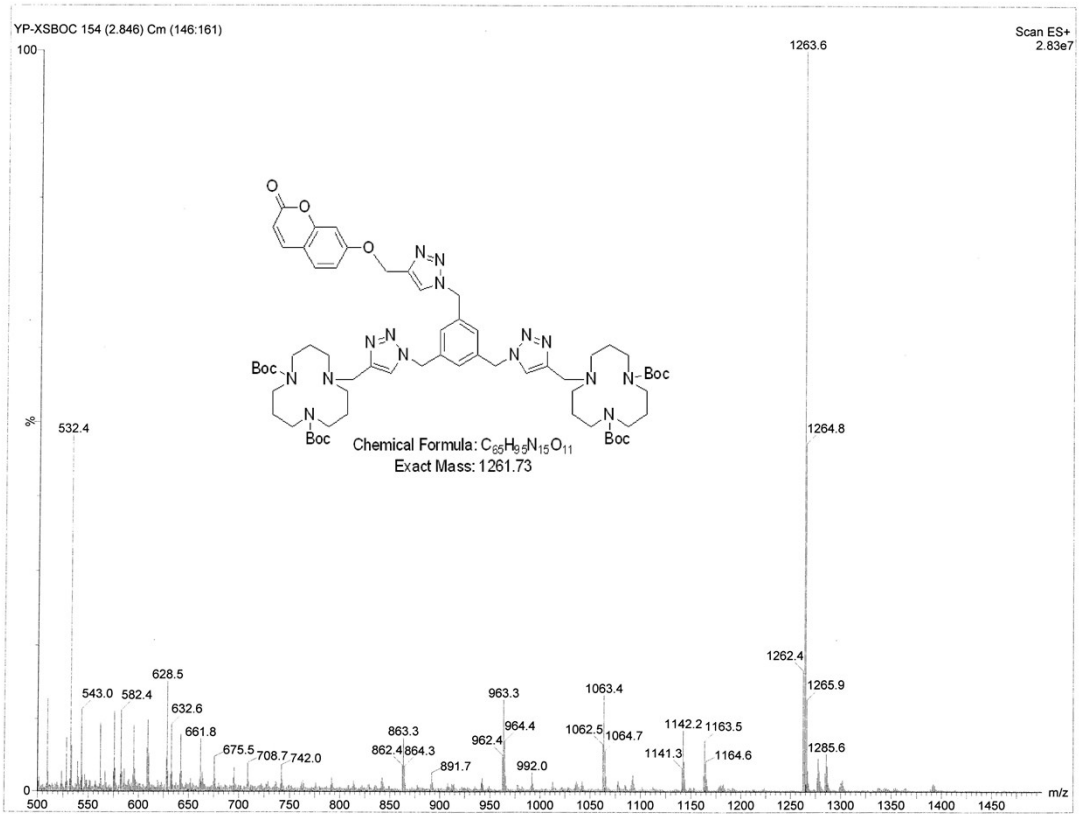
Minimum:				-1.5		
Maximum:		5.0	5.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
628.5770	628.5781	-1.1	-1.7	5.5	108.0	C40 H74 N3 O2
	628.5754	1.6	2.5	6.5	198.6	C36 H70 N9
	628.5741	2.9	4.6	1.5	254.1	C35 H74 N5 O4

7.13 Spectra data for compound **4Boc-4a**

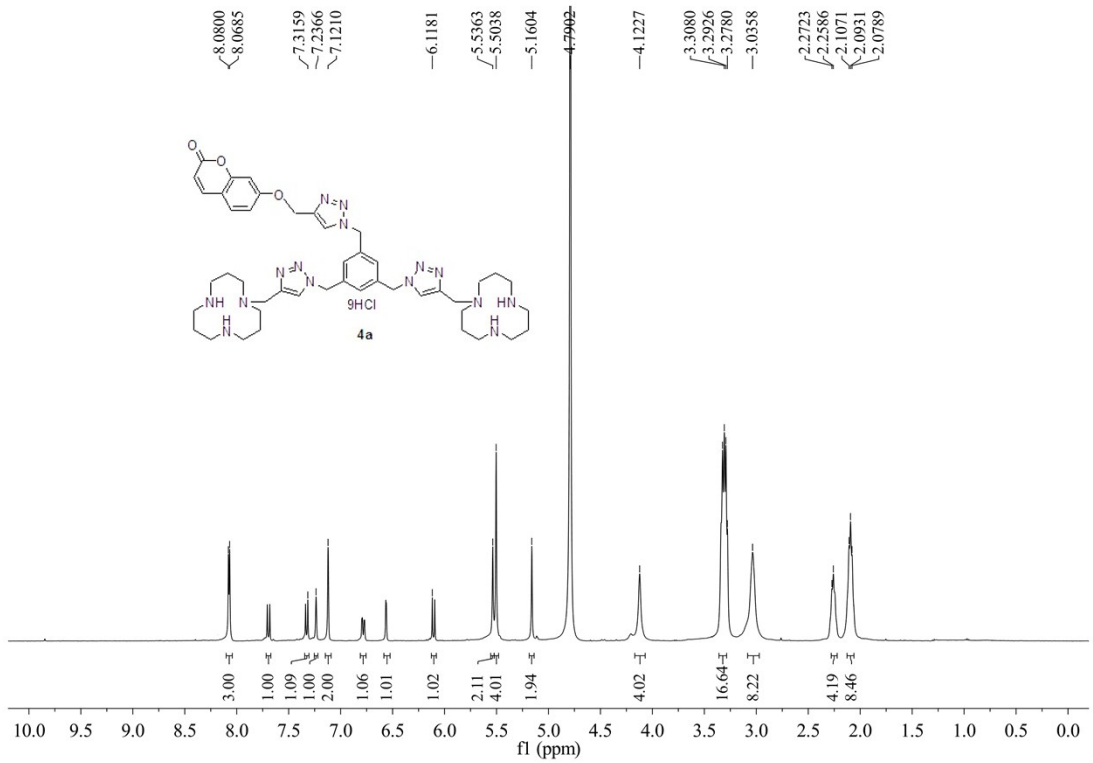


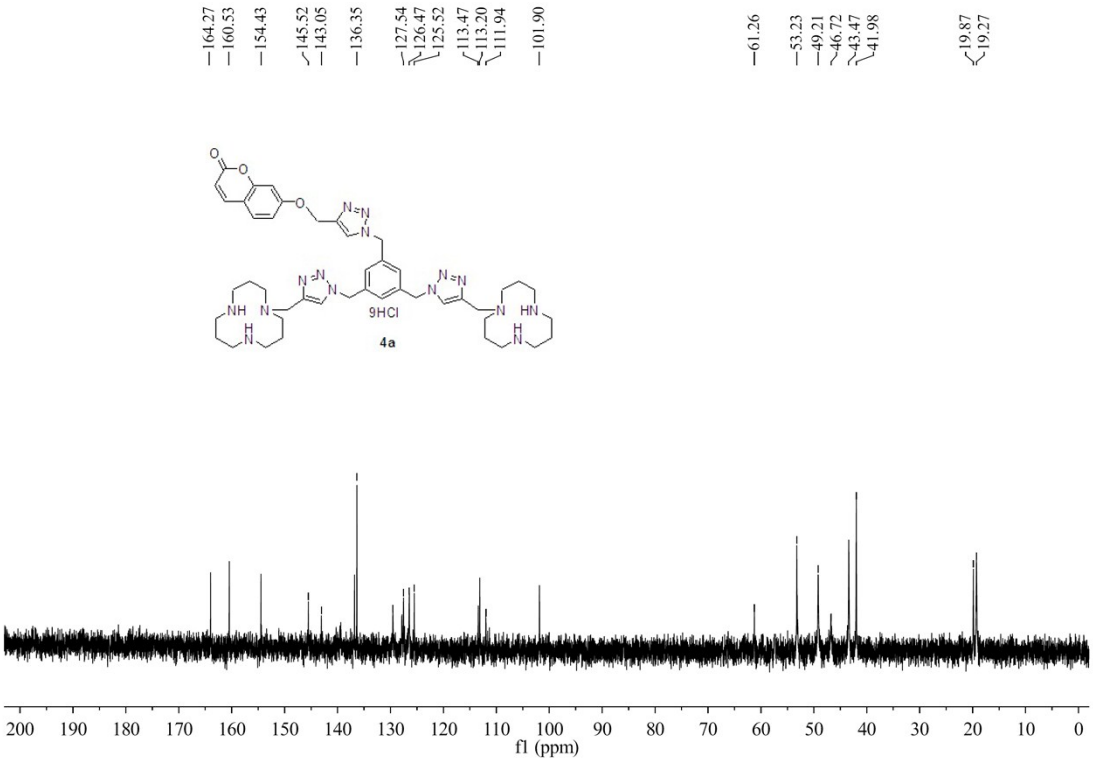


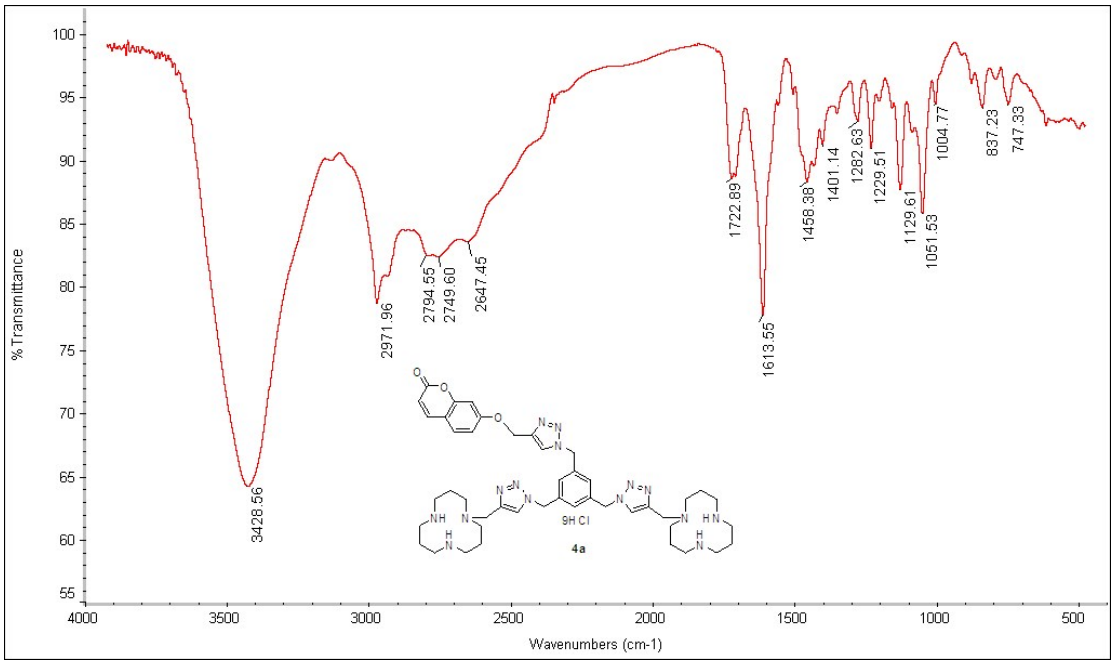

%Transmittance
Wavenumbers (cm-1)
3129.70
2975.19
2928.98
2869.47
1735.38
1688.58
1613.45
1552.06
1477.09
1457.18
1413.74
1365.61
1278.27
1251.17
1230.34
1164.81
1129.18
1049.48
863.53
839.14
776.79
4Boc-4a


YP-XSBOC 154 (2.846) Cm (146:161)
Scan ES+
2.83e7
Chemical Formula: C65H95N15O11
Exact Mass: 1261.73
m/z

7.14 Spectra data for compound **4a**





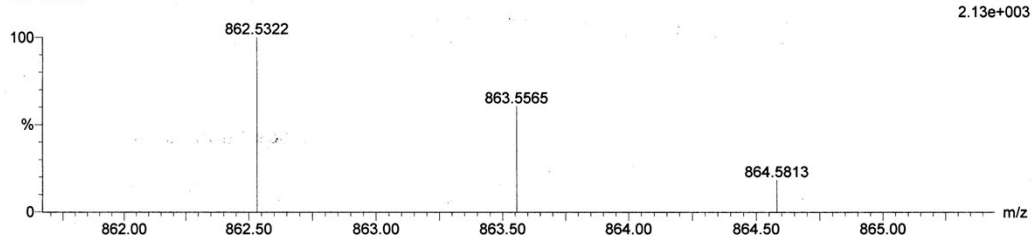


Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 3.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

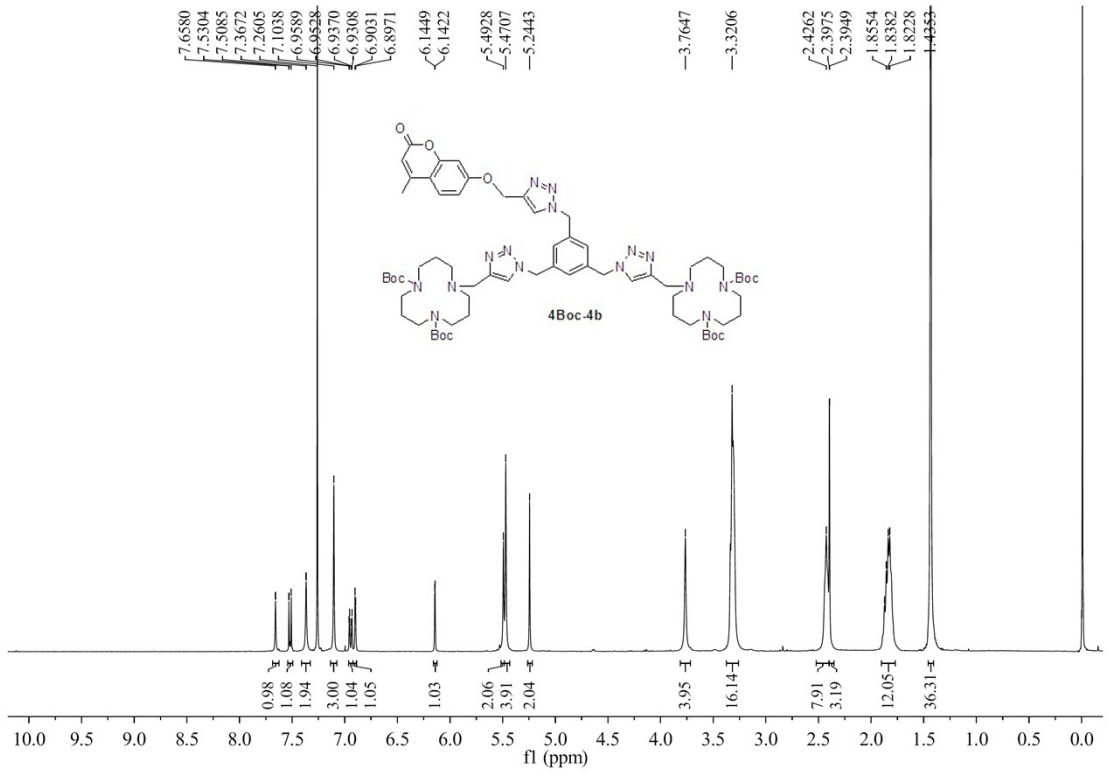
Monoisotopic Mass, Even Electron Ions
3445 formula(e) evaluated with 5 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-70 H: 0-80 N: 0-20 O: 0-5 Br: 0-3

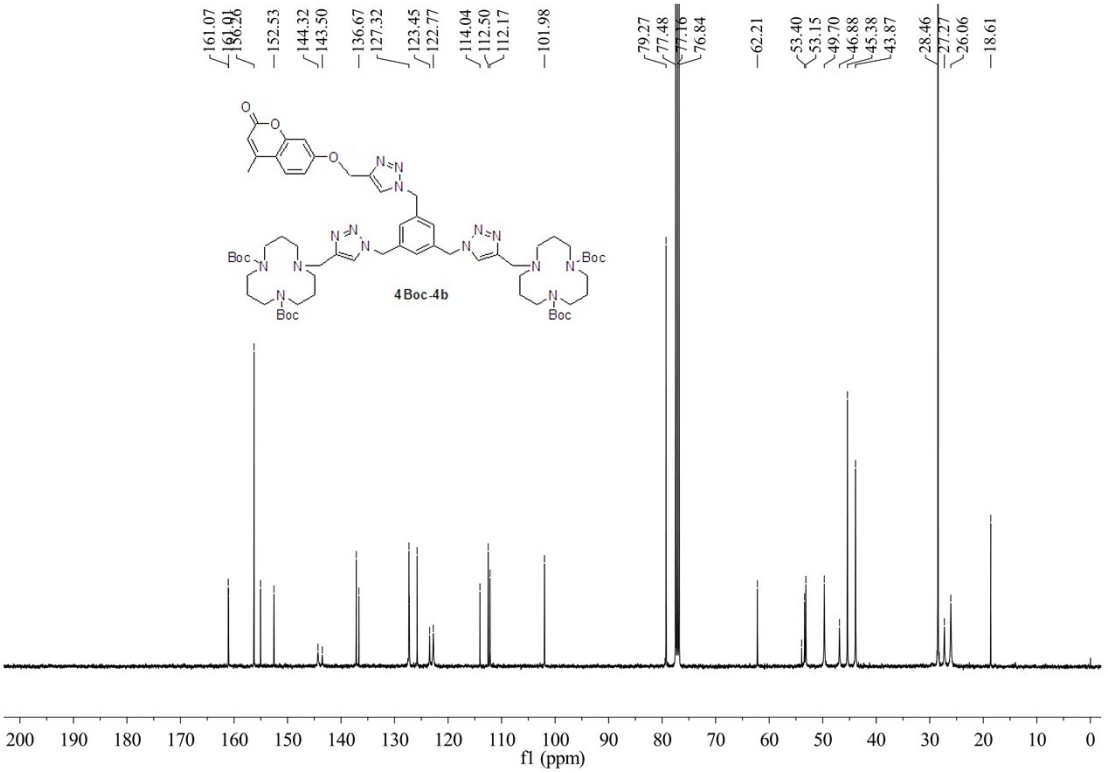
0J-XDS-HCL 5 (0.093)
TOF MS ES+

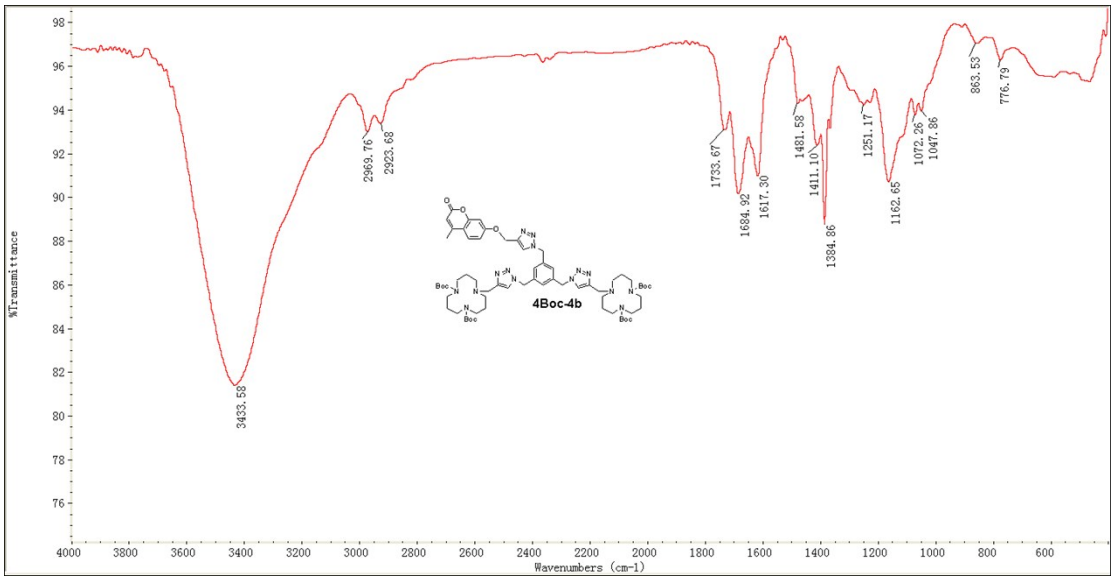


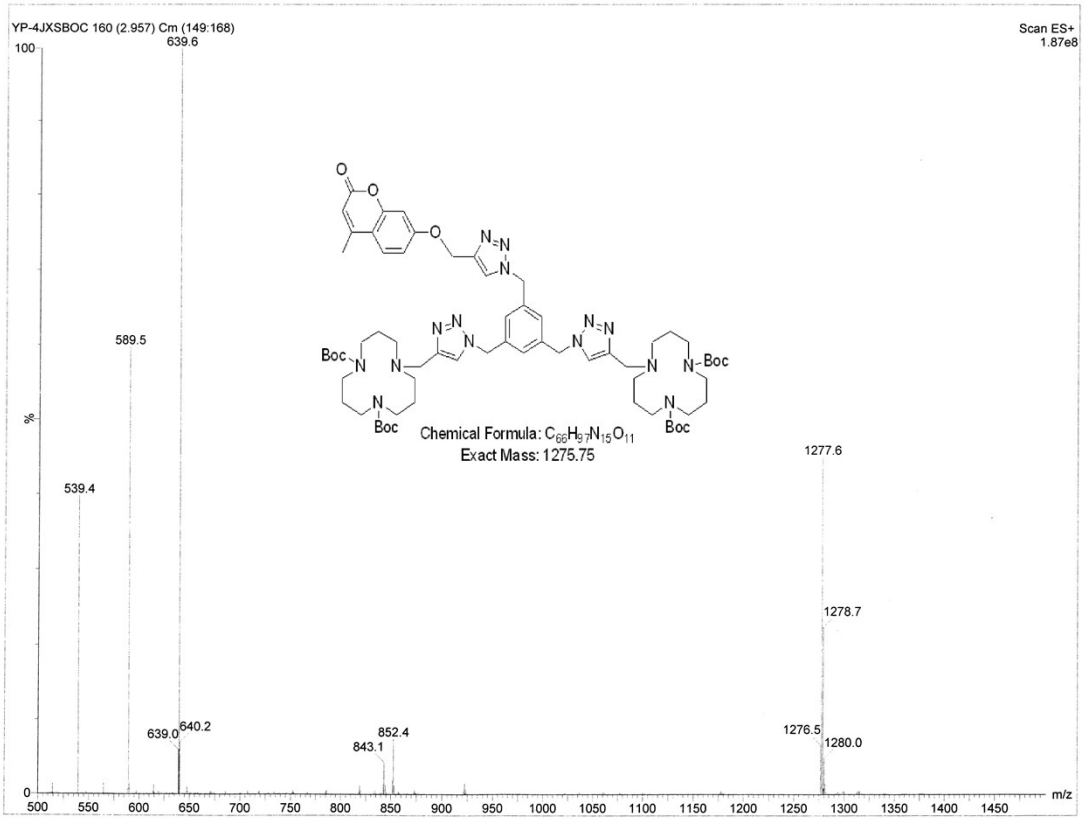
Minimum:				-1.5		
Maximum:		10.0	3.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
862.5322	862.5322	0.0	0.0	13.5	1003.3	C48 H77 N7 O2 Br
	862.5317	0.5	0.6	21.5	8.1	C45 H64 N15 O3 ✓
	862.5327	-0.5	-0.6	6.5	n/a	C33 H73 N19 O3 Br
	862.5312	1.0	1.2	28.5	21.2	C60 H68 N3 O2
	862.5343	-2.1	-2.4	20.5	4.5	C49 H68 N9 O5

7.15 Spectra data for compound **4Boc-4b**

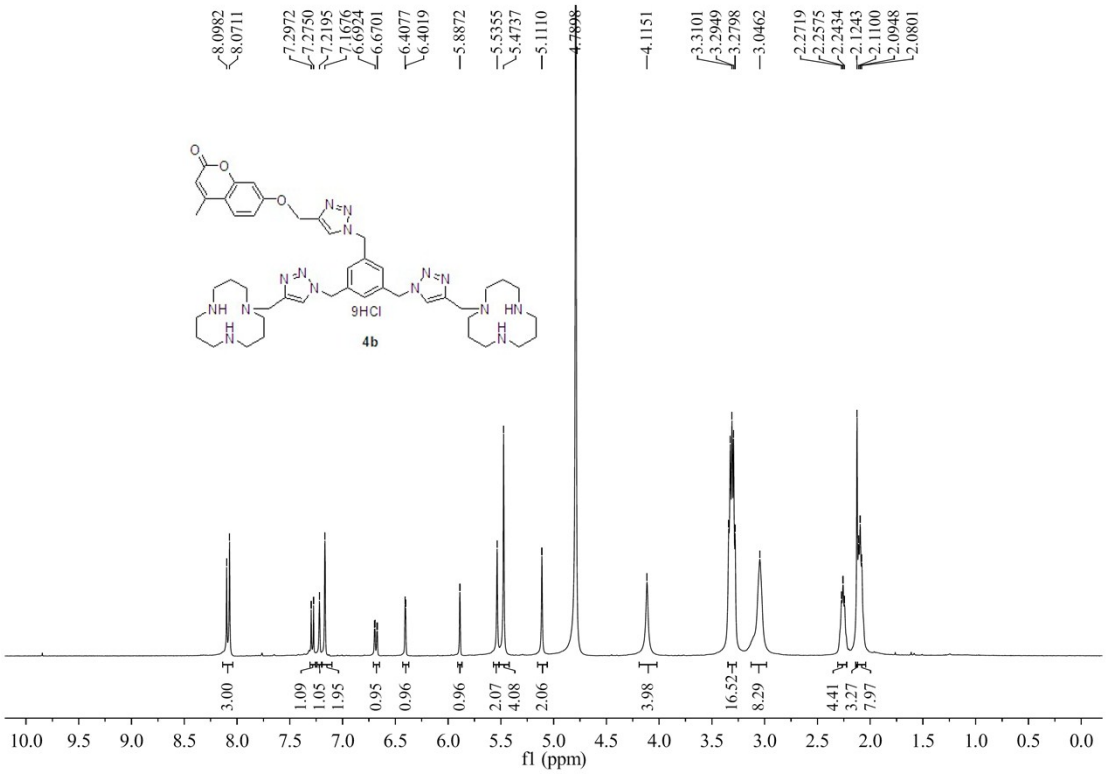


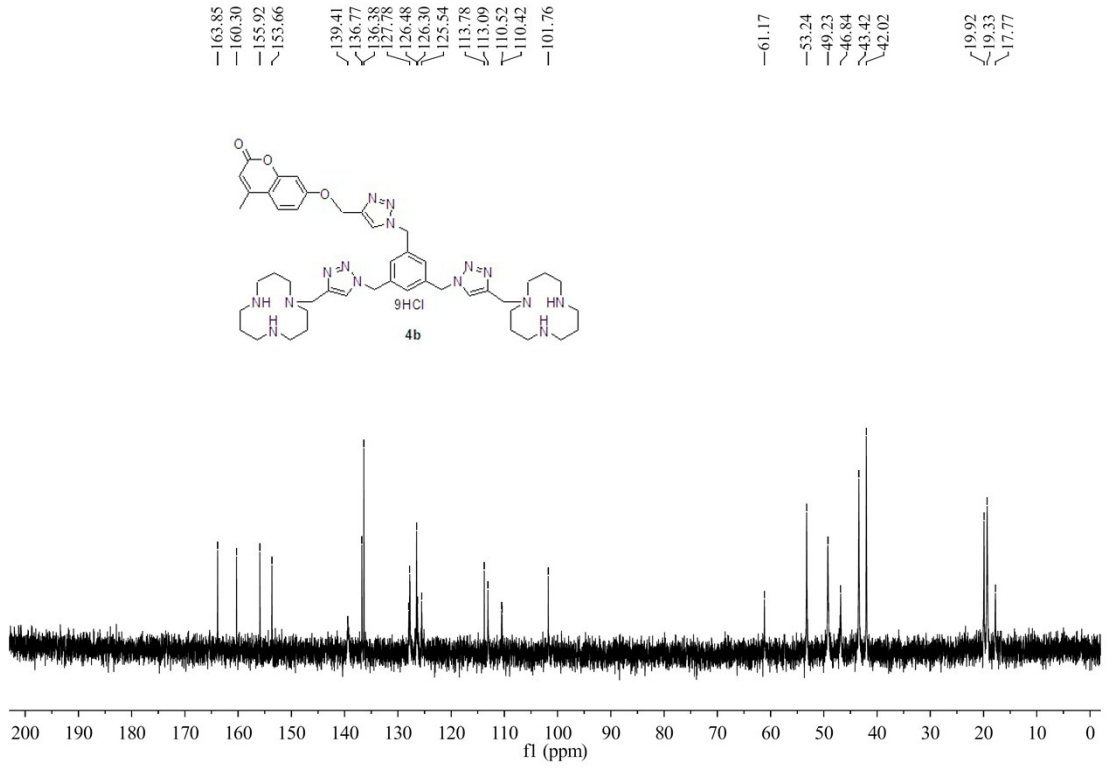


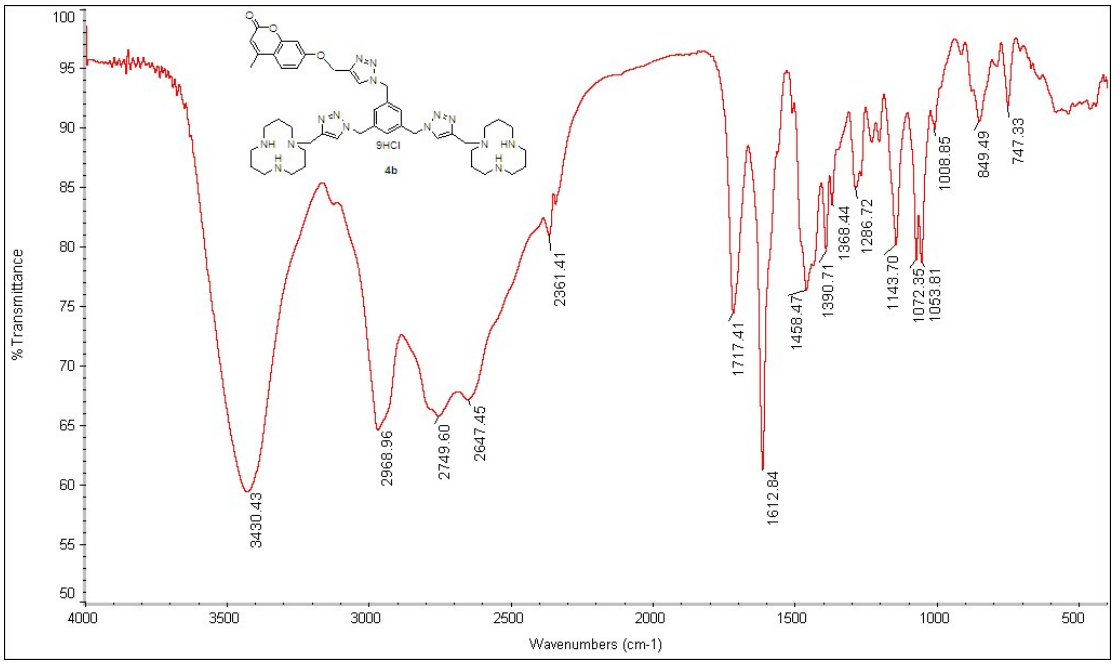

%Transmittance
Wavenumbers (cm-1)
3433.58
2969.76
2923.68
1733.67
1684.92
1617.30
1481.58
1411.10
1384.86
1251.17
1162.65
1072.26
1047.86
863.53
776.79
4Boc-4b


YP-4JXSBOC 160 (2.957) Cm (149:168)
Scan ES+
1.87e8
639.6
589.5
539.4
640.2
639.0
852.4
843.1
1277.6
1278.7
1276.5
1280.0
Chemical Formula: C66H97N15O11
Exact Mass: 1275.75
%
m/z

7.16 Spectra data for compound **4b**





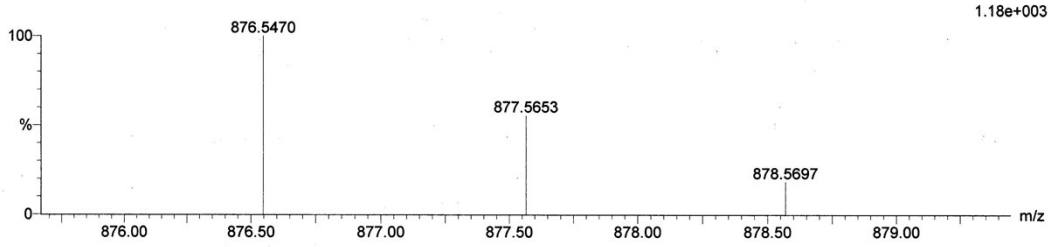


Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 3.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

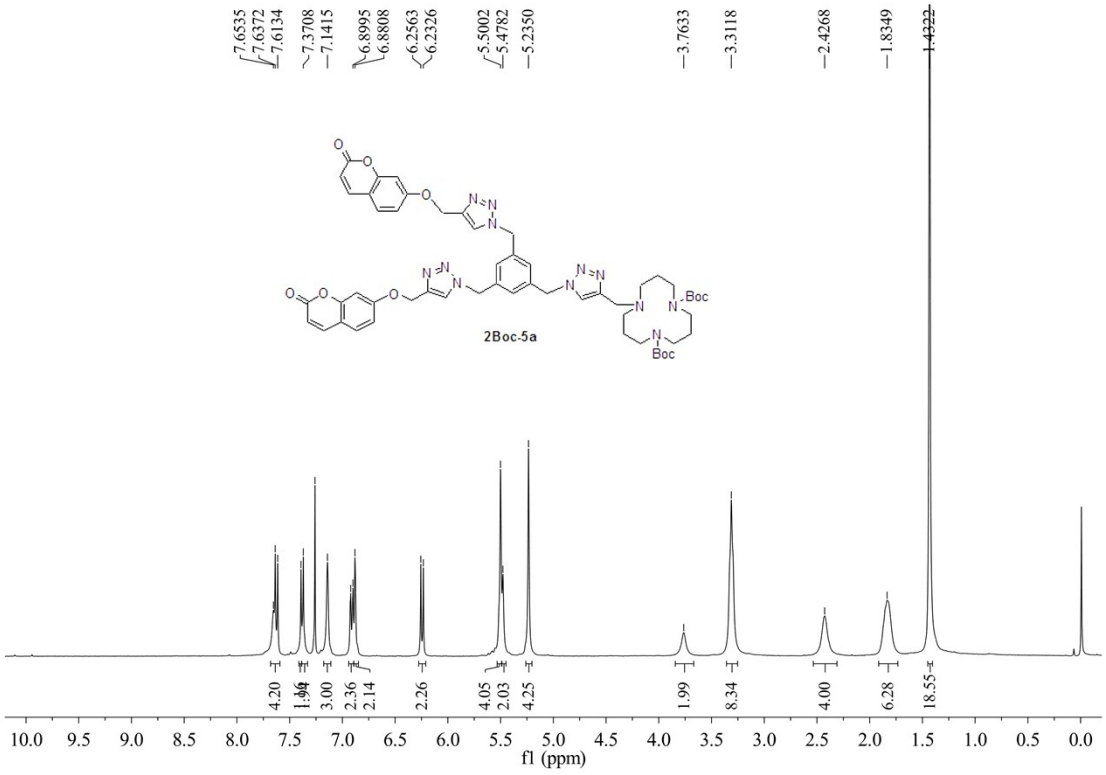
Monoisotopic Mass, Even Electron Ions
3452 formula(e) evaluated with 5 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-70 H: 0-80 N: 0-20 O: 0-5 Br: 0-3

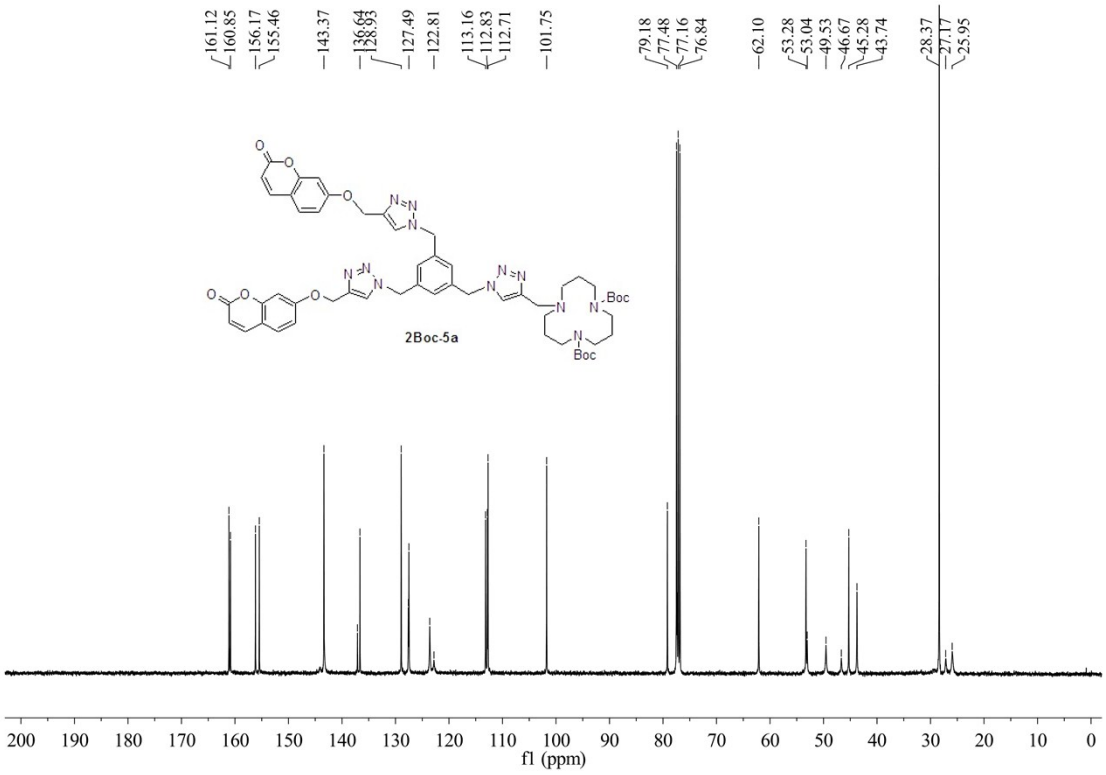
4J-XDS-HCL 4 (0.074)
TOF MS ES+

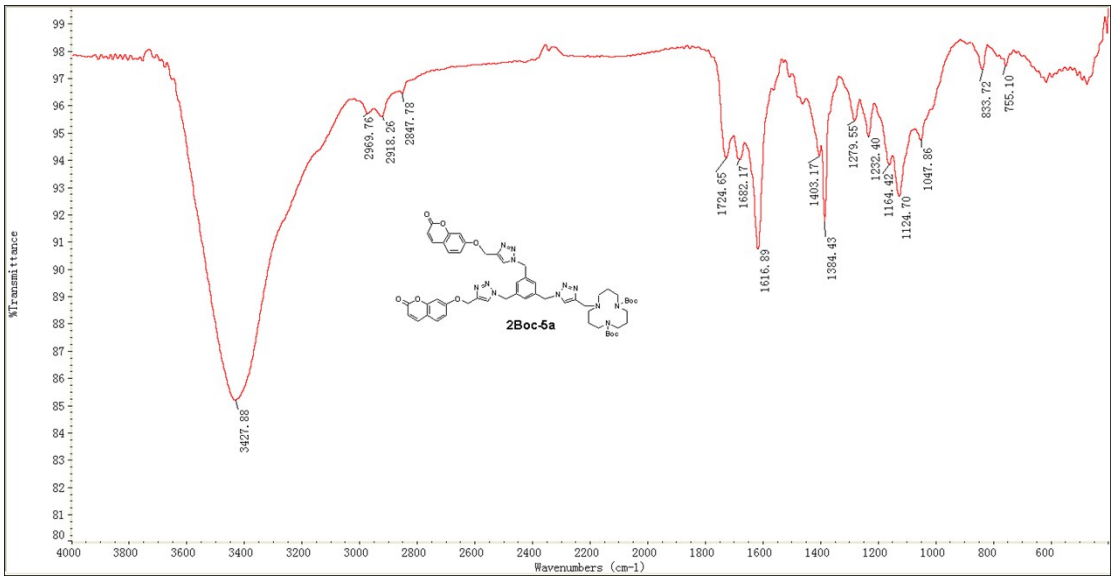


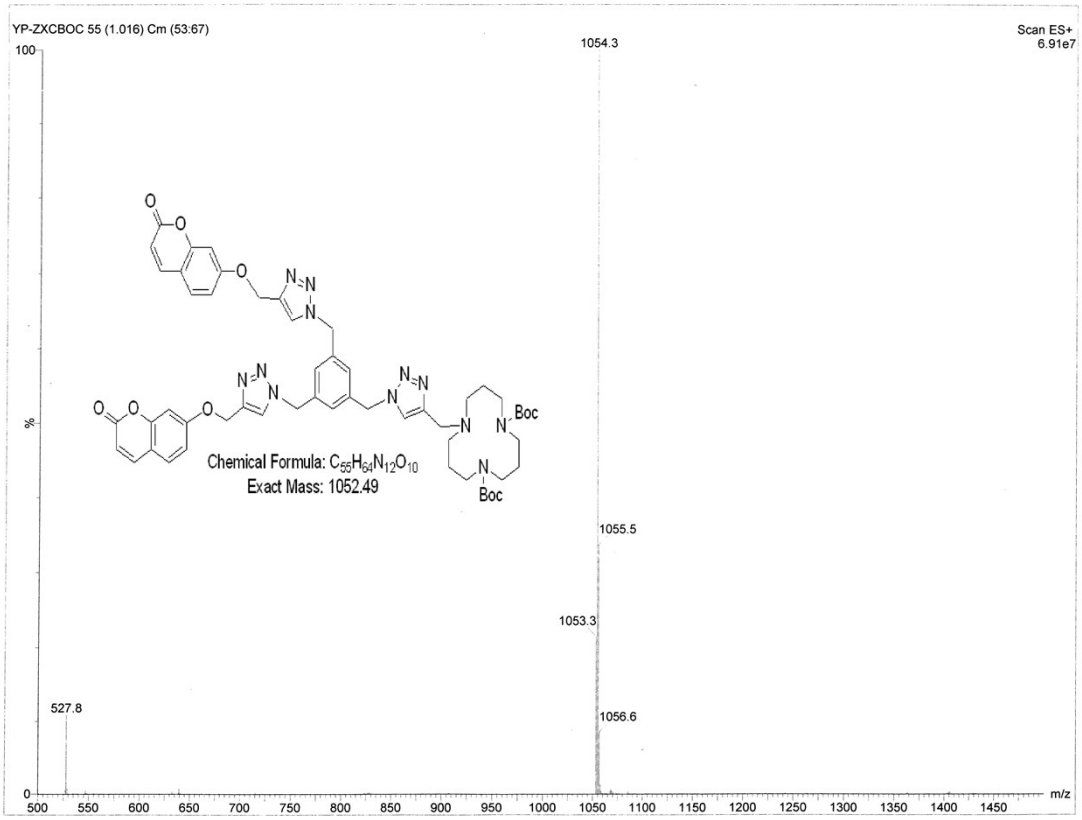
Minimum:				-1.5		
Maximum:		10.0	3.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
876.5470	876.5468	0.2	0.2	28.5	16.9	C61 H70 N3 O2
	876.5473	-0.3	-0.3	21.5	1.9	C46 H66 N15 O3
	876.5479	-0.9	-1.0	13.5	526.0	C49 H79 N7 O2 Br
	876.5484	-1.4	-1.6	6.5	536.7	C34 H75 N19 O3 Br
	876.5452	1.8	2.1	14.5	526.1	C45 H75 N13 Br

7.17 Spectra data for compound **2Boc-5a**

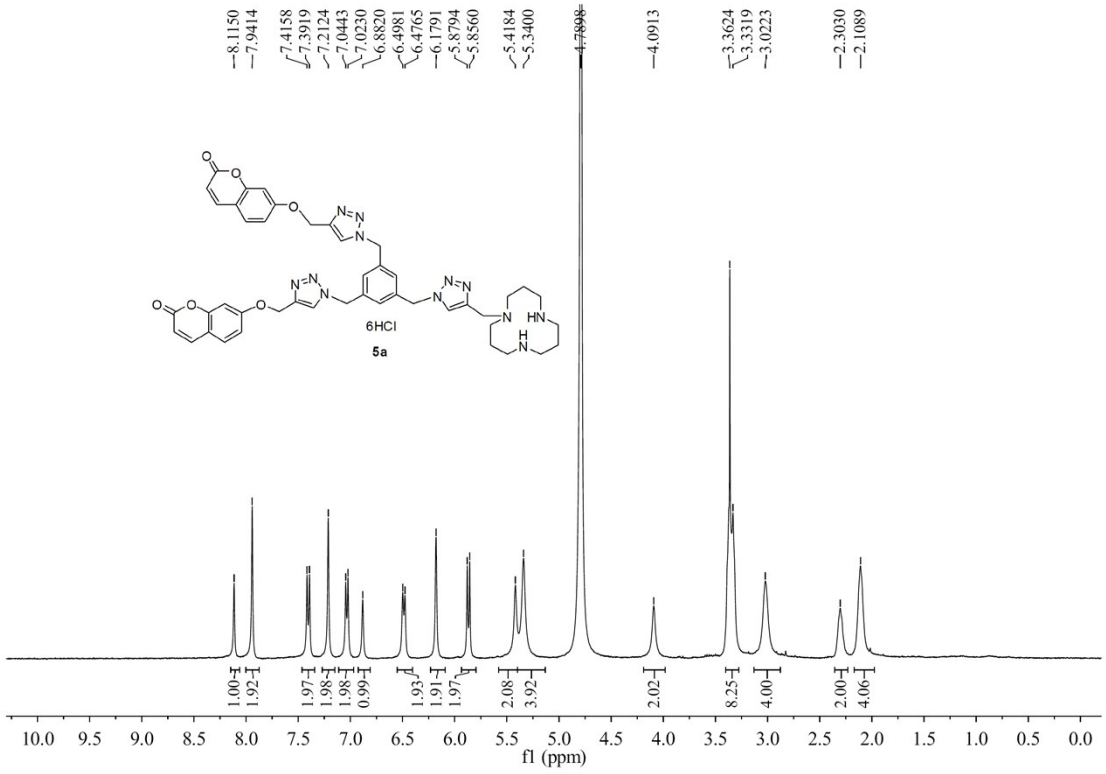


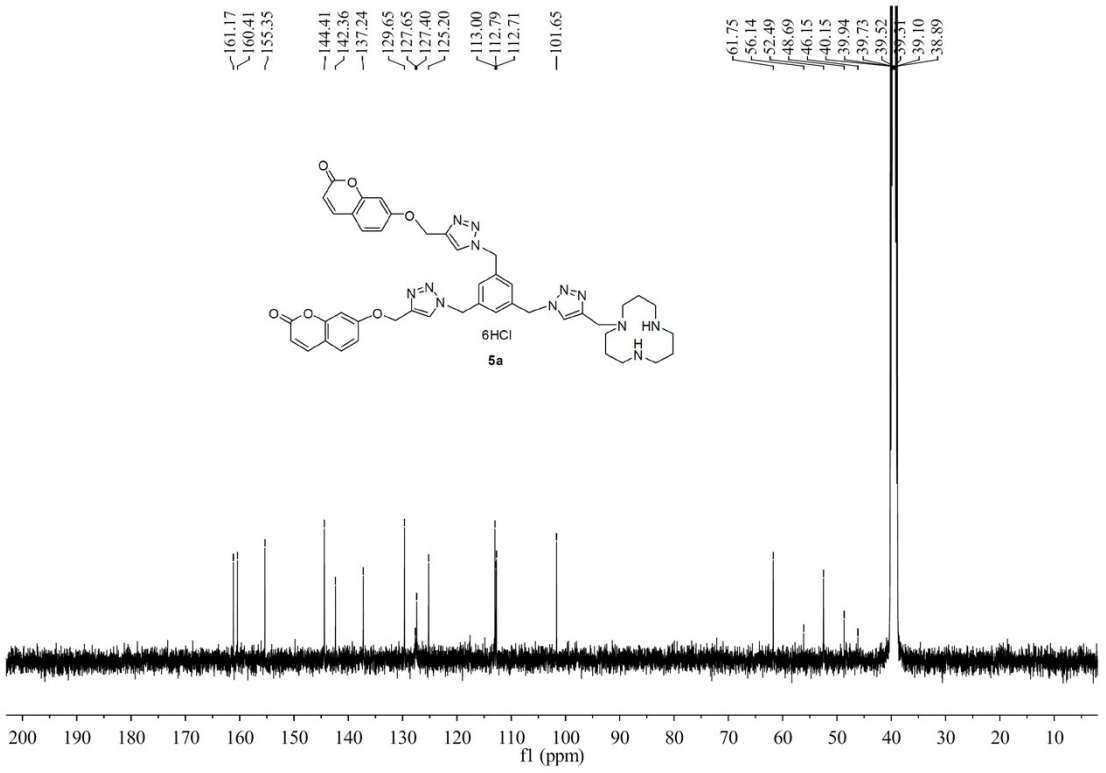


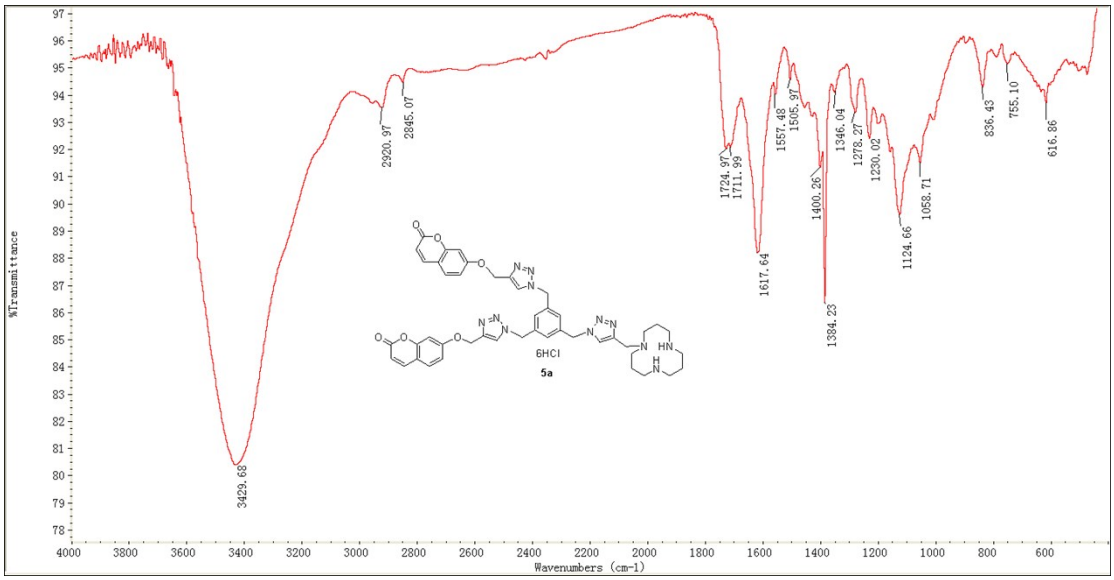

%Transmittance
Wavenumbers (cm-1)
3427.88
2969.76
2918.26
2847.78
1724.65
1682.17
1616.89
1403.17
1384.43
1279.55
1232.40
1164.42
1124.70
1047.86
833.72
755.10
2Boc-5a


YP-ZXCBOC 55 (1.016) Cm (53:67)
Scan ES+
6.91e7
1054.3
1055.5
1053.3
1056.6
527.8
Chemical Formula: C55H64N12O10
Exact Mass: 1052.49
Boc
Boc
%
m/z

7.18 Spectra data for compound **5a**



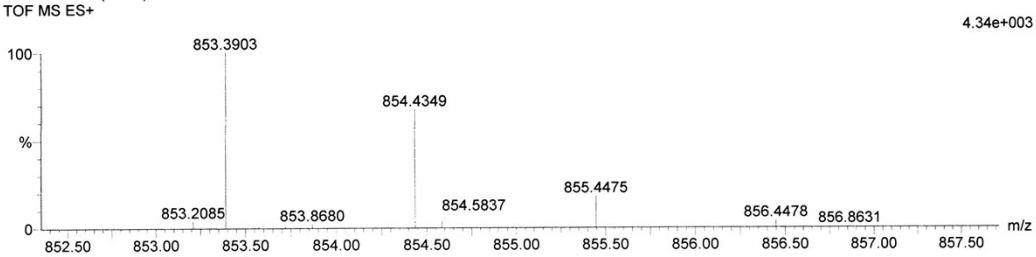




Elemental Composition Report **Page 1**

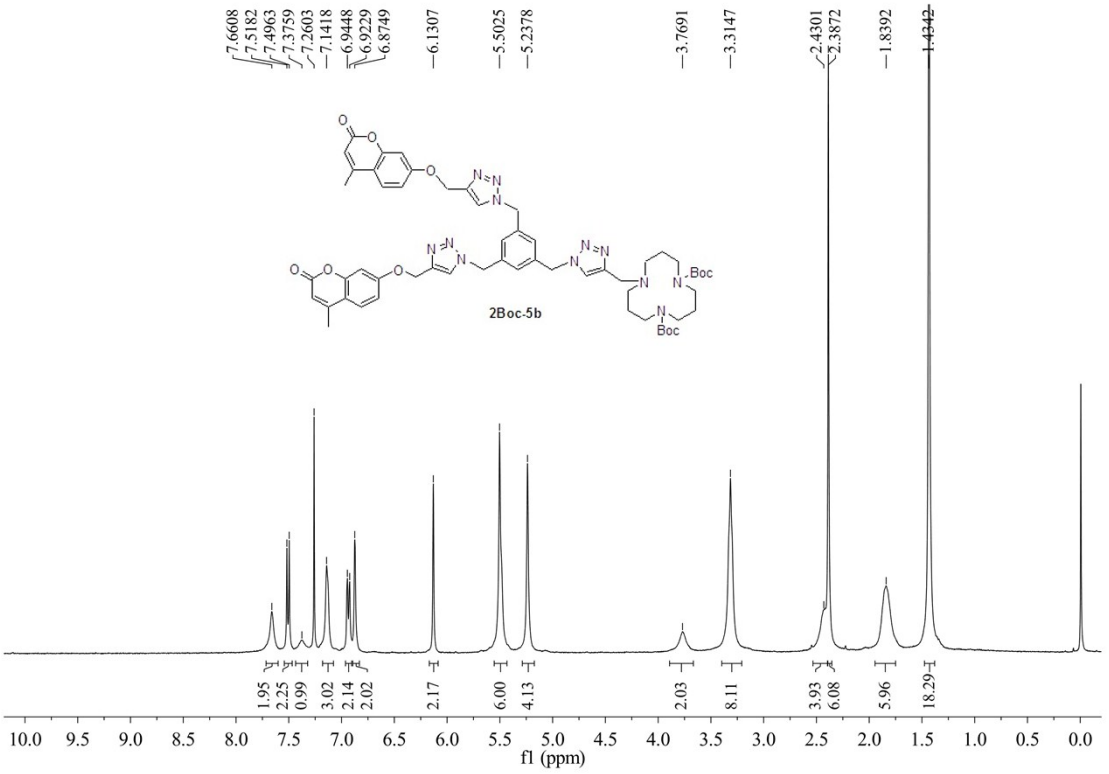
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

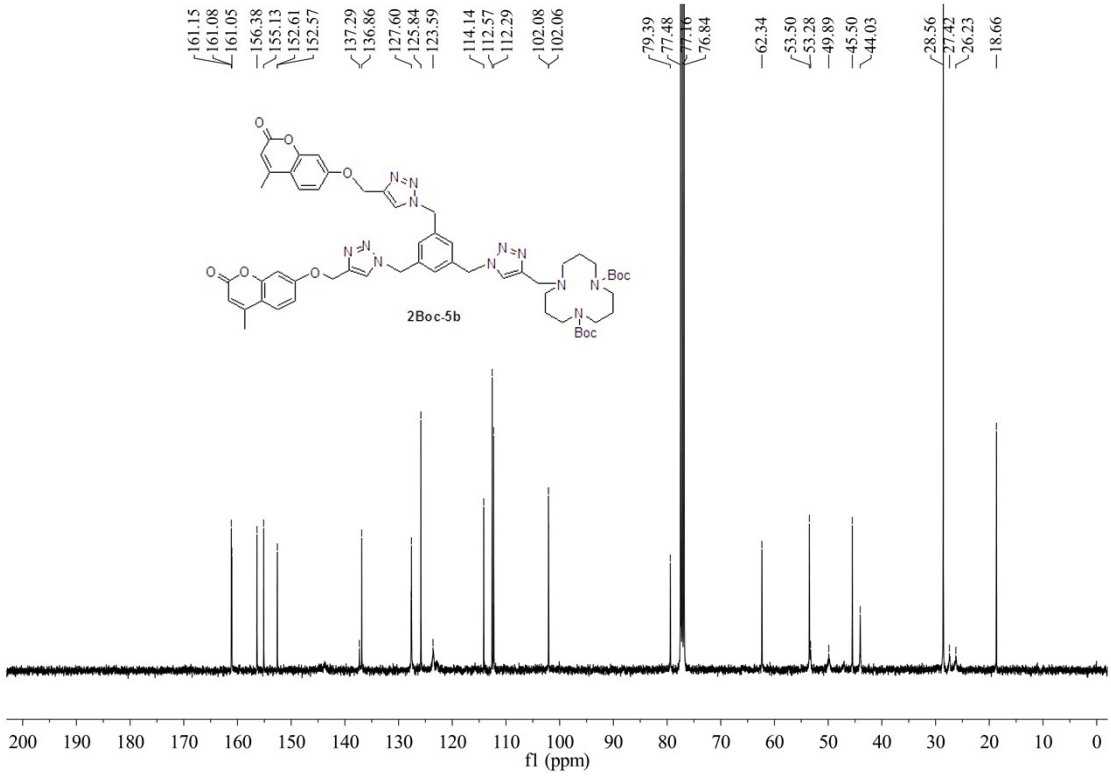
Monoisotopic Mass, Even Electron Ions
533 formula(e) evaluated with 4 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-50 H: 0-80 N: 0-15 O: 0-10
GZF-1-XDS 2 (0.037)
TOF MS ES+

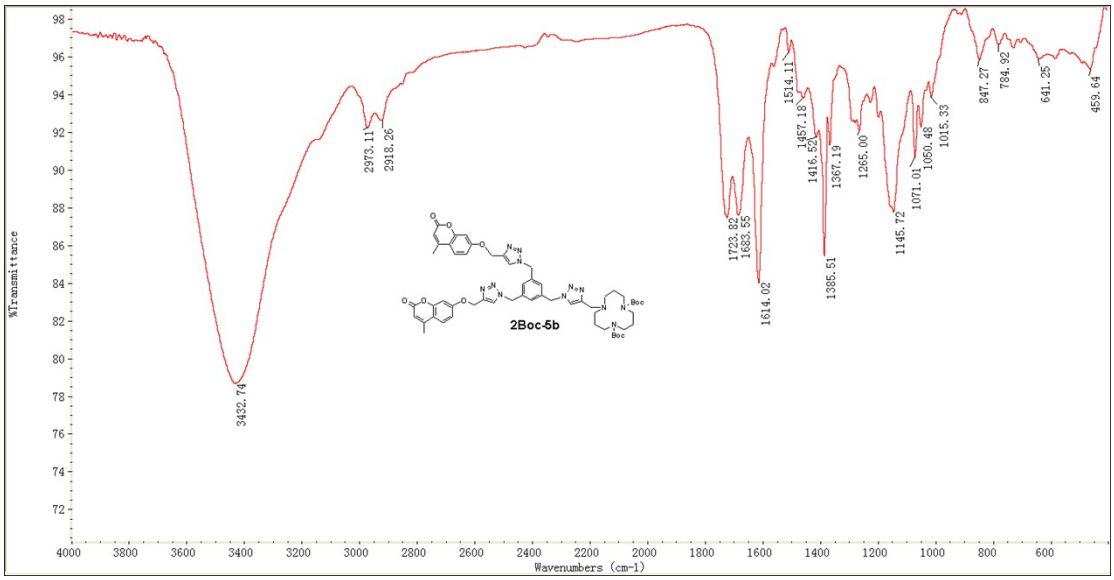


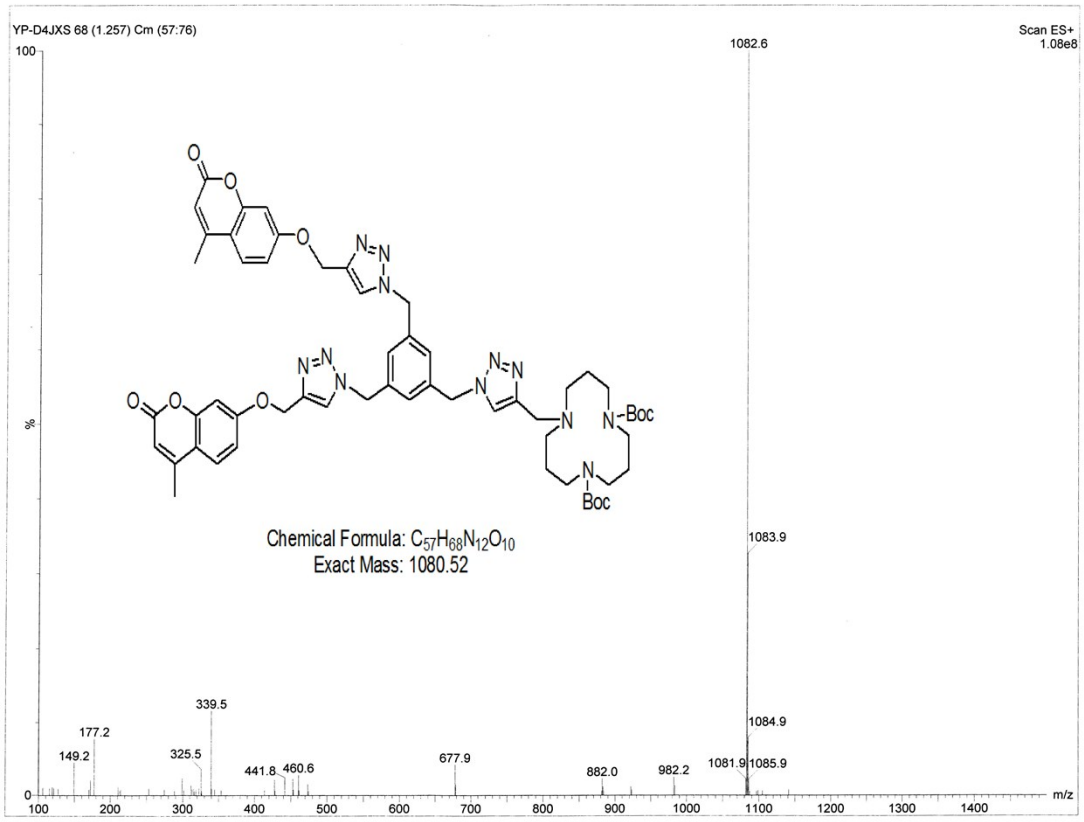
Minimum:				-1.5		
Maximum:		10.0	5.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
853.3903	853.3898	0.5	0.6	27.5	59.1	C45 H49 N12 O6 ✓ $[M+H]^+$
	853.3885	1.8	2.1	22.5	75.9	C44 H53 N8 O10
	853.3925	-2.2	-2.6	26.5	44.9	C49 H53 N6 O8
	853.3938	-3.5	-4.1	31.5	35.9	C50 H49 N10 O4

7.19 Spectra data for compound **2Boc-5b**

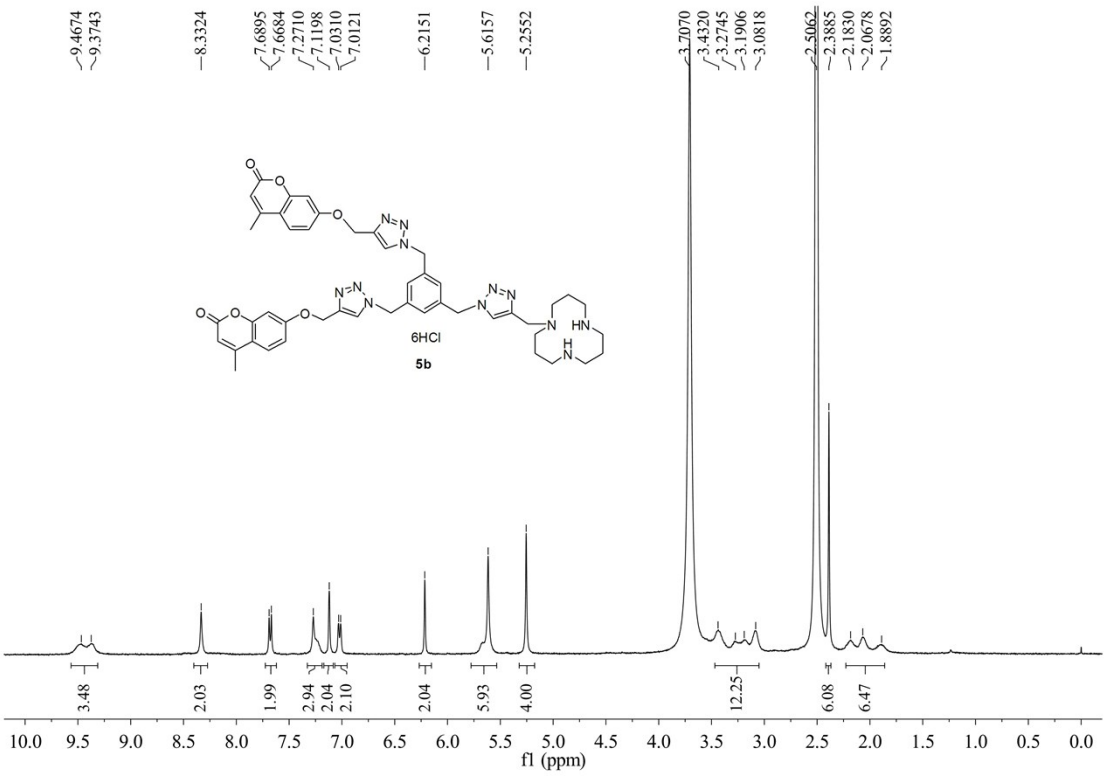


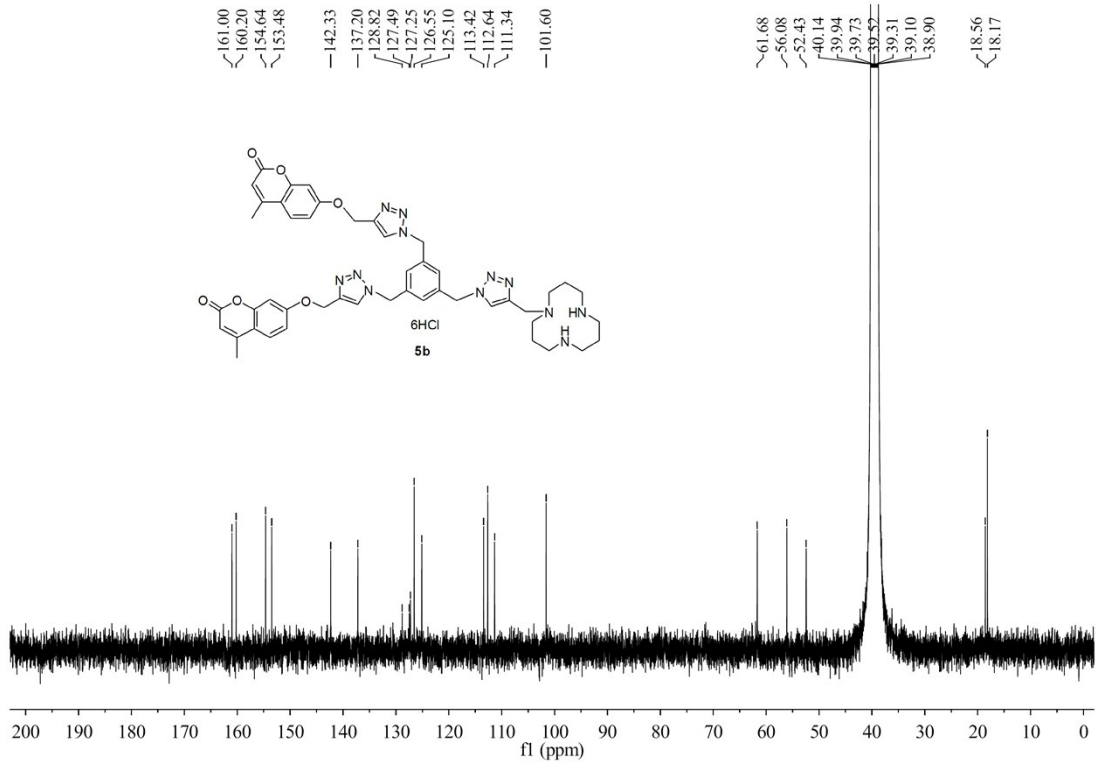


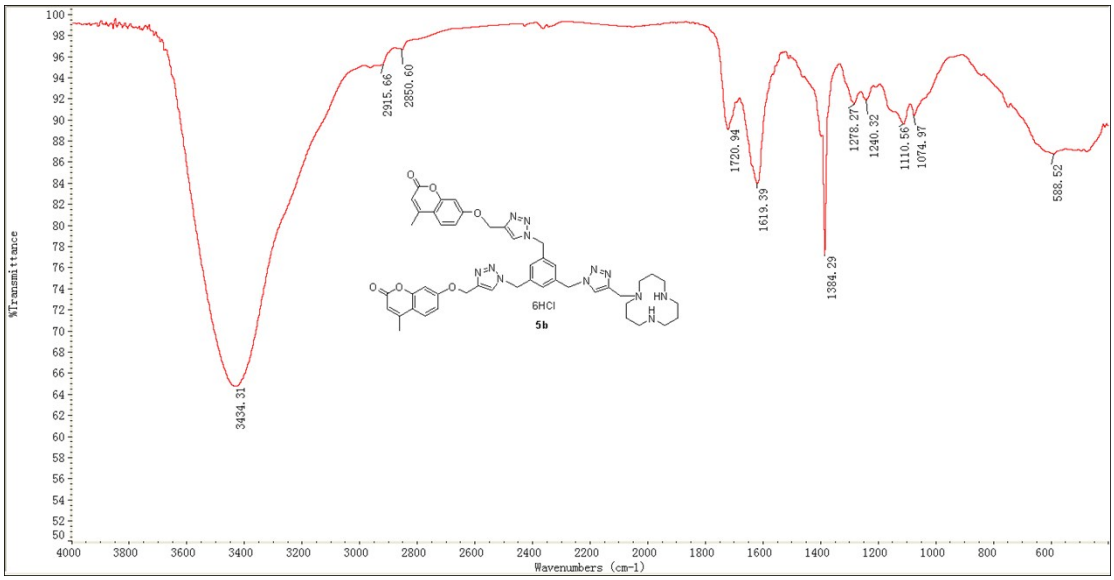

%Transmittance
Wavenumbers (cm-1)
3432.74
2973.11
2918.26
1723.82
1683.55
1614.02
1514.11
1457.18
1416.52
1385.51
1367.19
1265.00
1145.72
1071.01
1050.48
1015.33
847.27
784.92
641.25
459.64
2Boc-5b


YP-D4JXS 68 (1.257) Cm (57:76)
Scan ES+
1.08e8
1082.6
1083.9
1084.9
1085.9
1081.9
339.5
177.2
149.2
325.5
441.8
460.6
677.9
882.0
982.2
Chemical Formula: C57H68N12O10
Exact Mass: 1080.52
%
m/z

7.20 Spectra data for compound **5b**



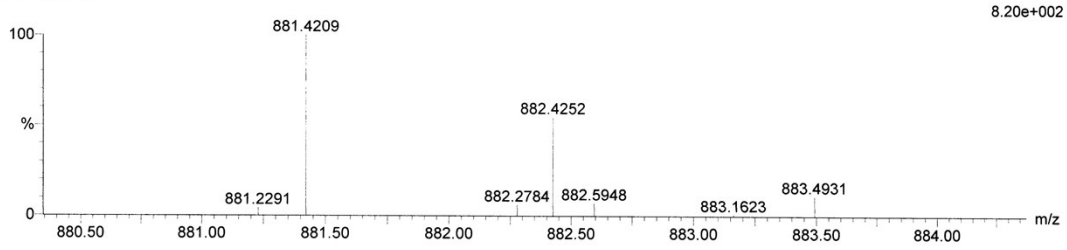




Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

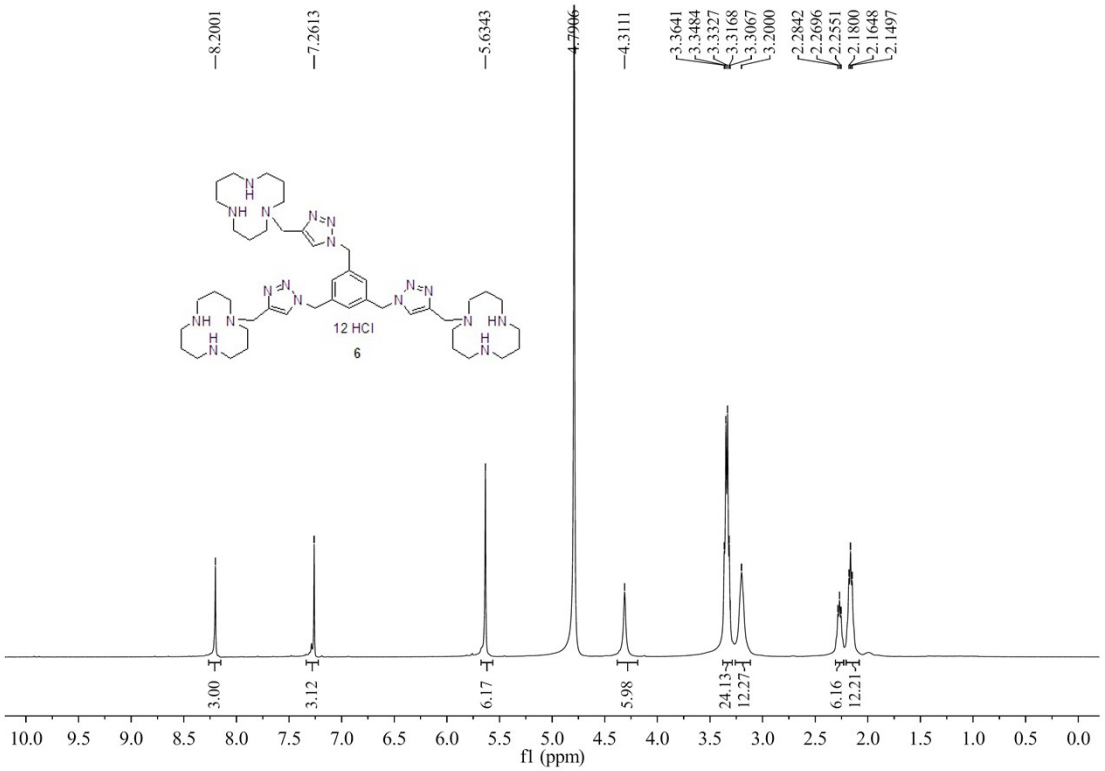
Monoisotopic Mass, Even Electron Ions
417 formula(e) evaluated with 3 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-50 H: 0-80 N: 0-15 O: 0-10
GZF-2-XDS 6 (0.111)
TOF MS ES+

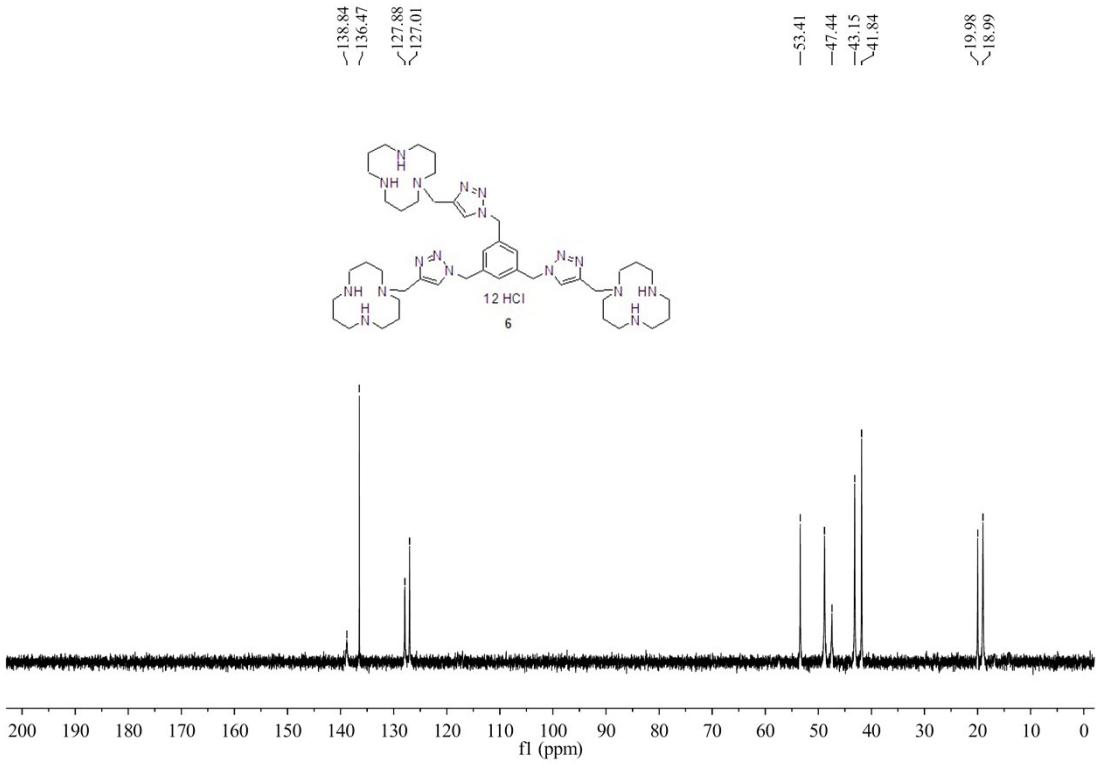


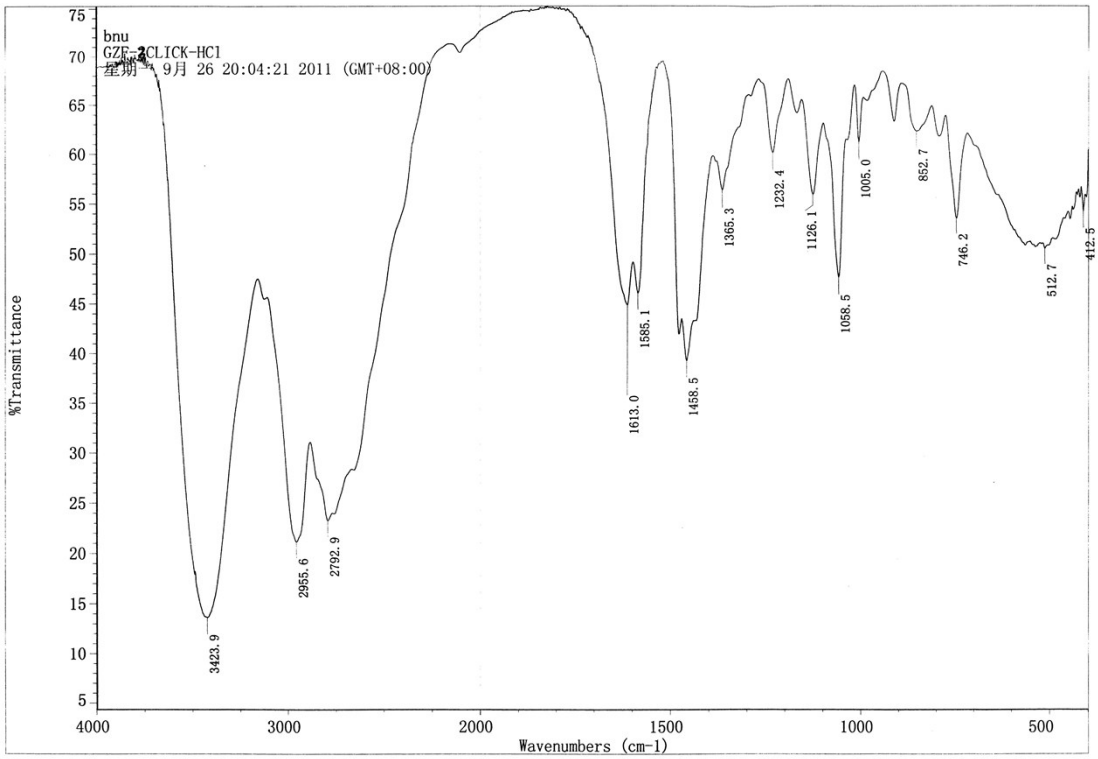
Minimum:				-1.5		
Maximum:		10.0	5.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
881.4209	881.4211	-0.2	-0.2	27.5	14.1	C47 H53 N12 O6 ✓
	881.4198	1.1	1.2	22.5	12.8	C46 H57 N8 O10
	881.4171	3.8	4.3	23.5	8.5	C42 H53 N14 O8

$[M+H]^+$

7.21 Spectra data for compound **6**





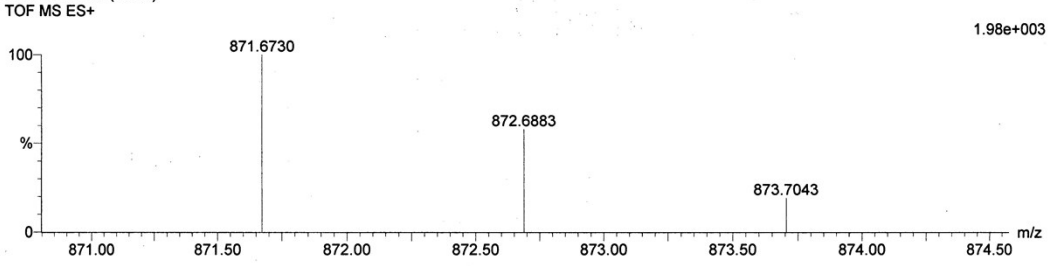


Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 3.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
3442 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-70 H: 0-80 N: 0-20 O: 0-5 Br: 0-3

3CLICK-HCL 6 (0.111)
TOF MS ES+



Minimum:				-1.5		
Maximum:		10.0	3.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
871.6730	871.6735	-0.5	-0.6	15.5	4.8	C45 H79 N18 ✓