Supporting information

New polyaminoisoprenyl antibiotics enhancers against two multidrug resistant Gram-negative bacteria *from Enterobacter and Salmonella species*

Aurélie Lieutaud[1], Cyril Pieri[1], Jean Michel Bolla[1]*and Jean Michel Brunel[1]*

[1] Aix Marseille Univ, INSERM, SSA, MCT, 13385 Marseille, France.

Table of Contents

Table S-1: MIC of the different compounds against enterobacteria

Table S-2: Efficiency Parameter (EP) of the different molecules used in the assay

Table S-3. MIC of doxycycline in a synergistic assay involving *Z/E*-**5r** mixture, *Z*-**5r**, *E*-**5r**, *Z*-**5r** (Hydrochloride salt), *Z*-**5r** (tartrate salt) against MDR strain EA289 (Average of 3 experiments)

Table S-4: Molecular formula strings of **8**, *Z*-**5r**, *E*-**5r** and **6j**

Fig. S-1. ^{1}H and ^{13}C NMR spectra of nerylchloride **8**

Fig. S-2. ^{1}H and ^{13}C of Z/E mixture of **5r**

Fig. S-3. ^{1}H and ^{13}C of Z/E mixture of **6j**

Fig. S-4. ^{1}H and ^{13}C of *cis*-**5r**

Fig. S-5. ^{1}H and ^{13}C of *cis*-**5r** tatrate salt

Fig. S-6. ^{1}H and ^{13}C of *trans*-**5r**

Fig. S-7. ^{1}H and ^{13}C of *trans*-**5r** tartrate salt

Table S-1: MIC of the different compounds against enterobacteria

Compounds	MIC µM (mg/L)		**MIC ratio**	MIC µM (mg/L)		**MIC ratio**
	Ea289	Ea289Δ*acrB*		BN10055	BN10055Δ*acrB*	
			Antibiotics			
CHL	3.2 (1024)	0.2 (64)	**16**	3.2 (1024)	0.2 (64)	**16**
NAL	17.6 (4096)	4.4 (1024)	**4**	17.6 (4096)	0.275 (64)	**64**
DOXY	0.072 (32)	0.00225 (1)	**32**	0.144 (64)	0.0045 (2)	**32**
ERY	0.35 (256)	0.011 (8)	**32**	0.35 (256)	0.0055 (4)	**64**
			Polyaminoisoprenyl derivatives			
5a	250	125	**2**	250	125	**2**
5b	500	250	**2**	500	250	**2**
5e	>250	>250	**-**	>250	>250	**-**
5f	>250	>250	**-**	>250	>250	**-**
5g	>250	>250	**-**	>250	>250	**-**
5j	>500	500	**-**	>500	500	**-**
5k	62.5	31.25	**2**	62.5	31.25	**2**
5l	>500	>500	**-**	>500	>500	**-**
5n	>500	>500	**-**	>500	>500	**-**
5p	125	125	**1**	125	125	**1**
5r	31.25	15.625	**2**	31.25	15.625	**2**
6a	125	62.5	**2**	125	62.5	**2**
6b	62.5	31.25	**2**	62.5	31.25	**2**
6c	62.5	31.25	**2**	31.25	31.25	**1**
6d	62.5	31.25	**2**	31.25	15.625	**2**
6e	>250	125	**-**	250	125	**2**
6g	250	250	**1**	250	125	**2**
6h	125	31.25	**4**	62.5	31.25	**2**
6i	31.25	15.625	**2**	31.25	15.625	**2**
6j	31.25	15.625	**2**	31.25	15.625	**2**
6m	31.25	15.625	**2**	15.625	15.625	**1**
6n	15.625	7.8125	**2**	15.625	15.625	**1**
6o	31.25	7.8125	**4**	62.5	3.90625	**16**
6p	31.25	15.625	**2**	62.5	15.625	**4**
6q	>250	250	**-**	>250	125	**-**
6r	31.25	31.25	**1**	31.25	31.25	**1**

CHL: Chloramphenicol, NAL: Nalidixic acid, DOXY: Doxycycline, ERY: Erythromycine. The results are the summary of at least three independent experiments for each molecule and each

strain. MIC ratio determine the ability of compounds to increase susceptibility to the considered antibiotic. A MIC ratio ≥ 4 is considered as significant, thus showing that the compound is active.

Table S-2: Efficiency Parameter (EP) of the different molecules used in the assay

Compounds	Used concentration[a]	LogP	Score[b]	Average score[c]	**Efficiency[d]**	IC_{50}[e]	**Index[f]**
5a	62.5	-	85	10.63	**0.17**	36.24	**0.58**
5b	125	-	120	17.10	**0.14**	150	**1.20**
5e	125	3.05	15	1.88	**0.02**	150	**1.20**
5f	125	-	10	1.25	**0.01**	150	**1.2**
5g	125	2.33	11	1.38	**0.01**	150	**1.2**
5j	2500	-	136	19.43	**0.01**	150	**0.06**
5k	15.625	-	208	26	**1.66**	30.92	**1.98**
5l	2500	-	30	7.5	**0**	150	**0.06**
5n	2500	-	43	6.14	**0**	150	**0.06**
5p	31.25	1.75	142	17.75	**0.57**	150	**4.8**
5r	7.8125	1.15	228	28.5	**3.65**	126.82	**16.13**
6a	31.25	3.26	284	35.5	**1.14**	32.30	**1.03**
6b	7.8125	3.36	608	76	**9.73**	32.06	**4.10**
6c	15.625	3.82	444	55.5	**3.55**	18.06	**1.16**
6d	15.625	4.23	162	20.25	**1.3**	38.38	**2.46**
6e	125	4.57	320	40	**0.32**	85.33	**0.68**
6g	62.5	3.86	124	15.5	**0.25**	57.64	**0.92**
6h	31.25	3.22	404	50.5	**1.62**	33.75	**1.08**
6i	7.8125	3.09	224	28	**3.58**	30.19	**3.86**
6j	7.8125	3.45	448	56	**7.17**	10.47	**1.34**
6m	7.8125	2.12	93	11.63	**1.49**	43.50	**5.57**
6n	3.9	1.75	90	11.25	**2.88**	41.71	**10.69**
6o	15.625	7.15	28	3.5	**0.22**	25.32	**1.62**
6p	15.6125	3.27	176	25.14	**1.61**	142.79	**9.15**
6q	125	3.26	48	6	**0.05**	150	**1.2**
6r	7.8125	2.68	221	27.63	**3.54**	150	**19.20**
PAßN	38		144	18	**0.47**	150	**3.95**

[a] Concentration used corresponds to MIC/4 (see Table S-1). When MIC is different from Ea289 to BN10005 the highest value is considered. When MIC is noticed >X, the concentration used for the adjuvant assay is X/2. [b] Score corresponds to the sum of the ratio of MIC shown in Table S-3. [c] Average score reported to the number of combinations tested. [d] Efficiency corresponds to the ratio between score and concentration used. [e] IC_{50} of each compound was determined on CHO cell line as described in the material and methods section. [f] Index corresponds to the ratio between IC_{50} and concentration used.

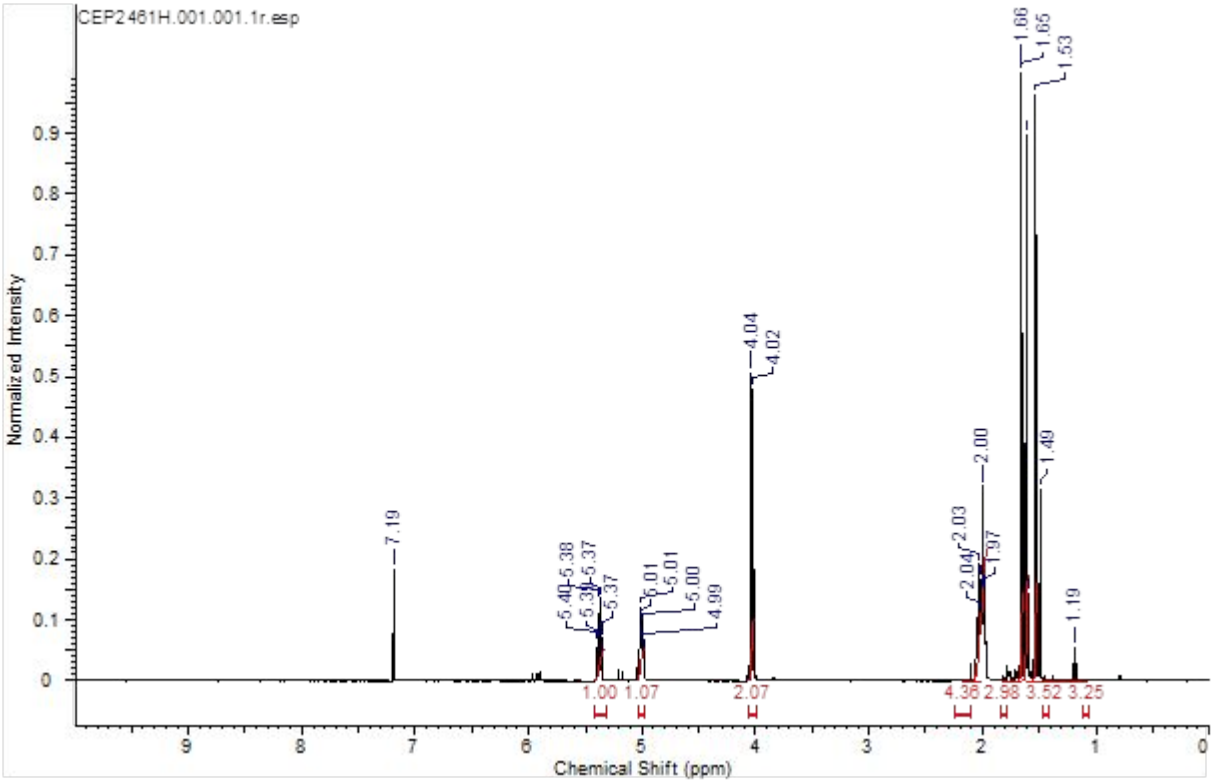
Table S-3. MIC of doxycycline in a synergistic assay involving *Z/E*-**5r** mixture, *Z*-**5r**, *E*-**5r**, *Z*-**5r** (Hydrochloride salt), *Z*-**5r** (tartrate salt) against MDR strain EA289 (Average of 3 experiments)

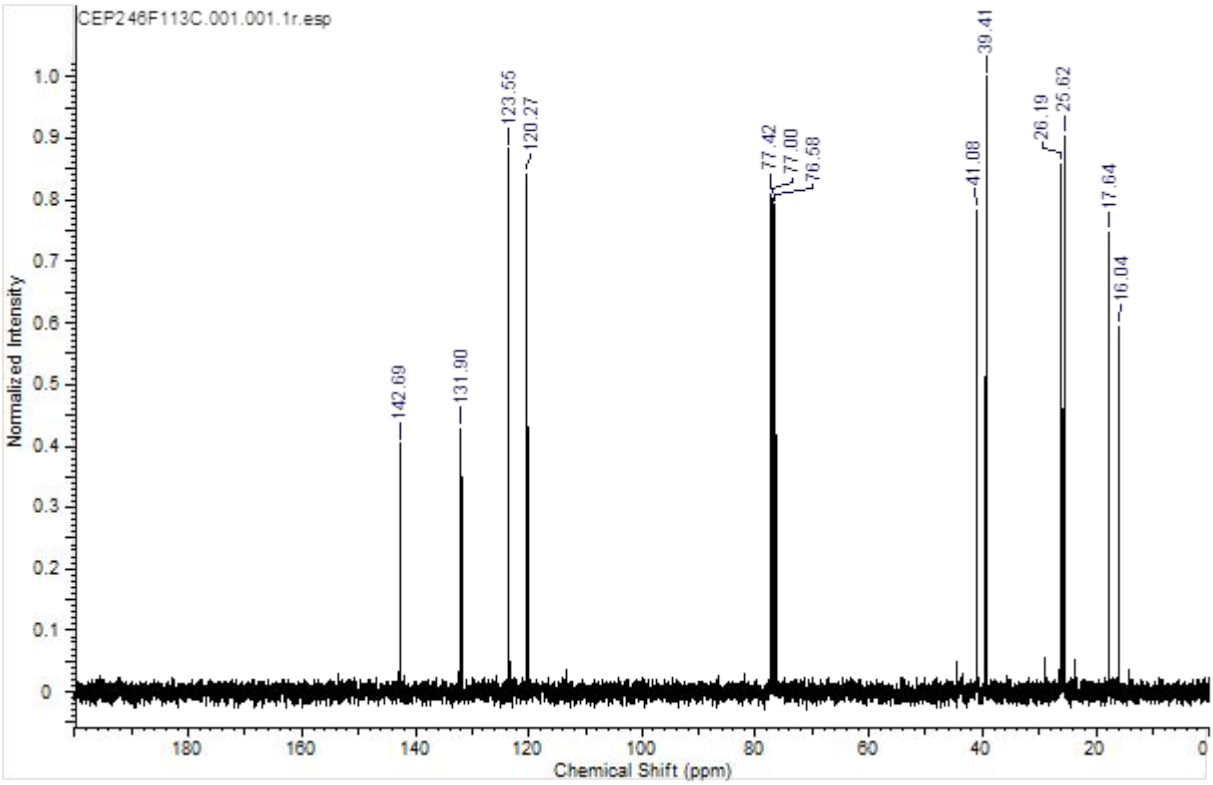
Compound used at 5 µM	*Z/E*-**5r** mixture	*Z*-**5r**	*E*-**5r**	*Z*-**5r** (Hydrochloride salt)	*Z*-**5r** (Tartrate salt)
MIC of doxycycline (µg/mL)	2	2	2.5	2.5	2

Table S-4. Molecular formula strings of **8**, ***Z*-5r, *E*-5r** and **6j**

Compound	**SMILES**
8	C/C(CC/C=C(C)/C)=C/CCl
***Z*-5r**	NCCCN(CCCN)CCCNC/C=C(CC/C=C(C)/C)\C
***E*-5r**	C/C(CC/C=C(C)/C)=C/CNCCCN(CCCN)CCCN
6j	C/C(CC/C=C(CC/C=C(C)\C)\C)=C\CNCCCN(C)CCCN

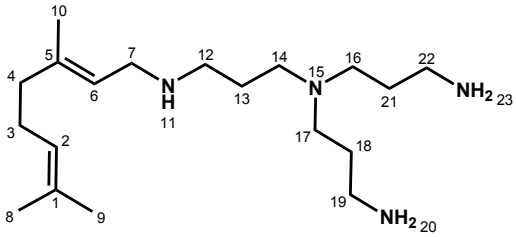
Fig. S-1 ^{1}H and ^{13}C NMR spectra of nerylchloride 8

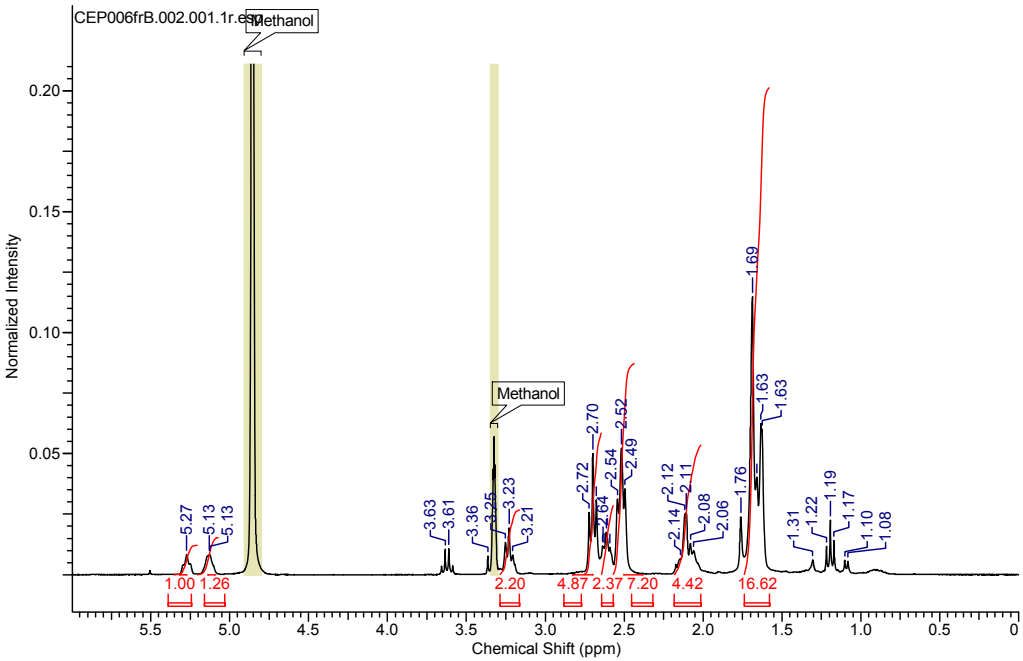


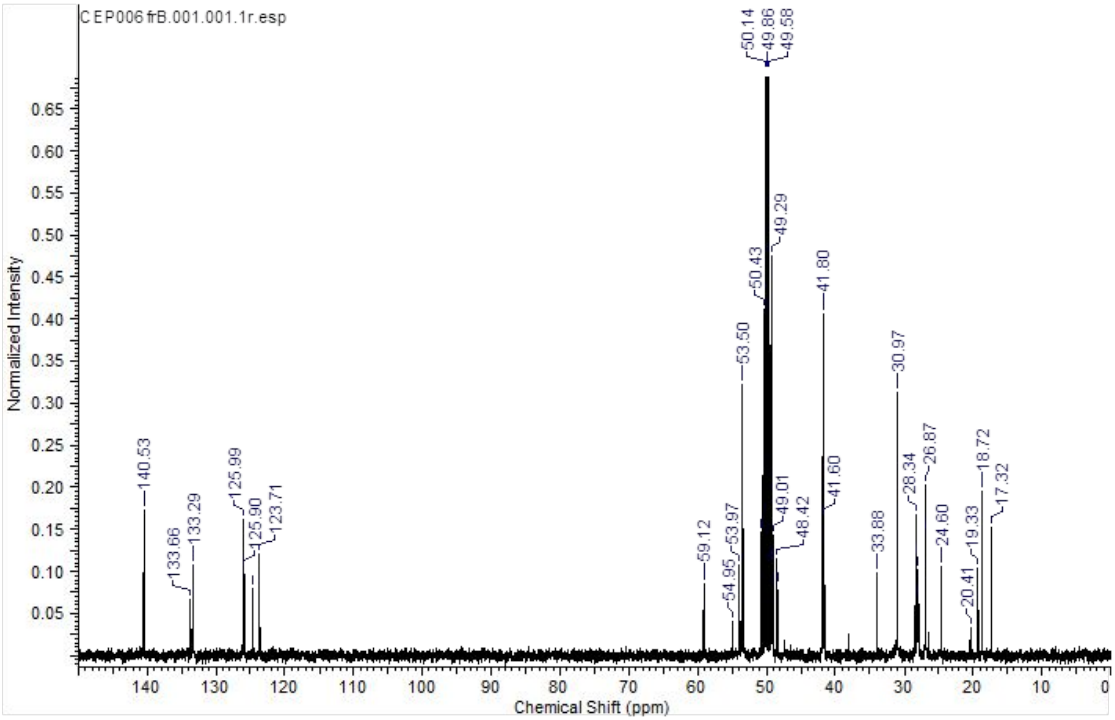


Starting material spectra in accordance with litterature

Fig. S-2 ^{1}H and ^{13}C of Z/E mixture of 5r







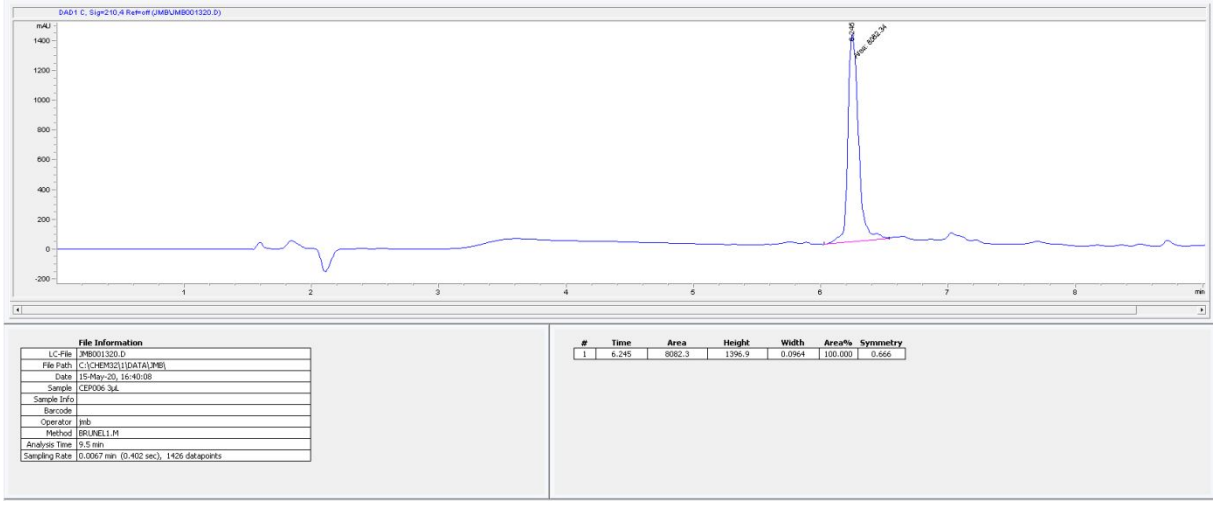
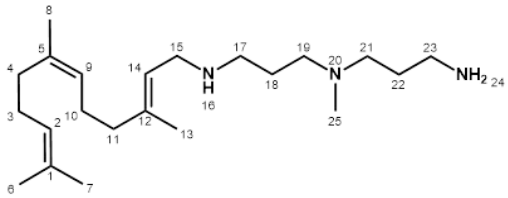
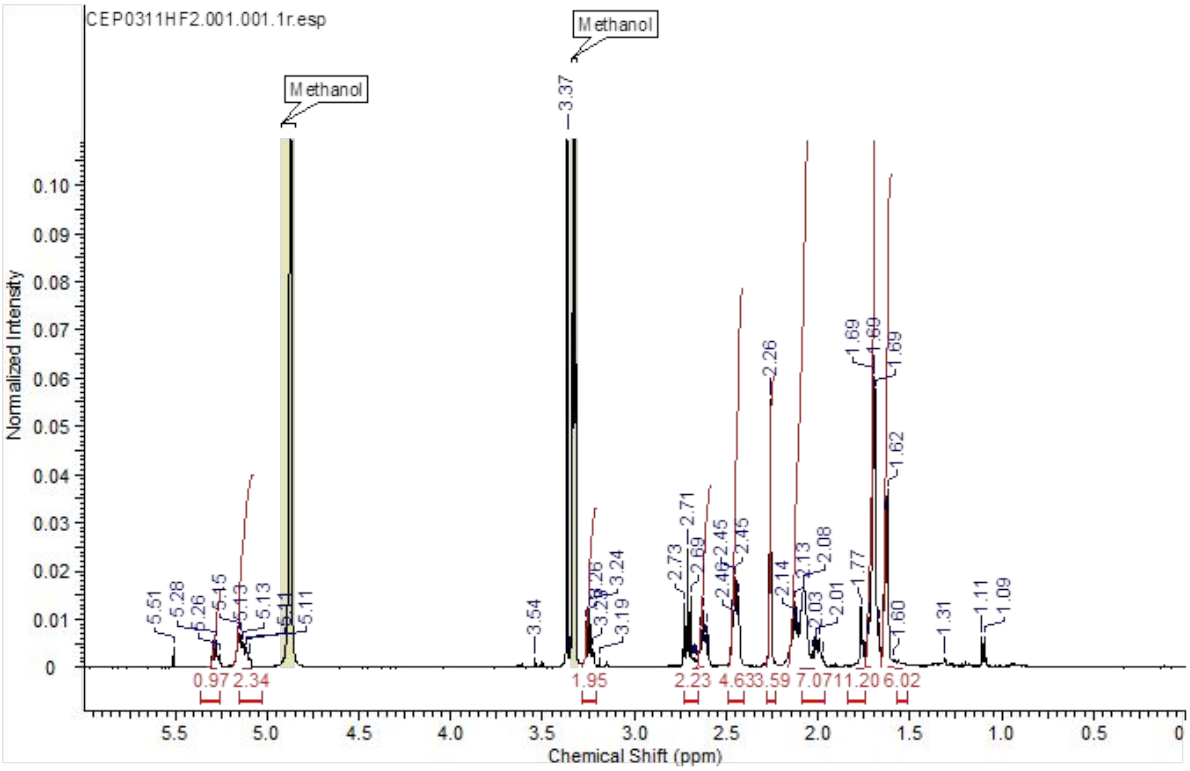
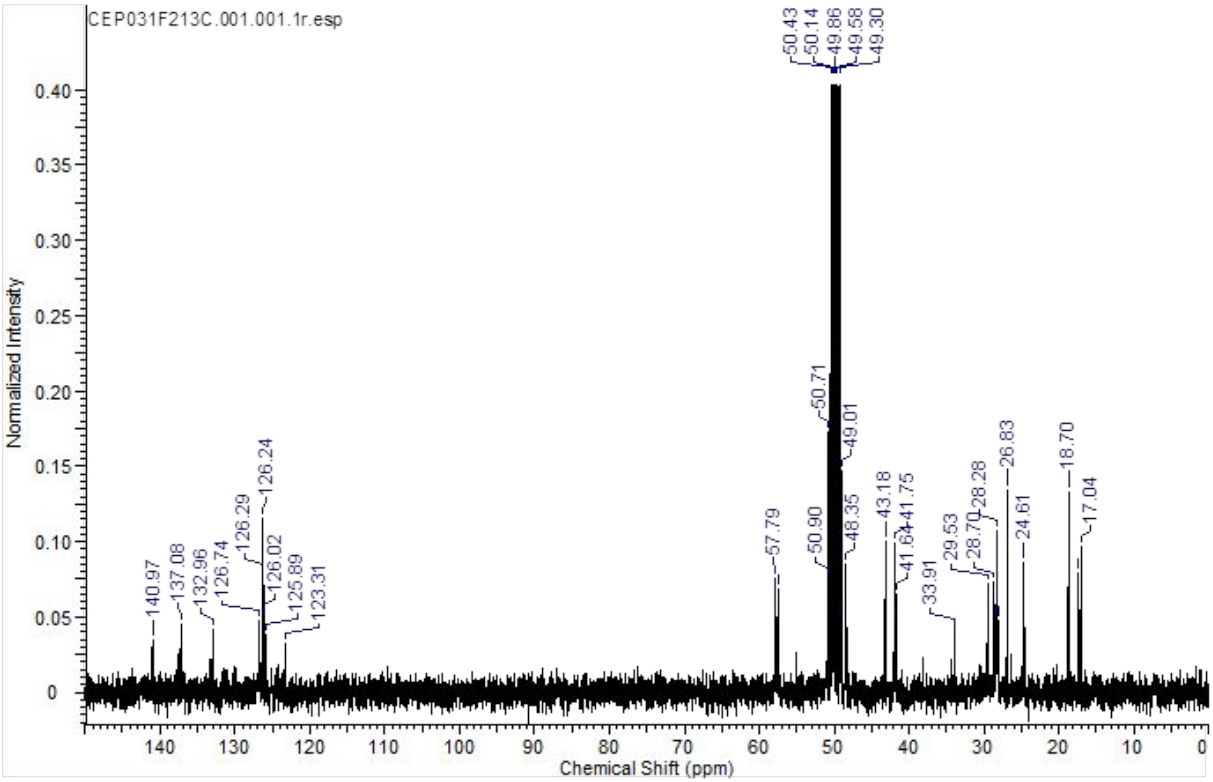
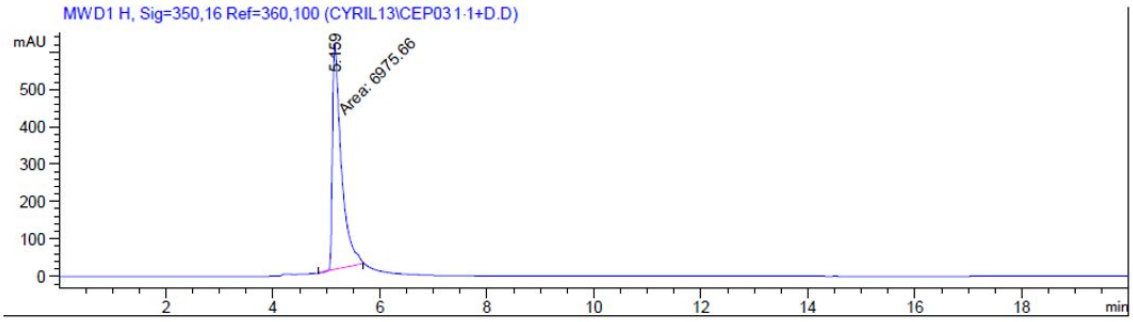


Fig. S-3 ^{1}H and ^{13}C of Z/E mixture of 6j









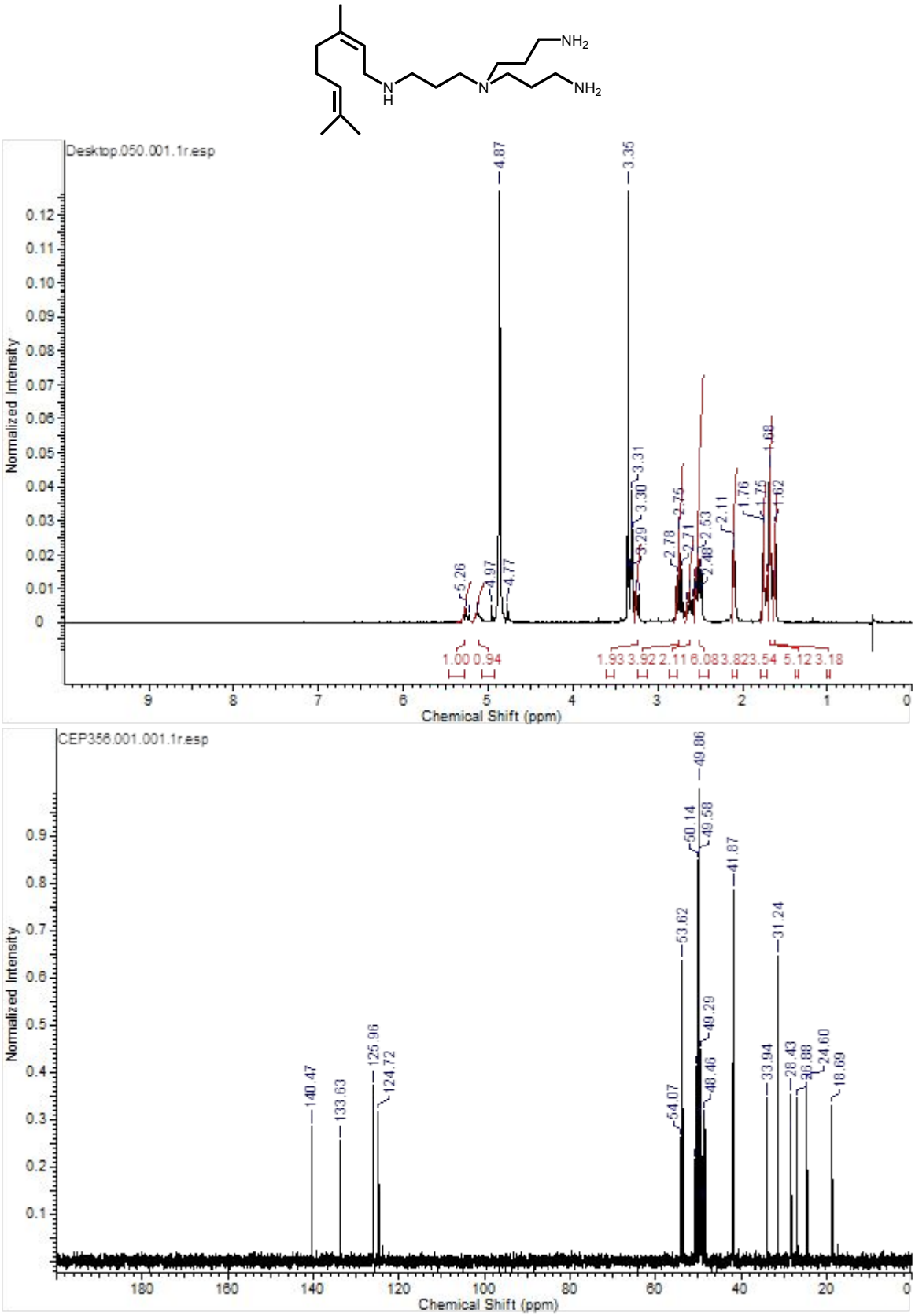

```
Signal 2: MWD1 H, Sig=350,16 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   5.159 MM    0.1928 6975.65820  602.99573 100.0000

Totals :                  6975.65820  602.99573


=====================================================================
                          *** End of Report ***
```

Fig. S-4 ^{1}H and ^{13}C of *cis*-5r



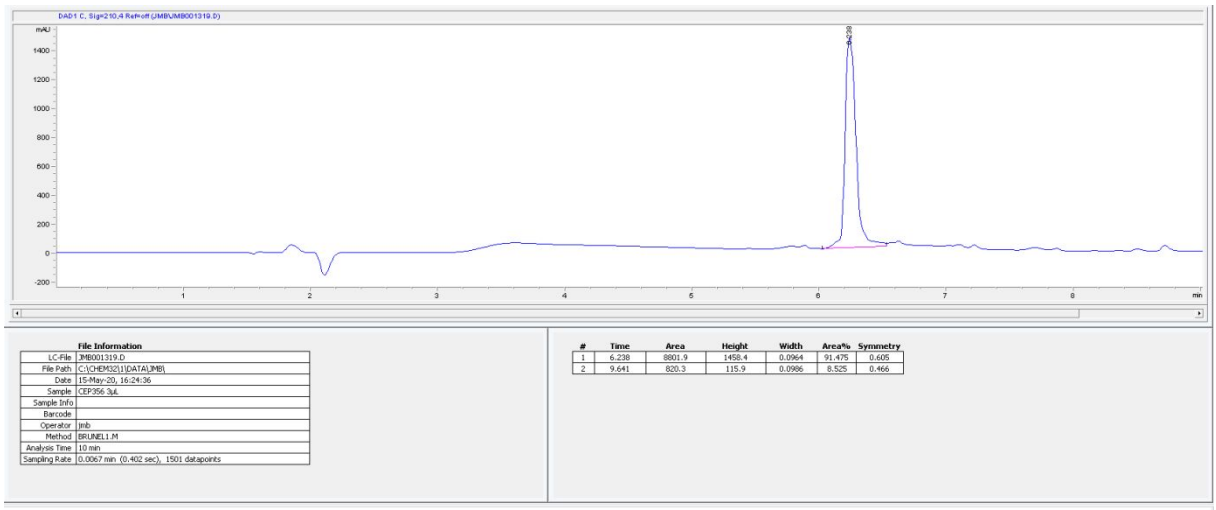
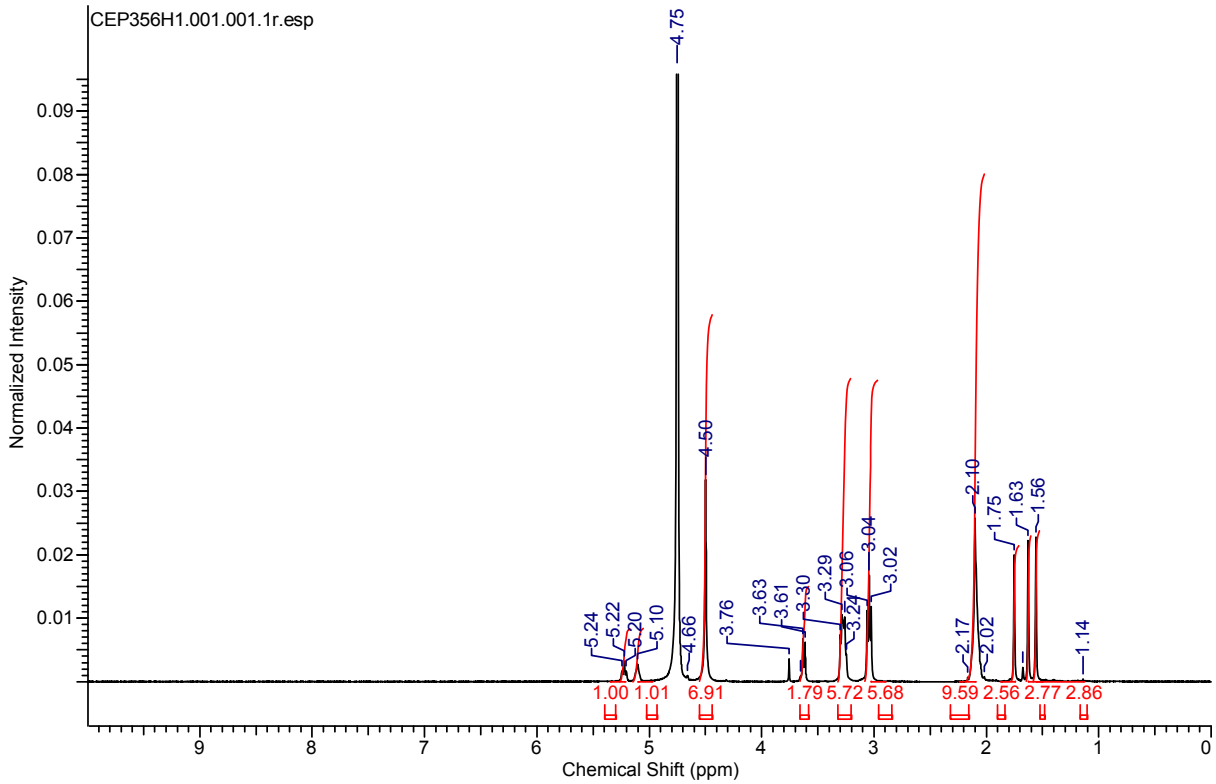


Fig. S-5 ^{1}H and ^{13}C of *cis*-5r tatrate salt



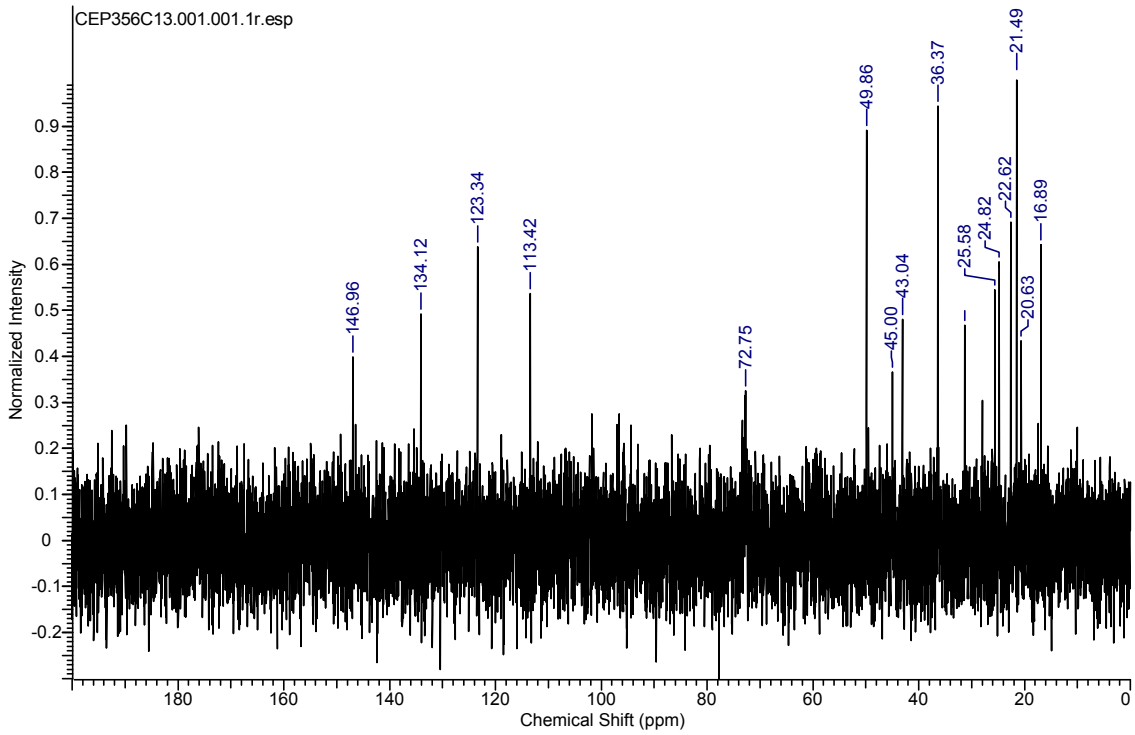
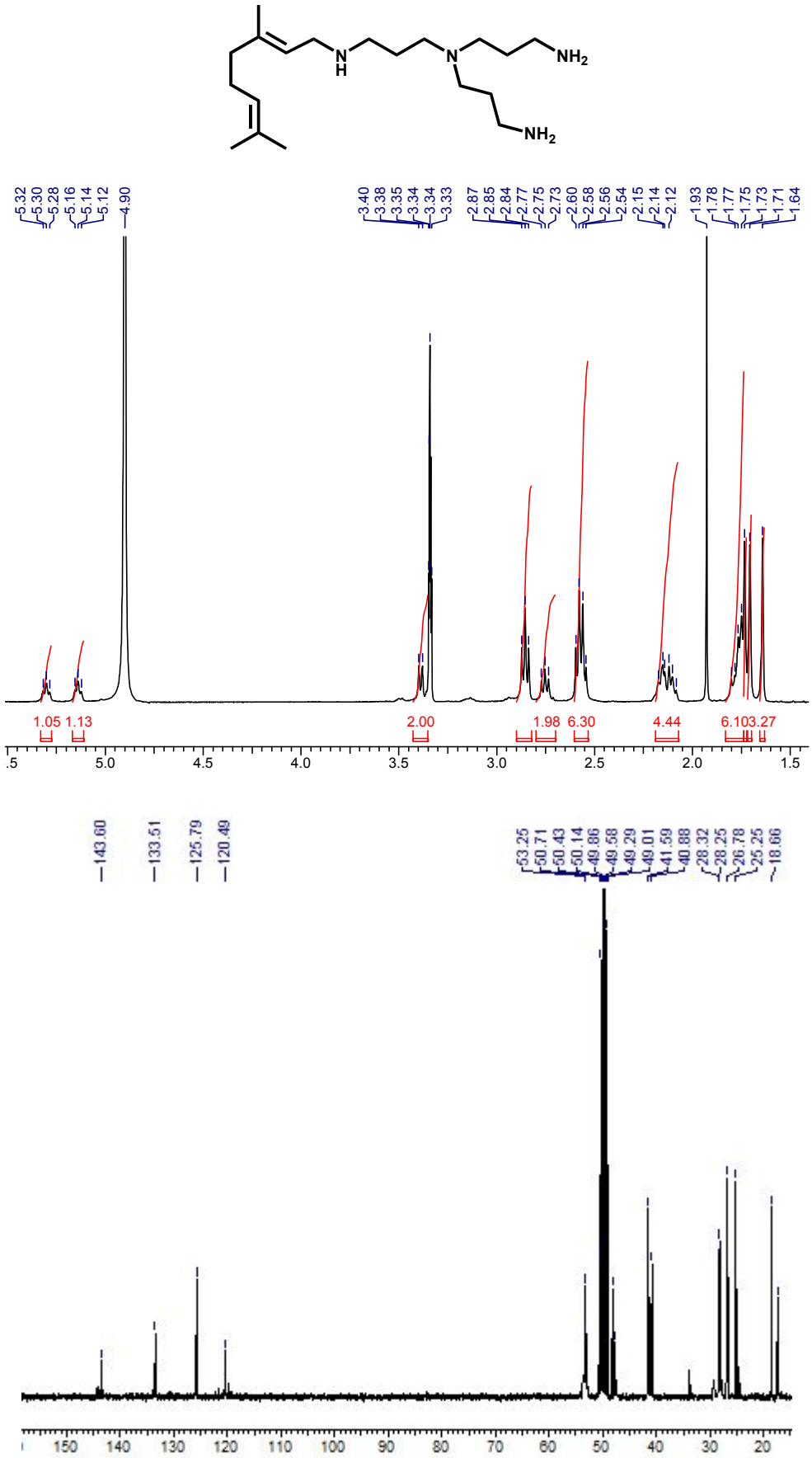

CEP356C13.001.001.1r.esp
Normalized Intensity
Chemical Shift (ppm)
146.96
134.12
123.34
113.42
72.75
49.86
45.00
43.04
36.37
25.58
24.82
22.62
21.49
20.63
16.89

Fig. S-6 ^{1}H and ^{13}C of *trans*-5r



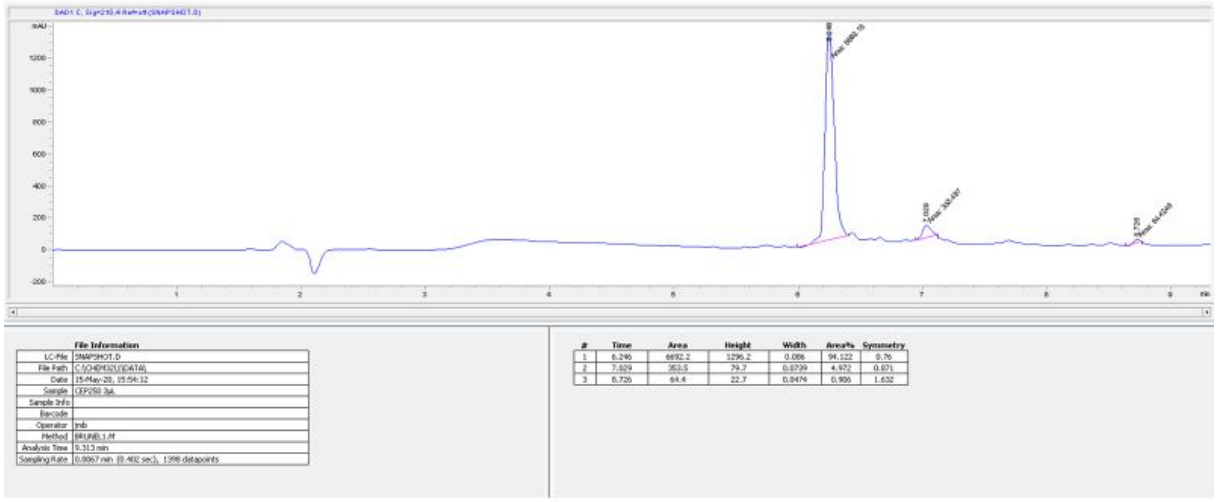
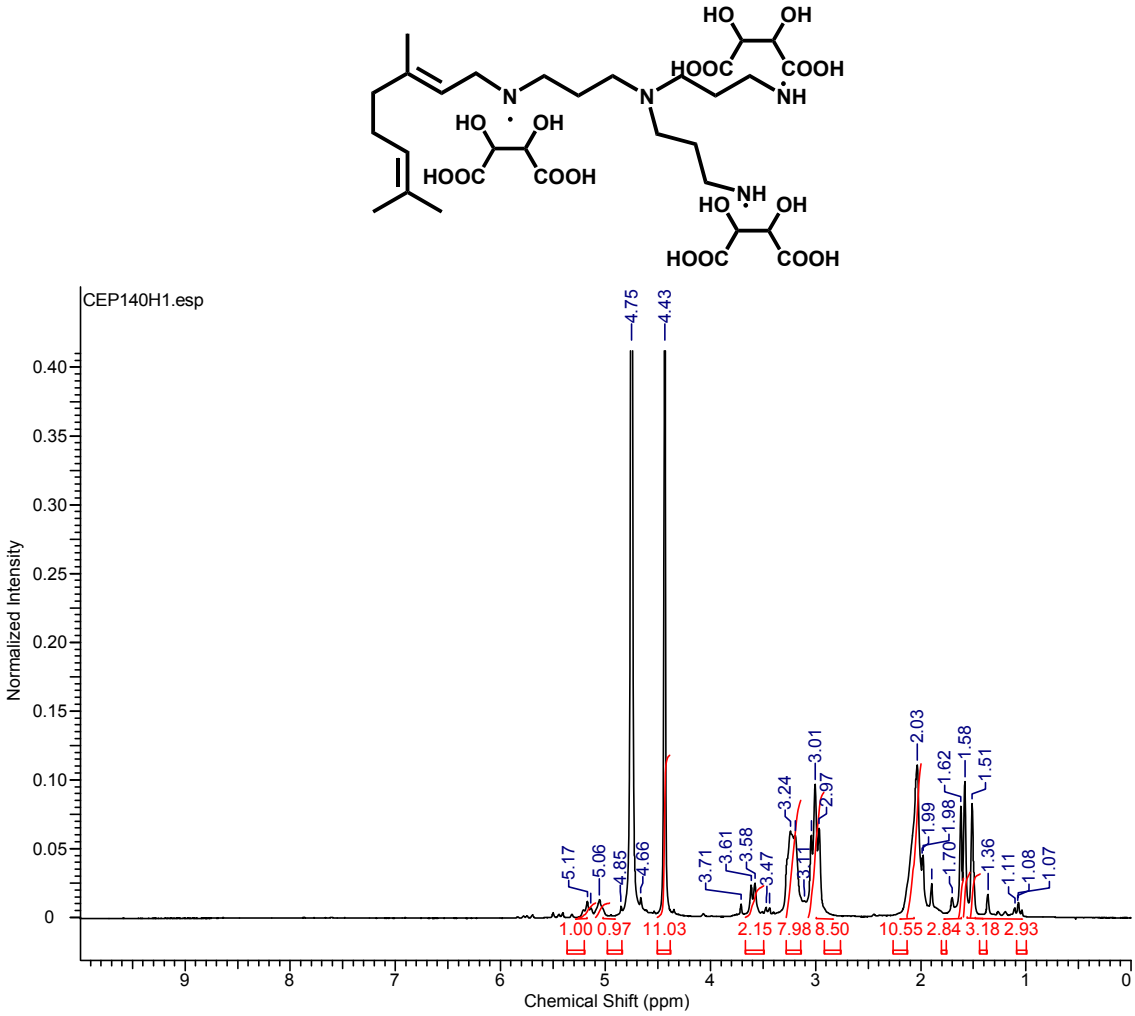
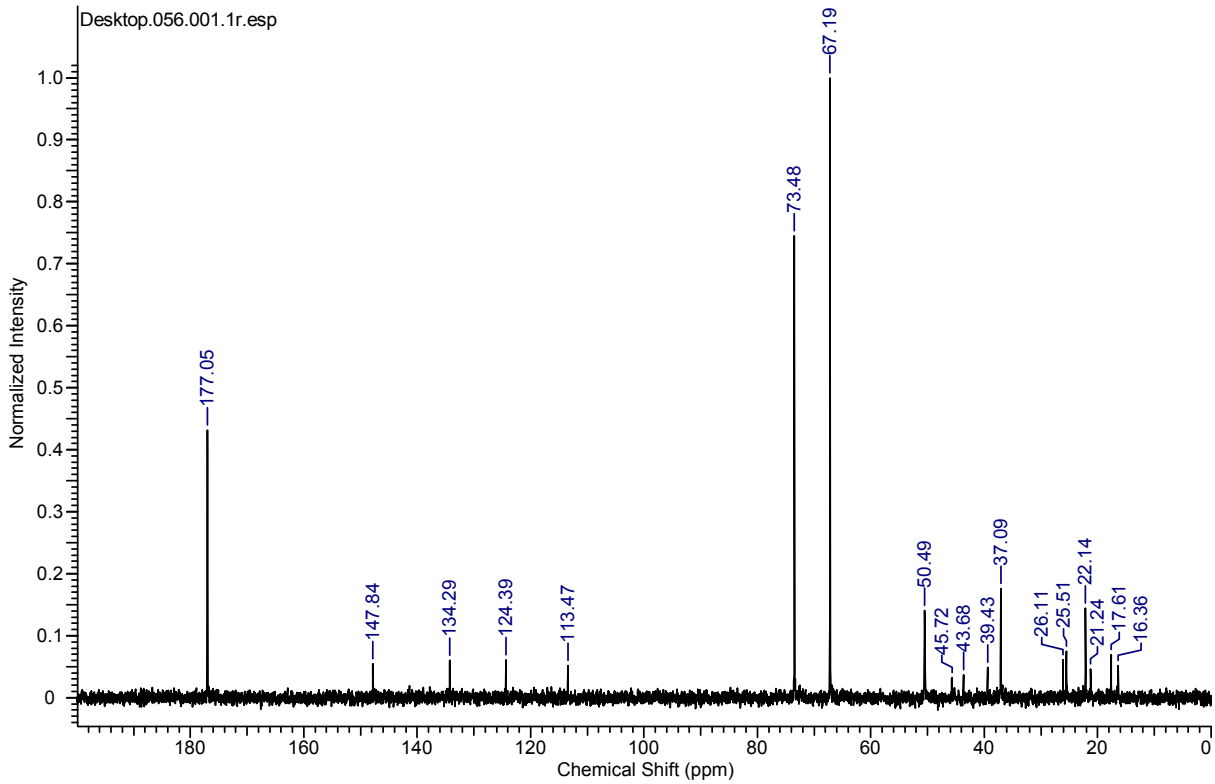


Fig. S-7 ^{1}H and ^{13}C of *trans*-5r tartrate salt



S15


Desktop.056.001.1r.esp
Normalized Intensity
Chemical Shift (ppm)
177.05
147.84
134.29
124.39
113.47
73.48
67.19
50.49
45.72
43.68
39.43
37.09
26.11
25.51
22.14
21.24
17.61
16.36