

Electronic Supplementary Information for:

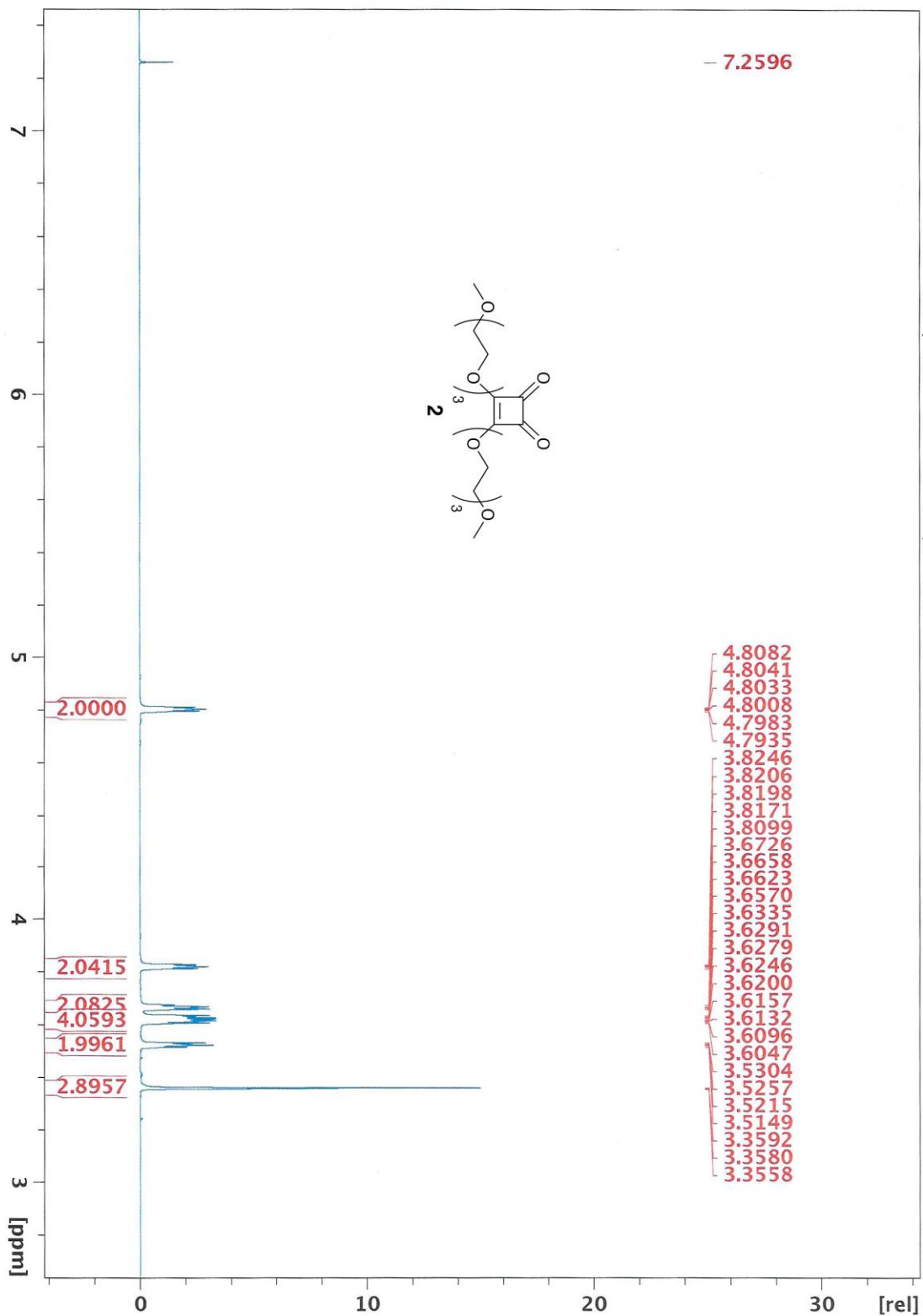
Conjugation of carbohydrates to proteins using of di(triethylene glycol monomethyl ether) squaric acid ester revisited

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g. S1 ¹H-NMR of compound 2

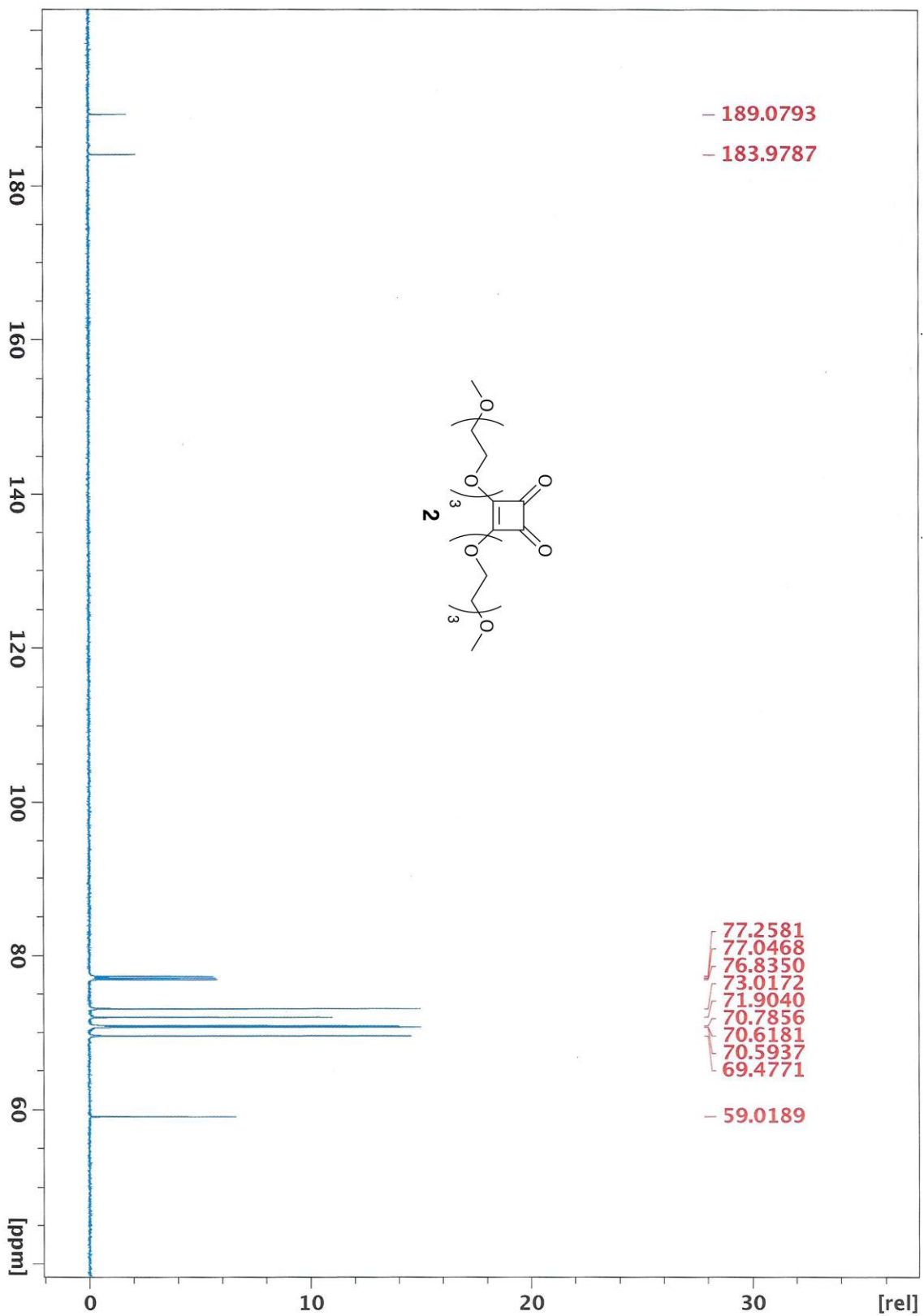
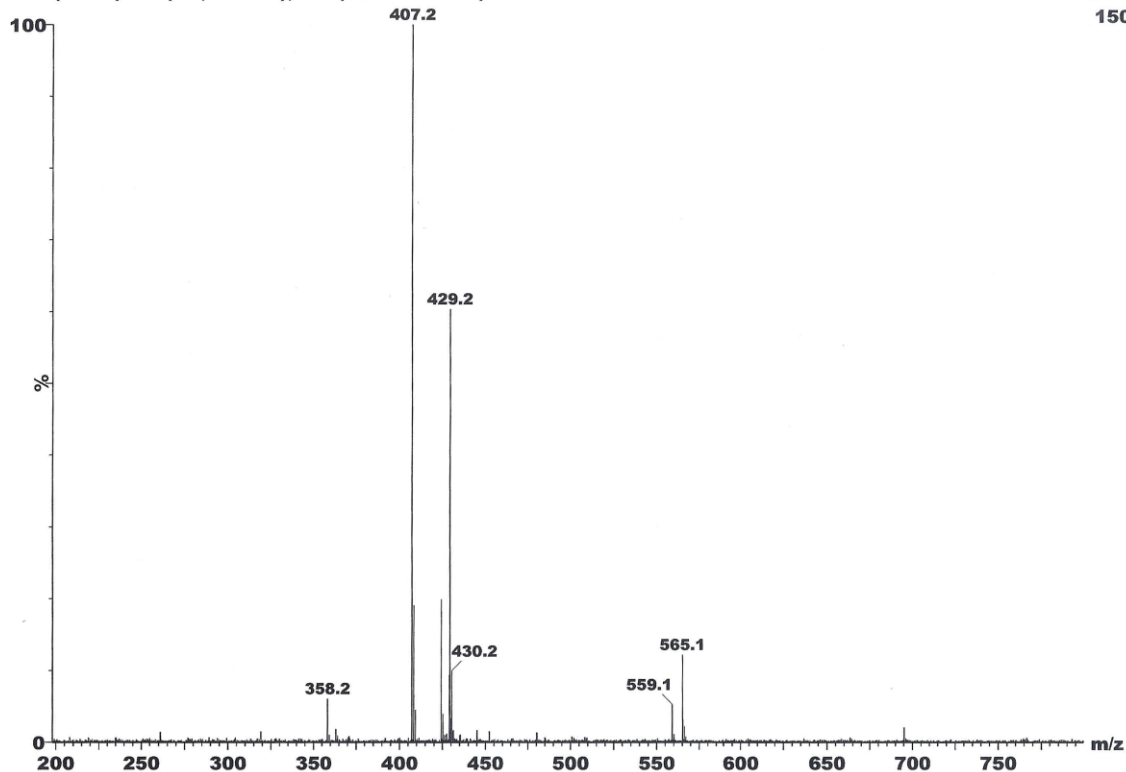


Fig. S2 ¹³C-NMR of compound 2

322 (5.490) Sm (SG, 3x5.00); Cm (322-18x5.000)

TOF MS ES+
150



Elemental Composition Report

Page 1

Single Mass Analysis

Tolerance = 25.0 mDa / DBE: min = -2.0, max = 500.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

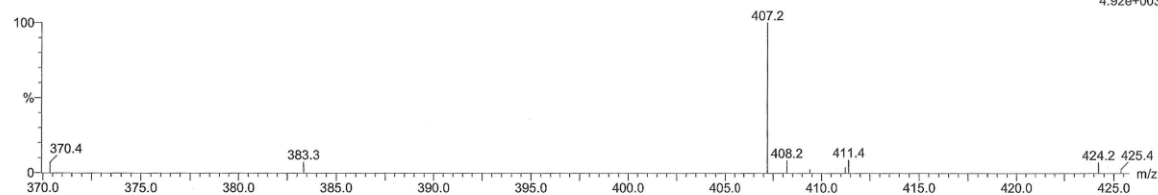
95 formula(e) evaluated with 8 results within limits (up to 19 closest results for each mass)

Elements Used:

C: 0-100 H: 0-200 O: 0-30

320 (5.456) Cn (Cen,7, 50.00, Ar); Sm (SG, 3x5.00); Sb (12,5.00)

TOF MS ES+
4.92e+003



Minimum:

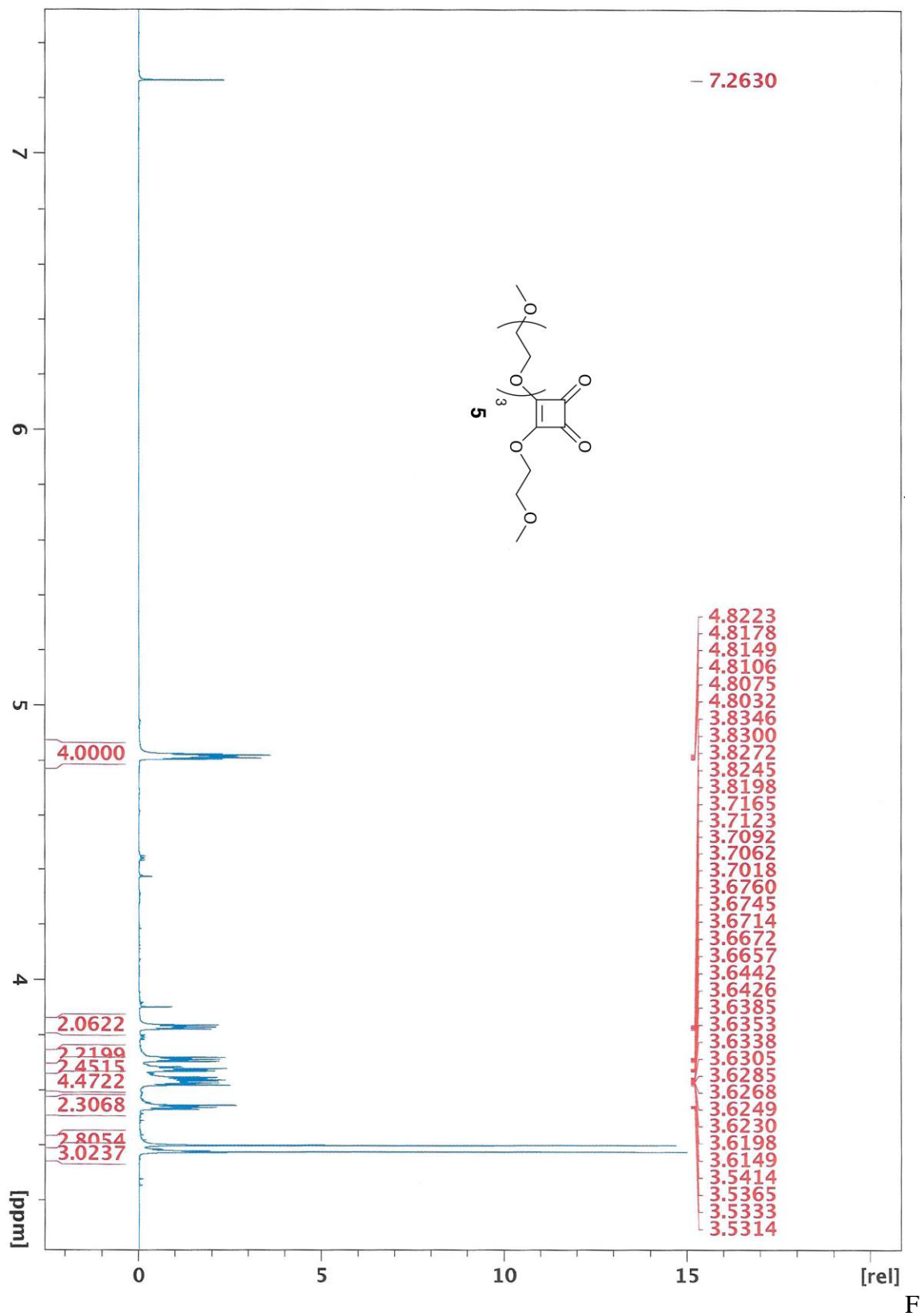
Maximum: 25.0 10.0 -2.0

Maximum:

25.0 10.0 500.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
407.1922	407.1917	0.5	1.2	3.5	386.3	C18 H31 O10
	407.1858	6.4	15.7	12.5	693.5	C25 H27 O5
	407.2011	-8.9	-21.9	16.5	850.3	C29 H27 O2
	407.1800	12.2	30.0	21.5	988.6	C32 H23
	407.2070	-14.8	-36.3	7.5	564.8	C22 H31 O7
	407.1765	15.7	38.6	-0.5	268.7	C14 H31 O13
	407.2129	-20.7	-50.8	-1.5	292.2	C15 H35 O12
	407.1706	21.6	53.0	8.5	571.6	C21 H27 O8

Fig. S3 HRMS of compound 2



ig. S4 ¹H-NMR of compound **5**

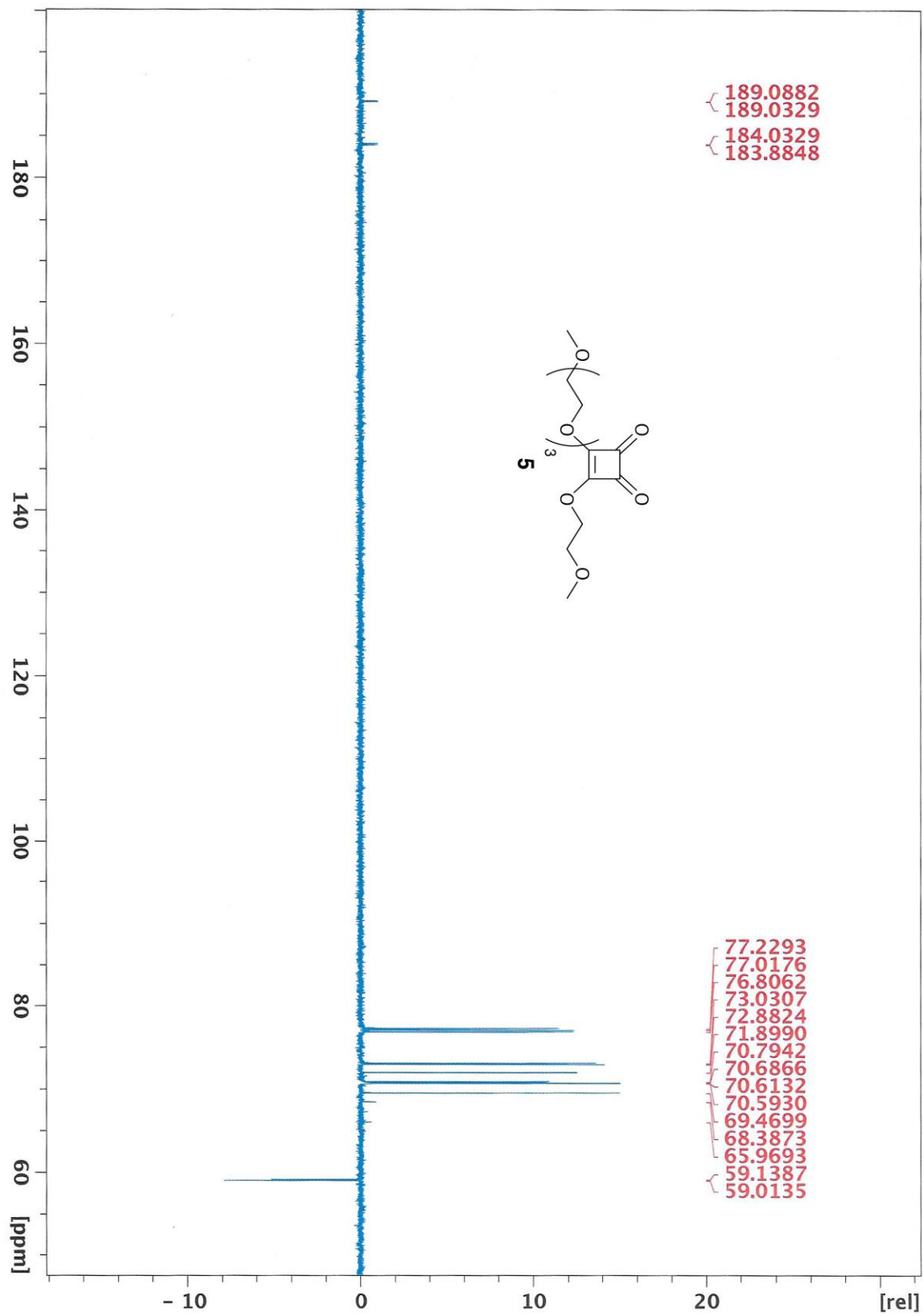
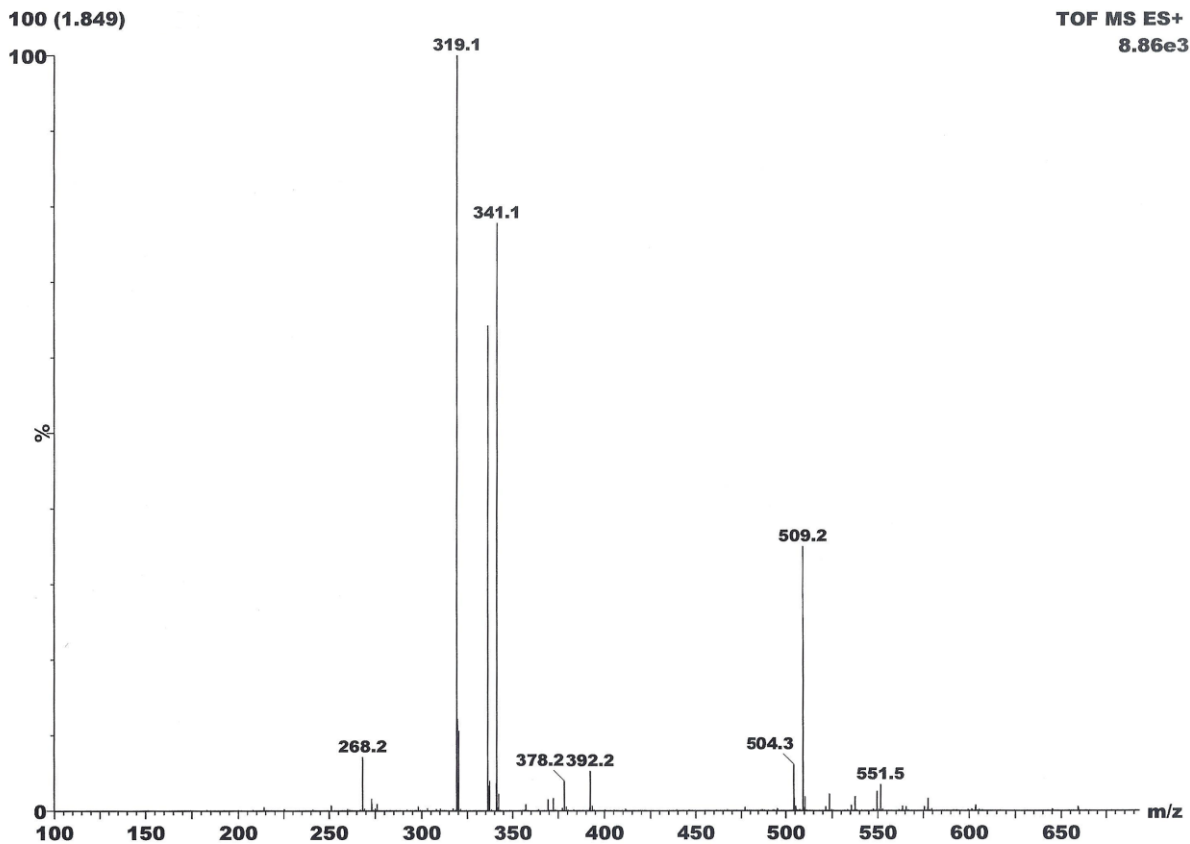


Fig. S5 ¹³C-NMR of compound 5



Elemental Composition Report

Page 1

Single Mass Analysis

Tolerance = 25.0 mDa / DBE: min = -2.0, max = 500.0
 Element prediction: Off
 Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

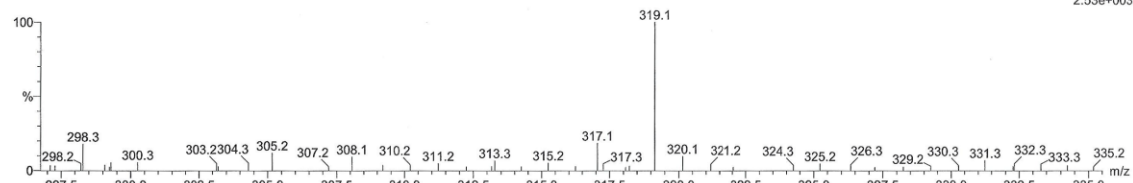
63 formula(e) evaluated with 7 results within limits (up to 19 closest results for each mass)

Elements Used:

C: 0-100 H: 0-200 O: 0-40

131 (2.423) Cn (Cen,5, 50.00, Ar); Sm (SG, 1x2.00); Sb (12,5.00)

TOF MS ES+
2.53e+003



Minimum: -2.0
 Maximum: 500.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
319.1393	319.1393	0.0	0.0	3.5	54.0	C14 H23 O8
	319.1334	5.9	18.5	12.5	180.5	C21 H19 O3
	319.1487	-9.4	-29.5	16.5	263.1	C25 H19
	319.1545	-15.2	-47.6	7.5	135.9	C18 H23 O5
	319.1240	15.3	47.9	-0.5	33.0	C10 H23 O11
	319.1182	21.1	66.1	8.5	139.4	C17 H19 O6
	319.1604	-21.1	-66.1	-1.5	51.6	C11 H27 O10

Fig. S6 HRMS of compound 5

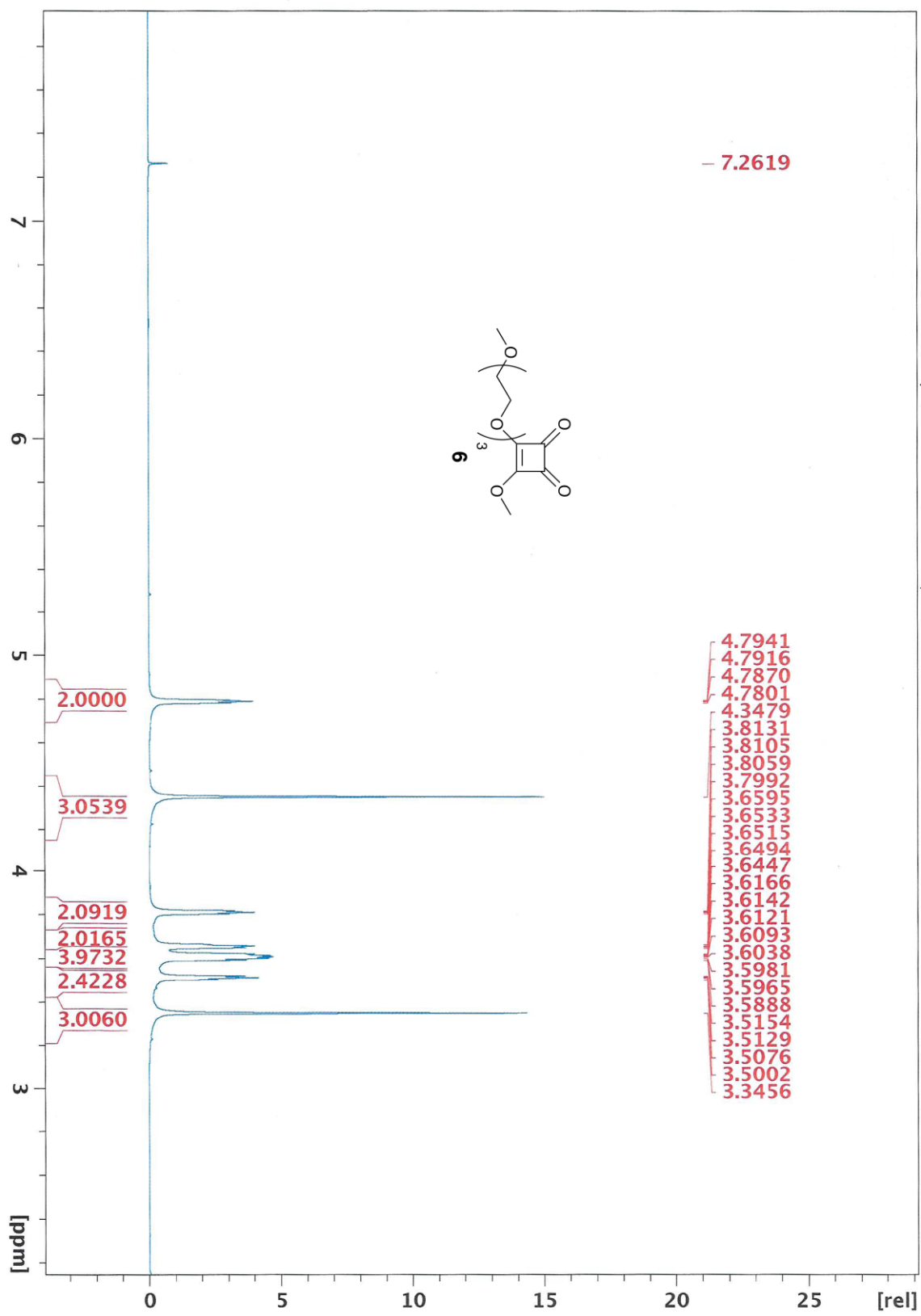


Fig. S7 ¹H-NMR of compound 6

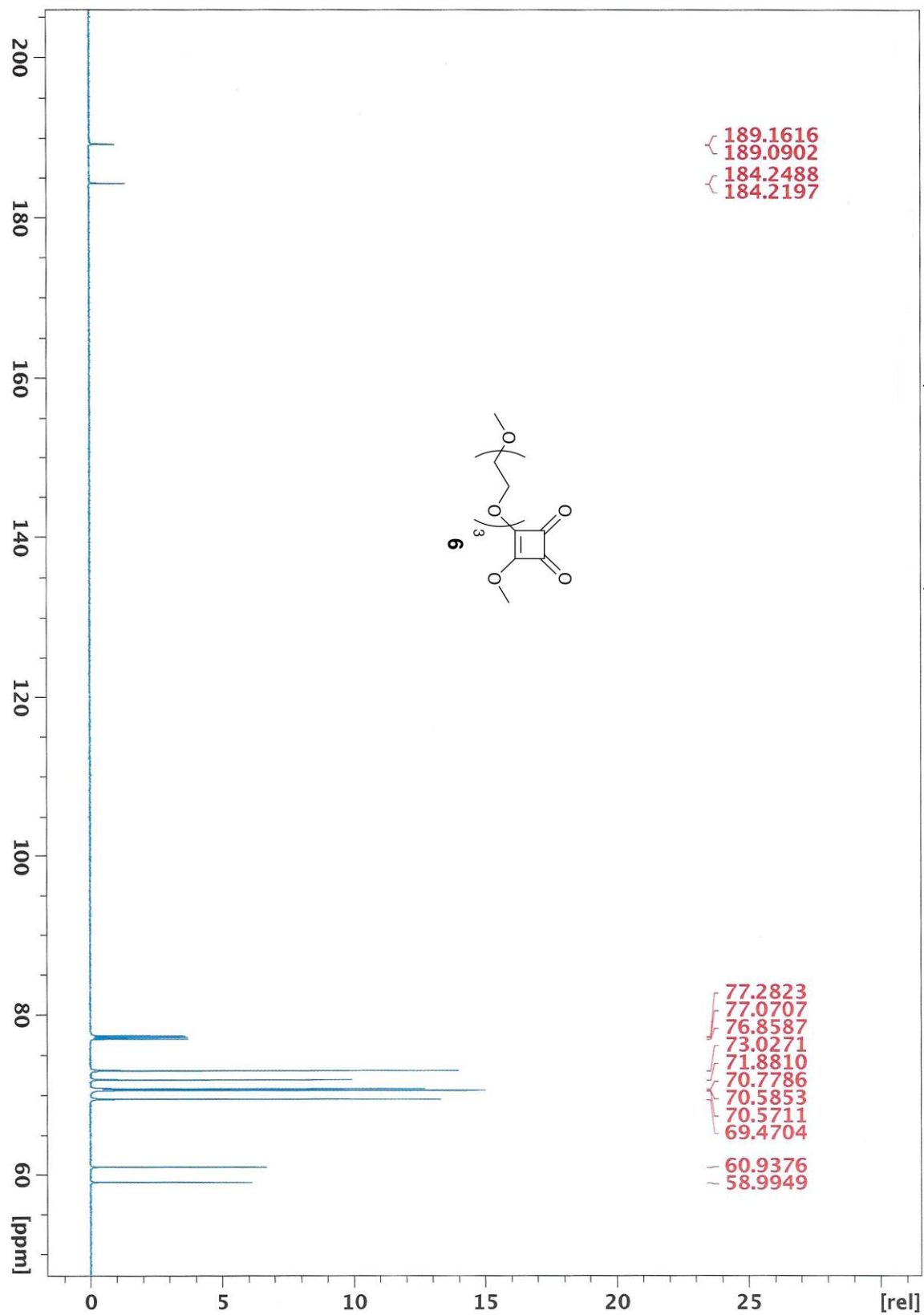
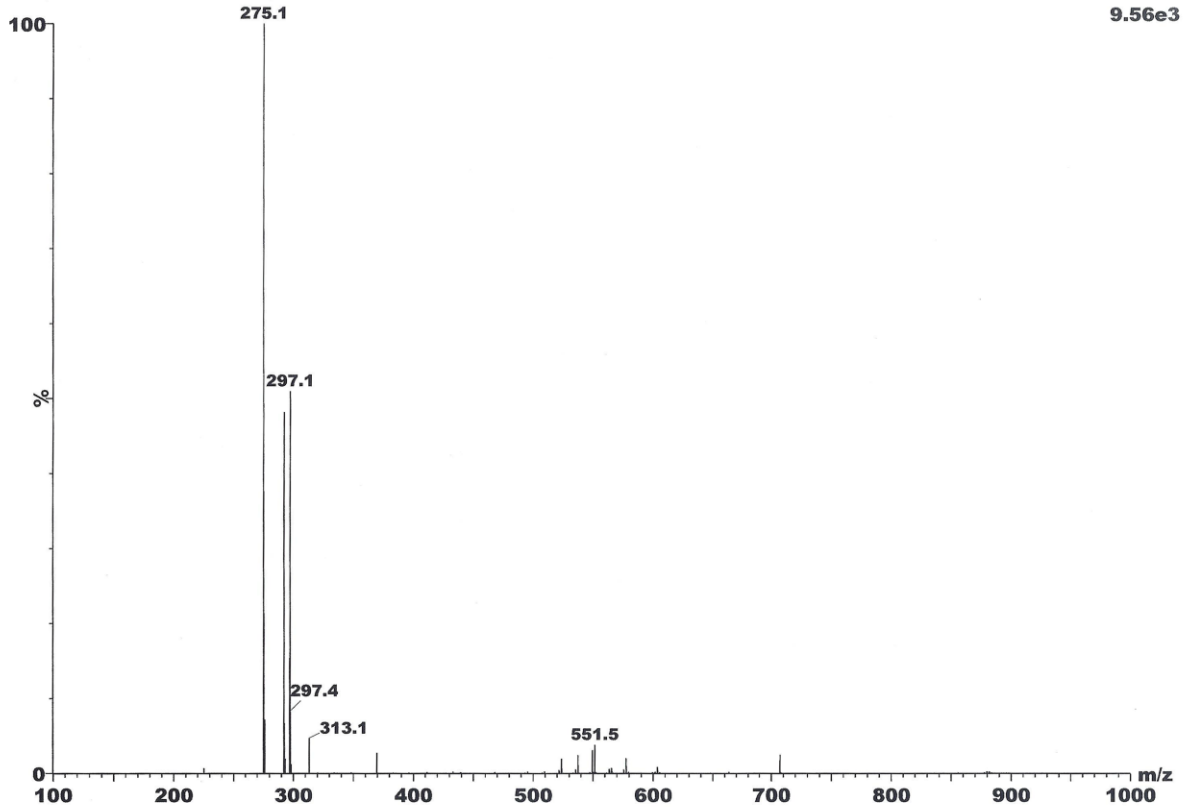


Fig. S8 ^{13}C -NMR of compound **6**

66 (1.220)

TOF MS ES+
9.56e3

Elemental Composition Report

Page 1

Single Mass Analysis

Tolerance = 25.0 mDa / DBE: min = -2.0, max = 500.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

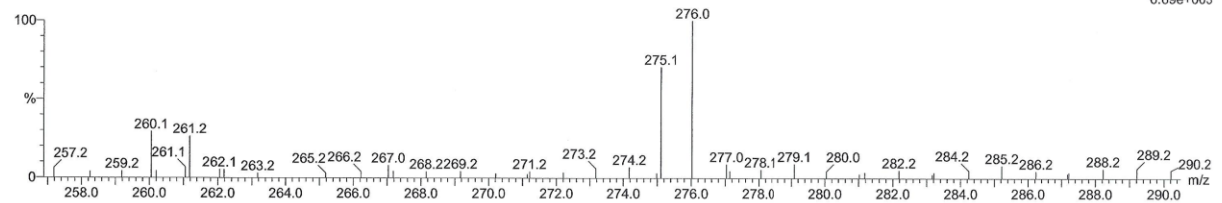
Monoisotopic Mass, Even Electron Ions

51 formula(e) evaluated with 6 results within limits (up to 19 closest results for each mass)

Elements Used:

C: 0-100 H: 0-200 O: 0-40

120 (2.219) Cn (Cen,5, 50.00, Ar); Sm (SG, 1x2.00); Sb (12,5.00)

TOF MS ES+
6.69e+003

Minimum: -2.0
Maximum: 25.0 10.0 500.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
275.1129	275.1131	-0.2	-0.7	3.5	4761.0	C12 H19 O7
	275.1072	5.7	20.7	12.5	4153.0	C19 H15 O2
	275.0978	15.1	54.9	-0.5	4392.4	C8 H19 O10
	275.1283	-15.4	-56.0	7.5	5326.8	C16 H19 O4
	275.0919	21.0	76.3	8.5	3861.4	C15 H15 O5
	275.1342	-21.3	-77.4	-1.5	6043.3	C9 H23 O9

Fig. S9 HRMS of compound 6

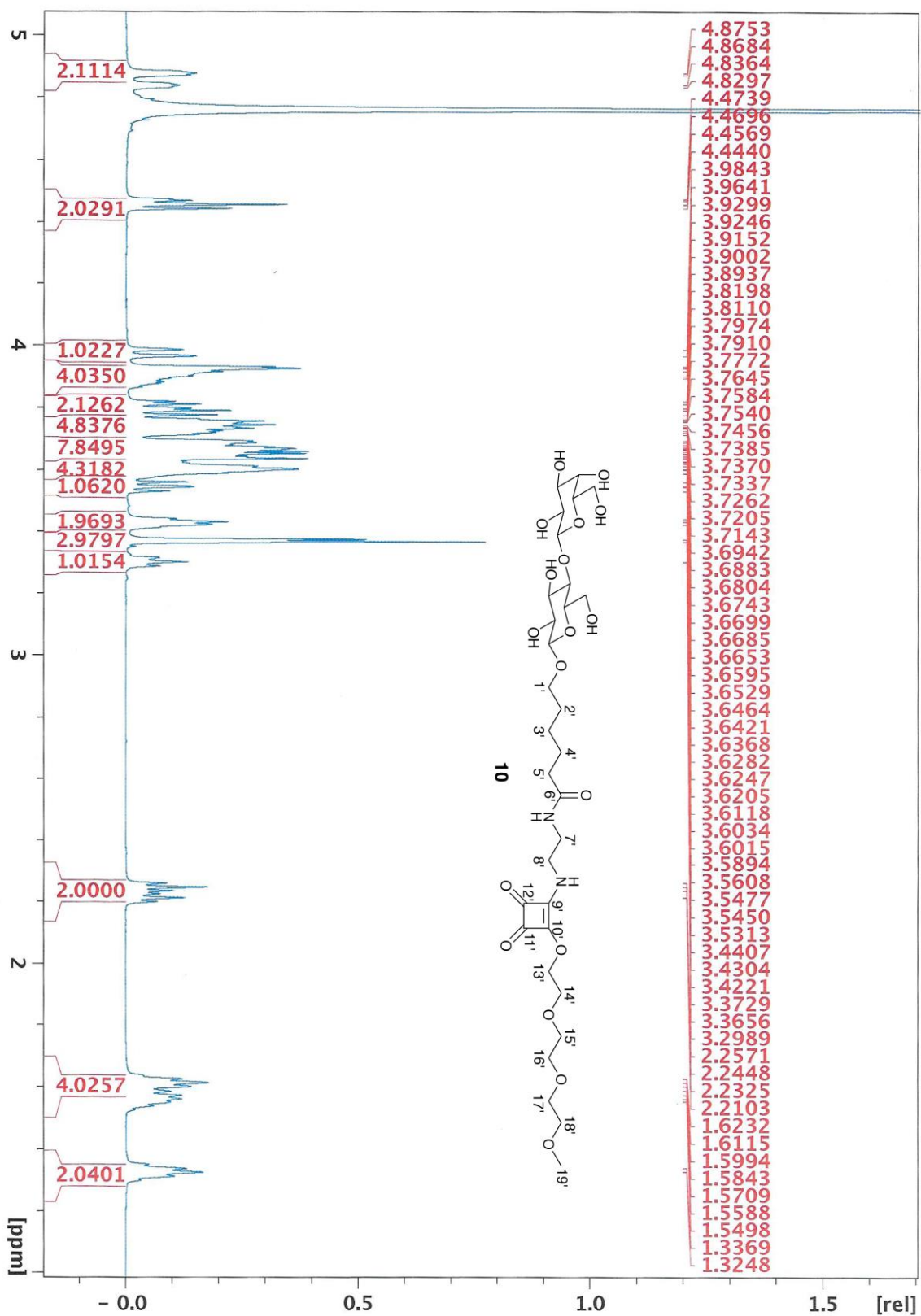


Fig. S10 $^1\text{H-NMR}$ of compound **10**

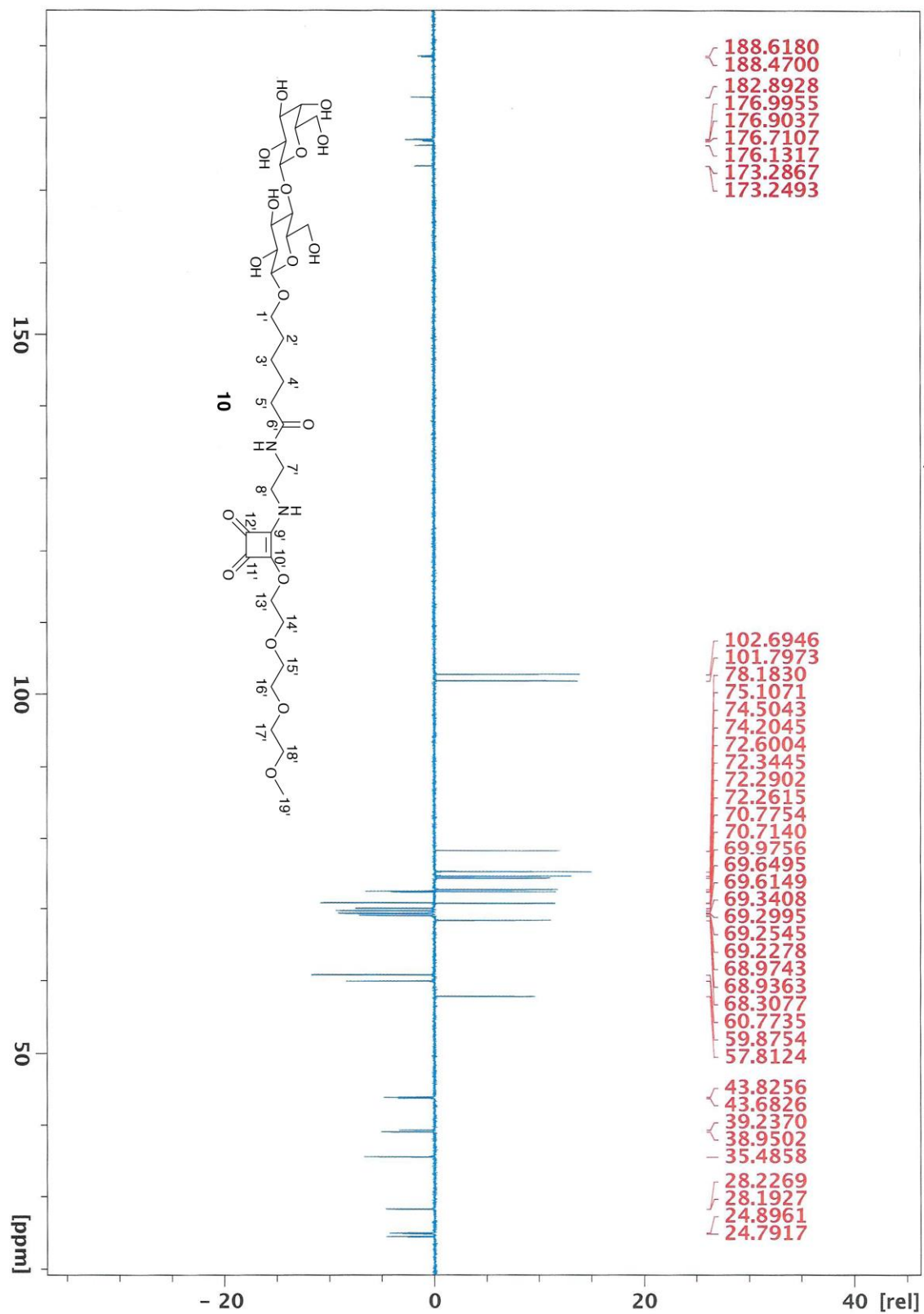
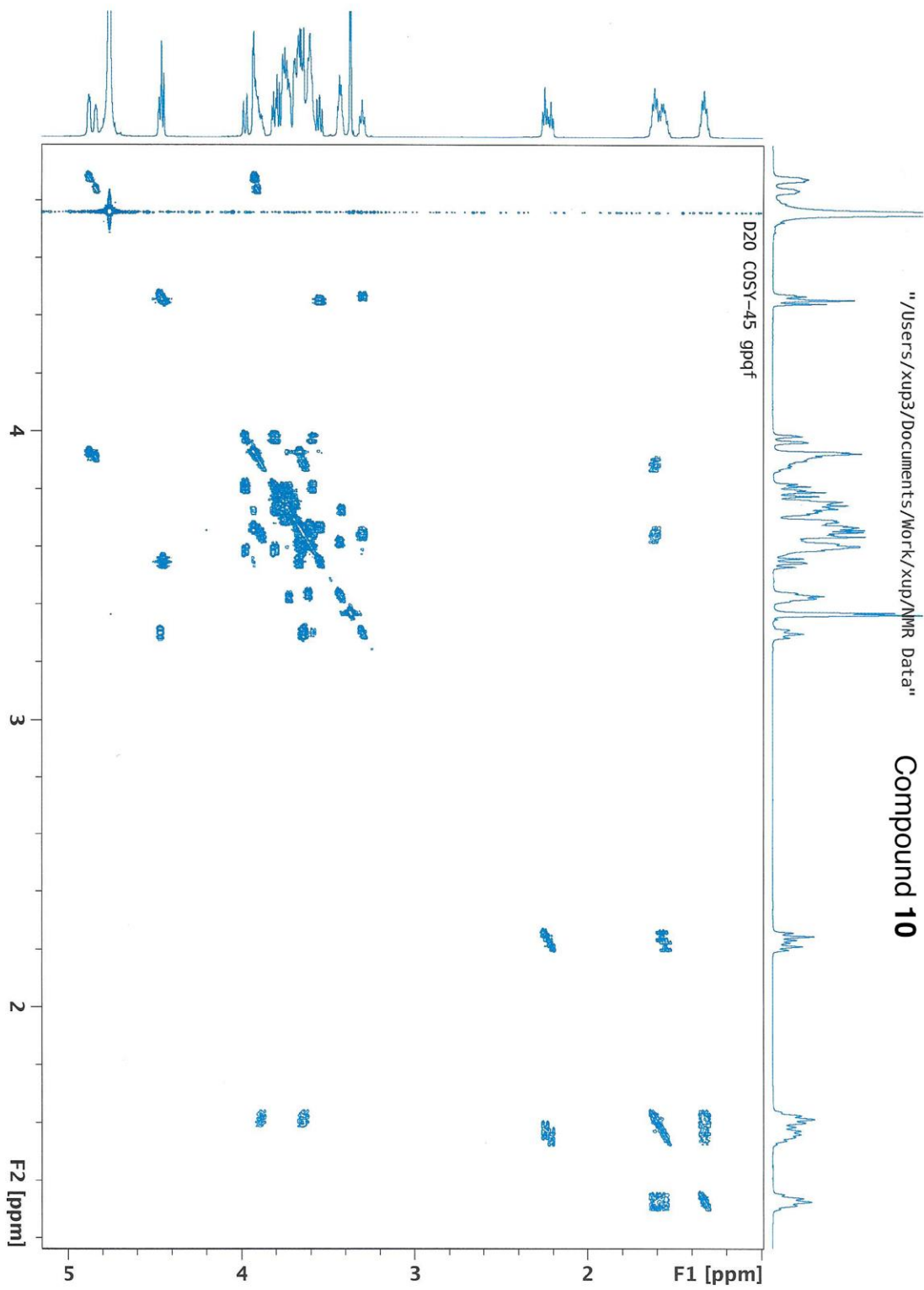


Fig. S11 ^{13}C -NMR of compound **10**



F

ig. S12 COSY of compound **10**

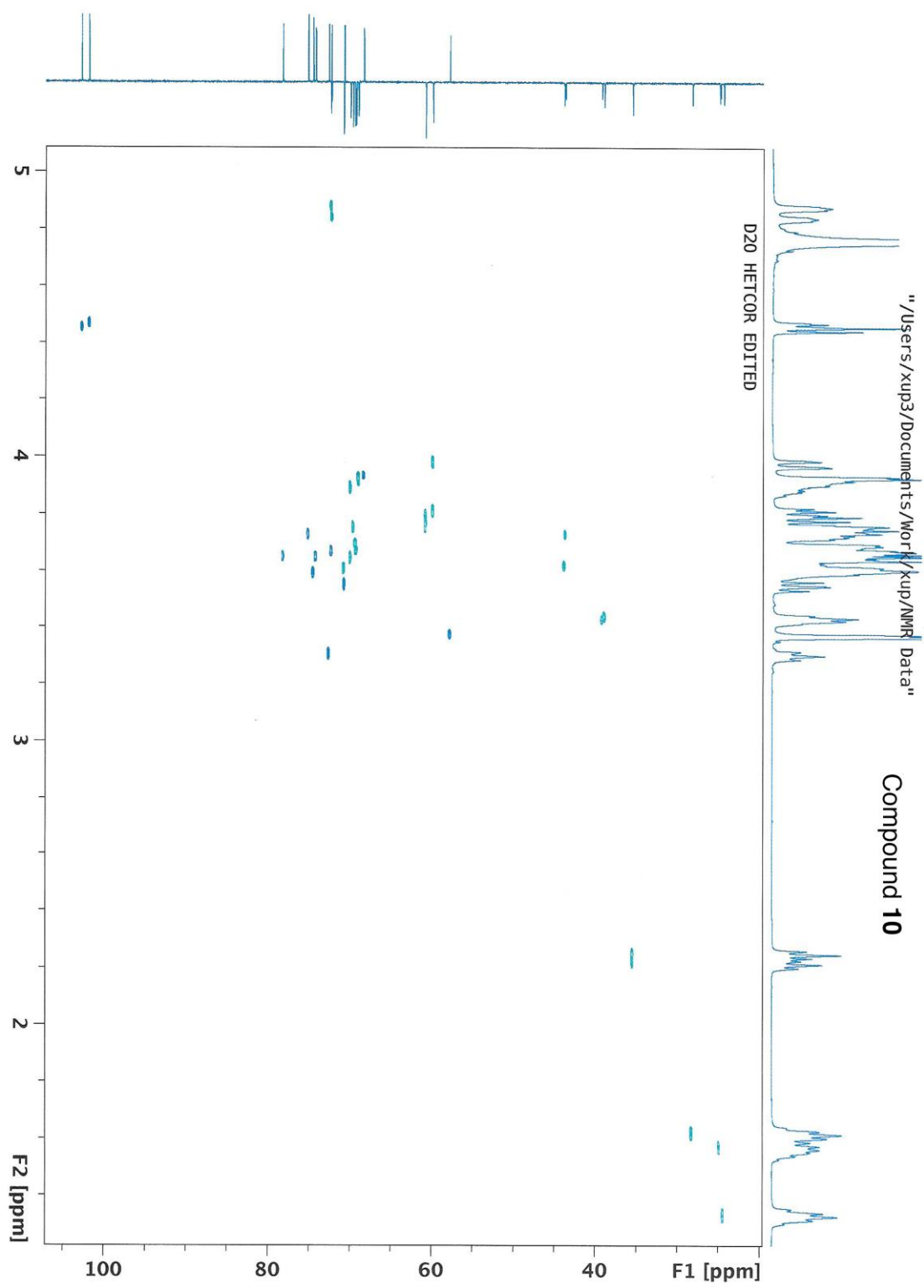
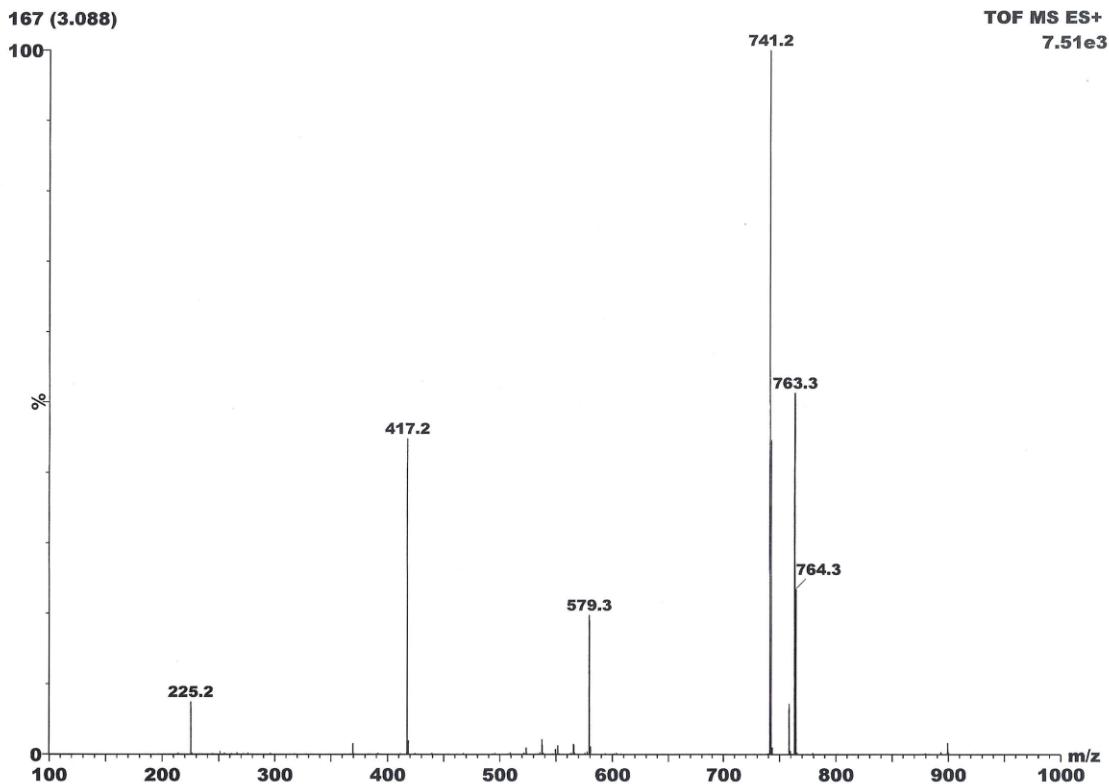


Fig. S13 HSQC of compound 10



Elemental Composition Report

Page 1

Single Mass Analysis

Tolerance = 25.0 mDa / DBE: min = -2.0, max = 500.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

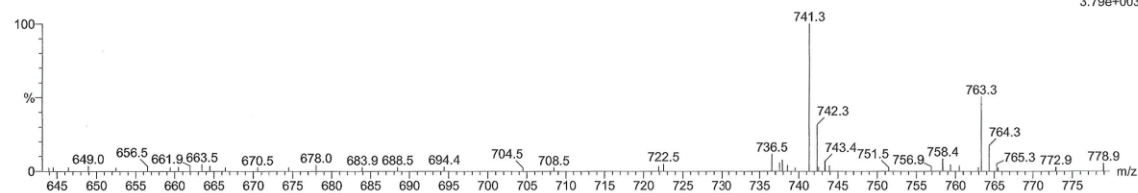
240 formula(e) evaluated with 13 results within limits (up to 19 closest results for each mass)

Elements Used:

C: 0-100 H: 0-200 N: 2-2 O: 0-40

170 (3.144) Cn (Cen,5, 50.00, Ar); Sm (SG, 1x2.00); Sb (12,5.00)

TOF MS ES+
3.79e+003



Minimum: -2.0
Maximum: 25.0 10.0 500.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
741.3292	741.3293	-0.1	-0.1	6.5	44.9	C31 H53 N2 O18
	741.3270	2.2	3.0	37.5	425.8	C56 H41 N2
	741.3328	-3.6	-4.9	28.5	291.8	C49 H45 N2 O5
	741.3235	5.7	7.7	15.5	118.9	C38 H49 N2 O13
	741.3387	-9.5	-12.8	19.5	174.4	C42 H49 N2 O10
	741.3176	11.6	15.6	24.5	227.8	C45 H45 N2 O8
	741.3141	15.1	20.4	2.5	33.4	C27 H53 N2 O21
	741.3446	-15.4	-20.8	10.5	84.3	C35 H53 N2 O15
	741.3117	17.5	23.6	33.5	359.4	C52 H41 N2 O3
	741.3481	-18.9	-25.5	32.5	372.9	C53 H45 N2 O2
	741.3082	21.0	28.3	11.5	86.5	C34 H49 N2 O16
	741.3505	-21.3	-28.7	1.5	35.4	C28 H57 N2 O20
	741.3540	-24.8	-33.5	23.5	250.4	C46 H49 N2 O7

Fig. S14 HRMS of compound 10

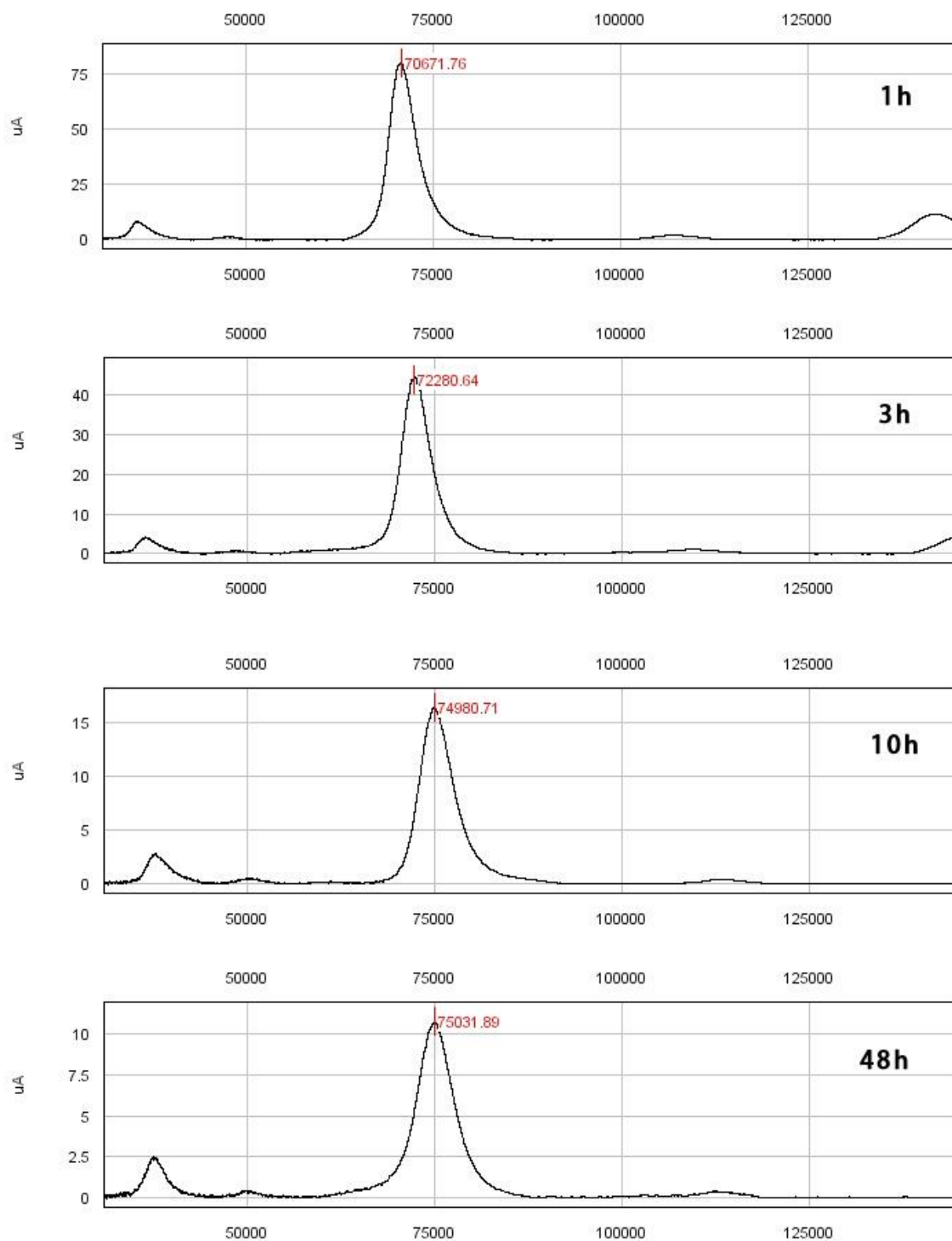


Fig. S15. SELDI-TOF monitoring of conjugation of **8** to BSA in buffer B

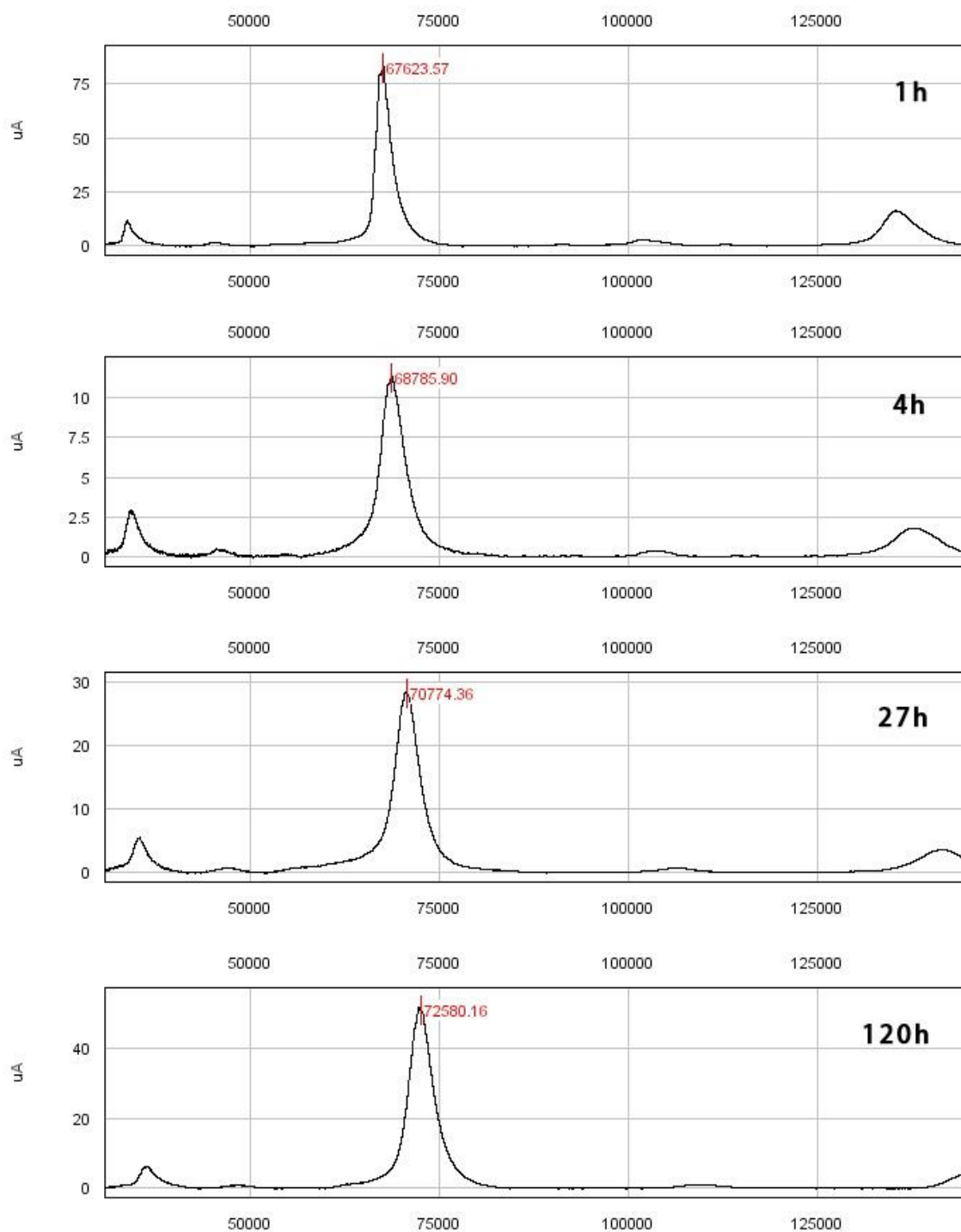


Fig. S16. SELDI-TOF monitoring of conjugation of **8** to BSA in buffer C

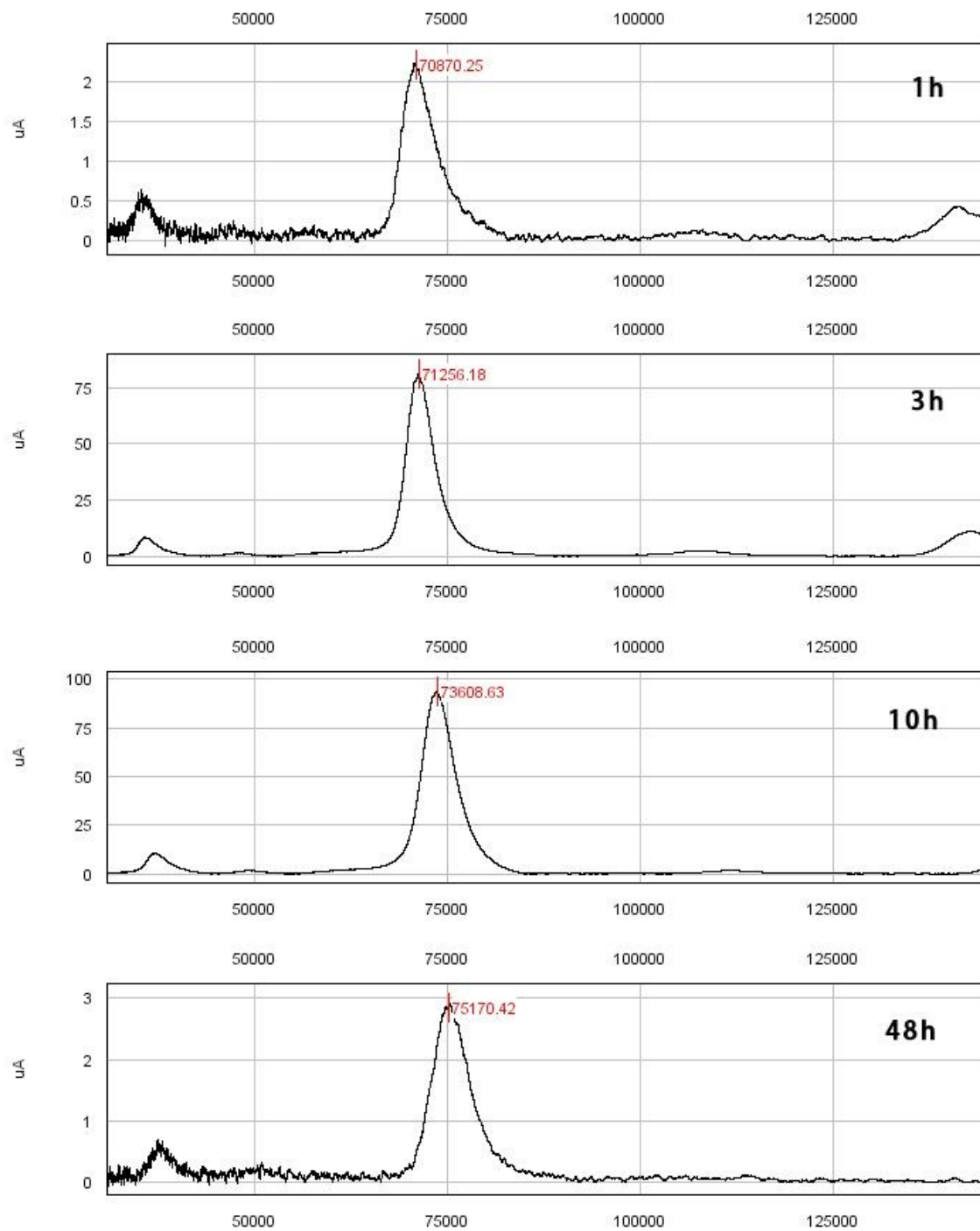


Fig. S17. SELDI-TOF monitoring of conjugation of **10** to BSA in buffer B

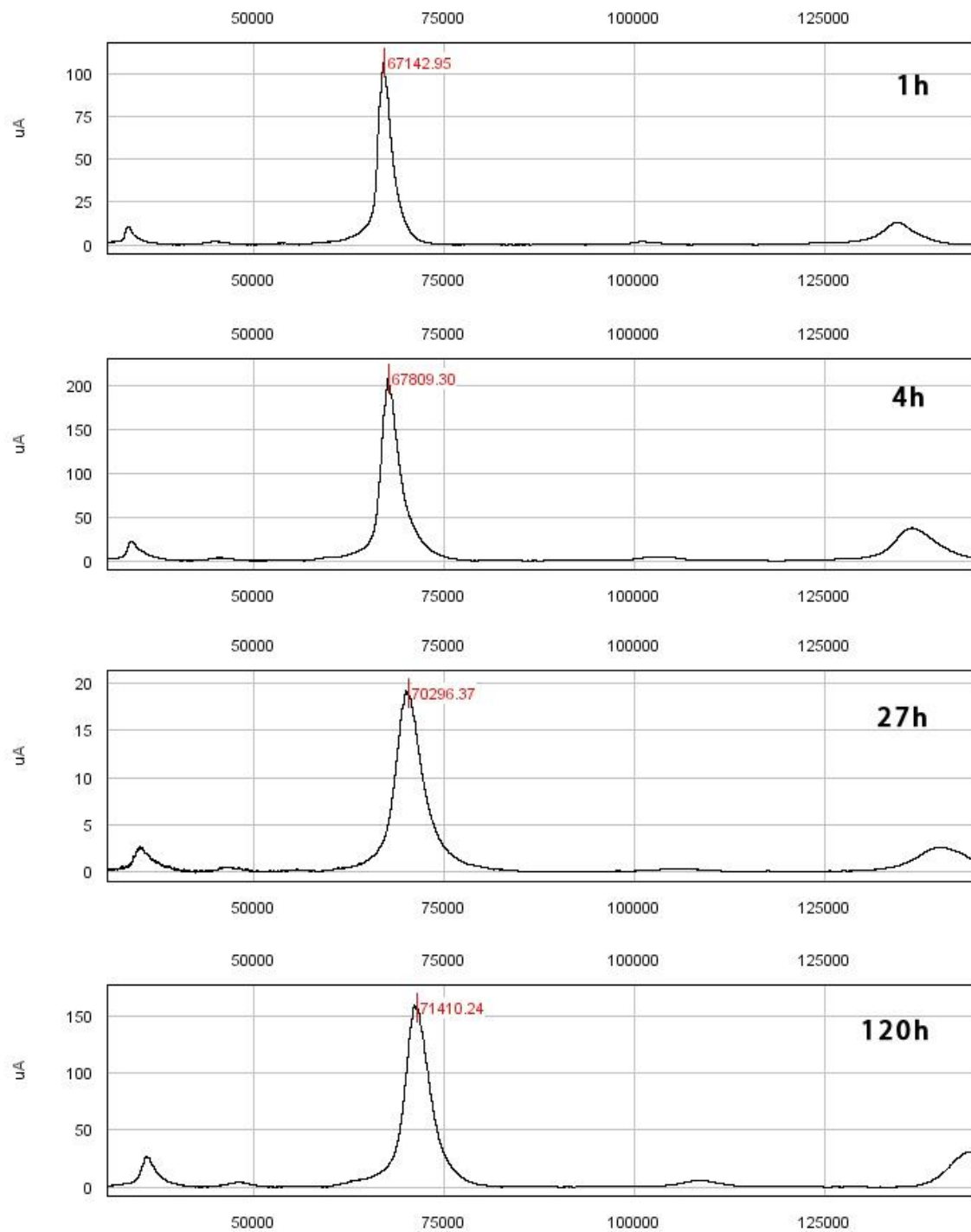


Fig. S18. SELDI-TOF monitoring of conjugation of **10** to BSA in buffer C