SUPPORTING INFORMATION

Targeting Triple Negative Breast Cancer Cells with Novel Cytotoxic Peptide-Doxorubicin Conjugates

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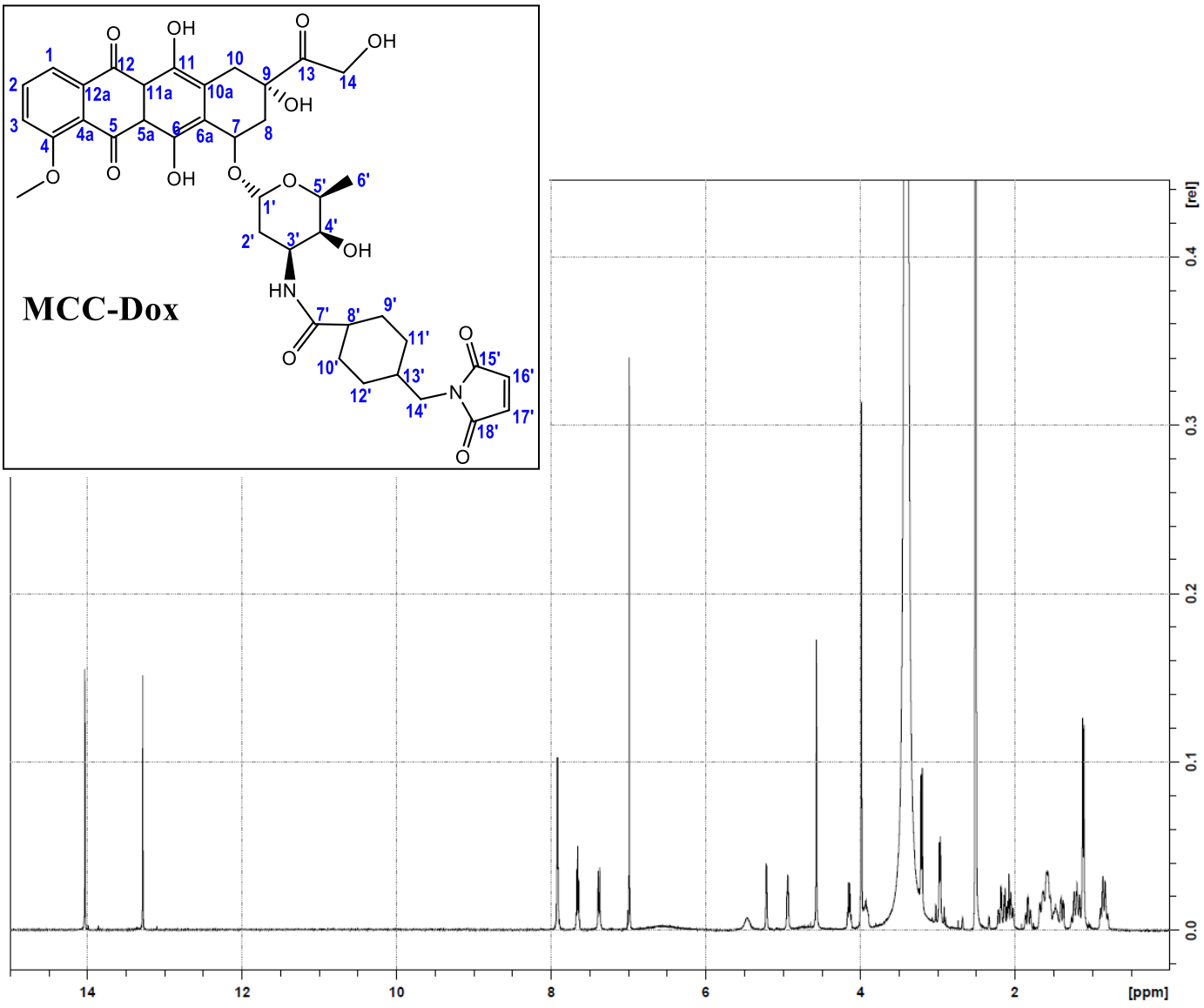
(a)



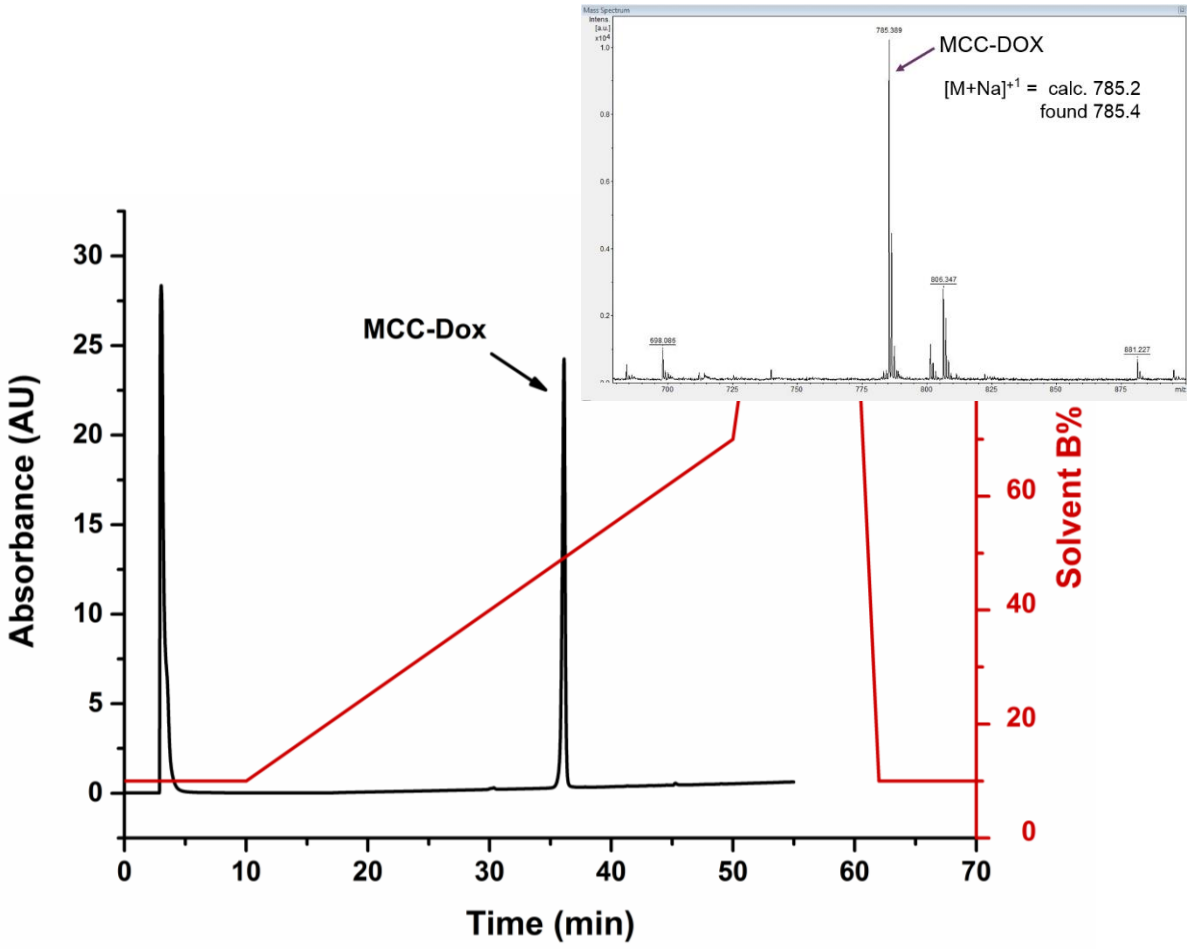
(b)

^{1}H NMR		
Number	**Chemical Shift**	**Proton**
11-OH	14.03	
6-OH	13.28	
1 and 2	7.92	2H, m
3	7.66	1H, m
NH	7.38	
16’ and 17’	6.99	2H, s
1’	5.22	1H, dd
7	4.94	1H, dd
14	4.57	2H, s
5’	4.14	1H, m
OMe	3.98	3H, s
3’	3.93	1H, m
4’	3.36	1H, m
14’	3.21	2H, d
10	2.97	2H, dd
DMSO	2.50	
8	2.14	2H, m
8’	2.06	1H, m
2’	1.83 and 1.39	2H, td and dd
9’ and 10’	1.66-1.55	4H, m
13’	1.48	1H, m
11’ and 12’	1.21 and 0.845	4H, m
6’	1.12	3H, d

^{13}C NMR	
Number	**Chemical Shift**
1	118.9
2	136.1
3	119.6
4	160.8
4a	119.1
5	186.5
5a	110.6
6	154.6
6a	134.0
7	70.0
8	36.5
9	75.0
10	32.1
10a	135.4
11	156.0
11a	110.8
12	186.5
12a	134.7
13 (C=O)	213.6
14	63.55
1’	100.0
2’	29.5
3’	44.6
4’	68.1
5’	66.7
6’	16.9
7’ (C=O)	174.1
8’	43.1
9’	28.3
10’	29.1
11’	28.1
12’	29.24
13’	35.8
14’	42.8
15’ and 18’	171.4
16’ and 17’	134.3

Figure S1. (a) Proton (^{1}H) NMR spectra for MCC-Dox in DMSO-d6. (b) Chemical shift assignments for MCC-Dox for the proton and carbon NMR spectra made using the HSQC and HMBC experiments.

(a)


MCC-DOX
[M+Na]+1 = calc. 785.2
found 785.4
MCC-Dox
Absorbance (AU)
Solvent B%
Time (min)

(b)

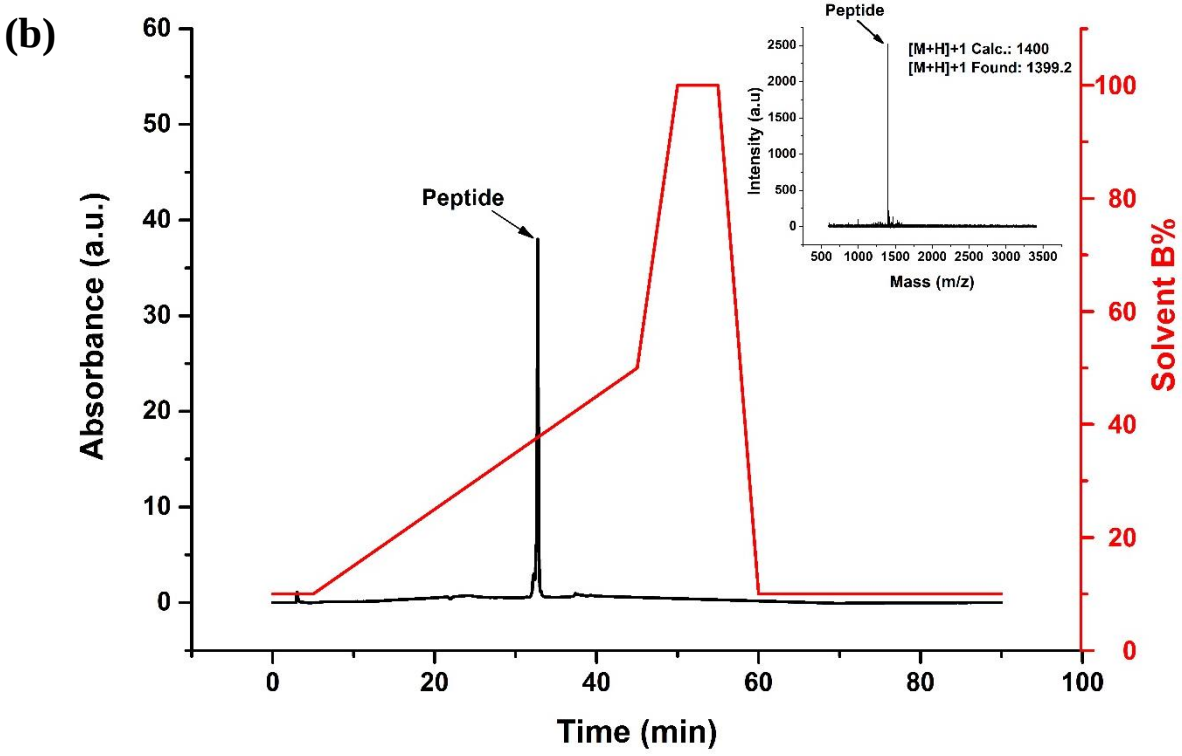
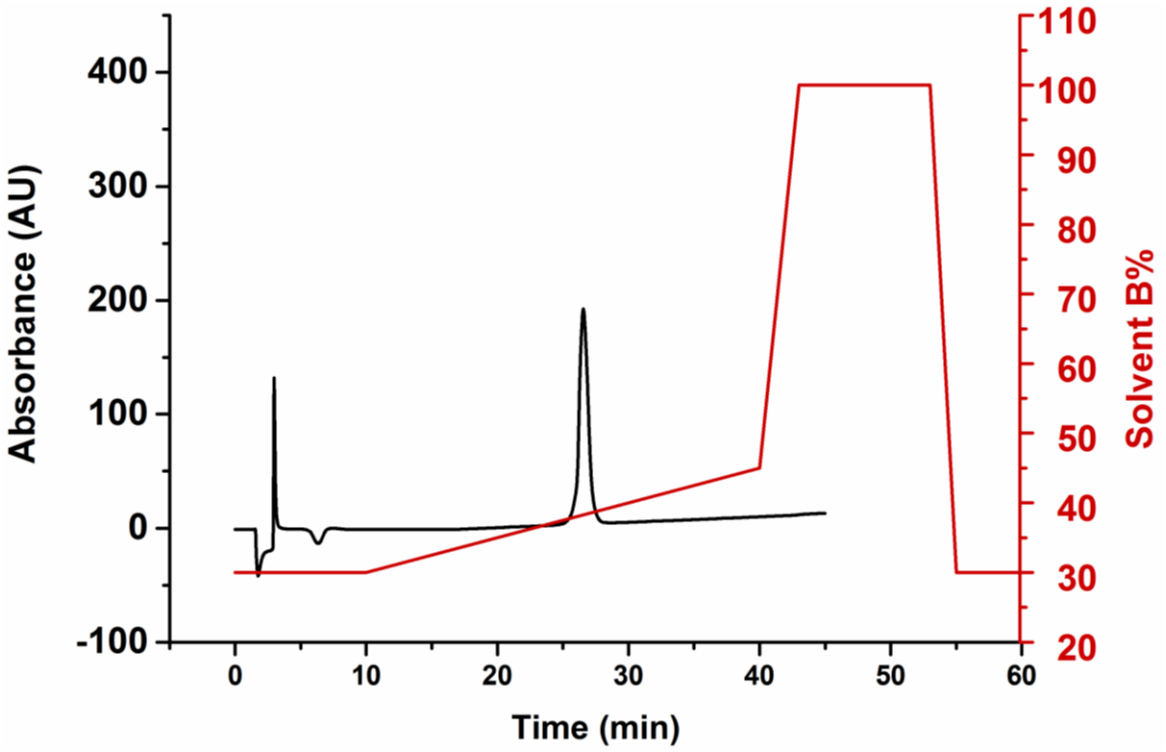
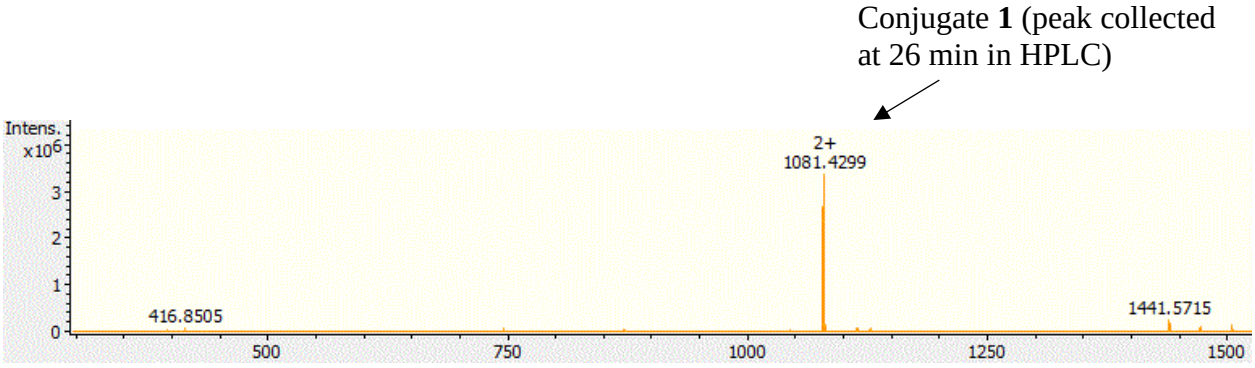

Peptide
[M+H]+1 Calc.: 1400
[M+H]+1 Found: 1399.2
Intensity (a.u)
Mass (m/z)
Peptide
Absorbance (a.u.)
Solvent B%
Time (min)

Figure S2. RP-HPLC chromatograms for (a) MCC-DOX and (b) peptide. A gradient of acetonitrile/water (0.05% TFA) was used in different ratios over 50 min with a flow rate of 1 mL/min on Vydac C18 semi-preparative column. The inset shows MALDI-TOF mass spectra of each of them showing the $[M+H]^{+1}$ or $[M+Na]^{+1}$ as the major peak.

(a)



(b)



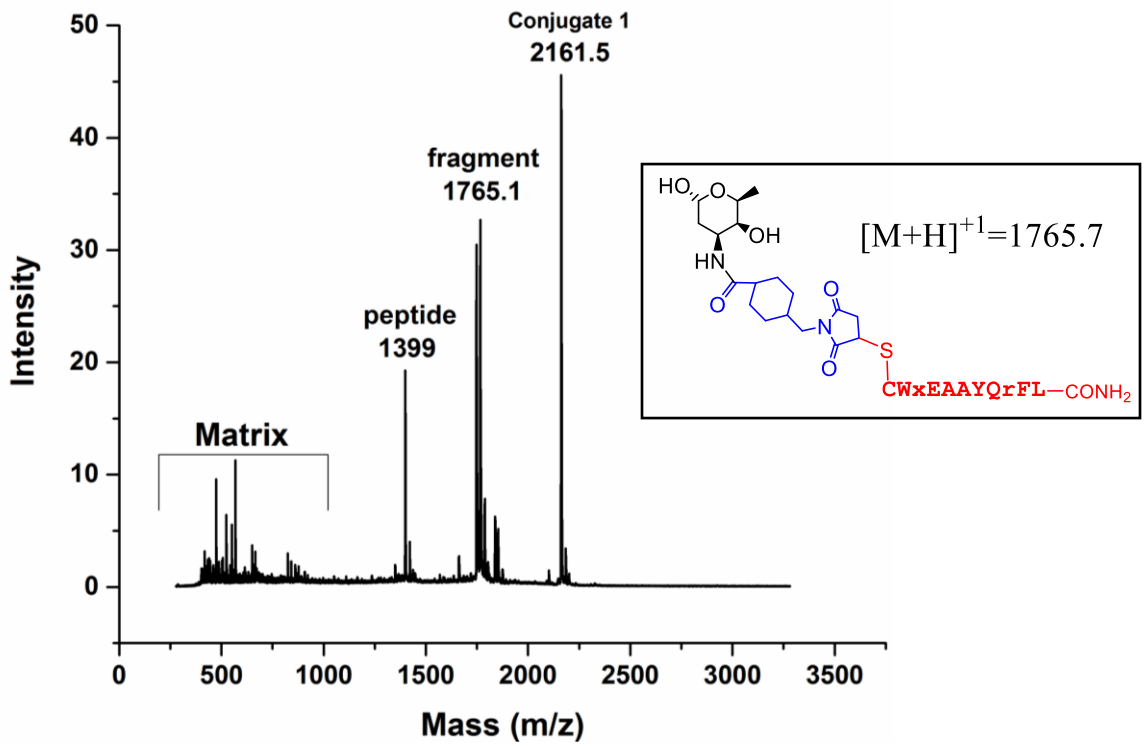


Figure S3. (a) RP-HPLC chromatogram of peptide-Dox conjugate **1**. Also shown are the MALDI-TOF (b) and Q-TOF (c) mass spectra for the conjugate showing the $[M+H]^{+1}$ as 2161.5 (calcd. M+H 2161.9) or (1081.43-1)*2 = 2160.86 (calcd M 2160.9) as the major peak. A gradient of acetonitrile/water (0.05% TFA) was used in different ratios over 50 min with a flow rate of 1 mL/min on Vydac C18 semi-preparative column.

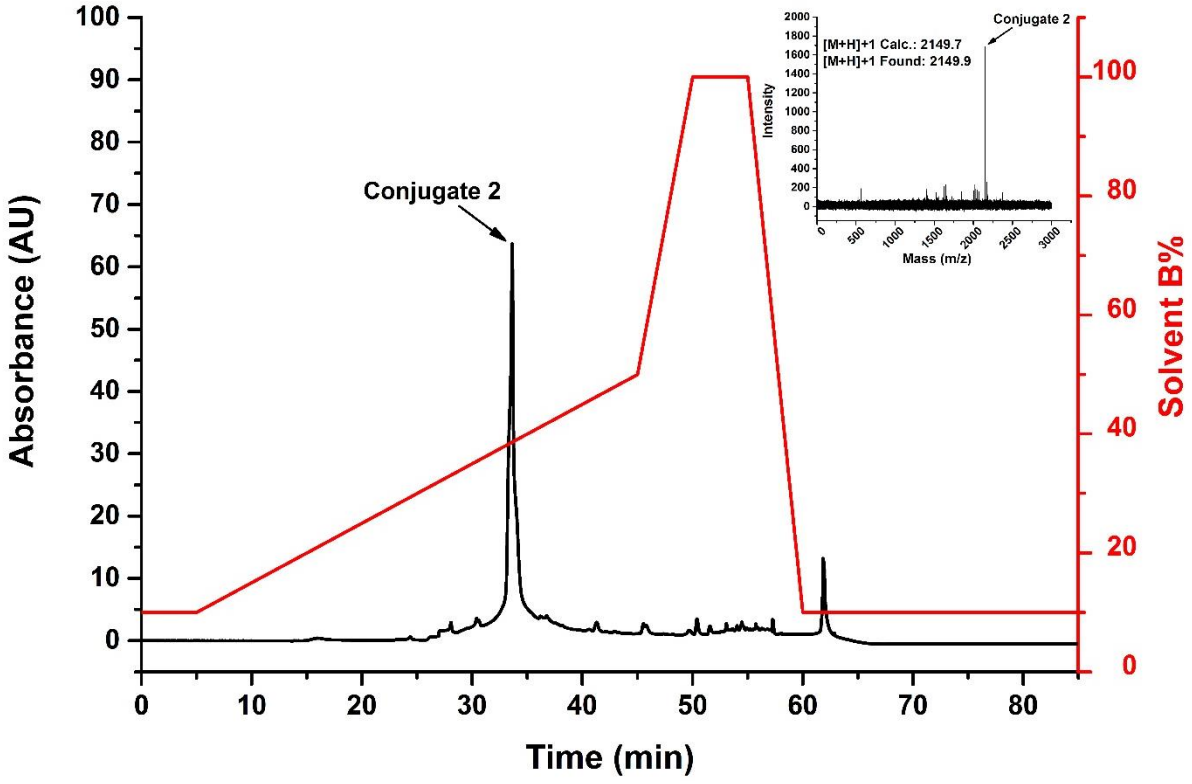


Figure S4. RP-HPLC chromatogram of conjugate **2**. A gradient of acetonitrile/water (0.05% TFA) was used in different ratios over 50 min with a flow rate of 1 mL/min on Vydac C18 analytical column. The inset shows MALDI-TOF mass spectrum showing the $[M+H]^{+1}$ as the major peak.

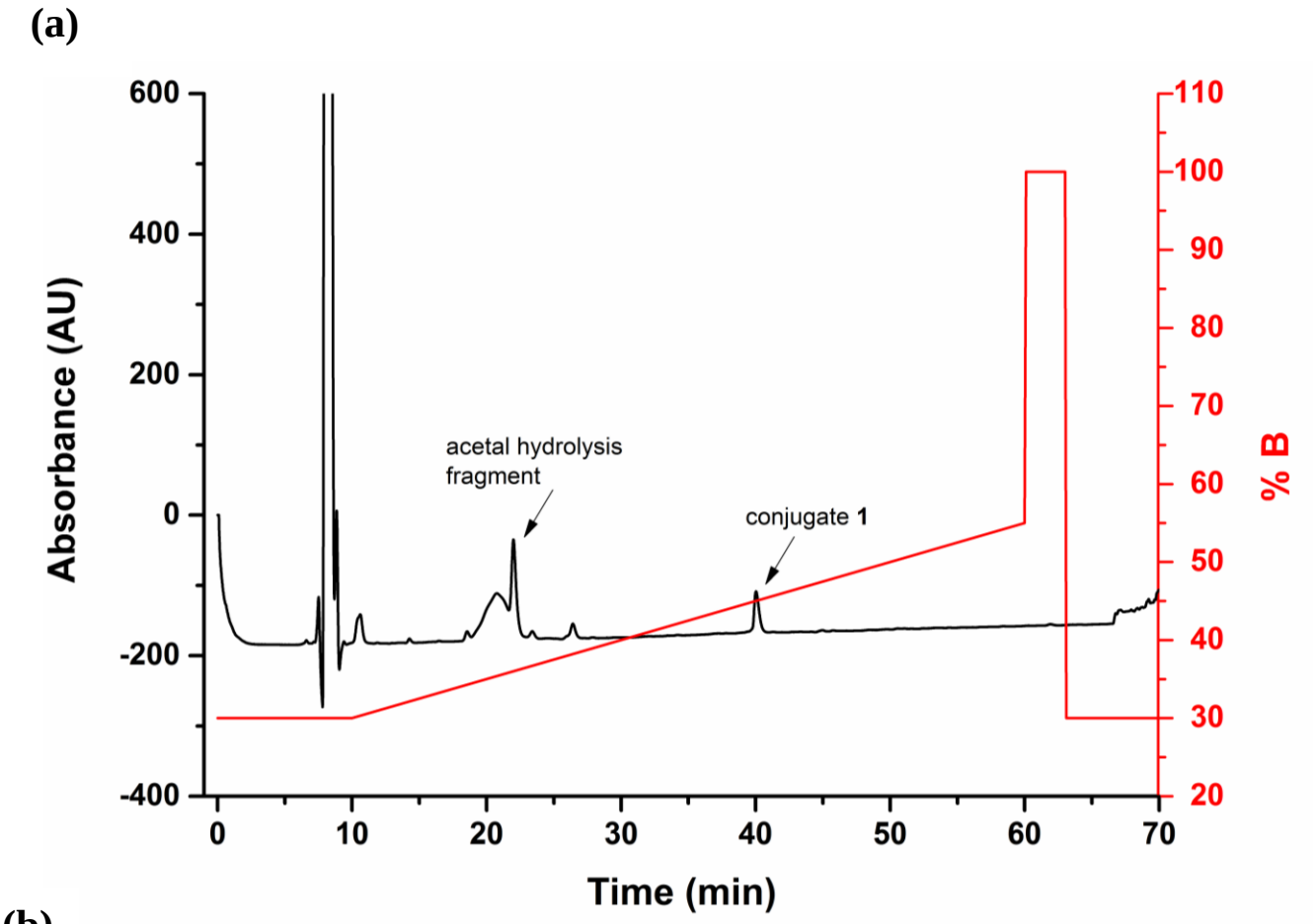


Figure S5. RP-HPLC chromatogram and mass spectrum (Q-TOF) of the aliquot (at 48 h) taken during acid stability experiment of conjugate **1**. (a) RP-HPLC chromatogram of aliquot at 48 h. A gradient of acetonitrile/water (0.05% TFA) was used in different ratios over 50 min with a flow rate of 1 mL/min on Vydac C18 semi-preparative column. (b) Q-TOF mass spectrum for the peak at 21 minutes in HPLC. The observed mass spec peak is 883.2 (+2 charge). This matches the calculated mass (1764.7) for the acid hydrolysis fragment i.e. (883.2-1)*2 = 1764.4.

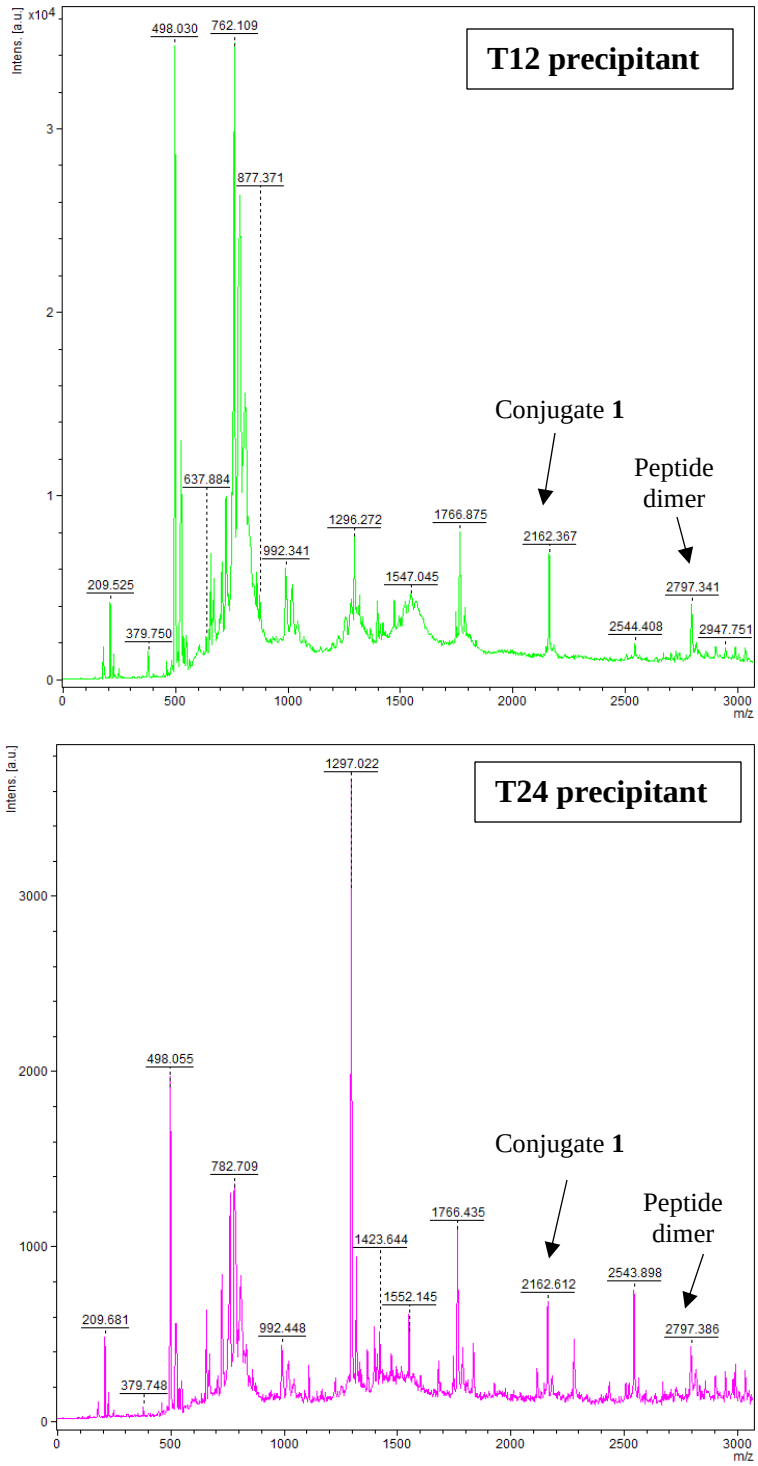


Figure S6. The stability of conjugate **1** in human serum at 37 °C. At different time intervals (0, 6, 12, and 24 hours), aliquots were removed and treated with methanol. Both the supernatant (see Figure 2b) and the precipitate were analyzed by RP-HPLC and MALDI-TOF to determine the fate of conjugate **1** in human serum. The MALDI-TOF of precipitate at 12 and 24 h are shown.

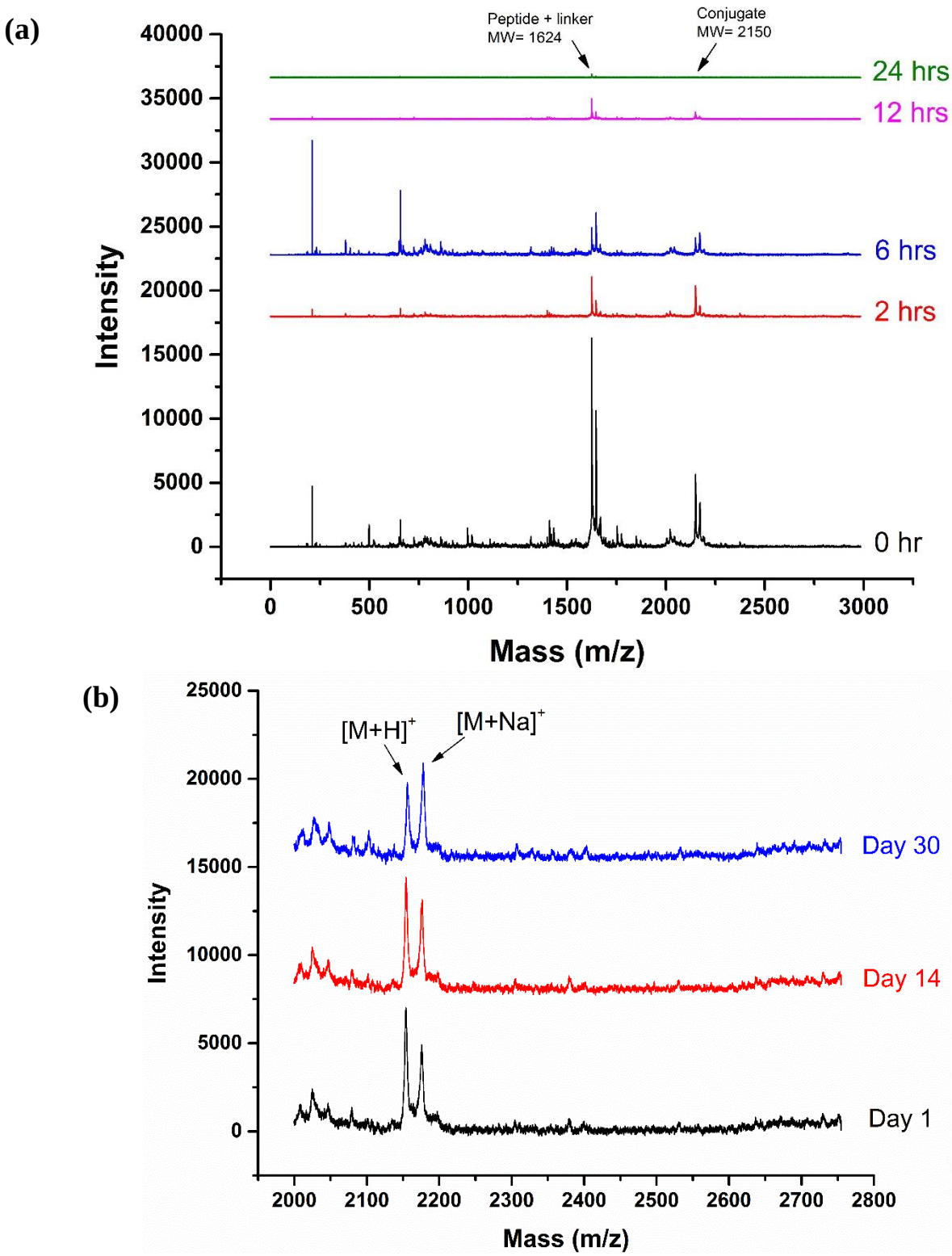


Figure S7. The stability of conjugate **2** in aqueous acidic solution pH 5 at 37 °C (a) and acetonitrile/water (9:1, v/v) at -20 °C (b). An aliquot was taken at different time intervals for MALDI-TOF mass analysis.