Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information

Synthesis and biological application of BKT-140 peptide modified polymer micelles for treating tumor metastasis with an enhanced cell internalization efficiency

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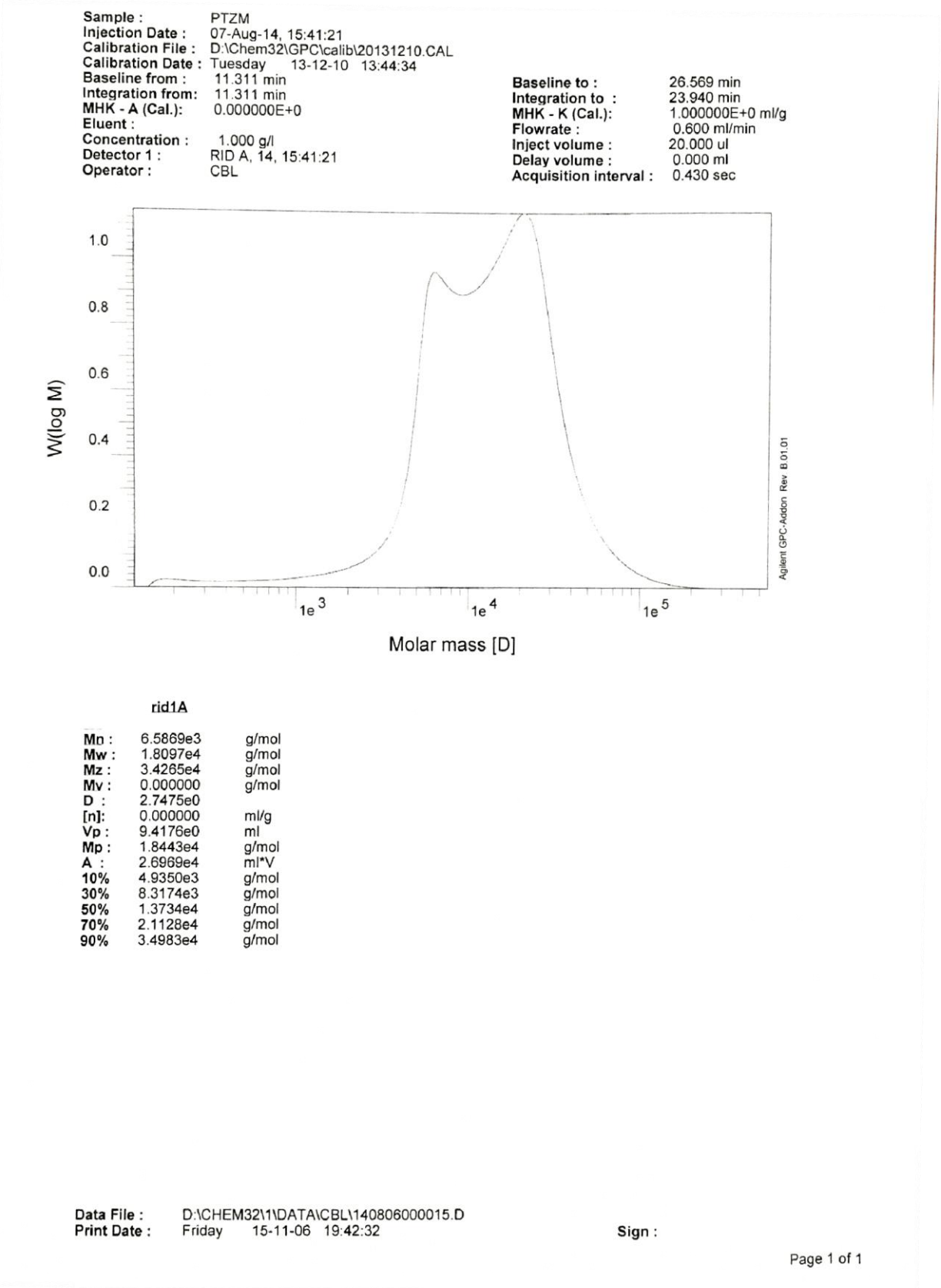
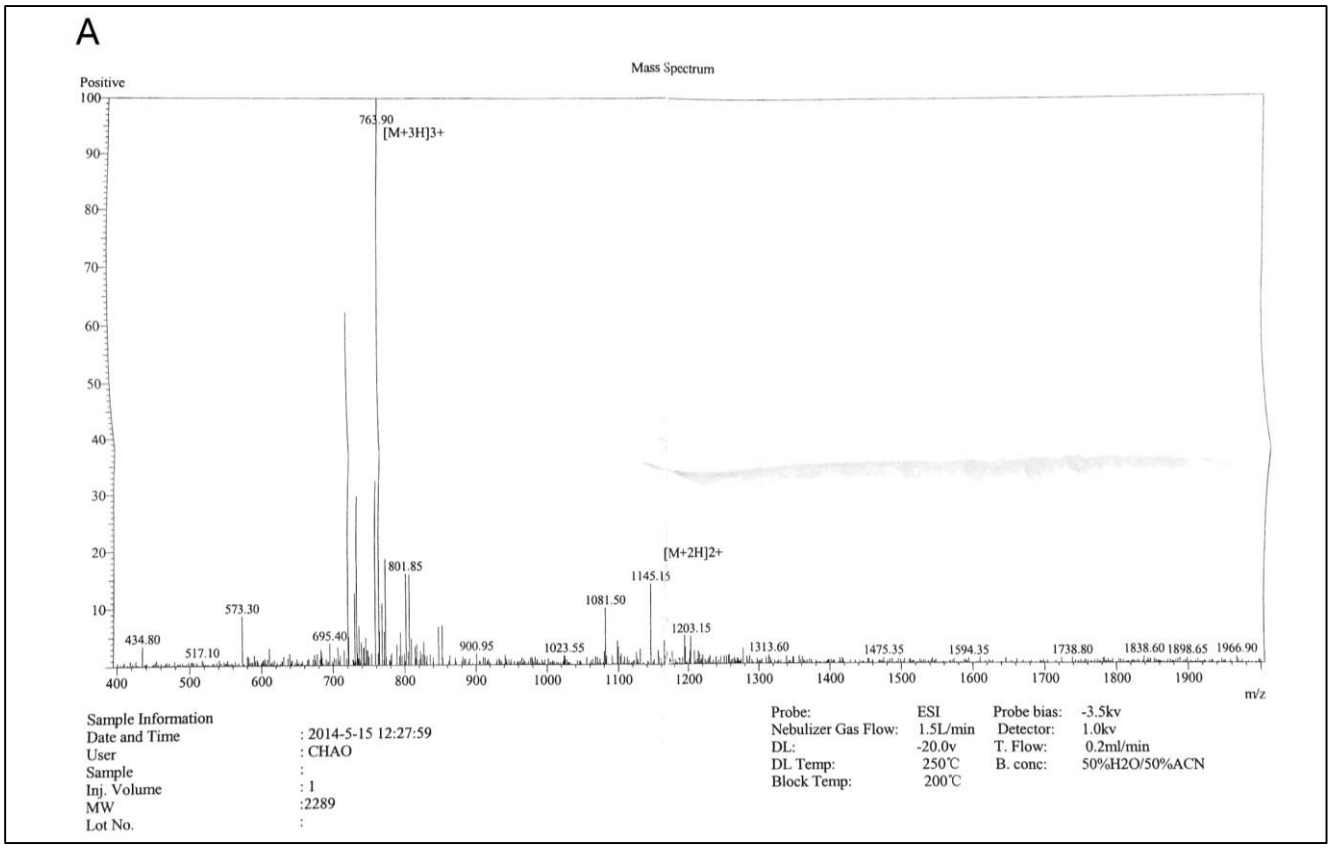


Figure S1. Report of Molecular weight (Mw) measurement of Chitosan Oligosaccharide by GPC. The chitosan (Mw 18.1kDa) used in this research was obtained by enzymatic degradation. The reaction time of hydrolysis was controlled by molecular weight measurement. The final reaction mixture was then filtered by

filter with 0.45 μm pole size, and then ultra-filtered by various molecular weight cut off ultrafiltration membrane (Millipore Labscale TFF system, Millipore Co., USA). The CSO was obtained by lyophilization. The molecular weight of final CSO was determined by gel permeation chromatography (GPC) with TSK-gel column (G3000SW, 7.5mm ID×30 cm) at 25 ◦C. The mobile phase is acetate buffer solution (pH 6.0) with a flow rate of 0.8ml/min. Master samples of polysaccharide with different molecular weight (Mw = 5.9, 11.8, 22.8, 47.3, 112, 212 kDa) were dissolved in acetate buffer solution (pH 6.0), and their final concentration was 1mg/ml. Calibration was performed by means of polysaccharide samples using the integral molecular weight distribution method. Figure S1 was the scan file of the original report.



B

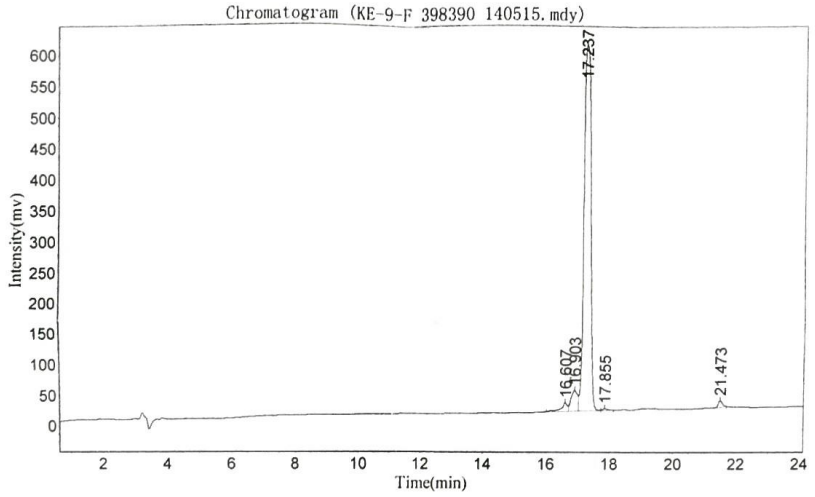
Date/Time: 2014-05-15, 14:10:02
Data File: E:\2014\KE-9-F 398390 140515.mdy

Analyst: HCM
Date/Time: 2014-05-15, 14:10:06

Sample Description:
Structure : KE-9
Number : 010250011
Lot No : P140505-HM398390
Column : 4.6×250mm, Kromasil 100-5C18
Solvent A : 0.1% trifluoroacetic in 100% acetonitrile
Solvent B : 0.1% trifluoroacetic in 100% water
Gradient :

	A	B
0.01min	8%	92%
25min	33%	67%
25.1min	100%	0%
30min	STOP	

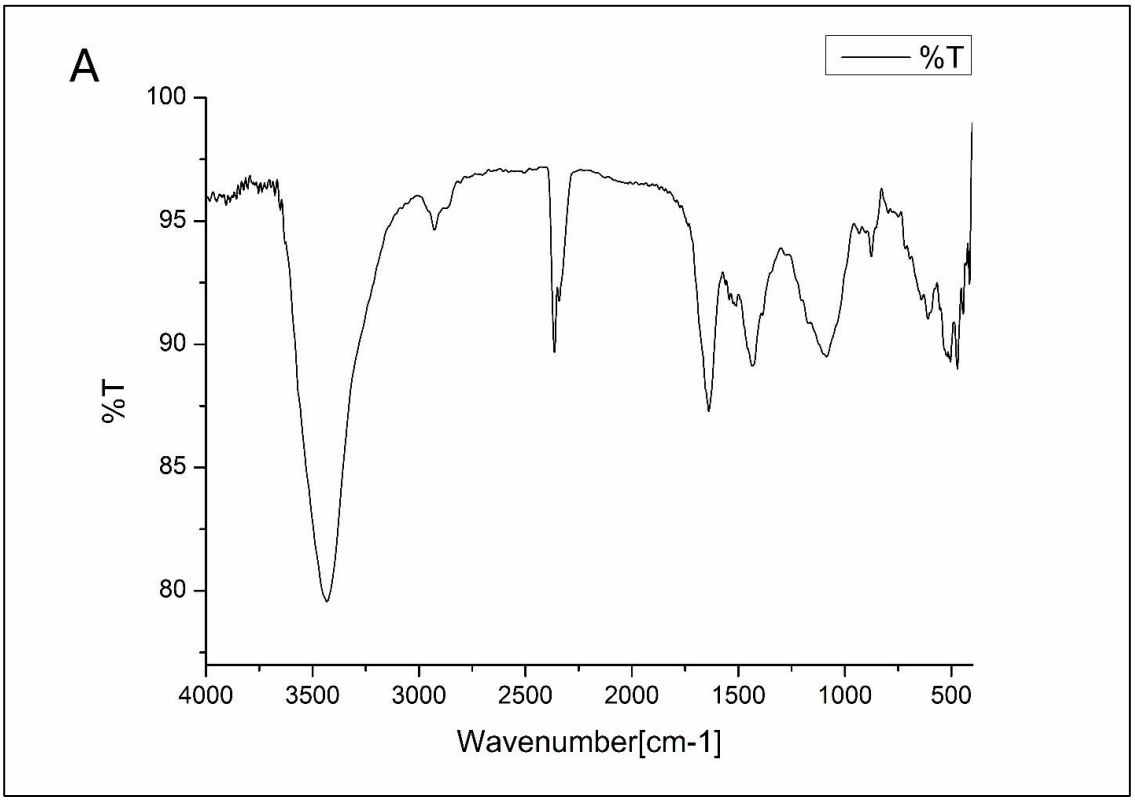
Flow rate : 1.0 mL/min
Wavelength : 220nm
Volume : 5ul

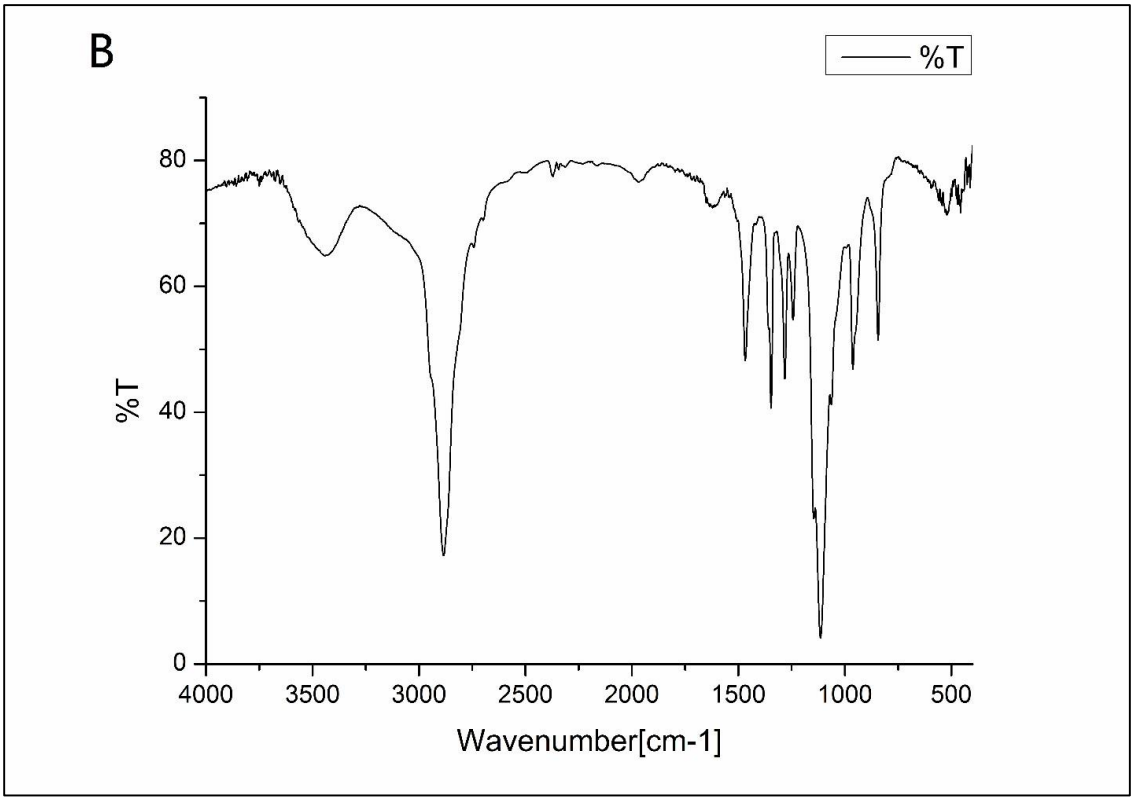


Results

Peak No.	Peak ID	Ret Time	Height	Area	Conc.
1		16.607	14297.675	196698.422	2.0505
2		16.903	34101.441	480019.563	5.0040
3		17.237	590841.125	8788924.000	91.6211
4		17.855	2955.336	29053.533	0.3029
5		21.473	11004.769	97990.430	1.0215
Total			653200.345	9592685.947	100.0000

Figure S2. (A)Molecular weight measurement by mass spectrum and (B) Purity Detection by HPLC of glu modified BKT-140. BKT-140 peptide (4F-benzoyl-Arg-Arg-Nal-Cys-Tyr-Cit-Lys-D-Lys-Pro-Tyr-Arg-Cit-Cys-Arg-NH_2) was synthesized by Hangzhou Dgpeptides Co., Ltd, China. In order not to interfere the function, we need to conjugate micelles and the peptide via a site distant from the pharmacophore. Therefore, CSOSA was conjugated with BKT-140 via the Glu added on D-Lys[8]. The molecular weight was measured by mass spectrum. Both Figure S2 (A) & (B) were the scan files of the original reports.


A
%T
100
95
90
85
80
%T
4000
3500
3000
2500
2000
1500
1000
500
Wavenumber[cm-1]


B
%T
80
60
40
20
0
%T
4000
3500
3000
2500
2000
1500
1000
500
Wavenumber[cm-1]

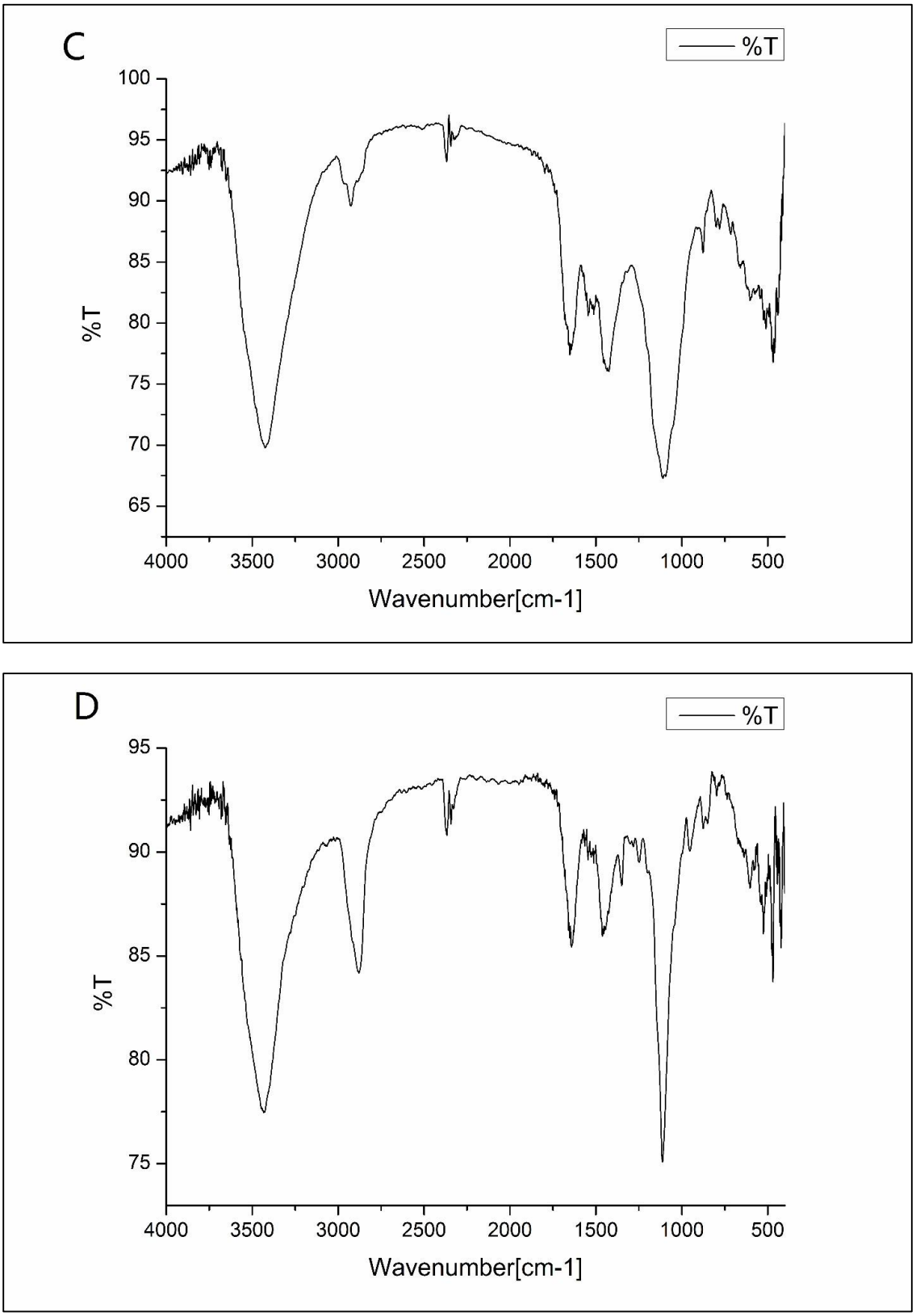
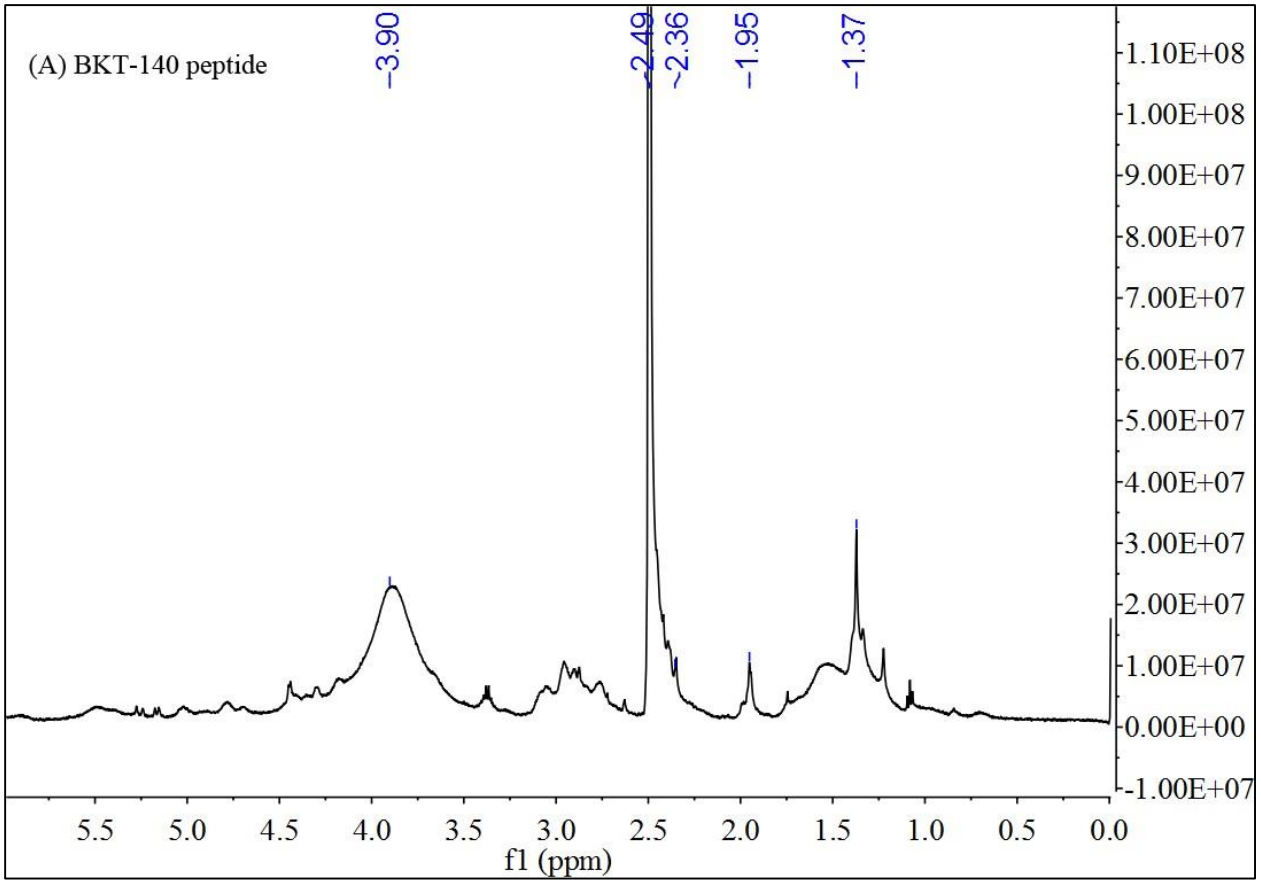
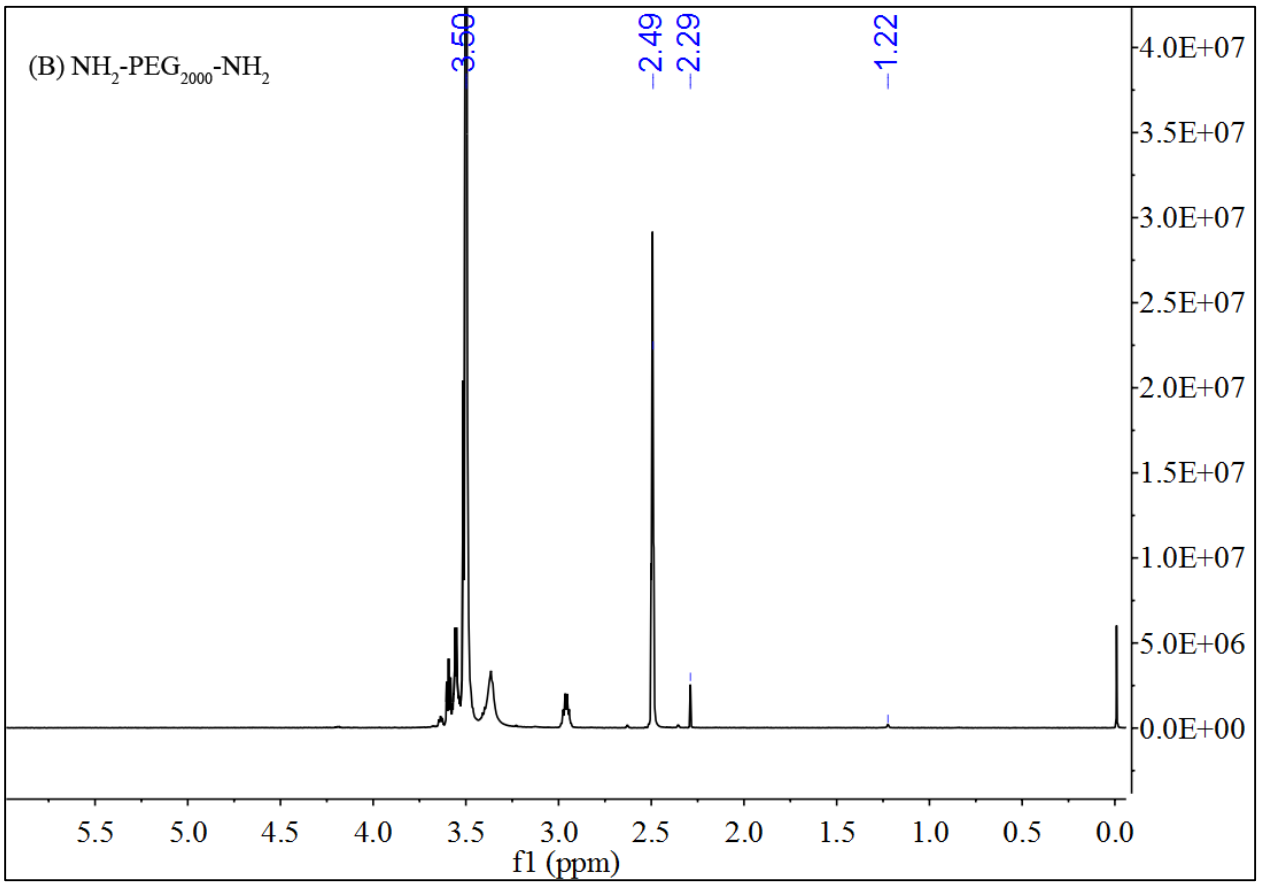
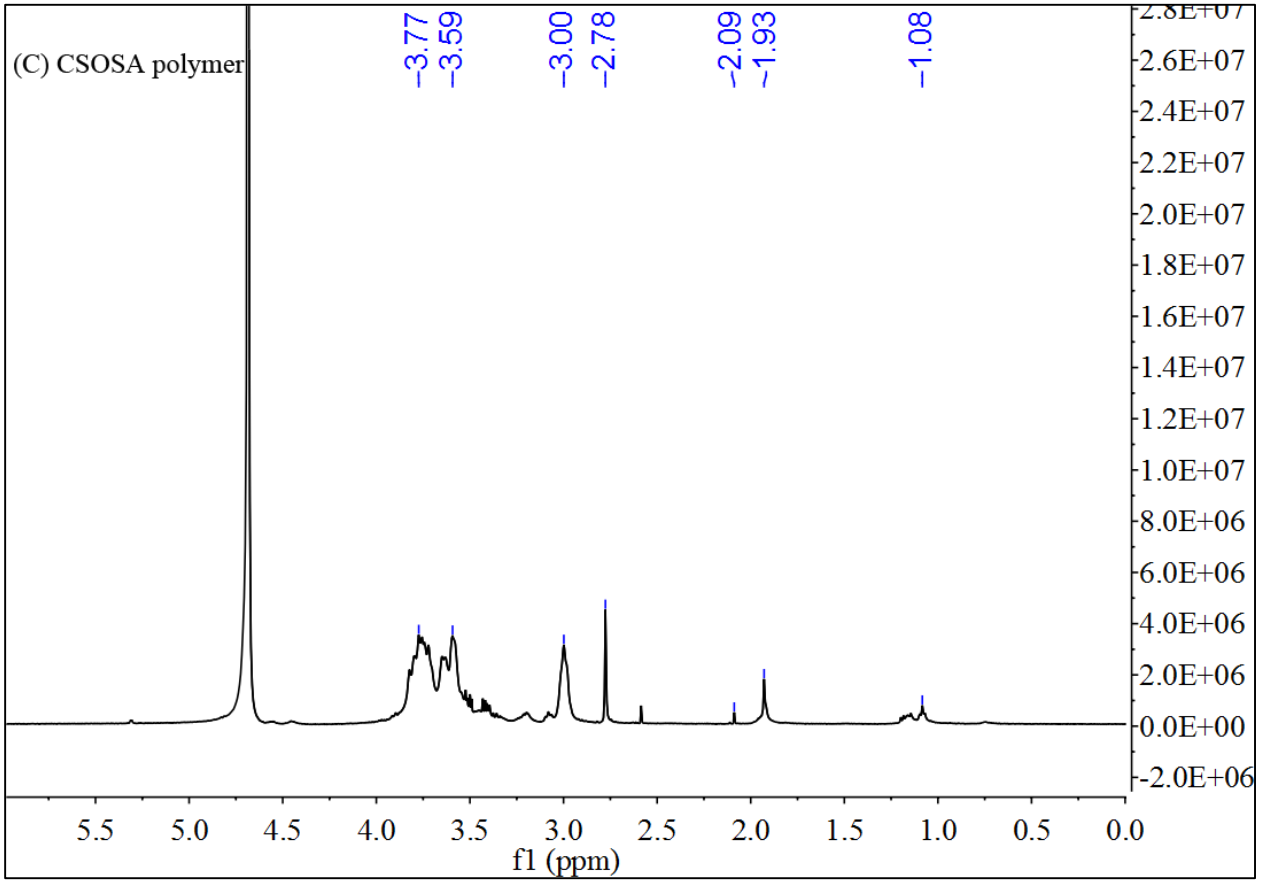
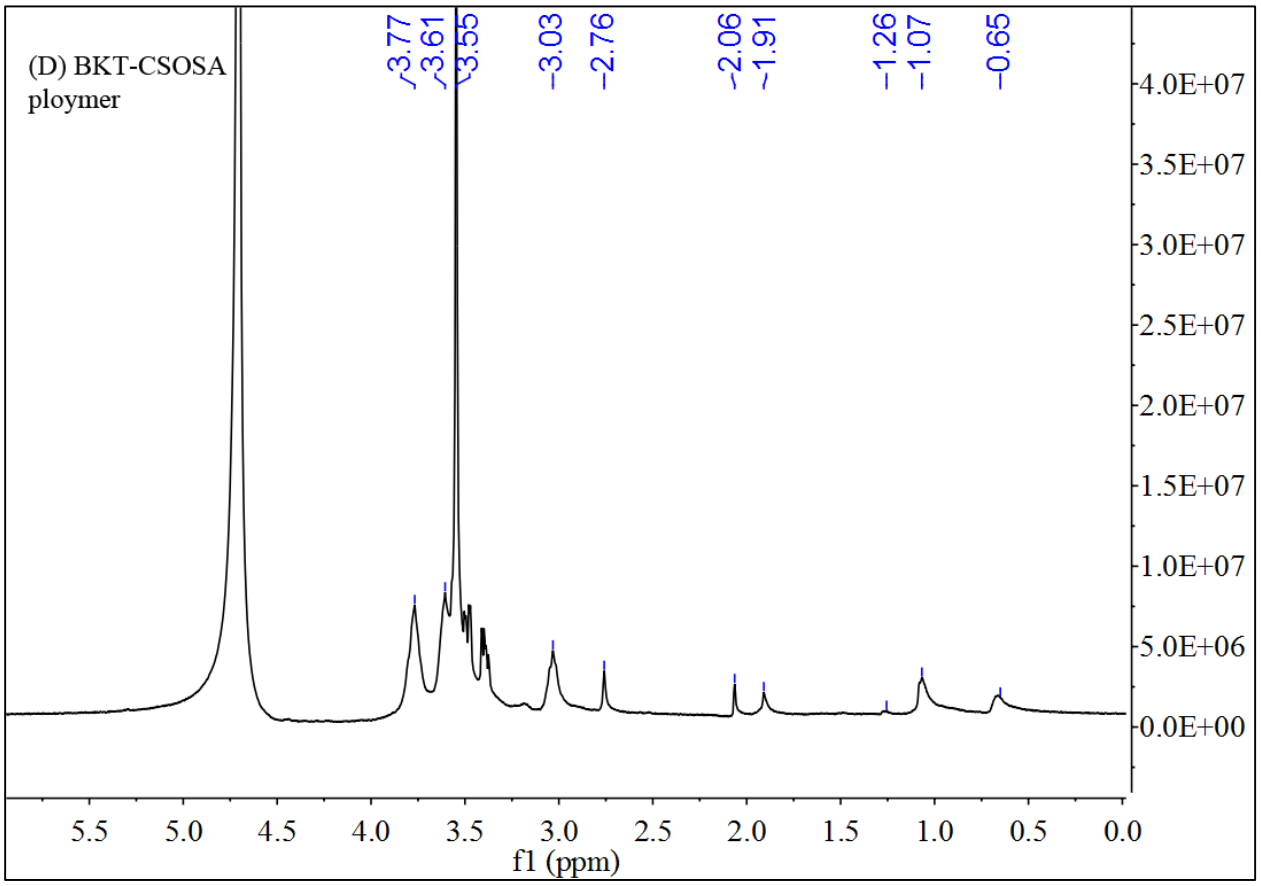


Figure S3. IR data of (A)BKT-140 peptide, (B)NH_2-PEG_{2000}-NH_2, (C) BKT-140 conjugated PEG_{2000} (BKT-PEG_{2000}-NH_2) and (D) BKT-140 peptide mixed with NH_2-PEG_{2000}-NH_2.


(A) BKT-140 peptide
3.90
2.49
2.36
1.95
1.37
1.10E+08
1.00E+08
9.00E+07
8.00E+07
7.00E+07
6.00E+07
5.00E+07
4.00E+07
3.00E+07
2.00E+07
1.00E+07
0.00E+00
-1.00E+07
5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


(B) NH2-PEG2000-NH2
3.50
2.49
2.29
1.22
4.0E+07
3.5E+07
3.0E+07
2.5E+07
2.0E+07
1.5E+07
1.0E+07
5.0E+06
0.0E+00
5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


(C) CSOSA polymer
3.77
3.59
3.00
2.78
2.09
1.93
1.08
2.6E+07
2.4E+07
2.2E+07
2.0E+07
1.8E+07
1.6E+07
1.4E+07
1.2E+07
1.0E+07
8.0E+06
6.0E+06
4.0E+06
2.0E+06
0.0E+00
-2.0E+06
5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


(D) BKT-CSOSA ploymer
3.77
3.61
3.55
3.03
2.76
2.06
1.91
1.26
1.07
0.65
4.0E+07
3.5E+07
3.0E+07
2.5E+07
2.0E+07
1.5E+07
1.0E+07
5.0E+06
0.0E+00
5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

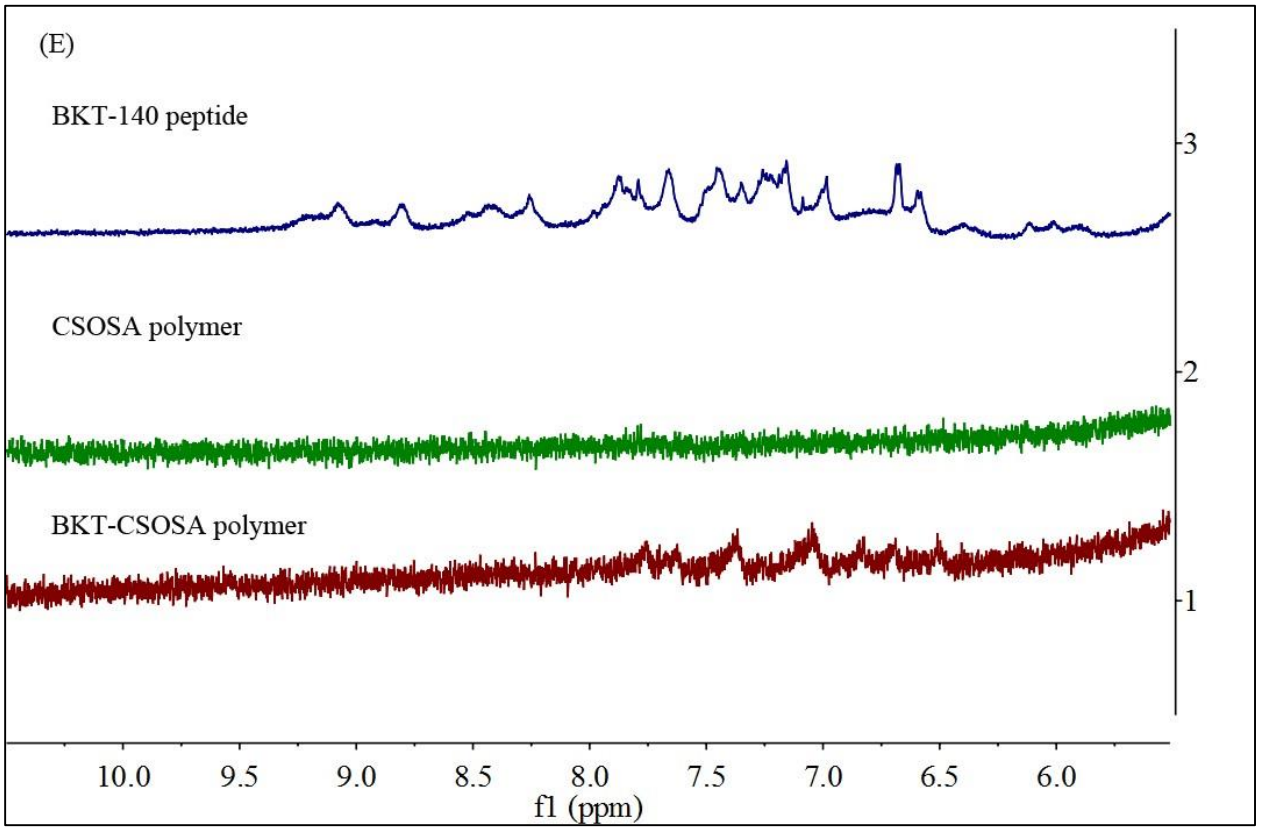


Figure S4. ^{1}H NMR spectrum of (A) BKT-140 peptide, (B) NH_2-PEG_{2000}-NH_2, (C) CSOSA polymer and (D) BKT-CSOSA. (E) shows the magnification of waves between 9.5-6.0 ppm. The chemical structures of BKT-140 peptide, NH_2-PEG_{2000}-NH_2, CSOSA polymer and BKT-CSOSA polymer were measured by ^{1}H NMR (Figure S4 (A) (B) (C) (D)). The peaks at about 1.94 ppm, 2.10 ppm and 3.76 ppm of BKT-CSOSA were attributed to -CH_3, -NH_2 and -COOH of CSOSA respectively. And the peaks at about 3.58 ppm of BKT-CSOSA were attributed to -CH_2CH_2O- of NH_2-PEG_{2000}-NH_2. The tiny but indelible waves near peaks at 6.68 ppm, 6.99 ppm and 7.78 ppm of BKT-CSOSA polymer (Figure S4 (E)) belong to BKT-140 peptide. These results indicated that BKT-140 peptide was conjugated to CSOSA.