Experimental supporting information

Preparation of peptide derivatives: The peptide derivative Chlorambucil-$G^DF^DFp^DY$ and Chlorambucil-$G^DF^DF^DY$ were prepared by solid phase peptide synthesis (SPPS) using 2-chlorotrityl chloride resin and the corresponding N-Fmoc protected amino acids with side chains properly protected by a tert-butyl group or t-butyl oxy carbonylgroup. The first amino acid (Fmoc-D-Tyr(OtBu)-OH or Fmoc-D-Tyr(H_2PO_3)-OH) were loaded on the resin at the C-terminal with the loading efficiency about 1.0 mmol/g. 20 % piperidine in anhydrous N, N'-dimethylformamide (DMF) was used during deprotection of Fmoc group. Then the next Fmoc-protected amino acid was coupled to the free amino group using O-(Benzotriazol-1-yl)-N, N, N', N'-tetramethyluroniumhexafluorophosphate (HBTU) as the coupling reagent. The growth of the peptide chain was according to the established Fmoc SPPS protocol. At the final step, Chlorambucil was used to couple with the peptide. After the last coupling step, excessive reagents were removed by a single DMF wash for 5 times (about 5 ml per gram of resin), followed by five steps of washing using DCM for 5 times (about 5 ml per gram of resin). The peptide derivatives were cleaved using 95% of trifluoroacetic acid (TFA) with 2.5 % of trimethylsilane (TIS) and 2.5 % of H_2O for 30 minutes. 20 mL per gram of resin of ice-cold diethylether was then added to cleavage reagent. Afterward the supernatant was decanted and the resulting crude product was purified by high performance liquid chromatography and lyophilized.

Characterization of Chloroambucil-$G^DF^DF^DY$: ^{1}H NMR (400 MHz, DMSO) δ 8.19 (dd, J = 15.0, 8.0 Hz, 2H), 8.01 – 7.87 (m, 2H), 7.23 (d, J = 4.3 Hz, 3H), 7.20 – 7.11 (m, 5H), 7.02 (t, J = 7.7 Hz, 3H), 6.65 (dd, J = 8.2, 5.9 Hz, 3H), 4.58 – 4.42 (m, 2H), 4.37 (dd, J = 13.5, 7.7 Hz, 1H), 3.81 – 3.58 (m, 8H), 3.52 (dd, J = 16.5, 5.5 Hz, 1H), 3.38 (q, J = 7.0 Hz, 1H), 3.09 – 2.87 (m, 3H), 2.80 (ddd, J = 19.3, 14.0, 8.9 Hz, 2H), 2.67 (dd, J = 13.7, 9.6 Hz, 1H), 2.42 (t, J = 7.5 Hz, 2H), 2.07 (d, J = 7.5 Hz, 2H), 1.77 – 1.63 (m, 2H). MS: calc. M^+ =818.30, $(M+H)^+$ =819.77.

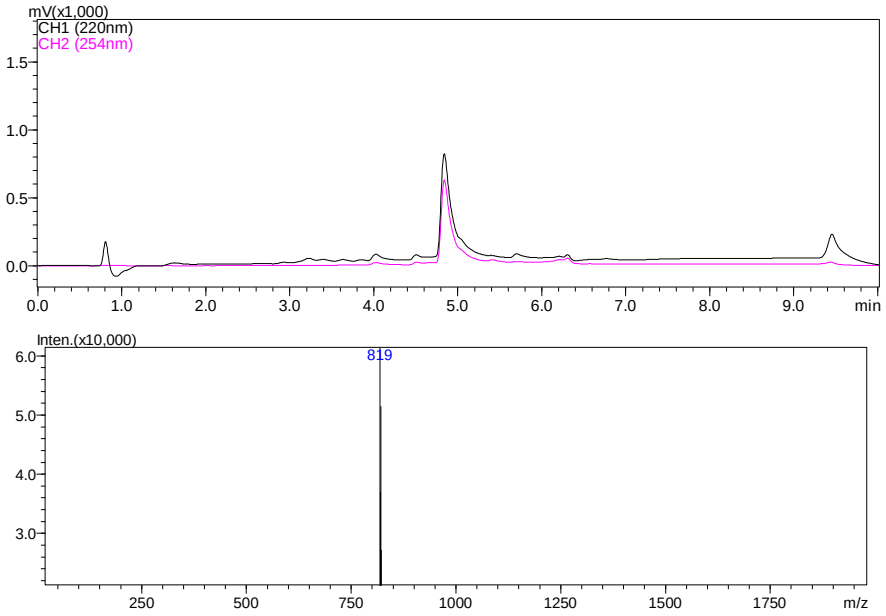

Figure S1. LC-MS spectrum of Chlorambucil-G^{D}F^{D}F^{D}Y

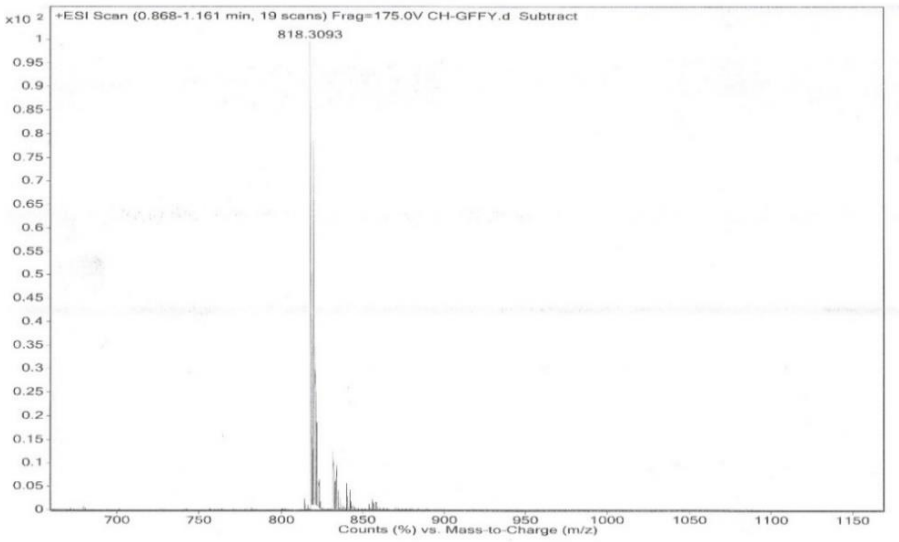

Figure S2. HR-MS of Chlorambucil-G^{D}F^{D}F^{D}Y

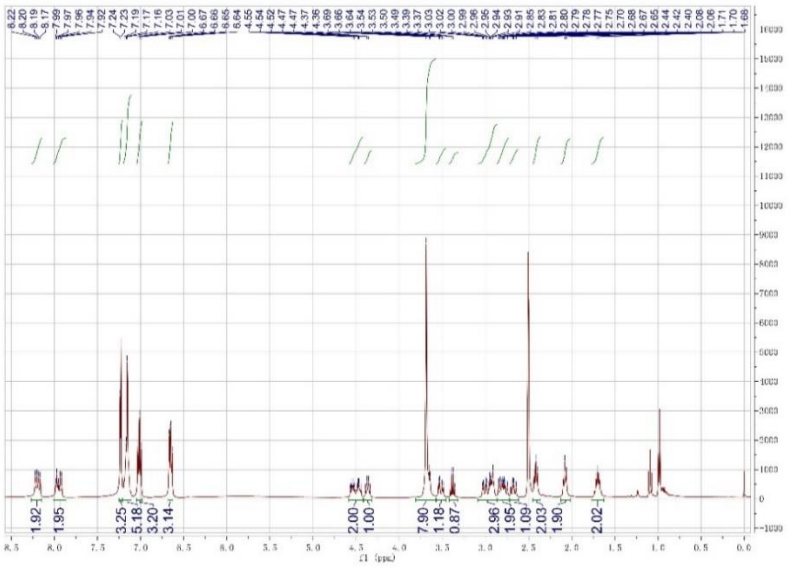

Figure S3. ^{1}H NMR of Chlorambucil-G^{D}F^{D}F^{D}Y

Characterization of Chloroambucil-G^{D}F^{D}F^{D}p^{D}Y: ^{1}H NMR (400 MHz, DMSO) δ 8.32 (d, J = 7.5 Hz, 1H), 8.22 (d, J = 8.1 Hz, 1H), 7.97 (d, J = 15.2 Hz, 2H), 7.29 – 7.16 (m, 9H), 7.04 (dd, J = 27.5, 8.1 Hz, 3H), 6.65 (d, J = 8.3 Hz, 2H), 4.55 (d, J = 8.9 Hz, 1H), 4.50 – 4.36 (m, 2H), 3.67 (d, J = 12.4 Hz, 5H), 3.53 (dd, J = 19.7, 12.5 Hz, 2H), 3.47 – 3.38 (m, 1H), 3.03 (d, J = 11.3 Hz, 2H), 2.97 – 2.88 (m, 2H), 2.84 – 2.63 (m, 3H), 2.54 (s, 1H), 2.46 – 2.35 (m, 2H), 2.08 (t, J = 7.3 Hz, 2H), 1.77 – 1.64 (m, 2H). M+ =897.27, obsvd. $(M+H)^{+}$ =898.2741, $(M+Na)^{+}$ =920.2544.

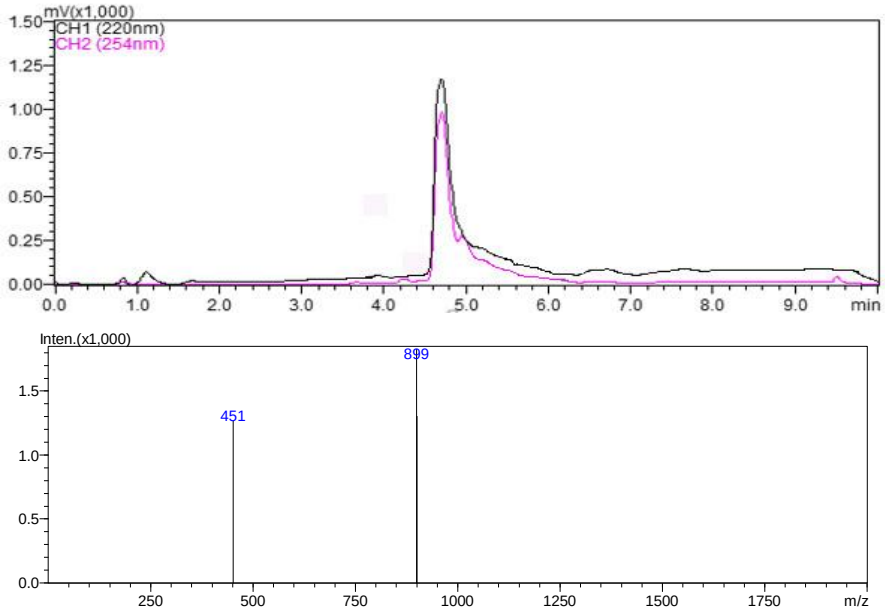

Figure S4. LC-MS spectrum of Chlorambucil-G^{D}F^{D}Fp^{D}Y

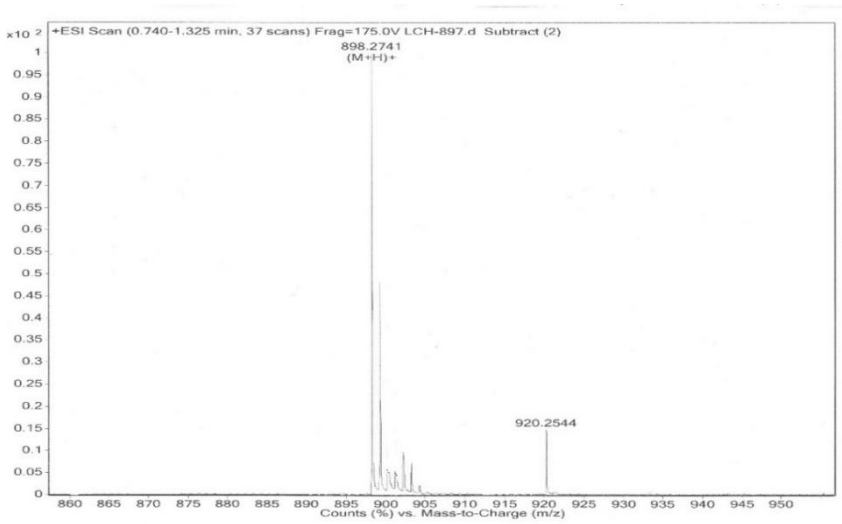

Figure S5. HR-MS of Chlorambucil-G^{D}F^{D}Fp^{D}Y

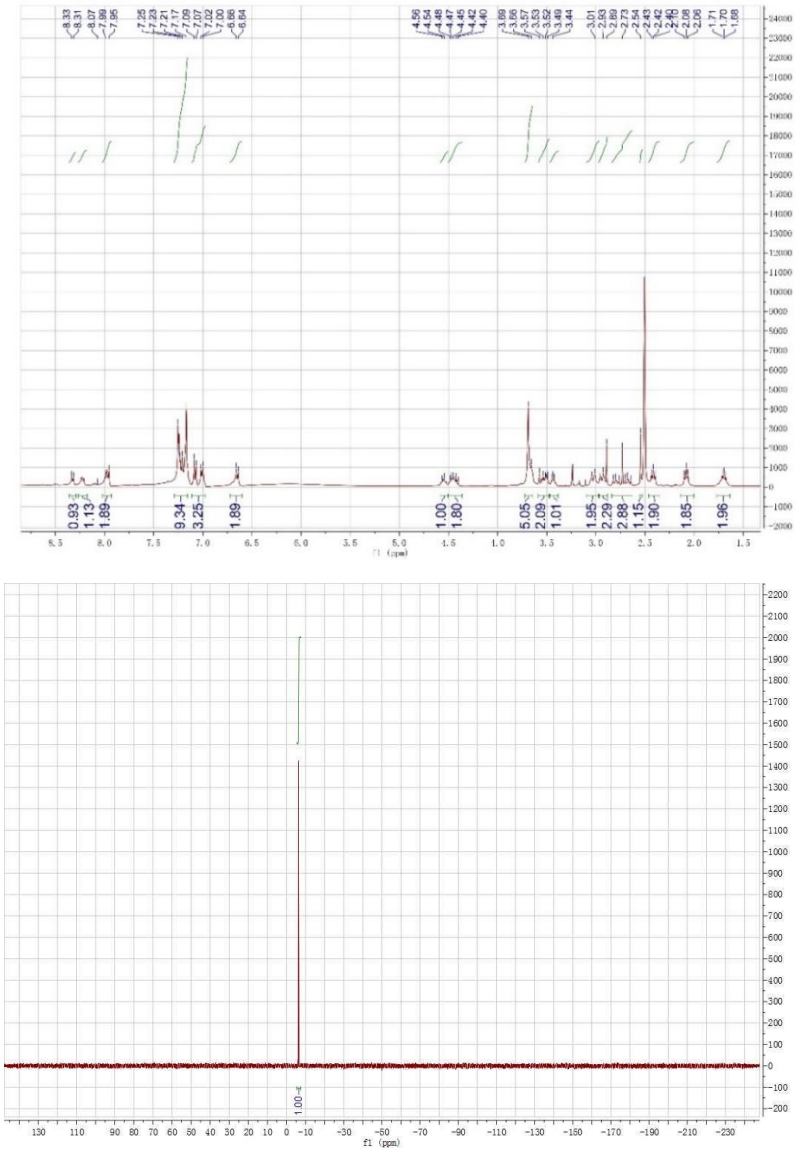

Figure S6. ^{1}H NMR and ^{31}P NMR of Chlorambucil-G^{D}F^{D}Fp^{D}Y

TEM of transition process of Chlorambucil-G^{D}F^{D}F^{D}Y in PBS:

1 mg of Chlorambucil-G^{D}F^{D}F^{D}Y was dissolved in 1 mL of PBS and stayed at room temperature for over 12 h. We chose three-time points to observe the transition process of diameter. We found that the diameter became bigger and bigger as the solution gradually became a precipitate. As is shown in Figure S7, 1 min after dissolving, the diameter of Chlorambucil-G^{D}F^{D}F^{D}Y is about 30-100 nm. 2 hours later, the diameter grew up to 200-300 nm. 10 hours later, the diameter reached over 500 nm and exhibited obvious hollow structure of vesicle. These observations indicated that this is a dynamic and continuous process in which nanoparticles gather into vesicles.

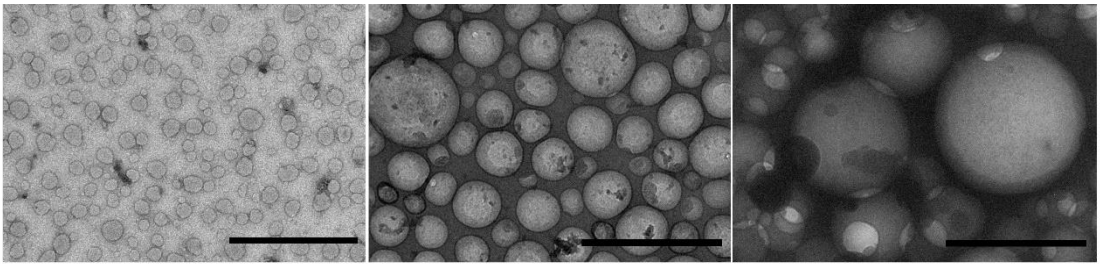

Figure S7. TEM images of transition process of CRB-G^{D}F^{D}F^{D}Y in PBS at room temperature (left: 1 min after dissolving, middle: 2 hours after dissolving, right: 10 hours after dissolving. All scale bars represent 500 nm).

Figure S8. Optical image of a precipitate of CRB-G^{D}F^{D}F^{D}Y in PBS at 10-hour time point

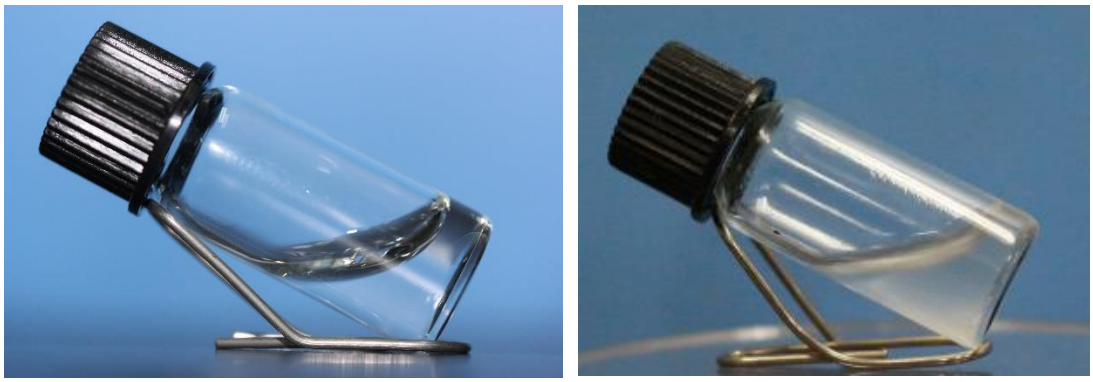

Figure S9. Optical image of the transparent solution when heating the hydrogel of CRB-G^{D}F^{D}F^{D}Y formed by EISA at 4 ℃ and the suspension after the solution cooling to room temperature.

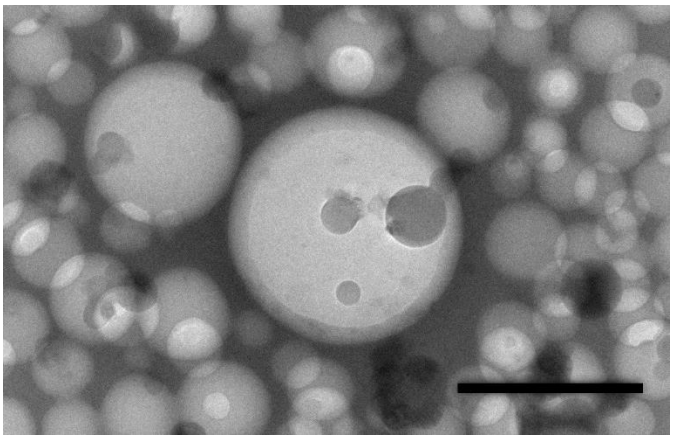

Figure S10. TEM image of the suspension of CRB-G^{D}F^{D}F^{D}Y produced by applying the heating-cooling cycle to the hydrogel of CRB-G^{D}F^{D}F^{D}Y formed by EISA at 4 ℃ (The scale bar represents 500 nm).

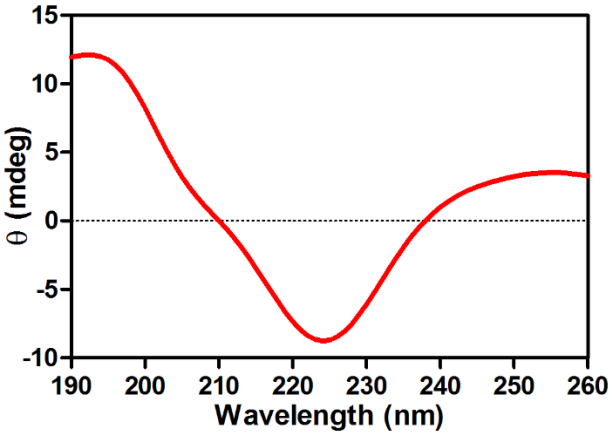

Figure S11. CD spectrum showed a β-sheet conformation of peptide in the suspension in Figure S9.

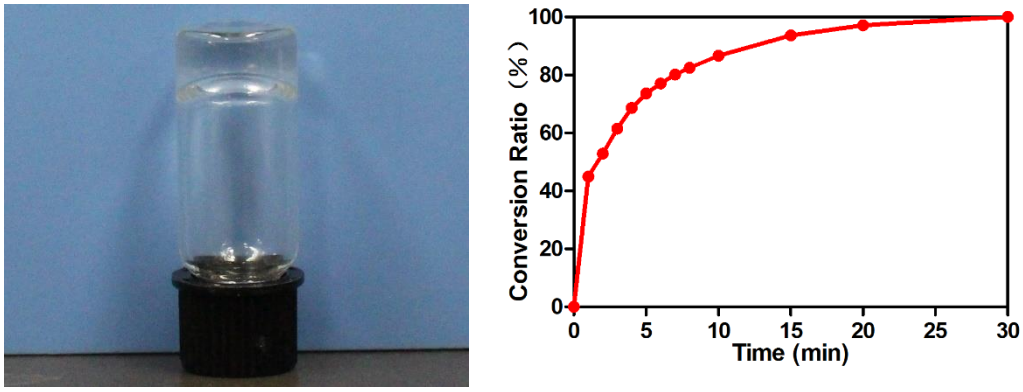

Figure S12. Optical image of the hydrogel of CRB-G^{D}F^{D}F^{D}Y and the conversion ratio of CRB-G^{D}F^{D}Fp^{D}Y by ALP at 37 ℃.

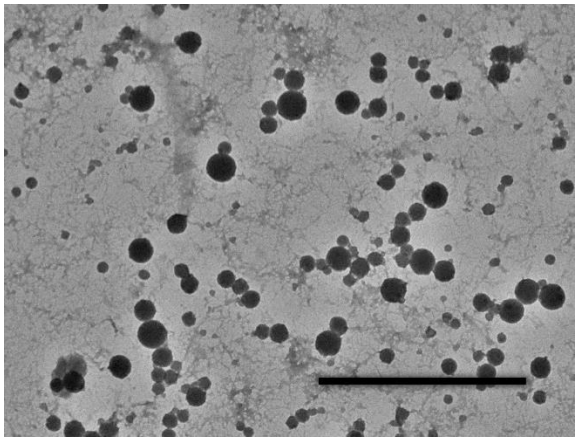

Figure S13. TEM image of the hydrogel of CRB-G^{D}F^{D}F^{D}Y formed by EISA at 37 ℃ (The scale bar represents 2.0 μm).

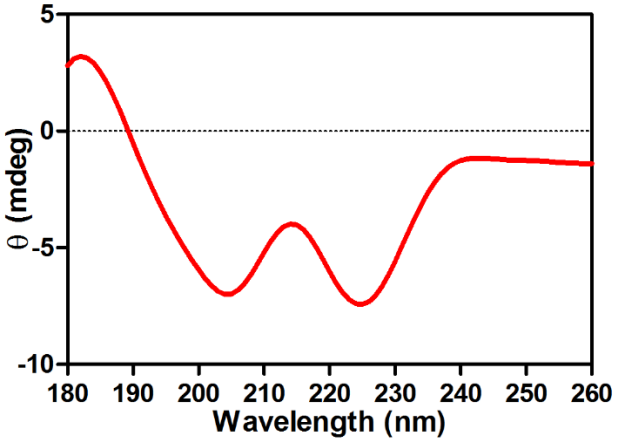

Figure S14. CD spectra of the hydrogel of CRB-G^{D}F^{D}F^{D}Y formed by EISA at 37 ℃.

Cell culture and Cellular uptake:

4T1, HepG2, MCF-7, A549 and HeLa cells were purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China), 4T1 cells were maintained in our lab. All kinds of cells were cultured in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% v/v fetal bovine serum (FBS), 100 U/mL penicillin and 100 g/mL streptomycin. All kinds of cells were cultured at 37 °C in a humidified atmosphere of 5% CO_2.

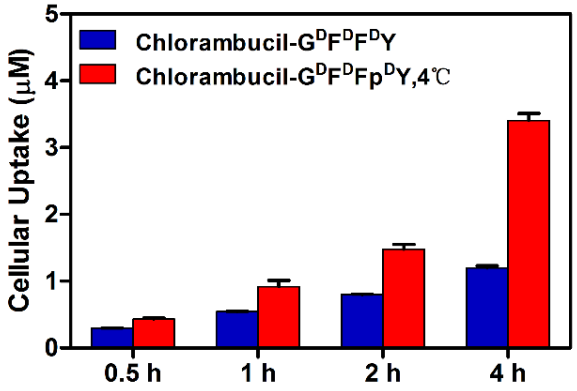

Figure S15. Cellular uptake by HeLa

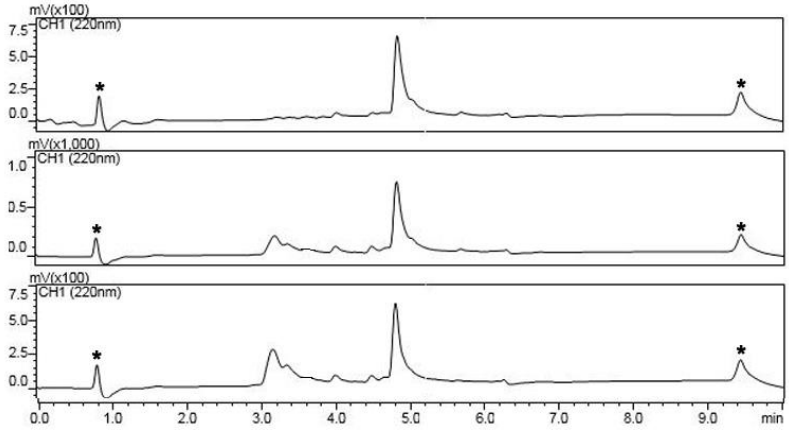

Figure S16. LC-MS traces of Chlorambucil-G^{D}F^{D}Fp^{D}Y (catalyzed by ALP at 4 ℃) digested by proteinase K (top: no proteinase K, middle: 12 hour after added proteinase K, bottom: 24 hour after added proteinase K). The stars represent systemic peaks.

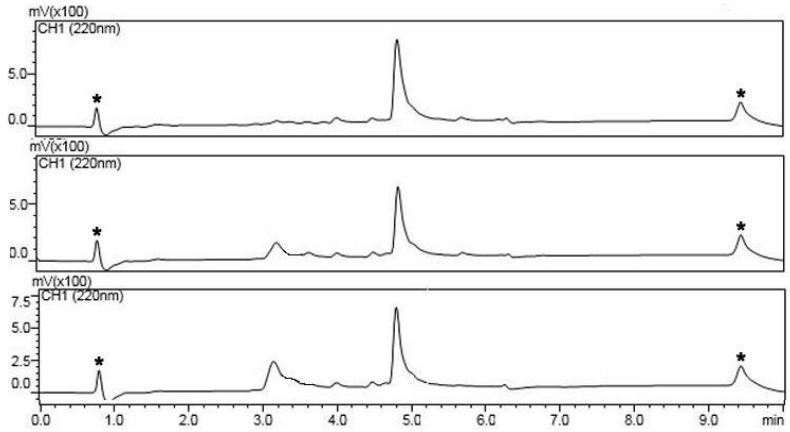

Figure S17. LC-MS traces of Chlorambucil-G^{D}F^{D}F^{D}Y digested by proteinase K (top: no proteinase K, middle: 12 hours after added proteinase K, bottom: 24 hours after added proteinase K). The stars represent systemic peaks.

Group	Drug	Dose
1	PBS	100 μL per mouse
2	Chlorambucil	1.5 mg/kg,100 μL per mouse
3	Chlorambucil-G^{D}F^{D}F^{D}Y	4 mg/kg,100 μL per mouse
4	Chlorambucil-G^{D}F^{D}Fp^{D}Y (ALP, 4 ℃)	4.4 mg/kg, 100 μL per mouse

Table S1. Drug formulations and doses for evaluation of tumor inhibition.

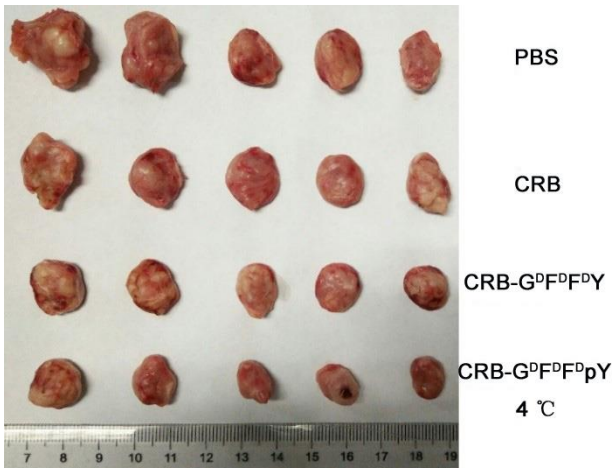

Figure S18. Ex vivo images of tumors extracted from 4T1 tumor-bearing BALB/C mice at day 23 after being i.v. injected with different drugs as dosage of Table S1.

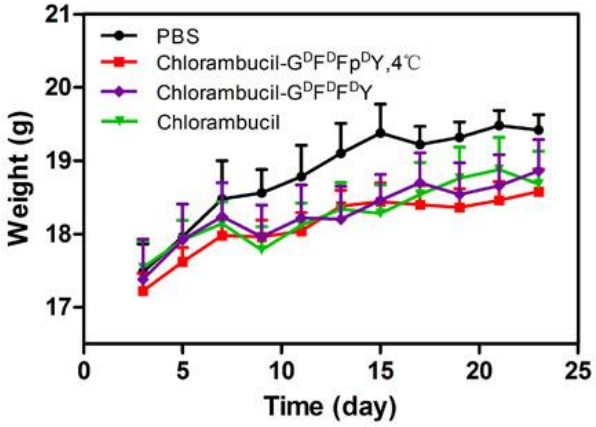

Figure S19. In vivo toxicity performance of different drugs compared with Normal Saline.

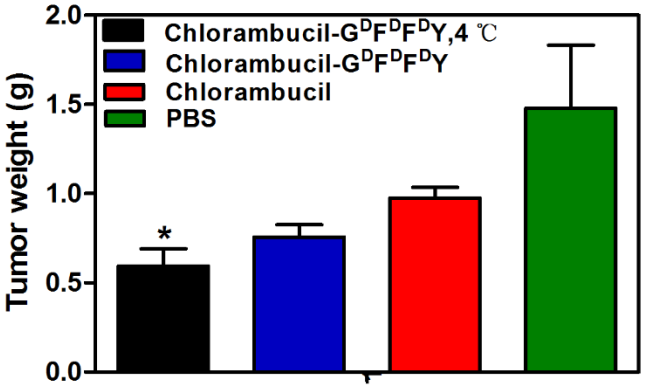

Figure S20. Weight of tumors extracted from 4T1 tumor-bearing BALB/C mice at day 22 after being i.v. injected with different drugs as dosage of Table S1. The data are shown as mean ± SEM (n = 5), * represents $P < 0.05$.

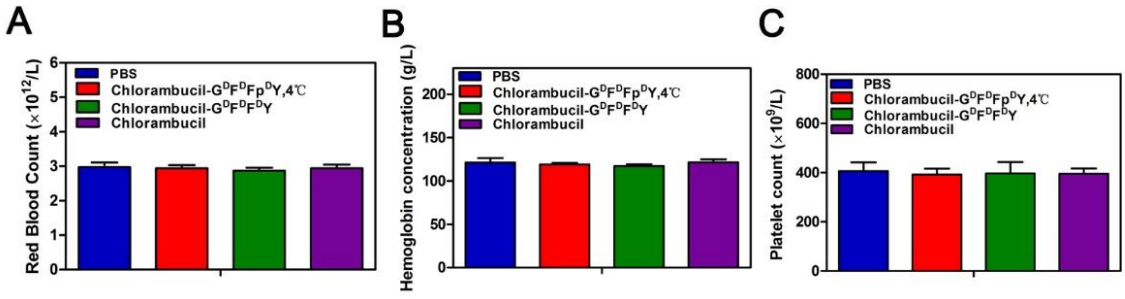

Figure S21. Index for Hematological examination. The data are shown as mean ± SEM (n = 6).

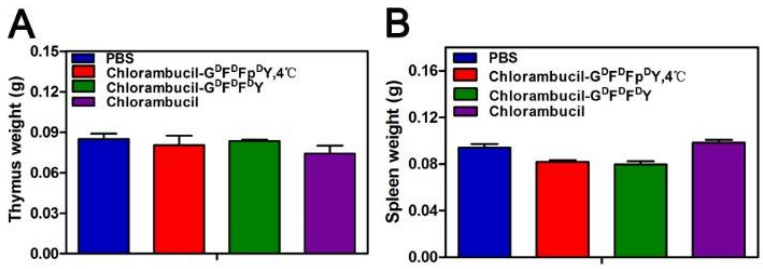

Figure S22. Weight of thymus and spleen. The data are shown as mean ± SEM (n = 6).

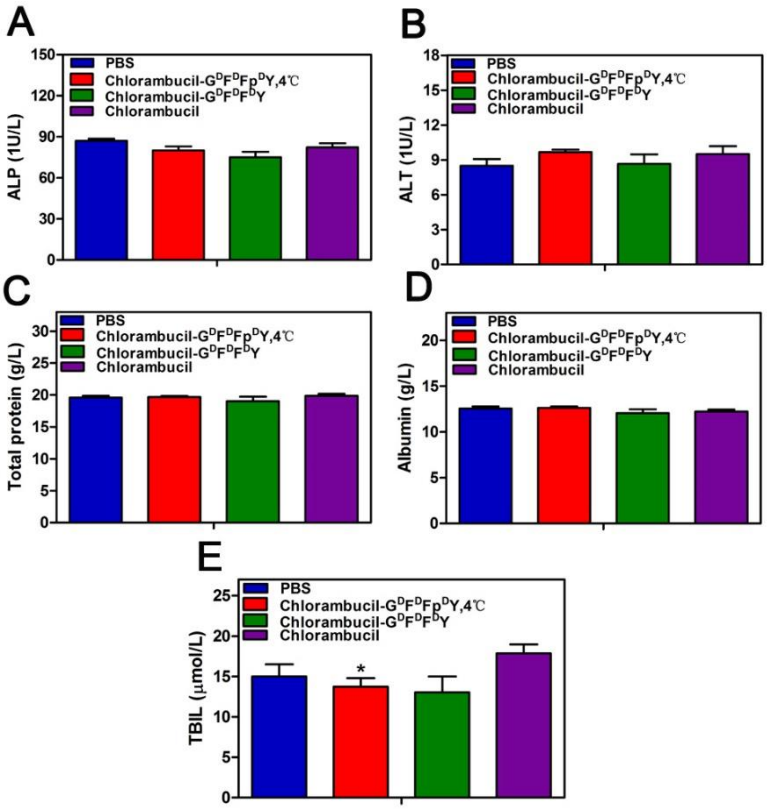

Figure S23. Index for liver function. The data are shown as mean ± SEM (n = 6). * represents $P < 0.05$.

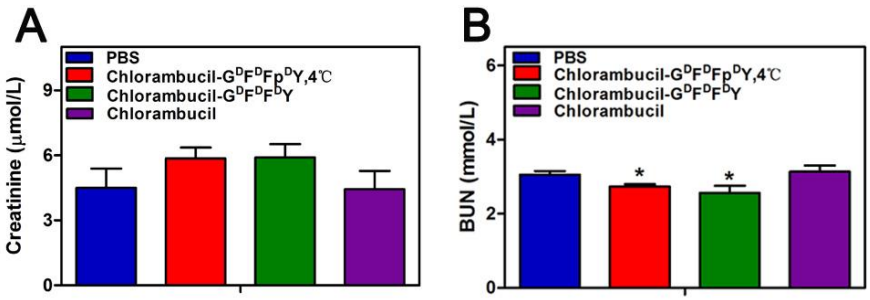

Figure S24. Index for kidneys function. The data are shown as mean ± SEM (n = 6). * represents $P < 0.05$.