Supplementary Information

Polymer-Engineered PROTAC Nanovehicles Amplify Synergistic Effects with Temozolomide by BRD4 Degradation

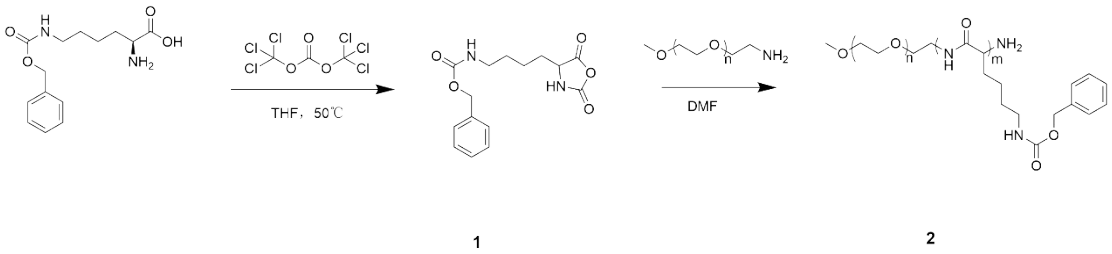

Figure S1. Synthetic route of polymer $mPEG_{45}$-pPhe-NH_2.

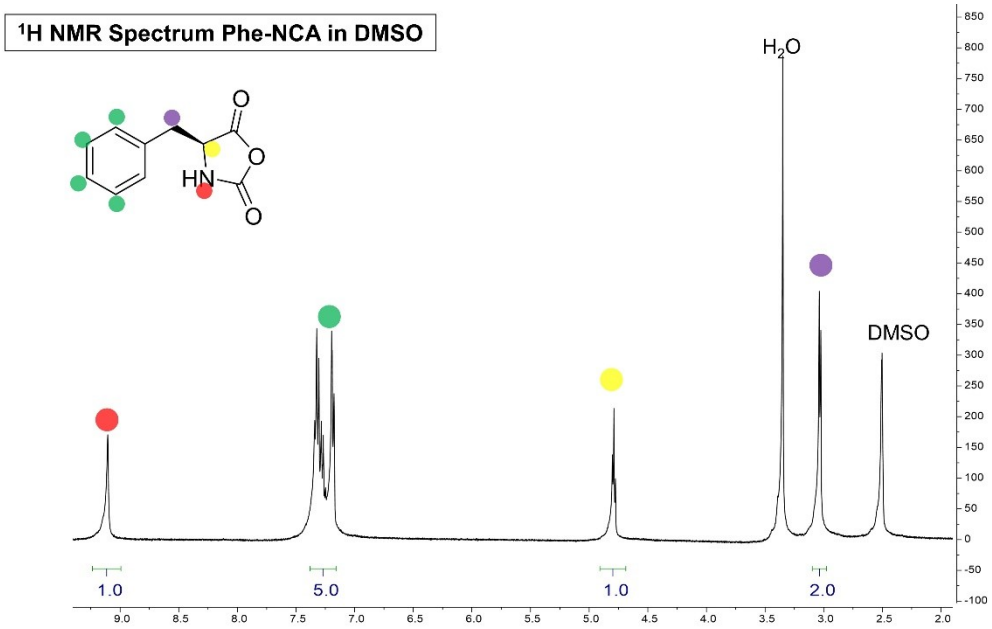

Figure S2. The ^1H NMR spectrum of compound 1 (Phe-NCA) in DMSO-d6.

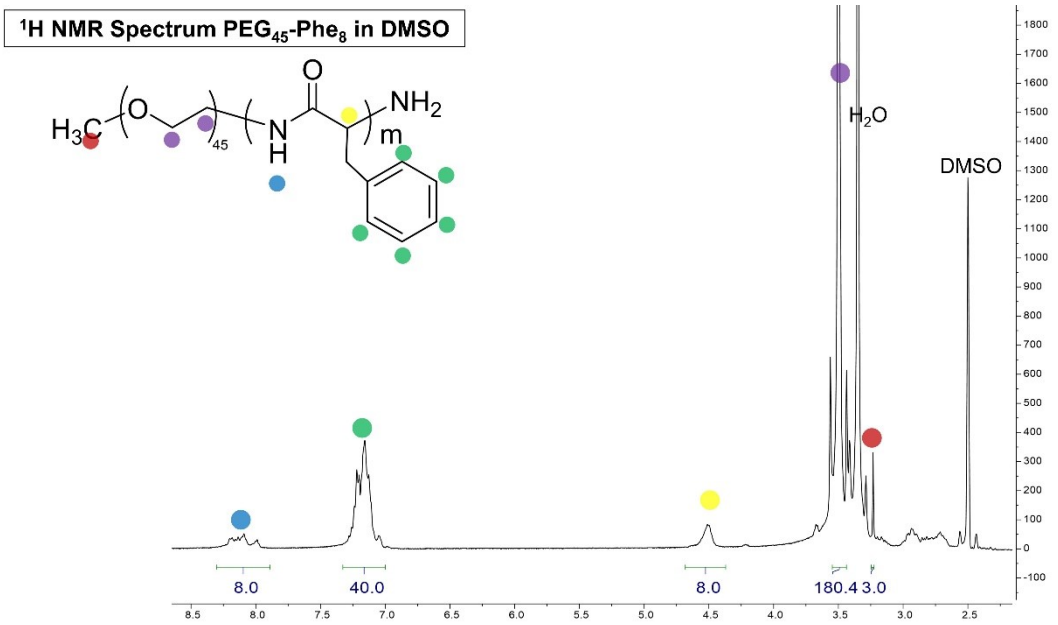

Figure S3. The ^{1}H NMR spectrum of mPEG$_{45}$-pPhe$_{8}$-NH$_{2}$ in DMSO-d6.

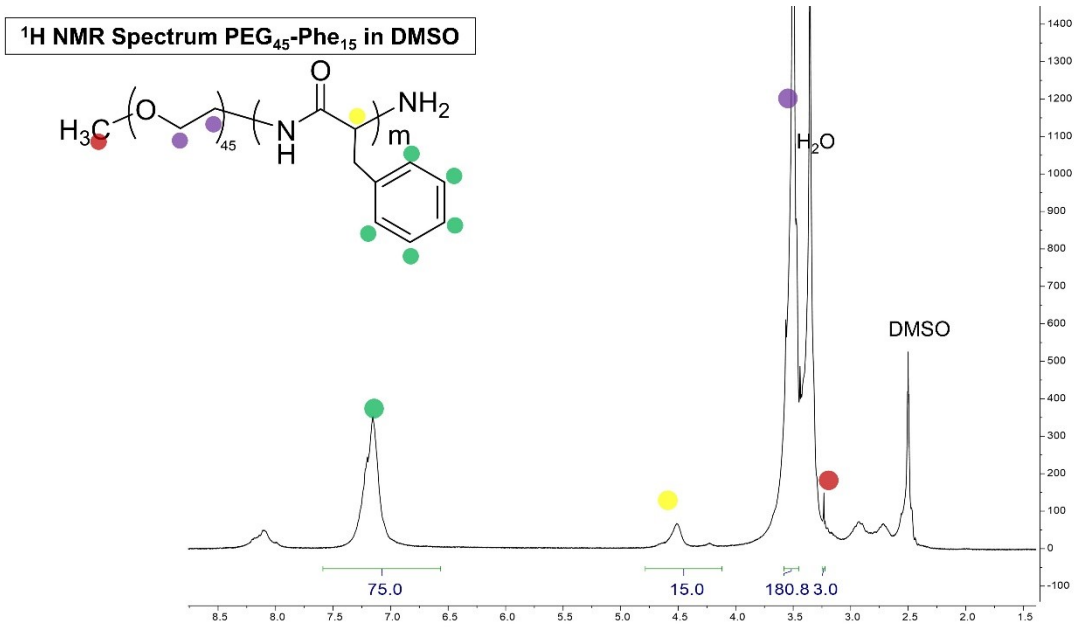

Figure S4. The ^{1}H NMR spectrum of mPEG$_{45}$-pPhe$_{15}$-NH$_{2}$ in DMSO-d6.

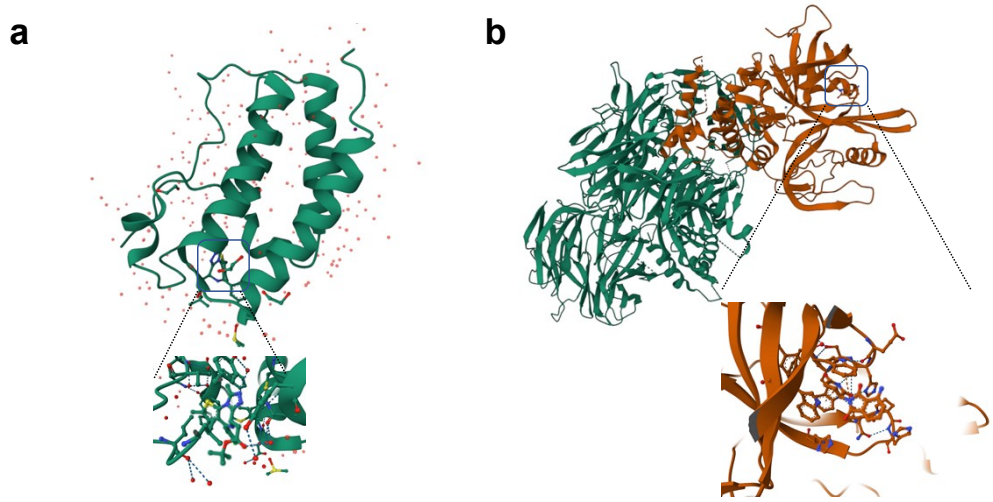

Figure S5. Design origins of on-demand designed cyclic peptides.

(a) Crystal Structure of the first bromodomain of human BRD4 in complex with the inhibitor JQ1. (RCSB. No. 3MXF)

(b) Structure of the DDB1-CRBN E3 ubiquitin ligase bound to Pomalidomide. (RCSB. No. 4CI3)

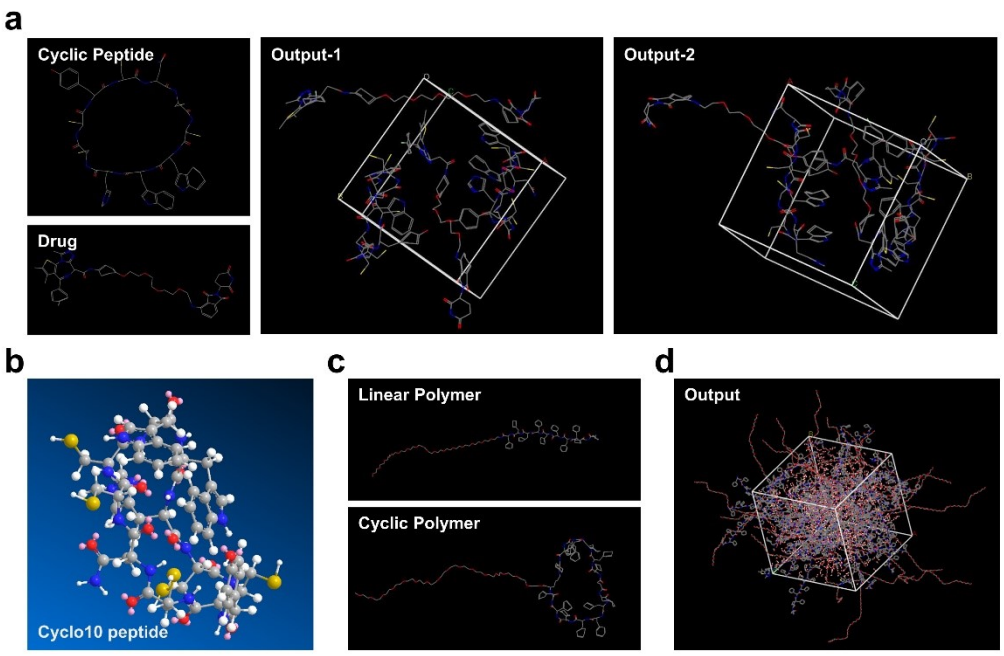

Figure S6. Encapsulation effect of computer-aided designed cyclic peptides on PROTAC.

(a) Schematic diagrams of the specific structures and output results of cyclic peptides and ARV-825 in simulations.

(b) Specific structure of the cyclic decapeptide specifically designed for ARV-825.

(c) Schematic diagrams of the structures of linear polymers and cyclic PEG-cyclic peptides.

(d) Output results of PEG-cyclic peptides and ARV-825 in simulation software.

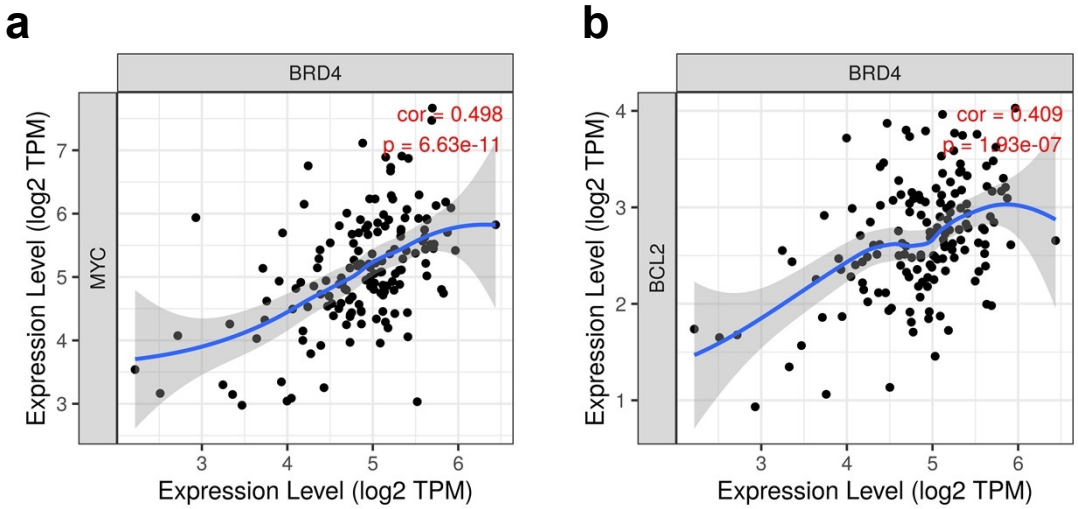

Figure S7. Gene correlation analysis in GBM (analyzed by TIMER: Tumor IMmune Estimation Resource).

(a) Correlation analysis between BRD4 and MYC.

(b) Correlation analysis between BRD4 and Bcl-2.

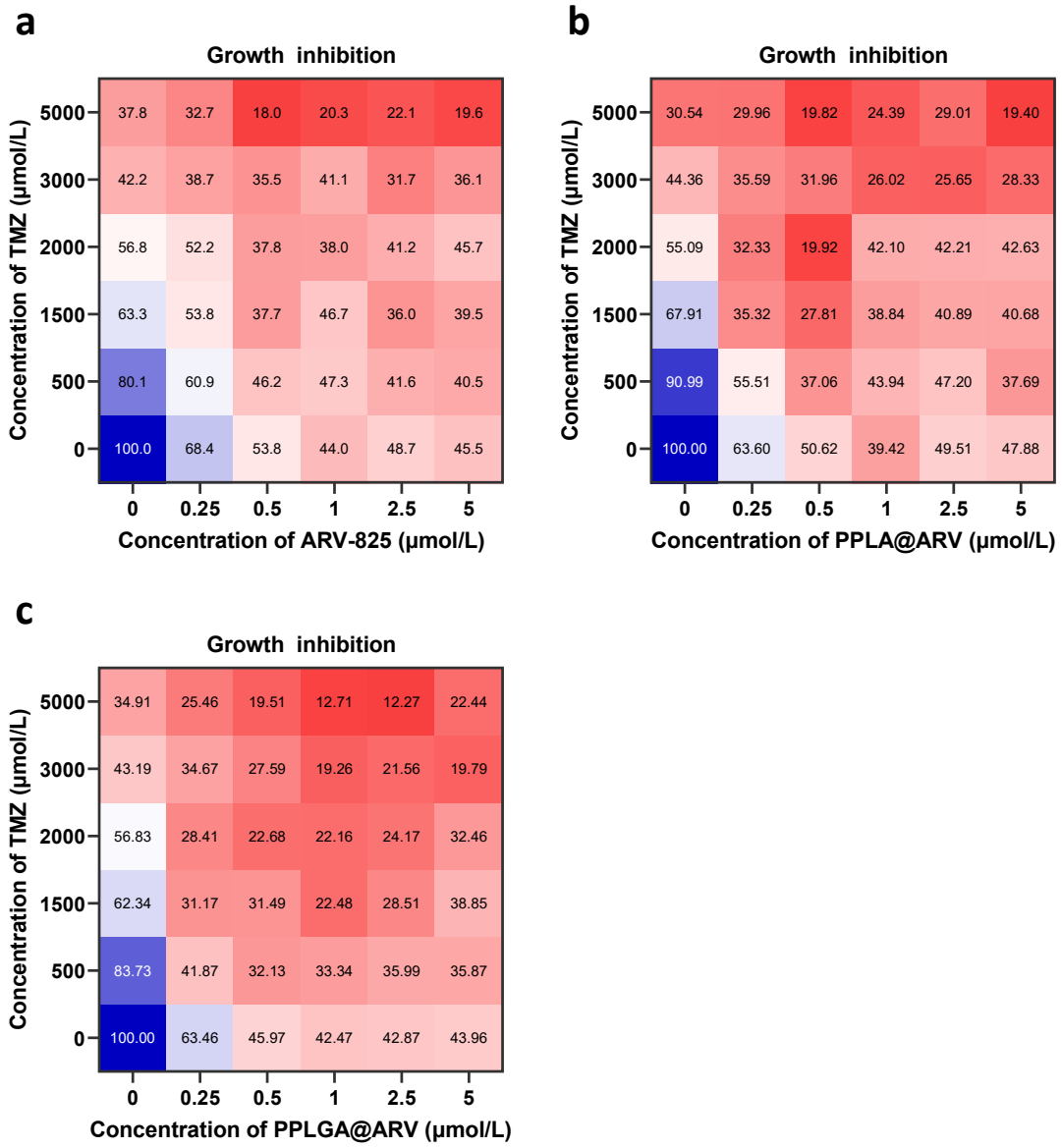

Figure S8. The specific cell viability data in Fig. 6d, Fig. 7c, and Fig. 7e.

(a) The cell viability data for TMZ in combination with ARV-825 across various concentration ratios in Fig. 6d.

(b) The cell viability data for TMZ in combination with PPLA@ARV across various concentration ratios in Fig. 7c.

(c) The cell viability data for TMZ in combination with PPLGA@ARV across various concentration ratios in Fig. 7e.

	Z-Average (nm)	PDI	Zeta Potential (mV)
PPLA@ARV	149.2±1.33	0.126±0.008	-13.68±0.024
PPLGA@ARV	118.7±1.03	0.202±0.013	-14.35±0.140

Table S1. Dynamic light scattering and zeta potential of PPLA@ARV and PPLGA@ARV. Statistics are presented as means ± SD (n=3).

1. Synthesis and characterization of polymers

1.1. Synthesis of compound 1, Phe-NCA

L-phenylalanine (3 g, 18.16 mmol, 2 equiv.) and triphosgene (2.7 g, 9.10 mmol, 1 equiv.) were dispersed in anhydrous THF (60 mL) and stirred under Ar at 50 ℃ for 3 hours. The resulting mixture was left undisturbed to permit a natural cooling process until it reached room temperature. Subsequently, the cooled mixture was passed through a filter to separate any solid particles from the liquid. Following filtration, the liquid was slowly added drop by drop to 600 mL of precooled anhydrous n-hexane while being subjected to intense agitation using a high-speed stirrer to ensure proper mixing and reaction. After suction filtration, a white solid was obtained and dried overnight, resulting in a 64% yield.

Compound 1 (Phe-NCA), ^{1}H NMR (400 MHz, DMSO-d6): δ 9.11 (s, 1H), 7.10-7.44 (ddd, 5H), 4.27 (t, J = 5.2 Hz, 1H), 3.03 (d, J = 5.1 Hz, 2H).

1.2. Synthesis of mEG_{45}-$pPhe_8$-NH_2

$mPEG_{45}$-NH_2 (500 mg, 0.25 mmol, 1 equiv.) and compound 1 (475 mg, 0.49 mmol, 10 equiv.) were dissolved in anhydrous DMF (20 mL) and stirred at 50 ℃ under Ar for 48 hours. The solution was cooled to room temperature and dialyzed against pure water using a dialysis bag (MWCO: 3500) for 24 hours. The mEG_{45}-$pPhe_8$-NH_2 was obtained after the solution was freeze-dried, as a white powder.

Compound mEG_{45}-$pPhe_8$-NH_2, ^{1}H NMR (400 MHz, DMSO-d6): δ 8.01-8.25 (d, 8H),

7.01-7.30 (d, 40H), 4.5 (s, 8H), 3.42-3.63 (s, 180H), 3.24 (s, 3H).

1.3. Synthesis of mEG_{45}-$pPhe_{15}$-NH_2

$mPEG_{45}$-NH_2 (140 mg, 0.07 mmol, 1 equiv.) and compound 1 (258.2 mg, 1.4 mmol, 20 equiv.) were dissolved in anhydrous DMF (20 mL) and stirred at 50 ℃ under Ar for 48 hours. The solution was cooled to room temperature and dialyzed against pure water using a dialysis bag (MWCO: 3500) for 24 hours. The mEG_{45}-$pPhe_{15}$-NH_2 was obtained after the solution was freeze-dried, as a white powder.

Compound mEG_{45}-$pPhe_{15}$-NH_2, ^{1}H NMR (400 MHz, DMSO-d6): δ 7.01-7.30 (d, 75H), 4.5 (s, 15H), 3.42-3.63 (s, 180H), 3.24 (s, 3H).