Supporting Information

Folic Acid-functionalized Graphene Oxide Nanocarrier: Synthetic Approaches, Characterization, Drug Delivery Study and Anti-tumor Screening

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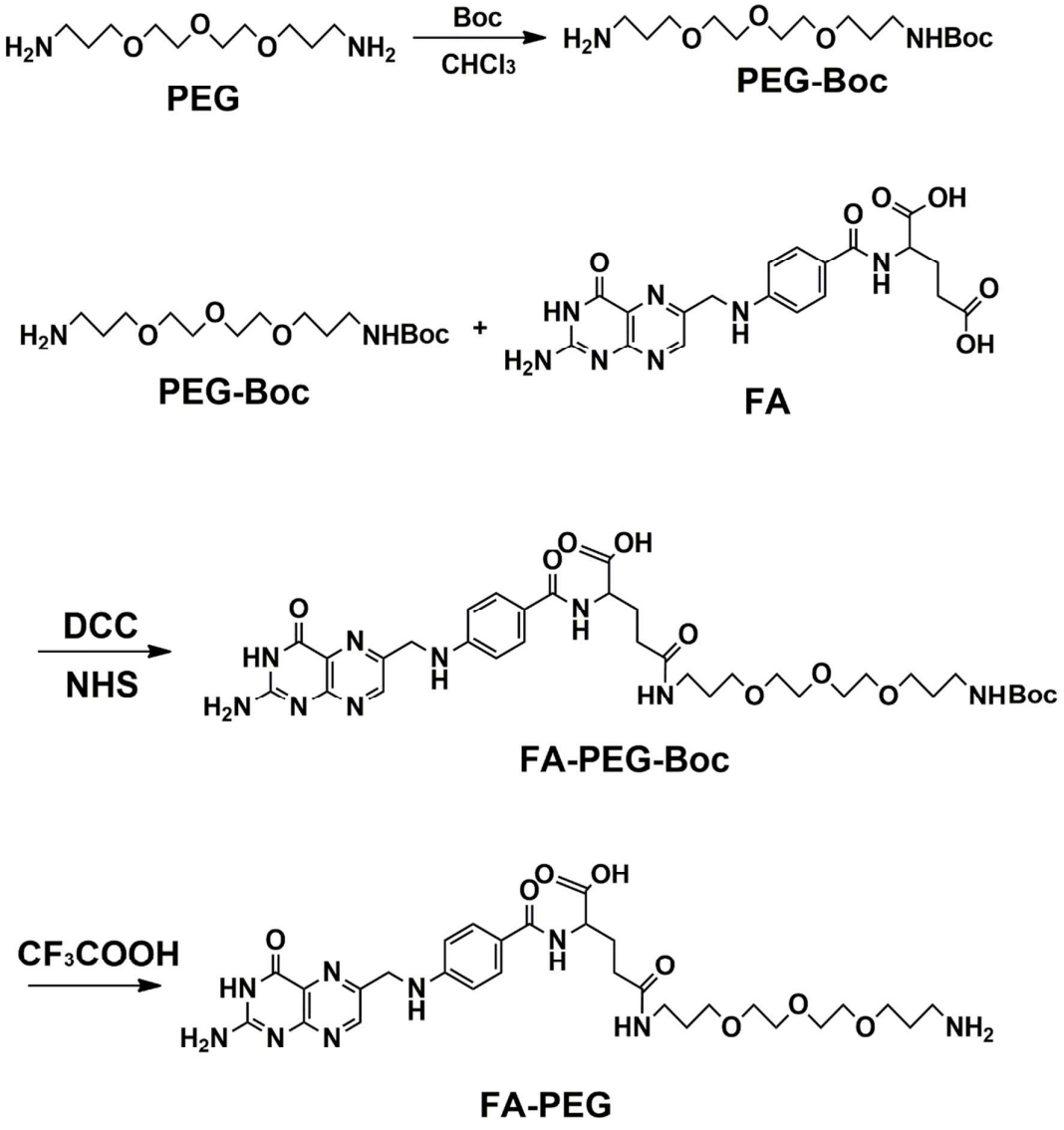


Figure S1. Reaction of the folic acid (FA) with PEG forming FA-PEG.

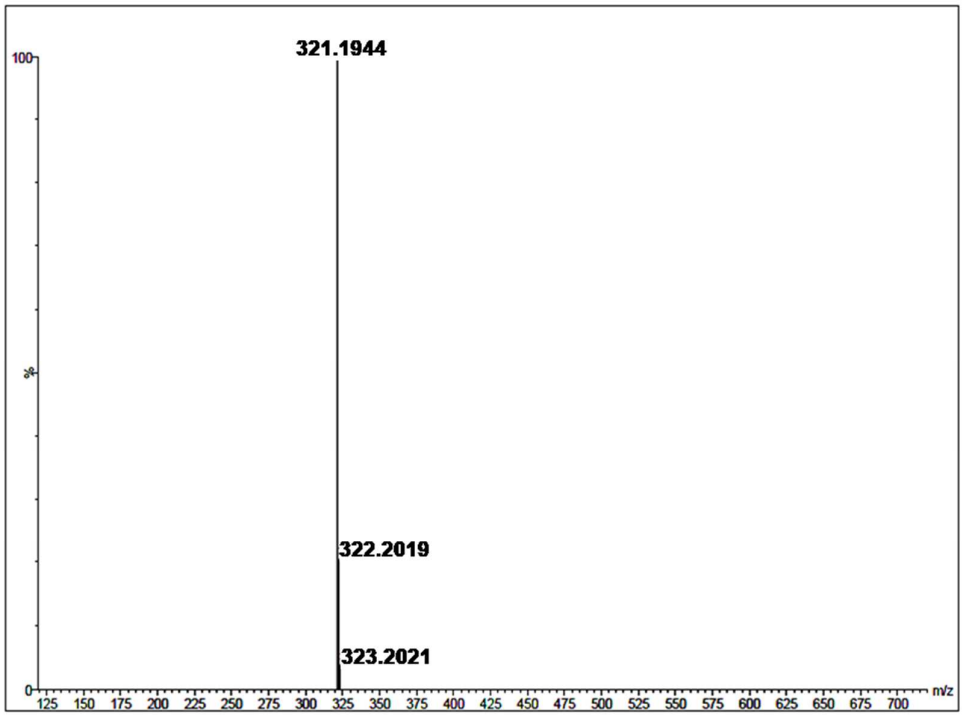
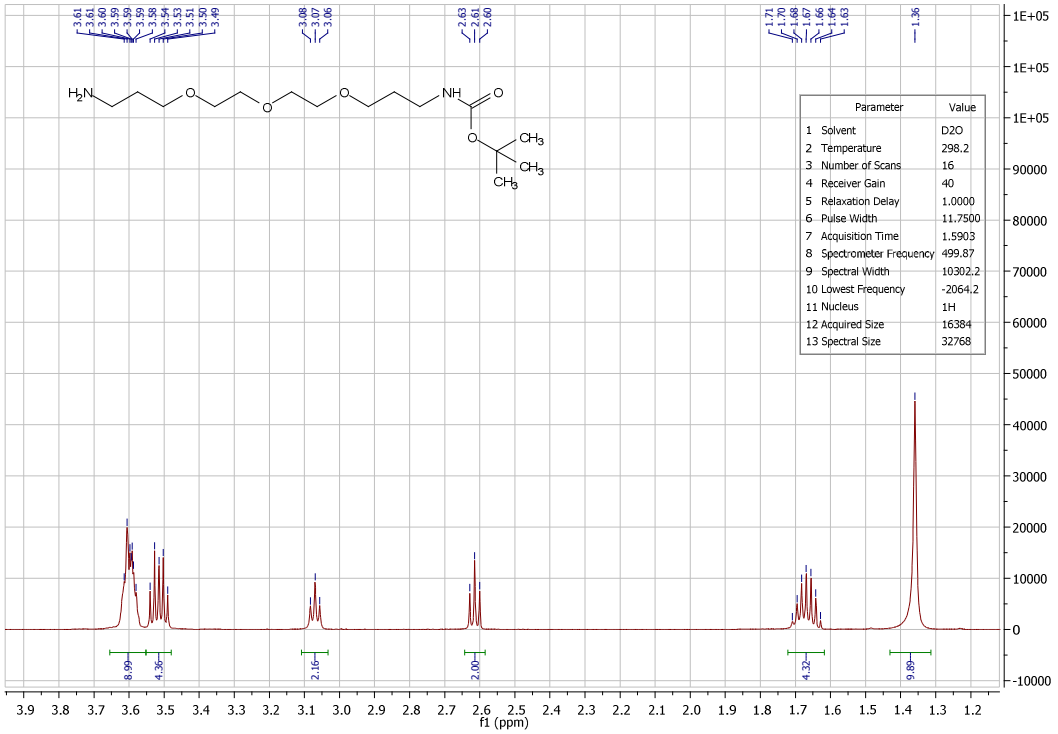


Figure S2. Positive-ion HRMS (m/z-ESI+) mass spectrum of the PEG-Boc ($C_{15}H_{32}N_2O_5$ [M] : 320.4250 g/mol). Calcd. for $C_{15}H_{33}N_2O_5$ [M+H] : 321.4329, found 321.1944.



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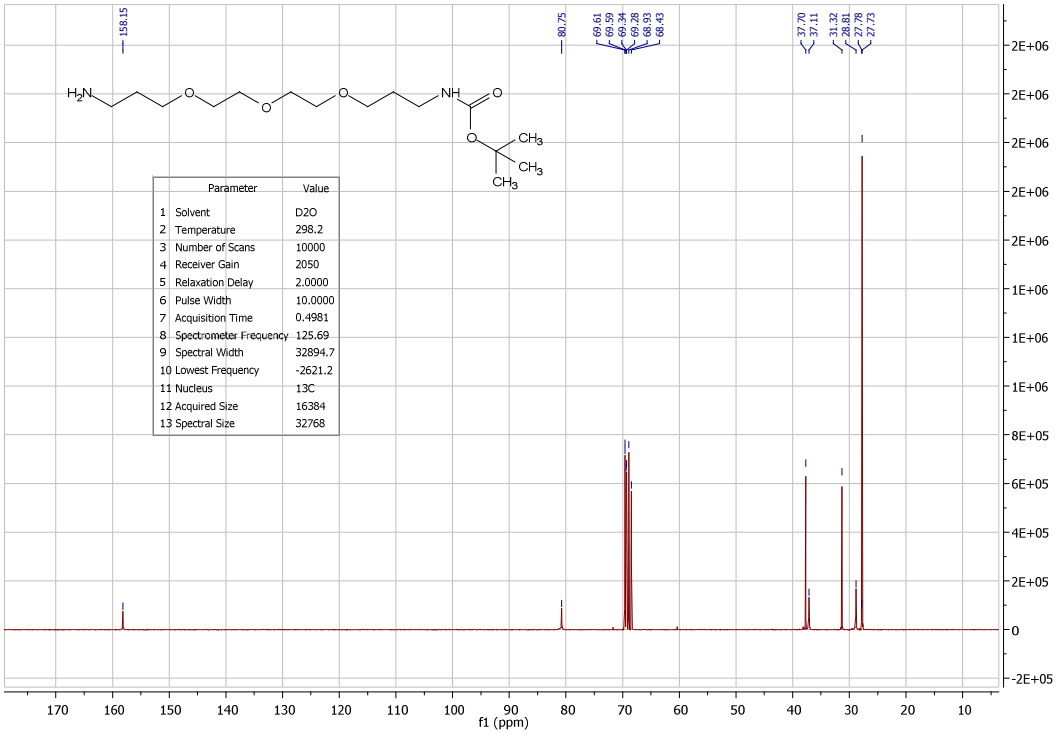
9	Spectral Width	10302.2
10	Lowest Frequency	-2064.2
11	Nucleus	1H
12	Acquired Size	16384
13	Spectral Size	32768

Figure S3. ^{1}H-NMR spectra of PEG-Boc. ^{1}H NMR (500 MHz, D_2O) δ 3.64 – 3.56 (m, 9H), 3.55 – 3.46 (m, 4H), 3.07 (t, J = 6.7 Hz, 2H), 2.61 (t, J = 7.1 Hz, 2H), 1.67 (dp, J = 13.6, 6.6 Hz, 4H), 1.36 (s, 9H).



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8	Spectrometer Frequency	125.69
9	Spectral Width	32894.7
10	Lowest Frequency	-2621.2
11	Nucleus	13C
12	Acquired Size	16384
13	Spectral Size	32768

Figure S4. ^{13}C-NMR spectra of PEG-Boc. ^{13}C NMR (126 MHz, D_2O) δ 158.15 (s), 80.75 (s), 69.60 (d, J = 1.7 Hz), 69.31 (d, J = 7.6 Hz), 68.93 (s), 68.43 (s), 37.70 (s), 37.11 (s), 31.32 (s), 28.81 (s), 27.76 (d, J = 6.5 Hz).

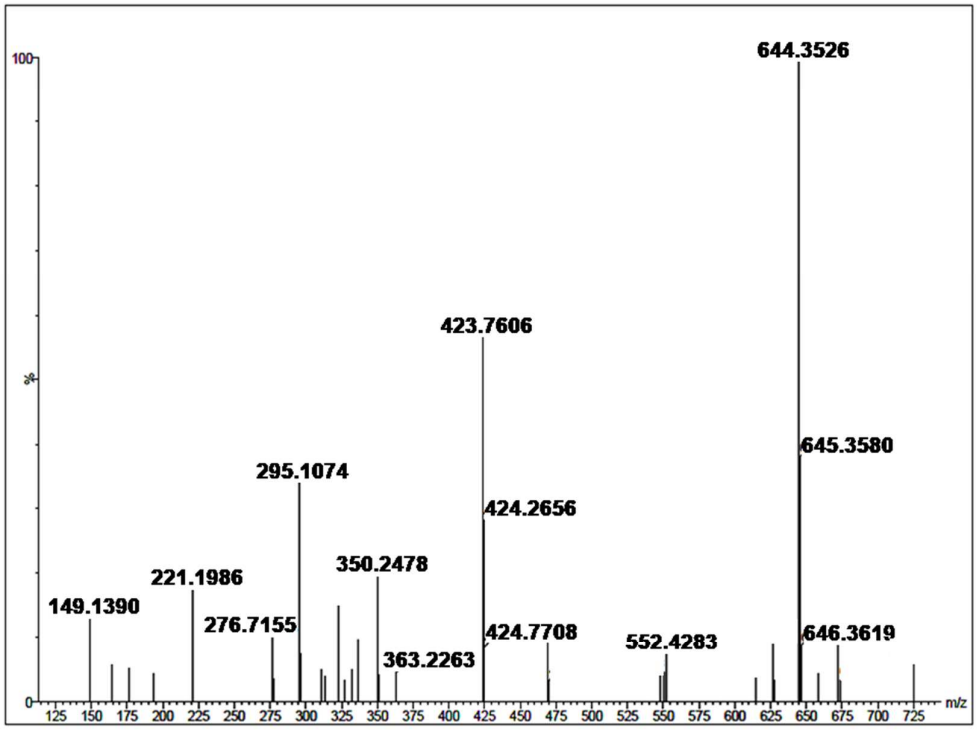


Figure S5. Positive-ion HRMS (m/z-ESI+) mass spectrum of the FA-PEG ($C_{29}H_{41}N_9O_8$ [M] : 643.6913 g/mol). Calcd. for $C_{29}H_{42}N_9O_8$ [M+H] : 644.6993, found 644.3526. The signal in 423.7608 comes from FA impurity ($C_{19}H_{19}N_7O_6$: 441,3975 g/mol) losing a H_2O molecule. Calcd. for FA-H_2O ($C_{19}H_{17}N_7O_5$), [M-H_2O] : 423.3822, found 423.7608. The FA impurity is confirmed by negative-ion HRMS (m/z-ESI-), Figure 5S. The m/z signals in 332.6769, 295.1074 and 221.1986 are due to degradation of the FA-PEG and FA in the moment of injection, as verified in the MS/MS spectrum of the Figure 6S and 7S. The other signals are due to small impurities that were not eliminated by purification and washing with ethanol and water. Purification attempts were performed using reverse phase column (C-18), however, the results were so better. These small impurities did not cause problems in the GO functionalization or biological tests.

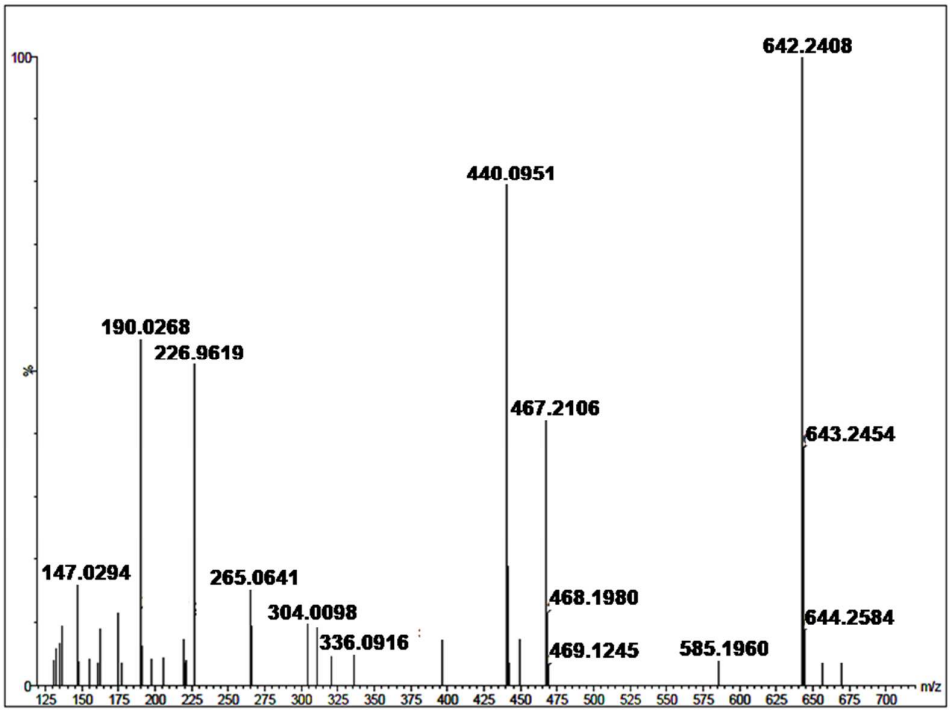


Figure S6. Negative-ion HRMS (m/z-ESI-) mass spectrum of FA-PEG to confirm the presence of FA in the mixture. FA-H ($C_{19}H_{18}N_7O_6$) calcd. for [M-H] : 440.3895, found 440.0951.

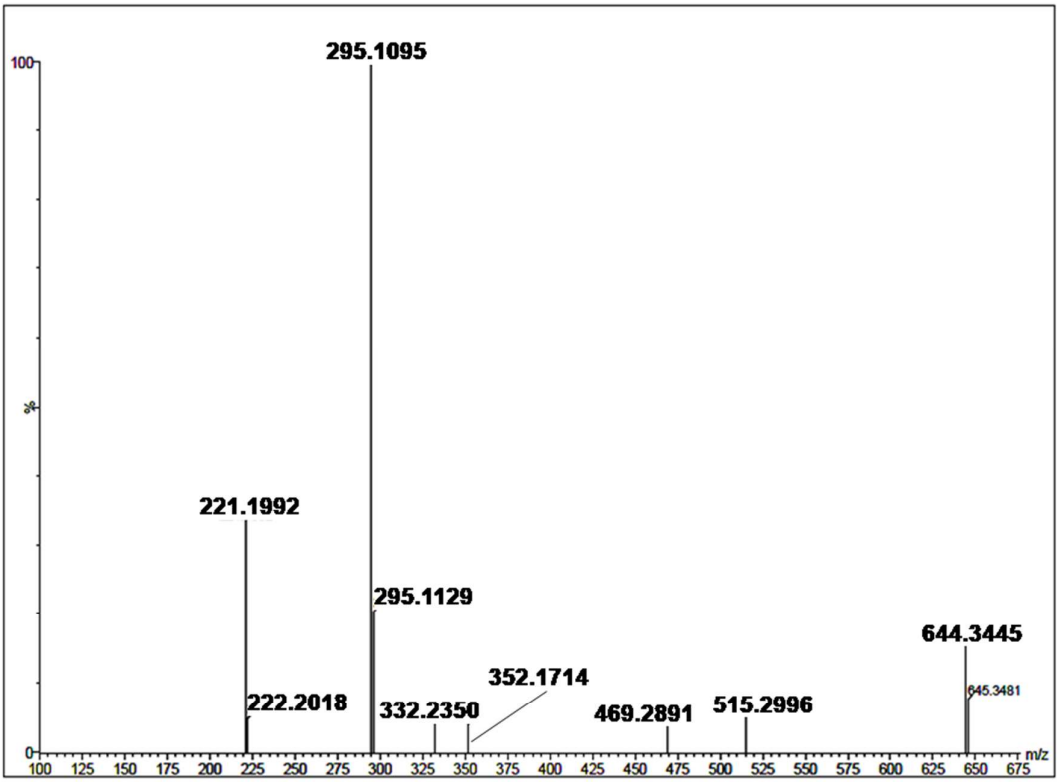


Figure S7. Positive-ion HRMS (m/z-ESI+) MS/MS spectrum for FA-PEG ($C_{29}H_{41}N_9O_8$ [M] : 643.6913 g/mol).

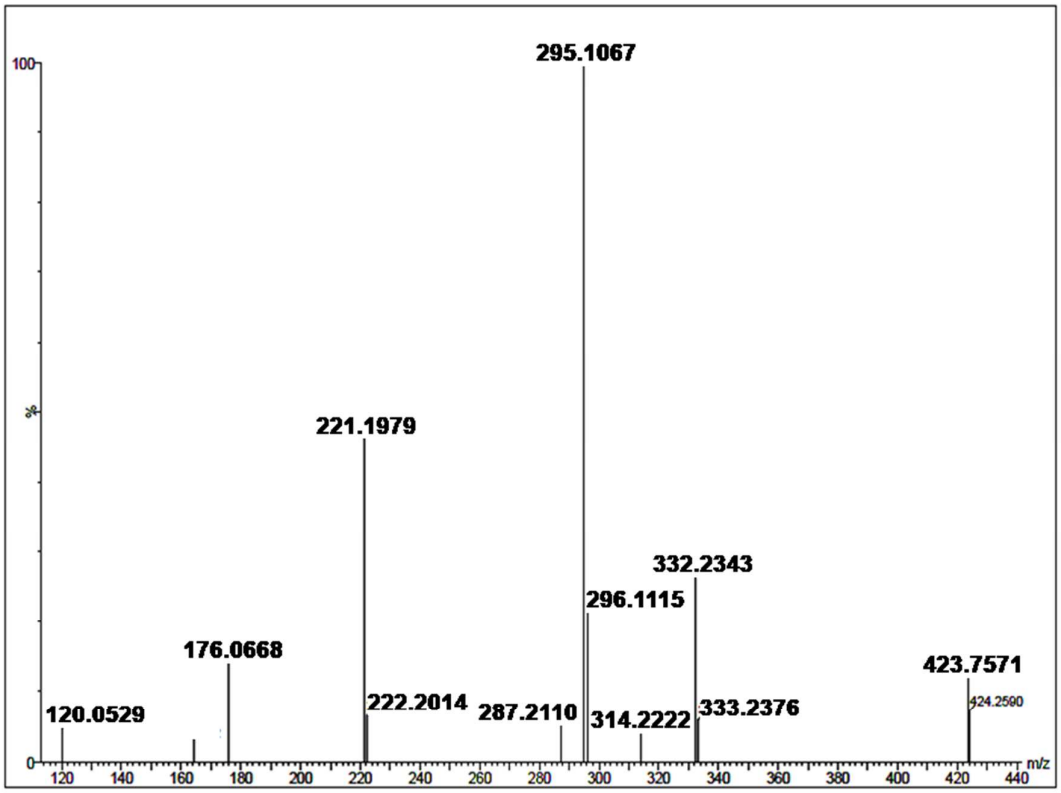
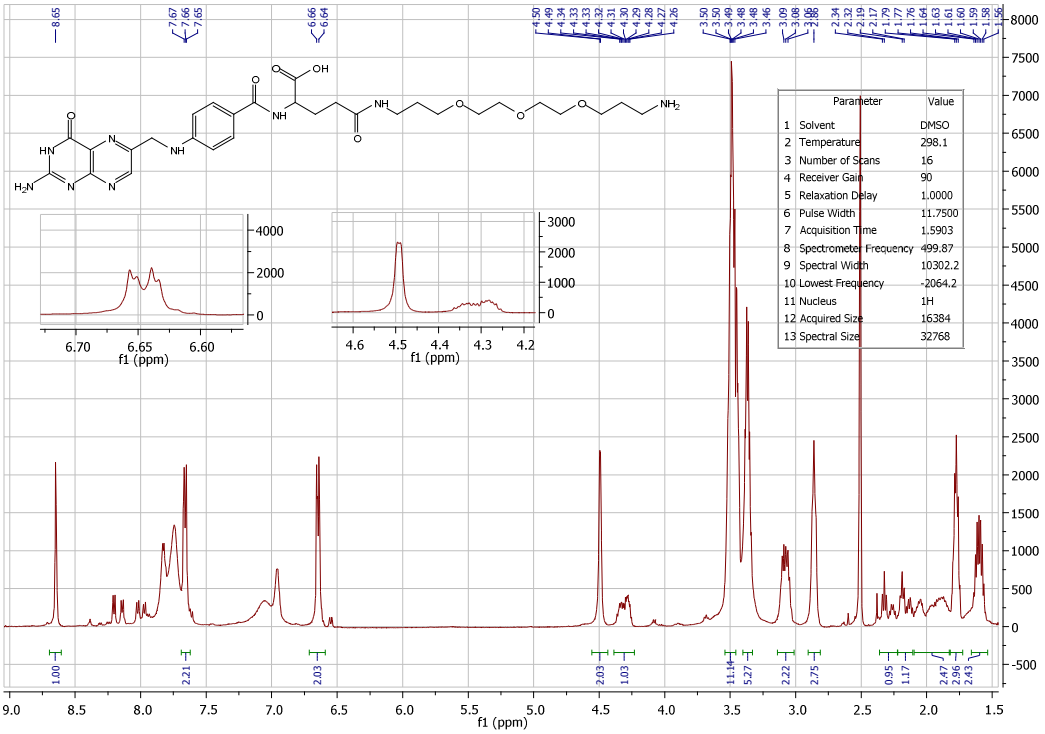


Figure S8. Positive-ion HRMS (m/z-ESI+) MS/MS spectrum for FA-H_2O ($C_{19}H_{17}N_7O_5$), [M-H_2O] : 423.3822.



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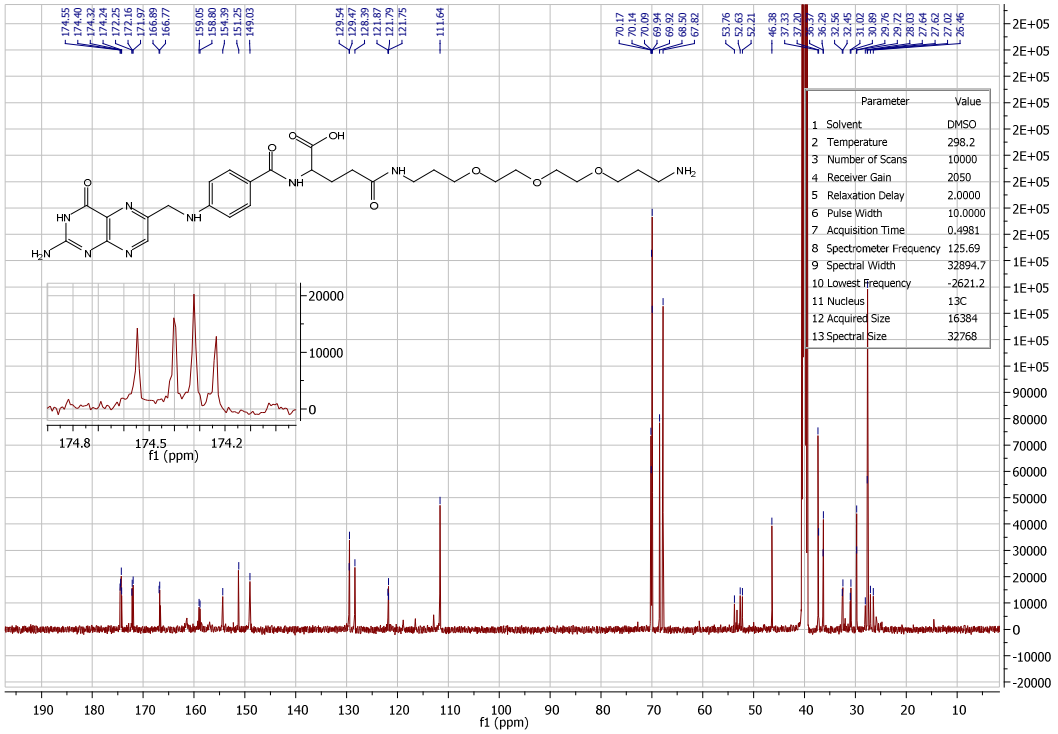
9	Spectral Width	10302.2
10	Lowest Frequency	-2064.2
11	Nucleus	1H
12	Acquired Size	16384
13	Spectral Size	32768

Figure S9. ^{1}H-NMR spectra of FA-PEG. ^{1}H NMR (500 MHz, DMSO) δ 8.65 (s, 1H), 7.67 – 7.65 (m, 2H), 6.65 (d, *J* = 8.7 Hz, 2H), 4.49 (d, *J* = 3.2 Hz, 2H), 4.39 – 4.23 (m, 1H), 3.54 – 3.35 (m, 16H)**,** 3.10-3.06 (dd, *J* = 14.2, 7.1 Hz, 2H), 2.86 (s, 2H), 2.32 – 2.10 (m, 2H), 2.10 – 1.82 (m, 2H), 1.83 – 1.73 (m, 2H), 1.66 – 1.53 (m, 2H).

The ^{1}H NMR for FA-PEG suggests the formation of isomers where the PEG molecule was linked either in γ-COOH or α-COOH and in lesser extent in both. The chemical shift at 6.65 ppm demonstrates this assumption, where it can be observed the overlapping of four signals, while the expected was only two. In other spectrum regions, this phenomenon can be observed, mainly in those related to the benzene rings, where the observed chemical shifts were twice of those expected. The chemical shifts between 3.39 ppm and 3.06 ppm corresponds to the region of the hydrogens of OCH_2. According to the molecule of the FA-PEG, it was expected twelve hydrogens in this region, but it was observed sixteen. This may occurred because in some cases two molecules of the PEG linked to one molecule of the FA.



	Parameter	Value
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3	Number of Scans	10000
4	Receiver Gain	2050
5	Relaxation Delay	2.0000
6	Pulse Width	10.0000
7	Acquisition Time	0.4981
8	Spectrometer Frequency	125.69
9	Spectral Width	32894.7
10	Lowest Frequency	-2621.2
11	Nucleus	13C
12	Acquired Size	16384
13	Spectral Size	32768

Figure S10. ^{13}C NMR (126 MHz, DMSO) δ 174.38 (dd, *J* = 24.3, 14.6 Hz), 172.25 (s), 172.07 (d, *J* = 23.5 Hz), 166.83 (d, *J* = 14.3 Hz), 159.36 – 159.12 (m), 158.92 (d, *J* = 30.6 Hz), 154.39 (s), 151.25 (s), 149.03 (s), 129.50 (d, *J* = 9.4 Hz), 128.39 (s), 122.18 – 121.64 (m), 111.64 (s), 70.48 – 69.81 (m), 68.50 (s), 67.82 (s), 53.76 (s), 52.63 (s),

52.21 (s), 46.38 (s), 37.26 (d, *J* = 15.5 Hz), 36.33 (d, *J* = 10.8 Hz), 32.51 (d, *J* = 14.9 Hz), 30.95 (d, *J* = 16.3 Hz), 29.74 (d, *J* = 5.0 Hz), 28.03 (s), 27.63 (d, *J* = 2.9 Hz), 27.02 (s), 26.46 (s).

It was observed several ^{13}C chemical shifts, demonstrating the presence of the isomers. This observation can be confirmed because the region at 174.38 ppm is showing four signals, and according to the literature the FA present only two signals in this region.[1S] The observation of the four signals is an evidence of the presence of two FA-PEG isomers in the mixture.

(1S) Bonechi, C.; Donati, A.; Lampariello, R.; Martini, S.; Picchi, M.P.; Ricci, M.; Rossi, C. Solution Structure of Folic Acid Molecular Mechanics and NMR Investigation. *Spectrochim. Acta. Mol. Biomol. Spectrosc.* **2004**, *60*, 1411-1419.

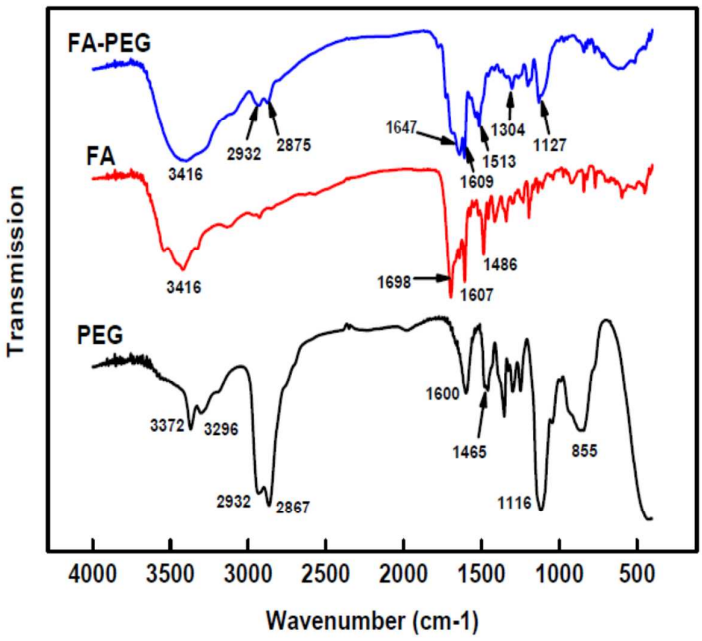

Figure S11. FTIR of FA-PEG and the counterparts.

FTIR spectra were obtained by KBr pellets of FA and FA-PEG, and with NaCl windows for PEG in the range between 400 and 4000 cm^{-1}, with 4 cm^{-1} with a resolution and 32 scans using in a FTLA 2000 spectrometer. The bands in 3416 cm^{-1} (νOH and νNH), 2932 cm^{-1} and 2875 cm^{-1} (νCH), 1131 cm^{-1} (νCO), 1600 cm^{-1} (δNH), 1465 m^{-1} (δCH), 1693 cm^{-1} (νO-C=O) and 1647 cm^{-1} (νN-C=O). The other bands are due to the νC-N, νC=C and bending are overlapping to these signals.

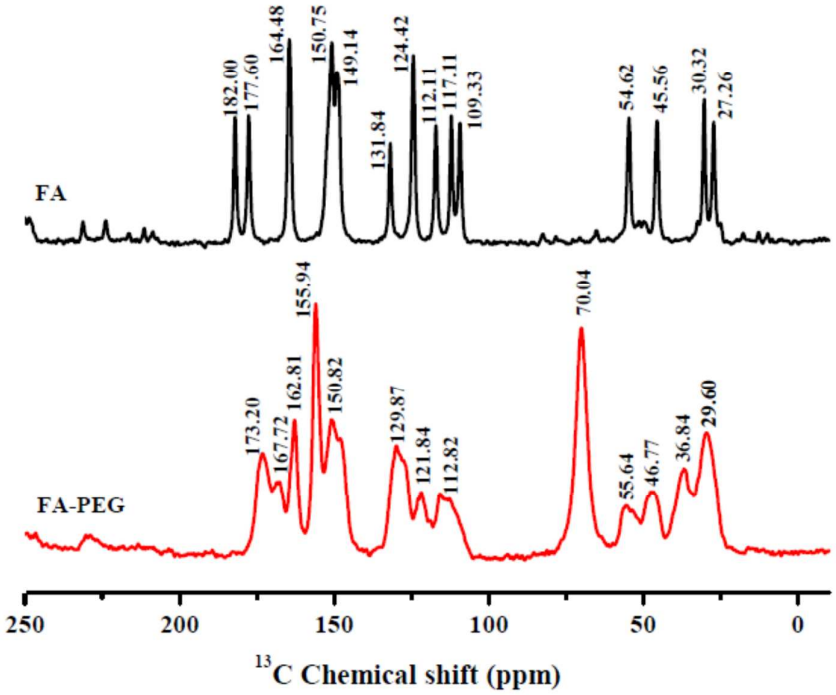

Figure S12. CP/MAS ^{13}C NMR spectra of FA-PEG and FA.

The chemical shifts of the FA are similar to the results of liquid ^{13}C NMR presented in the literature (Bonechi et al., 2004). The chemical shifts of the FA-PEG are larger when compared to those of the FA. By the profile of the spectra, it is reasonable to assume that the signals of FA at 27.26 ppm, 30.32 ppm, 45.56 ppm and 54.62 ppm correspond to the signals at 29.60 ppm, 36.84 ppm, 46.77 ppm and 55.64 ppm of the FA-PEG, respectively. According to the literature, the signals at 30.32 ppm and at 27.26 ppm from FA were attributed to the $-CH_2-$ carbons, where the first is attributed to the $-CH_2-$ closer to the γ-COOH and the other one is the $-CH_2-$ neighbor of this group. In the case of the FA-PEG, the chemical shift in 29.60 ppm is more intense than the chemical shift observed in 36.84 ppm, what is different of the behavior of the chemical shifts from FA observed in 27.26 ppm and 30.32 ppm. This information suggest that the –CH2– from PEG contributes to the increase of the intensity of the chemical shift in 29.60 ppm. The

chemical shift in 70.04 ppm comes from the C-O/C-N groups from PEG as discussed in the main text.

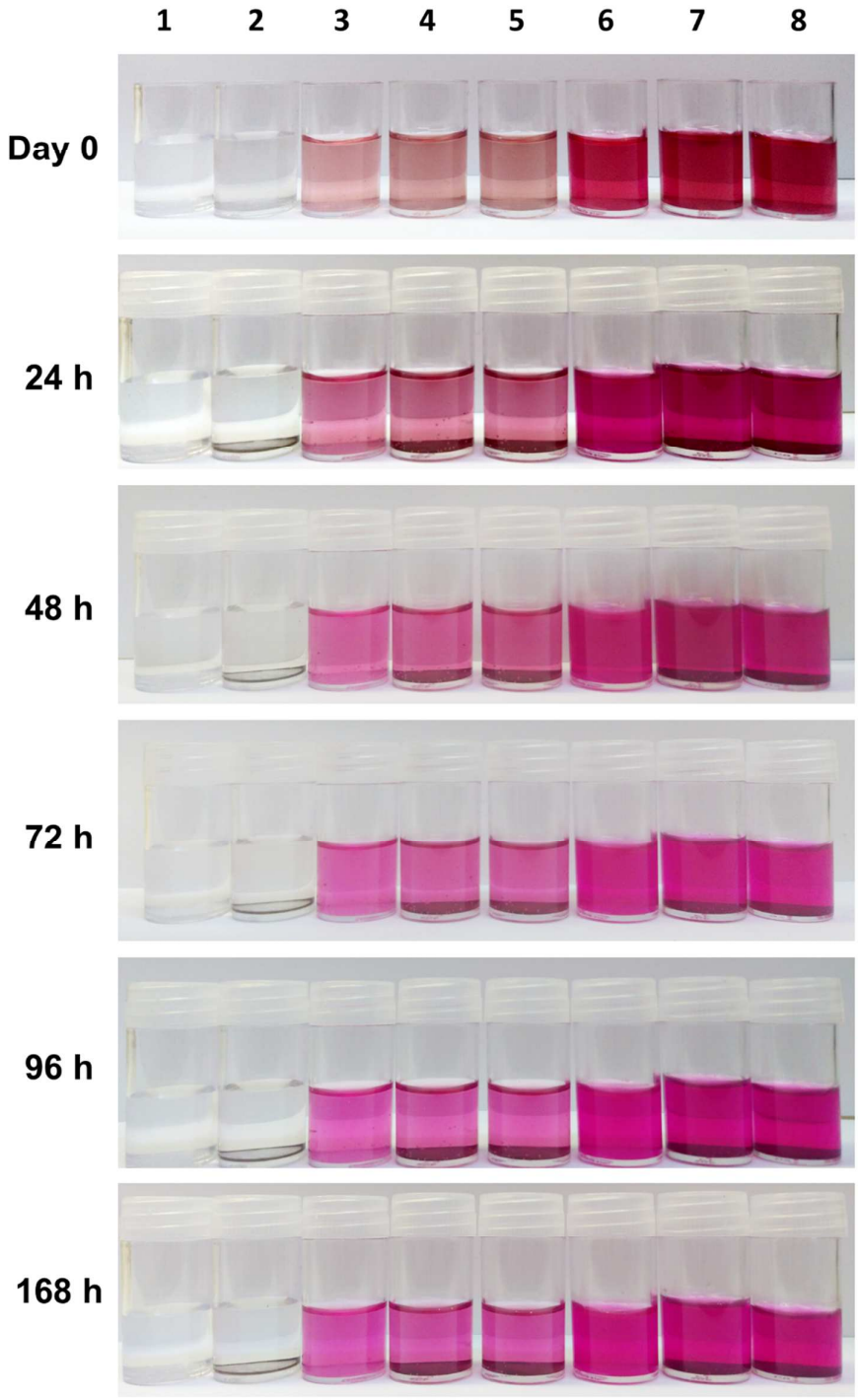

Figure S13. GO-FA nanocarrier stability over time. (1) Deionized water, (2) Deionized water + GO-FA (18.4 μg mL^{-1}), (3) RPMI cell culture media + 10% fetal bovine serum (FBS), (4 and 5) RPMI + 10% FBS + GO-FA (18.4 μg mL^{-1}), (6) DMEM high glucose + 10% FBS, (7 and 8) DMEM high glucose + 10% FBS + GO-FA (18.4 μg mL^{-1}).