Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information

Functionalization of filled radioactive multi-walled carbon nanocapsules by arylation reaction for in vivo delivery of radio-therapy

Agnieszka Gajewska,[‡a] Julie T.-W. Wang,[‡b] Rebecca Klippstein,[b] Markus Martincic,[c] Elzbieta Pach,[d] Robert Feldman,[e] Jean-Claude Saccavini,[e] Gerard Tobias,[c] Belén Ballesteros,[d] Khuloud T. Al-Jamal,*[b] and Tatiana Da Ros*[a]

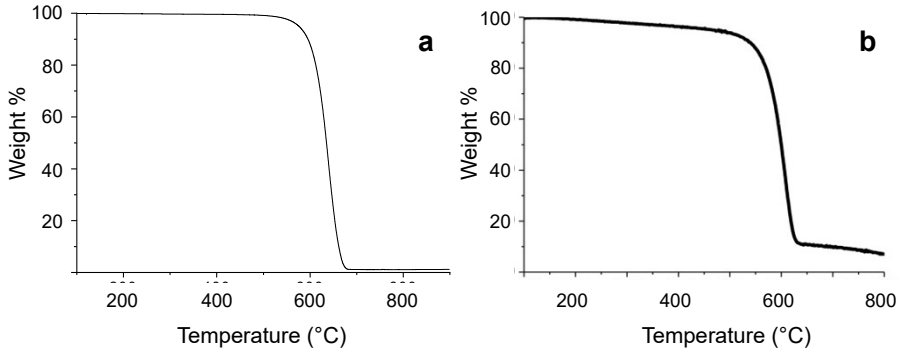


Figure S1. TGA of purified MWCNTs (a) and of filled MWCNTs (b) under flowing air.

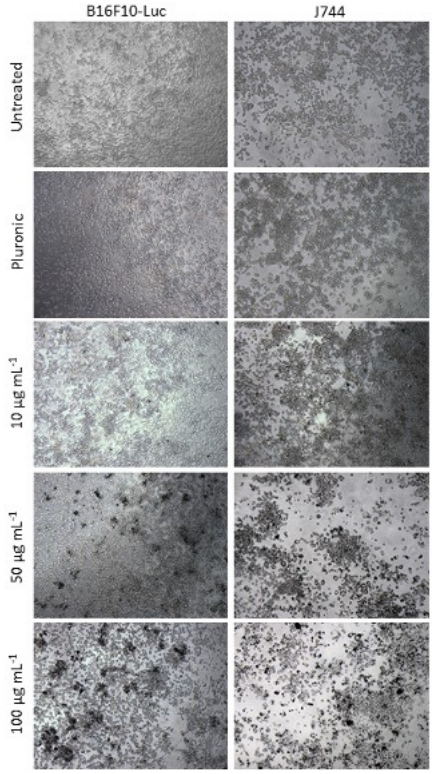


Figure S2. Light microscopy images of B16F10-Luc and J744 cells incubated for 72 h with $^{153}SmCl_3$@MWCNT-NH_2 dispersed in 1% Pluronic F127. Pictures were taken at 10 X magnification.

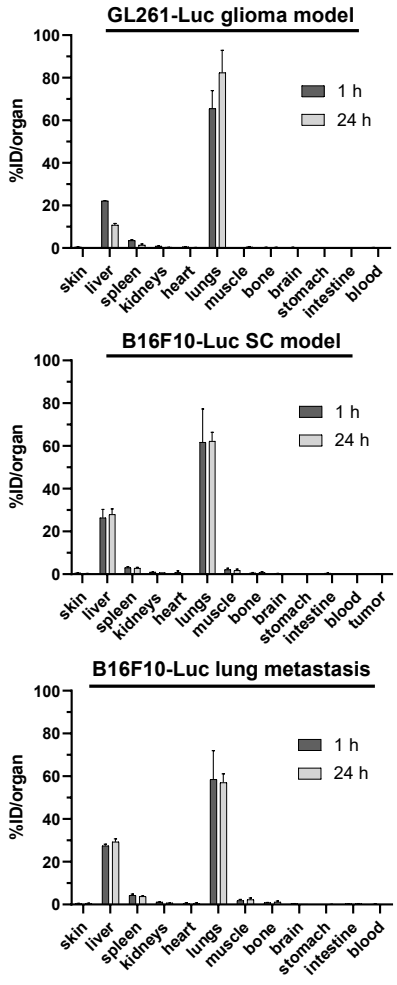
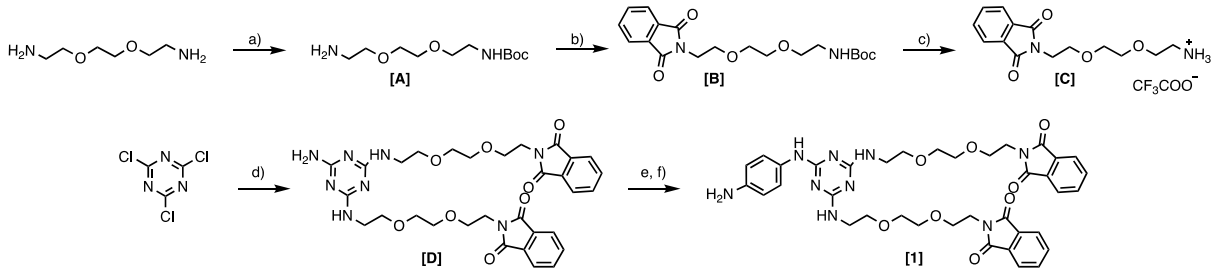


Figure S3. *In vivo* biodistribution of $^{153}SmCl_3$@MWCNT-NH_2 in tumor-bearing mice at 1 h and 24 h post-injection. Organ biodistribution profiles in intra-cranialGL261-Luc glioma model (top), subcutaneous B16F10-Luc (B16F10-Luc SC, middle) and B16F10-Luc lung metastasis model (bottom). Mice were injected with 200 μg of $^{153}SmCl_3$@MWCNT-NH_2 containing~1.5 MBq. The radioactivity of blood and major organs sampled at 1 h and 24 h post-injection was measured by γ-counting. The results are expressed as %ID/organ and presented as mean ± S.D. (n=3).

Preparation of compound 1



Scheme S1. Synthesis of compound **1**. a) di-*tert*-butyl dicarbonate, THF, 0 °C to r.t., 92%; b) phthalic anhydrite, toluene, reflux, overnight, 92%; c) TFA, DCM, 4 h, r.t., 98%; d) [**C**], DIEA, THF, 0 °C to r.t., 90%; e) *N*-Boc-*p*-phenylenediamine, THF, 80 °C, 4 h, 71%; f) TFA, DCM, 4 h, r.t., 88%.

Preparation of compound A

To a solution of 2,2'-(ethylene-dioxy)bis(ethylamine) (50 g, 0.34 mol) in THF (120 mL) a solution of di-*tert*-butyl dicarbonate (9.8 g, 0.045 mol) in THF (120 mL), was added dropwise over 40 min at 0 °C. The reaction mixture was stirred overnight at room temperature. Then, the mixture was concentrated under vacuum into white slurry, which was re-dissolved in water (200 mL). To remove not reacted di-*tert*-butyl dicarbonate, product was purified by extraction with diethyl ether and then extracted using DCM (3 x 50 mL). The combined organic layers were backwashed with water (3 x 200 mL), dried with anhydrous $MgSO_4$ and concentrated. The product was obtained as colorless viscous oil as expected. Yield: 10.5 g (94%). M/z (ES+) 249 ($M+H^+$, 100%); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.44 (s, 9H), 2.88 (t, *J*=5.2 Hz, 2H), 3.32 (q, *J*=4.9 Hz, 2H), 3.52 (t, *J*=5.3 Hz, 2H), 3.55 (t, *J*=5.1 Hz, 2H), 3.62 (s, 4H), 5.16 (bs, 1H). Characterization is in agreement with literature.[i]

Preparation of compound B

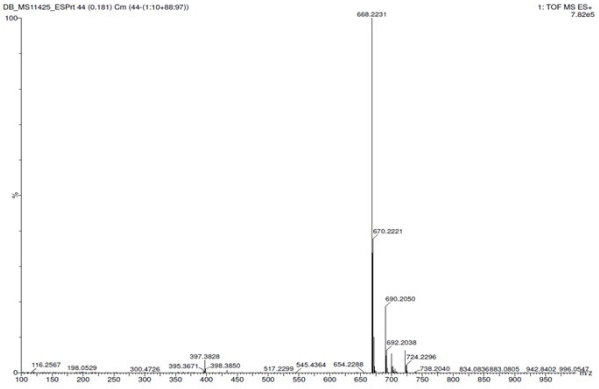
A toluene solution (60 mL) of N-*tert*-butoxycarbonyl-2,2'-ethylenedioxybis(ethylamine) (3 g, 0.012 mol) and phthalic anhydride (1.79 g, 0.012 mol) was stirred at reflux overnight. TLC (toluene:ethyl acetate 1:1) indicated the formation of the product (R_f = 0.6). The solvent was removed under reduced pressure. The product was purified by flash chromatography toluene:EtOAc 7:3. Yield: 3.5 g (92%); M/z (ES+) 378 ($M+H^+$, 100%); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.42 (s, 9H), 3.23 (m, 2H), 3.46 (m, 2H), 3.55 (m, 2H), 3.62 (m, 2H), 3.74 (m, 2H), 3.90 (m, 2H), 5.04 (s, 1H), 7.69 (m, 2H), 7.84 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 168.27, 155.98, 133.95, 132.11, 123.24, 79.12, 70.27, 69.90, 67.93, 40.35, 37.13, 28.41. Characterization is in agreement with literature.[ii]

Preparation of compound C

2.5 g (6.61 mmol) of compound **B** was dissolved in 5 mL of DCM and the solution was cooled down to 0 °C. Then 5 mL of TFA was added dropwise. The mixture was stirred at room temperature for 24 h. After evaporation of the solvent, the product was precipitated from methanol/cold diethyl ether bath as white crystals. After filtration, the product was dried under vacuum to give the pure compound **C**. The product was stored under vacuum. Yield: 2.5 g (98%). M/z (ES+) 279 ($M+H^+$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.21 (m, 2H), 3.60 (s, 4H), 3.72 (m, 4H), 3.88 (m, 2H), 7.71 (m, 2H), 7.84 (m, 2H), 8.22 (m, 2H);^{13}C NMR ($CDCl_3$, 100 MHz): δ 168.54, 161.88, 134.15, 131.88, 123.36, 118.62, 70.05, 69.62, 68.20, 66.52, 39.66, 37.29. Characterization is in agreement with literature.[ii]

Preparation of compound D

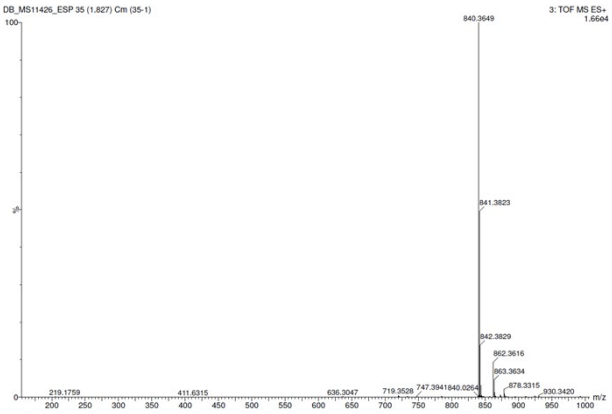
Compound **C** (1.27 g, 3.24 mmol) was dissolved in 30 mL of THF and cooled down to 0 °C. Then DIEA (563 µl, 3.240 mmol) was added. A solution of cyanuric chloride (299 mg, 1.62 mmol) in 30 mL of THF was added dropwise. The mixture was warmed to r.t. and stirred overnight. The reaction was monitored by TLC using EtOAc:DCM:MeOH/50:50:3 as eluent. Then the solvent was evaporated and product was purified by flash chromatography using EtOAc:DCM:MeOH/50:50:3 as eluent, affording 1.03 g of yellow oil. The product was stored under vacuum. Yield 1.03 g (95%). M/z (ES+) 668 ($M+H^+$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.54 (m, 12H), 3.63 (m, 4H), 3.74 (m, 4H), 3.90 (m, 4H), 5.91 (s, 1H), 6.04 (s, 1H), 7.69 (m, 4H), 7.84 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): δ 168.18, 165.43, 133.81, 131.95, 123.16, 123.07, 70.34, 70.10, 69.89, 69.52, 69.31, 67.97, 40.60, 37.09.



Preparation of compound E

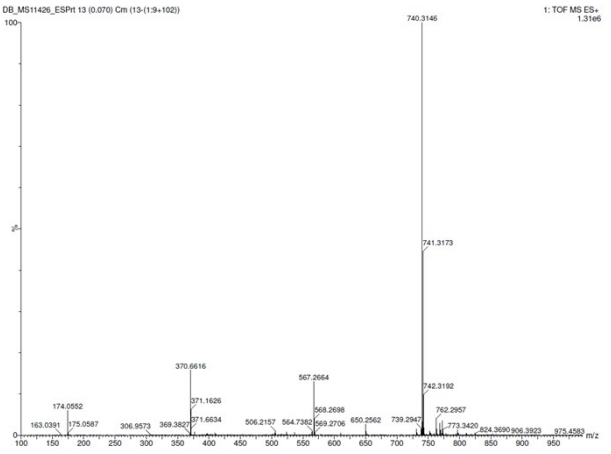
Compound **D** (900 mg, 1.35 mmol) was dissolved in THF (100 mL), DIEA (468 µl, 2.7 mmol) was added. *N*-Boc-*p*-phenylenediamine (270 mg, 1.26 mmol) was dissolved in 50 mL of THF and added dropwise to the solution of compound **D**. Reaction was stirred at 70 °C for 4 h. Product was purified by flash chromatography using EtOAc:DCM:MeOH/50:50:3 as eluent, affording 810 mg of pure product. Yield 810 mg (71%). M/z (ES^+) 840

(M+H$^+$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.51 (s, 9H), 3.52 (m, 4H), 3.59 (m, 8H), 3.65 (m, 4H), 3.76 (m, 4H), 3.92 (t, 4H), 5.98 (s, 2H), 6.55 (s, 1H), 7.31 (m, 2H), 7.50 (m, 2H), 7.68 (m, 4H), 7.83 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): δ 168.33, 133.91, 132.06, 123.22, 70.33, 70.00, 67.96, 37.20, 28.36.



Preparation of compound 1

Compound **E** (800 mg, 0.96 mmol) was dissolved in 40 mL of DCM and cooled down to 0 °C in ice bath. Then 5 mL of TFA was added drop wise. Reaction mixture was stirred at room temperature for 24 h to cleave of the Boc protecting group. After the solvent was evaporated and the product was triturated few times from toluene and then Et_2O to afford a brownish sticky oil. The product was stored under vacuum. Yield 750 mg, (88%). M/z (ES+) 740 (M+H$^+$);^{1}H NMR ($CDCl_3$, 100 MHz):δ 3.53 (m, 4H), 3.60 (m, 8H), 3.63 (m, 4H), 3.72 (m, 4H), 3.84 (t, 4H), 7.27 (m, 2H), 7.59 (m, 2H), 7.75 (m, 4H), 7.79 (m, 4H);^{13}C NMR ($CDCl_3$, 100 MHz): δ 168.31, 162.25, 161.96, 152.82, 134.04, 131.85, 123.23, 121.79, 117.17, 114.87, 70.16, 70.01, 68.70, 68.13, 40.63, 37.37.



[i] K. Kordatos, T. Da Ros, S. Bosi, E. Vázquez, M. Bergamin, C. Cusan, F. Pellarini, V. Tomberli, B. Baiti, D. Pantarotto, V. Georgakilas, G. Spalluto, and M. Prato; *J. Org. Chem.* **2001**, *66*, 4915–4920.

[ii] W. Wu, S. Wieckowski, G. Pastorin, M. Benincasa, C. Klumpp, J. P. Briand, R. Gennaro, M. Prato, A. Bianco; *Angew Chem. Int. Ed. Engl*. **2005**, *44*, 6358–6362.