Double functionalisation of carbon nanotubes for multimodal drug delivery

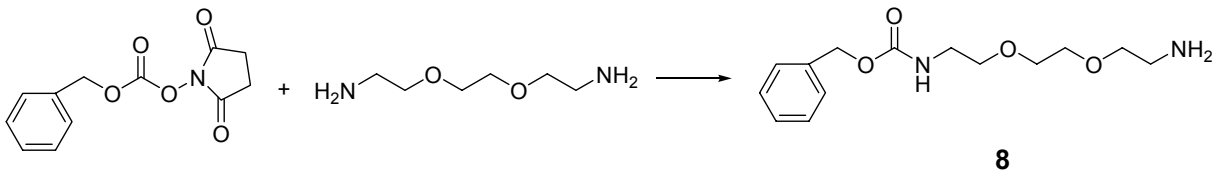
Giorgia Pastorin,[a] Wei Wu,[a] Sébastien Wieckowski,[a] Jean-Paul Briand,[a] Kostas Kostarelos,[b] Maurizio Prato*[c] and Alberto Bianco*[a]

Supporting Information

Experimental Section

General. The reactions were monitored by thin-layer chromatography (TLC) on silica gel (F_{254} Merck) and the products were visualised on aqueous potassium permanganate or ninhydrine spray followed by heating. Infrared spectra (IR) were measured on a Perkin Elmer Spectrum One ATR-FT-IR Spectrometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or CD_3OD using a Bruker DPX 300 spectrometer (300 MHz for proton and 75 MHz for carbon), the peak values were given as ppm (δ), using the tetramethylsilane or the residual deuterated solvent protons as reference. Chromatographic purifications were done with silica gel Merck (Kiesegel 60, 40-60 μm, 230-400 mesh ASTM) in standard column. Organic phases were dried with sodium sulphate. Transmission electron microscopy (TEM) analyses were performed on a TEM Hitachi 600 HS.

Synthesis of {2-[2-(2-Amino-ethoxy)-ethoxy]-ethyl}-carbamic acid benzyl ester (8)

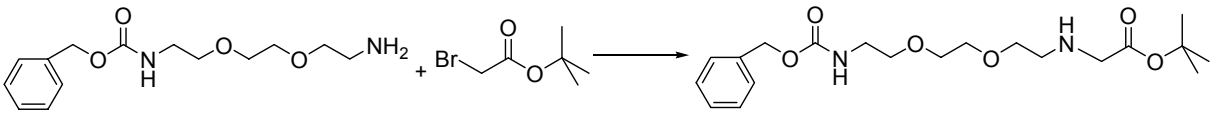


8

A solution of Z-OSu (1.68 g, 7 mmol) in 1,4-dioxane (30 ml), was added dropwise, over a period of 3 hours, to a solution of 2,2'-(ethylene-dioxy)bis(ethylamine) (10 g, 68 mmol) in 1,4-dioxane (50 ml). The reaction mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure and the residue dissolved in DCM (40 ml),

washed with water (3×100 ml), dried (Na_2SO_4) and evaporated. The unreacted diamine was removed by flash chromatography in DCM/ MeOH 7:2. Yield: 1.4 g (5 mmol, 70%). FT-IR: 3334, 1718 cm^{-1}.^{1}H NMR (300 MHz, $CDCl_3$): δ 1.78 (s, 2 H), 2.8-3.6 (m, 12 H), 5 (s, 2 H), 5.6 (bs, 1 H), 7.25-7.33 (m, 5 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 40.85, 41.56, 66.59, 70.02, 70.28, 73.14, 128.05, 128.30, 128.48, 136.63, 156.50.

Synthesis of {2-[2-(2-Benzyloxycarbonylamino-ethoxy)-ethoxy]-ethylamino}-acetic acid *tert*-butyl ester (9)

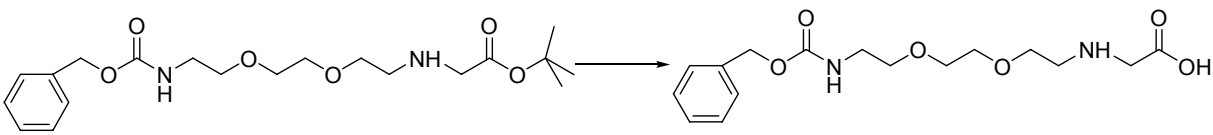


8 **9**

To a solution of {2-[2-(2-amino-ethoxy)-ethoxy]-ethyl}-carbamic acid benzyl ester **8** (1.4 g, 5 mmol) in 1,4-dioxane (30 ml) cooled to 0 °C, was added a solution of *tert*-butyl-bromoacetate (0.32 g, 1.7 mmol) in 1,4-dioxane (15 ml), dropwise over a period of 1.5 hours.

The reaction mixture was allowed to reach the room temperature and was stirred for 6 hours. The solvent was removed under reduced pressure and the residue dissolved in DCM (40 ml), washed with water (3×100 ml), dried (Na_2SO_4) and evaporated. The final product was purified by flash chromatography in DCM/MeOH 9.5:0.5. Yield: 0.5 g (1.3 mmol, 76%). FT-IR: 3334, 1718, cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.25 (s, 9 H) 2.1 (bs, 2 H), 2.5-3.5 (m, 14 H), 4.88 (s, 2 H), 5.93 (bs, 1H), 7.13 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 27.96, 40.77, 48.46, 51.38, 66.36, 69.95, 70.01, 70.12, 70.44, 80.99, 127.87, 127.94, 128,33, 136.66, 156.58, 171.42.

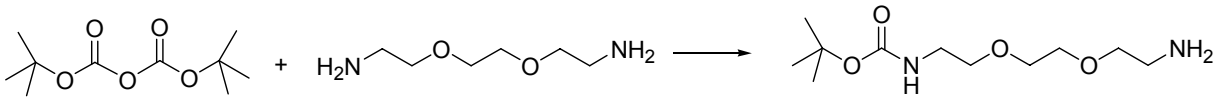
Synthesis of {2-[2-(2-Benzyloxycarbonylamino-ethoxy)-ethoxy]-ethylamino}-acetic acid (10)



9 **10**

To a solution of {2-[2-(2-benzyloxycarbonylamino-ethoxy)-ethoxy]-ethylamino}-acetic acid *tert*-butyl ester **9** (0.5 g, 1.3 mmol) in MeOH (10 ml), 2 ml of trifluoroacetic acid were added. The reaction mixture was stirred for 5 hours at room temperature. The solvent was removed under reduced pressure and the residue was precipitated in diethyl ether as trifluoroacetic acid salt. Yield: 0.4 g (1.3 mmol, 93 %). FT-IR: 3378, 1730, 1703 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.1 (bs, 1 H), 2.5-3.5 (m, 14 H), 5.06 (s, 2 H), 7.3 (m, 5H), 7.79 (bs, 1 H), 10.56 (bs, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 40.45, 47.16, 65.60, 66.52, 66.91, 69.70, 116.57, 127.05, 128.49, 128.81, 136.62, 157.16, 161.87, 169.29.

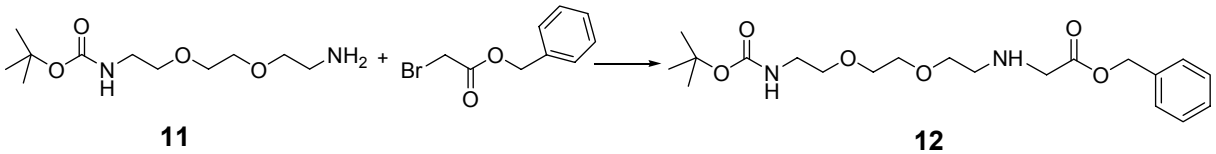
Synthesis of {2-[2-(2-Amino-ethoxy)-ethoxy]-ethyl}-carbamic acid *tert*-butyl ester[1] (11)



11

To a solution of 2,2'-(ethylene-dioxy)bis(ethylamine) (10 g, 68 mmol) in 1,4-dioxane (50 ml) was added a solution of Boc_2O (1.47 g, 6.8 mmol) in 1,4-dioxane (30 ml), dropwise over a period of 3 hours. The reaction mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure and the residue dissolved in DCM (40 ml), washed with water (3×100 ml), dried (Na_2SO_4) and evaporated. The unreacted diamine was removed by flash chromatography in DCM/MeOH 8:2. Yield: 1.4 g (5.6 mmol, 84 %). FT-IR: 3356, 1691 cm^{-1}.^{1}H NMR (300 MHz, $CDCl_3$): δ 1.39 (s, 9 H) 1.69 (bs, 2 H), 2.8-3.7 (m, 12 H), 5.1 (bs, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.27, 40.16, 41.51, 70.04, 73.14, 78.83, 155.92.

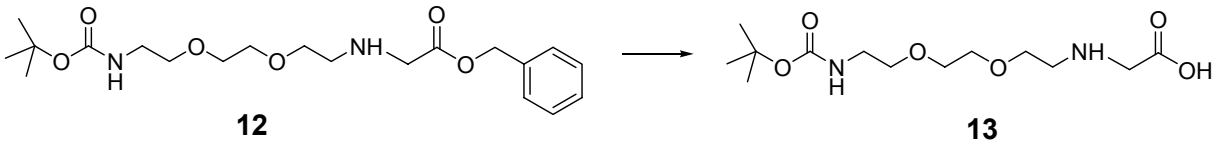
Synthesis of {2-[2-(2-Benzyloxycarbonylamino-ethoxy)-ethoxy]-ethyl}-carbamic acid *tert*-butyl ester[2] (12)



11 **12**

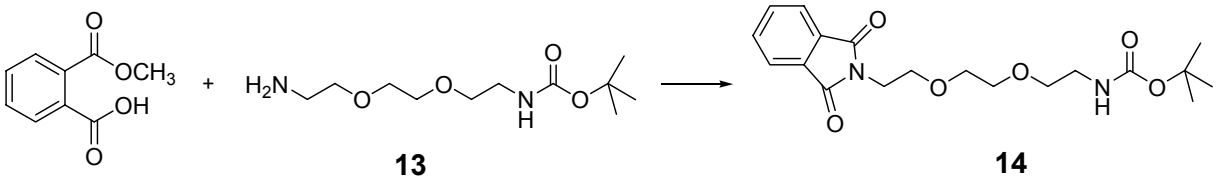
To a solution of {2-[2-(2-amino-ethoxy)-ethoxy]-ethyl}-carbamic acid *tert*-butyl ester **11** (1.4 g, 5.6 mmol) in 1,4-dioxane (30 ml) cooled to 0 °C, was added a solution of benzyl-bromoacetate (0.3 ml, 1.9 mmol) in 1,4-dioxane (15 ml), dropwise over a period of 1.5 hours. The reaction mixture was allowed to reach the room temperature and was stirred for 6 hours. The solvent was removed under reduced pressure and the residue dissolved in DCM (40 ml), washed with water (3×100 ml), dried (Na_2SO_4) and evaporated. The final product was purified by flash chromatography in DCM/ MeOH 9:1. Yield: 0.58 g (1.5 mmol, 78%). FT-IR: 3338, 1739, 1709 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.36 (s, 9 H), 2.1 (bs, 1 H), 2.7-3.6 (mt, 14 H), 5.1 (s, 2 H), 5.5 (bs, 1 H); 7.28 (m, 5 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.42, 40.36, 48.67, 50.79, 66.55, 70.14, 70.23, 70.55, 79.10, 128.37, 128.59, 135.58, 156.03, 172.07.

Synthesis of {2-[2-(2-*tert*-Butoxycarbonylamino-ethoxy)-ethoxy]-ethylamino}-acetic acid[2] (13)



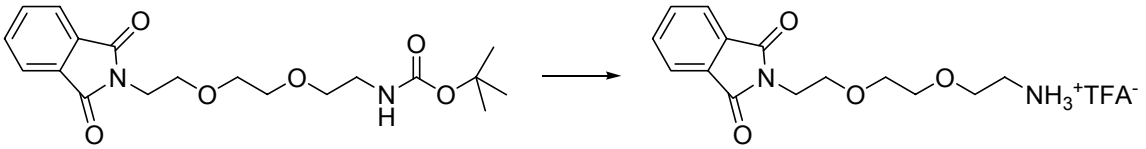
To a solution of {2-[2-(2-benzyloxycarbonylamino-ethoxy)-ethoxy]-ethyl}-carbamic acid *tert*-butyl ester **12** (0.58 g, 1.5 mmol) in methanol (20 ml), were added 50 mg of Pd/C (10%), in the presence of H_2. The reaction mixture was stirred for 5 hours at room temperature. The solution was filtered through a celite pad and the solvent was evaporated under reduced pressure. Yield: 0.4 g (1.4 mmol, 93%). FT-IR: 3346, 1694, 1626 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.39 (s, 9 H) 1.69 (bs, 2 H), 3.2-3.78 (m, 14 H), 7.8 (bs, 1 H), 10.56 (bs, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.46, 40.35, 46.54, 50.02, 66.54, 70.04, 70.15, 70.29, 79.05, 156.14, 170.83.

Synthesis of (2-{2-[2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-ethoxy]-ethoxy}-ethyl)-carbamic acid *tert*-butyl ester[3] (14)



A solution of {2-[2-(2-benzyloxycarbonylamino-ethoxy)-ethoxy]-ethyl}-carbamic acid *tert*-butyl ester **13** (1.5 g, 6 mmol), mono-methylphthalate (1 g, 5.76 mmol), BOP (2.55 g, 5.76 mmol) and DIEA (3 µl, 17.28 mmol) in CH_3CN (40 ml) was stirred at room temperature for 3 hours. A solution of Na_2CO_3 (1.2 g, 11.52 mmol) in water (20 ml) was subsequently added and the reaction was stirred for 5 hours at room temperature. The solvent was removed under reduced pressure. AcOEt was added and the solution was washed with saturated $NaHCO_3$, water, 1N $KHSO_4$ and water. The organic phase was dried with Na_2SO_4 and the solvent was evaporated. The product was obtained as an oil and used without further purifications. Yield: 1.66 g (4.4 mmol, 76%). FT-IR: 3388, 1774, 1707 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.36 (s, 9 H), 3.2-3.6 (m, 12 H), 7.5 (bs, 1 H), 7.75 (dd, 2 H), 7.81 (dd, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 27.28, 36.08, 39.24, 59.04, 66.66, 68.80, 69.02, 77.41, 121.98, 130.88, 132.93, 154.85, 166.84.

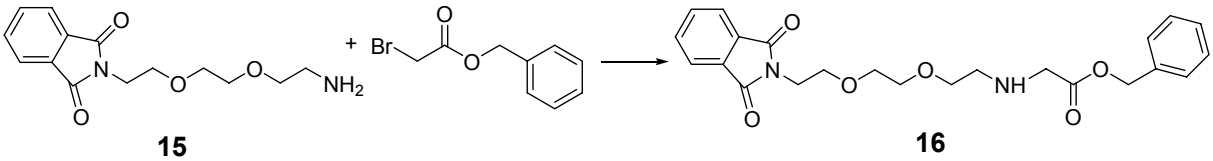
Synthesis of 2-{2-[2-(2-Amino-ethoxy)-ethoxy]-ethyl}-isoindole-1,3-dione (15)



14 **15**

To the derivative (2-{2-[2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-ethoxy]-ethoxy}-ethyl)-carbamic acid *tert*-butyl ester **14** (1.66 g, 4.4 mmol) was added a solution of TFA/CH_2Cl_2 (1:1 v/v). The reaction mixture was stirred at room temperature for 30 min. The solvent was removed under reduced pressure and the residue was precipitated several times from DCM/Et_2O as trifluoroacetic acid salt. Yield: 1.7 g (6.9 mmol, 93 %). FT-IR: 3438, 3332, 1771 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 3.2-3.87 (m, 12 H), 7.75 (dd, 2 H), 7.81 (dd, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 37.31, 39.68, 68.51, 68.24, 69.64, 70.05, 118.60, 123.37, 131.87, 134.17, 161.89, 168.57.

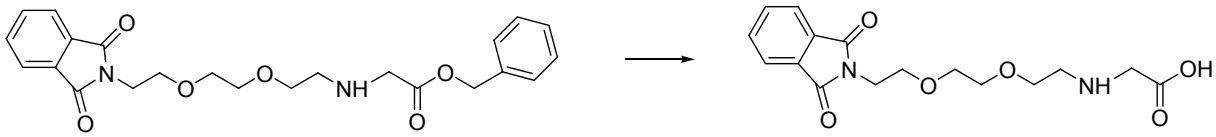
Synthesis of (2-{2-[2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-ethoxy]-ethoxy}-ethylamino)-acetic acid benzyl ester (16)



15 **16**

To a solution of 2-{2-[2-(2-amino-ethoxy)-ethoxy]-ethyl}-isoindole-1,3-dione **15** (1.7 g, 6.9 mmol) and DIEA (2 µl, 6 mmol) in 1,4-dioxane (20 ml) cooled to 0 °C was added benzyl-bromoacetate (0.4 ml, 2.3 mmol) in 1,4-dioxane (5 ml), dropwise over a period of 1.5 hours. The reaction mixture was allowed to warm to room temperature and stirred for 6 hours. The solvent was removed under reduced pressure. The final product was purified by flash cromathography in AcOEt/cyclohexane 8:2. Yield: 0.5 g (1.3 mmol, 55%). FT-IR: 3438, 3430, 1770, 1700 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.1 (bs, 1 H), 2.7-3.8 (m, 14 H), 4.95 (s, 2 H), 7.16 (s, 5 H), 7.49 (dd, 2 H), 7.62 (dd, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 37.09, 48.53, 50.70, 60.13, 67.72, 69.85, 70.09, 70.47, 122.99, 128.13, 128.40, 131.96, 133.76, 135.67, 167.94, 171.94.

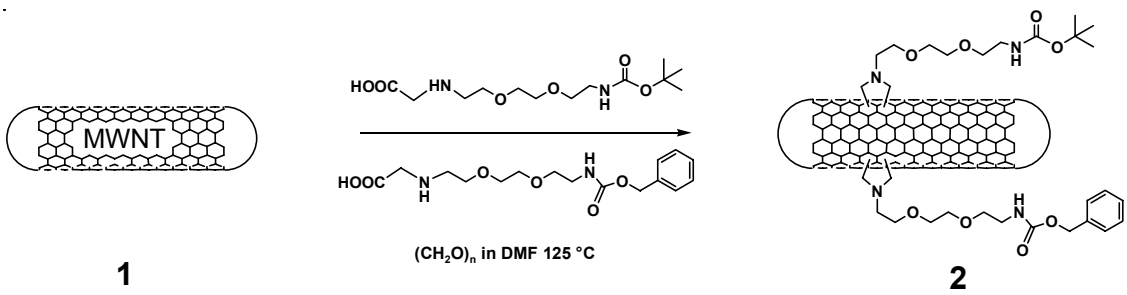
Synthesis of (2-{2-[2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-ethoxy]-ethoxy}-ethylamino)-acetic acid (17)



To a solution of (2-{2-[2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-ethoxy]-ethoxy}-ethylamino)-acetic acid benzyl ester **16** (0.5 g, 1.3 mmol) in methanol (20 ml), were added 50 mg of Pd/C (10%), in the presence of H_2. The reaction mixture was stirred for 5 hours at room temperature. The solution was filtered through a celite pad and the solvent was evaporate under reduced pressure. Yield: 0.4 g (1.2 mmol, 93%). FT-IR: 3438, 3332, 1771, 1701 cm^{-1}. ^{1}H NMR (300 MHz, CD_3OD): δ 3.18 (bs, 1 H), 3.24-3.86 (m, 14 H), 7.49 (dd, 2 H), 7.62 (dd, 2H), 10.56 (bs, 1 H). ^{13}C NMR (75 MHz, CD_3OD): δ 36.97, 46.87, 47.30, 65.59, 67.66, 69.77, 69.99, 122.75, 131.89, 134.04, 168.37.

Procedures for the double functionalisation of carbon nanotubes

Procedure 1: functionalisation with Boc- and Z-protected amino acids (2).



CNT (100 mg) and paraformaldehyde (3 × 150 mg every 24 hours) were suspended in 100 ml of DMF. 150 mg of Boc-NH-$(CH_2CH_2O)_2$-CH_2CH_2-$NHCH_2COOH$ (**13**) and 167 mg Z-NH-$(CH_2CH_2O)_2$-CH_2CH_2-$NHCH_2COOH$ (**10**) (1:1 molar ratio) were added and the reaction was heated at 125 °C for 72 hours. The heating was stopped. Unreacted CNT remained completely insoluble in DMF and were separated from the soluble functionalised tubes by centrifugation and filtration through a 0.22 μm PTFE filter. The filtrate was evaporated and the brown residue was dissolved in DCM, washed once with water and dried over Na_2SO_4. The solvent was evaporated and the product was reprecipitated several times from methanol/diethyl ether. MWNT **2** were characterised by TEM (Fig. S1) and ^{1}H-NMR (Fig. S2).

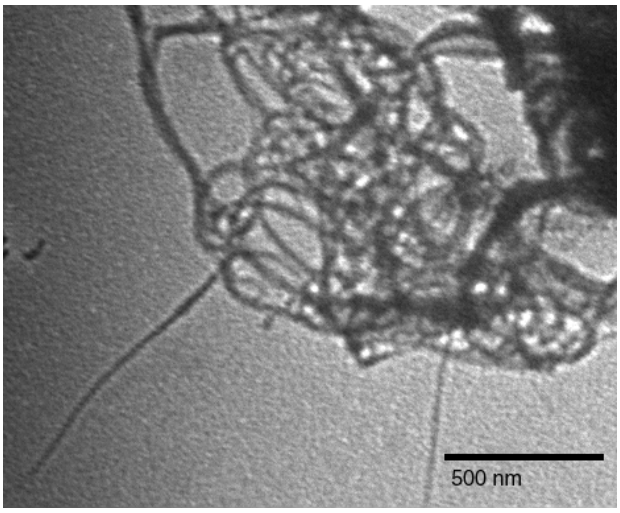


Figure S1. TEM image of MWNT **2**.

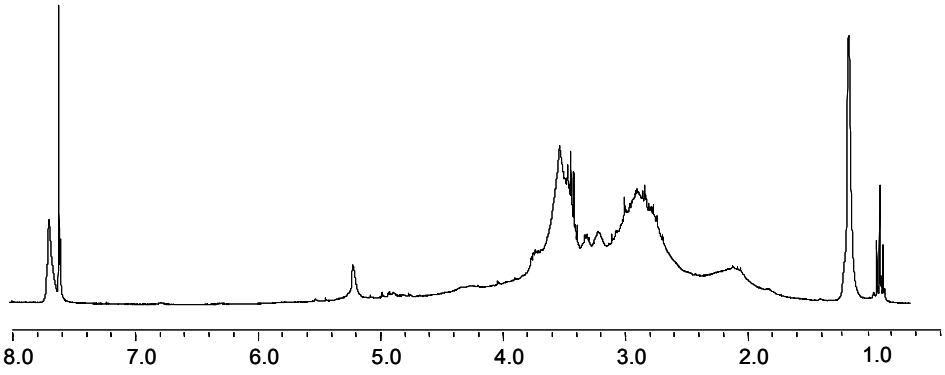
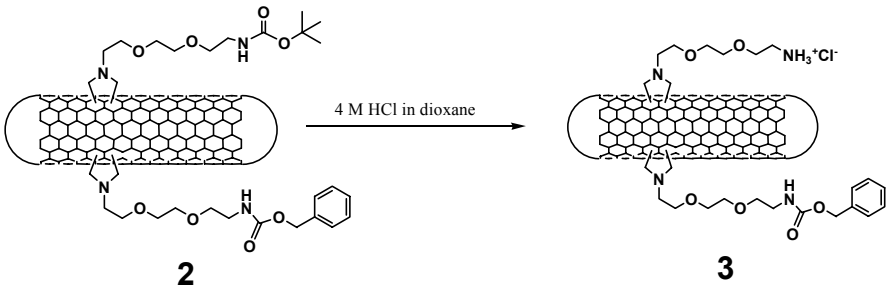


Figure S2. ^{1}H-NMR spectrum of MWNT **2**.

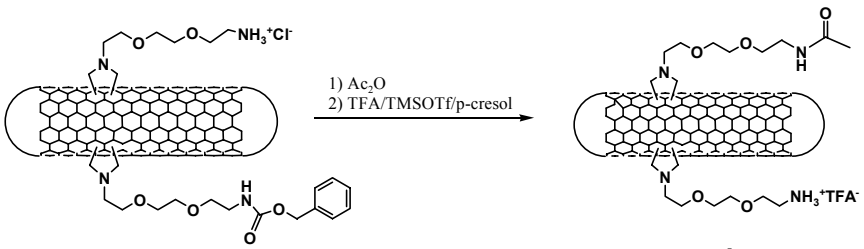
Cleavage of the Boc protecting group (3)



2 **3**

A solution of HCl 4 M in dioxane is added to MWNT **2** (5 mg) and the mixture was stirred for 5 hours at room temperature. The solvent was removed under reduced pressure and the product was reprecipitated several times from methanol/diethyl ether. MWNT **3** were characterised by TEM and ^{1}H-NMR. Quantitative Kaiser test: 0.35 mmol/g of free NH_2.

Acetylation of MWNT 3 and cleavage of Z protecting group[4] (4)

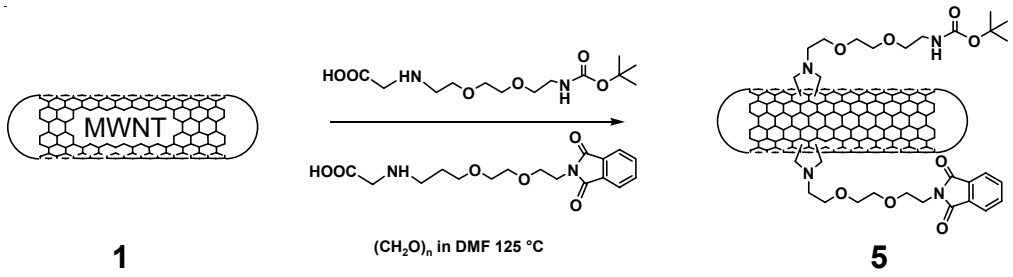


3 **4**

MWNT **3** (5 mg) were solubilised in 600 µl of DMF and 200 µl of acetic anhydride were added. The solution was stirred for 1.5 hours and then precipitated several times in cold

diethyl ether. Qualitative Kaiser test was negative. A solution (400 µl) of TFA/TMSOTf/*p*-cresol (325:87:42.5, µl/µl/mg) (TMSOTf, trimethylsilyltrifluoromethanesulfonate) was added to the acetylated MWNT **3** (5 mg) and the mixture was stirred for 12 hours at room temperature. The product was directly precipitated in diethyl ether and washed several times by reprecipitation from methanol/diethyl ether. MWNT **4** were characterised by TEM and ^{1}H-NMR. Quantitative Kaiser test: 0.35 mmol/g of free NH_2.

Procedure 2: functionalisation with Boc- and phthalimide-protected amino acids (5)



CNT (100 mg) and paraformaldehyde (3 × 150 mg every 24 hours) were suspended in 100 ml of DMF. 150 mg of Boc-NH-$(CH_2CH_2O)_2$-CH_2CH_2-$NHCH_2COOH$ (**6**) and 165 mg Pht-NH-$(CH_2CH_2O)_2$-CH_2CH_2-$NHCH_2COOH$ (**10**) (1:1 molar ratio) were added and the reaction was heated at 125 °C for 72 hours. The heating was stopped. Unreacted CNT remained completely insoluble in DMF and were separated from the soluble functionalised tubes by centrifugation and filtration through a 0.22 µm PTFE filter. The filtrate was evaporated and the brown residue was dissolved in DCM, washed once with water and dried over Na_2SO_4. The solvent was evaporated and the product was reprecipitated several times from methanol/diethyl ether. MWNT **5** were characterised by TEM (Fig. S3) and ^{1}H-NMR (Fig. S4).

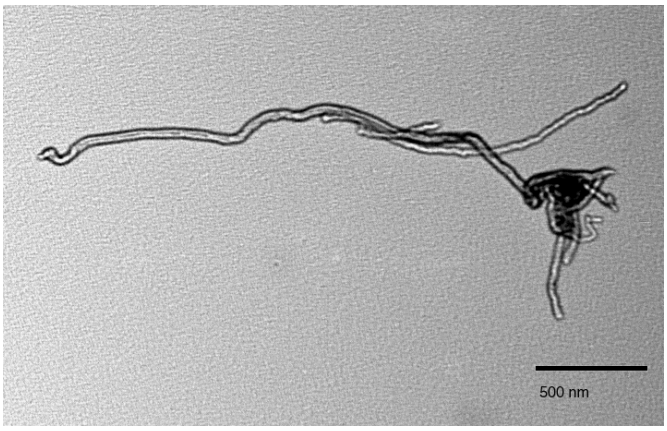


Figure S3. TEM image of MWNT **5**.

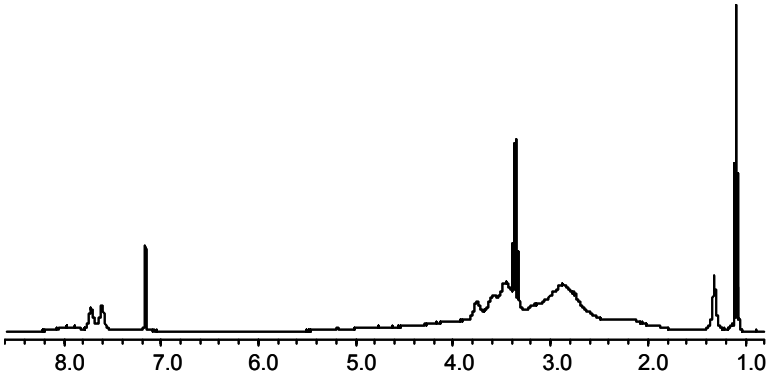
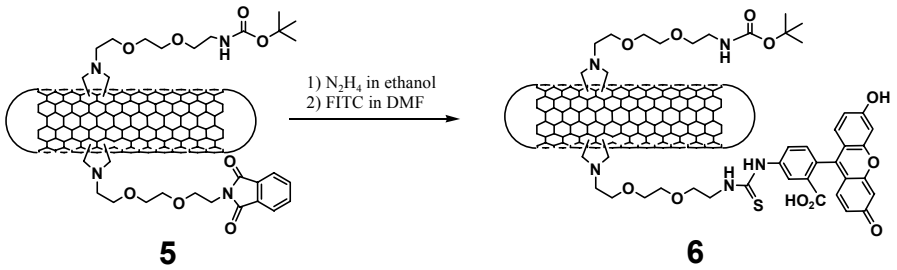


Figure S4. ^{1}H-NMR spectrum of MWNT **5**. The two multiplets at about 1.0 and 3.4 ppm correspond to residual diethyl ether.

Cleavage of the phthalimide protecting group and functionalisation with fluorescein isothiocyanate (6)



Hydrazine (36.5 µl, 752 µmol) in ethanol (10 ml) was added to double functionalised MWNT **5** (9.4 mg) and the mixture was stirred for 16 hours at room temperature under argon. The solvent was removed, methanol was added and the solution was centrifuged to eliminate phthalhydrazide. The product was reprecipitated several times from methanol/diethyl ether. Amino-functionalised MWNT **5** (7 mg, 4.2 µmol corresponding to the amount of free NH_2 measured by quantitative Kaiser test) were solubilised in 200 µl of DMF. A solution of fluorescein isothiocyanate (FITC) (4.9 mg, 12.6 µmol) in 200 µl of DMF was added and the solution was stirred overnight at room temperature. The solvent was removed and the product was precipitated several times from methanol/diethyl ether. Qualitative Kaiser test was negative. MWNT **6** were characterised by TEM (Fig. S5). Quantitative Kaiser test: 0.65 mmol/g of NH_2.

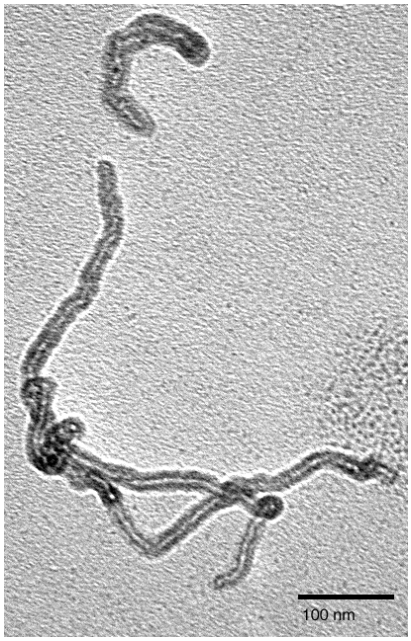
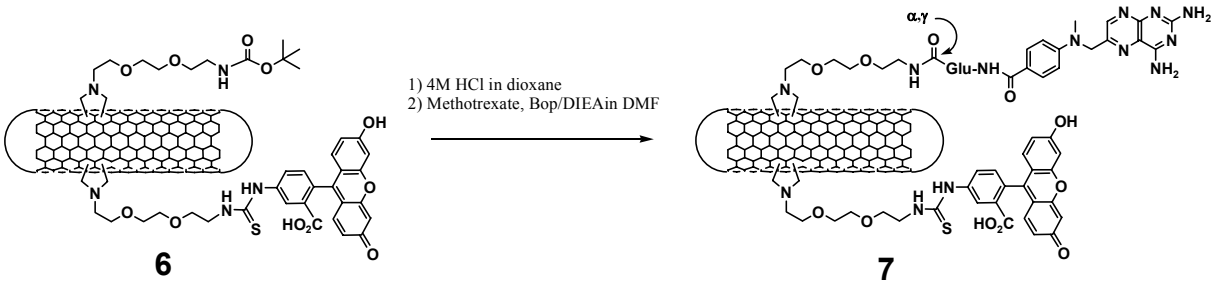


Figure S5. TEM image of MWNT **5** after the cleavage of Pht protecting group**.**

Reaction of fluorescein functionalised carbon nanotubes with methotrexate[5] (7)



A solution of HCl 4 M in dioxane is added to MWNT **6** (6 mg) and the mixture was stirred for 5 hours at room temperature. The solvent was removed under reduced pressure and the product was reprecipitated several times from methanol/diethyl ether. The functionalised carbon nanotubes were characterised by TEM and ^{1}H-NMR. Quantitative Kaiser test: 0.30 mmol/g of free NH_2. Methotrexate (650 μg, 1.44 μmol), DIEA (1 μl) and BOP (637 μg, 1.44 μmol) were mixed in 50 μl of DMSO. After stirring for 30 min, a solution of MWNT **6** without Boc (6 mg, 1.2 μmol corresponding to the amount of free NH_2 measured by quantitative Kaiser test) in DMSO and DIEA (1μl) was added. The mixture was stirred overnight at room temperature. The amino-PEGA resin (36 mg, 14.4 μmol) was added and the solution was stirred slowly for 2 hours. Then the resin was filtered and washed several

times (≥ 5) with MeOH. The solvent was removed and the product was precipitated in Et_2O. MWNT **7** were characterised by TEM and UV-Vis (Fig. S6).

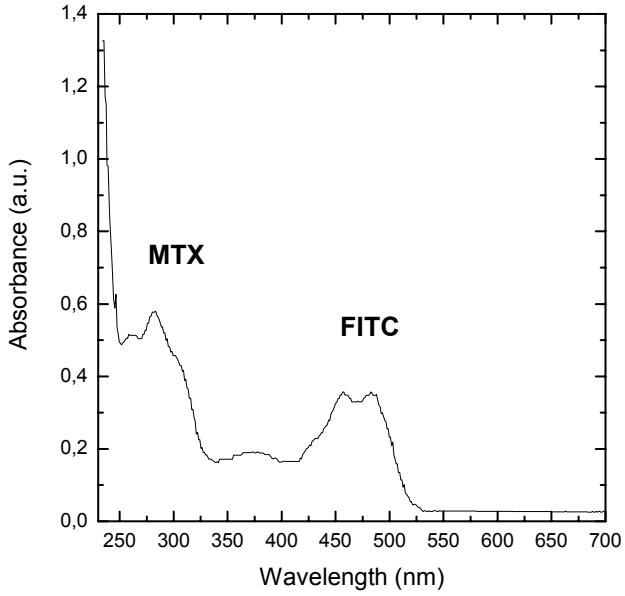


Figure S6. UV-Vis spectrum of **7** in methanol.

Cells and cell cultures

Preparation of MWNT 7

MWNT **7** were dissolved in DMSO/water (0.09/1, v/v) at different concentrations (0.05, 0.5, and 5 μg/ml). To have some controls, two other solutions of MWNT-FITC-NH_3^+, derived from MWNT **6** after Boc cleavage, (0.05, 0.5 and 5 μg/ml) and MTX alone (0.005, 0.05 and 0.5 μg/ml) were prepared in DMSO/water (0.09/1, v/v).

Cell culture and treatment with MWNT 7

Jurkat human T leukemia cell line was cultured in RPMI 1640 (Cambrex Bioscience) supplemented with gentamicin (10 μg/ml) and 10% of heat-inactivated fetal bovine serum. Cells were grown in suspension at 37 °C in a humidified atmosphere with 5% CO_2. For the tests cell suspensions were prepared at a final concentration of 5×10^5 cells/ml in 1 ml of medium containing 10 μl of indicated MWNT **7** solutions (final concentration: 0.05 to 5 μg/ml). As resulted from the dilution on cell medium the maximum final percentage of DMSO in cell culture is lower than 0.01%. The cells were then incubated at 37 °C for 1 and 24 hours. After incubation, all of the cells were washed twice with 1 ml of phosphate buffered

saline (PBS) and then analysed for intracellular MWNT **7** content by flow cytometry and fluorescence microscopy.

Flow cytometry measurements

After two washes in PBS, cells were resuspended in 300 µl of PBS and analysed with the flow cytometer FACSCalibur® operating at 488 nm excitation wavelength and detecting emission wavelengths with a 530/30 nm band-pass filter. At least 25,000 cells were acquired using the CellQuest 3.3 software (Becton & Dickinson) and distribution of the FITC fluorescence was analysed with the WinMDI 2.8 freeware (Joseph Trotter, Scripps Research Institute).

Epifluorescence and confocal microscopy

After two washes in PBS, cells were resuspended in ready to use Fluorescent Mounting Medium (DakoCytomation) and mounted between glass slide and coverslip. The distribution of fluorescence was analysed on an Olympus BX51 microscope with a FITC wide-band cube (460-490 nm excitation filter and 515-550 nm emission transmission range) using the AnalySIS 3.0 software. A Zeiss LSM 510 Meta confocal microscope was also used operating at 488 nm excitation wavelength and detecting emission wavelengths with a 505-550 nm band-pass filter. Data were analysed using the ImageJ 1.33u freeware (Wayne Rasband, National Institutes of Health) and the LSM Reader 3.2d plugin (Patrick Pirrotte, Yannick Krempp and Jerome Mutterer, Institute for Molecular Biology of Plants, Strasbourg, France).

Cell penetration of the precursor of MWNT 7

For comparison to the behaviour of MWNT **7**, we have verified the capacity of MWNT **6** without the Boc protecting group to penetrate into the cells by epifluorescence microscopy. Again human Jurkat cells were cultured using RPMI 1640 medium at 37 °C. Functionalised MWNT were added to the cells at room temperature in a range of concentration between 0.05 and 5 µg/ml, and the cells were incubated at 37 °C for 1 h. After this period, the cells were carefully rinsed, mounted on a microscope slide and observed under the microscope. Fig. S7 shows the bright light and epifluorescence images of the cells after being treated with 0.5 µg/ml of MWNT **6** without Boc. It is evident that CNT accumulated into the cell. In addition, we have studied the dose dependence of the internalisation process. Fig. S8 shows the flow cytometry analysis of the cells treated with three different amounts of MWNT **6** without Boc for 24 hours. Finally, we have measured the effect of time on the internalisation of MWNT **6** without Boc in comparison to MWNT **7** at the different doses (Fig. S9). The process was

followed between 0 and 24 hours. We have observed a linear correlation with a rapid kinetics of uptake for the highest dose used (5 µg/ml). The dose of 0.05 µg/ml was too low to detect a clear shift in the fluorescence signal after 24 hours.

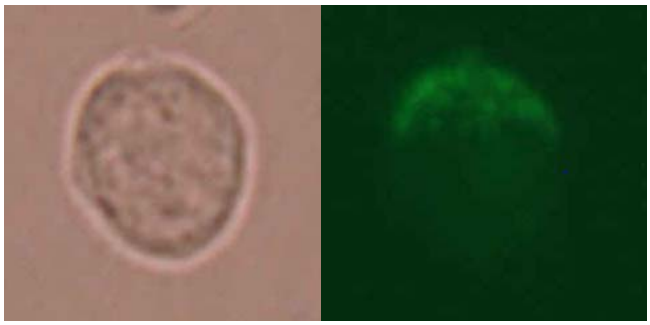


Figure S7. Bright field (left) and epifluorescence (right) images of Jurkat cells incubated for 1 h at 37 °C with 0.5 µg/ml of MWNT **6** after cleavage of Boc protecting group.

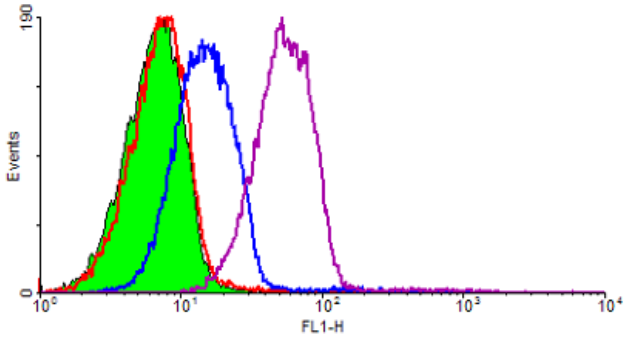


Figure S8. Dose-response of the internalisation after incubation of Jurkat cells for 24 h at 37 °C with increasing amount of MWNT **6** after cleavage of Boc protecting group [0.05 (red line), 0.5 (blue line) and 5 (magenta line) µg/ml]. FL1-H corresponds to FITC intensity.

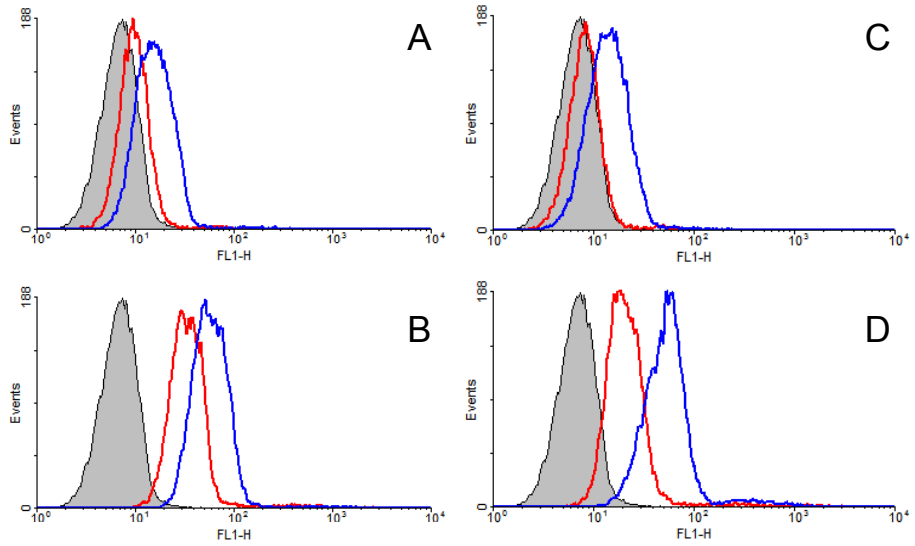


Figure S9. Effect of the time on the internalisation of MWNT **6** without Boc (A, B) and MWNT **7** (C, D) at 37 °C. A, C: 0.5 μg/ml. B, D: 5 μg/ml. Black line, 0 h; red line, 1 h; blue line, 24 h. FL1-H corresponds to FITC intensity.

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