Supporting Information

Catalytic Synthesis of Amino Acid- and Peptide-Derivatized Gadonanotubes

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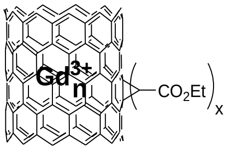
Chemicals and Instrumentation

All chemical reagents were commercially available and were used without further purification, except for toluene, which was refluxed over Na metal and then distilled from it. The RGD peptide was synthesized by the Baylor College of Medicine Protein Chemistry Core Laboratory on a Model 433A Peptide Synthesizer (Applied Biosystems, Foster City, CA) using standard Fmoc chemistry, purified by reverse phase HPLC and analysed by MALDI-TOF mass spectrometry and by amino acid analysis. Mass spectra were obtained using a *Bruker MS Reflex IV MALDI-TOF* instrument. Thermal gravimetric analysis (TGA) was performed on an *SDT 2960 Universal V3.4C TA* instrument. The experiments consisted of a 10° C/minute temperature ramp, from room temperature to 800° C, under argon. Fourier-transform infrared spectra were obtained using a *Nexus 670 FTIR Thermo-Nicolet* spectrometer in the range of 500–4000 cm^{-1}. Samples for IR were prepared by grinding with IR quality KBr, unless otherwise noted. The Raman spectroscopy measurements utilized a *Renishaw inVia Micro Raman* spectrometer with a 633 nm diode laser and a resolution of 2 cm^{-1}. The objective lens used was ×50 with a 0.55 µm aperture. XPS analysis was performed using a *PHI Quantera SXM scanning X-ray microprobe ULVac* with an Al mono 24.8 W X-ray source and a 100 µm X-ray spot size at 45.0° (26 eV for intervals of 20 min - 1 h). Data was analyzed with *MatLab 7.0* to estimate the relative peak areas and calculate the elemental composition.

Syntheses and Characterization



I

Ethyl 1H-cyclopropan-Gd@US-tube-1-carboxylate (**I**). In a typical experiment, 20 mg of purified Gd@US-tubes, dried under N_2(g) at 100° C for 12 hours, were dispersed in 100 ml of anhydrous toluene with bath sonication followed by the addition of a freshly prepared solution of 4 mg of rhodium carbonyl, $Rh_6(CO)_{16}$, in 1 ml of THF and 200 mg of ethyl diazoacetate. The suspension was stirred under nitrogen at 90-100° C for 4 days. Usually the coverage of the Gd@US-tube increases from 5-9 groups·nm^{-1} after 1 day of reaction to 14-28 groups·nm^{-1} after 4 days; further treatment did not increase coverage. The Gd@US-tube ester (**I**) was then collected by filtration, washed sequentially with toluene, methanol and water, and then dried under nitrogen at RT. The Gd@US-tube ester (**I**) was characterized by Raman spectroscopy (Fig. S 1), TGA (Fig. S 2) IR spectroscopy (Fig. S 3), and MS (Fig. S 4). Compound **I** decomposes between 340 and 360° C.

A large number of diazoesters cyclopropanation catalysts for alkenes have been described in recent years and we, therefore, tested a number of those catalysts including (1) Rh(II) triflate, (2) cyclopentadienyldicarbonyl(tetrahydrofuran) iron(II) tetrafluoroborate $Fe(Cp)(THF)(CO)_2{}^+BF_4{}^-$, (3) copper(I) triflate toluene complex $(CF_3SO_3Cu)_2{\cdot}C_6H_5CH_3$, (4) toluene-dispersed copper powder with 10 µm particle size, (5) triphenylphosphine-copper(I) hydride hexamer $[(C_6H_5)_3PCuH]_6$, (6) rhodium(II) acetate $[Rh(CH_3COO)_2]_2$, (7) triisopropylphosphite-copper(I) chloride $CuCl{\cdot}P(O\text{-}i\text{-}Pr)_3$, (8) bis(benzonitrile)palladium(II) dichloride $PdCl_2{\cdot}2PhCN$, and commercially-available rhodium carbonyls such as (9) $Rh_4(CO)_{12}$, (10) $Rh_4(CO)_{12}$, and (11) $Rh_2(CO)_4Cl_2$. After many trials we found rhodium carbonyl, $Rh_6(CO)_{16}$, to be the most appropriate catalyst, as it provided the SWNT cyclopropanation with coverage of 12-28 groups·nm^{-1} of SWNT length. The degree of coverage depends upon the reaction time and the amount of the catalyst. The reaction also appears to be influenced by the choice of the solvent, with toluene yielding the densest coverage, and dichlorobenzene the least.

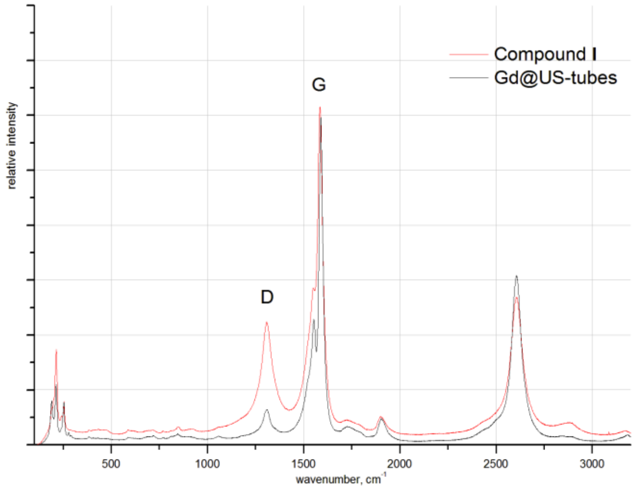


Fig. S 1. Raman spectra of the gadonanotubes and compound **I** with two bands, called G- (around 1590 cm^{-1}) and D- (around 1350 cm^{-1}). Since the intensity of the D-band is proportional to the types of carbon other than sp^2-carbons, it provides information about the degree of sidewall functionalization.

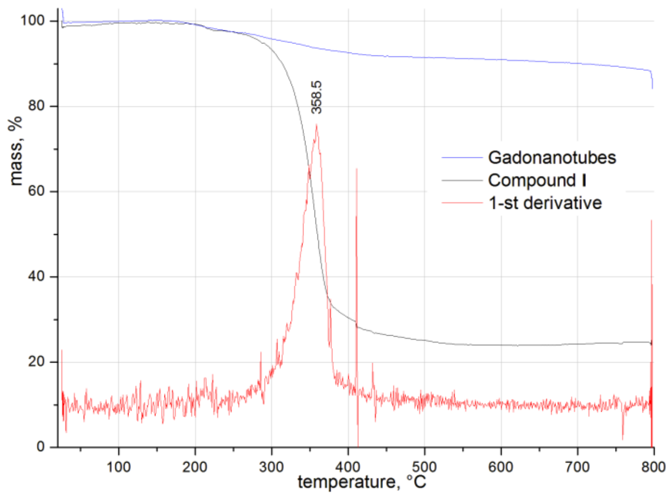


Fig. S 2. Typical TGA decomposition data for compound **I** under Ar. Unfunctionalized gadonanotubes TGA showed for comparison.

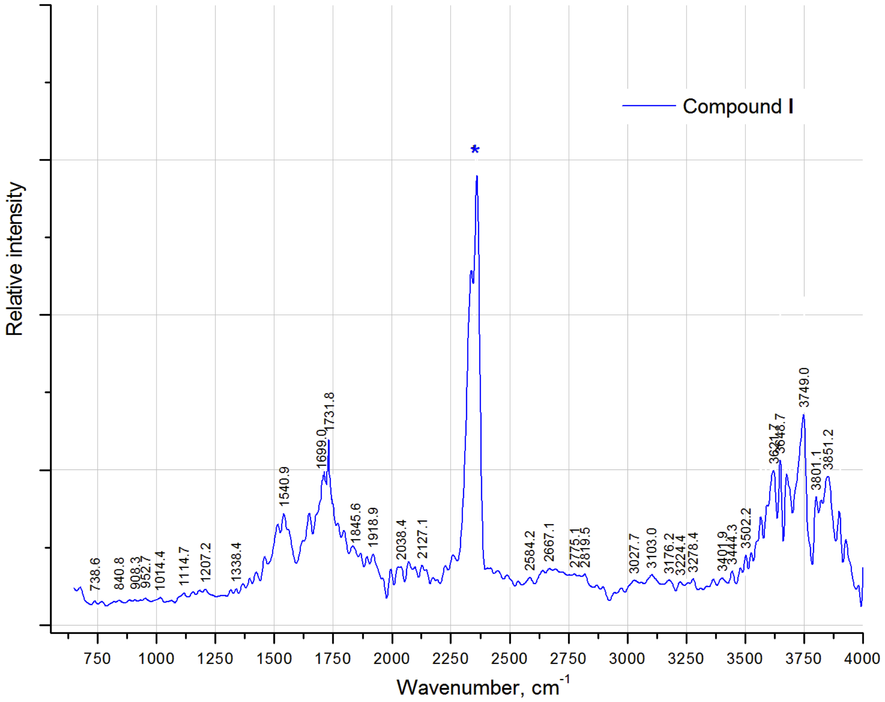


Fig. S 3. Baseline-corrected FT IR spectrum of **I** (nujol oil dispersion, peaks, labeled with * originate from oil background subtraction). The 1732 cm^{-1} peak corresponds to the C=O stretching of the ester group of compound **I**.

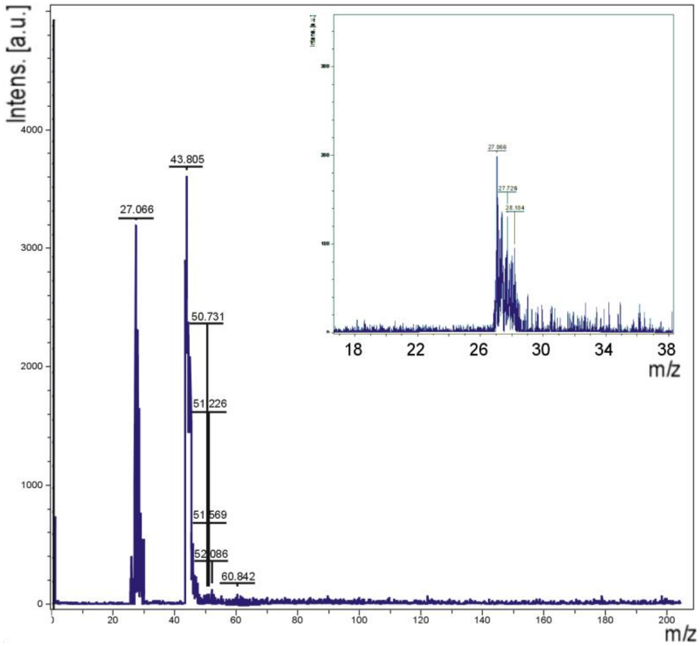
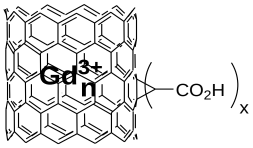


Fig. S 4. MS spectrum of compound **I**, showing peaks at m/z = 27-28 (for $CH_2{=}CH^+$ and $^{\bullet}CH_2\text{-}CH_2^+$ originating from ethyl groups), and 44-45 (for CO_2^+ and CO_2H^+ originating from the carboxyl groups).



II

<u>1H-cyclopropan-Gd@US-tube-carboxylic acid (**II**)</u>. 30 mg of purified I, dried under N_2(g) at 100° C for 12 hours, were dispersed in 20 ml of anhydrous CCl_4 by sonication, 0.1 ml of hexamethyldisilane (0.5 mmol) was then added, followed by 63 mg of I_2(s) (0.25 mmol). The suspension was stirred under nitrogen at 60° C for 1 day. All steps, excluding sonication, were performed in the drybox. After this, the Gd@US-tube intermediate was collected by filtration, washed with methanol and water, and resuspended in water and sonicated for another 2 hours, washed repeatedly with water and then dried under nitrogen at RT. The Gd@US-tube acid (**II**) was characterized by Raman spectroscopy (Fig. S 5), MS (Fig. S 6), FTIR (Fig. S 7), and by TGA (Fig. S 8). Compound **II** decomposes at ca. 330° C.

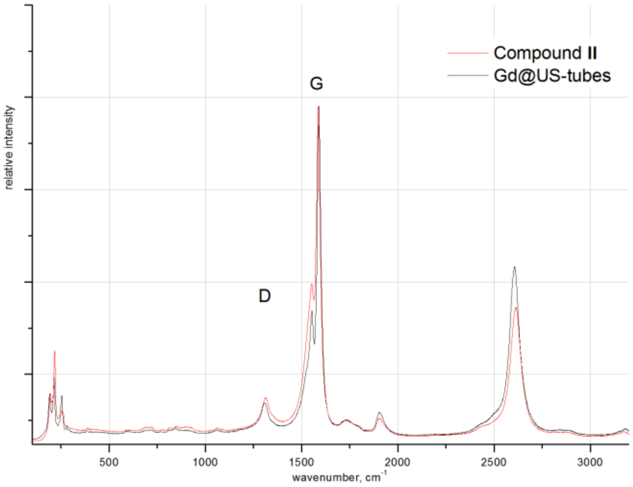


Fig. S 5. Raman spectra of the gadonanotubes and compound **II**, showing basically the same ratio between sp^2 and sp^3-types of carbon.

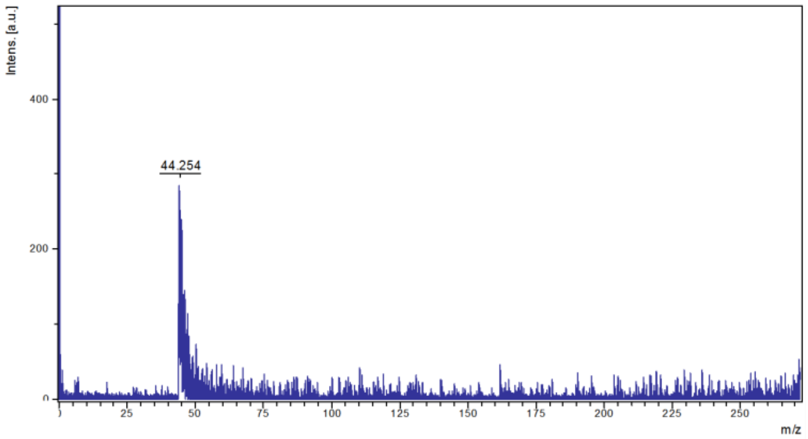


Fig. S 6. MS spectrum of compound **II**, showing major peak at m/z = 44-45 (for CO_2^+ and CO_2H^+ originating from the carboxyl groups).

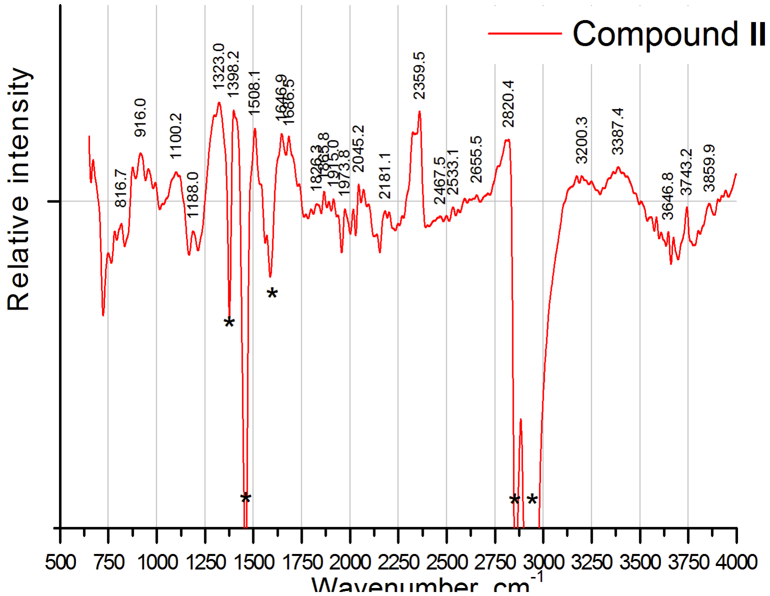


Fig. S 7. Baseline-corrected FT IR spectrum of **II** (ground with KBr crystals; peaks labeled with * originate from background subtraction). 1647 and 1687 cm^{-1} peaks correspond to the C=O stretching of the carboxylic groups.

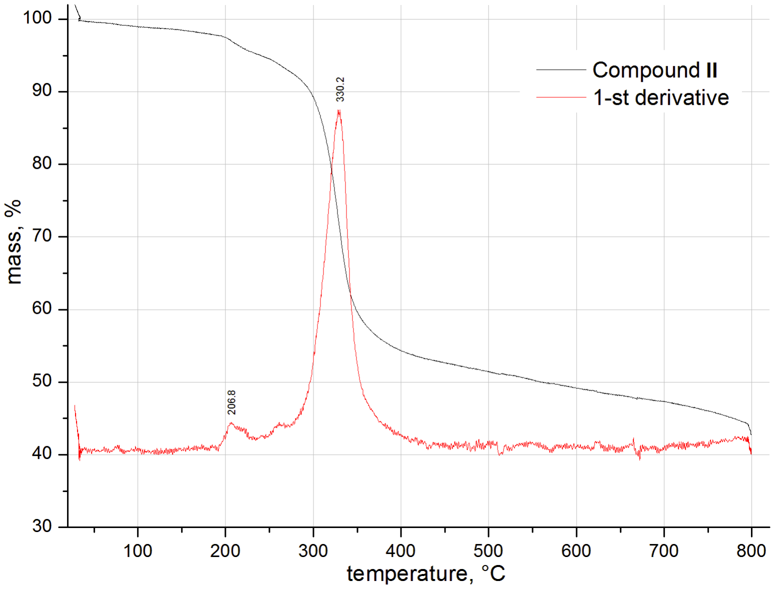
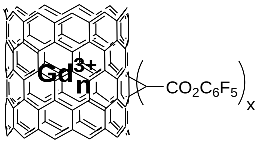
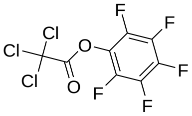


Fig. S 8. Typical TGA decomposition data for compound **II** under Ar.

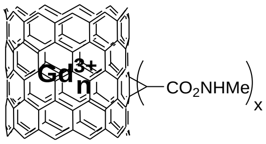


III

Pentafluorophenyl 1H-cyclopropan-Gd@US-tube-1-carboxylate (**III**). 10 mg of purified **II**, dried in the vacuum dessicator at RT for 3 days, was dispersed in 10 ml of anhydrous dioxane with bath sonication, 0.33 g of perfluorophenyl 2,2,2-trichloroacetate was added followed by 0.1 ml of anhydrous Et_3N. The sealed vial with the suspension was sonicated at 50° C for 2 hours and then stirred at the same temperature for 2 days. The Gd@US-tube derivative was collected by filtration and washed with dioxane on a PTFE filter. For the syntheses below, **III** was used suspended in dioxane. The compound must be stored under moisture-free conditions.



Perfluorophenyl α,α,α-trichloroacetate was prepared from trichloroacetyl chloride and pentafluorophenol according to [10]. Instead of recrystallyzing from hexane, as recommended, the product was sublimed onto a cold glass surface in vacuum (at 1-2 mm Hg) at 60° C. The compound should be stored under argon and direct sunlight should be avoided (turns yellow).



IVa

1H-cyclopropan-Gd@US-tube-carboxylic acid N-methylamide (**IVa**). 2 mg of purified **III** was suspended in dioxane (~2 ml) and 1 ml of a 2M solution of $MeNH_2$ in THF was then added. The sealed vial with the suspension was sonicated at 50° C for 2 hours, and then stirred at the same temperature for 2 hours. After this, the Gd@US-tube product was collected by filtration, washed with methanol and water, resuspended in water and sonicated again for few seconds, collected by filtration again and then dried under nitrogen at RT. The Gd@US-tube compound **IVa** was characterized by TGA (Fig. S 9) and Raman spectroscopy (Fig. S 10). Decomposition occurs at ca. 345° C. The coverage from 3 different samples was 12 to 19 groups nm^{-1} (TGA data), and 19 groups nm^{-1} (from XPS, average of 10 measurements, atomic ratio C : N=90 : 9.9).

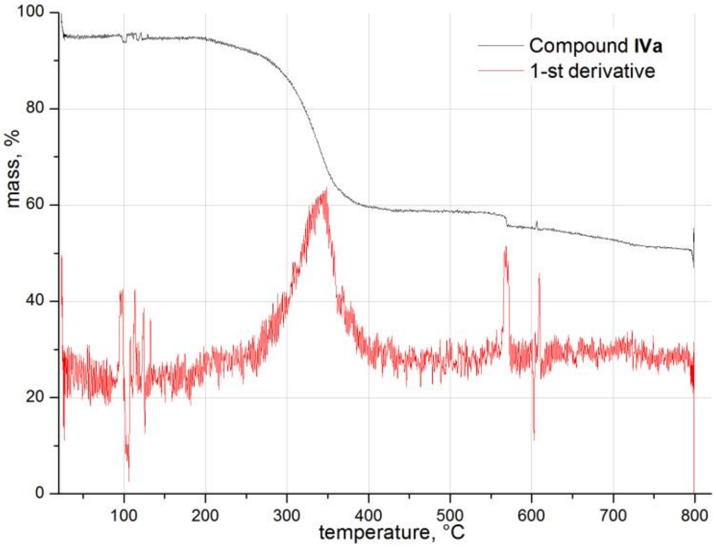


Fig. S 9. Typical TGA decomposition data for compound **IVa** under Ar.

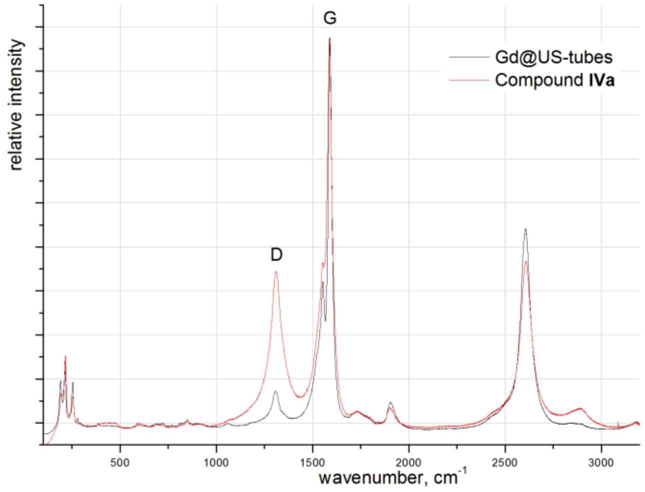
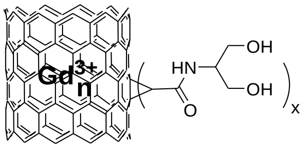


Fig. S 10. Raman spectra of the gadonanotubes (black) and compound **IVa** (red).



IVb

1H-cyclopropan-Gd@US-tube N-(1,3-dihydroxypropan-2-yl)-carboxamide (**IVb**, Serinol derivative). To 2 mg of purified **III**, suspended in dioxane (~2 ml), 50 mg of serinol (2-aminopropane-1,3-diol) was added in 1 ml of dioxane. The sealed vial with the suspension was sonicated at 50° C for 2 hours, then stirred at the same temperature for 4 hours, then the treatment cycle was repeated (sonication and stirring). After this, the Gd@US-tube product was collected by filtration, washed with methanol and water, resuspended in water and sonicated for a few seconds, filtered again and then dried under nitrogen at RT. The Gd@US-tube amide (**IVb**) was characterized by TGA (Fig. S 11) and Raman spectroscopy (Fig. S 12). The decomposition of compound **IVb** occurs at ca. 350° C. The coverage from 3 different samples was 8 to 12 groups nm^{-1} (TGA data), and 4 groups nm^{-1} (from XPS, average of 10 measurements, atomic ratio C : N=83 : 2.5).

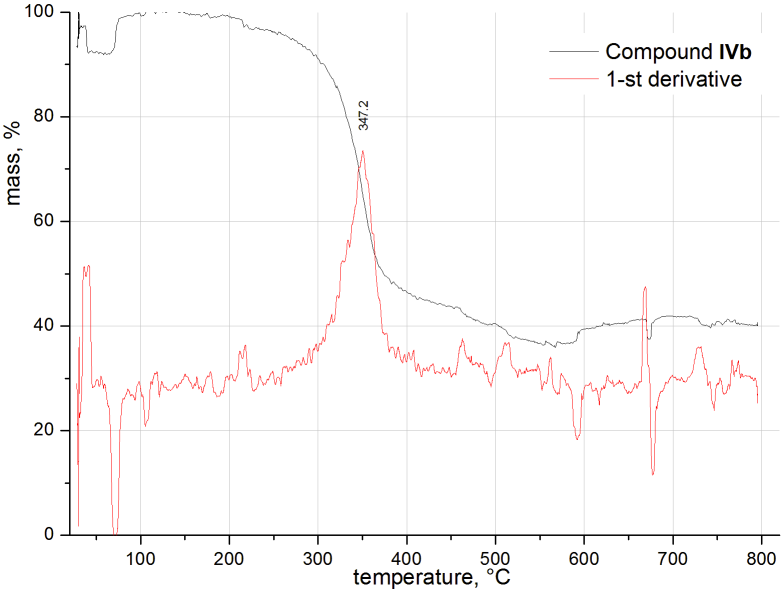


Fig. S 11. Typical TGA decomposition data for compound **IVb** under Ar.

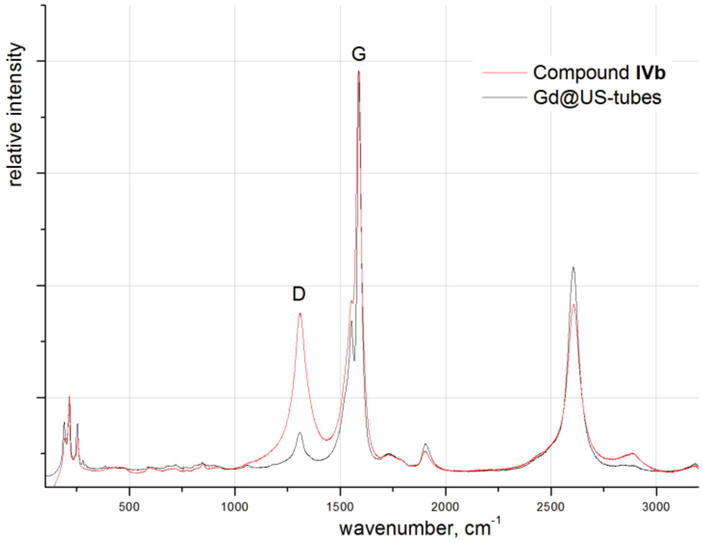
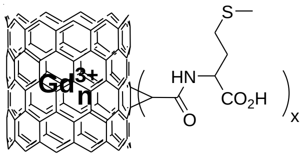


Fig. S 12. Raman spectra of the gadonanotubes (black) and compound **IVb** (red).



IVc

1H-cyclopropan-Gd@US-tube N-(1-carboxy-3-(methylthio)propyl)-carboxamide (**IVc**, Methionine derivative). 2 mg of purified **III**, suspended in dioxane (~2 ml), was added to a solution of 150 mg of L-methionine (*S*-2-amino-4-(methylthio)butanoic acid) in 2 ml of DMF : Et_3N (4 : 1) mixture. The sealed vial with the suspension was sonicated at 50° C for 2 hours, then stirred at the same temperature for 4 hours, then the treatment cycle was repeated (sonication and stirring). After this, the Gd@US-tube product was collected by filtration, washed with methanol and water, resuspended in water and sonicated for few seconds, filtered again and then dried under nitrogen at RT. The Gd@US-tube methionine derivative (**IVc**) was characterized by TGA (Fig. S 13) and Raman spectroscopy (Fig. S 14). Compound **IVc** decomposes at ca. 270° C. The coverage from 3 different samples was 9 to 19 groups nm^{-1} (TGA data), and 20 groups nm^{-1} (from XPS, average of 10 measurements, atomic ratio C : N : S = 64 : 5.1 : 4.9).

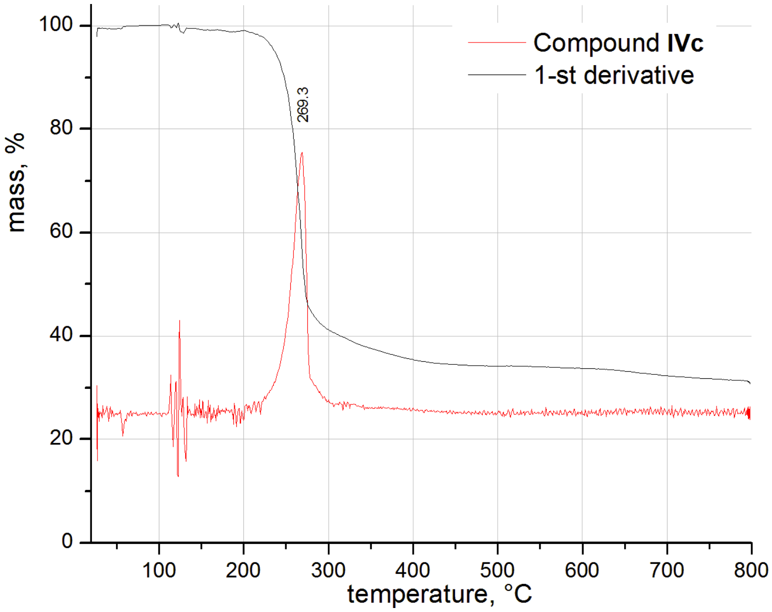


Fig. S 13. Typical TGA decomposition data for compound **IVc** under Ar.

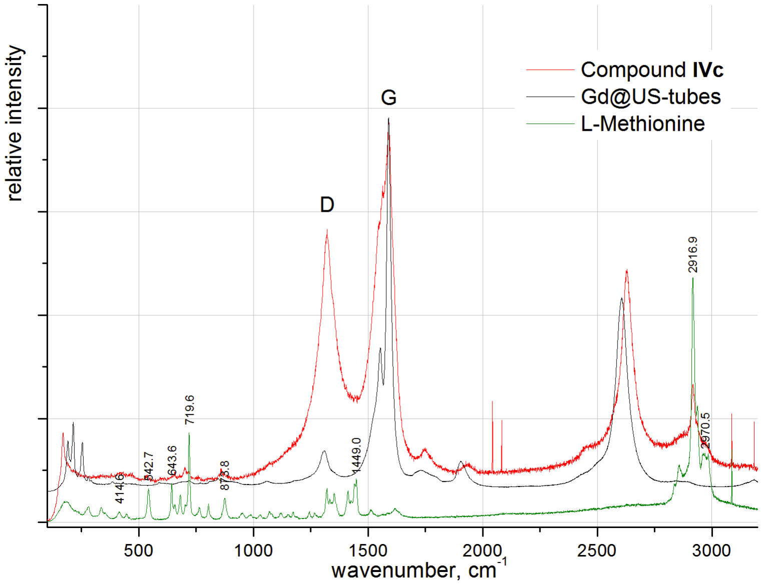
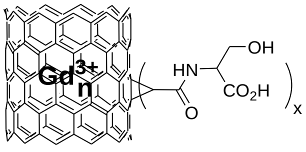


Fig. S 14. Raman spectra of the gadonanotubes (black) and compound **IVc** (red) with G- and D-bands. The spectra have been normalized using the G band. "Breathing modes" observed in the gadonanotube spectrum in the range 100-250 cm^{-1} are not present in compound **IVc** due to the debundling caused by sidewall functionalization. Some bands from L-Met found within the range of 540 to 720 cm^{-1} are also noticeable in the spectrum of compound **IVc** (bands at 542.7, 643.6 and 719.6 cm^{-1} due to S-CH_3 stretching, 873.8 cm^{-1} to the C-C skeletal stretch, 1449 cm^{-1} to the CH_3 deformation).



IVd

1H-cyclopropan-Gd@US-tube N-(1-Carboxy-2-hydroxy-ethyl-carboxamide), (**IVd**, Serine derivative). 2 mg of purified **III**, suspended in dioxane (~2 ml), was added to a solution of 150 mg of DL-serine (*2-Amino-3-hydroxypropionic acid*) in 2 ml of DMF : Et_3N (4:1) mixture. The sealed vial with the suspension was sonicated at 50° C for 2 hours, then stirred at the same temperature for 4 hours, then the treatment cycle was repeated (sonication and stirring). After this, the Gd@US-tube product was collected by filtration, washed with methanol and water, resuspended in water and sonicated for a few seconds, filtered again and then dried under nitrogen at RT. The Gd@US-tube serine derivative (**IVd**) has been characterized by FTIR (Fig. S 15), Raman spectroscopy (Fig. S 16) and by TGA (Fig. S 17, decomposition at ca. 240° C). The coverage from different samples was 12 to 16 groups nm^{-1} (from TGA data), and 14 groups nm^{-1} (from XPS, average of 10 measurements, atomic ratio C : N = 93 : 7.2).

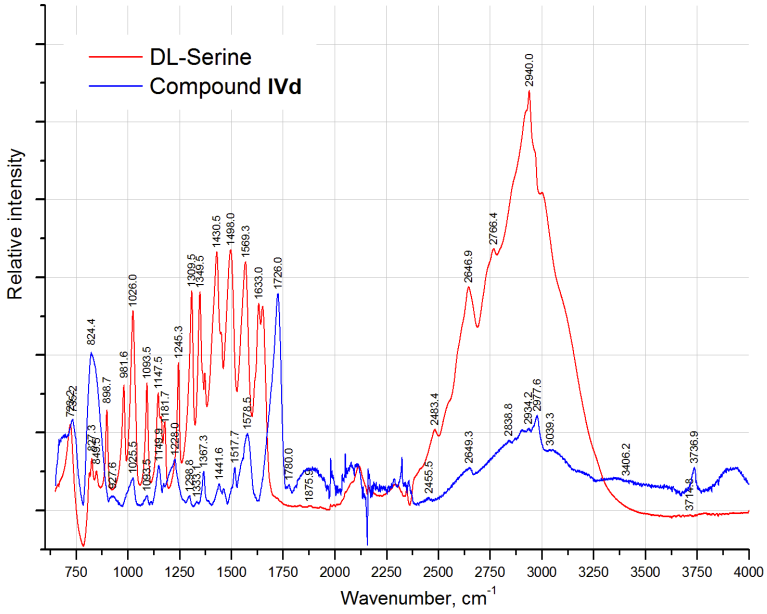


Fig. S 15. Baseline-corrected FT IR spectra of DL-serine and compound **IVd** (nujol oil dispersion, peaks, labeled with * originate from oil background subtraction). 1442 cm^{-1} peak in **IVd** corresponds to CH_2 scissoring of the -CH_2OH group of Ser, 1228 cm^{-1} - to CH_2 wagging; the group of peaks corresponding to CH_2 rocking, clearly visible in the spectrum of DL-serine at 815-900 cm^{-1}, appears as n intence broad peak in the spectrum of **IVd** at 800-870 cm^{-1}. The wide peaks around 2900-3000 cm^{-1}, clearly visible in both spectra, corresponds to the O-H stretching of the carboxylic groups of DL-serine.

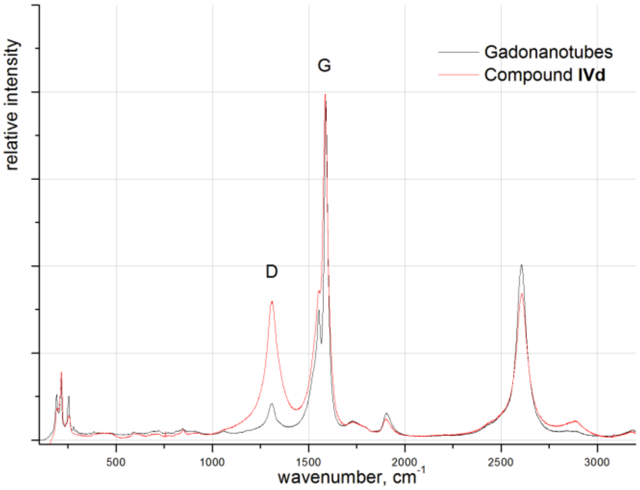


Fig. S 16. Raman spectra of the gadonanotubes (black) and compound **IVd** (red). The G/D band intensity ratio decreased from 8 to 2.6 upon formation of the serine derivative, which indicates the higher degree of disorder of the carbon nanotube two-dimensional π-type electron system.

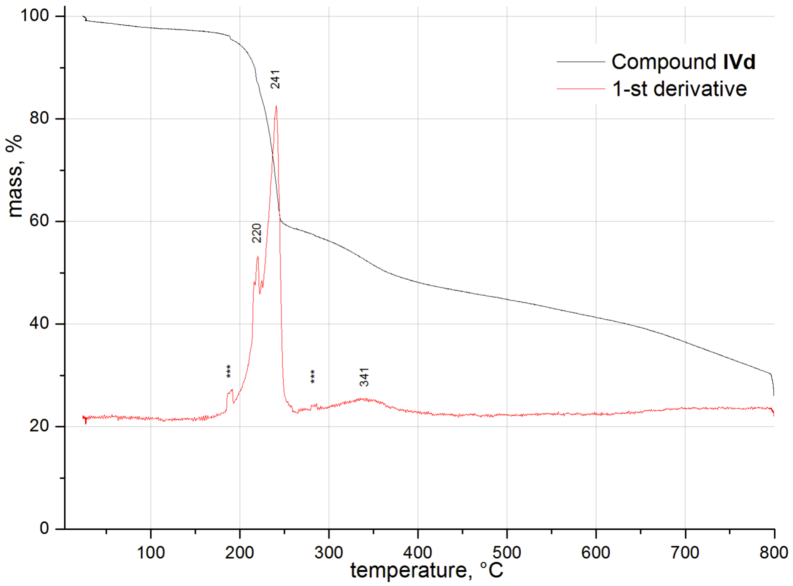
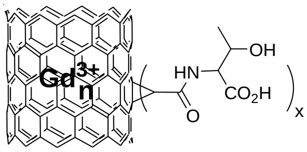


Fig. S 17. Typical TGA decomposition data for compound **IVd** under Ar.



IVe

1H-cyclopropan-Gd@US-tube N-(1-Carboxy-2-hydroxy-propyl-carboxamide), (**IVe**, Threonine derivative). 2 mg of purified **III**, suspended in dioxane (~2 ml), was added to a solution of 150 mg of DL-threonine (*2-Amino-3-hydroxy-butyric acid*) in 2 ml of DMF : Et$_3$N (4:1) mixture. The sealed vial with the suspension was sonicated at 50° C for 2 hours, then stirred at the same temperature for 4 hours, then the treatment cycle was repeated (sonication and stirring). After this, the Gd@US-tube product was collected by filtration, washed with methanol and water, resuspended in water and sonicated for a few seconds, filtered again and then dried under nitrogen at RT. The Gd@US-tube threonine derivative (**IVe**) has been characterized by TGA (Fig. S 18, decomposition at ca. 320° C), FTIR (Fig. S 19) and Raman spectroscopy (Fig. S 20). The coverage from different samples was 8 to 10 groups nm^{-1} (from TGA data), and 10 groups nm^{-1} (from XPS, average of 10 measurements, atomic ratio C : N = 77 : 4.5).

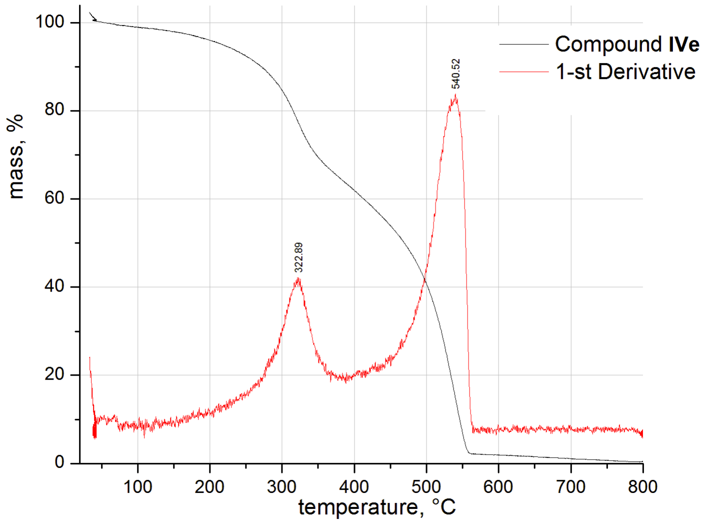


Fig. S 18. Typical TGA decomposition data for compound **IVe** under air.

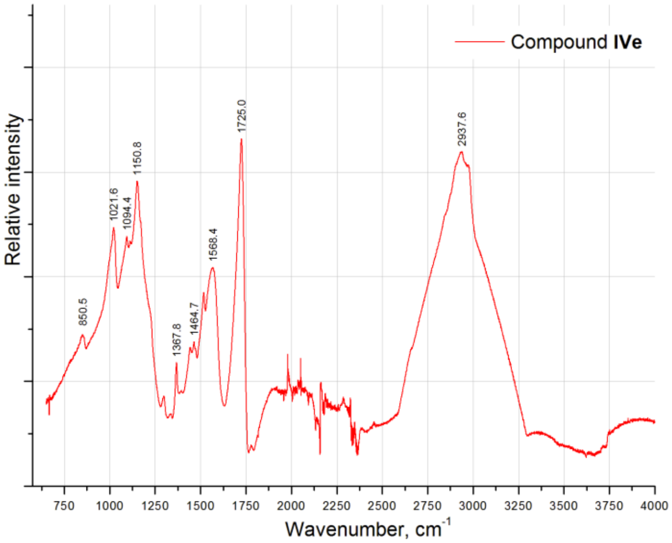


Fig. S 19. Baseline-corrected FT IR spectrum of compound **IVe** (nujol oil dispersion, peaks, labeled with * originate from oil background subtraction). The wide peaks around 2900-3000 cm^{-1} corresponds to the O-H stretching of the carboxylic groups.

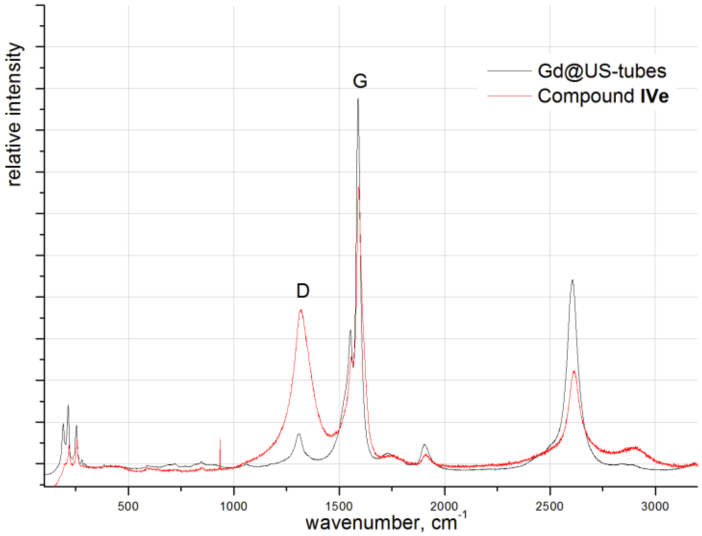
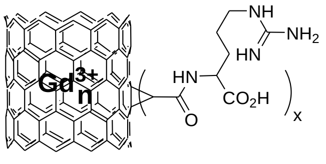


Fig. S 20. Raman spectra of the gadonanotubes (black) and compound **IVe** (red).



IVf

2-[(1H-cyclopropan-Gd@US-tube -1-carbonyl)-amino]-5-guanidino-pentanoic acid (**IVf**, L-Arginine derivative). 2 mg of purified **III**, suspended in dioxane (~2 ml), was added a solution of 150 mg of L-Arginine (L-*2-Amino-5-guanidino-pentanoic acid*) in 2 ml of DMF : Et_3N (4:1) mixture. The sealed vial with the suspension was sonicated at 50° C for 2 hours, then stirred at the same temperature for 4 hours, then the treatment cycle was repeated (sonication and stirring). After this, the SWNT product was collected by filtration, washed with methanol and water, suspended in 25 ml of water and sonicated for few seconds and then filtered again and dried under nitrogen at RT. The compound **IVf** was characterized by TGA (Fig. S 21, with a two-step decomposition, at ca. 240 and at 350° C) and IR spectroscopy (Fig. S 22). The coverage from 3 different samples was 9 to 19 groups nm^{-1} (TGA data), and 20 groups nm^{-1} (from XPS, average of 10 measurements, atomic ratio C : N = 77 : 23).

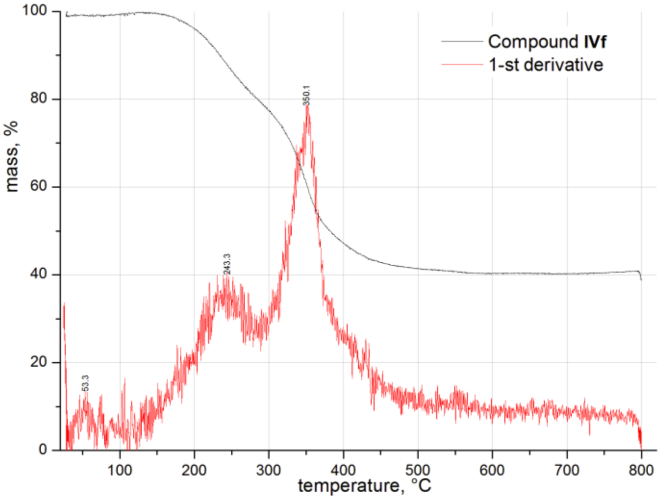


Fig. S 21. Typical TGA decomposition data for compound **IVf** under Ar.

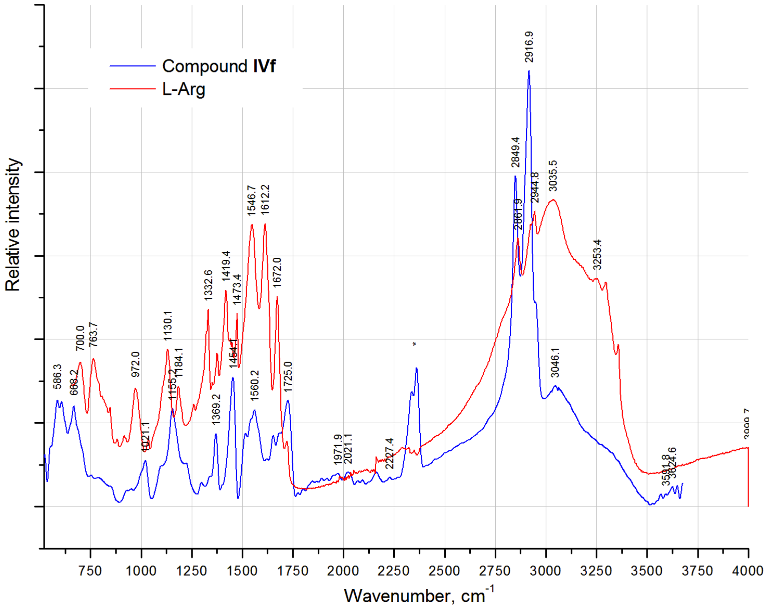
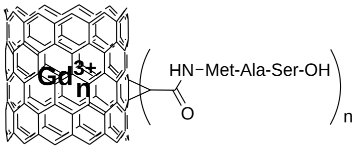


Fig. S 22. Baseline corrected FT IR spectra of L-arginine and compound **IVf** (nujol oil dispersion, peaks, labeled with * originate from oil background subtraction). A broad envelope is seen in both spectra between 2500 and 3400 cm^{-1} because of the hydrogen interaction with other atoms such as N–H stretching of NH_2 and C–H stretching of CH_2. It is observed due to the deprotonation of the CO_2H and H^+ shifted to amino group $[(NH_3)]^+$] and to guanidyl group $[(NH_2)_2C^+NH]$. Carboxylic group C=O stretching bands are visible at 1725 and 1672 cm^{-1}, peaks due to CH_2 wagging (around 1333 cm^{-1}) and CH_2 rocking (around 970 cm^{-1}), N-H in-plane bending (around 1547 cm^{-1}) are also seen in both spectra.



IVg

Gd@US-tube-Met-Ala-Ser derivative, **IVg**. 2 mg of purified **III**, suspended in dioxane (~2 ml), was added a solution of 150 mg of Met-Ala-Ser tripeptide in 2 ml of DMF : Et_3N (6:1) mixture. The sealed vial with the suspension was sonicated at 50° C for 2 hours, then stirred at the same temperature for 4 hours, then the treatment cycle was repeated (sonication and stirring). After this, the SWNT product was collected by filtration, washed with methanol and water, suspended in 25 ml of water and sonicated for few seconds and then filtered again and dried under nitrogen at RT. The compound **IVg** was characterized by TGA (Fig. S 21, with a two-step decomposition: at ca. 240 and at 350° C) and Raman spectroscopy (Fig. S 23). The coverage from 3 different samples was 9 to 19 groups nm^{-1} (from TGA data), and 19 groups nm^{-1} (from XPS, average of 10 measurements, atomic ratio C : N : S = 81 : 13 : 4.8).

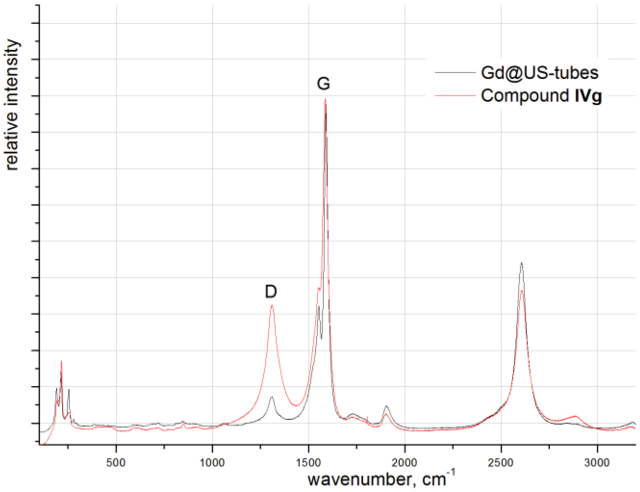


Fig. S 23. Raman spectra of the gadonanotubes (black) and compound **IVg** (red).

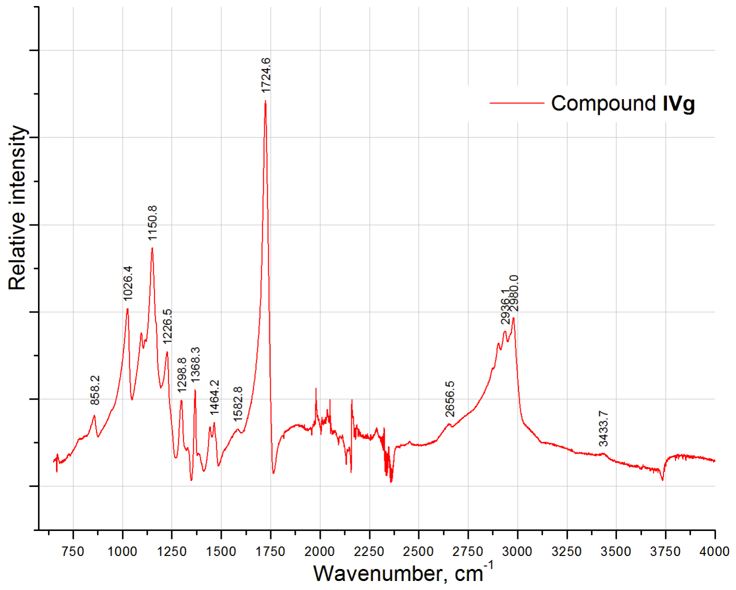
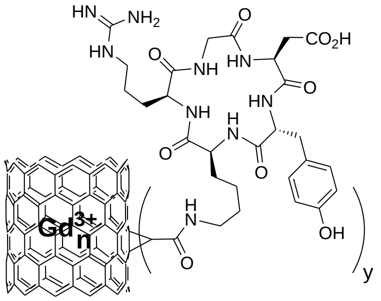
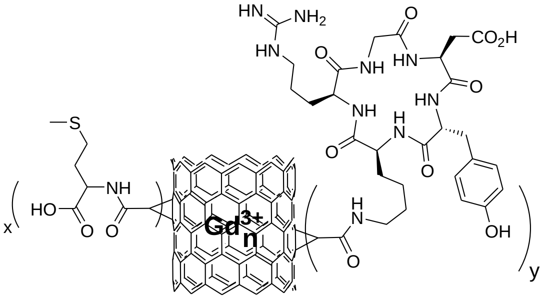


Fig. S 24. Baseline corrected FT IR spectrum of compound **IVg** (nujol oil dispersion, peaks, labeled with * originate from oil background subtraction).



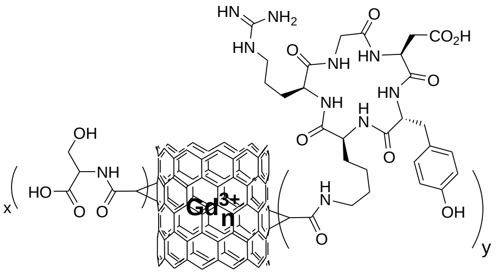
Va

Gd@US-tube-RGD derivative (**Va**). To 1 mg of purified **III**, suspended in dioxane (~1 ml), was added to a solution of 100 mg of RGD (*[8Z-(4-Amino-butyl)-11Z-(3-guanidino-propyl)-5E-(4-hydroxy-benzyl)-3,6,9,12,15-pentaoxo-1,4,7,10,13-pentaaza-cyclopentadec-2Z-yl]-acetic acid*) in 1 ml of DMF : Et$_3$N (4:1) mixture. The sealed vial with the suspension was sonicated at 35° C for 3 hours, then stirred at the same temperature for 12 hours, then the treatment cycle was repeated (sonication and stirring). After this, the Gd@US-tube product was collected by filtration, washed with methanol and water, resuspended in water and sonicated for few seconds, filtered again and then dried under nitrogen at RT. The Gd@US-tube RGD derivative (**Va**) from 3 different samples was 0.65 to 1 groups·nm^{-1} (TGA data), and up to 1 group·nm^{-1} (from XPS, average of 10 measurements for every sample, atomic ratio C : N= 15 : 1).



Vb

Gd@US-tube-RGD-Met derivative (**Vb**). To 1 mg of purified **III**, suspended in dioxane (~1 ml), was added to a solution of 100 mg of RGD in 1 ml of DMF : Et$_3$N (4:1) mixture. The sealed vial with the suspension was sonicated at 35° C for 3 hours, then stirred at the same temperature for 12 hours, then the treatment cycle was repeated (sonication and stirring). After this, the Gd@US-tube product was collected by filtration, again suspended in dioxane (~1 ml), was added to a solution of 150 mg of methionine (in 2 ml of DMF : Et$_3$N (4:1) mixture. The sealed vial with the suspension was sonicated at 35° C for 3 hours, then stirred at the same temperature for 12 hours, then the treatment cycle was repeated (sonication and stirring). After this, the Gd@US-tube product was collected by filtration, washed with methanol and water, resuspended in water and sonicated for few seconds, filtered again and then dried under nitrogen at RT. All steps prior to the methanol wash were performed under inert gas. The Gd@US-tube-RGD-Met derivative (**Vb**) from 3 different samples had *x* between 8 to 12 group nm^{-1}, and *y* up to 1 group nm^{-1} (from XPS, average of 10 measurements, atomic ratio C : N : S = 87 : 8.2 : 4.6).



Vc

Gd@US-tube-RGD-Ser derivative (**Vc**). To 1 mg of purified **III**, suspended in dioxane (~1 ml), was added to a solution of 100 mg of RGD in 1 ml of DMF : Et$_3$N (4:1) mixture. The sealed vial with the suspension was sonicated at 35° C for 3 hours, then stirred at the same temperature for 12 hours, then the treatment cycle was repeated (sonication and stirring). After this, the Gd@US-tube product was collected by filtration, again suspended in dioxane (~1 ml), was added to a solution of 150 mg of DL-serine (in 2 ml of

DMF : Et_3N (4:1) mixture. The sealed vial with the suspension was sonicated at 35° C for 3 hours, then stirred at the same temperature for 12 hours, then the treatment cycle was repeated (sonication and stirring). After this, the Gd@US-tube product was collected by filtration, washed with methanol and water, resuspended in water and sonicated for few seconds, filtered again and then dried under nitrogen at RT. All steps prior to the methanol wash were performed under inert gas. The Gd@US-tube-RGD-Ser derivative (**Vc**) from 3 different samples had x between 8 to 12 group nm^{-1}, and y up to 1 group nm^{-1} (from XPS, average of 10 measurements, atomic ratio C : N = 9 : 1, from TGA data the coverage was 12 to 16 groups nm^{-1}). Compound **Vc** was characterized by TGA (Fig. S 25).

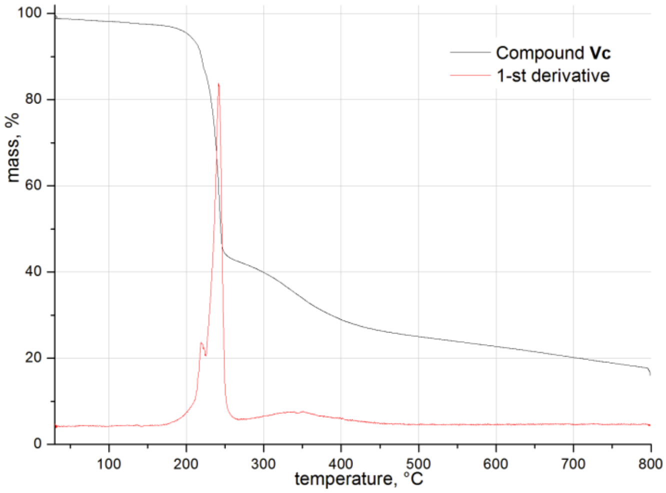


Fig. S 25. Typical TGA decomposition data for compound **Vc** under Ar.

Using TGA data to estimate the nanotube functionalization degree

We assumed that all functionalized gadonanotubes prepared in current work, decompose at elevated temperatures, resulting in loss of surface functionalities eventually leaving behind pristine gadonanotubes; in other words, the weight loss was provided by the loss of groups, attached to the exterior side. As an example, on Fig. S 2 the 1-st derivative of the TGA decomposition curve provides information about decomposition range – we may notice that the decomposition of compound I starts about 310° C, and ends about 380° C. The sample lost ~65% of its weight in this range (unfunctionalized gadonanotubes have only ~1% lost in this range). Assuming that 1 nm of length of typical HiP_{CO} nanotube has an average of 100 carbon atoms (depending upon typical diameter and geometry of HiP_{CO} nanotubes), and molecular weight of ~1200, the weight of side ester groups should be ~2200, or 25.9 groups·nm^{-1} of SWNT length. The accuracy of this method is limited by the inability to determine the exact temperatures when decomposition starts and ends, and also by inaccuracy of the TGA instrument.