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Synthesis of Water-Soluble PEGylated Single-Walled Carbon Nanotubes

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Carbon nanotube salts prepared by treating single-wall carbon nanotubes (SWNTs) with lithium in liquid ammonia react with *ω*-bromocarboxylic acids to yield sidewall-derivatized SWNTs. These can be reacted with amine-terminated polyethyleneglycol (PEG) chains (e.g., $H_2N-mPEG-OMe$, MW = 5000 Da) to yield water-soluble PEGylated SWNTs. Short nanotubes (average length ∼ 60 nm) functionalized by PEGylation of the carboxylic acid groups at the end of the nanotubes exhibit greater solubility in water than the longer (average length ∼ 300 nm) sidewall-PEGylated pristine nanotubes.

Introduction

Single-walled carbon nanotubes (SWNTs) promise to play an important role in the emerging field of biological nanotechnology.[1−3] However, applications have been impeded by low solubility of the nanotubes in water, a medium that is essential for studies in the presence of live cells. One route to soluble nanotubes involves chemical functionalization, either at the ends[4] or at the sidewalls[5−8] of the nanotubes. Polymeric functionalities[9−12] have also been employed to obtain soluble dispersions of carbon nanotubes. In this study, we report that carbon nanotube salts[13,14] react with *ω*-bromocarboxylic acids to yield functionalized SWNTs that can be PEGylated by amine-terminated PEG chains to give water-soluble nanotubes.[15]

Experimental Section

Materials. The SWNTs were prepared by a gas-phase process using carbon monoxide as the feedstock and iron pentacarbonyl as the catalyst (HiPco process). The crude SWNTs were purified as reported by Xu et al.[16] Studies were performed using both purified (average length ∼ 300 nm, residual metal less than 2% after purification) and “cut tubes” (average length ∼ 60 nm). The cut tubes were prepared by the controlled oxidation of purified SWNTs using a piranha[17] solution (4:1, v/v, 96% H_2SO_4/30% H_2O_2). Under these conditions cutting occurs at damaged (defect) sites.

Lithium (granules, 99%), DMAP (4-dimethylaminopyridine), DCC (*N,N′*-dicyclohexyl-carbodiimide), and *ω*-bromocarboxylic acids (*n* = 2, 4, 9, 10) were purchased from Aldrich. $H_2N-mPEG-OMe$ (MW = 5000 Da) was purchased from Nektar. The solvents used for the PEGylation reactions (DMF, DMSO) were dried according to literature procedure.[18]

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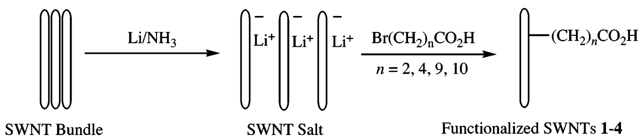
10.1021/cm0611082 CCC: $33.50

Scheme 1



General Procedure for the Synthesis of Carboxylic Acid Derivatized SWNTs. The derivatization reactions were carried out by adding SWNTs (4.17 mmol) under an atmosphere of argon to a dry 500 mL three neck round-bottomed flask fitted with a dry ice condenser. Ammonia (300 mL) was then condensed into the flask followed by the addition of lithium metal (41.7 mmol). The ω-bromocarboxylic acid[19] (12.5 mmol) was then added and the mixture stirred for 12 h as the ammonia evaporated. The flask was then cooled in an ice bath and the reaction mixture quenched by the slow addition of ethanol (15 mL) followed by water (20 mL). The reaction mixture was acidified with 10% HCl and the nanotubes were extracted into hexane and washed several times with water. The hexane layer was then filtered through a 0.2 μm PTFE membrane and washed successively with ethanol and chloroform. The functionalized SWNTs were dried overnight in vacuo at 80 °C.

HNO_3 Acid Treatment of SWNTs.[20] Fifty milligrams of purified SWNTs was refluxed overnight at 90 °C in 60 mL of 3 M nitric acid. The resulting mixture was cooled to room temperature, diluted with DI water, and collected by filtration using a 0.22 μm pore-sized polycarbonate membrane. The final product was washed thoroughly with DI water and dried overnight in vacuo at 50 °C.

General Procedure for PEGylation. In a typical procedure,[21] the carboxylic acid functionalized SWNTs (3.3 mmol) were dissolved in DMF (20 mL) and sonicated for ~10 min to achieve a homogeneous dispersion. DMAP (3.3 mol) in DMF (5 mL) and $H_2N-mPEG-OMe$ (MW = 5000 Da, 5×10^{-5} mmol) in DMF (10 mL) were then added to the reaction mixture. DCC (3.6 mmol) dissolved in a mixture of DMF (10 mL) and DMSO (15 mL) was added dropwise to this mixture over 1 h and the reaction mixture stirred at room temperature for 48 h. The solution was filtered through a 0.2 μm PTFE membrane and washed several times with chloroform. The product was then dried overnight in vacuo at 50 °C.

Characterization. The functionalized SWNTs were characterized by Raman spectroscopy, thermogravimetric analyses (TGA), FT-IR spectroscopy with ATR accessory, X-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM), and transmission electron microscopy (TEM). Raman spectra of solid samples were recorded using a Renishaw 1000 microraman system equipped with a 780 nm excitation laser source. Multiple spectra (3−5) were obtained, normalized to the G band, and averaged to give a comprehensive snapshot of the material. TGA experiments were carried out under argon using a SDT 2960 TA instrument. Samples were degassed at 80 °C and then heated at 10 °C/min to 800 °C and held there for 30 min. FT-IR spectra were obtained using a Nicolet spectrometer with the ATR accessory. XPS data were obtained using a physical electronics (PHI QUANTERA) XPS/ESCA system. The base pressure was at 5×10^{-9} Torr. A monochromatic Al X-ray source at 100 W was used with pass energy of 26 eV and with a 45° take-off angle. The beam diameter was 100.0 μm. Low-resolution survey scans and higher resolution scans of C, N, and O were taken. At least two separate locations were analyzed for each sample. AFM images were taken of an aqueous solution, spin-coated onto mica, using a Digital Instrument Nanoscope IIIa in tapping mode with a 3045 JVW piezo tube scanner. The tapping frequency was between 270 and 310 kHz. A TEM image was obtained using a JEOL 2010F instrument operating at 200 kV.

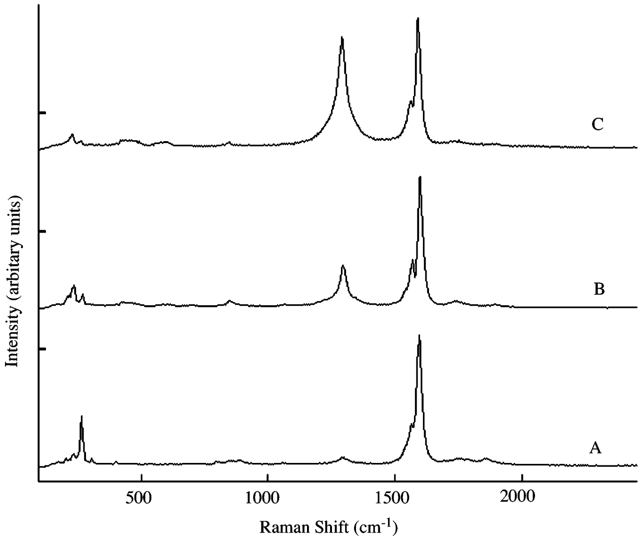


Figure 1. Raman spectra (780 nm excitation) of (A) purified SWNTs, (B) $SWNT(CH_2)_4COOH$, and (C) $SWNT(CH_2)_9COOH$.

Table 1. Weight Loss of Different Compounds Determined by TGA Analyses in Argon from 80 to 800 °C

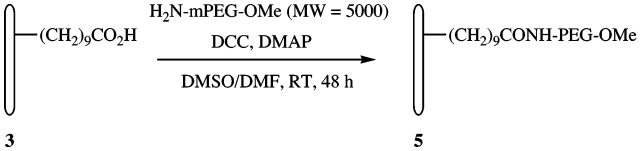
compounds	% weight loss observed	carbon/functional group ratio
$SWNT(CH_2)_2COOH$	13	40:1
$SWNT(CH_2)_4COOH$	14	46:1
$SWNT(CH_2)_9COOH$	28	25:1
$SWNT(CH_2)_{10}COOH$	26	28:1

Results and Discussion

Carbon nanotube salts can be reacted with ω-bromocarboxylic acids to yield SWNTs functionalized by carboxylic acids. Examples are presented in Scheme 1. As observed previously for alkylation[13] and arylation[14] reactions of carbon nanotube salts, nearly complete debundling of the SWNT ropes (bundles) is observed. This results in a high level of coverage by the radicals that are formed by electron transfer from the nanotube salts to the bromo acids. The functionalized SWNTs were used as starting materials for the PEGylation reactions that are described in this manuscript.

The SWNTs were characterized by Raman spectroscopy, TGA, and infrared spectroscopy (FT-IR/ATR). Evidence for covalent functionalization can be determined by inspection of the Raman spectra. As shown in Figure 1A, the Raman spectrum of the purified SWNTs exhibit a tangential mode (G band) at 1590 cm^{-1} and a weak disorder mode at 1290 cm^{-1} (D band) that probably arises from defects formed

Scheme 2



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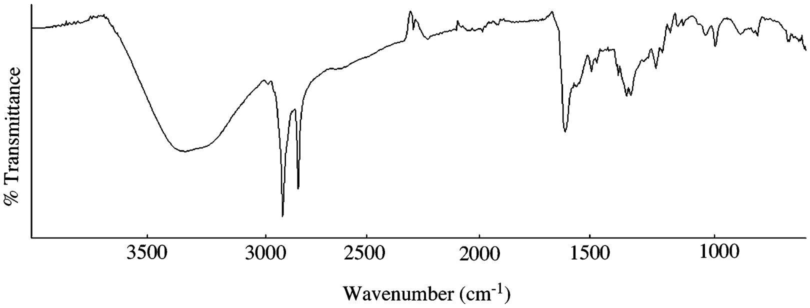


Figure 2. FT-IR spectrum (with ATR accessory) of functionalized SWNTs **3** ($n = 9$).

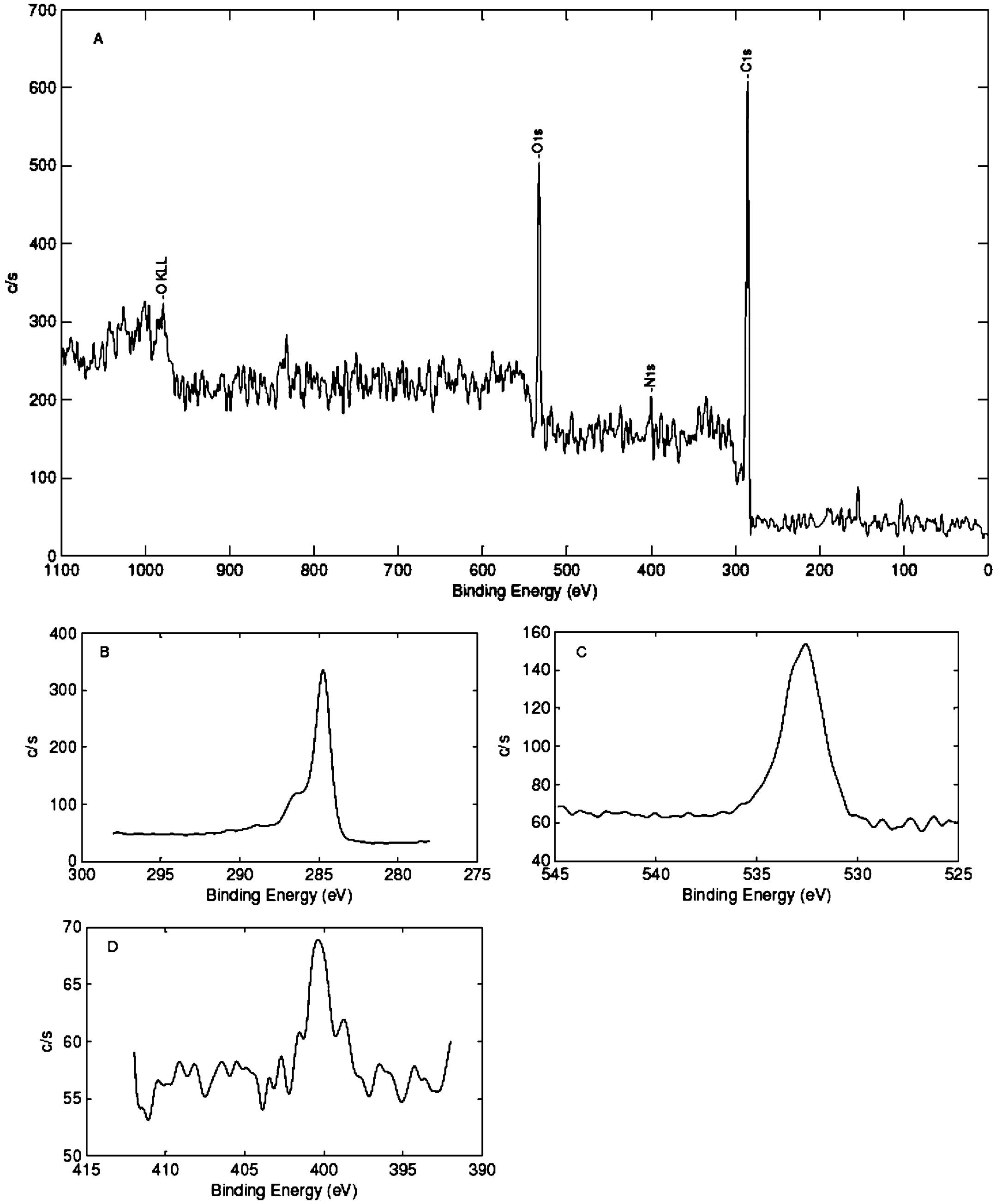
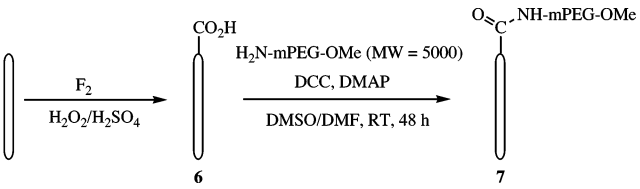


Figure 3. XPS of PEGylated SWNTs **5**. (A) Survey scans of the spectral region from 0 to 1100 eV; (B) the carbon 1s region, 76.7%; (C) the oxygen 1s region, 17.5%; and (D) the nitrogen 1s region, 5.8% (atomic percentage).

during either the synthesis or purification of the nanotubes. After functionalization, the relative intensity of the D band at 1290 cm^{-1} was observed to increase, confirming covalent attachment of the functional groups (Figures 1B and 1C). The larger D/G ratio for SWNT(CH$_2$)$_9$COOH implies that the reaction with Br(CH$_2$)$_9$COOH leads to a higher level of functionalization.

The carbon/functional group ratios determined by TGA analysis on the basis of weight loss of degassed samples are presented in Table 1. The results of these experiments are

Scheme 3



in agreement with the Raman spectra (Figure 1), confirming that a higher level of functionalization is realized when Br(CH_2)$_9$COOH and Br(CH_2)$_{10}$COOH are used.

The infrared spectrum of SWNT(CH_2)$_9$COOH is presented in Figure 2. The sp^3 C−H stretching absorptions that arise from the alkyl chain appear at ∼2840 and 2950 cm^{-1}. Absorptions assigned to the carboxylic acid group include the broad peak corresponding to the −OH group centered at 3350 cm^{-1} and the carbonyl absorptions at 1670 cm^{-1}.

PEGylation Reactions. PEGylation of **1**−**4** was carried out as illustrated in Scheme 2 for SWNT(CH_2)$_9$COOH.

The FT-IR spectrum of **5** exhibits the carbonyl stretching absorption at 1625 cm^{-1}. The shift to lower frequency is characteristic of amides.[22] The sp^3 C−H stretching frequency bands appear at 2850−3000 cm^{-1}.

X-ray photoelectron spectroscopy (XPS) provides direct evidence for the covalent linkage of nitrogen during the PEGylation reaction. XPS spectra of the region corresponding to 0−1100 eV are presented in Figure 3. Survey scans of **5** (Figure 3A) show the presence of carbon, nitrogen, and oxygen. The SWNT-functional group ratios obtained from XPS analysis compare favorably with those obtained from the TGA measurements. The high-resolution C 1s XPS spectrum (Figure 3B) shows two different binding energies. A small hump between 284 and 290 eV arises from overlap of four different but similar binding energies of carbon [C═O (287.8 eV), C−O (284.8 and 286.9 eV for polyethylene oxide), O═C−OH (289.1 eV), O═C−NH (288.4 eV)]. The O 1s XPS spectrum (Figure 3C) with a binding energy of 532.6 eV is shifted significantly verses other carboxylic acid functional groups (NIST XPS database). A distinct peak (Figure 3D) for nitrogen 1s at 399.7 eV (NIST XPS database) confirms the presence of the amide bond in the functionalized SWNTs.

Unfortunately, visual inspection of aqueous solutions of the PEGylated SWNTs shows only moderate solubility. Since the lengths of the starting SWNTs may affect solubility, PEGylation reactions of “cut” HiPco SWNTs were investigated with the expectation that the shortened tubes will exhibit greater solubility in water. The SWNTs used for this study were partially fluorinated to introduce defects and then cut using H_2O_2/H_2SO_4 solutions (Scheme 3) as described earlier.[16] Since this process involves a highly oxidizing medium in which the SWNTs are cut at defect sites, carboxylic acid groups are expected to reside at the ends of the tubes. Thus, “cut” tubes can be expected to yield water-soluble SWNTs by PEGylation of the carboxylic acid groups that are introduced during the cutting process. Indeed, water-soluble material was obtained when the cut tubes were PEGylated as illustrated in the scheme. The PEGylated cut-SWNTs show no indication of flocculation (Figure 4) after several weeks, whereas a solution of the purified pristine SWNTs tends to flocculate a few minutes after sonication.

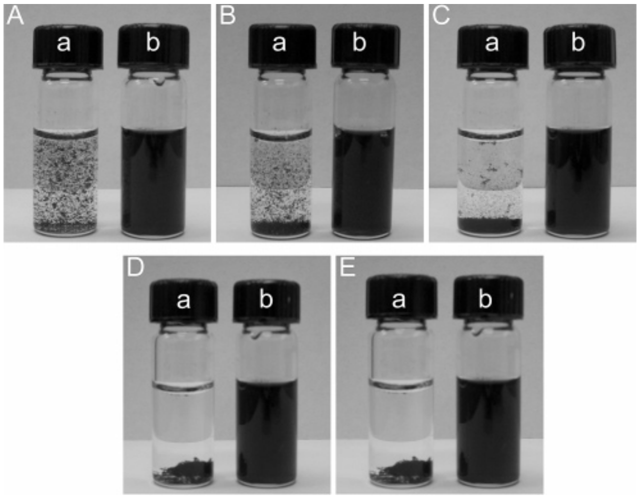


Figure 4. Photographs of (a) pristine SWNTs and (b) PEGylated cut-SWNT **7** in water. (A) Immediately after sonication, (B) after 10 min, (C) after 1 h, (D) after 24 h, and (E) after 10 days.

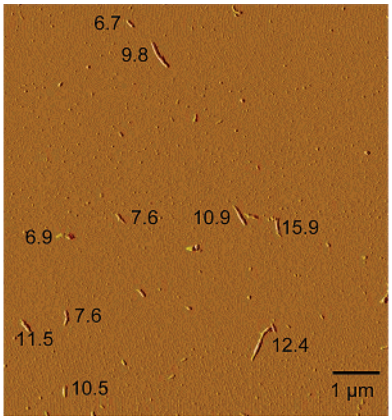


Figure 5. AFM images (amplitude) of PEGylated cut-SWNTs **7** spin-coated onto mica from water. The section analysis shows the height data in nanometers of the functionalized SWNTs. The vertical distance (shown as numbers, in nanometers) vary from ∼7 to 15 nm, indicating encapsulation of the nanotube core in a PEG shell.

Microscopy. Atomic force microscopy (AFM) images of the soluble SWNTs were recorded by spin coating small drops of the aqueous solution onto mica at room temperature. Figure 5 shows a tapping mode AFM image of compound **7**. The average diameter of ∼10 nm is attributed to encapsulation of the nanotube core into a PEG shell. PEGylated CNTs were termed “PEG-Eggs” by the late Richard Smalley.

Cut nanotubes that are more highly functionalized by PEG groups could be prepared as illustrated in Scheme 4. Average diameters of ∼8−10 nm were determined from the AFM images (Figure 6) of **9** (n = 2, 4).

Samples of **9** that have been dissolved in water are shown in Figure 7. The darker color of C indicates a higher level of solubility. This may be attributed to the longer chain length (n = 4) of the carboxylic acid that was used to functionalize the SWNTs.[23]

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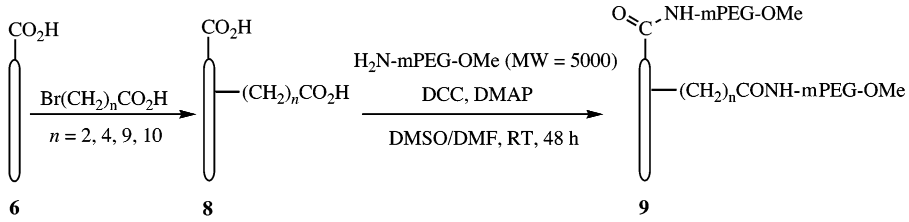
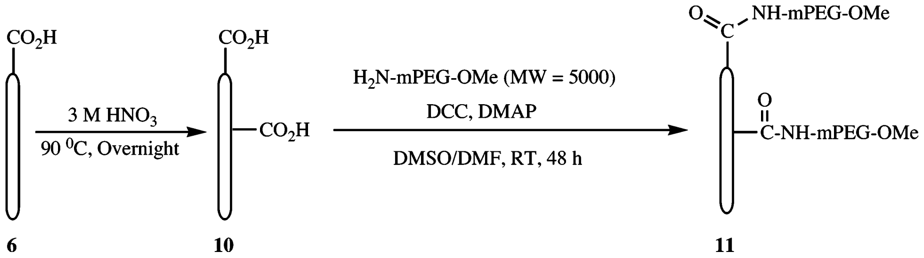
Scheme 4



Scheme 5



Cut nanotubes that have been oxidized by 3 M HNO_3[19] (Scheme 5) exhibit a high degree of solubility in water after PEGylation. The AFM image (Figure 8) of the PEGylated material was found to have an average diameter of ∼6−8 nm. These ultrashort (60 nm or less) nanotubes cannot be resolved easily by atomic force microscopy which has a tip scale of ∼30 nm.[24] However, the high-resolution cryo transmission electron microscope (cryo-TEM) image that is presented in Figure 1 (Supporting Information, SI 1) shows that most of the ultra short nanotubes are shorter than 60 nm.[25]

The PEGylated SWNTs prepared using Schemes 3, 4, and 5 are soluble in water at pH 3−11. The SWNTs precipitate below pH 3 or upon addition of sodium hydroxide (pH >11). We attribute this behavior to hydrolysis of the amide bonds that would occur readily under either acidic or basic conditions.

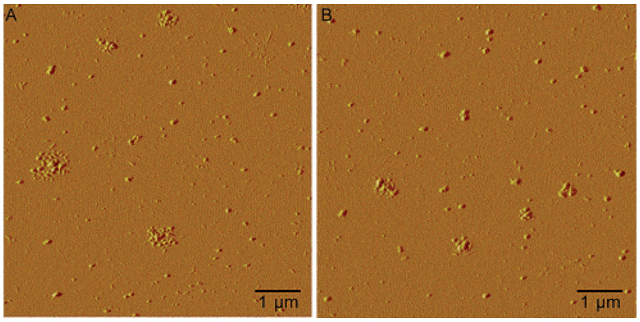


Figure 6. AFM images (amplitude) of PEGylated cut-SWNTs **9.** (A) $n = 2$ and (B) $n = 4$, spin-coated onto mica from an aqueous solution.

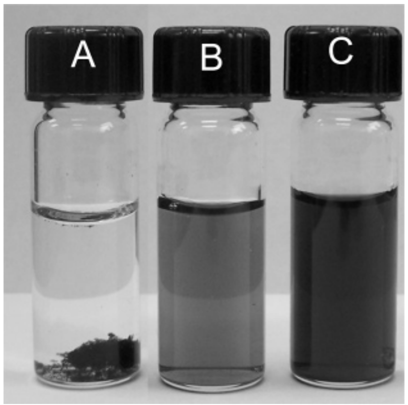


Figure 7. Photograph of (A) purified SWNTs, (B) PEGylated cut-SWNTs **9** ($n = 2$), and (C) PEGylated cut-SWNTs **9** ($n = 4$) in water. Solutions were allowed to stand for 10 days.

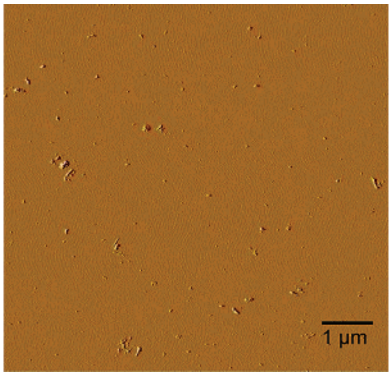


Figure 8. AFM image (amplitude) of PEGylated **11** spin-coated onto mica from an aqueous solution.

Conclusion

In summary, we describe an efficient route to carbon nanotubes that are functionalized by carboxylic acid groups. We further establish the aqueous solubility of the PEGylated derivatives of these sidewall-functionalized SWNTs. We are currently investigating applications of these water-soluble PEGylated SWNTs in a variety of medical applications including biocompatibility under physiologically relevant conditions.

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Supporting Information Available: (SI-1) Cryo-TEM image of compound **11**. This material is available free of charge via the Internet at http://pubs.acs.org.

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[25] A few were observed to be longer (100−200 nm).