Published in final edited form as:

Carbohydr Res. 2015 March 20; 405: 33–38. doi:10.1016/j.carres.2014.09.006.

Carbohydrate Conjugation through Microwave-Assisted Functionalization of Single-Walled Carbon Nanotubes Using Perfluorophenyl Azides

Na Kong^a, Manishkumar R. Shimpi^a, Jae Hyeung Park^b, Olof Ramström^a, and Mingdi Yan^{a,b}

Olof Ramström: ramstrom@kth.se; Mingdi Yan: Mingdi_Yan@uml.edu

^aKTH - Royal Institute of Technology, Department of Chemistry, Teknikringen 30, S-10044 Stockholm, Sweden

^bDepartment of Chemistry, University of Massachusetts Lowell, 1 University Ave., Lowell, MA 01854, United States

Abstract

Carbohydrate-functionalized single-walled carbon nanotubes (SWNTs) were synthesized using microwave-assisted reaction of perfluorophenyl azide with the nanotubes. The results showed that microwave radiation provides a rapid and effective means to covalently attach carbohydrates to SWNTs, producing carbohydrate-SWNT conjugates for biorecognition. The carbohydrate-functionalized SWNTs were furthermore shown to interact specifically with cognate carbohydrate-specific proteins (lectins), resulting in predicted recognition patterns. The carbohydrate-presenting SWNTs constitute a new platform for sensitive protein- or cell recognition, which pave the way for glycoconjugated carbon nanomaterials in biorecognition applications.

Keywords

Single-walled carbon nanotubes; microwave; perfluorophenyl azide; covalent functionalization; carbohydrate; lectin

Introduction

Single-walled carbon nanotubes have attracted much attention from a fundamental physics/chemistry perspective, as well as for applied fields such as nanoelectronics and nanomedicine. This is in part due to their unusual properties resulting from the pseudo-one-dimensional nanosize and the unique structure. However, several disadvantages limit their

© 2014 Published by Elsevier Ltd.

Correspondence to: Olof Ramström, ramstrom@kth.se; Mingdi Yan, Mingdi_Yan@uml.edu.

Supporting Information

Synthesis of Man-EG₂-NH₂ and Gal-EG₂-NH₂, additional Raman and characterization data.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

biological applications, such as poor water solubility, lack of reactive functionalities, and potentially high cytotoxicity.^{1, 2} An effective way to overcome these limitations is to introduce an organic coating on the carbon materials, for example, by using polar structures such as carbohydrates.^{3–7} Functionalization of carbon nanotubes (CNTs) with carbohydrates not only increases the biocompatibility and solubility, but also introduces molecular recognition features, which can impact cellular interactions and uptake of CNTs. These features of carbohydrate-functionalized carbon nanomaterials have been demonstrated in biomedical applications such as biosensing and drug delivery.^{3–5} For example, Zhang et al. wrapped SWNTs with boronic acid-derivatized phenoxy dextran. The resulting complex demonstrated a concentration-dependent riboflavin recognition resulting from the redshift of emission upon addition of riboflavin, which could be reversed by the addition of a riboflavin-binding protein.⁸ Torres and coworkers covalently functionalized fewer-layer graphene and SWNTs with α -D-mannosyl dendrons, and used the resulting glyco-SWNTs for the selective interaction with lectin.⁹ In the work of Sun and coworkers, monosaccharide-modified SWNTs were used to effectively bind *B. anthracis* spores in the presence of Ca²⁺.^{10, 11}

For CNTs and graphene, the most common way to achieve covalent functionalization is to use the oxidized forms. Oxidation generates oxygen-containing functional groups at the material surfaces, such as epoxides and carboxylic acids, which can subsequently be used to conjugate various entities such as amine-functionalized carbohydrates.^{10–13} Oxidation, however, may lead to extensive structural damage of the carbon materials, impacting the lattices and intrinsic properties. Milder conjugation methods are therefore desired. A number of alternative reactions have been developed for the chemical functionalization of CNTs, including radical additions via diazonium salts, alkyl or aryl peroxides, carbene or nitrene cycloadditions, as well as electrophilic and nucleophilic addition reactions.^{14, 15} However, owing to the relatively low reactivities of the pristine carbon materials, these reaction often require lengthy reaction times at elevated temperatures.

In this context, microwave radiation has become a standard technique in organic synthesis, inducing molecular collisions with direct impact on the reagents of the reaction. The efficient heating and milder reaction conditions generally result in faster kinetics, higher reaction yields and lower side products as compared to the traditional heating protocol.^{16, 17} Microwave radiation has also been successfully used in the process for the chemical modification of CNTs and fullerenes.^{9, 18–20}

Organic azides provide a particularly attractive functionalization method related to carbon nanomaterials.^{21–26} Azides may thus undergo cycloaddition reactions with reactive alkenes, eventually resulting in aziridines.²⁷ Of particular interest is the fact that thermal or light activation of organic azides leads to nitrogen extrusion, generating nitrene intermediates that add to the sp² carbons of the carbon material, forming aziridine adducts. Perfluorophenyl azides (PFPA) are especially useful, being among the most reactive aryl azides,²⁸ and resulting in singlet perfluorophenyl nitrenes that have longer lifetime than other aryl nitrenes, thus promoting efficient covalent adduct formation.^{29, 30} We have for example shown that pristine graphene can be effectively functionalized with PFPA either photochemically or thermally.^{21–23} In addition, Fréchet and coworkers used a PFPA

structure bearing an ATRP initiator entity to functionalize SWNT forests, and then generated polymer brushes by *in situ* polymerization.³¹ Besides light and heat, PFPAs can also be activated by other energy sources such as electrons and X-rays.^{28, 32}

In this work, we investigated the potential for microwave-assisted functionalization of SWNTs by PFPA derivatives, in order to generate functional nanoplatforams for carbohydrate presentation. Following conjugation, the biorecognition properties of the resulting glyconanomaterials were evaluated from analysis of the cognate lectin binding.

Results and Discussion

As-prepared SWNTs are usually contaminated with a variety of impurities including residual metal catalysts and carbonaceous impurities such as amorphous carbon and carbon nanoparticles.³³ The degree of contamination and the percent impurity vary depending on the preparation method and the manufacturers. Of the three common synthesis methods used to produce SWNTs, i.e., chemical vapor deposition (CVD), laser ablation and arc discharge, the lower temperature CVD technique has become a preferred production method owing to the better control over nanotube alignment, size, purity and density.³⁴ The HiPco SWNTs sample used in this study was produced by CVD with 85wt% purity, and the purity was further inspected by TEM before use. The TEM image shows large ropes which were bundled nanotubes, as well as dark particles that were mostly residual catalysts coated with amorphous carbon (Figure 1A).^{33, 35} To remove these impurities, chemical oxidation and physical purification methods are employed to purify as-prepared CNTs. The chemical oxidation selectively oxidizes carbonaceous impurities, however, the method often leads to structure damage by the oxidizing agent. In order to avoid damage of the pristine SWNTs, a mild, non-oxidative purification method was preferred. Thus, a differential centrifugation protocol was adopted to remove the metal catalysts, carbonaceous impurities or heavy bundles based on their weight difference. This process resulted in improved sample purity and smaller nanotube bundles (Figure 1B).

To functionalize SWNTs, the reaction was first carried out by heating a suspension of SWNTs and PFPA-NHS in 1,2-dichlorobenzene (DCB) at 130 °C in an oil bath. The azide functionality, showing a characteristic IR absorption band at $\sim 2130\text{ cm}^{-1}$, was used as a convenient way to monitor the reaction by FTIR (indicated by arrows in Figure 2A). The reaction was in this case relatively sluggish, where the PFPA was converted in 3 days (Figure 2A). The reaction was subsequently carried out in a microwave reactor under the same conditions, resulting in almost complete conversion in 6 h. Upon increasing the temperature to 150 °C, the reaction proceeded considerable faster, resulting in only trace amount of PFPA after 1 h radiation (Figure 2B). No other changes could be detected, indicating that the components were not further affected under the reaction conditions. These microwave reaction conditions (150 °C, 1 h) were thus chosen for the subsequent studies.

Further evidence for the covalent functionalization was provided by Raman spectroscopy. Conversion of sp^2 carbons to sp^3 carbon atoms is expected to lead to an increase in the intensity of the disorder (D) band ($1250\sim 1450\text{ cm}^{-1}$) along with a decrease in the graphite

(G) band (1500–1605 cm^{-1}). The intensity ratio of the D and G bands, I_D/I_G , is thus commonly used to evaluate the degree of functionalization; the larger the value, the higher the degree of functionalization.³⁶ In addition, covalent functionalization of SWNTs would reduce the intensity of the radial breathing mode (RBM, between 100–300 cm^{-1}), and this band would completely disappear for SWNTs having high degrees of functionalization.¹¹ In the present study, the Raman spectra were recorded for SWNTs before and after functionalization with PFPA-NHS (Figure 3). The I_D/I_G values were calculated to be 0.15 and 0.43 for purified SWNTs and PFPA-NHS-functionalized SWNTs, respectively. The increase in I_D/I_G value observed for the modified SWNTs compared to that of pristine SWNTs is consistent with the covalent functionalization of the nanotube lattice.³⁷ SWNTs functionalized by the conventional thermal reaction and the microwave-assisted reaction gave similar I_D/I_G values (Table 1S, Supporting Information), and in both case the RBM signals almost completely disappeared, demonstrating high degrees of functionalization.

The functionalized SWNTs prepared using the microwave-assisted reaction were also evaluated for solvation by sonicating the samples in acetone and allowing them to settle overnight. The results were in this case clear, and NHS-PFPA-functionalized SWNTs dispersed well in acetone, whereas the unfunctionalized SWNTs precipitated out of the solution to a large degree (Figure 4A). Furthermore, large bundles were observed in the TEM micrographs of the purified SWNTs (Figure 1B), whereas smaller bundles were present after functionalization with PFPA-NHS, likely due to the improved solubility (Figure 4B).

With NHS-PFPA-functionalized SWNTs at hand, we next addressed the carbohydrate functionalization of the SWNTs. To demonstrate this feasibility, two carbohydrate derivatives, Man-EG₂-NH₂ and Gal-EG₂-NH₂ were allowed to react with the PFPA-NHS-functionalized SWNTs in DMF to give Man- and Gal-presenting SWNTs, respectively (Scheme 1). Conjugation of the carbohydrates to SWNTs drastically increased their dispersity in water. While the pristine SWNTs were completely insoluble, Man-SWNTs and Gal-SWNTs were readily dispersed in water yielding homogeneous dispersions (Figure 4C). The dispersions were stable for over one week without obvious precipitation. In addition, the carbohydrate-conjugated SWNTs displayed improved debundling, and no large aggregates were observed by TEM (Figure 4D) compared with unfunctionalized SWNTs (Figure 1B).

The biorecognition properties of the carbohydrate-presenting SWNTs were furthermore evaluated by treating the carbohydrate-conjugated SWNTs with fluorescein-labeled Concanavalin A (FITC-Con A), a lectin specific for α -D-mannopyranosides and to a lower extent to α -D-glucopyranosides.^{38, 39} Con A exists as a homotetramer at neutral pH, and may induce significant agglomeration of multivalent scaffolds owing to the mutual crosslinking effect. This property was also observed in the present case, and when Man-SWNTs were treated with FITC-Con A, the fluorescence intensity of the solution decreased (Figure 5). This can be attributed to the interactions between multiple copies of Man on the Man-SWNTs and FITC-Con A causing aggregation between the two interaction partners. Upon examination of the agglomerates by fluorescence microscopy, bright fluorescence was also observed (Figure 1S, Supporting Information). These effects were selective for the

mannosylated glyco-SWNTs, whereas, for comparison, the Gal-SWNTs produced no agglomeration effect with FITC-Con A under the same conditions (Figure 5).

Conclusions

In summary, we have demonstrated an effective method to conjugate carbohydrate structures to pristine SWNTs using microwave-assisted reaction of perfluorophenyl azides. This is a first example of applying microwave radiation to PFPA activation, representing a new way to synthesize carbon nanotube-based glyconanomaterials. Our method used pristine SWNTs instead of oxidized SWNTs, avoiding extensive alteration to the lattice and the intrinsic properties of SWNTs. The resulting materials were less bundled and well dispersed in water compared to pristine SWNTs. In addition, the conjugated carbohydrates retained their binding selectivity toward cognate lectins, and furthermore induced extensive agglomeration resulting from the crosslinking of multiple carbohydrate ligands with multimeric lectins. The method developed here can be readily adapted to conjugate a variety of biomolecules and biomaterials facilitating the use of CNTs materials in bioanalytical and biomedical applications.

Experimental

Materials

The SWNTs were obtained from Unidym (HiPco, Lot# P2150). Methyl pentafluorobenzoate, D-(+)-mannose, β -D-(+)-galactose pentaacetate, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), sodium metabisulfide ($\text{Na}_2\text{S}_2\text{O}_5$) and palladium (10% on carbon) were purchased from Alfa Aesar. Boron trifluoride ethyl etherate ($\text{BF}_3 \cdot \text{Et}_2\text{O}$), *N*-hydroxysuccinimide (NHS), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDAC·HCl), 1, 2-dichlorobenzene (DCB), and bovine serum albumin (BSA) were obtained from Sigma-Aldrich. Sodium azide (NaN_3) and sodium methoxide (CH_3ONa) were acquired from Fluka. 2-[2-(2-Chloroethoxy)-ethoxy]ethanol was obtained from TCI Japan. Concanavalin A (fluorescein conjugate, lectin from Jack bean) was purchased from Molecular Probes (Eugene, Oregon). All chemicals were used as received without purification. Water used was from a Milli-Q ultrapure water purification system. PFPA-NHS was synthesized following previously reported procedures.^{40, 41} Man-EG₂-NH₂ and Gal-EG₂-NH₂ were synthesized following previously established procedures (see Supporting Information for detailed synthesis and characterization).^{42–44}

Instrumentation

¹H and ¹³C NMR spectra were recorded on a Bruker DMX 500 instrument at 500 MHz (¹H) or 125 MHz (¹³C) in CDCl_3 or D_2O . Thin layer chromatography (TLC) was performed on pre-coated Cromatofolios AL silica gel 60 F254 plates (Merck). Column chromatography was performed using silica gel 60, 0.040–0.063 mm (SDS). Microwave reactions were carried out using a Personal Chemistry Smith Creator Microwave Assisted Organic Synthesizer (Biotage Sweden AB). Infrared spectra were collected on a Mettler Toledo ReactIR™ iC 10 in situ FTIR spectrometer (Columbia, MD). Raman spectra were obtained using a Bio-Rad FTS6000 Raman spectrometer with an NdYAg-laser (1064 nm) as the

excitation source. Fluorescence measurements were conducted on a Varian Cary Eclipse fluorescence spectrophotometer (Agilent Technologies). TEM images were obtained on a JEOL 100CX transmission electron microscope operating at an accelerating bias voltage of 100 kV. Fluorescence microscopy images were obtained on a Nikon Eclipse E600 Epi-fluorescence microscope.

Purification of SWNTs

In a sealed conical flask, SWNT fine powder (100 mg) was suspended in DMF (300 mL). The mixture was sonicated for 3 h in an ultrasound bath (Model 2510, BransonTM) to break up the bundles and separate SWNTs from the impurities. The suspension was allowed to settle for 3 days. The supernatant was collected and then centrifuged at 5,000 rpm for 30 min. The precipitate was removed and the supernatant was centrifuged at 8,000 rpm for 30 min. After removal of the precipitate, the supernatant was centrifuged at 12,000 rpm for 1 h. The precipitate was collected as purified SWNTs (~25 mg), which was used in the subsequent studies.

Functionalization of SWNTs and Conjugation of Carbohydrates

The SWNTs were functionalized and subsequently conjugated with carbohydrates as shown in Scheme 1. For the conventional thermal method, purified SWNTs (5.0 mg) and PFPA-NHS (138 mg) were dispersed in DCB (5.0 mL) in a 25 mL round-bottom flask. The mixture was sonicated for 15 min to give a visually homogenous solution, which was heated to 130 °C for 3 days under nitrogen.

The microwave-assisted reaction was carried out as follows. In a 10 mL glass tube (Biotage Pressurized Reaction Vials), purified SWNTs (5.0 mg) and PFPA-NHS (138 mg) were dispersed in DCB (3.0 mL). The mixture was sonicated for 15 min, and then sealed with a Teflon cap prior to microwave irradiation. A time series of reactions were carried out at 130 °C under microwave irradiation for 15 min, 30 min, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h. In addition, reactions were carried out under microwave irradiation at different temperature (130 °C, 150 °C, 180 °C) for 1 h. The reaction progress was monitored by taking the IR spectra of the reaction mixtures. After the reaction, the brown reaction mixture was centrifuged at 14,000 rpm for 30 min. The precipitate was washed sequentially with DMF and acetone (3 x 14,000 rpm, 30 min) to remove unreacted SWNTs and excess PFPA-NHS until no brown color was observed in the solution. The precipitate was finally dried under reduced pressure to give NHS-PFPA-functionalized SWNTs (~3.5 mg).

The modified SWNTs (3.5 mg) were mixed with Man-EG₂-NH₂ (2.7 mg, Scheme 1) in DMF (3.0 mL) and stirred at RT overnight, after which the reaction mixture was centrifuged at 14,000 rpm for 30 min. The precipitate was washed with H₂O (3 x) followed by acetone (14,000 rpm, 30 min), and the precipitate was dried under reduced pressure to give Man-SWNTs (~2.6 mg). Gal-conjugated SWNTs was prepared using the same procedure to give Gal-SWNTs (~2.6 mg). Samples for TEM imaging were prepared by dropping an ethanol solution containing the SWNTs sample (10 µL) onto a 200 mesh copper grid, and dried the sample in air.

Binding of Glyco-SWNTs with Lectin

FITC-Con A was used to evaluate the binding affinity and selectivity of the carbohydrate-functionalized SWNTs. Man-SWNTs or Gal-SWNTs (1.0 mg) were incubated in a solution of BSA (3%) in pH 7.2 HEPES buffer (1.0 mL, 10 mM) for 30 min, and subsequently centrifuged. The precipitate was then incubated in a fresh pH 7.2 HEPES buffer for another 20 min and centrifuged. The resulting Man-SWNTs or Gal-SWNTs were subsequently treated with a solution of FITC-Con A in HEPES buffer (2.0 mL, 10 µg/mL) containing MnCl₂ (1.0 mM) and CaCl₂ (1.0 mM) under ambient condition for 1 h while shaking. The mixture was centrifuged to separate the supernatant that contained unbound FITC-Con A, and the precipitate was washed with fresh HEPES buffer to remove physically adsorbed FITC-Con A from the treated nanotubes.⁴⁵

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The study was in part supported by the Royal Institute of Technology, the National Institutes of Health (R01GM080295), and the National Science Foundation (CHE-1112436). N.K. thanks the China Scholarship Council for a special scholarship award. M.S. acknowledges support from the European Commission through the Erasmus Mundus India4EU program.

References

1. Kolosnjaj J, Szwarc H, Moussa F. *Adv Exp Med Biol.* 2007; 620:181–204. [PubMed: 18217344]
2. Porter AE, Gass M, Muller K, Skepper JN, Midgley PA, Welland M. *Nature Nanotech.* 2007; 2:713–717.
3. Chen Y, Star A, Vidal S. *Chem Soc Rev.* 2013; 42:4532–4542. [PubMed: 23247183]
4. Gorityala BK, Ma J, Wang X, Chen P, Liu XW. *Chem Soc Rev.* 2010; 39:2925–2934. [PubMed: 20585681]
5. Bandaru NM, Voelcker NH. *J Mater Chem.* 2012; 22:8748–8758.
6. Bhoi VI, Kumar S, Murthy CN. *Carbohydr Res.* 2012; 359:120–127. [PubMed: 22925774]
7. Yang L, Zhang B, Liang Y, Yang B, Kong T, Zhang LM. *Carbohydr Res.* 2008; 343:2463–2467. [PubMed: 18653174]
8. Zhang J, Landry MP, Barone PW, Kim JH, Lin S, Ulissi ZW, Lin D, Mu B, Boghossian AA, Hilmer AJ, Rwei A, Hinckley AC, Kruss S, Shandell MA, Nair N, Blake S, en F, en S, Croy RG, Li D, Yum K, Ahn JH, Jin H, Heller DA, Essigmann JM, Blankschtein D, Strano MS. *Nature Nanotech.* 2013; 8:959–968.
9. Ragoussi ME, Casado S, Ribeiro-Viana R, De la Torre G, Rojo JR, Torres T. *Chem Sci.* 2013; 4:4035–4041.
10. Wang H, Gu L, Lin Y, Lu F, Meziani MJ, Luo PG, Wang W, Cao L, Sun YP. *J Am Chem Soc.* 2006; 128:13364–13365. [PubMed: 17031942]
11. Luo PG, Wang H, Gu L, Lu F, Lin Y, Christensen KA, Yang ST, Sun YP. *ACS nano.* 2009; 3:3909–3916. [PubMed: 19911831]
12. Gu L, Luo PG, Wang H, Meziani MJ, Lin Y, Veca LM, Cao L, Lu F, Wang X, Quinn R, Wang W, Zhang P, Lacher S, Sun YP. *Biomacromolecules.* 2008; 9:2408–2418. [PubMed: 18712920]
13. Gu L, Elkin T, Jiang X, Li H, Lin Y, Qu L, Tzeng TRJ, Joseph R, Sun YP. *Chem Commun.* 2005; 7:874–876.
14. Singh P, Campidelli S, Giordani S, Bonifazi D, Bianco A, Prato M. *Chem Soc Rev.* 2009; 38:2214–2230. [PubMed: 19623345]

15. Karousis N, Tagmatarchis N, Tasis D. *Chem Rev.* 2010; 110:5366–5397. [PubMed: 20545303]
16. Kappe CO. *Angew Chem Int Ed.* 2004; 43:6250–6284.
17. Herrero MA, Kremsner JM, Kappe CO. *J Org Chem.* 2008; 73:36–47. [PubMed: 18062704]
18. Delgado J, de la Cruz P, Langa F, Urbina A, Casado J, Navarrete JTL. *Chem Commun.* 2004; 15:1734–1735.
19. Amiri A, Maghrebi M, Baniadam M, Zeinail Heris S. *Appl Surf Sci.* 2011; 257:10261–10266.
20. Leinonen H, Rintala J, Siitonen A, Lajunen M, Pettersson M. *Carbon.* 2010; 48:2425–2434.
21. Liu LH, Yan M. *Nano Lett.* 2009; 9:3375–3378. [PubMed: 19670850]
22. Liu LH, Lerner MM, Yan M. *Nano Lett.* 2010; 10:3754–3756. [PubMed: 20690657]
23. Liu LH, Yan M. *J Mater Chem.* 2011; 21:3273–3276.
24. Holzinger M, Vostrowsky O, Hirsch A, Hennrich F, Kappes M, Weiss R, Jellen F. *Angew Chem Int Ed.* 2001; 40:4002–4005.
25. Lee KM, Li L, Dai L. *J Am Chem Soc.* 2005; 127:4122–4123. [PubMed: 15783165]
26. Strom TA, Dillon EP, Hamilton CE, Barron AR. *Chem Commun.* 2010; 46:4097–4099.
27. Bräse S, Gil C, Knepper K, Zimmermann V. *Angew Chem Int Ed.* 2005; 44:5188–5240.
28. Liu LH, Yan M. *Acc Chem Res.* 2010; 43:1434–1443. [PubMed: 20690606]
29. Banks RE, Sparkes GR. *J Chem Soc, Perkin Trans.* 1972; 1:2964–2970.
30. Gritsan NP, Platz MS. *Chem Rev.* 2006; 106:3844–3867. [PubMed: 16967923]
31. Pastine SJ, Okawa D, Kessler B, Rolandi M, Llorente M, Zettl A, Fréchet JM. *J Am Chem Soc.* 2008; 130:4238–4239. [PubMed: 18331043]
32. Zorn G, Liu LH, Árnadóttir L, Wang H, Gamble LJ, Caster DG, Yan M. *J Phys Chem C.* 2014; 118:376–383.
33. Hou PX, Liu C, Chen HM. *Carbon.* 2008; 46:2003–2005.
34. Prasek J, Drbohlavova J, Chomoucka J, Hubalek J, Jasek O, Adam V, Kizek R. *J Mater Chem.* 2011; 21:15872–15884.
35. Thess A, Lee R, Nikolaev P, Dai H, Petit P, Robert J, Xu C, Lee YH, Kim SG, Rinzler AG, Colbert DT, Scuseria GE, Tomanek D, Fischer JE, Smalley RE. *Science.* 1996; 273:483–487. [PubMed: 8662534]
36. Basu-Dutt S, Minus ML, Jain R, Nepal D, Kumar S. *J Chem Educ.* 2012; 89:221–229.
37. Holzinger M, Abraham J, Whelan P, Graupner R, Ley L, Hennrich F, Kappes M, Hirsch A. *J Am Chem Soc.* 2003; 125:8566–8580. [PubMed: 12848565]
38. Sharon N. *J Biol Chem.* 2007; 282:2753–2764. [PubMed: 17145746]
39. Liu B, Bian HJ, Bao JK. *Cancer Lett.* 2010; 287:1–12. [PubMed: 19487073]
40. Yan M, Cai SX, Wybourne MN, Keana JFW. *Bioconjug Chem.* 1994; 5:151–157. [PubMed: 8031878]
41. Norberg O, Deng L, Yan M, Ramström O. *Bioconjug Chem.* 2009; 20:2364–2370. [PubMed: 19888719]
42. Zhou J, Butchosa N, Jayawardena HSN, Zhou Q, Yan M, Ramström O. *Bioconjug Chem.* 2014; 25:640–643. [PubMed: 24625204]
43. Deng L, Norberg O, Uppalapati S, Yan M, Ramström O. *Org Biomol Chem.* 2011; 9:3188–3198. [PubMed: 21423935]
44. Pei Z, Yu H, Theurer M, Waldén A, Nilsson P, Yan M, Ramström O. *Chem Bio Chem.* 2007; 8:166–168.
45. Wang X, Ramström O, Yan M. *Chem Commun.* 2011; 47:4261–4263.

Highlights

- A general and effective method to functionalize pristine SWNTs
- Microwave radiation greatly facilitated reaction kinetics
- Carbohydrate-SWNT conjugates showed good bioaffinity and binding selectivity

Figure 1. TEM images of (A) purchased SWNTs without purification, and (B) SWNTs after purification.

Figure 2.

IR spectra of the reaction of PFPA-NHS with SWNTs under (A) conventional oil bath heating at 130 °C for 3 days, and (B) microwave radiation at 150 °C for 1 h before (black) and after (red) reactions. The arrows indicate the azide absorption, which are enlarged in the insets.

Figure 3. Raman spectra of pristine SWNTs (black) and NHS-PFPA-functionalized SWNTs prepared using microwave-assisted reaction conditions (red).

Figure 4. (A) NHS-PFPA-functionalized SWNTs (left) and as-purified SWNTs (right) in acetone. (B) TEM image of NHS-PFPA-functionalized SWNTs. (C) Man-SWNTs (left) and as-purified SWNTs (right) in water. (D) TEM image of Man-SWNTs.

Figure 5. Fluorescence spectra of FITC-Con A (black), and after it was treated with Man-SWNTs (blue) and Gal-SWNTs (purple). Inset: percent of decrease in fluorescence intensity after FITC-Con A was treated with Man-SWNTs (blue column) and Gal-SWNTs (purple column).

Scheme 1.

Functionalization of SWNTs using PFPA-NHS and subsequent conjugation with carbohydrates.