

Functionalization of carbon nanotubes for applications in materials science and nanomedicine*

Ester Vázquez^{1,‡} and Maurizio Prato^{2,‡}

¹*Departament of Organic Chemistry, Faculty of Chemistry-IRICA, University of Castilla-La Mancha, Campus Universitario, 13071 Ciudad Real, Spain;* ²*Center of Excellence on Nanostructured Materials, Department of Pharmaceutical Science, INSTM, unit of Trieste, University of Trieste, Piazzale Europa 1, 34127 Trieste, Italy*

Abstract: Carbon nanotubes (CNTs) can be functionalized using a variety of efficient protocols. Especially, esterification and amidation reactions are exploited along with 1,3-dipolar cycloadditions. The use of microwaves (MWs) to activate the reactivity of CNTs is also reported. Innovative NMR methodologies can be introduced to investigate the covalent attachment of organic moieties to CNTs.

Keywords: carbon nanotubes; cycloadditions; fullerenes; microwaves; NMR.

INTRODUCTION

Carbon nanotubes (CNTs) represent a relatively new class of carbon-based nanomaterials, currently under deep scrutiny for applications in materials and biomedical fields. Multiwalled carbon nanotubes (MWNTs) were discovered in 1991 [1], followed in 1993 by their single-walled counterparts [2]. Single-walled nanotubes (SWNTs) possess the simplest geometry (i.e., a rolled-up graphene sheet that is closed by semi-fullerene-like caps), and their diameters typically range between 0.8 and 2 nm. MWNTs, on the other hand, are composed of a concentric arrangement of several cylinders, reaching diameters of up to 50 and sometimes even 100 nm. According to intense studies following their discovery, CNTs have proven to possess unique electronic, mechanical, and structural properties [3].

However, the difficult handling of CNTs has posed serious problems for their technological development. As a matter of fact, dissolution of CNTs may be necessary for proper chemical and physical processing. The ability to solubilize and separate discrete CNTs from their tight bundles would not only open new fields, but would also help in their purification, eventually allowing an easier manipulation. Recently, CNTs have received increasing attention as potential components in nanomedicine [4].

One of the most powerful methodologies to improve CNT handling is the covalent functionalization of their sidewalls and tips [5]. Among the various approaches, the most general ones include: (i) esterification or amidation of oxidized nanotubes and (ii) direct covalent attachment of functional groups, mainly by cycloadditions, nucleophilic or electrophilic or radical additions.

Within this review, we will briefly summarize our achievements in the field of synthesis and characterization of functionalized CNTs.

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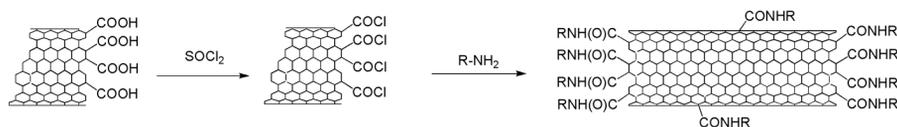
[‡]Corresponding authors

FUNCTIONALIZED CARBON NANOTUBES

Functionalization of oxidized nanotubes

CNTs are usually generated by arc discharge, laser ablation, or catalytic gas-phase growth starting from carbon monoxide or other carbon sources. The raw material contains CNTs contaminated with various amounts of amorphous carbon and/or catalytic metal particles. Consequently, the necessity exists to achieve an effective purification process prior to their use and/or further processing. Treatment of the crude materials, for example, under strong acidic and oxidative conditions constitutes a powerful approach. In particular, sonication in a mixture of concentrated nitric and sulfuric acid or heating in a mixture of sulfuric acid and hydrogen peroxide brings about the formation of shortened and open tubes [6]. The result of these oxidations is the generation of carboxyl groups, both at the caps and at the sidewalls. The presence of these functionalities opens the way for further modification of SWNTs or MWNTs, since the acid groups can react readily with alcohols or amines to give rise to ester or amide bonds, respectively.

Numerous reactions with oxidized CNTs have been reported, resulting in soluble functionalized materials [7] (Scheme 1).



Scheme 1 R-NH_2 can be virtually any amine. Many of these examples are known.

Scanning tunneling microscopy (STM) has been used to visualize the long-chain alkyl amines attached to SWNTs [8], Fig. 1.

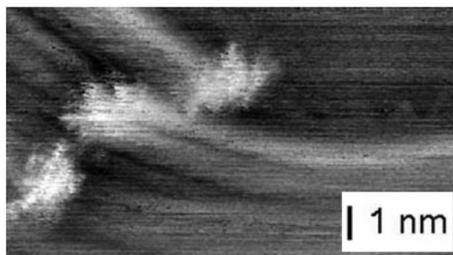


Fig. 1 STM image of oxidized/functionalized SWNTs (Scheme 1, $\text{R} = \text{octadecyl}$).

The typical STM images of oxidized and functionalized SWNTs (see structure in Scheme 1, $\text{R} = \text{CH}_3(\text{CH}_2)_{16}\text{CH}_2-$) show tiny bundles with brush-like tips, attributed to the aliphatic chains.

Transmission electron microscopy (TEM) images reveal that exfoliation of individual CNTs from large ropes can be achieved during this functionalization process. This is extremely important to allow an easy characterization of the soluble CNTs and to facilitate their manipulation. Functionalized SWNTs can also be characterized by proton NMR spectroscopy based on gradient-edited diffusion pulse sequences (1D-diffusion ordered spectroscopy, DOSY) [9]. In general, diffusion NMR experiments allow the separation of NMR signals of different species present in a mixture, according to their own diffusion coefficients, merging spectroscopy information with size analysis. Therefore, oxidized and functionalized SWNTs were subjected to DOSY analysis (Fig. 2).

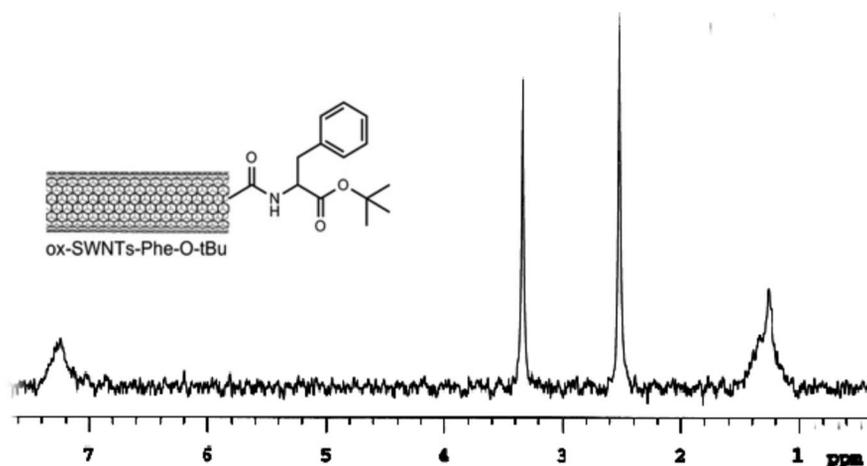


Fig. 2 1D-DOSY spectrum of ox-SWNTs-Phe-O-*t*Bu at $36.5 \text{ G}\cdot\text{cm}^{-1}$.

When a sufficiently strong gradient is applied, only macromolecules with low diffusion coefficients are seen in the NMR spectrum. Therefore, the signals present in a 1D-DOSY NMR spectrum can be confidently attributed to organic moieties covalently attached to SWNTs.

For instance, in the 1D-DOSY spectrum of the oxidized SWNTs conjugated with phenyl alanine *t*-butyl ester it is possible to clearly observe the peaks relative to the phenyl and the *t*-butyl groups, thus making this technique a valuable tool in the structural determination of functionalized CNTs.

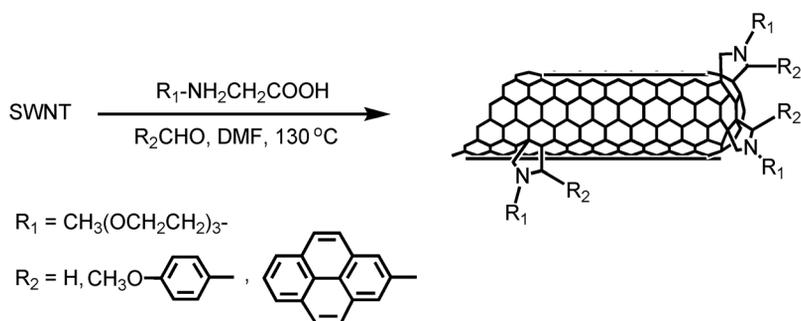
Covalent sidewall functionalization

The functionalization of SWNTs is not limited to the chemistry of carboxylic acids. More elaborate methods have been developed to attach organic moieties directly onto the nanotube sidewalls. These include cycloadditions, electrophilic and nucleophilic or radical additions.

In contrast to the esterification or amidation reaction of the acid groups in oxidized SWNTs, the functionalization via direct reactions is more difficult to accomplish as it requires the use of highly reactive species, such as free radicals. In general, addition reactions to carbon-carbon double bonds cause a transformation of sp^2 -hybridized into sp^3 -hybridized carbon atoms. Such changes are associated with the modification of the predominantly trigonal-planar local bonding geometry into a tetrahedral geometry. Importantly, this process is energetically more favorable in the cap region due to the pronounced 2D curvature, while the sidewalls reveal a comparatively low 1D curvature. In addition, nanotubes are not ideal structures, but rather contain defects formed during their synthesis. It is probable that reactions preferably occur near defect sites. Sidewall functionalization has been achieved either in pristine or oxidized and/or modified SWNTs.

Our group has developed a strategy for the functionalization and solubilization of CNTs based on the 1,3-dipolar cycloaddition of azomethine ylides, similar to that already applied in the fullerene field [10]. The main advantage of this reaction is the attachment to the sidewalls of CNTs of pyrrolidine rings substituted with functionalities that can lead to the construction of novel materials for diverse applications. The simplest approach for the in situ generation of azomethine ylides is the decarboxylation of immonium salts derived from condensation of α -amino acids with aldehydes. Thus, functionalized aldehydes can lead to 2-substituted pyrrolidine moieties located at the sidewalls of CNTs, while *N*-modified α -amino acids can lead to *N*-functionalized pyrrolidines.

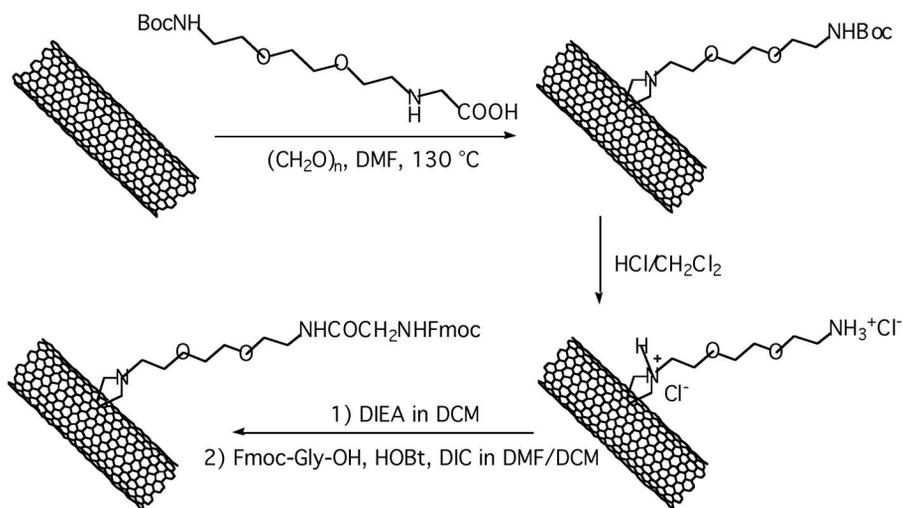
When the 1,3-dipolar cycloaddition of azomethine ylides was applied to various types of CNTs (e.g., oxidized, HiPco-SWNTs, MWNTs), the reaction was found to be very effective [11]. In the first



Scheme 2 1,3-dipolar cycloaddition of azomethine ylides to CNTs.

experiments, the triethylene-glycol group was chosen as *N*-substituent group of the α -amino acid due to its high solubilizing power (Scheme 2).

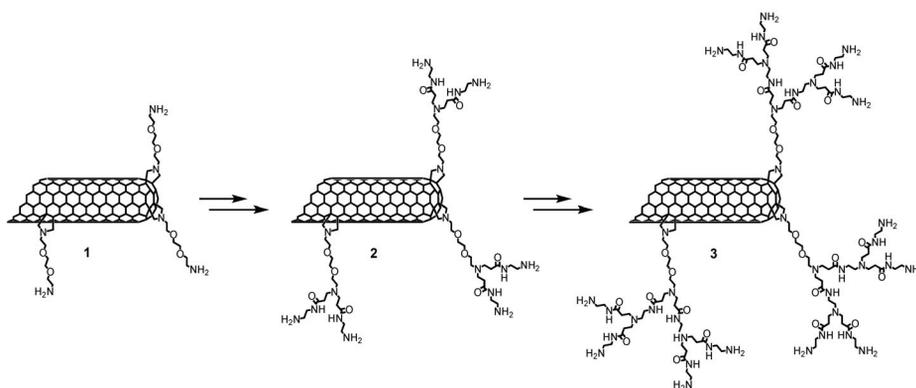
The use of an *N*-functionalized glycine, bearing a Boc (*tert*-butoxycarbonyl)-protected amino end group generates the corresponding functionalized SWNTs (f-SWNTs), which, after deprotection, lead to amine functionalities amenable to further transformations. Simple procedures in peptide chemistry can be adopted to generate f-SWNTs modified with amino acids and peptides. These hybrids are attractive tools for biomedical applications [12] (Scheme 3).



Scheme 3 Synthesis of a valuable intermediate amino-functionalized CNT.

Oxidized CNTs have also been functionalized by 1,3-dipolar cycloaddition in a new strategy for the double functionalization of CNTs to assess the characteristics of toxicity and uptake of CNTs functionalized with the antibiotic Amphotericin B (AmB) and fluorescein toward mammalian cells, and to evaluate the antifungal activity of CNT–AmB conjugates [13]. The results are very promising as they indicate that appropriate conjugation can increase the effectiveness of AmB while decreasing its toxicity on human cells.

The addition of azomethine ylides to CNTs is a relatively mild reaction, as it does not saturate too many double bonds in the conjugated system. However, in order to increase the number of functional groups present around the CNTs, without altering their electronic properties, dendrimers can be built

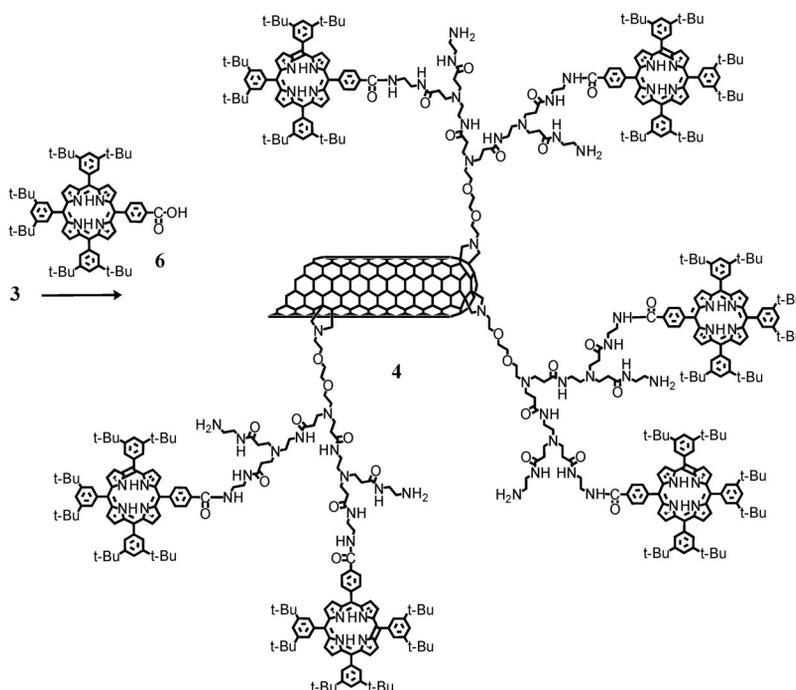


Scheme 4 Dendrimer construction on CNTs.

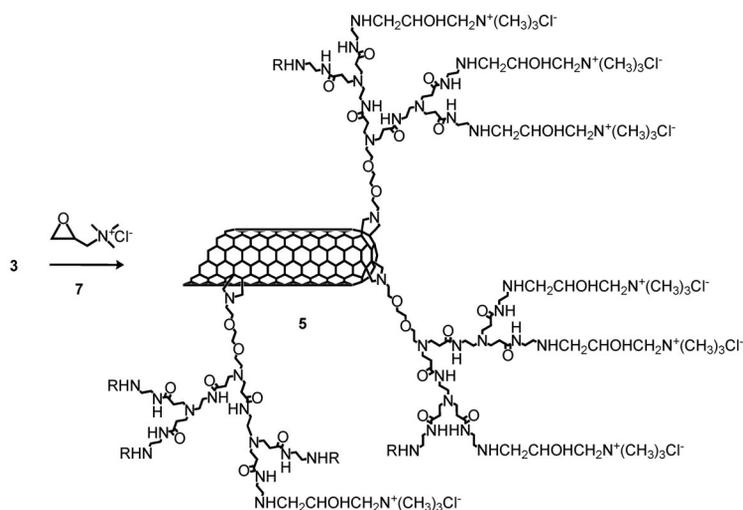
on the amino function. The poly(amido amine) (PAMAM) approach to dendrimers, reported in the past by Tomalia and colleagues, has been adopted in our case (Scheme 4).

First-generation dendron **2** and second-generation dendron **3** were prepared in high yield starting from **1**.

Compound **3** was condensed with porphyrin acid **6**, for the preparation of dyad **4** (Scheme 5), which was studied in photoinduced electron-transfer reactions [14]. Alternatively, dendron **3** was allowed to react with epoxide **7**, affording the polycationic species **5**, which was employed in promising gene silencing experiments (Scheme 6) [15].



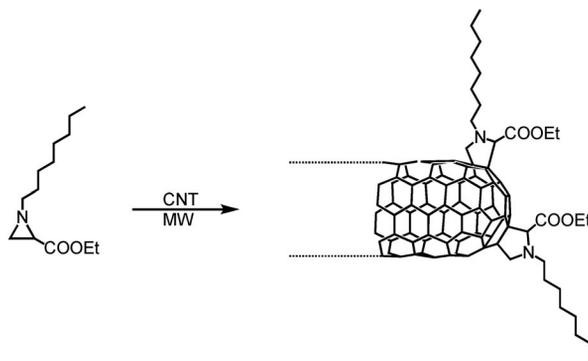
Scheme 5 Functionalization of CNT-PAMAM dendrimer with porphyrin units.



Scheme 6 Generation of quaternary ammonium salts on CNT-PAMAM dendrimers.

Still, a severe limitation for sidewall functionalization using conventional chemical techniques is that most of the reactions need refluxing and/or sonication of a great amount of solvents over long reaction times, which makes these protocols not suitable for large-scale applications. An attractive approach to solve these problems as well as to increase the number of groups covalently linked to the sidewalls is to use microwaves (MWs) to activate the reactivity of CNTs. It is known in organic chemistry that certain reactions can be MW-assisted to improve selectivity and to reduce reaction times [16]. When CNTs are exposed to MW radiation, strong absorptions are observed [17], and recent work has explored the use of this radiation to assist CNT functionalization [18]. Our group has shown that a solvent-free technique combined with MW irradiation produces functionalized nanotubes by using 1,3-dipolar cycloaddition in just 1 h of reaction [19]. This procedure provides a fundamentally different approach when considering the chemistry of CNTs, because in the absence of solvents, CNTs absorb directly the radiation and it is possible to take full advantage of the strong MW absorption typical of pristine nanotubes. Moreover, the solvent-free conditions pave the way to green protocols and large-scale functionalization.

Thus, CNTs (SWNTs and MWNTs) have been functionalized employing aziridines as starting materials using MW activation under solvent-free conditions (Scheme 7). SWNTs functionalized under MWs in 1 h, present a thermogravimetric analysis (TGA) loss of about 20 %, which corresponds to the



Scheme 7 MW-induced cycloaddition of aziridines to CNTs.

presence of one functional group for about 76 carbon atoms, while the same reaction carried out in dimethylformamide (DMF) under classical heating proceeds in 5 days and introduces only one functional group for about 138 carbon atoms of the CNT skeleton. Moreover, under MWs the overall sequence can be repeated twice in order to further increase the number of pyrrolidine rings attached to the sidewall of the tubes.

The methodology has been also applied to produce multifunctionalized CNTs using a combination of two different addition reactions, the 1,3-dipolar cycloaddition of azomethine ylides without solvent and the addition of diazonium salts in water, both via a simple, fast, and environmentally friendly method [20]. Multifunctional CNTs are very attractive for drug delivery applications [21], sensors [22], or in the preparation of composites [23].

The multiple functionalization can be efficiently followed using Raman spectroscopy. Figure 3 reports the Raman spectra of the pristine starting material, functionalized SWNTs by 1,3-dipolar cycloaddition and doubly functionalized SWNTs. Notably, the relative intensity of the D band increases as the degree of functionalization increases.

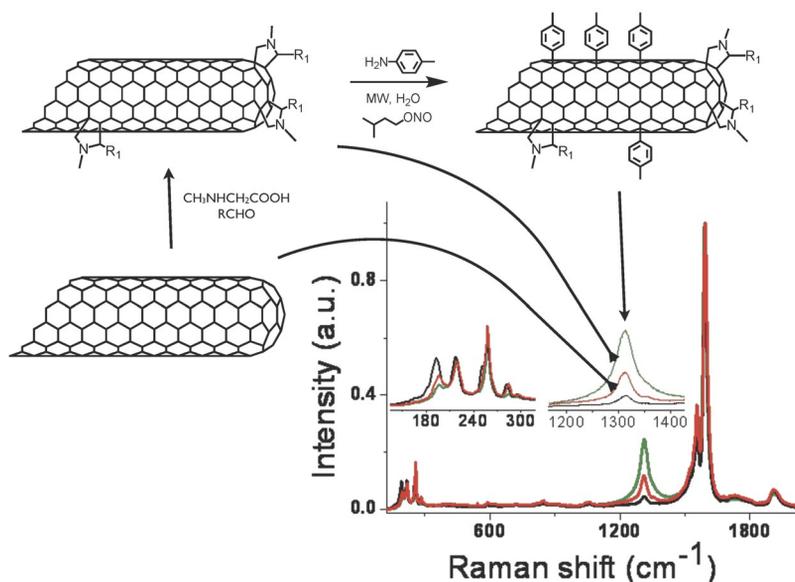


Fig. 3 Raman spectra ($\lambda_{\text{exc}} = 632.8 \text{ nm}$) of pristine SWNTs, functionalized SWNTs by 1,3-dipolar cycloaddition and doubly functionalized SWNTs.

Moreover, by using this procedure it is possible to obtain multifunctional SWNTs with a variety of groups that could be customized to fit a desired application. The two reactions can be performed in series. The results show that the radical arene addition saturates more reactive sites than the cycloaddition. Therefore, to attach two different functional groups to SWNTs, the order of reaction should be first the cycloaddition and then the arene addition. This gives rise to comparable functionalization degrees for the two additions. If only a few functional groups are required along with an excess of other organic/inorganic groups, then the functionalization order can be reversed. This latter could be the case of some biomedical applications, where, for example, much more drug molecules are needed with respect to a contrast agent.

CONCLUSIONS

The functionalization of CNTs is approaching maturity, as more methods become available and more analytical techniques allow a better understanding of the reaction outcomes.

With many powerful methodologies in hand, f-CNTs become a suitable building block for applications in materials science and nanomedicine.

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