Non-covalent functionalization of carbon nanotubes with glycolipids: glyconanomaterials with specific lectin-affinity†

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A strategy based on the utilization of neutral pyrene functionalized neoglycolipids I that interact with a CNT's surface giving rise to biocompatible nanomaterials which are able to engage specific ligand-lectin interactions similar to glycoconjugates on the cell membrane is reported.

The utilization of a nanomaterial wrapped in biologically relevant molecules to study and solve biomedical problems is a new and stimulating field of research.[1] One of the most salient features of using nanomaterials, such as nanoparticles, nanorods, nanowires and carbon nanotubes in biology is their ability to carry multiple copies of a single drug or various active principles with different, ideally synergistic, modes of action.[2] Consequently, those diseases or biological processes whose biological targets require a multivalent display of the active epitope, are expected to benefit from the application of a nanometric platform. Illustrative examples of such events are those mediated by carbohydrates, which include cell adhesion, inflammation, tumour cell metastasis, and pathogen infections.[3] It has been shown that the weak interaction between an individual ligand and the corresponding specific lectin is compensated by the multivalent display of carbohydrates through the so called cluster effect.[4] On the other hand, single-walled carbon nanotubes (SWCNTs) as interesting 1D nanomaterials, are actually being actively investigated as vehicles for the in vivo smart delivery of biologically relevant cargoes including drugs, proteins, and nucleic acids, as nanometric sensors, and for cancer treatment.[5] However, concerns about their potential toxicity have reduced much of the original enthusiasm about their promising clinical applications.[6] Nevertheless, recent investigations, including a pilot study, on the in vivo behaviour of SWCNTs, have concluded that conveniently functionalized water soluble SWCNTs are completely cleared from the body *via* the biliary and renal pathway, and are non toxic.[7] While covalent, and non-covalent, approaches have been followed for the dispersion of SWCNTs in aqueous media, the latter one is highly desirable as it conserves the nanotube structure, while the former one has been shown to disrupt their π-network, leading to possible losses in their mechanical, electrical, and biosensing properties.[8] In this Communication, we disclose our results on the utilization of carbon nanotubes as molecular platforms for a multivalent presentation of biologically relevant saccharide epitopes. Our strategy is based on the utilization of neutral pyrene functionalized neoglycolipids **I**[9] that interact with a CNT's surface giving rise to a nanometric material with a multivalent display of carbohydrates, much like the glycocalyx on the cell surface (Fig. 1).[10] Between the pyrene tail and the glycoligand, the designed amphiphilic compound **I** exhibits an advantageous variable spacer derived from tetraethylene glycol for the fine-tuning of the hydrophilic-hydrophobic balance of the pyrene-polyethlyene glycol-sugar (Py-PEG-Sugar) **I**.

The convergent construction of the neoglycolipids Py-PEG-Lac-**5** and Py-PEG-Man-**6** is realized efficiently starting from (2′-aminoethyl) per-O-acetylated-1-thio-glycosides **1** and **2**, for which a novel one step procedure has been developed (Scheme 1). Amide bond formation between amine **1** or **2** and the acid **3**, followed by [2 + 3] Huisgen cycloadition of the coupled azide product with the alkyne **4** affords the fully acetylated glyconjugates.[11] Zemplen deacetylation followed by purification on Sephadex G-20 yields the water soluble neoglycoconjugates Py-PEG-Lac-**5** and Py-PEG-Man-**6** in pure form.

By merely mixing the neutral lipid Py-PEG-Lac-**5** with SWCNTs or with MWCNTs in pure water without any additive, followed by sonication for 1 h, a stable black suspension is obtained, indicating the formation of stable SWCNT-Py-PEG-Lac-**5** as well as MWCNT-Py-PEG-Lac-**5** soluble aggregates, Fig. 2A (vials 2 and 3, respectively). In contrast, if the reaction is attempted in the absence of the glycolipid Py-PEG-Lac-**5**, the CNTs precipitate after a few minutes, Fig. 2A (vial 1).

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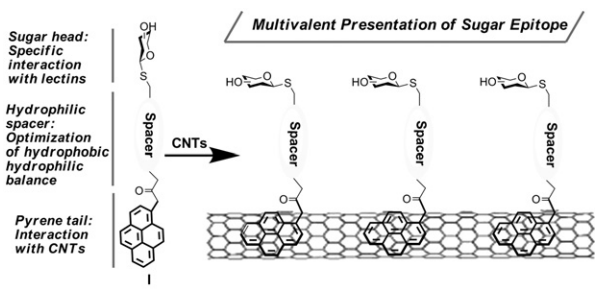
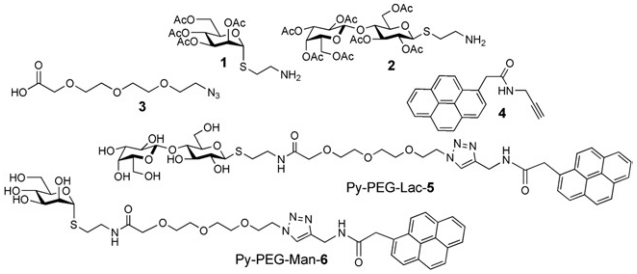


Fig. 1 Overview of the experimental strategy.



Scheme 1 Structure of (2′-aminoethyl) per-O-acetylated-1-thio-mannopyranoside **1** and lactopyranoside **2**, the bifunctional spacer **3**, and the alkyne **4** used for the synthesis of the pyrene-tethered amphiphilic 1-thiolactose, and 1-thiomannose glycoconjugates Py-PEG-Lac-**5** and Py-PEG-Man-**6**.

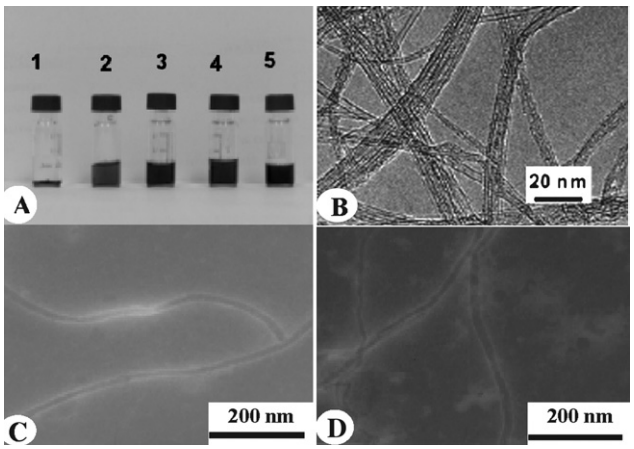


Fig. 2 (A) Photographs of vials with aqueous solutions of: **1**, as produced SWCNTs; **2**, SWCNTs coated with glycoconjugate Py-PEG-Lac-**5**; **3**, MWCNTs coated with glycoconjugate Py-PEG-Lac-**5**; **4**, SWCNTs coated with glycoconjugate Py-PEG-Man-**6**; **5**, MWCNTs coated with glycoconjugate Py-PEG-Man-**6**. (B) TEM image of as produced SWCNTs. (C) TEM image of MWCNTs-Py-PEG-Lac-**5**. (D) TEM image of SWCNTs-Py-PEG-Lac-**5**.

Similarly, stable black suspensions are obtained with glycolipid Py-PEG-Man-**6** derived from the monosaccharide mannose, when reacting with SWCNT and MWCNT Fig. 2A (vials 4 and 5, respectively). The characterization of SWCNT-Py-PEG-Lac-**5**/Py-PEG-Man-**6** as well as MWCNT-Py-PEG-Lac-**5**/Py-PEG-Man-**6** aggregates is carried out by transmission electron microscopy (TEM) and by scanning electron microscopy (SEM). TEM images of as produced SWCNTs as well as MW- and SWCNTs coated with neoglycolipid Py-PEG-Lac-**5** are given in Fig. 2B, 2C, and 2D, respectively. Due to the hydrophobic interactions, π-stacking and van der Waals forces among individual tubes, as produced SWCNTs self assemble into large bundles or ropes, Fig. 2B. Quite remarkably, simple functionalization with neoglycolipids Py-PEG-Lac-**5** or Py-PEG-Man-**6** in pure water and strictly neutral conditions, allowed the CNT packages to exfoliate affording small bundles and individual sugar coated nanotubes, as evidenced by TEM analysis (Fig. 2C). At the onset of this research was the question on the selectivity acquired by the CNT-carbohydrate aggregates toward specific receptors as a consequence of the multivalent display of sugar epitopes. In order to study this specific binding feature, critical for future biological applications of the prepared bio-nanomaterials, we make use of the known sugar-lectin specificity.[12a] Accordingly, the CNT-Py-PEG-Lac-**5** aggregates exhibiting lactose residues onto the nanotube surface, could be recognized by a lactose-specific receptor such as the Arachis hypogaea Peanut agglutinin (PNA) affording nanotube-epitope-lectine multicomposites, Fig. 3A-1.[12b] To test this hypothesis, freshly prepared MWCNTs-Py-PEG-Lac-**5** as well as SWCNTs-Py-PEG-Lac-**5** aggregates were incubated with fluorescein isothiocyanate conjugated lectine PNA-FITC in Tris buffer at pH 8. The excess of the fluorescent lectin is then removed by a series of centrifugations at 14 000 g followed by elimination of the supernatant, and re-suspension of the pellet in the same buffer. Subsequently, the formation of the complex CNT-Py-PEG-Lac-**5**-PNA-FITC

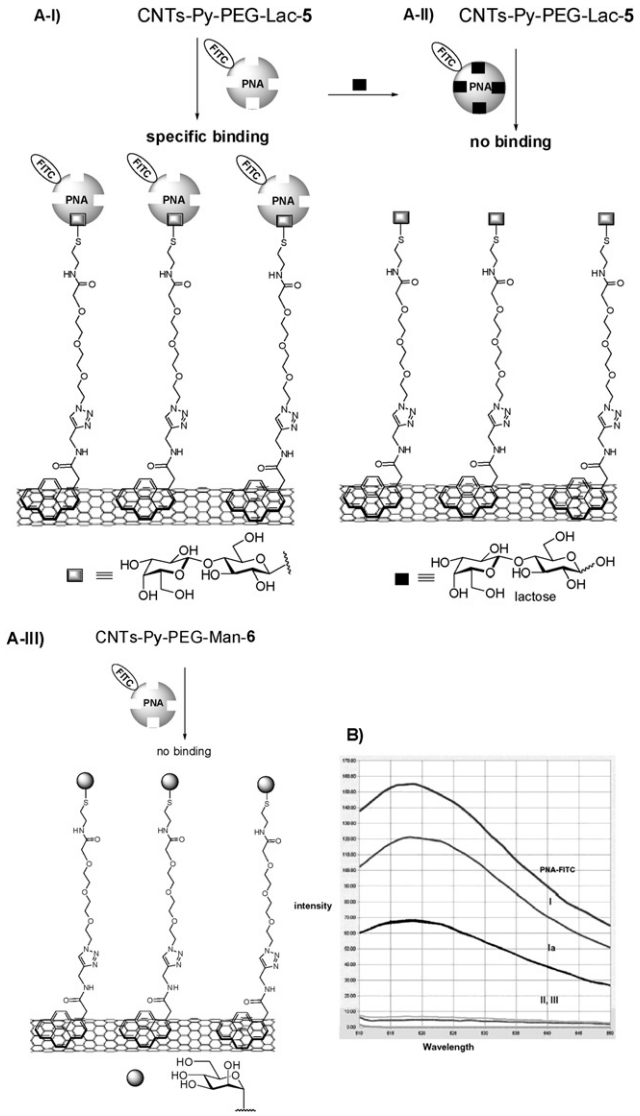


Fig. 3 Specificity acquired by the aggregates toward specific receptors. (A) Schematic representation of: selective recognition of CNT- Py-PEG-Lac-**5** (**I**), inhibition of lectin binding by adding monovalent lactose (**II**), absence of selective interaction of PNA-FITC with CNT-Py-PEG-Man-**6** displaying an α-mannose epitope on their surface (III). (B) Fluorescence spectra of **I**: PNA-FITC with MWCNTs-Py-PEG-Lac-**5**, **I-a**: PNA-FITC with SWCNTs-Py-PEG-Lac-**5**, **II**: MWCNTs-Py-PEG-Lac-**5** with PNA-FITC, previously incubated with lactose, **III**, PNA-FITC with MWCNTs-Py-PEG-Man-**6**. [PNA-FITC] = 0.82 μM.

multicomposite was determined by fluorescence spectroscopy, Fig. 3B. While both MWCNT-Py-PEG-Lac-**5** (Fig. 3B-I) and SWCNT- Py-PEG-Lac-**5** (Fig. 3B-Ia) samples exhibited positive responses, significant fluorescence was observed with the former system, attributed to higher bound fluorescent lectin, a consequence of major epitope exposition. This result indicates the higher efficiency of Py-PEG-Lac-**5** to disperse MWCNTs compared to SWCNTs which as a rope are cohesively held tightly together and thus more difficult to exfoliate.

Subsequently, two control experiments were conducted in order to confirm that the observed fluoresecence is a consequence of specific interaction of the PNA-lectin with its glycoligand lactose, and not to the well documented non specific protein-CNT interaction.[13] The initial one consisted of the preincubation of PNA lectin with free lactose prior to incubation with the aggregate, Fig. 3A-II. In this case the PNA-FITC-MWCNT-Py-PEG-Lac-**5** multicomposite formation was drastically inhibited (Fig. 3B-II), evidencing unambiguously that the fluorescence tagging was driven by the specific PNA-lactose interaction. In the second one, we performed the same experiment with MWCNT-Py-PEG-Man-**6** and SWCNT-Py-PEG-Man-**6** displaying an α-mannose epitope on their outer surface, which should not be recognized by PNA lectine, Fig. 3-III. This was indeed the case, as no fluorescence tagging (Fig. 3B-III) was detected, indicating that the biological receptor does not interact with aggregates bearing unspecific sugar epitopes. Taking these results all together indicates that once the CNTs are functionalized with glycoconjugates **I**, they acquire biological specificity towards protein receptors of the glycoligands exposed on their surface.

In summary, we have developed a mild and practical non covalent approach for the functionalization of carbon nanotubes. The obtained aggregates with a multivalent sugar exposition on their surface are able to engage specific ligand-lectin interactions similar to glycoconjugates on the cell membrane. Additionally, the designed convergent and modular synthetic strategy allowed a rapid preparation of a large number of neoglycoconjugates and the fine-tuning of their structure for an optimal and specific interaction with biologically relevant receptors. These studies are under investigation in our laboratories.

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