

Covalent functionalization of Carbon Nanotubes with an Azoic O-Glycoside



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Abstract

This report describes the coupling reaction between an azoic O-glycoside with a single wall nanotube functionalized with acyl chloride terminals, to generate the nanotube-azoic O-glycoside complex which was analysed by TEM and confocal fluorescence microscopy. The study showed the presence of bundles along the cylindrical nanotube, and fluorescence with maximum absorption of 595 y 696 nm, suggesting the functionalization through a covalent interaction with the azoic O-glycoside.

Keywords: Single-Walled Carbon Nanotubes; TEM Microscopy; Azoic Glycosides; Confocal Microscopy; Prodrugs

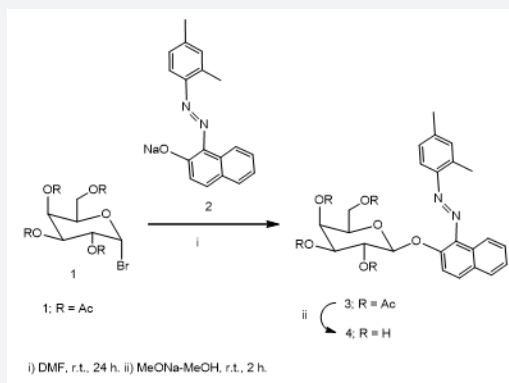
Introduction

There is an expanding interest in the functionalization of carbon nanotubes due their usefulness in diverse areas such as nanoelectronics [1], mechanical [2], biomedical [3], biosensor [4], and drug delivery [5]. In the field of bio nanotechnology novel tools have been implemented for understanding cell processes and in the design of nanodevices for disease diagnosis and treatment [6]. Although the carbon nanotubes toxicity is controversial in animal cells [7,8] there is an increasing evidence that appropriate surface chemistry or functionalization to solubilize CNTs, might also provide biocompatibility and low toxicity [9,10]. From the significant number of strategies for introducing covalent functionality to the carbon nanotubes, the carboxylation methods become one of the most extended methods since it can be converted to acyl chloride or alcohol derivatives [11]. Thus, a number of carboxyl derivatives such as esters and amides have been described with the aim of attaching small or large macromolecules to carbon nanotubes. Likewise, small molecules bound to carbon nanotubes has been evaluated as an option for drug delivery in particular as suicide molecules targeting cancer cells [12,13]. In addition, the attachment of fluorescent molecules to CNT has been of significant benefit for providing optical signals for imaging and localization of the CNT-drug conjugates [14]. Since a multiple functionalization of CNT seem to be a suitable strategy for improving, solubility, compatibility and low toxicity

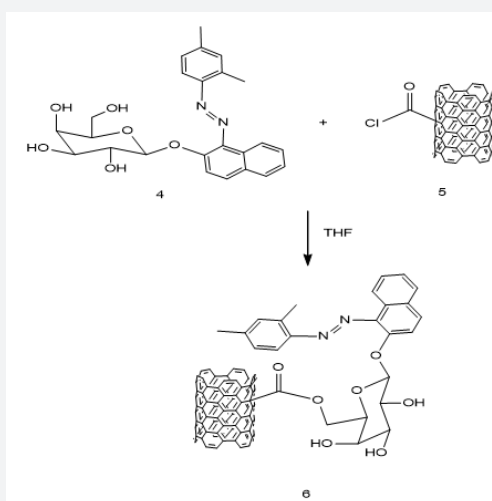
we propose the covalent attachment of an azoic glycoside having suitable partition coefficient with capacity to cross membranes, capable of being detected through fluorescence emission ultimately acting as prodrug [15]. Our hypothesis proposes that the synthesized nanotube-azoic O-glycoside complex will be enzymatically cleaved by glycosidases or azoreductases, allowing the release of the cytotoxic molecule at specific sites.

Methodology

The strategy employed for the preparation of covalently attached nanotube-azoic glycoside complex (6) start with the preparation of the azoic O-glycosides by condensation reaction between acetobromo glucose (1) with Sudan II dye (2) to furnish the protected glycoside Sudan II- β -D-galactopyranoside (3), which was subjected to final acetate removal affording Sudan II O-galactoside (4) as depicted in Scheme 1. The resulting azoic O-glycoside (4) was condensed with single walled nanotube which was derivatized to the acyl form (5) according to standard protocols [11]. The condensation reaction between (4) and (5) was carried out under sonication in THF at room temperature (Scheme 2). The resulting nanotube-azoic glycoside complex (6) was centrifuged and washed (3 x 2 mL) with MeOH-CH₂Cl₂ solution until the solution was colour less. The nanotubes obtained as pellet were dried in oven, suspended in isopropanol and analysed by TEM and fluorescence microscopy.



Scheme 1: Preparation of azoic O-glycoside (4).



Scheme 2: Preparation of nanotube-azoic glycoside complex (6).

Results and Discussion

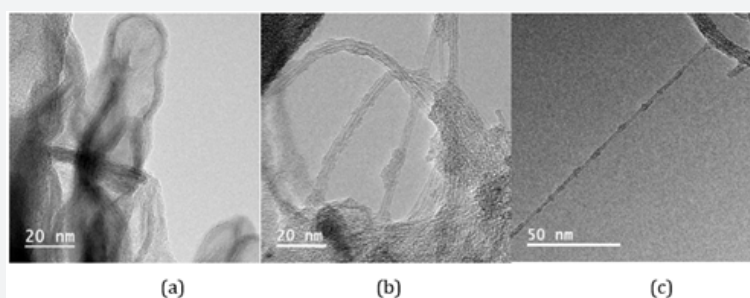


Figure 1: (a) Unreacted single wall carbon nanotube. (b,c) Single wall nanotube treated with azoic O-glycoside under sonication at different resolutions.

The azoic glycoside was synthesized by direct coupling reaction between acetobromo glucose with Sudan II under the Koenigs-Knorr conditions, and subsequently deprotected under Zemplen conditions [16]. The resulting azoic glycoside was condensed with nanotube previously derivatized to the acyl chloride form. The reaction was repeatedly washed and centrifuged to assure that

the nanotubes were free of unreacted glycoside. The suspended nanotubes were analysed by TEM microscopy, observing for the unreacted nanotube a uniform surface without the presence of aggregates around the cylinder. On the other hand, for the complex nanotube azoic O-glycosides it was observed the presence of small bundles around the cylindrical nanotubes which were analysed at

different resolutions and compared with unreacted SWNT (Figure 1a-c). The presence of this bundles has been described by other research groups which were able to incorporate glycodendrimers on the surface of nanotubes [3]. In order to provide evidence about the functionalization of the nanotube with the azoic O-glycoside we submitted the complex nanotube-azoic O-glycoside (6) to confocal

fluorescence microscopy, observing a maximum absorption at 598 and 657 nm (Figure 2a) in good agreement with the absorption spectra showed by the Sudan II dye. Moreover, the confocal image showed a fluorescence pattern as dispersed spots distributed randomly along the nanotube surface as it is observed in the 3D image (Figure 2b).

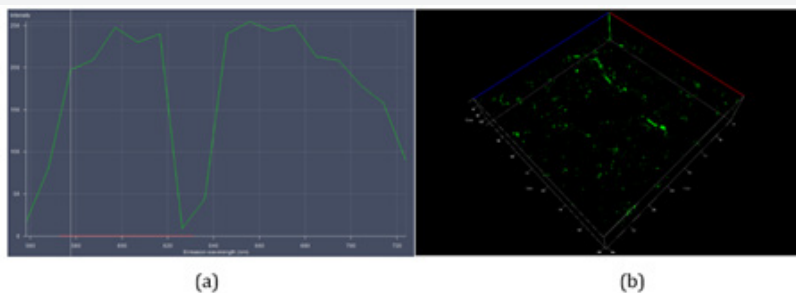


Figure 2: (a) Absorption spectra of the complex carbon nanotube-azoic O glycoside (6) and (b) 3D fluorescence confocal image.

Experimental Section

Materials and Methods

SWCNT 40-60 wt % carbon basis, diam. x L 2-10 nm x 1-5 μ m dimensions were purchased from Sigma-Aldrich Co, Milwaukee, WI, USA. Anhydrous solvents were distilled before use, thin layer chromatography was performed on silica gel 60 with fluorescent indicator UV254 aluminum sheets purchased from Macherey-Nagel GmbH Düren Germany. Nuclear magnetic resonance spectra were recorded on Varian Mercury 300 MHz spectrometer Varian Palo Alto CA. Confocal Microscopy was performed on Carl Zeiss Confocal Microscope with Laser LSM 710. Mass spectrometry was performed on Bruker micrOTOF-Q II Instrument. Bruker Co Westmidland UK, Transmission microscopy was performed with JEOL 1400 transmission electron microscope. Sudan II-2,3,4,6-tetraacetyl- β -D-O-galactopyranoside (3).

In a round bottom flask containing a NaOH solution (28 mg, 7.0 mmol) in 5 mL of water, was added portion wise Sudan II dye (1.93 g, 7.0 mmol) and stirred until complete dissolution. The mixture is evaporated under vacuo and the red solid dissolved with 20 mL of DMF and a solution of 1-bromo 2,3,4,6-tetraacetyl- α -D-O-galactopyranoside (2.87 g, 7.0 mmol) dissolved in DMF (3 mL) was added and the reaction kept at room temperature with stirring during 24 h. The reaction was dissolved with 50 mL of CH_2Cl_2 and washed with cold solution 1 N NaOH (3 x 30 mL) and water (50 mL). The organic phase is dried with anhydrous Na_2SO_4 and evaporated. The product is purified by column chromatography using ethyl acetate-hexane to furnish protected azoic O-glycoside (3) (1.98 g, 47 % yield) as a solid orange. ^1H NMR CDCl_3 : δ 1.95-2.15 (4s, 12H, AcO-), 2.33 y 2.65 (2s, 6H, $-\text{CH}_3$), 3.98-4.33 (m, H5, H6, H6'), 5.15 (dd, 1H, H-4), 5.20 (d, 1H, J = 7.8, H-1), 5.41-5.51 (m, 2H, H-2 y H-3), 7.11 (d, 1H, H-7, J = 8.4 Hz), 7.20 (s, 1H, H-15), 7.43-7.51 (m, 3H, H-14), 7.68 (d, 1H, H-10), 7.78 (d, 2H, H-9), 7.85

(d, 1H, H-8), 8.22 (d, 1H, H-13, J = 7.5 Hz). ^{13}C NMR dmsd- d_6 : δ 17.7, 20.1, 20.5, 20.6, 61.2, 61.5, 66.9, 68.7, 70.9, 76.6, 77.0, 77.4, 102.0, 115.2, 120.7, 123.7, 125.4, 127.1, 127.3, 127.4, 127.8, 129.4, 129.6, 131.0, 131.9, 138.8, 142.0, 145.1, 149.5, 169.3, 170.1, 170.4. HRESIMS: calc for $[\text{M} + \text{H}]^+$ $\text{C}_{32}\text{H}_{35}\text{N}_2\text{O}_{10}$: 607.2280; found: 607.2286. Sudan II β -D-O-galactopyranoside (4).

A round bottom flask containing the azoic O-glycoside (3) (0.5 g, 0.824 mmol) was added a sodium methoxide solution (128 mg, 0.32 mmol) and the reaction was stirred at room temperature during 2 hours. The reaction was neutralized with acidic ion exchange resin Dowex 50WX2-100 (H +) and filtered to yield 0.325 g (90 %) of deprotected azoic O-glycoside (4) as red solid which was purified by column chromatography using as elution system hexane-ethyl acetate-methanol. ^1H NMR (DMSO- d_6): δ 2.4 y 2.6 (2s, 6H, $-\text{CH}_3$), 3.14-3.70 (m, 6H, H-2, H-3, H-4, H-5, H-6 y H-6'), 5.04 (d, 1H, J = 7.8, H-1), 7.11 (d, 1H, H-7, J = 8.4 Hz), 7.20 (s, 1H, H-15), 7.43-7.51 (m, 3H, H-14), 7.68 (d, 1H, H-10), 7.78 (d, 2H, H-9), 7.85 (d, ^1H , H-8), 8.22 (d, 1H, H-13, J = 7.5 Hz). ^{13}C NMR dmsd- d_6 : δ 17.7, 20.1, 62.0, 69.7, 72.9, 73.8, 76.0, 97.3, 115.2, 120.7, 123.7, 125.4, 127.1, 127.3, 127.4, 127.8, 129.4, 129.6, 131.0, 131.9, 138.8, 142.0, 145.1, 149.5. HRESIMS: calc for $[\text{M} + \text{Na}]^+$ $\text{C}_{24}\text{H}_{26}\text{N}_2\text{NaO}$: 461.1677; found: 461.1683.

Conclusion

The synthesis of the galactoside β -D-O-Sudan II azoic glycoside was successfully achieved under the Koenigs-Knorr conditions and the resultin azoin O-glycoside (4) covalently attached to the single wall carbon nanotubes previously derivatized to the acyl chloride form. The complex carbon nanotube-azoic glycoside was Analyzed by TEM microscopy, observing the presence of bundles along the cylindrical shape of the nanotube, which provide evidence about the covalent glycoside attachment. Moreover, the modified nanotube was analyzed by confocal fluorescence microscopy,

observing the presence of fluorescent dots distributed randomly on the material surface.

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