1. Introduction

Many different and generally correct theories have been advanced concerning the biological roles of the oligosaccharide units of individual classes of glycoconjugates. Analysis of the available results indicates, however, that each of the current theories are not entirely satisfactory. Oligosaccharides are also involved in many important processes concerning molecular recognition. In particular, they selectively interact with some types of proteins. Prominent examples include carbohydrate-specific enzymes and anti-carbohydrate antibodies.

Lectins are proteins that are able to bind mono- and oligosaccharides with high specificity, and play an important role in biological processes. Carbohydrate–lectin interactions make up a crucial step in many biological processes, such as inflammation, embryogenesis, cell development, tumor progression, etc. Since the dissociation constants of carbohydrate–lectin complexes are in the millimolar range, multivalent systems (which involve the simultaneous binding of multiple ligands onto multiple receptors) are required for efficient recognition. Hence, in an effort to understand and mimic these biological phenomena, it is essential to prepare multivalent systems, in which multiple copies of the carbohydrate ligand are present.

With this goal in mind, chemists have synthesized several neoglycoconjugates, ranging from small molecules to macro- and supramolecular entities, in attempts to understand, mimic, and perturb natural multivalent carbohydrate–lectin interactions. For the various membrane-mimetic systems such as liposomes and vesicles, the use of cyclodextrins has many advantages. Graffing of sugar moieties onto the primary and/or secondary alcoholic groups of cyclodextrins may be performed by selective functionalization methodologies.

In recent years, there has been a plethora of systems reported for the investigation of carbohydrate–lectin interactions. In particular, sugar-functionalized carbon nanotubes (CNTs) have received considerable attention, in non-covalent as well as covalent systems since they are ideal platforms for the construction of multivalent architectures. Halloysite clay nanotubes (HNTs) are other naturally available nanosize systems. HNTs are tubular aluminosilicate clays with external diameters of 50–80 nm, lumens of 10–15 nm in diameter, and lengths of about 1000 nm. An important advantage of halloysite clays, as compared with platty clays such as montmorillonite, kaolin and laponite stacked in larger crystallites, is that the HNTs do not need exfoliation and can be easily dispersed in water or polar polymers.

Halloysite has been found to be a viable and inexpensive nanoscale container for the encapsulation of biologically active molecules such as biocides and drugs, as demonstrated by Price, Lyov and colleagues. Halloysite clay nanotubes (HNTs) have received considerable attention, in non-covalent as well as covalent systems since they are ideal platforms for the construction of multivalent architectures. Halloysite clay nanotubes (HNTs) are other naturally available nanosize systems. HNTs are tubular aluminosilicate clays with external diameters of 50–80 nm, lumens of 10–15 nm in diameter, and lengths of about 1000 nm. An important advantage of halloysite clays, as compared with platty clays such as montmorillonite, kaolin and laponite stacked in larger crystallites, is that the HNTs do not need exfoliation and can be easily dispersed in water or polar polymers.

In the present paper we describe a novel hybrid that could act as a molecular recognition system based on HNTs covalently linked to carbohydrate–cyclodextrin units. We opted for...
covalent instead of supramolecular binding of cyclodextrin to the HNT surface to make use of the major advantages of this approach, in particular the great stability of the hybrid material, the control over the degree of functionalization, and the data reproducibility.

In order to employ the new system as a drug delivery system we also studied its interaction with curcumin, a molecule with interesting biological activity.26

2. Results and discussion

Pristine halloysite was transformed, as described,27 into f-HNT-SH 1 and it was covalently linked to heptakis-6-(tert-butyldimethyloxysilyl)-2-allyloxy-β-cyclodextrin 2, which was synthesized by a thiol-ene reaction according to the procedure reported in the literature (Scheme 1).28

First of all, we investigated the photoinduced reaction of 2 with thiol halloysite 1. Experimental conditions similar to those used in the literature were adopted,28 i.e., irradiation with UV light from a Hg lamp. The experiment was carried out in a quartz vial at room temperature under argon atmosphere. Irradiation of a stirred dispersion of 1 and heptakis-6-(tert-butyldimethylsilyl) 2-allyloxy-β-cyclodextrin 2 in methanol, gave a product within 24 h, which was analysed by TGA, after filtration, in order to evaluate the percentage of the loading. This condition yielded a loading percentage of as large as 2.8% (Table 1, Entry 1).

Afterwards, we investigated the same reaction performed by microwave (MW) irradiation in solvent-free conditions for an irradiation time of 1 h at different temperatures, as reported in Table 1. We noticed that in these conditions (Entry 2) the loading of CD is only a little lower than that observed for the photochemical reaction (Entry 1). Therefore, we raised the operating temperature, which afforded a much more satisfactory loading of CD (Entry 3). The obtained results highlighted the importance of microwave radiation in improving this synthesis.

The use of microwaves and solvent-free conditions indeed improved the linkage of saccharides on the HNT external surface, and accomplished this linkage much more rapidly than does traditional synthesis. Since we have worked in solvent-free conditions, the reaction may be considered “green”.

Based on these results, we proceeded to graft the sugar onto 3 (Scheme 2). Hence, we carried out the photoinduced coupling of 3 with galactosylthiol 4, mannosylthiol 5 or lactosylthiol 6. After deacetylation of compounds 4, 5 and 6 to form the corresponding hydroxyl groups, the reactions of these compounds with compound 3 gave the corresponding per-glycocluster (7a-c) with a loading percentage of 3.8, 1.2 and 1.5% respectively (Table 2) as determined by the residual mass from TGA measurements.

We first studied the most suitable conditions for the synthesis of 7a. We noticed that, as for the synthesis of 3, the solvent-free microwave irradiation condition at 80 °C afforded a slightly lower loading as compared to the photochemical reaction. Raising the temperature for the microwave reaction up to 100 °C, however, resulted in the thermal decomposition of the sugar. Therefore, in contrast to the method chosen to synthesize 3, we chose to perform the syntheses of 7a-c using only the photochemical procedure.

Based on the loading reported in Table 2, the content of cyclodextrin and experimental conditions adopted, we can estimate that there are between three and six active sites on CD used for sugar grafting.

The new materials were characterized using IR spectroscopy, thermogravimetric analysis (TGA) and scanning electronic microscopy (SEM).

An FT-IR investigation of 7 shows that the vibrational bands of HNTs remain unaltered after the reactions. The frequency and assignments of each vibrational mode are based on previous reports on halloysite.

The pristine HNT shows two bands at 3622 and 3493 cm⁻¹ corresponding to O–H stretching vibrations of buried hydroxyl groups and inner surface hydroxyl groups, respectively.29

The bands at 3484 and 1645 cm⁻¹ are assigned to O–H stretching vibrations of adsorbed water. The bands at 1115 and 1031 cm⁻¹ are attributed to apical Si–O and Si–O–Si stretching vibrations, respectively. The band at 912 cm⁻¹ arises from O–H deformation vibration of inner Al–O–H groups. The band at 793 cm⁻¹ is attributed to the Si–O–Si stretching vibrations.

Table 1  Experimental conditions for the linkage of CD on HNT

<table>
<thead>
<tr>
<th>Entry</th>
<th>Method</th>
<th>Solvent</th>
<th>Time (h)</th>
<th>Temperature (°C)</th>
<th>Loading (wt %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>UV</td>
<td>MeOH</td>
<td>24</td>
<td>r.t.</td>
<td>2.8</td>
</tr>
<tr>
<td>2</td>
<td>MW</td>
<td>Free</td>
<td>1</td>
<td>80</td>
<td>2.5</td>
</tr>
<tr>
<td>3</td>
<td>MW</td>
<td>Free</td>
<td>1</td>
<td>100</td>
<td>4.1</td>
</tr>
</tbody>
</table>

* Cycloextrin content calculated from TGA results.

Scheme 1 Schematic representation for the linkage of cyclodextrin on HNT.

Scheme 2 Schematic representation of the linkage of sugar with compound 3.
cm\(^{-1}\) is assigned to Si–O symmetric stretching vibration. The bands at 753 and 690 cm\(^{-1}\) derive from perpendicular Si–O stretching vibrations (see ESI†).

Compared to pristine HNT, compounds 7a–c exhibit the vibration bands for C–H stretching of methylene groups around 2960 cm\(^{-1}\) and 2865 cm\(^{-1}\). These findings provide evidence for the presence of organic moieties in compounds 7a–c. Based on the unaltered frequency of the stretching bands of the OH of the inner-surface Al–OH groups, we can conclude that grafting has taken place only on the external surface of HNT.

Samples 7a–c were investigated by means of TGA to determine the amount of the organic moieties that were grafted. A mass lost in three steps was observed for 7a–c (see SI†). Based on the thermogram of the precursor 3, that of 7a–c show an additional mass loss in the range from 200 to 400 °C due to the degradation and volatilization of the carbohydrate grafted to the CD. Unfortunately, the pyrolytic processes overlap and the degradation temperature of each organic moiety could therefore not be determined.

Direct observations of the surface morphologies of 3 and 7 were carried out by scanning electron microscopy (SEM). Images show that the tubular shape of the nanoclay is not lost after the grafting of the organosilane and the binding of the CD and the sugar. Moreover, the lumen of the nanotube remains empty after functionalization, in agreement with previously reported data. Comparing the SEM images of 3 and 7 with that of the pristine material,\(^\text{28}\) we can notice that the average characteristic sizes of the nanoparticle are not altered (Fig. 1).

### 2.1. Dispersion in aqueous medium

Turbidimetric and UV-vis analyses were performed to highlight the influence of functionalization on the dispersion stability of HNT in aqueous media, which might be crucial for its application as a drug delivery system. Dispersions of compound 3 showed a higher stability in water than did pristine HNT. This result is a clear consequence of surface functionalization. The introduction of sugar moieties indeed increases the stability in water, owing to the enhanced hydrophilic character assumed by the external surfaces of the nanotubes (Fig. 2).

The absorbances at 400 nm of bare HNT and of 7a in aqueous medium at varying concentrations (0.1–1.3 mg L\(^{-1}\)) were measured (Fig. 3). As we can see, absorbances of both bare HNT and 7a suspensions increase linearly with concentration.

The straight lines in the figure describe the relationships between the absorbance and concentrations for the relatively bare HNT and 7a. The correlation coefficient between absorbance and concentration for 7a (\(R^2 = 0.9878\)) is higher than that for HNT (\(R^2 = 0.94477\)). It is clear that the 7a suspension well follows the Lambert–Beer’s law, which suggests that 7a exhibits good dispersion in the aqueous phase. Noticeably, sonication of 7a suspension exhibits no influence on the absorption peak intensity. This behavior indicates that β-CD and sugar have been grafted onto HNT surfaces via chemical interactions rather than physical sorption. The translational diffusion behavior of modified HNTs was characterized by dynamic light scattering (DLS). In particular the average diffusion coefficients (\(D\)) of 11 × 10\(^{-13}\) m\(^2\) s\(^{-1}\) and 19 × 10\(^{-13}\) m\(^2\) s\(^{-1}\) were obtained for compounds 3 and 7a–c. These values are slightly larger than that for pristine HNT (9.4 × 10\(^{-13}\) m\(^2\) s\(^{-1}\)),\(^\text{30}\) which shows that anchoring organic moieties slows down the translational diffusion.
diffusion of the nanotubes. This effect can be due to both the increase of the hydrodynamic size of the single particles or attractive interactions that determine the formation of the clusters. This interpretation also explains the further increase of D upon the introduction of carbohydrates.

2.2. Interaction with curcumin

Finally, we performed a preliminary test in order to verify the binding abilities of our new systems and the possibility to exploit them as carriers for biologically active molecules.

In particular, the inclusion equilibria between pristine HNT, β-CD, 7a and curcumin (Curc) were taken into account. Curcumin is a well-known molecule, has been shown in vitro to be a potentially active agent towards several tumor cell lines, and has also been tested for its efficacy towards HIV and cystic fibrosis, as well as its anti-microbial activity.26 Curcumin is a symmetric molecule with two aromatic moieties and an enolizable central core. Supramolecular inclusion of curcumin into CDs has been studied, and the formation of a 1 : 2 stoichiometry for Curc-CD complexes has been demonstrated.31 The interaction between Curc and pristine HNT has also been studied. This study showed curcumin both bound to the outer surface of HNT and incorporated into its cavity.32

The results of these reports led us to carry out a preliminary investigation of the possible interaction of Curc with our new functionalized HNTs (7a), using UV-vis spectroscopy. In particular, we recorded UV-vis spectra of a fixed concentration of curcumin (1 × 10⁻⁵ M) in the presence of increasing amounts of pristine HNT (0–1.8 mg mL⁻¹), β-CD (0–1.08 × 10⁻³ M) and 7a (0–1.4 mg mL⁻¹), all in water at 25 °C. Fig. 4 shows the spectra relative to free Curc and to the mixtures prepared with three representative HNT concentrations. The inset of this figure also shows the spectra relative to the 2 : 1 complex between Curc and native β-CD.

The UV-vis spectrum of free Curc shows a major absorption band centered at 420 nm, corresponding to an π–π* transition of the conjugate carbonyl moiety, and a secondary band centered at 260 nm corresponding to a n–π* transition.

As we can easily see, the interaction with 7a causes the former band to essentially disappear, and the intensity of the latter band to increase markedly. It is very important to notice that recorded spectra do not show any isosbestic point. This finding indicates that multiple step complexation equilibria occur in the system. In any case, absorbances recorded at 260 nm increase monotonically with increasing amounts of HNTs. It is also interesting to notice that the observed behavior differs significantly from the behaviors observed for the interaction of Curc with either native β-CD or pristine HNTs. In the former case, indeed only a hypsochromic shift of the Curc n–π* band can be observed; in contrast, in the latter case, a hypochromic effect on the same band occurs, together with the aforementioned hyperchromic effect on the π–π* band.

A possible model explaining the observed results is depicted in Fig. 5.

In particular we can hypothesize a multiple-step complexation process, by which Curc first interacts with one functionalized HNT unit, either by inclusion into a single CD unit (8a) or more loosely with the HNT outer wall (8b) or even in both ways (8c). TGA data may give some additional information about the inclusion site. For this purpose, the solid complex 7a/Curc was investigated and the thermogram is reported in ESL† It is well known that organic compounds entrapped into the HNT lumen show a strong increase of the degradation temperature due to the entrapment into the nanotube cavity.32,33 In our case the degradation of curcumin in the complex occurs in the range from 300 to 400 °C, which is similar to degradation temperature of the pristine curcumin (see thermograms in SI†). On this basis we can rule out the inclusion into the HNT lumen. In a subsequent step, on increasing the amount of HNT in the system, we can hypothesize that a sort of 1 : 2 complex is formed, in which a single Curc unit bridges two HNTs (9). The disappearance of the Curc n–π* band can be easily explained on the grounds of this scheme. In fact, it has been reported that when the polarity of medium is increased, the keto-enol equilibrium is shifted towards the enol, and spectral structure is lost.34 We can

---

**Fig. 4** UV-vis spectra of curcumin and 7a/Curc complexes. The inset shows the spectra relevant to the 2 : 1 complex between Curc and native β-CD.

**Fig. 5** Structural formulas and graphical representation of the hypothesized equilibria.
reasonably hypothesize that the highly functionalized hydrogen-bond donor–acceptor environment provided the sugar moieties linked to the CD scaffold with a large enhancement of the stability of the enol form of Curc, with consequent suppression of the absorption at 420 nm. This hypothesis is supported by IR results (Fig. 6). The spectrum of the solid complex indeed shows the disappearance of the C=O and C=C stretching bands at 1628, 1605 and 1509 cm\(^{-1}\), indicating the occurrence of deep structural changes for Curc upon formation of the solid complex with the HNT.

### 3. Experimental section

#### 3.1. Materials and method

All needed reagents were used as purchased (from Aldrich), without further purification. Thiol-functionalized HNTs, heptakis-6-(tert-butyldimethylsilyl) 2-allyloxy-β-cyclodextrin and thiol sugars were prepared according to previous reports.\(^{25,26}\)

Microwave-assisted syntheses were carried out with a CEM DISCOVER monomode system in a closed vessel.

An AESEM FEI QUANTA 200F microscope apparatus was used to study the morphology of the functionalized HNTs. Before each experiment, the sample was coated with gold under argon by means of an Edwards Sputter Coater S150A, in order to avoid charging under the electron beam.

Thermogravimetric analyses were performed on a Q5000 IR apparatus (TA Instruments) under a nitrogen flow of 25 cm\(^3\) min\(^{-1}\) for the sample and 10 cm\(^3\) min\(^{-1}\) for the balance. The weight of each sample was ca. 10 mg. Measurements were carried out by heating the sample from room temperature up to 900 °C at a rate of 10 °C min\(^{-1}\).

IR spectra (KBr) were recorded with an Agilent Technologies Cary 630 FT-IR spectrometer. Specimens for these measurements were prepared by mixing 5 mg of the sample powder with 100 mg of KBr.

DLS measurements were performed at 22.0 ± 0.1 °C in a sealed cylindrical scattering cell at a scattering angle of 90°, by means of a Brookhaven Instrument apparatus composed of a BI-9000AT correlator and a He–Ne laser (75 mW) at a wavelength (\(\lambda\)) of 632.8 nm. The solvent was filtered through a Millipore filter with a pore size of 0.45 μm. For all systems, the field-time autocorrelation functions were well described by a mono-exponential decay function, which provides the decay rate (\(\Gamma\)) of the single diffusive mode. For the translational motion, the collective diffusion coefficient at a given concentration is \(D_i = \Gamma / q^2\) where \(q\) is the scattering vector given by \(4\pi n\lambda^{-1} \sin(\theta/2)\). In this equation, \(n\) is the water refractive index and \(\theta\) is the scattering angle.

Turbidimetric measurements were performed with a Beckman DU 650 spectrometer.

The dispersions were sonicated with an ultrasound bath VWR Ultrasonic Cleaner (power 200 W, frequency 75 MHz).

#### 3.2. Synthesis of the HNT 3 modified by heptakis-6-(tert-butyldimethylsilyl) 2-allyloxy-β-cyclodextrin

**3.2.1 MW irradiation.** Thiol-functionalized HNTs (0.10 g) were weighed in a MW test tube provided with a cap and heptakis-6-(tert-butyldimethylsilyl) 2-allyloxy-β-cyclodextrin (0.4 mmol) was added. The mixture was inserted in the MW apparatus and the temperature varying between 80 and 100 °C and a power of 30 W, under constant stirring, for 1 h. The solid was filtered off, rinsed with CH\(_2\)Cl\(_2\) and dried at 80 °C under vacuum.

#### 3.3. Synthesis of compounds 7a–c

The appropriate thiol 4–6 (21 eq.) and modified HNT 3 (350 mg) were dissolved in distilled MeOH. A stream of Ar was bubbled through the solution for 15 min to thoroughly degas it. The solution, kept under an Ar atmosphere, was placed in front of an Hg lamp and stirred for 24 h. Following removal of solvent, the precipitate was washed with CH\(_2\)Cl\(_2\) and dried overnight at 80 °C under vacuum.

**3.3.1 Deacetylation of hydroxyl groups.** In a 10 mL round bottom flask compounds 6a–c were weighed, and 4 mL of MeOH were added under constant stirring. Then a solution of MeOH/MeOna was added dropwise at the dispersion in order to make the system slightly basic. The reaction mixture was stirred at room temperature for 5 days. After this time the dispersion was filtered, rinsed with MeOH and dried overnight at 80 °C in vacuo.

#### 3.4 General procedure to obtain 7a/curcumin dispersions

Various weighed amounts of 7a (from 2 to 14 mg) were dispersed by sonication for 5 minutes in H\(_2\)O, at an ultrasound power of 200 W and a temperature of 25 °C. 1 mL of an aqueous curcumin solution (1 × 10\(^{-4}\) M) was added to the 7a dispersions. The final volume was 10 mL.

#### 3.5 General procedure to obtain solid complexes

To a dispersion of 7a in 9 : 1 water–methanol (250 mL) were added 2.5 mL of a 10\(^{-3}\) M curcumin solution in methanol. The obtained dispersion was stirred for 24 h at r.t. and then was filtered, the powder was washed with small amounts of water and then dried at 60 °C under vacuum overnight.

In this way, we obtained a material with 7.2 wt% of curcumin.
4. Conclusions

The preparation of a halloysite-functionalized CD material has been successfully carried out by means of solvent-free microwave irradiation techniques. Then, the obtained material was further functionalized by decorating the CD units with thiosaccharide units, in such a way as to obtain new designs of biocompatible multivalent scaffolds for drug delivery, which enables the mimicking of the typical lectin–carbohydrate interactions occurring on the cell wall and implied in cellular recognition phenomena. Our materials were characterized by combined FT-IR, TGA, SEM and DLS techniques, which showed that the functionalization involves the outer surface only, and makes the composite more stable in aqueous suspension than pristine halloysite. The potential of our materials to carry out effective drug delivery was tested by studying their interactions with curcumin. In particular, UV-vis and FT-IR results allowed us to hypothesize a likely model for the interaction, involving the occurrence of both guest-CD and guest surface interactions, which result in the possibility that curcumin units might form a sort of 1 : 2 complex joining different material units; on the other hand, the possible internalization of the curcumin into the halloysite inner lumen may be ruled out. In this study we designed an advanced composite drug delivery system with two cavities available for encapsulation of different drug molecules or active species for imaging to be used in simultaneous applications.

Acknowledgements

The work was financially supported by the University of Palermo, PRIN 2010-2011 (prot. 2010329WPF) and FIRB 2012 (prot. RBFR12ETL5).

Notes and references


