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Glycosyl-nucleoside fluorinated amphiphiles as components of nanostructured hydrogels

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ABSTRACT

The synthesis of two novel glycosyl-nucleoside fluorinated amphiphiles (GNFs) derived from the 2*H*,2*H*,3*H*,3*H*-perfluoro-undecanoyl hydrophobic chain is described. The GNF amphiphiles, which feature either β-D-glucopyranosyl or β-D-lactopyranosyl moieties linked to a thymine base via a 1,2,3 triazole linker, were prepared using a 'double click' chemistry route. Surface tension measurements, gelation properties, and TEM studies show that GNFs spontaneously assemble into supramolecular structures. Similarly to their hydrocarbon analogues (GNLs), the GNFs have unique gelation properties in water. A minimum hydrogelation concentration of 0.1% (w/w), was determined in the case of the β-D-glucopyranosyl derivative. Cell viability studies indicate that fluorocarbon GNF **5** was not toxic for human cells (Huh7), whereas hydrocarbon analogue GNL is toxic above 100 μm.

Owing to their unique properties, hydrogels have attracted much attention for potential applications in nanoscale devices or systems.[1] These soft materials are present in numerous fields of research including chemistry, biology, and biomedicine.[2] For example, hydrogels can provide scaffolds for cell cultures,[3,4] matrix for tissue engineering,[5,6] or drug delivery systems.[7–9] Among the gelator molecules investigated so far, low molecular weight gelators (LMWGs)[10] are of particular interest for biomedical applications. Hence, due to their intrinsic properties, in particular the non-covalent nature of the molecular interactions at work, LMWGs feature several advantages to macromolecules such as polymers. Thus due to their supramolecular structure, the water gelation by small molecules is sensitive to external stimuli. These systems also allow an easier clearance from the body compared to polymeric gel. In this context, a critical challenge facing this field is how to develop non-toxic LMWGs, which can provide biocompatible supramolecular hydrogel scaffolds at low concentration. This is critical to develop new materials suitable for drug delivery and/or tissue engineering applications.

To this end, amphiphilic molecules derived from natural chemical structures appear as suitable small molecular building blocks to construct supramolecular hydrogels. Recently, bio-inspired-amphiphiles derived from amino acids have been reported to form supramolecular networks capable to stabilize gels.[11] Likewise, we have described that amphiphilic building blocks, appropriately functionalized with nucleoside and/or nucleotide moieties, form well-defined supramolecular systems with tunable physico-chemical properties and functions.[12] The combination of nucleic acids chemistry with supramolecular principles has been explored by several groups to prepare nucleoside-amphiphile based gels.[13–17] As hydrophobic parts, fluorocarbon chains can be inserted in the building blocks. An appealing part of the perfluorinated or semifluorinated hydrocarbon chains lies in their special properties related to the characteristics of the fluorine atom. It is well established that fluorinated chains are highly hydrophobic, chemically, and biologically stable and feature segregation behavior toward perhydrogenated compounds.[18–20] Numerous examples of fluorocarbon amphiphiles have been reported for different purposes, including gel stabilization,[21–24] and biomedical applications.[25–30] We previously reported that the combination of fluorocarbon chains with nucleoside has a significant impact on the properties of the supramolecular assemblies observed compared to their hydrocarbon analogues.[31] In parallel, we demonstrated that glycosyl-nucleoside-lipids (GNLs) self-assemble to give highly organized structures, which can stabilize hydrogels.[32,33] In the present study, we report the synthesis of two novel glycosyl-nucleoside fluorinated amphiphiles (GNFs), their self-assembly properties, the formation of hydrogels, and a study of their possible cytotoxicity.

We hypothesized that the use of a GNF based amphiphile would allow for the stabilization of non-toxic hydrogels. For this purpose, the 2H,2H,3H,3H-perfluoroundecanoyl chain was selected to provide a similar hydrophobicity character as the palmitoyl/stearoyl hydrocarbon chains.[34] This choice was also motivated by the non-detergency toward cell membranes of this fluorocarbon chain,[35] which may lead to a poor cytotoxicity.

The GNFs based amphiphiles **5** and **6** were synthesized in three steps starting from *N*-propargyl-2*H*,2*H*,3*H*,3*H*-perfluoroundecana-

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mide **1** and 5′-azido-2′-deoxythymidine **2** following a route as detailed in Scheme 1. Following a first 'click' reaction, the N-propargylated fluorocarbon **1** was reacted with the azido derivative **2** in the presence of $CuSO_4$ in a THF/H_2O mixture to afford the expected 2*H*,2*H*,3*H*,3*H*-perfluoroundecanamide-triazolyl-thymidine intermediate **3.** This fluorocarbon nucleoside (FN) intermediate **3** was treated at room temperature in the presence of potassium carbonate with 2 equiv of propargyl bromide to lead to N-propargylated thymidine derivative **4**. Then, either 1-azido-β-D-glucopyranosyl or 1-azido-β-lactosyl moieties were reacted with the *N*-propargyl derivative **4** in the presence of $CuSO_4$ following a second 'click' reaction to provide the expected non-ionic GNFs based amphiphiles (GNFs, compounds **5** and **6**).

One of the goals of the present study was to determine the self-assembly properties of GNFs **5** and **6**. The critical aggregation concentrations (CAC's) of GNFs were determined by air-solution surface tension (γ) measurements as a function of amphiphile concentration c. Example of (γ) versus (c) curves, measured at 25 °C, are shown in Figure 1. The GNF amphiphiles **5** and **6** gave breaks in γ versus Log (c) characteristic of CAC's of 5.89 ± 1.39 and 3.65 ± 0.16 μM, respectively. Note that CAC values for GNFs are similar in magnitude to the ones reported for GNLs bearing C_{18} hydrocarbon chains.[32] The surface tension measured at the CAC (γ_{cac}) indicates the effectiveness of the amphiphiles to stabilize

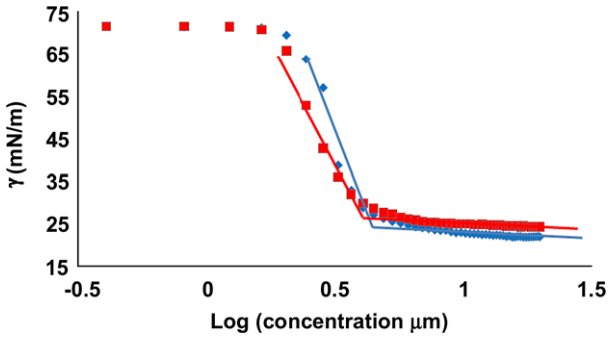
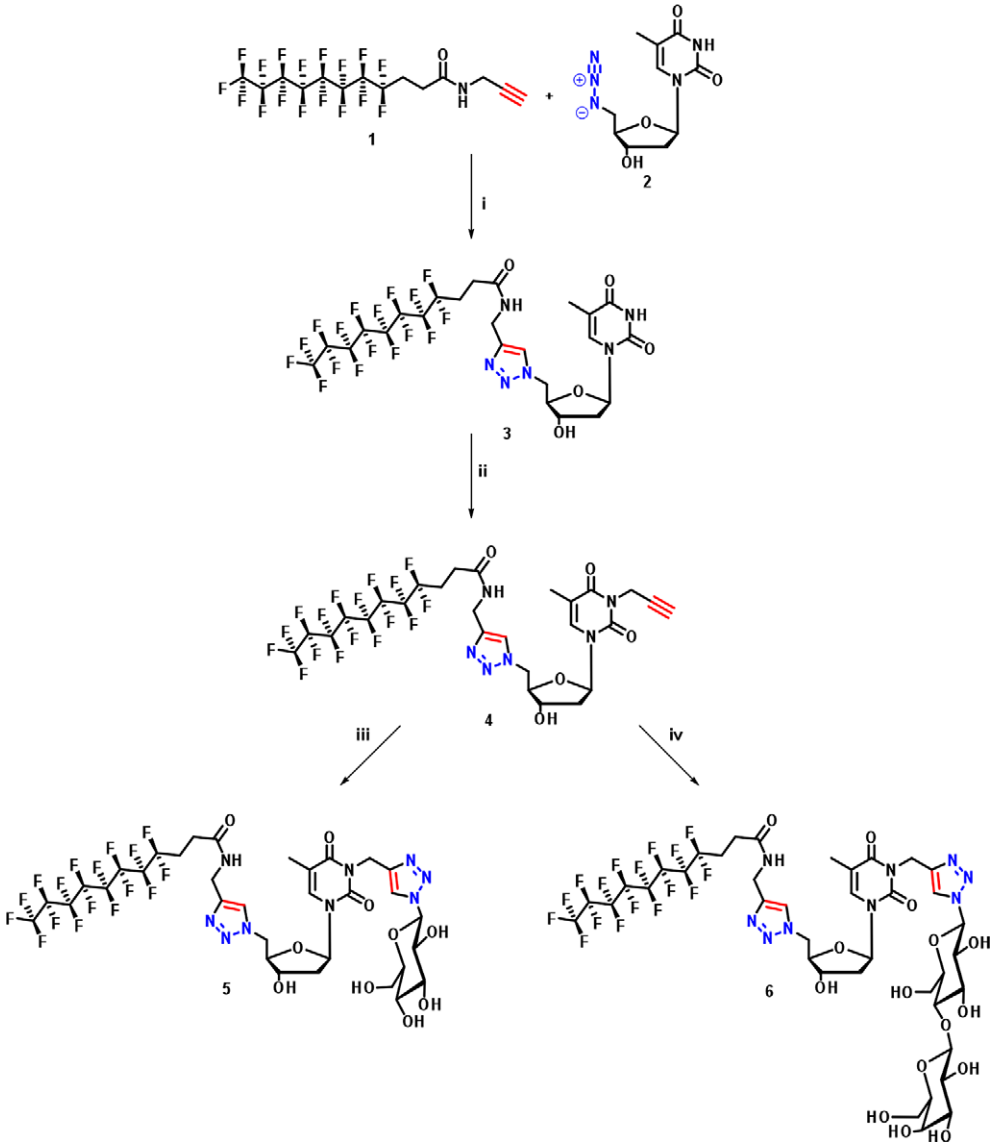


Figure 1. Air–water interfacial tension γ versus concentration for compound **5** (blue diamond) and compound **6** (red square) at 25 °C.



Scheme 1. Three step synthesis of the GNFs (**5**) and (**6**). Reagents and conditions: (i) 10 mol % $CuSO_4$, 20 mol % sodium ascorbate, THF/H_2O (50/50), 65 °C, 10 h; (ii) 2 equiv propargyl bromide, 2 equiv K_2CO_3, DMF, rt, overnight; (iii) 1 equiv 1-azido-β-D-glucopyranoside, 10 mol % $CuSO_4$, 20 mol % sodium ascorbate, THF/H_2O (50/50), 65 °C, 10 h; (iii′) 1 equiv 1-azido-β-lactoside , 10 mol % $CuSO_4$, 20 mol % sodium ascorbate, THF/H_2O (50/50), 65 °C, 10 h.

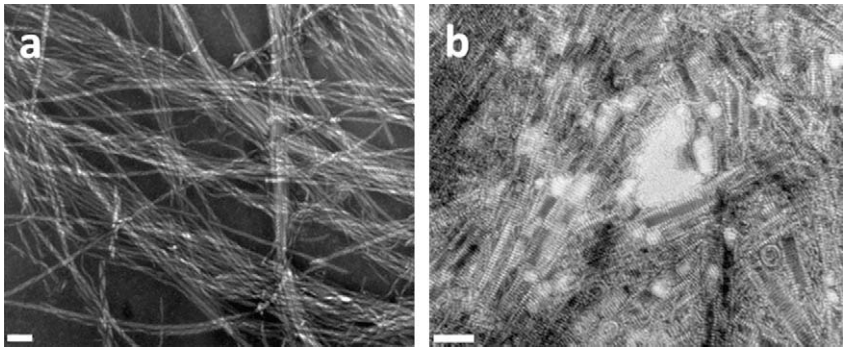


Figure 2. TEM images of supramolecular assemblies formed by GNFs **5** (a) and **6** (b) in water (scales: 100 nm).

the water–air interface. As expected the fluorocarbon chain decreases the γ_{cac} values ($\gamma_{cac} \approx 27$ and 25 mN m^{-1}, for **5** and **6**, respectively) compared to the C_{18} hydrocarbon GNLs ($\gamma_{cac} \approx 35$ mN m^{-1}).

Given our interest in LMWGs the gelation abilities of GNFs were studied both in pure water and in tissue culture medium (Dulbecco's modified Eagle medium, DMEM). Interestingly, clear gels were formed both in water and in DMEM for GNFs **5** and **6** (see Fig. SI5). The hydrogels obtained were stable at room temperature for several weeks. In water, the minimum gelation concentration was found to vary with the sugar moiety of the polar head. Similarly to its saturated C_{18} hydrocarbon analogue, the β-D-glucopyranoside GNF **5** is an excellent hydrogelator with a minimum gelation concentration of 0.1% w/w, whereas GNF **6** is less effective with a minimum gelation concentration of 2.5% w/w. Note that, contrary to hydrocarbon GNLs, which were not able to stabilize the hydrogels at low concentrations in the presence of DMEM, fluorocarbon GNFs stabilize gels at low concentrations in DMEM (0.1% and 1% w/w for **5** and **6**, respectively), indicating that these fluorocarbon derivatives can be used for cell culture environments. However, both GNFs **5** and **6** are not able to stabilize a gel in organic solvents such as *n*-hexane, *n*-butan-1-ol, toluene chloroform, DMSO, and perfluorohexane.

The morphology of the supramolecular network formed by GNFs was investigated through transmission electronic microscopy (TEM). GNFs gelators displayed different organization networks of varying morphologies (Fig. 2). In water, gelator **5** having β-D-glucopyranoside polar head formed entangled nanofibers of roughly 10–20 nm in diameter (Fig. 2a). A very compact system showing fibers helically organized reminiscent to springs (see Fig. SI4d for magnification of TEM images) was observed for β-D-lactopyranoside derivative **6** (Fig. 2b). These observations underline that the polar head structure has an impact on supramolecular organisations, and consequently on hydrogel macroscopic properties.

Another goal of this study was to determine the cytotoxicity of GNFs as compared to that of GNLs. The cell viability was assessed after a 5-day incubation of growing human cells (Huh-7, human hepatocarcinoma cell line) with increasing concentrations of GNF **5** or C_{18} GNL hydrocarbon analogue (see Fig. SI6 for GNL structure). Importantly, cell viability data presented in Figure 3 indicate that fluorocarbon GNF **5** are not toxic for these human cells at any of the tested concentrations, whereas hydrocarbon GNL becomes toxic at concentrations above 100 μM.

In conclusion, we report the synthesis of two fluorocarbon glycosyl-nucleoside fluorinated amphiphiles bearing a C_8F_{17} chain and either β-D-glucopyranoside or β-D-lactopyranoside sugar moieties. The presence of the fluorocarbon chain allows the formation of supramolecular systems, including either nanofibers or helical spring. Interestingly, GNFs also stabilize hydrogels at low concentrations (0.1% w/w for GNF **5**) both in the absence or presence of DMEM. The combination of the hydrogelation properties and the non-toxicity of GNFs demonstrate the great potential of these fluorocarbon amphiphiles for biomedical applications.

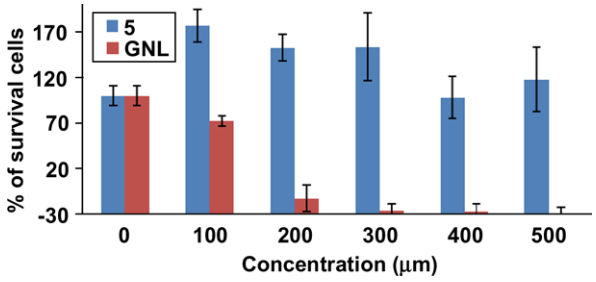


Figure 3. Cell viability in percent versus concentrations of GNF **5** and GNL (see Fig. SI6 for GNL structure).

Acknowledgments

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Supplementary data

Experimental procedures (synthesis of compounds **1**–**6**, Surface tension measurements, cells culture, cells survival), HRMS *spectra* for compounds **5** and **6**, TEM images, gel in water and DMEM images). Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.12.042.

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