Carbohydrate Polymers 86 (2011) 1093–1106

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Review

Sugar-labeled and PEGylated (bio)degradable polymers intended for targeted drug delivery systems

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ARTICLE INFO

Article history:
Received 23 March 2011
Received in revised form 26 May 2011
Accepted 5 June 2011
Available online 12 June 2011

Keywords:
Biocompatibility
Carbohydrate
PEGylation
Drug delivery systems
Long-circulating carriers
Active targeting

ABSTRACT

This paper aims at giving a comprehensive view of the research effort devoted to the preparation of sugar coated long-circulating degradable polymers intended for drug delivery applications. In the recent past, many research projects have focused on the controlled drug delivery and, therefore, on the design of drug carriers. Among them, polymeric carriers have great potential because they can be chemically modified to a large extent and so endowed with specific properties. For instance, depending on the selected polymer, either the circulation time in the bloodstream can be increased very significantly (long-circulating polymer) or the drug carrier can be completely degraded after administration. Moreover, active targeting, i.e., carriers bearing a ligand known for specific affinity for one tissue, has emerged as a method of choice in targeting the delivery of drugs. This concept is of the utmost importance because the large variety of receptors present in the body makes the selective targeting a must in order to prevent any healthy tissue from being damaged irreversibly. The purpose of this paper is to emphasize that carbohydrates are very promising pilot molecules for the next generation of drug delivery systems.

Contents

1. Introduction

Progress in the understanding of the molecular mechanism that underlies disease processes has led to the concept of one-disease one-target model. The idea is based on the complexation of a specific biochemical entity, the target, by a bioactive compound, such that this selective recognition enhances the biological activity to the point where a disease can be cured or at least effectively treated (cf. Section 2). Nowadays, computational chemistry and molecular modeling are methods of choice to design a drug and to fully exploit the aforementioned strategy. Once the bioactive compound has been designed, it is synthesized and tested. This three-steps cycle is repeated until the right molecule that triggers the desired

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doi:10.1016/j.carbpol.2011.06.004

cascade of events at the target is made available. (Barril, Hubbard, & Morley, 2004; Testa, 1997) At that point, the use of the optimized free drug in the body can raise problems related to systemic side effects and bioavailability. In this context, drug delivery must be controlled, which accounts for the search for strategies effective in liberating drugs at the right tissue so decreasing damages on healthy tissues. This manuscript aims at reporting on active targeting, i.e., decoration of the drug carrier surface with a pilot molecule selected for affinity for the tissue of interest. A special attention will be paid to carbohydrates because of quite valuable properties, including the ability to recognize receptors in the body and to target accordingly diseased tissues and organs. In order to fully exploit theses possibilities, new carriers have been searched for and polymeric carriers have emerged as materials of choice because of easy functionalization. The properties of the main polymers used for this purpose will be reported, with a special emphasis placed on degradable polymers. Finally, synthesis of long-circulating and (bio)degradable polymeric carriers and their decoration with sugar will be discussed.

2. Need for controlled drug delivery systems

It is mandatory to control the delivery of drugs in order to get rid of the problems raised by free drugs in the body. First of all, most drugs (organic molecule, proteins, nucleotides, etc.) are more or less rapidly degraded by enzymatic or metabolic processes. Therefore, drugs must be encapsulated for being protected against the external medium and for a large percentage of active compound to reach the pathological area. As a result, a lower amount of drug can be administered at the benefit of the production cost and the patient health (reduced side-effects). The poor solubility in water of most of the new drugs is an additional issue. Carriers consisting of amphiphilic (macro)molecules are then a way to increase the solubility of hydrophobic drugs and to improve their bioavailability. Interestingly, the rate of drug release can be tuned by the chemical composition of the carrier in order to fit the drug pharmacokinetics known for a curative effect. Proper control of release-rate and dose allows the drug concentration to be maintained between the sub-therapeutic drug level and the toxic level thus the treatment to be optimized.(Uhrich, Cannizzaro, Langer, & Shakesheff, 1999) Conversely, in case of conventional direct injection, the drug is rapidly metabolized and eliminated from the body, which results initially in an exceedingly high drug concentration at the site of action (toxic level) followed by a rapid decrease in the sub-therapeutic drug concentration domain. (Uhrich et al., 1999) Of course, controlled drug delivery enhances patient's comfort by avoiding regular intake. Another trouble caused by the injection of free drugs is the possible migration of the drug from the irrigation fluid into the subcutaneous tissues. This incident, known as extravasation, may have deleterious consequences whenever toxic drugs are used, such as doxorubicin in cancer therapy. Then, extravasation can lead to necrosis of tissues (Allen & Cullis, 2004). At the time being, a large variety of carriers, such as liposomes, polymer micelles (nanoparticles) and dendrimers, have been reported and currently used in controlled drug delivery (Letchford & Burt, 2007; Marcucci & Lefoulon, 2004). Biodegradable aliphatic polyesters, such as polylactide and polyglycolide, are well-accepted polymers in nanoparticles. These hydrophobic polymers are often associated to hydrophilic poly(ethylene oxide) (PEO), also designated as polyethylene glycol, is leading to amphiphilic constitutive components of nanoparticles (Torchilin, 2010). The added value of this association as block or graft copolymers, lies in the biocompatibility of the two polymeric partners, the degradability of the aliphatic polyesters and the bioelimination of PEO. Some of these copolymers have been approved by the FDA for application in drug delivery systems. Finally, the strong hydrophilicity and chain mobility of PEO accounts for well-established protein-repellent property, which is crucial whenever nanocarriers with long-circulating time in the blood-stream are concerned (Lee, Lee, & Andrade, 1995; Owens & Peppas, 2006). For all these reasons, only biodegradable amphiphilic copolymers containing poly(ethylene oxide) as the hydrophilic part will be considered in this paper as the building blocks of nanocarriers. Their synthesis, solution, properties including micellization and use as carriers in drug delivery systems have been reviewed elsewere (Kataoka, Harada, & Nagasaki, 2001; Mahmud, Xiong, Aliabadi Hamidreza, & Lavasanifar, 2007; Van Butsele, Jérôme, & Jérôme, 2007; van Vlerken, Vyas, & Amiji, 2007). Nevertheless, they are not selective as such towards diseased tissues, which is detrimental to the healthy ones. As mentioned in the Introduction, they must be modified for being operative in active targeting, which is based on ligand mediated interactions between the carrier and the targeted tissue (Marcucci & Lefoulon, 2004; Sutton, Nasongkla, Blanco, & Gao, 2007). The modification consists in grafting a pilot molecule onto the nanocarrier, which is selectively recognized by a receptor available on the surface of the targeted tissue. Once the pilot moiety is complexed with the receptor, the carrier is trapped within the tissue in which the drug must be released. In case of cancer treatment, the ligand recognition triggers the cell-mediated endocytosis leading to internalization of the drug loaded nanocarrier, which is a strategy much more selective than the passive targeting driven by the enhanced permeability and retention (EPR) effect (Torchilin, 2010). A variety of ligands have been tested, such as saccharides, peptides, antibodies and folic acid (Fahmy, Fong, Goyal, & Saltzman, 2005; Jagur-Grodzinski, 2009; Mahmud et al., 2007; Shi, Lu, & Shoichet, 2009; Sutton et al., 2007). Among them, sugars are receiving a steadily increasing attention and will be the focus of the next sections. Few examples of cells or tissues that contain sugar receptors and can be accordingly targeted will be first reported. Key properties of sugars and their potential as pilot molecules will then be discussed. Finally, physico-chemical methods able to probe the interactions between sugars and their protein-receptor, the lectins, will be considered.

3. Sugars as pilot molecules

3.1. Tissues and diseases possibly targeted by sugars

The purpose of this section is to establish that the use of sugars as pilot molecules in active targeting make sense. First, the most tissues that can be targeted by a selected-sugar labeled-carrier and thus the diseases that might be treated, will be considered. Then, the action mechanism at the molecular level will be tentatively approached.

The therapeutic effect of a sugar-labeled carrier depends however on sugar which actually dictates the receptors involved in the recognition process. Mannose is a very important and representative sugar used in the carriers labeling.

Mannose receptors are expressed on macrophages, which play a major role in the phagocytosis process responsible for the uptake of the particles, degradation and clearance from the bloodstream (Chellat, Merhi, Moreau, & Yahia, 2005). Therefore, targeting of macrophages is a way of treating bacterial infectious diseases, because macrophages are host cells of parasites and bacteria (Chellat et al., 2005). Because macrophages play a major role in the initiation and persistence of inflammation they can be targeted in the treatment of inflammatory diseases, such as rheumatoid arthritis (Feldmann, 2002), neuroinflammatory diseases (Misko, Trotter, & Cross, 1995), atherosclerosis (Rollins, 1997) and restenosis (Moreno et al., 1994). Genetic metabolic diseases, e.g., as Gaucher's disease and human immunodeficiency virus infection

(Everts et al., 2002), can also be treated by targeting macrophages, which are also active in the immune response to foreign antigens. Mannose receptors are also available on dendritic cells (DC) which are part of the immune system. Their main function is to capture antigens, to process them and to introduce them into native T cells, which then initiate the cellular immune response. DC plays a messenger role between the innate and adaptive immunity. Vaccine delivery system takes advantage of this characteristic feature to initiate adaptive immune response (Guermonprez, Valladeau, Zitvogel, Thery, & Amigorena, 2002; McGreal, Miller, & Gordon, 2005; van Vliet, Garcia-Vallejo, & van Kooyk, 2008). Interestingly enough, the bioadhesiveness of mannosylated nanoparticles might be exploited in for oral delivery (Fievez et al., 2009; Salman, Gamazo, Campanero, & Irache, 2006). Finally, alveolar macrophage makes possible administration by inhalation in the treatment of respiratory intracellular parasitic infections (Chono, Tanino, Seki, & Morimoto, 2008).

Receptor for galactose and lactose (a disaccharide of galactose and glucose) are highly expressed in hepatocytes liver cells (Ashwell & Harford, 1982). For instance, asialoglycoprotein (ASGR-R), which recognizes galactose and lactose, can be used in the treatment of diseases associated to liver, i.e., Wilson's disease, hereditary hemochromatosis and α1-antitrypsin deficiency (Pathak, Vyas, & Gupta, 2008). Moreover, liver is a primary location of tumor metastases, which explains that ASGP-R is targeted for the delivery of antitumor drugs. Galactose binding lectins (galectins) are overexpressed in a variety of tumor cells (Lahm et al., 2001), which paves the way to targeted systems in cancer therapy. Lactose receptors are also found on epithelial cells and are used for the treatment of cystic fibrosis (Allen et al., 1991). Moreover, galactose receptors are abundantly expressed in the lung (Powell, 1980; Zhang, Ma, & Sun, 2010) and used for lung-targeting gene delivery (Chen et al., 2008).

These representative examples illustrate the large range of tissues that can be targeted by a sugar moiety. The full exploitation of this opportunity requires however the availability of glycosylated carriers.

3.2. Sugar-receptor recognition

Success of the active targeting mainly relies on the communication between the carrier and the targeted cells, which is crucial in the human body at the cellular level. In this context, proteins have a key role. Indeed, after binding by a membrane receptor, a protein can trigger a cascade of fundamental biochemical responses in the cell. A lot of events are controlled by the decoding of this signal, from activation of genes to the cell proliferation. Less known, oligosaccharides or polysaccharides, referred as glycans, can also provide an efficient code system for biochemical signals (Gabius, 2008). Although the role of carbohydrates is well-known in energy storage and cell wall composition, it remains underestimated in information storage. Nevertheless, recognition processes are driven by "sugar language" based on a so-called "sugar code" as discussed hereafter (Gabius, André, Kaltner, & Siebert, 2002; Gabius, 2000).

3.2.1. Diversity of sugars

In the body, glycans are usually found on the outer surface of cells, where they have very important multifold roles. For example, N-linked glycans are known to target degradative lysosomal enzymes. Modification of a N-linked glycan with a mannose-6-phosphate residue serve as signal for the protein to which this glycan is attached, to move to the lysosome. In this case, the glycan associated to mannose-6-phosphate is effective not only in recognition but also in the trafficking of lysosomal enzymes. A glycan that designates a polysaccharide, an oligosaccharide or the carbohydrate portion of a glycoconjugate, e.g., a glycoprotein, a glycolipid and a proteoglycan, usually consists of O-glycosidic linkages of monosaccharides. For example, cellulose is a glycan composed of beta-1,4-linked D-glucose, whereas chitin is a glycan composed of beta-1,4-linked N-acetyl-D-glucosamine. Glycans can be homo- or hetero-polymers of monosaccharide residues, with a linear or a branched structure. The "sugar code" uses sugar as "letter" to form oligosaccharide as "word". Like in the written language, the change of letters order forms another word. The introduction of small substituents, such as sulfate and O-acetyl groups, generates an additional variability, equivalent to the "Umlaut" in the German language (Gabius, 2000). Only one parameter is affecting a given "word", i.e., the sequence of the "letters". In contrast, in the sugar code, four additional parameters of variability may be found in one oligosaccharide (Laine, 1997b): (i) each monosaccharide contains several hydroxyl groups for the oligomer modification by glycosidic bonds, including the C1 position, (ii) the branching of chains is common in polysaccharides, (iii) the anomeric position can be of the α or β type, (iv) the ring size can be changed, i.e., pyranose vs. furanose, which results in high isomer permutation and diversity of the oligosaccharide. A total isomer permutation of 1.44×10^{15} can be calculated for a hexamer containing 20 different sugars. For sake of comparison, 20 different amino acids would provide only 6.4×10^{7} possibilities (Laine, 1997a). This points out the high coding potential of oligosaccharides compared to peptides. An additional advantage of the "sugar code" is versatility in the construction of an impressive array of oligosaccharides while keeping the size of the active section small, which reduces the energetic cost of synthesis. However, the large variety of oligosaccharides makes the elucidation complex of their structure very difficult. During the last decades, sophisticated methods of isolation and analysis have been developed to tackle this issue. In parallel, techniques of oligosaccharides synthesis have been reported with the purpose to understand better their role in living organisms (Bernardes, Castagner, & Seeberger, 2009). Glycosylation is very common in constitutive proteins of eukaryotic and prokaryotic organisms, which led some authors to the conclusion that "more than half of all proteins in nature will eventually be found to be glycoproteins" (Apweiler, Hermjakob, & Sharon, 1999). Nevertheless, the relationship between structure and activity is not yet clear for each glycan (Varki, 1993; Varki et al., 2009).

Oligosaccharides of glycoproteins belong to either N-glycans or O-glycans. Whenever the oligosaccharide of the glycoprotein is bound to asparagine (Asn) residues by an N-glycosidic bond, the glycans is a N-glycan. Whenever it is bound to the hydroxyl group of an amino acid residue by O-glycosylation of serine or threonine, the glycan is an O-glycan. All the N-glycans have in common the following sequence: Mannose α1–6(Mannose α1–3) Mannose β1–4 N-acetylglucoseamine β1–4 N-acetylglucoseamine β1-asparagine (Varki et al., 2009). O-glycans have a higher structural diversity and the "common structure" being a single N-acetylgalactosamine. Moreover, the complexity of the structure is usually higher with formation of antennae which are available to intermolecular contact with suitable partner (Gabius, 2008). These carbohydrate antennae can be complexed by specific protein receptors, called lectins (Lis & Sharon, 1998).

3.2.2. Lectins as sugar receptors

Lectins can be found in most living organism i.e., virus, bacteria, plants, animals, humans. They are proteins that bind sugar reversibly in a highly specific manner (Barondes, 1988). Two classifications of lectins are reported in the scientific literature. The first one is based on specificity towards monosaccharides (Wu, Lisowska, Duk, & Yang, 2009). The second depends on sequence homologies and evolutionary relationships as proposed by Kurt Drickamer (Drickamer, 1995). Actually, in the amino acid sequence of lectins less than 200 amino acids belong to the carbohydrate

recognition domain (CRD) which may be a classification criterion. Each CRD is specific for a given oligosaccharide (Kilpatrick, 2002). This selective recognition relies on specific interactions, which are nothing but hydrogen bonds, between the amino acid sequence of the protein and the sugar. Indeed, the hydroxyl groups of the sugar can be involved in up to three hydrogen bonds through the two lone pairs of oxygen (acceptor) and the positively charged hydrogen (donor). Proteins contain groups in the main chain and/or inside chains, suited to hydrogen bonding (Lis & Sharon, 1998). A common hydrogen bonding scheme in which a sugar is involved, is the interaction of non-anomeric hydroxyl of the sugar with either, hydrogen donors of amides group or carbonyl or carboxylate acceptors of proteins (Naismith et al., 1994; Weis & Drickamer, 1996).

Despite their weakness, the Van der Waals forces are frequently involved in specific binding (e.g., DNA double strands) and they make a significant contribution as result of a cooperative effect (Lis & Sharon, 1998). In addition to H-bond forming groups, sugars contain aliphatic protons and carbons which are apolar patches able to contact intimately an aromatic residue. This type of interaction is frequently described as stacking, which indicates that the ring of the sugar is more or less parallel to the plane of the aromatic ring. Clearly, numerous Van der Waals contacts can be created between sugars and lectins (Solis et al., 2001).

Water is a common mediator of contacts between lectins and sugars. Indeed, water which is tightly bound to proteins, forms additional hydrogen bonds with sugars. In this case, the water molecule can be considered as fixed structural element. Moreover, depending on the lectin, divalent cations can also be essential in the complexation process either indirectly (structural role) or by direct interaction (as ligand) (Weis & Drickamer, 1996). The structural effect is common for several classes of lectins. Among them, legume lectins, such as concanavalin A (conA), use manganese and calcium cations to stabilize a protein structure well-suited to the binding of sugar. Ca^{2+} is coordinated to the carbonyl of an asparagine amino acid residue whose NH_2 group interacts with the hydroxyl group of the sugar by hydrogen bonding. The role of the manganese cation is to fix the calcium one in the right position (Kaushik, Mohanty, & Surolia, 2009).

The ligand effect is illustrated by the C-type lectin, which uses a calcium cation for interacting with a sugar. The cation thus interacts simultaneously with two stereochemically equivalent hydroxyl groups of the sugar and oxygen atoms of protein residues (Weis & Drickamer, 1996).

All the interactions make the binding of an (oligo)saccharide to its receptor very specific, quite similar to the lock-and-key complementarity, according to which a wrong key does not open a non-adaptable rigid lock. Any perturbation that prevents one hydroxyl group from forming an epimer causes the non-recognition of the sugar by the protein. Then, this hydroxyl group is no longer available to hydrogen bonding with amino acids or coordination with a metal cation. Moreover, the polarity can be concomitantly modified at the detrimental of Van der Waals interaction. It is thus of the utmost importance to know the actual structure of the sugar/lectin complex whenever a sugar has to be linked to a carrier. Indeed, any error in the linkage pattern can adversely affect the recognition process and the targeting effectiveness.

3.2.3. Thermodynamics of the sugar/lectin recognition

As a rule, the complexation is enthalpy driven, the entropy being unfavorable to the interaction. Actually, several effects contribute to the entropy: translational, rotational and conformational effects in addition to solvatation. The rotational and translational contributions are unfavorable, because of a loss of freedom caused by the interaction. Indeed, upon docking of the sugar in the binding site of the lectin, bonds are frozen including the torsion angle around the glycosidic bonds. Moreover, the two cannot translate independently anymore. Same effects and consequences hold for the conformational contribution. In contrast, the entropy of solvatation is favorable to the association. Indeed, lectin and sugar are hydrated before docking. This ordering organization of water molecules is lost upon complexation, at the benefit of the entropy (Mammen, Choi, & Whitesides, 1998). Usually, the magnitude of this favorable contribution, is too low for the sugar-lectin affinity to be entropy-driven, as is however the case for a bacterial lectin and fucose pair (Pokorna et al., 2006). To sum up, the binding of lectin and oligosaccharide in water results in the breaking of the hydrogen bonds responsible for the original solvatation of the protein and sugar and in the formation of hydrogen bonds between these two partners. The net binding energy, thus the difference of energy between these two events, is favorable to complexation (Lis & Sharon, 1998).

However, the specific sugar/lectin interaction is usually weak, the dissociation constant being in the millimolar range for monosaccharide (Lee & Lee, 1995). Nature succeeds in circumventing this loose binding by promoting a multivalency effect, related to the availability of several ligands (receptors) in one biological entity (Reynolds & Pérez, 2011). Glycans endowed with multiple antennae is an example. More than one mechanism can operated in a multivalent binding mode, such as chelate effect (adjacent binding sites on the receptor are occupied by a properly fitting multiligand), occupation of the primary binding site and subsite of the receptor, and the statistical clustering of the binding sites of receptors. The occurrence of such phenomena, referred to as "cluster glycoside effect" in the scientific literature (Lee & Lee, 1995), are the reasons for any enhanced affinity of a multivalent saccharide towards lectins compared to the affinity of a single isolated saccharide (on a "per mole" of saccharide basis or valence-corrected basis) (Lundquist & Toone, 2002).

Understanding the mechanism of this enhancement was the focus of many researches (Houseman & Mrksich, 2002; Lundquist & Toone, 2002; Mammen et al., 1998; Reynolds & Pérez, 2011). Translational and rotational entropy is lost during the docking of the sugar into the binding pocket of the lectin. This entropic penalty is paid for every interaction in the case of monovalent ligands binding to monovalent receptors. For multivalent interactions, this penalty is only paid for the first binding, the other one would not, favoring the overall binding entropy with as consequence an affinity enhancement for the other binding compared to the first binding. Effect of linker molecule conformation on the entropic contribution arises in multivalent interactions, since the linker possesses its own degrees of freedom. This additional term usually is unfavorable to the overall binding, since it loses flexibility by docking in the binding site of the receptor. However, this contribution is largely compensated by the gain in translational and rotational entropy of the entire molecule. Design of the linker affects also the enthalpy of binding. Size and rigidity/flexibility of the linker dictate how strained/unstrained is the linker during the binding. Thus, a strained linker (too long or too short) is unfavorable to the binding. Clearly, the design of the linker is decisive on the binding enhancement in multivalent interaction.

Effective concentration was proposed as alternative approach to explain the cluster glycoside effect. In this binding model, during multivalent interactions, the first binding forces the other ligands to come closer to the receptor. Therefore, the local concentration of the ligands is usually higher than in the bulk solution favoring the multivalent binding (Gargano, Ngo, Kim, Acheson, & Lees, 2001; Kramer & Karpen, 1998).

Therefore, a wide range of multivalent saccharide ligands of different architecture, sugar density and rigidity, have been prepared and their efficacy has been established and compared (Lundquist & Toone, 2002; Pieters, 2009; Ting, Chen, & Stenzel, 2010). Thermo-

dynamic and kinetic data have been collected by proper analytical tools of the complexation. Some of these physico-chemical techniques are briefly discussed hereafter.

3.3. Physico-chemical analysis of sugar/lectin interactions

Interactions between sugars or synthetic sugar-containing ligands and lectins have been analyzed by a wide range of qualitative (Turbidity, Inhibition Hemagglutination, Enzyme Linked Lectin Assay) and quantitative techniques. Those providing quantitative information about thermodynamics and kinetics of interaction are of high value. Isothermal Titration Calorimetry (ITC), Surface Plasmon Resonance (SPR) and Quartz Crystal Microbalance with Dissipation measurement (QCM-D) are very powerful tools, that emerge in the investigation of these interactions.

3.3.1. Isothermal Titration Calorimetry

Isothermal Titration Calorimetry (ITC) gives a rapid and accurate access to the thermodynamics of lectin/sugar interaction (Dam & Brewer, 2002; Leavitt & Freire, 2001). A solution of the investigated carbohydrate of known concentration is added at regular intervals (20–50 injections of the same volume) into a calorimeter that contains a lectin solution also of known concentration. The heat released (exothermic binding) or absorbed (endothermic binding) by the interaction is measured vs. time. Each recorded peak is proportional to the difference in temperature (ΔT) with respect to a reference cell supplied by a constant power. These ΔT are converted to power supplied by the interaction and the quantity of heat absorbed or released is directly proportional to the extent of binding, which decreases with the increasing number of injections. As the system reaches saturation, the heat signal indeed diminishes until only the dilution heat is monitored.

The binding curve is then compared to predictions by binding models proposed for the interaction under investigation (Langmuir, 1:2,..). When appropriate, the model provides the thermodynamic binding parameters of the interaction: binding association constant (K_a), change in enthalpy (ΔH) and stoiechiometry (n) (Brown, 2009) Free energy of binding (ΔG) is calculated by Eq. (1)

$$\Delta G = -RT \ln K_a \tag{1}$$

and the entropy of binding (ΔS) by Eq. (2)

$$\Delta G = \Delta H - T\Delta S \tag{2}$$

The same experiment must be repeated at different temperatures for the variation of heat capacity (ΔC_p) upon binding to be calculated by Eq. (3)

$$\Delta C_p = \frac{\delta \Delta H}{\delta T} \tag{3}$$

Interactions, whose association constant lies between 10^3 and 10^7 M^{-1}, can be studied by ITC. Moreover this technique gives a direct and accurate access to all the thermodynamic parameters of the binding from a single experiment. Moreover, it does not require any chemical modification or immobilization of the receptor. There is no restriction in the molecular weight of the species involved. Color or turbidity has no influence on the measurements. The major criticism might be lack of structural information and need of a large amount of materials.

3.3.2. Surface Plasmon Resonance

Surface Plasmon Resonance (SPR) is a popular optical technique (more than 1400 publications in 2008 (Rich & Myszka, 2010)) that monitors in real-time the binding of a ligand to a receptor anchored to a gold surface. The key physical phenomenon on which the technique is based, is the interaction of an incident light with the surface plasmon of a gold thin film. The evanescent longitudinal propagation wave of gold is excited by this incident polarized monochromatic light beam (SPR excitation) at a well-defined angle at constant wavelength or conversely at a well-defined wavelength at constant angle. The consequence of the SPR excitation is a minimum in the intensity of the reflected beam (Schasfoort & Tudos, 2008).

However, whenever the refractive index of a coating or a solution (i.e., a dielectric) in contact with the metal changes, the position of the minimum in reflectance changes accordingly. When the investigation of the ligand/receptor binding is concerned, one partner, designed as the ligand, is immobilized onto the gold surface (sensors decorated by chemical functions suitable for ligands anchoring are commercially available) and a solution of the other partner, the analyte, is flowed over the gold sensor. A, shift in the minimum position reflects rearrangement or binding at the immediate vicinity of the surface layer (1 Å–300 nm) (Schasfoort & Tudos, 2008; Su & Zhang, 2004).

The raw experimental data are usually reported as a real-time graph of response units (RU) against time. In case of proteins, 1000 RU correspond to about 1 ng bound to the flow cell surface. During the analyte injection, the position of the reflectivity minimum changes as result of association binding to the surface. When the addition of the analyte is complete, the buffer is flowed over the chip which triggers a change in the opposite direction typical of dissociation, until the surface is regenerated. Regeneration is evaluated empirically and it depends on the forces responsible for the binding of the analyte to the ligand. The experimental conditions must be such that the ligand activity is preserved. Would an ethylenediaminetetraacetic acid (EDTA) be used for regenerating complexed with a sugar, the calcium cations would be chelated by EDTA accounting for a loss of the lectin activity (Lameignere et al., 2008). Decomplexation of a ligated multivalent sugar is possible by competitive inhibition with free sugar (Jule, Nagasaki, & Kataoka, 2002). Free sugar competes for the binding site of the lectin with the bound ligand and can replace it. Its association to the lectin is usually weak and the buffer flow is sufficient enough to remove it from the surface. The association and dissociation phases make possible the calculation of the kinetic parameters of the binding, the dissociation and association rate constants and, from their ratio, the dissociation constant (Attie & Raines, 1995; Tang, Mernaugh, & Zeng, 2006).

Let us consider the equilibrium between sugar and lectin as schematized by Eq. (4)

$$A + L \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AL \tag{4}$$

where A is the analyte (the sugar or the lectin), L the immobilized ligand (the lectin or the sugar) and AL the complex.

The formation rate of the complex at time t may be expressed by Eq. (5)

$$\frac{d[AL]}{dt} = k_a[A][L] - k_d[AL] \tag{5}$$

Parallel to the complex formation, the concentration of the available binding sites of the immobilized ligand decreases as following: $[L] = [L]_0 - [AL]$, with $[L]_0$ the initial concentration of lectin, such that Eq. (5) can be rewritten as (Eq. (6)):

$$\frac{d[AL]}{dt} = k_a[A]([L]_0 - [AL]) - k_d[AL] \tag{6}$$

The experimental SPR data (R) are proportional to the extent of the complex AL formation and they reach a maximum (R_{max}) at saturation, R_{max} is of course proportional to the number of binding sites. The concentration of the analyte (C) is constant during the

experiment if no mass transport occurs. Eq. (6) then becomes (Eq. (7)):

$$\frac{dR}{dt} = k_a C(R_{max} - R) - k_d R = k_a C R_{max} - (k_a C + k_d)R \quad (7)$$

Eq. (7) can be simplified as (Eq. (8)):

$$\frac{dR}{dt} = k_w - k_s \cdot R \quad (8)$$

where $k_w = k_a C R_{max}$ and $k_s = k_a C + k_d$

The integrated form of the rate Eq. (8) is the following (Eq. (9)):

$$R = R_0 + \left(\frac{k_w}{k_s} - R_0\right)(1 - e^{-k_s.t}) \quad (9)$$

The response R thus changes exponentially with time during association, with R_0 the response at time zero. This equation is fitted by a software which is provided with the instrument and makes the association rate constant, k_a, available.

The dissociation process can be similarly monitored by SPR as soon as the flow of the analyte solution has been stopped and replaced by a flow of buffer. The rate of the complex dissociation fits Eq. (10)

$$\frac{dR}{dt} = k_d R \quad (10)$$

Eq. (11) is the integrated rate equation

$$R = R_a e^{-k_d.t} \quad (11)$$

with R_a is defined as the amplitude of the dissociation process.

The dissociation rate constant, k_d, can be extracted from Eq. (11). The equilibrium dissociation constant, K_d, is nothing but the ratio of k_d over k_a (Eq. (12)):

$$K_d = \frac{k_d}{k_a} \quad (12)$$

In a different approach, the equilibrium situation is considered, at with association and dissociation occur at the same rate (Eq. (13))

$$k_a C(R_{max} - R_{eq}) = k_d R_{eq} \quad (13)$$

R_{eq} is the SPR response at equilibrium. From $k_d \cdot k_a^{-1} = K_D$, Eq. (13) becomes (Eq. (14)):

$$\frac{1}{R_{eq}} = \frac{1}{R_{max}} + \frac{K_d}{R_{max}}\frac{1}{C} \quad (14)$$

$1/R_{max}$ is the intercept of the plot of $1/R_{eq}$ against $1/C$, whereas the slope is K_d/R_{max}, from which the dissociation constant, K_d, is obtained. These equations are very common for the analysis of receptor-ligand interactions and adaptable to a broad range of techniques that analyze the binding of ligands (Attie & Raines, 1995).

Repetition of the measurements at different temperatures allows the thermodynamic parameters to be determined. Nevertheless, the accuracy is much lower compared to ITC which remains the method of choice (Schasfoort & Tudos, 2008). SPR has the advantage to require very small amounts of materials for recording real time data. Needless to say that the immobilization of the receptor at the surface of the gold sensor is the key step of the method, because the activity of the bioreceptor must be totally preserved. At least 0.1% of the ligand must be occupied for being detected. Finally, non-specific interactions and small difference in the refractive index of the buffer and the analyte solutions can lead to unreliable results (Campbell & Kim, 2007; Schasfoort & Tudos, 2008).

In a representative experiment, Mann and co-workers (Mann, Kanai, Maly, & Kiessling, 1998) used a commercially available sensor chip with a hydrophobic surface, onto which glycolipid (with mannose residues) containing liposomes were self-assembled as a monolayer. ConA solutions of different concentrations were injected and sensorgrams were recorded. A binding association constant of $2.7 \pm 0.2 \times 10^4\ M^{-1}$ was calculated. Between each injection of ConA solution, the surface was regenerated by injection of free sugar, which competes with immobilized sugars for the binding site of ConA. Additionally, the inhibitor potential was assessed by competition inhibition with a series of ligands and their affinity was ranked. The ability of a sugar (e.g., mannose in this work) to inhibit the interaction of ConA with the immobilized sugar on the SPR surface is explored by the binding response in the presence of increasing concentrations of inhibitors. From the inhibition constants, sugars including neoglycopolymers, are easily compared (Mann et al., 1998).

3.3.3. Dissipative Quartz Crystal Microbalance for studies of sugar binding

Binding processes can also be monitored by a Quartz Crystal Microbalance with measurement of the energy Dissipation (QCM-D), although this method is less popular than the two previous ones. The general concept is quite simple. When a proper alternating electric field is applied to a piezoelectric quartz crystal sandwiched between electrodes, excitation occurs at the resonance frequency of the crystal, in direct dependence on the crystal mass. Deposition of a solid onto the surface changes the oscillation of the quartz crystal, whose resonance frequency decreases (Ferreira, da-Silva, & Tomé, 2009; Janshoff, Galla, & Steinem, 2000). This decrease is proportional to the deposited mass in the specific case of low amounts of a rigid compound, as expressed by the Sauerbrey relationship (Eq. (15)) (Janshoff et al., 2000; Sauerbrey, 1959).

$$\Delta f = -\frac{\omega}{2\pi}\frac{M}{m_q} \quad (15)$$

where Δf is the frequency shift, ω is the overtone number, M is the deposited mass and m_q is a constant including the characteristics of the quartz crystal.

However, when soft materials, such as proteins, are considered in a fluid environment, more appropriate Δf vs. M relationships have been made available (Voinova, Jonson, & Kasemo, 2002). In that case, measurement of the dissipation factor, D, gives information about the viscoelasticity of the soft material (Fredriksson, Kihlman, Rodahl, & Kasemo, 1998; Janshoff et al., 2000).

In a typical experiment, one partner the ligand is immobilized at the surface of the crystal, whereas the analyte is flowing over, as is the case in SPR. Real-time monitoring of the biorecognition and analysis of the kinetics are thus feasible (Mori, Toyoda, Ohtsuka, & Okahata, 2009). Moreover, the dissipation factor gives structural information about the complex. One drawback of this technique is sensitivity to environmental effect (temperature, pressure), which perturbs the solution viscosity (Kanazawa & Gordon, 1985).

As rule, the equations that express the affinity of binding sites for ligands and kinetics of the complexation are similar to those established in the previous section about SPR (Attie & Raines, 1995). Interaction between sugar and lectin has been investigated by QCM-D. However, because the frequency shift is (too) small when lectin is immobilized and sugar of low molar mass is flowing over, the sugar is preferably immobilized onto the surface rather than the lectin. To this end, a biotynilated sugar has been reacted with streptavidin immobilized at the quartz crystal surface (Mori et al., 2009). In another approach, a linker with a terminal alkyne function has been surface immobilized and reacted with an azido sugar by click chemistry (Zhang et al., 2006). In this experiment, solutions of lectin ConA of increased concentration was flowed over the surface and the frequency shift (Δf) was recorded. The association constant (K_a) was calculated from Eq. (16), which is an adaptation form of Eq. (13) established for SPR experiment.

$$\frac{C}{\Delta f} = \frac{C}{\Delta f_{max}} + \frac{1}{\Delta f_{max}}\frac{1}{K_a} \quad (16)$$

where C is the concentration of the ConA solution of, Δf is the frequency shift at equilibrium and Δf_{max} is the frequency shift at saturation.

A value of $8.7 \pm 2.8 \times 10^5\ M^{-1}$ was found for the association constant of ConA with mannose. Similarly to SPR, competition assay can be carried out by QCM-D. Pei, Anderson, Aastrup, & Ramstroem (2005) have reported about the inhibitory effect of several sugars towards the ConA/mannan binding.

3.4. Strategies for the sugar-decoration of PEGylated degradable polymeric carriers

As aforementioned, micelles consisting of amphiphilic copolymers of (bio)degradable polyesters and PEO have potential as drug delivery carriers (Jerome, 2010). It is thus worthy to review the main strategies reported for conjugating them with sugars.

In active targeting, the carrier is conjugated to a pilot molecule either chemically or by physical adsorption. Chemical binding is by far more desirable to control both the density and orientation of the sugar. Although a variety of coupling reactions are known, only few of them are applicable whenever chemically sensitive molecules and degradable materials are concerned. It may happen that the envisioned coupling is very challenging (Shi et al., 2009), because of the limited solubility of sugars in organic solvents, and the comparable reactivity of the hydroxyl groups of manosaccharides which makes the selective grafting of carbohydrates a very difficult task and a time consuming one when several protection/deprotection steps are required. Despite these limitations, an ideal coupling strategy should be straightforward, quantitative, site-selective, with formation of stable chemical bonds (Shi et al., 2009).

Two main strategies have been explored for the preparation of glycopolymers: (i) polymerization initiated by a sugar containing compound and copolymerization of a glycomonomer, (ii) post-functionalization of a preformed copolymer with the sugar of interest. The first strategy must be disregarded whenever the hydroxyl groups of the sugar interfere with the polymerization mechanism except if these groups are protected before polymerization and deprotected afterwards, which may be time consuming. The second strategy requires that (protected) groups are available at the end or along the polymer backbone and prone to reaction with the carbohydrate. Although the two approaches have been worked out, more attention has been paid to the second one.

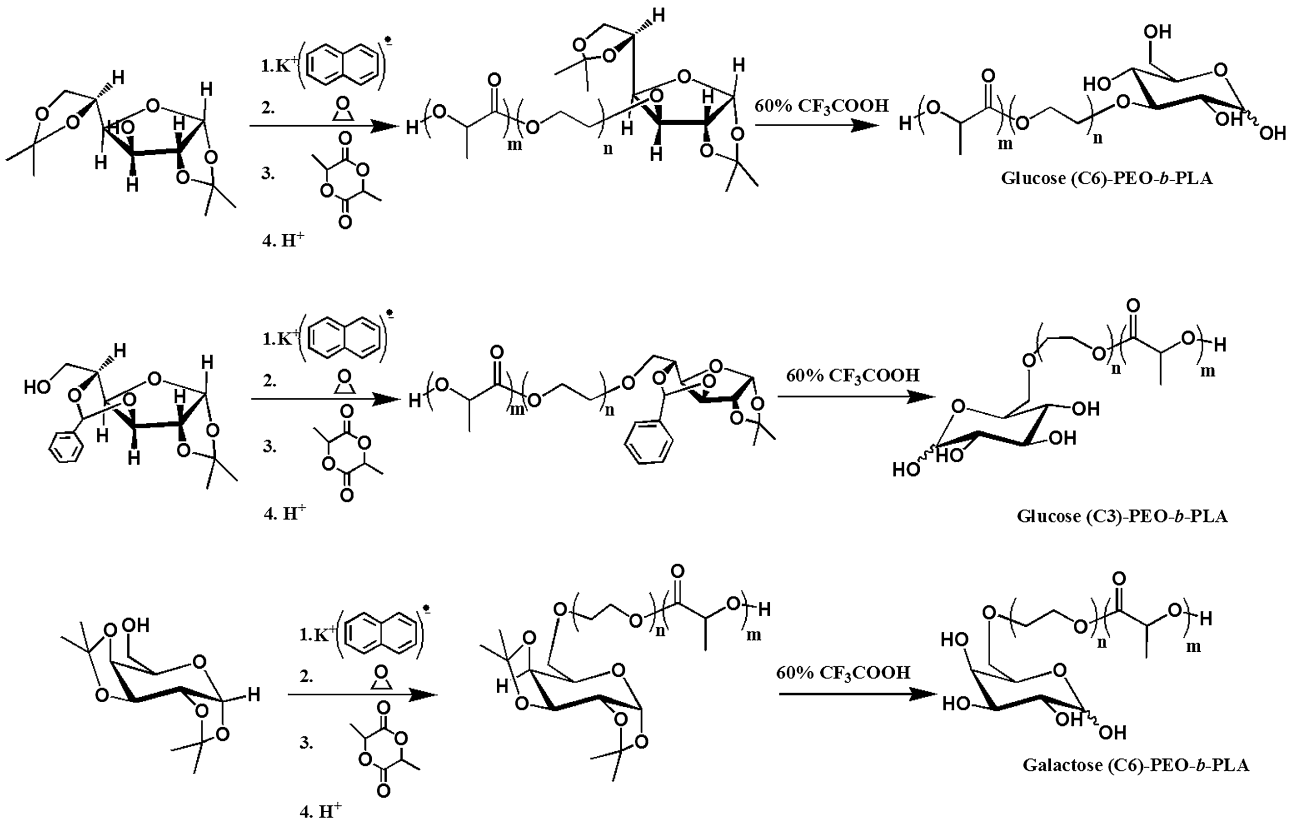
Kataoka et al. (Nakamura, Nagasaki, & Kataoka, 1998; Yasugi, Nakamura, Nagasaki, Kato, & Kataoka, 1999) reported on the anionic ring-opening polymerization of ethylene oxide followed by the anionic ring-opening polymerization of lactide. A metal alkoxide derivative of a protected sugar was used as an initiator (Scheme 1). Three different diblock copolymers were prepared with a sugar attached at the PEO chain-end: glucose through the C3 and C6 position, respectively, and galactose through the C6 position. These positions are less commonly used than other positions whose chemical modification is easier. The interest of these positions in targeting applications is not clear, although liver was shown to be targeted by N-(2-hydroxy-propyl) methacrylamide nanoparticles decorated by galactose linked at the C-6 position (Kopecek & Duncan, 1987). The major advantage of this strategy is the high degree of functionalization of the polymer chains. About 90% of the chains were capped by a sugar residue. Moreover, the deprotection was carried out under mild enough condition that prevented PLA from being degraded. The availability of the glucose residue at the surface of the copolymer micelles to interact with glucose-specific dimeric *Ricinus Communis Agglutinin* (RCA-I) lectin was proved by the interaction of these micelles preloaded with a fluorescent probe, with lectin immobilized in chromatographic column (Nagasaki, Yasugi, Yamamoto, Harada, & Kataoka, 2001).

We could not find another example of application of the first strategy. The post-polymerization strategy has the advantage to provide libraries of glycopolymers with the same macromolecular characteristics but bearing different sugar residues. That the copolymer contains groups reactive towards the sugar is a prerequisite, whereas the position of these groups determines the location of the sugar onto the copolymer.

An amphiphilic copolymer, poly(allyl glycidyl ether-*b*-lactide) (Scheme 2), was prepared by Hu et al. (Hu, Fan, & Zhang, 2010), followed by the free-radical coupling reaction of 2-mercaptoethyl-β-D-glucoside. This "thiol-ene click reaction" was carried out without degradation of PLA. The modified copolymer formed spherical micelles in water with a size of ca. 200 nm, thus larger than the micellar diameter currently observed in the 10–100 nm range (Letchford & Burt, 2007). No information was reported about the coupling efficiency and interaction with lectin. Lu et al. (2006) post-modified a poly(lactic acid)-*co*-[(glycolic acid)-*alt*-(L-glutamic acid)]}-*block*-poly(ethylene glycol)-*block*- poly (lactic acid)-*co*-[(glycolic acid)-*alt*-(L-glutamic acid)]} triblock with glucose residues. This copolymer was prepared by copolymerization of lactide and (3*s*)-benzoxylcarbonylethylmorpholine-2,5-dione (BEMD) initiated by the hydroxyl end-groups of poly(ethylene glycol) in the presence of $Sn(Oct)_2$ as catalyst (Scheme 3). It contained 4 mol% of protected L-glutamic acid. Deprotection was carried out by catalytic hydrogenation, i.e., under conditions that prevented PLA from being degraded even in the presence of glycolic acid (Park, 1995). The pendant carboxylic acids were then activated with *N*,*N*'-carbonyldiimidazole and reacted with the amine of 3-(2-aminoethylthio)propyl-α-D-glucopyranoside within very high yield (94.6%). The higher nucleophilicity of this amine compared to the hydroxyl groups of the sugar did not require the protection of these hydroxyl groups for the coupling to be selective. The availability of the glucose residue was proved by interaction with fluorescein isothiocyanate (FITC) labeled ConA.

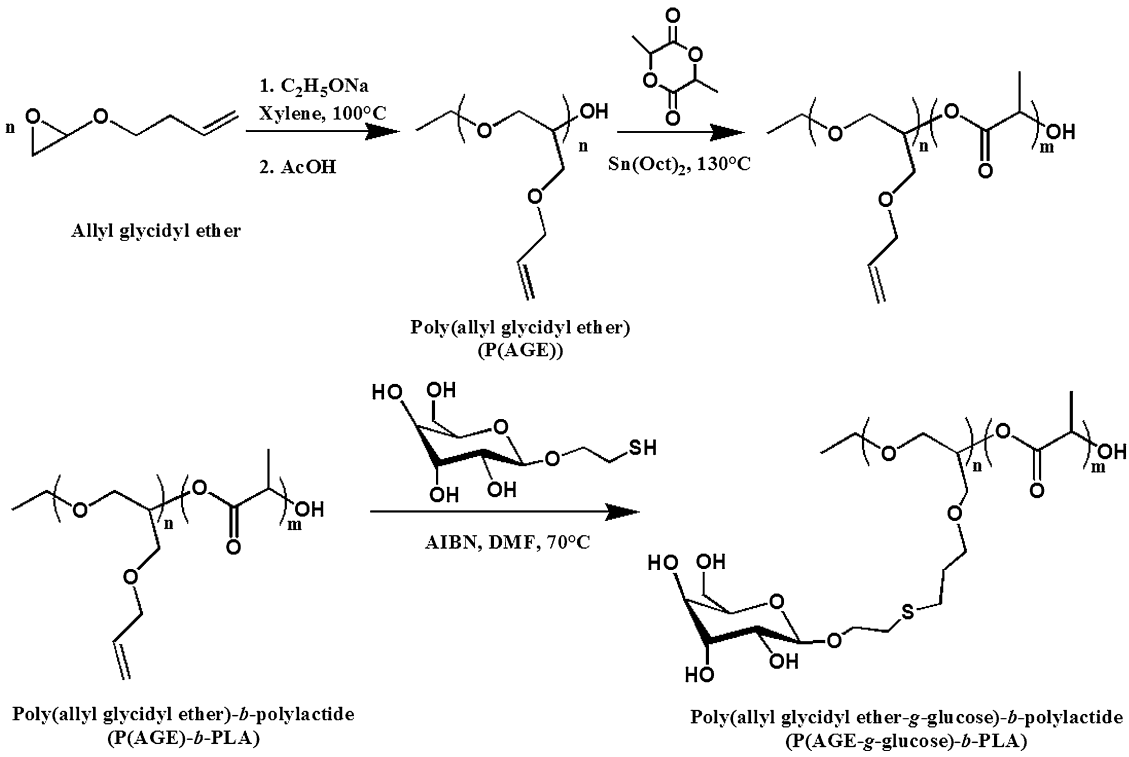
In an alternative approach, the sugar was attached the PEO as an end-groups. Sugar conjugated PEO-*b*-poly(γ-benzyl-L-glutamate) copolymers were prepared in two steps. A lactone containing sugar was first reacted with one of the two primary amine end-groups of α,ω-diamino PEO, followed by the initiation of the ring-opening polymerization of γ–benzyl-L-glutamate *N*-carboxyanhydride by the residual primary amine (Scheme 4) (Cho, Chung, Goto, Kobayashi, & Akaike, 1994; Jeong et al., 2005). An excess of sugar lactone was used in the first step in order to prevent unreacted α,ω-diamino PEO from being left. Remarkably, no protection of the sugar was necessary for the primary amine to be the actual initiator of the N-carboxyanhydride polymerization. Interestingly enough, the polypeptide block exhibit an α-helical structure (Cho et al., 1994). According to a similar strategy, Toyotama et al. synthesized a lactose-PEO-*b*-poly(γ-methyl-L-glutamate) copolymer (Toyotama, Kugimiya, Yamanaka, & Yonese, 2001). Big micelles were formed in water, the average diameter (~200 nm) being approximately two times larger compared to the micelles observed when the polypeptide was of the γ-benzyl-L-glutamate type (cf. supra (Cho et al., 1994; Jeong et al., 2005)). These two types of micelles could interact with lectin as proven by transition from clear to turbid solutions as result of crosslinking of the micelles by the RCA-I lectin. Jeong et al. (2005) investigated further the potential of the galactose-PEO-*b*-poly(γ-benzyl-L-glutamate) copolymer in biomedical applications. Once encapsulated within this copolymer, the cytotoxicity of Paclitaxel (TX) (concentration below 0.25 μg/ml) was low (*in vitro* testing) comparing to free TX. Moreover, the specific uptake by ASGPR-expressing cancer cell line SK-Hep02 was significantly higher than by an analogous non-ASGPR expressing cell line SK-Hep01.

Yoon, Nam, Kim, & Park (2002) functionalized a diblock copolymer of PEO and PLGA with a galactose residue. Their strategy relies

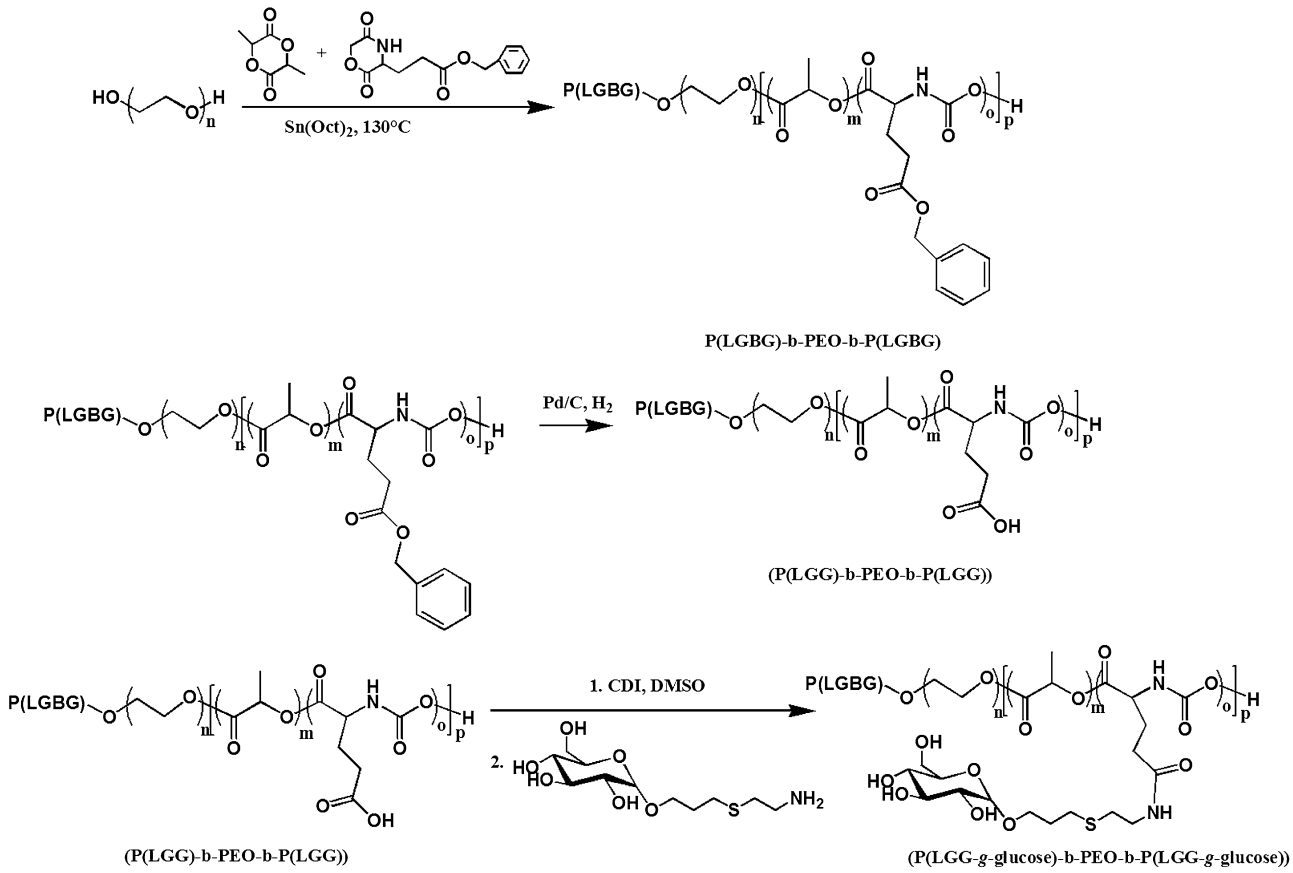


Scheme 1. A protected sugar is used to initiate the ethylene oxide polymerization, followed by the ring-opening polymerization of lactide and the subsequent deprotection of the carbohydrate (Nagasaki et al., 2001; Nakamura et al., 1998; Yasugi et al., 1999).

on the preparation of aminated PEO-*b*-PLGA by coupling diamine-PEO with dicyclohexylcarbodiimide (DCC)/*N*-hydroxysuccinimide (NHS) activated terminal carboxylic acid end-group of the PLGA and on the subsequent coupling of DCC/NHS activated lactobionic acid to the copolymer (Scheme 5). The availability of galactose on polymer film was proven by a protein assay using the colloidal gold solution method (Hatakeyama, Murakami, Miyamoto, & Yamasaki, 1996).



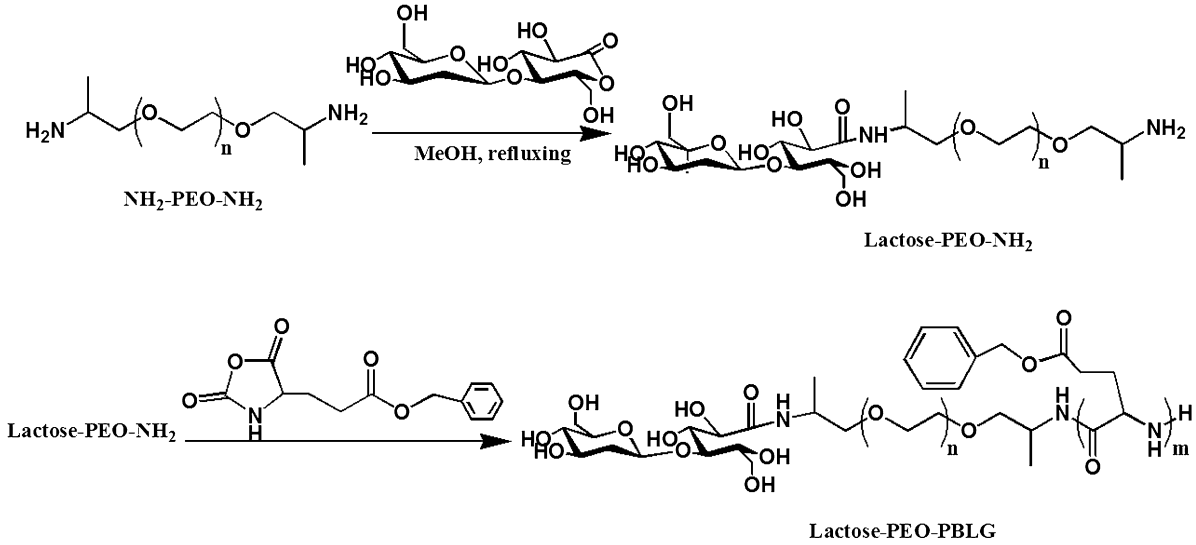
Scheme 2. Reaction pathway for the preparation of poly(allyl glycidyl ether-*g*-glucose)-*b*-polylactide (Hu et al., 2010).



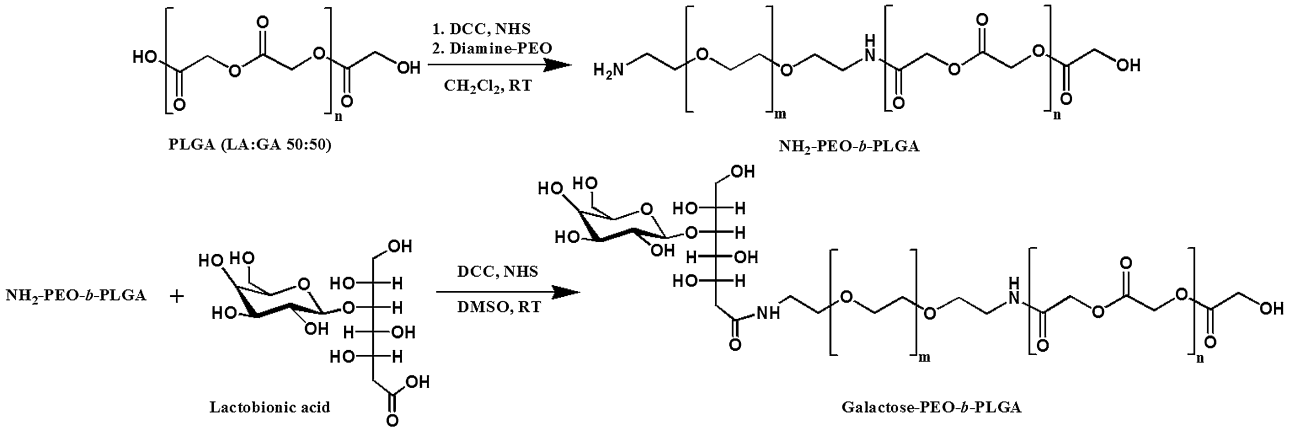
Scheme 3. Strategy by Lu et al. for the synthesis of PLGG-*b*-PEO-*b*-PLGG and P(LGG-g-glucose)-*b*-PEO-*b*-P(LGG-g-glucose) copolymer (Lu et al., 2006).

Rieger et al. (2007) prepared a poly(ethylene oxide)-*b*-poly(ε-caprolactone) copolymer (PEO-*b*-PCL), in which the PEO block was end-capped by a tertiary amine. This end-group was quaternized by a bromide derivative of mannose within high yield (90%) (Scheme 6). In contrast to the previous example, the sugar residue was attached to the preformed copolymer, which bypassed the problem of interference of the hydroxyl groups of the sugar in the synthesis of the second block (Jerome & Lecomte, 2008). Mannosylated micelles with a hydrodynamic diameter of 22 nm were formed in water and could interact with a lectin as monitored by ITC. Unexpectedly, comparison with free methyl-mannoside showed no affinity enhancement, which might have something to do with the evaluation protocol used (Corbell, Lundquist, & Toone, 2000; Lee & Lee, 2000). A drawback of this strategy by Rieger et al. is the positive charge on the mannosylated copolymer which is undesirable in drug delivery applications.

Ma et al. (2010) proposed a multistep approach for the synthesis of galactose end-capped PEO-*b*-PLA. Polymerization of ethylene



Scheme 4. Synthesis of a sugar end-capped PEO-*b*-poly(γ-benzyl L-glutamate) copolymer (Jeong et al., 2005).



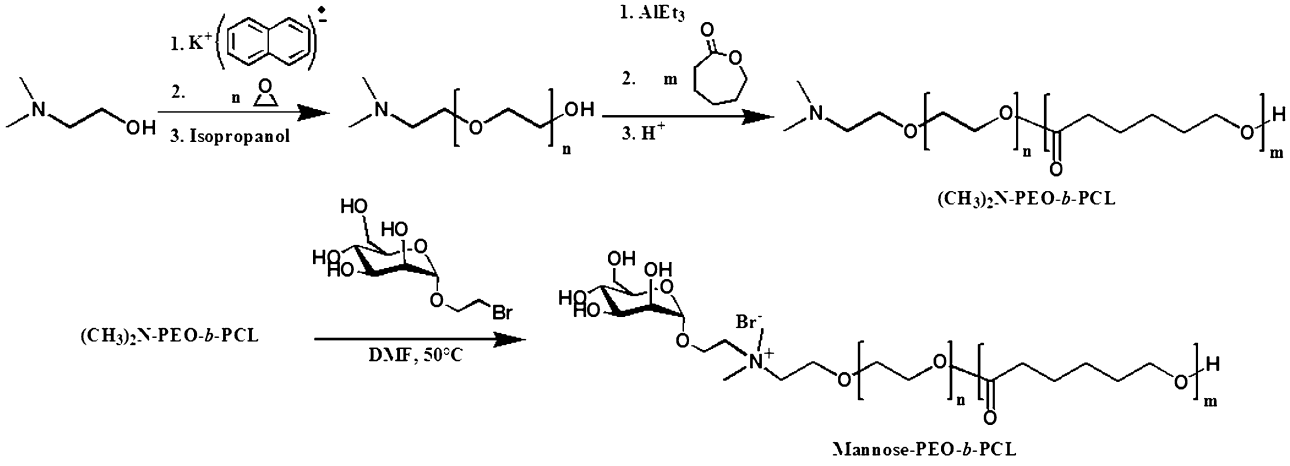
Scheme 5. Synthesis of a galactosylated PEO-*b*-PLGA copolymer (Yoon et al., 2002).

oxide was first initiated by potassium benzyl alkoxide with formation of α-benzyl ω-hydroxy PEO. The hydroxyl end-group was then converted into an amine by reaction with methyl sulfonyl chloride for 12 h followed by ammonium chloride for 3 days. The amine was protected in two steps by the well known Boc-protecting group. The accordingly collected α-hydroxy ω-Boc PEO was used as a macroinitiator for the lactide polymerization catalysed by $Sn(Oct)_2$. Deprotection of the primary amine by trifluoroacetic acid provided an amine-PEO-*b*-PLA copolymer. Finally, lactose was conjugated to this copolymer by reductive amination by $NaBH_3CN$ in DMSO at 45 °C. The coupling yield of the sugar was 60% at the end of this multistep strategy. The ability of the lactosylated copolymer to target overexpressed asialoglycoprotein receptors (ASGP-R) in the liver of mice was tested. Micelles of this copolymer added with rhodamine B labeled copolymer chain and a model drug (Lac+), were prepared and administered by vena tail injection. Fluorescent lactosylated micelles (Lac+) and micelles without lactose as ligand (Lac-f) were visualized in liver, spleen, kidney, heart and lung of mices. Fluorescence-based *in vivo* imaging system show the preferential accumulation ofthe lactosylated micelles in the liver. In contrast, micelles without the pilot lactose residue were evenly distributed among the different organs (Ma et al., 2010). A semi-quantitative analysis of the fluorescence intensities in the different organs confirms that Lac+ micelles preferentially accumulated in the liver compared not only to the other organs but also to the lac-f micelles over at least 48 h. The reticuloendothelial system metabolized the two types of micelles in the spleen and the liver, which accounts for their observation in these organs. Whereas the Lac+ content was systematically higher than that of Lac-f in the liver, the reverse situation prevailed in all the other organs, quite consistent with a specific interaction of the lactosylated micelles with lactose receptors in the liver.

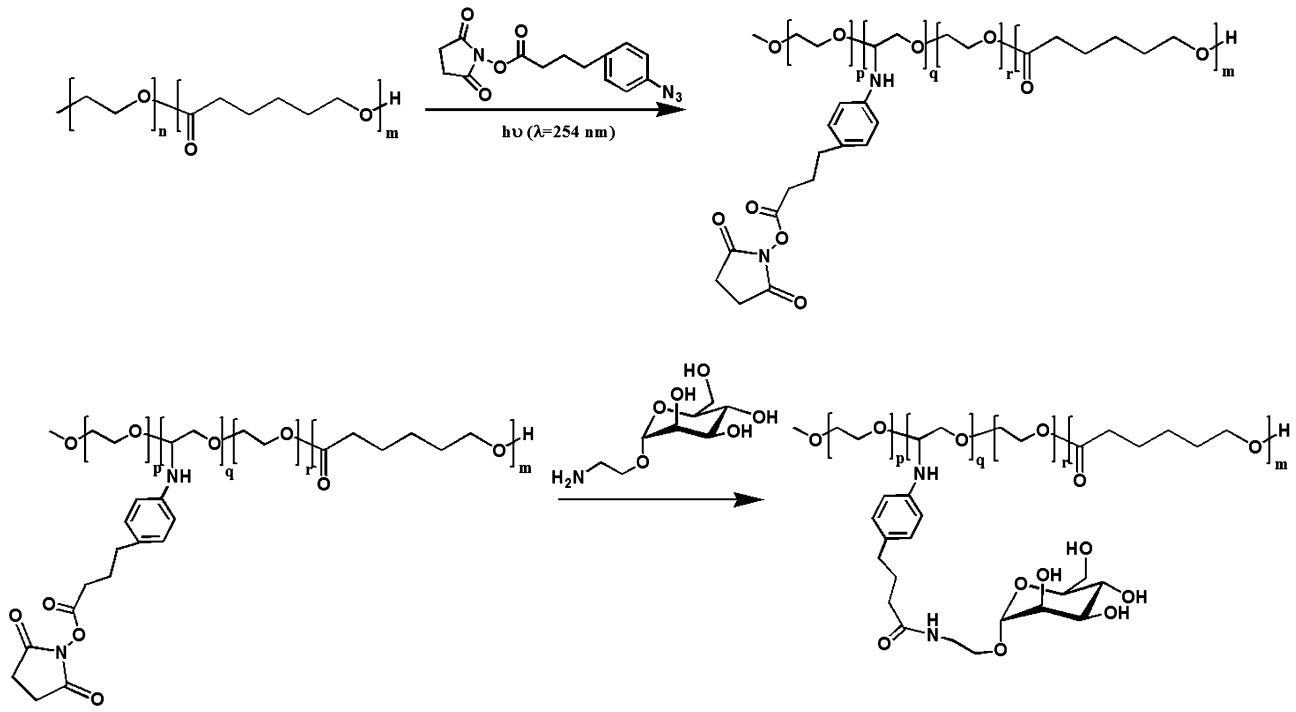
Finally, an original approach was recently reported by Pourcelle et al. (2009) based on the photografting of a functional group reactive towards an amino-mannose residue, onto an unmodified preformed PEO-*b*-PCL copolymer. An activated carboxylic acid containing nitrene was photodecomposed with statistical insertion within C–H bonds of ethylene oxide subunits. The so-grafted activated carboxylic acids were then reacted with an aminated-mannoside (Scheme 7). Approximately 10% of the copolymer chains were grafted by a mannoside residue, which is by far less than the yields reported for the other strategies herein discussed. However, this copolymer was tested *in vitro* and *in vivo* for oral immunization (Fievez et al., 2009). High immunization was observed with this mannosylated copolymer, which confirms its ability to target mannose receptors found in the intestine.

All the aforementioned strategies rely on sugar anchoring onto copolymers before micellization. Post-functionalization of preformed micelles has however been contemplated by several researchers, who showed a significant increase in the binding efficiency of the ligands. This observation suggested that ligand which are part of the copolymer before micellization can suffer from a shielding effect by longer PEO blocks if not buried within the micellar core (Sutton et al., 2007).

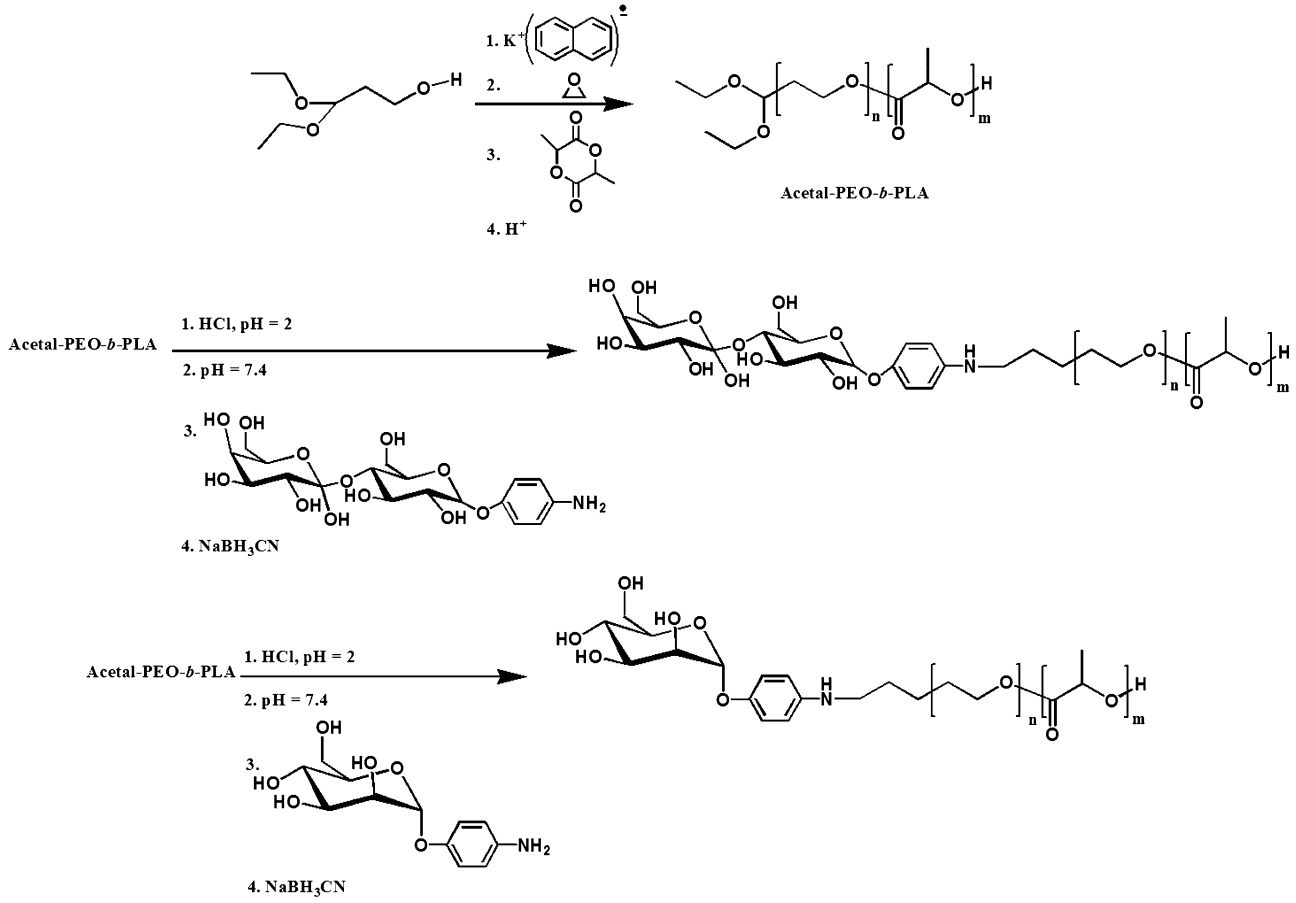
Another example of post-modification of micelles is worth being mentioned. It is based on a poly(ethylene oxide)-*b*-poly(D,L-lactide)



Scheme 6. Synthesis of a mannosylated PEO-*b*-PCL copolymer (Rieger et al., 2007).



Scheme 7. Synthesis of mannosylated PEO-*b*-PCL by photo-insertion of activated carboxylic acids followed by reaction with an aminated-mannose (Pourcelle et al., 2009).



Scheme 8. Synthesis of sugar labeled PEO-*b*-PLA by reductive amination (Nagasaki et al., 2001).

copolymer end-capped by an acetal on the PEO side. The copolymer was prepared by initiating the ethylene oxide polymerization with diethoxypropanol followed by the lactide polymerization (Nagasaki et al., 1998). The acetal-PEO-*b*-PLA was self-assembled into micelles in water and the acetal end-groups were converted to aldehyde by incubation at pH 2 and subsequent reaction with an amine-containing sugar. Stability of the accordingly formed Shiff base was enhanced by reduction with $NaBH_3CN$ (Scheme 8) (Jule, Nagasaki, & Kataoka, 2003; Nagasaki et al., 2001). Two different carbohydrates (lactose and mannose) were grafted by this method and their ability to interact with lectin was assessed by retention of the micelles on a lectin-immobilized affinity column (Nagasaki et al., 2001). A detailed study by surface plasmon resonance was conducted in order to know whether the galactosylated-copolymer could interact with lectin RCA-I simulating a cell surface (Jule et al., 2002, 2003). The sensorgrams points out a fast binding and a slow dissociation which facilitates binding and prolonged retention to the glyco-receptors. Furthermore, compared to free sugar, the strength of the association is higher and the binding constant, as well. The beneficial impact of the multivalent effect is clearly emphasized and attributed to the binding to additional surface receptors after initial binding.

In summary, synthesis of degradable glyco-PEGylated block copolymers is well-documented. Several strategies have been successfully tested for anchoring sugar residues onto the amphiphilic copolymers without chain degradation. Most of these reactions were performed in organic media. It is worth pointing out the direct anchoring of sugar residues onto the surface of micelles by reductive amination (Ma et al., 2010; Nagasaki et al., 2001). Amazingly enough, glycosylation of PEGylated graft copolymers with a (bio)degradable backbone has not been reported yet, at least to the best of our knowledge.

4. Conclusions

Design of "intelligent" drug carriers is nowadays a must for meeting the steadily more demanding requirements of controlled drug delivery. Polymers offer advantages over other possible constitutive materials, particularly biocompatible and (bio)degradable aliphatic polyesters and bioeliminable and protein repellent PEO. However, they are unable to target a diseased tissue, if no pilot molecule is attached to them. In this respect, pilot carbohydrates are receiving a special attention because of their recognition by numerous endogenous carbohydrate proteins and their enormous potential in information storage. Actually, much effort is devoted to the design of glycopolymers as key agents in drug delivery systems. This review has illustrated the variety of strategies that have been devised for attaching sugar residues either at the end or along the backbone of a hydrophilic PEO block associated to a biocompatible and biodegradable hydrophobic block in a diblock architecture.

Acknowledgements

The authors thank the "Region Wallonne" in the framework of the project VACCINOR and the "Interuniversity Attraction Poles Program (PAI 6/27) – Functional Supramolecular Systems" for financial support of this research. H.F. is grateful to "Grant F.R.S.-FNRS" for a research grant in the frame of "Télévie" program.

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