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Smart tools and orthogonal click-like reactions onto small unilamellar vesicles

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ABSTRACT

Click-based reactions were conducted at the surface of small unilamellar vesicles (SUVs) to provide onto-vesicle chemistry with efficient innovative ready-for-use tools. For that purpose, four amphiphilic molecules were designed to insert into bilayers while presenting a reactive functional head. In this manner, a dioleylglycero-ethoxy-ethoxy-ethoxy-ethanamine (DOG-PEG_4-NH_2) was chosen as a common platform while the reactive amine head was converted into several electrophilic functions. Thus, two dioleylglycerol-based cyclooctyne anchors were prepared: cyclooct-1-yn-3-glycolic acid-based anchor (DOG-COA) and 1-fluorocyclooct-2-ynecarboxylic acid-based anchor (DOG-FCOA). The last one differed from the first one in that a fluorine atom reinforces the electrophilic properties of the unsaturated bond. In addition, a third dioleylglycerol-based triphenylphosphine (DOG-PPh_3) was synthesized for the first time. These three innovative amphiphilic anchors were designed to react with any azide-based biomolecule following copper-free Huisgen 1,4-cycloaddition and Staudinger ligation, respectively. A fourth anchor bearing a 3,4-dibromomaleimide ring (DOG-DBM) was also unprecedentedly synthesized, to be further substituted by two thiols. Model reactions conducted in solution with either model biotinyl azide or model biotinyl disulfide gave good to total conversions and excellent isolated yields. The four new anchors were inserted into SUVs whose formula is classically used in *in vivo* biology. Stability and surface overall electrostatic charge were in the expected range and constant over the study. Then, the functionalized liposomes were ligated to biotin-based reagents and the experimental conditions were finely tuned to optimize the conversion. The biotinyl liposomes were demonstrated functional and totally accessible in an affinity test based on biotin scaffold quantification. Finally, DOG-FCOA's reactivity was confronted to that of DOG-DBM in a 'one-pot' orthogonal reaction. (Biotin-S)$_2$ and TAMRA-N_3 (tetramethylcarboxyrhodamine azide) were successively conjugated to the liposome suspension in a successful manner. These data implement and reinforce the interest of bioorthogonal click-like reactions onto lipid nanoparticles.

Abbreviations: AcOH, acetic acid; CH_2Cl_2, dichloromethane; Chol, cholesterol; DCC, dicyclohexylcarbodiimide; DIEA, diisopropylethylamine; DLS, dynamic light scattering; 4-DMAP, 4-*N*,*N*-dimethylaminopyridine; DMF, *N*,*N*-dimethylformamide; DOG-PEG_4-NH_2, dioleylglycero-ethoxy-ethoxy-ethoxy-ethanamine; EDCI, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride; Et_3N, triethylamine; EtOA, acetyl acetate; HATU, 2-(1H-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate; HBTU, *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyl-uronium-hexafluoro-phosphate; HMPA, hexamethylphosphoramide; HOBt, hydroxybenzotriazole; NHS, *N*-hydroxysuccinimide; PC, L-α-phosphatidylcholine; PDI, polydispersity index; PEG, polyethylene glycol; PG, L-α-phosphatidylglycerol; PTSA, para-toluene sulfonic acid; PyBOP, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate; SUVs, small unilamellar vesicles; TAMRA-N_3, tetramethylcarboxyrhodamine azide; TBAS, tetrabutylammonium sulfate; TCEP, *tris*(2-carboxyethyl)phosphine; THF, tetrahydrofurane; rt, room temperature.

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1. Introduction

The modification of the surface properties of liposomes by peptides, proteins or carbohydrates that convey either specificity or targeting has been extensively studied (Sankaram, 1994; Silvius and l'Heureux, 1994; Hojo et al., 1996; Boeckler et al., 1998; Schelté et al., 2000). For example, the stable anchorage of peptides into a phospholipid half-bilayer requires their branching by a lipid tail (Shahinian and Silvius, 1995; Epand, 1997) composed of at least one, or better, two alkyl chains (Martin and Papahadjopoulos, 1982; Kung and Redemann, 1986). However, it is a tedious task due to the poor water solubility of the resulting amphiphilic compounds. To overcome these difficulties, alternative strategies have been developed where the compound to be immobilized is covalently ligated to a functionalized lipid anchor already inserted in the membrane (Heeremans et al., 1992; Fleiner et al., 2001). In these strategies and until the mid-2000s, the chemical bond between anchor and either peptide, protein or carbohydrate compounds aimed at decorating the liposomes was chosen among amide bond (Kamps et al., 1996), thioether bond (Roth et al., 2004; Horak et al., 1999; Nakano et al., 2001), disulfide bridge (Zalipsky et al., 1995; Elliott and Prestwich, 2000) or imine bond (Harding et al., 1997). Later on, hydrazone and alpha-oxo hydrazone bonds were also developed and for the first time fully 'bioorthogonal' chemoselective ligations were available (Zalipsky, 1993; Chenevier et al., 2002; Bourel-Bonnet et al., 2005; Jolimaitre et al., 2007).

Then bioconjugate chemistry knew the boom of 'click chemistry' (Kolb et al., 2001; Torne et al., 2002; Rostovtsev et al., 2002). As a consequence several thousand papers and reviews can now be reached when typing these two words on a relevant research motor. Many authors now consider 'click chemistry' as a powerful tool for chemistry (Meldal and Torne, 2008), pharmaceutical sciences (Hein et al., 2008) and chemical biology (Speers et al., 2003; Boyce and Bertozzi, 2011; Beal and Jones, 2012). In this wake, in parallel to another pioneer work (Cavalli et al., 2006) and before a further work in this field (Mourtas et al., 2011), we have already proposed a Huisgen [1,3]-cyclo-addition – called 'click chemistry' by metonymy – to site-specifically immobilize a mannose-based tree onto preformed liposomes (Said Hassane et al., 2006; Frisch et al., 2010). The disadvantage of this method is the *in situ* generation of a reducing agent – Cu(I) – in excess. Moreover, the subsequent use of a water soluble Cu(I) chelator, such as bathophenanthroline disulfonate, was essential to obtain good yields (80%) in reasonable reaction times (1 h) while eliminating copper ions reputed toxic for living organisms. However, as assessed by agglutination experiments using concanavalin A, the mannose residues were perfectly accessible at the surface of the targeted vesicles. This first click chemistry onto preformed vesicles paved the way for further fully 'bioorthogonal' reactions. Nowadays, copper-free click chemistry is an outstanding and popular alternative and an increasing amount of experiments performed in the field of chemical biology (Jewett and Bertozzi, 2010; Shelbourne et al., 2012) are now conducted without copper to get a totally 'bioorthogonal' bond as the semantics puts it (Sletten and Bertozzi, 2009). As the use of this alternate route remained very rare in the field of chemical ligation involving liposomes or nanoparticles until very recently (Jolck et al., 2011; Tarallo et al., 2011; Bostic et al., 2012; Colombo et al., 2012; Koo et al., 2012; Wu et al., 2012; Emmetiere et al., 2013; Airoldi et al., 2014), we decided to implement our previous work by a further study. Here, we have designed four new anchors (Fig. 1) based on a dioleylglycero-ethoxy-ethoxy-ethoxy-ethanamine platform (DOG-PEG_4-NH_2, (**1**), whose amine has been converted into several electrophilic heads: cyclooctyne (DOG-COA, (**2**), fluorocyclooctyne (DOG-FCOA, (**3**), triphenylphosphine (DOG-PPh_3, (**4**) and dibromomaleimide (DOG-DBM, (**5**). These anchors were expected to react following copper-free Huisgen cycloaddition, Staudinger ligation and substitution respectively.

Biotin- and TAMRA-based tools were designed and synthesized (Fig. 2) to tune and monitor the experimental conditions leading to the expected decorated liposomes. The reactions could be perfectly conducted in solution and were transposed to SUVs suspensions. The biotinyl liposome adducts were proven functional in a biocompatible environment.

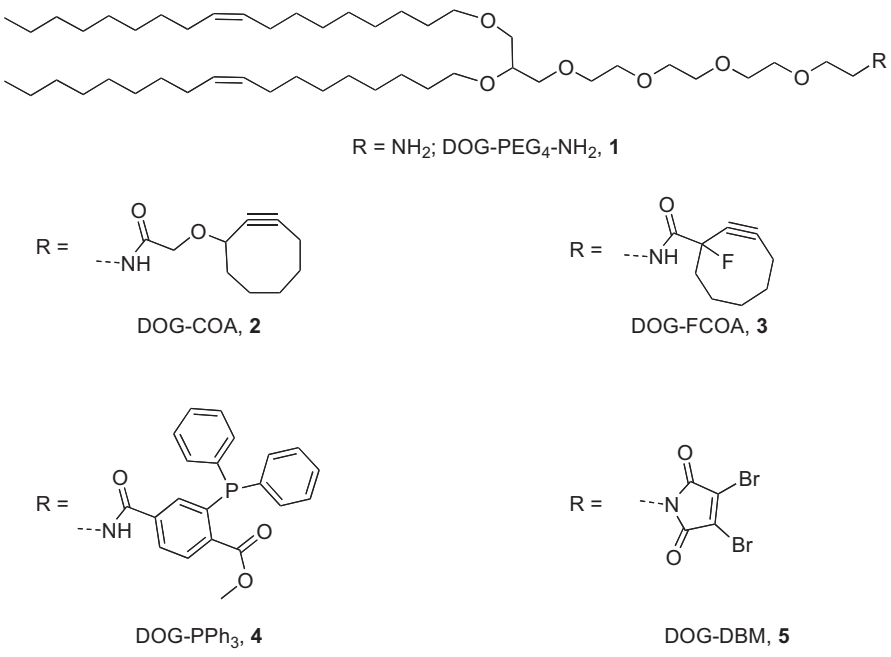

Fig. 1. Four anchors based on a DOG-PEG_4-NH_2 platform