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Tetraphenylethylene-based glycoclusters with aggregation-induced emission (AIE) properties as high affinity ligands of bacterial lectins

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Tetraphenylethylene (TPE) is a fluorescent through aggregation induced emission (AIE) in water. TPE was used as the core of glycoclusters targeting the bacterial lectins LecA and LecB of *Pseudomonas aeruginosa*. Synthesis of these TPE-based glycoclusters was accomplished by azide-alkyne "click" chemistry. The AIE properties of the resulting glycoclusters could be readily verified but imaging application could not be pursued due to fluorescence overlapping with cells and bacteria. Nonetheless, the glycoclusters displayed nanomolar affinities toward LecA and LecB. Further evaluation in a cell-based anti-adhesive assay highlighted a limited decrease in adhesion (20%) for the fucosylated glycocluster. These TPE-based glycoclusters are indeed LecA and LecB high affinity ligands. Nevertheless hypotheses for further applications for imaging or anti-adhesive therapy could not be verified.

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Introduction

Glycoclusters are composed of a multivalent core conjugated with a single copy of a (oligo)saccharide on each branching point. This perfect control of the molecular structure allows for an exact valency and provides a single and homogeneous molecule by opposition to glycopolymers or glyconanoparticles for which the valency is not fully controlled. While this statement is true for valency, controlling the exact tridimensional shape of glycoclusters can sometimes be more difficult. Glycoclusters have been designed with a large series of aromatic cores[1] among which several inherently fluorescent scaffolds.[2-12] We have recently applied perylenediimide (PDI) to the anti-adhesive strategy[13] against *Pseudomonas aeruginosa* (PA) and evaluated successfully PDI-based glyco-dots in the context of cancer cells detection.[14] As a continuation of these investigations, we report here the tetraphenylethylene(TPE)-based glycoclusters as multivalent lectin ligands and also for their anti-adhesive evaluation against PA. The aggregation-induced emission properties of TPE were investigated to capitalize on its fluorescence properties toward chemical biology and imaging of cells. Previous reports have used such TPE-based glycoclusters functionalized with different carbohydrates such as mannose,[15, 16] glucosamine,[17] or 6'-sialyl-lactose[18] for applications in lectins sensing, detection of alkaline phosphatase or influenza virus respectively.

Multivalency is a major strategy for the design of lectin ligands with high affinity.[19] The host-pathogen interaction of PA with the human host cell is mediated by LecA and LecB. These two soluble lectins are binding galactosides and fucosides respectively. While a large series of multivalent ligands of these lectins have been designed,[19] very few are incorporating a fluorescent core for further chemical biology applications.

Results and discussion

Synthesis of the TPE-based glycoclusters

The synthesis of the known tetrapropargylated tetraphenylethylene (TPE) core **1** was achieved through the McMurry reaction of 4,4'-dimethoxybenzophenone,[20-22] followed by demethylation with boron tribromide to the tetra-phenol[20, 23] then propargylation to the desired core **1**.[20, 23] Four azido-functionalized carbohydrates **2a-d**[24-27] were then conjugated to the tetra-alkynylated TPE core **1** under Meldal's conditions[28, 29] (CuI, *i*Pr$_2$NEt) using microwaves activation (Scheme 1). The resulting

acetylated glycoclusters **3a-d** were then deacetylated to afford the desired TPE-based glycoclusters **4a-d** in good isolated yields.
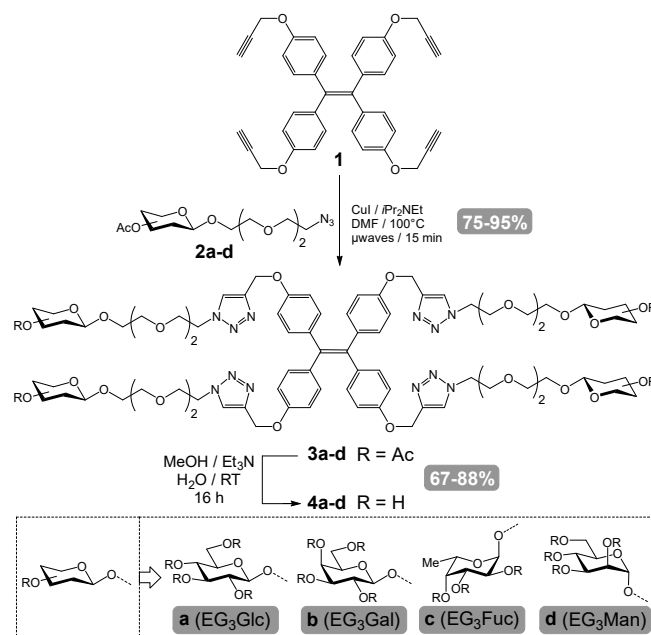
Scheme 1. Synthesis of the TPE-based glycoclusters

ITC binding studies

The affinity of the designed TPE-based glycoclusters have been measured by isothermal titration microcalorimetry (ITC) towards LecA or LecB with the corresponding galactosylated or fucosylated glycoclusters (Table 1). The glucosylated glycocluster **4a** was used as a negative control of affinity toward LecA and LecB. The high affinity of the galactosylated TPE-based glycocluster **4b** could be evidenced by an apparent dissociation constant (K_d) of 80 nM toward LecA. Moreover, the relative potency (β = 875) is among the best reported for such LecA ligands.[19] The stoichiometry of the complex (N = 0.275 ~ 1/4) implies that each of the four galactoside epitopes is involved in a binding interaction with the lectin. The fucosylated glycocluster **4c** displayed a nanomolar apparent K_d value but somewhat similar (β = 5) to the binding properties of the monovalent methyl fucoside used as a reference. Nevertheless, the cluster effect for multivalent LecB ligands is much more difficult to obtain given the tetrahedral geometry of the multimeric protein.[19] Again, all four fucoside epitopes could interact simultaneously with a LecB binding site (N = 0.197 ~ 1/4 or even 1/5).

Both glycoclusters **4b** and **4c** displaying nanomolar affinities for their lectins therefore appear as very promising candidates for anti-adhesive strategy against PA.

			for binding of TPE-based glycoclusters **4a-c** to LecA or LecB				
Ligand	Lectir	$N^{[a]}$	$-\Delta H$ (kJ/mol)	$-T\Delta S$ (kJ/mol)	$K_d^{[b]}$ (nM)	$\beta^{[c]}$
TPE-(EG₃Glc)₄ (4a)	LecA	No binding observed (see Supporting Information Figure S3)				
TPE-(EG₃Gal)₄ (4b)	LecA	0.275±0.005	111.0±0.5	70.5	80±5	875
TPE-(EG₃Glc)₄ (4a)	LecB	No binding observed (see Supporting Information Figure S4)				
TPE-(EG₃Fuc)₄ (4c)	LecB	0.197±0.001	119±2	79	84±5	5

defined as the number of glycoclusters per monomer of LecB. [b] The present dissociation constant is presented f
refore must be considered as apparent K_d values. [c] Relative potency: calculated using methyl β-D-galactopyran
r LecA[30] or methyl α-L-fucopyranoside (α-FucOMe) as a monovalent reference with K_d = 0.43 μM for LecB.[31]
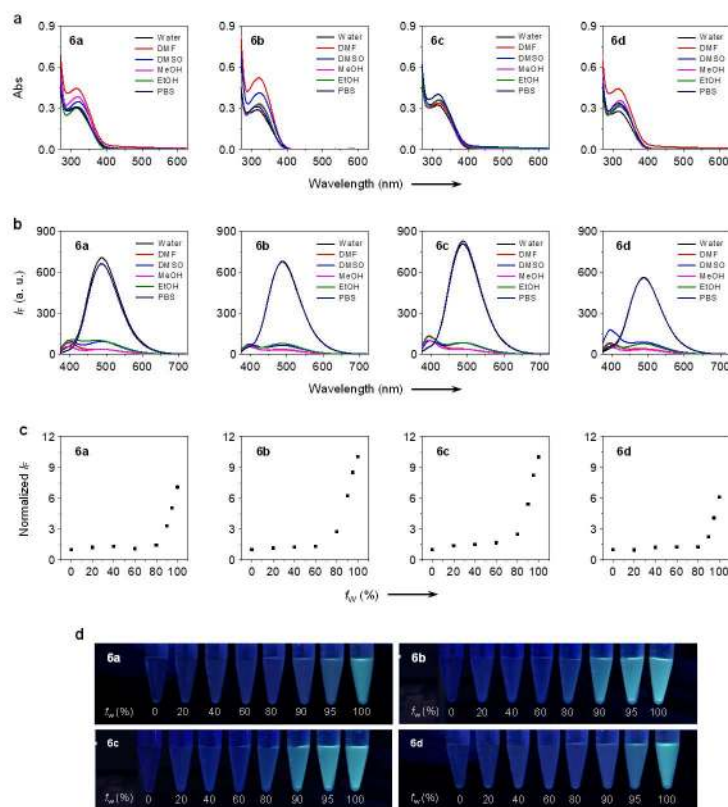

Figure 1. Photophysical properties of **4a-c.** (a) Stacked UV-vis absorbance spectra of **4a-d** (60 μM) in different organic solvents (DMSO, DMF, EtOH, MeOH), pure water and phosphate buffered saline (0.05 M, pH 7.4). The UV-vis absorbance measurements were carried out at room temperature by a Varian Cary 60 UV-vis spectrophotometer. (b) Stacked fluorescence spectra of **4a-d** (60 μM) in different organic solvents (DMSO, DMF, EtOH, MeOH), pure water and phosphate buffered saline (0.05 M, pH 7.4) with an excitation wavelength of 340 nm. The fluorescence measurements were carried out at room temperature by an Agilent Cary Eclipse fluorescence spectrophotometer. (c) Plotting the fluorescence intensity of **4a-d** (60 μM) in DMSO as a function of water fraction (fw%); the original intensities of the glycoclusters are normalized as 1.0. (d) Aggregation-induced-emission of **4a-d** in DMSO with increasing water fraction (fw%) photographed with a camera.

Photophysical properties

UV-vis spectra of TPE-based glycoclusters (Figure 1a) displayed an absorbance peak at ca. 340 nm which is characteristic of the TPE, respectively. Upon excitation at 340 nm, while we observed minimal fluorescence in organic solvents including DMF, DMSO, MeOH and EtOH, the glycoclusters exhibited a much stronger emission in pure water and a phosphate buffered saline (0.05 M, pH 7.4). This is in agreement with their typical aggregation-induced-emission (AIE) property,[32] because the amphiphilic TPE-based glycoclusters could form aggregates in water as demonstrated by increasing the water fraction of a DMSO solution of the TPE-based glycoclusters **4a-d** (Figure 1c and 1d). The difference in the fluorescence enhancement of the glycoclusters might be due to the structural difference of the carbohydrate epitopes conjugated on the TPE core.

The galactosylated glycocluster **4b** was then further investigated by dynamic light scattering (DLS) and high resolution transmission electron microscopy (HR-TEM) to better understand the aggregation of such TPE-based glycoclusters (Figure 2). The fluorescence intensity of the glycocluster increased drastically from 10 to 90 µM due to the AIE effect (Figure 1a) and then quenching of fluorescence occurred above these concentrations (from 120 to 600 µM) due to ACQ effect bringing the intensity back to almost zero. The DLS data collected for compound **4b** also confirmed the formation of larger aggregates upon increasing concentrations with typically 20 nm aggregates at 20 µM, 40 nm aggregates at 60 µM, 60 nm aggregates at 180 µM and 150 nm aggregates at 540 µM (Figure 2b). These observations could be further confirmed by HR-TEM (Figure 2c) in which large aggregates can be visualized at 180 or 540 µM.

PDI-based glycoclusters could provide weak fluorescence in cell adhesion assays, but yet not strong enough to allow potential application as imaging agents.[13] In comparison, TPE-based glycoclusters could not be applied in the same context since their fluorescence in the blue range was overlapping with the intrinsic fluorescence of PAO1.
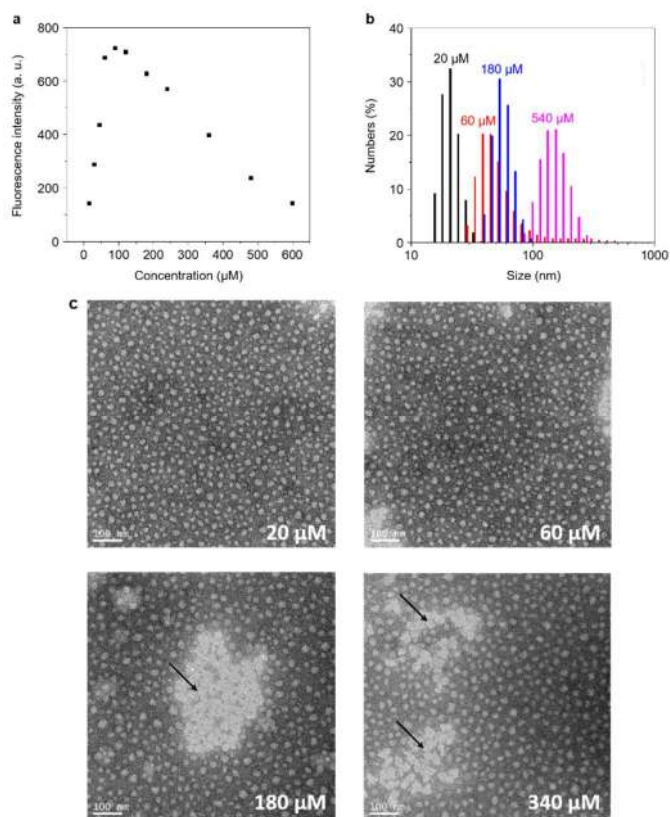

Figure 2. Concentration-dependent aggregation of the TPE-based glycocluster **4b**. (a) Plotting the fluorescence intensity (at 488 nm) of **4b** a function of its increasing concentration in deionized water. (b) Dynamic light scattering (DLS) of **4b** at different concentrations in deionized water. (c) Transmission electron microscopy images of **4b** at different concentrations in deionized water. The black arrows indicate **4b** aggregates at higher concentrations.

Cell adhesion assays

The involvement of PA lectins, namely LecA and LecB, in binding by TPE-based glycoclusters to host cells was further investigated in an *in vitro* cell culture model using A549 lung epithelial cells. Inhibition of adhesion reaching 20% with fucosylated glycocluster **4d** was clearly observed while a non-significant inhibition of adhesion was demonstrated with galactosylated glycocluster **4b** (Figure 3a). Adhesion assays performed with both *lecA* or *lecB* mutant strains (Figure 3b,c) confirmed the lack of inhibition of the galactosylated glycocluster **4b**. The fucosylated glycocluster **4c** had no inhibitory effect on adhesion of *lecA* or *lecB* mutant strains highlighting that its inhibition is dependent of both LecA and LecB. These results suggest a lack of specificity in the interaction of fucose with LecA probably due to non-specific interactions with LecA.
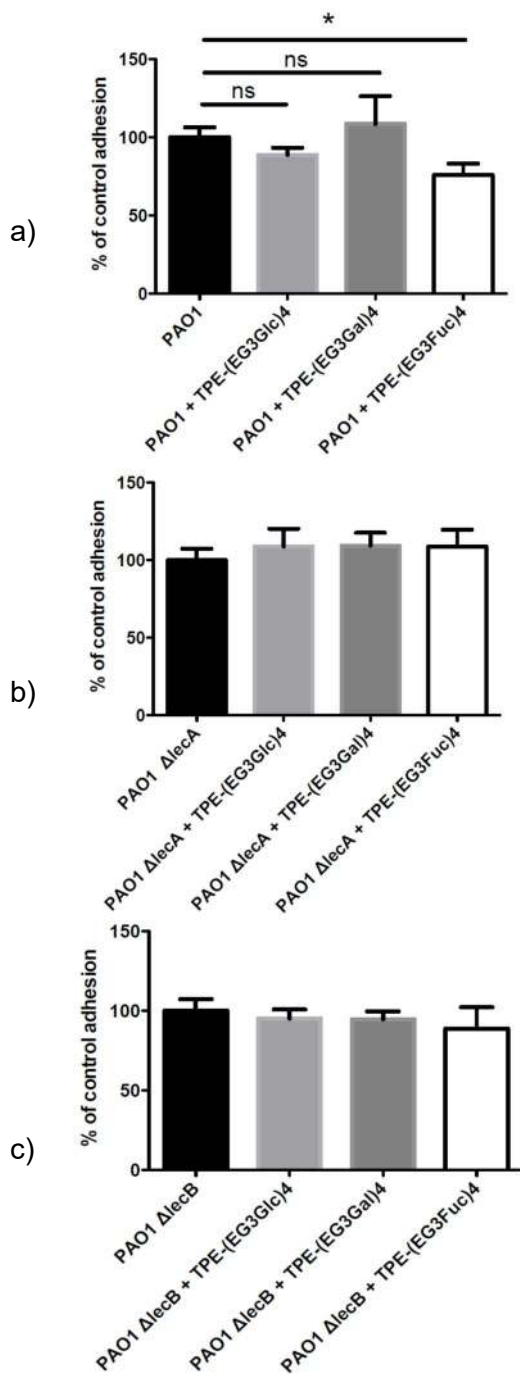
Figure 3. Bacterial adhesion to A549 cells (3 h infection, MOI 10) in the presence or absence of different TPE-based glycoclusters at increasing concentrations (µM) after washing off (5 times) excess non-adherent bacteria with PBS. (a) Inhibition of adhesion of wild-type PAO1. All results are compared to the first column without adhesion inhibitors (PAO1, positive control). (b) Inhibition of adhesion of Δ*lecA* strain. All results are compared to the second column without adhesion inhibitors (*PAO1ΔlecA*), the first column showing the PAO1 wild-type strain remains as a control. (c) Inhibition of adhesion of Δ*lecB* strain. All results are compared to the second column without adhesion inhibitors (*PAO1ΔlecB*), the first column showing the PAO1 wild-type strain remains as a control. All experiments were performed in triplicate. Results are mean±sem. (* $p <$ 0.05); ns: non significant.

Conclusions

TPE was incorporated as the core of glycoclusters to take advantage of its intrinsic fluorescence properties from AIE effect. The synthesis of TPE-based glycoclusters could be performed by azide-alkyne "click" chemistry and the resulting glycoclusters displayed the expected AIE effect but the blue fluorescence generated was overlapping with cells and bacteria and prevented their application as imaging agents. ITC binding studies with LecA and LecB as the two proteins involved in the adhesion of *Pseudomonas aeruginosa* to host cells provided strong binding properties towards both lectins with K_d values in the nanomolar range. Further evaluation as anti-adhesive agents proved less encouraging since only a very limited decrease of adhesion could be observed (20%) for the fucosylated glycocluster. These TPE-based glycoclusters therefore appear as high affinity ligands of the LecA and LecB bacterial lectins yet their AIE fluorescence properties could not be exploited for further biomedical applications.

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