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# Synthesis and self-assembly of amphiphilic polyphosphazene with controllable composition via two step thiol-ene click reaction

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ABSTRACT

Sequential thiol-ene click reaction is reported for amphiphilic glycosylated polyphosphazene. Poly[bis(allylamino)phosphazene] was used as precursor to go through UV irradiation with 2,3,4,6-tetra-O-acetyl-1-thiol-$\beta$-D-glucopyranose (SH-GlcAc$_4$) and 1-dodecanethiol in sequence. Variation of the reaction conditions, including click reaction time and the dose of photoinitiator, led to different hydrophilic/hydrophobic ratios. As a result, glycosylated polyphosphazenes were synthesized with 53.3%, 77.7% and 85.0% of glucose moieties. The different residual composition could give rise to different self-assembly behaviors. Micelles of amphiphilic polyphosphazenes were formed in aqueous solution and the CMC value ($0.79 \times 10^{-3}$–$4.00 \times 10^{-3}$ mg/mL) as well as mean diameter (170–220 nm) varied along with the hydrophilic glucose moiety/hydrophobic dodecyl moiety ratio.



## 1. Introduction

As well established, polyphosphazene possesses a backbone containing alternating nitrogen and phosphorus atoms, with organic or inorganic groups on each side of each phosphorus atom [1–3]. Owing to the tunability of side groups on the polyphosphazene platform, more than 700 different polyphosphazenes have been synthesized, which have been widely applied in tissue engineering [4–8], fire retardation [9,10], arctic rubber [11,12] and ion conductor materials [13,14]. Carbohydrate polyphosphazenes, with desirable hydrophilicity and biocompatibility, are of particular interest in biological processes such as carbohydrate–protein interaction [15–21]. Nonetheless, when it comes to practical use, the hydrophobic part is indispensable to control degradation rate, material strength and surface properties [22,23]. Moreover, self-assembly of glycopolymers can mimic cell surface of glycolipids, glycoproteins and glycans and participate in numerous biological events such as cellular recognition, adhesion, cancer cell [24]. Thus, the construction of amphiphilic glycosylated polyphosphazene with mutable hydrophilic/hydrophobic ratios should be highlighted. Carbohydrate polyphosphazenes were first synthesized by nucleophilic reaction involving sodium alkoxide, glucose, and poly(dichloro)phosphazene (PDCP) [25], which faced the unfavorable steric effect of glucose and required an intricate protection–deprotection method to avoid crosslinking and degradation of the polymer backbone.

Click chemistry is an emerging synthetic toolbox especially energetic suitable for end group or side group functionalization [26–30], crosslinking of polymeric matrices [31,32] and highly branched polymers [33–35]. In 2001, Sharpless et al. brought about the essence of the click reaction by reporting a class of heteroatom linkage processes conducted in various mild conditions [36]. Typically, the Cu(I)-catalyzed azide/alkyne cycloaddition reaction (CuAAC) has been universally applied in macromolecular engineering with near-quantitative conversion in both aqueous and organic media [35]. Other types of click reactions, such as thiol-ene and thiol-yne, are outstanding in terms of robustness, efficiency, and orthogonal conjugation [37–39]. These reactions could achieve quantitative yields, requiring only small concentrations of relatively benign catalysts, having rapid reaction rates with reactions occurring either in bulk or in environmentally benign solvents over a large concentration range [40]. As claimed by Campos et al. [26], the use of UV initiator in thiol-ene coupling reaction led to higher yields and shorter reaction time than thermal ones. Using click chemistry, controllable glycosylation and multifunctional architecture of polyphosphazene may be feasible.

Previous work in our lab accomplished the glycosylation of polyphosphazene by CuAAC using poly[di(propargylamine)phosphazene] as a precursor [41]. However, the residual copper at ppm levels in the product after tedious purification will place restrictions on biological application [42,43]. As a possible alternative, the thiol-yne reaction was conducted to synthesize

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carbohydrate polyphosphazene [44]. Owing to the fact that at most two bulky glucosyl groups might "click" to every alkyne group, not all C≡C bonds went through the two-step thiol-yne click reaction. For instance, Ren et al. [44] found that approximately 55% of the alkyne group and 8% of the alkene group did not participated in the click reaction even after 4 h of UV irradiation with excess 2,3,4,6-tetra-O-acetyl-1-thiol-β-D-glucopyranose (SH-GlcAc$_4$). At the mean time, the final glycosylated density was nearly 67.7%. With an aim to ease the steric repulsion among the induced groups and avoid the requirement for toxic copper cations, the thiol-ene reaction, in which poly[bis(allylamino)phosphazene] (PBAAP) was used as precursor, can be a potential route to design multigroup polyphosphazene by clicking two or more thiol agents in a row.

One-step click reaction was explored to synthesis amphiphilic polyphosphazene, however, weak controllability took up owing to the different thiol-ene reaction rates of SH-GlcAc$_4$ and 1-dodecanethiol. In this work, amphiphilic polyphosphazenes containing hydrophilic glucosyl and hydrophobic long alkyl groups with mutable ratio were synthesized by a two-step thiol-ene reaction. In the first step, a glucose moieties derived from SH-GlcAc$_4$ was immobilized on PBAAP. Controllable glycosylation could be reached by varying the reaction time or the dose of 2,2-dimethoxy-2-phenylacetophenone (DMPA) during UV irradiation. Then excess active 1-dodecanethiol was clicked to carbohydrate polyphosphazene to form the hydrophobic part. The ultimate poly(β-D-glucose-*co*-1-dodecyl)phosphazene with different density of glycosylation, P-37.1%, P-53.3%, P-77.7% and P-85.0%, were synthesized and studied. The resulting polyphosphazenes were amphiphilic and could self-assemble in water when the ratio of glucose group to dodecyl group was up to 50%. A larger proportion of glucose moieties caused a lower critical micelle concentration (CMC) value and micelle size expansion, according to the results from pyrene fluorescence spectrometry and dynamic light scattering (DLS). The morphology of micelles was intuitively observed by transmission electron microscopy (TEM).

## 2. Experimental section

### 2.1. Materials

Hexachlorocyclotriphosphazene (Bo Yuan New Materials & Technique, Ningbo, China) was purified by recrystallization from heptane and subsequent vacuum sublimation at 60 °C. Poly(dichlorophosphazene) was synthesized via the thermal ring-opening polymerization of the purified hexachlorocyclotriphosphazene in an evacuated Pyrex tubes at 250 °C. The polymer was treated with petroleum ether (Sinopharm Chemical Reagent, China) to remove unpolymerized hexachlorocyclotriphosphazene before use under a dry nitrogen atmosphere. Allylamine (Zoupin Mingxing Chemical Co., China) was purified by vacuum distillation. Tetrahydrofuran (THF) was dried by distillation from a Na–K alloy with benzophenone until a blue color was obvious. Triethylamine (TEA) was dried over $CaH_2$ prior to use. 2,2-Dimethoxy-2-phenylacetophenone (DMPA, Aladdin Reagent, China), trifluoroethanol (TFE, Aladdin Reagent, China, 99.5%), and 1-dodecanethiol were used without further purification. 2,3,4,6-Tetra-O-acetyl-1-thiol-β-D-glucopyranose (SH-GlcAc$_4$) was synthesized as previously reported [45].

### 2.2. Synthesis of poly[bis(allylamino)phosphazene] (PBAAP)

Poly(dichlorophosphazene) (2.00 g, 17.3 mmol) was dissolved in dry THF (250 mL) and the resulting solution was added in a drop-wise manner to a stirred solution of allylamine (5.2 mL, 69.6 mmol) and TEA (9.7 mL, 69.6 mmol) in dry THF (50 mL) under nitrogen. The mixture was stirred at 25 °C for 24 h and then heated to 40 °C and stirred for a further 18 h at that temperature. The mixture was filtered to remove the resulting triethylamine hydrochloride and the filtrate was concentrated by evaporation. The concentrated solution was added in a drop-wise manner to an excess of ethanol–water (1:1 by volume) to precipitate the polymer. The solid was subsequently filtered and the filter-cake washed several times with ethanol and dried under a vacuum to give the desired polymer product as a white fibrous solid (1.63 g, 60%).

### 2.3. Synthesis of poly(β-D-glucose-co-allyamine)phosphazene

PBAAP (150 mg) was dissolved in TFE at a concentration of 15 mg/mL and the mixture was then transferred to a quartz reactor. SH-GlcAc$_4$ (2 equiv. with respect to the double bonds) and DMPA (0.1 or 0.02 equiv. with respect to the double bonds) were added sequentially to the reactor and $N_2$ was then gently bubbled through the mixture for 10 min to eliminate dissolved oxygen. The thiol-ene reaction was initiated by UV irradiation (max = 365 nm, 0.6 mW/cm$^2$) and conducted for the described time (40 min, 2 h and 4 h) with stirring at ambient temperature. After the first click reaction, the acetyl protecting group was removed by the addition of a 1 M solution of $CH_3ONa$ in $CH_3OH$ to the polymer solution. The mixture was dialyzed against water for 2 days (molecular weight cut-off: 3.5 kDa) and purification of glycosylated phosphazene was conducted by freeze drying. The ratio of glucose moieties was calculated by integration for the signal at 3.66 ppm with 5.90 ppm.

### 2.4. Synthesis of poly(β-D-glucose-co-1-dodecyl)phosphazene

Glycosylated polyphosphazenes were dissolved in 2.25 mL DMF and 1-dodecanethiol (5 equiv. with respect to the double bonds) and DMPA (0.01 equiv.) were added to the solution. The mixture was under UV irradiation for 1 h and subsequently dialyzed against alcohol for 2 days and water for 1 day with the final product was obtained by freeze-drying. Take account for different proportion of glucose moieties, these amphiphilic polyphosphazenes can be named as P-53.3%, P-77.7%, P-85.0% and P-37.1% for short.

### 2.5. Self-assembly of poly(β-D-glucose-co-1-dodecyl)phosphazene by crew-cut method

Poly(β-D-glucose-*co*-1-dodecyl)phosphazenes with different glucose to dodecyl ratios were first dissolved in DMF (0.1 mg/mL), and then deionized water was gradually added at a constant rate (25 μL/min). The magnitude of water jump was 1 wt% per time. The solution was subjected to under moderate stirring at room temperature during water addition. The morphology of the micelles was quenched by adding a large amount of water after reaching the desired water content. DMF was removed by dialysis against water (molecular weight cut-off: 3.5 kDa).

### 2.6. Characterization

$^1$H NMR spectra were recorded on a Bruker Advance DMX500 in DMSO-d$_6$. A 2–5 wt % polymer solution was prepared in DMSO-d$_6$ for each analysis. $H_3PO_4$ in $D_2O$ was used as an external reference for the $^{31}$P NMR measurements. Number-average and weight-average molecular weights and molecular weight distributions were determined using gel permeation chromatography (GPC). FTIR spectra were recorded using a Bruker Vector 22 Fourier Transform Infrared Spectrometer, using samples pressed into potassium bromide pellets. Pyrene for fluorescence measurements (FLM) was dissolved in acetone solution (concentration $6.0 \times 10^{-7}$ mol/L), the whole mixture was then added to volumetric flask before acetone was separated from pyrene by evaporation.