**Acetyl-α-D-Mannopyranose based Cationic Polymer *via* RAFT Polymerisation for Lectin and Nucleic Acid Bindings**

S. R. Simon Ting\*, Eun Hee Min, Benjamin K. F. Lau and Gyorgy Hutvagner

Centre for Health Technologies (CHT), Faculty of Engineering and Information Technology, University of Technology Sydney (UTS), Ultimo NSW 2007, Australia

**\***Correspondence: Simon.Ting@uts.edu.au; Tel.: +61 02 9514 4507

**ABSTRACT**

Functional cationic polymers carrying mannose moieties were synthesised in a facile manner by employing RAFT polymerisation. Initially, a protected carbohydrate based monomer, [2-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyloxy)ethyl methacrylate (AcManEMA)], was prepared by the *O*-glycosylation of 2-hydroxyethyl methacrylate (HEMA). Subsequently, a macroRAFT agent of poly(2-(dimethyl)amino ethyl methacrylate) (PDMAEMA) was generated, and a further chain extension polymerisation with AcManEMA was carried out in dioxane to form a acetylated mannose cationic diblock copolymer, PDMAEMA-*b*-PAcManEMA. It was attained in high yields and displayed low dispersity (*Ð*). Acetylated mannose moieties on the polymer were deprotected with sodium methoxide and the amines from the DMAEMA block were protonated to yield a cationic diblock glycopolymer, PDMAEMA-*b*-PManEMA. The cationic property of polymers were characterised by mixing with a negatively charged siRNA duplex and a pDNA, and aggregates of 102 nm and 233 nm were obtained, respectively. Agarose gel shift assay revealed that the polymers were able to retain the nucleic acids as large polymer complexes. Lectin binding assay proved that the mannose residue on the polymers were only able to bind specifically with ConA. PNA lectin was employed as a control and did not show specific binding. The cationic glycopolymer could be advantageous in targeted nucleic acids delivery in specific cells.

**KEYWORDS**

RAFT polymerisation; glycopolymer; mannose; PDMAEMA; cationic polymer; lectin; nucleic acids; Concanavalin A (ConA); Peanut agglutinin (PNA); siRNA; pDNA

**INTRODUCTION**

Carbohydrates are biomolecules readily available on cell surfaces and they play an integral role in cell-cell recognitions and adhesions. They trigger signal transduction and in turn results in numerous biological processes which include, fertilisation, pathogen infection, immune system activation and attenuation and cell proliferation.[1](#_ENREF_1) It is therefore not surprising that natural polysaccharides and their synthetic counterparts ‘glycopolymers’ are intensively investigated as biomaterials. Due to the limited structures of polysaccharides obtained from their natural sources, they lack the versatility to interact with the biological systems and serve specific therapeutic delivery tasks.[2](#_ENREF_2) On the other hand, glycopolymers are well-defined synthetic polymers comprise of hydrocarbon backbones with pendant carbohydrate moieties which the latter can serve as ligand recognition sites for protein receptor binding.[**3**](#_ENREF_3)**,** [**4**](#_ENREF_4) Due to the vast potentials, there have been increasing efforts in investigating the synthesis of well-defined glycopolymers. One area of interest is their abilities to bind specific plant lectins. Significant efforts have been committed to study the ‘cluster glycoside effects’ of glycopolymers.[5](#_ENREF_5) Owing to the multiple carbohydrate pendant groups available on the polymer, glycopolymer exhibits the clustering effect. This can lead to enhance binding of polymer to specific targeted lectins or mammalian cells.[**6**](#_ENREF_6) Furthermore, glycopolymers have found to display low cytotoxicity.[7](#_ENREF_7) They can assist in shielding the charges from cationic polymers and reducing the level of toxicity in cells significantly.[7](#_ENREF_7), [8](#_ENREF_8)

Cationic glycopolymers containing galactose and glucose moieties have been widely synthesised by research groups working on nucleic acids delivery.[**9**](#_ENREF_9) Galactose based cationic polymers with different polymer structures and their bindings with lectins have been investigated as non-viral gene delivery systems.[10](#_ENREF_10), [11](#_ENREF_11) Other work on cationic glycopolymers with galactose moieties include the use of *N*-acetylgalactosamine based polymers to bind plasmids and increase specific delivery of polyplexes into the liver,[12](#_ENREF_12) the combined use of galactose as the carbohydrate moieties and lysine as the cationic moieties for gene delivery and intracellular responses,[13](#_ENREF_13) and degradable lactose and galactose based glycopolymer based nanogels were employed as carriers for intracellular delivery of DNA and enzyme.[14](#_ENREF_14) Although no binding with protein and nucleic acid were carried out, poly(3-*O*-methacryloyl-d-galactopyranose)-*b*-poly(2-(dimethyl)amino ethyl methacrylate) diblock copolymers were successfully synthesised via a protective route reaction.[15](#_ENREF_15) Trehalose which comprise of two linked (1→1) **-glucose units increased the stability of formed polyplexes for lyophilisation and the delivery of pDNA *in vivo*.[16](#_ENREF_16) In the same research group, poly(2-deoxy-2-methacrylamido glucopyranose)‑*b*‑poly(methacrylate amine) were synthesised and found that the glucose based cationic glycopolymers resulted in very effective pDNA delivery and polymers displayed low cytotoxicity profiles.[17](#_ENREF_17) However, there are only a few articles reporting the synthesis of mannose based cationic glycopolymers.[**18**](#_ENREF_18)**,** [**19**](#_ENREF_19) It has been reported that a possible way to delivery therapeutics to treat inflammatory diseases and promote wound healings is the use of the specific binding mechanism of mannose based glycopolymer with mannose receptors on alveolar macrophages.[**20**](#_ENREF_20) In a work by Chung and co-workers, it was found that a polystyrene derived mannose polymer was able to strongly bind to macrophage cells. They have suggested the use of these glycopolymers for gene delivery in macrophages.[**21**](#_ENREF_21)

Glycopolymers can be fabricated either by the polymerisation of glycomonomers,[22](#_ENREF_22) the postpolymerisation functionalisation of reactive repeating groups along a polymer[23](#_ENREF_23) or occasionally using a carbohydrate-derived initiator[**24**](#_ENREF_24). In the polymerisation of glycomonomer, the polymerisation of some protected galactose and unprotected mannose and glucose based glycomonomers have been widely studied. A galactose protected with acetal groups was synthesised using acryloyl chloride functionalised on the six position of the sugar configuration.[25](#_ENREF_25) In order to investigate the modification of sugar at different carbon positions, an acetate protected galactose was reacted with 2-hydroxyethyl methacrylate[26-28](#_ENREF_26) and 2-hydroxyethyl acrylate[29](#_ENREF_29) to yield an acrylate and methacrylate derivatives of glycomonomers both modified at the first position of the sugar configuration. Glycomonomers were also synthesised from their unprotected forms, mannose glycomonomer was enzymatically synthesised[27](#_ENREF_27), [30](#_ENREF_30) and glucose glycomonomer was developed using amine chemistry[31](#_ENREF_31), [32](#_ENREF_32) and both monomers were polymerised by the RAFT process. By employing commercially available glucose and galactose glycomonomers, glycopolymers bearing a DMAEMA block were synthesised *via* Atom transfer radical polymerization (ATRP) for nucleic acid delivery systems.[33](#_ENREF_33) Stimuli-reponsive spherical brushes comprising of 6-O-methacryloyl-1,2:3,4-di-O-isopropyli-dene-D-galactopyranose (MAIGal) and 2-(dimethylamino)ethyl methacrylate (DMAEMA) were also prepared by ATRP.[34](#_ENREF_34)

Glycopolymers are synthesized by the modifications of polymers with reactive functional unit, pentafluoro styrene.[35](#_ENREF_35), [36](#_ENREF_36) By employing more common chemistries such as the formations of ester and amide, glycopolymers were synthesised with silver and gold nanoparticles.[37](#_ENREF_37) A step-tandem post-polymerisation process was performed and the glycopolymers were found to increase affinity towards specific lectins up to 20-fold.[38](#_ENREF_38) Water-soluble conjugated glycopolymers were synthesised by click chemistry for the sensing of ConA.[39](#_ENREF_39)

These methods provide a wide range of synthetic pathways that can be tailored for specific chemistries. Obata *et al*[*19*](#_ENREF_19) has previously synthesised PDMAEMA-*b*-PManEMA by employing a unprotected chemistry synthetic route. In the present work, we present the synthesis of a protected mannose glycomonomer, 2-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyloxy)ethyl methacrylate (AcManEMA) using a acetylated α-D-mannose in the *O*-linked glycosylation of HEMA for the first time (Scheme 1). In order to incorporate cationic properties onto the polymer, a tertiary amine based methacrylated monomer, 2-(dimethyl)aminoethyl methacrylate (DMAEMA), was chosen.[**40**](#_ENREF_40) By employing reversible addition-fragmentation chain transfer (RAFT) polymerisation, a diblock copolymer comprised of PDMAEMA-*b*-PAcManEMA was synthesised *via* novel protective route chemistry. Deacetylation was carried out to afford mannosylated cationic polymers (Scheme 2). The cationic property of the polymers was examined by incubating them with a negatively charged small interfering RNA (siRNA) and a plasmid DNA (pDNA) and the formation of polyplexes were assayed. A new psiCheckTM-2 Vector Plasmid DNA based polyplexes were successfully developed here. Furthermore, the biofunctionality of the pendant mannose groups was investigated using a model lectin, Concanavalin A (ConA, *Canavalia ensiformis* from jack bean) that specifically binds with glucose and mannose.[41](#_ENREF_41)



Scheme 1. Schematic approach to the synthesis of 2-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyloxy)ethyl methacrylate (AcManEMA) glycomonomer. Reactions resulted in the *O*-glycosylation of HEMA with regioselective α-anomeric bond formation with AcMan.



Scheme 2. Schematic approach to the synthesis of cationic -D-mannose based glycopolymer by RAFT polymerisation and its deacetylation. PDMAEMA macroRAFT agent was first obtained and this was followed by the chain extension polymerisation of macroRAFT with AcManEMA to acquire the PDMAEMA-*b*-PAcManEMA diblock glycopolymer. Deacetylation of AcManEMA block was carried out using sodium methoxide solution and by dissolving the final polymer into acetate buffer (0.01M, pH = 5.5), DMAEMA block was protonated.

**EXPERIMENTAL**

**Materials**

1,4-Dioxane 98% anhydrous grade, chloroform-d 99.8% for NMR (CDCl3), dimethylacetamide 99.8% HPLC grade (DMAc), Sodium Methoxide 25% wt. in methanol, tetrahydrofuran 99.9% anhydrous (THF) and 4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl]pentanoic acid (CPDT), 4,4’-azobis(4-cyanovaleric acid) (ACVA), Concanavalin A (ConA) from Cananvalia ensiformis (Jackbean), Peanut agglutinin (PNA, *Arachis hypogaea*), methyl **-D-mannopyranoside and Luciferase (Luc) siRNA duplex (sense: 5′-GUGGAUUUCGAGUCGUCUUAA-3′ antisense: 5′-AAGACGACUCGAAAUCCACAU-3’) were purchased from Sigma-Aldrich. Agarose (BioRad), acetonitrile 99.9% HPLC grade (Honeywell Burdick and Jackson), diethyl ether 99% anhydrous (ChemSupply), dimethyl sulfoxide 99% anhydrous grade (DMSO) (ChemSupply), *n*-hexane 96% GC residue analysis (ChemSupply) and psiCheckTM-2 Vector Plasmid DNA with 6273 base pairs (Dual Luciferase Plasmid, MW = 4,077,450 g mol-1, Promega) were used directly from the suppliers.

For glycomonomer synthesis, 1,2,3,4,6-penta-*O*-acetyl-α-D-mannopyranose (α-D-mannose) (Carbosynth), and Boron trifluoride diethyl etherate 46%−51% synthesis grade (BF3⋅O(C2H5)2), 2-(dimethyl)aminoethyl methacrylate (DMAEMA, purified over basic alumina) and 2-Hydroxyethyl methacrylate (HEMA) were purchased from Sigma-Aldrich.

**Syntheses**

***Synthesis of 2-(2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyloxy)ethyl methacrylate (AcManEMA)***

With slight modifications, AcManEMA was synthesised as previously reported.[**42**](#_ENREF_42) 1,2,3,4,6-Penta-*O*-acetyl-*α*-d-mannopyranose (5.0 g, 17.8 mmol) and 2-Hydroxyethyl methacrylate (1.6mL, 29.1 mmol) were dissolved in dry acetonitrile (50mL) inside a 250mL one-neck round bottom flask. The reaction commenced when boron trifluoride diethyl etherate 9.7 mL (72 mmol) was introduced *via* a gastight syringe over 30 min whilst in nitrogen surroundings. The contents within the flask were stirred at ambient temperature of around 20°C for 26 h to provide sufficient time for maximum conversion. During the purification steps, the organic mixture was washed with MilliQ water (150mL × 2), saturated sodium bicarbonate (150mL × 2) followed by saturated sodium chloride (150mL × 2). Each wash caused a separation of phases consisting of an organic and aqueous phase. The top layer was the organic phase, as synthesis was conducted in acetonitrile, a solvent that has a less density than all the washing solutions aforementioned. The organic phase was kept after each washing and then dried under reduced pressure. This results in a yellow crude mixture that was purified with column chromatography (silica gel) using acetone/*n*-hexane (3:7 *v*/*v*) as the eluent. Fractions containing AcManEMA were identified with thin layer chromatography (TLC) using the same solvent system as the column chromatography and selected to dry under reduced pressure for 8 h. This gave a colourless translucent viscous product that was transferred into a 5mL flask and placed in a fridge overnight followed by a freezer for crystallisation that gave a white powder. Yield 2.3g (4.99 mmol).

***Homopolymerisation of 2-(2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyloxy)ethyl methacrylate (AcManEMA) via RAFT***

2-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyloxy)ethyl methacrylate (AcManEMA) (0.1 g, 0. 217 mmol), 4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl]pentanoic acid (CPDT) (0.00073 g, 0.0184 mmol) and 4,4’-Azobis(4-cyanovaleric acid) (ACVA) (0.0001 g, 0.0037 mmol) were dissolved with 0.72mL 1,4-dioxane in a pear shaped flask. The mixture was purged in nitrogen for 45 min and then submerged in a 70°C oil bath. Aliquots of 0.125mL were taken out at 2 h, 4 h and 8 h while maintaining a nitrogen environment and used to prepare samples for gel permeation chromatography (GPC) and nuclear magnetic resonance (NMR) analyses. Polymerisation kinetics were monitored at different time intervals where monomer conversions were determined using 1H NMR by dissolving small quantities (10L) of crude mixtures into CDCl3. Conversions were calculated by comparing one of the vinylic protons from AcManEMA at 6.11 ppm with the PAcManEMA hydrocarbon backbone protons at 0.82 ppm to 1.02 ppm.

***Homopolymerisation of 2-(dimethyl)aminoethyl methacrylate) (DMAEMA) via RAFT***

2-(Dimethyl)aminoethyl methacrylate (1.50 g, 9.54 mmol), 4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl]pentanoic acid (CPDT) (0.032 g, 0.08 mmol) and 4,4’-Azobis(4-cyanovaleric acid) (ACVA) were dissolved into 2.5mL of methanol in a pear shaped flask. The mixture was purged in nitrogen for 1 h. The flask containing the mixture was submerged in a 70°C oil bath to start the polymerisation. Polymerisation was terminated at 8 h by removing the pear shaped flask from the oil bath and allowing it to rest in an ice bath. During the purification steps, polymers were precipitated three times from THF and *n*-hexane. After each precipitation, polymers were centrifuged at 4,400 rpm for 8 min forming a pellet at the bottom. The supernatant was decanted and the pellet was allowed to dry in a vacuum oven (37°C) overnight. The product was a hard translucent yellow compound and the purified polymer was used for GPC and NMR analysis. Yield 1.31g (0.078 mmol). Monomer conversions were calculated by comparing one of the vinylic protons at 6.06 ppm from DMAEMA with the PDMAEMA hydrocarbon backbone protons at 0.81 ppm to 0.99 ppm.

***Synthesis of PDMAEMA-b-PAcManEMA via RAFT polymerisation***

2-(2,3,4,6-Tetra-*O*-acetyl-*α*-d-mannopyranosyloxy)ethyl methacrylate (AcManEMA) (0.100 g, 0.217 mmol), poly(2-(dimethyl)aminoethyl methacrylate) (0.102 g, 0.006 mmol), 4,4’-Azobis(4-cyanovaleric acid) (0.0041 g, 0.014 mmol) were dissolved with 1mL 1,4-dioxane in a 10mL pear shaped flask. The mixture was purged in nitrogen for 45 min and then submerged in a 70°C oil bath for 8 h. During the purification steps, polymers were precipitated three times from THF using *n*-hexane. After each precipitation, polymers were centrifuged at 4,400 rpm for 8 min forming a pellet at the bottom. The supernatant that comprised of solvents was decanted resulting in an off white pellet and it was placed in a 36°C vacuum oven overnight to dry. The final result was a pale yellow pellet that was stored at room temperature. Small quantities of the purified polymer were used for GPC and NMR analysis. Yield 0.950 g (0.031 mmol). Monomer conversions were calculated by comparing one of the un-polymerised AcManEMA vinylic protons at 5.6 ppm with the combined anomeric protons of AcManEMA and the polymerised PDMAEMA104-*b*-PAcManEMA at 4.9 ppm.

***Deacetylation of PDMAEMA-b-PAcManEMA***

PDMAEMA104-*b*-PAcManEMA31 (0.01 g, 3.2 × 10-4 mmol) copolymer was dissolved into 2mL of dry DMSO in a 5mL pear shaped flask and 0.08mL of 0.5 M sodium methoxide was added to the solution. The mixture was stirred for 30 min to allow maximum deacetylation. The reaction was quenched by promptly transferring the reaction mixture into a membrane (cut-off of 6,000–8,000 g mol-1) for dialysis against MilliQ water. Dialysis occurred for 18 h with 3−4 hourly water changes. The mixture was placed in the freezer overnight and then further chilled with liquid nitrogen prior to freeze-drying. The product was freeze-dried overnight to remove any water content and stored at room temperature. The deacetylated polymers were characterised using GPC, NMR, and zeta potential analyses. Yield 0.008g (0.0027mmol).

**Analyses**

***NMR characterisation of monomer and polymers***

All samples (liquid and solid) were prepared within the concentration range of 1−8 mg mL‑1 for analyses. Monomers or polymers were first dissolved in a glass vial along with 600μL of deuterated dimethyl sulfoxide (DMSO) or deuterated chloroform (CDCl3) and the solutions were transferred into NMR tubes. The NMR samples were placed in a queue in an Agilent 500 MHz NMR spectrometer. 1H-NMR analysis was conducted with the following settings: 1H, number of scans = 16. The results of the 1H NMR spectra data were processed using vnmrJ software. Peaks were selected and integrated to determine the structure of monomer and polymers.

***Gel permeation chromatography (GPC)***

Molecular weight distributions and dispersities of polymers were determined using a Waters 2790 HPLC system installed with an Empower personal GPCV software. The GPC columns were setup in the system’s oven to run at 40°C and DMAc [inhibited with lithium bromide (LiBr) and butylated hydroxytoluene (BHT)] was used as the eluent with a flow rate of 1mL min-1 and a sample injection volume of 100 μL. The GPC system is equipped with three Waters Styragel columns HR 0.5, 2 and 4 working in a series (with a separation range of 1 × 102–3 × 106 g mol-1), a Styragel guard column and a refractive index detector ERC-7510 (a white light and the wavelength from the tungsten filament). Polystyrene calibration provided calculations of weighted-average molar mass (*Mw*), number-average molar mass (*M­n*) and the dispersity (*Ð* = *Mw/Mn*). All polymer samples are prepared at concentrations of 2 mg mL-1 in inhibited DMAc. Samples were filtered through polyvinylidene fluoride (PVDF) 0.45 μM filters and loaded into the system for analysis. Data were processed with Empower Pro 2 software.

**Lectin binding assays**

***UV-vis Spectrophotometer***

A UV-vis spectrophotometer (Cary 300) equipped with a temperature controller was used for the turbidimetric assay of the ligand/protein conjugation. Absorbance data were recorded at 420 nm for 10 min at 1.2 Hz. 1 mg mL-1 of ConA or PNA was prepared in 0.01M phosphate-buffered saline (PBS) at pH of 7.4. 500L of lectin solution was pipetted into a low volume cuvette and inserted into the holding block to obtain a baseline. 300L of polymer solution (0.2 mg mL-1) was than mixed with 50L of lectin solution and immediately returned into the holding block where absorbance was taken over 20 min.

For a competition assay, a low volume cuvette containing PBS was first zeroed, and 300L of polymer solution (1 mg mL-1) was mixed with 300L of ConA solution (1 mg mL-1) and immediately returned to the holding block for conjugation over 15 min. This was followed by the addition of free mannose (500 mM) into the mixture with vigorously mixing and the cuvette was returned to the holding block for competition assay measurement.

***Dynamic light scattering (DLS)***

Particle sizes and zeta potential measurements were determined using a Malvern Zetasizer nano ZS with a laser of 4 mW (He-Ne),  = 632 nm, 173°, backscatter. The binding behaviour of ConA and PDMAEMA-*b*-PManEMA was investigated by measuring the change in size of particles. 1 mg mL-1 of ConA/PNA was first prepared in 0.01 M phosphate buffered saline (PBS) at 7.4 pH. Meanwhile a 0.2 mg mL-1 PDMAEMA-*b*-PManEMA solution was prepared by dissolving the polymers into PBS. An initial measurement of this polymer solution (300μL) was performed using a DLS analyser. 50μL of ConA/PNA solution was transferred into a low volume cuvette of polymer solution, along with gentle mixing to initiate the binding. This was immediately followed by placement into the holding block of the DLS analyser for measurements over duration of 20 min.

For a competition assay, 300L of polymer solution (1 mg mL-1) was mixed with 40L of ConA solution (1 mg mL-1) in a low volume cuvette and immediately returned to the holding block for measurements. Free mannose (500 mM), methyl **-D-mannopyranoside, was added and mixed vigorously into the mixture and measurements were taken. Polyplexes formed by PDMAEMA-*b*-PManEMA and pDNA were tested by mixing with 40L of ConA solution at 1 mg mL-1.

**Nucleic acid binding assays**

***Complexation of polymers with siRNA and pDNA***

To produce a polymer stock solution (5 mg mL-1) 0.001 g of PDMAEMA104-*b*-PManEMA31 or PDMAEMA polymers were dissolved into 200μL of acetate buffer (0.01 M) at a pH of 5.5. siRNA/pDNA stock solution was diluted in nuclease free water to adjust siRNA/pDNA concentration to 100 μM. The siRNA/pDNA solution was added to the polymer solution and mixed gently, resulting in different N/P ratios (defined as the ratio between the number of amine groups from the polymer and the number phosphate groups from the nucleic acids). These mixtures were incubated at room temperature for 45 min prior to gel shift assays, dynamic light scattering (DLS) or zeta potential measurements.

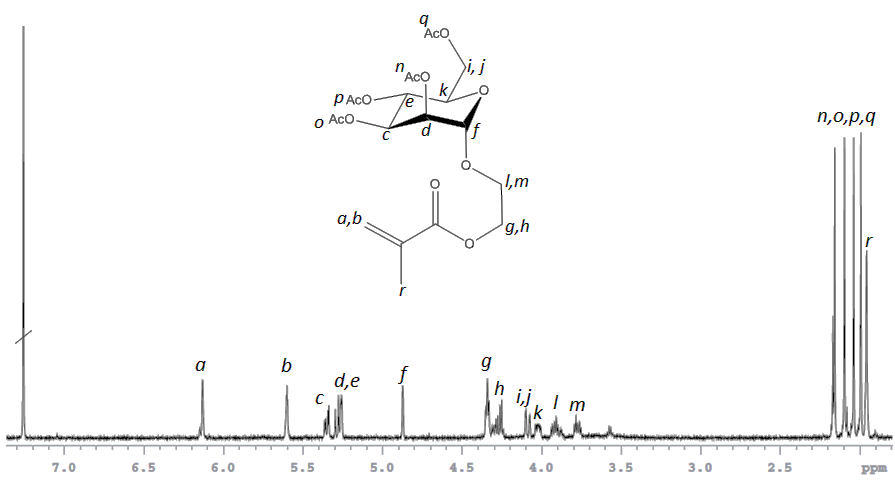
***Agarose gel retardation assay***

Prior to loading into agarose gel 9μL of polymer-pDNA complex solution was mixed with 5μL of loading dye. 8μL was taken from this mixture for the gel shift assay. Agarose powder was dissolved in hot 1 × Tris acetate-EDTA (TAE) buffer to produce 1 wt% agarose gel. The gel was subjected to electrophoresis at 90 mV for 40 min. The gels were stained with Gel Red and imaged using a UV transilluminator tube equipped with a digital camera in an enclosed dark hood.

**RESULTS AND DISCUSSION**

**Synthesis of AcManEMA**

AcManEMA was synthesised for the first time by the use of acetylated α-D-mannose in the *O*-linked glycosylation of HEMA with very similar yield when compared to previously synthesised AcMAnEMA from acetylated -D-mannose.[42](#_ENREF_42) The selection of protected glycomonomer was due the avoidance of multiple steps chemistry in synthesising an unprotected glycomonomer and the high degree of hydroylsis of ester bonds when strong base were used as a deaceylating agent.[42](#_ENREF_42), [43](#_ENREF_43) Whereas, straight forward deprotection and purification of resulting glycopolymers can be achieved using basic or acidic treatment and dialysied, respectively.[26](#_ENREF_26), [44](#_ENREF_44) AcManEMA synthesis was initially performed in anhydrous dichloromethane (DCM) which was previously carried out in the glycosylation of HEMA with protected acetylated galactose,[26](#_ENREF_26) but it did not give substantial yield and resulted in inseparable byproducts. Due to the poor solubility of reactants in DCM, anhydrous acetonitrile was eventually used as the solvent in the reaction.[42](#_ENREF_42) Purification steps during washings with saturated sodium bicarbonate and sodium chloride were essential as washings removed byproducts of water soluble salts. The separation of acyl transfer side products and unreacted mannose were separated by column chromatography. AcManEMA was obtained as a dry white solid after crystallisation overnight in the fridge. To confirm the chemical structure of AcManEMA, 1H NMR was employed. Figure 1 shows a NMR spectrum of the synthesised AcManEMA with proton signal assigned to a AcMAnEMA glycomonomer.

Figure 1. 1H NMR spectrum of the AcManEMA in deuterated chloroform (CDCl3) and its chemical structure. Proton signals in the spectrum are assigned as letters to hydrogen atoms on the chemical structure of AcManEMA.

**Homopolymerisation of AcManEMA via RAFT**

The investigation of RAFT homopolymerisation of AcManEMA under different reaction parameters was conducted. Before further reactions, this step was essential to ensure if AcMAnEMA was able to be controlled by the RAFT process. The RAFT polymerisation of AcManEMA was performed here for the first time. It is important that the selection of RAFT agent was carefully considered as the use of an amine based monomer, DMAEMA, can lead to a base degradation of RAFT agent. A trithiocarbonate RAFT agent, (4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl] pentanoic acid (CPDT)) was selected here. This was due to its resistant to base hydrolysis when compared to dithioester RAFT agents.[**45**](#_ENREF_45) Furthermore, in order to control the polymerisation of methacrylate based monomers, the RAFT agent with a tertiary carbon R-leaving group was also part of the intent.[**46**](#_ENREF_46) The pseudo-first order kinetic plot displayed in Figure 2 shows a linear correlation with respect to the duration of polymerisation. Moreover, polymerisation was successful in obtaining high conversion, greater than 80 %. The molecular weights increased linearly with conversion as indicated in Figure 2, which is a typical trend of a polymerisation governed by the reversible deactivation radical polymerisation (RDRP) mechanism.[**47**](#_ENREF_47) It should be noted that the experimental molecular weights of the protected glycopolymers were slightly higher than the theoretical molecular weights, indicated as dotted line in Figure 2B. This was due to the use of narrow polystyrene standards in the calibration of GPC. The hydrodynamic volumes (*Vh*) of glycopolymers are higher than narrow polystyrene standards in DMAc and resulted in slightly higher experimental values than the theoretical values. However, the values of *Ð* did not decrease with increasing conversion, remaining at approximately 1.2 (Figure 2). This is likely due to the relatively low chain transfer constant. The dodecanethiol Z-group of CPDT lowers the stability of the intermediate radical when compared with the more common phenyl typed Z-group leading to a low addition rate coefficient when the propagating radical is added to the C=S bond.[**32**](#_ENREF_32)**,** [**48**](#_ENREF_48) Anhydrous dioxane was chosen here because of its ability to dissolve the reactants. The use of dried dioxane was to ensure that there was no dissolved peroxide that could potential oxidise the RAFT agent and prevent the control of polymerisation.



**B**

**A**

Figure 2. Conversion versus time plot shows a typical radical polymerization curve which AcManEMA monomers were consumed at a high rate at the beginning of a reaction and slows down towards the end of reaction. Pseudo-first-order plot with *Ln[1/1-X)]* versus time shows a typical linear curve with a linear correlation coefficient value of 0.9907. (A) Molecular weights and *Ð* versus conversion for the polymerisation of AcManEMA with CPDT RAFT agent at 70°C in dioxane. (B) [AcManEMA] = 3.01 × 10-1 mol L-1, [CPDT] = 2.56 × 10-2 mol L-1 and [ACVA] = 5.14 × 10-1 mol L-1.

**Synthesis of PDMAEMA-b-PAcManEMA via RAFT polymerisation**

It was previously shown that the RAFT synthesis of diblock copolymer containing acetylated glycomonomer required the second polymerisation (chain extension) step to be the polymerisation of a glycomonomer. This was due to the highly unstable R radical generated from PAcManEMA due the attractive carbonyl groups from the acetyl units in the pre-equilibrium stage of RAFT polymerisation as reported previously.[26](#_ENREF_26), [49](#_ENREF_49) In the past, Lowe’s and Cameron’s groups had both reported the successful synthesis of cationic glycopolymers with PDMAEMA as the second block, but they did not face the issue of using acetylated glycopolymers as the first block.[15](#_ENREF_15), [27](#_ENREF_27) This greatly support that carbonyl groups from the reinitiating first polymer block can result in the unsuccessful diblock copolymers. Therefore, the cationic diblock glycopolymer was synthesised starting from the DMAEMA block. The same RAFT agent CPDT was also employed here. It was found that the bulk polymerisation of DMAEMA using CPDT was unsuccessful as it resulted in a biomodal molecular weight distribution with high molecular weight tailing. DMAEMA polymerisation was eventually conducted in solution with methanol as the solvent as suggested by Stenzel and co-workers.[50](#_ENREF_50) In contrast to the previous study on high molecular weight tailing of polymers, PDMAEMA synthesised here using ACVA as the initiator at 70°C did not result in side reactions. PDMAEMA yielded a low *Ð* of 1.11 at a percentage conversion of 87%. Figure 3 shows the molecular weight distributions of PDMAEMA macroRAFT agent and the chain extended polymer of PDMAEMA104-*b*-PAcManEMA31. The resulting cationic diblock glycopolymers retained a low *Ð* of 1.12 at a percentage conversion of 86%, evident of a RAFT controlled polymer with high uniformity. Cationic polymer block length of approximately 100 repeating units was found to be the ideal length for binding nucleic acids and therefore it was chosen as the targeted degree of polymerisation in this study.



Figure 3.Molecular weight distributions of PDMAEMA104 macroRAFT agent and its chain extension polymerisation with AcManEMA using ACVA as the initiator in 1,4-dioxane at 70°C yielding PDMAEMA104-*b*-PAcManEMA31 copolymer. [AcManEMA] = 2.17 × 10-1 mol L-1, [PDMAEMA104 macroRAFT] = 6.0 × 10-2 mol L-1 and [ACVA] = 1.4 × 10-1 mol L-1. PDMAEMA104 gave a *Ð* of 1.11 at 87% monomer conversion and PDMAEMA104-*b*-PAcManEMA31 gave a *Ð* of 1.12 at 86% monomer conversion.

**Deacetylation of PDMAEMA-b-PAcManEMA**

In order to expose the hydroxyl groups on mannose residues, the removal of acetylated groups on the protected mannose moieties were carried out using sodium methoxide (NaOMe). The possibility of decomposition of methacrylate ester along the polymer chain while using basic decetylating agent should be cautioned. To avoid the cleavage of ester groups, the reaction was stirred for short 30 min duration. A 1:1 ratio of dichloromethane and methanol was previously employed in the reaction but the solvent did not dissolve PDMAEMA104-*b*-PAcManEMA31 polymer, due to the presence of multiple highly polar amine groups from the PDMAEMA block. DMSO was chosen in this study as it is the best solvent in dissolving the cationic diblock glycopolymer. To confirm the deacetylation process, 1H NMR measurements before and after treatment with NaOMe were recorded using deuterated DMSO as a NMR solvent. The measurements confirmed that all the acetyl groups had been removed after the reaction as displays in Figure 4. The large proton signals at 1.9 to 2.1 ppm that were attributed by the acetylated groups of the protected carbohydrate block have diminished after deacetylation. An upfield shift of protons from the anomeric proton (5.5 ppm before and 4.7 ppm after reaction) and the protons within the six-membered sugar ring were present, evident that the mannose structures were still within the polymers. Molecular weight distributions of the polymers were analysed using the GPC. GPC curves showed that the PDMAEMA104-*b*-PManEMA31 polymer retained its uniformity and the *Ð* remained at a low of 1.12 (Figure 5). On the contrary, polymers after deacetylation gave a higher molecular weight distribution. Due the differences in *Vh* of hydrophilic and hydrophobic polymers in the same solvent, in this case DMAc, it seems the deprotected hydrophilic polymers turned out to have bigger *Vh* than the protected parent polymer.

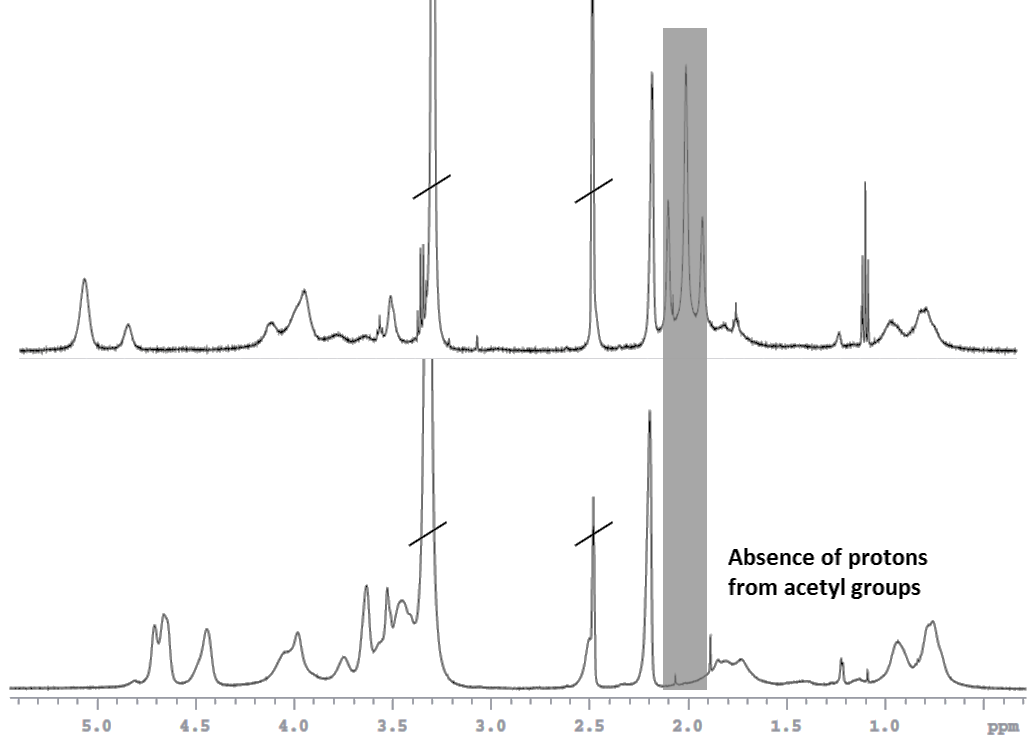


Figure 4. 1H NMR spectra of PDMAEMA104-*b*-PAcManEMA31 before deacetylation (Top) and PDMAEMA104-*b*-PManEMA31 after deacetylation (Bottom). Deuterated DMSO was used as the sampling solvent for both spectra. Signals at the region of 2.0 ppm, highlighted in grey, reflect the protons from the acetyl groups. The bottom spectrum show the disappearance of large proton signals from the acetyl groups after treatment with NaOMe.



Figure 5.Molecular weight distributions of PDMAEMA104-*b*-PAcManEMA31 (Before deacetylation) and PDMAEMA104-*b*-PManEMA31 (After deacetylation). GPC curves show that the uniformity of the polymers was retained after the treatment with NaOMe.

**Lectin binding**

The assessment of bioactivity of mannose residues on the polymers were performed by using a turbidimetric assay monitored with a UV-vis-spectrophotometer. The reduction in intensity in transmitted light owing to the scattering phenomenon was measured as absorbance at a wavelength of 420 nm. During the conjugation of polymer containing mannose ligands with specific lectins, large particles/aggregates will be formed and resulted in the loss of light intensity. The formation of aggregates is largely because of the multivalent interactions between multiple mannose pendant groups on the polymers and the four binding sites present on each lectin molecule that result in crosslinking of polymer chains on to the lectins.[3](#_ENREF_3) In order to test if the synthesised polymers bind specifically with mannose receptors, ConA with mannose and glucose receptors was chosen. On the other hand, PNA was also chosen as a lectin, which neither expresses mannose nor glucose receptors, to provide as a control experiment. Phosphate buffer solution (PBS) without Ca2+ and Mn2+ were used to prepare the polymer and lectin stock solutions. It has been reported that glucose based linear glycopolymer was unsuccessful in binding ConA when buffer solution without Ca2+ and Mn2+ was used. However, binding was achieved by increasing the glucose residues by using spherical glycopolymers.[32](#_ENREF_32), [51](#_ENREF_51) Figure 6 shows a turbidimetric assay of PDMAEMA104-*b*-PManEMA31 mixed with ConA and PNA. It was observed that the polymer only complexed specifically with ConA and resulted in an increased in absorbance at 420 nm. The successful binding between the PDMAEMA104-*b*-PManEMA31 and the ConA (without Ca2+ and Mn2+) greatly signified that mannose based linear glycopolymers has a higher affinity with ConA than glucose based linear glycopolymers. In the contrary, there was a slight reduction in absorbance at 420 nm when the polymer was mixed with PNA indicating no binding between the two and the reduction in absorbance was likely due to the increase solubility of polymers and lectin into the solution during the measurement.



Figure 6.Turbidimetric assay of absorbance at 420 nm versus conjugation time when PDMAEMA104-*b*-PManEMA31 polymer was mixed with ConA and PNA. In the case of polymer and ConA mixture, the increased in absorbance indicates the increased in turbidity in the measuring solution.

Further studies were conducted using a DLS analyser to prove if specific binding had occurred resulting in particles/aggregates. Online measurements monitored the size evolution of particles sizes with increasing complexation time (Figure 7). The particles of about 100 nm were formed when the polymers were mixed with ConA and the particles increase with time and reached a constant particle size (approx. 1.4 m) after 11 min. Saturation of ligand binding sites or the receptor binding sites might have resulted in the constant particle size evolution. On the other hand, when the polymers were mixed with PNA lectin solution no particles were detected by the analyser and further supported that the polymer could only bind specifically with ConA, which expresses mannose receptors. There is also a possibility that ConA lectin can bind to the cationic block of the polymers due the presence of positive charges acting as anion exchangers. To confirm that most of the bindings are due the interactions between mannose moieties on the polymer and ConA, a competition assay was conducted using methyl **-D-mannopyranoside as free mannose, a competing monosaccharide. Figure S1 shows a turbidimetric measurement when free mannose was added to the PDMAEMA104-*b*-PManEMA31/ConA complexes. The reduction in absorbance shows the dissociation of mannose moieties on the polymer and ConA complexes. DLS measurements were also performed to study the size of complexes after the addition of free mannose. Large PDMAEMA104-b-PManEMA31/ConA complexes of 1.18 m (number average) were recorded and after the incubation with free mannose the size was reduced to 42.1 nm (number average) indicating the dissociation of protein-carbohydrate bound complexes.



Figure 7. Dynamic light scattering (DLS) measurements of hydrodiameter (Δ*Dh*) (number average) versus conjugation time when PDMAEMA104-*b*-PManEMA31 polymers were mixed with lectins. Polymer with PNA did not indicate any binding as the particle size did not increase and remains at 0 nm.

**Nucleic acids binding**

To determine if the polymers were positively charged, for electrostatic interaction with negatively charged nucleic acids, zeta potential measurements were first performed. From table 1, it is obvious that the use of acetate buffer (0.01 M) was necessary to protonate the DMAEMA moieties on the polymers. For PDMAEMA104­ homopolymer, a zeta potential value of 26.5 mV in acetate buffer and a value of 15.4 mV in milliQ water were obtained signifying that the use of mild acidic buffer was crucial to protonate and generate a more positively charged cationic polymer. Homopolymer of PDMAEMA104 was finally compared with PDMAEMA104-*b*-PManEMA31 diblock glycopolymers. Due to the presence of hydrophilic ManEMA block on the diblock glycopolymer, it assisted in dissolving the polymer in milliQ water and led to the protonation of polymers in comparison to the homopolymer. Hence, a zeta potential value of 12.9 mV was obtained. The diblock glycopolymers however, gave a low zeta potential value when it was dissolved in acetate buffer as polymer solutions were prepared at 1 mg mL-1 of polymer of acetate buffer solution, therefore resulting in less cationic groups in the same volume of solutions. It has also been reported that excessive positive charges can lead to cytotoxicity and therefore the carbohydrate groups here can assist by lowing the toxicity and preventing colloidal aggregation in cell culture media.[17](#_ENREF_17)

TABLE 1: Zeta potential values of glycopolymers

|  |  |  |
| --- | --- | --- |
| Polymer | Zeta potential in MilliQ water (mV) | Zeta potential in Acetate buffer (0.01M), pH = 5.5 (mV) |
| PDMAEMA104 | 15.4 | 26.5 |
| PDMAEMA104-*b*-PManEMA31 | 12.9 | 17.5 |

Nucleic acid bindings were examined by recording the size of complexes, also termed as ‘polyplexes’, using the DLS. Negatively charged siRNA and pDNA were employed to study the effect of electrostatic complexation with cationic polymers. Unbound linear polymer produced a typical unimer value of 7.94 nm in solution[32](#_ENREF_32) and polyplexes from the polymer when bound with siRNA and pDNA yielded values of 102 nm and 233 nm, respectively (Table 2). The size differences were mainly due to the use of small siRNA and big pDNA molecules. In the case of siRNA which comprise of 21 base pairs gave a smaller complex and when compared to the larger pischeck2 DNA which has 6273 base pairs that resulted in larger complexes.

TABLE 2: DLS values of before and after pDNA and siRNA binding.

|  |  |  |
| --- | --- | --- |
| Sample | Number average (*Dh*/nm) | PDI*DLS* |
| 0.2 mg ml-1 PDMAEMA104-*b*-PManEMA31 | 7.94 | 0.706 |
| 0.2 mg ml-1 PDMAEMA104-*b*-PManEMA31 & siRNA  (N/P ratio = 8:1) | 102 | 0.239 |
| 0.2 mg ml-1 PDMAEMA104-*b*-PManEMA31 & pDNA  (N/P ratio = 8:1) | 233 | 0.338 |

As an additional test to show that the polymer binds to nucleic acid gel, retardation assay was employed. Figure 8 shows the polymer/pDNA polyplexes were gradually retained in the agarose gel when increasing amount of PDMAMEMA104-*b*-PManEMA31 polymer was mixed with the pDNA. The concentrations were calculated based on the number of amino groups on the polymers over the phosphate groups on the pDNA, denoted as N/P ratio in Figure 8. For all the wells, pDNA concentration was kept constant and the concentration of polymer was varied by increasing N/P ratios. In order to justify that the formed polyplexes were still able to bind ConA, DLS analysis were carried out to measure the size of pDNA/ PDMAMEMA104-*b*-PManEMA31 complexes before and after the addition of ConA. To ensure that there are no free polymer chains after the formation of polyplexes, the latter were generated with N/P ratio less than 1. DLS gave a polyplex size of 241 nm (*Z*-average) and after the addition of ConA, the size gradually increased over 20 min to 885 nm (*Z*-average) (Figure 9). The linear increment in the size shows that the polyplexes were able to bind ConA.

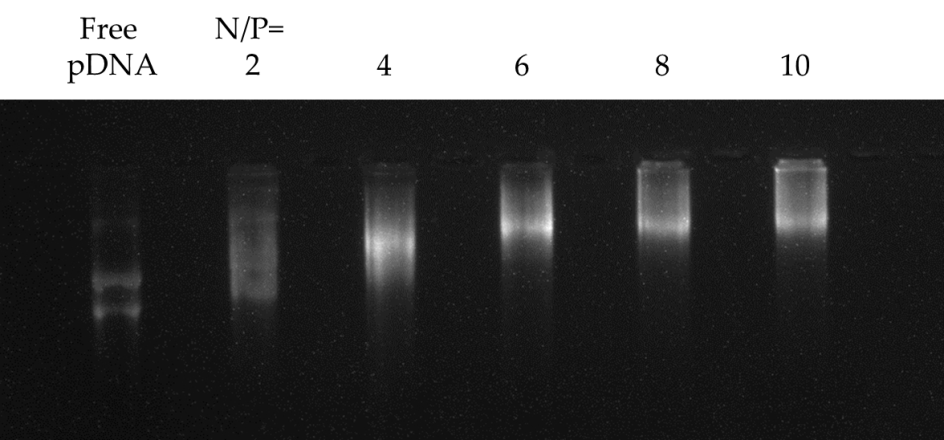


Figure 8. Gel retardation assay of PDMAEMA104-*b*-PManEMA31 bound pDNA with different N/P ratios. The loading wells are at the top of the image with the negatively charged end and the bottom with the positively charged end. The well with only free pDNA did not result in retardation when compared to polymer/pDNA complexes. At high N/P ratios, more pDNA was retained in the wells.



Figure 9. Dynamic light scattering (DLS) measurements of hydrodiameter (*Dh*) (*Z*-average) versus conjugation time when (pDNA + PDMAEMA104-*b*-PManEMA31) polyplexes were mixed with ConA. The linear increment indicates the binding between polyplexes and ConA.

**CONCLUSIONS**

Bioactive PDMAEMA104-*b*-PAcManEMA31 diblock glycopolymers with high uniformity had been successfully prepared using a trithiocarbonate RAFT agent. The use of acetylated glycomonomer, AcManEMA, allowed the polymerisation to proceed in a convenient reaction pathway. The choice of solvent and the purification steps in the synthesis of AcManEMA was crucial for a successful yield and protected cationic glycopolymer was deacetylated to produce a water soluble PDMAEMA104-*b*-PManEMA31 polymer. The biofunctionality of α-mannose moieties on the polymers were screened using a lectin, ConA, expressing mannose receptors. Results showed that the polymers were only specific in binding ConA lectins and did not showed binding with PNA lectins. Competitive assays performed by using free mannose further support the specific binding of polymannose on the polymers with ConA. Cationic property of the polymer was examined by binding with siRNA and pDNA. DLS measurements clearly showed that electrostatic binding between the cationic DMAEMA block and nucleic acids gave polyplexes of two different sizes. Moreover, gel retardation assay justified that the polymers were able to form polyplexes with pDNA and at higher N/P ratios polyplexes with tight bindings were achieved. The mannose moieties here will potentially act as ligands and can be advantageous for targeting cell surface mannose receptors. Polymers with different carbohydrate ligands and their specific bindings with lectins and cell receptors would warrant further examinations.

**SUPPORTING INFORMATION**

Figure S1: Competing turbidimetric assay is available online.

**ACKNOWLEDGEMENTS**

S. R. S. Ting acknowledged funding support from the National Health and Medical Research Council (NHMRC) fellowship (APP1054011), the UTS Chancellor’s Research Fellowship and the Ian Potter Foundation Grant. The authors thank the School of Mathematical and Physical Sciences and the School of Life Sciences at the Faculty of Science at UTS for the management of level 5 and 7 laboratories, and the support in maintaining the gel permeation chromatography. We thank Dr S. M. Bajan for the assistance with the initial setup of an agarose gel assay.

**REFERENCES**

1. Kiessling, L. L. and Grim, J. C. *Chemical Society Reviews* **2013**, *42*,4476-4491.

2. Ige, O. O., Umoru, L. E., and Aribo, S. *International Scholarly Research Notices: Materials Science* **2012**, *2012*,1-20.

3. Ting, S. R. S., Chen, G., and Stenzel, M. H. *Polymer Chemistry* **2010**, *1*,1392-1412.

4. Ghadban, A. and Albertin, L. *Polymers* **2013**, *5*,431.

5. Lundquist, J. J. and Toone, E. J. *Chemical Reviews* **2002**, *102*,555-578.

6. Miura, Y., Hoshino, Y., and Seto, H. *Chemical Reviews* **2016**, *116*,1673-1692.

7. Ingle, N. P., Malone, B., and Reineke, T. M. *Trends in Biotechnology* **2011**, *29*,443-453.

8. Taranejoo, S., Liu, J., Verma, P., and Hourigan, K. *Journal of Applied Polymer Science* **2015**, *132*,42096.

9. Ahmed, M. and Narain, R., *Synthetic cationic glycopolymers for gene delivery*, in *Polymers and Nanomaterials for Gene Therapy*. 2016. p. 81-98.

10. Ahmed, M. and Narain, R. *Biomaterials* **2011**, *32*,5279-5290.

11. Ahmed, M. and Narain, R. *Biomaterials* **2012**, *33*,3990-4001.

12. Dhande, Y. K., Wagh, B. S., Hall, B. C., Sprouse, D., Hackett, P. B., and Reineke, T. M. *Biomacromolecules* **2016**, *17*,830-840.

13. Sun, J., Sheng, R., Luo, T., Wang, Z., Li, H., and Cao, A. *Journal of Materials Chemistry B* **2016**, *4*,4696-4706.

14. Ahmed, M. and Narain, R. *Molecular Pharmaceutics* **2012**, *9*,3160-3170.

15. Lowe, A. B. and Wang, R. *Polymer* **2007**, *48*,2221-2230.

16. Tolstyka, Z. P., Phillips, H., Cortez, M., Wu, Y., Ingle, N., Bell, J. B., Hackett, P. B., and Reineke, T. M. *ACS Biomaterial Science and Engineering* **2016**, *2*,43-55.

17. Li, H., Cortez, M. A., Phillips, H. R., Wu, Y., and Reineke, T. M. *ACS Macro Letters* **2013**, *2*,230-235.

18. Obata, M., Kobori, T., Hirohara, S., and Tanihara, M. *Polymer* **2012**, *53*,4672-4677.

19. Obata, M., Kobori, T., Hirohara, S., and Tanihara, M. *Polymer Chemistry* **2015**, *6*,1793-1804.

20. Song, E.-H., Manganiello, M. J., Chow, Y.-H., Ghosn, B., Convertine, A. J., Stayton, P. S., Schnapp, L. M., and Ratner, D. M. *Biomaterials* **2012**, *33*,6889-6897.

21. Park, K.-H., Sung, W. J., Kim, S., Kim, D. H., Akaike, T., and Chung, H.-M. *Journal of Bioscience and Bioengineering* **2005**, *99*,285-289.

22. Ting, S. R. S. and Stenzel, M. H., *CHAPTER 2 Direct Synthesis of Glycopolymers Using Glycomonomers*, in *Glycopolymer Code: Synthesis of Glycopolymers and their Applications*. 2015, The Royal Society of Chemistry. p. 17-76.

23. Slavin, S., Burns, J., Haddleton, D. M., and Becer, C. R. *European Polymer Journal* **2011**, *47*,435-446.

24. Li, Y. and Yu, B. *Chemical Communications* **2010**, *46*,6060-6062.

25. Ting, S. R. S., Gregory, A. M., and Stenzel, M. H. *Biomacromolecules* **2009**, *10*,342-352.

26. Ting, S. R. S., Min, E. H., Escalé, P., Save, M., Billon, L., and Stenzel, M. H. *Macromolecules* **2009**, *42*,9422-9434.

27. Cameron, N. R., Spain, S. G., Kingham, J. A., Weck, S., Albertin, L., Barker, C. A., Battaglia, G., Smart, T., and Blanazs, A. *Faraday Discussions* **2008**, *139*,359-368.

28. Spain, S. G., Albertin, L., and Cameron, N. R. *Chemical Communications* **2006**,4198-4200.

29. Escalé, P., Ting, S. R. S., Khoukh, A., Rubatat, L., Save, M., Stenzel, M. H., and Billon, L. *Macromolecules* **2011**, *44*,5911-5919.

30. Ting, S. R. S., Granville, A. M., Quemener, D., Davis, T. P., Stenzel, M. H., and Barner-Kowollik, C. *Australian Journal of Chemistry* **2007**, *60*,405-409.

31. Min, E. H., Ting, S. R. S., Billon, L., and Stenzel, M. H. *Journal of Polymer Science Part A: Polymer Chemistry* **2010**, *48*,3440-3455.

32. Ting, S. R. S., Min, E. H., Zetterlund, P. B., and Stenzel, M. H. *Macromolecules* **2010**, *43*,5211-5221.

33. Munisso, M. C., Obika, S., and Yamaoka, T. *Carbohydrate Polymers* **2014**, *114*,288-296.

34. Arslan, H., Pfaff, A., Lu, Y., Stepanek, P., and Müller, A. H. E. *Macromolecular Bioscience* **2014**, *14*,81-91.

35. Valtola, L., Karesoja, M., Tenhu, H., Ihalainen, P., Sarfraz, J., Peltonen, J., Malinen, M., Urtti, A., and Hietala, S. *Journal of Applied Polymer Science* **2015**, *132*,41225.

36. Utama, R. H., Jiang, Y., Zetterlund, P. B., and Stenzel, M. H. *Biomacromolecules* **2015**, *16*,2144-2156.

37. Samoilova, N. A., Krayukhina, M. A., Babushkina, T. A., Yamskov, I. A., Likhosherstov, L. M., and Piskarev, V. E. *Journal of Applied Polymer Science* **2017**,44718.

38. Jones, M. W., Otten, L., Richards, S. J., Lowery, R., Phillips, D. J., Haddleton, D. M., and Gibson, M. I. *Chemical Science* **2014**, *5*,1611-1616.

39. Shi, J., Cai, L., Pu, K.-Y., and Liu, B. *Chemistry – An Asian Journal* **2010**, *5*,301-308.

40. Agarwal, S., Zhang, Y., Maji, S., and Greiner, A. *Materials Today* **2012**, *15*,388-393.

41. Chenette, H. C. S. and Husson, S. M. *Journal of Applied Polymer Science* **2015**, *132*,41437.

42. Obata, M., Shimizu, M., Ohta, T., Matsushige, A., Iwai, K., Hirohara, S., and Tanihara, M. *Polymer Chemistry* **2011**, *2*,651-658.

43. Suzuki, K., Koyama, T., Yingsakmongkon, S., Suzuki, Y., Hatano, K., and Matsuoka, K. *Bioorganic & Medicinal Chemistry* **2012**, *20*,446-454.

44. Zhao, J., Babiuch, K., Lu, H., Dag, A., Gottschaldt, M., and Stenzel, M. H. *Chemical Communications* **2014**, *50*,15928-15931.

45. Thomas, D. B., Convertine, A. J., Hester, R. D., Lowe, A. B., and McCormick, C. L. *Macromolecules* **2004**, *37*,1735-1741.

46. Perrier, S. and Takolpuckdee, P. *Journal of Polymer Science Part A: Polymer Chemistry* **2005**, *43*,5347-5393.

47. Shipp, D. A. *Polymer Reviews* **2011**, *51*,99-103.

48. Perrier, S., Takolpuckdee, P., Westwood, J., and Lewis, D. M. *Macromolecules* **2004**, *37*,2709-2717.

49. Moad, G., Rizzardo, E., and Thang, S. H. *Australian Journal of Chemistry* **2012**, *65*,985-1076.

50. Zhang, L., Nguyen, T. L. U., Bernard, J., Davis, T. P., Barner-Kowollik, C., and Stenzel, M. H. *Biomacromolecules* **2007**, *8*,2890-2901.

51. Yang, Q., Hu, M.-X., Dai, Z.-W., Tian, J., and Xu, Z.-K. *Langmuir* **2006**, *22*,9345-9349.

**GRAPHICAL ABSTRACT**

