Synthesis of Amphiphilic A_3B Mikto-Arm Copolymers from a Sugar Core: Combination of Hydrophobic PCL and Hydrophilic Glycopolymers for Biocompatible Nanovector Preparation

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ABSTRACT: Amphiphilic A_3B mikto-arm copolymers have been synthesized using a *t*-butyl-diphenyl silyl-based methylglucoside derivative. The latter has been first used as initiator for the polymerization of ε-caprolactone leading to three-arm star-shaped structures followed by several postpolymerization steps to obtain star-shaped poly(ε-caprolactone) macroinitiator. Atom transfer radical polymerization (ATRP) of diisopropylidene galactose methacrylate in THF at 60 °C using CuBr ligated with 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA) as catalytic complex allowed the formation of A_3B mikto-arm copolymers with different compositions and molecular weights. Selective deprotection of sugar protecting groups finally generated amphiphilic mikto-arm copolymers. The molecular characterization of those copolymers was performed by ^{1}H NMR spectroscopy and gel permeation chromatography (GPC) analysis. The self-assembly of the copolymers into micellar aggregates and the related critical micellization concentration (*CMC*) in aqueous media were determined by dynamic light scattering (DLS) and UV-visible spectroscopy, respectively. © 2010 Wiley Periodicals, Inc. J Polym Sci Part A: Polym Chem 48: 3271–3280, 2010

KEYWORDS: amphiphiles; glycopolymer; micelles; mikto-arm copolymer; ring-opening polymerization

INTRODUCTION The use of polymeric micelles as nanometric drug carrier systems has attracted much attention over the past decades. Indeed, polymeric micelles offer the advantage to trap drugs, such as anticancer agents, e.g., paclitaxel[1] and doxorubicin,[2,3] in their hydrophobic core. Such drug-loaded polymeric micelles have been exploited as therapeutic systems for anticancer treatment thanks to their nanometric size allowing them to escape from reticuloendothelial system (RES).[4,5] Indeed, one of the most important features in designing polymers for drug delivery applications relies upon the size of the resulting micelles. The latter should be in the range of 5 and 200 nm to allow long circulation time in the bloodstream, selective tumor targeting due to enhanced permeability and retention effect (EPR) in solid tumors, and avoid RES uptake.[6,7]

Practically, polymeric micelles are mostly obtained from amphiphilic random, block, or grafted copolymers.[8] Moreover, controlling polymer molecular parameters and architecture is of prime importance since a narrow dependence exists between material properties and related structure.[9] To that end, efforts have been invested to reach polymerization mechanisms allowing the control over the polymerization of a wide variety of monomers. In this field, controlled radical polymerization techniques (CRP),[10] such as atom transfer radical polymerization (ATRP),[11] nitroxide-mediated free radical polymerization (NMP),[12,13] and reversible addition-fragmentation chain transfer (RAFT)[14–16] have been widely studied and developed leading to countless possibilities taking into account variations in the monomer nature, chain-end functionality and polymer architecture along with the abilities to associate radical processes to other polymerization techniques such as ring-opening polymerization (ROP) of various cyclic monomers. This last option is of particular interest since a large range of functional materials can be obtained. As an example, Matyjaszewski et al. prepared cylindrical core-shell brushes from poly((2-hydroxyethyl methacrylate)-*graft*-poly((ε-caprolactone)-*block*-*n*-butyl acrylate)) (PHEMA-*g*-(PCL-*b*-PBA)) using a combination of ROP and ATRP processes.[17] Later, Wiltshire and Qiao prepared core-crosslinked star polymers by crosslinking hydroxyl 2-bromoisobutyrate telechelic PCL with ethylene glycol dimethacrylate followed by polymerization of methyl methacrylate by ATRP.[18] Crosslinking reaction was also extended to ATRP of *N,N*-dimethyl amino-2-ethylmethacrylate (DMAEMA) and αω-dimethacrylate PCL leading to the formation of hydrogels as reported by Mespouille et al.[19] Other ATRP/ROP combinations have allowed the formation of different structures as versatile as dendrimers,[20,21] brush-[22], stars[23] or comb-like polymers[24]

Additional Supporting Information may be found in the online version of this article. Correspondence to: O. Coulembier (E-mail: olivier.coulembier@umons.ac.be)

sometimes dealing with ring-opening metathesis polymerization (ROMP) as an additional technique. Besides, star-branched polymers having arms of different structures, also referred to as mikto-arm polymers recently attracted increasing interest thanks to their ability to highly change the morphology of their supramolecular structures,[25–28] resulting in a wide number of applications such as in nanotechnology, biological engineering, and drug/gene delivery.[29,30]

Herein we report the synthesis of three-arm star-shaped poly(ε-caprolactone) obtained by polymerization of (ε-caprolactone) (CL) from the three equatorial hydroxyl groups of an initially protected *tert*-butyldiphenylsilyl ether-based α-methylglucoside derivative. Post-polymerization derivatization of chain-end alcohols with acetic anhydride followed by selective hydrolysis of *tert*-butyldiphenylsilyl ether led to the formation of an acetylated star-shaped PCL bearing a single primary alcohol. Synthesis of such star-shaped PCL allowed the preparation of new star-shaped macroinitiator converting the primary alcohol into a 2-bromoisobutyrate moiety. This so-formed macroinitiator was used to initiate the ATRP polymerization of 1,2;3,4-di-O-isopropylidene-6-O-methacryloyl-D-galactopyranose (MAIGP) to produce perfectly controlled amphiphilic mikto-arm copolymers able to self-assemble in aqueous medium (Scheme 1).

EXPERIMENTAL

Materials

ε-Caprolactone (CL, from Acros, 99%) was dried over calcium hydride for 48 h at room temperature and distilled under reduced pressure just before use. Stannous octoate ($Sn(Oct)_2$) has been distilled under vacuum, diluted in dry toluene and stored in glass ampoules. *N,N*-dimethylamino-4-pyridine (DMAP, from Acros, 99%), 2-bromoisobutyryl bromide (from Aldrich, 98%), 1,1,4,7,10,10 hexamethylenetetramine (HMTETA, from Aldrich, 97%), tetrabutylammonium fluoride (TBAF, from Aldrich, 97%), *t*-butyl-diphenyl silyl chloride (TBDPSCl, from Fluka, 97%), methyl α-D-glucopyranoside (from Aldrich, 99%), pyridine (from Aldrich, 99.8%), formic acid (from Aldrich, 98%), acetic acid (from Aldrich, 99.7%), acetic anhydride (from Aldrich, 99%), and pyrene (from Aldrich, 98%) were used as received. Magnesium sulfate (from Acros, 99%) was dried at 100 °C for 48 h prior to use. Copper bromide (CuBr, from Fluka, 98%) was purified in acetic acid for 24 h and recrystallized in ethanol under inert atmosphere until a white powder was obtained. THF from Biosolve and chloroform from Chemlab were dried on a solvent purification system MB SPS-800 from MBraun. 1,2;3,4-Di-O-isopropylidene-6-O-methacryloyl-α-D-galactopyranose (MAIGP) has been synthesized prior to use applying previously reported method.[31]

*Synthesis of 6-O-*Tert*-Butyl Diphenylsilyl α-Methyl Glucoside*

In a flamed purged round-bottom flask were introduced 2.12 g of α-D-methyl glucoside (10.93 mmol), 0.2 g of DMAP (1.64 mmol), and 50 mL of pyridine. The mixture was heated to 50 °C and 2.6 mL of *tert*-butyl diphenylsilyl chloride (10 mmol) were added. The reaction evolution was monitored by TLC during 16 h using a heptane/ethyl acetate (4:1 v:v) mixture as eluent and a sulfuric acid/ethanol (1:9 v:v) mixture to reveal sugar derivatives. At the end of the reaction, 50 mL of a diethyl ether and ethyl acetate mixture (1:1 v:v) were added and the organic layer was washed twice with a 100 mL 1M HCl solution, once with 100 mL of a 0.1 M sodium carbonate aqueous solution and twice with 100 mL of deionized water. The product was recrystallized in diethyl ether, filtered, and dried till constant weight at 40 °C. Yield = 62%. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 0.97 (s, 9 H, Hm), 3.5–3.65 (m, 9 H, He + Hc + Hb + Hg + Hf + Hd), 4.6–4.9 (m, 4 H, Ha + Hj + Hi+ Hh), 7.35–7.85 (m, 10 H, Hk + Hl). ^{13}C-NMR (300 MHz, DMSO-d_6, δ ppm): δ 20, 28, 56, 72, 73, 74, 75, 101, 128–137.

*Synthesis of α-Hydroxyl ω-6-O-*t*-Butyl Diphenyl Silyl-α′-methyl Glucoside Three-Arm Star Poly(ε-caprolactone)*

In a previously flamed and purged round-bottom flask equipped with a three way stopcock and a rubber septum, were introduced 1.3 g of 6-O-*tert*-butyl diphenylsilyl α-methyl glucoside (3 mmol) previously dried by three successive azeotropic distillations by addition of THF. To the initiator were successively added 25.6 mL of THF and 8 mL of ε-CL (72.2 mmol). The mixture was allowed to warm up to 80 °C before adding a 0.31 M $Sn(Oct)_2$ solution (1.16 mL, 0.36 mmol) by using a previously flamed and nitrogen purged syringe. The polymerization reaction proceeded for 16 h and was stopped by the addition of 1M HCl aqueous solution (0.8 mL). The polymer was selectively precipitated in cold methanol, filtered, and dried till constant weight at 40°C. Yield = 77%. ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm: 1.05 (s, 9 H, Hn), 1.40 (m, 6 H, Hj), 1.65 (m, 12 H, Hi), 2.31 (t, 6 H, Hh), 3.35–3.45 (m, 6 H, Hg + Hf + Hb), 3.65 (t, 6 H, Hk′), 4.06 (t, 6 H, Hk), 4.7–5.5 (m, 4 H, Ha + Hc + Hd + He), 7.4–7.8 (m, 10 H, Hl + Hm)). M_n NMR = 2700 g mol^{-1}. M_w/M_n = 1.13.

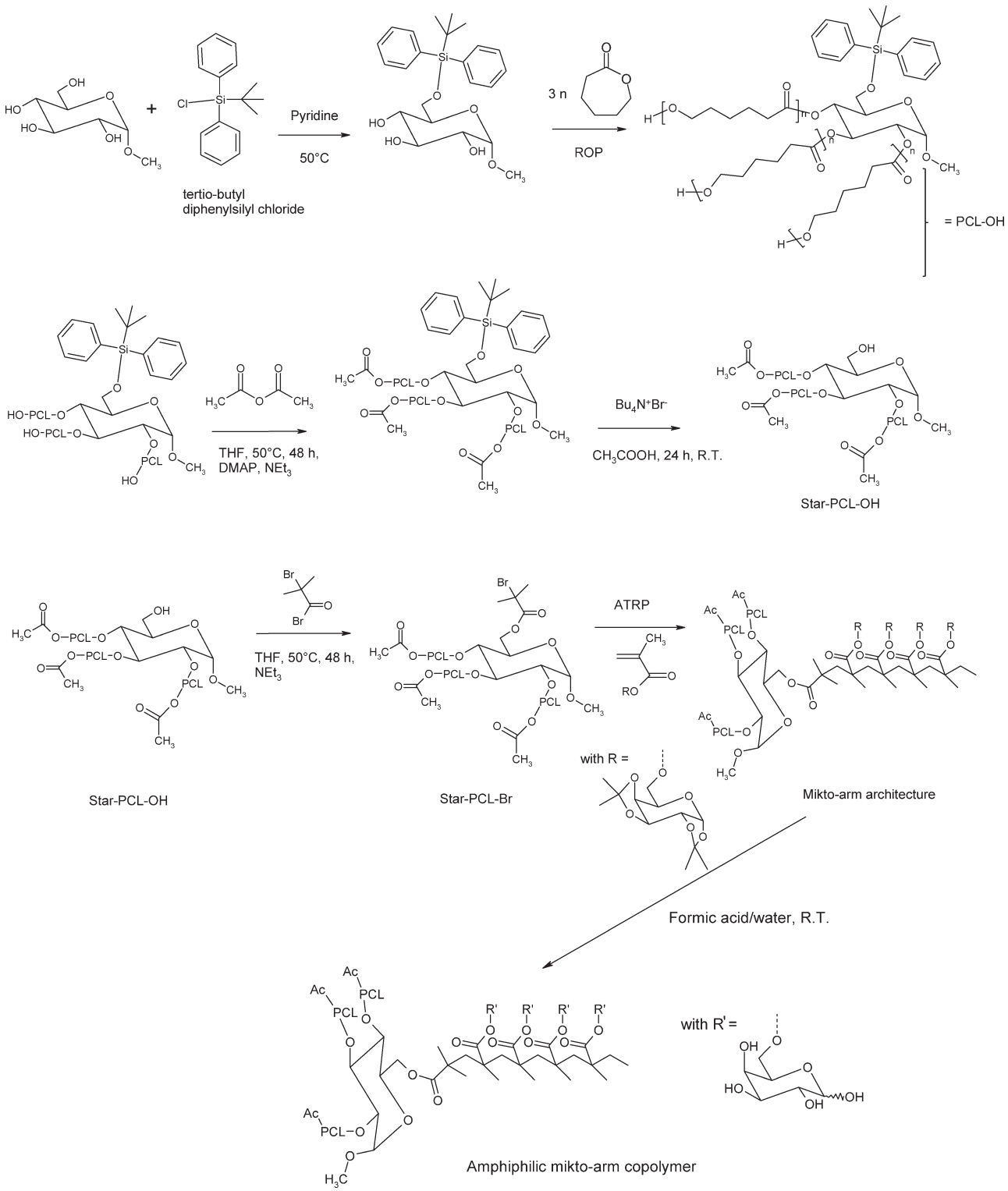
*Synthesis of α-Acetyl ω-6-O-*t*-Butyl Diphneyl Silyl-α′-methyl Glucoside Three-Arm Star Poly(ε-caprolactone)*

In a flamed and purged round-bottomed flask were introduced 6 g of three-arm star-PCL (M_n = 2700 g mol^{-1}, 2.22 mmol, 6.67 mmol OH), 30 mL of triethylamine (220 mmol) and 75 mL of THF. The mixture was heated to 50 °C when 19 mL of acetic anhydride (200 mmol) were added. After 48 h of reaction, the solvent was removed and the as-obtained crude solid was dissolved in 10 mL of THF. The polymer was selectively precipitated in cold methanol, filtered and dried till constant weight at 40 °C. Yield = 98%. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 1.05 (s, 9 H, Hn), 1.40 (m, 6 H, Hj), 1.65 (m, 12 H, Hi), 2.05 (s, 9 H, Hl), 2.31 (t, 6 H, Hh), 3.35–3.45 (m, 6 H, Hg + Hf + Hb), 4.06 (t, 6 H, Hk), 4.7–5.5 (m, 4 H, Ha + Hc + Hd + He), 7.4–7.8 (m, 10 H, Hm + Hn). M_n NMR = 2700 g mol^{-1}, M_n SEC = 2400 g mol^{-1}, M_w/M_n = 1.1.

Selective Deprotection of *tert*-Butyl Diphenylsilyl Ether

Synthesis of α-Acetyl ω-α′-Methyl Glucoside Three-Arm Star Poly(ε-caprolactone) (Star-PCL-OH)

In a PE container were introduced 4 g of α-acetyl ω-6-O-*t*-butyl diphneyl silyl-α′-methyl glucoside three-arm star



SCHEME 1 General synthetic route to access mikto-arm architecture where R represents 1,2;3,4-Di-O-isopropylidene-D-galactopyranose.

poly(ε-caprolactone) (M_n = 2700 g mol^{-1}, 1.41 mmol) and 85 mL of a 1 M TBAF solution (THF/acetic acid 85:15 v:v). After 24 h, the medium was concentrated before being dissolved in 10 mL of THF. The polymer was selectively precipitated in cold methanol, filtered, and dried till constant weight at 40 °C. Yield = 99%. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 1.40 (m, 6 H, Hj), 1.65 (m, 12 H, Hi), 2.05 (s, 9 H, Hl), 2.31 (t, 6 H, Hh), 3.45 (s, 3 H, Hb), 3.65–3.75 (m, 3 H, Hf

+ Hg), 4.1 (t, 6 H, Hk), 4.7–5.5 (m, 4 H, Ha + Hc + Hd + He). M_n NMR = 2800 g mol^{-1}, M_n SEC = 2400 g mol^{-1}, M_w/M_n = 1.1.

Synthesis of α-Acetyl ω-6-O-Bromoisobutyrate-α′-methyl Glucoside Three-Arm Star Poly(ε-caprolactone) (Star-PCL-Br)

In a flamed and purged round-bottom flask were introduced 2.5 g of star-PCL-OH (M_n = 2800 g mol^{-1}, 0.85 mmol), 20 mg of DMAP (0.16 mmol), 0.2 mL of triethylamine (1.45 mmol), and 15 mL of THF. The mixture was allowed to warm up to 50 °C before adding 0.15 mL of 2-bromoisobutyryl bromide (1.21 mmol). After 48 h, the medium was concentrated, solubilized by 5 mL of THF, and the crude polymer was selectively precipitated in cold methanol, filtered and dried till constant weight at 40 °C. Yield = 99%. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 1.40 (m, 6 H, Hj), 1.65 (m, 12 H, Hi), 1.95 (s, 6 H, Hm), 2.05 (s, 9 H, Hl), 2.31 (t, 6 H, Hh), 3.45 (s, 3 H, Hb), 3.75 (m, 1 H, Hf), 4.1 (t, 8 H, Hk + Hg), 4.7–5.5 (m, 4 H, Ha + Hc + Hd + He). M_n NMR = 2700 g mol^{-1}, M_n SEC = 2400 g mol^{-1}, M_w/M_n = 1.1.

Synthesis of A_3B Mikto-arm Copolymers

In a round-bottom flask A were introduced 5.7 mg of copper bromide (CuBr, 0.04 mmol) and 18.4 mg of 1,1,4,7,10,10-hexamethyl triethylene tetramine (HMTETA, 0.08 mmol). Three freezing/thawing cycles were performed to get rid of the trapped O_2. In a round-bottom flask B were introduced 0.11 g of star-PCL-Br (M_n = 2700 g mol^{-1}, 0.04 mmol), 0.66 g of 1,2;3,4-di-O-isopropylidene-6-O-methacryloyl-D-galactopyranose (MAIGP, 2 mmol) and 2 mL of THF. Nitrogen was bubbled through the solution before transferring it into flask A. The polymerization has been led at 60 °C for 4 h then stopped in liquid nitrogen and the polymer was selectively recovered after precipitation in heptane, filtered, and dried until constant weight at 40 °C. Yield = 99%. The copper catalyst was removed by passing the polymer solution in THF through a basic alumina column. The solvent was then evaporated to yield the pure polymer. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 0.88–1.03 (m, 3 H, Hi), 1.23–1.8 (m, 32 H, Hv + Hj + Hn + Hi), 1.64 (m, 4 H, Hd), 1.95 (s, 6 H, Hm), 2.05 (s, 9 H, Hl), 2.31 (t, 6 H, Hh), 3.43 (s, 3 H, Hb), 3.9–4.1 (m, 10 H, Hf + Hg + Ht + Hk), 4.37 (m, 4 H, Hq + Hs + Hu), 4.62 (s, 1 H, Hr), 4.8–5.55 (m, 5 H, Ha + Hc + Hd + He + Hp). M_n NMR = 20,000 g mol^{-1}, M_w/M_n = 1.28.

General Procedure for the Selective Removal of Isopropylidene Protective Groups from Sugar Units

Copolymer (1g) was dissolved in 50 mL of HCOOH/H_2O (4:1 v/v) and the solution was stirred at room temperature for 48 h. Formic acid was then removed by dialysis against water using a preswollen membrane (Spectra/Por, molecular weight cutoff size, 3500D) for 48 h replacing water every 4 h (when possible). Deprotected copolymer was finally recovered by freeze-drying. Yield = 75–90%.

Preparation of Aqueous Micellar Solutions for Dynamic Light Scattering Measurements

Micellar aggregates of the amphiphilic copolymers in water were prepared by the dissolution of the copolymers (1 mg) in dimethylsulfoxide (1 mL), followed by progressive addition (1 drop/10 s) of 10 mL of deionized water under vigorous stirring. Organic solvent was removed by transferring the solution in a preswollen membrane (Spectra/Por, molecular weight cutoff size, 3500 D), dialysis against water for 24 h. Solutions were then filtered through 1.2 μm Acrodisk® filter.

Solutions for the determination of the critical micellization concentration (CMC) of the polymers in deionized water were prepared by the dissolution of the copolymers (1 mg) in dimethylsulfoxide (1 mL), followed by progressive addition (1 drop/10 s) of 10 mL of deionized water under vigorous stirring. Organic solvent was removed by transferring the solution in a preswollen membrane (Spectra/Por, molecular weight cutoff size, 3500 D), followed by a dialysis against water for 24 h. The stock solution was then diluted to produce several solutions with various polymer concentrations ranging from 10^{-5} g L^{-1} to 1 g L^{-1}. To each solution (10 mL) was added 0.1 mL of a pyrene solution in acetone ($6 \cdot 10^{-5}$ mol L$^{-1)}$) and the acetone was finally slowly evaporated in mild conditions for 24 h. The final pyrene concentration was $6 \cdot 10^{-7}$ mol L^{-1} in all solutions.

Characterizations

^{1}H NMR spectra were recorded using a Bruker AMX-300 apparatus at r.t. in $CDCl_3$ (30 mg/0.6 mL) except for glucose-based initiator and deprotected galactose-based copolymers that were recorded in DMSO-d_6 (30 mg/0.6 mL). ^{13}C NMR spectra were recorded at higher concentrations (60 mg/0.6 ml). Size exclusion chromatography (SEC) was performed in THF at 35 °C using a Polymer Laboratories liquid chromatogram apparatus equipped with a PL-DG802 degasser, an isocratic HPLC pump LC 1120 (flow rate = 1 mL/min), a Marathon autosampler (loop volume = 200 μl, solution conc. = 1 mg/mL), a PL-DRI refractive index detector and three columns: a PL gel 10 μm guard column and two PL gel Mixed-B 10 μm columns (linear columns for separation of MW_{PS} ranging from 500 to 10^6 daltons). Polystyrene or poly (methyl methacrylate) standards were used for calibration. UV-visible absorption spectra have been recorded Cary-Win UV 50 spectrophotometer (from Varian) from 300 to 400 nm. Dynamic light scattering measurements were carried out using a BI-160 apparatus (Brookhaven Instruments) or a ZEN 3600 from Malvern Intruments with a He-Ne laser delivering a vertically polarized light (λ = 633 nm). The particle sizes and size distributions were calculated using CONTIN algorithms or NNLS algorithms. For all particle size measurements 5 runs were recorded and the data were reported as the average mean diameter for 5 runs.

RESULTS AND DISCUSSION

The synthesis of brush-like architectures based on saccharides was investigated via a "grafting through" approach. In the proposed strategy depicted in Scheme 1, the ring-opening polymerization (ROP) process takes place prior to the atom transfer radical polymerization (ATRP). The first step has then consisted on the synthesis of a saccharidic residue able to initiate the ROP reaction from some of dangling

TABLE 1 Molecular Characterizations of Star-Shaped Poly(ε-caprolactone)s

Entry	Time (h)	Yield (%)[a]	$M_{n\ th}$ (g mol^{-1})[b]	$M_{n\ exp\ NMR}$ (g mol^{-1})[c]	$M_{n\ exp\ NMR\ per\ chain}$ (g mol^{-1})[c]	$M_{n\ SEC}$ (g mol^{-1})[d]	M_w/M_n[d]
1	16	77	2,500	2,700	750	2,200	1.13
2	40	95	5,600	6,400	2,000	6,300	1.29

[a] As determined by gravimetry.
[b] Theoretical molecular weight: $M_{n\ th} = M_{w\ ini} + 114*DPth*conv.$ where $M_{w\ ini}$ is the initiator molecular weight.
[c] Experimental molecular weight as determined by ^{1}H-NMR (500 MHz) in $CDCl_3$.
[d] As determined by size exclusion chromatography (SEC) in THF at 35 °C using PS standards taking into account the Mark-Houwink-Sakurada parameters.

hydroxyl groups while providing another alcohol able to undergo adequate derivatization for further ATRP. To that end, α-methyl glucoside is well-suited since the pyranose sugar is composed of a primary alcohol and three secondary equatorial alcohols of same conformation and probability to initiate the lactone ROP. Finally, this sugar is blocked in its pyranosidic form by the presence of the methyl ether on the anomeric alcohol, preventing any mutarotation and degradation at common operating temperature during the polymerization reactions.

To promote the ROP from the secondary equatorial hydroxy groups, a first derivatization reaction of the primary alcohol has been realized. Purposely, *tert*-butyldiphenyl silyl (TBDPS) ether is perfectly adapted since it is known to selectively protect primary alcohols of pyranosidic sugars due to the bulky substituents on the silicon atom and to increase the relative solubility of the as-obtained compounds in organic solvents, thus being particularly useful in the case of polyhydroxylated sugars.[32,33]

The protection of the α-methyl glucoside molecule has been realized in pyridine with excess of *tert*-butyldiphenyl silyl chloride at 50 °C. After completion of the reaction (determined by thin layer chromatography, TLC), the medium is concentrated and purified by both multiple extractions in ethyl acetate and a recrystallization in diethyl ether leading to an overall yield of 62%. The ^{1}H NMR spectrum of the as-obtained 6-O-*tert*-butyldiphenyl silyl α-methyl glucoside highlights the presence of a new signal centered at about 1 ppm assigned to the methyl protons of the *tert*-butyl group (Hm) while peaks appearing between 7 and 8 ppm (Hk, Hl) are attributed to the phenyl protons giving credit to the expected ether formation (See Supporting Information Fig. S1). It is worth pointing out that integrations ratios between each functional group fitted the expected composition taking into account the inherent error of the NMR technique. The selectivity of this reaction was also attested by ^{13}C NMR spectroscopy highlighting a clear downfield shift from 61 (Hg′) to 64 ppm (Hg) upon silyl ether formation from the primary alcohol group (Supporting Information Fig. S2), which is more likely due to the strong electron-withdrawing effect caused by both diphenyl substituents.

The so-formed silyl ether glucoside has then been used as initiator for the CL ROP, promoting polymerization from all three secondary equatorial alcohol groups of the glucoside derivative. Tin octoate ($Sn(Oct)_2$) has been chosen as catalyst since its activity at 80 °C is well-suited to the thermal stability of the sugar-functionalized initiator. Indeed, as the anomeric alcohol is blocked by a methyl ether group, polymerizations under those conditions are not likely to undergo side reactions or degradation of the sugar moiety. As the TBDPS sugar-based initiator proved to be poorly soluble in toluene, polymerizations were conducted in THF under nitrogen pressure. The monomer concentration was set to 2 mol L^{-1} while $[Sn]_0/[OH]_0$ molar ratio fixed to 0.04 to get a catalytic amount of *in situ* generated tin alkoxide active species as elsewhere explained.[34] Two different sets of reactions were performed to get star-shaped PCL structures characterized by overall degree of polymerizations (DPs) of about 24 (Table 1, entry 1) and 48 (Table 1, entry 2). As expected and highlighted in Table 1, both polymerizations proceeded in a controlled manner with an exquisite correlation between theoretical and experimental molecular weights. As expected, both CL ROPs seemed to proceed from all three secondary alcohol groups of the glucosidic initiator since a complex set of peaks [Fig. 1(Ha,c,d, and e)] appears between 4.6 and 5.6 ppm as theoretically predicted, principally attributed to the three methine protons in the glucosidic ring (Hc,d, and e) undergoing a downfield shift upon ester formation due to the polymerization of CL. Quantitative evidence can be obtained from the comparison of the hydroxymethylene end-group protons signal ($I_{k'}$) and the ones corresponding to the aromatic phenyl protons of the initiator residue ($I_l + I_m$). Normalization of the respective signals allowed for the calculation of the number of PCL chains per sugar as: $(I_{k'}/2)/((I_l + I_m)/10)$. In very good agreement and close to an expected value of 3, experimental calculations of 3.3 and 3.2 were obtained for respective entries 1 and 2 in Table 1. Using both I_k and $I_{k'}$ attributed to repeating CL methylene protons and end-groups —CH_2—OH, respectively, allows for the calculation of the average molecular weight of each single PCL chain ($M_{nchain} = M_{w\ CL} \times (I_k + I_{k'})/I_{k'}$). An excellent correlation between theoretical and experimental molecular weights along with low polydispersity indices attests for a good control over the ROP of CL from the sugar-based multihydroxylated initiator. Polydispersity indices also attest for the homogeneity of all the single chains grafted on the sugar core.

In a next step, the functionalization of the three hydroxy methylene end-groups of both star-shaped PCL by acetylation reaction coupled with the selective deprotection of the

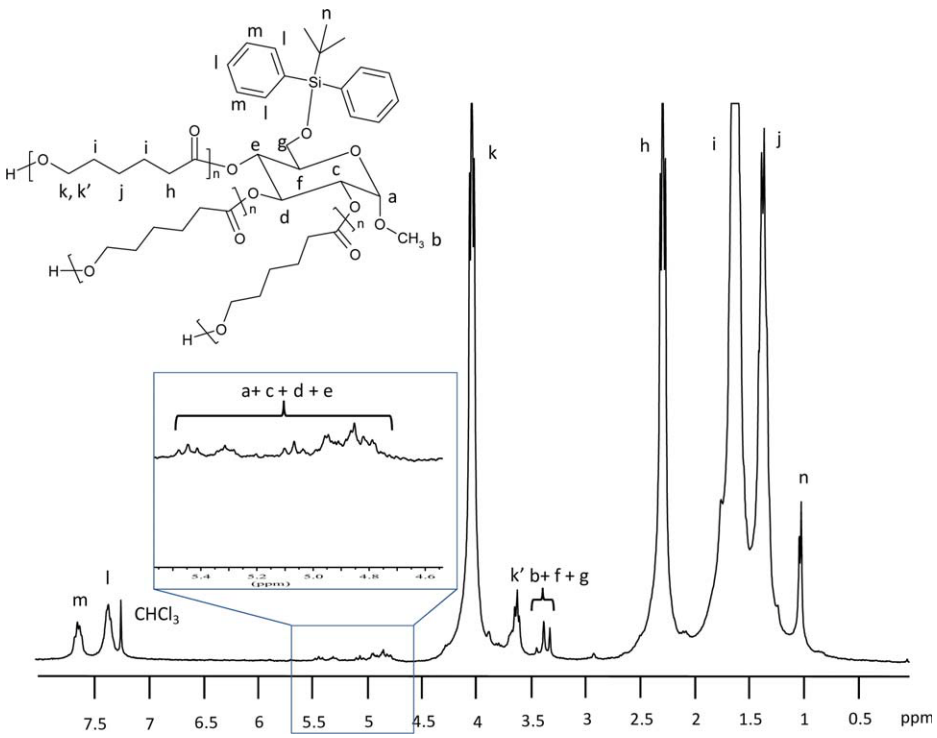


FIGURE 1 1H NMR spectrum of three-arm star-shaped PCL in CDCl3 (entry 2, Table 1) (signal k′ is attributed to the —CH_2—OH end-group). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

silyl ether functions has been realized. The acetylation reaction has been conducted in THF with a large excess of acetic anhydride ($[Ac_2O]_0/[OH]_0 = 30$) in the presence of *N,N′*-dimethylaminopyridine (DMAP) and triethylamine as catalyst and acidic quencher, respectively. Reactions proceeded at 50 °C for 48 h, then poured in a large excess of methanol to selectively precipitate the end-capped polymers. The effectiveness of the reaction has been evidenced by ^{1}H NMR by disappearance of the triplet initially present at 3.6 ppm corresponding to the α-hydroxymethylene end-group protons along with the appearance of a new signal at 2 ppm corresponding to the methyl protons (Hl) of the acetyl groups (Supporting Information Fig. S3). As far as SEC analyses are concerned, they have been performed using both RI and UV (260 nm) detectors. To that end the three-arm star-shaped PCL samples have been previously treated with phenyl isocyanate in THF. Phenyl isocyanate is known to readily react with any alcohol group if present, resulting in a phenyl carbamate formation.[35] The covalently linked phenyl groups are readily probed at 260 nm. Interestingly, UV detection did not show any absorption peak on the SEC polymer traces, then attesting for quantitative acetylation reactions.

The fourth step has consisted on the selective deprotection of all silyl ethers to regenerate the primary alcohol group on the glucoside moiety. Practically, the deprotection has been performed accordingly to a literature procedure, using a tetrabutyl ammonium fluoride (TBAF) solution in THF in the presence of acetic acid.[36] After 21 hours, polymers were selectively precipitated in cold methanol, filtered off and dried at 40 °C till constant weight. Recovering yields were between 85 to 95% while deprotection proved quantitative as attested by ^{1}H NMR with the disappearance of the signal initially present at about 1 ppm and between 7.3 and 7.8 ppm corresponding to the *tert*-butyl and the phenyl protons of the TBDPS group, respectively (Supporting Information Fig. S3). Interestingly, SEC traces present narrow molecular weight distributions without any trace of degradation ($M_{n\ app} = 2400$ g mol^{-1}; PDI = 1.1; $M_{n\ app} = 6400$ g mol^{-1}; PDI = 1.23 for entries 1 and 2, respectively, in Table 1). Finally, primary alcohol groups of the star-PCL-OH have been converted into a 2-bromoisobutyrate moiety by reaction with an excess of 2-bromo-2-methylpropionyl bromide (1.5 eq.) in THF at 50 °C for 48 h in presence of triethylamine and DMAP as quencher and catalyst, respectively. At the end of the reaction, triethylammonium bromide salts were filtered off and the functionalized polymers (star-PCL-Br) were selectively precipitated in cold methanol and dried until constant weight. Completion of the reaction has been evidenced by ^{1}H NMR in $CDCl_3$ from the disappearance of the hydroxymethylene protons at 3.6 ppm (Supporting Information Fig. S3, Hg) to the benefit of the new methyl protons appearing at 1.9 ppm (data not shown here).

The synthesis of mikto-arm copolymers has been performed by conducting the ATRP of the noncommercially available 1,2;3,4-di-O-isopropylidene-6-O-methacryloyl-D-galactopyranose (MAIGP) monomer using star-PCL-Br as macroinitiator and a catalytic complex of copper bromide (CuBr) ligated with 1,1,4,7,10,10-hexamethyltriethylene tetramine (HMTETA) ($[\text{star-PCL-Br}]_0/[CuBr]_0/[HMTETA]_0 = 1/1/2$). Polymerizations have been promoted in THF at 60 °C for different reaction times and three different polymerization degrees (DPs) in MAIGP (25, 50, and 100) (Table 2). As evidenced, the polymerization of MAIGP successfully proceeded from both star-PCL-Br macroinitiators with conversions higher than 75%.

TABLE 2 Molecular Characteristics of the Mikto-arm Copolymers as Obtained by ATRP of MAIGP from Star-PCL-Br

Entry	$M_{n\ star\text{-}PCL\text{-}Br}$ (g mol^{-1})	Time (h)	Targeted DP MAIGP	Conv. in MAIGP (%)[a]	$M_{n\ th}$ (g mol^{-1})[b]	$M_{n\ exp}$ (g mol^{-1})[c]	M_w/M_n[d]
1	2,700	2	25	75	8,600	8,700	1.19
2	2,700	4	50	99	18,700	20,000	1.28
3	6,400	4	50	91	21,200	20,900	1.34
4	6,400	8	100	86	34,500	35,100	1.22

[a] As determined by gravimetry. Conv. (%) = (M_{rec} – $M_{S\text{-}PCL\text{-}Br}$ – M_{cata})/M_{MAIGP} x 100 where M_{rec}, $M_{star\text{-}PCL\text{-}Br}$, M_{cata} are the masses of recovered crude copolymer, star-PCL-Br macroinitiator and catalytic complex, respectively.
[b] Theoretical molecular weight: $M_{n\ th} = M_{n\ star\text{-}PCL\text{-}Br} + (DP_{targeted} \times conv. \times Mw_{MAIGP})$.
[c] Experimental molecular weight as determined by ^{1}H-NMR (see text).
[d] As determined by size exclusion chromatography (SEC) in THF at 35 °C using PS standards.

Polymerization reactions took place in a well-controlled manner as attested for by the excellent correlation between theoretical and experimental number average molecular weights. ^{1}H NMR analysis allowed for determining the experimental M_n of the four samples by comparing the anomeric protons of the MAIGP repeating units at 5.5 ppm (Fig. 2, H_p) to the methyl-ether protons of the α-methylglucoside core clearly appearing at 3.4 ppm (H_b). Experimental average number molar masses have then been determined as:

$$M_{n\ exp} = I_p/(I_b/3) \times M_{w\ MAIGP} + M_{n\ star-PCL\text{-}Br}$$

Moreover, the control over the ATRP reactions using star-PCL-Br as macroinitiator is fully confirmed by monomodal SEC traces characterized by low polydispersity indices (from 1.19 to 1.34) and a clear shift to lower elution volumes attesting for the mikto-arm formation from both macroinitiators (Figs. 3 and 4).

The ultimate step has consisted in the complete and selective removal of the isopropylidene protective groups from the pendant sugars all along the polymethacrylate backbone to generate the amphiphilicity of the mikto-arm copolymers. Practically, mikto-arm copolymers were treated by an aqueous solution of formic acid (80%) for 48 hours and dialyzed against water. The white fluffy samples were recovered in good yields (75 to 90%) and characterized by ^{1}H NMR spectroscopy where the spectra showed the complete

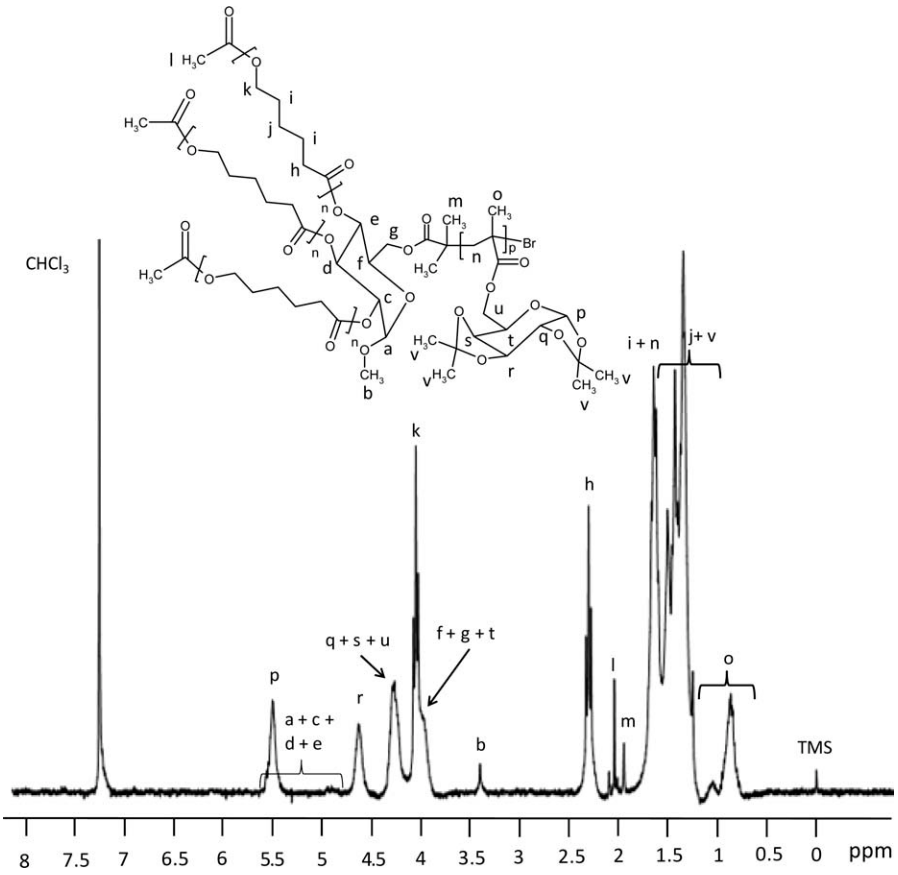


FIGURE 2 ^{1}H NMR spectrum ($CDCl_3$) of a mikto-arm copolymer based on three-arm star-shaped PCL and MAIGP (see entry 2, Table 2).

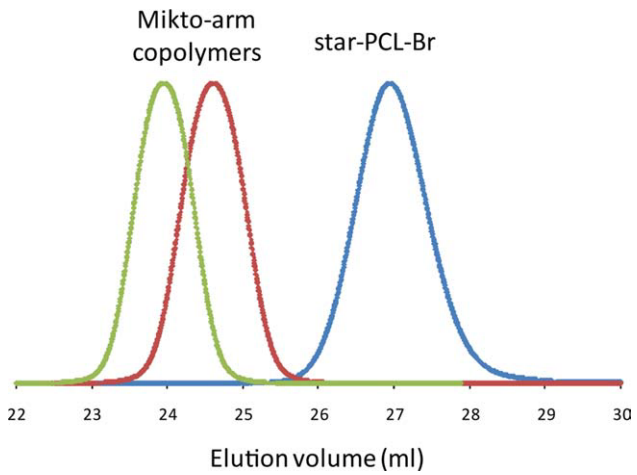


FIGURE 3 SEC traces of the star-shaped PCL-Br macroinitiator (M_n = 2700 g mol^{-1}; blue curve) and two corresponding mikto-arm copolymers obtained after MAIGP ATRP (red curve = entry 1, Table 2; green curve = entry 2, Table 2).

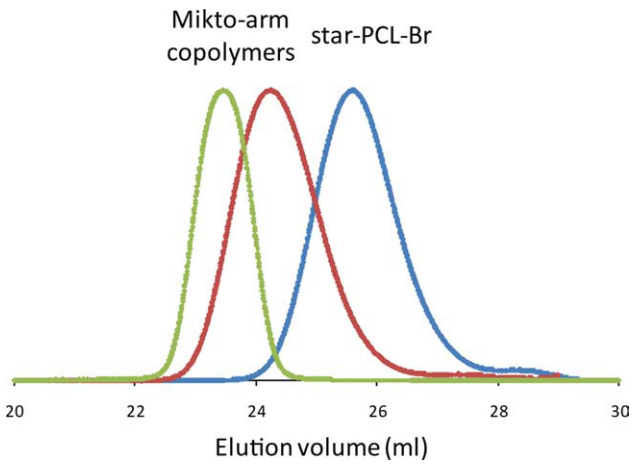


FIGURE 4 SEC traces of the initial three-arm star-shaped PCL macroinitiator (M_n = 6300 g mol^{-1}; blue curve) and two mikto-arm copolymers obtained via ATRP (red curve = entry 3, Table 2; green curve = entry 4, Table 2).

disappearance of the isopropylidene protons along with a general upfield shift of sugar ring protons without any modification of the other integrated signal values (data not shown here). Under such prevailing conditions FTIR spectroscopy evidenced for the selective deprotection of the isopropylidene protecting groups. FTIR spectrum of the deprotected mikto-arm copolymers confirms the presence of —OH groups as evidenced by a broad absorption around 3400 cm^{-1} along with isopropylidene ether signals disappearance (ν = 1160 cm^{-1}), while ester linkages (ν = 1730 cm^{-1}) remained untouched with no evidence of carboxylate signals (data not shown here).

The as-obtained amphiphilic mikto-arm copolymers were finally studied in aqueous solution to determine whether they self-organize or not as stable micelles with dimensions low enough to be potentially used as drug delivery systems. To that end the critical micellization concentrations (CMC) along with the size of the micelles have been investigated by UV analysis and dynamic light scattering (DLS) experiments. To determine the CMC of the amphiphilic copolymers, pyrene has been used as molecular probe.[37] Table 3 summarizes the CMC values obtained for amphiphilic copolymers along with their hydrophilic-lipophilic balance (HLB) as calculated by the Griffin relationship.[38] Although all CMCs are determined in the range of some mg/L, variations in compositions may be highlighted for the different samples. In Table 3, entries 1 and 2 focus on the CMC determination of samples based on the star-PCL-Br with the lower molar mass (M_n = 2700 g mol^{-1}; DP = 18) while entries 3 and 4 concern samples based on star-PCL-Br with the higher molar mass (M_n = 6400 g mol^{-1}; DP = 51). In both cases, increasing the length of the polymethacrylate backbone bearing the pendant hydrophilic sugars while maintaining a same PCL length resulted in an increase of the observed CMCs. This can be readily explained by a higher solubility of the resulting unimers in solution due to higher sugar content in the copolymers.

Copolymers (entries 2 and 3) are both composed by a polymethacrylate backbone with quite similar length ($DP_{polymeth}$ = 50 and 46, respectively), while the overall Mn_{PCL} increases. A higher PCL content thus reduces the solubility of the unimers in water, promoting self-organization and formation of micelles at lower concentration. Finally, copolymers with different compositions but having roughly the same HLB may also be compared. Comparison of samples in entries 1 and 3 highlights a lower CMC value for the second copolymer, thus with the higher overall molecular weight. Here we might consider that for the second sample, PCL chains are longer and thus less soluble in water. Moreover, longer PCL chains are also more likely to crystallize in the micelle core thus diminishing the CMC as well.

TABLE 3 Dependence of HLB, CMC, and Micelle Mean Diameter as a Function of Amphiphilic Mikto-arm Copolymer Composition

Entry	Mn_{PCL} (g mol^{-1})	$Mn_{Polymeth}$ (g mol^{-1})	HLB[a]	CMC (mg/L)[b]	D_h (nm)[c]
1	2,700	4,700	9.2	3.7	73 ± 0.19
2	2,700	12,400	11.9	5.4	61 ± 0.36
3	6,400	11,400	9.3	1.6	56 ± 0.26
4	6,400	21,300	11.2	2.4	46 ± 0.36

[a] As determined by the Griffin relationship: HLB = 20 x $M_H/(M_H + M_L)$ where M_H and M_L are the total molecular weights of the hydrophilic and lipophilic segments, respectively.

[b] As determined by pyrene encapsulation (Supporting Information Figs. S4 and S5).

[c] As determined by Dynamic Light Scattering with [Copolymer] = 50 mg/L.

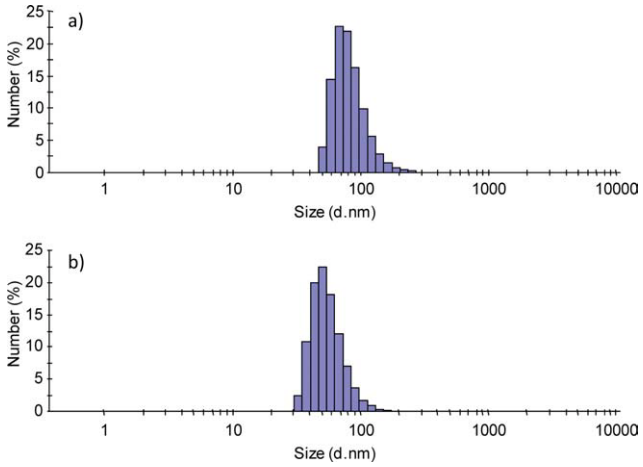


FIGURE 5 Size distributions of self-organized mikto-arm copolymers in water for deprotected polymer (a) entry 1, Table 3 and (b) entry 3, Table 3. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

In parallel with the CMC measurements, the average size of the resulting micelles has been determined by dynamic light scattering (DLS) in deionized water (Fig. 5). Taking into account the previously measured CMC of the different samples, the copolymer concentrations have been set to 50 mg/L. Interestingly, size of the as-obtained micelles are all relatively low with a average diameter below 80 nm. Thus their size is well under the common barrier of 200 nm for which RES uptake along with mechanical clearance by the lungs or the spleen usually occur.[39] Results listed in Table 3 show diameters slightly decreasing when increasing both PCL and polymethacrylate backbone lengths. Although further investigation for determining the shape of the micelles is required, it seems that longer chains tend to collapse thus forming smaller nanostructures. Nevertheless, it comes out that the studied amphiphilic mikto-arm copolymers exhibit interesting properties in aqueous solution with CMC in the range of some mg/mL along with average sizes below 80 nm making them excellent candidates for drug delivery systems.

CONCLUSIONS

To summarize, we reported an original synthetic strategy yielding new amphiphilic mikto-arm copolymers. ROP of CL has been achieved with excellent control from the secondary alcohol groups of a TBDPS protected α-methylglucose initiator leading to new three-arm star-shaped PCL. A series of postpolymerization protection-deprotection steps allowed for the synthesis of star-PCL-Br macroinitiator, which proved to be perfectly suited for the initiation of the ATRP of MAIGP monomers, thus leading to mikto-arm copolymers with no residual traces of initial macroinitiator. Well-defined copolymers with good control over molecular parameters were then selectively deprotected to recover the amphiphilic A_3B mikto-arms in good overall yields and also characterized by low CMCs along with low and narrow average sizes when forming micelles in solution.

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