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Exploring Strain-Promoted 1,3-Dipolar Cycloadditions of End Functionalized Polymers

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Abstract

Strain-promoted 1,3-dipolar cycloaddition of cyclooctynes with 1,3-dipoles such as azides, nitrones, and nitrile oxides, are of interest for the functionalization of polymers. In this study, we have explored the use of a 4-dibenzocyclooctynol (DIBO)-containing chain transfer agent in reversible addition–fragmentation chain transfer polymerizations. The controlled radical polymerization resulted in well-defined DIBO-terminating polymers that could be modified by 1,3-dipolar cycloadditions using nitrones, nitrile oxides, and azides having a hydrophilic moiety. The self-assembly properties of the resulting block copolymers have been examined. The versatility of the methodology was further demonstrated by the controlled preparation of gold nanoparticles coated with the DIBO-containing polymers to produce materials that can be further modified by strain-promoted cyclo-additions.

Keywords

alkynes; cycloaddition; gold; nanoparticles; polymerization

Introduction

In many application-driven material studies, there is a need for polymers bearing an addressable group at the chain-end for precise functionalization.^[1] An attractive approach for constructing such macromolecular architectures involves living polymerization combined with an initiator or a chain transfer agent (CTA) modified by a reactive group.^[2] Post-polymerization modification of the reactive group makes it possible to rapidly prepare a library of functional polymers from a common polymeric precursor thereby offering

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Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/chem.201402225>: Detailed experimental procedures, synthesis of polymers and azido derivatives (Schemes S1–S3), NMR spectra of compounds, GPC traces (Figure S1, S4), and UV/Vis and fluorescence spectra (Figures S3, S16–S17) of polymers and AuNPs and DLS traces of nanoparticles (Figures S5–S15) are provided in supporting information.

exciting opportunities to fine-tune the properties of materials.^[3] However, a challenge with such an endeavor is the identification of a reactive group that is compatible with polymerization conditions and can be modified by a wide range of functional entities. Here, we describe a CTA modified with 4-dibenzocyclooctynol (DIBO, 1) that can be utilized in living polymerization to give macromolecules terminating in a strained alkyne (Scheme 1). The latter functionality can easily be modified by a variety of functionalized 1,3-dipoles under metal-free conditions to give useful materials.

Reversible addition–fragmentation chain transfer (RAFT) polymerization has received increasing attention because of its compatibility with a wide range of monomers and polymerization conditions. Furthermore, this method offers control over molecular weights, dispersity, architecture, and functionalization of the resulting polymers.^[4] The RAFT method has been used to prepare polymers terminating in thiols, carboxylic acids, and amines. Although these reactive groups offer a possibility for post-polymerization modification, their scope is limited due to a lack of functional group tolerance.^[5] Terminal alkynes are a more attractive functional group for post polymerization modification because they tolerate a wide range of reaction conditions including polymerization techniques such as RAFT.^[6] Furthermore, in the presence of a copper(I) catalyst, terminal alkynes undergo facile 1,3-dipolar cycloadditions with azides to give stable triazoles.^[7] These reactions, which have been coined copper-catalyzed 1,3-dipolar azide-alkyne cycloadditions (CuAAC), are popular in polymer chemistry because of their excellent functional group tolerance.^[8] However, a limitation of CuAAC is that it cannot be used for applications that are sensitive to the presence of trace amounts of the toxic metal ions.^[9]

Strain-promoted azide–alkyne cycloadditions (SPAAC) offer a highly efficient functionalization method that does not need a metal catalyst.^[10] We have shown that derivatives of DIBO undergo SPAAC with azido-containing saccharides and amino acids with high efficiency and can be employed for the visualization of metabolically labeled glycans of living cells.^[11] We have extended the utility of SPAAC for controlled surface modification of polymeric micelles^[12] and the preparation of multifunctional comb type polymers^[13] that self-assemble into well-defined nanostructures. Recently, SPAAC has also been used for the derivatization of electrospun nanofibers,^[14] polymersomes,^[15] stealth polymeric vesicles,^[16] and dendrimers.^[17]

The purpose of this study was to examine whether strained alkynes such as DIBO can tolerate RAFT based free radical polymerization conditions to give polymeric building blocks having a α -terminal reactive functionality. The DIBO moiety of the resulting polymers can then be employed for modifications by metal-free cycloadditions using functionalized 1,3-dipoles such as azides, nitrones, and nitrile oxides (Scheme 1a). By careful selection of the functional entities, materials were obtained that self-assembled into spherical polymersomes when in water. Furthermore, we envisaged that the trithiocarbonate group on ω -chain end of the polymers would offer an orthogonal functional group that can also be employed for functionalization. The feasibility of such a bi-functionalization approach was examined by the preparation of polymer-stabilized gold nanoparticles having DIBO at their surface. The latter functionality was exploited for the attachment of functional entities to obtain water soluble particles.

Results and Discussion

Trithiocarbonates are versatile CTAs that offer control of polymerization of styrenes, acrylates, and methacrylates while having high hydrolytic stability. Commercially available 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid (DDMAT, **2**) was used as the starting material for the synthesis of the clickable RAFT agent **3** (Scheme 1).

Thus, DDMAT (**2**) was modified by DIBO (**1**) through an ester linkage in high yield using *N,N'*-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP). The product **3** was isolated as orange oil, which solidified upon standing in a yield of 83%. NMR and MALDI-TOF mass spectrometry confirmed the structure of the product (see Supporting Information for NMR spectra). With the DIBO-modified RAFT agent in hand, polymerization of styrene under standard conditions was attempted using 1,4-dioxane as the solvent and azobisisobutyronitrile (AIBN) as the initiator at 70 °C (Table 1, condition A). Although DIBO is rather labile, we were encouraged by a recent report that indicated that cyclooctynes tolerate free radical polymerization conditions.^[18] Furthermore, CTAs containing sensitive norbornene moieties which bear a strained double bond have also been used in free radical polymerizations.^[19]

A monomer to CTA to AIBN ratio of 100:1:0.1 was employed and after a reaction time of 20 h, the polymerization was terminated at 35% conversion. The end-functionalized polystyrene was isolated by precipitation into cold methanol and analysis of the resulting polymer by ¹H NMR showed successful installation of a DIBO moiety onto the α -chain end of polystyrene. Gel permeation chromatography (GPC) analysis of polymer showed a relatively high molar mass dispersity (D_M) of 1.44 and M_n of 3100 g mol⁻¹, which is lower than expected based on the conversion value (4250 g mol⁻¹). Furthermore, a high molecular weight shoulder was observed on the GPC chromatogram of the DIBO-modified polymer (Figure 1).

A kinetic study under the same polymerization condition using unmodified CTA agent **2** resulted in polystyrenes with a narrow D_M (Figure S1). Furthermore, literature reports on terminal alkyne-containing CTAs under similar reaction conditions resulted in higher conversions (30–65%) and narrow D_M .^[8b] Therefore, it was concluded that the DIBO moiety of CTA reagent **3** has an unfavorable impact on the outcome of the polymerization, probably due to the presence of the strained triple bond, which is susceptible to side reactions. Similar problems have been encountered by other groups when using norbornene-containing CTA. It was shown that unwanted side reaction at norbornene could be minimized by reducing the reaction temperature^[19b] or by increasing the monomer to CTA ratio.^[19a] Thus, we attempted the polymerization of the DIBO containing CTA at a reduced temperature of 50 °C (Table 1, condition B). As expected, the molar mass dispersity was lower compared to condition A, however, the conversion did not exceed 16%. NMR analysis confirms the presence of the DIBO and trithiocarbonate moieties. Further examination of end group fidelity by MALDI-TOF showed the presence of DIBO-terminating chains, but loss of trithiocarbonate functionality during ionization process (Figure S2). GPC analysis of the resulting polymer showed a low molecular weight. In order to increase the conversion at low temperature, the highly reactive initiator 2,2'-azobis(4-

methoxy-2,4-dimethyl valeronitrile) (V-70) (Table 1, condition C) was utilized. The use of this initiator resulted in an increase of the conversion to 29% after 20 h and the isolated polymer had M_n of 1550 g mol⁻¹ while maintaining a low $\bar{D}_M$ of 1.21. A small shoulder was visible in the GPC chromatogram, probably due to a higher free radical concentration. To further suppress the side reactions on the DIBO moiety, the monomer to CTA ratio was increased to 600:1 with AIBN as initiator at 70 °C. After 15 h, when a conversion of 20% was reached, the polymer was isolated by precipitation with cold methanol. GPC analysis showed that the resulting polystyrene had a relatively high M_n of 9450 g mol⁻¹ and low $\bar{D}_M$ of 1.31. Importantly, the molecular weight distribution was monomodal without an apparent shoulder indicating a good control over the polymerization.

Next, a kinetic study was performed by analyzing aliquots collected at different time points during the polymerization to examine the conversion and M_n , using ¹H NMR and GPC, respectively. GPC traces of aliquots taken at approximately 2 h intervals showed an increase in M_n of the polymer with time (Figure 2a). Furthermore, the pseudo-first-order kinetic plot and the plot of M_n as a function of conversion showed linearity, demonstrating a controlled nature of the polymerization mediated by CTA **3** and a decrease of chain termination due to side reactions (Figure 2b, c). After ~ 14 h, the polymer **P1** was isolated and analyzed by ¹H NMR, which showed the presence of a DIBO moiety (see Supporting Information for NMR spectra). Based on the integral intensity of the DIBO CH₂ signal at 2.75 ppm (one diastereotopic proton) and aromatic signals of styrene approximately 80 styrene units are terminated per DIBO moiety. This value is similar to the degree of polymerization obtained from GPC ($n = 85$). Collectively, these observations indicate the end group fidelity of **P1** is high. Similarly, we estimate the M_n of **P1** from NMR to be 14800 g mol⁻¹ based on integral intensity of trithiocarbonate signals at 0.8 and 2.75 ppm and CH-S signal at 4.8 ppm. Such discrepancy with M_n , obtained from GPC indicates partial loss of the trithiocarbonate moiety. We also found that the trithiocarbonate end group fidelity is further reduced at higher conversions/reaction times. Therefore in order to preserve control over the polymerization it was necessary to limit the conversion to 20%.

The DIBO moiety of the polymers offers an opportunity for functionalization with various 1,3-dipoles such as azides, nitrones, and nitrile oxides without the need for a catalyst to prepare libraries of amphiphilic molecules from hydrophilic and hydrophobic building blocks. Two types of hydrophilic moieties, polyethylene glycol, and the disaccharide lactose were chosen to prepare a range of amphiphilic polymeric conjugates. Polyethylene glycol could be easily derivatized with azide, nitron, and oxime groups to give compounds **4**, **6** and **7** for SPAAC, strain promoted alkyne-nitron cycloaddition (SPANOC) and strain promoted alkyne-nitrile oxide cycloaddition (SPANOC)-mediated derivatization of DIBO-PSt (**P1**), respectively. Azido-modified lactose **5** and lactose oxime **8** served as precursors for glycopolymer conjugates for SPAAC and SPANOC (Scheme 2).

These hydrophilic derivatives were attached to DIBO-containing polystyrene via a stable triazole, isoxazole and methylisoxazole linkages using strain promoted 1,3-dipolar cycloadditions. For the formation of amphiphilic PEG₇₅₀-*b*-PSt (**P2**) and lactose-PSt (**P3**) via SPAAC, the two components were reacted overnight in dichloromethane or a mixture of CH₂Cl₂ and methanol (MeOH), respectively (Scheme 3). The resulting amphiphilic

polymers **P2** and **P3** were isolated by precipitation with cold methanol. Similarly, nitrene-bearing polyethylene glycol **6** was coupled to DIBO-PSt (**P1**) in CH_2Cl_2 to give PEG₇₅₀-*b*-PSt (**P4**) with *N*-methylisoxazole linkage between two blocks after overnight SPANC reaction. Finally, the oxime-bearing PEG **7** and lactose oxime **8** could undergo SPANOC reaction with DIBO-PSt (**P1**) in presence of iodobenzene diacetate (BAIB). Again, CH_2Cl_2 and $\text{CH}_2\text{Cl}_2/\text{MeOH}$ solvent mixture were chosen to obtain PEG₇₅₀-*b*-PSt (**P5**) and lactose-PSt (**P6**). The conjugates were characterized with NMR spectroscopy, GPC and UV/Vis spectroscopy to confirm complete modification (See supporting information). The presence of CTA signatures in NMR and UV/Vis spectra confirmed the endurance of trithiocarbonate chain end to excellent chemoselectivity of SPAAC, SPANC, and SPANOC reactions. The successful modification was evident from disappearance of signature DIBO absorption at ~ 308 nm in UV/Vis spectra (Figure S3). GPC data are presented in Table 2. Since the change in molecular weight after modification was small, there was virtually no shift in peak position after conjugation (Figure 3). The small differences in molecular weights obtained from GPC of **P1**, **P2**, **P4** and **P5** are probably a result of conformational and solvation differences between triazole, isoxazole and *N*-methylisoxazole moieties. We have performed SPAAC with higher molecular weight PEG azides to further confirm that DIBO is indeed present on the polymer chain end. In this case, GPC analysis indicated that after the click reaction, the elution times decreased and the molecular weights increased demonstrating the presence of reactive DIBO chain ends on these polymers (Figure S4).

Next, the self-assembly properties of the amphiphilic polymers were studied using transmission electron microscopy (TEM) and dynamic light scattering (DLS). Solutions of the polymers **P2–P6** in tetrahydrofuran (10 mg mL^{-1}) were slowly added to distilled water while stirring to reach a final concentration of 1 mg mL^{-1} . Stirring was continued for 2 h to allow the organic solvent to evaporate. The resulting solutions were passed through a 0.8 mm filter and DLS (Figure S5–S9) of the resulting aqueous solutions showed formation of self-assembled materials. Polymer **P1** did not form self-assembled structures in water due to the absence of a hydrophilic segment. As expected, polymers **P2–P6**, containing hydrophilic lactose or PEG₇₅₀, formed polydisperse nanoparticles with mean diameters of 33–205 nm and polydispersities of 0.05–0.285 (Table 3, Figures S5–S9). The effective diameter as determined by DLS of **P2** and **P3**, having triazole linkages, was approximately 210 nm. On the other hand, the *N*-methylisoxazole linkage between styrene and PEG blocks in **P4** resulted in larger particles having effective diameter of 264 nm, whereas polymers **P5** and **P6**, containing an isoxazole linkage, produced smaller nanoparticles of 143 and 136 nm diameter. TEM was employed to gain further insight into self-assembly properties of **P2–P6**. In support of the DLS data (Supporting information, Figures S4–S9), **P2–P6** having terminal PEG₇₅₀ and lactose moieties assembled into polydisperse spherical polymersomes having a size range of 30 to 200 nm (Figure 3).

We envisaged that multifunctional polymers such as **P1** can be employed for the preparation of gold nanoparticles (AuNPs) that have DIBO at their surface for further modification. AuNPs offer opportunities for the preparation of biologically active platforms that combine biomolecules and nanoparticles for sensing, drug delivery, and imaging applications.^[20] The preparation of this type of nanoparticle often requires a coupling strategy for attachment of a

functional molecule of interest.^[21] The DIBO and trithiocarbonate terminal groups of polymers such as **P1**, offer unique handles for the controlled attachment to gold surfaces via thiol–gold linkages, resulting in particles that expose clickable DIBO groups at the surface. To this end, polymer coated AuNPs synthesis was explored with these materials (e.g., DIBO-PSt) (Scheme 4). Thus, a LiBH₄ solution in THF was added drop wise to a vigorously stirred solution of polymer **P1** (or **P2**, **P4** and **P5**) and HAuCl₄ in anhydrous THF under a nitrogen atmosphere. An immediate color change from yellow to deep purple ensued, indicating the formation of AuNPs. After a reaction time of 3 h, ethanol was added to quench the excess of LiBH₄.^[22] The solution was dialyzed against THF applying a 12–14 kDa molecular weight cutoff membrane to remove any unbound polymer. The TEM images (Figure 4 and Table 4) and DLS (Figures S10–S15) analysis showed a spherical morphology of AuNPs with a size ranging from 16 to 30 nm and polydispersity indexes ranging from 0.147 to 0.174. The UV/Vis absorption spectrum (Figure S16) showed λ_{max} at 537 nm, which is a characteristic surface plasmon resonance absorption band of AuNPs. These observations indicate that trithiocarbonate containing CTA can be reduced to act as stabilizing agent for AuNP synthesis thereby anchoring sulfur to gold cores to make gold-polymer hybrid NPs.^[22b] The AuNPs prepared from **P1** were modified by SPAAC using N₃-PEG₇₅₀ (Table 4 and Figure 4). The product, **GNP5** showed a mean diameter of 23.4 nm and an effective diameter of 65.7 nm. This is slightly higher than the precursor Au-PSt-DIBO nanoparticles, **GNP1**, which indicates that a copper-free azide–alkyne cycloaddition can be performed on DIBO functionalized AuNPs. The SPAAC modification was further confirmed by UV/Vis spectroscopy of **GNP1** and **GNP5** after treatment with a KI solution to dissolve the Au core.^[23] The resulting solution of **GNP1** contains DIBO and, showed $\lambda_{\text{max}} \sim 310$ nm, which is a characteristic feature of this functionality (Figure S17). On the other hand, KI treated of **GNP5**, which is modified by N₃-PEG₇₅₀, does not show the DIBO signature peak indicating a complete conversion of the triple bond (Figure S17). Next, **GNP1** was reacted with azido-modified carboxyfluorescein (see Supporting Information, S4) in THF for 24 h and then dialyzed against THF for 72 h to give **GNP7**. Dye conjugation was confirmed by recording fluorescence spectra of the resultant nanoparticles (Figure S17b). The degree of functionalization of **GNP7** was calculated to be 78% based on a standard curve of free carboxyfluorescein. It is important to point out that the functionalization may be higher as the fluorescence quenching by the gold core of the nanoparticles is unavoidable here. These results demonstrate that the trithiocarbonate terminal group of the polymers can be exploited for attachment to a gold core. Furthermore, the DIBO moiety is compatible with the reaction conditions used for the preparation of the GNPs and the resulting particles can be decorated with functional entities by SPAAC. The attraction of the approach described here is that in a single step, AuNPs can be prepared having a strained alkyne at its surface for further modification under metal free conditions.^[24]

Conclusion

We have developed a CTA modified by DIBO and have established reactions conditions for its utilization in controlled radical polymerizations using RAFT. The resulting DIBO-terminated polymer was successfully employed for post-polymerization modification via

strain-promoted cyclo-additions using different 1,3-dipoles. The resulting amphiphilic block copolymers exhibited self-assembly properties. The chemo-selectivity of SPAAC, SPANC, and SPANOC ensured the endurance of the trithiocarbonate chain end, which could be employed along with the DIBO moiety for bifunctionalization. To this end, polymer-coated gold NPs could be prepared to have reactive DIBO functionalities at their surface for first time in a single step. This transformation was achieved in a single step by in situ reduction of the trithiocarbonate moiety of the DIBO-bearing polymers. The reported synthetic approach for the preparation of bi-end-functional polymers offers opportunities for the attachment under metal free conditions of functional units such as therapeutics, targeting agents, or imaging agents for variety of material and biomedical applications.

Experimental Section

Materials and methods

All reagents were purchased from Sigma-Aldrich and used as received unless stated otherwise. CH_2Cl_2 was distilled over calcium hydride. Styrene was washed with 1 N NaOH, followed by water to remove inhibitors, dried over MgSO_4 and then purified by vacuum distillation over calcium hydride. AIBN was recrystallized from MeOH twice prior to use. Lactose-oxime **8** was synthesized according to previously reported procedures.^[25] The NMR spectra were recorded on a Varian Mercury 300 MHz or Varian Inova 500 MHz spectrometers using CDCl_3 as a solvent unless stated otherwise. ^1H NMR-based M_n of dodecyl trithiocarbonate-terminated polymers was calculated by comparing the integral areas under CH_{ar} peaks of repeating units with integrals of $\text{CH}_2\text{-S}$ signal of ω -chain end. The weight of CTA (566 g mol^{-1}) was then added to the sum of weights of repeating units. GPC analyses were performed on Shimadzu LC-20AD liquid chromatography instrument, equipped with RI detector. Two Waters Styragel columns (HR3 and HR4) were placed in series. THF was used as eluent at 1 mL min^{-1} flow rate; the column oven was set to 40°C . Molecular weights were calculated against polystyrene standards. TEM images were obtained on Philips/FEI Technai 20 instrument using copper TEM grids. A 2% (wt/vol) uranyl acetate solution was used to stain the polymeric nanoparticles before recording.

Synthesis of DIBO-DDMAT (3)

A solution of DCC (280 mg, 1.36 mmol) in CH_2Cl_2 (5 mL) was added drop wise to a stirred solution of 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid (508 mg, 1.36 mmol), 4-dibenzocyclooctynol (150 mg, 0.68 mmol), and DMAP (20 mg, 0.16 mmol) in CH_2Cl_2 (5 mL) and the resulting mixture was stirred for 18 h at room temperature. Upon the completion of the reaction, the mixture was filtered to remove dicyclohexylurea and the filtrate was concentrated under reduced pressure. The resulting residue was purified by column chromatography on silica gel using a gradient of 0 to 20% EtOAc in hexanes as an eluent to give pure DIBO-DDMAT (325 mg, 83%) as a yellow oil. ^1H NMR (500 MHz, CDCl_3): δ = 0.88 (t, J = 7.0 Hz, 3H, CH_3CH_2), 1.19–1.38 (m, 18 H, CH_3 (CH_2)₉), 1.61–1.67 (m, 2 H, $\text{CH}_2\text{CH}_2\text{S}$), 1.80 (s, 3H, CH_3C), 1.83 (s, 3H, CH_3C), 2.88 (dd, J = 15.1, 4.0 Hz, 1 H, CHHCHO), 3.18 (dd, J = 15.1, 2.0 Hz, 1 H, CHHCHO), 3.27 (app dt, J = 7.3, 4.2 Hz, 2 H, CH_2S), 5.50 (brs, 1 H, CH_2CHO), 7.25–7.39 (m, 7 H, $7\times\text{CH}_{\text{ar}}$), 7.61 (d, J = 8.0 Hz, 1 H, CH_{ar}); ^{13}C NMR (75.5 MHz, CDCl_3): δ = 14.1 (CH_2CH_3), 22.6 (CH_2CH_3), 25.3 (CH_3),

25.7 (CH₃), 27.9 (CH₂CH₂S), 28.9 (CH₂), 29.1 (CH₂), 29.3 (CH₂), 29.4 (CH₂), 29.5 (CH₂), 29.6 (2×CH₂), 31.9 (CH₂), 36.9 (CH₂S), 46.0 (CH₂CHO), 55.9 (C(CH₃)₂), 77.9 (OCHCH₂), 109.5 (C≡C), 113.0 (C≡C), 121.3 (C_{ar}), 123.5 (CH_{ar}), 124.1 (C_{ar}), 125.7 (CH_{ar}), 126.1 (C_{ar}), 126.9 (CH_{ar}), 127.1 (CH_{ar}), 127.8 (CH_{ar}), 128.1 (CH_{ar}), 130.2 (CH_{ar}), 150.9 (2×C_{ar}), 171.5 (C=O), 220.9 (SC=S); HRMS (MALDI): *m/z*: calcd for C₃₃H₄₃O₂S₃ [*M*+H⁺]: 567.24; found: 567.26.

Representative procedure for synthesis of DIBO-PSt (**P1**)

A dry Schlenk flask was charged with styrene (3.12 g, 30.0 mmol), AIBN (0.5 mL of stock solution (0.01 M) in dioxane, 0.005 mmol), **3** (28.8 mg, 0.05 mmol) and 1,4-dioxane (3 mL). The mixture was subjected to three freeze-pump-thaw cycles and then stirred at 70 °C. The 0.2 mL aliquots were taken out of the polymerization mixture using the syringe under the flow of argon at 3, 5, 7, 9, 11 and 13 h after beginning of polymerization. These aliquots were used to establish the polymerization conversion using NMR spectroscopy. The polymerization was terminated by submersion into liquid nitrogen after 13 h. Then the reaction mixture was diluted with THF (3 mL) and the polymer was purified by precipitation in cold MeOH (250 mL) twice to give polymer DIBO-PSt (**P1**) (0.5 g) as yellowish solid. ¹H NMR (500 MHz, CDCl₃): δ = 0.82–2.31 (m, CH₃CH₂, CH₂CH₂, CHCH₂, C(CH₃)₂), 2.65–2.85 (m, CHHCHO), 3.19–3.27 (m, CH₂S, CHHCHO), 4.65–5.17 (m, CHS), 5.31 (br s, CHO), 6.16–7.51 (m, CH_{ar}); *M_n* [g mol⁻¹] = 6050 (GPC), 12400 (NMR). *D_M* = 1.39(GPC).

General procedure for SPAAC reaction with DIBO-PSt (**P1**)

A solution of DIBO-terminated polymer **P1** (1 equiv) and corresponding azide (2 equiv) in CH₂Cl₂ (5 mL) (PEG-azide, **1**) or a MeOH/CH₂Cl₂ mixture (5 mL, 1:1, v/v) (lactose-azide, **2**) was stirred for 18 h at room temperature. The resulting polymer was purified by precipitation into cold MeOH (100 mL) twice.

PEG₇₅₀-b-PSt (P2**)** (34 mg) was prepared from polymer DIBO-PSt (**P1**) (50 mg, 0.008 mmol) and PEG₇₅₀N₃ (**1**) (12.5 mg, 0.016 mmol). ¹H NMR (500 MHz, CDCl₃): δ = 0.77–2.22 (m, CH₃CH₂, CH₂CH₂, CHCH₂, C(CH₃)₂), 2.57–3.27 (m, CH₂CHO, CH₂S), 3.40–3.80 (m, CH₃O, CH₂O), 3.91–5.18 (m, CH₂N, CHS), 6.02–7.66 (m, CH_{ar}, CHO); *M_n* [g mol⁻¹] = 5300 (GPC), *D_M* = 1.27(GPC).

Lactose-PSt (P3**)** (36 mg) was prepared from polymer DIBO-PSt (**P1**) (50 mg, 0.008 mmol) and lactose-azide **2** (7.2 mg, 0.016 mmol). ¹H NMR (500 MHz, CDCl₃): δ = 0.72–2.39 (m, NCH₂ (CH₂)₃CH₂O, CH₃CH₂, CH₂CH₂, CHCH₂, C (CH₃)₂), 2.63–3.38 (m, CH₂CHO, CH₂S), 3.38–4.65 (m, CHOH-lactose, CH₂OH-lactose, CHO-lactose), 4.65–5.42 (m, CH₂N, CHS), 5.88–7.62 (m, CHO, CH_{ar}); *M_n* [g mol⁻¹] = 6000 (GPC), *D_M* = 1.31 (GPC).

Preparation of PEG₇₅₀-b-PSt (**P4**) via SPANC

A solution of DIBO-terminated polymer **P1** (50 mg, 0.008 mmol) and PEG-nitrone **3** (13.6 mg, 0.016 mmol) in CH₂Cl₂ (5 mL) was stirred for 18 h at room temperature. The resulting polymer was purified by precipitation into cold MeOH (100 mL) twice to give PEG₇₅₀-b-PSt (**P4**) (36 mg) as an amorphous solid. ¹H NMR (500 MHz, CDCl₃): δ = 0.83–2.25 (m,

CH_3CH_2 , CH_2CH_2 , $CHCH_2$, $C(CH_3)_2$), 2.89–4.23 (m, CH_2CHO , CH_2S , CH_3O , CH_2O , CH_3N), 4.62–5.28 (m, CHS , CH), 5.93–7.54 (m, CH_{ar} , CHO); M_n [g mol⁻¹] = 5300 (GPC), $\bar{D}_M$ = 1.27(GPC).

Preparation of PEG₇₅₀-*b*-PSt (P5) via SPANOC

A solution of DIBO-terminated polymer **P1** (50.0 mg, 0.008 mmol), BAIB (5.1 mg, 0.016 mmol) and PEG-oxime **3** (13.6 mg, 0.016 mmol) in CH_2Cl_2 (5 mL) was stirred for 18 h at room temperature. The resulting polymer was purified by precipitation into cold MeOH (100 mL) twice to give PEG₇₅₀-*b*-PSt (**P5**) (38 mg) as an amorphous solid. ¹H NMR (500 MHz, $CDCl_3$): δ = 0.73–2.25 (m, CH_3CH_2 , CH_2CH_2 , $CHCH_2$, $C(CH_3)_2$), 2.63–4.25 (m, CH_2CHO , CH_2S , CH_3O , CH_2O), 4.62–5.21 (m, CHS), 6.27–7.48 (m, CH_{ar} , CHO); M_n [g mol⁻¹] = 6750 (GPC), $\bar{D}_M$ = 1.37(GPC).

Preparation of lactose-PSt (P6)

A solution of DIBO-terminated polymer **P1** (50 mg, 0.008 mmol), BAIB (5.1 mg, 0.016 mmol), and lactose-oxime **5** (5.7 mg, 0.016 mmol) in MeOH/ CH_2Cl_2 mixture (5 mL, 1:1, v/v) was stirred for 18 h at room temperature. The resulting polymer was purified by precipitation into cold MeOH (100 mL) twice to give lactose-PSt (**P6**) (30 mg) an amorphous solid. ¹H NMR (500 MHz, $[D_7]DMF$): δ = 0.73–2.45 (m, CH_3CH_2 , CH_2CH_2 , $CHCH_2$, $C(CH_3)_2$), 2.96–4.60 (m, CH_2CHO , CH_2S , lactose signals), 4.75–5.76 (m, CHS), 6.32–7.72 (m, CH_{ar} , CHO); M_n [g mol⁻¹] = 6500 (GPC), $\bar{D}_M$ = 1.34(GPC).

Representative procedure for AuNP synthesis

Polymer coated hybrid AuNPs were synthesized using **P1** or **P2** or **P4** or **P5**. Glassware used for the preparation of AuNPs was washed three times with copious amounts of nanopure water and dried in the oven for 24 h at 120 °C. To a solution of $HAuCl_4 \cdot 3H_2O$ (0.75 mg, 0.002 mmol) in anhydrous THF (10 mL) was added polymer **P1**, **P2**, **P4**, and **P5** (25 mg each). After stirring the mixture in the dark under a nitrogen atmosphere for 22 h, $LiBH_4$ in THF (1.5 mL, ~ 3 equiv to $HAuCl_4$) was added drop wise under vigorous stirring. After stirring the reaction mixture for 3 h, ethanol (10 mL) was added and stirring was continued for 12 h. The solution was dialyzed against THF applying a 12–14 kDa molecular weight cut-off membrane. The nanoparticles were then characterized by UV/Vis, TEM, and DLS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. GPC traces of polystyrenes obtained at different polymerization conditions

Figure 2.

Kinetic study on RAFT polymerization of Styrene in dioxane at 70 °C with a monomer to CTA to AIBN ratio of 600:1:0.1. a) GPC traces of polymers at different polymerization times. b) Kinetic plot of styrene polymerization using **3**. c) Evolution of the molecular weight and $\bar{D}_M$ with conversion.

Figure 3. TEM image of nanoparticles formed in water, scale bar is 100 nm. a) PEG₇₅₀-*b*-PSt (**P2**), b) lactose-PSt (**P3**), c) PEG₇₅₀-*b*-PSt (**P4**), d) PEG₇₅₀-*b*-PSt (**P5**), e) lactose-PSt (**P6**).

Figure 4.

TEM images of AuNPs synthesized using block copolymers. Scale bar is 100 nm. DIBO-PSt (**P1**) for AuPSt-DIBO (**GNP1**), PEG₇₅₀-*b*-PSt (**P2**) for Au-PSt-*b*-PEG₇₅₀ (**GNP2**), PEG₇₅₀-*b*-PSt (**P4**) for Au-PSt-*b*-PEG₇₅₀ (**GNP3**), PEG₇₅₀-*b*-PSt (**P5**) for Au-PSt-*b*-PEG₇₅₀ (**GNP4**), Au-PSt-DIBO clicked N₃-PEG₇₅₀ for Au-PSt-*b*-PEG₇₅₀ (**GNP5**) and AuPSt-DIBO clicked N₃-dye for Au-PSt-dye (**GNP7**).

Scheme 1.

RAFT polymerization using cyclooctyne-based CTA **3**. a) A general concept of polymerization and post-modification using clickable chain-transfer agent. b) Preparation of cyclooctyne-containing RAFT agent **3** and RAFT polymerization to give polymer **P1**.

Scheme 2.

PEG₇₅₀ and lactose derivatives **4–8** bearing various 1,3-dipoles for metal-free modification of DIBO-PSt.

Scheme 3.

Schematic representation of SPAAC, SPANC, and SPANOC cycloadditions of DIBO-PSI with hydrophilic moieties bearing azide (**4, 5**), nitron (**6**), and oxime groups (**7, 8**).

Scheme 4.

Schematic representation of AuNPs synthesized using block co-polymers: a) DIBO-PSt (**P1**) for Au-PSt-DIBO (**GNP1**), b) PEG₇₅₀-*b*-PSt (**P2**) for Au-PSt-*b*-PEG₇₅₀ (**GNP2**), PEG₇₅₀-*b*-PSt (**P4**) for Au-PSt-*b*-PEG₇₅₀ (**GNP3**), PEG₇₅₀-*b*-PSt (**P5**) for Au-PSt-*b*-PEG₇₅₀ (**GNP4**), Au-PSt-DIBO clicked N₃-PEG₇₅₀ for AuPSt-*b*-PEG₇₅₀ (**GNP5**).

Table 1

Results of RAFT polymerization using **3** at different conditions.

Condition	St: CTA:AIBN ^a	T [°C]	Initiator	t [h]	Conversion [b] [%]	M _n (theor)	M _n [g mol ⁻¹] [c] (GPC)	Đ _M (GPC)
A	100:1:0.1	70	AIBN	20	35	4250	3100	1.44
B	100:1:0.1	50	AIBN	20	16	2200	850	1.14
C	100:1:0.1	40	V-70	20	29	3600	1550	1.21
D	600:1:0.1	70	AIBN	15	20	13150	9450	1.31

^[a] Polymerizations were performed in 1,4-dioxane.

^[b] Conversions estimated by NMR analysis of reaction mixture aliquots.

^[c] Determined against narrow polystyrene standards at 40 °C using tetrahydrofuran as the mobile phase.

Table 2

Analytical data on DIBO-PSt and amphiphilic polymers prepared using post-polymerization modification via 1,3-dipolar cycloadditions.

Polymer	M_n (GPC) ^[a]	D_M ^[a]	M_w (GPC) ^[a]
DIBO-PSt (P1)	6100	1.39	8450
PEG ₇₅₀ - <i>b</i> -PSt (P2)	5300	1.27	6700
lactose-PSt (P3)	6000	1.31	7850
PEG ₇₅₀ - <i>b</i> -PSt (P4)	6050	1.30	7900
PEG ₇₅₀ - <i>b</i> -PSt (P5)	6750	1.37	9250
lactose-PSt (P6)	6500	1.34	8750

^[a] Determined against narrow polystyrene standards at 40 °C using tetra-hydrofuran as the mobile phase.

Table 3DLS data on nanoparticles prepared using amphiphilic block co-polymers **P2–P6**.

Polymer	Effective diameter [nm]	Mean diameter (Lognormal) [nm]	Mean diameter (MSD) [nm]	Poly-disper-sity
PEG ₇₅₀ - <i>b</i> -PSt (P2)	206.2	181.6	205.1	0.05
lactose-PSt (P3)	209.2	113.6	49.2	0.15
PEG ₇₅₀ - <i>b</i> -PSt (P4)	264.4	142.8	132.3	0.15
PEG ₇₅₀ - <i>b</i> -PSt (P5)	143.0	44.1	33.4	0.28
lactose-PSt (P6)	136.5	98.6	50.8	0.09

Table 4

DLS data on gold nanoparticles prepared using amphiphilic block copolymers in THF.

AuNPs	Effective diameter [nm]	Mean diameter [nm]	Poly-dispersity
Au-PSt-DIBO (GNP1)	62.6	16.4	0.168
Au-PSt- <i>b</i> -PEG ₇₅₀ (GNP2)	59.5	29.1	0.174
Au-PSt- <i>b</i> -PEG ₇₅₀ (GNP3)	56.7	30.1	0.156
Au-PSt- <i>b</i> -PEG ₇₅₀ (GNP4)	62.8	22.8	0.147
Au -PSt-DIBO clicked N ₃ -PEG ₇₅₀ (GNP5)	65.7	23.4	0.242
Au -PSt-DIBO clicked N ₃ -Dye (GNP7)	77.9	39.2	0.136