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Single-Chain Technology

Synthesis of Single-Chain Sugar Arrays**

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Numerous examples of functional objects composed of one or a few linear polymer chains exist in nature. Single-chain technology (SCT) aims to create similar functional systems based on discrete synthetic polymer chains.[1] Various single-chain objects, such as unimolecular micelles and single-chain nanoparticles, have been described.[2] In particular, Meijer and co-workers reported interesting examples of single-chain particles shaped by supramolecular interactions.[3] It was demonstrated that such self-assembled globular objects constructed with 3D morphological control could perform specific tasks, such as catalysis in water.[4] These recent results indicate that the field of SCT is developing rapidly.

However, future progress in SCT requires better control over the polymer chemical structure.[1] For example, it is mandatory that the positioning of functional elements along the polymer chains can be controlled precisely. However, for such a goal, the state of the art in polymer science remains in its infancy. Typically, functional groups can be attached at chain ends or directly to polymerizable monomer units. The outcome of the latter strategy is usually limited if two or more functional comonomers are used: in general, random or blocky comonomer sequences are obtained.[5] In this context, new approaches for the control of monomer sequences in synthetic polymerizations have been proposed.[6] For example, our research group has described a method for controlling monomer sequence distribution in controlled radical polymerizations.[7] In this approach, ultrareactive N-substituted maleimides (MIs) are placed at desired locations in a polystyrene chain by time-controlled monomer addition.[8] Although this method does not enable the synthesis of perfectly sequence defined primary structures, it can be applied to a wide variety of monomers and can therefore be used for the synthesis of monomer-sequence patterns of unprecedented complexity.[9] Thus, this approach opens up interesting options for SCT. For example, complex microstructures and topologies can be constructed.[10]

The objective of the present study was to illustrate further the relevance of sequence-controlled polymers for the preparation of tailored functional microstructures. In particular, we present herein a general concept for the preparation of polystyrene chains containing precisely positioned recognition biomolecules (e.g. peptides, oligonucleotides, or sugars). Such macromolecular objects are of fundamental interest, since they may participate in strong and specific interactions with biological systems. For example, single-chain bioarrays could be relevant for applications in diagnostics (e.g. molecular biochips) and biocatalysis (e.g. enzyme immobilization). Such materials could also be prepared on sequence-defined biopolymer backbones, such as peptides or nucleic acids. However, bioinert and biocompatible polystyrene chains are expected to be more technologically and industrially relevant.

As a proof of principle, the synthesis of single-chain sugar arrays is described herein. It has been shown that glycopolymers (i.e. macromolecules composed of a synthetic polymer backbone and pendant sugars) are interesting biohybrid structures for biotechnological applications owing to their recognition of lectins.[11] It is not possible to control the chain positioning of sugar moieties in glycopolymers if they are prepared by conventional approaches, such as chain-growth or step-growth polymerization. However, the use of oligomer backbones synthesized by iterative chemistry enables precise sugar positioning. Numerous examples of synthetic glycopeptides have been described.[12] Recently, Hartmann and co-workers reported the synthesis of short monodisperse glycopolymers based on non-natural oligoamide backbones.[13] However, straightforward methods that enable the synthesis of high-molecular-weight sequence-controlled glycopolymers are still lacking.

The sequence-controlled radical copolymerization of styrene and MIs was studied for the preparation of tailored sugar arrays.[14] Two distinct strategies can be proposed for the introduction of sugar moieties into the copolymer chains: 1) the direct copolymerization of sugar-functionalized MIs, and 2) postpolymerization site-selective functionalization with reactive MIs. The latter route was chosen in the present study (Figure 1). Indeed, postpolymerization modification approaches are versatile, since they are not restricted to one class of molecules (e.g. sugars) but can be applied to a wide variety of molecules. Copper-catalyzed azide–alkyne cycloaddition (CuAAC) was selected in this case as the modification method.[15] This reaction is bioorthogonal and has been shown to be robust for bioconjugation.[16] Figure 1 shows our general strategy. Three derivatives of *N*-propargylmaleimide (PMI) were synthesized. These monomers contain protecting

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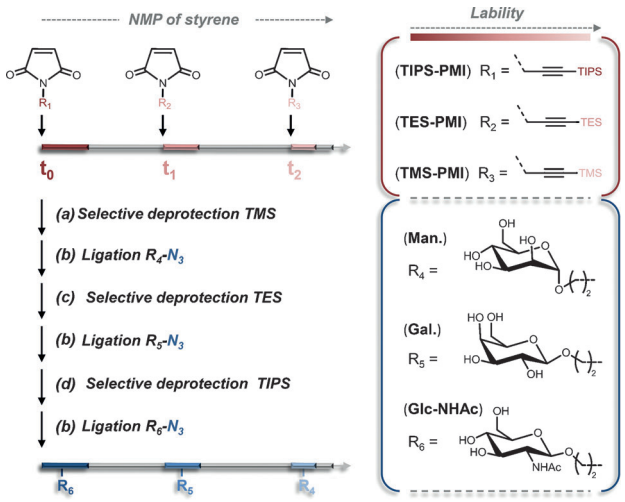

Figure 1. General strategy for the synthesis of single-chain sugar arrays. Experimental conditions: a) K_2CO_3, MeOH/H_2O/THF, 40 °C, 7 h; b) CuBr, 4,4′-di-*n*-nonyl-2,2′-bipyridine, DMF, room temperature; c) K_2CO_3, MeOH/H_2O/THF, 60 °C, 89 h; d) TBAF, THF, room temperature, overnight. DMF = *N*-dimethylformamide, NMP = nitroxide-mediated polymerization, TBAF = tetrabutylammonium fluoride.

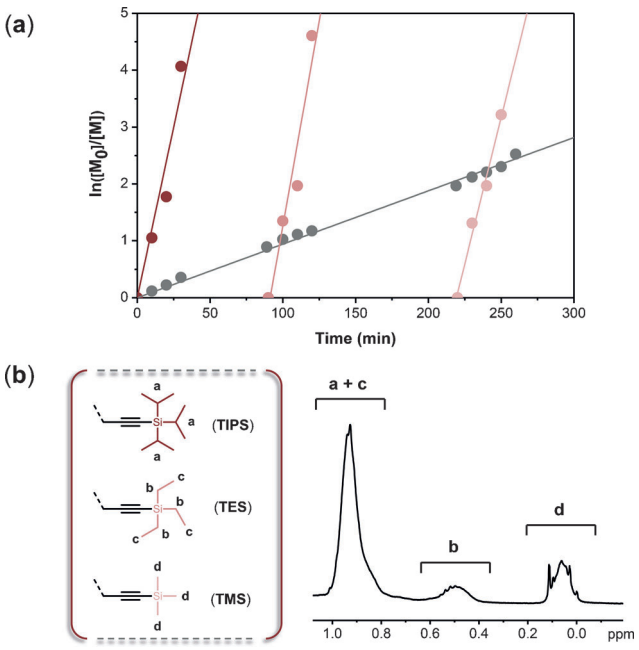

Figure 2. Synthesis of the sequence-controlled copolymers by NMP. a) Semilogarithmic plots of monomer conversion versus time as recorded for the copolymerization of styrene (gray) with TIPS-PMI (red), TES-PMI (dark pink), and TMS-PMI (light pink). This example corresponds to polymer $\mathbf{P_1}$. b) ^{1}H NMR spectrum (the region $\delta = -0.2$–1.1 ppm) of the purified copolymer $\mathbf{P_2}$.

groups of different lability, namely, trimethylsilyl (TMS), triethylsilyl (TES), and triisopropylsilyl groups (TIPS), which can be removed selectively (Figure 1) to enable stepwise modification of the copolymers with azide-functionalized molecules.[17]

The functional polymer backbones were synthesized by the nitroxide-mediated radical copolymerization of styrene (100 equiv) with the protected *N*-propargylmaleimides (2 or 4 equiv of each for polymer $\mathbf{P_1}$ and $\mathbf{P_2}$, respectively) using the commercial alkoxyamine BlocBuilder as the initiator. As described previously,[6a,9] the time-controlled addition of functional MIs during the nitroxide-mediated polymerization (NMP) of styrene enables the preparation of short local functional regions in linear polystyrene chains. According to this strategy, the three protected maleimides were introduced at different polymerization times (i.e. different styrene conversions). The addition times were optimized for the installation of the three functional regions at the beginning, in the middle, and close to the end of the polymer chains (Figure 1). The sequence of addition of the MIs was chosen on the basis of their potential sensitivity to the experimental conditions (Figure 2 A). The least labile monomer, TIPS-protected *N*-propargylmaleimide (TIPS-PMI), was introduced at the beginning of the reaction; at around 50 % styrene conversion, the TES-modified *N*-propargylmaleimide (TES-PMI) was added; and finally, the monomer with the most labile protecting group, that is, the TMS-protected *N*-propargylmaleimide (TMS-PMI), was introduced at the end of the reaction (i.e. at around 75 % styrene conversion). The kinetics of copolymerization confirmed the precise chain positioning of all three MIs in $\mathbf{P_1}$ and $\mathbf{P_2}$ (Figure 2 A; see also Table S1 in the Supporting Information). After purification, the copolymers formed were characterized by ^{1}H NMR spectroscopy (Figure 2 B). Three broad signals at $\delta = 0.05$, 0.44, and 0.87 ppm confirmed the presence of the three protected alkyne functionalities in the molecular structure of $\mathbf{P_1}$ and $\mathbf{P_2}$. Furthermore, analysis by size-exclusion chromatography indicated the formation of well-defined polymers with controlled molecular weights and molecular-weight distributions (see Table S1).

Next, we studied the stepwise orthogonal deprotection and functionalization of the three distinct alkyne sites. To verify the applicability of our strategy, we carried out a first series of experiments with $\mathbf{P_1}$ and three commercially available azide-functionalized model compounds, namely, 1-azidoadamantane, azidomethyl phenyl sulfide, and 11-azido-3,6,9-trioxaundecan-1-amine. We performed ^{1}H NMR spectroscopic analysis after each step of the synthesis to monitor the selective deprotection and functionalization of the alkyne functionalities (Figure 3). First, the TMS group was removed by treatment with K_2CO_3 in a methanol/water/THF mixture at 40 °C for 7 h. These experimental conditions were adapted from reported procedures and should be selective for the TMS group.[17,18] However, $\mathbf{P_1}$ is not fully solubilized under these conditions, but dispersed in the solvent mixture. Nevertheless, after this treatment, the TMS proton signals vanished from the NMR spectrum, whereas the TES and TIPS signals remained unchanged.[19] Consequently, 1-azidoadamantane reacted by CuAAC with the deprotected alkyne site. The cycloaddition was confirmed by the appearance of a new ^{1}H NMR signal at $\delta = 4.4$ ppm corresponding to the CH_2 group located at the α position to the triazole ring. The integration of this new signal suggested quantitative functionalization.

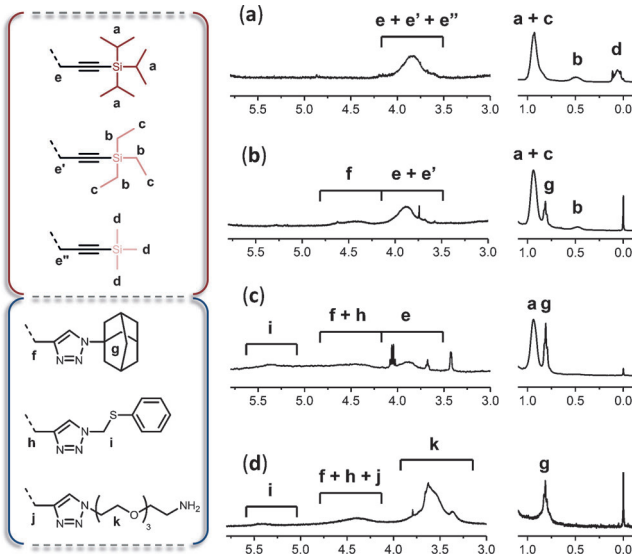

Figure 3. ^{1}H NMR spectra (regions $\delta = -0.1$–1.1 and 3.0–5.8 ppm) recorded at different stages of the modification of $\mathbf{P_1}$: a) initial state; b) after the removal of the TMS protecting group and the ligation of an adamantyl moiety (polymer $\mathbf{P_4}$); c) after removal of the TES group and the attachment of methyl phenyl sulfide (polymer $\mathbf{P_6}$); d) after removal of the TIPS group and final modification (polymer $\mathbf{P_f}$). For clarity, the regions $\delta = -0.1$–1.1 and 3.0–5.8 ppm are presented with different *y*-axis intensity.

The TES group was then removed by treatment with K_2CO_3 in a methanol/water mixture at a higher temperature and for a much longer time, that is, for 89 h at 60 °C. The selective removal of the TES group was confirmed by ^{1}H NMR spectroscopy (Figure 3c).[19] The bare alkyne functionality underwent reaction with azidomethyl phenyl sulfide. The appearance of a ^{1}H NMR signal at $\delta = 5.4$ ppm, which was attributed to the CH_2 group in α position to the sulfide group, confirmed the attachment of this moiety to the polymer backbone. The integration of this signal suggested quantitative site modification. Ultimately, the TIPS protecting group was cleaved by treatment with a solution of tetrabutylammonium fluoride in THF. The terminal alkyne then reacted quantitatively with 11-azido-3,6,9-trioxaundecan-1-amine, as confirmed by the appearance of ethylene oxide hydrogen-atom signals at $\delta = 3.6$ ppm in the ^{1}H NMR spectrum. After all modification steps, the ^{1}H NMR spectrum of the purified copolymer exhibited typical signals of the three covalently attached model compounds and thus indicated that the site-selective functionalization strategy is viable.

Following this successful model study, the site-selective modification strategy was used for the biofunctionalization of $\mathbf{P_1}$ and $\mathbf{P_2}$ with three azide-functionalized hexoses (Figure 1), namely, azidoethyl-α-D-mannopyranoside, azidoethyl-β-D-galactopyranoside, and azidoethyl-*N*-acetyl-β-D-glucosaminide. The TMS, TES, and TIPS protecting groups were cleaved selectively under the conditions described above. After removal of the TMS protecting group, the azidomannose derivative was attached to the chain by CuAAC. The covalent attachment of the mannose moiety was confirmed by the appearance of broad signals in the region $\delta = 3.1$–4.7 ppm of the ^{1}H NMR spectrum of the modified polymer (see Figure S1 in the Supporting Information). The TES group was then removed, and the alkyne was treated with the azido-galactose derivative. This transformation can be carried out in two steps as described above or by a one-pot deprotection/functionalization strategy.[10b,17,20] The ligation of the galactose moiety to the backbone was confirmed by a significant broadening of the signals in the region $\delta = 3.1$–5.0 ppm and by the appearance of additional peak deformations. After removal of the TIPS group and CuAAC with the azido-*N*-acetylglucosamine derivative, a further increase in the intensity of the signals in the region $\delta = 3.1$–5.0 ppm of the ^{1}H NMR spectrum was observed. The final purified polymers were analyzed by ^{1}H NMR and HSQC 2D NMR spectroscopy (see Figure S2). Both techniques confirmed that the polymers had been modified efficiently.

Monitoring of the deprotection and sugar modification of $\mathbf{P_1}$ and $\mathbf{P_2}$ by ^{1}H NMR spectroscopy suggested an efficient stepwise functionalization process. Nevertheless, it was important to demonstrate that the three hexoses retained their biological activity after polymer attachment. Thus, the interaction of the sequence-controlled glycopolymers with hexose-specific lectins was studied by quartz microbalance (QCM). Three different lectins were selected for this study: concanavalin A (Con A) obtained from jack bean, peanut agglutinin (PNA), and wheat-germ agglutinin (WGA), which are specific binding proteins for mannose, galactose, and *N*-acetylglucosamine, respectively. We chose the technique of quartz microbalance with dissipation monitoring (QCM-D) to monitor the sugar–lectin interactions (Figure 4). The QCM chambers were first washed with reverse-osmosis-purified water under a continuous constant flow until stabilization of

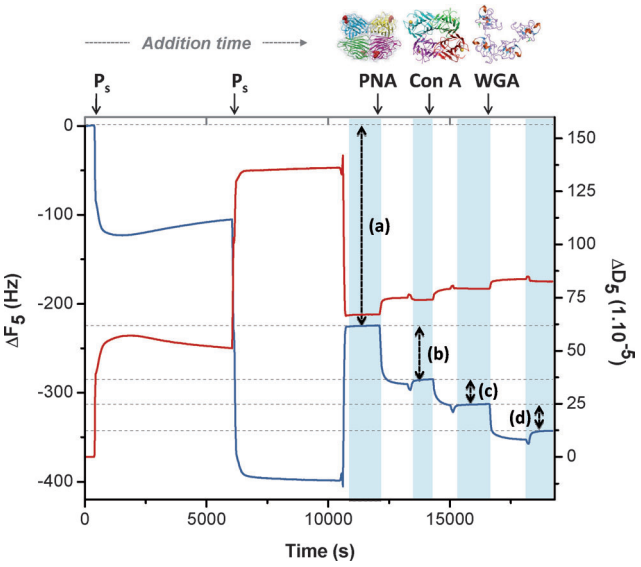

Figure 4. QCM-D plot of the frequency (blue) and dissipation shifts (red) versus time, as recorded for the sugar-functionalized polymer $\mathbf{P_s}$ in the presence of three different lectins (PNA, Con A, and WGA). The light-blue zones indicate rinsing with purified water. The marked differences (a), (b), (c), and (d) correspond to $\mathbf{P_s}$ adsorption, PNA binding, Con A binding, and WGA binding, respectively.

the baseline was observed. Then, the hydrophobic copolymer was adsorbed on the quartz crystal. Typically, a slightly heterogeneous solution of the polymer in a mixture of DMF and H_2O (3:1 v/v) was injected into the analysis chambers. The adsorption of the polymer on the crystal was evident from a significant decrease in frequency. To ensure the adsorption of a large amount of the copolymer, we injected a second polymer solution after 110 min. Before introducing the first lectin, we rinsed the system with water to remove DMF. We then injected a solution of PNA in PBS buffer (100 μL at 0.2 mg mL^{-1}; PBS = phosphate-buffered saline). The binding of this lectin to the galactose moieties of the copolymer was shown by a small decrease in frequency (Figure 4; see also Table S2). Moreover, further rinsing with water did not influence the QCM signal. This observation confirms the formation of strong noncovalent sugar–lectin associations. Similar trends in the QCM signal were observed for the binding of Con A and WGA to mannose and *N*-acetylglucosamine, respectively. A crystal coated with the unmodified precursor $\mathbf{P_1}$ was used as a negative control (Figure S3). In this case, no binding interactions with lectins were identified by QCM. Thus, the QCM-D technique clearly confirmed the accessibility of the biological ligands in the sequence-controlled copolymers.

In summary, it has been demonstrated that sugars can be placed at specific locations on bioinert polystyrene backbones. Such single-chain hexose arrays show specific supramolecular recognition of complementary proteins and suggest a strategy for the design of glycocalyx-mimicking glycopolymers. Moreover, since the density and number of carbohydrate segments in multivalent structures determine the affinity for lectins and their effector functionalities, the control offered by this original technology may enable the development of highly selective glycopolymer-based therapies or trapping systems for viruses and bacterial toxins. The stepwise bioorthogonal polymer-modification strategy described herein can most probably be extended to the synthesis of other biohybrid structures, such as peptide or oligonucleotide single-chain arrays.

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