Brush-First and ROMP-Out with Functional (Macro)monomers: Method Development, Structural Investigations, and Applications of an Expanded Brush-Arm Star Polymer Platform

Matthew R. Golder†, Hung V.-T. Nguyen†, Nathan J. Oldenhuis†, Julian Grundler†,‡, Ellane J. Park†,‡, and Jeremiah A. Johnson*,†

†Department of Chemistry, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, Massachusetts 02139, United States
‡Department of Chemistry, Rollins College, 1000 Holt Avenue, Winter Park, Florida 32789, United States

Abstract

The efficient synthesis of complex functional polymeric nanomaterials is often challenging. Ru-initiated ring-opening metathesis polymerization (ROMP) of multivalent macromonomers followed by cross-linking to form brush-arm star (BASP) polymers enables access to well-defined nano-structures with diverse functionality. This “brush-first” method leaves active Ru in the BASP microgel core, which could potentially be used in a subsequent “ROMP-out” (RO) step to introduce further modifications to the BASP structure via the addition of (macro)monomers. Here, we study this RO approach in depth. The efficiency of RO is assessed for a variety of BASP compositions using a combination of inductively coupled plasma mass spectrometry and gel permeation chromatography. To demonstrate the modularity of the RO process, arylboronic acid-functionalized BASPs were prepared; uptake of these RO-BASPs into hypersialylated cancer cells was enhanced relative to non-functionalized BASPs as determined by flow cytometry and fluorescence microscopy. In addition, the self-assembly of miktoarm BASPs prepared via brush-first and RO with different macromonomers is demonstrated. The combination of brush-first ROMP with RO provides a simple, modular strategy for access to a wide array of functional nanomaterials.

Graphical Abstract
Star polymers synthesized by living polymerization methods are privileged scaffolds in materials science and bio-medicine.\textsuperscript{1} Their three-dimensional architectures provide dense functionality and unique properties compared to their linear analogues.\textsuperscript{2} Most often, star polymers are synthesized through a “core-first” approach or an “arm-first” approach. Though they often allow for precise arm numbers, core-first techniques require the synthesis of a multifunctional core, and they can be limited to the synthesis of star polymers with a low arm number. In contrast, arm-first techniques can afford much greater arm numbers and densities. In addition, arm-first methods offer the ability to fully characterize the star arms before cross-linking, which provides additional insight into the structure of the resulting star polymers.\textsuperscript{1,2}

Inspired by reports from Matyjaszewski and co-workers where macroinitiators\textsuperscript{3–6} and/or macromonomers (MMs)\textsuperscript{7} were cross-linked via atom-transfer radical polymerization (ATRP) to generate narrowly dispersed star polymers following an arm-first strategy, and seeking to generate star-like architectures with exceptionally high arm and functional group density, we have developed the synthesis of brush-arm star polymers (BASPs) using a “brush-first” approach\textsuperscript{8} whereby norbornene-terminated MMs are first polymerized using Ru-initiated ring-opening metathesis polymerization (ROMP) to generate bottlebrush polymer arms that are subsequently cross-linked with bis-norbornene derivatives. Because of their modular functionality and unique nanoarchitecture, BASPs have proven to be unique scaffolds for drug delivery,\textsuperscript{9–12} magnetic resonance imaging,\textsuperscript{13,14} and self-assembly.\textsuperscript{15,16} Moreover, BASP synthesis is amenable to multiplexing,\textsuperscript{9–12} can be done under ambient conditions,\textsuperscript{17} and is readily reproducible on the >100 g scale.\textsuperscript{12}

Though our initial report of brush-first ROMP (Scheme 1) was, to our knowledge, the first example of using Ru-initiated ROMP in the context of arm-first star polymer synthesis, Schrock and co-workers had demonstrated much earlier the arm-first synthesis of star polymers bearing simple functional groups (e.g., esters, ethers, and nitriles) via ROMP using Mo and W alkylidene initiators, small norbornene monomers, and a bis-norbornene cross-linker.\textsuperscript{18,19} Others subsequently showed that addition of small-molecule norbornene-based monomers to living star polymers prepared by arm-first ROMP enables the growth of new arms from the core, thus providing a new handle for tuning the star polymer functionality.\textsuperscript{20–23} Such “in–out” processes have been demonstrated using other living polymerization techniques (e.g., ATRP\textsuperscript{24–30}) as well. Nonetheless, in the context of ROMP, these examples have invariably used small molecule monomers rather than MMs for the ROMP-in and
ROMP-out steps. Though the use of MMs in the ROMP-out step could prove to be challenging due to steric hindrance, if successful, it would provide a novel way to greatly increase the arm density and functional diversity of our BASPs. Herein, we report our studies on the development of a practical brush-first and then ROMP-out (RO) method for the synthesis of “RO-BASPs” using combinations of linear MMs, branched drug-conjugated MMs, and functional small molecule monomers. By leveraging controlled degradation of the RO-BASP core, we can correlate RO-BASP composition with the availability of active Ru initiators, providing new insights into the core environment of these nanostructures. Using electrophilic monomers, we prepare RO-BASPs that can be subsequently modified with various nucleophiles to install functionality that is not compatible with ROMP. Arylboronic acid-functionalized BASPs are synthesized via this approach; these materials are shown to display enhanced rates of cellular uptake in hypersialylated cancer cells compared to traditional BASPs and RO-BASPs lacking the same arylboronic acid functionality.

While we anticipated that RO from BASPs using MMs would be feasible, we were uncertain of what fraction of Ru in the BASP core would be active and available for RO. Indeed, it is possible that only a small fraction of Ru can participate in the RO step, which would critically impact the degree of polymerization of the newly formed RO arms. Matyjaszewski and co-workers have used degradation experiments and reaction kinetics to quantify core initiation efficiency in star polymers prepared by ATRP. Here, we exploit the acid degradability of Acetal-XL to investigate the RO initiation efficiency for BASPs.

We synthesized two sets of four different BASPs (eight samples in total) from a 3 kDa poly(ethylene glycol) MM (PEG-MM) and cross-linker Acetal-XL (Scheme 1). In one set, the bottlebrush backbone degree of polymerization, \( m \), was 7; the number of equivalents of cross-linker, \( N \), was 20; and the amount of MM added in the RO step, \( X \), was 0, 7, 14, or 21. In the second set, these values were \( m = 10, N = 10, \) and \( X = 0, 10, 20, \) or 30. Samples are referred to by their \( m-N-X \) values: 7–20-0, 7–20-7, 7–20-14, 7–20-21, 10–10-0, 10–10-10, 10–10-20, and 10–10-30. All ROMP reactions were quenched by addition of excess ethyl vinyl ether (EVE). The hydrodynamic diameters \( (D_h) \) of these BASPs were similar within each group (20–35 nm for 10–10-X and 35–50 nm for 7–20-X) (Table 1 and Figures S1–S8). We surmised that unlike “traditional” BASPs made by brush-first ROMP, which degrade to their constituent bottlebrush polymers of roughly equal size relative to their parent macroinitiators, RO-BASPs would degrade to a mixture of two unique bottlebrush polymers (Figure 1a). Specifically, chain ends within the BASP that do not undergo further polymerization in the RO step would produce bottlebrush polymers that are similar to the parent bottlebrush arms with additional short blocks derived from the cross-linker (Figure 1a, left), while RO-active chains would produce larger bottlebrush block copolymers that contain both the original bottlebrush arms and the short cross-linker blocks as well as the newly grown RO arms (Figure 1a, right). If the RO efficiency were 100%, we would expect the gel permeation chromatography (GPC) trace of the degraded BASP to show a single peak corresponding to the union of two bottlebrush polymers (of length \( m + N + X \)); however, if RO is not 100% efficient (as is the case for all of the examples presented herein), the RO-brush degradation product should be larger than its theoretical value. As a result, we...
expect that less efficient RO will lead to increasingly asymmetric star polymer architectures with mismatched brush lengths (Figure 2).

In the case of the 7–20-X series, each sample before degradation showed a major BASP peak along with small peaks for trace unreacted MM and brush polymer (Figure 1b, dotted lines). Degradation with excess trifluoroacetic acid (TFA) and GPC analysis (Figure 1b, solid lines) revealed the presence of two major peaks: one that decreases in retention time with X, which corresponds to the coupled brush products of effective RO, and another that remains at roughly the same retention time and corresponds to ineffective RO. A comparison of integrations between the higher molecular weight (MW) (“effective ROMP-out”) component and the lower MW shoulder (“ineffective ROMP-out”) in each GPC trace by curve fitting and integration (Figures S9−S11) reveals that the RO initiation efficiency was 30 ± 10% for the 7–20-X series of samples (see Supporting Information for details). GPC analysis of the 10–10-X series before (Figure 1c, dotted lines) and after (Figure 1c, solid lines) degradation showed a similar set of two major peaks in the latter case; curve fitting and integration (Figures S12−S14) revealed that the RO process was more efficient for this series of samples (50 ± 10%). The difference in RO initiation efficiency between these two sets of samples is likely due to the different amount of cross-linker used (N): the more highly cross-linked samples (7–20-X) have a more sterically crowded core, which leads to lower RO initiation efficiencies.

To further investigate the availability of Ru in the BASP core, we turned to ICP-MS to measure the concentration of Ru still present in BASP samples after quenching with EVE and dialysis against deionized water (Table 1). We reasoned that if the Ru is accessible and active following brush-first ROMP, then its reaction with EVE should cleave it from the BASP and allow for removal. Thus, any remaining Ru is likely inaccessible to EVE (either too sterically crowded or decomposed/inactive) and thus would represent Ru that could not have been accessible to more MM during RO. We note that Ru contamination could also occur due to noncovalent inter-actions between the EVE-quenched Ru Fischer carbene and the poly(norbornene) backbone; dialysis may not completely remove this product despite the fact that it represents available/active Ru. We also assume that inaccessible/inactive Ru is not removed by EVE and dialysis, which is reasonable given the mechanism of ROMP. With these caveats in mind, the data presented in Table 1 represent estimated maximum percentages of inaccessible/inactive Ru; that is, a given BASP sample following brush-first ROMP contains at most the stated amount of inaccessible/inactive Ru. We can readily calculate the fraction of inaccessible/inactive Ru by multiplying the concentration of Ru (measured by ICP-MS) by the total mass of all reagents added to a given reaction mixture (i.e., MM, XL, and initiator) and dividing that product by the mass of Ru added from the Grubbs III (eq 1).

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\% \text{Ru inactive/inaccessible} = \frac{[\text{Ru in sample}](\text{mass of all reagents})}{\text{theoretical mass of Ru from Grubbs III}}
\] (1)
The data reveal that there is no significant difference between accessible/active Ru content for the 7–20-0 and 10–10-0 BASPs; ca. <15% of the chain ends are inaccessible/inactive to EVE (Table 1, significance assessed using a two-tailed t test). Additionally, it should be noted that while the percentage of inactive Ru does not change significantly in either family following RO, the addition of more mass during RO effectively dilutes the total Ru concentration (w/w, ppm). These values are also noted in Table 1 and suggest that RO is an efficient way to lower residual Ru concentration (w/w).

Given the feasibility of RO from BASPs as described above, we next turned to using RO to install new functionality into BASPs via the use of functionalized (macro)monomers. Nomura and co-workers previously showed that after RO using small molecule monomers and Mo catalysis, it was possible to functionalize the chain-ends of their polymers via quenching with a variety of benzaldehydes.20 Rather than use quenching as a method to install functionality, we reasoned that it would be possible to further tune the properties of our BASPs through the use of a RO monomer containing a functional group for postpolymerization modification. To demonstrate this concept, we prepared a new set of RO-BASPs using PEG-MM and Acetal-XL where 2 equiv of p-nitrophenyl carbonate-functionalized norbornene monomer Nb-PNP (Scheme 2) were added following consumption of PEG-MM to extend the RO step and afford 7–20-7–2PNP and 10–10-10–2PNP RO-BASPs capable of functionalization by addition of an appropriate nucleophile (e.g., Cap-B(OH)_2 or Cap-Bn, Scheme 2). As we were particularly interested in developing a means by which we could enhance selective cellular uptake of BASPs,31,32 we envisioned treating 7–20-7–2PNP and 10–10-10–2PNP RO-BASPs with a suitable nucleophile that could mediate cell uptake (i.e., a cell-surface targeting ligand). Prior work from the Kataoka laboratory on nanostructures containing phenyl boronic acids33 showed that such materials are capable of highly selective sialic acid binding, which is believed to be orders of magnitude higher than that of other sugars due to a proximal coordinating amide.34–36 During growth, some cancer tissues experience hypersialylation (overexpression of sialic acid functionalized surface glycoproteins).37 Hence, we wondered whether BASPs labeled with phenylboronic acids would also be taken up more readily than their unlabeled RO-BASP and traditional BASP analogues through a receptor-mediated process.

Nickel-catalyzed Miyaura borylation38 of readily accessible 4-chlorobenzyl(N-Boc-amine) with tetrahydroxydiboron afforded the putative arylboronic acid, which was deprotected with TFA to afford Cap-B(OH)₂ in 34% yield over three steps. Treatment of crude 7–20-7–2PNP and 10–10-10–2PNP with excess Cap-B(OH)₂ (5 equiv relative to Nb-PNP used) and DIPEA (5 equiv) in 1:1 1,4-dioxane/DMSO afforded arylboronic acid-functionalized BASPs 7–20-7–2BA and 10–10-10–2BA with >92% conversion within 48 h based on HPLC analysis of p-nitrophenol production (Figure S15 and Table S1). To prove that any in vitro cell uptake results were due to the introduction of an arylboronic acid as opposed to the presence of carbamate or aryl functional groups, a control sample 10–10-10–2Bn was made using benzylamine (Cap-Bn) as the nucleophile. Importantly, when 10–10-10–2PNP was treated under identical functionalization conditions in the absence of a benzylic amine nucleophile, no p-nitrophenol was observed, suggesting a negligible rate of background PNP-carbamate hydrolysis by trace water. Excess amine nucleophile and p-nitrophenol were removed from the reaction mixture during the subsequent dialysis of the crude reaction mixture against
water. In addition to the functionalized 7–20-7–2BA and 10–10-10–2BA polymers, we also tested 7–20-0, 10–10-0, 7–20-7, and 10–10-10 BASPs. All polymers contained 1 mol % Cy5.5-MM to facilitate in vitro cell uptake quantification. Importantly, similar GPC retention times (Figure S16) and $D_h$ values (Figures S17 and S18) were observed for functionalized and unfunctionalized BASPs, suggesting that new molecular functionality could be installed without altering the size of the particles.

To begin to assess the effects of BASP structure on cell uptake, we incubated the aforementioned polymers with A549 (adenocarcinoma human alveolar basal epithelial cells) cells in DMEM (37 ºC, 5% CO$_2$) for 1, 6, or 24 h. Subsequent analysis by flow cytometry (Figure 3) revealed striking differences in cell uptake at each time point. In all cases, 10–10-X BASPs exhibited higher mean fluorescence than 7–20-X BASPs prepared from the same components, presumably due to differences in $D_h$ and/or PEG grafting densities. Interestingly, RO-BASPs without targeting ligands (i.e., 7–20-7 and 10–10-10) exhibited higher mean fluorescence than traditional BASPs (i.e., 7–20-0 and 10–10-0), perhaps due to an increase in PEG density for the RO-BASPs. The inclusion of phenylboronic acid groups (7–20-7–2BA and 10–10-10–2BA) resulted in up to a 9-fold increase in mean fluorescence within 24 h relative to the RO-BASPs 7–20-7 and 10–10-10. At all times points, 7–20-7–2BA and 10–10–10–2BA exhibited higher mean fluorescence compared to 7–20-7 and 10–10-10, respectively (P < 0.0001, two-tailed t test).

Further analysis reveals the effects of stoichiometry (i.e., 7–20-7 versus 10–10-10) on cellular uptake. For example, the ratio of the mean Cy5.5 fluorescence for 10–10-10 to 7–20-7 was 2.64 ± 0.19 after 6 h and 2.55 ± 0.11 after 24 h time points. In other words, for the unfunctionalized BASPs, 10–10-10 has a ~2.6-fold greater uptake than 7–20-7 at both time points. In contrast, the same ratios for the boronic acid-functionalized 10–10-10–2BA and 7–20-7–2BA RO-BASPs were 1.69 ± 0.07 and 1.67 ± 0.04, respectively. These values are significantly different (P < 0.001) from those of the unfunctionalized RO-BASPs, suggesting that the differences in uptake between the 10–10-X and 7–20-X BASPs are less pronounced after RO and installation of boronic acid groups; this effect is likely due to the lower RO initiator efficiency, and resulting longer RO arms, of the 7–20-7–2BA RO-BASP.

Confirming the role of the arylboronic acid ligand in active transport of RO-BASPs into cells, there was no significant difference in uptake between 10–10-10 and 10–10-10–2Bn BASPs (Figure 3). In addition, significant differences in mean fluorescence intensity were not observed for 7–20-7 and 7–20–7–2BA or 10–10-10 and 10–10–10–2BA when cells were incubated with these polymers for 1 h at 0 ºC where the rate of active transport is decreased (Figure S19).

Fluorescence microscopy was employed to ensure that our flow cytometry results were not due to cell surface adhesion (Figure 4). A549 cells were incubated as described above for 24 h prior to washing with PBS and fixing (1% paraformaldehyde) for imaging. Based on the merged DAPI/ Cy5.5 images under identical imaging conditions, boronic acid-labeled BASPs 7–20-7–2BA and 10–10–10–2BA are distributed throughout the cytosol while unlabeled BASPs 7–20-7 and 10–10-10 are not visible under the same imaging acquisition settings (Figure 4 and Figure S20).
We were also curious how BASPs would compare to their short star-like bottlebrush polymer analogues using similar cell uptake experiments. Two of such polymers \((n = 10)\) were synthesized using PEG-MM and 1 mol % Cy5.5-MM: 10–0–0 (note: this sample is identical to the macroinitiator for all 10–10–X BASPs) and 10–0–0–2BA (Figure 5a and Figure S16), the latter of which was prepared by sequential polymerization of MM followed by Nb-PNP and subsequent treatment with Cap-B(OH)₂. To our surprise, after incubating A549 cells with the two bottlebrush polymer samples alongside the BASP samples presented in Figure 4, both bottlebrush polymer samples showed very low minimal relative mean fluorescence intensity. In fact, 10–0–0–2BA had significantly lower mean fluorescence intensity than its unlabeled counterpart \((P < 0.0001)\). Not only did the boronic acid tag not influence bottlebrush uptake, it actually performed worse than when the boronic acid moiety was omitted \((10–0–0)\). In addition, perhaps counterintuitively, the boronic acids on the end of 10–0–0–2BA appear to be less effective than the boronic acids on 7–20–7–2BA and 10–10–10–2BA, despite the potential steric bulk in the latter examples. We are currently investigating these findings in more detail; nevertheless, these results should be informative for the design of bottlebrush- and star polymer-based polymeric materials for translational biomedical applications.

We also imagined that the RO approach could be used to access drug-conjugated BASPs in a divergent fashion. Rather than synthesizing “branched-MMs” (i.e., polymer–drug conjugate MMs) that would be subsequently used in traditional brush-first ROMP,\(^{39}\) we wondered if we could synthesize an analogous RO-BASP using a norbornene–drug conjugate and a living PEG BASP. This approach would allow for the generation of numerous RO-BASP prodrugs from a common, living BASP precursor after the addition of drug-conjugated monomers. Accordingly, we synthesized two BASPs conjugated to paclitaxel (PTX) by either our standard approach (polymerization of branched PTX-MM to form a 10–10–0 BASP, Figure 6a, top) or the RO approach (RO of PTX-monomer from a PEG BASP, 10–10–10 RO-BASP, Figure 6a, bottom). These BASPs were very similar in size as determined by GPC (Figures S21 and S22) and dynamic light scattering (DLS) \((D_h = 23 \pm 11 \text{ nm, respectively})\) (Figure 6b), though the 10–10–10 RO-BASP appeared to show slightly more aggregation. To assess the cytotoxicity of these BASPs, we measured \textit{in vitro} HeLa cell viability \((IC_{50})\) with the MTT/ formazan colorimetric assay after incubating the cells for 48 h with both BASPs. As shown in Figure 6c, both BASPs have similar \(IC_{50}\) values \((1.1 \pm 0.2 \mu g \text{ BASP/mL and } 1.3 \pm 0.2 \mu g \text{ RO-BASP/mL})\), suggesting that the difference between the traditional branched MM approach and the RO approach has minimal impact on \textit{in vitro} HeLa cell viability. We anticipate that this RO strategy will allow for modular access to BASP prodrugs in a complementary manner to the use of MM prodrugs and may enable novel strategies for the synthesis of multidrug/imaging agent-functionalized RO-BASPs.

Lastly, we sought to prepare miktoarm BASPs by using two different MMs in the brush-first and RO steps (Figure 7). Such an approach offers a new way to prepare miktoarm BASPs\(^{15,16}\) and simultaneously allows for another means to analyze the efficiency of the RO step by comparing the traditional BASP made from brush-first ROMP to one made via brush-first ROMP and then RO.\(^{26,28}\) A polydimethylsiloxane (PDMS)-based BASP (PDMS-BASP) was prepared via ROMP of PDMS-MM (Figure 7) followed by cross-linking with Acetal-XL and quenching with EVE. The properties of this PDMS-BASP were compared to

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that of a RO-BASP made by adding a polystyrene MM (PS-MM; Figure 7a) to the living PDMS-BASP prior to quenching (PDMS/PS-BASP; Figure 7b). GPC indicated that the conversion of both MMs was very high, and as expected, the molecular weight of PDMS/PS-BASP was greater than that of PDMS-BASP (Figure S23), suggesting that RO was successful. Despite the larger MW of PDMS/PS-BASP measured by GPC, DLS revealed (Figures S24 and S25) that the diameters of these two materials in tetrahydrofuran were similar (ca. 18 nm), suggesting that the addition of PS-MM did not significantly increase the size of the BASP. Small-angle X-ray scattering (SAXS) provided insight into the miktoarm BASP structure in the bulk state (Figure 7c). The scattering curve for PDMS-BASP possessed a single, broad peak in the low q region (d = 13.8 nm), which we attribute to the spacing between adjacent interdigitated PDMS-BASP. In contrast, PDMS/PS-BASP displays a lamellar morphology (q* = 26.2 nm), with peaks observed at 2q* and 3q*. It is likely that the immiscible PDMS and PS brush arms phase segregate within the miktoarm BASP forming Janus-type structures (Figure 7d) where the high arm density inhibits interdigitation.\textsuperscript{15,40–43}

Herein we describe detailed studies of RO from living BASPs. The efficiency of RO was quantified for various stoichiometries using ICP-MS and GPC. This approach provides new insights into the fraction of active chain ends within the core of BASPs and allows us to correlate differences in RO-BASP stoichiometry with RO efficiency. In all cases, while 7–20-X BASPs were taken up by cells less readily than 10–10-X BASPs due to their increased hydrophobicity, the inclusion of arylboronic acid tags increased uptake by up to 9-fold. The role of the arylboronic acid tags for sialic acid targeting was confirmed by both low temperature uptake studies and independent preparation of a RO-BASP bearing an unfunctionalized benzyl carbamate (10–10–10–2Bn). The importance of BASP architecture was further highlighted by comparison to bottlebrush polymer samples with (10–0–0–2BA) or without (10–0–0) boronic acids; the RO-BASPs greatly outperformed the bottlebrush polymers in terms of cell uptake. We utilized RO to synthesize a macromolecular PTX produg; BASPs synthesized by our traditional branched MM approach, and our new RO method had similar sizes and exhibited similar IC\textsubscript{50} values for HeLa cell viability. Finally, we expanded the structural diversity of RO-BASPs through a representative miktoarm BASP synthesis. The results presented in this work greatly expand upon the diversity of the BASP platform and open new avenues for the design of branched polymers prepared by ROMP.

**Supplementary Material**

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### ABBREVIATIONS

| Abbreviation | Description |
|--------------|-------------|
| ATRP         | Atom transfer radical polymerization |
| BASP         | Brush-arm star polymer               |
| DLS          | Dynamic light scattering             |
| EVE          | Ethyl vinyl ether                     |
| GPC          | Gel permeation chromatography         |
| ICP-MS       | Inductively coupled mass spectrometry |
| MM           | Macromonomer                          |
| PEG          | Poly(ethylene glycol)                 |
| PDMS         | Poly(dimethylsiloxane)                |
| PS           | Poly(styrene)                         |
| PTX          | Paclitaxel                             |
| RO           | ROMP-out                               |
| ROMP         | Ring-opening metathesis polymerization |

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Scheme 1. Schematic for “ROMP-Out” as a Strategy for the Installation of New Functionality into Brush-Arm Star Polymers (BASPs)\textsuperscript{a} 

\textsuperscript{a}First, brush-first ROMP is used to generate a BASP with active Ru present in the cross-linked core. Then, additional (macro)monomers are added to “ROMP-Out” (RO) from the BASP core generating a RO-BASP. Poly(ethylene glycol) macromonomer (PEG-MM) and cross-linker (Acetal-XL) used in this work to establish the brush-first and RO method. Representative sample names for the various BASPs and RO-BASPs prepared in this work are provided in bold text. Grubbs III = Grubbs 3rd generation bis-pyridyl complex.
Scheme 2. “ROMP-Out” Affords BASPs That Can Be Functionalized around the Core Periphery$^a$

$^a$Addition of 2 equiv of Nb-PNP after RO provides an activated electrophile capable of reacting with benzylic amines. Conversion for 10–10–10–2PNP to the substituted carbamate star polymer is monitored by HPLC; the appearance of p-nitrophenol is directly proportional to reaction progress. Cy5.5 is omitted from the BASP schematic for clarity.
Figure 1.
(a) Schematic representation of possible lower molecular weight (MW) and higher MW products formed after acid-mediated BASP degradation. Lower MW products arise from ineffective ROMP-out while higher MW products arise from effective ROMP-out. (b) GPC traces of 7–20-X BASPs before and after degradation. (c) GPC traces of 10–10-X BASPs before and after degradation. Direct estimation of RO efficiency is done by curve fitting and integration (see Table 1 for details). *Indicates peaks that represent small amounts of unfunctionalized/unconverted MM.
Figure 2.
(a) Schematic representation of 100% efficient RO where all chain ends in the core possess an active Ru alkylidene that is sterically accessible for RO. In this case, the average degree of polymerization for the RO arms, assuming 100% MM conversion, is equal to the amount of MM added in the RO step: X. (b) Schematic representation of 50% efficient RO where only half of the chain ends in the core possess an active Ru alkylidene that is sterically accessible for RO. In this case, assuming 100% MM conversion, the RO arms have a length of 2X. Thus, the MWs of the decomposition products of RO-BASPs reflect the RO efficiency.
Figure 3.
Quantification of BASP uptake (37 °C) into A549 cells as a function of time (1, 6, and 24 h) as determined by mean Cy5.5 fluorescence intensity using flow cytometry. Data are presented as mean ± SD (comparisons were made using a two-tailed t test, n = 3 data sets per sample per time point). ****P < 0.0001. Results are from at least 6000 independent events except for the following: 10–10-10–2BA at 24 h (n = 1 sets are from 4800 events), 10–10-10–2Bn at 24 h (n = 1 sets are from 4500 events), 10–10-10–2Bn at 6 h (n = 2 sets are from 5200 and 5300 events), 10–10-10–2BA at 1 h (n = 1 sets are from 5100 events), 10–10-10–2Bn at 1 h (n = 2 sets are from 4200 and 5200 events).
Figure 4.
Fluorescence microscopy images (30×) of nuclei (DAPI stained, blue, left), BASPs (Cy5.5, pink, middle), and merged DAPI/ Cy5.5 (right). For BASPs without boronic acid (BA) ligands (7–20-7 and 10–10-10, i.e., (a) and (b), virtually no Cy5.5 signal is detectable under these imaging conditions. Increased Cy5.5 fluorescence is observed for 7–20-7–2BA and 10–10-10–2BA (c, d). Exposures for each image: 69 ms (DAPI) and 440 ms (Cy5.5). Scale bar = 25 μm.
Figure 5.
(a) Synthesis of boronic acid end-functionalized bottlebrush polymer 10–0–0–2BA (note: 1% Cy5.5-MM not shown for clarity). (b) Comparison of BASP and bottlebrush polymer A549 cell uptake (37 °C, 24 h) as determined by mean Cy5.5 fluorescence using flow cytometry. Data are presented as mean ± SD (two-tailed t test, n = 3 data sets per sample per time point). ****P < 0.0001. Results are from at least 10000 independent events.
Figure 6.
(a) Synthesis of PTX-BASP from a paclitaxel (PTX)-conjugated branched MM. (b) Synthesis of PTX-RO-BASP via ROMP out. (c) DLS histograms showing that PTX-BASP and PTX-RO-BASP have similar hydrodynamic diameters ($D_h$). Note: the populations of aggregated BASPs represent <10% of the total sample by mass. (d) HeLa cell viability curves (from MTT assay) showing similar toxicity for PTX-BASP and PTX-RO-BASP. Data in (d) presented as mean ± SD ($n = 3$).
Figure 7.
Synthesis of miktoarm RO-BASPs. (a) MMs used in this study derived from poly(dimethylsiloxane) (PDMS-MM) and polystyrene (PS-MM). $M_n =$ number-average molar mass. (b) Synthesis of PDMS-BASP by brush-first ROMP followed by miktoarm PDMS/PS-BASP formation by RO. (c) 1D SAXS curves for PDMS-BASP and PDMS/PS-BASP. (d) Proposed bulk assembly of PDMS-BASP and PDMS/PS-BASP.
### Table 1.

**Physical Properties of Traditional and RO-BASPs**

| sample    | $M_w$ ($\text{kDa}$) | $D_h$ (nm) | Ru content (ppm) | inactive chain ends (%) |
|-----------|----------------------|------------|------------------|-------------------------|
| 7–20–0    | 1200                 | 33(15)     | 267(37)          | 9(1)                    |
| 7–20–7    | 2000                 | 39(16)     | 239(85)          | 14(5)                   |
| 7–20–14   | 2400                 | 46(18)     | 144(49)          | 11(4)                   |
| 7–20–21   | 2800                 | 50(9)      | 153(28)          | 16(3)                   |
| 10–10–0   | 220                  | 20(8)      | 308(65)          | 12(2)                   |
| 10–10–10  | 370                  | 26(9)      | 190(51)          | 13(4)                   |
| 10–10–20  | 620                  | 31(9)      | 117(26)          | 12(3)                   |
| 10–10–30  | 850                  | 37(12)     | 94(8)            | 13(1)                   |

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*a* Measured by GPC-MALS (0.025 M LiBr in DMF, $dn/dc = 0.044$).

*b* Measured by dynamic light scattering (DLS).

*c* Measured by ICP-MS. Data presented as mean standard deviation, SD for $n = 3$ independent measurements.

*d* Calculated using eq 1 based on Ru concentrations measured by ICP-MS ($n = 3$). Data presented as mean (SD) for $n = 3$ independent measurements.