Efficient click-addition sequence for polymer-polymer couplings

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Abstract

Controlled radical polymerization methods and click chemistry form a versatile toolbox for creating complex polymer architectures. However, the incompatibility between the functional groups required for click reactions and the reaction conditions of radical polymerization techniques often limits application. Here, we demonstrate how combining two complementary click reactions in a sequence circumvents compatibility issues. We employ isocyanate-amine addition on a polymer obtained by RAFT without purification, thus allowing us to work at exact equimolarity. The addition of commercially available amine-functional azido or strained alkyne compounds, yields orthogonally modified polymers, which can be coupled together in a subsequent strain promoted cycloaddition (SPAAC). The efficiency of this reaction sequence is demonstrated with different acrylate, methacrylate, and acrylamide polymers giving block copolymers in high yield. The resulting diblock copolymers remain active towards RAFT polymerization, thus allowing access to multiblock structures by simple chain extension. The orthogonality of the isocyanate-amine reaction, SPAAC and RAFT polymerization (both in terms of monomer and chain end groups) is a key advantage and offers access to functional and challenging polymer architectures without the need for stringent reaction conditions or laborious intermediate purifications.

Introduction

The last 20 years have dramatically changed the field of polymer synthesis, with the development of new synthetic methodologies enabling the production of a wealth of new materials with potential applications in engineering and life sciences. In particular, controlled radical polymerization techniques attracted considerable attention due to their extensive control of the polymer architecture without requiring stringent reaction conditions.[1-4] Nevertheless, access to certain structures remains challenging and requires alternative chemistry, for example in the synthesis of comb-like polymers, block copolymers with crystalline domains or the combination of vinyl ether based monomers with methacrylates.[5-7] The most powerful approach to achieve this is the coupling of independently made polymer chains *via* reactive linkers,[8, 9] although this route requires efficient reactions, typically based on click chemistry, working at equimolarity circumvents demanding purifications of the reaction product.[10] The most prominent examples of these click reactions are the radical thiol-ene reactions,[11] Diels-Alder couplings,[12] or the copper catalyzed azide-alkyne cycloaddition (CuAAC),[13] the latter being considered as the gold standard in click reactions. These reactions have been employed to produce a wealth of materials, including hyperbranched structures, to ligate polymers and to introduce functional side arms.[14-17] All these reactions have, however, limitations due to undesirable side reactions during radical polymerization or with the monomers.[18, 19] Furthermore, many reactions require the use of catalysts or high temperatures, which may intervene with other functionalities on the polymer, such as the active chain end in reversible addition−fragmentation chain-transfer (RAFT) polymerizations. In consequence, the orthogonality to controlled radical polymerization methods is lost and reactions cannot be conducted at the required equimolarity. Recently, new synthetic strategies have been shown to yield polymer-polymer couplings with high efficiency and purity.[20, 21] Barner-Kowollik et al. for example reported a hetero Diels-Alder reaction, which elegantly uses the RAFT chain end for a cycloaddition and proceeds to full conversion within minutes.[22] Other approaches also make use of cycloaddition reactions such as the Triazolinedione (TAD) coupling with dienes or the tetrazole-ene coupling.[23, 24] However, all these reactions still use at least one reagent that is incompatible with a radical polymerization and therefore has to be introduced in a post polymerization step. Unfortunately, these modifications require the purification of the polymers either prior to the functionalization due to incompatibility with residual monomer, or after the introduction of a reactive end group as the reagents have to be used in excess. These purification steps, in addition to being a laborious process, prevent working at equimolarity in

a polymer-polymer coupling reaction, since the exact number of polymeric chain end groups present in the reaction cannot be calculated due to the molar mass distribution of the polymeric reagents. Recently, we showed that RAFT polymerization combined with the addition of an amine to a tertiary isocyanate chain end group, fulfils all criteria of a polymer click reaction and can easily be performed at equimolarity, as the number of end-groups is determined by the initial quantity of chain transfer agent (CTA) and no purification is required prior to the coupling.[25]

Here, we demonstrate that this approach can be further expanded using a second, fully orthogonal click reaction – the strain promoted azide-alkyne cycloaddition (SPAAC) – to become a versatile and convenient tool for linking a variety of different polymers. SPAAC has so far been scarcely recognized in polymer chemistry, but it has attracted considerable attention in biomedicine and pharmacology for labelling or conjugation.[26, 27] This reactions proceeds without the need of catalysts or elevated temperatures to achieve high conversion and tolerates many functional groups as it can even be used for *in vivo* labelling.[28] However, the high reactivity of the strained alkyne towards radicals and the potential side reactions of azides with acrylate based monomers have limited the application of SPAAC in radical polymerization.[19, 29] These limitations can be overcome by combining SPAAC with our isocyanate chemistry, in order to introduce the strained alkyne and azide moiety in a post-polymerization step, using commercially available, amine-functionalized compounds (**Figure 1**).

We employ our previously described methodology to benchmark polymer conjugation by linking the corresponding homopolymers *via* this click addition sequence and calculate the efficiency of the coupling. Two commercially available strained alkynes (BCN-NH_2: N-[(1*R*,8*S*,9*s*)-Bicyclo[6.1.0]non-4-yn-9-ylmethyloxycarbonyl]-1,8-diamino-3,6-dioxaoctane and DBCO-NH_2: 3-Amino-1-(11,12-didehydrodibenzo[*b*,*f*]azocin-5(6*H*)-yl)propan-1-one) are tested for their stability and kinetics.[30, 31] Having established the procedure, several mixed block copolymers are prepared, and chain extension experiments prove that the CTA end-group remains intact.

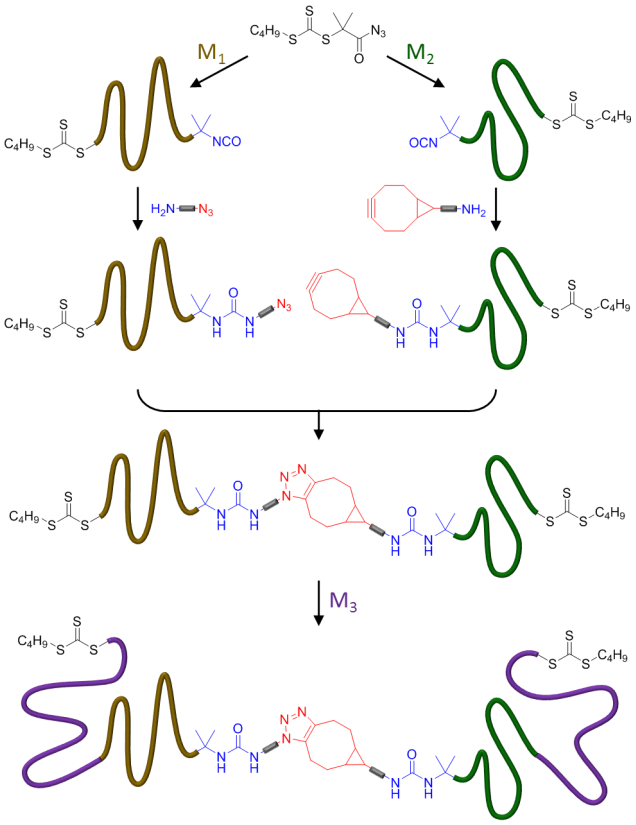

Figure 1. Schematic reaction procedure starting with 1-azido-2-methyl-1-oxopropan-2-yl butyl carbonotrithioate (BIAzTC) which undergoes a Curtius rearrangement in the first polymerizations (with monomers M_1 or M_2) to provide the isocyanate end-group. The azide and alkyne (BCN-NH_2 shown as example) are introduced *via* amine-isocyanate click to the individual polymers, which are subsequently combined for the formation of the block copolymer. To prove the orthogonality of the reactions to the CTA, the active chain ends are extended with a third monomer (M_3).

Results and discussion

We used 1-azido-2-methyl-1-oxopropan-2-yl butyl carbonotrithioate (BIAzTC) to prepare a variety of isocyanate functionalized polymers including hydrophobic poly(*n*-butyl acrylate) (pBA) and poly (methyl methacrylate) (pMMA), hydrophilic poly(4-acryloylmorpholine) (pNAM), and a sterically hindered poly(poly(ethylene glycol) methyl ether acrylate) (pPEGA) (summarized in **Table 1**).

Table 1. Polymerization conditions and results for the isocyanate precursor polymers used for coupling.

Sample	$[M]_0/[CTA]_0$	$[CTA]_0/[I]_0$	Monomer conversion[a]	$M_{n,th}$[b] (kg mol^{-1})	$M_{n,SEC}$[c] (kg mol^{-1})	$Đ$
$pNAM_{10}$	10	20	99%	1.7	1.9 [c]	1.11
$pNAM_{37}$	40	20	91%	5.5	4.7 [d]	1.14
pBA_{42}	50	20	84%	5.7	6.6 [d]	1.11
pBA_{206}	250	10	83%	26.7	25.5 [d]	1.14
$pPEGA_{38}$	50	10	76%	18.5	15.8 [d]	1.21
$pMMA_{27}$	50	10	54%	3.0	4.0 [d]	1.24

[a] Determined by ^{1}H NMR; [b] Calculated from $[M]_0/[CTA]_0$ and conversion; [c] Determined by SEC using Chloroform (2% Triethylamine) as eluent, calibrated with pMMA standards. [d] Determined by SEC using DMF (0.1% LiBr) as eluent, calibrated with pMMA standards.

To introduce the orthogonal functionalities BCN-NH_2 (obtained from Aldrich, purity > 95%) or DBCO-NH_2 (Jena Biosciences, purity > 95%) and azidopropylamine (Alfa Aesar, >98%), each bearing a primary amine group, the polymers, still in their original solution for polymerization, were reacted with exactly 1 equivalent (to CTA end group) of the respective compound. Remarkably, no extra precautions were made for avoiding water or oxygen, as standard solvents were used and solutions were not degassed. Furthermore, the NMR samples demonstrate that in all cases, except $pNAM_{10}$ (99% conversion), unconsumed monomers were still present (SI, Figure S1-S6).

Figure 2 illustrates the high yield of the reaction by determining the degree of functionalization of $pNAM_{10}$ by Electron Spray Ionisation-Time of Flight (ESI-ToF) measurements (**Figure 2**, full spectra in SI Figure S7-S10). NMR spectra of the products after reaction with the respective amines were also recorded (Figure S11-S12), but the overlap of signals with the polymer backbone or solvents prevents accurate quantification of the modification efficiency. In addition to ESI-ToF and NMR, we monitored the reaction with IR which showed a complete disappearance of the characteristic signal for the isocyanate at 2250 cm^{-1} (Figure S13).

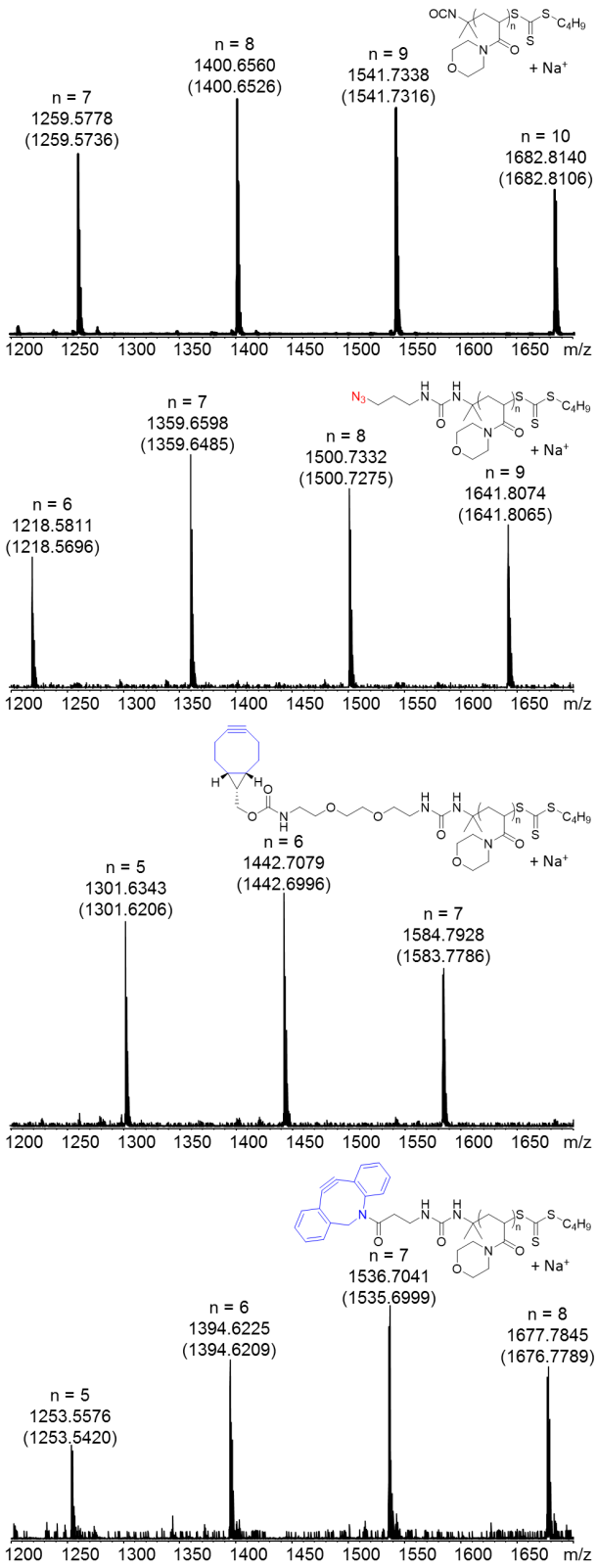

Figure 2. Electron spray ionisation-time of flight (ESI-ToF) measurements of the initial isocyanate modified polymer ($pNAM_{10}$-NCO) (a), after addition of 1 eq. of azidopropylamine (b), after addition of 1 eq. of BCN-NH_2 (c), and after addition of 1 eq. of DBCO-NH_2 (d). The calculated theoretical molecular weight values are given in brackets.

No side reactions were observed in the ESI-ToF or NMR spectra, proving the absence of any undesired side reactions of either the strained alkynes or the azides with the isocyanate groups, the CTA or the remaining monomer; a key requirement for efficient coupling of the polymer chains. Subsequently we combined the obtained polymers bearing orthogonal functionalities and tested the efficiency of the SPAAC. The reaction was examined for its conversion by size exclusion chromatography (SEC) as previously reported (**Figure 3**).[25] The respective number distribution plot is given in the SI (Figure S15). The tailing towards lower molecular weights is partially due to difficulties to correct the baseline being close to the lower limit of the separation range of the SEC.

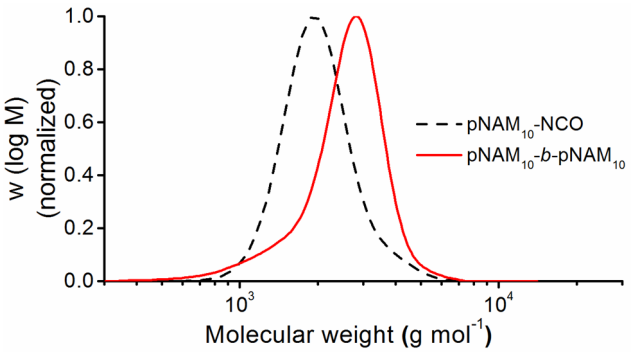

Figure 3. Normalized SEC traces (SEC: $CHCl_3$) of the initial polymer $pNAM_{10}$-NCO (dashed black line) and the polymer linked (red line) *via* SPAAC after modifying equal amounts of the precursor with 1 eq. of azidopropylamine or BCN-NH_2, respectively. The respective number distribution plot is given in the SI (Figure S15).

The SEC trace shifted towards higher molecular weight indicating that the polymers were coupled. Furthermore, comparison of the IR-spectra of the starting material and the coupling reaction after 4h revealed a complete disappearance of the azide signal at 2095 cm^{-1} indicating a high coupling efficiency (Figure S14). Encouraged by this result we applied the procedure to higher molecular weight pNAM and other types of polymers. The corresponding SEC traces are shown in **Figure 4** (The raw RI signal vs. retention time and the corresponding number distribution plots are given in Figures S16-20).

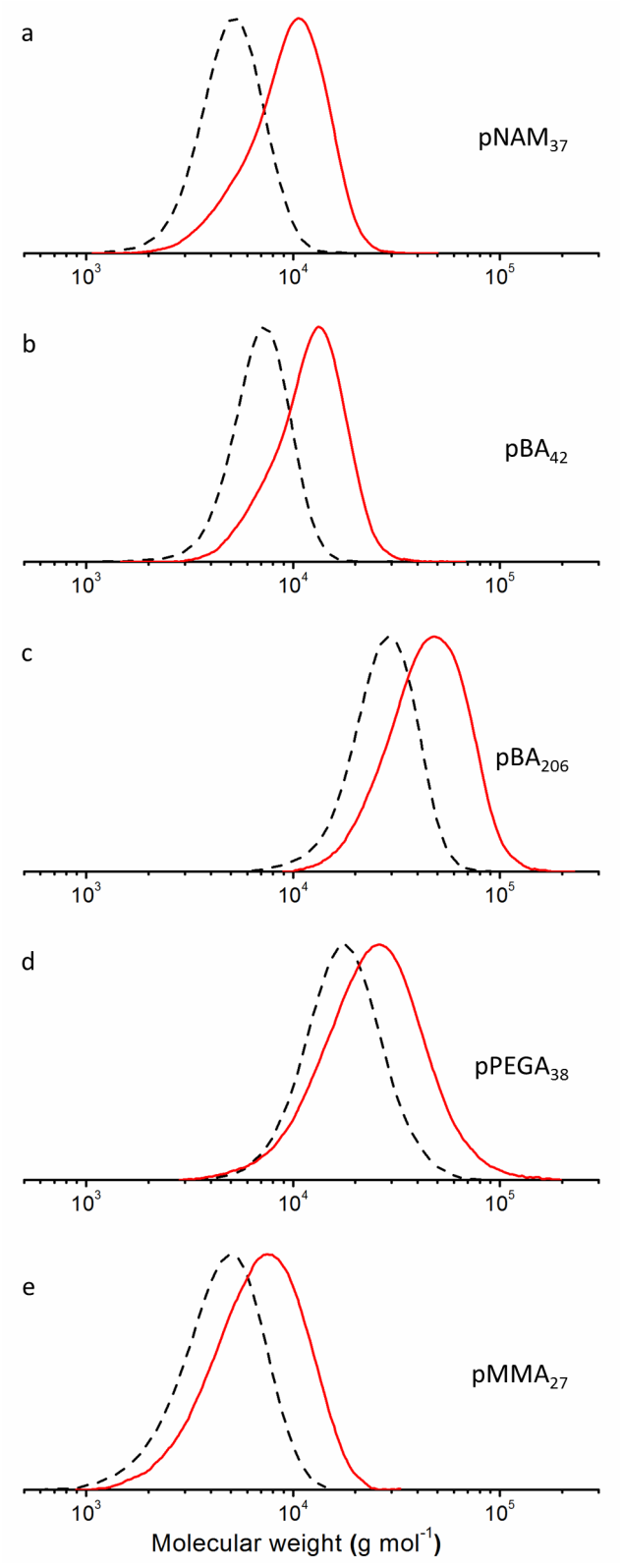

Figure 4. Normalized SEC traces of the initial precursors (dashed line) and the homocoupling (solid line) of $pNAM_{37}$-NCO (a), pBA_{42}-NCO (b), pBA_{207}-NCO (c), $pPEGA_{38}$-NCO (d), and $pMMA_{27}$-NCO (e) combining equal amounts of the polymers modified with exactly one equivalent of azidopropylamine or DBCO-NH_2, respectively.

According to the SEC traces, the homocoupling between the same polymers in all cases gave a significant shift of towards higher molecular weight and mostly monomodal distributions. Except for the sterically demanding pPEGA, a coupling efficiency of more than 90% was obtained for all different types of polymer (see SI for details of the calculation, Figures S21-S25, Table S1). Interestingly, increasing the degree of polymerization for pBA from 42 to 206 (5.7 kg/mol to 26.7 kg/mol) had no negative effect on coupling efficiency. These results clearly demonstrate the high efficiency of each step in this click addition sequence. As we did not observe any traces of side reactions we attribute any residual polymer to unavoidable dead chains from the RAFT polymerization, limitations in reaching a quantitative conversion in each step, or slight deviations in weighing the compounds precisely. It has to be kept in mind that a deviation of only 1% in the first step may cause a reduction of the efficiency of 5% in the final coupling step. Furthermore, the conversion to number distribution and the final deconvolution has limitations which may compound these errors. Nevertheless, such high efficiencies can only be reached if both reactions proceed to nearly quantitative conversion, and the results prove that this happens almost independently of the type of polymer and within a total time of less than 10 h.

A more detailed analysis of the reaction kinetics was undertaken, taking pBA_{42} as an example. For both strained alkynes, BCN-NH_2 and DBCO-NH_2, SEC samples of the click reaction with the azido modified counterpart were taken, diluted 100 fold and immediately measured (**Figure 5**).

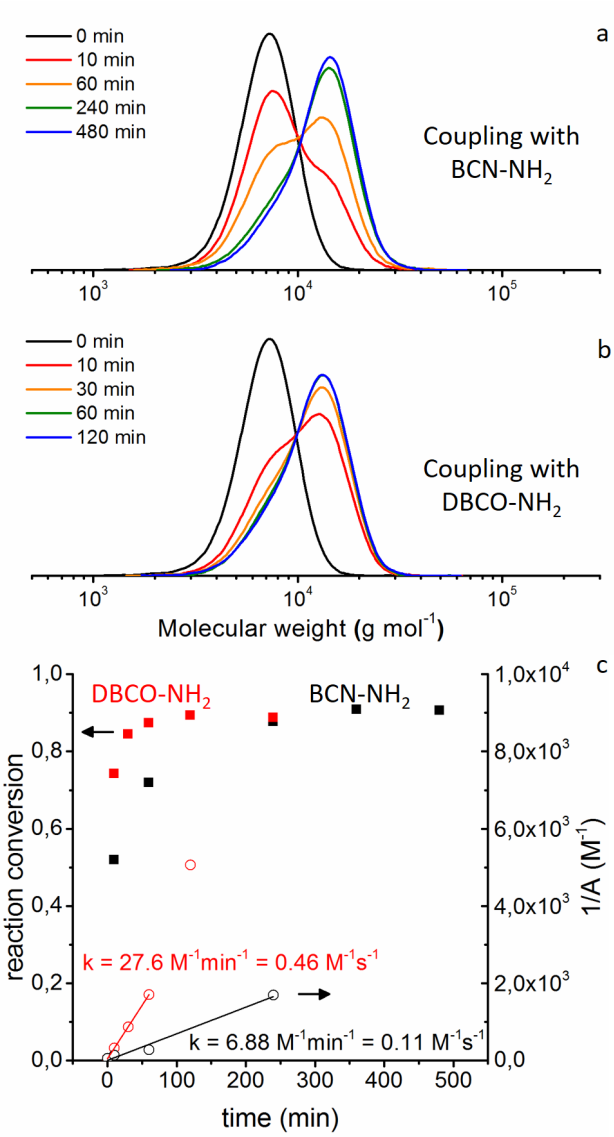

Figure 5. SEC traces of the samples taken from the reaction of pBA_{42}-BCN (a) and pBA_{42}-DBCO (b) with pBA_{42}-N_3. After deconvolution of the number distribution the calculated conversion (filled squares) and respective inverse concentrations (1/A, empty circles) of the reagents (red: DBCO-NH_2, black: BCN-NH_2) were plotted versus time (c). From the slope of the linear fits in the kinetics plot (1/A *vs* time) the rate constant k was calculated.

Both reactions reached high conversion (> 90%), however, the reaction with DBCO proceeded much faster reaching a remarkable 74% after only 10 min and with almost maximum conversion achieved in 1 h. The reaction of the BCN-NH_2 modified polymer is considerably slower and reaches the maximum conversion after only 6 h. Based on the calculated conversion and the initial concentration we tried to estimate the second-order reaction kinetics (**Figure 5c**). Unfortunately, only the early time points showed a linear trend, which most probably related to the increased error at determining high conversions. Nevertheless, this data allowed

us to estimate approximate rate constants (k) of 0.46 $M^{-1}s^{-1}$ and 0.11 $M^{-1}s^{-1}$ for DBCO and BCN, respectively. Despite the attached polymer chain, the apparent reaction rate of pBA-DBCO with the azide modified polymer is comparable to the reaction rate of the respective small molecules.[32] For BCN, rate constants of 0.29 $M^{-1}s^{-1}$ and 0.19 $M^{-1}s^{-1}$ (*endo* and *exo* form) were reported for reactions in water/acetonitrile mixtures.[30] These values are slightly higher than the observed rates, but expected due to the constraints of the bulky polymer chain. Despite this difference in reaction rates, it is noteworthy to mention that the strained precursor BCN-NH_2 proved considerably more stable than DBCO, when stored in solution (Figure S26-S27, SI).

To demonstrate the reactivity and orthogonality of the presented procedure, we attached BCN-NH_2 modified polymer chains to a diazido functionalized cyclic peptide (**Figure 6**). As previously reported, these materials form large tubular assemblies, which requires stringent optimization of reaction conditions to guarantee high yields in modification, especially with polymer chains.[33]

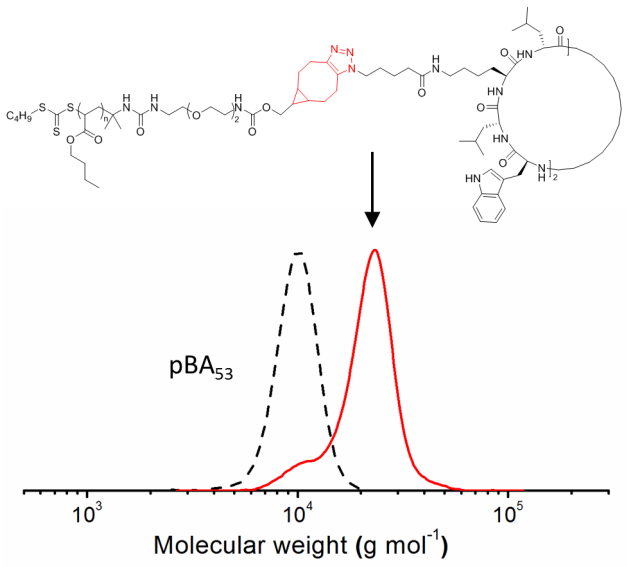

Figure 6. Normalized SEC traces of pBA_{53}-NCO and the cyclic peptide conjugate obtained after coupling to polymer chains *via* SPAAC.

Other reactions often require either an excess of polymer, high temperatures (100°C) or reaction times of up to 5 days to reach high conversions.[34, 35] The presented approach yields a coupling efficiency of more than 90% within 48 h, while no trace of a side reaction was observed. This result emphasises the speed, selectivity, and robustness of these click reactions.

A key advantage of the presented approach is the ability to combine various different RAFT polymers, while preserving the CTA chain end. **Table 2** summarizes several, exemplarily combinations of polymers using the click sequence.

Table 2. Summary of the coupling reactions combining different types of polymers.

Block copolymer	**DBCO precursor**	**N_3 precursor**	$M_{n,th}$[a] (kg mol^{-1})	$M_{n,SEC}$[b] (kg mol^{-1})	$Đ$
pBA_{42}-*b*-$pMMA_{27}$	pBA_{42}	$pMMA_{27}$	8.8	9.1	1.25
$pMMA_{27}$-*b*-$pNAM_{37}$	$pMMA_{27}$	$pNAM_{37}$	8.8	6.9	1.28
$pNAM_{37}$-*b*-$pPEGA_{38}$	$pNAM_{37}$	$pPEGA_{38}$	25.9	15.6	1.36
pBA_{42}-*b*-$pPEGA_{38}$	pBA_{42}	$pPEGA_{38}$	24.5	17.7	1.32

[a] *Determined form the theoretical M_n of the individual polymers and the molecular weight of the linkers;.*[b] *Determined by SEC using DMF (0.1% LiBr) as eluent, calibrated with pMMA standards.*

In all cases block copolymers with monomodal distributions were obtained. The SEC traces further show no measurable or only a very small amount of remaining starting material (**Figure 7**, the corresponding number distribution plots are given in Figure S28-31).

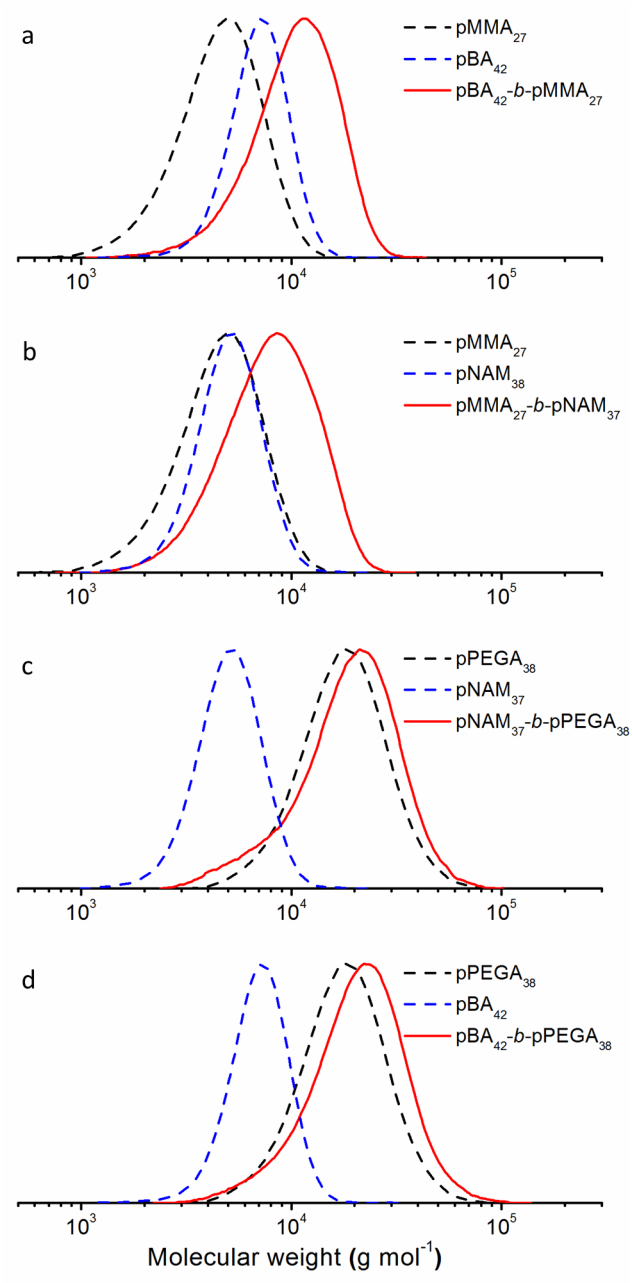

Figure 7. Normalized SEC traces of pBA-*b*-pMMA, pMMA-*b*-pNAM, pNAM-*b*-pPEGA, and pBA-*b*-pPEGA (solid lines). For comparison the traces of the respective isocyanate precursors are included (dashed lines).

No side reactions or limitations were observed in these reactions, despite the different character of the polymers and the presence of monomer in solution. A critical point in this context is the retention of the CTA end groups. These groups are known to be hydrolytically unstable, especially in presence of amines or thiols. In order to prove the preservation of the CTA end groups, we tried to chain extend the block copolymer pBA-*b*-pMMA using 4-acryloylmorpholine (NAM) (**Figure 8**).

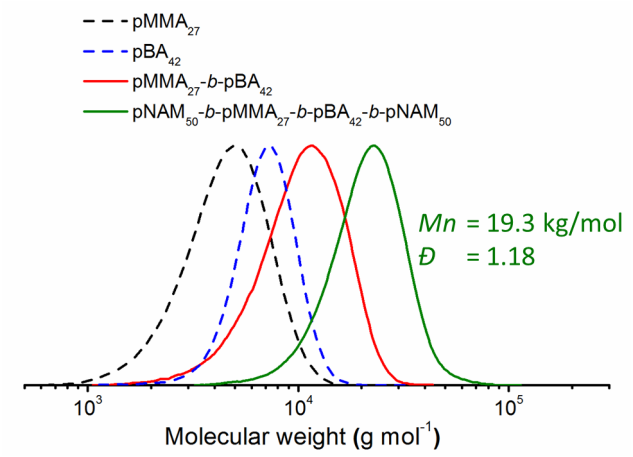

Figure 8. Normalized SEC traces of the block copolymer pBA-*b*-pMMA obtained by the click sequence and the resulting tetrablock after chain extension with NAM.

A clear shift of the SEC trace is observed, demonstrating the successful chain extension of the block copolymer pBA-*b*-pMMA. No significant tailing or residual polymer is visible in the SEC trace as would be expected from a partial cleavage of the CTA. We therefore assume that both CTA end groups are present and active after the click addition sequence, providing the ability to create asymmetric tetrablock copolymers with the sequence pNAM-*b*-pBA-*b*-pMMA-*b*-pNAM. Such a combination of acrylates, methacrylates and acrylamides is very challenging and hardly possible using common RAFT chain extension due to the difference in reactivity of methacrylates *versus* acrylates and acrylamides.

Conclusions

This study demonstrates the potential of combining efficient click reactions to effectively link various polymers created by the RAFT process. While the isocyanate chemistry has previously been shown to be an excellent tool and a robust click reaction for coupling polymers, the introduction of strained alkynes or azides bearing an amine group facilitates the creation of a variety of reactive polymers which can be combined to create well-defined block copolymers. Key elements, which guarantee the efficiency of this reaction sequence, are the pure addition character of all reactions and true orthogonality of the applied chemistry. Not only are there no major side reactions observable between the isocyanate-amine click and the SPAAC, but also no interaction with the CTA chain end and the residual vinyl groups of the monomer could be detected. In particular the latter provides the ability to work at exact equimolarity in the reaction, as no purification of the polymer is required. Another important feature of the reaction

sequence is the speed of reaction for each step. The isocyanate-amine click reaches full conversion within an hour, and the additional SPAAC also proceeds to high conversion in less than an hour, especially for the highly reactive DBCO. However, this increased reactivity comes at the cost of reagent stability. The BCN derivative requires longer, but still reasonable reaction times (< 6 h), yet it remains active even after storage in solution for several months. The tolerance to the CTA end group further allows the subsequent chain extension of the bifunctional, but asymmetrical block copolymer obtained by the click sequence. This procedure enables the formation of well-defined multiblocks combining methacrylates as central elements with pendant acrylate or acrylamide polymer chains, which is not possible using sequential controlled radical polymerization. In summary, this combination of controlled radical polymerization and click chemistry is a powerful and versatile tool to create functional and demanding polymer architectures. In addition, the effective introduction of strained alkynes to polymer chains, which are well-known for their bioorthogonality, may be useful for conjugation of proteins or other targets not only in reaction vessels, but also *in vitro* or even *in vivo*.

Experimental

Materials

All monomers, deuterated solvents for NMR and aluminum oxide were purchased from Sigma-Aldrich. If applicable, stabilizers were removed by passing the monomers through a short aluminum oxide column. Dimethyl 2,2'-azobis(2-methylpropionate) (V-601) was purchased from Wako Specialty Chemicals. All solvents were bought from commercial sources and used as received. The acyl azide chain transfer agent was synthesized according to previously published procedures.[36]

Characterization

NMR spectra were recorded on Bruker DPX-300, DPX-400 and HD-500 instruments. Mass spectrometry measurements were performed on a Bruker MicroToF for ESI ToF. Size exclusion chromatography (SEC) measurements were performed on an Agilent PL50 equipped with 2 Agilent Polargel Medium Columns eluting with dimethylformamide containing 0.1 M LiBr as an additive at 50°C. The flow rate was 1 mL/min and detection was achieved using simultaneous refractive index (RI) and UV (λ = 280 nm) detectors. As alternative an Agilent 1260 GPC-MDS fitted with differential refractive index (DRI), light scattering (LS), and viscometry (VS) detectors equipped with 2 × PLgel 5 mm mixed-D columns (300 × 7.5 mm),

1 × PLgel 5 mm guard column (50 × 7.5 mm) was used with the mobile phase being chloroform with 2% triethylamine at a flow rate of 1.0 mL/min. All molecular weights were calculated relative to narrow PMMA standards and every sample was passed through 0.45 μm PTFE filter before analysis. Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) spectra were recorded using a Bruker Alpha-E FTIR spectrometer fitted with a zinc-selenide crystal in the region between 4000 and 400 cm^{-1}. The resolution was set-up at 4 cm^{-1}, the scan speed at 0.5 $cm \cdot s^{-1}$ with 64 scans performed per sample.

Synthesis

Polymerizations

Typical protocol: chain transfer agent (CTA), monomer, initiator (V601) and DMF were introduced into a vial equipped with a magnetic stirrer and sealed with a rubber septum. The solution was degassed with constant stream of nitrogen for 10 min, the flask was then put in a thermostated oil bath set at 65°C. The polymerizations were stopped by cooling the flask and opening it to air. Conditions specific to each polymerization are detailed in Table 1. Conversions were determined by ^{1}H-NMR by comparison of the integration of the vinyl protons corresponding to the remaining monomer with the integration of polymer side chains signals. The final concentration (in mg/g solution) of CTA was determined gravimetrically weighing the empty vial with stirrer and subtracting this weight from the final mass of the vial with solution.

Modification with strained alkyne or azide

Exactly weighed aliquots of the polymerization solution (100-200 mg) were taken and either exactly one equivalent (stock solutions in DMF) of BCN (c = 33 mg/g), DBCO (c = 25 mg/g) or azidopropylamine (c = 10 mg/g) was added. The resulting mixture was agitated for 4 h at room temperature on a shaker to complete the amine-isocyanate addition.

Polymer-polymer coupling with strained promoted alkyne-azide cycloaddition

The alkyne or azide modified polymer precursors, respectively, were prepared as stated above, however to ensure equimolarity in the final polymer-polymer coupling reaction, the amount of initial polymerization solution was carefully weighed to ensure that equal number of end-groups were present in each reaction vessel. After modification the solutions were combined and stirred for 2 h (DBCO) or 8 h (BCN), respectively. For kinetic measurements, samples were taken at different time points and analysed using SEC. For the homocouplings the

conversion was determined by deconvolution of the SEC traces and comparison of the respective areas under the fitted curves.

Chain extension of linked polymers

The previously obtained pBA-*b*-pMMA was precipitated in a water/methanol mixture (1/1) to remove any residual monomer and dried in vacuum. The resulting block copolymer (0.0434 g, 5 x 10^{-6} mol, 1 eq.) was dissolved in dioxane (0.2 ml), and NAM (0.035 mg, 2.5 x 10^{-4} mol, 50 eq.) and V601 (1.14 x 10^{-4} g, 5 x 10^{-7} mol, 0.1 eq.) were added. After degassing for 10 min with constant stream of nitrogen, the polymerization was started by immersing the solution into a preheated oil bath at 65°C. The polymerization was stopped by cooling the flask and opening it to air. The chain extension was examined by SEC calibrated with PMMA standards: M_n = 19.3 kg/mol, $Đ$ = 1.18.

Polymer-cyclic peptide coupling with strain promoted alkyne-azide cycloaddition

The cyclic peptide was prepared according to previously published procedures.[33, 35] For the coupling reaction 0.099 g (3.67 x 10^{-6} mol, 2 eq.) of the reaction solution containing the previously BCN-modified pBA (c = 3.71 x 10^{-5} mol/g) were added to 2.4 x 10^{-3} g of cyclic peptide (1.84 x 10^{-6} mol, 1 eq.) previously dissolved in 0.25 mL *N*-methylpyrolidone. The mixture was agitated for 7 days and samples taken at different time points were analysed by SEC. The final conversion was calculated from the deconvolution of the traces.

Associated content

Electronic supplementary information.

Acknowledgements

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