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A New Methodology for Assessing Macromolecular Click Reactions and Its Application to Amine−Tertiary Isocyanate Coupling for Polymer Ligation

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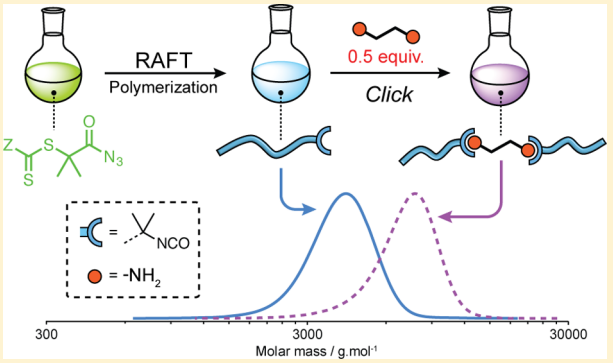
Supporting Information

ABSTRACT: Click reactions have provided access to an array of remarkably complex polymer architectures. However, the term "click" is often applied inaccurately to polymer ligation reactions that fail to respect the criteria that typify a true "click" reaction. With the purpose of providing a universal way to benchmark polymer−polymer coupling efficiency at equimolarity and thus evaluate the fulfilment of click criteria, we report a simple one-pot methodology involving the homodicoupling of α-end-functionalized polymers using a small-molecule bifunctional linker. A combination of SEC analysis and chromatogram deconvolution enables straightforward quantification of the coupling efficiency. We subsequently employ this methodology to evaluate an overlooked candidate for the click reaction family: the addition of primary amines to α-tertiary isocyanates (α-^{t}NCO). Using our bifunctional linker coupling strategy, we show that the amine−^{t}NCO reaction fulfills the criteria for a polymer−polymer click reaction, achieving rapid, chemoselective, and quantitative coupling at room temperature without generating any byproducts. We demonstrate that amine−^{t}NCO coupling is faster and more efficient than the more common amine−tertiary active ester coupling under equivalent conditions. Additionally, we show that the α-^{t}NCO end group is unprecedentedly stable in aqueous media. Thus, we propose that the amine−^{t}NCO ligation is a powerful new click reaction for efficient macromolecular coupling.



INTRODUCTION

Polymer science has benefited immensely from the development of highly efficient coupling reactions for post-polymerization modification.[1] Ideally, these reactions should proceed rapidly (<1 h) under mild conditions, require no catalyst, produce no byproducts, and, most importantly, not require an excess of either coupling partner (i.e., operate at equimolarity), since the purification of a mixture of macromolecules can be laborious and time consuming.[2] Click reactions, which satisfy these criteria by definition,[3] have been instrumental for the coupling of small molecules to each other[4] and to (bio)-macromolecular substrates.[5] However, relatively few small-molecule click reactions perform optimally when used for coupling one macromolecule to another, and often an excess of one coupling partner is required to drive the reaction to completion.[6] There are only a handful of reactions that enable highly efficient macromolecular coupling at equimolarity: the Heck coupling,[7] the nitrile imine-mediated tetrazole-ene cycloaddition reaction,[8] the photoinduced Diels−Alder reaction,[9] the RAFT hetero Diels−Alder reaction,[10] and CuAAC.[11] The development of new click reactions for post-polymerization modification therefore remains an important ongoing challenge in polymer chemistry.[12]

The nucleophile−isocyanate addition reaction has received little attention as a macromolecular ligation strategy despite its significance to the polymer and fine chemical industries.[13] Organic isocyanates are useful substrates due to their high reactivity toward amines,[14] thiols,[15] and alcohols,[16] often reacting rapidly and quantitatively to produce ureas, thioureas, and urethanes, respectively. Compared to functional groups encountered in other common coupling methodologies (e.g., thiol−ene/yne, CuAAC, active ester ligation), isocyanates are particularly attractive because they are unreactive toward radicals (unlike e.g., alkenes, alkynes, thiols, azides), their addition reactions with nucleophiles do not release any byproducts, and, in the case of amines, no catalysts or small-molecule promoters are required. The low prevalence of

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isocyanate-based ligation strategies in polymer chemistry can be attributed to a few perceived drawbacks of organic isocyanates: (i) synthetic routes involving highly toxic reagents (e.g., phosgene); (ii) a lack of chemoselectivity toward various nucleophiles; (iii) the need for a catalyst for coupling with alcohols and thiols; and (iv) high sensitivity to moisture, making them difficult to handle and store.[17]

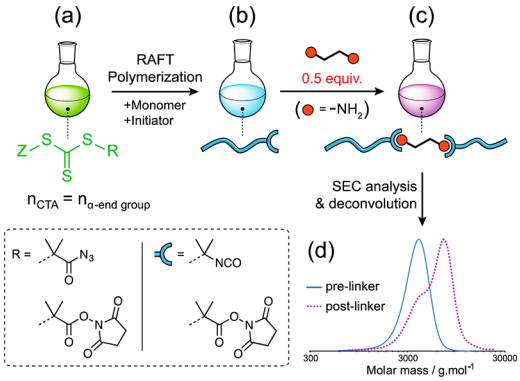
Recently, we developed a strategy for preparing α-tertiary isocyanate (α-^{t}NCO) end-functional polymers using a novel carbonyl-azide functionalized CTA,[18] which avoids the use of blocked isocyanates.[19] In this early work, we demonstrated how the carbonyl azide R group of a RAFT agent would rearrange into a tertiary isocyanate group during polymerization to form α-^{t}NCO polymers. Preliminary experiments suggested that the tertiary isocyanate group reacted efficiently with low molar mass alcohols and amines. However, a more demanding challenge in polymer chemistry is the coupling of end-functional polymers to other macromolecules at equimolarity. Indeed, this is a critical feature of a click reaction in the context of polymer chemistry. Presently, the performance of α-^{t}NCO polymers in macromolecule-to-macromolecule couplings remains an open question. We thus sought to determine the efficiency of this reaction for macromolecular coupling and to assess whether it meets the criteria of a click reaction.

Surprisingly, the existing literature lacks a universal methodology to evaluate whether a given macromolecular coupling reaction fulfills the criteria of a click reaction (e.g., efficiency at equimolarity, reaction time scale, modularity, chemoselectivity).[2b] Consequently, we have developed a simple one-pot strategy for quantifying the coupling efficiency at equimolarity of a polymer−polymer ligation reaction using a combination of SEC analysis and chromatogram deconvolution. We apply this methodology to assess whether the amine−α-^{t}NCO reaction is sufficiently chemoselective, efficient, and robust to be considered a click reaction in the context of macromolecular coupling. The efficiency of the amine−^{t}NCO reaction is also benchmarked against the amine−active-ester reaction (*N*-hydroxysuccin-imidyl ester, NHS), which is the most widely used reaction for attaching polymers to amine-bearing substrates.

RESULTS AND DISCUSSION

One-Pot Bifunctional Linker Strategy for Quantifying Polymer−Polymer Click Reaction Efficiency. In order to perform a polymer−polymer ligation reaction at equimolarity, one must know precisely the number of moles of reactive end-group in a sample. The molecular weight distribution of the polymer, however, obfuscates this information, as the average molecular weight does not represent the number of moles of end-group. To circumvent this limitation and to accurately assess the real efficiency of a polymer−polymer coupling reaction, we have designed a simple one-pot methodology that relies on the coupling of two α-end-functional polymers with a single bifunctional linker (Scheme 1). This approach hinges upon one-pot polymerization, as it avoids any mass loss due to purification. We are thus able to work at equimolarity since the number of moles of α-end functional group is equal to the number of moles of CTA initially introduced (or moles of initiator in other types of controlled polymerizations). In this way, it becomes straightforward to perform post-polymerization modification with an equimolar amount of a bifunctional linker (e.g., ethylenediamine), which simulates an ideal polymer−polymer dicoupling reaction.[11]

Scheme 1. One-Pot Bifunctional-Linker Coupling Strategy Used to Quantify Polymer−Polymer Coupling at Equimolarity[a]



[a]Workflow steps: (a) Moles of CTA initially introduced is equal to the final number of moles of α-end group after polymerization (R group of the CTA); (b) direct addition of 0.5 equiv bifunctional linker to the polymerization mixture produces (c) the polymer-linker-polymer conjugate; (d) analysis of the reaction mixture by SEC. Coupling efficiency is calculated by Gaussian multiple peak deconvolution of the number distribution chromatogram (SI Figure S1).

To quantify the coupling efficiency (i.e., coupled vs noncoupled polymer chains), we employ a simple SEC-based workflow whereby the SEC traces are plotted in terms of number distribution ($P(M)$ vs M) (SI Figure S1), then the underlying component distributions are extracted by multiple peak deconvolution of the overall SEC envelope. Conversion to number distribution is necessary because visual inspection of SEC distributions ($w(\log M)$ vs $\log M$) of partially successful coupling reactions can be misleading.[20,21] It is important to note that, in degenerative transfer RDRP techniques, polymerizations must be performed using a high $[CTA]_0/[initiator]_{consumed}$ ratio to ensure that the number of initiator-derived chains remains low,[22] as the initiator-derived chains will not possess an α-end functional group and thus cannot react with the diamine linker. Consequently, any initiator-derived chains will appear as apparently unconsumed α-end functional polymer after attempts at performing dimerization.

This general methodology can be applied to any ligation strategy involving α-end-functional polymers, enabling straightforward assessment of key click criteria such as coupling efficiency at equimolarity, reaction time, and chemoselectivity (by varying the functional groups of the linker). Consequently, it is possible to make quantitative comparisons between different coupling reactions using different polymer types.

Assessment of Amine−^{t}NCO Coupling As a Click Reaction for Polymer Ligation. Having established a methodology to assess some of the key click criteria in macromolecular coupling, our aim was to synthesize, using RAFT polyermzation, a range of α-^{t}NCO end-functional polymers and subsequently: (i) test the coupling efficiency of the amine−^{t}NCO reaction using different polymer types and chain lengths; (ii) examine the chemoselectivity of the α-^{t}NCO for primary amines over primary thiols and primary alcohols; (iii) ascertain the hydrolytic stability of the α-^{t}NCO end-group; and (iv) compare the efficiency of the amine−^{t}NCO reaction with the amine−^{t}NHS ester reaction under the same conditions.

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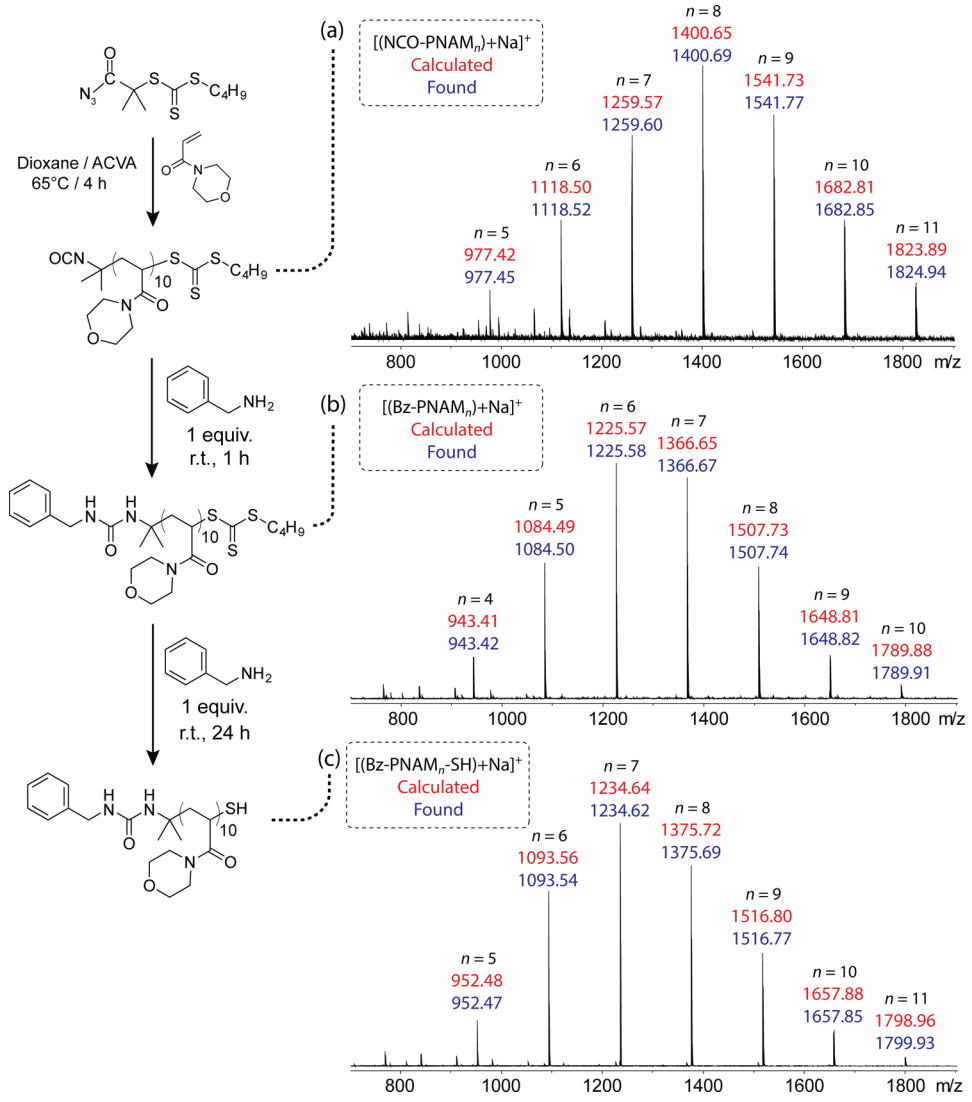

Figure 1. Experiment performed in a one-pot fashion to assess (a) the efficiency of the *in situ* Curtius rearrangement during RAFT polymerization and the difference in reactivity of benzylamine toward (b) the isocyanate or (c) the trithiocarbonate end group. HRMS analysis is detailed in SI Figures S6–S8.

Preliminary Considerations for Amine–Isocyanate Reaction. We employ a RAFT polymerization strategy in which a carbonyl-azide functional CTA undergoes an *in situ* Curtius rearrangement to a tertiary isocyanate group during polymerization (SI Figure S2).[18] In order to accurately assess the efficiency of the amine–isocyanate reaction, we must first ensure that the carbonyl-azide CTA is converted quantitatively into α-^{t}NCO polymer end-groups and that the rate of amine–^{t}NCO addition is faster than aminolysis of the trithiocarbonate (TTC) group. To verify that these criteria are satisfied in our system, a very short α-NCO-polymer (DP = 10) was prepared, and then, in a second step (i.e., directly after polymerization, without purification), 2 equiv (with respect to CTA) of benzylamine were added in successive 1 equiv aliquots separated by a 1 h interval (Figure 1). Addition of the first equivalent establishes a competition experiment between amine–^{t}NCO addition and aminolysis of the TTC group; the second equivalent consumes any isocyanate or TTC still remaining after the first addition. An interval of 1 h was chosen according to the kinetics of benzylamine addition to *tert*-butyl isocyanate, which was used as a model reaction for these experiments (SI Figure S4).

Results of this model reaction were highly encouraging: ESI-TOF-MS analysis of the mixture after 4 h of polymerization (99% monomer conversion) showed quantitative rearrangement of the acyl-azide CTA into an α-^{t}NCO-polymer (Figure 1a) with a single polymer population observed (NCO-PNAM-TTC, Na^+ adduct). After addition of the first equivalent of benzylamine (Figure 1b), only a single population (Bz-PNAM-TTC, Na^+ adduct) was again observed, corresponding to the coupling product between benzylamine and the α-NCO-polymer. Interestingly, no starting material (NCO-PNAM-TTC) and no aminolyzed polymer chains (NCO-PNAM-SH) were observed, indicating complete consumption of the α-^{t}NCO end-groups without aminolysis of the TTC. The high selectivity of amine–^{t}NCO coupling was further demonstrated by the addition of a second equivalent of benzylamine: After 2 h (SI Figure S5a) a mixture of aminolyzed (Bz-PNAM-SH) and nonaminolyzed α-functionalized polymers (Bz-PNAM-TTC) was observed; after 24 h (Figure 1c) only the aminolyzed α-functionalized population was present in the

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Table 1. Study of the Efficiency of the Amine–^{t}NCO and Amine–^{t}NHS Ester Coupling Reactions According to the Type of Amine Linker, the Type of Polymer, and the Polymer Chain Length[a]

	polymerization					post-polymerization modification		
run	polymer	conv. (%)	$M_{n,th}$ (g·mol^{-1})	Đ	$\alpha_{chain\text{-}end}$[b] (%)	solvent	reagent	coupling efficiency (%)
1	^{t}NCO-PNAM$_{40}$[c]	>99	5900	1.11	98.8	dioxane	ethylenediamine[h]	100
2	^{t}NCO-PMMA$_{30}$[d]	75	3300	1.24	96.3	dioxane	ethylenediamine[h]	93 ± 1.0
3	^{t}NCO-PtBA$_{33}$[e]	83	4500	1.07	99.4	toluene	ethylenediamine[h]	96 ± 1.2
4	^{t}NCO-PtBA$_{31}$[e]	78	4300	1.14	99.4	toluene	piperazine[i]	95 ± 1.7
5	^{t}NCO-PNAM$_{100}$[f]	>99	14500	1.15	98.4	dioxane	ethylenediamine[h]	91 ± 1.1
6	^{t}NCO-PNAM$_{200}$[g]	>99	27900	1.08	97.6	dioxane	ethylenediamine[h]	78 ± 1.0
7	^{t}NCO-PNAM$_{40}$[c]	>99	5900	1.13	98.8	dioxane	ethylenediamine[i]	93 ± 0.7
8	^{t}NHS-PNAM$_{40}$[c]	>99	5900	1.12	98.8	dioxane	ethylenediamine[i]	75 ± 2.0
9	^{t}NCO-PNAM$_{40}$[c]	>99	5900	1.11	98.8	DMSO/dioxane	cyclic peptide[j]	93 ± 7.0
10	^{t}NHS-PNAM$_{40}$[c]	>99	5900	1.11	98.8	DMSO/dioxane	cyclic peptide[j]	61 ± 13.0

[a]See SI, Table S1, for further details on the experimental conditions used. [b]Theoretical percentage of α-^{t}NCO end functional polymer chains calculated from the ratio $[CTA]_0/[initiator]_{consumed}$ (see SI, eq S2). [c]RAFT conditions: $[NAM]_0$ = 3 M; in dioxane at 65 °C for 4 h; $[NAM]_0/[acyl\ azide\ or\ NHS\ CTA]_0/[AIBN]_0$ = 40/1/0.05. [d]RAFT conditions: $[MMA]_0$ = 5.5 M; in dioxane at 65 °C for 7 h; $[MMA]_0/[acyl\ azide\ CTA]_0/[AIBN]_0$ = 40/1/0.1. [e]RAFT conditions: $[tBA]_0$ = 5 M; in toluene at 65 °C for 4 h; $[tBA]_0/[acyl\ azide\ CTA]_0/[AIBN]_0$ = 40/1/0.025. [f]RAFT conditions: $[NAM]_0$ = 3 M; in dioxane at 65 °C for 4 h; $[NAM]_0/[acyl\ azide\ CTA]_0/[AIBN]_0$ = 100/1/0.065. [g]RAFT conditions: $[NAM]_0$ = 3 M; in dioxane at 65 °C for 4 h; $[NAM]_0/[acyl\ azide\ CTA]_0/[AIBN]_0$ = 200/1/0.1. [h]0.5 equiv of ethylenediamine added as a single aliquot, allowed to react for 1 h. [i]0.1 equiv of ethylenediamine added every 1 h until 0.5 equiv added (5 h overall reaction time). [j]0.5 equiv of cyclic peptide in solution in DMSO with *N*-methylmorpholine (6 equiv) added one-shot and reaction for 4 days to 1 week.

mass spectrum (Bz-PNAM-SH). It is therefore clear that the amine–^{t}NCO coupling is faster than aminolysis of the TTC, conveniently preserving the latent thiol functionality of the polymer.

Amine–^{t}NCO Coupling for Polymer–Polymer Ligation. The effects of various macromolecular parameters on the efficiency of amine–^{t}NCO coupling were assessed in order to ascertain the utility of this reaction for polymer–polymer conjugation (Table 1): (i) the chemical nature of the polymer (comparison among poly(acrylamide), poly(methacrylate), and poly(acrylate)); (ii) the chemical nature of the linker (primary or secondary amine); (iii) the polymer chain length (comparison between DP 40, 100, and 200).

We observed highly efficient diamine-mediated dicoupling between α-^{t}NCO-polymers of 3–6 kDa bearing different repeating units (runs 1–4, Table 1). Remarkably, quantitative homodicoupling was achieved using α-NCO-PNAM$_{40}$ and ethylenediamine (run 1, Table 1, Figure 2a,b), clearly exemplifying the click nature of the amine–^{t}NCO reaction on a polymeric substrate. Chromatograms after 1 h coupling showed a clean shift to higher molar mass while maintaining a narrow and monomodal molar mass distribution (Đ = 1.11 before and after dimerization, Figure 2a). The isocyanate–amine reaction thus proceeds without any measurable side-reactions (e.g., isocyanate hydrolysis by adventitious water or aminolysis of the TTC) that would alter the theoretical α-end-group stoichiometry. We observed similarly high polymer–polymer coupling efficiencies of 93% and 96% using α-^{t}NCO-PMMA$_{30}$ (SI Figure S9) and α-^{t}NCO-PtBA$_{33}$ (SI Figure S10a), indicating that the coupling reaction is not sensitive to the chemical nature of the polymer. The lower apparent yield of coupling with the α-^{t}NCO-PMMA$_{30}$ (i.e., 93%) can be attributed to the relatively lower fraction of α-^{t}NCO chain-ends obtained at the end of the polymerization (96.3%, run 2, Table 1).

The type of diamine linker was observed to have little influence on the efficiency of polymer–polymer coupling: comparison of runs 3 and 4 (SI Figure S10a,b) both performed by homodicoupling of α-^{t}NCO-PtBA using either a primary amine linker (e.g., ethylenediamine) or a secondary amine linker (e.g., piperazine) showed high coupling yields of ca. 96% and 95%.

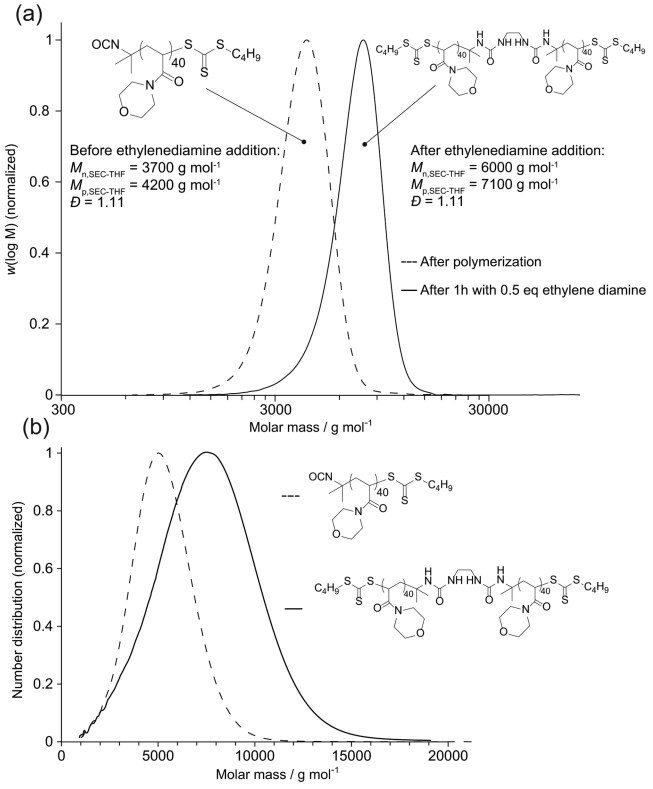

Figure 2. Chromatograms (SEC-THF) showing (a) the coupling reaction between NCO-PNAM$_{40}$ and ethylenediamine (0.5 equiv) in dioxane (25 °C, 1 h) performed directly after polymerization (one-pot reaction, run 6, Table 1) and (b) its number distribution representation.

As is typically the case with macromolecular ligation reactions, the length of the polymer chain was observed to have a strong influence on coupling efficiency. Comparison of

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Table 2. Comparative Study of the Efficiency of the Alcohol− and Thiol−Isocyanate Coupling Reaction via the Use of Two Different Linkers Ethylene Glycol or 1,2-Ethanedithiol[a]

	polymerization					post-polymerization modification		
run	polymer	conv. (%)	$M_{n,th}$ (g·mol^{-1})	Đ	$\alpha_{chain\text{-}end}$[b] (%)	solvent	reagent	coupling efficiency (%)
11	NCO-PNAM$_{40}$[c]	>99	5900	1.14	98.8	dioxane	1,2-ethanedithiol[d]	0
12	NCO-PNAM$_{40}$[c]	>99	5900	1.14	98.8	dioxane	1,2-ethanedithiol[e]	0
13	NCO-P*t*BA$_{39}$[f]	97	5200	1.12	98.8	toluene	ethylene glycol[g]	51 ± 2.0

[a]See SI for further details on the experimental conditions used, Table S2. [b]Theoretical percentage of α-NCO end functional polymer chains calculated from the ratio $[CTA]_0/[initiator]_{consumed}$ (see SI eq S2). [c]RAFT conditions: $[NAM]_0$ = 3 M; in dioxane at 65 °C for 4 h; $[NAM]_0/[acyl\ azide\ CTA]_0/[AIBN]_0$ = 40/1/0.05. [d]Reaction with 0.5 equiv of 1,2-ethanedithiol and 0.1 equiv of triethylamine at 25 °C. [e]Reaction with 0.5 equiv of 1,2-ethanedithiol and DBU (0.2 equiv) at 25 °C. [f]RAFT conditions: $[tBA]_0$ = 5 M; in toluene at 65 °C for 4 h; $[tBA]_0/[acyl\ azide\ CTA]_0/[AIBN]_0$ = 40/1/0.05. [g]Reaction with 0.5 equiv ethylene glycol for 48h at 25 °C.

Table 3. Study of the Stability of the α-NCO End-Functional Polymer in the Presence of Moisture[a]

	polymerization[b]				post-polymerization modification		
run	polymer	conv. (%)	$M_{n,th}$ (g·mol^{-1})	Đ	solvent	reagent	coupling efficiency (%)
14	^{t}NCO-PNAM$_{40}$	>99	5900	1.11	H_2O	no reagent	–
15	^{t}NCO-PNAM$_{40}$	>99	5900	1.12	100% H_2O	ethylene diamine	100 ± 0.5
16	^{t}NCO-PNAM$_{40}$[c]	98	5800	1.18	dioxane/H_2O	ethylene diamine	87 ± 0.4
17	^{t}NCO-PBA$_{35}$	88	4800	1.10	1/precipitation in H_2O/MeOH[d] 2/reaction in dioxane	ethylene diamine	89 ± 0.4

[a]See SI for further details on the experimental conditions used, Table S3. [b]The RAFT conditions use: $[acyl\ azide\ CTA]_0/[AIBN]_0$ = 1/0.05 at 65 °C for 4 h give a theoretical percentage of α-^{t}NCO functional polymer chains around 98.8% (see SI eq S2 for the calculation). [c]Polymerization performed in dioxane/H_2O 90/10 *v/v* (46 equiv of H_2O with respect to NCO group). [d]H_2O/MeOH 50/50 v/v.

the runs 1, 5, and 6 (Table 1) clearly shows that the coupling efficiency decreases drastically as the polymer chain length increases—presumably due to steric blocking of the tertiary isocyanate end-group—decreasing from 100% for α-^{t}NCO-PNAM$_{40}$ (Figure 2a,b) to 91% for α-^{t}NCO-PNAM$_{100}$ (SI Figure S11a) and to 78% for α-^{t}NCO-PNAM$_{200}$ (SI Figure S11b).

In conclusion, results from the first set of experiments (runs 1−6, Table 1) with a small diamine linker (ethylene diamine or piperazine) confirm that the amine−^{t}NCO reaction is rapid, reaching completion in <1 h at ambient temperature under equimolar coupling conditions and is compatible with a wide range of polymer types. Interestingly, polymer−polymer conjugation is as fast as the model reaction between benzylamine and *tert*-butyl isocyanate.

Assessing the Chemoselectivity of Amine−^{t}NCO Coupling. To be considered a true click reaction, the amine−^{t}NCO reaction must be orthogonal to the closely related thiol−^{t}NCO and alcohol−^{t}NCO addition reactions. In this section, we examine the reactivity of α-^{t}NCO functional polymers by employing the bifunctional linker strategy using ethylene glycol and 1,2-ethanedithiol (runs 11−13, Table 2).

The rate of thiol-^{t}NCO addition was initially modeled by observing the reaction between benzylmercaptan (1 equiv) and *tert*-butyl isocyanate in the presence of triethylamine (Et_3N, 1 equiv). *In situ* ^{1}H NMR reaction analysis (SI Figure S12) showed that thiol−^{t}NCO coupling was complete after 2 days at room temperature. This is in striking contrast to the analogous amine−^{t}NCO model reaction (SI Figure S4), which reached completion within 1 h at room temperature at the same concentration and without the use of a catalyst. Surprisingly, the reaction of NCO-PNAM$_{40}$ with 1,2-ethanedithiol as a reactive linker in the presence of either Et_3N or 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (runs 11 and 12, respectively) did not proceed at room temperature (SI Figure S13a,b, respectively) or upon addition of dibutyltin dilaurate (DBTDL) and heating at 50 °C. By contrast, isocyanate polymer−polymer conjugation using ethylene glycol as the reactive linker (run 13) in the presence of DBTDL produced a better result. After 48 h, the reaction between NCO-P*t*BA$_{39}$ and ethylene glycol at room temperature afforded the coupled product with a coupling efficiency of ca. 51% (SI Figure S14). Although the alcohol−isocyanate coupling reaction does proceed, the rate is very slow and the reaction requires a catalyst. It is therefore clear that the amine−^{t}NCO reaction is fully orthogonal to alcohol−^{t}NCO and thiol−^{t}NCO reactions.[14b,23]

Hydrolytic Stability of the Tertiary Isocyanate End-Group in Aqueous Medium. Isocyanates are powerful electrophiles and, consequently, are generally sensitive to moisture and often difficult to handle under ambient conditions.[17] It is therefore surprising that the α-NCO end-functional polymers obtained from a carbonyl-azide CTA can be prepared and functionalized using nonanhydrous solvents.[18]

We attribute the unprecedented water tolerance of the α-isocyanate polymer to the steric hindrance of the tertiary isocyanate group, which makes it less reactive toward nucleophiles, as explored in the previous section mainly with alcohols. Performing post-polymerization functionalization reactions in water is an area of great interest for the life-science applications of polymer chemistry. In this section, we aim to assess the scope and limitations of amine−isocyanate coupling for post-polymerization functionalization (e.g., bioconjugation) in the presence of water. Water-soluble ^{t}NCO-poly(4-acryloylmorpholine) (α-^{t}NCO-PNAM$_{40}$) was prepared in dioxane at 65 °C (run 14, Table 3). After polymerization and precipitation by dropwise addition into diethyl ether, α-^{t}NCO-PNAM$_{40}$ was dissolved in pure water (1230 equiv of H_2O with respect to ^{t}NCO end-group) at room temperature. Aliquots were extracted after 1 and 24 h, and the viability of the isocyanate group was measured both by SEC and FT-IR. With such a large excess of water, we expected that the isocyanate

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group would rapidly decompose, via a transient carbamic acid, into a primary amine (which itself might react with another α-^{t}NCO end-group and form a urea linkage) and gaseous carbon dioxide. Surprisingly, after 1 h in water the characteristic NCO stretch at 2275 cm^{-1} was still observed by ATR-FTIR, suggesting that hydrolysis of the isocyanate into an amine was much slower than expected (Figure 4a). After 24 h, the isocyanate signal had disappeared completely. SEC-THF analysis was highly informative (Figure 4a), showing a bimodal distribution after only 1 h in water with a characteristic population at twice the molar mass of the starting material (Figure 4b). This peak was attributed to the coupling of two polymer chains via urea-bond formation.

To confirm that the amine−^{t}NCO addition occurs preferentially over isocyanate hydrolysis, ^{t}NCO-PNAM$_{40}$ (1 equiv) was added to a solution of ethylenediamine (0.5 equiv) in pure water (1230 equiv, run 15, Table 3). After 1 h at room temperature, the SEC chromatogram revealed the presence of an almost pure polymer at the double of the molar mass (efficiency >99%) (Figure 4c). Comparison with the experiment without the diamine linker (run 14) confirmed that amine addition to the α-^{t}NCO group is very efficient and much faster than hydrolysis. It is remarkable that the amine−^{t}NCO coupling reaction proceeds perfectly in water, making this a very promising strategy for polymer bioconjugation reactions.

To further assess the tolerance of the α-^{t}NCO toward water, RAFT polymerization of NAM was carried out in dioxane with 10% (v/v) water (46 equiv with respect to NCO group) at 65 °C (run 16, Table 3). After 4 h, near complete monomer conversion was achieved. SEC analysis of the polymer revealed a small shoulder at twice the molar mass of the main distribution, which we attribute to the urea-linked dimer arising from an α-^{t}NCO-PNAM chain reacting with an α-amino-functional PNAM, derived from isocyanate hydrolysis (SI Figure S15a). However, the extent of degradation is minor, especially considering the large molar excess of water present (46 equiv per isocyanate) and the moderate temperature (i.e., 65 °C), thus emphasizing the good hydrolytic tolerance of the α-^{t}NCO end group. Ethylenediamine (0.5 equiv with respect to the theoretical amount of NCO group) was subsequently added to the polymerization mixture to confirm that the isocyanate end-group had indeed survived polymerization in the presence of water. The final chromatogram shows a coupling conversion of 87% (SI Figure S15b), clearly confirming that a large proportion of the isocyanate end-group is retained during RAFT polymerization in the presence of water.

The isocyanate end-group is also sufficiently robust to survive precipitation of the polymer into water-rich solvents. For example, after precipitation of α-^{t}NCO-PBA$_{35}$ in MeOH/H_2O 50/50 (v/v) (run 17, Table 3), characterization of the purified product by SEC-THF (SI Figure S16a) and FT-IR (SI Figure S16b) confirmed that no significant degradation of the NCO group has occurred. The addition of ethylenediamine (0.5 equiv) to the purified polymer afforded the coupled product in 89% yield after 1 h at room temperature. Nonquantitative coupling, which is obvious from the bimodal chromatographic profile (SI Figure S16c), most likely arises due to the incorrect coupling stoichiometry, since the number of moles of end-group in the purified polymer was determined from average molar mass measurements, which have inherent uncertainties (*vide supra*). This result confirms the excellent retention of the ^{t}NCO group after purification by precipitation into MeOH/H_2O, which is an unprecedented result that challenges the perception that isocyanate-bearing compounds are difficult to handle in the presence of adventitious water.

Comparing Efficiency of Amine−NHS and Amine−NCO Reactions for Polymer Ligation. The amine−active ester coupling reaction is a common strategy for attaching end-functional polymers to amine-bearing substrates. Despite their prevalence in polymer chemistry, active esters suffer from a key drawback in that their performance represents a trade-off between reactivity, chemoselectivity, and hydrolytic stability. To demonstrate that the amine−^{t}NCO reaction is superior to the more conventional amine−active ester reaction for polymer−polymer coupling, we compared the reactivity of ethylenediamine with α-^{t}NHS-PNAM$_{40}$ and α-^{t}NCO-PNAM$_{40}$ (runs 7 vs 8, Table 1). Unlike other previous coupling experiments, the ethylene diamine linker was added portion-wise instead of in a single aliquot in order to follow the coupling progress by SEC. Homodicoupling of α-^{t}NCO-PNAM$_{40}$ proceeds much faster than α-^{t}NHS-PNAM$_{40}$ (Figure 3a vs 3b) and achieves a higher coupling efficiency of 93% (Run

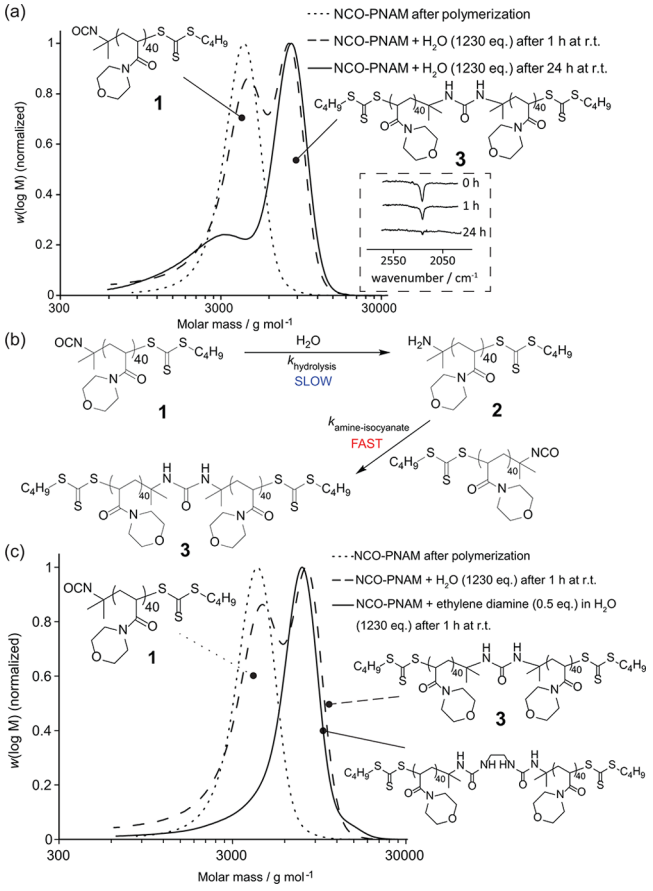

Figure 3. (a) Chromatograms (SEC-THF) showing the degradation of the α-^{t}NCO end-functional PNAM$_{40}$ in the presence of a large excess of water (1230 equiv). The peak at double molar mass is assigned to two polymer chains coupled by a urea bond and confirmed by the disappearance of the NCO peak in the FTIR spectrum (run 14, inset). (b) The large proportion of coupled product **3** observed when **1** is reacted in water suggests that amine−isocyanate coupling is much faster than isocyanate hydrolysis (i.e., $k_{hydrolysis} < k_{amine-NCO}$). Thus, **2** reacts with **1** as soon as it forms. (c) Chromatograms (SEC-THF) showing the reactivity/degradation of the α-^{t}NCO PNAM$_{40}$ (dotted line) in pure water with (plain line) or without (dashed line) 0.5 equiv ethylenediamine at room temperature after 1 h (run 15).

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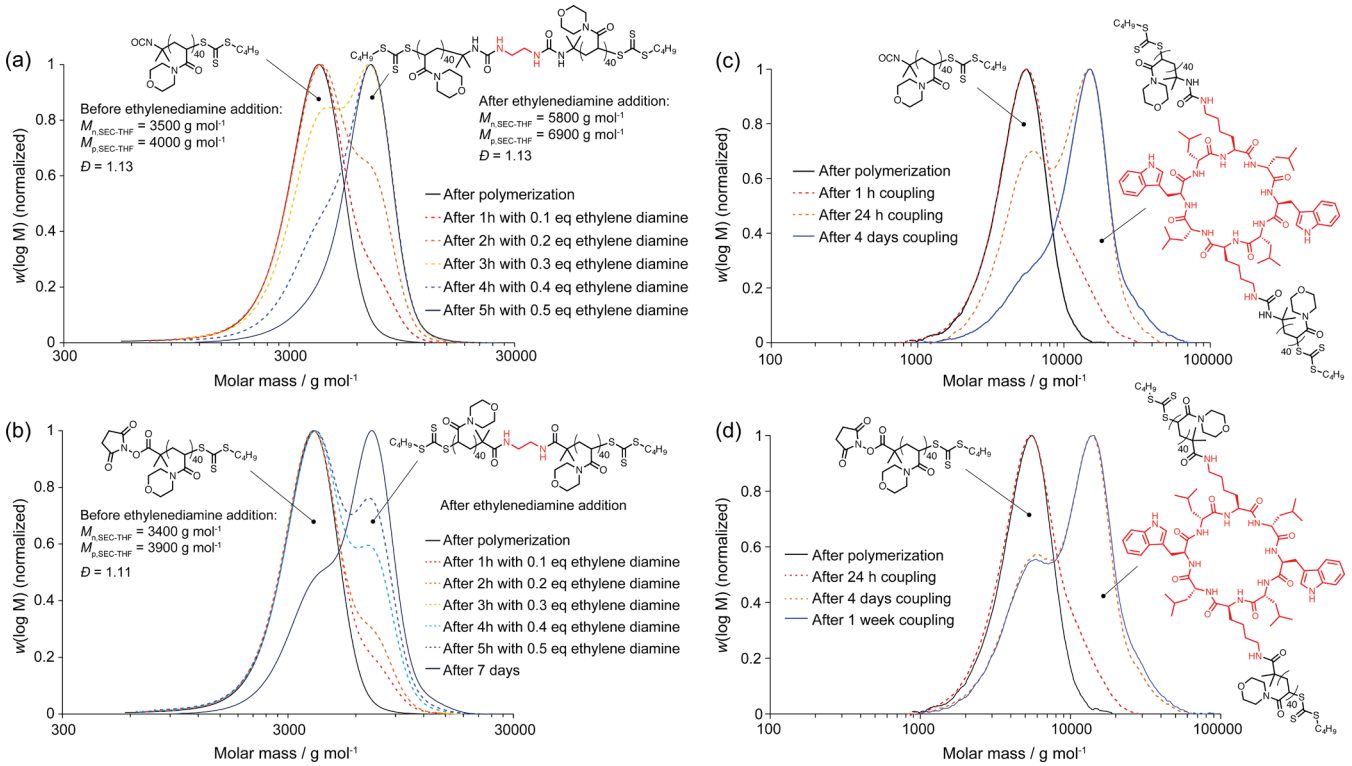

Figure 4. Comparison of the evolution of the SEC chromatograms showing the coupling reaction of (a) NCO-PNAM$_{40}$ (run 7, Table 1) and (b) α-NHS-PNAM$_{40}$ (run 8, Table 1) with a portion-wise addition of ethylenediamine (0.1 equiv per hour) performed at 25 °C in dioxane directly after polymerization; (c) α-NCO-PNAM$_{40}$ (run 9, Table 1) and (d) NHS-PNAM$_{40}$ (run 10, Table 1) with a single-aliquot addition diamino-cyclic peptide (0.5 equiv) at 25 °C in nonanhydrous DMSO/dioxane.

7, Table 1) compared with 75% for the active ester (Run 8, Table 1) even after prolonged reaction times (up to 7 days). It should be noted that withdrawing an aliquot from the reaction mixture for analysis before each new diamine linker addition slightly alters the overall reaction stoichiometry, which accounts for the lower coupling efficiency of run 7 compared to run 1 (100% via single aliquot addition). This result demonstrates that, under the identical conditions tested herein, the amine−^{t}NCO reaction outperforms the amine−^{t}NHS ester reaction.

Finally, we investigated the coupling reactions of α-^{t}NCO and α-^{t}NHS polymers to a more sophisticated diamine linker: a cyclic peptide synthesized from 8 alternating D- and L-amino acids, containing two nucleophilic lysine residues (runs 9 vs 10, Table 1). Irrespective of the coupling strategy (i.e., isocyanate or active ester), the one-pot diamine-linker approach that we have described herein allows us to precisely control the stoichiometry of the coupling partners (i.e., polymer and cyclic peptide). After polymerization of NAM to full conversion in dioxane, a solution of protonated cyclic peptide (0.5 equiv) in DMSO containing *N*-methylmorpholine (6 equiv) was added directly to the polymerization reaction mixture (SI Scheme S1 with the acyl azide CTA and SI Scheme S2 with the ^{t}NHS-CTA). Comparison of the SEC-chromatograms at different time intervals (Figure 3c,d) shows that the α-^{t}NCO-polymer reaches the same degree of coupling after 1 h as the α-^{t}NHS-polymer achieves in 24 h, again highlighting the superior rate of the amine−NCO reaction; neither reaction was observed to proceed beyond 4 days, at which point the coupling efficiencies were 93 ± 7% and 61 ± 13% for the α-NCO and α-NHS polymers, respectively. The lower coupling efficiencies observed with the cyclic peptide, compared to the small diamines, might be attributed to incipient self-assembly of the cyclic peptide into hydrogen-bonded supramolecular nanotubes.[24]

■ CONCLUSION

We have described an innovative methodology to quantify the efficiency at equimolarity of macromolecular conjugation reactions. This simple approach relies on the one-pot polymerization and subsequent homodicoupling of α-end-functional polymers in the presence of a bifunctional linker. This strategy is simple, effective, and can be adopted as a universal route to assess macromolecular coupling. Subsequently, we employed this protocol to demonstrate that the amine−^{t}NCO reaction is a highly efficient macromolecular ligation strategy for a wide range of vinyl-derived polymers. Indeed, the excellent performance of the amine−^{t}NCO reaction under the range of conditions described herein demonstrates that this coupling strategy fulfills several of the criteria of a click reaction for macromolecular coupling: It proceeds to completion rapidly (<1 h) at equimolarity under ambient conditions (e.g., room temperature in the presence of atmospheric moisture and oxygen) without generating any products or requiring any catalysts. Furthermore, the amine−^{t}NCO outperforms the ubiquitous amine−^{t}NHS coupling on comparable substrates. Although we have focused this study on the use of RAFT polymerization to enable a one-pot reaction strategy that facilitates the use of equimolar reactants, we anticipate the use of tertiary isocyanate can be extended to other types of polymers as a powerful and versatile click-type reaction.

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b11831.

Detailed experimental data (organic synthesis, coupling reactions and kinetics), ^{1}H NMR spectra, FTIR spectra, and size exclusion chromatography traces, ESI-ToF mass spectrometry (PDF)

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Notes

The authors declare no competing financial interest.

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