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Synthesis of Heterotelechelic α,ω Dye-Functionalized Polymer by the RAFT Process and Energy Transfer between the End Groups

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ABSTRACT: The synthesis of a vinyl polymer with two different fluorescent dye end groups using reversible addition−fragmentation chain transfer (RAFT) polymerization is described. Use of a pentafluorophenyl (PFP) activated ester chain transfer agent (CTA) provided a polymer with an α end group that was reactive toward amines and a dithioester ω end group. The α PFP ester was amidated with Oregon Green Cadaverin. This did not harm the ω dithioester, which was subsequently aminolyzed with an excess of *n*-propylamine in the presence of Texas Red-2-sulfonamidoethyl methanethiosulfonate, resulting in a disulfide bond connecting the second dye to the polymer chain. Excess dyes and side products were removed by thin layer chromatography (TLC). Gel permeation chromatography (GPC) using a UV−vis detector could verify the presence of each dye on the polymer chain and the absence of free dyes. The synthesis of the polymer by a living radical technique and the mild complementary conjugation methods conducted after polymerization at each end group allowed to introduce complex dye residues possessing high brightness and photostability. In particular, fluorescent dyes capable of acting as donor and acceptor for electronic excitation energy transfer were chosen. Time-resolved fluorescence measurements were used to determine the time constant of energy transfer between the end groups of isolated polymer chains. Assuming a Förster-type process, an average end-to-end distance of 4.5 nm was calculated, which was in reasonable agreement with data obtained from light scattering.

Introduction

Of the many possibilities to synthesize and employ functional polymers, especially heterotelechelic systems, i.e., polymer chains with two different functional end groups, are receiving an increasing amount of attention. Generally, they allow the preparation of ABC-type structures, where A and C are arbitrary groups kept at a specific distance by the polymer B. The polymer may provide solubility or a stimulus-dependent end group separation. One end group may as well be connected to a surface, with the polymer tethering the second end group to the surface but allowing it maximum distance and mobility. While end groups with high impact for biological research, such as two different proteins, have recently been reported,[1] polymers with functional end groups may also provide invaluable information for polymer physics. End-to-end distance, end group interactions, and polymer conformation may be calculated from energy transfer measurements between two fluorescent dye end groups.[2−6]

Fluorescence (or Förster) resonance energy transfer (FRET) is a useful tool for the investigation of molecular arrangements and fluctuations thereof on a nanometer scale, as the FRET efficiency is strongly distance dependent.[7,8] Two dyes located at strategic points of the same[9−17] or two different[18] molecules may thus provide extensive information on the distance and the relative orientation of these two points. Winnik and co-workers have extensively studied the end-to-end cyclization of polymers terminated with pyrene and dimethylaniline or benzil moieties prepared by anionic polymerization[19−21] or cationic ring-opening polymerization (CROP).[22] Only very few monomers are adequate for CROP, however. And as carbon anions are very reactive, the use of monomers and dye end groups in an anionic polymerization is greatly restricted to very inert molecules.[23−25] The dyes that have been employed in heterotelechelic polymers are hydrocarbon based, pyrene being the most prominent example. Pyrene and similar dyes, however, generally have a low solubility in organic solvents and a low emission yield and easily undergo excimer and exciplex formation.

It would therefore be of considerable interest to have synthetic access to heterotelechelic dye functionalized vinyl polymers without the harsh restrictions imposed by anionic polymerization or CROP. This would allow the use of more complex dye molecules with higher photostabilities, dyes suitable for single molecule spectroscopy,[26,27] or functional groups which could for instance provide water solubility. Such systems would not only be of interest for the investigation of polymer behavior—for instance a chain collapse, but also for the optoelectronic branch of modern science, where FRET finds many applications in light-harvesting arrays[28,29] or light-emitting diodes.[30]

The past decade has seen the vast and rapid ascent of controlled radical polymerization (CRP). Most prominent are nitroxide-mediated polymerization (NMP),[31] atom transfer radical polymerization (ATRP),[32,33] and reversible addition−fragmentation chain transfer polymerization (RAFT).[34−36] As the propagating species are radicals, less restrictions apply to the polymerization conditions, and a wide variety of monomers may easily be converted into polymers with excellent control over the molecular weight and very low polydispersity indices. As fairly

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new methods, however, the work on functionalized end groups, especially heterotelechelic polymers, has only just begun in the past few years. Several functionalized initiators and chain transfer agents allowing the introduction of one fluorescent dye per chain or the same dye at both end groups have been described for NMP,[37] ATRP,[38−41] and RAFT.[42−45] For polymers with two different functional end groups, generally two independent reactions for each end group modification have to be combined.[1,46−52]

In this paper, the preparation of an α,ω dye-functionalized polymer by the RAFT process is described. The synthesis featured a functionalized chain transfer agent providing a polymer with a pentafluorophenyl (PFP) activated ester at the α end group and a phenyl dithioester (DTE) as ω end group. The PFP esters were reacted with an amine-functionalized dye. As dithioesters are chromophores, and are known to interfere with dyes through exciplex formation and quenching,[53] the ω-terminal dithioesters were converted into functional disulfides carrying dye end groups by the use of a dye-functionalized methane thiosulfonate (MTS) reagent during aminolysis.[54,55] The dyes were chosen to be capable of electronic excitation energy transfer, and accordingly, the time constant of energy transfer between the end groups could be determined by recording the rise/decay time profile of the acceptor via time-correlated single photon counting (TCSPC).

Experimental Part

Materials and Methods. Oregon Green cadaverine was purchased from Molecular Probes. Texas Red methanethiosulfonate (Texas Red-2-sulfonamidoethyl methanethiosulfonate) was purchased from Toronto Research Chemicals. All other reagents and solvents were purchased from Acros, Aldrich, or Fluka. Thin layer chromatography (TLC) was performed on TLC aluminum sheets (20 × 20 cm, silica gel 60 F_{254}) purchased from Merck. Dialysis membranes (regenerated cellulose, 20 μm) were purchased from Roth and had a molecular weight cutoff (MWCO) of 12−14 kg/mol (in water; smaller pore size in MeOH).

Gel permeation chromatography was performed on 2 mg/mL THF solutions for UV−vis detection at 302 nm and for determination of molecular weight and polydispersity index. GPC measurements in DMF (1 mg/mL) were conducted for UV−vis detection at 502 and 580 nm because of a higher extinction coefficient of Oregon Green in DMF.

Static and dynamic light scattering measurements were performed with an ALV-SP86 goniometer, a Uniphase HeNe laser (22 mW output power at 632.8 nm wavelength), an ALV/High QE APD avalanche diode fiber-optic detection system, and an ALV-3000 correlator in linear mode. Prior to measurement, the solutions were filtered through 0.2 μm pore size Dimex filters (Millipore LG). The refractive index increment at $\lambda = 632.8$ nm was measured by a home-built Michelson interferometer.[56]

Steady state absorption and fluorescence measurements were performed with methanol solutions of the materials on an Omega 20 absorption spectrometer (Bruins Instruments, Germany) and a FluoroLog-3 (Instruments S.A., Jobin Yvon-Spex Division, Edison, NJ) spectrofluorometer, respectively. The concentration of the solutions for the steady state and time-resolved fluorescence studies was adjusted to $c \sim 10^{-7}$ mol/L. The fluorescence emission spectra ($\lambda_{ex} = 470$ nm) were corrected for the wavelength dependence of the detector, and the fluorescence excitation spectrum of the dyad ($\lambda_{ex} = 599$ nm) was corrected for the intensity variations of the excitation light source. Time-resolved fluorescence measurements were performed on a FluoroLog-3 spectrofluorometer connected to a FluoroHub TCSPC (time-correlated single photon counting) unit containing a time-to-amplitude converter (TAC) to construct the fluorescence decay histograms. In order to measure fast energy transfer times, a pulsed laser diode LDH−P-C-470 (PicoQuant, Berlin) with pulse duration of $\tau_p = 70$ ps was used for excitation ($\lambda_{ex} = 470$ nm, $\nu_{rep} = 10$ MHz), and a fast single photon avalanche diode (SPAD, Micro-Photon-Devices) was coupled to the exit slit of the emission monochromator. The overall time resolution of the setup was quantified by the fwhm (full width at half-maximum) of the instrumental response function (IRF) to ≈180 ps at $\lambda_{em} = 470$ nm. The fluorescence rise/decay time profile of the acceptor TR was fitted to a convolution of a biexponential model function with the IRF.

$$I_A(t) = \left[A_1 \exp\left\{-\frac{t}{\tau_{fl}}\right\} - A_2 \exp\left\{-\frac{t}{\tau_{EET}}\right\}\right] \otimes \mathrm{IRF}(t) + B \quad (1)$$

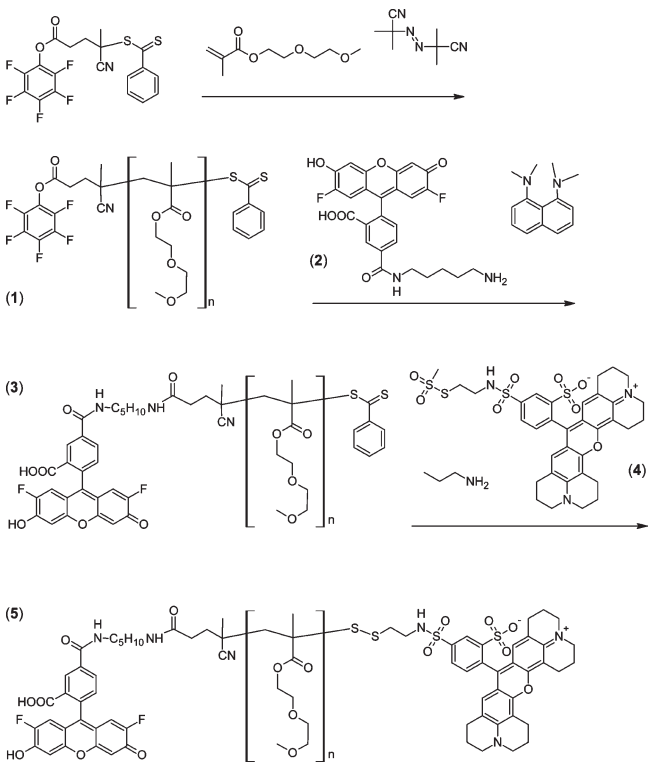
The model function consists of two time constants: the fluorescence lifetime τ_{fl} contributes with the positive amplitude A_1 and the energy transfer time τ_{ETT} with the negative amplitude A_2 (rise time). The parameter B accounts for background fluorescence and stray light contributions. Data processing was performed with an integrated data manipulation/visualization package (Igor Pro 6.04, Wavemetrics, Lake Oswego, OR).

Synthesis of α-Pentafluorophenyl Ester, ω-Dithioester Poly[diethylene glycol methacrylate] (PFP−PDEGMA−DTE, 1). The starting polymer was prepared according to a literature procedure.[57] As the dithioester has a characteristic absorbance band centered around 302 nm, the absence of free chain transfer could be confirmed by a gel permeation chromatography (GPC) with a UV−vis detector set to 302 nm. No signal at low molecular weight could be detected, indicating that all dithioester was located on polymer chains. The molecular weight of the polymer was determined by both GPC in tetrahydrofuran using a light scattering detector ($M_n = 6400$ g/mol) and by static light scattering (SLS) in methanol ($M_w = 6900$ g/mol). The radius of gyration R_g could not be determined by SLS indicating a value below 10 nm (no angular dependency of the scattering intensity, see Supporting Information), but this excluded the presence of high molar mass impurities. The SLS result is in accordance with dynamic light scattering (DLS) which yielded a hydrodynamic radius of 1.9 nm and a normalized second cumulant $\mu_2 = 0.08$ (scattering angle 20°), which is compatible with the polydispersity index of 1.11 given by GPC.

Reaction of PFP−PDEGMA−DTE (1) with Oregon Green Cadaverine (OG, 2). To 540 μL of a 18.65 mM solution of PFP−PDEGMA−DTE (**1**) in DMF (10.07 μmol), 5 mg (10.07 μmol) of Oregon Green Cadaverine and 4.75 mg (22.15 μmol) of 1,8-bis(dimethylamino)naphthalene (proton sponge) were added. The mixture was stirred at room temperature overnight in the dark. Afterward, the reaction mixture was dialyzed against methanol for 3 days with solvent changes twice a day. The mixture was removed from the membrane and dried under vacuum. The residue was dissolved in chloroform and extracted several times with water. After drying the organic phase (magnesium sulfate) and removing the solvent, OGC−PDEGMA−DTE (**3**) could be obtained in a 46% yield. A considerable product loss probably occurred during dialysis; however, the product was devoid of free dye which could be seen from a GPC measurement with the UV−vis detector set to 502 nm and from thin layer chromatography (TLC) (see Figure 3).

Reaction of OGC−PDEGMA−DTE (3) with Texas Red-Methanethiosulfonate (TR, 4). To 200 μL of a 3.36 mM solution of OG−PDEGMA−DTE (**3**) in DMF (0.672 μmol), first 5 mg (6.72 μmol) of Texas Red-2-sulfonamidoethyl methanethiosulfonate were added, and then 3.9 μL (47 μmol) of *n*-propylamine was injected. The mixture was stirred at room temperature overnight in the dark and was then dialyzed against methanol for 3 days with solvent changes twice a day. After that time, a black precipitate had formed inside the dialysis membrane, probably composed of free dye or the corresponding dye−dye

Scheme 1. Synthesis of Polymer with Two Functional End Groups and Successive Attachment of Fluorescent Donor and Acceptor Dyes



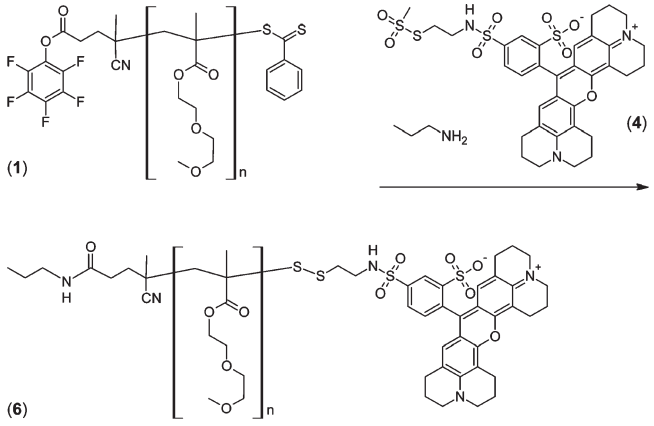
disulfide, which have a low solubility in methanol. The precipitate was removed by filtration. The solvent was removed under light vacuum, and water was added to the residue. Again, a black precipitate was removed by filtration. The water was removed under light vacuum, and the residue was dissolved in a minimum amount of dichloromethane. This raw product was further purified by thin layer chromatography (TLC) using methanol as solvent yielding the dye labeled polymer OG−PDEGMA−TR (**5**) in 34% yield. R_f (**5**): 0.75, R_f (free dye): 0.59, R_f (red side product, probably a dye−dye disulfide): 0.30.

Synthesis of PDEGMA−TR (6). α-*n*-Propylamide, ω-dithioester poly[diethylene glycol methacrylate] was synthesized in analogy to the synthesis OG−PDEGMA−TR (**5**) using PFP−PDEGMA−DTE (**1**) as starting material instead of polymer **3**. In this case, the excess of *n*-propylamine performed the aminolysis of both the PFP ester and the dithioester. The *n*-propylamide α-end group does not provide any fluorescence or influence the polymer's solubility or size compared to the native PFP ester end group. For purification, dialysis was omitted; DMF was removed from the reaction, and water was added. After filtration, the polymer was extracted from the aqueous phase with dichloromethane. The organic phase was dried with magnesium sulfate and concentrated on a rotovap. The raw product was purified by TLC with methanol as solvent. R_f values were the same as given above. Polymer **6** was obtained in 59% yield.

Results and Discussion

Method. Reversible addition−fragmentation chain transfer (RAFT)[34−36] polymerization was chosen as method to prepare a vinyl polymer with orthogonally reactive end groups, which were then reacted with functional dye molecules to obtain a donor/acceptor end group modified polymer. Because of the polymerization mechanism, monomers are inserted between the leaving group R and the dithioester (DTE) (see upper part of Scheme 1). Accordingly, the R group is retained as α end group, which is directly joined

Scheme 2. One-Step Synthesis of Polymer with Only Acceptor Dye



to the polymer chain, whereas the Z moiety in its subsequent role as ω end group is connected to the polymer chain via the dithioester. Incorporating functional[1,43,50,51,58,59] or reactive[36,49,57,59−65] sites into the R group of the CTA or employing functional[45,51] or reactive[49,66−68] Z groups can therefore produce polymers with one or two[1,49−51] defined end groups. In addition to being susceptible toward chemical decomposition,[36,69−71] e.g., during storage in tetrahydrofuran,[72] dithioesters are chromophores and are known to interfere with dyes through exciplex formation and quenching.[53] For these reasons, it is not favorable to attach a fluorescent dye to the Z group, whether into the CTA before polymerization or onto an appropriately reactive Z group after polymerization. Alternatively, a dithioester may also be understood as a protected thiol. Aminolysis is a very common method to release the terminal thiols[42,73−78] which may regrettably undergo side reactions.[54,79,80] A strategy can be the simultaneous aminolysis and the reaction with a thiol-reactive reagent. While 2,2′-dithiodipyridine[81,82] and divinylsulfone[83] have been used to produce polymers with terminal thiol-reactive sites, functional maleimides[81,84] or functional methane thiosulfonates (MTS)[54] have been employed to introduce a terminal functionality in one step.

In the present paper, we use an MTS reagent carrying the fluorescent dye Texas Red to introduce this dye at the ω end group. This method for ω functionalization was combined with a pentafluorophenyl (PFP) activated ester α end group which was installed into the polymer via a chain transfer agent carrying a PFP modified R group (see Scheme 1). Both literature results[59] and preliminary experiments conducted for this study (please see Supporting Information for details) showed a sufficient difference of reactivity of activated esters versus dithioesters with primary amines and thus the possibility to quantitatively convert PFP esters with one equivalent of amine in the presence of the dithioester at the ω position, which stayed intact. Both end groups—α PFP and ω DTE—are reactive toward amines. This fact also invites progressing via a one-step reaction course where an excess of amine performs both reactions at the two end groups. However, as some complex functional amines, such as e.g. the dye-functionalized amine **2**, might have solubility issues, might be disadvantageous nucleophiles due to steric hindrance, or might simply be too expensive to be used in large excess, a two-step reaction may be favored. In this paper, we describe both the one-step and the two-step reaction pathways. The syntheses are outlined in Schemes 1 and 2.

Dyes and Polymer. Texas Red (TR), introduced as MTS reagent (**4**), served as energy acceptor. As donor, Oregon Green (OG) Cadaverine (**2**) was chosen, which was reacted

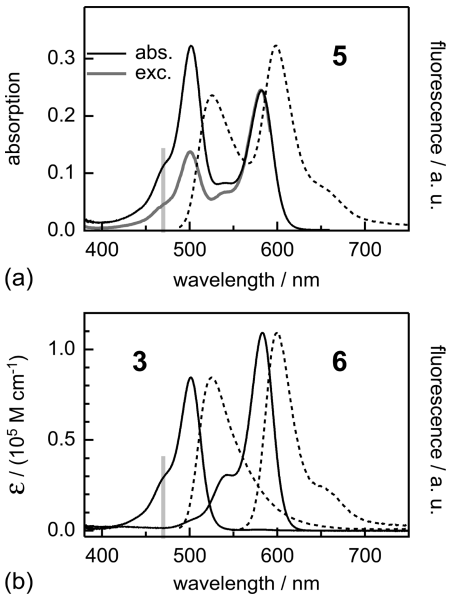


Figure 1. Absorption (black lines) and fluorescence emission spectra (dashed lines) of (a) polymer **5** and (b) the one-dye-only polymer compounds **3** and **6** in methanol solutions. Additionally, in (a) the fluorescence excitation spectrum of polymer **5** (λ_{em} = 599 nm) is displayed (gray line). The excitation wavelength of λ_{ex} = 470 nm is marked with a gray bar.

Table 1. Polymer Molecular Weight and Dimensions

	obtained from	value
M_w[a]	SLS[g]	6900 g/mol
M_n[b]	GPC[h]	6400 g/mol
M_w	GPC	7050 g/mol
PDI[c]	GPC	1.11
r_H[d]	DLS[i]	1.9 nm
r_{DA}[e]	TCSPC[j]	4.5 nm
R_0[f]		6.1 nm

[a] Weight-average molecular weight. [b] Number-average molecular weight. [c] Polydispersity index = M_w/M_n. [d] Hydrodynamic radius (methanol). [e] Average donor−acceptor distance. [f] Förster radius. [g] Static light scattering. [h] Gel permeation chromatography. [i] Dynamic light scattering. [j] Time-correlated single photon counting.

with its primary amine. This donor−acceptor couple allowed for selective excitation of the donor and selective detection of the emission of the acceptor (see Figure 1). The Förster radius was calculated to be R_0 = 6.1 nm (for details, see below). Since at this separation the energy transfer efficiency is 50%, we aimed for a polymer size (end-to-end distance) around or below this value to enable an efficient energy transfer between the end groups. Poly[diethylene glycol monomethyl ether methacrylate] (PDEGMA) was chosen as spacer between the dyes as it is very well soluble in polar solvents. As an energy transfer between the polymer end groups was intended, a narrowly distributed, low molecular weight polymer was synthesized. Molecular weights and size of the polymer determined by GPC and light scattering are given in Table 1.

Synthesis of Heterotelechelic Polymer. The two-step synthesis of the donor/acceptor labeled polymer OG-PDEGMA-TR (**5**) is described in Scheme 1. In order to avoid decomposition of the DTE during the modification of the PFP ester, only 1 equiv of donor amine (OG Cadaverine, **2**) was employed. Additionally, 1,8-bis(dimethylamino)naphthalene was used as non-nucleophilic scavenger for the acidic pentafluorophenol. The product **3** of this first step was purified by dialysis and extraction. It was intensely colored, indicating a successful dye attachment, and still showed the proton

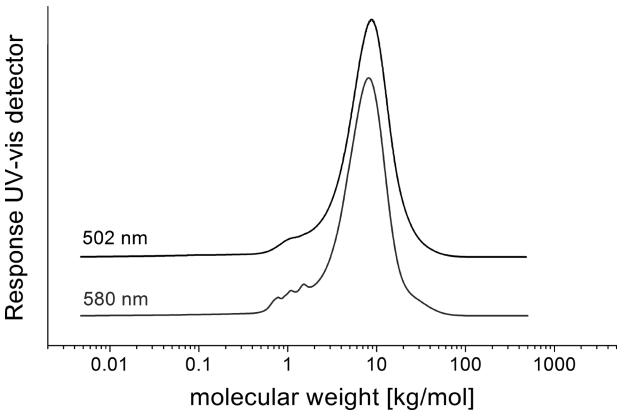


Figure 2. Gel permeation chromatograms (DMF) of donor/acceptor labeled polymer **5** with the UV−vis detector set to 502 nm (upper curve, selective detection of donor) and 580 nm (lower curve, selective detection of acceptor). The position of the monomodal peaks and the absence of other signals showed the attachment of each dye to the polymer and the absence of excess free dyes.

signals of the dithiobenzoyl group in ^{1}H and HSQC NMR, showing the presence of the desired DTE end group and confirming the higher reactivity of PFP esters toward amines compared to DTE (spectra are given in Supporting Information). In the second step, the DTE of the OG labeled polymer **3** was aminolyzed with a 70-fold excess of *n*-propylamine in the presence of a 10-fold excess of Texas Red-MTS (**4**). The product was purified by dialysis and preparative thin layer chromatography (TLC) with methanol as solvent. This technique yielded a spot of orange color, as to be expected from a mixture of red and green emission, as well as two spots of red color. One of these spots corresponded to the reagent Texas Red-MTS, whereas the second one was assumed to originate from a dye−dye disulfide caused by hydrolysis and oxidative coupling of two MTS species. The orange spot yielded a material, which showed both red and green absorbance in UV−vis spectroscopy (solvent methanol) (see Figure 1a). This product was characterized further by gel permeation chromatography (GPC, in DMF). Two measurements were performed, the only difference being the wavelength the UV detector was set to. Setting it to 502 nm allowed the selective detection of the Oregon Green dye which has its absorbance maximum at that wavelength (in DMF) because the polymer itself has no absorbance in the visible range. The elution curve (Figure 2, upper graph) showed a monomodal peak around 9 kg/mol (polystyrene standard), indicating that the green dye now had the molecular weight of the polymer because they had been covalently joined together, as was already known from the analysis of the intermediate product (**3**). No peak in the low molecular weight region was detected, suggesting the complete removal of unbound dye. Moreover, the elution curve showed no signal of a double molecular weight material, confirming that no polymer−polymer disulfide of the structure OG-PDEGMA-S-S-PDEGMA-OG had been formed. In contrast, GPC analysis with the UV−vis detector set to 580 nm allowed the selective detection of the Texas Red dye, as neither polymer nor Oregon Green absorbs at this wavelength (Figure 2, lower graph). The monomodal peak at the molecular weight of the polymer gave evidence that the MTS reaction had successfully introduced a second dye onto the ω end group of the α-modified polymer. Again, the absence of strong signals of low molecular weight or double molecular weight suggested that the vast majority of red dye in the sample had been attached to the narrowly size

distributed polymer. This data thus showed that the applied combination of PFP esters and MTS chemistry could successfully introduce two terminal fluorescent dyes onto a polymer chain.

For energy transfer evaluations, measurements of systems with only the donor or only the acceptor are conducted and used for comparison. As green-only polymer, the intermediate **3** was employed. To have an appropriate red-only reference, a one-step reaction yielding a polymer PDEGMA-TR (**6**) was carried out. This way, the red-only material had the same solubility and diffusion properties as the donor−acceptor pair **5** and was not prone to hydrolysis and subsequent oxidation, in contrast to a supposable Texas Red-MTS reagent reference. The synthesis is outlined in Scheme 2. The starting α-PFP, ω-DTE PDEGMA (**1**) was subjected to an excess of *n*-propylamine and Texas Red-MTS (**4**). In this case, the amine formed the propylamide at the α end group and released the terminal ω-thiol in one step. For purification, precipitation and preparative thin layer chromatography (TLC) were applied. TLC could also be employed to probe the successful attachment of dyes to the polymer as well as the absence of free dyes. Both reactant dyes Oregon Green Cadaverine (**2**) and Texas Red-MTS (**4**), the three dye-labeled polymers (with both donor and acceptor **5**, only donor **3**, only acceptor **6**), and the starting polymer **1** were run on a TLC plate with chloroform/methanol/aqueous ammonia 3:2:1 drop as eluent. Figure 3 shows a photograph of the TLC plate under UV light (365 nm) irradiation. In the solvent mixture, all polymers had an R_f value of 1, whereas the free dyes were not transported all the way to the top. The orange color of the spot caused by the donor/acceptor labeled polymer (**5**, Figure 3, spot 3) suggested the presence of both green and red dye within the polymer. The one-dye polymers **3** and **6** gave spots of distinct green and red color. No spots corresponding to free dyes could be seen for polymer samples. The starting polymer (**1**, Figure 3, spot 6) did not have any emission and could not be seen under this illumination. Using a wavelength of 254 nm, however, a spot at $R_f = 1$ could be seen due to the fluorescent labeling of the TLC plate.

The end group conversions were calculated from the absorbance of solutions with defined concentrations, considering the molecular weight of the polymer and the molar extinction coefficient of each dye (please see Supporting Information for details). There was, however, an uncertainty about the exact molecular weight of the polymer. The conversion with Oregon Green Cadaverine on polymers **3** and **5** was thus estimated to be at least 71%, still in the same range as conversions previously reported with a PFP α-end group.[57] The functionalization with Texas Red employing MTS chemistry was at least 47% in the case of polymer **5** prepared in two steps and at least 48% in case of polymer **6** synthesized in one step. These values are lower than expected from previous reports on quantitative conversions.[54,55] Noteworthy, both conversions of the MTS reactions are in the same range. It can therefore be ruled out that the α end group amidation in the two-step process had caused a significant loss of ω-DTE of polymer **3**, as even in the one-step reaction, where the complete presence of DTE of the starting polymer **1** can be assumed, no quantitative conversion was reached. A likely reason for the lower conversion is the rather poor solubility of the Texas Red MTS reagent. Because of this, a lower absolute amount (10 compared to 20 equiv[55]) of MTS reagent and also a significant lower polymer concentration (3.36 compared to 42 mM[55]) were employed. The lower end group conversions should thus be attributed to the intricacy of this MTS reagent. Poor solubility of reagents can thus limit the scope of otherwise robust, efficient, and orthogonal functionalization methods.[85] Polymer sample **5** thus also contained polymer chains labeled with only an acceptor or only a donor dye. However, spectroscopic techniques discussed below were designed to be sensitive only to polymers carrying both dyes; thus, the presence of polymer bearing only one of the two chromophores was not an issue. The general method, however, of combining MTS conversions with PFP activated esters allows the orthogonal α/ω end group functionalization of RAFT polymers in one or two steps and thus presents a synthetic route toward a broad range of possible end group modifications, including even challenging molecules such as complex fluorescent dyes. In the present project, the next step was the spectroscopic exploitation of polymer **5** with its strategically located dyes.

Spectroscopy. The absorption and fluorescence emission spectra of the donor−acceptor-labeled polymer **5** and the donor-only (acceptor-only) polymers **3** (**6**) in methanol solutions are shown in Figure 1. Within the experimental accuracy, no difference of the spectral positions and the shape of the spectra of free (data not shown) and polymer-coupled chromophores (**3**, **5**, **6**) could be observed. After selective excitation of the donor in polymer **5** at 470 nm (gray bar in Figure 1a), the emission spectrum showed donor as well as acceptor emission, suggesting the occurrence of excitation energy transfer. Compared to the singly labeled polymers (**3**, **6**) with the same dye concentrations, the emission spectrum showed a decreased donor emission and an increased acceptor emission (see Supporting Information). In addition to the absorption spectrum, also the excitation spectrum of **5** using a detection wavelength of 599 nm (selective acceptor emission) is shown in Figure 1a (gray curve). The obvious discrepancy between the absorption and excitation spectra was due to the presence of singly labeled polymer (or trace amounts of free donor) in the solution. Because of the low concentration of the components, however, diffusion-controlled energy transfer from donor-only labeled polymer (via interchain energy transfer) only played a minor role, leading to a higher donor contribution in the absorption spectrum. Accordingly, by selective excitation of the donor and selective detection of the acceptor emission, we could sort out to a high degree those polymer chains carrying both the donor and acceptor chromophore and being capable of energy transfer (via intrachain energy transfer). As the concentration of the polymer solution could not be determined precisely, the molar extinction coefficients of the free dyes Oregon Green (**2**) and Texas Red-MTS (**4**) were used to scale the absorption spectra of **3** and **6** (Figure 1b). Because of the low solubility of Texas Red-MTS (**4**) in methanol, the extinction coefficient was measured in DMF. For the following considerations, it was assumed that the coupling of the dyes to the polymer as well as the solvent change (in the case of Texas Red) had only a minor influence on the molar extinction coefficients. According to Förster theory,[7] the time constant of energy transfer τ_{EET} and the transfer efficiency E_{EET} can be calculated by the following equations

$$\tau_{EET}^{-1}(r) = k_{EET}(r) = \frac{1}{\tau_D}\left(\frac{R_0}{r_{DA}}\right)^6 \quad \text{and}$$

$$E_{EET}(r) = 1/(1 + (r_{DA}/R_0)^6) \qquad (2)$$

where τ_D is the donor fluorescence lifetime (in the absence of acceptor) and r_{DA} is the center-to-center distance of donor and acceptor. Please note that eq 2 is derived for the case in

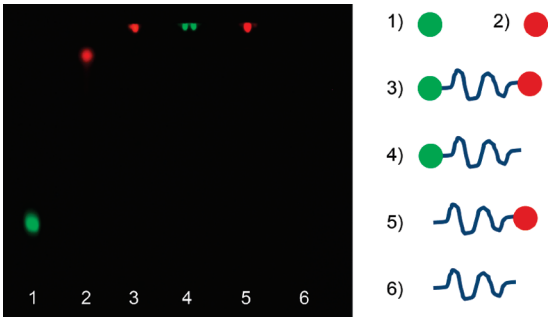


Figure 3. Photograph of thin layer chromatography plate under UV irradiation (365 nm). Running direction was from bottom to top with chloroform/methanol/aqueous ammonia 3:2:1 drop as eluent. (1) donor reagent Oregon Green Cadaverine (**2**); (2) acceptor reagent Texas Red-MTS (**4**); (3) donor/acceptor labeled polymer **5**; (4) donor-only polymer **3**; (5) acceptor-only polymer **6**; starting polymer **1**.

which an ensemble of dyes are all separated by a common distance r_{DA}. The characteristic Förster radius R_0 is given by the following expression:[18]

$$R_0 = 0.211[\kappa^2 n^{-4} Y_{fl}^{D} J(\lambda)]^{1/6} \quad (3)$$

For the donor−acceptor couple considered here, the value of the spectral overlap integral $J(\lambda) = 2.96 \times 10^{-22}$ m^6 mol^{-1} was obtained from a convolution of the absorption spectrum of **6** with the area-normalized emission spectrum of **3** (Figure 1b). For the orientation factor, we assumed a value of $\kappa^2 = 2/3$,[18] corresponding to a random orientation of the transition dipoles of both chromophores due to rotational diffusion which seems to be a reasonable approximation for the situation in **5** in solution. The fluorescence lifetime of the isolated donor **3** was measured via time-correlated single photon counting (TCSPC) to be $\tau_D = 4.0$ ns (data not shown). With a fluorescence quantum yield $Y_{fl}^{D} = 0.92$ for Oregon Green (**2**)[86] and the refractive index $n = 1.33$ of methanol, a Förster radius R_0 of 6.1 nm was obtained.

The energy transfer time could be directly measured with TCSPC by recording the rise/decay time profile of the acceptor after selective pulsed excitation of the donor.[87] As pointed out before, this approach allowed to sort out the doubly labeled polymers capable of energy transfer. In contrast, recordings of the donor decay curves would be contaminated by contributions from donor-only labeled polymer. The fluorescence rise/decay profile of **5** recorded at an emission wavelength of 599 nm ($\lambda_{ex} = 470$ nm) is shown in Figure 4 (red curve) together with the instrumental response function (IRF). The rise/decay profile could be described by eq 1, resulting in a fluorescence lifetime of the acceptor of $\tau_{fl} = 4.4$ ns (positive amplitude) and an energy transfer time of $\tau_{EET} = 671$ ps (negative amplitude). Since the fluorescence lifetime of the acceptor-only polymer **6** was determined to be $\tau_A = 4.4$ ns, the presence of the donor obviously did not influence the acceptor decay. The fluorescence rise time curve of the acceptor could be fitted by a single exponential, although there ought to be a distribution of donor−acceptor distances in **5**. One reason could be that due to the limited number of data points in the rise profile several components are not easily distinguished. Inserting the energy transfer time into eq 2, a donor−acceptor distance of $r_{DA} = 4.5$ nm was obtained, which translated into a transfer efficiency of $E_{EET}(r) = 0.85$. This high value of $E_{EET}(r)$ and a donor−acceptor distance which is smaller than the Förster radius suggested that in a large fraction of polymers **5** energy was transferred quite efficiently from the donor to the acceptor. Hence, we assumed that the contributions

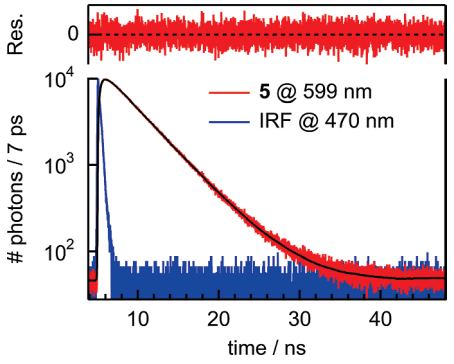


Figure 4. Fluorescence rise/decay time profile for polymer **5** (red curve) at $\lambda_{em} = 599$ nm and the instrumental response function (IRF) recorded at $\lambda_{em} = 470$ nm (blue curve). An iterative reconvolution fit (solid line) according to eq 1 delivered a fluorescence lifetime $\tau_{fl} = 4.4$ ns of the acceptor and an energy transfer time of $\tau_{EET} = 671$ ps.

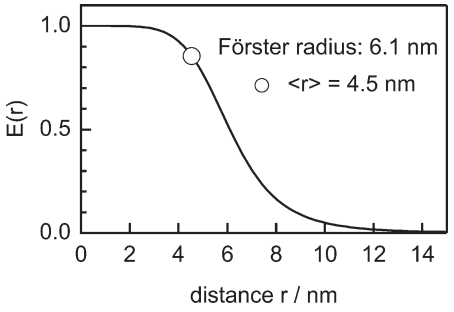


Figure 5. Energy transfer efficiency $E_{EET}(r)$ as a function of the donor−acceptor distance with the Förster Radius $R_0 = 6.1$ nm of polymer **5**. The transfer efficiency determined by TCSPC (○) is shown.

from such chains dominated the acceptor rise curve, this being another cause for an apparent single exponential behavior. Accordingly, it is reasonable to assume that the energy transfer time and the quantities derived from it ($E_{EET}(r)$, r_{DA}) represent average values of an at present unknown distribution. In general, distance distributions can be inferred from the energy transfer efficiency assuming a specific polymer model.[88] Such studies, which are beyond the scope of this paper, will be especially helpful when polymer chains of different lengths are studied and distance distributions directly determined by single molecule studies.

From the DLS experiments, the hydrodynamic radius of the polymer was found to be $r_H = 1.9$ nm, corresponding to a diameter of 3.8 nm. To compare these values to the TCSPC measurements, which directly give information on end-to-end-distances (i.e., donor−acceptor distances), the hydrodynamic radius would have to be converted into an end-to-end distance distribution, which again would call for a specific polymer model not available at present. Nevertheless, we feel that a diameter of 3.8 nm (from DLS) at least qualitatively is in agreement with the results from the energy transfer measurements ($r_{DA} = 4.5$ nm).

In Figure 5, the energy transfer efficiency $E_{EET}(r)$ calculated as a function of the donor−acceptor distance r_{DA} for the donor−acceptor couple used in this study (Förster radius $R_0 = 6.1$ nm) is shown. At a value corresponding to the Förster radius ($R_0 = 6.1$ nm) the transfer efficiency is 50%. In addition, given in the plot is the (average) donor−acceptor distance of polymer **5** as determined by TCSPC. As methanol is a good solvent, PDEGMA is assumed to adopt an extended conformation. By inducing a coil collapse, the average donor−acceptor distance r_{DA}

should decrease, and the energy transfer efficiency should increase. From Figure 5, however, it can be estimated that even a reduction of the donor−acceptor distance in the 1 nm range would lead to only a small change in the transfer efficiency for the given combination of D−A couple and polymer, being difficult to detect within the error margins of the experiment. Therefore, it would be advantageous to use either a longer polymer chain or a different donor−acceptor couple (decreased spectral overlap) to shift the transfer efficiency of the noncollapsed state into the 50% regime, where distance variations lead to strong changes in the transfer efficiency. With the successful synthesis of a heterotelechelic dye-labeled polymer, we could envision to study a stimulus-induced chain collapse at the level of single polymer molecules.

Conclusion

Two methods to functionalize the end groups of RAFT polymers were combined to generate α,ω heterotelechelic vinyl polymers carrying two different fluorescent dyes at their end groups. Pentafluorophenyl (PFP) activated ester α end groups were employed to be reacted with the amino group of Oregon Green Cadaverine, which subsequently acted as energy donor. The success of the independent ω-functionalization relied on the polymers retaining their dithioester after the mild PFP ester conversion. Aminolysis in the presence of a methane thiosulfonate carrying the dye Texas Red introduced the energy acceptor onto the ω end groups via disulfide linkage. Through this method, the easy decomposable dithioester is not retained in between the polymer and the ω-functionality, and the conversion at the ω end group (or also both end groups) may be carried out in a single step. Compared to anionic polymerization or cationic ring-opening polymerization, fewer restrictions for monomer or dye end group choice applied, allowing the introduction of high-performance dyes. These end groups allowed for an easy detection of the successful attachment and the purification by TLC through GPC. Reference materials carrying only one of the two chromophores were synthesized.

The fluorescence excitation spectrum showed that energy was transferred between the end groups of isolated polymer chains, which could be verified and quantified by time-resolved fluorescence measurements. Following the Förster formalism, Förster radius and average dye−dye end group distance were calculated, the latter being in agreement with data on the polymer size obtained from dynamic light scattering. The synthetic approach described here opens the possibility to investigate the stimulus induced chain collapse of polymers on single chains.

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Supporting Information Available: ^{19}F NMR and UV−vis absorbance data showing the selective conversion of an α-PFP ester in the presence of an ω-DTE; HSQC and UV−vis data showing the presence of α-dye and ω-DTE in polymer **3**; calculations of end group conversions; light scattering experiments and results, UV−vis and fluorescence spectra of polymers **3**, **5**, **6** with same dye concentrations. This material is available free of charge via the Internet at http://pubs.acs.org.

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