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Combining RAFT and Staudinger Ligation: A Potentially New Synthetic Tool for Bioconjugate Formation

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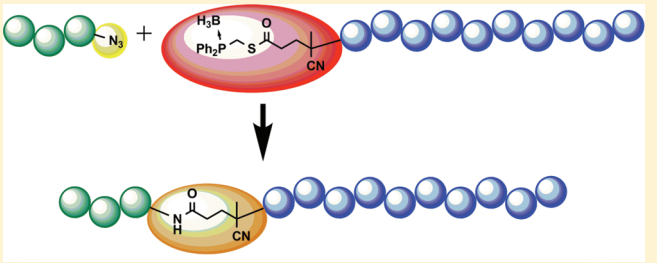
Supporting Information

ABSTRACT: We report a new route for biocompatible polymer end-group modification by means of the Staudinger ligation. This reaction allows the formation of a peptide bond in aqueous media between a phosphine-containing ester functionality and an azide group. Esterification of the two carboxylic acid-containing chain transfer agents (CTAs), 2-(dodecylsulfanylthiocarbonylsulfanyl)-2-methylpropionic acid (**1**) and 4-cyano-4-(dodecylsulfanylthiocarbonylsulfanyl)pentanoic acid (**2**), with different appropriate phosphines gave phosphine-containing CTAs. They allowed us to synthesize polystyrene of medium molecular weight via "reversible addition−fragmentation chain transfer" (RAFT) polymerization. 3,6,9-Trioxodecyl azide (**TOD-N$_3$**) was then used as model compound to study the Staudinger ligation with the corresponding polymers. Among all CTAs tested, the phosphine-functionalized **CTA-4**, prepared from **2** and *P*-borane-(diphenylphosphanyl)methanethiol (**6**), not only proved to be suitable for RAFT polymerization of styrene but the polymer-bound *P*-borane-(diphenylphosphanyl)methyl thioester group also showed the best performance in the subsequent polymer analogous Staudinger ligation.



INTRODUCTION

During the past years strong efforts have been put in the synthesis of well-defined polymer materials. Specifically, the development of controlled radical polymerization (CRP) techniques such as nitroxide-mediated radical polymerization (NMRP),[1] atom transfer radical polymerization (ATRP),[2] and reversible addition−fragmentation chain transfer (RAFT)[3] has prompted further the design of a large number of macromolecular assemblies for distinct applications. Because of the good structural control that can be achieved as well as the tolerance toward many functional groups, these processes certainly belong to the most rapidly expanding themes in polymer science. Highly efficient reactions, often called "click" reactions, are another important topic especially in polymer chemistry where specific features have to be addressed.[4] In particular, the combination of CRP with "click" chemistry is foreseen to have a striking impact on material science. Click reactions like the Cu(I)-catalyzed 1,3-dipolar cycloaddition reactions[5,6] between alkynes and azides have been successfully employed not only for the construction of telechelic polymers[7−9] but also in the orthogonal side-group modification[10−12] and the synthesis of hyperbranched materials.[13] Meanwhile the field has grown significantly, and several review articles have been published.[14−21] Nonetheless, the emergence of azide−alkyne click chemistry in polymer science also provoked a search for alternate reactions that can be counted as click reactions with similar simplicity and versatility.[22−26] In many cases, traditional organic reactions are reconsidered and combined with controlled radical polymerizations to allow for the design of tailor-made materials.[27,28] Clearly spoken, the inspiration for a proper manipulation of macromolecules on a functional as well as architectural level can be drawn from well-established, modified techniques in organic chemistry.

With this philosophy, the traceless Staudinger ligation[29,30] is proposed to be a synthetic tool for reliable polymer analogous reactions. The Staudinger ligation has to be regarded as a special case of an aza-Wittig reaction[31] where an intermediately formed aza-ylide can be converted with a plethora of electrophiles. Usually, the aza-ylide is being formed by the reaction of a phosphine with an organic azide, which in general proved to be the subject of very prosperous chemistry.[32] Some years ago, Bertozzi[33,34] and Raines[35] designed a system where such aza-Wittig reactions can be conducted in aqueous media without the reduction of the azide into the corresponding amine, i.e., Staudinger reduction. In this concept, the electrophile is placed at the vicinity of a phosphine which can be easily reacted with an azide to form the aza-ylide. A subsequent intramolecular reaction links the former azide covalently to the electrophile while eliminating the phosphine in its oxidized form. This seminal work has drawn a lot of attention, and especially at the interface of organic chemistry and life science a vast progress in the development of organic−bioorganic conjugates has been launched.[36−41] The big advantage of this method over a classical click reaction is that one is not restricted to the formation of a rather non-biogene triazole ring. Depending on the nature of the

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electrophile, the resulting link is an amide, imine, carbodiimide bond, etc.[30] Moreover, Cu(I) catalysis is entirely abandoned, and the reactions can be carried out selectively in water and organic media. In fact, phosphines have been developed that orthogonally stimulate the acylation of organic azides rather than amines which might be present in a reaction mixture.[42] Therefore, with the same right, the Staudinger ligation might be assigned to the methodology of click chemistry.[4]

In the present work we demonstrate for the first time the unique combination of RAFT polymerization and Staudinger ligation. Novel RAFT reagents were synthesized that allowed for the synthesis of well-defined polymers with adjustable molecular weights and low polydispersities. The utilized chain transfer agent transferred a fragment into the macromolecule that could be used for polymer analogous Staudinger ligation, giving rise to the design of hybrid polymeric materials.[43]

EXPERIMENTAL SECTION

Measurements. NMR spectra were recorded on a DRX 500 spectrometer (Bruker, Germany) operating at 500.13 MHz for ^{1}H, 125.75 MHz for ^{13}C, and 202.40 MHz for ^{31}P using $CDCl_3$ as solvent. The spectra were referenced on the solvent signal ($\delta(^1H)$ = 7.26 ppm; $\delta(^{13}C)$ = 77.0 ppm) and on external phosphoric acid ($\delta(^{31}P)$ = 0 ppm). The signal assignments were deduced from the combination of 1D and 2D NMR spectra.

Gel permeation chromatography (GPC) with narrow dispersed polystyrene standards (PL EasiCal PS-1, Polymer Laboratories) was used to determine molar masses and polydispersity indices (PDI) of the polymers. GPC measurements with THF as eluent were performed employing a Polymer Laboratories PL-GPC 50 Plus Integrated GPC system equipped with a microvolume double piston pump. Measurements were conducted with a PL-RESIPORE 300 × 7.5 mm column using a RI or a LS detector. In all GPC measurements the flow rate was set to 1 mL/min.

Matrix-assisted laser desorption/ionization−time-of-flight (MALDI-TOF) mass spectra of the polymers were acquired on a Biflex II MALDI-TOF MS system (Bruker Daltonics, Germany). The measurements were carried out in the positive ion reflectron mode, and a continuous ion acceleration voltage of 20 kV was used. 1,8,9-Trihydroxyanthracene (dithranol) and 2-(4-hydroxyphenylazo)benzoic acid (HABA) were used as matrices and dissolved in acetone, respectively. Sodium azide was used as cationizing agent. The samples were exposed on a target to the desorption/ionization process induced by pulsed N_2 laser (337 nm). The ionized molecules were accelerated and reflected by electric fields and projected on a mass-sensitive detector. Each mass spectrum represents the accumulation of about 300 single-laser-spot spectra.

Chemicals. The monomer styrene was filtered through basic alumina oxide to remove the stabilizer. 2-(Diphenylphosphanyl)phenol (**3**),[44] thioacetic acid *S*-[*P*-borane-(diphenylphosphanyl)methyl] ester (**5**),[45,46] 2-(dodecylsulfanylthiocarbonylsulfanyl)-2-methylpropionic acid (**1**),[47] and 4-cyano-4-(dodecylsulfanylthiocarbonylsulfanyl)pentanoic acid (**2**)[48] were synthesized according to the corresponding literature procedures. All other reagents were purchased from Aldrich and used without further purification. Further experimental details and characterization results on compounds **CTA-1**, **CTA-2**, **CTA-3**, *P*-borane-2-(diphenylphosphanyl)phenol (**4**), *P*-borane-(diphenylphosphanyl)-methanethiol (**6**), and 3,6,9-trioxodecyl azide (**TOD-N$_3$**) can be found in the Supporting Information.

CTA-4. **CTA-4** was prepared in a two-step procedure. First, **2** was transformed into the activated ester **2-A**, i.e., the pentafluorophenyl ester. Then, the activated ester was reacted with phosphine **6**. For the first step, a round-bottom flask was charged under an argon atmosphere with 4-cyano-4-(dodecylsulfanylthiocarbonylsulfanyl)pentanoic acid (**2**) (2.0 g, 5 mmol), CDI (1.07 g, 5.94 mmol), and a catalytic amount of DMAP. The mixture was dissolved in dry CH_2Cl_2 (60 mL) and stirred at room temperature for 1 h. Then, pentafluorophenol (1.23 g, 6.7 mmol) in 3 mL of CH_2Cl_2 was added, and the mixture was left to react overnight. CH_2Cl_2 was evaporated, and the crude product was purified by silica column chromatography with hexane/CH_2Cl_2 (1:2) as eluent. Finally, 0.57 g of a yellow oily product was obtained (yield: 20%). In the second step, a round-bottom flask was charged under an argon atmosphere with the activated ester **2-A** (0.53 g, 0.93 mmol) and a catalytic amount of DMAP and dry CH_2Cl_2 (10 mL). Then, *P*-borane-(diphenylphosphanyl)methanethiol (**5**) (0.24 g, 0.96 mmol) in 10 mL of CH_2Cl_2 was added, and the mixture was left to react overnight. The solvent was evaporated, and the crude product was purified by silica column chromatography with hexane/CH_2Cl_2 (1:2) as eluent. Finally, 0.1 g of a yellow-orange oily product was obtained (yield: 17%). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.88 (3H, t, H_{19}), 1.26 (16H, H_{11-18}), 1.40 (2H, m, H_{10}), 1.69 (2H, m, H_9), 1.77 (3H, s, H_5), 2.24 (1H, m, H_{3a}), 2.38 (1H, m, H_{3b}), 2.74 (2H, m, H_2), 3.33 (2H, t, H_8), 3.73 (2H, d, H_{20}, $^2J_{HP}$ = 6.4 Hz), 7.47 (4H, m, H_{23}), 7.53 (2H, m, H_{24}), 7.70 (4H, m, H_{22}). The typical broad signal of the protons originating from the borane protecting group appears between 0.7 and 1.4 ppm and is superposed with the signal of the $C_{12}H_{25}$ alkyl chain. ^{13}C NMR ($CDCl_3$): δ (ppm) = 14.06 (C_{19}), 22.63 (C_{18}), 23.69 (C_{20}, $^1J_{PC}$ = 34.5 Hz), 24.74 (C_5), 27.62 (C_9), 28.8−29.6 (C_{10-16}), 31.85 (C_{17}), 33.77 (C_3), 37.08 (C_8), 38.77 (C_2), 46.11 (C_4), 118.69 (C_6), 127.39 (C_{21}, $^1J_{PC}$ = 55.4 Hz), 128.86 (C_{23}, $^3J_{PC}$ = 10.1 Hz), 131.82 (C_{24}, $^4J_{PC}$ = 2.2 Hz), 132.51 (C_{22}, $^2J_{PC}$ = 9.0 Hz), 194.44 (C_1, $^3J_{PC}$ = 1.8 Hz), 216.62 (C_7). ^{31}P NMR ($CDCl_3$): δ (ppm) = 20.25 ($Ph_2P(BH_3)CH_2S$−).

PS-1c. A flame-dried Schlenk tube was charged with AIBN (0.1 M in anisole, 192 μL, 19 μmol), **CTA-1** (121.3 mg, 194 μmol), styrene (2.037 g, 19.5 mmol), and PBu_3 (6 mg, 30 μmol). The solution was degassed by four freeze−pump−thaw cycles and polymerized at 70 °C under a N_2 atmosphere for 17 h. From the crude product the conversion was determined by ^{1}H NMR (65%). Generally, the styrene conversion was determined from the integral of the vinyl protons signal of the residual styrene and the integral of the aromatic protons signal taking into account the integral contribution from one overlapping vinyl proton. The product was thereafter isolated by repeated precipitation in methanol. According to ^{31}P NMR, 5% of the phosphine moieties were oxidized. ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.72 and 0.86 (H_3), 0.89 (H_{16}), 1.1−2.3 (H_6−H_{15}, CH and CH_2 of the backbone), 3.25 (H_5), 4.6−5.0 (−C(S)−S−C**H**Ph−), 6.3−7.5 (H_o, H_m, H_p, aromatic protons of the phosphine group). ^{13}C NMR ($CDCl_3$): δ (ppm) = 14.1 (C_{16}), 22.7 (C_{15}), 24.5−26.2 (C_3), 27.9 (C_6), 28.5−29.5 (C_7−C_{13}), 31.8 (C_{14}), 36.7 (C_5), 40.0−41.0 (CH of the backbone), 41.0−47.0 (C_2, CH_2 of the backbone), 52.9 and 53.2 (−C(S)−S−**C**HPh−), 122.2 (C_{22}), 125.0−127.0 (C_{20}, C_p), 127.0−129.0 (C_{25}, C_o, C_p), 128.8 (C_{26}), 129.5 (C_{18}, C_{21}), 133.3 (C_{19}), 133.9 (C_{24}), 135.9 (C_{23}), 144.0−147.0 (C_i), 152.9 (C_{18}), 175.2 (C_1), 222.3 (C_4). ^{31}P NMR ($CDCl_3$): δ (ppm) = −15.5 to −16.0 ($Ph_2PC_6H_4O$−, 95%) and 27.8 ($Ph_2P(O)C_6H_4O$−, 5%). SEC (THF): M_n = 6900 g mol^{-1}; M_w = 7600 g mol^{-1}; PDI = 1.10.

PS-1a. The same procedure was followed as for **PS-1c**, but no PBu_3 was added: AIBN (1.0 mg, 6 μmol) **CTA-1** (30.3 mg, 48 μmol), and styrene (1.00 g, 9.7 mmol) at 70 °C for 25 h. Conversion: 62%. According to ^{31}P NMR, 30% of the phosphine moieties were oxidized. SEC (THF): M_n = 11 800 g mol^{-1}; M_w = 12 800 g mol^{-1}; PDI = 1.09.

PS-1b. The same procedure was followed as for **PS-1c**, but no PBu_3 was added: AIBN (0.1 M in anisole, 96 μL, 9.6 μmol), **CTA-1** (60.3 mg, 96 μmol), and styrene (1.04 g, 10 mmol) at 70 °C for 70 h. Conversion: 82%. SEC (THF): M_n = 8600 g mol^{-1}; M_w = 9400 g mol^{-1}; PDI = 1.09.

PS-2b. A flame-dried Schlenk tube was charged with AIBN (0.1 M in anisole, 100 μL, 10 μmol), **CTA-2** (60 mg, 90 μmol), styrene (1 g, 9.6 mmol), and PBu_3 (5 mg, 25 μmol). After degassing via four freeze−pump−thaw cycles, the mixture was heated to 70 °C under a N_2

atmosphere and left to polymerize overnight (15 h). After twice being precipitated from MeOH, 263 mg of slightly yellow **PS-2b** was obtained. Conversion = 69%. According to ^{31}P NMR, 25% of the phosphine functionalities were oxidized. ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.89 (H_{19}), 0.77 and 0.97 (H_5), 1.1−2.3 (H_3, H_9−H_{18}, CH and CH_2 of the backbone), 2.23 (H_2), 3.25 (H_8), 4.6−5.0 (−C(S)−S−C**H**Ph−), 6.3−7.5 (H_o, H_m, H_p, aromatic protons of the phosphine group). ^{13}C NMR ($CDCl_3$): δ (ppm) = 14.0 (C_{19}), 22.6 (C_{18}), 23.5−24.5 (C_5), 27.8 (C_9), 28.5−29.5 (C_{10}−C_{16}), 29.6 (C_2), 31.8 (C_{17}), 34.0−36.0 (C_3, C_4), 36.7 (C_8), 40.0−41.0 (CH of the backbone), 41.0−47.0 (CH_2 of the backbone), 52.8 and 53.2 (−C(S)−S−**C**HPh−), 122.3 (C_{21}), 122.9 and 123.9 (C_6), 125.0−127.0 (C_{23}, C_p), 127.0−129.0 (C_{28}, C_o, C_p), 129.0 (C_{29}), 129.8 (C_{22}), 130.1 (C_{25}), 133.8 (C_{24}), 133.9 (C_{27}), 135.3 (C_{26}), 144.0−147.0 (C_i), 152.4 (C_{20}), 170.0 (C_1), 222.3 (C_7). ^{31}P NMR ($CDCl_3$): δ (ppm) = −15.0 to −15.6 ($Ph_2PC_6H_4O$−; 75%) and 26.5 ($Ph_2P(O)C_6H_4O$−; 25%). SEC (THF): M_n = 8500 g mol^{-1}; M_w = 9000 g mol^{-1}; PDI = 1.06.

PS-2a. The same procedure was followed as for **PS-2b**, but no PBu_3 was added: AIBN (0.1 M in anisole, 48 μL, 4.8 μmol), **CTA-2** (60 mg, 90 μmol), and styrene (1.00 g, 9.6 mmol) at 70 °C for 9 h. According to ^{31}P NMR, 40% of the phosphine functionalities were oxidized. SEC (THF): M_n = 7600 g mol^{-1}; M_w = 8400 g mol^{-1}; PDI = 1.10.

PS-3. A flame-dried Schlenk tube was charged with AIBN (0.1 M in anisole, 52 μL, 5.2 μmol), **CTA-3** (19 mg, 52 μmol), and styrene (339 mg, 3.26 mmol). After degassing via four freeze−pump−thaw cycles, the mixture was polymerized at 70 °C under an argon atmosphere overnight (16 h). After twice being precipitated from MeOH, 66 mg of slightly yellow **PS-3** was obtained (yield: 26%). Conversion = 64%. According to ^{31}P NMR, 49% of the phosphine functionalities were oxidized, 33% were unprotected, and 18% were present in their borane-protected state. ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.77 and 0.97 (H_5), 0.90 (H_{19}), 1.10−2.70 (H_2, H_3, H_9−H_{18}, CH and CH_2 of the backbone), 3.26 (H_8), 4.6−5.0 (−C(S)−S−C**H**Ph−), 6.30−7.85 (H_o, H_m, H_p, aromatic protons of the phosphine group). ^{31}P NMR ($CDCl_3$): δ (ppm) = −15.6 ($Ph_2PC_6H_4O$−, 33%), 18.7 ($Ph_2P(BH_3)C_6H_4O$−, 18%), 25.9 ($Ph_2P(O)C_6H_4O$−, 49%). SEC (THF): M_n = 5400 g mol^{-1}; M_w = 5900 g mol^{-1}; PDI = 1.08.

PS-4b. A flame-dried Schlenk tube was charged with AIBN (0.1 M in anisole, 176 μL, 17.6 μmol), **CTA-4** (111 mg, 176 μmol), and styrene (1.16 g, 11.1 mmol). After degassing via four freeze−pump−thaw cycles, the mixture was heated to 60 °C under an argon atmosphere and left to polymerize overnight (19 h). After twice being precipitated from MeOH, 447 mg of slightly yellow **PS-4b** was obtained. Conversion = 40%. According to ^{31}P NMR, 12% of the phosphine functionalities were oxidized, 6% were unprotected, and 82% were present in their borane-protected state. ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.80 and 0.95 (H_5), 0.90 (H_{19}), 1.1−2.5 (H_2, H_3, H_9−H_{18}, CH and CH_2 of the backbone, BH_3), 3.26 (H_8), 3.68 (H_{20}), 4.6−5.0 (−C(S)−S−C**H**Ph−), 6.3−7.8 (H_{22}−H_{24}, H_o, H_m, H_p). ^{13}C NMR ($CDCl_3$): δ (ppm) = 14.1 (C_{19}), 22.6 (C_{18}), 23.5 (C_{20}, $^{1}J_{PC}$ = 34.7 Hz), 23.8 and 24.3 (C_5), 27.9 (C_9), 28.8−29.6 (C_{10-16}), 31.9 (C_{17}), 34.0−35.2 (C_3), 35.2−36.2 (C_4), 36.7 (C_8), 38.9 and 39.1 (C_2), 40.0−41.0 (CH of the backbone), 41.0−47.0 (CH_2 of the backbone), 52.8 and 53.2 (−C(S)−S−**C**HPh−), 122.4−123.2 (C_6), 125.0−126.0 (C_p), 127.0−129.0 (C_{21}, C_o, C_m), 128.8 (d, C_{23}), 131.7 (C_{24}), 132.5 (d, C_{22}), 138.5−141.0 (C_i at −S−CHPh−), 144.5−146.0 (C_i), 195.2 (C_1), 222.2 (C_7). ^{31}P NMR ($CDCl_3$): δ (ppm) = −14.2 (Ph_2PCH_2S−, 6%), 20.1 ($Ph_2P(BH_3)CH_2S$−, 82%), 29.3 ($Ph_2P(O)CH_2S$−, 12%). SEC (THF): M_n = 3200 g mol^{-1}; M_w = 3400 g mol^{-1}; PDI = 1.08.

PS-4a. The same procedure was followed as for **PS-4b**: AIBN (0.1 M in anisole, 30 μL, 3 μmol), **CTA-4** (19 mg, 30 μmol), and styrene (193 mg, 1.85 mmol) at 70 °C for 15 h. Conversion = 45%. Yield = 31 mg. According to ^{31}P NMR, 21% of the phosphine functionalities were oxidized, 4% were unprotected, and 75% were present in their borane-protected state. SEC (THF): M_n = 4500 g mol^{-1}; M_w = 5000 g mol^{-1}; PDI = 1.11.

PS-1c-TOD. A round-bottom flask was charged with a solution of **PS-1c** (204 mg, 30 μmol) and 3,6,9-trioxodecyl azide (**TOD-N$_3$**) (36.6 mg, 193 μmol) in 4.4 mL of THF/H_2O (9:1). After stirring at room temperature overnight a small quantity was taken out to determine the conversion by ^{1}H NMR (15%). Thereafter, the solution was heated at 50 °C, stirred for another 48 h, and worked up by repeated precipitation in water and methanol. According to ^{1}H NMR, the amide bond was formed to an extent of 43%. Yield: 134 mg. SEC (THF): M_n = 7100 g mol^{-1}; M_w = 7800 g mol^{-1}; PDI = 1.10.

PS-2b-TOD. A round-bottom flask was charged with **PS-2b** (100 mg, 12 μmol) and 3,6,9-trioxodecyl azide (**TOD-N$_3$**) (24 mg, 127 μmol) in 2 mL of THF/H_2O (9:1). The mixture was stirred overnight. The solvents were then evaporated, and the polymer was twice precipitated in MeOH. According to ^{1}H NMR, the amide bond was formed to an extent of 30%. Yield: 76 mg. SEC (THF): M_n = 9400 g mol^{-1}; M_w = 10 300 g mol^{-1}; PDI = 1.10.

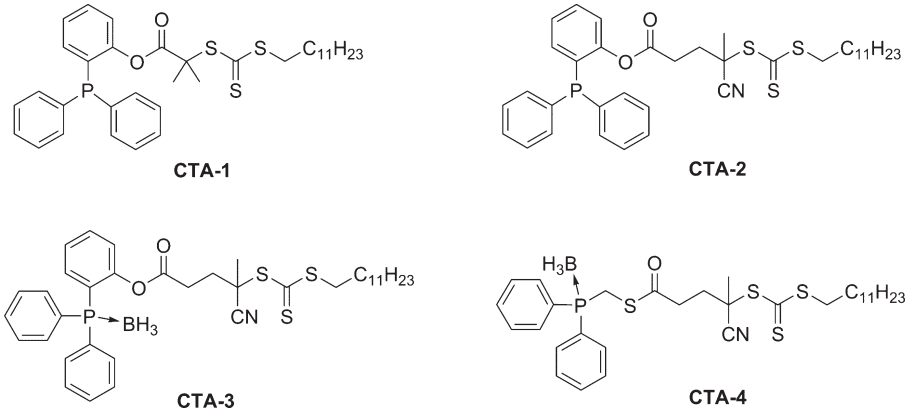
PS-4b-TOD. A round-bottom flask was charged with **PS-4b** (380 mg, 0.12 mmol), 1,4-diazabicyclo[2.2.2]octane (DABCO) (271 mg, 2.4 mmol), and 3,6,9-trioxodecyl azide (**TOD-N$_3$**) (441 mg, 2.33 mmol) in 10 mL of THF/H_2O (9:1) under an argon atmosphere. The mixture was stirred at 40 °C for 22 h overnight. The solvents were then evaporated, and the polymer was twice precipitated from MeOH. According to ^{1}H NMR, the amide bond was formed to an extent of 82%. Phosphorus-containing moieties could not be proved by ^{31}P NMR. Yield: 312 mg. ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.84 and 1.03 (H_5), 0.89 (H_{19}), 1.1−2.3 (H_2, H_3, H_9−H_{18}, CH and CH_2 of the backbone), 3.25 (H_8), 3.37 (H_g), 3.40 (H_a), 3.5−3.6 (H_b, H_f), 3.63 (H_c, H_d, H_e), 4.6−5.0 (−C(S)−S−C**H**Ph−), 5.9−6.1 (CON**H**), 6.3−7.3 (H_o, H_m, H_p). ^{13}C NMR ($CDCl_3$): δ (ppm) = 14.1 (C_{19}), 22.6 (C_{18}), 23.5−24.5 (C_5), 27.9 (C_9), 28.8−29.6 (C_{10}−C_{16}), 31.7 (C_2), 31.8 (C_{17}), 34.7−36.2 (C_3, C_4), 36.7 (C_8), 39.2 (C_a), 40.0−41.0 (CH_2 of the backbone), 41.0−47.0 (CH of the backbone), 52.8 and 53.2 (−C(S)−S−**C**HPh−), 58.9 (C_g), 69.7 (C_b), 70.1, 70.4, 70.5 (C_c, C_d, C_e), 71.9 (C_f), 123.0−123.7 (C_6), 125.0−127.0 (C_p), 127.0−129.0 (C_o, C_m), 138.5−141.0 (C_i at −S−**C**HPh−), 144.5−146.0 (C_i), 171.2 (C_1), 222.2 (C_7). SEC (THF): M_n = 3200 g mol^{-1}; M_w = 3500 g mol^{-1}; PDI = 1.09.

RESULTS AND DISCUSSION

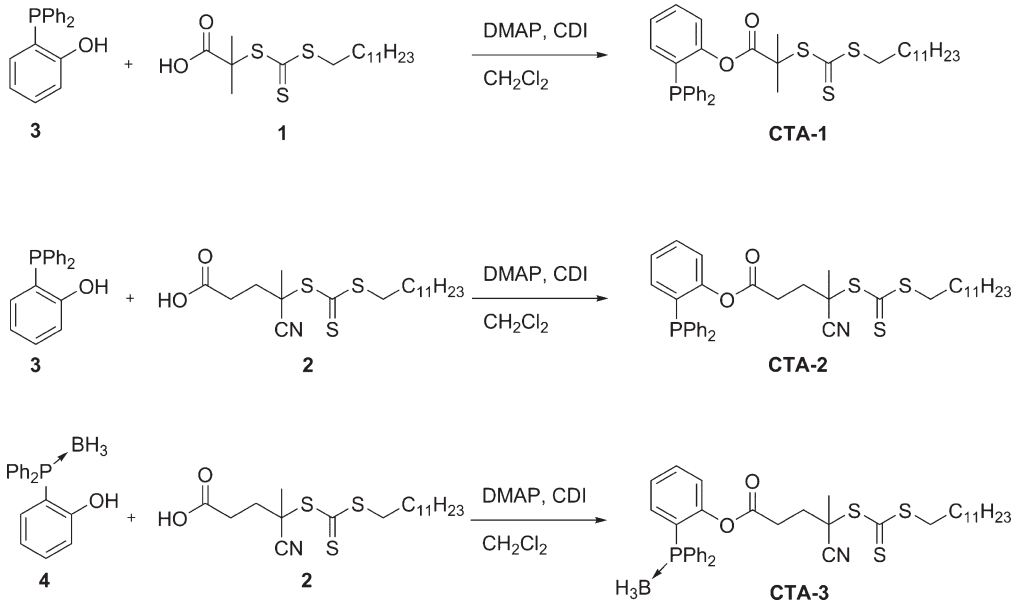
Nowadays, a large variety of chain transfer agents (CTAs) can be found in the literature.[3,49] In order to prepare a RAFT reagent prone to polymer analogous Staudinger ligation, it seems reasonable to resort to carboxyl-functionalized CTAs since promising phosphine groups for Staudinger ligation[42] can be linked to the substrate in an esterification reaction via their hydroxyl or thiol groups. We chose 2-(dodecylsulfanylthiocarbonylsulfanyl)-2-methylpropionic acid (**1**) since it is simple to synthesize in a one-pot procedure[47] and 4-cyano-4-(dodecylsulfanylthiocarbonylsulfanyl)pentanoic acid (**2**).[48] The phosphine 2-(diphenylphosphanyl)phenol (**3**) could be obtained in a rather elaborated procedure[44] and was converted with **1** and **2** to yield the phosphine-functionalized chain transfer agents **CTA-1** and **CTA-2**, respectively (Scheme 1).

In both cases the esterification with **3** was conducted in dichloromethane at room temperature overnight using 1,1′-carbonyldiimidazole (CDI) and 4-(dimethylamino)pyridine (DMAP) as activating agents (Scheme 2). The products were purified by column chromatography and obtained in acceptable yields (see Supporting Information).

Scheme 1. Structures of the Different Chain Transfer Agents Prepared in This Work



Scheme 2. Preparation of CTA-1, CTA-2, and CTA-3 Using Standard Steglich Esterification



The controlled radical polymerization (CRP) of styrene in bulk was mediated with **CTA-1** and **CTA-2**, respectively (Scheme 3). As radical source, AIBN was used and the reaction temperature of 70 °C provided its acceptable decomposition. The kinetic experiment with **CTA-1** clearly demonstrated the controlled nature of the polymerization process. This can be deduced from the molecular weight evolution that followed linear dependency and the low polydispersity values throughout the polymerization process (Supporting Information, Figure SI7). A test experiment using **1** as chain transfer agent was conducted under the same conditions to yield polystyrene **PS**. The reaction followed the same kinetics; hence, we assume that the phosphine group present in **CTA-1** does not interfere with the polymerization process. For all polymers the molar masses could be adjusted as desired, and the isolated products exhibited narrow molecular weight distributions (Table 1).

Nonetheless, one issue arose concerning the phosphine moiety. The ^{31}P NMR spectra indicate that besides the phosphine moiety (∼−15 ppm) another phosphorus-containing structure with $\delta(^{31}P) \sim 26.5$ ppm appears (Figure SI10). This pronounced low-field shift indicates that the phosphine has been oxidized upon reaction.[50] Obviously, at elevated temperature in the presence of a radical source, the formation of the corresponding phosphine oxide occurred. This situation is, of course, not acceptable since it reduces the amount of accessible phosphine groups for the Staudinger ligation.

Although there are methods known in the literature to reduce a phosphine oxide efficiently to the phosphine species,[51] our interests aimed for the avoidance of phosphine oxide formation. This, we thought, can be accomplished by either vigorous degassing of the polymerization batch or addition of an antioxidizing agent. It is well-known that trialkylphosphines are oxidative less stable than triarylphosphines. In fact, tributylphosphine even tends to ignite under ambient atmosphere. Hence, in a competing process of oxidation the trialkylphosphine should be predominately oxidized compared to triarylphosphine. As expected,

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Scheme 3. Controlled Radical Polymerization of Styrene with Different Chain Transfer Agents CTA-1, CTA-2, and CTA-3, Respectively, and Subsequent Polymer Analogous Modification of the Obtained Polystyrenes with TOD-N_3 in a Staudinger Ligation

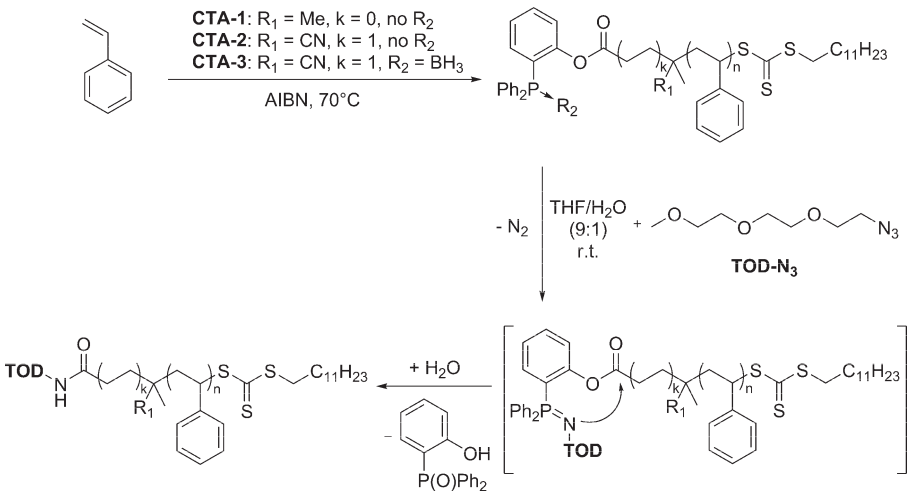

Table 1. Characteristics of Polystyrenes Obtained by RAFT Polymerization Using Different CTAs[a]

entry	CTA	molar ratio styrene/CTA/AIBN	X_p[d] [%]	$M_{n,cal}$[e] [g mol^{-1}]	$M_{n,SEC}$ [g mol^{-1}]	PDI	oxidized phosphine groups [%]
PS	**1**	100/0.5/0.05	97	22300	22500	1.10	
PS-1a	**CTA-1**	100/0.5/0.05	62	13100	11800	1.09	30
PS-1b	**CTA-1**	100/1/0.1	82	8900	8600	1.09	n.d.
PS-1c[b]	**CTA-1**	100/2/0.2	85	6900	6900	1.10	5
PS-2a	**CTA-2**	100/1/0.1	60	7400	7600	1.10	40
PS-2b[b]	**CTA-2**	100/1/0.1	69	8300	8400	1.07	25
PS-3	**CTA-3**	63/1/0.1	64	4900	5400	1.08	49
PS-4a	**CTA-4**	62/1/0.1	45	3600	4500	1.11	21
PS-4b[c]	**CTA-4**	63/1/0.1	40	3300	3200	1.08	12

[a] The polymerization was carried out in bulk at 70 °C. [b] Addition of PBu_3 (1.5 and 2.5 equiv with respect to AIBN). [c] Carried out at 60 °C. [d] Conversion of monomer. [e] Theoretical molar mass determined from monomer conversion, n.d. = not determined.

upon addition of tributylphosphine the amount of phosphine oxide could be drastically reduced for the RAFT polymerization utilizing **CTA-1**. In the ^{31}P NMR spectrum the remaining signal of phosphine oxide was determined to be 5% only (**PS-1c**, Figure SI10). In contrast, if the feed solution was extensively degassed prior to polymerization, once more 30% side product formation was detected (**PS-1b**).

As promising as the polymerizations with **CTA-1** were, the polymer analogous end-group modifications were rather disappointing. The reaction of **PS-1a** with 3,6,9-trioxodecyl azide (**TOD-N_3**) (Scheme 3, bottom), as simple model compound for a proof of principle reaction, conducted in a 9:1 mixture of THF/H_2O at room temperature overnight only yielded 15% of the desired amido glycol **PS-1a-TOD**. Heating improved this situation only slightly. After 48 h at 50 °C a conversion of 43% was reached. However, this scenario is in good accordance with the literature.[42] Hereby, it was found that Staudinger ligations proceeded fast and under smooth conditions when the electrophile resembled a glycyl structure. The rate of reaction drastically decreased if non-glycyl motifs were used, i.e., when a α-methyl group was present in the electrophile and the conversions could go as low as 27%.

In order to obtain higher yield, we decided to avoid the sterical hindrance in the α-position of the ester group by using **CTA-2** as transfer agent. Unfortunately, it appears that in this case the phosphine group is more prone to oxidation than it was in the case when **CTA-1** had been used. After the synthesis and the unavoidable purification of **CTA-2** by column chromatography, 15% of the phosphine groups were transformed into phosphine oxide. Again, this evolution could be easily monitored by ^{31}P NMR spectroscopy (Figure SI5). The subsequent controlled radical polymerization of styrene with **CTA-2** yielded the desired polystyrenes (**PS-2a**, **PS-2b**) with control over molecular weight and low polydispersity indices (PDI = 1.10 and 1.07, respectively). Similar to the polymerization with **CTA-1**, the addition of PBu_3 reduced the formation of phosphine oxide, in this case from 40% to 25%. Knowing that 15% of **CTA-2** was already oxidized before the polymerization, another 10% of phosphine oxide had been formed (Figure SI11). Nevertheless, polymer **PS-2b** was used to perform a Staudinger ligation with **TOD-N_3** in a 9:1 mixture of THF/H_2O at room temperature, stirred for 20 h. Analysis via ^{1}H and ^{31}P NMR spectroscopy revealed that, on the one hand, the amount of phosphine oxide had risen to 35% but, on the other, 30% of the ester groups were

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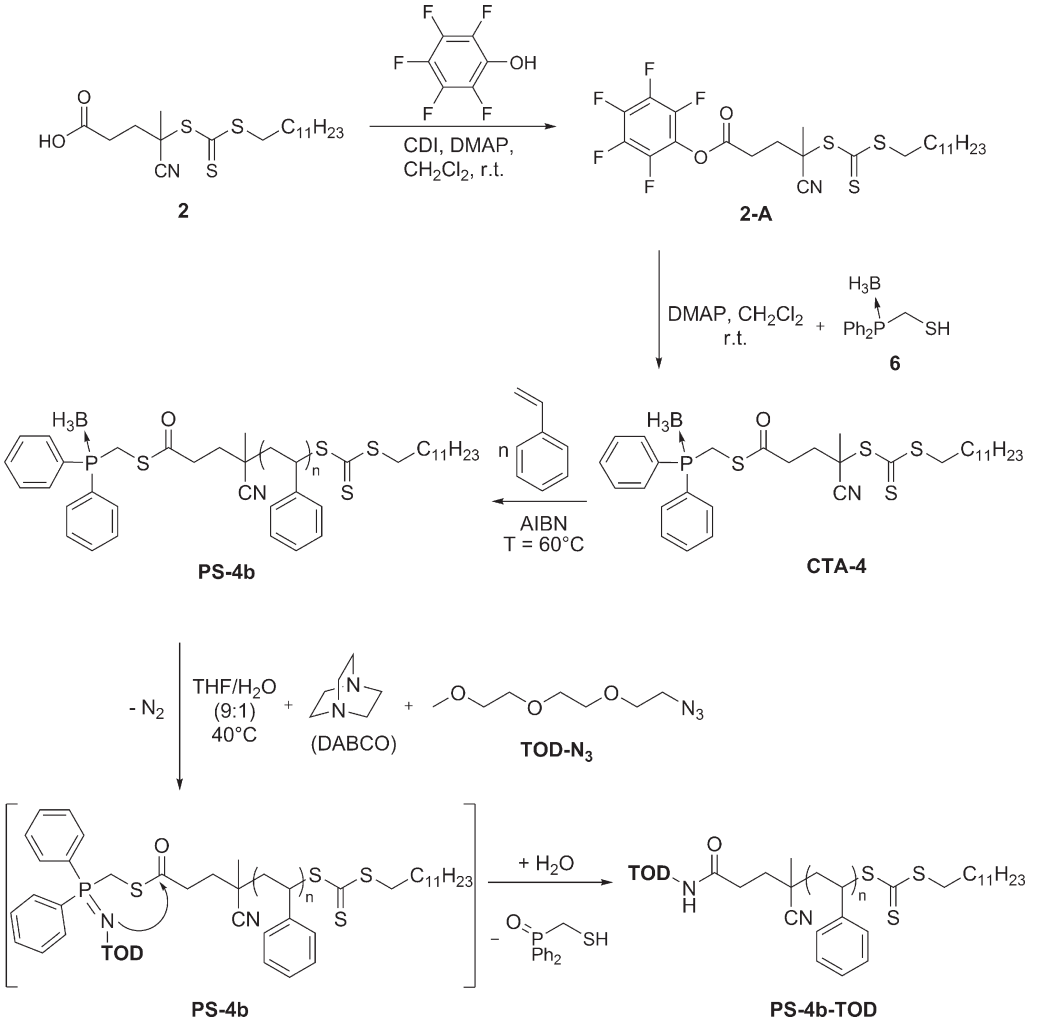
Scheme 4. Two-Step Preparation of CTA-4, RAFT Polymerization of Styrene Using CTA-4, and Subsequent Polymer Analogous Staudinger Ligation with TOD-N_3



successfully transformed into the corresponding amide function to yield polymer **PS-2b-TOD**. At room temperature, the less sterically hindered ester derived from **CTA-2** was converted into a peptide bond with slightly higher yields than the ester function derived from **CTA-1**. This is even more remarkable if we keep in mind that 25% of the phosphine groups in **PS-2b** were already inactive before carrying out the Staudinger ligation, compared to 5% in **PS-1a**. This confirms the assumption that the Staudinger ligation is sensitive to sterically hindering groups in the α-position of the ester group. Nevertheless, the rapid oxidation of **CTA-2** during its synthesis, purification, as well as the polymerization and the Staudinger ligation is a major drawback.

On the basis of these initial results, we resorted to phosphine groups that are inherently more stable toward oxidation and also broadened the spectrum of phosphine groups used in this study. From the literature, it is well-known that, besides 2-diphenylphosphanylphenol (**3**), diphenylphosphanylmethanethiol shows one of the best reactivity profiles among the phosphines tested until today.[30,42] Therefore, diphenylphosphanylmethanethiol was included in our consideration. The compound is often handled in a form where the phosphorus atom in the phosphine group is protected by a borane group to prevent it from oxidation. This form is here labeled as *P*-borane-(diphenylphosphanyl)-methanethiol (**6**). We decided to apply the same borane complexation strategy to the 2-diphenylphosphanylphenol (**3**). For this purpose, **3** was treated with borane dimethyl sulfide complex solution at −15 °C, and the complex *P*-borane-2-(diphenylphosphanyl)phenol (**4**) was obtained in excellent yield. *P*-Borane-(diphenylphosphanyl)methanethiol (**6**) was prepared following a modified procedure from Raines and coworkers.[46] The thiol was thereby freshly liberated from its acyl protecting group prior to its use in the esterification reaction. On the basis of the comparison of **CTA-1** and **CTA-2** in terms of sterical hindrance within the Staudinger ligation, 4-cyano-4-(dodecylsulfanylthiocarbonylsulfanyl)pentanoic acid (**2**) was retained as basic CTA structure to prepare the functionalized CTAs. *P*-Borane-2-(diphenylphosphanyl)phenol (**4**) was reacted with **2** to give **CTA-3** in acceptable yield (Scheme 2). The same esterification strategy using classical Steglich conditions did not work out for the preparation of **CTA-4** starting from **2** and *P*-borane-(diphenylphosphanyl)methanethiol (**6**). A major side reaction emerged consisting in a concurrent nucleophilic attack

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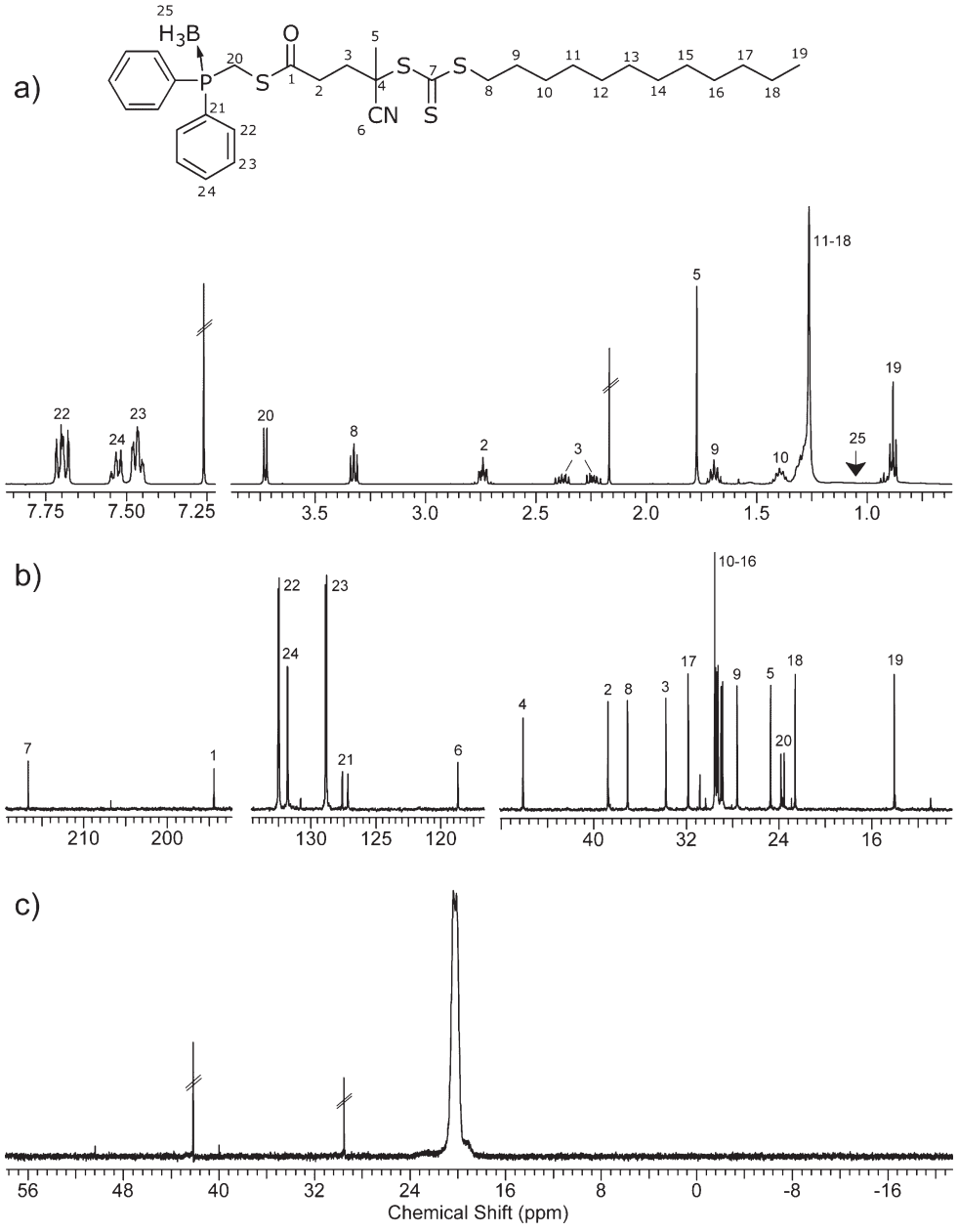

Figure 1. ^{1}H (a), ^{13}C (b), and ^{31}P (c) NMR spectra of **CTA-4** in $CDCl_3$.

of the thiol group of **6** at the trithiocarbonate group in **2**. We tried to circumvent this side reaction by rendering the carboxyl group in **2** more reactive. Therefore, the more reactive pentafluorophenyl ester **2-A** was prepared and reacted with **6** to give **CTA-4** in acceptable yield and relatively high purity (Scheme 4). The side reaction still appeared but could be suppressed to a large extent. The ^{1}H, ^{13}C, and ^{31}P NMR spectra of **CTA-4** are depicted in Figure 1.

The two new CTAs, **CTA-3** and **CTA-4**, were then tested in the controlled radical polymerization of styrene. Again, AIBN was used as initiator and the polymerization was carried out in bulk. Polystyrenes with control over molar masses and low polydispersity indices were obtained (Table 1). Furthermore, a kinetic experiment was conducted using **CTA-4**. The controlled nature of the polymerization process could be deduced from the rather linear dependency of $\ln(c/c_0)$ on polymerization time (Figure 2a) and the linear molecular weight evolution on monomer conversion as well as the low polydispersity values (Figure 2b).

However, for **PS-3**, which had been obtained using **CTA-3**, ^{31}P NMR spectroscopy (Figure SI12) revealed that only 17% of the phosphine groups were present in their borane-protected state. 50% had been oxidized, and 33% were present in the unprotected, but yet not oxidized, form. It appears that the coordinative bond between the borane and the phosphine was, in this case, not strong enough to survive the polymerization process at 70 °C. In contrast, **PS-4a**, obtained upon mediation of **CTA-4** in the RAFT process, showed only 21% of oxidized phosphine groups and a rather small amount of 4% of unprotected phosphine groups. This means that in total 79% of the phosphine groups in the polymer had survived the RAFT process and were still active groups for Staudinger ligation. This result

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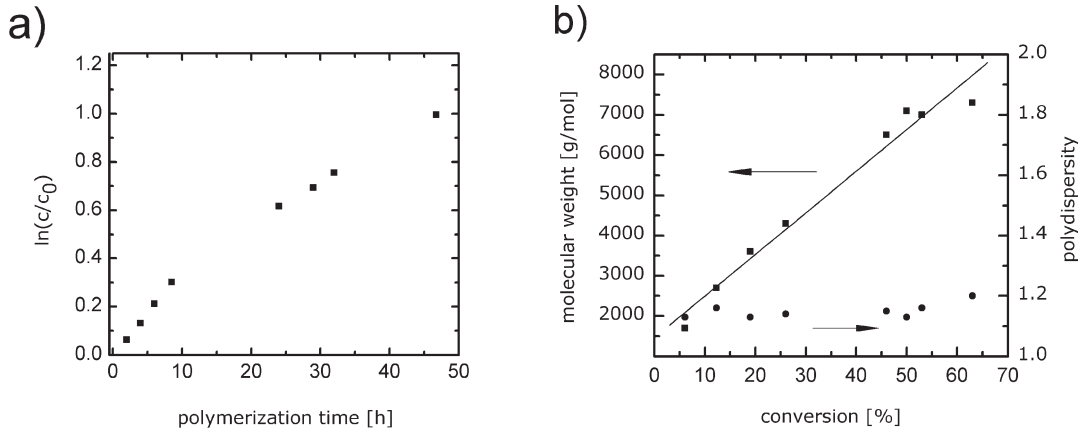

Figure 2. Kinetic plot of the controlled polymerization of styrene mediated with **CTA-4** (a). Dependency of molecular weight from conversion and polydispersity indices over conversion (b).

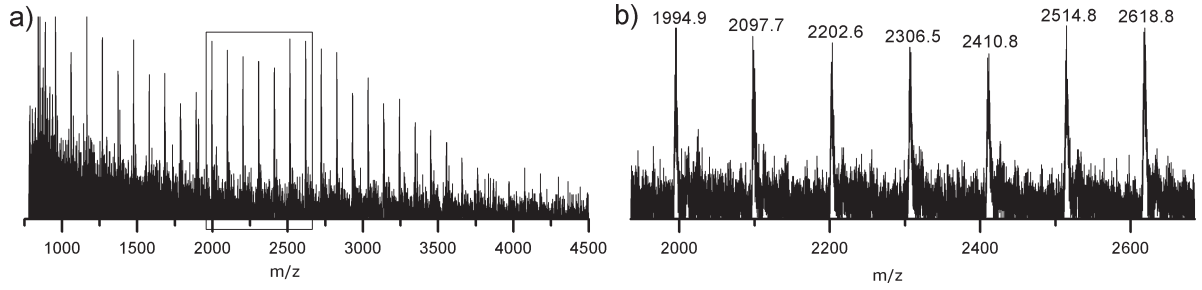

Figure 3. MALDI-TOF mass spectrum (reflectron mode) of **PS-4b** with NaN_3/dithranol matrix: global spectrum (a) and enlargement (b).

appears even more striking if one recalls the fact that unprotected diphenylphosphanylmethanethiol is much more prone to oxidation than it is the case for 2-(diphenylphosphanyl)phenol (**3**). The coordinative bond between borane and phosphine seems to be much stronger in this case. Reducing the reaction temperature within the RAFT polymerization from 70 to 60 °C resulted in polystyrene (**PS-4b**) with an even lower content of oxidized phosphine groups (12%) as shown in Figure SI13.

PS-4b was additionally investigated by MALDI TOF mass spectrometry (Figure 3). The measurement was carried out in the positive ion reflectron mode using dithranol as matrix material. Sodium azide was added to enhance the ionization of the polymer by cationization. The major peak series corresponds to polystyrene as evidenced by the consecutive peak distance of 104 g/mol, which corresponds to the mass of one styrene unit. Furthermore, the starting group can been assumed consisting of the unprotected phosphine form originating from the reinitiating group of **CTA-4** and the end group of dodecyltrithiocarbonate derived from **CTA-4** as exemplarily shown for the peak at 2202.6 ($104.06 \times 15 + 340.1 + 277.1 + 23 = 2201.1$). There is a good chance that the cleavage of the borane protecting groups from the phosphine groups is due to the high laser energy entry inside the MALDI-TOF mass spectrometer during the measurement.

PS-4b was then used to perform a Staudinger ligation with **TOD-N_3** in a 9:1 mixture of THF/H_2O at 40 °C (Scheme 4). 1,4-Diazabicyclo[2.2.2]octane (DABCO) was added as a tertiary base to cleave the borane protecting group from the phosphine group prior to ligation. The cleavage process as well as the subsequent consumption of the unprotected phosphine groups had been followed before via ^{31}P NMR spectroscopy in an online kinetic experiment (Figure SI8) using **PS-4a** and **TOD-N_3**. Thereby, it was found that the cleavage process was accomplished after 7 h. The content of the unprotected phosphine groups reached a maximum after about 3 h, and complete conversion was found after 13 h. Besides the ^{31}P NMR signal of released phosphine oxide thiol as known byproduct during the Staudinger ligation, several other signals were detected but could not be assigned to specific byproducts up to now. For a more detailed mechanistic study, including discussion of side reactions, the reader is referred to a paper published by Soellner et al.[42]

The ^{1}H NMR spectra of **PS-4b**, **TOD-N_3**, and **PS-4b-TOD** are depicted in Figure 4. The phosphine moiety of **PS-4b** can be well identified by the signals of H_{20} and $H_{22}-H_{24}$ (Figure 4a). These signals completely disappear in the spectrum of the modified polymer **PS-4b-TOD**, indicating that there are no phosphine groups left in the polymer (Figure 4c). This is confirmed by a ^{31}P NMR spectrum showing no signals. In addition, the ^{1}H NMR signal of the CH_2 group adjacent to the thioester group (H_2) shifts from 2.43 to 2.12 ppm due to a change in chemical environment from thioester to amide group. Finally, the appearance of the signals of H_a-H_g between 3.33 and 3.70 ppm originating from the TOD group and the NH signals at ~6.00 ppm prove the successful modification of the chain end. For **PS-4b-TOD**, the degree of conversion by polymer analogous Staudinger ligation was determined to be 82% by comparing the integral value of signals H_a-H_g (representing 15 H) to the one of signal H_8 (representing 2 H) (Figure 4c). Taking into account that 82% of the phosphine groups at the end of each polymer chain in **PS-4b** were present in the borane-protected state and assuming that the 6% of the phosphine groups originally present

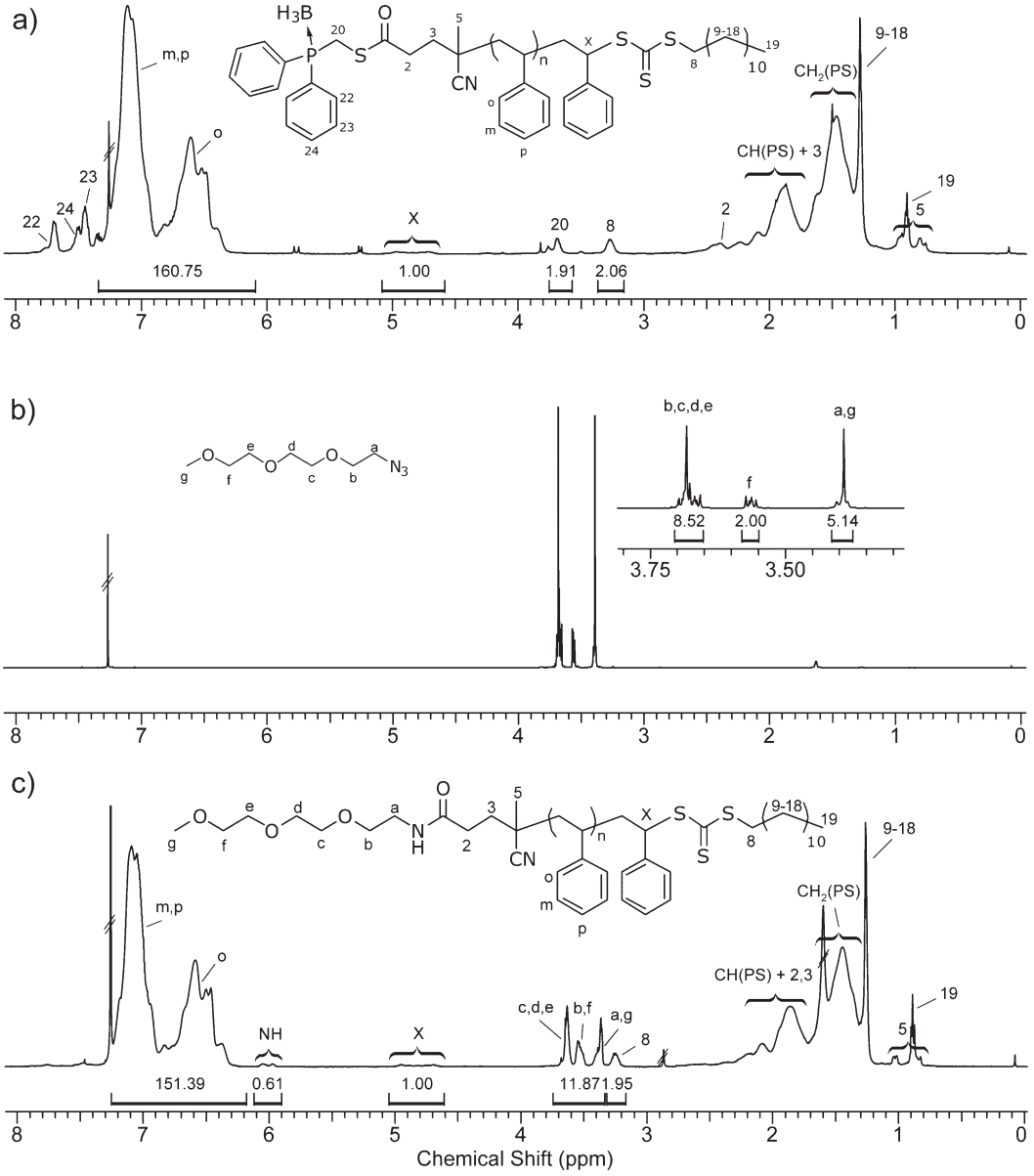

Figure 4. ^{1}H NMR spectra of **PS-4b** (a), **TOD-N$_3$** (b) and **PS-4b-TOD** (c) in $CDCl_3$.

in the unprotected state might have been oxidized while storing, one can assume that the polymer analogous ligation has been accomplished in *quantitative manner*. The comparison of GPC curves of **PS-4b** and **PS-4b-TOD** did not show any significant difference (Figure SI9), indicating that no side reaction with significant change of molecular weight had occurred. This is, apart from a high degree of conversion, generally seen as a prerequisite for a polymer analogous reaction in order to be widely applicable.

■ CONCLUSION

New phosphine-containing chain transfer agents were synthesized and used in RAFT polymerization to obtain the corresponding polystyrenes displaying low polydispersity and high control over molar mass. Staudinger ligation of the thus-obtained products was investigated. Thereby, it was found that sterical hindrance at the ester function as well as the phosphine groups' sensitivity toward oxidation presented the major drawbacks for the breakthrough of the Staudinger ligation as a new efficient polymer analogous reaction. Sterical hindrance could be avoided by using a CTA structure where the ester group is bound to a methylene group. The sensitivity toward oxidation was significantly reduced by using the borane protected form of the phosphine moiety. Decrease in temperature during the polymerization process lowered the amount of oxidized phosphine groups even more. Finally, for the proof of principle, the Staudinger ligation of polystyrene prepared by RAFT using **CTA-4** and **TOD-N$_3$** as simple model compound could be accomplished in almost quantitative manner. We believe that this concept, the combination of Staudinger ligation and RAFT polymerization, will become an appropriate method for the preparation of bioconjugates. Future work will be dedicated to further improvements in terms of oxidation stability and the reaction pathways toward the phosphine-functionalized CTAs.

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■ ASSOCIATED CONTENT

Supporting Information. Synthetic procedures and NMR data of **TOD-N$_3$**, **4**, **6**, phosphine-functionalized CTAs **CTA-1** to **CTA-4**, ^{1}H, ^{13}C, and ^{31}P NMR spectra of polymers, GPC curves of **PS-4b** and **PS-4b-TOD**, MALDI-TOF mass spectrum of **PS-4b-TOD**, kinetic plot of the RAFT polymerization using **CTA-1**, and the online kinetic experiment of the Staudinger ligation using ^{31}P NMR spectroscopy. This material is available free of charge via the Internet at http://pubs.acs.org.

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