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Three-Fold Metal-Free Efficient ("Click") Reactions onto a Multifunctional Poly(2-oxazoline) Designer Scaffold

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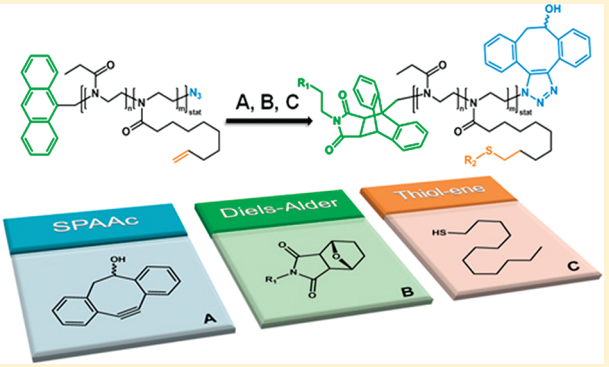
Supporting Information

ABSTRACT: The synthesis of a new multifunctional copoly(2-oxazoline) scaffold containing *a*-anthracene and *ω*-azide termini as well as pendant alkene groups in the side chain is described. With its three different functionalities this system represents the first polymer scaffold that can be applied in triple orthogonal ("click") post-modification reactions. Thus, the functional groups were exploited for sequential 3-fold metal-free efficient reactions encompassing Diels–Alder cycloaddition (DA), thiol–ene coupling (TE) and strain-promoted azide alkyne cycloaddition (SPAAC) reactions. Each reaction was optimized individually to determine the preferential reaction order for the sequential three-step functionalization of the polymer scaffold: SPAAC–DA–TE. The successful progress of the successive reactions was confirmed by ^{1}H NMR spectroscopy, MALDI–TOF MS spectrometry, (online) infrared spectroscopy and UV–Vis spectroscopy, respectively. Furthermore, a one-pot three-step reaction for the 3-fold modification of the polymer scaffold is reported. Finally, the potential of the triple post-modifications for the preparation of functional nanoparticles by nanoprecipitation is presented using various functional groups to tune the overall solubility of the copolymer, to attach cell penetrating or targeting groups and to prepare labeled systems demonstrating the versatility of this approach for the preparation of multifunctional nanoparticles.



INTRODUCTION

In the past decade, the combination of living/controlled polymerization techniques and efficient ("click") reactions has opened a new research area for the preparation of new highly functionalized materials.[1−7] In particular, the opportunity to design systems with defined molecular structure, controllable architecture and composition as well as a high degree of functionalization and, thus, adjustable properties are major factors for the enormous interest developed in this field. The "click chemistry" concept was introduced in 2001 by Sharpless and co-workers, facilitating a modular methodology for the preparation of tailor-made materials.[8] The most popular and studied "click" reaction is the Cu(I)-catalyzed azide alkyne 1,3-dipolar cycloaddition (CuAAC),[9] which already found a wide range of applications in different research fields. Recently, the "click" terminology was extended to several other reaction types meeting the basic criteria for a "click" reaction, such as high yields, high selectivity accompanied by a high tolerance of different functional groups and the lack of side reactions. The necessity for the development of new quantitative and orthogonal synthetic "click" approaches emerged from the need for copper-free "click" strategies with regard to biological applications.[1,10−12] In terms of highly functionalized materials, controlled and living polymerization techniques do not only provide control over composition and backbone length, but also enable a straightforward functionalization of the corresponding polymers either at the chain ends or in the side chains. The usage of functional initiators and terminating agents separately or in conjunction leads to polymers with *a*- or *ω*-chain termini and telechelic polymers, respectively.[13−16] This approach has been numerously employed in case of controlled radical polymerizations.[17] Another strategy for the incorporation of functionalities into a polymer is the copolymerization with functional monomers.[18−21] Both approaches result in the formation of versatile polymeric scaffolds, which can be modified by simple post-modification reactions when introducing appropriate, e.g., "clickable", reactive groups. Even though the combination of *a*-, *ω*-, and side-chain functionalities into one single polymer

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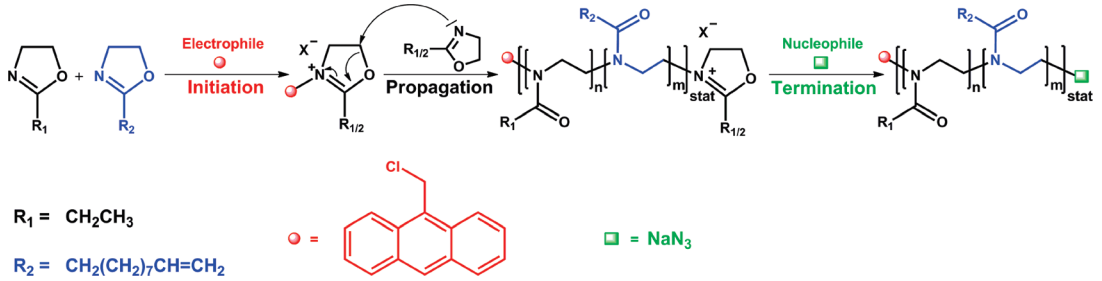
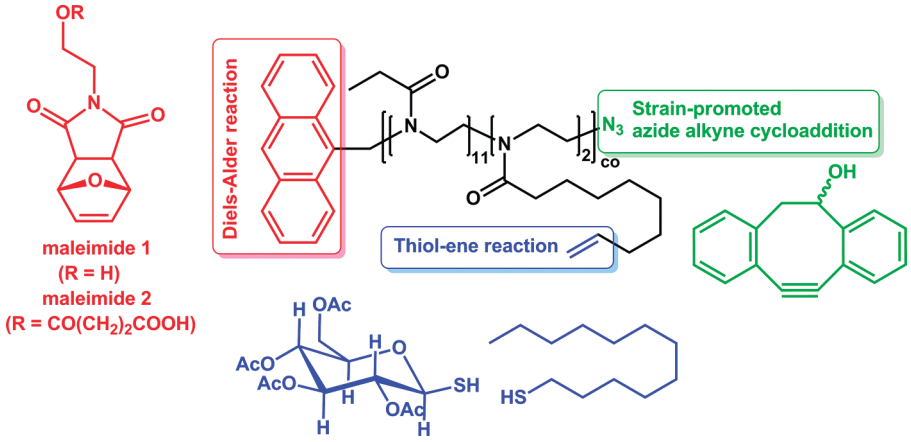
Scheme 1. Schematic Representation of the Mechanism of the Cationic Ring-Opening Copolymerization of 2-Oxazolines Highlighting the Incorporation of α-Anthracene, ω-Azido, and Side-Chain Alkene Functionalities



Scheme 2. Schematic Representation of the Polymer Scaffold Exhibiting Different Functionalities for the Incorporation of Various Residues by Three Orthogonal Efficient Metal-Free ("Click") Reactions



chain holds great promise, we are not aware of any reports on such multifunctional polymer scaffolds which allow an easy triple orthogonal post-modification process. Such a multifunctional polymer scaffold represents a "designer polymer" that can be easily modified for a diverse range of potential applications. It is challenging, however, to identify three reactive groups that are compatible with the applied polymerization conditions while at the same time they should ideally possess an orthogonal and highly efficient reactivity allowing the stepwise and simultaneous modification of the polymer scaffold.[22] These requirements thus point toward the incorporation of metal-free "clickable" groups to be able to apply the polymer scaffold for a wide range of applications, including biomedical ones.

A highly promising platform for the development of such a multifunctional polymer scaffold is provided by poly(2-oxazoline)s that can be prepared by a living cationic ring-opening polymerization (CROP) process allowing the introduction of functional end-groups by initiation and termination steps as well as functional side-chains by incorporation of a functional monomer (Scheme 1).[23−26] In recent years, the incorporation of various "clickable" side groups, including alkene[19,27,28] and alkyne,[29,30] as well as end-groups, such as alkyne[31,32] and azide,[33,34] into poly(2-oxazoline)s have been demonstrated by various research groups.[35] However, the combination of three or even just two different orthogonal "clickable" functional groups into a single poly(2-oxazoline) has not been reported to the best of our knowledge.

To design a multifunctional poly(2-oxazoline) polymer scaffold allowing efficient metal-free post-modification, we targeted the incorporation of an anthracene moiety for Diels−Alder (DA) cycloadditions during initiation, an azide moiety for strain-promoted azide alkyne cycloadditions (SPAAC) during termination as well as terminal alkenes for radical thiol−ene reactions by incorporation of 2-(dec-9-enyl)-2-oxazoline (DecEnOx) as depicted in Schemes 1 and 2. The synthesis of this multifunctional polymer scaffold based on water-soluble and biocompatible poly(2-ethyl-2-oxazoline) will be discussed.[36,37] The three post-modification methods will be addressed individually serving as basis for sequential triple post-modification of the polymer scaffold. Finally, the potential of the multifunctional polymer scaffold toward biomedical applications is demonstrated by the formation of FITC labeled nanoparticles.

EXPERIMENTAL SECTION

Materials. 2-Ethyl-2-oxazoline, 9-chloromethylanthracene, and methyl tosylate were obtained from Acros Chemicals, distilled to dryness over barium oxide (BaO), and stored under argon. Dichloromethane, 2,2-dimethoxy-2-phenylacetophenone (DMPA), dodecanethiol, *exo*-3,6-epoxy-1,2,3,6-tetrahydrophthalic anhydride and fluorescein 5(6)-isothiocyanate were purchased from Sigma-Aldrich. 4-Dibenzocyclooctynol,[12] the protected maleimides **1** and **2**[38,39] and 2-(dec-9-enyl)-2-oxazoline (DecEnOx)[28] were prepared according to literature procedures. 2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-glycopyranose (>99%) was purchased from GLYCON Biochem GmbH.

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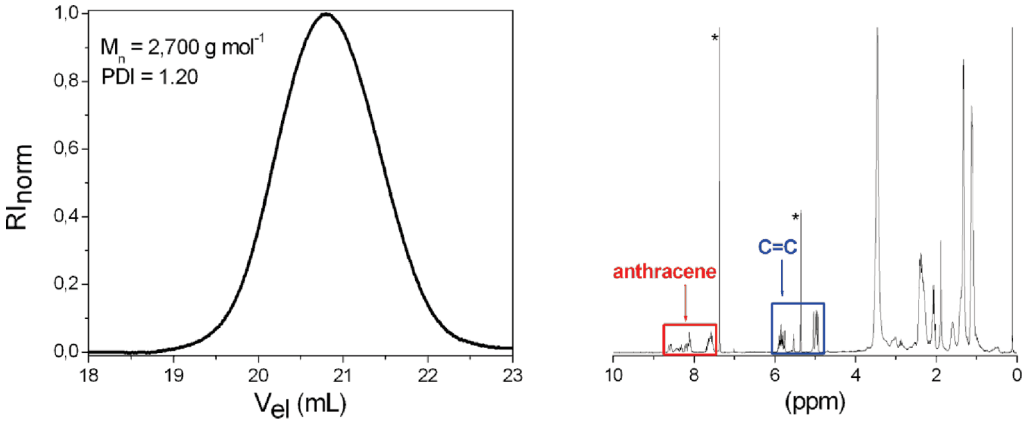


Figure 1. Characterization of the multifunctional polymer scaffold. Left: SEC trace (eluent: DMA with 0.1% LiCl). Right: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz). Asterisk indicates residual solvent.

General Methods and Instrumentation. The Initiator Sixty single-mode microwave synthesizer from Biotage, equipped with a noninvasive IR sensor (accuracy: ± 2%), was used for polymerizations under microwave irradiation. Microwave vials were heated overnight to 110 °C and allowed to cool to room temperature under argon before usage. All polymerizations were carried out with temperature control. Size exclusion chromatography measurements were performed on an Agilent system equipped with a diode array detector and a refractive index detector. Two PSS SDV (5 μm pore size) columns were placed in series. DMA with 5 mmol of LiCl was used as eluent at 1 mL/min flow rate, and the column oven was set to 50 °C. Molar masses were calculated against polystyrene standards. For preparative SEC, Bio-Beads S-X1 (cross-linked polystyrene beads) from Bio-Rad were used. ^{1}H NMR spectra were recorded on a Bruker AC 300 MHz spectrometer at room temperature, with $CDCl_3$ as the solvent. The chemical shifts are given in ppm relative to the signal from residual nondeuterated solvent. For the MALDI measurements an Ultraflex III TOF/TOF apparatus (Bruker Daltonics, Bremen, Germany) equipped with a Nd:YAG laser and a collision cell was used. All spectra were measured in the positive reflector or linear mode. The instrument was calibrated prior to each measurement with an external PMMA standard from PSS Polymer Standards Services GmbH (Mainz, Germany). DLS measurements were carried out on a Zetasizer Nano ZS (Malvern Instrument, Malvern, U.K.) using a He/Ne-laser (λ = 633 nm) and a scattering angle of 173°. The mean particle size was approximated as the effective (Z average) diameter and the width of the distribution as the polydispersity index ($PDI_{particle}$) obtained by the cumulants method assuming a spherical shape.

Microwave-Assisted Synthesis of the Polymer Scaffold An−(PEtOx-*stat*-PDecEnOx)−N_3. A solution of 9-chloromethylanthracene (**I**; 0.25 g; 1.1 mmol), 2-ethyl-2-oxazoline (EtOx; 1.203 g; 12.1 mmol), and 2-(dec-9-enyl)-2-oxazoline (DecEnOx; 0.521 g; 2.5 mmol) was prepared in dichloromethane (3.15 g; 37 mmol) followed by the addition of sodium iodide (0.165 g; 1.1 mmol). The total monomer concentration was adjusted to 3.5 M with a [EtOx]:[DecEnOx]:[**I**] ratio of 11:2:1. The vial was heated to 140 °C in the microwave synthesizer for 20 min. After cooling, a 5-fold excess of sodium azide (with respect to the initiator) was added and the polymerization mixture was stirred at room temperature overnight. Subsequently, the white precipitate was filtered off and the polymer solution was washed three times with water and brine and was dried over $MgSO_4$. After the solution was concentrated under reduced pressure, the polymer was precipitated in ice-cold diethyl ether yielding a slight yellowish product. SEC (DMAc, LiCl): M_n = 2,700 g mol^{-1}, PDI = 1.2. ^{1}H NMR (300 MHz, CD_2Cl_2): δ 8.8−8.0 (anthracene), 7.8−7.4 (anthracene), 5.85 (*HC* = CH_2), 5.76, 5.53 (CH_2−anthracene), 5.1−4.9 (HC=CH_2), 3.8−3.1 (N−CH_2), 2.7−2.17 (CH_2 EtOx, CH_2 DecEnOx), 2.17−1.98 (CH_2 DecEnOx), 1.72−1.5 (CH_2 DecEnOx), 1.5−1.2 (CH_2 DecEnOx), 1.2−0.75 (CH_3 EtOx) ppm.

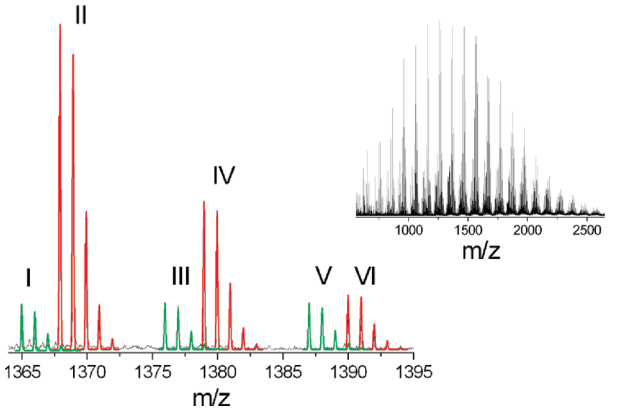


Figure 2. MALDI−TOF MS spectrum (matrix: DCTB, ionization agent: NaI, solvent: $CHCl_3$) of the multifunctional polymer scaffold (top right) with detailed assignment of a selected part of the spectrum (bottom left) $(C_{15}H_{11}(C_5H_9NO)_m(C_{13}H_{23}NO)_nN_3 + Na^+$: II, m = 11, n = 2; IV, m = 9, n = 3; VI: m = 7, n = 4. $H(C_5H_9NO)_m(C_{13}H_{23}NO)_nN_3 + Na^+$: I, m = 11, n = 1; III, m = 9, n = 2; V, m = 7, n = 3).

Strain-Promoted Azide Alkyne Cycloaddition (SPAAC) of the Polymer Scaffold with 4-Dibenzocyclooctynol. The reaction was monitored with the ReactIR iC10 from Mettler Toledo. The polymer scaffold (50 mg; 1 equiv) was dissolved in 1.75 mL of chloroform. The IR measurement was paused and 4-dibenzocyclooctynol (7.9 mg; 1.2 equiv) was added. After restarting and normalizing the measurement, stirring was continued for about 30 min at room temperature. Eventually, the product was obtained by precipitation in ice-cold diethyl ether. SEC (DMAc, LiCl): M_n = 3,300 g mol^{-1}, PDI = 1.15; ^{1}H NMR (300 MHz, CD_2Cl_2): δ 8.8−8.0 (anthracene), 8.0−7.0 (anthracene, dibenzocyclooctynol), 5.85 (*HC*=CH_2), 5.76, 5.54 (CH_2−anthracene), 5.1−4.9 (HC=CH_2), 3.8−3.1 (N−CH_2), 2.7−2.17 (CH_2 EtOx, CH_2 DecEnOx), 2.17−1.98 (CH_2 DecEnOx), 1.72−1.5 (CH_2 DecEnOx), 1.5−1.2 (CH_2 DecEnOx), 1.2−0.75 (CH_3 EtOx) ppm.

Thiol−Ene Photoaddition (TE) of the Polymer Scaffold with 2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-glyco-pyranose (Ac_4GlcSH). A stock solution of the polymer scaffold (50 mg; 1 equiv) and Ac_4GlcSH (37 mg; 1.2 equiv) was prepared in 1.2 mL of tetrahydrofuran and divided over two vials. 2,2-Dimethoxy-2-phenylacetophenone (DMPA; 0.2 equiv) was added to one of the solutions. After degassing the solutions for 30 min,

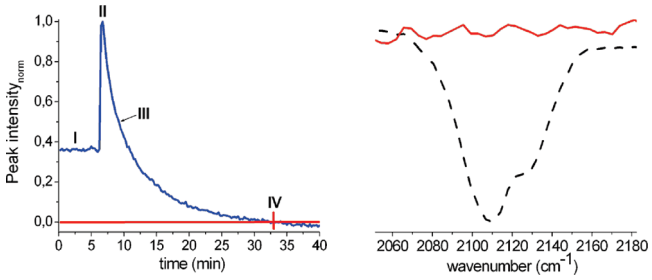


Figure 3. Online IR monitoring of the SPAAC of 4-dibenzocyclooctynol to the multifunctional polymer scaffold. Left: Change in azide signal (2110 cm^{-1}) with reaction time (I, building block dissolved, constant azide signal intensity; II, addition of 4-dibenzocyclooctynol and normalization, start of the reaction; III, reaction monitoring; IV, end of the reaction). Right: Azide peak before (--) and after (red line) the cycloaddition.

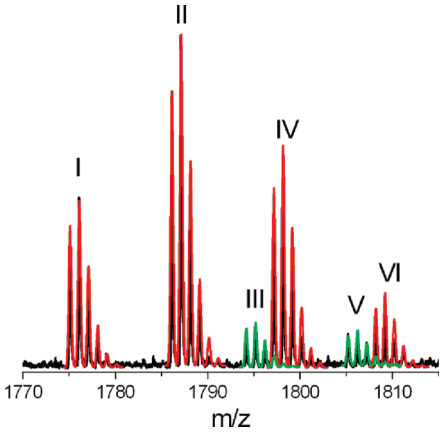


Figure 4. Detailed analysis of a selected part of the MALDI−TOF MS spectrum (matrix: DCTB, ionization agent: NaI, solvent: $CHCl_3$) of the product of the SPAAC reaction of 4-dibenzocyclooctynol to the multifunctional polymer scaffold. $(C_{15}H_{11}(C_5H_9NO)_m(C_{13}H_{23}NO)_nN_3C_{16}H_{12}O + Na^+$: I, m = 11, n = 1; II, m = 9, n = 2; IV, m = 7, n = 3; VI, m = 5, n = 4. $H(C_5H_9NO)_m(C_{13}H_{23}NO)_nN_3C_{16}H_{12}O + Na^+$: III, m = 11, n = 2; V, m = 9, n = 3).

the solutions were exposed to UV light (356 nm) for 3 h. An additional reaction was performed with a solution of the polymer scaffold (24 mg; 1 equiv), 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glycopyranose (19 mg; 1.2 equiv) and DMPA (0.2 equiv) in 0.6 mL tetrahydrofuran. This solution was degassed for 30 min and subsequently irradiated at 354 nm for 6 h. SEC (DMAc, LiCl): M_n = 5900 g mol^{-1}, PDI = 1.35; ^{1}H NMR (300 MHz, CD_2Cl_2): δ 8.8−8.0 (anthracene), 7.8−7.4 (anthracene), 5.76, 5.54 (CH_2−anthracene), 5.3−3.8 (glucose), 3.8−3.1 (N−CH_2), 2.9−2.5 (CH_2 DecEnOx), 2.5−2.2 (CH_2 EtOx, CH_2 DecEnOx), 2.1−1.9 (acetate), 1.8−1.5 (CH_2 DecEnOx), 1.5−1.2 (CH_2 DecEnOx), 1.2−0.75 (CH_3 EtOx) ppm.

Diels−Alder Reaction (DA) of the Polymer Scaffold with Protected Maleimides. The polymer scaffold (21 mg; 1 equiv) and protected maleimide **1** (4.9 mg; 2 equiv) were dissolved in 1 mL *N*,*N*′-dimethylformamide (DMF). The solution was degassed for 25 min and subsequently stirred at 120 °C for 48 h. The product was isolated by precipitation in ice-cold diethyl ether. SEC (DMAc, LiCl): M_n = 3,000 g mol^{-1}, PDI = 1.21. ^{1}H NMR (300 MHz, CD_2Cl_2): δ 8−6.9 (DA product), 5.85 (*HC* = CH_2), 5.1−4.9 (HC=CH_2), 3.8−3.1 (N−CH_2), 2.5−2.2 (CH_2 EtOx, CH_2 DecEnOx), 2.1−1.9 (CH_2 DecEnOx), 1.8−1.5 (CH_2 DecEnOx), 1.5−1.2 (CH_2 DecEnOx), 1.2−0.75 (CH_3 EtOx) ppm.

Sequential Procedure for Successive SPAAC−DA−TE on the polymer scaffold. The polymer scaffold (150 mg; 1 equiv) and 4-dibenzocyclooctynol (23.7 mg; 1.2 equiv) were dissolved in 5.25 mL chloroform and stirred for 45 min at room temperature. Subsequently, the product (**SA1**) was obtained by precipitation in ice-cold diethyl ether. **SA1** (50 mg; 1 equiv) and protected maleimide **2** (15.5 mg; 2 equiv) were dissolved in 2 mL *N*,*N*′-dimethylformamide (DMF). The solution was degassed for 25 min and subsequently stirred at 120 °C for 48 h. The product (**SA2**) was isolated by precipitation in ice-cold diethyl ether. **SA2** (25 mg; 1 equiv), dodecanethiol (22.5 mg; 10 equiv) and DMPA (0.6 mg; 0.2 equiv) were dissolved in 0.6 mL tetrahydrofuran and the solution was degassed for 30 min under exclusion of light. After irradiating the solution for 8 h at 356 nm the product (**SA3**) was purified by preparative size exclusion chromatography. **SA1**. SEC (DMAc, LiCl): M_n = 3400 g mol^{-1}, PDI = 1.15. ^{1}H NMR (300 MHz, CD_2Cl_2): δ 8.8−8.0 (anthracene), 8.0−7.0 (anthracene, dibenzocyclooctynol), 5.85 (*HC*=CH_2), 5.76, 5.54 (CH_2−anthracene), 5.1−4.9 (HC=CH_2), 3.8−3.1 (N−CH_2), 2.7−2.17 (CH_2 EtOx, CH_2 DecEnOx), 2.17−1.98 (CH_2 DecEnOx), 1.72−1.5 (CH_2 DecEnOx), 1.5−1.2 (CH_2 DecEnOx), 1.2−0.75 (CH_3 EtOx) ppm. **SA2**. SEC (DMAc, LiCl): M_n = 4,600 g mol^{-1}, PDI = 1.19. ^{1}H NMR (300 MHz, CD_2Cl_2): δ 8−6.9 (DA product, dibenzocyclooctynol), 5.85 (*HC*=CH_2), 5.1−4.9 (HC=CH_2), 3.8−3.1 (N−CH_2), 2.5−2.2 (CH_2 EtOx, CH_2 DecEnOx), 2.1−1.9 (CH_2 DecEnOx), 1.8−1.5 (CH_2 DecEnOx), 1.5−1.2 (CH_2 DecEnOx), 1.2−0.75 (CH_3 EtOx) ppm. **SA3**. SEC (DMAc, LiCl): M_n = 4800 g mol^{-1}, PDI = 1.21. ^{1}H NMR (300 MHz, CD_2Cl_2): δ 8.0−6.9 (DA product, dibenzocyclooctyne), 3.8−3.1 (N−CH_2), 2.9−2.5 (CH_2 DecEnOx), 2.5−2.2 (CH_2 EtOx, CH_2 DecEnOx), 1.9−1.2 (CH_2 DecEnOx, CH_3-(CH_2)S), 1.2−0.75 (CH_3 EtOx) ppm.

One Pot—Three Step Reaction of SPAAC/DA−TE on the Polymer Scaffold. The polymer scaffold (50 mg; 1 equiv), 4-dibenzocyclooctynol (7.9 mg; 1.2 equiv), and protected maleimide **1** (11.6 mg; 2 equiv) were dissolved in 1.25 mL DMF and stirred for 45 min at room temperature. Subsequently, the solution was degassed for 30 min and stirring was continued at 120 °C for 48 h. After cooling the reaction mixture to room temperature, a stock solution of Ac_4GlcSH (31.5 mg; 3 equiv) and DMPA (1.5 mg; 0.2 equiv) in 0.5 mL DMF was added. The combined solutions were degassed for 30 min and irradiated at 356 nm for 10 h. The product was isolated by preparative size exclusion chromatography. SEC (DMAc, LiCl): M_n = 5,500 g mol^{-1}, PDI = 1.23. ^{1}H NMR (300 MHz, CD_2Cl_2): δ 8.0−6.9 (DA product, dibenzocycloctyne), 5.3−3.8 (glucose), 3.8−3.1 (N−CH_2), 2.7−2.5 (CH_2 DecEnOx), 2.5−2.1 (CH_2 EtOx, CH_2 DecEnOx), 2.1−1.9 (acetate), 1.9−1.2 (CH_2 DecEnOx, CH_3(CH_2)S), 1.2−0.75 (CH_3 EtOx) ppm.

Labeling with Fluorescein 5(6)-Isothiocyanate. A 10 mg sample of **SA3** was dissolved in 0.5 mL of DMF and fluorescein 5(6)-isothiocyanate (0.5 equiv) was added. After stirring for 3 days at room temperature the solvent was evaporated. The polymer was redissolved in chloroform, washed three times with water and brine and dialyzed for 5 days against water. The surrounding water was exchanged 10 times until no free dye could be detected in the dialysis water anymore.

Nanoparticle Preparation and DLS Measurement. Three mg of the fluorescein labeled **SA3** were dissolved in 2.5 mL of THF and 10 mL of distilled water were added dropwise to the solution. Subsequently, the stirring was continued at 70 °C to evaporate the THF. The nanoparticle suspension was characterized by means of dynamic light scattering (DLS) measurements: 100 μL of suspension was diluted with filtered demineralized water, and three measurements were performed at 25 °C for 150 s.

RESULTS AND DISCUSSION

Polymer Scaffold Synthesis. For the synthesis of the multifunctional polymer scaffold 9-chloromethylanthracene was used

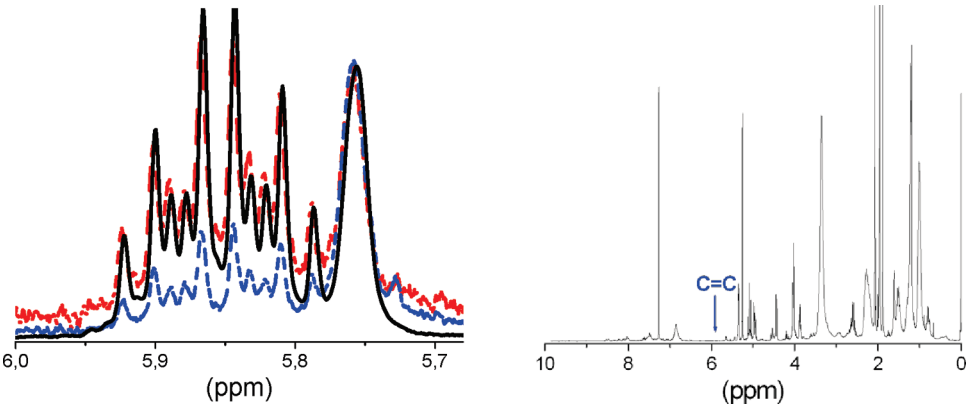


Figure 5. ^{1}H NMR spectra (CD_2Cl_2; 300 MHz) of the thiol−ene photoaddition of the multifunctional polymer scaffold with Ac_4GlcSH at 354 nm. Left: Evaluation of the alkene signal with and without a photoinitiator (−, polymer scaffold; red dashed line, 3 h, without DMPA; blue dashed line, 3 h, with DMPA). Right: Product after 6 h irradiation time.

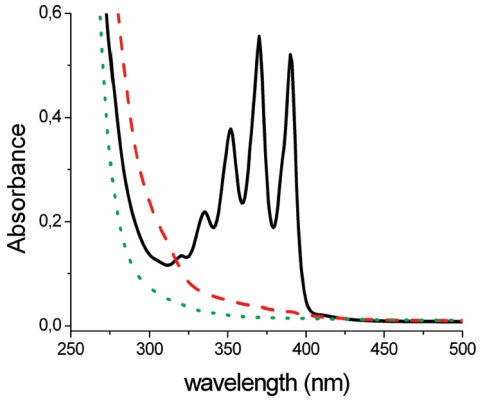


Figure 6. UV−vis spectrum of the multifunctional polymer scaffold (−), the protected maleimide **1** (green dotted line) and of the Diels−Alder cycloaddition product (red dashed line).

as initiator accompanied by the addition of sodium iodide for an anion exchange to accelerate the polymerization and to improve the control over the polymerization compared to pure 9-chloromethylanthracene. The monomers (EtOx and DecEnOx) were copolymerized in a ratio of 11:2 (EtOx:DecEnOx) in equivalence to the initiator to incorporate on average two terminal alkene groups in the side chains of the polymer scaffold. By adding sodium azide after the polymerization the living polymer chains were effectively end-capped with an azide moiety. SEC measurements of the precipitated polymer scaffold (Figure 1 left) revealed a monomodal and narrow molar mass distribution (PDI = 1.20).

The ^{1}H NMR spectrum (Figure 1 right) confirms the incorporation of the anthracene end group (7.5−9 ppm) as well as of the pendant alkene groups (5.8−6 ppm, 5 ppm). The degree of anthracene functionalization was calculated to be >90% based on the ratio between the aromatic anthracene signals and the polymer backbone signal.[40] Even though the functionalization is not quantitative, most likely caused by chain-transfer reactions during the polymerization, the anthracene moieties allow for efficient modifications in Diels−Alder approaches. In addition, the calculated EtOx/DecEnOx ratio is in good agreement with the feed ratio. Further analysis of the molecular composition by MALDI−TOF MS measurements revealed main polymer distributions (II, IV, VI) which are in agreement with the expected composition containing both, the anthracene and azide end groups as well as both monomers (Figure 2). Furthermore, minor secondary distributions (I, III, V) were observed indicative of the occurrence of minimal chain transfer reactions during the polymerization process resulting in hydrogen-initiated polymer chains, but still containing the azide end groups, as was also observed by ^{1}H NMR spectroscopy.

Evaluation of Individual Functionalization Reactions (SPAAC, TE, DA). Further proof for the successful incorporation of an azide end group is provided by the IR spectrum (Figure 3, right) showing the corresponding azide band at 2110 cm^{-1}, which was exploited for an online monitoring of the metal-free SPAAC reaction using 4-dibenzocyclooctynol (Figure 3, left). After the addition of the 4-dibenzocyclooctynol (stage II) an immediate drop of the azide signal intensity was observed demonstrating the consumption of more than 80% of the azide within 3 min (stage III). After 25 min, the signal intensity reached 0% (stage IV) indicative of full conversion of the polymer azide groups as supported by the complete disappearance of the entire azide band as depicted in Figure 3 right. The main distributions (I, II, IV, VI) in the corresponding MALDI−TOF MS spectrum are consistent with the attachment of 4-dibenzocyclooctynol to the polymer scaffold bearing the anthracene moiety (Figure 4). Furthermore, the minor fraction of chain transfer products present in the polymer scaffold also underwent complete cycloaddition (III, V).

In order to calculate the degree of functionalization, the additional aromatic signals in the ^{1}H NMR spectrum, related to the phenyl substituents of the 4-dibenzocyclooctynol, were exploited. Integration of the anthracene and 4-dibenzocyclooctynol signals with respect to the polymer backbone yielded a functionalization of >92%.[40]

The suitability of the multifunctional polymer scaffold for thiol−ene reactions was explored by the photoaddition of 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glycopyranose (Ac_4GlcSH). The necessity of a photoinitiator was tested by running two parallel reactions with and without 2,2-dimethoxy-2-phenylacetophenone (DMPA). After 3 h reaction timse a decrease in the alkene signal could be observed consistent with a conversion of 67% and 12% for the reactions with and without DMPA, respectively (Figure 5 left). Thus, further thiol−ene reactions were performed

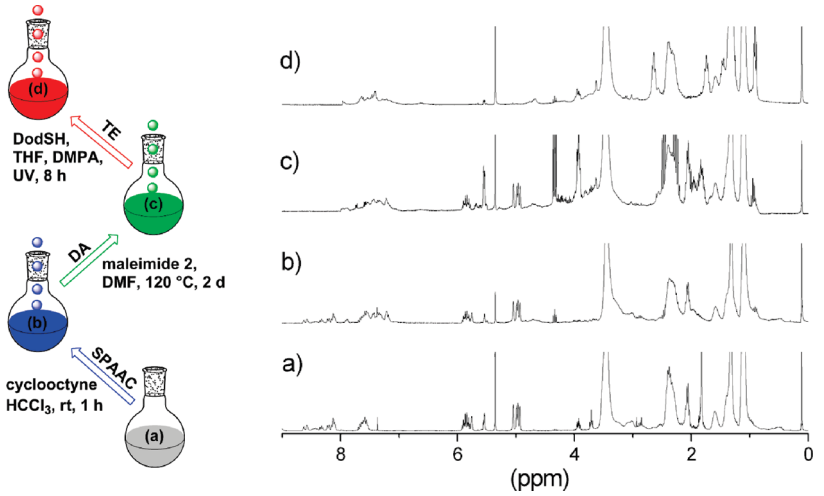


Figure 7. Left: Schematic representation of the order of the sequential efficient ("click") functionalization reactions. Right: ^{1}H NMR spectra (CD_2Cl_2; 300 MHz) of the products of the sequential addition of 4-dibenzocyclooctynol (b), the protected maleimide **2** (c), and dodecanethiol (d) to the multifunctional polymer scaffold (a).

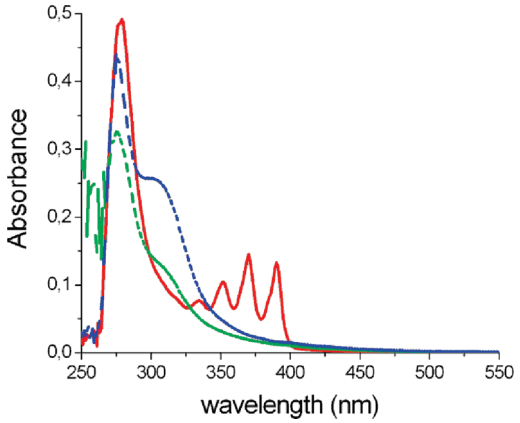


Figure 8. UV−vis spectra of the products obtained after sequential strain-promoted alkyne azide cycloaddition (red line), Diels−Alder cycloaddition reaction (blue dashed line), and thiol−ene photoaddition (green dashed line) to the multifunctional polymer scaffolds.

in the presence of DMPA. Extending the reaction time to 6 h provided full modification of the polymer scaffold as demonstrated by the disappearance of the alkene signal at about 5.85 ppm in Figure 5 right. Moreover, the successful coupling is confirmed by the presence of the glucose signals between 3.8 and 5.3 ppm as well as the protecting groups (acetate) of the glucose units at about 2 ppm in the ^{1}H NMR spectrum. In addition, the UV light irradiation had no influence on the azide as demonstrated by a stable azide signal during online IR monitoring (data not shown). Nonetheless, the irradiation at a wavelength above 300 nm may induce dimerization of the anthracene.[41]

The anthracene moiety used for the initiation of the polymerization is known to undergo an efficient Diels−Alder cycloaddition with diverse electron-poor alkenes, such as maleimides.[42,43] Since anthracene exhibits a characteristic absorption spectrum in the range of 300 to 400 nm (Figure 6), UV−Vis spectroscopy is perfectly suited to investigate the Diels−Alder cycloaddition. Due to the disruption of the extended aromatic anthracene structure upon Diels−Alder cycloaddition, the corresponding product does not exhibit UV bands in this region anymore demonstrating the success of the Diels−Alder cycloaddition (Figure 6). Because of the overlap of the signals in the ^{1}H NMR spectrum it is not possible to determine the actual degree of functionalization of the DA modification reaction.

Sequential Three-Step Functionalization of the Polymer Scaffold. On the basis of the successful individual post-modification reactions of the multifunctional polymer scaffold, a strategy for the sequential triple modification was developed. Because of its rapid reaction at ambient temperature the SPAAC was chosen as the first reaction leading to consumption of the azide end group, which might also potentially interfere with the other reactions. Second, the Diels−Alder cycloaddition will be performed in order to prevent a possible intramolecular Diels−Alder coupling of two anthracene moieties under UV light.[41] Thus, the TE photoaddition will be the final post-modification step. The order of the sequential reactions is summarized in Figure 7, left.

To demonstrate the versatility and potential of this triple post-modification concept different strategies and reactants were exploited for the sequential addition reactions, that is the introduction of functional groups by a hydroxy-functional 4-dibenzocyclooctyne and an acid-functionalized protected maleimide **2** while dodecanethiol was incorporated as hydrophilicity/hydrophobicity modifier. All reactions were performed successively, whereby intermediate products were purified by precipitation and the final product by preparative size exclusion chromatography. The success of the reactions was investigated by ^{1}H NMR and UV−Vis spectroscopy. Figure 7 (right) shows the ^{1}H NMR spectra after each sequential post-modification step. The successful addition of 4-dibenzocyclooctynol to the polymer scaffold is demonstrated by the appearance of the aromatic proton signals around 7.5 ppm and is supported by the disappearance of the azide band in the respective IR spectrum (data not shown). An additional change in the aromatic region of the ^{1}H NMR spectrum was observed after the DA reaction. The disappearance of the signals above 8 ppm demonstrates the disruption of

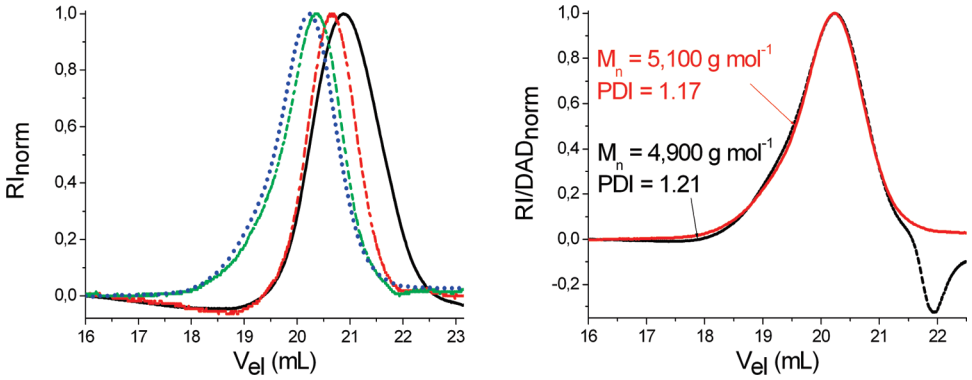
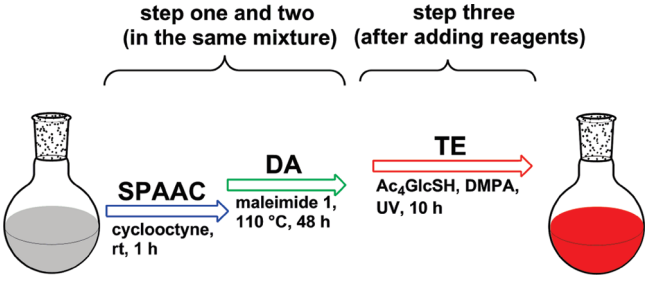


Figure 9. SEC traces (solvent: DMA + 0.1% LiCl) of the products from the sequential addition reactions. Left: polymer scaffold (−), after strain-promoted alkyne azide cycloaddition (red dashed line), Diels−Alder reaction (green dashed line) and thiol−ene photoaddition (blue dotted line). Right: Final labeled copolymer measured in RI (red solid line) and DAD (--; λ = 488 nm) mode.

Scheme 3. Schematic Representation of the One-Pot Three-Step Functionalization of the Polymer Scaffold



the anthracene structure and, thus, confirms the successful cycloaddition reaction with the maleimide. Since the protons of the maleimide itself overlap with proton signals of the polymer scaffold a further proof for the successful DA reaction is provided by UV−Vis spectroscopy. Figure 8 illustrates that the characteristic anthracene UV pattern is maintained after the SPAAC while it disappeared after the reaction with the maleimide. On the basis of the separated ^{1}H NMR signals of the pendant alkene groups at 5.8 and 5.0 ppm it can be concluded that they are not affected by the SPAAC and the DA reactions. Moreover, the final TE reaction is evidenced by the disappearance of these signals.

In addition to these structural changes, the reactions caused a change in the hydrodynamic volume of the polymer scaffold as demonstrated by a shift of the SEC trace to slightly lower elution volumes, also indicating successful post-modification (Figure 9, left). The presence of the SPAAC product in the final polymer structure was further evidenced by labeling the 4-dibenzocyclooctynol hydroxyl group with fluorescein 5(6)-isothiocyanate (FITC), a dye with a strong absorption band at 494 nm making it a popular biological marker. SEC traces obtained with the refractive index detector (RID) and the diode-array detector (DAD; λ = 488 nm) revealed almost perfect overlap demonstrating successful attachment of the FITC to the polymer scaffold.

One-Pot Three-Step Functionalization. Ideally, the 3-fold functionalization of the polymer scaffold should be achievable in an one-pot approach. Under the conditions presented so far, an one-pot one-step reaction is limited by the concurrent reaction of the thiol compound with the deprotected maleimide (Michael addition). As a consequence, the SPAAC and DA cycloaddition reactions were performed prior to the addition of the thiol for the TE photoaddition (Scheme 3).

The isolated product was characterized by IR, ^{1}H NMR and UV−Vis spectroscopy. The disappearance of the azide band in the IR spectrum demonstrated the successful SPAAC reaction. The success of the DA cycloaddition reaction was confirmed by UV−Vis measurements revealing the disappearance of the characteristic anthracene band (Figure 10 right). Furthermore, the full conversion of the pendant alkene groups in the UV-induced TE reaction was assured by the absence of the alkene signals (Figure S1, Supporting Information) as well as the appearance of the Ac_4GlcSH signals (Figure 10 left).

Both approaches, the sequential three-step as well as the one-pot three-step functionalization, provided similar near-quantitative conversions, as demonstrated by the complete disappearance of the respective signals including the disappearance of the azide signal in the IR spectrum (SPAAC), the disappearance of the characteristic UV absorption band (DA) as well as the disappearance of the alkene signals in the ^{1}H NMR spectrum (TE).

Nanoparticles of the Fluorescein-Labeled Triple Functionalized Copolymer. The high functionality of the polymer scaffold does not only allow the direct attachment of dyes, cell penetrating, and/or targeting groups as well other polymer chains, but also enables the tuning of the overall solubility of the copolymer. The incorporation of large hydrophobic groups renders the copolymer water-insoluble, making it well-suited for the preparation of nanoparticles by the nanoprecipitation technique.[44] Nanoparticles are especially interesting since they are particularly taken up by dendritic cells that have evolved to react to bacteria and viruses. The final FITC-labeled copolymer, resulting from sequential 3-fold modification and FITC labeling, comprises dodecanethiol groups attached to the pendant alkenes making it water-insoluble. The thus prepared stable fluorescent dispersion was analyzed with dynamic light scattering, which revealed the formation of relatively defined particles with a diameter of 452 nm and a $PDI_{particle}$ value of 0.145 (Figure 11).

CONCLUSION

The synthesis of a versatile multifunctional poly(2-oxazoline) scaffold is described. Equipped with three orthogonal functional groups, namely an anthracene moiety, pendant alkene groups

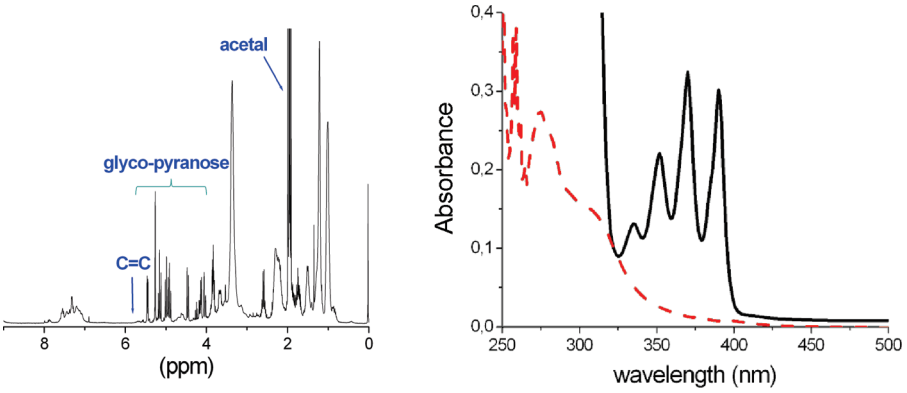


Figure 10. ^{1}H NMR spectrum (CD_2Cl_2, 300 MHz) (left) and UV−vis spectra (right) of the one-pot three-step reaction product (red dashed line).

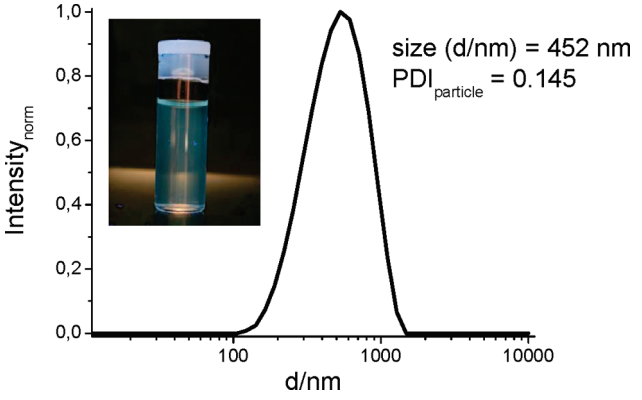


Figure 11. DLS size distribution (CONTIN) of nanoparticles formed by nanoprecipitation of the fluorescein-labeled triple functionalized copolymer. The inset shows the appearance of the nanoparticles solution demonstrating the stability of the dispersion and the successful fluorescein incorporation.

and an azide end group, this polymer scaffold allows efficient triple metal free post-modification by strain-promoted azide alkyne cycloaddition as well as radical thiol−ene and Diels−Alder cycloaddition reactions. The feasibility of each single post-modification reaction was demonstrated before the polymer scaffold was utilized for successful straightforward 3-fold functionalization in a sequential manner. Furthermore, efficient triple post-modification of the polymer scaffold by a successive one pot three-step approach is reported. Taking everything into account this multifunctional polymer scaffold offers the possibility for the preparation of a large variety of functional nanoparticles by using appropriate reactants as demonstrated by the nanoprecipitation of a FITC-labeled copolymer.

The developed approach shows great promise for the selective attachment of different dyes, cell penetrating and/or targeting groups to a multifunctional polymer scaffold in order to tailor the properties for diverse applications, including the preparation of smart nanoparticles. First cell studies of labeled nanoparticles are currently under investigation.

■ ASSOCIATED CONTENT

Supporting Information. Magnification of the ^{1}H NMR spectra of the polymer scaffold and the one-pot three-step product. This material is available free of charge via the Internet at http://pubs.acs.org/.

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