Functional Materials

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Multifunctional Poly(ethylene glycol)s

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bioconjugates · epoxides · multivalency · poly(ethylene glycol) · polyethers

In the rapidly evolving multidisciplinary field of polymer therapeutics, tailored polymer structures represent the key constituent to explore and harvest the potential of bioactive macromolecular hybrid structures. In light of the recent developments for anticancer drug conjugates, multifunctional polymers are becoming ever more relevant as drug carriers. However, the potentially best suited polymer, poly(ethylene glycol) (PEG), is unfavorable owing to its limited functionality. Therefore, multifunctional linear copolymers (mf-PEGs) based on ethylene oxide (EO) and appropriate epoxide comonomers are attracting increased attention. Precisely engineered via living anionic polymerization and defined with state-of-the-art characterization techniques—for example real-time ^{1}H NMR spectroscopy monitoring of the EO polymerization kinetics—this emerging class of polymers embodies a powerful platform for bio- and drug conjugation.

1. Introduction

Polymer therapeutics represent a growing field of modern multidisciplinary research ranging from organic and polymer synthesis to biomedicine, including clinical studies. Since Helmut Ringsdorf envisioned his concept of bioactive polymer–drug conjugates in the 1970s[1,2] a number of conjugates has already arrived on the market over the last two decades. In this context, the proclamation of a "dawning era of polymer therapeutics"[3,4] by Ruth Duncan in the turn of the century can be considered a symptomatic description for one of the major challenges in modern polymer chemistry. To harvest the potential of bioactive macromolecular hybrid structures, that is, conjugates of bioactive molecules and synthetic polymers, biomedicine and polymer chemistry, with innovative synthetic procedures to design the macromolecular structure and analytic tools to determine the properties will have to go hand in hand. Polymer therapeutics, which are usually prepared by the combination of an appropriate polymer and biologically active molecules, such as small drugs, proteins, labels, or targeting moieties, can exhibit considerably improved pharmacokinetics compared to the native bioactive compounds.[5–13] The positive effects stemming from the macromolecular features include increased blood circulation times and stability[14–17] and passive tumor targeting through the enhanced permeability and retention (EPR) effect, studied intensively by Maeda and co-workers and by other groups.[18–20] Adjusting polymer parameters such as molecular weight, topology, polydispersity, composition, functional groups, and solubility therefore also serves to adjust the pharmacokinetics of the resulting conjugates.

However, in view of the recent innovations in polymer chemistry, what specifically is the challenge? For successful medical application, the requirements for the polymer segment will depend not only on its chemical nature but also on its toxicity and biodegradability. In addition, considerations concerning the specific pathophysiology of the targeted disease, potency of the drug, and the desired administration route of the macromolecular conjugate are essential, resulting in a vast variety of factors for optimization of the overall system with the careful choice of the polymer as the key element.

Most polymer–drug conjugates commercialized within the last two decades are based on poly(ethylene glycol) (PEG).[21–26] To date, PEG, also referred to as poly(ethylene oxide) (PEO) for molecular weights above 20000 g mol^{-1}, is the established reference polymer for pharmaceutical and biomedical applications, because of 1) its excellent solubility in both aqueous and organic media, 2) the fact that it displays no immunogenicity, antigenicity, or toxicity and 2) the high flexibility and hydration of the main chain.[27–30] Often PEG is

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employed as a simple additive by the pharmaceutical and cosmetic industry for a wide range of applications.[31] Furthermore, based on the pioneering work of Davis, Abuchowski et al. in the late 1970s,[32,33] the covalent conjugation of PEG (PEGylation) has emerged as a valuable tool to overcome many of the deficiencies, particularly of protein- and peptide-based drugs, by increasing the molecular weight and shielding them from proteolytic degradation and immune response.[21,34–37] These PEG–protein conjugates represent convincing examples for the application and the synergistic power of polymer therapeutics.

However, functional polymers with their "defined copolymer composition" and "unusual binding capabilities"[1] permit adjustment of the conjugate's features by covalent attachment of solubilizing or targeting moieties to the backbone and provide superior drug loading capacity in comparison to the established PEGylation. And indeed, this concept has been put into practice utilizing *N*-(2-hydroxypropyl)-methacrylamide copolymers (PHPMA) as drug carriers. Poly(2-oxazoline)s,[38] poly(glycerol)s,[40,65,66] and poly(amino acid)s[41,42] represent other alternatives. Currently, various clinical trials of conjugates of PHPMA with anticancer drugs (e.g., doxorubicin or paclitaxel) are in different phases, exploiting the passive targeting effect.[43,44] For these cases of low-molecular-weight drugs, the application of the potentially best-suited polymeric candidate PEG is unfavorable, as only two functional groups (the end groups) are available for drug loading. It is self-evident that high loading capacity, a general goal for polymeric drug delivery, and feasible access to the polymer properties correspond to a high number of functional groups per macromolecule.

Innovative strategies developed in recent years to overcome the intrinsically low loading capacity of PEG have been dendronization of PEG[45,46] or the synthesis of star- or dendrimer-like PEGs.[39,47–50] As in the case of block copolymers based on PEG combined with monomers other than epoxides, for example, *N*-carboxy anhydrides, either the polymer structure or the chemical composition are significantly different from the established PEG homopolymer.[51–56] Considering the desired features, it is surprising that both linear block and random copolymers of ethylene oxide (EO) and appropriate epoxide comonomers represent a rather neglected class of polymers.

Although first reported in the mid 1990s,[57,58] multifunctional PEG derivatives have received increasing attention only in recent years, with emphasis on polymer therapeutics. Considering the predominance of PEG, which has been approved by the US Food and Drug Administration (FDA), has a well-established safety profile for drug administration, but is limited by low loading capacity, multifunctional PEG copolymers promise vast potential. They also enable multivalent interactions with biological surfaces[59–61] and application in combinational therapy.[8] Applications of multifunctional PEG copolymers are not confined to pharmaceutics and biomedical purposes. Another important field is catalysis. Today the most popular soluble support for catalysts is PEG monomethyl ether with a molecular weight of 5000 g mol^{-1} (MPEG-5000). By "PEGylation of the catalyst" the homogeneous reaction kinetics of low-molecular-weight compounds

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Frederik Wurm was born in Wiesbaden, Germany in 1981. He studied chemistry in Mainz and conducted his doctoral studies in the group of Holger Frey in macromolecular chemistry at the Johannes Gutenberg-Universität Mainz. He finished his PhD in summer 2009 focusing on syntheses of linear and branched macromolecules. Currently, he is working as a postdoc at the Ecole Polytechnique Fédérale in Lausanne, Switzerland in the group of Harm-Anton Klok, studying novel bioconjugates. He is supported by the Alexander von Humboldt Stiftung as a Feodor-Lynen fellow.

Holger Frey (born 1965) studied Chemistry at the University of Freiburg. Following a stay at Carnegie Mellon-University (Pittsburgh, USA) he obtained his PhD at the University of Twente (NL). After his Habilitation (University of Freiburg, 1998) on polycarbosilanes, he moved to the Johannes Gutenberg-Universität Mainz in 2001. Since 2003 he has held a full professorship there in organic and macromolecular chemistry. His research is directed at novel linear and branched functional polymer structures, microreactor-based syntheses, and biomedical materials in general.

are combined with the advantageous separation properties of heterogeneous catalysts.[62–64] Again, the beneficial properties of PEG are accompanied by limited loading capacity.

Preserving the desirable characteristics of the "gold standard" PEG, linear copolymers of EO and an appropriate comonomer represent precisely controlled macromolecules with an adjustable number of functional groups at the backbone (Compare Figure 1 and Figure 2 for an overview of possible polymer structures; see below for synthetic details). The structure can be viewed as a PEG backbone with functional side chains substituting several hydrogen atoms of the ethylene glycol repeating unit (Figure 1). Thus, in particular for low comonomer contents, we consider it reasonable to describe linear copolymers with various functional groups based on EO and other epoxide monomers with the term "multifunctional PEGs" (*mf*-PEGs). An emphasis must be placed on the difference to the structurally related poly(glycerol)s.[40,65,66] In spite of their proven biocompatibility,[67–69] without EO repeating units their chemical nature is clearly different from PEG. In contrast to branched or linear poly(glycerol)s with one functional group per repeating unit, *mf*-PEGs reduce the amount of often expensive comonomer to a minimum, and the functionality can be tailored for each case individually. Herein, we highlight the development and state of the art of *mf*-PEGs and present selected recent examples of this emerging class of polymers for different applications with focus on the potential of *mf*-PEGs for polymer therapeutics.

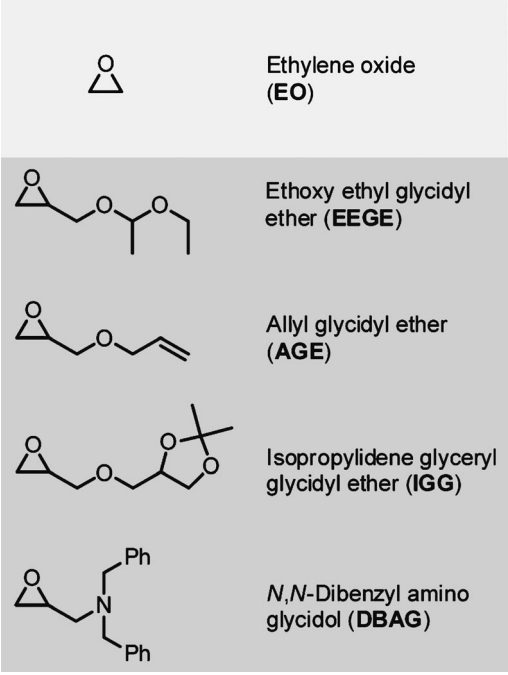


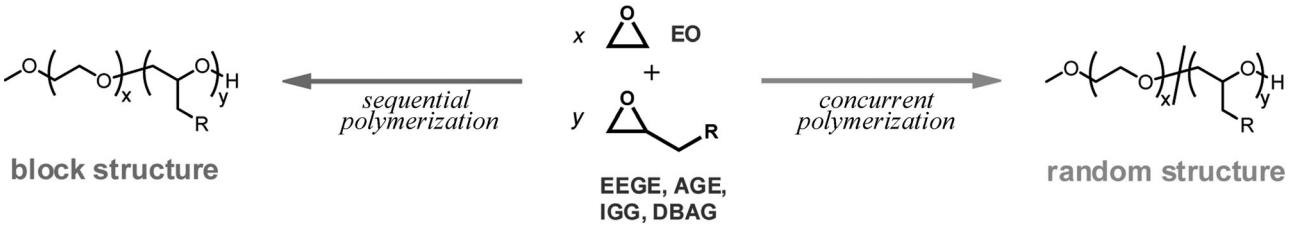
Figure 1. Monomer building blocks for preparation of linear multifunctional poly(ethylene oxide)s (*mf*-PEGs) with random or block structure.

2. Synthetic Strategies

The key for the synthesis of *mf*-PEGs is an appropriate epoxide comonomer for the ring-opening polymerization (Scheme 1). Random copolymerization of a mixture of the gaseous EO (b.p. 11 °C) and the respective comonomer in an appropriate solvent can be initiated by an alkali-metal alkoxide. The composition of the monomer feed determines the number of functional groups per polymer chain. Di- or triblock *mf*-PEGs are prepared by sequential polymerization of EO and the respective comonomer or directly by using commercially available PEG as a macroinitiator.[70–73] A potential drawback in this case is often a limitation in molecular weight of the functional segment owing to chain-transfer reactions.[74,75]

Appropriate comonomers provide an inherent functional group—to date only the direct introduction (i.e. avoiding protective groups) of allyl groups has been realized[58,76–85]—that is accessible by post-polymerization modification by two different routes: 1) removal of a protective group and/or 2) further organic transformation steps. Crucial requirements for a suitable protective group are stability under the harsh basic conditions of the anionic polymerization, few chain transfer reactions, and facile cleavage after the polymerization.

The ethoxy ethyl acetal protective group, first applied to glycidol by Fitton et al., fulfils these requirements most perfectly.[86] Thus, linear copolymers of EO and 1-ethoxy ethyl glycidyl ether (EEGE), yielding random poly(ethylene oxide-*co*-glycerol) (P(EO-*co*-G)) after acidic deprotection, represent the most common access to *mf*-PEGs.[57,70,74,75,87–94] The living copolymerization of EO and EEGE guarantees excellent control over molecular weights and narrow molecular-weight distributions with polydispersity indices (M_w/M_n) commonly below 1.10. Most interestingly, the expected biocompatibility of hydroxy *mf*-PEG was demonstrated in



Scheme 1. Common synthetic routes to linear multifunctional poly(ethylene glycol)s with block or random structures based on ethylene oxide and an appropriate comonomer by anionic ring-opening copolymerization. Left: sequential copolymerization leading to *mf*-PEG block copolymers; right: concurrent copolymerization providing *mf*-PEG random copolymers.

vitro and in vivo just recently.[95] As a valuable alternative to EEGE, the novel monomer 1,2-isopropylidene glyceryl glycidyl ether (IGG) provides one primary and one secondary hydroxy group per comonomer unit upon acidic deprotection.[96,97]

Furthermore, use of an initiator with an orthogonal functional group that can be selectively addressed can be of special value with respect to bioconjugation of *mf*-PEGs.[97–99] Besides hydroxy groups, to date only the introduction of amino groups, through *N*,*N*-dibenzyl amino glycidol (DBAG),[100] has been reported by direct copolymerization and subsequent hydrogenolytic deprotection. *mf*-PEGs with other functional groups have to be synthesized by transformation of *mf*-PEGs bearing hydroxy, amino, or allyl groups. For instance, starting from hydroxy-functionalized *mf*-PEGs, Li and Chau reported the preparation of a broad library of *mf*-PEGs with various types of functionalities by transforming the hydroxy groups in one or multiple synthetic steps (Figure 2).[101]

However, post-polymerization modifications can result in reduced yields, incomplete conversions, and the formation of byproducts if standard organic reactions are used. Their application at the macromolecular level is often more demanding and can lead to insufficiently defined materials, with potential negative effects on toxicity and biocompatibility. Precisely tailored *mf*-PEGs are most effectively obtained if reactions exhibiting very high yields, high selectivity, versatility, and simplicity—in recent years designated "click reactions"—are applied.[102–109] The transformation of copolymers of EO and allyl glycidyl ether (AGE) through thiol–ene coupling (TEC)[110–113] represents a powerful modular platform for precisely tailored *mf*-PEGs.[58,76–85] Through commercial AGE as a comonomer, an adjustable number of allyl ethers can be introduced at the PEG backbone (in a random or block fashion), which are accessible for TEC. AGE also reacts by way of a living (co)polymerization, which provides excellent control of molecular weights and narrow molecular-weight distributions.

In this case TEC cannot be regarded as a "click reaction" in a strict sense, since an excess of thiol is necessary to suppress crosslinking.[114] However, the metal-free reactions grant access to *mf*-PEGs with versatile functionalities, if the respective, often inexpensive, heterobifunctional thiol is available. The functional group is connected through an ether and thioether linkage to the backbone. Studies based on AGE copolymers are discussed below.

2.1. Copolymer Structure

Whereas linear diblock copolymers of EO and an appropriate epoxide monomer are directly obtained with a defined structure, the synthesis of random copolymers gives rise to a crucial issue: Is the comonomer randomly distributed along the backbone or does the resulting polymer exhibit a gradient or even a blocklike structure?

From the steric and electronic differences between EO and the glycidyl ether type epoxide comonomer, different reactivities might be anticipated. Considering the specific properties of *mf*-PEGs, a combination of the following analytical techniques has turned out to be effective for the determination of the comonomer incorporation and consequently the polymer structure.

The ^{13}C NMR chemical shift of the monomer units varies depending on both of the neighboring monomer units of a so-called "monomer triad", a chain segment consisting of three monomer units. ^{13}C NMR spectroscopy enables analysis of the distribution of the different triad sequences and therefore leads to a detailed understanding of the microstructure of the polyether backbone.[115–117] A typical phenomenon for a random structure is the decreasing intensity of the homo EO triad (EO-EO-EO, that is, **E**) with increasing comonomer content, as illustrated in the enhanced backbone region in Figure 3 for different AGE-based *mf*-PEGs.

Despite the plethora of studies involving PEG, only recently a facile experimental procedure was developed that allowed for the first time the monitoring of (co)polymerizations of the toxic and gaseous EO by ^{1}H NMR spectroscopy in real time (Figure 4).[100] At all stages of the polymerization and at various temperatures, the monomer consumption and the composition of the monomer feed can be analyzed in a detailed manner by integration of the isolated epoxide signals of each monomer. Interestingly, for AGE as well as for IGG, and to a lesser extent for *N*,*N*-dibenzyl amino glycidol, completely random comonomer incorporation was found for comonomer fractions of 10–20%. This observation was independent of the polymerization temperature applied (25–70 °C; Figure 5).

As a consequence of the degree of crystallization of PEG, the incorporation of random comonomer "defects" is also evident from the thermal properties characterized by differential scanning calorimetry (DSC). Only random incorporation leading to a homogenous average PEG homopolymer segment length within the polymer chains results in gradual changes in thermal behavior upon variation of the comonomer ratio.[97,98,100] Nonrandom comonomer incorporation and block formation leads to microphase separation, and the melting point depression is less pronounced.

Random copolymerization is a key feature for the tailoring of *mf*-PEGs and is of central importance for the synthesis of precisely defined conjugates. The random comonomer distribution and resulting constant average spacing between neighboring conjugation sites is a precondition for the systematic investigation of structure–response relationships and guarantees that materials properties are not influenced by structural inhomogeneity effects.

3. Selected Biomedical Applications

3.1. Random Cisplatin–(mf-PEG) Conjugates

For clinical treatment of several cancers, such as ovarian, bladder, neck, or lung cancer, cisplatin (*cis*-diamminedichloroplatinum or CDDP) is a well-established therapeutic.[118] Many of its drawbacks, such as acute nephrotoxicity and chronic neurotoxicity, rapid inactivation in the plasma, and poor solubility in water can characteristically be reduced

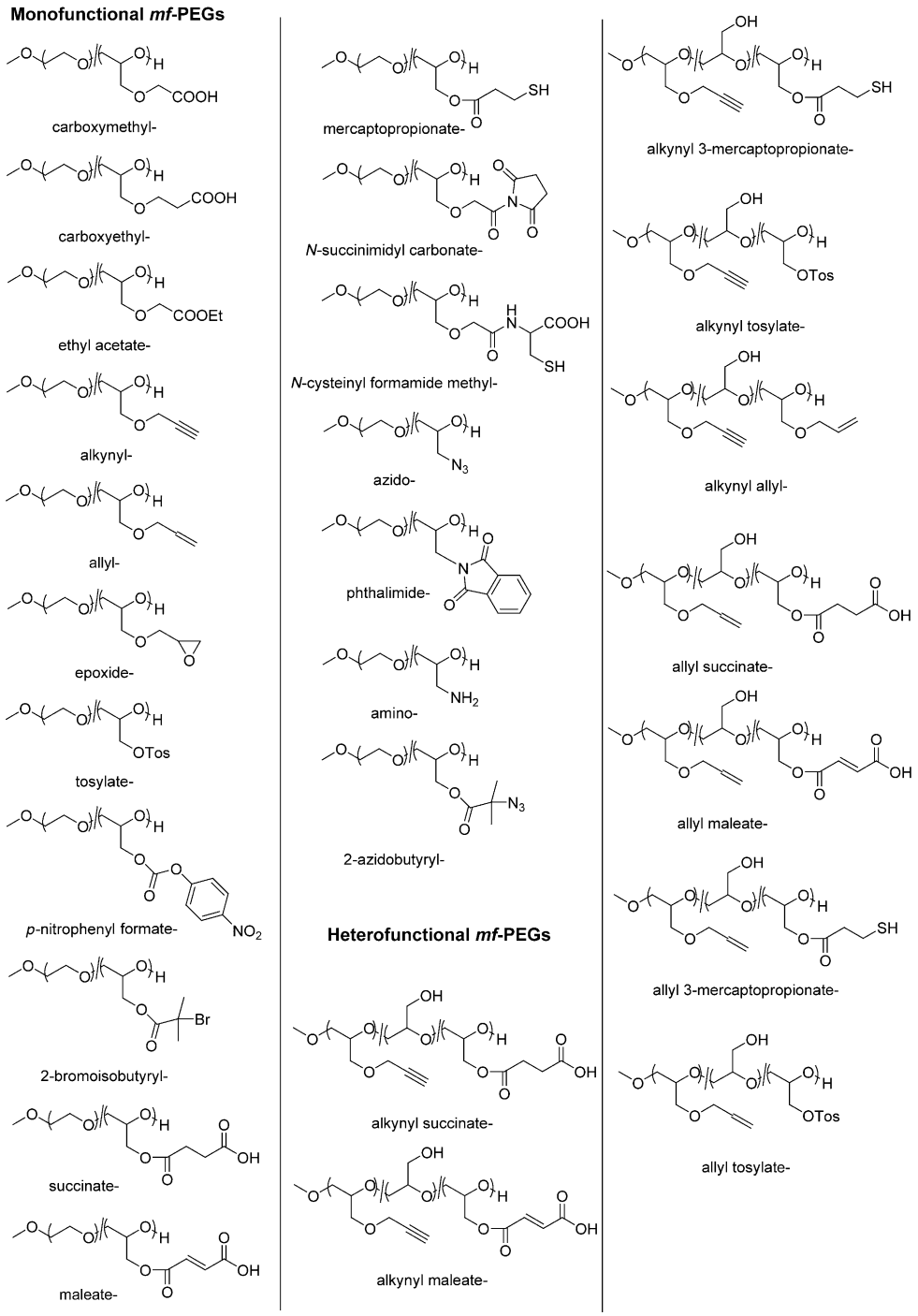


Figure 2. Library of *mf*-PEGs with functional groups derived from random poly(ethylene glycol-*co*-glycerol) via post-polymerization modifications. (From Ref. [101]).

through polymer conjugation.[119,120] In 2010 Zhou et al. reported the preparation of an anticancer polymer–drug conjugate, for the first time based on the biocompatible random hydroxy *mf*-PEG P(EO-*co*-G), obtained by copolymerization of EO and EEGE and subsequent removal of the acetal protective groups.[95] Modification of the hydroxy groups with malonate derivatives allowed for the reversible conjugation of cisplatin through a six-membered, chelate-type dicarboxylate coordination bond (Scheme 2). As expected, loading capacities were four to eight times higher than with the corresponding monofunctional PEG, and solubility was significantly increased compared to unsupported cisplatin.

With respect to polymer therapeutics, the non-biodegradability of *mf*-PEGs is an important issue giving rise to a special biological rationale concerning the molecular weight. For intravenous injection, the molecular weight needs to be small enough to guarantee renal excretion (threshold ca. 40000 g mol^{-1})[121] but still has to be sufficiently high to profit

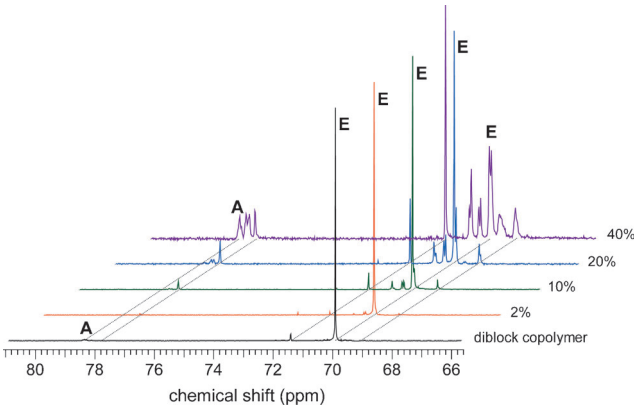


Figure 3. ^{13}C NMR spectral (75.5 MHz, [D$_6$]DMSO) region of backbone signals of MPEG$_{113}$-*b*-PAGE$_9$ diblock copolymer and random P(EO-*co*-AGE) copolymers with AGE contents of 2%, 10%, 20%, and 40%. **E** refers to EO-**EO**-EO triad, **A** to AGE-**AGE**-AGE triad. (From Ref. [85]).

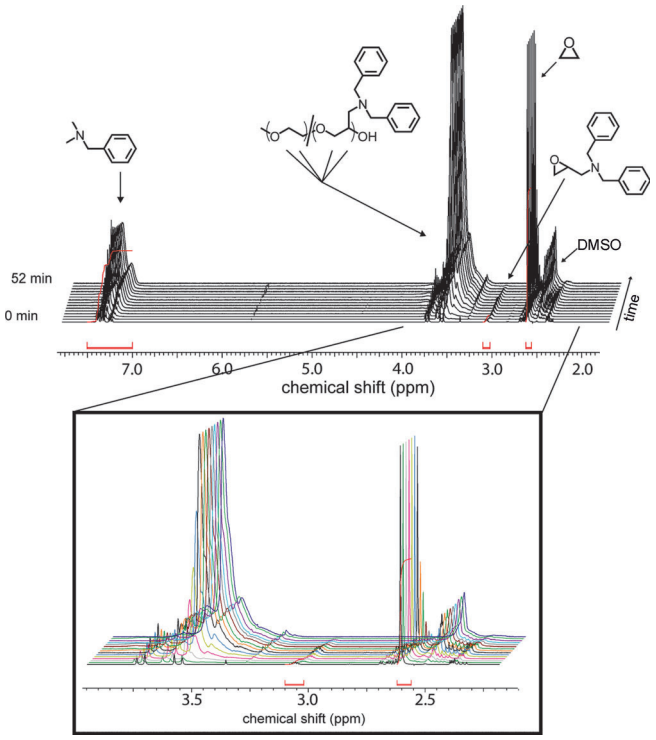


Figure 4. Time-resolved 400 MHz ^{1}H NMR spectra and details with relevant *N*,*N*-dibenzyl amino glycidol ($\delta = 3.02$ ppm), ethylene oxide ($\delta = 2.61$ ppm), and backbone signals for copolymerization of ethylene oxide and *N*,*N*-dibenzyl amino glycidol (15%) at 50°C, monitored in [D$_6$]DMSO for 52 min. (From Ref. [100]).

from the macromolecular properties (EPR effect)[18–20] and the multifunctionality.

In this context it is an important fact that higher tumor accumulation was found already for PEGs with molecular weights above 10000 g mol^{-1}.[122] Zhou et al. adjusted the molecular weights of the conjugates to a range of 12300–22400 g mol^{-1} and observed in vivo an antitumor activity similar to free cisplatin, but with reduced loss of body weight in nude mice bearing human nasopharyngeal carcinoma (HONE-1) xenografts (Figure 6). In vitro antitumor activity was observed for HONE-1 and human breast cancer, albeit at a potency lower than free cisplatin, possibly a specific result of the different uptake of the conjugate combined with a lack of uptake of the platin species, which is already released extracellularly owing to the rather weak coordinative bonding. In summary, the study gives initial but substantial evidence for the suitability of *mf*-PEGs as high-capacity carriers for low-molecular-weight (cytostatic) drugs.

3.2. pH-Sensitive Doxorubicin–(mf-PEG Block Copolymer) Conjugates

Vetvicka et al. presented the covalent conjugation of doxorubicin, one of the most promising cytostatic drugs, to *mf*-PEG diblock copolymers based on AGE and EO. A pH-sensitive linker was introduced by thiol–ene coupling (Scheme 3).[76,77] In blood plasma the amphiphilic diblock conjugates aggregate into micelles that slowly disintegrate to release doxorubicin in the acidic environment of the tumor tissue upon linker cleavage. The system exhibits almost 20 times lower systemic toxicity than free doxorubicin and long blood circulation times with half the dose after 24 h. Significant tumor accumulation was demonstrated by fluorescence whole-body imaging in mice with EL-4 T-cell lymphoma. Remarkably, about 75% of tumor-bearing mice were completely cured, and treatment in cured mice induced tumor-specific resistance (Figure 7). In early 2011, Zhong and co-workers reported an analogous system based on random copolymers.[123]

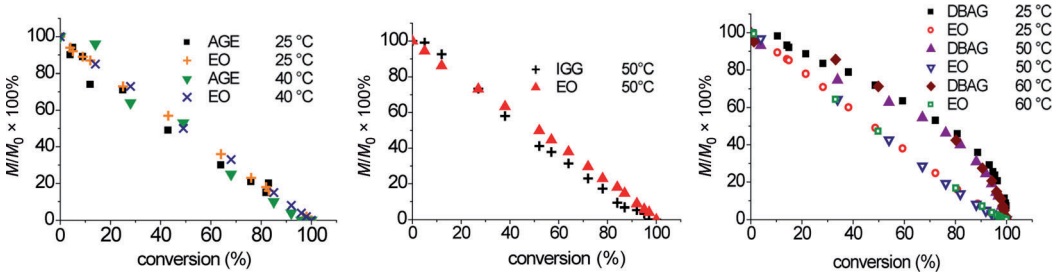
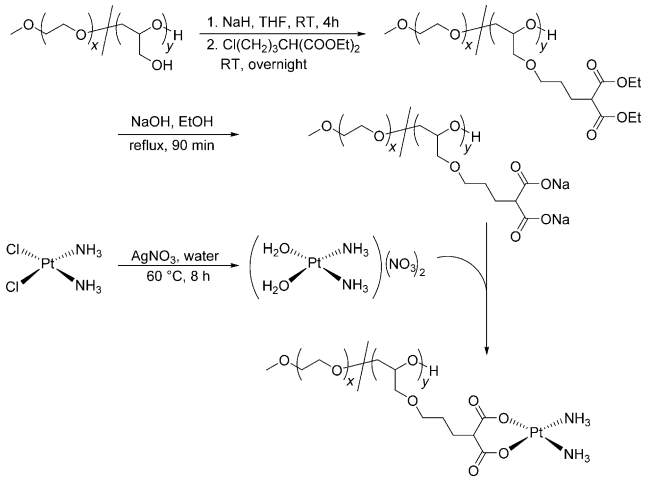


Figure 5. Percentage of initial monomer concentration for copolymerizations of ethylene oxide, allyl glycidyl ether (10%), isopropylidene glyceryl glycidyl ether (23%), and *N*,*N*-dibenzyl amino glycidol (15%) versus conversion for copolymerizations carried out in [D$_6$]DMSO, characterized by time-resolved NMR spectroscopy as shown in Figure 4.(From Ref. [85, 97, 100]).



Scheme 2. Synthesis of poly(ethylene glycol-*co*-glycerol) platinate based on random hydroxy *mf*-PEG, which was obtained from anionic copolymerization of EO and ethoxy ethyl glycidyl ether and subsequent deprotection. (From Ref. [95]).

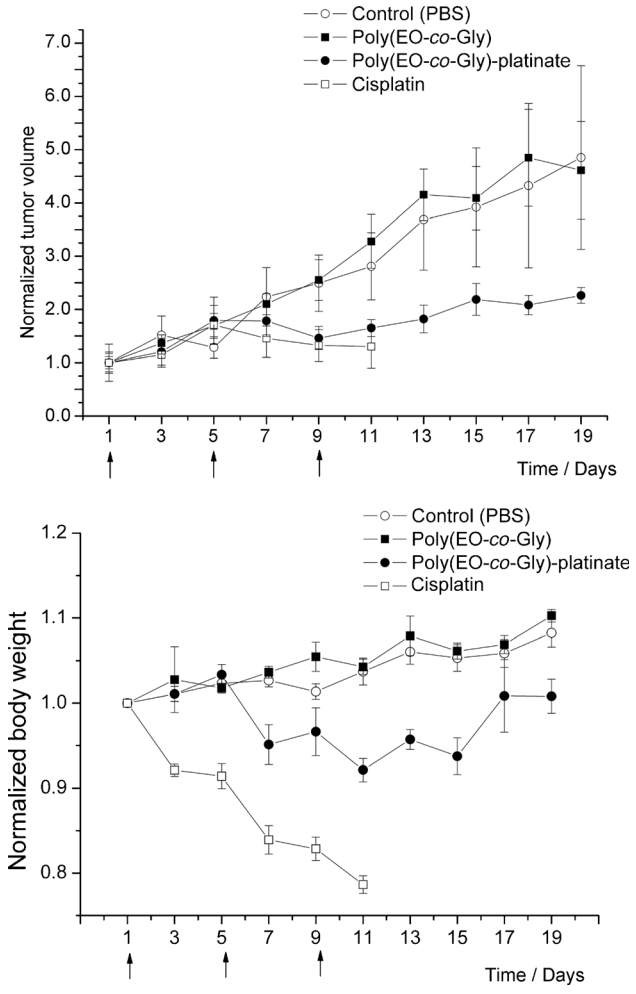
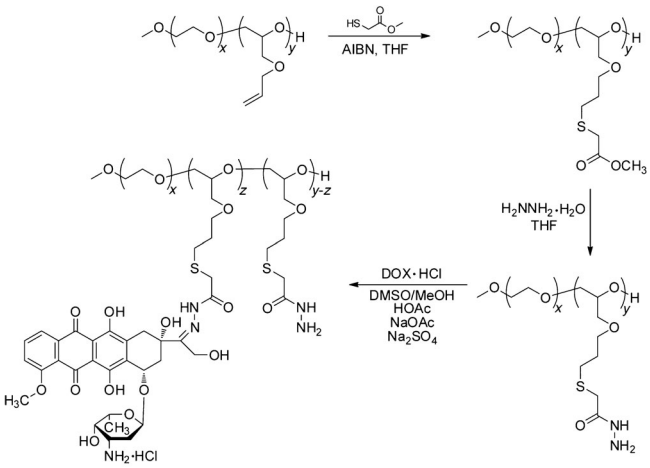


Figure 6. In vivo antitumor activity (top) and change of normalized body weight (bottom) of nude mice bearing human nasopharyngeal carcinoma xenografts after intravenous injections of poly(ethylene glycol-*co*-glycerol) and of poly(ethylene glycol-*co*-glycerol)-platinate conjugate and free cisplatin three times in four-day intervals (arrows). Mice in the free cisplatin group were sacrificed on day 11 due to severe weight loss. PBS = phosphate-buffered saline. (From Ref. [95]).



Scheme 3. Synthesis of doxorubicin conjugates based on poly(ethlyene glycol-*block*-allyl glycidyl ether). AIBN = azobisisobutyronitrile. (From Ref. [76]).

3.3. In Vivo and In Vitro Nonviral Gene Transfection

The success of nonviral gene transfection by ternary DNA/polycation/polyanion assemblies can distinctively depend on a polyanion covering the formed DNA/polycation complex and thus determining stability and solution behavior in the blood stream. Sakae et al. used the transformation of random P(EO-*co*-AGE)s via TEC for the preparation of highly negatively charged *mf*-PEGs for recharging DNA/polycation complexes to prevent their nonspecific interaction with proteins or cells (Scheme 4).[81] In addition, the multifunctionality was utilized to attach multiple RGD peptide side chains for active tumor targeting of the complex. The functionalized *mf*-PEG coated plasmid/PEI complex resulted in more than three times increased reporter protein activity on cultured B16 cells and very high gene expression in tumor, lung, and liver after injection into mice (Figure 8).

3.4. High-Capacity Peptide Conjugation

The combination of peptides and synthetic polymers results in the formation of hybrid structures, merging unique attributes of the biological and the synthetic elements.[124–129] For biological applications, the close resemblance of *mf*-PEG to the PEG homopolymer is a bonus. Frey and Obermeier recently demonstrated an efficient route to PEG-based bioconjugates with multiple peptides attached homogeneously to the *mf*-PEG backbone.[85] Without further modification, random P(EO-*co*-AGE) was directly conjugated with multiple units of the tripeptide glutathione using cysteine as the coupling amino acid for TEC (Scheme 5). Test reactions with *N*-acetyl-L-cystein methyl ester demonstrated that narrow molecular-weight distributions were maintained (M_w/M_n below 1.2). Detailed 1D and 2D NMR spectroscopic analysis showed virtually quantitative conversion of allyl ether side chains for a comonomer content of approximately 10%. With 11 linked glutathione moieties, the precisely defined novel PEG-based peptide conjugates exhibited superior peptide

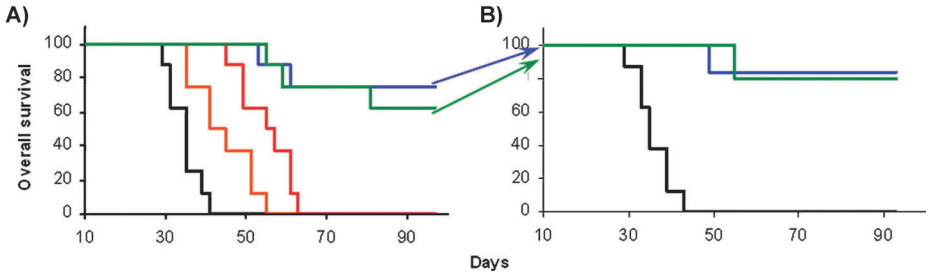
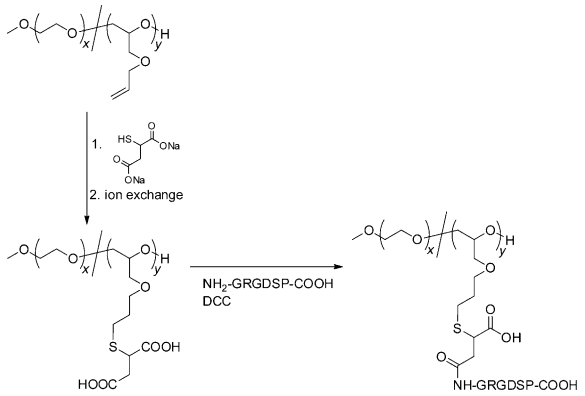


Figure 7. A) Survival of mice bearing EL-4 T-cell lymphoma and treated with diblock *mf*-PEG-doxorubicin conjugates (black: control; orange: 2×5 mg (DOX) kg^{-1}; red: 1×75 mg (DOX equiv) kg^{-1}; blue: 2×75 mg (DOX equiv) kg^{-1}; green: 1×150 mg (DOX equiv) kg^{-1}). B) Cured mice were retransplanted with a lethal dose of the same cancer cells and left without treatment (From Ref. [76]).



Scheme 4. Synthesis of carboxy *mf*-PEGs from random poly(ethlyene glycol-*co*-allyl glycidyl ether) using mercaptosuccinic acid and attachment of RGD peptide side chain for deposition onto DNA/poly(ethylene imine) (PEI) complexes and targeting of malignant cell surfaces. (From Ref. [81]).

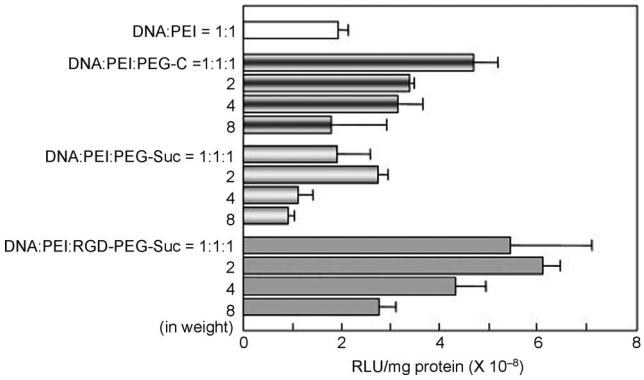
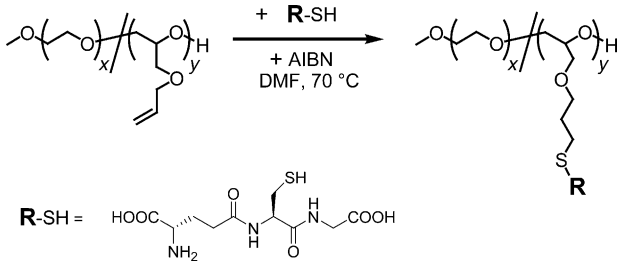


Figure 8. Transgene expression efficiency of DNA/poly(ethylene imine) coated with poly(ethlyene glycol-*co*-allyl glycidyl ether) with succinic acid (SUC) side chains and attached RGD peptide side chains on B16. RLU = relative light units. (From Ref. [81]).

loading and might be viewed as an example of "high-capacity PEGylation".

4. Conclusion and Outlook

Motivated by the evolving multidisciplinary field of polymer therapeutics and the status of PEG, with a documented (safety) profile over decades, the development of *mf*-PEGs is attracting increasing attention. By introducing functionalities at the polyether backbone, the intrinsically low loading capacity of PEG can be overcome. Structurally very close to PEG, linear copolymers based on EO and an appropriate comonomer provide a versatile platform for the preparation of bioactive hybrid polymer



Scheme 5. Thiol–ene coupling of the tripeptide glutathione to random poly(ethylene oxide-*co*-allyl glycidyl ether). (From Ref. [85]).

structures. As EO is the main structural component, the comonomer fraction can be reduced to the minimum amount necessary to achieve the desired functionality. This renders *mf*-PEGs attractive from an industrial point of view as well. Capitalizing on a combination of living anionic polymerization and advanced characterization techniques such as real-time ^{1}H NMR spectroscopic monitoring of EO polymerization kinetics, the macromolecular structure can be precisely designed. These special features render *mf*-PEGs highly interesting for applications in catalysis, as novel hybrids for materials' sciences, multifunctional cross-linking agents, or polyvalent functional materials in general. The first studies, presented in recent years, illustrate the versatility of *mf*-PEGs and their suitability for polymer therapeutics, along with evidence of the expected biocompatibility of random hydroxy *mf*-PEGs (P(EO-*co*-G). The structure of *mf*-PEGs in general indicates biocompatibility for several functional groups, of course for each one and for the respective linker and conjugate, biocompatibility will have to be evaluated individually. In addition to the *mf*-PEGs presented herein, the development of *mf*-PEGs exhibiting two or more orthogonal groups is also feasible by random polymerization of three different monomers. In this context, increasingly sophisticated protective-group monomers can play an important role, but also the further extension of the concept of α-ω-heterotelechelic polymers, that is, defined functionalities at the chain end that are different from those within the backbone, to *mf*-PEGs. In summary, *mf*-PEGs represent a powerful platform for polyvalent conjugation in general and are ideal candidates for polymer therapeutics in particular. However, it is a safe bet that the highly functional PEG structures will also play a role in coordination chemistry,

catalysis, surface modification, and for polymer-supported reagents.

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