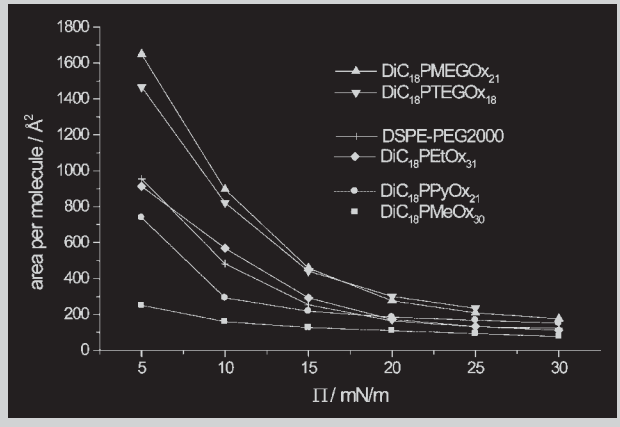
Summary: We present the synthesis of novel 2-oxazoline monomers with different 2-substituents and their consecutive conversion into lipopolymers by living cationic polymerization. The side functions of these monomers were varied to realize different steric needs and hydrogen bonding interactions of the polymer side chains. 2-(2′-*N*-pyrrolidonylethyl)-2-oxazoline, 2-(3′-methoxymonoethyleneglycol)propyl-2-oxazoline, and 2-(3′-methoxytriethyleneglycol)propyl-2-oxazoline were synthesized. All of the monomers could be converted into the corresponding lipopolymers by living cationic polymerization using 2,3-di-*O*-octadecyl-1-trifluormethansulfonyl-*sn*-glycerol as the initiator. The characterization of the 2,3-di-*O*-octadecyl-glycerol-poly(2-oxazoline) lipopolymers by NMR spectroscopy, IR spectroscopy, and gel permeation chromatography revealed that the targeted molar masses and compositions can be controlled by the initial initiator/monomer ($[M]_0/[I]_0$) ratio for all the synthesized lipopolymers. The polydispersities were found to be narrow (polydispersity indices from 1.06–1.3). The amphiphilic lipopolymers were spread at the air-water interface (Langmuir-Blodgett film balance) and the effect of the polymer side groups and chain lengths upon the Π-area (*A*) isotherms of the corresponding lipopolymer monolayers were compared and analyzed. The impact of the polymer side functionalities on a 2D gel formation was examined using an interfacial rheometer operated in an oscillating stress-strain mode. Interestingly enough, none of the newly synthesized lipopolymers showed a rheological transition. This somewhat surprising result not only verified that these 2D gels are not established by hydrogen bonding among hydrophilic polymer moieties, as earlier proposed, but also supported the concept of jammed surface micelles as the more likely origin for the gelation phenomenon.



Lipopolymers from New 2-Substituted-2-Oxazolines for Artificial Cell Membrane Constructs

This contribution is dedicated to Prof. Dr. *Rolf C. Schulz* on the occasion of his 85th birthday

Karin Lüdtke,[1] *Rainer Jordan,**[1,2] *Peter Hommes,*[1a] *Oskar Nuyken,*[1] *Christoph A. Naumann*[3]

[1] Lehrstuhl für Makromolekulare Stoffe, Department Chemie, Technische Universität München, Lichtenbergstr. 4, 85748 Garching, Germany
Fax: +49 89 289 13562; E-mail: Rainer.Jordan@ch.tum.de
[2] Department of Chemistry Chemical Engineering and Materials Science, Polytechnic University, Six Metrotech Center, Brooklyn, New York 11201, USA
[3] Department of Chemistry, Indiana University Purdue University Indianapolis, Indianapolis, Indiana 46202-3274, USA

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Introduction

Lipopolymers are amphiphiles composed of a linear hydrophilic polymer chain and a terminal hydrophobic lipid moiety.[1,2] In contrast to low molar mass soaps or phospholipids, their hydrophilic headgroup is not only a single ionic group or ion pair, but represents a bulkier headgroup consisting of a polymer chain of higher steric need and conformational freedom. Lipopolymers can be viewed as a synthetic variation of, e.g., natural glycolipids found in cell membranes. They have been synthesized and used in combination with lipids mainly for the preparation and study of artificial membrane constructs, such as vesicles, liposomes, black lipid membranes, or as tethers for lipid bilayer membranes on solid supports.[3–6] In such

[a] Current address: BASF Coatings AG, Paradiesstraße 18-22, 97080 Würzburg, Germany.

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constructs, the addition of lipopolymers increases stability and improves compatibility towards complex biological matrices such as blood or serum ('stealth liposomes') by the so-called steric repulsion mechanism.[7–9]

Recently, we demonstrated the great potential of functionalized lipopolymers based on poly(2-oxazoline)s to serve as defined tethers for the construction of stable, solid-supported membranes.[10,11] In this study, it is shown that the fine-tuning of the lipopolymer tethers in terms of the hydrophilic-lipophilic balance (HLB) is crucial for the morphology, the stability, and the physical properties (e.g., lipid diffusivity, mobile lipid fraction) of the membrane. Notably, in lipopolymer-supported model membranes, the length of the polymer tether defines the membrane-substrate spacing, thus allowing for the incorporation of large biologically important transmembrane proteins, such as an integrin cell receptor, into the phospholipid bilayer of the model membrane. Furthermore, we found that the successful preparation of the polymer-tethered lipid bilayer construct by Langmuir-Blodgett (LB)-transfer and consecutive vesicle fusion depends on many important lipopolymer-related factors, including the nature of the hydrophobic lipid-like moiety, the chemistry of the hydrophilic polymer, the ratio between hydrophobic and hydrophilic moieties of the molecule, and the terminal polymer functionality as well as on co-lipid-related factors like the type and concentration of matrix co-lipids. The appearance of dissipative structures (lipopolymer-rich stripes during LB-transfer of lipid/lipopolymer mixtures) allows the preparation of microscopically defined membrane structures. In other words, a tailored lipopolymer is crucial to the preparation of stable and homogeneous artificial membranes.[12] Single molecule tracking experiments recently showed that such model membrane systems can provide new, important insight into the lateral diffusion of lipids and membrane proteins obstructed by individual polymer-tethered lipids, which is a problem of great biological relevance.[13]

Based on these previous experiments, it has become more and more obvious that more realistic mimetics of biomembranes require a thorough understanding of the relationship between structural properties of membrane constituents and their subtle interplay via mostly weak intermolecular forces. Obviously, a much deeper understanding of the structure-function relationship of relevant membrane molecules is needed to mimic even selected aspects of membrane-associated properties of biological importance.

In the field of research for the development of more sophisticated biomembrane models, we have contributed lipopolymers of tailored compositions and defined structural variations. Unlike the popular and by now commercially available lipopolymers containing poly(ethylene glycol) (PEG), we developed poly(2-oxazoline)-based lipopolymers.[1,2,11,14–17]

Their synthesis via living cationic ring-opening polymerization allows not only a precise control of the degree of polymerization (length) by the monomer/initiator ratio and low polydispersities, but also the introduction of specific end groups.[18] For example, the initiator defines the lipid moiety, termination reaction the terminal functionalization with e.g. surface grafting or introduction of fluorescence labels.[11,14,15,19] Furthermore, the preparation of deuterated/hydrogenated block colipopolymers [20] for structural investigations with neutron scattering[21] as well as the fine tuning of the assembly behavior by the use of different side functionalities as in the case of poly(2-methyl- and 2-ethyl-oxazoline) lipo- and block copolymers can be achieved.[2,22,23] In contrast to the PEG systems, the polymer side group of poly(2-oxazolines) can be varied by the polymerization of different 2-substituted-2-oxazolines. However, in the past, only 2-methyl- and 2-ethyl-oxazoline have been used for the preparation of lipopolymers as tethers in polymer-supported bilayers, because the resulting polymers must be very water soluble and of good water storage capacity[24] to create a water cushion between the substrate and the membrane, which is advantageous for the bilayer properties.[10] The propionyl side group of the poly(2-ethyl-2-oxazoline) has resulted in a noticeable amphiphilic character of the monomer units as well as differences in the formation of hydrogen bonding.[2] In systematic studies, we found that the poly(2-ethyl-2-oxazoline) segment in lipopolymers as well as in amphiphilic block copolymers behaves as a nonionic polysoap and reversibly adsorbs at the air-water interface.[23] The amphiphilic nature of each monomer unit gives rise to a temperature-insensitive plateau region in the Π-*A* isotherms, thus being similar to PEG-based lipopolymers.[25] Minor changes, such as the introduction of an additional methylene group in the polymer side group of the lipopolymers, results in significant differences of the physical behavior, especially, at interfaces.

Another interesting aspect is related to the packing and collective association behavior of lipopolymers in two dimensions under confined conditions. For example, we found that PEG and poly(2-oxazoline)-based lipopolymers form 2D gels at the air/water interface if the monolayer is compressed to higher molecular densities.[23] Interestingly enough, it has been found that the gelation transition is dependent on both the polymer molecular weight[26] and the type of lipid anchor,[23] thus suggesting an important role for the steric mismatch between hydrophobic and hydrophilic moieties of lipopolymer molecules in the monolayer. Based on this concept, we recently suggested that the gelation transition is caused by the jamming of surface micelles.[23] Here, it is assumed that the formation of surface micelles is necessary for the gelation transition. However, the experimental data also indicate that the gel formation cannot only be explained by the steric mismatch between hydrophobic and hydrophilic part, but is also related to alkyl chain condensation among lipid moieties of lipopolymers.[23] It is less clear, however, whether the 2D

gelation also includes the formation of attractive linkages among polymer chains of adjacent lipopolymers. Particularly, inter- and intramolecular hydrogen bonding within the polymer part may contribute to or influence the gelation process. In previous studies, a possible influence of the hydrophilic polymer part could not be treated due to the structural similarity of available lipopolymers. By broadening the choice of different side groups of poly(2-substituted-2-oxazoline)s, this study helps to clarify the still open topic of attractive linkages among polymer chains of lipopolymers. To address this, the current work not only reports on the synthesis of new 2-substituted-2-oxazoline monomers with different side groups and the preparation of the corresponding lipopolymers by living cationic polymerization, but also describes film balance and surface rheometry experiments on monolayer systems of these lipopolymers at the air-water interface.

Experimental Part

Instruments and Methods

FTIR spectra were recorded on a Bruker IFS 55. NMR spectra were obtained on a Bruker ARX 300 (^{1}H: 300.13 MHz and ^{13}C: 75.47 MHz) with TMS as internal standard at 300 K in $CDCl_3$. Analytical gel permeation chromatography (GPC) was carried out on Waters 510 (column: 10, 50 nm; eluent: $CHCl_3$). Melting points were measured with a Mettler FP51 FP5 (heating rate: 5 °C/min). Salt suspensions were filtered through a PTFE membrane (pore diameter 45 nm) with a Sartorius 16249 stainless steel pressure filtration device. It was equipped with glass filter. Staining of nonfluorescent substances was achieved by dipping in a Haynes-Isherwood reagent (5 mL 60% perchloric acid, 1 g ammonium molybdate, 10 ml 1$\underline{N}$ hydrochloric acid filled up to 100 mL) and drying and heating to 80 °C.

The LB experiments were carried out on a small film balance (91 cm^2, 12.64 cm length, 7.2 cm width) equipped with a Wilhelmy system. Monolayers were formed by dissolving the lipopolymers in $CHCl_3$ and spreading of the solution on distilled and deionized water (pH = 5.5, 18.2 MΩ-cm). The compression rate was 10 cm^2/min. The isotherms are composed of two individual measurements. All the isotherms were measured several times to ensure reproducibility. For each polymer, compression-expansion cycles were performed as well as steady pressure experiments to verify film stability.

A more detailed description of the surface rheology experiments was given recently in ref.[26] Briefly, a Camtel CIR-100 Interfacial Rheometer (Camtel, UK) was used, which operates based on an oscillating Pt/Ir De Noüy ring attached to a virtually frictionless suspension wire. The system's drive unit is controlled by the drive unit coil, which operates similarly to a taut band galvanometer. The movement of the ring is detected by a sensor that reflects light off a target that rests on the saddle of the ring. All experiments were conducted in normalized resonance mode where the feedback control system forces the system into phase resonance. Working in this mode, G'_s and G''_s can be calculated independent of instrumental factors. The CIR-100 was operated with a small Labcon Molecular Photonics 700 Series LB film balance (Labcon, UK), thus allowing the control of the surface pressure and the area per molecule of the monolayer. The amphiphiles were spread at the air-water interface of the small LB trough in the rheometer at 20 °C. The film pressure was set to the desired pressure with an accuracy of ±0.06 mN/m, and the film was presented to the Pt/Ir De Noüy ring of the rheometer. Following a 5-min incubation period, the dynamic moduli were measured using a frequency of 2 Hz and the amplitude of 1 500 μrad. Each data point was averaged over eight cycles.

Materials

Purification of the monomers (before polymerization), the initiator and the terminating agent (piperidine) was performed as reported elsewhere.[10,15,19] 2-Methyl-2-oxazoline (**MeOx**) was received from Henkel KGaA, Germany. DSPE-PEG2000 was purchased from Avanti Polar Lipids, 2,3-di-*O*-octadecyl-*sn*-glycerol from Bachem. All other chemicals were purchased from Aldrich and used as received.

Monomers

2-(2′-*N*-Pyrrolidonylethyl)-2-oxazoline, **PyOx**

The reaction was carried out according to Seeliger et al.[28] using 58.1 g (0.42 mol) 3-(N-pyrrolidonyl)propionitrile (**7**) and 27.5 g (0.45 mol) ethanolamine (**6**). The product was isolated by vacuum distillation (bp = 115–120 °C at p = 0.008 mbar). A colorless oil (36.7 g, 48%) was obtained.

^{1}H NMR: δ = 2.02 (mp, 2H, –$CH_2C\underline{H}_2CH_2CONR$), 2.36 (t, 2H, ^3J = 8.0 Hz, –$CH_2CH_2C\underline{H}_2CONR$), 2.51 (t, 2H, ^3J = 7.6 Hz, –$C\underline{H}_2CH_2CH_2CONR$), 3.43 (t, 2H, ^3J = 7.1 Hz, –$NCH_2C\underline{H}_2C$=N–), 3.59 (t, 2H, ^3J = 7.1 Hz, –$NC\underline{H}_2CH_2C$=N), 3.81 (t, 2H, ^3J = 9.4 Hz, –$OCH_2C\underline{H}_2N$=C–), 4.23 (t, 2H, ^3J = 9.5 Hz, –$OC\underline{H}_2CH_2N$=C–).

^{13}C NMR: δ = 18.36 (–$CH_2\underline{C}H_2CH_2CONR$), 26.76 (–$N\underline{C}H_2CH_2C$=N), 31.21 (–$CH_2CH_2\underline{C}H_2CONR$), 39.76 (–$NCH_2\underline{C}H_2C$=N), 47.71 (–$\underline{C}H_2CH_2CH_2CONR$), 54.78 (–$OCH_2\underline{C}H_2N$=C–), 67.75 (–$O\underline{C}H_2CH_2N$=C–), 166.43 (–$OCH_2CH_2N$=$\underline{C}$–), 175.40 (–$CH_2C\underline{H}_2CH_2\underline{C}ONR$).

IR (film): 2 940, 1 682, 1 494, 1 463, 1 425, 1 171 cm^{-1}.

$C_9H_{14}N_2O_2$ (182.22): Calcd. C 58.59, H 7.78, N 15.52; Found C 59.32, H 7.74, N 15.37.

2-(3′-Methoxymonoethyleneglycol)propyl-2-oxazoline, **MEGOx**

First, 2-(3′-Methoxymonoethyleneglycol)butyronitrile (**4**) was synthesized in the following way. KOH (100 g) was dissolved in 150 ml of water. At 0 °C, 26.64 g 2-methoxyethanol (0.35 mol), 50 g 4-bromobutyronitrile (0.338 mol) and 2 g tetrabutylammonium bromide were added. The mixture was vigorously stirred for 14 h at room temperature. All of the insoluble material was removed, and the resulting mixture was extracted with methylene chloride. The collected organic phases were dried (Na_2SO_4), and the solvent removed. 2-(3′-Methoxymonoethyleneglycol)butyronitrile (**4**) was purified by vacuum distillation as a colorless oil was obtained (bp = 57–60 °C at p = 0.6 mbar).

MEGOx was synthesized according to Seeliger et al.[28] using 28.3 g (0.2 mol) **4** and 12.2 g (0.2 mol) of **6**. The final

product was isolated by vacuum distillation (bp = 120 °C at $p = 0.3$ mbar) as a colorless oil (16.4 g, 26%).

^{1}H NMR: $\delta = 1.88-1.98$ (mp, 2H, –OCH$_2$C$\underline{\text{H}}_2$CH$_2$C=N), 2.37 (t, 2H, $^3J = 7.6$ Hz, –OCH$_2$CH$_2$C$\underline{\text{H}}_2$C=N), 3.38 (s, 3H, –OC$\underline{\text{H}}_3$), 3.50–3.59 (mp, 6H, –C$\underline{\text{H}}_2$OC$\underline{\text{H}}_2$C$\underline{\text{H}}_2$OMe), 3.81 (t, 2H, $^3J = 9.3$ Hz, –OCH$_2$C$\underline{\text{H}}_2$N=C–), 4.22 (t, 2H, $^3J = 9.3$ Hz, –OC$\underline{\text{H}}_2$CH$_2$N=C–).

^{13}C NMR: $\delta = 24.69$ (–OCH$_2$C$\underline{\text{H}}_2$CH$_2$C=N), 25.95 (–OCH$_2$CH$_2$C$\underline{\text{H}}_2$C=N), 54.40 (–OCH$_2$C$\underline{\text{H}}_2$N=C–), 59.09 (CH$_2$O$\underline{\text{C}}$H$_3$), 67.20 (–O$\underline{\text{C}}$H$_2$CH$_2$N=C–), 70.05, 70.35 (–$\underline{\text{C}}$H$_2$O$\underline{\text{C}}$H$_2$CH$_2$OMe), 71.99 (–CH$_2$OCH$_2$$\underline{\text{C}}H_2$OMe), 166.28 (–OCH$_2CH_2$N=$\underline{\text{C}}$–).

IR (film): 2 879, 1 668, 1 632, 1 117 cm^{-1}.

$C_9H_{17}NO_3$ (187.24): Calcd. C 57.75, H 9.23, N 7.73; Found C 57.73, H 9.15, N 7.48.

2-(3′-Methoxytriethylenegycol)propyl-2-oxazoline, **TEGOx**

4-(Methoxytriethylenoxy)butyronitrile (**5**) was first synthesized as described above using 57.43 g (0.35 mol) methoxytriethyleneglycole. Colorless oil (bp = 110–112 °C at $p = 0.35$ mbar).

TEGOx was then synthesized accordingly using 53.17 g (0.23 mol) of **5** and 14.9 g (0.23 mol) **6**. The final product was isolated by vacuum distillation (bp = 135–140 °C at $p = 0.0025$ mbar) as a colorless oil (16.6 g, 18%).

^{1}H-NMR: $\delta = 1.87-1.96$ (mp, 2H, –OCH$_2$C$\underline{\text{H}}_2$CH$_2$C=N), 2.36 (t, 2H, $^3J = 7.6$ Hz, –OCH$_2$CH$_2$C$\underline{\text{H}}_2$C=N), 3.38 (s, 3H, –OC$\underline{\text{H}}_3$), 3.49–3.66 (mp, 14H, –C$\underline{\text{H}}_2$(OC$\underline{\text{H}}_2$C$\underline{\text{H}}_2$)$_3$OMe), 3.81 (t, 2H, $^3J = 9.3$ Hz, –OCH$_2$C$\underline{\text{H}}_2$N=C–), 4.22 (t, 2H, $^3J = 9.3$Hz, –OC$\underline{\text{H}}_2$CH$_2$N=C–).

^{13}C-NMR: $\delta = 24.67$ (–OCH$_2$C$\underline{\text{H}}_2$CH$_2$C=N), 26.0 (–OCH$_2$CH$_2$C$\underline{\text{H}}_2$C=N), 54.38 (–OCH$_2$C$\underline{\text{H}}_2$N=C–), 59.04 (CH$_2$O$\underline{\text{C}}$H$_3$), 67.21 (–O$\underline{\text{C}}$H$_2$CH$_2$N=C–), 70.15, 70.21, 70.27, 70.49, 70.55, 70.65 (–$\underline{\text{C}}$H$_2$(O$\underline{\text{C}}$H$_2$C$\underline{\text{H}}_2$)$_2$O$\underline{\text{C}}$H$_2$CH$_2$OMe), 71.99 (–OCH$_2$$\underline{\text{C}}H_2$OMe), 166.28 (–OCH$_2CH_2$N=$\underline{\text{C}}$–).

IR (film): 2 877, 1 669, 1 632, 1 117 cm^{-1}.

$C_{13}H_{25}NO_5$ (275.34): Calcd. C 56.62, H 9.11, N 5.48; Found C 56.71, H 9.15, N 5.09.

Initiator

2,3-di-*O*-Octadecyl-1-trifluormethansulfonyl-*sn*-glycerol, **DiC$_{18}$OTf**

The synthesis of 2,3-di-*O*-octadecyl-1-trifluormethansulfonyl-*sn*-glycerol, **DiC$_{18}$OTf**, was performed as reported elsewhere.[14,15]

Lipopolymers

The living cationic polymerization of all 2-oxazolines was performed as reported elsewhere.[15] After work-up, all polymers were obtained as a colorless solid.

DiC$_{18}$PMEGOx$_n$

^{1}H-NMR: $\delta = 0.86-0.93$ (mp, 6H, C$\underline{\text{H}}_3$–(CH$_2$)$_{15}$–), 1.25–1.45 (mp, 60H, –CH$_2$–(C$\underline{\text{H}}_2$)$_{15}$–CH$_2$–), 1.4–1.7 (mp, 10H, –N$^{\text{piperazine}}$–CH$_2$–C$\underline{\text{H}}_2$–C$\underline{\text{H}}_2$, –C$\underline{\text{H}}_2$–CH$_2$–O–), 1.8–2.1 (mp, 2nH, –CO–CH$_2$–C$\underline{\text{H}}_2$–CH$_2$–), 2.25–2.6 (mp, 2nH, –CO–CH$_2$–CH$_2$–C$\underline{\text{H}}_2$–O–), 3.2–3.9 (mp, 13nH + 15H, –N–C$\underline{\text{H}}_2$–C$\underline{\text{H}}_2$–, –N$^{\text{piperazine}}$–C$\underline{\text{H}}_2$–, –CO–C$\underline{\text{H}}_2$–CH$_2$–CH$_2$–, –O–C$\underline{\text{H}}_2$–C$\underline{\text{H}}_2$–O–CH$_3$, CH$_2$–C$\underline{\text{H}}_2$–O–, –O–CH–C$\underline{\text{H}}_2$–O–, –O–C$\underline{\text{H}}$–CH$_2$–O–, –(C$\underline{\text{H}}_2$)$_{15}$–C$\underline{\text{H}}_2$–O–), 3,36 (s, 3nH, –O–C$\underline{\text{H}}_3$).

^{13}C-NMR: $\delta = 14.12$ (CH$_3$–), 22.69 (CH$_3$–$\underline{\text{C}}$H$_2$–), 25.19 (–CO–CH$_2$–$\underline{\text{C}}$H$_2$–CH$_2$–O–), 26.11 (–O–CH$_2$–CH$_2$–$\underline{\text{C}}$H$_2$–), 29.0–29.7 (C^2, C^4–C$^{15\text{alkyl}}$), 30.93 (CH$_3$–CH$_2$–$\underline{\text{C}}$H$_2$–, –CO–CH$_2$–CH$_2$–$\underline{\text{C}}$H$_2$–O–), 42.9–45.1 (–N–$\underline{\text{C}}$H$_2$–$\underline{\text{C}}$H$_2$–), 58.99 (–O–CH$_3$), 69.9–70.6 (–CO–$\underline{\text{C}}$H$_2$–CH$_2$–CH$_2$–O–, –O–CH$_2$–$\underline{\text{C}}$H$_2$–O–), 71.94 (–O–$\underline{\text{C}}$H$_2$–CH$_2$–O–), 173.10 (–N–$\underline{\text{C}}$O–).

DiC$_{18}$PTEGOx$_n$

^{1}H-NMR: $\delta = 0.86-0.92$ (mp, 6H, C$\underline{\text{H}}_3$–(CH$_2$)$_{15}$–), 1.15–1.35 (mp, 60H, –CH$_2$–(C$\underline{\text{H}}_2$)$_{15}$–CH$_2$–), 1.35–1.65 (mp, 10H, –N$^{\text{piperazine}}$–CH$_2$–C$\underline{\text{H}}_2$–C$\underline{\text{H}}_2$, –C$\underline{\text{H}}_2$–CH$_2$–O–), 1.9–2.0 (mp, 2nH, –CO–CH$_2$–C$\underline{\text{H}}_2$–CH$_2$–), 2.25–2.6 (mp, 2nH, –CO–CH$_2$–CH$_2$–C$\underline{\text{H}}_2$–O–), 3.2–3.9 (mp, 21nH + 15H, –N–C$\underline{\text{H}}_2$–C$\underline{\text{H}}_2$–, –N$^{\text{piperazine}}$–C$\underline{\text{H}}_2$–, –CO–C$\underline{\text{H}}_2$–CH$_2$–CH$_2$–, –O–C$\underline{\text{H}}_2$–C$\underline{\text{H}}_2$–O–CH$_3$, CH$_2$–C$\underline{\text{H}}_2$–O–, –O–CH–C$\underline{\text{H}}_2$–O–, –O–C$\underline{\text{H}}$–CH$_2$–O–, –(C$\underline{\text{H}}_2$)$_{15}$–C$\underline{\text{H}}_2$–O–), 3.36 (s, 3nH, –O–C$\underline{\text{H}}_3$).

^{13}C-NMR: $\delta = 14.18$ (CH$_3$–), 22.68 (CH$_3$–$\underline{\text{C}}$H$_2$–), 25.22 (–CO–CH$_2$–$\underline{\text{C}}$H$_2$–CH$_2$–O–) 26.11 (–O–CH$_2$–CH$_2$–$\underline{\text{C}}$H$_2$–), 29.1–29.7 (C^2, C^4–C$^{15\text{alkyl}}$), 31.92 (CH$_3$–CH$_2$–$\underline{\text{C}}$H$_2$–, –CO–CH$_2$–CH$_2$–$\underline{\text{C}}$H$_2$–O–), 43.1–45.3 (–N–$\underline{\text{C}}$H$_2$–$\underline{\text{C}}$H$_2$–), 59.01 (–O–CH$_3$), 70.0–70.6 (–CO–$\underline{\text{C}}$H$_2$–CH$_2$–CH$_2$–O–, –O–CH$_2$–$\underline{\text{C}}$H$_2$–O–), 71.94 (–O–$\underline{\text{C}}$H$_2$–CH$_2$–O–), 172.78 (–N–$\underline{\text{C}}$O–).

DiC$_{18}$PTEGOx$_n$

^{1}H-NMR: $\delta = 0.86-0.93$ (mp, 6H, C$\underline{\text{H}}_3$–(CH$_2$)$_{15}$–), 1.25–1.45 (mp, 60H, –CH$_2$–(C$\underline{\text{H}}_2$)$_{15}$–CH$_2$–), 1.45–1.7 (mp, 10H, –N$^{\text{piperazine}}$–CH$_2$–C$\underline{\text{H}}_2$–C$\underline{\text{H}}_2$, –C$\underline{\text{H}}_2$–CH$_2$–O–), 1.9–2.2 (mp, 2nH, –N$^{\text{pyrrolidone}}$–CO–CH$_2$–C$\underline{\text{H}}_2$–CH$_2$–), 2.2–2.5 (mp, 2nH + 6H, –N$^{\text{pyrrolidone}}$–CO–C$\underline{\text{H}}_2$–CH$_2$–CH$_2$–, –N$^{\text{piperazine}}$–C$\underline{\text{H}}_2$–CH$_2$–C$\underline{\text{H}}_2$), 2.5–2.8 (mp, 2nH, –N–CO–C$\underline{\text{H}}_2$–CH$_2$–) 3.2–3.8 (mp, 8nH + 15H, –N–C$\underline{\text{H}}_2$–C$\underline{\text{H}}_2$–, –C$\underline{\text{H}}_2$–N$^{\text{pyrrolidone}}$–C$\underline{\text{H}}_2$–, –O–CH–C$\underline{\text{H}}_2$–O–, –O–C$\underline{\text{H}}$–CH$_2$–O–, –(C$\underline{\text{H}}_2$)$_{15}$–C$\underline{\text{H}}_2$–O–).

^{13}C-NMR: $\delta = 14.12$ (CH$_3$–), 18.13 (–N$^{\text{pyrrolidone}}$–CO–CH$_2$–CH$_2$–$\underline{\text{C}}$H$_2$–), 22.70 (CH$_3$–$\underline{\text{C}}$H$_2$–), 26.06 (–O–CH$_2$–CH$_2$–$\underline{\text{C}}$H$_2$–), 29.0–29.7 (C^2,C^4–C$^{15\text{alkyl}}$), 30.8–31.1 (–N$^{\text{pyrrolidone}}$–CO–CH$_2$–$\underline{\text{C}}$H$_2$–), 31.92 (CH$_3$–CH$_2$–$\underline{\text{C}}$H$_2$–), 39.30 (–N–CO–CH$_2$–$\underline{\text{C}}$H$_2$–), 43.5–48.0 (–N$^{\text{pyrrolidone}}$–CO–$\underline{\text{C}}$H$_2$–, –N–CO–CH$_2$–CH$_2$–$\underline{\text{C}}$H$_2$–, –N–$\underline{\text{C}}$H$_2$–$\underline{\text{C}}$H$_2$–), 171.26 (–N–$\underline{\text{C}}$O–), 175.15 (–N$^{\text{pyrrolidone}}$–$\underline{\text{C}}$O).

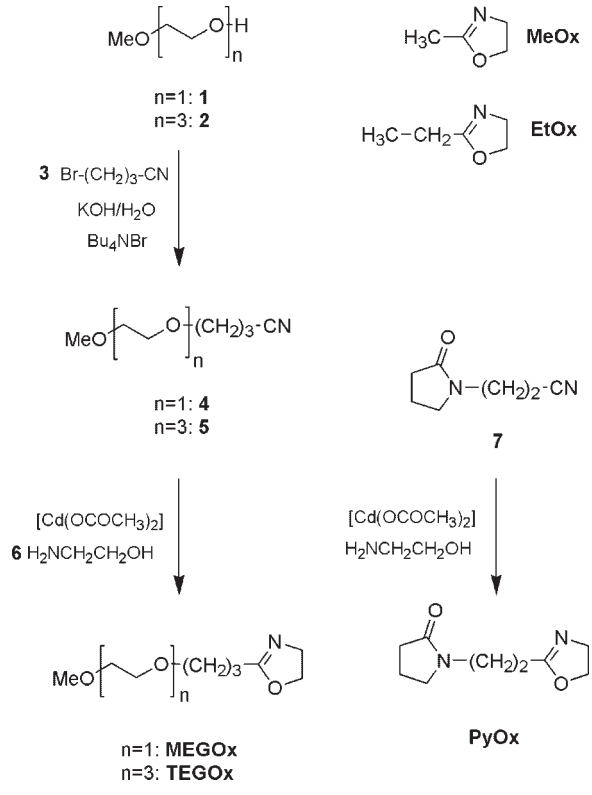
Results and Discussion

Monomer Synthesis

Beside the commercially available 2-methyl- (**MeOx**) and 2-ethyl-2-oxazoline (**EtOx**) monomers used in this study, we synthesized 2-(3′-methoxymonoethylene glycol)propyl-2-oxazoline (**MEGOx**) and 2-(3′-methoxytriethylene glycol)propyl-2-oxazoline (**TEGOx**) to combine the ethylene glycol motif with the poly(2-oxazoline) main chain. The latter results in short, comb-like or better 'bottle-brush'-like copolymers with a poly(2-oxazoline) backbone decorated with oligo(ethylene glycol) (o-EG) side-chains.

This affects the coil dimension of the hydrophilic polymer segment and, thereby, also the 'stealth performance' of the resulting lipopolymers when incorporated into liposomes, vesicles, or other membrane constructs. Furthermore, a terminal *N*-pyrrolidone moiety is featured in the new 2-(2′-*N*-pyrrolidonylethyl)-2-oxazoline (**PyOx**) monomer. Polymerization results in lipopolymers with different cross-sectional areas due to the different steric needs of the side chains and different motifs for inter- and intramolecular hydrogen bonding. The *N*-pyrrolidone and ethylene glycol (EG) side functions are known to be compatible with biological systems. The interaction of poly(2-oxazoline)s with biological systems have not been investigated thoroughly, but all the data available indicate low toxicity, low interaction with protein, and good excretion from blood circulation.[3,27]

The three monomers were synthesized according to Seeliger et al.[28] using the corresponding nitriles, **4, 5** and **7**. Although the 3-propylnitrile ethers are synthetically readily accessible via Michael addition to acrylnitrile, they were not suitable for the monomer synthesis. During the consecutive reaction to the 2-oxazolines, they underwent retro Michael addition and resulted in the corresponding alcohol and other side products. Hence, the corresponding nitriles were prepared from 4-bromobutyronitrile (**3**) for the synthesis of **MEGOx** and **TEGOx** and the alcohol derivative of the o-EG compounds **1** and **2** by ether synthesis[29] (Scheme 1).



Scheme 1. Synthesis of 2-substituted-2-oxazolines from the corresponding nitrile compounds according to ref.[28]

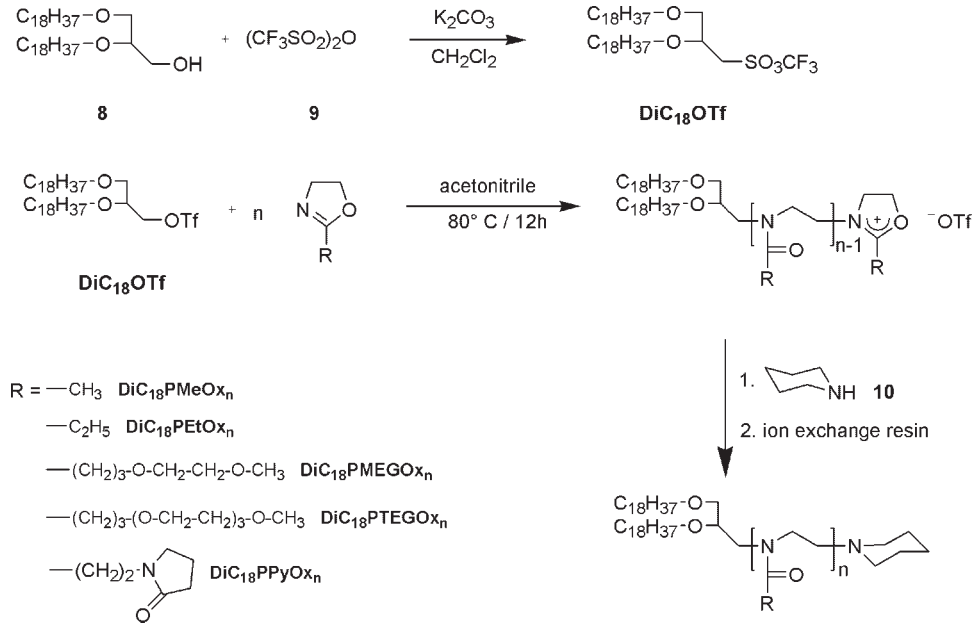
Living Cationic Polymerization with Lipid Triflates

All the monomers could be converted into the respective lipopolymers by living cationic polymerization using 2,3-di-*O*-octadecyl-1-trifluormethansulfonyl-*sn*-glycerol (**DiC$_{18}$OTf**) as the initiator (Scheme 2). A lipid moiety was quantitatively introduced into the polymer by the initiation method to result in the respective amphiphilic lipopolymers.[15] The synthesis of the initiator from the ether lipid, **8**, is shown in Scheme 2. Due to their much higher reactivity, as compared to tosylates, triflates are first-choice sulfonates for fast initiation ($k_{initiation} \gg k_{polymerization}$) and a propagation of the 2-oxazoline polymerization via a living ionic mechanism.[14,15] In contrast to other lipopolymer systems, an ether-linked lipid in the *sn*-1 and *sn*-2 position of the glycerol was chosen because of its better chemical stability. In model membrane systems, the 'chemical factory' of natural eukaryotic or prokaryotic cells that constantly controls its membrane lipid composition by enzymatic synthesis and decomposition of fatty acid esters is not available. Hence, long-term stability of artificial membrane constructs are better achieved by the use of lipids as found in archaebacteria.[30]

The resulting lipopolymers were characterized by ^{1}H- and ^{13}C-NMR spectroscopy and gel permeation (GPC). The results are summarized in Table 1.

The lipopolymers, **DiC$_{18}$PPyOx$_n$**, of the hydrophilic monomer, **PyOx**, feature a structure comparable to poly(*N*-vinylpyrrolidone) (PVP) but with a hydrophilic polymer backbone containing a tertiary amide function. It is reasonable to assume that these polymers exhibit similar properties in vivo, thus making them interesting candidates for biomedical applications,[31] for the complexation of metal ions, or for the combination with silica in sol-gel materials. In contrast to PVP, which can only be synthesized by free-radical polymerization, this monomer system allows the precise control of the polymer chain length and mass and, therefore, may be an 'alternative PVP' polymer for systematic studies of structure-properties relationships and for in vivo applications (e.g., artificial blood serum, stabilization of liposomal drug carriers).

The polymerization of **MEGOx** and **TEGOx** resulted in lipopolymers of low molar mass distribution and controlled chain length. The lipopolymers containing **MEGOx** show slightly higher polydispersities in comparison to those containing **TEGOx**. With the exception of **DiC$_{18}$PMEGOx$_n$**, GPC traces revealed that the polymer mass distributions are narrow and monomodal. Due to the very similar structure of both monomers, we assume that traces of impurities cause the bimodality in **DiC$_{18}$PMEGOx$_n$** rather than any side reaction of the monomer functionality during the polymerization. The lipopolymers containing the monomers,



Scheme 2. Synthesis of the triflated etherlipid **DiC$_{18}$OTf** from the corresponding primary alcohol and polymerization of the various 2-oxazoline monomers.

MEGOx and **TEGOx**, are the very first examples of lipopolymers with a comb-, or better said, 'bottle-brush'-like architecture. The easy variation of the side chain and the polymer length is promising in terms of the optimization of the coil and 'stealth performance' of these lipopolymers.

Langmuir-Blodgett Monolayers of Lipopolymers

To study the Langmuir-Blodgett monolayers of lipopolymers at the air-water interface, lipopolymers were dissolved in chloroform and spread at the air-water interface of a

Table 1. Analytical data for the synthesized lipopolymers as obtained by NMR spectroscopy and GPC. For details and nomenclature, please refer to main text.

Polymer	$\overline{DP}_n$, calc.[a)]	$\overline{DP}_n$, ^{1}H NMR[b)]	$\overline{M}_n$,^{1}H NMR[c)]	$\overline{M}_n$, GPC[d)]	PDI, GPC[e)]
DiC$_{18}$PPyOx$_{20}$	17	20	4 360	3 060	1.27
DiC$_{18}$PPyOx$_{24}$	23	24	5 120	4 450	1.21
DiC$_{18}$PPyOx$_{33}$	30	33	6 750	5 390	1.33
DiC$_{18}$PPyOx$_{38}$	43	38	7 610	8 120	1.17
DiC$_{18}$PMEGOx$_{13}$	14	13	3 040	4 000	1.30
DiC$_{18}$PMEGOx$_{21}$	20	21	4 650	5 910	1.14
DiC$_{18}$PMEGOx$_{29}$	28	29	6 100	6 900	1.37
DiC$_{18}$PMEGOx$_{31}$	30	31	6 430	7 470	1.37
DiC$_{18}$PTEGOx$_{8}$	10	8	2 970	4 030	1.16
DiC$_{18}$PTEGOx$_{18}$	16	18	5 560	6 270	1.12
DiC$_{18}$PTEGOx$_{18}$	19	18	5 560	6 640	1.13
DiC$_{18}$PTEGOx$_{25}$	30	25	7 620	7 270	1.21
DiC$_{18}$PMeOx$_{30}$	30	30	3 220	3 020	1.06
DiC$_{18}$PEtOx$_{31}$	33	31	3 710	4 750	1.09

[a)] Initial monomer/initiator feed.
[b)] Degree of polymerization calculated from ^{1}H-NMR spectra (end group analysis).
[c)] Number average from ^{1}H-NMR spectra.
[d)] Number average from gel permeation chromatography (GPC) (calibrated with linear polystyrene).
[e)] Polydispersity index ($\overline{M}_w/\overline{M}_n$). Yields were not determined due to substance losses during the work-up process. Furthermore, the polymers contain considerable amounts of solvent, which could not removed by vacuum drying, but the good control of the molar mass indicates quantitative conversion.

Langmuir-Blodgett trough; pressure-area isotherms were recorded at room temperature (20 °C) and interfacial rheology experiments were conducted as a function of film pressure. In all cases, stable lipopolymer monolayers were observed and repeated compression/expansion cycles did not indicate a significant loss of material into the water subphase. **$DiC_{18}PPyOx_n$** and **$DiC_{18}PMEGOx_n$** can be compressed up to $\Pi \approx 30$ mN/m; **$DiC_{18}PTEGOx_n$** forms stable monolayers up to $\Pi \approx 25$ mN/m before an uncontrolled film collapse is observed.

Π-*A* Isotherms

LB-film balance experiments were carried out with the lipopolymers, **$DiC_{18}PMEGOx_{21}$**, **$DiC_{18}PTEGOx_{18}$** and **$DiC_{18}PPyOx_{21}$**, which had a similar degree of polymerization; they were compared to **$DiC_{18}PEtOx_{31}$**, **$DiC_{18}PMeOx_{30}$**, and the commercially available **DSPE-PEG2000** ($n = 45$) (Figure 1).

As shown in Figure 1, the side chains have a significant influence on the required space per molecule. As expected, the lipopolymers with ethylene glycol moieties display a larger cross-sectional area than those with pyrrolidone, methyl, or ethyl side groups. However, the differences between **$DiC_{18}PMEGOx_n$** and **$DiC_{18}PTEGOx_n$** are surprisingly small.

Interestingly enough, the more dominant differences between the various lipopolymers are not due to the differences in the steric needs, but in the adsorption behavior of the polymer part at the air-water interface. Whereas the pure hydrophilic **$DiC_{18}PMeOx_{30}$** and **$DiC_{18}PPyOx_{21}$** display a slight but monotone pressure increase upon compression at relatively low molecular densities (continuous stretching of solvated polymer chains in the water subphase), the Π-area isotherms of all other lipopolymers feature a plateau region between 1 000 and ca. 400 Å^2/ molecule. In earlier studies, we demonstrated that such plateaus originate from the desorption of the polymer part from the air-water interface upon compression.[22,23] This only occurs if the 'hydrophilic' polymer moiety of lipopolymers has a slight amphiphilic motif within each monomeric subunit. The appearance of a similar plateau for the o-EG side chain-functionalized **$DiC_{18}PMEGOx_{21}$** and **$DiC_{18}PTEGOx_{18}$** can, therefore, be attributed to the adsorption of the o-EG side chains at the air-water interface.

The pressure-induced alkyl chain condensation of the lipid moiety causes the previously observed phase transition in the Π-*A* isotherms of **$DiC_{18}PMeOx$** and **$DiC_{18}PEtOx$** monolayers to occur at a film pressure of ca. 22 and 30 mN/m, respectively, and of **DSPE-PEG2000** at 17.5 mN/m (all data taken at room temperature).[2,23] In contrast to the low-pressure transition characterizing the amphiphilic character of polymer chains, the alkyl chain condensation transition is dependent on temperature. Notably, the novel oligo-EO side chain-substituted lipopolymers, **$DiC_{18}PMEGOx_n$** and **$DiC_{18}PTEGOx_n$**, do not display any alkyl chain condensation transition upon compression to the film collapse. It is reasonable to conclude that the higher steric need of the polymer side chains and the resulting larger cross-sectional area of the polymer part prevent the crystallization of the hydrophobic alkyl moiety. This is of great interest for the design of cell membrane constructs, because lipid crystallization impairs the performance of artificial biomembranes by affecting the lateral diffusion of lipid and membrane proteins[13] and by introducing local physical defects and thus compromising the barrier function of such supramolecular assemblies.

The varying impact of the side chain functionality on the steric needs of the lipopolymer and their compressibility is summarized in Figure 2, which shows the area-per-molecule-film pressure plots for several lipopolymers of comparable polymer chain length. Figure 2 reveals that the two most hydrophilic polymer moieties

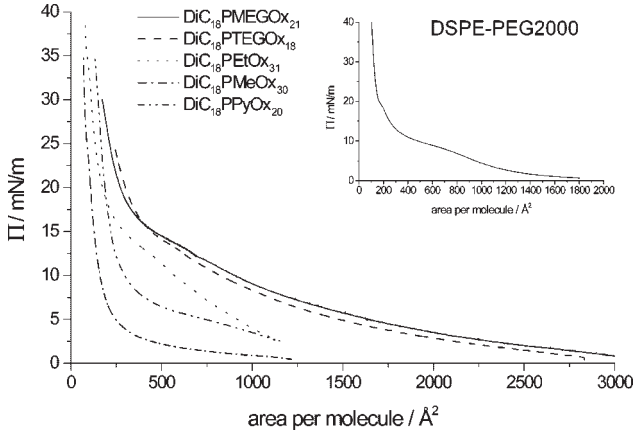


Figure 1. Π-area isotherms of **$DiC_{18}PPyOx_{20}$**, **$DiC_{18}PMeOx_{30}$**, **$DiC_{18}PEtOx_{31}$**, **$DiC_{18}PTEGOx_{18}$**, and **$DiC_{18}PMEGOx_{21}$** recorded at room temperature. The isotherm of **DSPE-PEG2000** ($n = 45$) is included for comparison (inset).

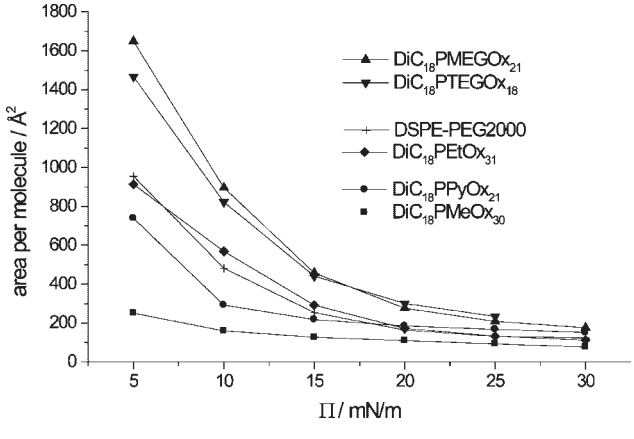


Figure 2. Area per molecule versus the given surface pressure plotted for the different lipopolymers presented in Figure 1.

of the lipopolymer amphiphiles, **$DiC_{18}PMeOx_{30}$** and **$DiC_{18}PPyOx_{21}$**, naturally display the smallest cross-sectional area. **$DiC_{18}PEtOx_{31}$** and **DSPE-PEG2000** behave very similar and not only occupy a similar interfacial area per molecule, but show an almost identical adsorption/desorption behavior at the air-water interface due to their slight amphiphilicity of their polymer chains. With respect to the degree of polymer amphiphilicity, DSPE-PEG2000 is followed by the mono- and tri-EG substituted lipopoly(2-oxazoline)s, **$DiC_{18}PTEGOx_{18}$**, and **$DiC_{18}PMEGOx_{21}$**, with even stronger interfacial attraction up to relatively high film pressures. A closer look at Figure 1 reveals the extended plateau of these lipopolymers at intermediate pressures. If compared at an elevated film pressure of 25 mN/m (above the low-pressure transition), all lipopolymers of comparable chain length fall in order depending on their steric need of the side functionalities: **$DiC_{18}PTEGOx_{18} > DiC_{18}PMEGOx_{21} > DiC_{18}PPyOx_{21} > DiC_{18}PEtOx_{31} > DiC_{18}PMeOx_{30}$**.

Effect of Polymer Chain Length

To illustrate the effect of the polymer chain length upon the compression behavior of the lipopolymer monolayers, three LB-isotherms of **$DiC_{18}PMEGOx_n$** with $n = 13$, 21, and 31 are displayed in Figure 3a, along with a row of **$DiC_{18}PTEGOx_n$** with comparable degrees of polymerization ($n = 8$, 18, and 25) in Figure 3b. For such short polymers, an increase in the degree of polymerization by 8 to 10 monomer units results in significantly higher Π-onset values. This simply means that the first effective intermolecular interaction of the amphiphiles at the interface, the polymer-polymer interaction, is a direct function of the polymer chain length. A closer look at the actual values reveals that the polymer chains do not form a solvated random coil conformation after submersion in the aqueous subphase, but remain partially adsorbed at the air-water interface (see previous section). Hence, polymer-polymer interactions begin to affect the Π-*A* isotherm at much higher Å^2/molecule values. As earlier mentioned, the polymer desorption from the air-water interface is responsible for the plateau, which becomes more prominent with increasing polymer chain length. However, at higher compressions, after desorption of the polymer part and compression of the submerged polymer from a coil/mushroom to a brush conformation, all the lipopolymers can be compressed to almost the same cross-sectional area mainly defined by the steric requirements of the terminal lipid moiety.

Whereas all the other o-EG-substituted lipopoly(2-oxazoline)s display a more or less pronounced intermediate plateau, **$DiC_{18}PTEGOx_8$** displays only a monotone increase in the Π-area isotherm (Figure 3b). One possible explanation is that the submerged amphiphilic polymer part is too short and too compact to adjust its backbone conformation to reach the air-water interface.

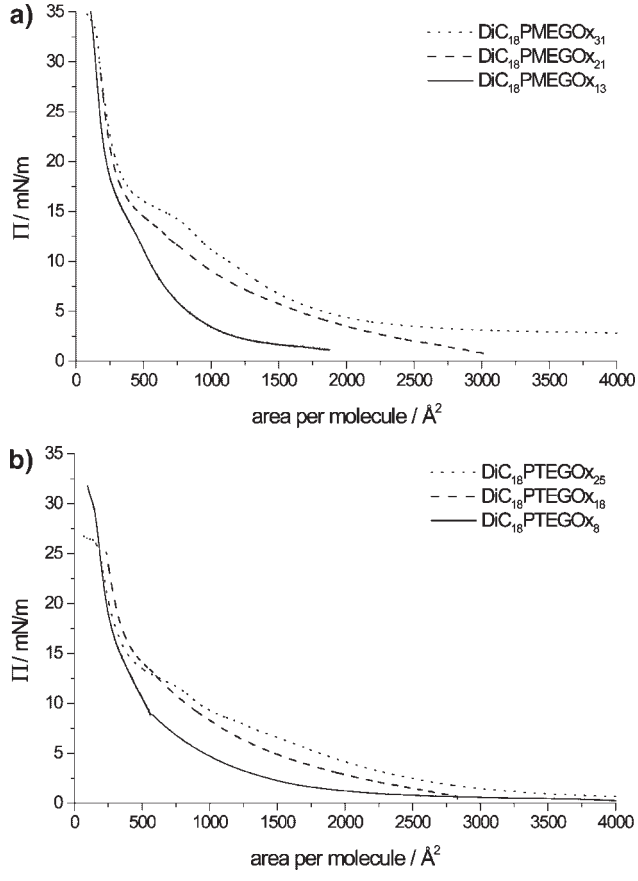


Figure 3. a) Π-area isotherms of the oligo-EG substituted lipopoly(2-oxazoline)s **$DiC_{18}PMEGOx_n$** with increasing degrees of polymerization ($n = 13$, 21, 31) and b) **$DiC_{18}PTEGOx_n$** for ($n = 8$, 18, 25). Note, the monotone isotherm of **$DiC_{18}PTEGOx_8$**

Surface Rheology

In addition to the LB-experiments, the synthesized lipopolymer monolayers were investigated using surface rheometry. This technique has previously been successfully applied to examine the formation of 2D gels among lipopolymers at the air-water interface.[26] As stated earlier, the similarity among lipopolymers studied prevented a clear understanding about the polymer-polymer interaction in the gel state, in particular, via hydrogen bonding. To explore the possible role of hydrogen bridges among the polymer moieties of lipopolymers for the 2D gel formation, this work sought to compare the viscoelastic properties of lipopolymers with different side chains. We hoped that by introducing of side chains within the hydrophilic polymer part, a comblike polymer structure or various hydrogen bonding sites or both could be created. If 2D gels of lipopolymers are stabilized via hydrogen bonds, the introduced side chains should have a notable impact on the 2D gel formation. In addition, the impact of the polymer chain length can be investigated to evaluate the additional aspect of the number of possible interaction sites as well as

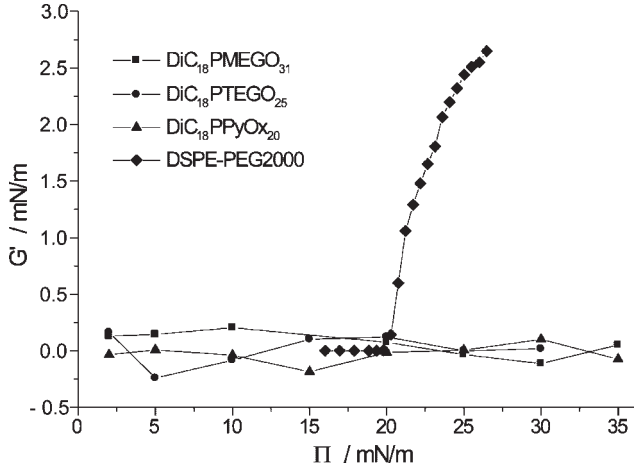


Figure 4. Plots of storage modulus (G') versus film pressure (Π) for **$DiC_{18}PMEGO_{31}$**, **$DiC_{18}PTEGO_{31}$**, **$DiC_{18}PPyOx_{20}$**, and **DSPE-PEG2000**. While the PEG-lipopolymer **DSPE-PEG2000** shows a pronounced gel transition at ca. 21mN/m, the other lipopolymers lack such a transition within the range of film pressures observed. (Data for **DSPE-PEG2000** are taken from our previous account.[23])

contributions due to entanglement. Thus, we investigated selected lipopolymers of the low and high degrees of polymerization: **$DiC_{18}PMEGO_n$** ($n = 13$, 31), **$DiC_{18}PTEGO_n$** ($n = 8$, 25) and **$DiC_{18}PPyOx_n$** ($n = 20$, 38).

Figure 4 shows representative plots of the storage modulus, G', versus film pressure for these lipopolymers (only higher molecular weight systems shown) together with corresponding data from **DSPE-PEG2000**. While **DSPE-PEG2000** (as well as **$DiC_{18}EtOx_n$** and **$DiC_{18}MeOx_n$**) shows a 2D gel transition,[23a] the side group-modified lipopolymers, **$DiC_{18}PMEGOx_n$**, **$DiC_{18}PTEGOx_n$**, and **$DiC_{18}PPyOx_n$**, completely lack such a transition, because G' is totally unaffected by the compression state of the monolayer.

This leads to the important result that the 2D gel formation of lipopolymers are not stabilized by attractive hydrogen bonds between polymer moieties of these amphiphiles. In addition, this study verifies our previous suggestion that alkyl chain condensation is a necessary precursor for 2D gel formation (Π-area isotherms of **$DiC_{18}PMEGOx_n$**, **$DiC_{18}PTEGOx_n$**, and **$DiC_{18}PPyOx_n$** show no plateau region at elevated film pressures) and that the 2D gelation is the result of jammed surface micelles.[23]

Conclusion

The synthesis of novel hydrophilic 2-oxazoline monomers with different 2-substituents is presented. The functionalities were chosen by their hydrophilicity, biocompatibility, and degree of hydrogen bonding. All monomers could be converted into the corresponding lipopolymers by living cationic polymerization using a lipid triflate initiator (**$DiC_{18}Tf$**). An interesting aspect is the preparation of **PyOx**, an *N*-(vinyl)pyrrolidone analog monomer, and its polymerization by living cationic polymerization in a defined fashion. All the lipopolymers were found to form stable monolayers at the air-water interface. Furthermore, it was established that the interfacial properties of the o-EG polymers are a combination of the poly(2-oxazoline) and PEG lipopolymers. In particular, the observed (reversible) adsorption/desorption behavior of the 'hydrophilic' polymer moieties at lower pressures could be attributed to the slightly amphiphilic behavior of the monomer units. In contrast, the cross-sectional areas at high pressures reflect the steric needs of the polymer side functionalities in the extended polymer chain. Our study showed that bulky functionalities prevent the alkyl chain condensation of the lipid moieties and that the 2D gels are not stabilized by hydrogen bonds between polymer chains of neighboring lipopolymers.

It is important to note that the above experimental data have indicated that some of the presented lipopolymers are potential candidates for the construction of tailored model cell membranes (polymer-tethered phospholipid bilayers), similarly to PEG and poly(2-oxazoline) lipopolymers used before. Corresponding experiments on polymer-tethered membranes are currently ongoing in our laboratories.

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