Unexpected Interactions of an Alternating Poly(ether-ester) with Artificial and Biological Bilipidic Membranes

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Summary: The anionic polymerization of a spiro monomer containing both an ester-activated cyclopropane moiety and a 1,4,7,10,13-pentaoxacyclohexadecane-14,16-dione crown ether bislactone unexpectedly yielded a linear alternating poly(ether-ester) via the ring-opening polymerization of the crown ether cycle. Ion conductivity measurements using black lipid membranes as model systems showed that oligomers of this structure are able to permeabilize bilipidic membranes, with single-ion channel behaviors being observed. Biological assays on fibroblast cells indicated a significant cytotoxicity, probably related to the above permeabilization mechanism.

Keywords: Poly(ether-ester), bilipidic membrane, pore, ion transport, cytotoxicity.

Introduction

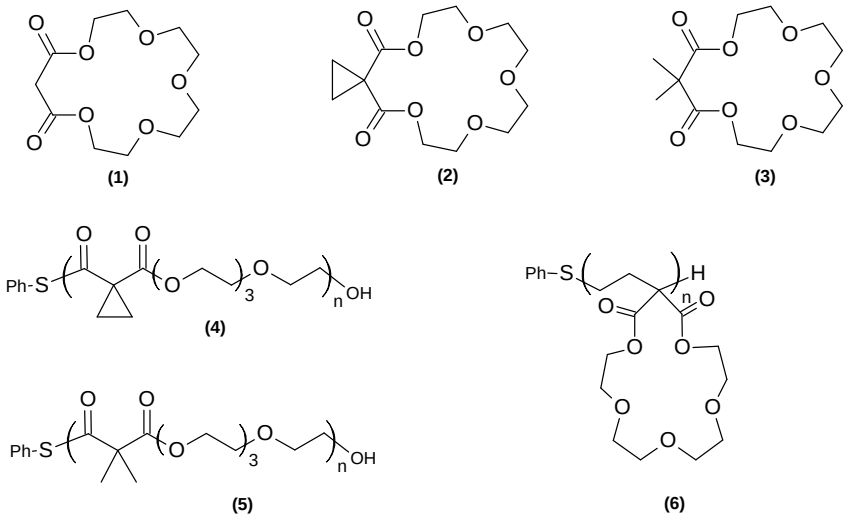
Ion transport across cell membranes, as executed in Nature by ion-channel proteins, is an essential biological mechanism, being involved in several critical life processes such as in energy metabolism or the excitability of nerves and muscles. In addition to fundamental studies aimed at better understanding the molecular aspects of protein-based ion channels, major efforts have also been devoted to design synthetic permeabilizers capable of generating pores of defined sizes within natural or artificial lipidic bilayer membranes.[1-2] Synthetic ion channels and pores designed thus far are generally based on molecular assemblies (*e.g.,* via the stacking of cyclic peptides[3] or using rigid-rod barrels molecules[4]) and on unimolecular architectures, including non-peptidic

macrocycles, that exhibit ion-complexing ligands in their structure like crown-ethers.[5-6]

Other polymer-based systems are also able to form pores within lipid bilayer membranes.[7] In particular, some amphilic polymers have been reported to cause lipid membrane disruption[8-9] and cell lysis.[9-11] Well-defined pore formation induced by polymers has also been observed in a few specific cases (*e.g.,* amphipols[12]), using conductivity measurements[12-13] as well as ion transport activity measurements in artificial bilayers.[14]

In that context, the original objective of the presently described project targeted a highly substituted carbon-chain polymer **6**, whose synthesis could theoretically be achieved via the living anionic ring-opening polymerization of **2**, a cyclopropane-1,1-dicarboxylate monomer bearing a crown ether pendant group as a malonate-containing spiro subunit. Previous studies had already demonstrated the excellent polymerizability of this family of strained and ester-activated cyclopropyl rings.[15-17] It was expected that a polymer such as **6**, with lateral substituents located on every third carbon, could be obtained by this technique, leading to an optimum inter-substituent distances between next neighbors of about 0.585 nm as suggested by structural results described elsewhere.[18]

Initial results reported here indicate that the polymerization of this spiro monomer proceeds via the previously unreported ring-opening polymerization of the crown-ether bislactone (leading to **4**) rather than via the normal cyclopropane ring-opening polymerization (leading to **6**). Interaction of the obtained oligomers with artificial and biological bilipidic membranes displayed unexpected activities that will be discussed in this communication.



Scheme 1: Structure of the low molecular weight molecules **(1-3)** and oligomers **(4-6)** described in this report.

Experimental section

Materials. Malonyl dichloride (99%, Fluka), tetraethylene glycol (99,5%, Acros), anhydrous dimethyl sulfoxide (≥ 99.9%, Aldrich), 1,2-dibromoethane (99%, Aldrich), potassium carbonate (≥ 99.9%, Aldrich), methyl iodide (99%, Acros) and thiophenol (≥ 99%, Aldrich) were used as received. Sodium thiophenolate (PhSNa) was synthesized as previously described.[15] 1,4,7,10,13-Pentaoxacyclohexadecane-14,16-dione **(1)** was synthesized according to a procedure reported in the literature.[19]

Techniques. ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ or CD_3COCD_3 using a Bruker 400 MHz NMR spectrometer. Size Exclusion Chromatography (SEC) experiments were performed in chloroform (1mL.min^{-1}) at room temperature, using a Spectra Physics P100 pump and two PLgel Polymer Laboratories columns (5 μm Mixed-C). A Wyatt Technology Optilab Rex interferometric refractometer was used as detector. Calibration was obtained with polystyrene standards. IR spectra were recorded on a Bruker Tensor 27 instrument. Elemental analysis was performed by the microanalysis department of I.C.S.N. (Gif sur Yvette, France).

Monomer synthesis.

5,8,11,14,17-Pentaoxaspiro[2,15]octadecane-4,18-dione (2). A mixture of 1,4,7,10,13-pentaoxacyclohexadecane-14,16-dione **(1)** (1.00 g, 3.8 mmol), 1,2-dibromoethane (1.504 g, 8.0 mmol), anhydrous potassium carbonate (3.162 g,

22.9 mmol) and DMSO (4 mL) was stirred vigorously for 3 days at room temperature. 15 mL of water were added to the resulting mixture, and the aqueous phase was extracted with five 10 mL ether fractions. The combined ether extracts were dried overnight over sodium sulphate and the ether was evaporated. **2** was recovered after drying during 3 days under secondary vacuum (55-60% yield).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 1.44 (4H, s, CH_2 cyclopropane), 3.68 (8H, m, O-C**H**$_2$-C**H**$_2$-O), 3.81 (4H, t, COO-CH_2-C**H**$_2$-), 4.31 (4H, t, COO-C**H**$_2$-). ^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 17.01 (CH_2, cyclopropane), 28.28 (C, cyclopropane), 65.06 (COO**C**H_2-), 68.92 (COOCH_2-**C**H_2-), 70.29, 70.89 (O-CH_2-CH_2-O), 170.21 (COO). IR (ATR): 2892, 1712, 1312, 1203, 1130, 938 cm^{-1}, mp 45.5°C ; Anal. Calcd. for $C_{13}H_{20}O_7$ (M_r = 288.29): C, 54.16; H, 6.99; O, 38.85. Found: C, 54.40; H, 7.12; O, 38.67.

15,15-Dimethyl-1,4,7,10,13-pentaoxacyclohexadecane-14,16-dione (3). A mixture of 1,4,7,10,13-pentaoxacyclohexadecane-14,16-dione **(1)** (1.00 g, 3.8 mmol), methyl iodide (5.39 g, 38 mmol), anhydrous potassium carbonate (3.162 g, 22.9mmol) and DMSO (4 mL) was stirred vigorously for 5 days at room temperature. 15 mL of water were added to the resulting mixture, and the aqueous phase was extracted with five 10 mL ether fractions. The combined ether extracts were dried overnight over sodium sulphate and the ether was evaporated (25% yield). **3** is recovered as a pale yellow viscous liquid.

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 1.44 (CH_3, s), 3.66 (8H, m, O-C**H**$_2$-C**H**$_2$-O), 3.77 (4H, t, COO-CH_2-C**H**$_2$-), 4.28 (4H, t, COO-C**H**$_2$-).^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 22.89 (CH_3), 49.72 (C), 64.52 (COO**C**H_2-), 68.73 (COOCH_2-**C**H_2-), 70.29, 70.36, 71.05 (O-CH_2-CH_2-O), 172.81 (COO).

Polymers 4 synthesis. Monomer **2** (1.00 g, 3.5 mmol) was introduced under argon and in a glove-box into a polymerization tube fitted with a Rotaflo®. A solution of sodium thiophenolate (9.17 mg, 0.069 mmol) in DMSO (0.25 mL) was added at room temperature. The polymerization was then stirred at a given temperature until the medium became viscous. The reaction was quenched with a 12 mol.L^{-1} HCl solution. Polymers were purified either by precipitation in cold methanol or by dialysis (membrane cut-off of 1000 Da). **4** is recovered as a viscous liquid after drying overnight at 60°C under vacuum.

(4) ^{1}H NMR (CD_3COCD_3, 400 MHz): δ (ppm) 1.28 (4H, s, cylopropane), 3.48 (8H, m, O-C**H**$_2$-C**H**$_2$-O), 3.58 (4H, t, COO-CH$_2$-C**H**$_2$-), 4.13 (4H, t, COO-C**H**$_2$-). ^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 16.89 (CH_2, cyclopropane), 28.07 (C, cyclopropane), 64.37 (COO**C**H$_2$-), 68.91 (COOCH$_2$-**C**H$_2$-), 70.58, 70.63 (O-CH$_2$-CH$_2$-O), 169.49 (COO). IR (ATR): 2871, 1720, 1316, 1204, 1103, 856 cm^{-1}.

Polymer **5** was obtained by the same procedure, using **3** as the monomer. Purification was performed by dialysis (membrane cut-off of 1000 Da).

(5) ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 1.43 (4H, s, cylopropane), 3.48 (8H, m, O-C**H**$_2$-C**H**$_2$-O), 3.55 (4H, t, COO-CH$_2$-C**H**$_2$-), 4.26 (4H, t, COO-C**H**$_2$-). ^{13}C NMR ($CDCl_3$, 400 MHz): δ (ppm) 22.69 (CH_3), 49.84 (C, malonyl), 64.26 (COO**C**H$_2$-), 68.77 (COOCH$_2$-**C**H$_2$-), 70,52 (O-CH$_2$-CH$_2$-O), 172.52 (COO).

BLM conductance measurements. The bilayer was formed according to standard techniques, in a hole-perforated vessel (Warner Instruments, Hamden, CT) and using a home-made experimental set-up as previously described.[20] The pinhole size of about 150 µm was coated by carefully painting a micro-droplet of 1,2-diphytanoyl-glycero-3-phosphocholine decane solution on its external side. Decane evaporated over 20-30 min of drying at room temperature. The vessel was introduced into a two-chamber apparatus, and both chambers were filled with 1 mL of a 1 mol.L^{-1} KCl solution. A pipet was dipped into the lipid solution and used to produce an air bubble to brush the coated aperture. The bilayer formation was monitored by measuring the current between two Ag-AgCl electrodes, a strong current variation from the saturation value of the amplifier to about 0 pA indicating bilayer formation. From 10 to 50 µL of an oligomer stock solution (10 mg.mL^{-1} in a 1 mol.L^{-1} KCl solution) were added to both chambers. The original membrane was then ruptured, and a new one generated by the above procedure to allow for the oligomer insertion. The current flow through the bilayer membrane was measured with a BLM 120 amplifier. Data are filtered at 10 kHz by a five-pole output filter. The acquisition card is a 16 bit PCI-6014 model (National Instrument, U.S.A.). The sampling rate is adjusted to 50 kHz.

Crystal violet assays. Murine fibroblasts NIH-3T3 cells, cultivated in Dulbecco's Modified Medium (DMEM) supplemented by 10% of fetal calf serum (Invitrogen, Cergy Pontoise, France) were seeded in 24-multiwell plates (2.10^4 per well) and incubated in humidified atmosphere of 7% at 37℃ for 24 hours for

adhesion. Cells were then treated with the tested substance (**2** and **4** at respectively 0.087 µM / 0.87 µM and 0.1µM / 1µM), and further incubated at 37°C, 7% CO_2. After 72 hours, they were stained by crystal violet (0.02 % solution in 2% v/v ethanol) for 30 minutes at room temperature. Afterward, they were washed three times with Phosphate Buffered Saline (PBS), dried and the cristal violet was solubilised with 100 µL of 1% Sodium Dodecyl Sulfate (SDS) for 1 h at room temperature. Cell quantification was obtained by measuring the absorbance at 595 nm with an ELISA plate reader.

Results and discussion

Monomers **2** and **3** were synthesized by techniques adapted from the literature[15-17;19] and provide a low melting point solid and a liquid, respectively. ^{1}H and ^{13}C NMR spectra are in full agreement with the expected structures. Monomer **2** was also characterized by elemental analysis and IR spectroscopy. The results confirm the molecular structure. In particular a band at 1200 cm^{-1}, characteristic of the cyclopropane ring, can be observed in the IR spectrum.

As mentioned in the introduction, anionic polymerization of **2** using sodium thiophenolate as an initiator is expected to yield polymer **6**, via the opening of the cyclopropyl ring during both initiation and propagation steps. Polymerization according to procedures known to open the cycle cleanly and to provide “living” polymers led in this case to oligomers of moderate molecular weights (M_n = 3.0-19.0 x 10^3) and high polydispersity indices (M_w/M_n < 2.7) (Table 1). The obtained polymers were pale yellow viscous liquids, soluble in DMSO and chlorinated solvents, sparingly soluble in water, and insoluble in alkanes.

Table 1: Polymerization of **(2)**; V_{DMSO} = 0.25 mL, **(2)** 3.5 mmol, [**2**] / [PhSNa] = 50. Molecular weights M_n and polydispersity index M_w/M_n were determined by SEC in $CHCl_3$ (IR detector) and using polystyrene standards. The residual amount of monomer was determined by ^{1}H NMR.

Polymer	T (°C)	$\overline{M_n}$	$\overline{M_w}/\overline{M_n}$	$\%_{mass}$ monomer
POLY 1	115	11000	1.8	13
POLY 2	120	7000	2.7	<1
POLY 3	140	6000	1.5	20
POLY 4	140	3500	2.3	3
POLY 5*	80	11600	1.9	0
POLY 6*	80	18900	1.7	0

* Purified by dialysis

The observed signals in the ^{13}C-NMR spectrum reveal a structure incompatible with the ring-opening of the cyclopropane, and strongly suggest that the cyclopropyl rings are still part of the polymeric structure. In particular, a comparison with polymers obtained from dialkyl cyclopropane-1,1- dicarboxylates [15-17] indicates that the three carbons originally part of the three-membered ring should be significantly shifted to higher chemical shifts when incorporated in the main chain of a hypothetical structure **6**, with a δ_{CH2} around 26 ppm (instead of the observed 17 ppm) and δ_C around 56 ppm (instead of the observed 28 ppm). On the other hand, the observed resonances at 17 and 28 ppm are fully compatible with the presence of an unreacted cyclopropyl ring. In addition a band at 1200 cm^{-1}, characteristic of the cyclopropane ring, can still be observed in the IR spectrum. If we further consider the ^{1}H-NMR data, it can be concluded that both ^{1}H- and ^{13}C-spectra support the formation of the linear alternating poly(ether-ester) **4** whose structure is schematically depicted in Figure 1, along with assignments of the peaks observed in the ^{1}H- and ^{13}C-NMR spectra.

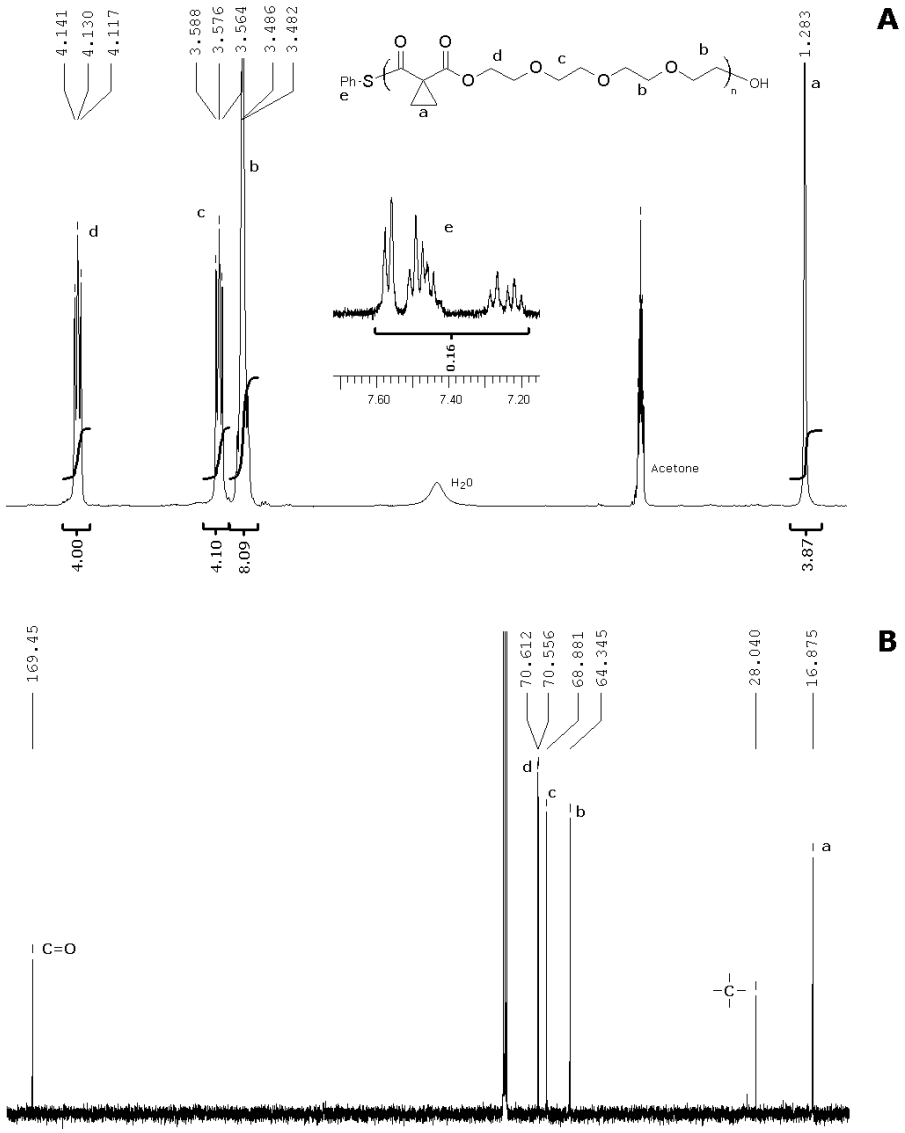
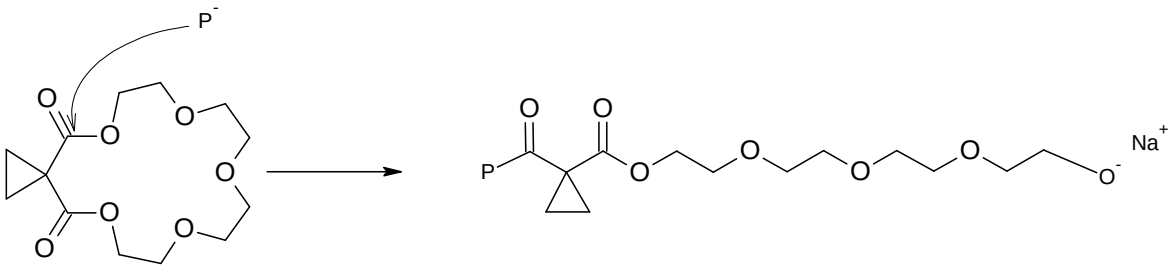


Figure 1: NMR data for oligomers **4: (A)** ^{1}H NMR spectrum in CD_3COCD_3 (POLY 5); **(B)** ^{13}C NMR spectrum in $CDCl_3$ (POLY 6).

This assignment implies that a competitive ring-opening involving the crown-ether bislactone is taking place. To the best of our knowledge, such a polymerization had never been observed before. To test the above assumption, a monomer **3**, similar to **2** but with the cyclopropyl ring replaced by two methyl groups, was synthesized and mixed with PhSNa under the same polymerization conditions as the ones previously described. A polymer was obtained (M_n = 6.0 x 10^3, M_w/M_n = 2), whose spectroscopic data are compatible with an alternating poly(ether-ester) **5**, confirming the viability of a ring-opening polymerization for the crown-ether macrocycle. It seems reasonable to assume that the site of attack during the nucleophilic attack of the propagating anion is one of the two carbonyls

of the malonate bislactone substructure included in the crown ether ring (Scheme 2).



Scheme 2: Proposed mechanism for the propagation step involved in the anionic ring-opening polymerization of dicarboxylate crown-ether bislactones, as illustrated with monomer **2**. P^- is the propagating chain.

Previous studies, based on hydrolysis kinetic experiments, have demonstrated that the carbonyl groups in crown ether bislactone cycles such as **1** are significantly activated by the complexation of the cycle with metal ions such as Na^+ and K^+.[21-22] This observation suggests that the competition between the two ring-openings might be shifted toward the unexpected bislactone ring-opening because of the complexation of the sodium counter-ions. As the ring-opening of the cyclopropyl unit should still be possible, polymerization of **2** can be expected to favor the formation of gels under appropriate conditions. Such an event was indeed observed in some polymerization experiments, but never for polymerizations of monomer **3** that lacks the crucial cyclopropyl unit.

BLM potassium ion conductance experiments were performed with samples of POLY 3 (M_n = 6.0 x 10^3, M_w/M_n = 1.5) (Table 1). The experiment provides data on the permeability induced by the molecules inserted in a planar bilipidic membrane by measurement of its conductance. It must be mentioned that POLY 3 samples were contaminated by small amounts of monomer **2** (Table 1), explaining why control experiments were carried out on the monomer.

In the absence of polymer, no current was detected. Similarly, no current was observed even after several hours when the membrane was built in the presence of monomer **2**. By applying a 100 mV potential, we measure a null average current with a 10 pA standard deviation due to the thermal noise with a 10 kHz bandwidth filter (Figure 2A).

No conduction signal could be observed when oligomer **4** was placed in the presence of an already built membrane, suggesting that spontaneous insertion in

this type of membrane is slow or impossible. Nevertheless, when the membrane was built in presence of oligomers, two general responses could be observed, a regular and discrete one corresponding to well-characterized channels that last for a few milliseconds to several seconds or even minutes before closing (Figure 2B-2D) and a flickering and irregular one (Figure 2E). The first type of activity was the most common mode observed, and shows generally high current levels (up to 300 pA). As shown in Figure 2C, several well-defined quantified jumps corresponding to single-channel openings and closures were also observed. The histogram of the current value measured at 100 mV is plotted in Figure 2F. The two peaks correspond to the quantified current jumps. By fitting each peak with a gaussian function, the unitary current jump, i_{unit}, was estimated to 3.5 pA. This value increases linearly with the applied voltage (-100 mV < ΔV < 100 mV) (Figure 2G). Assuming the validity of Ohm's law and the fact the channel is a homogeneously conducting cylindrical pore, the channel diameter was estimated to be 0.15 nm according to the following relationship $D = \sqrt{\frac{4\varepsilon_0 \varepsilon g S}{\pi K C}}$, where ε_0 is the vacuum permittivity (8,854.10^{-12} F.m^{-1}), ε is the dielectric constant of a biological membrane (~2), S is the nominal pinhole surface, K is the conductivity of a 1 M KCl solution (11,2 S.m^{-1}), C is the membrane capacitance and g is the channel conductance.

These results demonstrate the channel behavior induced by the alternating poly(ether-ester) POLY 3 is due to the formation of conducting pathways by pores rather than by carriers. Indeed, the smallest single-channel conductance from Figure 2G corresponds to a ion flow rate of 2.2 x 10^7 ion.s^{-1}, which is much faster than the ion transfer rate of any known carrier.

It has been shown that simple poly(ethylene glycol) (PEG) is not able to permeabilize bilipidic membranes [23-24], whereas amphiphilic polyether-based compounds such as Pluronics block-copolymers are able to induce membrane permeation, with evidences for well-defined pore formation having been observed by BLM experiments.[23;25] Random amphiphilic polymers based on hydrophobically-modified polyacrylic acids (amphipols) have also been reported to form distinct pores in bilipidic membranes.[12] The literature in this area also

suggests that usually only a small fraction of hydrophobic moieties (a few mol-%) is needed within the polymer to induce the efficient formation of a pore.[7]

By analogy, the permeation performances of oligomer **2** may be attributed to the hydrophobic nature of pendant cyclopropyl rings that induce amphiphily to the otherwise hydrophilic polymer. In the presently reported case, pore sizes ranged from 0.2 nm to 6.0 nm. The lowest value corresponds to a single-polymer molecule inserted into the membrane. Higher values could be attributed either to pores formed by supramolecular aggregates of several molecules according to the so-called “barrel-stove” mechanism or to the generation of transient pores by the “carpet” mechanism. In the last case, the pore is induced by a strong change in the membrane curvature.[7]

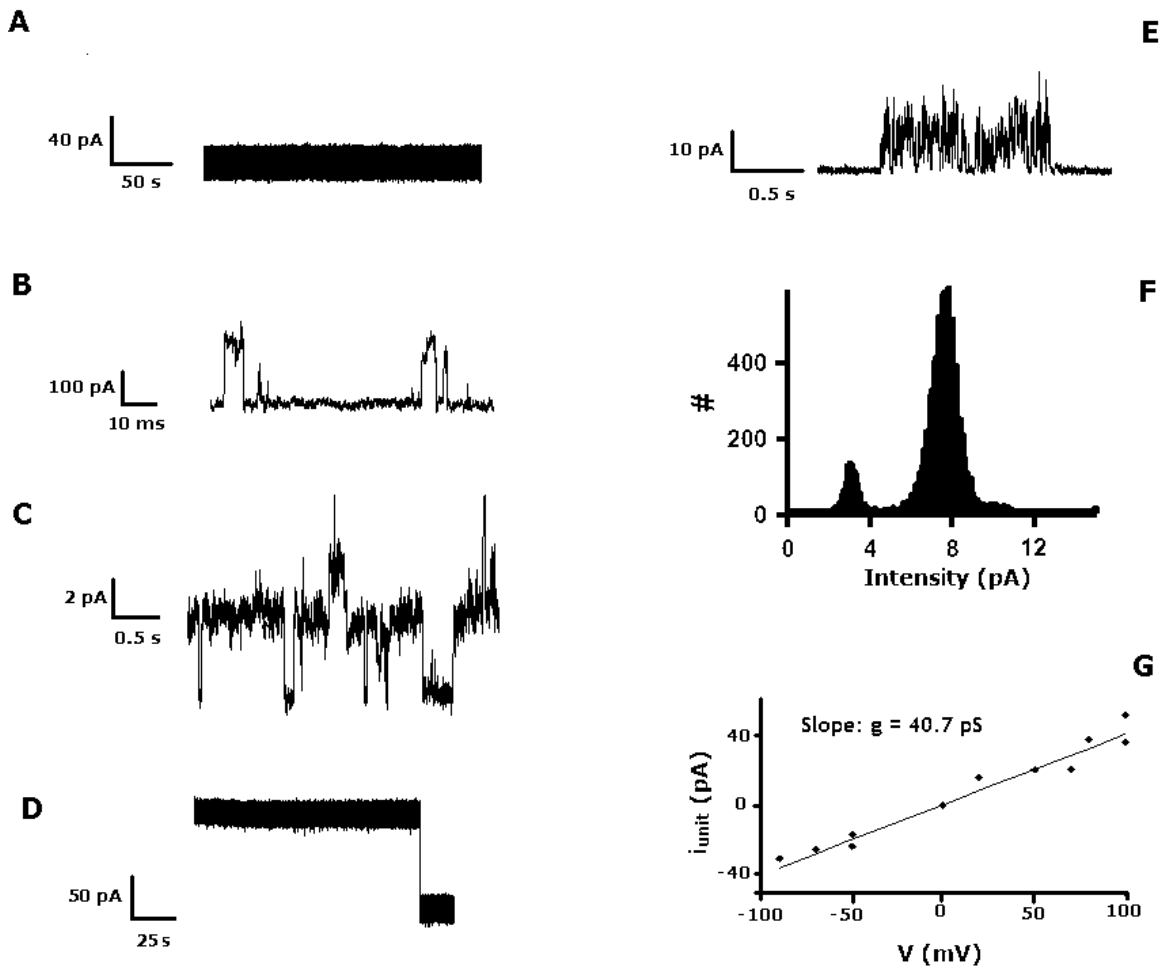


Figure 2. BLM conductance measurements (bilayer thickness: ~ 6 nm); (A-F) Current traces of a planar lipid bilayer with an applied voltage V=100mV. (A) Control experiment with monomer **(2)** (80 µM); (B-E) Oligomer 80 µM (POLY 3, M_n = 6.10^3 g/mol, M_w/M_n=1.5). (B-D) Regular and discrete current jumps; (E)

flickering and irregular current fluctuations; (F) Point amplitude histogram analysis of a trace of discrete channel steps ; (G) The unit current versus the applied voltage. The slope of the line corresponds to the channel conductance.

Cytotoxicity

Cristal violet staining assays were used to investigate the ability of the oligomers to modulate cell proliferation. In this assay, cells number after treatment was determined by cell staining with cristal violet and quantified by absorbance measurements. Control experiments were performed with the solvent (DMSO) needed to initially dissolve the oligomer and with monomer **2**.

As shown in Figure 3, oligomers induce a significant decrease of cell number as compared to a control performed with DMSO alone (polymer solvent). At 3 days post-seeding, 20% to 30 % inhibition of cell proliferation can be observed, that is slightly more significant at 1µmol.L^{-1} than at 0.1 µmol.L^{-1} concentration in polymer. Interestingly, no significant effect of monomer **2** could be observed, suggesting that the inhibition of cell proliferation is due indeed to the macromolecular compound.

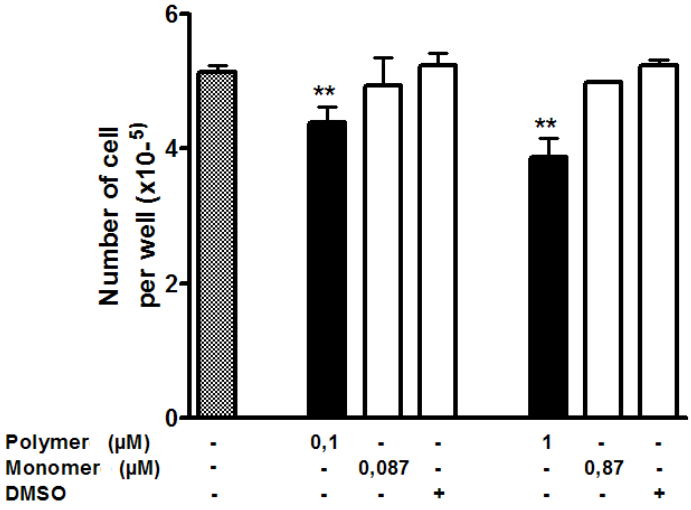


Figure 3: Crystal violet staining assay; Effect of **(4)** (POLY 1, M_n = 11.10^3 g/mol, M_w/M_n =1.8) on the proliferation of murine fibroblasts NIH-3T3 cells. Investigated concentrations: **(2)**, 0.087 µM and 0.87 µM; **(4)**, 0.1 µM and 1 µM. Control experiments with DMSO. ** < p=0,01, by unpaired t-test (probability test).

Conclusions

An alternating poly(ether-ester) copolymer containing small PEG-type units in the backbone was serendipitously obtained when attempting to polymerize a spiro monomer containing both an activated cyclopropane and a crown ether

bislactone. It was discovered that oligomers with this structure are capable of generating pores within bilipidic membranes and of partially inhibiting cell proliferation at rather low concentrations. This is the first time that an alternating hydrophobically modified polyether based compound is reported to induce bilipidic membrane permeation.

The preliminary results described in this contribution suggest that interactions of other oligomers of this type, such as **5**, should also be considered to fully assess the potentialities of this new class of poly(ether-ester)s as membrane permeabilizers. The occurrence of an alternative route to the "normal" cyclopropane ring-opening polymerization, when the attached esters can competitively react with the attacking nucleophilic propagating center, prevents the formation of polymers such as **6** as precursors to multichannel-like structures. The observed reactivity pattern implies that monomers substituted by crown ethers via an alkyl spacer should be considered as an alternative route.

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[1] S. Matile, A. Som, N. Sorde, *Tetrahedron* **2004**, *60*, 6405–6435.
[2] A. Sisson, M. R. Shah, S. Bhosale, S. Matile, *Chem. Soc. Rev*. **2006**, *35*, 1269-1286.
[3] J. D. Hartgerink, T. D. Clark, M. R. Ghadiri, *Chem. Eur.J*.**1998**, *4*, 1367-1372.
[4] (a) B. Baumeister, N. Sakai, S. Matile, *Angew. Chem.-Int. Edit.* **2000**, *39*, 1955-1958 (b) P. Talukdar, G. Bollot, J. Mareda, N. Sakai, S. Matile, *J. Am. Chem. Soc* **2005**, *127*, 6528-6529.
[5] (a) L. Jullien, J-M. Lehn, *Tetrahedron Lett.* **1988**, *29* (31), 3803-3806; (b) M. J. Pregel, L. Jullien, J. Canceill, L. Lacombe, J-M. Lehn, *J. Chem. Soc. Perkin Trans. II* **1995**, 417-426.
[6] (a) A. Nakano, Q. Xie, J. V. Mallen, L. Eschegoyen, G. W. Gokel, *J. Am. Chem. Soc*. **1990**, *112*, 1287-1289; (b) W. M. Leevy, J. E. Huettner, R. Pajewski, P. H. Schlesinger, G. W. Gokel, *J. Am. Chem. Soc*. **2004**, *126*, 15747-15753.
[7] W. H. Binder, *Angew. Chem.-Int. Edit*. **2008**, *47*, 3092-3095.
[8] F. Vial, S. Rabhi, C. Tribet, *Langmuir* **2005**, *21*, 853-862.
[9] (a) M. F. Ilker, H. Schule, E. B. Coughlin, *Macromolecules* **2004**, *37*, 694-700;(b) M. F. Ilker, K. Nüsslein, G. N. Tew, E. B. Coughlin, *J. Am. Chem. Soc*. **2004**, *126*, 15870-15875.
[10] N. Lavignac, M. Lazenby, P. Foka, B. Malgesini, I. Verpilio, P. Ferruti, D. Duncan, *Macromol. Biosci*. **2004**, *4*, 922–929.
[11] K. Kuroda, W. F. DeGrado, *J. Am. Chem. Soc*. **2005**, *127*, 4128-4129.
[12] F. Vial, A. G. Oukhaled, L. Auvray, C. Tribet, *Soft Matter* **2007**, *3*, 75-78.
[13] J. C. Chung, D. J. Gross, J. L. Thomas, D. A. Tirrell, L. R. Opsahk-Ong, *Macromolecules* **1996**, *29*, 4636-4641.
[14] M. F. M. Roks, R. M. Nolte, *Macromolecules.* **1992**, *25*, 5398-5407.

[15] J. Penelle, H. Herion, T. Xie, P. Gorissen, *Macromol. Chem. Phys.* **1998**, *199*, 1329-1336.
[16] J. Penelle, T. Xie, *Macromolecules* **2000**, *33*, 4667-4672.
[17] J. Penelle, T. Xie, *Macromolecules* **2001**, *34*, 5083-5089.
[18] P. Sikorski, E. D. T. Atkins, L. C. Kagumba, J. Penelle, *Macromolecules* **2002**, *35*, 6975-6984.
[19] J. S. Bradshaw, C. T. Bishop, S. F. Nielsen, R. E. Asay, D. K. R. Masihdas, E. D. Flanders, L. D. Hansen, R. M. Izatt, J. J. Christensen, *J. Chem. Soc. Perkin Trans. I* **1976**, 2505-2508.
[20] L. Bacri, A.Ben-Khaled, P. Guegan, L. Auvray, *Langmuir* **2005**, *21*, 5842-5846.
[21] D. S. Baker, V. Gold, *J. Chem. Soc. Perkin Trans. II* **1983**, 1129-1132.
[22] R. J. M. Hedderwick, F. Hibbert , V. Gold, *J. Chem. Soc. Perkin Trans. II* **1991**, 811-815.
[23] J. Gau-Racine, J. Lal, M. Zeghal, L. Auvray, *J. Phys. Chem. B* **2007**, *111*, 9900-9907.
[24] T. Demina, I. Grozdova, O. Krylova, A. Zhirnov, V. Istratov, H. Frey, H. Kautz, N. Melik-Nubarov, *Biochemistry* **2005**, *44*, 4042-4054.
[25] O. Krylova, P. Pohl, *Biochemistry* **2004**, *43*, 3696-3703.