# Articles

## Modular Norbornene Derivatives for the Preparation of Well-Defined Amphiphilic Polymers: Study of the Lipid Membrane Disruption Activities

**M. Firat Ilker, Hanna Schule, and E. Bryan Coughlin***

*Polymer Science and Engineering Department, University of Massachusetts Amherst, 120 Governors Drive, Amherst, Massachusetts 01003*

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ABSTRACT: Novel 2,3-disubstituted-7-alkylidene norborn-2-ene derivatives (**1**−**4**) were prepared through Diels−Alder cycloaddition of fulvene derivatives and appropriate dienophiles. The ring-opening metathesis polymerization of this type of modular monomer was studied using a molybdenum alkylidene catalyst (**7**) and three different ruthenium carbene catalysts (**8**−**10**). Through the choice of monomer and catalyst it was possible to obtain polymers with a range of molecular weights and narrow polydispersities (1.08−1.20). The resulting polymers were quantitatively transformed to water-soluble amphiphilic polymers through simple chemical modifications. By changing the monomer structure, it was possible to tune the balance of hydrophobicity and ionic nature of the final polymer. The interaction of these polymers with phospholipid membranes was studied using large unilamellar vesicles that entrap a fluorescent dye. To probe the effect of polymer molecular weight, ionic nature, and hydrophobic character on membrane disruption activity, polymer-induced dye leakage from the large unilamellar vesicles was measured.

### Introduction

One interesting aspect of amphiphilic macromolecules is their interactions with phospholipid membranes. Depending on the interaction between the membrane and the amphiphilic macromolecule, various deformations such as pore formation, tube formation, or complete disruption of phospholipid membranes have been reported.[1] Because phospholipid membranes are an important component of living organisms, amphiphilic polymers and oligomers have been studied for applications such as drug delivery,[2] gene delivery,[3] and antibacterial agents.[4] Amenable synthetic approaches for the preparation of amphiphilic polymers with well-controlled structures are expected to broaden the interface between macromolecular science and biological sciences. In addition, development of facile techniques for the preparation of these types of polymeric materials may allow them to be useful for practical and widespread applications.

Ring-opening metathesis polymerization (ROMP), employing well-defined homogeneous catalyst systems, is a versatile technique for preparing macromolecules. It provides broad flexibility over the choice of functional groups on the monomer unit and a high level of control over the macromolecular architecture.[5] The highly active molybdenum-based metathesis catalyst system introduced by Schrock and co-workers allows for controlled ROMP of various cyclic olefins in the absence of certain polar functionalities and in inert reaction media.[6] Grubbs and co-workers introduced ruthenium-based catalyst systems that are tolerant to various polar functional groups.[7] These type of catalysts exhibit not only very high activities but also excellent control over polymer architecture.[6−8] Very recently, a derivative of Grubbs' catalyst bearing labile 3-bromopyridine ligands has been reported.[9] This latest catalyst exhibits very high activities in addition to fast initiation rates that allow for the preparation of narrow polydispersity polymers. Therefore, with the availability of powerful metathesis catalysts and the suitable choice of monomer, amphiphilic polymers with controlled molecular weights and narrow molecular weight distributions can be prepared.

Functionalized norbornene derivatives have been shown to be excellent monomers for ROMP as they have been used in the preparation of a wide range of polymeric structures.[10] Because of the strained nature of the norbornene ring, these are active monomers for living ROMP, resulting in narrow polydispersity polymers in addition to tolerating the presence of large side groups. Using various norbornene derivatives, polymers bearing a variety of side groups have been prepared via ROMP. Examples include polynorbornene derivatives carrying oligopeptides,[11] oligonucleotides,[12] anticancer drugs,[13] saccharides,[14] dendrons,[15] and polymeric side groups.[16] In addition, functionalized norbornene derivatives are readily prepared via Diels−Alder cycloaddition of a diene, most generally furan or cyclopentadiene, to a dienophile possessing desired functional group.[10] This procedure affords a 2- or 2,3-functionalized norbornene derivative. In the current study, the task of preparing a monomer structure with dual functionality, in this

* To whom correspondence should be addressed: e-mail Coughlin@mail.pse.umass.edu.

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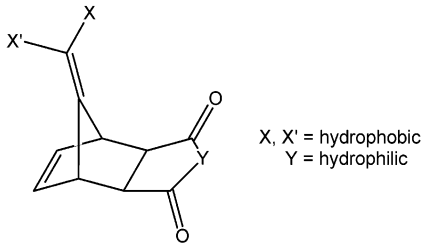



**Figure 1.** General structure of amphiphilic modular norbornene derivatives.

particular case a hydrophobic and a hydrophilic group, lead us to investigate the preparation and polymerization of modular norbornene derivatives with an additional functionality on the 7-position of the ring (Figure 1). Using this general strategy, two complementary functionalities can be introduced into the monomer structure, and the properties of the resulting amphiphilic polymer can thus be fine-tuned.

## Experimental Section

**Materials**. 2,6-Diisopropylphenylimidoneophylidenemolybdenum(VI) bis(hexafluoro-*tert*-butoxide) (**7**),[6] $RuCl_2$(=CHPh)-$(PCy_3)_2$ (**8**),[7b] and (tricyclohexylphosphine)(1,3-dimesitylimidazolidine-2-ylidine)benzylideneruthenium dichloride (**9**)[8b] were purchased from Strem Chemical. Stearoyloleoylphosphatidylcholine (SOPC) and phosphatidylserine (SOPS) were purchased from Avanti Polar-Lipids, Inc. Cyclopentadiene for the synthesis of fulvene derivatives was obtained by the thermally induced cracking of dicyclopentadiene followed by distillation. Fulvene derivatives,[17] compound **1**,[18] compound **6**,[3c] and [($H_2$-Imes)(3-Br-py)$_2$-$(Cl)_2$Ru=CHPh] (**10**)[9a] were prepared according to literature procedures. All other reagents were obtained from Aldrich. Deuterated chloroform, dichloromethane, and toluene were passed through columns of basic activated alumina prior to use.

**Instrumentation**. $^1$H (300 MHz) and $^{13}$C NMR (75 MHz) spectra were obtained on a Bruker DPX-300 NMR spectrometer. Gel permeation chromatography (GPC) was performed with a Polymer Lab LC1120 high-performance liquid chromatography (HPLC) pump equipped with a Waters differential refractometer detector. The mobile phase was tetrahydrofuran (THF) or dimethylformamide (DMF) with a flow rate of 1.0 and 0.5 mL/min, respectively. Separations were performed with $10^5$, $10^4$, and $10^3$ Å Polymer Lab columns. Molecular weights were calibrated vs narrow molecular weight polystyrene standards. Aqueous GPC setup consisted of Kratos Spectroflow 400 Pump, Shimadzu RID-6A RI detector, and TSK-GEL column set (2x GMPWXL, 1x G3000PWXL, and 1x G2000SW). Phosphate buffer (0.035 M, pH = 8.2, $I$ = 0.4) was used as an eluent at a flow rate of 1.0 mL/min. System was calibrated with narrow poly(ethylene oxide) standards. MALDI−TOF analysis was performed with a Bruker Daltonics Reflex III MALDI−TOF mass spectrometer. The MALDI matrix was dihydroxybenzoic acid (saturated solution in THF). Fluorescence spectroscopy was recorded with a Perkin-Elmer LS50B luminescence spectrometer.

**Preparation of 2.** A literature procedure for the cobalt-catalyzed maleic anhydride−maleimide transformation was adapted for the synthesis of monomer **2**.[19] Monoprotected diamine **6** (1.57 g, 9.8 mmol) was added to **1** (1 g, 4.9 mmol) in DMAc (*N*,*N*-dimethylacetamide, 6 mL) at 60 °C and stirred for 20 min. A catalytic amount of cobalt acetate (0.1 mmol) dissolved in DMAc was added to this mixture followed by the addition of acetic anhydride (5 mmol), and the reaction mixture was stirred for 4 h at 80 °C. After cooling to room temperature the solution was diluted with ethyl acetate, washed with water and dilute HCl, dried, and evaporated under reduced pressure to afford 92% yield of an *exo*−*endo* (88:12) mixture of **2**. Recrystallization from cold diethyl ether afforded pure *exo* isomer **2** (56%). $^1$H NMR (300 MHz, $CDCl_3$, ppm): $\delta$ 6.42 (2H, t, $J$ = 2.0 Hz), 4.78 (1H, s), 3.72 (2H, t, $J$ = 1.9 Hz), 3.56 (2H, t, $J$ = 5.6 Hz), 3.20 (2H, q, $J$ = 5.3 Hz), 2.74 (2H, s), 1.53 (6H, s), 1.43 (9H, s). $^{13}$C NMR (75 MHz, $CDCl_3$, ppm): $\delta$ 177.8, 155.8, 140.8, 137.8, 115.5, 79.6, 48.0, 45.7, 39.5, 38.4, 28.4, 19.7. Elemental analysis for $C_{19}H_{26}N_2O_4$ (346.43 g/mol) calculated: C, 65.92; H, 7.51; N, 8.09. Found: C, 65.73; H, 7.48; N, 7.95.

**Preparation of 3.** The Diels−Alder reaction between isopropylfulvene[17] and maleic anhydride was performed in ethyl acetate at 90 °C for 12 h in a sealed pressure tube. Upon removal of ethyl acetate under reduced pressure, the adduct (**3**) was obtained in high yield as an oil with a 80:20 *exo*−*endo* ratio. $^1$H NMR (300 MHz, $CDCl_3$, ppm): $\delta$ 6.46 (2H, m), 4.82 (*exo*, 1H, d, $J$ = 9.7 Hz), 4.64 (*endo*, 1H, d, $J$ = 9.4 Hz), 3.94 (*endo*, 1H, s), 3.87 (*exo*, 1H, s), 3.60 (*endo*, 1H, s), 3.55 (*endo*, 2H, dd, $J$ = 3.0 Hz, 4.2 Hz), 3.51 (*exo*, 1H, s), 3.05 (*exo*, 2H, dd, $J$ = 8.1 Hz, 7.7 Hz), 2.30 (1H, m), 0.91 (6H, d, $J$ = 5.2 Hz). $^{13}$C NMR (75 MHz, $CDCl_3$, ppm): $\delta$ 171.4, 171.2, 171.1, 167.4, 149.5, 142.8, 137.7, 137.2, 135.9, 135.2, 121.5, 117.9, 49.0, 48.9, 48.5, 46.5, 46.3, 44.1, 28.2, 23.2, 22.9. HRMS (EI) calcd for $C_{13}H_{14}O_3$: 218.0943. Found: 218.0942.

**Preparation of 4.** The same procedure that was used for the preparation of **2** from **1** was used for the preparation of **4** from **3** to afford 90% yield of an *exo*−*endo* (85:15) mixture of the product **4**. Recrystallization in cold diethyl ether afforded pure *exo* isomer of **4** (40%). $^1$H NMR (300 MHz, $CDCl_3$, ppm): $\delta$ 6.42 (2H, m), 4.81 (1H, s), 4.68 (1H, d, $J$ = 9.4 Hz), 3.72 (1H, s), 3.55 (2H, t, $J$ = 5.6 Hz), 3.36 (1H, s), 3.29 (2H, broad), 2.76 (2H, dd, $J$ = 10.2 Hz, 7.5 Hz), 2.24 (1H, m), 1.42 (9H, s), 0.88 (3H, d, $J$ = 6.7 Hz), 0.79 (3H, d, $J$ = 6.7 Hz). $^{13}$C NMR (75 MHz, $CDCl_3$, ppm): $\delta$ 177.8, 156.0, 144.4, 138.0,137.3, 120.7, 79.5, 49.0, 47.9, 44.6, 39.2, 38.7, 28.5, 28.2, 23.6, 23.2. Repeated elemental analyses resulted in lower carbon content than what was calculated. Elemental analysis for $C_{20}H_{28}N_2O_4$ (360.43 g/mol) calculated: C, 66.64; H, 7.83; N, 7.77. Found: C, 65.96, 65.66; H, 7.92, 7.74; N, 7.70, 7.68. HRMS (EI) calcd for $C_{20}H_{28}N_2O_4$: 361.213. Found: 361.214.

**Preparation of 5.** The same procedure that was used for the preparation of **2** from **1** was used for the preparation of **5** from *exo*-7-oxanorbornene-2,3-dicarboxylic anhydride[20] to afford 86% yield of **5** as the pure *exo* isomer. $^1$H NMR (300 MHz, $CDCl_3$, ppm): $\delta$ 6.52 (2H, s), 5.27 (2H, s), 4.82 (1H, s), 3.64 (2H, t, $J$ = 5.6 Hz), 3.30 (2H, dt, $J$ = 10.9 Hz, 5.3 Hz), 2.86 (2H, s), 1.42 (9H, s). $^{13}$C NMR (75 MHz, $CDCl_3$, ppm): $\delta$ 176.5, 156.1, 136.6, 81.1, 79.5, 47.5, 38.9, 38.6, 28.5. Repeated elemental analyses resulted in lower carbon content than what was calculated. Elemental analysis for $C_{15}H_{20}N_2O_5$ (308.34 g/mol) calculated: C, 58.43; H, 6.54; N, 9,09. Found: C, 57.29, 57.23; H, 6.54, 6.52; N, 9.10, 9.10. HRMS (EI) calcd for $C_{15}H_{21}N_2O_5$: 309.145. Found: 309.260.

**Polymerization of 1.** The procedure for the polymerization of **1** is a representative procedure for all other monomers, and exceptions will be noted. Catalyst **10** was used for the polymerizations of **1**. A solution of catalyst was added to a dichloromethane (0.5 mL) solution of **1** (0.3 mmol, 61 mg) at room temperature, under an inert atmosphere. Catalyst to monomer molar ratios ranging from 1/10 (0.03 mmol) to 1/50 (0.006 mmol) were employed depending on the targeted molecular weight. The mixture was allowed to react for 0.5−1 h depending on the catalyst-to-monomer ratio during which precipitation of poly(**1**) was observed. Ethyl vinyl ether (0.2 mL) was added, and the precipitated solid was filtered and washed with pentane. The polymers were dried overnight under reduced pressure at room temperature. The isolated yields were between 88 and 90% (54−55 mg). Poly(**1**) was dissolved in DMF or deuterated DMSO for characterization. $^1$H NMR (300 MHz, d-DMSO, ppm): $\delta$ 5.60−5.10 (2H, br), 3.69 (2H, br), 3.46 (2H, br), 1.66 (6H, s).$^{13}$C NMR (75 MHz, d-DMSO, ppm): $\delta$ 173.9, 134.3 (br), 132.2 (br), 131.0 (br), 130.3 (br), 51.8 (br), 49.3, 48.9, 47.8, 21.1.

**Polymerization of 2.** Catalyst-to-monomer molar ratios ranging from 1/5 to 1/45 were employed. The polymerization was terminated by addition of ethyl vinyl ether (0.2 mL) followed by precipitation in 10 mL of pentane. The isolated yields were between 85 and 90%. $^1$H NMR (300 MHz, $CDCl_3$, ppm): $\delta$ 5.60−5.24 (2H, trans, br), 5.22−4.80 (2H, cis, br), 3.66

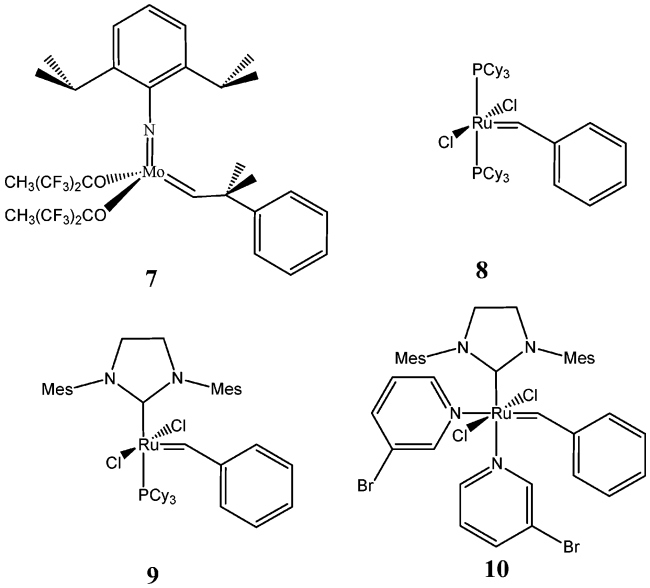



**Figure 2.** Catalysts **7**, **8**, **9**, and **10**.

(2H, s), 3.57 (2H, s), 3.26 (2H, s), 3.08 (2H, s), 1.67 (6H, s), 1.40 (9H, s). $^{13}$C NMR (75 MHz, $CDCl_3$, ppm): $\delta$ 178.9, 155.7, 135.1 (br), 131.2 (br), 130.0 (br), 79.1, 51.9, 50.9, 48.1, 43.7, 38.4 (br), 28.0, 21.1.

**Polymerization of 3.** Polymerizations of **3** were carried out using catalysts **9** and **10**. When catalyst **9** was used, polymerization solutions were heated to 40−50 °C. The isolated yields were between 90 and 94%. $^1$H NMR (300 MHz, d-DMSO, ppm): $\delta$ 5.60−5.00 (2H, br), 4.00−3.20 (5H, br), 2.65−2.30 (1H, br), 0.84 (6H, s). $^{13}$C NMR (75 MHz, d-DMSO, ppm): $\delta$ 173.2 (br), 140.34 (br), 137.8 (br), 134.1 (br), 132.8 (br), 52.0 (br), 51.1 (br), 50.2 (br), 49.2 (br), 46.8 (br), 27.9, 23.2.

**Polymerization of 4.** Polymerizations of monomer **4** were carried out using catalysts **7**, **8**, **9**, and **10** (Figure 2). Dichloromethane was vacuum-distilled from $CaH_2$ to be used for the polymerizations that employed catalyst **7**. Catalyst **8** was used in toluene solutions. Catalyst-to-monomer molar ratios ranging from 1/5 to 1/150 were employed. In the case of catalysts **7**−**9,** polymerization solutions were heated to 40−50 °C for 0.5−2 h depending on the catalyst-to-monomer ratio. Catalyst **10** was used at room temperature. Polymerizations were stopped by the addition of 0.2 mL of ethyl vinyl ether for catalysts **8**−**10** or 0.2 mL of benzaldehyde for catalyst **7**, followed by the precipitation of the polymer into pentane. The isolated yields were between 85 and 95%. $^1$H NMR (300 MHz, $CDCl_3$, ppm): $\delta$ 5.70−5.30 (2H, trans, broad d, $J$ = 51.6), 5.30−4.85 (2H, cis, br), 4.10−3.95 (broad s), 3.95−3.80 (broad s), 3.80−3.50 (broad s), 3.40−3.20 (broad s), 3.20−2.85 (broad s), 2.70−2.30 (1H, br), 1.37 (9H, s), 0.89 (6H, s). $^{13}$C NMR (75 MHz, $CDCl_3$, ppm): $\delta$ 179.2, 156.1, 139.8 (br), 136.6 (br), 132.5 (br), 79.5, 51.8 (br), 50.2, 46.9 (br), 38.9 (br), 28.5, 27.0, 26.4, 23.2.

**Polymerization of 5.** Polymerizations of **5** were carried out using catalysts **9** and **10** at room temperature. $^1$H NMR (300 MHz, $CDCl_3$, ppm): $\delta$ 6.05 (trans, s), 5.78 (cis, s) (2H, cis/trans = 44/56), 5.19 (1H, s), 5.02 (2H, cis, s), 4.51 (2H, trans, s), 3.59 (2H, s), 3.32 (4H, s), 1.39 (9H, s). $^{13}$C NMR (75 MHz, $CDCl_3$, ppm): $\delta$ 176.0, 156.3, 131.5 (br), 131.0 (br), 79.7, 53.5, 52.3, 39.2, 38.5, 28.5.

**Deprotection of Poly(2), Poly(4), and Poly(5).** Polymers bearing *t*-BOC protected primary amine groups resulting from the synthetic procedures described earlier were deprotected by dissolution in trifluoroacetic acid and stirring at 45 °C for 8 h. Polymers were recovered in quantitative yield either by evaporation of trifluoroacetic acid under reduced pressure or by precipitation of polymers into dichloromethane followed by filtration and drying overnight under reduced pressure at room temperature. Dep-poly(**2**), $^1$H NMR (300 MHz, $D_2O$, ppm): $\delta$ 5.90−5.10 (2H, br), 4.00−3.60 (4H, br), 3.50−3.00 (4H, br), 1.70 (6H, s). $^{13}$C NMR (75 MHz, $D_2O$, ppm): $\delta$ 181.3 (br), 163.7, 163.3, 162.8, 162.3, 134.9 (br), 131.5 (br), 130.6 (br), 122.6, 118.7, 114.9, 111.0, 52.9, 51.6 (br), 48.5 (br), 44.1, 37.8, 36.7, 21.1. Dep-Poly(**3**), $^1$H NMR (300 MHz, $D_2O$, ppm): $\delta$ 5.90−5.05 (2H, br), 3.81 (4H, br), 3.20 (4H, br), 2.44 (1H, br), 0.87 (6H, br). $^{13}$C NMR (75 MHz, $D_2O$, ppm): $\delta$ 180.8 (br), 163.5, 163.1, 162.6, 162.2, 139.5 (br), 136.0 (br), 132.2 (br), 122.8, 118.8, 114.9, 111.1, 51.6 (br), 50.1 (br), 46.5 (br), 37.8, 36.6, 29.0 (br), 22.6. Dep-Poly(**5**), $^1$H NMR (300 MHz, $D_2O$, ppm): $\delta$ 6.08 (trans, s), 5.88 (cis, s) (2H, cis/trans = 44/56), 4.98 (2H, cis, s), 4.63 (2H, trans, s), 3.80 (2H, s), 3.61 (2H, s), 3.20 (2H, s). $^{13}$C NMR (75 MHz, $D_2O$, ppm): $\delta$ 178.1, 163.9, 163.4, 162.9, 162.5, 132.2 (br), 122.8, 118.9, 115.1, 76.8, 53.3, 52.5,

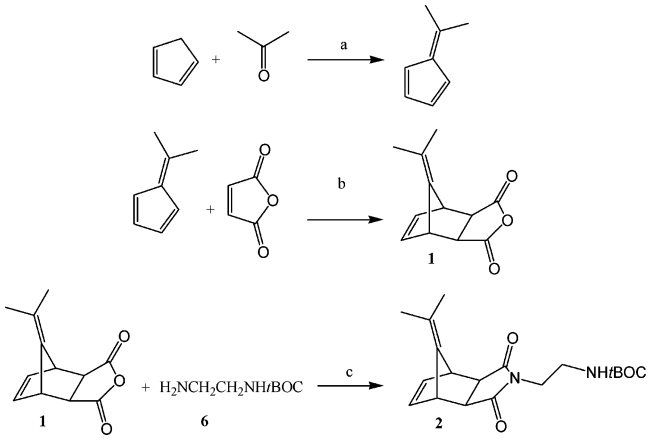
**Hydrolysis of Poly(1) and Poly(3).** Polymers bearing anhydride groups were hydrolyzed by dissolution in dilute NaOH solution and stirring for 3 h. The polymers were recovered either by lyophilization or by precipitation into DMF followed by centrifugation. The precipitated polymers were washed by THF and dried overnight under reduced pressure at room temperature. Dep-Poly(**1**), $^1$H NMR (300 MHz, $D_2O$, ppm): $\delta$ 5.41 (2H, broad s), 3.55 (2H, broad s), 2.82 (2H, broad s), 1.62 (2H, s). $^{13}$C NMR (75 MHz, $D_2O$, ppm): $\delta$ 182.1, 137.9 (br), 132.8 (br), 127.5 (br), 57.2, 56.4, 49.5 (br), 46.2, 21.4. Dep-Poly(**3**), $^1$H NMR (300 MHz, $D_2O$, ppm): $\delta$ 5.90−4.95 (2H, br), 3.90−3.20 (2H, br), 3.05−2.70 (2H, br), 2.65−2.25 (1H, br), 0.84 (6H, s). $^{13}$C NMR (75 MHz, $D_2O$, ppm): $\delta$ 174.0 (br), 173.5 (br), 140.4 (br), 137.7 (br), 134.3 (br), 132.8 (br), 52.1 (br), 51.2 (br), 50.2 (br), 49.3 (br), 46.8 (br), 28.0, 23.3.

**Determination of Polymer-Induced Leakage of Vesicle Contents.** The lipid vesicles were prepared with slight modifications of literature procedures.[4d,h,j] Chloroform solutions of SOPC (12.5 mg) and SOPS (1.5 mg) were mixed, and the chloroform was subsequently removed under a nitrogen stream followed by drying under reduced pressure for 3 h at room temperature to obtain the lipid mixture as a dry film. The dried film was hydrated by addition of 2 mL of $H_2O$ containing calcein (40 mM) and sodium phosphate (10 mM, pH 7.0). The suspension was vortexed for 10 min. The suspension was sonicated three times in a bath type sonicator (Aquasonic 150 HT) at room temperature and freeze−thawed after each sonication. The nontrapped calcein was removed by eluting through a size exclusion Sephadex G-25-150 column with 90 mM sodium chloride, 10 mM sodium phosphate buffer (pH 7) as eluent. Vesicle suspension was diluted 10-fold with the elution buffer prior to the polymer addition. The polymer-induced leakage was monitored by recording the increase of calcein fluorescence intensity at 515 nm (excitation at 490 nm, slit width 3.0). Phospholipid vesicles that were suspended in buffer solutions (pH 7) were stable, and no increase of fluorescence was observed before the addition of the polymer. Complete vesicle disruption was achieved by addition of 50 $\mu$L of 0.2% TRITON-X 100 (polyoxyethylene(10) isooctylphenyl ether), a strong surfactant, after 3 min from the addition of the polymer, into the 3 mL of vesicle suspension, and the corresponding fluorescence intensity was used as 100% leakage. The lysis caused by the polymer was reported as "% lysis", which is a fraction of the total lysis caused by TRITON-X.

## Results and Discussion

Synthetic amphiphilic polymers are generally prepared through block, random, or alternating copolymerizations of polar and nonpolar monomers. In the case of amphiphilic block copolymers, the amphiphilicity is at the macromolecular level, resulting in unique properties such as solvophobically driven micelle formation.[21] Random or alternating copolymers exhibit amphiphilic properties along their backbone where hydrophobic and hydrophilic groups are in close proximity.[22] However, for these copolymerizations, the choice of comonomers and the level of control over the molecular weights bring additional complications when compared to homopolymerizations. Homopolymerizations will provide broader flexibility over the hydrophobic and hydrophilic character if both attributes are present in the monomer. The

**Scheme 1. Representative Preparation of Modular Norbornene Derivatives: (a) Ref 17; (b) Ref 18; (c) $CoAc_2$, $Ac_2O$, DMAc, 80 °C, 4 h, 56% Yield**



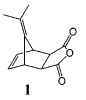
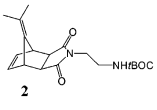
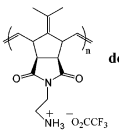
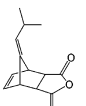
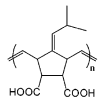
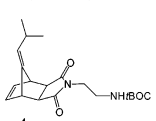
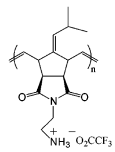

focus of the current study is the homopolymerization of monomers with amphiphilic character where the amphiphilicity of the resulting polymer is tuned at the repeating unit level, giving rise to a polymer backbone structure with regularly spaced hydrophilic and hydrophobic groups.

**Monomer Synthesis.** Fulvene derivatives were used as functionalized dienes for the Diels−Alder cycloaddition reaction with an appropriate dienophile to obtain the modular norbornene structures (Scheme 1). Several different fulvene derivatives were prepared through a simple synthetic methodology in high yields.[17] Hence, the hydrophobic character of the monomer and the resulting polymer can be tuned by the choice of fulvene as diene precursor for the monomer.

Maleic anhydride was used as the dienophile, allowing for further functionalization following the assembly of the norbornene skeleton. Cycloaddition of 6,6′-dimethylfulvene or 6-isopropylfulvene to maleic anhydride at elevated temperatures and moderate concentrations afforded quantitative yields of the corresponding norbornene derivatives. To achieve high level of control over polymerizations and resulting polymer microstructures, the preparation of pure isomers of the monomers was targeted. When maleic anhydride was used as the dienophile, *exo*−*endo* mixture of the cycloaddition adducts were obtained that were not always separable by selective recrystallizations. Although compounds **1**, **2**, and **4** were separated from their *endo* isomers through selective recrystallization to yield white crystalline solids, the *exo*−*endo* Diels−Alder adducts of 6-isopropylfulvene and maleic anhydride (**3**) could not be separated and remained as a brown oil. Cobalt-catalyzed transformation of the anhydride into a substituted imide linkage resulted in the protected amine-functionalized monomer structure in excellent yield. For both monomers **2** and **4** pure *exo* isomer was isolated by successive recrystallizations from cold ether, lowering the overall yield to between 40 and 56%.

**Polymerization Studies.** The initial target of the current study was to prepare amphiphilic polymers with well-defined architectures. Because the amphiphilic character was already dictated in the monomer unit, the target in the polymerization study of modular norbornene derivatives was to achieve controlled polymerization and obtain narrow polydispersities. Hence, the polymerization of monomer **4** was tested using four different metathesis catalysts, **7**−**10,** in order to screen

**Table 1. Examples of Modular Norbornene Derivatives and Amphiphilic Polymers Resulting from Corresponding Polymerizations[a] and Deprotections**

| Monomer | Deprotected Polymer | Theo. $M_n$[b] | Obs. $M_n$[c] | PDI |
|---|---|---|---|---|
|  **1** |  **dep-poly(1)** | 2,000 | 2,900[d] | 1.15 |
| | | 5,100 | 7,000[d] | 1.14 |
| | | 10,200 | 10,000[e] | 1.17 |
|  **2** |  **dep-poly(2)** | 2,400 | 1,950 | 1.13 |
| | | 5,900 | 7,000 | 1.08 |
| | | 19,800 | 17,900 | 1.11 |
| | | 29,900 | 24,100 | 1.13 |
|  **3** (*exo*-*endo*) |  **dep-poly(3)** | 10,900 | 9500 | 1.63 |
| | | 21,900 | 19,500 | 1.49 |
|  **4** |  **dep-poly(4)** | 1,900 | 1,800 | 1.20 |
| | | 8,800 | 8,600 | 1.10 |
| | | 31,100 | 27,000 | 1.13 |

[a] Polymers were prepared using catalyst **10**. [b] Theoretical molecular weights were calculated based on the catalyst-to-monomer ratio assuming full conversion. [c] Determined by THF GPC relative to polystyrene standards prior to the deprotection of polymer. [d] Determined by water GPC relative to poly(ethylene oxide) standards. [e] Determined by DMF GPC relative to polystyrene standards prior to the hydrolysis of polymer.

the polymerizability and the effect of catalyst on the resulting polydispersities. The polymerization of **4** using catalysts **7**−**9** required elevated temperatures between 40 and 55 °C, whereas catalyst **10** allowed the polymerization at room temperature. Desired molecular weights ranging between 1600 and 75 000 g/mol ($M_n$) were obtained by adjusting the catalyst-to-monomer ratio for all four types of catalysts. For a targeted number-average molecular weight of 8800 g/mol at complete conversion, the polymerization of **4** using catalysts **7**−**10** resulted in polydispersity values of 1.23, 1.27, 1.96, and 1.10, respectively. Following these results the polymerization of monomers **1**−**3** were studied using catalyst **10** (Table 1). Poly(**1**) precipitated from the polymerization solution. Despite the early precipitation during polymerization, 88−90% yield of poly(**1**) was isolated with polydispersity values ranging between 1.14 and 1.17 ($M_n$ ranging from 2900 to 10 000 g/mol). From the polymerization of monomer **2** using catalyst **10**, poly(**2**) was obtained in 85−90% yield with polydispersity values ranging between 1.08 and 1.13. When the pure *endo* isomer of **2** was prepared through the Diels−Alder reaction of a protected amine-functionalized maleimide derivative[19] and 6,6′-dimethylfulvene, it was observed that this monomer does not undergo ROMP using catalyst **9** or **10** even at elevated temperatures. In the case of monomer **3** the *exo*−*endo* mixture was polymerized in good yields into high molecular weight polymers using catalysts **9** and **10**; however, the resulting polydispersities were broader when compared to the other monomers. For all monomers the obtained molecular weights were in agreement with the targeted molecular weights as observed from GPC results. The slight discrepancy between the targeted and observed molecular weights of the polymers in Table 1 was expected due to the differences in hydrodynamic volume

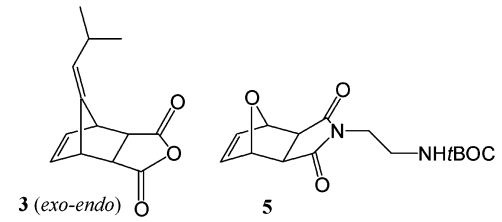



**Figure 3.** Compounds **3** and **5**.

of these polymers vs narrow polydispersity polystyrene and poly(ethylene oxide) GPC standards. $^1$H NMR end group analysis was performed to confirm the match between the targeted and observed number-average molecular weights for samples below 9000 g/mol ($M_n$). The relative integrations of the peaks from polymer's repeat unit vs the multiplet from styrenic end group at 7.32 ppm revealed good agreement with the targeted molecular weight for each type of polymer. This result was also confirmed by MALDI−TOF analysis of low molecular weights poly(**2**) and poly(**4**). MALDI−TOF analysis revealed that all polymer chains bear well-defined end groups, a benzylidene and a methylidene group, as a result of well-controlled initiation and termination.

The *t*-BOC protected pendant primary amine groups of poly(**2**) and poly(**4**), and the anhydride functionalities of poly(**1**) and poly(**3**) provide a nonionic and hydrophobic character to these polymers that allows for controlled ROMP and subsequent characterization of the polymers in a wide range of organic solvents. To obtain the final amphiphilic nature of the polymers, these groups were deprotected into their ionic forms, resulting in water-soluble polymers. Protected primary amine functionalities of different molecular weight samples of poly(**2**), poly(**4**), and poly(**5**) were deprotected quantitatively by dissolution in warm TFA to obtain dep-poly(**2**), dep-poly(**4**), and dep-poly(**5**) as observed by $^1$H NMR recorded in $D_2O$ solutions. $^1$H NMR spectra of these polymers also showed that carbon−carbon double bonds on the polymer backbone remain unaffected after treatment with TFA. Anhydride functionalities of poly(**1**) and poly(**3**) were hydrolyzed successfully by dissolution of polymers in NaOH solutions to obtain dep-poly(**1**) and dep-poly(**3**). After these processes narrow polydispersity well-defined amphiphilic polymers with a desired anionic or cationic character and hydrophobic character were obtained.

**Disruption of Phospholipid Membranes.** One important aspect of amphiphilic macromolecules is their ability to increase permeability through, or disrupt, lipid membranes.[1−4] To test the disruption activity of above-described amphiphilic polymers against negatively charged phospholipid membranes, a fluorescent dye (calcein) encapsulated within large unilamellar vesicles (LUV) were prepared from 1:9 (molar ratio) mixtures of phosphatidylserine (anionic) and phosphatidylcholine (zwitterionic). The phospholipid bilayer membrane of these vesicles provide a rough model for the negatively charged bacterial cell membranes in terms of the lipid content and surface charge.[1a,g,h,4d]

Dep-poly(**4**) of 13 500 g/mol number-average molecular weight ($M_n$) caused 100% lysis of phospholipid vesicles at concentrations as low as 5 $\mu$g/mL (Figure 4). Lysis was dose and molecular weight dependent (Figure 5). When a series of molecular weights of dep-poly(**4**) ranging between monomer and 64 000 g/mol ($M_n$) were studied, it was observed that the membrane disruption

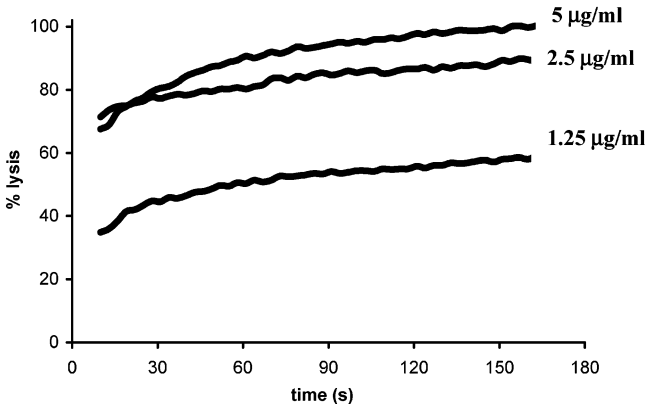



**Figure 4.** Lysis of anionic lipid vesicles in the presence of different concentrations of dep-poly(**4**) ($M_n$ = 13 500 g/mol). Lysis was obtained by measuring the increase of the fluorescence from the solutions after an average of 10 s from the addition of the polymer. The fluorescence before the addition was accepted as 0% lysis, and the fluorescence after the addition of TRITON-X was accepted as 100% lysis.

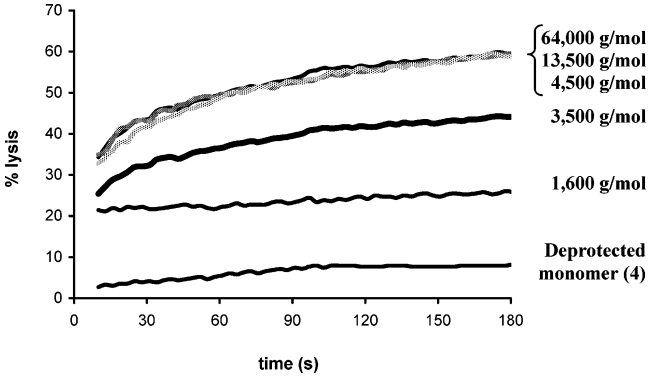



**Figure 5.** Lysis of anionic lipid vesicles caused by different number-average molecular weight ($M_n$) samples of dep-poly(**4**) at the concentration of 1.25 $\mu$g/mL. All polymers were prepared using catalyst **10**. Their polydispersity values are narrow changing between 1.17 and 1.30, except the polymer with molecular weight of 64 000 that was prepared using catalyst **9** and have a polydispersity value of 1.96. $M_n$ values were measured prior to the deprotection of polymers using GPC with THF as mobile phase.

activity was low for the monomer and short oligomers. Dep-poly(**4**) of molecular weights above 4500 g/mol up to 64 000 g/mol showed very high activities independent of molecular weight in this range. This result suggests that dep-poly(**4**) needs to reach a certain molecular weight to obtain maximum membrane disruption activity. The living nature of ROMP allows for the precise targeting of the desired molecular weight and hence allows for tuning the membrane activity of oligomers and polymers of **4**. Above a molecular weight of 4500 g/mol the activity does not change upon an increase in molecular weight. It should be noted that the concentration of the polymer that was added into vesicle suspension was calculated in terms of mass/volume. When the corresponding molar concentrations are calculated, the dep-poly(**4**) sample with a number-average molecular weight of 64 000 g/mol has 14 times fewer, but longer, chains than the dep-poly(**4**) sample of 4500 g/mol at the same mass/volume concentration.

To observe the effect of the ionic charge of lipid membranes on the activity of the dep-poly(**4**) neutral, zwitterionic phospholipid vesicles were prepared from only phosphatidylcholine. Although this particular polymer structure of various molecular weights was still

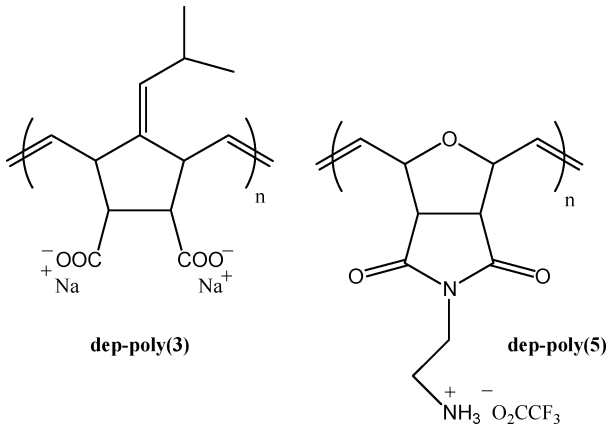



**Figure 6.** Anionic analogue dep-poly(**3**) (left, $M_n$ = 22 000 g/mol) and nonamphiphilic analogue dep-poly(**5**) (right, $M_n$ = 25 000 g/mol).

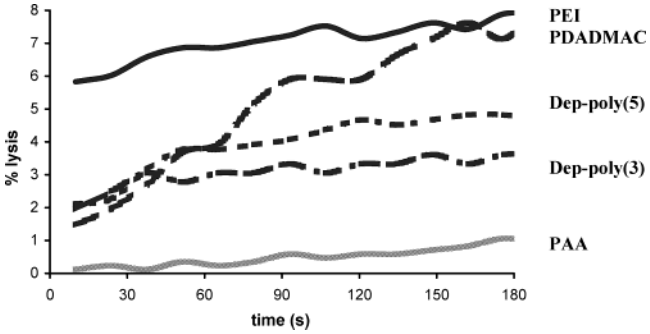



**Figure 7.** Lysis of anionic lipid vesicles caused by dep-poly(**3**), dep-poly(**5**), polyethylenimine (PEI, $M_n$ = 400 000 g/mol), poly(diallyldimethylammonium chloride) (PDADMAC, $M_n$ = 75 000), and poly(allylamine) (PAA, $M_n$ = 25 000). All samples are at a concentration of 15 $\mu$g/mL.

active against neutral vesicles, its activity was observed to be 50−100% less than what was observed for anionic vesicles at the same polymer concentration.

In a control experiment when the anionic dep-poly(**3**) ($M_n$ = 25 000 g/mol) or a cationic but nonamphiphilic dep-poly(**5**) ($M_n$ = 22 000 g/mol) was added to the phospholipid vesicle suspensions, no lysis was observed at comparable concentrations (Figure 6). Three commercially available cationic polymers, poly(ethylenimine) (PEI, $M_n$ = 400 000 g/mol), poly(dimethyldiallylammonium dichloride) (PDADMAC, $M_n$ = 75 000 g/mol), and poly(allylamine) (PAA, $M_n$ = 25 000 g/mol), were also tested as control experiments. These polymers were observed to be far less active in the lysis of the lipid vesicles when compared to dep-poly(**4**) (Figure 7). These results suggest that cationic amphiphilic polymer structures have the highest activity for disruption of phospholipid membranes among the polymers studied.

## Conclusions

Synthesis and ROMP of modular norbornene derivatives possessing dual character, hydrophilic and hydrophobic, have been developed and studied. This approach has been used for the preparation of novel amphiphilic polymers with a high level of structural control at the repeating unit level as well as control over polymer molecular weight and polydispersity. These amphiphilic polymers have been studied for their phospholipid membrane disruption activities. The level of control over the amphiphilic character on the repeating unit and molecular weight of polymers has been shown to play an important role in tuning the membrane disruption activities. The presence, and balance, of a hydrophobic group and a cationic group have been shown to be critical to achieve high activities. The membrane disruption activity of cationic amphiphilic polymers was found to reach a maximum at a critical molecular weight. These results suggested a cooperative action of these polymers in disrupting the phospholipid membranes. These interesting results from the initial studies on membrane disruption activities of the above-described amphiphilic polymers warrant further exploration. Applications for this type of polymeric materials, such as antibacterial activity, are currently under investigation.

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