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Combining Spontaneous Polymerization and Click Reactions for the Synthesis of Polymer Brushes: A "Grafting Onto" Approach

Andrea Cappelli,*[a] Giorgio Grisci,[a] Marco Paolino,[a] Federica Castriconi,[a] Germano Giuliani,[a] Alessandro Donati,[a] Stefania Lamponi,[a] Raniero Mendichi,[b] Antonella Caterina Boccia,[b] Filippo Samperi,[c] Salvatore Battiato,[c] Eugenio Paccagnini,[d] Mariangela Gentile,[d] Mariano Licciardi,[e] Gaetano Giammona,[e] and Salvatore Vomero[a]

Abstract: Two novel benzofulvene monomers bearing propargyl or allyl groups have been synthesized by means of readily accessible reactions, and were found to polymerize spontaneously by solvent removal, in the apparent absence of catalysts or initiators, to give the corresponding polybenzofulvene derivatives bearing clickable propargyl or allyl moieties. The clickable propargyl and allyl groups were exploited in appropriate click reactions to develop a powerful and versatile "grafting onto" synthetic methodology for obtaining tailored polymer brushes.

Keywords: click reactions · molecular recognition · photografting · polymers · spontaneous polymerization

Introduction

Molecular brushes are macromolecules formed by polymeric side chains densely grafted onto a linear polymeric backbone. They show peculiar features, which are regulated by the grafting density and by the properties of the components (backbone and side chains), such as the side-chain length. Moreover, the conformational behavior of brushlike macromolecules can be affected by the characteristics of the surrounding environment, such as solvent properties, temperature, pH, and ionic strength.[1] A brushlike architecture can be synthesized by means of three different approaches: the polymerization of macromonomers (grafting through, GT), the addition to a polymeric backbone of separately prepared side chains (grafting onto, GO), and the polymerization of side chains from a macroinitiator backbone (grafting from).[1,2]

According to the original definition by Sharpless and coworkers, click reactions are efficient and versatile chemical transformations capable of producing chemical substances rapidly, quantitatively, without byproducts and side reactions, under mild reaction conditions, and with broad tolerance to functional groups and applicability.[3] Among them, the most popular reactions are the Cu^{I}-catalyzed 1,3-dipolar cycloaddition between organic azides and alkynes (CuAAC)[4] and the addition of thiols to alkenes, which is commonly called thiol–ene coupling.[5,6]

The application of click reactions to macromolecular science has allowed the rapid synthesis of a large variety of polymers.[7–16] Indeed, the presence of clickable groups, along the backbone, in the side chains, or at chain termini, can be exploited for postpolymerization modifications (i.e., functionalization) leading to polymers bearing functionalities incompatible with the polymerization conditions.[7] Another interesting potential of the application of click reactions to macromolecular science is that a large number of different functionalized polymers can be prepared starting from a single clickable polymer.[7] A key step in the postpolymerization modification approach is represented by the insertion of the clickable moieties.

Self-initiating spontaneous polymerization has been demonstrated to occur in a number of different vinyl monomers. In our prototypical benzofulvene derivative **BF1**

[a] Prof. A. Cappelli, Dr. G. Grisci, Dr. M. Paolino, Dr. F. Castriconi, Dr. G. Giuliani, Prof. A. Donati, Dr. S. Lamponi, Prof. S. Vomero
Dipartimento Farmaco Chimico Tecnologico and
European Research Centre for Drug Discovery and Development
Università degli Studi di Siena
Via A. Moro, 53100 Siena (Italy)
Fax: (+39) 0577-234333
E-mail: andrea.cappelli@unisi.it

[b] Dr. R. Mendichi, Dr. A. C. Boccia
Istituto per lo Studio delle Macromolecole (CNR)
Via E. Bassini 15, 20133 Milano (Italy)

[c] Dr. F. Samperi, Dr. S. Battiato
Istituto di Chimica e Tecnologia dei Polimeri (CNR)
Viale A. Doria 6, 95125 Catania (Italy)

[d] Dr. E. Paccagnini, Dr. M. Gentile
Dipartimento di Biologia Evolutiva
Università degli Studi di Siena
Via A. Moro, 53100 Siena (Italy)

[e] Dr. M. Licciardi, Prof. G. Giammona
Dipartimento di Scienze e Tecnologie Molecolari e Biomolecolari (STEMBIO)
Università degli Studi di Palermo
Via Archirafi 32, 90123 Palermo (Italy)

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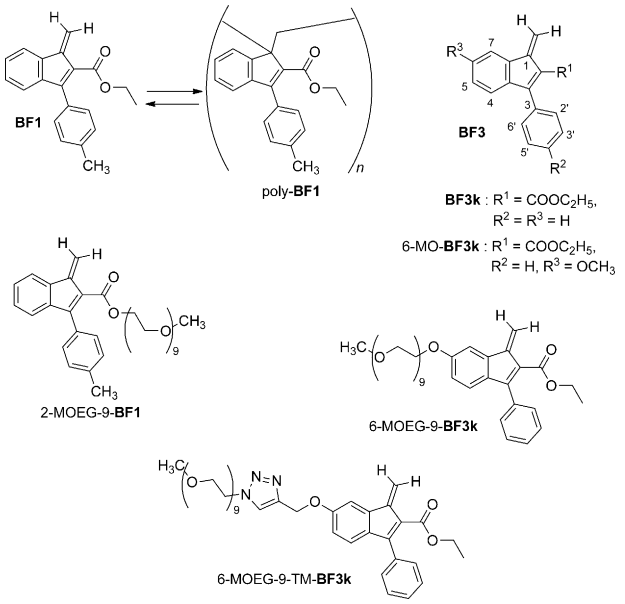


Scheme 1. Selected benzofulvene derivatives.

(Scheme 1), self-initiating spontaneous polymerization was shown to occur by solvent removal in the apparent absence of catalysts or initiators to give poly-**BF1**, a hydrophobic polymer showing vinyl structure stabilized by aromatic stacking interactions.[17,18] These results required the exploration of the reactivity of benzofulvene derivatives **BF3**[19] and the subsequent introduction of monomethyl oligo(ethylene glycol) (MOEG) side chains onto the polybenzofulvene backbone of poly-**BF1** and poly-**BF3k** (Scheme 1), which led to the development of a new family of polymer brushes including poly-2-MOEG-9-**BF1**,[20] poly-6-MOEG-9-**BF3k**,[21] and poly-6-MOEG-9-TM-**BF3k-GT** (TM = triazolylmethoxy).[22]

These macromolecules showed interesting properties and significantly different behaviors in the interaction with the water environment, which ranged from the formation of a compact physical hydrogel (poly-2-MOEG-9-**BF1**)[20] to the apparent water solubility of poly-6-MOEG-9-TM-**BF3k-GT**.[22] The results obtained suggest that MOEG-9 side chains and their location in the monomeric unit play an important role in determining polymer features, such as intermolecular interactions and aggregation properties. The latter component of the family (poly-6-MOEG-9-TM-**BF3k-GT**) was considered to be particularly attractive because it demonstrated that click can be used as an easy access to polybenzofulvene derivatives tailored for specific applications and, more generally, to the enlargement of the accessible chemical space around polybenzofulvene derivatives. However, the reaction conditions used in the methylation/dehydration procedure of the published GT approach may limit the variety of the functional groups present in the side chains.

To develop a powerful and versatile GO methodology for the synthesis of tailored polymer brushes, benzofulvene monomers bearing propargyl or allyl groups were designed, synthesized, and induced to polymerize spontaneously by solvent removal to give the corresponding polybenzofulvene derivatives bearing clickable propargyl or allyl moieties. These were exploited in the appropriate click reactions, such as the CuAAC reaction and thiol–ene coupling.

The work described herein demonstrates the viability of the GO approach to polybenzofulvene brushes. Moreover, several features of the spontaneous polymerization of the bifunctional benzofulvene monomers appear to fit the original definition of click reactions by Sharpless and co-workers.

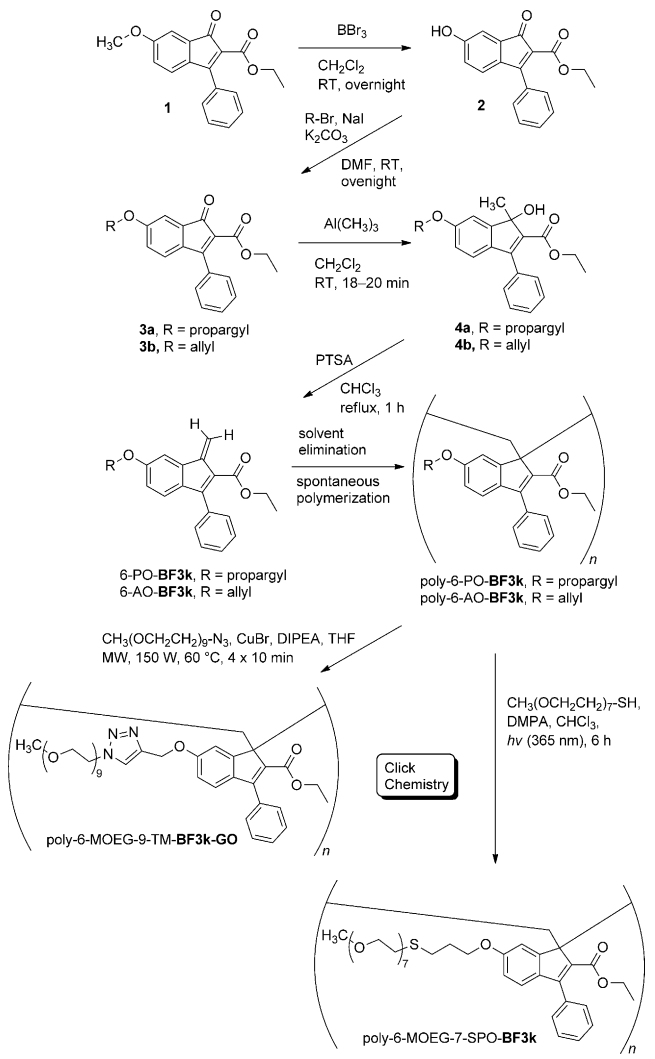
Results and Discussion

Synthesis: Benzofulvene monomers 6-PO-**BF3k** and 6-AO-**BF3k** were prepared starting from indenone **1**,[23] which was demethylated with BBr_3 and alkylated with propargyl bromide[22] or allyl bromide to obtain compounds **3a**[22] or **3b** (Scheme 2). These indenone derivatives were submitted to the usual methylation/dehydration procedure to afford benzofulvene monomers 6-PO-**BF3k** and 6-AO-**BF3k**, which polymerized, in the apparent absence of catalysts, upon solvent removal to give the corresponding polymers.

With the aim of developing a versatile synthetic methodology for the functionalization of the polybenzofulvene backbone with a wide variety of side chains, poly-6-PO-**BF3k** was reacted with a MOEG-9 chain bearing an azide group (MOEG-9-N_3)[22] as a model, in THF in the presence of CuBr and DIPEA, to afford poly-6-MOEG-9-TM-**BF3k-GO**, which could be compared with its analogous polymer brush prepared previously by means of a "grafting through" (GT) approach (poly-6-MOEG-9-TM-**BF3k-GT**).[22] The optimization of the conditions of the click CuAAC reaction required hard work because poly-6-PO-**BF3k** is liable to depolymerize when heated in the presence of solvents. After several attempts, we found that in the very simple conditions reported above, the CuAAC reaction proceeded even at room temperature and became virtually exhaustive by microwave irradiation without any significant depolymerization.

On the other hand, thiol–ene functionalization of poly-6-AO-**BF3k** with a MOEG-7 chain bearing a thiol group (MOEG-7-SH) was more difficult to optimize because the conditions used to generate the sulfenyl radical produced detectable amounts of the monomer. The best results were obtained by means of UV excitation in the presence of photoinitiator molecules, such as 2,2-dimethoxy-2-phenyl acetophenone (DMPA), but the thiol–ene photoreaction could not be considered exhaustive (see below).

Properties of the new polybenzofulvene derivatives: All the newly synthesized polybenzofulvene derivatives showed an excellent solubility in the most common organic solvents. In dichloromethane or chloroform the polymers dissolved rapidly, whereas the solubility in water can be considered as a distinctive feature between the couple of macromolecular precursors poly-6-PO-**BF3k**/poly-6-AO-**BF3k** and function-



Scheme 2. Synthesis and click functionalization of poly-6-PO-**BF3k** and poly-6-AO-**BF3k**. Reagents: i) BBr_3, CH_2Cl_2; ii) for compound **3a**: propargyl bromide, K_2CO_3, NaI, DMF; for compound **3b**: allyl bromide, K_2CO_3, NaI, DMF; iii) $Al(CH_3)_3$, CH_2Cl_2; iv) PTSA, $CHCl_3$; v) (spontaneous polymerization) solvent elimination; vi) $CH_3(OCH_2CH_2)_9N_3$, CuBr, DIPEA, THF; vii) $CH_3(OCH_2CH_2)_7SH$, DMPA, $CHCl_3$. PTSA = *p*-toluenesulfonic acid monohydrate, DIPEA = *N*,*N*-diisopropylethylamine, DMPA = 2,2-dimethoxy-2-phenylacetophenone.

alized polymers poly-6-MOEG-9-TM-**BF3k-GO**/poly-6-MOEG-7-SPO-**BF3k**. The presence of clickable groups in poly-6-PO-**BF3k** and poly-6-AO-**BF3k** is compatible with the easy dissolution into the organic solvents commonly used in click reactions, whereas the properties of the functionalized polymers poly-6-MOEG-9-TM-**BF3k-GO** and poly-6-MOEG-7-SPO-**BF3k** are governed by the side chains, as expected.

For example, poly-6-MOEG-9-TM-**BF3k-GO** is soluble in water and the dissolution was complete after a few hours at room temperature producing a transparent solution. Remarkably, this behavior is consistent with that observed with homopolymer poly-6-MOEG-9-TM-**BF3k** obtained by spontaneous polymerization of macromonomer 6-MOEG-9-TM-**BF3k** (poly-6-MOEG-9-TM-**BF3k-GT**).[22] On the other hand, poly-6-MOEG-7-SPO-**BF3k** interacts with water to produce a pale yellow hydrogel, which is broken rapidly by ultrasound to provide a milky dispersion. In static conditions, the dispersion generated an opalescent sediment easy to be newly suspended in water by gentle stirring. Thus, the behavior shown by poly-6-MOEG-7-SPO-**BF3k** in the interaction with the water environment appears to be similar to that shown by previously reported poly-6-MOEG-9-**BF3k**,[21] and this close analogy is easily explained in terms of structural similarity between the side chains of these polybenzofulvene brushes.

Characterization of molecular weight distribution: The molecular characterization of the new polybenzofulvene derivatives was performed by means of an absolute multiangle laser light scattering (MALS) detector online to a size-exclusion chromatography (SEC) apparatus. A MALS detector online to a SEC system furnishes for each polymeric eluting fraction the molecular weight (M) and the size of the macromolecules, denoted as radius of gyration R_g. Consequently, a SEC-MALS system furnishes the molecular weight distribution (MWD) and the radius of gyration distribution (RGD) of the polymer. Furthermore, if the MWD of the polymer is relatively broad the conformation plot, that is, the scaling law $R_g = f(M)$, is obtained from a single injection into the chromatographic system. The slope α of the conformation plot, obtained directly from the SEC-MALS system, shows the conformation of the macromolecules.

The different solubility properties of the polymers required the use of two different SEC mobile phases. In particular, pure THF was used for the couple of macromolecular precursors poly-6-PO-**BF3k**/poly-6-AO-**BF3k**. A solvent mixture of THF and DMF + 0.01 M LiBr (80:20) was employed in the case of functionalized polymers poly-6-MOEG-9-TM-**BF3k-GO** and poly-6-MOEG-7-SPO-**BF3k**. The most important results of SEC-MALS molecular and conformational characterization by means of these two mobile phases are summarized in Table 1. Basically, the data show that spontaneous polymerization of 6-PO-**BF3k** and 6-AO-**BF3k** afforded polymers characterized by MWD features very similar to those of parent polymer poly-**BF3k**. In particular, poly-6-PO-**BF3k** and poly-6-AO-**BF3k** show ultrahigh molecular weight values (M_w = 1794 and 1497 kg mol^{-1}, respectively) and relatively broad polydispersity indexes (PDI = M_w/M_n = 2.2 and 2.6, respectively), which appear to be significantly different from those shown by poly-6-MO-**BF3k** (M_w = 347 kg mol^{-1}; PDI = 4.3).[21]

When poly-6-PO-**BF3k** was reacted with MOEG-9-N_3 in THF in the presence of CuBr and DIPEA to afford poly-6-MOEG-9-TM-**BF3k-GO**, we observed both a decrease in the molecular weight (M_w = 821 kg mol^{-1}) and an increase in PDI (M_w/M_n = 8.9) without the production of significant monomer concentrations. These observations suggest that the polybenzofulvene backbone is susceptible to breaking under the conditions used in the CuAAC reaction. Probably,

Table 1. Macromolecular features of the new polybenzofulvene derivatives compared with those shown by previously reported poly-6-MOEG-9-TM-**BF3k-GT** (obtained by the GT approach), poly-6-MO-**BF3k**, and poly-**BF3k**.

Polymer	M_p [kg mol^{-1}][a]	M_w [kg mol^{-1}][b]	PDI[c]	R_g [nm][d]	K [nm][e]	α[e]
poly-6-PO-**BF3k**	2030	1794	2.2	51.5	0.0101	0.58
poly-6-AO-**BF3k**	1864	1497	2.6	47.7	0.0231	0.52
poly-6-MO-**BF3k**	312	347	4.3	19.4	0.0058	0.61
poly-**BF3k**	1900	1506	3.4	49.9	0.0066	0.60
poly-6-MOEG-9-TM-**BF3k-GO**	484	821	8.9	33.6	0.0249	0.50
poly-6-MOEG-9-TM-**BF3k-GT**	193	358	3.9	15.5		

[a] M_p: peak molecular weight. [b] M_w: weight-average molecular weight. [c] Polydispersity index PDI = M_w/M_n, in which M_n denotes the number-average molecular weight. [d] R_g: radius of gyration, that is, the dimension of the macromolecules. [e] K, α: intercept and slope of conformation plot $R_g = K M^{\alpha}$.

multiple ruptures occurred randomly along the backbone up to the production of macromolecules showing a molecular weight quite similar to that shown by poly-6-MOEG-9-TM-**BF3k-GT** obtained by spontaneous polymerization of the macromonomer.

Similarly, the results of MWD characterization of poly-6-MOEG-7-SPO-**BF3k** performed by SEC-MALS suggest that the polybenzofulvene backbone is also susceptible to multiple ruptures under the conditions used in the photografting reaction. In fact, when poly-6-AO-**BF3k** was reacted with MOEG-7-SH by UV excitation in the presence of DMPA to afford poly-6-MOEG-7-SPO-**BF3k**, we obtained a decrease in the molecular weight (M_w around 70 kg mol^{-1}) and in the PDI ($M_w/M_n = 1.7$; for details see the Supporting Information). The comparison of the degree of polymerization shown by macromolecular precursor poly-6-AO-**BF3k** (around 5000) with the corresponding value shown by poly-6-MOEG-7-SPO-**BF3k** (around 100) suggests that a considerable number of ruptures occurred during the photografting reaction. Moreover, the relatively low polydispersity of both poly-6-MOEG-7-SPO-**BF3k** samples (for details see the Supporting Information) is reminiscent of a living radical character of the backbone segmentation products.

Structure of the new polybenzofulvene derivatives

NMR spectroscopy: The ^{13}C NMR spectra of the couple of macromolecular precursors poly-6-PO-**BF3k** and poly-6-AO-**BF3k** were compared with that of parent macromolecule poly-6-MO-**BF3k** (Figure 1) to ascertain the correspondence between the structure of these polybenzofulvene derivatives and the presence of intact clickable groups in the new polymers poly-6-PO-**BF3k** and poly-6-AO-**BF3k**. The comparison of both the low-field portion containing C-1′ and C-3 signals and the upfield region containing C-1 and C-D (which may be affected by the competing 1,4-polymerization) confirms the retention of the spontaneous 1,2-polymerization mechanism. Moreover, the presence of the additional signals attributed to propargyl (poly-6-PO-**BF3k**) or allyl (poly-6-AO-**BF3k**) side chains demonstrates that 1,2-polymerization involves selectively the benzofulvene exocyclic double bond.

When poly-6-PO-**BF3k** was reacted with MOEG-9-N_3 in THF in the presence of CuBr and DIPEA to afford poly-6-MOEG-9-TM-**BF3k-GO**, we observed the complete disappearance of the signals attributed to the propargyl moiety from the ^{13}C NMR spectra of the latter polymer (Figure 2). This result suggests that the conditions used in the CuAAC reaction allowed exhaustive grafting to be obtained.

Moreover, the correspondence between the ^{13}C NMR spectrum of poly-6-MOEG-9-TM-**BF3k-GO** obtained by functionalization of poly-6-PO-**BF3k** (GO approach) and the one of the same polymer obtained by spontaneous polymerization of macromonomer 6-MOEG-9-TM-**BF3k-GT** (GT approach)[22] was considered to be the final evidence required to close the circle.

On the other hand, ^{13}C NMR analysis of poly-6-MOEG-7-SPO-**BF3k** revealed the presence of minor signals (marked

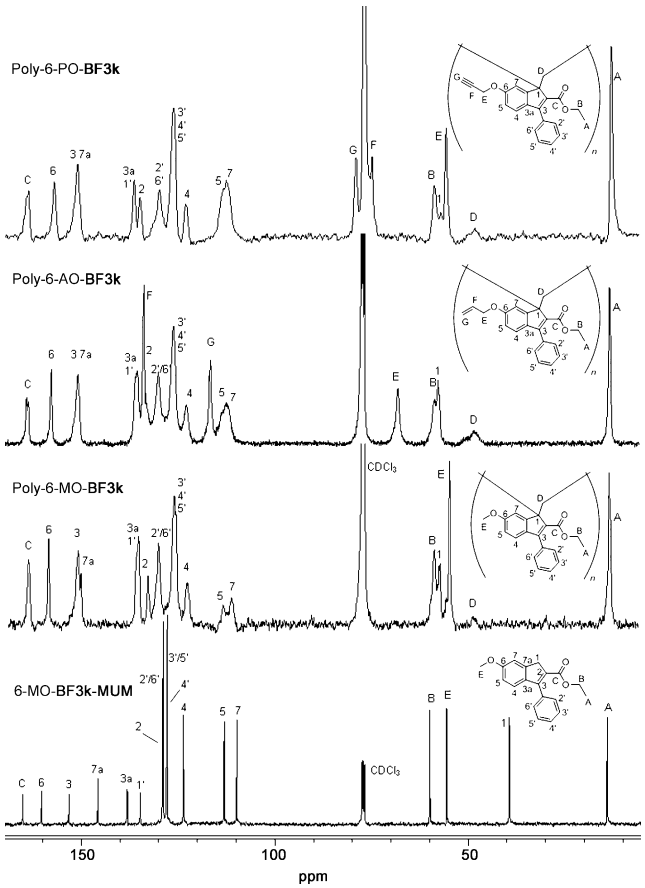


Figure 1. ^{13}C NMR spectra ($CDCl_3$) of newly synthesized poly-6-PO-**BF3k** and poly-6-AO-**BF3k** and, for comparison, those of previously reported poly-6-MO-**BF3k** and 6-MO-**BF3k**-MUM (monomeric unit model) representing the model of its monomeric unit.[21,22]

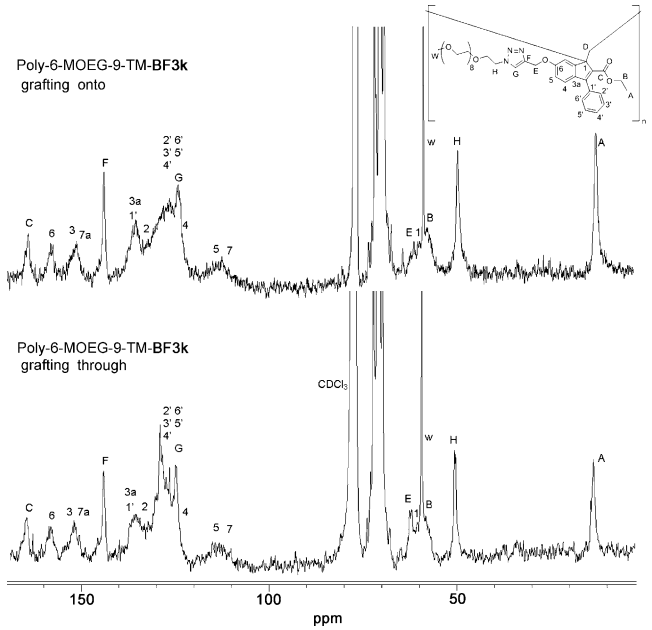


Figure 2. ^{13}C NMR spectrum ($CDCl_3$) of poly-6-MOEG-9-TM-**BF3k-GO** obtained by functionalization of poly-6-PO-**BF3k** (grafting onto) and, for comparison, that of the same polymer obtained by spontaneous polymerization of macromonomer 6-MOEG-9-TM-**BF3k-GT** (grafting through).

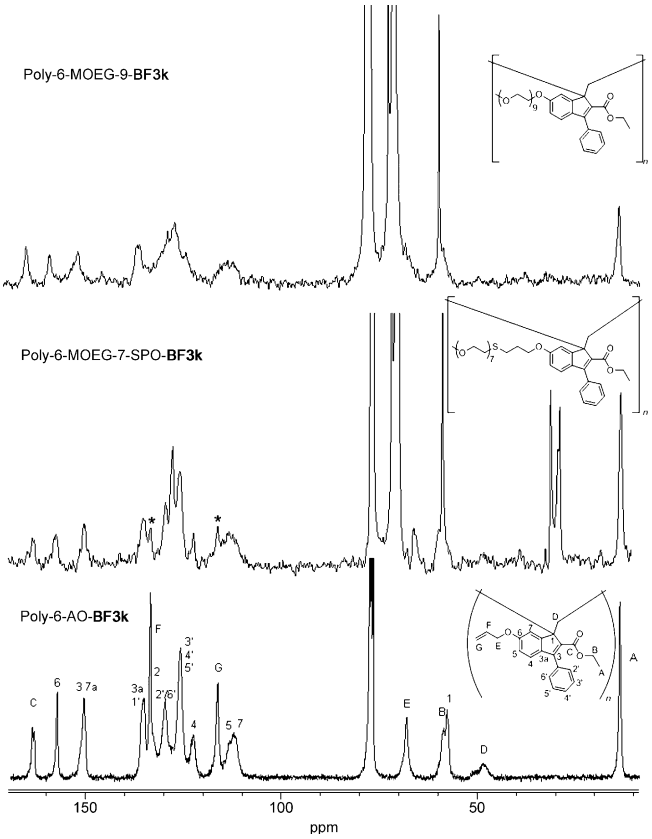


Figure 3. ^{13}C NMR spectra ($CDCl_3$) of newly synthesized poly-6-MOEG-7-SPO-**BF3k** and poly-6-AO-**BF3k** and, for comparison, that of previously reported poly-6-MOEG-9-**BF3k**.[21] The signals marked with asterisks were assigned to the residual vinyl groups remained unmodified after the exposition to the thiol–ene photoreaction conditions.

with asterisks in Figure 3) attributable to the residual vinyl groups, thus suggesting that a relatively small amount of these reactive functionalities remained unmodified after prolonged exposure to the thiol–ene photoreaction conditions.

Depolymerization studies: The thermoreversible polymerization behavior, typical of the polybenzofulvene derivatives,[17,19–21] was evaluated in the couple of macromolecular precursors poly-6-PO-**BF3k** and poly-6-AO-**BF3k** as well as in functionalized polymers poly-6-MOEG-9-TM-**BF3k-GO** and poly-6-MOEG-7-SPO-**BF3k** by using our standard protocol. Thus, solutions of the polymers (5.0 mg in 0.5 mL of [D_5]nitrobenzene) were heated at 150 °C and the ^1H NMR spectra were recorded at regular time intervals.

The experiments performed with poly-6-PO-**BF3k** and poly-6-AO-**BF3k** showed the expected behavior for the macromolecular precursors. The most significant steps ($t=0$ and 24 h) of the depolymerization processes are summarized in Figure 4. The presence of intact clickable groups in the formed monomers suggests that they are not involved in the thermoreversible polymerization/depolymerization mechanism.

Interestingly, the thermoreversible polymerization can be exploited to evaluate the grafting degree obtained by means of our coupling procedures. The results of the depolymerization experiments, summarized in Figure 5, show the usual depolymerization behavior for poly-6-MOEG-9-TM-**BF3k-GO** obtained by the GO strategy. The expected monomer was obtained in its pure form at significant concentrations after 2 h at 150 °C, and its concentration increased until the decomposition phenomenon became apparent (96 h). This behavior is similar to that previously observed with the same polymer obtained by spontaneous polymerization of macromonomer 6-MOEG-9-TM-**BF3k** (GT approach)[22] and can be interpreted in terms of close structural similarity. The absence of the signals attributable to monomer 6-PO-**BF3k** (e.g., the peaks at 2.89 and 4.87 ppm of the propargyl moiety, compare Figure 5 with Figure 4) substantiates the high grafting density assumed for poly-6-MOEG-9-TM-**BF3k-GO** on the basis of its ^{13}C NMR spectrum. Finally, the persistence of the thermoreversible properties after functionalization opens the way to the production of new tailored monomers (e g., bioactive monomers).

On the other hand, the depolymerization experiments performed with poly-6-MOEG-7-SPO-**BF3k** (Figure 6) showed the presence of a relatively small amount of monomer 6-AO-**BF3k**, thus confirming that the conditions applied in the thiol–ene photoreaction were inadequate to produce an exhaustive grafting of poly-6-AO-**BF3k** with a MOEG-7 chain bearing a thiol group (MOEG-7-SH).

MALDI-TOF mass spectrometry: The structures and the end groups of the newly prepared polybenzofulvene derivatives were also studied by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS)[24,25] in positive-ion mode, using different matrices.

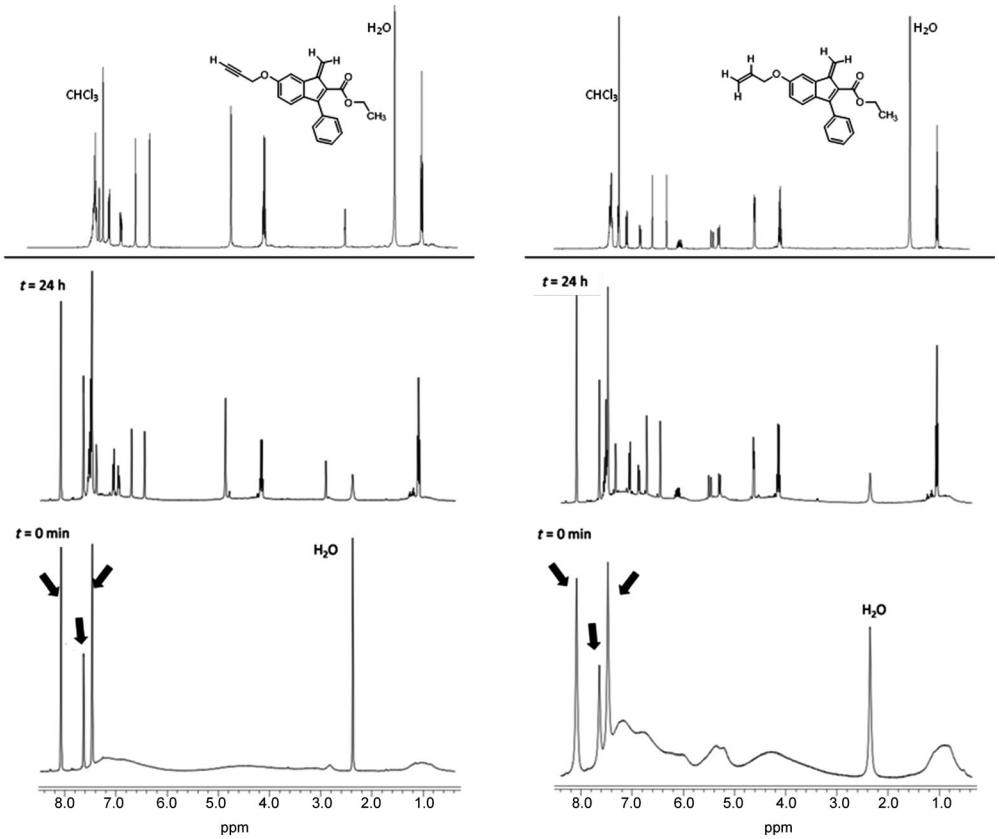
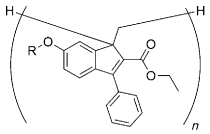
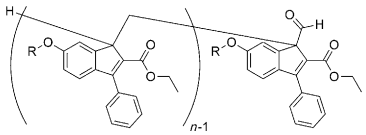
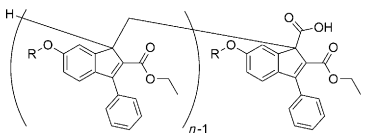


Figure 4. Thermoinduced depolymerization of poly-6-PO-**BF3k** (left) and of poly-6-AO-**BF3k** (right) as followed using ^{1}H NMR spectroscopy (400 MHz). A solution of the polymer (5.0 mg) in [D_5]nitrobenzene (0.5 mL) was heated at 150 °C, and the ^{1}H NMR spectra were recorded at regular time intervals. Only the spectra at $t=0$ and 24 h are displayed. The arrows indicate the nitrobenzene peaks and H_2O the water peak. The spectra of the corresponding monomers in $CDCl_3$ are given at the top for the sake of comparison.

Table 2. Structural assignments of the peaks observed in the MALDI-TOF spectra of poly-6-PO-**BF3k**, poly-6-AO-**BF3k**, and poly-6-MOEG-9-TM-**BF3k-GO** samples.

Species	Structures[a]	$[M^+]$[b] (n)		
		Poly-6-PO-	Poly-6-AO-	Poly-6-MOEG-9-
A_n		993.2 (3) 1323.6 (4) 1654.0 (5) 1984.4 (6) 2314.8 (7) 2645.2 (8) etc.	999.2 (3) 1331.6 (4) 1664.0 (5) 1996.4 (6) 2328.8 (7) 2661.2 (8) etc.	1569.8 (2) 2353.7 (3) 3137.6 (4) 3921.5 (5) 4705.4 (6) 5489.3 (7)
B_n		1007.2 (3) 1337.6 (4) 1668.0 (5) 1998.4 (6) 2328.8 (7) 2659.6 (8) etc.	1013.2 (3) 1344.6 (4) 1678.0 (5) 2010.4 (6) 2342.8 (7) 2675.2 (8) etc.	1583.8 (2) 2367.7 (3) 3151.6 (4) 3935.5 (5) 4719.4 (6) 5503.3 (7)
C_n		1023.2 (3) 1353.6 (4) 1684.0 (5) 2014.4 (6) 2344.8 (7) 2675.2 (8) etc.	1029.2 (3) 1361.6 (4) 1694.0 (5) 2026.4 (6) 2358.8 (7) 2691.2 (8) etc.	1599.8 (2) 2383.7 (3) 3167.6 (4) 4735.4 (5) 5519.3 (6) 6303.2 (7)

[a] R = propargyl for poly-6-PO-**BF3k**; R = allyl for poly-6-AO-**BF3k**; R = MOEG-9-TM for poly-6-MOEG-9-TM-**BF3k-GO**. [b] The mass of the repeat unit of poly-6-PO-**BF3k** is 330.4 Da, that of poly-6-AO-**BF3k** is 332.4 Da, and that of poly-6-MOEG-9-TM-**BF3k-GO** is 783.9 Da. The mass accuracy was better than (±0.5) and (±0.2) Da in the mass spectra recorded in linear and reflectron mode, respectively.

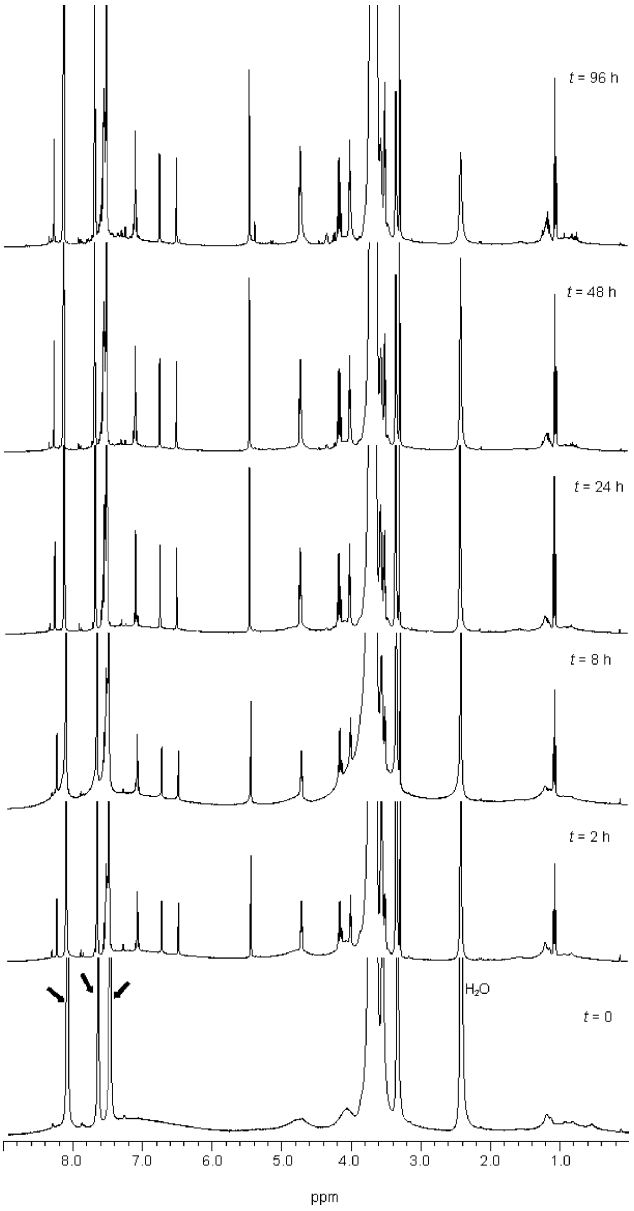


Figure 5. Thermoinduced depolymerization of poly-6-MOEG-9-TM-**BF3k-GO** (grafting onto) as followed using ^{1}H NMR spectroscopy (400 MHz). A solution of poly-6-MOEG-9-TM-**BF3k-GO** (5.0 mg) in [D_5]nitrobenzene (0.5 mL) was heated at 150 °C, and the ^{1}H NMR spectra were recorded at regular time intervals. To appreciate the variation in monomer concentration, the signals attributable to the vinylene group were integrated, and the integral values were compared with that of the lowest field signal of nondeuterated nitrobenzene, which was considered as an internal standard. Integrals are omitted for the sake of clarity. The arrows indicate the solvent peaks and H_2O the water peak.

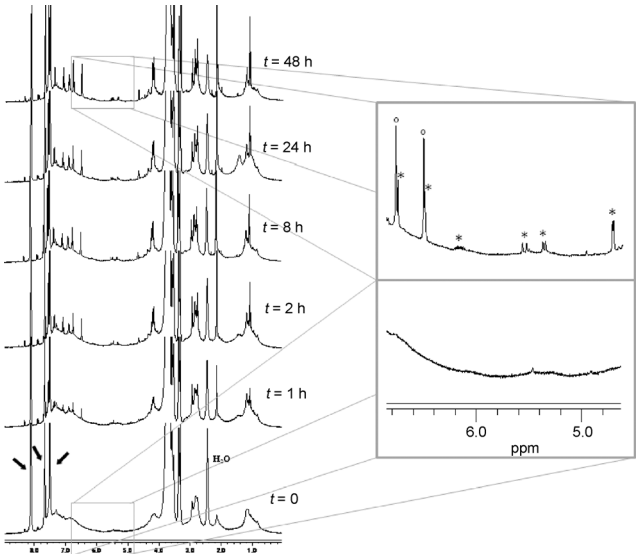


Figure 6. Thermoinduced depolymerization of poly-6-MOEG-7-SPO-**BF3k** as followed by ^{1}H NMR spectroscopy (400 MHz). A solution of poly-6-MOEG-7-SPO-**BF3k** (5.0 mg) in [D_5]nitrobenzene (0.5 mL) was heated at 150 °C, and the ^{1}H NMR spectra were recorded at regular time intervals. The arrows indicate the solvent peaks and H_2O the water peak. In the inset, the signals marked with asterisks were assigned to 6-AO-**BF3k** and those marked with circles to monomer 6-MOEG-7-SPO-**BF3k**.

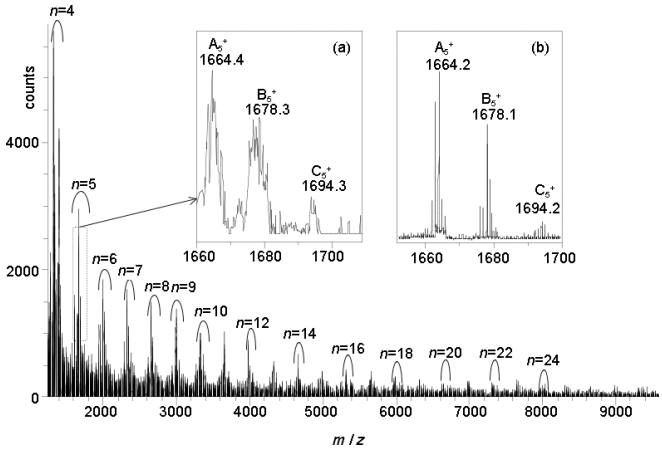


Figure 7. MALDI-TOF mass spectrum of poly-6-AO-**BF3k** recorded in linear mode. Insets show enlarged sections of the mass spectrum recorded in linear (a) and reflectron (b) mode.

Malononitrile (DCTB, see Experimental Section) has been found to be the best matrix, and the mass spectrum of the poly-6-AO-**BF3k** sample, recorded in linear mode, is displayed in Figure 7.

The spectrum shows, in the mass range within m/z 1000 and 10000, a series of three peaks separated from each other of (332.4 ± 0.3) Da, which corresponds to the mass of the repeating unit. The peak series correspond to the molecular ions (M^+) of linear polybenzofulvene oligomers from trimer (M_3) up to 30-mer (M_{30}). Signals due to polybenzofulvene macromolecules with molar mass higher than 15000 Da were not observed in the MALDI mass spectrum because, as often occurs, a discrimination effect has been observed in the MALDI-TOF MS analysis of these polydisperse polymers.[24,25] Generally, low-molecular-mass species are more easily desorbed than high-molecular-mass ones, and therefore species with molar mass higher than 10000–15000 Da were not revealed. MALDI-TOF MS gives abso-

lute determinations of average molar masses (M_w and M_n) only for polymers with a narrow dispersity ($PDI = M_w/M_n < 1.1$); it succeeds independently of their structure, but fails for polymeric materials with a higher polydispersity ($PDI > 1.1$) as well as for the polymers investigated in the present work.

The inset of Figure 7 shows that the most intense peaks correspond to the molecular ions (M^+) of linear polybenzofulvene oligomers terminated with hydrogen at both ends (H/H; species A_n in Table 2 and Figure 7). The second intense peak series belongs to the molecular ions of linear chains having an aldehyde moiety (H/CHO; species B_n in Table 2 and Figure 7), whereas peaks corresponding to the polybenzofulvene oligomers terminated with one carboxylic group (H/COOH; species C_n in Table 2 and Figure 7) appear with weak intensity.

High-resolution mass spectra were also recorded for poly-6-PO-**BF3k** and poly-6-MOEG-9-TM-**BF3k-GO** samples in reflectron mode. The enlarged sections of their mass spectra are displayed in Figure 8, whereas the mass spectra recorded

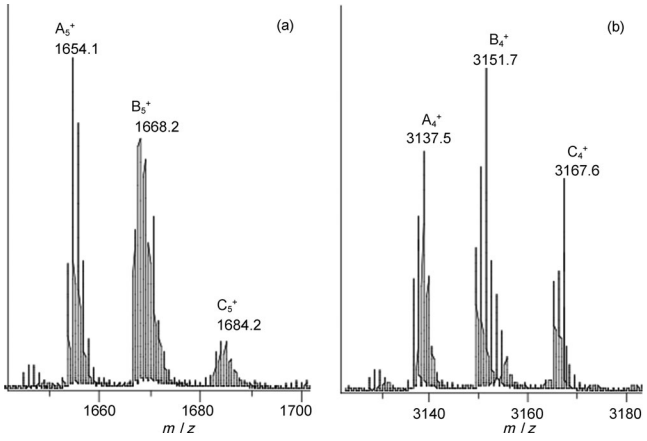


Figure 8. Enlarged sections of MALDI-TOF mass spectra of a) poly-6-PO-**BF3k** and b) poly-6-MOEG-9-TM-**BF3k-GO**, recorded in reflectron mode.

in linear mode are displayed in the Supporting Information (Figures 2S and 3S).

Similarly to the mass spectrum of poly-6-AO-**BF3k**, the mass spectra of both samples recorded in linear mode show a series of three peaks (in the mass range from m/z 1000 up to m/z 15000) separated from each other of (330.4 ± 0.4) Da (in the case of the poly-6-PO-**BF3k** sample) or of (783.9 ± 0.5) Da (for the poly-6-MOEG-9-TM-**BF3k-GO** sample), which correspond to the mass of their repeating units. The most intense peaks in the poly-6-PO-**BF3k** spectrum were attributed to polybenzofulvene chains terminated with H at both ends (species A_n). The other two peak series were assigned to the oligomers terminated with H/CHO and H/COOH groups (species B_n and C_n, respectively). The relative intensity of peaks belonging to the families A_n and B_n is comparable with that observed in the spectra of the poly-6-AO-**BF3k** sample, whereas the peaks belonging to oligomers terminated with the carboxyl group (species C_n) appear with more intensity (compare Figures 7 and 8). The relative intensity of peaks corresponding to poly-6-PO-**BF3k** chains terminated with H/H, H/CHO, and H/COOH is about 54:39:7, as calculated from 20 mass spectra recorded in linear mode. On the other hand, in the MALDI mass spectra of poly-6-MOEG-9-TM-**BF3k-GO**, the most intense peaks were assigned to linear chains terminated with H/CHO groups (species B_n in Figure 8b), whereas those terminated with H/H (species A_n) and H/COOH (species C_n) groups appear with similarly lower intensity. Thus, the relative intensity of peaks belonging to chains terminated with H/H, H/CHO, and H/COOH groups is 30:42:28 with a variance of ± 2.5. By way of comparison, previously synthesized poly-6-MOEG-9-TM-**BF3k-GT** showed a relative intensity of 70:28:2 determined with a variance of ± 2.5 by using the intensities of the corresponding peaks present in the mass range 1000–8000 of 12 mass spectra recorded using different laser intensities.

*Macromolecular structure of poly-6-MOEG-9-TM-**BF3k-GO***: To obtain information about the aggregation state of poly-6-MOEG-9-TM-**BF3k-GO** in water, dynamic light scattering (DLS) studies were carried out by using aqueous dispersions of the polymer in the concentration range of 0.1–1.0 mg mL^{-1} and at 25 and 37 °C. The analysis of DLS data obtained with clear solutions at 25 °C suggested the presence of objects showing average dimensions of 57 nm. These dimensions are significantly smaller than those previously observed with the same polymer synthesized by spontaneous polymerization of macromonomer 6-MOEG-9-TM-**BF3k** (poly-6-MOEG-9-TM-**BF3k-GT**, 245 nm)[22] and agree with the radius of gyration as measured by SEC-MALS analysis (ca. 34 nm).

Furthermore, DLS dimensional data showed the predominant formation of aggregates at 37 °C and at a minimum polymer concentration of 0.5 mg mL^{-1}. These aggregates

Table 3. Size distribution and Z-potential values of poly-6-MOEG-9-TM-**BF3k-GO** water dispersions (0.5 mg mL^{-1}) at 25 and 37 °C.

Temperature [°C]	d_{av} [nm]	PDI	Z-potential [mV]
25	57	0.38	−7.23
37	290	0.17	−7.50

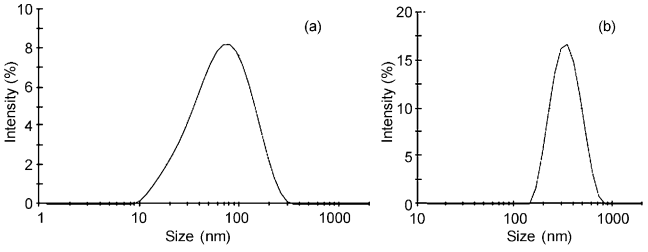


Figure 9. Size distribution curves of poly-6-MOEG-9-TM-**BF3k-GO** recorded at 25 (a) and 37 °C (b) by DLS (sample concentration 0.5 mg mL^{-1}).

showed an average diameter of 290 nm and PDI of 0.17 (see Table 3 and Figure 9). Actually, this finding is consistent with the hypothesis that the polymer dispersion, at the analyzed concentration, is composed of a homogeneous aggregate population at 37 °C. Zeta (Z)-potential values measured at 25 and 37 °C did not vary significantly, which indicated that any modification of the exposed surface of the polymer occurred as a consequence of the polymer aggregation induced by temperature variation.

Briefly, the water solutions of newly synthesized poly-6-MOEG-9-TM-**BF3k-GO** show the features of thermoresponsive colloidal dispersions similar to those observed with previously published poly-6-MOEG-9-TM-**BF3k-GT**, with the difference that the polymer synthesized by the GO strategy appears to be molecularly dissolved at room temperature, probably because of its higher Z-potential value (−7.23 mV versus −1.24 mV shown poly-6-MOEG-9-TM-**BF3k-GT**).

The data obtained by DLS studies agree with the images obtained by transmission electron microscopy (TEM) of poly-6-MOEG-9-TM-**BF3k-GO** water solutions processed at room temperature. In fact, Figure 10 shows the presence of

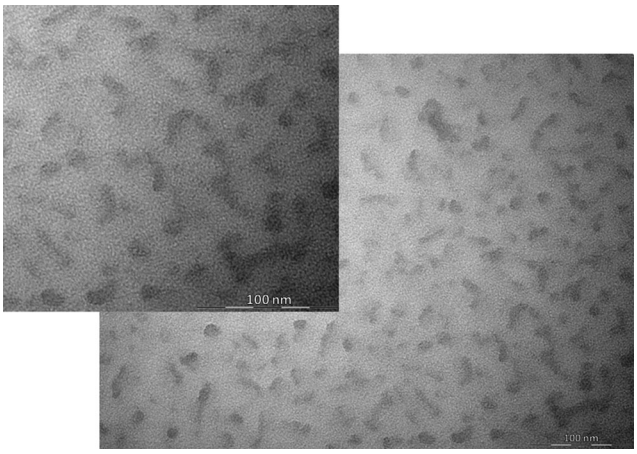


Figure 10. Structure of brush macromolecules and aggregates found by TEM analysis of poly-6-MOEG-9-TM-**BF3k-GO** solution in water.

wormlike objects, which can be assumed to be isolated macromolecules, together with relatively abundant globular species presumably resulting from intramolecular collapse, and rare small aggregates confirming the low propensity towards aggregation shown by poly-6-MOEG-9-TM-**BF3k-GO**.[21,22]

Cytotoxicity of poly-6-MOEG-9-TM-BF3k-GO: The exhaustive grafting observed in poly-6-MOEG-9-TM-**BF3k-GO** taken together with its appreciable water solubility and the low liability to aggregation led us to envision potential biological or biotechnological applications for this polymer brush. Previous fluorescence microscopy studies suggested that poly-6-MOEG-9-TM-**BF3k-GT** is unable to interact, cross the membrane, and alter the morphology of HEK293T cells, thereby suggesting the absence of toxic effects. The result was tentatively rationalized in terms of aggregation properties and dimension of the aggregates in water.[22] On the basis of the smaller dimension of the objects generated by poly-6-MOEG-9-TM-**BF3k-GO** in aqueous medium, the potential cytotoxicity of the newly synthesized polymer was evaluated in two different cell lines (i.e., immortalized mouse fibroblasts NIH3T3 and human neuroblastoma IMR-32 cells). IC_{50} values were found to be >77 μM (NIH3T3) and 13 μM (IMR-32) and confirmed a low cytotoxic potential for the tested polymer towards both NIH3T3 and IMR-32 cell lines. The low cytotoxicity shown by poly-6-MOEG-9-TM-**BF3k-GO** was considered to be in good agreement with its low propensity to interact and cross the cytoplasmic membrane. In fact, preliminary fluorescence microscopy studies showed a weak fluorescence uptake in both the different cell lines used, even after a prolonged incubation time.

Conclusion

The work described herein shows that benzofulvene monomers bearing propargyl or allyl groups can be synthesized by means of straightforward reactions and induced to polymerize spontaneously, in the apparent absence of catalysts or initiators, by solvent removal to give the corresponding polybenzofulvene derivatives showing very high molecular weight values. In particular, the results of SEC-MALS experiments suggest that spontaneous polymerization of monomers 6-PO-**BF3k** and 6-AO-**BF3k** produces polybenzofulvene derivatives showing MWD features similar to those of parent polymer poly-**BF3k**. NMR studies substantiate the retention of the spontaneous 1,2-polymerization mechanism, and the presence in the ^{13}C NMR spectra of additional signals attributed to propargyl or allyl side chains (clickable groups) suggests that 1,2-polymerization involves the benzofulvene exocyclic double bond selectively. Moreover, depolymerization experiments confirmed that the clickable groups are not involved in the thermoreversible polymerization/depolymerization mechanism. Thus, the presence of allyl or propargyl groups does not affect the spontaneous polymerization of benzofulvene monomers in terms of MWD and incorporation of allyl or propargyl groups into the polybenzofulvene backbone. We assume that the benzofulvene derivatives are capable of forming columnar aggregates in concentrated solutions. When the intermonomer distance becomes compatible with the formation of covalent bonds among the monomers, the spontaneous polymerization starts and proceeds rapidly as a domino reaction leading to macrobiradical species, which undergo termination. MALDI-TOF experiments substantiate that the main termination pathway of the macrobiradical species produced by spontaneous polymerization of 6-PO-**BF3k** and 6-AO-**BF3k** is hydrogen addition at both ends of the polybenzofulvene backbone. Moreover, the interaction of the macrobiradical species with molecular oxygen was assumed to produce the

oxidation of the terminal methylene group with the formation of aldehydic or carboxylic groups.

With the aim of developing a powerful and versatile GO methodology to obtain tailored polymer brushes, the clickable propargyl and allyl groups were exploited in appropriate click reactions. In particular, the CuAAC reaction was used to transform poly-6-PO-**BF3k** into poly-6-MOEG-9-TM-**BF3k-GO**, which showed a ^{13}C NMR spectrum virtually indistinguishable from that of the homopolymer obtained by spontaneous polymerization of macromonomer 6-MOEG-9-TM-**BF3k**.[22] This high similarity with the spectrum of the homopolymer suggests that the CuAAC coupling reaction led to a very high grafting density, substantiated also by the lack of signals attributable to the propargyl moiety as well as by the results of the depolymerization studies. On the contrary, thiol–ene photografting of poly-6-AO-**BF3k** with a MOEG-7 chain bearing a thiol group (MOEG-7-SH) was less exhaustive, as suggested by the presence of minor signals attributable to the residual vinyl groups in the ^{13}C NMR spectrum of poly-6-MOEG-7-SPO-**BF3k**. These preliminary results suggest that, of the two click reactions investigated in the present work, the CuAAC coupling is superior to the thiol–ene one, and additional work is probably required to make the thiol–ene coupling as efficient as the CuAAC reaction in the GO approach to polybenzofulvene brushes.

SEC-MALS measurements showed that the transformation of poly-6-PO-**BF3k** into poly-6-MOEG-9-TM-**BF3k-GO** produces both a decrease in the molecular weight and an increase in the PDI, thus suggesting that the polybenzofulvene backbone is susceptible to breaking under the conditions used in the CuAAC reaction. We assumed that multiple ruptures occurred randomly along the backbone up to the production of macromolecules showing a molecular weight quite similar to that shown by poly-6-MOEG-9-TM-**BF3k-GT** obtained by spontaneous polymerization of the macromonomer. Probably, this behavior is the consequence of the balance between the increasing steric hindrance around the polybenzofulvene backbone and its relative stability to covalent bond scission. In other words, because of the high grafting density, MOEG-9 side chains are assumed to repel each other and stretch the polybenzofulvene backbone up to its scission,[26] which can be facilitated by the relative stability of the indenyl radical formed by homolytic cleavage.

DLS analysis suggested for the aqueous solutions of poly-6-MOEG-9-TM-**BF3k-GO** that the properties of thermoresponsive colloidal dispersions are similar to those observed with previously published poly-6-MOEG-9-TM-**BF3k-GT**,[22] with the difference that the polymer synthesized by the GO strategy appears to be molecularly dissolved at room temperature. This difference can be explained by the presence of a relatively high number of chains terminated with carboxylic acid groups in poly-6-MOEG-9-TM-**BF3k-GO**, thereby leading to a Z-potential value (−7.23 mV) higher than that measured for poly-6-MOEG-9-TM-**BF3k-GT** (−1.24 mV), in which chains terminated with COOH groups were not observed.[22] Finally, the lack of any toxic effect and cell viability impairment found with poly-6-MOEG-9-TM-**BF3k-GO** demonstrates that our polybenzofulvene derivatives bearing clickable groups can be functionalized by a suitable methodology to produce nanostructured materials potentially useful in a wide range of biological and biotechnological applications.

Experimental Section

Synthesis: Melting points were determined in open capillaries in a Gallenkamp apparatus and are uncorrected. UV/Vis spectra were recorded with a Shimadzu 260 spectrophotometer and the emission spectra were obtained by means of a Perkin–Elmer LS45 instrument. Merck silica gel 60 (230–400 mesh) was used for column chromatography. Merck TLC plates, silica gel 60 F254, were used for TLC. NMR spectra were recorded with a Bruker AC200, Varian Mercury-300, Bruker DRX-400 AVANCE, Bruker DRX-500 AVANCE, or Bruker DRX-600 AVANCE spectrometer in the indicated solvents (TMS as internal standard): the values of the chemical shifts are expressed in ppm and the coupling constants (J) in hertz. An Agilent 1100 LC/MSD instrument operating with an electrospray source was used in mass spectrometry experiments.

*Ethyl 1-methylene-3-phenyl-6-(prop-2-ynyloxy)-1*H*-indene-2-carboxylate (6-PO-**BF3k**)*: A mixture of indenol derivative **4a** (0.87 g, 2.5 mmol) in $CHCl_3$ (50 mL) with *p*-toluenesulfonic acid monohydrate (PTSA; 0.47 g, 2.5 mmol) was heated under reflux for 1 h and cooled to room temperature. The reaction mixture was washed with a saturated solution of $NaHCO_3$ and dried over sodium sulfate to afford a stock (about 0.05 M) solution of the monomer 6-PO-**BF3k** that was stored under an argon atmosphere. ^{1}H NMR (400 MHz, [D]$CHCl_3$, 25 °C, TMS): δ = 1.04 (t, J = 7.1 Hz, 3H), 2.53 (t, J = 2.3 Hz, 1H), 4.11 (q, J = 7.1 Hz, 2H), 4.75 (d, J = 2.3 Hz, 2H), 6.34 (s, 1H), 6.62 (s, 1H), 6.90 (dd, J = 8.3 Hz, 2.3, 1H), 7.13 (d, J = 8.3 Hz, 1H), 7.33 (d, J = 2.3 Hz, 1H), 7.41 ppm (m, 5H); MS (ESI): m/z: 353 [M+Na$^+$].

*Ethyl 6-(allyloxy)-1-methylene-3-phenyl-1*H*-indene-2-carboxylate (6-AO-**BF3k**)*: A mixture of compound **4b** (0.48 g, 1.37 mmol) in chloroform (28 mL) with PTSA (0.26 g, 1.37 mmol) was heated under reflux for 1 h and cooled to room temperature. The reaction mixture was washed with a saturated solution of $NaHCO_3$ and dried over sodium sulfate to afford a stock (about 0.05 M) solution of benzofulvene monomer 6-AO-**BF3k**, which was stored under an argon atmosphere. ^{1}H NMR (400 MHz, [D]$CHCl_3$, 25 °C, TMS): δ = 1.04 (t, J = 7.1 Hz, 3H), 4.11 (q, J = 7.1 Hz, 2H), 4.60 (d, J = 5.2 Hz, 2H), 5.29 (dd, J = 10.5, 1.1 Hz, 1H), 5.43 (dd, J = 17.2, 1.1 Hz, 1H), 6.07 (m, 1H), 6.32 (s, 1H), 6.60 (s, 1H), 6.84 (dd, J = 8.4, 2.3 Hz, 1H), 7.10 (d, J = 8.4 Hz, 1H), 7.27 (d, J = 2.3 Hz, 1H), 7.41 ppm (m, 5H); MS (ESI): m/z: 333 [M+H$^+$].

*Poly[ethyl 1-methylene-3-phenyl-6-(prop-2-ynyloxy)-1*H*-indene-2-carboxylate] (poly-6-PO-**BF3k**)*: A solution of benzofulvene monomer 6-PO-**BF3k** in chloroform was concentrated under reduced pressure to give a viscous oil, which was dissolved in chloroform and newly evaporated (the dissolution/evaporation procedure was repeated three times). The polymer was purified by precipitation with ethanol (bad solvent) from a solution of the final residue in chloroform (good solvent) and dried under reduced pressure to obtain poly-6-PO-**BF3k** (0.61 g, yield 74%) as a white solid. ^{1}H NMR (400 MHz, [D]$CHCl_3$, 25 °C, TMS): δ = 0.3–1.1 (br m, 3H), 2.3–2.5 (br m, 1H), 2.6–4.0 (br m, 4H), 4.1–4.9 (br m, 2H), 5.8–7.3 ppm (br m, 8H).

*Poly[ethyl 6-(allyloxy)-1-methylene-3-phenyl-1*H*-indene-2-carboxylate] (poly-6-AO-**BF3k**)*: A solution of monomer 6-AO-**BF3k** in chloroform was concentrated under reduced pressure to give a viscous yellow oil, which was dissolved in chloroform and newly evaporated (the dissolution/evaporation procedure was repeated three times). The polymer was purified by precipitation with ethanol from a solution of the final residue in chloroform and dried under reduced pressure to obtain poly-6-AO-**BF3k** as a white solid (0.36 g, yield 79%). ^{1}H NMR (400 MHz, [D]$CHCl_3$,

25 °C, TMS): $\delta = 0.2–1.1$ (br m, 3H), 1.7–4.7 (br m, 6H), 4.8–5.5 (br m, 2H), 5.6–7.8 ppm (br m, 9H).

*Poly(ethyl 6-[[1-(2,5,8,11,14,17,20,23,26-nonaoxaoctacosan-28-yl)-1*H*-1,2,3-triazol-4-yl]methoxy]-1-methylene-3-phenyl-1*H*-indene-2-carboxylate) (poly-6-MOEG-9-TM-**BF3k-GO**)*: In a microwave tube, a mixture of poly-6-PO-**BF3k** (0.15 g, 0.45 mmol in monomer base unit) in THF (5.0 mL) containing 28-azido-2,5,8,11,14,17,20,23,26-nonaoxaoctacosane (MOEG-9-N_3, 0.41 g, 0.90 mmol), CuBr (0.013 g, 0.091 mmol), and DIPEA (0.016 mL, 0.092 mmol) was stirred at room temperature for 5 h, exposed to microwave irradiation in a CEM Discover instrument for 40 min (4×10 min, $T = 60$ °C, W = 150), and finally evaporated under reduced pressure. The residue was dissolved with water (15 mL) and 33% NH_4OH (4.0 mL) to obtain a clear solution after overnight stirring at room temperature. The aqueous solution was extracted with chloroform and the organic layer was dried over sodium sulfate and concentrated under reduced pressure. The polymer was purified by precipitation with *n*-hexane (50 mL) from a solution of the final residue in dichloromethane (5 mL) and dried under reduced pressure to obtain poly-6-MOEG-9-TM-**BF3k-GO** as a pale-yellow sticky solid (0.27 g, yield 77%). ^{1}H NMR (300 MHz, [D]$CHCl_3$, 25 °C, TMS): $\delta = 0.1–1.1$ (br m, 3H), 3.3 (s, 3H), 3.4–5.5 (br m, 42H), 5.6–8.2 ppm (br m, 9H).

*Poly[ethyl 6-(2,5,8,11,14,17,20-heptaoxa-23-thiahexacosan-26-yloxy)-1-methylene-3-phenyl-1*H*-indene-2-carboxylate] (poly-6-MOEG-7-SPO-**BF3k**)*: In a vial, poly-6-AO-**BF3k** (0.030 g, 0.090 mmol in monomer base unit) was dissolved in $CHCl_3$ (1.0 mL), and *O*-(2-mercaptoethyl)-*O*′-methyl-hexa(ethylene glycol) (MOEG-7-SH, 0.16 g, 0.45 mmol) and DMPA (0.011 g, 0.043 mmol) were added. The vial was sealed with a screw cap fitted with a PTFE septum and the reaction mixture was purged with argon for 5–10 min. Irradiation with a UV lamp (365 nm) was carried out at room temperature for 6 h. The reaction mixture was then concentrated under reduced pressure and the residue was washed with *n*-hexane and dried under reduced pressure to obtain poly-6-MOEG-7-SPO-**BF3k** as a pale-yellow sticky solid (0.047 g).

SEC-MALS: The MWD characterization of the new polybenzofulvene derivatives was performed by a MALS light scattering photometer online to a SEC system. The SEC-MALS system and the corresponding experimental conditions were identical to those used in our previous studies[18,19] and are not reported in detail herein.

Mass spectrometry: MALDI-TOF mass spectra of the new polybenzofulvenes were recorded in linear mode with a Voyager DE-STR (Perseptive Biosystem) mass spectrometry instrument equipped with a nitrogen laser emitting at 337 nm, with a 3 ns pulse width and working in positive mode. The accelerating voltage was 25 kV, and the grid voltage and delay time were optimized for each sample to achieve better mass resolution, expressed as the molecular weight of a given ion divided by the full width at half maximum (fwhm). The irradiance was maintained slightly above the threshold. External calibration was performed using a home-made low-molar-mass ($M_n = 3000$ g mol^{-1}) hydroxy-ended poly(bisphenol A carbonate) (PC-OH). Mass spectra in reflectron mode were also recorded with a 4800 Proteomic Analyzer (Applied Biosystems), a MALDI-TOF/TOF instrument equipped with a Nd:YAG laser at a wavelength of 355 nm with < 500 ps pulse and 200 Hz firing rate. External calibration was performed using an Applied Biosystems calibration mixture consisting of polypeptides with different molecular weight values. The irradiance was maintained slightly above the threshold, to obtain a mass resolution of about 6000–8000 fwhm; isotopic resolution was observed throughout the entire mass range detected (from *m/z* 1000 up to *m/z* 6000). Mass accuracy was about 50 ppm. All measurements were performed in positive mode; approximately 1500 laser shots were accumulated for each mass spectrum. For the analysis of each polymer, four different matrices were used: α-cyano-4-hydroxycinnamic acid, 1,8,9-anthracenetriol (dithranol), 2-(4′-hydroxybenzeneazo)benzoic acid, and *trans*-2-[3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB), 0.1 M in THF, with and without the addition of cationization salts (i.e., CF_3COONa or CF_3COOAg; 10^{-3} M in THF). The better spectra were recorded using DCTB without salts. Mass spectra recorded in linear mode showed intense mass-resolved peaks with a fwhm of about 800 within *m/z* 1000 and 7000, and also poorly resolved peaks in the mass range between *m/z* 7000 and 15000. On the other hand, spectra recorded in reflectron mode showed significantly intense peaks only in the mass range lower than *m/z* 6000. Samples for MALDI analysis were prepared by the dried-droplet method, in which a mixture of matrix and sample was deposited onto the target plate and dried at room temperature under inert atmosphere (N_2 flow). Typically, a THF solution (0.5 μL) of the polymer (10 mg mL^{-1}) was mixed with a matrix solution (0.5 or 1.5 μL), and then spotted on the MALDI sample holder and dried slowly to allow matrix crystallization. The better spectra were recorded using a (matrix solution)/(polymer solution) ratio of 3:1 v/v. The mass peaks corresponding to the macromolecular species were measured with an accuracy of (±0.5) Da in linear mode and (±0.2) Da in reflectron mode.

Dynamic light scattering studies and Z-potential measurements: DLS studies and Z-potential measurements were performed at 25 and 37 °C by means of a Malvern Zetasizer NanoZS instrument fitted with a 532 nm laser at a fixed scattering angle of 90°. The polymer samples were molecularly dissolved into doubly distilled water (pH ≈6) at concentrations ranging from 0.1 to 1.0 mg mL^{-1} and the intensity-average hydrodynamic diameter (size in nm) and polydispersity index (PDI) were obtained by cumulant analysis of the correlation function. The Z-potential (mV) was calculated from the electrophoretic mobility by using the Smoluchowsky relationship and assuming $Ka \gg 1$ (in which K and a are the Debye–Hückel parameter and particle radius, respectively). Each experiment was performed in triplicate.

Transmission electron microscopy: A drop of poly-6-MOEG-9-TM-**BF3k-GO** solution in water was placed onto a 300 mesh formvar-coated copper grid and observed in a Philips CM10 transmission electron microscope at an acceleration voltage of 80 kV.

Cytotoxicity experiments: NIH3T3 and IMR-32 cells were employed for cytotoxicity experiments. NIH3T3 cells were kept in Dulbecco's modified Eagle's medium (DMEM) and IMR-32 cells in Eagle's minimal essential medium (EMEM) at 37 °C in a humidified atmosphere containing 5% CO_2. The culture media were supplemented with 10% fetal calf serum, 1% L-glutamine–penicillin–streptomycin solution, and 1% MEM Non-Essential Amino Acid Solution. Once at confluence, cells were washed with phosphate-buffered saline (PBS, 0.1 M), taken up with trypsin–ethylenediaminetetraacetic acid solution, and then centrifuged at 1000 rpm for 5 min. The pellet was resuspended in medium solution (dilution 1:15). Cell viability after 24 h of incubation with the polymer was evaluated by Neutral Red Uptake assay (Sigma–Aldrich, Switzerland) by the procedure reported previously.[27] The data processing included Student's *t* test with $p < 0.05$ taken as significance level.

Interaction with live cells: NIH3T3 and IMR-32 cells were plated on coverslips and allowed to grow overnight. The coverslips were washed and incubated with poly-6-MOEG-9-TM-**BF3k-GO** (0.01 mg mL^{-1}) in PBS for 12 h at room temperature. Cells on coverslips were washed with PBS, fixed with 4% paraformaldehyde, and mounted for observation with a Nikon epifluorescence microscope (Zeiss).

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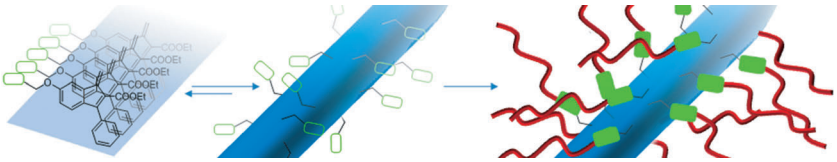

A clean sweep: Two novel benzofulvene monomers bearing either propargyl or allyl groups were found to polymerize spontaneously by solvent removal in the absence of catalysts or initiators to give the corresponding polybenzofulvene derivatives bearing clickable propargyl or allyl moieties. These derivatives were modified using click reactions, thus demonstrating a "grafting onto" methodology for the synthesis of tailored polymer brushes (see figure).

Click Reactions

A. Cappelli, G. Grisci, M. Paolino, F. Castriconi, G. Giuliani, A. Donati, S. Lamponi, R. Mendichi, A. C. Boccia, F. Samperi, S. Battiato, E. Paccagnini, M. Gentile, M. Licciardi, G. Giammona, S. Vomero* . . ■■■■–■■■■

Combining Spontaneous Polymerization and Click Reactions for the Synthesis of Polymer Brushes: A "Grafting Onto" Approach