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MALDI-TOF Analysis of Dendrimer-like Poly(ethylene oxide)s

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Introduction

Poly(ethylene oxide) (PEO), often referred to as PEG for poly(ethylene glycol), has many potential applications in biomedical and pharmaceutical areas owing to its water solubility, nontoxicity, ion-transporting ability, and nonrecognition by the immune system.[1,2] PEO is also an efficient and inert support for liquid-phase organic synthesis.[3] However, low molar mass linear PEO suffers from its inherent low loading capacities. Indeed, only one or at best two reacting sites, depending on the chemical nature of the end groups, can be employed to derivatize linear PEO precursors. Different solutions have been proposed to increase the loading capacity of PEO's, including the synthesis of functionalized starlike PEO's,[4] hyperbranched PEO's,[5] arborescent PEO's,[6−8] or dendrimer-like PEO's.[9−11]

Dendrimer-like polymers exhibit similar molecular features to that of regular dendrimers, including the presence of a central core, a precise number of branching points, and outer terminal functions, but dendrimer-like polymers possess true oligomeric/polymeric segments between their branching points.[10−13] Such polymers combine, in principle, some of the features of linear polymers such as crystallinity and chain entanglements with those of dendrimers.[13] From a synthetic point of view, dendrimer-like polymers are obtained by a combination of "controlled/living" polymerization techniques with selective branching reactions, following in most cases a divergent approach. In contrast to the large number of publications on "regular" dendrimers, only a few examples of dendrimer-like (co)polymers have been described in the recent literature.[13] Very recently, the Gnanou group has developed an easy access to dendrimer-like PEO based on an iterative strategy combining anionic ring-opening polymerization of ethylene oxide from multihydroxylated precursors and a two-step sequence of branching reactions. In this way, dendrimer-like PEO's could be obtained up to the eighth generation with a the total number-average molecular weight (M_n) reaching 900 000 g/mol for PEO branches exhibiting an average molar mass of 1500 g/mol and a polydispersity index (PDI) lower than 1.2 and comprising not less than 384 peripheral hydroxy functions.[11]

A crucial issue to obtain well-defined dendritic compounds is the quantitative introduction of the branching points, which requires highly selective branching reactions of the peripheral groups. However, there are only a few analytical means to monitor such chemical modifications of polymer chain ends. Among these, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is certainly the most suitable technique to account for the perfection or defaults within dendrimers. MALDI-TOF MS has become an important technique to characterize the chemical structure of many different analytes, including synthetic polymer materials.[14−16] It can generate important data on the repeat units, end groups, and average molar mass of these materials. Despite the wide use of MALDI-TOF to analyze linear homo- and copolymers, not many papers described the analysis of star-shaped or branched polymers with MALDI-TOF. Moore et al. could characterize the molecular weight of monodisperse phenylacetylene dendrimers with MALDI-TOF and matrix-assisted laser desorption Fourier transform mass spectrometry (MALDI-FTMS) up to a molecular weight of 30 000 Da, but isotopic resolution could only be obtained for a mass up to 3700 Da.[17,18] Penczek et al. described the MALDI-TOF analysis of star-shaped multibranched PEO.[7] However, because of the complex nature of the samples, it was only possible to measure spectra up to a molar mass of 4500 Da. Kricheldorf et al. were able to explain the reaction mechanism for the preparation of hyperbranched polyesters by MALDI-TOF, but again only spectra with low resolution in the low molar mass region (up to 6000 Da) were obtained.[19] Finally, Hillmyer et al. were able to analyze hydroxyl-functionalized polyisoprene star polymers with molar masses up to 9000 g mol^{-1}, but spectra with isotopic resolution were not obtained.[20] In general, branched polymers are often too complex to be analyzed with MALDI-TOF due to a distribution in the number of arms, differences in end-group functionality, and broad molar mass distributions (MMD).

In this Note, we report the detailed MALDI-TOF analysis of second- and third-generation dendrimer-like PEO structures. To our knowledge, this is the first demonstration of MALDI-TOF analysis of polydisperse highly branched structures with relatively high molar mass.

Experimental Section

Synthesis of the Dendrimer-like Poly(ethylene oxide)s. The synthesis of the dendrimer-like poly(ethylene oxide)s was described earlier.[11] The materials and preparation methods can be found in the Supporting Information.

Materials. For the MALDI-TOF measurements, dithranol, *trans*-2-[3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene]malononitrile (BMPM), NaI, and analytical-grade solvents were purchased form Sigma Aldrich and were used as received.

MALDI-TOF Analysis. MALDI-TOF mass spectra were recorded on an Applied Biosystems Voyager DE STR MALDI-TOF mass spectrometer equipped with 2 m linear and 3 m reflector flight tubes and a 337 nm nitrogen laser (3 ns pulse). All mass spectra were obtained averaging 100 shots with an accelerating potential of 20 kV in positive ion mode and in reflector mode. Dithranol (20 mg/mL in tetrahydrofuran (THF)) was used as a matrix for the second-generation PEO samples, and BMPM (20 mg/mL in THF) was used for the

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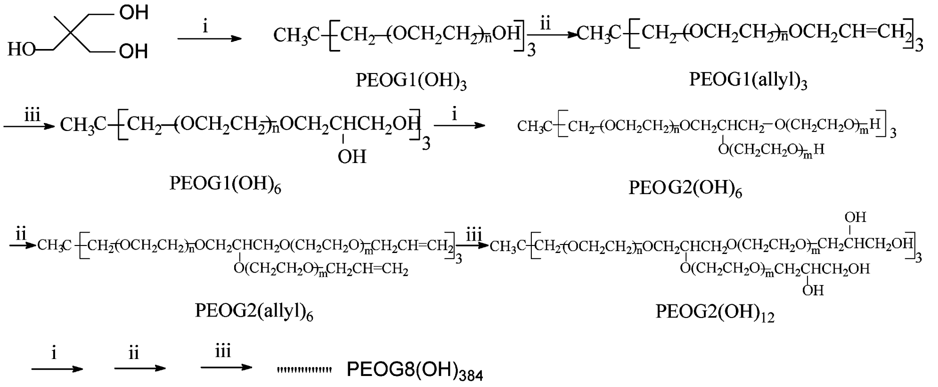
Scheme 1. Preparation of Dendrimer-like Poly(ethylene oxide)



i: diphenylmethylpotassium / dimethyl sulfoxide; ethylene oxide
ii: CH_2=$CHCH_2Cl$, NaOH / H_2O, tetrabutylammonium bromide, 50°C
iii: OsO_4, N-methylmorpholine-N-oxide, *tert*-butanol, CH_3COCH_3 / H_2O

Table 1. Molecular Data for the Dendrimer-like Poly(ethylene oxide) Samples[a]

sample code	M_n MALDI (g mol^{-1})	M_n SEC (g mol^{-1})	PDI SEC
PEOG1$(OH)_3$	2800	3400	1.08
PEOG1$(allyl)_3$	2900	4000	1.07
PEOG1$(OH)_6$	3000	3400	1.07
PEOG2$(OH)_6$	8500	9000	1.09
PEOG2$(allyl)_6$	9400	11000	1.11
PEOG2$(OH)_{12}$	9900	10000	1.12
PEOG3$(OH)_{12}$	*b*	18000	1.13
PEOG3$(allyl)_{12}$	21400	18000	1.15
PEOG3$(OH)_{24}$	20500	17800	1.13

[a] SEC = size exclusion chromatography; M_n = number-average molecular weight; PDI = polydispersity index. [b] Not measured.

third-generation PEO samples, NaI (1 mg/mL in THF) was used as a cationating agent, and polymer samples were dissolved in methanol (2 mg/mL). Analyte solutions were prepared by mixing 10 μL of the matrix, 5 μL of the salt, and 5 μL of the polymer solution. Subsequently, 0.5 μL of this mixture was spotted on the sample plate, and the spots were dried in air at room temperature. Poly(ethylene oxide) standards (M_n = 5000 or 10 000 g mol^{-1}) were used for calibration. All data were processed using the Data Explorer (Applied Biosystems), while the Polymerix (Sierra Analytics) software package was used to determine M_n.

Details of other methods of analysis (NMR and size exclusion chromatography (SEC)) can be found in the Supporting Information.

Results and Discussion

Synthesis. The dendrimer-like PEO structures were prepared following a procedure recently reported.[11] Briefly, this could be achieved by repeating several times a sequence of three reactions that were (i) the anionic ring-opening polymerization of ethylene oxide from a precursor possessing a precise number of hydroxyl groups followed by (ii) the introduction of allylic functions at the PEO chain ends and finally (iii) the bishydroxylation of these allylic end groups to introduce two OH functions per PEO arm. This synthetic methodology and the nomenclature of the different compounds are illustrated in Scheme 1.

First of all, a three-arm hydroxy-ended PEO star denoted as PEOG1$(OH)_3$, synthesized from 1,1,1-tris(hydroxymethyl)ethane as precursor, was obtained with a low PDI as determined by size exclusion chromatography (SEC) (Table 1) and a molar mass that was very close to the expected value, confirming the living/controlled character of polymerization of EO.

The introduction of the branching points at the PEO termini was performed by reaction of PEOG1$(OH)_3$ with allyl chloride, leading to a three-arm PEO star with allylic end groups, denoted as PEOG1$(allyl)_3$. The latter star compound was in turn submitted to a bis-hydroxylation reaction using OsO_4 and *N*-methylmorpholine-*N*-oxide. This allowed us to obtain three-arm PEO stars that were end-functionalized with a primary and a secondary hydroxyl group at each arm, further denoted as PEOG1$(OH)_6$ (Scheme 1). By repeating this three-step procedure, dendrimer-like structures up to the eighth generation could be obtained.

The effectiveness of the branching reactions was monitored by ^{1}H NMR spectroscopy (see both Supporting Information and ref 11). Given the accuracy of MALDI-TOF mass spectrometry, it appeared essential to us to also analyze the dendrimer-like PEO samples by the latter technique. The molecular weight data of all investigated samples are given in Table 1.

MALDI-TOF Analysis. In Figure 1, the MALDI-TOF mass spectra of second-generation PEO dendrimers with different end groups are depicted. MALDI-TOF mass spectra of the second-generation samples were recorded using dithranol as a matrix, and NaI was used as cationating agent. All three polymers showed quite narrow PDI's. Figure 1 shows that the distributions shift to slightly higher mass, in the region between 8500 and 9500 Da, with increasing mass of the end groups. This observation is also confirmed by M_n(MALDI-TOF) values, which increase in the order PEOG2$(OH)_6$, PEOG2$(allyl)_6$, and PEOG2$(OH)_{12}$. This increase mirrors the total molar mass of all end groups, which varies from 342.2 Da for PEOG2$(OH)_6$, to 582.4 Da for PEOG2$(allyl)_6$, and to 786.4 Da for PEOG2$(OH)_{12}$. The values of the end groups are the result of the sum of the atom masses in the structure, which are not part of the repeating unit.

To confirm the quantitative end-group functionalization, the isotope distributions of the signals in the MALDI-TOF mass spectra were investigated. The magnifications of the spectra of PEOG2$(OH)_6$, PEOG2$(allyl)_6$, and PEOG2$(OH)_{12}$ are presented in Figure 2. Despite the relatively high molar mass of all branched structures, all spectra could be recorded with sufficiently high resolution to allow a detailed analysis. For PEOG2$(OH)_6$ and PEOG2$(allyl)_6$ a resolving power up to respectively 12 600 and 10 200 could be obtained, which resulted in the observation of resolved isotopes. For all

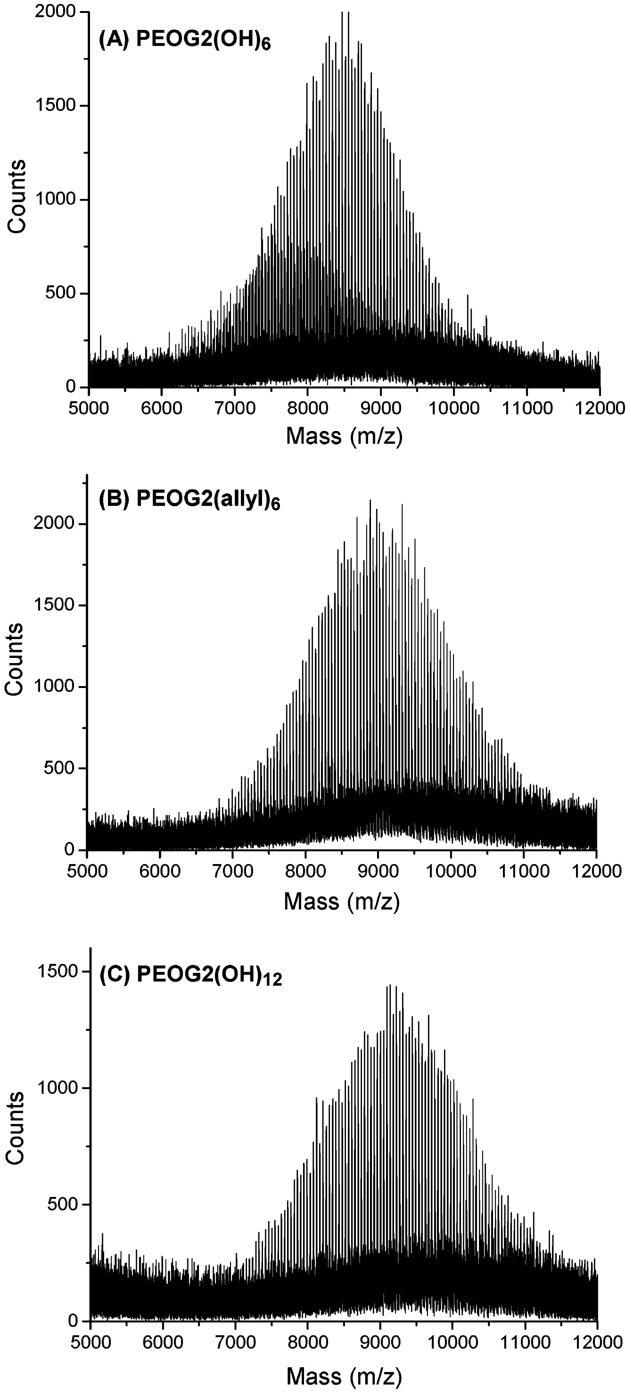


Figure 1. Matrix-assisted laser desorption/ionization time-of-flight mass spectra of second-generation poly(ethylene oxide) (PEO) dendrimers with (A) 6 OH (PEOG2(OH)$_6$), (B) 6 allyl (PEOG2(allyl)$_6$), and (C) and 12 OH (PEOG2(OH)$_{12}$) end groups. All spectra were measured using dithranol as a matrix and NaI as cationating agent.

three polymers, the distance between the individual peaks is 44 Da, equal to the molar mass of the repeating unit in PEO. For PEOG2(OH)$_6$, two different series could be detected. From the comparison between the measured and the theoretical isotope distributions, both signals could be attributed to the sodium and potassium adduct of the expected hydroxyl-functionalized PEO dendrimer. The presence of the potassium adduct could be explained by the use of the latter cation during synthesis. No other series of products with defects in their dendrimer structure could be detected. This again illustrates on one hand the well-controlled synthesis of

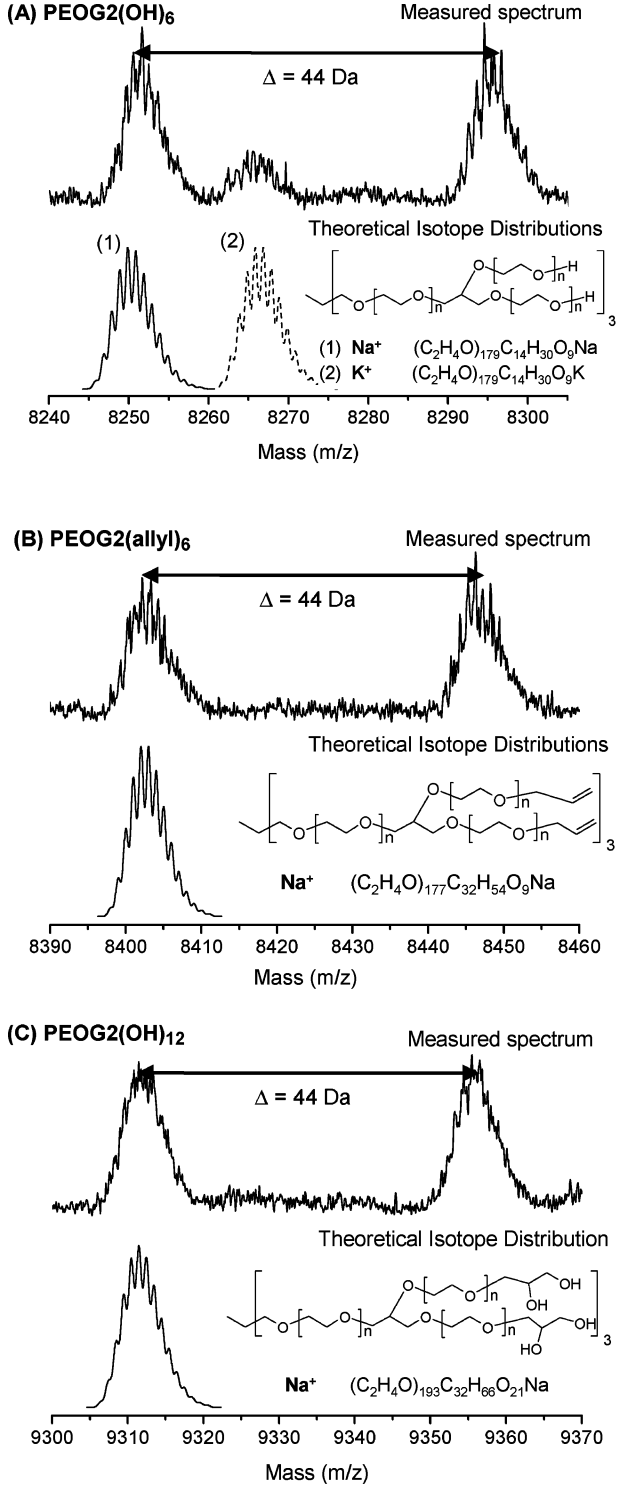


Figure 2. Magnification of the matrix-assisted laser desorption/ionization time-of-flight mass spectra of second-generation poly(ethylene oxide) (PEO) dendrimers with (A) 6 OH (PEOG2-(OH)$_6$), (B) 6 allyl (PEOG2(allyl)$_6$), and (C) and 12 OH (PEOG2-(OH)$_{12}$) end groups. Experimental and theoretical isotope distributions are shown for each sample. All spectra were measured using dithranol as a matrix and NaI as cationating agent.

those complex molecules and on the other hand the power of MALDI-TOF to analyze such structures.

The complete end-group transformation of PEOG2-(OH)$_6$ to allyl-functionalized PEO (PEOG2(allyl)$_6$) stars can also be observed in Figure 2b. No series of the starting product or product with partially remaining OH groups could be detected. Finally, the subsequent quan-

Table 2. Measured and Theoretical Masses of the Second-Generation Poly(ethylene oxide) (PEO) Dendrimers with (A) 6 OH (PEOG2(OH)$_6$), (B) 6 Allyl (PEOG2(allyl)$_6$), and (C) and 12 OH (PEOG2(OH)$_{12}$) Groups

PEOG2(OH)$_6$[a]		PEOG2(allyl)$_6$[b]		PEOG2(OH)$_{12}$[c]	
theor masses (*m/z*)	measd masses (*m/z*)	theor masses (*m/z*)	measd masses (*m/z*)	av theor mass (*m/z*)	av measd mass (*m/z*)
8246.911	8246.897	8400.013	8400.210	9312.080	9312.029
8247.900	8247.908	8401.016	8401.175		
8248.893	8248.913	8402.019	8402.211		
8249.888	8249.985	8403.022	8403.151		
8250.882	8250.925	8404.025	8404.144		
8251.879	8251.902	8405.029	8405.212		

[a] Values for the signal with formula $(C_2H_4O)_{179}C_{14}H_{30}O_9Na$. [b] Values for the signal with formula $(C_2H_4O)_{177}C_{32}H_{54}O_9Na$. [c] Values for the signal with formula $(C_2H_4O)_{193}C_{32}H_{66}O_{21}Na$.

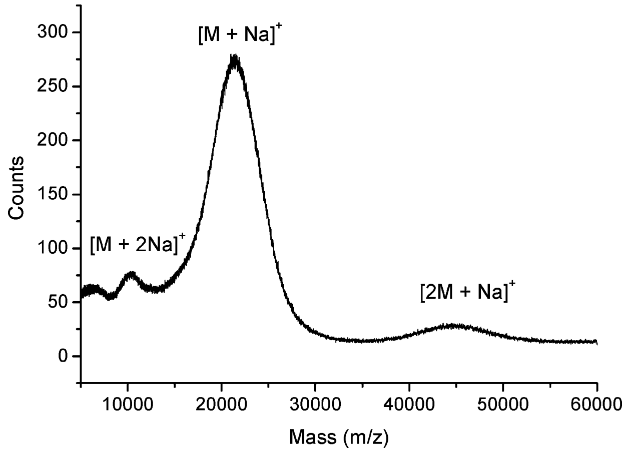


Figure 3. Matrix-assisted laser desorption/ionization time-of-flight mass spectrum of third-generation poly(ethylene oxide) (PEO) dendrimer with 24 OH end groups (PEOG3-(OH)$_{24}$). This spectrum was measured using *trans*-2-[3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene]malononitrile as a matrix and NaI as cationating agent.

titative reaction from PEOG2(allyl)$_6$ to PEOG2(OH)$_{12}$ could also be confirmed (Figure 2c). Again, only one series of the expected product was observed.

In Table 2, the measured and theoretical masses of the six isotopes with the highest intensity of one measured signal are compared for PEOG2(OH)$_6$ and PEOG2(allyl)$_6$. Mass errors in the low ppm range (about 20 ppm) were obtained. For PEOG2(OH)$_{12}$, it was not possible anymore to obtain isotopic resolved mass spectra. However, the measured average mass was in good agreement with the theoretically expected one (Table 2).

In Figure 3, the mass spectrum of the third-generation dendrimer-like PEO with 24 OH groups (PEOG3-(OH)$_{24}$) is depicted. Similar spectra were obtained for the other products of third generation (see Supporting Information). The mass spectra have been recorded using BMPM as a matrix and NaI as cationating agent. By using BMPM, the laser intensity could be reduced, which allowed the analysis of high molecular weight compounds.[21,22] Three distributions could be observed, of which the main distribution at 20 500 g mol^{-1} corresponds to the expected molecular weight of PEOG3-(OH)$_{24}$. The two smaller distributions at 10 000 and 40 000 g mol^{-1} can be ascribed to respectively a double-charged molecule and a single-charged dimer. Similar MALDI-TOF observations have been described earlier for other polymer structures.[18,23] Because of the high molecular weight of the samples, no resolution of the signals could be obtained. Nevertheless, the confirmation of the molecular weight by MALDI-TOF gives additional proof of the effectiveness of the synthetic method.

Conclusion

MALDI-TOF mass spectrometry was used to analyze dendrimer-like PEO's of second and third generation, which were synthesized in a controlled way with different end groups. It was shown that all polymer structures exhibited narrow molar mass distributions. Detailed analysis of the isotope distributions of the polymers confirmed that well-defined structures were prepared with quantitative end-group functionality. Hence, it was shown that MALDI-TOF is indeed a powerful technique to characterize complex branched macromolecules.

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Supporting Information Available: Experimental information for the preparation of the dendrimer-like poly(ethylene oxide)s. This material is available free of charge via the Internet at http://pubs.acs.org.

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