Synthesis of Heterotelechelic Poly(ethylene glycol) Macromonomers. Preparation of Poly(ethylene glycol) Possessing a Methacryloyl Group at One End and a Formyl Group at the Other End

Yukio Nagasaki, Ryutaro Ogawa, Sei Yamamoto, Masao Kato, and Kazunori Kataoka*

Department of Materials Science and Technology, Science University of Tokyo, Noda 278, Japan

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ABSTRACT: Poly(ethylene glycol) (PEG) having a polymerizable methacryloyl group at one end and an aldehyde group at the other end was quantitatively synthesized. Potassium 3,3-diethoxy-1-propanoxide initiated the anionic polymerization of ethylene oxide to form an acetal-ended PEG having potassium alkolate at the ω chain end. By adding methacrylic anhydride to the reaction mixture, the ω end was quantitatively converted to the methacryloxy group. When the PEG with an acetal group at one end and a methacryloyl group at the other end was treated with 90% acetic acid solution, the hydrolysis of the acetal end group proceeded to form a PEG macromonomer possessing an aldehyde end group. The end groups were characterized by ^{1}H NMR and MALDI-TOF-MS spectroscopies. Copolymerization of the aldehyde-PEG macromonomer with methacrylate monomers quantitatively gave the PEG graft polymer with an aldehyde group at the free end of the graft chain.

Introduction

Recently, end-reactive poly(ethylene glycol)s (PEG) have become more and more important in a variety of fields such as biology, biomedical science, and surface chemistry, due to their unique properties such as solubility and flexibility of the chains and basicity of the ether oxygens in the main chain.[1,2] One of the most important utilizations of PEG is the construction of polymer brushes,[3] a densely packed layer of tethered polymers anchored on the surface utilizing the end-functionality of the polymer chain. Such a PEG brush significantly changes the surface properties. For example, a PEGylated surface, which means that the poly(ethylene glycol) chains are densely packed on a surface and attached by the end of the polymer chain, shows effective rejection of protein adsorption resulting in a good blood compatibility.[1,4−6] A PEGylated surface is also utilized as a capillary for high performance capillary electrophoresis.[7,8]

To construct polymer brushes on the surface, two major approaches are available. One is direct chemical bonding by the end of the polymer chain.[1,9] The other is physical adsorption of the block and/or graft copolymers.[1,10] In the latter case, a segment other than PEG adsorbs on the surface by certain forces such as hydrophobic and ionic interactions to form a PEG brush. Semitelechelic PEGs (including macromonomers) have been utilized for the preparation of these block or graft copolymers. In general, commercially available methoxy-ended PEGs having a hydroxyl group at the other terminal are utilized as the starting material for the semitelechelic PEG preparations. Thus, these PEG surface brushes possess inert free end groups. If certain reactive groups can be introduced to the free ends of the brush, an opportunity for the PEG brush will be expanded. For example, the introduction of an affinity ligand to the brush free end changes the surface to be utilized for affinity separation, keeping a low nonspecific adsorption.

Few approaches have been done to introduce reactive groups at the brush free ends. Most of the approaches are based on the conjugation of one of the two identical end groups of the homotelechelic PEG on the surface. In these cases, however, several defects in the tethered structure, such as loop formation through the reaction of both ends of the PEG, may be unavoidable. Such defects may have a serious negative effect on the performance of the PEG brush surface.

To create a PEG surface brush having a reactive group at free brush ends, a new molecular design must be required for PEG telechelics and their copolymers. For these objectives, a heterobifunctional PEG, which is defined as a PEG having a reactive group at one end and another reactive group at the other chain end,[11] will become a powerful tool. There are several reports on the synthesis of heterobifunctional PEGs using homotelechelic PEG as the starting material.[12] The synthetic methods, however, are complicated because they have to use several reaction steps to derivatize the PEG terminus. In addition, the efficiency for derivatizations is not very high, meaning that the resulting PEG is a partial mixture of starting homotelechelics and the resulting heterotelechelics.

Ito et al. reported the preparation of α-hydroxy-ω-methacryloxy-PEG macromonomers.[13] This is an important tool for tethered chain preparation, as stated above. The hydroxyl groups at the free chain ends of the tethered chains, however, do not show enough reactivity in terms of versatile modification reactions. Our idea was to incorporate reactive groups at the free chain ends of the tethered chains. For this objective, we started to prepare a poly(ethylene glycol) possessing an aldehyde group at one end and a methacryloyl group at the other end. The aldehyde group is known to show a high reactivity with a primary amino group and thiol group and to be stable in aqueous media. Our strategy for heterotelechelic polymer synthesis is starting from a polymerization of ethylene oxide (EO) using new initiators containing defined functionalities.

So far, we have synthesized several types of heterotelechelic PEGs such as NH_2-PEG-OH, CHO-PEG-

* To whom all correspondence should be addressed.

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OH, etc.[11,14−16] This synthetic technique can be applicable to new reactive-PEG brush surface creation. In this paper, we describe the synthesis of aldehyde-ended PEG macromonomers that possess a polymerizable methacryloyl group at the other chain end as well as synthesis of a comb-type graft copolymer using aldehyde-ended PEG macromonomers as one of the comonomers. The results of MALDI-TOF-MS analysis of the obtained aldehyde-ended PEG macromonomers are also described.

Experimental Part

Materials. Commercial tetrahydrofuran (Wako, THF), methanol (Wako), 3,3-diethoxy-1-propanol (Aldrich), methacrylic anhydride (Aldrich), and methyl methacrylate (Wako) were purified by conventional methods.[17] EO (Saisan) was dried over calcium hydride and distilled under an argon atmosphere. Potassium naphthalene was prepared according to a previous paper[18] and the concentration determined by titration. Azobis(isovaleronitrile) (V-65) was recrystallized by methanol and then dried under a vacuum. Other reagents were used as received.

Polymerization Procedure. A glass vessel equipped with a three-way stop cock was degassed and argon gas introduced. This cycle was repeated three times; then 45 mL of THF, 0.47 mL of 3,3-diethoxy-1-propanol (0.44 g, 3.0 mmol), and 8.0 mL of potassium naphthalene (3 mmol, 0.38 mol/L) were added via a syringe. After a few minutes agitation to form potassium 3,3-diethoxy-1-propanoxide, 11.8 mL of EO (10.5 g, 238 mmol) was added via a cooled syringe. After the mixture was allowed to react for 2 days at room temperature 2.2 mL of methaclylic anhydride (2.3 g, 15 mmol) was added and the solution stirred for a further 24 h. The obtained polymer was precipitated in cooled 2-propanol and separated by centrifugation (5000 rpm; 40 min, −4 °C). The polymer was finally dried by freeze-drying with benzene.

Acid Hydrolysis of the Acetal End Group. A 1.5 g sample of the acetal-ended PEG was dissolved in a 90% acetic acid aqueous solution and allowed to react for 5 h at 30 °C. After the hydrolyzed polymer was extracted into chloroform, the polymer was precipitated in ether. The polymer thus obtained was dried by freeze-drying with benzene.

Copolymerization of Aldehyde-Ended PEG Macromonomers with Methacrylates. A 0.30 g sample of the aldehyde-ended PEG macromonomers (8.5×10^{-2} mmol), 0.3 g of MMA (3.0 mmol), V-65 (7.5 mg; 3.0×10^{-2} mmol), and 3.0 mL of benzene were placed in a glass vessel, the mixture was freezed and degassed, and the glass vessel was sealed for the polymerization.

Analysis. GPC measurements were carried out using a Shimadzu 6A liquid chromatograph equipped with a Shodex gel permeation column (Shodex KD-806M·2) and an internal RI detector (RID-6A). DMF containing 10 mmol L^{-1} of lithium bromide was used as the eluent at a flow rate of 1.0 mL min^{-1} at 40 °C. ^{1}H NMR spectra were obtained using chloroform-*d* solutions (1.0 wt %) with a JEOL EX400 spectrometer at 400 MHz. Chemical shifts relative to $CHCl_3$ (^{1}H: $\delta = 7.26$) were employed. MALDI-TOF-MS spectra were recorded using Bruker Reflex II. 2,5-Dihydroxybenzoic acid (DHB) was used as the matrix for the ionization operated in the reflection mode. Cytochrom C was used for the calibration of the detected ions.

Results and Discussion

After construction of PEG brushes on the surface, reactive groups at the free ends must be usually utilized in aqueous media for conjugation with specific compounds such as proteins. A formyl group is very useful for conjugation with protein due to its stability in water and its rapid reactivity with primary amino groups. In addition, no charge variation takes place by the modification because the resulting Schiff base can be easily converted to a secondary amino group by reduction. No change in charge distribution is considered to keep the modified protein much more native than other techniques. Therefore, an aldehyde group was selected for the introduction as a reactive group at the PEG chain end. For introduction of an aldehyde group at the α-terminal of a PEG chain, potassium 3,3-diethoxypropanoxide was used as the initiator.

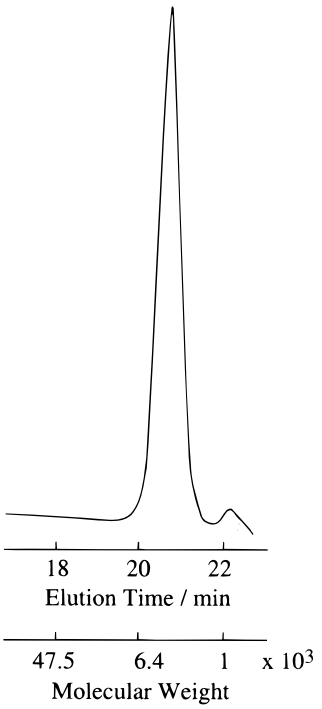


Figure 1. GPC of an acetal-ended PEG macromonomer.

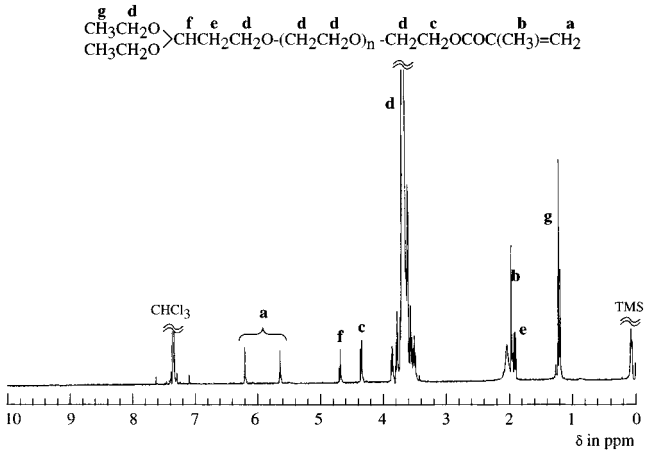
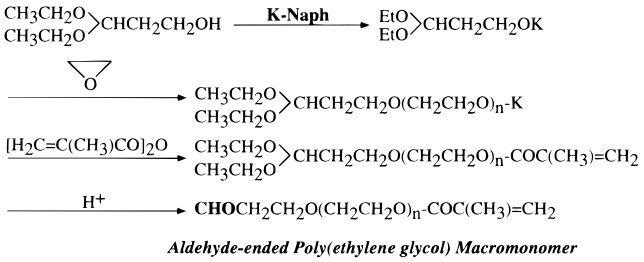


Figure 2. ^{1}H NMR spectrum of the acetal-ended PEG macromonomer (same sample as in Figure 1).

The anionic polymerizations of EO initiated with potassium 3,3-diethoxypropanoxide were carried out in THF solution at room temperature. After a 2 day reaction, an excess amount of methacrylic anhydride was added to the reaction mixture and the mixture was stirred for a further 24 h. Figure 1 shows the GPC profile of the obtained polymer. The MW of the polymer determined from GPC [M_n(GPC) = 3180] was close to that from the initial monomer/initiator ratio [M_n(calc.) = 3660], indicating effective initiation ability of potassium 3,3-diethoxypropanoxide without any side reaction. The closeness of the MW distribution factor to unity ($M_w/M_n = 1.10$) indicated a rapid initiation process by potassium 3,3-diethoxypropanoxide to form a uniformly sized PEG.

Since the polymer chain end is potassium alkanolate if there is no side reaction, methacrylic anhydride as

Scheme 1



Aldehyde-ended Poly(ethylene glycol) Macromonomer

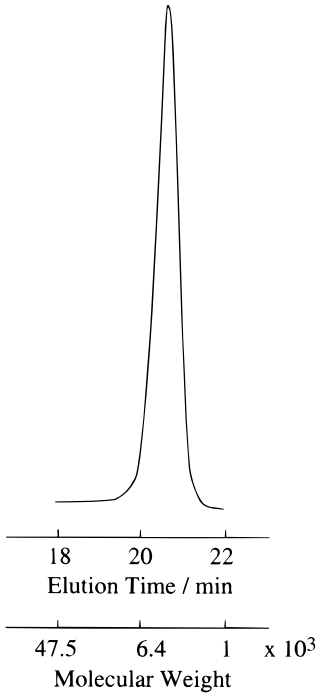


Figure 3. GPC of an aldehyde-ended PEG macromonomer.

an electrophile was added to introduce the methacryloyl group at the ω-chain end. As can be seen in the ^{1}H NMR spectrum of the obtained polymer shown in Figure 2, it is clear that olefin protons appear in the higher frequency field, where the assignments were carried out using MMA, PEG, and 3,3-diethoxy-1-propanol and are described in the figure. The MWs determined from the NMR assuming one methacryloyl per each ω chain end [Mn(NMR-ω)] and one acetal group at the α-terminal [Mn(NMR-α)] were 3820 and 3800, respectively. These results indicate the quantitative delivertization of each end group.

On the basis of these results, it is concluded that heterotelechelic PEG macromonomers having an acetal at one end and a methacryloyl at the other end were quantitatively obtained (Scheme 1).

It is well-known that an acetal group can be easily converted to an aldehyde group by acid treatment.[19] Strong acid treatment of the heteroPEG macromonomers, however, induced serious side reactions. When the acetal-PEG macromonomers were treated with 1 N HCl, the MW distribution in GPC became bimodal, indicating dimerization of the macromonomers, which was attributable to an aldol condensation of two aldehyde end groups in strong acid media. To avoid the intermolecular aldol condensation, hydrolyses of the acetal end group were examined under several reaction conditions. Figure 3 shows the GPC diagram of the PEG macromonomer after the hydrolysis by 90% acetic acid solution for 5 h. Under these conditions, no

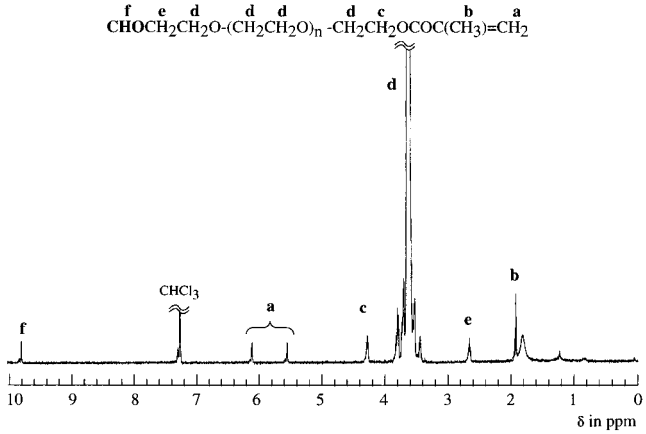


Figure 4. ^{1}H NMR spectrum of the aldehyde-ended PEG macromonomer (same sample as in Figure 3).

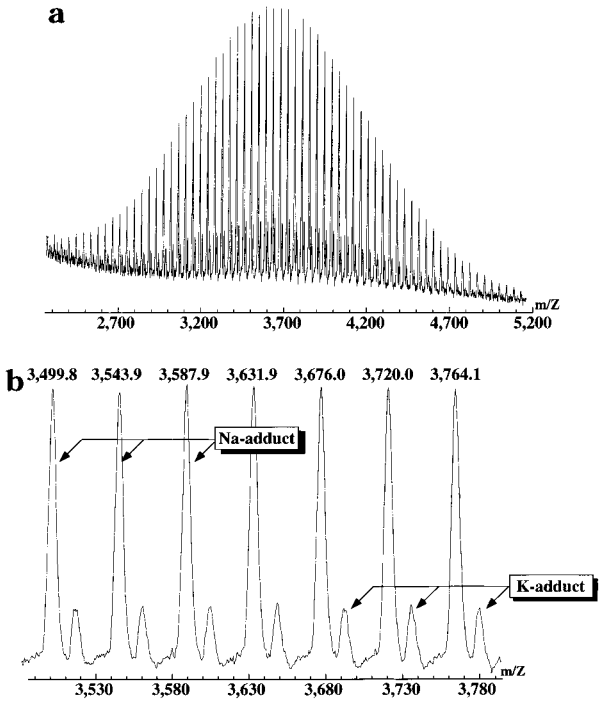


Figure 5. MALDI-TOF mass spectrum of the acetal-ended PEG macromonomer (a) and its expanded spectrum in the region of 3500−3800 (b).

intermolecular reaction took place. In the ^{1}H NMR spectrum of the acetic acid hydrolyzed polymer shown in Figure 4, the acetal protons completely disappeared, indicating complete hydrolysis of the acetal end group. Instead of the acetal protons, a new signal appears around 9.8 ppm, which was attributable to aldehyde protons at the end of the polymer chain. The integral ratio of the aldehyde proton versus olefin protons was 1/2, indicating quantitative conversion to the aldehyde end group.

All the data determined from the calculation using the initial molar ratio, the GPC pattern, the ^{1}H NMR assuming one α-acetal (and/or aldehyde) end group per each polymer molecule, and the ^{1}H NMR assuming one ω-methacryloyl group per each polymer molecule agreed well with each other. From these results, heterotelechelic aldehyde-ended PEG macromonomers must be obtained. However, there is no obvious evidence that each polymer molecule possesses both one aldehyde (and/or acetal) group at the α-chain end and one methacryloyl group at the ω-chain end. The MALDI-

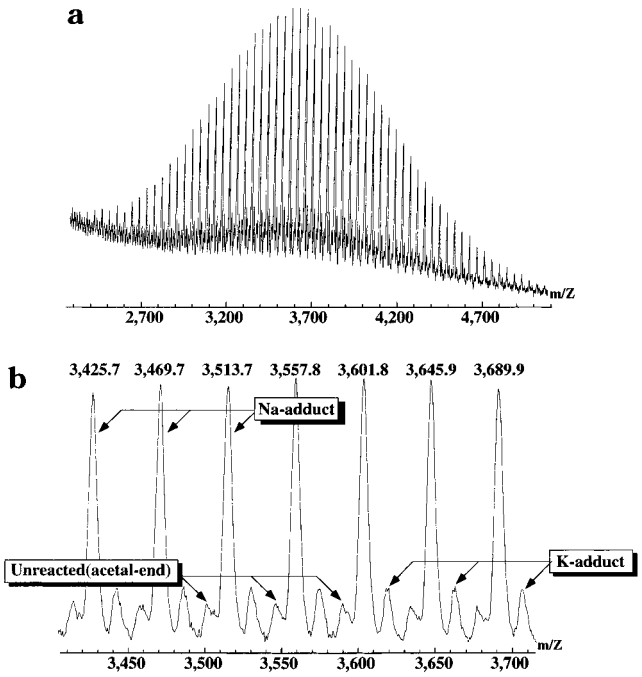


Figure 6. MALDI-TOF mass spectrum of the aldehyde-ended PEG macromonomer (a) and its expanded spectrum in the region of 3400−3750 (b).

Table 1. Molecular Weight Data of APM Determined by Several Analytical Methods

	M_n				M_w		M_w/M_n	
	GPC	NMR-a	NMR-w	MS	GPC	MS	GPC	MS
acetal-APM	3180	3800	3820	3550	3500	3680	1.10	1.04
aldehyde-APM	3560	3780	3710	3620	3810	3720	1.07	1.03

TOF-MS[20] analysis makes it feasible by analyzing the molecular mass of the individual polymer molecule.

Figure 5 shows the MALDI-TOF-MS spectra of acetal-PEG-methacryloyl. From the MALDI-TOF-MS analysis, only parent ions of each polymer molecule are generally observed, meaning no fragment signal can be detected. From the spectrum shown in Figure 5a, the mass of the products appears around 3700 ($M_n = 3550$; $M_w/M_n = 1.03$), which is in good accord with the GPC results. The difference in mass of each signal was roughly estimated as 44, indicating the signals were assignable to the PEG homologues.

In consideration of the MW of the EO monomer and both end groups, the MW of each heterotelechelic PEG should be expressed by the following equation.

$$\underset{\text{EO}}{44.053n} + \underset{\text{acetal}}{147.194} + \underset{\text{methacryloyl}}{69.083} \quad (1)$$

The detected signals were 23 mass units larger than those calculated from eq 1, which are generally known as sodium adduct ions of the products. For example, the center peak in the expanded spectrum (Figure 5b) showed a mass of 3631.9, which agreed well with 77 mers. Small signals appearing at 17 mass units higher than each large signal can be assignable to the potassium adduct, which originated from the initiator for the polymerization.

The conversion of the end acetal group to the aldehyde group could also be monitored by the MALDI-TOF-MS analysis. Figure 6 shows the MALDI-TOF-MS spectra of the heteroPEG after the acid hydrolysis reaction. As mentioned above, the mass distribution of the obtained polymer after acid treatment was the same as that before the acid treatment. No intermolecular reaction based on the aldol condensation reaction was observed.

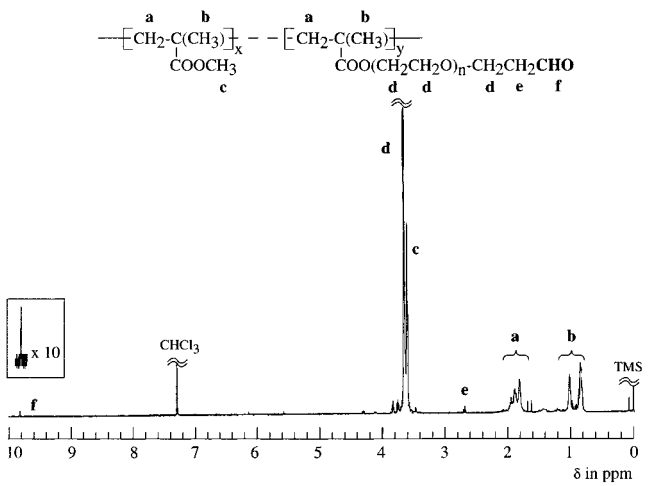


Figure 7. ^{1}H NMR spectrum of poly(MMA-*graft*-PEG-CHO).

The major series of the molecular masses of the polymer is expressed in eq 2, though a small amount of unreacted polymer (acetal-PEG-methacryloyl) was observed in this case as shown in Figure 6b.

$$\underset{\text{EO}}{44.053n} + \underset{\text{aldehyde}}{73.071} + \underset{\text{methacryloyl}}{69.083} + \underset{\text{sodium}}{22.990} \quad (2)$$

On the basis of these results, it is concluded that the poly(ethylene glycol) that possesses an aldehyde group at one end and a methacryloyl group at the other chain end was actually synthesized in this technique. The molecular weight data of aldehyde-ended PEG macromonomers determined from several analyses are summarized in Table 1.

In order to verify if the aldehyde-ended PEG macromonomers thus obtained can be incorporated into a polymer matrix as the graft chain, retaining aldehyde free end groups, radical copolymerization with MMA was investigated. Radical copolymerization of the aldehyde-ended PEG macromonomers with MMA homogeneously proceeded. The obtained polymer was soluble in several organic solvents such as THF, toluene, and benzene and was insoluble in water. From the GPC profile of the obtained polymer, it was found that the polymer with a higher MW ($M_w = 8.8 \times 10^4$) than that of the macromonomer was formed, though a small amount of the macromonomers remained. Figure 7 shows the ^{1}H NMR spectrum of the obtained copolymer. Based on the quantitative analysis of the composition of the obtained polymer using the yield of the polymer, the integrated ratio of the macromonomers and PMMA signals in Figure 7, MMA conversion determined by GC, ca. two macromonomer chains were incorporated in each 100 MMA units of the obtained copolymer. Aldehyde groups were found to remain intact after the copolymerization with MMA, which was verified in the NMR spectrum; *viz.*, the protons of the aldehyde groups appear at 9.8 ppm in Figure 7.

In conclusion, aldehyde-ended PEG macromonomers were quantitatively prepared, which can be introduced into the polymethacrylate backbone as a graft chain. The copolymer thus obtained possesses a reactive aldehyde group at each graft chain end.

Therefore, the heterotelechelic poly(ethylene glycol) macromonomer prepared using this methodology can be

anticipated as one of the new methods for creation of tethered PEG brushes that possess a reactive group at the free end of the tethered chain. This molecular design can be utilized for a high-performance surface design that shows specific interaction with a biomolecule without nonspecific interaction.

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