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Adamantyl-terminated dendronized molecules: synthesis and interaction with β-cyclodextrin-functionalized poly(dimethylsiloxane) interface†

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The supramolecular interaction between the host molecule β-cyclodextrin (β-CD) and the guest molecule adamantane is extensively applied in several disciplines. However, recent studies on the applications of this molecular recognition mainly focused on glass, silicon, and gold substrates. Few studies have explored the field of poly(dimethylsiloxane) (PDMS), an optically transparent elastomer widely used in biological microfluidic devices. In the present study, various functional group-modified adamantyl-terminated dendronized molecules were synthesized *via* an efficient and facile route. The binding of adamantyl-terminated dendronized molecules onto β-CD-conjugated PDMS surfaces and their reversible dissociation from PDMS surfaces through the competitive mechanism of β-CD-N_3 were studied. The results showed that the dissociation is relatively slow compared with the binding, and the former process can be accelerated through exchanging the original β-CD-N_3 solution with a fresh one. Afterward, more complex multilayer assemblies were constructed on PDMS surfaces using the host–guest interaction between adamantane and β-CD as well as between biotin and streptavidin. A further study on the association and dissociation of the established multilayer assemblies showed that the assembly constructed with a biotinylated monoadamantyl-terminated molecule has a reversible property, whereas that with a biotinylated triadamantyl-terminated molecule has an irreversible property. These results demonstrated that the reversible properties of the fabricated assembly can be easily modulated on the PDMS surfaces by regulating the valence of the supramolecular interaction between adamantane and β-CD.

Introduction

In recent years, β-cyclodextrin (β-CD)-based supramolecular chemistry has been extensively applied in several fields, such as chromatography,[1] controlled drug release,[2] and biosensors,[3] which stem from the strong combination of β-CD with a variety of hydrophobic organic molecules through different binding affinities.[4] Compared with the classical methods of covalently conjugating molecules, the fabrication of a highly ordered molecular system through supramolecular interaction has exploited new prospects for the modulation of molecular interactions and the construction of novel functional materials.[5] Adamantane, commonly used as a guest molecule of β-CD, can precisely anchor into the hydrophobic β-CD cavity.[6] This supramolecular association can also be detached in an β-CD aqueous solution *via* a competitive mechanism.[7] More importantly, β-CD has low toxicity, is biodegradable and biocompatible, and hardly has a negative impact on biological activity.[8] Moreover, this binding strength between β-CD and adamantane can be tuned by varying the valence of the host–guest interaction. The monovalent association constant of this host–guest interaction is approximately 3×10^4 M^{-1} (T = 298 K).[6a,9] The multivalent association of the host–guest interaction involves simultaneous interactions between multiple functionalities on one entity and complementary functionalities on another, which is typically several orders of magnitude stronger than the monovalent one.[7a,10] The application of the multivalent host–guest interaction makes the formation of a kinetically

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stable assembly possible. This kind of interaction has gained increasing interest, particularly in biochemistry, because it provides an approach to finely tune the overall interaction strengths and has a pivotal role in sundry vital movement.[11] To date, the supramolecular interaction between β-CD and adamantane has been utilized to build nanometer arrays of functional light-harvesting antenna complexes,[12] immobilize desired proteins,[13] control the shape of a peptide-decorated vesicle,[14] detect anti-gliadin autoantibodies in celiac patient samples or the anthrax biomarker,[3a,15] and so on. All of these studies have greatly shown the potential of the host–guest chemistry in constructing various bioanalytical systems. However, recent studies on the applications of this molecular recognition mainly focused on glass, silicon, and gold substrates. Few studies have explored the field of poly(dimethylsiloxane) (PDMS), an optically transparent elastomer widely used in biological microfluidic devices because of its prominent advantages, such as non-toxicity, easy fabrication, practical scalability, and gas permeability.[16] In addition, previous bioanalytical studies indicated that occupying the hydrophobic cavity of β-CD with monovalent supramolecular blocking agent hexa(ethylene glycol) mono(adamantyl ether)[17] or adding a certain concentration of β-CD in a protein solution[18] is always needed in advance to suppress nonspecific protein adsorption on β-CD-conjugated surfaces. In our previous work,[19] we presented a strategy for the preparation of a dually functionalized PDMS surface (PDMS-PEG-CD) by conjugating β-CD onto PDMS surfaces *via* click chemistry and surface-initiated atom transfer radical polymerization of poly(ethylene glycol) (PEG) units. The prepared PDMS-PEG-CD surface possesses a reversible property; however, it also has a good protein-repelling property because of the introduction of PEG units in the β-CD-based supramolecular chemistry system.

To realize various biological applications on β-CD-functionalized interfaces through the supramolecular interaction between β-CD and adamantane, adamantane must be linked with the desired molecules to indirectly anchor them on β-CD-functionalized interfaces. Previous studies used carboxymethyl cellulose as a bridge to conjugate adamantane and the desired molecules[20] because it possesses some typical properties of polysaccharides, such as effectively preventing nonspecific protein adsorption, excellent biocompatibility, as well as low toxicity.[16b,21] For example, O'Sullivan *et al.* built a nanostructure using the interfacial self-assembly of bifunctionalized carboxymethyl cellulose bearing adamantane unit and an antigenic fragment onto a CD-containing support and realized its applicability in the detection of anti-gliadin antibodies.[3a] As a potential alternative to carboxymethyl cellulose, PEG also has the same advantages in protein repelling.[22] Reinhoudt *et al.* used 3,5-dihydroxybenzonitrile as the molecule core and PEG as the linker spacing to prepare the supramolecular patterning of β-CD monolayers on silicon oxide *via* microcontact printing and dip-pen nanolithography.[23] However, they used Raney cobalt as a catalyst and hydrogen as a reducing reagent under relatively harsh conditions to convert the nitrile functionality to an amino group, a prerequisite to further conjugate desired molecules.

Considering the abovementioned analyses, as well as our previous studies on PDMS surface modification,[16,19] an efficient and facile route to synthesize various adamantyl-terminated dendronized molecules, including fluorescent molecule-, carbamate nitrophenyl ester-, and biotin-containing adamantane derivatives, is described in this study. Meanwhile, the reversible dissociation of the adamantane derivatives from the pre-prepared PDMS-PEG-CD surfaces was investigated through the competitive mechanism of β-CD-N_3. Finally, using the supramolecular interaction between adamantane and β-CD and between biotin and streptavidin (SAv), more complex assemblies were constructed on PDMS surfaces. The reversible property of the constructed assembly on the PDMS surfaces was also studied by adjusting the valence of the supramolecular interaction, that is, using a biotinylated triadamantyl-terminated or a monoadamantyl-terminated molecule in the assembly.

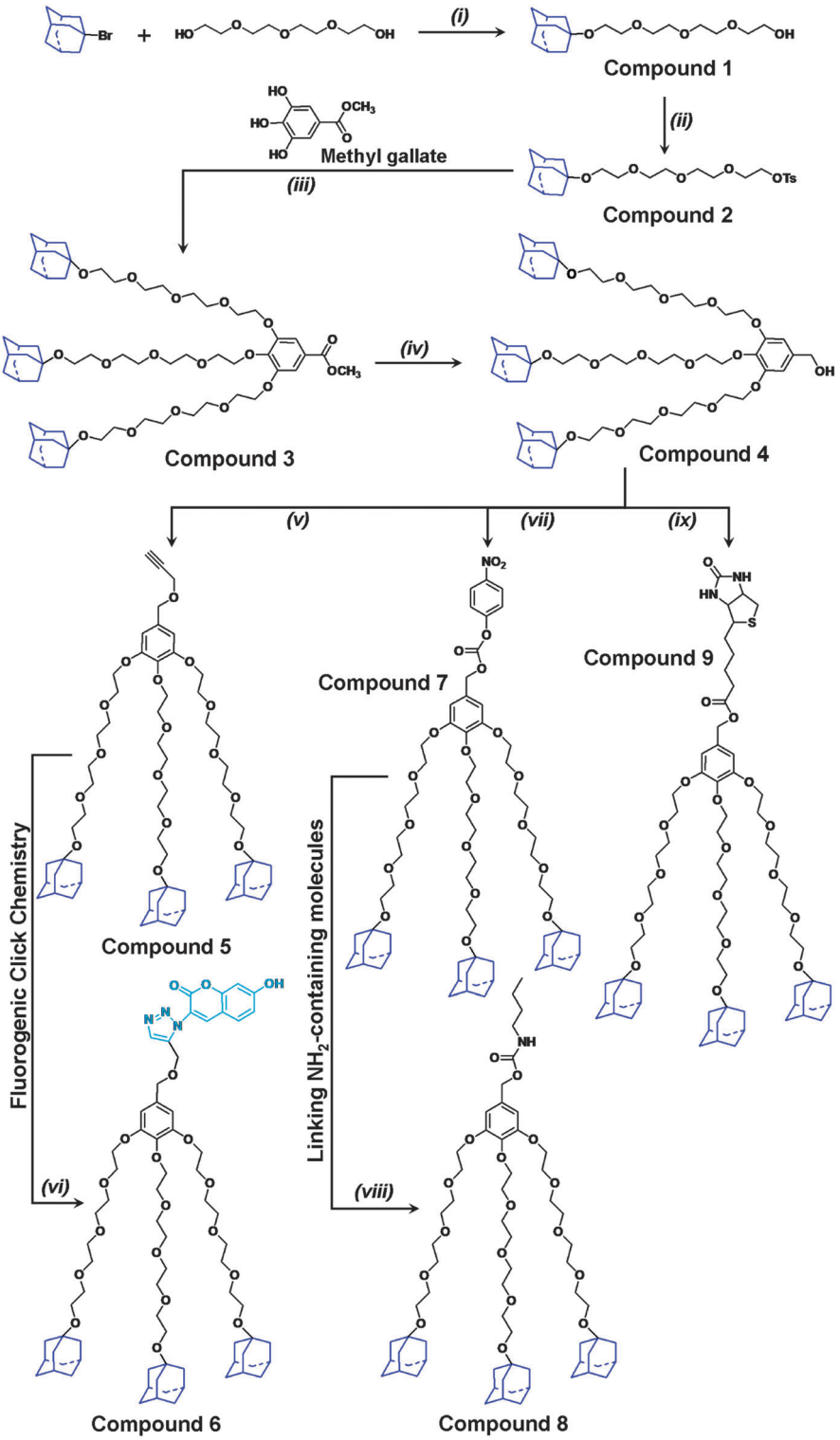
Results and discussion

Synthesis of functionalized adamantyl-terminated dendronized molecules

In this study, methyl gallate was used as the starting material to synthesize various functionalized adamantyl-terminated dendronized molecules because it has three phenolic hydroxyl groups at its 3-, 4-, and 5-positions (which can be used to link three adamantane moieties on its benzene ring) and one methyl ester (which can be used to link one desired molecule on its benzene ring). The dendritic wedge was synthesized *via* a convergent growth strategy (Scheme 1), *i.e.*, starting from the adamantane periphery and working inward toward what is to be further derived to link another molecule.

We first synthesized 2-(2-(2-(2-(adamantyl-1-oxy)ethoxy) ethoxy)ethoxy)ethanol *p*-toluenesulfonate (Scheme 1, Compound **2**) through a two-step reaction.[7c] The PEG unit in this structure has three functions: (1) improves the solubility of the target molecules in an aqueous solution; (2) effectively prevents or decreases nonspecific interaction in a further biological application because of its well-hydrated nature; and (3) allows the multiple complexation with β-CD to take place for enough chain length.[5,24] Several reaction conditions were tested to simultaneously link three adamantane moieties onto one methyl gallate skeleton (Scheme 1, Compound **3**). The results indicated that the reaction can be completed in 24 h with a high yield of 84% under dry potassium carbonate as base, 18-crown-6 as phase transfer catalyst, dry acetone as solvent, and the 4 : 1 ratio of compound **2** and methyl gallate. Finally, lithium aluminum hydride (LAH) was used as the reducing reagent to transform methyl ester to alcoholic hydroxyl group (Scheme 1, Compound **4**), which can further react with several other functional groups to covalently link another molecule onto the benzene ring of the compound **3**.

Fluorescence imaging is a powerful and sensitive microscopic technique that allows the study of molecules at the monolayer level and provides the possibility of imaging sites on a restricted area for the detection of on-surface reaction.[25] We introduced the fluorescent moiety 3-azido-7-hydroxy coumarin



Scheme 1 Synthesis of various functionalized triadamantyl-terminated dendronized molecules: (i) Et_3N, 180 °C, 24 h; (ii) Et_3N, DMAP, CH_2Cl_2, TsCl, rt; (iii) K_2CO_3, 18-crown-6, acetone, reflux, 24 h; (iv) LAH, THF, 50 °C, 6 h. (v) NaH, propargyl bromide, THF, overnight, rt; (vi) CuBr, PMDETA, 3-azido-7-hydroxy coumarin, DMF, rt, 24 h; (vii) 4-nitrophenyl chloroformate, Et_3N, CH_2Cl_2, rt, 12 h; (viii) DIPEA, *n*-butylamine, THF, 5 h; (ix) D-biotin, EDC, DMAP, DMF, rt, overnight.

onto the triadamantyl-terminated dendrimeric compound **4** (Scheme 1) *via* fluorogenic click chemistry to visually detect the interaction between adamantane and β-CD-anchored PDMS-PEG-CD surfaces.[26] First, the alkynyl group was introduced to compound **4** using propargyl bromide as the electrophilic reagent; this reaction was smoothly completed overnight with a high yield of 91% (Scheme 1, compound **5**). Afterward, 3-azido-7-hydroxy coumarin was conjugated onto the alkynyl-ended compound

5 as the fluorogenic substrate through click chemistry using CuBr/PMDETA as the catalysis system; we obtained the fluorescent target product as a pale yellow liquid (Scheme 1, compound **6**). However, when the reaction flask was placed under an ultraviolet lamp (365 nm), only a slight fluorescence was observed. The reason for this phenomenon is that Cu(II) can quench the fluorescence of the triazole product of fluorogenic click chemistry.[27] Therefore, the organic phase of this reaction was thoroughly washed using the Cu(II)-chelator ethylenediaminetetraacetic acid (EDTA) aqueous solution (log K = 18.76) until the blue aqueous phase became colorless before purification *via* column chromatography.[28]

Thereafter, the photophysical property of compound **6** was extensively studied. The results (Fig. S1, S2 and S3, ESI†) demonstrated that this compound has the following photophysical properties: (1) a maximum emission at 478 nm with a very large Stokes shift ($\Delta\lambda$ = 7.81 × 10^3 cm^{-1}) in 10 μM β-CD solution (Fig. S1, ESI†) that can effectively decrease the interference of excitation light;[29] (2) a linear correlation (r = 0.99) in the concentration range of 0.05 to 1.0 μM (Fig. S2, ESI†); and (3) no obvious photobleaching over a long period (20 min) under a continuous intensive excitation with a 150 W xenon lamp (Fig. S3, ESI†). These characteristics proved that compound **6** is suitable for studying the interaction between the adamantane and the PDMS-PEG-CD surface using fluorescence imaging.

We also tried to activate the benzyl alcohol group of compound **4** using 4-nitrophenylchloroformate by forming a carbonate group (Scheme 1, compound **7**) to conveniently link other molecules on the triadamantyl-terminated molecule because the terminal carbonate group reacts with various amino-ended molecules (*e.g.*, proteins) to form stable urethane linkages.[30] As an example, we used *n*-butylamine as a reactant model because its similar structure with the side chain of lysine, the sole amino acid with the side chain of primary amine in all proteins.[31] Under the catalysis of diisopropylethylamine (DIPEA), this reaction can be completed in 5 h, accompanied with the appearance of yellow 4-nitrophenol, indicating the success of this reaction (Scheme 1, compound **8**). Moreover, this reaction can also proceed at room temperature, importantly, in sodium phosphate buffer and pH 8.0 (verified by thin layer chromatography). This condition benefits the real application of ligation between the desired proteins and compound **7** because a common organic solvent is usually destructive and can cause the denaturation of proteins.

Given that the biotin–SAv affinity couple represents a widely used system for various bioconjugations,[32] the biotinylated triadamantyl-terminated dendronized molecule in the current study was also prepared. Using compound **4** as a starting material and carbodiimide chemistry as the conjugating method, we obtained biotin-functionalized triadamantyl-terminated dendronized molecule (Scheme 1, compound **9**).

Study of the reversible interaction between triadamantyl-terminated dendronized molecule and PDMS-PEG-CD interface

After identifying the photophysical property of compound **6**, the supramolecular interaction between compound **6** and PDMS-PEG-CD interfaces[19] was investigated under a fluorescence microscope (Fig. 1, left). The pre-prepared PDMS-PEG-CD slides (0.5 × 0.5 cm) were first immersed in 0.2 mM β-CD-containing aqueous solution (50 μM) of compound **6** for a certain time in the dark. After washing with deionized water, the fluorescence images of the post-interaction PDMS-PEG-CD surfaces were captured, and their fluorescence intensity was analyzed. Curve i in Fig. 2A shows the relationship between the incubation time of the PDMS-PEG-CD slides with the fluorescent compound **6** and the fluorescence intensity of these post-interaction

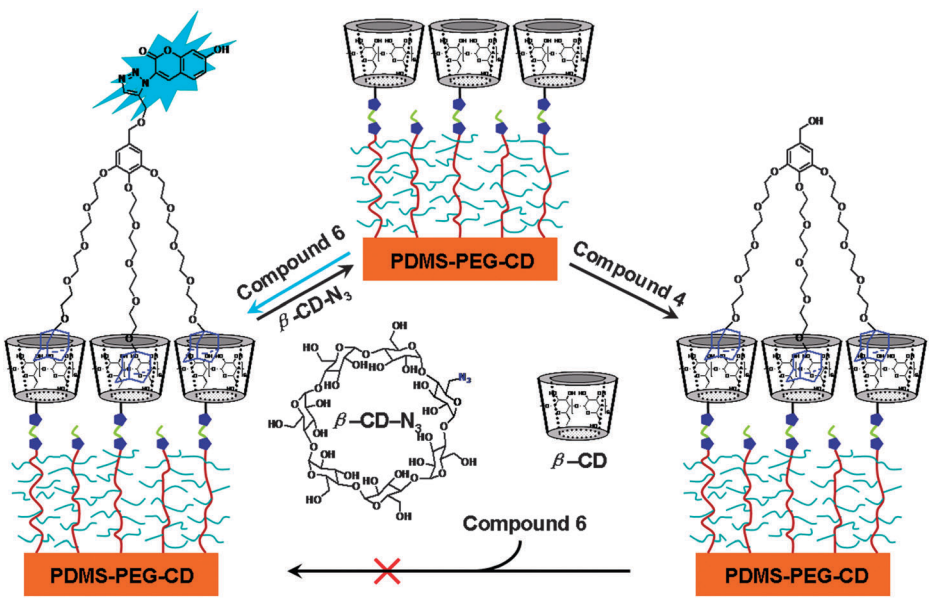


Fig. 1 Illustration of the reversible supramolecular interaction between PDMS-PEG-CD interface and triadamantyl-terminated dendronized molecules through the competitive mechanism of β-CD-N_3.

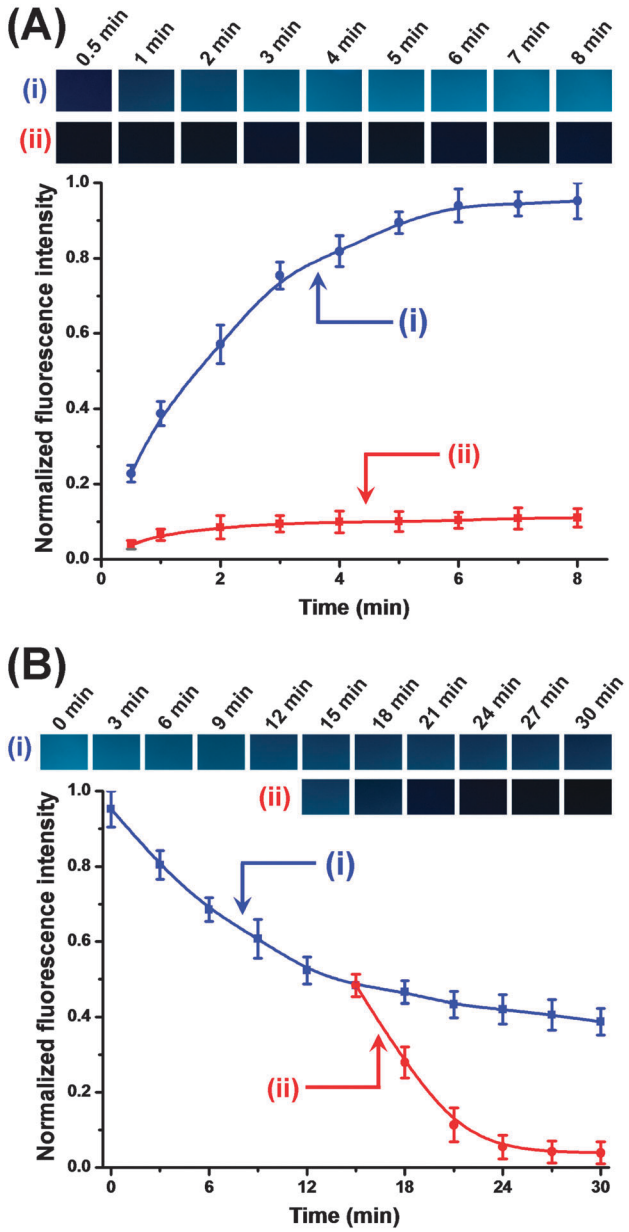


Fig. 2 (A) Effect of incubation time between fluorescent triadamantyl-terminated molecule (compound **6**) and PDMS-PEG-CD surfaces on the surface fluorescence intensity. The top images are their corresponding fluorescence images at different time points. (i) No pre-incubation of the PDMS-PEG-CD surfaces with nonfluorescent triadamantyl-terminated molecule compound **4**; (ii) pre-incubation of the PDMS-PEG-CD interfaces with nonfluorescent triadamantyl-terminated molecule compound **4**. (B) Effect of incubation time between β-CD-N_3 aqueous solution and post-interaction PDMS-PEG-CD surfaces on the surface fluorescence intensity. The top images are their corresponding fluorescence images at different time points. (i) No alteration of the β-CD-N_3 solution at 15 min; (ii) alteration of the β-CD-N_3 solution at 15 min. Normalization of the fluorescence intensity was performed as follows: the fluorescence intensity of the nonreacted PDMS-PEG-CD interface was first subtracted from those of compound **6** (or β-CD-N_3)-treated PDMS-PEG-CD surfaces, and then divided by the maximum fluorescence intensity obtained in the whole analysis.

surfaces. This curve indicates that the fluorescence intensity of these surfaces increased with prolonged incubation time, and the adsorption was fast because no obvious increase in the intensity was detected after 6 min exposure to the compound **6** solution.

The native PDMS-PEG-CD slides were first incubated with 0.2 mM β-CD-containing aqueous solution of nonfluorescent compound **4** (50 μM) for 8 min to confirm that this result was due to the supramolecular interaction between compound **6** and β-CD conjugated on the PDMS surface, not the physical adsorption of compound **6** on the PDMS-PEG-CD surfaces. After rinsing with deionized water, the slides were then incubated with compound **6** for a certain time. After rinsing with deionized water again, the fluorescence images of the surfaces were also captured and analyzed following the procedures described earlier (Fig. 1, right and bottom). Adamantane derivatives form highly stable inclusion complexes with β-CD;[10c] thus the adamantane moiety of compound **4** should occupy the hydrophobic cavity of β-CD conjugated on PDMS-PEG-CD surfaces and prevent the possible host–guest interactions between the adamantane moiety of compound **6** and PDMS-PEG-CD surfaces. The result (curve ii in Fig. 2A) showed that the fluorescence intensity of these surfaces only had a slight increase with prolonged incubation time, suggesting that merely a small partial replacement of nonfluorescent compound **4** by fluorescent compound **6** occurred, similar with related studies reported previously.[12,17] The results showed that the immobilization of compound **6** on PDMS-PEG-CD surfaces was obviously achieved through the specific interaction between the adamantane moiety of compound **6** and the β-CD conjugated on the PDMS-PEG-CD surfaces.

The PDMS-PEG-CD surfaces were subsequently immersed in the aqueous solution of β-CD-N_3 (5 mL, 50 mM) with gentle shaking for a certain time in the dark after reaction with fluorescent compound **6** to investigate the reversibility and the kinetic stability of the interaction between compound **6** and PDMS-PEG-CD surface. After rinsing with deionized water, the fluorescence images of the β-CD-N_3-treated PDMS-PEG-CD surfaces were also captured and analyzed following the procedures described earlier. Curve i in Fig. 2B shows the relationship between the incubation time of the compound **6**-treated PDMS-PEG-CD surfaces with the β-CD-N_3 aqueous solution and the fluorescence intensity of these post-reaction surfaces. This curve shows that the fluorescence intensity of the surfaces decreased with prolonged incubation time. However, compared with the association process (Curve i in Fig. 2A), this dissociation was very slow because the fluorescence intensity decreased to approximately 40% of the pristine value after exposure to β-CD-N_3 aqueous solution for 30 min and the reduced magnitude of the latter 15 min was slower compared with that of the former 15 min. These results reflect that a trend to reach a thermodynamic equilibrium between rebinding and dissociation exists. We replaced the original β-CD-N_3 aqueous solution with a fresh one at 15 min to break this equilibrium to suppress rebinding and accelerate the dissociation; the fluorescence intensity rapidly decreased to approximately 97% of the pristine value (curve ii in Fig. 2B). We hypothesized that if the replacement frequency of β-CD-N_3 aqueous solution is further improved, complete dissociation can be achieved in a relatively shorter time.

Construction of multilayer assembly on the PDMS-PEG-CD surface *via* host–guest interaction and biotin–SAv interaction

After verifying the reversible assembly on the PDMS-PEG-CD interface *via* the host–guest interaction between triadamantyl-terminated molecule and β-CD conjugated on PDMS-PEG-CD surfaces, we tried to fabricate a more complex multilayer assembly on the PDMS surface through two kinds of noncovalent attachments: the host–guest interaction between β-CD and adamantane and the biotin–SAv interaction. Previous studies demonstrated that SAv can attach to the biotin monolayer on a certain surface through two binding sites of one SAv, and the remaining two binding sites of SAv can bind to other biotinylated molecules.[18,33] Therefore, the biotinylated triadamantyl-terminated compound **9** (Scheme 1) was synthesized as the orthogonal linker, in which the adamantane moiety binds it to the β-CD-functionalized PDMS surface (PDMS-PEG-CD) through the host–guest interaction and the biotin moiety binds it to SAv through biotin–SAv interaction. The construction procedure is illustrated in Fig. 3. PDMS-PEG-CD slide was first incubated with compound **9** for 8 min to obtain biotinylated PDMS interface (step i in Fig. 3). After thorough washing, the biotinylated PDMS interface was then incubated with SAv for 30 min to obtain SAv-functionalized PDMS interface (step ii in Fig. 3). The SAv-functionalized PDMS slide was further incubated for 30 min with fluorescent biotin compound **10** to examine whether SAv was attached on the PDMS surface (step iii in Fig. 3). The synthesis and characterization of compound **10** are presented in Scheme S1 and Fig. S4 of the ESI.† The fluorescence images of the obtained PDMS-PEG-CD surfaces were captured and analyzed following the procedures described earlier. At the same time, two negative control experiments were also conducted; one is that compound **9** was omitted and the other is that SAv was omitted in the assembly. The results (Fig. 4A) showed that the fluorescence intensities of the two controls were significantly lower than that of the unabridged assembly after incubation with the fluorescent biotin compound **10**. These results indicated that the biotin- and adamantane-bifunctionalized molecule and SAv are two essential linkers to fabricate this multilayer assembly.

The fluorescent PDMS surfaces obtained from the interaction of compound **10** and the SAv-functionalized PDMS interfaces were immersed in the aqueous solution of β-CD-N_3 (5 mL, 50 mM) with gentle shaking for a certain time in the dark to test whether such a fabricated multilayer assembly has stimulus-responsive reversibility under the competitive

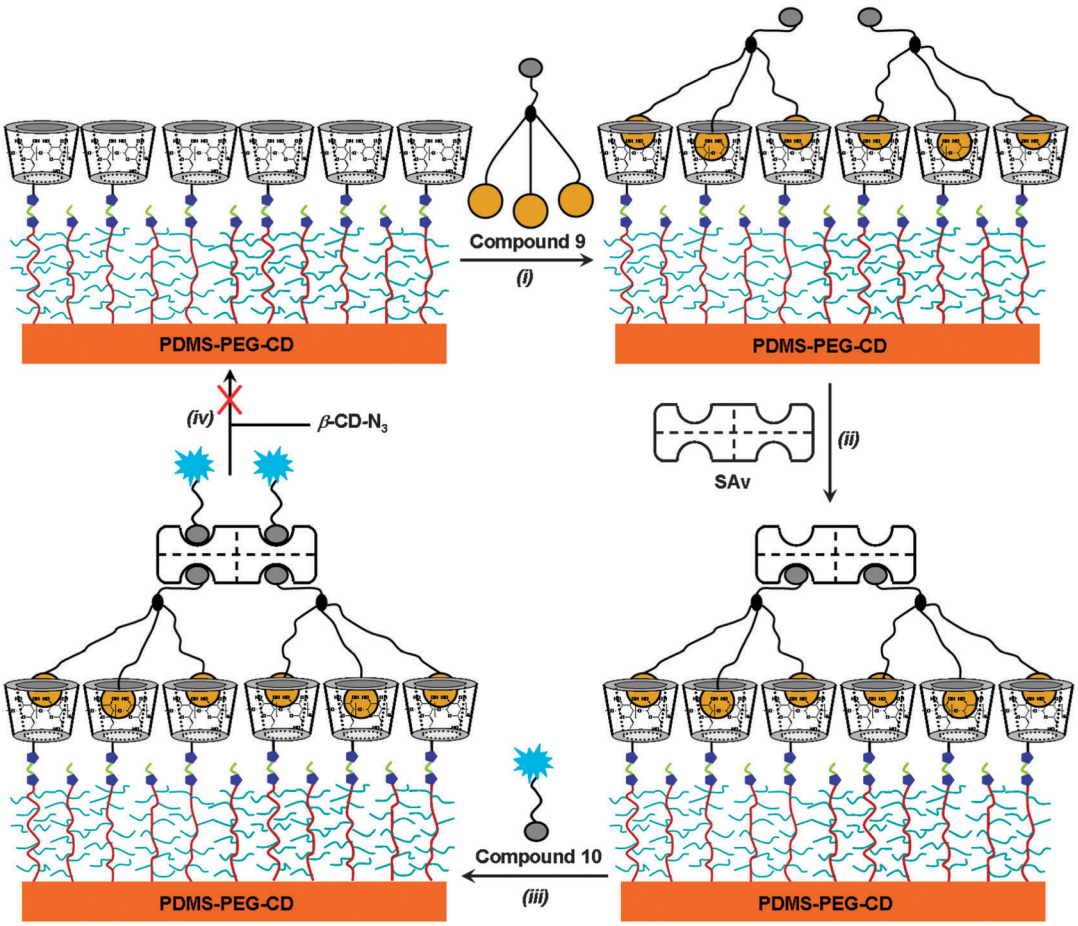


Fig. 3 Illustration for fabricating the irreversible multilayer assembly *via* host–guest interaction and biotin–SAv interaction, using biotinylated triadamantyl-terminated molecule (compound **9**) as linker and fluorescent biotin (compound **10**) as indicator (i → ii → iii); and its irreversible property under the competitive mechanism of β-CD-N_3 (iv).

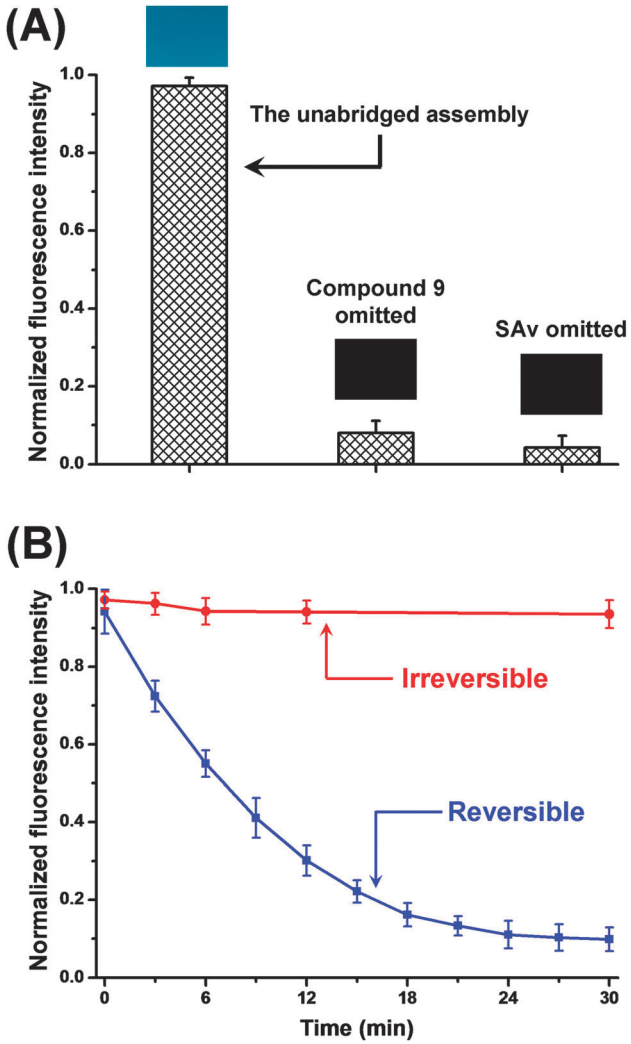


Fig. 4 (A) Relationship between the fluorescence intensity of the PDMS surfaces and the process of the multilayer assembly on the PDMS surfaces. The top images are the corresponding fluorescence images of these tests: the left for the unabridged assembly, including biotinylated triadamantyl-terminated molecule (compound **9**), SAv, and fluorescent biotin; the middle for the assembly without compound **9**; the right for the assembly without SAv. (B) Effect of incubation time between the post-interaction fluorescent PDMS surfaces and β-CD-N_3 aqueous solution on the fluorescence intensity of PDMS surfaces: the top line corresponds to the multilayer assembly fabricated *via* biotinylated triadamantyl-terminated compound **9**; the bottom line corresponds to the multilayer assembly fabricated *via* biotinylated monoadamantyl-terminated compound **11**.

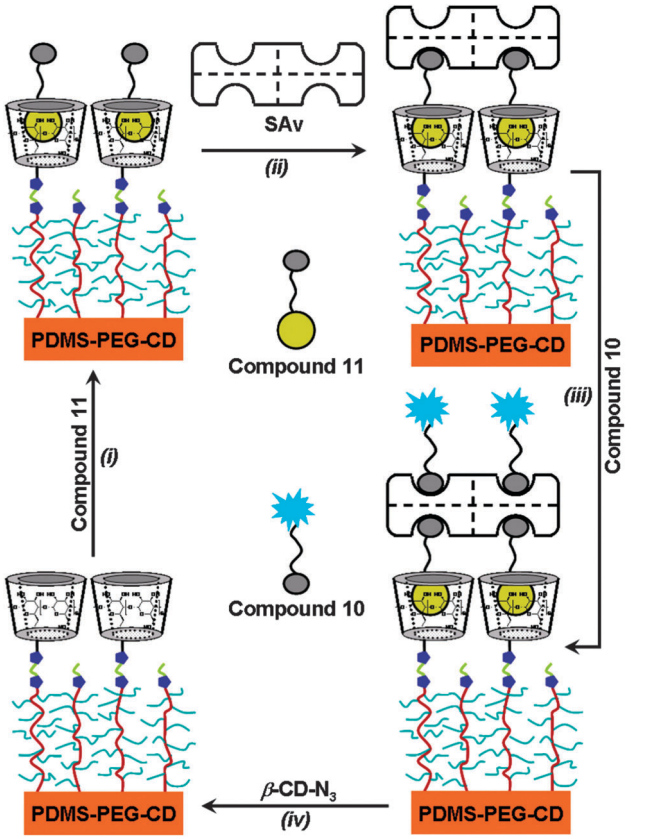


Fig. 5 Illustration for fabricating the reversible multilayer assembly *via* host–guest interaction and biotin–SAv interaction, using biotinylated monoadamantyl-terminated molecule (compound **11**) as linker and fluorescent biotin (compound **10**) as indicator (i → ii → iii); and its reversible property under the competitive mechanism of β-CD-N_3 (iv).

mechanism of β-CD-N_3. The results (the top curve in Fig. 4B) showed that the fluorescence intensity of these surfaces had almost no detectable change with prolonged incubation time, suggesting that such a multilayer assembly has kinetically stable properties.[10a,34] These results may be explained as follows. Two SAv binding sites of one SAv oriented towards biotinylated PDMS-PEG-CD surface resulted in more than trivalent β-CD–adamantane interaction, which reinforced the supramolecular interaction between β-CD and adamantane and made the removal of the multilayer assembly from PMDS surface impossible.[10]

We also synthesized biotinylated monoadamantyl-terminated molecule compound **11** to further verify the abovementioned explanation and simultaneously construct a reversible assembly on the PDMS-PEG-CD surface (Scheme S2, ESI†). The construction procedure of this reversible assembly using the monoadamantyl-terminated molecule compound **11** is shown in Fig. 5, which was different from the construction of the irreversible one (Fig. 3). A monoadamantyl-terminated molecule was used to construct this reversible assembly. After the PDMS-PEG-CD slide was incubated in turn with compound **11**, SAv, and fluorescent compound **10**, the obtained PDMS slides were then immersed in the aqueous solution of β-CD-N_3 (5 mL, 50 mM) with gentle shaking for a certain time in the dark. The results (the bottom curve in Fig. 4B) showed that the fluorescence intensity of these surfaces decreased sharply with prolonged incubation time. After 24 min, the fluorescence intensity of these surfaces decreased to approximately 11% of the pristine value. In this reversible assembly, two SAv binding sites of one SAv oriented towards biotinylated PDMS-PEG-CD surface resulted in no more than bivalent β-CD–adamantane interaction, which is weaker than the multivalent β-CD–adamantane interaction in the above irreversible assembly and made the removal of this assembly from the PMDS surface easier. This result indirectly verified that the irreversible property of the multilayer assembly fabricated through biotinylated triadamantane-contained molecules (Fig. 3) was caused by the

more than trivalent β-CD–adamantane interaction. Moreover, this result implied that the dissociation is directly related to the valence of the interaction, wherein, the adjustment of the irreversibility or reversibility of the multilayer assembly can be conveniently realized using only a biotinylated triadamantyl-terminated or a monoadamantyl-terminated molecule.

Conclusion

In this study, starting from the commercially available and inexpensive material methyl gallate, we successfully synthesized various functionalized triadamantyl-terminated dendronized molecules *via* a convergent growth strategy. Through the competitive mechanism of β-CD-N_3, the reversible association of adamantyl-terminated molecules onto β-CD-functionalized PDMS surfaces and subsequent dissociation from the PDMS surfaces were realized. Meanwhile, more complex multilayer assemblies were also successfully constructed on the PDMS surfaces *via* the β-CD–adamantyl and the biotin–SAv interactions. Further studies demonstrated that the reversible property of the fabricated multilayer assembly on PDMS interfaces can be controlled by adjusting only the valence of biotinylated adamantane. Many biomolecules or molecules can be easily biotinylated, thus, they can be conveniently conjugated onto PDMS surfaces through the method developed in this study, which paves the route for further study on the construction of various functionalized PDMS surfaces *via* β-CD-based supramolecular chemistry, and for further exploring the wide-ranging applications of these surfaces in PDMS microfluidic device-based biomedical fields.

Experimental

Materials and physical methods

1-Bromoadamantane, *p*-nitrophenyl chloroformate, and 18-crown-6 were purchased from Alfa Aesar (Lancaster, England). Cuprous bromide (CuBr), methyl gallate, *N,N*-dimethylpyridine-4-amine (DMAP), *N*,*N*,*N*′,*N*′′,*N*′′-pentamethyldiethylene-triamine (PMDETA), *n*-butylamine, tetraethylene glycol, D-biotin, Streptavidin (SAv), and ethyl diisopropyl amine (DIPEA) were purchased from Aladdin (Shanghai, China). 2,4-Dihydroxy benzaldehyde, *N*-acetylglycine, and anhydrous sodium acetate were purchased from Ouhe Technology Co. Ltd (Beijing, China). 1-Ethyl-3-(3-(dimethylamino) propyl) carbodiimide (EDC) was purchased from GL Biochem Ltd. (Shanghai, China). Solvents were purified by standard procedures. All other materials were purchased from local commercial suppliers and were of analytical reagent grade, unless otherwise stated. The preparation of PDMS-PEG-CD surfaces followed the method reported by ourselves.[19] All reactions were monitored by thin-layer chromatography (TLC) with detection by UV or by iodine. ^{1}H and ^{13}C NMR spectra of the products were recorded on a Bruker 500 MHz NMR spectrometer in $CDCl_3$ solution using tetramethylsilane (TMS) as the internal standard (chemical shifts in ppm). Mass spectra (MS) were recorded on a Bruker instrument using standard conditions (electron spray ionization, ESI). The fluorescence spectra were recorded on a fluorospectrophotometer (F-4500). The synthesis of 3-azido-7-hydroxy coumarin, compound **1**, and compound **2** were performed according to previous literatures, respectively.[7c,35] ^{1}H NMR for 3-azido-7-hydroxy coumarin: δ 10.55 (s, 1H), 7.61 (s, 1H), 7.50 (d, J = 8.50 Hz, 1H), 6.83 (dd, J = 8.50 and 2.00 Hz, 1H), and 6.78 (d, J = 2.00 Hz, 2H); ^{1}H NMR for compound **1**: δ 3.72 (t, J = 4.75 Hz, 2H), 3.70 to 3.65 (m, 8H), 3.63 to 3.57 (m, 6H), 2.88 (bs, 1H), 2.14 (s, 3H), 1.75 (s, 6H), and 1.65 to 1.57 (m, 6H); ^{1}H NMR for compound **2**: δ 7.80 (d, J = 8.00 Hz, 2H), 7.34 (d, J = 8.00 Hz, 2H), 4.16 (t, J = 4.75 Hz, 2H), 3.69 (t, J = 5.00 Hz, 2H), 3.64 to 3.56 (m, 12H), 2.45 (s, 3H), 2.14 (br, 3H), 1.74 (s, 6H), and 1.65 to 1.56 (m, 6H); all of which were similar previously reported data.

Specific procedure for synthesizing triadamantyl-terminated dendronized molecule compound 4

Compound **4** was synthesized through a two-step reaction (Scheme 1). A suspension of compound **2** (4.90 g, 10 mmol), methyl gallate (0.46 g, 2.5 mmol), dry potassium carbonate (1.66 g, 12 mmol), and 18-crown-6 (0.32 g, 1.2 mmol) in dry acetone (50 mL) was refluxed for 24 h under nitrogen atmosphere. After the complete disappearance of methyl gallate monitored through TLC, the solvent was evaporated, and the residue was partitioned between water (30 mL) and CH_2Cl_2 (60 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 30 mL) and the combined organic layer was dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue was purified by column chromatography (eluent: CH_2Cl_2/MeOH, 50 : 1, v/v) to afford compound **3** as a colorless liquid (2.34 g, 84%). MS (ESI): $[M+Na]^+$ calculated for $C_{62}H_{98}O_{17}Na$, 1137.68, found 1137.62; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.29 (s, 2H), 4.23–4.18 (m, 6H), 3.89 to 3.85 (m, 7H), 3.80 (t, J = 4.75 Hz, 2H), 3.72 to 3.70 (m, 6H), 3.68 to 3.62 (m, 18H), 3.60 to 3.57 (m, 12H), 2.15 (br, 9H), 1.74 (br, 18H), and 1.65 to 1.57 (m, 18H); ^{13}C NMR (125 MHz, $CDCl_3$): 166.59, 152.29, 142.57, 124.96, 109.05, 72.42, 72.27, 71.29, 70.82, 70.66, 70.57, 69.62, 68.86, 41.48, 36.47, and 30.52.

Under nitrogen atmosphere, LAH (76 mg, 2 mmol) was added in portions at 0 °C to the solution of compound **3** (1.12 g, 1 mmol) in dry THF (20 mL). The resulting mixture was stirred for 1 h at this temperature, gradually warmed to room temperature, and then stirred for another 6 h at 50 °C. The reaction was quenched by sequential drop-wise addition of water (5 mL), 10% NaOH (10 mL), and water (5 mL). The resulting precipitate was filtered off, and THF was evaporated. The residue was dissolved in CH_2Cl_2 (40 mL), and then washed with brine solution. After drying over Na_2SO_4, the residue was purified by column chromatography (eluent: CH_2Cl_2/MeOH, 30 : 1, v/v) to obtain compound **4** as a colorless liquid (956.3 mg, 88%). MS (ESI): $[M+Na]^+$ calculated for $C_{61}H_{98}O_{16}Na$, 1109.69, found 1109.78; ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.64 (s, 2H), 4.57 (s, 2H), 4.17 (t, J = 4.75 Hz, 4H), 4.13 (t, J = 4.50 Hz, 2H), 3.83 (t, J = 4.50 Hz, 4H), 3.78 (t, J = 4.50 Hz, 2H), 3.72 to 3.68 (m, 6H), 3.68 to 3.60 (m, 18H), 3.59 to 3.55 (m, 12H), 2.13 (br, 9H), 1.74 (br, 18H), and 1.65 to 1.57 (m, 18H); ^{13}C NMR (125 MHz, $CDCl_3$): 152.68, 137.85, 136.84, 106.80, 72.34, 72.27,

71.27, 70.79, 70.69, 70.58, 70.50, 69.84, 68.90, 65.13, 59.26, 41.48, 36.47, and 30.52.

Specific procedure for synthesizing fluorescent triadamantyl-terminated molecule compound 6

Compound **6** was obtained through a two-step reaction (Scheme 1). First, a solution of compound **4** (1.63 g, 1.5 mmol) in THF (20 mL) was added drop-wise to the THF suspension of NaH (0.12 g, 60% suspension in oil, 3.0 mmol, 2.0 equiv.) at 0 °C under nitrogen atmosphere. The reaction mixture was gradually warmed to room temperature for 2 h, and propargyl bromide (0.28 g, 2.3 mmol, 1.5 equiv.) was subsequently added drop-wise. The resulting mixture was stirred at room temperature for 12 h, and then cooled to 0 °C. Saturated NH_4Cl solution was slowly added to the reaction mixture to terminate the reaction. After THF was evaporated under vacuum, the residue was extracted with CH_2Cl_2 three times. The organic phase was washed with saturated NaCl solution and dried over anhydrous Na_2SO_4. The crude product was purified through column chromatography (eluent: CH_2Cl_2/MeOH, 50 : 1, v/v) to obtain alkynyl-contained molecule compound **5** as a colorless liquid (1.13 g, 91%). MS (ESI): $[M+Na]^+$ calculated for $C_{64}H_{100}O_{16}Na$, 1147.70, found 1147.68; ^1H NMR (500 MHz, $CDCl_3$): δ 6.59 (s, 2H), 4.49 (s, 2H), 4.20 to 4.10 (m, 8H), 3.83 (t, J = 5.00 Hz, 4H), 3.78 (t, J = 5.00 Hz, 2H), 3.73 to 3.68 (m, 6H), 3.68 to 3.60 (m, 18H), 3.59 to 3.55 (m, 12H), 2.54 (t, J = 2.50 Hz, 1H), 2.13 (br, 9H), 1.74 (br, 18H), and 1.65 to 1.57 (m, 18H); ^{13}C NMR (125 MHz, $CDCl_3$): 152.63, 138.07, 132.71, 107.60, 79.55, 74.82, 72.27, 72.18, 70.79, 70.69, 70.58, 70.50, 69.84, 68.90, 65.13, 59.23, 56.97, 41.45, 36.44, and 30.47.

Compound **5** (0.26 g, 0.23 mmol), 3-azido-7-hydroxy coumarin (60 mg, 0.29 mmol), PMDETA (5 μL, 0.023 mmol), and dried DMF (10 mL) were added to a Schlenk tube equipped with a magnetic stirring bar. The resulting mixture was degassed *via* three freeze-thaw cycles, and CuBr (3.3 mg, 0.023 mmol) was added under a nitrogen atmosphere. After stirring the mixture at room temperature for 24 h, DMF was removed under vacuum. The residue was dissolved in 30 mL of CH_2Cl_2, the organic phase was washed with EDTA [1% (w/v), 2 × 15 mL] and water successively, and then dried over anhydrous Na_2SO_4. The crude product was purified through column chromatography (eluent: CH_2Cl_2/MeOH, 25 : 1, v/v) to obtain compound **6** as a yellow liquid (253.5 mg, 83%). MS (ESI): $[M+Na]^+$ calculated for $C_{73}H_{105}N_3O_{19}Na$, 1350.73, found 1350.54; ^1H NMR (500 MHz, $CDCl_3$): 8.47 (s, 1H), 8.43 (s, 1H), 7.48 (d, J = 9.00 Hz, 1H), 6.97 (br, 2H), 6.55 (s, 2H), 5.30 (s, 2H), 4.74 (s, 2H), 4.13 (t, J = 5.00 Hz, 4H), 4.09 (t, J = 5.00 Hz, 2H), 3.83 (t, J = 5.00 Hz, 4H), 3.74 (t, J = 5.00 Hz, 2H), 3.74 to 3.65 (m, 6H), 3.67 to 3.62 (m, 18H), 3.60 to 3.55 (m, 12H), 2.12 (br, 9H), 1.73 (br, 18H), and 1.65 to 1.55(m, 18H); ^{13}C NMR (125 MHz, $CDCl_3$): 162.58, 156.44, 154.85, 154.79, 152.47, 134.69, 133.66, 130.24, 124.00, 115.30, 115.20, 110.61, 107.16, 103.29, 103.24, 72.67, 71.27, 71.20, 70.66, 70.57, 70.51, 70.35, 69.66, 68.58, 59.27, 41.44, 36.42, and 30.51.

General procedure for linking amino-ended molecule with triadamantyl-terminated molecule

A two-step reaction is required to link an amino-ended molecule with triadamantyl-terminated molecule (Scheme 1). For example, the synthesis of compound **8** was as follows: first, under nitrogen atmosphere, *p*-nitrophenyl chloroformate (0.402 g, 2 mmol) and compound **4** (1.09 g, 1 mmol) were dissolved in 20 mL of dry dichloromethane, and then triethylamine (2 mmol, 280 μL) was added drop-wise. The reaction mixture was stirred at room temperature for 12 h until the disappearance of compound **4**, which was confirmed *via* TLC. Dichloromethane was evaporated, and the residue was purified through column chromatography (eluent: CH_2Cl_2/MeOH, 50 : 1, v/v) to obtain compound **7** as a pale yellow liquid (1.09 g, 87%). MS (ESI): $[M+Na]^+$ calculated for $C_{68}H_{101}NO_{20}Na$, 1274.69, found 1274.27; ^1H NMR (500 MHz, $CDCl_3$): 8.28 (d, J = 9.00 Hz, 2H), 7.40 (d, J = 9.00 Hz, 2H), 6.68 (s, 2H), 5.18 (s, 2H), 4.17 (t, J = 5.00 Hz, 6H), 3.86 (t, J = 4.50 Hz, 4H), 3.79 (t, J = 4.50 Hz, 2H), 3.74 to 3.69 (m, 6H), 3.68 to 3.60 (m, 18H), 3.62 to 3.55 (m, 12H), 2.13 (br, 9H), 1.74 (br, 18H), and 1.65 to 1.55 (m, 18H); ^{13}C NMR (125 MHz, $CDCl_3$): 155.52, 152.83, 152.34, 145.38, 139.14, 129.41, 125.26, 121.76, 108.54, 72.17, 71.26, 70.82, 70.64, 70.57, 70.51, 69.69, 68.99, 59.24, 41.47, 36.45, and 30.48.

The corresponding carbamate nitrophenyl ester compound **7** (125.1 mg, 0.1 mmol) was dissolved in 5 mL of dry THF, to which, *n*-butylamine (20 μL, 0.2 mmol) and DIPEA (35 μL, 0.2 mmol) were sequentially added drop-wise. The resulting mixture was stirred for 5 h at room temperature. The solvent was evaporated under vacuum and the residue was purified through column chromatography (eluent: CH_2Cl_2/MeOH, 40 : 1, v/v) to obtain compound **8** as a colourless liquid (98.4 mg, 83%). MS (ESI): $[M+NH_4]^+$ calculated for $C_{66}H_{111}N_2O_{17}$, 1203.75, found 1204.34; ^1H NMR (500 MHz, $CDCl_3$): 6.59 (s, 2H), 4.97 (s, 2H), 4.16 to 4.13 (m, 6H), 3.84 (t, J = 5.00 Hz, 4H), 3.78 (t, J = 5.00 Hz, 2H), 3.73 to 3.70 (m, 6H), 3.68 to 3.62 (m, 18H), 3.60 to 3.55 (m, 12H), 3.19 (q, J = 6.50 Hz, 2H), 2.13 (br, 9H), 1.74 (br, 18H), 1.65 to 1.55 (m, 18H), 1.51 to 1.47 (m, 2H), 1.37 to 1.30 (m, 2H), and 0.92 (t, J = 7.00 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): 152.59, 132.14, 125.99, 115.81, 107.73, 72.32, 59.22, 41.44, 36.44, and 30.48.

Specific procedure for synthesizing biotinylated triadamantyl-terminated molecule compound 9

A solution of 4-DMAP (25.0 mg, 0.2 mmol) in 2 mL of DMF was first added drop-wise to an ice-cooled mixture solution of compound **4** (217.5 mg, 0.2 mmol) and D-biotin (48.9 mg, 0.2 mmol) in 10 mL of DMF. After stirring for 5 min at room temperature, EDC (46 mg, 0.24 mmol) was sequentially added upon stirring the reaction mixture. The reaction mixture was kept in an ice-water bath for another 10 min, and then stirred overnight at room temperature. DMF was removed under vacuum and the residue was resolved in CH_2Cl_2 and washed with 1 M HCl to remove 4-DMAP. After drying over anhydrous Na_2SO_4, the organic layer was purified through silica gel column chromatography (eluent: CH_2Cl_2/MeOH, 15 : 1, v/v) to afford compound **9** as a colourless liquid (264.8 mg, 91%). MS (ESI): $[M+Na]^+$ calculated for $C_{81}H_{134}N_2O_{18}SNa$, 1477.94, found 1477.86; ^1H NMR (500 MHz, $CDCl_3$): 6.45 (s, 1H), 6.19 (s, 1H), 5.77 (s, 1H), 4.84 (s, 2H), 4.30 (br, 1H), 4.10 (br, 1H), 4.00

(br, 6H), 3.70 (br, 6H), 3.57 (t, J = 2.80 Hz, 3H), 3.60 to 3.40 (m, 36H), 2.96 (br, 1H), 2.68 (br, 1H), 2.57 to 2.50 (m, 1H), 2.23 (t, J = 7.20 Hz, 1H), 1.98 (s, 1H), 1.59 (s, 18H), 1.55 to 1.40 (m, 22H), and 1.28 (br, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): 173.21, 163.78, 152.54, 138.26, 131.27, 107.90, 72.19, 71.99, 71.12, 70.66, 70.51, 70.44, 70.39, 70.35, 69.70, 69.58, 68.82, 66.41, 66.05, 61.78, 60.00, 59.14, 55.47, 53.62, 41.36, 40.31, 36.34, 28.23, 29.09, 24.69, and 15.07.

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