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Protein-Resistant Surfaces through Mild Dopamine Surface Functionalization

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Abstract: The synthesis and evaluation of new dopamine-based catechol anchors coupled to poly(ethylene glycol) (PEG) for surface modification of TiO_2 are reported. Dopamine is modified by dimethylamine–methylene (**7**) or trimethylammonium–methylene (**8**) groups, and the preparation of mPEG-Glu dopamine polymer **11** is presented. All these PEG polymers allow stable adlayers on TiO_2 to be generated through mild dip-and-rinse procedures, as evaluated both by variable angle spectroscopic ellipsometry and X-ray photoelectron spectroscopy. The resulting surfaces substantially reduced protein adsorption upon exposure to full human serum.

Keywords: biofouling · biomimetic strategies · natural products · surface chemistry · synthesis design

Introduction

The nonspecific attachment of macromolecules of biological nature, such as proteins, or of microorganisms, such as bacteria or fungi, to surfaces (so-called biofouling) is a fundamental problem in areas ranging from medical devices and biosensors[1] to protein production, handling, and storage.[2] Surfaces suffer from biofouling as soon as they are exposed to biological fluids or protein solutions. This process[3] is often considered to be the first step in nosocomial infections,[4] which are a serious problem in hospital settings nowadays.

The direct approach towards preventing biofouling is the generation of antifouling surfaces,[5] that is, coatings that repel the adhesion of proteins and cells. Over recent years, effective antifouling polymers have been developed, including poly(ethylene glycol)[6] polyglycerol,[7] poly(ethylene oxide)–poly(propylene oxide)/pluronics,[8,9] peptoids,[10] and poly-2-methyl-2-oxazoline.[11] The remaining general challenge consists of attaching these polymers to surfaces in a mild, rapid and efficient way. Several approaches have been developed based on polyelectrolytes,[12] silanes,[13] or thiols[14] on gold. All of these approaches suffer from limitations regarding the scope, stability or reactivity of the anchoring groups. Recently, catechols have been presented as promising alternative adhesives in surface modifications.[15] These anchors are found in mussel-adhesive proteins (MAPs),[16] which are responsible for the very strong wet adhesion of mussels to surfaces. MAPs have been shown to contain up to 27% of the catechol dihydroxyphenylalanine (DOPA), which is the key constituent for adhesion. Based on early work by Waite and co-workers,[15a] and then Grätzel and co-workers,[15b] Messersmith and co-workers[15c–h] demonstrated the usefulness of catechols for surface functionalization. In these systems, however, oligomers of DOPA, such as **2**, are required to achieve sufficient adhesion stability and protein resistance. We were able to overcome this limitation by introducing anchor **3**[17a] for effective single-site attachment based on the iron chelator anachelin.[18] This biomimetic strategy allowed PEG-based, protein-resistant,[17a] and cell-resistant[17b] surfaces to be generated, with the drawback that the anchor required multistep organic synthesis, involving heavy-metal-based[18a,b] or enzymatic[18c,d] conversions. We sought to structurally simplify this anchor and, while retaining its benefits, make its preparation more straightforward. Herein, we present structurally simple, easy-to-produce anchors that allow protein-resistant TiO_2 surfaces to be generated through straightforward dip-and-rinse procedures.

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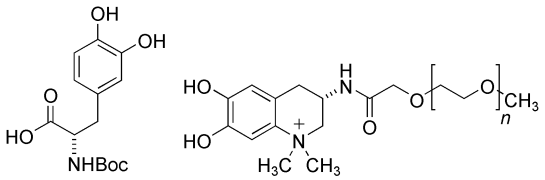
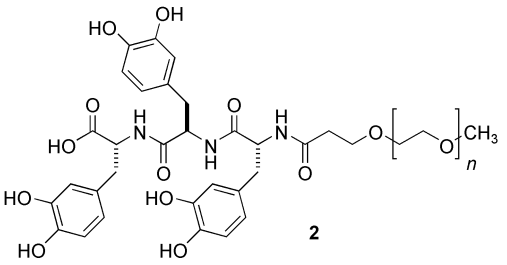
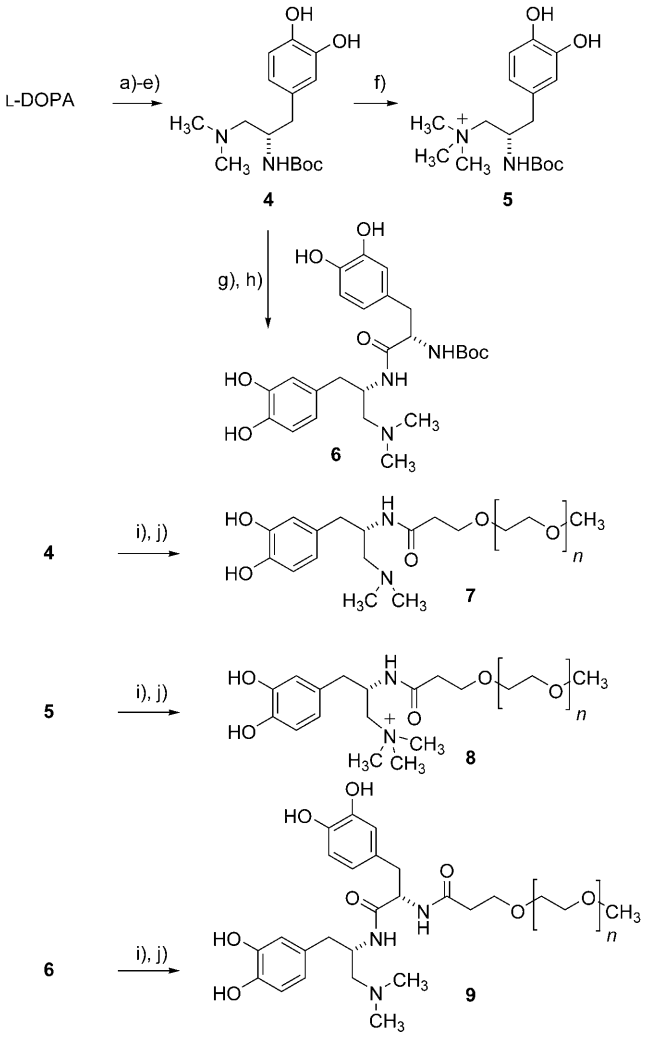




Results and Discussion

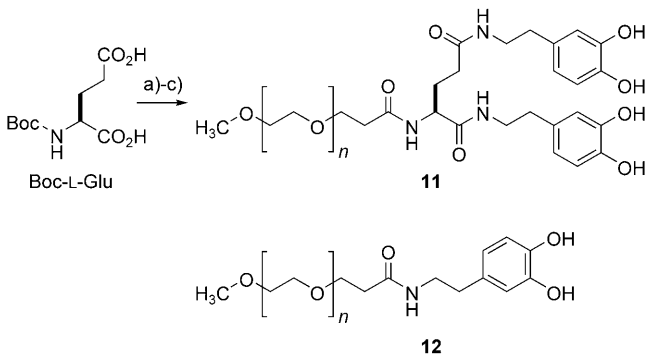
The goal of this investigation was the design of an effective catechol surface anchor that could generate protein-resistant surfaces and that was readily available by a short synthetic route. We initially proposed that the key to successful surface immobilization through single-site attachment is the combination of both catechol and a positive ammonium group, such as that in **3**.[17a] These functional units should synergistically act on surface binding with the catechol as the anchoring group in a similar fashion to MAPs and the positive charge inducing favorable interactions with the negatively charged surface of the metal oxide. Therefore, we turned our attention to compound **4**, which is readily available from Boc-L-DOPA.[18a,b] This monoprotected catechol diamine exists in a charged state under physiological conditions, thus displaying the desired properties of catechol and a positive charge. A permanent, pH-independent positive charge was introduced by N-methylation of **4** in one step with excellent yield (97%; Scheme 1). The resulting compound **5** can therefore be obtained through a short synthetic sequence from DOPA. In addition to the monocatechol anchors, such as **4** and **5**, we were interested in dicatechol anchors because of their increased adhesion stability on surfaces.[15g] Thus, dicatechol–diamine **6** was prepared in two steps from **4**, simply through deprotection of **4** and coupling to Boc-L-DOPA (Boc = *tert*-butoxycarbonyl). The three anchors (**4**–**6**) were deprotected and coupled to poly(ethylene glycol) (PEG-5000) through the corresponding PEG-succinidyl esters and purified by size exclusion chromatography. The resulting polymers (**7**–**9**) were thus readily available for surface functionalization.

In addition, we prepared didopamine polymer **11** in three steps from Boc-L-Glu, through coupling to dopamine, deprotection and PEGylation with mPEG succinidyl ester (Scheme 2). Compound **11** was designed in view of beneficial properties through bidentate anchoring of the catechols on the surface, while also being readily available through a short three-step synthetic sequence. From a mechanistic point of view, this compound could also allow the role of the amino group in **9** to be investigated with respect to the two catechol units. Compound **11** should also display increased binding properties when compared with dopamine PEG polymer **12**, as noted previously for DOPA-based polymers.[10,15g]



Scheme 1. Preparation of catechol-PEG conjugates for surface modification: a) Boc_2O, NaOH (aq), dioxane, RT, 16 h; b) 1) *i*BuOCOCl, THF, −35 °C, 0.5 h; 2) $HNMe_2$, THF, −35 °C to RT, 16 h, 68% (over three steps); c) Cs_2CO_3, BnBr, acetone, reflux, 4 h, 83%; d) 1) trifluoroacetic acid (TFA), CH_2Cl_2, 0 °C, 1 h; 2) BH_3·THF, THF, 0 °C to RT, 16 h; 3) Boc_2O, NaOH (aq), dioxane, RT, 16 h, 53% (over 3 steps); e) H_2, Pd/C, AcOH, MeOH, 14 h, RT, 99%; f) CH_3I, CH_2Cl_2/MeOH 2:1, 6 h, RT; g) TFA, CH_2Cl_2, 0 °C, 1 h; h) Boc-L-DOPA, NEt_3, 1-hydroxybenzotriazole (HOBt), *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide (EDC), 14 h, RT, 54% (over two steps); i) HCl (4 M) in dioxane, dioxane, 0 °C, 2 h; j) methoxy(polyethylene glycol) *N*-hydroxysuccinidylpropionate (mPEG-SPA), *N*-methylmorpholine (NMM), CH_2Cl_2/DMF 1:1, RT, 64% (for **7**), 44% (for **8**), 60% (for **9**).

The generation of protein-resistant surfaces was then carried out by means of spontaneous adsorption (self-assembly) by using a dip-and-rinse protocol. TiO_2 was chosen as the surface in view of its relevance in the field of biomedical implants and biosensors.[19] Thus, clean TiO_2 surfaces were



Scheme 2. Preparation of mPEG didopamine polymer **11**: a) dopamine hydrochloride, NEt_3, HOBt, EDC, 14 h, RT, $CHCl_3$; b) HCl (4 M) in dioxane, dioxane, 0 °C, 2 h; c) mPEG-SPA, NMM, CH_2Cl_2/DMF 1:1, RT, 58 %.

dipped in dilute solutions of **7**–**9** and **11**–**12** (polymers (0.1 mg mL^{-1}) in high salt buffer (0.1 M MOPS/0.6 M NaCl/ 0.6 M K_2SO_4)) for 4 h at 50 °C. These cloud-point conditions were chosen to achieve dense packing of PEG and thus maximize PEG surface density.[5a] After incubation, the resulting surfaces were briefly rinsed with water and the thickness of the adlayer was measured by variable angle spectroscopic ellipsometry (VASE). This measured thickness is proportional to the amount of polymer adsorbed and thus directly reflects the mass of PEG on the surface. From these values (Figure 1, ●), it is evident that polymer **8**, featuring the quaternary ammonium anchor, as well as didopamine polymer **11** displayed the highest mass adsorbed. Among the monocatechol polymers, the performance of **8** is comparable to anachelin chromophore polymer **3** and to that measured for dopamine-derived **12**. Clearly, the nonpermanently charged polymer **7** displayed a lower adlayer thickness. As a next step, the polymers were equilibrated by exposing the surfaces to physiological buffer (HEPES 2; pH 7.4) for 24 h. At the same time, the PEG chains become rehydrated in this process. The adlayer thickness of all polymers was again measured by VASE (Figure 1, □). Quaternary ammonium polymer **8** again displayed a high value, with a measured thickness of roughly 17 Å. This is comparable to control polymer **3** and dopamine-derived **12**. Tertiary amine polymer **7** showed a significantly lower thickness, with a measured value of 9.8 Å.

We next investigated the adlayer thickness of divalent didopamine derivatives **9** and **11** (Figure 1). Interestingly, derivative **9**, featuring additional amino groups, displayed a similar low initial adlayer thickness when compared to the parent monovalent polymer **7** and after 24 h exposure to the buffer an adlayer thickness of 8.8 Å was determined. Divalent dopamine derivative **11**, however, gave the highest adlayer thickness, both after adsorption (ca. 25 Å) and after 24 h incubation in buffer (ca. 18 Å).

From these data, the following conclusions can be drawn: 1) A permanent positive charge, present, for example, in **8**, results in higher values for the adlayer thickness (on negatively charged surfaces), and therefore, a higher adsorbed mass for the monovalent compounds. Thus, polymers such as **8** and control **3** have superior adsorption properties when compared with nonpermanently charged aminodopamines such as **7**. 2) Didopamine polymer **11** resulted in the highest adlayer thickness, as determined by VASE. Clearly, an optimal arrangement of both catechols is beneficial for surface attachment. In contrast, diamino dopamine polymer **9** performed poorly, with layer thicknesses similar to its monovalent counterpart **7**. Increased steric hindrance expected for molecule **9**, when compared with **11**, is possibly the reason for the observed lower adlayer thickness. Molecular modeling results supports this view, in which the dimethylamino group in **9** appears to separate the catechol units spatially and thus prevent multivalent attachment (data not shown). From the adsorption data after incubation for 24 h under physiological conditions, it can be concluded that the performance regarding adlayer thickness of the group of polymers **3**, **8**, **11**, and **12** are superior to **7** and **9**.

Next, we investigated protein adsorption to the TiO_2 surfaces coated with the different polymers prepared as described above. The resulting surfaces were subsequently exposed to human serum for 20 min, rinsed with buffer and ultra-pure water, and the resulting increase in layer thickness was measured by VASE (Figure 2). These results dem-

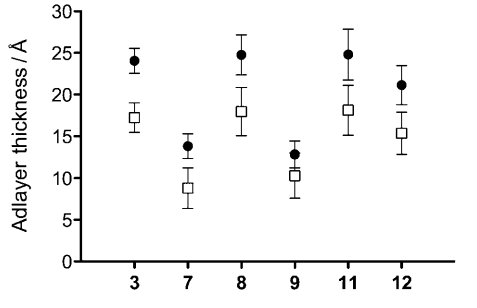


Figure 1. Adlayer thickness of polymers **3** (8), **7** (10), **8** (9), **9** (10), **11** (6), and **12** (8) as measured by VASE (numbers in parentheses refer to the number of independent experiments, error bars denote the standard deviation). ■: values measured directly after adsorption in cloud point buffer, □: values measured after equilibration in physiological buffer at pH 7.4.

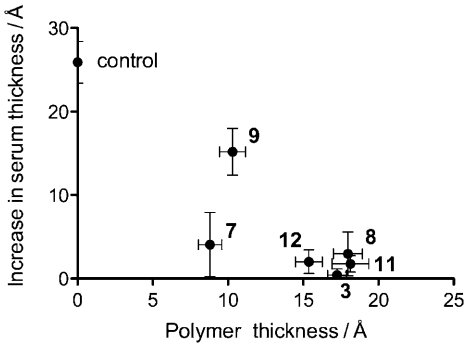


Figure 2. Measured increase in layer thickness due to serum adsorption on polymer adlayers of **3**, **7**, **8**, **9**, **11**, and **12** plotted against polymer adlayer thickness after equilibration for 24 h in physiological buffer. Control refers to the bare TiO_2 surface.

onstrate that coatings of **7** and **8** substantially reduce non-specific serum adsorption when compared with bare TiO_2 (Figure 2, control). The measured protein resistance values for **7** and **8** are slightly lower than that of anachelin chromophore **3**, which reflects their lower polymer adlayer thicknesses (Figure 1). In addition, whereas the introduction of a permanent charge, such as in **8**, leads to increased PEG surface coverage, as determined by VASE (Figure 1), it has no influence on protein resistance (Figure 2) because both polymers **7** and **8** are equally efficient towards this goal.

Surprisingly, the diamino catechol polymer **9** coating was inefficient at reducing the protein-adsorbed mass (Figure 2), in contrast to the expectation given the adlayer thickness of 10 Å after buffer incubation (Figure 1). The reason for this unexpected result is unclear at present. Didopamine polymer **11** showed a significantly better performance in this respect, with over 95% reduction of protein adsorption when compared with the control. Thus, polymer **11** as well as the charged monovalent **8** generally display the best values with regard to performance in adlayer thickness and protein resistance.

The data obtained from VASE experiments were further corroborated by X-ray photoelectron spectroscopy (XPS) measurements. For protein-resistant surfaces, the chemical composition of the adsorbate layer is expected to remain unchanged, whereas surfaces that adsorb proteins should show a substantial increase in the surface nitrogen concentration as well as changes in the composition of the carbon components of the C1s signal. The XPS data confirmed that surfaces identified by VASE as protein resistant, for example, **3**, **11**, and **12**, also show the smallest change in their chemical composition (shown in the Supporting Information). The combined XPS and VASE findings for these three polymers clearly demonstrate that the measured layer thickness after the serum test mostly reflects the polymer film and that adsorption or exchange of polymer molecules by proteins has not taken place to a significant degree. In contrast, the films obtained from **7** and **9** show a substantial increase in the nitrogen and carbon surface concentrations. The XPS data also indicate that thicker polymer layers (corresponding to a higher C/Ti atomic concentration ratio) with correspondingly higher PEG-chain surface density result in more favorable protein resistance, which is in agreement with the ellipsometry data (Figure 2).

Conclusion

We have presented the synthesis and evaluation of a series of new dopamine-based anchors for surface functionalization. Two anchors with excellent properties were identified. Dopamine **8** with a permanent positive charge displayed a comparable adlayer thickness as reference **3**. Didopamine polymer **11** was identified as the anchor of choice because this compound showed the highest adlayer thickness while displaying a large reduction in protein attachment. In addition, the didopamine anchor of **11** can be prepared from Boc-L-Glu and dopamine in a straightforward and convenient one-step process. Benefits of the new anchors included in **8** and **11** are 1) ease of synthesis compared with previously reported systems such as **3**, 2) operationally simple dip-and-rinse protocols for the generation of protein-resistant surfaces, and 3) mild surface functionalization through catechols, which are compatible with many functional groups. Applications of the novel surface-active molecules for the generation of functional surfaces are currently underway in our laboratories.

Experimental Section

Materials and methods: Chemicals were purchased from Fluka, ABCR, or Acros and used without further purification. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel 60 F254 plates (0.25 mm thickness) precoated with a fluorescent indicator. The developed plates were examined under UV light and stained with ceric ammonium molybdate followed by heating. All ^1H and ^{13}C NMR spectra were recorded by using a Bruker DPX 400 MHz (^1H) or 100 MHz (^{13}C) FT spectrometer at RT; chemical shifts (δ) are given in ppm and coupling constants (J) in Hz. IR spectra were recorded by using a Varian 800 FT-IR ATR spectrometer. The absorptions are reported in cm^{-1} and the IR bands were assigned as strong (s), medium (m), or weak (w). All mass spectra were recorded by the Mass Spectroscopy service of EPF Lausanne on a MICROMASS (ESI) Q-TOF Ultima API instrument. Melting points were determined by using a Büchi B-545 apparatus in open capillaries and are uncorrected. Compound **4** was prepared according to the literature,[18] and procedures and data are reported in the Supporting Information.

TiO_2 surfaces and surface preparation: Silicon wafers (Si-Mat Silicon Materials Landsberg/Deutschland) were coated with TiO_2 (15/20 nm) by magnetron sputtering (PSI Villingen, Switzerland). Metal oxide coated wafers were subsequently sawn into 1 cm × 1 cm pieces. Prior to polymer modification, TiO_2-coated silicon wafers were sonicated in toluene twice for 10 min, followed by sonication in 2-propanol twice for 7 min, dried under a stream of nitrogen, and finally exposed to O_2 plasma (Harrick Scientific Corporation, Ossining, NY) for 3 min to remove adventitious contamination from the surface.

Ellipsometry (VASE): The adlayer thickness on the TiO_2 wafers was measured by M-2000F Variable angle spectroscopic ellipsometry (J. A. Woollam Co.) at 65, 70, and 75 °C by using wavelengths from 370 to 1000 nm. VASE spectra were fitted with multilayer modes by using a custom analysis software (WVASE 32).

X-ray photoelectron spectroscopy (XPS): XPS data were acquired on a SIGMA probe thermo XPS system spectrophotometer. The instrument was equipped with a multichannel detector and an Alpha 110 hemispherical analyzer. All spectra were acquired at 300 W with an $Al_{K\alpha}$ X-ray source (1486.6 eV) with a large area spot size. High-resolution spectra (C 1s, N 1s) were recorded with 0.1 eV step size and 25 eV pass energy. Recorded spectra were referenced to the aliphatic hydrocarbon C1s signal at 285.0 eV. Data were analyzed by using the program CasaXPS (Version 2.3.5 http://www.casaxps.com). The signals were fitted by using Gaussian–Lorentzian functions and an Marquardt–Levenberg optimization algorithm following Shirley iterative background subtraction.

Preparation of trimethylammonium dopamine 5: Compound **4** (44 mg, 0.14 mmol, 1.0 equiv) was dissolved in CH_2Cl_2/MeOH 2:1 (1 mL). MeI (44 μL, 0.71 mmol, 5.0 equiv) was added and the solution was stirred at RT for 6 h under N_2. The solvent was removed under reduced pressure to give compound **5** (62 mg, 0.14 mmol, 97%). ^1H NMR (CD_3OD, 400 MHz): δ = 1.36–1.39 (s, 9H; Boc rotamers), 2.61–2.74 (m, 2H), 3.17 (s, 9H), 3.49 (m, 2H), 4.20–4.23 (m, 1H), 6.59–6.61 (m, 1H), 6.72–6.74 ppm (m, 2H); ^{13}C NMR (CD_3OD, 100 MHz): δ = 27.5, 27.6 (Boc rotamers), 40.7, 48.4, 54.8, 73.8, 79.5, 115.6, 116.8, 123.2, 132.0, 144.6, 145.8,

155.6 ppm; HRMS: *m/z*: calcd for $C_{17}H_{29}N_2O_4$ $[M+H]^+$: 325.2122; found: 325.2127.

Preparation of compound 6: Compound **4** (250 mg, 0.81 mmol, 1.0 equiv) was suspended in CH_2Cl_2 (4 mL) and cooled to 0 °C under N_2. TFA (2 mL) was added dropwise and the solution stirred at 0 °C for 1 h and at RT for 1 h. The reaction mixture was then concentrated, redissolved in toluene (1 mL), concentrated, and dried under high pressure. The residue was dissolved in CH_2Cl_2 (10 mL) before Et_3N (450 μL, 4.0 equiv) and HOBt (130 mg, 1.2 equiv) were added and the solution was stirred for 5 min at RT. EDC·HCl (165 mg, 1.2 equiv) and a solution of BOC-L-DOPA (259 mg, 1.1 equiv) in CH_2Cl_2 (3 mL) were added. The solution was stirred at 0 °C for 1 h and at RT for 18 h under N_2 in the dark. The reaction mixture was concentrated, diluted with Et_2O and washed with 1 M HCl (2×10 mL), saturated $NaHCO_3$ (2×10 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$), concentrated, and the residue purified by chromatography on SiO_2 (CH_2Cl_2/MeOH 8:1) to give **6** (287 mg, 0.63 mmol, 54 %).

Preparation of mPEG-SPA: mPEG-propionic acid (150 mg, 30 μmol, 1.0 equiv) was dissolved in $CHCl_3$ (3 mL) at 0 °C under N_2. Dicyclohexylcarbodiimide (DCC; 7 mg, 33 μmol, 1.1 equiv) was added and the solution stirred for 15 min at 0 °C. *N*-Hydroxysuccinimide (NHS; 4 mg, 33 μmol, 1.1 equiv) was added and the solution was stirred for 1 h at 0 °C and for 14 h at RT under N_2. The reaction mixture was kept at 4 °C for 4 h and filtered. The solvent was removed and the residue was dissolved in Et_2O (30 mL) and stored at 4 °C for 1 h. Filtration gave mPEG-SPA (146 mg, 29 μmol, 97 %), which was used without further purification.

Preparation of polymer 7: Compound **4** (14 mg, 30 μmol, 3.0 equiv) was dissolved in CH_2Cl_2 (1 mL), cooled at 0 °C under N_2, and TFA (1 mL) was added dropwise. The solution was stirred for 1 h at 0 °C and for 1 h at RT. The solvent was removed and the residue was dissolved in toluene and concentrated. The residue was dissolved in CH_2Cl_2/DMF 1:1 (1 mL) under N_2. NMM (50 μL) was added dropwise and the solution was stirred at RT for 15 min. mPEG-SPA (50 mg, 10 μmol, 1.0 equiv) was added and the solution was stirred for 14 h at RT under N_2. The solution was filtered, diluted with Et_2O (30 mL), and stored at 4 °C for 4 h. Filtration and purification by chromatography on Sephadex LH-20 (MeOH) gave **7** (22 mg, 4 μmol, 44 %).

Preparation of polymer 8: Compound **5** (9 mg, 30 μmol, 3.0 equiv) was dissolved in CH_2Cl_2 (1 mL), cooled at 0 °C under N_2, and TFA (1 mL) was added dropwise. The solution stirred for 1 h at 0 °C and for 1 h at RT. The solvent was removed and the residue was dissolved in toluene and concentrated. The residue was dissolved in CH_2Cl_2/DMF 1:1 (1 mL) under N_2. NMM (50 μL) was added dropwise and the solution was stirred at RT for 15 min. mPEG-SPA (50 mg, 10 μmol, 1.0 equiv) was added and the solution was stirred for 14 h at RT under N_2. The solution was filtered, diluted with Et_2O (30 mL), and stored at 4 °C for 4 h. Filtration and purification by chromatography on Sephadex LH-20 (MeOH) gave **8** (32 mg, 6 μmol, 64 %).

Preparation of polymer 9: Compound **6** (15 mg, 30 μmol, 3.0 equiv) was dissolved in CH_2Cl_2 (1 mL), cooled at 0 °C under N_2, and TFA (1 mL) was added dropwise. The solution stirred for 1 h at 0 °C and for 1 h at RT. The solvent was removed and the residue was dissolved in toluene and concentrated. The residue was dissolved in CH_2Cl_2/DMF 1:1 (1 mL) under N_2. NMM (50 μL) was added dropwise and the solution stirred at RT for 15 min. mPEG-SPA (50 mg, 10 μmol, 1.0 equiv) was added and the solution was stirred for 14 h at RT under N_2. The solution was filtered, diluted with Et_2O (30 mL), and stored at 4 °C for 4 h. Filtration and purification by chromatography on Sephadex LH-20 (MeOH) gave **9** (30 mg, 5 μmol, 60 %).

Preparation of polymer 11: Boc-L-Glu (300 mg, 1.2 mmol, 1.0 equiv) was dissolved in $CHCl_3$ (3 mL) and cooled to 0 °C (ice bath). NEt_3 (750 μL, 5.3 mmol, 4.0 equiv), HOBt (360 mg, 2.4 mmol, 2.0 equiv), EDC hydrochloride (510 mg, 2.7 mmol, 2.2 equiv), and dopamine hydrochloride (690 mg, 3.6 mmol, 3.0 equiv) were sequentially added. The reaction mixture stirred at 0 °C for 1 h and at RT for 16 h. The mixture was diluted with $CHCl_3$ (10 mL), washed with 1 N HCl (2×10 mL), a saturated aqueous solution of $NaHCO_3$ (2×10 mL), and brine (10 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated. The residue was purified by chromatography on SiO_2 (CH_2Cl_2/MeOH 9:1) to give (*S*)-*tert*-butyl-1,5-bis(3,4-dihydroxyphenethylamino)-1,5-dioxopentan-2-ylcarbamate (477 mg, 0.92 mmol, 76 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.41–1.44 (s, 9H; Boc rotamers), 1.76–1.87 (m, 2H), 2.19 (t, J = 7.10 Hz, 2H), 2.62–2.68 (m, 4H), 3.31–3.37 (m, 4H), 3.64–3.77 (m, 2H), 3.94–4.00 (m, 1H), 6.56–6.61 (m, 2H), 6.63 (d, J = 0.96 Hz, 2H), 6.79 ppm (d, J = 8.32 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 27.2, 28.2, 29.6, 34.6, 34.8, 40.4, 41.1, 51.8, 80.4, 115.3, 115.5, 115.7, 115.8, 120.5, 120.6, 130.5, 130.7, 143.0, 143.1, 144.0, 144.1, 155.9, 171.9, 173.9 ppm; IR 3402 (m), 3010 (m), 1780 (s), 1580 (s), 1273 (s), 1151 (s).

(*S*)-*tert*-Butyl 1,5-bis(3,4-dihydroxyphenethylamino)-1,5-dioxopentan-2-ylcarbamate (15 mg, 30 μmol, 3.0 equiv) was dissolved in CH_2Cl_2 (1 mL), cooled at 0 °C under N_2, and CF_3CO_2H (1 mL) was added dropwise. The solution stirred for 1 h at 0 °C and for 1 h at RT. The solvent was removed and the residue was dissolved in toluene and concentrated. The residue was dissolved in CH_2Cl_2/DMF 1:1 (1 mL) under N_2. NMM (50 μL) was added dropwise and the solution was stirred at RT for 15 min. mPEG-SPA (50 mg, 10 μmol, 1.0 equiv) was added and the solution was stirred for 14 h at RT under N_2. The solution was filtered, diluted with Et_2O (30 mL), and stored at 4 °C for 4 h. Filtration and purification by chromatography on Sephadex LH-20 (MeOH) gave **11** (30 mg 5 μmol, 56 %).

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