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Electronic Supplementary Information

A Degradable Polydopamine Coating Based on Disulfide-Exchange Reaction

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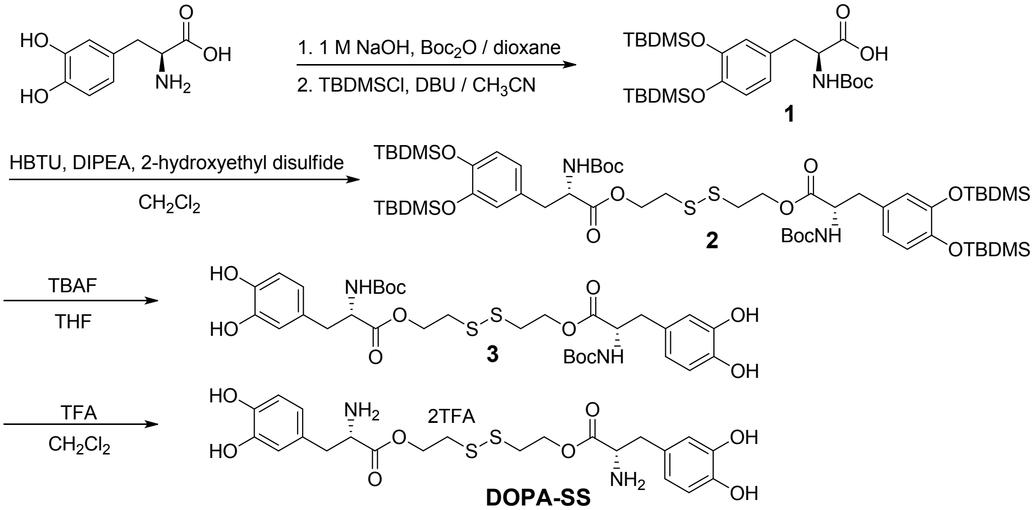
Experimental Details

Materials. 3-(3,4-Dihydroxyphenyl)-L-alanine (L-DOPA, >98.0%, Tokyo Chemical Industry), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 98%, Aldrich), di-*tert*-butyl dicarbonate (Boc_2O, ReagentPlus®, 99%, Aldrich), *tert*-butyldimethylsilyl chloride (TBDMSCl, reagent grade, 97%, Aldrich), tetrahydrofuran (THF, anhydrous, ≥99.9%, Sigma-Aldrich), 2-hydroxyethyl disulfide (technical grade, Aldrich), *N,N,N′,N′*-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU, ≥98%, Aldrich), *N,N*-diisopropylethylamine (DIPEA, ≥99.9%, Sigma-Aldrich), tetrabutylammonium fluoride solution (TBAF, Aldrich), trifluoroacetic acid (TFA, ReagentPlus®, 99%, Sigma-Aldrich), L-glutathione reduced (GSH, ≥98%, Sigma-Aldrich), doxorubicin hydrochloride (HPLC, Sigma), dioxane (anhydrous, 99.8%, Sigma-Aldrich), acetonitrile (anhydrous, 99.8%, Sigma-Aldrich), ammonium chloride (extra pure, Junsei Chemical), calcium hydride (reagent grade, 95%, Sigma-Aldrich), ethanol (absolute, Merck), ethyl acetate (95%, Samchun Chemicals), hexane (95%, Samchun Chemicals), magnesium sulfate ($MgSO_4$, anhydrous, 99.5%, Junsei Chemical), sodium chloride (99.5%, Junsei Chemical), hydrochloric acid (HCl, 35%, Junsei Chemical), diethyl ether (≥99.9%, Daejung), sodium hydroxide (NaOH, 96%, Junsei Chemical), aluminum oxide (Al_2O_3, 99.9% metals basis, Alfa Aesar), zinc (Zn, foil, 0.25-mm thick, 99.9%, Sigma-Aldrich), vanadium (V, foil, 0.127-mm thick, 99.7% metals basis, Aldrich), nickel (Ni, foil, 0.125-mm thick, ≥99.9%, Aldrich), indium tin oxide (ITO, Aldrich), copper (Cu, foil, Kumjeong), and nylon (Goodfellow) were used as received. Polycarbonate (PC), polyethylene (PE), polypropylene (PP), and acryl plate (AC) were purchased from Q-Tech. Polished silicon (100) wafers were purchased from Tasco. Gold (Au) or titanium dioxide (TiO_2) substrates were prepared by coating Au or TiO_2 onto silicon wafers with the thickness of 100 nm by using a thermal evaporator. Dichloromethane (HPLC grade, J. T. Baker) was distilled with calcium hydride before its use for the synthetic reactions. Deionized (DI) water from the Human Ultrapure System (Human Corp.) was used.



Synthesis of DOPA-SS. L-DOPA (6.0 g, 30.42 mmol) was dissolved in dioxane (10.0 mL), and to the L-DOPA solution were added 1 M NaOH (30.42 mL, 30.42 mmol) and Boc_2O (6.6 g, 30.42 mmol) at 0 °C. The mixture solution was stirred for 24 h at room temperature. The solvent was evaporated, and the resultant was washed with 1 M HCl, DI water, and brine, and then dried over $MgSO_4$. The product was concentrated *in vacuo* to give Boc-DOPA without further purification. To the acetonitrile solution (30.0 mL) of Boc-DOPA (2.4 g, 8.1 mmol) was added TBDMSCl (13.3 g, 88.22 mmol). The mixture was cooled to 0 °C, and DBU (13.7 mL, 91.16 mmol) was added dropwise over 10 min. The resulting mixture was stirred at room temperature for 16 h and then concentrated under reduced pressure. The crude product was purified by silica-gel column chromatography (ethyl acetate:hexane = 1:2) to give 5.6 g (35%) of **1** as a yellow oil. ^1H NMR (400 MHz, $CDCl_3$): δ 6.76-6.66 (m, 3H), 4.89 (d, J = 8.0 Hz, 1H), 4.52 (m, 1H), 3.00 (m, 2H), 1.42 (s, 9H), 0.97 (s, 18H), 0.18 (s, 12H). 2-Hydroxyethyl disulfide (1.0 g, 3.24 mmol) and the compound **1** were dissolved in dichloromethane, and to the solution was added HBTU (4.3 g, 11.3 mmol). The mixture was cooled to 0 °C, and DIPEA (2.3 mL, 12.96 mmol) was added dropwise over 10 min. The resulting mixture was stirred at room temperature for 15 h, followed by brine wash. The organic phase was separated, and the aqueous phase was extracted with dichloromethane. The combined organic phase was dried over $MgSO_4$. The organic layer was concentrated under reduced pressure and purified by silica-gel column chromatography (ethyl acetate:hexane = 1:10) to give 2.1 g (56%) of **2**. ^1H NMR (400 MHz, $CDCl_3$): δ 6.75-6.56 (m, 6H), 4.95 (d, J = 8.0 Hz, 2H), 4.53 (m, 2H), 4.36 (m, 4H), 2.96 (m, 4H), 2.87 (m, 4H), 1.42 (s, 18H), 0.98 (s, 36H), 0.18 (s, 24H). TBAF (9.1 mL, 9.1 mmol) was added to the solution of **2** (2.1 g, 1.82 mmol) in THF (20 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and then quenched with saturated ammonium chloride solution (40 mL). The resulting solution was concentrated under reduced pressure and purified by silica-gel column chromatography (ethyl acetate:hexane = 1:1) to give 0.92 g (72%) of **3**. ^1H NMR (400 MHz, $CDCl_3$): δ 6.68-6.50 (m, 6H), 4.31 (m, 6H), 2.85 (m, 8H), 1.39 (s, 18H). The solution of **3** (0.92 g, 1.32 mmol) in dichloromethane (30 mL) was added to TFA (3 mL, 39.5 mmol) at 0 °C. The mixture was stirred at room temperature for 4 h. The organic layer was concentrated under reduced pressure and recrystallized by diethyl ether to give 0.87 g (90%) of DOPA-SS. ^1H NMR (400 MHz, $CDCl_3$): δ 6.78-6.57 (m, 6H), 4.49 (m, 4H), 4.23 (t, 2H), 3.10 (m, 4H), 3.00 (m, 4H). MS: m/z $[M+H]^+$ calcd for $C_{22}H_{29}N_2O_8S_2$ 513.14, found 513.14.

Film formation and degradation. All substrates were washed thoroughly with ethanol before coating processes. The buffer solution (10 mM Tris, pH 8.5) of DOPA-SS (2.5 mM) was prepared and carefully poured over a substrate for coating. After 3 h, the substrate was washed with DI water and dried under a stream of argon gas. The substrate was coated again with a freshly prepared Tris solution of DOPA-SS for 3 h, and washed with DI water, and dried under a stream of argon gas. The buffer solution (10 mM Tris, pH 7.4) of GSH (1, 5, or 20 mM) was used for DOPA-SS film degradation. DOPA-SS-coated substrates were immersed in the prepared GSH solution for predetermined time, washed with DI water, and dried under a stream of argon gas.

Release profiles of doxorubicin. The buffer solution (10 mM Tris, pH 8.5) of DOPA-SS (2.5 mM) and doxorubicin hydrochloride (125 μM) was prepared, and carefully poured over TiO_2 substrates for coating. After 3 h, the TiO_2 substrates were washed with DI water and dried under a stream of argon gas. For GSH-mediated film degradation and release of doxorubicin, the substrates were incubated in a buffer solution (10 mM Tris, pH 7.4) of GSH (1, 5, or 20 mM) for predetermined time. An aliquot was taken from the solution, and its fluorescence intensity was measured by fluorescence spectrometry (λ_{ex} = 480 nm; λ_{em} = 580 nm) for the quantification of released doxorubicin.

QCM-D analysis of DOPA-SS-coated films. Quartz crystal microbalance with dissipation (QCM-D) measurements for frequency change, *Δf*, and dissipation change, *ΔD*, were performed with a Q-sense E4 system (Biolin Scientific). A titanium-coated crystal was used (Biolin Scientific). Tris buffered solution (10 mM, pH 7.4) was used as a flow (flow rate: 50 μL/min). The DOPA-SS-coated crystal was mounted at room temperature and prewashed with a buffer solution for 20 min. The degradation of the DOPA-SS films was performed by injecting the buffer solution that contained GSH (20 mM), and time-course changes in the *Δf* and *ΔD* were measured simultaneously at the seven different overtones for 1 h. The crystal was excited at its fundamental frequency (5 MHz), and the measurements were performed at the first, third, fifth, seventh, ninth, eleventh, and thirteenth overtones, corresponding to 5, 15, 25, 35, 45, 55, and 65 MHz, respectively. For the analysis, *Δf* and *ΔD* of the third, fifth, and seventh overtone of a QCM crystal were used.

Characterizations. All synthesized compounds were characterized by nuclear magnetic resonance spectroscopy (NMR, Inova) operated at the ultrashield of 400 MHz. High resolution mass spectrometry (HR-MS, Bruker Daltonik) was used for the characterization of DOPA-SS. The X-ray photoelectron spectroscopy (XPS) study was performed with a VG-Scientific spectrometer (model: Sigma Prove) with a monochromatized Al Kα X-ray source (1486.6 eV). Emitted photoelectrons were detected by a multi-channel detector at a take-off angle of 90^{o} relative to the surface. During the measurements, the base pressure was 8.0×10^{-8} Torr. Survey spectra were obtained at a resolution of 1 eV from 2 scans, and high-resolution spectra were acquired at a resolution of 0.05 eV from 15 scans. Contact angle measurements were performed with Phoenix 300 apparatus (Surface Electro Optics Co.) equipped with a video camera. The static contact angles of 2-μL water droplets were measured at more than five different locations on each sample, and the average values were reported in this paper. The fluorescence intensities of doxorubicin (λ_{ex} = 480 nm; λ_{em} = 580 nm) were measured with a Varioskan Flash Multimode Reader (Thermo Scientific). The thickness of the DOPA-SS films was measured with a Gaertner L116s ellipsometer (Gaertner Scientific Corporation) equipped with a He-Ne laser (632.8 nm) at a 70° angle of incidence. A refractive index of 1.46 was used for all the films. FT-IR spectra were obtained in vacuum with an IFS-66v/S FTIR spectrometer (Bruker).

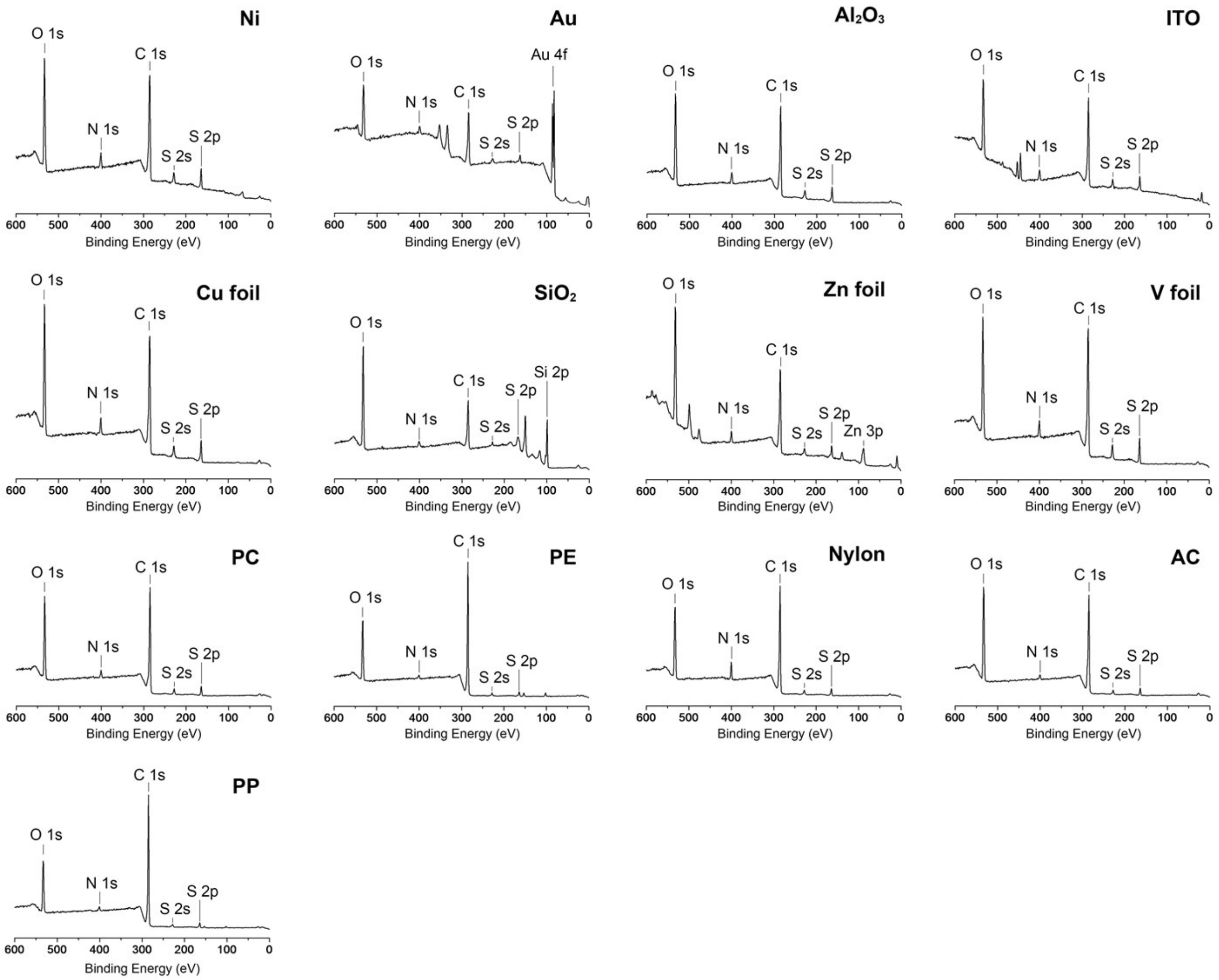


Fig. S1 XPS characterizations of DOPA-SS-coated surfaces. Wide-scan XPS spectra of DOPA-SS-coated surfaces. Nickel (Ni), gold (Au), aluminum oxide (Al_2O_3), indium tin oxide (ITO), copper foil (Cu foil), silicon oxide (SiO_2), zinc foil (Zn foil), vanadium foil (V foil), polycarbonate (PC), polyethylene (PE), nylon, acryl plate (AC), and polypropylene (PP).

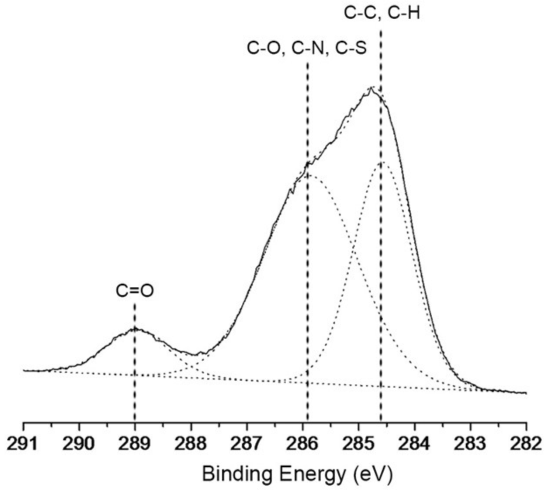


Fig. S2 The C(1s) region of the XPS spectrum of DOPA-SS-coated TiO_2. The characteristic peaks of DOPA-SS at 285.9 (C-O, C-N, and C-S) and 289.0 eV (C=O) were confirmed.

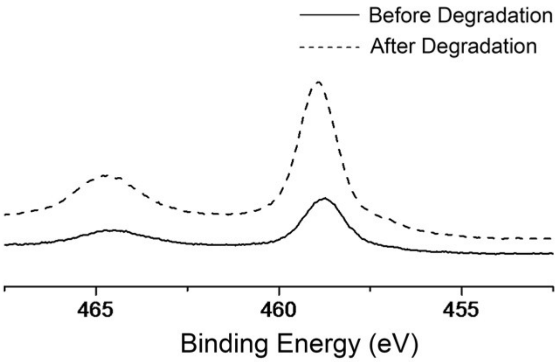


Fig. S3 The Ti(2p) region of the XPS spectra of DOPA-SS-coated TiO_2. The signal intensities of TiO_2 (Ti $2p_{3/2}$, 458.5 eV; $2p_{1/2}$, 464.5 eV) were recovered after degradation (20 mM of GSH, 1 h).

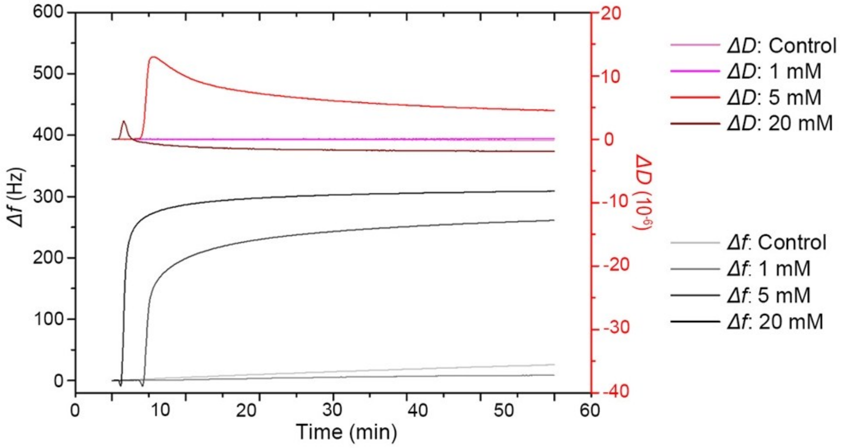
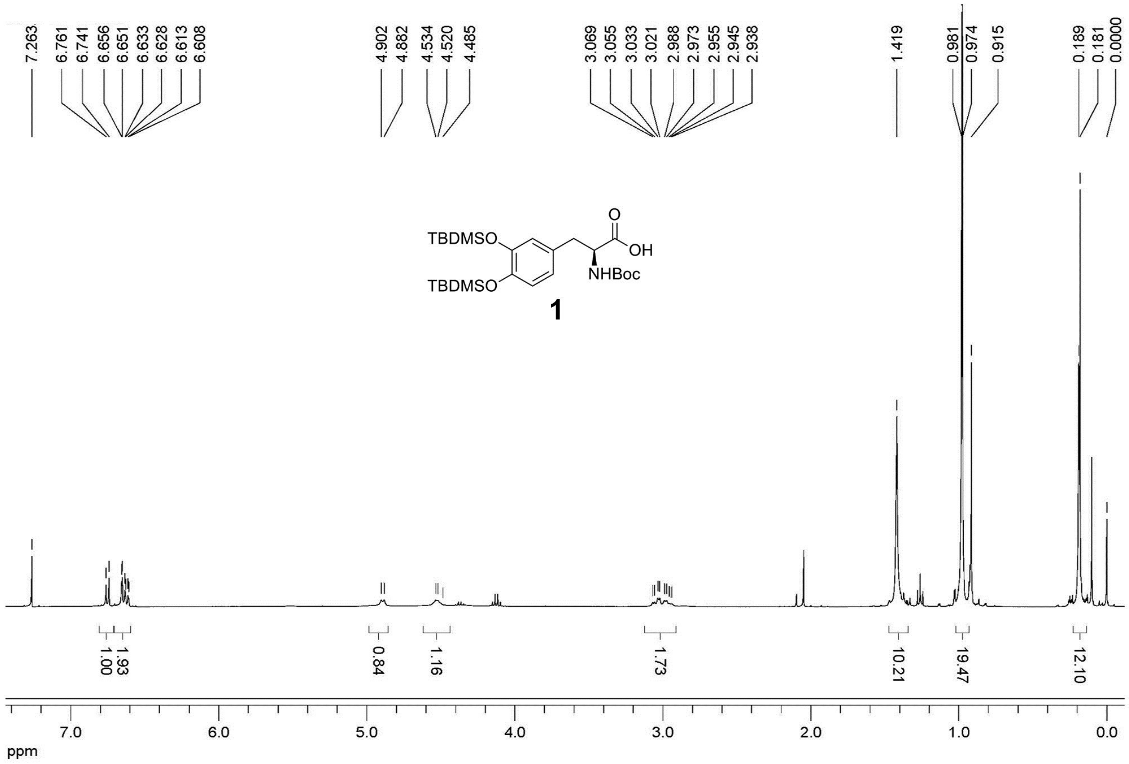


Fig. S4 QCM-D spectra of DOPA-SS-coated TiO_2. Degradation behaviors of DOPA-SS films were monitored by varying the concentration of GSH.

Fig. S5 ^{1}H NMR spectra of the synthesized compounds.



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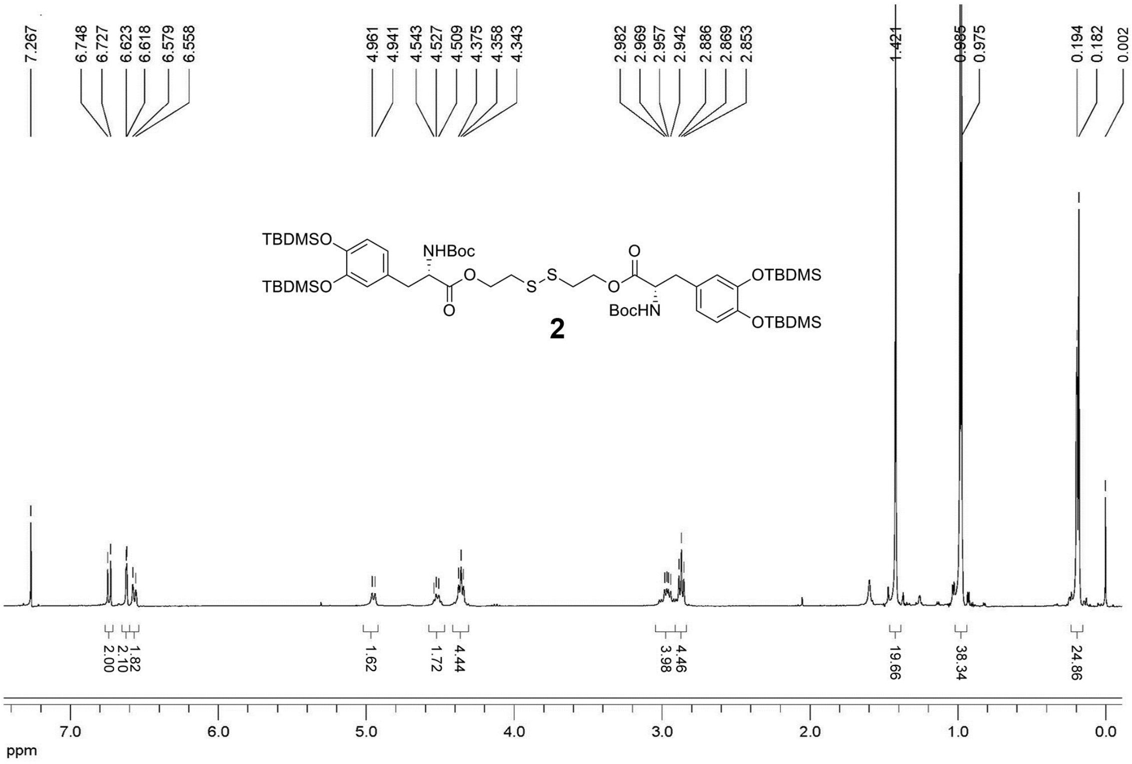

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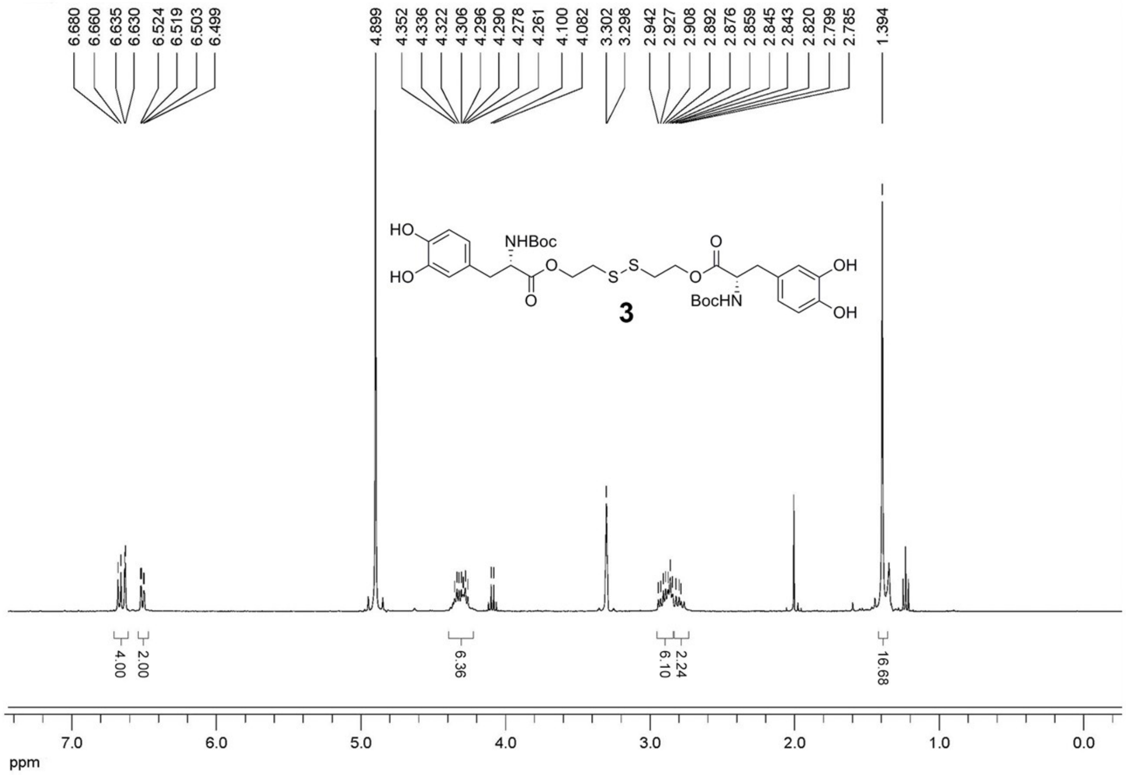
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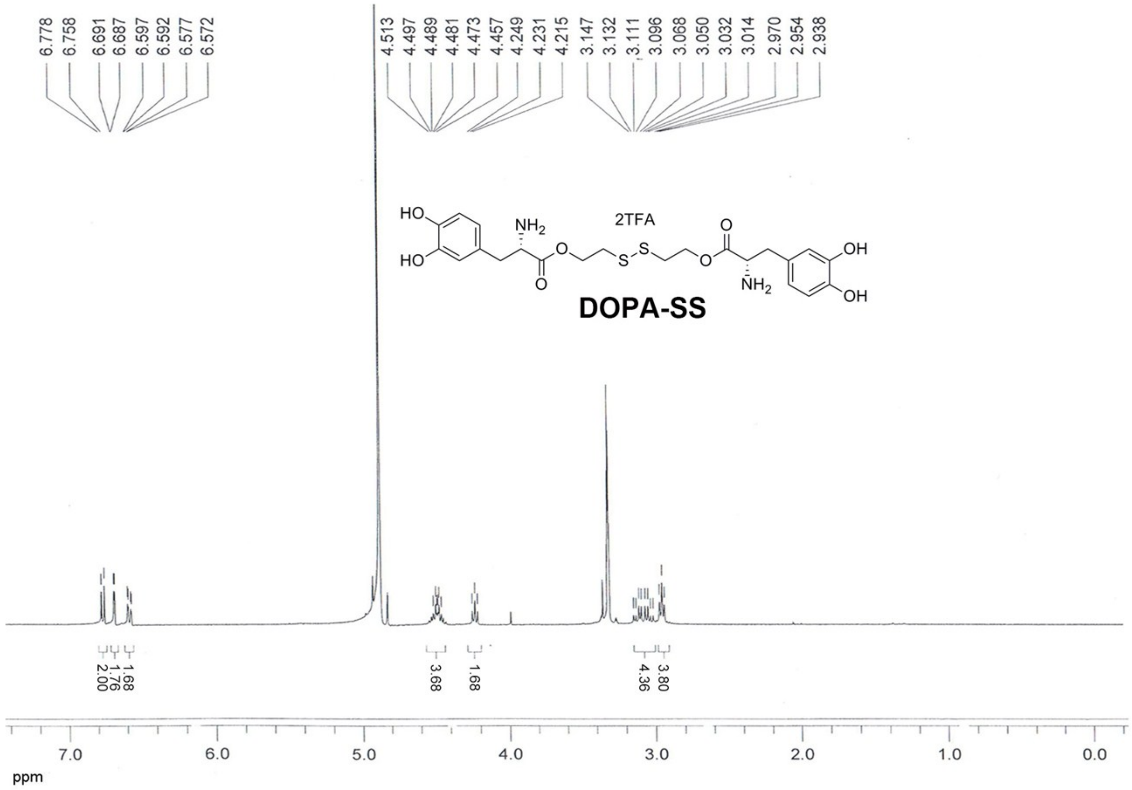
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ppm

DOPA-SS