Electronic Supplementary Information (ESI) for Chemical Communications

Amine-selective affinity resins based on pH-sensitive reversible formation of covalent bonds

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Table of contents

Materials

Triethyl-2-phosphonopropionate, tetraethyl orthosilicate (TEOS) and dimethyl-2-oxoglutarte were purchased form TCI (Japan), sodium hydride (NaH, 60% in mineral oil), dichloromethane (DCM, anhydrous), Tetrahydrofuran (THF, anhydrous), Oxalyl chloride (2M in DCM), 3-aminopropyl triethoxysilane (APTES), triethylamine (TEA), trifluoroacetic acid (TFA) cetyltrimethylammonium bromide (CTAB), benzyl amine and phenyl acetic acid were purchased form Aldrich (USA). NH_4Cl, KOH, HCl, ethanol (EtOH), benzyl alcohol, poly(ethylene glycol)(M_w =2000) and magnesium sulfate ($MgSO_4$) were purchased form Daejung (South Korea). Urea, cyclohexane n-butanol, toluene and acetonitrile (ACN) were purchased form Samchun Chemical (South Korea). Amino functionalized poly(ethylene glycol) (mPEG-amine, M_w =2000) and succinic acid functionalized poly(ethylene glycol) (mPEG-COOH, M_w =2000) were purchased from SunBio (south korea)

Synthesis of carboxy dimethyl maleic anhydride (CDM) (2)

CDM was synthesized by the method in a previous report[1]. Briefly, triethyl-2-phosphonopropionate (1.64 g, 6.89 mmol) was added into anhydrous THF (200 mL) under a nitrogen atmosphere at 0°C. NaH (0.37 g, 9.2 mmol) was added slowly into the solution. The evolution of hydrogen gas stopped after 30-min stirring. Dimethyl-2-oxoglutarte (1.00 g, 5.74 mmol) was added to the solution. After the reaction completion was checked by TLC, excess NaH was quenched by a saturated aqueous solution of NH_4Cl. Following the removal of the THF content in the mixture by evaporation, the remaining aqueous phase was extracted with ethyl acetate (EA) several times. The combined organic phase was washed with deionized water and brine, and dried over MgSO4. After removal of most of organic solvents, the remaining residue was purified by the silica gel chromatography with an eluent of EA/hexane. After evaporation, 4-ethyl 1,3-dimethyl (Z)-pent-3-ene-1,3,4-tricarboxylate (**1**) was obtained as a colorless oil (Yield: 94%). ^{1}H-NMR spectrum of 1 is shown in Figure S6, Supporting Information.

Compound 1 (1.4 g, 5.4 mmol) was dissolved in a 2 M KOH solution in ethanol (14 mL), and allowed to reflux at 80°C for 2 h. The hot reaction mixture was cooled to ambient temperature. After removal of ethanol by evaporation, the mixture was acidified to pH 2 using concentrated HCl. The aqueous phase was then extracted with EA several times. The combined organic phase was dried over $MgSO_4$ and concentrated under reduced pressure to produce CDM (2) as a white solid (Yield: 65%). ^{1}H-NMR spectrum of **2** is shown in Figure S7, Supporting Information.

Synthesis of APTES-CDM (3)

Oxalyl chloride (2 M in DCM, 1.8 mmol), was added dropwise to a solution of CDM (300 mg, 1.6 mmol) in DCM (9 mL) in a glove box. The resulting solution was stirred at ambient temperature for 6 hr and remaining oxalyl chloride was removed under reduced pressure. The resulting oil was suspended in DCM (9 mL), and APTES (420 µL, 1.8 mmol) and TEA (250 µL, 1.8 mmol) were added to the suspension. After 4 hr-stirring, the mixture was concentrated under reduced pressure. The remaining mixture was dissolved in EA and washed with 0.1 M HCl solution (× 2) and brine. The organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel chromatography with an eluent of EA/hexane. APTES-CDM (**3**) was obtained as a colorless oil (Yield: 22%). ^{1}H-NMR spectrum of **3** is shown in Figure S8, Supporting Information.

Synthesis of Wrinkled Silica Nanoparticles

Wrinkled silica nanoparticles (WSN) were synthesized by a previously reported method[2]. First, CTAB (28 g, 78 mmol) and urea (18 g, 0.3 mol) were dissolved in deionized water (900 mL). Subsequently, cyclohexane (900 mL) and n-butanol (33 mL, 0.36 mol) were added to the solution. With vigorous stirring, TEOS (80 mL, 0.36 mol) was added to the mixture. After stirring for 30 min at ambient temperature, the reaction mixture was heated to 70°C and further stirred for 16 h. The reaction mixture was centrifuged and washed with acetone and ethanol. An aqueous HCl (10 M, 300 mL) was added to the mixture for the removal of CTAB from WSN, and the mixture was further stirred for 24 h. The WSN dispersion was centrifuged for 15 min at 8000 rpm, and the precipitate was re-dispersed in EtOH. This centrifugation-re-dispersion procedure was repeated three times. After vacuum drying, WSNs were calcinated in the furnace at 600°C for 6 h. The white product (WSNs) was then obtained (Yield: 84%).

Preparation of WSN-CDM

Synthesized WSNs (400 mg) were dispersed in toluene (40 mL) with sonication for 30 min. APTES-CDM (106 mg) in EtOH (3 mL) was added to the dispersion. The reaction mixture was heated to 80°C, and stirred vigorously for 15 h. The mixture was concentrated under reduced pressure and the remaining solid was washed with ethanol (× 4) and collected by centrifugation. Finally, the precipitate was washed by an aqueous HCl (0.01 M). White product (WSN-CDM) was then obtained by lyophilization (Yield: 79%).

Scanning Electron Microscopy (SEM)

The surface morphology of WSN and WSN-CDM was analyzed by a FT-SEM instrument (Hitachi S-4300, Japan) operated at 15.0 kV. Each sample was coated with Pt by vacuum sputtering for 60 s using an ion sputter (Hitachi E-1030, Japan).

Fourier Transform Infrared (FT-IR) spectroscopy

Functional groups of WSN and WSN-CDM were analyzed by the FT-IR spectroscopy. The FT-IR spectrum was recorded using a spectrometer (Thermo Scientific Model iS10, USA). For the measurement, WSN and WSN-CDM were pressed into KBr pellets.

Thermogravimetric analysis (TGA)

The thermogravimetric analysis of WSN and WSN-CDM was conducted in an N_2 gas stream (TA instruments Model TGA Q50, USA). Each sample was heated from 30°C to 800°C at a heating rate of 10°C/min.

High performance liquid chromatography (HPLC)

Model molecules were quantified by using an Agilent HPLC system (Model 1100 series) which is assembled with a binary pump (G1312A), a column oven (G1316A), and a UV detector (G1365B). A XDB-C18 reverse phase column (5 μm, Agilent) was used for the analysis. A gradient-based ACN/water mixture containing 0.1% TFA was used as the mobile phase for the quantification of benzyl amine, benzyl alcohol and phenyl acetic acid. An 8% MeOH/water mixture containing 0.1% TFA was used as the mobile phase for the quantification of benzyl amine, N,N-dimethyl amino benzene and benzyl trimethyl ammonium chloride.

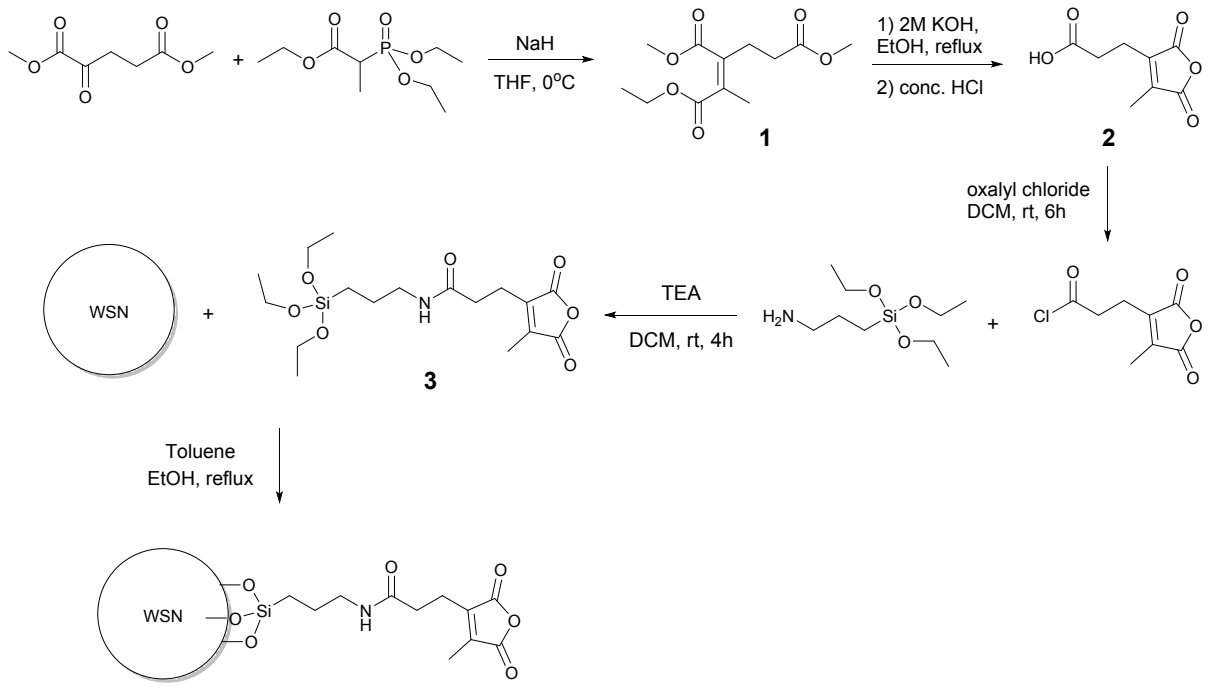
MALDI-TOF MS

MALDI-TOF mass spectrometry was carried out for the analysis of polymer purification. A Bruker DE/micro flex LT mass spectrometer equipped with a nitrogen UV laser (337 nm) was used for the analysis. Generally, dithranol was used as the matrix.

General method for the separation of amine-containing molecules

The BBP and BDT mixture sample were prepared as an ACN solution. An approximate concentration of each component in the mixture was 0.4 mM. TEA was added to the solution at the final concentration of 50 mM for alkaline circumstances. WSN-CDM (20 mg) was dispersed in the mixture solution (1 mL) and stirred for 2 hr at ambient temperature. After the formation of covalent bonds between amines and WSN-CDM, WSN-CDM with amine-containing molecules was separated from the mixture by centrifugation, and washed with a TEA solution in ACN (50 mM) (× 2) and with NH_4Cl (× 2) for removal of non-specifically adsorbed molecules. WSN-CDM with amine-containing molecules was dried by vacuum. For releasing amine-containing molecules from WSN-CDM, the dried WSN-CDM was dispersed in 0.1% TFA solution in ACN for 2hr at ambient temperature. After centrifugation, the supernatant was analyzed by HPLC.

The PEG-mixture sample was prepared by mixing of three PEG in ACN (0.33 mM each, containing 50 mM TEA). For the PEG binding, 0.0165 μmol of each PEG was loaded to 1 mg of the WSN-CDM resin. The polymeric mixture was analysed by MALDI-TOF MS during the purification.



Scheme S1. Synthetic scheme of WSN-CDM

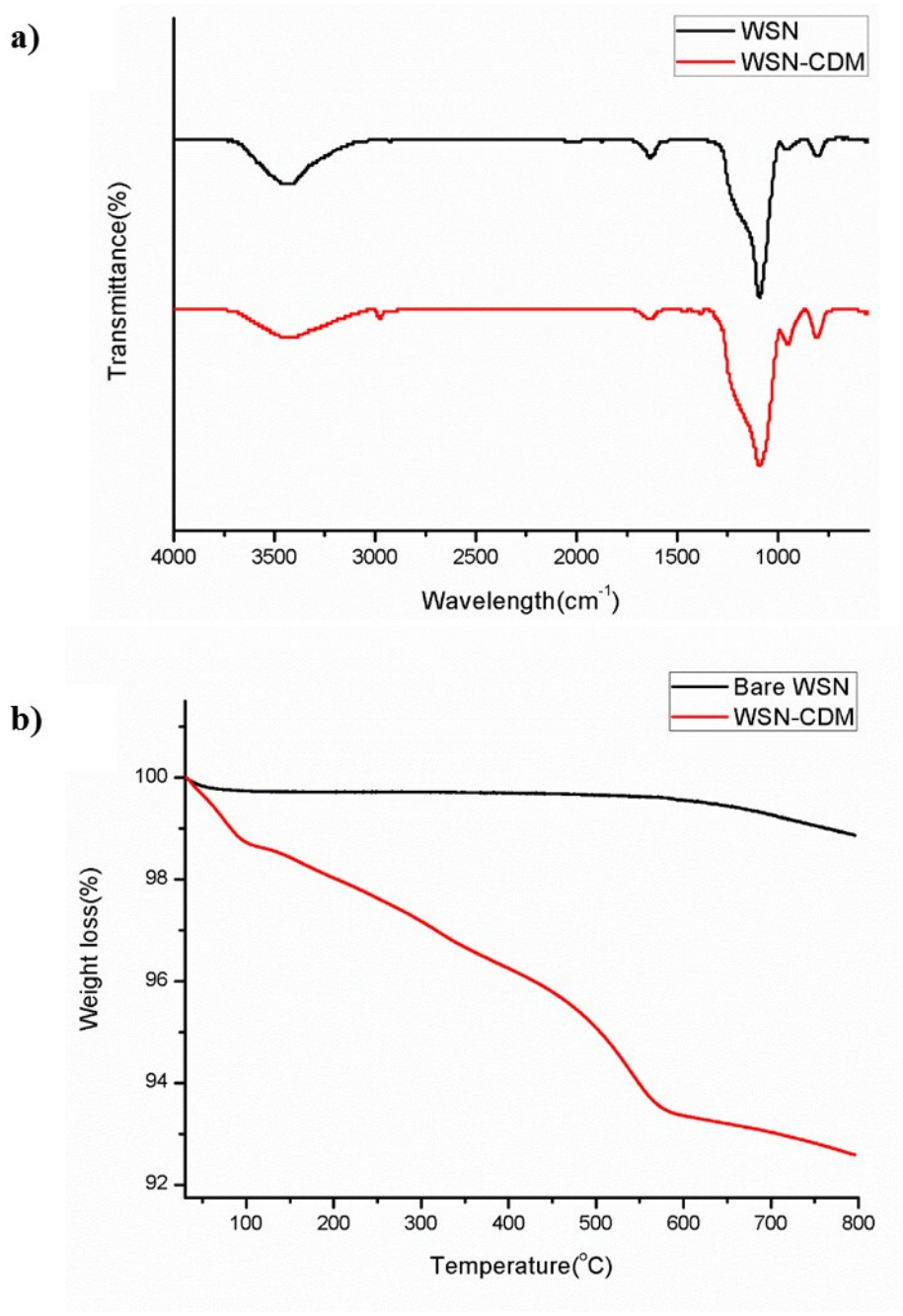


Figure S1. Characterization WSN-CDM. FT-IR spectra (a) and TGA analyses (b) under an N_2 atmosphere of bare WSN (black) and WSN-CDM (red).

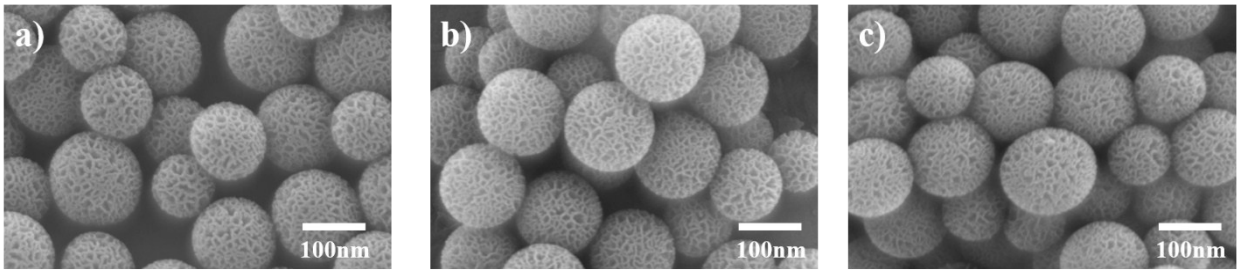


Figure S2. Scanning electron microscope (SEM) images of bare WSN (a), calcined WSN (b) and WSN-CDM (c).

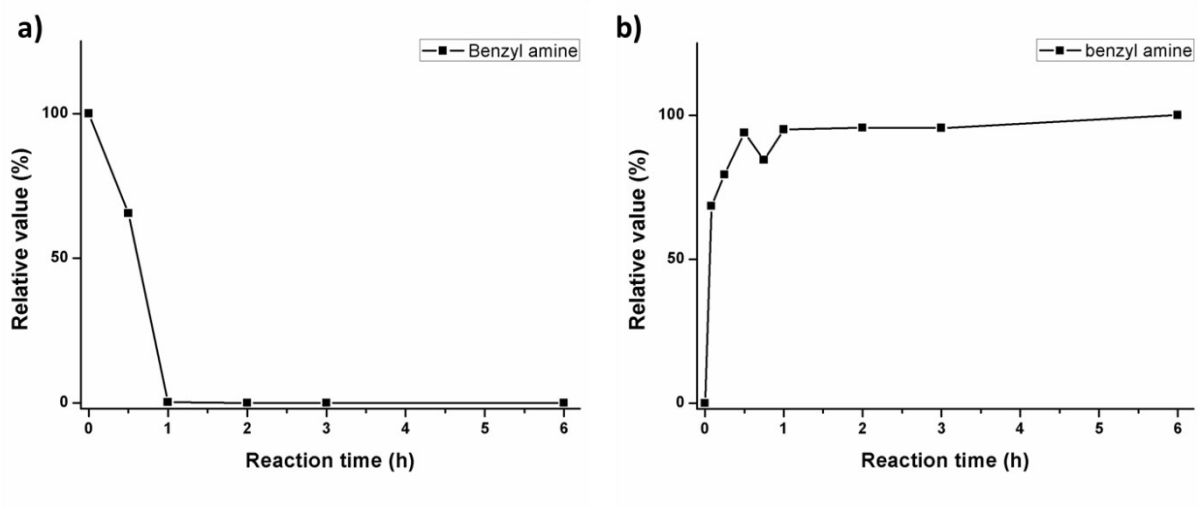


Figure S3. Time dependence of binding (a), and releasing (b) chromatography of Benzyl amine.

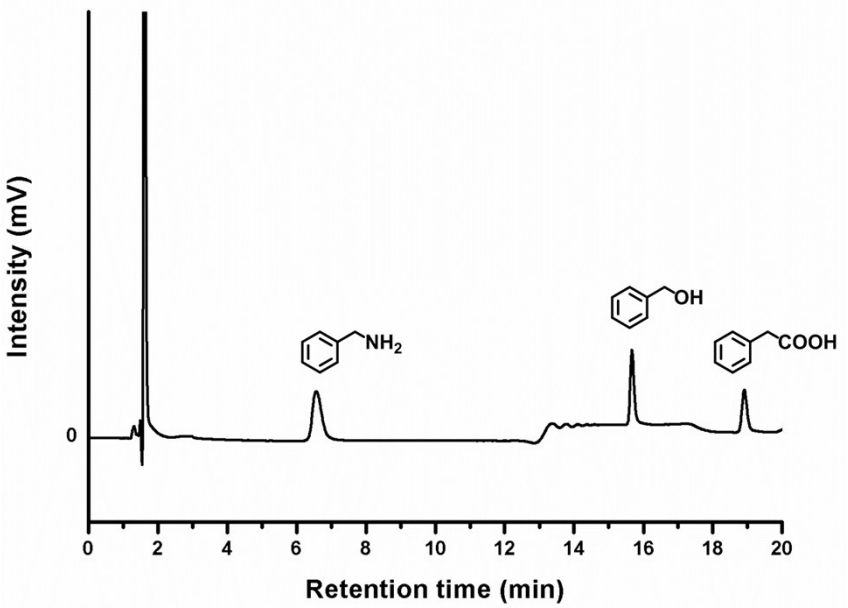


Figure S4. Chromatography of benzyl amine, benzyl alcohol and phenyl acetic acid (BBP mix).

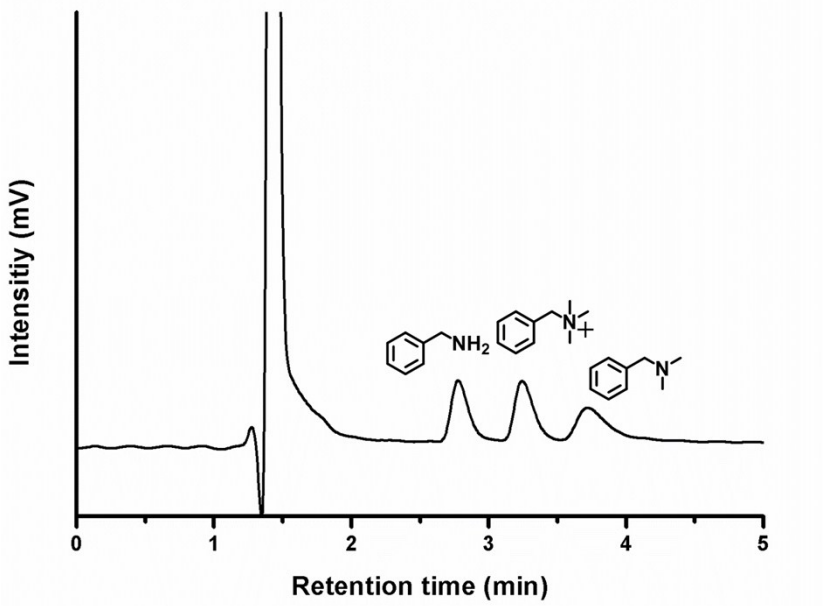


Figure S5. Chromatography of benzyl amine, N,N-dimethyl benzyl amine, benzyl trimethyl ammonium (BDT mix)

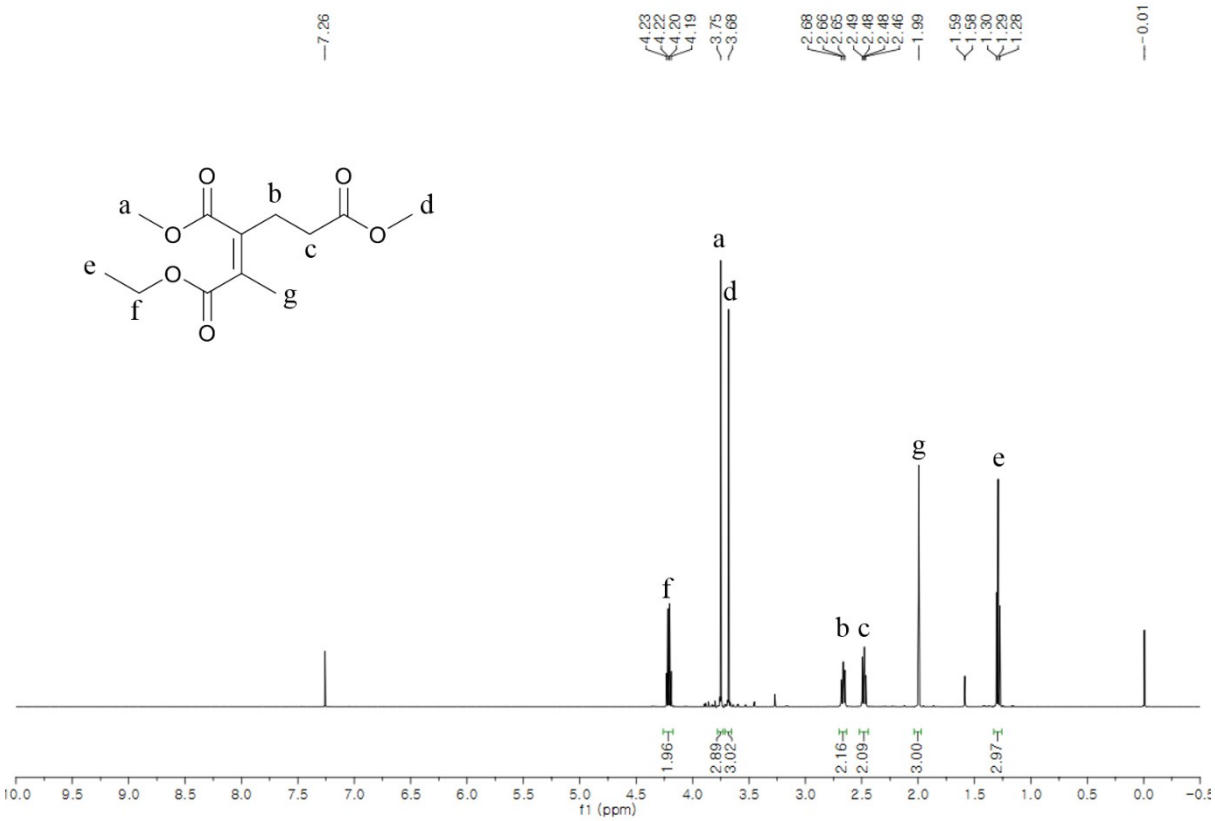


Figure S6. ^{1}H-NMR spectra of 4-ethyl 1,3-dimethyl (Z)-pent-3-ene-1,3,4-tricarboxylate.

δH (500 MHz; $CDCl_3$; Me_4Si) 4.2 (2 H, q, CH_3CH_2OC), 3.75 (3 H, s, CH_3OCOC), 3.68 (3 H, s, CH_2COOCH_3), 2.6 (2 H, t, CCH_2CH_2COO), 2.5 (2 H, t, CCH_2CH_2COO), 2.0 (3 H, s, CCH_3) and 1.3 (3 H, t, CH_3CH_2OC)

Figure S7. ^{1}H-NMR spectra of CDM

δH (300 MHz; $CDCl_3$; Me_4Si) 2.77 (4 H, s, C*CH*$_2$*CH*$_2$CO$_2$H), and 2.12 (3 H, s, COC*CH*$_3$C)

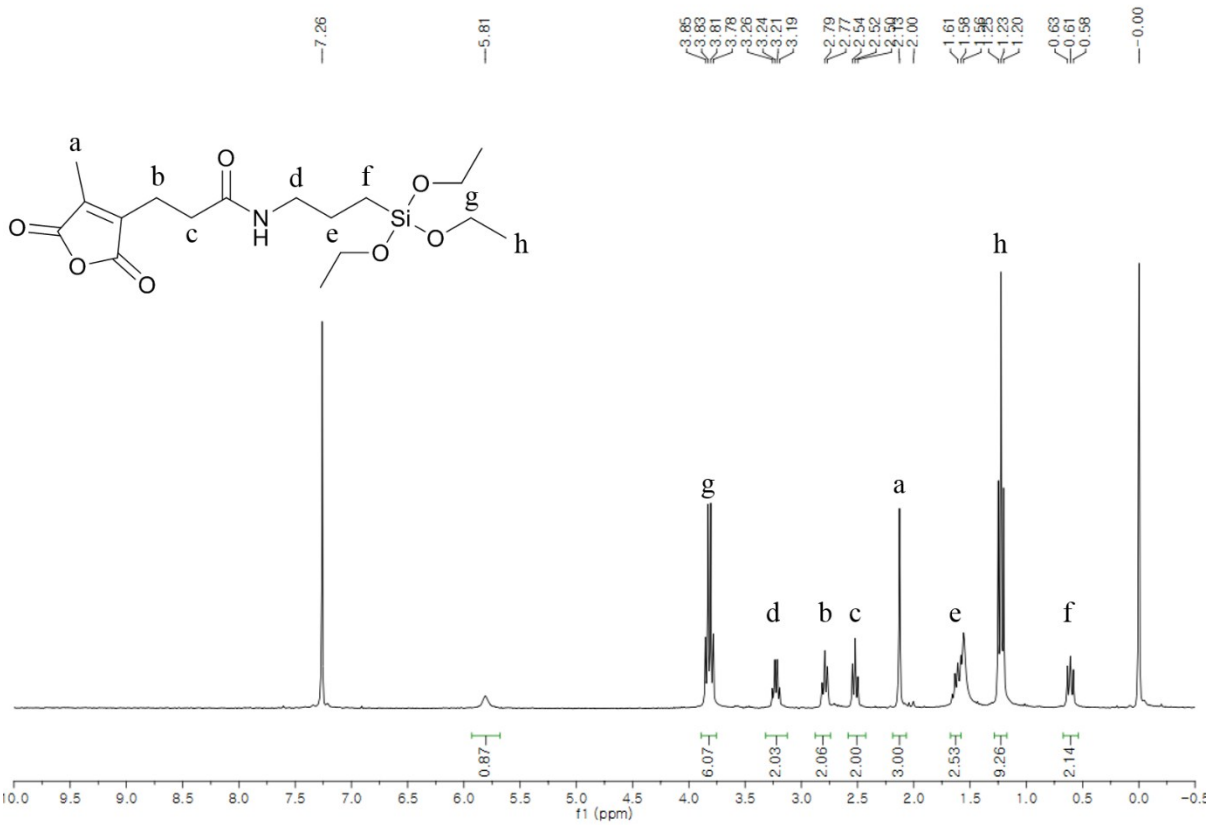


Figure S8. ^{1}H-NMR spectra of APTES-CDM

δH (300 MHz; $CDCl_3$; Me_4Si) 5.8 (1 H, s, CH_2*NH*CO), 3.8 (6 H, q, SiO*CH_2*CH_3), 3.2 (2 H, q, CH_2*CH_2*NHCO), 2.7 (2 H, t, NHCOCH$_2$*CH_2*C), 2.5 (2 H, t, NHCO*CH_2*CH_2C), 2.1 (3 H, s, COC*CH_3*C), 1.6 (2 H, m, SiCH$_2$*CH_2*CH_2), 1.2 (9 H, t, SiOCH$_2$*CH_3*), 0.6 (2 H, t, Si*CH_2*CH_2)

Table S1. Binding and recovery efficiency of benzyl amine according to the loading amount.

Molecules	Loading (μmol/mg)	Bound		Released	
		Amount (μmol/mg)[a]	Binding Efficiency (%)[b]	Amount (μmol/mg)[c]	Recovery Efficiency (%)[d]
B. amine	0.034	0.026	76	0.019	73
	0.012	0.010	83	0.009	92

[a]The binding amount was calculated from the non-bound amount in the supernatant, and includes non-covalently bound molecules. [b]The binding efficiency is the ratio between the binding amount and the loading amount. [c]The amount obtained after removal of non-covalently bound molecules by washing and treatment with TFA. [d]The release efficiency is the ratio between the released amount and the bound amount.

References

1. S. Kang, Y. Kim, Y. Song, J. U. Choi, E. Park, W. Choi, J. Park and Y. Lee, *Bioorganic & Medicinal Chemistry Letters*, 2014, **24**, 2364-2367.
2. D.-S. Moon and J.-K. Lee, *Langmuir*, 2012, **28**, 12341-12347.