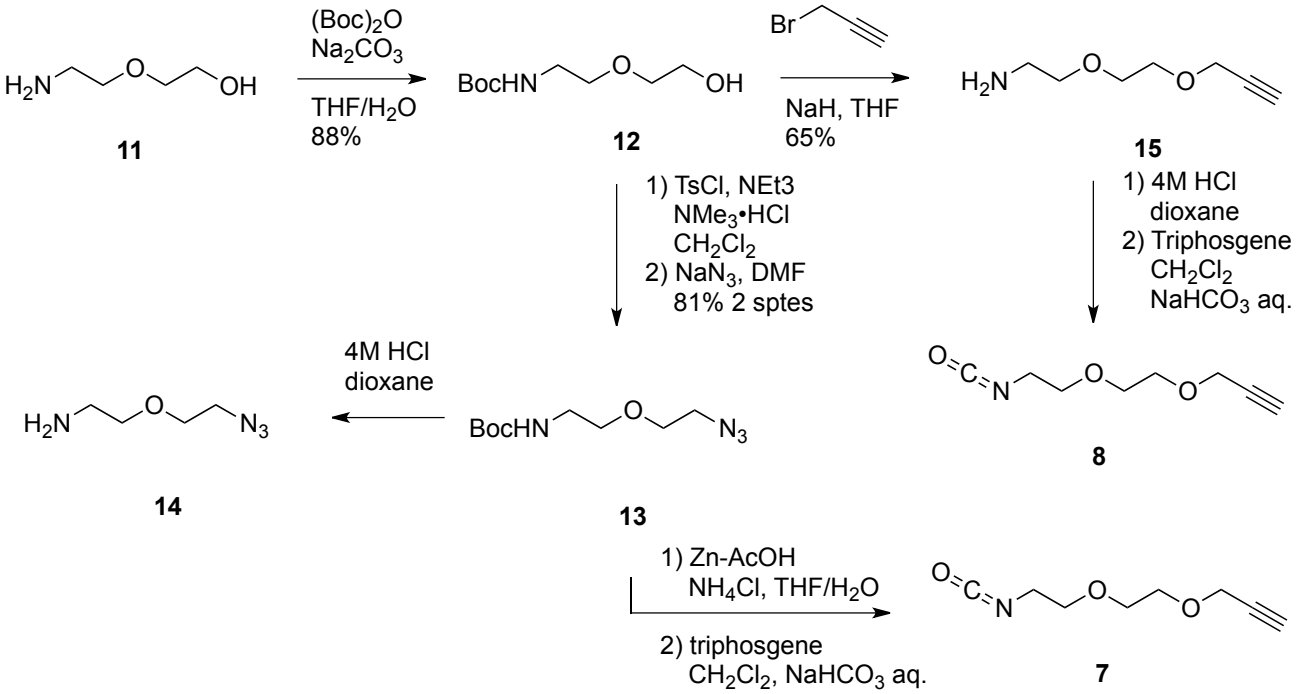
Electronic Supplementary Material (ESI) for RSC Advances

Supporting Information

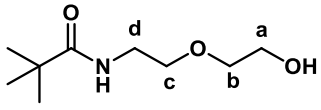
Preparation of the PEG-acetylene (8) and PEG-NHBoc (7)



***tert*-butyl 2-(2-hydroxyethoxy)ethylcarbamate (12)**

To a stirred solution of 2-(2-aminoethoxy)ethanol (**11**) (9.97 mL, 100 mmol, 1.00 eq.) in THF (100 mL) and H_2O (100 mL) was added $(Boc)_2O$ (24.0 g, 110 mmol, 1.10 eq.) and Na_2CO_3 (31.8 g, 300 mmol, 3.00 eq.) at 0 °C. After being stirred at room temperature for 24 h, the reaction mixture was poured into ice-cooled 3 M HCl. The aqueous layer was extracted with two portions of ethtyl acetate. The combined extract was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was chromatographed on silica gel with 97 : 3 chloroform : methanol to give *tert*-butyl 2-(2-hydroxyethoxy) ethylcarbamate (**12)** (18.1 g, 88.0 mmol, 88%) as a colorless neat.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.23 (br-s, 1H, N*H*), 3.68 (td, 2H, H-a, $J_{a,OH}$ = 5.3 Hz, $J_{a,b}$ = 4.4 Hz), 3.53-3.48 (m, 4H, H-b, c), 3.27 (m, 2H, H-d), 3.04 (t, 1H, O*H*, $J_{OH,a}$ = 5.3 Hz), 1.39 (s, 9H, *t*Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.1, 79.2, 72.2, 70.2, 61.5, 40.3, 28.3; IR (neat); 3419, 2978, 1695, 1172 cm^{-1}; HRMS (ESI-TOF) $[M+H]^+$ calcd. 206.1392, found 206.1389.

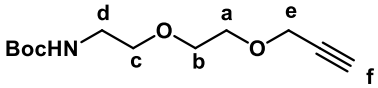


***tert*-butyl 2-(2-(prop-2-ynyloxy)ethoxy)ethylcarbamate (15)**

To a stirred 63% sodium hydride (850 mg, 22.3 mmol, 2.00 eq.), washed twice with dry hexane, was added a solution of *tert*-butyl 2-(2-hydroxyethoxy)ethylcarbamate (**12**) (2.29 g, 11.2 mmol, 1.00 eq.) in THF (30.0 mL) at 0 °C and the reaction mixture was stirred at the same

temperature for 10 min. Then propagyl bromide (967 μL, 11.7 mmol, 1.05 eq.) was added to the reaction mixture at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was poured into ice-cooled 1 M HCl. The aqueous layer was extracted with two portions of ethtyl acetate. The combined extract was washed with saturated aq. $NaHCO_3$, brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was chromatographed on silica gel with 97 : 3 chloroform : methanol to give *tert*-butyl 2-(2-(prop-2-ynyloxy)ethoxy)ethylcarbamate (**15**) (1.77 g, 7.26 mmol, 65 %) as a colorless neat.

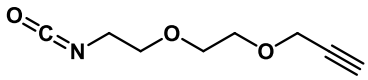
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.01 (br-s, 1H, N*H*), 4.21 (d, 2H, H-e, $J_{e,f}$ = 2.4 Hz), 3.71-3.68 (m, 2H, H-b or c), 3.67-3.64 (m, 2H, H-b or c), 3.55 (t, 2H, H-a, $J_{a,b}$ = 5.3 Hz), 3.35-3.30 (m, 2H, H-d), 2.46 (t, 1H, H-f, $J_{f,e}$ = 2.4 Hz), 1.45 (s, 9H, *t*Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.8, 155.9, 79.5, 79.1, 74.6, 70.2, 70.1, 69.0, 58.4, 40.3, 28.3; IR (neat); 3356, 2875, 2116, 1713, 1520, 1251, 1170, 773 cm^{-1}; HRMS (ESI-TOF) $[M+H]^+$ calcd. 244.1549, found 244.1543.



3-(2-(2-isocyanatoethoxy)ethoxy)prop-1-yne (**8**)

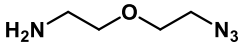
To a stirred solution of *tert*-butyl 2-(2-(prop-2-ynyloxy)ethoxy)ethylcarbamate (**15**) (1.09 g, 4.49 mmol, 1.00 eq.) in 1,4-dioxane (5.00 mL) was added 4 N HCl in 1,4-dioxane (15.0 mL) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was concentrated *in vacuo*. The residue was used for the next reaction without further purification.

To a stirred solution of the above residue in CH_2Cl_2 (20.0 mL) and saturated aq. $NaHCO_3$ (20.0 mL) was added triphosgene (454 mL, 1.53 mmol, 0.340 eq.) at 0 °C. After being stirred at same temperature for 15 min, the reaction mixture was poured into water and CH_2Cl_2. The aqueous layer was extracted with two portions of CH_2Cl_2. The combined extract was washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was used for the next reaction without further purification.



2-(2-azidoethoxy)ethanamine (**14**)

To a stirred solution of *tert*-butyl 2-(2-azidoethoxy)ethylcarbamate (**13**) (230 mg, 1.00 mmol, 1.00 eq.) in 1,4-dioxane (2.00 mL) was added 4 N HCl in 1,4-dioxane (4.00 mL) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was concentrated *in vacuo*. The residue was used for the next reaction without further purification.



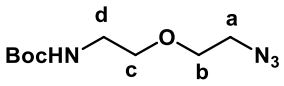
***tert*-butyl 2-(2-azidoethoxy)ethylcarbamate** (**13**)

To a stirred solution of *tert*-butyl 2-(2-hydroxyethoxy)ethylcarbamate (**12**) (9.33 g, 45.5 mmol, 1.00 eq.) in CH_2Cl_2 (100 mL) was added *p*-toluenesulfonyl chloride (9.50 g, 50.0 mmol, 1.10 eq.), triethylamine (12.8 ml, 90.9 mmol, 2.00 eq.) and a catalytic amount of trimethylamine

hydrochloride at 0 °C. After being stirred at room temperature for 2 h, the reaction mixture was poured into ice-cooled 1 M HCl. The aqueous layer was extracted with two portions of ethtyl acetate. The combined extract was washed with saturated aq. $NaHCO_3$, brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was used for the next reaction without further purification.

To a stirred solution of the above residue in DMF (100 mL) was added sodium amide (3.14 g, 48.4 mmol, 1.10 eq.) at room temperature. After being stirred at 60 °C for 2 h, the reaction mixture was poured into ice-cooled 1 M HCl. The aqueous layer was extracted with two portions of ethtyl acetate. The combined extract was washed with saturated aq. $NaHCO_3$, brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was chromatographed on silica gel with 50 : 50 ethylacetate : hexane to give *tert*-butyl 2-(2-azidoethoxy)ethylcarbamate (**13**) (8.49 g, 36.9 mmol, 81% in 2 steps) as a colorless neat.

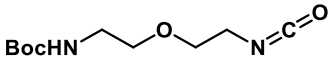
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.92 (br-s, 1H, N*H*), 3.57 (m, 2H, H-b or c), 3.49-3.44 (m, 2H, H-b or c), 3.30 (t, 2H, H-a, $J_{a,b}$ = 5.3 Hz), 3.24 (t, 2H, H-d, $J_{d,c}$ = 5.3 Hz), 1.37 (s, 9H, *t*Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.8, 79.0, 70.1, 69.7, 50.4, 40.2, 28.2; IR (neat); 3364, 2978, 2108, 1710, 1515, 1279 cm^{-1}; HRMS (ESI-TOF) $[M+Na]^+$ calcd. 253.1277, found 253.1278.



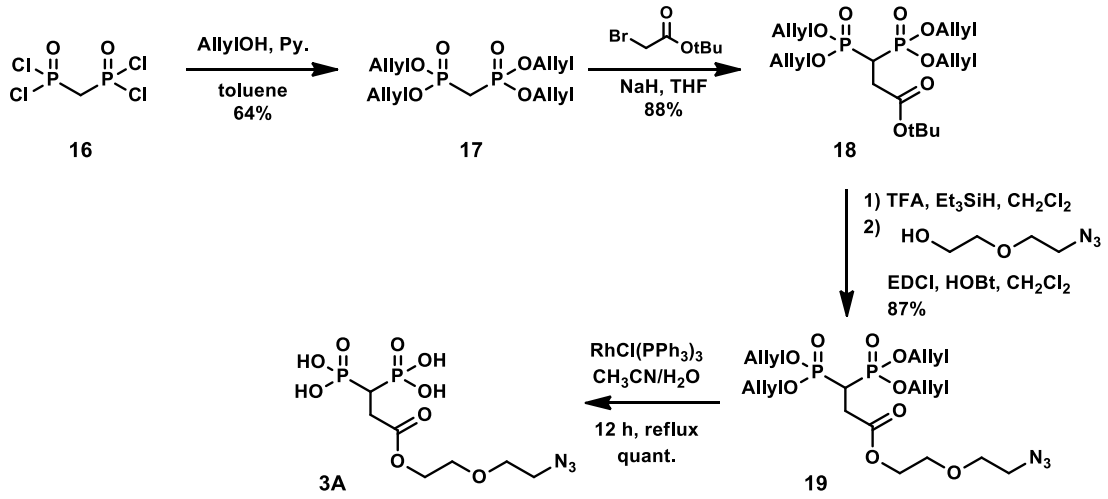
***tert*-butyl 2-(2-isocyanatoethoxy)ethylcarbamate** (**7**)

To a stirred solution of *tert*-butyl 2-(2-azidoethoxy)ethylcarbamate (**13**) (241 mg, 1.05 mmol, 1.00 eq.) in THF (9.00 mL) and H_2O (1.00 mL) was added Zn dust (205 mg, 3.14 mmol, 3.00 eq.) and NH_4Cl (168 mg, 3.14 mmol, 3.00 eq.) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was filtered and concentrated *in vacuo*. The residue was used for the next reaction without further purification.

To a stirred solution of the above residue in CH_2Cl_2 (10.0 mL) and $NaHCO_3$ aq. (10.0 mL) was added triphosgene (106 mL, 0.357 mmol, 0.340 eq.) at 0 °C. After being stirred at same temperature for 15 min, the reaction mixture was poured into water and CH_2Cl_2. The aqueous layer was extracted with two portions of CH_2Cl_2. The combined extract was washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was used for the next reaction without further purification.



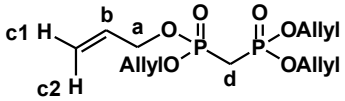
Preparation of the phosphrous unit (3A)



Tetraallyl methylene bisphosphonate (17)

To a stirred solution of bis(phosphonic dichloride) (**16**) (5.00 g, 20.0 mmol, 1.00 eq.) in toluene (10.0 mL) was added a solution of allyl alcohol (6.80 mL, 100 mmol, 5.00 eq.) in pyridine (6.00 mL) over 80 min at 0 ^{o}C. After being stirred at room temperature for 48 h, the reaction mixture was poured into ice-cooled 1 M HCl. The aqueous layer was extracted with two portions of ethtyl acetate. The combined extract was washed with saturated aq. $NaHCO_3$, brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was chromatographed on silica gel with 97 : 3 chloroform : methanol to give tetraallyl methylene bisphosphonate (**17)** (5.72 g, 17.0 mmol, 85 %) as a colorless neat.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.90-5.81 (m, 4H, H-b), 5.28 (dd, 4H, H-c1, $J_{c1,b}$ = 15.6 Hz, $J_{c1,c2}$ = 1.5 Hz), 5.16 (dd, 4H, H-c2, $J_{c2,b}$ = 15.6 Hz, $J_{c2,c1}$ = 1.5 Hz), 4.54-4.50 (m, 8H, H-a), 2.47 (t, 2H, H-d, $J_{d,p}$ = 21.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 132.4, 118.1, 66.82, 66.79, 66.77, 25.8 (t, d, $J_{Cd,p}$ = 137 Hz); ^{31}P NMR (100 MHz, $CDCl_3$) δ 20.5; IR (neat); 3484, 2894, 1258, 1023 cm^{-1}; HRMS (ESI-TOF) $[M+H]^+$ calcd. 337.0970, found 337.0964.

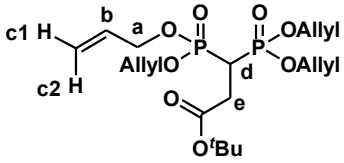


***tert*-butyl 3,3-bis(bis(allyloxy)phosphoryl)propanoate (18)**

To a stirred 55% sodium hydride (781 mg, 17.9 mmol, 1.05 eq.), washed twice with dry hexane, was added a solution of tetraallyl methylene bisphosphonate (**17**) (5.72 g, 17.0 mmol, 1.00 eq.) in dry THF (50.0 mL) at 0 ^{o}C and the reaction mixture was stirred at the same temperature for 10 min. Then *t*-butyl bromoacetate (2.64 mL, 17.9 mmol, 1.05 eq.) was added to the reaction mixture at 0 ^{o}C. After being stirred at room temperature for 12 h, the reaction mixture was poured into ice-cooled 1 M HCl. The aqueous layer was extracted with two portions of ethtyl acetate. The combined extract was washed with saturated aq. $NaHCO_3$, brine, dried over $MgSO_4$, filtered and

concentrated *in vacuo*. The residue was chromatographed on silica gel with 97 : 3 chloroform : methanol to give *tert*-butyl 3,3-bis(bis(allyloxy)phosphoryl)propanoate (**18**) (6.89 g, 15.3 mmol, 90 %) as a colorless neat.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.90-5.81 (m, 4H, H-b), 5.28 (br-d, 4H, H-c1, $J_{c1,b}$ = 16.9 Hz), 5.15 (br-d, 4H, H-c2, $J_{c2,b}$ = 10.6 Hz), 4.59-4.50 (m, 8H, H-a), 3.11 (tt, 1H, H-d, $J_{d,p}$ = 24.2 Hz, $J_{d,e}$ = 6.3 Hz), 2.72 (td, 1H, H-e, $J_{e,p}$ = 16.4 Hz, $J_{e,d}$ = 6.3 Hz), 1.37 (s, 9H, *t*Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.50, 169.41, 169.32, 132.69, 132.58, 117.9, 81.3, 67.10, 67.05, 66.97, 66.91, 34.3, 32.9, 31.5, 31.22, 31.18, 31.14, 27.89, 27.84; ^{31}P NMR (100 MHz, $CDCl_3$) δ 23.9; IR (neat); 3484, 2982, 1734, 1251, 1024, 830 cm^{-1}; HRMS (ESI-TOF) $[M+H]^+$ calcd. 451.1651, found 451.1655.

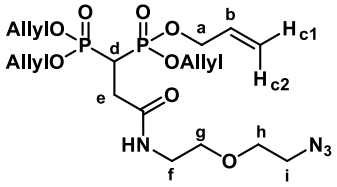


tetraallyl 3-(2-(2-azidoethoxy)ethylamino)-3-oxopropane-1,1-diyldiphosphonate (19)

To a stirred solution of *tert*-butyl 3,3-bis(bis(allyloxy)phosphoryl)propanoate (**18**) (2.00 g, 4.44 mmol, 1.00 eq.) in CH_2Cl_2 (10.0 mL) was added to trifluoroacetic acid (10.0 mL) at 0 °C. After being stirred at room temperature for 6 h, the reaction mixture was concentrated *in vacuo*. The residue was azeotroped with toluene and CH_2Cl_2 twice to give 3,3-bis(bis(allyloxy)phosphoryl)propanoic acid (1.75 g, 4.44 mmol, quant.) as a colorless neat.

To a stirred solution of 3,3-bis(bis(allyloxy)phosphoryl)propanoic acid (1.77 g, 4.53 mmol, 1.00 eq.) and 2-(2-azidoethoxy)ethanamine (**3B**) (884 mg, 6.80 mmol, 1.50 eq.) in CH_2Cl_2 (20.0 mL) was added EDCI-HCl (1.30 g, 6.80 mmol, 1.50 eq.) and HOBt anhydride (918 mg, 6.80 mmol, 1.50 eq.) at 0 °C. After being stirred at room temperature for 6 h, the reaction mixture was poured into water and CH_2Cl_2. The aqueous layer was extracted with two portions of CH_2Cl_2. The combined extract was washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was chromatographed on silica gel with 97 : 3 chloroform : methanol to give tetraallyl 3-(2-(2-azidoethoxy)ethylamino)-3-oxopropane-1,1-diyldi phosphonate (**19**) (2.00 g, 3.94 mmol, 87%) as a colorless neat.

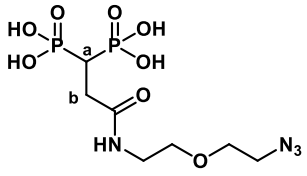
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.21 (br-s, 1H, N*H*), 5.99-5.89 (m, 4H, H-b), 5.36 (dd, 4H, H-c1, $J_{c1,b}$ = 16.9 Hz, $J_{c1,c2}$ = 1.5 Hz), 5.23 (br-d, 4H, H-c2, $J_{c2,b}$ = 10.6 Hz), 4.68-4.55 (m, 8H, H-a), 3.66 (t, 2H, H-g or h, *J* = 4.8 Hz), 3.56 (t, 2H, H-g or h, *J* = 4.8 Hz), 3.48-3.44 (m, 2H, H-f), 3.39-3.36 (m, 2H, H-i), 3.32 (t, 1H, H-d, $J_{d,e}$ = 5.8 Hz), 2.75 (td, 1H, H-e, $J_{e,p}$ = 16.4 Hz, $J_{e,d}$ = 6.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.2 (t, *J* = 8.4 Hz), 132.7 (t, *J* = 7.6 Hz), 118.0, 69.7 (d, *J* = 35.0 Hz), 67.1 (dd, *J* = 28.9, 6.9 Hz), 50.5, 39.4, 33.9, 32.6, 31.8 (t, *J* = 4.6 Hz), 31.3; ^{31}P NMR (100 MHz, $CDCl_3$) δ 24.1; IR (neat); 3302, 2288, 2106, 1675, 1553, 1426, 1251, 1060, 831 cm^{-1}; HRMS (ESI-TOF) $[M+H]^+$ calcd. 507.1774, found 507.1776.



3,3-diphosphonopropanoic acid-PEG-N_3 (3A)

To a stirred solution of tetraallyl 3-(2-(2-azidoethoxy)ethylamino)-3-oxopropane-1,1-diyl diphosphonate (**19**) (686 mg, 1.35 mmol, 1.00 eq.) in CH_3CN (6.00 mL) and H_2O (4.00 mL) was added a catalytic amount of $Rh[(PPh_3)_3Cl]$ at room temperature. After being stirred at 90 ^oC for 12 h, the reaction mixture was concentrated *in vacuo*. The residue was poured into water and CH_2Cl_2. The aqueous layer was extracted with two portions of CH_2Cl_2. To a stirred aqueous layer was added a large amount of Dowex. After being stirred for 10 min, the reaction mixture was filtered and residual water was removed by lyophilization to give 3,3-diphosphonopropanoic acid-PEG-N_3 (**3A**) (464 mg, 1.35 mmol, quant.) as a colorless solid.

^1H NMR (400 MHz, D_2O) δ 3.59 (t, 2H, PEG, J = 5.4 Hz), 3.54 (t, 2H, PEG, J = 5.4 Hz), 3.37 (t, 2H, PEG, J = 5.4 Hz), 3.30 (t, 2H, PEG, J = 5.4 Hz), 2.72-2.58 (m, 3H, H-a, b); IR (solid); 3319, 2954, 2110, 1644, 1171, 1069, 917, 750, 532 cm^{-1}; HRMS (ESI-TOF) $[M+H]^+$ calcd. 347.0522, found 347.0529.



Functionalization of Dextran

Acetalated-Dextran (**8a**), (**8b**), (**8c**)

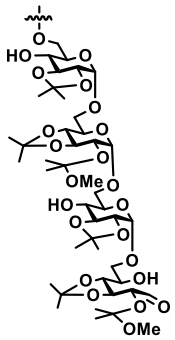
General procedure

A dried flask was charged with **dextran** (**10a**), (**10b**), (**10c**) and complete dissolution of dextran in DMSO (20.0 mL) was observed. A catalytic amount of pyridinium *p*-toluenesulfonate was added followed by 2-methoxypropene (10.0 mL). The flask was placed under a positive pressure of argon, then sealed to prevent evaporation of 2-methoxypropene. After being stirred for 12 h, the reaction mixture was quenched with triethylamine (1.00 mL) and the modified dextran was precipitated in water. The product was isolated by centrifugation for 10 min and the resulting pellet was washed thoroughly with water by vortexing and sonication followed by centrifugation and removal of the supernatant. Residual water was removed by lyophilization, yielding **acetalated-dextran** (**8a**), (**8b**), (**8c**) as a fine white solid.

dextran (**10a**) (Mw = 40,000 g/mol, 4.00 g)
acetalated-dextran (**8a**) (6.23 g)
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.18 (br-s, 1H), 4.97 (br-s, 1H), 4.20-3.20 (m, 17H), 1.43 (br-s, 16H); IR (solid) 3377, 2932, 2118, 1640, 1369, 1033, 835, 584 cm^{-1}.

dextran (**10b**) (Mw = 15,000 g/mol, 4.50 g)
acetalated-dextran (**8b**) (6.20 g)
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.17 (br-s, 1H), 4.95 (br-s, 1H), 4.01-3.24 (m, 19H), 1.40 (br-s, 21H); IR (solid) 3377, 2932, 2118, 1640, 1369, 1033, 835, 584 cm^{-1}.

dextran (**10c**) (Mw = 6,000 g/mol, 4.50 g)
acetalated-dextran (**8c**) (7.13 g)
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.16 (br-s, 1.2H), 4.93 (br-s, 1H), 4.00-3.23 (m, 24H), 1.40 (br-s, 27H); IR (solid) 3377, 2932, 2118, 1640, 1369, 1033, 835, 584 cm^{-1}.



Acetylation of Acetalated-Dextran

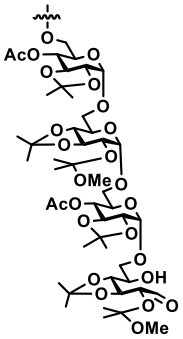
General procedure

To a stirred solution of **acetalated-dextran** (**8a**), (**8b**), (**8c**) (Mw = 40,000, 15,000, 6,000: 150 mg) in CH_2Cl_2 (2.00 mL) was added triethylamine (0.500 mL) and acetic anhydride (0.500 mL) at room temperature. After being stirred at the same temperature for 1 h, the reaction mixture was concentrated *in vacuo*. The residue was done redeposit from hexane and chloroform, then the precipitate was filtered, washed with hexane and dried over *in vacuo* to give **acetyl-acetyl-dextran** as a white solid. The introduction percentage of acetyl group was determined by ^{1}H NMR analysis.

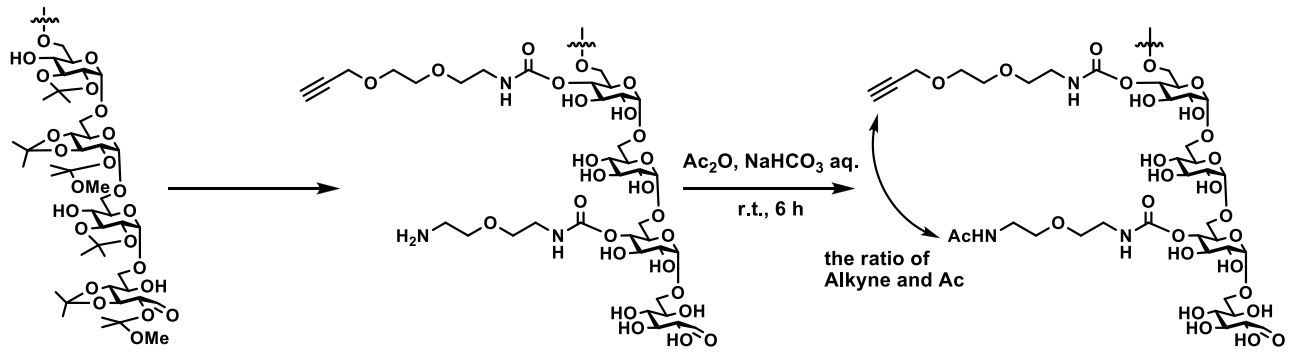
acetalated-dextran (**8a**) (150 mg) (Mw = 40,000)
acetyl-acetyl-dextran (**9a**) (125 mg, Ac : 32.1 mol%)
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.23-4.77 (m, 10H), 4.20-3.24 (m, 34H), 2.20-1.95 (m, 15H, Ac), 1.58-1.25 (m, 40H).

acetalated-dextran (**8b**) (150 mg) (Mw = 15,000)
acetyl-acetyl-dextran (**9b**) (156 mg, Ac : 49.1 mol%)
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.47-4.78 (m, 10H), 4.14-3.02 (m, 30H), 2.13-1.89 (m, 18H, Ac), 1.54-1.43 (m, 25H).

acetalated-dextran (**8c**) (150 mg) (Mw = 6,000)
acetyl-acetyl-dextran (**9c**) (171 mg, Ac : 29.9 mol%)
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.31-4.77 (m, 10H), 4.11-3.24 (m, 41H), 2.14-1.98 (m, 14H, Ac), 1.54-1.39 (m, 38H).



Various Ratio of Acetylene-Amino-Dextran (4a)-(4c), (5a)-(5c)



General procedure

To a stirred solution of acetalated-dextran (**8a**), (**8b**), (**8c**) (500 mg) in CH_2Cl_2 (5.00 mL) was added a solution of **3-(2-(2-isocyanatoethoxy)ethoxy)prop-1-yne** (**7**) and ***tert*-butyl 2-(2-isocyanato ethoxy)ethylcarbamate** (**6**) in CH_2Cl_2 (5.00 mL) and a large amount of DBU at room temperature. After being stirred at the same temperature for 1 h, the reaction mixture was concentrated *in vacuo*. The residue was done redeposit from hexane and chloroform, then the precipitate was filtered, washed with hexane and dried over *in vacuo* to give a white solid. The residue was used for the next reaction without further purification.

To a stirred solution of the above residue in H_2O (10.0 mL) was added 4 N HCl in dioxane (5.00 mL) at room temperature. After being stirred at the same temperature for 6 h, the reaction mixture was concentrated *in vacuo*. The residue was done redeposit from IPA and H_2O to give acetylene-amino-dextran (**4a**)-(**4c**) as a white solid.

To a stirred solution of acetylene-amino-dextran (**4a**)-(**4c**) in H_2O (2.00 mL) was added acetic anhydride (100 μL) and $NaHCO_3$ (50.0 mg) at room temperature. After being stirred at the same temperature for 6 h, the reaction mixture was purified by PD-10 to give acetylene-AcNH-dextran (**5a**)-(**5c**) as a white solid. The introduction percentage of PEG-acetylene and PEG-NH_2 was determined by ^{1}H NMR analysis.

Mw=40,000 (PEG-acetylene and -NH_2 : 26.9 mol%)
3-(2-(2-isocyanatoethoxy)ethoxy)prop-1-yne (**7**) (352 mg, 1.45 mmol)
***tert*-butyl 2-(2-isocyanatoethoxy)ethylcarbamate** (**6**) (258 mg, 1.12 mmol)
PEG-acetylene (17.6 mol%), **PEG-NH_2** (9.31 mmol%)
acetylene-amino-dextran (**4a**): ^{1}H NMR (400 MHz, D_2O) δ 5.20-4.54 (m, 40H), 4.23 (s, 2.3H, H-b), 3.98-3.19 (m, 33H), 2.92 (s, 1H, H-a).
acetylene-AcNH-dextran (**5a**): ^{1}H NMR (400 MHz, D_2O) δ 5.20-4.92 (m, 1.9H), 4.77-4.64 (m, 42H), 4.60-4.51 (m, 0,72H), 4.23 (s, 2H, H-b), 4.04-3.35 (m, 28H), 2.93 (s, 0.67H, H-a), 1.99 (s, 1.6H, Ac).

Mw = 15,000 (PEG-linker : 33.7 mol%)
3-(2-(2-isocyanatoethoxy)ethoxy)prop-1-yne (**7**) (1.13 g, 4.66 mmol)
***tert*-butyl 2-(2-isocyanatoethoxy)ethylcarbamate** (**6**) (359 mg, 1.56 mmol)

PEG-acetylene (25.9 mol%), **PEG-NH_2** (7.76 mmol%)

acetylene-amino-dextran (**4b**): ^{1}H NMR (400 MHz, D_2O) δ 5.15-4.90 (m, 1.2H), 4.70-4.54 (m, 14H), 4.24 (s, 2.2H, H-b), 4.10-3.19 (m, 19H), 2.94 (s, 1H, H-a).

acetylene-AcNH-dextran (**5b**): ^{1}H NMR (400 MHz, D_2O) δ 5.10-4.95 (m, 1.1H), 4.68-4.51 (m, 0.71H), 4.24 (s, 2H, H-b), 4.07-3.35 (m, 18H), 2.95 (s, 0.41H), 1.99 (s, 0.90H, Ac).

Mw = 6,000 (PEG-linker : 28.8 mol%)

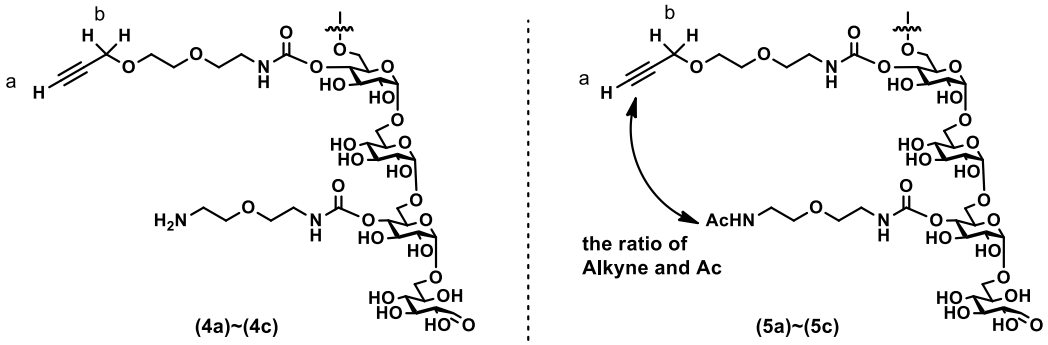
3-(2-(2-isocyanatoethoxy)ethoxy)prop-1-yne (**7**) (620 mg, 2.55 mmol)

***tert*-butyl 2-(2-isocyanatoethoxy)ethylcarbamate** (**6**) (145 mg, 0.629 mmol)

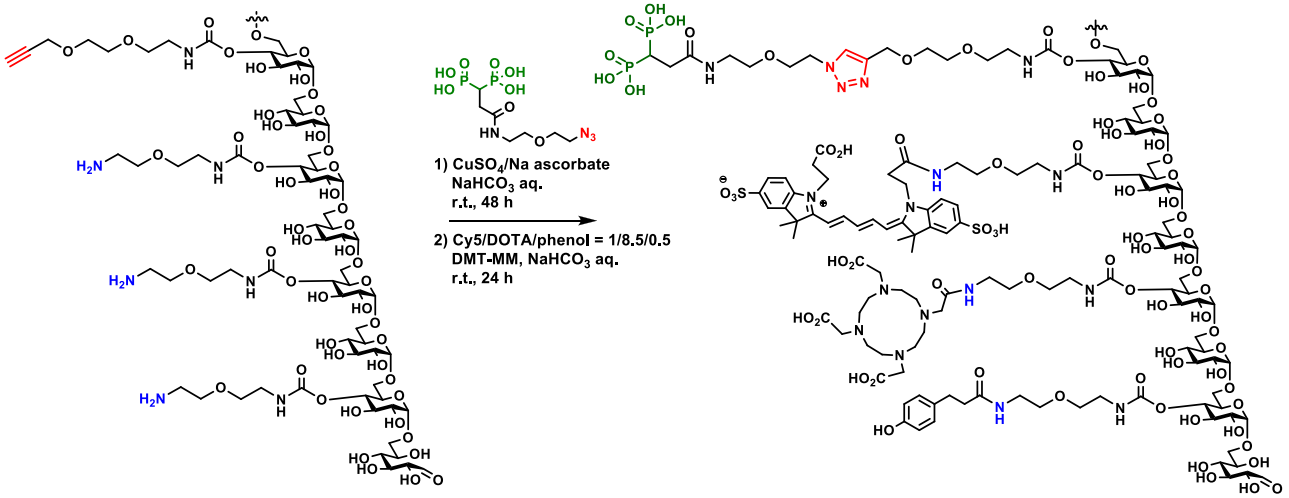
PEG-acetylene (23.8 mol%), **PEG-NH_2** (5.02 mmol%)

acetylene-amino-dextran (**4c**): ^{1}H NMR (400 MHz, D_2O) δ 5.20-4.90 (m, 1.4H), 4.70 (s, 20H), 4.60-4.51 (m, 0.43H), 4.24 (s, 2.3H, H-b), 4.10-3.19 (m, 22H), 2.93 (s, 1H, H-a).

acetylene-AcNH-dextran (**5c**): ^{1}H NMR (400 MHz, D_2O) δ 5.09-4.95 (m, 1.3H), 4.66-4.50 (m, 0.55H), 4.24 (s, 2H, H-b), 4.09-3.35 (m, 21H), 2.95 (s, 0.046H), 1.99 (s, 0.64H, Ac).



Phosphrous-Cy5-DOTA-Phenol-Dextran (1aA)-(1cA)



General Procedure

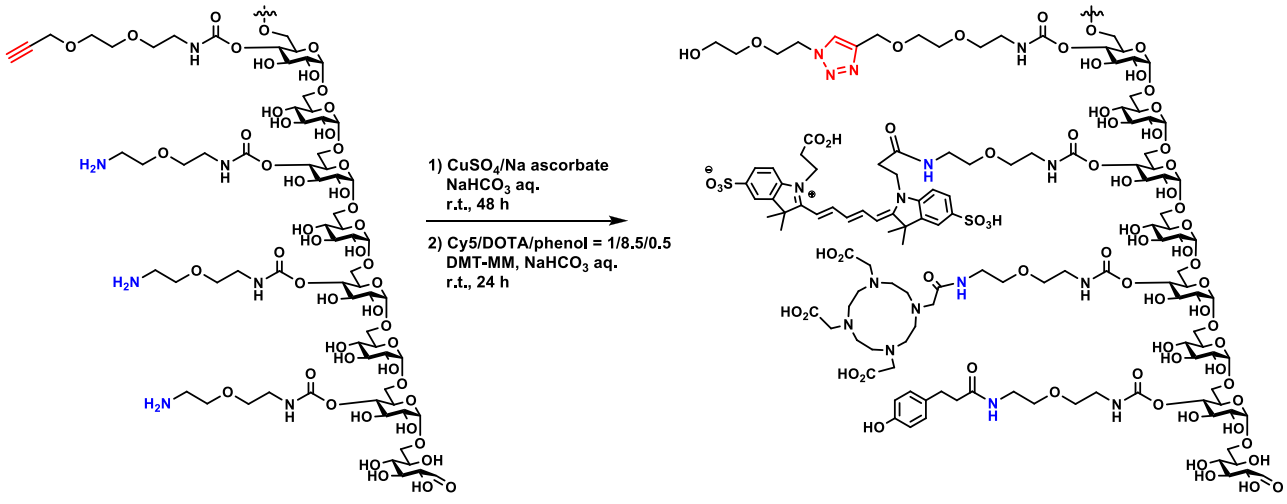
To a stirred solution of Acetylene-H_2N-PEG-Dextran (**4a**)-(**4c**) (Mw = 40,000, 15,000, 6,000: 50.0 mg) and 3-(2-(2-azidoethoxy)ethylamino)-3-oxopropane-1,1-diyldiphosphonic acid (**3A**) (100 mg) in $NaHCO_3$ aq. (2.00 mL) was added a catalytic amount of 0.5 M $CuSO_4$ (50.0 μL) and 0.5

M Na ascorbate (50.0 μL) at room temperature. After being stirred at the same temperature for 48 h, the reaction mixture was purified by PD-10. The residue water was removed by lyophilization to give **Phosphrous-H_2N-Dextran** as a white solid.

To a stirred solution of **Phosphrous-H_2N-PEG-Dextran** (Mw=40,000, 15,000, 6,000: 15.0 mg) and Cy5-CO_2H (1.00 mg), DOTA-CO_2H (5.23 mg) and 3-(4-hydroxyphenyl)propanoic acid (0.127 mg) in $NaHCO_3$ aq. (1.00 mL) was added DMT-MM (60.0 mg) at room temperature. After being stirred at the same temperature for 12 h, the reaction mixture was purified by PD-10. The residue water was removed by lyophilization to give **Phosphrous-Cy5-DOTA-Phenol-Dextran** (**1aA**)-(**1cA**) as a white solid.

Phosphrous-Cy5-DOTA-Phenol-Dextran (**1aA**) (Mw = 40,000, 20.1 mg)
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (br-s, 1H, triazole), 7.84 (br-s, 0.13H), 7.30-7.12 (m, 0.22H), 5.20-4.60 (m, 99H), 4.07-3.10 (m, 52H), 2.89 (s, 5.05H), 2.75-2.60 (m, 2.54H), 2.55-2.41 (m, 1.12H), 1.96-1.86 (m, 0.75H), 1.69-1.57 (m, 0.56H), 1.25 (t, 0.31H, J = 7.3 Hz); IR (solid) 3392, 2933, 2112, 1706, 1646, 1463, 1370, 1262, 1112, 1051, 897, 610 cm^{-1}.
Phosphrous-Cy5-DOTA-Phenol-Dextran (**1bA**) (Mw = 15,000: 21.7 mg)
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (br-s, 1H, triazole), 7.27-7.11 (m, 0.26H), 4.98-4.60 (m, 66H), 4.06-3.10 (m, 30H), 2.89 (s, 1.57H), 2.71-2.61 (m, 1.59H), 2.55-2.42 (m, 0.71H), 1.96-1.86 (m, 0.12H), 1.69-1.57 (m, 0.25H), 1.25 (t, 0.13H, J = 7.3 Hz); IR (solid) 3321, 1642, 1106, 874, 752, 603 cm^{-1}.
Phosphrous-Cy5-DOTA-Phenol-Dextran (**1cA**) (Mw = 6,000: 23.1 mg)
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (br-s, 1H, triazole), 7.29-7.12 (m, 0.18H), 5.07-4.51 (m, 70H), 4.07-3.16 (m, 32H), 2.89 (s, 1.25H), 2.71-2.61 (m, 1.72H), 2.55-2.41 (m, 0.89H), 1.69-1.57 (m, 0.33H), 1.25 (t, 0.13H, J = 7.3 Hz); IR (solid) 3366, 1646, 1026 cm^{-1}.

(OH-PEG)-Cy5-DOTA-Phenol-Dextran (1aB), (1bB), (1cB)



General Procedure

To a stirred solution of the Acetylene-H_2N-PEG-Dextran **(4a), (4b), (4c)** (Mw = 40,000, 15,000, 6,000: 50.0 mg) and 2-(2-azidoethoxy)ethanol (**3B**) (40.0 mg) in $NaHCO_3$ aq. (2.00 mL) was added a catalytic amount of **0.5 M $CuSO_4$** and **0.5 M Na ascorbate** at room temperature. After being stirred at the same temperature for 48 h, the reaction mixture was purified by PD-10. The residue water was removed by lyophilization to give **(OH-PEG)-H_2N-PEG-Dextran** as a white solid.

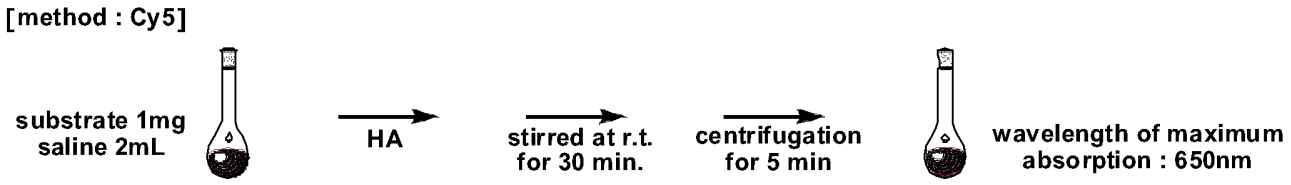
To a stirred solution of **(OH-PEG)-H_2N-PEG-Dextran** (15.0 mg) and Cy5-CO_2H (1.00 mg), DOTA-CO_2H (5.23 mg) and 3-(4-hydroxyphenyl)propanoic acid (0.127 mg) in $NaHCO_3$ aq. (1.00 mL) was added DMT-MM (60.0 mg) at room temperature. After being stirred at the same temperature for 12 h, the reaction mixture was purified by PD-10.

(OH-PEG)-Cy5-DOTA-Phenol-Dextran (**1aB**) (Mw = 40,000, Sample-b: 21.4 mg)
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (s, 1H, triazole), 7.28-7.11 (m, 0.16H), 5.08-4.60 (m, 145H), 3.93-3.25 (m, 32H), 2.56-2.45 (m, 0.22H), 1.67-1.58 (m, 0.47H); IR (solid) 3298, 2923, 2117, 1698, 1362, 1030, 923, 817, 624 cm^{-1}.

(OH-PEG)-Cy5-DOTA-Phenol-Dextran (**1bB**) (Mw = 15,000: 19.7 mg)
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (s, 1.0H, triazole), 7.27-7.11 (m, 0.089H), 5.05-4.61 (m, 24.6H), 3.93-3.25 (m, 26H), 2.56-2.4 (m, 0.077H), 1.67-1.58 (m, 0.21H); IR (solid) 3320, 2928, 1700, 1557, 1463, 1361, 1257, 1020, 919, 773, 648 cm^{-1}.

(OH-PEG)-Cy5-DOTA-Phenol-Dextran (**1cB**) (Mw = 6,000: 23.4 mg)
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (s, 1H, triazole), 7.28-7.12 (m, 0.097H), 5.07-4.42 (m, 34.6H), 3.94-3.25 (m, 32.5H), 2.56-2.4 (m, 0.14H), 1.67-1.58 (m, 0.34H); IR (solid) 3317, 2920, 1701, 1550, 1365, 1257, 1019, 771, 646, 520 cm^{-1}.

Evaluation of binding affinity for hydroxyapatite



Hydroxyapatite beads were suspended in 2.00 mL of saline and mixed with 1.00 mg of DEX-Cy5 The mixture was placed at room temperature for 30 min with continuous mixing and then centrifuged at the same temperature for 10 min. The supernatant was carefully transferred to UV cell.

Preparation of mouse model of ectopic bone formation

A biodegradable hydrogel with a water content of 97.8% was prepared through the chemical crosslinking of aqueous gelatin solution with glutaraldehyde. Briefly, 3.00 wt% gelatin aqueous solution containing 0.160 wt% glutaraldehyde was cast into a polypropylene dish (138 x 138 mm^2) followed by crosslinking reaction at 4 ^oC for 12 h. The crosslinking hydrogel prepared was punched out to obtain the discs in 6 nm diameter. The hydrogel discs were stirred in 100 mM aqueous glycine solution at 37 ^oC for 1 h to block the residual aldehyde groups of glutaraldehyde. Following washing three times with DDW, the hydrogels were freeze-dried and sterilized with ethylene oxide gas.

To prepared gelatin hydrogel incorporating BMP-2, 20.0 μL of aqueous solution containing of BMP-2 was dropped onto the freeze-dried hydrogel, followed by leaving them at 4 ^oC overnight. Then, the gelatin hydrogels incorporating BMP-2 was implanted into the back subcuits of ddY mice (male, 6W) to prepare a mouse model of ectopic bone formation. The mice prepared were used in the following imaging and histochemistry studies.

Radioisotope imaging

Dextran derivatives **1aA-1cB** (0.100 mg) were suspended in 0.5 M NaCl aq. (0.100 mL) and 0.5 M potassium buffer (0.100 mL) and mixed with NaI^{125} in NaOH aq. (5.00 μL) and chloramine-T in 0.5 M NaCl aq. (0.100 mL) and and 0.5 M potassium buffer (0.100 mL, 0.200 mg/mL). After being stirred at room temperature for 2 min, $Na_2S_2O_5$ in MilliQ (0.100 mL, 4.00 mg/mL) was added. After being stirred at room temperature for 2 min, the residue was purified by PD-10 to give Radioisotope-labeled dextran derivatives. The **Products** (100 μL) was injected to the mice through the tail vein 2 weeks after the hydrogels implantation, and 6 h later, the mice were observed. Figure 1 shows the accumulation of dextran derivatives **1aA-1cB** in each organ, and Figure 2 the percentage of remaining dextran derivatives in blood of mice.

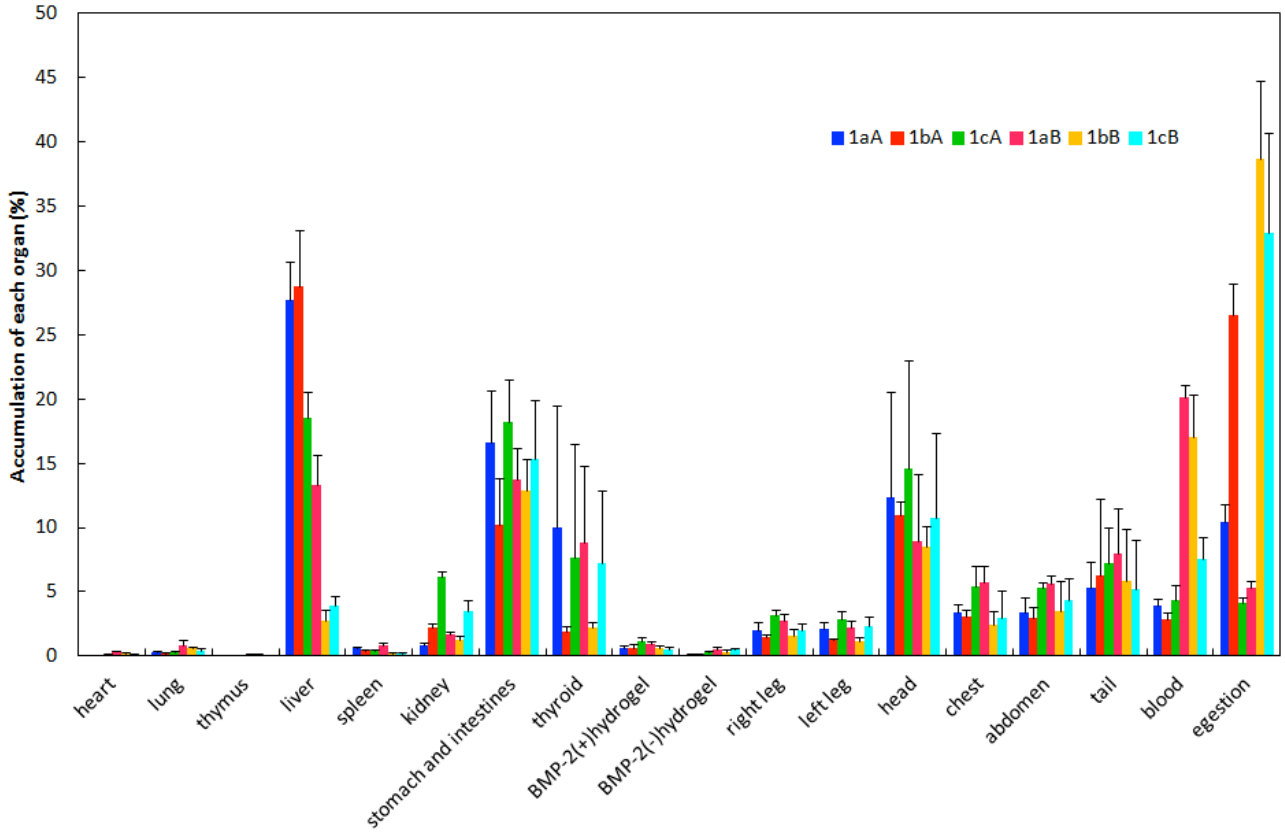


Figure 1: Accumulation of **1aA-1cB** in each organ.

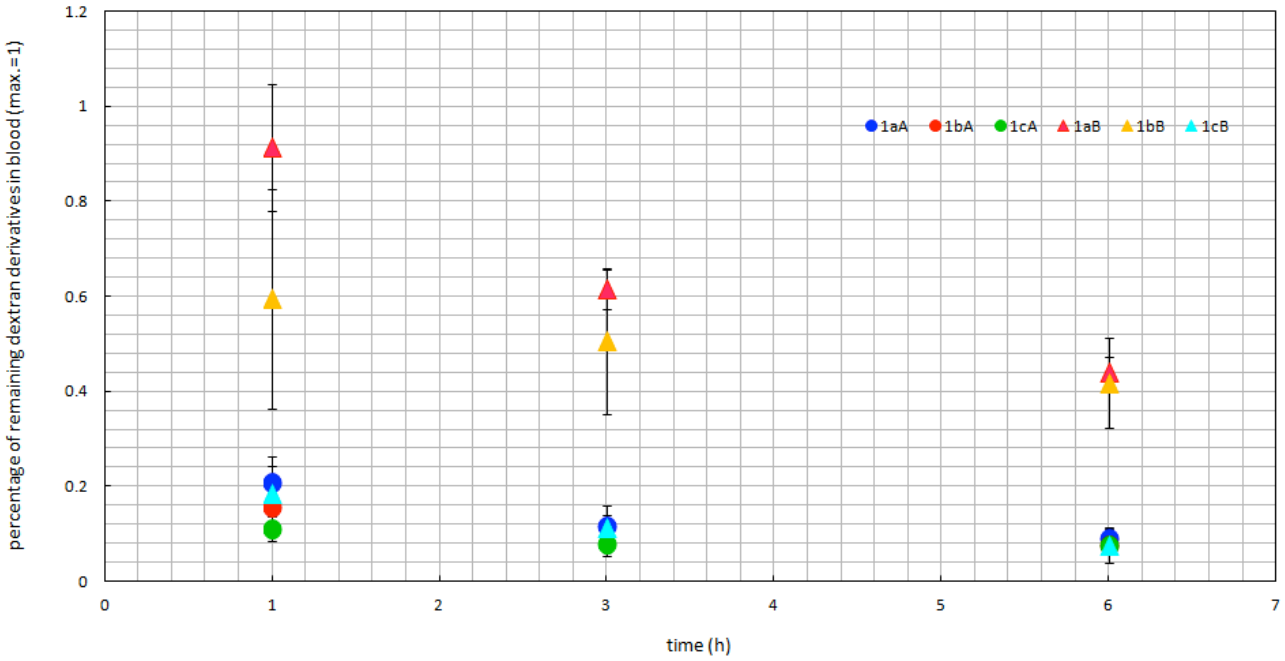


Figure 2: The percentage of remaining dextran derivatives in blood of mice.

Optical imaging of the BMP-2 containing hydrogel in mice

In vivo optical imaging was performed before or 1, 2, 3, 4, and 6 h after intravenous injection (100 μL) of Gd-containing BP-tracer **[Gd^{3+}]-1cA** ([Gd^{3+}] = 20 mM) to the mice 3 weeks after hydrogel implantation. Images were acquired using a fluorescent imager (Maestro EX) with the following parameters: excitation filter = 576–621 nm; emission filter = 635 nm longpass; acquisition setting = 630–800 nm in 10 nm steps; acquisition time = 300 ms; and FOV = 12.0 × 12.0 cm^2. Prior to and during the MRI scan, all mice were anaesthetized through a facemask with 2.0% isoflurane (Mylan Japan, Tokyo, Japan). After acquisition, unmixed fluorescence information was extracted from the fluorescence spectrum using the Maestro software package (PerkinElmer).

MRI imaging of the BMP-2 containing hydrogel in mice

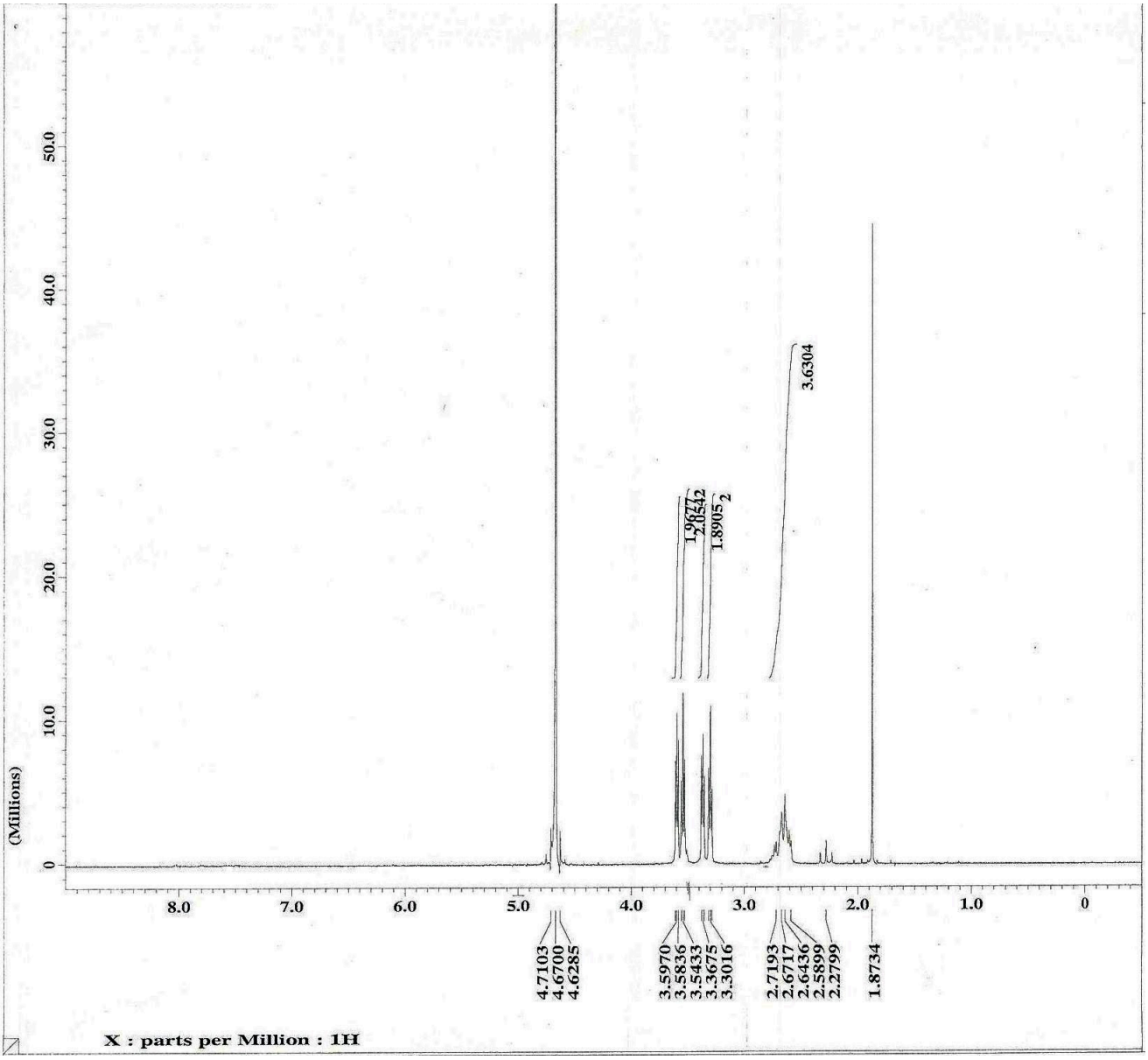
For the in vivo MRI study, 100 μL of Gd-containing BP-tracer **[Gd^{3+}]-1cA** ([Gd^{3+}] = 20 mM) was administered to the mice 3 weeks after hydrogel implantation. The MRI acquisitions were performed in a 7.0 T, 40 cm bore horizontal magnet (Kobelco and Jastec, Kobe, Japan) interfaced to a Bruker Avance console (Bruker Biospin, Ettlingen, Germany) with a volume coil for transmission and reception (35 mm internal diameter, Rapid Biomedical, Rimpar, Germany). All mice were anaesthetized through a facemask with 2.0% isoflurane prior to and during the MRI scan. Polyethylene catheter (PE-10, Becton Dickinson, MD) were placed in the tail vein for the injection of **[Gd^{3+}]-1cA**. Rectal temperature was continuously monitored and automatically maintained at 36.5 ± 0.5°C using a warm-air heating system (laboratory made) throughout all experiments. Trans-axial multi-slice T_1-weighted MR images (T_1WI) were acquired with a spin-echo sequence, (TR/TE = 400/9.57 ms, 4 slices, slice thickness = 1.0 mm, matrix = 256 × 256, FOV = 25.6 × 25.6 mm^2, average = 4, scan time = 6 m 50s.). Image reconstruction and analysis were performed using ParaVision (Bruker Biospin) and MRVision (Ver. 1.5, MRVision Co., MA). The experimental group is composed of 3 mice. Gd-DTPA (gadopentetate dimeglumine, Magnevist® of clinical use) was diluted to 20 mM with saline and used as a control.

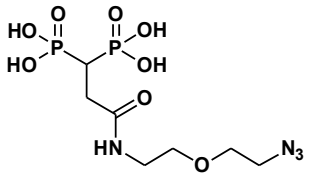
Histochemical evaluation of bone tissue ectopically formed

Histological examination was performed for tissue around the hydrogel implanted of mice receiving dextran derivatives **1cA** and **1cB**. For mice 4 weeks after the hydrogel implantation, dextran derivatives were injected intravenously and 6 h later, the mice were sacrificed and the skin tissue including the hydrogel implanted was taken out. After the confirmation of bone generation by soft X-ray photography, the subcutaneous tissue including the gelatin hydrogel incorpolating BMP-2 was embedded into Tissue-Tek (Sakura, Co., Ltd., Aichi, Japan), followed by freezing in liquid nitrogen. After fixation with ethanol, serial cryo-sections of the tissues (5 μm thicknes) were prepared for the following staining and microscopic viewing.

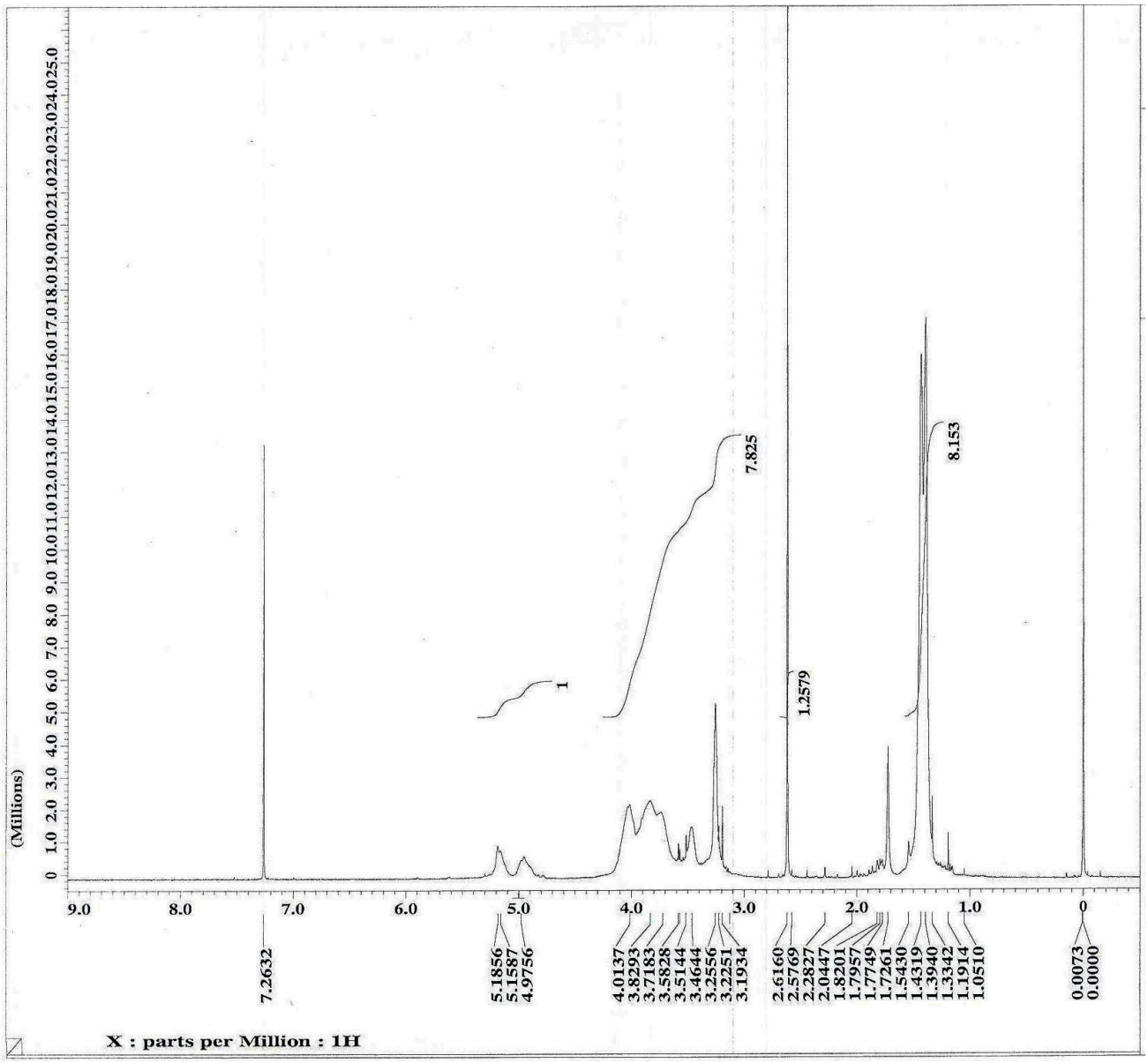
The calcium deposition of tissues was visualized by the von Kossa staining according to the method reported by Drury et al[1]. Briefly, the sections were stained with silver nitrate with the irradiation of UV light for 20 min, followed by washing with sodium thiosulphate and DDW. The sections prepared were counter-stained by nuclear fast red and histologically viewed on a light microscope (AX-80, Olympus, Japan). In addition, the conventional hematoxylin and eosin (H&E) stain was also performed. On the other hand, the tissue sections of mice receiving the injection of dextran derivatives were viewed by using a confocal laser scanning microscopy system (LSM-510, Carl Zeiss, Jena, Germany) for the localization of conjugates while the control experiment was done.

[1] R. Durry, E. Wallington, Demonstration of calcium salts, Carleton's Histological Techniques, 5th ed., Oxford University Press, 1980.





3A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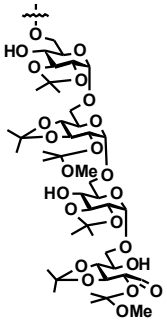
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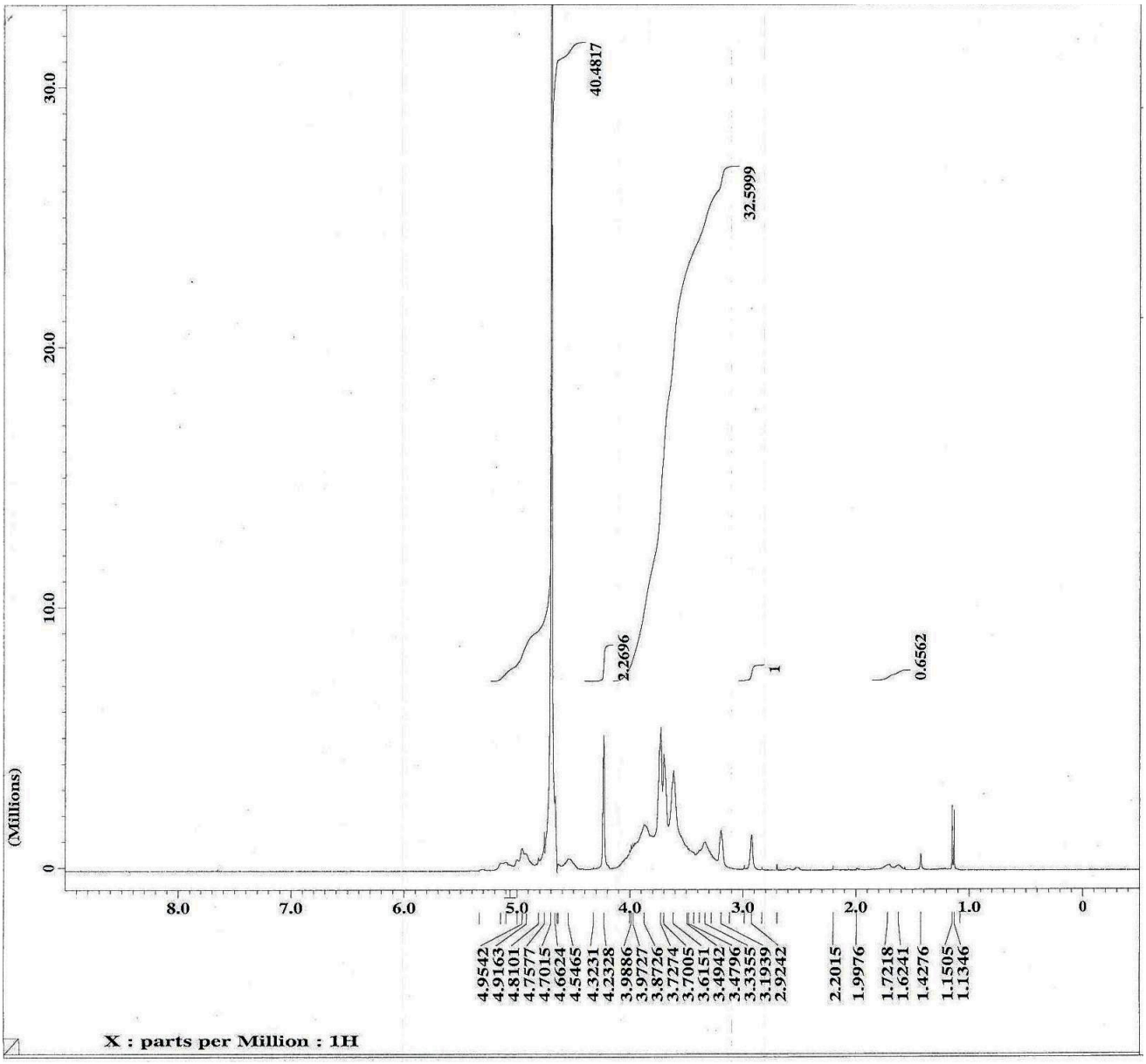
OMe

10a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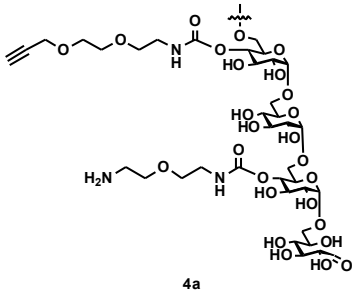
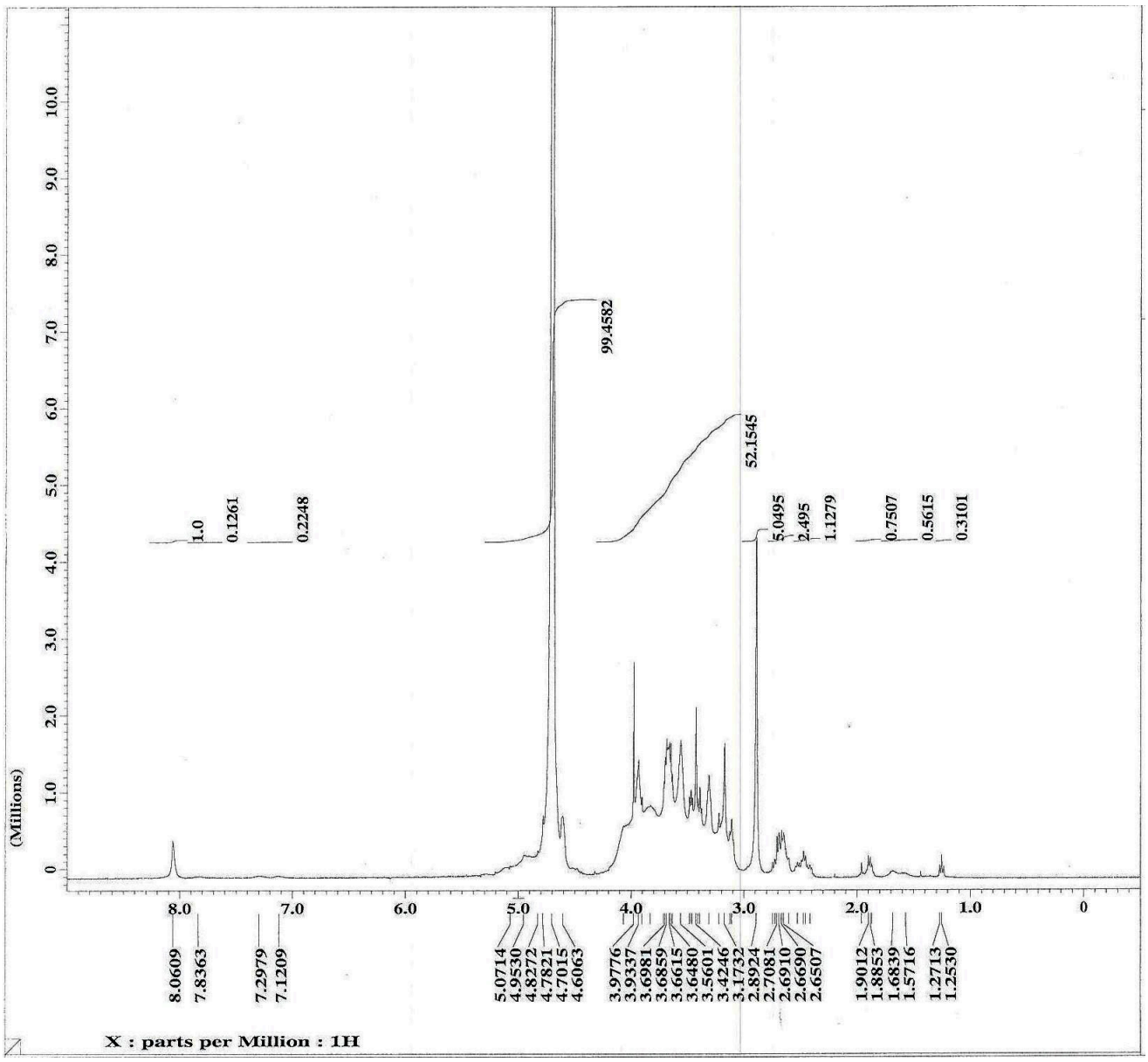


4a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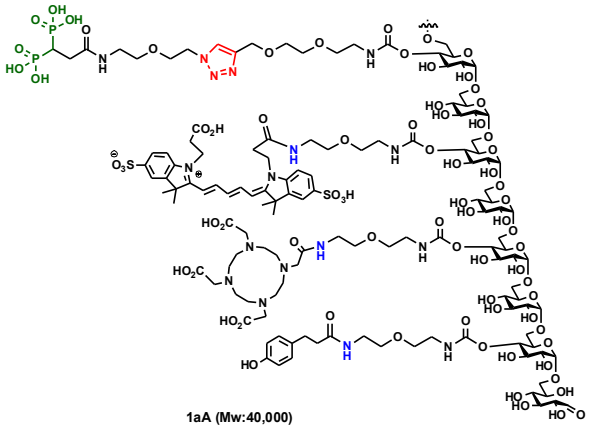
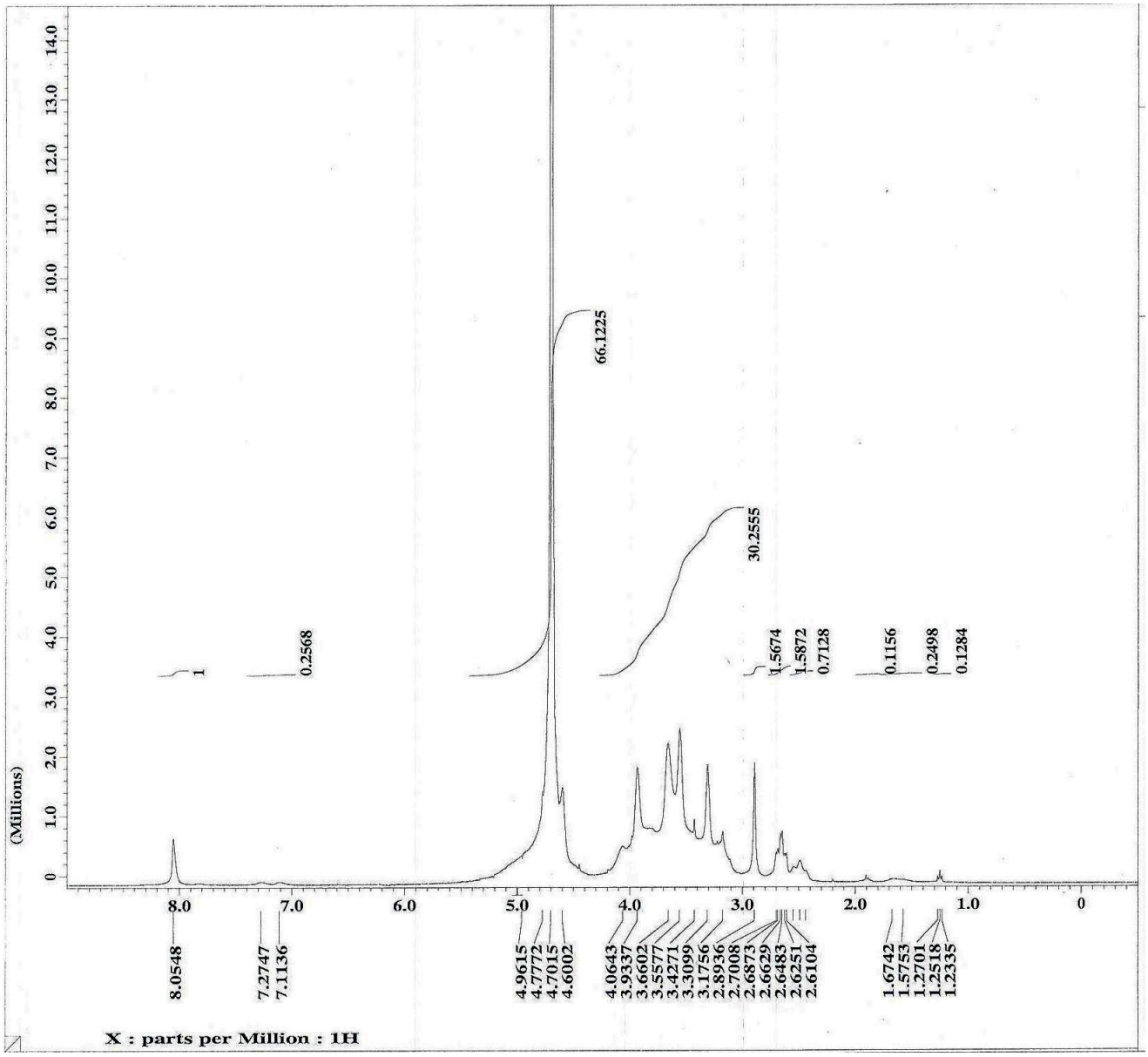


1aA (Mw:40,000)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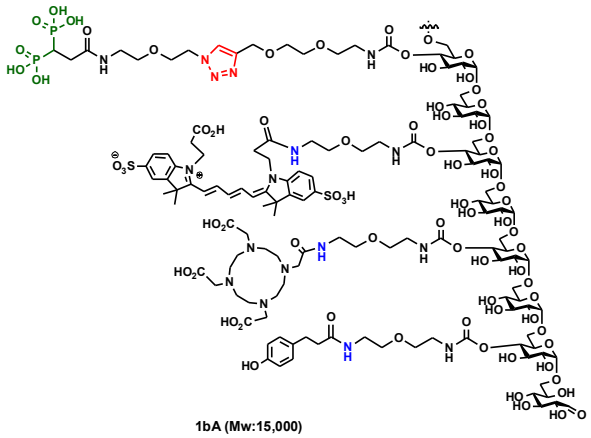
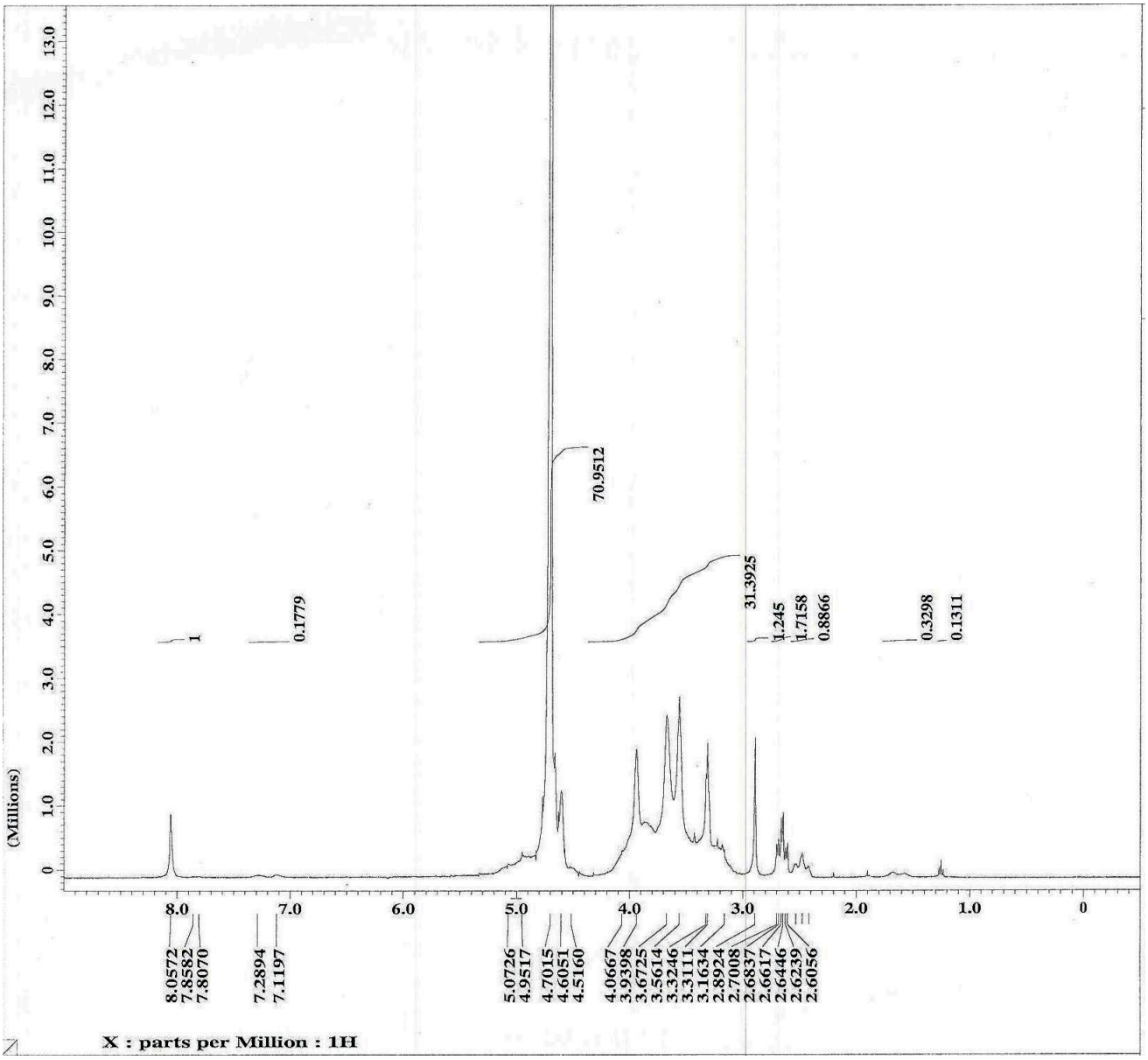
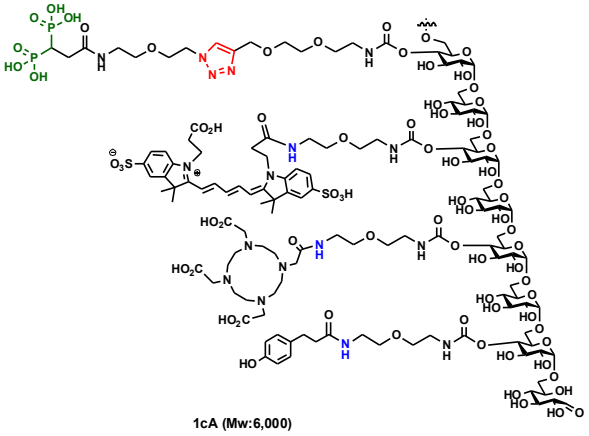
8.0548
7.2747
7.1136
4.9615
4.7772
4.7015
4.6002
4.0643
3.9337
3.6602
3.5577
3.4271
3.3099
3.1756
2.8936
2.7008
2.6873
2.6629
2.6483
2.6251
2.6104
1.6742
1.5753
1.2701
1.2518
1.2335
X : parts per Million : 1H


1bA (Mw:15,000)


(Millions)
70.9512
31.3925
1
0.1779
1.245
1.7158
0.8866
0.3298
0.1311
8.0572
7.8582
7.8070
7.2894
7.1197
5.0726
4.9517
4.7015
4.6051
4.5160
4.0667
3.9398
3.6725
3.5614
3.3246
3.3111
3.1634
2.8924
2.7008
2.6837
2.6617
2.6446
2.6239
2.6056
X : parts per Million : 1H



1cA (Mw:6,000)