Supplementary Information

Oriented Crystallization of Hydroxyapatite by the Biomimic Amelogenin Nanospheres from the Self-Assemblies of Amphiphilic Dendrons

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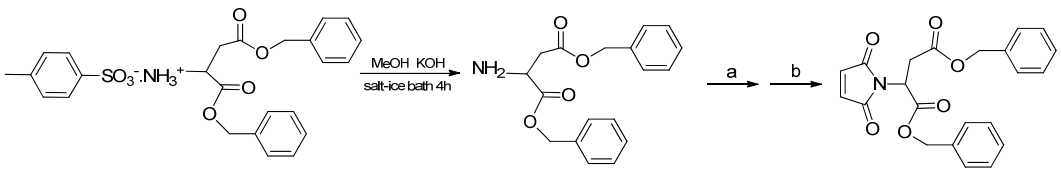
General

Unless stated otherwise, all reagents and common solvents were obtained from commercial sources and used as received. ^{1}H NMR and ^{13}C NMR spectra were recorded on Brucker 300 MHz spectrometer. Electrospray ionization mass spectrometry (ESI-MS) was used for detected molecular weight. FTIR spectra were obtained using a Bruker VECTOR22 IR spectrometer. High resolution transmission electron microscopy (HRTEM) was performed on a Hitachi H-9000 NAR TEM and operated at 100 keV. X-ray diffraction (XRD) patterns were obtained with a Rigaku D/max-2400 X-ray diffractometer from 3° to 60° at a rate of 4°/min, using Cu-Kα radiation (K = 0.1541 nm). The tube voltage was 40 kV and the tube current was 100 mA.

Synthesis and Characterization

1. Synthesis of 3(L-Asp-maleinimide, M-AspBZ)



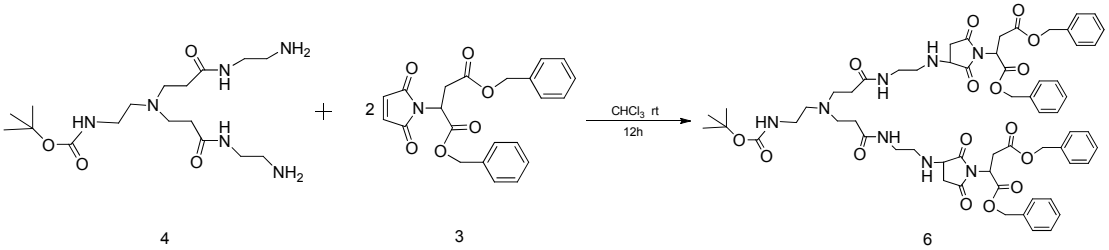
1 2 3

1 was synthesized according to the reference[1]. A MeOH (2 mL) solution containing KOH (0.7g,

12.5mM) was added dropwise into a MeOH solution of 1 (6.1g, 12.5mM) below 0℃. After the reaction mixture was stirred for 4 hours, the residue was filtered repeatedly to remove the white precipitates and rotary evaporated to wipe out MeOH. The product was dissolved in a small amount of $CHCl_3$ and dried with anhydrous sodium sulfate. Filtered and evaporated again, 2 was obtained. L-Asp-maleinimide (3) was obtained by two steps of reactions. Firstly, 2 was reacted with maleic anhydride in $CHCl_3$ at room temperature for 1 hour, then the temperature was increased to 80℃ for 30 min with the addition of acetic anhydride sodium and acetate anhydrous. The crude solid with orange color was purified by a silica column with MeOH/ petroleum ether (2:3) as the eluant. Yield: 71.5%.

^{1}H NMR(300 MHz, RT, $CDCl_3$): δ =7.35-7.24 (m, 10H, Ar-H), 6.66 (s, 2H, CH=CH), 5.27-5.22(m, 1H, $NCHCH_2CO$), 5.14-5.08 (d, 4H, $COCH_2C_6H_5$), 3.36-3.3.09(m, 2H, $CHCH_2CO$) ppm. ^{13}C NMR (300 MHz, RT, $CDCl_3$): δ =169.57-167.82 (CH_2CO), 135.27-134.7 ($CH_2C_6H_5$), 134.1 (CH_2=CH), 128.52-128.09 (CH2C6H5), 76.40-76.56 ($OCH_2C_6H_5$), 67.92-66.81 (NCHCO), 33.89 ($CHCH_2CO$) ppm. ESI-MS（$C_{22}H_{19}NO_6$）m/z Cala.: 393.1, found: [M + H] 394.1; [M + Na] 416.1; [M + K] 432.1.

2. Synthesis of 6 (Boc-PAMAM-AspBZ)

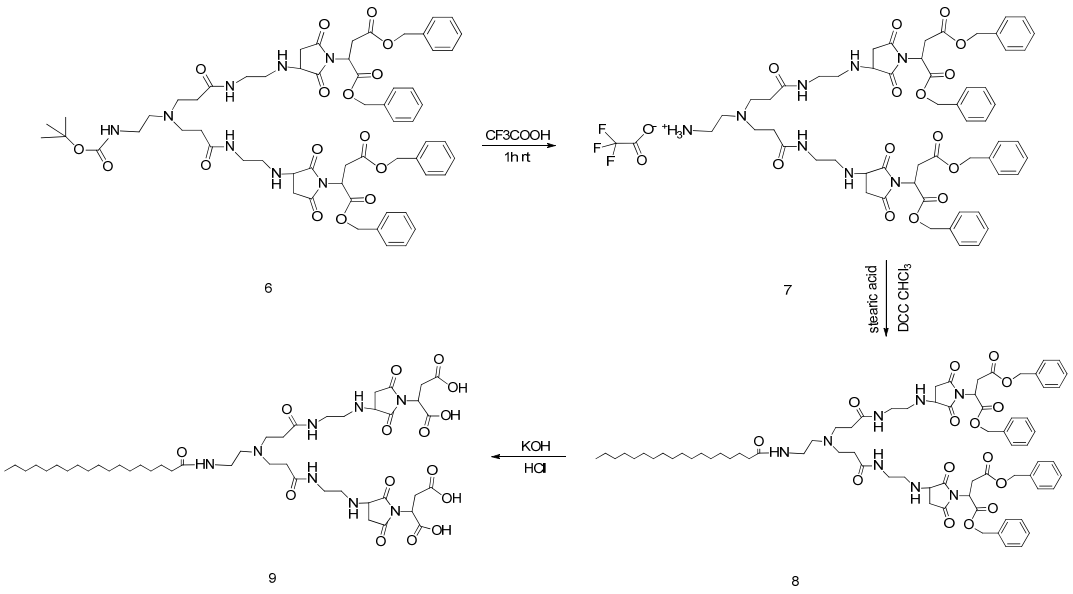


4 3 6

PAMAM Dendron G1 (4) was synthesized according to the procedures reported previously[2]. 3 was grafted on the periphery of 4 via Michael addition reaction. 3 (2.11g, 5.37mM) was dissolved in 3ml $CHCl_3$ solution, then 4 (1.04, 2.68mM) was drop into the solution of 3. The mixture was kept at room temperature for 12 hours under nitrogen atmosphere. The crude product 6 was subjected to a silica column with MeOH/ ethyl acetate (1:4) as the eluant. Yield: 52.2%.

^{1}H NMR(300 MHz, RT, $CDCl_3$): δ =7.39-7.20 (m, 20H, Ar-H), 5.27-5.21(m, 2H, $NCHCH_2CO$), 5.13-5.03 (d, 8H, $COCH_2C_6H_5$), 3.33-2.04(m, 35H, PAMAM-H), 1.42(s, 9H, CCH_3) ppm. ESI-MS（$C_{61}H_{74}N_8O_{16}$）m/z Cala.: 1174.5, found: [M + H] 1175.5; [M + Na] 1197.5.

3. Synthesis of 9 (Sa-PAMAM-Asp)



A mixed solution of dichloromethane solution (3 mL) and trifluoroacetic acid (1/1, v/v) was added dropwise to a dichloromethane solution (10 ml) of 6 (2.04g 1.74mM). The reaction mixture was stirred for 1 h at room temperature. After evaporating the solvent under vacuum, the crude product 7 was obtained and purified by dissolving in dichloromethane and precipitated in anhydrous diethyl ether with vigorous stirring repeatedly. The white precipitate 7 was collected. Yield: 85.5%.

^{1}H NMR(300 MHz, RT, $CDCl_3$): δ = 7.26-7.20 (m, 20H, Ar-H), 5.27-5.21(m, 2H, $NCHCH_2CO$), 5.01-4.89 (d, 8H, $COCH_2C_6H_5$), 3.33-2.04 (m, 35H, PAMAM-H) ppm.

The trichloromethane solution (10ml) of 7 (2.37g, 1.99mM) was mixed with 2ml triethylamine at salt-ice bath. After stirring for 15min, the trichloromethane solution (2ml) of stearic acid (1.70g, 5.98mM) was added dropwise into the mixture. Then a trichloromethane solution (2ml) of DCC (1.36g, 6.59mM) was added dropwise into the mixture. All the reaction mixture was stirring for 48h at room temperature. After evaporating the solvent under vacuum, the crude product 8 was obtained and purified by a silica column with MeOH/ ethyl acetate (1:5) as the eluant. Yield: 51.6%.

^{1}HNMR ($CDCl_3$, ppm): δ=7.38-7.26 (m, 20H, Ar-H), 5.26-5.23(m, 2H, $NCHCH_2CO$), 5.13-5.06 (d, 8H, $COCH_2C_6H_5$), 3.33-2.04(m, 35H, PAMAM-H), 2.16-2.12(m, 2H, CH_2), 1.59-1.47(m, 2H,

CH_2), 1.25-1.24(s, 28H, CH_2), 0.90-0.85(m, 3H, CH_3) ppm. ESI-MS（$C_{74}H_{100}N_8O_{15}$）m/z Cala: 1340.7, found: [M + H] 1341.7; [M + Na] 1363.7.

A MeOH solution (5ml) of 8 (1.01g, 0.75 mM) was mixed with a MeOH solution (1ml) of KOH (0.42g, 7.5mM). The mixture was stirred for 30 min. Then the solvent was evaporated under vacuum, the crude product was obtained, then 2 ml deionized water and 0.5 ml 37% HCl were added under stirred. The white precipitate 9 was collected and dried under vacuum. Yield: 85.5%. ^{1}HNMR (d_6-DMSO, ppm): δ =4.56-4.52(m, 2H, $NCHCH_2CO$), 3.61-2.44(m, 39H, PAMAM-H), 2.11-2.06(m, 2H, CH_2), 1.47(m, 2H, CH_2), 1.30-1.15(s, 28H, CH_2), 0.88-0.83(m, 3H, CH_3) ppm. ESI-MS（$C_{46}H_{76}N_8O_{15}$）m/z Cala.: 980.5, found: [M + $2H_2O$] 1017.5.

Fluorescence spectra and CAC (Critical Aggregation Concentration) curves of Sa-PAMAM-Asp

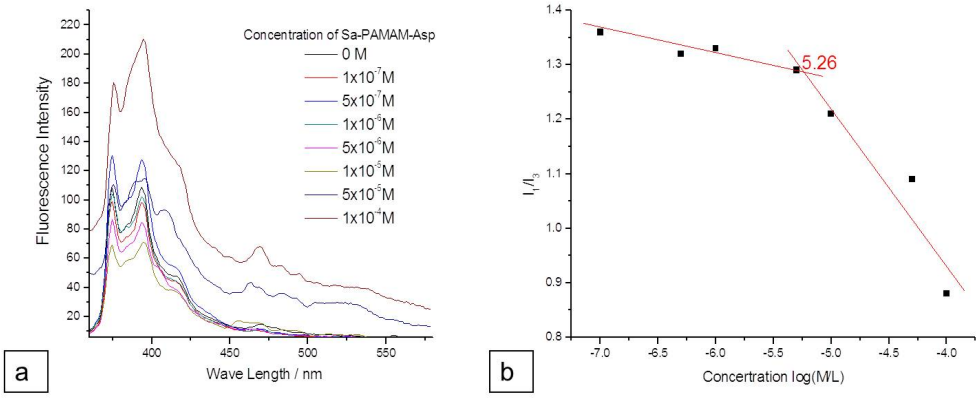


Fig. S1 (a) Fluorescence spectra of Sa-PAMAM-Asp at different concentrations; (b) Fluorescence intensity I_1/I_3 ratio of pyrene as a function of Sa-PAMAM-Asp concentration in aqueous solution containing pyrene (1.0×10^{-6} M).

The excitation spectra of Pyrene in the solutions were recorded on a Hitachi F-4500 fluorescence spectrometer. The procedures were referenced to the literature[3]. The emission wavelengths were set at 390 nm (for monomer) and 480 nm (for excimer), respectively. Both excitation and emission slit widths were set at 5.0 nm. The excitation spectra were recorded from 300 to 360 nm at a scanning rate of 240 nm/min. 100 μL of pyrene in THF solution (1.0×10^{-3} M) was added into a 100 mL volumetric flask, and the solvent was evaporated under a nitrogen flow. 100 mL deionized aqueous solution was then added, and the obtained solution was equilibrated for 2 days (adjusted pH value to 7.4). The aqueous solutions of Sa-PAMAM-Asp with various concentrations were mixed with the solution of pyrene at 37℃. The final concentration of pyrene in all of the solutions was 1.0×10^{-6} M unless otherwise indicated. I_1/I_3 was defined as the intensity ratio of the first (ca. 375 nm) to the third (ca. 385 nm) bands in the emission spectrum. In order to reduce the magnitude of this influence, the intensities of the first (I_1) and third (I_3) bands were subtracted by the intensity at 362 nm for calculating the ratio of I_1/I_3.

High resolution transmission electron microscopy (HRTEM) of the Aggregates of Sa-PAMAM-Asp

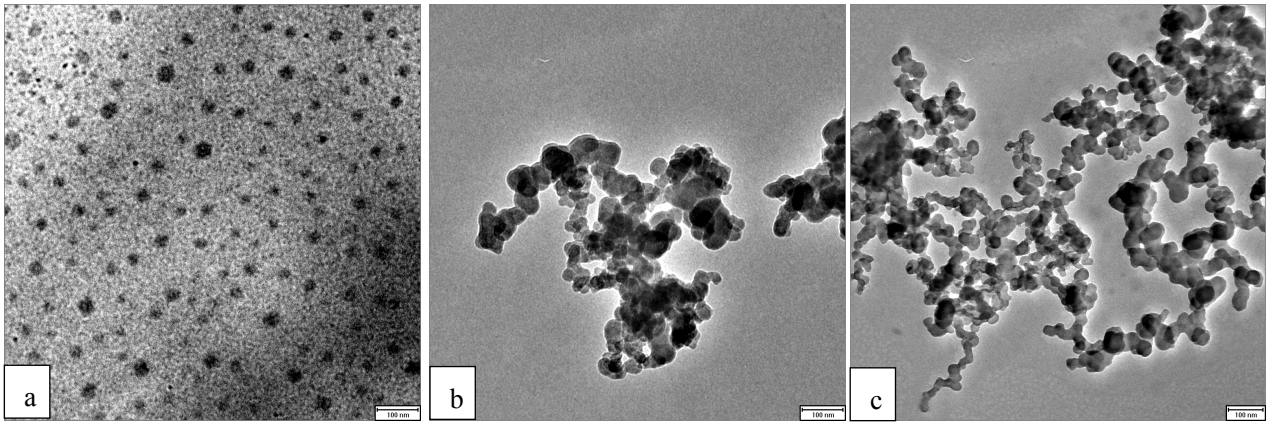


Fig. S2 TEM images of the assemblies from Sa-PAMAM-Asp at different concentrations (the freshly prepared samples); (a) 6.0×10^{-6}M; (b) 4.0×10^{-5} M; (c) 8.0×10^{-5} M. (Scale bar: 100nm)

The possible mechanism of formation of linear chain assemblies

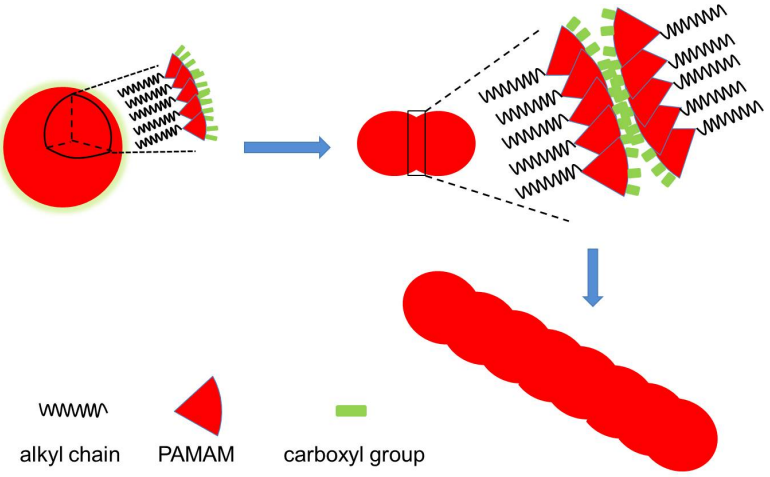


Fig. S3 Schematic illustration of the linear chain formation

The possible mechanism behind the formation of linear chains might be explained as follows: the formation of nanospheres could be attributed to the amphiphilic structure of dendrons, i.e., hydrophilic dendritic branches and hydrophobic alkyl chain at the focal point. The hydrophilic branches of dendrons tumble out to occupy more room in water. To minimize the energy in water, the alkyl chains shrink and pack together in parallel with the aid of hydrophobic interaction to form the hydrophobic interphase in the center of the aggregates. Because the hydrophilic dendritic branches with the end group of carboxyl in the outer layer, these nanospheres are very adhesive. They are readily attached by H-bonding interaction and the entanglement of the branches, such leading to the formation of linear chains by fusion of several individuals.

The mineralization was implemented according to the references[4, 5]. 32 μl DMSO solution with Sa-PAMAM-Asp (0.01M) were mixed with 1ml aqueous solution containing $Ca(NO_3)_2 \cdot 4H_2O$(0.1 M) at 37℃, and the mixture became cloudy immediately. After incubation at 37℃ for 0.5 hour, the 1ml solution of $(NH_4)_2HPO_4$ (0.06M) was added into the above solution. The final concentration of Sa-PAMAM-Asp was 1.6×10^{-4} M. The resulting solution was transferred into two tubes and kept at pH 7.4 and 37℃ for 7 and 14 days, respectively.

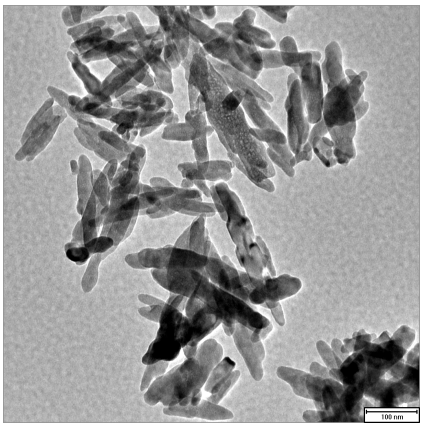


Fig. S4 TEM image of the HAP (100nm -600nm in length and 50-100nm in wide) developed in the absent of the assemblies of Sa-PAMAM-Asp. (Scale bar: 100nm)

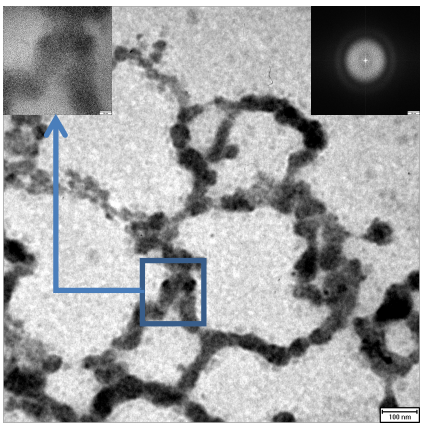


Fig. S5 TEM image of the mixture of Sa-PAMAM-Asp and mineralized solution at 37℃(Scale bar: 100nm). The electron diffraction pattern of the blue marked region indicated that the ions of Ca^{+2} were deposited on the linear chains initially by chelating with carboxyl groups on the assemblies (Scale bar: 20nm).

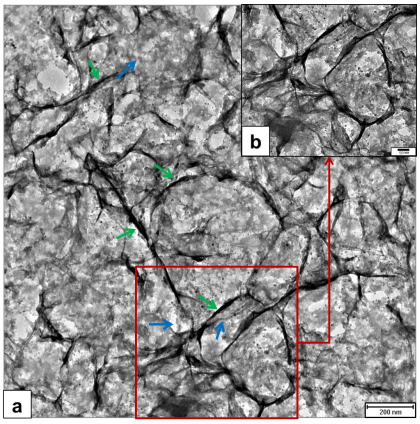


Fig. S6 TEM images of HAP crystals together with the assemblies of Sa-PAMAM-Asp at the concentration of 1.6×10^{-4} M (in 7 days). The Blue and green arrows indicate the assemblies and the crystals respectively. Image (b) is the magnification of image (a). (a) Scale bar: 200 nm; (b) Scale bar: 50 nm.

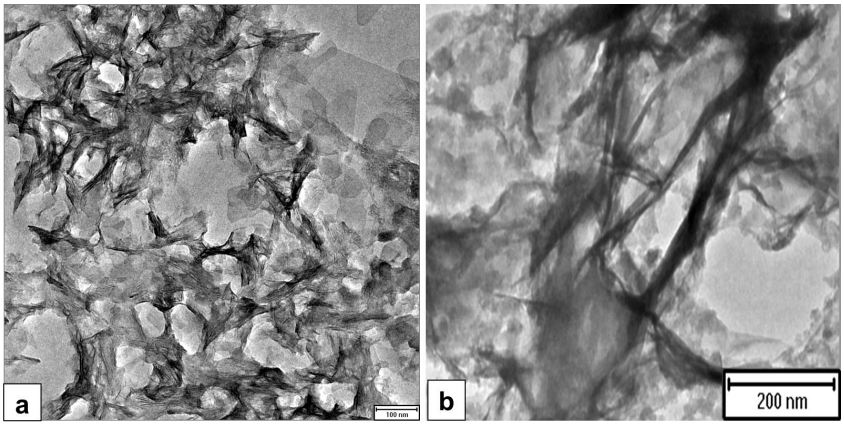


Fig. S7 TEM images of oriented HAP crystals generated in the present of the assemblies of Sa-PAMAM-Asp at the concentration of 1.6×10^{-4} M (in 7 days, the images obtained from different samples with the same preparation conditions). (a) Scale bar: 100 nm; (b) Scale bar: 200 nm

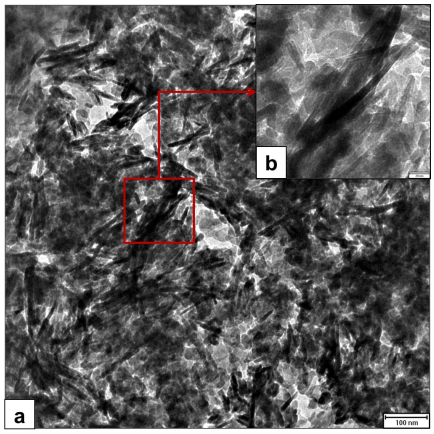


Fig. S8 TEM images of oriented HAP crystals generated in the present of the assemblies of Sa-PAMAM-Asp at the concentration of 1.6×10^{-4} M (in 14 days, scale bar: 100 nm). Inset of Fig. S8: enlarged TEM image of a. (scale bar: 20 nm).

XRD detection

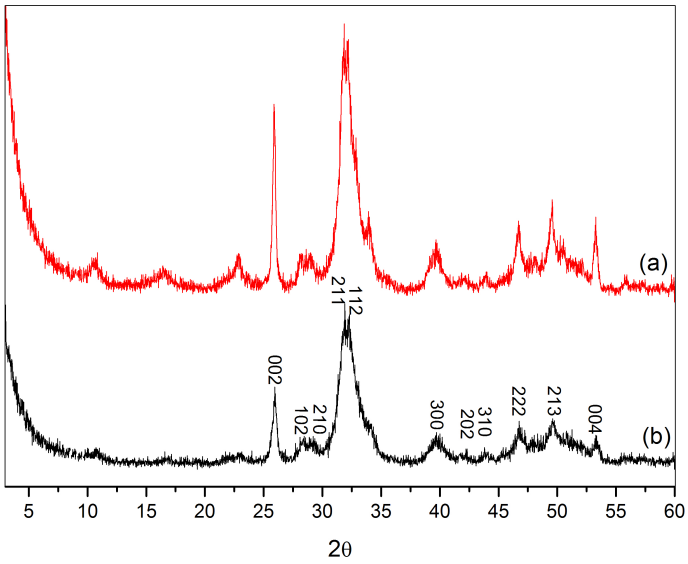


Fig. S9 XRD detection of HAP (a) with and (b) without adding Sa-PAMAM-ASP. The peaks at 25.8° and 31.8° were in good agreement with HAP's (002) and (211) direction (JCPDS card No. 09-0432).

FTIR

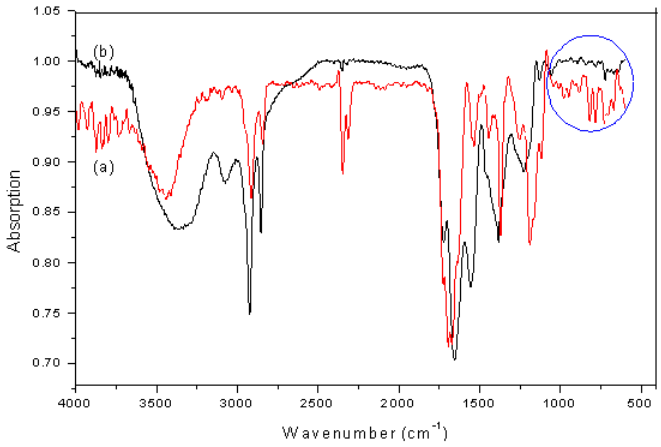


Fig. S10 FTIR spectra of synthesized molecule 8 (a) and synthesized molecule 9 (b) (before and after removing benzyl groups, blue ring marked)

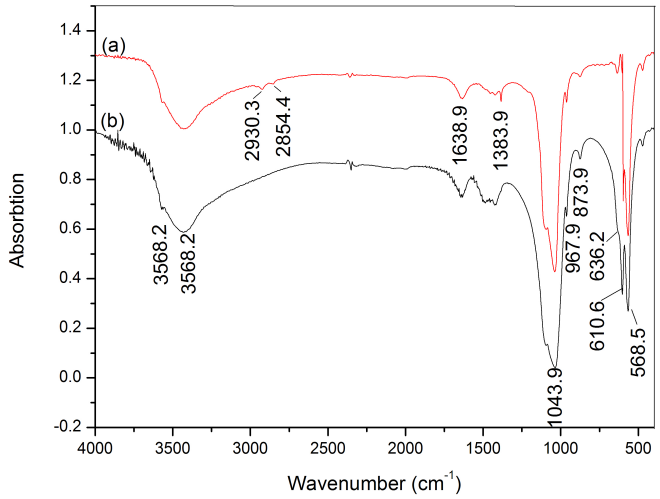


Fig. S11 FTIR spectra of (a) Sa-PAMAM-Asp-HAP and (b) HAP without adding Sa-PAMAM-Asp

References

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