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# Design and synthesis of novel amphiphilic Janus dendrimers for bone-targeted drug delivery

Junzhu Pan [a], Min Wen [a], Dongqin Yin [a,b], Bo Jiang [a], Dongsheng He [a], Li Guo [a,*]

[a] Key Laboratory of Drug Targeting and Drug Delivery System of Education Ministry, Department of Medicinal Chemistry, West China School of Pharmacy, Sichuan University, Chengdu 610041, China
[b] Laboratory of Stem Cell Biology, West China Hospital, West China Medical School, Sichuan University, Chengdu 610041, China

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ABSTRACT

In this paper, we synthesized a range of amphiphilic Janus dendrimers, which consisted of acidic amino acid and naproxen molecules as the peripheral groups, as novel potential bone-targeting dendritic drug delivery. These dendrimers take advantage of a dendritic display to carry multiple drug molecules and targeting moieties simultaneously. All of the dendrimers exhibited more than 80% binding rates to hydroxyapatite (HAP), especially the [$G_2$]-dendrimers (**2a** and **2b**) showed dramatic binding rates (>95%). Moreover, the solubility of naproxen was remarkably enhanced by the dendritic drug delivery system, especially the naproxen concentration of **2b** achieved 5.37 mg/ml, which is more than 28-fold over that of native drug. Furthermore, cell viability studies showed that all the dendrimers exhibited no significant cytotoxicity against HEK293 cells. These results provided an effective entry to the development of new bone-targeting drugs.



## 1. Introduction

Dendrimers are new artificial macromolecules, which have monodisperse, highly branched structure with well-defined three-dimensional architecture. The features of dendrimers, such as multivalency, well-defined and large globular structure, mono- or low polydispersity, make them have many properties that are significantly different from those of corresponding linear polymers.[1–3] With the development of dendritic macromolecules, it is an art and also a challenge to design dendrimer with much larger number and more types of sophisticated functional groups, which are precisely placed on different areas of the surface. Recently, Janus dendrimers, also called surface-block co-dendrimers or bow-tie dendrimers, characterized by two differently functionalized segments on opposite sides, have been prepared. These Janus dendrimers were designed for drug delivery and related therapy,[4,5] recognition-detection agents,[6] gene transfection,[7] fluorescent label,[8] magnetic resonance imaging (MRI),[9] dye,[10] anti-bacterial agents,[11] and new material or carrier for potential application of science.[12–15] Meanwhile, new synthetic strategy[16,17] and extraordinary properties (such as thermal behavior,[14,18,19] interfacial behavior[20]) of Janus dendrimers also have been expanded. Particular interest was focused on the amphiphilic Janus dendrimers, which consisted of two totally different dendrons (hydrophilic and hydrophobic segments) because these dendrimers can be used for self-assembly or as liquid crystals.[7,15,21–23]

Our research about this kind of dendrimers was focused on their biomedical applications as drug delivery systems. Amphiphilic Janus dendrimers are promising candidates for developing drug carriers for several reasons. First, they can assume two, or even more, different tasks such as targeting a particular site and transporting a medicinally active drug to this site. Furthermore, the amphiphilic structure is potential to self-assemble into nanoscale polymeric micelles (50–500 nm) in aqueous solutions.[24] Then, they may decrease the unwanted side effects, prolong the circulation time, and reduce the uptake by the reticuloendothelial system (RES).[25]

Acidic oligopeptides, which pose repetitive aspartic acid (Asp) or glutamic acid (Glu) sequences, are well-known bone-targeting moieties reported by both our group[26] and other researchers.[27–29] According to Sarig's research,[30] the mechanisms that the acidic oligopeptides showed bone-affinity because the multi-carboxy groups of peptides provide ionic interaction between negative charges and calcium ions in the mineral component of bone (HAP), and this kind of affinity only depends on the number of amino acid residues rather than the optical characters (L or D) of the peptides or

*Abbreviations:* DIPEA, *N*,*N*-diisopropylethylamine; DMAP, dimethylamino pyridine; EDCI·HCl, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; IBCF, isobutyl chloroformate; HBTU, *o*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HOBt, 1-hydroxy benzotriazole; NMM, *N*-methylmorpholine.

* Corresponding author. Tel.: +86 (0)28 85503777; fax: +86 28 85502609; e-mail address: rosaguoli2000@yahoo.com.cn (L. Guo).

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their species (Asp or Glu). Besides, peptides have been attached to multivalent scaffolds to obtain a higher affinity for their biological marker through multivalent interactions.[31] Inspired by the affinity mechanism and the dendritic effect, we reported peptide dendrimers with Asp or Glu periphery as novel bone-targeting moieties.[32] Compared with linear peptides, the dendritic peptides have more negatively charged groups (carboxy groups) in periphery, which benefit both affinity to bone and water solubility.

In this paper, we designed and synthesized a series of bis-functionalized amphiphilic Janus dendrimers, which consisted of multiple polar L-Asp or L-Glu (bone-targeting moieties), and multiple nonpolar naproxen (model drug of poorly water-soluble). We hope that this sort of dendrimers can be a satisfying bone-targeting prodrug with increased bone-targeting effect, water solubility, and low cytotoxicity.

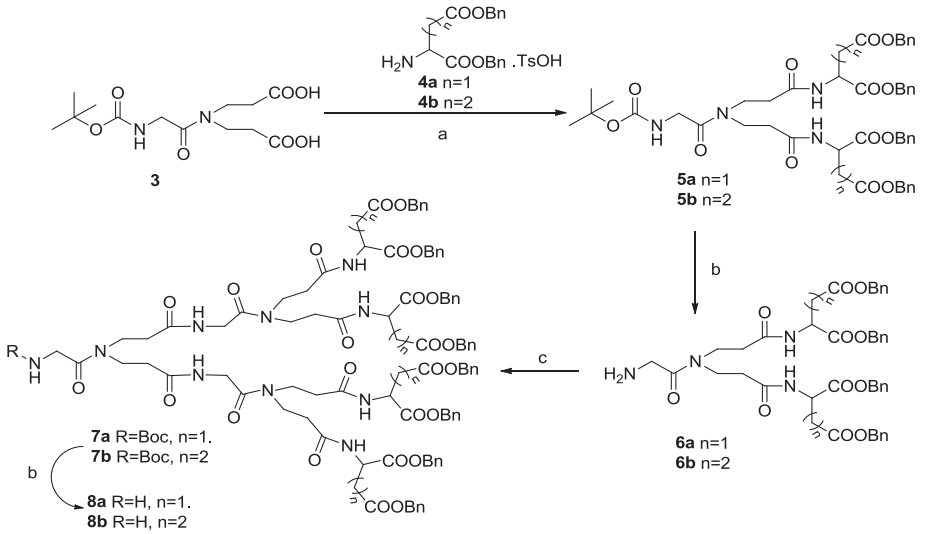
## 2. Results and discussion

### 2.1. Chemistry

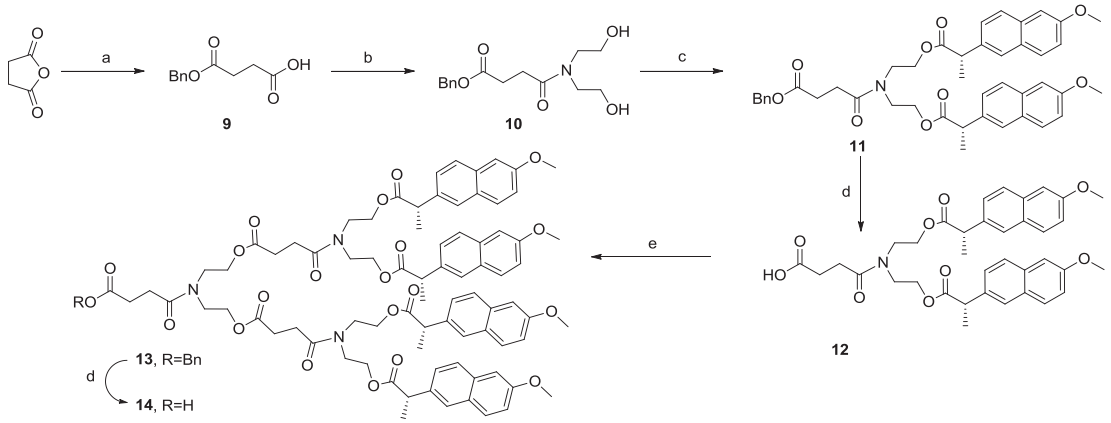
The synthetic procedure of the Janus dendrimers involved two steps: the synthesis of dendritic Asp/Glu (Scheme 1) and dendritic naproxen (Scheme 2), respectively, and the assembling of these two functional dendrons together (Scheme 3).

First, both of the two types of dendrons were prepared through a convergent synthesis, which involves several iterative generation-proliferation and deprotection steps. For the synthesis of dendritic Asp (or Glu), the core **3** was coupled with benzyl protected L-Asp (or L-Glu) in the presence of EDCI/HOBt to afford [$G_1$]-dendritic Asp (or Glu) **5a**,**b**, respectively. Formation of the second-generation dendron was achieved in a two-step sequence involving TFA-mediated removal of the Boc group from [$G_1$] dendron **5a**,**b** to afford **6a**,**b**, and subsequent generating with core **3**/HBTU/DIPEA to afford [$G_2$]-dendritic Asp (or Glu) **7a**,**b**, respectively. Then, **8a**,**b** were obtained through the removal of Boc group from **7a**,**b**. The strategy for synthesis of dendritic naproxen is similar to the preparation of dendritic Asp (or Glu). The synthesis of [$G_1$]-dendritic Nap **11** was carried out by utilizing EDCI and DMAP as the coupling reagents between core **10** and naproxen. Then, the benzyl groups were removed from **11** by catalytic hydrogenolysis to afford **12**. Subsequently, [$G_2$]-dendritic Nap **13** was prepared by treating **12** with core **10**/EDCI/DMAP. The activation of the focal point was done by the removal of benzyl group from **13** by catalytic hydrogenolysis giving compound **14**.

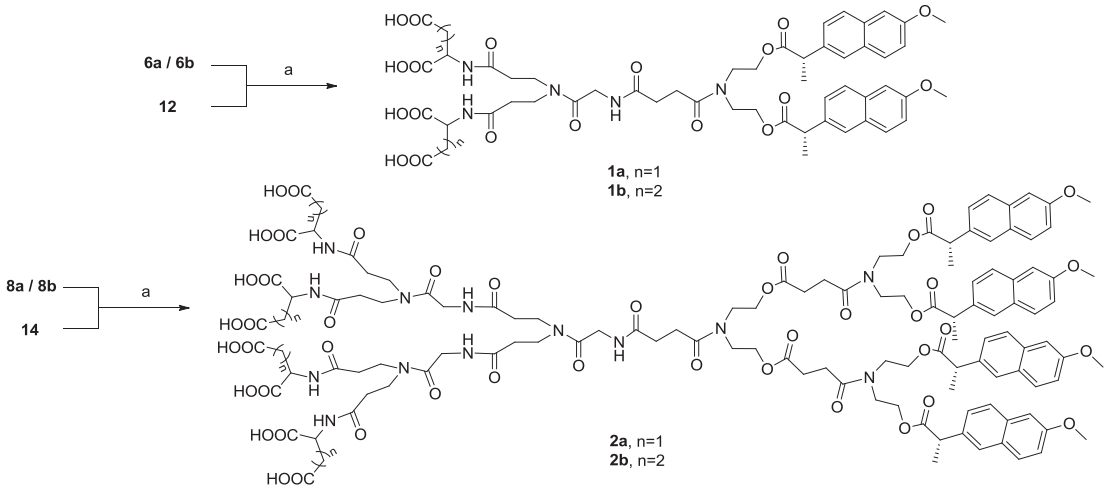
The key point of the synthesis of Janus dendrimers is the coupling of two different dendrons by connecting their cores. Such a reaction is challenging due to the steric hindrance at dendron focal points, which may render coupling inefficient leading to low




**Scheme 1.** Preparation of the dendritic Asp (Glu). Conditions: (a) EDCI/HOBt, $CH_2Cl_2$, rt, 24 h. Yields: **5a**, 81%; **5b**, 78%. (b) TFA, $CH_2Cl_2$, rt, 5 h. Yields: 99%. (c) **3**, HBTU/DIPEA, $CH_2Cl_2$, rt, 24 h. Yields: **7a**, 72%; **7b**, 65%.




**Scheme 2.** Preparation of the dendritic naproxen. Conditions: (a) benzyl alcohol, DMAP, THF, 50 °C, 5 h Yield: 85%. (b) Diethanolamine, NMM, IBCF, acetone, −15 °C to rt, 2 h. Yield: 70%. (c) Naproxen, EDCI/DMAP, $CH_2Cl_2$, rt, 24 h. Yield: 66%. (d) $H_2$, Pd/C (10%, wt%), $CH_2Cl_2$/MeOH, rt, 24 h. Yield: 99%. (e) Compound **10**, EDCI/DMAP, $CH_2Cl_2$, rt, 24 h. Yield: 41%.




**Scheme 3.** Preparation of the Janus dendrimer. Conditions: (a) (i) NMM, IBCF, THF, −15 °C to rt, 24 h; (ii) $H_2$, Pd/C (10%, wt %), MeOH, rt, 24 h. Yields: **1a**, 71%; **1b**, 75%; **2a**, 66%; **2b**, 63%.

yields and loss of dendron. For assembling of the dendritic Asp (or Glu) and dendritic naproxen, we attempted to use several coupling reagents, such as IBCF/NMM, EDCI/HOBt, HBTU/DIPEA. For the reaction between [$G_1$]-dendritic Asp (or Glu) and [$G_1$]-dendritic Nap, the use of all the coupling reagents mentioned above could give the product acceptable yields, while IBCF/NMM provided the best yield. For the similar reaction between the second-generation dendrons, steric hindrance had become a more significant problem because of the increased generation of dendrons. Only the use of IBCF/NMM gave the product an acceptable yield, while other coupling reagents mentioned above gave extremely poor results. Finally, after removal of the protected groups (benzyl) by catalytic hydrogenolysis, the target molecules **1a**,**b** and **2a**,**b** were obtained.

The high degree of symmetry in these dendrons and dendrimers enabled facile confirmation of both structure and purity by NMR techniques. In the $^1$H NMR spectrum of dendrons **5a**,**b**, **7a**,**b**, **11**, **13**, the structures can be confirmed by calculating the integration of the respective areas of the core protons and periphery protons. For instance: resonance signals at 1.4 ppm from Boc-*H* (core) and 4.7 ppm from Glu-α-C*H*, 4.9 ppm from Asp-α-C*H* (periphery); 5.1 ppm from ph-C$H_2$ (core) and 1.4 ppm from −CHC$H_3$ (periphery), which were clearly distinguishable from other resonances signals, areas confirmed the perfect jointing. Furthermore, the structures of these dendrons and dendrimers were further verified by ESI MS or ESI TOF MS. Moreover, elemental analysis was also in good agreement with those of the key intermediates and target structures.

## 2.2. HAP binding assay in vitro

With an overview of Fig. 1, two general characters of these dendrimers were revealed. First, all of the compounds exhibited some HAP affinity, whereas the parent drug naproxen was at best negligibly bound. The binding rates of these compounds approximated or evidently exceeded 80%. Moreover, **2a** and **2b** showed dramatic binding rates, which were more than 95%. The other distinguishing feature was that the binding rates culminated rapidly. To compare certain compounds' HAP binding rates of 1 h and 24 h, they did not present any divergence. This comparison indicated that the dendrimers loaded with dendritic Asp (or Glu) could bind to HAP and approach their binding plateaus in a short time (1 h), which was coincident with our group's previous work.[32] Besides, comparing the HAP affinity of dendrimers of the same generation, the compounds containing Asp or Glu as periphery showed no significant difference.

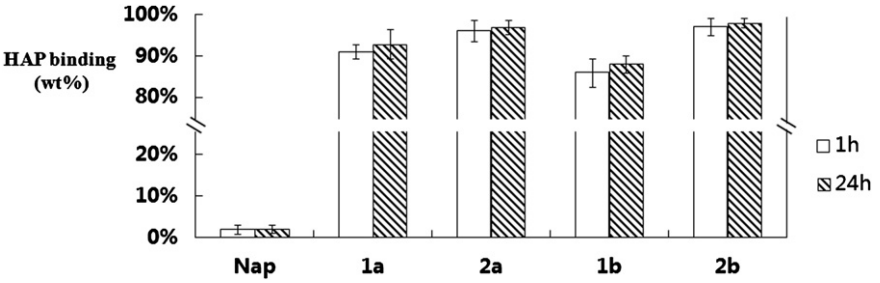



**Fig. 1.** Percentage of dendrimers bound to HAP. Percentage of dendrimers **1a**,**b**, **2a**,**b** bound to HAP. Values are represented as mean±SD ($n$=3).

can be used to ensure the complete coupling of these dendrons. In the $^1$H NMR spectrum of Janus dendrimers **1a**,**b**, **2a**,**b**, though a number of resonance signals were overlapped because of the complicated structures, the peaks at 1.41−1.44(m) ppm (Nap−CHC$H_3$) and 4.49−4.55(m)/4.18−4.20(m) ppm (α-C*H* of Asp/Glu) were distinguishable from other resonances signals and assigned to the two opposite sides, dendritic naproxen and dendritic Asp/Glu, respectively. Integration of the above respective

## 2.3. Solubility assessment in PBS (pH=7.4)

The data presented in Table 1 clearly demonstrated the solubility of these dendrimers. It was observed that the solubility of these Janus dendrimers is significantly better than naproxen, which is a poor-soluble molecule by itself. This highly increased solubility could be due to the surface carboxy groups that were available to interact with water molecules.[33] The dendrimers had 4.75−14.52 mg/ml solubility

**Table 1**
Water solubility of these dendrimers in PBS (pH=7.4)

| Compound | Numbers of −COOH | Water solubility *C* (mg/ml) | Numbers of loading naproxen $N_{Nap}$ | Naproxen concentration[a] (mg/ml) |
|---|---|---|---|---|
| Naproxen | 1 | 0.19 | — | 0.19 |
| **1a** | 4 | 6.01 | 2 | 2.61(13.7[b]) |
| **1b** | 4 | 4.75 | 2 | 2.01(10.5[b]) |
| **2a** | 8 | 11.58 | 4 | 4.29(22.4[b]) |
| **2b** | 8 | 14.52 | 4 | 5.37(28.1[b]) |

[a] Calculated by formula: $C\times N_{Nap}\times 230.26\times 1000/Mw_{dendrimer}$.
[b] Equivalent to native naproxen.

in water, which was equivalent to as high as 2.01–5.37 mg/ml of naproxen. By comparison of enhanced solubility, the naproxen concentration of [$G_2$] dendrimers (4.29–5.37 mg/ml) was more than two times as much as the [$G_1$] ones (2.01–2.61 mg/ml), thus the [$G_2$] dendrimers seemed to be a more effective vehicle to enhance the solubility of insoluble drugs. It presumably resulted from the fact that the [$G_2$] molecules, which have more and condensed carboxy groups at the surface area, have a higher ability to interact with water molecule than the [$G_1$] ones.[34] In summary, the solubility data indicated that amphiphilic Janus dendrimer is a favorable approach to deliver poor water-soluble drugs.

### 2.4. Cytotoxicity assay

In spite of extensive applicability in biological system, the use of dendrimers for biomedical applications is constrained because of the inherent toxicity associated with them. Surface engineering, such as PEGylation, carbohydrate and peptide/amino acids conjugation, is an effective way for improving the biocompatibility of dendrimers.[35] In this paper, natural acid amino acids, rather than other classical bone-targeting moieties (tetracycline, bisphosphonate and their derivates), were chosen to build drug delivery system to bone. It was expected to provide bone-affinity and benefit the biocompatibility of dendrimers simultaneously by introducing natural amino acid. Fig. 2 showed the MTT assay results of cytotoxicity of naproxen and dendrimers in HEK293 cell lines. All dendrimers did not show any cytotoxicity in the concentration range of 100–250 nM/ml naproxen equivalents ($Eq_{nap}$). With the augment of tested concentration (500–1000 nM/ml $Eq_{nap}$), the cytotoxicity of these compounds increased by only a slight degree. Moreover, we did not observe a significant difference in the toxicity of dendrimers with different amino acids block. As a summary, all the synthetized dendrimers were non-toxic or low-toxic in tested concentration.

## 3. Conclusion

Site-specific drug delivery via a dendrimer approach has generated considerable interest for enhancing the target-site affinity and drug potency by dendritic effect. In this study, we have designed and prepared a range of amphiphilic Janus dendrimers, which consisted of L-Asp/L-Glu and naproxen molecules as the peripheral groups, as novel potential bone-targeting dendritic drug delivery. These dendrimers take advantage of a dendritic display to carry multiple drug molecules and targeting moieties simultaneously. The resulting dendrimers were evaluated for the affinity to HAP, water solubility, and cytotoxicity on HEK293. HAP binding assay highlighted the strong affinity and fast initial binding of Janus dendrimers to bone, as opposed to the negligible bone affinities of the parent drugs. Although the water solubility of naproxen was poor, these dendrimers showed enhanced solubility in water, which indicated that amphiphilic Janus dendrimer was a favorable approach to delivery poor water-soluble drug. Moreover, cell viability studies showed that all dendrimers exhibited no significant cytotoxicity against HEK293 cells. The preliminary results are very promising and we are continuing to develop more suitable animal models for in vivo comprehensive evaluation.

## 4. Experimental section

### 4.1. General

All reactions requiring anhydrous conditions were performed under an Ar or $N_2$ atmosphere. Thin layer chromatography (TLC): silica gel plates $GF_{254}$; compounds were visualized by irradiation with UV light and/or by treatment with a solution of ninhydrin (0.2% in ethanol) followed by heating. Column chromatography was performed by using silica gel with eluent given in parentheses. $^1H$ NMR analysis was performed by the INOVA VARIAN 400 MHz

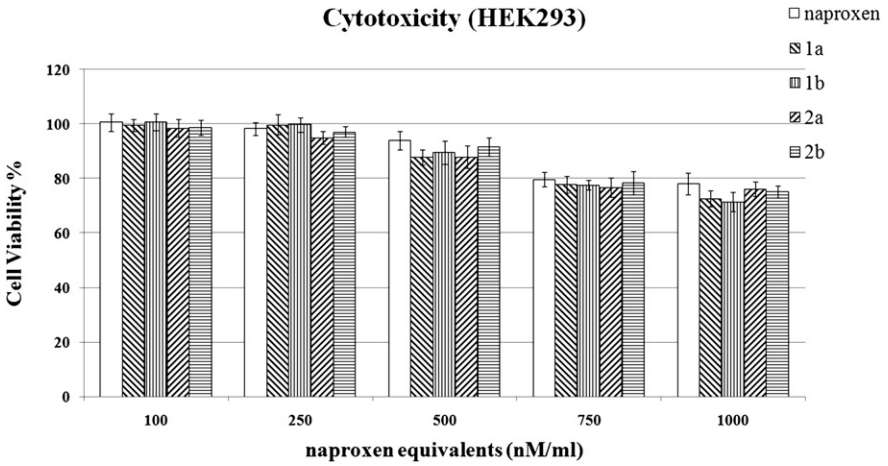



**Fig. 2.** Cell viability upon addition of dendrimers in HEK293 cells. Cytotoxicity of dendrimers against HEK293 cells over a 24 h incubation period determined by MTT assay. Cell viability was expressed as a percentage of the control cell culture. Values are represented as mean±SD ($n$=3).

spectrometer using $CDCl_3$ or DMSO-$d_6$ as a solvent at room temperature. The chemical shifts are expressed in relative to TMS (=0 ppm) and the coupling constant *J* in hertz. MS spectra were performed on Agilent 1964B spectrometer. ESI TOF MS were recorded with Shimadzu LCMS-IT-TOF. Elemental analysis was performed by Atlantic Microlab, Atlanta, GA. Melting points (°C) were determined with X-4 digital melting-point apparatus and are uncorrected. UV spectroscopy was performed on Perkin–Elmer Lambda 35. The MTT assay was carried on Bio-Rad 680 microplate reader.

Chemicals and solvents were either A.R. grade or purified by standard techniques. Hydroxyapatite (HAP) was purchased from Shanghai Institute of Biochemistry with surface area 9.12 $m^2/g$ and average particle size 15 μm.

## 4.2. Synthesis

*4.2.1. Synthesis of core **3**.* Core **3** was synthesized by literature methods of our group's work previously.[36]

*4.2.2. Synthesis of the dendron **5a**.* Core **3** (0.95 g, 3 mmol) was dissolved in $CH_2Cl_2$ (30 ml), EDCI·HCl (6.5 mmol), HOBt (6.5 mmol), and L-Aspartic acid dibenzyl ester 4-toluenesulfonate salt **4a** (3.15 g, 6.5 mmol) were added into the solution. The reaction mixture was stirred under $N_2$ at room temperature for 24 h. The solution was then concentrated under vacuum, and the residue was taken up in ethyl acetate (30 ml) and washed with 1 M HCl (10 ml), 1 M $NaHCO_3$ (10 ml), and brine (10 ml). The organic layer was dried over anhydrous $Na_2SO_4$. After concentration, the residue was purified by silica-gel column chromatography ($CH_2Cl_2$/MeOH) to obtain **5a** as colorless oil (2.21 g, yield: 81%). $^1H$ NMR ($CDCl_3$) $\delta$: 1.44 (s, 9H, Boc-*H*), 2.43–2.49 (m, 4H, $NCH_2CH_2\times2$), 2.85–3.07 (m, 4H, Asp-β-$CH_2\times2$), 3.47–3.68 (m, 4H, $NCH_2CH_2\times2$), 4.00 (s, 2H, Gly-$CH_2$), 4.92–4.94 (m, 2H, Asp-α-$CH\times2$), 5.00–5.14 (m, 8H, ph-$CH_2\times4$), 7.26–7.34 (m, 20H, ph-*H*). ESI MS (*m*/*z*): calcd for $C_{49}H_{56}N_4O_{13}$: 908.38; obsd 931.43 ($[M+Na]^+$). Anal. Calcd for C, 64.75; H, 6.21; N, 6.16; O, 22.88. Found: C, 64.61; H, 6.12; N, 6.11; O, 22.79.

*4.2.3. Synthesis of the dendron **5b**.* The same procedure as described above for preparation of **5a**. Dendron **5b**: colorless oil (2.19 g, yield: 78%). $^1H$ NMR ($CDCl_3$) $\delta$: 1.43 (s, 9H, Boc-*H*), 1.91–2.24 (m, 4H, Glu-β-$CH_2\times2$), 2.31–2.62 (m, 8H, $NCH_2CH_2\times2$+Glu-γ$CH_2\times2$), 3.38–3.78 (m, 4H, $NCH_2CH_2\times2$), 3.96–4.11 (m, 2H, Gly-$CH_2$), 4.57–4.70 (m, 2H, Glu-α-$CH\times2$), 5.06–5.16 (m, 8H, ph-$CH_2\times4$), 7.27–7.33 (m, 20H, ph-*H*). ESI MS (*m*/*z*): calcd for $C_{51}H_{60}N_4O_{13}$: 936.42; obsd 959.80 ($[M+Na]^+$). Anal. Calcd for C, 65.37; H, 6.45; N, 5.98; O, 22.20. Found: C, 65.25; H, 6.33; N, 5.91; O, 22.15.

*4.2.4. Synthesis of the dendron **7a**.* To the solution of trifluoroacetic acid (TFA)/anhydrous $CH_2Cl_2$ (1:1, v/v), **5a** (2.27 g, 2.5 mmol) was added at room temperature. When the protecting groups were completely removed, the solvent was rotary evaporated to obtain **6a** (yield: 99%) without further purification. Core **3** (0.32 g, 1 mmol) was dissolved in $CH_2Cl_2$ (30 ml), HBTU (2.5 mmol), DIPEA (2.5 mmol), and **6a** were added into the solution. The reaction mixture was stirred under $N_2$ at room temperature for 24 h. The solution was then concentrated under vacuum, and the residue was taken up in ethyl acetate (30 ml) and washed with 1 M HCl (10 ml), 1 M $NaHCO_3$ (10 ml), and brine (10 ml). The organic layer was dried over anhydrous $Na_2SO_4$. After concentration, the residue was purified by silica-gel column chromatography ($CH_2Cl_2$/MeOH) to obtain **7a** as colorless oil (1.37 g, yield: 72%). $^1H$ NMR ($CDCl_3$) $\delta$: 1.40 (s, 9H, Boc-*H*), 2.48–2.54 (m, 12H, $NCH_2CH_2\times6$), 2.86–3.04 (m, 8H, Asp-β-$CH_2\times4$), 3.54–3.60 (m, 12H, $NCH_2CH_2\times6$), 4.07 (s, 6H, Gly-$CH_2\times3$), 4.93 (br s, 4H, Asp-α-$CH\times4$), 5.00–5.12 (m, 16H, ph-$CH_2\times8$), 7.26–7.31 (m, 40H, ph-*H*). ESI MS (*m*/*z*): calcd for $C_{101}H_{114}N_{10}O_{27}$: 1898.79; obsd 1922.44 ($[M+Na]^+$). Anal. Calcd for C, 63.85; H, 6.05; N, 7.37; O, 22.74. Found: C, 63.76; H, 6.00; N, 7.31; O, 22.62.

*4.2.5. Synthesis of the dendron **7b**.* The same procedure as described above for preparation of **7a**. Dendron **7b**: colorless oil (1.27 g, yield: 65%). $^1H$ NMR ($CDCl_3$) $\delta$: 1.40 (s, 9H, Boc-*H*), 1.92–2.20 (m, 8H, Glu-β-$CH_2\times4$), 2.43 (br s, 20H, $NCH_2CH_2\times6$+Glu-γ$CH_2\times4$), 3.51–3.66 (m, 12H, $NCH_2CH_2\times6$), 3.96–4.08 (m, 6H, Gly-$CH_2\times3$), 4.64 (br s, 4H, Glu-α-$CH\times4$), 5.08–5.13 (m, 16H, ph-$CH_2\times8$), 7.17–7.32 (m, 20H, ph-*H*). ESI TOF MS (*m*/*z*): calcd for $C_{105}H_{122}N_{10}O_{27}$: 1954.85; obsd 1978.89 ($[M+Na]^+$). Anal. Calcd for C, 64.47; H, 6.29; N, 7.16; O, 22.08. Found: C, 64.38; H, 6.18; N, 7.12; O, 22.01.

*4.2.6. Synthesis of the core **10**.* Succinic acid monobenzyl ester **9** was prepared by the literature methods.[37] Compound **9** (10 mmol, 2.0 g) dissolved in anhydrous acetone (25 ml) was cooled at −15 °C, then added NMM (10 mmol) and IBCF (10 mmol), stirred for 10 min, diethanolamine (15 mmol, 1.6 g) dissolved in anhydrous acetone (10 ml) was added dropwise to the reaction. The reaction mixture was vigorously stirred at room temperature for 2 h. The solution was then concentrated under vacuum, and the residue was taken up in ethyl acetate (30 ml) and washed with 1 M HCl (10 ml), 1 M $NaHCO_3$ (10 ml), and brine (10 ml). The organic layer was dried over anhydrous $Na_2SO_4$. After concentration, the residue was purified by silica-gel column chromatography ($CH_2Cl_2$/MeOH) to obtain **10** as colorless oil (2.1 g, yield: 70%). $^1H$ NMR ($CDCl_3$) $\delta$: 2.77 (br s, 4H, succinic acid-$CH_2CH_2$), 3.55–3.59 (m, 4H, $NCH_2\times2$), 3.81–3.86 (m, 4H, $CH_2OH\times2$), 5.16 (s, 2H, ph-$CH_2$), 7.37–7.40 (m, 5H ph-*H*). ESI MS (*m*/*z*): calcd for $C_{15}H_{21}NO_5$: 295.14; obsd 318.35 ($[M+Na]^+$).

*4.2.7. Synthesis of the dendron **11**.* Core **10** (1.48 g, 5 mmol) was dissolved in $CH_2Cl_2$ (30 ml), EDCI·HCl (15 mmol), DMAP (2 mmol), and naproxen (2.76 g, 12 mmol) were added into the solution. The reaction mixture was stirred under $N_2$ at room temperature for 24 h. The solution was then concentrated under vacuum, and the residue was taken up in ethyl acetate (30 ml) and washed with 1 M HCl (10 ml), 1 M $NaHCO_3$ (10 ml), and brine (10 ml). The organic layer was dried over anhydrous $Na_2SO_4$. After concentration, the residue was purified by silica-gel column chromatography (petroleum ether/ethyl acetate) to obtain **11** as white solid (2.37 g, yield: 66%). Mp=107–110 °C. $^1H$ NMR ($CDCl_3$) $\delta$: 1.50–1.53 (m, 6H, $CHCH_3\times2$), 2.22–2.36 (m, 4H, succinic acid-$CH_2CH_2$), 2.93–3.39 (m, 4H, $NCH_2\times2$), 3.84–3.89 (m, 8H, $OCH_3\times2$+$CHCH_3\times2$), 3.98–4.14 (m, 4H, $NCH_2CH_2\times2$), 5.08 (s, 2H, ph-$CH_2$), 7.06–7.13 (m, 5H, ph-*H*), 7.29–7.37 (m, 6H, Nap-ph*H*), 7.58–7.66 (m, 6H, Nap-ph*H*). ESI MS (*m*/*z*): calcd for $C_{43}H_{45}NO_9$: 719.31; obsd 742.52 ($[M+Na]^+$). Anal. Calcd for C, 71.75; H, 6.30; N, 1.95; O, 20.00. Found: C, 71.66; H, 6.21; N, 1.88; O, 19.91.

*4.2.8. Synthesis of the dendron **13**.* The mixture of **11** (1.44 g, 5 mmol) and 10% Pd/C (100 mg) in methanol/$CH_2Cl_2$ (5:1 v/v 30 ml) was stirred at the room temperature under hydrogen atmosphere. After 24 h the mixture passed through a membrane filter to remove the catalyst, then evaporated under reduced pressure to afford **12** as white foam (yield: 99%). Without further purification, **12** was dissolved in $CH_2Cl_2$ (30 ml), EDCI·HCl (5 mmol), DMAP (1 mmol), and core **10** (2 mmol, 0.59 g) were added into the solution. The reaction mixture was stirred under $N_2$ at room temperature for 24 h. The solution was then concentrated under vacuum, and the residue was taken up in ethyl acetate (30 ml) and washed with 1 M HCl (10 ml), 1 M $NaHCO_3$ (10 ml), and brine (10 ml). The organic

layer was dried over anhydrous $Na_2SO_4$. After concentration, the residue was purified by silica-gel column chromatography (petroleum ether/ethyl acetate) to obtain **13** as colorless oil (1.24 g, yield: 41%). $^1$H NMR ($CDCl_3$) $\delta$: 1.50–1.52 (m, 12H, CHC$H_3$×4), 2.21–2.68 (m, 12H, succinic acid-C$H_2$C$H_2$×3), 2.92–3.73 (m, 12H, NC$H_2$×6), 3.82–3.89 (m, 16H, OC$H_3$×4+C$H$CH$_3$×4), 3.99–4.17 (m, 12H, NCH$_2$C$H_2$×6), 5.10 (s, 2H, ph-C$H_2$), 7.06–7.66 (m, 7H, ph-$H$+Nap-ph$H$), 7.26–7.32 (m, 10H, Nap-ph$H$), 7.59–7.66 (m, 12H, Nap-ph$H$). ESI MS ($m/z$): calcd for $C_{87}H_{95}N_3O_{21}$: 1517.65; obsd 1539.42 ([M+Na−H]$^+$). Anal. Calcd for C, 68.80; H, 6.31; N, 2.77; O, 22.12. Found: C, 68.72; H, 6.25; N, 2.73; O, 22.01.

*4.2.9. Synthesis of the dendrimer **1a**.* Compound **12** (315 mg, 0.5 mmol) dissolved in anhydrous THF (25 ml) was cooled at −15 °C, then added NMM (10 mmol) and IBCF (10 mmol), stirred for 10 min, **6a** (404 mg, 0.5 mmol) dissolved in anhydrous THF (10 ml) was added dropwise to the reaction. The reaction mixture was vigorously stirred at room temperature for 2 h. The solution was then concentrated under vacuum, and the residue was taken up in ethyl acetate (30 ml) and washed with 1 M HCl (10 ml), 1 M $NaHCO_3$ (10 ml), and brine (10 ml). The organic layer was dried over anhydrous $Na_2SO_4$. After concentration, the residue was purified by silica-gel column chromatography ($CH_2Cl_2$/MeOH) to obtain a white waxy solid. Pd/C of 10% (100 mg) was added to a solution of the obtained white waxy solid in methanol/$CH_2Cl_2$ (3:1 v/v 30 ml). The reaction mixture was stirred under hydrogen atmosphere for 24 h, filtered through membrane filter, and concentrated under reduced pressure to afford **1a** as white foam (376 mg, yield: 71%). $^1$H NMR (DMSO-$d_6$) $\delta$: 1.41–1.44 (m, 6H, CHC$H_3$×2), 2.31–2.70 (m, 12H, succinic acid C$H_2$×2+Asp-β-C$H_2$×2+NCH$_2$C$H_2$CONH×2), 3.19–3.45 (m, 8H, NC$H_2$CH$_2$O×2+NC$H_2$CH$_2$CONH×2), 3.86 (s, 6H, OC$H_3$×2), 3.94–4.06 (m, 8H, NCH$_2$C$H_2$O×2+C$H$CH$_3$×2+Gly-C$H_2$×1), 4.50–4.52 (m, 2H, Asp-α-C$H$×2), 7.12–7.33 (m, 4H, Nap-ph$H$), 7.64–7.78 (m, 6H, Nap-ph$H$), 7.80 (br s, 1H, CON$H$), 8.24 (br s, 1H, CON$H$), 8.37 (br s, 1H, CON$H$), 12.57 (br s, 4H, Asp-COO$H$×4). ESI MS ($m/z$): calcd for $C_{52}H_{61}N_5O_{19}$: 1059.4; obsd 1059.52 ([M]$^-$). Anal. Calcd for C, 58.92; H, 5.80; N, 6.61; O, 28.68. Found: C, 58.83; H, 5.69; N, 6.49; O, 28.60.

*4.2.10. Synthesis of the dendrimer **1b**.* The same procedure as described above for preparation of **1a**. Dendrimer **1b**: white foam (408 mg, yield: 75%). $^1$H NMR (DMSO-$d_6$) $\delta$: 1.42–1.44 (m, 6H, CHC$H_3$×2), 1.71–1.96 (m, 4H, Glu-β-C$H_2$×2), 2.26 (br s, 4H, Glu-γC$H_2$×2), 2.31–2.47 (m, 8H, succinic acid C$H_2$×2+NCH$_2$C$H_2$CONH×2), 3.19–3.47 (m, 8H, NC$H_2$CH$_2$O×2+NC$H_2$CH$_2$CONH×2), 3.88 (s, 6H, OC$H_3$×2), 3.93–4.06 (m, 8H, NCH$_2$C$H_2$O×2+C$H$CH$_3$×2+Gly-C$H_2$×1), 4.17–4.22 (m, 2H, Glu-α-C$H$×2), 7.12–7.34 (m, 4H, Nap-ph$H$), 7.67–7.81 (m, 6H, Nap-ph$H$), 7.81 (br s, 1H, CON$H$), 8.16 (br s, 1H, CON$H$), 8.28 (br s, 1H, CON$H$), 12.43 (br s, 4H, Asp-COO$H$×4). ESI MS ($m/z$): calcd for $C_{54}H_{65}N_5O_{19}$: 1087.43; obsd 1087.89 ([M]$^-$). Anal. Calcd for C, 59.61; H, 6.02; N, 6.44; O, 27.94. Found: C, 59.49; H, 5.93; N, 6.32; O, 27.88.

*4.2.11. Synthesis of the dendrimer **2a**.* To the solution of trifluoroacetic acid TFA/anhydrous $CH_2Cl_2$ (1:1, v/v), **7a** (475 mg, 0.25 mmol) was added at room temperature. When the protecting groups were completely removed, the solvent was rotary evaporated to obtain **8a** (yield: 99%). The mixture of **13** (379 mg, 0.25 mmol) and 10% Pd/C (100 mg) in methanol/$CH_2Cl_2$ (5:1 v/v 30 ml) was stirred at the room temperature under hydrogen atmosphere. After 24 h the mixture passed through a membrane filter to remove the catalyst, then evaporated under reduced pressure to afford **14** (yield: 99%).

Without further purification, **14** dissolved in anhydrous THF (25 ml) was cooled at −15 °C, then added NMM (0.25 mmol) and IBCF (0.25 mmol), stirred for 10 min, **8a** dissolved in anhydrous THF (10 ml) was added dropwise to the reaction. The reaction mixture was vigorously stirred at room temperature for 2 h. The solution was then concentrated under vacuum, and the residue was taken up in ethyl acetate (30 ml) and washed with 1 M HCl (10 ml), 1 M $NaHCO_3$ (10 ml), and brine (10 ml). The organic layer was dried over anhydrous $Na_2SO_4$. After concentration, the residue was purified by silica-gel column chromatography ($CH_2Cl_2$/MeOH) to obtain a white waxy solid. Pd/C of 10% (100 mg) was added to a solution of the obtained white waxy solid in methanol/$CH_2Cl_2$ (3:1 v/v 30 ml). The reaction mixture was stirred under hydrogen atmosphere for 24 h, filtered through membrane filter and, concentrated under reduced pressure to afford **2a** as white foam. (411 mg, yield: 66%). $^1$H NMR (DMSO-$d_6$) $\delta$: 1.40–1.42 (m, 12H, CHC$H_3$×4), 2.26–2.75 (m, 32H, succinic acid C$H_2$×6+Asp-β-C$H_2$×4+NCH$_2$C$H_2$CONH×6), 3.17–3.66 (m, 24H, NC$H_2$CH$_2$O×6+NC$H_2$CH$_2$CONH×6), 3.83 (s, 12H, OC$H_3$×4), 3.93–4.12 (m, 22H, NCH$_2$C$H_2$O×6+C$H$CH$_3$×4+Gly-C$H_2$×3), 4.49–4.55 (m, 4H, Asp-α-C$H$×4), 7.11–7.33 (m, 12H, Nap-ph$H$), 7.64–7.75 (m, 12H, Nap-ph$H$), 7.85 (br s, 1H, CON$H$), 7.95 (br s, 1H, CON$H$), 8.08 (br s, 1H, CON$H$), 8.26 (br s, 2H, CON$H$×2), 8.39 (br s, 2H, CON$H$×2), 12.55 (br s, 8H, Asp-COO$H$×8). ESI TOF MS ($m/z$): calcd for $C_{120}H_{145}N_{13}O_{45}$: 2487.95; obsd 2487.85 ([M]$^-$). Anal. Calcd for C, 57.89; H, 5.87; N, 7.31; O, 28.92. Found: C, 57.75; H, 5.74; N, 7.26; O, 28.84.

*4.2.12. Synthesis of the dendrimer **2b**.* The same procedure as described above for preparation of **2a**. Dendrimer **2b**: white foam (401 mg, yield: 63%). $^1$H NMR (DMSO-$d_6$) $\delta$: 1.41–1.42 (m, 12H, CHC$H_3$×4), 1.77–1.97 (m, 8H, Glu-β-C$H_2$×4), 2.27 (br s, 8H, Glu-γC$H_2$×4), 2.36–2.51 (m, 24H, succinic acid C$H_2$×6+NCH$_2$C$H_2$CONH×6), 3.24–3.67 (m, 24H, NC$H_2$CH$_2$O×6+NC$H_2$CH$_2$CONH×6), 3.84 (s, 12H, OC$H_3$×4), 3.94–4.12 (m, 22H, NCH$_2$C$H_2$O×6+C$H$CH$_3$×4+Gly-C$H_2$×3), 4.18–4.20 (m, 4H, Glu-α-C$H$×4), 7.11–7.33 (m, 12H, Nap-ph$H$), 7.65–7.75 (m, 12H, Nap-ph$H$), 7.86 (br s, 1H, CON$H$), 7.96 (br s, 1H, CON$H$), 8.09 (br s, 1H, CON$H$), 8.18 (br s, 2H, CON$H$×2), 8.31 (br s, 2H, CON$H$×2), 12.40 (br s, 8H, Asp-COO$H$×8). ESI TOF MS ($m/z$): calcd for $C_{124}H_{153}N_{13}O_{45}$: 2544.01; obsd 2544.92 ([M]$^-$). Anal. Calcd for C, 58.51; H, 6.06; N, 7.15; O, 28.28. Found: C, 58.43; H, 6.00; N, 7.06; O, 28.15.

### 4.3. In vitro HAP binding assay

The dendrimers **1a**,**b**, **2a**,**b** were dissolved in PBS (pH=7.4), respectively, with various precise concentrations and the adsorption amounts were determined by an UV spectrophotometer at 273 nm to obtain the $A$–$C$ linear regression equation. Then, in tubes, 0.05 M **1a**,**b**, **2a**,**b** were dissolved in 10 ml PBS (pH=7.4), respectively ($C_0$=5 mmol/ml), and incubated with HAP 20 mg in a shaker bath at 37 °C. Each tube was cultured for 1 h and 24 h. After the prescribed time, tubes were centrifuged for 3 min at 5000 rpm. The adsorption amounts were determined by UV at 273 nm and $C$ was obtained by the special equation. The bound percentage was calculated by $(C_0-C)/C_0\times100\%$ and the result was presented in Fig. 1.

### 4.4. Solubility assessment

The solubility of the Janus dendrimers was determined at pH=7.4 in PBS. The method used for sample preparation was similar to each system,[38] i.e., $A$–$C$ linear regression equation was obtained in 4.3. Excess compounds **1a**,**b**, **2a**,**b** were added to 1 ml of the PBS (pH=7.4) solution, respectively, to ensure the compound solution reaching saturation. The solutions were mechanically shaken for 10 min at 37 °C and centrifuged at 10,000 rpm for 10 min. Supernatant of 200 μl was pipetted and diluted by PBS (pH=7.4) as proper multiple to make the concentration of the diluted solution in the range of the obtained $A$–$C$ linear regression equation. The adsorption amounts were determined by UV at

273 nm. Then $C_1$ (diluted solution) was obtained by the special equation and $C$ (saturated solution) was calculated by $C_1 \times N$ (diluted multiple). The data were presented in Table 1.

### 4.5. Cytotoxicity evaluation

The cytotoxicity of these Janus dendrimers **1a**,**b**, **2a**,**b** was evaluated using the MTT assay in human embryonic kidney (HEK293) cell lines. The cell lines were cultured in Dulbecco's modified eaglemedium (DMEM), supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 units/mL of penicillin and 100 μg/ml of streptomycin at 37 °C, under 5% $CO_2$, and 95% relative humidity atmosphere. The cells were seeded in a 96-well microtiter plate at a density of 100 cells/well and incubated in 100 μl of DMEM/well for 24 h. The culture media were replaced with fresh culture media containing serial dilutions of free naproxen and dendrimers (100–1000 nM/ml naproxen equivalents), and the cells were incubated for 24 h. The culture media were replaced with fresh media. Then, 20 μl of sterile-filtered MTT stock solution in PBS (5 mg/ml) was added to each well, reaching a final MTT concentration of 0.8 mg/ml. After 4 h, the unreacted dye was removed by aspiration. The produced formazan crystals were dissolved in DMSO (100 μl/well). The absorbance was measured using a microplate reader at a wavelength of 570 nm. The cell viability (%) relative to control cells cultured in media without dendrimers was calculated from [*A*]test/[*A*]control×100%, where [*A*]test and [*A*] control are the absorbance values of the wells (with the dendrimers) and control wells (without the dendrimers), respectively. For each sample, the final absorbance was the average value measured from six wells in parallel. The result was presented in Fig. 2.

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