



and concentrated to a pale yellow oil. The crude product was purified by flash column chromatography (gradient elution with 0%, 2%, 4%, 6% acetone/hexanes) to provide the product as a clear oil (10.33 g, 89%). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 3.55 (t, 2H); 3.20 (bm, 4H); 2.20 (dt, 2H); 1.94 (t, 1H); 1.75-1.50 (m, 8H); 1.45 (s, 9H). HRMS (C<sub>15</sub>H<sub>26</sub>ClNO<sub>2</sub>) calcd 288.1730, found 288.1729.

#### Synthesis of Compound 7

Compound 6 (Scheme S1; 10.33 g, 35.88 mmol) in 100 mL EtOAc was treated with HCl gas for 45 min. The reaction mixture was capped and stirred at rt overnight. The white precipitate that formed was filtered, rinsed with Et<sub>2</sub>O and dried under vacuum (7.46 g, 93%). <sup>1</sup>H NMR (D<sub>2</sub>O): δ 3.54 (m, 2H); 2.97 (m, 4H); 2.27 (bs, 1H); 2.17 (m, 2H); 1.75-1.67 (m, 6H); 1.49 (m, 2H). HRMS (C<sub>10</sub>H<sub>18</sub>ClN) calcd 188.1206, found 188.1203.

#### Synthesis of Compound 8

Compound 7 (Scheme S1; 0.68 g, 3.05 mmol) in DCM (10 mL) was cooled to 4°C and was treated with POCl<sub>3</sub> (0.34 mL, 3.66 mmol, 1.2 equiv). Diisopropylethylamine (1.2 mL, 7.02 mmol, 2.3 equiv) was added and the reaction came gradually to rt. After 18h the reaction mixture was quenched with saturated NH<sub>4</sub>Cl solution. The phases were separated and the organic phase was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to an amber oil. The crude product was purified by flash column chromatography (gradient elution 0 to 15% Et<sub>2</sub>O/hexanes) (0.72 g, 78%). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 3.56 (t, 2H); 3.30-3.20 (m, 4H); 2.25 (dt, 2H); 1.97 (t, 1H); 1.97-1.71 (m, 6H); 1.55 (m, 2H). <sup>31</sup>P NMR (CDCl<sub>3</sub>): δ -7.60 (s). HRMS (C<sub>10</sub>H<sub>17</sub>Cl<sub>3</sub>NOP) calcd 304.0192, found 304.0193.

#### Synthesis of Compound 9

Nitrofurfuryl alcohol (0.72 g, 2.36 mmol, 1.05 equiv) in THF (10 mL) was cooled to -78°C. LiHMDS (1M/THF, 2.6 mL, 2.60 mmol, 1.1 equiv) was added by syringe. A solution of Compound 8 (Scheme S1; 0.72 g, 2.36 mmol) in THF (10 mL) was prepared, cooled to -78°C, and was then treated with the alkoxide solution. After 3h at -78°C the reaction mixture was quenched with saturated NH<sub>4</sub>Cl solution and after warming to rt was extracted with EtOAc (3 x 25 mL). The combined organic extracts were dried over Na<sub>2</sub>SO<sub>4</sub> and were concentrated to a dark amber oil. Purification by flash column chromatography (gradient elution 0%, 10%, 20%, 30%, 40% Et<sub>2</sub>O/hexanes) provided the product as a pale yellow oil (0.56 g, 57%). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.27 (d, 1H); 6.71 (d, 1H); 5.13 (m, 2H); 3.52 (t, 2H); 3.18-3.01 (m, 4H); 2.19 (dt, 2H); 1.97-1.93 (t, 1H); 1.77-1.65 (m, 6H); 1.49 (m, 2H). <sup>31</sup>P NMR (CDCl<sub>3</sub>): δ -8.20 (s). HRMS (C<sub>15</sub>H<sub>21</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>5</sub>P) calcd (M + Na) 433.0463, found 433.0468.

#### Synthesis of Compound 10

Tert-butylfarnesol [2] (0.28 g, 1.05 mmol) dissolved in DCM (2 mL) was added to Compound 9 (Scheme S1; 0.43 g, 1.05 mmol) in DCM (11 mL). The mixture was cooled to 4°C and NEt<sub>3</sub> (0.18 mL, 1.26 mmol, 1.2 equiv) and 1M TiCl<sub>4</sub> in CH<sub>2</sub>Cl<sub>2</sub> (0.21 mL, 0.21 mmol, 0.2 equiv) were added. The reaction was capped and kept in the refrigerator for 2 days. Solvent was removed and the residue was applied to a silica gel column and was eluted first with 20% hexanes/DCM, then with 3% Et<sub>2</sub>O/DCM to isolate the product as a yellow oil (0.35 g, 52%). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.26 (d, 1H); 6.64 (d, 1H); 5.21 (t, 1H); 5.16 (m, 2H); 4.98 (d, 2H); 4.72 (m, 2H); 3.55 (t, 2H); 3.07-3.01 (m, 4H); 2.20 (t, 2H); 2.05 (bs, 1H); 1.99-1.94 (m, 8H); 1.76-1.48 (m, 8H); 1.67 (s, 3H); 1.60 (s, 6H); 1.12 (s, 9H). <sup>31</sup>P NMR (CDCl<sub>3</sub>): δ -15.23 (s). HRMS (C<sub>33</sub>H<sub>52</sub>ClN<sub>2</sub>O<sub>6</sub>P) calcd (M + Na)<sup>+</sup> 661.3149, found 661.5154.

#### Synthesis of Compound 3

Compound 10 (Scheme S1; 0.35 g, 0.55 mmol) in *tert*-butanol (5.6 mL) was treated with 3-(3-azidopropylcarbamoyl)propanoic acid [5] (0.11 g, 0.55 mmol, 1.0 equiv). 1.8 mL of 0.08M CuSO<sub>4</sub> was added

to the azide and alkyne followed by sodium ascorbate (0.04 g, 0.22 mmol, 0.4 equiv). The reaction was capped and stirred at rt overnight. Organic solvent was removed by rotovap, 5 mL of EtOAc was added, and the reaction mixture was filtered through celite. The filtrate was diluted with 5 mL of H<sub>2</sub>O and was extracted with EtOAc (3 x 10 mL). Combined extracts were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to a gold-colored syrup. Purification by column chromatography with 10%Et<sub>2</sub>O/DCM followed by 4% MeOH/DCM provided the product as a yellow oil (0.36 g, 76%). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.48 (s, 1H); 7.25 (d, 1H); 6.64 (d, 1H); 5.15-5.06 (m, 3H); 4.96 (d, 2H); 4.68 (m, 2H); 4.36 (t, 2H); 3.51 (t, 2H); 3.22 (m, 2H); 3.06-2.96m, 4H); 2.67 (m, 4H); 2.45 (t, 2H); 2.06-1.95 (m, 10H); 1.74-1.52 (m, 8H); 1.65 (s, 3H); 1.57 (s, 6H); 1.09 (s, 9H). <sup>31</sup>P NMR (CDCl<sub>3</sub>): δ-14.77 (s). HRMS (C<sub>41</sub>H<sub>67</sub>ClN<sub>5</sub>O<sub>11</sub>P) calcd 872.4342, found 872.4358.

### *Synthesis of prodrug FTI dendrimers*

To increase the hydrophilicity of the tert-butylfarnesyl monophosphate prodrugs we conjugated them to PAMAM G4, which contains 64 surface primary amino groups. A generalized dendrimer-prodrug conjugate is outlined in Figure 1 in the main manuscript. Guanidinium groups were incorporated to enhance hydrophilicity and cellular permeability [6]. Prodrugs were connected to the dendrimer via a linker, and unmodified surface amines were capped to minimize cation-induced cytotoxicity. Optimization of guanidinium groups (x), prodrug moieties (y), and capped surface amines (z) was explored through synthesis of various analogs as outlined in Scheme S1.

The number of guanidinium residues per dendrimer-prodrug conjugate was reliably determined using <sup>1</sup>H NMR integration values of the Boc protons of the protected guanidinylated dendrimers 11 (Scheme S1). However, determination of y, the number of prodrug residues per dendrimer (see Figure 1 in the main manuscript), by <sup>1</sup>H NMR integration was less reliable since the corresponding <sup>1</sup>H NMR spectra are complicated and resonances are broad and overlapping. For this reason UV analysis was used to assess prodrug loading of the dendrimer-prodrug conjugates. The extinction coefficient of 3 was measured at 310 nm, and the UV absorbance at 310 nm of the dendrimer-prodrug conjugates in solutions of defined concentrations were used according to the Beer-Lambert Law to determine prodrug loading as μmol/mg conjugate.

### Synthesis of Compound 11

PAMAM-G4 as a 10% solution in MeOH (2 mL, 0.2 g, 0.014 mmol dendrimer, 0.90 mmol NH<sub>2</sub> groups) was treated with N,N'-di-Boc-N''-triflylguanidine (0.09 g, 0.22 mmol) prepared as described by Feichtinger et al. [7]. The reaction mixture was capped and stirred at rt for 18h. Solvent was removed by rotovap and the resulting white solid was coevaporated with DCM (3 x 2mL). A small portion of the crude was purified by dialysis for analysis and the remainder was used without further purification (0.24 g, 93%). The number of di-Boc guanidine groups was assessed to be 17 by integration of the <sup>1</sup>H NMR. <sup>1</sup>H NMR (D<sub>2</sub>O): δ 3.24 (248H); 3.06-2.73 (m, 376H); 2.54 (m, 123H); 2.33 (m, 248H); 1.39 (s, 155H); 1.471.33 (s, 155H).

It should be noted that the number of guanidine moieties per PAMAM-G4 dendrimer could be varied by altering the concentration of N,N'-di-Boc-N''-triflylguanidine, and different batches of compound 11, (Scheme S1) with varied numbers of guanidine moieties, were used in the synthesis of prodrug FTI PAMAM-G4 conjugated dendrimers.

### Synthesis of Compound 12

Compound 11 (Scheme S1; 0.15 g, 0.007 mmol) was treated with 25% TFA/CHCl<sub>3</sub>. The suspension was capped and stirred at rt for 2 days. Solvent was removed and the residue was triturated with CH<sub>2</sub>Cl<sub>2</sub> (3 x 2

mL). Purification by dialysis against pure H<sub>2</sub>O provided the product as a white solid (0.12 g, 57%). <sup>1</sup>H NMR (D<sub>2</sub>O): δ 3.40 (m, 64H); 3.24 (m, 250H); 3.05 (m, 64H); 2.80 (m, 248H); 2.61(m, 124H); 2.37 (s, 248H).

### Synthesis of Compound 13

Compound 11 (Scheme S1; 1.25 g, 0.069 mmol) in 10 mL MeOH was treated with 10 mL 6M HCl/MeOH (1/1). The solution was capped and stirred at rt overnight. Solvents were removed under reduced pressure and the residue was co-evaporated with CH<sub>3</sub>CN (3 x 10 mL). The remaining solid was triturated with DCM (3 x 5 mL) and the material was dried under vacuum to give a white foam (0.83 g, 62%). This material was used without further purification; however, a small portion of the product was further purified by dialysis against pure water to provide a sample for analysis. <sup>1</sup>H NMR (D<sub>2</sub>O): δ 3.44 (m, 245H); 3.30-2.93 (m, 500H); 2.60 (m, 248H).

### Synthesis of Compound 14 (aka IG 6)

Compound 13 (x = 16) (Scheme S1; 0.05 g, 3.2 μmol) in 2 mL MeOH was treated with 0.2 mL Ac<sub>2</sub>O and 0.3 mL NEt<sub>3</sub>. The solution was capped and stirred at rt overnight. Solvents were removed under reduced pressure and the residue was taken up in 1 mL H<sub>2</sub>O and was dialyzed against pure water. After three water changes, the dialysis mixture was acidified with 0.5 mL 6M HCl/MeOH, and the resulting mixture was passed through a column of sephadex LH-20 with MeOH as eluent. Fractions containing only baseline spots on thin-layer chromatography when eluted with 5% MeOH/EtOAc were collected, concentrated, taken up in 3 mL H<sub>2</sub>O and lyophilized to give a white foam (0.04 g, 71%). <sup>1</sup>H NMR (MeOH-d<sub>4</sub>): δ 3.90-3.20 (m, 993H); 2.90 (m, 248H); 2.09 (s, 138H).

### *General procedure for the synthesis of dendrimer prodrug conjugates by conjugation of Compounds 12 and 3 using HOBt/HATU to yield Compounds IG 1, IG 3, IG 5*

Compound 12 (Scheme S1; 0.20 g, 0.0070 mmol dendrimer, 0.17 mmol NH<sub>2</sub> groups) was dissolved in 0.6 mL dry DMF. In a separate vial, compound 3 (0.22 g, 0.26 mmol) and HOBt (0.04 g, 0.29 mmol, 1.1 equiv) in 0.4 mL dry DMF were treated with HATU (0.11 g, 0.29 mmol, 1.1 equiv). The reaction vial was swirled for 45 s and the contents were added to the dendrimer solution. The mixture was stirred under Ar overnight. As much DMF as possible was removed under reduced pressure and the resulting sticky oil was taken up in MeOH (2 mL) and propylene oxide (1 mL) was added. The vial was capped and stirred at rt overnight. The reaction mixture was concentrated under reduced pressure and the residue was passed through a column of sephadex LH-20 with MeOH/DCM (1/1) as eluent. Fractions containing only baseline spots on thin-layer chromatography when eluted with 5% MeOH/EtOAc were collected and concentrated to yield 0.22g of material which still showed some HOBt/HATU by-products by <sup>1</sup>H NMR. Repeated sephadex chromatography was required to provide the pure CF<sub>3</sub>CO<sub>2</sub>H salts. To obtain acetate salts, the partially-purified CF<sub>3</sub>CO<sub>2</sub>H salts were dissolved in H<sub>2</sub>O, shaken with Dowex 1-X10 anion exchange resin, and washed extensively with 1N HCl and then 1N HOAc. After 30 min the resin was filtered and washed with H<sub>2</sub>O (2 x 5 mL). The filtrate was lyophilized to provide the acetate salt as an amber-colored foam of 0.132 g.

*Integration values for the compounds below are based on setting the prodrug nitrofurfuryl vinyl protons at 1.*

**IG 1.** <sup>1</sup>H NMR (MeOH-d<sub>4</sub>): δ 7.78 (s, 1H); 7.44 (d, 1H); 6.82 (d, 1H); 5.23-5.02 (m, 5H); 4.74 (t, 1H); 4.40 (t, 1H); 3.57 (t, 1H); 3.19 (bs, 3H); 2.89-2.60 (m, 9H), 2.49-2.39 (m, 14 H); 2.06-1.93 (m, 10H); 1.71-1.47 (m, 22H); 1.13 (s, 9H). <sup>31</sup>P NMR (MeOH-d<sub>4</sub>): δ -13.94 (s).

**IG 3.** <sup>1</sup>H NMR (MeOH-d<sub>4</sub>): δ 7.77 (s, 1H); 7.44 (d, 1H); 6.82 (d, 1H); 5.23-5.02 (m, 5H); 4.74 (t, 1H); 4.40 (t, 1H); 3.56 (t, 1H); 3.07 (bs, 3H); 2.78-2. (m, 9H), 2.57-2.36 (m, 13H); 2.05-1.95 (m, 10H); 1.65-1.58 (m, 15H); 1.13 (s, 9H). <sup>31</sup>P NMR (MeOH-d<sub>4</sub>): δ -13.71 (s).

**IG 5.** <sup>1</sup>H NMR (MeOH-d<sub>4</sub>): δ 7.76 (s, 1H); 7.43 (d, 1H); 6.80 (d, 1H); 5.23-4.92 (m, 5H); 4.73 (t, 1H); 4.39 (t, 1H); 3.55 (t, 1H); 3.29-2.91 (m, 6 H), 2.69-2.61 (m, 4 H); 2.61-2.48 (m, 12H); 2.05-1.95 (m, 8H); 1.68-1.58 (m, 22H); 1.13 (s, 9H). <sup>31</sup>P NMR (MeOH-d<sub>4</sub>): δ -14.89 (s).

*General procedure for the synthesis of dendrimer prodrug conjugates by conjugation of Compounds 13 and 3 using EDC·HCl to generate Compounds IG 2, IG 4*

Compound **13** (Scheme S1; 0.12 g, 0.0063 mmol dendrimer, 0.29 mmol NH<sub>2</sub> groups) was dissolved in H<sub>2</sub>O (0.75 mL). In a separate vial compound **3** (Scheme S1; 0.16 g, 0.182 mmol) was dissolved in acetone (1 mL) to make a cloudy solution that was added to the dendrimer. To this mixture was added EDC·HCl (0.04 g, 0.22 mmol, 1.2 equivalent) and DIEA (0.07 mL, 0.41 mmol, 2.3 equiv). The cloudy solution was capped and stirred at rt overnight. Solvents were removed under reduced pressure and the residue was taken up in MeOH (1.5 mL) and was treated with Ac<sub>2</sub>O (0.1 mL) and NEt<sub>3</sub> (0.3 mL). After 20 h solvent was removed, the residue was taken up in H<sub>2</sub>O (10 mL) and the solution was purified by dialysis against pure H<sub>2</sub>O. The product was obtained as an amber-colored foam, 0.07 g.

*Integration values for the compounds below are based on setting the prodrug nitrofurfuryl vinyl protons at 1.*

**IG 2.** <sup>1</sup>H NMR (MeOH-d<sub>4</sub>): δ 7.78 (s, 1H); 7.43 (d, 1H); 6.81 (d, 1H); 5.23-5.02 (m, 5H); 4.75 (t, 1H); 4.40 (t, 1H); 3.56 (t, 1H); 3.19 (bs, 3H); 2.87-2.70 (m, 10H), 2.70-2.68 (m, 7H); 2.52-2.43 (m, 20H); 2.06-1.95 (m, 13H); 1.74-1.59 (m, 17H); 1.20 (s, 9H). <sup>31</sup>P NMR (MeOH-d<sub>4</sub>): δ -13.68 (s).

**IG 4.** <sup>1</sup>H NMR (MeOH-d<sub>4</sub>): δ 7.77 (s, 1H); 7.44 (d, 1H); 6.81 (d, 1H); 5.23-5.01 (m, 5H); 4.76 (t, 1H); 4.39 (t, 1H); 3.57 (t, 1H); 3.21 (bs, 4H); 2.86-2.74 (m, 12H), 2.59-2.43 (m, 32H); 2.06-1.95 (m, 26H); 1.74-1.53 (m, 17H); 1.13 (s, 9H). <sup>31</sup>P NMR (MeOH-d<sub>4</sub>): δ -14.37 (s).

*General procedure for synthesis of dendrimer-prodrug conjugates by conjugation of Compounds 13 and 3 using 1,1'-carbonyldiimidazole (CDI)*

Compound **13** (Scheme S1; 0.47 g, 0.024 mmol dendrimer, 1.16 mmol NH<sub>2</sub> groups) was dissolved in H<sub>2</sub>O (9 mL). Imidazole was added to bring the pH to 7. In a separate vial, compound **3** (0.43 g, 0.51 mmol) was dissolved in acetone (9 mL) and was treated with CDI (0.20 g, 1.23 mmol, 2.4 equivalent). A small aliquot was removed and concentrated after 40 min. Examination by <sup>1</sup>H NMR showed a complete downfield shift of the methylene next to the carboxylic acid group in compound **3**. Thus, the activated acid was added to the dendrimer and the cloudy mixture was capped and stirred at rt overnight. Ac<sub>2</sub>O (0.55 mL) was added to the mixture. After 5 min the pH was found to be approximately 5 and imidazole was added to bring the pH to 7. After stirring at rt for 20 h, the acetone was removed and the cloudy aqueous solution was washed multiple times with Et<sub>2</sub>O to remove the bulk of unreacted Ac<sub>2</sub>O and imidazole. The remaining material was passed through a sephadex LH-20 column with MeOH as eluent to provide the product as a brownish-yellow colored foam, 0.40 g.

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