Supplementary Material for Steffensen and Simanek

General. Solvents were reagent grade and used without further purification. Cyanuric Chloride, isonipecotic acid, diethylamine, *p*-toluidine, n-butylamine, and 1-(2-amioethyl)-piperazine (Acros), diisopropylethylamine, 2-(2-aminoethoxy)ethanol, *p*-aminobenzylamine, 4,7,10-trioxa-1,13-tridecanediamine, 1-methylpiperazine, *p*-methylbenzylamine, benzylamine (Aldrich), 4-(aminomethyl)piperidine, 1-acetylpiperazine (TCI), morpholine (Fluka), and piperidine (Advanced ChemTech) were used as received without further purification.

Supplemental Table 1. Measured product distributions.

Trial	Temperature	Amines	Product Ratio
1	RT	**A, B, C**	55:34:11
2	RT	**A, D, F**	98.2:1.2:0.6 [a]
3	RT	**C, D, F**	75:17:8
4	70	**A, D, F**	95.6:3:1.4 [a]
5	70	**C, D, F**	70:19:11
6	70	**D, E, G**	47:35:18
7	70	**D, G, H**	72:28:0 [b]
8	120	**D, G, H**	68:32:0 [b]

[a] Accurate values for these competitions are not required and would be difficult given the product ratios. Better estimates of relative reactivity are provided by comparisons of amines with similar reactivities.

[b] Not observed by ^{1}H NMR or ESI-MS.

Useful Additional Observations on Dendrimer Preparation not Germane to the Manuscript

Aminoalcohols. Aminoalcohols make ideal surface groups for the convergent synthesis of dendrimers based on melamine. The hydroxyl group remains unreactive under normal reaction conditions with a monochlorotriazine – and importantly – trichlorotriazine, which is used subsequently.

Isonipecotic acid. Isonipecotic acid, 4-piperidine carboxylic acid (INP), is a useful focal group, although there may be limitations to its general use. The use of INP on the surface is precluded by side reactions between the carboxylic acid groups and trichlorotriazine, a reaction that produces the active ester. In independent studies, the INP group on the *N*-terminus of peptides has been shown to be more reactive towards the triazine than Pro or *N*-methylglycine on the *N*-terminus or Lys, Arg, and Glu sidechains.[1]

Aminoethylpiperazine. Aminoethylpiperazine (AEP) provides an opportunity to incorporate groups that are cationic at low pHs. The ability to affect the sequestration potential of dendrimers based on AEP by changing the hydrophobicity of the dendrimer interior with pH is now under investigation.

Piperazine derivatives of amino acids. Piperazine derivatives of amino acids have also been shown to react offering the opportunity for the incorporation of chirality into dendrimers based on melamine. The product distributions for these reactions have not been systematically examined. We predict that *C*-terminal AMP groups would proceed more selectively.

Triamines. Reaction with triamines containing two primary and a secondary amine, *i.e.* $(H_2NCH_2CH_2)_2N$, did not proceed to give a major product as predicted by the relative reactivity difference of 1.5 and the polarity of the compounds precluded convenient separation.

[1]Pattarawarapan, M.; Reyes, S.; Xia, Z.; Burgess, K. Texas A&M University. *Manuscript in preparation.*

[2] A pH responsive dendrimer: Krämer, M.; Stumbé, J.F.; Türk, H.; Krause, S.; Komp, A.; Delineau, L.; Prokhorova, S.; Kautz, H.; Haag, R. *Angew. Chem. Int. Ed.* **2002**, *41*, 4252-4256.

Typical Competition Reaction (Trial 1). Piperidine **A** (179 mg, 2.10 mmol), *N*-methyl piperazine **B** (210 mg, 2.10 mmol), and *N*-acetyl piperazine **C** (269 mg, 2.10 mmol) were added to a Parr vessel with THF (10mL). To this solution **2** (200 mg, .700 mmol) was added, and the reaction was left to stir for 18 h. [For those reactions at elevated temperature the solution was brought to ~ 50 °C before addition of **2**, and then the vessel was sealed.] TLC confirmed the absence of starting material **2** for all reactions. The solvent was then removed and the residue was passed through a silica gel column with CH_2Cl_2:MeOH (9:1) to remove excess amines. All fractions containing UV-active compounds that were not positive to ninhydrin staining (those excluding benzylic amines or anilines) were combined and analyzed using ^{1}H NMR.

For Trials 7 and 8, unreacted *p*-toluidine, **H**, could not be separated from the desired UV-active compounds that did not stain with ninhydrin, so NMR was taken of the crude mixture. No large shift of protons corresponding to the addition product of **H** to **2** was observed, and to further rule out reaction, ESI-MS analysis was performed. While the $(M + H)^+$ for the addition of amine **D** (calcd for $C_{15}H_{26}N_6O_2$ 322.41; found 323.22 $(M + H)^+$) and **G** (calcd for

$C_{19}H_{26}N_6O_2$ 370.46; found 371.22 ($M + H^+$)) to **2** were readily observed, no peak for the addition of amine **H** (calcd for $C_{18}H_{24}N_6O_2$ 356.43; nothing found) to **2** was found in either trial.

Experimental

Dendron 1. In a Parr vessel, **4** (300 mg, .30 mmol) was dissolved in 20 mL of CH_2Cl_2:MeOH (1:1) and Hunig's base (0.2 mL, 1.2 mmol) was added. The reaction was cooled in an ice bath before addition of cyanuric chloride (27 mg, 0.15 mmol). The reaction stirred at room temperature for 24 h before isonipecotic acid (INP, 77 mg, .60 mmol) and 1 mL NH_4OH (to solubilize INP) were added. The vessel was sealed and heated at 50 °C for 24 hours. The residue obtained after cooling and evaporation of the solvent was purified by silica gel chromatography using CH_2Cl_2:MeOH (8:2) with 1% NH_4OH to give a white solid (0.28 g, 87%). ^{1}H NMR (500MHz, DMSO) δ=7.64 (br, 8H), 7.17 (br, 8H), 4.65 (br, 4), 4.47 (br, 2H), 4.36 (br, 8H), 3.42 (br, 64H), 3.13 (br, 4H), 2.85 (br, 6H), 2.47 (br, 1H), 1.82 (br, 4H), 1.72 (br, 4H), 1.42 (br, 2H), 1.08 (br, 4H); ^{13}C NMR (125 MHz,): δ=176.52, 166.27, 165.96, 164.78, 164.62, 164.43, 164.35, 138.95, 134.23, 127.68, 119.86, 72.39, 69.54, 60.68, 45.61, 43.17, 42.27, 41.24, 39.92, 36.83, 36.44, 29.96, 28.13; MS (MALDI-TOF): calcd for $C_{99}H_{146}N_{42}O_{18}$: 2212.54; found 2212.73 ($M + H)^+$.

Dimorpholinomonochlorotriazine 2. To a stirred solution of cyanuric chloride (5.00 g, 27.1 mmol) in THF (250 mL) at 0 °C, morpholine (4.75 mL, 54.2 mmol) and Hunig's base (10.4 mL, 59.8 mmol) were added. After stirring for 6 h, the solution was filtered and the solvent was removed. The crude product was recrystallized twice from methanol to give a white solid (6.85 g, 88%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.68 (br t, *J*=4.5 Hz, 8H), 3.60 (t, *J*=4.5 Hz, 8H). ^{13}C NMR (75 MHz,DMSO-d_6) δ 169.42, 164.17, 66.41, 43.63. MS (ESI): calcd for $C_{11}H_{16}ClN_5O_2$: 285.75; found 286.10 ($M + H)^+$.

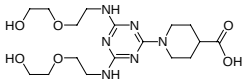
Intermediate 3. To a Parr vessel containing 75 mL THF, 2-(2-aminoethoxy)ethanol (2.20 mL, 21.7 mmol) and Hunig's base (3.80 mL, 21.9 mmol) were added. The mixture was cooled in an ice bath before addition of cyanuric chloride (2.00 g, 10.9 mmol). The reaction was warmed to room temperature and stirred for 24 hrs. To this solution *p*-aminobenzylamine (5.00 mL, 43.5 mmol) was added and the vessel was sealed and heated to 70 °C for 24 h. Upon cooling, the solids were filtered and the solvent was removed by evaporation. The residue was purified by silica gel chromatography using CH_2Cl_2:MeOH (8:2) with 1% NH_4OH to give a yellow oil (4.06 g, 92%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.95 (d, *J*=7.5 Hz, 2H), 6.48 (d, *J*=7.5 Hz, 2H), 4.20 (s, 2H), 3.52 (br, 8H), 3.46 (t, *J*=4.8 Hz, 4H), 3.39 (br t, 4H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.23, 147.70, 128.95, 114.36, 72.70, 69.89, 60.79, 43.52. ^{13}C NMR (75 MHz, CD_3OD) δ 166.95, 147.41, 130.96, 129.41, 116.56, 73.37, 70.91, 62.13, 41.28; MS (FAB): calcd for $C_{18}H_{29}N_7O_4$: 407.48; found 408.23 ($M + H)^+$.

Intermediate 4. Intermediate **3** (3.93 g, 9.64 mmol) was dissolved in 100 mL CH_2Cl_2:MeOH (1:1) followed by addition of Hunig's base (1.6 mL, 9.2 mmol) in a Parr vessel. The solution was cooled in an ice bath before cyanuric chloride (890 mg, 4.84 mmol) was added. The reaction was stirred for 24 h at room temperature before 4-aminomethylpiperidine (2.3 mL, 19 mmol) was added. After stirring for an additional 24 h, the solvent was removed and the residue was purified by silica gel chromatography using CH_2Cl_2:MeOH (8:2-7:3) with 1% NH_4OH to give a white solid (4.11g, 85%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.62 (d, 4H), 7.15 (d, 4H), 4.66 (d, 2H), 4.35 (s, 4H), 3.40 (br m, 32H), 2.81 (br, 2H), 2.43 (br d, 2H), 1.74(br d, 2H), 1.55 (br, 1H), 1.01 (br q, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 166.24, 164.88, 164.64, 139.15, 134.62, 128.11, 120.23, 72.65, 69.82, 60.80, 47.33, 43.61, 40.00, 30.07; MS (ESI): calcd for $C_{45}H_{69}N_{19}O_8$: 1004.17; found 1004.57 ($M + H)^+$.

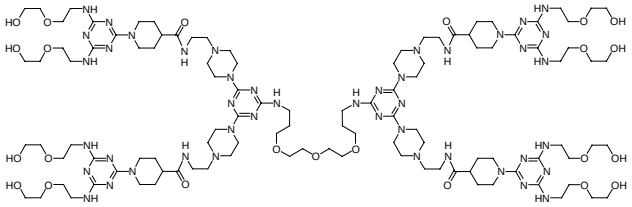
Tetraamine core 5. To a 100 mL THF:MeOH solution (9:1), 4, 7, 10-trioxa-1,13-tridecanediamine (598 mg, 2.72 mmol) and Hunig's base (3.00 mL, 17.3 mmol) were added. Upon cooling in an icebath, cyanuric chloride (1.00 g, 5.43 mmol) was added. After 5 min the TLC showed consumption of cyanuric chloride and a single UV active spot. No free amines were detected by ninhydrin staining. The reaction was then cooled in a dry ice/isopropanol bath and 1-(2-aminoethyl)piperazine (4.20 g, 32.6 mmol) was added drop-wise. The reaction was left to stir in the ice bath for 1 hour, then was slowly warmed to room temperature over 4 h. After stirring for an additional 12 h, the solids were removed by filtration and the solvent was

evaporated. The residue was purified by silica gel chromatography using CH_2Cl_2:MeOH (8:2) with 3% NH_4OH to give a white solid (2.41 g, 76%). ^{1}H NMR (500 MHz, $CDCl_3$): δ=3.61 (br, 16H), 3.52 (m, 4H), 3.47 (m, 4H), 3.42 (t, *J*=6 Hz, 4H), 3.30 (t, *J*=6.5 Hz, 4H), 2.66 (t, *J*=6.5 Hz, 8H), 2.33 (m, 24H), 1.70 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$): δ= 165.95, 164.70, 70.30, 69.92, 69.03, 60.13, 52.90, 42.64, 37.83, 37.71, 37.64, 29.98, 29.33; MS (ESI): calcd. for $C_{40}H_{78}N_{20}O_3$: 887.20; found: 887.67 $(M+H)^+$.

Supplementary Intermediate A. Cyanuric chloride (2.00 g, 10.9 mmol) was dissolved in 75 mL of 9:1 THF:MeOH in a Parr vessel. After cooling in an ice bath, 2-(2-aminoethoxy)ethanol (2.20 mL, 21.7 mmol) and Hunig's base (6.0 mL, 34.5 mmol) were added. The reaction was stirred at RT for 8 hours before isonipecotic acid (1.4 g, 10.9 mmol) and 5 mL aqueous NH_4OH were added. The reaction was heated at 60 °C for 12 hours. The residue, obtained after filtration of the reaction mixture and evaporation of the solvent, was washed with water and hot CH_3CN to give a white solid (4.32g, 96%). ^{1}H NMR (300 MHz, CD_3OD) δ 4.58 (br, 2H), 3.67 (m, 4H), 3.60 (m, 4H), 3.55 (m, 8H), 2.98 (br t, 2H), 2.53 (t, 1H), 1.91 (m, 2H), 1.57 (m, 2H) ; ^{13}C NMR (75 MHz, CD_3OD) δ 179.45, 165.34, 73.44, 70.88, 62.23, 43.86, 43.06, 41.44, 29.47; MS (ESI): calcd. for $C_{17}H_{30}N_6O_6$: 414.47: found: 415.33 $(M+H)^+$.



Supplementary Dendrimer B. Intermediate **A** (350 mg, .845 mmol) was dissolved in DMF (8 mL) and cooled in an ice bath. PyBOP (450 mg, .865 mmol) and Hunig's base (0.2 mL, 1.2 mmol) were added to the solution, followed by **5** (184 mg, .207 mmol) dissolved in DMF (5 mL). The solution was stirred at RT for 18 hrs. The solvent was removed, and the residue was purified by silica gel chromatography with DCM:MeOH (8:2) and 2% NH_4OH to yield a white solid (418 mg, 82% yield).^{1}H NMR (500 MHz, CD3OD) δ 4.74 (br, 8H), 3.75 (br, 16H), 3.66 (m, 16H), 3.62 (m, 4H), 3.58 (m, 20H), 3.53 (m, 36H), 3.39 (t, *J*=6.5 Hz, 4H), 3.35 (t, *J*=6.5 Hz, 8H), 2.80 (br t, 8H), 2.52 (m, 24H), 2.42 (m, 4H), 1.76 (m, 12H), 1.59 (m, 8H). ^{13}C NMR (75 MHz, CD_3OD) δ 176.62, 166.85, 166.28, 165.61, 165.15, 72.96, 71.12, 70.75, 70.53, 69.82, 61.77, 57.71, 53.56, 44.04, 43.38, 40.90, 38.53, 36.64, 30.17, 29.13; MS (MALDI-TOF): calcd. for $C_{108}H_{190}N_{44}O_{23}$: 2473.00; found: 2472.74 $(M+H)^+$.



Supplementary Dendrimer C: Dendron **1** (200 mg, .090 mmol) was dissolved in DMF and the reaction was cooled to 0 °C. To the cooled solution HATU (100 mg, .263 mmol) and Hunig's base (0.1 ml, .575 mmol) were added, and the solution turned yellow. Next, 4,7,10-trioxa-1,13-tridecanediamine (.01 mL, .045 mmol), was added and the reaction left to stir and warm to RT overnight. To hydrolyze any esters that may have formed 0.5 mL of 3 M NaOH solution was added and stirred for 1 hour. The product was precipitated from solution by addition of *i*-PrOH, and isolated by filtration. MALDI-TOF showed evidence for the presence of dimer (calcd. for $C_{208}H_{312}N_{86}O_{37}$: 4609.36; found: 4612.42) and addition of the diamine to **1**, but due to the polarity of the compounds a satisfactory separation of the product from the impurities could not be obtained.