Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information for:

2021 Pioneering Investigators issue: Guanidium-Functionalized Cationic Molecular Umbrellas as Antibacterial Agents

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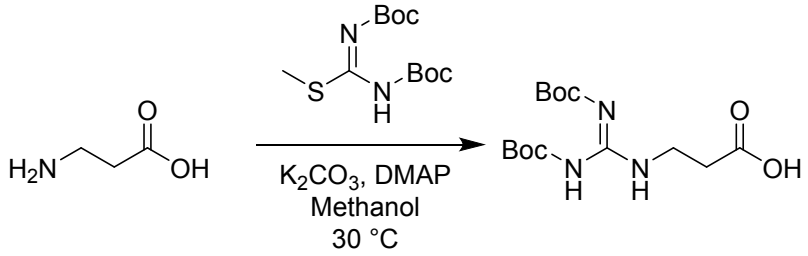
1. Synthesis Procedure, NMR and ESI MS

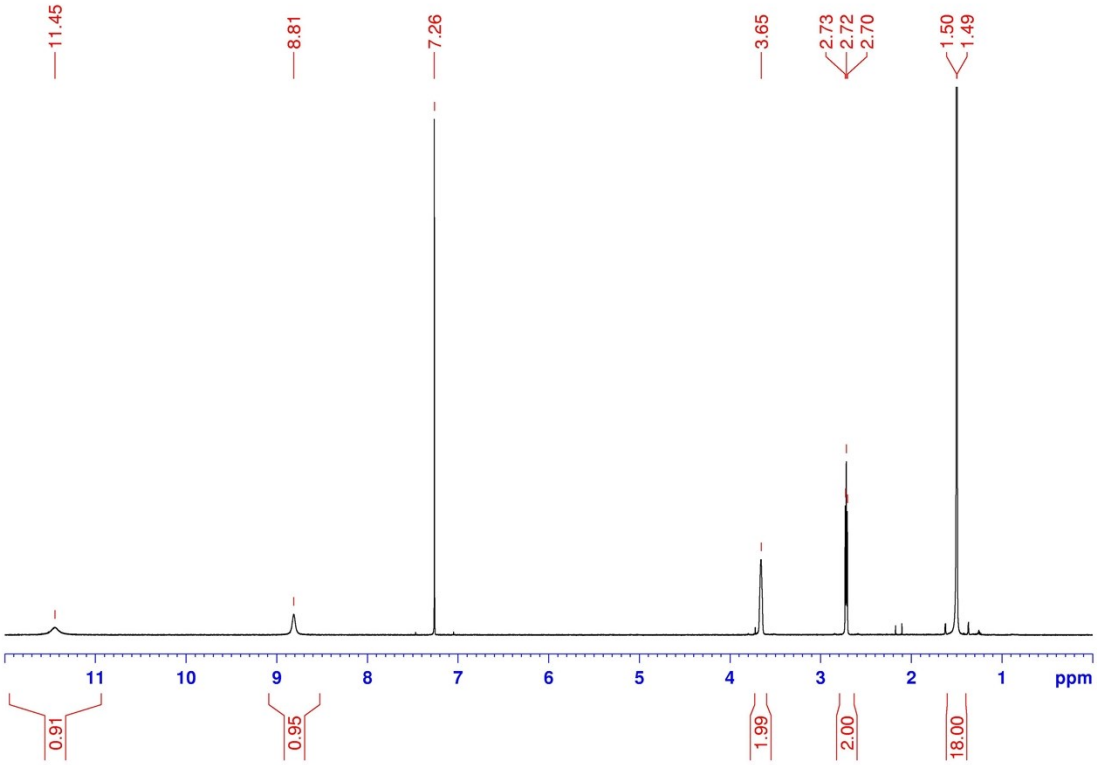
The detailed synthesis of Acetonide-(2,2-bis(hydroxymethyl)propionic Acid, G1 and G2 dendron series with acetonide protecting groups and deprotection of acetonide protecting groups, please refer to the procedure previously described.[1]

Synthesis of 3-(DiBoc)Guanidino Propanoic Acid



Scheme S1: Synthetic Route of 3-(DiBoc)Guanidino Propanoic Acid.

3-(DiBoc)Guanidino Propanoic Acid was synthesized as previously reported.[2] *N*,*N*'-Bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea (1 *equiv.*, 2.00 g, 6.89 mmol) and *β*-alanine (2 *equiv.*, 1.23 g, 13.8 mmol) were dissolved in methanol. K_2CO_3 (1 *equiv.*, 0.952 g, 6.89 mmol) and DMAP (0.1 *equiv.*, 0.084 g, 0.69 mmol) were added to the above solution. The solution was stirred at 30 °C until total conversion of *N*,*N*'-Bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea (tracked with TLC using 1: 9 ethyl acetate: hexane *vol.* ratio). Methanol was evaporated and the residue was dissolved in diethyl ether (120 mL) and washed with 0.2 M acetic acid (150 mL). The water phase was extracted again with 40 mL diethyl ether. The organic phases were combined and washed with brine and dried over Na_2SO_4. The solvent was finally evaporated to afford white powder. Yield 91%. ^{1}H NMR ($CDCl_3$): 1.49 (s, 9H, Boc), 1.50 (s, 9H, Boc), 2.72 (t, 2H, -$COCH_2CH_2$-, J = 5.9 Hz), 3.65 (s, 2H, -$COCH_2CH_2$-), 8.81 (s, 1H, -CH_2NHC-) and 11.45 (s, 1H, -NHBoc). ^{13}C NMR ($CDCl_3$): 28.01, 28.15, 35.66, 36.36, 80.15, 83.79, 152.93, 156.55, 162.38, 174.30.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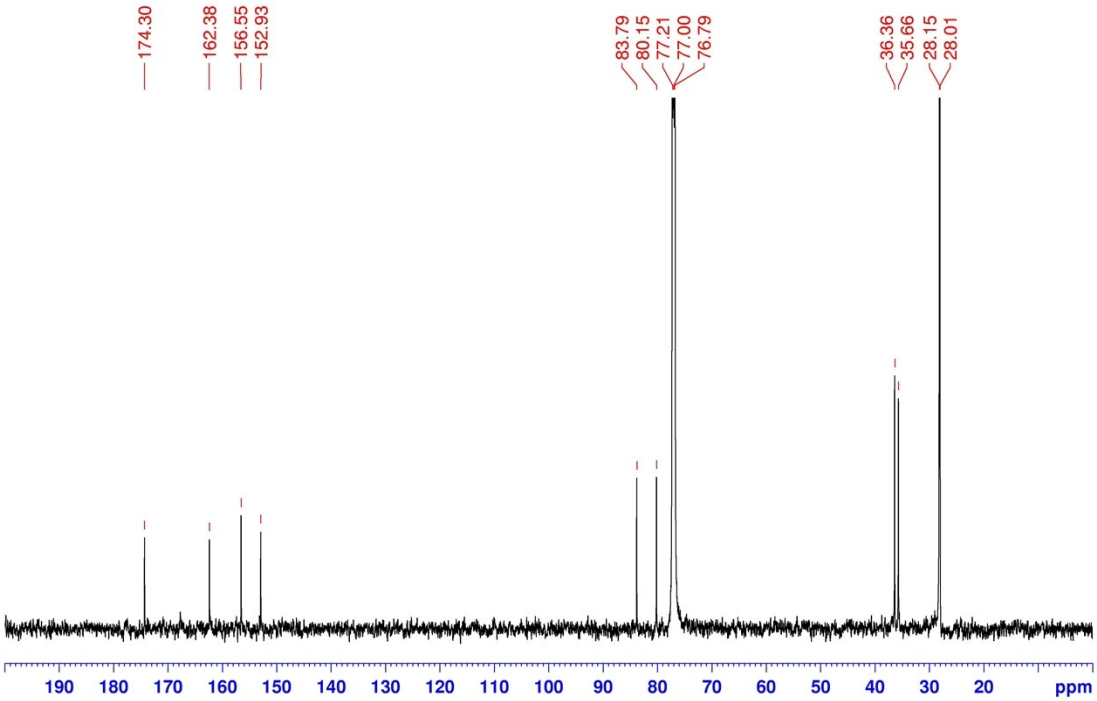
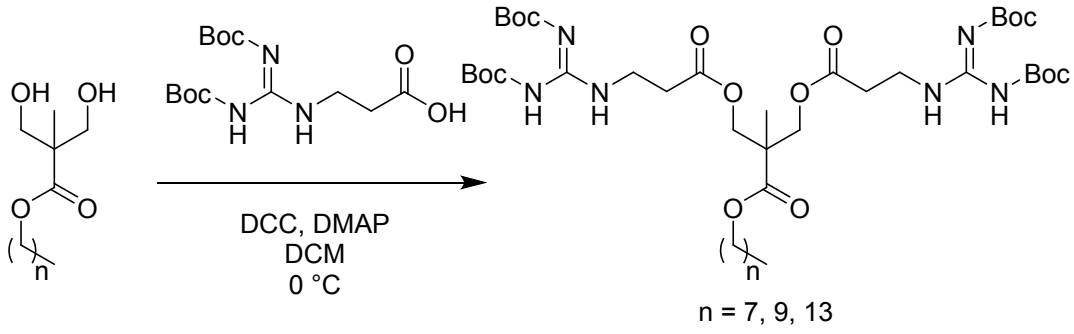


Figure S1: ^{1}H NMR (upper) and ^{13}C NMR (lower) Spectra of 3-(DiBoc)Guanidino Propanoic Acid in $CDCl_3$.

Synthesis of G1-3-(DiBoc)Guanidino Propanoic Acid Dendron Series

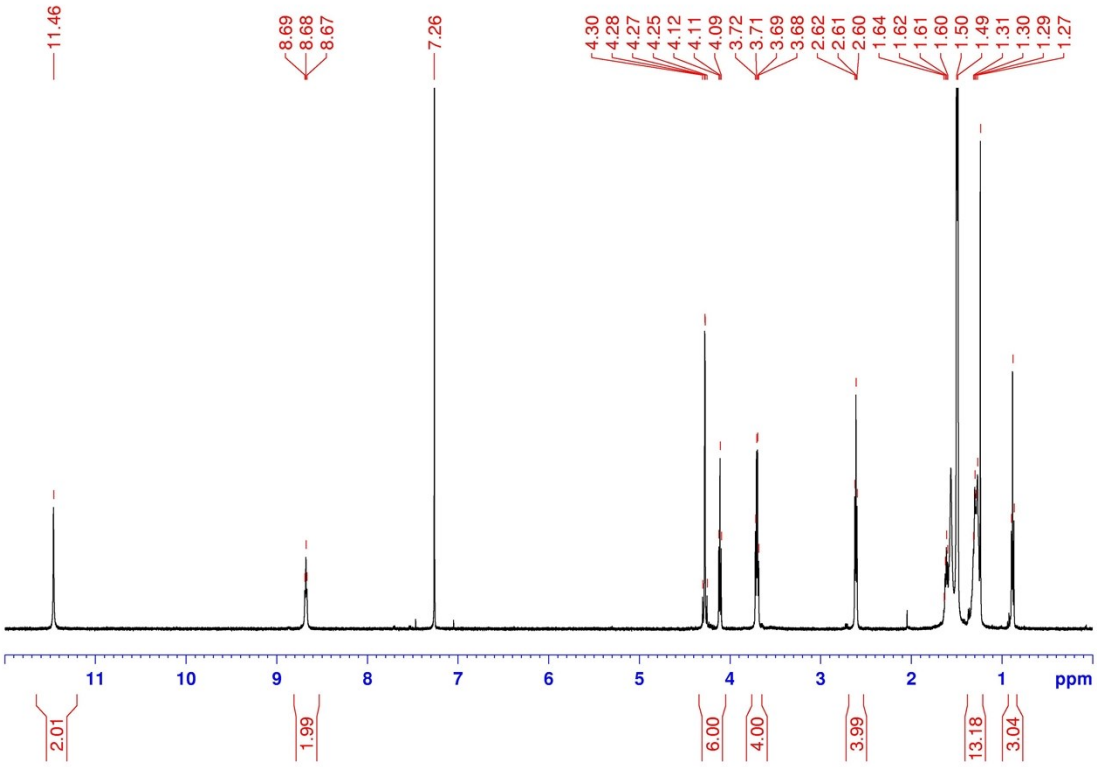


Scheme S2: Synthetic Route of G1-3-(DiBoc)Guanidino Propanoic Acid Dendron Series.

General Procedure:

3-(DiBoc)Guanidino Propanoic Acid (0.600 g, 18.1 mmol), G1-OH dendron (0.75 mmol) and DMAP (0.037 g, 0.30 mmol) were dissolved in 2.5 mL dry CH_2Cl_2. DCC (0.602 g, 1.80 mmol) was dissolved in 2.5 mL CH_2Cl_2 and dropwise added into the above solution stirred in an ice bath. After the addition of DCC, the reaction was gradually allowed to warm to room temperature and stirred overnight. The urea was filtered and the filtrate was concentrated. The concentrated crude product was then concentrated and purified using column chromatography (eluent: ethyl acetate: hexane *vol.* ratio 1: 2).

G1-3-(DiBoc)Guanidino Propanoic Acid-Octanol: viscous oil. Yield: 70%. ^{1}H NMR ($CDCl_3$): 0.88 (t, 3H, -CH_2CH_3, J = 7.1 Hz), 1.24 (s, 3H, -CCH_3, 1st generation), 1.27 – 1.31 (m, 10H, -$OCH_2CH_2(CH_2)_5CH_3$), 1.49 (s, 18H, Boc group), 1.50 (s, 18H, Boc group), 1.61 (m, 2H, -OCH_2CH_2-, J = 6.5 Hz), 2.61 (t, 4H, -$COOCH_2CH_2NH$-, J = 6.2 Hz), 3.70 (q, 4H, -$COOCH_2CH_2NH$-, J = 6.2 Hz), 4.11 (t, 2H, -OCH_2CH_2-, J = 6.8 Hz), 4.28 (m, 4H, -CH_2O-), 8.68 (t, 2H, -$COOCH_2CH_2NH$-, J = 5.8 Hz), 11.46 (s, 2H, -NHBoc). ^{13}C NMR ($CDCl_3$): 14.07, 17.78, 22.60, 25.78, 28.05, 28.29, 28.48, 29.13, 31.75, 33.70, 35.98, 46.23, 65.43, 65.66, 79.28, 83.08, 152.97, 156.19, 163.53, 171.59, 172.58.



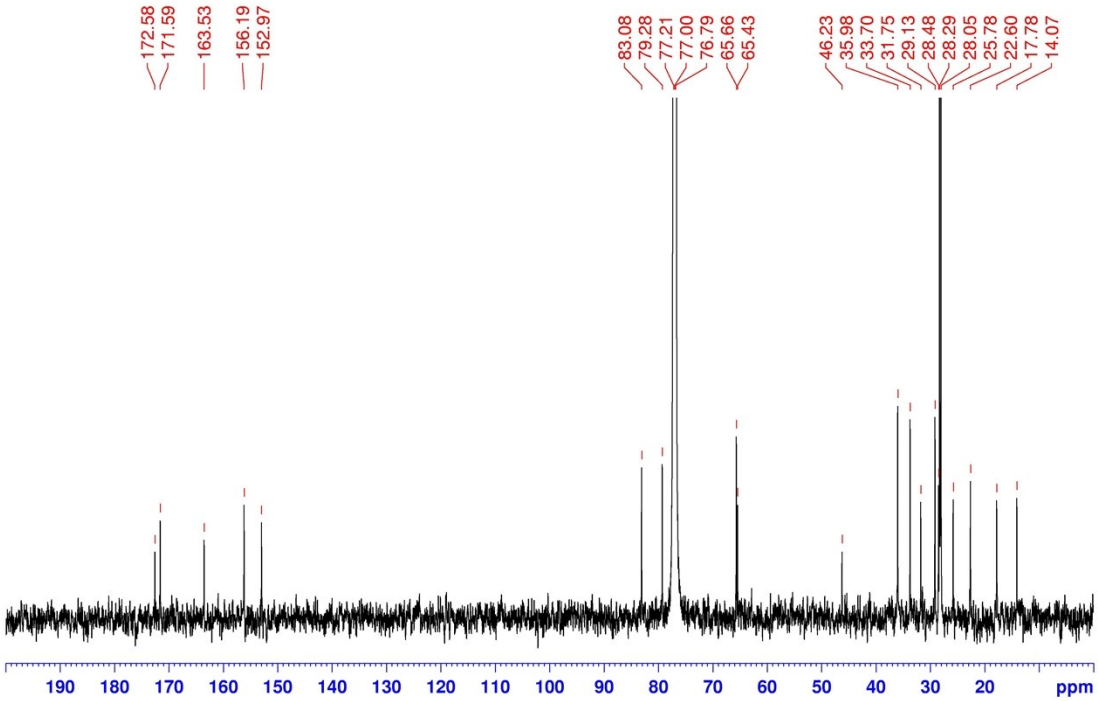
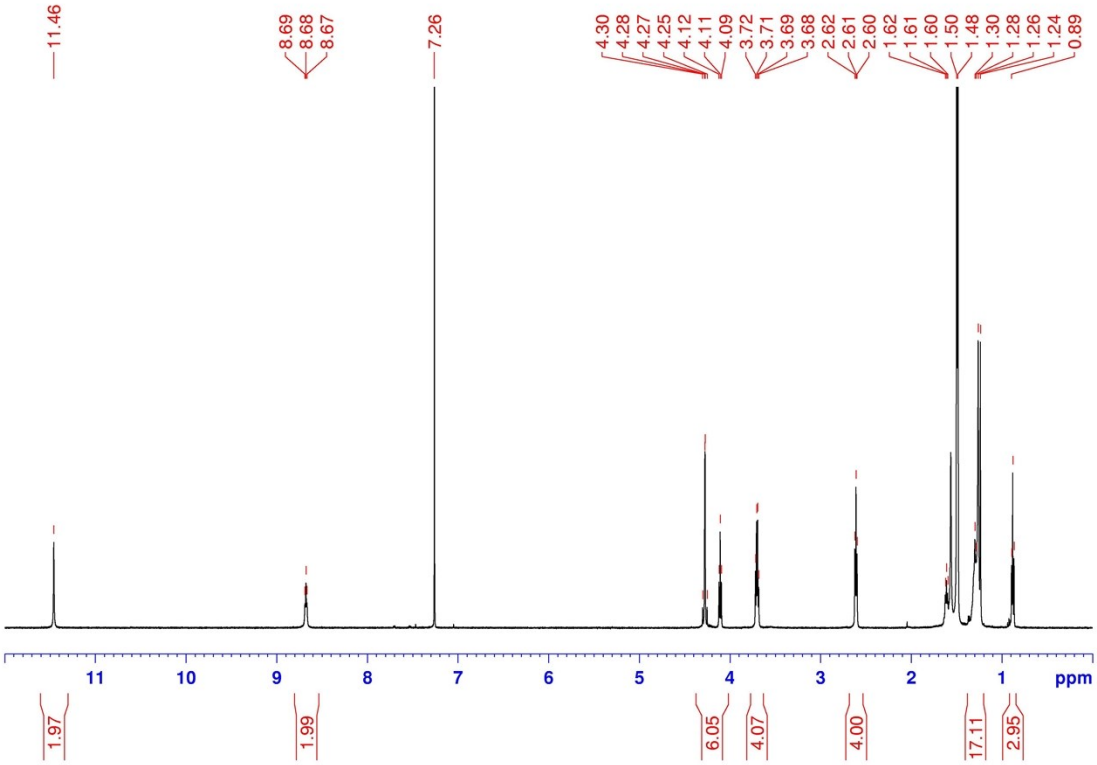


Figure S2: ^{1}H (upper) and ^{13}C NMR (lower) Spectra of G1-3-(DiBoc)Guanidino Propanoic Acid-Octanol in $CDCl_3$.

G1-3-(DiBoc)Guanidino Propanoic Acid-Decanol: viscous oil. Yield: 64%. ^{1}H NMR ($CDCl_3$): 0.88 (t, 3H, -CH_2CH_3, J = 7.2 Hz), 1.24 (s, 3H, -CCH_3, 1st generation), 1.26 – 1.30 (m, 14H, -$OCH_2CH_2(CH_2)_7CH_3$), 1.48 (s, 18H, Boc group), 1.50 (s, 18H, Boc group), 1.61 (m, 2H, -OCH_2CH_2-, J = 7.2 Hz), 2.61 (t, 4H, -$COOCH_2CH_2NH$-, J = 6.2 Hz), 3.70 (q, 4H, -$COOCH_2CH_2NH$-, J = 6.1 Hz), 4.11 (t, 2H, -OCH_2CH_2-, J = 6.8 Hz), 4.28 (m, 4H, -CH_2O-), 8.68 (t, 2H, -$COOCH_2CH_2NH$-, J = 5.9 Hz), 11.46 (s, 2H, -NHBoc). ^{13}C NMR ($CDCl_3$): 14.10, 17.78, 22.66, 25.79, 28.05, 28.29, 28.48, 29.18, 29.27, 29.50, 31.87, 33.70, 35.98, 46.23, 65.44, 65.65, 79.28, 83.08, 152.97, 156.18, 163.53, 171.59, 172.57.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1.62
1.61
1.60
1.50
1.48
1.30
1.28
1.26
1.24
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10
9
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2.95

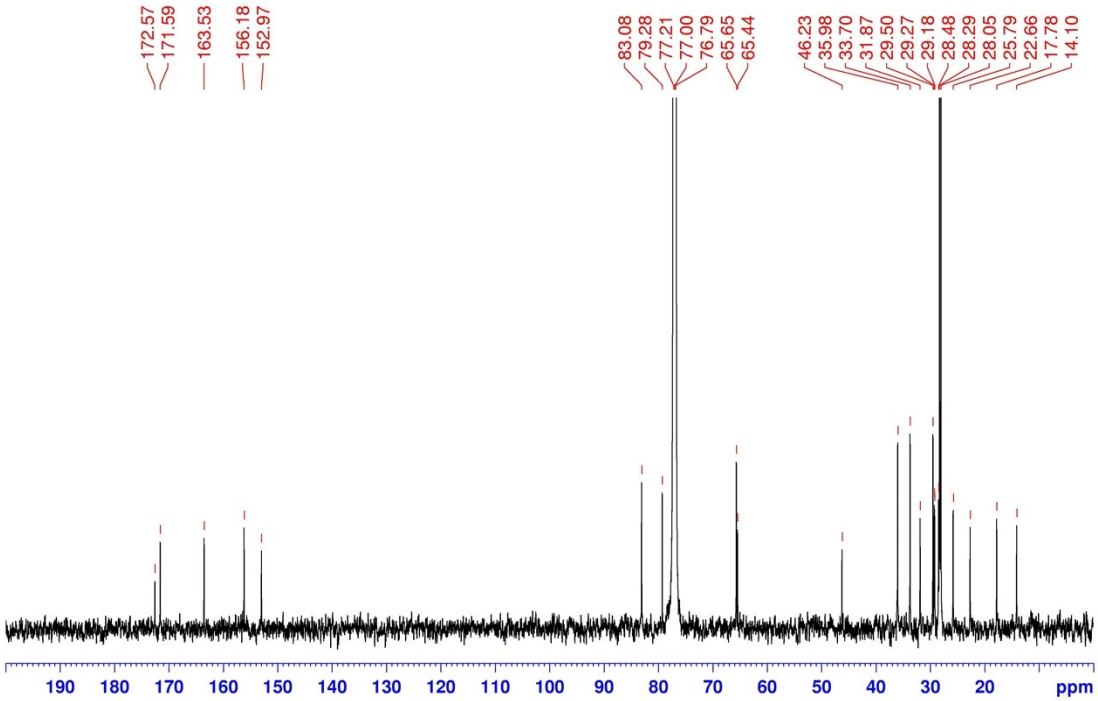
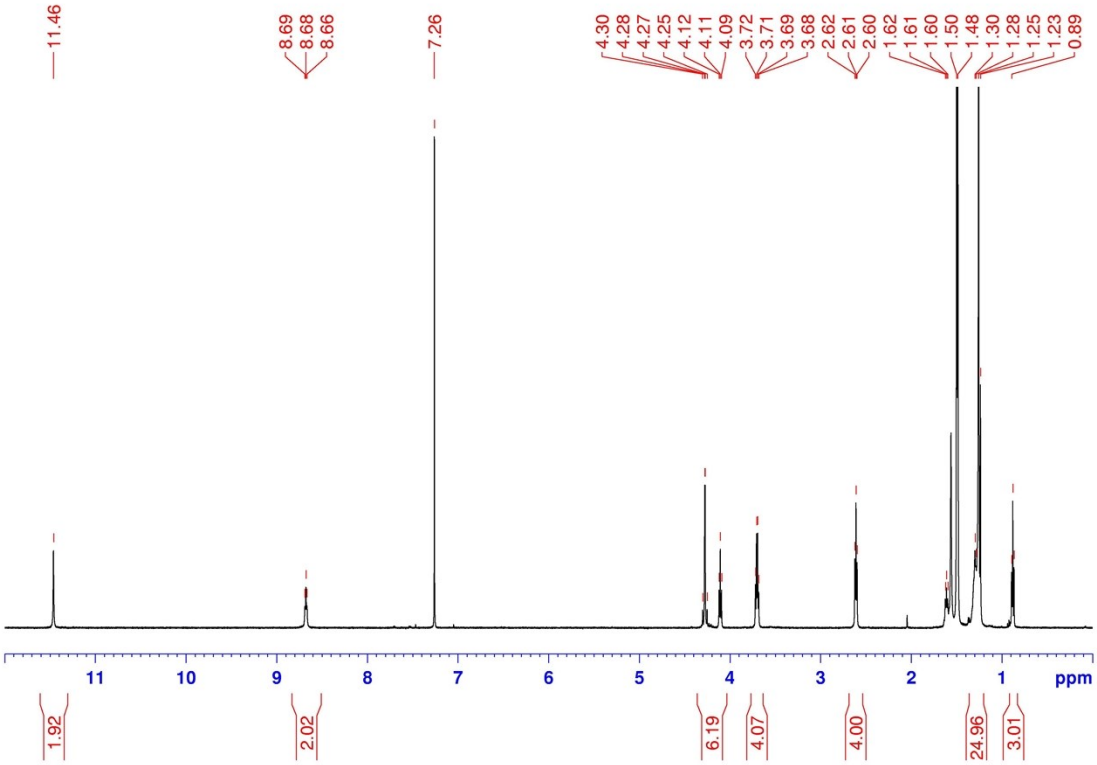


Figure 3: ^{1}H (upper) and ^{13}C NMR (lower) Spectra of G1-(DiBoc)Guanidino Propanoic Acid-Decanol in $CDCl_3$.

G1-(DiBoc)Guanidino Propanoic Acid-Tetradecanol: viscous oil. Yield: 68%. ^{1}H NMR ($CDCl_3$): 0.88 (t, 3H, -CH_2CH_3, J = 7.1 Hz), 1.23 (s, 3H, -CCH_3, 1st generation), 1.25 – 1.30 (br, 22H, -$(CH_2)_{11}CH_2CH_3$), 1.48 (s, 18H, Boc group), 1.50 (s, 18H, Boc group), 1.61 (m, 2H, -OCH_2CH_2-, J = 6.4 Hz), 2.61 (t, 4H, -$COOCH_2CH_2NH$-, J = 6.2 Hz), 3.70 (q, 4H, -$COOCH_2CH_2NH$-, J = 6.0 Hz), 4.11 (t, 2H, -OCH_2CH_2-, J = 6.8 Hz), 4.28 (m, 4H, -CH_2O-), 8.68 (t, 2H, -$COOCH_2CH_2NH$-, J = 6.1 Hz), 11.46 (s, 2H, -NHBoc). ^{13}C NMR ($CDCl_3$): 14.10, 17.78, 22.68, 25.79, 28.05, 28.29, 28.49, 29.19, 29.35, 29.51, 31.97, 33.70, 35.98, 46.23, 65.44, 65.65, 79.28, 83.07, 152.97, 156.18, 163.53, 171.59, 172.57.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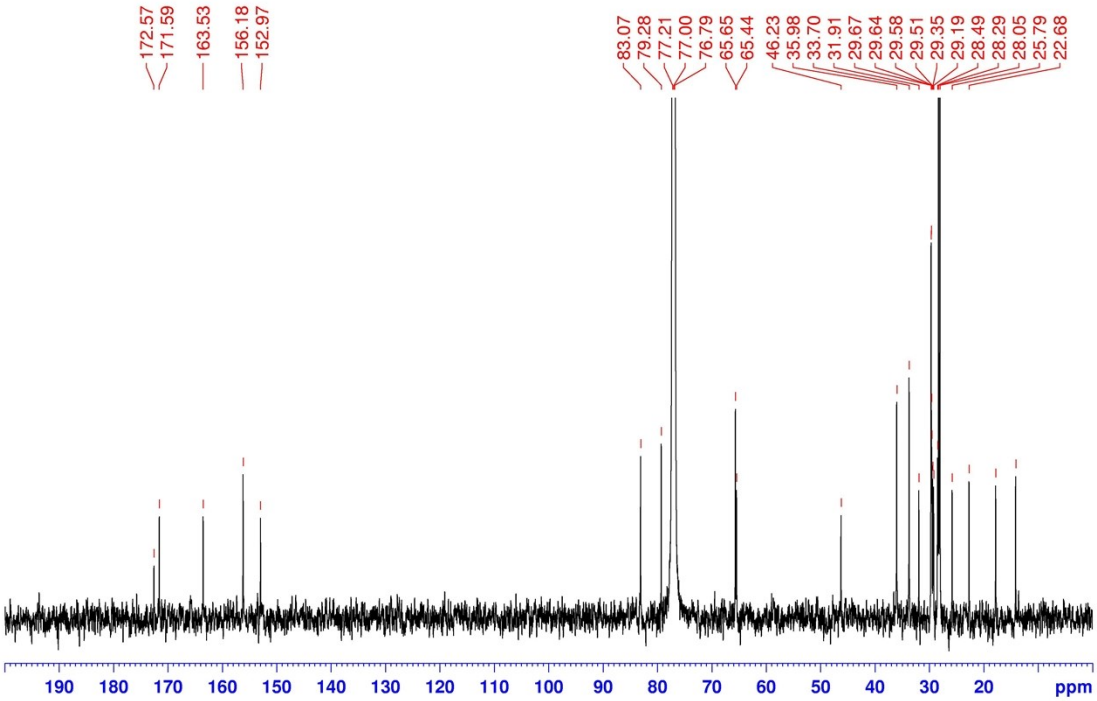
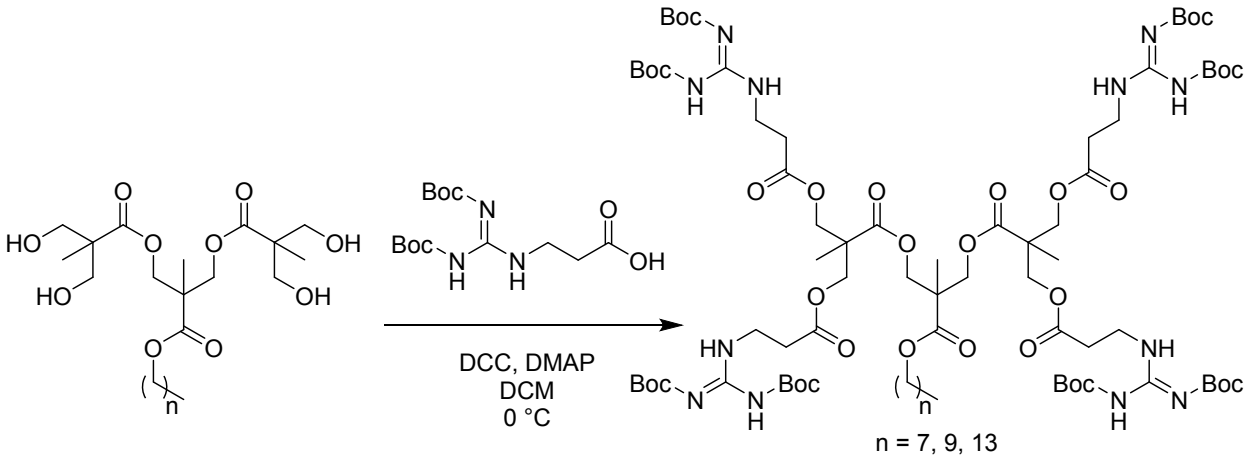


Figure S4: ^{1}H (upper) and ^{13}C NMR (lower) Spectra of G1-(DiBoc)Guanidino Propanoic Acid-Decanol in $CDCl_3$.

Synthesis of* G2-(DiBoc)Guanidino Propanoic Acid *Dendron Series:



Scheme S3: Synthetic Route of G2-(DiBoc)Guanidino Propanoic Acid Dendron Series.

General Procedure:

(DiBoc)Guanidino Propanoic Acid (0.500 g, 1.50 mmol), G2-OH dendron (0.300 mmol) and DMAP (0.031 g, 0.25 mmol) were dissolved in 2 mL dry CH_2Cl_2. 1.5 mmol DCC (0.310 g) was dissolved in 2 mL CH_2Cl_2 and dropwise added into the above solution stirred in an ice bath. After the addition of DCC, the reaction was gradually allowed to warm to room temperature and stirred overnight. The urea was filtered and the filtrate was concentrated. The concentrated crude product was then concentrated and purified using column chromatography (eluent: ethyl acetate: hexane *vol.* ratio 2: 3).

G2-(DiBoc)Guanidino Propanoic Acid-Octanol: solid. Yield: 56%. ^{1}H NMR ($CDCl_3$): 0.88 (t, 3H, -CH_2CH_3, J = 7.1 Hz), 1.22 (s, 6H, -CCH_3, 2nd generation), 1.23 (s, 3H, -CCH_3, 1st generation), 1.26 – 1.30 (m, 10H, -$OCH_2CH_2(CH_2)_5CH_3$), 1.48 (s, 36H, Boc group), 1.49 (s, 36H, Boc group), 1.62 (m, 2H, -OCH_2CH_2-, J = 7.4 Hz), 2.62 (t, 8H, -$COOCH_2CH_2NH$-, J = 6.3 Hz), 3.71 (q, 8H, -$COOCH_2CH_2NH$-, J = 6.1 Hz), 4.09 (t, 2H, -OCH_2CH_2-, J = 6.8 Hz), 4.24 (m, 12H, -CH_2O-), 8.67 (t, 4H, -$COOCH_2CH_2NH$-, J = 5.7 Hz), 11.46 (s, 4H, -NHBoc). ^{13}C NMR ($CDCl_3$): 14.08, 17.59, 17.73, 22.61, 25.84, 28.05, 28.29, 28.49, 29.15, 31.75, 33.61, 35.96, 46.34, 46.57, 65.37, 65.63, 65.79, 79.25, 83.04, 152.95, 156.17, 163.53, 171.56, 171.85, 172.00.

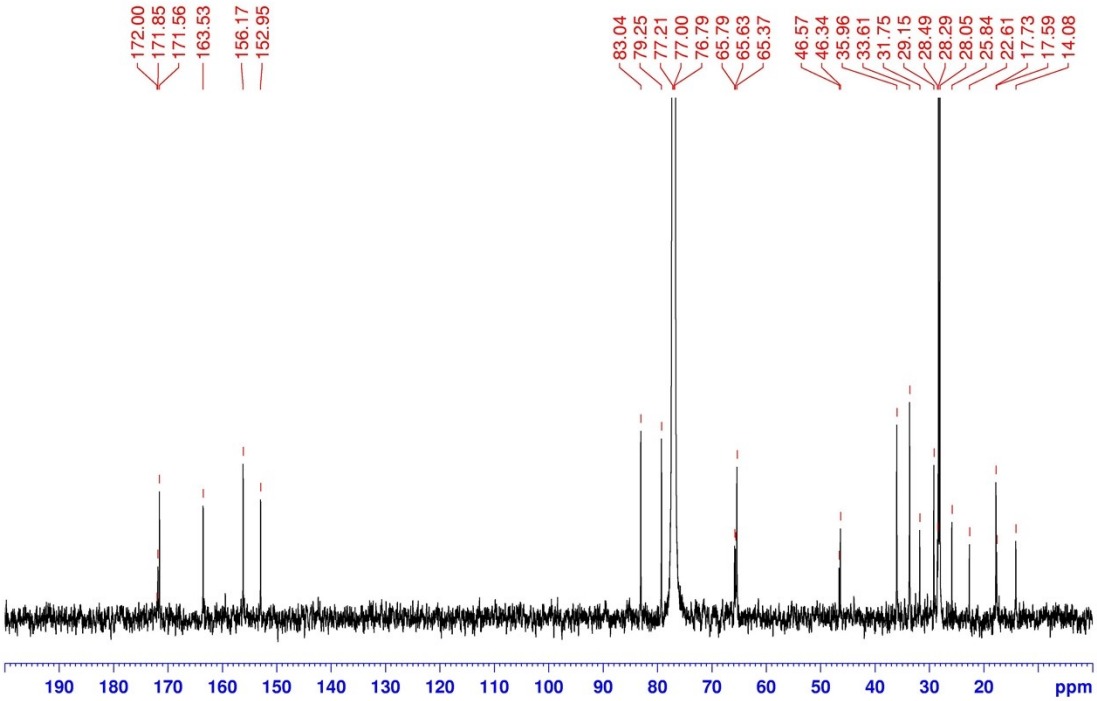


Figure S5: ^{1}H NMR (upper) and ^{13}C NMR (lower) Spectra of G2-(DiBoc)Guanidino Propanoic Acid-Octanol in $CDCl_3$.

G2-(DiBoc)Guanidino Propanoic Acid-Decanol: solid. Yield: 58%. ^{1}H NMR ($CDCl_3$): 0.88 (t, 3H, -CH_2CH_3, J = 7.1 Hz), 1.23 (s, 6H, -CCH_3, 2nd generation), 1.24 (s, 3H, -CCH_3, 1st generation), 1.26 – 1.31 (m, broad, 14H, -$(CH_2)_7CH_2CH_3$), 1.48 (s, 36H, Boc group), 1.49 (s, 36H, Boc group), 1.62 (m, 2H, -OCH_2CH_2-, J = 7.4 Hz), 2.62 (t, 8H, -$COOCH_2CH_2NH$-, J = 6.3 Hz), 3.71 (q, 8H, -$COOCH_2CH_2NH$-, J = 6.1 Hz), 4.09 (t, 2H, -OCH_2CH_2-, J = 6.8 Hz), 4.24 (m, 12H, -CH_2O-), 8.67 (t, 4H, -$COOCH_2CH_2NH$-, J = 5.6 Hz), 11.46 (s, 4H, -NHBoc). ^{13}C NMR ($CDCl_3$): 14.10, 17.59, 17.73, 22.66, 25.85, 28.05, 28.29, 28.51, 29.22, 29.28, 29.51, 31.87, 33.61, 35.97, 46.33, 46.57, 65.35, 65.64, 65.79, 79.25, 83.04, 152.95, 156.18, 163.53, 171.57, 171.85, 172.04.

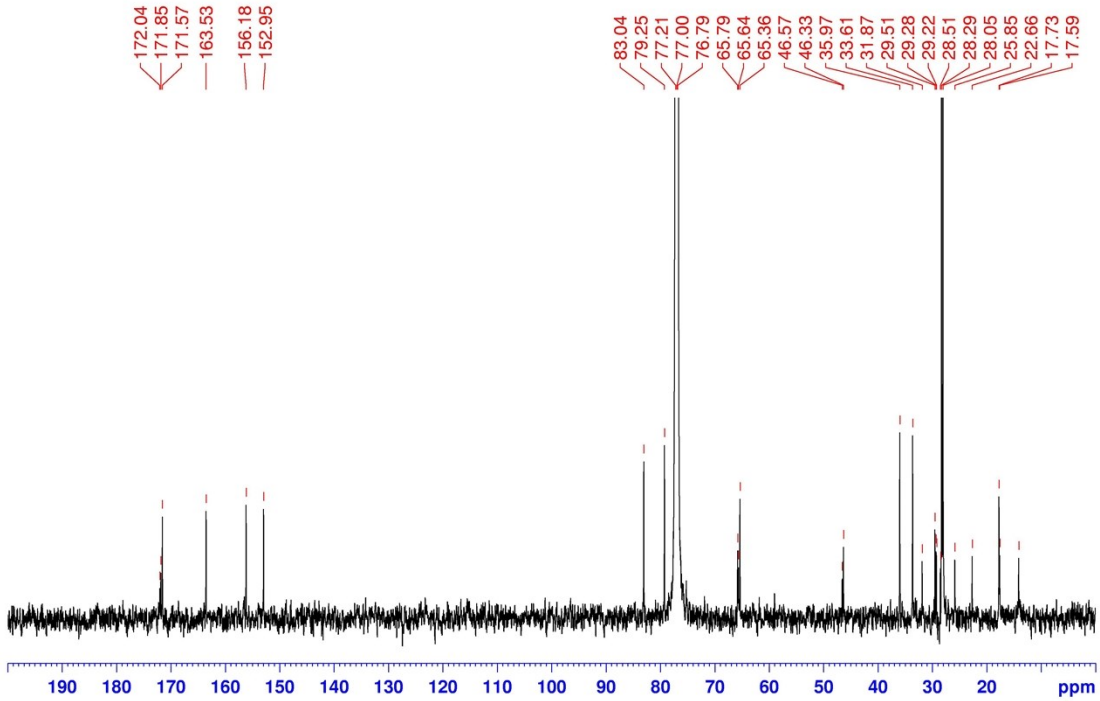


Figure S6: ^{1}H (upper) and ^{13}C NMR (lower) Spectra of G2-(DiBoc)Guanidino Propanoic Acid-Decanol in $CDCl_3$.

G2-(DiBoc)Guanidino Propanoic Acid-Tetradecanol: solid. Yield: 53%. ^{1}H NMR ($CDCl_3$): 0.88 (t, 3H, -CH_2CH_3, J = 7.1 Hz), 1.23 (s, 6H, -CCH_3, 2nd generation), 1.24 (s, 3H, -CCH_3, 1st generation), 1.25 – 1.31 (m, 22H, -$OCH_2CH_2(CH_2)_{11}CH_3$), 1.48 (s, 36H, Boc group), 1.49 (s, 36H, Boc group), 1.62 (m, 2H, -OCH_2CH_2-, J = 7.2 Hz), 2.62 (t, 8H, -$COOCH_2CH_2NH$-, J = 6.3 Hz), 3.71 (q, 8H, -$COOCH_2CH_2NH$-, J = 6.1 Hz), 4.09 (t, 2H, -OCH_2CH_2-, J = 6.8 Hz), 4.24 (m, 12H, -CH_2O-), 8.67 (t, 4H, -$COOCH_2CH_2NH$-, J = 5.6 Hz), 11.46 (s, 4H, -NHBoc). ^{13}C NMR ($CDCl_3$): 14.11, 17.59, 17.73, 22.68, 25.86, 28.05, 28.29, 28.52, 29.24, 29.35, 29.53, 29.62, 29.66, 29.68, 31.91, 33.61, 35.96, 46.33, 46.56, 65.36, 65.64, 65.78, 79.25, 83.03, 152.95, 156.18, 163.53, 171.56, 171.85, 172.03.

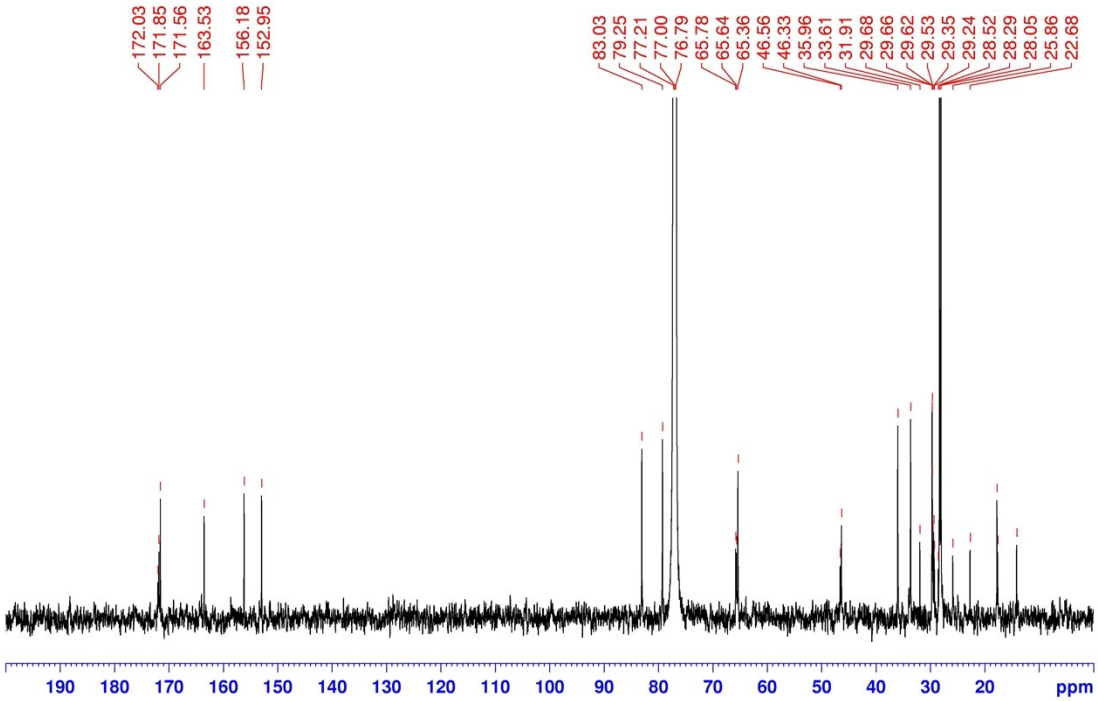
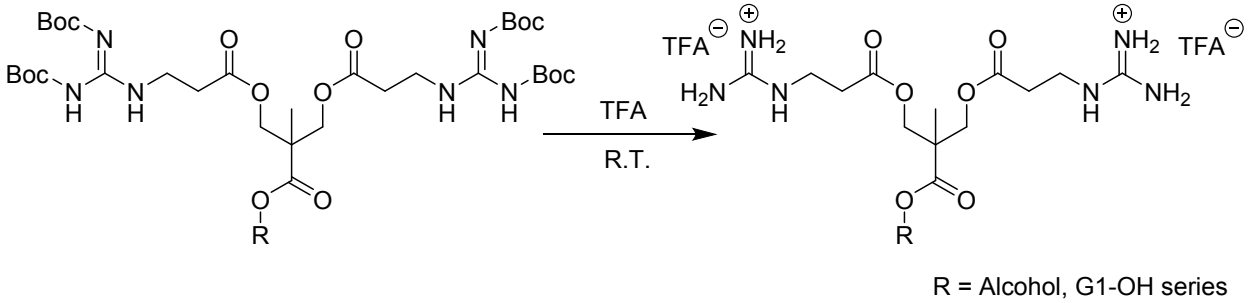


Figure S7: ^{1}H (upper) and ^{13}C NMR (lower) Spectra of G2-(DiBoc)Guanidino Propanoic Acid-Tetradecanol in $CDCl_3$.

Deprotection of Boc Group

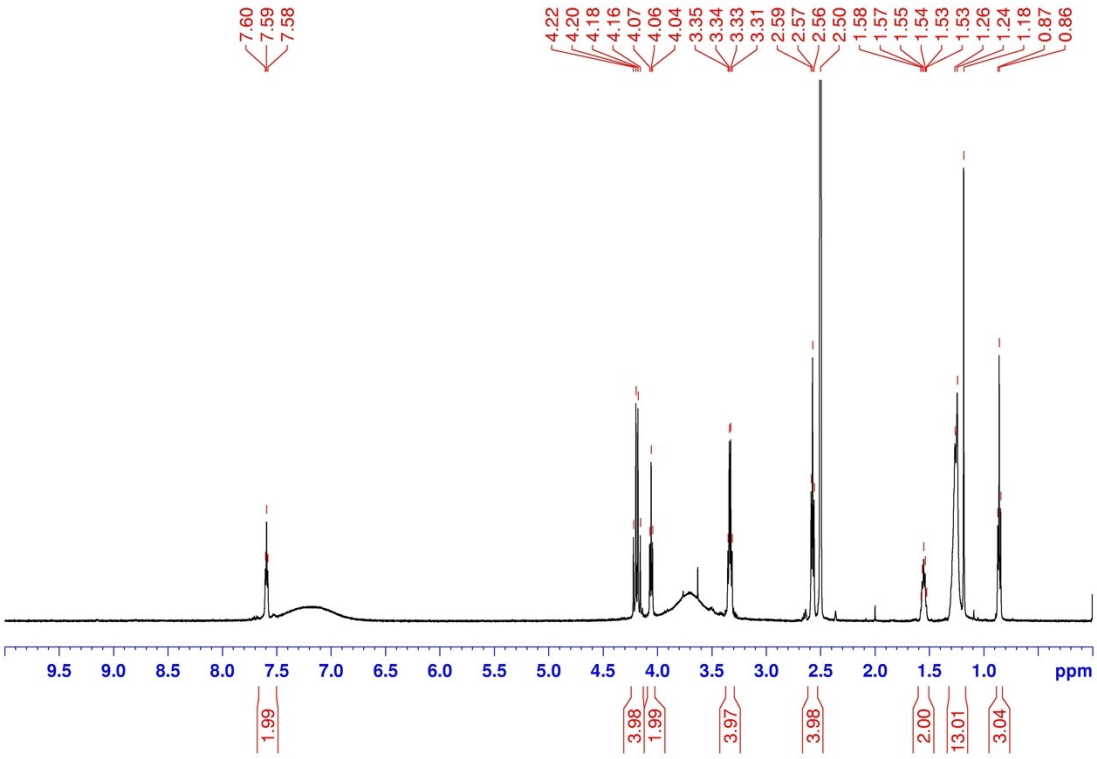
0.2 g of each dendrons was treated with 2 mL trifluoroacetic acid (TFA) and kept stirring for 30 min. TFA was then evaporated under high vacuum. Water was then added and the product were obtained after lyophilization.



Scheme S4: Synthetic Route of the Deprotection of Boc Group.

Generation 1:

C_8G_1: viscous oil, quantitative. ^{1}H NMR (DMSO-d6): 0.86 (t, 3H, -CH_2CH_3, J = 7.1 Hz), 1.18 (s, 3H, -CCH_3), 1.24 – 1.26 (m, broad, 10H, -$(CH_2)_5CH_2CH_3$), 1.53 – 1.58 (m, 2H, -CH_2CH_3, J = 7.0 Hz), 2.57 (t, 4H, -CH_2CH_2NH-, J = 6.7 Hz), 3.34 (q, 4H, -CH_2CH_2NH-, J = 6.5 Hz), 4.06 (t, 2H, -OCH_2CH_2-, J = 6.5 Hz), 4.19 (q, 4H, -CH_2O-, J = 10.4 Hz), 7.20 (broad, 8H, -$CNH_2NH_2^+$), 7.59 (t, 2H, -CH_2CH_2NH-, J = 5.7 Hz). ^{13}C NMR (DMSO-d6): 13.94, 17.14, 22.04, 25.21, 27.92, 28.49, 28.55, 31.16, 32.88, 36.48, 45.95, 64.71, 65.38, 156.73, 170.51, 172.15. ESI: $[M+H]^+$ calcd for $[C_{21}H_{40}O_6N_6+H]^+$ = 473.31, found: 473.3085.



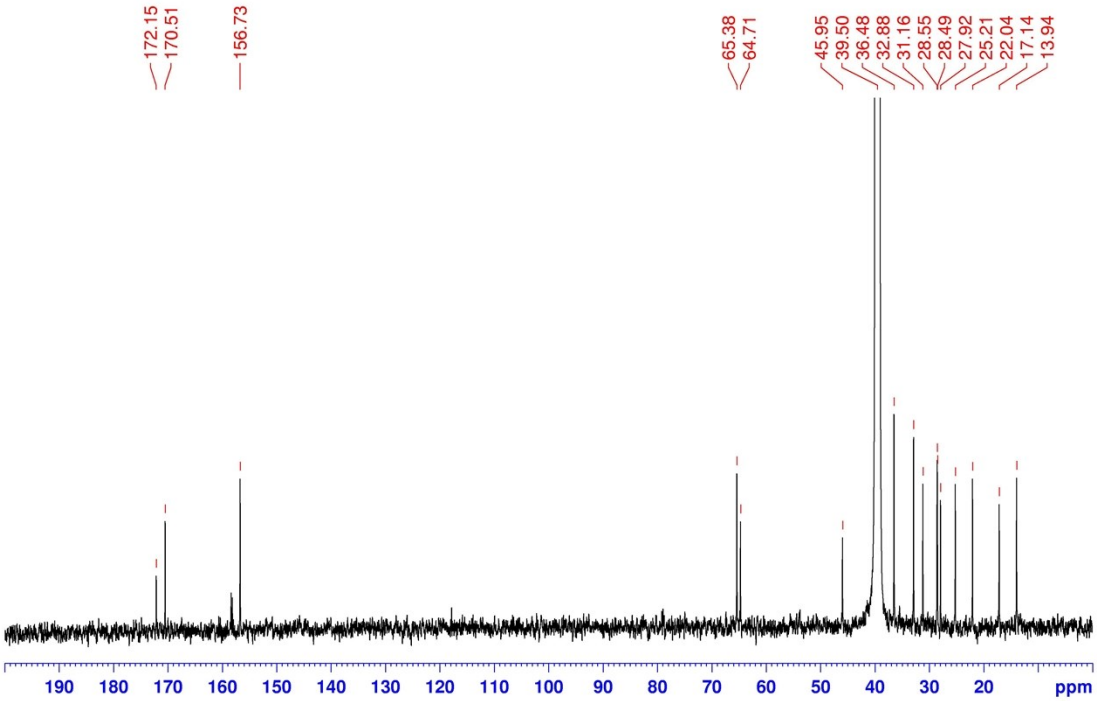
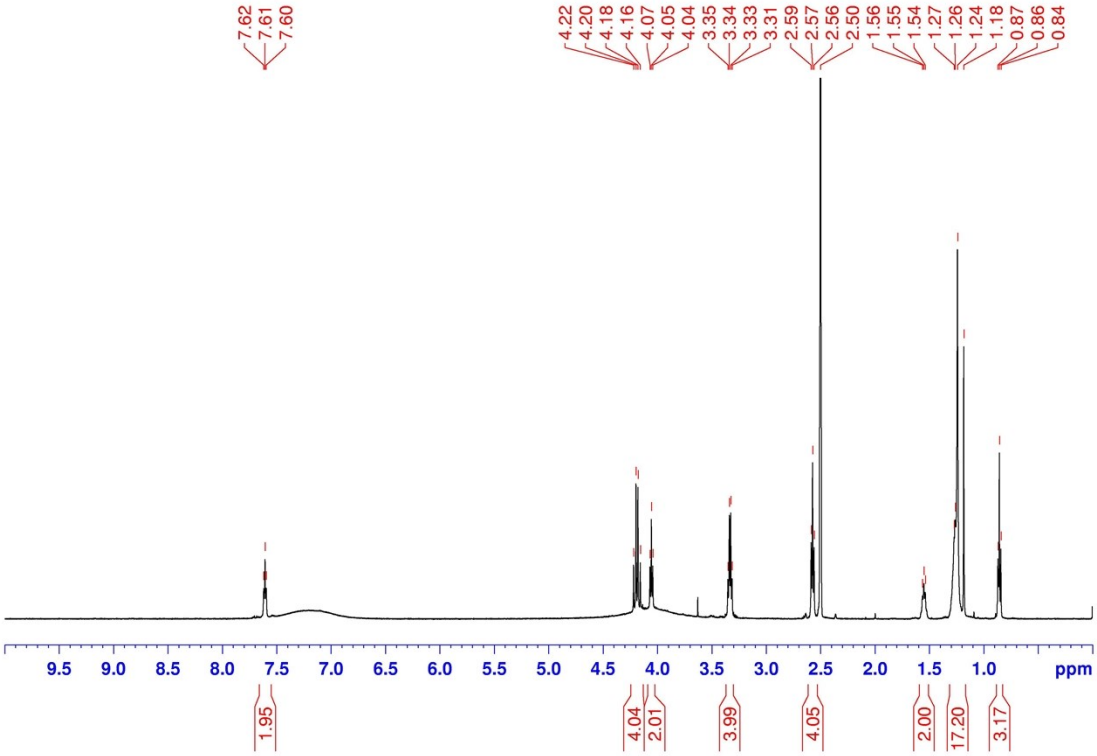


Figure S8: ^{1}H NMR (upper) and ^{13}C NMR (lower) of C_8G_1 in DMSO-d6.

$C_{10}G_1$: viscous oil, quantitative. ^{1}H NMR (DMSO-d6): 0.86 (t, 3H, -CH_2CH_3, J = 7.1 Hz), 1.18 (s, 3H, -CCH_3), 1.24 – 1.27 (br, 14H, -$(CH_2)_7CH_2CH_3$), 1.54 – 1.56 (m, 2H, -CH_2CH_3, J = 6.7 Hz), 2.57 (t, 4H, -CH_2CH_2NH-, J = 6.8 Hz), 3.34 (q, 4H, -CH_2CH_2NH-, J = 6.2 Hz), 4.05 (t, 2H, -OCH_2CH_2-, J = 6.5 Hz), 4.19 (q, 4H, -CH_2O-, J = 10.2 Hz), 7.20 (broad, 8H, -$CNH_2NH_2^+$), 7.61 (t, 2H, -CH_2CH_2NH-, J = 5.8 Hz). ^{13}C NMR (DMSO-d6): 13.95, 17.14, 22.08, 25.21, 27.93, 28.53, 28.66, 28.90, 31.28, 32.88, 36.48, 45.95, 64.71, 65.37, 156.74, 170.51, 172.15. ESI: $[M+H]^+$ calcd $[C_{23}H_{44}O_6N_6+H]^+$ = 501.34, found: 501.3400.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8.5
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6.0
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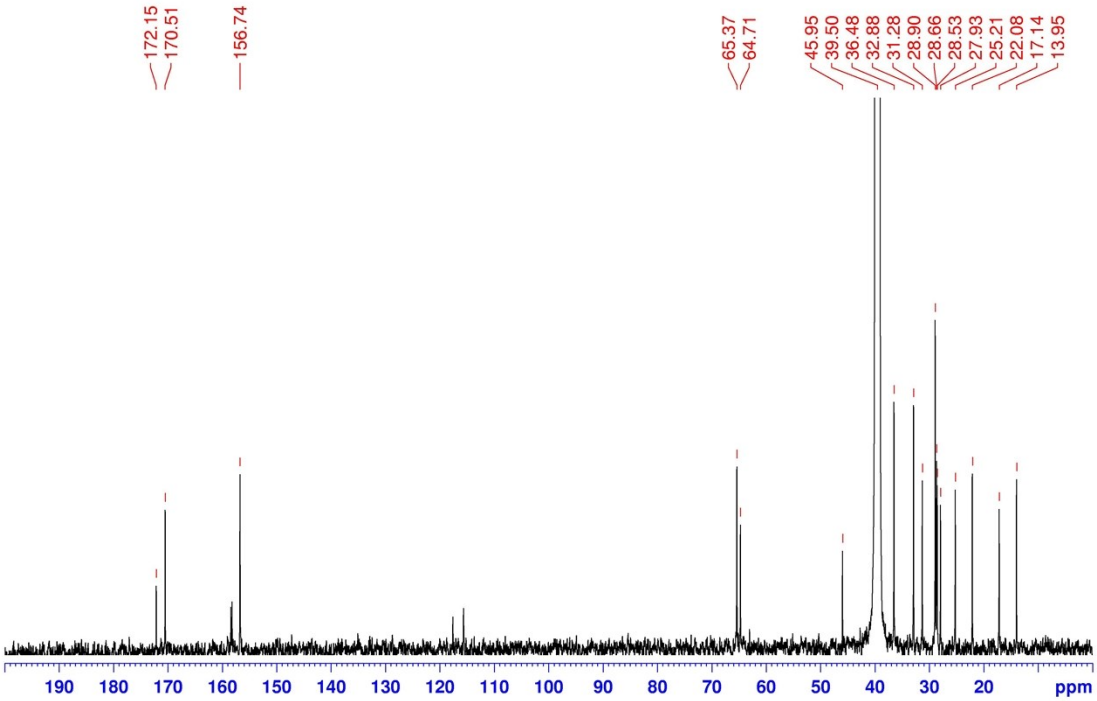
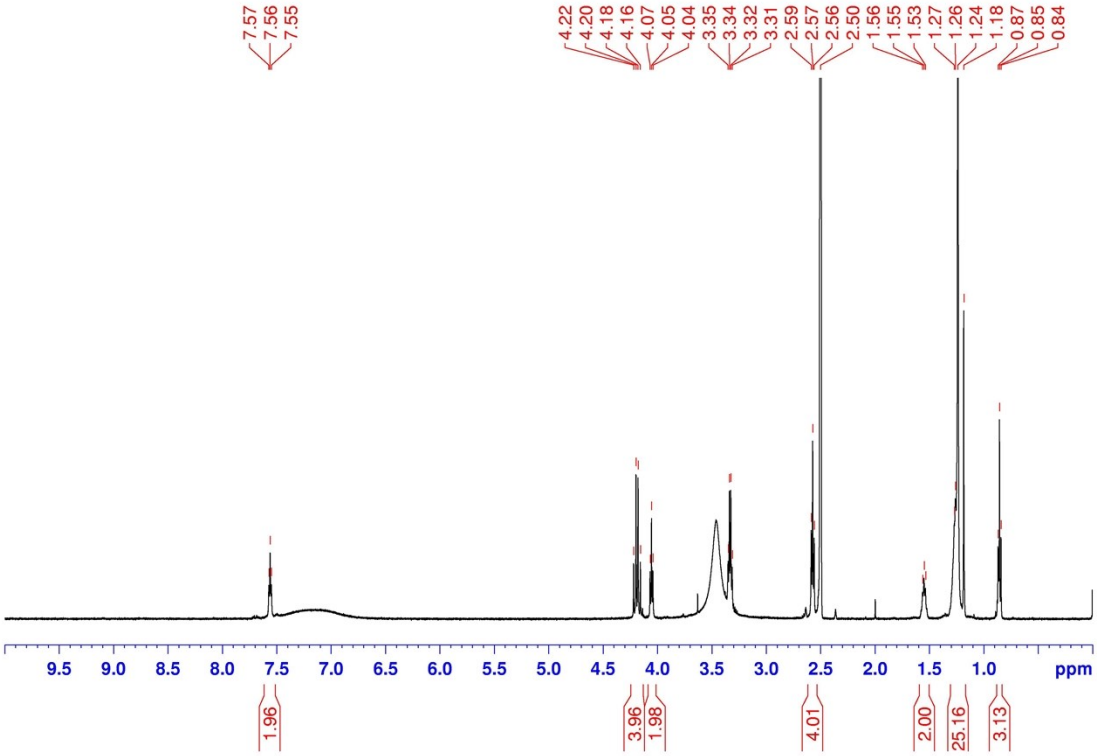


Figure S9: ^{1}H NMR (upper) and ^{13}C NMR (lower) Spectra of $C_{10}G_1$ in DMSO-d6.

$C_{14}G_1$: viscous oil, quantitative. ^{1}H NMR (DMSO-d6): 0.85 (t, 3H, -CH_2CH_3, J = 7.2 Hz), 1.18 (s, 3H, -CCH_3), 1.24 – 1.27 (m, broad, 22H, -$(CH_2)_{11}CH_2CH_3$), 1.53 – 1.56 (m, 2H, -CH_2CH_3, J = 6.5 Hz), 2.57 (t, 4H, -CH_2CH_2NH-, J = 7.2 Hz), 3.33 (q, 4H, -CH_2CH_2NH-, J = 6.2 Hz), 4.05 (t, 2H, -OCH_2CH_2-, J = 6.5 Hz), 4.19 (q, 4H, -CH_2O-, J = 9.7 Hz), 7.20 (broad, 8H, -$CNH_2NH_2^+$), 7.56 (t, 2H, -CH_2CH_2NH-, J = 5.9 Hz). ^{13}C NMR (DMSO-d6): 13.95, 17.14, 22.08, 25.21, 27.93, 28.53, 28.69, 28.90, 28.94, 29.00, 29.03, 31.27, 32.88, 36.48, 45.94, 64.71, 65.38, 156.70, 170.51, 172.15. ESI: $[M+H]^+$ calcd $[C_{25}H_{48}O_6N_2+H]^+$ = 557.40, found: 557.4024.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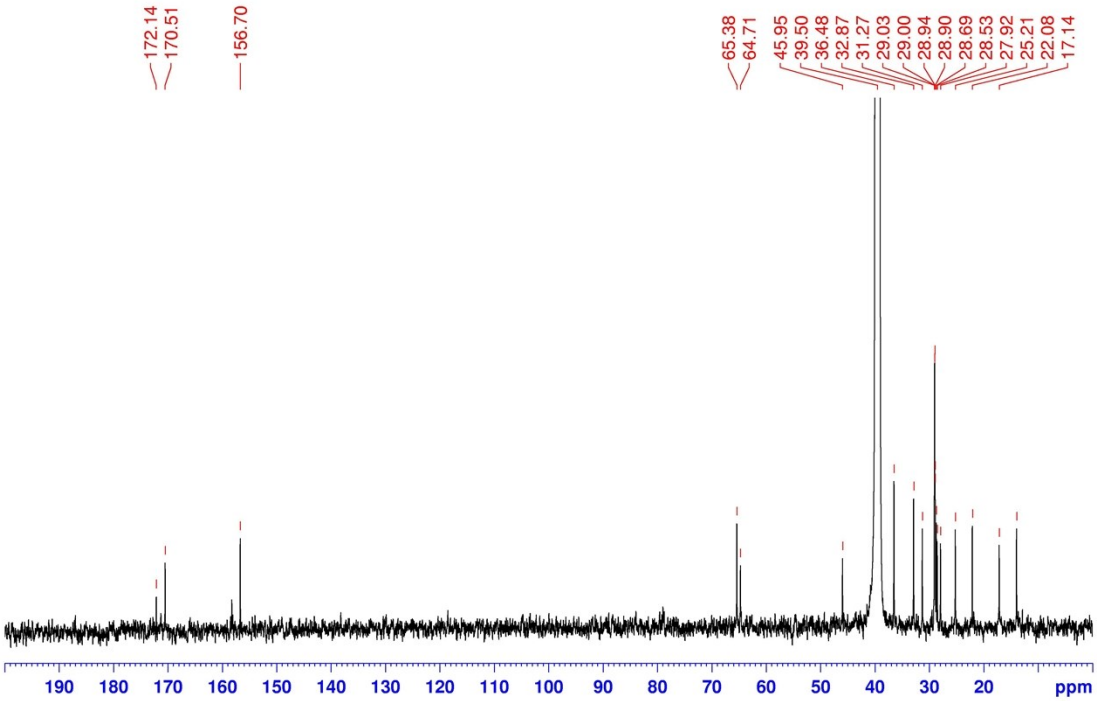
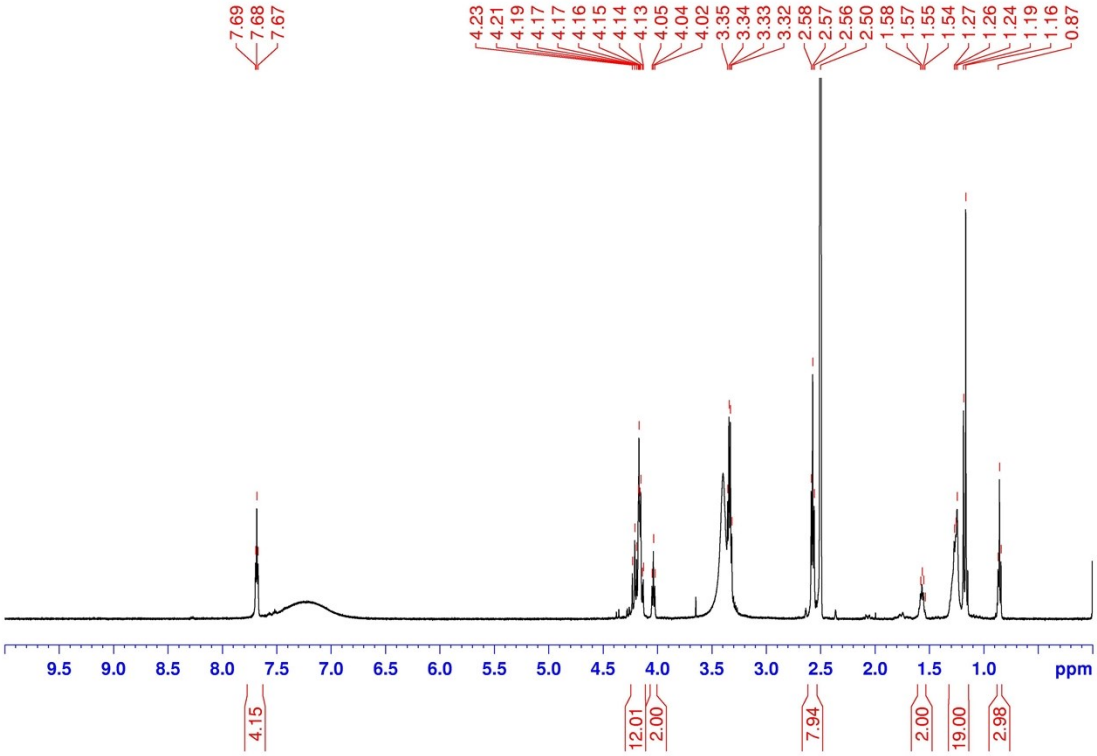


Figure S10: ^{1}H NMR (upper) and ^{13}C NMR (lower) Spectra of $C_{14}G_1$ in DMSO-d6.

Generation 2:

C_8G_2: solid, quantitative. ^{1}H NMR (DMSO-d6): 0.86 (t, 3H, -CH_2CH_3, J = 7.1 Hz), 1.16 (s, 6H, -CCH_3, 2nd gen), 1.19 (s, 3H, -CCH_3, 1st gen), 1.24 – 1.27 (m, broad, 10H, -$(CH_2)_5CH_2CH_3$), 1.54 – 1.58 (m, 2H, -CH_2CH_3, J = 6.5 Hz), 2.57 (t, 8H, -CH_2CH_2NH-, J = 6.8 Hz), 3.34 (q, 8H, -CH_2CH_2NH-, J = 6.4 Hz), 4.04 (t, 2H, -OCH_2CH_2-, J = 6.6 Hz), 4.13 – 4.23 (m, 12H, -CH_2O-), 7.20 (broad, 16H, -$CNH_2NH_2^+$), 7.68 (t, 4H, -CH_2CH_2NH-, J = 5.8 Hz). ^{13}C NMR (DMSO-d6): 13.92, 16.86, 17.06, 22.04, 25.29, 27.91, 28.51, 31.16, 32.78, 36.44, 45.96, 46.17, 64.86, 65.05, 65.72, 156.78, 170.52, 171.62, 171.97. ESI: $[M+2H]^{2+}/2$ calcd $[C_{39}H_{70}O_{14}N_{12}+2H]^{2+}/2$ = 466.26, found: 466.2652. Note, the majority peak is z = 2.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2.00
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19.00
2.98

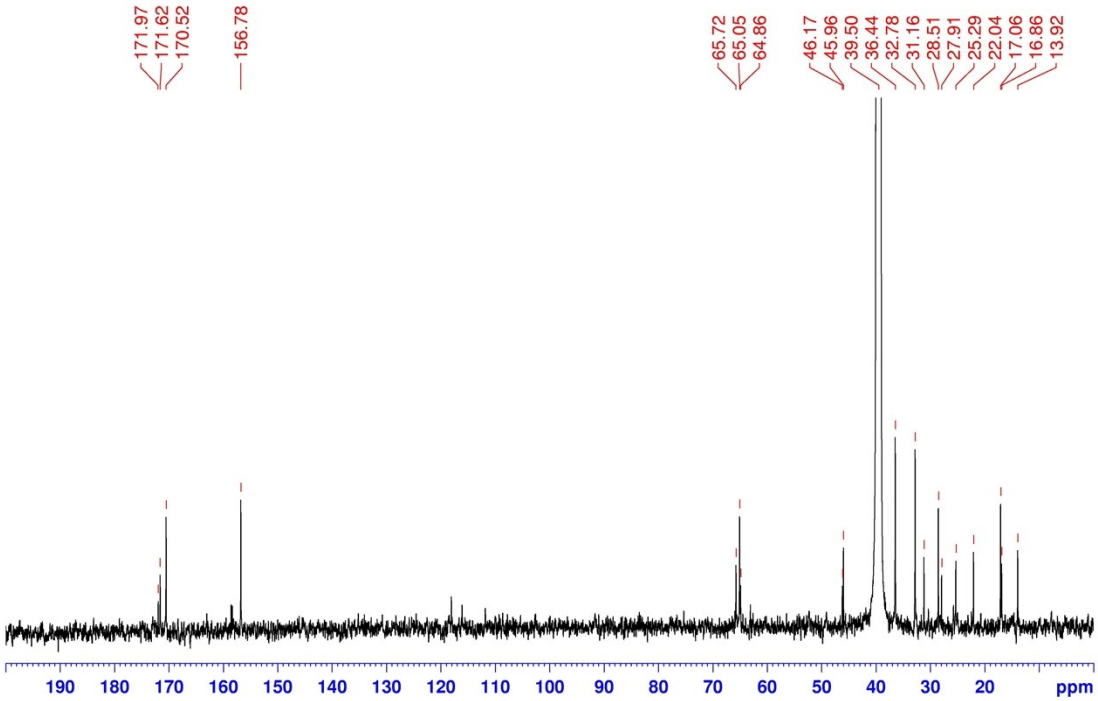
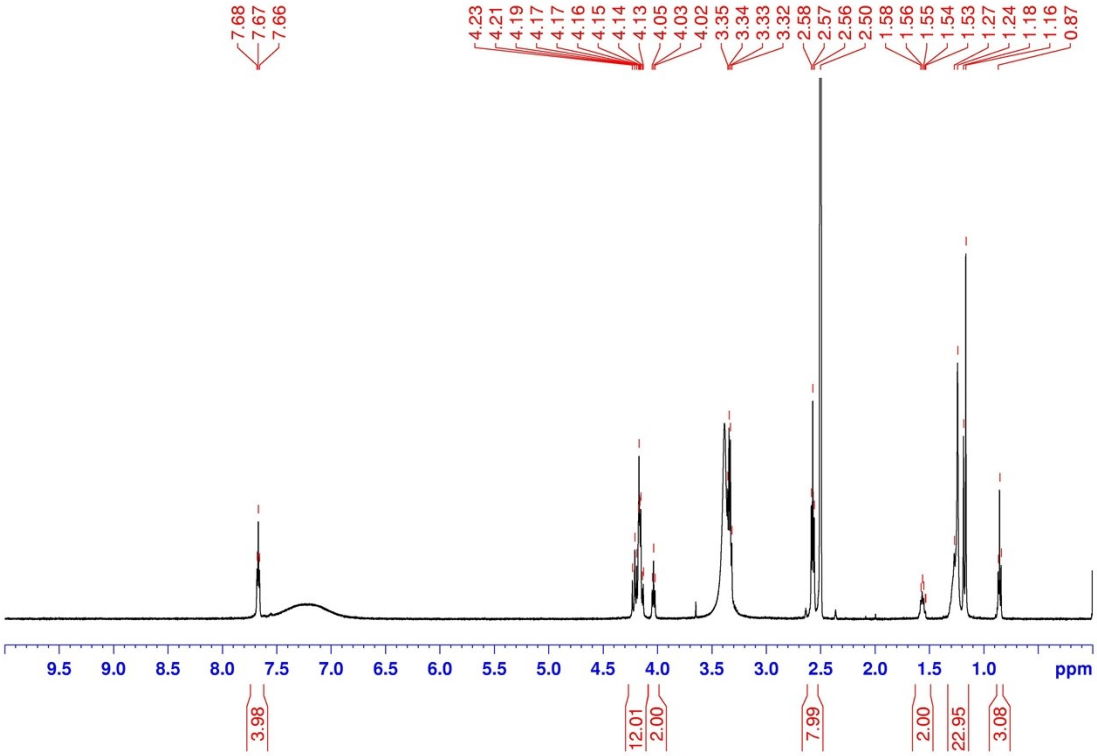


Figure S11: ^{1}H NMR (upper) and ^{13}C NMR (lower) Spectra of C_8G_2 in DMSO-d6.

$C_{10}G_2$: solid, quantitative. ^{1}H NMR (DMSO-d6): 0.86 (t, 3H, -CH_2CH_3, J = 7.1 Hz), 1.16 (s, 6H, -CCH_3, 2nd gen), 1.19 (s, 3H, -CCH_3, 1st gen), 1.24 – 1.27 (m, broad, 14H, -$(CH_2)_7CH_2CH_3$), 1.53 – 1.58 (m, 2H, -CH_2CH_3, J = 6.5 Hz), 2.57 (t, 8H, -CH_2CH_2NH-, J = 6.7 Hz), 3.34 (q, 8H, -CH_2CH_2NH-, J = 6.2 Hz), 4.03 (t, 2H, -OCH_2CH_2-, J = 6.5 Hz), 4.13 – 4.23 (m, 12H, -CH_2O-), 7.20 (broad, 16H, -$CNH_2NH_2^+$), 7.67 (t, 4H, -CH_2CH_2NH-, J = 5.7 Hz). ^{13}C NMR (DMSO-d6): 13.93, 16.87, 17.07, 22.07, 25.29, 27.92, 28.55, 28.66, 28.89, 31.26, 32.78, 36.44, 45.96, 46.17, 64.87, 65.05, 65.72, 156.77, 170.52, 171.62, 171.96. ESI: $[M+2H]^{2+}/2$ calcd $[C_{41}H_{74}O_{14}N_{12}+2H]^{2+}/2$ = 480.28, found: 480.2809.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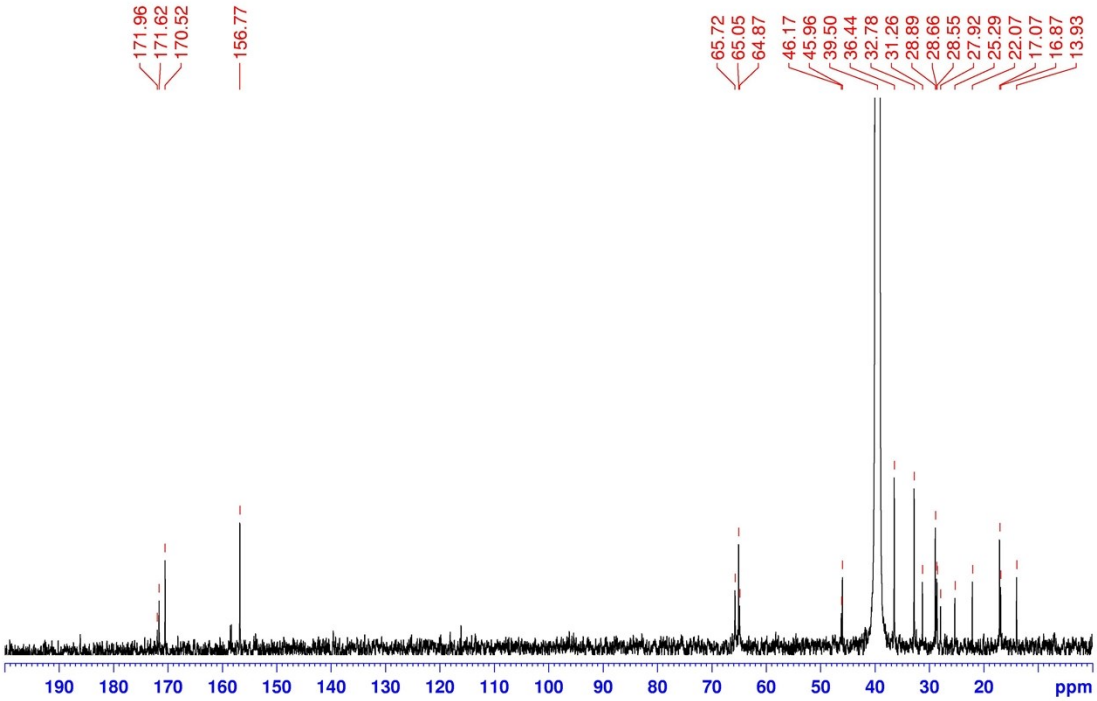
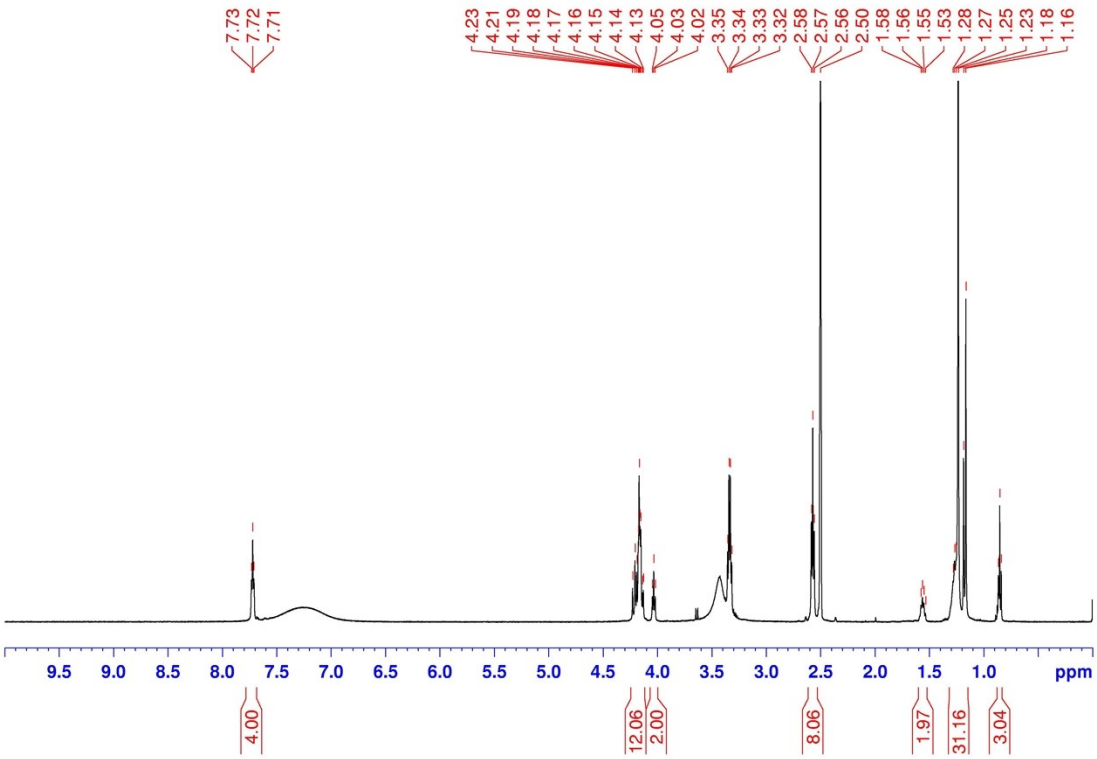


Figure S12: ^{1}H NMR (upper) and ^{13}C NMR (lower) Spectra of $C_{10}G_2$ in DMSO-d6.

$C_{14}G_2$: solid, quant. ^{1}H NMR (DMSO-d6): 0.86 (t, 3H, -CH_2CH_3, J = 7.1 Hz), 1.16 (s, 6H, -CCH_3, 2nd gen), 1.18 (s, 3H, -CCH_3, 1st gen), 1.23 – 1.28 (m, broad, 22H, -$(CH_2)_{11}CH_2CH_3$), 1.53 – 1.58 (m, 2H, -CH_2CH_3, J = 6.8 Hz), 2.57 (t, 8H, -CH_2CH_2NH-, J = 6.7 Hz), 3.34 (q, 8H, -CH_2CH_2NH-, J = 6.1 Hz), 4.03 (t, 2H, -OCH_2CH_2-, J = 6.5 Hz), 4.13 – 4.23 (m, 12H, -CH_2O-), 7.20 (broad, 16H, -$CNH_2NH_2^+$), 7.72 (t, 4H, -CH_2CH_2NH-, J = 5.7 Hz). ^{13}C NMR (DMSO-d6): 13.93, 16.87, 17.07, 22.08, 25.29, 27.92, 28.57, 28.69, 28.89, 28.95,29.03, 31.28, 32.78, 36.44, 45.96, 46.17, 64.86, 65.05, 65.70, 156.81, 170.51, 171.63, 171.96. ESI: $[M+2H]^{2+}/2$ calcd $[C_{45}H_{82}O_{14}N_{12}+2H]^{2+}/2$ = 508.31, found: 508.3118.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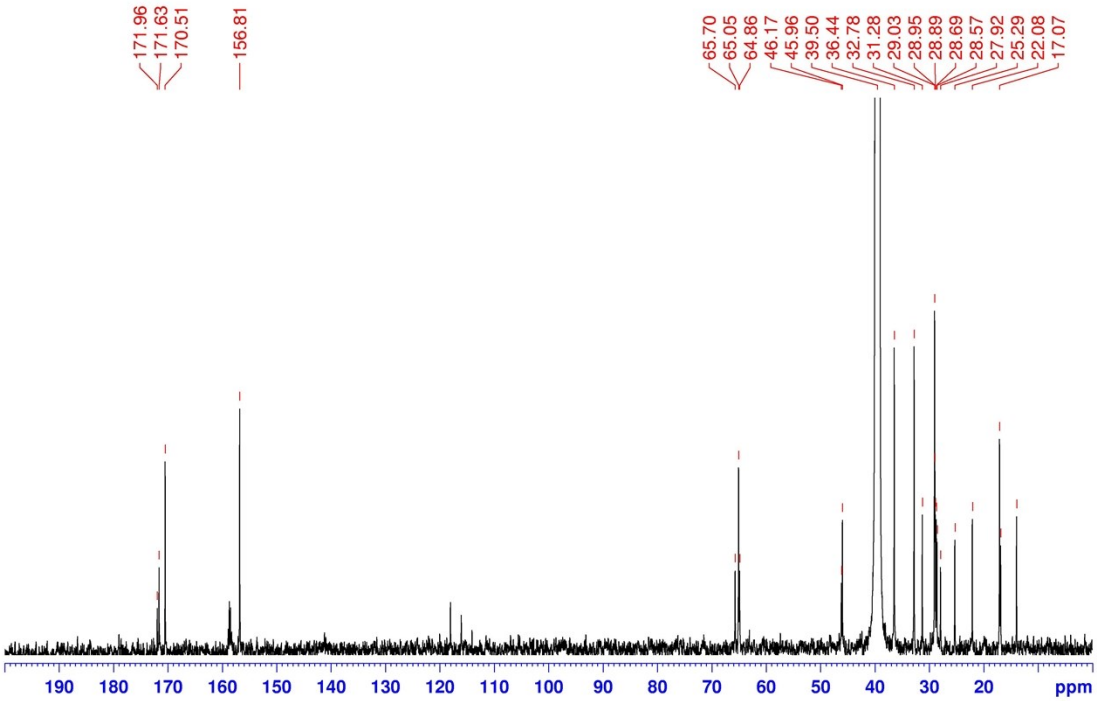

Figure S13: ^{1}H NMR (upper) and ^{13}C NMR (lower) Spectra of $C_{14}G_2$ in DMSO-d6.

2. Solution Properties

2.1 Pyrene Emission Assay

Fluorescence spectroscopy of pyrene was measured on a Molecular Devices SpectraMax M2 multimode microplate spectrophotometer. A series of serial two-fold dilutions of molecular umbrella solution, ranging from 50 mg/mL to 39.1 µg/mL, were prepared in water. 10 µL of the above dilutions were added to each well of a black 96-well plate and 90 µL of the pyrene-saturated PBS solution was then added to each well and thoroughly mixed. The final compound concentration on the microplate ranges from 5 mg/mL down to 3.9 µg/mL. The fluorescence emission from each well was measured, with excitation wavelength set at 334 nm and emission at 373 (I_1) and 384 nm (I_3) recorded. The quotient of I_1/I_3 was plotted against the concentration of the dendrons in a semi-log plot and curve-fitting was done with the empirical Hill equation.

2.2 Pyrene I_1/I_3 Dose-Responsive Curves

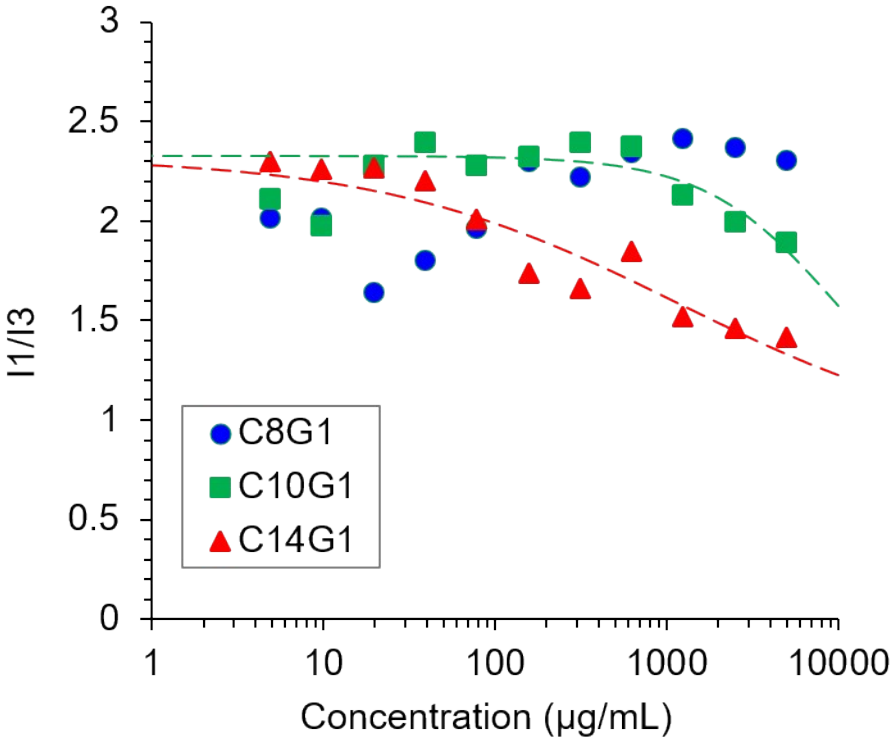

Figure S14: Pyrene I_1/I_3 Dose-Responsive Curves of the G_1 Dendron Series.

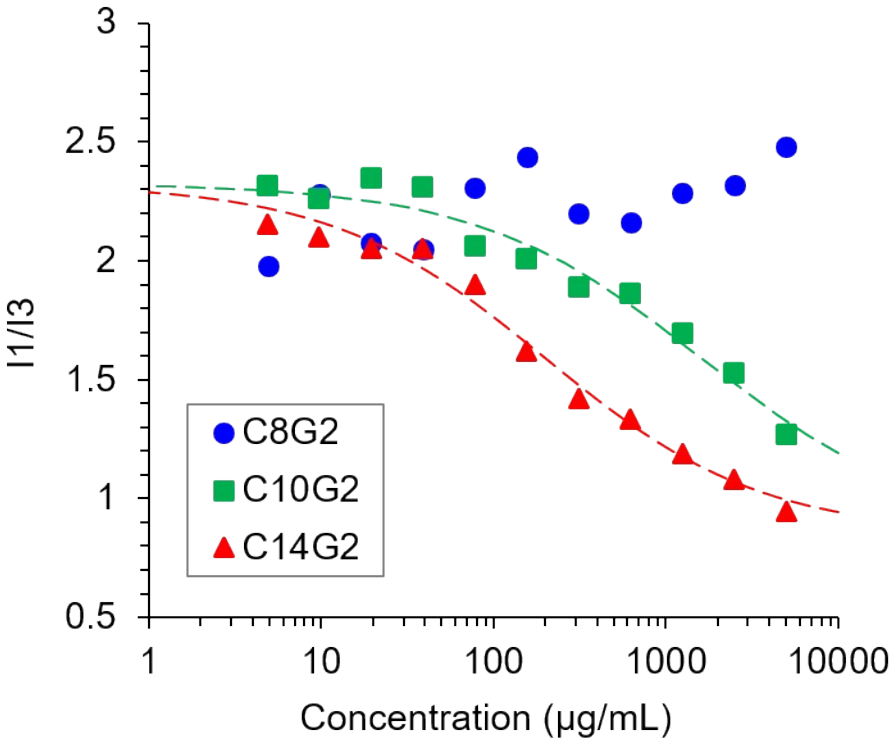

Figure S15: Pyrene I_1/I_3 Dose-Responsive Curves of G_2 Dendron Series.

3. Biological Activity

3.1 Minimum Inhibitory Concentration (MIC) Assay

Stock solutions (20 mg/mL) were prepared by dissolving each molecular umbrella in milliQ water. Then, two-fold serial dilutions were prepared to cover the range of concentration from 10 mg/mL to 0.25 μg/mL. Bacteria was first streaked on MH agar plates and incubated at 37 °C overnight. One colony was picked and inoculated in 10 mL MH broth and incubated at 37 °C on a shaker at 500 rpm overnight. The overnight culture was diluted using MH broth till OD600 reached 0.1 and incubated for another 90 min to reach mid-log phase. The culture at mid-log phase was then diluted again using MH broth till OD600 was 0.1 (~5×10^7 CFU/mL for *E. coli* and ~6×10^7 CFU/mL for *S. aureus*). The culture was diluted by a factor of 10^2 to afford the density of *E. coli* at ~5×10^5 CFU/mL and *S. aureus* at ~6×10^5 CFU/mL. 10 μL of the dilutions of molecular umbrella solutions were added into 96-well plates, and then 90 μL of the final culture was added and mixed. The 96-well plates were sealed with parafilm and incubated at 37 °C overnight. The lowest concentration of the molecular umbrella solution that inhibit visual growth of bacteria is the minimum inhibitory concentration. All tests were performed three times in triplicate.

3.2 MIC in Molar Units

Table S1: MIC of Dendrons against *E. coli* and *S. aureus* in Molar Units

Cmpd.	Gen.	C_n	MW (g/mol)	MIC (μM)	
				E. coli	*S. aureus*
C_8G_1	G1	8	701	357	713
$C_{10}G_1$		10	729	85.7	85.7
$C_{14}G_1$		14	785	9.9	9.9
C_8G_2	G2	8	1387	721	180
$C_{10}G_2$		10	1415	44.2	44.2
$C_{14}G_2$		14	1471	5.3	5.3

3.3 Hemolysis Assay

10% sheep red blood cell (RBC) suspension was centrifuged (2000 rpm, 5 min) and washed with PBS three times. The washed RBCs were resuspended in 10 mL PBS to reach 1% RBC suspension. To each well of the round-bottom 96-well plate, 10 μL of the dendron solutions were added. To the wells of positive control was added 10 μL 0.1% v/v Triton X solution, and to the negative control wells was added 10 μL PBS. 90 μL of the 1% RBC suspension in PBS was then mixed with the molecular umbrella solution. The 96-well plate was sealed with parafilm and incubated at 37 °C for 1 h agitated at 200 rpm to avoid the deposit of RBCs. After incubation, the plate was centrifuged at 1000 rpm for 10 min. 10 μL supernatant of each well was transferred into a flat-bottom 96-well plate and diluted with 90 μL PBS. The absorbance of each well was measured at 415 nm. The percentage of hemolysis (H) was calculated using the equation below:

$$H\ (\%) = \frac{OD_{415}(Dendron) - OD_{415}(PBS)}{OD_{415}(TritonX) - OD_{415}(PBS)} \times 100\% \quad (1)$$

HC_{50} was calculated as the concentration of dendrons causing 50% hemolysis by fitting the data with function:

$$H = \frac{1}{1 + \frac{HC_{50}}{[C_nG_x]^n}} \quad (2)$$

where HC_{50} and n are the curve fitting variables that represent the characteristic hemolytic concentration and the Hill coefficient (indicative of the steepness of the dose-response transition). All tests were performed three times in triplicate.

3.4 Hemolytic Activity in Molar Units

Table S2: HC_{50} of Dendrons against Sheep Red Blood Cells in Molar Units

Cmpd.	Gen.	C_n	MW (g/mol)	HC_{50} (μM) RBC
C_8G_1		8	701	625
$C_{10}G_1$	G1	10	729	383
$C_{14}G_1$		14	785	29.2
C_8G_2		8	1387	1120
$C_{10}G_2$	G2	10	1415	231
$C_{14}G_2$		14	1471	79.6

3.5 Hemolysis Dose-Response Curves

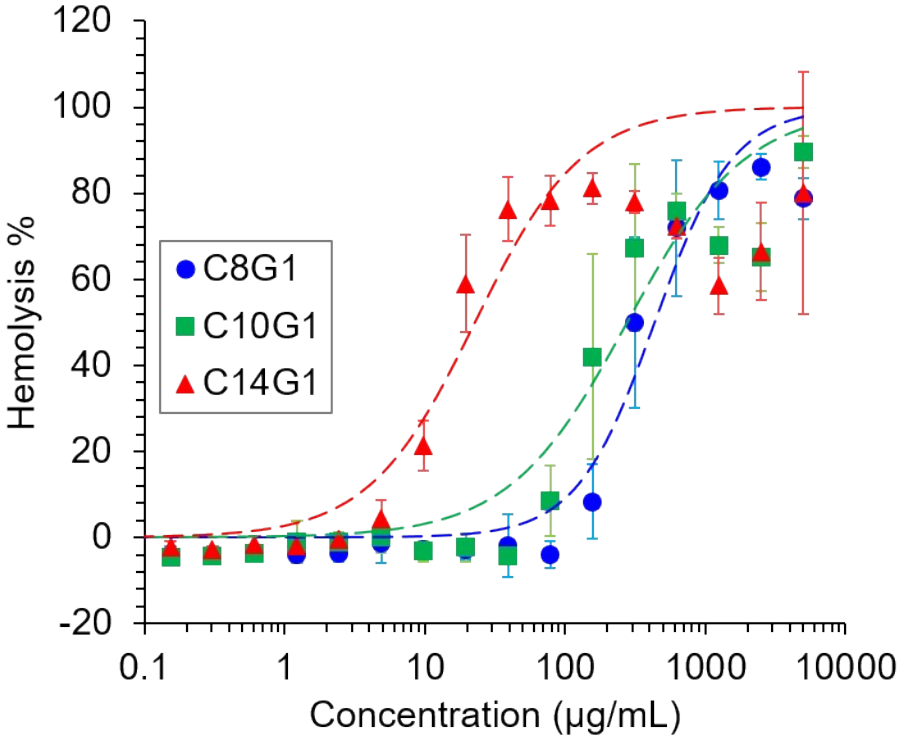

Figure S16: Hemolysis Dose-Response Curves of G_1 Dendron Series.

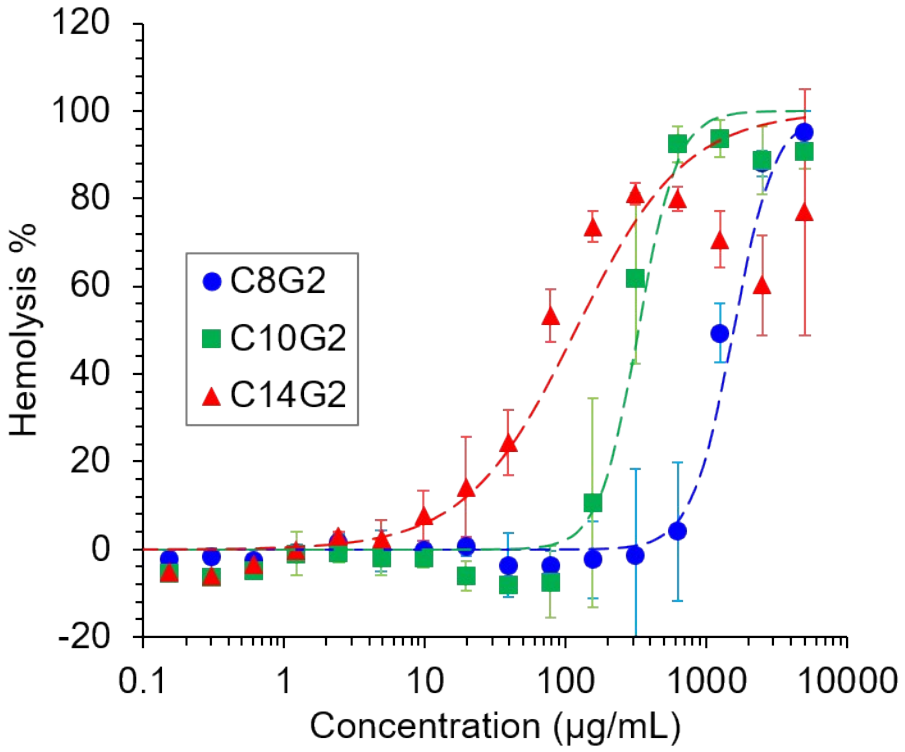

Figure S17: Hemolysis Dose-Response Curves of G_2 Dendron Series.

3.6 Selectivity of Molecular Umbrellas of *S. aureus* over Red Blood Cells

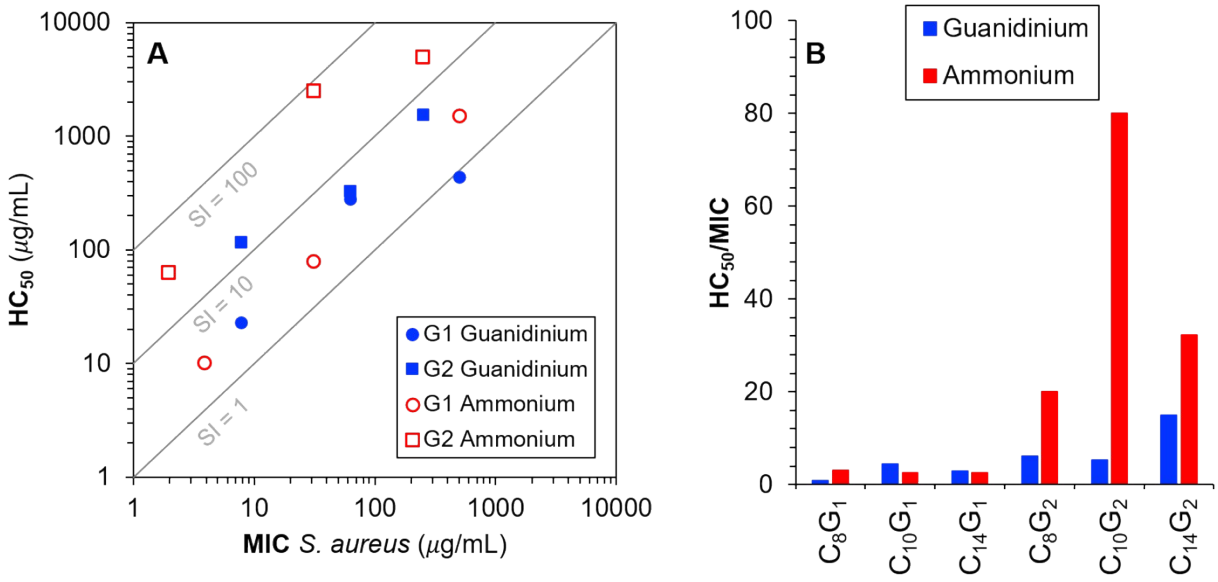

Figure S18: (A) Log-log Ashby plot of hemolytic activity against sheep red blood cells versus antimicrobial activity against *S. aureus* and (B) bar chart of selectivity index, HC_{50}/MIC, for the

1st and 2nd generation molecular umbrellas as a function of the chemical structure of their cationic charges.

3.7 Live/Dead Assay

A suspension of *S. aureus* or *E. coli* at mid-log phase was obtained through the same procedure described in MIC assay. The bacteria were washed with PBS three times, resuspended in PBS and adjusted to OD600 = 0.1. Two $C_{14}G_2$ aqueous solutions were prepared with concentrations respectively at 320 μg/mL. 40 μL of the 320 μg/mL dendron solution was added into 3.6 mL of either *E. coli* or *S. aureus* suspension to reach the final concentration of dendron at 32 μg/mL (4×MIC). The suspensions were incubated at 37 °C agitated at 500 rpm for 3 h. Bacteria were pelleted at 2000 rpm for 10 min and resuspended in 100 μL PBS. The above suspension was stained with Live/Dead Baclight Stain according to the instruction manual. 5 μL of the stained suspension was trapped between a glass slide and coverslip and observed under a Zeiss LSM 510 Meta laser scanning confocal microscope. SYTO9 was excited using Argon laser (488 nm) and its emission at 510-540 nm was recorded. Propidium iodide was excited using HeNe1 laser (543 nm) and emission at 620-650 nm was recorded. Images were taken in 1024×1024 pixel format.

3.8 Confocal Microscopy of *S. aureus*

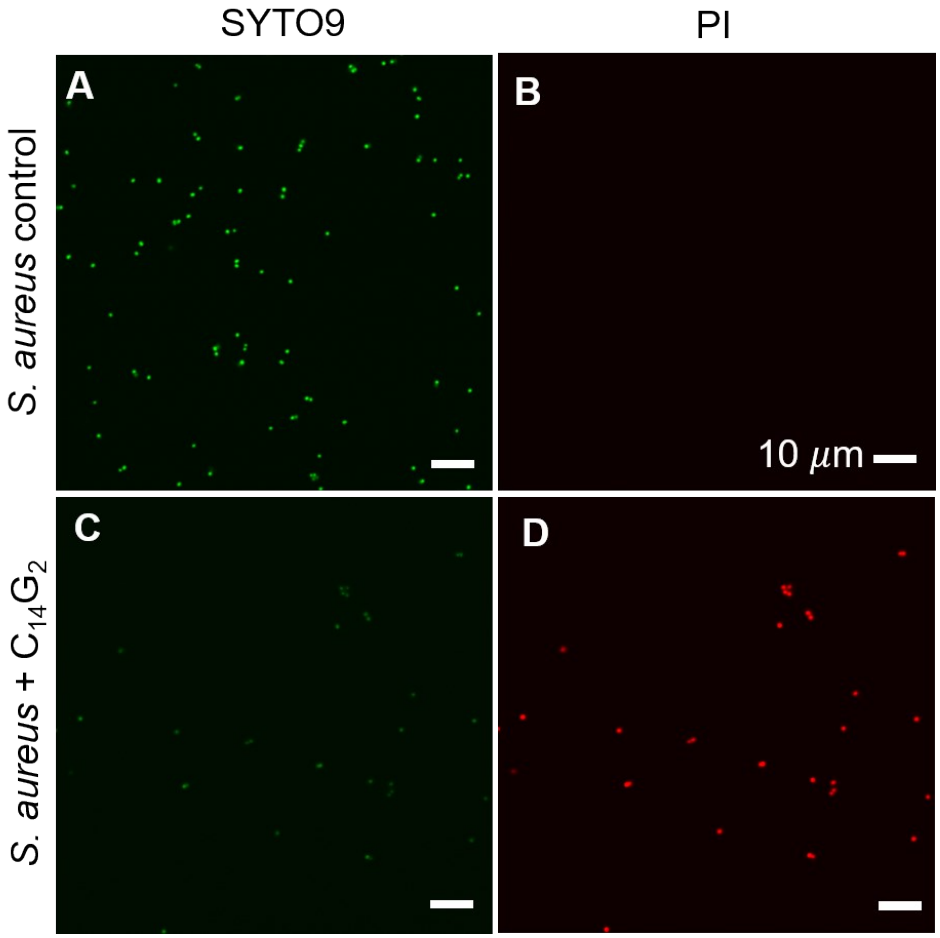

Figure S19: Confocal laser scanning microscopy images of (A, B) *S. aureus* in PBS and (C, D) *S. aures* exposed to $C_{14}G_2$ at 32 μg/mL

4. SEM Characterization

4.1 Sample Preparation Method

Bacteria treated 32 μg/mL of guanylated G_2C_{14}, as well as non-treated controls, were harvested by centrifugation. They were washed three times with sterile PBS and fixed with 2.5% glutaraldehyde in PBS solution at 4˚C for at least 4 hours. They were then washed three more times with sterile PBS and adhered using poly-L-lysine to microscope glass slides cut into 1 cm x 1 cm squares. The cells were then dehydrated in 24-well plates in a fume hood at 20% relative humidity. Cells were treated with a series of solutions comprised of ethanol in purified water at volumetric concentrations of 20%, 40%, 60%, 80%, and three times at 100%, followed by a solution of 1:1 volumetric HMDS: ethanol and then pure HMDS. The cells were dried under air and immediately

sputter coated with 10 nm of platinum. Bacterial morphologies were qualitatively observed with an FEI Versa 3-D Dual Beam SEM using an acceleration voltage of 5.0 kV and a 4.0 spot size.

4.2 SEM Images of *S. aureus*

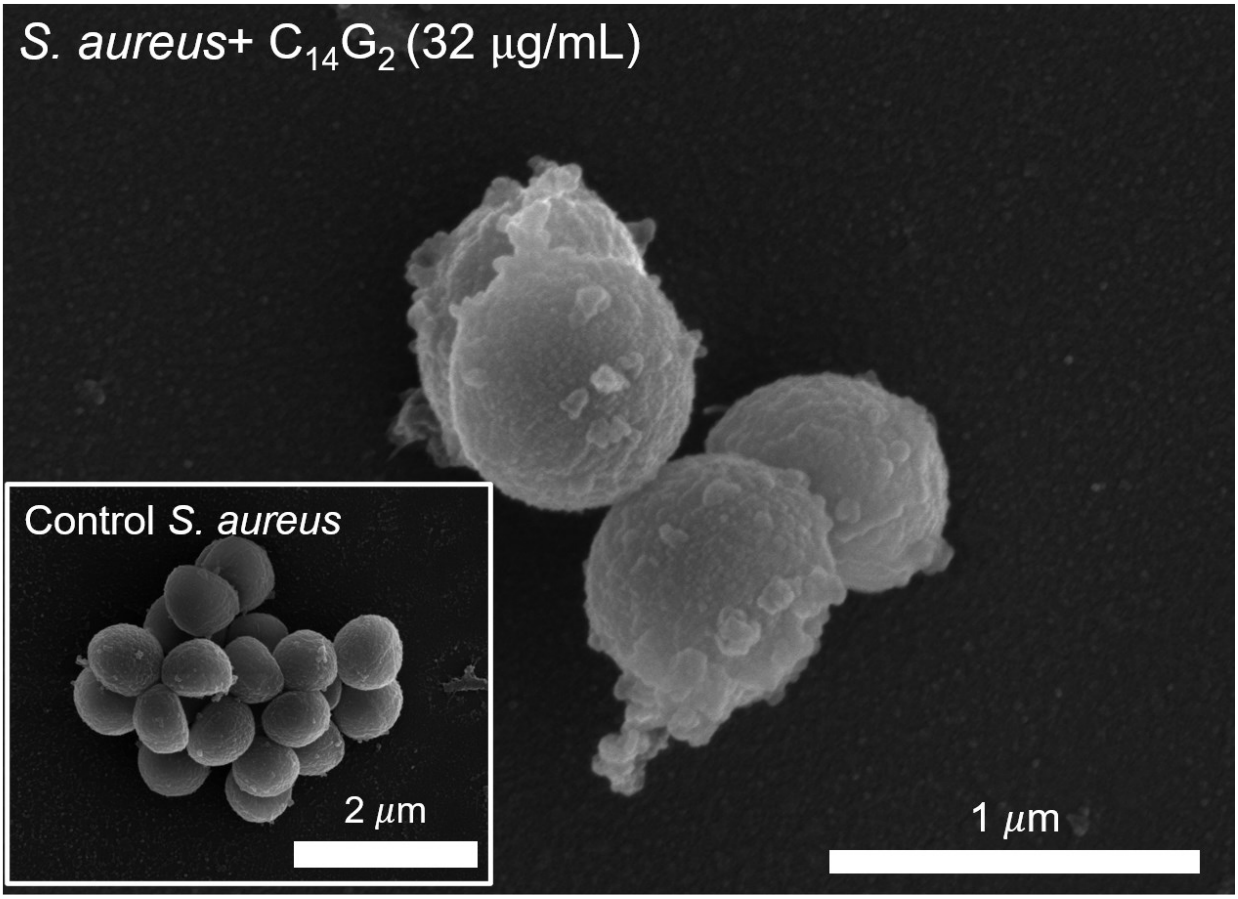

Figure S20: SEM images of *S. aureus* exposed to $C_{14}G_2$ at 32 μg/mL compared to (inset) *S. aureus* alone, both after fixation

5. Reference:

1. Chen, A.; Karanastasis, A.; Casey, K. R.; Necelis, M.; Carone, B. R.; Caputo, G. A.; Palermo, E. F., Cationic Molecular Umbrellas as Antibacterial Agents with Remarkable Cell-Type Selectivity. *ACS Appl. Mater. Interfaces* **2020,** *12* (19), 21270-21282.
2. Schmidt, M. F.; Korb, O.; Abell, C., MicroRNA-Specific Argonaute 2 Protein Inhibitors. *ACS Chem. Biol.* **2013,** *8* (10), 2122-2126.