Type III-C Rotaxane Dendrimers: Synthesis, Dual Size Modulation and In Vivo Evaluation

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General Methods

Unless otherwise stated, all the chemicals were purchased from Sigma-Aldrich, Acros, IL, Fluka, J&K, and Dieckmann. The AR grade solvents were purchased commercially and used without further purification. All the reactions were conducted in anhydrous, high purity nitrogen or argon atmospheric protection. Thin-layer chromatography (TLC Silica gel 60, Merck) was performed on aluminum plate. The plates were observed under 254 nm UV light, visualized by staining with phosphomolybdic acid (PMA) solution or ninhydrin solution with general heating. Column chromatography was carried out on silica gel SiO₂ 60F (Merck 9385, 0.040–0.063 mm, Germany) as the stationary phase. ¹H NMR, ¹³C NMR, ³¹P NMR, were recorded at 298K with Bruker Avance-III (¹H: 400 MHz, ¹³C: 101 MHz, ³¹P: 162 MHz). Chemical shifts of solvent were calibrated to the solvent residue reference peak, for ¹H NMR (CDCl₃ = 7.26 ppm, CD₂Cl₂ = 5.32 ppm, CD₃CN = 1.94 ppm, DMSO-d₆ = 2.50 ppm), for ¹³C NMR (CDCl₃ = 77.16 ppm, CD₂Cl₂ = 54.00 ppm, CD₃CN = 1.39 ppm, DMSO-d₆ = 39.51 ppm). Coupling constants (J) are reported in hertz (Hz). Standard abbreviations indicate the multiplicity of the peaks (s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, m = multiplet, br = broad, dd = doublet of doublets, ddd = doublet of doublet of doublets, etc.). High-resolution mass spectrometry (HRMS) was carried out with Bruker Autoflex spectrometer (MALDI-TOF), and Thermofinnigan MAT 95 XL spectrometer (ESI-MS) with quadrupole ion trap (QIT).
Synthesis of di-functionalized cis-DB24C8-NHS

The intermediates before S1 were synthesized according to the literature report.[S1]

Preparation of S1: cis-DB24C8-diester (5 g, 9 mmol) was dissolved in 180 mL water containing KOH (5 g, 90 mmol). The reaction mixture was refluxed overnight to give a clear solution. The reaction mixture was cooled to low temperature, and conc. HCl was added until no further precipitation. The white precipitate was then filtered, washed with water (50 mL x 3), air dried overnight to give a white solid in quantitative yield. 1H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 7.54 (d, \(J = 8.7\) Hz, 2H), 7.43 (s, 2H), 7.03 (d, \(J = 8.7\) Hz, 2H), 4.12 (d, \(J = 14.6\) Hz, 8H), 3.72 (m, 16H). 13C NMR (101 MHz, DMSO-\(d_6\)) \(\delta\) 167.03, 152.22, 147.70, 123.46, 123.12, 113.82, 112.39, 70.49, 70.45, 70.00, 69.02, 68.91, 68.79, 68.71. HRMS (MALDI-TOF): C\(_{26}\)H\(_{32}\)O\(_12\) [M+Na]\(^+\): calcd 599.1786; found 599.1770.

Preparation of S2: cis-DB24C8-diacid S1 (3 g, 5.5 mmol) was dissolved in excess oxayl chloride (20 mL) with one drop of DMF. The orange milky solution was refluxed for 4 h. Excess oxayl chloride was removed by distillation to give a yellow solid. The yellow solid was redissolved in THF (120 mL) containing \(N\)-hydroxysuccinimide (3.2 g, 28 mmol). Et\(_3\)N (4 mL, 28 mmol) was added finally to give a pale-yellow turbid solution and stirred overnight. The reaction mixture was filtered and concentrated in vacuum. The residue was redissolved in CHCl\(_3\) (100 mL) and extracted with 1 M HCl (50 mL). The combined organic layers were dried over anhydrous MgSO\(_4\) and concentrated in vacuum to give a pale-yellow solid. The yellow liquid was purified by column chromatography (SiO\(_2\); CH\(_2\)Cl\(_2\)/MeOH 10:1) yielding a yellow solid (3.2 g, 80%). 1H NMR (400 MHz, CD\(_3\)CN) \(\delta\) 7.75 (dd, \(J = 8.5, 2.1\) Hz, 2H), 7.56 (d, \(J = 2.1\) Hz, 2H), 7.06 (d, \(J = 8.6\) Hz, 2H), 4.23 – 4.15 (m, 8H), 3.87 – 3.79 (m, 8H), 3.69 (d, \(J = 6.9\) Hz, 8H), 2.83 (s, 8H). 13C NMR (101 MHz,
Acetonitrile-d3) δ 171.38 , 162.65 , 155.61 , 149.64 , 125.88 , 117.73 , 114.94 , 113.50 , 71.88 , 71.81 , 70.33 , 70.13 , 70.04 , 26.49 . HRMS (MALDI-TOF): C_{34}H_{38}N_{2}O_{16} [M+Na]^+: calcd 753.2113; found 753.2151.

**Synthesis of 1-H·PF₆**

[Diagram of the synthesis of 1-H·PF₆]

Azido dendron was synthesized according to the literature report.[82]

Preparation of S₃: Azido dendron (4.5g, 1.0 mmol) and 4-(prop-2-yn-1-yl oxy)benzaldehyde (1.5 g, 1.0 mmol) were dissolved in CH₂Cl₂, followed with the addition of CuI (5 g, 26 mmol) and DIPEA (2 mL, 11 mmol). The reaction mixture was stirred at room temperature overnight. After the reaction, the reaction mixture was filtered through a plug of Celite. The filtrate was extracted with 50 mL 2M NaCN(aq). The combined organic layers were dried over anhydrous MgSO₄, concentrated in vacuum, and purified by column chromatography (SiO₂; Hexane/EtOAc 2 : 1) yielding a yellow oil (4 g, 66%). ¹H NMR (400 MHz, CDCl₃) δ 9.86 (s, 1H), 7.83 (d, J = 8.7 Hz, 2H), 7.57 (s, 1H), 7.41 (d, J = 8.4 Hz, 4H), 7.33 (d, J = 8.3 Hz, 4H), 7.10 (d, J = 8.7 Hz, 2H), 6.60 (t, J = 2.2 Hz, 1H), 6.50 (d, J = 2.2 Hz, 2H), 5.46 (s, 2H), 5.30 – 5.24 (m, 2H), 4.94 (s, 4H), 1.33 (s, 16H). ¹³C NMR (101 MHz, CDCl₃) δ 190.85 , 163.26 , 160.75 , 151.46 , 143.78 , 136.53 , 133.40 , 132.12 , 130.51 , 127.71 , 125.75 , 123.01 , 115.22 , 107.28 , 102.17 , 70.25 , 62.36 , 54.50 , 34.76 , 31.47 . HRMS (MALDI-TOF): C_{39}H_{43}N_{3}O_{4} [M]^+: calcd 618.3326; found 618.3305.

Preparation of S₄: S₃ (3g, 4.8 mmol) and (4-(prop-2-yn-1-yl oxy)phenyl)methanamine (0.78 g, 4.8 mmol) was dissolved in MeOH, and the reaction mixture was refluxed overnight. It was then cooled to 0 °C followed with the addition of NaBH₄. The reaction mixture was stirred at room temperature overnight. After the reaction, MeOH was removed in vacuum, and the residue was redissolved in EtOAc and extracted with
water. The combined organic layers were dried over anhydrous MgSO$_4$ and concentrated in vacuum to give a yellow oil. The yellow oil was purified by column chromatography (SiO$_2$; Hexane/EtOAc 1:2) yielding a pale-yellow oil (2.8 g, 76%). $^1$H NMR (400 MHz, CDCl$_3$) δ 7.54 (s, 1H), 7.41 (d, $J = 8.4$ Hz, 4H), 7.33 (d, $J = 8.3$ Hz, 4H), 7.27 (s, 1H), 7.24 (d, $J = 9.0$ Hz, 3H), 6.93 (dd, $J = 8.7$, 1.6 Hz, 4H), 6.59 (t, $J = 2.2$ Hz, 1H), 6.50 (d, $J = 2.2$ Hz, 2H), 5.44 (s, 2H), 5.18 (s, 2H), 4.94 (s, 4H), 4.68 (d, $J = 2.4$ Hz, 2H), 3.72 (d, $J = 5.5$ Hz, 4H), 2.51 (t, $J = 2.4$ Hz, 1H), 1.32 (s, 16H).

$^{13}$C NMR (101 MHz, CDCl$_3$) δ 160.71, 157.42, 156.73, 151.41, 144.88, 136.70, 133.46, 129.52, 129.47, 127.72, 125.74, 122.76, 114.96, 114.83, 107.25, 102.20, 78.71, 75.57, 70.23, 62.33, 56.02, 54.44, 52.59, 34.76, 31.48. HRMS (MALDI-TOF): C$_{49}$H$_{54}$N$_4$O$_4$ [M]$^+$: calcd 762.4140; found 762.4189.

Preparation of 1-H·PF$_6$: S4 (2 g, 2.6 mmol) was dissolved in MeOH/CH$_2$Cl$_2$ (2:1) solution. Concentrated HCl was added until the pH reached 2. The MeOH was removed in vacuum. The residue was redissolved in 50 mL acetone, and saturated NH$_4$PF$_6$ solution was added until all solid was dissolved, and stirred for 30 minutes. The reaction mixture was then concentrated in vacuum to give yellow gel. The residue was redissolved in CH$_2$Cl$_2$ and extracted with water. The combined organic layers were dried over anhydrous MgSO$_4$ and concentrated in vacuum to give a pale-yellow solid in quantitative yield. $^1$H NMR (400 MHz, CD$_3$CN) δ 7.88 (s, 1H), 7.45 – 7.36 (m, 8H), 7.32 (d, $J = 8.4$ Hz, 4H), 7.05 (dd, $J = 15.2$, 8.7 Hz, 4H), 6.54 (d, $J = 2.2$ Hz, 1H), 6.51 (d, $J = 2.2$ Hz, 2H), 5.45 (s, 2H), 5.16 (s, 2H), 4.98 (s, 4H), 4.76 (d, $J = 2.4$ Hz, 2H), 4.12 (d, $J = 4.0$ Hz, 4H), 2.83 (t, $J = 2.4$ Hz, 1H), 1.30 (s, 16H). $^{13}$C NMR (101 MHz, CD$_3$CN) δ 161.24, 160.31, 159.39, 152.09, 144.37, 139.06, 134.98, 132.81, 132.79, 128.76, 126.44, 125.06, 124.39, 123.90, 116.12, 116.08, 107.97, 102.58, 77.15, 70.56, 62.47, 56.57, 54.44, 51.84, 51.75, 35.23, 31.55.

**Synthesis of 2-H·PF$_6$ and 3-H·PF$_6$**

Preparation of 2-H·PF$_6$: 1-H·PF$_6$ (0.40 g, 0.86 mmol), S2 (0.68 g, 0.93 mmol), and
**azido dendron** (0.81 g, 1.80 mmol) were dissolved in 8 mL degassed CH$_2$Cl$_2$. The reaction mixture was stirred for 2 hours before the addition of Cu(MeCN)$_4$PF$_6$ (0.66 g, 1.78 mmol). The reaction was then stirred for 2 days at room temperature. The reaction mixture was diluted with 80 mL CH$_2$Cl$_2$, and extracted with (1) 50 mL 2 M NaCN$_{aq}$, (2) 50 mL 1 M HCl$_{aq}$, and (3) NH$_4$PF$_6$_{aq}. The combined organic layers were dried over anhydrous MgSO$_4$ and concentrated in vacuum to give a yellow solid. The yellow solid was purified by column chromatography (SiO$_2$; CHCl$_3$/MeOH 40 : 1 à 30 : 1) yielding a white solid (1.30 g, 72 %). $^1$H NMR (400 MHz, CD$_3$CN) δ 7.84 (s, 2H), 7.64 (dd, $J = 8.5, 2.0$ Hz, 2H), 7.40 (t, $J = 7.5$ Hz, 12H), 7.31 (d, $J = 8.3$ Hz, 8H), 7.24 (d, $J = 8.7$ Hz, 4H), 6.81 (d, $J = 8.7$ Hz, 2H), 6.74 (d, $J = 8.6$ Hz, 4H), 6.55 (d, $J = 2.2$ Hz, 2H), 6.52 (d, $J = 2.2$ Hz, 4H), 5.44 (s, 4H), 5.00 (s, 4H), 4.97 (s, 8H), 4.57 (t, $J = 6.1$ Hz, 4H), 4.06 (d, $J = 5.2$ Hz, 8H), 3.82 – 3.73 (m, 8H), 3.64 (d, $J = 5.5$ Hz, 8H), 2.76 (s, 8H), 1.29 (s, 36H). $^{13}$C NMR (101 MHz, CD$_3$CN) δ 171.70 , 162.96 , 161.70 , 154.41 , 152.52 , 148.92 , 144.89 , 139.54 , 135.39 , 132.17 , 129.12 , 126.83 , 126.17 , 125.76 , 125.32 , 116.06 , 114.47 , 113.60 , 108.45 , 102.97 , 79.56 , 72.19 , 71.26 , 71.15 , 71.00 , 69.90 , 69.64 , 62.76 , 54.80 , 53.11 , 35.61 , 31.96 , 26.86 . HRMS (MALDI-TOF): C$_{112}$H$_{128}$N$_9$O$_{22}$P$_6$ [M–PF$_6$]$^+$: calcd 1951.9200; found 1951.8650.

Preparation of 3-H·PF$_6$: 2-H·PF$_6$ (0.80 g, 0.38 mmol) was dissolved in 6 mL CH$_2$Cl$_2$, (4-(azidomethyl)phenyl)methanamine (0.20 g, 1.20 mmol) was added. The reaction mixture was stirred overnight at room temperature. The reaction mixture was diluted with 20 mL CH$_2$Cl$_2$ and extracted with 20 mL 1 M HCl$_{aq}$ and then NH$_4$PF$_6$_{aq}. The combined organic layers were dried over anhydrous MgSO$_4$ and concentrated in vacuum to give a yellow solid. The yellow solid was purified by column chromatography (SiO$_2$; CH$_2$Cl$_2$/MeOH 25 : 1), yielding a pale yellow solid (0.70 g, 84 %). $^1$H NMR (400 MHz, CD$_3$CN) δ 7.84 (s, 2H), 7.70 (t, $J = 6.0$ Hz, 2H), 7.40 (d, $J = 8.4$ Hz, 8H), 7.35 – 7.27 (m, 16H), 7.22 (dd, $J = 8.7, 2.5$ Hz, 10H), 6.71 (t, $J = 8.1$ Hz, 6H), 6.53 (s, 6H), 5.41 (s, 4H), 4.96 (s, 8H), 4.92 (s, 4H), 4.56 (t, $J = 6.7$ Hz, 4H), 4.45 (d, $J = 6.0$ Hz, 4H), 4.27 (s, 4H), 4.01 (d, $J = 5.5$ Hz, 8H), 3.73 (s, 8H), 3.62 (d, $J$
= 3.8 Hz, 8H), 1.28 (s, 36H). $^1$H NMR (101 MHz, CD$_3$CN) δ 167.21, 161.32, 159.64, 152.14, 150.75, 148.05, 144.38, 140.84, 139.03, 135.46, 135.00, 131.78, 129.50, 128.84, 128.76, 128.23, 126.47, 125.47, 125.10, 121.23, 115.54, 112.47, 112.16, 108.17, 102.63, 71.82, 71.74, 71.05, 70.62, 68.98, 68.83, 62.14, 54.90, 54.51, 52.71, 43.72, 35.25, 31.72, 31.60. 

HRMS (MALDI-TOF): C$_{120}$H$_{138}$N$_{15}$O$_{16}$PF$_6$ [M–PF$_6$]$^+$: calcd 2046.0519; found 2046.5208.

**Synthesis of G1-H$_3$·3PF$_6$**

Preparation of G1-H$_3$·3PF$_6$: 3-H·PF$_6$ (0.65 g, 0.30 mmol), DB24C8 (0.470 g, 1.00 mmol), and 1-H·PF$_6$ (0.55 g, 0.60 mmol) were dissolved in 8 mL degassed CH$_2$Cl$_2$. The reaction mixture was stirred for 3 hours before the addition of Cu(MeCN)$_4$PF$_6$ (0.25 g, 0.67 mmol). The reaction was then stirred for 4 days at room temperature. The reaction mixture was diluted with 70 mL CH$_2$Cl$_2$, and extracted with (1) 50 mL 2 M NaCN(aq), (2) 50 mL 1 M HCl(aq), and (3) NH$_4$PF$_6$(aq). The combined organic layers were dried over anhydrous MgSO$_4$ and concentrated in vacuum to give a yellow solid. The yellow solid was purified by column chromatography (SiO$_2$; CH$_2$Cl$_2$/EtOAc 1 : 1) yielding a white solid (0.92 g, 63 %). $^1$H NMR (400 MHz, CD$_3$CN) δ 7.84 (s, 2H),
7.82 (s, 2H), 7.78 (s, 2H), 7.69 (t, J = 6.1 Hz, 2H), 7.41 – 7.37 (m, 18H), 7.31 – 7.27 (m, 18H), 7.27 (s, 6H), 7.24 – 7.19 (m, 20H), 6.82 – 6.78 (m, 8H), 6.75 – 6.72 (m, 12H), 6.71 – 6.66 (m, 8H), 6.52 (s, 12H), 5.43 (s, 8H), 5.38 (s, 4H), 5.01 (s, 4H), 4.95 (d, J = 9.9 Hz, 20H), 4.89 (s, 4H), 4.53 (d, J = 6.3 Hz, 12H), 4.42 (d, J = 6.0 Hz, 4H), 4.02 – 3.94 (m, 24H), 3.74 – 3.66 (m, 24H), 3.61 (d, J = 11.9 Hz, 8H), 3.50 (s, 16H), 1.27 (d, J = 5.1 Hz, 72H).

13C NMR (101 MHz, CD3CN) δ 161.24, 159.61, 159.55, 152.07, 150.69, 148.33, 147.95, 144.44, 144.29, 144.09, 139.05, 138.97, 135.36, 134.93, 134.91, 131.84, 131.71, 129.10, 128.88, 128.72, 128.17, 126.42, 125.51, 125.47, 125.40, 125.10, 124.91, 124.79, 122.47, 122.16, 121.17, 115.52, 115.48, 113.32, 112.39, 112.06, 108.11, 108.04, 102.55, 71.56, 71.08, 70.56, 68.77, 62.24, 54.44, 54.20, 52.65, 35.22, 31.58.


Synthesis of Me6G1-H3·9PF6

Preparation of Methylated G1 type III-C [4]rotaxane dendrimer: G1-H3·3PF6 (0.118 g, 0.002 mmol) was dissolved in excess MeI (3 mL) and stirred at room temperature for 5 days. After the reaction, MeI was removed in vacuum. The residue was redissolved in CH2Cl2 and saturated NH4PF6 solution was added. The reaction mixture was stirred for 6 hours, and extract with CH2Cl2. The combined organic layers were dried over anhydrous MgSO4 and concentrated in vacuum to give a yellow solid in quantitative yield. 1H NMR (400 MHz, CD3CN) δ 8.52 (s, 2H), 8.42 (d, J = 3.2 Hz, 4H), 7.43 (dd, J = 8.9, 7.2 Hz, 18H), 7.40 – 7.26 (m, 44H), 6.82 – 6.75 (m, 28H), 6.67 (s, 12H), 5.69 – 5.61 (m, 12H), 5.14 – 5.07 (m, 12H), 5.03 (s, 8H), 5.00 (s, 8H), 4.64 (s, 12H), 4.43 (d, J = 6.1 Hz, 4H), 4.23 (s, 6H), 4.21 (s, 6H), 4.18 (s, 6H), 4.09 – 4.01 (m, 24H), 3.84 – 3.75 (m, 24H), 3.70 (d, J = 10.2 Hz, 8H), 3.62 (s, 16H), 1.29 (d, J = 6.1 Hz, 72H). 13C NMR (101 MHz, CD3CN) δ 166.11, 160.16, 160.14, 157.12, 157.09, 150.91, 149.56, 147.00, 146.63, 141.04, 139.63, 139.54, 139.44, 133.80, 133.44, 130.75, 130.69, 130.23, 129.13, 128.99, 128.96, 128.92, 127.76, 127.44, 126.62, 125.52, 125.39, 125.15, 120.75, 120.11, 114.16, 111.95, 111.28, 110.48, 107.74, 102.15, 102.10, 70.32, 69.82, 69.60, 69.41, 69.39, 67.72, 67.39, 57.70, 57.63, 56.68, 56.65, 56.51, 54.00, 51.30, 42.21, 38.34,
38.26 , 38.23 , 33.88 , 30.19 . HRMS (ESI): C_{272}H_{330}N_{23}O_{40}P_{9}F_{54} [M–9PF_{6}–H]^{8+}: calcd 570.6875; found 570.0549.

**Synthesis of 4-H·3PF_{6} and 5-H·3PF_{6}**

Preparation of 4-H·3PF_{6}: 3-H·PF_{6} (0.80 g, 0.37 mmol), S2 (0.82 g, 1.10 mmol), and 1-H·PF_{6} (0.67 g, 0.74 mmol) were dissolved in 7 mL degassed CH_{2}Cl_{2}. The reaction mixture was stirred for 3 hours before the addition of Cu(MeCN)_{4}PF_{6} (0.3 g, 0.80 mmol). The reaction was then stirred for 7 days at room temperature. The reaction mixture was diluted with 70 mL CH_{2}Cl_{2}, and extracted with (1) 50 mL 2 M NaCN(aq), (2) 50 mL 1 M HCl(aq), and (3) NH_{4}PF_{6}(aq). The combined organic layers were dried over anhydrous MgSO_{4} and concentrated in vacuum to give a yellow solid. The yellow solid was purified by column chromatography (SiO_{2}; CH_{2}Cl_{2}/EtOAc 1:1) yielding a white solid (1.03 g, 53 %). ^{1}H NMR (400 MHz, CD_{3}CN) δ 7.86 – 7.79 (m, 6H), 7.60 (s, 2H), 7.41 – 7.35 (m, 2H), 7.32 – 7.27 (m, 2H), 7.22 (d, J = 8.6 Hz, 16H), 7.17 (d, J = 7.8 Hz, 4H), 6.78 (d, J = 8.7 Hz, 2H), 6.74 – 6.66 (m, 12H), 6.54 – 6.51 (m, 12H), 5.40 (d, J = 17.7 Hz, 12H), 4.98 (s, 4H), 4.97 – 4.92 (m, 20H), 4.88 (s,
4.55 (s, 12H), 4.41 (s, 4H), 4.00 (d, \(J = 18.7\) Hz, 24H), 3.73 (s, 24H), 3.65 – 3.58 (m, 24H), 2.75 (s, 16H), 1.27 (d, \(J = 5.8\) Hz, 72H). \(^{13}\)C NMR (101 MHz, CD\(_3\)CN) \(\delta\)

171.34 , 162.53 , 161.23 , 159.62 , 153.94 , 152.07 , 150.69 , 148.43 , 147.93 , 144.43 , 141.02 , 139.12 , 138.96 , 135.42 , 134.93 , 134.90 , 132.83 , 131.73 , 126.42 , 125.75 , 125.40 , 125.13 , 124.99 , 121.21 , 115.56 , 115.44 , 113.97 , 113.14 , 112.38 , 112.03 , 108.10 , 107.97 , 102.54 , 102.47 , 71.76 , 71.69 , 70.98 , 70.83 , 70.73 , 70.55 , 69.43 , 69.14 , 68.91 , 68.74 , 62.25 , 62.03 , 55.37 , 54.46 , 54.39 , 54.16 , 52.65 , 43.63 , 35.22 , 31.58 .

HRMS (ESI): C\(_{286}\)H\(_{324}\)N\(_2\)O\(_5\)P\(_3\)F\(_{18}\) [M–3PF\(_6\)]\(^{3+}\): calcd 1678.4518; found 1678.4464.

Preparation of 5-H\(_3\)·3PF\(_6\): 4-H\(_3\)·3PF\(_6\) (0.92 g, 0.17 mmol) was dissolved in 5 mL CH\(_2\)Cl\(_2\), (4-(azidomethyl)phenyl)methanamine (0.16 g, 0.98 mmol) was added. The reaction mixture was stirred overnight at room temperature. The reaction mixture was diluted with 20 mL CH\(_2\)Cl\(_2\) and extracted with 20 mL 1 M HCl(aq) and then NH\(_4\)PF\(_6\)(aq). The combined organic layers were dried over anhydrous MgSO\(_4\) and concentrated in vacuum to give a yellow solid. The yellow solid was purified by column chromatography (SiO\(_2\); CH\(_2\)Cl\(_2\)/MeOH 20 : 1) yielding a pale yellow solid (0.80 g, 83 %). \(^1\)H NMR (400 MHz, CD\(_3\)CN) \(\delta\) 7.84 (s, 2H), 7.81 (d, \(J = 3.7\) Hz, 4H), 7.75 (s, 4H), 7.40 – 7.36 (m, 18H), 7.31 – 7.24 (m, 34H), 7.21 – 7.18 (m, 24H), 7.14 (s, 4H), 6.69 – 6.63 (m, 14H), 6.54 – 6.50 (m, 12H), 5.42 – 5.36 (m, 12H), 4.93 (d, \(J = 7.5\) Hz, 20H), 4.89 (s, 4H), 4.86 (d, \(J = 3.1\) Hz, 4H), 4.53 (s, 12H), 4.42 (d, \(J = 5.9\) Hz, 8H), 4.39 (d, \(J = 6.2\) Hz, 4H), 4.25 (s, 8H), 4.00 – 3.91 (m, 24H), 3.69 (d, \(J = 20.0\) Hz, 24H), 3.59 (d, \(J = 15.6\) Hz, 24H), 1.26 (d, \(J = 5.0\) Hz, 72H). \(^{13}\)C NMR (101 MHz, CD\(_3\)CN) \(\delta\) 167.20 , 161.24 , 159.55 , 159.52 , 152.07 , 150.67 , 147.94 , 144.27 , 144.07 , 140.75 , 138.95 , 135.38 , 135.24 , 134.91 , 134.90 , 131.69 , 129.44 , 129.11 , 128.82 , 128.74 , 128.72 , 128.21 , 126.42 , 125.39 , 125.12 , 124.96 , 121.20 , 115.44 , 112.40 , 112.08 , 108.11 , 107.96 , 102.53 , 71.78 , 71.67 , 70.98 , 70.56 , 68.91 , 68.72 , 62.04 , 55.37 , 54.84 , 54.46 , 54.21 , 52.63 , 43.69 , 35.22 , 31.58 .

Synthesis of $\text{G2-H}_7\cdot\text{7PF}_6$

Preparation of $\text{G2-H}_7\cdot\text{7PF}_6$: $\text{5-H}_3\cdot\text{3PF}_6$ (0.59 g, 0.10 mmol), DB24C8 (0.28 g, 0.62 mmol), and $\text{1-H-PF}_6$ (0.38 g, 0.42 mmol) were dissolved in 6 mL degassed CH$_2$Cl$_2$. The reaction mixture was stirred for 3 hours before the addition of Cu(MeCN)$_4$PF$_6$ (0.15 g, 0.40 mmol). The reaction was then stirred for 7 days at room temperature. The reaction mixture was diluted with 70 mL CH$_2$Cl$_2$, and extracted with (1) 50 mL 2 M NaCN$_{aq}$, (2) 50 mL 1 M HCl$_{aq}$, and (3) NH$_4$PF$_6$$_{aq}$. The combined organic layers were dried over anhydrous MgSO$_4$ and concentrated in vacuum to give a yellow solid. The yellow solid was purified by column chromatography (SiO$_2$; CH$_2$Cl$_2$/EtOAc 1 : 1) yielding a white solid (0.62 g, 56 %). $^1$H NMR (400 MHz, CD$_3$CN) $\delta$ 7.89 – 7.77 (m,
20H), 7.42 – 7.20 (m, 142H), 6.79 (d, J = 26.6 Hz, 54H), 6.55 (s, 22H), 5.47 – 5.36 (m, 28H), 5.04 – 4.88 (m, 60H), 4.56 (br, 24H), 4.42 (br, 12H), 3.99 (br, 58H), 3.77 – 3.56 (m, 80H), 3.52 (br, 30H), 1.32 – 1.25 (m, 144H).

13C NMR (101 MHz, CD3CN) δ 165.85, 159.92, 159.92, 158.29, 158.23, 150.75, 149.36, 147.01, 146.63, 143.11, 142.93, 139.72, 137.71, 135.01, 130.51, 130.35, 127.75, 127.52, 127.37, 125.08, 124.20, 124.13, 123.80, 123.58, 123.50, 120.83, 119.87, 112.00, 110.67, 106.79, 106.73, 101.23, 70.22, 69.74, 69.63, 69.23, 67.59, 67.44, 60.91, 60.69, 54.00, 53.08, 52.84, 51.30, 42.28, 33.86, 30.22.


**Synthesis of Me14G2-H·22PF6**

Preparation of Methylated G2 type III-C [8]rotaxane dendrimer: G2-H·7PF6 (0.10 g, 9 µmol) was dissolved in excess MeI (3 mL) and stirred at room temperature for 5 days. After the reaction, MeI was removed in vacuum. The residue was redissolved in CH2Cl2 and saturated NH4PF6 solution was added. The reaction mixture was stirred for 10 hours, and extract with CH2Cl2. The combined organic layers were dried over anhydrous MgSO4 and concentrated in vacuum to give a yellow solid in quantitative yield. 1H NMR (400 MHz, CD3CN) δ 8.52 (d, J = 3.6 Hz, 4H), 8.48 (s, 2H), 8.43 (d, J = 1.8 Hz, 8H), 7.47 – 7.29 (m, 140H), 6.82 – 6.75 (m, 50H), 6.69 – 6.61 (m, 28H), 5.69 – 5.60 (m, 28H), 5.11 (d, J = 9.3 Hz, 22H), 5.01 (d, J = 16.1 Hz, 38H), 4.63 (br, 24H), 4.45 – 4.44 (br, 12H), 4.23 – 4.18 (m, 42H), 4.12 – 3.98 (m, 58H), 3.87 – 3.66 (m, 80H), 3.62 (s, 28H), 1.29 (d, J = 7.5 Hz, 144H). 13C NMR (101 MHz, CD3CN) δ 170.33, 166.11, 160.16, 160.13, 157.12, 157.09, 150.92, 149.56, 147.00, 146.04, 141.03, 139.65, 139.53, 139.44, 133.79, 133.45, 130.76, 130.69, 130.23, 129.12, 128.99, 128.93, 127.80, 127.73, 127.44, 126.63, 125.52, 125.15, 120.75, 120.13, 114.37, 114.16, 111.95, 111.31, 107.74, 102.16.
102.10, 70.32, 69.82, 69.41, 67.73, 67.39, 59.64, 57.70, 56.68, 56.51, 54.00, 51.30, 42.23, 38.34, 38.27, 36.23, 38.20, 33.88, 30.19.

HRMS (ESI): C$_{608}$H$_{734}$N$_{55}$O$_{92}$P$_{21}$F$_{126}$ [M−10PF$_6$]$^{10+}$: calcd 1188.2124; found 1187.7024.

**Deprotonation of Rotaxane Dendrimers**

**General procedure:** Rotaxane dendrimers were dissolved in 1 mL MeCN, BEMP resin (2-tert-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine, polymer-bound (Sigma-Aldrich)) was added and stirred overnight at room temperature. CH$_2$Cl$_2$ was added to redissolve the deprotonated neutral rotaxane dendrimer, BEMP resin was filtered, and the combined organic layers were concentrated in vacuum to afford the deprotonated G(n) rotaxane dendrimer as a yellow solid in quantitative yield.

![Figure S1. Deprotonation of G1-H$_3$·3PF$_6$ to G1.](image1)

![Figure S2. Deprotonation of Me$_6$G1-H$_3$·9PF$_6$ to Me$_6$G1-6PF$_6$.](image2)
Figure S3. Deprotonation of $\text{G}_2\text{H}_7\cdot7\text{PF}_6$ to $\text{G}_2$.

Figure S4. Deprotonation of $\text{Me}_{14}\text{G}_2\text{H}_7\cdot21\text{PF}_6$ to $\text{Me}_{14}\text{G}_2\cdot14\text{PF}_6$. 
Stacked $^1$H NMR spectra

Figure S5. Stacked $^1$H NMR spectra (400 MHz, CD$_3$CN) of a) 2-H·PF$_6$ and b) 3-H·PF$_6$. The peaks highlight as blue corresponding to the characteristic peaks. Asterisk: solvent residue signal.
Figure S6. Stacked $^1$H NMR spectra (400 MHz, CD$_3$CN) of a) 4-H·PF$_6$ and b) 5-H·PF$_6$. The peaks highlight as blue corresponding to the characteristic peaks.

Figure S7. Stacked $^1$H NMR spectra (400 MHz, CD$_3$CN) of a) G1 type III-C [4]rotaxane dendrimer, and b) G2 type III-C [8]rotaxane dendrimer.
Figure S8. $^1$H NMR spectra (400 MHz, CD$_2$Cl$_2$) of a) G$_2$·H$_7$·7PF$_6$ and b) G$_2$.

Figure S9. $^1$H NMR spectra (400 MHz, CD$_2$Cl$_2$) of a) Me$_{14}$G$_2$·H$_7$·21PF$_6$ and b) Me$_{14}$G$_2$·14PF$_6$. 
Acid-base switching of Rotaxane Dendrimers

Figure S10. Stacked $^1$H NMR spectra (400 MHz, CD$_2$Cl$_2$) G1 [4]rotaxane dendrimer (G1-H$_3 \cdot 3$PF$_6$) after cumulative addition of DBU (Concentration: 1 mM). Blue color represents the triazole, red color represents the protons adjacent to DBA.
Figure S11. Stacked $^1$H NMR spectra (400 MHz, CD$_2$Cl$_2$) Neutral G1 [4]rotaxane dendrimer (G1) after cumulative addition of TFA (Concentration: 1 mM). Blue color represents the triazole, red color represents the protons adjacent to DBA, green color represents the benzyl protons next to triazoles.
Figure S12. Stacked $^1$H NMR spectra (400 MHz, CD$_2$Cl$_2$) G2 [8]rotaxane dendrimer (G2-H$_7$·7PF$_6$) after cumulative addition of DBU (Concentration: 1 mM). Blue color represents the triazole, red color represents the protons adjacent to DBA.
Figure S13. Stacked $^1$H NMR spectra (400 MHz, CD$_2$Cl$_2$) Neutral G2 [8]rotaxane dendrimer (G2) after cumulative addition of TFA (Concentration: 1 mM). Blue color represents the triazole, red color represents the protons adjacent to DBA, green color represents the benzyl protons next to triazoles.
Figure S14. Stacked $^1$H NMR spectra (400 MHz, CD$_2$Cl$_2$) G1 methylated [4]rotaxane dendrimer (Me$_6$G1-H$_3$·9PF$_6$) after cumulative addition of DBU (concentration: 1 mM). Blue color represents the triazole, red color represents the protons adjacent to DBA, green color represents the methyl protons on triazolium.
Figure S15. Stacked $^1$H NMR spectra (400 MHz, CD$_2$Cl$_2$) G1 methylated [4]rotaxane dendrimer (Me$_6$G1-6PF$_6$) after cumulative addition of TFA (concentration: 1 mM). Blue color represents the triazole, red color represents the protons adjacent to DBA, green color represents the methyl protons on triazolium.
Figure S16. Stacked $^1$H NMR spectra (400 MHz, CD$_2$Cl$_2$) G2 methylated [8]rotaxane dendrimer ($\text{Me}_{14}\text{G2-H}_7\cdot\text{21PF}_6$) after cumulative addition of DBU (concentration: 1 mM). Blue color represents the triazole, red color represents the protons adjacent to DBA, green color represents the methyl protons on triazolium.
Figure S17. Stacked $^1$H NMR spectra (400 MHz, CD$_2$Cl$_2$) G2 methylated [8]rotaxane dendrimer ($\text{Me}_{14}\text{G2-14PF}_6$) after cumulative addition of TFA (concentration: 1 mM). Blue color represents the triazole, red color represents the protons adjacent to DBA, green color represents the methyl protons on triazolium.
Figure S18. Stacked $^1$H NMR spectra (400 MHz, CD$_3$CN) of G1 [4]rotaxane dendrimer (G1-H$_3$·3PF$_6$) after alternatively addition of DBU and TFA with 5 cycles (concentration: 1 mM).
Figure S19. Stacked $^1$H NMR spectra (400 MHz, CD$_3$CN) of G1 methylated [4]rotaxane dendrimer (Me$_6$G1-6PF$_6$) after alternatively addition of DBU and TFA with 5 cycles (concentration: 1 mM).
Figure S20. Stacked $^1$H NMR spectra (400 MHz, CD$_3$CN) of G2 [8]rotaxane dendrimer (G2-H$_7$·7PF$_6$) after alternatively addition of DBU and TFA with 3 cycles (concentration: 1 mM).

Figure S21. The appearance of NMR titration of G2 [8]rotaxane dendrimer (G2-H$_7$·7PF$_6$) in CD$_3$CN. After the addition of DBU, the G2 not dissolved in CD$_3$CN thus the obtained NMR showed very board peaks. After the addition of TFA, G2 turned to G2-H$_7$·7PF$_6$ and dissolved in CD$_3$CN again.
**Figure S22.** Stacked $^1$H NMR spectra (400 MHz, CD$_2$Cl$_2$) of G2 [8]rotaxane dendrimer (G2-H$_7$·7PF$_6$) after alternatively addition of DBU and TFA with 5 cycles (concentration: 1 mM).

**Figure S23.** The appearance of NMR titration of G2 [8]rotaxane dendrimer (G2-H$_7$·7PF$_6$) in CD$_2$Cl$_2$. After the addition of DBU, the G2 dissolved well in CD$_2$Cl$_2$, which is better to observed the switching cycles in CH$_2$Cl$_2$. 
**Figure S24.** Stacked $^1$H NMR spectra (400 MHz, CD$_2$Cl$_2$) of G2 methylated [8]rotaxane dendrimer ($\text{Me}_{14}\text{G2-H}_7\cdot21\text{PF}_6$) after alternatively addition of DBU and TFA with 5 cycles (concentration: 1 mM).

**Figure S25.** The appearance of NMR titration of G2 methylated [8]rotaxane dendrimer ($\text{Me}_{14}\text{G2-H}_7\cdot21\text{PF}_6$) in CD$_3$CN. In comparison to G2 [8]rotaxane dendrimer ($\text{G2-H}_7\cdot7\text{PF}_6$), the deprotonated, highly charged rotaxane dendrimer was solvated better in CD$_3$CN.
Figure S26. Stacked $^1$H NMR spectra (400 MHz, CD$_2$Cl$_2$) G1 (Concentration: 1 mM) after cumulative addition of chlorambucil. Red color represents the proton signal of guest (chlorambucil) molecules. Green color represents the proton signal adjacent to DBA. Blue color represents the proton signal of triazole.
Figure S27. Stacked $^1$H NMR spectra (400 MHz, CD$_2$Cl$_2$) G2 (Concentration: 1 mM) after cumulative addition of chlorambucil. Red color represents the proton signal of guest (chlorambucil) molecules. Green color represents the proton signal adjacent to DBA. Blue color represents the proton signal of triazole.
**Figure S28.** Stacked $^1$H NMR spectra (400 MHz, CD$_2$Cl$_2$) G1 (Concentration: 1 mM) after cumulative addition of lithocholic acid. Red color represents the proton signal of guest (lithocholic acid) molecules. Green color represents the proton signal adjacent to DBA. Blue color represents the proton signal of triazole.
Figure S29. Stacked $^1$H NMR spectra (400 MHz, CD$_2$Cl$_2$) G2 (Concentration: 1 mM) after cumulative addition of lithocholic acid. Red color represents the proton signal of guest (lithocholic acid) molecules. Green color represents the proton signal adjacent to DBA. Blue color represents the proton signal of triazole.

The binding of two guest molecules (chlorambucil and lithocholic acid) with deprotonation type III-C rotaxane dendrimers have been studied by NMR titration. Chlorambucil is an anti-cancer drug to treat leukemia, while lithocholic acid$^{53}$ is a bile acid which showed the anticancer activity toward breast cancer. Both chlorambucil and lithocholic acid contain carboxylic acid group, which is able to bind to the amine groups inside the rotaxane dendrimers through electrostatic interaction and hydrophobic interaction.

In G1, by titrating chlorambucil the unique hydrogen-bonded DBA proton signals were restored (green color) until 3.0 equivalents of guest molecules, indicating the DB24C8 were still located at the triazoles, while the DBA interacts with the carboxylate (chlorambucil). When excess guest (acid) was added, the carboxylate reprotonated to carboxylic acid and the DB24C8 moved from triazoles back to the original site DBA. From the NMR titration result, G1 was able to bind with 2 chlorambucil and G2 was able to bind with 6 guest molecules.

Similar NMR shifting have been observed in lithocholic acid, revealing lithocholic acid was also able to bind with rotaxane dendrimers. From the NMR result,
G1 was able to bind with 3 lithocholic acid while G2 was able to bind with 7.

**AFM Analysis**

Figure S30. AFM images of type III-C rotaxane dendrimers on mica surface (A–F) (1 µm x 1 µm) or silicon surface (G–H) (1 µm x 1 µm) and height profiles.
**DLS Experiment**

Dynamic light scattering (DLS) was performed by Beckman Coulter DelsaMax CORE, and the experiment was performed in dichloromethane.

![DLS Experiment Diagram](image)

**Figure S31.** Dynamic light scattering (DLS) diagram of G1-H3·3PF6, G1, G2-H7·7PF6 and G2 in CH2Cl2.

![DLS Experiment Diagram](image)

**Figure S32.** Dynamic light scattering (DLS) diagram of G1-H3·3PF6 and MeG1-H3·pPF6 (A) and G2-H7·7PF6 and MeG2-H7·21PF6 in CH2Cl2.

**Table S1.** The hydrodynamic diameter of rotaxane dendrimer from DLS.

<table>
<thead>
<tr>
<th>G(n) rotaxane dendrimer</th>
<th>Diameter (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1-H3·3PF6</td>
<td>2.2</td>
</tr>
<tr>
<td>G1</td>
<td>2.3</td>
</tr>
<tr>
<td>Me6G1-H3·6PF6</td>
<td>2.1</td>
</tr>
<tr>
<td>G2-H7·7PF6</td>
<td>5.6</td>
</tr>
<tr>
<td>G2</td>
<td>4.8</td>
</tr>
<tr>
<td>Me14G2-H7·21PF6</td>
<td>5.3</td>
</tr>
</tbody>
</table>
Brief Summary of DLS Experiment

The DLS result suggested that after the average size of all G1-related rotaxane dendrimers were about 2.1-2.2 nm. This indicated that after the deprotonation or methylation, the size of G1-H$_3$·3PF$_6$ was kept similar. While in G2-related rotaxane dendrimers, the deprotonation of G2-H$_7$·7PF$_6$ to G2 will lead to a decrease in size from 5.6 nm to 4.8 nm due to the squeezing movement of the periphery mechanical bonds to the center. The methylation of G2-H$_7$·7PF$_6$ to MeG2-H$_7$·21PF$_6$ was not give a significant change in size. Since the mechanical bonds deprotonated Me$_6$G1-6PF$_6$ and Me$_{14}$G2-14PF$_6$ were oscillating in solution, it cannot give a satisfactory result, thus we have not report its DLS result here. In conclusion, all the above results were in consistence to AFM analysis suggested that the size modulation of rotaxane dendrimers have been successfully demonstrated after the pH changes.
In vivo Experiment

Animals experiment
Male C57BL/6J mice (6 weeks old) were purchased from the Chinese University of Hong Kong. Animal experiments were carried out in accordance with the Guidelines and approved by the Animal Ethics Committee of Hong Kong Baptist University. After 12 h of fasting, G1 and G2 solution (physiological saline, 10 % DMSO) were administered by intraperitoneal injection at a single dose of 33 mg kg⁻¹. The control mice were administered with physiological saline (10 % DMSO). The mice were killed and dissected after 12h, 24h, 36h and 48h intraperitoneal injection. Lung, kidney, spleen, liver, heart and brain samples were collected. The organs were dissected immediately after the sacrifice, washed with saline to remove residual blood, and snap-frozen in liquid nitrogen before transferring to store at –80 ºC until sample preparation.

General procedures for bio-distribution of rotaxane dendrimers using MALDI-TOF MS
The organs (brain, heart, kidney, spleen, lung and liver) of the mice were isolated tissue was cut and then homogenized and extracted (according to its weight in 1 : 9 (CH₂Cl₂) ratio) by bead beater with analytical grade CH₂Cl₂. The extract was centrifuged and the supernatant was combined and concentrated following i.p. administration. MALDI-TOF experiments with standards were carried out by using dried-droplet sample preparation and spotting onto steel MALDI target plates. The sample solution was mixed with the matrix (DCTB, 10 mg/mL) solution at ration of 1 : 1, and 1 µL of the resulting mixture was then pipetted on the MALDI target plate, following by drying in a stream of nitrogen gas at room temperature. Moreover, homogeneous deposition of MALDI matrix onto tissue surface is essential to obtain the high quality MALDI MSI data.

Figure S33. Schematic diagram of in vivo experiment.
Matrix selection for the MALDI-TOF experiment

**Figure S34.** Matrix selection for G1 (A) and G2 (B) MALDI-TOF experiment.

All experiments were done in positive ion mode. The choice of an appropriate matrix is important to increase the ionization efficiency, especially for those biological samples. We have screened 10 matrix to determine the most suitable matrix for G1 and G2 analysis and these included CA, SA1, SA2, BPF, 3-HPA, DHB, THAP, FA, CHCA and DCTB. The experimental results indicated that DCTB was the most suitable matrix for MALDI-TOF analysis of the target compounds. Matrix DCTB is able to strongly interact with the target compounds (G1 and G2), efficiently transfer energy absorbed from the laser and promote ionization, meaning no low ionization rate was occurred during the MALDI-TOF experiments.

Matrix abbreviations:
Caffeic acid (CA), Sinapic acid (SA1), Succinic acid (SA2), Bisphenol F (BPF), 3-Hydroxypicolinic acid (3-HPA), 2,5-Dihydroxybenzoic acid (DHB), 2,4,6-Trihydroxyacetophenone (THAP), 4-Hydroxy-3-methoxycinnamic acid (FA), a-Cyano-4-hydroxycinnamic acid (CHCA), trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB)
Figure S35. G1 MALDI-MS spectral profiles of standard (top) and organs obtained from G1 intraperitoneal injected mice (24h). The zoom (right panel) showed the enlarged region of the mass spectra (left panel) of m/z 4470.97 ± 0.15 and 2235.09 ± 0.15. The single charged (▲) and double charged (■) ions species were labeled.
Figure S36. MALDI-MS spectral profiles of standard (top) and organs obtained from control group mice (24h). The zoom (right panel) showed the enlarged region of the mass spectra (left panel) of m/z 4470.97 ± 0.15 and 2235.09 ± 0.15. The single charged (▽) and double charged (●) ions species were labeled.
Figure S37. G2 MALDI-MS spectral profiles of standard (top) and organs obtained from G2 intraperitoneal injected mice (24h). The zoom (right panel) showed the enlarged region of the mass spectra (left panel) of m/z 10069.83 ± 0.15.
**Figure S38.** G2 MALDI-MS spectral profiles of standard (top) and organs obtained from control group mice (24h). The zoom (right panel) showed the enlarged region of the mass spectra (left panel) of m/z 10069.83 ± 0.15.

**Figure S39.** G1 MALDI-MS spectral time profiles of heart (A), brain (B) and lung (C) obtained from G1 intraperitoneal injected mice (12h, 24h, 36h and 48h).
Figure S40. G1 MALDI-MS spectral time profiles of kidney obtained from G1 intraperitoneal injected mice (12h, 24h, 36h and 48h).

Figure S41. G1 MALDI-MS spectral time profiles of liver obtained from G1 intraperitoneal injected mice (12h, 24h, 36h and 48h).
Figure S42. G1 MALDI-MS spectral time profiles of spleen obtained from G1 intraperitoneal injected mice (12h, 24h, 36h and 48h).

Figure S43. G2 MALDI-MS spectral time profiles of heart (A) and brain (B) obtained from G2 intraperitoneal injected mice (12h, 24h, 36h and 48h).
**Figure S44.** G2 MALDI-MS spectral time profiles of kidney obtained from G2 intraperitoneal injected mice (12h, 24h, 36h and 48h).

**Figure S45.** G2 MALDI-MS spectral time profiles of liver obtained from G2 intraperitoneal injected mice (12h, 24h, 36h and 48h).
Figure S46. G2 MALDI-MS spectral time profiles of lung obtained from G2 intraperitoneal injected mice (12h, 24h, 36h and 48h).

Figure S47. G2 MALDI-MS spectral time profiles of spleen obtained from G2 intraperitoneal injected mice (12h, 24h, 36h and 48h).
Summary of In Vivo Experiment

The Type III-C RDs were administrated by intraperitoneal injection and dissected after 12h, 24h, 36h, 48h. Since charged compounds were not stable in MALDI-TOF-MS, in this study, we can only use the neutral G1 and G2. In G1, peaks were clearly detectable in the kidney, spleen and liver after administration of 24h, however, cannot be found in the brain, heart and lung (Figure S27). The abundance of G1 in the spleen and liver was more predominant than that of the kidney. Interestingly, in G2, it was found in the kidney, liver, spleen and lung, while G1 cannot be observed in lung (Figure S29). As the molecular weight of G2 was much higher than G1, larger molecules administered might trapped in the lungs on the first pass effect. The amount of G1 and G2 in organ was increased gradually from 12h to 24h, and started to decrease after 36h to 48h due to the excretion from organs. Only trace amount of RDs were found in the spleen and not detectable in other organs after 48h, suggesting the retention-time of RDs in mice body was about 48h and it were able to excrete from the organs. Moreover, G1 and G2 did not undergo degradation in vivo, fragment ions or its metabolites cannot be observed in the spectra (12h to 48h) indicating that both G1 and G2 were stable in physiological environment.
NMR Spectra of Selected Compounds

Figure S48. $^1$H NMR spectrum of (400 MHz, $d_6$-DMSO) S1.

Figure S49. $^{13}$C NMR spectrum of (101 MHz, $d_6$-DMSO) S1.
Figure S50. $^1$H NMR spectrum of (400 MHz, CD$_3$CN) S2.

Figure S51. $^{13}$C NMR spectrum of (101 MHz, CD$_3$CN) S2.
Figure S52. $^1$H NMR spectrum of (400 MHz, CDCl$_3$) S3.

Figure S53. $^{13}$C NMR spectrum of (101 MHz, CDCl$_3$) S3.
Figure S54. $^1$H NMR spectrum of (400 MHz, CDCl$_3$) S4.

Figure S55. $^{13}$C NMR spectrum of (101 MHz, CDCl$_3$) S4.
Figure S56. $^1$H NMR spectrum of (400 MHz, CD$_3$CN) 1-H·PF$_6$.

Figure S57. $^{13}$C NMR spectrum of (101 MHz, CD$_3$CN) 1-H·PF$_6$. 
Figure S58. $^1$H NMR spectrum of (400 MHz, CD$_3$CN) 2-H·PF$_6$ (Asterisk: solvent residual signal).

Figure S59. $^{13}$C NMR spectrum of (101 MHz, CD$_3$CN) 2-H·PF$_6$. 
**Figure S60.** $^1$H NMR spectrum of (400 MHz, CD$_3$CN) 3-H-PF$_6$ (Asterisk: solvent residual signal).

**Figure S61.** $^{13}$C NMR spectrum of (101 MHz, CD$_3$CN) 3-H-PF$_6$. 
Figure S62. $^1$H NMR spectrum of (400 MHz, CD$_3$CN) G1-H$_3$·PF$_6$ (Asterisk: solvent residual signal).

Figure S63. $^{13}$C NMR spectrum of (101 MHz, CD$_3$CN) G1-H$_3$·PF$_6$. 
Figure S64. $^1$H NMR spectrum of (400 MHz, CD$_3$CN) Methylated G1 type III-C [4]rotaxane dendrimer Me$_6$G1-H$_3$·9PF$_6$ (Asterisk: solvent residual signal).

Figure S65. $^{13}$C NMR spectrum of (101 MHz, CD$_3$CN) Methylated G1 type III-C [4]rotaxane dendrimer Me$_6$G1-H$_3$·9PF$_6$. 
Figure S66. $^1$H NMR spectrum of (400 MHz, CD$_3$CN) 4-H·PF$_6$ (Asterisk: solvent residual signal).

Figure S67. $^{13}$C NMR spectrum of (101 MHz, CD$_3$CN) 4-H·PF$_6$. 

S59
Figure S68. $^1$H NMR spectrum of (400 MHz, CD$_3$CN) 5-H·PF$_6$ (Asterisk: solvent residual signal).

Figure S69. $^{13}$C NMR spectrum of (101 MHz, CD$_3$CN) 5-H·PF$_6$. 
Figure S70. $^1$H NMR spectrum of (400 MHz, CD$_3$CN) G2-H$_7$·7PF$_6$ (Asterisk: solvent residual signal).

Figure S71. $^{13}$C NMR spectrum of (101 MHz, CD$_3$CN) G2-H$_7$·7PF$_6$. 
Figure S72. $^1$H NMR spectrum of (400 MHz, CD$_3$CN) Methylated G2 type III-C [8]rotaxane dendrimer $\text{Me}_{14}\text{G}2\text{-H}_7\cdot\text{21PF}_6$ (Asterisk: solvent residual signal).

Figure S73. $^{13}$C NMR spectrum of (101 MHz, CD$_3$CN) Methylated G2 type III-C [8]rotaxane dendrimer $\text{Me}_{14}\text{G}2\text{-H}_7\cdot\text{21PF}_6$. 
Figure S74. $^1$H NMR spectrum of (400 MHz, CD$_2$Cl$_2$) Neutral G1 type III-C [4]rotaxane dendrimer G1.

Figure S75. $^1$H NMR spectrum of (400 MHz, CD$_2$Cl$_2$) methylated and deprotonated G1 type III-C [4]rotaxane dendrimer Me$_6$G1-6PF$_6$. 

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Figure S76. $^1$H NMR spectrum of (400 MHz, CD$_2$Cl$_2$) Neutral G2 type III-C [8]rotaxane dendrimer G2.

Figure S77. $^1$H NMR spectrum of (400 MHz, CD$_2$Cl$_2$) methylated and deprotonated G2 type III-C [8]rotaxane dendrimer Me$_{14}$G2-14PF$_6$. 
Figure S78. $^{31}$P NMR spectrum (162 MHz, CD$_2$Cl$_2$) of G1-H$_3$·3PF$_6$.

Figure S79. $^{31}$P NMR spectrum (162 MHz, CD$_2$Cl$_2$) of G1.
Figure S80. $^{31}$P NMR spectrum (162 MHz, CD$_2$Cl$_2$) of G2-H$_7$·7PF$_6$.

Figure S81. $^{31}$P NMR spectrum (162 MHz, CD$_2$Cl$_2$) of G2.
Figure S82. $^{31}P$ NMR spectrum (162 MHz, CD$_2$Cl$_2$) of methylated and deprotonated G1 type III-C [8]rotaxane dendrimer Me$_6$G1-6PF$_6$.

Figure S83. $^{31}P$ NMR spectrum (162 MHz, CD$_2$Cl$_2$) of methylated and deprotonated G2 type III-C [8]rotaxane dendrimer Me$_{14}$G2-14PF$_6$.
Mass Spectra of Selected Compounds

Figure S84. HRMS MALDI-TOF of S1.

Figure S85. HRMS MALDI-TOF of S2.
Figure S86. HRMS MALDI-TOF of S3.

Figure S87. HRMS MALDI-TOF of S4.
Figure S88. HRMS MALDI-TOF of 2-H·PF$_6$.

Figure S89. HRMS MALDI-TOF of 3-H·PF$_6$. 
Figure S90. HRMS ESI of G1-H$_3$-3PF$_6$.

Figure S91. HRMS ESI of 4-H$_3$-3PF$_6$. 
Figure S92. HRMS ESI of 5-H₃·3PF₆.

Figure S93. HRMS ESI of G2-H₇·7PF₆.
Figure S94. HRMS ESI of Me₆G1-H₃·9PF₆.

Figure S95. HRMS ESI of Me₄G2-H₇·21PF₆.
References


