Supporting information for:

Synthetic glycopolypeptides: Synthesis and self-assembly of poly(γ-benzyl-L-glutamate)-glycosylated dendron hybrids

Ariane Peyret\textsuperscript{a}, John F. Tran\textsuperscript{b}, Colin V. Bonduelle\textsuperscript{a,d}, Khalid Ferji\textsuperscript{a}, Namrata Jain\textsuperscript{b}, Sebastien Lecommandoux\textsuperscript{a*}, and Elizabeth R. Gillies\textsuperscript{b,d*}

\textsuperscript{a}Université. Bordeaux, LCPO, UMR 5629, F-33600, Pessac, France CNRS, LCPO, UMR 5629, F-33600, Pessac, France Bordeaux INP, LCPO, UMR 5629, F-33600, Pessac, France
\textsuperscript{b}Department of Chemistry, The University of Western Ontario, 1151 Richmond Street, London, Ontario, Canada N6A 5B7
\textsuperscript{c}current address: CNRS, LCC (Laboratoire de Chimie de Coordination (UPR8241)), 205 route de Narbonne, F-31077, Toulouse, France.
\textsuperscript{d}Department of Chemical and Biochemical Engineering, The University of Western Ontario, 1151 Richmond Street, London, Ontario, Canada N6A 5B9

Table of contents:

1. Additional synthesis procedures……………………………………………………pg. S2-7
2. NMR spectra of dendrons……………………………………………………..pg. S8 - S16
3. Characterization data (NMR, SEC, IR) for PBLG………………………………pg. S17 - S19
4. IR spectra for linear-dendritic block copolymers………………………………pg. S19
5. NMR spectra of linear-dendritic block copolymers……………………………pg. S20 - S23
6. Representative DLS data ………………………………………………………pg. S24 – S25
7. Additional TEM images…………………………………………………………..pg. S26 - S27
8. CAC data………………………………………………………………………….pg. S27
9. References…………………………………………………………………………pg. S28
Scheme S1. Synthesis of G3 and G4 α-galactose-functionalized dendrons.

Experimental procedures

Third generation α-galactose-functionalized dendron (12)

Amine-functionalized third generation dendron S1, prepared from G3-OH (10) by a published route\(^1\) (270 mg, 0.12 mmol) was dissolved in 5 mL of anhydrous DMF under nitrogen. Isothiocyanate 4 (crude product containing 800 mg, 3.2 mmol, 26.4 equiv. based on \(^1\)H spectroscopy) was dissolved in DMF (13 mL) in a round-bottomed flask under nitrogen equipped with a magnetic stirring bar. Freshly distilled DIPEA (4 mL, 23 mmol) was added to the isothiocyanate solution and the resulting solution was
transferred to the solution of dendron via cannula. The reaction mixture was stirred at ambient temperature for 24 hours, then the temperature was increased to 35 °C for an additional 48 hours. The solution was then concentrated to dryness and triturated with diethyl ether and dichloromethane successively to provide 1.0 g of crude material. This was redissolved in DMF (12 mL) and dialysed against DMF (2 kg/mol MWCO) for 12 hours. The dialysis solution was then changed to 70:30 DMF-pH 6.8 PBS (0.1 M) and the product was dialysed for an additional 12 hours. Finally, the product was dialysed against pure water for 4 hours, and then lyophilized to afford dendron 10 as an off-white solid (200 mg, 49% yield). $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta_{ppm}$ 7.49 (br s, 6H), 7.36 (br s, 6H), 4.77-4.72 (m, 10H), 4.60 (d, $J = 5.0$ Hz, 8H), 4.52-4.49 (m, 8H), 4.34 (d, $J = 4.54$ Hz, 8H), 4.26-4.13 (m, 28H), 3.80-3.78 (m, 8H), 3.73-3.70 (m, 8H), 3.68-3.63 (m, 8H), 3.62-3.40 (m, 40H), 2.62 (m, 1H), 2.57 (t, $J = 6.28$ Hz, 16H), 1.80-1.65 (m, 16H), 1.20 (s, 9H), 1.17 (s, 12H); IR $\nu_{max}$ (film from MeOH): 3550, 2953, 2370, 1761, 1722, 1457 cm$^{-1}$; MALDI-TOF MS: Calculated for C$_{134}$H$_{220}$N$_{16}$O$_{70}$S$_8$: 3429. No molecular ion peak was observed. Largest observed peak (1629.8) was observed corresponding to the molecular ion remaining after the cleavage of one ester group losing 4 galactosyl moieties and related dendrimer components (Calcd. for C$_{63}$H$_{105}$N$_8$O$_{33}$S$_4$: 1629.6).

**Fourth generation Boc-protected dendron (S2)**

Dendron 11$^2$ (0.50 g, 0.25 mmol, 1.9 equiv.) and Boc-protected β-alanine (6) (1.50 g, 8.2 mmol, 32 equiv.), were dissolved in distilled pyridine (8.4 mL, 40 equiv.). EDC·HCl (1.3 g, 8.2 mmol, 32 equiv.) and DMAP (1.0 g, 8.2 mmol, 32 equiv.) were then added. Anhydrous CH$_2$Cl$_2$ (20 mL) was added and the mixture was stirred at room temperature for 36 hours. The solution was then diluted with 20 mL of CH$_2$Cl$_2$, and sequentially washed with H$_2$O (1 x 20 mL), 1M HCl (3 x 20 mL), 1M Na$_2$CO$_3$ (2 x 20 mL), and brine (1 x 20 mL). The organic phase was then dried with anhydrous magnesium sulfate, filtered and concentrated in vacuo. Column chromatography (1:1 hexanes:ethyl acetate to pure ethyl acetate) of
the resulting residue provided dendron S2 as an amorphous white solid (0.87 g, yield = 76%). \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta_{\text{ppm}}\) 5.27 (br s, 1H), 4.73 (d, \(J = 2.3\) Hz, 2H), 4.27-4.16 (m, 60H), 3.40-3.34 (m, 32H), 2.65-2.63 (m, 1H), 2.53 (t, \(J = 6.6\) Hz, 32H), 1.42 (s, 144H), 1.33 (s, 3H), 1.27 (s, 6H), 1.26 (s, 12H), 1.25 (s, 24H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta_{\text{ppm}}\) 171.9, 171.8, 171.6, 171.3, 171.2, 155.8, 79.3, 64.9, 53.5, 46.7, 46.4, 36.1, 34.4, 28.4, 17.8, 17.6, 17.5, 17.4; IR \(\nu_{\text{max}}\) (film from CHCl\(_3\)): 3276, 2977, 2922, 2120, 1735, 1701, 1514 cm\(^{-1}\). MALDI-TOF MS could not be obtained for this molecule due to the tendency for the Boc groups to be cleaved in the presence of the acidic MALDI matrix.

Fourth generation amine-functionalized dendron (trifluoroacetate salt) (S3)

Dendron S2 (0.25 g, 0.050 mmol) was dissolved in 1 mL of 1:1 TFA:CH\(_2\)Cl\(_2\) and the solution was stirred at room temperature for 4 hours. The solvent was removed \textit{in vacuo} to provide dendron S3 as a viscous oil (223 mg, 94% yield). The materials was stored at -4 °C. \(^1\)H NMR (400 MHz, CD\(_3\)OD) \(\delta_{\text{ppm}}\): 4.80 (d, \(J = 2.3\) Hz, 2H), 4.35-4.28 (m, 60H), 3.25 (t, \(J = 6.4\) Hz, 32H), 2.82 (t, \(J = 7.0\) Hz, 32H), 2.75 (t, \(J = 2.3\) Hz, 1H), 1.37 (s, 3H), 1.34 (s, 6H), 1.31 (s, 12H), 1.29 (s, 24H); \(^{13}\)C NMR (150 MHz, 1:1, CD\(_3\)OD-CDC\(_3\)) \(\delta_{\text{ppm}}\): 173.9, 173.7, 173.6, 172.3, 172.2, 79.7, 78.9, 70.5, 67.6, 67.5, 55.0, 48.7, 48.4, 48.3, 37.1, 37.0, 33.5, 29.6, 19.7, 19.4, 19.3, 17.4; IR \(\nu_{\text{max}}\) (film from CHCl\(_3\)): 3516, 3244, 3000, 2852, 1741, 1681 cm\(^{-1}\); MALDI-TOF MS: Calculated for C\(_{126}\)H\(_{204}\)N\(_{16}\)O\(_{62}\)Na (M+Na): 2956.32. Found: 2955.8.

Fourth generation \(\alpha\)-galactose-functionalized dendron (13)

Dendron S4 (400 mg, 90 \(\mu\)mol) was dissolved in 5 mL of anhydrous DMF under nitrogen. Isothiocyanate 4 (crude product containing 1.2 g, 4.8 mmol, 54 equiv. based on \(^1\)H spectroscopy) was dissolved in DMF (20 mL) under nitrogen. Freshly distilled DIPEA (5.5 mL, 32 mmol) was added to the isothiocyanate solution and the resulting mixture was transferred to the solution of dendron via cannula. The reaction mixture was stirred at ambient temperature for 24 hours, then the temperature was
increased to 35 °C for an additional 48 hours. The solution was then concentrated to dryness and triturated with diethyl ether and dichloromethane successively to provide 2.1 g of crude material. This material was redissolved in DMF (12 mL) and dialysed against DMF (MWCO 2 kg/mol) for 12 hours. The dialysis solution was then changed to 70:30 DMF:pH 6.8 phosphate buffered saline (0.1 M) and the product was dialysed for an additional 12 hours. Finally, the product was dialysed against pure water for 4 hours, and then lyophilized to afford dendron 11 as an off-white solid (380 mg, 64% yield).

**1H NMR** (400 MHz, DMSO-<i>d</i><sub>6</sub>): δ ppm 7.50 (br s, 11H), 7.36 (br s, 11H), 4.78 (d, <i>J</i> = 4.1 Hz, 16H), 4.72 (br s, 2H), 4.64 (d, <i>J</i> = 4.3 Hz, 16H), 4.54 (br s, 16H), 4.38 (d, <i>J</i> = 2.3 Hz, 16H), 4.27-4.05 (m, 60H), 3.83-3.65 (m, 16H), 3.75-3.69 (m, 16H), 3.70-3.62 (m, 16H), 3.62-3.37 (m, 80H), 2.68-2.64 (m, 1H), 2.63-2.52 (m, 32H), 1.82-1.65 (m, 32H), 1.29-1.10 (m, 45H); IR ν<sub>max</sub> (film from MeOH): 3450, 2955, 2368, 1753, 1726, 1642, 1468 cm<sup>-1</sup>; MALDI-TOF MS: Calculated for C<sub>270</sub>H<sub>444</sub>N<sub>32</sub>O<sub>142</sub>S<sub>16</sub>: 6918.4037. No molecular ion peak was observed. A peak (4532.57) was observed corresponding to the molecular ion remaining after the cleavage of two ester groups losing a combined 6 galactosyl moieties and related dendrimer components (Calcd for C<sub>178</sub>H<sub>289</sub>DN<sub>20</sub>O<sub>94</sub>S<sub>10</sub>: 4532.57).

**Synthesis of PBLG-14**

The same procedure described for PBLG-28 was used except that 110 mg (1.1 mmol) of 1-azido-3-aminopropane was used (2.7 g, 80% yield). 1H NMR (400 MHz, 85/15 CDCl<sub>3</sub>/TFA) δ ppm = 7.85-7.80 (m, 13H), 7.34-7.21 (m, 89H), 5.17-5.03 (m, 28H), 4.60 (br s, 14H), 3.40-3.25 (m, 4H), 2.65-2.40 (m, 29H), 2.20-1.70 (m, 28H). IR ν<sub>max</sub>: 3290, 3033, 2948, 2094, 1727, 1649, 1541, 1453 cm<sup>-1</sup>. SEC: M<sub>n</sub> = 13950, <i>D</i> = 1.13.

**Synthesis of PBLG-54**

The same procedure described for PBLG-28 was used except that 30 mg (0.30 mmol) of 1-azido-3-aminopropane was used (2.4 g, 72% yield). 1H NMR (400 MHz, 85/15 CDCl<sub>3</sub>/TFA) δ ppm 7.85-7.80 (m,
Synthesis of PBLG-14-G2-Gal

The same procedure described for the synthesis of PBLG-28-G2-Gal was used. The starting materials were dendron 9 (29 mg, 0.017 mmol, 1.1 equiv) and PBLG-14 (50 mg, 0.016 mmol, 1.0 equiv). Yield = 61 mg, 80%. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta_{ppm}$ 8.35-8.05 (m, 13H), 7.30-7.15 (m, 69H), 5.15 (br s, 2H), 5.10-4.85 (m, 28H), 4.70-4.35 (m, 5H), 4.35-4.05 (m, 22H), 3.95-3.35 (m, 34H), 3.10-2.95 (m, 4H), 2.40-1.75 (m, 60H), 1.15-1.01 (m, 9H). IR $\nu_{max}$: 3289, 3034, 2941, 2096, 1729, 1651, 1548, 1452 cm$^{-1}$. SEC: $M_n = 26400$, $D = 1.04$.

Synthesis of PBLG-28-G3-OH

The same procedure described for the synthesis of PBLG-28-G2-Gal was used. The starting materials were dendron 10 (25 mg, 0.018 mmol, 1.8 equiv) and PBLG-28 (100 mg, 0.016 mmol, 1.0 equiv). Yield = 81 mg, 71%. $\delta_{ppm}$ 8.35-8.05 (m, 24H), 7.30-7.15 (m, 141H), 5.15 (br s, 2H), 5.10-4.85 (m, 56H), 4.63 (t, $J = 5.4$ Hz, 8H), 4.38 (br s, 2H), 4.25-4.08 (m, 18H), 3.95-3.80 (m, 23H), 3.45-3.35 (m, 16H), 2.25-1.80 (m, 72H), 1.18 (s, 3H), 1.12 (s, 5H), 1.02 (s, 11H). IR $\nu_{max}$: 3291, 3033, 2948, 1730, 1649, 1545, 1498, 1453 cm$^{-1}$.

Synthesis of PBLG-28-G3-Gal

The same procedure described for the synthesis of PBLG-28-G2-Gal was used. The starting materials were dendron 12 (39 mg, 0.012 mmol, 1.4 equiv) and PBLG-28 (50 mg, 8.0x10$^{-3}$ mmol, 1.0 equiv). Yield = 56 mg, 72%. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta_{ppm}$ 8.35-8.00 (m, 23H), 7.30-7.15 (m, 137H), 5.18 (br s, 2H), 5.10-4.85 (m, 56H), 4.63 (br s, 2H), 4.35-3.75 (m, 54H), 3.65-3.40 (m, 16H), 2.40-1.75 (m, 92H), 1.15-1.01 (m, 19H). IR $\nu_{max}$: 3293, 2939, 1731, 1652, 1545, 1453 cm$^{-1}$. 

S6
Synthesis of PBLG-28-G4-Gal

The same procedure described for the synthesis of PBLG-28-G2-Gal was used. The starting materials were dendron 13 (72 mg, 0.010 mmol, 1.3 equiv) and PBLG-28 (50 mg, 8.0x10^{-3} mmol, 1.0 equiv). Yield = 76 mg, 72%. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta_{ppm}$ 8.35-8.00 (m, 21H), 7.30-7.15 (m, 121H), 5.10-4.85 (m, 56H), 4.63 (br s, 7H), 4.35-3.75 (m, 78H), 3.65-3.40 (m, 41H), 2.35-1.75 (m, 74H), 1.15-1.01 (m, 38H). IR $\nu_{max}$: 3294, 2940, 1731, 1652, 1546, 1454 cm$^{-1}$.

Synthesis of PBLG-54-G3-OH

The same procedure described for the synthesis of PBLG-28-G2-Gal was used. The starting materials were dendron 10 (8.0 mg, 9.2x10^{-3} mmol, 1.1 equiv) and PBLG-54 (100 mg, 8.4x10^{-3} mmol, 1.0 equiv). $\delta_{ppm}$ 8.35-8.05 (m, 39H), 7.30-7.15 (m, 278H), 5.18 (br s, 2H), 5.10-4.85 (m, 105H), 4.63 (t, J = 5.3 Hz, 7H), 4.39 (br s, 2H), 4.20-4.10 (m, 17H), 3.95-3.80 (m, 48H), 3.49-3.35 (m, 16H), 2.25-1.80 (m, 164H), 1.18 (s, 3H), 1.12 (s, 6H), 1.02 (s, 11H). IR $\nu_{max}$: 3290, 3034, 2943, 1729, 1651, 1545, 1498, 1453 cm$^{-1}$.

Synthesis of PBLG-54-G3-Gal

The same procedure described for the synthesis of PBLG-28-G2-Gal was used. The starting materials were dendron 12 (32 mg, 9.2x10^{-3} mmol, 1.1 equiv) and PBLG-54 (100 mg, 8.4x10^{-3} mmol, 1.0 equiv). $\delta_{ppm}$ 8.40-8.05 (m, 42H), 7.30-7.15 (m, 270H), 5.18 (br s, 3H), 5.10-4.85 (m, 107H), 4.35 (br s, 5H), 4.25-3.75 (m, 70H), 3.65-3.40 (m, 19H), 2.40-1.75 (m, 137H), 1.15-1.01 (m, 20H). IR $\nu_{max}$: 3289, 1728, 1648, 1542, 1498, 1452 cm$^{-1}$. 
Figure S1. $^1$H NMR (600 MHz, CD$_3$OD) spectrum of compound 2.

Figure S2. $^{13}$C NMR (100 MHz, CD$_3$OD) spectrum of compound 2.
Figure S3. $^1$H NMR (600 MHz, CD$_3$OD) spectrum of compound 3.

Figure S4. $^{13}$C NMR (150 MHz, $^1$H-coupled, CD$_3$OD) spectrum of compound 3.
Figure S5. $^1$H NMR (400 MHz, DMSO-$_d_6$) spectrum of compound 4.

Figure S6. $^{13}$C NMR (100 MHz, DMSO-$_d_6$) spectrum of compound 4.
Figure S7. $^1$H NMR (400 MHz, $CDCl_3$) spectrum of dendron 7.

Figure S8. $^{13}$C NMR (100 MHz, $CDCl_3$) spectrum of dendron 7.
Figure S9. $^1$H NMR (400 MHz, $CD_3OD$) spectrum of dendron 8.

Figure S10. $^{13}$C NMR (100 MHz, $CD_3OD$) spectrum of dendron 8.
Figure S11. $^1$H NMR (400 MHz, DMSO-$d_6$) spectrum of dendron 9.

Figure S12. $^1$H NMR (400 MHz, DMSO-$d_6$) spectrum of dendron 12.
Figure S13. $^1$H NMR (400 MHz, CDCl$_3$) spectrum of dendron S2.

Figure S14. $^{13}$C NMR (100 MHz, CDCl$_3$) spectrum of dendron S2.
Figure S15. $^1$H NMR (400 MHz, $CD_3OD$) spectrum of dendron S3.

Figure S16. $^{13}$C NMR (150 MHz, $CD_3OD/CDCl_3$ 1/1) spectrum of dendron S3.
Figure S17. $^1$H NMR (400 MHz, DMSO-$d_6$) spectrum of dendron 13.
Figure S18. $^1$H NMR (400 MHz, CDCl$_3$/TFA 85/15) spectra of: a) PBLG-54, b) PBLG-28; c) PBLG-14. The degree of polymerization was determined based on the relative integrations of the peaks corresponding to the initiator at ~3.4 ppm, and the $\alpha$-proton of the amino acid at ~4.6 ppm.

Figure S19. Infrared spectra of: a) PGLG-14; b) PBLG-28; c) PBLG-54. Arrows indicate the peak corresponding to the azide stretch at ~2100 cm$^{-1}$. 
Figure S20. SEC trace for PBLG-14 in DMF. Consistent with previously reported results, the peak is bimodal, which can likely be attributed to the coexistence of random coil and \( \alpha \)-helical conformations.\(^3\)

Figure S21. SEC trace for PBLG-28 in DMF.
**Figure S22.** SEC trace for PBLG-54 in DMF.

<table>
<thead>
<tr>
<th>Dist Name</th>
<th>Mn</th>
<th>Mw</th>
<th>MP</th>
<th>Mz</th>
<th>Mz+1</th>
<th>Mw</th>
<th>Polydispersity</th>
<th>Mw Marker 1</th>
<th>Mw Marker 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26411</td>
<td>27462</td>
<td>27672</td>
<td>28465</td>
<td>29418</td>
<td>1.039793</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure S23.** Infrared spectra of linear-dendritic block copolymers: a) PBLG-28-G3-OH; b) PBLG-54-G3-OH; c) PBLG-14-G2-Gal; d) PBLG-28-G2-Gal; e) PBLG-28-G3-Gal; f) PBLG-28-G4-Gal; g) PBLG-54-G3-Gal. In each case, no peak corresponding to the azide stretch at 2100 cm$^{-1}$ was observed, suggesting complete coupling.
Figure S24. $^1$H NMR (400 MHz, $DMSO-d_6$) spectrum of PBLG-28 for comparison with the spectra of the copolymers below, which were also obtained in $DMSO-d_6$.

Figure S25. $^1$H NMR (400 MHz, $DMSO-d_6$) spectrum of PBLG-14-G2-Gal.
Figure S26. $^1$H NMR (400 MHz, DMSO-$d_6$) spectrum of PBLG-28-G3-OH.

Figure S27. $^1$H NMR (400 MHz, DMSO-$d_6$) spectrum of PBLG-28-G2-Gal.
Figure S28. $^1$H NMR (400 MHz, DMSO-<em>d</em><sub>6</sub>) spectrum of PBLG-28-G3-Gal.

Figure S29. $^1$H NMR (400 MHz, DMSO-<em>d</em><sub>6</sub>) spectrum of PBLG-28-G4-Gal.
Figure S30. $^1$H NMR (400 MHz, DMSO-$d_6$) spectrum of PBLG-54-G3-OH.

Figure S31. $^1$H NMR (400 MHz, DMSO-$d_6$) spectrum of PBLG-54-G3-Gal.
Figure S32. Autocorrelation functions and relaxation time distributions (determined at 50° scattering angle) obtained by multi-angle DLS for assemblies: a) PBLG-28-G4-Gal (DMSO into water, fast); b) PBLG-54-G3-OH (water into DMSO, 64 mL/h), c) PBLG-28-G3-OH (DMSO into water, fast); d) PBLG-28-G4-Gal (DMF into water, fast); e) PBLG-28-G3-OH (water into DMSO, 64 mL/h); f) PBLG-54-G3-OH (DMSO into water, fast).
Figure S33. Autocorrelation functions and relaxation time distributions (determined at 90° scattering angle) obtained by single-angle DLS for assemblies: a) PBLG-28-G4-Gal + 5wt% PBLG (DMSO into water, fast); b) PBLG-28-G3-OH (water into DMSO, fast); c) PBLG-54-G3-OH (water into DMSO, fast); d) PBLG-54-G3-OH (water into DMSO, 8 mL/h).
Figure S34. TEM image of assemblies obtained by fast injection of DMF solution of PBLG28-G4-Gal into water.

Figure S35. TEM image of assemblies obtained by slow (64 mL/h) injection of water into a DMSO solution of PBLG28-G3OH. The sample is unstained.
**Figure S36.** TEM images of assemblies obtained from PBLG-54-G3OH by a) fast injection of DMSO solution of polymer into water; b) slow (64 mL/h) injection of water into a the DMSO solution of polymer. The samples are unstained.

**Fig. S37.** Critical aggregation concentration (CAC) for PBLG-28-G4-Gal, PBLG-28-G3-OH and PBLG-54-G3-OH: determined as the concentration at the intercept of the lines for the two linear regions of the above graphs.
References