

A Modular Synthetic Approach for Adjusting the Disassembly Rates of Enzyme-Responsive Polymeric Micelles

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Instrumentation and materials:

Instrumentation:

HPLC: All measurements were recorded on a Waters Alliance e2695 separations module equipped with a Waters 2998 photodiode array detector. All solvents were purchased from Bio-Lab Chemicals and were used as received. All solvents are HPLC grade. **¹H and ¹³C NMR:** spectra were recorded on Bruker Avance I and Avance III 400 MHz spectrometers as indicated. Chemical shifts are reported in ppm and referenced to the solvent. The molecular weights of the PEG-dendron hybrids were determined by comparison of the areas of the peaks corresponding to the PEG block (3.63 ppm in CDCl₃) and the protons peaks of the dendrons. **GPC:** All measurements were recorded on Viscotek GPCmax by Malvern using refractive index detector and PEG standards (purchased from Sigma-Aldrich) were used for calibration. **Infrared spectra:** All measurements were recorded on a Bruker Tensor 27 equipped with a platinum ATR diamond. **Fluorescence spectra:** All measurements were recorded on an Agilent Technologies Cary Eclipse Fluorescence Spectrometer using quartz cuvettes. **CMC:** All measurements were recorded on a TECAN Infinite M200Pro device. **MALDI-TOF MS:** Analysis was conducted on a Bruker AutoFlex MALDI-TOF MS (Germany) and also on a Waters MALDI synapt (USA). DHB matrix was used. **TEM:** Images were taken by a Philips Tecnai F20 TEM at 200 kV, **DLS:** All measurements were recorded on a Corduan Technology VASCO⁷ particle size analyzer.

Materials:

Poly (Ethylene Glycol) methyl ether ($M_n = 5$ kDa), 2-(Boc-amino)-ethanethiol, 2,2-dimethoxy-2-phenylacetophenone (DMPA), Penicillin G Amidase from Escherichia coli (PGA), Allyl bromide, Propargyl bromide (80% in toluene), N,N'-dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP), phenylacetic acid and Sephadex® LH20 were purchased from Sigma-Aldrich. Cystamine hydrochloride, potassium hydroxide and Diisopropylethylamine (DIPEA) were purchased from Merck. Gallic acid was purchased from Chem Impex. 3,5 dihydroxy benzoic acid was purchased from Apollo Scientific Ltd. Anhydrous potassium carbonate was purchased from J. T. Baker. Silica Gel 60Å, 0.040-0.063mm, Sodium Hydroxide and all solvents were purchased from Bio-Lab and were used as received. Deuterated solvents for NMR were purchased from Cambridge Isotope Laboratories (CIL), Inc.

Synthesis

Synthesis of branching units:

Synthesis of PNP-AB₃:

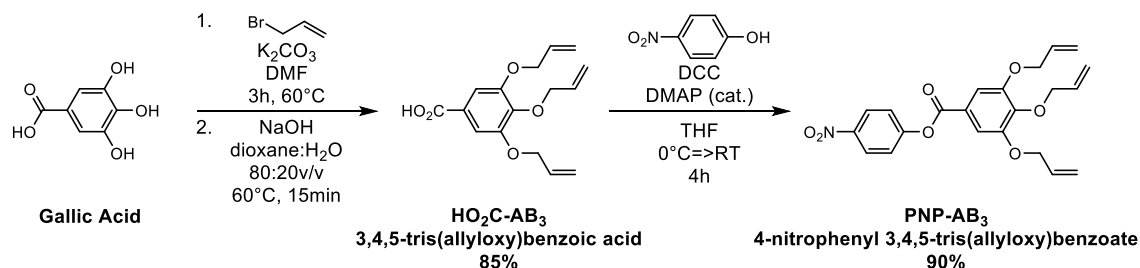


Figure S1. Preparation of 4-nitrophenyl 3,4,5-tris(allyloxy)benzoate (PNP-AB₃).

3, 4, 5-tris(allyloxy)benzoic acid: Gallic acid (1.7 g, 10 mmol), anhydrous K_2CO_3 (11.1 g, 80 mmol, 8 eq.) and allyl bromide (6.9 ml, 80 mmol, 8 eq.) were reacted in DMF (30 mL) and then treated with 4N NaOH (5 mL, 20 mmol, 2 eq.) in dioxane:H₂O 80:20v/v (60 mL) as described in previously reported procedure.¹ The product was obtained as an off-white solid in 85% yield (2.47 g) and its characterizations correlated with previous reports.²

¹H-NMR (DMSO-d₆): δ 7.22 (s, 2H, arom H), 5.96-6.10 (m, 3H, vinylic H), 5.41 (dd, $J = 17.3\text{Hz}$, 1.7Hz, 2H, *E* vinylic H), 5.32 (dd, $J = 17.2\text{Hz}$, 1.7Hz, 1H, *E* vinylic H), 5.27 (dd, $J = 10.6\text{Hz}$, 1.6Hz, 2H, *Z* vinylic H), 5.17 (dd, $J = 10.4\text{Hz}$, 1.7Hz, 1H, *Z* vinylic H), 4.62 (d, $J = 4.9\text{Hz}$, 4H, allylic H), 4.52 (d, $J = 5.6\text{Hz}$, 2H, allylic H); ¹³C-NMR (DMSO-d₆): δ 166.9, 151.8, 140.6, 134.5, 133.6, 125.9, 117.5, 117.2, 108.0, 73.2, 69.1;

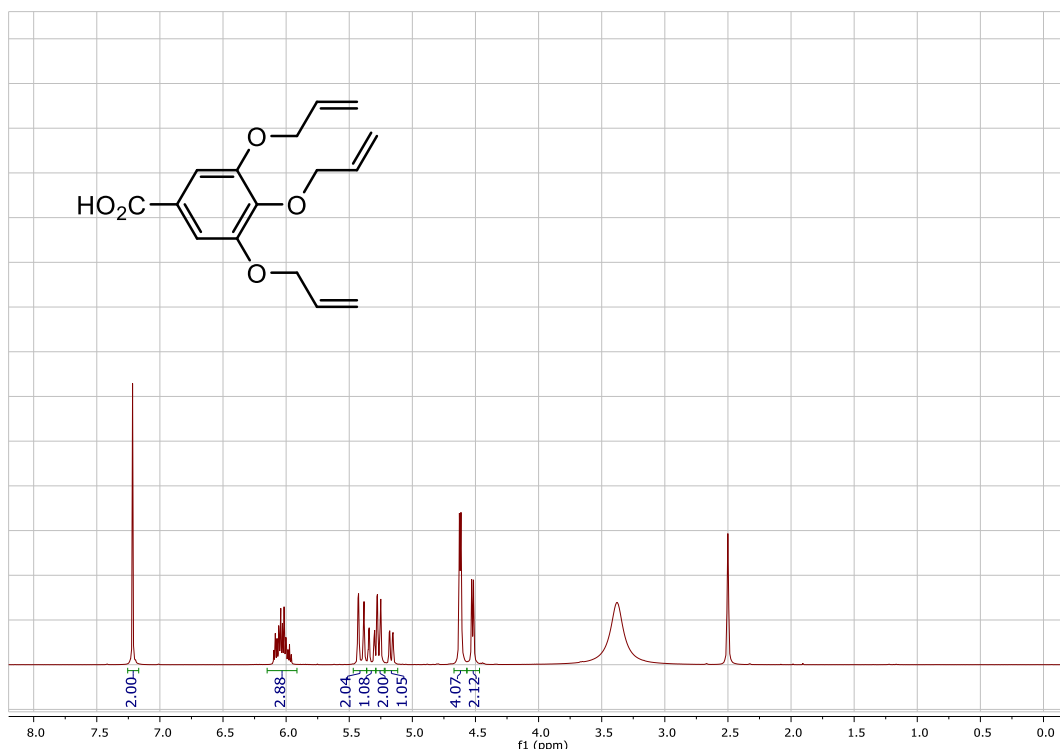


Figure S2. $^1\text{H-NMR}$ spectrum of 3,4,5-tris(allyloxy)benzoic acid in DMSO- d_6 .

4-nitrophenyl 3, 4, 5-tris(allyloxy)benzoate: 3, 4, 5-tris(allyloxy)benzoic acid (1.16 g, 4 mmol), 4-nitrophenol (612 mg, 4.4 mmol, 1.1 eq.), DCC (908 mg, 4.4 mmol, 1.1 eq.) and DMAP (49 mg, 0.4 mmol, 0.1 eq.) were reacted in THF (25 mL) as described in previously reported procedure.¹ The product was obtained as a fluffy white solid in 90% yield (1.49 g).

$^1\text{H-NMR}$ (CDCl_3): δ 8.33 (d, $J = 9.2\text{Hz}$, 2H, arom H), 7.42 (s, 2H, arom H), 7.39 (d, $J = 9.2\text{Hz}$, 2H, arom H), 6.04-6.15 (m, 3H, vinylic H), 5.46 (dd, $J = 17.3\text{Hz}$, 1.5Hz, 2H, *E* vinylic H), 5.30-5.38 (m, 3H, *E* vinylic H + *Z* vinylic H), 5.22 (dd, $J = 10.3\text{Hz}$, 1.6Hz, 1H, *Z* vinylic H), 4.65-4.69 (m, 6H, allylic H); $^{13}\text{C-NMR}$ (CDCl_3): δ 164.0, 155.9, 152.7, 145.6, 143.2, 134.1, 132.9, 125.4, 123.3, 122.8, 118.3, 118.1, 109.7, 74.3, 70.2; FT-IR, $\nu(\text{cm}^{-1})$: 1735, 1614, 1558, 1516, 1495, 1456, 1421, 1364, 1345, 1327, 1208, 1125, 1071, 1022, 982, 933, 877, 858; High resolution MS (EI, positive/negative mode, eV): calculated mass: 411.1318, found: 411.1317;

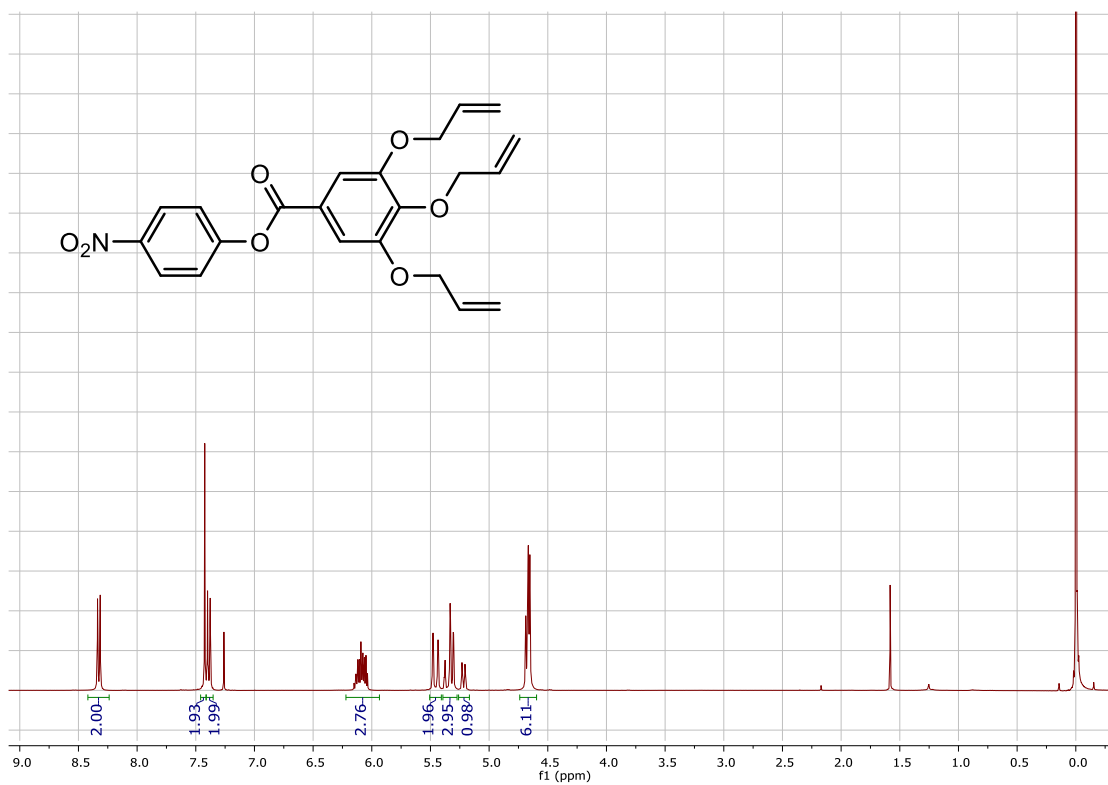


Figure S3. ¹H-NMR spectrum of 4-nitrophenyl 3,4,5-tris(allyloxy)benzoate in CDCl₃.

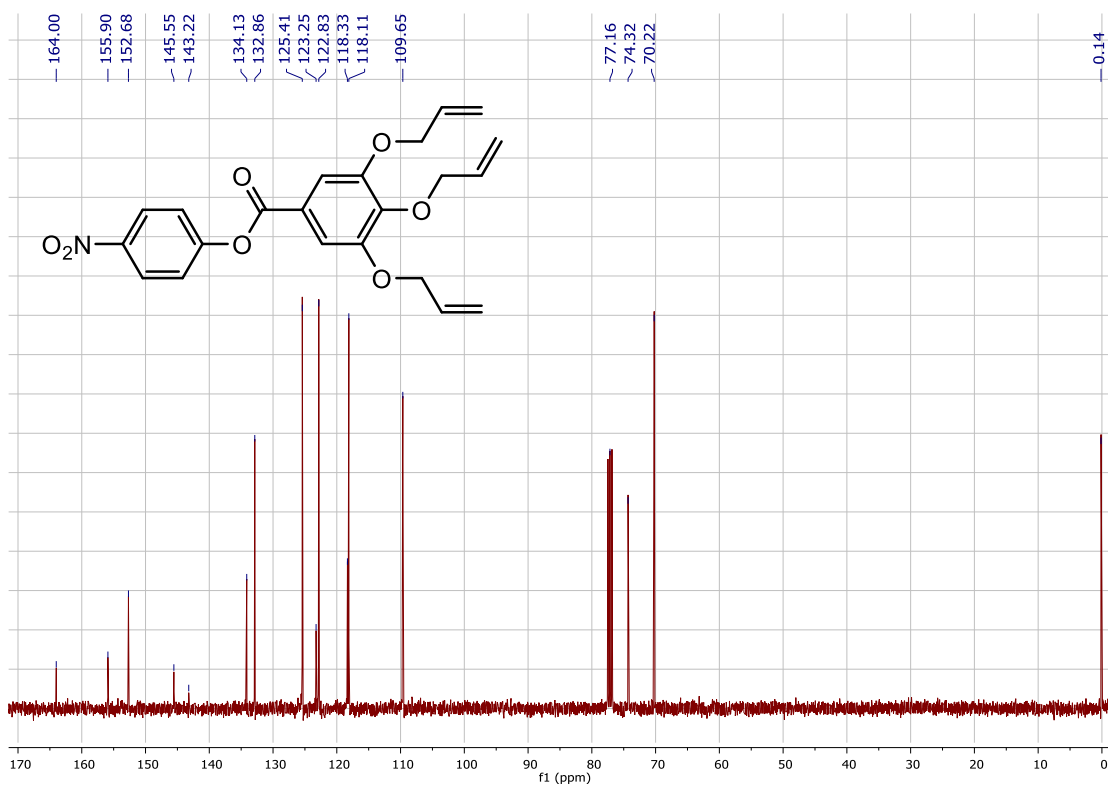


Figure S4. ¹³C-NMR spectrum of 4-nitrophenyl 3,4,5-tris(allyloxy)benzoate in CDCl₃.

Synthesis of PNP-AB₄:

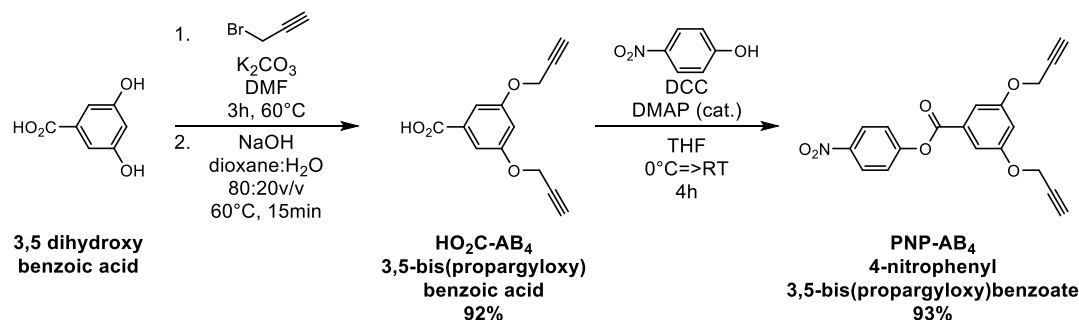


Figure S5. Preparation of 4-nitrophenyl 3,5-bis(propargyloxy)benzoate (PNP-AB₄).

3,5-bis(propargyloxy) benzoic acid: 3,5 dihydroxy benzoic acid (1.54 g, 10 mmol), anhydrous K_2CO_3 (8.3 g, 60 mmol, 6 eq.) and 80% propargyl bromide in toluene (6.4 mL, 60 mmol, 6 eq.) were reacted in DMF (25 mL), and then treated with 4N NaOH (5 mL, 20 mmol, 2 eq.) in dioxane:H₂O 80:20v/v (50 mL) as described in previously reported procedure.¹ The product was obtained as an off-white solid in 92% yield (2.1 g).

¹H, ¹³C-NMR and FT-IR correlated with previous reports.³

4-nitrophenyl 3,5-bis(propargyloxy)benzoate: 3,5-bis(propargyloxy)benzoic acid (0.69 g, 3 mmol), 4-nitrophenol (0.46 g, 3.3 mmol, 1.1 eq.), DMAP (37 mg, 0.3 mmol, 0.1 eq.) and DCC (0.52 g, 3.3 mmol, 1.1 eq.) were reacted in THF (15 mL) as described in previously reported procedure.¹ The product was obtained as a fluffy white solid in 93% yield (0.98 g).

¹H, ¹³C-NMR and FT-IR correlated with previous reports.³

Synthesis of PNP-AB₆:

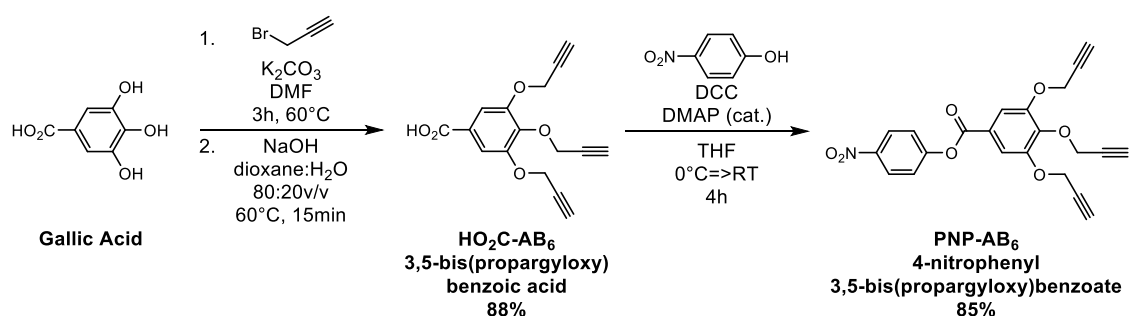


Figure S6. Preparation of 4-nitrophenyl 3,4,5-tris(propargyloxy)benzoate (PNP-AB₆).

3, 4, 5-tris(propargyloxy)benzoic acid: Gallic acid (1.7 g, 10 mmol), anhydrous K_2CO_3 (11.1 g, 80 mmol, 8 eq.) and 80% propargyl bromide in toluene (8.5 mL, 80 mmol, 8 eq.) were reacted in DMF (30 mL) and then treated with 4N NaOH (5 mL, 20 mmol, 2 eq.) in dioxane:H₂O 80:20v/v (60 mL) as described in previously reported procedure.¹ The product

was obtained as an off-white solid in 88% yield (2.51 g) and its characterizations correlated with previous reports.⁴

¹H-NMR (DMSO-d₆): δ 7.38 (s, 2H, arom H), 4.89 (d, *J* = 2.4Hz, 4H, meta -O-CH₂-C≡C), 4.71 (d, *J* = 2.4Hz, 2H, para -O-CH₂-C≡C), 3.61 (t, *J* = 2.4Hz, 2H, meta -C≡CH), 3.47 (t, *J* = 2.4Hz, 1H, para -C≡CH); ¹³C-NMR (DMSO-d₆): δ 166.7, 150.9, 139.7, 126.5, 108.9, 79.1, 78.9, 78.8, 78.3, 59.5, 56.5;

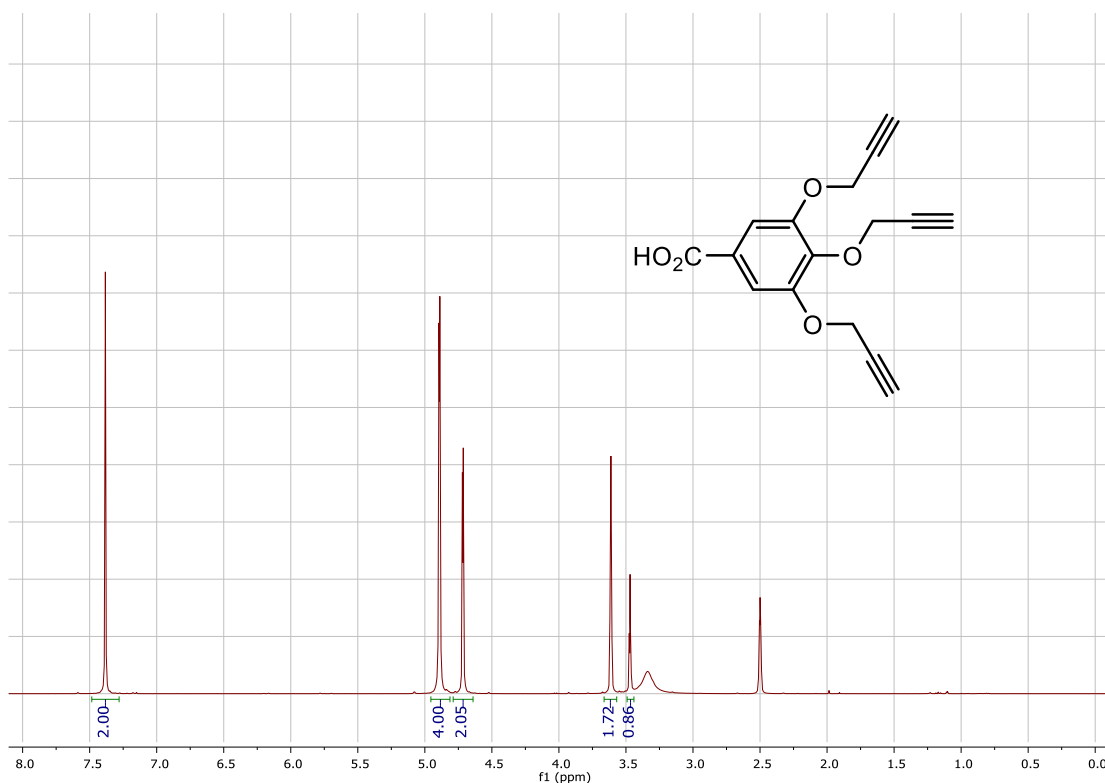


Figure S7. ¹H-NMR spectrum of 3, 4, 5-tris(propargyloxy)benzoic acid in DMSO-d₆.

4-nitrophenyl 3, 4, 5-tris(propargyloxy)benzoate: 3, 4, 5-tris(propargyloxy)benzoic acid (1.42 g, 5 mmol), 4-nitrophenol (765 mg, 5.5 mmol, 1.1 eq.), DCC (1.14 g, 5.5 mmol, 1.1 eq.) and DMAP (61 mg, 0.5 mmol, 0.1 eq.) were reacted in THF (30 mL) as described in previously reported procedure.¹ The product was obtained as a fluffy white solid in 85% yield (1.72 g).

¹H-NMR (DMSO-d₆): δ 8.37 (d, *J* = 9.1Hz, 2H, arom H), 7.63 (d, *J* = 9.1Hz, 2H, arom H), 7.60 (s, 2H, arom H), 4.97 (d, *J* = 2.3Hz, 4H, meta -O-CH₂-C≡C), 4.81 (d, *J* = 2.4Hz, 2H, para -O-CH₂-C≡C), 3.65 (t, *J* = 2.3Hz, 2H, meta -C≡CH), 3.50 (t, *J* = 2.4Hz, para -C≡CH); ¹³C-NMR (DMSO-d₆): δ 163.2, 155.5, 151.2, 145.2, 141.1, 125.3, 123.5, 123.3, 109.8, 79.0, 78.9, 78.7, 78.6, 59.6, 56.8; FT-IR, ν(cm⁻¹): 1739, 1592, 1541, 1521, 1506, 1491, 1360, 1315, 1267, 1234, 1216, 1184, 1171, 1101, 1017, 993, 942, 921, 862; High resolution MS (EI, positive/negative mode, eV): calculated mass: 405.0849, found: 405.0855.

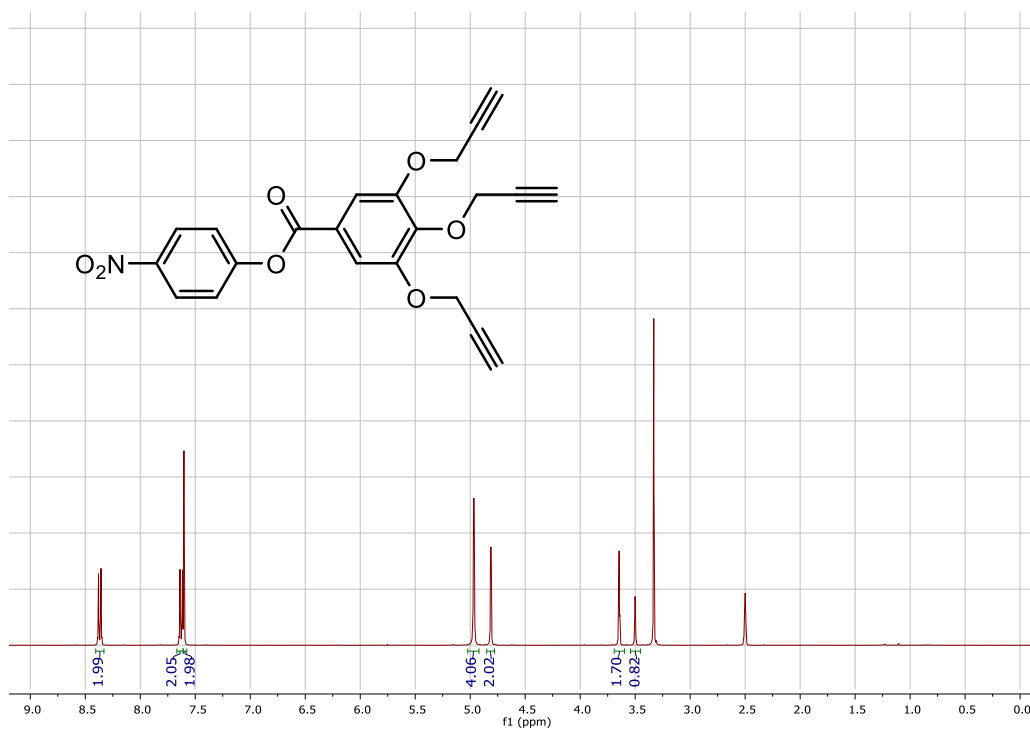


Figure S8. ¹H-NMR spectrum of 4-nitrophenyl 3,4,5-tris(propargyloxy)benzoate in DMSO-d₆.

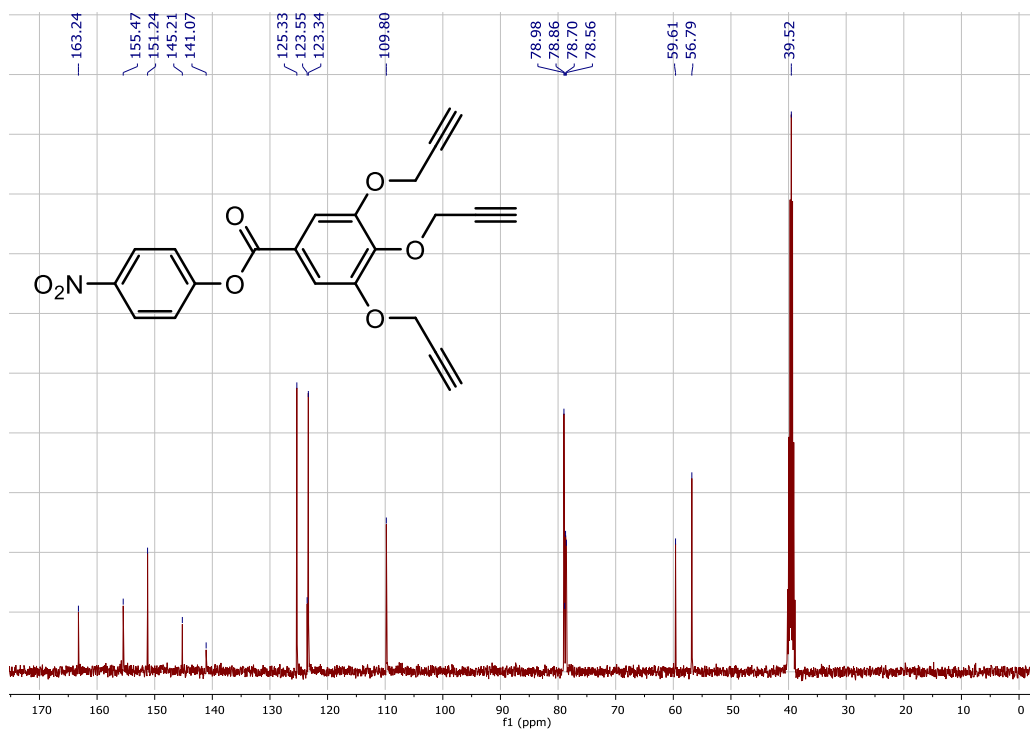


Figure S9. ¹³C-NMR spectrum of 4-nitrophenyl 3,4,5-tris(propargyloxy)benzoate in DMSO-d₆.

Synthesis of PEG building blocks:

Synthesis of mPEG_{5kDa}-NH₂:

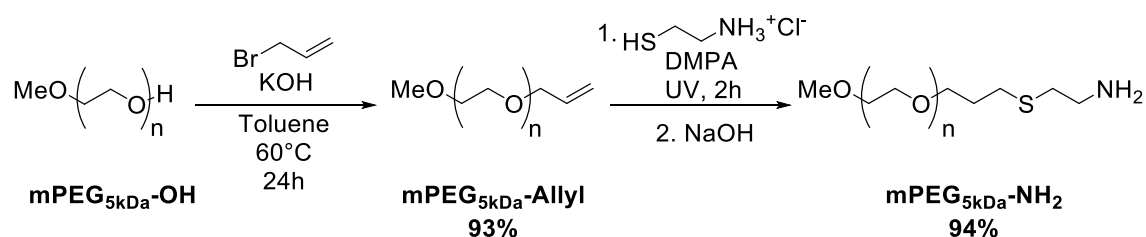


Figure S10. Preparation of mPEG_{5kDa}-NH₂.

mPEG_{5kDa}-allyl and mPEG_{5kDa}-NH₂ were prepared as was described in previously reported procedure¹ and the spectroscopic characterization correlated well with these reports.

Synthesis of mPEG_{5kDa}-(NH₂)₂:

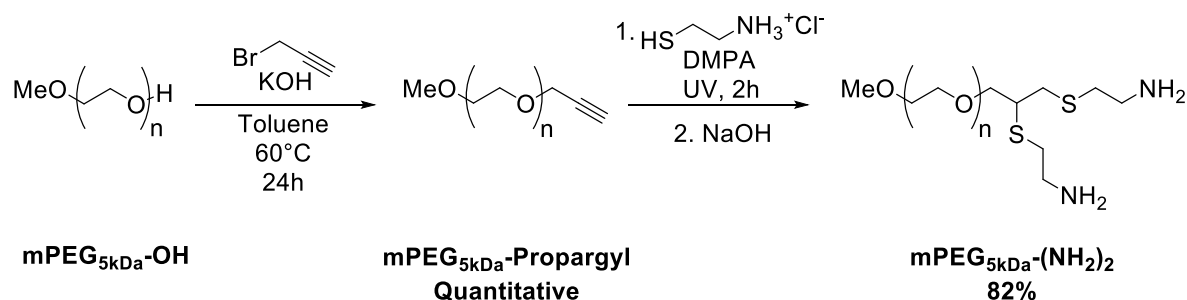


Figure S11. Preparation of mPEG_{5kDa}-(NH₂)₂.

mPEG_{5kDa}-propargyl: methoxy poly(ethylene glycol) ($M_n = 5$ kDa, 5.00 g, 1 mmol), KOH (1.68 g, 30 mmol, 30 eq.) and 80% propargyl bromide in toluene (3.25 mL, 30 mmol, 30 eq.) were reacted in toluene (50 mL) as described in previously reported procedure.¹ The product was obtained as an off-white to light yellowish solid in quantitative yield (4.97 g).

¹H-NMR (CDCl₃): δ 4.17 (d, $J = 2.4$ Hz, 2H, -O-CH₂-C \equiv), 3.78-3.46 (m, 505H, PEG backbone), 3.35 (s, 3H, H₃C-O-), 2.43 (t, $J = 2.4$ Hz, 1H, -C \equiv CH); ¹³C-NMR (CDCl₃): δ 74.6, 72.0, 70.6, 69.2, 59.1, 58.5; FT-IR, $\nu(\text{cm}^{-1})$: 2883, 1558, 1541, 1522, 1508, 1472, 1466, 1457, 1360, 1319, 1280, 1241, 1146, 1106, 1061, 962, 842; GPC (DMF + 25 mM NH₄Ac): $M_n = 5.0$ kDa, PDI = 1.03, Expected $M_n = 5.0$ kDa;

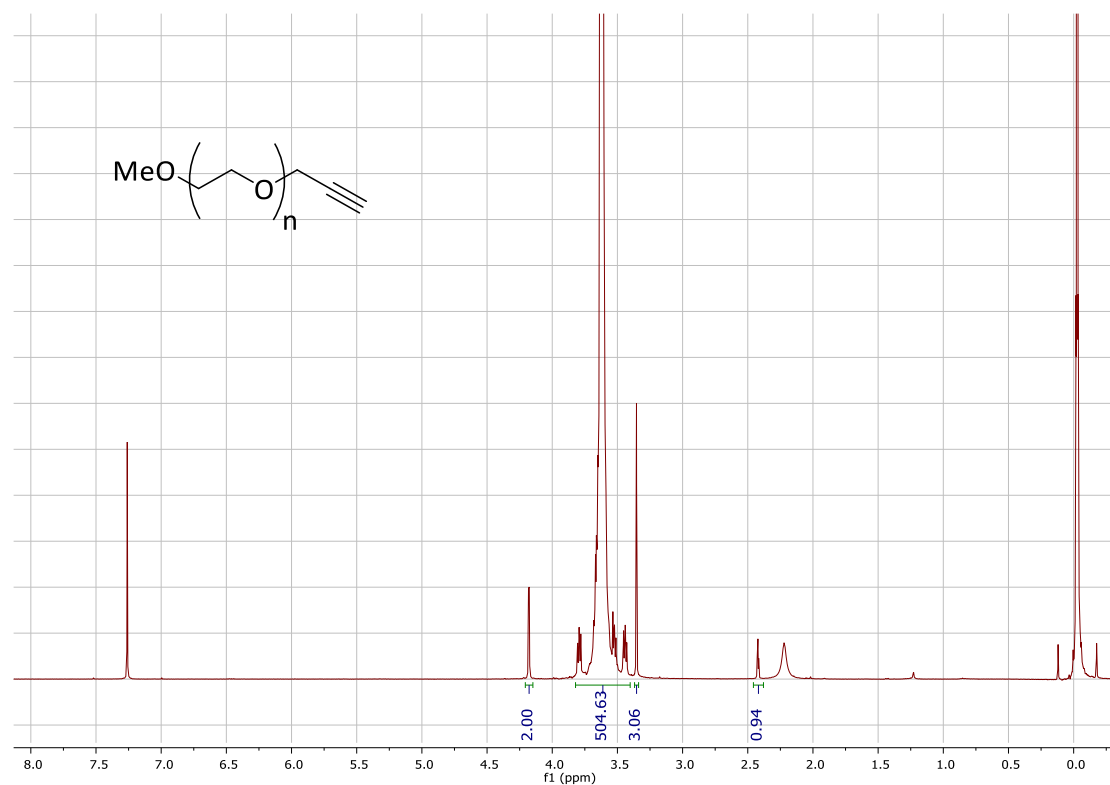


Figure S12. $^1\text{H-NMR}$ spectrum of $\text{mPEG}_{5\text{kDa}}$ -Propargyl in CDCl_3 .

$\text{mPEG}_{5\text{kDa}}-(\text{NH}_2)_2$: $\text{mPEG}_{5\text{kDa}}$ -propargyl (1.26 g, 0.25 mmol), cysteamine hydrochloride (1.14 g, 10 mmol, 40 eq.) and DMPA (26 mg, 0.1 mmol, 0.4 eq.) were reacted in MeOH (6 mL) as described in previously reported procedure.¹ The product was obtained as an off-white solid in 82% yield (1.06 g).

$^1\text{H-NMR}$ (CDCl_3): δ 3.79-3.41 (m, 503H, PEG H), 3.34 (s, 3H, $\text{H}_3\text{C-O-}$), 2.96-2.61 (m, 11H, -S- CH_2 - + - CH_2 - NH_2); $^{13}\text{C-NMR}$ (CDCl_3): δ 73.3, 72.0, 70.6, 59.1, 45.8, 41.7, 41.3, 37.4, 35.9, 34.8; FT-IR, $\nu(\text{cm}^{-1})$: 2883, 1558, 1541, 1521, 1508, 1472, 1457, 1360, 1342, 1280, 1241, 1147, 1102, 1061, 962, 843; GPC (DMF + 25 mM NH_4Ac): $M_n = 5.3$ kDa, PDI = 1.03, Expected $M_n = 5.3$ kDa;

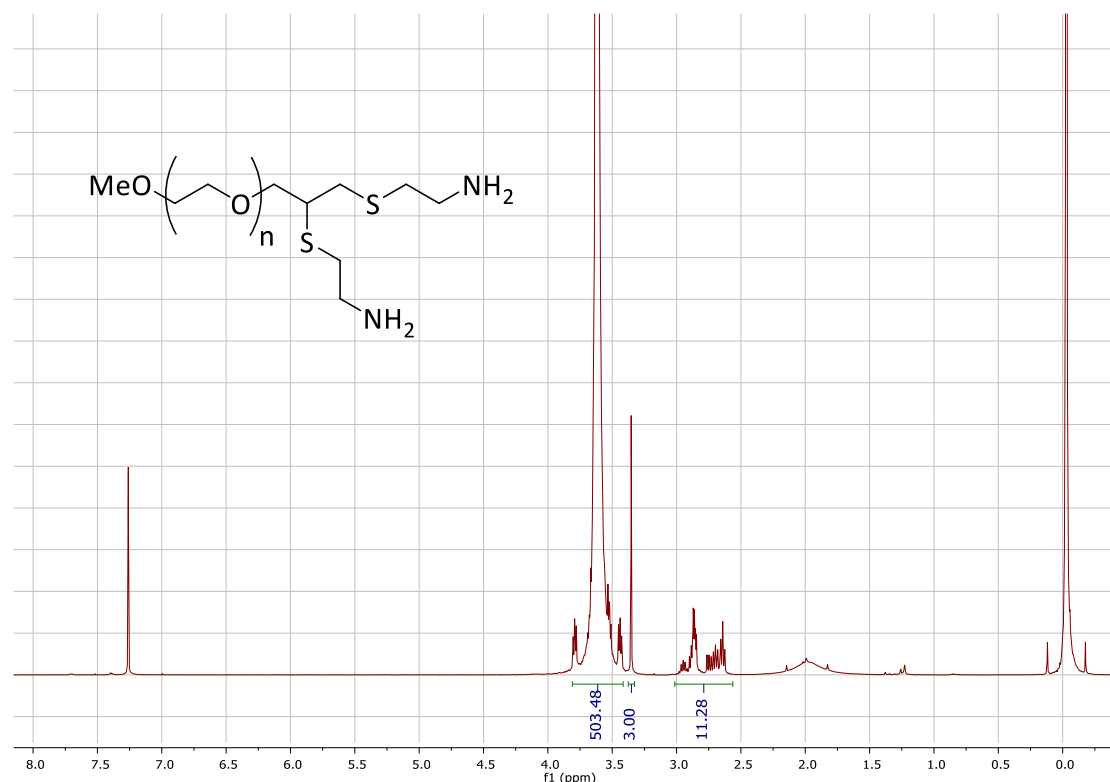


Figure S13. $^1\text{H-NMR}$ spectrum of $\text{mPEG}_{5\text{kDa}}-(\text{NH}_2)_2$ in CDCl_3 .

Synthesis of PEG-dendron hybrids:

General procedures for preparation of hybrids:

General procedure #1: conjugation of $\text{mPEG}_{5\text{kDa}}-\text{NH}_2$ / $\text{mPEG}_{5\text{kDa}}-(\text{NH}_2)_2$ to PNP- AB_x

$\text{mPEG}_{5\text{kDa}}-\text{NH}_2$ or $\text{mPEG}_{5\text{kDa}}-(\text{NH}_2)_2$ were dissolved in $\text{DCM}:\text{DMF}$ 1:1 v/v (1 mL per 100 mg PEG). DIPEA (10 eq. per amine) and the desired 4-nitrophenyl ester of the branching unit (PNP- AB_x , 3 eq. per amine) were added and the reaction was allowed to stir overnight at room temperature. Then, the reaction mixture was loaded as-is on a LH20 (Sephadex®) size exclusion column and eluted with MeOH. Fractions that contained the product (identified by UV light and/or I_2 staining) were unified and MeOH was evaporated to dryness. In order to facilitate the solidification of the product, the oily residue was redissolved with DCM (1 mL per 100 mg PEG) and hexane (5 mL per 100 mg PEG) was added. Organic solvents were evaporated to dryness and the obtained solid was dried under high vacuum.

General procedure #2: thiol-ene/yne reaction with 2-(Boc-amino)ethanethiol

The PEG reactant, 2-(Boc-amino)ethanethiol (20 eq. per double bond or 40 eq. per triple bond) and DMPA (1 mol % with respect to the thiol) were dissolved in MeOH (0.5 mL per 100 mg PEG). The solution was purged with N_2 for 15 minutes and then placed under UV

light (365 nm) for 2 hours. The product was isolated and purified as described in general procedure #1.

General procedure #3: Boc removal and amidation with 4-nitrophenyl 2-phenylacetate:

The PEG reactant was dissolved in DCM (0.5 mL per 100 mg PEG) and TFA was added (0.5 mL per 100 mg PEG). After 30 minutes, all organic solvents were evaporated to dryness and the obtained oily residue was dried under high vacuum for 1 hour. Then, the deprotected PEG was redissolved with DCM:DMF 1:1v/v (1 mL per 100 mg PEG), DIPEA (10 eq. per amine) and 4-nitrophenyl 2-phenylacetate³ (3 eq. per amine) were added, and the reaction was allowed to stir overnight at room temperature. The product was isolated and purified as described in general procedure #1.

Synthesis of mPEG_{5kDa}-dend-(3Ph) (Hybrid 1):

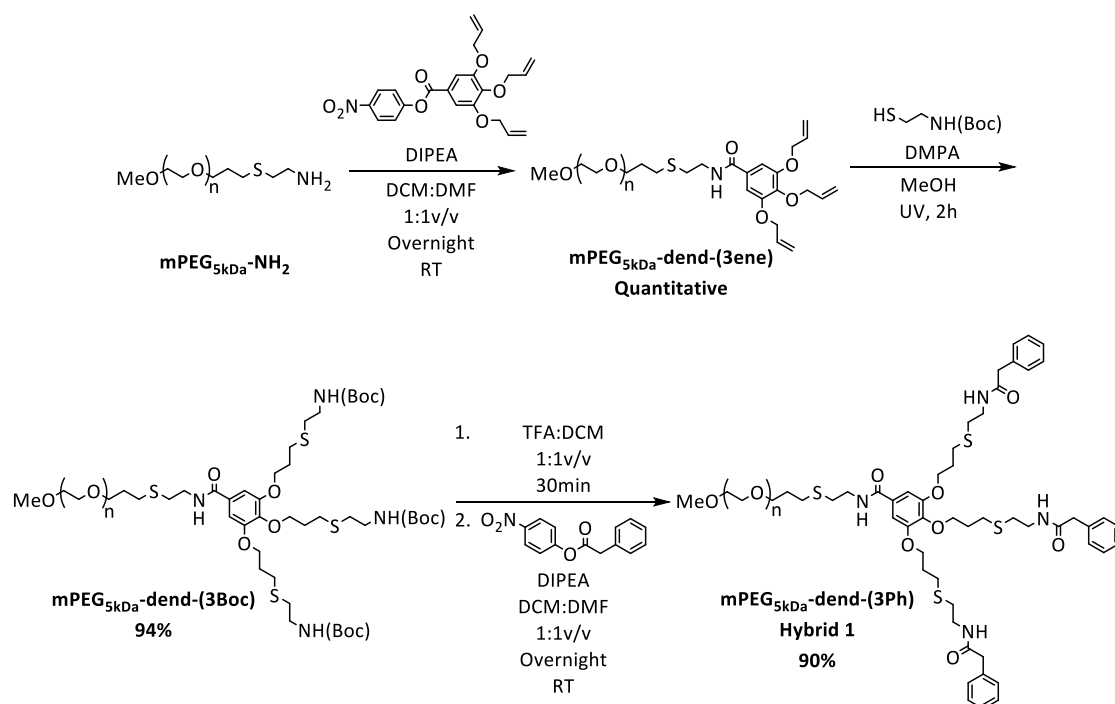


Figure S14. Preparation of mPEG_{5kDa}-dend-(3Ph) (Hybrid 1).

mPEG_{5kDa}-dend-(3ene): mPEG_{5kDa}-NH₂ (205 mg, 0.04 mmol), DIPEA (63 μ L, 0.36 mmol, 9 eq.) and PNP-AB₃ (49 mg, 0.12 mmol, 3 eq.) were reacted in DCM:DMF 1:1v/v (2 mL) according to the described general procedure. The product was obtained as a white solid in quantitative yield (210 mg).

¹H-NMR (CDCl₃): δ 7.00 (s, 2H, arom H), 6.73 (t, J = 5.5Hz, 1H, -NH-CO-), 5.97-6.09 (m, 3H, vinylic H), 5.39 (dd, 2H, J = 17.3Hz, 1.6Hz, 2H, *E* vinylic H), 5.23-5.30 (m, 3H, *E* vinylic H + *Z* vinylic H), 5.14 (dd, J = 10.3Hz, 1.5Hz, 1H, *Z* vinylic H), 4.58 (dt, J = 5.2Hz, 1.4Hz, 4H, allylic H), 4.54 (dt, J = 6.0Hz, 1.2Hz, 2H, allylic H), 3.41-3.79 (m, 491H, PEG

H), 3.33 (s, 3H, H₃C-O-), 2.72 (t, $J = 6.6\text{Hz}$, 2H, -S-CH₂-), 2.61 (t, $J = 7.2\text{Hz}$, 2H, -S-CH₂-), 1.83 (qui, $J = 6.7\text{Hz}$, 2H, -O-CH₂-CH₂-CH₂-S-); ¹³C-NMR (CDCl₃): δ 167.0, 152.6, 140.8, 134.3, 133.2, 129.8, 117.9, 117.7, 106.7, 74.1, 72.0, 70.6, 70.3, 70.2, 69.5, 59.1, 39.0, 31.8, 29.7, 28.3; FT-IR, $\nu(\text{cm}^{-1})$: 2869, 1570, 1558, 1541, 1522, 1507, 1497, 1489, 1473, 1457, 1437, 1419, 1396, 1374, 1348, 1281, 1245, 1095, 993, 948, 843; GPC (DMF + 25 mM NH₄Ac): $M_n = 5.5$ kDa, PDI = 1.03, Expected $M_n = 5.4$ kDa;

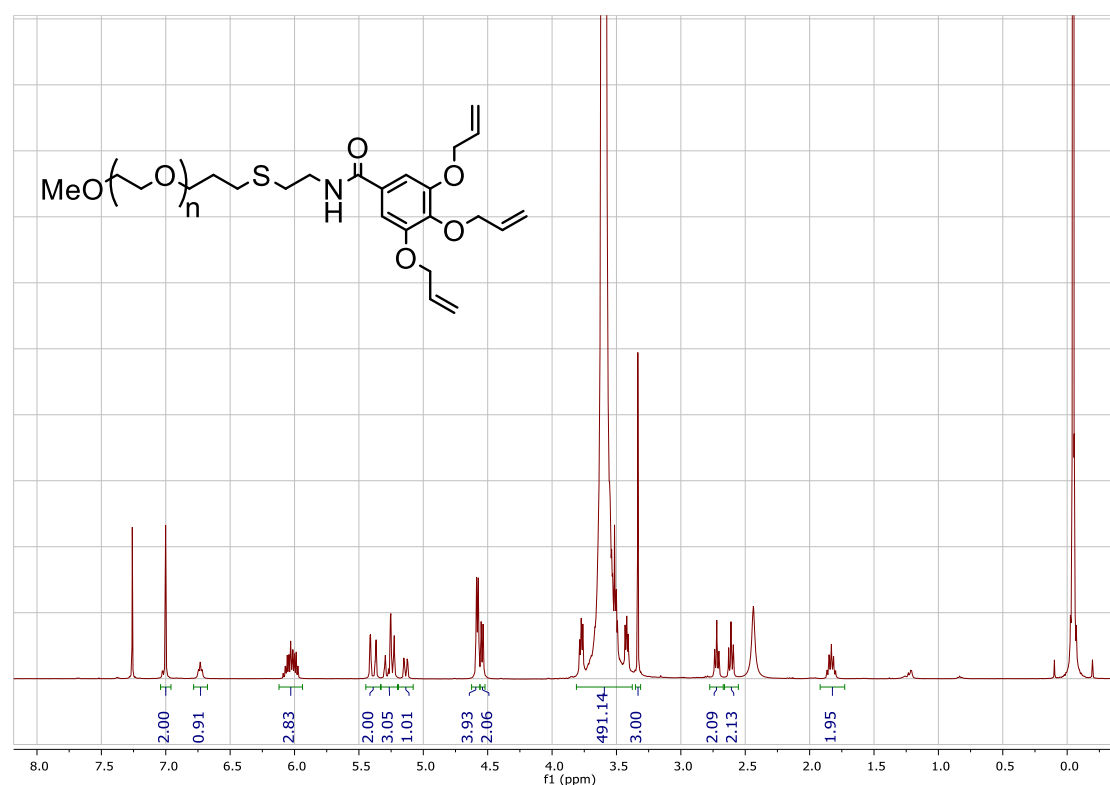


Figure S15. ¹H-NMR spectrum of mPEG_{5kDa}-dend-(3ene) in CDCl₃.

mPEG_{5kDa}-dend-(3Boc): mPEG_{5kDa}-dend-(3ene) (162 mg, 0.03 mmol), DMPA (5 mg, 0.018 mmol, 0.6 eq.) and 2-(Boc amino)ethanethiol (315 μL , 1.8 mmol, 60 eq.) were reacted in MeOH (810 μL) according to the described general procedure. The product was obtained as a white solid in 94% yield (167 mg).

¹H-NMR (CDCl₃): δ 7.00 (s, 2H, arom H), 6.75-6.91 (m, 1H, -NH-CO-), 4.89-5.21 (m, 3H, -NH-(Boc)), 4.08 (t, $J = 6.0\text{Hz}$, 4H, Ar-O-CH₂-), 4.03 (t, $J = 5.9\text{Hz}$, Ar-O-CH₂-), 3.40-3.78 (m, 496H, PEG H), 3.33 (s, 3H, H₃C-O-), 3.19-3.26 (m, 6H, -CH₂-NH-(Boc)), 2.67-2.77 (m, 8H, -CH₂-S-), 2.55-2.63 (m, 8H, -CH₂-S-), 2.04 (qui, $J = 6.5\text{Hz}$, 4H, -O-CH₂-CH₂-CH₂-S-), 1.93 (qui, $J = 6.5\text{Hz}$, 2H, -O-CH₂-CH₂-CH₂-S-), 1.83 (qui, $J = 6.7\text{Hz}$, 2H, -O-CH₂-CH₂-CH₂-S-), 1.39 (s, 27H, -CO-O-C-(CH₃)₃); ¹³C-NMR (CDCl₃): δ 167.1, 155.9, 152.7, 140.7, 130.0, 106.2, 82.1, 79.5, 72.0, 71.7, 70.6, 70.3, 69.5, 67.6, 59.1, 39.9, 39.2, 32.4, 32.3, 31.7, 30.5, 29.8, 29.7, 29.4, 28.5, 28.4; FT-IR, $\nu(\text{cm}^{-1})$: 2869, 1569, 1558, 1541, 1522, 1508, 1498, 1489,

1466, 1457, 1437, 1419, 1396, 1360, 1342, 1279, 1241, 1146, 1104, 1061, 993, 962, 842;
 GPC (DMF + 25 mM NH₄Ac): M_n = 6.0 kDa, PDI = 1.03, Expected M_n = 5.9 kDa;

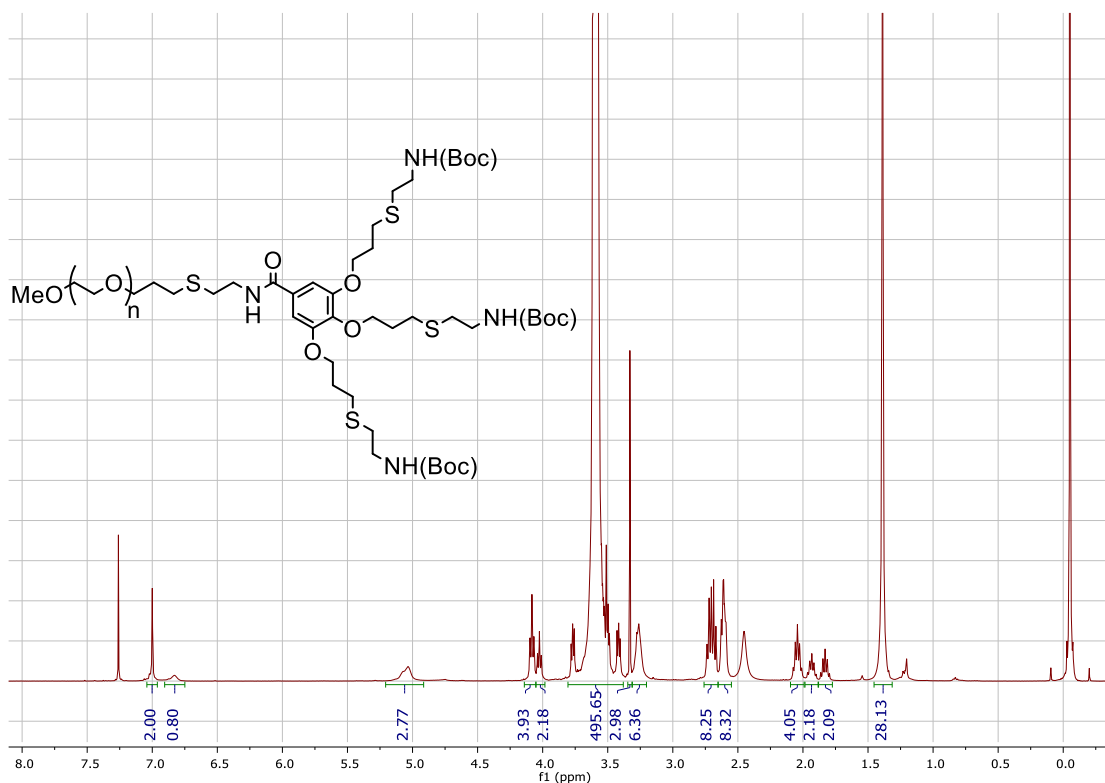


Figure S16. ¹H-NMR spectrum of mPEG_{5kDa}-dend-(3Boc) in CDCl₃.

mPEG_{5kDa}-dend-(3Ph) (Hybrid 1): The Boc groups of mPEG_{5kDa}-dend-(3Boc) (118 mg, 0.02 mmol) were removed with TFA and then the deprotected PEG was reacted with DIPEA (95 μL, 0.54 mmol, 30 eq.) and 4-nitrophenyl 2-phenylacetate (46 mg, 0.18 mmol, 9 eq.) in DCM:DMF 1:1v/v (1.2 mL) according to the described general procedure. The product was obtained as a white solid in 90% yield (107 mg).

¹H-NMR (CDCl₃): δ 7.17-7.25 + 7.27-7.36 (m, 15H, arom H), 7.11 (t, *J* = 5.5Hz, 1H, -NH-CO-Ar), 7.04 (s, 2H, arom H), 6.17 (t, *J* = 5.6Hz, 1H, -NH-CO-), 6.08 (t, *J* = 5.3Hz, 2H, -NH-CO-), 4.06 (t, *J* = 6.1Hz, 4H, Ar-O-CH₂-), 4.01 (t, *J* = 5.8Hz, 2H, Ar-O-CH₂-), 3.55-3.81 (m, 488H, PEG backbone), 3.33-3.40 (m, 9H, H₃C-O + -CH₂-NH-CO-), 2.52-2.77 (m, 16H, -CH₂-S-), 2.00 (qui, *J* = 6.5Hz, 4H, -O-CH₂-CH₂-CH₂-S-), 1.81-1.93 (m, 4H, -O-CH₂-CH₂-CH₂-S-); ¹³C-NMR (CDCl₃): δ 171.3, 171.2, 167.1, 152.6, 140.6, 135.0, 134.9, 130.1, 129.5, 129.02, 128.97, 127.4, 127.3, 106.3, 72.0, 71.7, 70.7, 70.3, 69.6, 67.5, 59.1, 43.79, 43.76, 39.4, 39.0, 38.9, 31.8, 31.7, 31.6, 30.4, 29.8, 29.5, 28.4, 28.3; FT-IR, ν(cm⁻¹): 2868, 1569, 1558, 1541, 1522, 1508, 1497, 1489, 1473, 1457, 1437, 1419, 1396, 1374, 1348, 1277, 1248, 1146, 1093, 1039, 949, 845; GPC (DMF + 25 mM NH₄Ac): M_n = 5.9 kDa, PDI = 1.04, Expected M_n = 6.0 kDa;

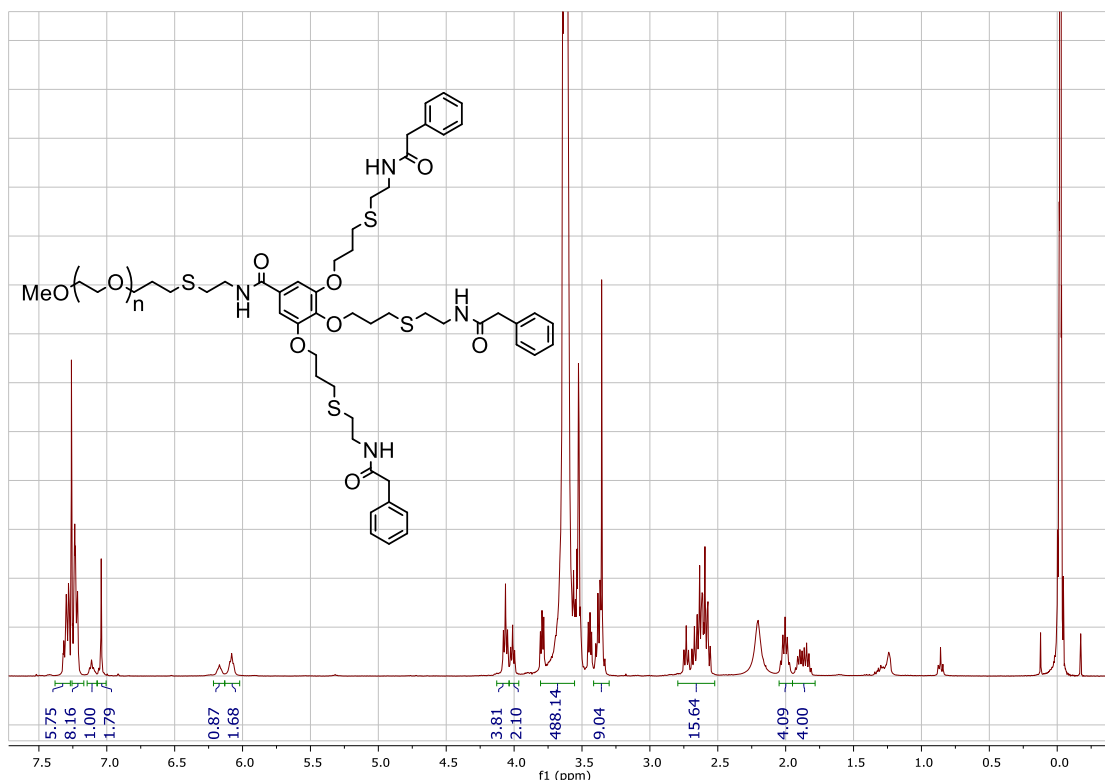


Figure S17. $^1\text{H-NMR}$ spectrum of $\text{mPEG}_{5\text{kDa}}\text{-dend-(3Ph)}$ (Hybrid 1) in CDCl_3 .

Synthesis of $\text{mPEG}_{5\text{kDa}}\text{-dend-(4Ph)}$ (Hybrid 2):

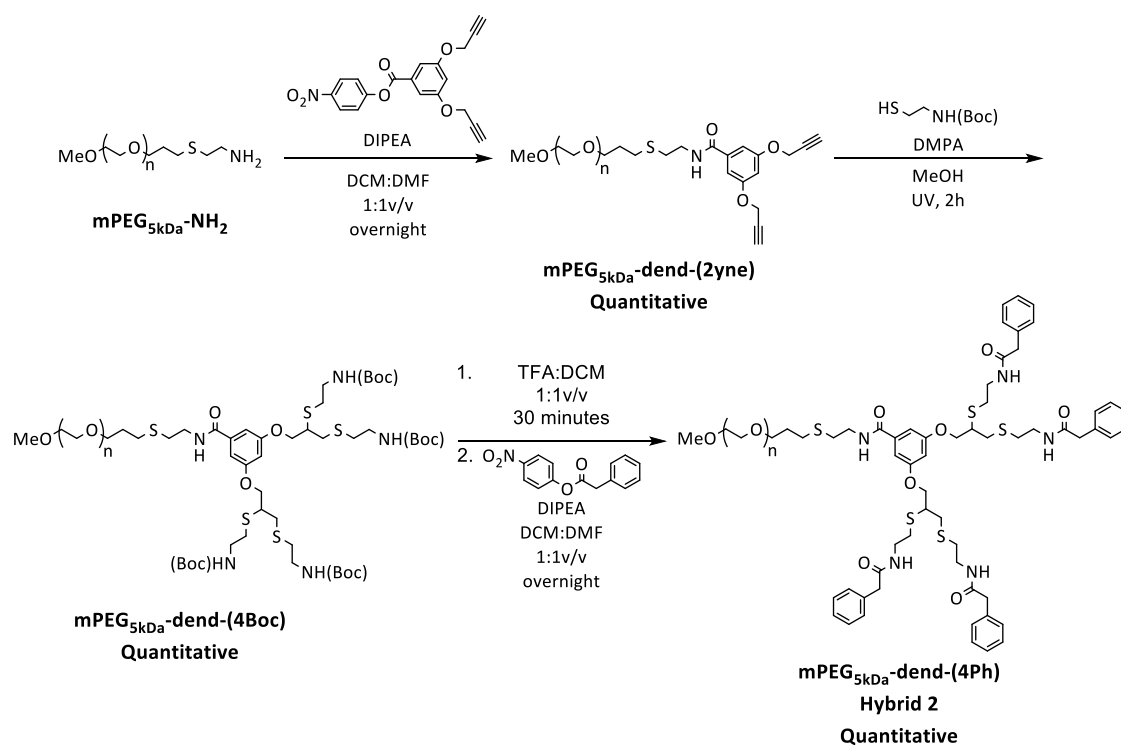


Figure S18. Preparation of $\text{mPEG}_{5\text{kDa}}\text{-dend-(4Ph)}$ (Hybrid 2).

mPEG_{5kDa}-2yne, mPEG_{5kDa}-4Boc and mPEG_{5kDa}-4Ph (Hybrid 2) were prepared according to the described general procedures. Their characterizations (¹H and ¹³C NMR, IR, GPC and MALDI) correlated with previously reported data.¹

Synthesis of mPEG_{5kDa}-dend-(6Ph) (Hybrid 3):

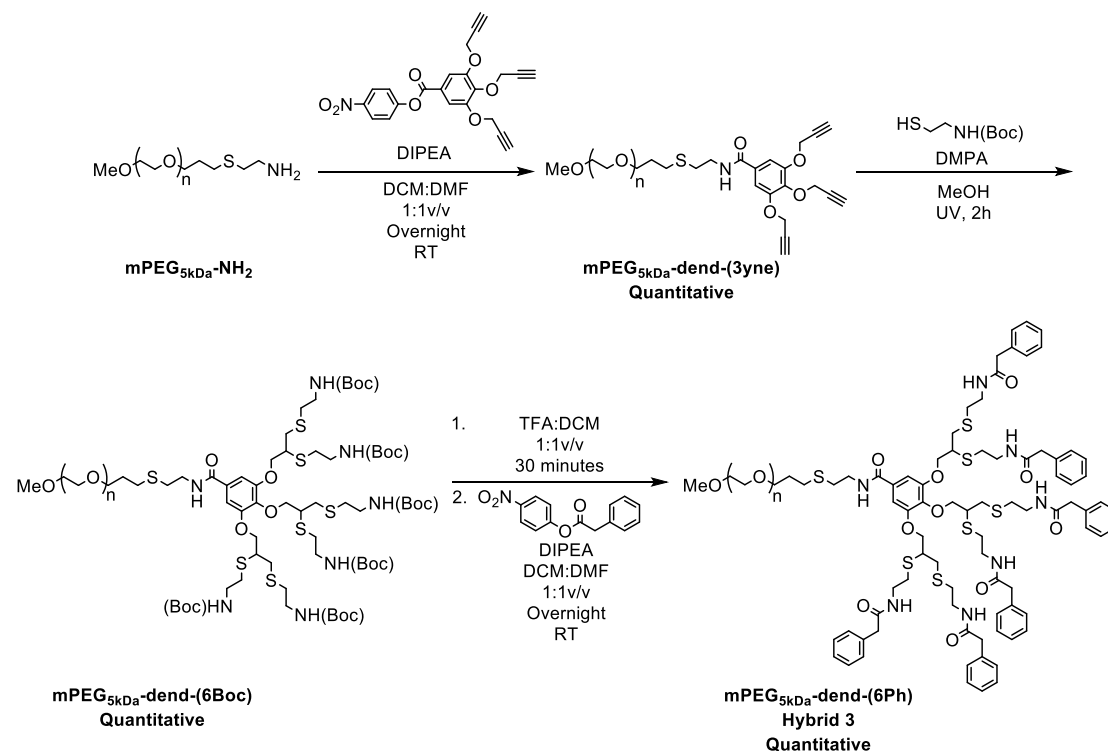


Figure S19. Preparation of mPEG_{5kDa}-dend-(6Ph) (Hybrid 3).

mPEG_{5kDa}-dend-(3yne): mPEG_{5kDa}-NH₂ (205 mg, 0.04 mmol), DIPEA (63 μ L, 0.36 mmol, 9 eq.) and PNP-AB₆ (49 mg, 0.12 mmol, 3 eq.) were reacted in DCM:DMF 1:1v/v (2 mL) according to the described general procedure. The product was obtained as a white solid in quantitative yield (209 mg).

¹H-NMR (CDCl₃): δ 7.23 (s, 2H, arom H), 6.86 (t, J = 5.4Hz, 1H, -NH-CO-), 4.81 (d, J = 2.3Hz, 4H, -O-CH₂-C \equiv), 4.79 (d, J = 2.4Hz, 2H, -O-CH₂-C \equiv), 3.44-3.82 (m, 513H, PEG backbone), 3.37 (s, 3H, H₃C-O-), 2.77 (t, J = 6.4Hz, 2H, -S-CH₂-CH₂-NH-CO-), 2.65 (t, J = 7.2Hz, 2H, -O-CH₂-CH₂-CH₂-S-), 2.56 (t, J = 2.3Hz, 2H, -C \equiv CH), 2.46 (t, J = 2.4Hz, 1H, -C \equiv CH), 1.87 (qui, J = 6.7Hz, 2H, -O-CH₂-CH₂-CH₂-S-); ¹³C-NMR (CDCl₃): δ 166.7, 151.7, 140.1, 130.5, 108.0, 78.9, 78.3, 76.5, 75.7, 72.1, 70.7, 70.3, 69.5, 60.5, 59.2, 57.4, 39.1, 31.9, 29.8, 28.4; FT-IR, ν (cm⁻¹): 2869, 1569, 1558, 1541, 1522, 1508, 1498, 1489, 1466, 1457, 1437, 1419, 1396, 1360, 1342, 1279, 1241, 1146, 1104, 1061, 993, 962, 842;; GPC (DMF + 25 mM NH₄Ac): M_n = 5.4 kDa, PDI = 1.04, Expected M_n = 5.4 kDa;

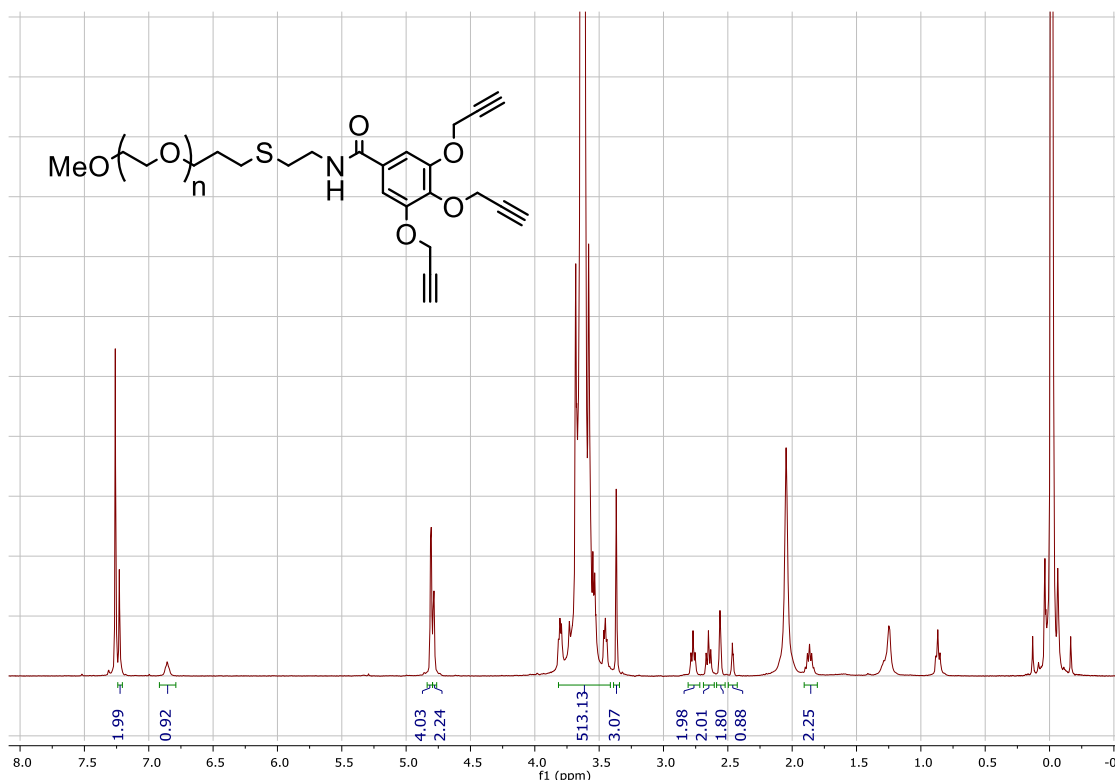


Figure S20. $^1\text{H-NMR}$ spectrum of $\text{mPEG}_{5\text{kDa-dend-(3yne)}}$ in CDCl_3 .

mPEG_{5kDa-dend-(6Boc)}: $\text{mPEG}_{5\text{kDa-dend-(3yne)}}$ (185 mg, 0.034 mmol), 2-(Boc amino)ethanethiol (715 μL , 4.1 mmol, 120 eq.) and DMPA (11 mg, 0.041 mmol, 1.2 eq.) were reacted in MeOH (925 μL) according to the described general procedure. The product was obtained as a white solid in quantitative yield (217 mg).

$^1\text{H-NMR}$ (CDCl_3): δ 7.12 (s, 2H, arom H), 5.08-5.48 (m, 6H, -NH-CO-O-), 4.07-4.36 (m, 6H, Ar-O-CH₂-), 3.43-3.81 (m, 482H, PEG backbone), 3.24-3.39 (m, 15H, H₃C-O- + -CH₂-NH-CO-O-), 2.59-3.23 (m, 25H, -CH₂-S- + CH-S-), 1.86 (qui, $J = 6.7\text{Hz}$, 2H, -O-CH₂-CH₂-CH₂-S-), 1.41 (s, 54H, -CO-O-C-(CH₃)₃); $^{13}\text{C-NMR}$ (CDCl_3): δ 165.7, 155.1, 155.0, 151.0, 139.2, 129.2, 105.5, 81.2, 78.5, 78.4, 73.5, 73.1, 73.0, 71.0, 70.9, 69.6, 69.3, 68.6, 67.4, 66.2, 62.8, 58.1, 45.3, 44.1, 39.6, 39.2, 38.5, 38.4, 33.6, 32.1, 31.0, 30.6, 28.7, 27.6, 27.4; FT-IR, $\nu(\text{cm}^{-1})$: 2867, 1569, 1558, 1540, 1522, 1508, 1497, 1489, 1472, 1457, 1437, 1419, 1397, 1374, 1348, 1277, 1246, 1146, 1093, 1040, 949, 842; GPC (DMF + 25 mM NH₄Ac): $M_n = 6.5$ kDa, PDI = 1.03, Expected $M_n = 6.4$ kDa;

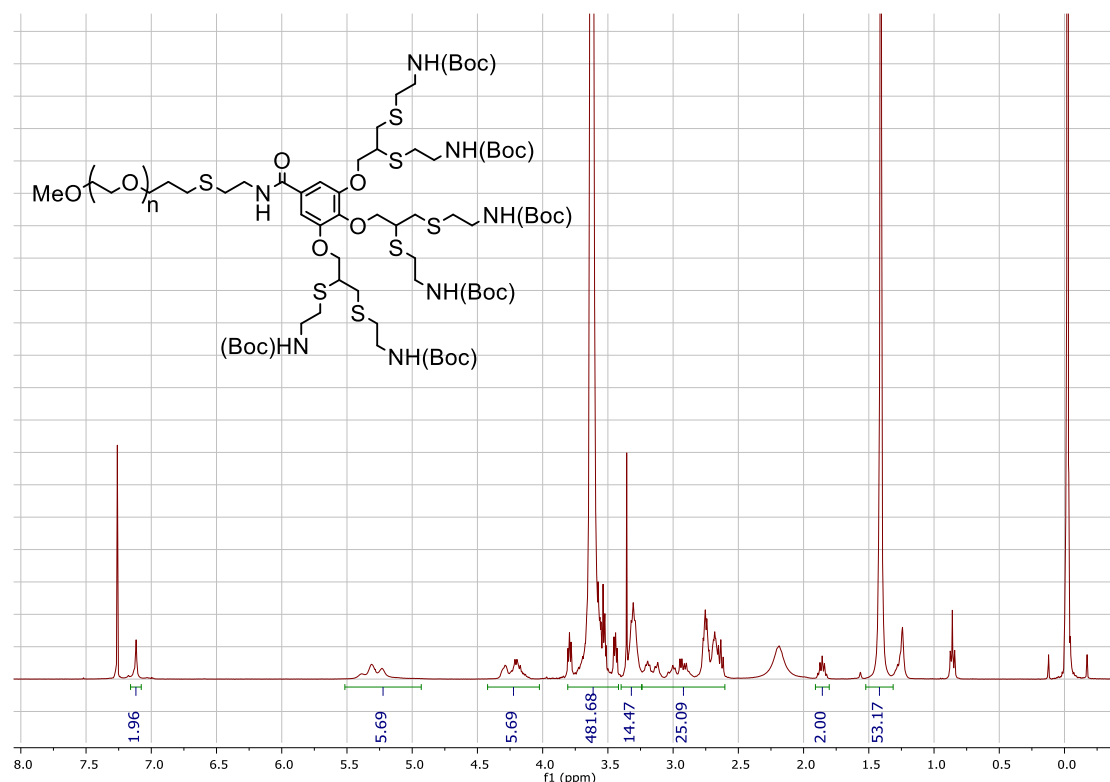


Figure S21. $^1\text{H-NMR}$ spectrum of $\text{mPEG}_{5\text{kDa-dend-(6Boc)}}$ in CDCl_3 .

mPEG_{5kDa-dend-(6Ph)} (Hybrid 3): $\text{mPEG}_{5\text{kDa-dend-(6Boc)}}$ (148 mg, 0.023 mmol) was deprotected with TFA and then reacted with DIPEA (220 μL , 1.24 mmol, 54 eq.) and 4-nitrophenyl 2-phenylacetate (106 mg, 0.41 mmol, 18 eq.) as described in the general procedure. The product was obtained as a white solid in quantitative yield (145 mg).

$^1\text{H-NMR}$ (CDCl_3): δ 7.71 (t, $J = 5.1\text{Hz}$, 1H, -NH-CO-Ar), 7.11-7.25 + 7.27-7.36 (m, 32H, arom H), 6.80 (t, $J = 5.0\text{Hz}$, 1H, -NH-CO-), 6.69 (t, $J = 5.2\text{Hz}$, 1H, -NH-CO-), 6.59 (t, $J = 5.2\text{Hz}$, 2H, -NH-CO-), 6.51 (t, $J = 5.4\text{Hz}$, 2H, -NH-CO-), 3.99-4.31 (m, 6H, Ar-O-CH₂-), 3.50-3.81 (m, 523H, PEG backbone), 3.24-3.40 (m, 15H, H₃C-O- + -CH₂-NH-CO-), 2.48-3.18 (m, 25H, -CH₂-S- + -CH-S-), 1.83 (qui, $J = 6.7\text{Hz}$, 2H, -O-CH₂-CH₂-CH₂-S-); $^{13}\text{C-NMR}$ (CDCl_3): δ 171.7, 171.6, 166.9, 151.9, 140.0, 135.2, 135.1, 130.4, 129.4, 128.9, 128.82, 128.80, 127.25, 127.22, 127.15, 127.13, 72.0, 70.6, 70.2, 69.7, 59.1, 46.1, 45.0, 43.63, 43.59, 43.55, 39.8, 39.7, 39.5, 39.3, 34.5, 32.5, 32.3, 31.5, 31.0, 29.7, 28.5; FT-IR, $\nu(\text{cm}^{-1})$: 2866, 1569, 1558, 1540, 1522, 1507, 1496, 1488, 1472, 1457, 1437, 1419, 1397, 1366, 1348, 1277, 1246, 1146, 1102, 1040, 949, 845; GPC (DMF + 25 mM NH₄Ac): $M_n = 6.5$ kDa, PDI = 1.06, Expected $M_n = 6.6$ kDa;

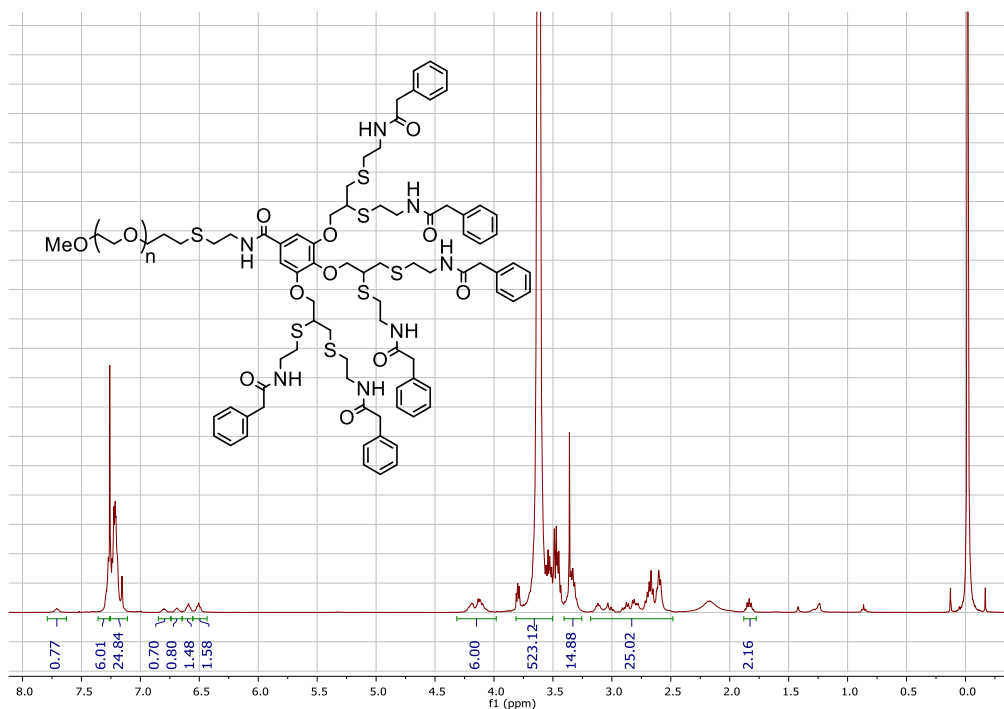


Figure S22. $^1\text{H-NMR}$ spectrum of $\text{mPEG}_{5\text{kDa}}\text{-dend-(6Ph)}$ (Hybrid 3) in CDCl_3 .

Synthesis of $\text{mPEG}_{5\text{kDa}}\text{-[dend-(3Ph)]}_2$ (Hybrid 4):

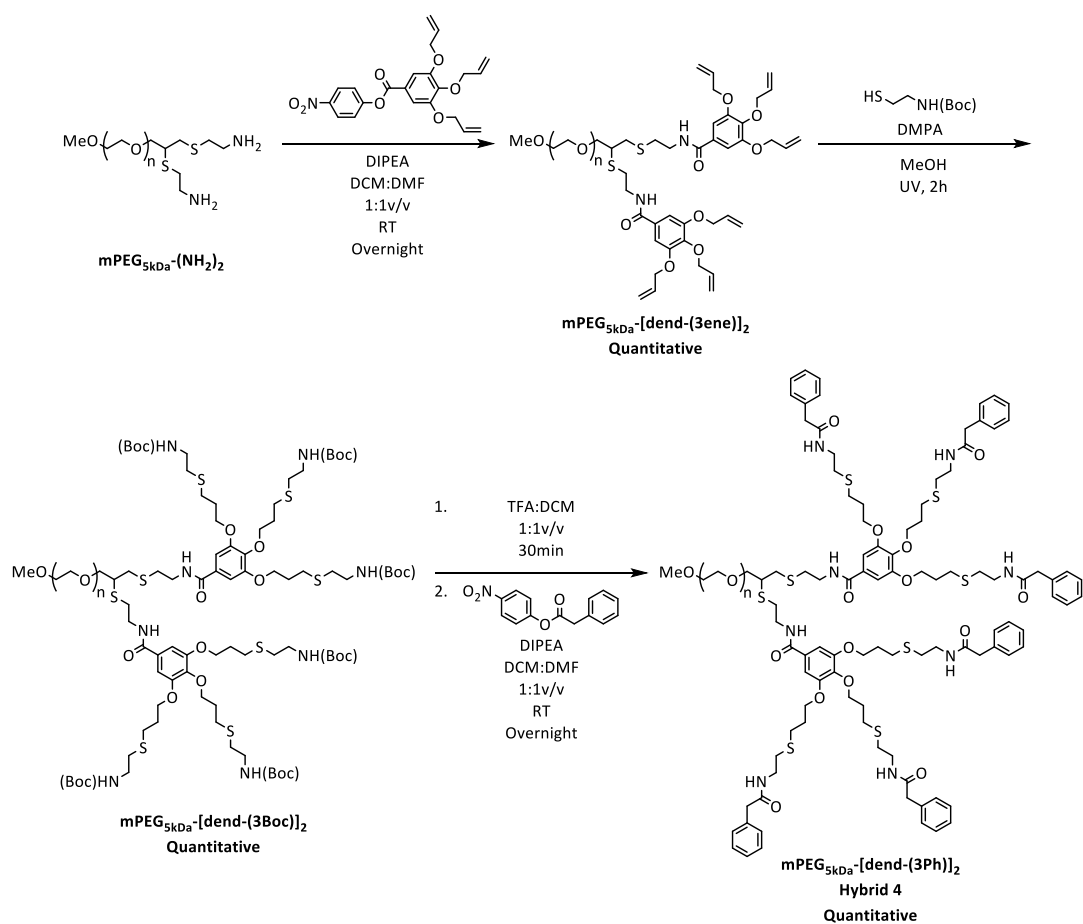


Figure S23. Preparation of $\text{mPEG}_{5\text{kDa}}\text{-[dend-(3Ph)]}_2$ (Hybrid 4).

mPEG_{5kDa}-[dend-(3ene)]₂: mPEG_{5kDa}-(NH₂)₂ (260 mg, 0.05 mmol), DIPEA (175 μL, 1 mmol, 20 eq.) and PNP-AB₃ (123 mg, 0.3 mmol, 6 eq.) were reacted in DCM:DMF 1:1v/v (2.5 mL) as described in the general procedure. The product was obtained as a white solid in quantitative yield (283 mg).

¹H-NMR (CDCl₃): δ 7.33 (t, *J* = 5.6Hz, 1H, -NH-CO-), 7.04 (s, 4H, arom H), 5.85-6.10 (m, 6H, vinylic H), 5.30-5.39 (m, 4H, *E* vinylic H), 5.23-5.30 (m, 2H, *E* vinylic H), 5.16-5.23 (m, 4H, *Z* vinylic H), 5.09-5.16 (m, 2H, *Z* vinylic H), 4.39-4.56 (m, 12H, allylic H), 3.41-3.79 (m, PEG backbone, 507H), 3.33 (s, 3H, H₃C-O-), 3.04 (qui, *J* = 6.1Hz, 1H, -CH-S-), 2.65-2.86 (m, 6H, -CH₂-S-); ¹³C-NMR (CDCl₃): δ 167.2, 167.1, 152.5, 146.0, 134.3, 133.1, 129.5, 117.8, 117.6, 106.69, 106.65, 74.1, 73.1, 72.0, 70.6, 70.0, 59.1, 45.2, 40.1, 39.5, 34.0, 31.8, 31.0; FT-IR, ν(cm⁻¹): 2866, 1569, 1558, 1540, 1522, 1508, 1496, 1489, 1473, 1457, 1437, 1419, 1397, 1367, 1348, 1277, 1246, 1146, 1100, 1040, 945, 843; GPC (DMF + 25 mM NH₄Ac): M_n = 5.6 kDa, PDI = 1.04, Expected M_n = 5.5 kDa;

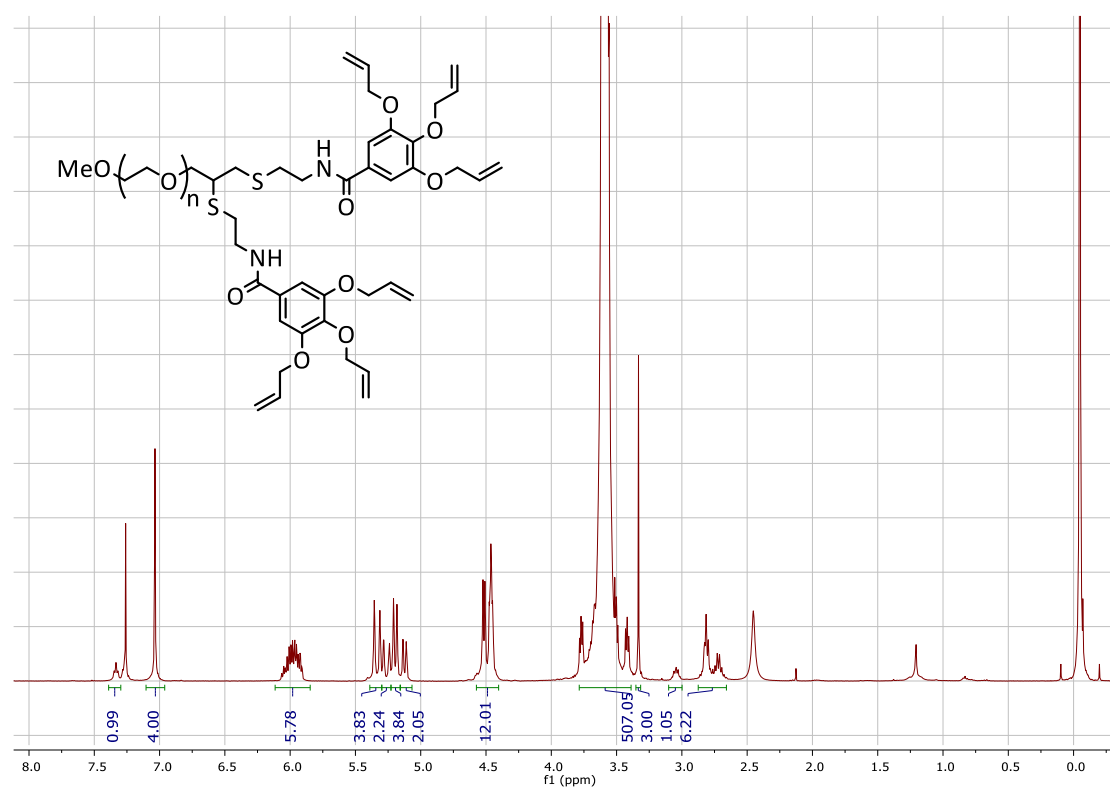


Figure S24. ¹H-NMR spectrum of mPEG_{5kDa}-[dend-(3ene)]₂ in CDCl₃.

mPEG_{5kDa}-[dend-(3Boc)]₂: mPEG_{5kDa}-[dend-(3ene)]₂ (229 mg, 0.04 mmol), 2-(Boc amino)ethanethiol (835 μL, 4.8 mmol, 120 eq.) and DMPA (12 mg, 0.048 mmol, 1.2 eq.) were reacted in MeOH (1.15 mL) as described in the general procedure. The product was obtained as a white solid in quantitative yield (260 mg).

$^1\text{H-NMR}$ (CDCl_3): δ 7.64-7.83 (m, 2H, -NH-CO-), 7.09 (s, 4H, arom H), 4.96-5.28 (m, 6H, -NH(Boc)), 3.89-4.08 (m, 12H, Ar-O-CH₂-), 3.41-3.81 (m, 507H, PEG backbone), 3.36 (s, 3H, H₃C-O-), 3.20-3.33 (m, 12H, -CH₂-NH(Boc)), 3.05-3.16 (m, 1H, -CH-S-), 2.54-2.96 (m, 30H, -CH₂-S-), 1.88-2.07 (m, 12H, -O-CH₂-CH₂-CH₂-S-); $^{13}\text{C-NMR}$ (CDCl_3): δ 167.1, 156.0, 152.7, 140.6, 129.6, 106.1, 79.5, 72.9, 71.7, 70.7, 67.5, 59.1, 44.8, 40.1, 39.9, 32.3, 30.5, 29.4, 28.5, 28.4, 28.3; FT-IR, $\nu(\text{cm}^{-1})$: 2868, 1570, 1558, 1540, 1522, 1508, 1496, 1489, 1473, 1457, 1436, 1419, 1398, 1367, 1348, 1277, 1246, 1146, 1095, 1040, 942, 842; GPC (DMF + 25 mM NH₄Ac): $M_n = 6.7$ kDa, PDI = 1.07, Expected $M_n = 6.8$ kDa;

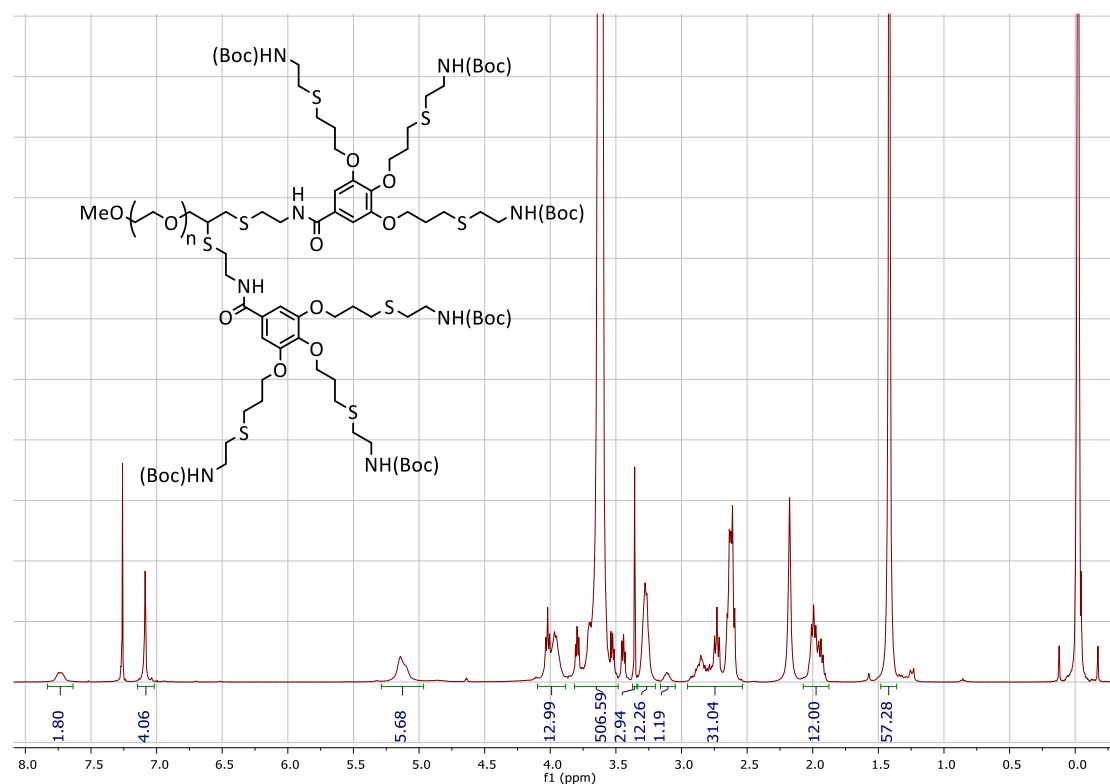


Figure S25. $^1\text{H-NMR}$ spectrum of mPEG_{5kDa}-[dend-(3Boc)]₂ in CDCl_3 .

mPEG_{5kDa}-[dend-(3Ph)]₂ (Hybrid 4): mPEG_{5kDa}-[dend-(3Boc)]₂ (187 mg, 0.028 mmol) was deprotected with TFA and then reacted with DIPEA (285 μL , 1.65 mmol, 60 eq.) and 4-nitrophenyl 2-phenylacetate (127 mg, 0.5 mmol, 18 eq.) as described in the general procedure. The product was obtained as an off-white solid in quantitative yield (188 mg).

$^1\text{H-NMR}$ (CDCl_3): δ 8.15-8.36 (m, 2H, -NH-CO-), 7.27-7.35 + 7.17-7.25 (m, 30H, arom H), 7.11 (s, 4H, arom H), 6.50-6.59 (m, 4H, -NH-CO-), 6.27-6.40 (m, 2H, -NH-CO-), 3.96 (t, $J = 5.7$ Hz, 4H, *para* Ar-O-CH₂-), 3.83-3.92 (m, 8H, *meta* Ar-O-CH₂-), 3.54-3.76 (m, 482H, PEG backbone), 3.29-3.39 (m, 15H, H₃C-O- + -CH₂-NH-CO-), 3.02-3.19 (m, 1H, -CH-S-), 2.49-2.94 (m, 30H, -CH₂-S-), 1.78-1.98 (m, 12H, -O-CH₂-CH₂-CH₂-S-); $^{13}\text{C-NMR}$ (CDCl_3): δ 171.5, 171.3, 167.3, 167.2, 152.6, 140.4, 135.0, 129.7, 129.5, 129.0, 127.4, 127.3, 106.2, 73.0, 72.0, 71.7, 67.4, 59.1, 43.7, 39.0, 31.7, 31.6, 30.4, 29.4, 28.4, 28.3; FT-IR, $\nu(\text{cm}^{-1})$: 2866,

1570, 1559, 1540, 1520, 1508, 1496, 1487, 1473, 1457, 1432, 1419, 1398, 1367, 1350, 1277, 1246, 1146, 1095, 1040, 944, 845; GPC (DMF + 25 mM NH₄Ac): M_n = 6.9 kDa, PDI = 1.05, Expected M_n = 6.9 kDa;

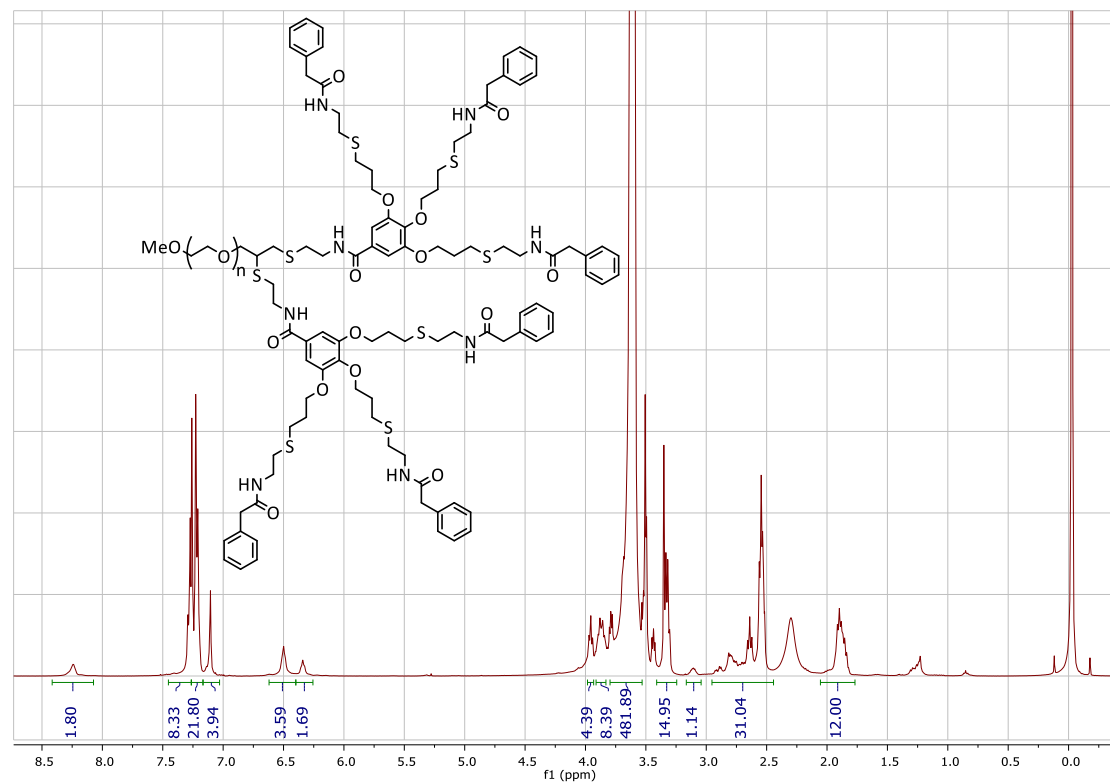


Figure S26. ¹H-NMR spectrum of mPEG_{5kDa}-[dend-(3Ph)]₂ (Hybrid 4) in CDCl₃.

Synthesis of mPEG_{5kDa}-[dend-(4Ph)]₂ (Hybrid 5):

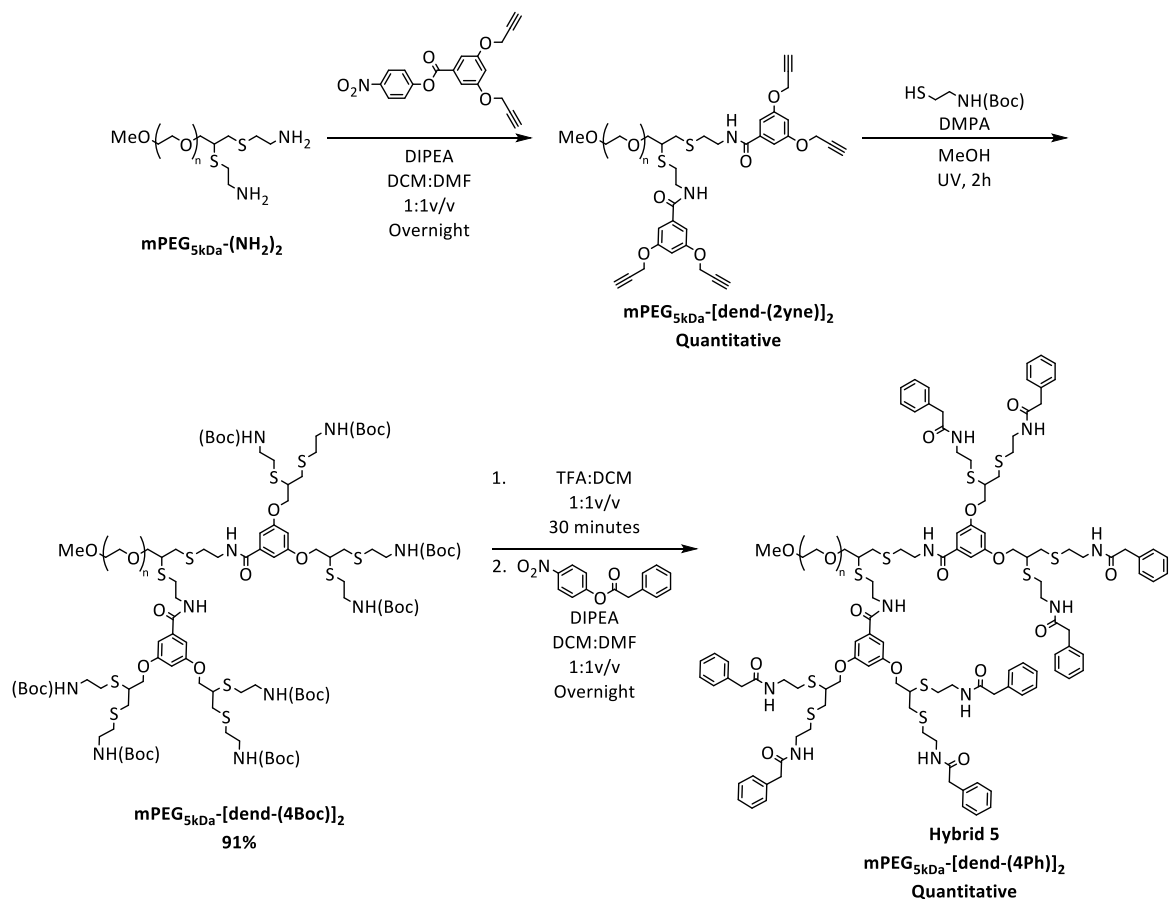


Figure S27. Preparation of $m\text{PEG}_{5\text{kDa}}-[\text{dend}-(4\text{Ph})]_2$ (Hybrid 5).

$m\text{PEG}_{5\text{kDa}}-[\text{dend}-(2\text{yne})]_2$: $m\text{PEG}_{5\text{kDa}}-(\text{NH}_2)_2$ (260 mg, 0.05 mmol), DIPEA (175 μL , 1 mmol, 20 eq.) and PNP-AB₄ (105 mg, 0.3 mmol, 6 eq.) were reacted in DCM:DMF 1:1 v/v (2.5 mL) as described in the general procedure. The product was obtained as an off-white solid in 91% yield (257 mg).

¹H-NMR (CDCl₃): δ 7.41 (t, $J = 5.5\text{Hz}$, 1H, -NH-CO-), 7.30 (t, $J = 5.5\text{Hz}$, 1H, -NH-CO-), 6.96-7.11 (m, 4H, arom H), 6.65-6.75 (m, 2H, arom H), 4.57-4.70 (m, 8H, Ar-O-CH₂-), 3.43-3.80 (m, 520H, PEG backbone), 3.35 (s, 3H, H₃C-O-), 2.99-3.11 (m, 1H, -CH-S-), 2.65-2.93 (m, 6H, -CH₂-S-), 2.54 (t, $J = 2.3\text{Hz}$, 4H, -C \equiv CH); ¹³C-NMR (CDCl₃): δ 167.0, 166.9, 158.7, 136.7, 106.80, 106.77, 105.58, 105.57, 78.1, 76.1, 73.1, 72.0, 70.6, 59.0, 56.1, 45.4, 40.0, 39.5, 34.2, 31.9, 31.0; FT-IR, $\nu(\text{cm}^{-1})$: 2868, 1570, 1559, 1540, 1521, 1508, 1496, 1487, 1473, 1457, 1433, 1418, 1398, 1365, 1348, 1277, 1246, 1143, 1098, 1045, 943, 842; GPC (DMF + 25 mM NH₄Ac): $M_n = 5.5$ kDa, PDI = 1.04, Expected $M_n = 5.6$ kDa;

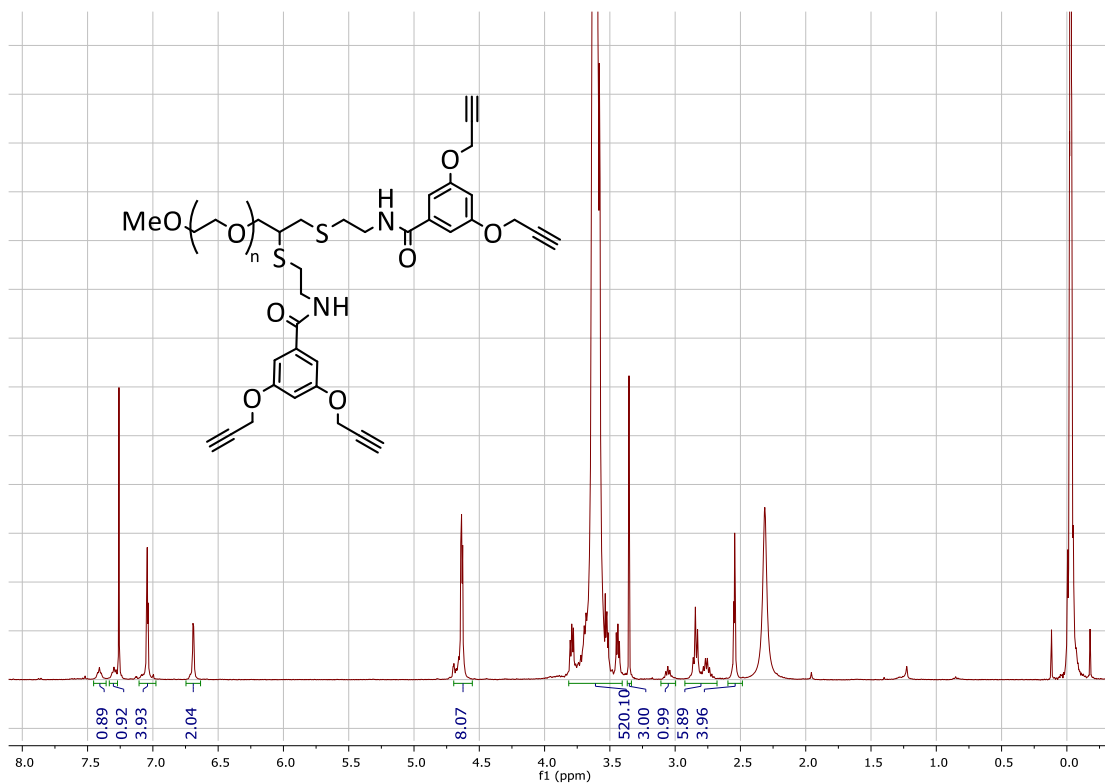


Figure S28. $^1\text{H-NMR}$ spectrum of $\text{mPEG}_{5\text{kDa}}\text{-[dend-(2yne)]}_2$ in CDCl_3 .

mPEG_{5kDa}-[dend-(4Boc)]₂: $\text{mPEG}_{5\text{kDa}}\text{-[dend-(2yne)]}_2$ (193 mg, 0.034 mmol), 2-(Boc amino)ethanethiol (960 μL , 5.5 mmol, 160 eq.) and DMPA (14mg, 0.055mmol, 1.6 eq.) were reacted in MeOH (1 mL) as described in the general procedure. The product was obtained as an off-white solid in quantitative yield (238 mg).

$^1\text{H-NMR}$ (CDCl_3): δ 7.51-7.88 (m, 2H, -NH-CO-), 6.89-7.07 (m, 4H, arom H), 6.45-6.62 (m, 2H, arom H), 5.05-5.47 (m, 8H, -NH(Boc)), 3.97-4.20 (m, 8H, Ar-O-CH₂), 3.49-3.79 (m, 554H, PEG backbone), 3.20-3.35 (m, 19H, H₃C-O- + -CH₂-NH(Boc)), 3.00-3.12 (m, 5H, -CH-S-), 2.56-2.94 (m, 30H, -CH₂-S-), 1.39 (s, 72H, -O-C(CH₃)₃); $^{13}\text{C-NMR}$ (CDCl_3): δ 167.09, 167.06, 159.5, 156.03, 156.00, 136.6, 106.2, 105.1, 79.5, 74.1, 73.0, 72.0, 71.8, 70.6, 69.7, 69.5, 67.2, 63.7, 59.1, 45.1, 45.0, 40.6, 40.2, 40.1, 39.7, 34.4, 34.0, 33.0, 32.0, 31.8, 30.9, 28.5; FT-IR, $\nu(\text{cm}^{-1})$: 2867, 1569, 1559, 1542, 1520, 1508, 1496, 1487, 1473, 1458, 1435, 1419, 1398, 1365, 1348, 1277, 1246, 1143, 1098, 1043, 943, 842; GPC (DMF + 25 mM NH₄Ac): $M_n = 6.9$ kDa, PDI = 1.1, Expected $M_n = 7.0$ kDa;

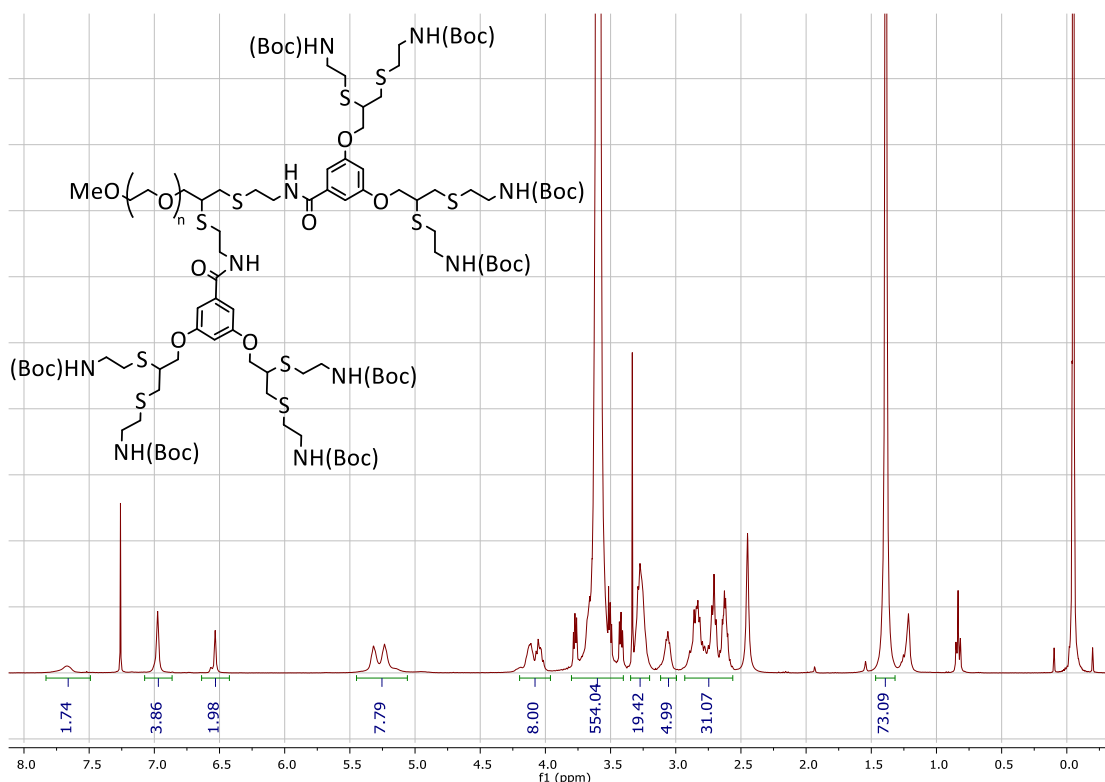


Figure S29. $^1\text{H-NMR}$ spectrum of $\text{mPEG}_{5\text{kDa}}\text{-[dend-(4Boc)]}_2$ in CDCl_3 .

$\text{mPEG}_{5\text{kDa}}\text{-[dend-(4Ph)]}_2$ (Hybrid 5): $\text{mPEG}_{5\text{kDa}}\text{-[dend-(4Boc)]}_2$ (175 mg, 0.025 mmol) was deprotected with TFA and then reacted with DIPEA (315 μL , 1.8 mmol, 72 eq.) and PNP-PhAcAcid (154 mg, 0.6 mmol, 24 eq.) as described for $\text{mPEG}_{5\text{kDa}}\text{-3Ph}$. The product was obtained as a white solid in quantitative yield (177 mg).

$^1\text{H-NMR}$ (CDCl_3): δ 7.86-8.16 (m, 2H, -NH-CO-), 7.11-7.43 (m, 40H, arom H + CHCl_3), 6.99 (s, 4H, arom H), 6.21-6.74 (m, 10H, arom H + -NH-CO-), 3.89-4.15 (m, 8H, Ar-O- CH_2 -), 3.51-3.73 (m, 552H, PEG backbone), 3.44-3.50 (m, 16H, -CO- CH_2 -Ph), 3.20-3.41 (m, 19H, - CH_2 -NH-CO- + $\text{H}_3\text{C-O}$ -), 2.40-3.15 (m, 35H, - CH_2 -S- + -CH-S-); $^{13}\text{C-NMR}$ (CDCl_3): δ 171.5, 171.4, 167.2, 159.4, 136.6, 135.1, 129.4, 128.9, 127.2, 106.4, 105.0, 72.8, 72.0, 71.8, 70.6, 69.7, 59.1, 44.8, 43.6, 39.6, 39.5, 39.2, 39.1, 34.2, 32.2, 31.2; FT-IR, $\nu(\text{cm}^{-1})$: 2868, 1569, 1560, 1542, 1521, 1509, 1496, 1485, 1473, 1458, 1435, 1420, 1398, 1365, 1348, 1277, 1246, 1145, 1098, 1043, 942, 845; GPC (DMF + 25mM NH_4Ac): $M_n=7.1\text{kDa}$, PDI=1.09, Expected $M_n=7.2\text{kDa}$;

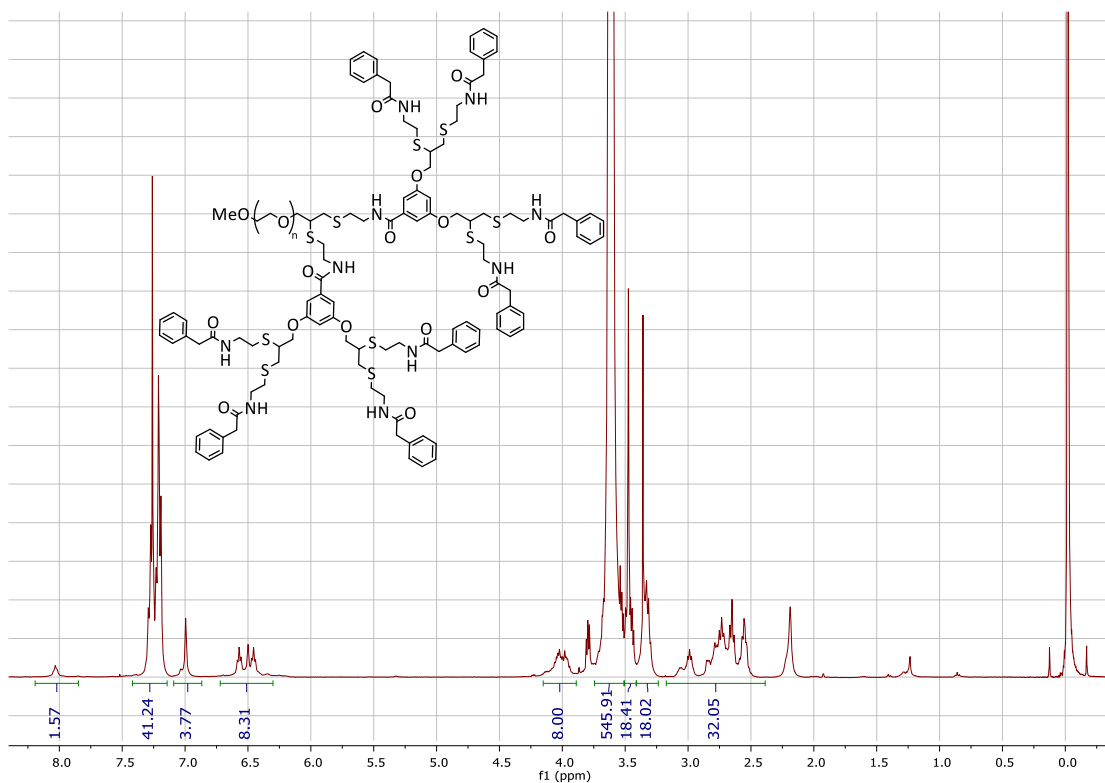


Figure S30. $^1\text{H-NMR}$ spectrum of $\text{mPEG}_{5\text{kDa}}\text{-[dend-(4Ph)]}_2$ (Hybrid 5) in CDCl_3 .

Synthesis of $\text{mPEG}_{5\text{kDa}}\text{-[dend-(6Ph)]}_2$ (Hybrid 6):

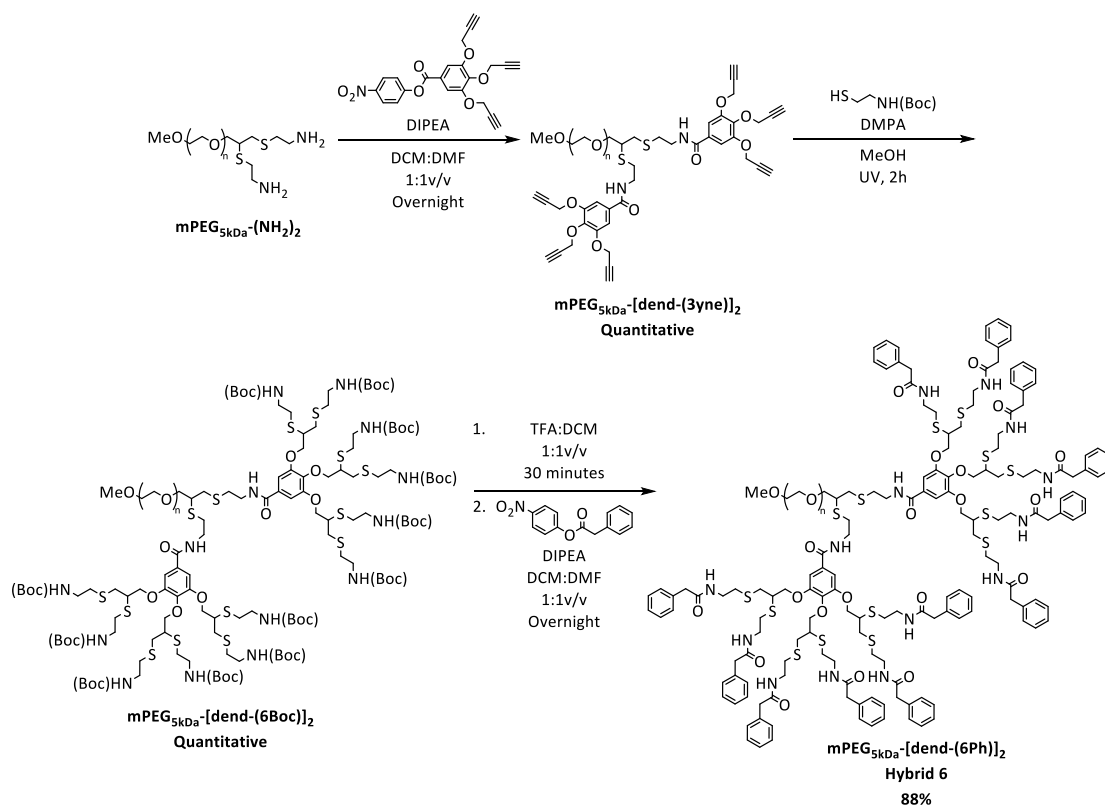


Figure S31. Preparation of $\text{mPEG}_{5\text{kDa}}\text{-[dend-(6Ph)]}_2$ (Hybrid 6).

mPEG_{5kDa}-[dend-(3yne)]₂: mPEG_{5kDa}-(NH₂)₂ (260 mg, 0.05 mmol), DIPEA (175 μL, 1 mmol, 20 eq.) and PNP-AB₆ (122 mg, 0.3 mmol, 6 eq.) were reacted in DCM:DMF 1:1v/v (2.5 mL) as described in the general procedure. The product was obtained as an off-white solid in quantitative yield (278 mg).

¹H-NMR (CDCl₃): δ 7.46 (t, *J* = 5.6 Hz, 1H, -NH-CO-), 7.37 (t, *J* = 5.6 Hz, 1H, -NH-CO-), 7.23 (s, 4H, arom H), 4.74 (d, *J* = 2.4 Hz, 4H, *para* Ar-O-CH₂-), 4.66-4.72 (m, 8H, *meta* Ar-O-CH₂-), 3.41-3.80 (m, 525H, PEG backbone), 3.33 (s, 3H, H₃C-O-), 3.06 (qui, *J* = 6.2 Hz, 1H, -CH-S-), 2.69-2.87 (m, 6H, -CH₂-S-), 2.52 (t, *J* = 2.3 Hz, 4H, *meta* -C≡CH), 2.45 (t, *J* = 2.4 Hz, 2H, *para* -C≡CH); ¹³C-NMR (CDCl₃): δ 166.8, 166.7, 151.5, 139.8, 130.2, 107.8, 78.8, 78.2, 76.4, 75.7, 73.1, 71.9, 70.6, 60.3, 59.1, 57.1, 45.2, 40.2, 39.6, 34.1, 31.8, 31.0; FT-IR, ν(cm⁻¹): 2866, 1570, 1559, 1542, 1521, 1509, 1496, 1485, 1473, 1458, 1435, 1420, 1398, 1365, 1348, 1277, 1246, 1148, 1103, 1043, 945, 842; GPC (DMF + 25 mM NH₄Ac): M_n = 5.8 kDa, PDI = 1.04, Expected M_n = 5.7 kDa;

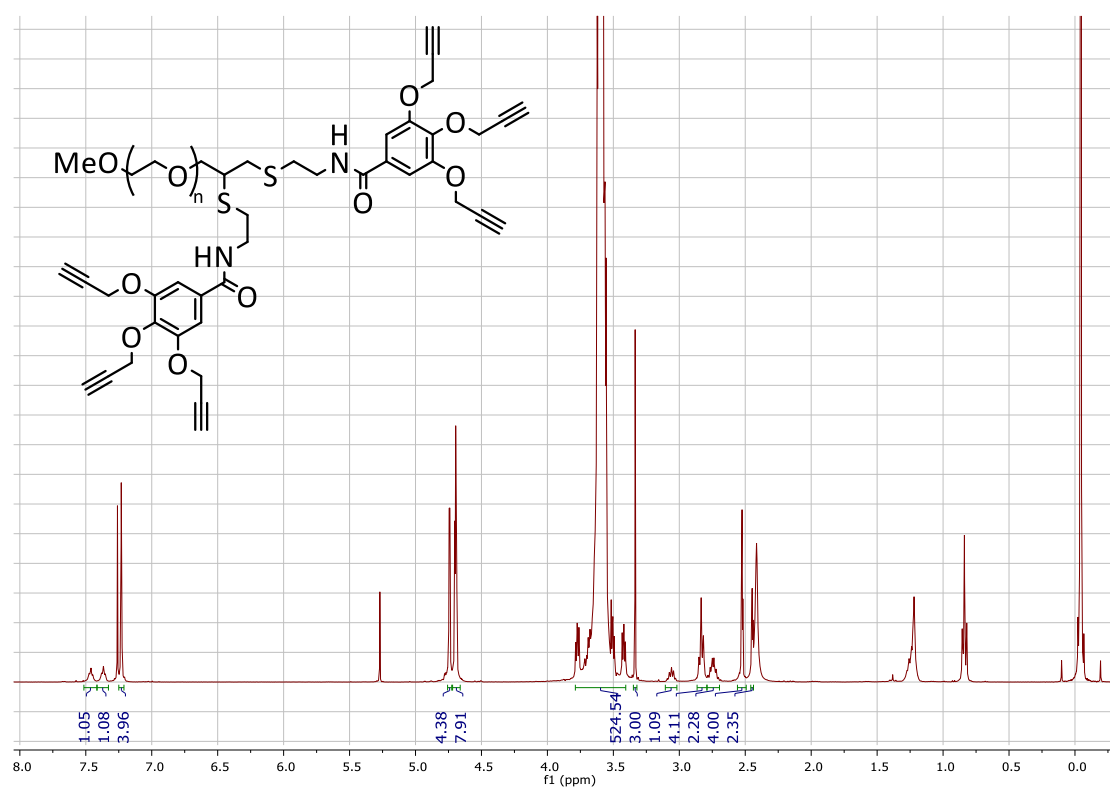


Figure S32. ¹H-NMR spectrum of mPEG_{5kDa}-[dend-(3yne)]₂ in CDCl₃.

mPEG_{5kDa}-[dend-(6Boc)]₂: mPEG_{5kDa}-[dend-(3yne)]₂ (196 mg, 0.034 mmol), 2-(Boc amino)ethanethiol (1.5 mL, 8.2 mmol, 240 eq.) and DMPA (21 mg, 0.082 mmol, 2.4 eq.) were reacted in MeOH (1 mL) as described in the general procedure. The product was obtained as an off-white solid in quantitative yield (261 mg).

$^1\text{H-NMR}$ (CDCl_3): δ 7.57-7.99 (m, 2H, -NH-CO-), 7.17 (s, 4H, arom H), 5.18-5.57 (m, 12H, -NH-CO-O-), 4.02-4.33 (m, 12H, Ar-O-CH₂-), 3.40-3.82 (m, 539H, PEG backbone), 3.20-3.37 (m, 27H, H₃C-O- + -CH₂-NH-CO-O-), 2.59-3.19 (m, 49H, -CH-S- + -CH₂-S-), 1.40 (s, 108H, -C-(CH₃)₃); $^{13}\text{C-NMR}$ (CDCl_3): δ 166.8, 156.1, 156.0, 152.1, 140.1, 129.9, 106.4, 79.4, 74.5, 74.1, 73.1, 72.0, 71.8, 70.7, 69.5, 67.2, 63.8, 59.1, 46.3, 45.2, 40.6, 40.3, 40.1, 39.7, 34.6, 33.1, 31.9, 28.6; FT-IR, $\nu(\text{cm}^{-1})$: 2866, 1570, 1559, 1543, 1521, 1509, 1496, 1485, 1473, 1459, 1435, 1422, 1398, 1365, 1348, 1277, 1248, 1148, 1103, 1043, 945, 842; GPC (DMF + 25 mM NH₄Ac): $M_n = 7.9$ kDa, PDI = 1.08, Expected $M_n = 7.9$ kDa;

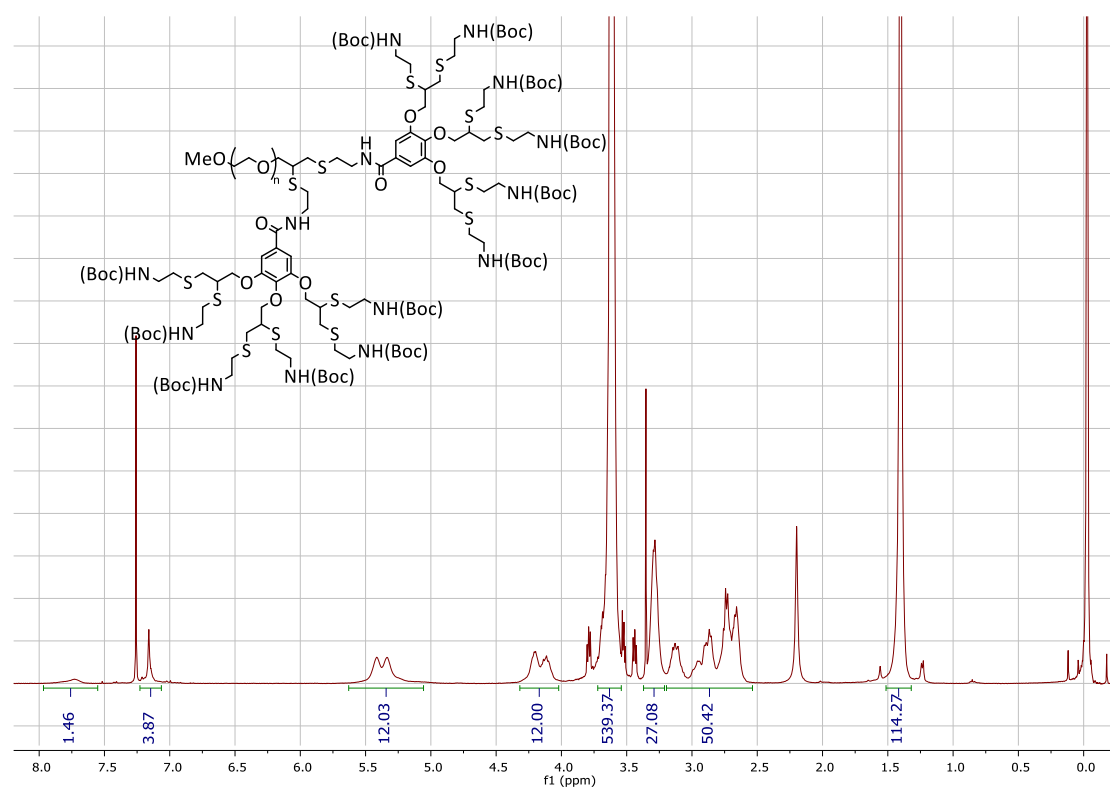


Figure S33. $^1\text{H-NMR}$ spectrum of $\text{mPEG}_{5\text{kDa}}\text{-[dend-(6Boc)]}_2$ in CDCl_3 .

mPEG_{5kDa}-[dend-(6Ph)]₂ (Hybrid 6): $\text{mPEG}_{5\text{kDa}}\text{-[dend-(6Boc)]}_2$ (120 mg, 0.015 mmol) was deprotected with TFA and then reacted with DIPEA (315 μL , 1.8 mmol, 120 eq.) and 4-nitrophenyl 2-phenylacetate (142 mg, 0.55 mmol, 36 eq.) as described in the general procedure. The product was obtained as an off-white solid in 93% yield (115 mg).

$^1\text{H-NMR}$ (CDCl_3): δ 8.03-8.41 (m, 2H, -NH-CO-), 7.03-7.50 (m, 64H, arom H), 6.48-6.99 (m, 12H, -NH-CO-), 3.88-4.26 (m, 12H, -Ar-O-CH₂-), 3.50-3.88 (m, 534H, PEG backbone), 3.18-3.39 (m, 27H, -CH₂-NH-CO- + H₃C-O-), 2.39-3.15 (m, 49H, -CH₂-S- + -CH-S-); $^{13}\text{C-NMR}$ (CDCl_3): δ 171.8, 171.6, 166.9, 152.0, 139.8, 135.23, 135.19, 135.16, 129.8, 129.4, 128.8, 127.14, 127.10, 106.4, 74.5, 74.0, 72.9, 72.0, 70.6, 69.4, 69.1, 67.1, 63.7, 59.1, 46.1,

45.0, 43.5, 43.4, 39.7, 39.5, 39.3, 34.4, 32.3, 32.2, 30.9; FT-IR, $\nu(\text{cm}^{-1})$: 2868, 1570, 1559, 1543, 1522, 1509, 1496, 1485, 1473, 1459, 1435, 1421, 1399, 1365, 1348, 1277, 1248, 1148, 1101, 1043, 944, 844; GPC (DMF + 25 mM NH_4Ac): $M_n = 8.1$ kDa, PDI = 1.12, Expected $M_n = 8.1$ kDa;

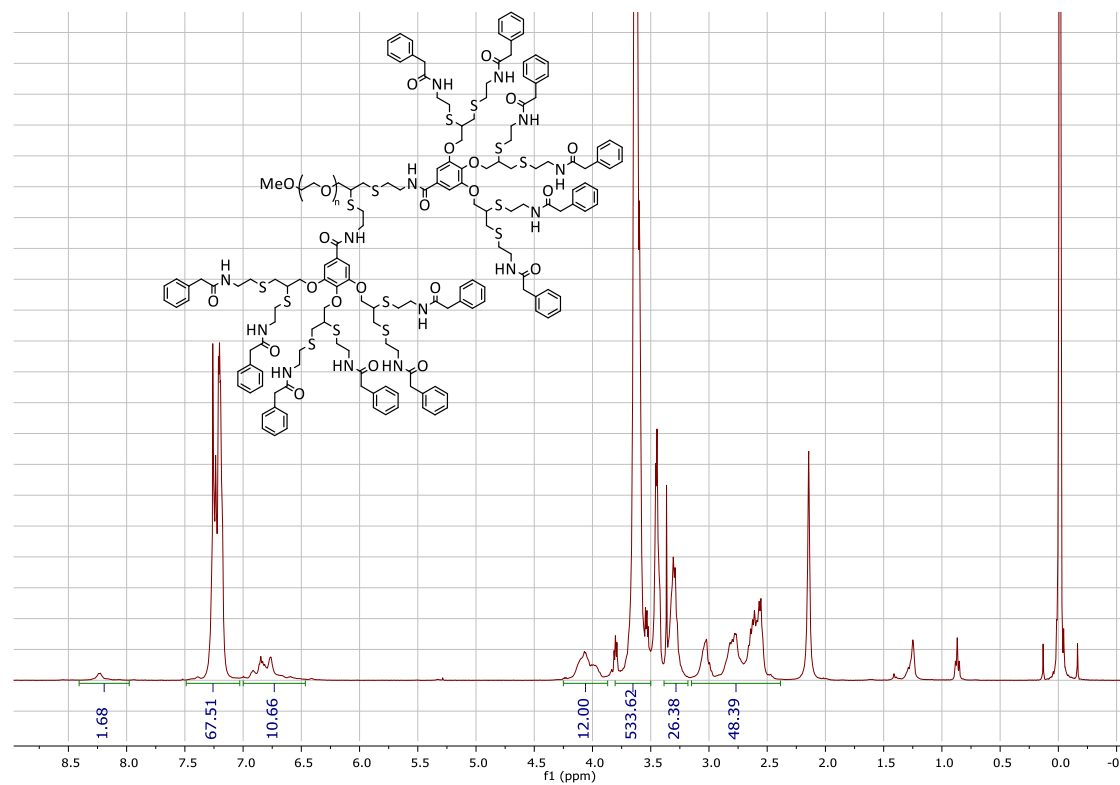


Figure S34. ¹H-NMR spectrum of mPEG_{5kDa}-[dend-(6Ph)]₂ (Hybrid 6) in CDCl₃.

Characterizations of PEG-dendron hybrids

Gel permeation chromatography (GPC)

Instrument: Malvern VISCOTEK GPCmax

Columns: 2 x PSS GRAM 1000 Å + PSS GRAM 30 Å

Columns' temperature: 50 °C

Mobile phase: DMF + 25 mM ammonium acetate

Flow rate: 0.5 ml/min

Injection time: 90 minutes

Needle wash: DMF

Detector: Viscotek VE3580 RI detector

Detector's Temperature: 50 °C

Sample preparation:

Typically, the PEG sample was dissolved in the mobile phase to afford a final concentration of 10 mg/ml. Solution was filtered through 0.22µm PTFE syringe filter and then 50 µL were injected to the system.

GPC monitoring of the synthesis of mPEG_{5kDa}-dend(3Ph) (Hybrid 1)

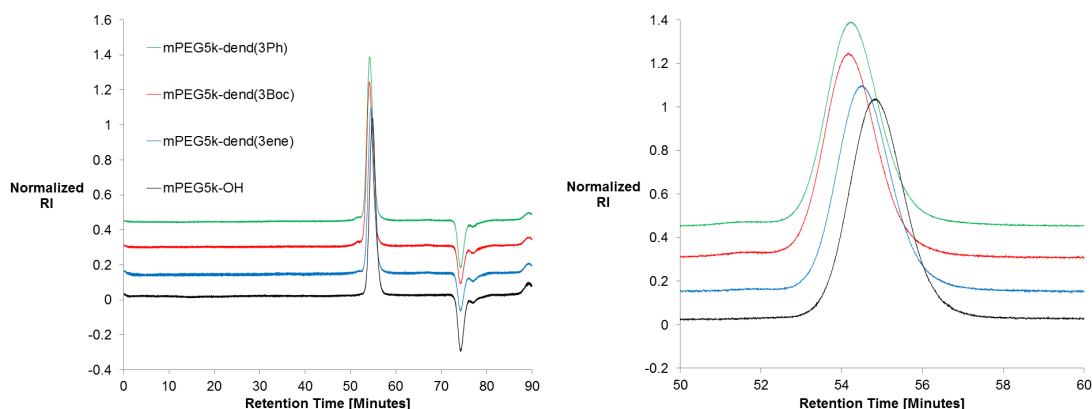


Figure S35. GPC overlay for preparation of mPEG_{5kDa}-dend(3Ph) (Hybrid 1). The starting 5kDa PEG (in black) was placed first as a reference.

GPC monitoring of the synthesis of mPEG_{5kDa}-dend(4Ph) (Hybrid 2)

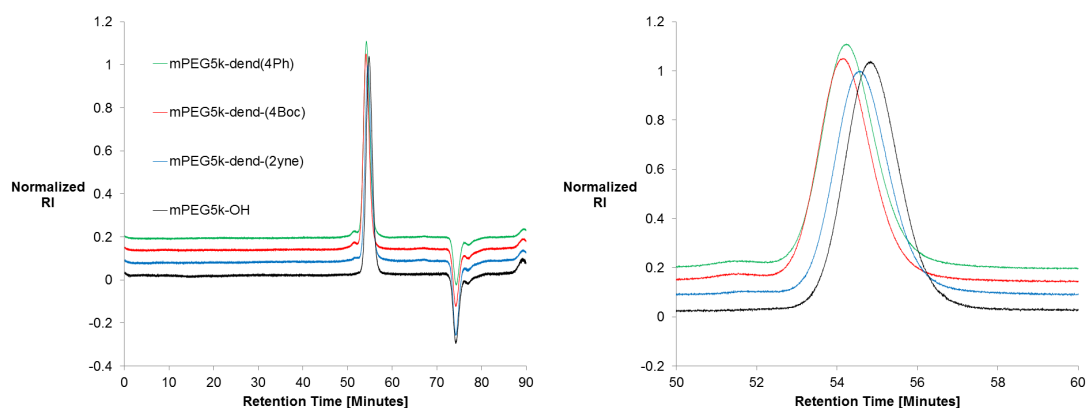


Figure S36. GPC overlay for preparation of mPEG_{5kDa}-dend(4Ph) (Hybrid 2). The starting 5kDa PEG (in black) was placed first as a reference.

GPC monitoring of the synthesis of mPEG_{5kDa}-dend(6Ph) (Hybrid 3)

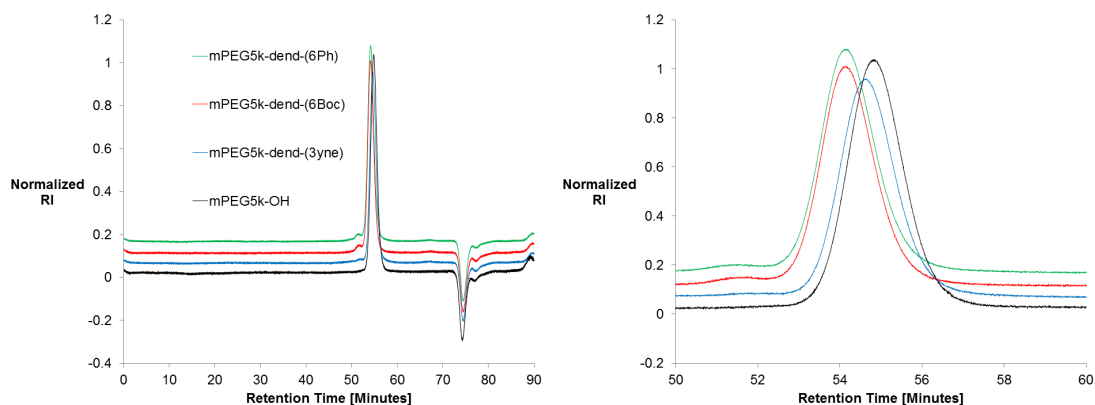


Figure S37. GPC overlay for preparation of mPEG_{5kDa}-dend(6Ph) (Hybrid 3). The starting 5kDa PEG (in black) was placed first as a reference.

GPC monitoring of the synthesis of mPEG_{5kDa}-[dend(3Ph)]₂ (Hybrid 4)

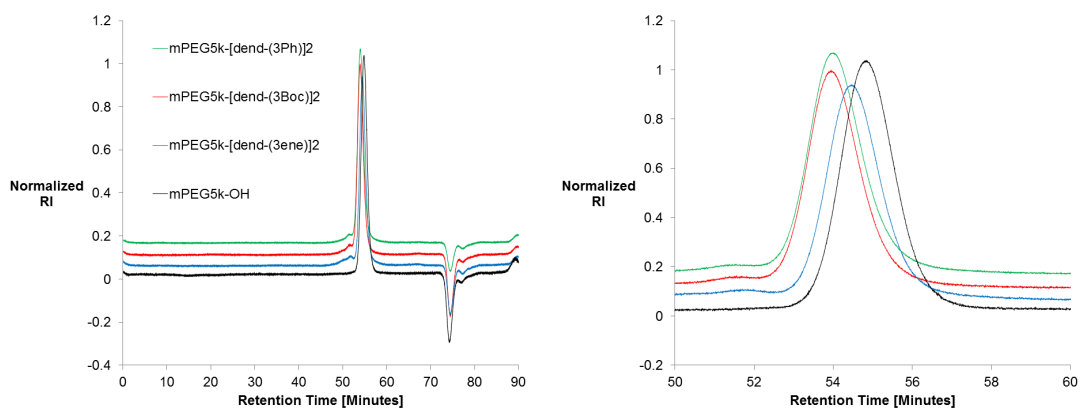


Figure S38. GPC overlay for preparation of mPEG_{5kDa}-[dend(3Ph)]₂ (Hybrid 4). The starting 5kDa PEG (in black) was placed first as a reference.

GPC monitoring of the synthesis of mPEG_{5kDa}-[dend(4Ph)]₂ (Hybrid 5)

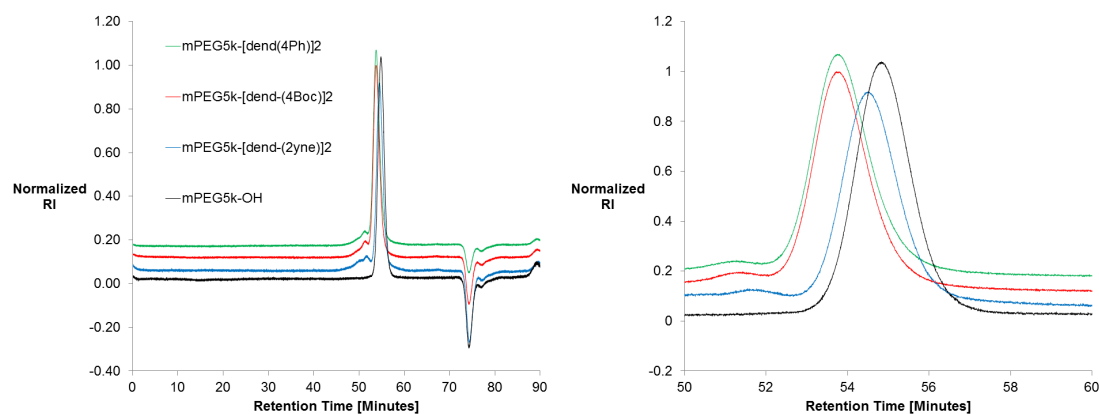


Figure S39. GPC overlay for preparation of mPEG_{5kDa}-[dend(4Ph)]₂ (Hybrid 5). The starting 5kDa PEG (in black) was placed first as a reference.

GPC monitoring of the synthesis of mPEG_{5kDa}-[dend(6Ph)]₂ (Hybrid 6)

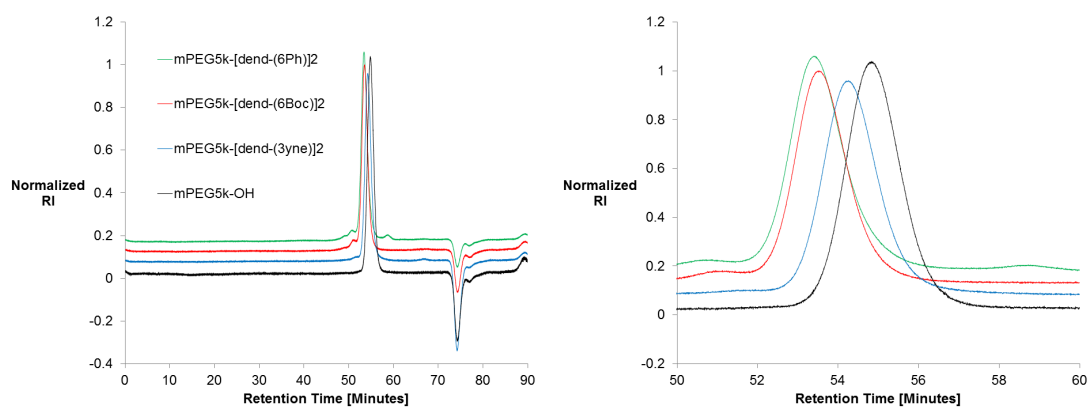


Figure S40. GPC overlay for preparation of mPEG_{5kDa}-[dend(6Ph)]₂ (Hybrid 6). The starting 5kDa PEG (in black) was placed first as a reference.

MALDI measurements

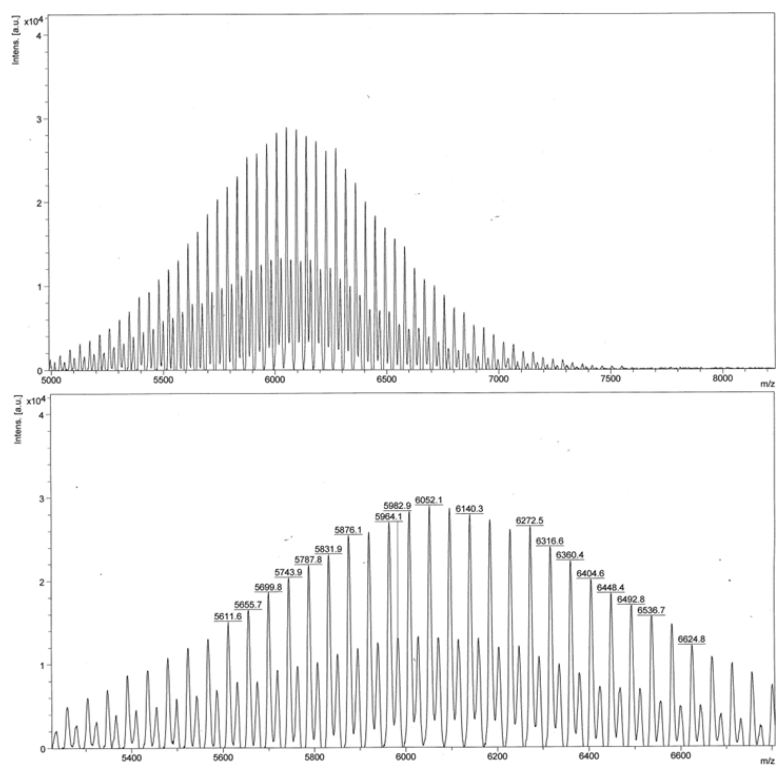


Figure S41. MALDI measurement of Hybrid 1. $M_n = 6.0$ kDa, Expected $M_n = 6.0$ kDa.

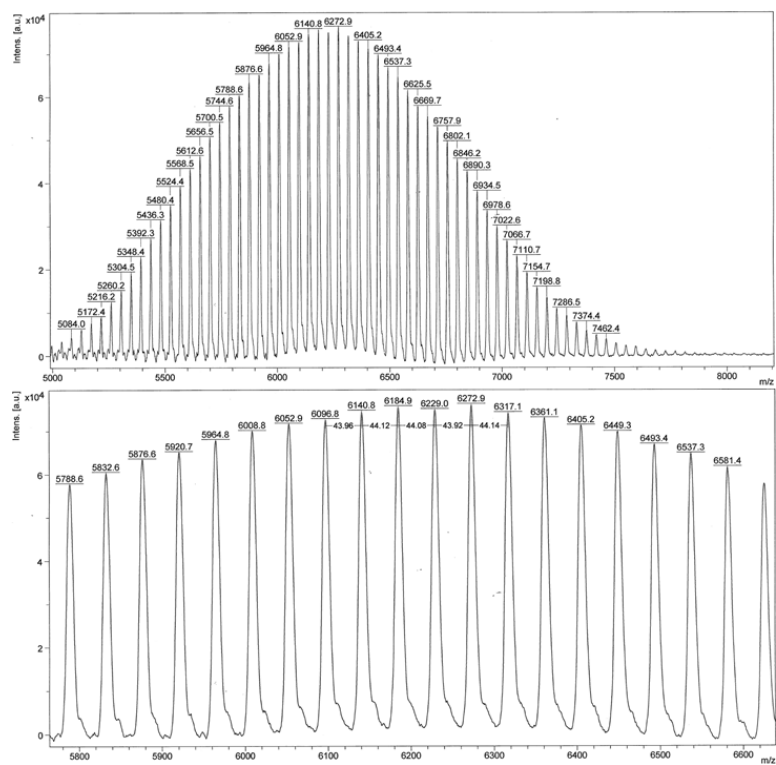


Figure S42. MALDI measurement of Hybrid 2. $M_n = 6.2$ kDa, Expected $M_n = 6.1$ kDa.

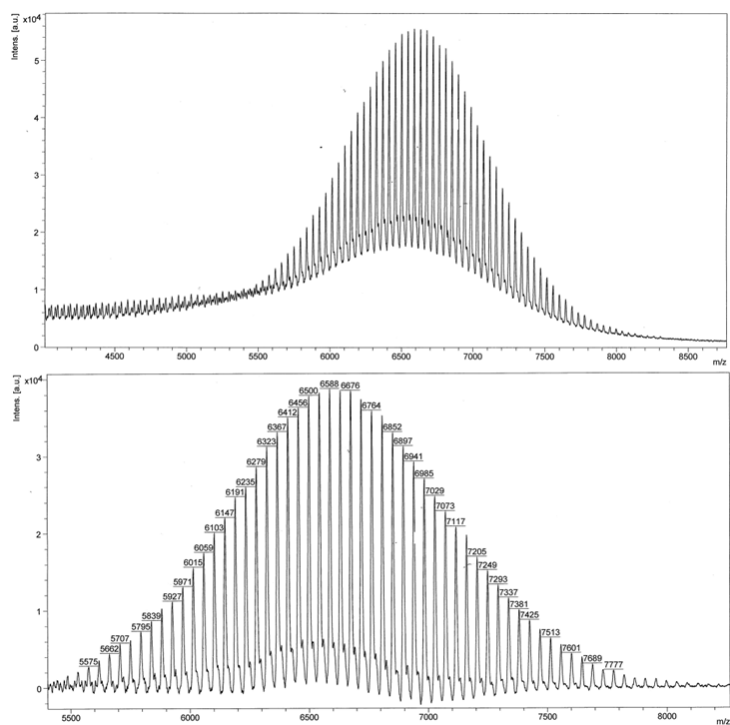


Figure S43. MALDI measurement of Hybrid 3. $M_n = 6.6$ kDa, Expected $M_n = 6.6$ kDa.

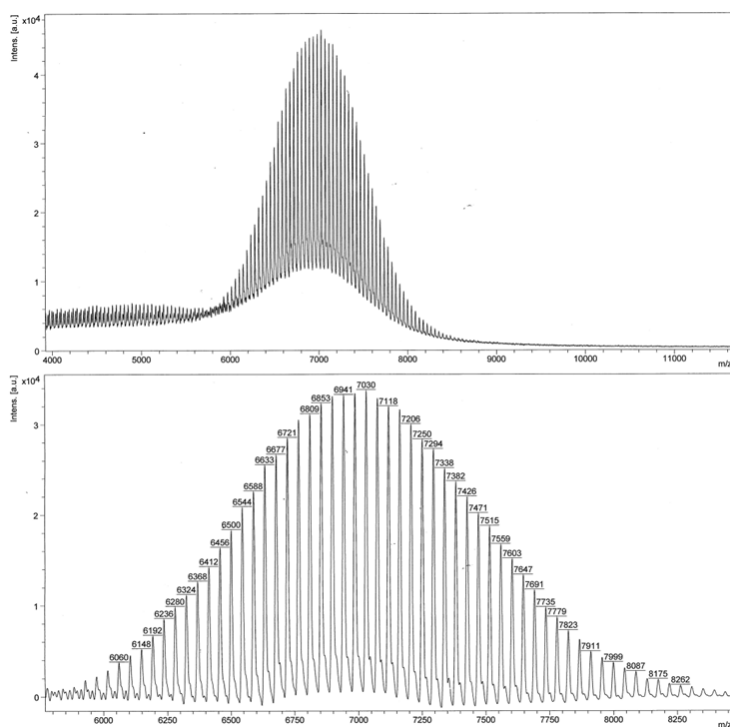


Figure S44. MALDI measurement of Hybrid 4. $M_n = 7.0$ kDa, Expected $M_n = 6.9$ kDa.

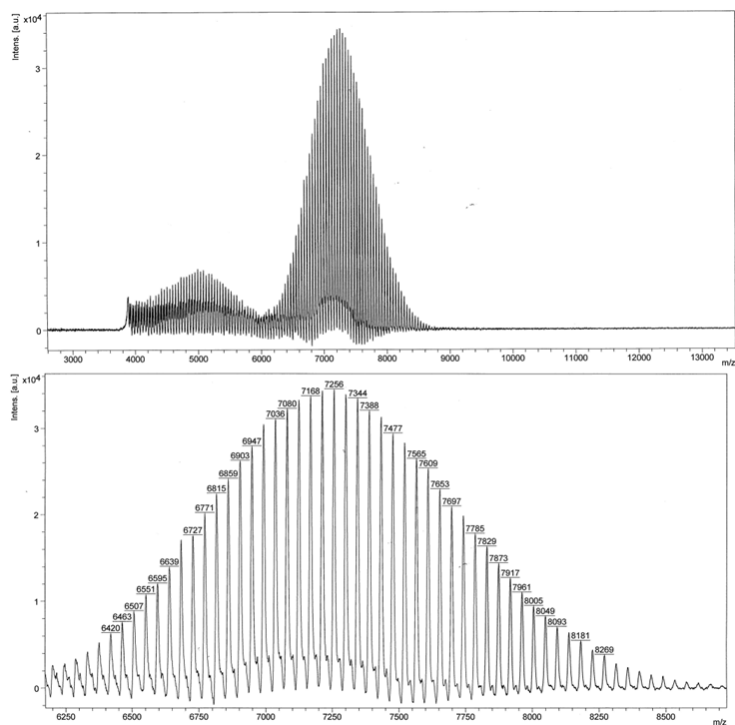


Figure S45. MALDI measurement of Hybrid 5. $M_n = 7.2$ kDa, Expected $M_n = 7.1$ kDa.

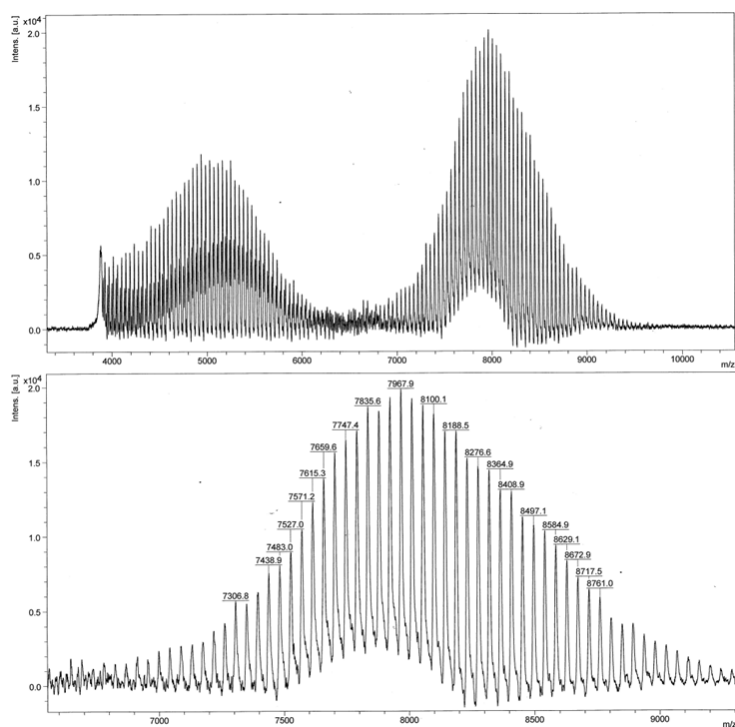


Figure S46. MALDI measurement of Hybrid 6. $M_n = 8.0$ kDa, Expected $M_n = 8.1$ kDa.

Critical micelles' concentration (CMC)

Instrument: TECAN Infinite M200Pro

Excitation: 550nm

Emission intensity scan: 580-800nm

Step: 2nm

Number of flashes: 15

Preparation of diluent:

Nile Red stock solution (0.88 mg/ml in ethanol) was diluted into phosphate buffer (pH 7.4) to give a final concentration of 1.25 μ M.

Preparation and measurement of samples:

The PEG-dendron hybrid was directly dissolved in the diluent to give a concentration of 500 μ M. This solution was repeatedly diluted by a factor of 1.5 with the diluent to yield a series of 24 solutions in total. 150 μ L of each solution was loaded into a 96 well plate and a fluorescence emission scan was performed for each well. In order to determine the hybrid's CMC, the maximum emission of Nile Red (at about 630nm) was plotted as a function of the hybrid's concentration. Each hybrid was measured at least three times.

CMC measurement for Hybrid 1:

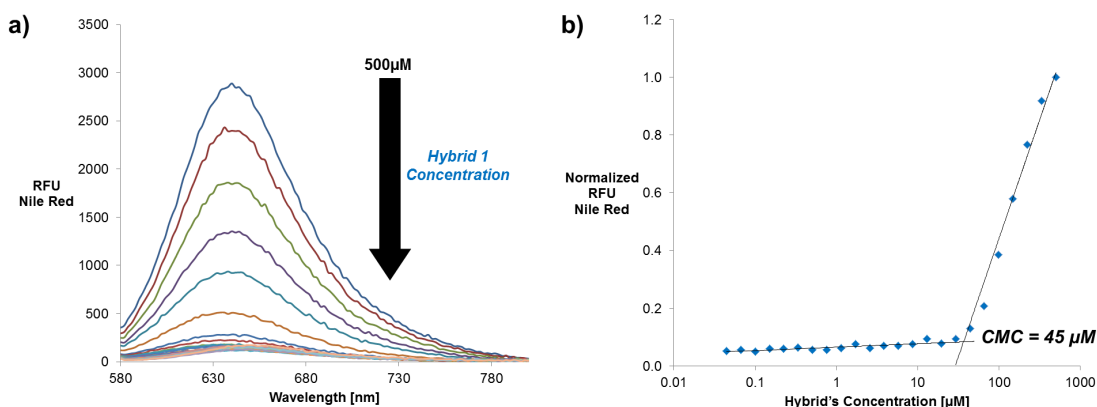


Figure S47. a) Spectra overlay of Nile Red's emission at different hybrid's concentrations. **b)** The maximum emission of each spectrum was plotted as a function of hybrid's concentration and the CMC was determined as the marked cross-section.

CMC measurement for Hybrid 2:

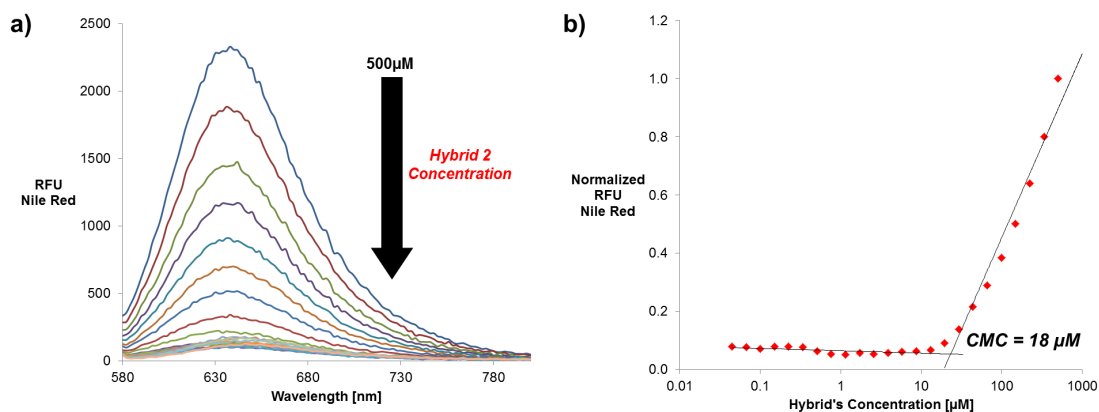


Figure S48. a) Spectra overlay of Nile Red's emission at different hybrid's concentrations. **b)** The maximum emission of each spectrum was plotted as a function of hybrid's concentration and the CMC was determined as the marked cross-section.

CMC measurement for Hybrid 3:

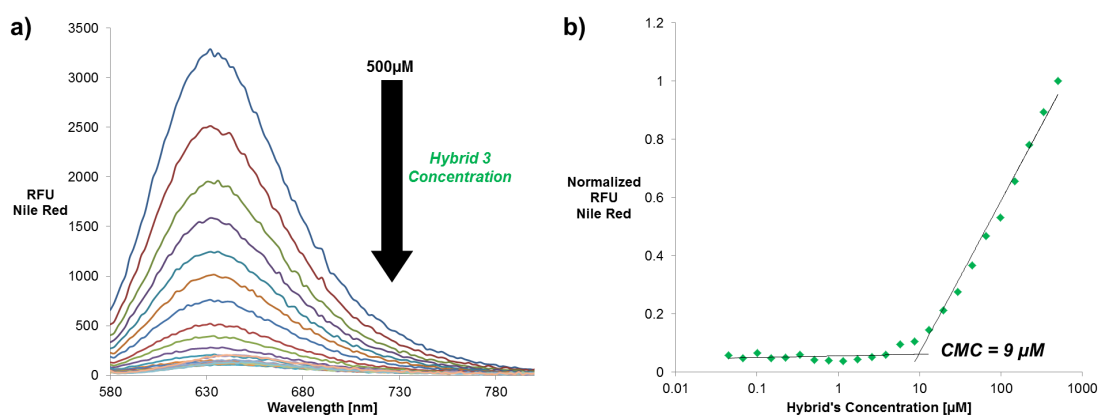


Figure S49. a) Spectra overlay of Nile Red's emission at different hybrid's concentrations. **b)** The maximum emission of each spectrum was plotted as a function of hybrid's concentration and the CMC was determined as the marked cross-section.

CMC measurement for Hybrid 4:

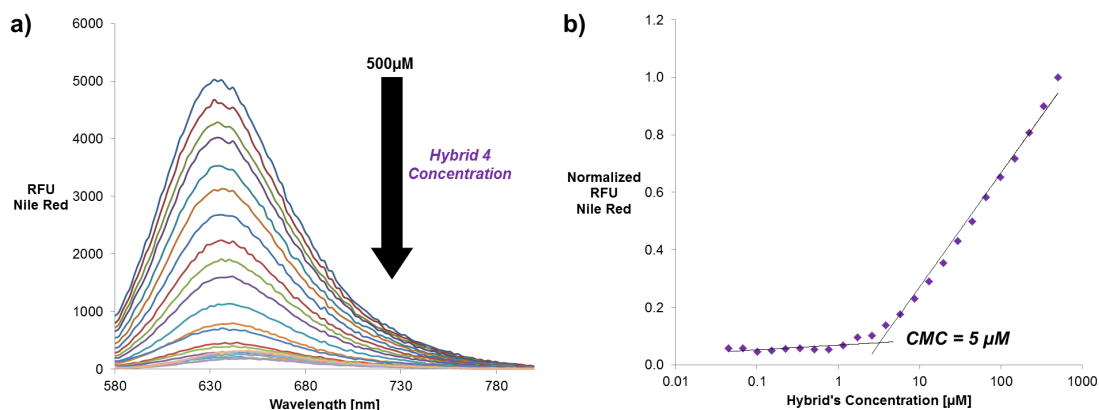


Figure S50. a) Spectra overlay of Nile Red's emission at different hybrid's concentrations. **b)** The maximum emission of each spectrum was plotted as a function of hybrid's concentration and the CMC was determined as the marked cross-section.

CMC measurement for Hybrid 5:

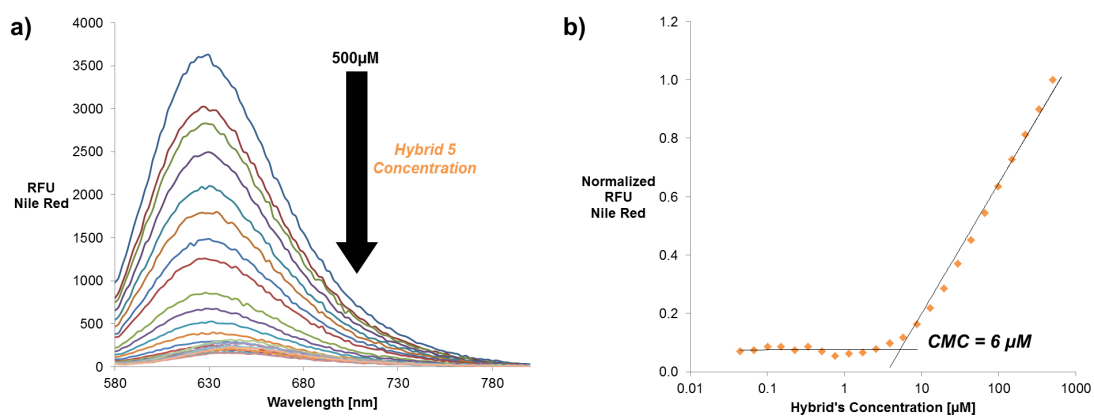


Figure S51. a) Spectra overlay of Nile Red's emission at different hybrid's concentrations. **b)** The maximum emission of each spectrum was plotted as a function of hybrid's concentration and the CMC was determined as the marked cross-section.

CMC measurement for Hybrid 6:

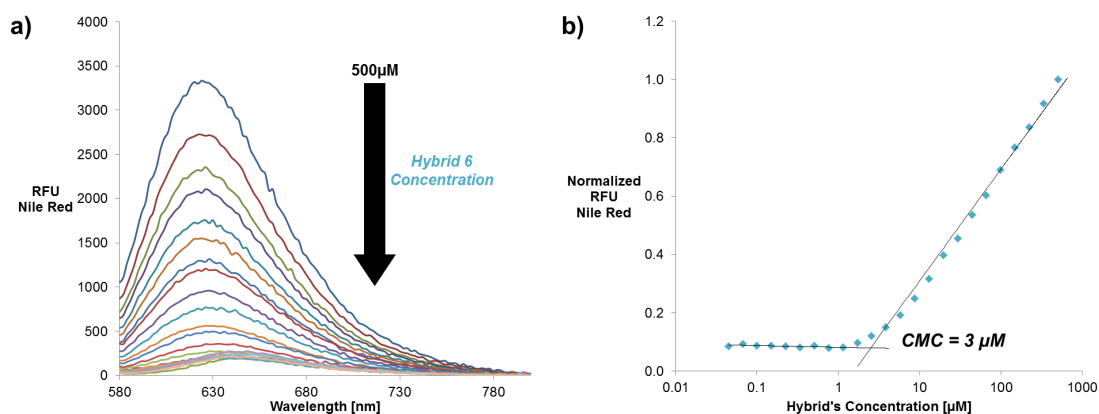


Figure S52. a) Spectra overlay of Nile Red's emission at different hybrid's concentrations. **b)** The maximum emission of each spectrum was plotted as a function of hybrid's concentration and the CMC was determined as the marked cross-section.

Transmittance electron microscopy (TEM) measurements:

Sample preparation:

Hybrids 1-6 were dissolved in phosphate buffer (pH 7.4) to afford a final concentration of 160 μM. 10 μL of each solution were dropped cast onto carbon copper grids. The excessive solvent of the droplet was wiped away using a solvent-absorbing filter paper after 1 minute and the sample grids were left to dry in air at room-temperature for 1 hour. Then, grids were inspected in transmission electron microscope (TEM), operated at 200kV (Philips Tecnai F20).

Enzymatic disassembly of the micelles

Preparation of PGA stock 1:

250 μL of *penicillin G amidase* (Sigma #76427) were added to 1600 μL of phosphate buffer pH 7.4 to give a final concentration of 250 μM.

Preparation of PGA stock 2:

PGA stock 1 was diluted into phosphate buffer pH 7.4 by a factor of 50 to give a final concentration of 5 μM.

Fluorescence of Nile Red measurements

Instrument: Agilent Technologies Cary Eclipse Fluorescence Spectrometer

Excitation wavelength: 550 nm

Emission scan: 580 - 800 nm

Scan rate: 600 nm/min

Averaging time: 0.1 sec

Excitation slit: 10 nm

Emission slit: 20 nm

PMT detector voltage: a typical value of about 600 V was used

Preparation of diluent:

Nile Red stock solution in ethanol (0.88 mg/ml) was diluted into phosphate buffer pH 7.4 to yield a final concentration of 1.25 μM .

Preparation and measurement of samples:

The PEG-dendron hybrid was directly dissolved in the diluent in order to afford a final concentration of 80 or 160 μM . Solution was vortexed, sonicated for 15 minutes and then vortexed again until all solids were completely dissolved. 685 μL of the tested solution was transferred into a quartz cuvette and incubated at 37 $^{\circ}\text{C}$ for at least 2 hours. Samples that contained hybrid concentration of **160 μM** were stimulated with 14 μL of **PGA stock 2**, while samples with a concentration of **80 μM** were stimulated with 14 μL of **PGA stock 1**. The cuvette was properly mixed and the emission intensity of Nile Red was measured over time. In order to derive the disassembly rate of the micelles, the maximum intensity of each spectrum (at about 630 nm) was plotted as a function of time.

Fluorescence measurement of Hybrid 1:

[Hybrid 1] = 160 μM , [PGA] = 0.1 μM , [Nile Red] = 1.25 μM , T = 37 $^{\circ}\text{C}$

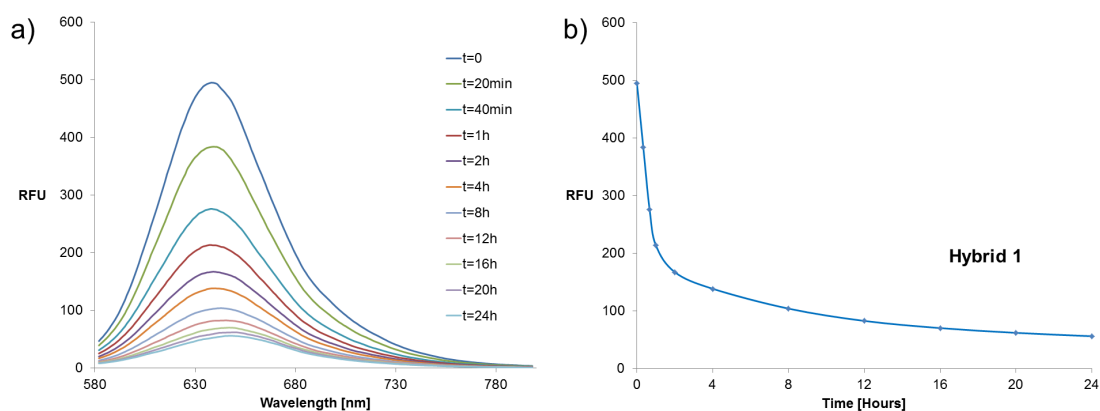


Figure S53. a) Emission intensity scans of Nile Red over time. b) Maximum intensity of each spectrum was plotted as a function of time.

Fluorescence measurement of Hybrid 2:

[Hybrid 2] = 160 μM , [PGA] = 0.1 μM , [Nile Red] = 1.25 μM , T = 37 $^{\circ}\text{C}$

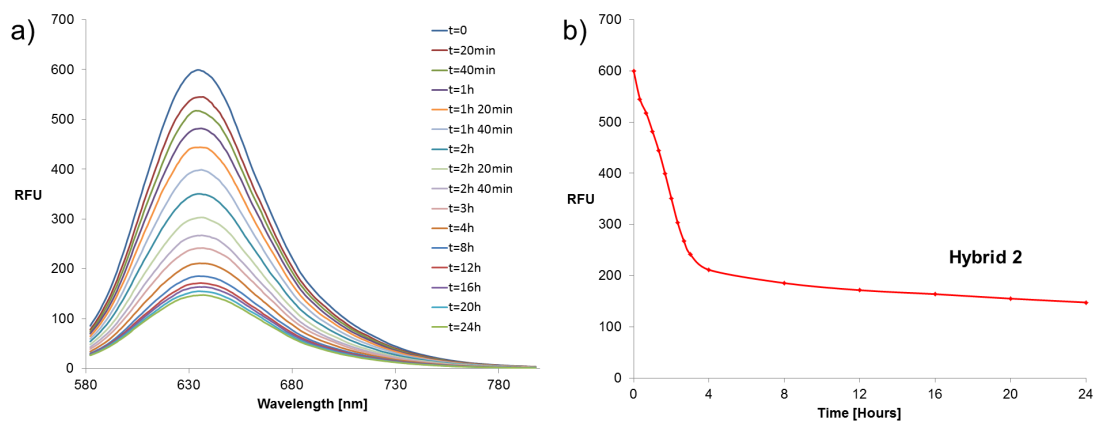


Figure S54. a) Emission intensity scans of Nile Red over time. b) Maximum intensity of each spectrum was plotted as a function of time.

Fluorescence measurements of Hybrid 3:

[Hybrid 3] = 160 μM , [PGA] = 0.1 μM , [Nile Red] = 1.25 μM , T = 37 $^{\circ}\text{C}$

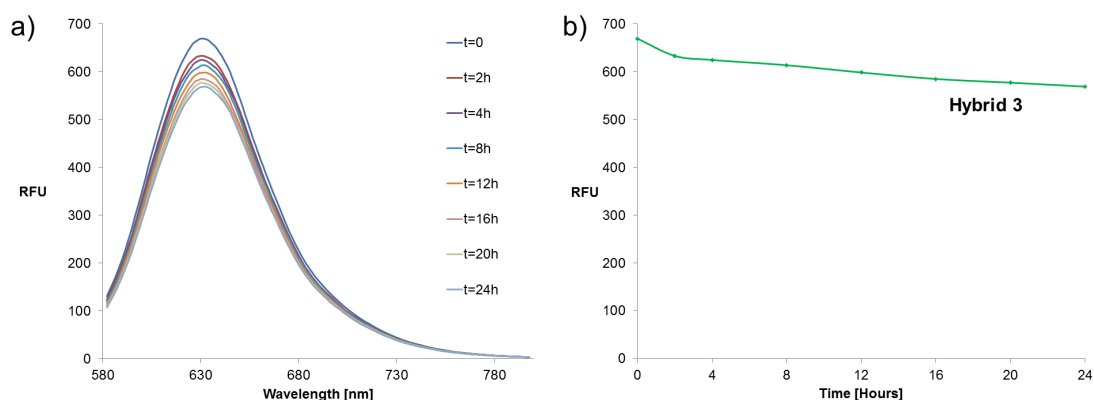


Figure S55. a) Emission intensity scans of Nile Red over time. b) Maximum intensity of each spectrum was plotted as a function of time.

[Hybrid 3] = 80 μM , [PGA] = 5 μM , [Nile Red] = 1.25 μM , T = 37 $^{\circ}\text{C}$

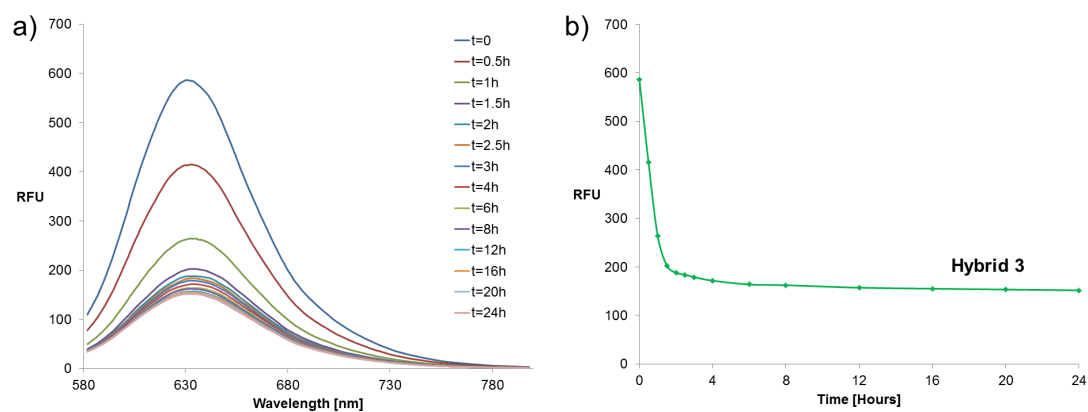


Figure S56. a) Emission intensity scans of Nile Red over time. b) Maximum intensity of each spectrum was plotted as a function of time.

Fluorescence measurements of Hybrid 4:

[Hybrid 4] = 160 μM , [PGA] = 0.1 μM , [Nile Red] = 1.25 μM , T = 37 $^{\circ}\text{C}$

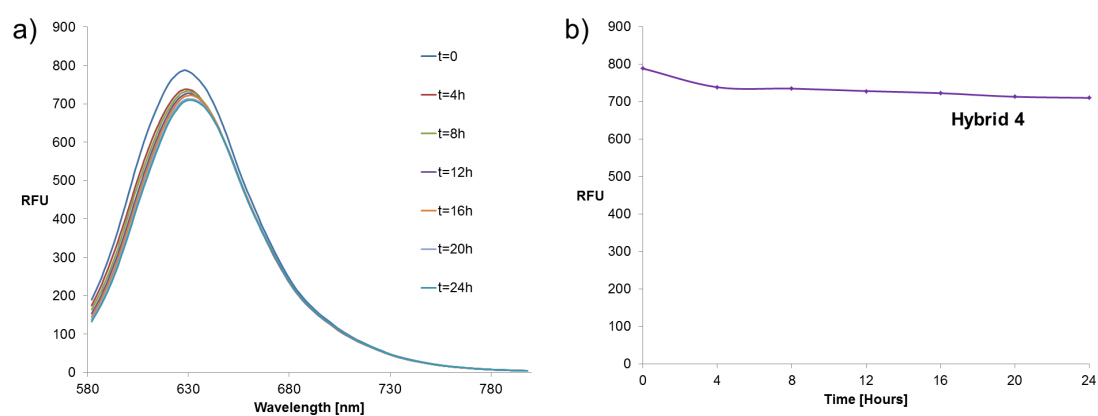


Figure S57. a) Emission intensity scans of Nile Red over time. b) Maximum intensity of each spectrum was plotted as a function of time.

[Hybrid 4] = 80 μM , [PGA] = 5 μM , [Nile Red] = 1.25 μM , T = 37 $^{\circ}\text{C}$

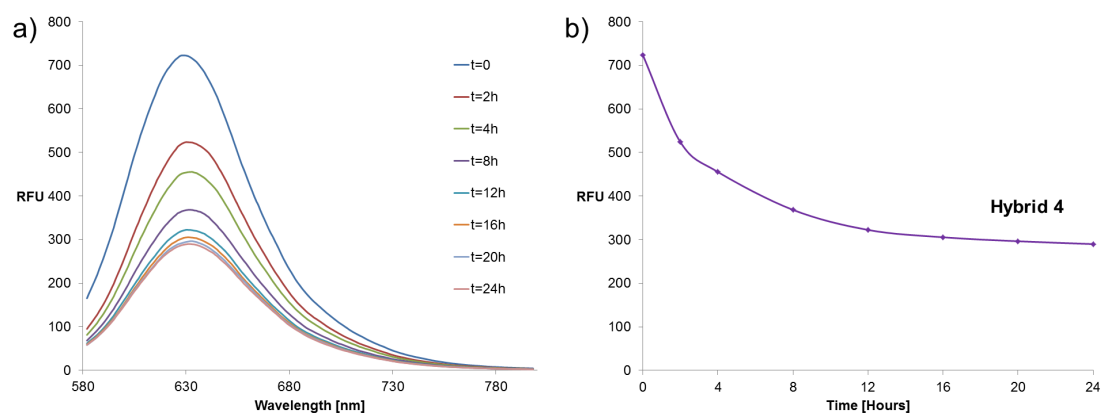


Figure S58. a) Emission intensity scans of Nile Red over time. b) Maximum intensity of each spectrum was plotted as a function of time.

Fluorescence measurement of Hybrid 5:

[Hybrid 5] = 80 μM , [PGA] = 5 μM , [Nile Red] = 1.25 μM , T = 37 $^{\circ}\text{C}$

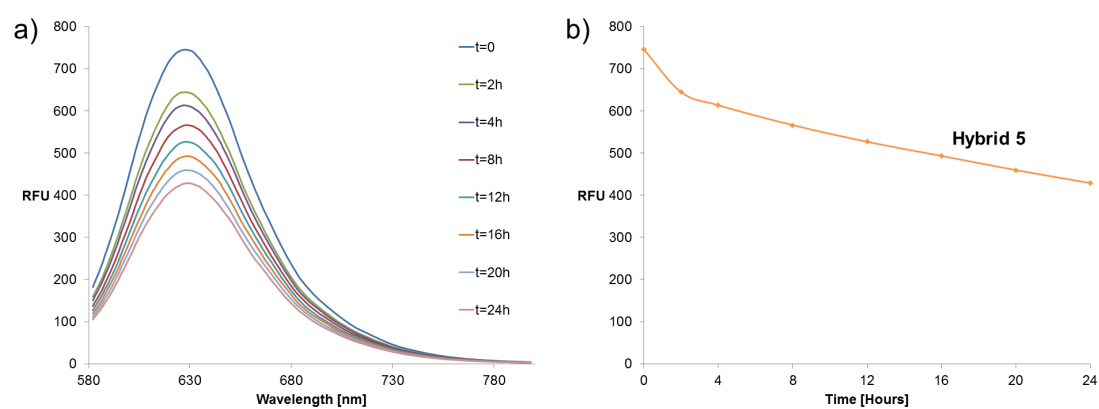


Figure S59. a) Emission intensity scans of Nile Red over time. b) Maximum intensity of each spectrum was plotted as a function of time.

Fluorescence measurement of Hybrid 6:

[Hybrid 6] = 80 μM , [PGA] = 5 μM , [Nile Red] = 1.25 μM , T = 37 $^{\circ}\text{C}$

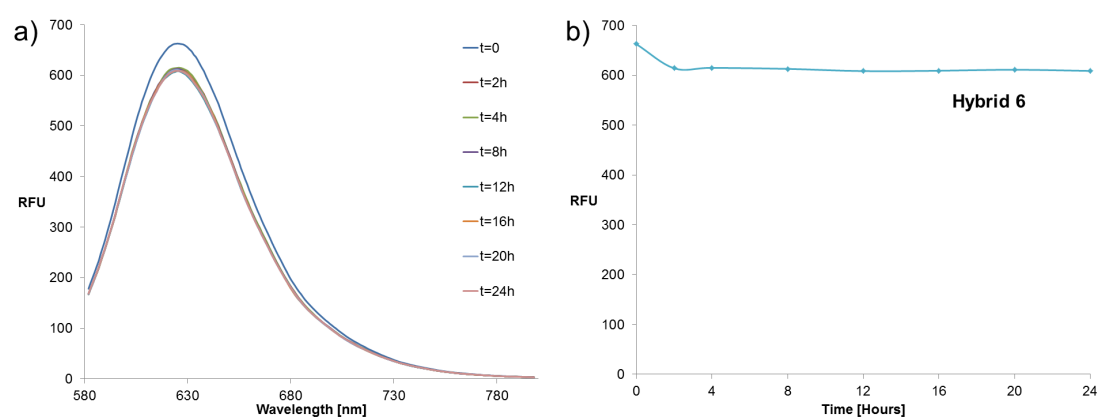


Figure S60. a) Emission intensity scans of Nile Red over time. b) Maximum intensity of each spectrum was plotted as a function of time.

HPLC measurements

Instrument: Waters Alliance e2695

Column: Aeris WIDEPORÉ, C4, 3.6 µm, 150 x 4.6 mm

Column temperature: 30 °C

Sample temperature: 37 °C

Solution A: 0.1% HClO₄:ACN 95:5v/v

Solution B: 0.1% HClO₄:ACN 5:95v/v

Solution C: THF

Flow rate: 1.0 mL/min

Gradient program for 30 minutes injection:

Time [minutes]	Sol. A [%]	Sol. B [%]	Sol. C [%]
0.0	95	0	5
1.0	95	0	5
20.0	0	95	5
23.0	0	95	5
23.1	95	0	5
30.0	95	0	5

Seal wash: H₂O:MeOH 90:10v/v

Needle wash: MeOH

Detector: Waters 2998 photodiode array detector

Sampling rate: 2 points/sec

Preparation and measurement of samples:

The PEG-dendron hybrid was directly dissolved in phosphate buffer pH 7.4 in order to afford a final concentration of 80 or 160 μM . The obtained solution was vortexed, sonicated for 15 minutes and then vortexed again until all solids were completely dissolved. Then, 1500 μL were transferred to a HPLC vial and the sample was incubated at 37 $^{\circ}\text{C}$ for at least 2 hours. 30 μL of the sample were injected to the system as $t = 0$ and then 30 μL of PGA stock solution (**stock 1** for 80 μM samples and **stock 2** for 160 μM samples) were added. The vial was properly mixed and repeatedly injected over time. In order to evaluate the degradation rate of hybrids in response to the enzyme, the peak area of the parent hybrids was plotted as a function of time.

HPLC measurement of Hybrid 1:

[Hybrid 1] = 160 μM , [PGA] = 0.1 μM , T = 37 $^{\circ}\text{C}$, $\lambda = 260 \text{ nm}$

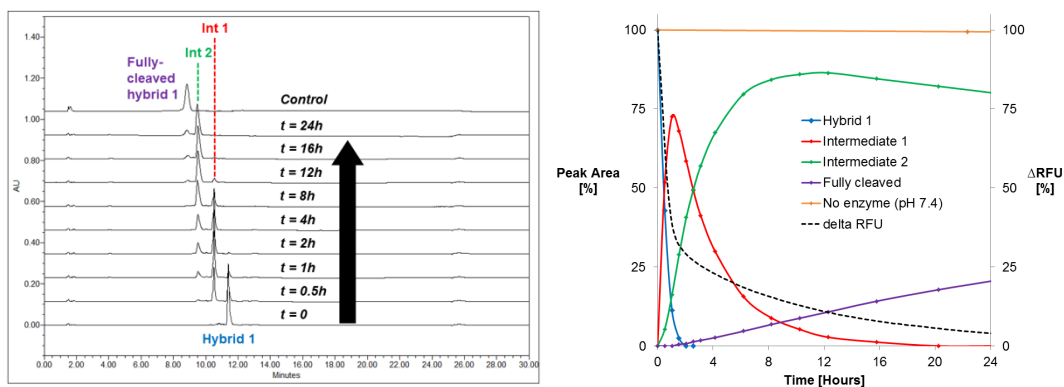


Figure S61. Left: HPLC overlay of the enzymatic degradation of Hybrid 1 and its partially-cleaved intermediates. **Right:** HPLC degradation profile and the change of Nile Red's emission as a function of time. No degradation was observed in the absence of enzyme.

HPLC measurement of Hybrid 2:

[Hybrid 2] = 160 μ M, [PGA] = 0.1 μ M, T = 37 $^{\circ}$ C, λ = 297 nm

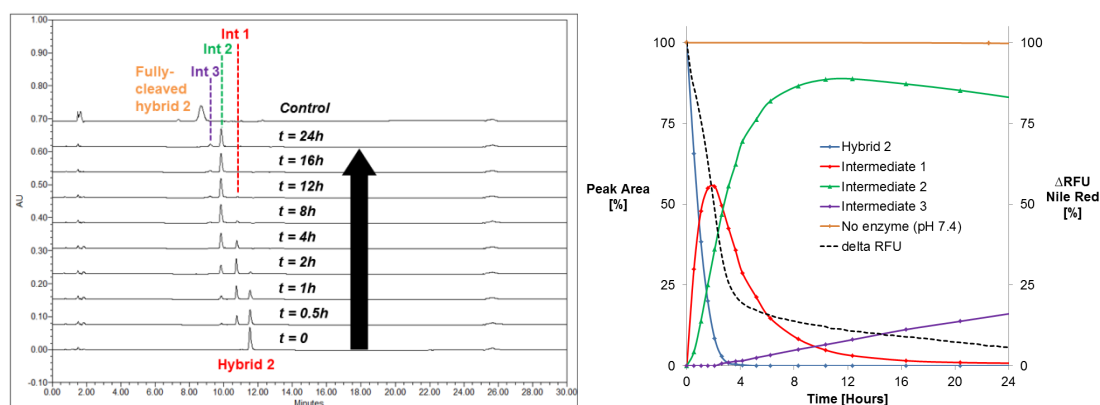


Figure S62. Left: HPLC overlay of the enzymatic degradation of Hybrid 2 and its partially-cleaved intermediates. **Right:** HPLC degradation profile and the change of Nile Red's emission as a function of time. No degradation was observed in the absence of enzyme.

HPLC measurements of Hybrid 3:

[Hybrid 3] = 160 μ M, [PGA] = 0.1 μ M, T = 37 $^{\circ}$ C, λ = 260 nm

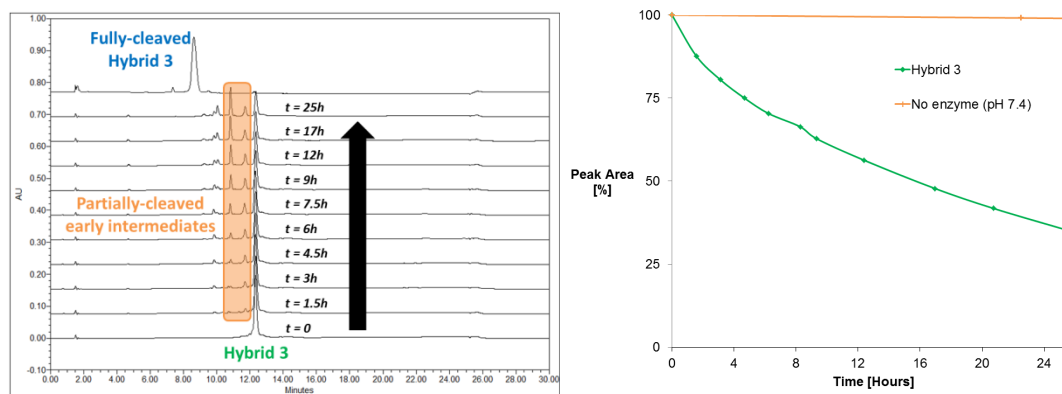


Figure S63. Left: HPLC overlay of the enzymatic degradation of Hybrid 3 and its partially-cleaved intermediates. **Right:** HPLC degradation of the parent hybrid as a function of time. No degradation was observed in the absence of enzyme.

[Hybrid 3] = 80 μ M, [PGA] = 5 μ M, T = 37 $^{\circ}$ C, λ = 260 nm

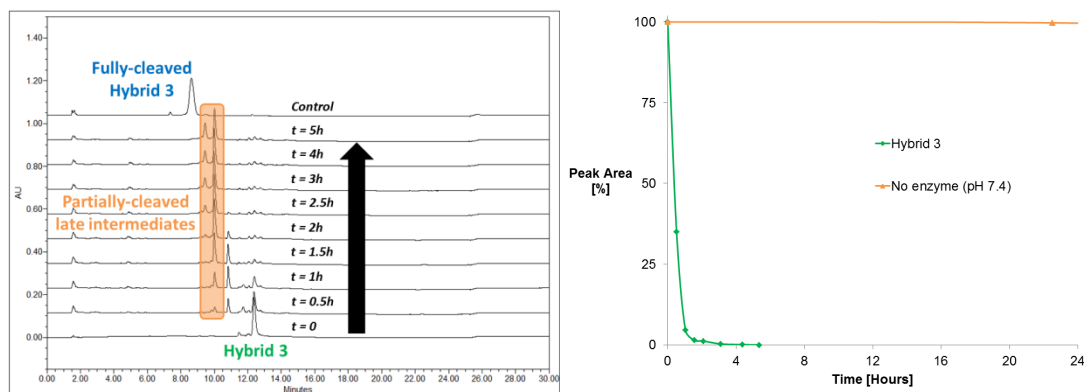


Figure S64. Left: HPLC overlay of the enzymatic degradation of Hybrid 3 and its partially-cleaved intermediates. **Right:** HPLC degradation of the parent hybrid as a function of time. No degradation was observed in the absence of enzyme.

HPLC measurements of Hybrid 4:

[Hybrid 4] = 160 μ M, [PGA] = 0.1 μ M, T = 37 $^{\circ}$ C, λ = 260 nm

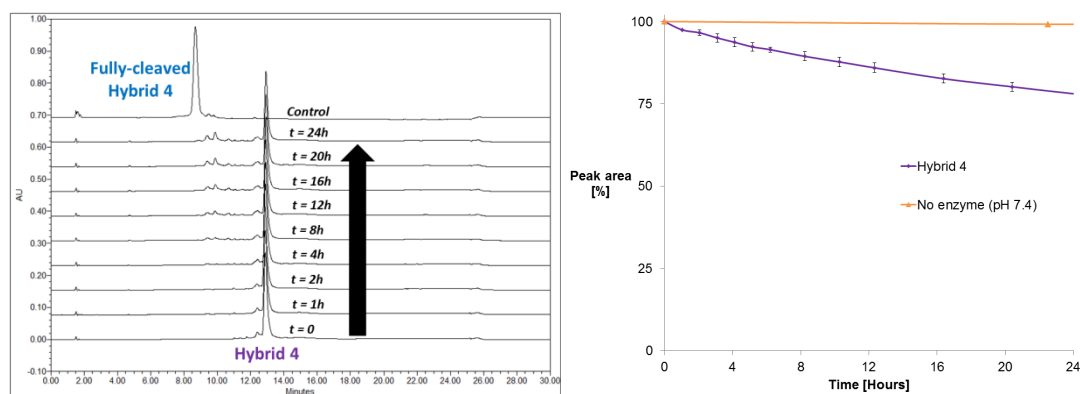


Figure S65. Left: HPLC overlay of the enzymatic degradation of Hybrid 4 and its partially-cleaved intermediates. **Right:** HPLC degradation of the parent hybrid as a function of time. No degradation was observed in the absence of enzyme.

[Hybrid 4] = 80 μ M, [PGA] = 5 μ M, T = 37 $^{\circ}$ C, λ = 260 nm

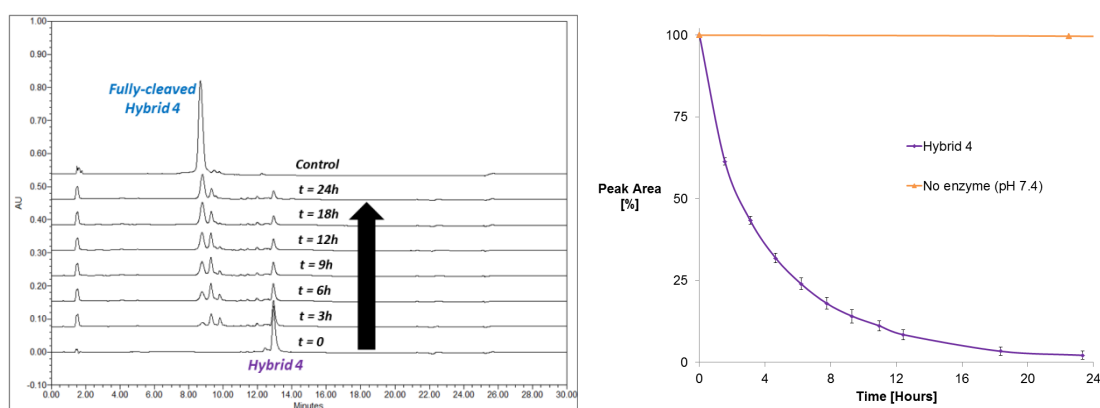


Figure S66. Left: HPLC overlay of the enzymatic degradation of Hybrid 4 and its partially-cleaved intermediates. **Right:** HPLC degradation of the parent hybrid as a function of time. No degradation was observed in the absence of enzyme.

HPLC measurements of Hybrid 5:

[Hybrid 5] = 80 μ M, [PGA] = 5 μ M, T = 37 $^{\circ}$ C, λ = 297 nm

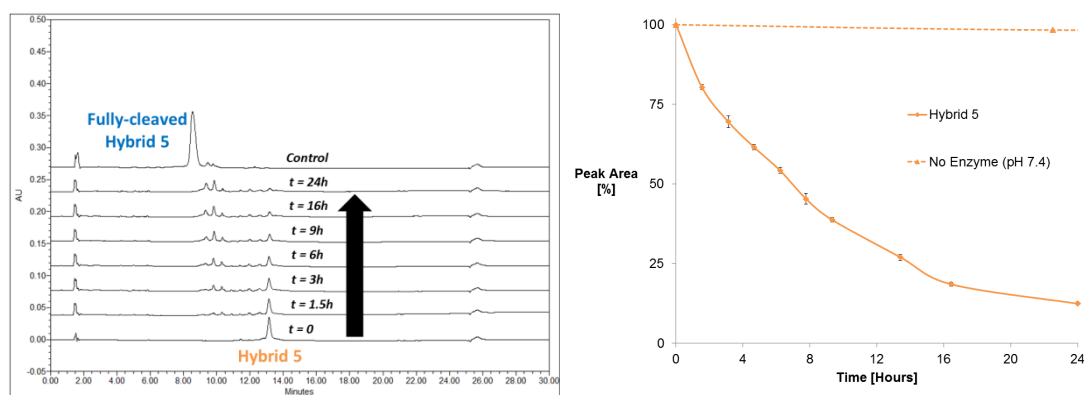


Figure S67. Left: HPLC overlay of the enzymatic degradation of Hybrid 5 and its partially-cleaved intermediates. **Right:** HPLC degradation of the parent hybrid as a function of time. No degradation was observed in the absence of enzyme.

HPLC measurements of Hybrid 6:

[Hybrid 6] = 80 μ M, [PGA] = 5 μ M, T = 37 $^{\circ}$ C, λ = 260 nm

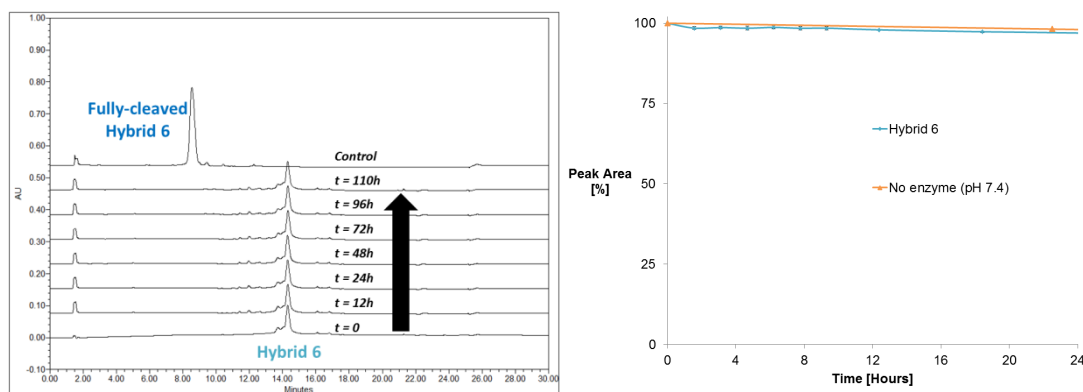


Figure S68. Left: HPLC overlay of the enzymatic degradation of Hybrid 6 and its partially-cleaved intermediates. **Right:** HPLC degradation of the parent hybrid as a function of time. No degradation was observed in the absence of enzyme.

Dynamic light scattering (DLS) measurements

Instrument: Corduan Technology VASCO^y particle size analyzer

Typical parameters for measurement of samples:

Cell Temperature: 37 $^{\circ}$ C

Time interval: 10-15 μ sec

Number of channels: 200-300

Laser Power: 50-100%

Preparation and measurement of samples:

The PEG-dendron hybrid was directly dissolved in phosphate buffer (pH 7.4) to afford a final concentration of 80 or 160 μ M. Solution was vortexed, sonicated and then vortexed again until all solids were completely dissolved. Then, solution was filtered through 0.22 μ m nylon syringe filter and immediately measured using the instrument. Micellar diameters were recorded in nm as a function of volume %.

Stability of *penicillin G* amidase (PGA)

PGA's stability under the applied measurements' conditions (phosphate buffer pH 7.4 at 37 °C) was confirmed by comparing the enzymatic degradation of N-(4-nitrophenyl)-2-phenylacetamide⁵ (4N-PhAcAm) without incubation of the enzyme and after 24 hours incubation at 37 °C in phosphate buffer pH 7.4 using HPLC.

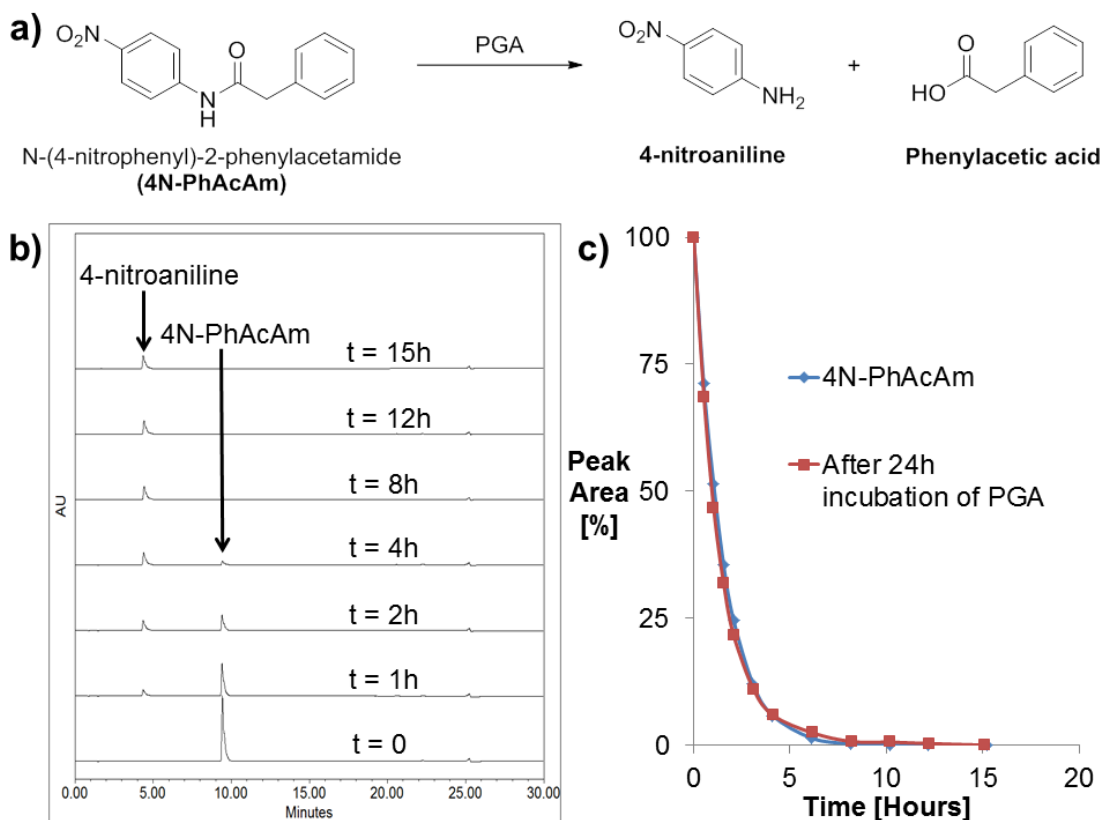


Figure S69: a) 4N-PhAcAm goes through enzymatic hydrolysis of the amide bond to release 4-nitroaniline and phenylacetic acid. b) HPLC overlay of the reaction under the following conditions: [4N-PhAcAm] = 250 μM in phosphate buffer pH 7.4, [PGA] = 0.013 μM , T = 37 °C, λ = 320 nm. c) The peak area of 4N-PhAcAm was plotted as a function of time for two separate experiments: 1. PGA was not incubated. 2. PGA's stock solution was incubated for 24h in phosphate buffer at 37 °C and the experiment was repeated. No change in the enzymatic degradation rate was observed.

References:

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