Tuning the molecular weight of polymeric amphiphiles as a tool to access micelles with a wide range of enzymatic degradation rates

Gadi Slor, a,b Nitsan Papo, a,b Uri Hananel b,c and Roey J. Amir a,b,d

a Department of Organic Chemistry, School of Chemistry, Faculty of Exact Sciences, Tel-Aviv University, Tel-Aviv 6997801, Israel.

b Tel Aviv University Center for Nanoscience and Nanotechnology, Tel-Aviv University, Tel-Aviv 6997801, Israel.

c Department of Physical Chemistry, School of Chemistry, Faculty of Exact Sciences, Tel-Aviv University, Tel-Aviv 6997801, Israel.

d BLAVATNIK CENTER for Drug Discovery, Tel-Aviv University, Tel-Aviv 6997801, Israel.

Supplementary Information

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 400MHz 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) 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: 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). α-Cyano-4-hydroxycinnamic acid matrix was used. High resolution MS: Analysis was conducted on Autospec HRMS (EI) Micromass (UK) or Synapt High Definition MS (ESI), Waters Inc. (USA). DLS: All measurements were recorded on a Corduran technology VASCOγ – particle size analyzer. TEM: Images were taken by a Philips Tecnai F20 TEM at 200kV.
Materials:

Poly (Ethylene Glycol) methyl ether (5kDa and 10kDa), 2-mercaptoethanol (98%), cystamine dihydrochloride (96%), hexanoic acid (99.5%), 2,2-dimethoxy-2-phenylacetophenone (DMPA, 99%), 4-(Dimethylamino)pyridine (DMAP, 99%), Porcine liver esterase (PLE), Penicilin G amidase (PGA) and Sephadex® LH20 were purchased from Sigma-Aldrich. Anhydrous NaSO₄ (granular, 10-60 mesh) was purchased from Macron. Propargyl bromide (80% in toluene), allyl bromide (99%), chlorotriphenylmethane (Trt-Cl, 98%), 4-nitrophenol (99%), triethylsilane (98%), N,N'-dicyclohexylcarbodiimide (DCC, 99%), phenyl acetyl chloride (98%) and Anhydrous K₂CO₃ (99%) were purchased from Alfa Aesar. 3,5-dihydroxy benzoic acid was purchased from Apollo scientific. Potassium hydroxide and DIPEA were purchased from Merck. Silica Gel 60Å, 0.040-0.063 mm, NaOH 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 triple bond containing branching unit:

Figure S1: Preparation of 4-nitrophenyl 3,5-bis(propargyloxy)benzoate (compound 1).

3,5-bis(propargyloxy)benzoate was synthesized as previously reported¹ and the spectroscopic characterization correlated well with these reports.
Synthesis of thiolated enzymatically cleavable end-groups:

2-(tritylthio)ethanol (2): Trt-Cl (7.15 gr, 25.7 mmol) and 2-mercaptoethanol (1.5 ml, 1.67 gr, 21.4 mmol) were dissolved in 20 ml THF and stirred overnight at ambient temperature. Then, solvents were removed in vacuum and product was purified using flash silica chromatography (DCM:EtOAc 90:10). Product was obtained as white solid in 87% yield (5.99 gr).

\[ \text{\(^1\)H-NMR (CDCl}_3\): } \delta \text{ 7.43-7.48 (m, 6H, Trt), 7.28-7.34 (m, 6H, Trt), 7.21-7.26 (m, 3H, Trt), 3.40 (t, } J = 6.2Hz, 2H, -CH}_2-OH\), 2.50 (t, } J = 6.2Hz, 2H, Trt-S-CH}_2-\); \[ \text{\(^{13}\)C-NMR (CDCl}_3\): } \delta \text{ 144.3, 129.1, 127.5, 126.3, 66.2, 60.4, 34.8; FT-IR (ATR), } \nu(\text{cm}^{-1}): 3320, 3061, 2924, 2782, 1590, 1485, 1440, 1357, 1317, 1293, 1280, 1181, 1063, 1034, 1012, 842.}

Figure S2: Preparation of 2-mercaptoethyl hexanoate (compound 4).

Figure S3: \(^1\)H-NMR spectrum of compound 2 in CDCl\(_3\).
2-(tritylthio)ethyl hexanoate (3): Compound 2 (3.2 gr, 10 mmol) and hexanoic acid (1.38 ml, 1.28 gr, 11 mmol) were dissolved in 40 ml THF and flask was cooled to 0°C. Then DCC (2.27 gr, 11 mmol) was added followed by DMAP (0.37 gr, 3 mmol). Reaction was heated to room temperature and stirred at ambient temperature overnight. Reaction mixture was filtered through celite and the filtrate was evaporated to dryness under vacuum and product was purified using flash silica chromatography (Hex:DCM 10:90). Product was obtained as white solid in 84% yield (3.50 gr).

\[ \text{Product was obtained as white solid in 84% yield (3.50 gr)} \]

\[ \text{1H-NMR (CDCl}_3\text{: } \delta 7.41-7.46 \text{ (m, 6H, Trt), 7.28-7.33 \text{ (m, 6H, Trt), 7.21-7.26 \text{ (m, 3H, Trt), 3.89 \text{ (t, } J=6.7\text{Hz, 2H, } -\text{CH}_2\text{-O-CO-}, \text{), 2.48 \text{ (t, } J=6.7\text{Hz, 2H, } \text{Trt-S-CH}_2\text{-), 2.27 \text{ (t, } J=7.6\text{Hz, 2H, } -\text{O-CO-CH}_2\text{-), 1.61 \text{ (quin., } J=6.5\text{Hz, 2H, } -\text{O-CO-CH}_2\text{-CH}_3\text{), 1.25-1.37 \text{ (m, } 4\text{H, } -\text{CH}_2\text{-CH}_2\text{-CH}_3\text{), 0.90 \text{ (t, } J=6.9\text{Hz, 3H, } -\text{CH}_2\text{-CH}_3\text{) ; } ^{13}\text{C-NMR (CDCl}_3\text{: } \delta 173.0, 144.1, 129.1, 127.5, 126.3, 66.3, 62.1, 33.7, 30.8, 30.4, 24.1, 21.8, 13.4; FT-IR (ATR), } \nu(\text{cm}^-1): 3057, 3030, 2955, 2929, 2859, 1735, 1653, 1636, 1489, 1458, 1380, 1244, 1164, 1083, 1034, 1001, 970, 886, 850; \text{ High resolution MS (EI, positive mode, 70eV): calculated mass: 441.1859, found: 441.1866 (C}_{27}\text{H}_{30}\text{O}_2\text{SNa).} \]

\[ \text{Product was obtained as white solid in 84% yield (3.50 gr)} \]

2-mercaptoethyl hexanoate (4): Compound 3 (3.48 gr, 8.31 mmol) was dissolved in 40 ml DCM and 10 ml TFA were added followed by triethylsilane (1.59 ml, 1.16 gr, 9.98 mmol). Reaction was stirred for 30 minutes at ambient temperature and solvents were evaporated to dryness under vacuum. Product was purified using flash silica chromatography (Hex:DCM 1:1) (product was identified on TLC using KMnO\textsubscript{4}). Product was obtained as colorless oil in 81% yield (1.18 gr).

\[ \text{Product was obtained as colorless oil in 81% yield (1.18 gr)} \]

\[ \text{1H-NMR (CDCl}_3\text{: } 4.18 \text{ (t, } J=6.6\text{Hz, 2H, } -\text{CH}_2\text{-O-CO-}, \text{), 2.71-2.77 \text{ (m, 2H, HS-CH}_2\text{-), 2.32 \text{ (t, } J=7.5\text{Hz, 2H, } -\text{O-CO-CH}_2\text{-CH}_2\text{-), 1.63 \text{ (quin., } J=6.5\text{Hz, 2H, } -\text{O-CO-CH}_2\text{-CH}_2\text{-), 1.48 \text{ (t, } J=8.5\text{Hz, 1H,} \]

\[ \text{Product was obtained as colorless oil in 81% yield (1.18 gr)} \]
HS-CH₂), 1.26-1.36 (m, 4H, -CH₂-CH₂-CH₃), 0.89 (t, J = 6.8Hz, 3H, -CH₂-CH₃) ; ¹³C-NMR (CDCl₃): δ 13C NMR (101 MHz, CDCl₃) δ 173.7, 65.6, 34.3, 31.4, 24.8, 23.5, 22.4, 14.0; FT-IR (ATR), ν(cm⁻¹): 2956, 2931, 2861, 2569, 1734, 1541, 1508, 1458, 1418, 1382, 1351, 1295, 1241, 1163, 1098, 1026, 996, 890, 855.

Figure S5: ¹H-NMR spectrum of compound 3 in CDCl₃.

Figure S6: Preparation of N-(2-mercaptoethyl)-2-phenylacetamide.

N-(2-mercaptoethyl)-2-phenylacetamide was synthesized as previously reported⁴ and the spectroscopic characterization correlated well with these reports.
Figure S7: $^1$H-NMR spectrum of compound N-(2-mercaptoethyl)-2-phenylacetamide in CDCl$_3$.

Synthesis of mPEG$_{5kDa}$-NH$_2$ from mPEG$_{5kDa}$-OH:

mPEG$_{5kDa}$-allyl and mPEG$_{5kDa}$-NH$_2$ were prepared as was described in previously reported procedure$^1$ and the spectroscopic characterization correlated well with these reports.
Synthesis of mPEG$_{10\text{kDa}}$-(NH$_2$)$_2$ from mPEG$_{10\text{kDa}}$-OH:

\[ \text{mPEG}_{10\text{kDa}} \xrightarrow{\text{Br}} \text{mPEG}_{10\text{kDa}} \xrightarrow{\text{KOH}} \xrightarrow{\text{Toluene, 60°C, 24h}} \text{mPEG}_{10\text{kDa}}-\text{propargyl} \xrightarrow{\text{1. HS-\text{NH}_2^+\text{Cl}^-}} \xrightarrow{\text{DMPA, UV, 2h}} \xrightarrow{\text{2. NaOH}} \text{mPEG}_{10\text{kDa}}-\text{(NH}_2)_2 \xrightarrow{95\%} \]

Figure S9: Preparation of 10 kDa methoxy di-amino PEG.

mPEG$_{10\text{kDa}}$-propargyl and mPEG$_{10\text{kDa}}$-(NH$_2$)$_2$ were prepared as was described in previously reported procedure$^5$ using mPEG$_{10\text{kDa}}$-OH as starting material.

**mPEG$_{10\text{kDa}}$-propargyl:** mPEG$_{10\text{kDa}}$-OH (2.58 gr, 0.26 mmol), propargyl bromide (0.92 gr, 7.74 mmol) and potassium hydroxide (0.43 gr, 7.74 mmol) were reacted in toluene (30 ml) according to the previously reported procedure$^5$. The product was obtained as a white solid in 95% yield (2.47 gr).

$^1$H-NMR (CDCl$_3$): $\delta$ 4.16 (d, $J$ = 2.4Hz, 2H, -O-CH$_2$-C≡), 3.80-3.40 (m, 9H, PEG backbone), 3.34 (s, 3H, CH$_3$-O-PEG-), 2.41 (t, $J$ = 2.4Hz, 1H, -C≡CH); $^{13}$C-NMR (CDCl$_3$): $\delta$ 74.5, 72.1, 70.5, 69.2, 59.1, 58.6; FT-IR, $\nu$ (cm$^{-1}$): 2885, 1557, 1543, 1521, 1509, 1471, 1468, 1457, 1361, 1321, 1282, 1243, 1145, 1104, 1064, 960, 842; GPC (DMF + 25 mM NH$_4$Ac): Mn = 10.1 kDa, PDI = 1.04, Expected Mn = 10.1 kDa;

Figure S10: $^1$H-NMR spectrum of compound mPEG$_{10\text{kDa}}$-propargyl in CDCl$_3$. 
**mPEG10kDa-(NH₂)₂**: mPEG₁₀kDa-propargyl (1.00 gr, 0.099 mmol), cysteamine hydrochloride (0.67 gr, 5.93 mmol) and DMPA (15 mg, 0.06 mmol) were reacted in MeOH (5 ml) according to the previously reported procedure⁵. The product was obtained as a white solid in 88% yield (0.90 gr).

¹H-NMR (CDCl₃): δ 3.38-3.80 (m, 938H, PEG backbone), 3.33 (s, 3H, CH₃O-PEG⁻), 2.92 (quin., 1H, -S-CH₂⁻), 2.58-2.88 (m, 10H, -S-CH₂⁻ + -CH₂⁻NH₂⁻); ¹³C-NMR (CDCl₃): δ 73.4, 71.9, 70.5, 59.2, 45.7, 41.6, 41.3, 37.4, 36.0, 34.7; FT-IR, ν (cm⁻¹): 2882, 1560, 1540, 1520, 1510, 1473, 1457, 1363, 1345, 1276, 1240, 1150, 1101, 1059, 960, 842; GPC (DMF + 25 mM NH₄Ac): N.A. due to presence of amines.

*Figure S11*: ¹H-NMR spectrum of compound mPEG₁₀kDa-(NH₂)₂ in CDCl₃.
Synthesis of PEG-dendron hybrids:

General procedures for preparation of hybrids:

General procedure for conjugation of mPEG_{5kDa-NH₂} / mPEG_{10kDa-(NH₂)₂} to 4-nitrophenyl ester of triple bond containing branching unit:

mPEG_{5kDa-NH₂} or mPEG_{10kDa-(NH₂)₂} were dissolved in DMF (1 ml per 100 mg PEG). DIPEA (9 eq. per amine) and compound 1 (3 eq. per amine) were added and the reaction was heated to 40°C and stirred overnight. Then, 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 I₂ 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 for thiol-yne reaction with thiolated enzymatically cleavable end-groups:

The PEG reactant, desired thiol (20 eq. per triple bond) and DMPA (1 mol % with respect to the thiol) were dissolved in DMF (1 ml). The solution was purged with N₂ for 15 minutes and then stirred under UV light (365 nm) for 2 hours. The product was isolated and purified as described in the previous general procedure.
Synthesis of mPEG_{5kDa}-(dend-Ph_{4})

**Figure S12**: Preparation of mPEG_{5kDa}-(dend-Ph_{4}).

**mPEG_{5kDa}-(dend-yne_{2})**: mPEG_{5kDa}-NH\_2 (200 mg, 0.039 mmol), compound 1 (41 mg, 0.117 mmol, 3eq) and DIPEA (63 µL, 0.352 mmol, 9 eq) were reacted in DMF (2 ml) according to the general procedure. The product was obtained as a white solid in quantitative yield (208 mg). GPC (DMF + 25mM NH\_4Ac): Mn = 5.4 kDa, PDI = 1.06, Expected Mn = 5.3 kDa; the spectroscopic characterization correlated well with previously reported procedure.\(^5\)

**mPEG_{5kDa}-(dend-Ph_{4})**: mPEG_{5kDa}-(dend-yne_{2}) (150 mg, 0.028mmol), N-(2-mercaptoethyl)-2-phenylacetamide (220 mg, 1.126 mmol, 40eq) and DMPA (2.9 mg, 0.011 mmol, 0.4eq) were reacted in DMF (1 ml) according to the general procedure. The product was obtained as a white solid in quantitative yield (173 mg). GPC (DMF + 25mM NH\_4Ac): Mn = 6.2 kDa, PDI = 1.08, Expected Mn = 6.1 kDa; the spectroscopic characterization correlated well with previously reported procedure.\(^5\)
**Synthesis of mPEG\textsubscript{5kDa}-\{dend-Hex\}_4**

![Synthesis of mPEG\textsubscript{5kDa}-\{dend-Hex\}_4](image)

**Figure S13: Preparation of mPEG\textsubscript{5kDa}-\{dend-Hex\}_4.**

**mPEG\textsubscript{5kDa}-\{dend-Hex\}_4:** mPEG\textsubscript{5kDa}-\{dend-yne\}_2 (150 mg, 0.028 mmol), compound 4 (201 mg, 1.141 mmol, 40 eq) and DMPA (2.9 mg, 0.011 mmol, 0.4 eq) were reacted in DMF (1 ml) according to the general procedure. The product was obtained as a white solid in 95% yield (163 mg). GPC (DMF + 25mM NH\textsubscript{4}Ac): Mn = 6.2 kDa, PDI = 1.06, Expected Mn = 6.0 kDa; the spectroscopic characterization correlated well with previously reported procedure.\textsuperscript{6}
Synthesis of mPEG_{10kDa-(dend-Ph)}_{2}

**Figure S14:** Preparation of mPEG_{10kDa-(dend-Ph)}_{2}.

mPEG_{10kDa-(dend-yne)}_{2}; mPEG_{10kDa-(NH}_{2})_{2} (200 mg, 0.020 mmol), compound 1 (42 mg, 0.118 mmol, 6 eq) and DIPEA (63 µL, 0.355 mmol, 9 eq) were reacted in DMF (2 ml) according to the general procedure. The product was obtained as a white solid in quantitative yield (205 mg).

^{1}H-NMR (CDCl_{3}): δ 7.35 (t, J = 5.9 Hz, 1H, -NH-CO-), 7.25 (m, 1H, -NH-CO- + CHCl_{3}), 7.00-7.06 (m, 4H, Ar-H), 6.65-6.71 (m, 2H, Ar-H), 4.62 (t, J = 2.4 Hz, 8H, Ar-O-CH_{2}-), 3.40-3.81 (m, 89H, PEG backbone), 3.34 (s, 3H, CH_{2}-O-PEG-), 3.00-3.09 (m, 1H, -CH-S-), 2.69-2.89 (m, 6H, -CH_{2}-S-), 2.54 (t, J = 2.4 Hz, 4H, -C≡CH); ^{13}C-NMR (CDCl_{3}): δ 167.5, 167.0, 158.7,
136.7, 106.84, 106.81, 105.6, 78.2, 76.1, 73.0, 72.0, 70.6, 59.1, 56.1, 45.5, 40.0, 39.5, 34.3, 32.0, 31.1; FT-IR, \( \nu \) (cm\(^{-1}\)): 2869, 1571, 1560, 1539, 1521, 1509, 1495, 1485, 1472, 1455, 1433, 1417, 1397, 1362, 1348, 1277, 1246, 1143, 1098, 1045, 943, 842; GPC (DMF + 25 mM NH\(_4\)Ac): Mn = 10.8 kDa, PDI = 1.12, Expected Mn = 10.6 kDa;

**Figure S15**: \(^1\)H-NMR spectrum of compound mPEG10kDa-(dend-yne\(_2\))\(_2\) in CDCl\(_3\).

**mPEG\(_{10kDa}\)-(dend-Ph\(_4\))\(_2\)**: mPEG10kDa-(dend-yne\(_2\))\(_2\) (149 mg, 0.014 mmol), N-(2-mercaptoethyl)-2-phenylacetamide (221 mg, 1.131 mmol, 80 eq) and DMPA (2.8 mg, 0.011 mmol, 0.8 eq) were reacted in DMF (1 ml) according to the general procedure. The product was obtained as a white solid in 98% yield (166 mg).

\(^1\)H-NMR (CDCl\(_3\)): \( \delta \) 8.02-8.09 (m, 2H, -NH-CO-), 7.17-7.34 (m, 40H, Ar-H + CHCl\(_3\)), 7.01 (s, 4H, Ar-H), 6.58 (t, \( J = 5.9\)Hz, 4H, -NH-CO-), 6.52 (Brs, 2H, Ar-H), 6.46 (t, \( J = 5.9\)Hz, 4H, -NH-CO-), 3.94-4.10 (m, 8H, Ar-O-CH\(_2\)-), 3.52-3.84 (m, 972H, PEG backbone), 3.48-3.51 (m, 16H, -CO-CH\(_2\)-Ph), 3.30-3.39 (m, 19H, -CH\(_2\)-NH-CO- + CH\(_3\)-O-PEG-), 2.50-3.11 (m, 35H, -CH\(_2\)-S- + -CH-S-); \(^{13}\)C-NMR (CDCl\(_3\)): \( \delta \) 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, \( \nu \) (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 + 25 mM NH\(_4\)Ac): Mn = 12.8 kDa, PDI = 1.18, Expected Mn = 12.1 kDa;
Figure S16: $^1$H-NMR spectrum of compound mPEG$_{10\text{kDa}}$(dend-Ph)$_2$ in CDCl$_3$. 
Synthesis of mPEG10kDa-(dend-Hex$_4$)$_2$

Figure S17: Preparation of mPEG$_{10kDa}$-(dend-Hex)$_4$$_2$. 

mPEG$_{10kDa}$-(dend-Hex)$_4$$_2$: mPEG$_{10kDa}$-(dend-yne)$_2$$_2$ (150 mg, 0.014 mmol), compound 4 (200 mg, 1.135 mmol, 80 eq) and DMPA (2.9 mg, 0.011 mmol, 0.8 eq) were reacted in DMF (1 ml) according to the general procedure. The product was obtained as a white solid in 98% yield (166 mg).

$^1$H-NMR (CDCl$_3$): δ 7.32 (t, $J = 6.6$ Hz, 1H, -NH-CO-), 7.22-7.26 (m, 1H, -NH-CO-), 6.96-7.02 (m, 4H, Ar-H), 6.56-6.60 (m, 2H, Ar-H), 4.09-4.30 (m, 24H, Ar-O-CH$_2$- + -S-CH$_2$-CH$_2$-O-CO-), 3.41-3.85 (m, 1012H, PEG backbone), 3.36 (s, 3H, CH$_3$-O-PEG-), 2.72-3.21 (m, 35H, -CH$_2$-S- + -CH-S-), 2.23-2.33 (m, 16H, -O-CO-CH$_2$-CH$_2$- + H$_2$O), 1.59 (quin, $J = 7.4$ Hz, 16H, -O-CO-CH$_2$-CH$_2$-CH$_2$-), 1.24-1.33 (m, 32H, -CO-CH$_2$-CH$_2$-CH$_2$-CH$_2$-), 0.87 (t, $J = 6.9$ Hz, 24H, -CH$_2$-CH$_3$) ; $^{13}$C-NMR (CDCl$_3$): δ 171.5, 171.4, 167.2, 159.4, 136.6, 135.0, 129.4, 128.9, 127.3, 106.4, 105.0, 73.00, 72.0, 71.8, 63.7, 59.1, 45.1, 44.8, 43.6, 39.6, 39.2, 34.3, 32.3, 31.7, 31.2; FT-IR, $\nu$ (cm$^{-1}$): 2880, 2745, 1735, 1730, 1592, 1467, 1453, 1413, 1379, 1360, 1342, 1280,
1241, 1147, 1097, 1060, 961, 842; GPC (DMF + 25mM NH₄Ac): Mn = 12.5 kDa, PDI = 1.13, Expected Mn = 12.0 kDa;

Figure S18: ¹H-NMR spectrum of compound mPEG10kDa-(dend-Hex)₂ in CDCl₃.

Characterization of PEG-dendron hybrids

Gel permeation chromatography (GPC)

Instrument method:

Instrument: Malvern Viscotek GPCmax

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

Columns temperature: 50°C

Flow rate: 0.5ml/min

Injection time: 90 min

Injection volume: 50 µL from a 10 mg/ml sample

Diluent + mobile phase: DMF + 25mM NH₄Ac
Needle wash: DMF

Detector: Viscotek VE3580 RI detector

**Figure S19:** GPC overlay of commercial 5kDa methoxy PEG (blue), mPEG_{S5kDa}-(dend-yne_2) (red), mPEG_{S5kDa}-(dend-Ph_4) (green) and mPEG_{S5kDa}-(dend-Hex_4) (purple).
Figure S20: GPC overlay of commercial 10kDa methoxy PEG (blue), mPEG10kDa-propargyl (red), mPEG10kDa-(dend-ynen)2 (purple), mPEG10kDa-(dend-Ph4)2 (Turquoise) and mPEG10kDa-(dend-Hex4)2 (orange)

MALDI measurements
Figure S21: MALDI measurement of mPEG\textsubscript{5kDa-}(dend-yn)e\textsubscript{2}. $M_n = 5.4$ kDa, Expected $M_n = 5.3$ kDa.

Figure S22: MALDI measurement of mPEG\textsubscript{5kDa-}(dend-Ph\textsubscript{4}). $M_n = 6.2$ kDa, Expected $M_n = 6.1$ kDa.

Figure S23: MALDI measurement of mPEG\textsubscript{5kDa-}(dend-Hex\textsubscript{4}). $M_n = 6.1$ kDa, Expected $M_n = 6.0$ kDa.
Figure S24: MALDI measurement of mPEG$_{10k}$-(dend-yne)$_2$. $M_n = 10.4$ kDa, Expected $M_n = 10.6$ kDa.

Figure S25: MALDI measurement of mPEG$_{10k}$-(dend-Ph)$_2$. $M_n = 11.5$ kDa, Expected $M_n = 12.2$ kDa.
Figure S26: MALDI measurement of mPEG_{10kDa}{(dend-Hex)}_2. \( M_n = 11.9 \text{ kDa}, \text{ Expected } M_n = 12.0 \text{ kDa.} \)

Critical micelles' concentration (CMC)

General procedure of measurement:

Preparation of diluent:

Nile Red stock solution (0.88 mg/ml in ethanol) was diluted into a phosphate buffer (100mM, pH 7.4) to afford a final concentration of 1.25 µM.

Preparation and measurement of samples:

The PEG-dendron hybrid was directly dissolved in the diluent to give a final concentration of 250 µM for the 10 kDa based hybrids or 500 µM for the 5 kDa based hybrids. Solution was sonicated for 15 minutes and vortexed vigorously until the hybrid was completely dissolved. This solution was consecutively diluted by a factor of 1.5 with the diluent to afford a series of 24 samples. 150 µL of each sample was loaded onto 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 630 nm) was plotted as a function of the hybrid’s concentration.
Instrument method:

Instrument: TECAN Infinite M200Pro
Excitation: 550 nm
Emission intensity scan: 580-800 nm
Step: 2 nm
Number of flashes: 15
Gain: 100

Figure S27: CMC measurement mPEG_{5kDa-}(dend-Ph₄) Nile Red method.
Figure S28: CMC measurement of mPEG_{10kDa}-(dend-Ph$_4$)$_2$ using Nile Red method.

Figure S29: CMC measurement of mPEG$_{5kDa}$-(dend-Hex$_4$) using Nile Red method.
Figure S30: CMC measurement of mPEG$_{10kDa}$-(dend-Hex$_4$)$_2$ using Nile Red method.

$^1$H-NMR in D$_2$O

Figure S31: $^1$H-NMR of mPEG$_{5kDa}$-(dend-Ph$_4$) (1 mg/ml) in CDCl$_3$ (bottom) and in D$_2$O (top).
Figure S32: $^1$H-NMR of mPEG$_{10000}$-(dend-Ph)$_2$ (1 mg/ml) in CDCl$_3$ (bottom) and in D$_2$O (top).

Figure S33: $^1$H-NMR of mPEG$_{5000}$-(dend-Hex)$_2$ (1 mg/ml) in CDCl$_3$ (bottom) and in D$_2$O (top).
Figure S34: $^1$H-NMR of mPEG$_{10KDa}$-$(dend-Hex_4)_2$ (1 mg/ml) in CDCl$_3$ (bottom) and in D$_2$O (top).

Dynamic light scattering

Instrument method:

**Instrument**: Corduan technology VASCOγ – particle size analyzer

**Time interval**: 10µsec

**Number of channels**: 400

**DTC position**: down

**Laser power**: 50-80%

**Cell temperature**: 37°C
Figure S35: DLS of mPEG$_{10kDa}$-(dend-Ph)$_2$ (purple) and mPEG$_{10kDa}$-(dend-Hex)$_2$ (red) before (solid lines) and after (dashed lines) incubation with PGA (500 nM) and PLE (1400 nM), respectively.

**HPLC measurements**

**Instrument:** Waters Alliance e2695

**Column:** Aeris WIDEPORE, C4, 3.6µm, 150x4.6mm

**Column temperature:** 30°C

**Sample temperature:** 37°C

**Solution A:** 0.1% HClO$_4$:ACN 95:5v/v

**Solution B:** 0.1% HClO$_4$:ACN 5:95v/v

**Solution C:** THF

**Flow rate:** 1ml/min
Gradient program for 30 minutes injection:

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>95</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>1.0</td>
<td>95</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>20.0</td>
<td>0</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>23.0</td>
<td>0</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>23.1</td>
<td>95</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>30.0</td>
<td>95</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

Injection volume: 30 µL

Seal wash: H₂O:MeOH 90:10 v/v

Needle wash: MeOH

Detector: Waters 2998 photodiode array detector

Sampling rate: 2 points/sec

Figure S36: HPLC overlay of mPEG₅kDa-(dend-Ph₄) (1 mg/ml) with PGA (5 nM).
Figure S37: HPLC overlay of mPEG$_{10kDa}$-(dend-Ph)$_2$ (1 mg/ml) with PGA (5 nM).

Figure S38: HPLC overlay of mPEG$_{10kDa}$-(dend-Ph)$_2$ (1 mg/ml) with PGA (500 nM).
Figure S39: HPLC overlay of mPEG\textsubscript{5kDa}-(dend-Hex\textsubscript{4}) (1 mg/ml) with PLE (14 nM).

Figure S40: HPLC overlay of mPEG\textsubscript{10kDa}-(dend-Hex\textsubscript{4})\textsubscript{2} (1 mg/ml) with PLE (14 nM).
CPT encapsulation procedure:

The tested hybrid was dissolved in DCM (1 mg/ml). 1 ml of hybrid solution was mixed with 200µl CPT solution (1 mg/ml in DCM). DCM was removed in vacuum forming a thin film, which was further dried on high vacuum for 30 minutes. Then, 1 ml phosphate buffer were added and vial content was stirred vigorously and placed in an ultrasonic bath for 30 minutes. Undissolved CPT was filtered off using 0.45µm filter and the clear solution was analyzed by HPLC. CPT concentration was calculated by calibration curve at 360nm.

TEM imaging:

Sample preparation:

The desired hybrid was dissolved in phosphate buffer (pH 7.4) to afford a final concentration of 1 mg/ml. 10 µl of the hybrid solution were dropped 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 8 hour. Then, grids were inspected in transmission electron microscope (TEM), operated at 200kV (Philips Tecnai F20).
References:


