

Supplementary information

Penetration of Nanobody-Dextran Polymer Conjugates through Tumor Spheroids

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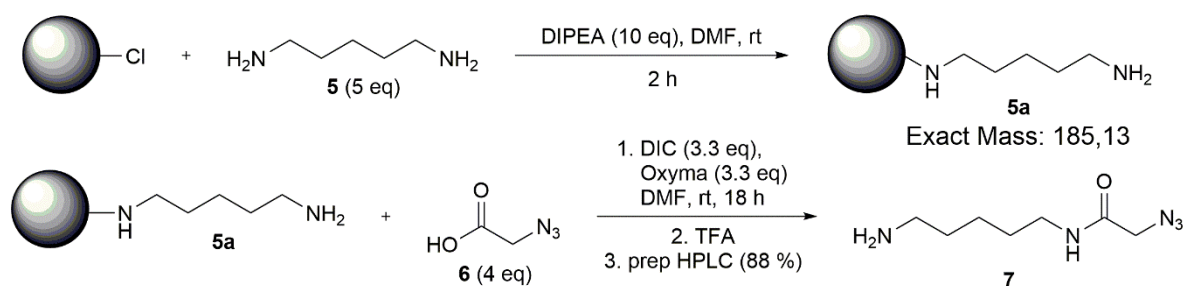
† These authors contributed equally to this work.

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1. Synthesis of compounds

1.1. Synthesis of cadaverine-azide linker



Scheme S 1. Synthesis of the N₃-Cad-Linker (*N*-(aminopentyl)-2-azidoacetamide).

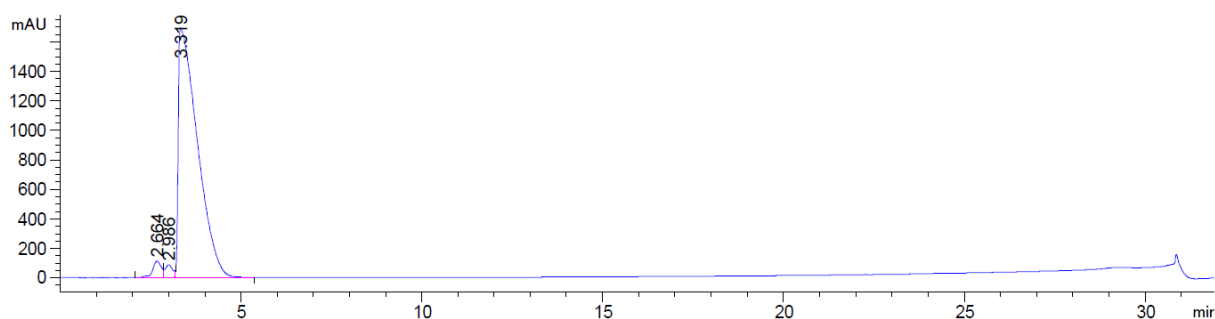


Figure S 1. HPLC-diagram of the N₃-Cad-Linker (0to80 % Eluent B, 220 nm).

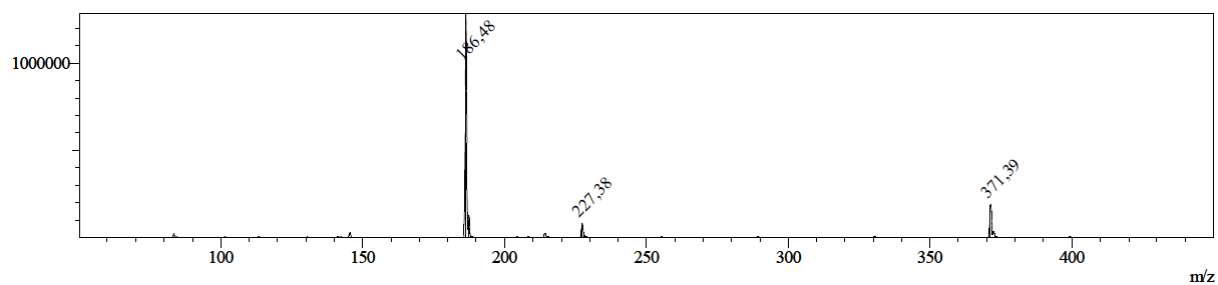
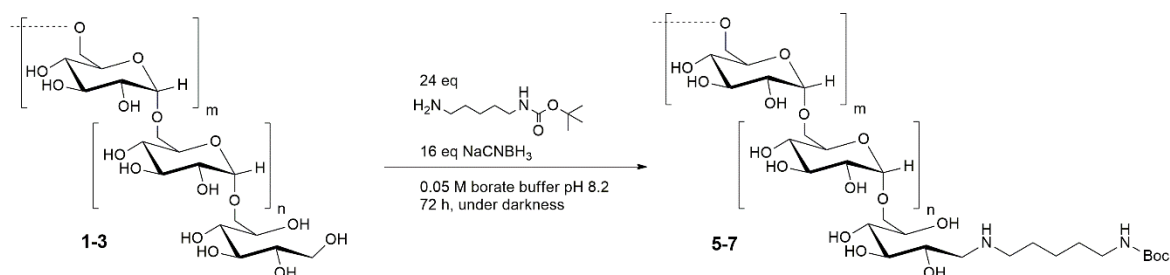


Figure S 2. LC-MS spectrum of the N₃-Cad-Linker (exact mass = 185.13 m/z). As method, a gradient from 10to100 % Eluent B was used over 20 min with a flow rate of 0.7 mL/s.

1.2. Synthesis of dextran-*N*-Boc-cadaverine



Scheme S 2. Reductive amination of dextran with *N*-Boc-cadaverine.

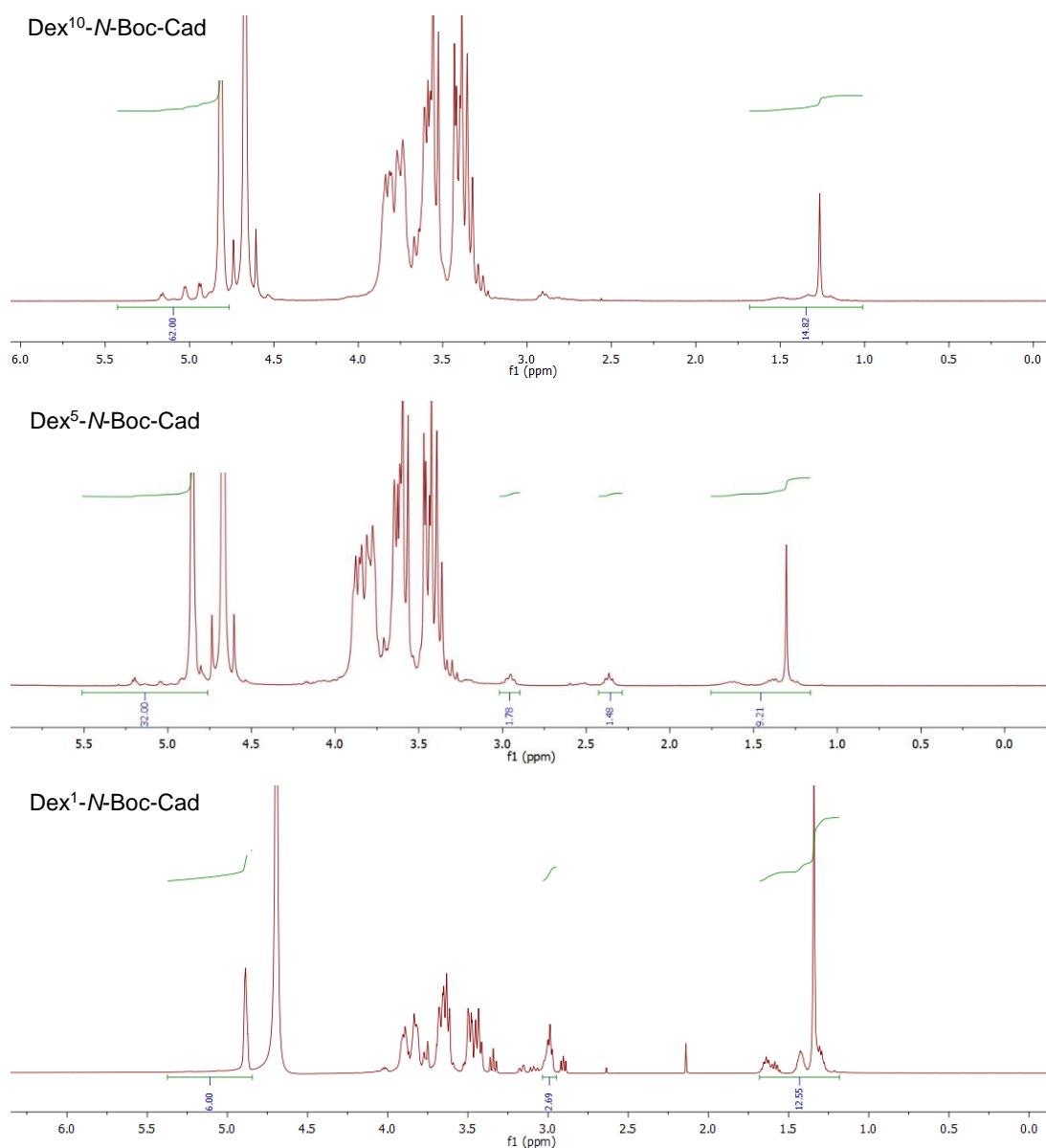
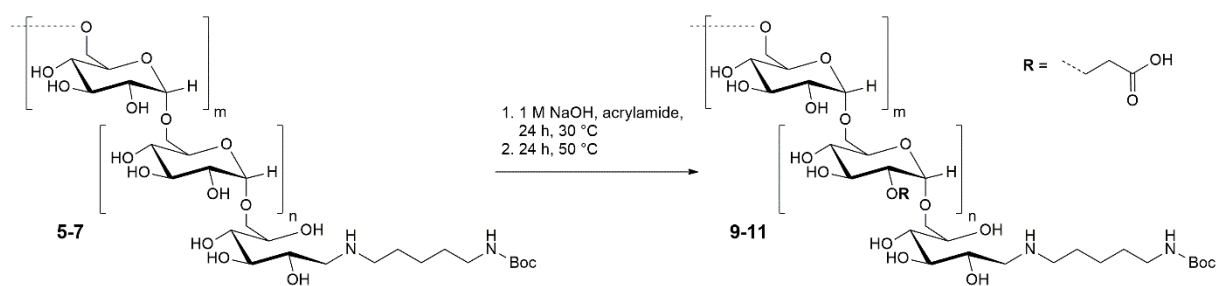


Figure S 3. ¹H-NMR spectra of Dex-*N*-Boc-Cad variants. ¹H-NMR (300 MHz, deuterium oxide) δ = 5.36 – 4.80 (m, 1 H, C(1)H), 4.20 – 3.22 (m, C(2-6)H (glucose units)), 3.05 – 2.81 (m, 4 H, CH₂NH-Boc, CH₂(CH₂)₄NHBoc), 1.73 – 1.19 (overlapped m, 15H, CH₂(CH₂)₃NH-Boc, CH₂(CH₂)₂NH-Boc, CH₂(CH₂)₁NH-Boc, 3 CH₃(Boc)) ppm.

1.3. Carboxyethylation of dextran

Table S 1. Calculated CE-ratios per dextran.

compound	CE-groups per dextran	Yield [%]
2-CED ¹⁰ -N-Boc-Cad	2.4	65
2-CED ⁵ -N-Boc-Cad	2.4	73
2-CED ¹ -N-Boc-Cad	4.3	45



Scheme S 3. Carboxyethylation of Dex-N-Boc-Cad to 2-CED-N-Boc-Cad.

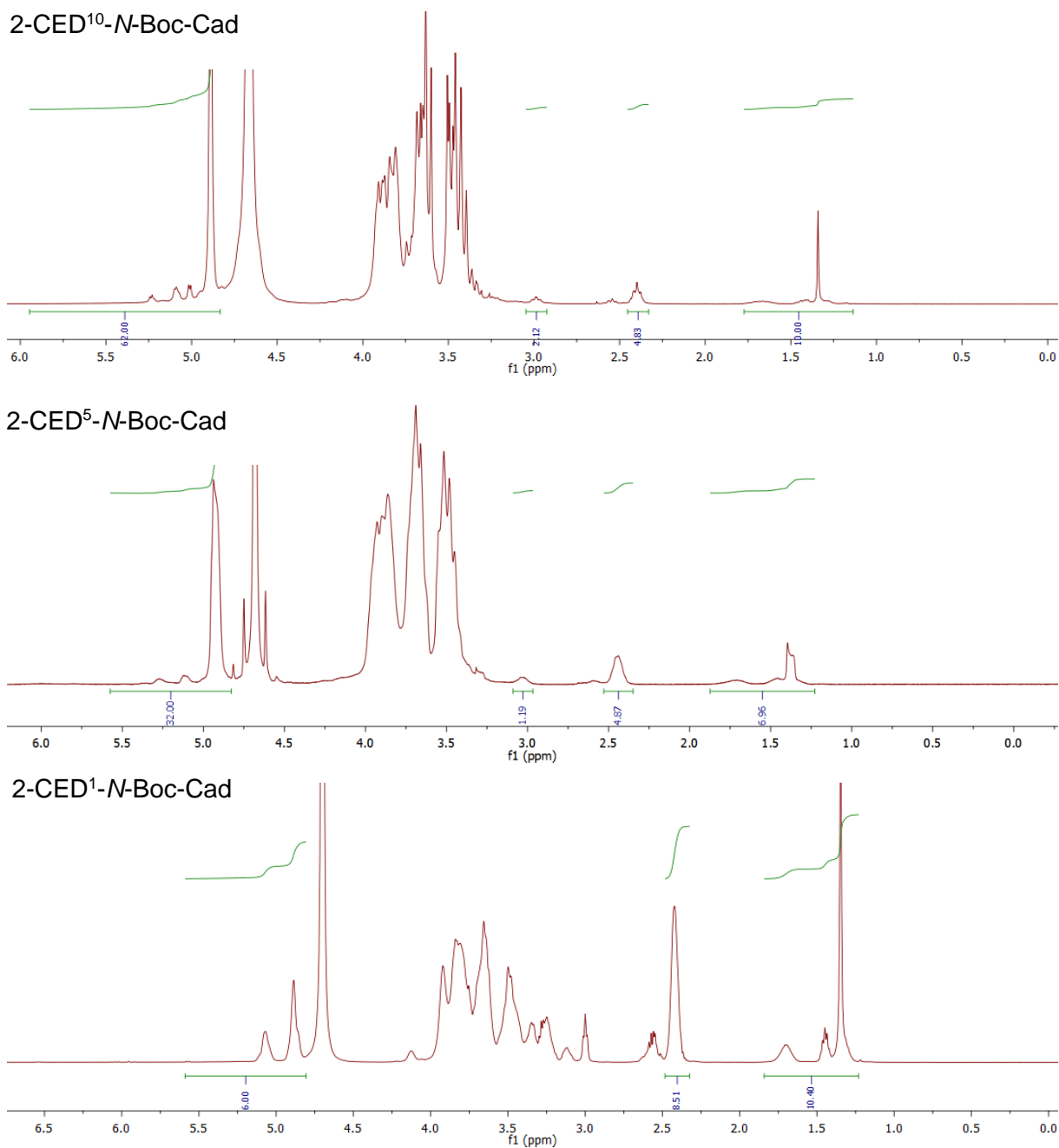
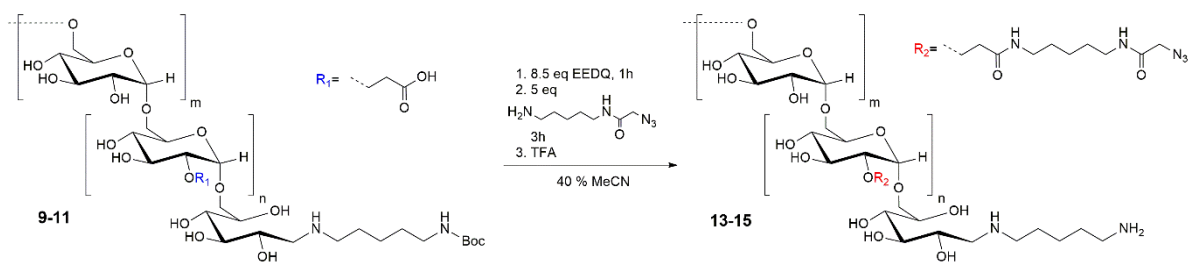


Figure S 4. ¹H-NMR spectra of 2-CED¹⁰-N-Boc-Cad variants. ¹H-NMR of CE-dextrans (300 MHz, D₂O) δ = 6.09 – 4.79 (m, 1 H, C(1)H), 4.34 – 3.16 (m, C(2-6)H (glucose units); CH₂CH₂COOH), 3.14 – 2.91 (m, 4 H, CH₂NH-Boc, CH₂(CH₂)₄NH-Boc), 2.52 – 2.29 (t, CH₂COOH), 1.82 – 1.24 (overlapped m, 15 H, CH₂(CH₂)₃NH-Boc, CH₂(CH₂)₂NH-Boc, CH₂(CH₂)₁NH-Boc, 3 CH₃(Boc)) ppm.

1.4. Synthesis of N₃-dextran-*N*-Boc-cadaverine



Scheme S 4. N₃-functionalization of 2-CED-*N*-Boc-Cad towards N₃-Dex-Cad.

Table S 2. Yields of N₃-functionalization of 2-CED-*N*-Boc-Cad with the N₃-cad-linker and determined number of N₃-groups per dextran (by ¹H-NMR).

compound	N ₃ -groups per dextran	Yield [%]
N ₃ -Dex ¹⁰ -Cad	2.4	70
N ₃ -Dex ⁵ -Cad	2.4	65
N ₃ -Dex ¹ -Cad	4.3	40

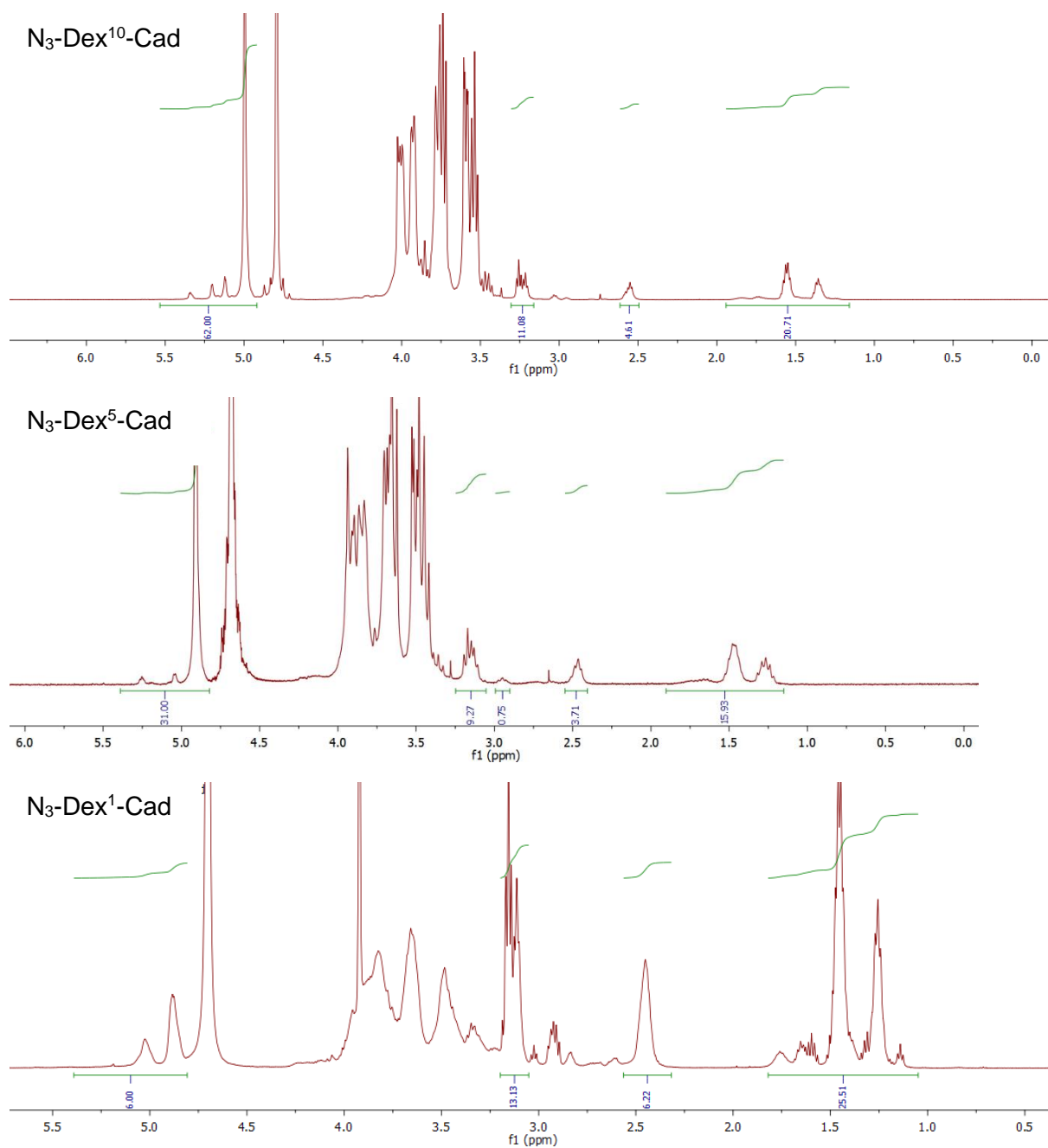


Figure S 5. ¹H-NMR spectra of N₃-Dex-Cad variants. ¹H-NMR (300 MHz, D₂O) δ = 6.24 – 4.80 (m, 1 H, C(1)H), 4.23 – 3.25 (m, C(2-6)H (glucose units); CH₂CH₂COOH; CH₂-N₃), 3.23 – 3.07 (m, NHCO-CH₂-N₃; CH₂-(CH₂)₄-NHCO-CH₂-N₃), 3.07 – 2.96 (m, 4 H, CH₂NH-BOC, CH₂(CH₂)₄NH-Boc), 2.73 – 2.34 (m, CH₂COOH), 1.80 – 1.19 (overlapped m, 15 H, CH₂(CH₂)₃NH-Boc, CH₂(CH₂)₂NH-Boc, CH₂(CH₂)₁NH-Boc, 3 CH₃(BOC); (1.80 – 1.37 CH₂-CH₂-NHCO-CH₂-N₃, CH₂-(CH₂)₃-NHCO-CH₂-N₃, 1.31 – 1.03 CH₂-(CH₂)₂-NHCO-CH₂-N₃) ppm.

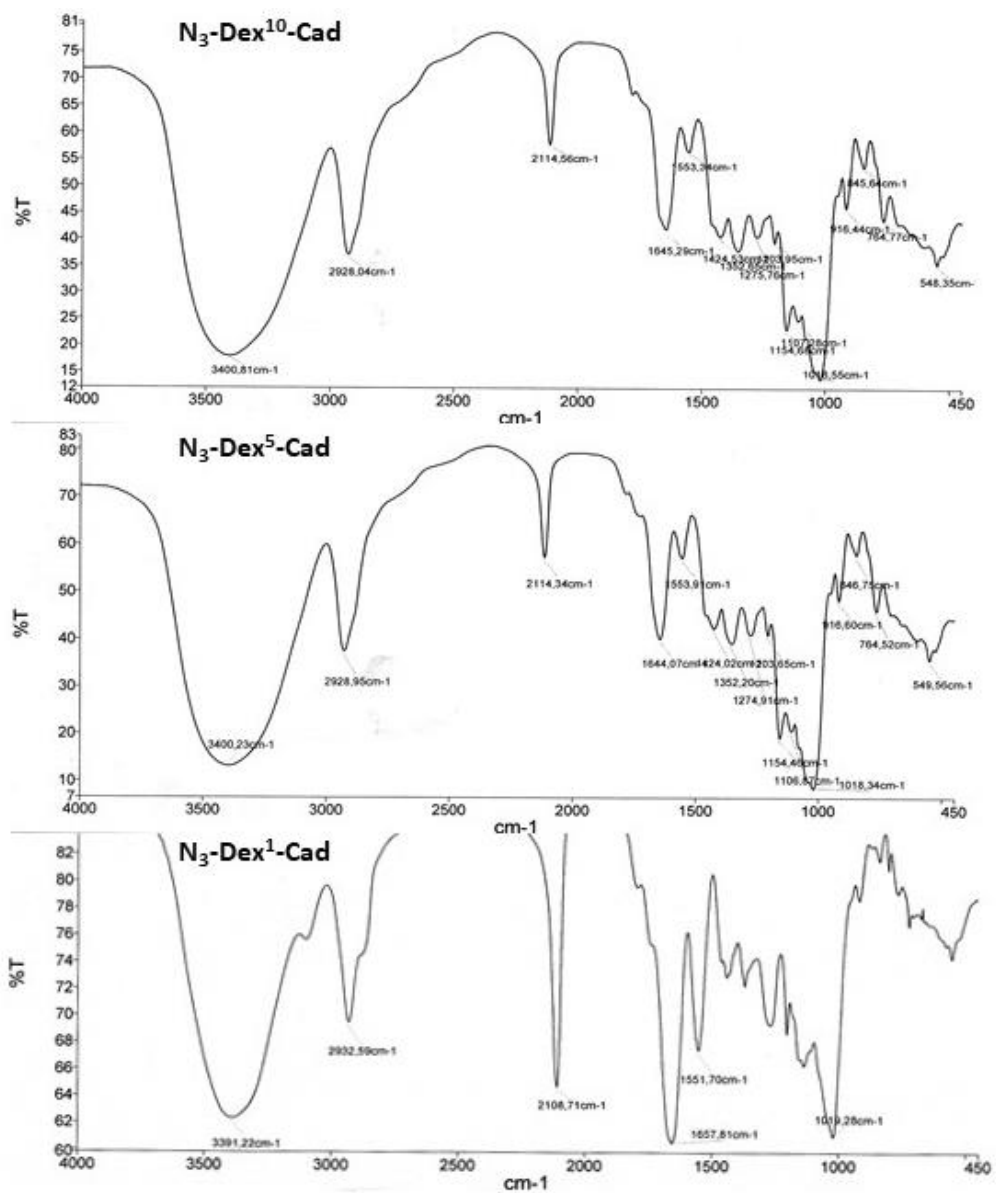
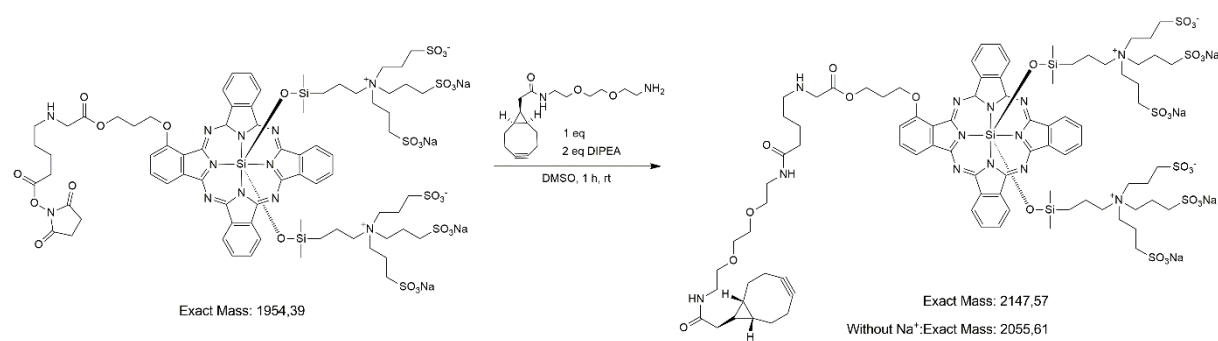


Figure S 6. IR-Spectra of N₃-Dex¹⁰-Cad variants. N₃-stretching vibrational band: 2110 – 2115 cm⁻¹³.

1.5. Synthesis of BCN-IRDye700DX



Scheme S 5 Synthesis of BCN-IRDye700DX.

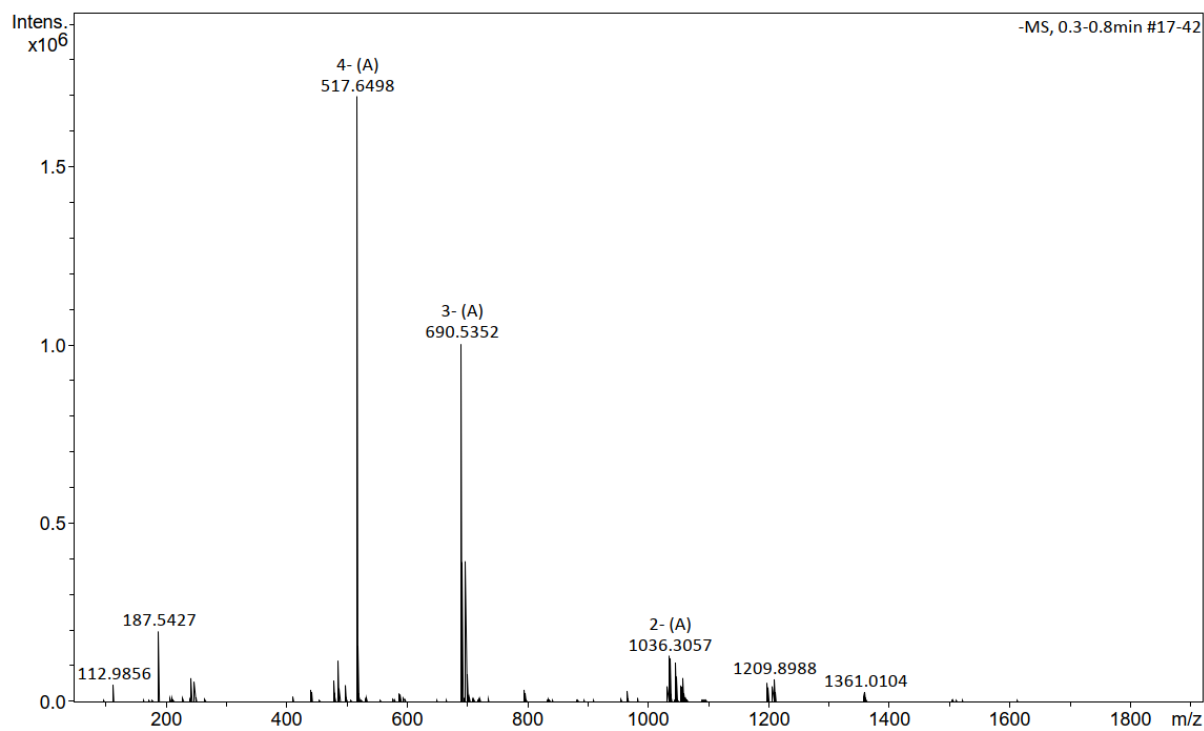
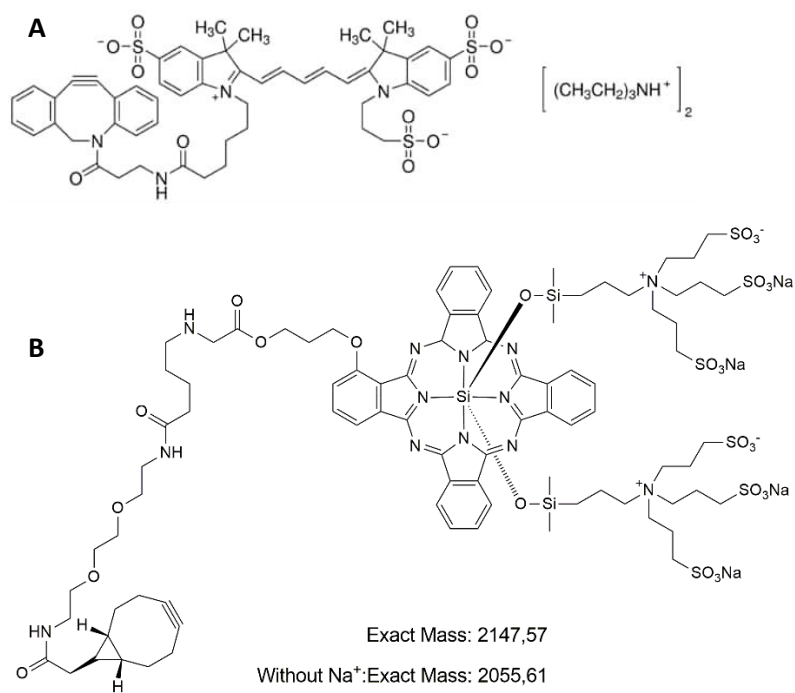


Figure S 7. ESI-MS spectrum of BCN-IRDye700DX®.

1.6. Structural formulas of utilized labels



Scheme S 6. Utilized labels for SPAAC. **A:** DBCO-Cy5. **B:** BCN-IRsy700DX.

1.7. Generation of dextranoknobs

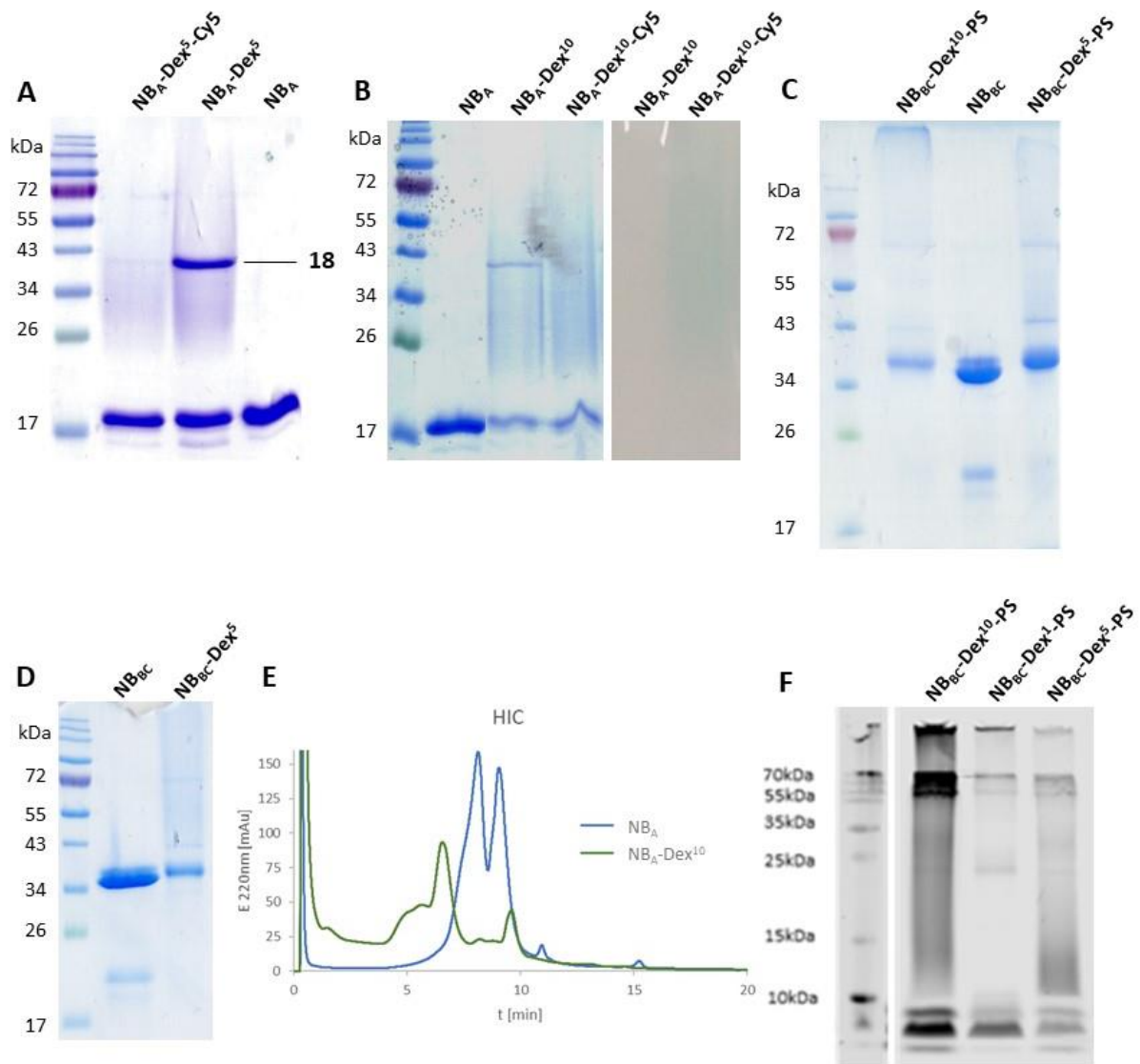


Figure S 8. A-D: Coomassie stained SDS-Gels of generated dextranoknobs. E: HIC chromatogram of NB_A-Dex¹⁰. F: SDS-Gel imaged with fluorescence reader (700 nm). Bands show excited IRDye700DX®.

2. Supplementary figures cell assays

2.1. Cellular binding assays

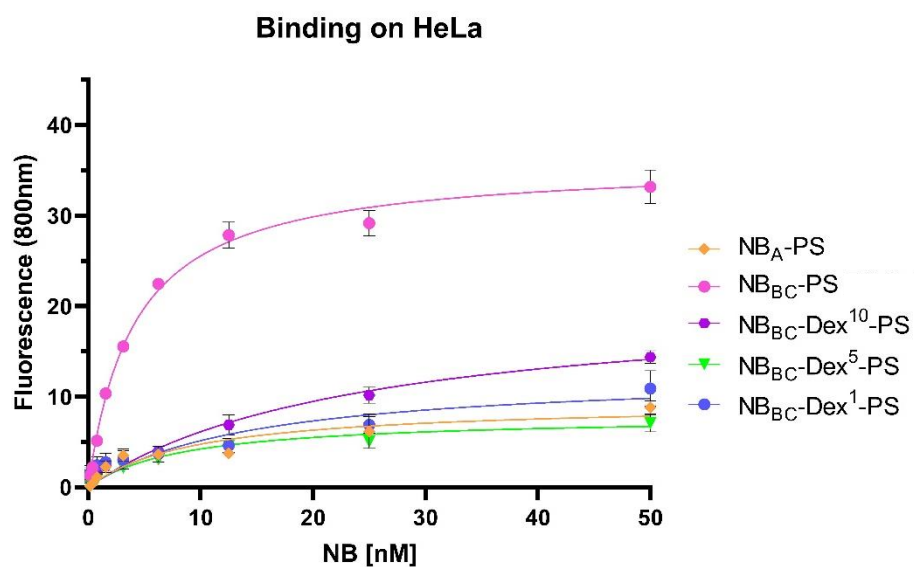


Figure S 9. Data of binding assay of NB-PS conjugates on HeLa cell line.

Table S 3. K_D -values of tested conjugates on HeLa cells.

K_D HeLa [nM]	
NB _{BC} -Dex ¹⁰ -PS DOC 1.2	24.3
NB _{BC} -Dex ⁵ -PS DOC 0.7	8.7
NB _{BC} -Dex ¹ -PS DOC 1.1	14.8
NB _A -PS-DOC 0.9	9.7
NB _{BC} -PS-DOC 1.5	4.1

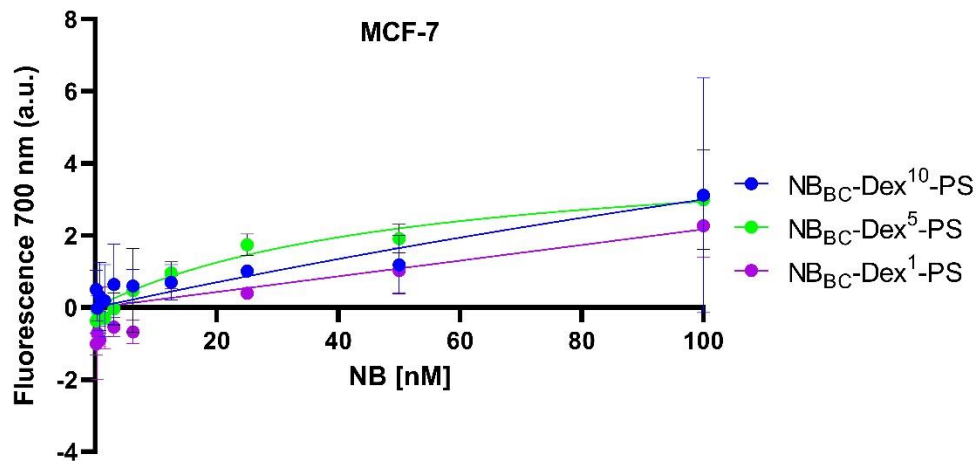


Figure S 10. Data of binding assay of NB_{BC}-Dex-PS conjugates on MCF-7 cells.

2.2. Confocal microscopy of spheroids

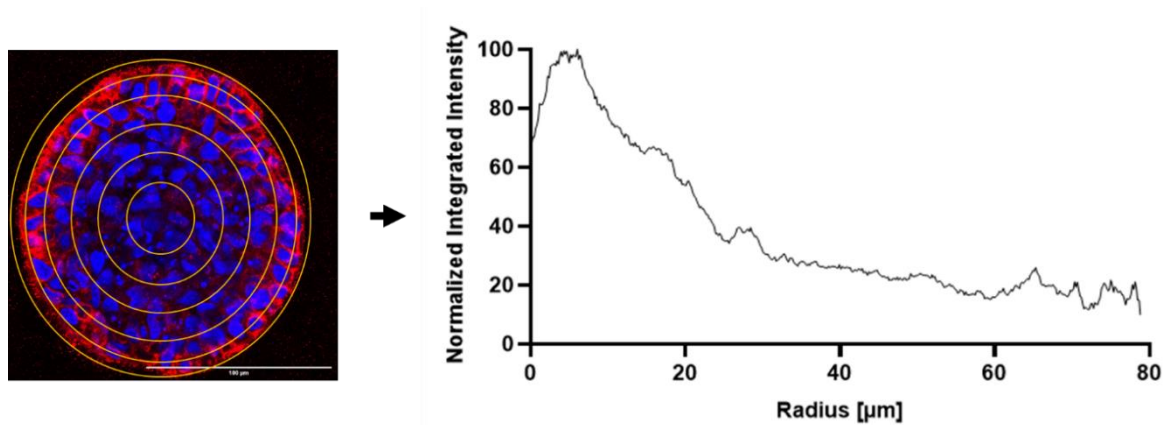


Figure S 11. Schematic representation of the analysis of confocal images taken of spheroids using ImageJ radial angle plug-in to create normalized integrated intensity plots of the NB signal along the radius of the spheroids.⁷

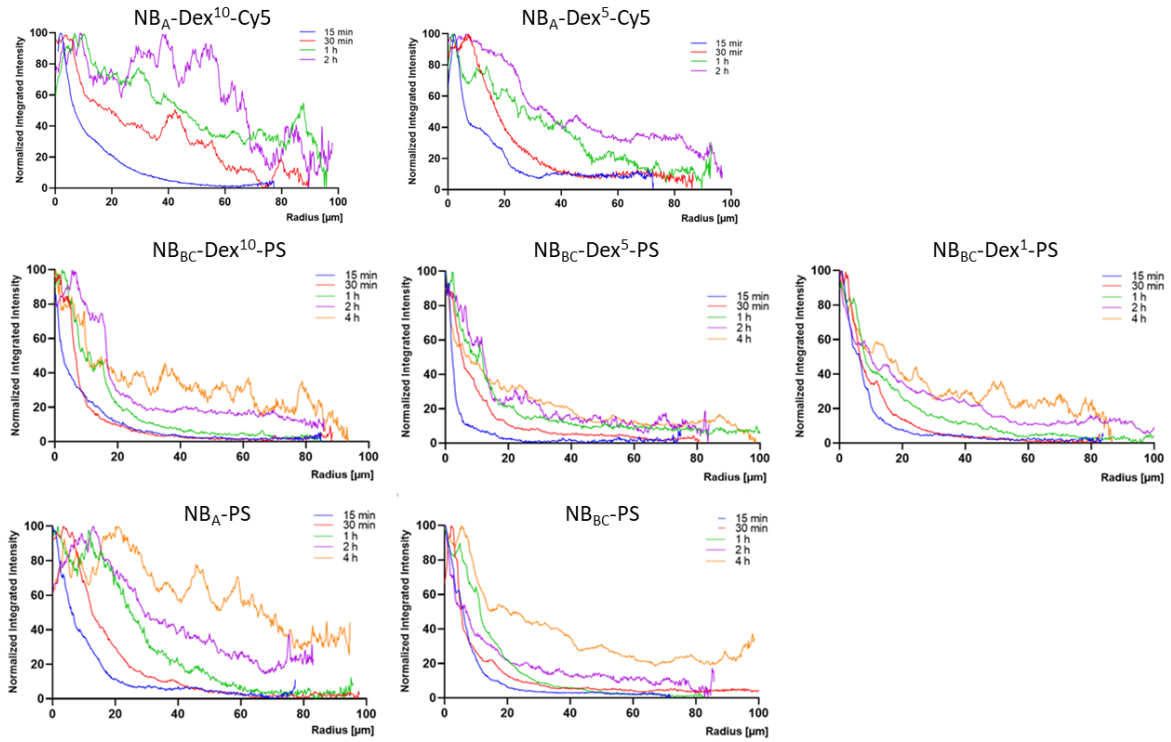


Figure S 12. Normalized integrated intensity plots of all conjugates at time points 15 min, 30 min, 1 h and 2 h. PS-conjugates were also measured at 4 h.

2.3. Nanobody-targeted photodynamic therapy (in vitro) on 2D monolayer cell culture

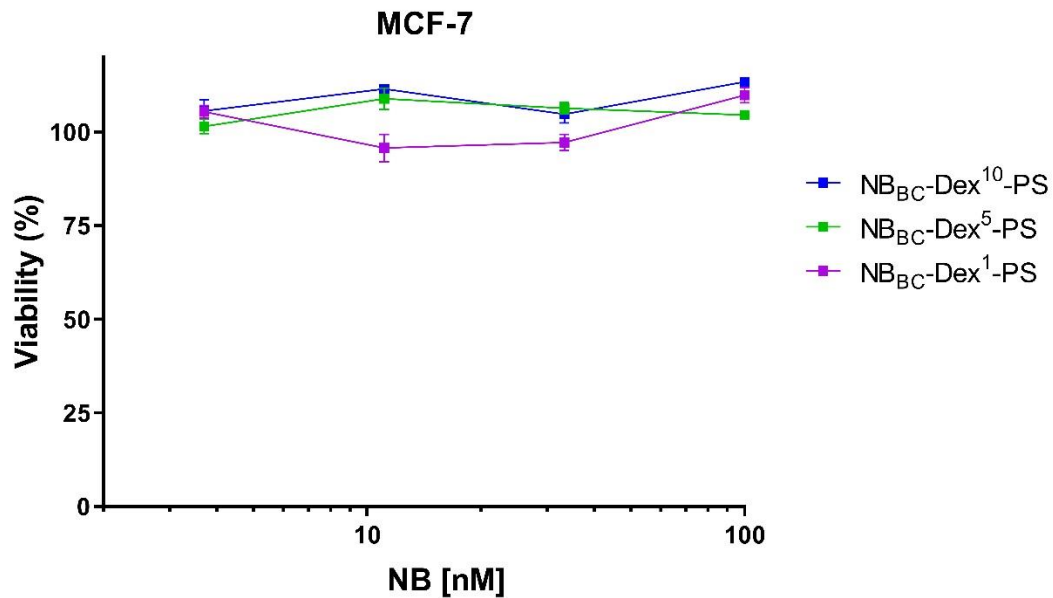


Figure S 13. Percentages (%) of cell viability after a 10 J/cm² light dose relative to untreated cells.

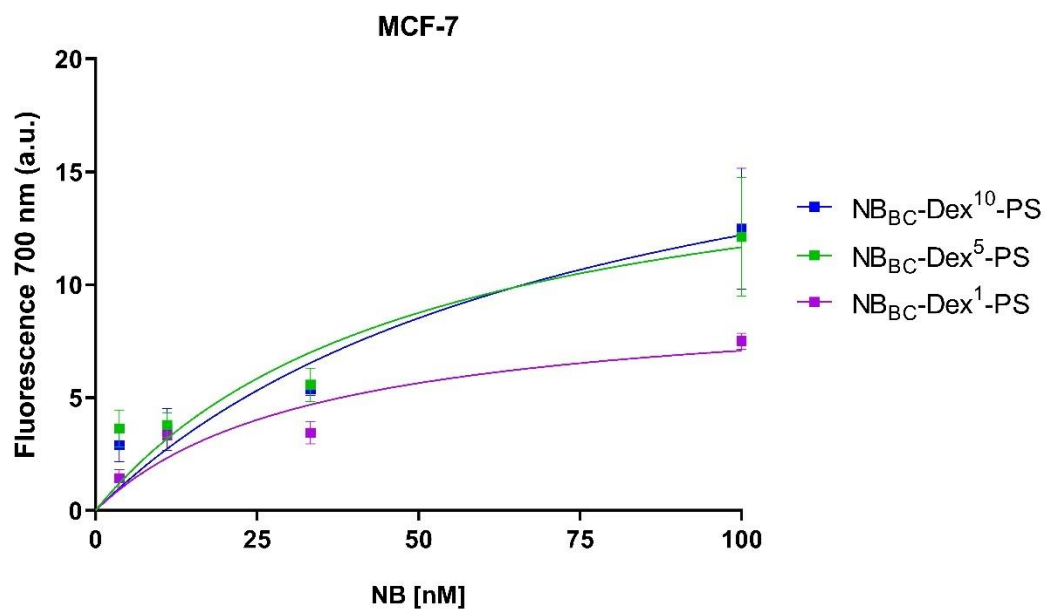


Figure S 14. Fluorescence intensities of NB_{BC}-Dex--PS conjugates bound to cells after 30 min pulse incubation with a concentration range of the conjugates.

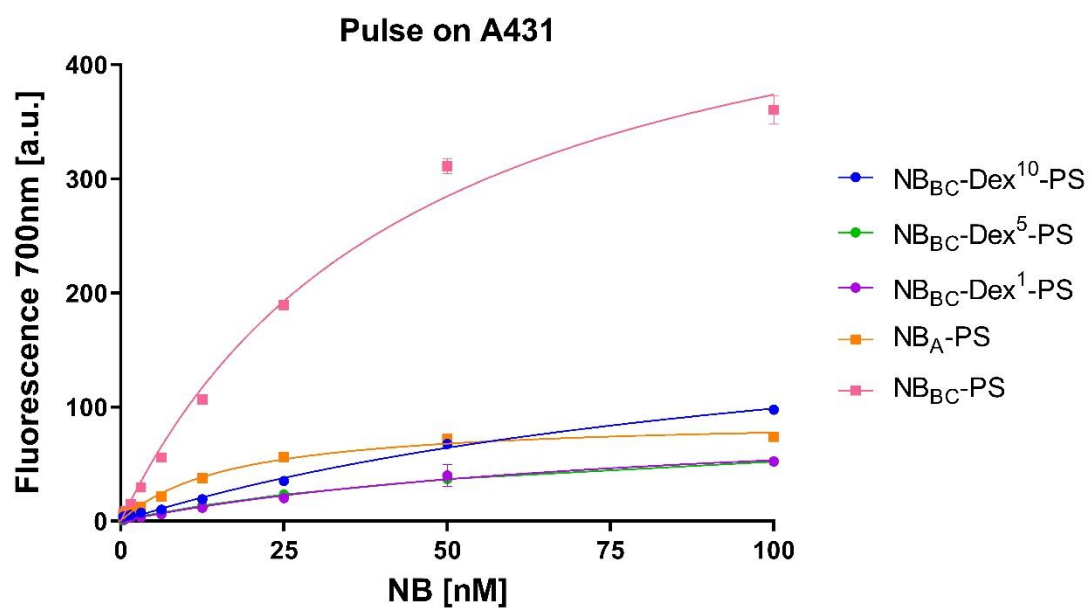


Figure S 15. Fluorescence intensities of NB-PS conjugates bound to cells after 30 min pulse incubation with a concentration range of the conjugates.

2.4. NB-targeted PDT on 3D spheroid cell culture

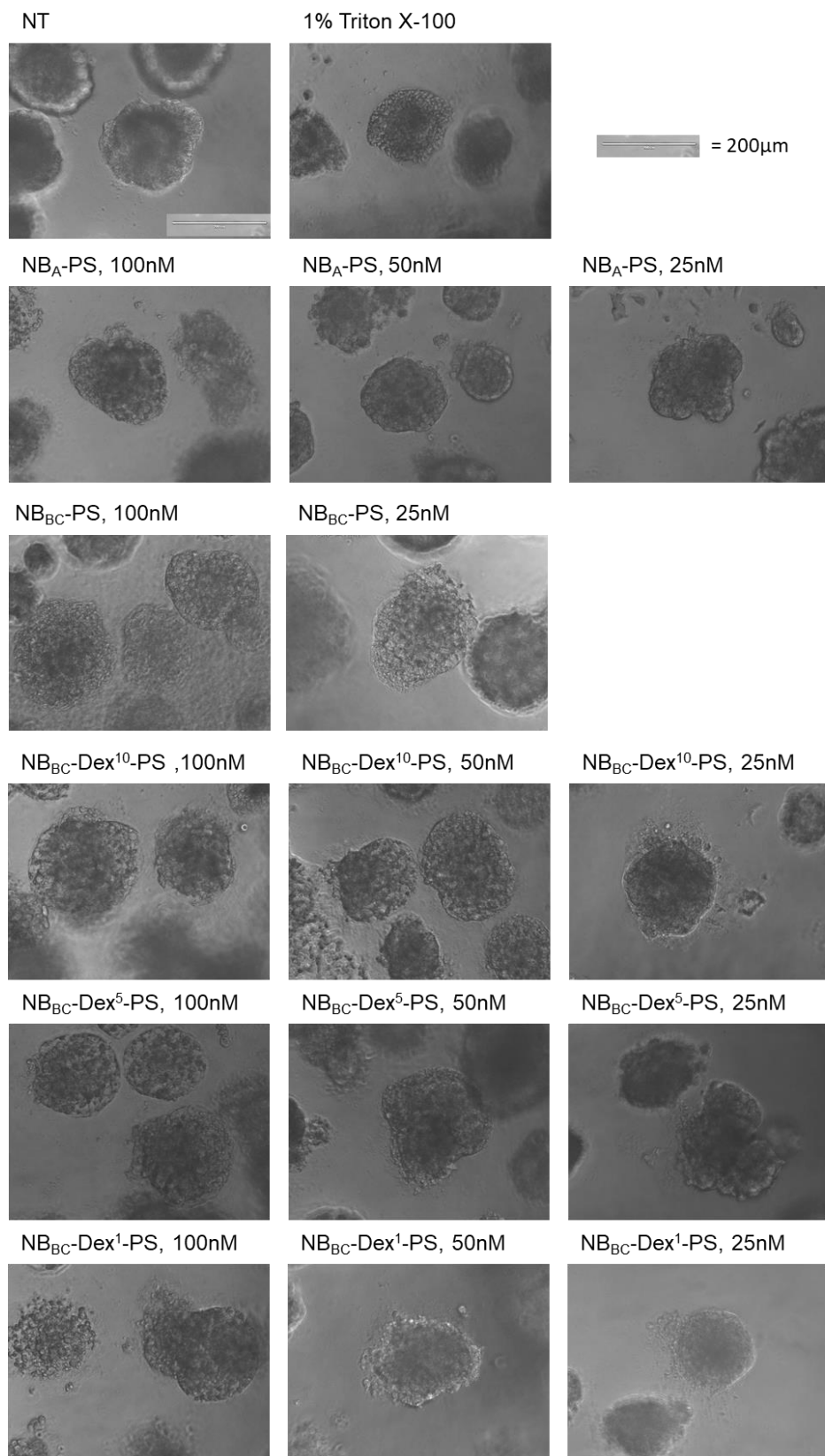


Figure S 16. 3D PDT 20J/cm², phase-contrast microscopy images taken 24h post treatment/irradiation.