Supporting Information

Building Block Based Construction of Membrane-Organelle Double Targeted Nanosystem for Two-Drug Delivery

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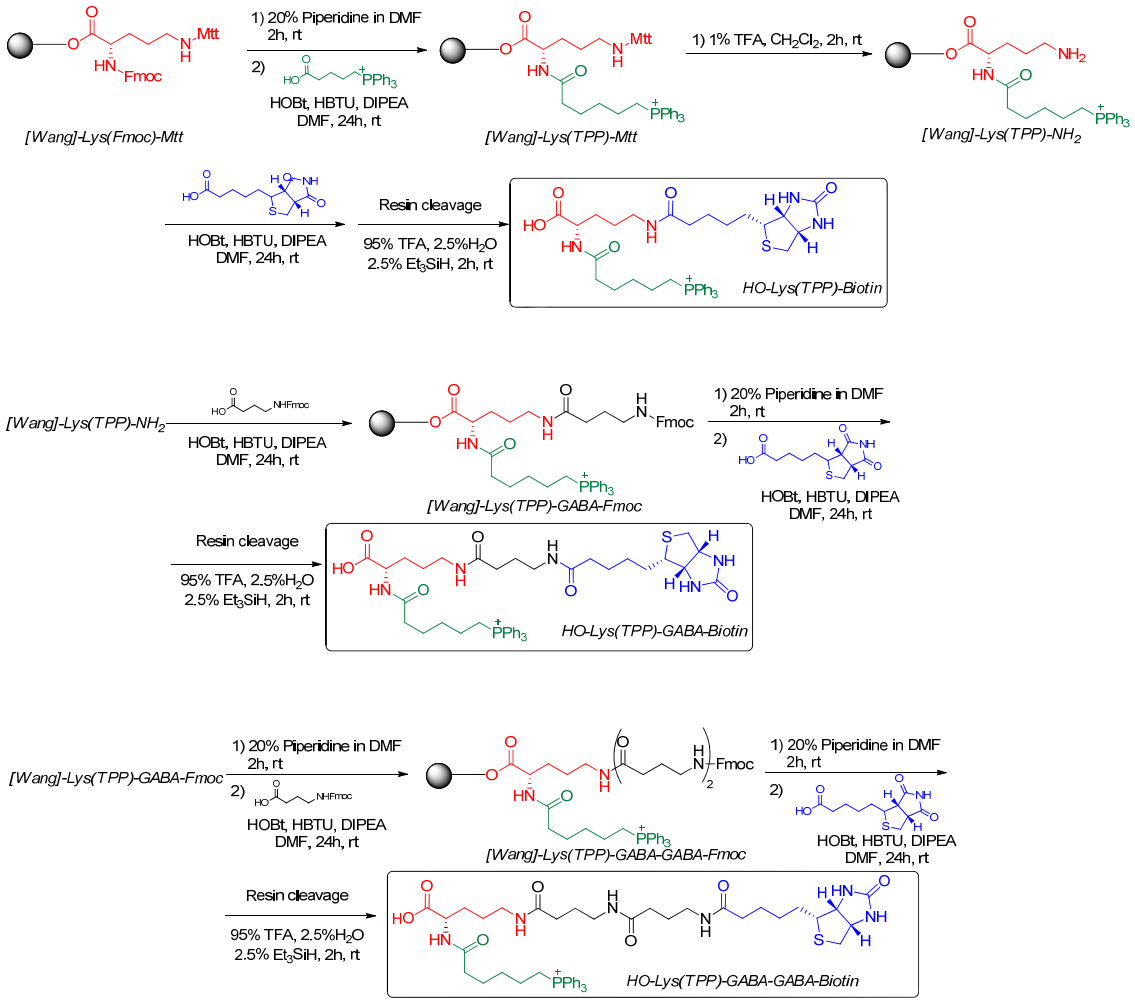
General scheme and procedures for solid phase synthesis of dual-targeted ligands.



The synthesis of the dual-targeted ligands was carried out employing well-known processes developed for solid phase. Particularly, these compounds were prepared employing the commercially available Wang Resin functionalized with an orthogonally protected Lysine: [Wang]-Lys(Fmoc)Mtt. From this starting material, upon applying the corresponding coupling, deprotection or cleavage protocol there were obtained the targeted fragments with acceptable yields and purities. The different synthetic procedures, described for 0.10 mmol resin scale, could be scaled up to 0.30 mmol without modifications on the materials or reaction conditions. The solid phase synthesis was accomplished in 10 to 30mL luer glass reactors able to fit in the Visiprep® solid phase sample processor in which where realized all the washing processes. All the different synthetic steps are carried out employing a rotatory shaker at room temperature for the indicated time and 0.10mmol [Wang]-Lys(Fmoc)Mtt scale; detailed reagents and conditions are specified below.

1) Fmoc deprotection: The corresponding Fmoc-functionalized resins are treated with a solution of piperidine (20%) in DMF (5mL) for 2h, to produce the protecting group cleavage. Then, the reaction medium is washed out from the solid resin, which is thoroughly washed with DMF (3x10mL) and taken to the next synthetic step.

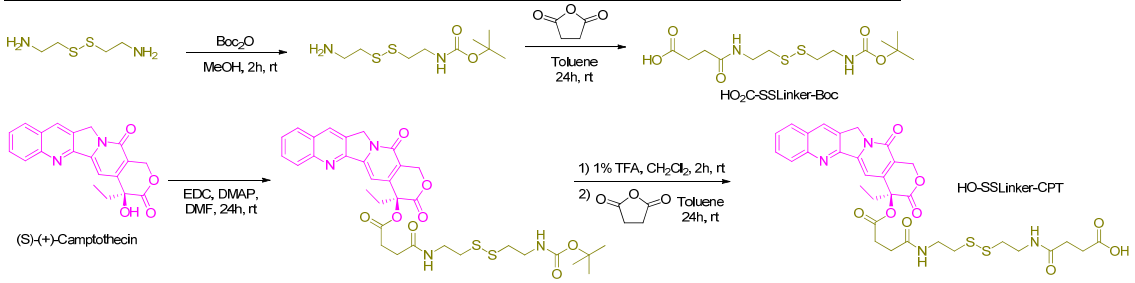
2) Boc and Mtt deprotection: The corresponding acid-cleavable protected resins are pre-treated with 3 washing cycles with CH_2Cl_2 to remove the coupling solvent (DMF) and then treated with a solution of TFA (1%) in CH_2Cl_2 (5mL) during 2h. Once the deprotection step is completed, the reaction medium is washed out from the solid resin, which is washed with DMF (4x10mL) and taken to the next step.

3) Coupling protocol with HOBt/HBTU: In an independent reaction vessel is prepared a mixture of the carboxylic acid to incorporate (0.20mmol, 2.0 equiv.), HOBt (40.5 mg, 0.30 mmol, 3 equiv.), HBTU (75.8 mg, 0.20 mmol, 2 equiv.) and DIPEA (105 µL, 0.60 mmol, 6 equiv.) in 5mL of DMF. This mixture is allowed to react for 10 minutes at RT and then is added into the reactor which contains the properly

conditioned free-amino resin to bind. The coupling reaction is maintained for a minimum of 24h at RT under rotatory shaker. When concluded, the reaction medium is filtered off and the resin loaded with the coupled compound thoroughly washed with DMF (4x10mL) before proceeding to the next step.

4) Peptide sequence cleavage and purification: The corresponding Wang-resin bounded sequence is cleaved employing 5mL of a TFA (95%), H_2O (2.5%) and Et_3SiH (2.5%) solution for 2h at RT. After this treatment, the corresponding cleavage which contains the final compound is collected and the resin washed with 2x10mL of CH_2Cl_2; these combined washing fractions are evaporated under vacuum to provide a crude oil. The prepared peptide sequence is recovered from the crude by dissolving it in the minimum amount of MeOH followed by precipitation with cold Et_2O, yielding the peptide as a white precipitate. The peptide is purified throughout a Sephadex-G 25 exclusion column employing water as mobile phase, obtaining the pure compound after lyophilization of the adequate fractions.

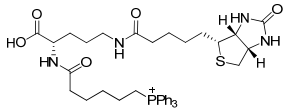
General scheme for the synthesis of Camptothecin substituted cleavable linker.



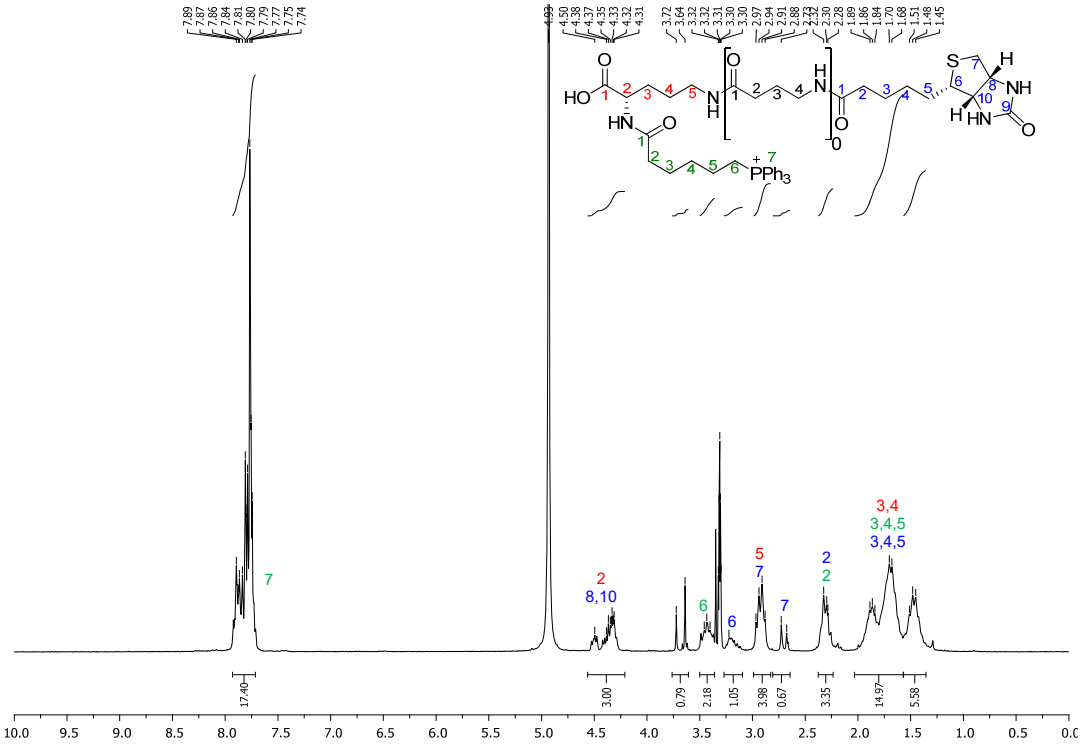
The synthesis of HO_2C-SS_{Linker}-Boc[1,2] was done as reported in the literature. The first step, the cystamine desymmetrization to obtain *N-tert*-Butoxycarbonyl)cystamine was accomplished following the reported protocol: Di-*tert*-butyldicarbonate (3.21 g, 14.7 mmol, 1 equiv.) and triethylamine (6.1 mL, 44 mmol, 3 equiv.) were added to a methanol solution (60 mL) of cystamine bis hydrochloride (3.31 g, 14.7 mmol, 1 equiv.). After 20 min the solvent was evaporated, and 1 M NaH_2PO_4 was added (10 mL, pH 4.2). The aqueous solution was extracted with ether to remove the di-Boc-cystamine. The aqueous solution was basified to pH 9 by 1 M NaOH and extracted with EtOAc (4 x 5 mL). The combined organic phases were dried over $MgSO_4$ and evaporated to yield the product (1.74 g, 46%) as a white solid which was employed on the next step without further purification. The reaction with the succinic anhydride was carried out as reported in the literature too. Briefly, from to a solution of *N-tert*-Butoxycarbonyl)cystamine (1.74g, 6.89 mmol) in 80 mL DCM, were added succinic anhydride (0.694 g, 6.89 mmol) and a catalytic amount of DMAP. This mixture was stirred for overnight and then washed with 100 mL of 1M $KHSO_4$, followed by a brine wash and drying over anhydrous $MgSO_4$. Then, the organic solvent was removed under vacuum to obtain final product as a white solid (2.06 g, 85%).

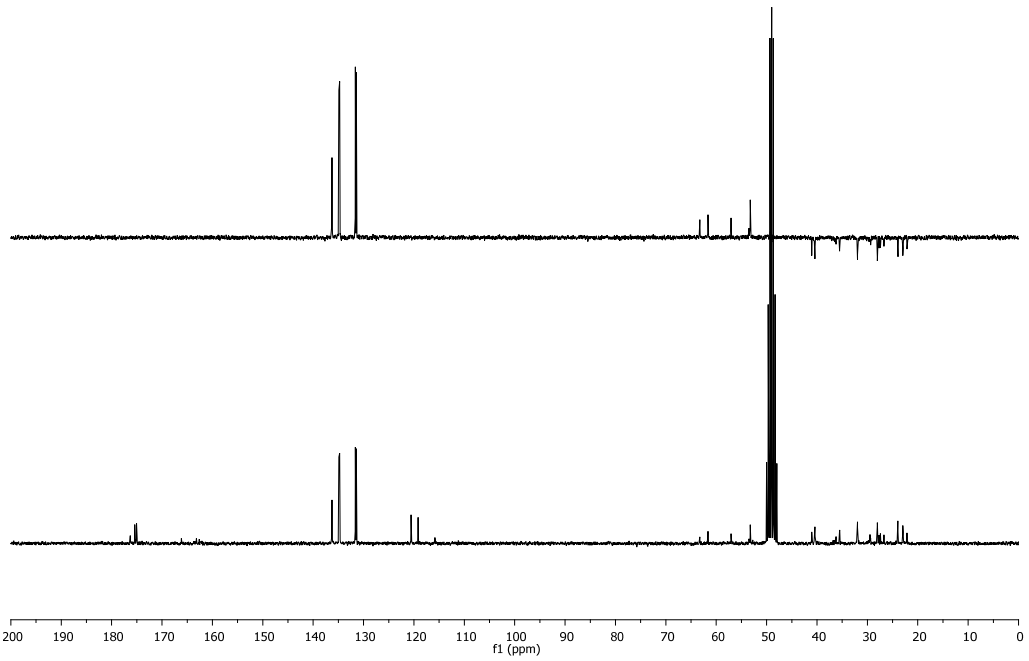
The reaction between the cleavable disulfide-containing linker and (*S*)-Camptothecin was done employing a methodology reported for a similar process. [3] The coupling reaction was carried out adding a solution of (*S*)-Camptothecin (80 mg, 0.23 mmol) in 2 mL DMF over a pre-reacted mixture of *N-tert*-Butoxycarbonyl)cystamine (85 mg, 0.24 mmol), DMAP (28 mg, 0.23 mmol) and EDC (72 mg, 0.24 mmol) in 5 mL DMF for 5 minutes. The resulting mixture was stirred at RT for 48h. When the coupling was completed, the reacting mixture was diluted with EtOAc, washed with NH_4Cl and brine and dried over $MgSO_4$. Purification of this step was carried out after evaporation of the solvent throughout a SiO_2 flash chromatography with $CHCl_3$-MeOH (95:5) as mobile phase. The desired *Boc-SSLinker-CPT* was obtained as a yellowish solid. For the deprotection and later transformation into the final acid-ended derivate there was followed a two-step one-pot process: First the *N*-Boc protected Camptothecin derivate (20 mg, 0.029 mmol) was deprotected employing a 1:1 TFA-CH_2Cl_2 mixture (1 mL) for 20 min at RT. The mixture was then evaporated to dryness to provide the intermediate amino free Camptothecin-linker which was used without further purification. For the next step the crude was reacted with a solution of succinic anhydride (2.9 mg, 0.029 mmol) in toluene (3 mL) for overnight. The reacted mixture was evaporated and the resulting crude purified by SiO_2 flash chromatography employing $CHCl_3$-EtOH (9:1) as mobile phase. The final acid ended Camptothecin linker (*HO-SSLinker-CPT*) was obtained as a yellow solid after evaporation and precipitation with Et_2O.

HO-Lys(TPP)-Biotin

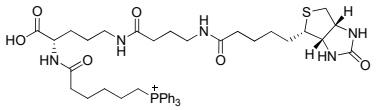


IR: 3271, 3059, 2931, 1666, 1538, 1438, 1176, 1127, 996, 831, 798, 720, 689, 529, 495, 434. ^{1}H NMR (250 MHz, MeOD) δ 7.97 – 7.65 (m, 15H), 4.55 – 4.25 (m, 3H), 3.68 (d, *J* = 21.0 Hz, 1H), 3.52 – 3.35 (m, 2H), 3.26 – 3.09 (m, 1H), 2.99 – 2.84 (m, 3H), 2.70 (d, *J* = 12.7 Hz, 1H), 2.39 – 2.23 (m, 3H), 1.98 – 1.57 (m, 12H), 1.56 – 1.35 (m, 4H). ^{13}C NMR (63 MHz, MeOD) δ 176.3, 175.4 (2C), 175.1, 136.3 (d, *J* = 2.9 Hz, 3C), 134.8 (d, *J* = 9.9 Hz, 6C), 131.5 (d, *J* = 12.6 Hz, 6C), 119.9 (d, *J* = 86.5 Hz, 3C), 63.3, 61.6, 57.1, 53.2, 41.1, 40.4, 36.2, 35.5, 32.0, 29.5, 28.0, 27.7, 27.4, 26.7, 24.0, 23.0, 22.1. HRMS (ESI, TOF/TOF): Calculated for $C_{39}H_{50}N_4O_5PS^+$: 717.3234 [M^+]; found 717.3207.

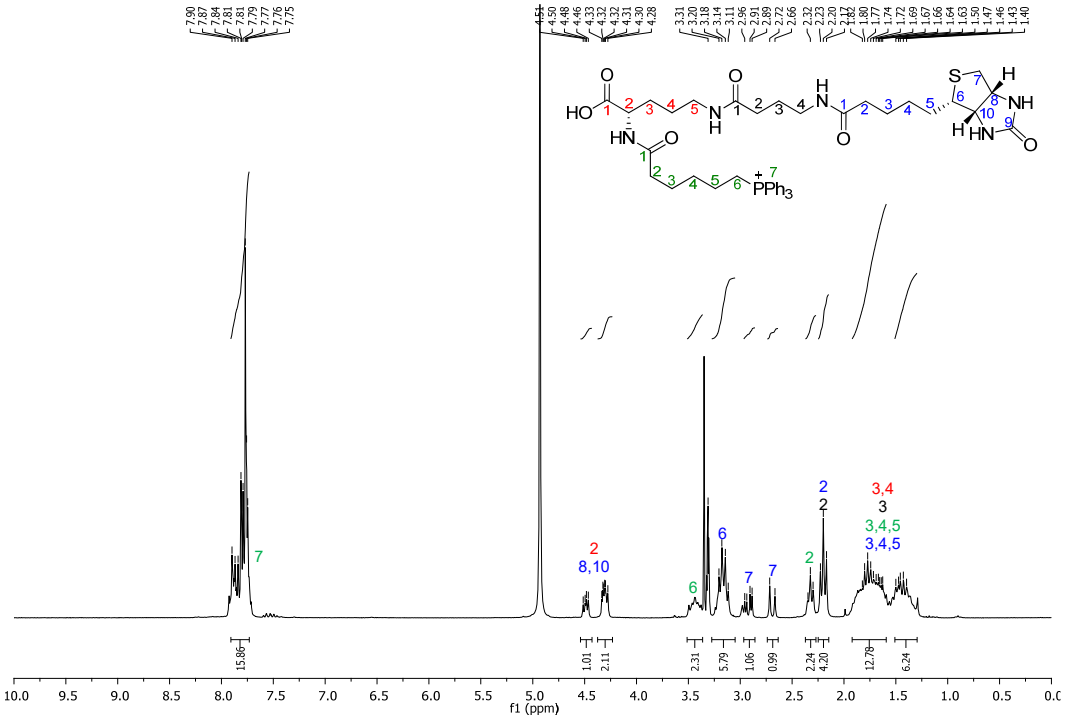


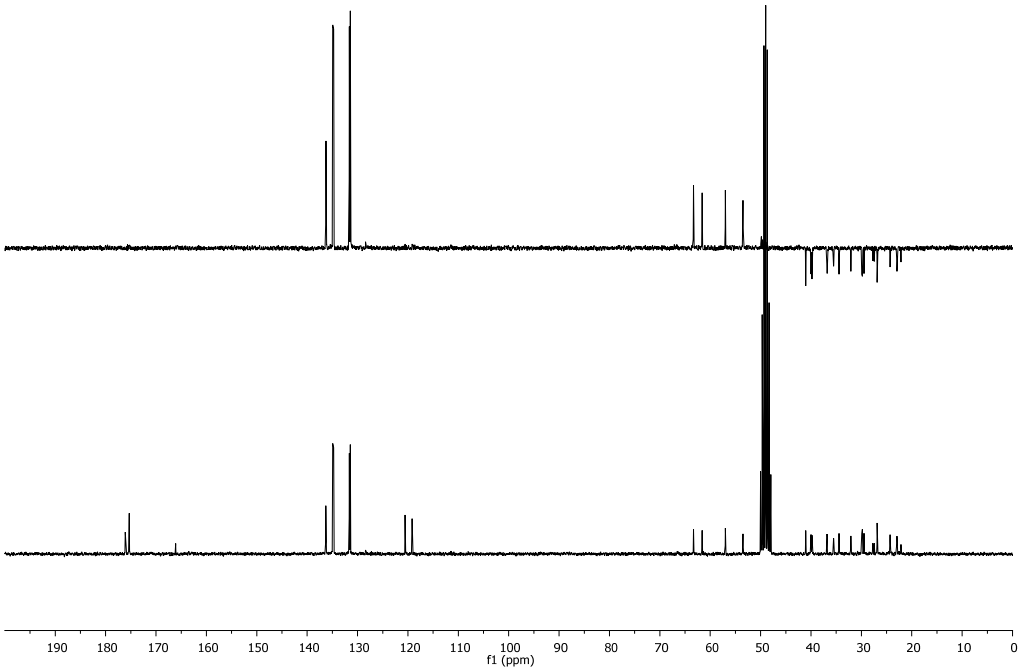


HO-Lys(TPP)-GABA-Biotin

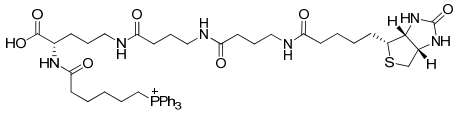


IR: 3271, 3059, 3064, 2929, 2862, 1637, 1541, 1437, 1175, 1111, 1026, 996, 827, 798, 720, 687, 528, 492. ^{1}H NMR (250 MHz, MeOD) δ 7.94 – 7.69 (m, 15H), 4.49 (dd, *J* = 7.8, 4.5 Hz, 1H), 4.37 – 4.24 (m, 2H), 3.51 – 3.36 (m, 2H), 3.24-3.08 (m, 5H), 2.92 (dd, *J* = 12.8, 4.9 Hz, 1H), 2.69 (d, *J* = 12.7 Hz, 1H), 2.32 (t, *J* = 6.3 Hz, 2H), 2.20 (t, *J* = 7.4 Hz, 4H), 1.94 – 1.56 (m, 12H), 1.55 – 1.26 (m, 6H). ^{13}C NMR (63 MHz, MeOD) δ 176.1, 175.4, 175.3 (2C), 166.1, 136.3 (d, *J* = 3.0 Hz, 3C), 134.8 (d, *J* = 10.0 Hz, 6C), 131.5 (d, *J* = 12.6 Hz, 6C), 119.9 (d, *J* = 86.4 Hz, 3C), 63.3, 61.6, 57.0, 53.5, 41.1, 40.0, 39.8, 36.8, 35.5, 34.4, 32.1, 29.9, 29.8, 29.5, 27.7, 27.5, 26.9, 24.3, 23.0, 22.1. HRMS (ESI, TOF/TOF): Calculated for $C_{43}H_{57}N_5O_6PS^+$: 808.9808 [M^+]; found 808.3803.

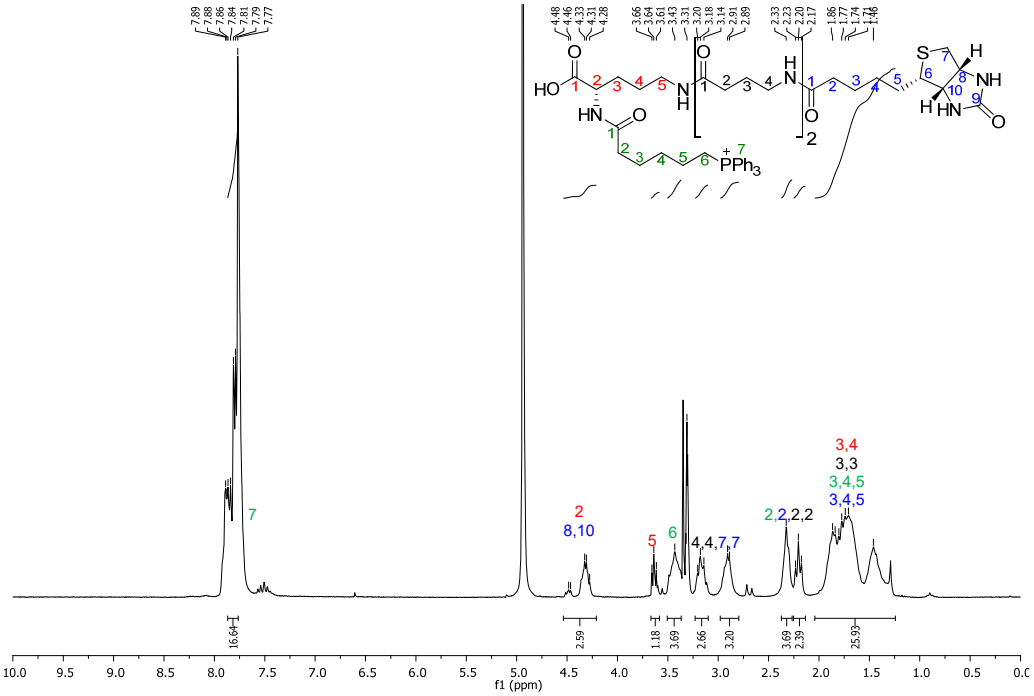


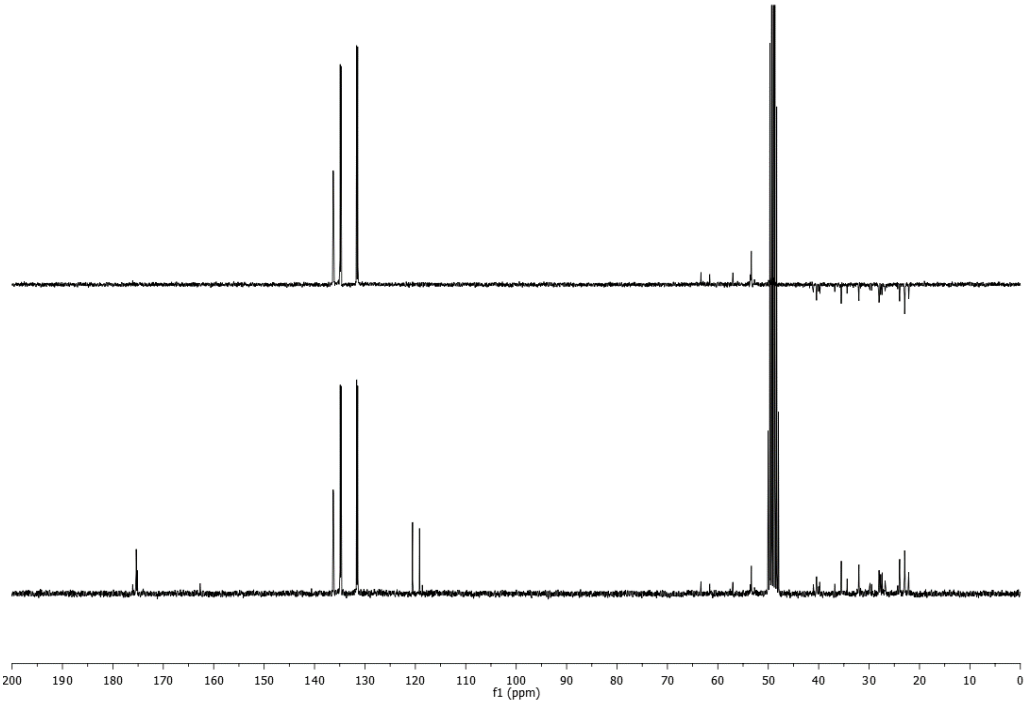


HO-Lys(TPP)-GABA-GABA-Biotin

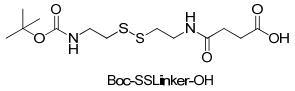


IR: 3265, 3059, 2930, 1644, 1540, 1438, 1173, 1111, 996, 827, 797, 718, 687, 528, 492. ^{1}H NMR (250 MHz, MeOD) δ 7.93 – 7.69 (m, 15H), 4.53 – 4.23 (m, 3H), 3.64 (dd, *J* = 6.2, 4.9 Hz, 1H), 3.52 – 3.36 (m, 3H), 3.22 – 3.10 (m, 2H), 2.99 – 2.81 (m, 3H), 2.38-2.26 (m, 3H), 2.20 (t, *J* = 7.3 Hz, 2H), 1.96 – 1.29 (m, 25H). ^{13}C NMR (63 MHz, MeOD) δ 176.1, 175.4, 175.3 (3C), 166.1, 136.3 (d, *J* = 3.0 Hz, 3C), 134.8 (d, *J* = 10.0 Hz, 6C), 131.5 (d, *J* = 12.6 Hz, 6C), 119.9 (d, *J* = 86.4 Hz, 3C), 63.3, 61.6, 57.0, 53.4, 41.1, 40.4, 40.0, 39.8, 36.8, 35.5, 34.4, 32.0, 29.8, 29.5, 28.0, 27.7, 27.4, 26.8 (2C), 23.9, 23.0, 22.9, 22.1. HRMS (ESI, TOF/TOF): Calculated for $C_{47}H_{64}N_6O_7PS^+$: 887.4289 [M^+]; found 887.4278.

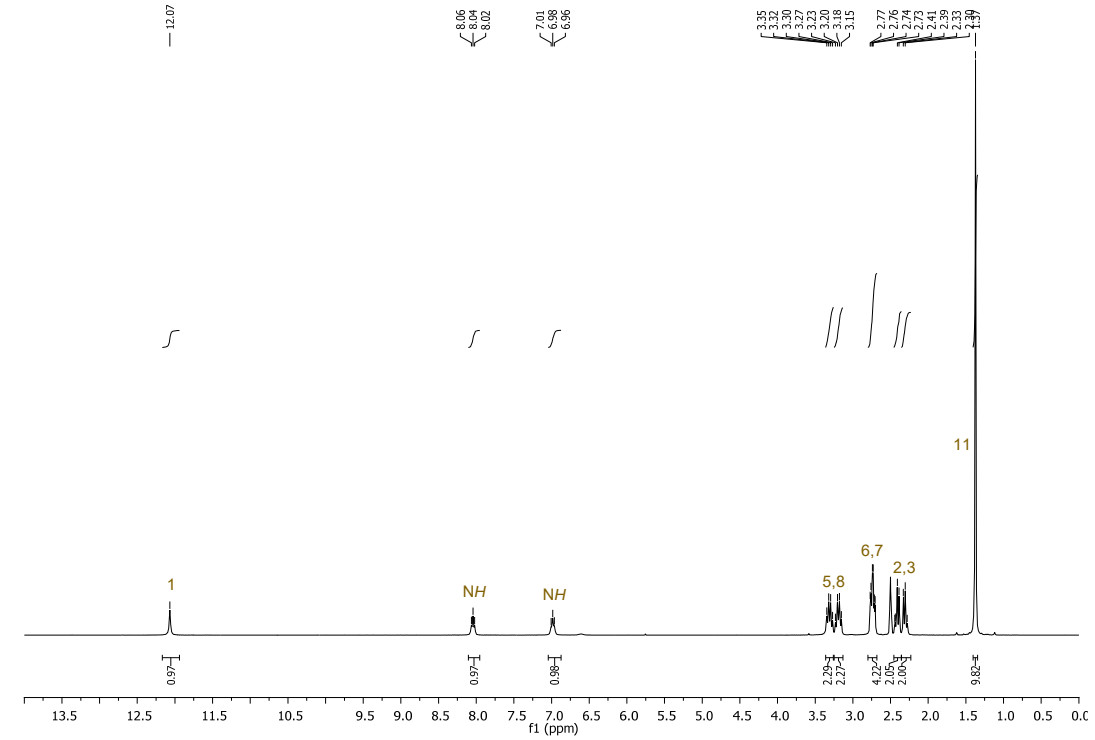


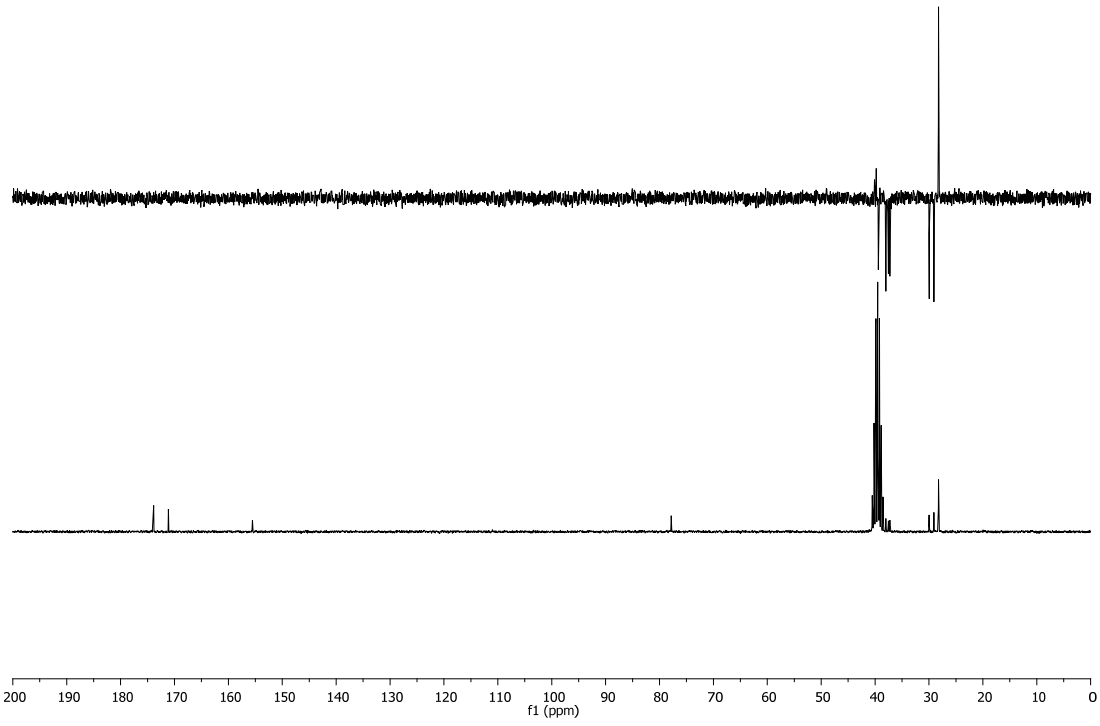


***Boc-SSLinker-OH*: 4-((6-((tert-butoxycarbonyl)amino)-3,4-dithiahexyl)amino)-4-oxobutanoic acid** [2]

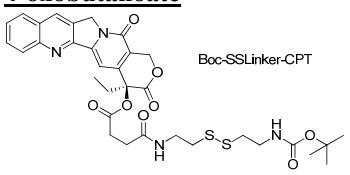


IR: 3354, 3335, 2977, 2937, 1686, 1650, 1524, 1425, 1364, 1273, 1251, 1165, 1040, 945, 872, 627, 584. ^{1}H NMR (250 MHz, DMSO-*d*6) δ 12.07 (s, 1H), 8.04 (t, *J* = 5.4 Hz, 1H), 6.98 (t, *J* = 5.3 Hz, 1H), 3.31 (dd, *J* = 12.7, 6.4 Hz, 2H), 3.19 (dd, *J* = 13.1, 6.3 Hz, 2H), 2.78-2.69 (m, 4H), 2.42 (dd, *J* = 10.5, 3.8 Hz, 2H), 2.30 (dd, *J* = 10.6, 3.9 Hz, 2H), 1.37 (s, 9H). ^{13}C NMR (63 MHz, DMSO-*d*6) δ 173.8, 171.1, 155.5, 77.8, 38.0, 37.5, 37.3, 30.0, 29.1, 28.2.

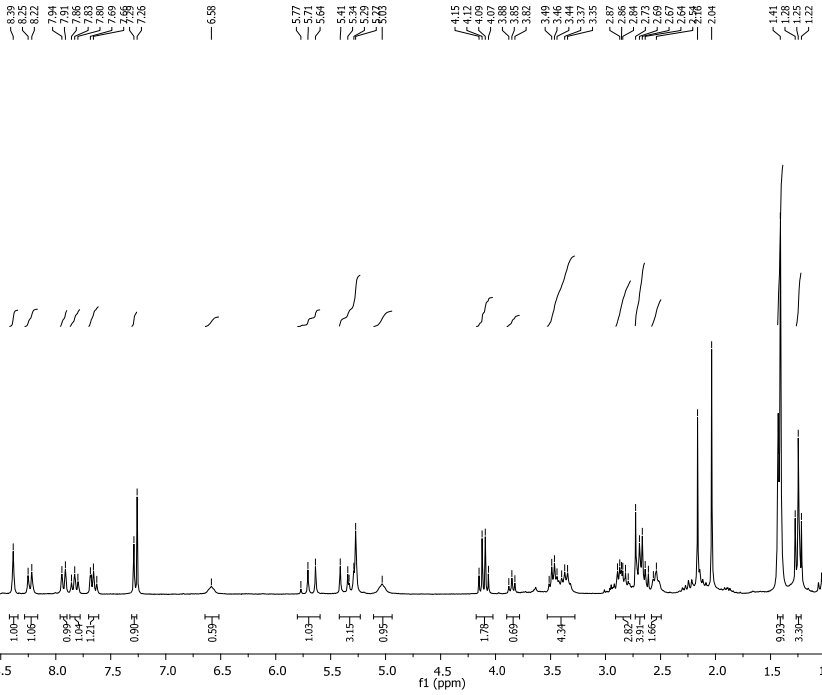


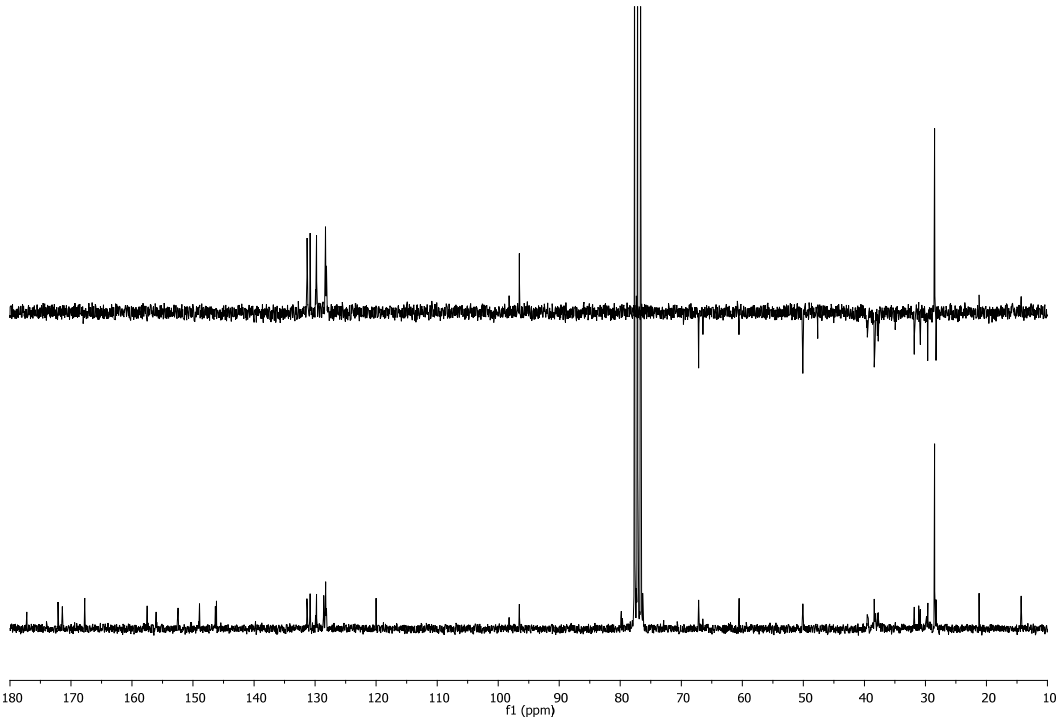


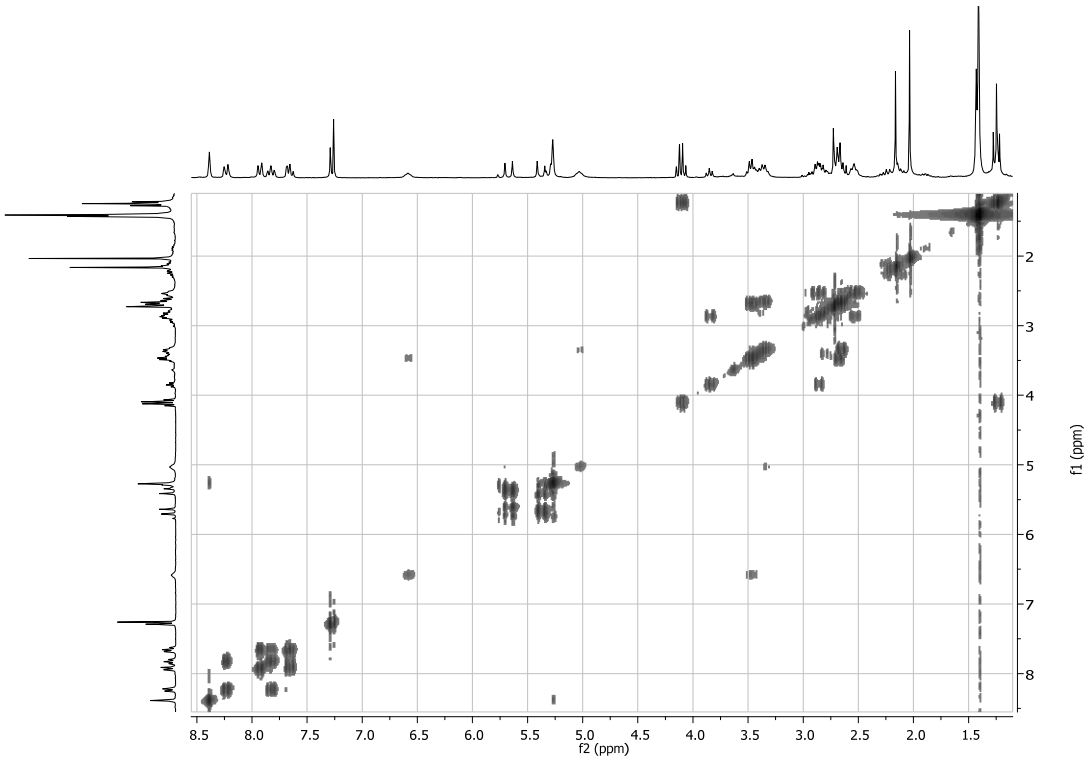
***Boc-SSLinker-CPT*: [4*O*-(*S*)-Capmtothecinyl]-4-((6-((*tert*-butoxycarbonyl)amino)-3,4-dithiahexyl) amino)-4-oxobutanoate**



IR: 3336, 2975, 2926, 1746, 1697, 1659, 1616, 1501, 1400, 1364, 1249, 1147, 1086, 1043, 945, 750, 723, 664, 590, 558, 428. ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.39 (s, 1H), 8.24 (d, *J* = 8.4 Hz, 1H), 7.93 (d, *J* = 8.0 Hz, 1H), 7.83 (t, *J* = 7.2 Hz, 1H), 7.66 (t, *J* = 7.3 Hz, 1H), 7.27 (d, *J* = 7.4 Hz, 2H), 6.58 (bs, 1H), 5.71 (t, *J* = 16.7 Hz, 1H), 5.44 – 5.22 (m, 3H), 5.03 (bs, 1H), 4.11 (q, *J* = 7.1 Hz, 2H), 3.85 (t, *J* = 6.9 Hz, 1H), 3.52 – 3.29 (m, 4H), 3.01 – 2.75 (m, 2H), 2.75 – 2.59 (m, 3H), 2.54 (bt, *J* = 6.8 Hz, 1H), 1.41 (s, 9H), 1.25 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (63 MHz, $CDCl_3$) δ 177.3, 172.1, 171.4, 167.8, 157.5, 156.1, 152.4, 148.9, 146.4, 146.1, 131.3, 130.8, 129.8, 128.6, 128.3, 128.2, 120.0, 96.5, 79.8, 67.2, 60.5, 50.1, 39.5, 38.4, 37.7, 31.8, 31.1, 30.8, 29.6, 28.5 (3C), 21.2. HRMS (ESI, TOF/TOF): Calculated for $C_{33}H_{38}N_4O_8S_2 \cdot H^+$: 682.2131 [M+H$^+$]; found 682.2193



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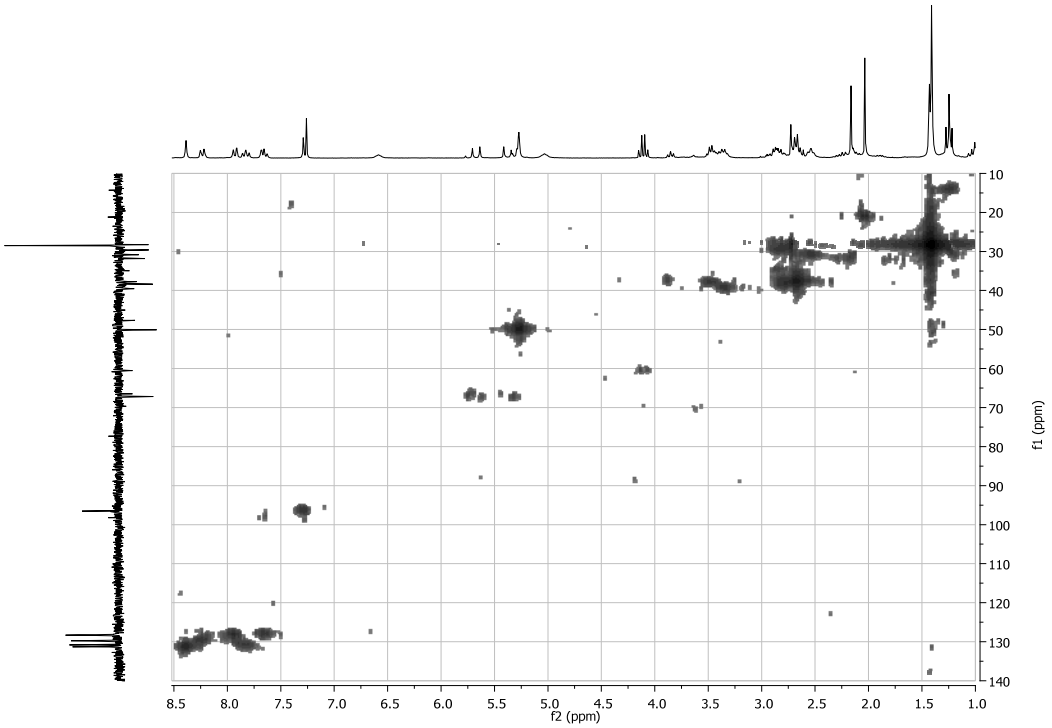
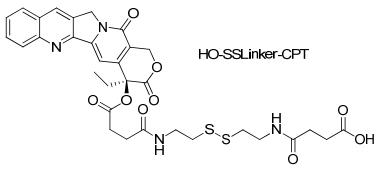
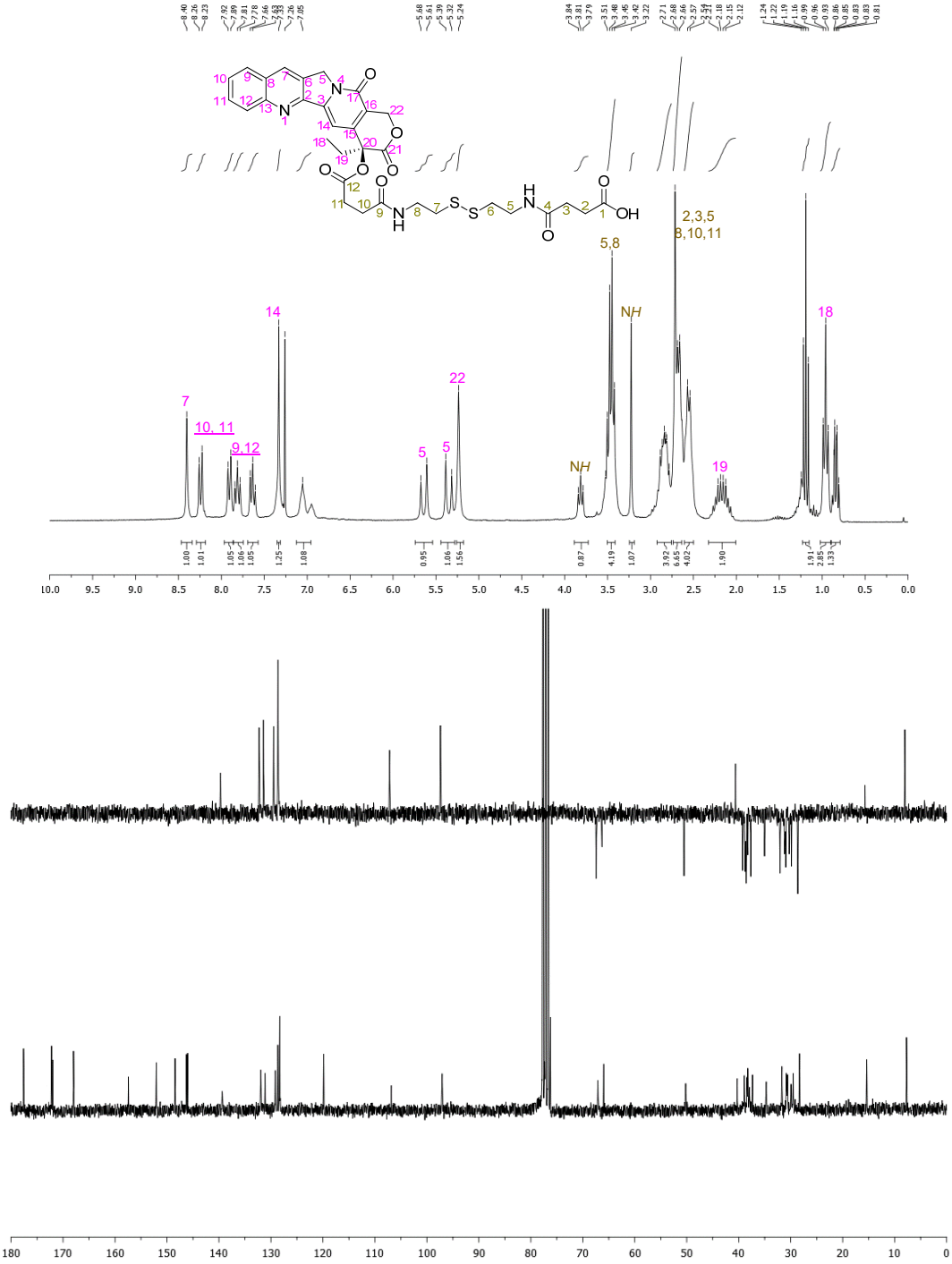
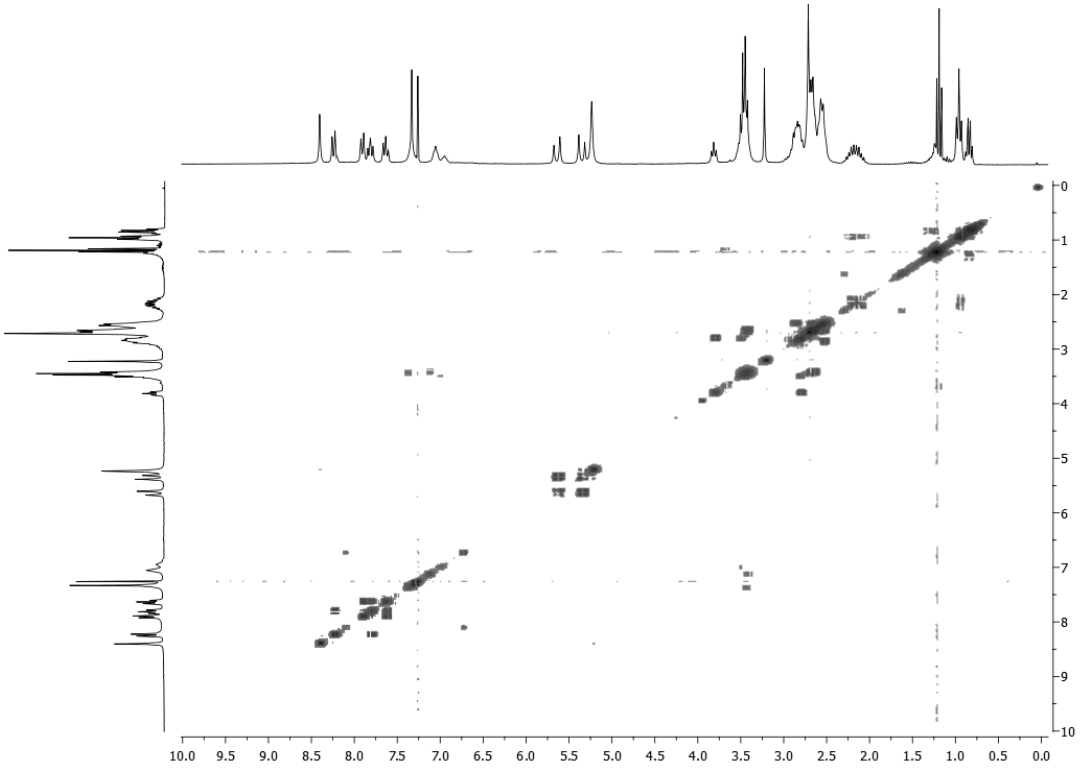

f1 (ppm)
f2 (ppm)

***HO-SSLinker-CPT*: [4*O*-(*S*)-Capmtothecinyl]-4-oxobutanamido)ethyl)disulfanyl)ethyl)amino)-4-oxobutanoic acid**



IR: 3294, 2921, 1737, 1698, 1650, 1599, 1555, 1400, 1228, 1147, 1085, 1052, 999, 814, 787, 747, 723, 665, 590, 406. ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.40 (s, 1H), 8.24 (d, *J* = 8.5 Hz, 1H), 7.91 (d, *J* = 8.1 Hz, 1H), 7.81 (t, *J* = 7.5 Hz, 1H), 7.63 (t, *J* = 7.3 Hz, 1H), 7.33 (br. s, 1H), 7.05 (br. s, 1H), 5.64 (d, *J* = 17.2 Hz, 1H), 5.35 (d, *J* = 17.2 Hz, 1H), 5.24 (s, 1H), 3.81 (t, *J* = 7.0 Hz, 1H), 3.49 (d, *J* = 9.6 Hz, 2H), 3.43 (d, *J* = 9.6 Hz, 2H), 3.22 (s, 1H), 2.92 – 2.75 (m, 3H), 2.73 – 2.63 (m, 4H), 2.60 – 2.50 (m, 3H), 2.17 (qd, *J* = 13.9, 7.3 Hz, 2H), 1.19 (t, *J* = 7.0 Hz, 2H), 0.96 (t, *J* = 7.3 Hz, 3H), 0.89 – 0.79 (m, 1H). ^{13}C NMR (63 MHz, $CDCl_3$) δ 177.6, 172.2, 172.0, 168.0, 157.4, 152.0, 148.4, 146.3, 146.0, 139.4, 131.9, 131.1, 129.2, 128.3, 128.3, 119.8, 106.9, 97.0, 76.2, 67.1, 50.2 40.3, 38.9, 38.4, 37.4, 34.7, 31.7, 30.6, 29.5, 28.3, 15.4, 7.7. HRMS (ESI, TOF/TOF): Calculated for $C_{32}H_{35}N_4O_9S_2 \cdot H^+$: 683.1845 [M+H$^+$]; found 683.1919

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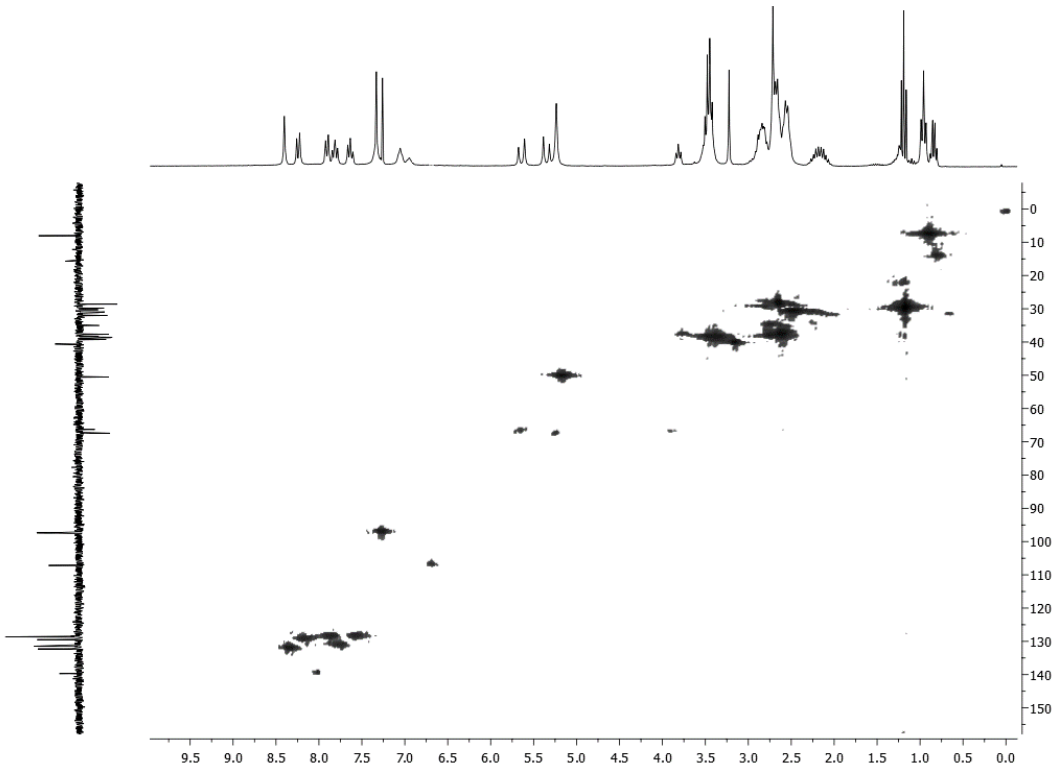
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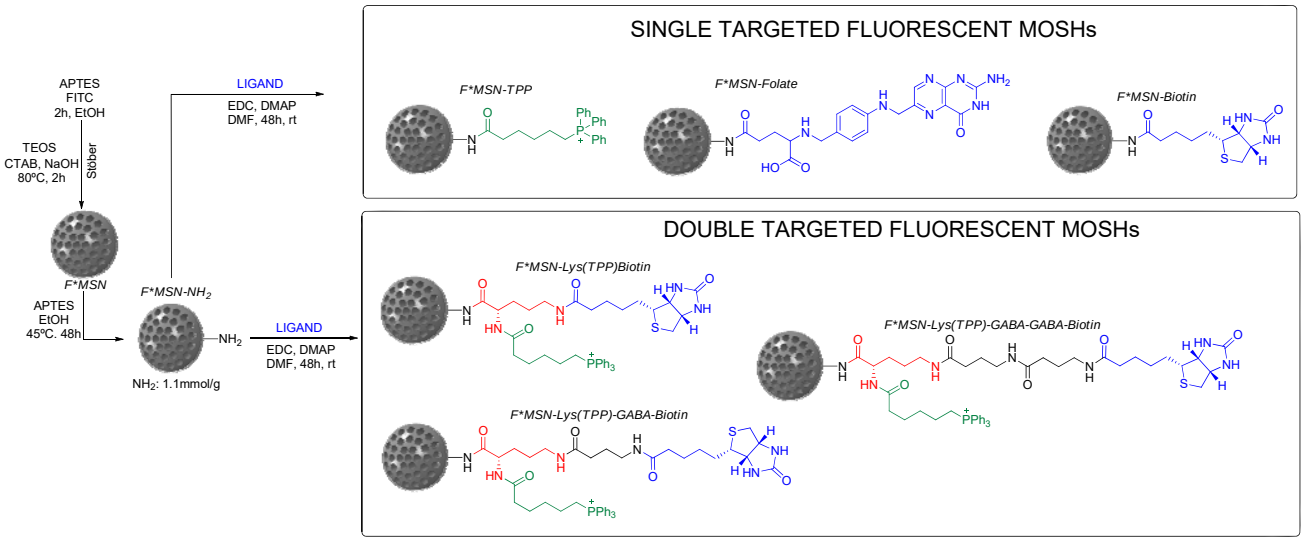
Synthetic scheme for the preparation of targeted Mesoporous Organosilica Hybrids (MOSHs).



Amide bonds formation onto MSNs were successfully accomplished employing the following described methodologies; which are adjusted for a 100 mg MSNs scale (5 mL of a 20 mg/mL DMF suspension, 1.1 mmol NH_2 per mg of *F*MSN-NH₂*).

1) Coupling protocol A: The corresponding carboxylic acid functionalized fragment (1.5 equiv., 0.17 mmol) was reacted with HOBt (3 equiv., 0.33 mmol, 44.6 mg), HBTU (2 equiv., 0.22 mmol, 83.4 mg) and DIPEA (4 equiv., 0.44 mmol, 77µL) in dry DMF (25 mL) for 10 minutes at RT in order to form the reactive intermediate species; then to this mixture was added the amino-functionalized MSNs in DMF suspension. The reaction was gently stirred with the aid of an orbital shaker for overnight, centrifuged as usually and washed repeatedly with DMF (3x25 mL) to remove reagents' excess, finally the obtained particles were dispersed back to 20 mg mL^{-1} with 5 mL DMF.

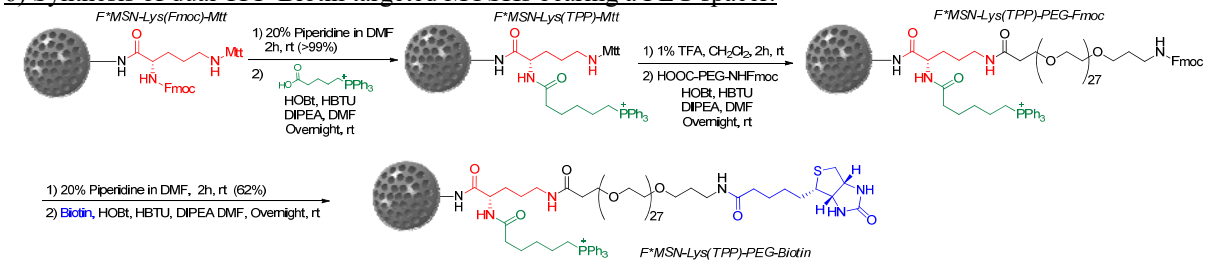
2) Coupling protocol B: The corresponding carboxylic acid functionalized ligand (1.5 equiv., 0.17 mmol) was reacted with EDC (2 equiv., 0.22 mmol, 42.2 mg) and DMAP (4 equiv., 0.44 mmol, 53.8 mg) in dry DMF (25 mL) for 10 minutes at RT, followed by the addition of MSNs in DMF. The reaction was stirred overnight, centrifuged as usually and washed with DMF and dispersed back to work concentration (20 mg mL^{-1}) with DMF.

3) Folic acid coupling protocol: MSNs were washed with 2x20 mL of DMSO and dispersed to a final volume of 20 mL. Meanwhile, Folic acid (1.5 equiv., 0.17 mmol, 66.2 mg), EDC (2 equiv., 0.22 mmol, 42.2 mg) and DMAP (4 equiv., 0.44 mmol, 53.8 mg) were dissolved in dry DMSO (5 mL), mixing up both mixtures when done. The reaction was stirred for 48h at 60ºC, washed with 3x10 mL DMSO and further processed as other analog MOSHs.

4) Mtt deprotection: For the deprotection of methyltrityl group, the dispersant was changed to CH_2Cl_2 with 2x20 mL cycles of centrifugation/dispersion to remove the DMF. Then the particles were dispersed in a mixture of TFA (1%) and CH_2Cl_2 (5 mL) and shaken for 2h. When completed, the particles were recovered by centrifugation, washed with DMF (2x20 mL) and dispersed back to work concentration.

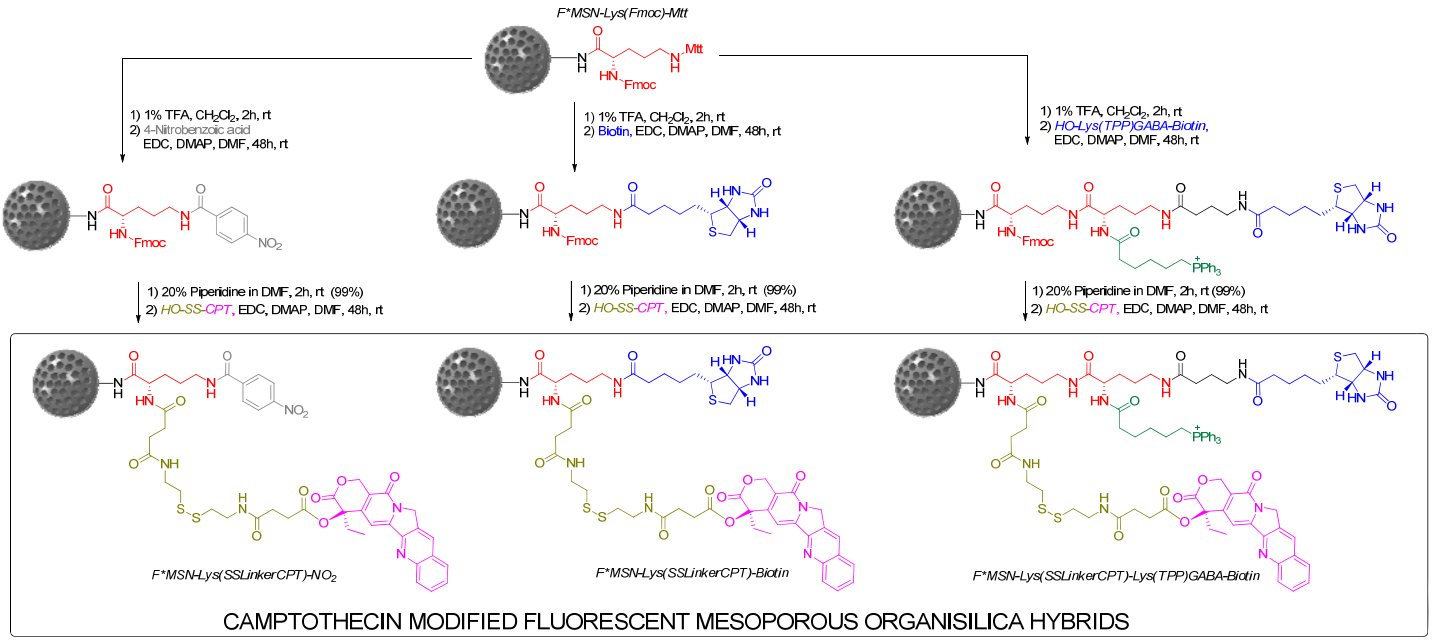
5) Fmoc deprotection: To cleave the Fmoc group, the corresponding particles were centrifuged and redispersed in a mixture of 20% piperidine in DMF (10 mL), maintaining the reaction for 2h. When completed, the particles were recovered by centrifugation, washed with DMF (2x20 mL) and dispersed back to work concentration. The fluorenyl rich reaction supernatants were recurrently analyzed by UV-Vis (301 nm) to determine the progress of the synthesis by comparison between the initial amount of NH_2 and the current NH-Fmoc groups incorporated.

6) Synthesis of dual TPP-Biotin targeted MOSHs bearing a PEG spacer:



In this case, because of the harsh cleaving conditions employed for solid-phase synthesis of dual targeted ligands, the preparation of the PEG-spaced dual targeted MOSHs was achieved directly from the starting *F*MSN-NH$_2$* material in a four-step couplings and deprotection. Briefly, 100 mg of starting material in working concentration were first coupled with *HO-Lys(Fmoc)Mtt* protected amino acid under conditions A, followed by Fmoc deprotection. Then, TPP was coupled to the cleaved peptidic amino group employing again conditions A. The cleavage of the side-chain amino group with Mtt deprotection protocol allowed the incorporation of *HO-PEG$_{27mer}$-NHFmoc* throughout coupling conditions A during 48h. Finally, after Fmoc deprotection and quantification (62% PEG substitution), Biotin was incorporated to the final free NH_2 groups employing coupling protocol B. The resulting PEG containing MOSHs were isolated by centrifugation (14000 rpm, 20 min) and washed with DMF (2x20 mL) and ethanol (2x20 mL) to provide the PEG containing dual targeting MOSHs which were stored refrigerated at work concentration (20 mg/mL) in EtOH.

Synthesis of Camptothecin-modified targeted MOSHs



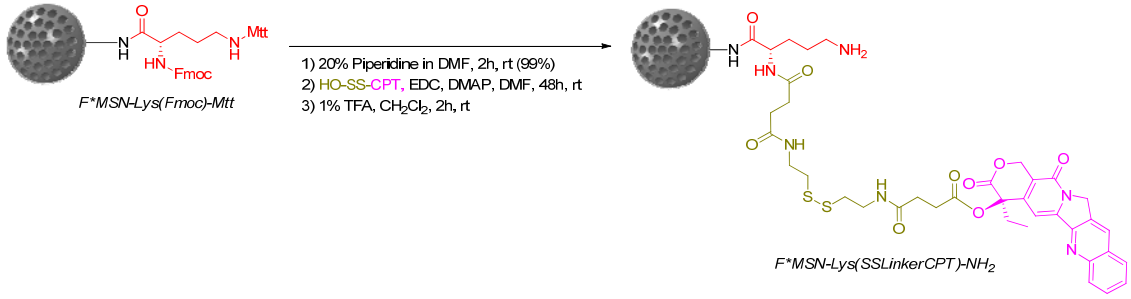
1) Mtt deprotection protocol: To cleave the Mtt group the starting *F*MSN-Lys(Fmoc)Mtt* particles were treated with a mixture of 1% CF_3CO_2H in CH_2Cl_2 (5 mL), maintaining the reaction for 2h. When completed, the MOSHs were recovered by centrifugation, washed with CH_2Cl_2 (2x20 mL) and DMF (2x20 mL) and dispersed to work concentration (20 mg mL^{-1}) with DMF.

2) Ligand Coupling protocol: Then, the carboxylic acid containing ligand (1.5 equiv., 0.17 mmol) was reacted with EDC (2 equiv., 0.22 mmol, 42.2 mg) and DMAP (4 equiv., 0.44 mmol, 53.8 mg) in dry DMF (25 mL) for 10 minutes at RT, followed by the addition of *F*MSN-Lys(Fmoc)NH$_2$* in DMF prepared in step 1. The reaction was stirred overnight, centrifuged as usually and washed with DMF (4x10mL) and dispersed back to work concentration (20 mg mL^{-1}) with DMF.

3) Fmoc deprotection: To cleave the Fmoc group the corresponding MOSHs were redispersed in a mixture of 20% piperidine in DMF (10 mL), maintaining the reaction for 2h. When completed, the particles were recovered by centrifugation, washed with DMF (2x20 mL) and dispersed back to work concentration.

4) *CPT-SSLinker-OH* coupling protocol: The CPT containing linker (1.5 equiv., 0.17 mmol) was reacted with EDC (2 equiv., 0.22 mmol, 42.2 mg) and DMAP (4 equiv., 0.44 mmol, 53.8 mg) in dry DMF (25 mL) for 10 minutes at RT, followed by the addition of the corresponding MOSHs in DMF. The reaction was stirred for 72h, centrifuged as usually and washed with DMF (2x10mL), EtOH (2x10mL) and Et_2O (1x10mL) and dried under vacuum at low temperature.

5) Preparation of *F*MSN-Lys(SSLinkerCPT)NH$_2$*:



The untargeted MOSHs were prepared employing the previously described protocols. Briefly, the starting *F*MSN-Lys(Fmoc)Mtt* particles were submitted to Fmoc deprotection (3) step, followed by *CPT-SSLinker-OH* coupling (4) and ending with Mtt deprotection protocol (1). The so prepared MOSHs were dispersed back to work concentration (20 mg mL^{-1}) with EtOH and stored refrigerated.

Quantification of NH_2 groups on MSNs

The determination of the progress of the reaction as well as the amount of reagents needed for following steps could be adjusted by measuring the amount of Fmoc released on each deprotection step (UV-Vis absorbance at 301 nm) with the aid of a previous calibration. Briefly, the determination of incorporated fragments (amino groups) onto MSNs was by washing out all reagents after reaction completion by repetitive cycles of centrifugation and redispersion in DMF. Once ensured all reagents and byproducts were removed, the particles were dispersed in 5 mL of 20% piperidine in DMF and shaked for 2h. When finished, the particles were collected by centrifugation and used for the next step after two more washing cycles, while the Fmoc absorbance of the supernatant was recorded. The determined yields for different reaction steps are noted on the *Synthetic scheme for the preparation of targeted MSNs* between brackets. Amino quantification for prepared amino-capped fluorescent MSNs is of 1.1 mmol g^{-1}

Porous properties of prepared MSNs/MOSHs.

Pore width and volume of the prepared fluorescent amino-capped MSNs (red curve, Figure S1) showed a typical N_2 adsorption isotherm with a type IV isotherms and pore widths of about 2.4 nm according to the Brunnauer-Emmet-Teller (BET) approach. The ordered structure of these particles prepared fluorescent amino-capped MSNs was confirmed throughout small angle X-rays diffraction, which showed the typical pattern for a MCM-41 porous silica (red curve, Figure S2).

The functionalization of those amino-capped fluorescent MSNs was done exclusively throughout amide bonds, which ensured the integrity of mesopores. This is demonstrated when the graphics for N_2 adsorption isotherm and SAXS of raw MSNs and the heavily modified *Lys(SSLinker-CPT)-Lys(TPP)GABA-Biotin* particles are compared (blue lines, Figures S1 and S2). In both cases, independently of the extensive surface modification applied onto MSNs to obtain the dual targeted-drug hybrids, SAXS and BET show unaltered patterns. Those values were of importance because ensured the possibility of hosting doxorubicin for dual therapy purposes.

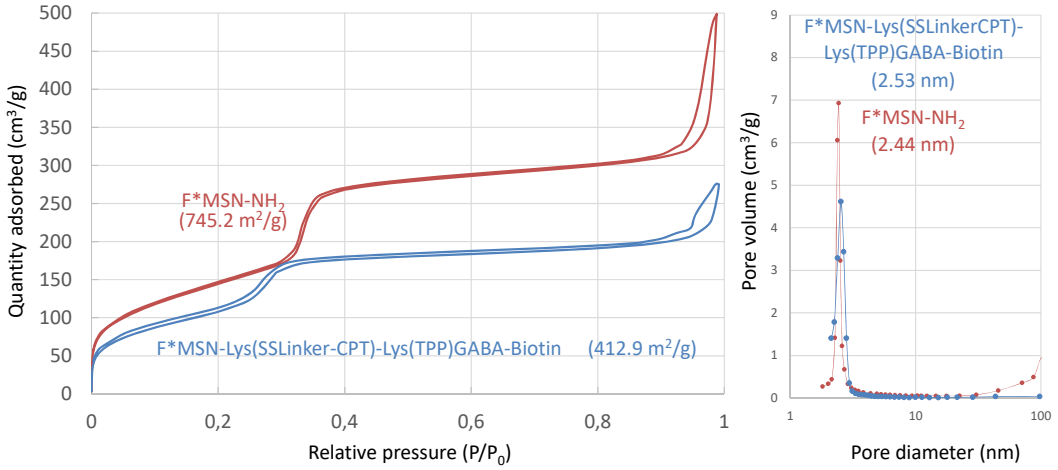


Figure S1: Comparative N_2 adsorption isotherms (BET) of prepared *F*MSN-NH₂* (in red) and their final targeting-drug containing Mesoporous Organo-Silica Hybrids (MOSHs, *F*MSN-Lys(SSLinker-CPT)-Lys(TPP)GABA-Biotin*, in blue). In brackets are noted the values for surface area obtained according to BET calculations.

Small-angle X-ray scattering prepared MSNs/MOSHs.

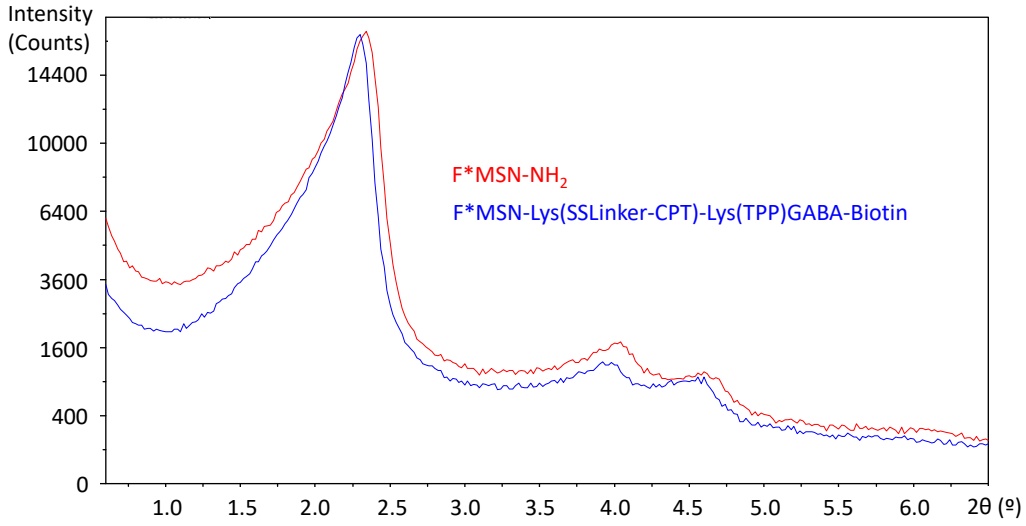


Figure S2: SAXS patterns of the fluorescent aminopropyl-modified MCM-41 mesoporous silica nanoparticles (*F*MSN-NH₂*, red) and the modified MOSHs (*F*MSN-Lys(SSLinker-CPT)-Lys(TPP)GABA-Biotin*, blue) employed on the present work. Measures were recorded from θ to 2θ in a diffractometer fitted with a monochromator and a collimator on the diffracted beam. 2θ = 0.6 to 6.5º, step size= 0.02º, time per step =5s, Cu K_α = 1.54Å.

Hydrodynamic size, surface potential and morphology of prepared MOSHs.

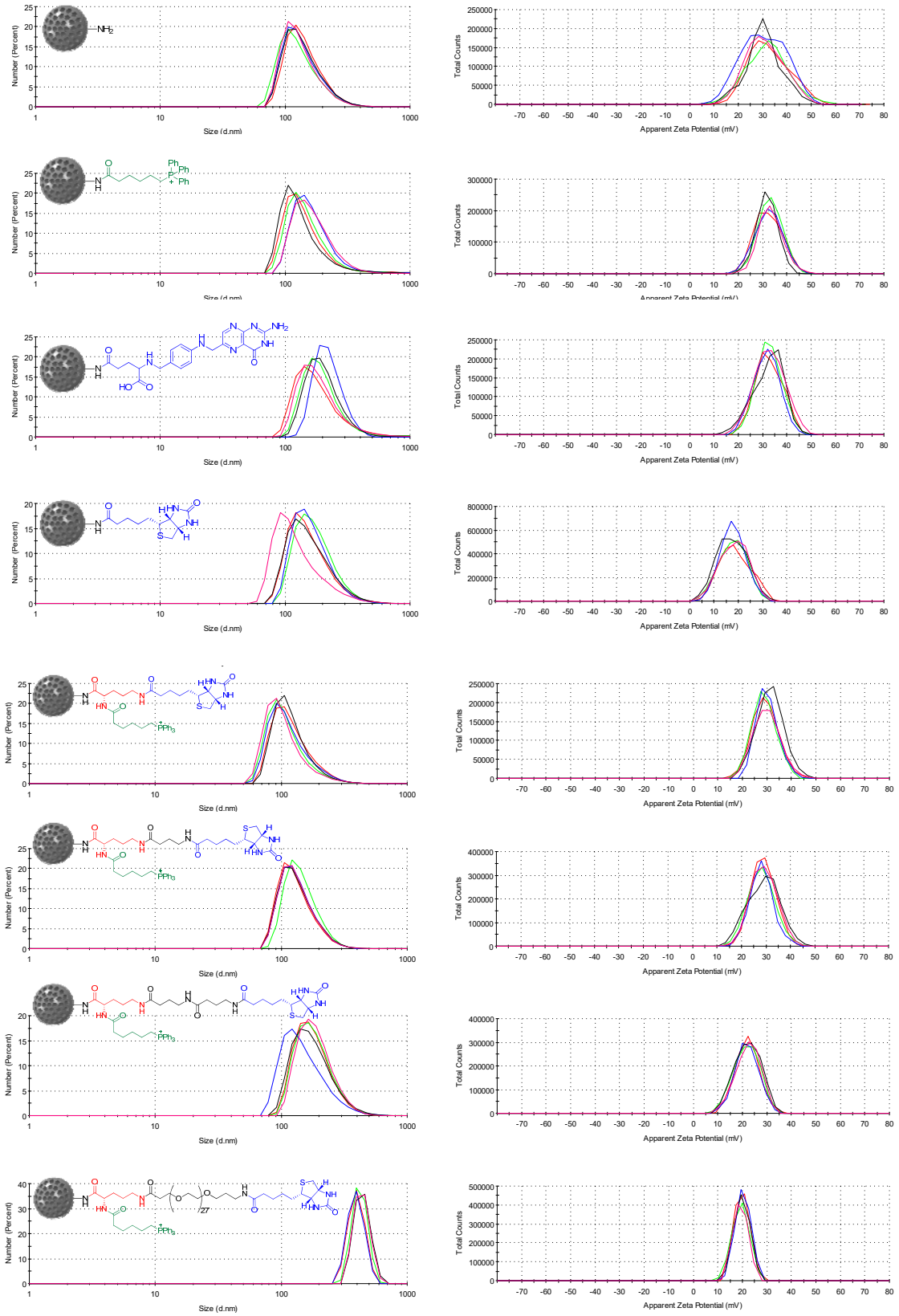


Figure S3: DLS and z-potential analysis of targeted MOSHs studied. Showed curves correspond to 5 sequential but independent records performed in distilled water.

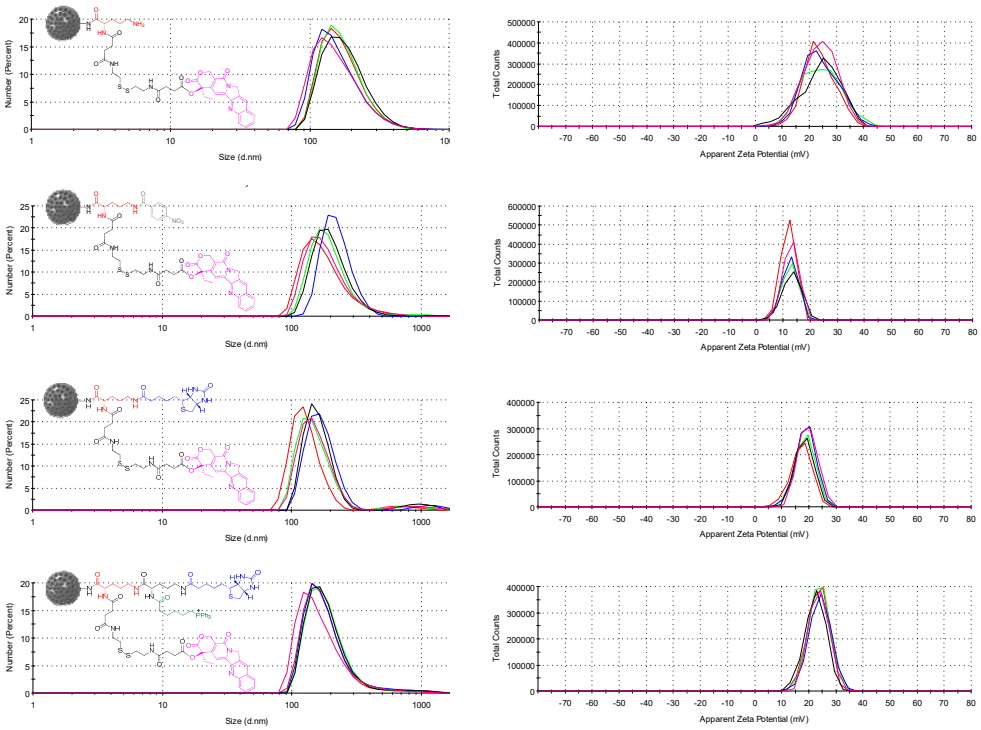


Figure S4: DLS and z-potential analysis of Camptothecin-modified targeted MSNs tested. Showed curves correspond to 5 sequential but independent records performed in distilled water.

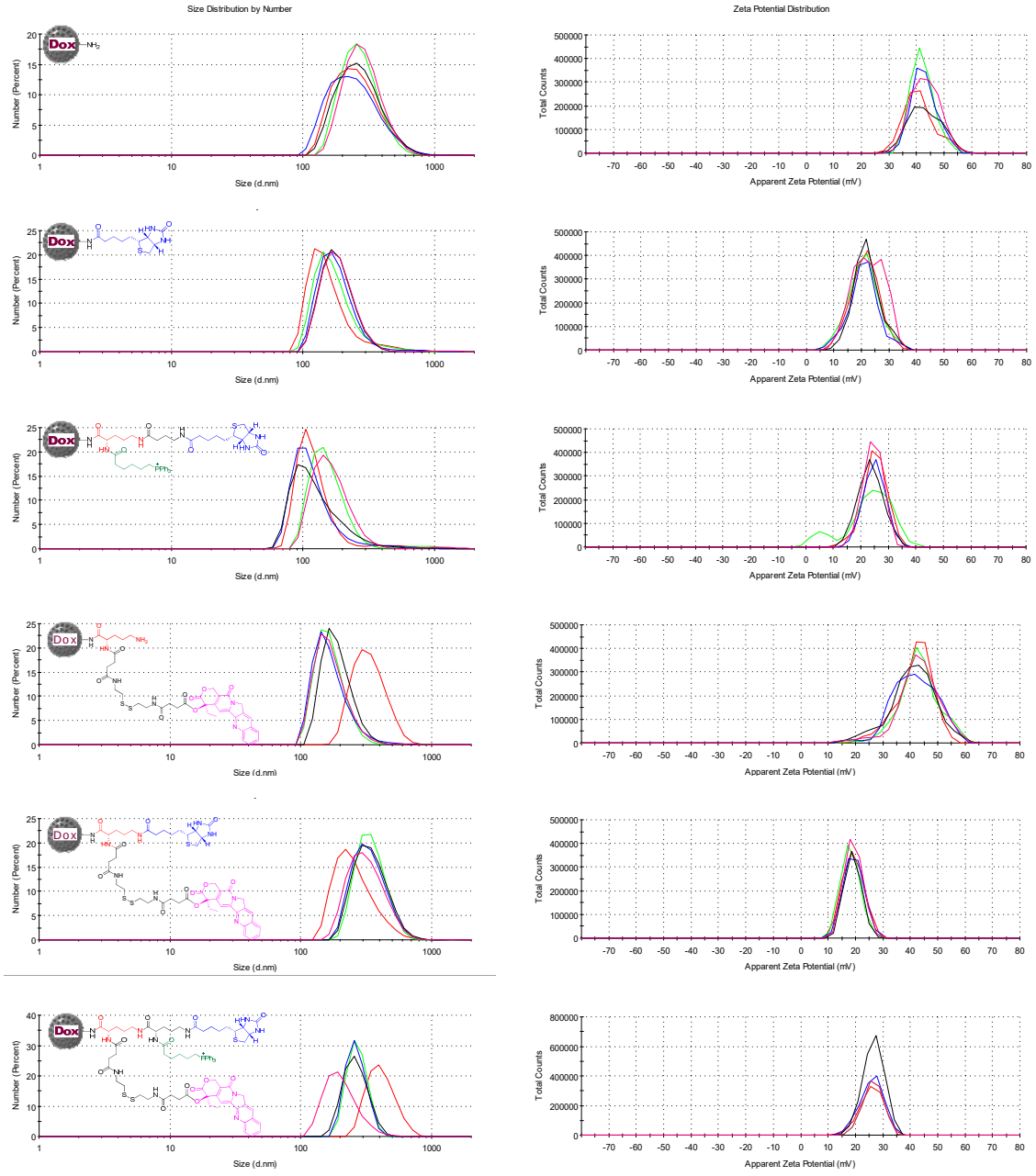


Figure S5: DLS and z-potential analysis of Doxorubicin-loaded MOSHs tested. Showed curves correspond to 5 sequential but independent records performed in distilled water.

Transmission Electron microscopy images of prepared MOSHs

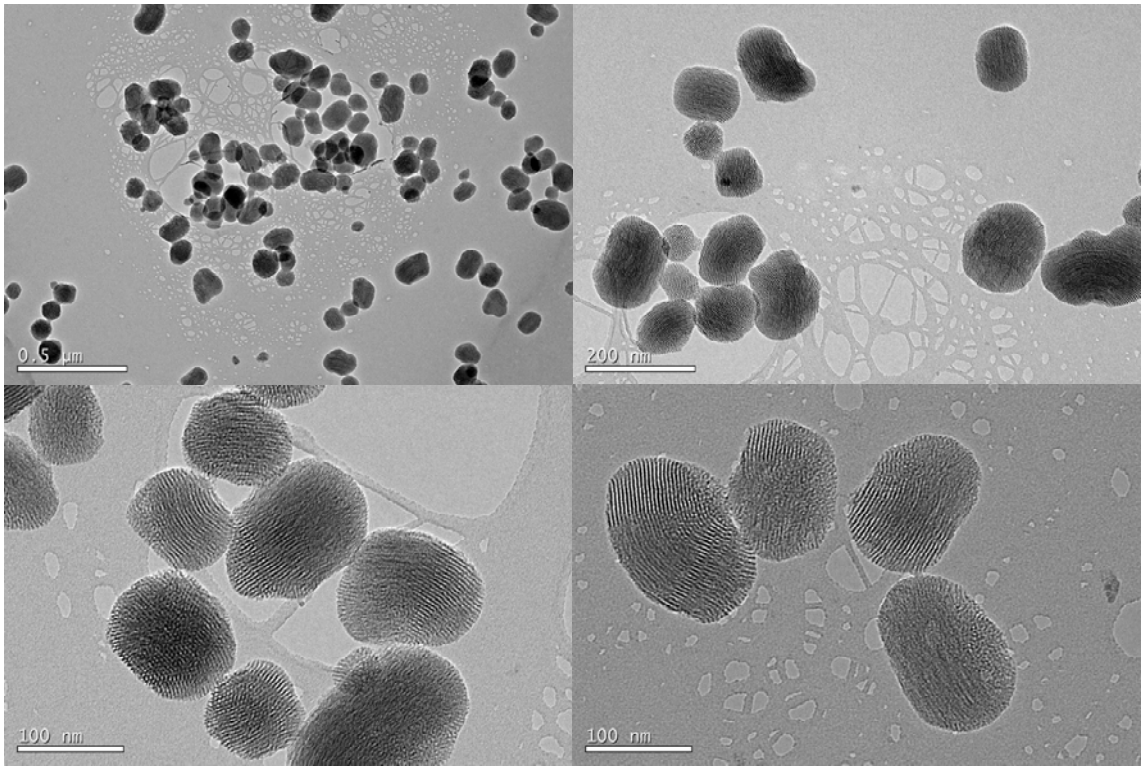


Figure S6: TEM micrographs of the prepared fluorescent aminopropyl-functionalized mesoporous silica nanoparticles (*F*MSN-NH_2*) employed for the present work.

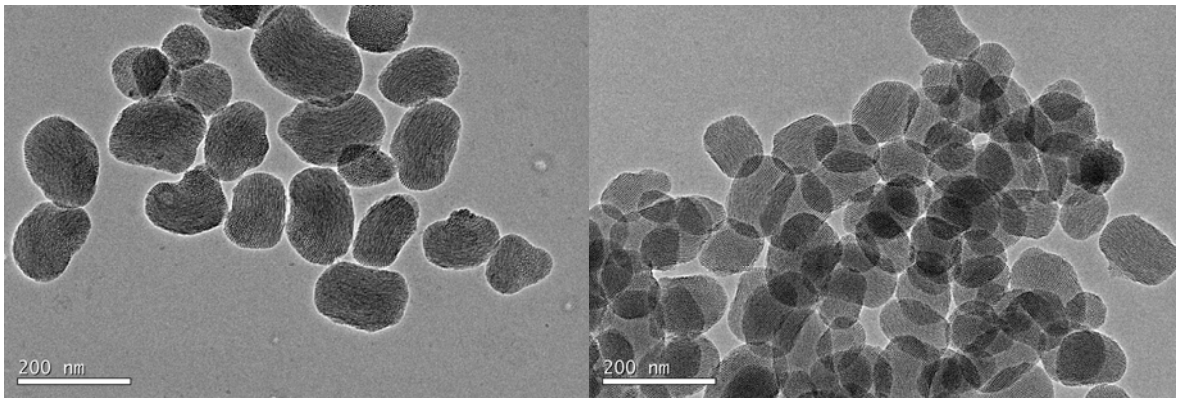


Figure S7: TEM micrographs of the prepared *F*MSN-Lys(SSLinker-CPT)-Lys(TPP)GABA-Biotin*.

IR spectra of prepared MOSHs.

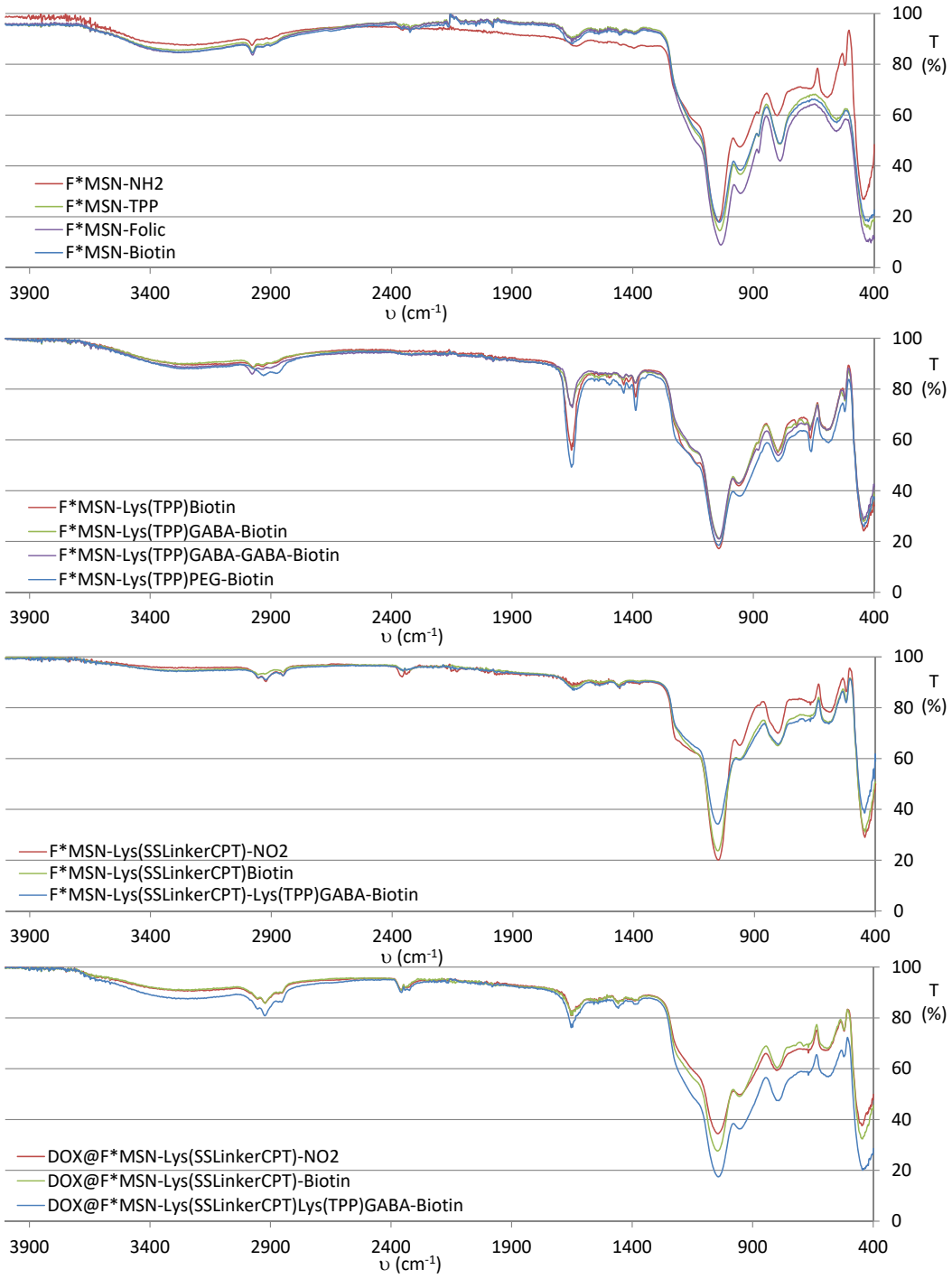


Figure S8: Comparative infrared spectra grouped by similarity (from up to down: mono-targeted, dual-targeted, camptothecin-functionalized and doxorubicin-loaded camptothecin-functionalized MOSHs) of representative modifications performed onto MSNs.

Thermogravimetric analysis of prepared MOSHs.

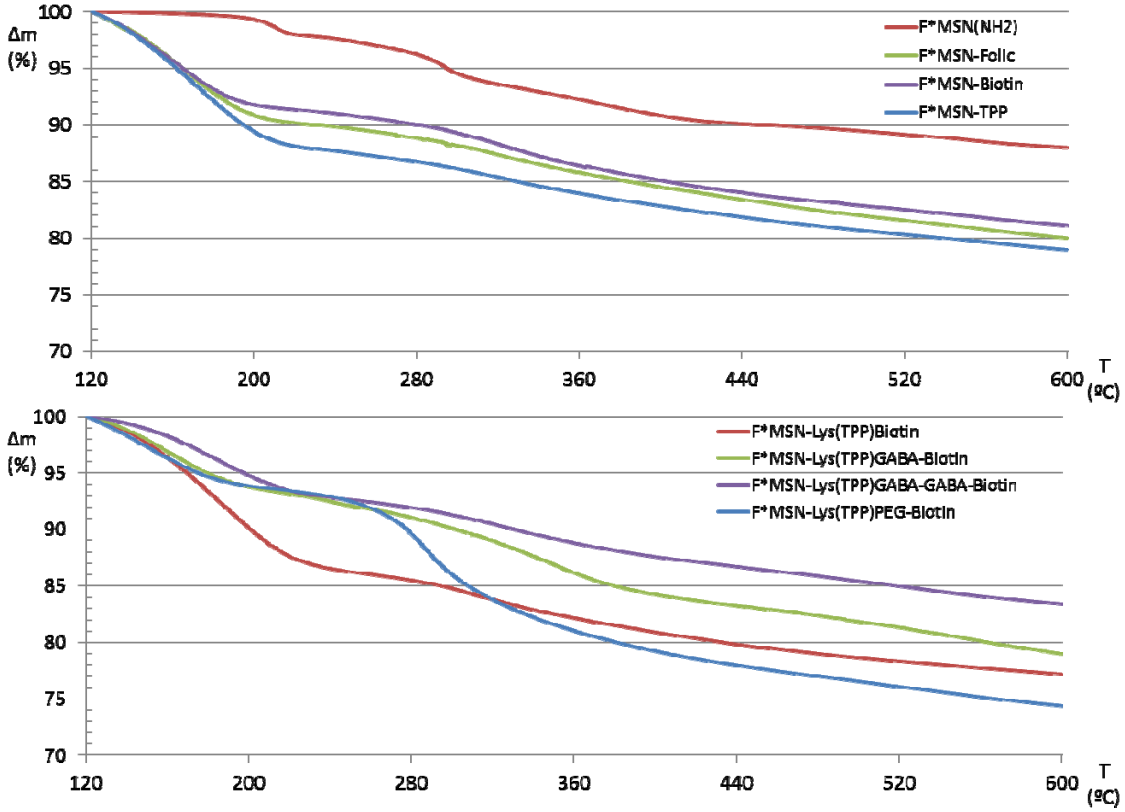


Figure S9: Thermogravimetric analysis of the different targeted MSNs prepared. The upper graphic compares the raw -NH_2 capped particles (mass loss ≈ 12 %) with the single targeted MSNs (TPP: 21%, Folate: 20 %, Biotin: 19 %). On the lower graphic are compared the different spacers employed to separate TPP and Biotin on double targeted MOSHs (no separation: 23 %, mono-GABA: 21 %, bis-GABA: 17 %, PEG: 26 %).

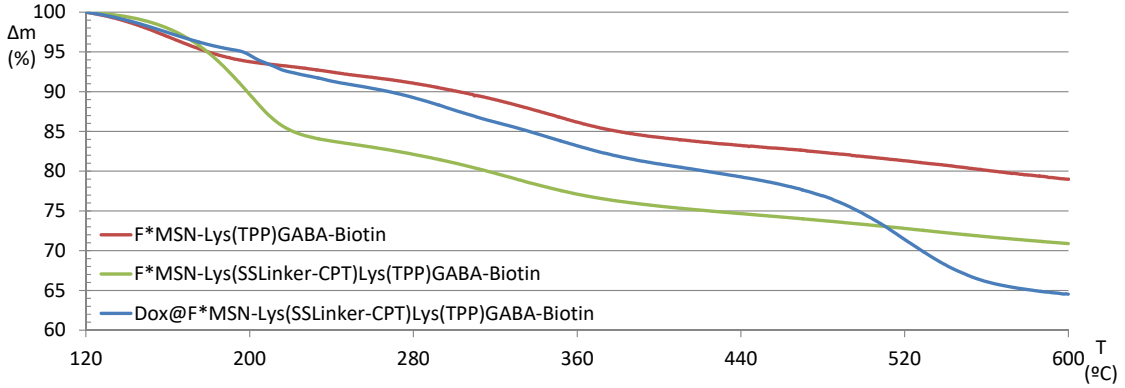


Figure S10: Comparison between the different stages of drug loading for the optimal targeted MOSHs. There are extrapolated amounts of 8 and 6 % in weight for modified camptothecin and doxorubicin respectively.

Additional *in vitro* experiments

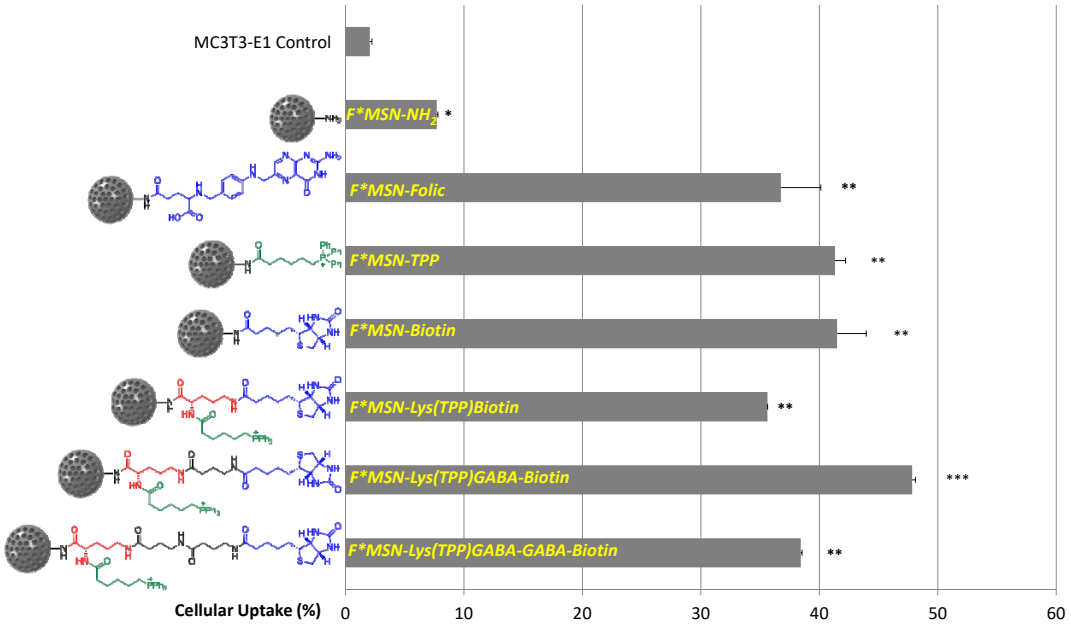


Figure S11. Cellular uptake of fluorescein-labeled nanoparticles measured by flow cytometry at 2h of internalization in MC3T3-E1 cells. Data are mean ± SEM of 3 independent experiments performed in triplicate *p < 0.05 *vs* MC3T3-E1 control;**p < 0.05 *vs* *F*MSN-NH*$_2$*; ***p < 0.01 *vs* *F*MSN-NH*$_2$*.

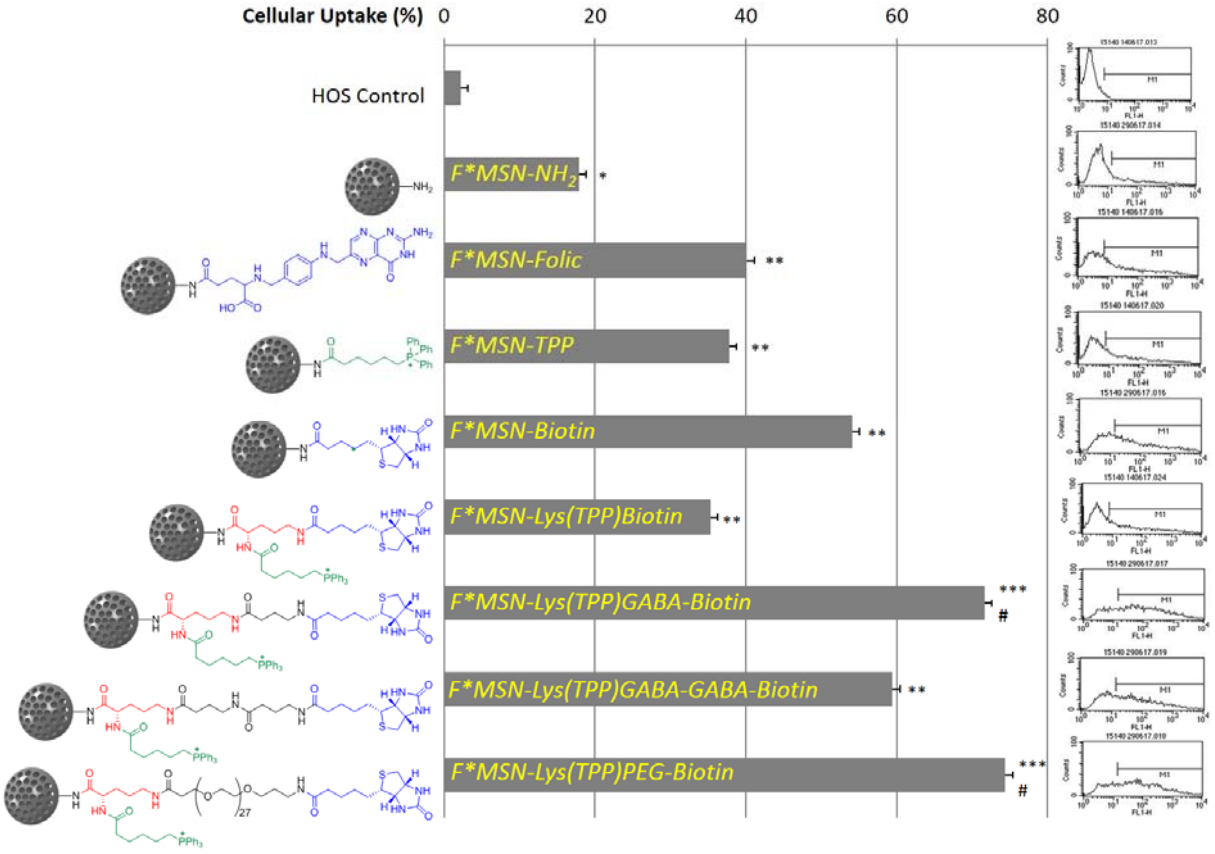


Figure S12. Cellular uptake of fluorescein labeled nanoparticles measured by flow cytometry at 2 h of internalization in HOS cells. Representative flow cytometry images are shown. Data are mean ± SEM of 3 independent experiments performed in triplicate *p < 0.05 *vs* HOS control;**p < 0.05 *vs* *F*MSN-NH*$_2$*; ***p < 0.01 *vs* *F*MSN-NH*$_2$*; #p < 0.01 *vs* all groups.

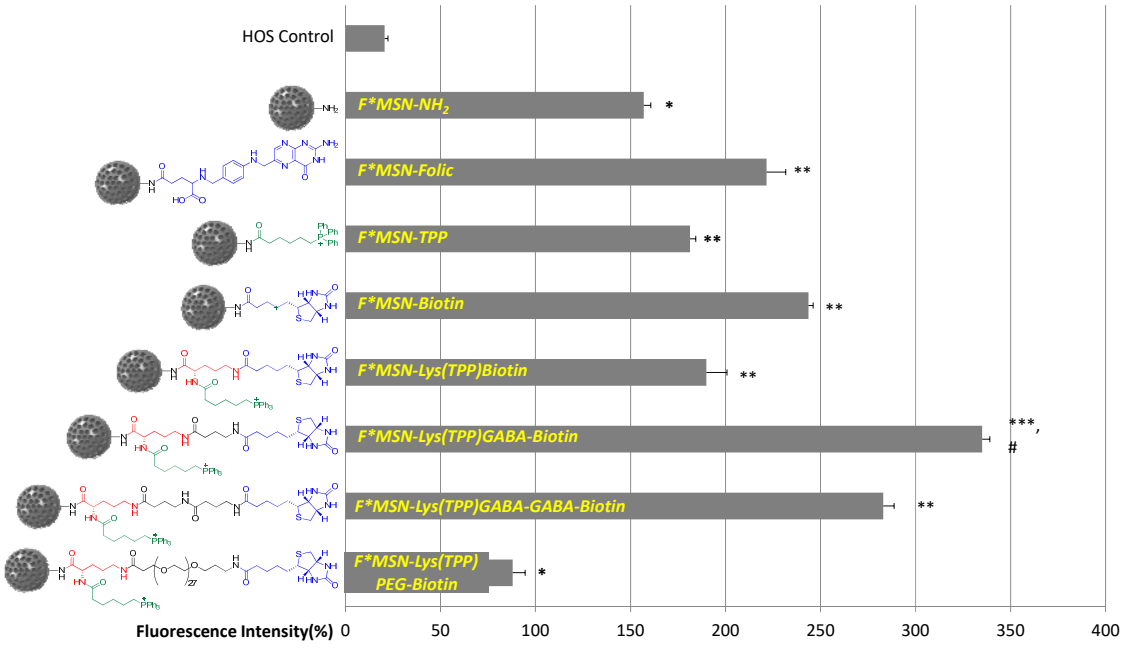


Figure S13. Fluorescence intensity of fluorescein-labeled nanoparticles measured by flow cytometry at 2h of internalization in HOS cells. Data are mean ± SEM of 3 independent experiments performed in triplicate *p < 0.05 *vs* HOS control; **p < 0.05 *vs* *F*MSN-NH*$_2$; ***p < 0.01 *vs* *F*MSN-NH*$_2$; #p < 0.01 *vs* all groups.

Bibliography for Supporting Information.

(1) Blanco M. D., Teijon C., Olmo R. M., Teijo J. M., (2012) Recent Advances in Novel Drug Carrier Systems: Targeted Nanoparticles for Cancer Therapy. *Recent Advances in Novel Drug Carrier Systems* (Ali Demir Sezer Ed.) pp. 255–263, Chapter 9, IntechOpen, London.

(2) Li S., Zeng D., (2007) Chemoenzymatic Enrichment of Phosphotyrosine-Containing Peptides, *Angew. Chemie Int. Ed.*, 46, 4751–4753.

(3) Cline E. N., Li M.-H., Choi S.K., Herbstman J.F., Kaul N., Meyhöfer E., Skiniotis G., Baker J.R., Larson R.G., Walter N.G., (2013) Paclitaxel-Conjugated PAMAM Dendrimers Adversely Affect Microtubule Structure through Two Independent Modes of Action, *Biomacromolecules*, 14, 654–664.