Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information

New targeting agents for selective drug delivery nanocarriers for treating neuroblastoma.

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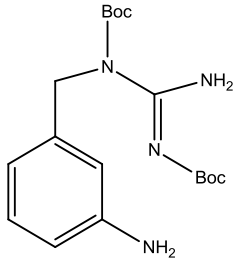
A.1 Synthesis of MIBG analogues.

MABG-BOC (1)



Benzyl alcohol (1 g, 8.12 mmol) in 10 mL of anhydrous THF was stirred under argon with *N*,*N* - bis(tert-butyloxycarbonyl)guanidine (2.32 g, 8.93 mmol) and TPP (4.26 g, 16.24 mmol). DIAD (3.20 mL, 16.24 mmol) was added drop-wise, and the resulting yellow heterogeneous mixture was stirred overnight at room temperature. The solvent was evaporated, and the resulting crude was reconstituted in 15 mL of EtOAc, washed with 3 x 15 mL of water and 2 x 20 mL of brine, dried, and concentrated. Pure white powder was afforded by flash chromatography isolation. Yield 60-70%.

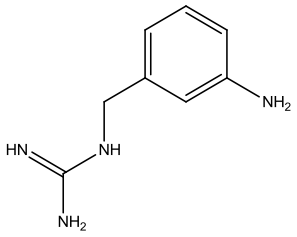
^{1}H NMR (250 MHz, $CDCl_3$) δ 9.41 and 9.35 (s, br, 2H, NH_2), 7.18 – 7.04 (m, 1H, CH (Ar)), 6.64 – 6.52 (m, 3H, CH (Ar)), 5.09 (s, 2H, CH_2), 3.62 (s, 2H, NH_2), 1.48 (s, 9H, 3xCH_3(BOC)), 1.32 (s, 9H, 3xCH_3(BOC)).

^{13}C NMR (63 MHz, CDCl3) δ 173.98 (C=O, BOC), 173.93 (C=O, BOC), 155.42 (C=N), 146.69 (C (Ar)), 140.44(C (Ar)), 129.47 (CH (Ar)), 117.56 (CH (Ar)), 114.10 (CH (Ar)), 113.81 (CH (Ar)), 84.58 (2xC(BOC)), 48.11 (CH_2), 28.70 (3xCH3(BOC)), 28.19 (3xCH_3(BOC)).

FTIR (cm-1): 3458; 3369; 3060; 2967; 2920; 1715; 1588; 1495; 1283; 1140; 1107; 977; 589.

ESI(-): 363m/z [M-1]

1-(3-aminobenzyl)guanidine (MABG)



To a solution of compound (1) (100 mg, 0.27439 mmol) in DCM (2mL) at inert atmosphere, TFA (1 mL) diluted in DCM (3 mL) was added drop-wise at room temperature. When the addition finalized the temperature was increased at 65 ºC and the reaction mixture was stirred overnight. The mixture was concentrated and the resulting crude was recovered in ethyl acetate and filtrated, the solution was concentrated and the resulted crude was triturated with chloroform. The resulting sticky solid was dried in reduce pressure oven at 40 ºC. The reaction resulted quantitative.

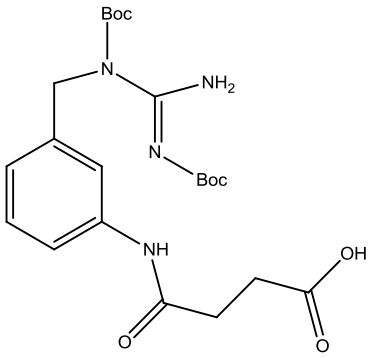
^{1}H NMR (250 MHz, MeOD) δ 7.81 (s, 1H, C=NH), 7.42 – 7.30 (m, 1H (Ar)), 7.12 (dd, J = 14.8, 7.4 Hz, 3H (Ar)), 4.35 (s, 2H, CH_2).

^{13}C NMR (63 MHz, MeOD) δ 159.29 (C=NH), 140.64 (C (Ar)), 136.86 (C (Ar)), 131.93 (CH (Ar)), 126.74 (CH (Ar)), 122.26 (CH (Ar)), 121.38 (CH (Ar)), 45.74 (CH_2).

FTIR (cm-1): 3359; 3179; 2960; 2641; 2356; 1788; 1655; 1436; 1356; 1180; 845; 795; 722; 682; 595; 519; 430.

ESI(+): 165 [M+1]

(*E*)-4-((3-((1,2-bis(tert-butoxycarbonyl)guanidino)methyl)phenyl)amino)-4-oxobutanoic acid (2)



A solution of succinic anhydride (35 mg, 274, 4mmol) in toluene (10mL) was treated drop-wise with a solution (5 mL) of product **1** (100 mg) also in toluene with gentle stirring under inert conditions. The resulting mixture was stirred overnight at room temperature until the reaction finished. When the reaction was over, white powder was suspended in the solution and was filtered and washed with water (30 mL). The resulting white powder was dried in reduced pressure oven at 40ºC. The process resulted quantitative.

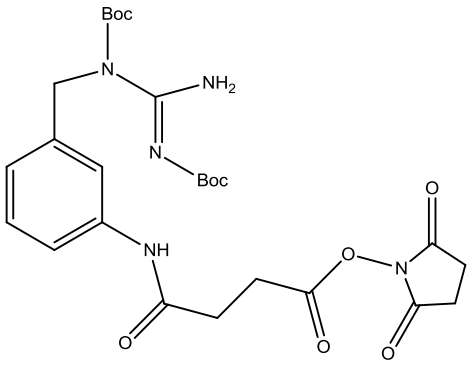
^{1}H NMR (250 MHz, $CDCl_3$) δ 9.38 and 9.08 (s, br, 2H, NH_2), 8.09 (s, 1H, NH, amide), 7.42 (d, *J* = 7.8 Hz, 1H, CH (Ar)), 7.16 – 7.08 (m, 2H, CH (Ar)), 6.80 (d, *J* = 7.5 Hz, 1H, CH (Ar)), 5.03 (s, 2H, CH_2), 2.67 – 2.51 (m, 4H, 2xCH_2), 1.40 (s, 9H, 3xCH_3(BOC)), 1.25 (s, 9H, 3xCH_3(BOC)).

^{13}C NMR (63 MHz, $CDCl_3$) δ 175.85 (C=O), 175.56 (C=O), 170.56 (C=O), 154.95 (C=N), 143.16 (C=O), 139.56 (C (Ar)), 137.99 (C (Ar)), 128.99 (CH (Ar)), 122.30 (CH (Ar)), 118.60 (CH (Ar)), 117.75 (CH (Ar)), 84.75 (2xC(BOC)), 47.62 (CH_2), 31.81 (CH_2), 29.51 (CH_2), 28.38 (3xCH_3(BOC)), 27.89 (3xCH_3(BOC)).

FTIR (cm^{-1}): 3372; 3329; 2977; 2927; 1721; 1625; 1625; 1608; 1286; 1246; 1137; 1120; 981; 944; 615; 504.

ESI(-): 462.9 m/z [M-1]

(*E*)-2,5-dioxopyrrolidin-1-yl 4-((3-((1,2-bis(tert butoxycarbonyl) guanidino) methyl) phenyl) amino)-4-oxobutanoate (3)

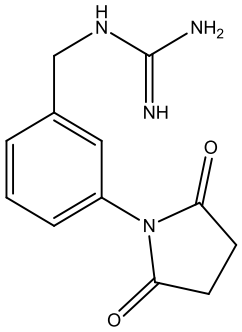


A solution of compound **(3)** (50 mg, 0.107 mmol, in 4 mL of DCM) was treated with N,N'-methanediylidenebis(propan-2-amine) (50.0 µL, 0.323 mmol) and N-hydroxysuccinimide (24.8 mg, 0.215 mmol). When the addition was finished, the reaction mixture was stirred overnight at room temperature. The resulting solution was washed with 5x15 mL of $NaHSO_4$ 20% solution and 2x10 mL of brine.

Organic layer was dried with $NaSO_4$ anhydride and concentrated in order to check by ^{1}H-NMR the presence of the desired product. Crude can be use, just recovering in THF for NP´s functionalization without further purification.

^{1}H NMR (250 MHz, $CDCl_3$) δ 9.49 (s, 2H, NH_2), 7.77 (s, 1H, amide), 7.53 (d, *J* = 8.2 Hz, 1H, Ar), 7.18 (dd, *J* = 16.7, 8.5 Hz, 2H, (Ar)), 6.90 (d, *J* = 7.6 Hz, 1H, (Ar)), 5.13 (s, 2H, CH_2), 3.03 (t, *J* = 7.1 Hz, 2H, CH_2 succ), 2.84 (s, 4H, 2xCH_2, (NHS)), 2.76 (t, *J* = 7.1 Hz, 2H, CH_2 succ), 1.47 (s, 9H, 3xCH_3(BOC)), 1.32 (s, 9H, 3xCH_3(BOC)).

1-(3-(2,5-dioxopyrrolidin-1-yl)benzyl)guanidine (MPyBG)



To a solution of compound (**3**) (130 mg, 0.280 mmol) in DCM (2mL) at inert atmosphere, TFA (1 mL) diluted in DCM (3 mL) was added drop-wise at room temperature. When the addition finalized the temperature was increased at 65 ºC and the reaction mixture was stirred 48 hours. The mixture was concentrated and the resulting crude was recovered in ethyl acetate and filtrated, the solution was concentrated and the new crude triturated with chloroform. The resulting sticky solid was dried in reduce pressure oven at 40 ºC. Quantitative yield was afforded but also some impurities are still in the crude as traces.

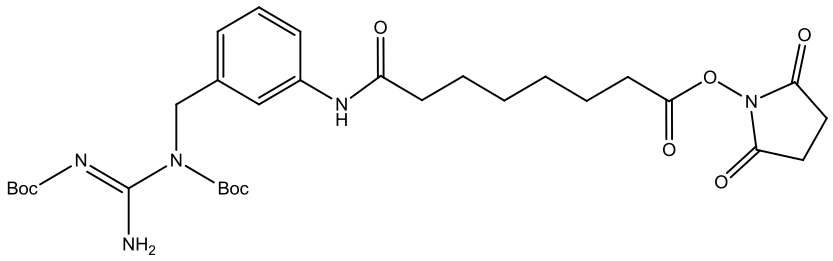
^{1}H NMR (250 MHz, MeOD) δ 7.93 (s, 2H, C=NH protonated imine), 7.53 (dt, *J* = 7.6, 4.2 Hz, 1H, amide), 7.46 – 7.34 (m, 1H, CH (Ar)), 7.36 – 7.26 (m, 2H, 2xCH (Ar)), 6.90 (d, *J* = 6.3 Hz, 1H, CH (Ar)), 4.48 (s, 2H, CH_2), 2.89 (s, 4H, 2xCH_2, (pyrroidin)).

^{13}C NMR (63 MHz, MeOD) δ 179.33 (2xC=O), 166.44 (C=N), 139.36(C (Ar)), 134.98 (C (Ar)), 130.98 (CH (Ar)), 128.65(CH (Ar)), 128.12(CH (Ar)), 127.30 (CH (Ar)), 45.91 (CH_2), 29.87(2xCH_2).

FTIR (cm-1): 3342; 3183; 2934; 2864; 2469; 2386; 1702; 1655; 1609; 1426; 1389; 1183; 1120; 832; 798; 712.

ESI(+): 247.0 m/z [M+1]

(*E*)-2,5-dioxopyrrolidin-1-yl8-((3-((1,2-bis(tertbutoxycarbonyl)guanidino)methyl) phenyl) amino)-8-oxooctanoate (4)



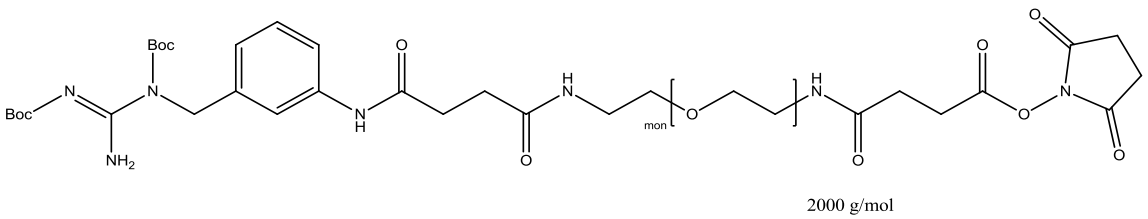
To a solution of DSS (101 mg, 2 mL DCM), compound **1** was added as DCM solution (100 mg in 2 mL of DCM and 115 µL of Et_3N) drop-wise. When the addition was finished, the reaction mixture was heated at 65 ºC overnight. The resulting solution was washed with 5x15 mL of $NaHSO_4$ 20% solution and 2x10 mL of brine. Organic layer was dried with $NaSO_4$ anhydride and concentrated in order to check the presence of the desired product by ^{1}H-NMR and ESI. The crude can be use, just recovering it in DCM for NP´s functionalization without further purification.

^{1}H NMR (250 MHz, $CDCl_3$) δ 9.49 (s, 2H, NH_2), 7.58 (s, 1H (CH (Ar)), 7.28 – 7.20 (m, 2H, (CH (Ar)), 6.94 (d, *J* = 7.0 Hz, 1H (CH (Ar)), 5.19 (s, 2H, CH_2), 2.87 (s, *J* = 2.4 Hz, 4H, 2xCH_2(NHS)), 2.70 – 2.58 (m, 2H, CH_2), 2.38 (t, *J* = 5.6 Hz, 2H, CH_2), 1.86 – 1.69 (m, 4H, 2xCH_2), 1.51 (s, 9H, 3xCH_3(BOC)), 1.48 – 1.40 (m, 4H, 2xCH_2), 1.36 (s, 9H, 3xCH_3(BOC)).

^{13}C NMR (63 MHz, $CDCl_3$) δ 173.35 (C=O, amide), 170.77 (C=O), 170.48 (C=O, (NHS)), 168.18 (C=O (ester,NHS), 153.89 (2C; C=N, C=O (BOC)), 145.65 (C=O, (BOC)), 138.52 (C, (Ar)), 137.28 (C, (Ar)), 127.73 (CH, (Ar)), 121.05 (CH, (Ar)), 117.11 (CH, (Ar)), 116.50 (CH, (Ar)), 83.25 (C, (BOC)), 78.08 (C, (BOC)), 52.43 (CH_2), 46.42 (CH_2-CON, Suberic), 43.88 (CH_2-CO-NHS, Suberic), 27.26 (3xCH_3, (BOC)), 26.74 (3xCH_3, (BOC)), 24.58 (CH_2, Suberic), 24.56 (CH_2, Suberic), 24.37 (CH_2, (NHS)), 24.31 (CH_2, (NHS)).

ESI(+): 640.5m/z [M+Na].

MABG-PEG2000-NHS (5)



2000 g/mol

To a solution of $(NHS)_2PEG$ (2000 g/mol) (55 mg, 5 mL DCM), compound **1** was added as DCM solution (10 mg in 2 mL of DCM and 58 µL of Et_3N) drop-wise. When the addition was finished, the reaction mixture was heated at 65 ºC overnight, extra Et_3N (58 µL) was added at 24 h and the mixture was again stirred at 65 ºC overnight. The reaction was followed by TLC (AcOEt/Hex 1:1). The reaction is over 48 h after first addition. The resulting solution was washed with 5x15 mL of $NaHSO_4$ 20% solution and 2x10 mL of brine. Organic layer was concentrated and the crude precipitated by diethyl ether addition. The resulting solid can be use, for NP´s functionalization without further purification.

^1H NMR (250 MHz, $CDCl_3$) δ 9.49 and 9.27 (s, broad, NH_2) 8.20 (s, NH, amide) 7.47 (2xCH (Ar)), 7.01 (CH (Ar)), 6.96 (CH (Ar)), 5.67 (CH_2, guanidine moiety), 3.66 (CH_2, PEG), 2.73 (CH_2, (NHS)), 2.28 (CH_2, broad, succinic moiety), 1.33 (s, 3xCH_3 (BOC)), 1.26 (s, 3xCH_3 (BOC)).

^{13}C NMR (63 MHz, $CDCl_3$) δ 177.37 (C=O, amide), 170.97 (C=O), 167.69 (C=N), 127.59 (CH, (Ar), (there are some non-resolved down-field signals) 87.90 (C, (BOC) 70.68 (CH_2, PEG), 69.98 (CH_2, PEG), 67.26 (CH_2, PEG), 62.83 (CH_2, PEG), 58.53 (CH_2, guanidine moiety), 45.42 (CH_2-CON), 37.98 (CH_2-CON), 28.17 (6xCH_3 (BOC) (there are some non-resolved aliphatic signals).

FTIR (cm-1): 3543; 2877; 2741; 2691; 1967; 1768; 1698; 1462; 1336; 1309; 1283; 1236; 1193; 1090; 1054; 957; 838; 569; 529.

HPLC/GPC:

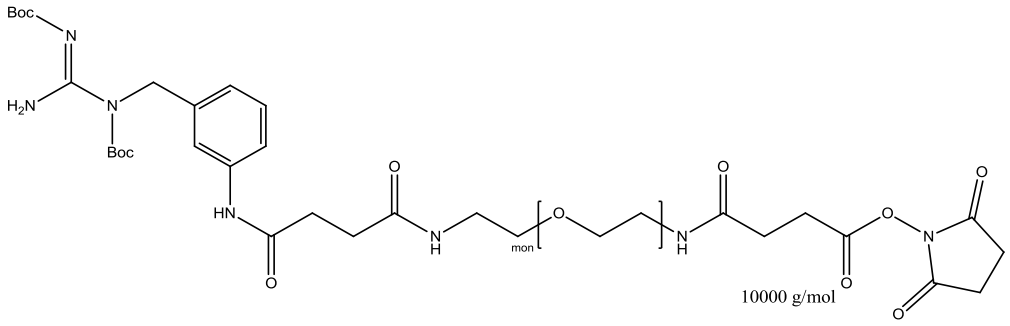
-$(NHS)_2$PEG2000 (Starting Material)

Migration Time	Mn	**Mw**	MP	Mz	Mz+1	Polydispersity	Mz/Mw	Mz+1/Mw	Area	% Area	Height
10,998	1651	**2048**	1836	2506	3054	1,240353	1.223888	1.491386	36440559	100	912676

-MABG-PEG2000-NHS (5)

Migration Time	Mn	Mw	**MP**	Mz	Mz+1	Polydispersity	Mz/Mw	Mz+1/Mw	Id	Area	**% Area**	Height
10,417			**4250**						0	1059367	**2,96**	60415
10,859	2099	2339	**2486**	2532	2696	1,114256	1,082608	1,152513	1485	34690629	**97,04**	1126405

MABG-PEG10000-NHS (6)



To a solution of $(NHS)_2$PEG (10000 g/mol) (274.4 mg, 5 mL DCM), compound **1** was added as DCM solution (10 mg in 2 mL of DCM and 58 µL of Et_3N) drop-wise. When the addition was finished, the reaction mixture was heated at 65 ºC overnight. Extra Et_3N (58 µL) was added at 24 h and the mixture was again stirred at 65 ºC overnight. The reaction was followed by TLC (AcOEt/Hex 1:1). The reaction is over 48 h after first addition. The resulting solution was washed with 5x15 mL of $NaHSO_4$ 20% solution and 2x10 mL of brine. Organic layer was

concentrated and the crude precipitated by diethyl ether addition. The resulting solid can be use, for NP´s functionalization without further purification.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.02 (s, NH amide), 7.54 – 7.50 (m, CH (Ar)), 7.28 – 7.24 (m, CH (Ar)), 7.16 – 7.11 (m, CH (Ar)), 6.90 – 6.81 (m, CH (Ar)), 5.11 – 5.06 (s, broad, CH_2 guanidine moiety), 3.85 (m, CH_2 PEG), 3.74 (d, *J* = 2.9 Hz, CH_2 PEG), 3.58 (s, CH_2 PEG), 3.29 (m, CH_2 succinic moiety), 2.64 (s, CH_2, NHS), 2.61 – 2.54 (m, CH_2 succinic moiety), 2.52 – 2.39 (m, CH_2 succinic moiety), 1.26 (s, 3xCH_3 (BOC)), 1.19 (s, 3xCH_3 (BOC)).

HPLC/GPC:

-$(NHS)_2$PEG10000 (Starting Material)

Migration Time	Mn	**Mw**	MP	Mz	Mz+1	Polydispersity	Mz/Mw	Mz+1/Mw	Id	Area	**% Area**	Height
8,934			18896						0	2547858	**7,54**	93594
9,529	8460	**9477**	10451	10152	10674	1,120122	1,071306	1,126313	1487	31248507	**92,46**	879940

-MABG-PEG10000-NHS (6)

Migration Time	Mn	**Mw**	MP	Mz	Mz+1	Polydispersity	Mz/Mw	Mz+1/Mw	Id	Area	**% Area**	Height
8,91			19369						0	2035698	**5,77**	76645
9,5	8775	**9743**	10739	10408	10932	1,110247	1,068307	1,122032	1483	33246772	**94,23**	957764

MALDI-TOF

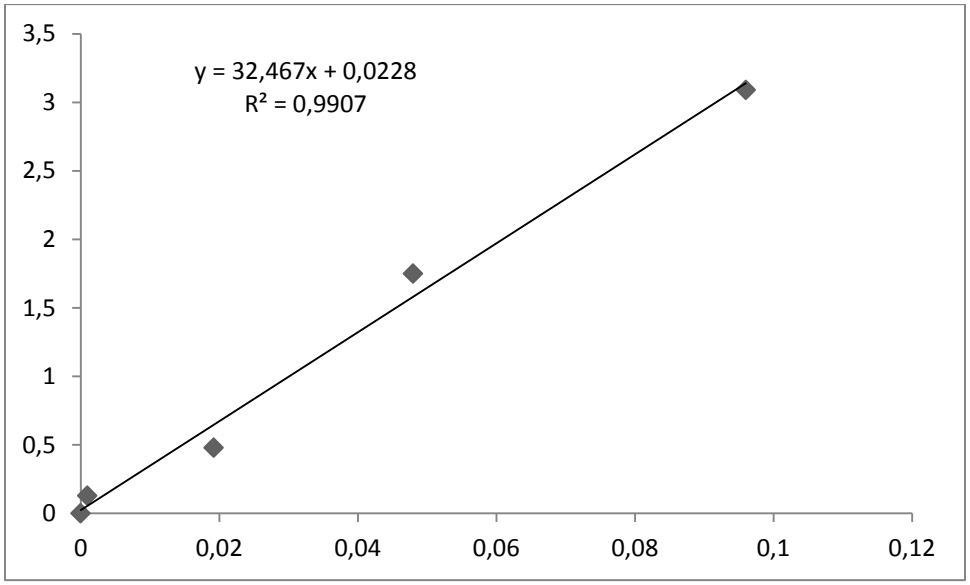
$(NHS)_2$PEG10000; 11076.831 m/z [M^+]

MABG-PEG10000-NHS (6); 11440.146 m/z [M^+]

11440.146 m/z-11076.831 m/z = **364 [M^++1] MABG-BOC (1)**

A.2 Determination of MAmideBG content in PEG analogues (5) and (6) by UV/Vis Spectrometry

Quantification of guanidine moiety in PEG´s using as standard compound (*E*)-2,5-dioxopyrrolidin-1-yl8-((3-((1,2-bis(tertbutoxycarbonyl)guanidino)methyl) phenyl) amino)-8-oxooctanoate **(5)** as standard in from of MABG-PEG analogues.



mg/mL	A
0.096	3.089
0.048	1.748
0.0192	0.479
0.00096	0.128
0	0

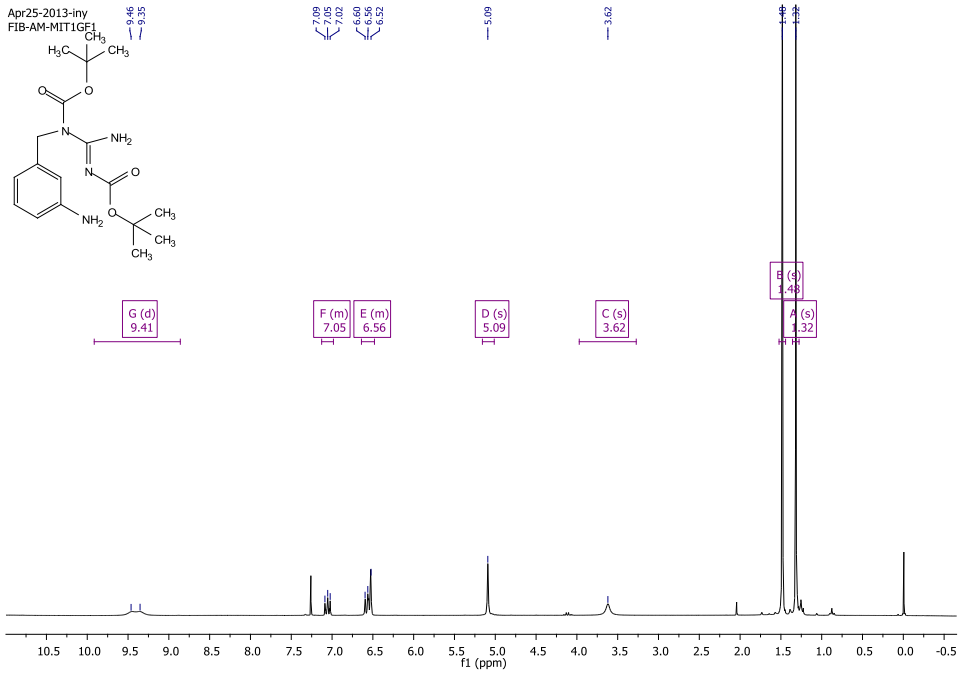
MABG-BOC-PEG10000NHS (6) A= 0.442 (1.1 mg/mL) 0.01234 mg/mL--------- 1.2% w/w

MABG-BOC-PEG2000NHS (5) A= 2.149 (0.85 mg/mL) 0.06555 mg/mL---------7.7% w/w

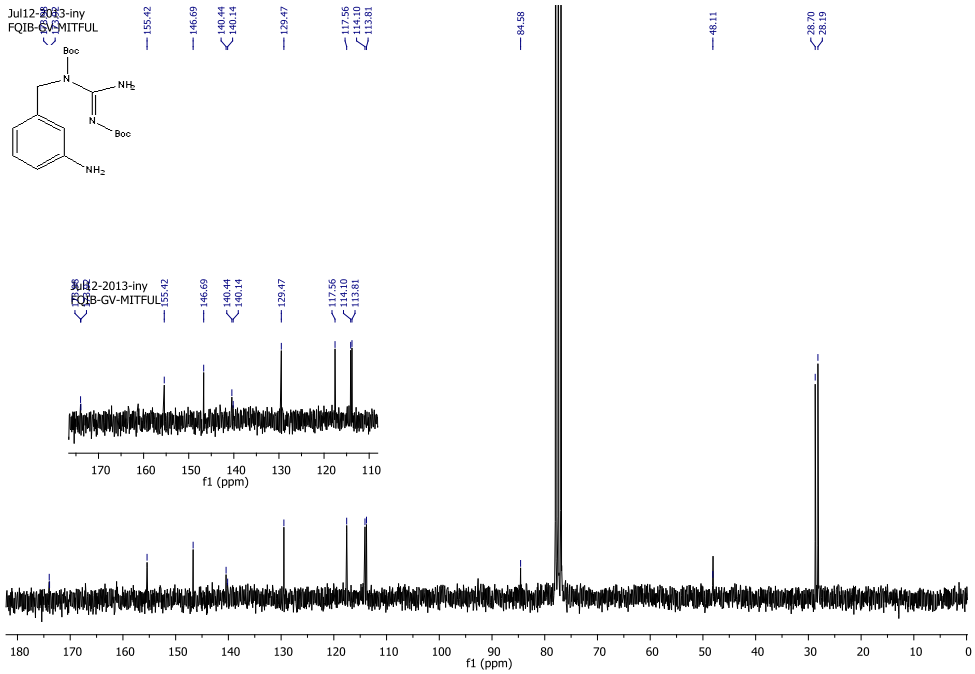
A.3 Spectra

MABG-BOC (1)

-NMR

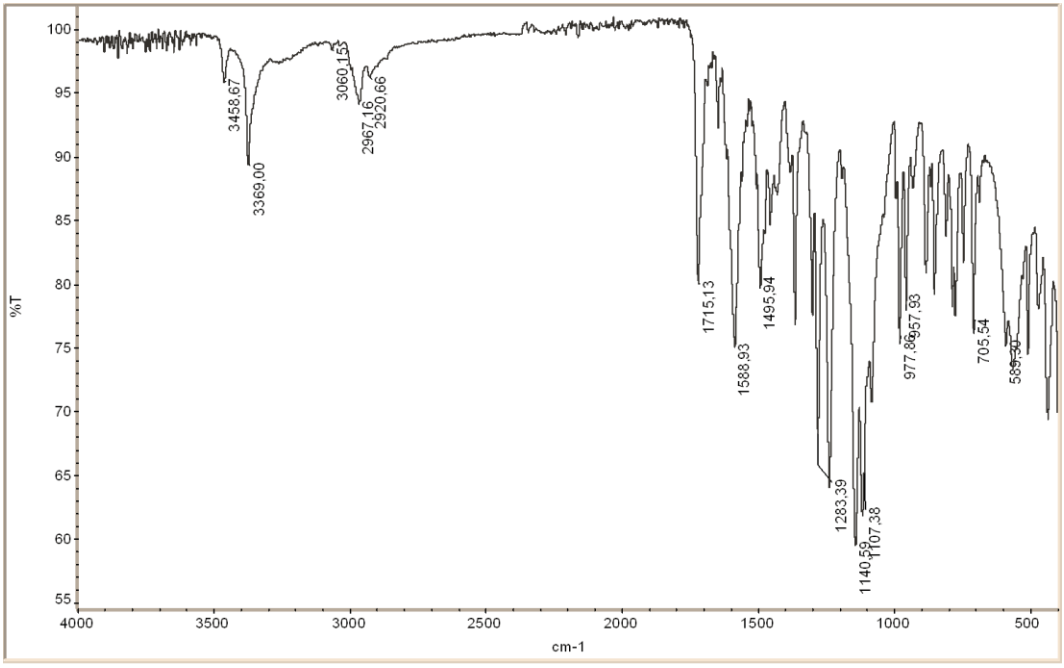


S 1. ^{1}H-NMR MABG-BOC (1)



S 2. ^{13}C-NMR MABG-BOC (1)

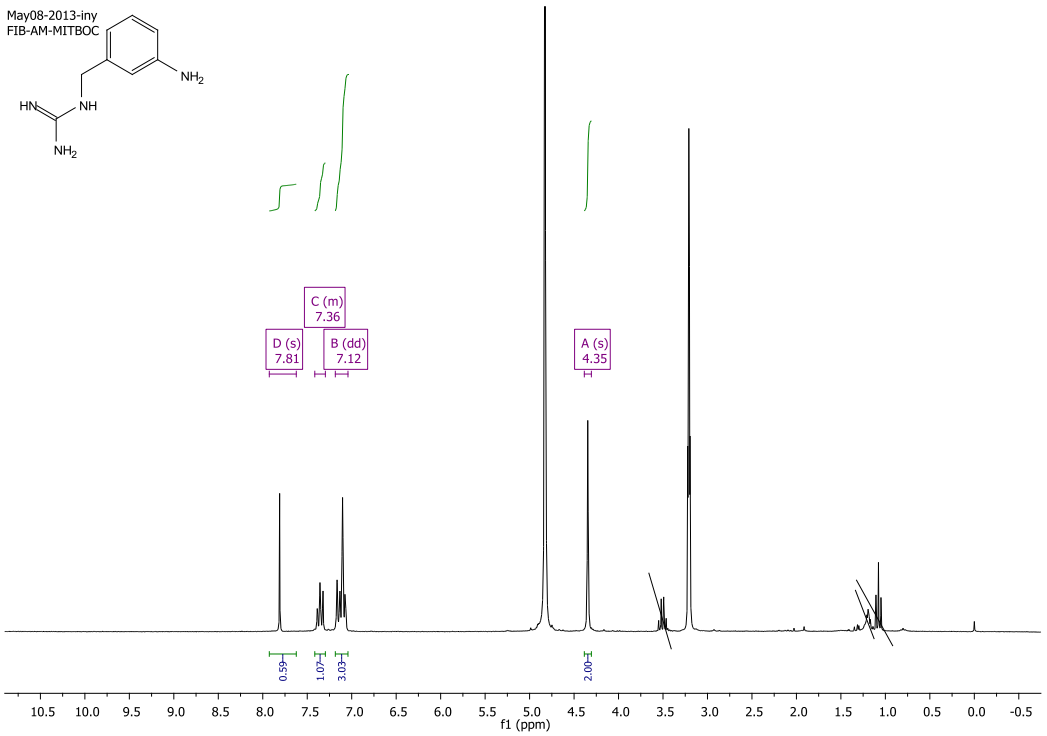
-IR



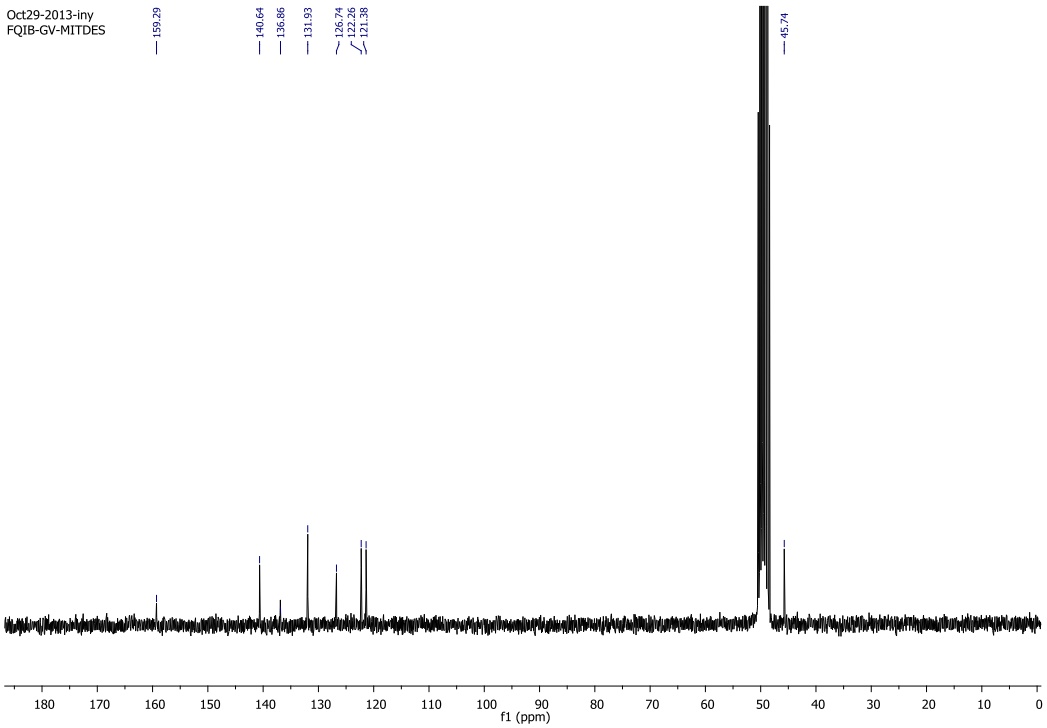
S 3. IR MABG-BOC (1)

MABG

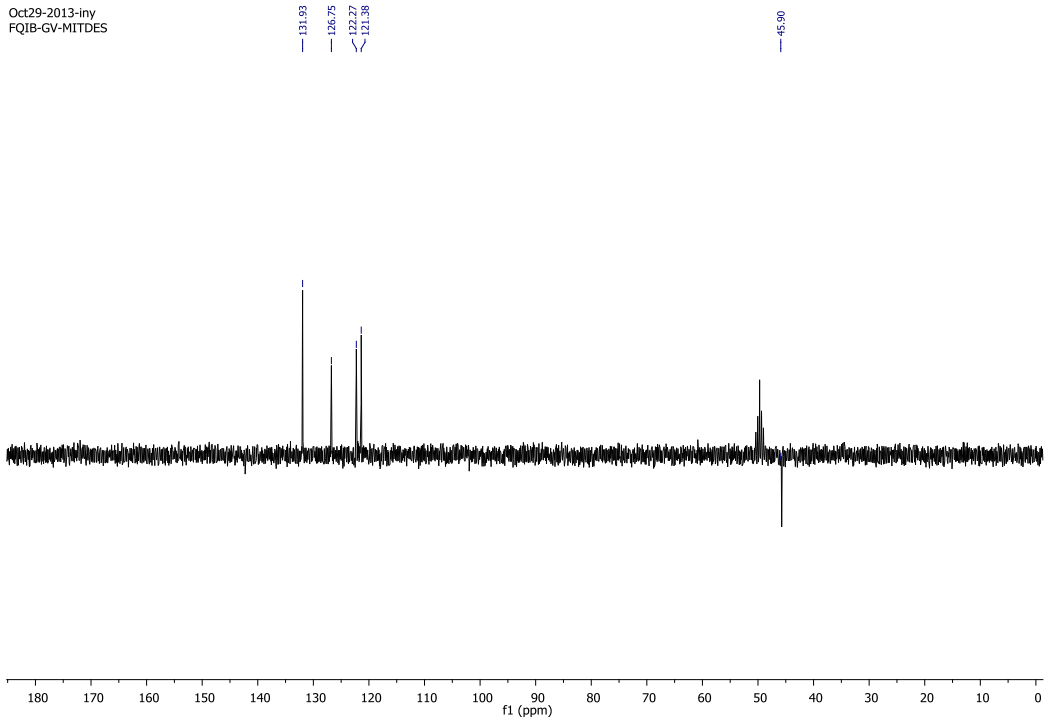
-NMR



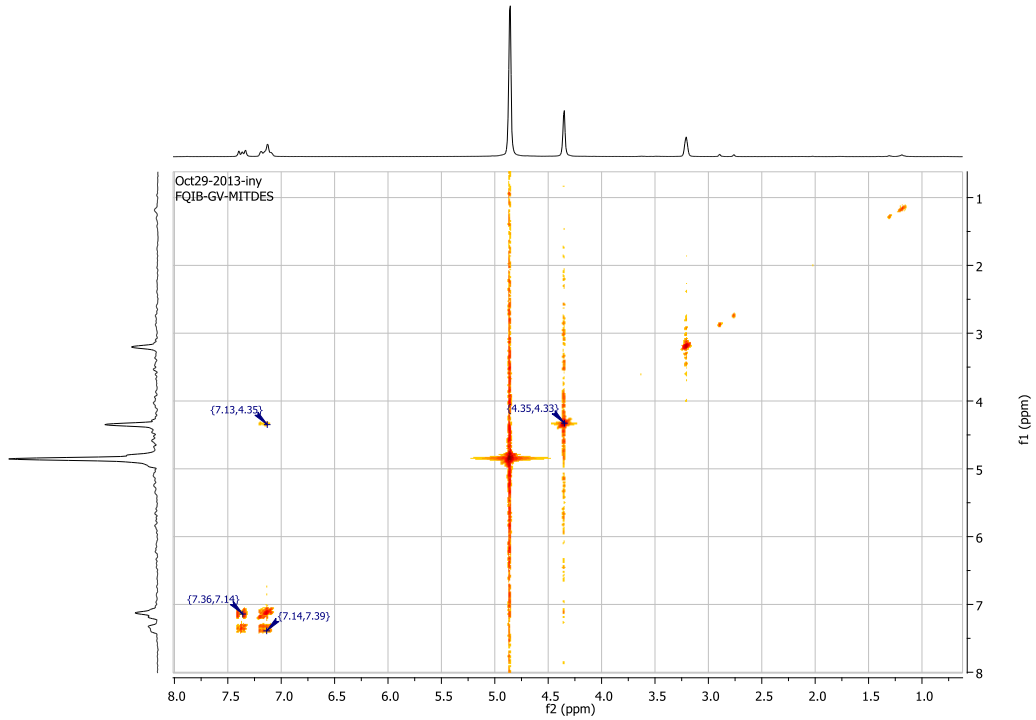
S 4. ^{1}H-NMR MABG



S 5. ^{13}C-NMR MABG

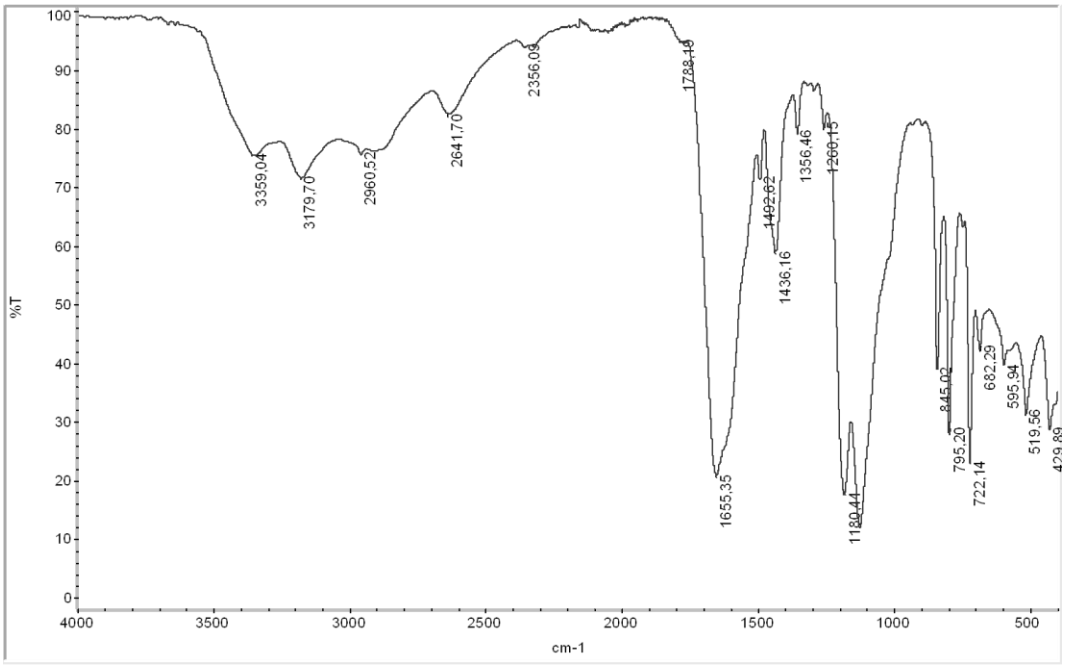


S 6. ^{13}C-NMR DEPT MABG



S 7. ^{1}H-NMR COSY MABG

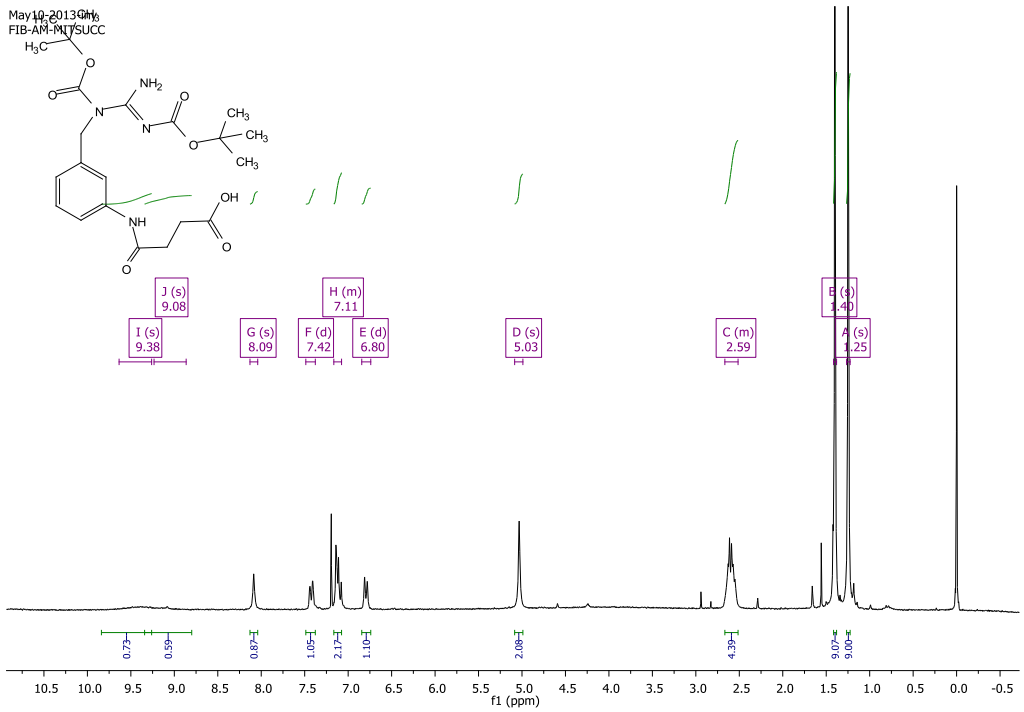
-IR


%T
cm-1
3359.04
3179.70
2960.52
2641.70
2356.09
1788.18
1655.35
1492.62
1436.16
1356.46
1260.15
1180.41
845.02
795.20
722.14
682.29
595.94
519.56
429.89

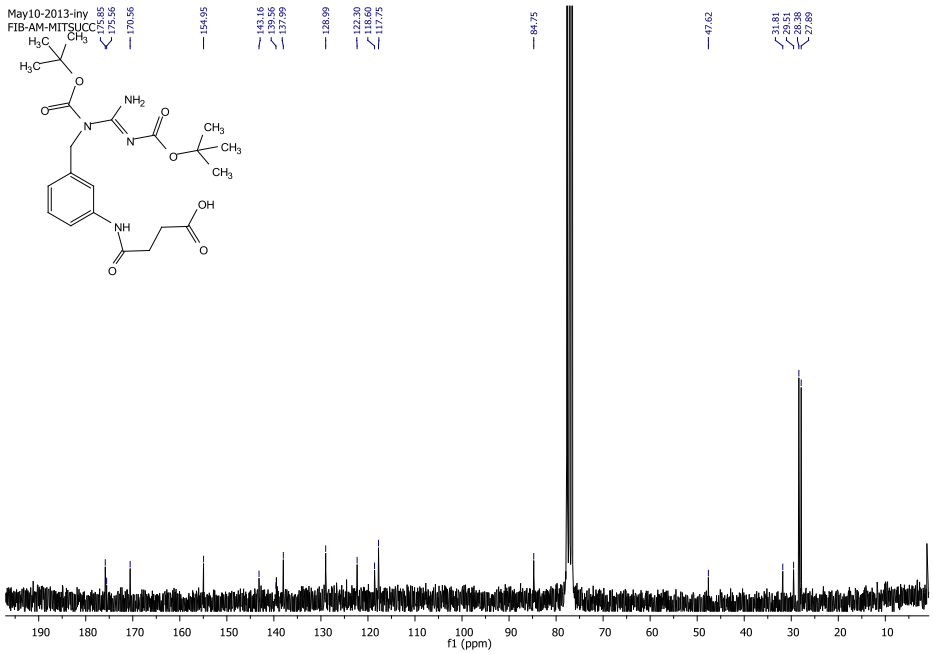
S 8. IR MABG

(*E*)-4-((3-((1,2-bis(tert-butoxycarbonyl)guanidino)methyl)phenyl)amino)-4-oxobutanoic acid (2)

-NMR

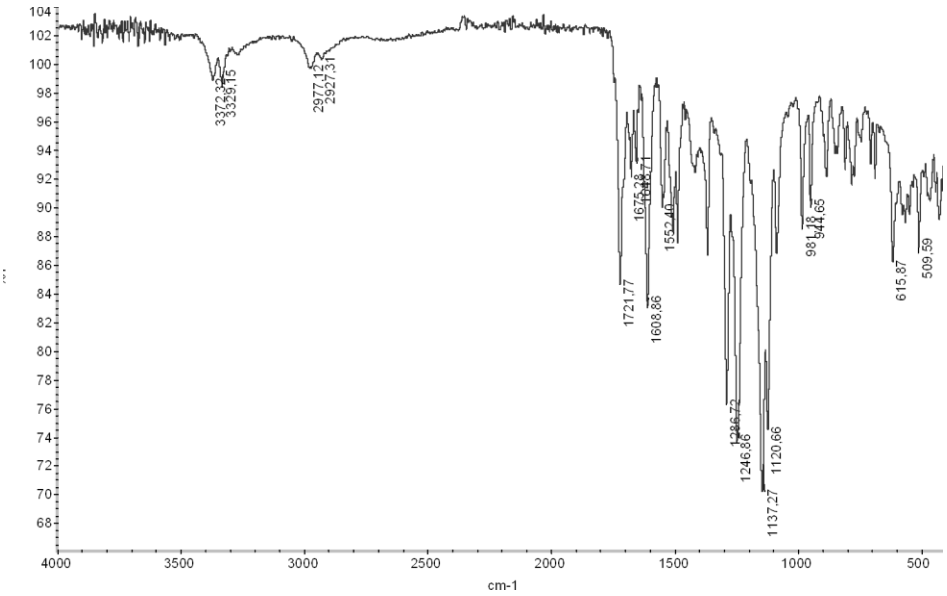


S 9. ^{1}H-NMR (2)



S 10. ^{13}C-NMR (2)

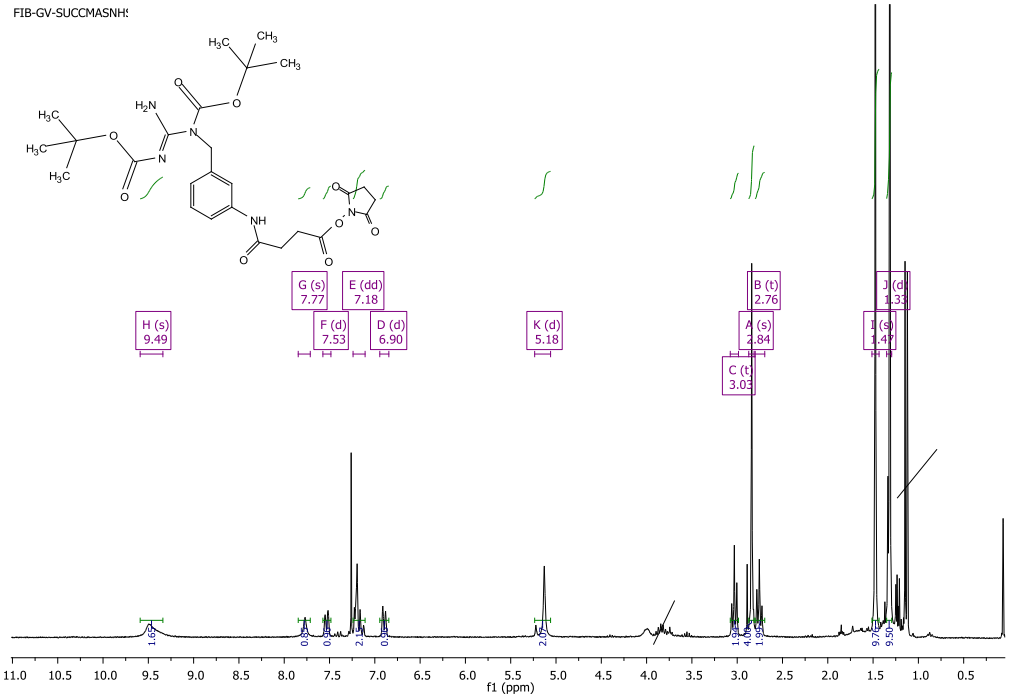
-IR


3372.34
3329.15
2977.12
2927.31
1721.77
1675.08
1648.11
1608.86
1552.40
1286.72
1246.86
1137.27
1120.66
981.18
944.65
615.87
509.59
cm-1

S11. IR (2)

(*E*)-2,5-dioxopyrrolidin-1-yl 4-((3-((1,2-bis(tert butoxycarbonyl) guanidino) methyl) phenyl) amino)-4-oxobutanoate (3)

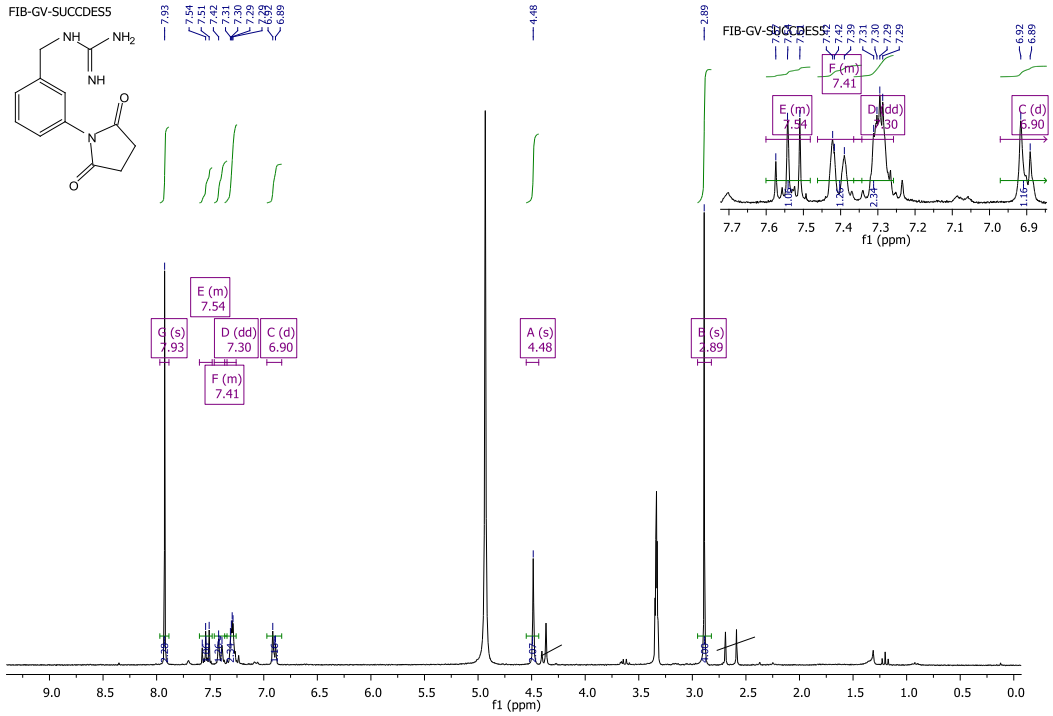
-NMR



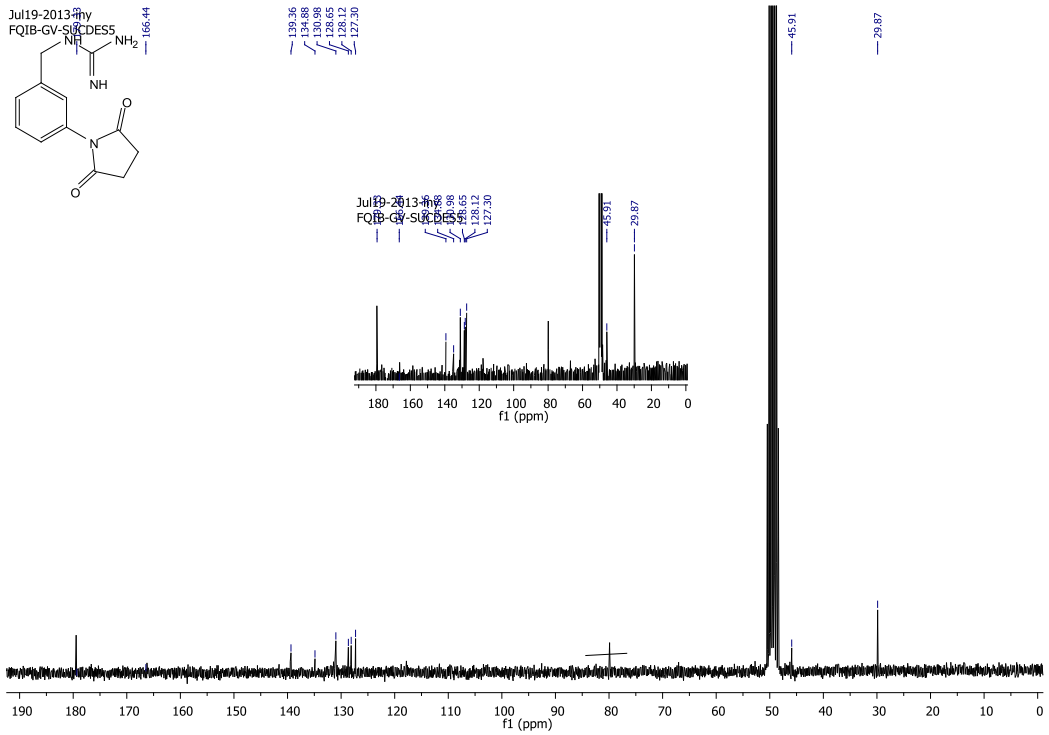
S12. ^{1}H-NMR (3)

1-(3-(2,5-dioxopyrrolidin-1-yl)benzyl)guanidine (MPyBG)

-NMR

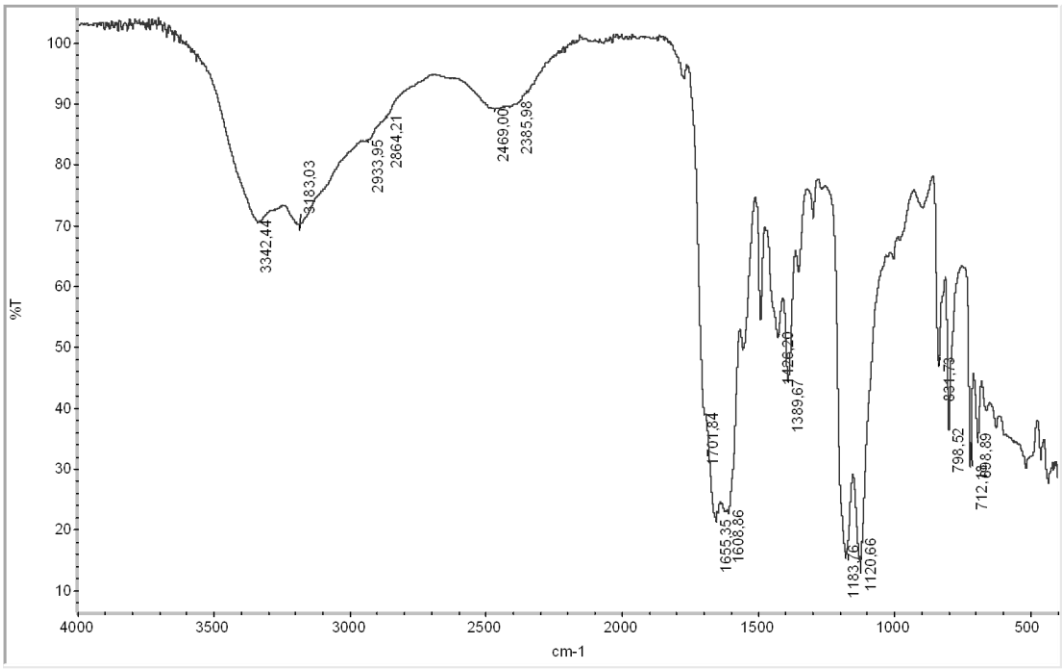


S13. ^{1}H-NMR MPyBG



S14. ^{13}C-NMR MPyBG

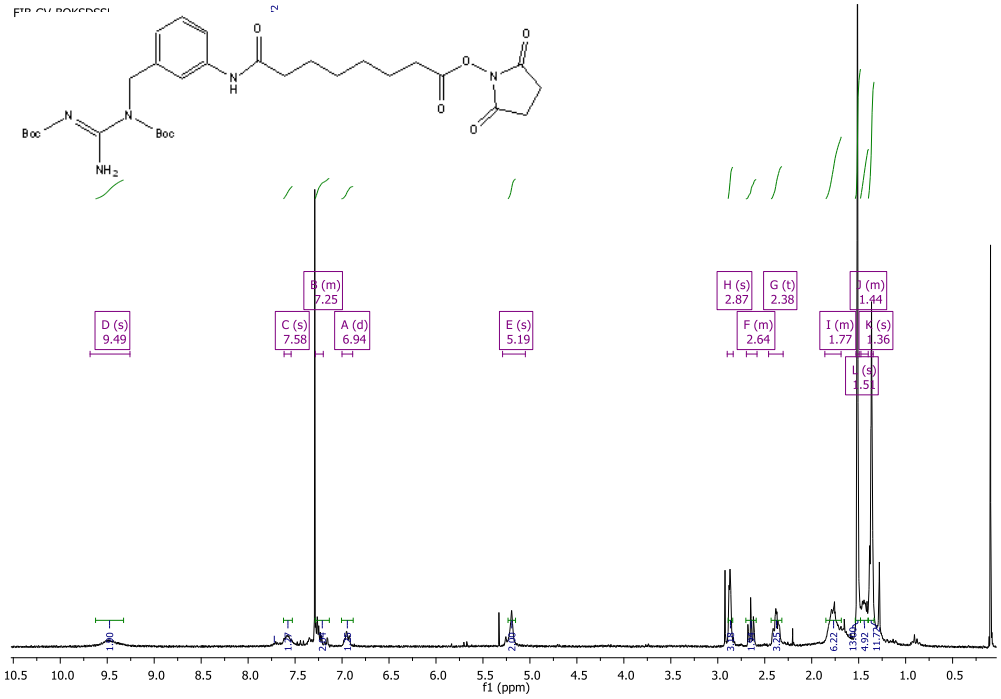
-IR



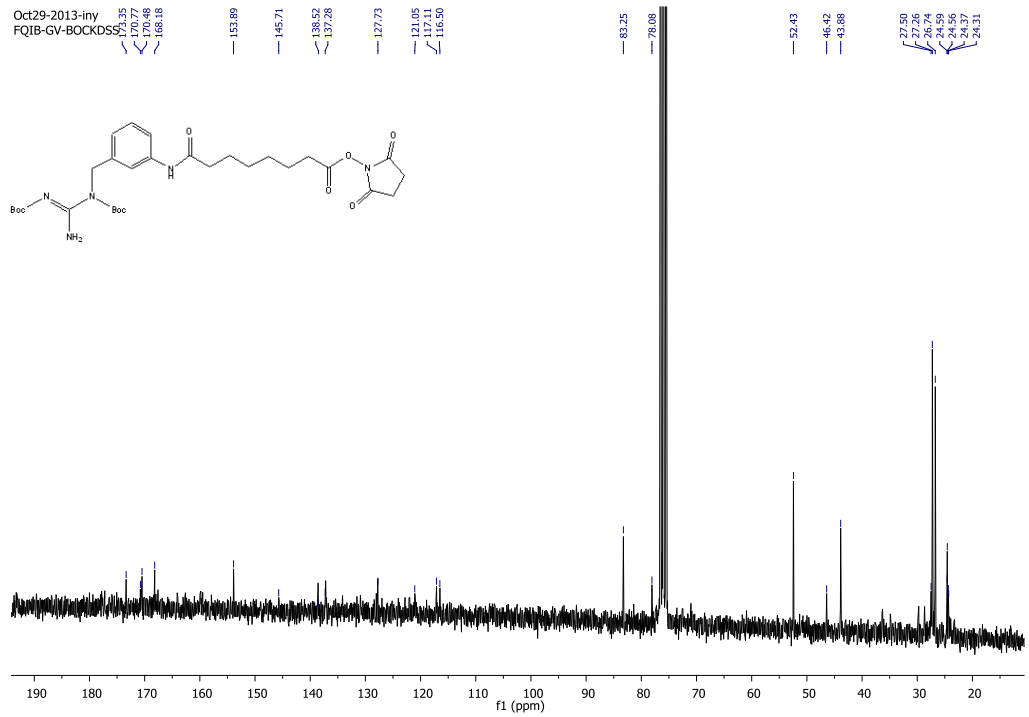
S15. IR MPyBG

(*E*)-2,5-dioxopyrrolidin-1-yl8-((3-((1,2-bis(tertbutoxycarbonyl)guanidino)methyl) phenyl) amino)-8-oxooctanoate (4)

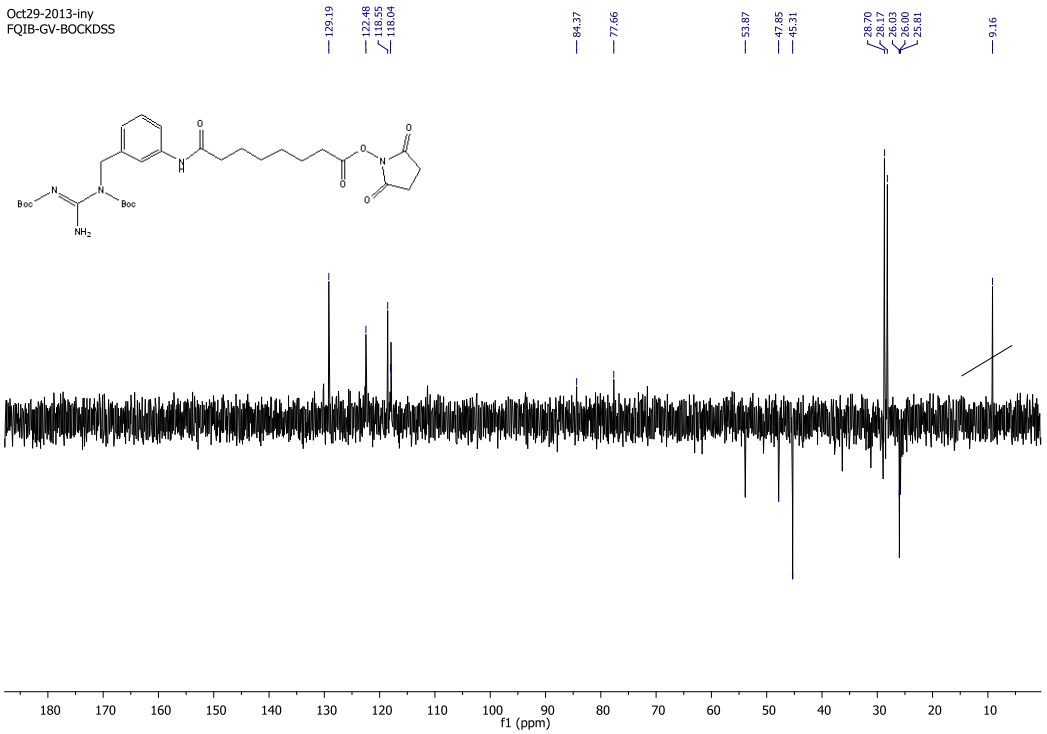
-NMR



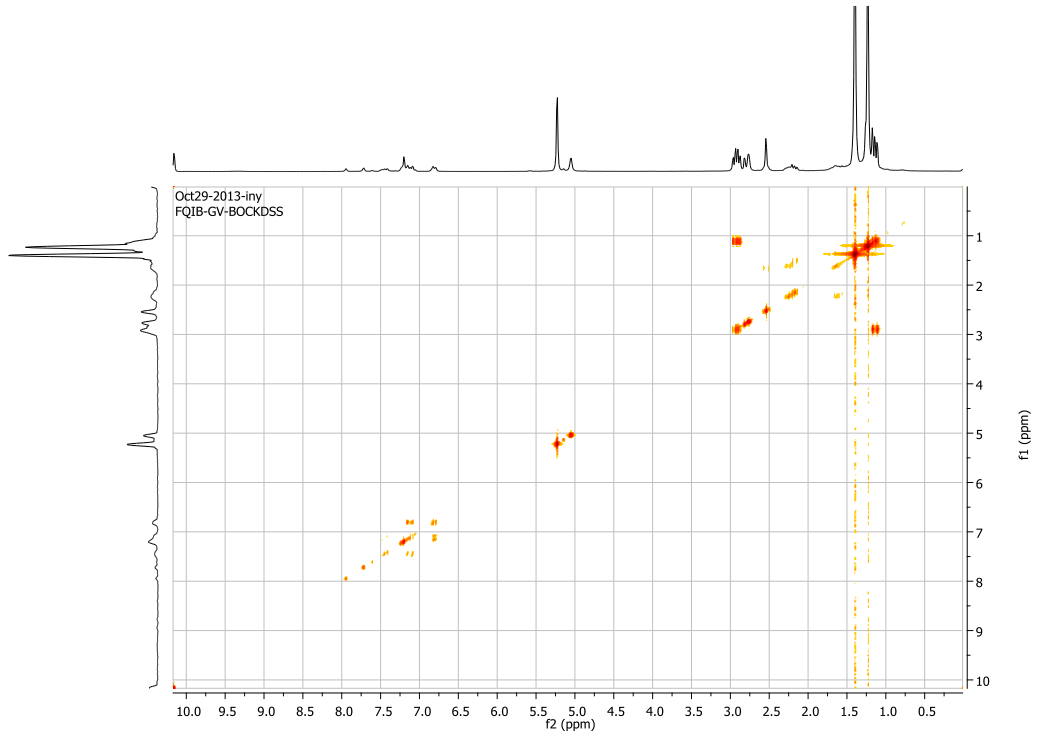
S16. ^{1}H-NMR (4)



S17. ^{13}C-NMR (4)



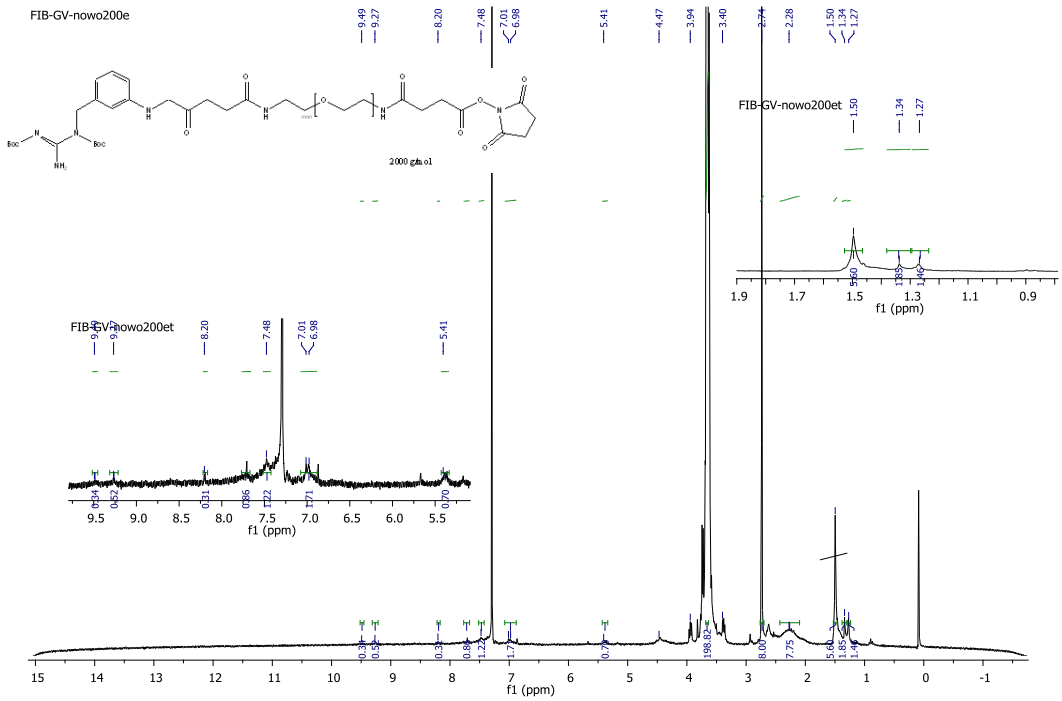
S18. ^{13}C-NMR DEPT (4)



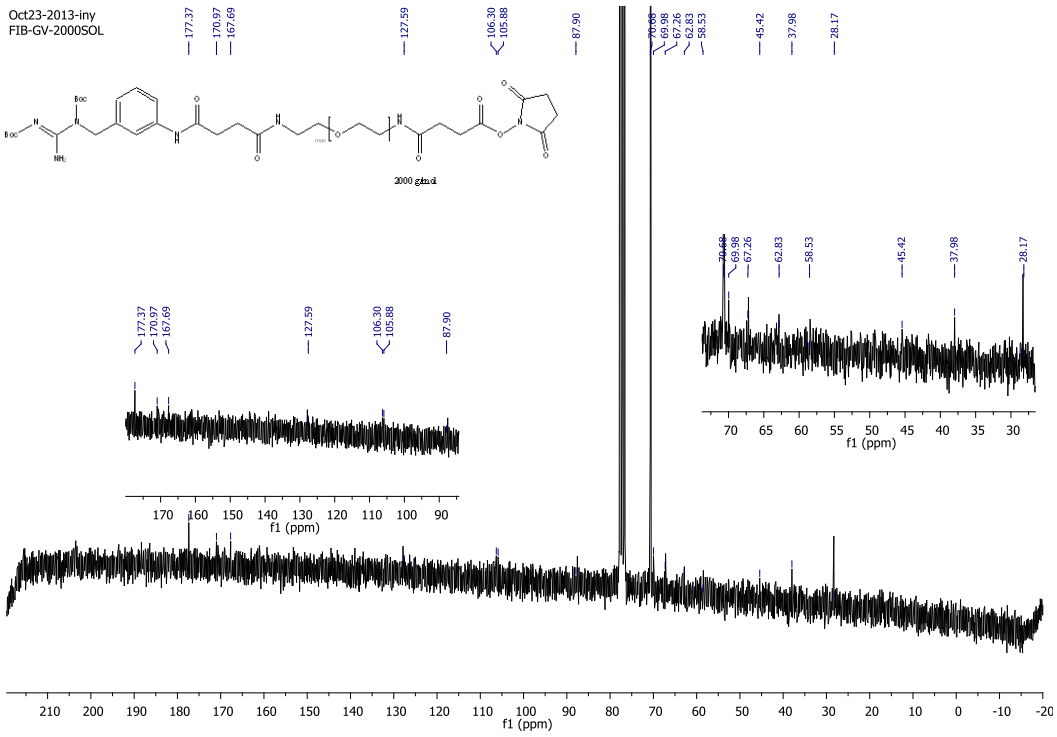
S19. ^{1}H-NMR COSY (4)

MABG-PEG2000-NHS (5)

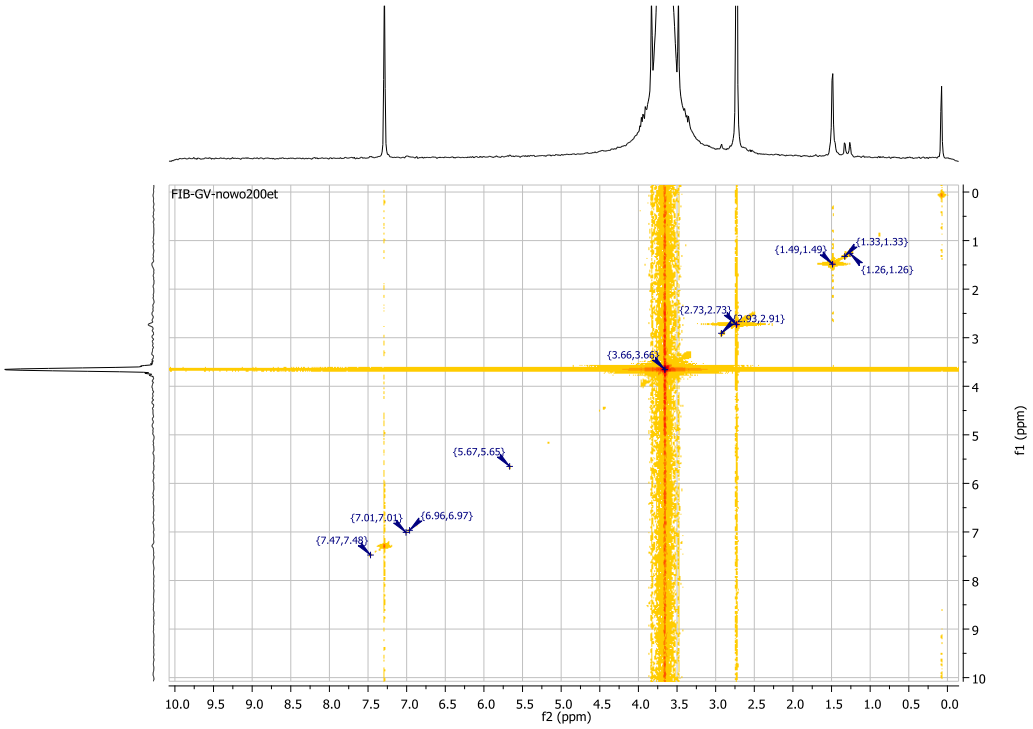
-NMR



S20. ^{1}H-NMR (5)



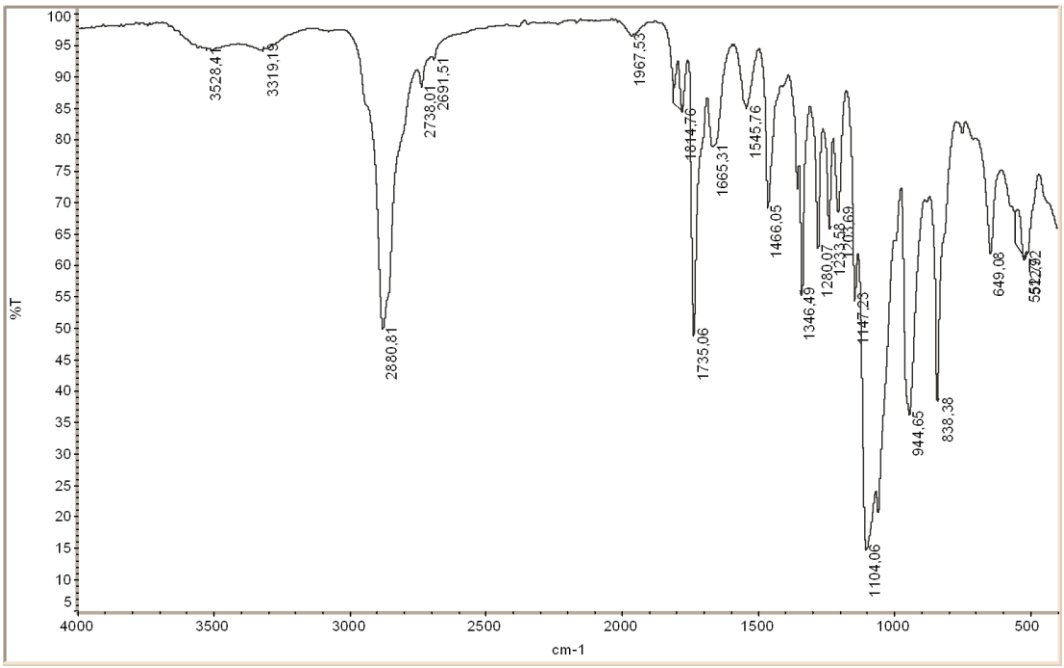
S22. ^{13}C-NMR (5)



S22. ^{1}H-NMR COSY (5)

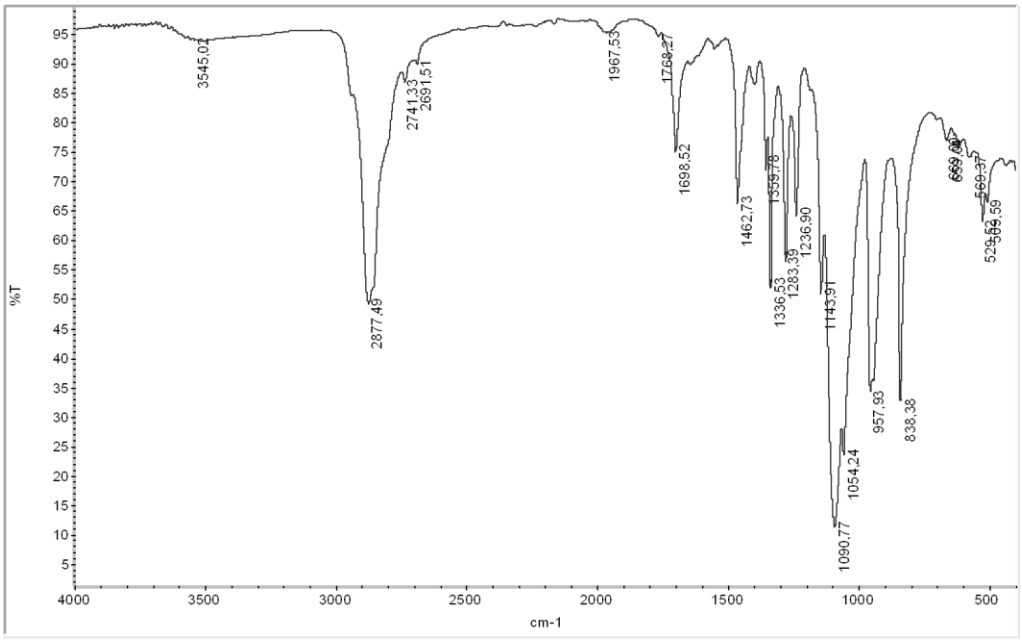
-IR

$(NHS)_2$PEG2000



S23. IR Starting Material PEG2000

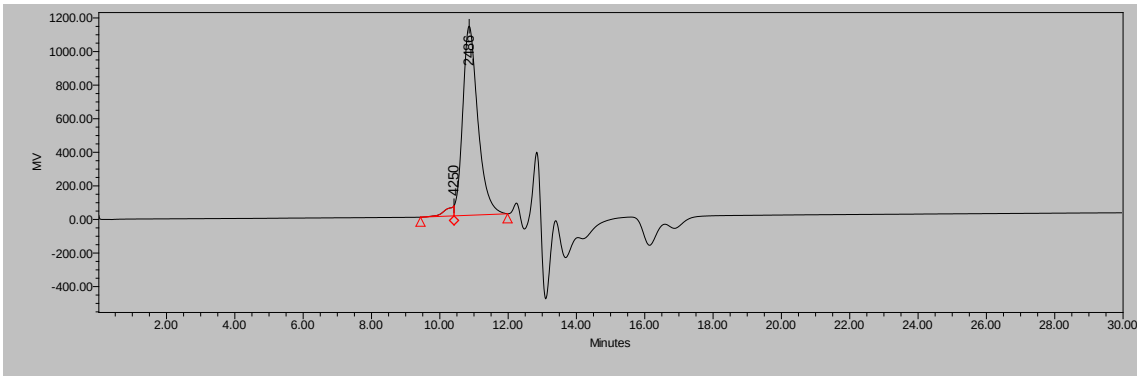
MABG-PEG2000-NHS (5)



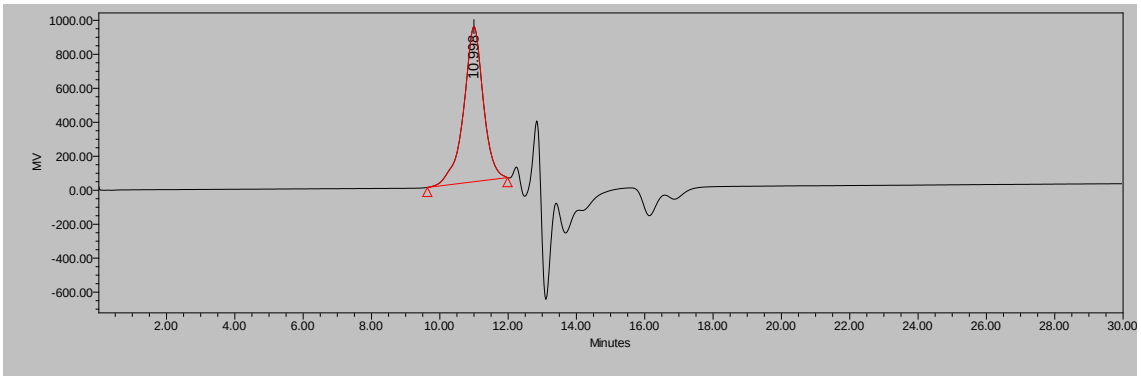
S23. IR (5)

-GPC HPLC

MAmBG-PEG2000-NHS (5)

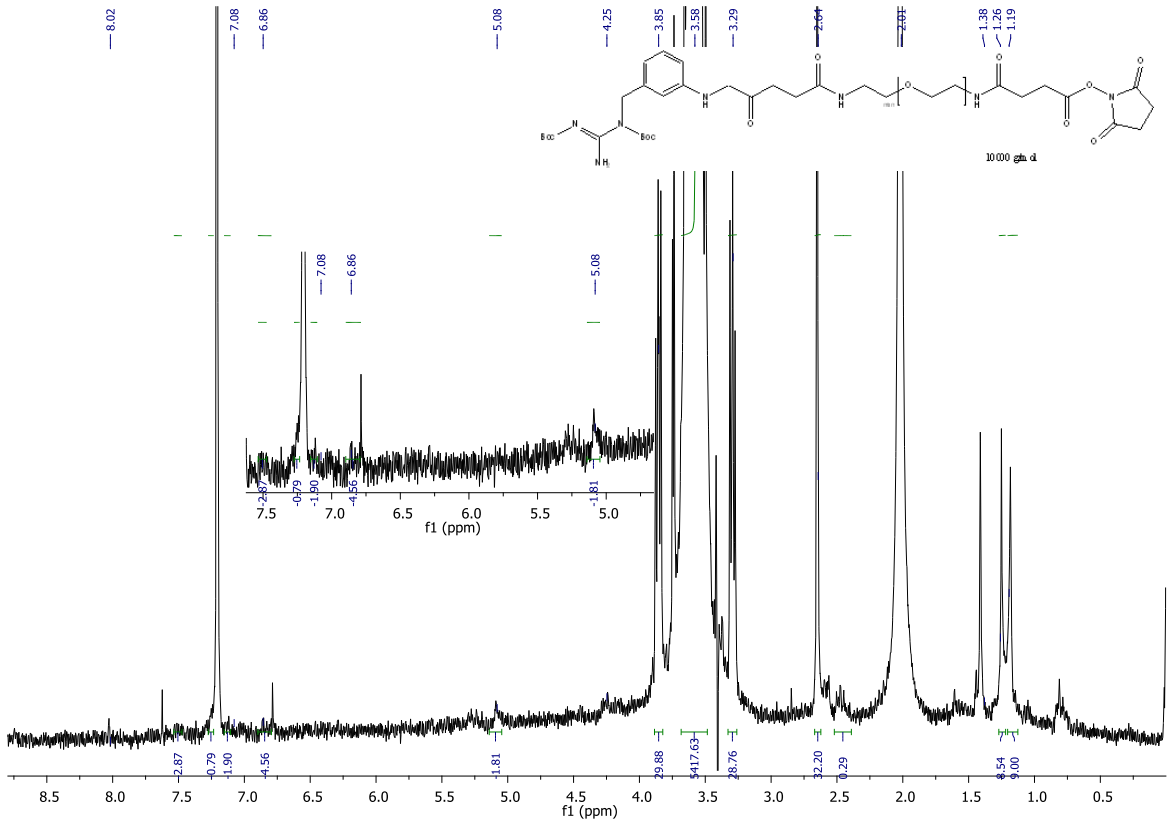


$(NHS)_2PEG2000$



MAmBG-PEG10000-NHS (6)

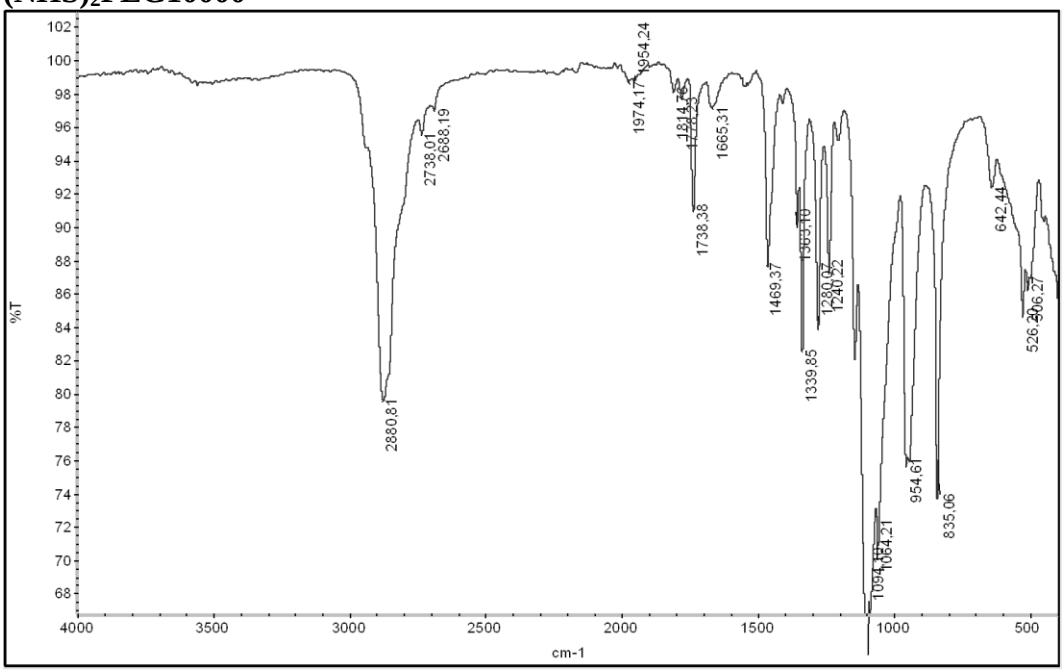
-NMR



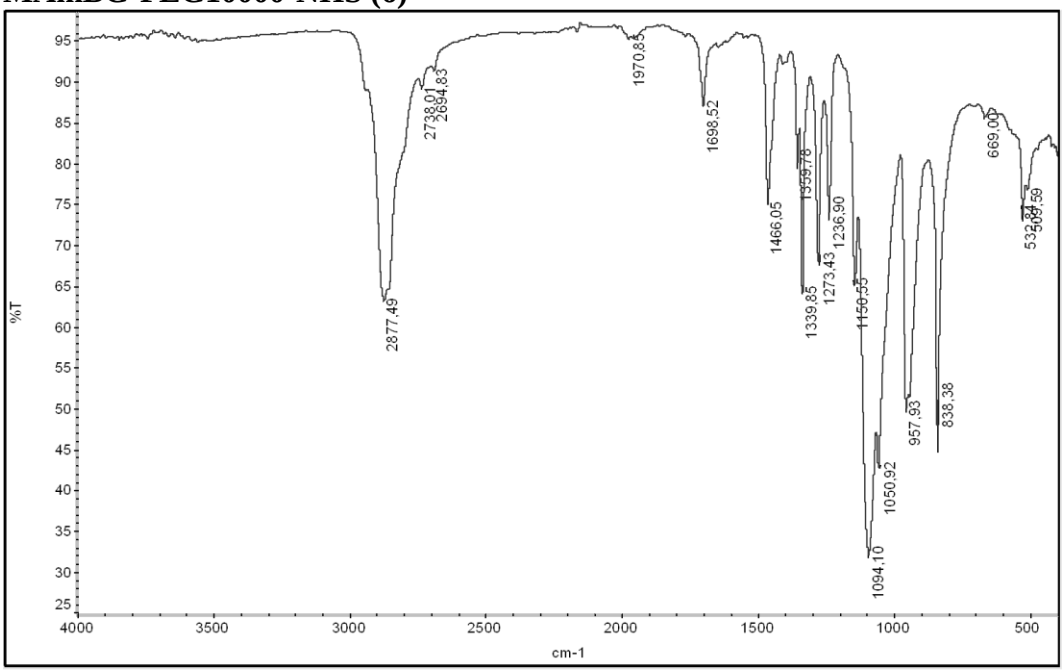
S24. ^{1}H-NMR (6)

-IR

$(NHS)_2$PEG10000

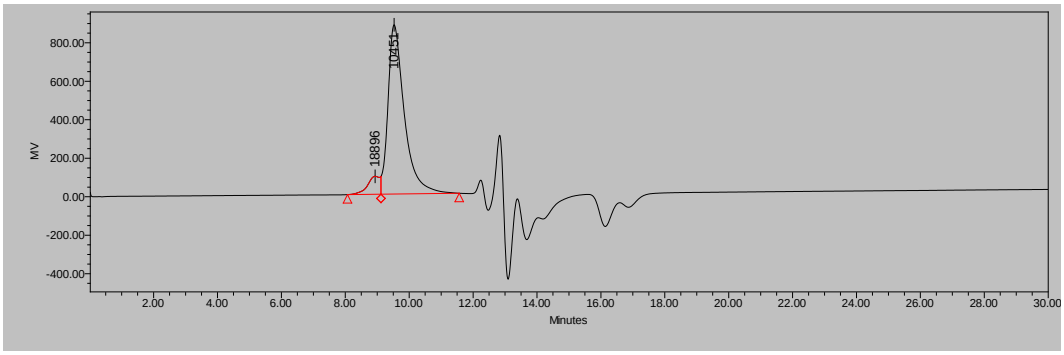


MAmBG-PEG10000-NHS (6)

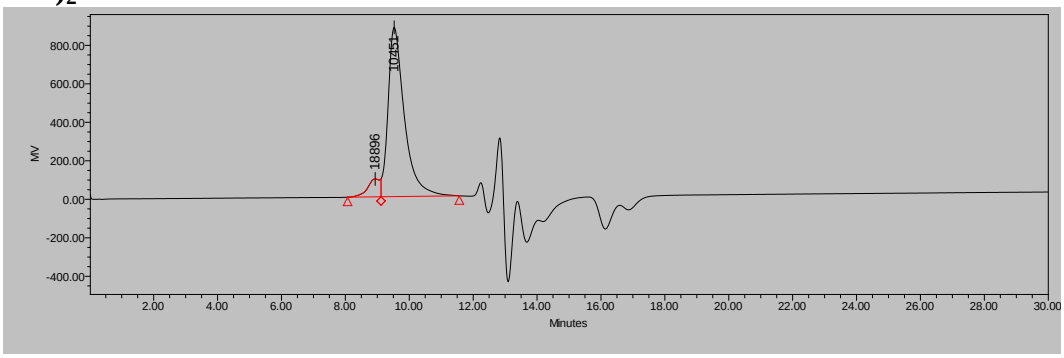


GPC/UV HPLC

MAmBG-PEG10000-NHS (6)



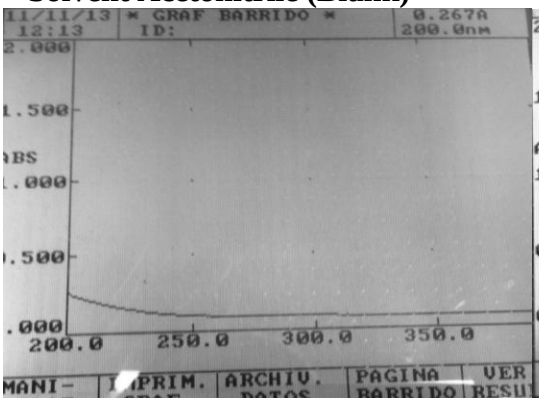
$(NHS)_2PEG10000$



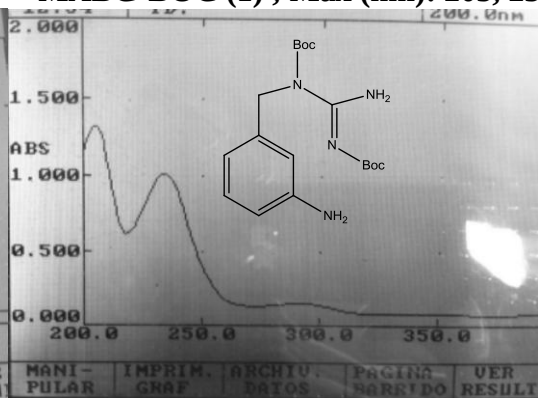
Determination of MABG content in PEG analogues (5) and (6) by UV/Vis Spectrometry

-MAmBG-PEG2000-NHS (5)

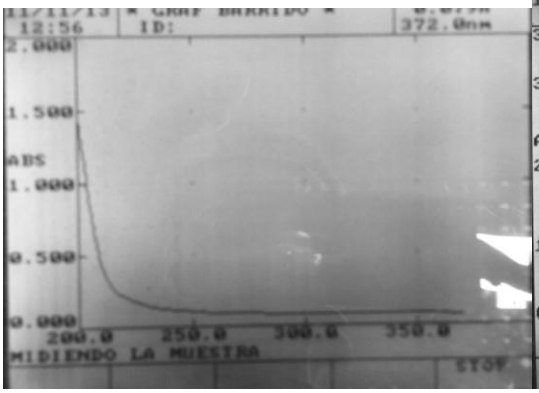
Solvent Acetonitrile (Blank)



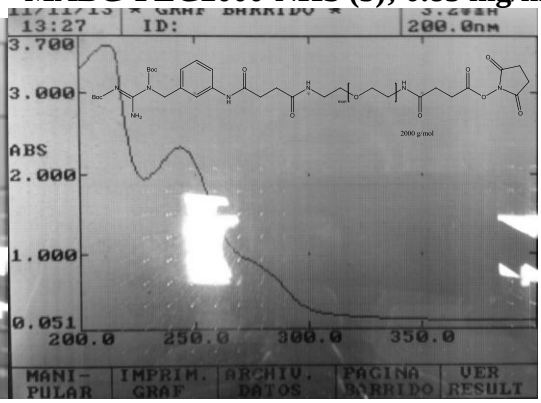
MABG-BOC (1) ; Max (nm): 205, 234, 293.



$(NHS)_2$-PEG2000 Starting material



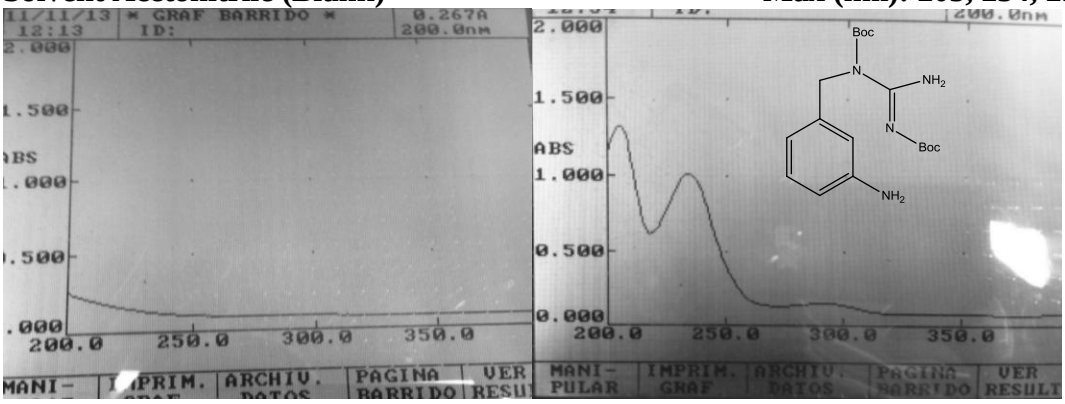
MABG-PEG2000-NHS (5); 0.85 mg/mL



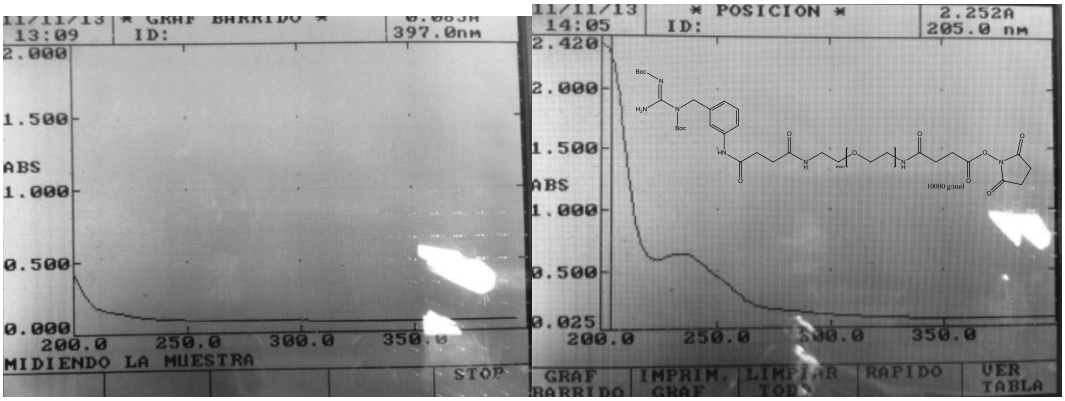
Max:
212 nm (3.594 A)
234 nm (2.340 A)
277 nm (0.887 A)

-MAmBG-PEG10000-NHS (6)

Solvent Acetonitrile (Blank) **Max (nm): 205, 234, 293.**



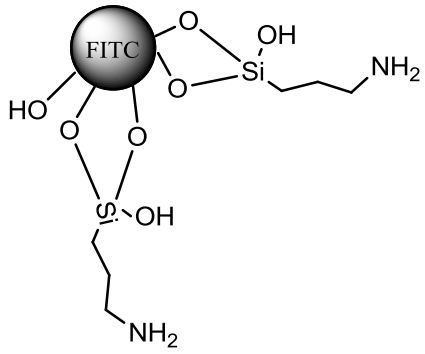
$(NHS)_2$-PEG10000 Starting material **MABG-PEG10000-NHS (6) (1.1 mg/mL)**



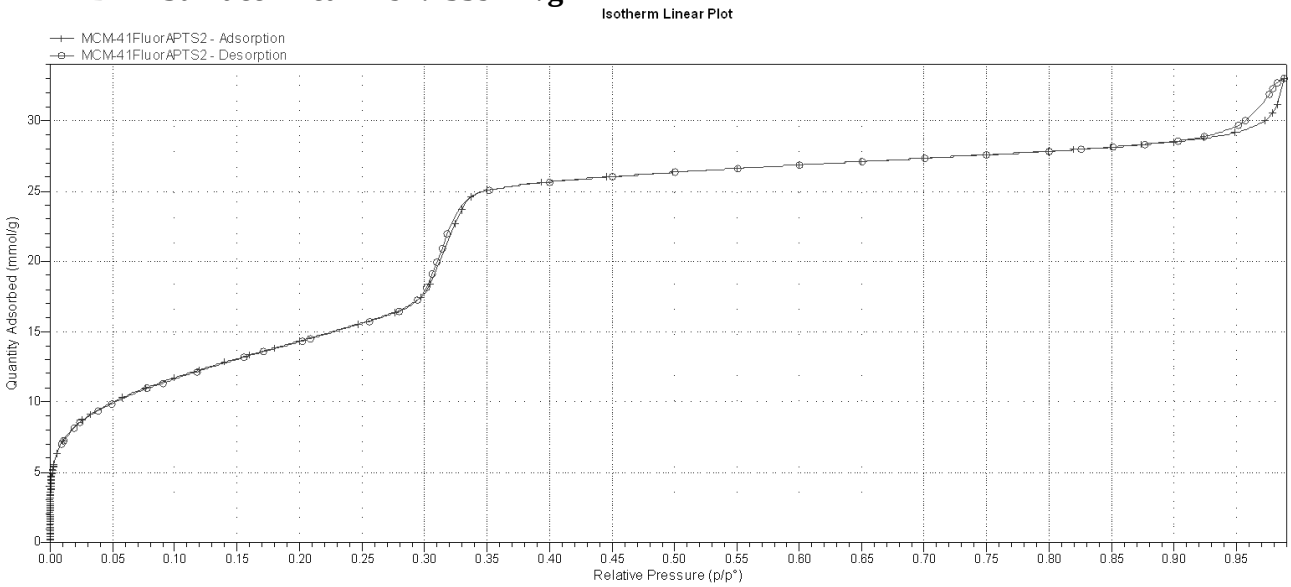
205 nm (2.252 A)
235 nm (0.557 A)

B. Synthesis of Functionalized nanoparticles

- B.1 MSN-F-NH_2

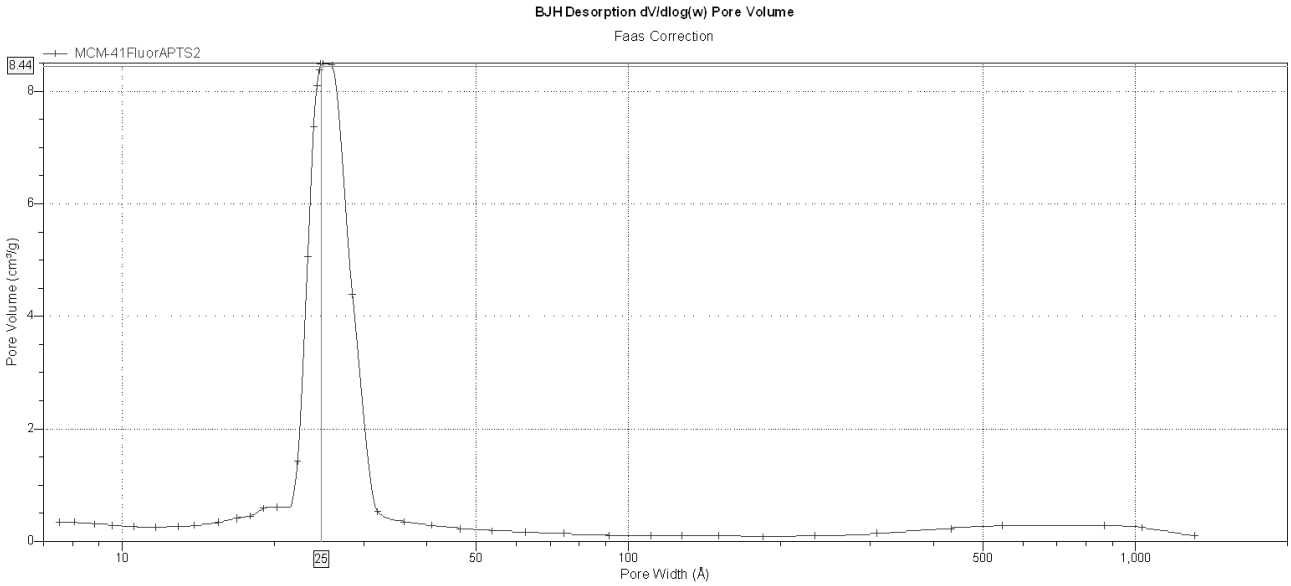


- **BET Surface Area 1192.4339 m^2/g**



S25. BET MSN-F-NH_2

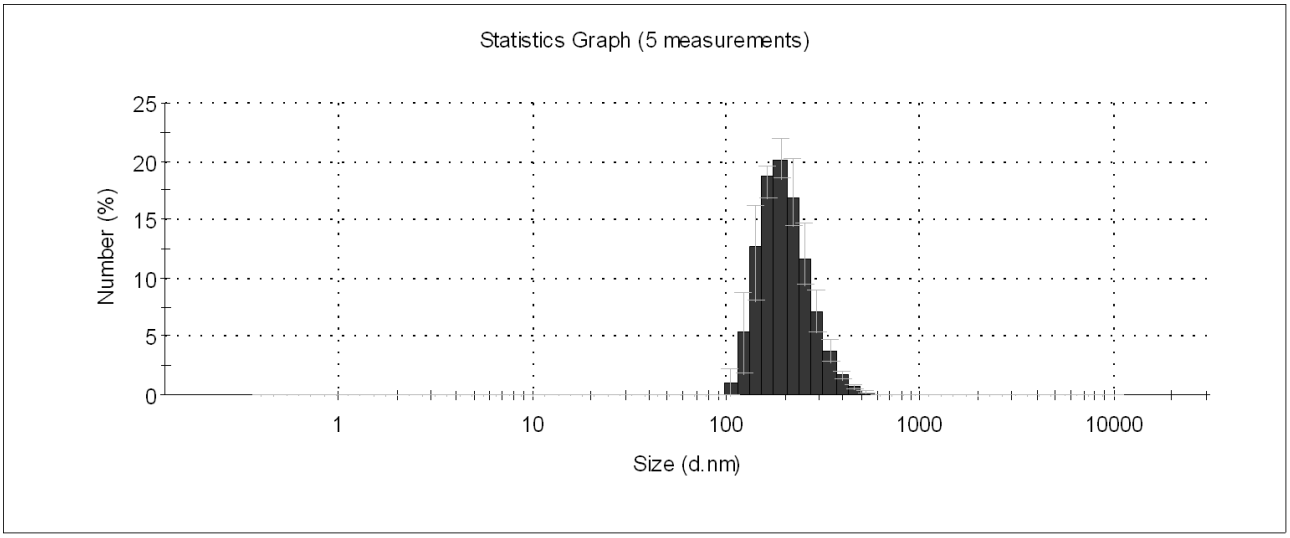
- **Pore Width 2.5 nm**



S26. Pore Width MSN-F-NH_2

- **Size**

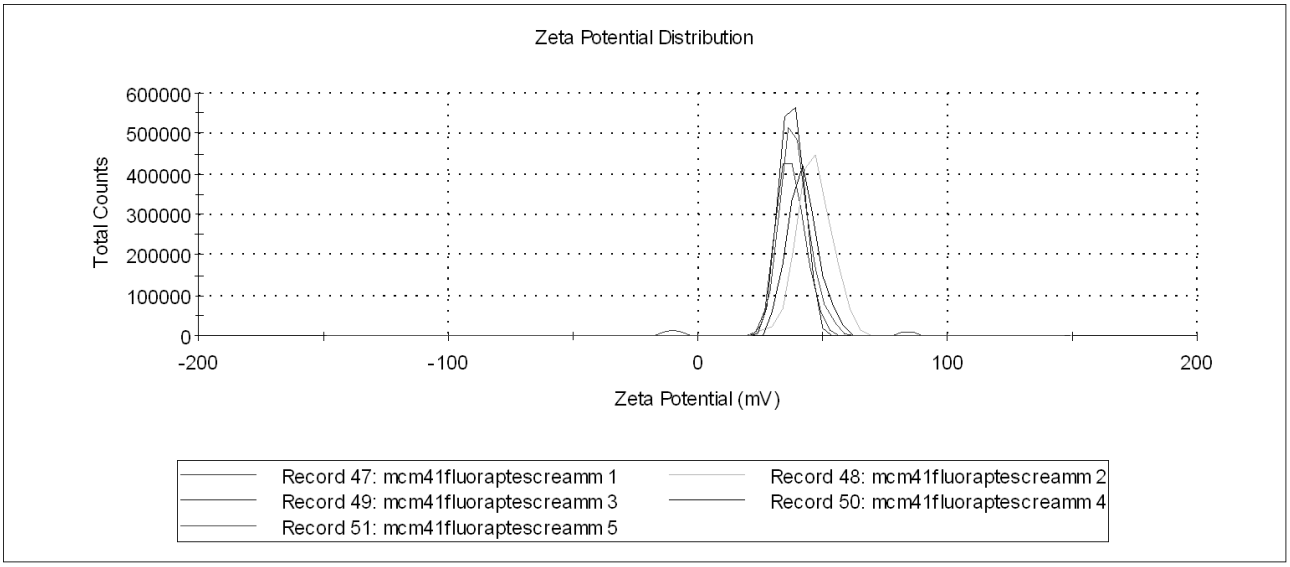
Mean (5)	**189.8**
Std Dev	1.276
RSD %	0.672



- **S27. Size MSN-F-NH_2**

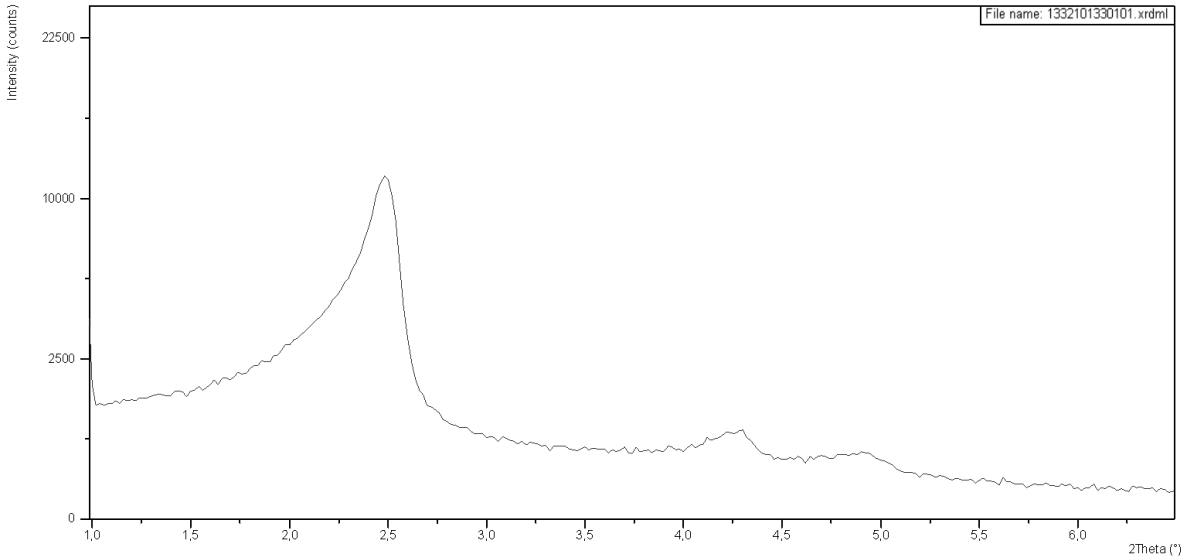
- **Z-Pot**

Mean (5) mV	+40.4
Std Dev	3.97
RSD %	9.81



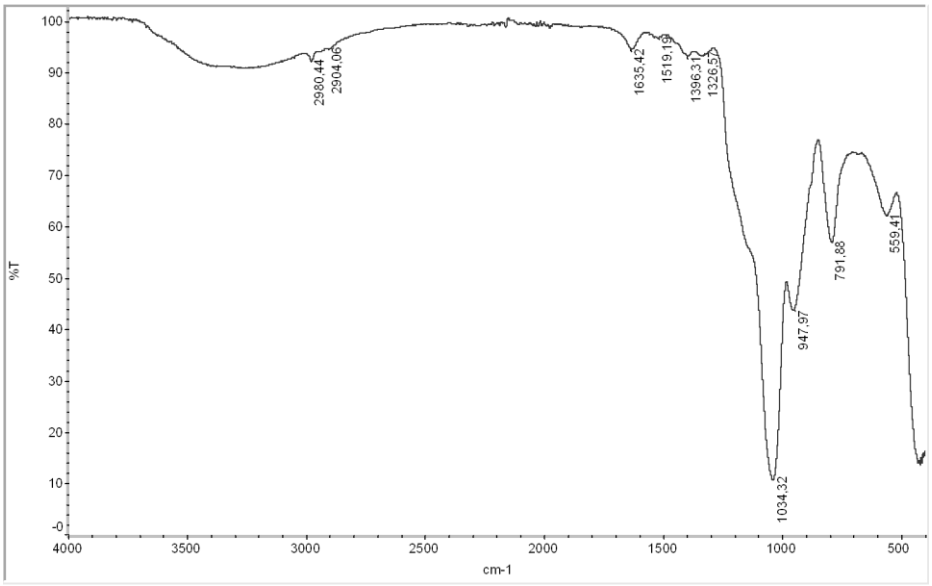
- **S28. Z-Pot MSN-F-NH_2**

- **XRD powder**



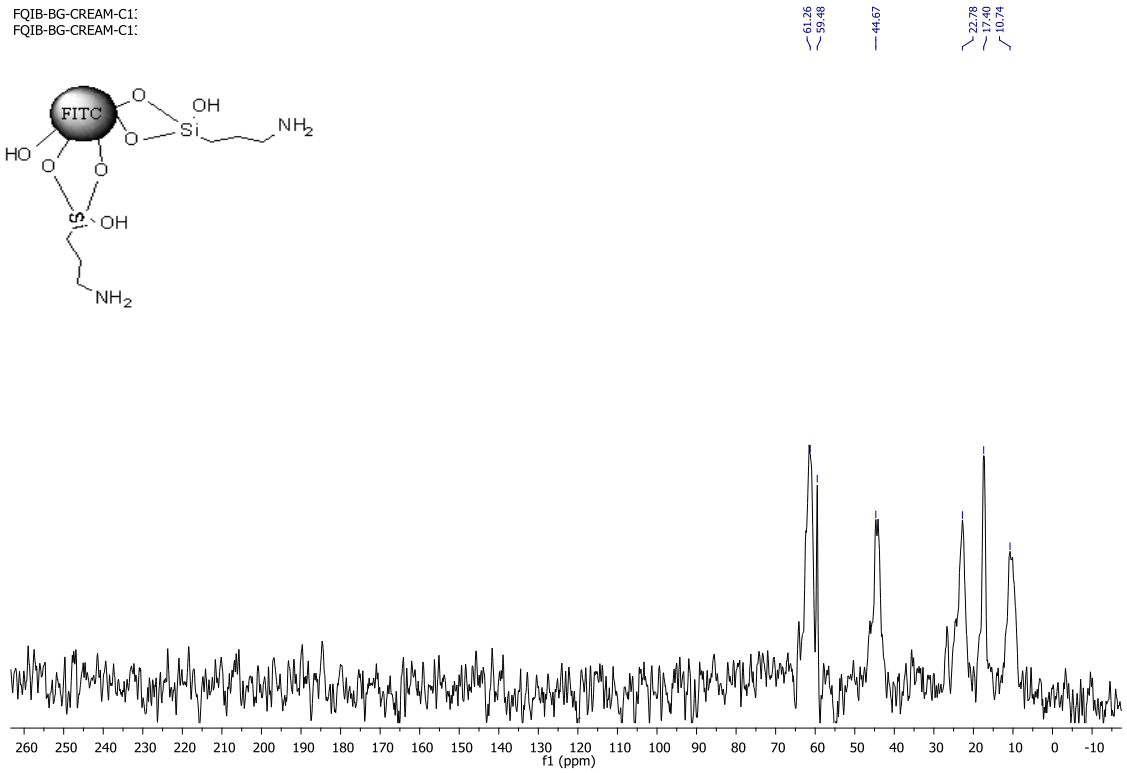
S29. XRD MSN-F-NH_2

- **FTIR** (υ; cm-1): 2980.4; 2904.1; 1635.4; 1519.2; 1396.3; 1326.6; 1034.3; 948.0; 791.9; 555.4.



S30. FTIR MSN-F-NH_2

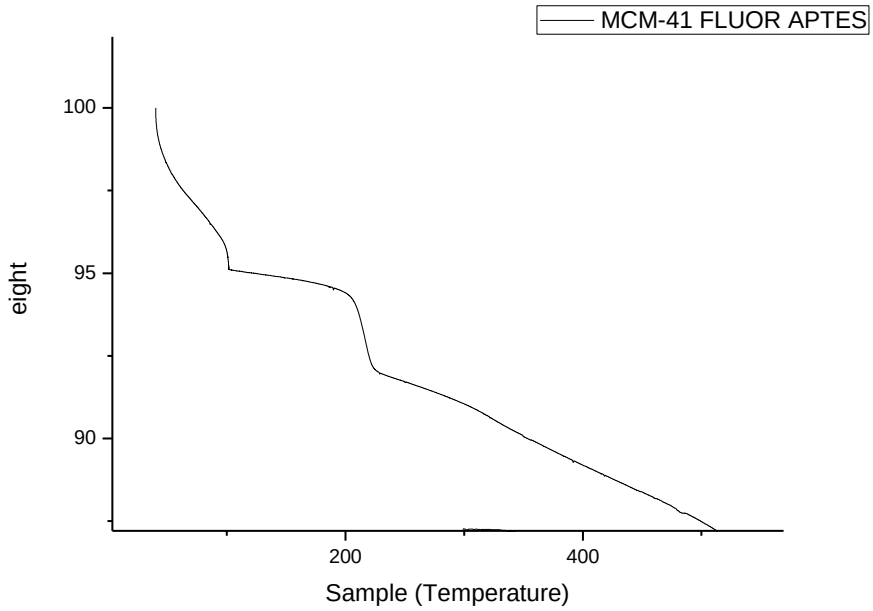
- ^{13}C-NMR MAS



- **S31. ^{13}C-NMR MAS MSN-F-NH_2**

^{13}C NMR (101 MHz, None) δ 61.26 (CH_3-$\underline{CH_2}$-O-Si), 59.48 (CH_3-$\underline{CH_2}$-O-Si), 44.67 ($\underline{CH_2}$-NH_2), 22.78 (CH_2-$\underline{CH_2}$-CH_2), 17.40 ($\underline{CH_3}$-CH_2-O -Si), 10.74 (CH_2-Si).

- **TG**

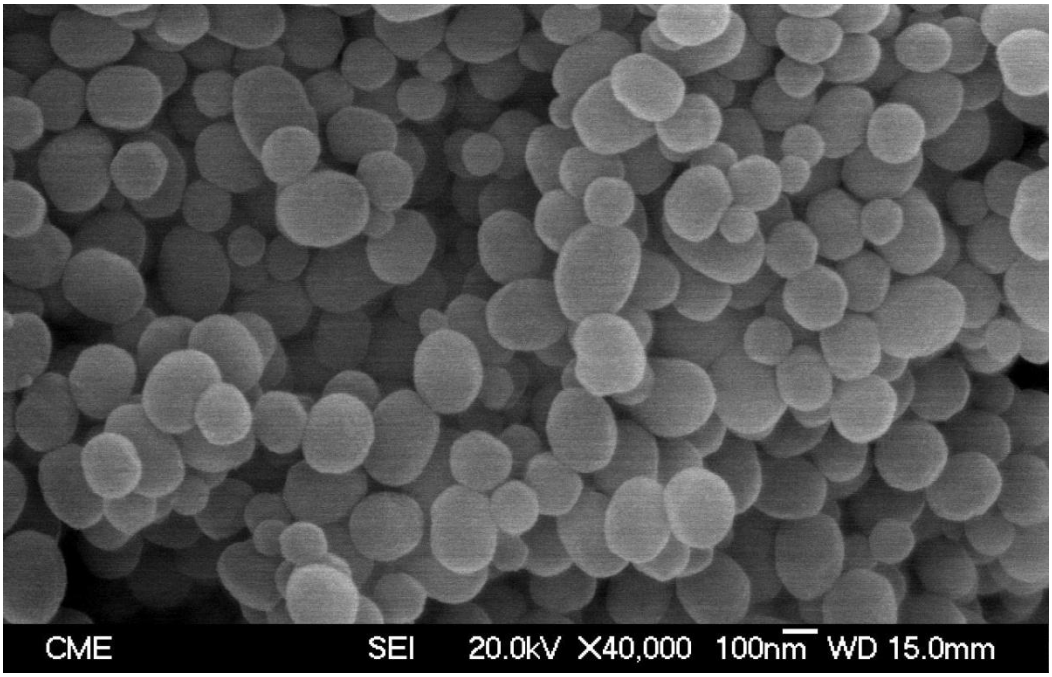


- **S32. TG MSN-F-NH_2**

100 ºC ……95.664 %

500 ºC…….87.48 % Lost 8.18 % or organic material.

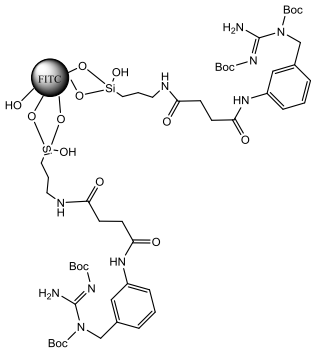
- **SEM**



S33. SEM MSN-F-NH_2

B.2 Functionalization of nanoparticles

-MSN-F-MAmBG-3-BOC



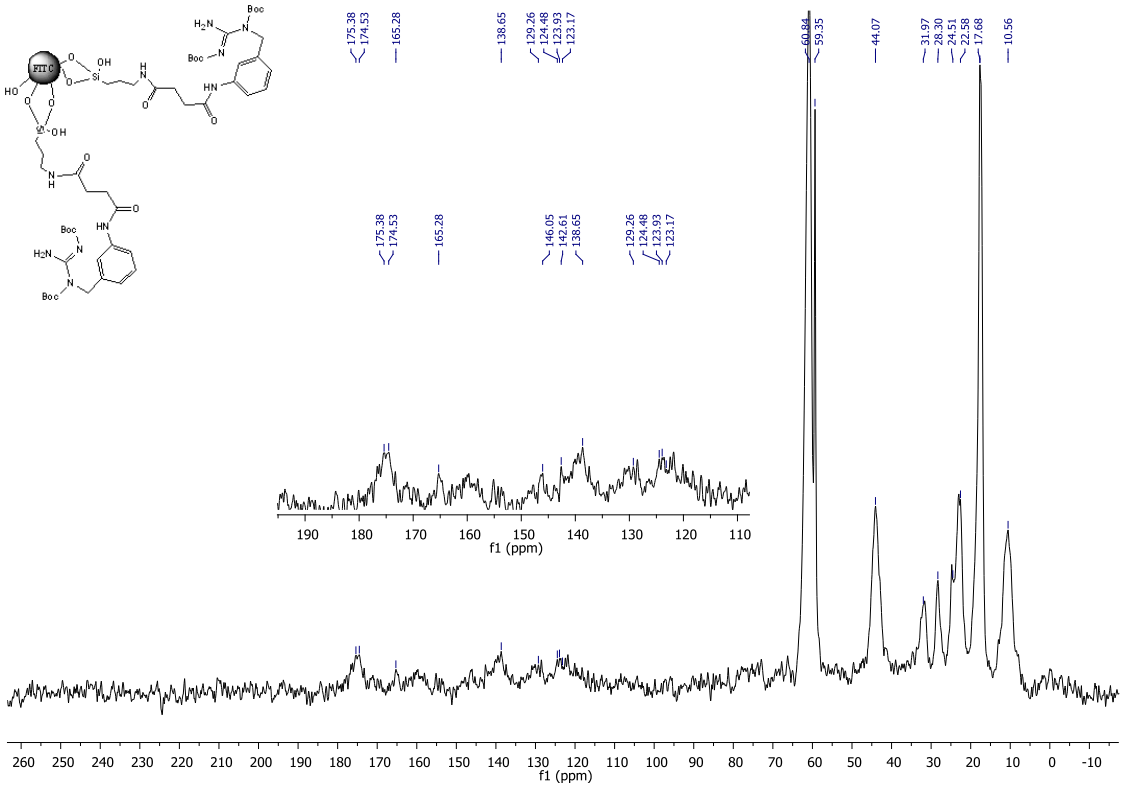
- **Size**

Mean (5)	214.3
Std Dev	5.288
RSD %	5.288

- Z-Pot

Mean (5)	36.6
Std Dev	0.983
RSD %	2.69

- **FTIR** (υ; cm-1) : 2980; 2933; 1947; 1847; 1642; 1555; 1200; 951; 791.
- ^{13}C-NMR MAS



- **S34. ^{13}C-NMR MAS MSN-F-MAmBG-3-BOC**

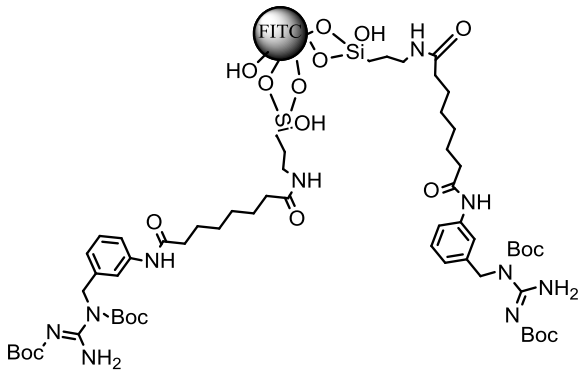
^{13}C NMR (101 MHz, None) δ 175.38(C=O), 174.53(C=O), 165.28(C=N), (aromatic signals)146.05, 138.65, 129.26, 124.48, 123.93, 123.17, 121.82, 60.84 ($\underline{CH_3}$-CH_2-O-Si), 59.35 ($\underline{CH_3}$-CH_2-O-Si), 44.07 ($\underline{CH_2}$-NH_2), 31.97 (CH_2-C=O), 28.30 (CH_2-$\underline{CH_2}$-CH_2 APTES, CH_3,BOC), 22.58(CH_2-$\underline{CH_2}$-CH_2 APTES, CH_3BOC), 17.68 ($\underline{CH_3}$-CH_2-O -Si), 10.56 (CH_2-Si).

- **TG**

100 ºC ……95.09431 %

500 ºC…….76.38038% Lost 18.71393 % or organic material.

-MSN-F-MAmBG-4-BOC



- **Size**

Mean (5)	188.6
Std Dev	4.386
RSD %	2.33

- **Z-Pot**

Mean (5)	24.1
Std Dev	0.329
RSD %	1.36

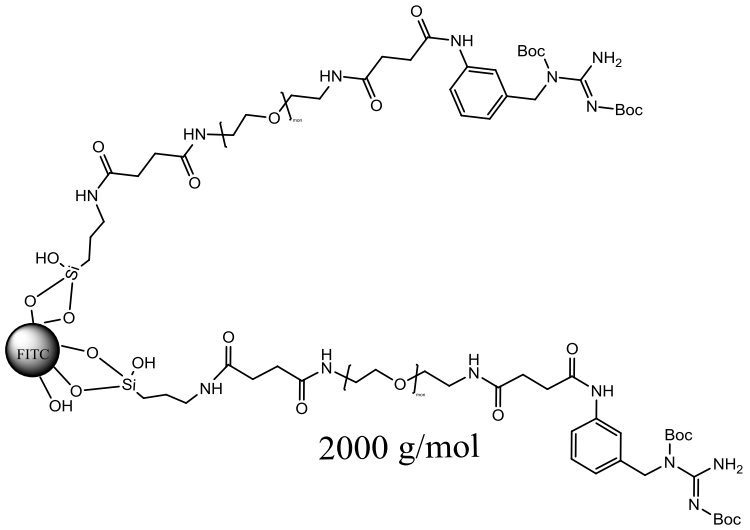
- **FTIR** (υ; cm-1): 2977; 2943; 1851; 1636; 1448; 1386; 1216; 1070; 954; 808.

- **TG**

100 ºC……96.12715 %

500 ºC……84.84642 %.....Lost 11.28075% of organic material

-MSN-F-MAmBG-4-BOC



- **Size**

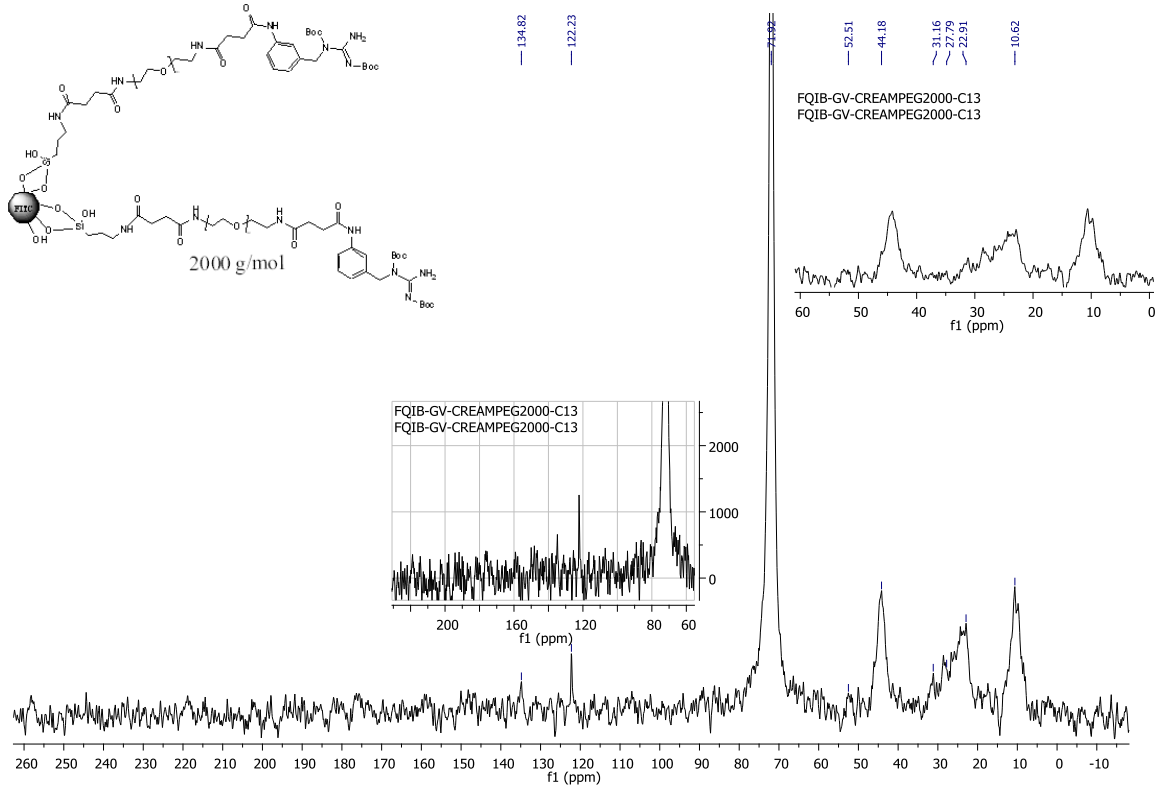
Mean (5)	**207.6**
Std Dev	7.685
RSD %	3.7

- **Z-pot**

Mean (5)	**19.1**
Std Dev	0.552
RSD %	2.89

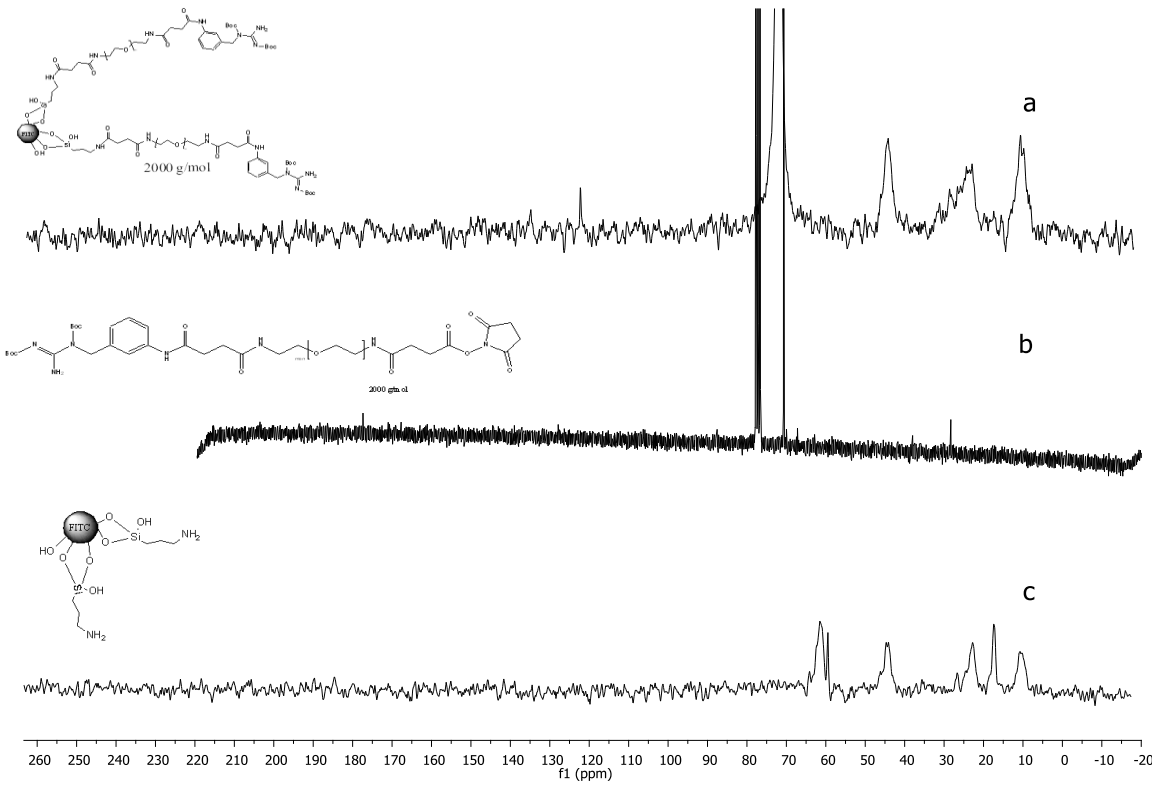
- FTIR (υ; cm-1) : 2937; 2870; 2283; 1980; 1695; 1632; 1535; 1412; 1343;1200; 947; 801; 559.

- ^{13}C-NMR MAS



- **S35. ^{13}C-NMR MAS MSN-F-MAmBG-4-BOC**

^{13}C-NMR (101 MHz, None) δ 134.82 (C_{Ar}), 122.23 (CH_{Ar}), 71.92(CH_2, PEG), 52.51(CH_3-$\underline{CH_2}$-O-Si, APTES; CH_2, guanidine), 44.18 ($\underline{CH_2}$-NH_2, APTES), 31.16 (CH_2-CO-NH), 27.79((CH_2-$\underline{CH_2}$-CH_2, APTES; CH_3, BOC; CH_2, succinic moiety), 22.91(CH_2-$\underline{CH_2}$-CH_2, APTES), 10.62(CH_2-Si, APTES).



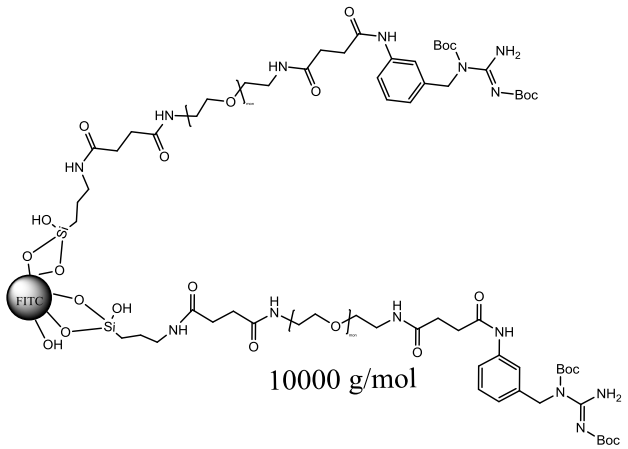
S36. ^{13}C-NMR MAS; a) MNS-F-MAmBG-5, b) compound (5), c) MSN-F-NH_2.

- **TG**

  100 ºC…… 96.58964%

  500 ºC……80.10971 %.....Lost 16.47993 % of organic material

-MSN-F-MAmBG-4-BOC



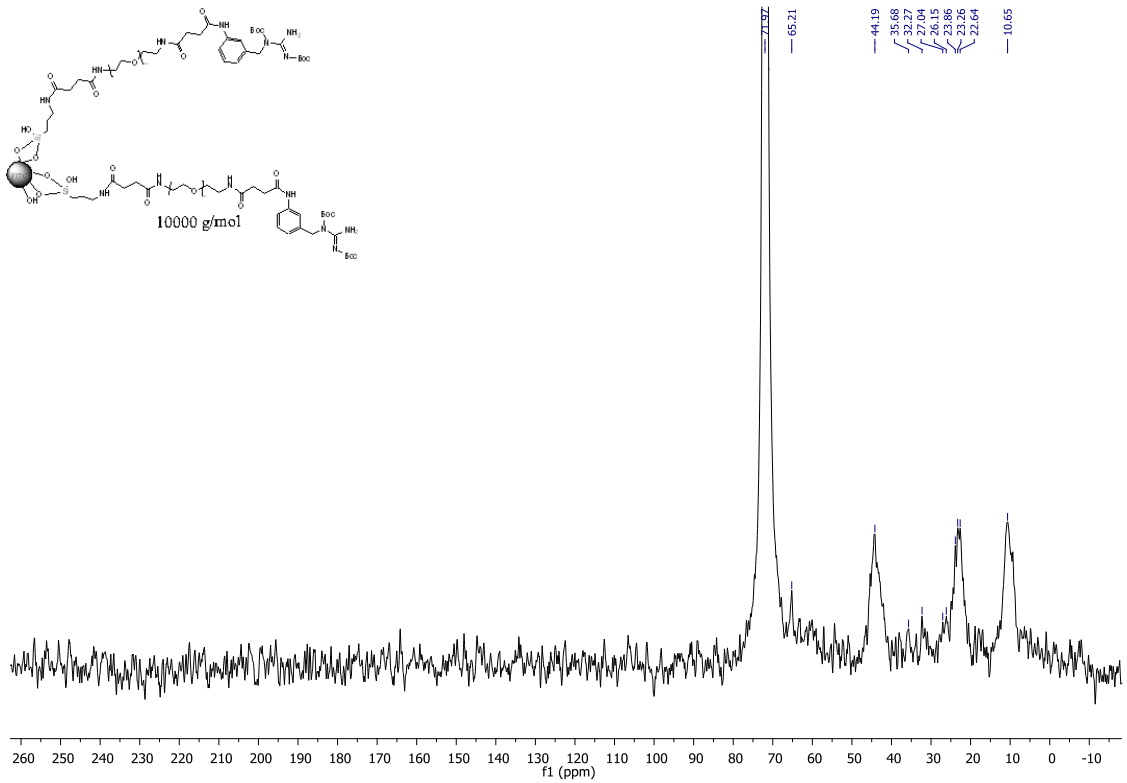
- **Size**

Mean (5)	**214.6**
Std Dev	14.03
RSD %	6.54

- Z-pot

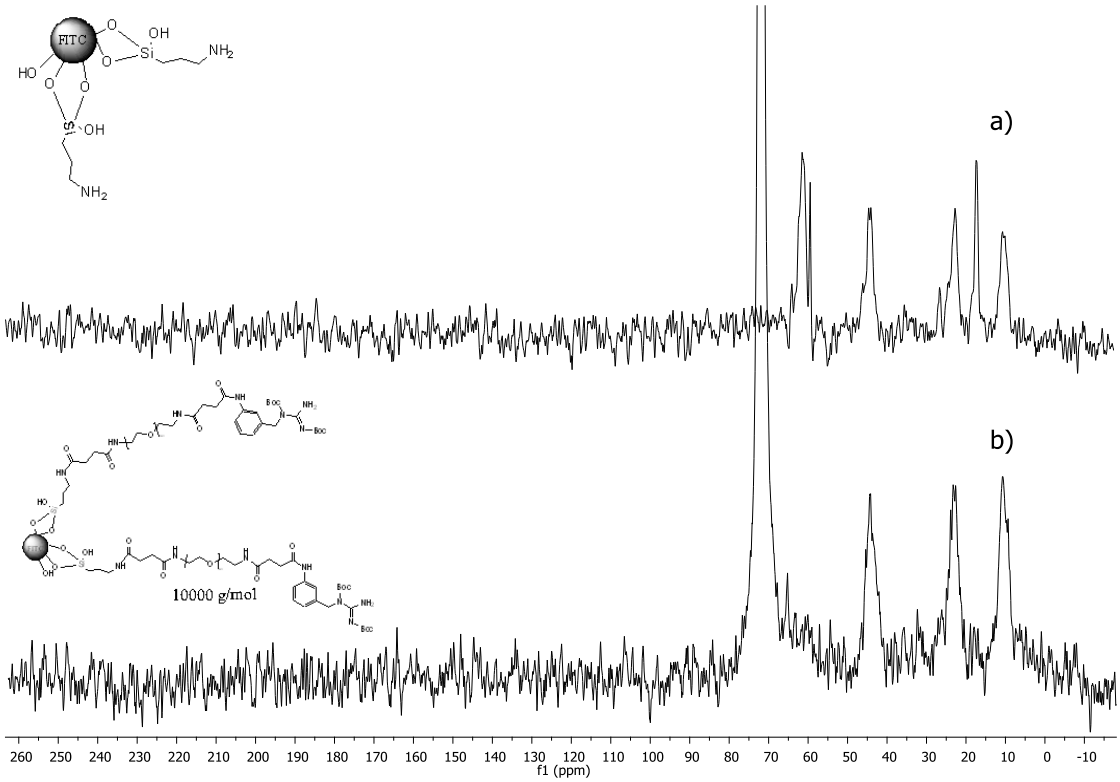
Mean (5)	12.1
Std Dev	0.607
RSD %	5

- ^{13}C-NMR MAS



S37. ^{13}C-NMR MAS MNS-F-MAmBG-6.

^{13}C NMR (101 MHz, None) δ 71.97 (CH_2, PEG), 65.21 (CH_2, PEG), 44.19 ($\underline{CH_2}$-NH_2, APTES), 35.91 (CH_2-CO-NH), 32.27 (CH_2-CO-NH), 27.04 (CH_3, BOC; CH_2-$\underline{CH_2}$-CH_2, APTES; CH_2, succinic moiety), 26.15 (CH_3, BOC; CH_2-$\underline{CH_2}$-CH_2, APTES; CH_2, succinic moiety), 23.86 (CH_2-$\underline{CH_2}$-CH_2, APTES), 23.26 (CH_2-$\underline{CH_2}$-CH_2, APTES), 22.64 (CH_2-$\underline{CH_2}$-CH_2, APTES), 10.65 (CH_2-Si, APTES).



S38. ^{13}C-NMR MAS; a) MSN-F-NH_2, b) MNS-F-MAmBG-6.

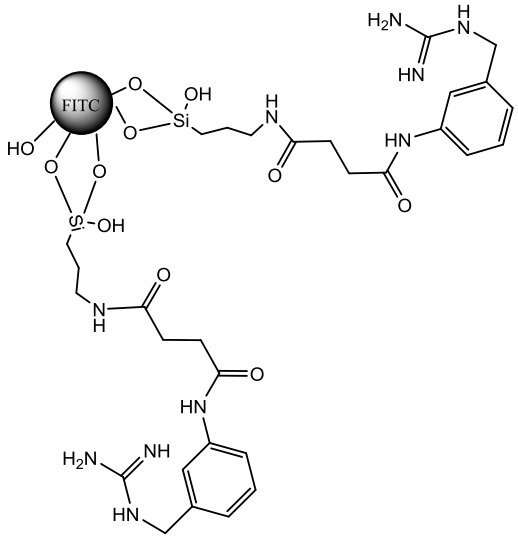
- FTIR (υ; cm-1) : 2923; 2877; 1977; 1635; 1532; 1456; 1353; 1200; 947; 788; 559.

- **TG**

100 ºC……99.13686 %
500 ºC…… 78.91454%.....Lost 20.22232% of organic material

B.3 Nanomaterial guanidine deprotected

-MSN-F-MAmBG-3



- **Size**

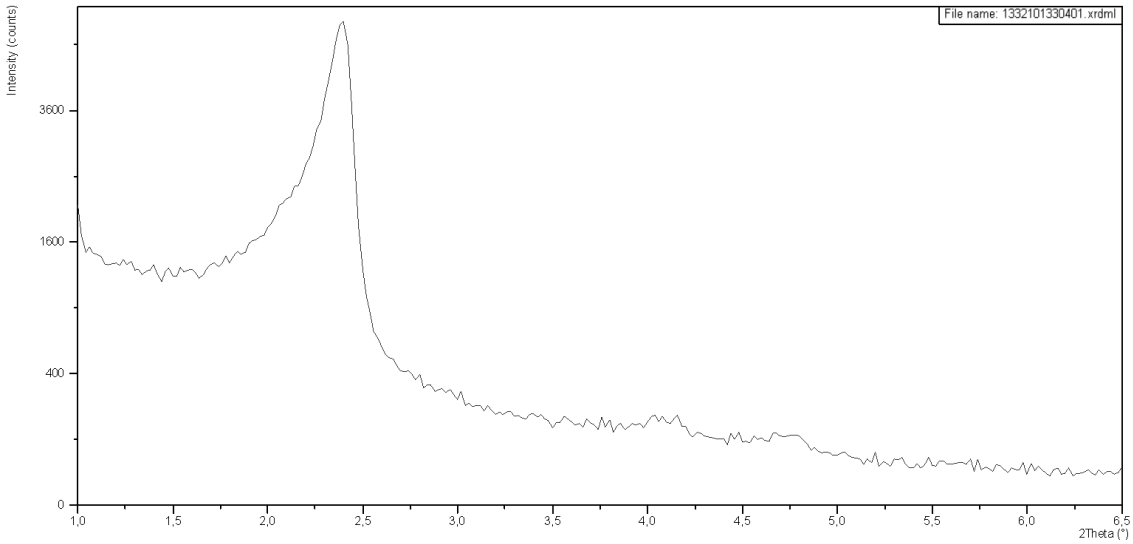
Mean (5)	231.4
Std Dev	14.74
RSD %	6.36

- **Z-Pot**

Mean (5)	**29.0**
Std Dev	1.78
RSD %	2.27

- **FTIR** (υ; cm-1) : 2967; 1977; 1661; 1542; 1260; 1040; 954; 851; 805; 715; 552.

- **XRD powder**

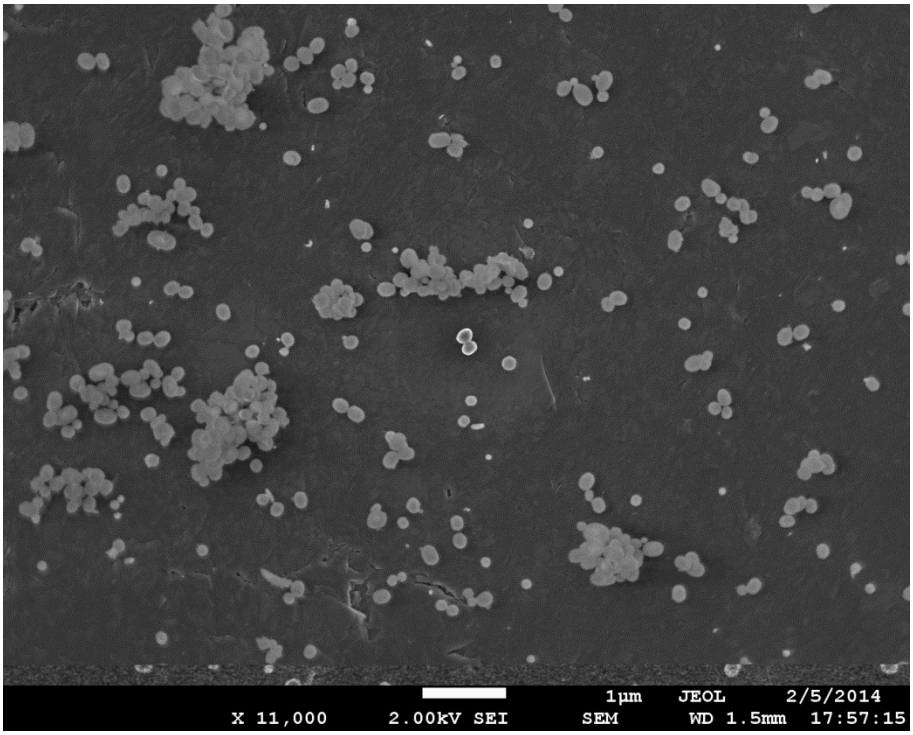


S39. XRD MSN-F-MAmBG-3

- **TG**

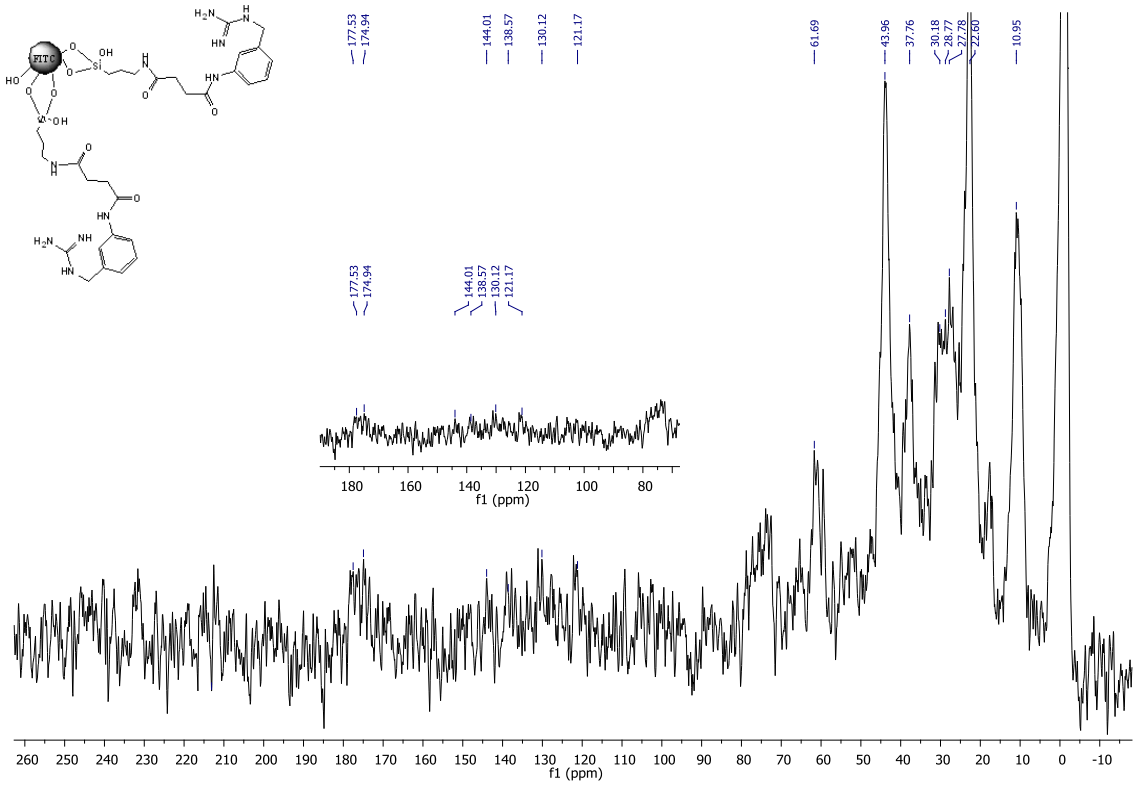
100 ºC…… 98.01542 %
500 ºC…… 79.85624 %.....Lost 18.15918 % of organic material.

- **SEM**



S40. SEM MSN-F-MAmBG-3

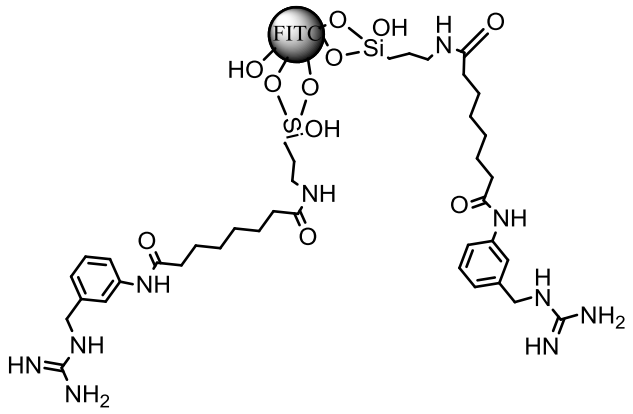
- ^{13}C-NMR MAS



- **S41. ^{13}C-NMR MAS MSN-F-MAmBG-3**

^{13}C NMR (101 MHz, None) δ, 177.53(C=O), 174.94(C=O), (aromatic signals)144.01, 138.57, 130.12, 121.17, 61.69($\underline{CH_3}$-CH_2-O-Si), 43.96($\underline{CH_2}$-NH_2), 37.76(CH_2-C=O), 30.18(CH_2-C=O), 28.77 (CH_3, BOC; CH_2-$\underline{CH_2}$-CH_2, APTES; CH_2, succinic moiety), 27.78 (CH_3, BOC; CH_2-$\underline{CH_2}$-CH_2, APTES; CH_2, succinic moiety), 22.60 (CH_2-$\underline{CH_2}$-CH_2), 10.95 (CH_2-Si).

- MSN-F-MAmBG-4



- **Size**

Mean (5)	176.5
Std Dev	3.756
RSD %	2.13

- **Z-Pot**

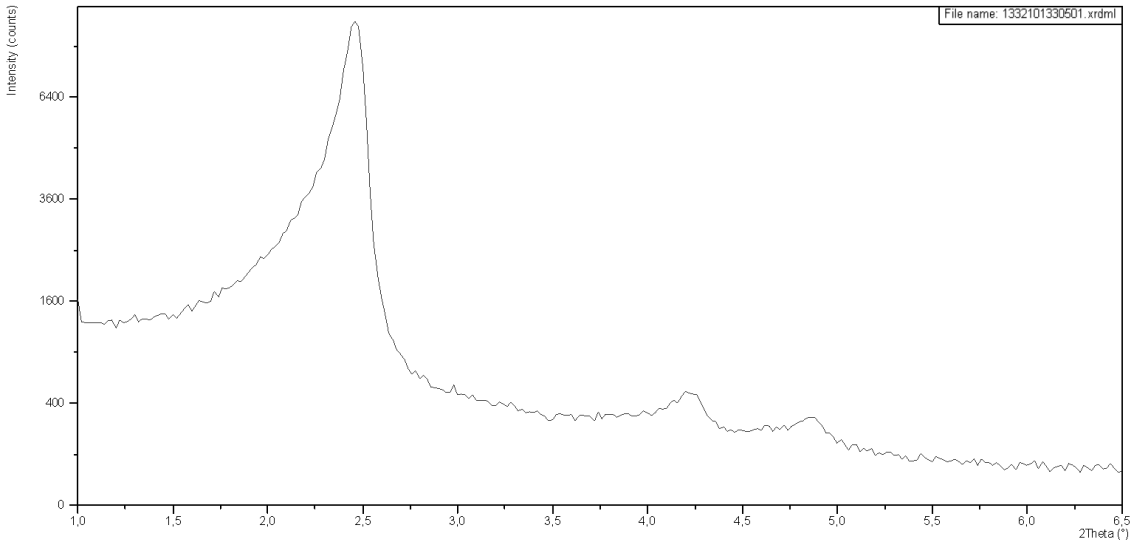
Mean (5)	35.6
Std Dev	1.03
RSD %	2.88

- **TG**

100 ºC…… 97.64331 %

500 ºC…… 82.14358 %.....Lost 15,49981 % of organic material

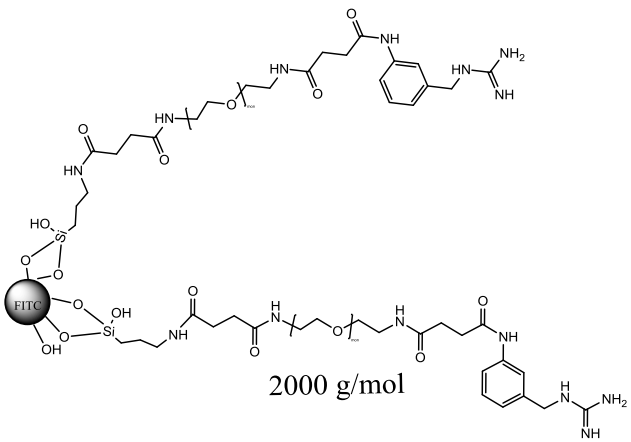
- **XRD powder**



S42. XRD MSN-F-MAmBG-4

- **FTIR** (υ; cm-1): 2973; 1648; 1442; 1403; 1150; 1047; 954; 795; 549.

- MSN-F-MAmBG-5



- **Size**

Mean (5)	237.9
Std Dev	19.89
RSD %	8.36

- **Z-pot**

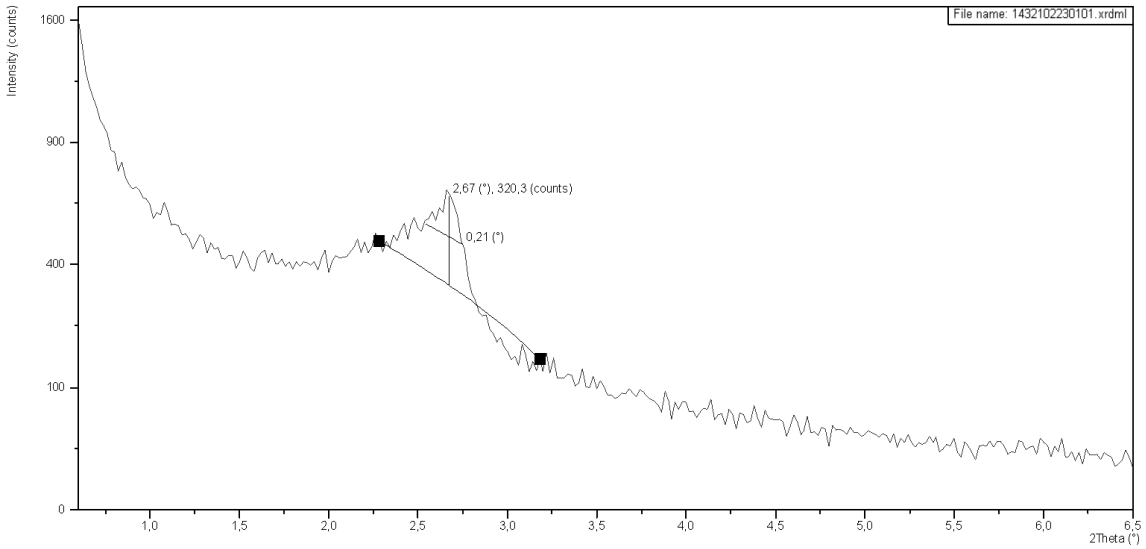
Mean (5)	24.2
Std Dev	0.524
RSD %	2.17

- **TG**

100 ºC……95.81783 %
500 ºC…… 83.37829%.....Lost 12.43954% of organic material

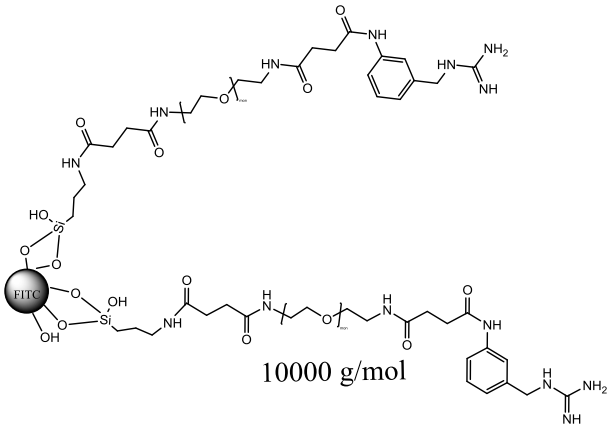
- **FTIR** (υ; cm-1): 2973; 2920; 1980; 1678; 1635; 1150; 1064; 954; 798; 443.

- **RX powder**



S43. XRD MSN-F-MAmBG-5

- MSN-F-MAmBG-6



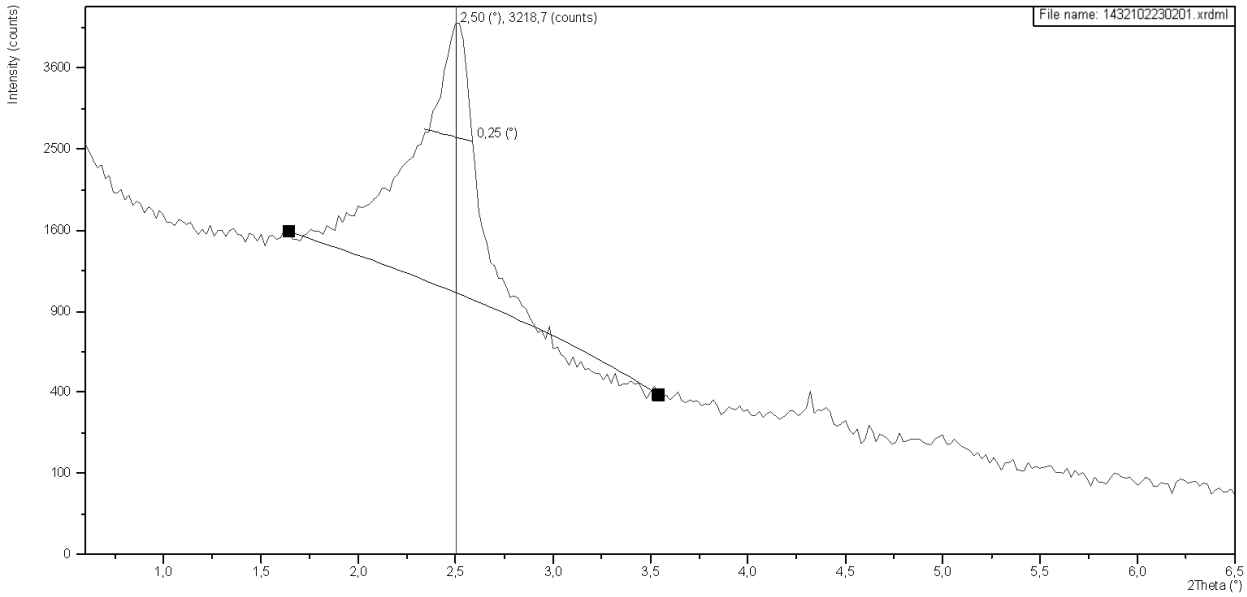
- **Size**

Mean (5)	**226.3**
Std Dev	32.65
RSD %	14.4

- **Z-pot**

Mean (5)	**35.3**
Std Dev	0.517
RSD %	1.46

- **FTIR** (υ; cm-1): 2977; 2910; 1665; 1648; 1462; 1393; 1153; 1050; 957; 801; 565; 443.
- **RX powder**



S44. XRD MSN-F-MAmBG-6

- TG

100 ºC……93.59214 %

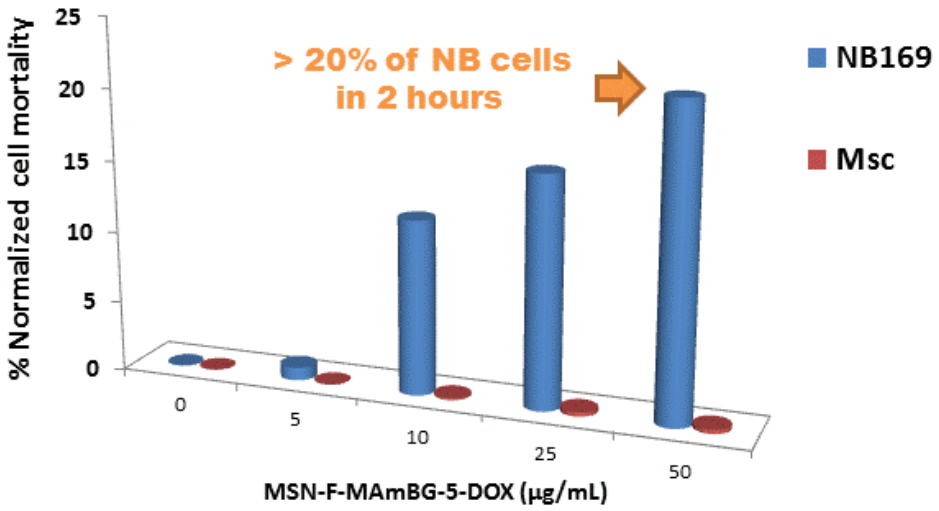
500 ºC…… 78.14645%.....Lost 15.44569% of organic material

C.1 Cytotoxicity In vitro assays

The capacity to deliver cytotoxic drugs to NB cells was evaluated using PEGylated material MSN-F-MAmBG-5 which exhibited remarkable targeting ability. The targeted nanovehicle was loaded with doxorubicin hydrochloride by suspension of 11.6 mg of MSN-F-MAmBG-5 particles in 1.5 mL of 5.3 mg/mL Dox PBS solution. The suspension was stirred for 24 h and the nanomaterial were scrupulously washed with H_2O twice (10mL x 2) and dried in vacuum.

NB1691-luc and human-bone marrow mesenchymal stem cells (MSC) were seeded in 24-well plates at 5000 cells/cm^2. Cells underwent a dose response test ranging from 0 to 50 µg/ml of MSN-F-AmBG-5 or DOXO-MSN-F-AmBG-5 for 2 hours at 37ºC, 5% CO2 and 95% humidity. Cells were then washed and harvested. Cell viability was evaluated by flow cytometry with 7AAD (Biolegends, San Diego, CA.) following the manufacturer instructions. Percentages of dead cell populations are shown as the normalized mean of two independent replicates.

The cytotoxicity of the doxorubicine loaded material MSN-F-MAmBG-5-DOX was studied in increasing concentrations for both mesenchymal (Msc) and neuroblastoma cells culture. (Figure S.45).



S45. Cytotoxic effects of nanocarriers. Data normalized with percentage of basal mortality without of citotoxic loaded nanoparticles, in 2 hours.

Mesenchymal cells were chosen as negative control because they do not exhibit NET on their membrane and they are present in the tumor-surrounding as supportive cells. The targeted vehicle showed excellent selective cytotoxic effect in all concentrations studied. Tumor cells mortality correlated with the increase of nanoparticles concentration: more than 20% NB cells

died at concentration of 50 µg/mL in short period of time (2 hours). On the other hand, mechenchymal cells were barely affected; even at the highest carrier concentration (50 µg/mL).