Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.
This journal is © The Royal Society of Chemistry 2014

Electronic Supplementary Information (ESI)

Title: Thermo-responsive and Shape Transformable Amphiphilic Scaffolds for Loading and Delivering Anticancer Drugs

Authors: Smita Kashyap and Manickam Jayakannan*

Synthesis of *tert*-butyl (2-(3-pentadecy-8-en-1-yl) phenoxy) ethyl) carbamate (CAR-amine-Boc): Compound **2** (3g, 18.6mmol)), Cardanol (5.62g, 18.6mmol) and triphenylphosphine (5.34g, 20.4mmol) was dissolved in of dry tetrahydrofuran (40 ml). The reaction mixture was then kept in ice-cooled bath for next 10 min along with N_2 purging. Diisopropyl azodicarboxylate (3.62ml, 17.7mmol) was added drop wise and reaction mixture was stirred at 25 ˚C for 24 h. The mixture was directly loaded in silica gel column of 60-120 and was eluted using 2.5% ethyl acetate in hexane as eluent.Yield = 4.1g (49 %).^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.19 ppm (t, 1H, Ar-**H**), 6.83-6.73 ppm (m, 3H, Ar-**H**),5.38 ppm (d,2H, C**H**=C**H**), 5.02 ppm (s, 1H, N**H**), 4.03 ppm (t, 2H, Ar-OC**H**$_2$), 3.53 ppm (t, 2H, C**H**$_2$-N), 2.58 ppm (t, 2H, Ar-C**H**$_2$), 2.05 ppm (m, C**H**$_2$ CH=CHC**H**$_2$), 1.46 ppm (s, 9H, OC-C(C**H**$_3$)$_3$, 1.6-0.88 ppm (m, 21H, Aliphatic H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 158.62(Ar-C), 155.98 (**C**O-O), 144.85, 129.29, 121.32, 114.75, 111.40 (Ar-C), 130.03 (**C**H=**C**H), 79.57 (O**C** $(CH_3)_3$, 67.08 (Ar-OCH_2), 40.26(C**H**$_2$-N), 36.09, 32.00, 29.76, 26.47, 22.77, 14.20. FT-IR (cm^{-1}): 3396, 2916, 2850, 1690, 1590, 1512, 1453, 1362, 1250, 1157, 1060, 959, 866, 778, 690.MALDI-TOF-MS: m/z calculated for $C_{28}H_{47}NO_3$: 445.68 and Found: 468.23 (M^+ + Na^+).

Synthesis of 2-(2-(2-methoxyethoxy)ethoxy)ethyl 4-oxo-4-((2-(3-pentadec-8-en-1-yl)phenoxy)ethyl)amino)butanoate (CAR-TEG):): Trifluoroacetic acid (14.81 ml, 67.4 mmol) was added drop wise in to Car-amine-Boc (2.87 g, 6.45 mmol) in dichloromethane (7 ml). After string the reaction mixture for 1 h at 25 ˚C the solvent was removed by rotavapour. Fresh dichloromethane (5mL) was added to the product and washing was repeated for 3 times to remove TFA. The content was poured in ice-cooled diethyl ether (15 mL). The yellow liquid (0.64 g, 1.85 mmol) was dissolved in dry dichloromethane (15 ml) under N_2atmosphere.. To this reaction mixture **1c** (0.443 g, 1.68 mmol) was added and purging was continued for another 15 minutes. Then DCC (0.414 g, 2.06 mmol) and DMAP (0.021 g, 0.168 mmol) were added to the reaction mixture under nitrogen atmosphere and the reaction was continued for 24 h at 25 ˚C. The mixture was poured into water (30 mL) and extracted with chloroform (20 mL). The organic layer obtained was neutralized with 2N HCl (2 mL), washed with aqueous 5% $NaHCO_3$ (50 mL) and brine. After drying over anhydrous sodium sulphate, the solvent was removed to obtain pale yellow liquid as product. It was further purified by passing through silica gel column of 60-120 mesh using 25% methanol in chloroform as eluent. Yield = 0.22 g (23.0 %).^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.16 ppm (t,

1H, Ar-**H**), 6.78-6.68 ppm (m, 3H, Ar-**H**),6.32 (CO-N**H**), 5.33 ppm (d,2H, C**H**=C**H**), 4.21 ppm (t, 2H,COO-C$\mathbf{H}_2$), 4.01 ppm (t, 2H, Ar-OC$\mathbf{H}_2$), 3.67-3.63 ppm (m, 10H, O-C$\mathbf{H_2}$-C$\mathbf{H}_2$),3.54 ppm (t, 2H, C$\mathbf{H}_2$-N), 2.70 ppm (t, 2H, Ar-C$\mathbf{H}_2$), 3.36 ppm (s, 3H, CH_2-OC$\mathbf{H}_3$), 2.57 ppm (t, 2H, NH-CO-C$\mathbf{H}_2$), 2.50 ppm (t, 2H, C$\mathbf{H}_2$-COO), 2.00 ppm(m, C$\mathbf{H}_2$ CH=CHC$\mathbf{H}_2$), 1.6-0.88 ppm (m, 21H, Aliphatic H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 172.89(NH-**C**O),171.66(**C**O-O), 158.53,144.84, 129.90, 121.40, 114.70, 111.45 (Ar-C), 130.04(**C**H=**C**H), 71.97 (**C**H_2-OCH_3), 70.61 (O-**C**H_2-**C**H_2),69.06, 66.64(Ar-OCH_2), 63.84 (COO-**C**H_2), 59.10(O-CH_3), 39.17(C$\mathbf{H}_2$-N), 36.09, 32.00, 31.50, 29.77, 29.07, 22.74, 14.20.FT-IR (cm^{-1}): 3309, 2848, 2915, 1741, 1640, 1552, 1454, 1405, 1351, 1293, 1249, 1203, 1166, 1106, 1045, 952, 857, 777, 696. MALDI-TOF-MS: m/z calculated for $C_{34}H_{59}NO_7$: 591.41 and Found: 614.41 ($M^+ + Na^+$).

Synthesis of 2-(2-(2-methoxyethoxy)ethoxy)ethyl 4-(dodecylamino)-4-oxobutanoate (DD-TEG):. Dodecyl amine (1.31 g, 8.33 mmol) was dissolved in dry dichloromethane (15 mL) and purged with nitrogen for 15 minutes. To this **1c** (2.0 g, 7.5 mmol) was added and the content was purged under nitrogen for 15 minutes DCC (1.87 g, 9.09 mmol) and diisopropylethylamine (0.09 g, 0.75 mmol) was added to the reaction mixture under nitrogen atmosphere and the reaction was continued for 24 h at 25 ˚C. The mixture was poured into water (30 mL) and extracted with chloroform (20 mL). The organic layer obtained was neutralized with 2N HCl (2 mL), washed with aqueous 5% $NaHCO_3$ (50 mL) and brine. After drying over anhydrous sodium sulphate, the solvent was removed to obtain yellow liquid as product. It was further purified by passing through silica gel column of 60-120 mesh using 80% ethyl acetate in hexane as eluent. Yield = 1.5 g (46.0 %).^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.20 ppm (t, 2H,COO-C$\mathbf{H}_2$), 3.93 ppm (t, 2H, COOC$\mathbf{H}_2$C$\mathbf{H}_2$), 3.67-3.61 ppm (m, 8H, O-C$\mathbf{H_2}$-C$\mathbf{H}_2$),3.53 ppm (t, 2H, C$\mathbf{H}_2$-N), 3.35 ppm (s, 2H, CH_2-OC$\mathbf{H}_3$), 2.70 ppm (t, 2H, NH-CO-C$\mathbf{H}_2$), 2.63 ppm(t, 2H, C$\mathbf{H}_2$-COO), 1.87-0.72 ppm (m, 23H, Aliphatic H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 172.75(NH-**C**O),171.52(**C**O-O), 158.42, 144.74, 129.20, 121.27, 114.58, 111.32 (Ar-C), 71.85 (**C**H_2-OCH_3), 70.48 (O-**C**H_2-**C**H_2), 68.92, 66.52(Ar-OCH_2), 63.70 (COO-**C**H_2), 58.96(O-CH_3), 39.05(C$\mathbf{H}_2$-N), 35.97, 31.87, 31.38, 29.64, 29.32, 22.64, 14.08. FT-IR (cm^{-1}): 3309, 2848, 2915, 1741, 1640, 1552, 1454, 1405, 1351, 1293, 1249, 1203, 1166, 1106, 1045, 952, 857, 777, 696. MALDI-TOF-MS: m/z calculated for $C_{34}H_{59}NO_7$: 431.32 and Found: 509.23 ($M^+ + K^+$)

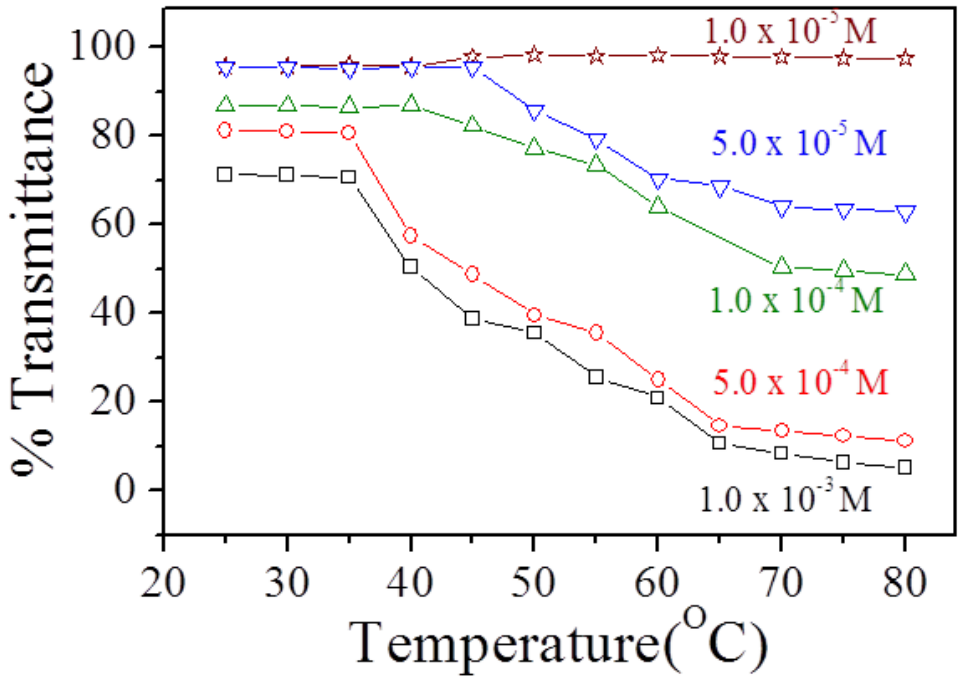


Fig. S1. %Transmittance of PDP-TEG at various concentrations (heating cycle)

Note: The above plot of %transmittance at various concentration of PDP-TEG corresponds to heating cycle. The plot implies that the deformation process of the aggregates in heating cycle is slow at all concentrations.

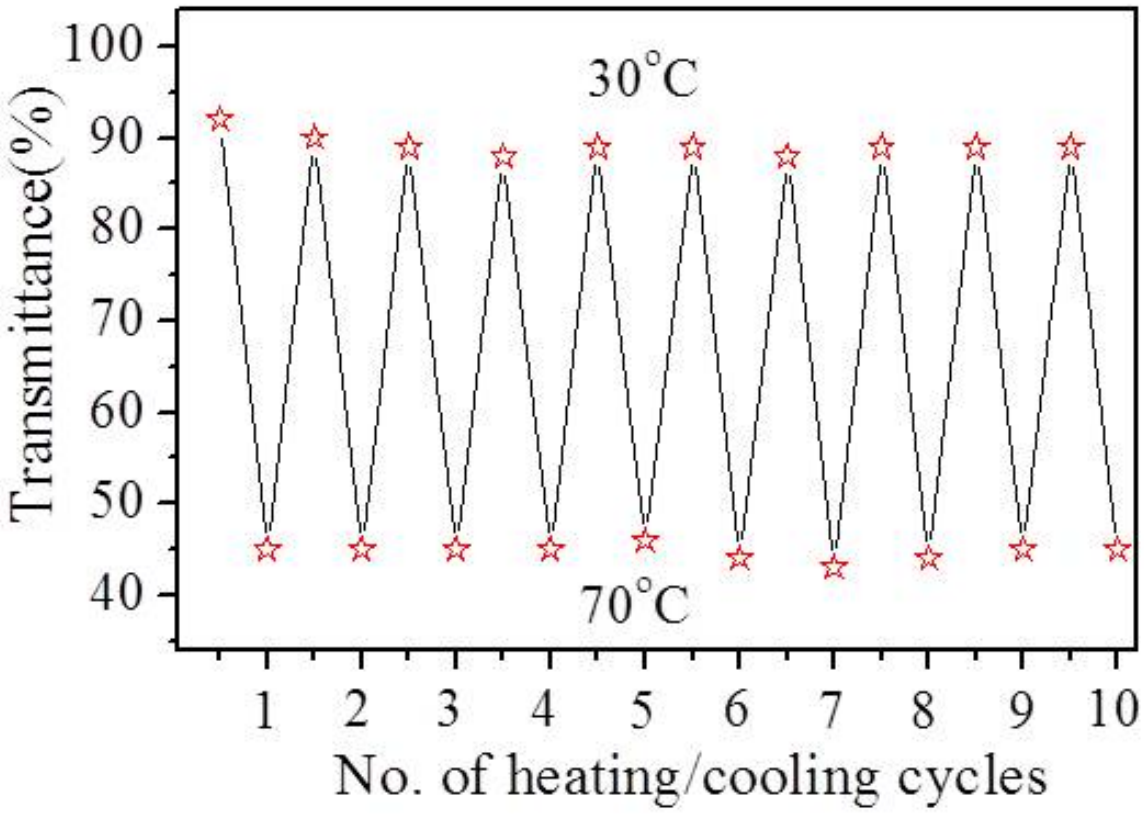


Fig. S2. Reversible phase transition phenomena in heating and cooling cycles.

Note: Transmittance (%) of the amphiphile (10^{-4}M) was recorded in water both at temperature above and below LCST in ten consecutive cycles. The amphiphile shows complete reversibility in the self-assembly process in heating and cooling cycle.

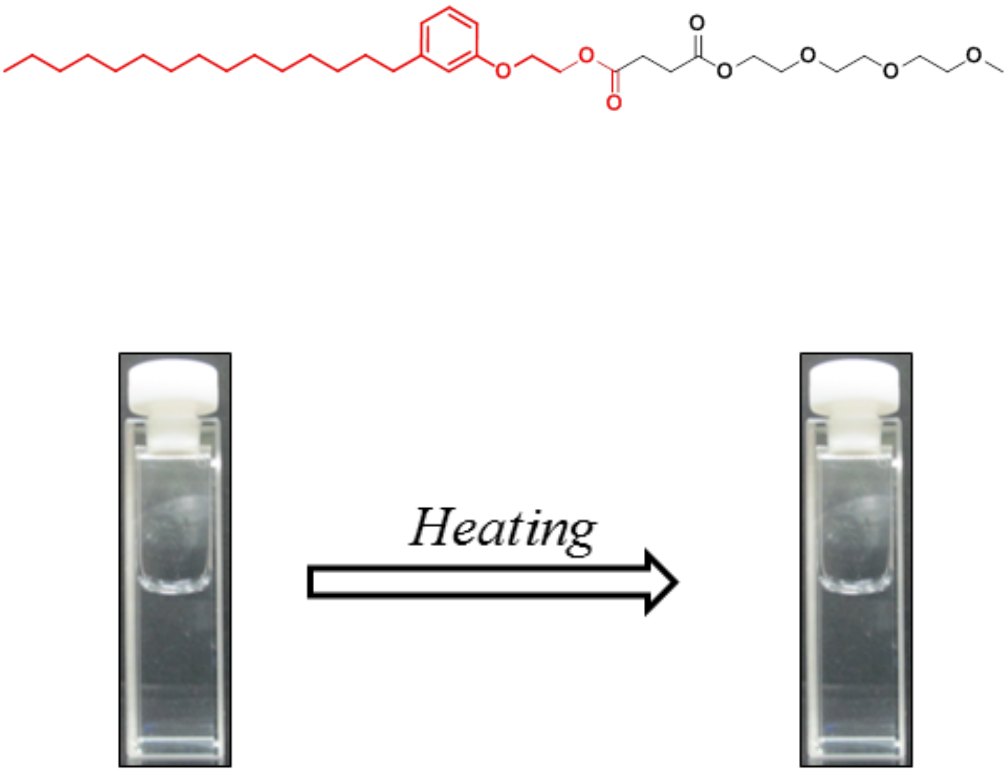


Fig. S3. Image of PDP-TEG having ester linkage.

Note: The amphiphile PDP-TEG having ester linkage does not undergoes phase –transition phenomena upon heating.

Ref: this amphiphile was reported in ref. smita et al. ref. 28

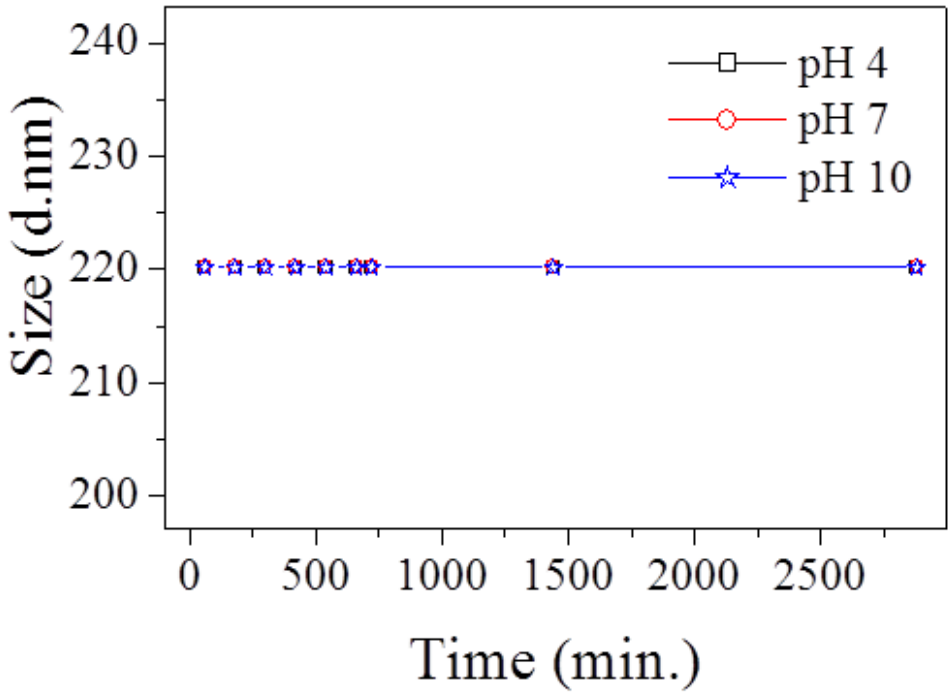


Fig. S4. Aggregate size of PDP-TEG vs time at various pH.

Note: The aggregate size was monitored for 48hrs at various pH such as 2.0, 4.0 and 10.0. As no changes in aggregate size was observed which indicated that these aggregates are quiet stable in acidic, neutral and as well as at basic pH.

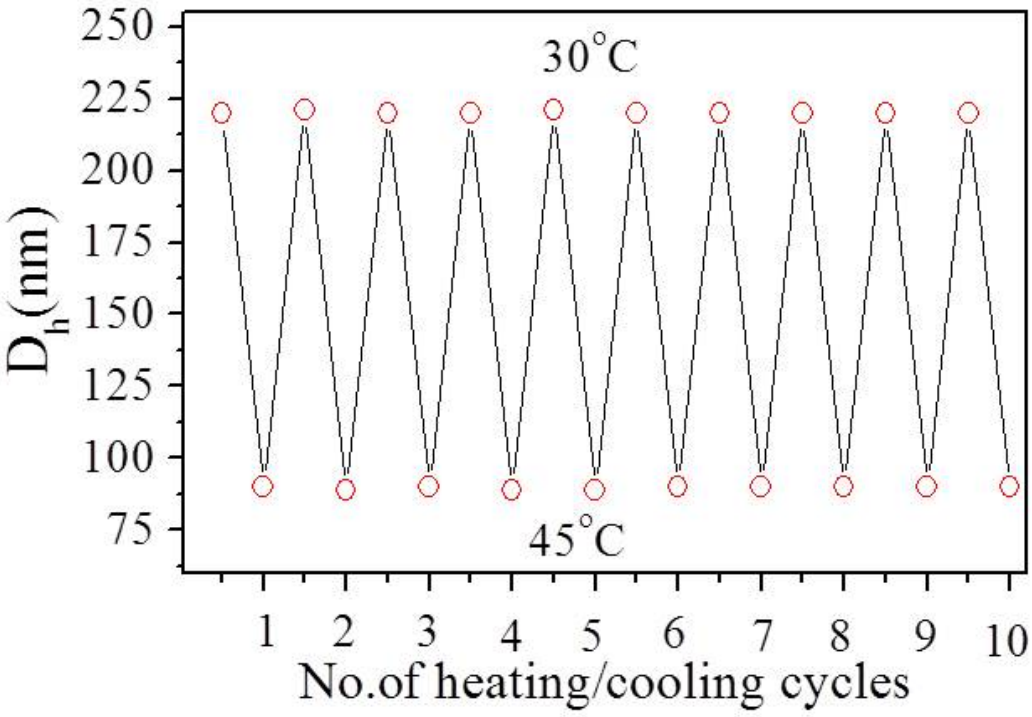


Fig.S5. Hydrodynamic diameter oscillation of the nanoparticles in the heating and cooling cycle.

Note: The hydrodynamic diameter of the amphiphile in water was recorded at temperature above and below LCST in ten consecutive cycles. The plot reveals that the change in size of the nanoparticles from 220nm (below LCST) to 90 nm (above LCST) was completely reversible in nature.

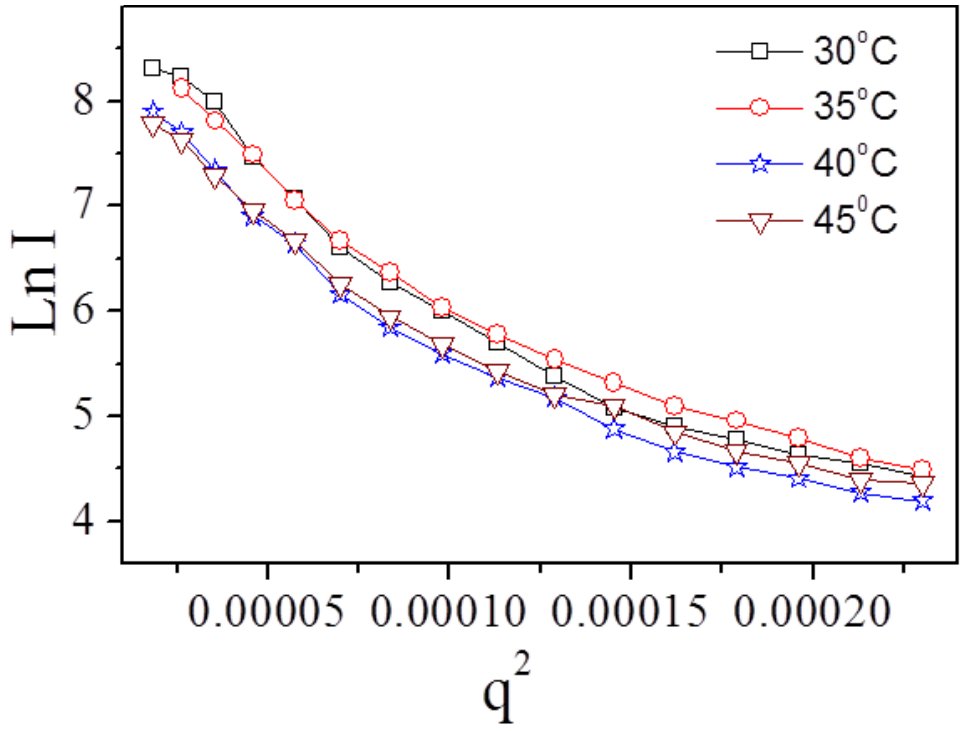


Fig.S6. Guinier plot of PDP-TEG in water at 10^{-4}M at various temperatures.

Note: The scattering of the nanoparticle was monitored at various temperatures i: e below LCST and above LCST. The scattering intensity changes at temperature above LCST as compared to scattering intensity at temperature below LCST thereby, indicating the transformation of core-shell morphology to rod-like structure.

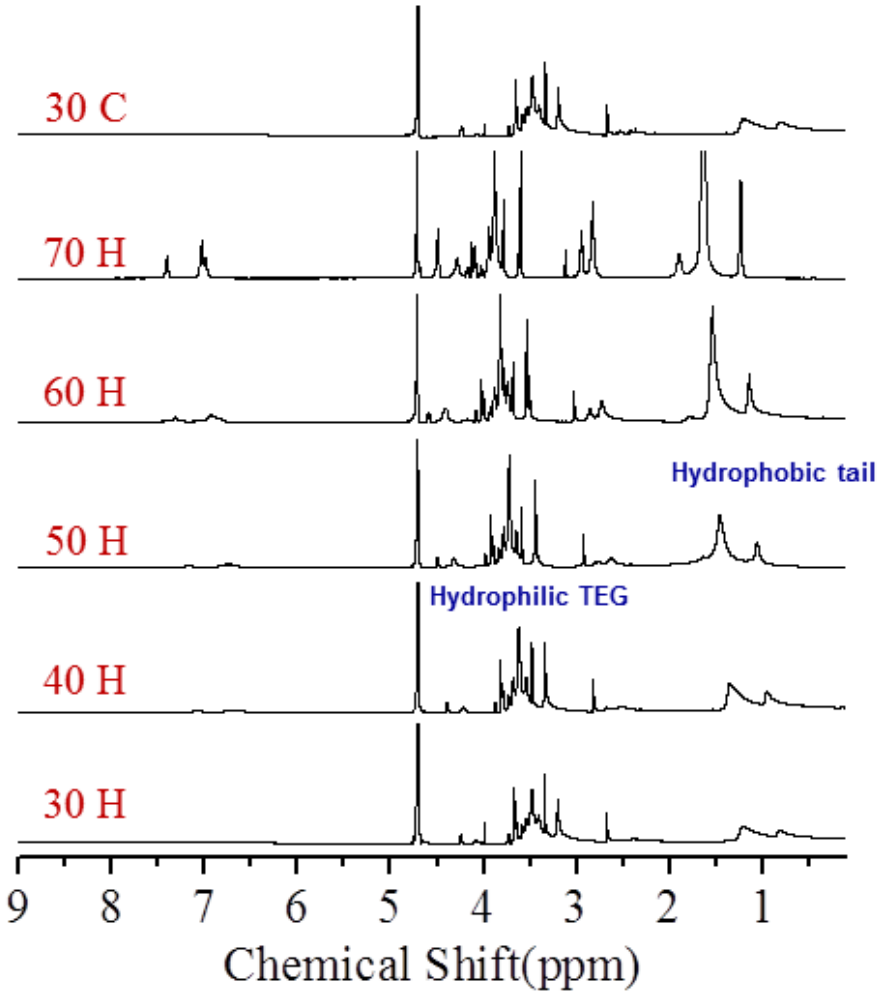


Fig. S7 *Variable temperature H^1 NMR of PDP-TEG in D_2O.* H-heating and C-cooling

Note: The H^1 NMR of the amphiphile in water was recorded at various temperatures. Initially the sample was heated from 30° to 70 °C and subsequently cooled to 30 °C. The low intensity of the signals corresponding to the hydrophobic part (below 1.50 ppm) indicates shielding of the protons of hydrophobic region from the solvent at temperature below LCST. Above LCST, signal intensity increases due to increase in chain mobility as result of breaking of hydrogen bond between amide-linkage and water molecules.

Table-ST1: Unit cell parameters of PDP-amine-Boc molecule

Compound	**PDP-amine-Boc**
Formula	$C_{27}H_{44}O_5$
recrystn solv	DCM/MeOH
mol wt	447
Colour, habit	Colourless, rectangular
temp(K)	100
system	Triclinic
space group	P-1
a, (Å)	5.39
b, (Å)	10.53
c, (Å)	23.86
α, (deg)	83.74
β, (deg)	88.42
γ, (deg)	86.78
V, Å^3	1346.30
d_{cacl} ,g cm^{-1}	1.203
μ(mm^{-1})	0.08
GOF	0.708
no. of unique reflections	4663
Reflections collected	6781
θ range	1.34 to 24.04
No. of refined parameters	293
R_1 (on F, I>2σ (I))	0.0554
wR_2 (on F^2, all data)	0.1960

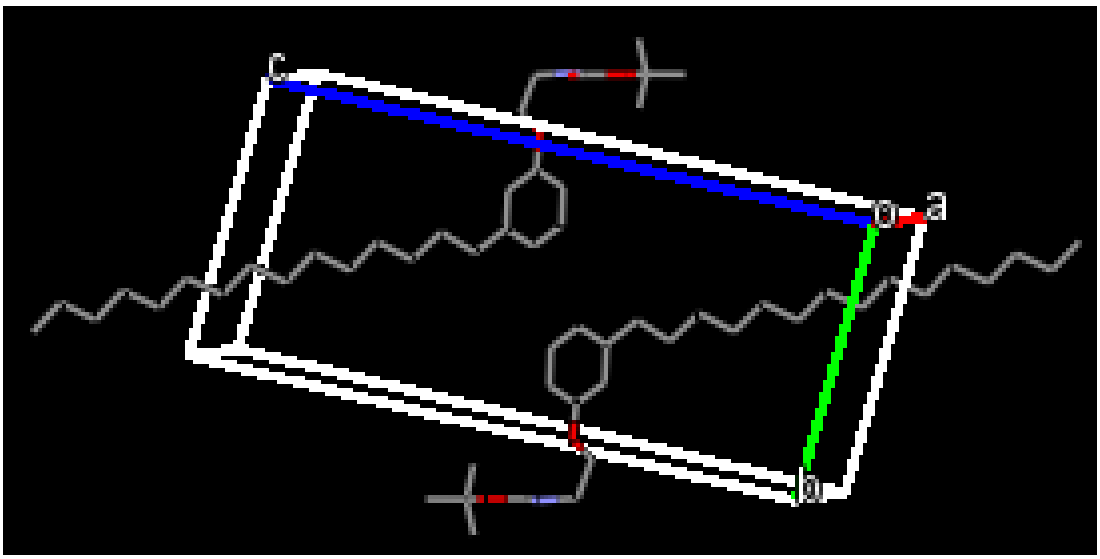
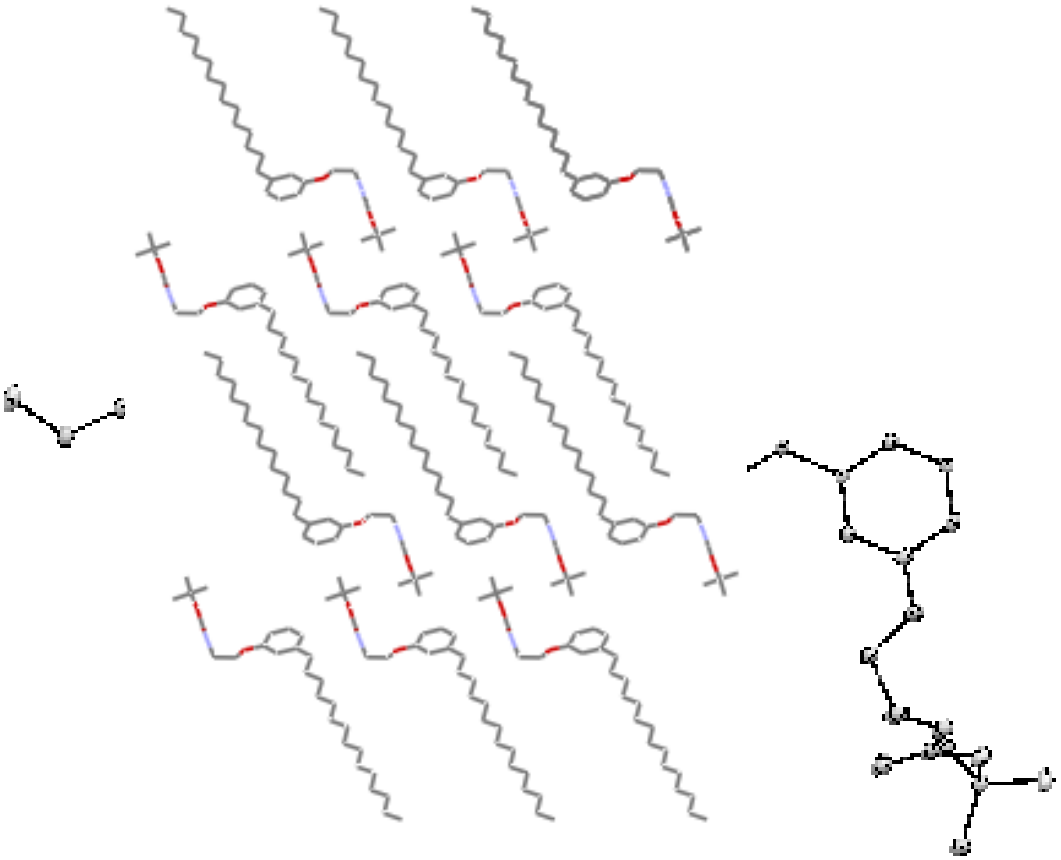

c
a
b

Fig. S8. Unit cell and ORTEP diagram for the single crystal of compound 3 (PDP-amine-Boc)



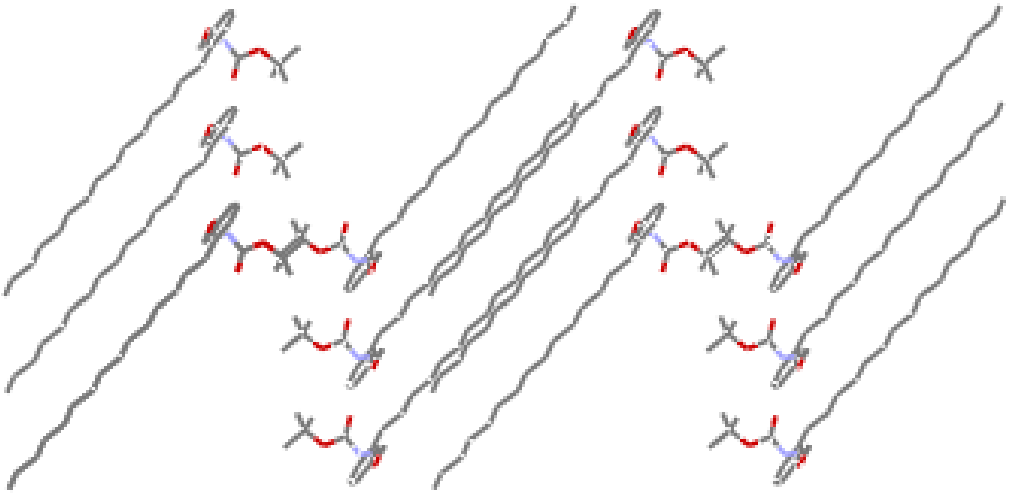


Fig. S9. View of the molecular packing diagram for compound 3(PDP-amine-Boc) along a and b axis respectively.

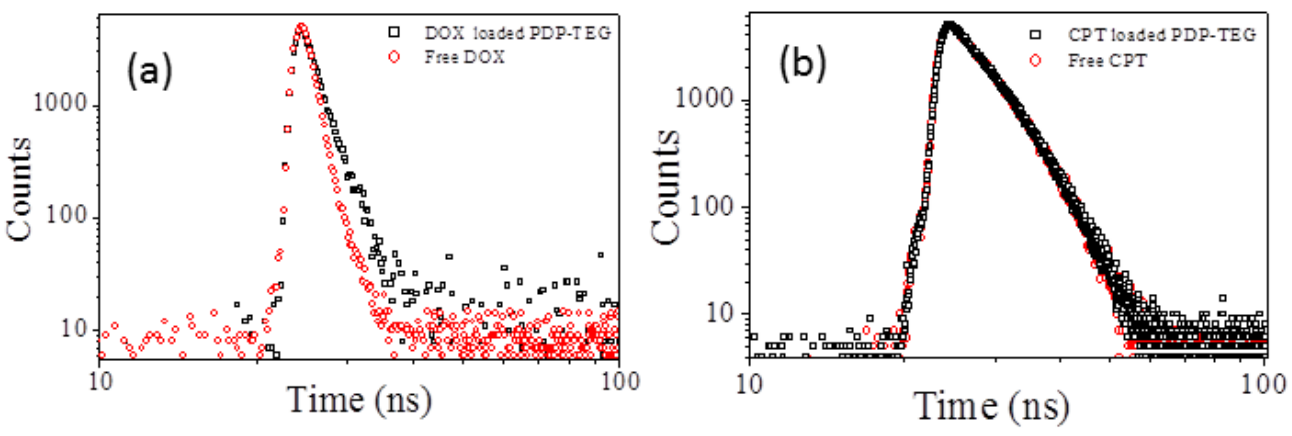


Fig. S10. TCSPC Decay profiles of (a) DOX loaded nanoparticle and free DOX. (b) CPT loaded nanoparticle and free CPT.

Table-ST2: Life time values of drug loaded PDP-TEG nanoparticles. The values in the brackets indicate the amplitudes.

Drug loaded nanoparticles	**Collected at emission wavelength**		
	τ_1 (ns)	**τ_2 (ns)**	**χ^2**
DOX loaded nanoparticle	1.49 (0.90)	5.06 (0.10)	1.07
Free DOX	0.95 (0.95)	2.40 (0.05)	1.29
CPT loaded nanoparticle	4.66 (0.57)	0.62(-0.43)	1.15
Free CPT	4.59 (0.56)	0.67 (-0.44)	0.99

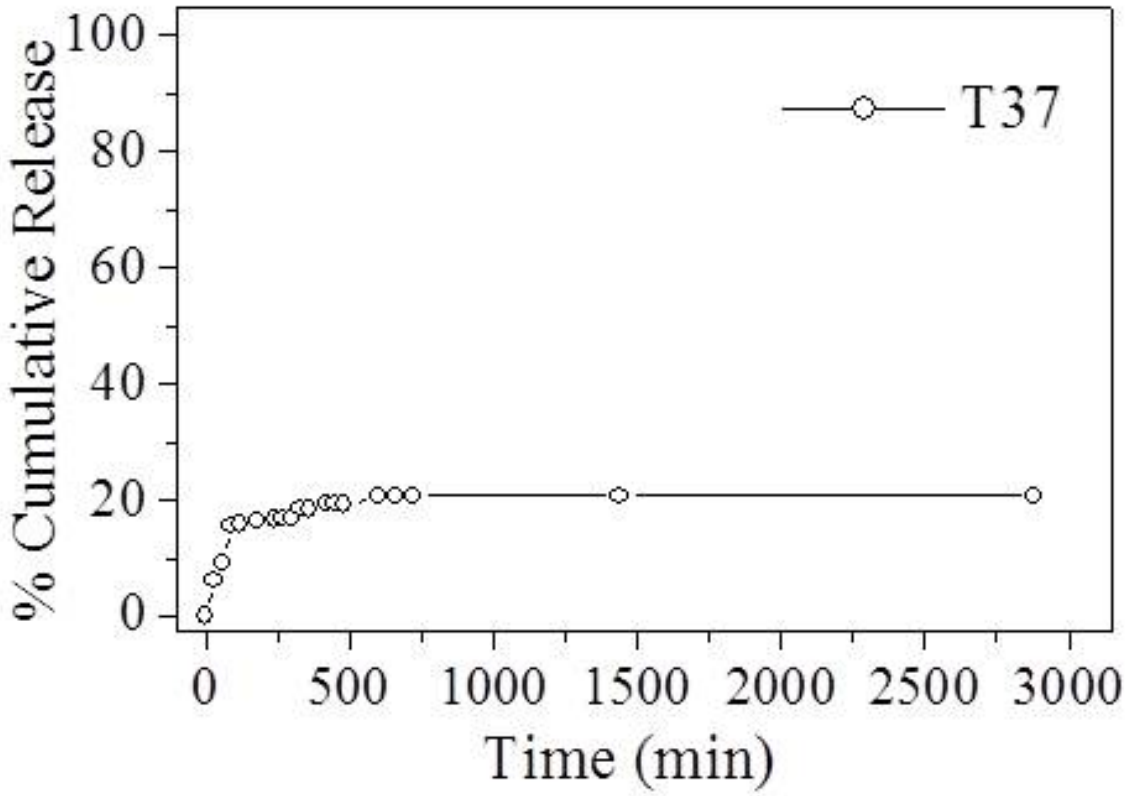
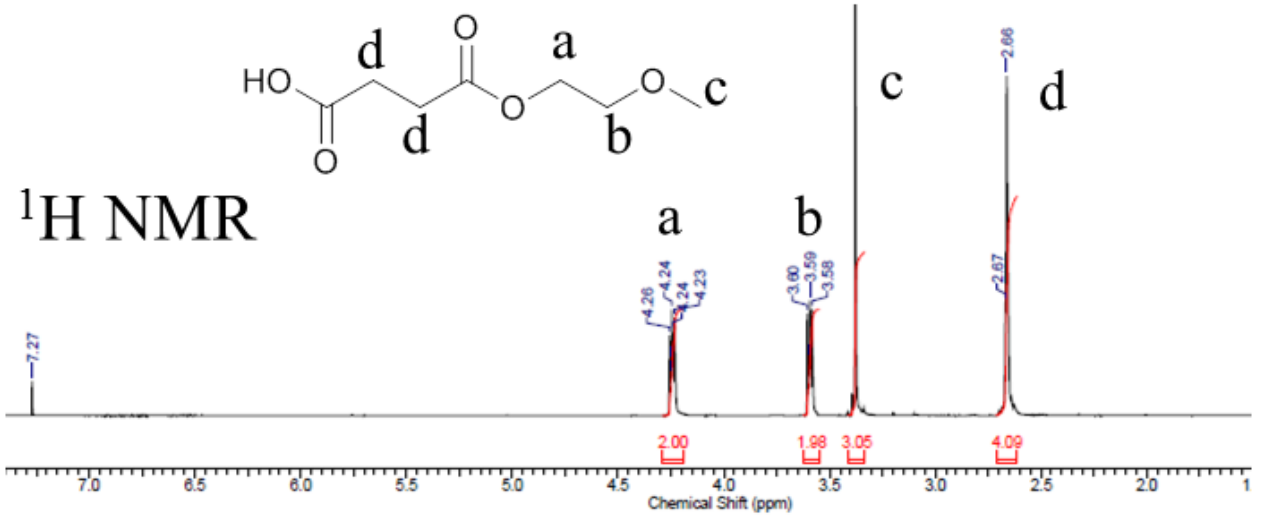
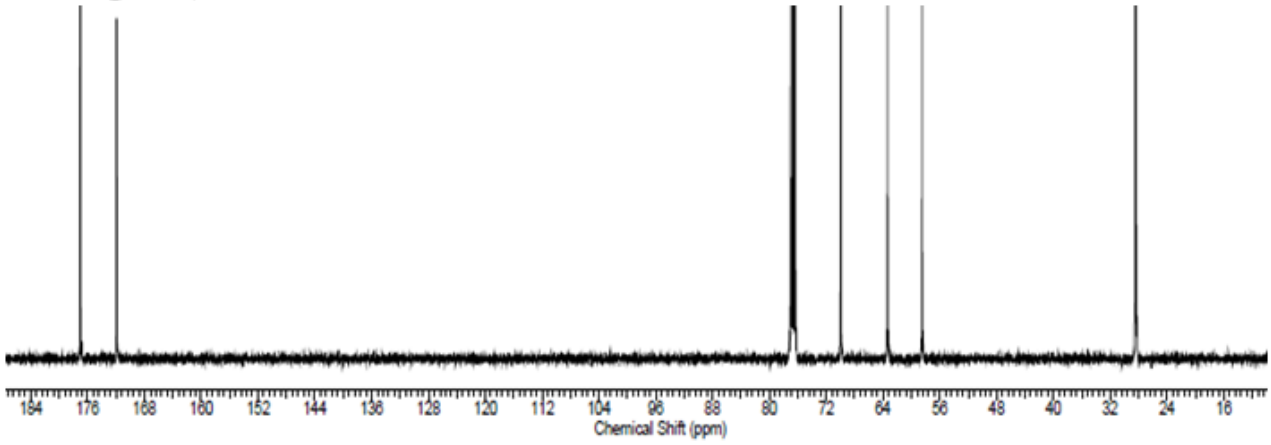


Fig. S11. %Cumulative Release of DOX loaded nanoparticle at pH= 6.8 and at 37 °C.

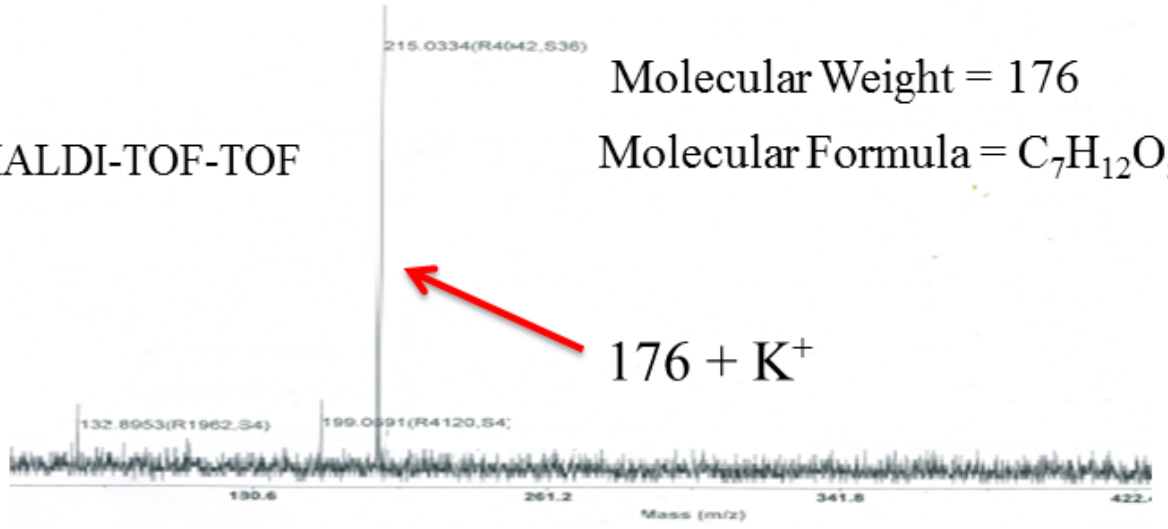
Note: The drug release profile of DOX loaded scaffold at 37 °C in PBS having pH= 6.8 was studied. The plot reveals that the nanoparticle was very stable and is capable of preserving the drug at pH= 6.8 and 7.4 under physiological conditions.

Compound 1a





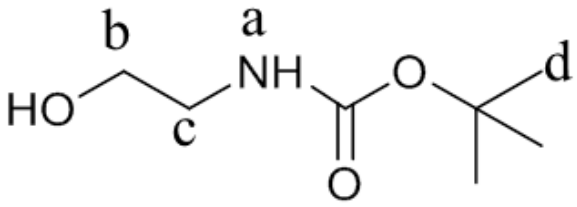
MALDI-TOF-TOF



Molecular Weight = 176

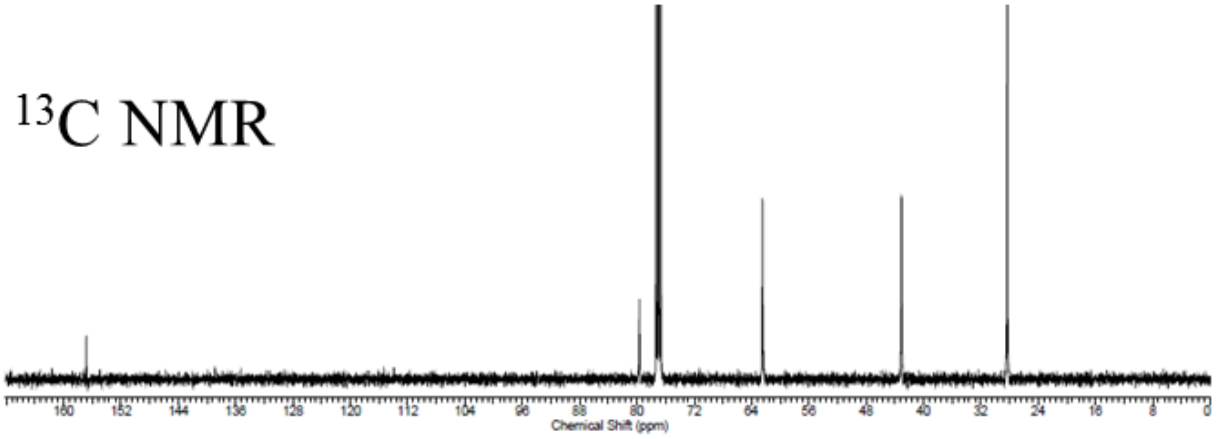
Molecular Formula = $C_7H_{12}O_5$

Compound 2

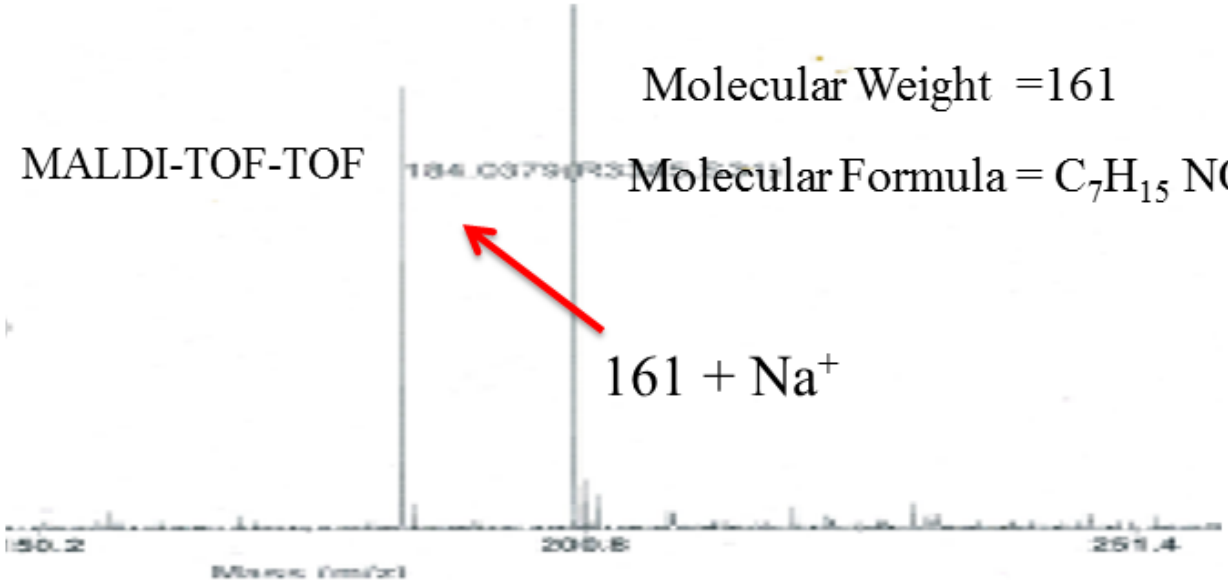


^{1}H NMR

^{13}C NMR



MALDI-TOF-TOF

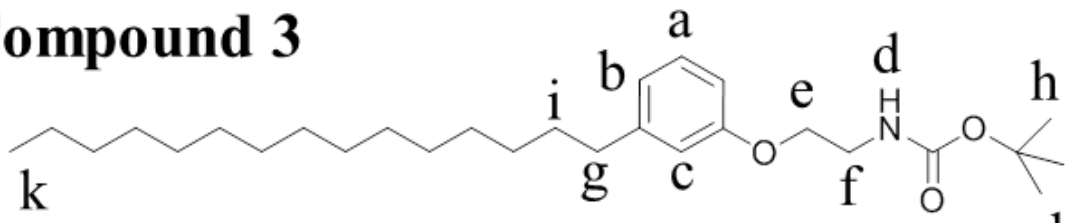


Molecular Weight =161

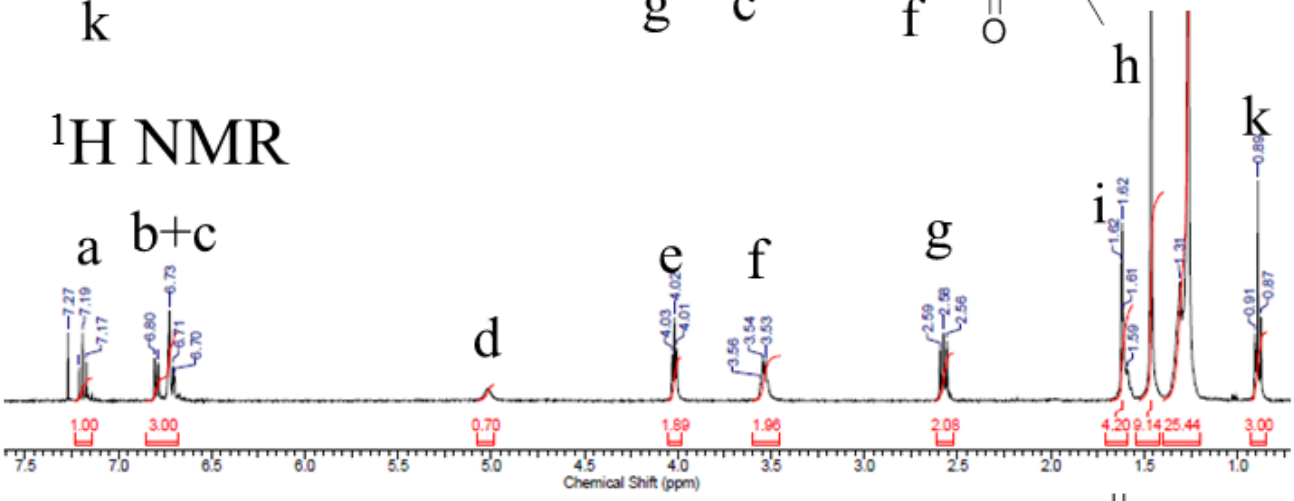
Molecular Formula = $C_7H_{15}NO_3$

161 + Na^+

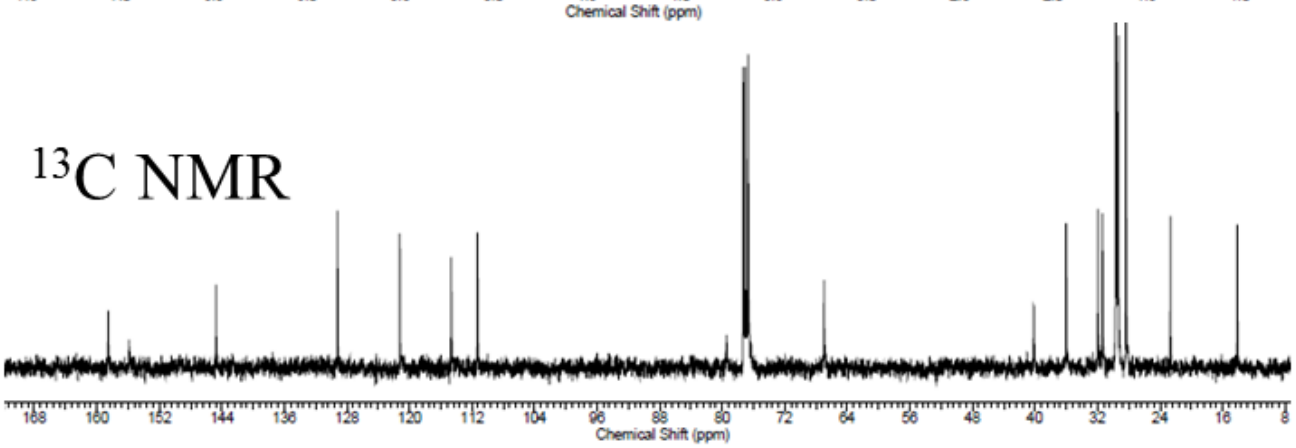
Compound 3



^{1}H NMR



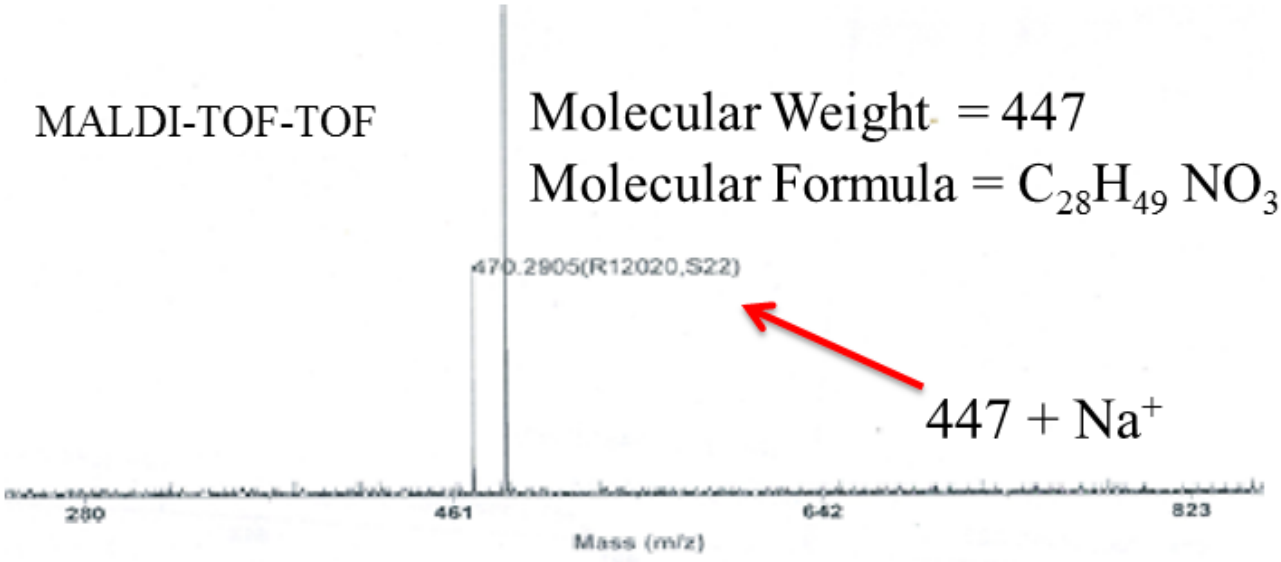
^{13}C NMR



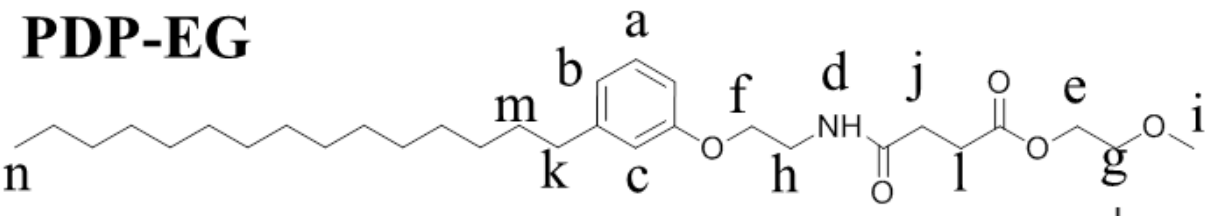
MALDI-TOF-TOF

Molecular Weight = 447

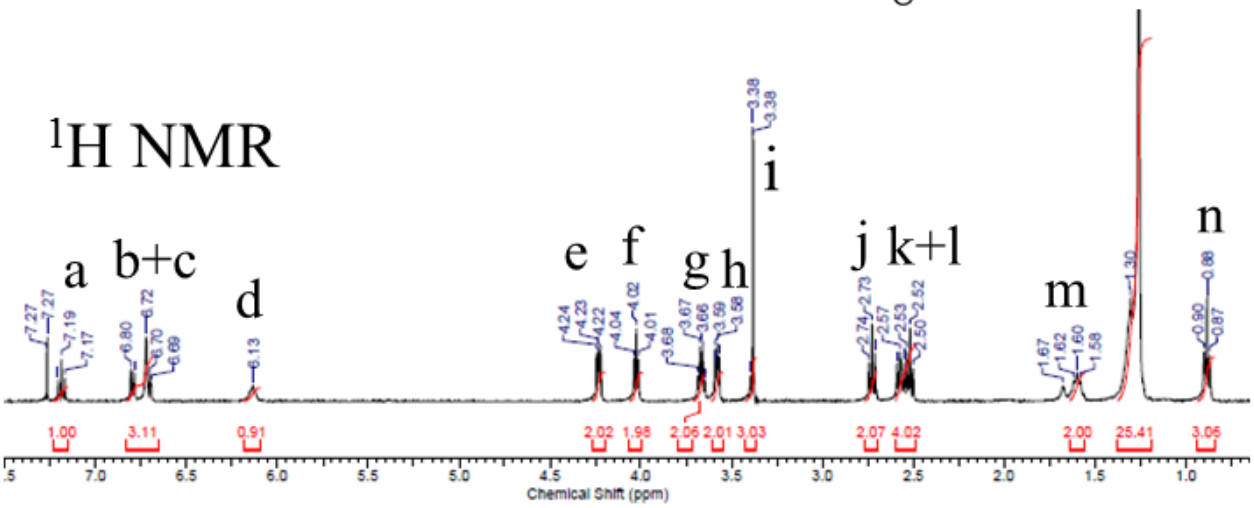
Molecular Formula = $C_{28}H_{49}NO_3$



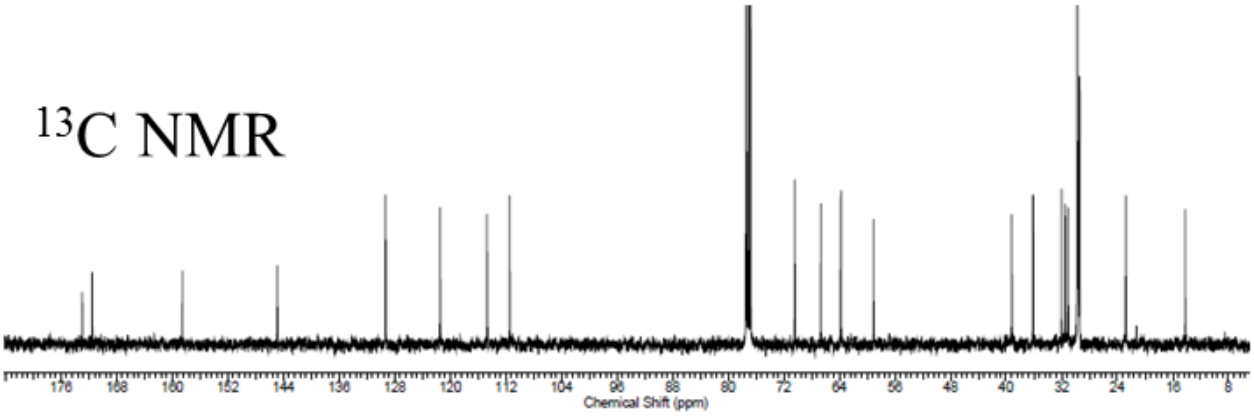
PDP-EG



^{1}H NMR



^{13}C NMR

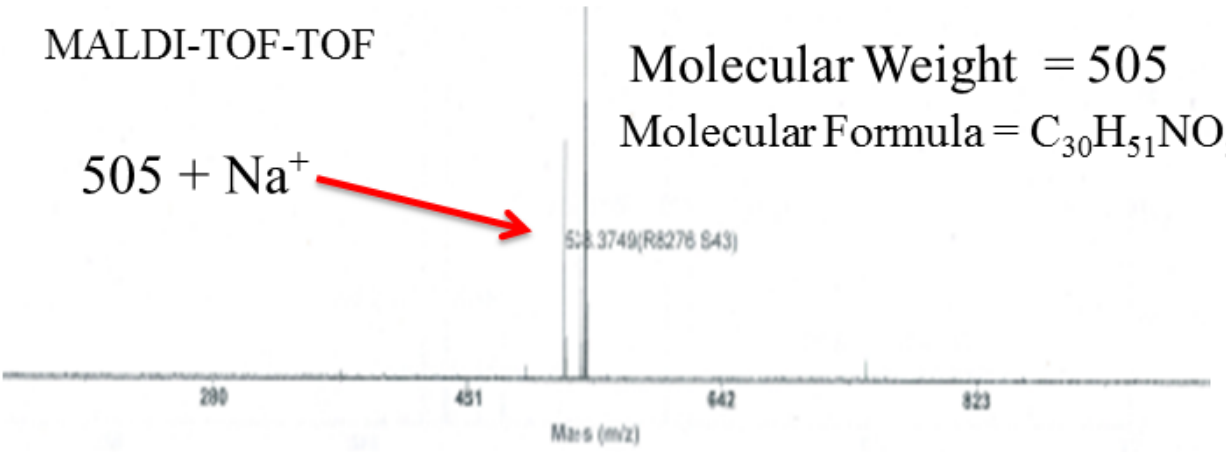


MALDI-TOF-TOF

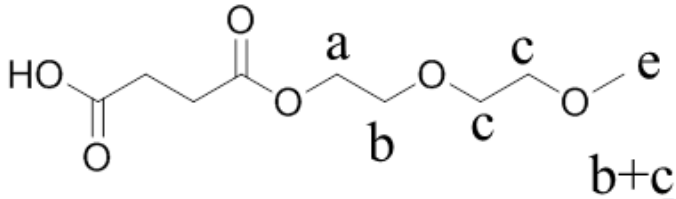
Molecular Weight = 505

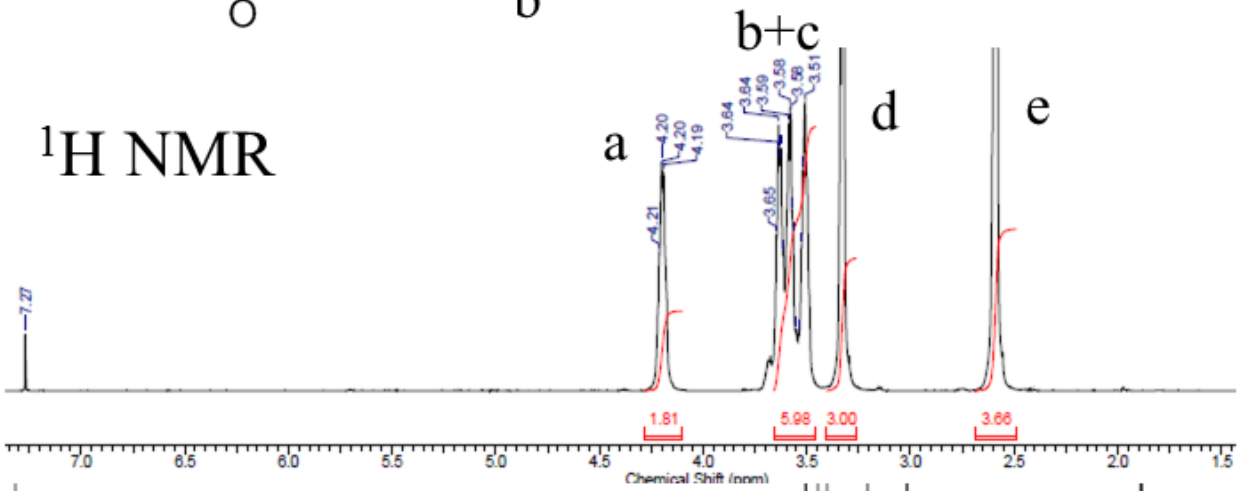
Molecular Formula = $C_{30}H_{51}NO_5$

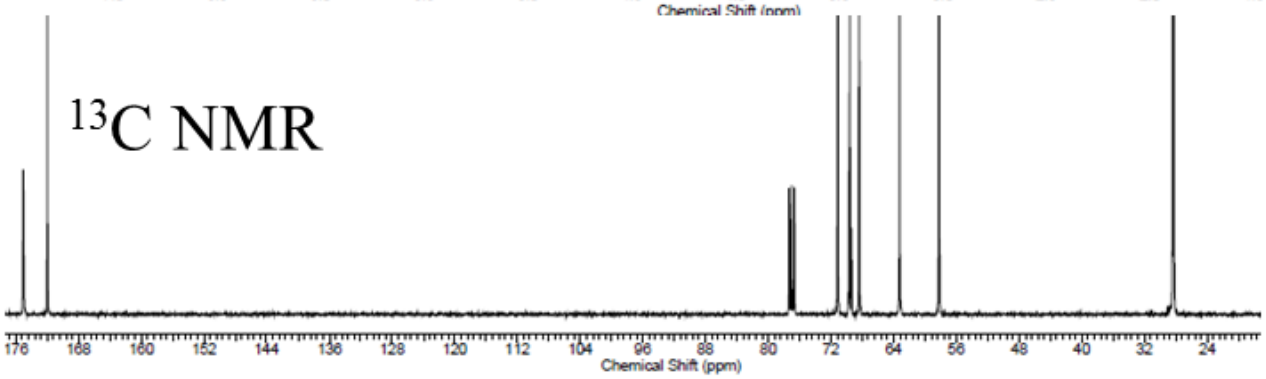
505 + Na^+



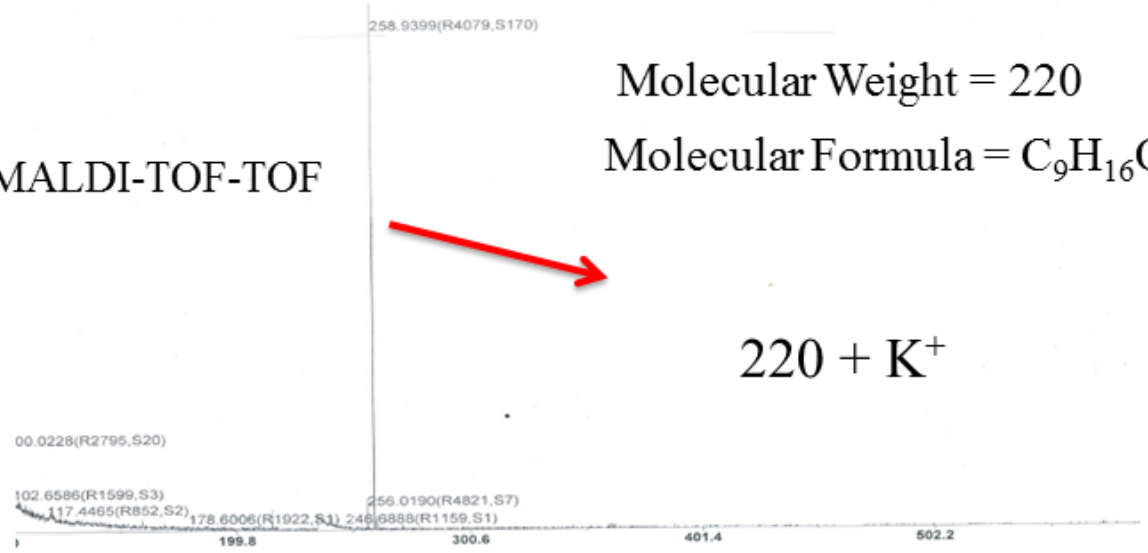
Compound 1b







MALDI-TOF-TOF

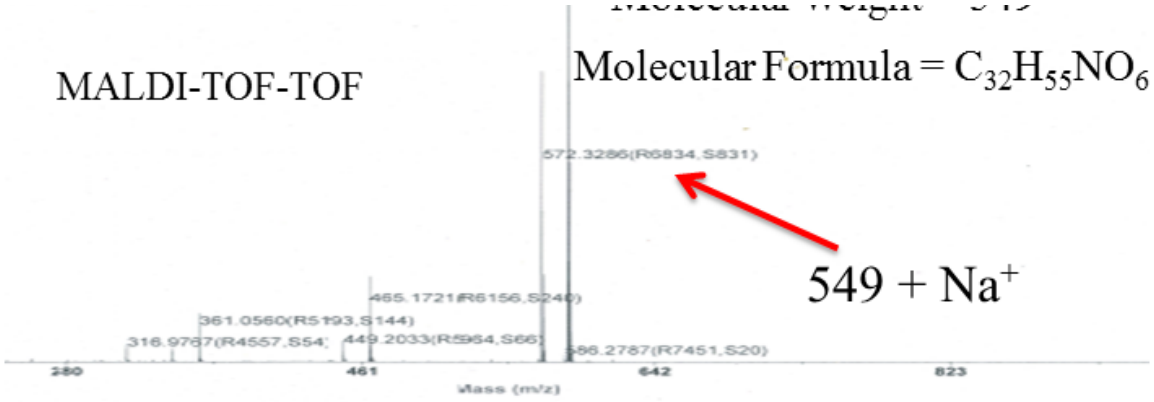


Molecular Weight = 220

Molecular Formula = $C_9H_{16}O_6$

220 + K^+

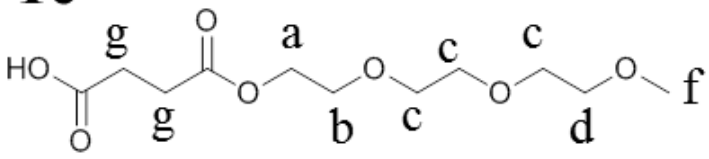
MALDI-TOF-TOF



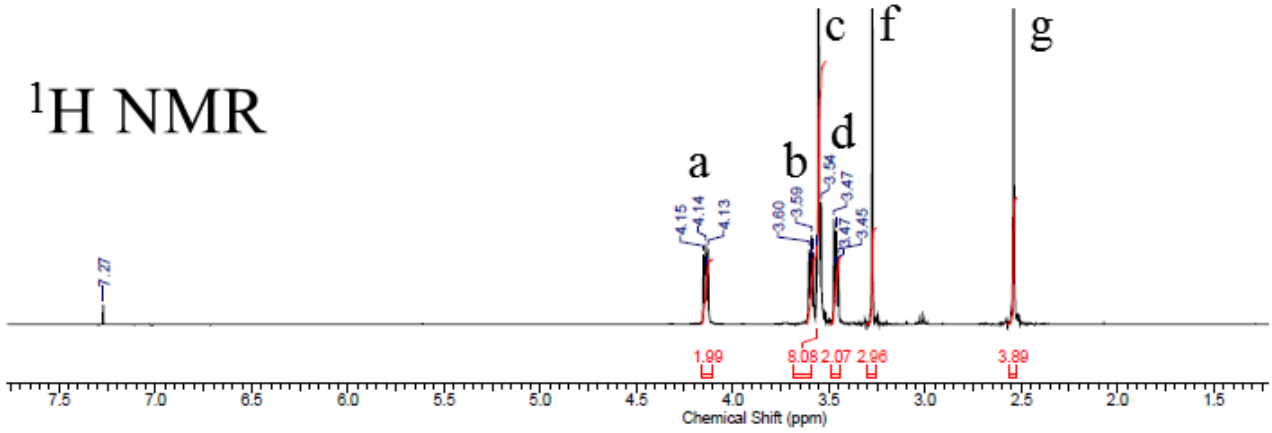
Molecular Formula = $C_{32}H_{55}NO_6$

549 + Na^+

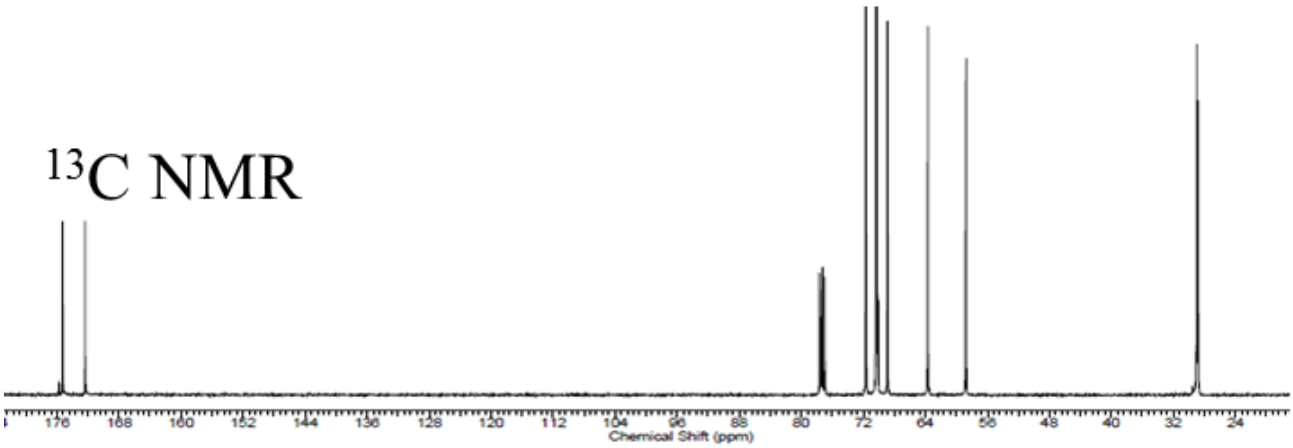
Compound 1c



^{1}H NMR



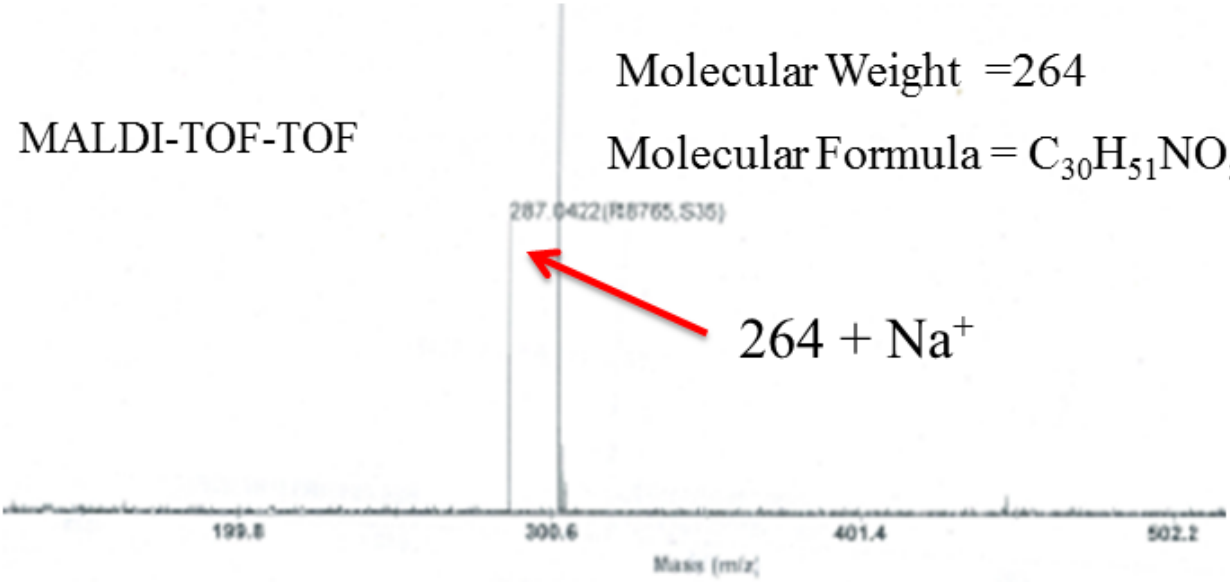
^{13}C NMR



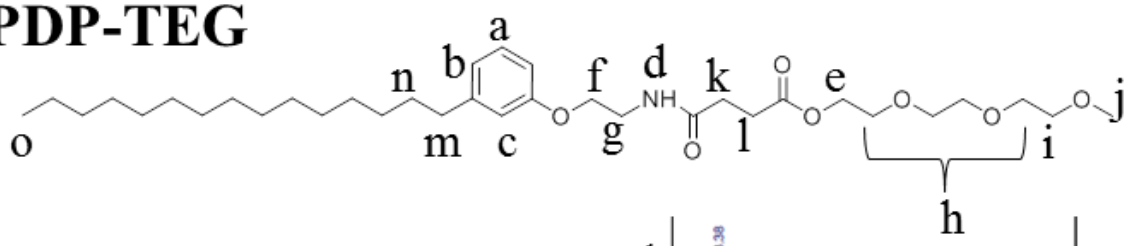
MALDI-TOF-TOF

Molecular Weight =264

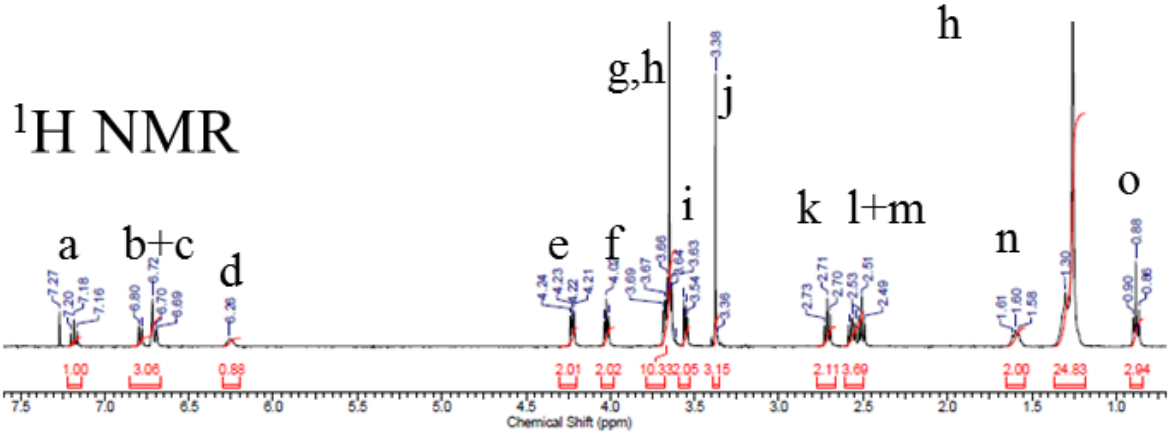
Molecular Formula = $C_{30}H_{51}NO_5$



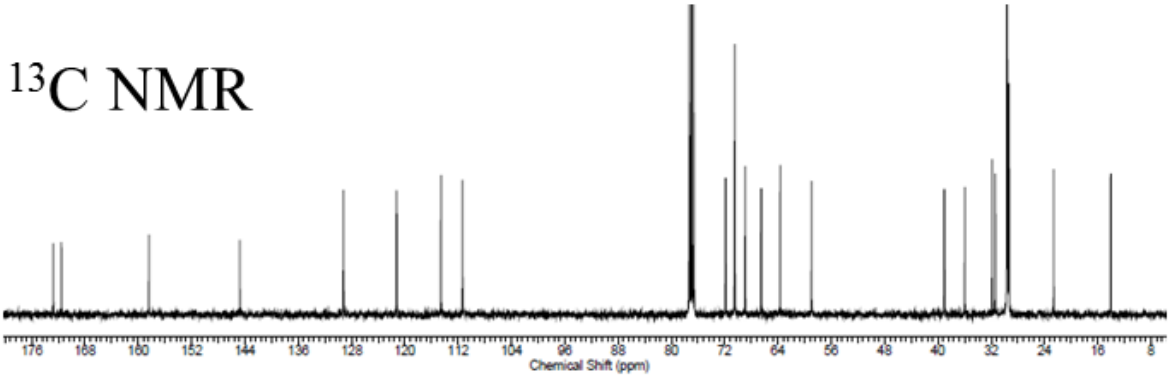
PDP-TEG



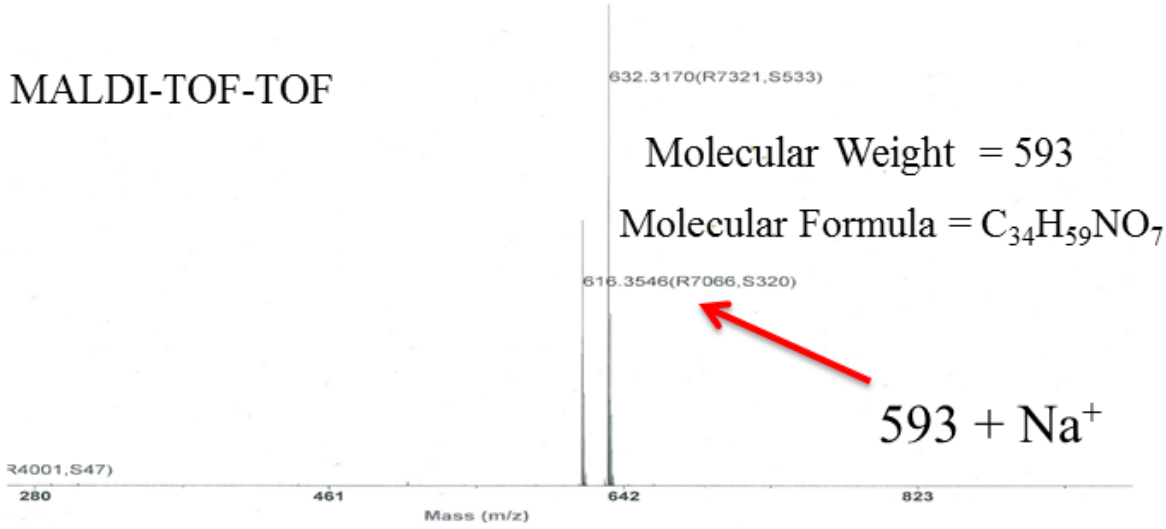
^{1}H NMR



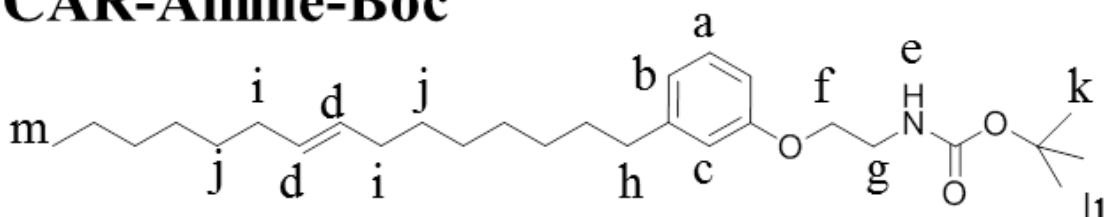
^{13}C NMR



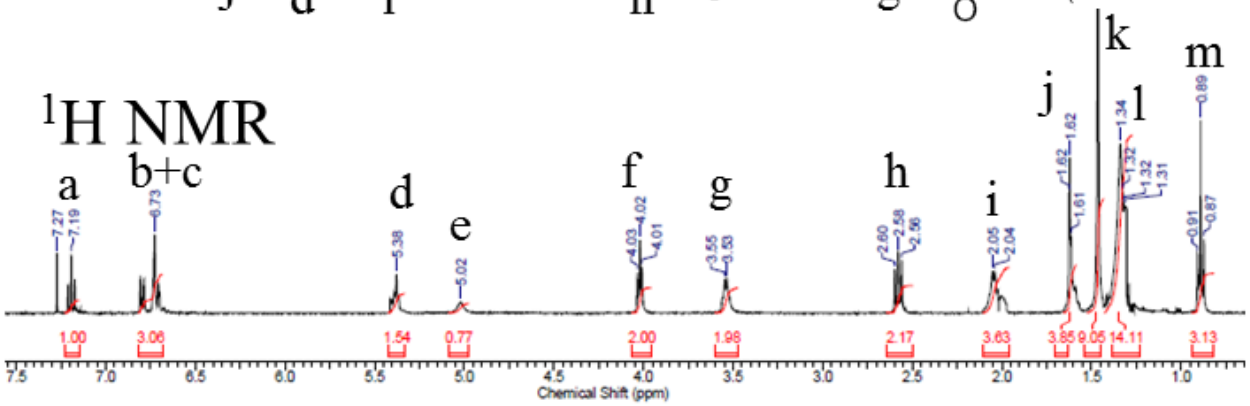
MALDI-TOF-TOF



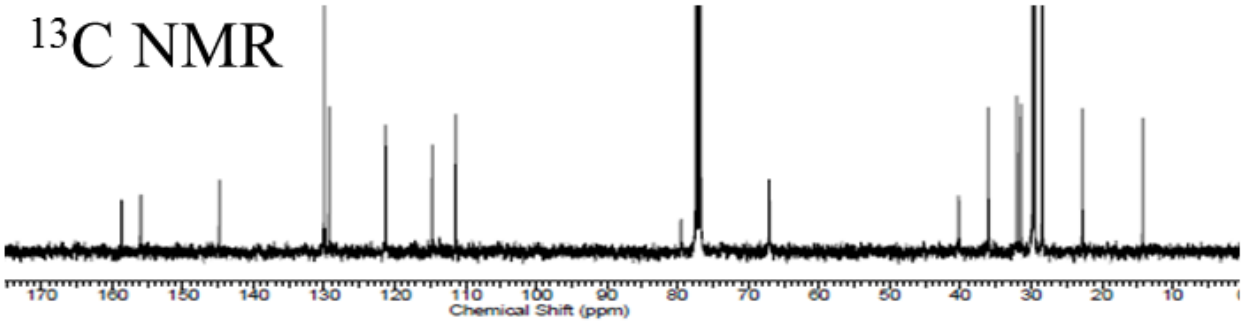
CAR-Amine-Boc

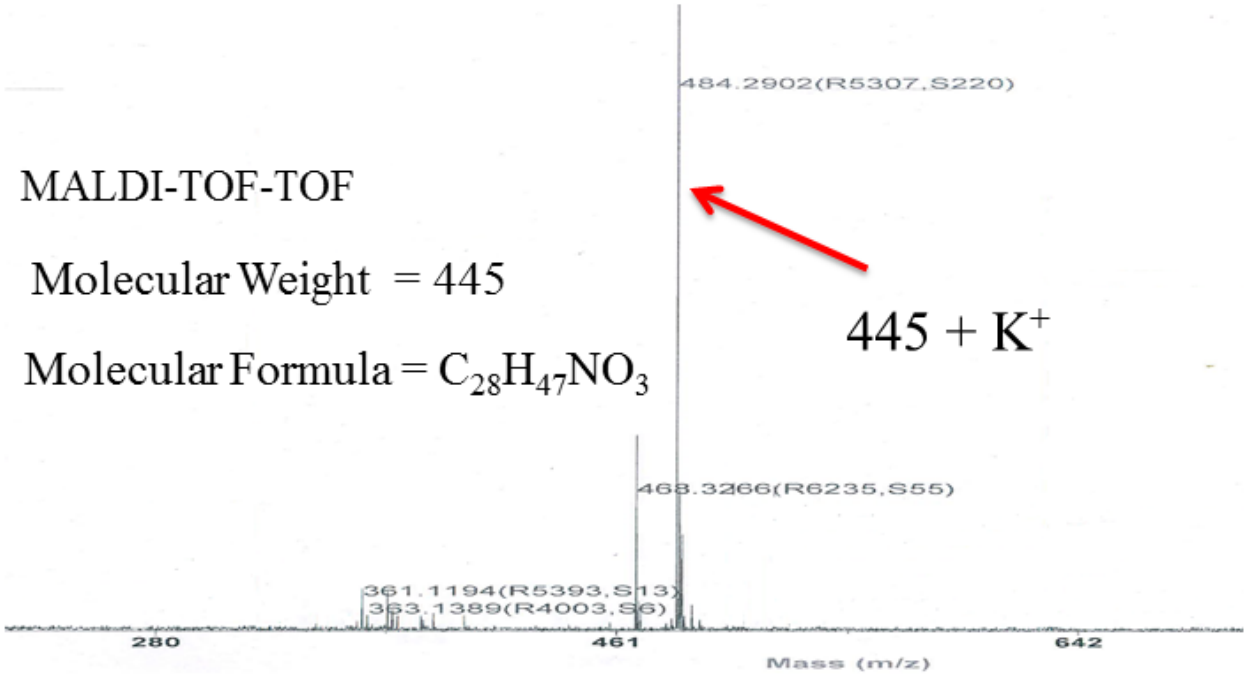


^{1}H NMR



^{13}C NMR





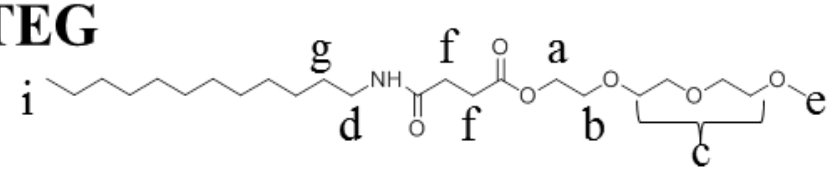
MALDI-TOF-TOF

Molecular Weight = 445

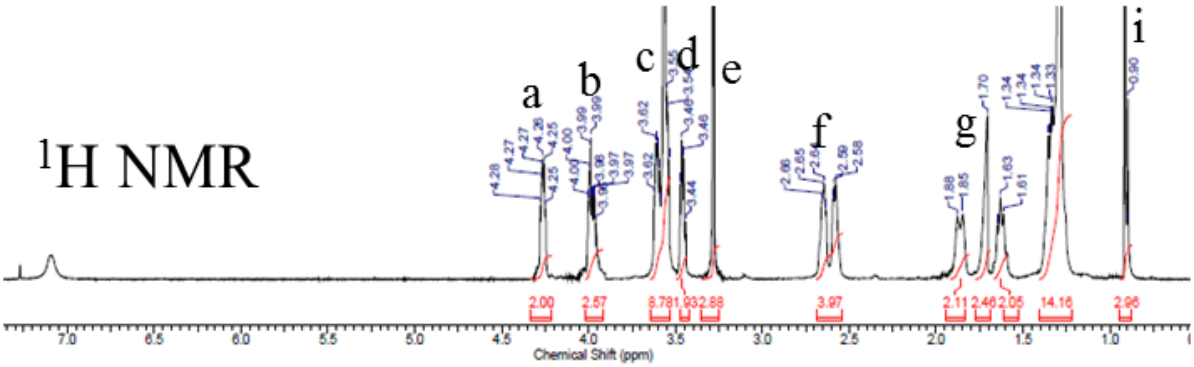
Molecular Formula = $C_{28}H_{47}NO_3$

445 + K^+

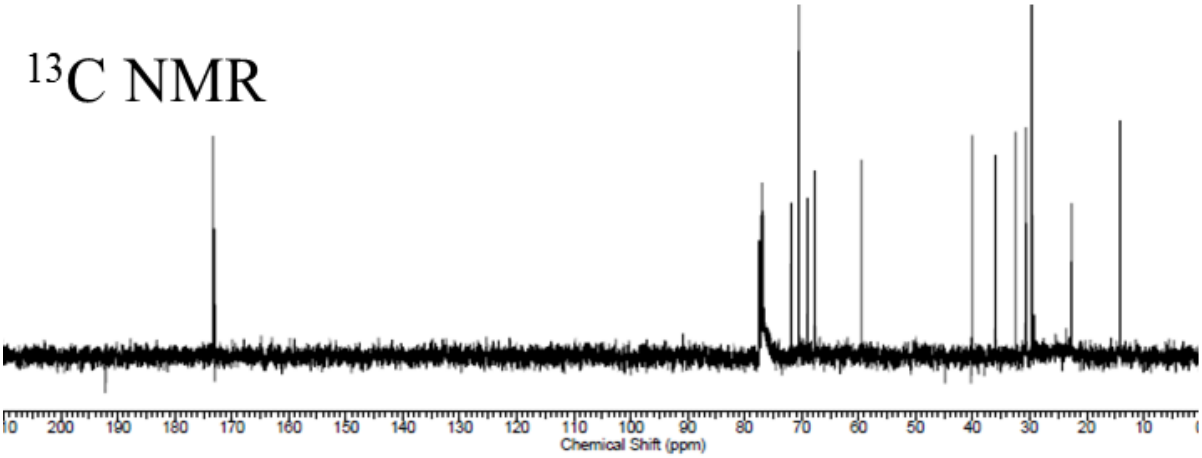
DD-TEG



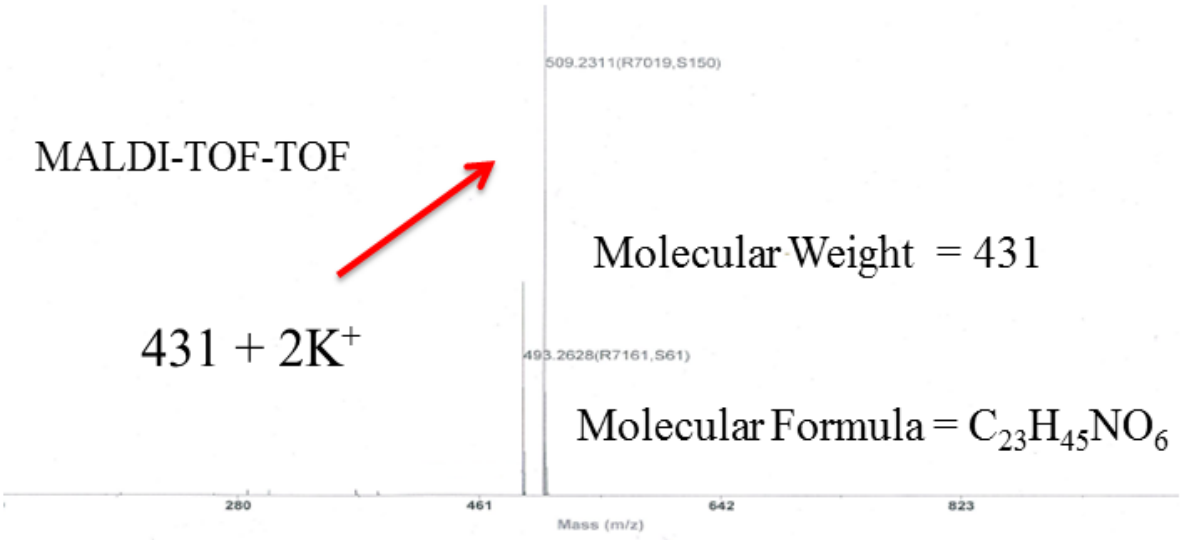
^{1}H NMR



^{13}C NMR



MALDI-TOF-TOF

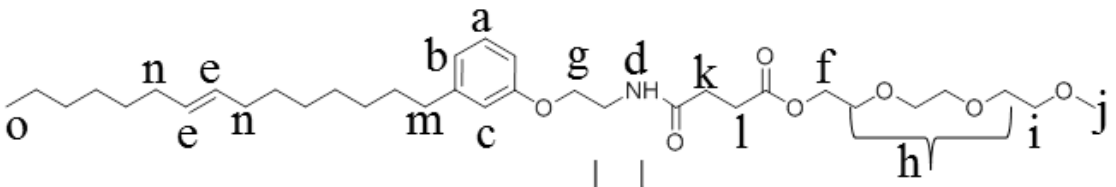


Molecular Weight = 431

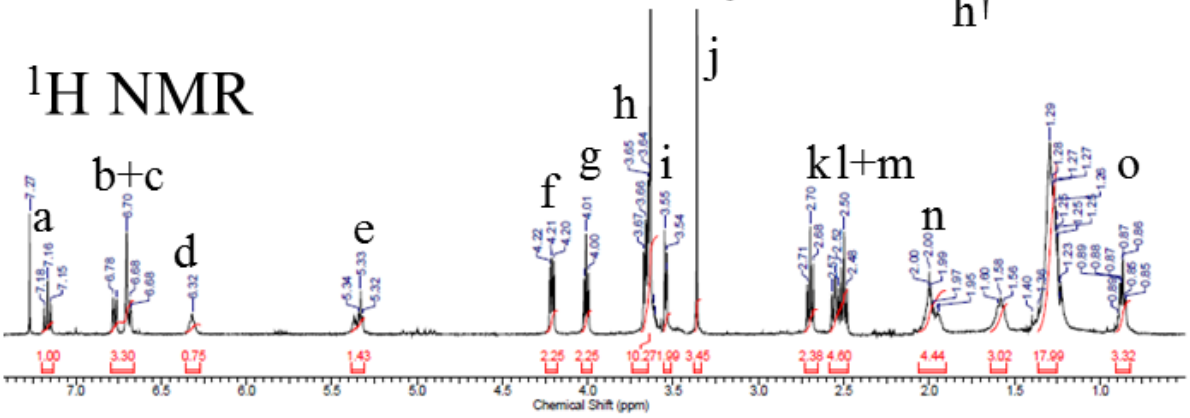
$431 + 2K^{+}$

Molecular Formula = $C_{23}H_{45}NO_6$

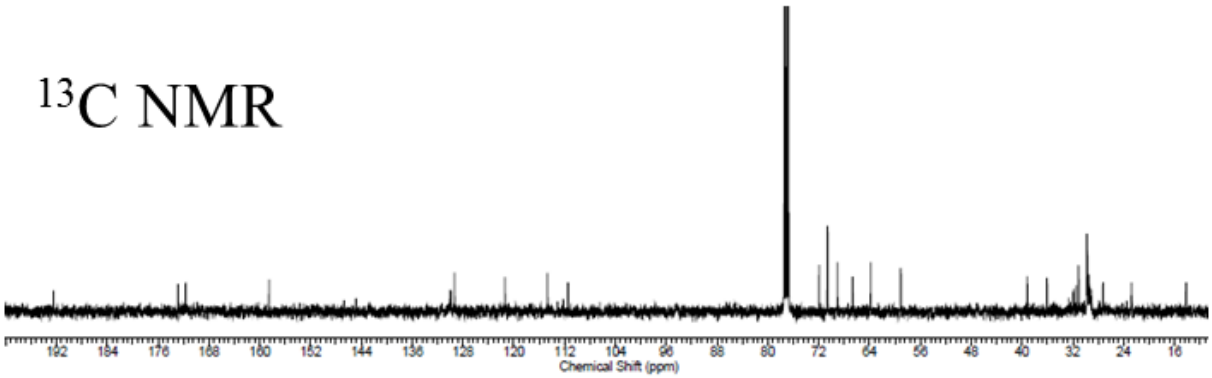
CAR-TEG



^{1}H NMR



^{13}C NMR



MALDI-TOF-TOF

Molecular Weight = 591

Molecular Formula = $C_{34}H_{57}NO_7$

591 + K^+