Electronic Supporting Informations (ESI)

Dual Stimuli Polysaccharide Nanovesicles for Conjugated and Physically Loaded Doxorubicin Delivery in Breast Cancer Cells

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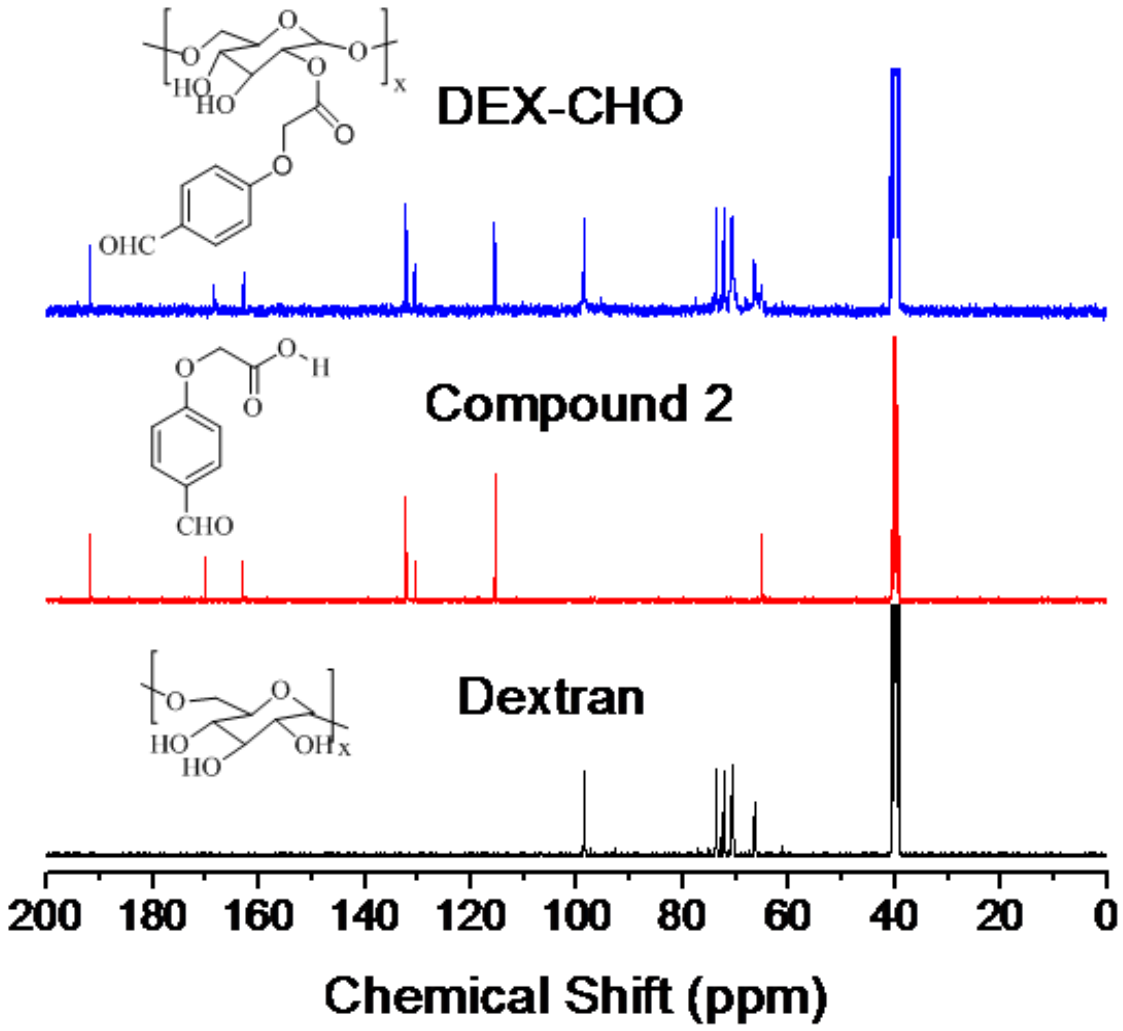


Figure SF-1: ^{13}C NMR spectra of Dextran, Compound 2 and DEX-CHO in DMSO (d_6)

Note: The appearance of peak at 65.11 ppm and 168.41 ppm corresponds to ester carbon atom, Ar-O**C**H_2-COO and Ar-OCH_2-**C**OO respectively in ^{13}C-NMR spectrum of DEX-CHO, substantiates the formation of coupled product.

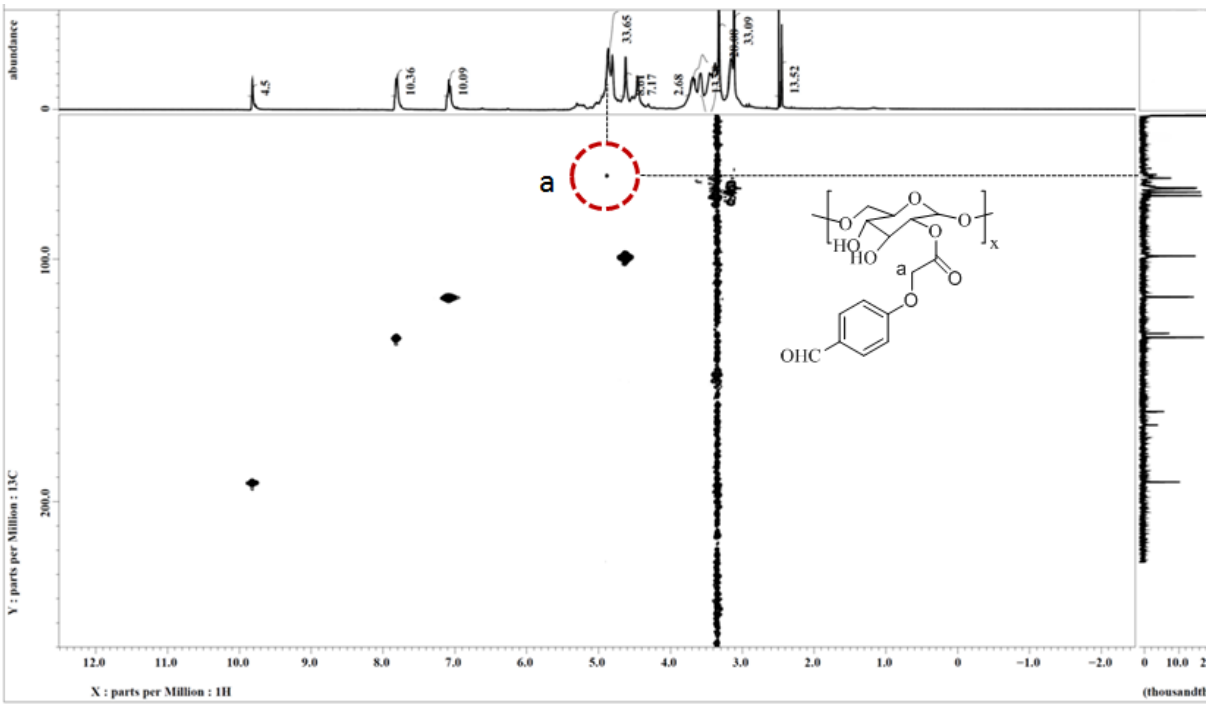


***Figure SF-2**: 2-D NMR-HSQC spectrum of DEX-CHO in DMSO (d6)*

Note: The contour highlighted as “a” in the spectrum belongs to Ar-O-**<u>CH$_2$</u>**-COO-dex

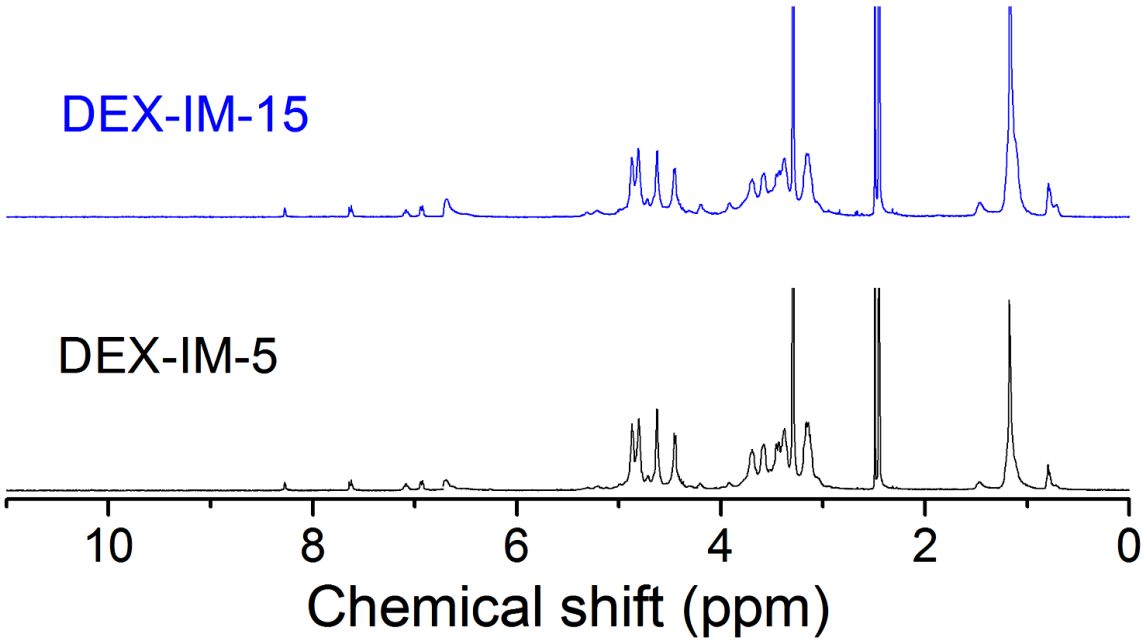


Figure SF-3: *^{1}H NMR spectra of DEX-IM-5 and DEX-IM-15*

Note: DEX-CHO-x was coupled with PDP-amine and the degree of substitution of hydrophobic unit on dextran back bone was estimated by comparing the peak intensities of the anomeric protons in dextran at 4.62 ppm and PDP aromatic protons at 7.2 ppm.

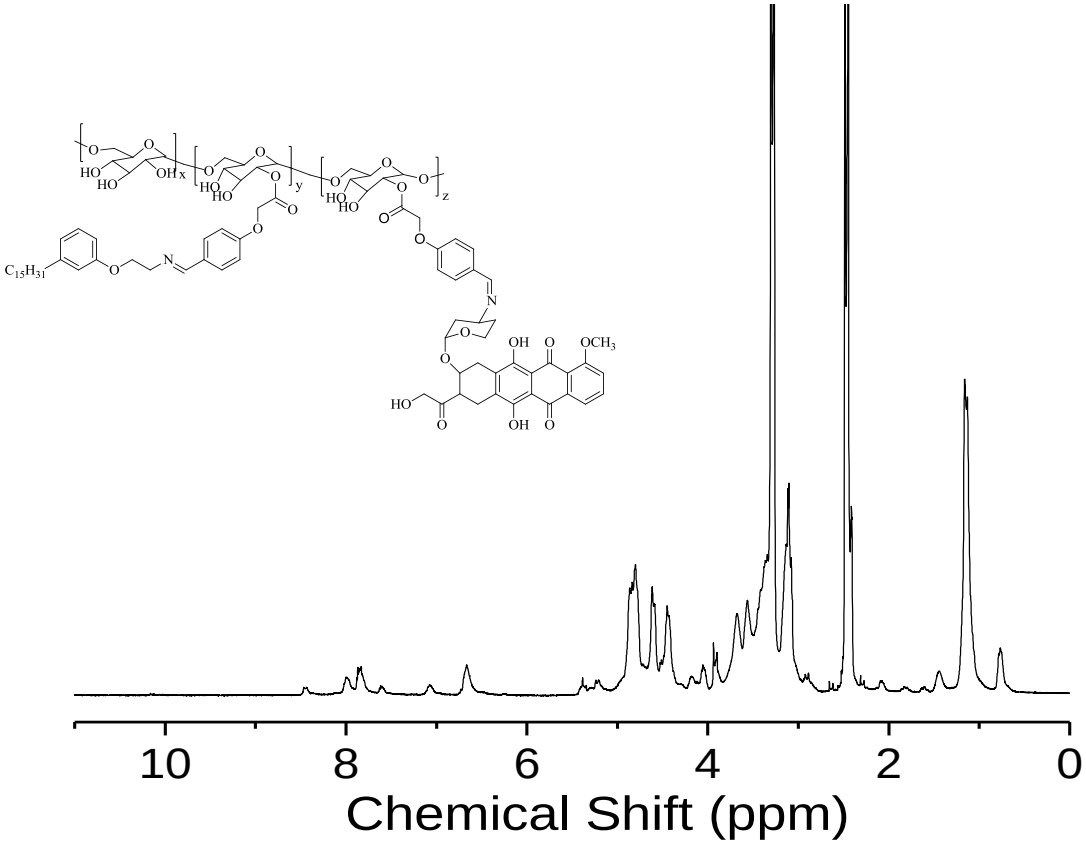


Figure SF-4. *1H NMR spectra of DEX-IM-DOX*

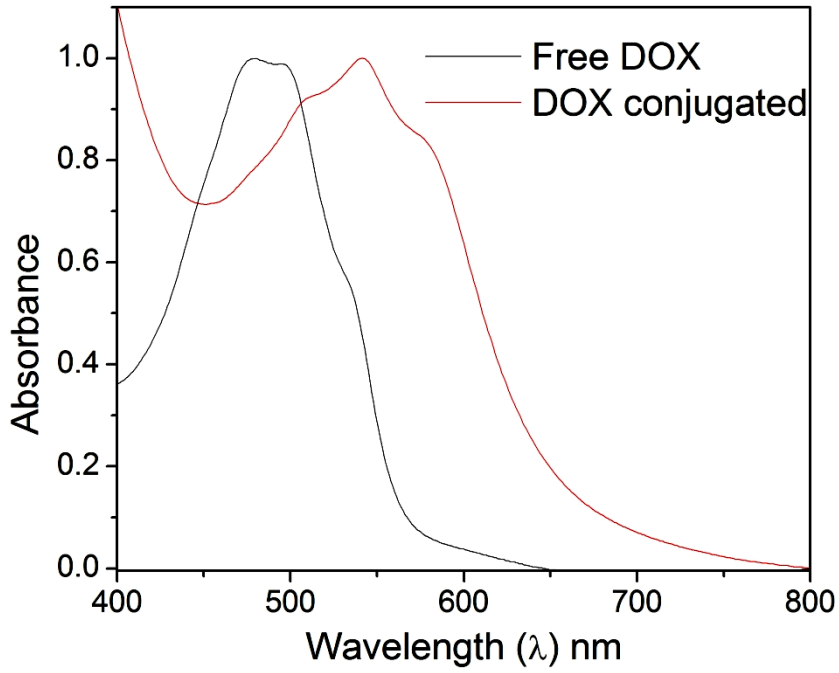


Figure SF-5. *Absorbance Spectra of Free DOX and DOX conjugated DEX-IM-5*

Type of Polymer	Mn (g/mol)	Mw (g/mol)	PDI
Dextran	16,800	18,000	1.07
DEX-CHO-5	22,600	26,000	1.15
DEX-IM-5	38,300	50,200	1.31
DEX-IM-DOX	46,400	51,200	1.10

Table ST-1. *Table depicting molecular weight of Dextran derivatives determined using GPC in DMF solvents with polystyrene standard.*

Note: Separation of polymers in Gel Permeation Chromatography is dependent on the solvated size rather than actual molecular weight. Here molecular weight of dextran derivatives was found to be overestimated by GPC. This is due to the solvation by polar DMF solvent and thereby changes in the effective hydrodynamic radius of dextran polymers with respect to polystyrene standard.

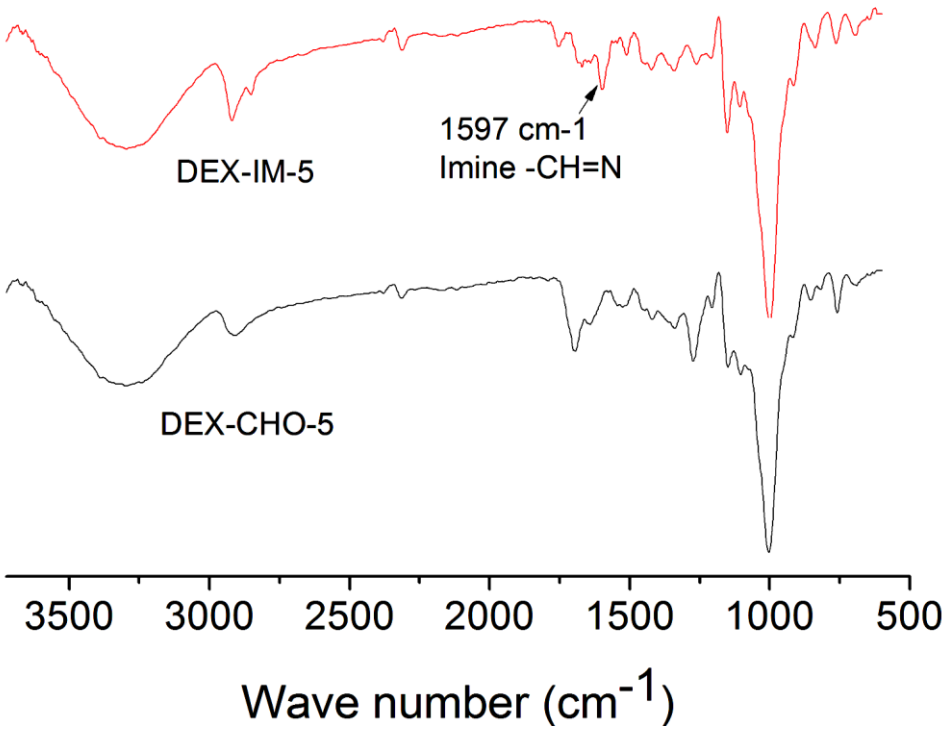


Figure SF-6. *FTIR spectra of DEX-CHO-5 and DEX-IM-5*

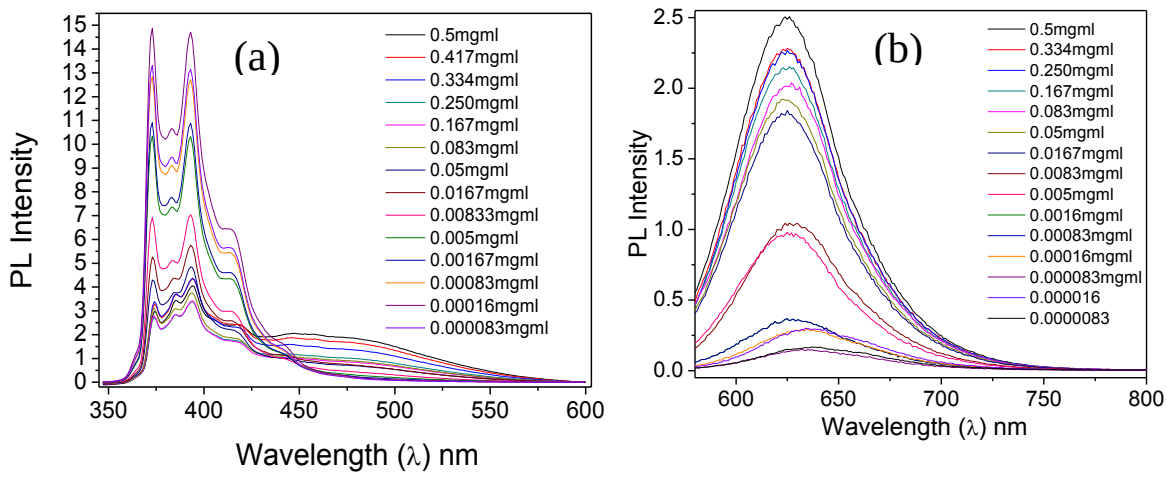


Figure SF-7: *Pyrene (a) and Nile red (b) emission spectra at different concentration of DEX-IM-10 polymer*

Note: Critical Vesicular concentrations (CVCs) of DEX-IM-10 were determined independently using pyrene (a) and Nile red (b) as fluorescent probe. The concentration of pyrene (0.6 µM) and nile red (1µM) was fixed and the concentration of polymer varied. In case of pyrene the ratio of PL intensity at 375 nm and 386 nm was plotted against concentration. The PL intensity of nile red at 626 nm was also plotted against concentration. The CVC of DEX-IM-10 was estimated to be 0.016 mg/ml from the deflection point in the plots.

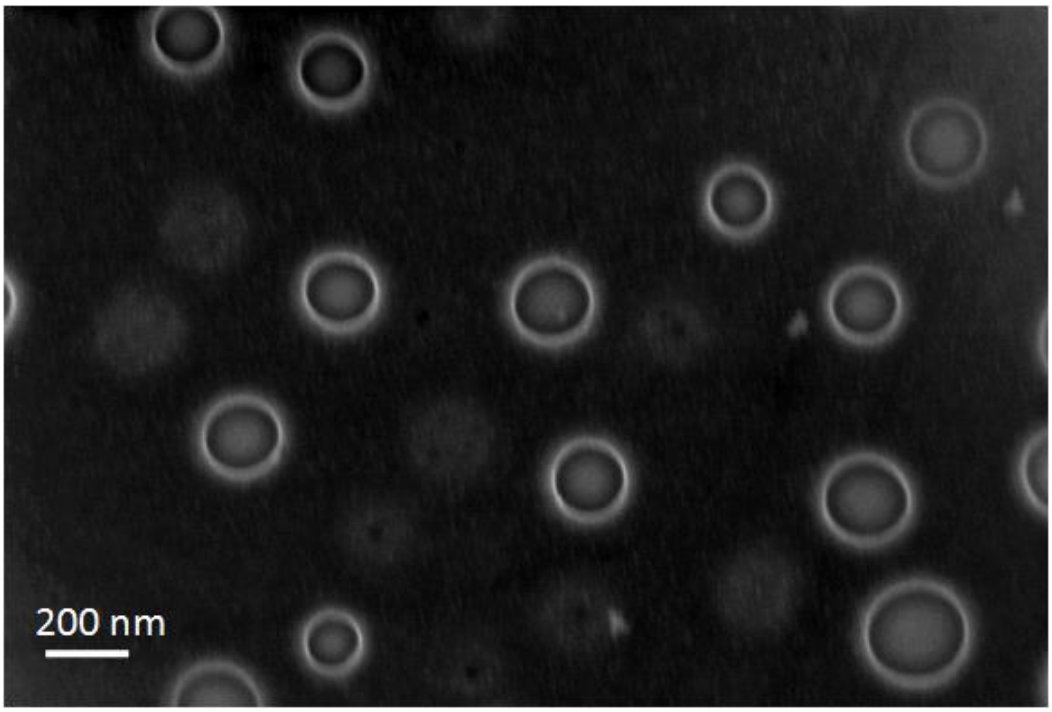


Figure SF-8: *FESEM image of DOX loaded DEX-IM-5.*

Note: FESEM image proved the retention of vesicular geometry of DEX-IM-5 after encapsulation of doxorubicin.

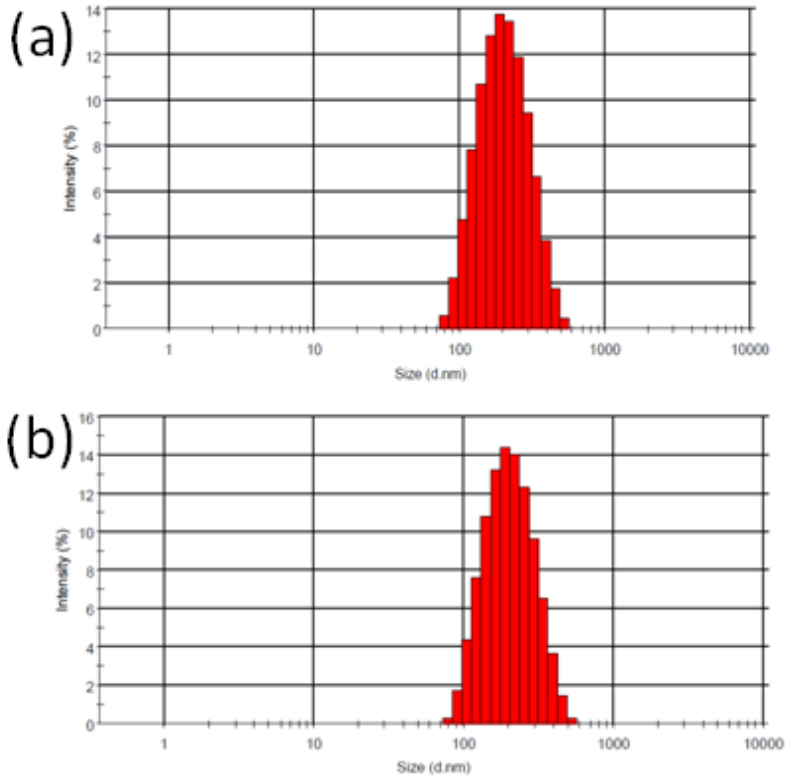


Figure SF-9: *DLS histogram of DEX-IM-5 at pH 7.4 day 1 (a) and day 7 (b).*

Note: DLS histogram of DEX-IM-5 dispersed in PBS (pH 7.4) were collected at different time interval from day 1 to day 7. The size of the particle was remained unchanged indicated the stability of DEX-IM-5 at physiological pH conditions.

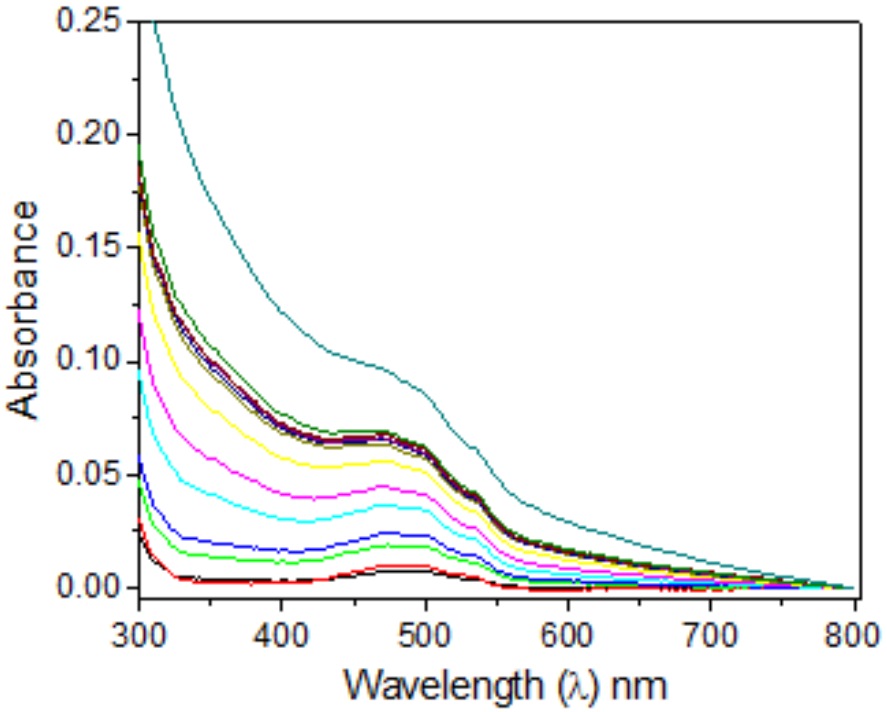


Figure SF-10: *Absorbance spectra of DOX released from dialysis containing DOX physically loaded DEX-IM -5 vesicles.*

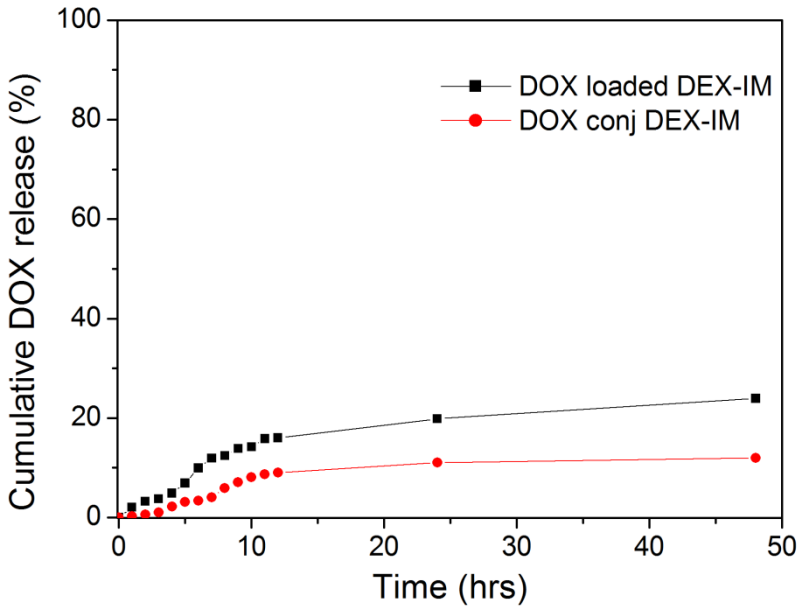


Figure SF-11: *In-vitro DOX releases from DOX loaded and DOX conjugated vesicles in the presence of serum (FBS).*

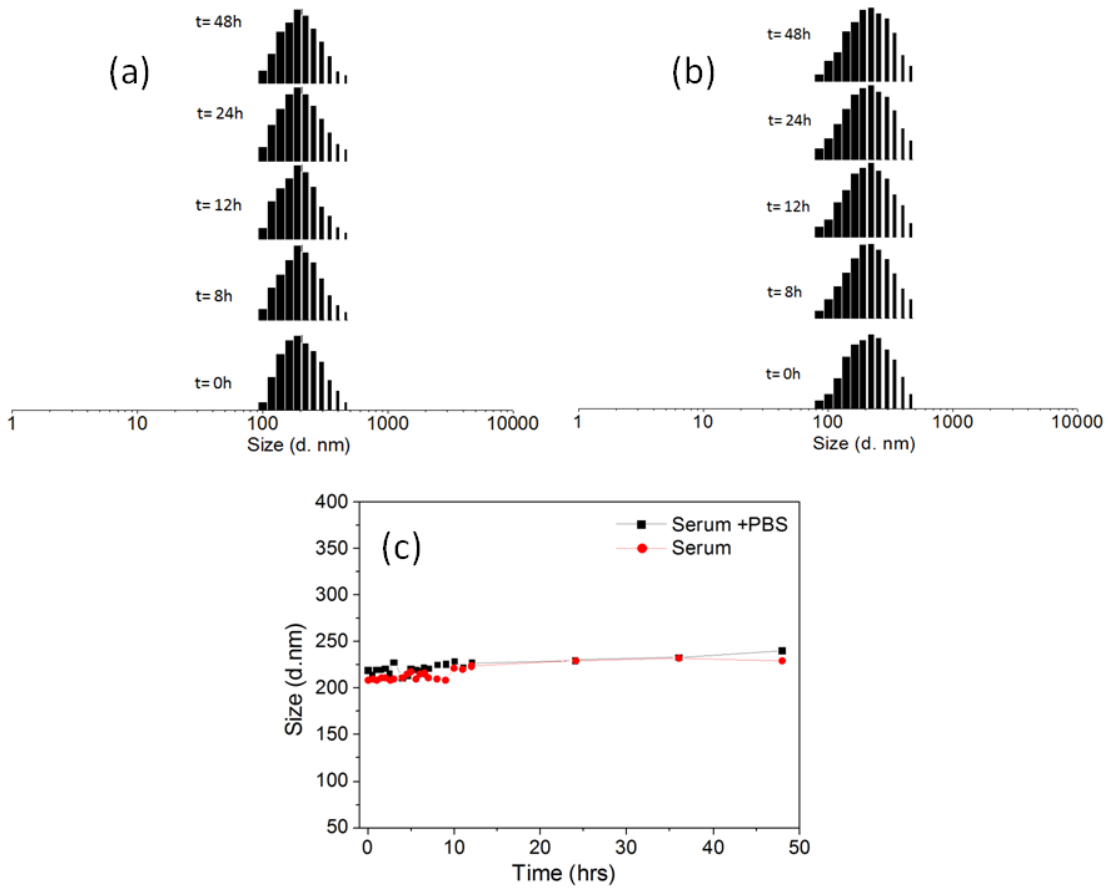


Figure SF-12: *DLS histograms of DOX loaded DEX-IM vesicles incubated (a) in FBS, (b) in FBS and PBS (pH 7.4) at different time interval. Hydrodynamic size (diameter) Vs time profile of DOX loaded DEX-IM vesicles in serum and serum + PBS.*

Sl No.	Condition Employed	k (μs^{-1}) for DOX loaded vesicles	k (μs^{-1}) for DOX conj. vesicles
1	pH 7.4	6.5	2.4
2	pH 6.5	8.4	4.0
3	pH 6.0	16.5	7.7
4	pH 5.5	22.6	11.9
5	pH 5.0	59.5	17.3
6	pH 7.4 and Esterase	19.1	11.7
7	pH 6.0 and Esterase	58.8	22
8	pH 5.5 and Esterase	63.0	30.7

Table-*ST-2:* *Rate constant of DOX release from DEX-IM loaded vesicles and conjugated vesicles at various conditions*

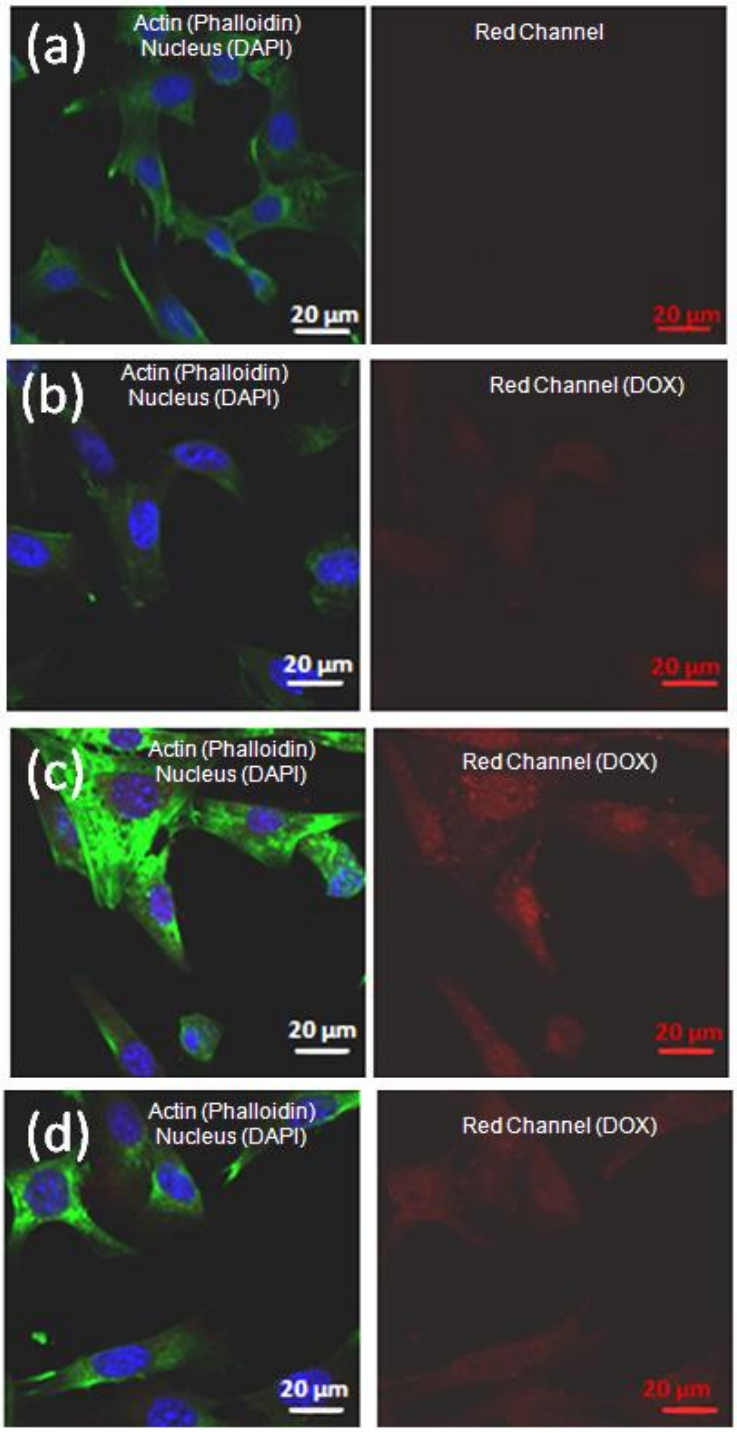
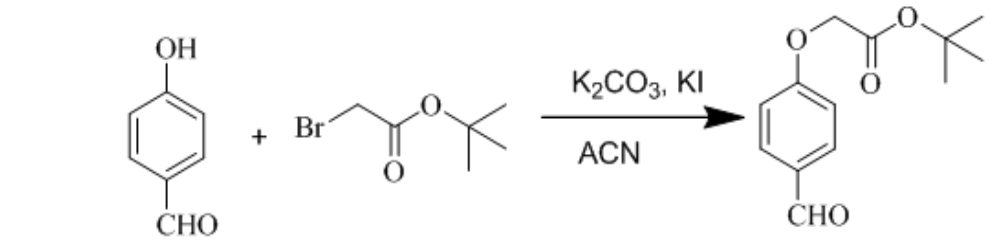


Figure SF-13: *Confocal microscopic image of (a) control, (b) free DOX, (c) DOX loaded DEX-IM vesicles and (d) DOX conjugated DEX-IM treated WTMEFS cells in second set of experiment. The data was exactly matched as that in Figure 8. The nucleus was counter stained with DAPI (blue), actin cytoskeletal network in cells is stained with phalloidin (green). The cells were observed through red channel to locate DOX fluorescence (red). Scale bar in each pannel represents 20 µm.*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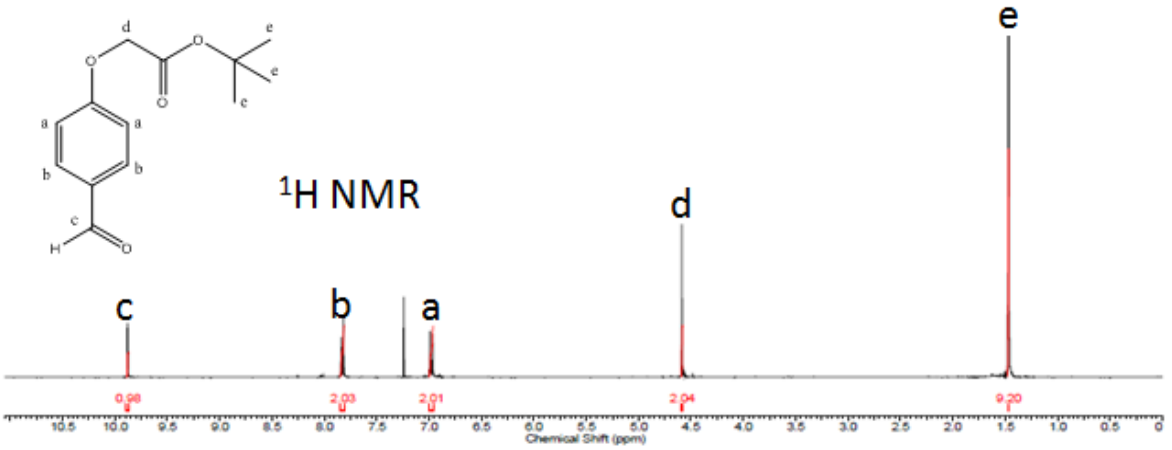
CHO
Br
O
O
K_2CO_3, KI
ACN
O
O
O
CHO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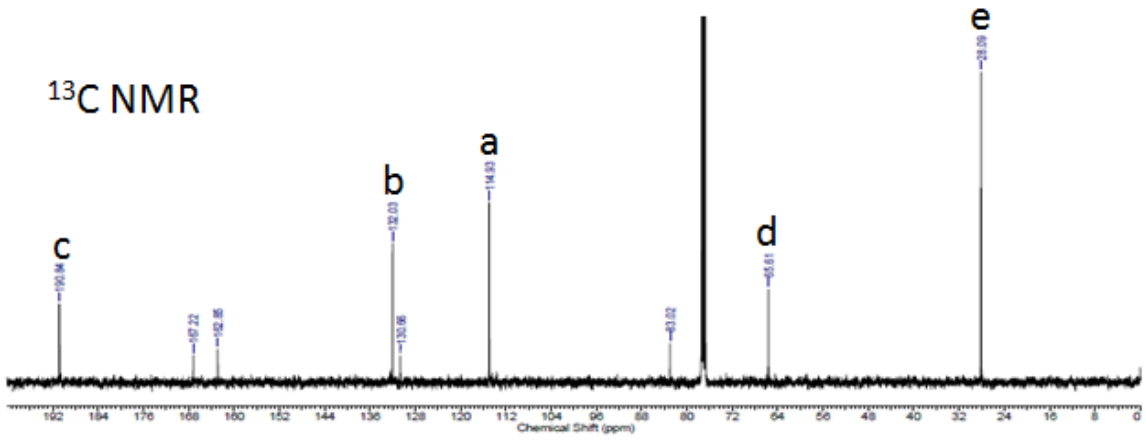
a
b
c
d
e
Chemical Shift (ppm)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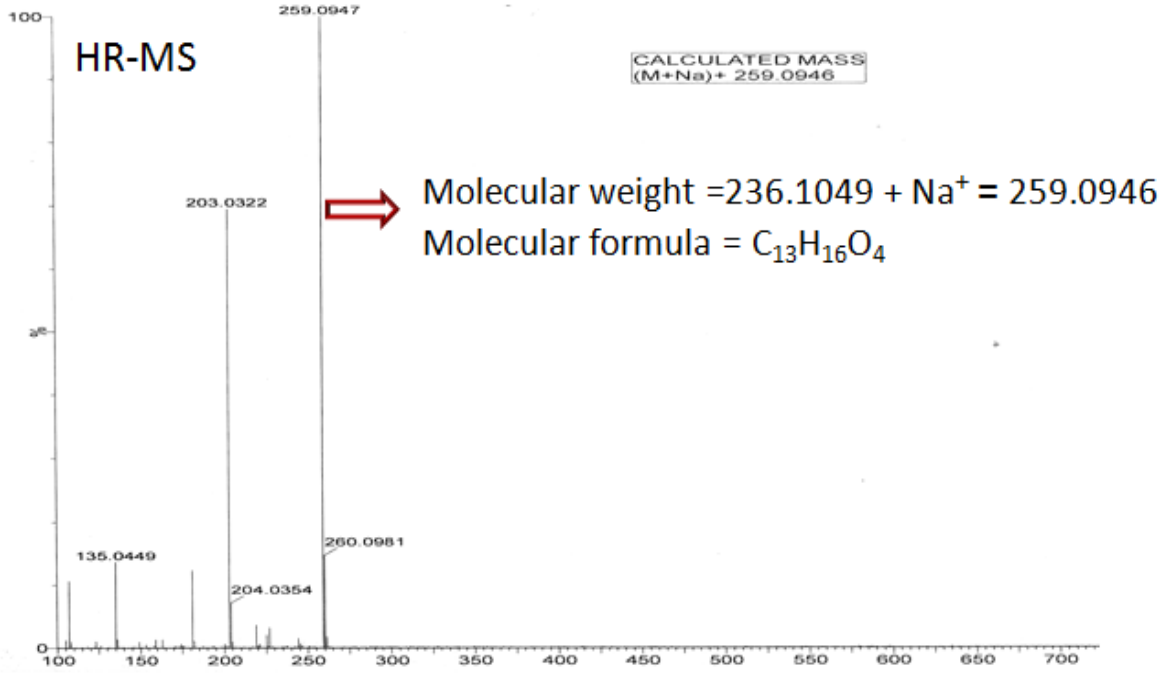
a
b
c
d
e
Chemical Shift (ppm)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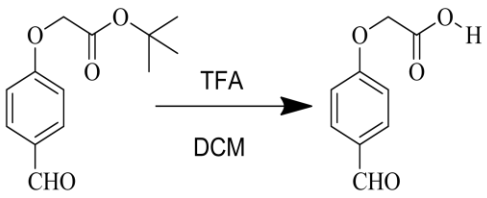
260.0981
135.0449
204.0354
Molecular weight =236.1049 + Na^+ = 259.0946
Molecular formula = $C_{13}H_{16}O_4$



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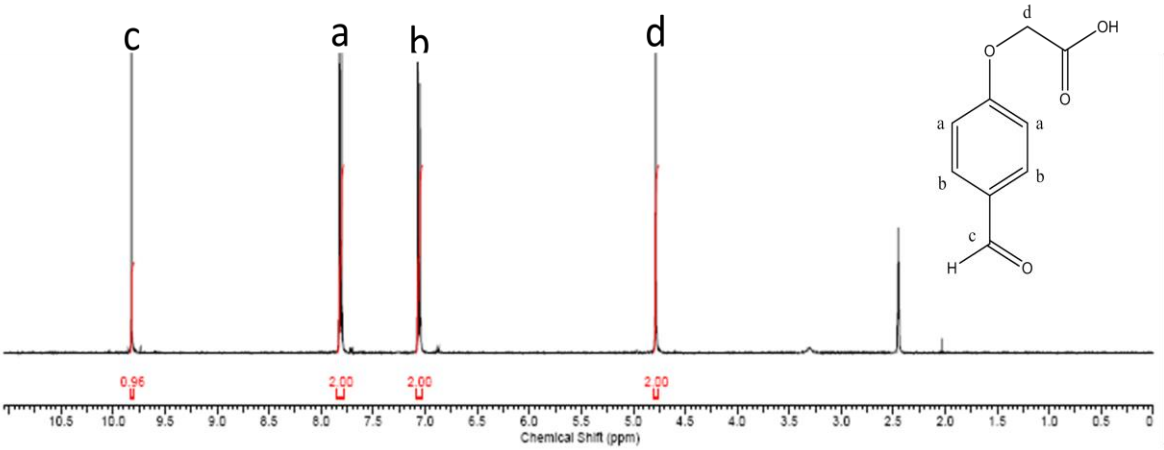

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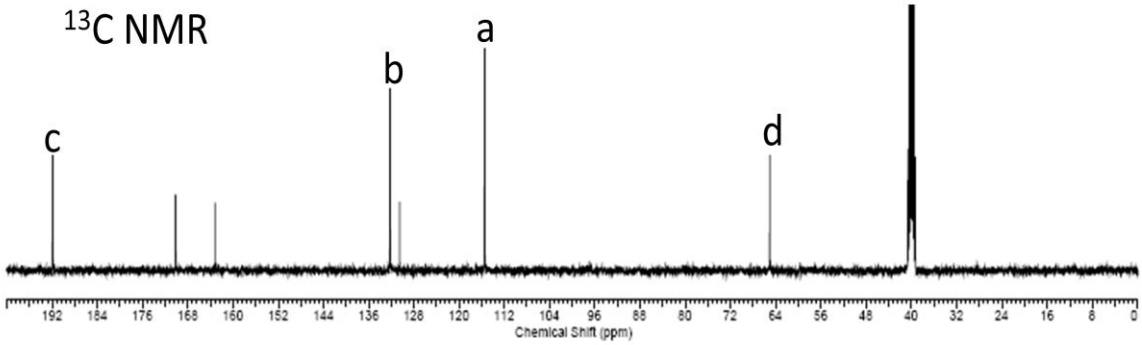

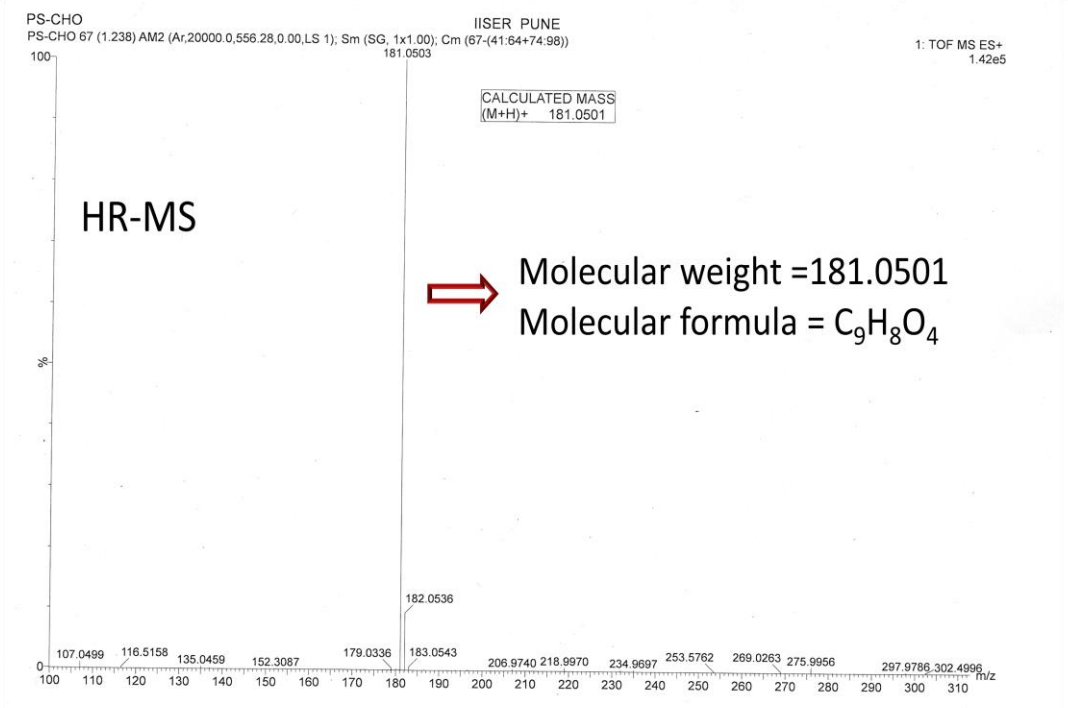

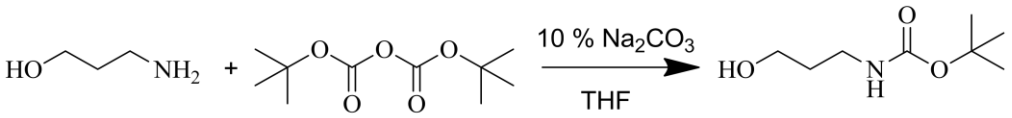
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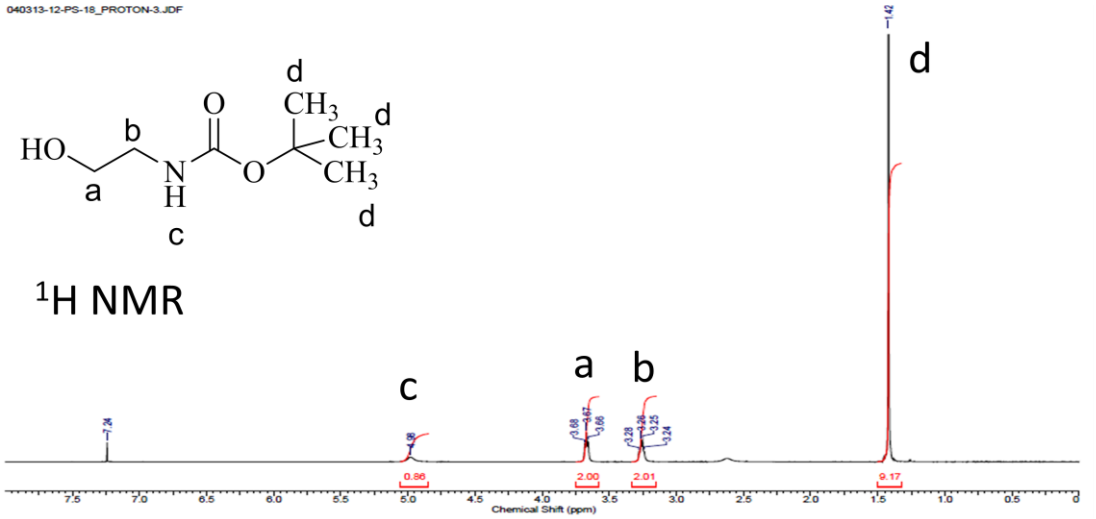
N
H
O
O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a
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H
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O
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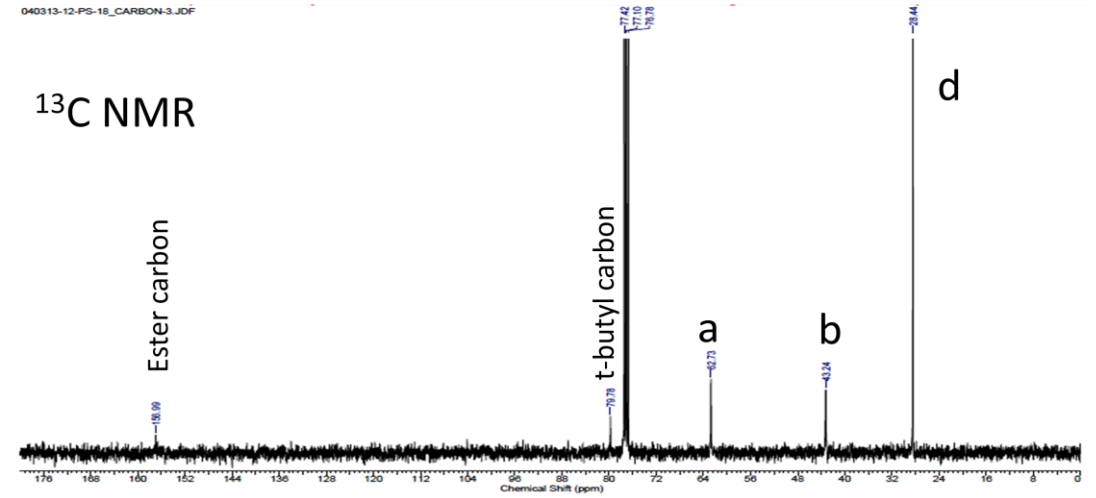
CH3
CH3
CH3
d
d
1H NMR
c
a
b
d
Chemical Shift (ppm)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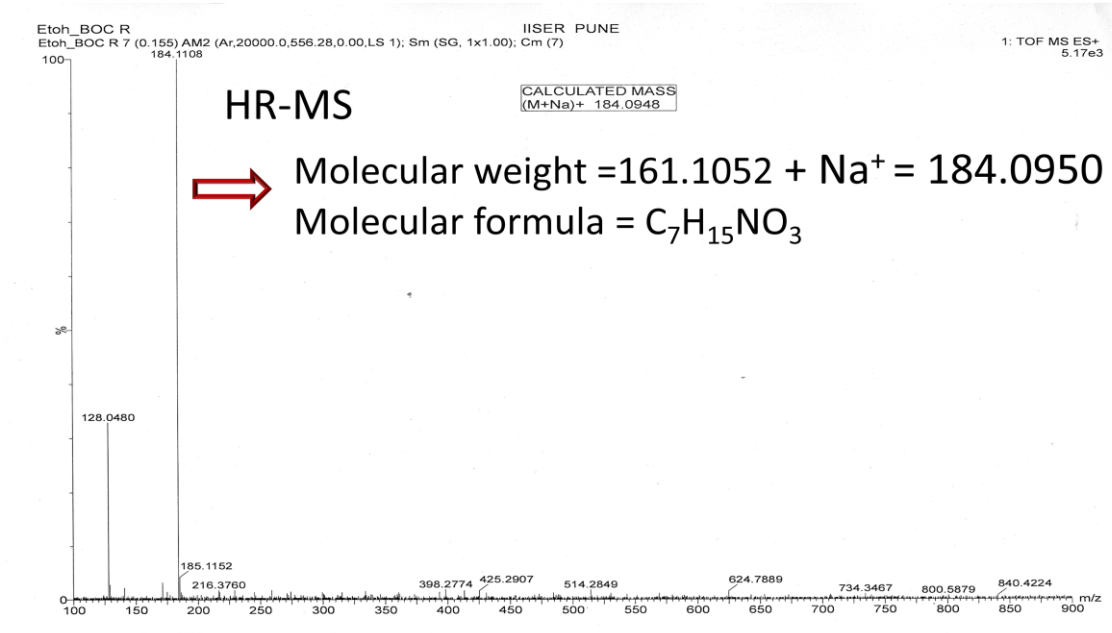
Ester carbon
t-butyl carbon
a
b
d
Chemical Shift (ppm)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IISER PUNE
Etoh_BOC R 7 (0.155) AM2 (Ar,20000.0,556.28,0.00,LS 1); Sm (SG, 1x1.00); Cm (7)
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HR-MS
CALCULATED MASS
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Molecular formula = C7H15NO3
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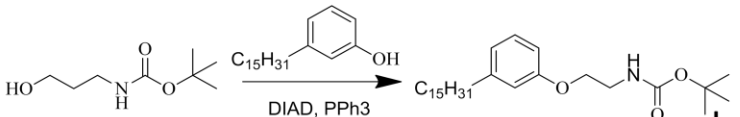
m/z



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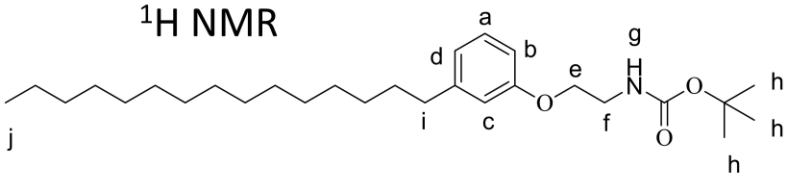

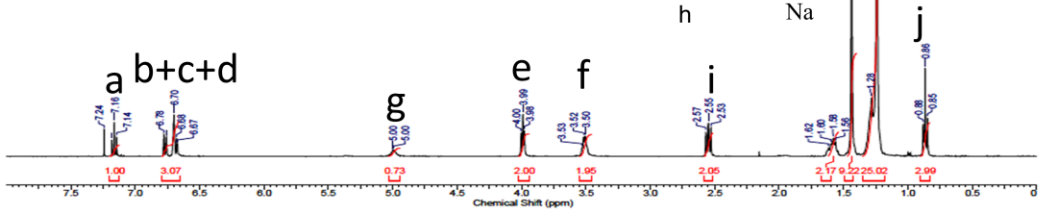

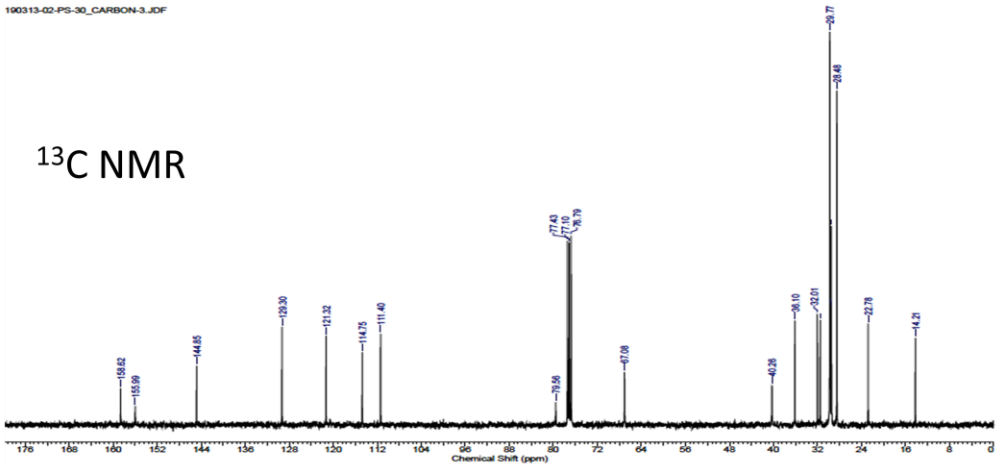

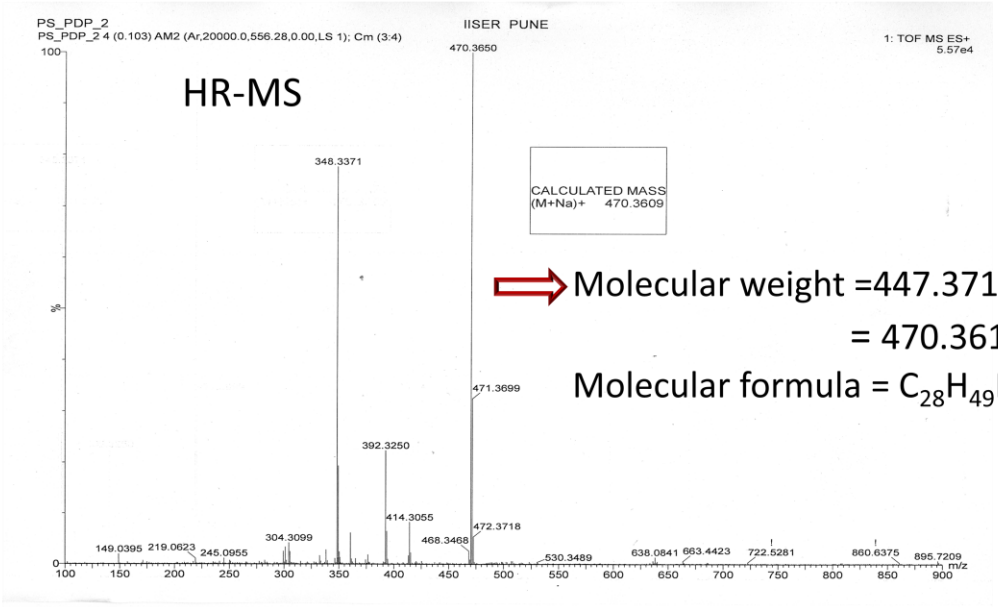

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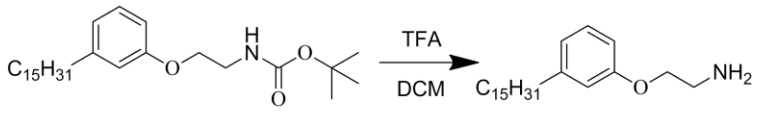
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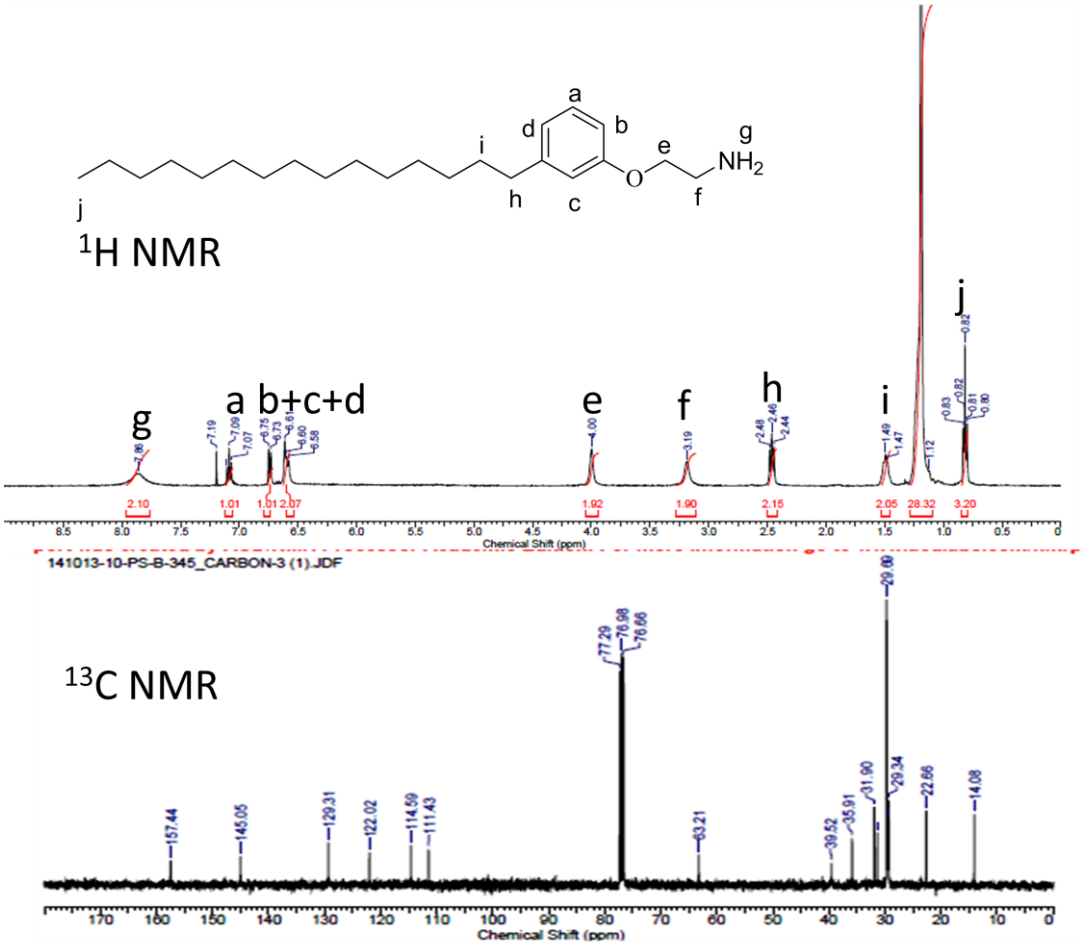
NH_2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b
c
d
e
f
g
h
i
j
NH_2
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g
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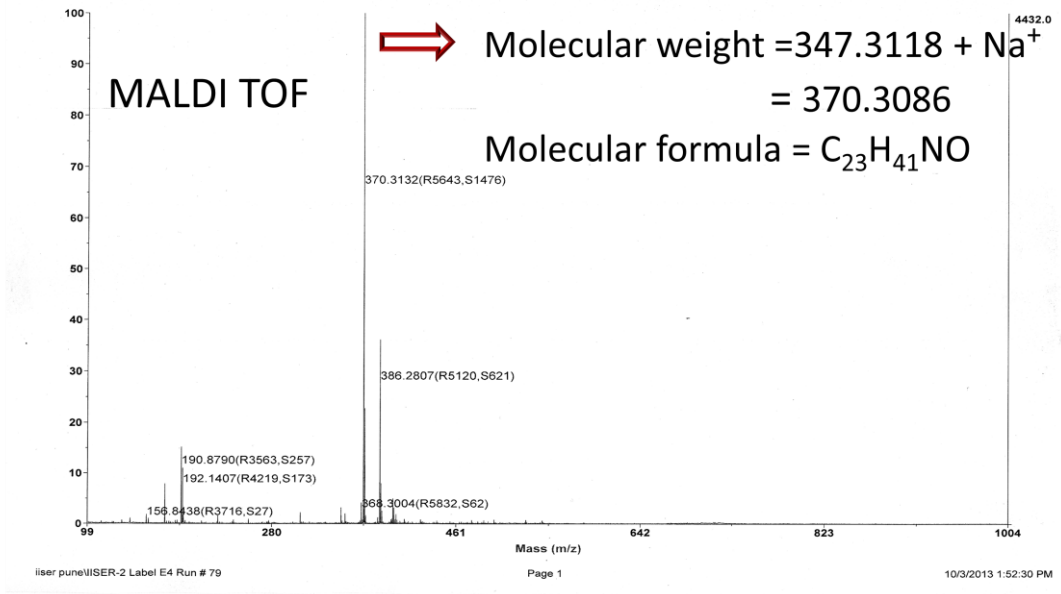
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Molecular formula = $C_{23}H_{41}NO$
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