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Synthesis, characterization and *in vitro* evaluation of novel vitamin D3 nanoparticles as versatile platform for drug delivery in cancer

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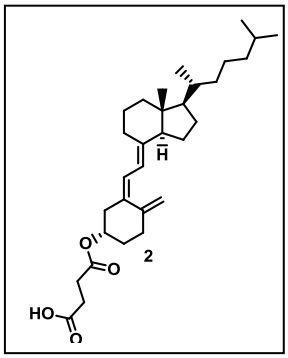
^{1}H-NMR, ^{13}C-NMR and HR-MS spectral data for compound 2:

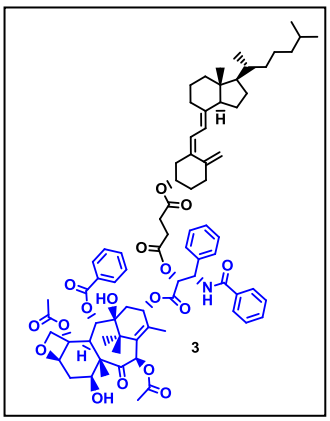


^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.22-6.19 (d, 1H, *J* = 12 Hz), 6.03-6.01 (d, 1H, *J* = 8 Hz), 5.056-5.050 (d, 1H, *J*= 2.4 Hz), 5.00-4.94 (m, 1H), 4.835-4.829 (d, 1H, *J* = 2.4Hz), 2.68-2.66 (m, 2H), 2.62-2.60 (m, 2H), 2.42-2.33 (m, 2H), 2.02-1.92 (m, 4H), 1.54-1.44 (m, 4H), 1.35-1.25 (m, 14H), 1.17-1.07 (m, 5H), 0.92-0.91 (d, 3H, *J*= 4 Hz), 0.87-0.86 (d, 3H, *J*= 4 Hz), 0.855-0.851 (d, 3H, *J* = 1.6 Hz).

^{13}C NNMR (100 MHz, $CDCl_3$): δ= 177.9, 171.7, 144.6, 142.7, 134.2, 122.7, 117.6, 112.9, 72.4, 56.7, 56.5, 46.0, 42.2, 40.7, 39.6, 36.3, 32.2, 31.9, 29.8, 29.3, 29.2, 29.1, 28.1, 27.8, 24.0, 23.7, 22.9, 22.7, 22.4, 18.9, 12.1.

HRMS (ESI): m/z: for $C_{31}H_{49}O_4^+$ $[M+H]^+$: calculated = 485.3553, observed = 385.3632 and for $C_{31}H_{48}O_4Na^+$ $[M+Na]^+$: calculated = 507.3450; observed = 507.3445.

^{1}H-NMR, ^{13}C-NMR and HR-MS spectral data for compound 3:



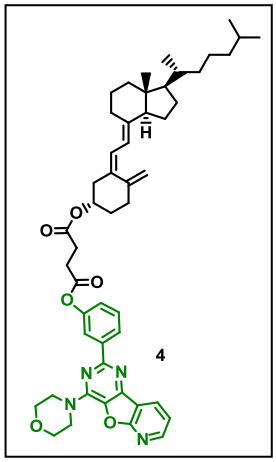
^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.18-8.10 (m, 1H), 7.83-7.76 (m, 1H), 7.72-7.57 (m, 1H), 7.56-7.46 (m, 2H), 7.46-7.31 (m, 5H), 7.30-7.17 (m, 4H), 7.17-7.08 (m, 1H), 6.31-6.14 (m, 2H), 6.07-5.91 (m, 1H), 5.75-5.65 (m, 1H), 5.52-5.42 (m, 1H), 5.40-5.21 (m, 4H), 5.18-5.06 (m, 1H), 5.05-4.89 (m, 2H), 4.85-4.73 (m, 1H), 4.50-4.38 (m, 1H), 4.35-4.25 (m, 2H), 4.23-4.17 (m, 1H), 4.16-4.09 (m, 1H), 4.08-4.02 (m, 1H), 3.84-3.77 (m,

1H), 2.83-2.68 (m, 3H), 2.51-2.41 (m, 5H), 2.61-2.53 (m, 2H), 2.37-2.25 (m, 8H), 1.62-1.58 (m, 7H), 2.09-1.95 (m, 12H), 1.23-1.18 (m, 14H), 0.88-0.86 (m, 9H).

^{13}C NNMR (100 MHz, $CDCl_3$): δ= 203.9, 173.3, 171.4, 170.8, 169.9, 168.2, 167.1, 144.4, 142.9, 142.7, 137.0, 134.0, 133.7, 132.7, 131.9, 130.3, 129.9, 129.5, 129.0, 128.7, 128.6, 128.0, 127.3, 126.5, 124.3, 122.6, 117.4, 115.5, 114.1, 112.9, 81.1, 79.1, 75.6, 75.1, 74.3, 72.5, 72.0, 71.8, 68.9, 62.1, 58.5, 56.6, 56.4, 52.7, 45.9, 45.5, 43.2, 42.3, 41.9, 39.7, 39.5, 37.1, 36.1, 35.5, 34.1, 33.8, 33.6, 31.4, 29.7, 29.4, 29.1, 27.9, 27.2, 26.8, 24.9, 24.7, 22.7, 22.6, 20.8, 18.8, 17.7, 16.0, 14.8, 14.1, 12.0, 9.6, 1.0.

HRMS (ESI): m/z: for $C_{78}H_{98}NO_{17}$ $[M+H]^+$: calculated = 1320.6757, observed = 1320.6881, for $C_{78}H_{97}NO_{17}Na$ $[M+Na]^+$: calculated = 1342.6757, observed = 1342.6703 and for $C_{78}H_{97}NO_{17}K$ $[M+K]^+$: calculated = 1358.6757, observed = 1358.6611

^{1}H-NMR, ^{13}C-NMR and HR-MS spectral data for compound 4:

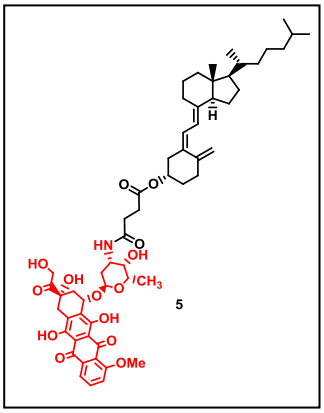


^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.61-8.59 (m, 2H), 8.37-8.35 (m, 1H), 8.19-8.18 (m, 1H), 7.48-7.43 (m, 1H), 7.21-7.20 (m, 1H), 7.19-7.18 (m, 1H), 5.05-5.02 (m, 1H), 5.01-4.94 (m, 1H), 4.83-4.81 (m, 1H), 4.23-4.21 (t, 4H, *J* = 4.6 Hz), 3.93-3.90 (t, 4H, *J* = 4.8 Hz), 2.95-2.92 (t, 2H, *J* = 6.6 Hz), 2.78-2.75 (t, 2H, *J*= 6.8 Hz), 2.61-2.56 (m, 1H), 2.44-2.34 (m, 1H), 2.04-1.92 (m, 2H), 1.62-1.52 (m, 10H), 1.28-1.19 (m, 15H), 0.91-0.85 (m, 9H).

^{13}C NNMR (100 MHz, $CDCl_3$): δ= 170.9, 162.4, 158.7, 150.9, 149.6, 148.8, 147.2, 144.5, 142.6, 139.9, 134.1, 133.4, 131.9, 129.3, 125.6, 123.1, 122.6, 121.2, 120.3, 117.4, 115.4, 112.8, 72.4, 72.0 66.9, 56.6, 56.5, 45.9, 45.8, 42.1, 40.5, 39.5, 36.1, 32.1, 32.0, 29.7, 29.6, 28.8, 28.02, 23.9, 23.7, 22.8, 22.6, 22.3, 18.8, 14.0, 11.9.

HRMS (ESI): m/z: for $C_{50}H_{63}N_4O_6$ $[M+H]^+$: calculated= 815.4748, observed= 815.4752.

^{1}H-NMR, ^{13}C-NMR and HR-MS spectral data for compound 5:



^{1}H NMR (400 MHz, $CDCl_3$): 8.06-8.04 (dd, 1H, *J* = 1 Hz, 4.4 Hz), 7.81-7.77 (t, 1H, *J* = 8.2 Hz), 7.41-7.38 (d, 1H, *J* = 8 Hz), 6.19-6.17 (m, 1H), 6.01-5.98 (m, 1H), 5.92-5.89 (m, 1H), 5.15-5.49 (m, 1H), 5.31-5.28 (m, 1H), 5.04-5.02 (m, 1H), 4.97-4.89 (m, 1H), 4.81-4.80 (m, 1H), 4.76-4.74 (m, 2H), 4.57-4.56 (m, 1H), 4.17-4.10 (m, 2H), 4.08 (s, 3H), 3.69-3.64 (m, 1H), 3.32-3.26 (m, 1H), 3.06-2.96 (m, 2H), 2.82-2.77 (m, 1H), 2.68-2.51 (m, 2H), 2.40-2.31 (m, 5H), 2.24-2.21 (m, 1H), 2.19-2.12 (m, 2H), 2.00-1.94 (m, 2H), 1.82-1.78 (m, 2H), 1.61-1.59 (m, 11H), 1.20-1.25 (m, 13H), 0.92-0.85 (m, 9H).

^{13}C NMR (100 MHz, $CDCl_3$): δ= 219.5, 195.1, 182.7, 176.5, 171.3, 161.2, 156.4, 155.8, 147.7, 147.2, 143.0, 142.6, 139.4, 135.9, 133.7, 131.0, 128.9, 124.6, 124.1, 123.6, 122.7, 120.0, 119.2, 118.5, 117.6, 116.0, 114.2, 112.9, 101.0, 79.7, 72.7, 72.4, 71.8, 69.8, 69.1, 68.4, 67.9, 67.2, 65.7, 63.3, 56.8, 56.5, 46.1, 45.4, 42.2, 40.6, 39.6, 37.2, 36.9, 36.3, 35.0, 33.9, 32.1, 31.5, 29.8, 29.5, 22.8, 14.4

HRMS (ESI): m/z: for $C_{58}H_{75}NO_{14}Na$ $[M+Na]^+$: calculated = 1032.5083, observed= 1032.5110.

Absorbance vs concentration calibration graph of paclitaxel, PI103 and doxorubicin:

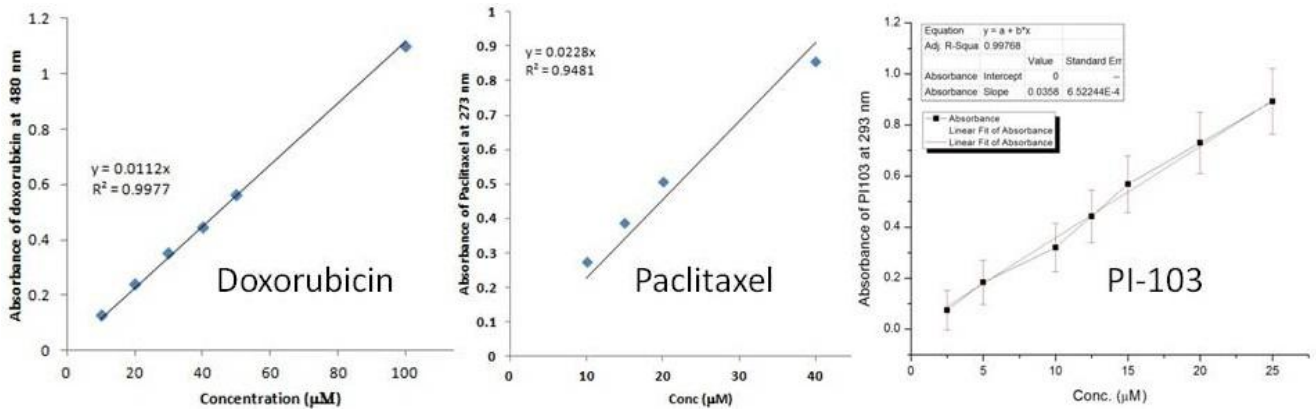


Fig. S1. Concentration vs absorbance calibration curve for doxorubicin, paclitaxel and PI103 at λ_{max} = 480 nm, 273 nm and 293 nm respectively.

Size and morphology of the self-assembled nanoparticles by FE-SEM and TEM:

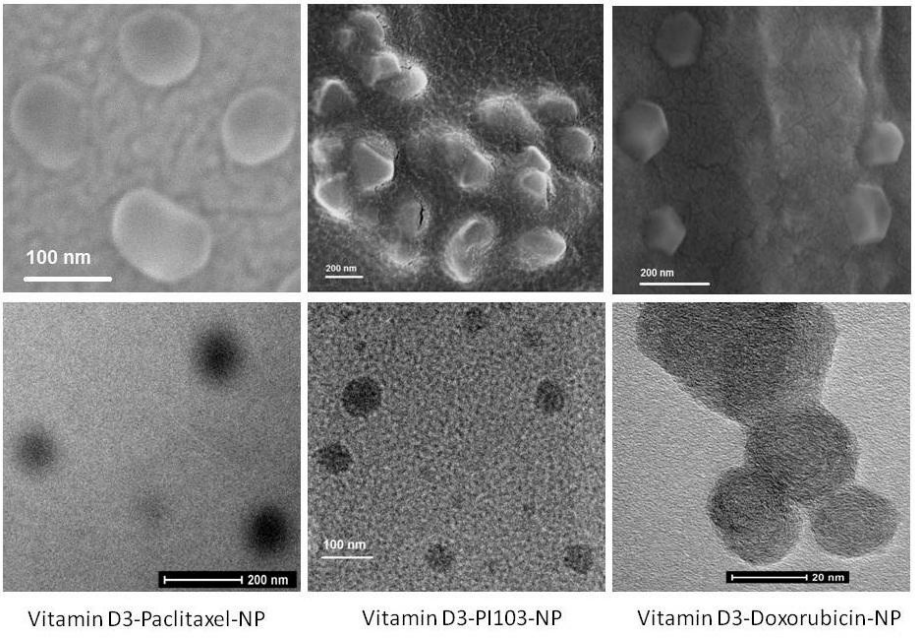


Fig. S2: Size and shape determination of vitamin D3-NPs by FE-SEM and TEM.

Size and morphology of the self-assembled nanoparticles by atomic force microscopy (AFM):

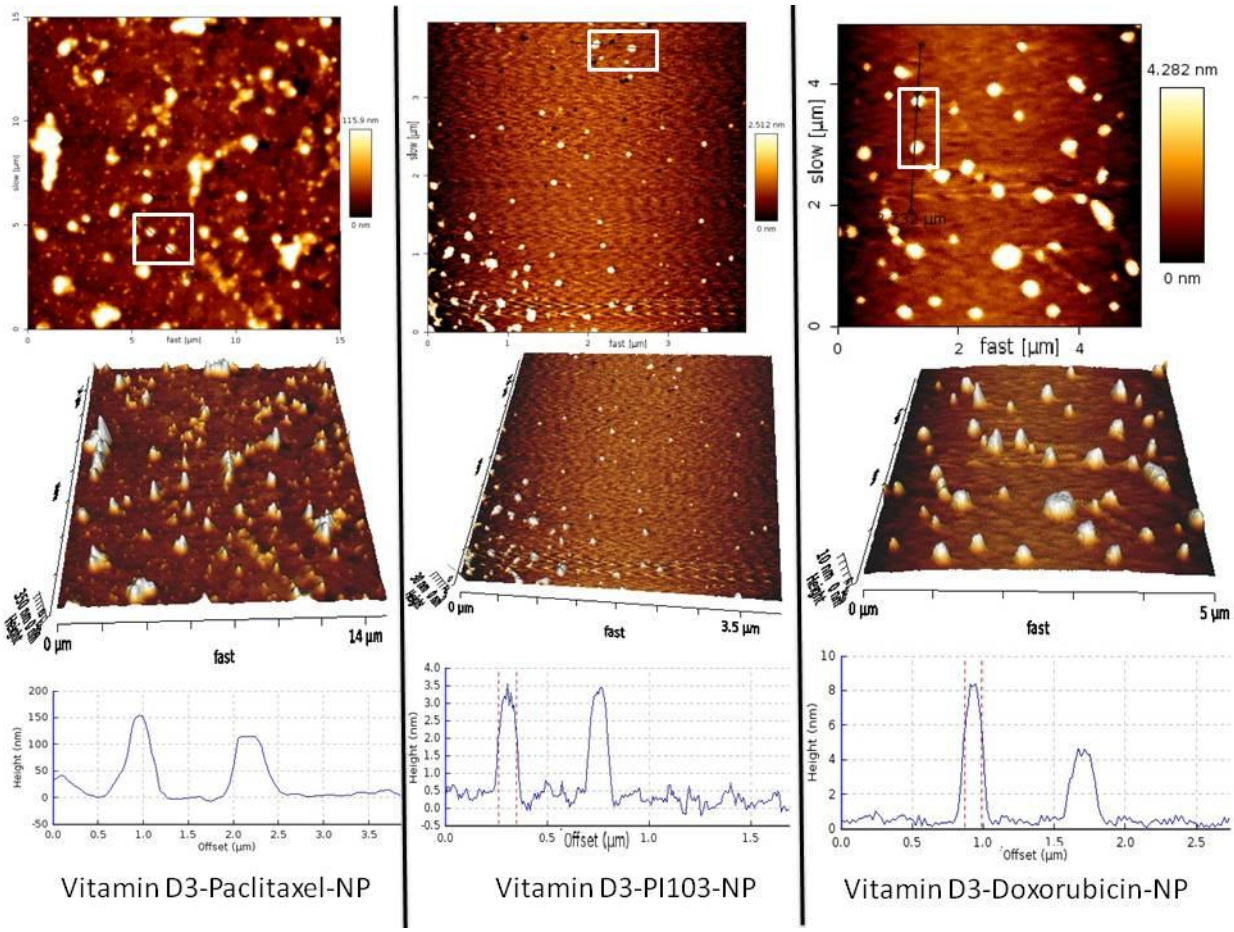


Fig. S3: Size and morphology determination of vitamin D3-NPs by AFM.

Size and loading of drug encapsulated vitamin D3-NPs:

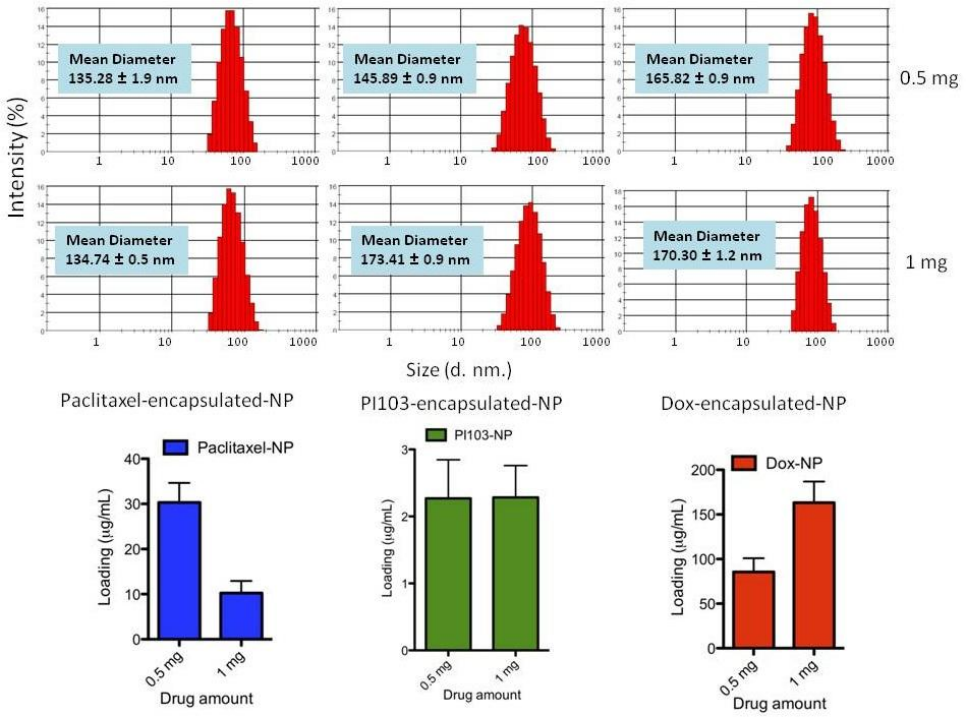


Fig. S4: Size and loading of different drugs in vitamin D3-NPs.

Size and PDI of drug loaded vitamin D3-NPs by DLS at 37 °C:

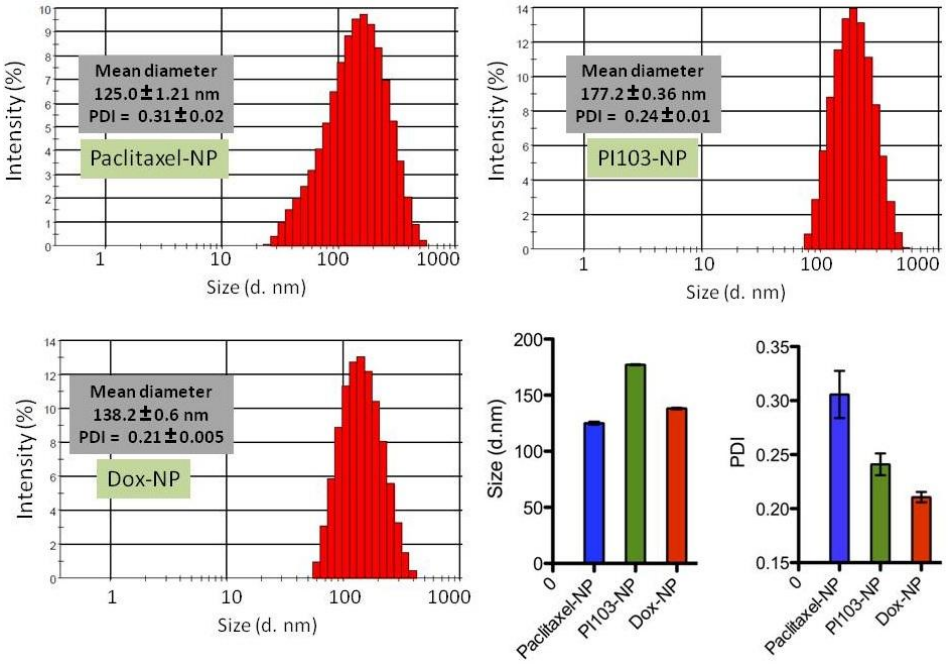


Fig. S5: Size and PDI of different drug loaded vitamin D3-NPs by DLS at 37 °C.

Size and PDI of drug loaded vitamin D3-NPs using 100 nm polycarbonate membrane by DLS:

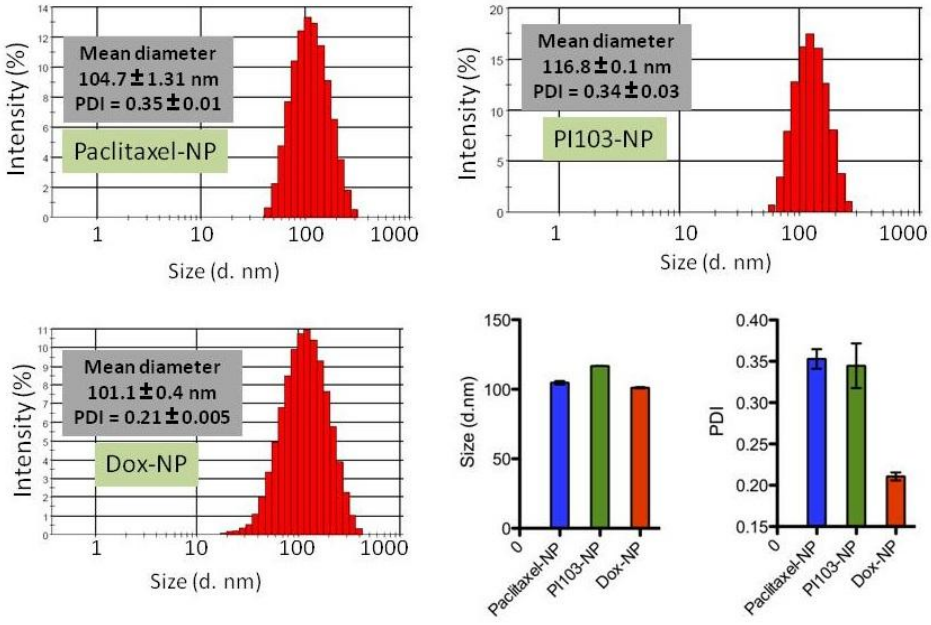


Fig. S6: Size distribution and PDI of different drug loaded vitamin D3-NPs using 100 nm polycarbonate membrane. Size and PDI were measured by DLS.

Stability of drug-loaded vitamin D3-NPs:

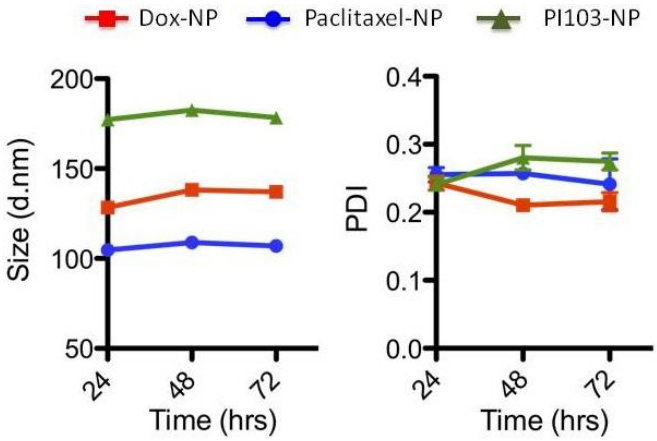


Fig. S7: Stability of drug-loaded vitamin D3-NPs in FBS at 37 °C for 72 h by DLS.

Characterization of free doxorubicin released from doxorubicin-NP in release kinetics by MALDI-TOF:

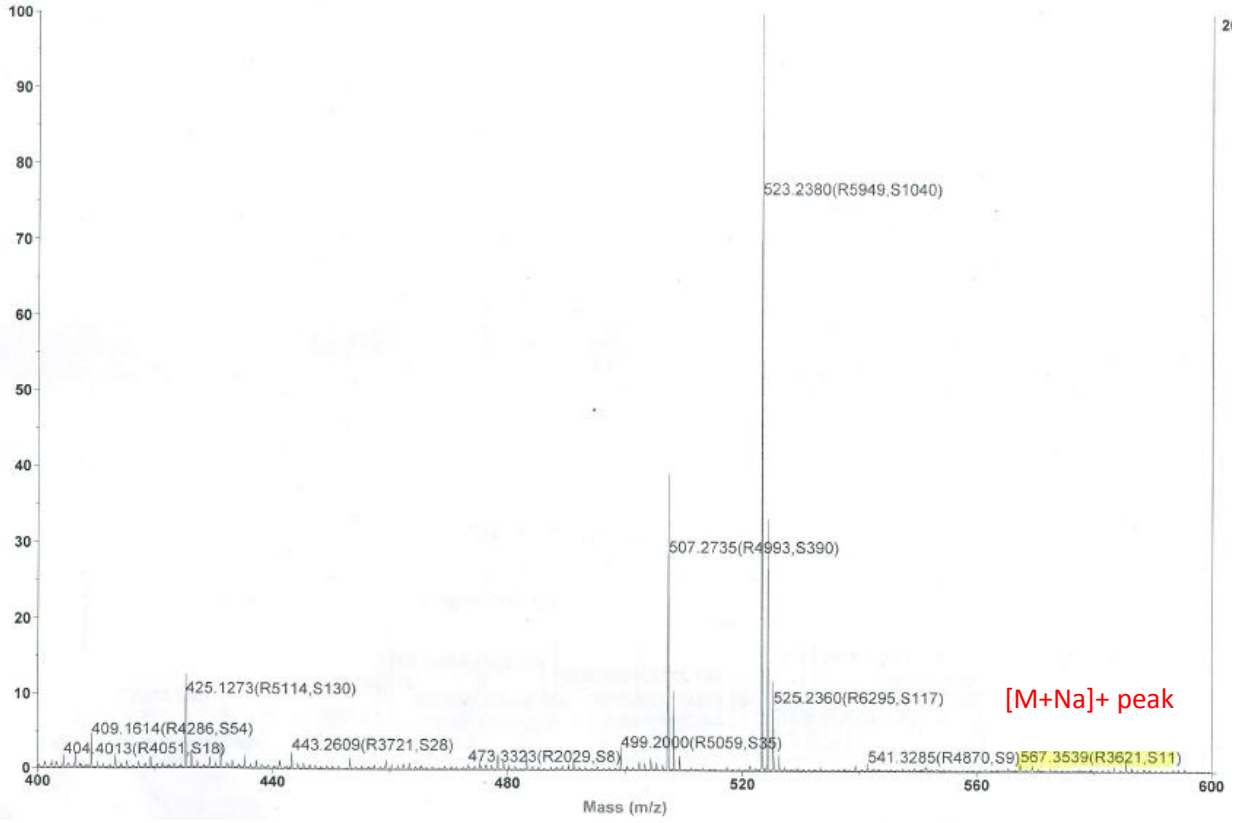


Fig. S8: MALDI-TOF of released doxorubicin from doxorubicin-NP showing the presence of free doxorubicin.

Characterization of free PI103 released from PI103-NP in release kinetics by MALDI-TOF:

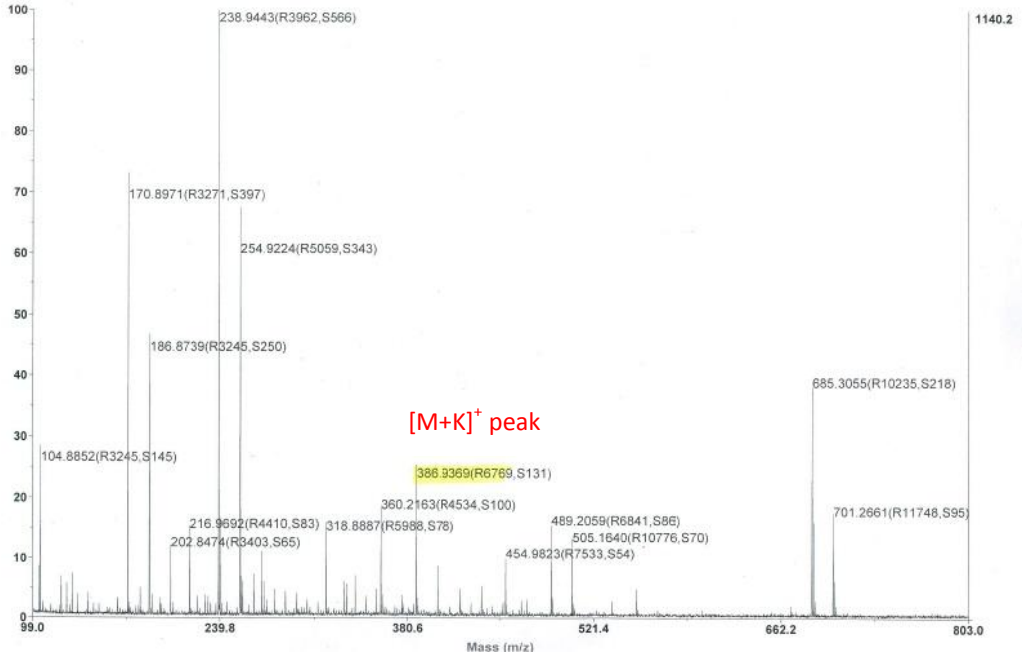


Fig. S9: MALDI-TOF of released PI103 from PI103-NP showing the presence of free PI103.

Characterization of free paclitaxel released from paclitaxel-NP in release kinetics by MALDI-TOF:

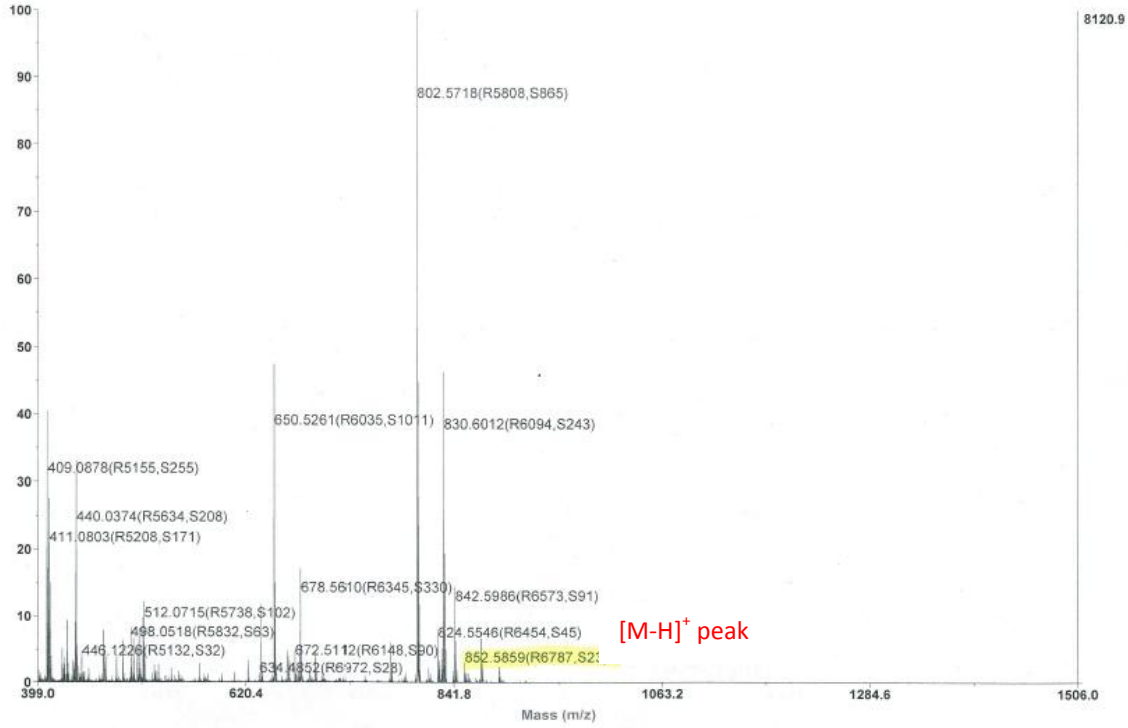


Fig. S10: MALDI-TOF of released paclitaxel from paclitaxel-NP showing the presence of free paclitaxel.

Confocal Laser Scanning Microscopy (CLSM) images of time dependent internalization of free doxorubicin in HeLa cells:

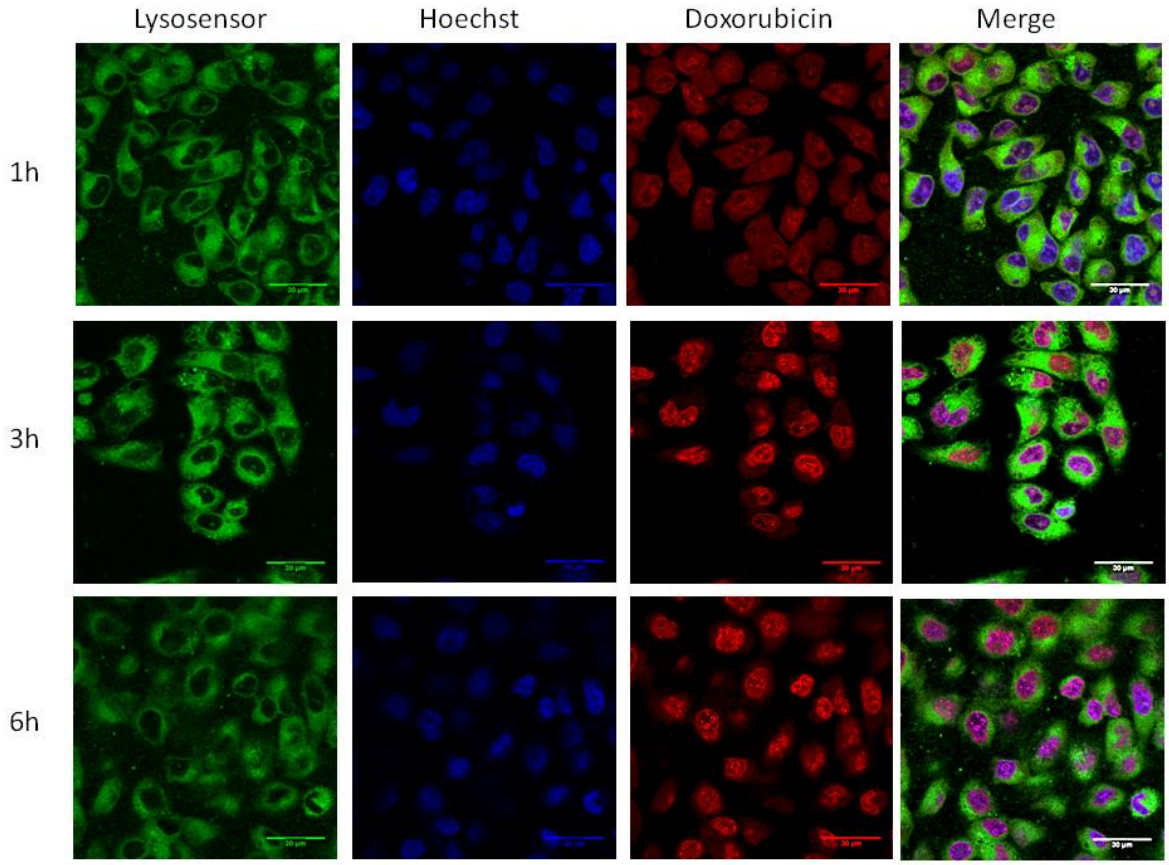


Fig. S11: Confocal laser scanning microscopy (CLSM) images of free doxorubicin internalization in HeLa cells in 1 h, 3 h and 6 h time points. Low pH lysosomal compartments and nucleus were stained with LysoSensor^{TM} Green DND-153 (green) and Hoechst 33342 (blue) respectively. In merged images purple color showed the colocalization of free doxorubicin (red) in nucleus in a time dependent manner.

Characterization of the products 2, 3, 4 and 5 by $^{1}H/^{13}C$ NMR and HR-MS:

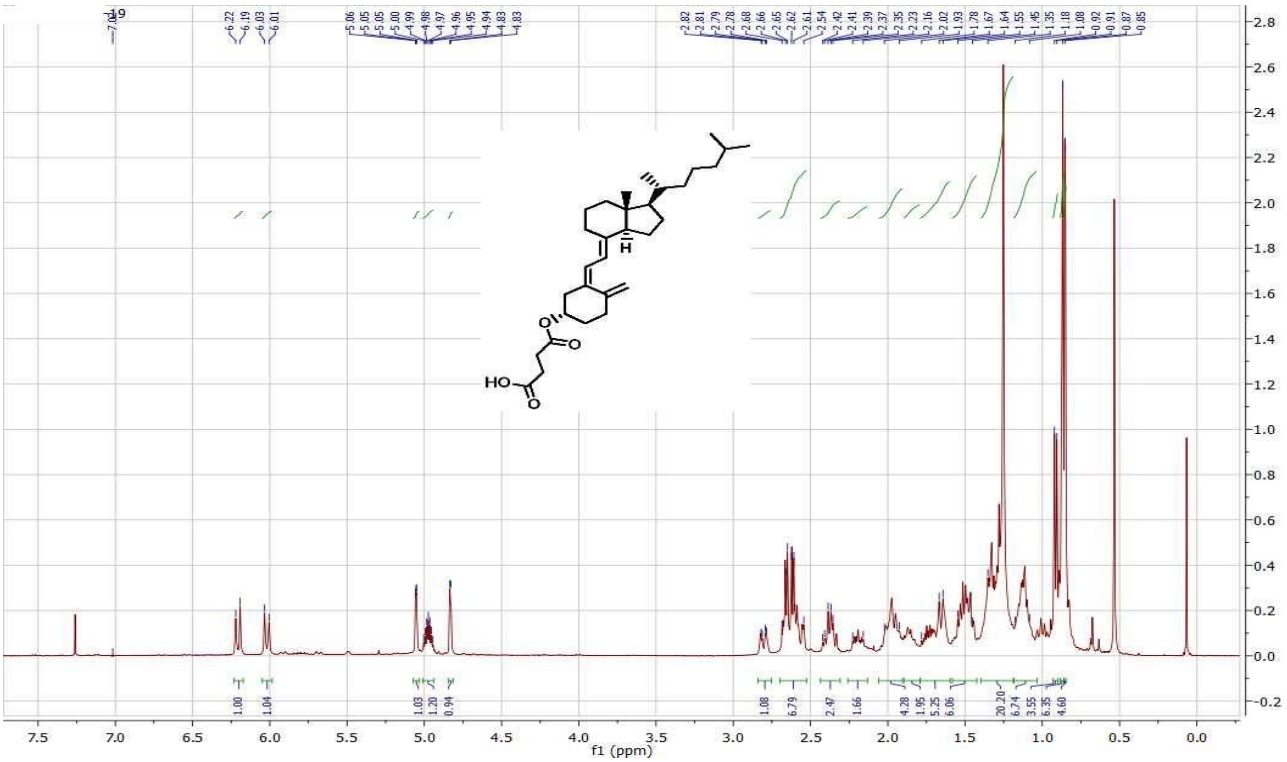


Fig. S12: ^{1}H NMR spectra of vitamin D3-succinic acid conjugate (**2**).

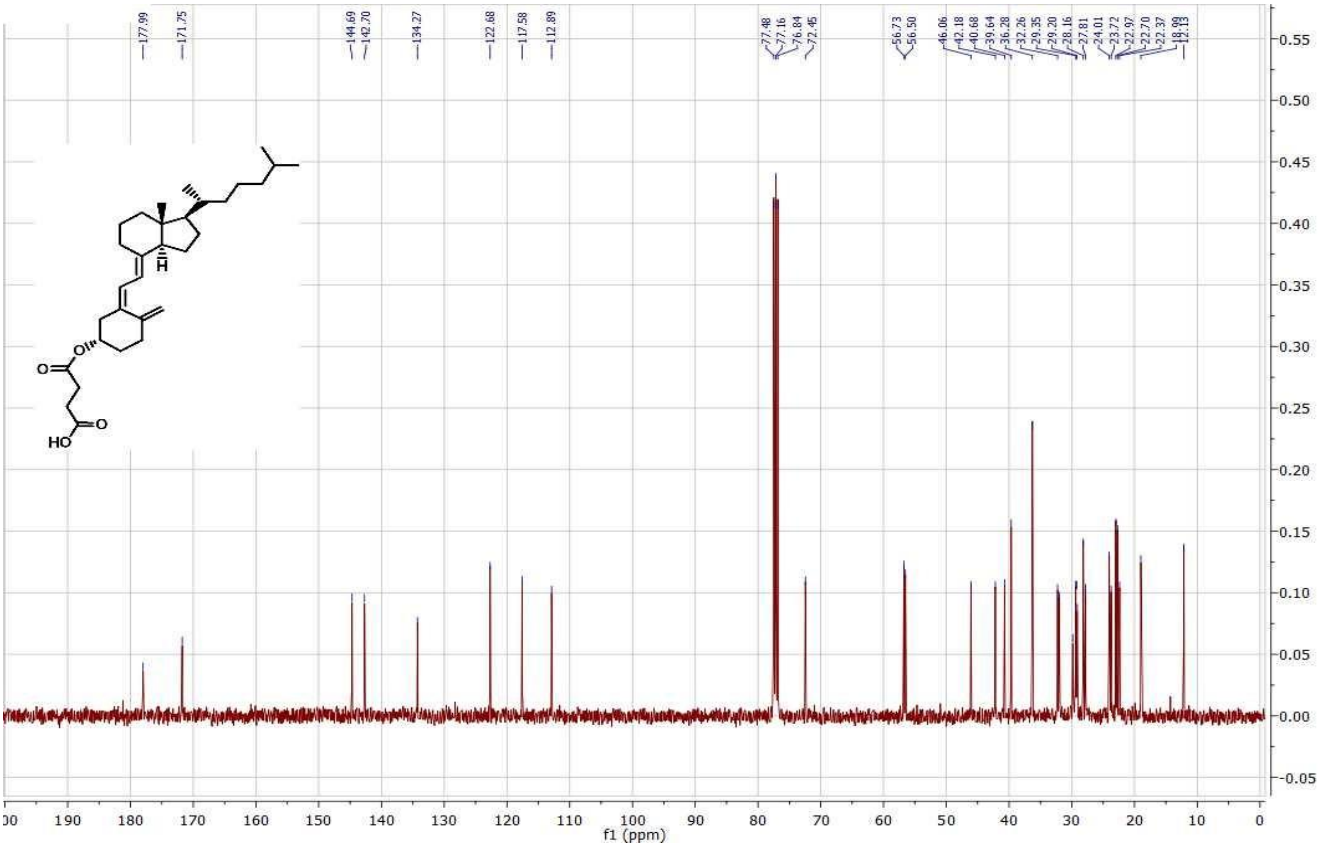


Fig. S13: ^{13}C NMR spectra of vitamin D3-succinic acid conjugate (**2**).

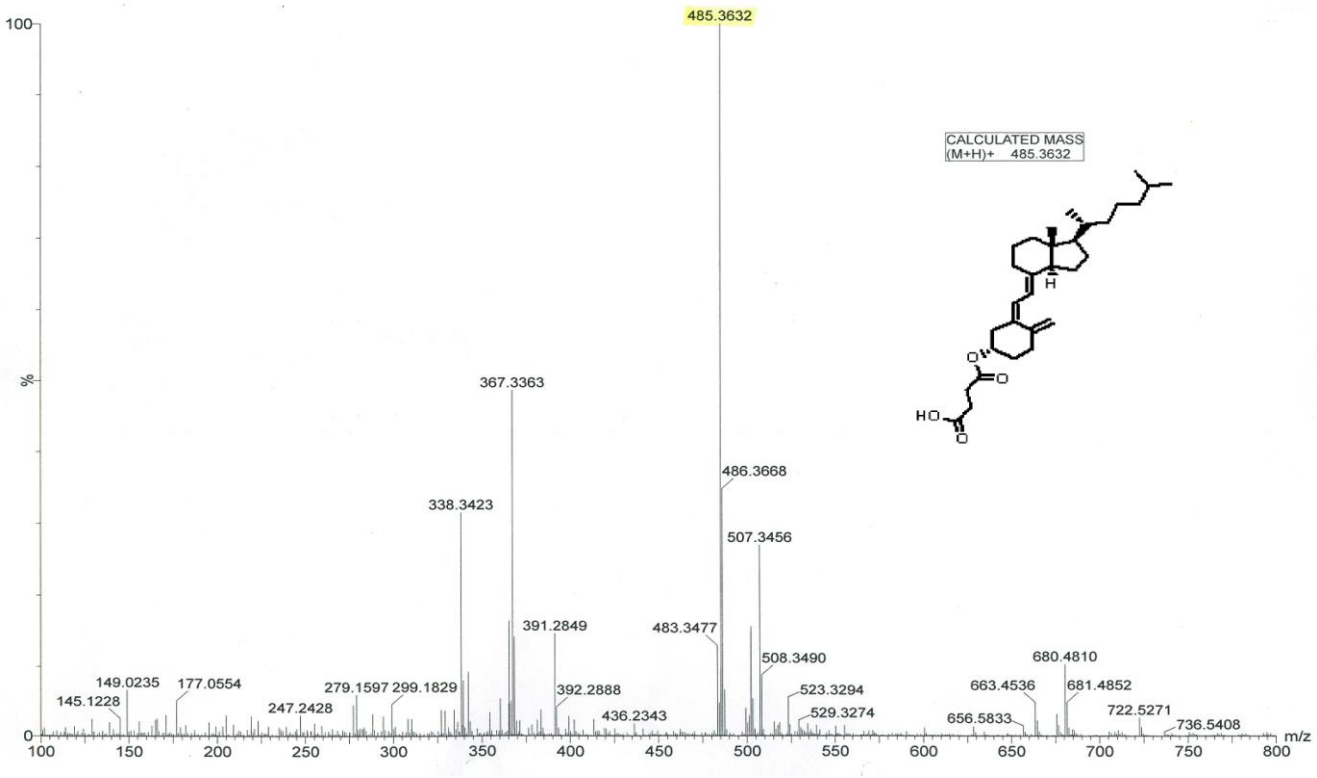


Fig. S14: HR-MS spectra of vitamin D3-succinic acid conjugate (**2**).

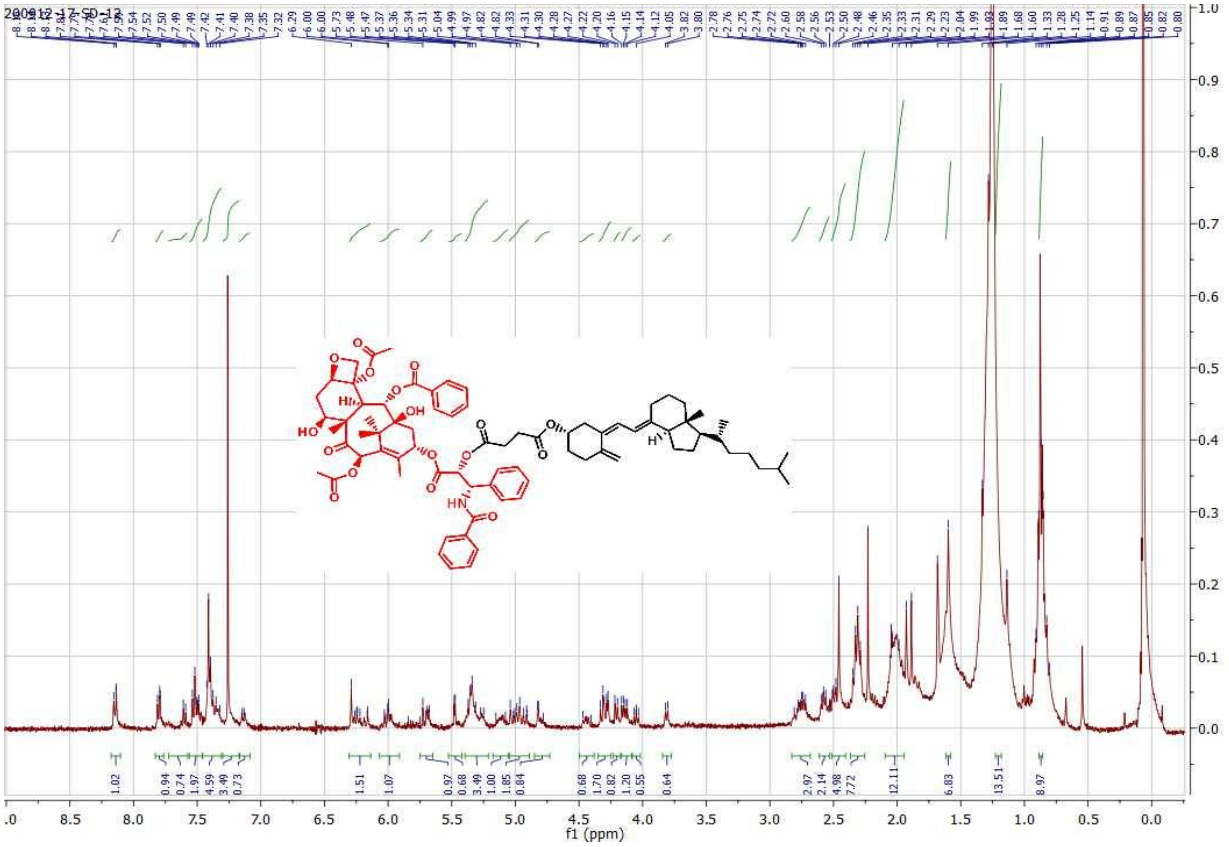


Fig. S15: ^{1}H NMR spectra of vitamin D3-succinic acid-paclitaxel conjugate (**3**).

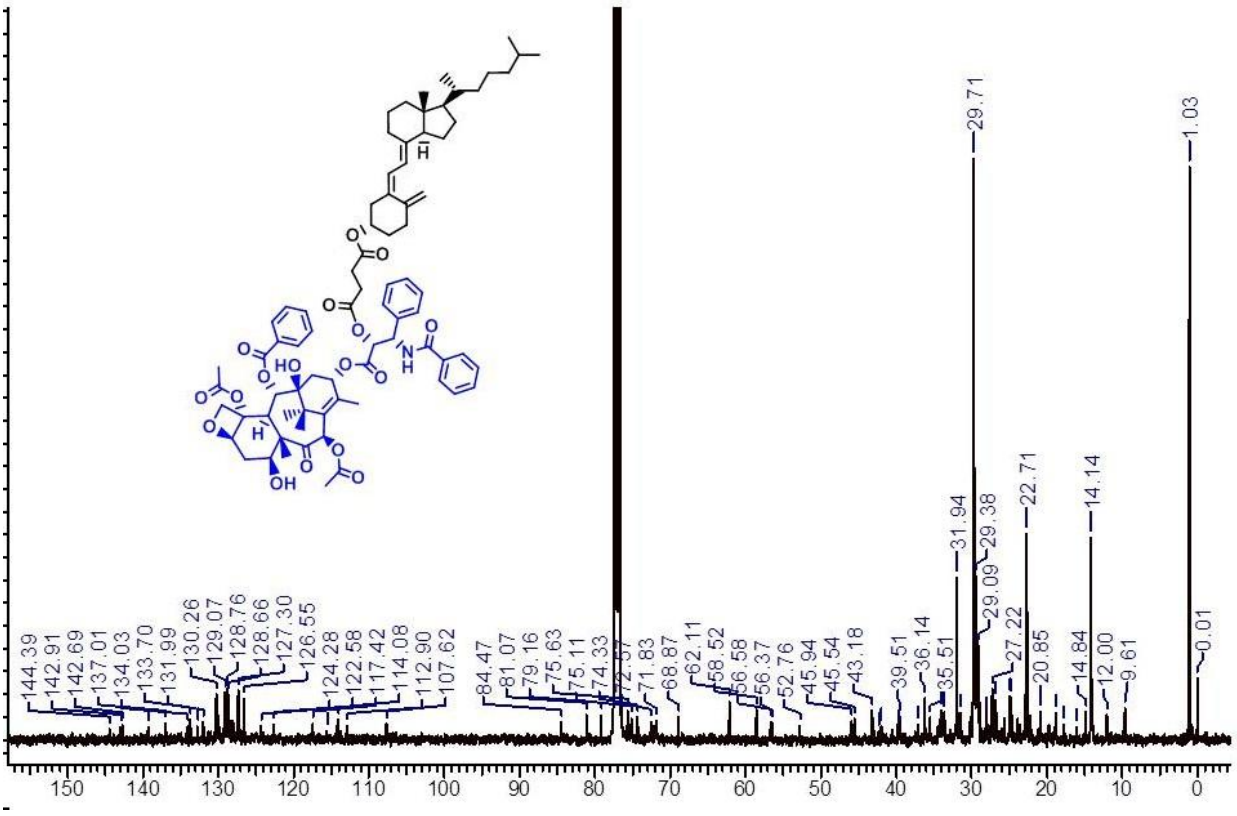


Fig. S16: ^{13}C NMR spectra of vitamin D3-succinic acid-paclitaxel conjugate (**3**).

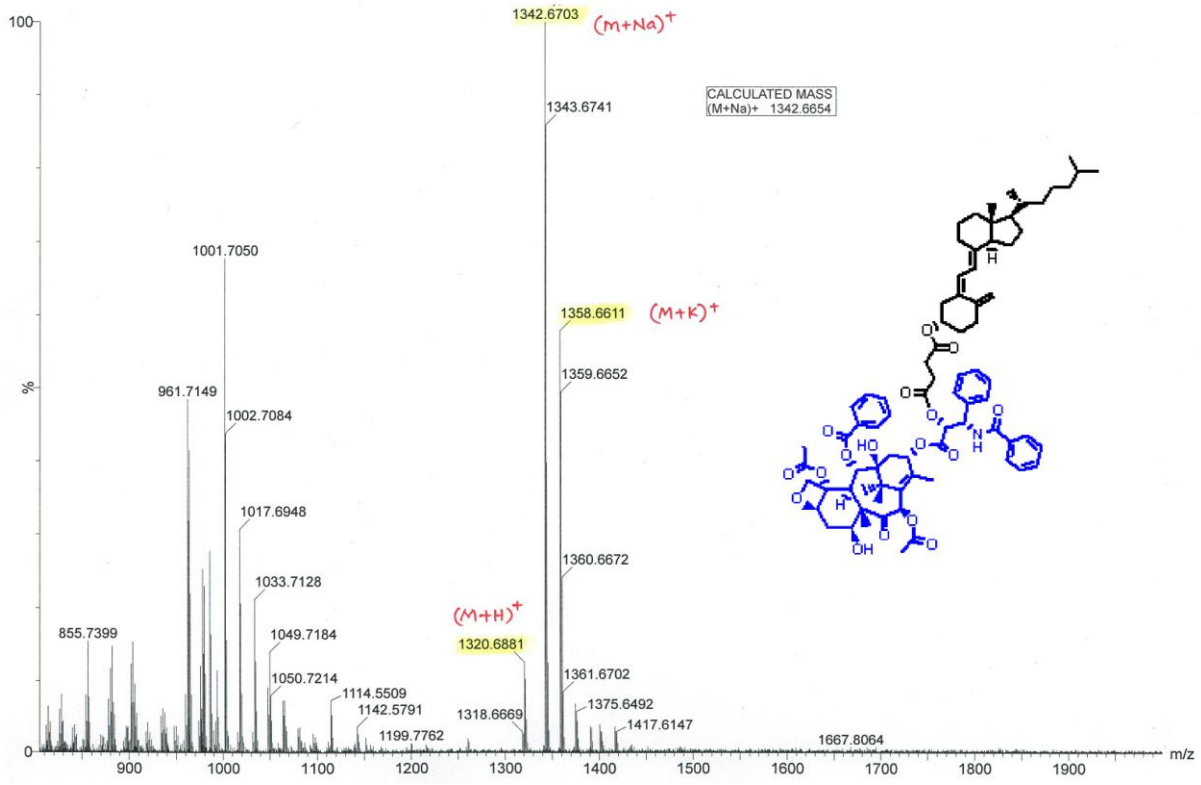


Fig. S17: HR-MS spectra of vitamin D3-succinic acid-paclitaxel conjugate (**3**).

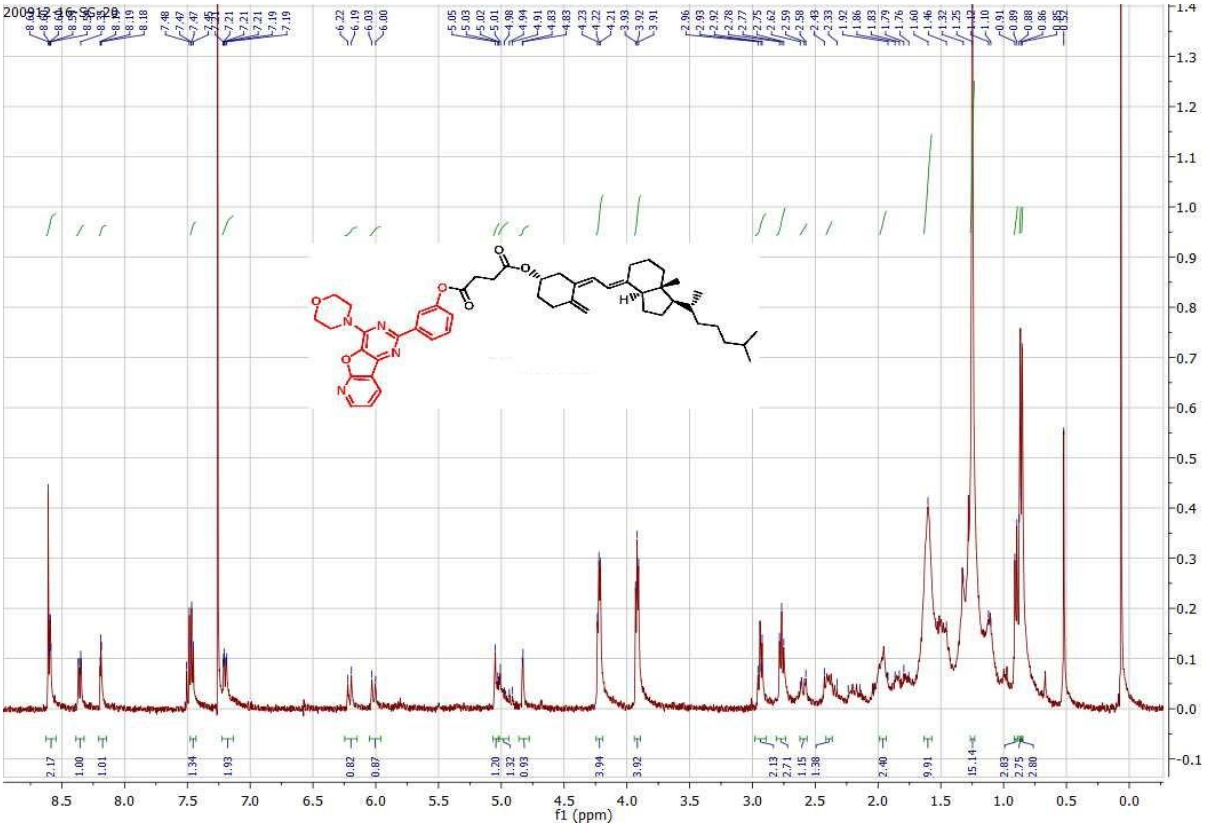


Fig. S18: ^{1}H NMR spectra of vitamin D3-succinic acid-PI103 conjugate (**4**).

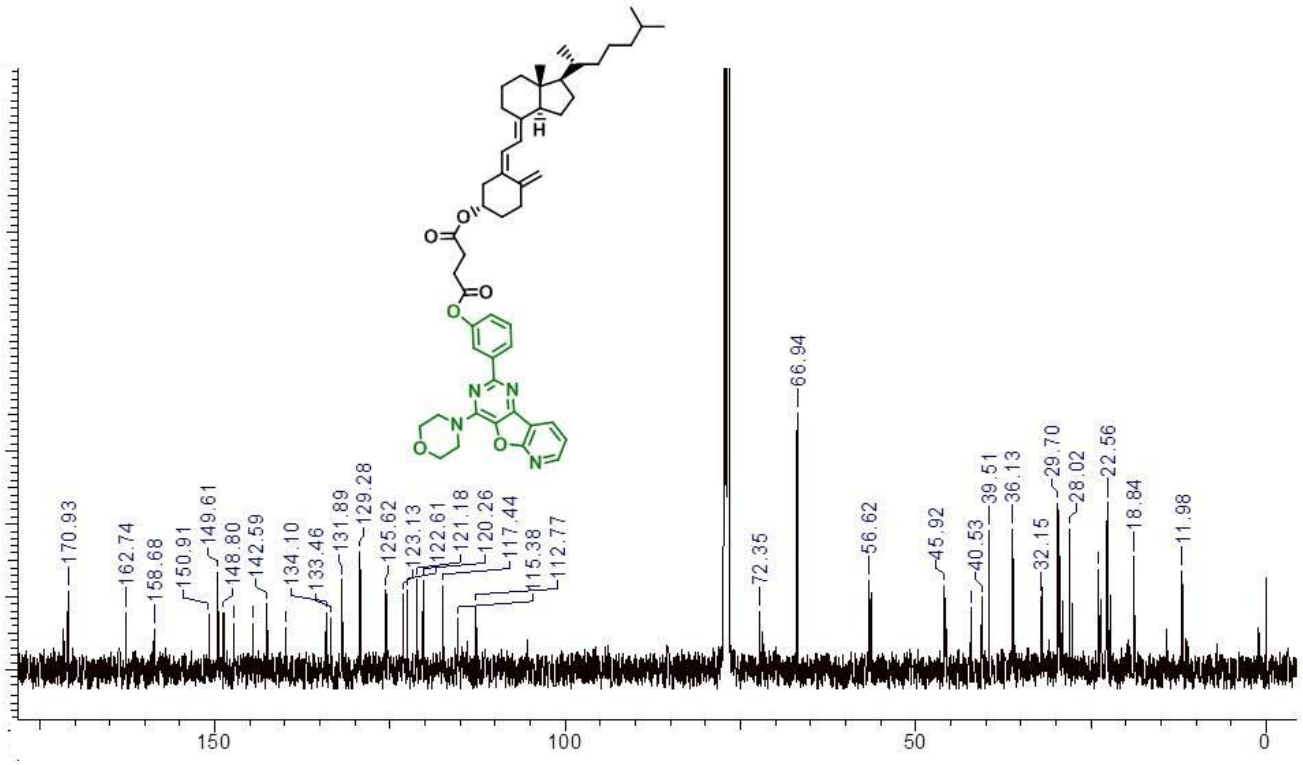


Fig. S19: ^{13}C NMR spectra of vitamin D3-succinic acid-PI103 conjugate (**4**).

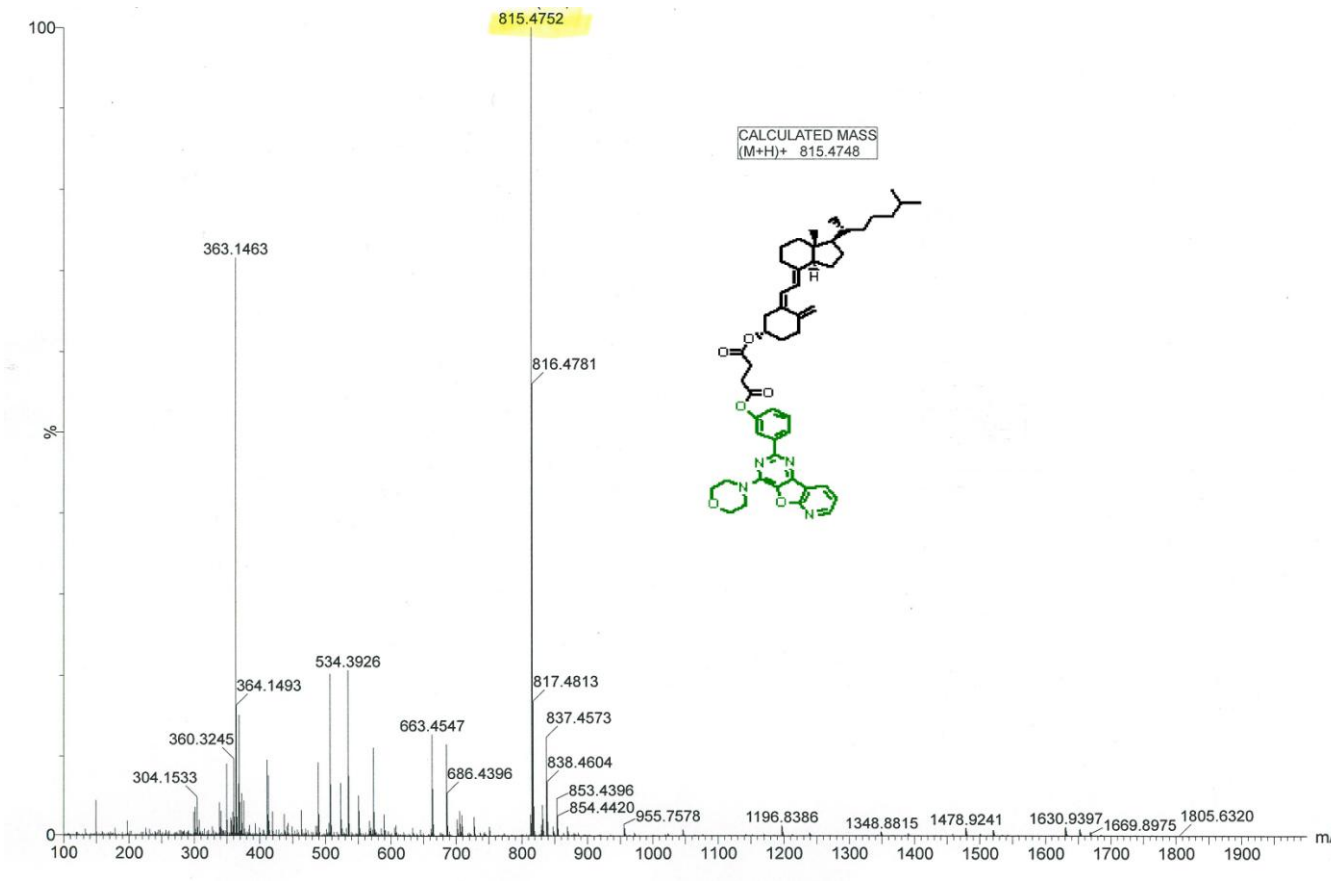


Fig. S20: HR-MS spectra of vitamin D3-succinic acid-PI103 conjugate (**4**).

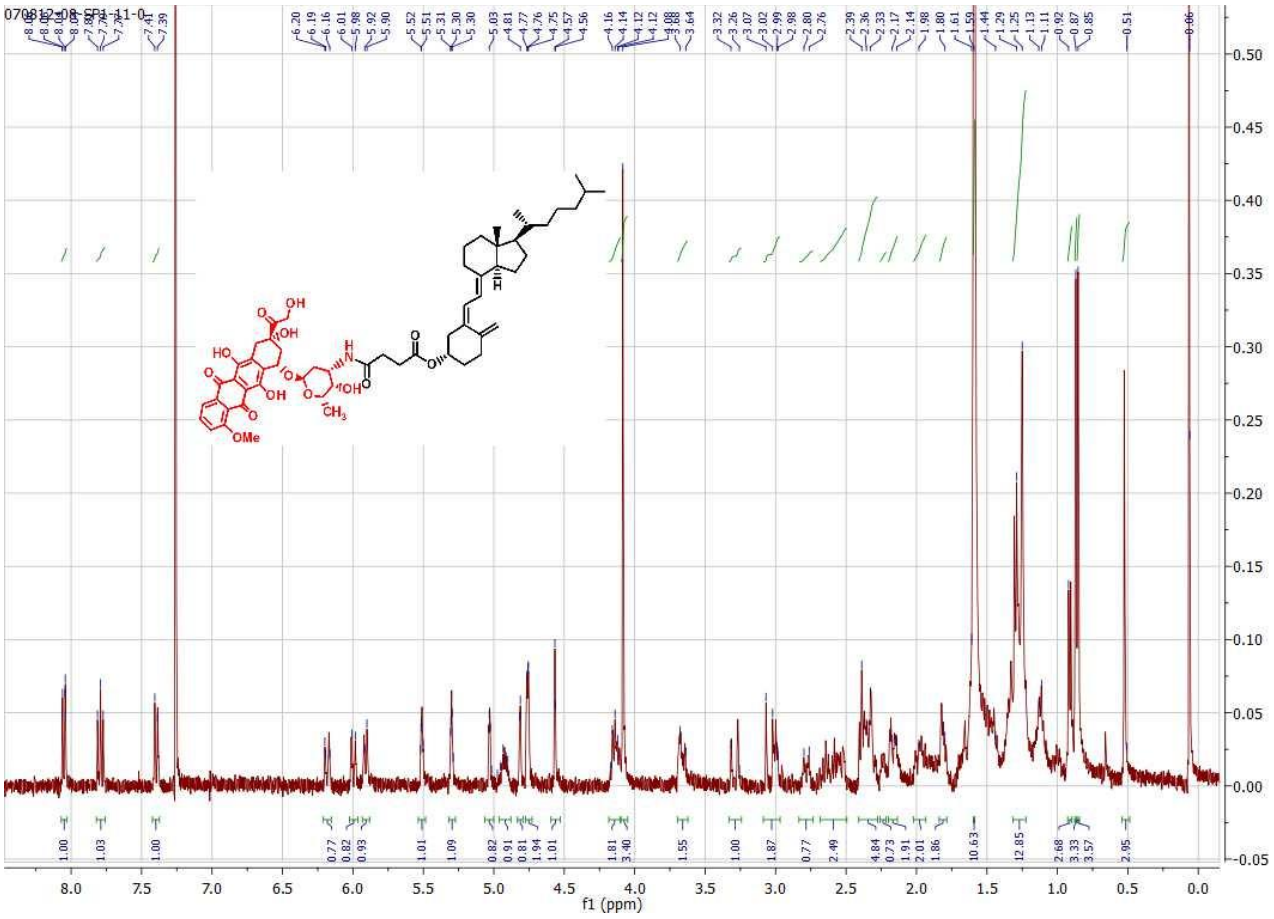


Fig. S21: ^{1}H NMR spectra of vitamin D3-succinic acid-doxorubicin conjugate (**5**).

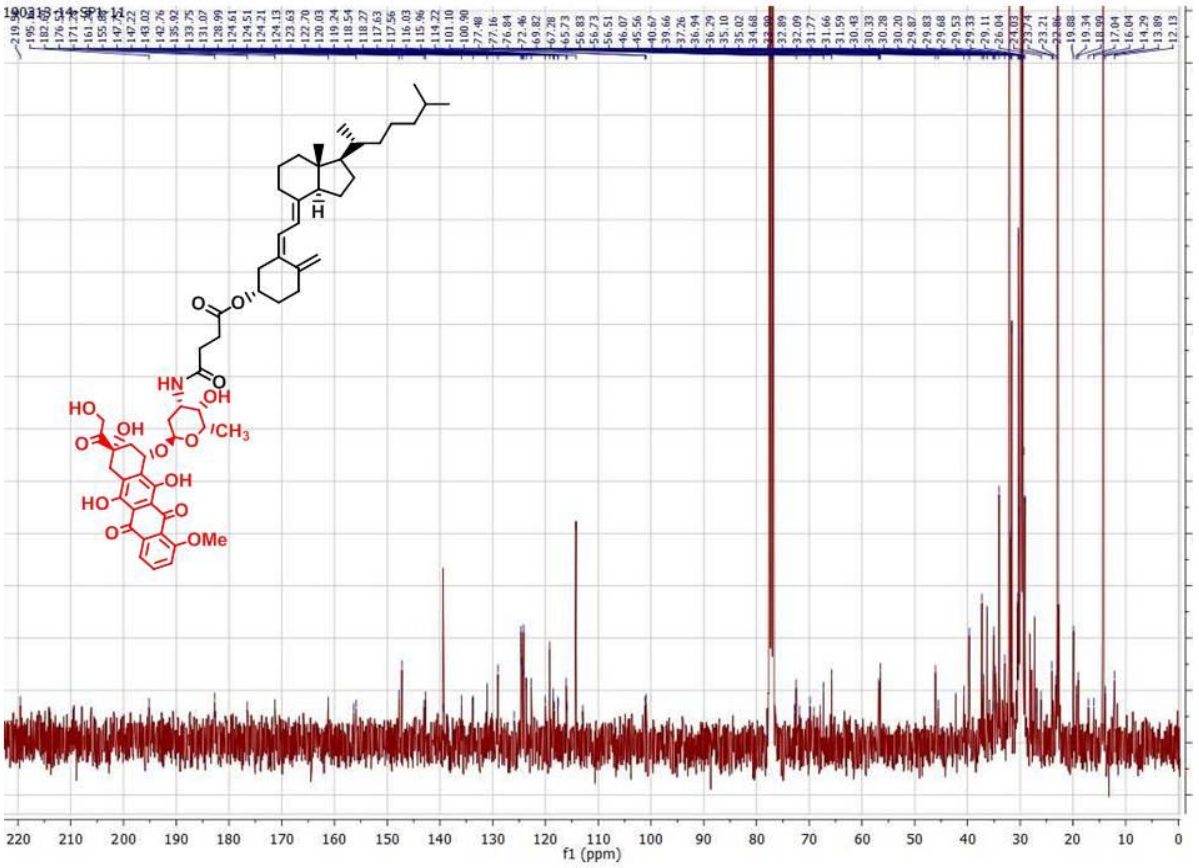


Fig. S22: ^{13}C NMR spectra of vitamin D3-succinic acid-doxorubicin conjugate (**5**).

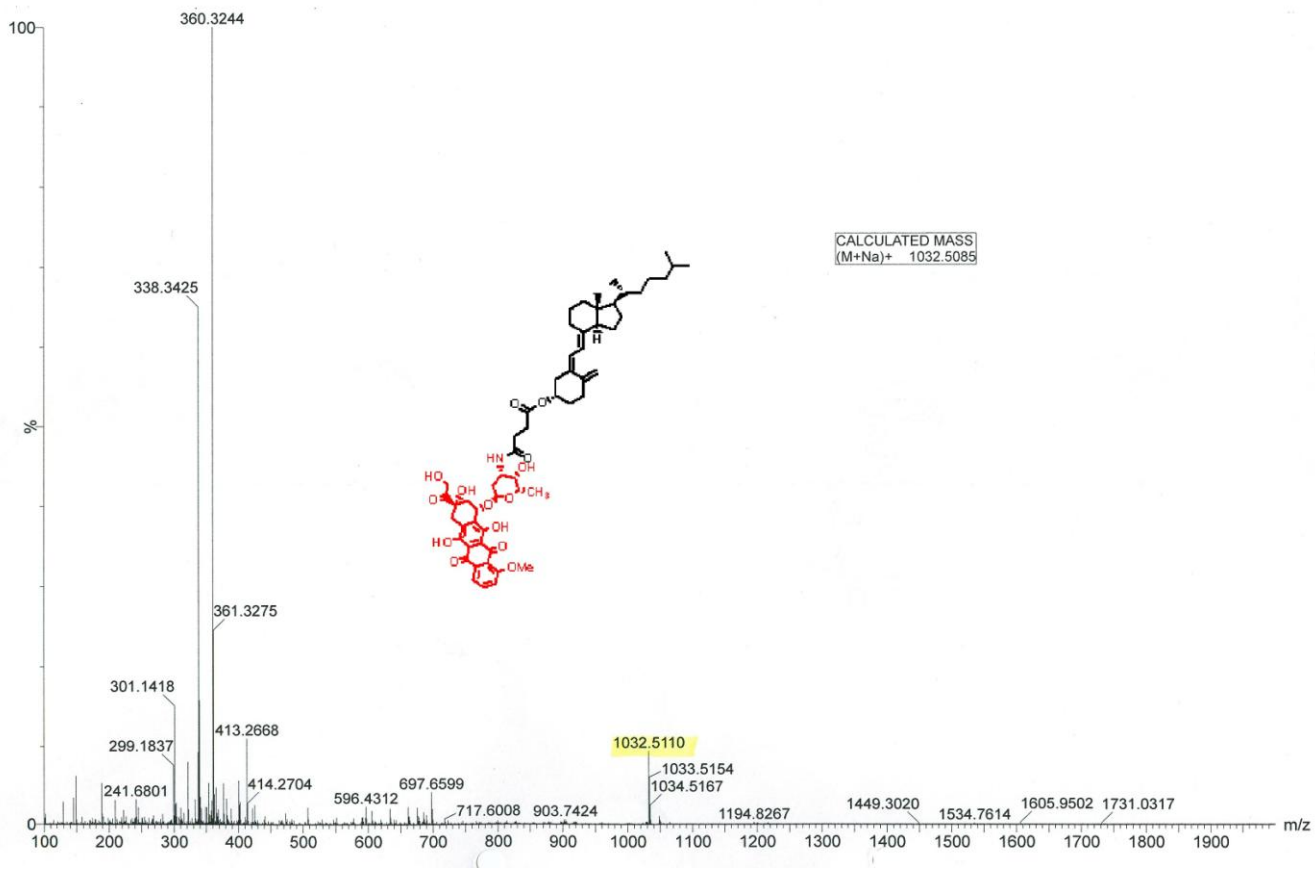


Fig. S23: HR-MS spectra of vitamin D3-succinic acid-doxorubicin conjugate (**5**).