

Supporting Information

for

Highly Sensitive Method for Analysis of 7-Dehydrocholesterol for the Study of Smith-Lemli-Opitz Syndrome (SLOS)

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Figure S-1: Standard curves for LC-MS/MS analysis of desmosterol PTAD products. Different amounts of desmosterol (ranging from 10 ng to 2000 ng, with 25 ng of *d*₇-7DHC internal standard) were added for the PTAD incubations, respectively, and 10 μL of each reaction mixture was injected.

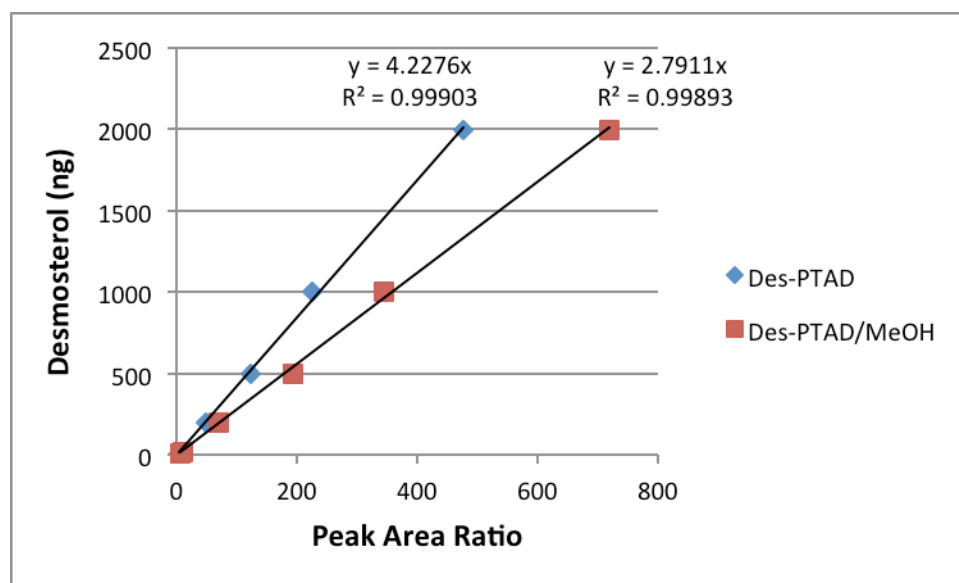
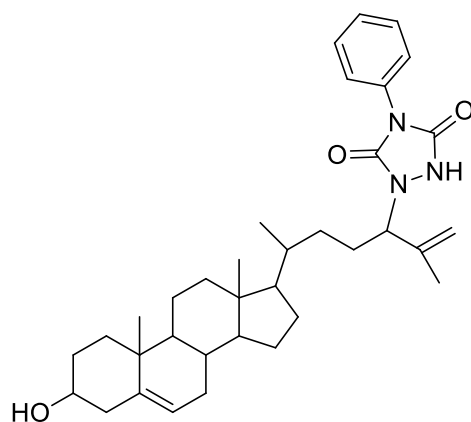


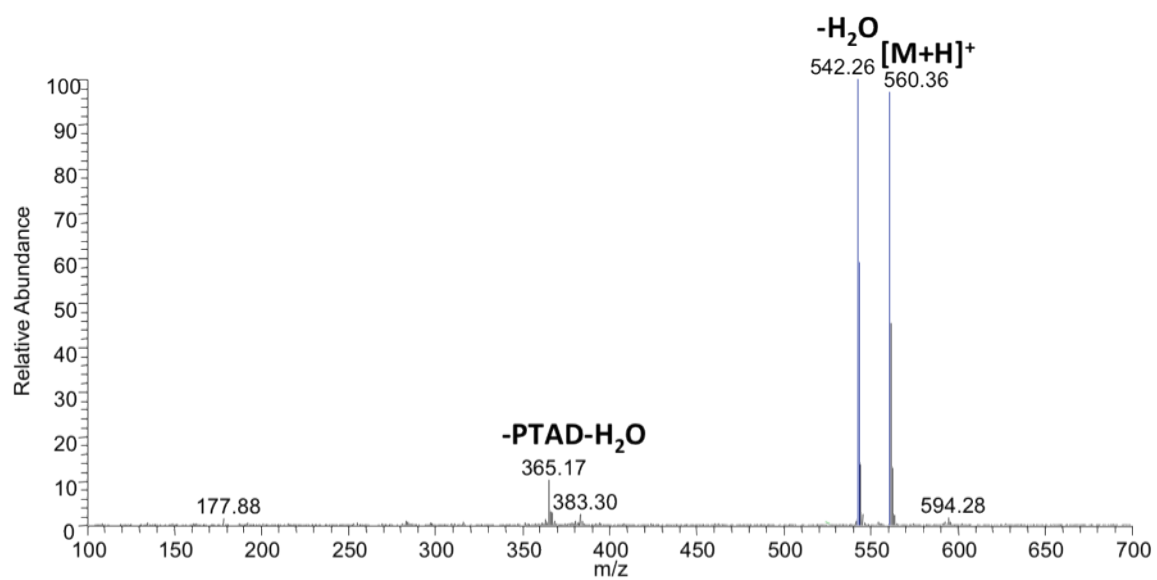
Figure S-2: MS and NMR for desmosterol-PTAD derivatives

ene product **Des-PTAD:**

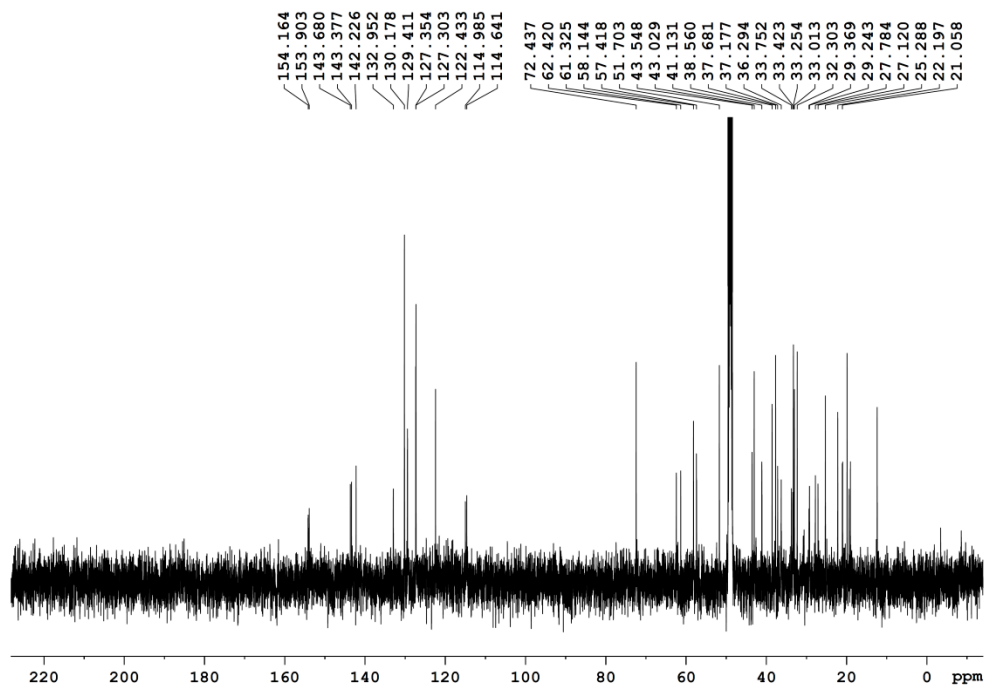


Exact Mass: 559.38

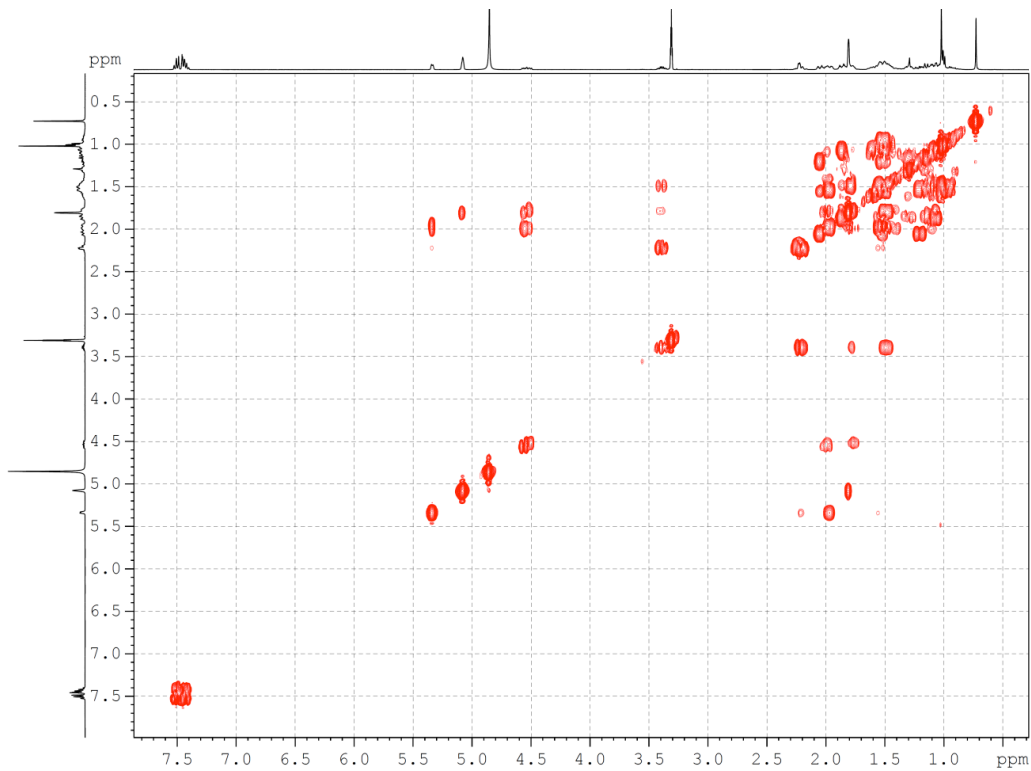
MS¹:



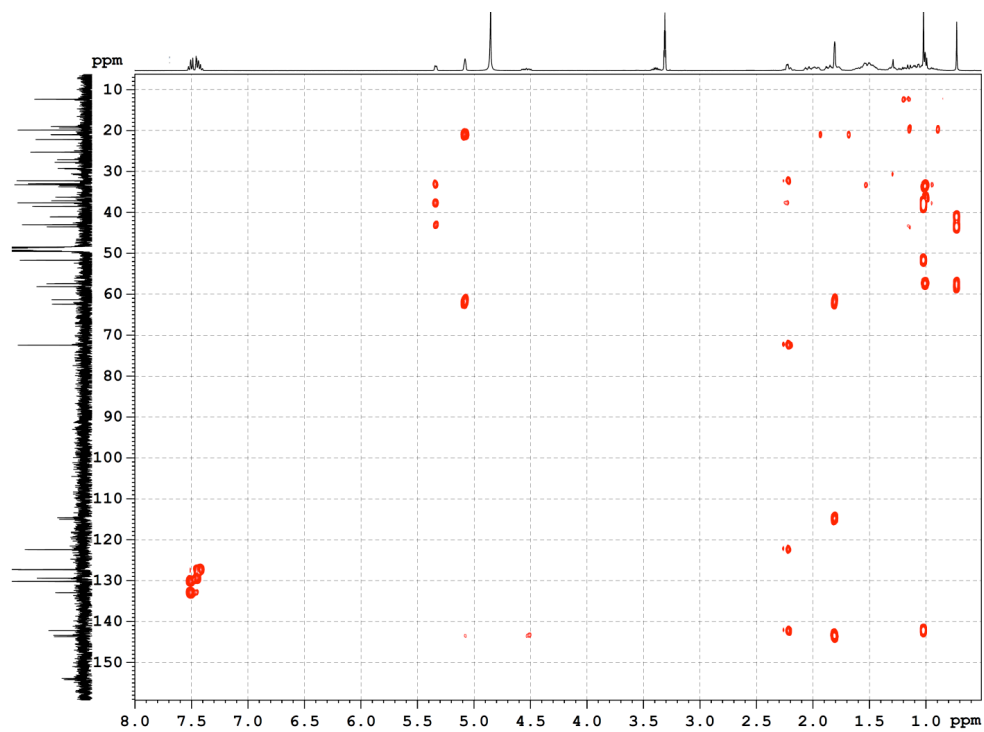
^{13}C NMR:



COSY:



HMBC:



HSQC:

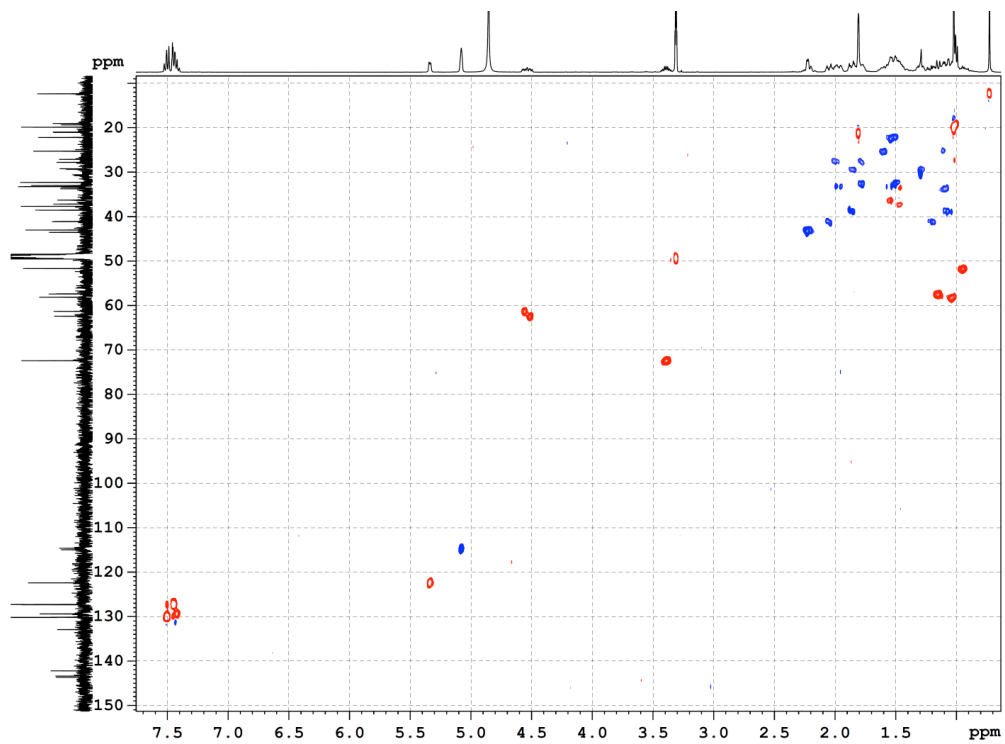
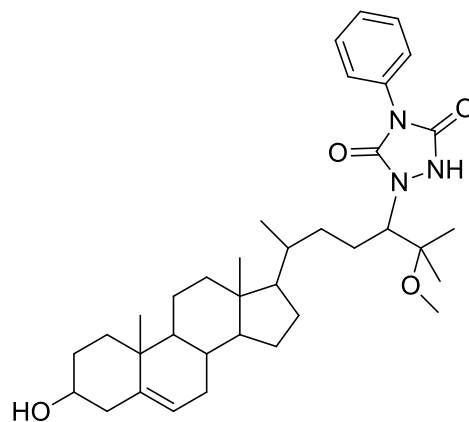


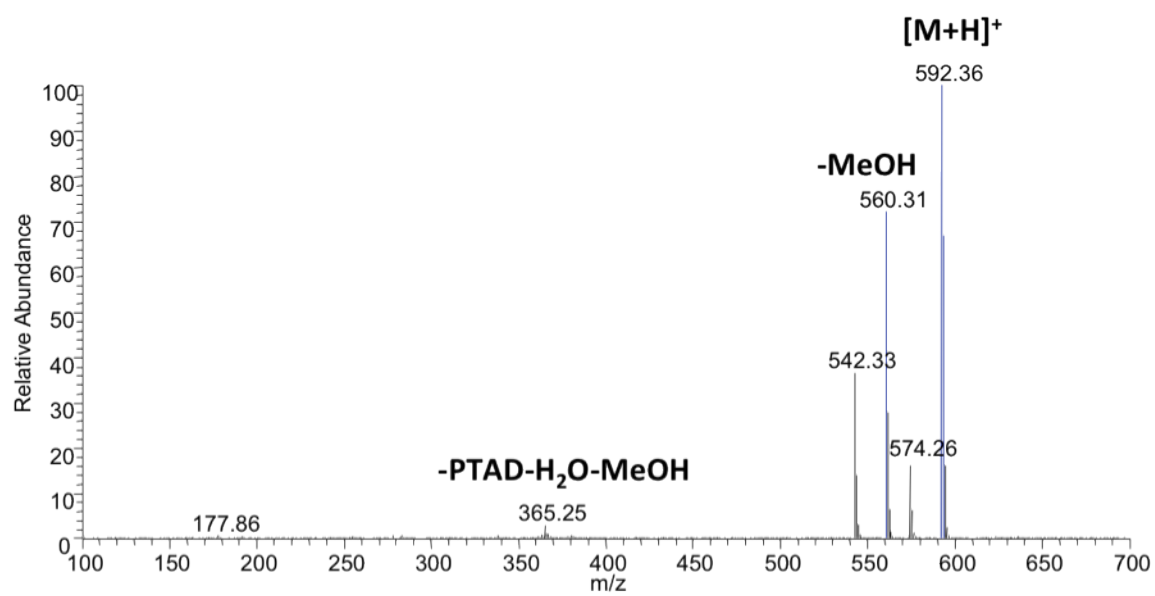
Figure S-3: MS and NMR for desmosterol-PTAD derivatives

solvent addition product **Des-PTAD/MeOH:**

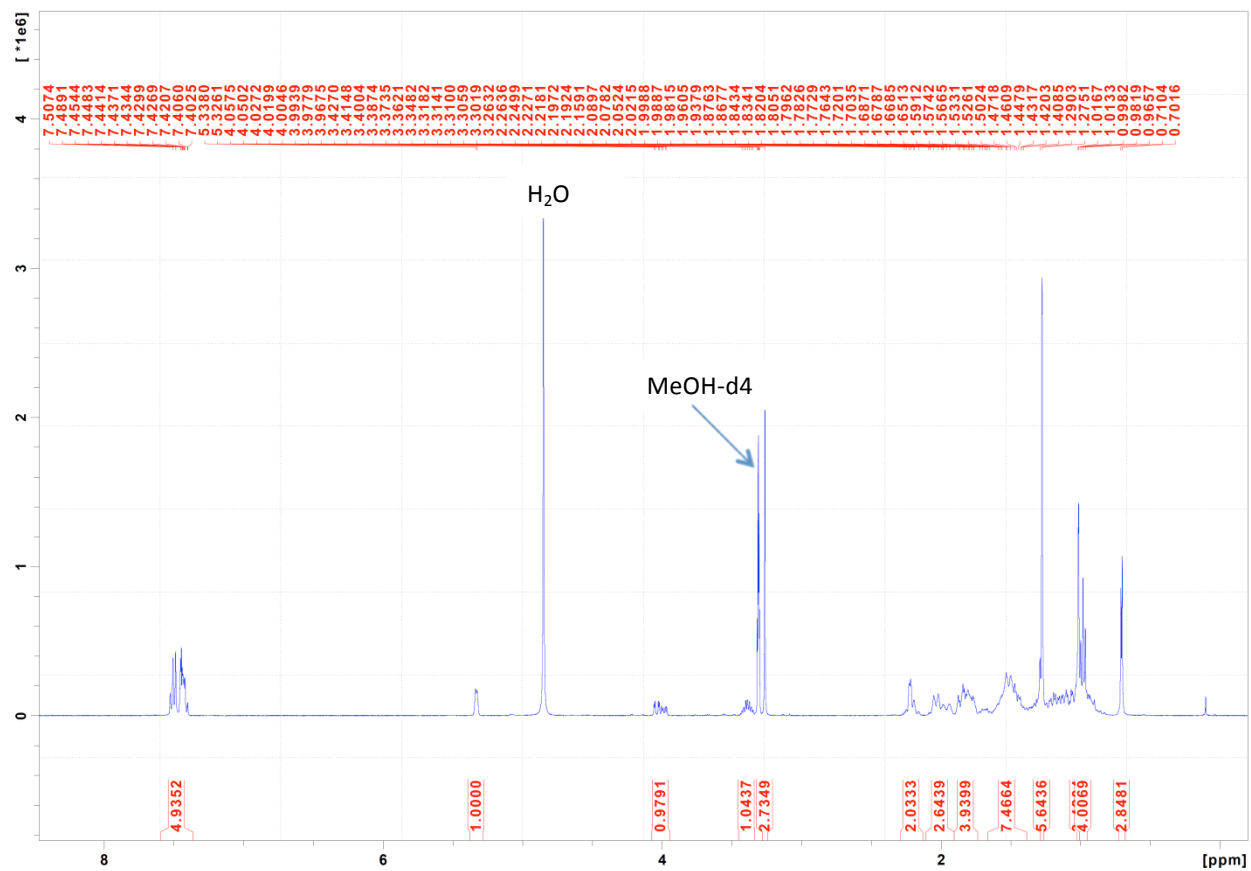


Exact Mass: 591.40

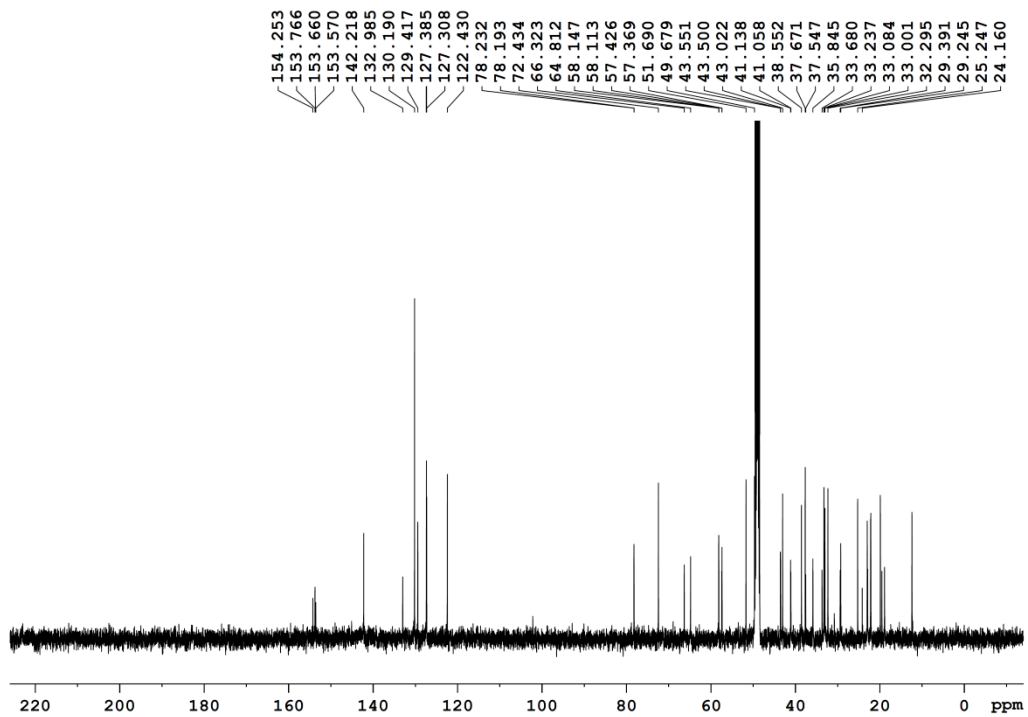
MS¹:



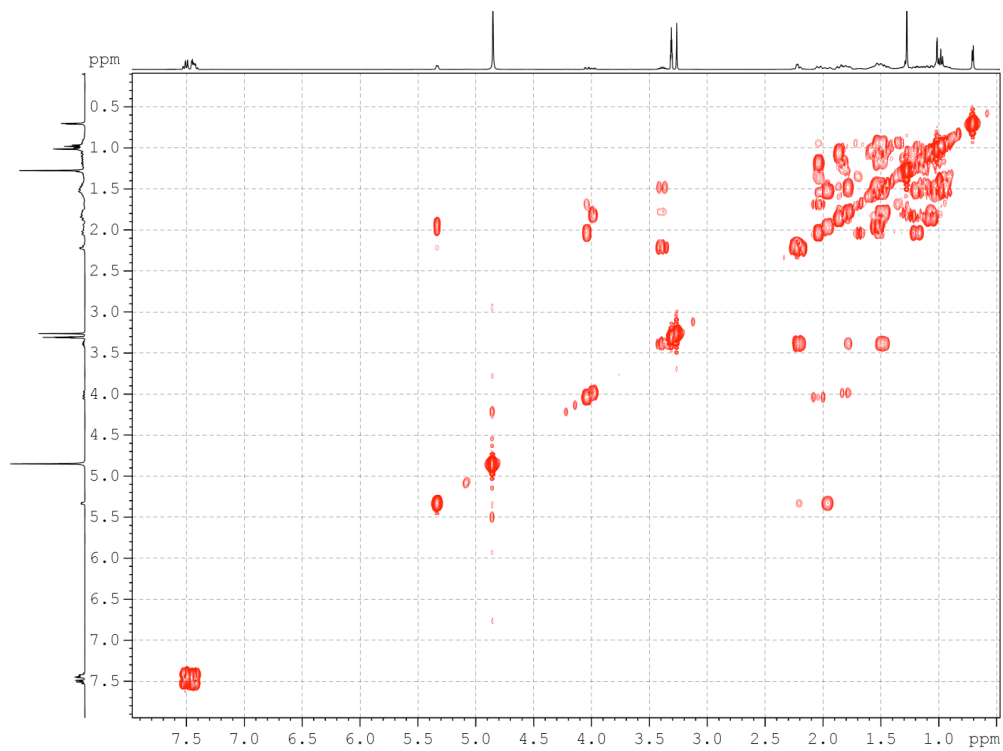
¹H NMR:



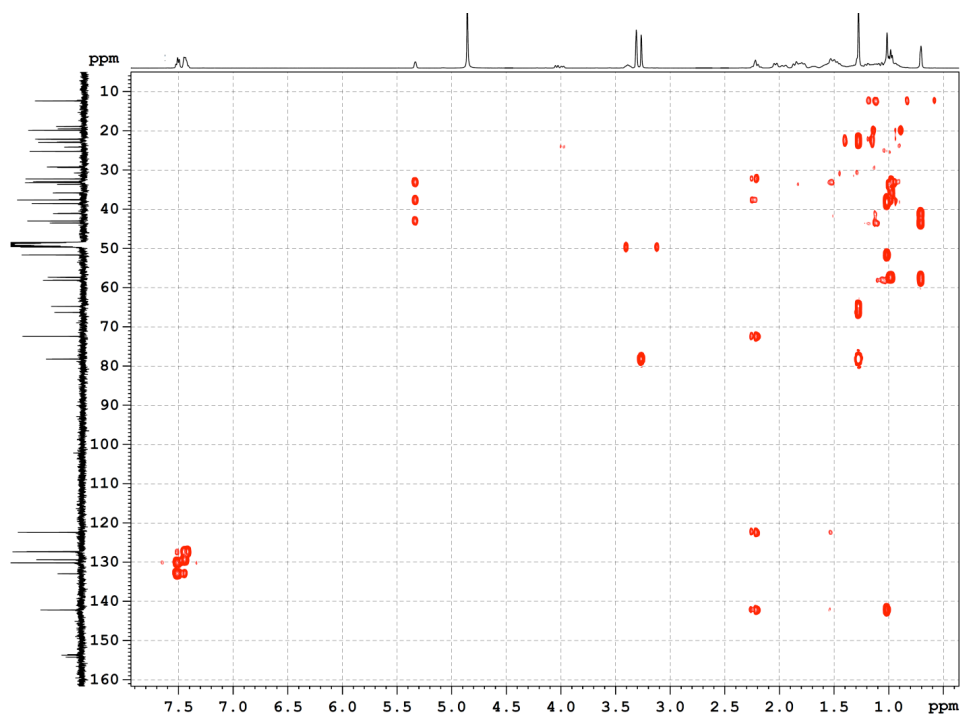
^{13}C NMR:



COSY:



HMBC:



HSQC:

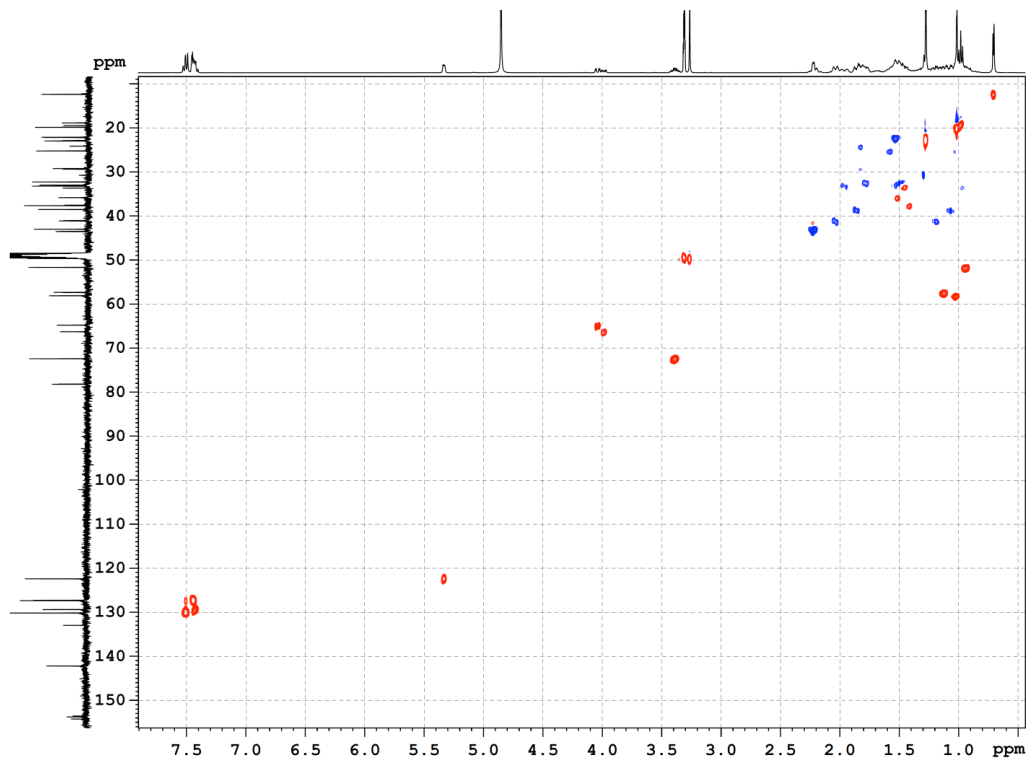
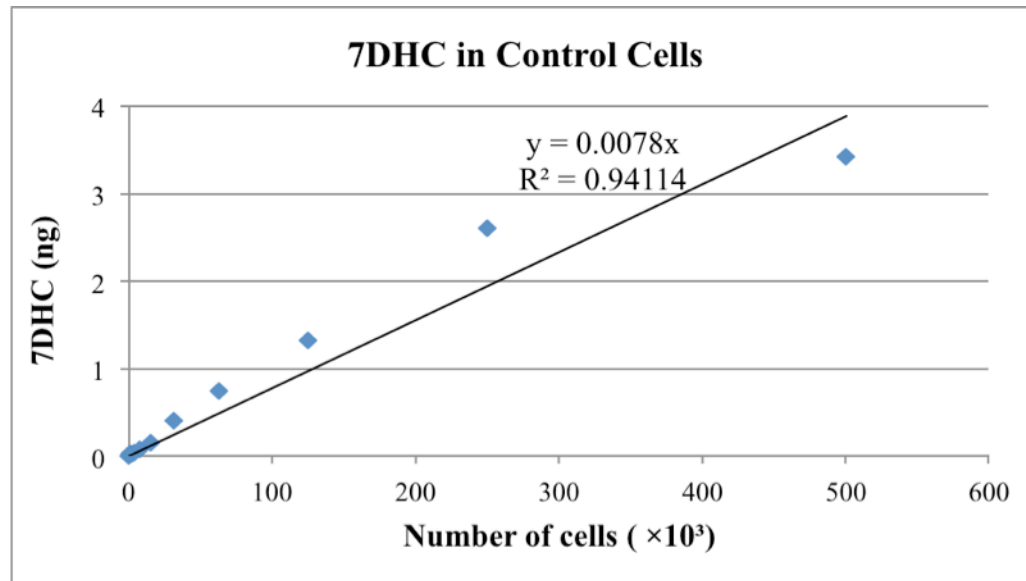


Table S-1. 7-DHC analysis in a specified number of control and *Dhcr7*-deficient Neuro2a cells with PTAD derivatization. Extracts from different numbers of cells (ranging from 125 to 500,000 cells, with 13 ng of *d*₇-7DHC internal standard) were incubated with PTAD, respectively, and 10 μL of each reaction mixture was injected. a: blank sample, d0 impurity form *d*₇-7DHC; b: hard interference of d0 impurity form *d*₇-7DHC for less than 4,000 control cells.

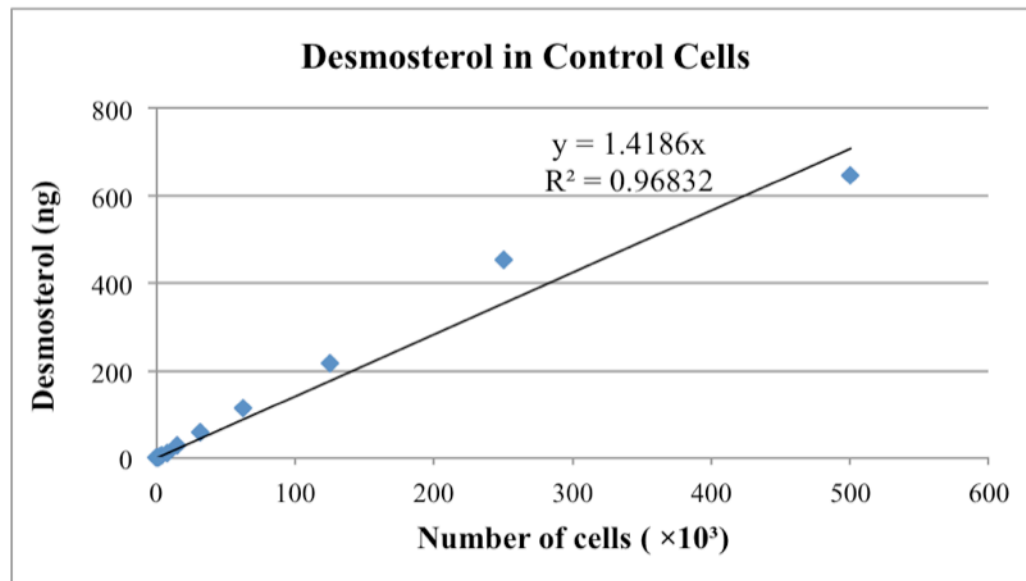
Number of cells	Control cell		<i>Dhcr7</i> -deficient Cell	
	7DHC (ng)	Desmosterol (ng)	7DHC (ng)	Desmosterol (ng)
0	0.018 ^a	--	0.019 ^a	--
125	0.011 ^b	0.21	0.25	0.02
250	0.018 ^b	0.38	0.53	0.08
500	0.031 ^b	0.73	1.09	0.11
1,000	0.029 ^b	1.49	1.92	0.20
1,850	0.035 ^b	3.27	4.59	0.58
3,750	0.046 ^b	6.70	8.83	1.13
7,500	0.077	11.7	19.2	2.25
15,000	0.15	29.1	41.6	4.52
31,250	0.41	59.4	94.7	9.05
6,2500	0.74	113.1	189.1	18.2
125,000	1.33	217.5	386.1	35.4
250,000	2.61	452.1	792.6	76.6
500,000	3.43	646.3	1239.2	121.8

Figure S-4: Correlations of 7-DHC and desmosterol with number of cells (ranging from 125 to 500,000 cells, with 13 ng of *d*₇-7DHC internal standard). A, B: in control Neuro2a cells; C, D: in *Dhcr7*-deficient Neuro2a cells.

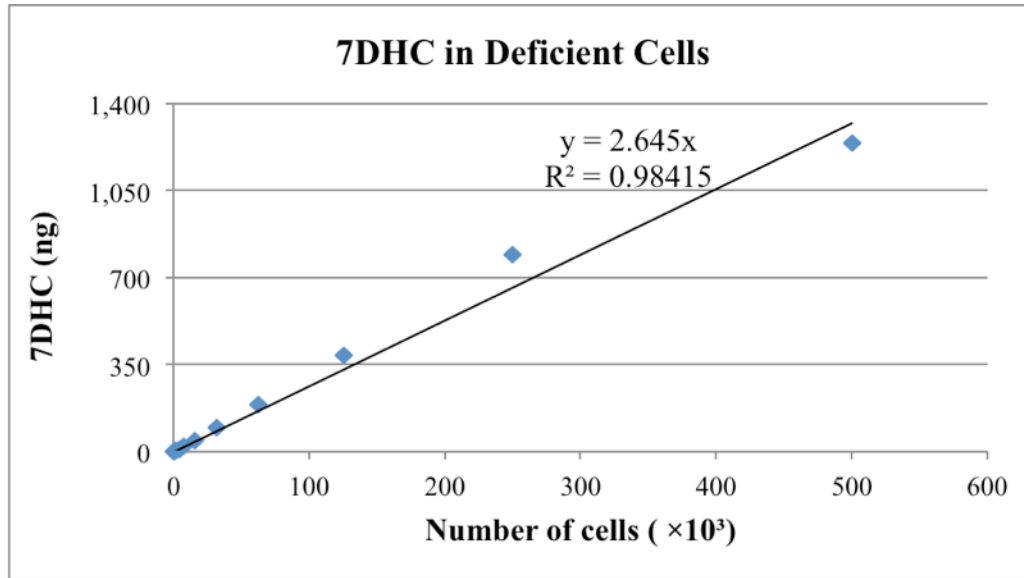
A



B



C



D

