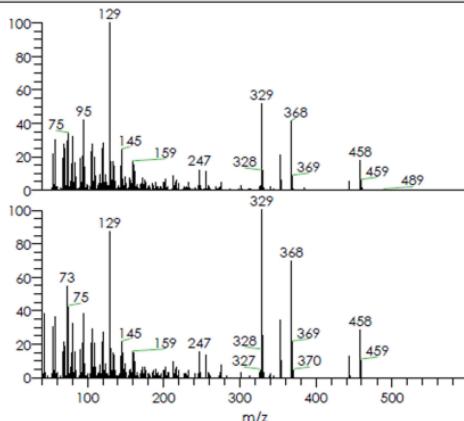
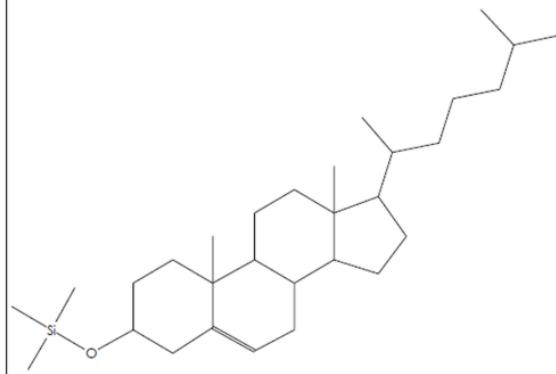


**Supplemental Figure 1:** Growth curve of *Lic* promastigotes. The average and standard deviation of nine independent experiments are presented. Each culture was started with stationary phase promastigotes diluted at an initial cell density of  $1 \times 10^6$  cells/ml (day 0) and incubated in cell culture medium for 9 days. Cells were microscopically enumerated daily. The number of cell divisions (n) and the doubling time (h) for each day of cell culture were calculated and are listed below the graph.

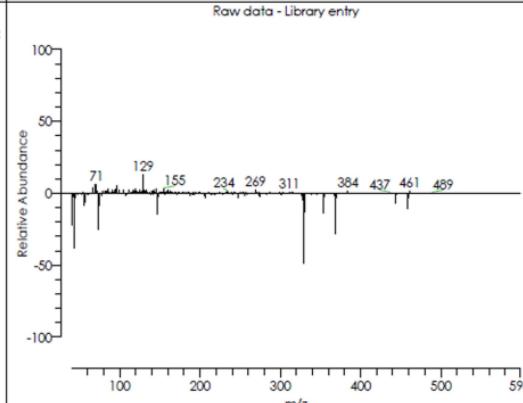
Hit	SI	RSI	Prob	Name	Library Name
1	891	910	72.52	Cholesterol trimethylsilyl ether	mainlib
2	888	898	72.52	Cholesterol trimethylsilyl ether	replib
3	883	907	72.52	Cholesterol trimethylsilyl ether	replib
4	861	897	72.52	Cholesterol trimethylsilyl ether	replib
5	849	889	16.27	3 $\alpha$ ,5-Bis(trimethylsiloxy)-5 $\alpha$ -cholestane	mainlib
6	829	837	7.41	3 $\alpha$ -(Trimethylsiloxy)cholest-4-ene	mainlib
7	815	878	16.27	3 $\alpha$ ,5-Bis(trimethylsiloxy)-5 $\alpha$ -cholestane	replib
8	792	862	1.80	3 $\alpha$ -(Trimethylsiloxy)cholest-5-ene	mainlib
9	785	819	72.52	Cholesterol trimethylsilyl ether	replib
10	713	750	0.20	Cholest-5-ene, 3-methoxy-, (3 $\alpha$ )-	replib
11	704	796	0.14	Cholesta-3,5-diene	replib
12	704	744	0.20	Cholest-5-ene, 3-methoxy-, (3 $\alpha$ )-	mainlib
13	703	756	0.14	Cholest-5-ene, 3-ethoxy-, (3 $\alpha$ )-	mainlib
14	703	762	0.14	Cholesterol 3 $\alpha$ -O-[2-chloroethyl]- ether	mainlib
15	696	722	0.10	Cholesterol	replib
16	690	822	0.14	Cholesta-3,5-diene	replib
17	690	786	0.08	Cholest-5-en-3-ol (3 $\alpha$ )-, nonanoate	mainlib
18	689	779	0.08	Cholest-5-en-3-ol (3 $\alpha$ )-, tetradecanoate	mainlib
19	688	714	0.07	Cholesterol margarate	mainlib
20	687	713	0.07	5-Cholesten-3 $\beta$ -yl isobutyl carbonate	mainlib
21	687	716	0.07	Silane, [[[3 $\alpha$ ,6 $\alpha$ ,6 $\beta$ ]-cholestane-3,6-diy]bis(ox	mainlib
22	686	833	0.14	Cholesta-3,5-diene	replib
23	685	710	0.07	Cholesteryl benzoate	mainlib
24	685	817	0.07	Cholest-5-en-3-ol (3 $\alpha$ )-, propanoate	mainlib
25	685	773	0.07	Cholest-5-ene, 3-(1-oxobutoxy)-	mainlib

Cholesterol trimethylsilyl ether  
 Formula C<sub>30</sub>H<sub>54</sub>O<sub>5</sub>, MW 458, CAS# 1856-05-9, Entry# 139760  
 Silane, [[(3 $\alpha$ )-cholest-5-en-3-yl]oxy]trimethyl-



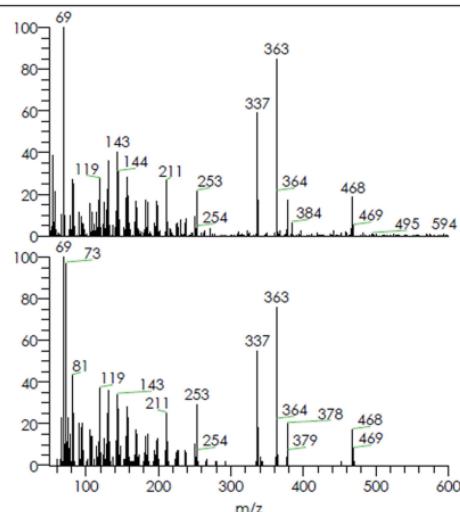
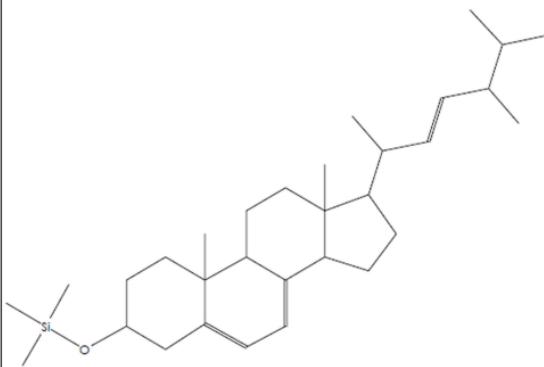
NL: 9.99E2  
 V5220812#841 RT: 17.33 AV:  
 1 S8: 8 19.48-19.60 T: [0.0] +  
 c El det=349.00 Full ms [  
 50.00-600.00]

NL: 9.99E2  
 SI 891, RSI 910, mainlib,  
 Entry# 139760, CAS#  
 1856-05-9, Cholesterol  
 trimethylsilyl ether



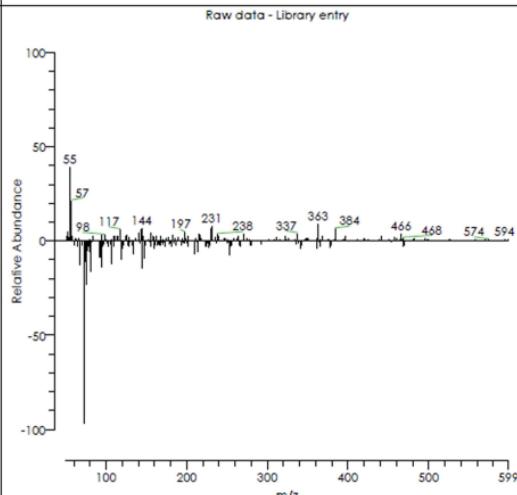
Hit	SI	RSI	Prob	Name	Library Name
1	759	837	88.42	Silane, (ergosta-5,7,22-trien-3 $\alpha$ -yloxy)trimethyl-	mainlib
2	669	760	7.56	Ergosterol	repib
3	661	706	7.56	Ergosterol	mainlib
4	659	732	7.56	Ergosterol	repib
5	629	755	1.75	Ergosta-5,8,22-trien-3-ol, (3 $\alpha$ ,22E)-	mainlib
6	580	656	0.36	Ergosta-9(11),20(22)-diene-3,6-diol, diacetate, (:	mainlib
7	577	726	0.31	3 $\alpha$ ,5-Cyclo-5 $\alpha$ -ergosta-6,8(14),22-triene	mainlib
8	570	608	0.24	5,7,22-Ergostatriene, 3-(methoxymethoxy)-	mainlib
9	569	701	0.23	3,5-Cyclo-6,8(14),22-ergostatriene	mainlib
10	563	739	0.18	Ergost-4,7,22-trien-3 $\alpha$ -ol	mainlib
11	550	579	0.11	5,8-(4-Phenyl-1,2,4-triazolidine-3,5-dione-1,2-diy)mainlib	mainlib
12	547	661	0.10	Cholesta-9(11),20(22)-dien-23-one, 3,6-bis(acet)	mainlib
13	546	634	0.10	Ergosta-5,7,22-trien-3-ol, acetate, (3 $\alpha$ ,22E)-	repib
14	533	640	0.06	Ergost-9(11)-ene-3,6,20-triol, 3,6-diacetate, (3 $\alpha$ ,5	mainlib
15	527	616	0.05	19-Norbrassicaster-3-one, 7,8,9,10-detetrahydro	repib
16	527	675	0.05	17-(1,5-Dimethyl-hex-2-enyl)-10,13-dimethyl-2,3,	mainlib
17	524	636	0.04	Cholan-24-oic acid, 3,7,12-tris(acetoxy)-, meth	mainlib
18	524	601	0.04	Ergostetraenoil, trimethylsilyl	mainlib
19	521	604	0.04	1,10-Secobergosta-5,7,9,22-tetraen-3-ol, acetate	mainlib
20	516	584	0.03	Ergocalciferol	mainlib

Silane, (ergosta-5,7,22-trien-3 $\alpha$ -yloxy)trimethyl-  
Formula C<sub>31</sub>H<sub>52</sub>OSi, MW 468, CAS# 2625-45-8, Entry# 27623  
(22E)-3-[Trimethylsilyloxy]ergosta-5,7,22-triene #



NL: 9.99E2  
V5220812#946-960 RT:  
19.12-19.36 AV: 15 SB: 8  
19.48-19.60 T: {0.0} + c El  
det=349.00 Full ms [  
50.00-600.00]

NL: 9.99E2  
SI 759, RSI 837, mainlib, Entry#  
27623, CAS# 2625-45-8, Silane,  
(ergosta-5,7,22-trien-3 $\alpha$ -yloxy)trimethyl-

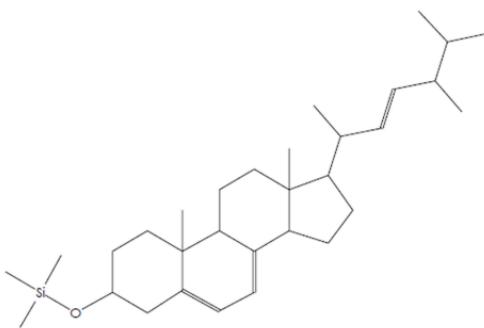


m/z

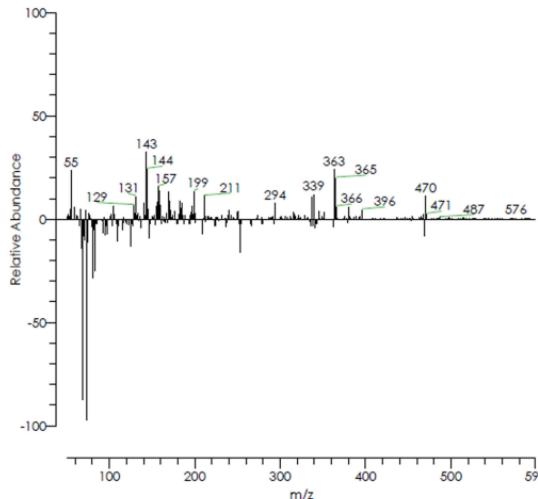
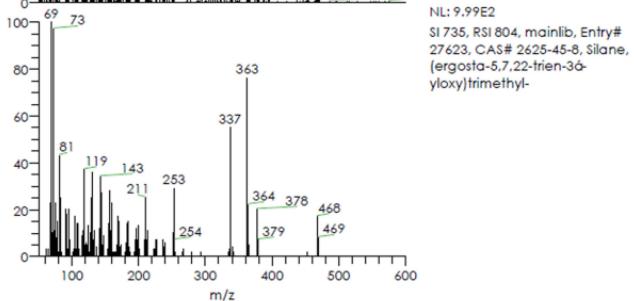
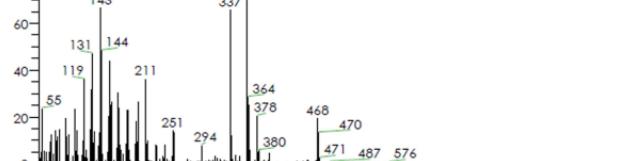
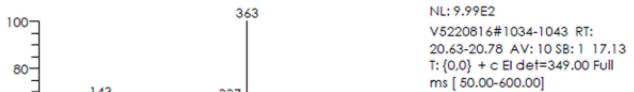


Hit	SI	RSI	Prob	Name	Library Name
1	735	804	89.59	Silane, (ergosta-5,7,22-trien-3 $\alpha$ -yloxy)trimethyl-	mainlib
2	641	738	7.33	Ergosterol	replib
3	629	697	7.33	Ergosterol	replib
4	619	652	7.33	Ergosterol	mainlib
5	600	718	1.67	Ergosta-5,8,22-trien-3-ol, (3 $\alpha$ 22E)-	mainlib
6	562	632	0.40	Ergosta-9(11),20(22)-diene-3,6-diol, diacetate	mainlib
7	543	575	0.19	5,7,22-Ergostatriene, 3-(methoxymethoxy)-	mainlib
8	539	565	0.16	5,8-(4-Phenyl-1,2,4-triazolidine-3,5-dione-1,2-methyl-	mainlib
9	525	636	0.10	Cholesta-9(11),20(22)-dien-23-one, 3,6-bis( $\alpha$ )-	mainlib
10	521	673	0.08	Cholesta-5,7-dien-3-ol, acetate, (3 $\alpha$ )-	replib
11	516	530	0.07	Spiro(1,3-dithiane)-2,16-(androst-5-en-3,17-	mainlib
12	512	649	0.05	3,5-Cyclo-6,8(14),22-ergostatriene	mainlib
13	504	675	0.04	Ergost-4,7,22-trien-3 $\alpha$ -ol	mainlib
14	500	648	0.03	Cholestan-3,7,12,25-tetrol, tetraacetate, (3 $\alpha$ )-	mainlib
15	499	623	0.03	Ergosta-5,7,22-trien-3-ol, acetate	mainlib
16	498	604	0.03	Cholan-24-oic acid, 3,7,12-tris(acetyloxy)-	mainlib
17	487	636	0.02	Cholesta-5,7-dien-3 $\alpha$ -ol, 3,5-dinitrobenzoate	replib
18	484	583	0.02	Pregna-5,7-dien-3-ol, 20-(3-butynyl)-	mainlib
19	481	530	0.01	Bufa-4,20,22-trienolide, 6-(acetyloxy)-3-( $\delta$ -D-	mainlib

Silane, (ergosta-5,7,22-trien-3 $\alpha$ -yloxy)trimethyl-  
Formula C<sub>31</sub>H<sub>52</sub>O<sub>5</sub>Si, MW 468, CAS# 2625-45-8, Entry# 27623  
(22E)-3-[Trimethylsilyl]oxy)ergosta-5,7,22-triene #



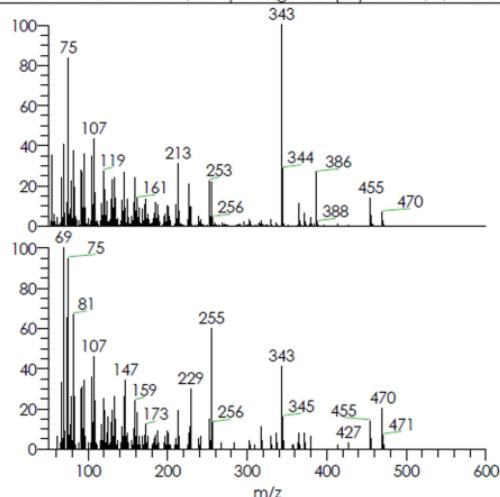
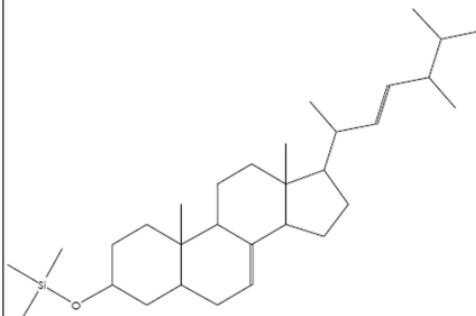
Raw data - Library entry



IV

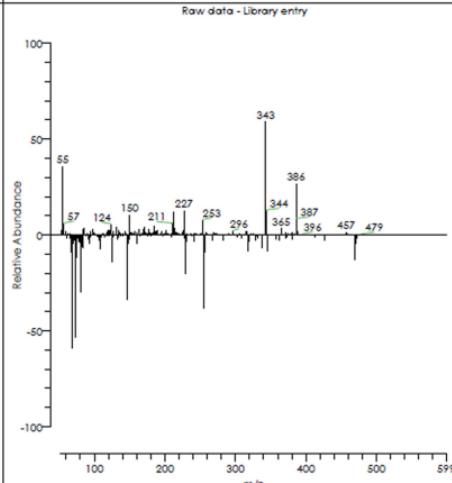
Hit	SI	RSI	Prob	Name	Library Name
1	791	841	79.07	Silane, [[[3 $\alpha$ ,22E]-ergosta-7,22-dien-3-yl]oxy]trimethyl-	mainlib
2	730	755	13.77	Silane, [[[3 $\alpha$ ,5 $\alpha$ ]-ergosta-8,24(28)-dien-3-yl]oxy]trimethyl-	mainlib
3	692	736	3.30	Silane, [[[3 $\alpha$ ,5 $\alpha$ ]-ergosta-7,22-dien-3-yl]oxy]trimethyl-	mainlib
4	647	806	0.70	Silane, trimethyl[[[(3 $\alpha$ )-stigmasta-7,24(28)-dien-3-yl]oxy]-	mainlib
5	639	669	0.52	Silane, trimethyl[(4 $\alpha$ -methylergosta-7,24(28)-dien-3 $\alpha$ -yl)ox	mainlib
6	639	760	0.52	Silane, trimethyl[[[(3 $\alpha$ ,4 $\alpha$ ,5 $\alpha$ )-4-methylcholesta-8,24-dien-3]	mainlib
7	638	746	0.50	Silane, [[[3 $\alpha$ ,5 $\alpha$ ]-cholesta-8,24-dien-3-yl]oxy]trimethyl-	mainlib
8	613	767	0.15	Cholest-7-en-3-ol, (3 $\alpha$ ,5 $\alpha$ )-	replib
9	609	687	0.12	Ergosta-5,22-dien-3-ol, acetate, (3 $\alpha$ ,22E)-	mainlib
10	596	649	0.08	Cholic acid	mainlib
11	593	751	0.07	Cholest-8(14)-en-3-ol, (3 $\alpha$ ,5 $\alpha$ )-	mainlib
12	593	615	0.08	Cholic acid	replib
13	581	728	0.04	25-Hydroxy-24-methylcholesterol, di-TMS	mainlib
14	580	623	0.04	17-(1,5-Dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,	mainlib
15	579	760	0.04	Cholest-8-en-3-ol, (3 $\alpha$ )-	mainlib
16	579	652	0.04	2-[3-Hydroxy-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12	mainlib
17	579	631	0.04	Methyl cholate	replib
18	578	655	0.04	Androst-9(11)-en-17-one, 3-[[trimethylsilyl]oxy]-, O-methyl	mainlib
19	576	600	0.04	5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 9,9a-bis(ac $\epsilon$	mainlib
20	574	614	0.03	Cholan-24-oic acid, 3,6,7-tris(acetyloxy)-, methyl ester, (3-	mainlib
21	573	632	0.03	9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate,	mainlib

Silane, [[[3 $\alpha$ ,22E]-ergosta-7,22-dien-3-yl]oxy]trimethyl-  
Formula C<sub>31</sub>H<sub>54</sub>O<sub>5</sub>, MW 470, CAS# 55527-93-0, Entry# 27626  
(22E)-3-[[trimethylsilyl]oxy]ergosta-7,22-diene #



NL: 9.99E2  
V5220812#1050-1057 RT:  
20.90-21.02 AV: 8 SB: 8  
19.48-19.60 T: {0.0} + c El  
det=349.00 Full ms [  
50.00-600.00]

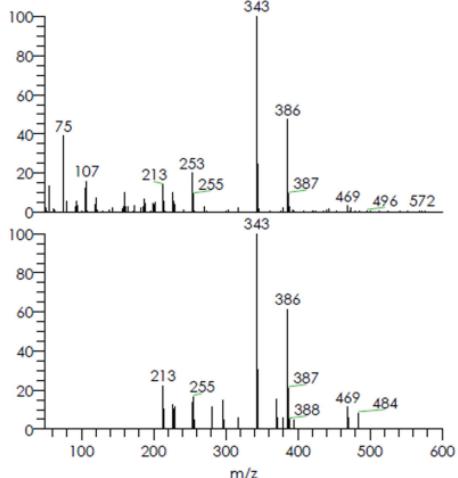
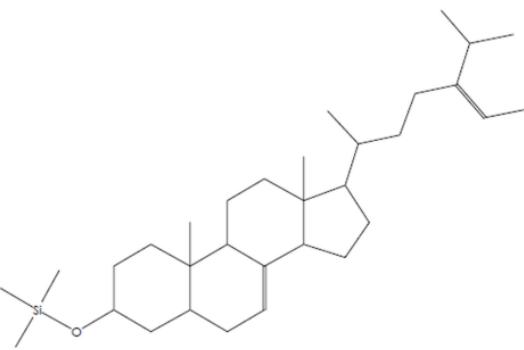
NL: 9.99E2  
SI 791, RSI 841, mainlib, Entry# 27626, CAS# 55527-93-0, Silane,  
[[[(3 $\alpha$ ,22E)-ergosta-7,22-dien-3-yl]oxy]trimethyl-



V

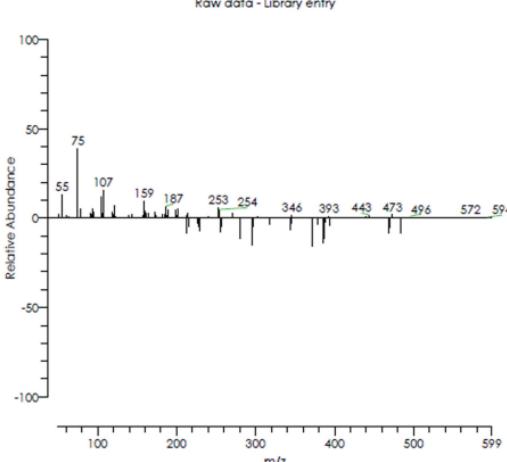
Hit	SI	RSI	Prob	Name	Library Name
1	737	790	97.74	Silane, trimethyl [[(3 $\alpha$ )-stigmasta-7,24(28)-dien-3-yl]oxy]-	
2	548	585	1.48	Cobalt, 1,2-bis(diisopropylphosphino)ethane-cyclo mainlib	
3	471	490	0.18	5-Acetoxy-2-(3-acetoxy-4-methoxyphenyl)-3,7-dim	mainlib
4	446	577	0.05	2-Methyl-1-(3,4-dimethoxyphenyl)-1-(2,4-diisoprop	mainlib
5	438	505	0.04	Silane, [[[3 $\alpha$ ,22E]-ergosta-7,22-dien-3-yl]oxy]trimeth	mainlib
6	433	472	0.03	Cholestan-1-one	repfib
7	428	524	0.02	5-(p-Aminophenyl)-4-(4-biphenyl)-2-thiazolamine	mainlib
8	427	475	0.02	Androst-7-en-6-one, 2,3,17-tris(acetyloxy)-, (2 $\alpha$ ,3 $\alpha$ ,5-	mainlib
9	427	629	0.02	2H,8H-Benzo[1,2-b:5,4-b']dipyran-10-propanoic aci	mainlib
10	426	442	0.03	Cholestan-1-one	mainlib
11	426	450	0.02	6H-Dibenzo[b,d]pyran, 6 $\alpha$ ,7,8,10a-tetrahydro-6,6,9-	repfib
12	425	483	0.02	10-(Acenaphthelen-1-yl)-9-phenanthryl acetate	mainlib
13	424	450	0.02	Chol-4-en-24-oic acid, 3-oxo-, methyl ester	mainlib
14	421	599	0.02	Cholest-7-en-3-ol, (3 $\alpha$ ,5 $\beta$ )-	mainlib
15	417	606	0.02	2H,8H-Benzo[1,2-b:5,4-b']dipyran-10-propanoic aci	repfib
16	415	479	0.01	Silane, [[[3 $\alpha$ ,17 $\alpha$ ]-17-methylandrost-5-ene-3,17-diy] mainlib	
17	411	575	0.01	Cholest-8(14)-en-3 $\alpha$ -ol	mainlib
18	410	433	0.01	Cholan-24-oic acid, 3-[(ethoxycarbonyl)oxy]-7,12-c	mainlib
19	409	616	0.01	Cholan-16-one, 23-methyl-, (5 $\alpha$ ,20.xi)-	mainlib
20	406	434	0.01	Thymolphthalein	mainlib
21	404	419	0.02	6H-Dibenzo[b,d]pyran, 6 $\alpha$ ,7,8,10a-tetrahydro-6,6,9-	mainlib

Silane, trimethyl|[[(3 $\alpha$ )-stigmasta-7,24(28)-dien-3-yl]oxy]-  
Formula C<sub>32</sub>H<sub>56</sub>OSi, MW 484, CAS# 55527-95-2, Entry# 141100  
(24Z)-3-[(Trimethylsilyl)oxy]stigmasta-7,24(28)-diene #



NL: 9.99E2  
V5220812#1217-1224 RT:  
23.75-23.87 AV: 8 SB: 6  
19.53-19.62 T: {0.0} + c El  
det=349.00 Full ms [  
50.00-600.00]

NL: 9.99E2  
SI 737, RSI 790, mainlib, Entry# 141100, CAS# 55527-95-2,  
Silane,  
trimethyl|[[(3 $\alpha$ )-stigmasta-7,24(28)-dien-3-yl]oxy]-



Raw data - Library entry

**Supplemental Figure 2.** Sterol identification. Mass spectrum of each GC peak was used to search against small molecule library of the National Institute of Standards and Technology Library (2001 Version) that was purchased with the GC-MS and the library search program is part of its operating software, Xcalibur. A sterol was positively identified if its mass spectrum fairly matched up with one in the library (score of SI and RSI matching factors  $\geq 800$ ) with a probability score  $\geq 70$ . Details about SI, RSI and probability may be found in Library Search Results Window of Xcalibur. There is a main library (mainlib) and also a replicate library (replib) for redundant spectra. So for cholesterol, there is the “mainlib” hit and 3 very similar hits in the repolib and the all give basically the same SI, RSI, and Prob numbers. I: cholesterol; II and III: two ergosterol isoform stereoisomers termed #1 and #2, respectively; IV: ergosta-7,22-dien-3b-ol; and V: stigmasta-7-24(28)-dien-3b-ol.



**Supplemental Figure 3. Released MSPs and MLP.** Proteins identified by LC-MS/MS were aligned by CLUSTALW software. Shown in boxes are unique peptides for individual proteins. P15706, A4HUF6 and A4HUG0 are all MSPs. A4I3D1 is MLP.