Supplemental Figure 1

α-GalCer stabilizes CD1d: heavy chain/β_2m complexes at 37°C in C1R:CD1d transfectants.

Panel A. C1R:hCD1d transfectants were surface biotinylated and lysed with 0.5% Triton X-100 pH 7.4. Lysates were incubated for 2 hours at 4ºC (lanes 1-5) or 37ºC (lanes 6-24) with different concentrations of α-GalCer (lanes 11-30) or without lipid (lanes 1-10). CD1d HC/β_2m complexes were immunoprecipitated with anti-CD1d antibody 42.1 (lanes 2, 7, 12, 17, 22 and 27; solid line arrows) or anti-β_2m antibody BBM.1 (lanes 4, 9, 14, 19, 24 and 29; dashed line arrows). Free CD1d HC were immunoprecipitated with antibody 75.10 (lanes 3, 8, 13, 18, 23 and 28; dotted line arrows) and MHC class II with antibody L242 (lanes 5, 10, 15, 20, 25 and 30). Mouse IgG1 and IgG2a were used as isotype controls. Immunoprecipitates were analyzed by SDS-PAGE (15%) in reducing conditions and immunoblotted with streptavidin-HRP. **Panel B.** Quantification of the intensitiy of CD1d HC and β_2m bands immunoprecipitaed with 42.1, BBM1 and 75.10 antibodies. Bars indicate the relative change in signal of CD1d HC (grey bars) and β_2m (white bars) in samples incubated for 2 hours at 37ºC in presence of α-GalCer or at 4ºC without lipid (black bars). Lower right graph shows the ratio between associated (42.1) and free (75.10) forms of CD1d HC. Intensities of bands were measured by densitometry with ImageJ software and relative change of signal was calculated as proportion of HC or β_2m signal at 37ºC with or without α-GalCer to HC or β_2m signal at 4ºC.

Supplemental Figure 2

C80 TMM does not influence the stability CD1d HC/β_2m complexes at 37°C.

CD1d-transfected HeLa cells were surface biotinylated and lysed with 0.5% Triton X-100 pH 7.4. Lysates were incubated for 2 hours at 4ºC or 37ºC in the absence of lipid (**Panel A**) or the presence of increasing concentrations of α-GalCer (**Panel B**) or C80 TMM (**Panel C**). CD1d: HC/β_2m complexes were immunoprecipitated with anti-CD1d antibody 42.1, free CD1d HC with antibody 75.10 and MHC class I with antibody W6/32. Immunoprecipitates were analyzed by SDS-PAGE (15%) in reducing conditions and immunoblotted with streptavidine-HRP. Bar graphs show the quantification of the ratio between associated and free forms of CD1d heavy. Intensity of bands was measured by densitometry with ImageJ software. Ratio between associated versus free form of HC was calculated as proportion of HC signal immunoprecipitaded with 42.1 Ab to signal obtained with 75.10 Ab in particular experimental conditions.

Supplemental Figure 3

α-GalCer does not influence the stability of CD1b HC/β_2m complex at 37°C.

Panel A. CD1d-transfected C1R cells were surface biotinylated and lysed with 0.5% Triton X-100 pH 7.4 (lanes 1-3) or pH 5.5 (lanes 4-11). α-GalCer was added in increasing concentrations to samples run in lanes 7-11. Lysates were incubated for 15 hours at 4ºC (lane 1 and 4), 15 hours at 37ºC (lane 2 and 5) or 3 hours at 37ºC followed by change in pH to 5.5 (lane 3) or 7.4 (lanes 6-11) and additional 12-hour incubation at 37ºC. CD1d heavy chain (HC)/β_2m complexes were immunoprecipitated with anti-β_2m antibody BBM.1. Immunoprecipitates were analyzed by SDS-PAGE (15%) in reducing conditions and immunoblotted with streptavidin-HRP. **Panel B.** Quantification of intensities of bands corresponding to CD1b HC immunoprecipitated with BBM.1 antibody. Bars indicate the relative change of CD1d HC signal in samples incubated at different conditions (see below graph) compared to incubation at 4ºC without lipid. Intensities of bands were measured by densitometry using ImageJ software. Relative change of signal was calculated as proportion of HC signal at 37ºC with or without α-GalCer to HC signal at 4ºC and pH 7.4.

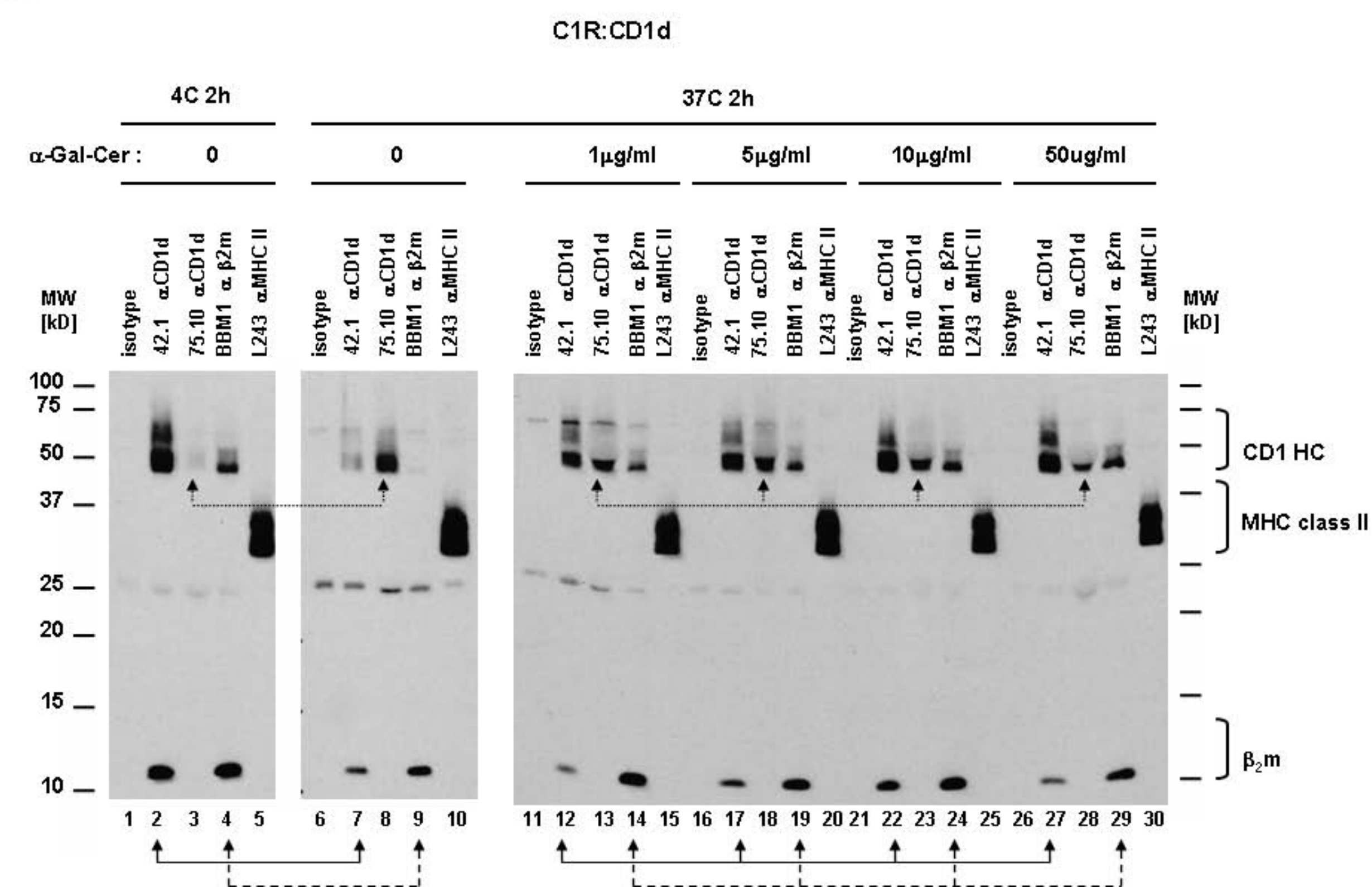
Supplemental Figure 4

Chemical structures of lipids used in experimental system.

Panel A. Chemical structure of alpha-Galactosylceramide (α-GalCer); **Panel B.** Chemical structure of C80 trehalose monomycolate (C80 TMM); **Panel C.** Chemical structure of C32 glucose monomycolate (C32 GMM).

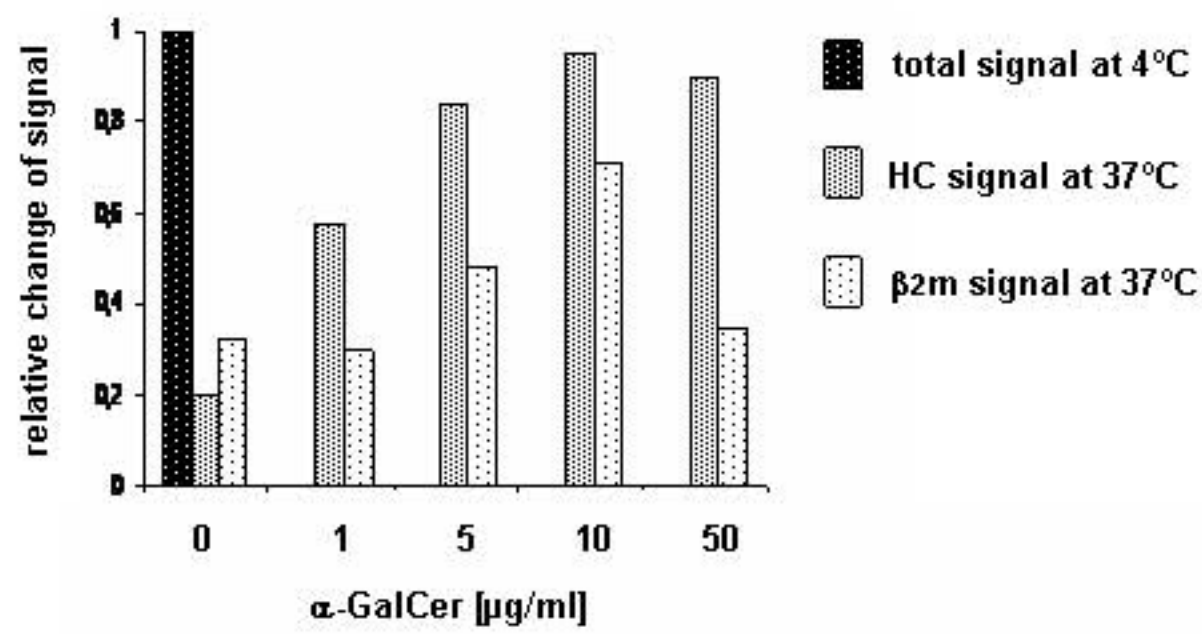
SUPPLEMENTAL FIGURE 1

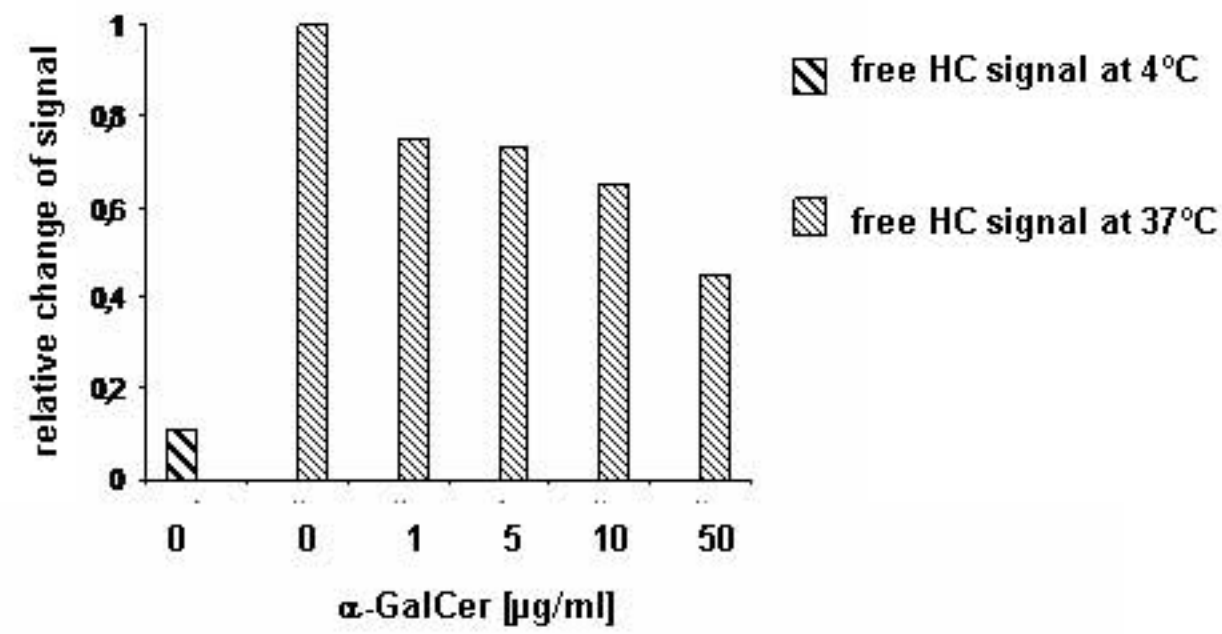
A

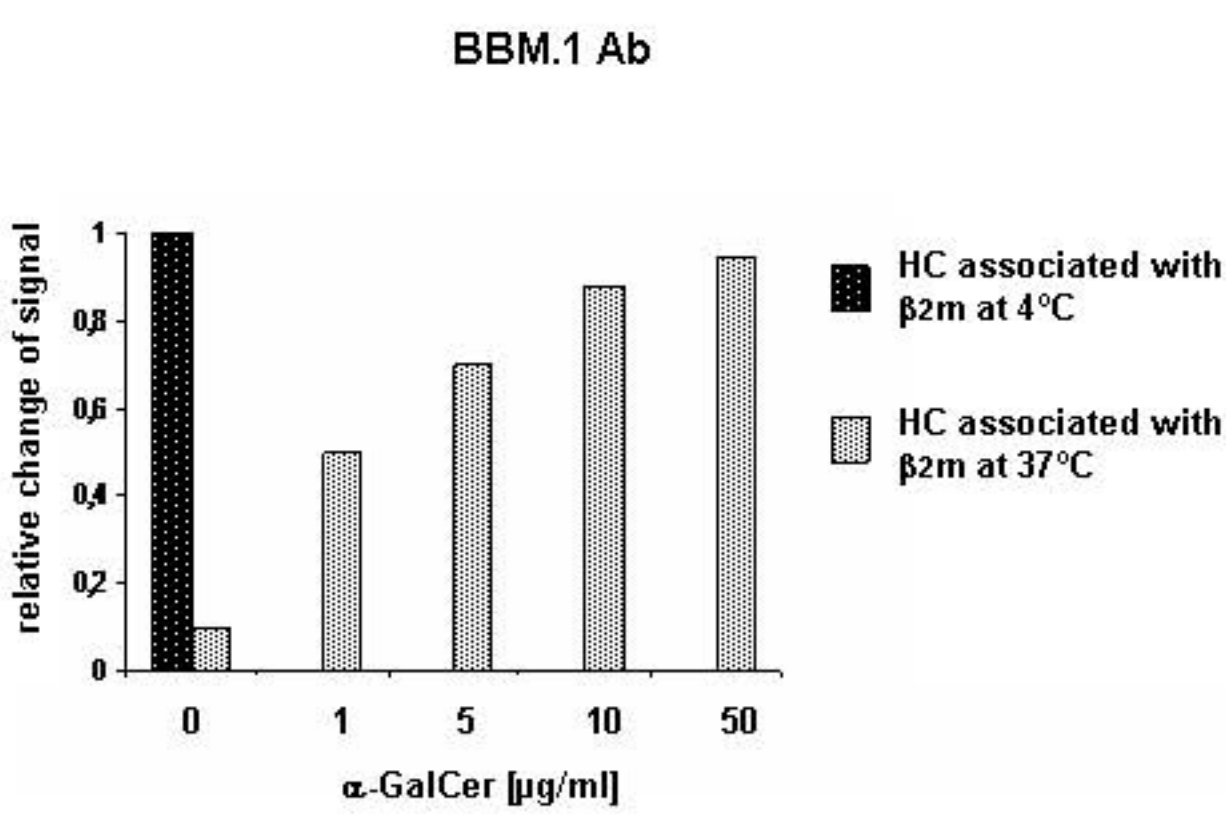


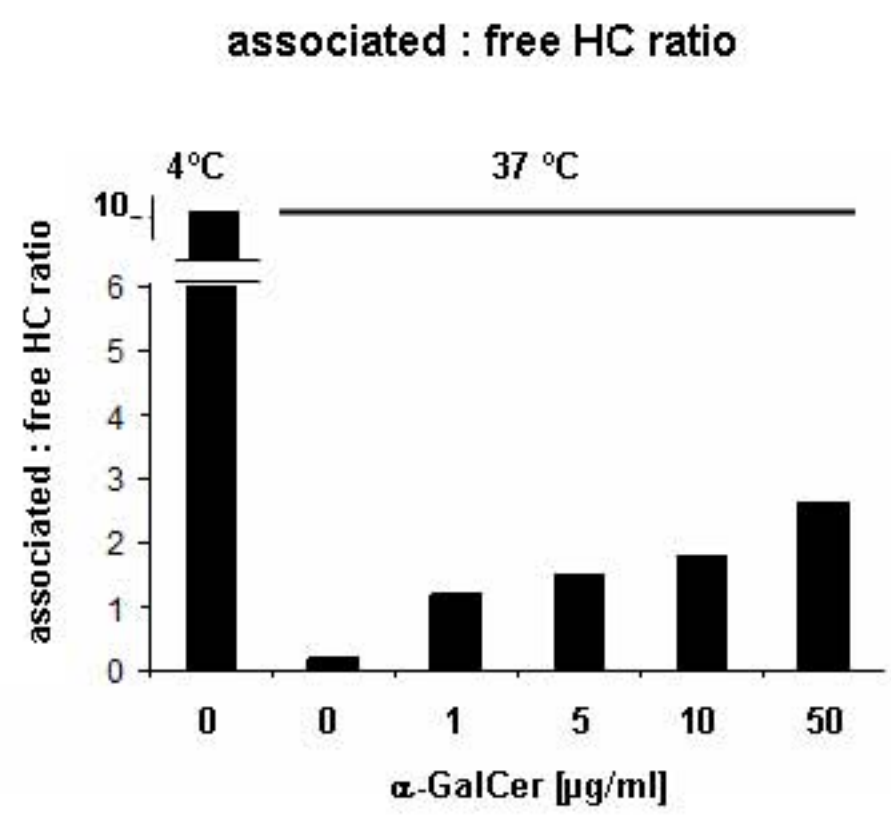
B

Supplemental Figure 1 Continued

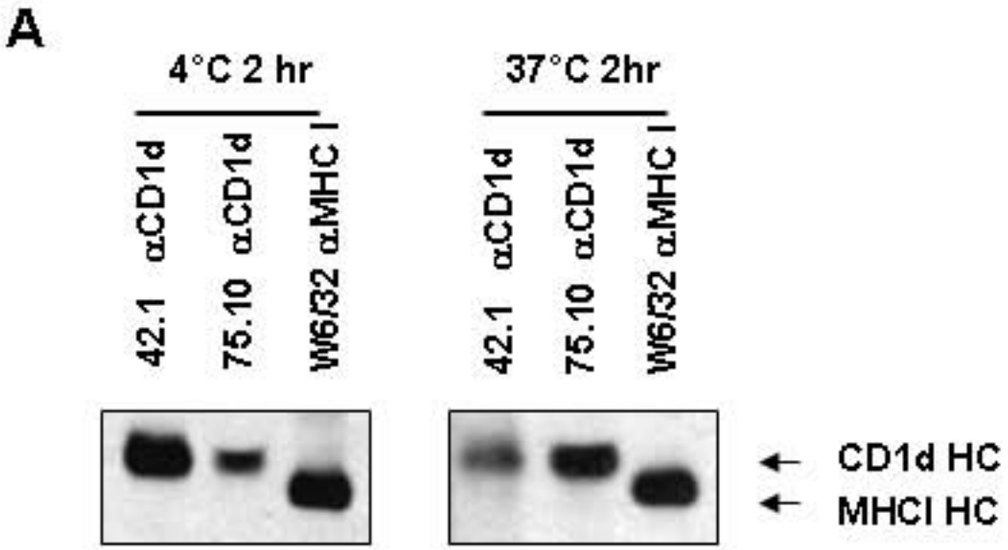


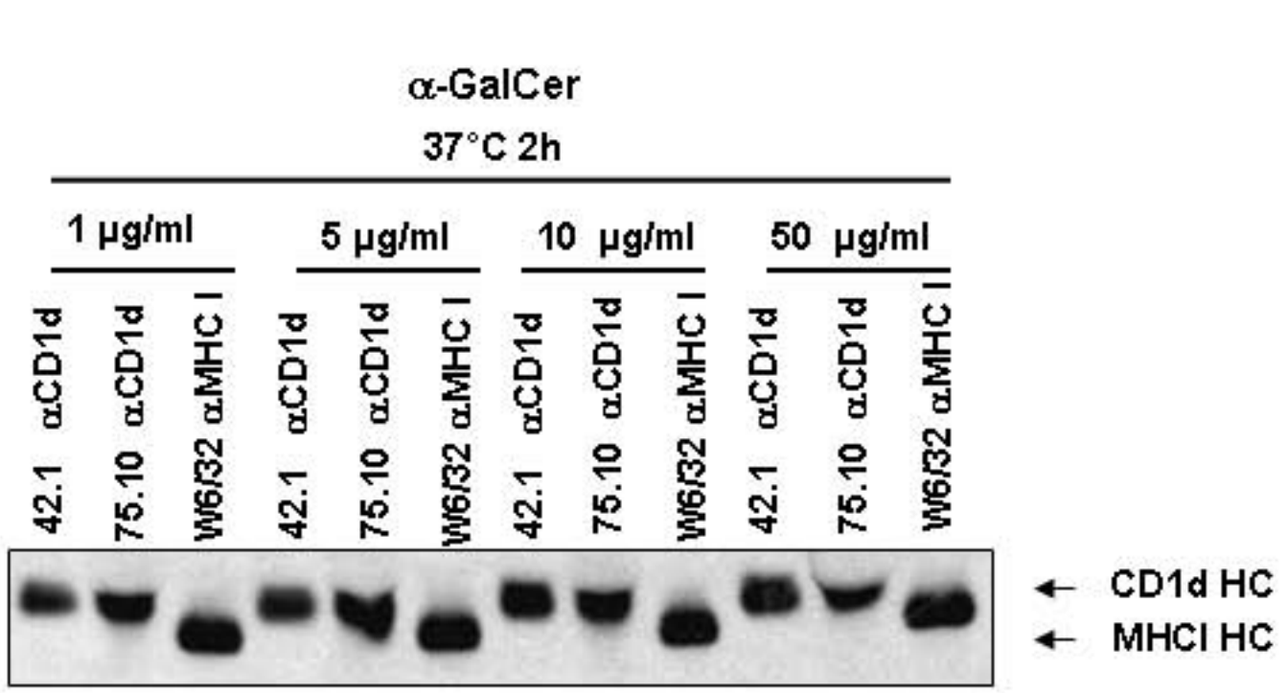


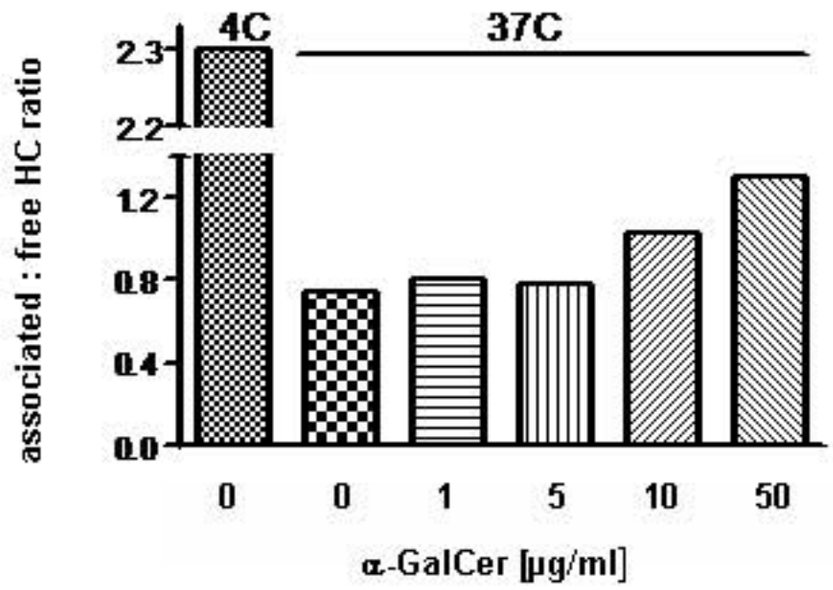


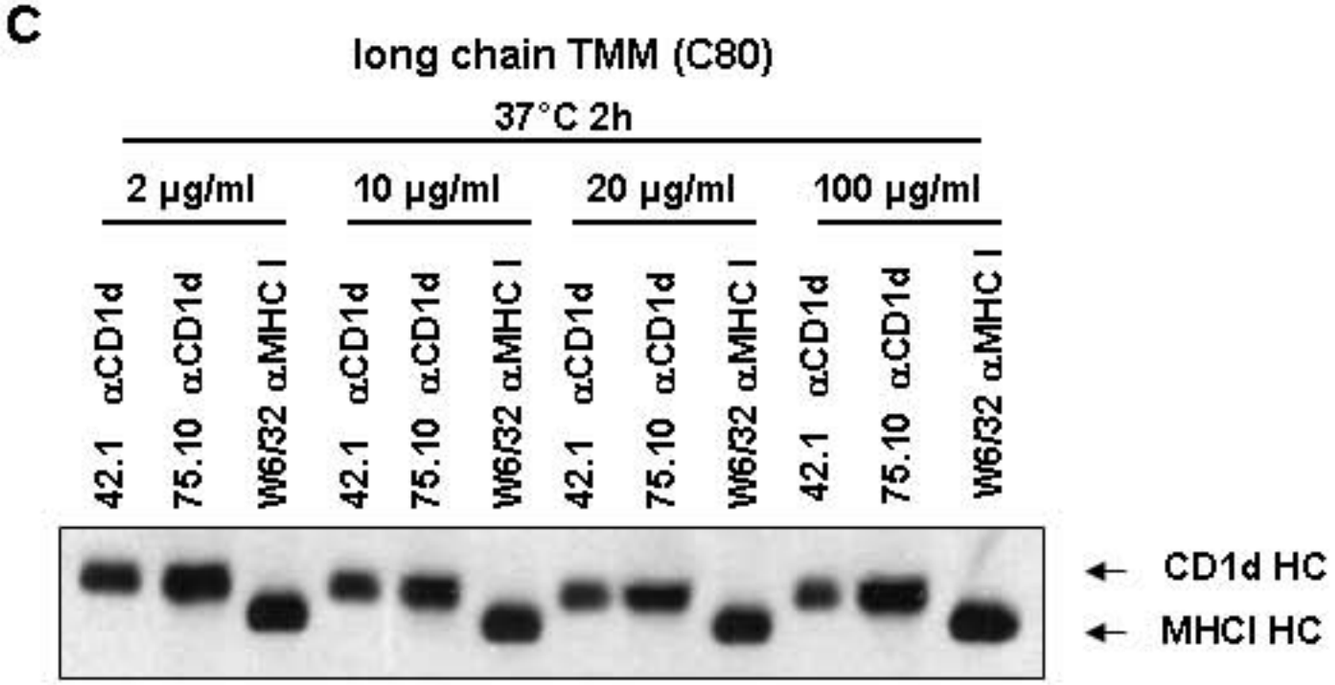


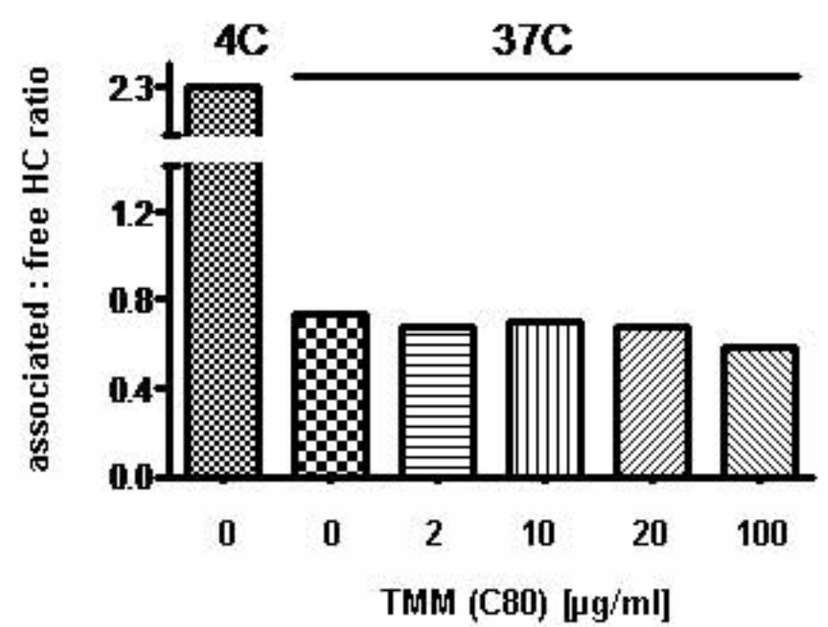
SUPPLEMENTAL FIGURE 2



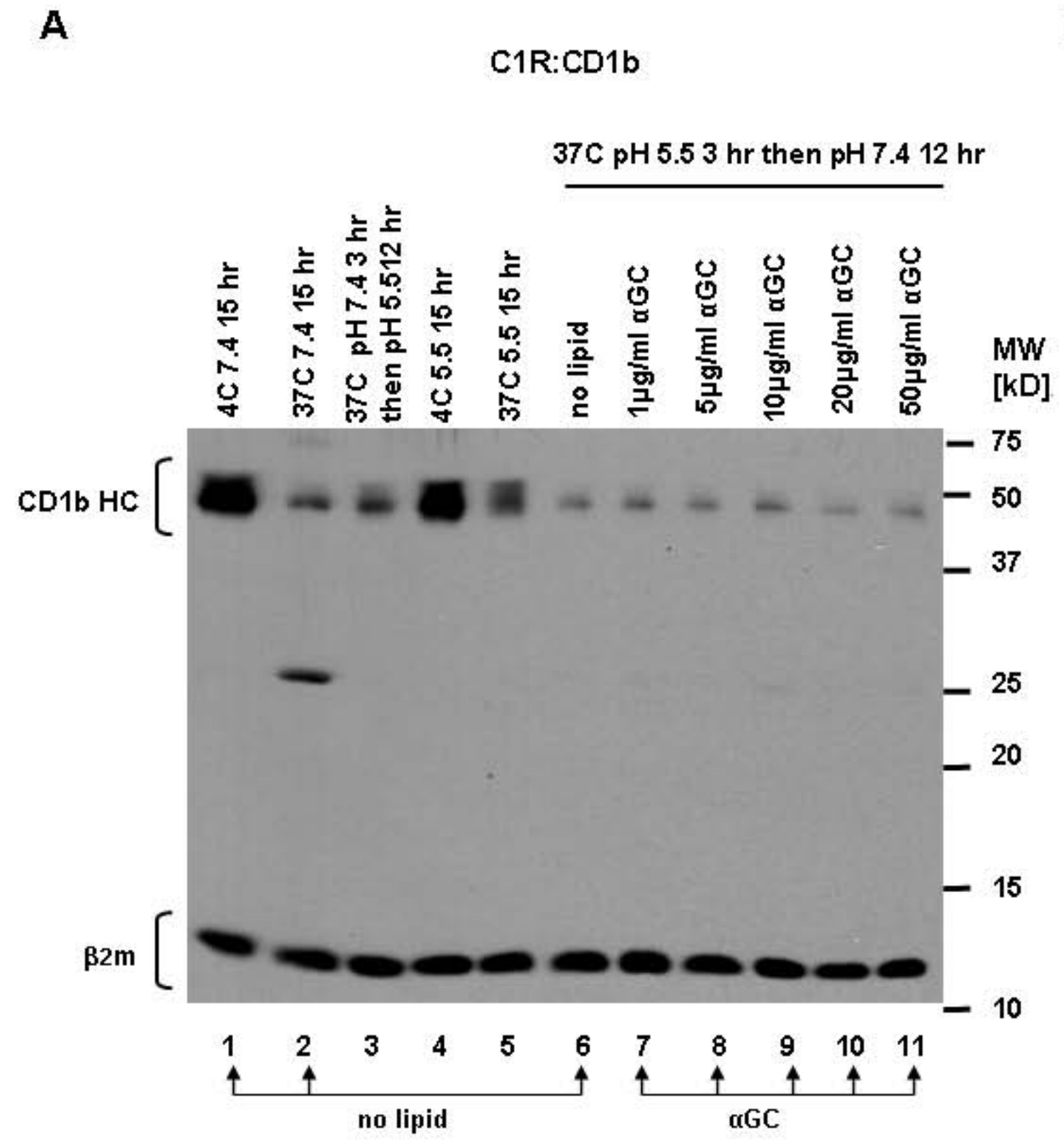


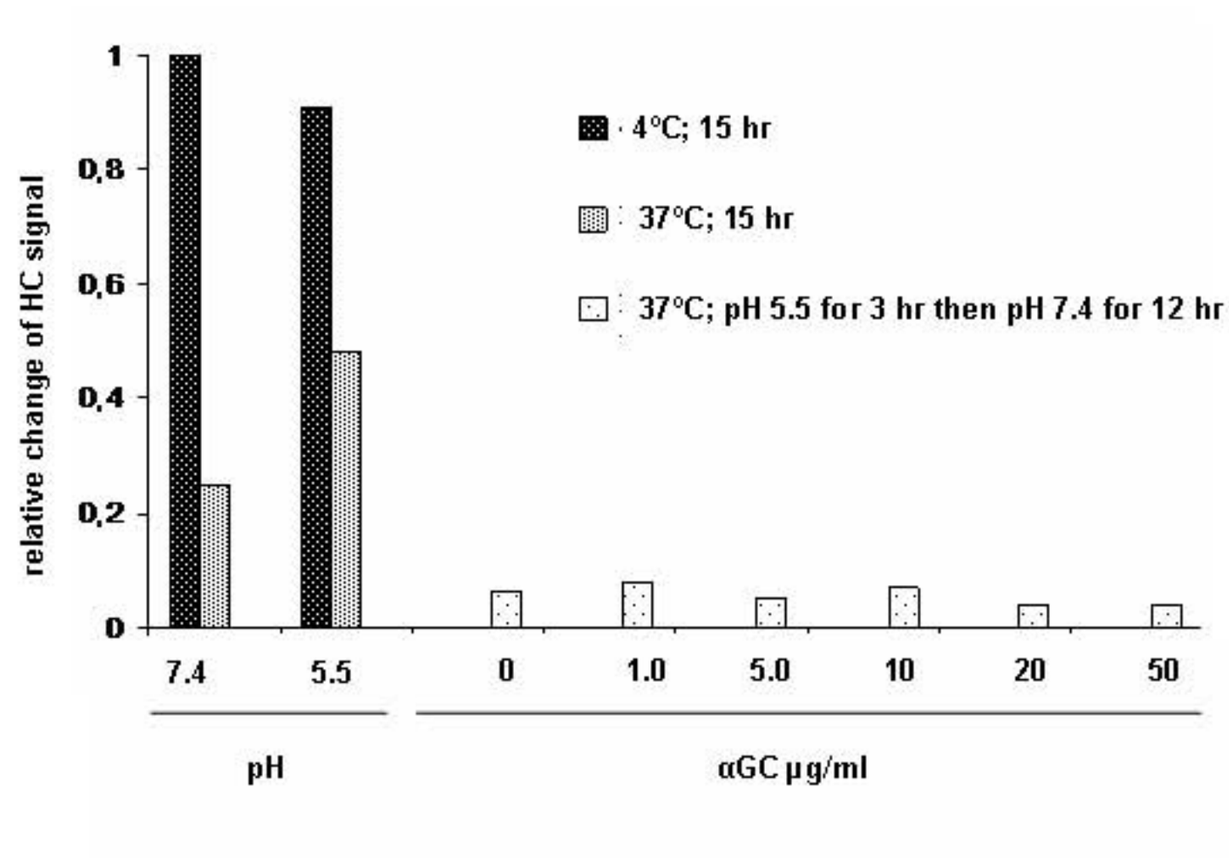






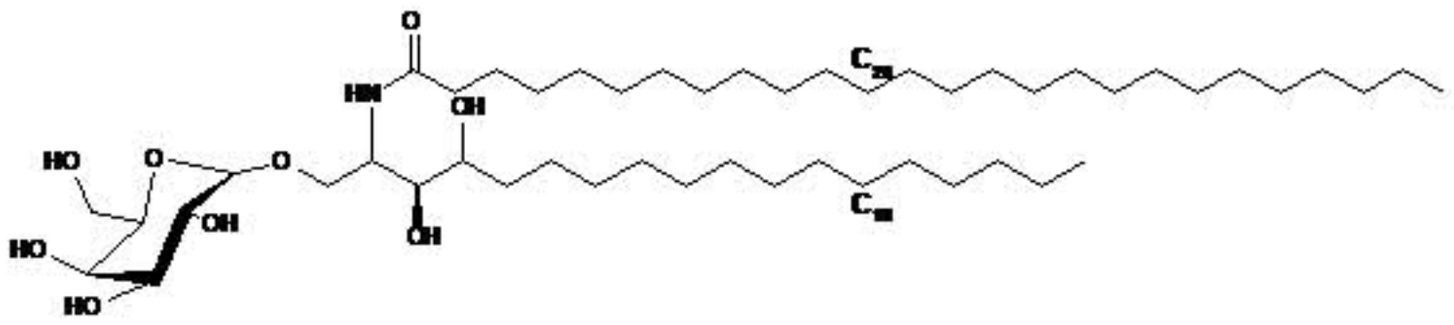
SUPPLEMENTAL FIGURE 3





SUPPLEMENTAL FIGURE 4

A



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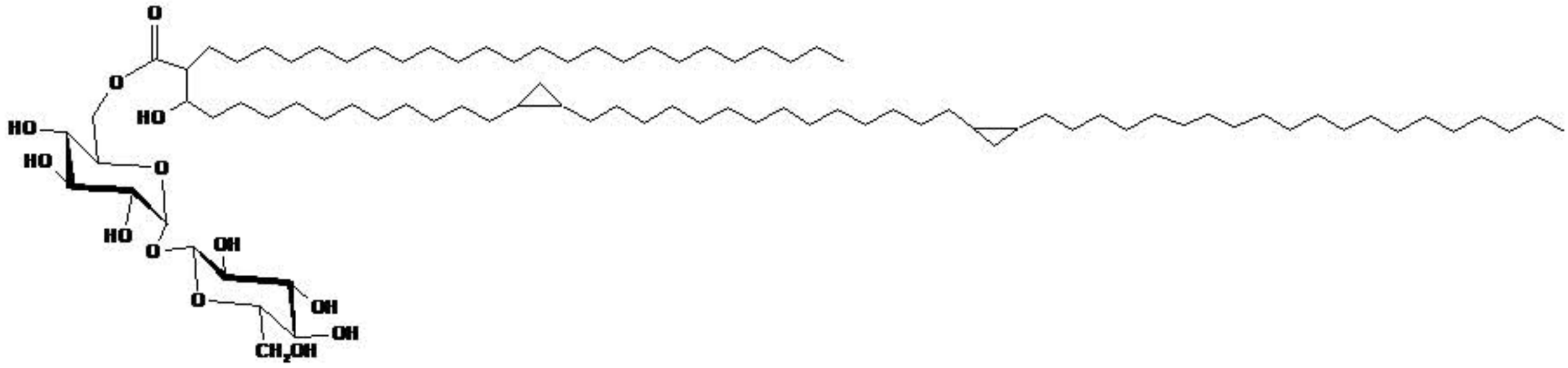

C