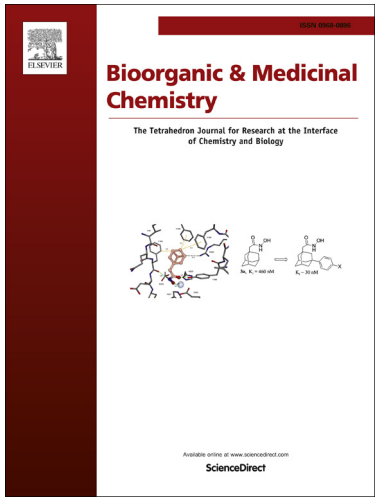

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Synthesis of α-L-Rhamnosyl Ceramide and Evaluation of its Binding with Anti-Rhamnose Antibodies

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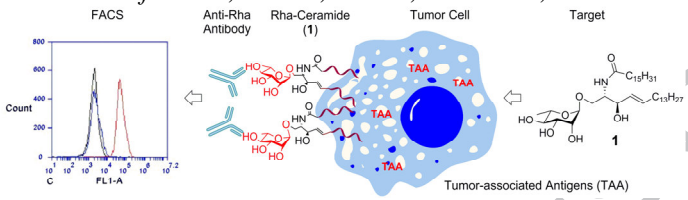

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Synthesis of α-L-Rhamnosyl Ceramide and Evaluation of its Binding with Anti-Rhamnose Antibodies

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ABSTRACT

An α-L-rhamnosyl ceramide (**1**, α-L-RhaCer) has been prepared that was recognized by anti-L-rhamnose (anti-Rha) antibodies. During these studies we explored the use of an α-L-rhamnosyl thioglycoside and a trichloroacetimidate as a glycosyl donors. Subsequently, the acceptors desired for glycosylation, 3-*O*-benzoylazidosphingosine or 3-*O*-alloxycarbonylsphingosine, were prepared from D-xylose. The thioglycoside donor, 2,3,4-tri-*O*-acetyl-1-(4-tolyl)thio-α-L-rhamnopyranoside, and the trichloroacetimidate donor, 2,3,4-tri-*O*-acety-1-(2,2,2-trichloroethanimidate)-α-L-rhamnopyranoside, were synthesized in 50% and 78% yield overall, respectively. The synthesis of the glycosylation acceptor employed an addition-fragmentation olefination that was successfully carried out in 53% yield. With the successful synthesis of key intermediates, α-L-RhaCer (**1**) was prepared without any insurmountable obstacles. Anti-Rha antibodies were prepared in BALB/c mice by immunizing them with rhamnose-Ficoll with Sigma Adjuvant System (SAS) and the anti-L-Rha antibodies were isolated from the blood sera. Liposomes and EL4 tumor cells were used as model systems to demonstrate the ability of **1** to insert into a lipid bilayer. The interaction of the liposomes or the EL4 cells with α-L-RhaCer (**1**) and anti-Rha antibodies were investigated by fluorescence microscopy and flow cytometry, respectively, to confirm the ability of glycolipid **1** to be displayed on the tumor cell surface as well as the ability to be recognized by anti-Rha antibodies.

1. Introduction

A protective anti-tumor immune response can be evoked by tumor associated antigens (TAA) following anti-cancer vaccination via adequate uptake of the TAA by antigen presenting cells (APC), which can take up, process and display the TAA resulting in activation of cytotoxic T cells. However, naturally occurring tumor cells often lack surface markers that allow for uptake by APC. One strategy for overcoming this problem has been to decorate tumor cells with natural antibodies which can interact with Fcγ receptors (FcγR) on APC and induce tumor cell uptake, Figure 1.[1] For example, injection of heterogenous glycolipids isolated from rabbit red blood cells that contain terminal α-gal epitopes have been injected into melanoma tumors implanted in mice that express a high level of anti-α-gal antibodies.[1] The resulting tumor cells displayed the α-gal epitopes on their membranes and an anti-tumor response was demonstrated.

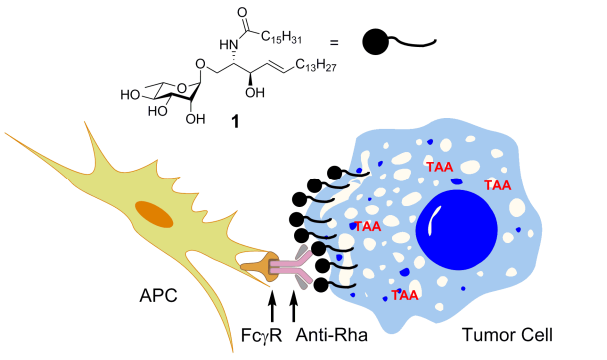

Figure 1. Structure of α-L-rhamnosyl ceramide (**1**) and model for anti-Rha-antibody-mediated uptake of TAA in tumor cells

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Potential drawbacks of this approach are the need to isolate α-gal glycolipids from rabbits and heterogeneity of the isolates. Further, naturally occurring antibodies more abundant than anti-α-gal antibodies have been identified; e.g., anti-rhamnose (anti-Rha) antibodies.[2] Model systems for generating and studying anti-α-gal antibodies also require α-gal KO mice. To circumvent the problem of heterogeneity, we designed a homogeneous α-L-RhaCer (**1**) which we envisioned could be injected into tumor cells. Another advantage of studying the response to anti-Rha antibodies is that they can be generated in common laboratory mice rather than in proprietary mice.[3]

Spontaneous insertion of α-L-RhaCer (**1**) into phoshopholipid-based cell walls is expected to occur because the fatty acid tails are thermodynamically more stable when surrounded by other lipids as opposed to water.[1] This insertion causes the anti-Rha epitopes to be displayed on the tumor cells which would allow binding of anti-Rha antibodies to Rha epitopes. After binding occurs, the Fc portions of the anti-Rha antibodies would be expected to interact with the FcγR on APC analogous to what has been shown with α-gal glycolipids and anti-α-gal antibodies.[1] These interactions allow the tumor cells to be accessible for effective uptake by the APC, resulting in an anti-TAA immune response. We also note, there is substantial interest in the literature related to coating cells, viruses or bacteria with small molecules that can recruit antibodies including anti-Rha antibodies.[4]

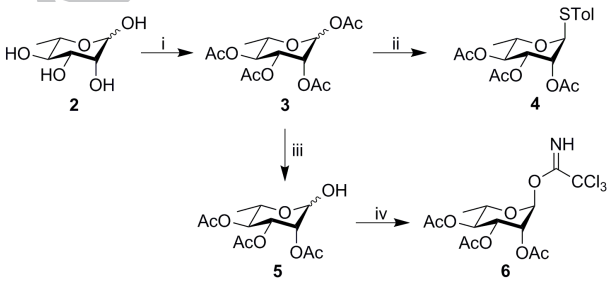
In order to design a homogeneous glycolipid we focused on naturally occurring ceramides. Ceramides have varying *N*-acyl chain lengths, from 14 to 26 carbons (C14 to C26) in mammals, with the most abundant being C16 (16:0) and C24 (24:0 and 24:1).[5] With this rationale for target selection taken into consideration, an interest in synthesizing an anomerically pure glycolipid, as opposed to extraction from a natural source, using L-rhamnose and ceramide (C16 or C24) for the development of an anti-cancer vaccine was created.

2. Results

*2.1. Synthesis of glycosyl donors 2,3,4-tri-O-acetyl-1-(4-tolyl)thio-α-L-rhamnopyranoside (**4**) and 2,3,4-O-triacetyl-α-L-rhamnopyranoside trichloroacetimidate (**6**)*

To prepare target **1**, the first requirement was to obtain fully protected rhamnosyl donors (**Scheme 1**). L-Rhamnose **2** was peracetylated and reacted with *p*-thiocresol to afford known thioglycoside **4** in moderate yield.[6] We also obtained a trichloroacetimidate donor **6** by deacetylating L-rhamnose **3** at the anomeric position with hydrazine acetate to afford intermediate **5** in 92%.[7] Known imidate **6** was then accessed in good yield by reaction at the anomeric position of compound **5** with CCl_3CN in the presence of DBU.[7]

Scheme 1. Synthesis of glycosyl donors **4** and **6**

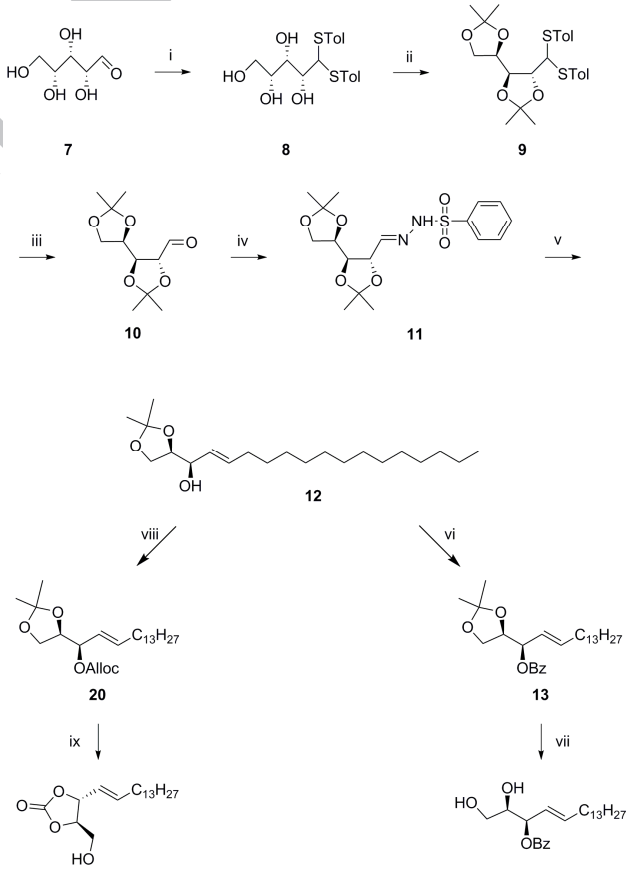


Reagents and Conditions: i. Ac_2O, pyridine, DMAP, RT, 16 h, quant.; ii. *p*-thiocresol, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 0 °C – RT, 18 h 52%; iii. hydrazine acetate, DMF, 55 °C, 4 h, 91%; iv. CCl_3CN, DBU, CH_2Cl_2, 0 °C – RT, 2 h, 85%.

*2.2. Synthesis of ceramide acceptor (2R, 3R, 4E)-3-O-benzoyloxyoctadec-4-ene-1,2-diol (**14**) and (2R, 3R, 4E)-carbonate-octadec-4-ene-1,2,3-triol (**21**)*

The synthesis of the ceramide acceptor started from D-xylose (**7**) (**Scheme 2**). D-xylose (**7**) was converted into dithioacetal **8** by reaction with *p*-thiocresol in 90% TFA.[8] Reaction of compound **8** with 2,2-dimethoxypropane (2,2-DMP) and catalytic *p*-toluenesulfonic acid (*p*-TSA) afforded isopropylidene **9**.[9] Deprotection of the thiol group was carried out in high yield with $HgO/HgCl_2$ to afford aldehyde **10**.[10] Without further purification, aldehyde **10** was reacted with *p*-toluenesulfonylhydrazide (*p*-$TsNHNH_2$) which afforded hydrazone **11**.[11] In order to synthesize the required *trans* double bond, an addition-fragmentation procedure was employed that reported the exclusive formation of the *trans* alkene; however, the yield of this reaction was moderate.[11] The olefination procedure required accessing the Grignard reagent tridecylmagnesium bromide ($C_{13}H_{27}MgBr$), followed by addition to hydrazine **11** to afford alkene **12**.[11] Alkene **12** was then protected using benzoyl chloride (BzCl) to afford benzoate **13** in 77% yield.[12] The deprotection of the isopropylidene group was attempted with 1 *N* HCl at 60 °C [11a]; however, this led to undesired side reactions. Ultimately, treatment of isopropylidene

Scheme 2: Synthesis of intermediates **14** and **21**



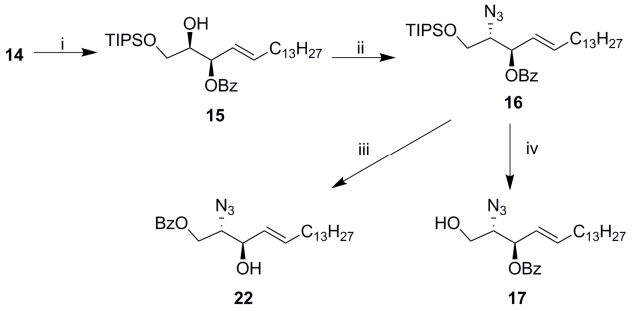
Reagents and Conditions: i. *p*-thiocresol, 90% TFA, 55 °C for 15 min, then RT for 0.5 h, quant.; ii. 2,2-DMP, *p*-TSA, ACN, RT, 3 h, 45%; iii. HgO (yellow), $HgCl_2$, acetone/H_2O, 12 h, 55 °C, 98%; iv. *p*-$TsNHNH_2$, MeOH, 12 h, RT, 80%; v. $C_{13}H_{27}MgBr$, Et_2O, 0 °C for 0.5 h, then RT overnight, 52%; vi. BzCl, pyridine, 0 °C – RT, 12 h, 77%; vii. 3:1:1 AcOH-THF-H_2O, 55 °C, 13 h, 89%; viii. AllocCl, 3:2 CH_2Cl_2-pyridine, -10 °C, 3 h, 92%; ix. 3:1:1 AcOH-THF-H_2O, 55 °C, 12 h, 64%.

13 with 3:1:1 AcOH-THF-H_2O mixture at 55 °C[13] produced compound **14** in an optimized 89% yield.[13]

*2.3. Preparation of protected sphingosine acceptor **17***

Compound **14** was protected at the primary hydroxyl position with triisopropylsilyl chloride (TIPSCl) in the presence of imidazole to give silane **15 (Scheme 3)**.[14] A Mitsunobu reaction was employed to install the azide directly at the free secondary hydroxyl position in compound **15** in the presence of diisopropyl azodicarboxylate (DIAD) at 0 °C.[14] The conversion of **15** into azide **16** took place in 2 hours with isolated 92% yield. After the azide group was in place, we attempted to remove the TIPS protecting group using tetrabutylammonium fluoride (TBAF). However, close inspection of the NMR revealed we isolated a rearrangement product, alcohol **22** (Figure S9). Ultimately we found we could remove the TIPS group without rearrangement by treating compound **16** for 24 hours with HF-pyridine [15] to obtain the target sphingosine derivative acceptor **17** in 94% yield.

Scheme 3: Preparation of the sphingosine derivative acceptor



Reagents and Conditions: i. TIPSCl, imidazole, THF, 0 °C, 24 h, 91%; ii. PPh_3, DIAD, HN_3, THF, 4 Å, 0 °C, 2 h, 92%; iii. 1M TBAF, THF, 0 °C – RT, 10 min, 80%; iv. HF-pyridine, THF, RT, 24 h, 94%

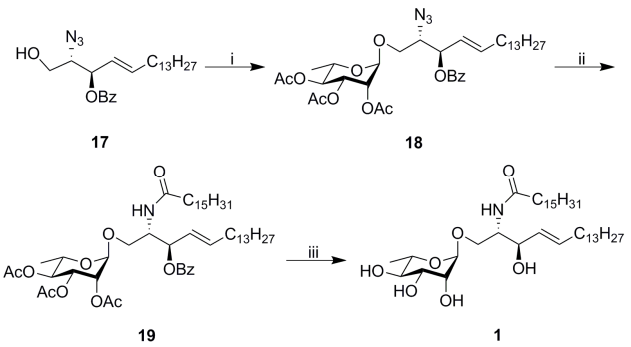
*2.4. Synthesis of α-rhamnosyl ceramide (**1**)*

Initially we attempted a selective glycosylation on partially protected acceptor substrates using thioglycoside **4**; however, complications, discussed-latter, forced us to investigate the use of the glycosyl trichloroacetimidate **6** (**Scheme 4**). Imidate **6** and sphingosine derivative **17** were glycosylated with $BF_3.OEt_2$ promotion within 30 minutes in a very clean and high yielding reaction to afford **18**.[16] The azide group of compound **18** was then subjected to a Staudinger reduction to form the amine. The amine, without purification, was subsequently *N*-acylated with palmitic acid ($C_{15}H_{31}COOH$) in the presence of EDC.HCl, HOBt and Et_3N to give **19** in 84% yield over two steps.[17] Glycoside **19** was treated with sodium methoxide to give the target α-L-RhaCer (**1**).[16]

*2.5. Synthesis of ceramide control **24***

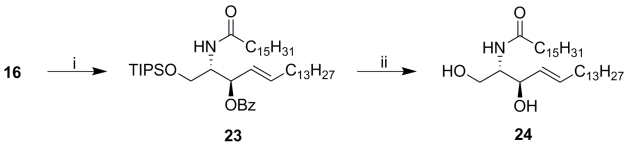
Once α-L-RhaCer (**1**) was in hand, we focused on accessing its des-rhamnosyl analogue **24** using a similar strategy **(Scheme 5)**. Starting with the TIPS protected azide **16**, Staudinger reduction followed by *N*-acylation with palmitic acid in the presence of EDC·HCl, HOBt and Et_3N led to the intermediate **23** in 50% yield over two steps.[16] The TIPS deprotection was achieved in 10 minutes by treating **23** with 1M TBAF at room temperature.[14] The crude product was subjected to a NaOMe-mediated benzoyl deprotection to obtain the target non-rhamnose analogue **24** in 86.5% yield over two steps.[16]

Scheme 4: Synthesis of α-L-rhamnosyl ceramide (**1**)



Reagents and Conditions: i. Donor **6**, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 4 Å MS, -20 °C, 0.5 h, 98.6%; ii. (a) PPh_3, H_2O, THF, 45 °C, 3 h; (b) $C_{15}H_{31}COOH$, EDC·HCl, HOBt, Et_3N, CH_2Cl_2, RT, 10 h, 84% over two steps; iii. NaOMe, MeOH-CH_2Cl_2, pH=9, RT, 10 h, 93%.

Scheme 5: Synthesis of ceramide control **24**

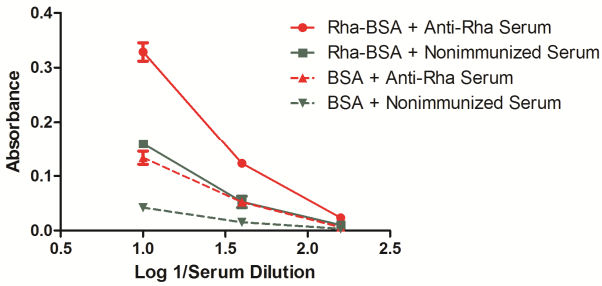


Reagents and Conditions: i. (a) PPh_3, H_2O, THF, 45 °C, 3 h; (b) $C_{15}H_{31}COOH$, EDC·HCl, HOBt, Et_3N, CH_2Cl_2, RT, 10 h, 50% over two steps; ii. (a) 1M TBAF, THF, 0 °C – RT, 10 min; (b) NaOMe, MeOH-CH_2Cl_2, pH=9, RT, 10 h, 86.5% over two steps.

2.6. Anti-rhamnose antibody preparation

In order to prepare anti-rhamnose antibodies, two BALB/cJ mice were immunized and boosted with 1 µg rhamnose ovalbumin conjugate (Rha-Ova)[3a,c] with Sigma Adjuvant System (1:1 ratio). The production of anti-Rha antibody was confirmed by ELISA (**Figure 2**). On day 38 following priming, blood was pooled from the two mice and the sera were isolated. The antibodies were isolated by precipitation from the sera at 40% saturated ammonium sulfate solution (pH ~7) followed by dialysis against phosphate buffered saline (PBS).

Figure 2. Anti-rhamnose antibody titer of Balb/CJ mice immunized with rhamnose ovalbumin conjugate (after 1st boost)



Rha-BSA: Rhamnose conjugated with bovine serum albumin (BSA). Anti-Rha Serum: Serum isolated from mice immunized with rhamnose-ovalbumin. Nonimmunized serum: Serum isolated from nonimmunized mice.

*2.7. Anti-rhamnose antibody binding to surface exposed α-L-RhaCer (**1**) on liposomes*

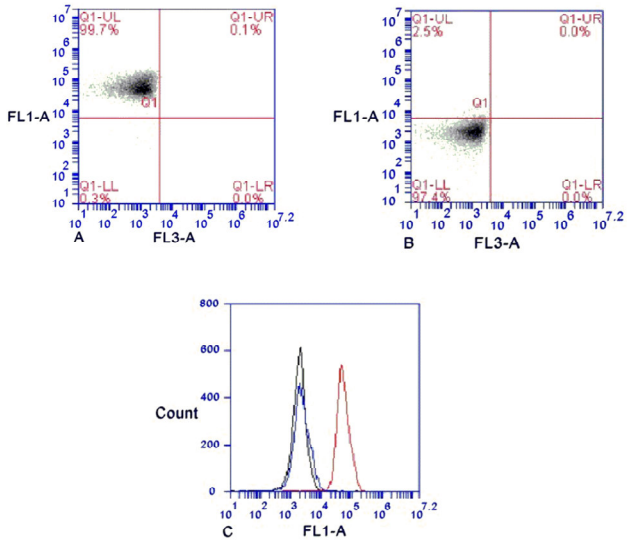
As preliminary model study to verify that synthetic α-L-RhaCer (**1**) can be recognized by anti-rhamnose antibodies, we prepared liposomes of approximately 100 nm in size that display α-L-RhaCer (**1**) or control ceramide **24** on their surface, as a model for a cell membrane. 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) (80%) and cholesterol (20%) were used to prepare the liposome.[3c] The liposomes were formulated by the extrusion method in a total lipid concentration of 30 mM. Three batches of liposomes were prepared, the first displaying the α-L-RhaCer (**1**, 10 nM) and the second displaying the ceramide **24** (10 nM). The third batch of liposomes did not contain any ceramide and was used as a background.

In order to determine if rhamnosyl-ceramide can interact with surface exposed anti-Rha antibodies, liposomes displaying the different ceramides on their surface were incubated with either anti-Rha antibody or antibodies collected from non-immunized mice sera. The resulting complexes were incubated with FITC-conjugated goat anti-mouse IgG/IgM secondary antibody. The fluorescence imaging (**Figure S11**) shows specific binding of rhamnosyl-ceramide displayed on the liposome surface to anti-rhamnose antibodies.

*2.8. Anti-rhamnose antibody binding to surface exposed α-L-RhaCer (**1**) on tumor cells*

Once we demonstrated that the α-L-RhaCer (**1**) displayed on liposome surfaces could successfully bind to anti-rhamnose antibodies, the next step was to see whether it could be recognized on real cancer cells. EL4 tumor cells were used as the model cancer cells. The cells were exposed to either α-L-RhaCer (**1**) or ceramide **24** followed by anti-Rha or non-Rha antibodies. After a final incubation with FITC-conjugated goat anti-mouse IgG/IgM secondary antibody, flow cytometry was performed. The flow data clearly indicates that the synthetic α-L-RhaCer (**1**) can be displayed on tumor cell surfaces and forms a complex with anti-Rha antibodies (Figure 3).

Figure 3. Flow cytometry of EL4 tumor cells



FL1-A = FITC (**A**) EL4 with α-L-RhaCer and anti-rhamnose antibodies (**B**) EL4 with α-L-RhaCer and non-rhamnose antibodies (**C**) Black peak: EL4 cells with α-L-RhaCer and non-Rha antibodies; blue peak: EL4 cells with Cer and anti-Rha antibodies; red peak: EL4 cells with α-L-RhaCer and anti-Rha antibodies.

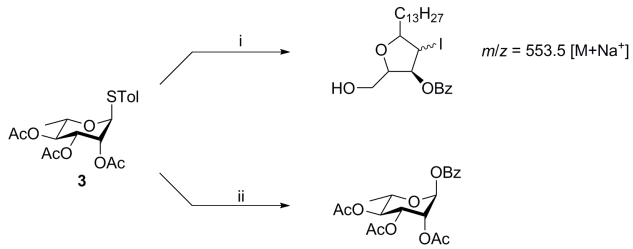
3. Discussion

During the course of the synthesis, accessing the ceramide acceptor by simpler and more expedient methods with higher yields was quite challenging. To transform isopropylidene **9** into the aldehyde **10**, in order to avoid the use of mercury compounds, attempts to remove the thiol protecting groups were explored using I_2, CH_3I and Br_2. However, in each case, the results were unsatisfactory.

As previously noted, issues arose during the deprotection of benzoate **13**. While working through the deprotection issues we also explored a different protecting group strategy for comparison. Rather than benzoyl, we investigated the use of the allyl carbonate (Alloc) group (**Scheme 2**). For the Alloc protection, alkene **12** was protected using allyl chloroformate to afford allyloxycarbonylate **20** in 93% yield.[18] Compound **20** was then subjected to our optimized deprotection conditions utilizing 3:1:1 AcOH-THF-H_2O at 55 °C. However, the only isolable product from this reaction sequence was carbonate **21**. This result led us to maintain the benzoyl protecting group in our synthetic strategy.

Further, a regioselective glycosylation was investigated in order to introduce the rhamnosyl group on the primary hydroxyl of diol **14**. Initially, glycosylation was carried out with thioglycoside **4** using *N*-iodosuccinimide (NIS) and trimethylsilyl trifluoromethanesulfonate (TMSOTf) to promote the reaction.[19] These reaction conditions led to the formation of a cyclic ether adduct with mass spectral data corresponding to the compound shown in **Scheme 6**. To avoid the use of the electrophilic iodine reagent, dimethyl(methylthio)sulfonium triflate (DMTST) was also explored as a glycosyl promoter. Spectral data showed that these conditions resulted in an anomerically benzoylated rhamnose as the major product as shown in **Scheme 6**. No isolatable desired product was formed from this procedure. These results drove us to both reconsider the selective glycosylation as well as the use of the thioglycoside donor **4**. Thus, we prepared the glycosyl donor **6** to avoid the problems created by the *S*-tolyl donor. To modify the acceptor, compound **14** was protected at the primary hydroxyl position with triisopropylsilyl chloride (TIPSCl) to afford silane **15** as noted in Scheme 4.

Scheme 6: Attempted glycosylation of *S*-tolyl donor and sphingosine acceptor.



Reagents and Conditions: i. Acceptor **14**, NIS, TMSOTf, 4 Å MS, -45 °C; ii. Acceptor **14**, DMTST, CH_2Cl_2, 4 Å MS, 0 °C.

The next step was forming the azide at the free secondary hydroxyl position. Originally, our approach consisted of mesylation followed by the backside attack of the azide, to our chagrin the conditions for these steps were sluggish and it had been reported to require an overnight reaction to form the mesylate followed by a 4 day reaction at 95 °C to install the azide for a similar compound.[17] Also, another group has reported that this procedure led to no conversion at low temperature and compound decomposition at high temperatures.[14] Therefore, an

alternate approach was taken to directly install the azide via the Mitsunobu reaction.[14] After the azide installation, the TIPS deprotection was attempted with 1M TBAF and the reaction was completed in less than 10 minutes. But, after a close examination of the spectral data, it was found that the benzoyl group had migrated to the primary position aided by the strongly basic fluorinating agent to afford compound **22** as the major product. Although interesting, this result was undesirable and a different approach was taken using HF-pyridine.[15] HF-pyridine led to the desired **17** in 94% yield; however, the reaction time increased significantly to 24 hours. This procedure still produced compound **22** but in negligible amounts (13.3:1 desired to rearranged ratio as opposed to 1:4 with TBAF). However, TBAF worked well in the course of obtaining **24** from **23** (**Scheme 5**).

Interpretation of the binding studies is straightforward. Only cells exposed to α-L-RhaCer (**1**) bound anti-Rha antibodies while the antibodies did not bind to cells exposed to control ceramide **24**. It should be noted that initial experimental conditions containing lipid bind proteins prevented the coating of tumor cells with our lipids. Switching to alternate culture media resolved this problem.

4. Conclusion

We have successfully synthesized α-L-RhaCer (**1**) via an efficient synthetic route, taking time to optimize the synthesis of several key intermediates. We have also demonstrated the incorporation of α-L-RhaCer (**1**) on the surface of a liposomal phospholipid bilayer and demonstrate that anti-Rha antibodies recognize the Rha motif. We then demonstrated that the same lipid could coat or label a model tumor cell and those cells bind anti-Rha antibodies. Thus, we hypothesize/speculate that the reinjection of these modified tumor cells, after inactivation such as freeze-thaw cycles, back into animals would produce an antitumor immune response in an EL4 mouse tumor model mediated through anti-Rha antibodies and FcγRs on APCs.

5. Experimentals

5.1. Materials

All fine chemicals such as L-rhamnose monohydrate, D-(+)-xylose, $BF_3{\cdot}Et_2O$, *p*-thiocresol, *p*-toluenesulfonic acid monohydrate, acetic anhydride, pyridine, anhydrous solvents such as anhydrous methanol and *N*,*N*-dimethylformamide were purchased from Acros and used without further purification. Tridecyl bromide was acquired from Sigma-Aldrich. Trifluoroacetic acid, sodium bicarbonate, common solvents such as hexanes, ethyl acetate, acetone, and acetonitrile were purchased from Fisher. Acetonitrile and acetone were dried following standard procedures and distilled before use in reactions. Mercuric oxide was obtained from Hawaii Chemical and Scientific. Silica (230-400 mesh) for column chromatography was obtained from Sorbent Technologies; thin-layer chromatography (TLC) precoated plates were from EMD. TLCs (silica gel 60, f_{254}) were visualized under UV light or by charring (5% H_2SO_4 – MeOH). Flash column chromatography was performed on silica gel (230-400 mesh) using solvents as received. ^{1}H NMR were recorded either on INOVA 600 MHz spectrometer in $CDCl_3$, CD_3OD or DMSO-d_6 using residual undeuterated solvent or TMS as the internal reference. ^{13}C NMR were recorded on a VXRS 400, INOVA 600 or AVANCE 600. High resolution mass spectrometry (HRMS) was performed on a micro mass Q-TOF2 instrument. Low resolution mass spectra were taken on Esquire-LC electrospray ionization (ESI) mass spectrometer operated in the positive ion mode. Fluorescence microscopy was performed on a Nikon TiU microscope. Flow cytometry was performed in BD Accuri C6 flow cytometer. 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) was obtained from Avanti Polar Lipids. Ovalbumin and horseradish peroxidase (HRP) goat anti-mouse IgG/ IgM were purchased from Sigma. FITC goat anti-mouse IgG/IgM was acquired from BD-Biosciences (San Jose, CA). Female, 6-8 week old BALB/cJ mice were obtained from the Jackson Laboratory (Bar Harbor, ME) and maintained in the Animal Care Facility of the University of Toledo. All animal care procedures and experimental protocols have been approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Toledo. C57BL/6 EL4 lymphoma cells were from the ATCC (TIB-39).

5.2. Synthesis

5.2.1. 1,2,3,4-*O*-Tetraacetyl-α,β-L-rhamnopyranoside (3).[6] L-rhamnose monohydrate (2.50 g, 13.7 mmol) and a catalytic amount of 4-dimethylaminopyridine were dissolved in anhydrous pyridine (8.9 mL) followed by the addition of acetic anhydride (10.4 mL, 110 mmol). The solution was stirred for 16 hours at room temperature under N_2 atmosphere. Upon completion, the solution was concentrated and the excess pyridine was co-evaporated with toluene (4x). The crude product was dissolved in ethyl acetate and washed with 1.0 N HCl (2x), followed by sat. aq. $NaHCO_3$ (2x), and then brine (2x). The organic layer was dried ($MgSO_4$), concentrated and co-evaporated with toluene (3x). The product was dried under reduced pressure to provide a product as a colorless glass. Yield = 4.57 g (100%); mass spectrum (ESIMS), *m/z* = 355.3 $[M+Na]^+$ $C_{14}H_{20}O_9Na$ requires 355.3.

5.2.2. 2,3,4-Tri-*O*-acetyl-1-(4-tolyl)thio-α-L-rhamnopyranoside (4).[6] 1,2,3,4-*O*-Tetraacetyl-α,β-L-rhamnopyranoside (3.66 g, 11.0 mmol) and *p*-thiocresol (1.78 g, 14.3 mmol) were dissolved in dry CH_2Cl_2 (15 mL). The solution was stirred under N_2 atmosphere and cooled to 0 °C followed by the drop-wise addition of $BF_3{\cdot}OEt_2$ (1.88 mL, 15.2 mmol). The solution was stirred at room temperature for 18 hours. The reaction was monitored by thin layer chromatography using 1:1 ethyl acetate-hexanes. Upon completion, the crude product was diluted with CH_2Cl_2 (30 mL) and washed with sat. aq. $NaHCO_3$ (2x) followed by water. The organic layer was dried (Na_2SO_4), concentrated, and crystallized from hot ethyl acetate/hexanes. The product was recrystallized from hot ethyl acetate/hexanes, filtered, washed with cold hexanes, and then dried under reduced pressure to provide an off-white solid. Yield = 2.124 (52.4%); R_f = 0.68 (1:1 ethyl acetate-hexanes); m.p. = 116.5-117.5 °C; mass spectrum (ESIMS), *m/z* = 435.5 $[M+K]^+$ $C_{19}H_{24}O_7SK$ requires 435.5. (3.66 g, 11.0 mmol) and *p*-thiocresol (1.78 g, 14.3 mmol) were dissolved in dry CH_2Cl_2 (15 mL). The solution was stirred under N_2 atmosphere and cooled to 0 °C followed by the drop-wise addition of $BF_3{\cdot}OEt_2$ (1.88 mL, 15.2 mmol). The solution was stirred at room temperature for 18 hours. The reaction was monitored by thin layer chromatography using 1:1 ethyl acetate-hexanes. Upon completion, the crude product was diluted with CH_2Cl_2 (30 mL) and washed with sat. aq. $NaHCO_3$ (2x) followed by water. The organic layer was dried (Na_2SO_4), concentrated, and crystallized from hot ethyl acetate/hexanes. The product was recrystallized from hot ethyl acetate/hexanes, filtered, washed with cold hexanes, and then dried under reduced pressure to provide an off-white solid. Yield = 2.124 (52.4%); R_f = 0.68 (1:1 ethyl acetate-hexanes); m.p. = 116.5-117.5 °C; mass spectrum (ESIMS), *m/z* = 435.5 $[M+K]^+$ $C_{19}H_{24}O_7SK$ requires 435.5.

5.2.3. 2,3,4-*O*-Triacetyl-α,β-L-rhamnopyranoside (5).[7] In a round bottom flask, 1,2,3,4-*O*-tetraacetyl-α,β-L-

rhamnopyranoside (4.56 g, 13.7 mmol) was dissolved in DMF (50 mL) and hydrazine acetate (1.42 g, 15.4 mmol) was added. The reaction was heated to 55 °C and stirred under N_2 atmosphere for 4 hours. The reaction was monitored by thin layer chromatography using 1:1 hexanes-ethyl acetate. Upon completion, the reaction mixture was cooled to room temperature, quenched with sat. aq. $NaHCO_3$, extracted with ethyl acetate (3x), and filtered through Celite. The filtrate was washed with H_2O (2x) and then brine, dried (Na_2SO_4), filtered, co-evaporated with toluene (2x) and concentrated under reduced pressure. The concentrated crude product was then recrystallized from $CHCl_3$-hexanes to provide a white amorphous solid. Yield = 3.61 g (90.7%); TLC R_f = 0.50 (1:1 hexanes-ethyl acetate); ^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.23 (d, J = 6.0 Hz, 3H, CH_3), 2.00 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), 2.17 (s, 3H, CH_3), 3.06 (s, 1H, OH), 4.14 (m, 1H, H-5), 5.09 (dd, 1H, H-4, J = 10.2, 10.2 Hz), 5.18 (m, 1H, H-1), 5.28 (d, J = 1.8 Hz, 1H, H-2), 5.38 (dd, J = 3.0, 10.2 Hz, 1H, H-3); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 17.7, 21.0, 21.1, 21.2, 66.6, 69.0, 70.4, 71.3, 92.4, 170.3, 170.5, 170.6; Mass spectrum (HRMS), m/z = 313.0884 $[M+Na]^+$ $C_{25}H_{44}O_5Na$ requires 313.0899.

5.2.4. 2,3,4-*O*-Triacetyl-α-L-rhamnopyranoside Trichloroacetimidate (6).[7] In a round bottom flask, 2,3,4-*O*-triacetyl-α,β-L-rhamnopyranoside (0.203 g, 0.792 mmol) was dissolved in dry CH_2Cl_2 (5 mL) and then cooled to 0 °C. After cooling, DBU (15.39 µL, 0.103 mmol) and trichloroacetonitrile (93.0 µL, 0.927 mmol) were added and the reaction was allowed to warm to room temperature and stirred under N_2 atmosphere for 12 hours. The reaction was monitored by thin layer chromatography using 1:1 hexanes-ethyl acetate. Upon completion, the reaction mixture was diluted with CH_2Cl_2, washed with brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The concentrated crude product was loaded onto silica gel and the remaining solvent was evaporated under reduced pressure. The dry slurry was purified by flash column chromatography (15 x 2.5 cm) on a silica gel (230 x 400 mesh) eluting with 2:1 hexanes-ethyl acetate. The product fractions were combined, concentrated, and then dried under reduced pressure to provide **6** as a colorless oil. Yield = 0.295 g (85.7%); TLC R_f = 0.58 (1:1 hexanes-ethyl acetate); Mass spectrum (ESIMS), m/z = 457.8 $[M+Na]^+$ $C_{14}H_{18}Cl_3NO_8Na$ requires 457.7.

5.2.5. D-xylose di(*p*-tolyl) dithioacetal (8). D-(+)-Xylose (1.00 g, 6.66 mmol) and *p*-thiocresol (1.74 g, 14.0 mmol) were added portion-wise to vigorously stirred 90% trifluoroacetic acid (5 mL). An air-cooled reflux condenser was attached to the flask and the solution was heated to 50-60 °C under N_2 atmosphere until all starting materials dissolved. After complete dissolution, the flask was removed from heat and allowed to stir for 30 minutes. The reaction was monitored by thin layer chromatography using 1:1:2 ethyl acetate-acetone-hexanes. Upon completion, the solution was concentrated under reduced pressure at 40 °C. The concentrated crude product was loaded onto silica gel and any remaining solvent was evaporated under reduced pressure. The dry slurry was purified by flash column chromatography (16 x 3 cm) on silica gel (230 x 400 mesh) eluting with 1:1:2 ethyl acetate-acetone-hexanes. The product fractions were combined, concentrated under reduced pressure, and then dried under reduced pressure to provide **8** as a yellow syrup. Yield = 1.96 g (77.4 %); R_f = 0.25 (1:1:2 ethyl acetate-acetone-hexanes); ^{1}H NMR ($CDCl_3$, 600 MHz): δ 2.28 (br.s, 6H, 2 CH_3), 3.39-3.62 (hump, 4H, 4 OH), 3.68 (m, 3H, H-2, H-3, H-5), 3.79 (m, 1H, H-5), 4.21 (m, 1H, H-4), 4.51 (d, 1H, H-1, J = 7.8 Hz), 7.06 (d.d, 4H, Ph, J = 5.4, 7.8 Hz), 7.28 (d, 2H, Ph, J = 7.8 Hz), 7.33 (d, 2H, Ph, J = 8.4 Hz); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 21.3, 63.8, 64.1, 70.7, 73.3, 73.7, 128.9, 129.8, 130.0, 133.5, 134.0, 138.3, 138.6; mass spectrum (HRMS), m/z = 403.1021 $[M+Na]^+$ $C_{19}H_{24}O_4S_2Na$ requires 403.1014.

5.2.6. 2,3:4,5-Di-*O*-isopropylidene-1,1-di(*p*-tolyl)-dithioacetal-D-xylose (9). D-(+)-Xylose (3.0 g, 19.98 mmol) and *p*-thiocresol (5.211 g, 41.96 mmol) were added portion-wise to vigorously stirred 90% trifluoroacetic acid (15 mL). An air-cooled reflux condenser was attached to the flask and the solution was heated to 50-60 °C under N_2 atmosphere until all sugar dissolved. After complete dissolution, the flask was removed from heat and stirred for 30 minutes. The reaction was monitored by thin layer chromatography using 1:1:2 ethyl acetate-acetone-hexanes. Upon completion, the solution was concentrated under reduced pressure, co-evaporated with toluene (3x), and then dried under reduced pressure to give a yellow syrup. The crude syrup was dissolved in dry acetonitrile (60 mL). After dissolution, 2,2-dimethoxypropane (10.16 mL, 81.92 mmol) and a catalytic amount of *p*-toluenesulfonic acid were added to the solution. The solution was stirred at room temperature under N_2 atmosphere for 3.5 hours. The reaction was monitored by thin layer chromatography using 4:1 hexanes-ethyl acetate. Upon completion, the solution was neutralized with 30% NH_4OH and then concentrated under reduced pressure. The crude product was taken up in CH_2Cl_2 and washed with water. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (3x). The organic extracts were combined and washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. The concentrated crude product was loaded onto silica and any remaining solvent was evaporated under reduced pressure. The dry slurry was purified by flash column chromatography (18 x 2 cm) on silica gel (230 x 400 mesh). Elution was with 19:1 hexanes-ethyl acetate. The product fractions were combined, concentrated, and then dried under reduced pressure to provide a colorless oil. Yield = 4.206 g (45.7% over two steps); R_f = 0.61 (4:1 hexanes-ethyl acetate); ^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.37 (s, 6H, 2 CH_3), 1.46 (s, 3H, CH_3), 1.53 (s, 3H, CH_3), 2.32 (s, 6H, 2 CH_3), 3.84 (d.d, 1H, H-5, J = 7.8**,** 7.8 Hz), 3.95 (d.d, 1H, H-5, J = 6.6, 7.8 Hz), 4.28-4.32 (m, 2H, H-4, H-3), 4.40-4.43 (m, 2H, H-2, H-1), 7.09 (d, 4H, Ph, J = 8.4), 7.34 (d, 4H, Ph, J = 8.4); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 21.1, 25.6, 26.0, 27.2, 62.4, 65.6, 75.5, 78.2, 78.7, 109.5, 110.4, 129.7, 129.72, 130.2, 130.4, 133.2, 133.4, 138.1, 138.2; mass spectrum (HRMS), m/z = 483.1638 $[M+Na]^+$ $C_{25}H_{32}O_4S_2Na$ requires 483.1640.

5.2.7. 2,3:4,5-Di-*O*-isopropylidene-D-xylose (10).[10] In a round bottom flask, 2,3:4,5-di-*O*-isopropylidene-1,1-di(*p*-tolyl)-dithioacetal-D-xylose (1.97 g, 4.27 mmol) was dissolved in acetone (9.0 mL) and water (0.9 mL). Subsequently, yellow HgO (2.96 g, 13.65 mmol) followed by $HgCl_2$ (2.896 g, 10.665 mmol) were added to the solution with vigorous stirring. The reaction mixture was refluxed at 55 °C for 12 hours. The reaction was monitored by thin layer chromatography using 4:1 hexanes-ethyl acetate. Upon consumption of all starting material, the reaction mixture was filtered and then concentrated under reduced pressure. The resulting residue was taken up in $CHCl_3$ and filtered into a separatory funnel. The organic layer was washed with a 1 *N* KI solution (2x), water (2x), dried ($MgSO_4$), and then evaporated under reduced pressure to give an off-white semisolid. Yield = 0.963 g (98.1%).

5.2.8. 2,3:4,5-Di-*O*-isopropylidene-D-xylose tosylhydrazone (11).[11] In a round bottom flask, 2,3:4,5-di-*O*-isopropylidene-D-xylose (2.85 g, 12.4 mmol) and *p*-toluenesulfonylhydrazide (2.31 g, 12.4 mmol) were dissolved in anhydrous methanol (6.5 mL). The solution was stirred at room temperature under N_2 atmosphere for 8 hours. The reaction was monitored by thin layer

chromatography using 1:1 hexanes-ethyl acetate. Upon completion, the solution was concentrated under reduced pressure and then triturated with hexanes. The solid was filtered, washed with cold Et_2O, and then dried under reduced pressure to give an off-white solid. Yield = 4.074 g (82.6%); R_f = 0.44 (1:1 hexanes-ethyl acetate); mass spectrum (ESIMS), *m/z* = 421.6 $[M+Na]^+$ $C_{18}H_{26}N_2O_6SNa$ requires 421.5.

5.2.9. (2*R*, 3*R*, 4*E*)-1,2-*O*-isopropylideneoctadec-4-ene-1,2,3-triol (12).[11] In a two-neck round bottom flask, Mg metal turnings (0.210 g, 8.64 mmol) were moistened with a small amount of anhydrous Et_2O. In a separate pierce flask, tridecyl bromide (1.84 mL, 7.2 mmol) was dissolved in anhydrous Et_2O (10 mL) and added drop-wise to the Mg turnings until the reaction had begun. The remaining tridecyl bromide was added drop-wise to give a constant reflux. As the Grignard reaction was taking place, anhydrous Et_2O (10 mL) was added to 2,3:4,5-di-*O*-isopropylidene-D-xylose tosylhydrazone (0.465 g, 1.17 mmol) in a separate flask to give a stirred suspension, which was then cooled to 0 °C. After the majority of the Mg metal had been consumed, the tridecylmagnesium bromide solution was added drop-wise to the tosylhydrazone suspension. The resulting mixture was stirred at 0 °C under N_2 atmosphere for 30 minutes and then allowed to warm to room temperature and stirred overnight. The reaction was monitored by thin layer chromatography using 1:1 ethyl acetate-hexanes. Upon completion, the solution was quenched with 1 *M* NH_4Cl, filtered, and concentrated under reduced pressure. The concentrated crude product was loaded onto silica and any remaining solvent was evaporated under reduced pressure. The dry slurry was purified by flash column chromatography (15 x 2 cm) on silica gel (230 x 400 mesh). Elution was with 19:1 hexanes-ethyl acetate. The product fractions were combined, concentrated, and then dried under reduced pressure to provide a colorless oil. Yield = 0.204 g (51.4%); TLC R_f = 0.71 (1:1 hexanes-ethyl acetate); Mass spectrum (ESIMS), *m/z* = 363.7 $[M+Na]^+$ $C_{21}H_{40}O_3Na$ requires 363.54.

5.2.10. (2*R*, 3*R*, 4*E*)-3-*O*-Benzoyloxy-1,2-*O*-isopropylideneoctadec-4-ene-1,2-diol (13).[12] In a round bottom flask, (2*R*, 3*R*, 4*E*)-1,2-*O*-isopropylideneoctadec-4-ene-1,2,3-triol (0.460 g, 1.35 mmol) was dissolved in anhydrous pyridine (8 mL) and cooled to 0 °C. After cooling, benzoyl chloride (0.251 mL, 2.16 mmol) was added drop-wise. The solution was then allowed to warm to room temperature and stirred at that temperature for 12 hours under N_2 atmosphere. The reaction was monitored by thin layer chromatography using 4:1 hexanes-ethyl acetate. Upon completion, the reaction was quenched with MeOH and concentrated under reduced pressure. The residue was taken up in CH_2Cl_2 and washed with water (3x) followed by brine. The organic phase was separated, dried (Na_2SO_4), and co-evaporated with toluene (3x). The concentrated crude product was loaded onto silica and any remaining solvent was evaporated under reduced pressure. The dry slurry was purified by flash column chromatography (18 x 3 cm) on silica gel (230 x 400 mesh). Elution was with 49:1→24:1 hexanes-ethyl acetate. The product fractions were combined, concentrated, and then dried under reduced pressure to provide a colorless oil. Yield = 0.460 g (76.6%); TLC R_f = 0.62 (4:1 hexanes-ethyl acetate); Mass spectrum (ESIMS), *m/z* = 467.7 $[M+Na]^+$ $C_{28}H_{44}O_4Na$ requires 467.7.

5.2.11. (2*R*, 3*R*, 4*E*)-3-*O*-Benzoyloxyoctadec-4-ene-1,2-diol (14).[13] In a round bottom flask, (2*R*, 3*R*, 4*E*)-3-*O*-benzoyloxy-1,2-*O*-isopropylideneoctadec-4-ene-1,2-diol (0.080 g, 0.18 mmol) was dissolved in a 1:1 THF:H_2O mixture (0.6 mL) and cooled to 0 °C. After cooling, glacial acetic acid (0.9 mL) was added drop-wise. The solution was then heated to 55 °C and stirred for 12 hours with a water-cooled reflux condenser. The reaction was monitored by thin layer chromatography using 4:1 hexanes-ethyl acetate. Upon completion, the reaction was quenched with saturated aq. $NaHCO_3$ and extracted with ethyl acetate (3x). The extracts were combined, dried ($MgSO_4$), and concentrated under reduced pressure. The concentrated crude product was loaded onto silica and any remaining solvent was evaporated under reduced pressure. The dry slurry was purified by flash column chromatography (10 x 1 cm) on silica gel (230 x 400 mesh). Elution was with 9:1→4:1 hexanes-ethyl acetate. The product fractions were combined, concentrated, and then dried under reduced pressure to provide an off-white residue. Yield = 0.065 g (89.3%); TLC R_f = 0.18 (4:1 hexanes-ethyl acetate); Mass spectrum (ESIMS), *m/z* = 427.8 $[M+Na]^+$ $C_{25}H_{40}O_4Na$ requires 427.6.

5.2.12. (2*S*, 3*R*, 4*E*)-3-*O*-Benzoyloxy-1-*O*-triisopropylsilyloxy-octadec-4-ene-2-ol (15). In a round bottom flask, 3-*O*-benzoyloxyoctadec-4-ene-1,2-diol (0.295 g, 0.729 mmol) was dissolved in dry THF (5 mL). After dissolution, imidazole (0.065 g, 0.948 mmol) was added and the reaction cooled to 0 °C. After cooling, triisopropylsilyl chloride (0.172 mL, 0.802 mmol) was added drop-wise. The solution was allowed to warm to room temperature and stirred at that temperature for 24 hours under N_2 atmosphere. The reaction was monitored by thin layer chromatography using 3:1 hexanes-ethyl acetate. Upon completion, the reaction was quenched with saturated aq. NH_4Cl and diluted with ethyl acetate. The aqueous layer was separated and extracted with ethyl acetate (3x). The extracts were combined, dried (Na_2SO_4), and concentrated under reduced pressure. The concentrated crude product was loaded onto silica and any remaining solvent was evaporated under reduced pressure. The dry slurry was purified by flash column chromatography (18 x 2 cm) on silica gel (230 x 400 mesh). Elution was with 3% ethyl acetate in hexanes. The product fractions were combined, concentrated, and then dried under reduced pressure to provide a colorless oil. Yield = 0.327 g (82.0%); TLC R_f = 0.81 (3:1 hexanes-ethyl acetate); ^{1}H NMR ($CDCl_3$, 600 MHz): δ 0.89 (t, 3H, CH_3, *J* = 6.6 Hz), 1.03-1.14 (m, 22H, 11CH_2), 1.25 (m, 18 H, 6 CH_3), 1.37 (m, 3H, 3 C*H*(CH_3)$_2$), 2.06 (m, 2H, CH=CHC*H*$_2$), 2.68 (br. s, 1H, OH), 3.79 (m, 1H, C*H*OH), 3.84 (m, 2H, SiOC*H*$_2$), 5.61 (m, 2H, C*H*OBz, C*H*=CHCH$_2$), 5.93 (d.t, 1H, CH=C*H*CH$_2$, *J* = 7.8, 14.5 Hz), 7.43 (t, 2H, Ph, *J* = 7.2 Hz), 7.54 (t, 1H, Ph, *J* = 7.8 Hz), 8.08 (d, 2H, Ph, *J* = 7.2); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 12.1, 12.5, 14.3, 17.8, 17.9, 18.1, 22.9, 29.0, 29.4, 29.6, 29.7, 29.8, 29.86, 29.9, 32.1, 32.6, 64.0, 73.5, 76.0, 124.7, 128.4, 129.8, 130.7, 133.0, 137.3, 166.0; Mass spectrum (HRMS), *m/z* = 583.4160 $[M+Na]^+$ $C_{34}H_{60}O_4SiNa$ requires 583.4159.

5.2.13. (2*R*, 3*R*, 4*E*)-2-Azido-3-*O*-benzoyloxy-1-*O*-triisopropylsilyloxy-octadec-4-ene (16). In a round bottom flask, sodium azide (3.25 g) was stirred in 3.25 mL of water and 20 mL of toluene. The suspension was cooled to 0 °C and concentrated H_2SO_4 (0.675 mL) was added drop-wise. The reaction mixture was stirred slowly for 30 minutes and then the toluene layer was decanted into a cooled conical flask in ice, dried over sodium sulfate, and stored in a freezer. To determine the concentration, 0.3 mL of the solution and 0.3 mL of water were stirred in a vial and one drop of a phenolphthalein-solution (1% in MeOH) was added, and the solution was titrated using NaOH (standardized to 0.249 *N*) to give a calculated 0.852 *N* HN_3 solution. In a round bottom flask, triphenylphosphine (275 mg, 1.05 mmol) was dissolved in dry THF (7.5 mL) with crushed 4 Å molecular sieves under N_2 atmosphere and the solution was cooled to 0 °C. DIAD (0.208 mL, 1.05 mmol) and 1.23 mL of the

previously prepared HN_3 solution were added. After stirring for 1 min, a solution of (2*S*, 3*R*, 4*E*)-3-*O*-benzoyloxy-1-*O*-triisopropylsilyloxy-octadec-4-ene-2-ol (0.147 mg, 0.262 mmol) in THF (4 mL) was added rapidly. The reaction was allowed to come to room temperature and stirred overnight at that temperature. The reaction was monitored by thin layer chromatography using 4:1 hexanes-ethyl acetate. Upon completion, the reaction was quenched with MeOH, filtered through Celite, and concentrated under reduced pressure. The concentrated crude product was loaded onto silica and any remaining solvent was evaporated under reduced pressure. The dry slurry was purified by flash column chromatography (18 x 3 cm) on silica gel (230 x 400 mesh). Elution was with 2→3% ethyl acetate in hexanes. The product fractions were combined, concentrated, and then dried under reduced pressure to provide a colorless oil. Yield = 0.141 g (91.9%); TLC R_f = 0.81 (4:1 hexanes-ethyl acetate); ^{1}H NMR ($CDCl_3$, 600 MHz): δ 0.90 (t, 3H, CH_3, *J* = 6.6 Hz), 1.04-1.16 (m, 22H, $11CH_2$), 1.27 (m, 18 H, 6 CH_3), 1.41 (m, 3H, 3 C*H*$(CH_3)_2$), 2.10 (m, 2H, CH=CHCH_2), 3.80-3.86 (m, 3H, C*H*N_3, SiOCH_2), 5.61 (d.d, 1H, C*H*=CHCH_2, *J* = 8.4, 15.6 Hz), 5.69 (m, 1H, C*H*OBz), 5.96 (d.t, 1H, CH=C*H*CH_2, *J* = 7.2, 14.5 Hz), 7.46 (t, 2H, Ph, *J* = 7.2 Hz), 7.57 (t, 1H, Ph, *J* = 7.2 Hz), 8.08 (d, 2H, Ph, *J* = 8.4); ^{13}C NMR ($CDCl_3$, 400 MHz): δ12.1, 14.3, 17.8, 17.9, 18.1, 22.9, 29.0, 29.3, 29.6, 29.7, 29.75, 29.8, 29.89, 29.9, 32.1, 32.6, 63.5, 66.3, 74.7, 74.8, 123.4, 123.45, 128.6, 129.9, 130.3, 133.3, 138.67, 138.71, 165.4; Mass spectrum (HRMS), *m/z* = 608.4202 $[M+Na]^+$ $C_{34}H_{59}N_3O_3SiNa$ requires 608.4223.

5.2.14. (2*S*, 3*R*, 4*E*)-2-Azido-3-*O*-benzoyloxy-octadec-4-ene-1-ol (17).[15] In a polypropylene tube, (2*R*, 3*R*, 4*E*)-2-azido-3-*O*-benzoyloxy-1-*O*-triisopropylsilyloxy-octadec-4-ene (59.0 mg, 0.101 mmol) was dissolved in THF (2.5 mL). After dissolution, HF-pyridine (120 µL) was added drop-wise and the reaction was stirred at room temperature under N_2 atmosphere for 24 hours. The reaction was monitored by thin layer chromatography using 4:1 hexanes-ethyl acetate. Upon completion, the reaction mixture was slowly poured into saturated aq. $NaHCO_3$ and extracted with CH_2Cl_2 (4x). The extracts were combined, washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. The concentrated crude product was loaded onto silica and any remaining solvent was evaporated under reduced pressure. The dry slurry was purified by flash column chromatography (15 x 1.5 cm) on silica gel (230 x 400 mesh). Elution was with 7% ethyl acetate in hexanes. The product fractions were combined, concentrated, and then dried under reduced pressure to provide a colorless oil. Yield = 34.5 mg (94.0%); TLC R_f = 0.36 (4:1 hexanes-ethyl acetate); Mass spectrum (ESIMS), *m/z* = 452.2871 $[M+Na]^+$ $C_{25}H_{39}N_3O_3Na$ requires 452.2889.

5.2.15. (2*S*, 3*R*, 4*E*)-2',3',4'-Tri-*O*-acetyl-α-L-rhamnopyranosyl-(1'→1)-2-azido-3-*O*-benzoyloxy-octadec-4-ene-1-ol (18).[16] In a round bottom flask, (2*S*, 3*R*, 4*E*)-2-azido-3-*O*-benzoyloxy-octadec-4-ene-1-ol (41.0 mg, 0.095 mmol) and 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranose trichloroacetimidate (54.0 mg, 0.124 mmol) were dissolved in dry CH_2Cl_2 (3 mL) under N_2 atmosphere and stirred with crushed 4Å molecular sieves for 30 minutes. The reaction mixture was then cooled to -20 °C and $BF_3{\cdot}OEt_2$ (11.8 µL, 0.0954 mmol) was added drop-wise. The reaction mixture was stirred at this temperature for 30 minutes. The reaction was monitored by thin layer chromatography using 4:1 hexanes-ethyl acetate. Upon completion, the reaction mixture was quenched with Et_3N and filtered through Celite. The filtrate was concentrated and loaded onto silica and any remaining solvent was evaporated under reduced pressure. The dry slurry was purified by flash column chromatography (15 x 2 cm) on silica gel (230 x 400 mesh). Elution was with 17% ethyl acetate in hexanes. The product fractions were combined, concentrated, and then dried under reduced pressure to provide a colorless oil. Yield = 66.96 mg (98.6%); TLC R_f = 0.33 (4:1 hexanes-ethyl acetate); Mass spectrum (ESIMS), *m/z* = 724.2 $[M+Na]^+$ $C_{37}H_{55}N_3O_{10}Na$ requires 724.4.

5.2.16. (2*S*, 3*R*, 4*E*)-2',3',4'-Tri-*O*-acetyl-α-L-rhamnopyranosyl-(1'→1)-2-(hexadecaneoylamido)-3-*O*-benzoyloxy-octadec-4-ene-1-ol (19).[17] In a round bottom flask, (2*S*, 3*R*, 4*E*)-2',3',4'-Tri-*O*-acetyl-α-L-rhamnopyranosyl-(1'→1)-2-azido-3-*O*-benzoyloxy-octadec-4-ene-1-ol (53.0 mg, 0.0755 mmol) was dissolved in THF (2 mL). After dissolution, triphenylphosphine (59.43 mg, 0.2266 mmol) and H_2O (27 µL) were added, and the reaction was heated to 45 °C with a cold-water condenser under N_2 atmosphere. The reaction was stirred at this temperature for 4 hours. The reaction was monitored by thin layer chromatography using 3:1 hexanes-ethyl acetate. Upon completion, the reaction mixture was quenched with Et_3N and filtered through Celite. The filtrate was concentrated and co-evaporated with toluene (1x) and used for further reaction without purification. The crude product was dissolved in dry CH_2Cl_2. After dissolution, HOBt (15.03 mg, 0.0982 mmol), EDC·HCl (18.82 mg, 0.0982 mmol), palmitic acid (19.36 mg, 0.0755 mmol), and Et_3N (15.80 µL, 0.1133 mmol) were added to the solution. The reaction mixture was stirred under N_2 atmosphere at room temperature for 10 hours. The reaction was monitored by thin layer chromatography using (2:1 hexanes-ethyl acetate). After completion, the solution was concentrated under reduced pressure. The concentrated crude product was loaded onto silica and any remaining solvent was evaporated under reduced pressure. The dry slurry was purified by flash column chromatography (16 x 2.5 cm) on silica gel (230 x 400 mesh). Elution was with 15→25% ethyl acetate in hexanes. The product fractions were combined, concentrated, and then dried under reduced pressure to provide a colorless gel. Yield = 58.0 mg (84.1% over two steps); TLC R_f = 0.29 (2:1 hexanes-ethyl acetate); Mass spectrum (ESIMS), *m/z* = 937.0 $[M+Na]^+$ $C_{53}H_{87}NO_{11}Na$ requires 937.2.

5.2.17. (2*S*, 3*R*, 4*E*)-α-L-Rhamnopyranosyl-(1'→1)-2-(hexadecaneoylamido)-4-octadecene-1,3-diol (1).[16] In a round bottom flask, (2*S*, 3*R*, 4*E*)-2',3',4'-Tri-*O*-acetyl-α-L-rhamnopyranosyl-(1'→1)-2-(hexadecaneoylamido)-3-*O*-benzoyloxy-octadec-4-ene-1-ol. (41.0 mg, 0.0954 mmol) was dissolved in a dry 2:1 MeOH:CH_2Cl_2 mixture (6 mL) under N_2 atmosphere. After dissolution, sodium metal was added directly to the reaction until the pH was approximately 9. The reaction mixture was then stirred at room temperature for 10 hours. The reaction was monitored by thin layer chromatography using 15:1 $CHCl_3$-MeOH. Upon completion, the reaction mixture was neutralized with Amberlite IRC-50 C.P. ion exchange resin and filtered. The filtrate was concentrated and loaded onto silica and any remaining solvent was evaporated under reduced pressure. The dry slurry was purified by flash column chromatography (15 x 2 cm) on silica gel (230 x 400 mesh). Elution was with 15:1 $CHCl_3$-MeOH. The product fractions were combined, concentrated, and then dried under reduced pressure to provide a colorless oil. Yield = 40.3 mg (93.0%); TLC R_f = 0.3 (15:1 $CHCl_3$-MeOH); ^{1}H NMR (CD_3OD, 600 MHz): δ 0.09-.08 (m, 6H, $2CH_3$), 1.25 (d, 3H, *J* = 6.6 Hz, Rha CH_3), 1.26-1.1.32 (m, 42H, $21CH_2$), 1.42-1.36 (m, 2H, CH_2), 1.62-1.56 (m, 2H, CH_2), 2.03 (q, 2H, *J* = 7.2 Hz, CH_2), 2.18 (t, 2H, *J* = 7.2 Hz, CH_2), 3.67 (t, 1H, *J* = 9 Hz), 3.52 (d, 1H, *J* = 7.2 Hz), 3.66-3.60 (m, 2H), 4.04-3.98 (m, 2H), 4.66-4.36 (m, 1H), 5.48-5.42 (m, 1H, C*H*=CHCH_2CH_2), 5.72-5.65 (m, 1H, CH=C*H*CH_2); ^{13}C NMR (CD_3OD, 600 MHz): δ 14.50, 18.03, 23.80, 30.47, 30.57, 30.89,

33.14, 33.49, 37.26, 48.58, 48.86, 49.00, 49.28, 49.43, 54.30, 67.19, 69.81, 72.09, 73.16, 73.96, 101.35, 131.51, 134.97, 176.08; Mass spectrum (ESIMS), *m/z* = 706.7 $[M+Na]^+$ $C_{40}H_{77}NO_7Na$ requires 707.0.

5.2.18. (2*R*, 3*R*, 4*E*)-3-*O*-Alloxycarbonyloxy-1,2-*O*-isopropylideneoctadec-4-ene-1,2-diol (20). In a round bottom flask, (2*R*, 3*R*, 4*E*)-1,2-*O*-isopropylideneoctadec-4-ene-1,2,3-triol (0.232 g, 0.681 mmol) was dissolved in a dry 3:2 CH_2Cl_2:pyridine mixture (10 mL) and cooled to -10 °C. After cooling, allyl chloroformate (0.253 mL, 2.38 mmol) was added drop-wise. The solution was stirred at this temperature for 2 hours under N_2 atmosphere. The reaction was monitored by thin layer chromatography using 4:1 hexanes-ethyl acetate. Upon completion, the reaction was quenched with MeOH and concentrated under reduced pressure. The concentrated crude product was loaded onto silica and any remaining solvent was evaporated under reduced pressure. The dry slurry was purified by flash column chromatography (15 x 2 cm) on silica gel (230 x 400 mesh). Elution was with 9.5:0.5 hexanes-ethyl acetate. The product fractions were combined, concentrated, and then dried under reduced pressure to provide a colorless oil. Yield = 0.261 g (93.3%); TLC R_f = 0.64 (4:1 hexanes-ethyl acetate); ^{1}H NMR ($CDCl_3$, 600 MHz): δ 0.88 (t, 3H, CH_3, *J* = 7.2 Hz), 1.24-1.33 (m, 22H, $11CH_2$), 1.36 (s, 3 H, CH_3CCH_3), 1.44 (s, 3 H, CH_3CCH_3), 2.05 (m, 2H, CH=CHCH_2), 3.74 (d.d, 1H, OCH_2, *J* = 6.6, 8.4 Hz), 3.98 (d.d, 1H, OCH_2, *J* = 7.8, 8.4 Hz), 4.22 (m, 1H, OC*H*CH_2), 4.62 (m, 2H, OCH_2CH=CH_2), 5.06 (d.d, 1H, CHOAlloc, *J* = 7.8, 7.8 Hz), 5.26 (d, 1H, OCH_2CH=CH_2, *J* = 10.2 Hz), 5.34-5.39 (m, 2H, OCH_2CH=CH_2, C*H*=$CHCH_2$), 5.89-5.97 (m, 2H, OCH_2C*H*=CH_2, CH=C*H*CH_2); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 14.3, 22.8, 25.6, 26.6, 28.4, 28.8, 29.2, 29.4, 29.5, 29.58, 29.6, 29.7, 29.81, 29.82, 29.84, 32.1, 32.5, 56.5, 65.9, 68.6, 77.5, 80.5, 110.4, 118.9, 123.3, 131.7, 139.0, 154.4; Mass spectrum (HRMS), *m/z* = 447.4 $[M+Na]^+$ $C_{25}H_{44}O_5Na$ requires 447.6.

5.2.19. (2*R*, 3*R*, 4*E*)-2,3-*O*-Carbonate-octadec-4-ene-1,2,3-triol (21). In a round bottom flask, (2*R*, 3*R*, 4*E*)-3-*O*-alloxycarbonyloxy-1,2-*O*-isopropylideneoctadec-4-ene-1,2-diol (0.255 g, 0.621 mmol) was dissolved in a 1:1 THF:H_2O mixture (3 mL) and cooled to 0 °C. After cooling, glacial acetic acid (4.5 mL) was added drop-wise. The solution was then heated to 55 °C and stirred for 12 hours with a water-cooled reflux condenser. The reaction was monitored by thin layer chromatography using 4:1 hexanes-ethyl acetate. Upon completion, the reaction was quenched with saturated aq. $NaHCO_3$ and extracted with ethyl acetate (3x). The extracts were combined, dried ($MgSO_4$), and concentrated under reduced pressure. The concentrated crude product was loaded onto silica and any remaining solvent was evaporated under reduced pressure. The dry slurry was purified by flash column chromatography (15 x 2.5 cm) on silica gel (230 x 400 mesh). Elution was with 4:1 hexanes-ethyl acetate. The product fractions were combined, concentrated, and then dried under reduced pressure to provide an off-white solid. Yield = 129.0 mg (63.6%); TLC R_f = 0.05 (4:1 hexanes-ethyl acetate); ^{1}H NMR ($CDCl_3$, 600 MHz): δ 0.89 (t, 3H, CH_3, *J* = 7.2 Hz), 1.26-1.32 (m, 20H, $10CH_2$), 1.40 (m, 2H, CH=CHCH_2CH_2), 1.95 (d.d, 1H, OH, *J* = 6.0, 7.8 Hz), 2.11 (m, 2H, CH=CHCH_2), 3.70 (m, 1H, HOCH_2), 4.00 (m, 1H, HOCH_2), 4.38 (m, 1H, $HOCH_2$C*H*OC=O), 5.00 (d.d, 1H, OC*H*CH=CH, *J* = 7.8, 7.8 Hz), 5.52 (d.d, 1H, C*H*=$CHCH_2$, *J* = 7.8, 15.0 Hz), 5.97 (d.t, 1H, CH=C*H*CH_2, *J* = 7.8, 15.3 Hz); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 14.3, 22.9, 28.7, 29.3, 29.5, 29.6, 29.7, 29.8, 32.1, 32.3, 60.4, 79.4, 82.4, 123.9, 140.6, 155.2; Mass spectrum (HRMS), *m/z* = 349.2352 $[M+Na]^+$ $C_{19}H_{34}O_4Na$ requires 349.2355.

5.2.20. (2*R*, 3*R*, 4*E*)-2-Azido-1-*O*-benzoyloxy-octadec-4-ene-3-ol (22). In a round bottom flask, (2*R*, 3*R*, 4*E*)-2-azido-3-*O*-benzoyloxy-1-*O*-triisopropylsilyloxy-octadec-4-ene (0.138 g, 0.236 mmol) was dissolved in THF (3.5 mL) and cooled to 0 °C. After cooling, 1M TBAF (0.637 mL, 0.637 mmol) was added drop-wise and the reaction was allowed to come to room temperature. The reaction was stirred at this temperature for 10 minutes under N_2 atmosphere. The reaction was monitored by thin layer chromatography using 4:1 hexanes-ethyl acetate. Upon completion, the reaction was quenched with sat. aq. $NaHCO_3$. The aqueous layer was then extracted with ethyl acetate (4x) and the extracts were combined, washed with brine, dried ($MgSO_4$), and concentrated under reduced pressure. The concentrated crude product was loaded onto silica and any remaining solvent was evaporated under reduced pressure. The dry slurry was purified by flash column chromatography (22 x 2.5 cm) on silica gel (230 x 400 mesh). Elution was with 9:1 hexanes-ethyl acetate. The product fractions were combined, concentrated, and then dried under reduced pressure to provide a colorless oil. Yield = 99.0 mg (78.0%); TLC R_f = 0.15 (4:1 hexanes-ethyl acetate); ^{1}H NMR ($CDCl_3$, 600 MHz): δ 0.89 (t, 3H, CH_3, *J* = 7.2 Hz), 1.26-1.1.32 (m, 20H, $10CH_2$), 1.39 (m, 2H, CH=CHCH_2CH_2), 2.08 (m, 2H, CH=CHCH_2), 2.29 (d, 1H, OH, *J* = 3.6 Hz), 3.83 (m, 1H, CHN_3), 4.26 (m, 1H, C*H*OH), 4.42 (m, 1H, BzOCH_2), 4.56 (m, 1H, BzOCH_2), 5.57 (d.d, 1H, C*H*=$CHCH_2$, *J* = 7.2, 15.0 Hz), 5.84 (d.t, 1H, CH=C*H*CH_2, *J* = 7.8, 15.3 Hz), 7.47 (t, 2H, Ph, *J* = 7.8 Hz), 7.59 (t, 1H, Ph, *J* = 7.8 Hz), 8.08 (d, 2H, Ph, *J* = 8.4); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 14.3, 22.9, 29.1, 29.4, 29.5, 29.7, 29.8, 29.84, 29.85, 29.87, 29.88, 32.1, 32.5, 64.5, 65.2, 72.8, 127.6, 128.7, 129.8, 130.0, 133.5, 136.5, 166.5; Mass spectrum (ESIMS), *m/z* = 452.5 $[M+Na]^+$ $C_{25}H_{39}N_3O_3Na$ requires 452.6.

5.2.21. (2*S*,3*R*,*E*)-2-palmitamido-1-((triisopropylsilyl)oxy)octadec-4-en-3-yl benzoate (23).[16] In a round bottom flask, (2*R*, 3*R*, 4*E*)-2-Azido-3-*O*-benzoyloxy-1-*O*-triisopropylsilyloxy-octadec-4-ene (100.0 mg, 0.1708 mmol) was dissolved in THF (4 mL). After dissolution, triphenylphosphine (134.0 mg, 0.5124 mmol) and H_2O (61.5 µL) were added, and the reaction was heated to 45 °C with a cold-water condenser under N_2 atmosphere. The reaction was stirred at this temperature for 3.5 hours. The reaction was monitored by thin layer chromatography using 9.5:0.5 hexanes-ethyl acetate. Upon completion, the reaction mixture was quenched with Et_3N and filtered through Celite. The filtrate was concentrated and co-evaporated with toluene (1x) and used for further reaction without purification. The crude product was dissolved in dry CH_2Cl_2. After dissolution, HOBt (34.0 mg, 0.222 mmol), EDC·HCl (42.56 mg, 0.222 mmol), palmitic acid (43.8 mg, 0.1708 mmol), and Et_3N (35.73 µL, 0.2562 mmol) were added to the solution. The reaction mixture was stirred under N_2 atmosphere at room temperature for 7 hours. The reaction was monitored by thin layer chromatography using 9.5:0.5 hexanes-ethyl acetate. After completion, the solution was concentrated under reduced pressure. The concentrated crude product was loaded onto silica and any remaining solvent was evaporated under reduced pressure. The dry slurry was purified by flash column chromatography (15 x 2 cm) on silica gel (230 x 400 mesh). Elution was with 1→5% ethyl acetate in hexanes. The product fractions were combined, concentrated, and then dried under reduced pressure to provide a colorless gel. Yield = 64.0 mg (45.8% over two steps); TLC R_f = 0.17 (9.5:0.5 hexanes-ethyl acetate); Mass spectrum (HRMS), *m/z* = 820.6636 $[M+Na]^+$ $C_{50}H_{87}NO_4SiNa$ requires 820.6615.

5.2.22. *N*-((2*S*,3*R*,*E*)-1,3-dihydroxyoctadec-4-en-2-yl)palmitamide (24).[16] In a round bottom flask, (2*S*,3*R*,*E*)-2-

palmitamido-1-((triisopropylsilyl)oxy)octadec-4-en-3-yl benzoate (0.138 g, 0.236 mmol) was dissolved in THF (3.5 mL) and cooled to 0 °C. After cooling, 1M TBAF (0.637 mL, 0.637 mmol) was added drop-wise and the reaction was allowed to come to room temperature. The reaction was stirred at this temperature for 10 minutes under N_2 atmosphere. The reaction was monitored by thin layer chromatography using 4:1 hexanes-ethyl acetate. Upon completion, the reaction was quenched with sat. aq. $NaHCO_3$. The aqueous layer was then extracted with ethyl acetate (4x) and the extracts were combined, washed with brine, dried ($MgSO_4$), and concentrated under reduced pressure. The concentrated crude product was dissolved in a dry 2:1 MeOH:CH_2Cl_2 mixture (6 mL) under N_2 atmosphere. After dissolution, sodium metal was added directly to the reaction until the pH was approximately 9. The reaction mixture was then stirred at room temperature for 10 hours. The reaction was monitored by thin layer chromatography using 15:1 $CHCl_3$-MeOH. Upon completion, the reaction mixture was neutralized with Amberlite IRC-50 C.P. ion exchange resin and filtered. The filtrate was concentrated and loaded onto silica and any remaining solvent was evaporated under reduced pressure. The dry slurry was purified by flash column chromatography (15 x 2 cm) on silica gel (230 x 400 mesh). Elution was with 1:1 hexane-ethyl acetate. The product fractions were combined, concentrated, and then dried under reduced pressure to provide the compound **24**. Yield = 86.5% (over two steps); TLC R_f = 0.197 (1:1 hexanes-ethyl acetate). Mass spectrum (ESIMS), *m/z* = 560.7 $[M+Na]^+$ $C_{34}H_{67}NO_3Na$ requires 560.51.

5.3. Binding Study

5.3.1. Anti-Rhamnose Antibody Binding to Surface Exposed Rhamnosyl Ceramide 1 on EL4 cells; Fluorescence-activated cell sorting (FACS): EL4 lymphoma cells were cultured in DMEM medium with 10% fetal calf serum. Cells suspended in DMEM without serum were incubated with α-L-RhaCer (**1**) or ceramide **24** for 90 min at 37 °C. The cells were then washed and taken in Hank's balanced salt solution (HBSS) and then incubated with anti-Rha antibodies or non-Rha antibodies for one hour at 0 °C. The cells were washed and further incubated with FITC-conjugated goat anti-mouse IgG/IgM secondary antibody for one hour at 0 °C. The cells were then washed and taken in HBSS and flow cytometry was performed on an Accuri c6 flow cytometer.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/.