Rapid Preparation of Glycolipid Libraries by Cross Metathesis

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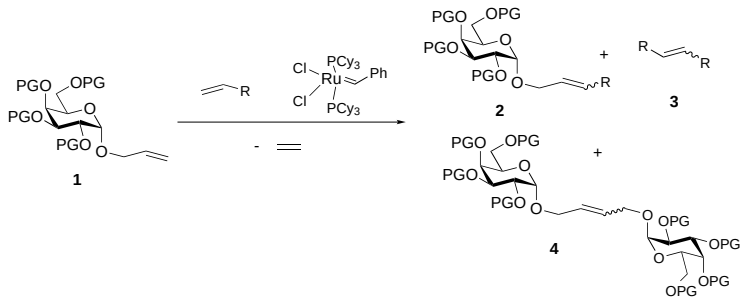
Abstract: A new strategy for the synthesis of a well-defined glycolipid library is presented. A highly selective cross metathesis reaction is used to diversify the lipid moiety of anomerically pure α- or β-glycosides, and subsequent functionalization of the double bond allows further expansion of the diversity. These glycolipids are being investigated for their effect on the immune system, especially the CD1-mediated T-cell activation.

Keywords: cross metathesis; glycolipid library; parallel synthesis

Carbohydrates conjugated with proteins or lipids play a crucial role in various cellular processes, including bacterial and viral infection, cancer metastasis, modulation and activation of the immune system, tissue differentiation and development, and many other intercellular recognition events.[1] Development of new methods for the synthesis of glycolipids with various structures on the lipid moiety is of current interest, as the lipid moiety of glycolipids is critical for cell-membrane and cell-wall assemblies and for various cellular signaling processes. One area of particular interest to us is the glycolipid and CD1-receptor interaction that activates killer T-cells.[2] Understanding the molecular requirement of glycolipids involved in this immuno-response may lead to development of new anti-cancer and anti-infective agents. Here we report the synthesis of α-galactosyl lipids with variation in the lipid moiety as an effort to identify novel glycolipids to activate T-cells and to study the structural requirements for binding to CD1.

α-Galactosylceramide has been reported to bind to the CD1d− receptor through the lipid-protein interaction. The sugar moiety is then presented to the natural killer T-cell to stimulate the T-cell activation. The structure and function study of such glycolipids has been very limited due to the difficult access to this class of molecules.[2] It is, however, clear that the α-isomer is responsible for the T-cell activation; the β-isomer shows no activity, though it is bound to CD1 as well as is the α-isomer. Conventional glycosidation, especially in the absence of participating neighboring groups, usually leads to an α/β mixture and requires tedious chromatographic purification. To avoid any formation of interfering β-products, we turned our attention to the use of anomerically pure α-galactosides with a short olefinic group for cross metathesis to create glycolipids with a high degree of diversity on the lipid moiety (Scheme 1).

Unlike the widely used ring-closing metathesis, cross metathesis has only recently gained more attention in organic synthesis, mainly due to the fact that up to six different reaction products could be generated in the



Scheme 1. Glycolipid synthesis via cross metathesis.

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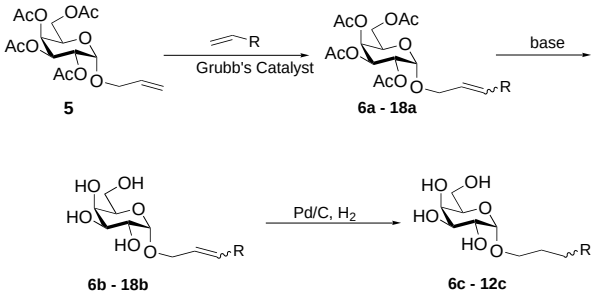
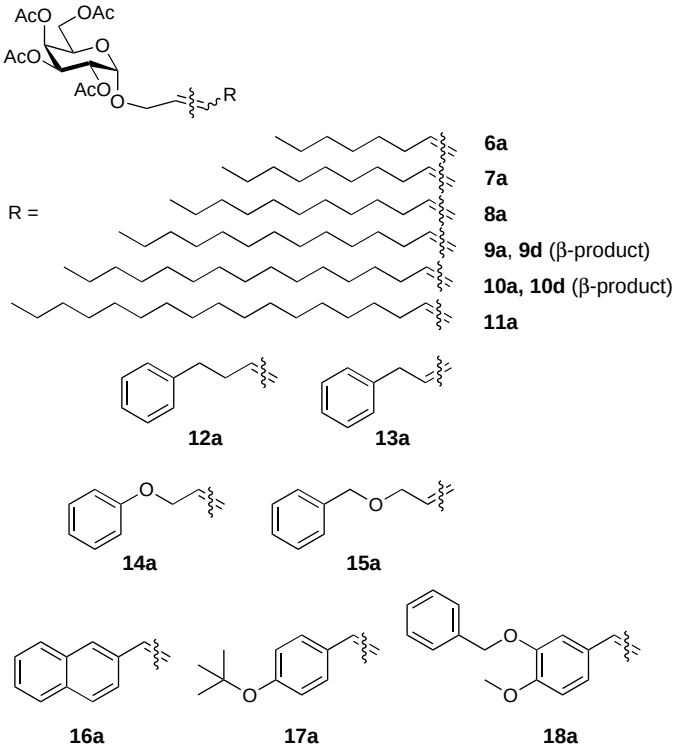


Scheme 2. Cross metathesis followed by deprotection and hydrogenation.

Table 1. Compounds prepared by cross metathesis.



Product	Yield [%]	*E/Z* ratio
6a	74	10:1:1
7a	73	6.6:1
8a	77	7.1:1
9a	73	7.7:1
9d	62	6.6:1
10a	77	7.6:1
10d	64	6.9:1
11a	73	6.2:1
12a	80	6.7:1
13a	69	5.2:1
14a	77	4.0:1
15a	69	6.8:1
16a	53	4.2:1
17a	72	20.2:1
18a	44	*E* only

reaction. Besides the desired product **2**, which is usually obtained as a mixture of *E* and *Z* isomers, the two products **3** and **4**, resulting from self-metathesis of each olefin could be observed, each one being a mixture of *E* and *Z* isomers. To be useful in synthesis, a method has to be developed to obtain a well-defined product without presence of by-products.

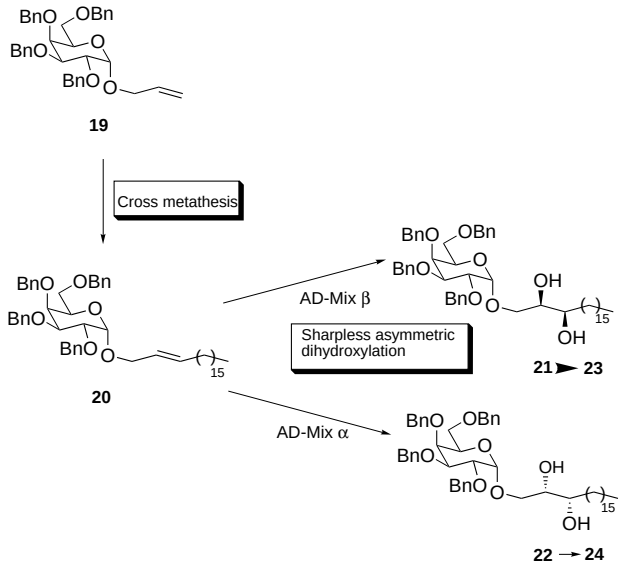
In the field of carbohydrate chemistry, although metathesis has been used,[3] there are only few examples for the use of cross-metathesis, most of which are methodology studies.[4–6] The advantage of this method is that we are able to have a complete stereo-control of the anomeric center, as a small contamination of the β-isomer could affect the biological activity. The allyl galactosides used in this study are either commercially available or easily accessible and easy to purify. Another advantage of this method is its general applicability and simplicity; one can use various readily available olefins as the component to create a library of glycolipids. Also the double bond bears potential for further functionalization of the molecule (Scheme 2).

Dimerization of the carbohydrate moiety, as observed by Roy and coworker,[5] leading to compound **4**, could be suppressed successfully by employment of 4 equivalents of the lipid-olefin and heating the reaction mixture under reflux in dichloromethane for at least 16 h. The excess of the nonpolar (dimerized) reagent could be removed by elution with hexanes upon chromatography of the reaction mixture (Scheme 2).

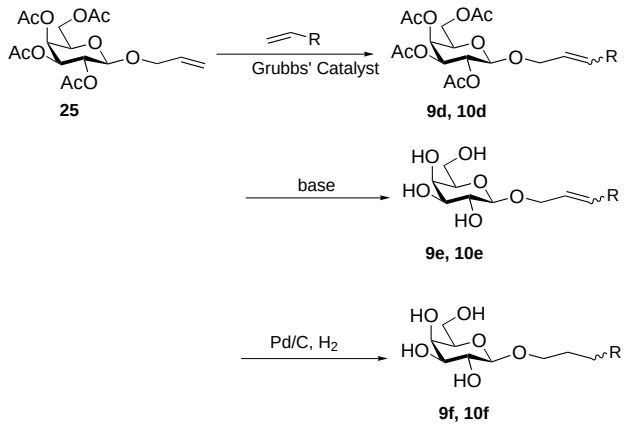
The unsaturated glycolipids were obtained in good yields and the *E/Z*-ratios were mostly higher than 6:1 (see Table 1). The compounds were deprotected to furnish unsaturated derivatives **6b** – **18b**. It should be noted that deprotection of the ester groups of the labile compounds **17a** and **18a** required mild reaction conditions (MeOH/water/NEt_3 instead of sodium methoxide in methanol). By hydrogenation, the saturated compounds **6c** – **12c** were obtained. We have not only introduced simple, non-functionalized olefins, but also styrene or allylic ether derivatives to the galactose anomeric position using this straight-forward method (compounds **14** – **18**).

When the double bond of the resulting olefin **2** is hydrogenated, the *E/Z* ratio of the product is deemed meaningless. However, for diastereoselective functionalization of the yielded product, we felt it would be useful to have a method to give access to the pure *trans* olefin.

We found that by using perbenzylated allyl galactoside **19**[7] as a starting material, only *E*-product can be obtained when non-functionalized olefins are used. This observation allowed further functionalization of the double bond to give diastereomerically pure products. We have, for example, successfully dihydroxylated compound **20** with AD-Mix α and β. The products were obtained in good diastereoselectivity (95% de for **21**, 83% de for **22**). The traces of minor diastereomer



Scheme 3. Cross metathesis followed by asymmetric dihydroxylation.



Scheme 4. Cross metathesis of â-allylgalactose followed by deprotection and hydrogenation.

could be removed by chromatography to yield pure galactosyl lipids **21** and **22**. These compounds were deprotected by hydrogenation using Pd on charcoal as catalyst to give **23** and **24**, respectively.

For examination of the influence of the anomeric center on the cross metathesis reaction, certain β-galactosides (**9d** and **10d**) were synthesized as well, following the same method, starting from β-*O*-allyl galactoside **25**.[8] (Scheme 4) The yields and *E/Z* ratios were, however, comparable to those of the α-galactosides (Table 1). The compounds were liberated as described for the α-series to give **9e**/**10e** and **9f**/**10f**, respectively.

In summary, a structurally diverse galactolipid library has been synthesized rapidly via cross metathesis and further functionalization. This method is simple, effective, and suitable for parallel and/or automated synthesis of glycolipid libraries as well as for large-scale processing. The obtained glycolipids are well suited for further transformation into more complex structures, since this method leads to structurally well-defined olefins.

Using the described glycolipid library, we were able to perform several studies. For example we have identified optimal lipid linkers for the non-covalent attachment of glycolipids to microtiter plates.[9] Thus, the saturated hydrocarbon chain with 15 to 17 carbons interacts with the plate surface more strongly than does BSA (bovine serum albumin) and completely withstands the aqueous washing conditions. Also **23** and **24** did show interesting results in a CD1d-based immunoassay. Both biological studies will be published separately.

Experimental Section

General Remarks

Reagents of commercial quality were purchased from Aldrich or Sigma; Grubbs' catalyst from Strem Chemicals, Inc., dichloromethane (CH_2Cl_2) was distilled over calcium chloride. All reactions were carried out under an argon atmosphere with dry solvents under anhydrous conditions, unless otherwise noted. Analytical thin-layer chromatography was performed using silica gel 60 F_{254} glass plates (Merck), compound spots were visualized by UV light (254 nm) and by staining with a yellow solution containing $Ce(NH_4)_2(NO_3)_6$ (0.5 g) and $(NH_4)_6Mo_7O_{24} \cdot 4\,H_2O$ (24.0 g) in 6% H_2SO_4 (500 mL). Flash chromatography was performed on silica gel 60 Geduran (35 – 75 μm, EM Science). High resolution mass spectra were recorded on a VG ZAB-ZSE instrument with fast atom bombardment (FAB). ^{1}H NMR and ^{13}C NMR spectra were obtained on Bruker AMX 400, DRX 500 and DRX 600 MHz instruments.

Glycolipid Library by Cross Metathesis

One mmol of tetra-*O*-acetyl α-D-allyl galactoside **5** (or tetra-*O*-acetyl β-D-allyl galactoside **25**) was dissolved in 5 mL of dry dichloromethane and put in an oven-dried flask. Four equivalents of the corresponding coupling partner and 0.07 mmol of Grubbs' catalyst, dissolved in 5 mL of dry dichloromethane, were added via syringe. The reaction mixture was refluxed rapidly for about 16 h. Conversion of the starting material could either be detected by TLC or by direct NMR measurements of an aliquot of the reaction mixture (e.g., using APT spectra). The solution was concentrated under vacuum and directly chromatographed on a silica gel column. An elution step with hexanes was performed first to remove excess of the coupling partner, subsequently the column was eluted with hexanes:ethyl acetate mixtures to give the described products **6a – 18a, 9d**, and **10d**. The *E/Z* ratio was determined by ^{1}H NMR spectroscopy. Representative data are given below.

2,3,4,5-Tetra-*O*-acetyl-α-pentadec-2-enyl D-galactoside (9a): ^{1}H NMR (MeOD, 400 MHz): δ = 5.61 (dt, 1H, *J* = 15.0, 7.0 Hz), 5.38 (m, 1H), 5.35 (dd, 1H, *J* = 3.3, 1.0 Hz), 5.26 (m,

1H), 5.05 – 5.00 (m, 2H), 4.15 (t, 1H, $J = 6.6$ Hz), 4.07 – 3.94 (m, 3H), 3.85 (dd, 1H, $J = 12.2$, 6.9 Hz), 2.03 (s, 3H), 1.99 – 1.92 (m, 2H), 1.97 (s, 3H), 1.94 (s, 3H), 1.87 (s, 3H), 1.26 (m, 2H), 1.21 – 1.11 (m, 18H), 0.77 (t, 3H, $J = 6.5$ Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): $\delta = 170.01$, 169.95, 169.92, 169.63, 135.94, 124.46, 94.70, 68.40, 67.94, 67.91, 67.46, 66.06, 61.45, 32.07, 31.68, 29.44, 29.40, 29.35, 29.25, 29.11, 29.98, 28.78, 22.45, 20.48, 20.38, 20.34, 13.85; MALDI-FTMS: calcd. for $C_{29}H_{48}O_{10}Na^+$: 579.3139; found: 579.3143.

Deacetylation of Galactosyl Lipids

The acetylated product was dissolved in 10 mL of methanol and 1 mL of NaOMe in MeOH (10%) was added. The solution was stirred for 3 h. A small amount of ion exchange resin (Amberlyst 15, H^+-form) was added to remove sodium ions. The reaction mixture was diluted with methanol and ethyl acetate and the resin was filtered off and washed thoroughly. The filtrate was taken to dryness to give the corresponding unsaturated galactolipids **6b – 16b**, **9e**, and **10e** as white solids. Representative data are given below.

α-Pentadec-2-enyl D-galactoside (9b): ^{1}H NMR (MeOD, 500 MHz): $\delta = 5.71$ (dt, 1H, $J = 15.0$, 6.9 Hz), 5.56 (m, 1H), 4.82 (d, 1H, $J = 3.3$ Hz), 4.12 (dd, 1H, $J = 11.6$, 5.7 Hz), 3.94 (dd, 1H, $J = 12.1$, 7.0 Hz), 3.85 (dd, 1H, $J = 2.0$, 1.1 Hz), 3.77 (t, 1H, $J = 5.9$ Hz), 3.74 – 3.62 (m, 4H), 3.27 (m, OH), 2.01 (q, 2H, $J = 7.0$ Hz), 1.35 (m, 2H), 1.31 – 1.18 (m, 18H), 0.86 (t, 3H, $J = 7.0$ Hz); ^{13}C NMR (MeOD, 125 MHz): $\delta = 135.98$, 127.10, 99.04, 72.31, 71.52, 71.03, 70.20, 69.13, 62.69, 33.35, 33.04, 30.77, 30.74, 30.73, 30.72, 30.61, 30.45, 30.31, 30.27, 23.70, 14.45; MALDI-FTMS: calcd. for $C_{21}H_{40}O_6Na^+$: 411.2717; found: 411.2715.

Mild Deacetylation

The acetylated galactosyl lipid was dissolved in a 3:3:1 mixture of methanol, water, and triethylamine. The reaction mixture was stirred overnight, evaporated to dryness, and the product was purified on a short silica gel column using ethyl acetate/methanol/water (9:1:0.1) as eluent to give the unsaturated galactosyl lipids **17b** and **18b**.

α-3-[(4-*tert*-Butoxy)-phenyl]-prop-2-enyl D-galactoside (17b): ^{1}H NMR (MeOD, 500 MHz): $\delta = 7.10$ (d, 2H, $J = 8.1$ Hz), 6.68 (d, 2H, $J = 8.1$ Hz), 6.39 (d, 1H, $J = 15.8$ Hz), 6.04 (dt, $J = 16.1$, 6.2 Hz), 4.68 (d, 1H, $J = 3.3$ Hz), 4.11 (ddd, 1H, $J = 12.7$, 5.7, 1.1 Hz), 3.94 (ddd, 1H, $J = 13.0$, 6.1, 0.8 Hz), 3.67 (dd, 1H, $J = 2.2$, 0.8 Hz), 3.62 (t, 1H, $J = 6.1$ Hz), 3.55 (t, 2H, $J = 3.1$ Hz), 3.48 (dd, 2H, $J = 5.9$, 4.0 Hz), 1.06 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz): $\delta = 156.29$, 133.51, 133.33, 128.10, 125.53, 125.13, 99.44, 79.67, 72.47, 71.47, 71.01, 70.18, 69.23, 62.72, 29.20; MALDI-FTMS: calcd. for $C_{19}H_{28}O_7Na^+$: 391.1727; found: 391.1726.

Hydrogenation of Galactosyl Lipids

The catalyst Pd on charcoal (50 mg, 10%) was suspended in 20 mL of methanol. The suspension was set under hydrogen and stirred vigorously for 15 min. The unsaturated galactosyl lipid (100 mg), dissolved or suspended in methanol, was added and the reaction mixture was stirred overnight. The reaction mixture was diluted with methanol and ethyl acetate, filtered through Celite, and evaporated to dryness to yield the saturated galactosyl lipids **6c – 12c**, **9f**, and **10f**. Representative data are given below.

α-Pentadecyl D-galactoside (9c): ^{1}H NMR ($CDCl_3$, 500 MHz): $\delta = 4.77$ (s, 1H), 3.97 (d, 1H, $J = 1.8$ Hz), 3.77 (t, 1H, $J = 6.1$ Hz), 3.75 – 3.65 (m, 5H), 3.41 (m, 1H), 1.60 (m, 2H), 1.40 – 1.21 (m, 24H), 0.87 (t, 3H, $J = 7.0$ Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): $\delta = 100.26$, 72.21, 71.52, 71.00, 70.24, 69.20, 62.64, 33.04, 30.78, 30.74, 30.64, 30.45, 27.32, 23.70, 14.47; MALDI-FTMS: calcd. for $C_{21}H_{42}O_6Na^+$: 413.2873; found: 413.2870.

Preparation of Dihydroxylated Compounds 23 and 24

Compound **20** was prepared by cross metathesis as described for the synthesis of compounds **6a – 18a** using α-allyl-2,3,4,6-tetra-*O*-benzyl D-galactoside[8] as the glycosidic component. The isomerically pure compound was isolated by column chromatography. AD-Mix [α or β] (1.4 g) was dissolved in a 9 mL of a mixture of *tert*-butanol and water (ratio 4:5). The mixture was stirred until phases went clear. Methanesulfonamide (95 mg, 1 mmol) was added and the solution was cooled to 4 °C. The starting material (0.5 mmol) dissolved in *tert*-butanol (1 mL) was added to the reaction mixture and stirring was continued until complete conversion was detected by TLC. Sodium sulfite (1.4 g, 11.1 mmol) was added and the solution was allowed to warm to room temperature. The reaction mixture was diluted with EtOAc and the aqueous layer extracted with EtOAc (3×). The combined organic layers were extracted with saturated NaCl and dried over sodium sulfate. After evaporation of the solvent, the major diastereomer was obtained in pure form by column chromatography and subsequently deprotected by hydrogenation as described above for compounds **6 – 12c**.

α-[(2*S*,3*S*)-2,3-Dihydroxynonadecyl)] D-galactoside (23): ^{1}H NMR (pyridine-d_5, 500 MHz): $\delta = 5.88$ (br, OH), 5.52 (d, 1H, $J = 3.7$ Hz), 4.70 (dd, 1H, $J = 9.8$, 3.8 Hz), 4.60 (m, 2H), 4.54 (dd, 1H, $J = 9.8$, 3.0 Hz), 4.51 – 4.46 (m, 1H), 4.43 (m, 2H), 4.26 (m, 1H), 4.11 – 4.10 (m, 1H), 1.95 – 1.81 (m, 2H), 1.79 – 1.69 (m, 1H), 1.60 – 1.50 (m, 1H), 1.40 – 1.15 (m, 27H), 0.85 (t, 3H, $J = 6.9$ Hz); ^{13}C NMR (pyridine-d_5, 125 MHz): $\delta = 101.66$, 73.51, 72.92, 72.22, 71.80, 71.74, 71.011, 70.67, 62.65, 34.33, 32.08, 30.22, 30.05, 29.97, 29.95, 29.89, 29.57, 26.70, 22.90, 14.25; MALDI-FTMS: calcd. for $C_{25}H_{50}O_8Na^+$: 501.3398; found: 501.3393.

Supporting Information

Complete characterization for compounds **6a – 18a**, **6b – 18b**, **6c – 12c**, **9d**, **10d**, **9f**, **10f**, and **20 – 24** is available on the WWW under http://www.wiley-vch.de/home/asc/.

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